Anti-ovarian cancer and collagenase, α-amylase, and aldose reductase inhibition properties of 2′-hydroxy-5′-methoxyacetophenone with molecular modeling studies

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Abstract

Introduction: This work investigated the inhibition effect of 2′-hydroxy-5′-methoxyacetophenone on aldose reductase, α-amylase, and also collagenase enzymes. Also, molecular modeling calculations were performed comparing the biochemical activity of the 2′-hydroxy-5′-methoxyacetophenone molecule with these enzymes.
Material and methods: The enzymes used in these calculations are aldose reductase (AR), α-amylase (Alpha-Amy), and collagenase from *Clostridium histolyticum* (Coll). After the docking calculations were performed, ADME/T analysis parameters were examined in order to calculate the properties of the methoxyacetophenone molecule to be utilized as a drug in the future. For determining anti-ovarian cancer properties of 2′-hydroxy-5′-methoxyacetophenone, the MTT assay was used on normal (HUVEC), PA-1, Caov-3, and SK-OV-3 cell lines.
Results: The IC_{50} values of 2′-hydroxy-5′-methoxyacetophenone against PA-1, Caov-3, and SK-OV-3 cell lines were found to be 271, 326 and 405 µg/ml, respectively.
Conclusions: 2′-hydroxy-5′-methoxyacetophenone was found to have significant antioxidant and anti-ovarian cancer properties. It appears that the anti-ovarian cancer effect of 2′-hydroxy-5′-methoxyacetophenone is due to its antioxidant effects.

Key words: 2′-hydroxy-5′-methoxyacetophenone, enzymes inhibition, anti-ovarian cancer, molecular docking.

Introduction

As it is the first enzyme involved in the initiation of the polyol pathway, aldose reductase (AR) represents as an attractive pharmacological target in the prevention of diabetic complications. On the other hand, among some factors (such as AR activity) high blood sugar is related to glycation of significant biocompounds and the organization of advanced glycation and products, resulting in secondary complications including visual impairment, kidney failure, ischemic heart disease, diabetes, and stroke [1–4]. Reactive oxygen species (ROS) accumulate in the skin af-

ter exposure, which can activate enzymes such as collagenase and elastase that degrade and also break down the collagen and elastin respectively. Thus, collagenase and elastase synthesis promotes premature skin aging, as evidenced by symptoms such as wrinkles, pallor, freckles, deep grooves or severe atrophy, and limpness [5, 6]. α-Amylase breaks down starch via cleavage of α-1.4 glycosidic bonds in starch molecules, thereby leading to release of products such as maltose and glucose [7–10].

Studies in recent years show that researchers use both experimental results and theoretical results to compare the bioactivities of compounds [11–14]. In calculations using theoretical methods, molecular modeling is the most popular assay utilized to compare the biological activities of compounds against enzymes. Theoretically, biochemical activities of compounds are calculated by molecular modeling. In this way, their biochemical activities can be compared with other molecules. As a result, many parameters are obtained from the interaction of molecules and enzyme. These parameters provide important information about the bioactivities of molecules. After the docking, the ADME (metabolism, distribution, absorption, toxicity, and excretion) mechanism of the molecule was performed [15].

Additionally, we extended the application of our molecule in the bio-assay against ovarian cancer using the common ovarian cancer cell lines PA-1, Caov-3, and SK-OV-3. We think that the results obtained in the studies will contribute to the pharmacological applications and design of drugs to be used for therapeutic purposes. Additionally, In this work, we investigated the *in vitro* inhibition effects of 2′-hydroxy-5′-methoxyacetophenone on the enzymes aldose reductase, α-amylase, and collagenase.

Material and methods

Enzyme studies

Determination of the α-amylase inhibitory property was as described by Adefegha *et al.* [16] and according to the previous studies [17]. Collagenase enzyme activity of 2′-hydroxy-5′-methoxyacetophenone was investigated based on the assay described by Wang *et al.* [18] with several modifications and conforming to previous studies [19]. The supernatant part of some of the test tube was measured at 550 nm (Tecan Trading AG, Sunrise TW, Switzerland, Männedorf). On the other hand, AR enzyme activity of 2′-hydroxy-5′-methoxyacetophenone was investigated by measuring the reduction of NADPH at 340 nm dl glyceraldehyde as the substrate and according to prior studies [20].

