

# Anti-human lung cancer activities and survey of glutathione reductase and glutathione S transferase inhibition properties with molecular modeling studies of 2'-hydroxy-5'-methyl-3'-nitroacetophenone: a pre-clinical trial study

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## Abstract

**Introduction:** The molecular docking method was found to calculate the biological activity of the 2'-hydroxy-5'-methyl-3'-nitroacetophenone (2'-H-5'-M-3'-N) molecule against the enzymes studied.

**Material and methods:** In these calculations, the enzymes used were glutathione reductase (GR) and glutathione S-transferase (GT). After the modeling calculations were completed, the ADME/T parameters were examined to calculate the future drug use properties of the 2'-H-5'-M-3'-N molecule. To survey the antioxidant properties of 2'-H-5'-M-3'-N, the DPPH test was used. Several human lung adenocarcinoma cell lines, i.e., lung moderately differentiated adenocarcinoma (LC-2/ad), lung poorly differentiated adenocarcinoma (PC-14), and lung well-differentiated bronchogenic adenocarcinoma (HLC-1) cell lines, were used to determine the anticancer properties of the molecule.

**Results:** Cell viability of 2'-H-5'-M-3'-N was very low against PC-14, LC-2/ad, and HLC-1 cell lines without any cytotoxicity towards the normal cell line. The IC<sub>50</sub> values of 2'-H-5'-M-3'-N against LC-2/ad, PC-14, and HLC-1 cell lines were 475, 250, and 691 µg/ml, respectively. The best anti-human lung cancer properties of 2'-H-5'-M-3'-N against the above cell lines were observed in the case of the PC-14 cell line.

**Conclusions:** 2'-H-5'-M-3'-N was found to have significant antioxidant and anti-human lung cancer properties. It appears that the anti-human lung carcinoma effect of 2'-H-5'-M-3'-N is due to its antioxidant effects.

**Key words:** 2'-hydroxy-5'-methyl-3'-nitroacetophenone, human lung cancer, enzyme inhibition, molecular modeling.

## Introduction

Glutathione S-transferase (GST) is an enzyme involved in the detoxification of the liver that can catalyze the binding reaction of glutathione

(GSH) with various exogenous toxic compounds. It is found mainly in the liver and kidney, and in small amounts in the human stomach, ovary, breast and other organs. In addition to binding to GSH, it acts as a catalyst for conjugate reactions in protein purification. Given the importance of GST in biology and clinical practice, people have begun to devote themselves to the research of GST detection methods [1–4]. Glutathione reductase (GR) is the only enzyme that catalyzes the recovery of GSH from GSSG in an NADPH-dependent manner. Inhibition or activation of GR causes problems in the antioxidant system and many enzymatic reactions; studies report that this condition may be associated with aging and certain diseases such as cystic fibrosis, HIV, neurodegenerative disorders, and cancer. Compared to other enzymes in the glutathione system, GR activity has been found to decrease significantly with age and in various diseases [5–7].

When many studies in recent years are examined, the results of experimental and theoretical studies are seen together [8]. It has been observed that the studies made in this way sound better quality and realistic, because it has been observed that the results obtained support each other, thus increasing the accuracy of the results. In these studies, it was observed that the numerical values of the results obtained from theoretical studies were very close to each other. Therefore, when theoretical calculations are made before experimental studies, it has been seen that time will be an important guide for experimental studies. In this direction, it is possible to synthesize more effective and active compounds with theoretical calculations [9]. In theoretical calculations, molecular docking is the best method to compare the biochemical activities of molecules against enzymes [10]. In the calculations made by the molecular docking method, theoretical biochemical activity values of molecules against enzymes are found. It is possible to compare the biological activities of other molecules with the numerical values obtained as a result of the calculation. Many parameters are obtained in the calculations of the 2'-hydroxy-5'-methyl-3'-nitroacetophenone (2'-H-5'-M-3'-N) molecule against enzymes.

