Remedial Effects and Molecular Docking Studies of Nevadensin: Antiglucosidase, Anti-cholinesterase, and Anti-Human Oral Squamous Cell Carcinoma Potentials

**Type**
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**Keywords**
Nevadensin, Acetylcholine esterase, alpha-glucosidase, Therapeutic activities

**Abstract**

Introduction
Nevadensin has a variety of pharmacological effects, including effects of anti-mycobacterium tuberculosis, antitussive, anti-hypertensive, anti-inflammatory, and free radical-scavenging activities. In this study, we investigated for their anticholinergics, antidiabetic, and anti-human oral squamous cell carcinoma potentials for nevadensin.

Material and methods
The antioxidant activities of nevadensin were elucidated by using various bioanalytical assays. On the other hand, IC50 values were calculated for acetylcholine esterase, α-glucosidase inhibition effects of nevadensin. For determining of anti-human oral squamous cell carcinoma properties of nevadensin, MTT assay was used on HUVEC, HSC-3, HSC-4, and Ca9-22 cell lines. The molecular docking method used to compare the biological activities of the nevadensin molecule against enzymes was used. Afterwards, the ADME/T analysis was performed to investigate the drug availability of the nevadensin molecule and the obtained parameters from ADME/T analysis were examined.

Results
The cell viability of nevadensin was very low against human oral squamous cell carcinoma cell lines without any cytotoxicity on the human normal (HUVEC) cell line. The IC50 of the nevadensin against HSC-3, HSC-4, and Ca9-22 were 316, 273, and 399 µg/mL, respectively. Thereby, the best cytotoxicity results and anti-human oral squamous cell carcinoma potentials of our nevadensin was observed in the case of the HSC-4 cell line.

Conclusions
Maybe the anti-human lung carcinoma properties of nevadensin are related to their antioxidant effects.
Remedial Effects and Molecular Docking Studies of Nevadensin: 
Antiglucosidase, Anti-cholinesterase, and Anti-Human Oral Squamous Cell Carcinoma Potentials

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ABSTRACT

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1. INTRODUCTION

Nevadensin (5,7-dihydroxy-6,8,4′-trimethoxyflavone, Figure 1), the main active component that existed in the Maxim Lysionotus pauciflorus plant. Lysionotus Maximus Pauciflorus. It is a species of the Lysionotus genus Gesneriaceae, widely distributed in southern China, and an important raw herb used in traditional Chinese medicines (TCMs) (Tong et al., 2011). It has been used in treating tuberculosis of the lymph node, tachypnoea cough and rheumatic pains (Liu et al., 1998). Nevadensin has a variety of pharmacological effects, including effects of anti-mycobacterium tuberculosis, antitussive, anti-inflammatory, anti-hypertensive, and free radical-scavenging activities (Murillo et al., 2003; Suksamrarn et al., 2003).

Having referred to previous literature, we find out some literatures about the effectiveness of nevadensin, such as the treatment of lymphoid tuberculosis, the reduction of blood pressure and antibacterial, etc. (Song et al., 1985; Xu et al., 1979)

Acetylcholine esterase (AChE; EC 3.1.1.7) creates optimum conditions for the electron carriers by removing the chemicals that accumulate in the nerve end over time (Mahdavi et al., 2019; Nematpour et al., 2020). There is no definitive treatment method for Alzheimer's disease as in Parkinson's disease. The current treatment methods are only aimed at minimizing the effects of the disease or improving the quality of life. For this purpose, AChE inhibitors (Donepezil, Rivastigmin) are widely used in order to ensure a better quality of life for patients (Mahdavi et al., 2019; Nematpour et al., 2020). These frequently used drugs may have side effects such as gastrointestinal disorders and hepatotoxicity. For this reason, in recent years, natural AChE inhibitors, which are both high inhibition capacity and reliable, have become the focus of attention. They widely use AChE inhibitors in agricultural and medical fields. Since both branches of science are directly linked to living things, the interest in inhibitors, which fall into the category of natural products, is increasing day by day in order to eliminate side effects (Mahdavi et al., 2019; Nematpour et al., 2020).

Alpha-glucosidase inhibitors (AGIs; acarbose, miglitol, voglibose) are widely used in the treatment of patients with type 2 diabetes. AGIs delay the absorption of carbohydrates from the small intestine and thus have a lowering effect on postprandial blood glucose and insulin levels (Van De Laar et al., 2005).

