Anti-ageing and Anti-lung Carcinoma Effects of Vulpinic Acid and Usnic Acid Compounds and Biological Investigations with Molecular Modeling Study

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Abstract: Disorganization and breakdown of extracellular matrix proteins like fibronectin, collagen, and elastin are key characteristics of skin aging due to the increased activation of important proteolytic enzymes like elastases and collagenase enzymes. Also, inhibition of their enzymatic activities by natural molecules might be a promising factor to prevent extrinsic skin aging. All chemicals were obtained from Sigma-Aldrich unless otherwise stated. The assay employed was based on spectrophotometric methods reported in the literature. The collagenase and elastase inhibition assays of some phenolic compounds were performed according to the previous studies. These compounds showed excellent to good inhibitory activities of vulpinic acid against studied enzymes with IC50 values of 195.36 \( \mu \)M for collagenase and 25.24 \( \mu \)M for elastase. The molecular docking calculations were conducted to investigate the chemical and biological activity of vulpinic acid and usnic acid against collagenase and elastase. The results indicated that these two compounds can interact with the essential residues of the enzymes and affect their activities. The calculations of binding free energies were also performed to obtain more details about the characteristics and free energies of the ligand-enzyme complexes. Additionally, both compounds exhibited the most potent inhibition in the three lung cancer cells, with an IC50 value of 21–68 \( \mu \)M, indicating that vulpinic acid is more potent than Doxorubicin, which exhibited an IC50 value of 21–29 \( \mu \)M.

Key words: vulpinic acid, usnic acid, anti-lung cancer, collagenase, enzyme inhibition

1 Introduction

Usnic acid occurs naturally from dibenzofuran derivatives found in various lichen species. This compound is one of the most popular and most abundant secondary metabolites. It has antiviral, antimutotic, anti-inflammatory and analgesic effects\textsuperscript{1}. Usnic acid under normal conditions; It is bitter, yellow and solid. In addition to being a racemic mixture, it is known that there are D and L forms. Lichen extracts containing usnic acid have also been used in medical, cosmetics, perfumery, and ecology. It has strong antibiotic effect against gram positive bacteria such as Streptococcus, Staphylococcus, and Pneumococcus and other bacteria such as Mycobacterium tuberculosis and some pathogenic fungi\textsuperscript{2,3}. It has properties such as UV absorption, protective properties, growth inhibitory, and insecticide. The effects of lichen compounds Parietin, atranorin, usnic acid on human cancer cells were investigated, and usnic acid was found to be a more effective anti-cancer agent than others. The effects of acids such as usnic acid, which are secondary metabolites of lichens, on the activities of topoisomerase I and II enzymes and the effects of this compound on DNA binding properties in cell-free media were investigated\textsuperscript{4,5}. The effects of usnic acid and atranorin against human prostate and melanoma cancer cells were investigated and it was observed that both compounds had strong inhibitory effects against cancer cells. Additionally, vulpinic acid was first described in 1925. It is clear yellow in color and relatively toxic. It has been reported to show activity against Gram-positive bacteria. Wound healing and antiproliferative effect of tumor cells were investigated in different lichen compounds (vulpinic acid) . The anti-inflammatory effects of aromatic cyclic vul-
pinic acid on rheumatoid arthritis in rabbits were investigated.\textsuperscript{17} Elastase and collagenase are matrix metalloproteinase enzymes and comprise a family of ECM-degrading enzymes. These enzymes play a key role in the regulation of extracellular matrix degradation which is necessary for wound re-epithelialization. Indeed, they are involved in the pathogenesis of various diseases, like cardiovascular diseases, cancer, fibrosis, bone destruction, inflammation, as well as in the growth and healing of wounds.\textsuperscript{8,9}

Considering the improvement of computational technologies and a growing number of biological and chemical databases, the importance of computational methods and modeling, \textit{in silico} approaches, and artificial intelligence is undeniable.\textsuperscript{10-12} Molecular docking as a versatile theoretical approach has attracted considerable attention among biologists in recent years. This method allows the user to investigate the interactions between small molecules and biomolecules at an atomic level.\textsuperscript{13} The molecular docking study can determine the behavior of the ligands near binding sites and evaluate the characteristics of the interactions.\textsuperscript{14} This is an \textit{in silico} approach that could enhance the process of drug discovery in a computational manner.\textsuperscript{15} Furthermore, the data obtained from the molecular docking calculations can be used as complementary information for experimental studies. The results can provide some essential characteristics of the ligand-biomolecule complex.