Significant information is provided about the biochemical activities of compounds with the parameters obtained as a result of these calculations. After these calculations, ADME/T (distribution, metabolism, excretion, absorption, and toxicity) analysis of the 2'-H-5'-M-3'-N molecule was performed. With the ADME/T analysis, attempts are made to predict theoretically the effects and reactions of drug molecules in human metabolism in cells and tissues. Attempts are made to predict

these effects and responses with the numerical values of the parameters found by the ADME/T calculations in the molecular docking calculations. The numerical value of each parameter obtained gives important information about the action and reaction of molecules in different organs or tissues. These results give the properties of the molecule to be used as a drug in the future [11].

We investigated 2'-H-5'-M-3'-N in cytotoxicity studies against common human lung cancer cell lines, i.e., LC-2/ad, PC-14, and HLC-1 cell lines, and *in vitro* enzyme inhibition with molecular modeling studies.

## Material and methods

### Material

Bovine serum, antimycotic antibiotic solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), decamplmaneh fetal, 4-(dimethylamino) benzaldehyde, hydrolysate, Ehrlich solution, borax-sulfuric acid mixture, and DMED were obtained from Sigma-Aldrich (USA).

### Biological assays

Inhibition assays of GST. Phosphate buffer (415  $\mu$ l, 0.1 M pH 6.6), GST (10  $\mu$ l, 0.148 mg/ml), and inhibitor (12.5  $\mu$ l in DMSO) were added to a 500  $\mu$ l cuvette, and the solution was mixed well. After incubation at ambient temperature for 5 min, CDNB (12.5  $\mu$ l, 40 mM in EtOH) and GSH (50  $\mu$ l, 10 mM) were added and quickly mixed well. Absorbance was measured at 340 nm at 20°C for 5 min [12] and performed conforming to previous studies [13–15].

### Docking studies

An important method used to calculate the theoretical biochemical activities of compounds against enzymes is molecular modeling. In the calculations made by the molecular docking method, many parameters are obtained to compare the biochemical activities of compounds [16]. These parameters are very significant parameters to explain the theoretical biological activities of compounds [17]. In order to calculate the biological activity of the 2'-H-5'-M-3'-N molecule, calculations were made with many enzymes. In this study, the enzymes glutathione reductase (GR) and glutathione S-transferase (GT) were used for the 2'-H-5'-M-3'-N molecule. Molecular docking calculations to calculate the biochemical activity of the 2'-hydroxy-5'-nitro-methyl-3'-nitroacetophenone molecule were performed using the Maestro Molecular modeling platform (version 12.2) by Schrödinger. Proteins and 2'-H-5'-M-3'-N molecules must be prepared for calculations using the Maestro Mo-

molecular modeling platform (version 12.2) by the Schrödinger program. In docking calculations, each stage is completed by carrying out different procedures for molecules and enzymes. Firstly, the Gaussian software program [18] was used to obtain optimized structures of molecules. Using these optimized structures, all calculations were made with the Maestro Molecular modeling platform (version 12.2) by Schrödinger, LLC [19], which comprises many modules. In the first one, the protein preparation module [20] is used to prepare the enzymes formed by proteins for calculations. In the next module, the LigPrep module [21] was used to prepare the 2'-H-5'-M-3'-N molecule for calculations.

In the next module, the Glide ligand docking module [22] was used to interact with the molecule and enzymes. OPLS3e assay was used in all calculations for docking calculations of proteins and compounds in all modules used. After the docking calculations, ADME/T analysis (distribution, metabolism, absorption, excretion and toxicity) was performed to examine the future drug properties of the molecule. The Qik-prop module [23] of the Schrödinger software was used for ADME/T analysis.