The molecular docking method, the most common among the theoretical methods, was used to compare the biological activities of nevadensin molecule against enzymes (Tüzün et al., 2018; Tüzün and Sarıpınar, 2020; Bytyqi-Damoni et al., 2020; Kisa et al., 2020). These enzymes are human alpha-galactosidase (α-Gly) (PDB ID: 1R47) (Garman and Garboczi, 2004), human acetylcholinesterase (AChE) (PDB ID: 4M0E) (Cheung et al., 2013), Afterwards, numerical
values of the parameters obtained by using ADME/T analysis were examined in order to analyze the drug availability properties of the nevadensin molecule. With this ADME/T analysis, the biological effects and reactions of this molecule in human metabolism were theoretically examined.

Figure 1. The chemical structure of nevadensin.

2. Experimental
2.1. Molecular docking method

Theoretical calculations are one of the most widely used methods in recent years to compare the activities of biological and chemical of molecules (Ojha et al., 2020; Bilgiçli et al., 2020a, 2020b, 2020c; Douche et al., 2020; Taslimi et al., 2020). With these calculations, theoretically, a lot of information is obtained about the molecule. Consequently, theoretical calculations provide great convenience. In this way, it saves both time and money. The results found guide the molecules to be newly synthesized. In this study, theoretically, the biological activity of the molecule will be compared against enzymes. The enzymes used in this study are human alpha-galactosidase (PDB ID: 1R47), human acetylcholinesterase (PDB ID: 4M0E). The biological activity of nevadensin molecule was compared against these enzymes by molecular docking calculations. For this investigation, firstly, it was obtained from Gaussian software program (Frisch et al., 2009) to find the optimized structures of molecules, using these structures, files with the extension *.sdf were created. Using these files, calculations were made with Maestro Molecular modeling platform (version 12.2) by Schrödinger, LLC (Schrodinger, 2019a). The Maestro Molecular modeling platform (version 12.2) by Schrödinger came together with many modules. In the first module, the protein preparation module (Schrodinger, 2019b; Du et al., 2020) was used for the preparation of proteins. The enzymes studied consist of many proteins. The crystal structures of these proteins have been downloaded from the protein data bank site. These enzymes were initially minimized and the water molecules in the structure were removed.
In the next step, the active sites of the enzymes were determined, in which the proteins in this active zone were given freedom of movement. Therefore, these proteins interacted more easily with molecules. In the next step, the molecule was prepared for calculations, the LigPrep module (Sastry et al., 2013; Schrodinger, 2019c) was used for this process. Calculations were made to find high energy isomers in the 3D structures and the correct protonation states of the nevadensin molecule.

In the next step, the prepared protein and molecule were docked with each other. OPLS3e method was used to calculate the molecule and proteins. The Glide ligand docking module (Friesner et al., 2006; Du et al., 2020) was used for this step. Molecules and enzymes were interacted with this module. Following the docking calculations, ADME/T analysis (absorption, distribution, metabolism, excretion and toxicity) was performed to examine the ability of the molecule to be used as a drug in the future. The Qik-prop module (Schrodinger, 2020) of the Schrödinger software was used for ADME/T analysis.

2.2. Anti-human oral squamous cell carcinoma studies
In this study, the human oral squamous cell carcinoma cell lines i.e., HSC-3, HSC-4, and Ca9-22 are being used to investigate the cytotoxicity and anti-human oral squamous cell carcinoma effects of the Nevadensin using the common cytotoxicity test i.e., MTT assay. 15 ml of RPMI 1640 medium containing 10% FSC (10 mg/ml penicillin and 100 mg/ml streptomycin) in a culture flask, placed in a CO2 incubator for 2 hours to equilibrate the medium. Under safe conditions (using insulated gloves and goggles) the frozen cell vial was removed from the nitrogen storage tank. In order to avoid the possibility of explosion of the vial (due to the possible entry of liquid nitrogen into the vial), loosen the lid, after disinfecting the outer surface of the vial with 70% alcohol, under the hood to remove nitrogen gas. Close the vial lid again and immediately melt it in a pan at 37 °C. The melting process should be completed in about 1 minute and the cells should be avoided from overheating. The medium was added dropwise to the vial and then its contents were taken out and centrifuged with the medium in 15 cc sterile test tubes. After centrifugation, the supernatant was removed and the cells were suspended again in the medium and transferred to a pre-prepared flask containing the medium and FBS and incubated (Hemmati et al., 2020; Jalalvand et al., 2020; Liu et al., 2020; Mahdavi et al., 2019; Nematpour et al., 2020; Zangeneh et al., 2019).