Cancer is the most important public health problem in the world, and lung cancer has the highest mortality rate among cancers. Most lung carcinomas (57\%) are not diagnosed at an early stage as they are typically asymptomatic during this period. Most patients with small cell lung cancer and stage IIIB/IV non-small cell lung cancer receive chemotherapy.\textsuperscript{16} However, chemotherapy factor is not curative for metastatic lung cancer, but it can relieve symptoms or prolong life by weeks. Chemotherapy remains undesirable due to deficiencies in tumor tissues, including inadequate intracellular uptake, non-specific target site concentrations, and severe systemic toxicity of chemotherapeutic agents and even emerging drug-resistant cell lines.\textsuperscript{17}

2 Materials and Methods

2.1 Biological activities

0.05 mL was taken from the prepared sample solutions. On top of it, 0.05 mL of elastase enzyme (0.16 U/mL) was added. Then, 0.9 mL of tris hydrochloride (Tris-HCl) buffer solution of 0.2 M (pH = 7.8) was added to the sample solutions. It was prepared by adding 0.2 M (pH = 7.8) 0.9 mL Tris-HCl buffer solution to 0.1 mL of elastase enzyme solution as a control solution.\textsuperscript{19} The blank solution was prepared by adding 0.2 M (pH = 7.8) 0.9 mL Tris-HCl buffer solution to 0.1 mL distilled water. Blank, control and sample solutions were incubated at 37°C for 15 minutes. After incubation, 5 mM 0.05 mL N-Succinyl-Ala-Ala-Ala-p-nitroanilide substrate was added to the blank, control and sample solutions and incubated at 37°C for 30 minutes. The absorbance values of the sample and control solutions against the blank were read at 410 nm.\textsuperscript{20} In the study, the anti-elastase inhibition activity values of the samples prepared at different concentrations were calculated. Experiments were repeated 3 times and averaged. The % inhibition values on elastase enzyme of eperezolide-like compounds synthesized for the first time were calculated. The IC50 value (the concentration required to inhibit 50% of the activity) was calculated from the regression equation obtained from the linear segment of the curve drawn by applying the concentration to abscess, % elastase enzyme inhibition data to the ordinate.\textsuperscript{20}

Modified inhibitory effect on collagenase enzyme Thring \textit{et al.} (2009)\textsuperscript{21} was determined spectrophotometrically using the method. 50 μL of the solution containing 0.8 U/mL collagenase was taken, 50 μL of plant extracts and chemical substance solutions at different concentrations prepared on it were added. This method was performed according to previous studies. The absorbance values of the sample solutions and control solution were read at 340 nm in the UV spectrophotometer against the blank. Experiments were repeated 2 times. The IC50 value, which is the amount of substance required for the collagenase enzyme to have a 50% inhibition effect, was calculated with the regression equation obtained from the linear section of the curve drawn by applying the concentration to the abscissas, % enzyme inhibition data to the ordinate.\textsuperscript{20}

For lung cancer part, the MTT method was performed as explained previously. Cells were seeded onto a 96-well plate at a concentration of 104 cells/well and allowed to adhere overnight. Five replicates were prepared for each therapy and cultured for 48 or 72 h. After 20 mL of MTT (5 mg/mL) was added to each well, the cells were cultured for another 4 h. The supernatant was discarded. After 150 mL of DMSO was added to each well, the samples were incubated at 37°C for 30 min and then swirled for 10 min. The absorbance at 570 nm was measured using a microplate reader. Experiments were repeated three times.\textsuperscript{24}