#### Determination of the antioxidant activities of 2'-H-5'-M-3'-N

Analysis of antioxidant capacity of the DPPH radical method is a well-known test for measuring the antioxidant power of various compounds. The method is based on the reduction of free radical DPPH by antioxidants in the absence of other free radicals in the environment. A compound is generally compared to a known antioxidant compound such as butylated hydroxytoluene (BHT). Analysis of antioxidant capacity by the DPPH method is a test that has received much attention in the fields of food, pharmaceuticals and biotechnology and is used to develop and introduce new antioxidants. The method is based on the reduction of free radical DPPH by antioxidants in the absence of other free radicals in the environment, which results in color in the environment whose intensity can be measured by spectroscopy. DPPH is a stable free radical that has an unpaired electron on one of the nitrogen bridge atoms. Radical inhibition of DPPH is the basis for assessing antioxidant capacity [24]:  $\text{DPPH}^\bullet (\text{purple}) + \text{H} - \text{A} \rightarrow \text{DPPH} (\text{yellow}) + \text{A}^\bullet$ .

DPPH is a stable radical whose methanolic solution has a purple color that shows the highest light absorption at 519–595 nm. The basis of this method is that the DPPH radical acts as an electron acceptor of a donor molecule such as an antioxidant, thus converting DPPH to DPPH<sub>2</sub>. In this case, the purple color of the environment

turns yellow, so the absorption intensity decreases to 595 nm. Antioxidant properties can be determined by measuring the decrease in adsorption intensity by spectroscopy [24].

In the present study to measure the antioxidant properties of 2'-H-5'-M-3'-N, 2 ml of DPPH (100 μM) was dissolved in methanol with 2 ml of 2'-H-5'-M-3'-N at the concentrations of μg/ml. The resulting mixture was held at 25°C for 30 min. Then the samples absorbance was measured at 520 nm by a spectrophotometer (Spectramax Gemini XS; Molecular Devices, Sunnyvale, CA) and the amount of antioxidant effect was determined by the following formula [24]: %Inhibition =  $[(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$ .

The blank sample consisted of a mixture of 2 ml of 2'-H-5'-M-3'-N and 2 ml of methanol, and a sample containing 2 ml of DPPH and 2 ml of 2'-H-5'-M-3'-N with the concentrations used was considered as a negative control. BHT was also used as a positive control [24].

Calculating the half maximal inhibitory concentration ( $IC_{50}$ ) is an excellent way to compare drug activity. The dose in which 50% of the final activity of the drug occurs is the criterion for measurement and comparison. In this test, the value of  $IC_{50}$  for different repetitions of the test was also calculated and was compared with  $IC_{50}$  of the BHT molecule, which is an indicator of antioxidant activity. The closer  $IC_{50}$  is to BHT, the stronger the antioxidant activity. In the following experiments, the  $IC_{50}$  of 2'-H-5'-M-3'-N was calculated by plotting the inhibition percentage curve against the 2'-H-5'-M-3'-N concentration. In the next step, a serial dilution was prepared from each sample and  $IC_{50}$  of three separate samples was measured and its mean was calculated. All experiments were performed three times [24].

#### Determination of anti-human lung cancer effects of 2'-H-5'-M-3'-N

In this assay, different human lung cancer cell lines i.e., lung poorly differentiated adenocarcinoma (PC-14), lung moderately differentiated adenocarcinoma (LC-2/ad), and lung well-differentiated bronchogenic adenocarcinoma (HLC-1) cell lines and also a normal cell line (HUVEC) were used to study the cytotoxicity and anti-human lung cancer potential of the 2'-H-5'-M-3'-N using the common cytotoxicity test, i.e., MTT assay in *in vitro* condition. 15 ml of RPMI 1640 medium containing 10% FCS (10 mg/ml penicillin and 100 mg/ml streptomycin) in a culture flask was placed in a CO<sub>2</sub> incubator for 2 h 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 possi-

ble 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 min and the cells should not be overheated. The medium was added dropwise to the vial and then its contents were taken out and centrifuged with the medium in 15 ml 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 [25, 26].

