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Computational Modelling of Anti-Carcinogenic Effects of Naringenin

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Abstract

Naringenin is a bioactive polyphenol derived from citrus fruits, known for its various beneficial effects on human health. This study focuses on naringenin as a polyphenolic scavenger of nine ultimate chemical carcinogens: 2-cyanoethylene oxide, aflatoxin B1 exo-8,9-epoxide, glycidamide, ethylene oxide, propylene oxide, styrene oxide, vinyl carbamate epoxide, chloroethylene oxide, and β-propiolactone. The aim was to calculate the activation free energies and elucidate the molecular mechanisms of alkylation reactions between naringenin and these genotoxic chemical carcinogens, using the quantum-mechanical Hartree-Fock method in combination with two flexible basis sets, 6-31G(d) and 6-311++G(d,p). Activation free energy calculations were performed using Gaussian 09 in a vacuum and with two implicit solvation models: Polarisable Continuum Model and Langevin Dipoles. To assess naringenin's scavenging efficacy, the activation free energies calculated using these solvation models, were compared to the experimental values of the activation free energies between the same ultimate chemical carcinogens and the most reactive DNA base, guanine. The findings indicated naringenin's efficacy as a polyphenolic scavenger of six ultimate chemical carcinogens, particularly β-propiolactone, vinyl carbamate epoxide, and propylene oxide. For chloroethylene oxide, aflatoxin B1 exo-8,9-epoxide, and ethylene oxide, naringenin also demonstrated significant anti-carcinogenic potential, as the calculated activation free energies were comparable to experimentally determined values for guanine. However, naringenin's protective activity was less potent against 2-cyanoethylene oxide, glycidamide, and styrene oxide, where the calculated activation free energies were significantly higher than the experimental values for guanine.

Keywords

Naringenin, ultimate chemical carcinogens, activation free energies, Hartree-Fock method, implicit solvation models

1 Introduction

Cancer is a disease that affects the genome of cells, causing genetic instability. As the tumour progresses, various genetic mutations accumulate within the cell genome, and structural changes also occur.[1] Despite extensive research into numerous therapies, cancer remains a leading cause of mortality.[2]

A healthy human body consists of approximately 30 trillion cells, functioning as a mutually reliant community. Continuous communication between cells ensures that each tissue maintains the appropriate size and structure. However, cancer cells deviate significantly from this pattern and become resistant to the usual control mechanisms of cell growth, leading to uncontrolled proliferation. Tumours composed of such malignant cells gradually become more aggressive and begin to impair the function of vital tissues and organs essential for the organism's survival.[3]

Although significant progress has been made in understanding the molecular basis of cancer, advanced forms of this disease remain incurable, and a definitive cure is still elusive.[4] Currently, chemotherapy and radiotherapy are the most prevalent methods of cancer treatment.[5] Unfortunately, these treatments often do not yield the desired results, as they fail to completely eliminate the source cancer cells, and frequently damage healthy cells.[4]

Natural polyphenols from the flavonoid group are gaining increasing attention in cancer prevention and treatment due to their safety and beneficial biological effects, including antioxidant, anti-inflammatory, and antimicrobial effects.[6] Given the promising role of flavonoids in cancer prevention, flavanone naringenin, a polyphenolic compound found in citrus fruits, was selected for study. Naringenin has proven pharmacological properties, such as anti-inflammatory, antioxidant, neuroprotective, hepatoprotective, and anti-carcinogenic effects.[7]