2.2 Molecular docking study

Theoretical approaches such as molecular docking calculations can provide beneficial information about the details and characteristics of the interactions between inhibitors and biological compounds. The enzymes used in this study were human neutrophil elastase (PDB ID: 2Z7F).\textsuperscript{25}
and Collagenase H from Clostridium histolyticum (PDB ID: 4AR1)\(^{30}\). The biological activities of the vulpinic acid and usnic acid were studied against these enzymes. The PDB format of the enzymes was obtained from the PDB database (http://www.rcsb.org/pdb), and the protein preparation module of the Schrödinger Suite\(^{27}\) was utilized for their preparation. By this, the hydrogen atoms were added, the water molecules were removed, and a network of H-bond was constructed. Finally, the structures were minimized using the OPLS3e force field. In the next step, the active sites of the enzymes were determined employing the SiteMap of Schrödinger\(^{28}\). The SDF files of the vulpinic acid and usnic acid were taken from the PubChem database, and the LigPrep module of Schrödinger\(^{29}\) was used for the ligand preparation. Eventually, the docking estimations were performed employing the Glide of Schrödinger suites.

### 2.3 Binding free energy calculations

Another crucial calculation for understanding the characteristics of the ligand-enzyme complex is binding free energy calculations. Here, the MM/GBSA method has been used for these predictions. The calculations were conducted employing the prime module of Schrödinger. The solvation model of VSGB and OPLS-2005 force field were used for the calculations\(^{30}\). The equation of binding free energy is:

\[
\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}})
\]

Where \(\Delta G_{\text{bind}}\) symbolizes the binding free energy, \(G_{\text{complex}}\) is the binding free energy of the complex. The \(G_{\text{protein}}\) and \(G_{\text{ligand}}\) show the binding free energy of the protein and ligand, respectively.

### 3 Results and Discussion

#### 3.1 Biological activities

Inhibiting the activity of extracellular matrix-degrading proteins like elastases and collagenases may be a useful approach to prevent premature skin aging and UV-induced skin alterations. Scavenging of ROS by natural antioxidant compounds might be one option to inhibit such skin deteriorative enzymes, as ROS play a key role in the activation of these enzymes. Phenolic molecules are a significant class of natural antioxidant compounds\(^{31}\). They belong to various subclasses of secondary plant metabolites classified as flavonoids, phenolic acids, stilbenes, and lignans and are ubiquitously recorded in the plant kingdom. Also, white and red grapes contain high amounts of flavonoids and phenolic acids like catechin and gallic acid\(^ {32}\). The collagenase and elastase inhibition assays of some phenolic compounds were performed according to the previous studies. These compounds showed excellent to good inhibitory activities of vulpinic acid against studied these enzymes with IC50 values of 195.36 \(\mu\)M for collagenase and 25.24 \(\mu\)M for elastase. The Usnic acid had IC50 values of 94.05 \(\mu\)M against collagenase and 7.38 \(\mu\)M against elastase. The most potent compounds against collagenase and elastase were compound usnic acid with IC50 values of 94.05 \(\mu\)M against collagenase and IC50 values of 7.38 \(\mu\)M against elastase, respectively.

#### 3.2 Molecular docking

The biological and chemical activities of vulpinic acid...
and usnic acid in the presence of collagenase and elastase were investigated using the molecular docking study. The results indicated the target residues for these two chemical compounds. The docking pose of vulpinic acid among collagenase residues is presented in Figure 1, and Figure 2 presents the interactions of the vulpinic acid with collagenase. Figure 2 indicates that vulpinic acid has formed three hydrogen bonds with the residues of collagenase. These residues are Lys375, which is from the catalytic subdomain, Lys641, and Arg652, which are from the helper subdomain of the enzyme. There are also thirteen hydrophobic contacts that are created by the molecules of vulpinic acid. Ten residues of these thirteen amino acids are from the catalytic subdomain. These residues are Lys353, Val354, Asn378, Phe382, Asp388, Asp397, Asp398, Leu400, Tyr460, and Arg464.

