Wogonoside: Anti-human colon cancer activities and survey of HMG-CoA reductase inhibition properties with molecular modeling

Type
Research paper

Keywords
wogonoside, HMG-CoA reductase, colon cancer, molecular docking, enzyme activity

Abstract
Introduction
The biological activities and interactions of wogonoside in the presence of HMG-CoA reductase were investigated using the molecular docking study as a versatile theoretical approach. Wogonoside showed a considerable binding affinity to the enzyme with a docking score of -7.582 kcal/mol.

Material and methods
The in vitro cytotoxic and anti-colon carcinoma effects of biologically synthesized Wogonoside against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines were assessed.

Results
The results indicated that the compound makes hydrophobic contacts with essential residues of the catalytic domain of the enzyme. Therefore, wogonoside could be considered as a potential inhibitor for HMG-CoA reductase. The IC50 of the Wogonoside were 105, 198, 173, 382, 71, and 183 µg/mL against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines. The anti-colon carcinoma properties of the Wogonoside could significantly remove GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines in a time and concentration-dependent manner by MTT assay.

Conclusions
It appears that the anti-human colorectal carcinoma effect of recent nanoparticles is due to their antioxidant effects. We have obtained results for the HMG-CoA Reductase enzyme at the micromolar level. In our study, inhibition result of on HMG-CoA reductase showed lower values 28.70±4.73 micromolar.
Wogonoside: Anti-human colon cancer activities and survey of HMG-CoA reductase inhibition properties with molecular modeling

Jiamiao Liu1,*, Huifang Tan1, Qing Zeng1

Surgical, Yichun Traditional Chinese Medicine Hospital, lingquan street, Yichun City, Jiangxi Province, 336000, China

*Corresponding author: Jiamiao Liu: liu1958791739@163.com
Abstract

The biological activities and interactions of wogonoside in the presence of HMG-COA reductase were investigated using the molecular docking study as a versatile theoretical approach. Wogonoside showed a considerable binding affinity to the enzyme with a docking score of -7.582 kcal/mol. The results indicated that the compound makes hydrophobic contacts with essential residues of the catalytic domain of the enzyme. Therefore, wogonoside could be considered as a potential inhibitor for HMG-COA reductase. The in vitro cytotoxic and anti-colon carcinoma effects of biologically synthesized Wogonoside against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines were assessed. The IC$_{50}$ of the Wogonoside were 105, 198, 173, 382, 71, and 183 µg/mL against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines. The anti-colon carcinoma properties of the Wogonoside could significantly remove GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines in a time and concentration-dependent manner by MTT assay. It appears that the anti-human colon carcinoma effect of recent nanoparticles is due to their antioxidant effects. We have obtained results for the HMG-CoA Reductase enzyme at the micromolar level. In our study, inhibition result of on HMG-CoA reductase showed lower values 28.70±4.73 micromolar.

**Keywords:** wogonoside, HMG-COA reductase, colon cancer, molecular docking, enzyme activity
1. Introduction

Cancer, one of the deadliest causes of death in the world, kills more than 7.5 million people annually. Genetic factors and environmental factors are among the most important factors involved in the development of cancer. On the other hand, environmental factors such as diet, lifestyle, geographical conditions, stressors, age and obesity are involved in the incidence of this disease. Clinicians use a variety of methods to diagnose and treat a variety of cancers, the most important of which are chemotherapy, radiation therapy, surgery, and hormone therapy, but the most important way to fight cancer is chemotherapy, which causes many side effects for the patient, including fatigue, nausea and hair loss, etc [1-4]. These complications can have a great effect on the patient's life quality. Because many of the chemical drugs used to treat cancer cause gastrointestinal disorders, kidney damage, etc. Scientists are looking for drugs with fewer side effects than chemical materials, in which natural compounds and molecules have received much attention [4-7]. Natural compounds and molecules have fewer side effects than chemical materials due to the combination of other compounds with a specific drug effect. Many natural compounds and molecules contain anti-cancer agents that can exert their effects at different stages of the onset and growth of cancer cells. The main goal in preventing cancer with natural or chemical substances is to slow down or inhibit the carcinogenic process. This approach focuses purposefully on abnormal intracellular pathways that lead to abnormal cellular function [5-9].