Cell line used in RPMI 1640 medium containing penicillin (100 IU / ML), streptomycin (100 IU / ML), glutamine (2 mmol) and 10% fetal bovine serum (FBS). They were incubated at 37 °C and in an atmosphere containing 0.5 CO2. Cells began to grow in 75 cm² T-flasks in 15 ml
medium with an initial number of 1-2 × 10^6 cells. After three days and covering the flask bed with the cell, the adhesive layer to the bottom of the flask was separated enzymatically using trypsin-verseon and transferred to a sterile test tube for 10 minutes at 1200 rpm. The cells were then suspended in a fresh culture medium with the help of a Pasteur pipette and the suspension was poured into 100-well plate flat wells (for cell culture) using an 8-channel sampler of 100 µl. One column of wells was kept cell-free and as a plank containing only culture medium. In another column, it was considered to contain culture medium and healthy cells and in other columns, it was considered to contain culture medium and cell line cells. One of these columns, which contained culture medium and cells and did not contain Nevadensin, was considered as a control (Hemmati et al., 2020; Jalalvand et al., 2020; Liu et al., 2020; Mahdavi et al., 2019; Nematpour et al., 2020; Zangeneh et al., 2019).

The plates were incubated in the incubator for 24 hours to return the cells to normal from the stress of trypsinization. After this time, suitable dilutions of the prepared Nevadensin (0-1000 µl / ml) and 100 µl of each dilution were added columnarly to the plate wells (Thus, the final concentration of the studied compound in the wells was halved. Therefore, the concentrations were prepared twice as much to reach the final concentration after being added to the well). The cells were incubated for 37 hours at 37 °C and 5% CO2 in the atmosphere. After 72 hours, 20 µl of MTT solution (5 mg/ml) was added to each well. The plates were incubated for 3 to 4 hours and then the residue was removed and 100 µl of DMSO was added to each well to dissolve the resulting formazan. After 10 minutes, using shaking the plates, the optical absorption of Formazan at 570 nm was read using a plate reader. Wells containing cells without Nevadensin were considered as control and the optical density of wells without cells and only culture medium were considered as blank. The percentage of cell viability was calculated using the following formula (Hemmati et al., 2020; Jalalvand et al., 2020; Liu et al., 2020; Mahdavi et al., 2019; Nematpour et al., 2020; Taslimi et al., 2020; Türkan et al., 2020; Zangeneh et al., 2019):

\[
\text{Cell viability (\%)} = \frac{\text{Sample A.}}{\text{Control A.}} \times 100
\]
3. Result and discussion
3.1. Enzymes Results

Inhibition of some enzymes was investigated, and their results were reported as follows.

Nevadensin was effective inhibiting AChE as metabolic enzyme. $K_i$ values for AChE were obtained to be 17.54±4.72 nM (Table 1). Also, the Galanthamine molecule was used as AChE enzyme control molecule; it had $K_i$ values of 27.43±3.45 nM. Nevadensin and Galanthamine values IC$_{50}$ were: Nevadensin (26.63 nM) < Galanthamine (38.03 nM, $r^2$: 0.9988) for AChE. AChE inhibitor compound is a neurotoxic molecule capable of causing central, peripheral, or both peripheral and central cholinergic crises. The molecule investigated in the present study can record application as medicinal products developed to treat myasthenia gravis and AD. On the other hand, Nevadensin shown as IC$_{50}$ and $K_i$ values are 4.65 nM, $r^2$:0.9602 and 5.16±0.53 (Table 3). For the $\alpha$-glucosidase present on cells lining, and the intestine, hydrolyzing monosaccharides are absorbed through the intestine. The results of the $\alpha$-glucosidase assay showed that Nevadensin has an effective $\alpha$-glucosidase inhibition profile compared to that of acarbose (IC$_{50}$: 8.81 nM, $K_i$:11.53± 2.73) as a standard $\alpha$-glucosidase inhibitor (Tao et al., 2013). The inhibition of $\alpha$-glucosidase digestive enzyme was of great importance for the treatment and prevention of diabetes, postprandial glucose levels and hyperglycemia.