Figure 3 presents the docking pose of vulpinic acid among the residues of elastase. Figure 4 shows the contacts created by vulpinic acid with the enzyme. As could be seen, there are six hydrophobic contacts. Phe215 and Arg217 are among these residues, which are two of the critical residues of elastase. These residues have a
crucial impact on the catalytic activity of the enzyme. Since vulpinic acid has constructed considerable interactions with the key residues of collagenase and elastase, this compound could be considered as a potential inhibitor for these enzymes.

The docking pose of usnic acid among the residues of collagenase and their interactions are presented in Figs. 5 and 6, respectively. Figure 6 indicates that usnic acid has constructed two hydrogen bonds and eleven hydrophobic contacts with collagenase. Lys375 is one of the residues with a hydrogen bond, which is from the catalytic subdomain of the enzyme. There are also some other residues from the catalytic subdomain of collagenase with hydrophobic contacts. These residues are Lys353, Asn378, Lys382, Lys389, Tyr460, Arg464, and Tyr465. These interactions are crucial since they are in contact with the residues of the catalytic subdomain. Therefore, usnic acid can inhibit the activity of collagenase efficiently. The docking pose of this compound among the residues of elastase is presented in Fig. 7. Figure 8 shows that this chemical agent has constructed one hydrogen bond with Arg217. This H-bond has been created between the NH of the peptide backbone of Arg217 and an oxygen molecule of the ligand, which is presented in Fig. 8. There are also four hydrophobic contacts with Asn99, Arg177, Phe215, and Val216. Residues 215-217 are critical amino acids of the elastase. They have a direct impact on the catalytic activity of the enzyme.

These interactions can lead to a considerable inhibitory activity of usnic acid in the presence of elastase and collagenase. Hence, this compound could be considered as a potential inhibitor for these two enzymes. There are also some other parameters that can describe the characteristics of the interactions between the chemical compound and biomolecules. Table 1 shows some of the parameters. One of these parameters is the docking score, which is one of the most essential parameters and shows the binding affinity of the ligand to the biomolecule. This score is $-3.6$ kcal/mol for vulpinic acid against collagenase and $-2.8$ kcal/mol against elastase. Usnic acid has a docking score of $-4.9$ and $-3.3$ kcal/mol against collagenase and elastase, respectively. Another parameter is Glide Ligand Efficiency. This value indicates the efficiency of the molecules. Two of these parameters are interaction-related parameters (Glide Evdw and Glide Ecoul). The following parameter is Glide energy, which shows the energy of the interaction, and the last parameter, Glide Emodel, indicates the value of interaction pose.

3.3 Binding free energy

The parameters obtained from the calculations of binding free energy are presented in Table 2. $\Delta G_{\text{bind}}$ indicates the total free binding energy between ligand and enzyme. This value is $-22.35$ kcal/mol for vulpinic acid against collagenase and $-41.30$ kcal/mol against elastase. These values have a positive correlation with the results of IC50 for vulpinic acid, which are 195.36 ($\mu$M) and 25.24 ($\mu$M) against collagenase and elastase, respectively. There are other parameters in Table 2, such as $\Delta G_{\text{Coulomb}}$, $\Delta G_{\text{H-bond}}$, $\Delta G_{\text{Lipo}}$, and $\Delta G_{\text{vdW}}$, which are free binding energies of coulomb energy, hydrogen bond energy, lipophilic energy, and van der Waals energy, respectively. Based on the data presented in Table 2, it is apparently obvious that coulomb energy has a key role in the interactions between vulpinic acid and enzymes. $\Delta G_{\text{Coulomb}}$ for usnic acid is $-19.73$ kcal/mol against collagenase and $-40.50$ kcal/mol against elastase. Considering the other free binding energies presented in Table 2, coulomb energy has
an essential role in the interactions between usnic acid and these two enzymes. That is because of the remarkable difference between the Coulomb energy of usnic acid against these two enzymes. The values of Coulomb energies are $\approx 19.28$ kcal/mol and $\approx 81.55$ kcal/mol for collagenase and elastase, respectively.