The gradual maturation of biotechnology has been considered not only for treating cancer but also for a wide variety of applications, especially for drug delivery and diagnostic and imaging cases. There are many types of molecules available and choosing the right carriers according to demand is a key issue [7-12]. Molecules are very close in size to biological molecules in terms of size and can easily penetrate into the cell, for this reason, one of the goals of biotechnology is to
mount molecules and drugs on molecules and transfer them to the target cell [7-13]. It is also possible to create different surface properties for molecules by attaching protective ligands to increase the molecules resistance to the immune system and increase their presence in the bloodstream, and even binding ligands to specifically bind the molecules to the target tissue [11-15].

Hypercholesterolemia, known as high cholesterol, is a common disease in which excess fat and fatty acids accumulate in the blood. Hypercholesterolemia is one of the major risk factors that cause atherosclerosis and coronary heart diseases. Especially increased low-density lipoprotein (LDL) and triglyceride levels, which cause hypercholesterolemia, also lead to diseases such as obesity, diabetes and cancer. HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase enzyme catalyzes the reaction in which HMG-CoA is converted to mevalonate through the mevalonate pathway in cholesterol biosynthesis. HMG-CoA reductase is the rate-limiting enzyme that regulates cholesterol synthesis in the body [16-18].

Theoretical studies like molecular docking are beneficial methods that can provide a reliable insight into the interactions and biological activities of various compounds in the presence of biomolecules [19]. The outcomes of such studies can help the biologists to find the mechanisms in which the chemical inhibitors would have in the vicinity of related enzymes [20]. In general, the theoretical approaches are practical complementary information that can support the results of experimental studies.

In the recent study, also the properties of Wogonoside against common colon carcinoma cell lines i.e. GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM were evaluated.

2. Experimental
Enzyme assay

HMG-CoA Reductase activity according to the method described by Takahashi S. et al.;

\[
\text{(DLHMG-CoA + 2NADPH + 2H} \rightarrow \text{(R) -mevalonate + 2NADP + +}
\]
was measured spectrophotometrically by using the absorbance change of NADPH at 340 nm in accordance with the reaction. 3. NaN\(_3\): 2M; 4. Phosphate buffer: pH: 7.2; 50 mM: Experiment Conducting Spectrophotometer After zero adjustment against distilled water at 340 nm, phosphate buffer is added to the test tubes separately as blank and sample for each sample, NaN\(_3\), NADPH and the sample were added and mixed thoroughly and the absorbance change was followed for 1 minute [21]. Then HMG-CoA was added to the cuvette and the absorbance change was monitored for 3 minutes. HMG CoA activity was calculated using the coefficient of NADPH. \(\varepsilon: 6.22\times10^3 \text{ L} / \text{mol.cm.}\) is given as IU / L. activities are measured, then we started the IC50 study after the control value was found. For the IC50 study, we used six different inhibitor concentrations and wrote down the activities and then plotted the IC50. After obtaining the (concentration-percent activity) equation, we calculated the IC50 value. We repeated this task 3 times and calculated the standard deviation and found that our work was correct [22].

Molecular docking study

Molecular docking was used for the assessment of biological activities of wogonosid against the HMG-COA reductase activity. The enzyme structure 2.22Å and X-RAY diffraction method was obtained from the PDB database (http://www.rcsb.org/pdb) (PDB ID: 1HWK) [23]. The enzyme was subjected to some preparation steps before molecular docking calculations. At the first step, the addition of hydrogen molecules was performed. After that, the water molecules were removed from the structure, and a network of H-bond was created using the optimization step. Finally, the minimization step was applied to the structure utilizing the OPLS3e force field. This
preparation process was performed with the protein preparation module of the Schrödinger Suite [24]. The prepared protein was then assessed for the prediction of the binding site, and the active sites of the protein were predicted using SiteMap of Schrödinger [25]. The structure of wogonoside as an SDF file was downloaded from the PubChem database and then prepared with the LigPrep module of Schrödinger [26] to produce the correct molecular geometric and suitable protonation states. A grid box of 20 × 20 ×20 (Å³) was generated around the first active site, and the molecular docking was conducted using the Glide of Schrödinger suites.