Cell lines were 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 CO<sub>2</sub>. Cells began to grow in 75 cm<sup>2</sup> T-flasks in 15 ml of medium with an initial number of 1–2 × 10<sup>6</sup> cells. After three days and covering the flask bed with the cells, the adhesive layer on the bottom of the flask was separated enzymatically using trypsin-*verson* and transferred to a sterile test tube for 10 min 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 as a blank containing only culture medium. Another column contained culture medium and healthy cells and other columns contained culture medium and cell line cells. One of these columns, which contained culture medium and cells and did not contain 2'-H-5'-M-3'-N, was considered as a control [25].

The plates were incubated in the incubator for 24 h to return the cells to normal from the stress of trypsinization. After this time, suitable dilutions of the prepared 2'-H-5'-M-3'-N (0-1000 µl/ml) and 100 µl of each dilution were added in columns to the plate wells. (Thus, the final concentration of the studied compound in the wells was halved. Therefore, the concentrations were prepared twice as high to reach the final concentration after being added to the well.) The cells were incubated for 37 h at 37°C and 5% CO<sub>2</sub> in the atmosphere. After 72 h, 20 µl of MTT solution (5 mg/ml) was added to each well. The plates were incubated for 3 to 4 h and then the residue was removed and 100 µl of DMSO was added to each well to dissolve the resulting formazan. After 10 min, using shaking of the plates, the optical absorption of formazan at 570 nm was read using a plate reader. Wells containing cells without 2'-H-5'-M-3'-N were considered as a control and the optical density of wells without cells and only culture medium were considered as a blank. The

percentage of cell viability was calculated using the following formula [26]:

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

### Qualitative measurement

After collecting data, Minitab statistical software was used for statistical analysis. Evaluation of antioxidant results in a completely randomized design and comparison of means was by Duncan post-hoc test with a maximum error of 5%. To measure the percentage of cell survival in factorial experiments with the original design of completely randomized blocks and compare the means, the Duncan post-hoc test with a maximum error of 5% was used. The 50% cytotoxicity (IC<sub>50</sub>) and 50% free radical scavenging (IC<sub>50</sub>) were estimated with ED50 plus software (INER, V: 1.0). Measurements were reported as mean ± standard deviation.

