A Pre-clinical Trial Study: Anti-human Colon Cancer Effect of Thalassiolin B in vitro with Enzymes Inhibition Effects and Molecular Docking Studies

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Abstract: In this study, it is recorded the inhibition effect of Thalassiolin B on aldose reductase, alpha-glucosidase and alpha-amylase enzymes. In the next step, the molecular docking method was used to compare the biological activities of the Thalassiolin B molecule against enzymes formed from the assembly of proteins. In these calculations, the enzymes used are Aldose reductase, Alpha-Amylase, and Alpha-Glucosidase, respectively. After the docking method, ADME/T analysis of Thalassiolin B molecule was performed to be used as a drug in the pharmaceutical industry. In the MTT assay, the anti-human colon cancer properties of Thalassiolin B against EB, LS1034, and SW480 cell lines were investigated. The cell viability of Thalassiolin B was very low against human colon cancer cell lines without any cytotoxicity on the human normal (HUVEC) cell line. The IC₅₀ of the Thalassiolin B against EB, LS1034, and SW480 were 483, 252, and 236 µg/mL, respectively. Thereby, the best cytotoxicity results and anti-human colon cancer potentials of our Thalassiolin B were observed in the case of the SW480 cell line. Maybe the anti-human colon cancer properties of Thalassiolin B are related to their antioxidant effects.

Key words: Thalassiolin B, enzyme inhibition, molecular docking studies, colon cancer, cytotoxicity

1 Introduction

Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications⁷. Additionally, alpha-glucosidase (maltase, glucosidoucercase, glucoinvertase, alpha-glucopyranosidase, maltase-glucoamylase, alpha-D-glucosidase, alpha-1,4-glucosidase, glucosidoinvertase, alpha-D-glucoside glucohydrolase, alpha-glucoside hydrolase) is a glucosidase located in the brush border of the small intestine that acts upon α(1→4) bonds.

It has been seen in many studies that the numerical values obtained from experimental and theoretical studies are quite compatible with each other²⁴. Therefore, it has been observed that the theoretical calculations made before experimental studies will be more useful. As a result, it is possible to synthesize more effective and active molecules with theoretical calculations made before experimental studies⁷. The results of these calculations direct

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the experimental studies to be conducted. As a result, huge savings will be made for experimental studies. In theoretical calculations, the most widely used method is the molecular docking method. Many parameters are calculated due to calculations made with the molecular docking method. These parameters provide important information about the biological activities of molecules. After the docking calculations, ADME/T (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis of the molecule was performed. With the ADME/T analysis, the effects and reactions of drug molecules in human metabolism in cells and tissues are tried to be predicted theoretically. These effects and responses are tried to be predicted by numerical values of the parameters obtained due to the calculations. Each parameter obtained is tried to be predicted the effect and response in different organs or tissues. These results give the properties of the molecule to be used as a drug in the future.

Cancer is a genetic disease that is ultimately the result of the effects of environmental factors. In 2010, more than 14,000,000 people were diagnosed with cancer and about 7,000,000, or 50%, died. Since last year, cancer has been ranked number one in the world in terms of mortality. Until now, cardiovascular disease has been the number one concern. The highest percentages of cancers are breast cancer, hepatocarcinoma, colorectal cancer, gastric cancer, and lung adenocarcinoma in women and ovarian cancer in men, respectively. The highest rates of cancer in children include blood, brain, and lymph nodes. The highest risk factor for cancer is aging. The older you get, the greater your risk of developing cancer. 93% of cancers are caused by the environment, 30% by cigarette smoke, 35% by diet, 25% by infectious diseases, and 10% by ion and non-ion rays. Cancers are caused by a series of mutations in human genes and each mutation causes a new change in the cell. Chemicals cause cancer cells called carcinogens. There are more than 100,000 types of chemicals in nature that directly or indirectly affect the cytoplasm and the nucleus of cells and lead to genetic disorders that cause mutant cup heads. Various viruses, bacteria, and radiation, in turn, produce inherited cancers, which account for about 7% of all cancerous tissue: Blood, lymph nodes, sarcoma, carcinoma, embryonic cells, and germ cells. Cancer is a disease that disrupts intercellular relationships and disrupts vital and key genes. These molecular irregularities affect the cell division cycle and lead to a lack of cell differentiation. Cancer can be treated in several ways: surgery, chemotherapy, radiation therapy, immunotherapy, gene therapy, or a combination of these. Due to the relative inefficiency and very severe side effects of chemotherapy drugs, researchers and scientists have been looking for new formulations of various compounds, especially antioxidant molecules.