**Anti-human colon carcinoma properties of Wogonoside**

The process of the controlled culture of prokaryotic or eukaryotic cells in a filtered or unfiltered flask or cell culture plate by a suitable culture medium is called. This term is mostly used for culturing multicellular cells. Special culture media are used to culture cells. The cells are usually cultured at 37 °C in equipment such as CO₂ incubators. Cell culture should be performed under aseptic (disinfected) conditions because the growth of these cells is much slower than the growth of bacteria and yeasts and there is a possibility of contamination of the culture medium. Antibiotics such as penicillin, streptomycin, or gentamicin are sometimes used to stop the growth of bacteria. In order for cells to proliferate well in culture medium, their density in culture medium must be low. For this purpose, the cells should be passed to the fresh culture medium from time to time. One of the goals of cell culture is to study cells in terms of how they grow, their nutritional needs, and the reasons they stop growing, each of which can have a profound effect on the morphology of the cells we see under a microscope. Therefore, to study the cell growth cycle, develop methods to control the growth of cancer cells and modulate the expression of genes, it is necessary to cultivate these cells in the external environment [27]. With the help of
cell culture, cells can be prepared that are in different stages of differentiation and can be differentiated into other cells with the help of hormones and growth factors. With the help of cell culture, homogenous cells can be prepared and intracellular activities such as DNA replication, DNA transcription synthesis, RNA and protein synthesis and other details related to metabolism can be studied. It is also possible to examine the subsequent events and intracellular currents, such as the displacement of these complexes, the type of intracellular messages, and how the messages are transmitted, after connecting different molecules to the corresponding membrane receptor. The cultured cells can be stored frozen at very low temperatures. Such conditions will maintain the growth rate or genetic composition of these cells and can be thawed and used again at the appropriate time. This prevents the aging of cells, while it is currently not possible to prevent the aging of animals. When working with laboratory animals, systemic changes due to the effect of the animal's natural homeostasis or the stress of the experiments on the results should be considered, while the use of cell culture eliminates this problem. In addition, standardizing laboratory tests is easier and more practical than tests on living organisms. In laboratory environments, it is much easier to control the physical and chemical factors in the living environment of cells, including acidity, heat, osmotic pressure, and the pressure of gases such as oxygen and carbon dioxide. Cells that are taken directly from the individual are known as primer cells and have a limited lifespan. Most cells have a limited lifespan, except for those taken from a tumor. An immortal cell line can proliferate indefinitely by creating a random or targeted mutation (such as artificial expression of the genus and be established as a representative of specific cell types [27].

In this research, we used Human Umbilical Vein Endothelial Cells (HUVECs) to determine the cytotoxicity potentials of Wogonoside using MTT. Also, the in vitro anti-colon carcinoma effects
of biologically synthesized Wogonoside against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cancer cell lines were evaluated.

In the recent study, the cells were cultured in medium (RPMI1640 = Roswell Park Memory Institute1640) with 10% FBS combined with penicillin and streptomycin antibiotics in an incubator containing 5% CO₂ in a flask (T25). After three passages for purification, the cells were used to perform the next steps. Cell count and the number of viable cells were performed with a homocytometer slide using trypan blue. Evaluation of the cytotoxic effect of the Wogonoside was performed by the modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric test. In this method, MTT, which is yellow, is converted to insoluble and formazan purple dye by the dehydrogenase enzymes in the mitochondria of active cells. The adsorption of this compound can be measured after dissolving at 570-540 nm. After two days and covering the flask bottom with cell, the cell layer adhering to the flask bottom was isolated enzymatically using trypsin-EDTA (5%) (Tetraacetic acid ethylenediamine), after transfer to sterile test tubes, it was centrifuged at 2000 rpm for 10 minutes. The cells were then resuspended in a fresh culture medium with the help of a pasteur pipette and cell suspension (10⁶ ml/μg) was prepared from them. 40 μl of this cell suspension (equivalent to 10⁴×4 cells) was poured into 96-well plate flat-bottomed wells (for cell culture). Then the final volume of each well with 10% FBS medium reached 200 μl. The first-row containing cell suspension was considered as negative control (control). After incubation for 18-24 hours to remove cells from the stress caused by trypsinization, the supernatant was removed slowly and carefully, A new medium was added to all rows with different concentrations of the Wogonoside (only a new medium was added to the negative and positive control rows), so that the diluted Wogonoside with concentrations of 1-1000 μg/ml was added to the third to sixth rows, respectively, the plate
was incubated in CO2 for 48, 24 and 72 hours. After the incubation time, the plate was taken out of the incubator and 20 μl of MTT (Sigma) was added to all wells, and incubated for 3 hours. The supernatant was then gently removed and 100μl of DMSO was added to the wells and pipetted to dissolve the formazan crystals. The amount of light absorption (OD) according to the intensity of the blue color of formazan at 540 nm was read by Eliza reader. To convert OD to the percentage of living cells, the following formula was used and the percentage of cell life at each concentration was calculated after 48, 24 and 72 hours [27].

\[
\text{Cell viability (\%)} = \frac{\text{Sample A. COntrol A.}}{x 100}
\]

The concentration of the tested compounds that reduced the percentage of cell life by half was considered as IC50 (The half maximal inhibitory concentration) [27].