## Results and Discussion

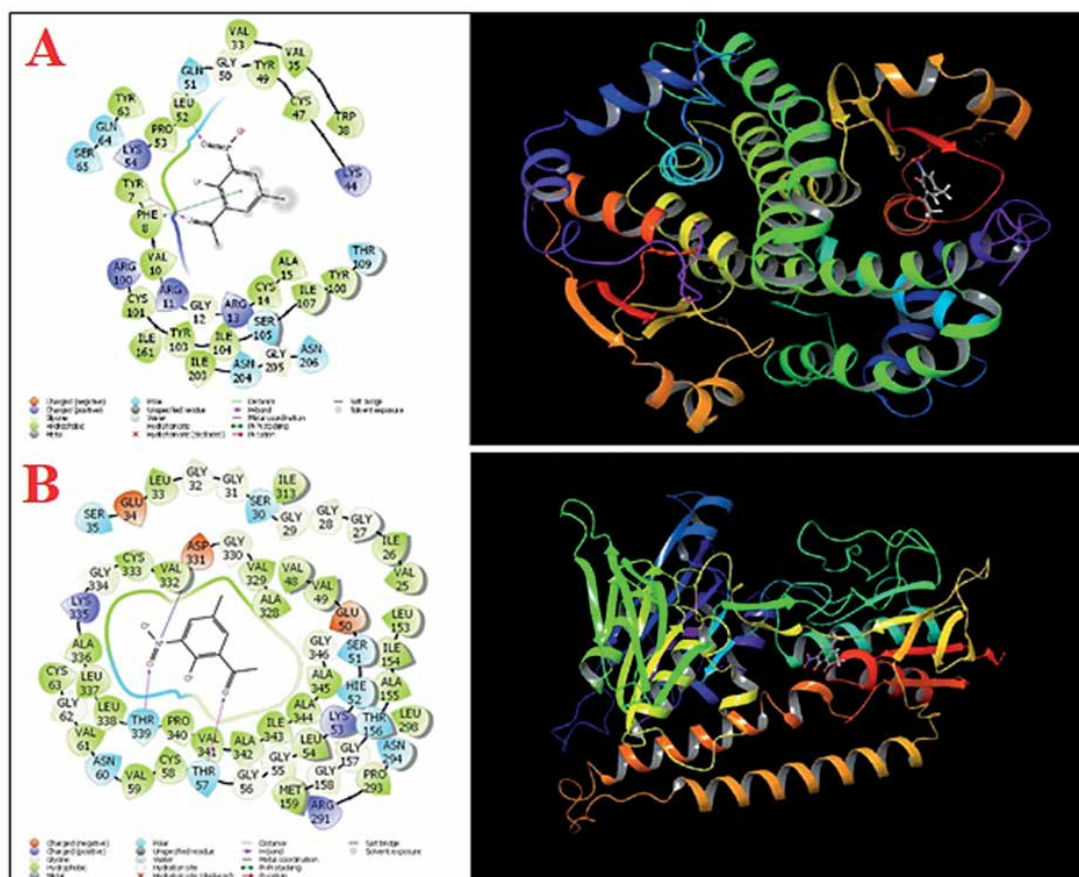
### Enzyme results

IC<sub>50</sub> values for these two enzymes were obtained at the micromolar level. IC<sub>50</sub> values for GST and GR were determined as 165.31 and 98.71 µmol, respectively. GSTs are a broad family of dimeric enzymes responsible for cell detoxification, thus protecting the cell from cytotoxic and oxidative stress. They catalyze the conjugation of GSH to a wide variety of xenobiotic electrophiles such as prostaglandins, quinones, and base propeptals, making them more soluble in water and easily removed from the cell. GSTs are a potential drug target in cancer therapy, where resistance to chemotherapeutic drugs is directly associated with overexpression of GSTs in tumor cells and parasitic diseases such as schistosomiasis and malaria [27, 28]. Glutathione reductase (GR) is an important antioxidant enzyme required to maintain the GSH/GSSG ratio by catalyzing the recovery of reduced glutathione (GSH) from oxidized glutathione (GSSG). Because of this vital task, inhibition of GR is an important target in the treatment of many diseases, so we aimed to identify natural and novel GR inhibitors to guide drug design [29, 30].

### Molecular modeling results

As a result of molecular docking calculations, many parameters of the 2'-H-5'-M-3'-N molecule against enzymes were found [31]. As a result of the calculations, the most important parameter found is the docking score. It should be well known that the molecule with the most negative numerical value of the docking score parameter obtained as a result of the interaction between molecules and enzymes has higher biological ac-





**Figure 1.** Presentation of interactions of nitroacetophenone with glutathione S-transferase (A) and glutathione reductase (B)

tivity than other molecules [32]. As the interaction between the 2'-H-5'-M-3'-N molecule and the enzyme increases, the increase in the biological activity value of the 2'-H-5'-M-3'-N molecule shows both experimental and theoretical results. These interactions are given in Figure 1.

Enzymes used for this comparison are glutathione reductase (GR) (PDB ID: 4GRT) and glutathione S-transferase (GT) (PDB ID: 2GSS). The parameters of the molecule obtained as a result of interactions with all enzymes are given in Table I.

Many other parameters were found in the calculations. These parameters are used to explain the interaction between 2'-H-5'-M-3'-N and the enzyme. Other parameters found as a result of the calculations are Glide hbond, Glide evdw, and Glide ecoul parameters, which give numerical values of hydrogen bonding, Van der Waals, and Coulomb interactions, which are many interactions between molecules and enzymes [33]. On the other hand, the last remaining parameters are Glide emodel, Glide energy, Glide einternal, and Glide posenum, which give numerical values about the interaction between molecule and enzyme [34].