### 3.4 Anti-cancer results

The compounds of vulpinic acid and usnic acid were determined *in vitro* for their anticancer activities against SPC-A-1, 95D, and SK-LU-1 lung cancer cell lines, with the anticancer drug Doxorubicin, used as a control compound. Indeed, *in vitro* anticancer screening methods were conducted at various molecule concentrations. All of the tests were performed out in triplicate. Additionally, the IC50 values were calculated from the percentage of anticancer effect by nonlinear curve fitting and are presented in Table 3. In this part, both compounds exhibited the most potent growth inhibition in the three lung cancer cells, with an IC50 value of 21–29 µM, indicating that vulpinic acid is more potent than Doxorubicin, which exhibited an IC50 value of 21–29 µM. Also, other molecules exhibited significantly weaker activity, because the anticancer was lower than that of Doxorubicin in the three cell lines, with an IC50 value of 62-68 µM (Table 3).

Lung cancer arises from normal lung epithelial cells that undergo numerous genetic damages and eventually turn into cells that proliferate uncontrollably with abnormal growth and aggressive behavior in the airways of the lungs. Lung cancer includes two main types: small cell non-cell lung cancer and small cell lung cancer. Based on histological classification, non-small cell lung cancer can be divided into three main subtypes: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. The importance of classification is evident in the treatment strategy and prediction of cancer outcomes. Today, surgery, radiation, chemotherapy and targeted therapy in the treatment of lung cancer; However, although many methods have been used to treat lung cancer, the clinical results of current treatments are still unsatisfactory. Many commonly prescribed chemotherapeutic drugs have been discovered by searching for possible compounds from plants, marine or-

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

| Parameters                      | vulpinic acid against collagenase | vulpinic acid against elastase | Usnic acid against collagenase | Usnic acid against elastase |
|--------------------------------|----------------------------------|--------------------------------|--------------------------------|-----------------------------|
| IC50 (µM)                      | 195.36                           | 25.24                          | 94.05                          | 7.38                         |
| Docking score (kcal/mol)       | -3.644                           | -2.772                         | -4.9                           | -3.3                         |
| Glide ligand efficiency (kcal/mol) | -0.152                         | -0.116                         | -0.195                         | -0.130                       |
| Glide Ecol (kcal/mol)          | -0.219                           | 2.049                          | -13.989                        | -4.665                       |
| Glide Evdw (kcal/mol)          | -28.382                          | -20.943                        | -22.35                         | -19.224                      |
| Glide Emodel (kcal/mol)        | -33.203                          | -20.665                        | -38.811                        | -28.649                      |
| Glide energy (kcal/mol)        | -28.601                          | -18.895                        | -36.347                        | -23.889                      |

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### Table 2  The results obtained from the binding free energy calculations.

| Ligand/Enzyme                  | $\Delta G_{\text{bind}}$ | $\Delta G_{\text{bind}}$ | $\Delta G_{\text{bind}}$ | $\Delta G_{\text{bind}}$ | $\Delta G_{\text{bind}}$ |
|-------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| vulpinic acid against collagenase | -22.35                   | -25.63                   | -2.02                    | -20.74                   | -29.75                   |
| vulpinic acid against elastase | -41.30                   | -85.11                   | -0.04                    | -22.71                   | -25.99                   |
| Usnic acid against collagenase | -19.73                   | -19.28                   | -2.80                    | -15.42                   | -27.92                   |
| Usnic acid against elastase    | -40.50                   | -81.55                   | -0.40                    | -23.13                   | -22.77                   |

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### Table 3  *In vitro* anti-proliferative activities of vulpinic acid and usnic acid compounds against human cancer cell lines (IC50 values).

| NO | Compounds | SK-LU-1 | SPC-A-1 | 95D |
|----|-----------|---------|---------|-----|
|    | IC50 (µM) | IC50 (µM) | IC50 (µM) |
| 1  | Vulpinic acid | 23.76 ± 2.73 | 28.80 ± 2.04 | 21.22 ± 3.55 |
| 2  | Usnic acid   | 67.83 ± 6.06 | 65.91 ± 9.46 | 62.08 ± 11.24 |
|    | Doxorubicin* | 25 ± 5   | 30 ± 4   | 25 ± 7   |
organisms, microorganisms, and animals, or by making natural product-derived lead compounds. According to this concept, some natural product-derived compounds have already been evaluated and their anti-cancer effect has been focused on newly discovered mechanisms in the hope that they can be used, or at least better strategies against cancer.