Docking studies

Studies in recent years show that the most popular method used by researchers to compare the bioactivities of molecules is molecular modeling [21, 22]. A comparison for their factor can be made from the numerical value of the interactions of enzymes with any molecule studied by molecular modeling assay. Indeed, many of these parameters are determined in the molecular docking calculations [23, 24]. Comparison of the parameters of the compounds from the numerical value to the biological activities is made. Aldose reductase (AR), α-amylase (alpha-Amy), and collagenase from *Clostridium histolyticum* (Coll) enzymes were used in this work to compare the bioactivities of the methoxyacetophenone compound.

Molecular calculations to determine the biochemical activity of the methoxyacetophenone molecule were recorded using the Maestro Molecular docking platform by Schrödinger. Proteins and methoxyacetophenone molecules should be prepared for calculations using this program. In docking calculations, a diverse process is recorded for molecules and enzymes at each stage [25–32].

Anti-ovarian cancer studies

In this work, the ovarian cancer cell lines PA-1, Caov-3, and SK-OV-3 were utilized to investigate the anti-ovarian cancer and cytotoxicity effects of 2′-hydroxy-5′-methoxyacetophenone using a popular cytotoxicity test, the MTT method.

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% CO_2 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 min. 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 an equal proportion of trypan blue and counting them 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 [33].

To investigate the effect of 2′-hydroxy-5′-methoxyacetophenone on cancer cell proliferation, the tetrazolium (MTT) salt colorimetric method was used. For this test, 10^4 cells were added to each 96-well plate well. After 24 h of incubation, concentrations of 1–1000 µg/ml were applied to cancer and normal cells for 24, 48, and 72 h.

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 CO_2 incubator at 37°C for 4 h in the dark. After this time, 100 µl 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 [33]: Cell viability (%) = (Sample A/Control A) × 100.

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 $p < 0.05$ was considered significant.

Results and discussion

Enzyme results

α-Amylase inhibitors (α-AI) have great potential to treat obesity [34]. The polyol pathway is a biochemical pathway which is involved in the progression of diabetic complications. Its increased activity during hyperglycemia causes oxidative stress in cells. Therefore, researchers are interested in developing AR inhibitor compounds to treat and also manage diabetic complications [35, 36].

The significant goal of this work is to investigate selective potent inhibitor compounds for the enzymes aldose reductase and collagenase. Indeed, inhibitory action of these important enzymes was found with 2′-hydroxy-5′-methoxyacetophenone to control the diabetic complications. IC_{50} values were recorded for AR, α-amylase, and collagenase. The results of this study for inhibitory activity of the studied 2′-hydroxy-5′-methoxyacetophenone molecule are presented in Table I. IC_{50} values of these enzymes were 20.046, 0.982, and 3.264 µM, respectively. The collagenases and gelatinases play an important role in cancer-related progression, angiogenic, and metastasis events. Additionally, their inhibitors can be an effective factor for treatment and cancer prevention.

With the increase in the activity of aldose reductase, the amount of certain coenzymes (NADPH and NADH) changes in the cell and the gene expression of enzymes belonging to the antioxidant defense system is inhibited. This leads to oxidative stress in cells. In addition, sorbitol accumulated in the cell can cause tissue damage by causing osmotic stress, and it also reduces the formation of phosphoinositide signals by decreasing the amount of myoinositol [35]. Thus, the conduction velocity in nerve cells slows down and can lead to various diabetic microvascular complications. The AR inhibitors are structurally divided into three main classes. These are: the carboxylic acid group (epalrestat, tolrestat and zopolrestat), the cyclic imide group (fidarestat, sorbinil), and sulfonyl nitromethane derivatives. The interactions between the polar ends of the inhibitors and their active sites constitute an important condition in the stability of the enzyme inhibitor complex [36].

Docking results

Through the higher values of some parameters recorded by the Maestro program utilized to calculate the bioactivities of the methoxyacetophenone molecule, the biochemical activities of the compounds are compared. By using these calculated parameters, also bioactivities of compounds with other molecules are compared. The key enzymes utilized for this comparison are aldose reductase (PDB ID: 3V36) (AR) [37], α-amylase (PDB ID: 3BAJ) (AA) [38], and collagenase enzyme from *Clostridium histolyticum* (PDB ID: 4U6T) [39] (Table II).