After the docking calculations made to compare the biological activities of the molecules, the

**Table I.** Numerical values of the docking parameters of molecule against enzymes

| Parameter               | GR     | GT     |
|-------------------------|--------|--------|
| Docking score           | -5.96  | -4.96  |
| Glide ligand efficiency | -0.43  | -0.35  |
| Glide hbond             | -0.19  | -0.32  |
| Glide evdw              | -23.77 | -16.55 |
| Glide ecoul             | -1.85  | -5.73  |
| Glide emodel            | -36.58 | -31.44 |
| Glide energy            | -25.62 | -22.27 |
| Glide einternal         | 0.08   | 0.03   |
| Glide posenum           | 66     | 58     |

drug properties of the 2'-H-5'-M-3'-N molecule were investigated. As a result of the ADME/T analysis, many parameters were found for the molecule. These calculated parameters give the effects and responses of molecules in tissues or organs in human metabolism [35]. As a result of this analysis, each parameter has a numerical value in different organs or tissues.

Among all ADME/T parameters, two important parameters are the rule of five and the rule of three. The rule of five [36] and rule of three [37] parameters are more important than any other parameter. The numerical value of these two parameters is expected to be zero. These are the two most important parameters in ADME/T calculations. If the conditions for these two parameters are not met, the molecule is considered unsuitable to be a drug.

### Therapeutic capacities of 2'-H-5'-M-3'-N

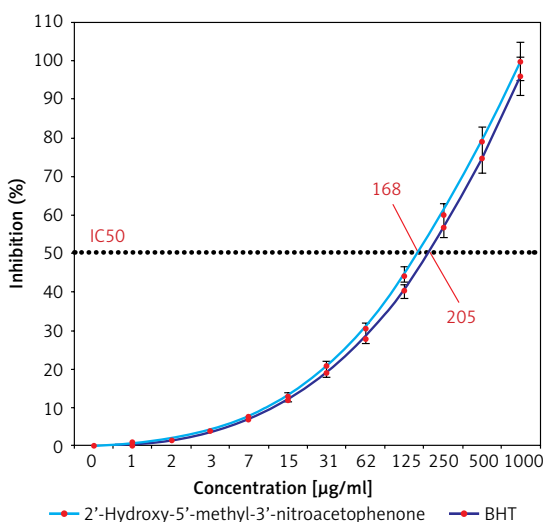
Free radicals are highly active compounds with single or unpaired electrons. These compounds are made by metabolic functions in the body during the reaction of oxygen with certain molecules. Free radicals tend to gain or lose an electron so that the number of electrons is even. Free radical damage occurs when radicals collide with other molecules to find electrons [1]. Free radicals often take an electron from a nearby molecule and convert it into a new free radical, and then the new free radical repeats the reaction chain sequentially, causing cell damage and DNA damage. DNA damage can lead to a variety of effects, including premature aging and cancer. Free radicals are produced in the body due to the body's natural response to pathogens, metabolism, especially lipid oxidation, stress, air pollutants, high-energy electromagnetic waves such as X-rays, foods such as hydrogenated vegetable oils and some drugs such as doxorubicin [24].

In order to fight the free radicals produced, the cells in the body have intracellular defense due to the presence of the enzymes superoxide dismutase, catalase and glutamine peroxidase. In addition, compounds such as vitamins and minerals (selenium and zinc) with their antioxidant