In the present study, we investigated the anti-colon cancer potentials of the Thalassiolin B against EB, LS1034, and SW480 cell lines. The antioxidant properties of the Thalassiolin B were calculated against DPPH free radicals. In this paper, we have evaluated that the in vitro inhibition effects of the Thalassiolin B on AR, alpha-glucosidase and alpha-amylase enzymes, also, molecular docking of enzymes results were studied.

2 Experimental

2.1 Enzymes assays

Determination of the alpha-amylase inhibitory property of the ligands was carried out following procedures described by Adefegha et al. and according to the previous studies. Aldose reductase activity was measured according to the previous studies and measured the decrease of NADPH at 340 nm spectrophotometrically using the D-glucose substrate. Alpha-glycosidase inhibitory effect of Justiciresinol was evaluated using p-NPG as the substrate, according to the procedure of Tao et al. and according to other study.

2.2 Docking studies

Many studies show that the most commonly used method to compare the biological activities of molecules is molecular docking. By molecular docking method, comparison of biological activities is made from the numerical value obtained from the interactions of molecules and enzymes. The active areas of the molecules are determined by calculations. In this study, molecular docking calculations were made to compare the biological activities of the Thalassiolin B molecule. Molecular docking calculations to calculate the biological activity of the Thalassiolin B molecule were performed using the Maestro Molecular modeling platform (version 12.2) by Schrödinger. Proteins and Thalassiolin B molecules must be prepared for these calculations. In docking calculations, a different process is performed for molecules at each stage. First, it was used from Gaussian software program to obtain optimized structures of molecules, files with extension *.sdf were created using these structures. Using these files, all calculations were made with the Maestro Molecular modeling platform (version 12.2) by Schrödinger, LLC. The Maestro Molecular modeling platform (version 12.2) by Schrödinger comes together from many modules. In the first module used, the protein preparation module was used to prepare the studied proteins for calculations. It should be well known that the enzymes studied are composed of many small proteins. The crystal structures of these enzymes studied have been downloaded from the protein data bank site. The enzymes studied were first minimized and the water molecules in their crystal structures were removed. In the next step, the active sites of the enzymes were de-
A Pre-clinical Trial Study

J. Oleo Sci. 71, (2) 267-276 (2022)

termined for calculations, in which freedom of movement was given to all proteins in this active site. Thus, these proteins have been enabled to interact more easily with molecules. In the next step, the LigPrep module was used to prepare the working molecules for calculations.

Calculations were made to find 3D structures at physiological pH values of the Thalassiolin B molecule and high energy isomers in the correct protonation conditions. In the next step, the prepared protein and molecules were docked with each other. The Glide ligand docking module was used for this step. In this module, the OPLS3e method was used in all calculations for docking calculations of molecules and proteins. Numerical values of many parameters obtained due to molecular docking calculations using this module are used. After the docking calculations, ADME/T analysis (absorption, distribution, metabolism, excretion and toxicity) was performed to examine the future drug properties of the molecule. The Qik-prop module of the Schrödinger software was used for ADME/T analysis.

2.3 Assessment of antiproliferative effect by MTT assay

In this assay, following human colon cancer and normal cell lines were used to study the cytotoxicity and anticancer potential of human colon over the Thalassiolin B and Cisplatin (As a common positive control) using the common cytotoxicity test i.e., MTT assay in vitro condition:

1) Human colon cancer cell lines: EB, LS1034, and SW480.
2) Normal cell line: HUVEC.

These cells were maintained in a DMEM medium with 10% bovine embryos and 1% penicillin/streptomycin antibiotic (to prevent fungal growth). Prerequisites for cell growth at 37°C are 5% CO₂ with 95% moisture, which was provided by the NUVE incubator (EC160 model). For MTT assay, when the cells reached at least 70% cell growth, they were separated from the bottom of the flask by trypsin-ethyldiamine tetraacetic acid and centrifuged at 1700 rpm for 6-1 minutes. Cell precipitate was prepared in suspension in 1 mL of culture medium. The viability of cells in cell suspension was determined by mixing it with a neobar slide under a light microscope. After confirming that the cells were not infected, cells with a viability of more than 90% were used for testing.