In the results of molecular modeling, more parameters were found for the methoxyacetophenone molecule. The most key parameter among these parameters is the modeling score. It can be observed that the compound with a highly negative numerical value of the docking score parameter has higher biochemical activity than other compounds [40]. As the interaction between the methoxyacetophenone molecule and the enzyme increases, it is seen that the biochemical activity value of the compound increases. These interactions are shown in Figures 1–3.

Apart from this parameter, many parameters are calculated to explain the interactions between molecules and enzymes. In the results of these docking studies, the obtained Glide evdw, Glide hbond, and Glide ecoul parameters obtained numerical values of Van der Waals, hydrogen bonding, and Coulomb interactions that show good interactions between the molecules and these en-

Table I. Enzyme action results of 2′-hydroxy-5′-methoxyacetophenone against, α-amylase, aldose reductase and collagenase

Compound	IC_{50} (micromolar)					
	Aldose reductase	r^2	Alpha amylase	r^2	Collagenase	r^2
2′-hydroxy-5′-methoxyacetophenone	20.046	0.9268	0.982	0.9045	3.264	0.9889

Table II. Numerical values of the docking parameters of molecule against enzymes

Variable	Aldose reductase	α-Aamylase	Collagenase
Docking score	–6.32	–4.30	–5.56
Glide ligand efficiency	–0.53	–0.36	–0.46
Glide hbond	0.00	0.00	0.00
Glide evdw	–18.35	–18.15	–19.78
Glide ecoul	–0.84	–1.67	–6.40
Glide emodel	–32.75	–31.42	–40.13
Glide energy	–19.18	–19.81	–26.18
Glide einternal	3.63	0.47	1.45
Glide posenum	221	13	115