At least three independent replications were performed for each data and the result was presented as mean ± SD. Data statistical analysis was done with SPSS software version 22 and Anova Way One and Duncan tests. Significance was considered at the level of \( P \leq 0.05 \).

3. Results and discussion

Enzyme result

HMG-CoA reductase inhibitor compounds are mostly prescribed for lipid-lowering to treat hypercholesterolemia. Additionally, they are well tolerated; their pharmacological interactions with other drugs can give rise to some inverse clinical consequences [28,29]. The two main classes of these enzyme inhibitors are constituted by competitive and oxysterols inhibitors [30]. Oxysterol molecules, which are oxidative factors of cholesterol can play an important role in the
regulation of enzyme activity in mammalian cells. Additionally, oxysterols exhibit a diversity of effects independent of the inhibition of this important enzyme. In our study, inhibition result of wogonoside on HMG-CoA reductase showed lower values 28.70±4.73.

**Docking results**

The biological activities of wogonoside in the proximity of HMG-COA reductase were investigated using the molecular docking study. The docking pose of wogonoside among the residues of the enzyme is shown in Fig. 1, and Fig. 2 shows the interactions between this chemical compound and HMG-COA reductase. It is apparent that the ligand has created several hydrophobic contacts and hydrogen bonds with the enzyme residues. The residues with hydrogen bonds are Gly560 and Arg590. For the hydrogen bond of Gly560, this residue is a hydrogen bond donor, while in the case of Arg590, the amino acid is hydrogen bond acceptor. It shows that wogonoside can make interactions with biomolecules in both hydrogen bond acceptor and hydrogen bond donor manner. There are also thirteen hydrophobic contacts between wogonoside and the enzyme, which could lead to an adequate inhibitory activity for the compound. One of the residues with hydrophobic contact is Glu559, which has previously been reported to be one of the crucial members of catalytic residues of the enzyme [31]. Another residue with hydrophobic contact is Asp767, and like Glu559, this amino acid is among the catalytic residues of the enzyme [32]. These hydrophobic contacts can increase the inhibitory activity of wogonosid against HMG-COA reductase. Various parameters obtained from the docking calculations are presented in Table 1. As could be seen, the compound has a remarkable binding affinity to the enzyme with a docking score of -7.582 kcal/mol. The other parameters show the characteristics of the ligand-enzyme complex. For instance, Glide Ligand Efficiency shows the binding energy between the molecule and the binding partner. Glide Evdw and Glide Ecoul
present the Van der Waals and Coulomb energy, respectively. The value of interaction pose is numerically calculated with Glide Emodel. The position of the first active site in the enzyme structure is presented in Fig.3. Two essential parameters for the evaluation of an active site are Dscore and site score. Dscore for the first active site is 1.040, and the site score is 1.072. Both of the scores are greater than one, which shows the reliability of the predicted active site. These scores also indicate the suitable drugability of this site. This drugability approves this site is able to bind to the drug-like molecules tightly. In Fig. 3, the blue color shows the hydrogen bond donor regions and the red color indicates the hydrogen bond acceptor areas. The hydrophobic regions are presented with yellow color. The residues that are engaged in the first active site are presented in Table 2. In general, wogonoside is able to create several hydrophobic contacts and hydrogen bonds with various amino acids of the HMG-COA reductase in both hydrogen bond donor areas and hydrogen bond acceptor regions. In addition to the experimental studies, these results can also approve that wogonoside could be considered as a potential inhibitor for this enzyme.

Fig 1. The docking pose of wogonoside among HMG-COA reductase residues.
Fig. 2. The interactions of wogonoside and HMG-COA reductase. Green dashed lines indicate the hydrogen bonds, and semicircles show the hydrophobic contacts.
**Fig. 3.** The position of the first active site of the HMG-COA reductase.