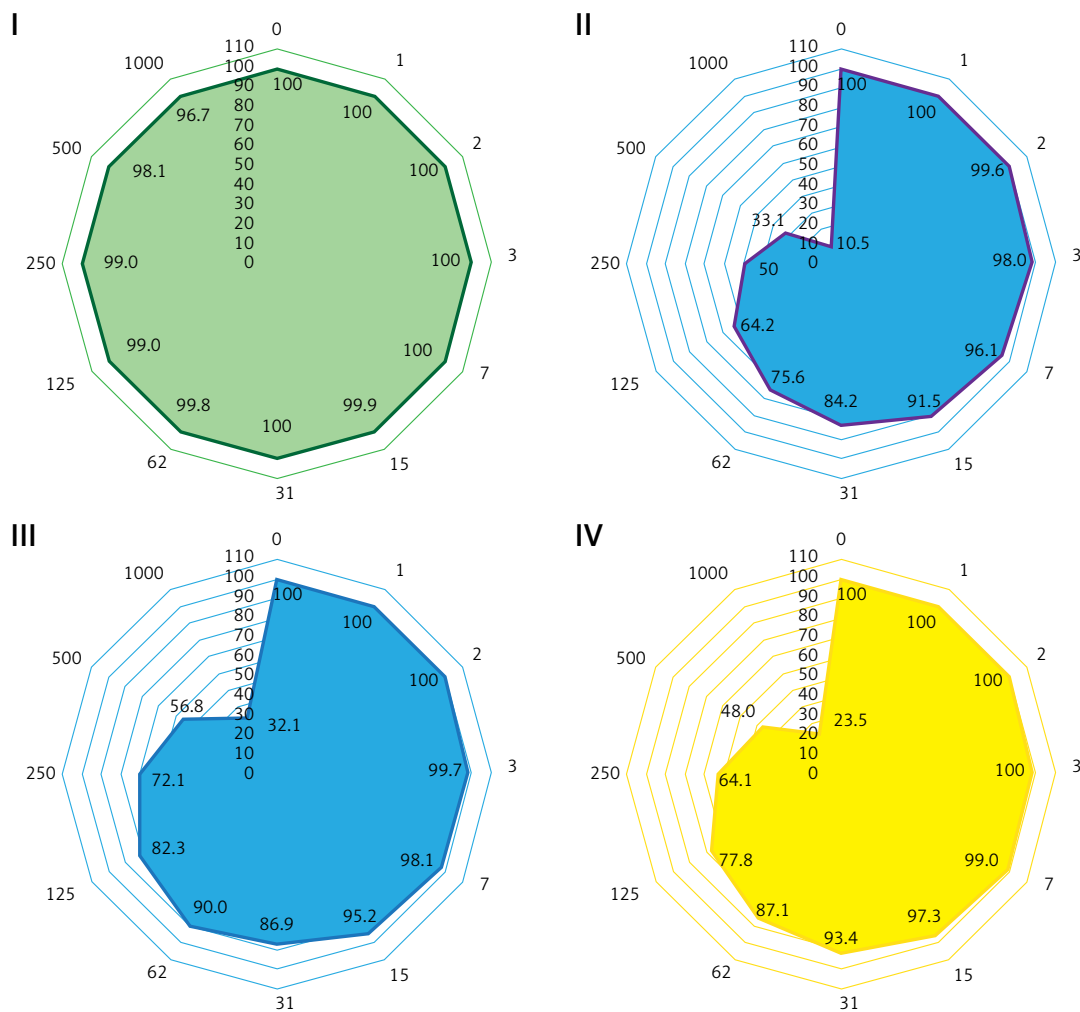
behavior cause the elimination of free radicals and thus reduce their harmful effects on the body [38]. Antioxidants inhibit the free radicals' activity or cause them to be removed, and protect the cells of the body from the damaging effects of the free radicals. Hence, they fight the aging process and various diseases. These substances can inhibit the formation of free radicals in the body and, if formed, reduce their impact on the body [1, 24]. In fact, antioxidants are compounds that are used to prevent or slow down the damage caused by oxidative reactions in the body, and they act as neutralizers of free radicals and therefore prevent damage from these compounds in the body [24, 38]. These compounds, on the one hand, reduce the risk of cardiovascular disease and stroke in the first place, and on the other hand, prevent the progression of cancers that cause DNA damage. Despite the presence of various antioxidants in plasma, the body's immune system alone is not able to eliminate free radicals created in the body; therefore, it needs to provide antioxidants from external sources that are provided through food sources. Therefore, there is a need for strong antioxidants with lower toxicity and greater effectiveness. Antioxidants are also used in industry as food preservatives to prevent spoilage and discoloration of foods, thus increasing the shelf life of foods. Plant extracts are rich in antioxidant compounds [39–41].