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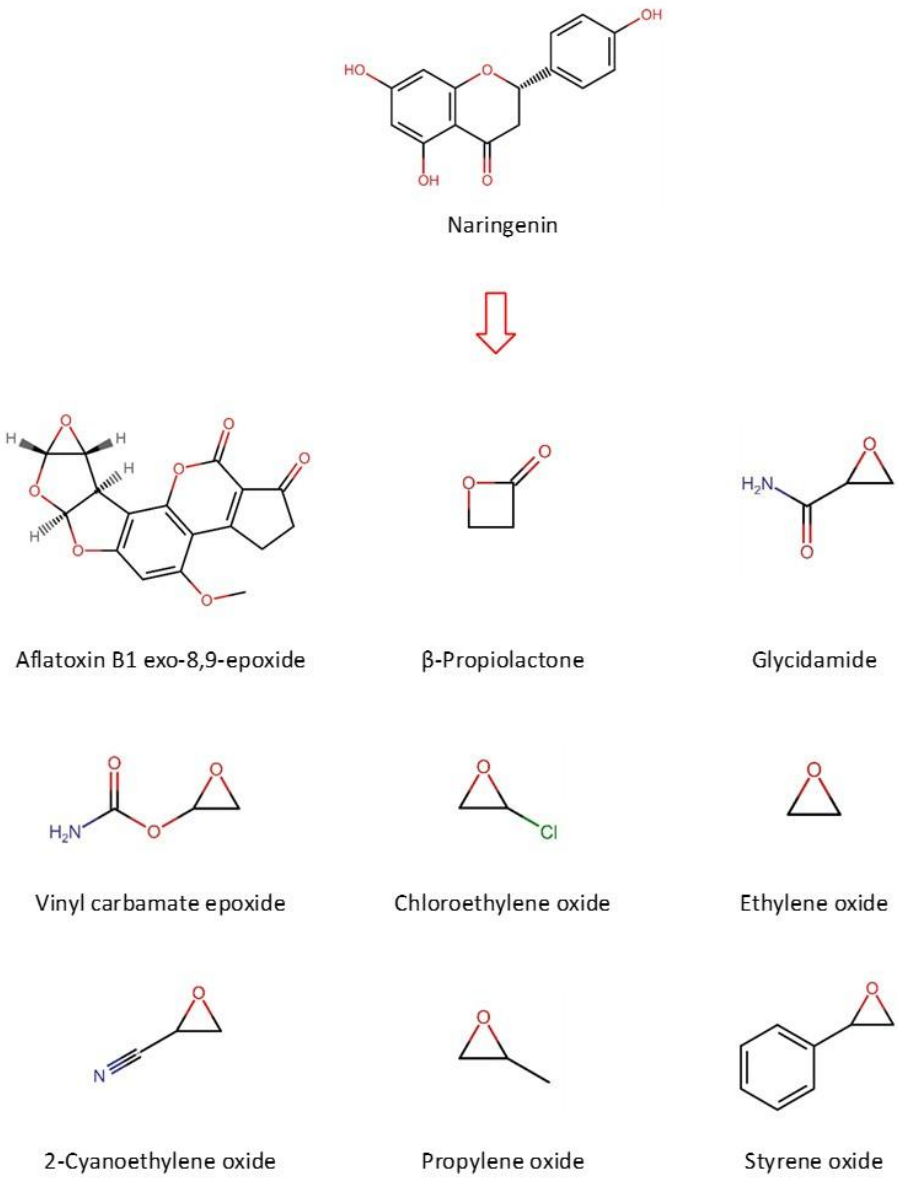


Fig. 1 – Chemical structures of (*S*)-naringenin, and nine ultimate chemical carcinogens: aflatoxin B1 exo-8,9-epoxide, β-propiolactone, glycidamide, vinyl carbamate epoxide, chloroethylene oxide, ethylene oxide, 2-cyanoethylene oxide, propylene oxide, and styrene oxide. The structural formulas were prepared in the MarvinJS program.[8]

Slika 1 – Strukture (*S*)-naringenina i devet karcinogenih tvari: aflatoksin B1 ekso-8,9-epoksid, β-propiolakton, glicidamid, epoksid vinil-karbamat, kloretilen oksid, etilen oksid, 2-cijanoetilen oksid, propilen oksid i stiren oksid. Strukturne formule pripremljene su u MarvinJS programu.[8]

Naringenin is considered to hold great potential in preventing cancer initiation, and the aim of this study was to investigate the molecular mechanisms of naringenin as a scavenger of nine genotoxic chemical carcinogens: 2-cyanoethylene oxide, aflatoxin B1 exo-8,9-epoxide, glycidamide, ethylene oxide, propylene oxide, styrene oxide, vinyl carbamate epoxide, chloroethylene oxide, and β-propiolactone. Their chemical structures are presented in Fig. 1. 2-Cyanoethylene oxide is the carcinogenic metabolite of acrylonitrile,[9] a key industrial monomer.[10] Aflatoxin B1 exo-8,9-epoxide is the metabolite of aflatoxin B1, the most genotoxic procarcinogen known to humans, produced by the fungus *Aspergillus flavus*, which grows on peanuts, grains, and milk under warm and humid conditions.[11] Glycidamide, the carcinogenic form of acrylamide, forms when starchy foods are heated above 120 °C and is also present in tobacco smoke.[12] Ethylene oxide, a metabolic product of ethylene, is found in various consumer