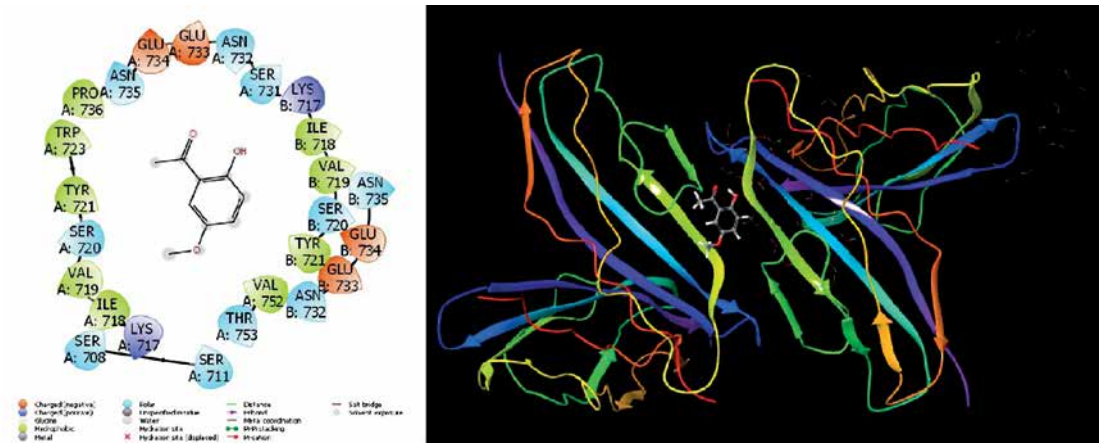

Figure 1. Presentation of interactions of methoxyacetophenone with α-amylase

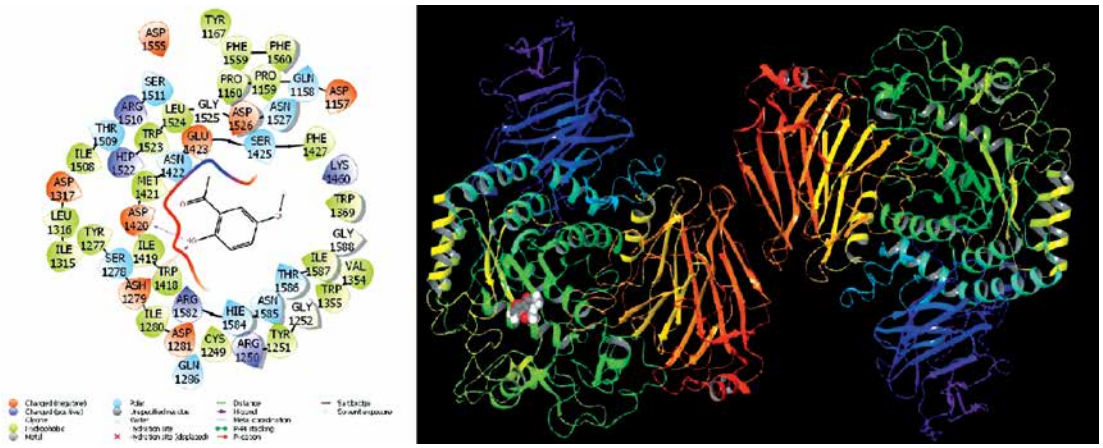

Figure 2. Presentation of interactions of methoxyacetophenone with collagenase

zymes [41, 42]. Additionally, Glide emodel, Glide energy, Glide einternal, and some other parameters give numerical descriptions of some of the interactions between the compound and these enzymes [43–45].

After molecular modeling calculations, the properties of compounds to be used as drugs were examined. As a result, many parameters were determined. All parameters calculated are given in Table III in detail.

Among all ADME/T parameters, another two important parameters are RuleOfThree and RuleOfFive. Indeed, the RuleOfFive [46] and RuleOfThree [47] parameters are more significant than any another parameter. The RuleOfFive parameter, also known as Lipinski's, is Pfizer's fifth rule.

Results of anti-ovarian cancer effects

With the advances in life sciences, measuring the rate of proliferation, survival, and cell mortal-

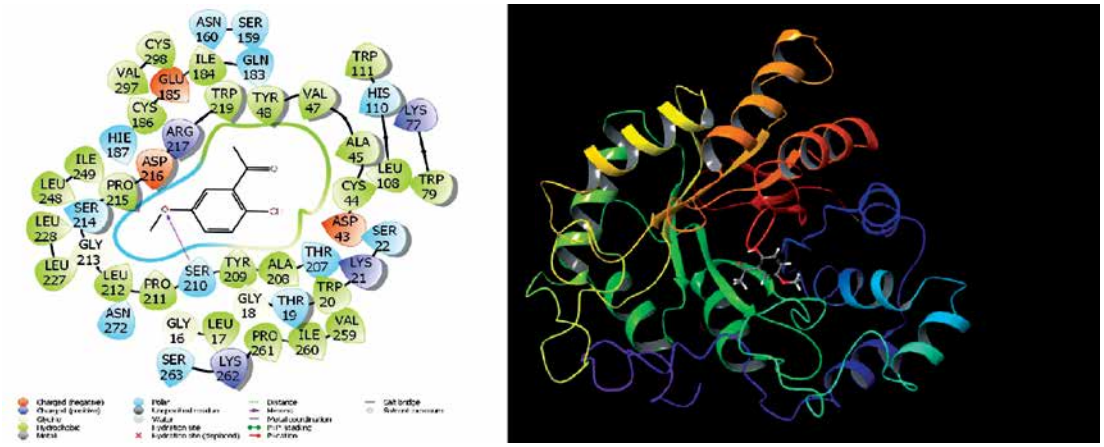

Figure 3. Presentation of interactions of methoxyacetophenone with aldose reductase

ity under different conditions has become very important. In this regard, MTT analysis has greatly contributed to the study of biocompatibility of various materials by providing a highly safe non-radioactive colorimetric system. Cytotoxicity tests examine the side effects of various compounds on the cell. These processes take place in the environment outside the human body. Most of these processes also use cell culture. In MTT analysis according to the ISO 10993-5 international standard, different types of equipment are tested for cytotoxicity; if they do not have toxic effects, they will obtain the necessary standards and licenses and enter the buying and selling market. 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 [33]. 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 anticancer drugs, may cause necrosis or apoptosis (death) of cells or slow down the rate of proliferation and growth or even loss of cell structure [33]. Proper analysis of the MTT test can evaluate many of these behaviors. The MTT analysis 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 properties.