**Table 1.** The parameters obtained from the molecular docking calculations.

| Parameters                                | HMG-CoA reductase |
|-------------------------------------------|-------------------|
| IC₅₀ (mM)                                 | 28.70             |
| Docking score (kcal/mol)                  | -7.582            |
| Glide ligand efficiency (kcal/mol)        | -0.230            |
| Glide Ecoul (kcal/mol)                    | -8.768            |
| Glide Evdw (kcal/mol)                     | -38.848           |
| Glide Emodel (kcal/mol)                   | -59.335           |
| Glide energy (kcal/mol)                   | -47.615           |

**Table 2.** The residues of the first active site with Dscore of 1.040 and site score of 1.072.
Anti-Cancer Part

Cancer is recognized as one of the leading causes of death in today's society and several drugs have been introduced to treat this disease, but, most common cancers are not yet controllable and this disease imposes huge costs on the patient and society. The main factor in the development and progression of cancer has not yet been precisely identified, however, the available data suggest that metabolic disorders in the tissue and immune disorders may be involved in the development and exacerbation of this disease [33]. In addition, metabolic disorders in the production and excretion of oxygen free radicals are important factors affecting cancer cells. Free radicals are destructive compounds that are produced as a by-product by the body's chemical reactions and are destroyed by the body's defense system and enzyme system and antioxidants. However, in cases where the body's metabolic disorders and the production of free radicals are high and they are not destroyed by the neutralizing system, due to their instability, these compounds have a strong tendency to react with a variety of molecules in the body [34]. It is estimated that each cell in the human body is exposed to free radicals 10,000 times a day and DNA strands 5,000 times a day. Damage to cell components includes proteins (genetic disorder), fats (lipid oxidation), and cell membranes (permeability disorder) that if the damage is not repaired, it leads to disruption of the chemical reaction and normal proteinization of the cell and

| Chain | The number of predicted residues of the first active site |
|-------|---------------------------------------------------------|
| Chain A | Residues: 536,556,557,558,559,560,561,562,565,735,751,752,755,758,759,762,853,857 |
| Chain B | Residues: 525,526,590,628,652,653,654,655,656,657,658,659,683,684,686,688,689,690,691,692,735,762,765,766,767,768,769,770,801,802,803,805,806,807,808,809,810,814,826,831 |
the formation of harmful compounds and sometimes cancer cells in the body [35]. It is reported that thousands of cancer cells are produced daily in the human body that are killed by the body’s defense system. In some cases, due to dysfunction of the above systems, cancer cells proliferate and conditions for cancer development in different tissues. According to the above, antioxidants play a vital role in preventing disorders caused by the effects of free radicals and thus the prevention and treatment of cancer. Antioxidants are a wide range of molecular compounds with complex properties that combine with and neutralize free radicals. The results show that more than 60,000 types of molecular antioxidants have been identified so far. Antioxidants can be effective in three known ways to prevent and treat cancer; 1. Destruction of free radicals 2. Strengthen the immune system to destroy cancer cells. Prevent the adhesion of cancer cells to other cells and prevent their proliferation [36].

In this study, the treated cells with different concentrations of the present Wogonoside were assessed by MTT assay for 48h about the cytotoxicity properties on normal (HUVEC) and colon malignancy cell lines i.e. GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM (Figures 1,2).
Fig. 1. The anti-human colon carcinoma properties (Cell viability (%)) of Wogonoside (Concentrations of 0-1000 µg/mL) against normal (HUVEC: a) and human colon carcinoma (GP5d (b), MDST8 (c), and HCA-46 (d)) cell lines. The numbers indicate the percent of cell viability at the concentrations of 0-1000 µg/mL of Wogonoside against several human colon carcinoma cell lines.
Fig. 2. The anti-human colon carcinoma properties (Cell viability (%)) of Wogonoside (Concentrations of 0-1000 µg/mL) against human colon carcinoma (HT115 (b), LS174T (c), and COLO 320DM (d)) cell lines.

The numbers indicate the percent of cell viability at the concentrations of 0-1000 µg/mL of Wogonoside against several human colon carcinoma cell lines.
The viability of malignant colon cell lines reduced dose-dependently in the presence of Wogonoside. The IC50 of Wogonoside were 105, 198, 173, 382, 71, and 183 µg/mL against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cell lines, respectively (Table 1). The absorbance rate was evaluated at 570 nm, which represented viability on normal cell line (HUVEC) even up to 1000µg/mL for Wogonoside (Table 1 and Figures 1,2).