Table 1. The enzyme inhibition results of Nevadensin against AChE and $\alpha$-glucosidase ($\alpha$-Glu) enzymes

| Compounds  | IC$_{50}$ (nM) | $K_i$ (nM) |
|------------|----------------|------------|
|            | AChE | $\alpha$-Glu | AChE | $\alpha$-Glu |
| Nevadensin | 26.63±3.54 | 4.65±1.12 | 17.54±4.72 | 5.16±0.53 |
| Galanthamine | 38.03±6.87 | - | 27.43±3.45 | - |
| Acarbose | - | 8.81±2.25 | - | 11.53±2.73 |
3.3. Molecular Docking Results

It is a method used to estimate the chemical and biological activities of molecules before experimental processes with theoretical calculations (Ojha et al., 2020; Bilgiçli et al., 2020a, 2020b, 2020c; Douche et al., 2020; Taslimi et al., 2020). One of the most common and effective methods used to compare the biological activities of the molecules is molecular docking. Docking calculations are an important guide for experimental studies. In this way, both experimental procedures have shortened the time and caused the costs to decrease. In the calculations made with the molecular docking method, as a result of the interaction of the molecule and the protein, many parameters were found. Numerical values of these parameters are used to compare the biological activities of molecules. It should be known very well that the most important factor affecting the biological activities of molecules is the interactions between molecules and enzymes. These interactions have many interactions such as hydrogen bonds, polar and hydrophobic interactions, $\pi-\pi$ and halogen bonds (Jayarajan et al., 2020; Sayin and Karakaş 2017; 2018a; 2018b; Sayin and Üngördü, 2018; 2019a; 2019b).

The numerical values of the parameters obtained as a result of the calculations are given in Table 2. There are many parameters in this table. All of these parameters are used to explain interactions in molecular docking calculations. The interactions between molecules and enzymes are given in Figure 1, 2. The most important parameter among the parameters obtained as a result of the calculations is the docking score, which has the lowest biological activity of the molecule whose numerical value is the highest positive. Many isomers have been found in the ligprep module of the molecule, these isomers docking at different points in the active region of the protein. However, another parameter is the Glide Ligand Efficiency, which is a numerical value of the effectiveness of the molecules. The parameters consisting of Glide Hbond, Glide Evdw, and Glide Ecoul are interaction related parameters. These interactions are

|                      | $\alpha$-Glu | $\text{AChE}$ |
|----------------------|--------------|---------------|
| Docking Score        | -3.63        | -6.65         |
| Glide Ligand Efficiency | -0.15       | -0.27         |
| Glide Hbond          | 0.00         | -0.17         |
| Glide Evdw           | -27.62       | -32.05        |
| Glide Ecoul          | -2.74        | -8.66         |
| Glide Emodel         | -39.44       | -61.35        |
| Glide Energy         | -30.37       | -40.71        |
| Glide Einternal      | 5.23         | 4.03          |

Table 2. Numerical values of the parameters obtained from interaction of molecule with enzymes
Nevadensin molecule interacted with many enzymes. In the interactions obtained as a result of the calculations, it is seen that the nevadensin molecule makes more hydrogen bonds with
enzymes. After examining the interactions of nevadensin molecule with enzymes, ADME/T analysis (Çelik et al., 2020) was performed to examine the ability of this molecule to become a drug in the future. The parameters obtained in this analysis have obtained numerical values of the effects and reactions of the molecule on human metabolism.

Many parameters were obtained from ADME/T analysis to investigate the medicinal properties of nevadensin molecule. If the numerical values of these parameters are within a certain range, this molecule can be used as a medicine in the future. The first parameter for this is Solute Molecular Weight, which shows the numerical value of the molecular weight of the drug molecule for human metabolism. Another parameter is Solute as Donor-Hyrogen Bonds, which is an estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution. Another parameter is QP log p for octanol/water, which gives the estimated octanol/water ratio as a result of the calculation. Another parameter is QPlogHERG, which is the estimated value of IC50 for blockage of HERG K channels. Another parameter is QPPCaco, which is used to estimate the permeability of the intestinal blood barrier in nm/sec. The numerical value of this parameter is expected to be <25 poor, > 500 high (in table "*"). Another important parameter is QPlogBB, which is a coefficient of the brain / blood barrier. The drug is expected to be in the range required to pass this barrier. Another important parameter is QPPMDCK, which is the numerical value of Madin-Darby Canine Kidney (MDCK) cells transmittance in nm/sec unit. The numerical value of this parameter should be <25 poor,> 500 great (in table "*"").