To investigate the effect of 2’-Hydroxy-5’-methoxyacetophenone on cancer cell proliferation, tetrizolium (MTT) salt colorimetric method was used. For this test, 10⁶ cells were added to each 96-well plate well. After 24 hours of incubation, concentrations of 1-1000 μg/mL were treated on cancer and normal cells for 24, 48, and 72 hours. After these times, 20 μL of MTT solution and 200 μL of base culture medium were added to each well. The plate was placed in a dark CO₂ incubator at 37°C for 4 hours in the dark. After this time, 100 microliters of DMSO were added to each well. 492 and 630 nm optical readings were placed in the ELISA reader (DANA model DA3200). The cell viability was computed by the following formula:

Cell viability (%) = \frac{Sample A}{Control A} \times 100

2.4 Evaluation of the antioxidant property of the Thalassiolin B

The ability of hydrogen atoms or electrons to give off different compounds and antioxidant molecules in this test is measured by the degree of decolorization of the 2 and 2-diphenyl-1-picryl-hydrazyl purple solution in methanol. In this method, DPPH (Sigma-Aldrich) was used as a stable radical compound. Thus, 100 μL of various dilutions of Thalassiolin B in methanol was added to 10 mL of 0.005% DPPH solution in methanol. After 1 hour of incubation at the absorption room temperature, the samples were read against Blank at 518 nm. The DPPH inhibition percentage was computed by the following formula:

Inhibition (%) = \frac{Sample A}{Control A} \times 100

In this formula, “Control A” shows the negative control of light absorption that lacks nanoparticles, and “Sample A” expresses the amount of light absorption of different concentrations of nanoparticles.

2.5 Qualitative measurement

To compare the results, in addition to the formula mentioned above, which was calculated as an average of 5 repetitions of experiments. The results were analyzed using SPSS software version 22 and the statistical differences between the treatments were examined by t-test and p ≤ 0.01 was considered significant.

3 Results

3.1 Enzymes results

The important purpose of this study is to determine efficacious, selective and more potent inhibitors for aldose reductase, alpha-glucosidase and alpha-amylase enzymes. Hence, inhibition analysis of these enzymes was recorded with Thalassiolin B to control diabetic complications. Researchers often list an IC₅₀ value to describe inhibitory effects, but the Ki constant is the more suitable for measurement. Both the Ki and IC₅₀ parameters of compounds were performed in the current study from Lineweaver-Burk graphs (1/V-1/[S]) and Activity (%) - [Inhibitor] graphs respectively. IC₅₀ and Ki values were calculated for AR, alpha-glucosidase and alpha-amylase. The results for inhibitory activity of the studied Thalassiolin B compound are shown in Table 1. IC₅₀ of these enzymes were 24.82, 187.06, 3.91, and 194.42 μM, respectively. It showed that Ki values order of compound showing inhibitory potency was 21.48
The results showed that Thalassiolin B had efficient IC$_{50}$ values than that acarbose.

### 3.2 Molecular modeling results

The biological activities of molecules are compared with many parameters obtained by the Maestro program, which is used to calculate the biological activities of molecules\(^{[1]}\). With this method, many parameters are calculated due to the interaction of molecules with enzymes\(^{[2]}\). By using these calculated parameters, the biological activities of molecules with other molecules are compared. Enzymes used for this comparison are Aldose reductase (PDB ID: 3V36) (AR), Alpha-Amylase (PDB ID: 3BAJ) (Alpha-Amy), and Alpha-Glucosidase (PDB ID: 3TOP) (alpha-Gly), respectively. The parameters obtained due to interactions with these enzymes are given in **Table 2**.

The most important parameter among the many parameters obtained for the Thalassiolin B molecule due to molecular docking calculations is the Docking score parameter of the Thalassiolin B molecule.\(^{[3]}\) This parameter obtained is the numerical value of the interactions between Thalassiolin B molecule and enzyme. Due to calculations, the molecule with the most negative numerical value of this parameter has higher biological activity than other molecules. It should be well known that the more interaction between any molecule and enzyme, the more the numerical value of this parameter decreases. Therefore, the most important factor affecting the biological activities of molecules is the interactions between molecules and proteins in enzymes. These interactions have many interactions such as hydrogen bonds, polar and hydrophobic interactions, \(\pi-\pi\) and halogen.\(^{[4]}\) These interactions are given in Figs. 1, 2, and 3.