Table III. ADME properties of molecule

Variable	Methoxyacetophenone	Reference range
Mol_MW	166	130–725
Dipole (D)	7.3	1.0–12.5
SASA	384	300–1000
FOSA	166	0–750
FISA	104	7–330
PISA	114	0–450
WPSA	0	0–175
Volume (A^3)	598	500–2000
DonorHB	0	0–6
AccptHB	2.5	2.0–20.0
Glob (Sphere = 1)	0.9	0.75–0.95
QPpolrz (A^3)	16.9	13.0–70.0
QPlogPC16	5.2	4.0–18.0
QPlogPoct	7.5	8.0–35.0
QPlogPw	3.9	4.0–45.0
QPlogPo/w	1.7	–2.0–6.5
QPlogS	–1.8	–6.5–0.5
CIQPlogS	–1.8	–6.5–0.5
QPlogHERG	–3.8	
QPPCaco [nm/s]	1016	
QPlogBB	–0.5	–3.0–1.2
QPPMDCK [nm/s]	503	
QPlogKp	–2.8	Kp in cm/hr
IP (ev)	9.0	7.9–10.5
EA (eV)	0.5	–0.9–1.7
#metab	2	1–8
QPlogKhsa	–0.4	–1.5–1.5
Human Oral Absorption	3	–
Percent Human Oral Absorption	91	
PSA	54	7–200
RuleOfFive	0	Maximum is 4
RuleOfThree	0	Maximum is 3
Jm	5.9	–