products such as antifreeze, detergents, and plastics.[13] Propylene oxide is an important industrial chemical essential for polyurethane synthesis.[14] Styrene oxide is the carcinogenic form of styrene, commonly used in the plastics industry.[15] Urethane, formed during various fermentation processes,[16] is metabolised by cytochrome P450 2E1 to produce the carcinogenic vinyl carbamate epoxide.[17] Vinyl chloride, used in polyvinyl chloride synthesis,[18] is metabolically activated by cytochrome P450 2E1 to form the ultimate carcinogen, chloroethylene oxide.[19] β-Propiolactone, a colourless liquid, is often used as an inactivating agent in the production of viral vaccines.[20] All these ultimate chemical carcinogens are reactive electrophilic compounds that interact with DNA bases, particularly guanine.

However, additional factors must be considered regarding the role of these ultimate chemical carcinogens in human cells. To interact with DNA, chemical carcinogens must first pass through the cell membrane and enter the nucleus. Along this path, they may encounter antioxidants present in the cytoplasm.[21] Naringenin, with its well-documented antioxidant properties,[22] can neutralise these carcinogens, reducing their potential to reach and damage DNA. Moreover, even if chemical carcinogens enter the nucleus, the concentration of guanine is much higher than that of naringenin, given that guanine is a fundamental component of DNA.[23] This elevated guanine concentration enhances the likelihood of DNA damage. Therefore, the consumption of dietary supplements containing natural polyphenols like naringenin is recommended. It is also important to note that not every interaction between a chemical carcinogen and a nucleotide leads to a carcinogenic mutation. Cancer development is closely associated with the formation of DNA adducts by chemical carcinogens, but not all adducts lead to mutations.[24] Additionally, the presence of detoxification enzymes, such as glutathione S-transferase, in cells help reduce the likelihood of mutations.[25] Finally, it is crucial to highlight naringenin's low bioavailability[22] and low solubility in water.[26] These factors significantly influence naringenin's efficacy in cancer prevention. Therefore, the appropriate encapsulation of naringenin before consumption should be carefully considered.[27]

2 Experimental

The experimental part of this study was based on the fundamental principles of molecular modelling and quantum mechanical calculations, using the Gaussian 09 software package and the Hartree-Fock method, combined with two flexible basis sets, 6-31G(d) and 6-311++G(d,p), as well as two implicit solvation models.[17] Naringenin was examined in its nucleophilic (monoanionic) form at a physiological pH of 7.4. In the absence of experimental data, the p*K*a value of 7.86 for the most acidic proton of naringenin was calculated using the MarvinSketch software,[28] which was consistent with experimental data, predicting the monoanionic form as most dominant under physiological conditions.[29]

The input files for quantum-mechanical calculations in Gaussian 09 were created for the geometry optimisation and frequency analysis of reactants of nine ultimate genotoxic chemical carcinogens with naringenin. Initial three-dimensional molecular structures were prepared using the Avogadro program.[17]

A relaxed potential energy surface (PES) scan was employed to locate initial conformations of transition states, which were subsequently subjected to geometry optimisation and frequency analysis.[17]

To better approximate the reactions between naringenin and the nine ultimate chemical carcinogens to the cell environment, two implicit solvation models were incorporated in the calculations: the Polarisable Continuum Model (PCM) and the Langevine Dipole (LD) model. PCM treats the solvent as a dielectric continuum defined by the dielectric constant ε. In this model, cavity formation is designed to handle the solute through overlapping spheres with defined point charges on their surfaces.[30] Self-Consistent Reaction Field (SCRF) is the key term used to incorporate the solvation effect into quantum-mechanical calculations in Gaussian 09. The LD model represents the distribution of solvent molecules using point dipoles of the Langevin type, positioned on a cubic lattice surrounding the solute, enclosed by a continuum with a dielectric constant ε.[17]

Most of the experimental work, including modelling initial molecular structures and input file preparation, was conducted on a personal computer, while quantum mechanical calculations using Gaussian 09 were performed on the VRANA supercomputer cluster at the National Institute of Chemistry in Ljubljana. After completing the calculations, the output log files were saved in appropriate folders on personal computers, where they were analysed using the Molden programme.[17]