Many parameters obtained from the docking calculations of the Thalassiolin B molecule were obtained. Each parameter evaluates the interaction between molecule and enzyme from different angles. The Glide hbond, Glide

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**Table 1** The enzyme inhibition results of the Thalassiolin B against aldose reductase, Alpha-Amylase and alpha-glucosidase enzymes.

| Compounds    | IC$_{50}$ Aldose reductase (µM) | IC$_{50}$ Alpha-Amylase (µM) | IC$_{50}$ Alpha-Glucosidase (µM) | Ki Aldose reductase (µM) | Ki Alpha-Amylase (µM) | Ki Alpha-Glucosidase (µM) |
|--------------|---------------------------------|------------------------------|---------------------------------|--------------------------|----------------------|-------------------------|
| Thalassiolin B | 24.82 ± 4.35                    | 187.06 ± 15.76               | 134.42 ± 28.04                  | 21.48 ± 4.70             | 168.31 ± 14.62       |
| Acarbose     | -                               | 255.80 ± 23.05               | 176.04 ± 13.66                  | -                        | 203.83 ± 25.08       |

\(\mu\)M for AR, and 168.31 \(\mu\)M for alpha-glucosidase enzyme.
A Pre-clinical Trial Study

J. Oleo Sci. 71, (2) 267-276 (2022)

evdw, and Glide ecoul parameters of the Thalassiolin B molecule provide information about the chemical interactions of the molecule with the enzyme. Each parameter evaluates the interaction between molecule and enzyme from different perspectives.

Another important parameter is the QPlogHERG, which is the numerical value of the estimated IC₅₀ value when HERG K channels are blocked. It is seen that this parameter of the Thalassiolin B molecule does not meet the required conditions. The next parameter is QPPCaco, which is Caco-2 cell permeability at the gut-blood barrier for inactive transport. It is seen that the numerical value of this parameter is too little for this molecule.

3.3 Anti-human colon cancer potentials of the Thalassiolin B

The MTT set is the best-known test for cell viability. The main purpose of this test is to evaluate the toxicity of compounds, drugs, or other supplements on the cell. Of course, it may also be mentioned in articles as a process for examining cell proliferation or counting. MTT analysis can differentiate between living and dead cells by affecting intracellular organs. In this method, the cells, after being cultured in the laboratory, are "treated" with the desired substances to evaluate their toxicity. At the end of this test, for each concentration of the substance, the cell viability is determined. Although this method is primarily for water-soluble solutions and compounds, it is currently used for other compounds soluble in organic solvents and molecules. The behavior and rate of cell proliferation may increase or not change at all under the influence of hormones, growth factors, cytokines, and mitogens. Also, some drugs and cytotoxic (toxic) substances, such as anti-cancer drugs, may cause necrosis or apoptosis (death) of cells or slow down the rate of proliferation and growth or even loss of cell structure. Proper analysis of the MTT test can evaluate many of these behaviors. The MTT analysis basis is based on mitochondrial activity. This activity is usually stable in living cells. Hence, any change in several active and living cells is linked to mitochondrial property. This examination is a colorimetric way based on the breakdown and reduction of yellow tetrazolium crystals by the succinate dehydrogenase, and the formation of insoluble purple crystals perform the final analysis. Unlike other methods, MTT analysis eliminates the cells washing and shrinking steps, which usually causes the loss of cells part and increases the work error. That is, all the steps of the experiment, from the cell culture, beginning to reading and analyzing the findings with a photometer, are done in a completely compact way and a "microplate". Hence the sensitivity, accuracy, and repeatability of the test are high.

In the present study, the cytotoxicity of the Thalassiolin B and Cisplatin was explored by studying their interaction with normal (HUVEC) and EB, LS1034, and SW480 cell
lines by MTT assay for 48 h. The interactions being expressed as cell viability (%) were observed at different Thalassiolin B and Cisplatin concentrations (0–1000 μg/mL) with the four cell lines which have been shown in detail in Figs. 4-6. In all the cases, the % cell viability gets reduced with increasing Thalassiolin B and Cisplatin samples concentrations.