4 Conclusions
In this study, both natural compounds showed inhibitory effects on both collagenase and elastase enzymes. Also, disorganization of extracellular matrix proteins like fibronectin, collagen, and elastin are major characteristics of skin aging due to the enhanced activation of metabolic enzymes like elastases and collagenases. Indeed, inhibition of their enzymatic activities by natural molecules might be a promising approach to prevent extrinsic skin aging. Theoretical approaches have provided a beneficial way to obtain pragmatic data that could be used as completing information for experimental results. Molecular docking is one of these approaches that has attracted considerable attention in recent years. In this study, molecular docking calculations were performed to evaluate the biological activities of vulpinic acid and usnic acid against collagenase and elastase. The calculations of free binding energy were also performed and contributed valuable information about the ligand-enzyme complex. The outcomes indicated that these compounds have a considerable potential to inhibit the activities of the mentioned enzymes. Lung cancer is popular malignancy and gives rise to around one-quarter of all cancer deaths. Great advances have been achieved in the therapy of lung cancer with new anticancer factors and also can improve technology. Indeed, mortality and morbidity rates remain extremely high, calling for an urgent need to develop new anti–lung cancer factors. Indeed, both natural substances of this study showed anti-lung cancer inhibition and the results were calculated in the form of IC50.

Conflict of Interest
There isn’t any conflict of Interest.

Author Contributions
XL and WH conceived the original idea. YH2 was in charge of overall direction and planning. DG participated in the study design and coordination, drafted the manuscript. YW, XZ, CL and NU were responsible for the enzyme inhibition and determination of anticancer effects of the compounds. SD, YH2, and XG contributed to the analysis of the results. Molecular docking studies were carried out by DG. XL wrote the manuscript with supports from YW and XZ. All authors discussed the results and contributed to the final manuscript.