### 4. Conclusion

Theoretical approaches can give valuable information about the details of the experimental study. It can help biologists to understand the mechanisms of biological activities. Molecular docking is one of the popular methods among these approaches. In this study, the results of docking calculation revealed that wogonoside can be considered as a promising inhibitor for HMG-COA reductase with a considerable binding affinity to the binding site of the enzyme. Due to the advantages of molecules such as the ability to carry drugs, reduce toxicity, controlled drug release and specific drug delivery to the target tissue, these structures have been able to attract the attention of many researchers. As a result of these features, biotechnology has great potential for cancer treatment that can move from a research laboratory to a patient's bedside. One possible concern that limits the administration of some molecules in treating cancer is their toxicity,
which needs further investigation. However, molecules-based cancer therapies will continue to be developed to improve treatment outcomes. The viability of malignant colon cell lines reduced dose-dependently in the presence of Wogonoside. The IC50 of Wogonoside were 105, 198, 173, 382, 71, and 183 µg/mL against GP5d, MDST8, HCA-46, HT115, LS174T, and COLO 320DM cell lines, respectively. After clinical study, Wogonoside can be utilized as an efficient drug in the treatment of colon carcinoma in humans.

References

1. Zhang Y, Zhang X, Zhang L, Alarfaj AA, Hirad AH, Alsabri AE. Green formulation, chemical characterization, and antioxidant, cytotoxicity, and anti-human cervical cancer effects of vanadium nanoparticles: A pre-clinical study. Arab J Chem. 2021; 14(6): 103147.
2. Xinli DHZS. Applications of nanocarriers with tumor molecular targeted in chemotherapy. Chemistry. 2012; 75(7):621–627.
3. Yang F, Jin C, Jiang Y, et al. Liposome based delivery systems in pancreatic cancer treatment: from bench to bedside. Cancer Treat Rev. 2011; 37(8):633-642.
4. Li YN, G F. Recent progress in doxorubicin nano-drug delivery systems for reserving multidrug resistitance. 2014; 11(3):177-181.
5. Mohammed MI, Makky AM, Teaima MH, Abdellatif MM, Hamzawy MA, Khalil MA. Transdermal delivery of vancomycin hydrochloride using combination of nano-ethosomes and iontophoresis: in vitro and in vivo study. Drug Deliv. 2016;23(5):1558-1564.
6. Gao J, Wang Z, Liu H, Wang L, Huang G. Liposome encapsulated of temozolomide for the treatment of glioma tumor: preparation, characterization and evaluation. Drug Discov Ther. 2015; 9(3):205-212.
7. Matsumura, Y.; Hamaguchi, T.; Ura, T.; Muro, K.; Yamada, Y.; Shimada, Y.; Shirao, K.; Okusaka, T.; Ueno, H.; Ikeda, M.; et al. Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelleencapsulated doxorubicin. Br. J. Cancer 2004, 91, 1775-1781.
8. Zhang, Y.; Huang, Y.; Li, S. Polymeric micelles: Nanocarriers for cancer-targeted drug delivery. AAPS PharmSciTech, 2014, 15, 862–871.
9. Pranali P Deshpande, Current trends in the use of liposomes for tumor targeting, Nanomedicine, 2013; 8(9), 1509–1528.
10. Torchilin VP. Targeted pharmaceutical nanocarriers for cancer therapy and imaging. AAPS J. 2007; 9:E128-E147.
11. Byrne JD, Betancourt T, Brannon-Peppas L. Active targeting schemes for nanoparticle systems in cancer therapeutics. Adv. Drug Del. Rev. 2008; 60(15):1615–1626.
12. Allen TM. Ligand-targeted therapeutics in anticancer therapy. Nat. Rev. Cancer. 2002; 2(10):750–763.
13. Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov. 2008; 7(9):771-82.

14. Gao Z, Lukyanov AN, Singhal A, Torchilin VP. Diacyllipid-polymer micelles as nanocarriers for poorly soluble anticancer drugs. Nano Lett. 2002; 2(9):979–82.