The IC_{50} of the Thalassiolin B against EB, LS1034, and SW480 were 483, 252, and 236 μg/mL, respectively. Thereby, the best cytotoxicity results and anti-human colon cancer potentials of our Thalassiolin B were observed in the case of the SW480 cell line (Table 3).

3.4 Antioxidant potentials of the Thalassiolin B

Maybe the antiproliferative effect of the Thalassiolin B is linked to its antioxidant activity (Fig. 7 and Table 4). Similar reports have indicated the antioxidant materials such as biological molecules reduce the volume of tumors by removing free radicals⁷. In detail, the high presence of free radicals in the normal cells make many mutations in their DNA and RNA, destroy their gene expression and then accelerate the proliferation and growth of abnormal cells or cancerous cells⁸, ⁹.

4 Discussion

After the enzymes of the Thalassiolin B molecule were activated, ADME/T analysis was performed to theoretically calculate the effects and responses of the Thalassiolin B molecule on human metabolism in cells and tissues. Due to this analysis, many parameters have been obtained. The parameters obtained as a result of the calculations of the Thalassiolin B molecule are given in Table 3. The first parameter in this table is Solute Molecular Weight, which gives information about the molecular weight of the molecule being studied. Among all ADME/T parameters, another two important parameters are RuleOfFive and RuleOfThree. The RuleOfFive and RuleOfThree parameters are more important than any other parameter. The numerical value of these two parameters is expected to be zero. The RuleOfFive parameter, also known as Lipinski’s fifth rule, is Pfizer’s fifth rule. The rules are: mol MW<500, QPlogP o/w<5, donorHB≤5, acceptHB≤10. However, the RuleOfThree parameter is known as the three of Jorgensen’s rule. The three rules are: QPlogS>−5.7, QP PCaco>22 nm/s, #Primary Metabolites<7. If the numerical value of the RuleOfThree parameter is zero, this molecule can be used orally as a drug. The last and another important parameter is Jm, which is the predicted maximum transdermal trans-

![Fig. 4 The anti-human colon cancer properties of the Thalassiolin B against EB (a), LS1034 (b), and SW480 (c) cell lines.](image-url)
A Pre-clinical Trial Study

Fig. 5 The anti-human colon cancer properties of the Cisplatin against EB (a), LS1034 (b), and SW480 (c) cell lines.

Table 3 The IC$_{50}$ of the Thalassiolin B and cisplatin in the anti-human colon cancer test.

|          | Thalassiolin B (µg/mL) | Cisplatin (ng/mL) |
|----------|------------------------|-------------------|
| IC$_{50}$ against EB | 483 ± 0$^b$          | 722 ± 0$^b$      |
| IC$_{50}$ against LS1034 | 252 ± 0$^c$      | 575 ± 0$^c$      |
| IC$_{50}$ against SW480  | 236 ± 0$^c$          | 500 ± 0$^c$       |

The different words indicate the significant difference ($p$ ≤ 0.01) between several groups.

Fig. 6 The cytotoxicity effects of the Thalassiolin B against Normal (HUVEC) cell line.

The anticancer potential of therapeutic compounds against human colon cancer cell lines is immensely related to their antioxidant properties. Several earlier reports have uncovered that therapeutic compounds with strong antioxidant capacity significantly inhibit the growth of cancer cells by removing free radicals$^{8}$. This is our earnest effort in exploiting biological molecule (Thalassiolin B) towards the adenocarcinoma studies and the corresponding results,
we believe, will open up a wing towards anticancer research in future.

Now, turning our attention to investigate the bioactivity of the Thalassiolin B molecule, a concentration-dependent DPPH radical scavenging effect of molecule was observed against BHT as a reference. The interaction between the Thalassiolin B and DPPH might have occurred by transferring electrons and hydrogen ions. The scavenging capacity of the Thalassiolin B and BHT at different concentrations, expressed in terms of percentage inhibition, has been shown in Fig. 6. In the antioxidant test, the IC₅₀ of butylated hydroxytoluene and Thalassiolin B were 175 and 133 μg/mL, respectively (Table 4). Biological molecules show higher antioxidant effects against free radicals formation into the living system. The biological molecules have excellent redox properties and have a significant role in free radical deactivating.