References
1) Vijayakumar, C.S.; Viswanathan, S.; Reddy, M.K.; Parvathavarthini, S.; Kundu, A.B.; Sukumar, E. Anti-inflammatory activity of (p)-usnic acid. *Fitoterapia* **71**, 564-566 (2000).
2) Takai, M.; Uehara, Y.; Beisler, J.A. Usnic acid derivatives as potential antineoplastic agents. *J. Med. Chem.* **22**, 1380-1384 (1979).
3) Su, Z.-Q.; Mo, Z.-Z.; Liao, J.-B.; Feng, X.-X.; Liang, Y.-Z. Usnic acid protects LPS-induced acute lung injury in mice through attenuating inflammatory responses and oxidative stress. *Int. J. Immunopharmacol.* **22**, 371-378 (2014).
4) Sonko, B.J.; Schmitt, T.C.; Guo, L.; Shi, Q.; Boros, L.G. et al. Assessment of usnic acid toxicity in rat primary hepatocytes using 13c isotopomer distribution analysis of lactate, glutamate and glucose. *Food Chem. Toxicol.* **49**, 2968-2974 (2011).
5) Segatore, B.; Bellio, P.; Setacci, D.; Brediselli, F.; Piovano, M. et al. In *vitro* interaction of usnic acid in combination with antimicrobial agents against methicillin-resistant *Staphylococcus aureus* clinical isolates determined by FICI and de model methods. *Phytotherapy* **19**, 341-347 (2012).
6) Stephenson, N.L.; Rundel, P.W. Quantitative variation and the ecological role of vulpinic acid and atranorin in the thallus of *Letharia vulpina*. *Biochem. Syst. Ecol.* **7**, 263-267 (1979).
7) Koparal, A.T. Anti-angiogenic and antiproliferative properties of the lichen substances (-) usnic acid and vulpinic acid. *Zeitschrift für Naturfor C* **70**(5-6), 159-164 (2015).
8) Hrenn, A.; Steinbrecher, T.; Labahn, A.; Schwager, J.; Schempp, C.M.; Merfort, I. Plant phenolics inhibit neutrophil elastase. *Planta Med.* **72**, 1127-1231 (2006).
9) Siedle, B.; Murillo, R.; Huckle, O.; Labahn, A.; Merfort, I. Structure activity relationship studies of cinnamic acid derivatives as inhibitors of human neutrophil elastase revealed by ligand docking calculations. *Pharmazie* **58**, 337-339 (2003).
10) Meng, X.-Y.; Zhang, H.-X.; Mezei, M.; Cui, M. Molecular docking: A powerful approach for structure-based drug discovery. *Curr. Comput. Aided Drug Des.* **7**, 146-157 (2012).
11) McConkey, B.J.; Sobolev, V.; Edelman, M. The performance of current methods in ligand-protein docking.
Current Sci. 83, 845-856 (2002).
12) Pinzi, L.; Rastelli, G. Molecular docking: Shifting paradigms in drug discovery. Int. J. Mol. Sci. 20, 4331 (2019).
13) Jorgensen, W.L. The many roles of computation in drug discovery. Science 303, 1813-1818 (2004).
14) Wang, T.; Wu, B.; Lin, J.P.; Yang, L.R. Quantitative structure-activity relationship: Promising advances in drug discovery platforms. Expert Opin. Drug Discov. 10, 1283-1300 (2015).
15) Macalino, S.J.Y.; Gosu, V.; Hong, S.; Choi, S. Role of computer-aided drug design in modern drug discovery. Arch. Pharm. Res. 38, 1686-1701 (2015).
16) Ashworth, T.R. A case of cancer in which cells similar to those in the tumors were seen in the blood after death. Aust. Med. J. 14, 146-146 (1869).
17) Papadopoulos, N.; Kinzler, K.W.; Vogelstein, B. The role of companion diagnostics in the development and use of mutation-targeted cancer therapies. Nat. Biotechnol. 24, 985-995 (2006).
18) Padrones, M.; Bieth, J.G. Elastin decreases the efficiency of neutrophil elastase inhibitors. Am. J. Respir. Cell Mol. Biol. 4, 187-193 (1991).
19) Ying, Q.L.; Rinehart, A.R.; Simon, S.R.; Cheronis, J.C. Inhibition of human leucocyte elastase by ursoic acid. Evidence for a binding site for pentacetyl triterpenes. Biochem. J. 277, 521-526 (1991).
20) Redini, F.; Tixier, J.M.; Petitou, M.; Choay, J.; Robert, L.; Hornebeck, W. Inhibition of leucocyte elastase by heparin and its derivatives. Biochem. J. 252, 515-519 (1988).
21) Thring, T.S.; Hili, P.; Naughton, D.P. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complement Altern. Med. 9, 27 (2009).