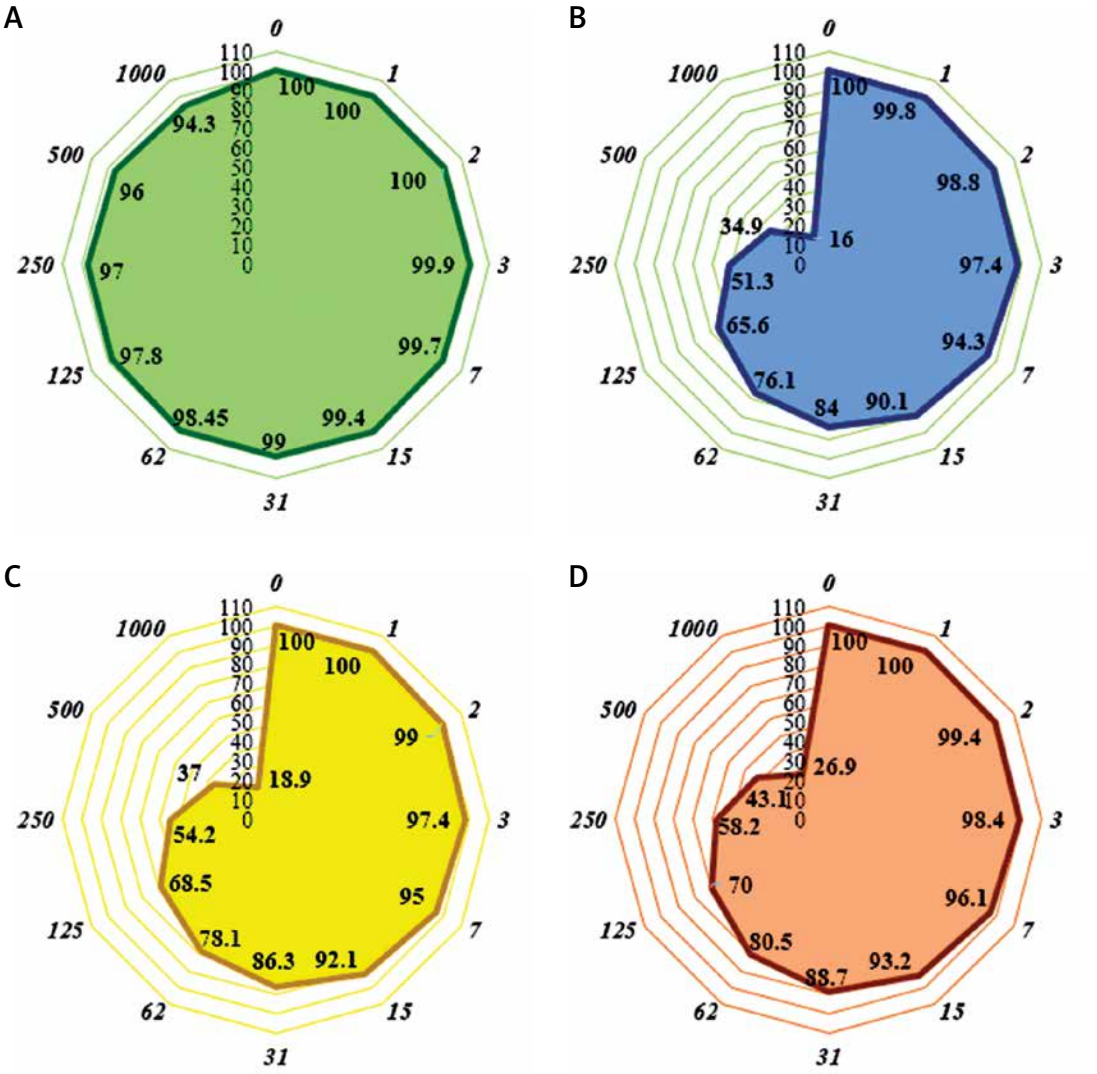

Figure 4. Anti-ovarian cancer properties (cell viability (%)) of 2′-hydroxy-5′-methoxyacetophenone (concentrations of 0–1000 µg/ml) against normal (HUVEC: **A**), PA-1 (**B**), Caov-3 (**C**), and SK-OV-3 (**D**) cell lines

Table IV. IC_{50} of 2′-hydroxy-5′-methoxyacetophenone in the anti-ovarian cancer test

Variable	PA-1	Caov-3	SK-OV-3
IC_{50} [µg/ml]	271	326	405

This examination is a colorimetric way based on the breakdown and reduction of yellow tetrazolium crystals by succinate dehydrogenase, and the formation of insoluble purple crystals is involved in the final analysis. Unlike other methods, MTT analysis eliminates the cell washing and shrinking steps, which usually causes the loss of part of cells and increases the work error. That is, all the steps of the experiment, from the cell culture, beginning to read 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 [33].

In this work, the cytotoxicity of 2′-hydroxy-5′-methoxypethenophenone was investigated by treating different concentrations of PA-1, Caov-3, and SK-OV-3 cancer cells with MTT for 48 h. Cell viability (%) was plotted against the concentration of 2′-hydroxy-5′-methoxetatophenone (0–1000 µg/ml) with the three cell lines recorded in Figure 4. In all cases, increasing the doses of IC_{50} 2′-hydroxy-5′-methoxetatophenone against PA-1, Caov-3, and SK-OV-3 cells with increasing doses of 2750, 326 and 405 µg/ml, respectively, was observed (Table IV). Thus, the best results of cytotoxicity and anti-ovarian cancer potentials of our molecule were observed in PA-1 cells.

In conclusion, the biochemical activity of the methoxyacetophenone compound toward enzymes was found as a result of modeling calculations. With these calculations, it is possible to compare the methoxyacetophenone molecule with other molecules in the future. In the next step, ADME/T analysis of the methoxyacetophenone molecule was performed. With this analysis, ADME/T param-

eters of the methoxyacetophenone molecule show that it is safe to use it as a drug in the future. In this direction, the methoxyacetophenone molecule will progress towards becoming a drug with future *in vivo* and *in vitro* studies. 2′-Hydroxy-5′-methoxyacetophenone also revealed significant cytotoxic activities against common ovarian cancer cell lines, i.e., PA-1, Caov-3, and SK-OV-3. The IC_{50} values of 2′-hydroxy-5′-methoxyacetophenone against PA-1, Caov-3, and SK-OV-3 cell lines were found to be 271, 326 and 405 µg/ml, respectively.

Conflict of interest

The authors declare no conflict of interest.