5 Conclusions

Biological activities of the Thalassiolin B molecule against enzymes were compared. Afterwards, a theoretical ADME/T analysis of this molecule was performed. The calculated parameters of the Thalassiolin B molecule were examined. The numerical values obtained from ADME/T calculations for the Thalassiolin B molecule show that some ADME/T parameters of the Thalassiolin B molecule do not meet the necessary conditions; theoretically, this molecule is not recommended to be used as a drug. But it should be kept in mind that experimental conditions differ from theoretical computational conditions. The numerical values of the parameters obtained from this study are used in the future in vivo and in vitro studies, providing a great deal for new drug candidate discovery.

The oncological part of the recent study was revealed the cytotoxicity and anti-human colon cancer properties of Thalassiolin B against EB, LS1034, and SW480 cell lines in the in vitro condition. The IC₅₀ of Thalassiolin B against EB, LS1034, and SW480 were 483, 252, and 236 μg/mL, respectively. The antioxidant properties of Thalassiolin B were determined against DPPH free radicals. The IC₅₀ of Thalassiolin B was 133 μg/mL. Maybe the anti-human colon cancer properties of Thalassiolin B are related to their antioxidant effects.

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Authors’ Contributions

All authors have had a same role in preparing, designing, doing experiments, analyzing, writing, and submitting the recent manuscript.

References

1) Beking, K.; Vieira, A. Flavonoid intake and disability-adjusted life years due to Alzheimer’s and related dementias: A population-based study involving twenty-three developed countries. Public Health Nutr. 13,
A Pre-clinical Trial Study

1403-1409 (2010).

2) Kim, T.H.; Kim, J.K.; Kang, Y.H.; Lee, J.Y.; Kang, I.J.; Lim, S.S. Aldose reductase inhibitory activity of compounds from Zea mays L. Biomed. Res. Int. 2013, 727143 (2013).

3) Zhang, L.; Hogan, S.; Li, J.; Sun, S.; Canning, C. et al. Grape skin extract inhibits mammalian intestinal α-glucosidase activity and suppresses postprandial glycemic response in streptozocin-treated mice. Food Chem. 126, 466-471 (2011).

4) Worsztynowicz, P.; Napierala, M.; Bialas, W.; Grajek, W.; Olkowicz, M. Pancreatic α-amylase and lipase inhibitory activity of polyphenolic compounds present in the extract of black chokeberry (Aronia melanocarpa L.). Process Biochem. 49, 1457-1463 (2014).

5) Zhang, Y.; Zeng, Z.; Zeng, G.; Liu, X.; Liu, Z. et al. Effect of Triton X-100 on the removal of aqueous phenol by laccase analyzed with a combined approach of experiments and molecular docking. Colloids Surf. B 97, 7-12 (2012).

6) Cassidy, C.E.; Setzer, W.N. Cancer-relevant biochemical targets of cytotoxic Lonchocarpus flavonoids: A molecular docking analysis. J. Mol. Model. 16, 311-326 (2010).

7) Kameshwar, A.K.S.; Barber, R.; Qin, W. Comparative modeling and molecular docking analysis of white, brown and soft rot fungal laccases using lignin model compounds for understanding the structural and functional properties of laccases. J. Mol. Graphics Modell. 79, 15-26 (2018).

8) Mo, D.; Zeng, G.; Yuan, X.; Chen, M.; Hu, L. et al. Molecular docking simulation on the interactions of laccase from Trametes versicolor with nonylphenol and octylphenol isomers. Bioprocess Biosyst. Eng. 41, 331-343 (2018).

9) Thun, M.J.; Hannan, L.M.; Adams-Campbell, L.L.; Boffetta, P.; Buring, J.E. et al. Lung cancer occurrence in never-smokers: an analysis of 13 cohorts and 22 cancer registry studies. PLoS Med. 5, e185 (2008).

10) Taylor, R.; Najaﬁ, F.; Dobson, A. Meta-analysis of studies of passive smoking and lung cancer: effects of study type and continent. Int. J. Epidemiol. 36, 1048-1059 (2007).