RuleOfFive and RuleOfThree are the most important parameters showing the feature of being a drug among the parameters obtained as a result of ADME analysis. These two parameters are more important than other parameters. The numerical value of these two parameters is required to be zero. The RuleofFive parameter (Lipinski, 2004; Lipinski et al., 1997) is also known as Lipinski's fifth rule of Pfizer. The rules are: mol MW <500, QPlogP o/w <5, donorHB ≤ 5, acceptHB ≤ 10. Compounds that satisfy these rules are considered druglike. On the other hand, the RuleOfThree parameter (Jorgensen and Duffy, 2002) is known as the number of violations of Jorgensen’s rule of three. The three rules are: QPlogS> -5.7, QP PCaco> 22 nm / s, #Primary Metabolites <7. Molecules with a numerical value of this RuleofThree parameter closer to zero can be taken orally as drugs.
2.3. Anti-human oral squamous cell carcinoma effects results

The MTT assay is a procedure of colorimetric based on reducing and breaking of yellow tetrazolium crystals by the enzyme succinate dehydrogenase to form insoluble purple crystals. In this method, unlike other methods, the steps of washing and collecting cells, which often cause the loss of a number of cells and increase the work error, have been eliminated and all test steps from the beginning of cell culture to reading the results with a photometer are performed on a microplate, so the repeatability, accuracy and sensitivity of the test are high (Lipinski et al., 1997). If the test is performed on cells attached to the plate, an appropriate number of cells (about 2,000 cells) must first be cultured in each of the wells. Then we select the control and test wells and add the appropriate amount of mitogen or drug to the test wells and place the plate in the incubator for the required time so that the desired substance affects the cells (Hemmati et al., 2020; Jalalvand et al., 2020; Liu et al., 2020; Mahdavi et al., 2019; Nematpour et al., 2020; Taslimi et al., 2020; Türkan et al., 2020; Zangeneh et al., 2019). At the end of the incubation time, discard the supernatant and add 200 μl of culture medium containing half an mg/ml of MTT solution to each well and put it again in a carbon dioxide incubator for 2 to 4 hours at 37 °C. During incubation, MTT is regenerated by one of the enzymes of the mitochondrial respiratory cycle i.e., succinate dehydrogenase. The regeneration and breakage of this ring produce purple-blue crystals of formazan that are easily detectable under a microscope. At the end, the optical absorption of the resulting solution can be read at 570 nm and the cells number can be calculated using a standard curve. For each cell line, there is a linear relationship between the number of cells and the light absorption of the final solution. Therefore, to examine each cell type, a standard curve related to the same cell line must be drawn and used (Lipinski et al., 1997).

In the current study, the cytotoxicity of Nevadensin was investigated by treating variable concentrations of it towards the affected HSC-3, HSC-4, and Ca9-22 cancer cells lines by MTT.
assay for 48h. The cell viability (%) was plotted against Nevadensin concentrations (0-1000 μg/mL) with the three cell lines which have been shown in Figure 3. In all the cases the cell viability gets reduced with increasing concentration doses. The IC$_{50}$ values of Nevadensin against HSC-3, HSC-4, and Ca9-22 cell lines were found 316, 273 and 399 μg/mL, respectively (Table 3) without any cytotoxicity effects on normal (HUVEC) cell line. Thereby, the best cytotoxicity results and anti-human oral squamous cell carcinoma potentials of our molecule was observed in the case of the HSC-4 cell line.

Oxidation from reactive oxygen species can cause cell membrane disintegration, damage to membrane proteins, and DNA mutation that the result is the onset or exacerbation of many diseases such as cancer, liver damage, and cardiovascular disease. Although the body has a defense system, constant exposure to chemicals and contaminants can lead to an increase in the number of free radicals outside the body's defense capacity and irreversible oxidative damage (Lipinski et al., 1997). Therefore, antioxidants with the property of removing free radicals play an important role in the prevention or treatment of oxidation-related diseases or free radicals.