References

1. Zhang L, Zhang H, Zhao Y, et al. Inhibitor selectivity between aldo-keto reductase superfamily members AKR1B10 and AKR1B1: role of Trp112 (Trp111). FEBS Letters 2013; 587: 3681-6.
2. Srivastava S, Chandra A, Bhatnagar A, Srivastava SK, Ansari NH. Lipid peroxidation product, 4-hydroxynonenal and its conjugate with GSH are excellent substrates of bovine lens aldose reductase. Biochem Biophys Res Commun 1995; 217: 741-6.
3. Ramasamy R, Liu H, Oates PJ, Schaefer S. Attenuation of ischemia induced increases in sodium and calcium by the aldose reductase inhibitor zopolrestat. Cardiovasc Res 1999; 42: 130-9.
4. Mylari BL, Larson ER, Beyer TA, et al. Novel, potent aldose reductase inhibitors: 3, 4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl] methyl]-1-phthalazineacetic acid (zopolrestat) and congeners. J Med Chem 1991; 34: 108-22.
5. Van Wart HE, Steinbrink DRA. A continuous spectrophotometric assay for Clostridium histolyticum collagenase. Anal Biochem 1981; 113: 356-65.
6. Thring TS, Hili P, Naughton DP. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complement Altern Med 2009; 9: 27.
7. Pereira PJ, Lozanov V, Patthy A, et al. Specific inhibition of insect α-amylases: yellow meal worm α-amylase in complex with the amaranth α-amylase inhibitor at 2.0 å resolution. Structure 1999; 7: 1079-88.
8. Nguyen PQ, Wang S, Kumar A, Yap LJ, Luu TT, Lescar J. Discovery and characterization of pseudocyclic cystine-knot α-amylase inhibitors with high resistance to heat and proteolytic degradation. FEBS J 2014; 281: 4351-66.
9. Nguyen PQ, Luu TT, Bai Y, Nguyen GK, Pervushin K, Tam JP. Allotides: proline-rich cystine knot α-amylase inhibitors from allamanda cathartica. J Nat Prod 2015; 78: 695-704.
10. Lu S, Deng P, Liu X, et al. Solution structure of the major α-amylase inhibitor of the crop plant amaranth. J Biol Chem 1999; 274: 20473-8.
11. Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. Nucl Acids Res 2014; 42 (W1): W32-8.
12. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discov 2004; 3: 935-49.
13. Ojha LK, Tüzün B, Bhawsar J. Experimental and theoretical study of effect of allium sativum extracts as corrosion inhibitor on mild steel in 1 M HCl medium. J Bio Tribo Corros 2020; 6: 39.
14. Kroemer RT. Current protein. Peptide Sci 2007; 8: 312-28.
15. Bekhit AA, Fahmy HTY, Rostoma SAF, Bekhit AEA. Baraka AM. Design and synthesis of some substituted 1H-pyrazolyl-thiazolo[4,5-d]pyrimidines as anti-inflammatory-antimicrobial agents. Eur J Med Chem 2003; 38: 27-36.
16. Adefegha SA, Oboh G, Oyeleye SI, Ejakpovi I. Erectogenic, antihypertensive, antidiabetic, anti-oxidative properties and phenolic compositions of almond fruit (Terminalia catappa L.) parts (hull and drupe) – in vitro. J Food Biochem 2017; 41: e12309.
17. Lozanov V, Guarnaccia C, Patthy A, Foti S, Pongor S. Synthesis and cystine/cysteine-catalyzed oxidative folding of the amaranth α-amylase inhibitor. J Pept Res 1997; 50: 65-72.
18. Wang L, Lee W, Oh JY, Cui YR, Ryu B, Jeon YJ. Protective effect of sulfated polysaccharides from Celluclast-assisted extract of Hizikia fusiforme against ultraviolet B-induced skin damage by regulating NF-κB, AP-1, and MAPKs signaling pathways in vitro in human dermal fibroblasts. Mar Drugs 2018; 16: 239.
19. Sin BY, Kim HP. Inhibition of collagenase by naturally-occurring flavonoids. Arch Pharm Res 2005; 28: 1152-5.
20. Mylari BL, Larson ER, Beyer TA, et al. Novel, potent aldose reductase inhibitors: 3, 4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetic acid (zopolrestat) and congeners. J Med Chem 1991; 34: 108-22.
21. Morris GM, Goodsell DS, Halliday RS, et al. Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. J Comp Chem 1998; 19: 1639-62.
22. Gilad Y, Senderowitz H. Docking studies on DNA intercalators. J Chem Inf Model 2013; 54: 96-107.
23. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem 2009; 30: 2785-91.
24. Avdović EH, Milanović ŽB, Živanovića MN, et al. Synthesis, spectroscopic characterization, biological activity, DFT and molecular docking study of novel 4-hydroxycoumarine derivatives and coresponding palladium(II) complexes. Inorg Chim Acta 2020; 504: 119465
25. Frisch MJ, Trucks GW, Schlegel HB, et al. Gaussian 09, revision D.01. Gaussian Inc, Wallingford CT 2009.
26. Schrodinger L. Small-Molecule Drug Discovery Suite (2019). 2019-4.
27. 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.
28. Friesner RA, Murphy RB, Repasky MP, et al. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem 2006; 49: 6177-96.
29. Sastry GM, 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 2013; 27: 221-34.
30. Schrödinger Release 2019-4: LigPrep, Schrödinger, LLC, New York, NY, 2019.
31. 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 Structure Dynam 2020; 38: 625-33.