11) Hecht, S.S. Lung carcinogenesis by tobacco smoke. Int. J. Cancer 131, 2724-2732 (2012).

12) Collins, L.G.; Haines, C.; Perkel, R.; Enck, R.E. Lung cancer: Diagnosis and management. Am. Fam. Physician 75, 56-63 (2007).

13) Alsharari, N.A. The effects of dietary supplements on asthma and lung cancer risk in smokers and non-smokers: A review of the Literature. Nutrient 11, 725 (2019).

14) Stahl, M.; Mariette, C.; Haustermans, K.; Cervantes, A.; Arnoed, D. Oesophagel cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann. Oncol. 24 (Suppl), 51-56 (2013).

15) Murugan, K.; Dinesh, D.; Kavithaa, K.; Paulpandi, M.; Ponraj, T. et al. Parasitol. Res. 115, 1085-1096 (2016).

16) Radini, I.A.; Hasan, N.; Malik, M.A.; Khan, Z. Biosynthesis of iron nanoparticles using Trigonella foenum-graecum seed extract for photocatalytic methyl orange dye degradation and antibacterial applications. J. Photochem. Photobiol. B 183, 154-163 (2018).

17) Klein, S.; Sommer, A.; Distel, L.H.; Hazemann, J.-L.; Kröner, W. et al. Superparamagnetic iron oxide nanoparticles as novel X-ray enhancer for low-dose radiation therapy. J. Phys. Chem. B 118, 6159-6166 (2014).

18) El-Sayed, I.H.; Huang, X.; El-Sayed, M.A. Selective laser photo-thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. Cancer Lett. 239, 129-135 (2006).

19) Adefegha, S.A.; Oboh, G.; Omojokun, O.S.; Jimoh, T.O.; Oyeleye, S.I. In vitro antioxidant activities of African birch (Anogeissus leiocarpus) leaf and its effect on the α-amylase and α-glucosidase inhibitory properties of acarbose. J. Taibah Univ. Med. Sci. 11, 236-242 (2016).

20) Xiao, Z.; Storms, R.; Tsang, A. A quantitative starch-iodine method for measuring alphaamylase and glucoamylase activities. Anal. Biochem. 351, 146-148 (2006).

21) Kadam, A.; Dawane, B.; Pawar, M.; Shegokar, H.; Patil, K. et al. Development of novel pyrazolone derivatives as inhibitors of aldose reductase: an ecofriendly one-pot synthesis, experimental screening and in silico analysis. Bioorg. Chem. 53, 67-74 (2014).

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

23) Teng, H.; Chen, L.; Fang, T.; Yuan, B.; Lin, Q. Rb2 inhibits α-glucosidase and regulates glucose metabolism by activating AMPK pathways in HepG2 cells. J. Funct. Foods 28, 306-313 (2017).

24) Huczynski, A.; Majcher, U.; Maj, E.; Wietrzyk, J.; Janczak, J. et al. Synthesis, antiproliferative activity and molecular docking of Colchicine derivatives. Bioorg. Chem. 64, 103-112 (2016).

25) Schrödinger, L. Small-Molecule Drug Discovery Suite 2019-4 (2019).

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

27) Friesner, R.A.; Murphy, R.B.; Repasky, M.P.; Frye, L.L.; Greenwood, J.R. et al. Extra precision glide: Docking
and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J. Med. Chem. 49, 6177-6196 (2006).

28) Sastry, G.M.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. J. Comput. Aided Mol. Des. 27, 221-234 (2013).

29) Schrödinger Release 2019-4: LigPrep, Schrödinger, LLC, New York, NY, 2019.

30) Du, Q.; Qian, Y.; Yao, X.; Xue, W. Elucidating the tight-binding mechanism of two oral anticoagulants to factor Xa by using induced-fit docking and molecular dynamics simulation. J. Biomol. Struct. Dyn. 38, 625-633 (2020).

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

32) Baghayeri, M.; Mahdavi, B.; Hosseinpor-Mohsen Abadi, Z.; Farhadi, S. Green synthesis of silver nanoparticles using water extract of Salvia lavifolia: Antibacterial studies and applications as catalysts in the electrochemical detection of nitrate. Appl. Organomet. Chem. 32, e4057 (2018).