Extensive molecular cell research on cancer cells has developed a targeted approach to the biochemical prevention of cancers that the goal is to stop or return cells to their pre-cancerous state without any toxic doses through nutrients and drugs. Numerous studies have been performed on the use of natural compounds as anti-cancer agents in relation to appropriate antioxidant activity (Lipinski et al., 1997). It seems the high anti-human oral squamous cell carcinoma properties of Nevadensin are related to its antioxidant activities. Our successful efforts to utilize Nevadensin in carcinoma studies certainly shed light on future studies in this area.
Fig. 3. The anti-human oral squamous cell carcinoma properties (Cell viability (%)) of Nevadensin (Concentrations of 0-1000 µg/mL) against HUVEC (A), HSC-3 (B), HSC-4 (C), and Ca9-22 (D) cell lines.

The numbers indicate the percents of cell viability in the concentrations of 0-1000 µg/mL of Nevadensin against several human oral squamous cell carcinoma cell lines.

**Table 3.** The IC$_{50}$ of Nevadensin in the anti-human oral squamous cell carcinoma test.

|         | HUVEC | HSC-3 | HSC-4 | Ca9-22 |
|---------|-------|-------|-------|--------|
| IC$_{50}$ (µg/mL) | -     | 316   | 273   | 399    |
CONCLUSIONS

Biological activities of nevadensin molecule against enzymes were compared. Afterwards, ADME/T analysis of this molecule was done and it was theoretically investigated in the future. This molecule was found to provide all ADME/T analysis parameters except two parameters. Nevadensin also revealed significant cytotoxic activities against common human oral squamous cell carcinoma cell lines i.e., HSC-3, HSC-4, and Ca9-22. This study will be a great guide for future in vivo and in vitro studies.

Reference

Alterio V., Monti S.M., Truppo E., Pedone C., Supuran C.T., De Simone, G. (2010). The first example of a significant active site conformational rearrangement in a carbonic anhydrase-inhibitor adduct: the carbonic anhydrase I–topiramate complex. Organic & Biomolecular Chemistry, 8(15), 3528-3533.

Cheung J., Gary E. N., Shiomi K., Rosenberry T. L. (2013). Structures of human acetylcholinesterase bound to dihydrotanshinone I and terrettrem B show peripheral site flexibility. ACS medicinal chemistry letters, 4(11), 1091-1096.

Celik İ, Erol M., Arpacı O.T., Senol F.S., Orhan I.E., (2020). Evaluation of activity of some 2, 5-disubstituted benzoazolate derivatives against acetylcholinesterase, butrylcholinesterase and tyrosinase: ADME prediction, DFT and comparative molecular docking studies. Polycyclic Aromatic Compounds, 1-12.

Douche D., Elmsellem H., Guo L., Hafez B., Tüzün B., El Louzi A., Bougrin K., Karrouch K., Himmi, B. (2020). Anti-corrosion performance of 8-hydroxyquinoline derivatives for mild steel in acidic medium: Gravimetric, electrochemical, DFT and molecular dynamics simulation investigations. Journal of Molecular Liquids, 308, 113042.

Du Q., Qian Y., Yao X., Xue W., (2020) Elucidating the tight-binding mechanism of two oral anticoagulants to factor Xa by using induced-fit docking and molecular dynamics simulation. Journal of Biomolecular Structure and Dynamics, 38(2) 625-633.

Garman S.C., Garboczi D.N. (2004). The molecular defect leading to Fabry disease: structure of human a-galactosidase. Journal of molecular biology, 337(2), 319-335.

Friesner R.A., Murphy R.B., Repasky M.P., Frye L.L., Greenwood J.R., HalgrenT.A., Sanschagrin P.C., Mainz D.T., (2006) Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes. Journal of medicinal chemistry. 49 6177–6196.