15. Nie S, Xing Y, Kim GJ, Simons JW. Nanotechnology applications in cancer. Annu Rev Biomed Eng. 2007; 9:257-88.

[16] Guymer RH, Dimitrov PN, Varsamidis M, Lim LL, Baird PN, Vingrys AJ, Robman L (2008). Can HMG Co-A reductase inhibitors (statins) slow the progression of age-related macular degeneration? The agerelated maculopathy statin study (ARMSS). Clin. Interv. Aging, 3(3): 581-593.

[17] Guymer RH, Chiu AW, Lim L, Lim LL, Baird PN, Vingrys AJ, Robman L (2005). HMG CoA reductase inhibitors (statins): do they have a role in age-related macular degeneration? Surv. Ophthalmol. 50(2): 194-206.

[18] Connolly PJ, Westin CD, Loughney DA, Minor LK. HMG-CoA reductase inhibitors: design, synthesis, and biological activity of tetrahydroindazole-substituted 3,5-dihydroxy-6-heptenoic acid sodium salts. J Med Chem. 1993 Nov 12;36(23):3674-85. doi: 10.1021/jm00075a024. PMID: 8246237.

[19] Ü. M. Koçyiğit, P. Taslimi, B. Tüzün, H. Yakan, H. Muğlu, and E. Güzel, “1,2,3-Triazole substituted phthalocyanine metal complexes as potential inhibitors for anticholinesterase and antidiabetic enzymes with molecular docking studies,” J Biomol. Struct. Dyn., Dec. 2020.

[20] C. H. Jhong, J. Riyaphan, S. H. Lin, Y. C. Chia, and C. F. Weng, “Screening alpha-glucosidase and alpha-amylase inhibitors from natural compounds by molecular docking in silico,” BioFactors, vol. 41, no. 4, pp. 242–251, Jul. 2015.

[21] Alberts, A.W. Effects of HMG CoA Reductase Inhibitors on Cholesterol Synthesis. Drug Invest 2, 9–17 (1990).

[22] Willey JZ, Elkind MSV. 3-Hydroxy-3-methylglutaryl–Coenzyme A Reductase Inhibitors in the Treatment of Central Nervous System Diseases. Arch Neurol. 2010;67(9):1062–1067.

[23] E. S. Istvan and J. Deisenhofer, “Structural mechanism for statin inhibition of HMG-CoA reductase,” Science (80-. )., vol. 292, no. 5519, pp. 1160–1164, May 2001.

[24] “Schrödinger Release 2020-4: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY 2016; Impact, Schrödinger, LLC, New York, NY 2016; Prime, Schrödinger, LLC, New York, NY 2020.”

[25] A. Poustforoosh, H. Hashemipour, B. Tüzün, A. Pardakhty, M. Mehrabani, and M. H. Nematollahi, “Evaluation of potential anti-RNA-dependent RNA polymerase (RdRP) drugs against the newly emerged model of COVID-19 RdRP using computational methods,” Biophys. Chem., vol. 272, p. 106564, 2021.

[26] “Schrödinger Release 2020-4: LigPrep, Schrödinger, LLC, New York, NY 2020.”

[27] Luo W, Wen Q, Zhou M, Ma L. [An anti-human ovarian carcinoma and CD3 bispecific single-chain antibody mediates CDR3 spectratype drift of T cell receptor alpha and beta chains]. Nan Fang Yi Ke Da Xue Xue Bao. 2012 Jun;32(7):919-23.

[28] B. E. Haines, O. Wiest, and C. V. Stauffacher, “The Increasingly Complex Mechanism of HMG-CoA Reductase,” Acc. Chem. Res., vol. 46, no. 11, pp. 2416–2426, Nov. 2013.
[29] Youssef S, Stüve O, Patarroyo JC, et al. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. Nature. 2002;420(6911):78-84

[30] Obi C, Wysokinski W, Karnicki K, Owen WG, McBane RD II. Inhibition of plateletrich arterial thrombus in vivo: acute antithrombotic effect of intravenous HMGCoA reductase therapy. Arterioscler Thromb Vasc Biol. 2009;29(9):1271-1276.

[31] Trott O. Olson A.J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 2010; 31: 455-461.

[32] Kirshner DA, Nilmeier JP, Lightstone FC. Catalytic site identification—a web server to identify catalytic site structural matches throughout PDB. Nucleic Acids Res. 2013;41(Web Server issue):W256-W265. doi:10.1093/nar/gkt403