32. Schrödinger Release 2020-1: QikProp, Schrödinger, LLC, New York, NY, 2020.
33. Zangeneh MM, Bovandi S, Gharehyakheh S, Zangeneh A, Irani P. 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. Appl Organometal Chem 2019; 33: e4961.
34. Ramasubbu N, Paloth V, Luo Y, Brayer GD, Levine MJ. Structure of human salivary α-amylase at 1.6 Å resolution: implications for its role in the oral cavity. Acta Crystallogr D Biol Crystallogr 1996; 52: 435-46.
35. Balendiran GK, Sawaya MR, Schwarz FP, et al. The role of Cys-298 in aldose reductase function. J Biol Chem 2011; 286: 6336-44.
36. Khan S, Bhardwaj T, Somvanshi P, et al. Inhibition of C298S mutant of human aldose reductase for antidiabetic applications: evidence from in silico elementary mode analysis of biological network model. J Cell Biochem 2018; 119: 6961-73.
37. Zheng X, Zhang L, Chen W, Chen Y, Xie W, Hu X. Partial inhibition of aldose reductase by nitazoxanide and its molecular basis. Chem Med Chem 2012; 7: 1921-23.
38. Maurus R, Begum A, Williams LK, et al. Alternative catalytic anions differentially modulate human α-amylase activity and specificity. Biochemistry 2008; 47: 3332-44.
39. Ren L, Qin X, Cao X, Wang L, Bai F, Bai G, Shen Y. Structural insight into substrate specificity of human intestinal maltase-glucoamylase. Protein Cell 2011; 2: 827-36.
40. Avdović EH, Milenković D, Dimitrić Marković JM, et al. Synthesis, spectroscopic characterization (FT-IR, FT-Raman, and NMR), quantum chemical studies and molecular docking of 3-(1-(phenylamino)ethylidene)-chroman-2,4-dione. Spectrochim Acta A 2018; 195: 31-40.
41. Zhao L, Liu J, Guo R, Sun Q, Yang H, Li H. Investigating the interaction mechanism of fluorescent whitening agents to human serum albumin using saturation transfer difference-NMR, multi-spectroscopy, and docking studies. RSC Adv 2017; 7: 27796-806.
42. Laskar K, Alam P, Khan RH, Rauf A. Synthesis, characterization and interaction studies of 1,3,4-oxadiazole derivatives of fatty acid with human serum albumin (HSA): a combined multi-spectroscopic and molecular docking study. Eur J Med Chem 2016; 122: 72-8.
43. Siddiqi M, Nusrat S, Alam P, et al. Investigating the site selective binding of busulfan to human serum albumin: biophysical and molecular docking approaches. Int J Biol Macromol 2018; 107: 1414-21.
44. Shu Y, Xue W, Xu X, et al. Interaction of erucic acid with bovine serum albumin using a multi-spectroscopic method and molecular docking technique. Food Chem 2015; 173: 31-7.
45. Lipinski CA. Lead-and drug-like compounds: the rule-of-five revolution. Drug Discovery Today Technologies 2004; 1: 337-41.
46. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001; 46: 3-26.
47. Jorgensen WJ, Duffy EM. Prediction of drug solubility from structure. Adv Drug Deliv Rev 2002; 54: 355-66.