Frisch M.J., Trucks G.W., Schlegel H.B., Scuseria G.E., Robb M.A., Cheeseman J.R., Scalmani G., Barone V., Mennucci B., Petersson G.A., Nakatsuji H., Caricato M., Li X., Hratchian H.P., Izmaylov A.F., Bloino J., Zheng G., Sonnenberg J.L., Hada M., Ehara M., Tokuyama K., Fukuda R., Hasegawa J., Ishida M., Nakajima T., Honda Y., Kitao O., Nakai H., Vreven T., Montgomery J.A., Peralta J.E., Ogliaro F., Bearpark M., Heyd J.J., Brothers E., Kudin K.N., Staroverov V.N., Kobayashi R., Normand J., Raghavachari K., Rendell A., Burant J.C., Iyengar S.S., Tomasi J., Cossi M., Rega N., Millam J.M., Klene M., Knox J.E., Cross J.B., Bakken V., Adamo C., Jaramillo J., Gomperts R., Stratmann R.E., Yazyev O., Austin A.J., Cammi R., Pomelli C., Ochterski J.W., Martin R.L., Morokuma K., Zakrzewski V.G., Voth G.A., Salvador P., Dannenberg J.J., Dapprich S., Daniels A.D., Farkas O., Foresman J.B., Ortiz J.V., Cioslowski J., Fox D.J. (2009) Gaussian 09, revision D.01. Gaussian Inc, Wallingford CT.
Sayin K., Üngördü A., (2018) Investigation of anticancer properties of caffeinated complexes via computational chemistry methods. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 193 147-155.

Sayin K., Üngördü A., (2019a) Investigations of structural, spectral and electronic properties of enrofloxacin and boron complexes via quantum chemical calculation and molecular docking. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 220 117102.

Sayin K., Üngördü A., (2019b) Quantum chemical calculations on sparfloxacin and boron complexes. *Chemical Physics Letters*, 733 136677.

Schrodinger, L. (2019a). Small-Molecule Drug Discovery Suite 2019-4

Schrödinger Release 2019-4 (2019b): Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY, 2016; Impact, Schrödinger, LLC, New York, NY, 2016; Prime, Schrödinger, LLC, New York, NY, 2019.

Schrödinger Release 2019-4 (2019c): LigPrep, Schrödinger, LLC, New York, NY, 2019

Schrödinger Release 2020-1 (2020): QikProp, Schrödinger, LLC, New York, NY, 2020.

Song, J., He, X., Chen, X., Hu, J., Luo, G., and Mo, Y. (1985). Hypotensive effect of nevadensin. *Zhongguo yao li xue bao= Acta pharmacologica Sinica* 6, 99.

Suksamrarn, A., Poomsing, P., Aroonrer, N., Punjanon, T., Suksamrarn, S., and Kongkun, S. (2003). Antimycobacterial and antioxidant flavones from Limnophila geoffrayi. *Archives of pharmacal research* 26, 816-820.

Supuran, C. T. (2008). Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nature reviews Drug discovery* 7, 168-181.

Tao, Y., Zhang, Y., Cheng, Y., and Wang, Y. (2013). Rapid screening and identification of α-glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with HPLC/MS and NMR. *Biomedical chromatography* 27, 148-155.

Tong, X., Xiao, X., and Li, G. (2011). On-line coupling of dynamic microwave-assisted extraction with high-speed counter-current chromatography for continuous isolation of nevadensin from Lyeicnotus pauciflorus Maxim. *Journal of Chromatography B* 879, 2397-2402.

Van De Laar, F. A., Lucassen, P. L., Akkermans, R. P., Van De Lisdonk, E. H., Rutten, G. E., and Van Weel, C. (2005). α-Glucosidase inhibitors for patients with type 2 diabetes: results from a Cochrane systematic review and meta-analysis. *Diabetes care* 28, 154-163.

Xu, Y., Hu, Z.-B., Feng, S. C., and Fan, G. (1979). Studies on the anti-tuberculosis principles from Lysionotus pauciflorus Maxim. I. Isolation and identification of nevadensin (author's transl). *Yao xue xue bao= Acta pharmaceutica Sinica* 14, 447.

Zangeneh, M. M., Bovandi, S., Gharehyakhe, S., Zangeneh, A., and Irani, P. (2019). Green synthesis and chemical characterization of silver nanoparticles obtained using Allium saralicum aqueous extract and survey of in vitro antioxidant, cytotoxic, antibacterial and antifungal properties. *Applied Organometallic Chemistry* 33, e4961.