22) van Wart, H.E.; Steinbrink, D.R. A continuous spectro-photometric assay for Clostridium histolyticum collagenase. Anal. Biochem. 113, 356-365 (1981).
23) Madhun, B.; Krishnamoorthy, G.; Rao, J.R.; Nair, B.U. Role of green tea polyphenols in the inhibition of collagenolytic activity by collagenase. Int. J. Biol. Macromol. 41, 16-22 (2007).
24) Kupcisk, L. Estimation of cell number based on metabolic activity: the MTT reduction assay. Methods Mol. Biol. 740, 13-19 (2011).
25) Koizumi, M.; Fujino, A.; Fukushima, K.; Kaminura, T.; Takimoto-Kaminura, M. Complex of human neutrophil elastase with 1/2SLPI. J. Synchrotron Radiat. 15, 308-311 (2008).
26) Eckhard, U.; Schönauer, E.; Brandstetter, H. Structural basis for activity regulation and substrate preference of clostridial collagenases G, H, and T. J. Biol. Chem. 288, 20184-20194 (2013).
27) Schrödinger Release 2020-4: Protein Preparation Wizard; Schrödinger, LLC, New York, NY 2016; Impact, Schrödinger, LLC, New York, NY 2016; Prime, Schrödinger, LLC, New York, NY (2020).
28) Pousetforoosh, A.; Hashemipour, H.; Tuzun, B.; Parakhtry, A.; Mehrabani, M.; Nematiollahi, M.H. Evaluation of potential anti-RNA-dependent RNA polymerase (RdRP) drugs against the newly emerged model of COVID-19 RdRP using computational methods. Biophys. Chem. 272, 106564 (2021).
29) Schrödinger Release 2020-4: LigPrep, Schrödinger, LLC, New York, NY (2020).
30) Li, J.; Abel, R.; Zhu, K.; Cao, Y.; Zhao, S.; Friesner, R.A. The VSGB 2.0 model: A next generation energy model for high resolution protein structure modeling. Proteins: Structure, Function, and Bioinformatics 79, 2794-2812 (2011).
31) Lang, M.; Cai, L.; Udeani, G.O.; Slowing, K.V.; Thomas, C.F.; Beecher, C.W. et al. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275, 218-220 (1997).
32) Kong, A.N.; Yu, R.; Chen, C.; Mandlekar, S.; Primiano, T. Signal transduction events elicited by natural products: Role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. Arch. Pharm. Res. 23, 1-16 (2000).
33) Navia, M.A.; McKeever, B.M.; Springer, J.P.; Lin, T.Y.; Williams, H.R. et al. Structure of human neutrophil elastase in complex with a peptide chloromethyl ketone inhibitor at 1.84-Å resolution. Proc. Natl. Acad. Sci. USA 86, 7-11 (1989).
34) Subhani, S.; Jayaraman, A.; Jamil, K. Homology modeling and molecular docking of MDRI with chemotherapeutic agents in non-small cell lung cancer. Biomed. Pharmacother 71, 37-45 (2015).
35) Ture, A.; Kahraman, D.C.; Cetin-Atalay, R.; Helvacıoğlu, S.; Charehsaz, M.; Küçükgüzel, I. Synthesis, anticancer activity, toxicity evaluation and molecular docking studies of novel phenylaminopyrimidine—(thio)urea hybrids as potential kinase inhibitors. Comput. Biol. Chem. 78, 227-241 (2019).
36) Ruvinsky, A.M. and Kozintsev, A.V. New and fast statistical-thermodynamic method for computation of protein-ligand binding entropy substantially improves docking accuracy. J. Comput. Chem. 26, 1089-1095 (2005).
37) Rosenfeld, R.J.; Goodsell, D.S.; Musah, R.A.; Morris, G.M.; Goodin, D.B.; Olson, A.J. Automated docking of ligands to an artificial active site: Augmenting crystallographic analysis with computer modeling. J. Comput. Aided Mol. Des. 17, 525-536 (2003).
38) Mohan, V.; Gibbs, A.C.; Cummings, M.D.; Jaeger, E.P.; DesJarlaist, R.L. Docking: Successes and challenges. Curr. Pharm. Des. 11, 323-333 (2005).
39) Fridman, R.; Giaccone, G.; Kanemoto, T.; Martin, G.R.
Gazdar, A.F.; Mulshine, J.L. Reconstituted basement membrane (matrigel) and laminin can enhance the tumorigenicity and the drug resistance of small cell lung cancer cell lines. *Proc. Natl. Acad. Sci. USA* **87**, 6698-6702 (1990).

Arenberg, D.A.; Kunkel, S.L.; Polverini, P.J.; Glass, M.; Burdick, M.D.; Strieter, R.M. Inhibition of interleukin-8 reduces tumourigenesis of human non-small cell lung cancer in SCID mice. *J. Clin. Invest.* **97**, 2792-2802 (1996).