Now, turning our attention to investigate the bioactivity of 2'-H-5'-M-3'-N a concentration-dependent DPPH radical scavenging effect of 2'-H-5'-M-3'-N was observed against BHT as a reference. In the antioxidant test, the  $IC_{50}$  values of butylated hydroxytoluene and 2'-H-5'-M-3'-N were 168 and 205  $\mu\text{g}/\text{ml}$ , respectively (Figure 2).

Oxidation from reactive oxygen species can cause cell membrane disintegration, damage to membrane proteins, and DNA mutation, the result of which 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 [42]. 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 whose 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 activ-



**Figure 2.** Antioxidant properties of 2'-H-5'-M-3'-N and BHT against DPPH



**Figure 3.** Anti-human lung cancer properties (cell viability (%)) of 2'-H-5'-M-3'-N (concentrations of 0-1000 µg/ml) against HUVEC (I), lung poorly differentiated adenocarcinoma (PC-14: II), lung well-differentiated bronchogenic adenocarcinoma (HLC-1: III), and lung moderately differentiated adenocarcinoma (LC-2/ad: IV) cell lines. The numbers indicate the percent of cell viability in the concentrations of 0-1000 µg/ml of 2'-H-5'-M-3'-N against several human lung cancer cell lines

ity [42-44]. It seems the high anti-lung adenocarcinoma properties of 2'-H-5'-M-3'-N are related to its antioxidant activities. Our successful efforts to utilize 2'-H-5'-M-3'-N in adenocarcinoma studies certainly shed light on future studies in this area.

In this present study, the cytotoxicity of 2'-H-5'-M-3'-N was explored by studying its interaction with normal (HUVEC), LC-2/ad, PC-14, and HLC-1 cell lines by MTT assay for 48 h. The interactions expressed as cell viability (%) were observed at different 2'-H-5'-M-3'-N concentrations (0-1000 µg/ml) with the four cell lines which are shown in Figure 3.

In all the cases the % cell viability decreases with increasing 2'-H-5'-M-3'-N concentrations. The IC<sub>50</sub> values of 2'-H-5'-M-3'-N against LC-2/ad, PC-14, and HLC-1 cell lines were 475, 250, and 691 µg/ml, respectively (Table II). Thus, the best cytotoxicity results and anti-human lung cancer

potential of our 2'-H-5'-M-3'-N was observed in the case of the PC-14 cell line.

In conclusion, the biological activity of the 2'-H-5'-M-3'-N molecule against enzymes was determined by docking calculations. The numerical values of these parameters were compared with other molecules. Then, ADME/T analysis of the 2'-H-5'-M-3'-N molecule was performed. With this analysis, the ADME/T parameters of the 2'-H-5'-M-3'-N molecule show that it is safe to use it as a drug in the future. In this direction, the 2'-hydroxy-5'-methyl-3'-nitroacetophenone molecule will progress to become a drug with future *in vivo*

**Table II.** IC<sub>50</sub> of 2'-H-5'-M-3'-N in the anti-human lung cancer test

|                          | HUVEC | PC-14                | HLC-1                | LC-2/ad              |
|--------------------------|-------|----------------------|----------------------|----------------------|
| IC <sub>50</sub> [µg/ml] | -     | 250 ± 0 <sup>a</sup> | 691 ± 0 <sup>c</sup> | 475 ± 0 <sup>b</sup> |

and *in vitro* studies. 2'-H-5'-M-3'-N was also assessed in biological applications such as radical scavenging and anticancer (adenocarcinoma) activities. In the antioxidant test, the IC<sub>50</sub> values of butylated hydroxytoluene and 2'-H-5'-M-3'-N were 168 and 205 µg/ml, respectively. 2'-H-5'-M-3'-N exhibited good antioxidant properties, even better than the reference standard molecule. It also showed significant cytotoxic activities against common human lung cancer cell lines, i.e., LC-2/ad, PC-14, and HLC-1.

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### Conflict of interest

The authors declare no conflict of interest.

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