33) Namvar, F.; Rahman, H.S.; Mohamad, R.; Baharara, J.; Mahdavi, M. et al. Cytotoxic effect of magnetic iron oxide nanoparticles synthesized via seaweed aqueous extract. Int. J. Nanomedicine 9, 2479 (2014).

34) Majcher, U.; Klejborowska, G.; Moshari, M.; Maj, E.; Wietrzyk, J. et al. Antiproliferative activity and molecular docking of novel double-modified colchicine derivatives. Cells 7(11), 192 (2018).

35) Majcher, U.; Urbaník, A.; Maj, E.; Moshari, M.; Delgado, M. et al. Synthesis, antiproliferative activity and molecular docking of thiocolchicine urethanes. Bioorg. Chem. 81, 553-566 (2018).

36) Zheng, X.; Zhang, L.; Chen, W.; Chen, Y.; Xie, W.; Hu, X. Partial inhibition of aldose reductase by nitazoxanide and its molecular basis. ChemMedChem 7, 1921-1923 (2012).

37) Maurus, R.; Begum, A.; Williams, L.K.; Fredriksen, J.R.; Zhang, R. et al. Alternative catalytic anions differentially modulate human α-amylase activity and specificity. Biochemistry 47, 3332-3344 (2008).

38) Ren, L.; Qin, X.; Cao, X.; Wang, L.; Bai, F. et al. Structural insight into substrate specificity of human intestinal maltase-glucosamylase. Protein Cell 2, 827-836 (2011).

39) Sayin, K.; Üngördağ, A. Investigations of structural, spectral and electronic properties of enrofloxacin and boron complexes via quantum chemical calculation and molecular docking. Spectrochim. Acta A Mol. Biomol. Spectrosc. 220, 117102 (2019).

40) Sayin, K.; Karakaş, D. Investigation of structural, electronic properties and docking calculations of some boron complexes with norfloxacin: A computational research. Spectrochim. Acta A Mol. Biomol. Spectrosc. 202, 276-283 (2018).

41) Sayin, K.; Karakaş, D. Quantum chemical investigation of levofloxacin-boron complexes: a computational approach. J. Mol. Struct. 1158, 57-65 (2018).

42) Sayin, K.; Karakaş, D. Determination of structural, spectral, electronic and biological properties of tosufloxacin boron complexes and investigation of substitution effect. J. Mol. Struct. 1146, 191-197 (2017).

43) Chatterjee, A.K.; Sarkar, R.K.; Chattopadhyay, A.P.; Aich, P.; Chakraborty, R.; Basu, T. A simple robust method for synthesis of metallic copper nanoparticles of high antibacterial potency against E. coli. Nano. Technology 23, 085103 (2012).

44) Soni, A.; Krishnamurthy, R. Plants- The next generation treatment of Leukemia. Indian J. Plant. Sci. 2, 117-125 (2013).

45) Katata-Seru, L.; Moremedi, T.; Aremu, O.S.; Bahadur, I. Green synthesis of iron nanoparticles using Moringa oleifera extracts and their applications: Removal of nitrate from water and antibacterial activity against Escherichia coli. J. Mol. Liq. 256, 96-304 (2018).

46) Sangani, S.; Manu, M. Synthesis of Green Iron Nanoparticles using Laterite and their application as a Fenton-like catalyst for the degradation of herbicide Ametryn in water. Environ. Technol. Innov. 8, 150-163 (2017).

47) Beheshtkhoo, N.; Kouhbanani, M.A.J.; Savardashtaki, A.; Amani, A.; Taghizadeh, S. Green synthesis of iron oxide nanoparticles by aqueous leaf extract of Daphne mezereum as a novel dye removing material. Appl. Phys. A 124, 363-369 (2018).

48) Radini, I.A.; Hasan, N.; Malik, M.A.; Khan, Z. Biosynthesis of iron nanoparticles using Trigonella foenumgraecum seed extract for photocatalytic methyl orange dye degradation and antibacterial applications. J. Photochem. Photobiol. B 183, 154-163 (2018).

49) Oganesyan, G.; Galstyan, A.; Mnatsakanyan, V.; Paronyan, R.V.; Ter-Zakharyan, Y.Z. et al. Phenolic and flavonoid compounds of Ziziphus clinopodioides. Chem. Nat. 27, 247-247 (1991).