To determine the effectiveness of naringenin as a scavenger of the nine ultimate genotoxic chemical carcinogens, the calculated activation free energies for reactions between each chemical carcinogen and naringenin were compared to the experimentally determined activation free energies for reactions between the same chemical carcinogen and the most reactive deoxyribonucleic acid (DNA) base, guanine, as presented in Fig. 2. If the activation free energy for a reaction between a specific ultimate chemical carcinogen and naringenin was lower than that for the reaction between the ultimate chemical carcinogen and guanine, it indicated that the reaction between naringenin and the selected carcinogen will occur more rapidly, thereby allowing naringenin to prevent DNA damage.

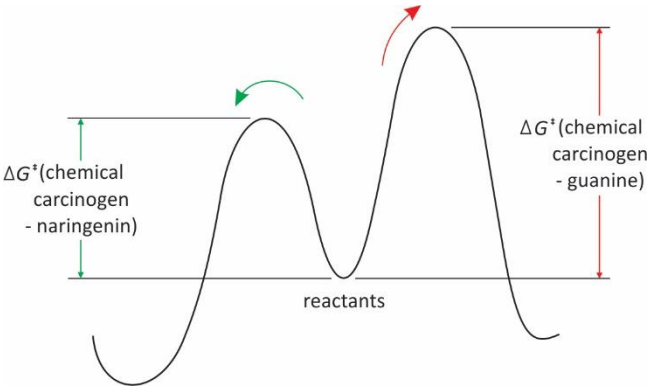


Fig. 2 – Comparison of activation free energies for reactions between guanine and naringenin with ultimate chemical carcinogens. The activation free energies were calculated as the difference between the Gibbs free energy of the corresponding transition state and the Gibbs free energy of the corresponding reactants according to the equation:

$$\Delta G^{\ddagger} = G_{\text{transition state}} - G_{\text{reactants}}$$

Slika 2 – Usporedba energija aktivacije za reakcije gvanina i naringenina s odabranim karcinogenima. Energija aktivacije odgovara razlici Gibbsove energije aktiviranog kompleksa i Gibbsove energije odgovarajućih reaktanata temeljem jednadžbe:

$$\Delta G^{\ddagger} = G_{\text{prijelazno stanje}} - G_{\text{reaktanti}}$$

3 Results and discussion

3.1 Alkylation reactions between naringenin and nine ultimate chemical carcinogens

The optimised structures of the reactants and transition states of the alkylation reactions between naringenin and the nine ultimate chemical carcinogens are presented in Fig. 3. The optimised structures of the reactants and transition states illustrate the formation of a new C−O covalent bond between the nucleophilic oxygen of naringenin and the achiral electrophilic carbon of the ultimate chemical carcinogen, characteristic of alkylation reactions. Among the studied chemical carcinogens, 2-cyanoethylene oxide, glycidamide, chloroethylene oxide, and vinyl carbamate epoxide contained a chiral centre in the epoxide ring. However, it should be noted that all alkylation reactions were characterised by the formation of a C−O covalent bond when the nucleophilic oxygen of naringenin approached the achiral electrophilic carbon of the ultimate chemical carcinogen, leading to the opening of the epoxide ring. These alkylation reactions between naringenin and nine ultimate chemical carcinogens follow an S_N2 mechanism. The images were created using the Avogadro programme, and the structures of the reactants and transition states were obtained using the Hartree-Fock method, combined with the 6-311++G(d,p) basis set. Results for alkylation reactions between naringenin and ultimate chemical carcinogens, obtained using the Hartree-Fock method with two flexible basis sets, 6-31G(d) and 6-311++G(d,p), are presented in Table 1. The table includes the following data:

$\Delta G^{\ddagger}$ – activation energy in a vacuum, kcal mol^{-1}

$\Delta\Delta G^{\ddagger}_{\text{SCRF}}$ – activation free energy (SCRF method), kcal mol^{-1}

$\Delta\Delta G^{\text{SCRF}}_{\text{hydr}}$ – difference between the hydration free energy of the transition state and the hydration free energy of the reactants, kcal mol^{-1}

ω^{R} – lowest positive vibrational frequency of the reactants, cm^{-1}

ω^{TS} – value of the imaginary vibrational frequency corresponding to the transition state, i cm^{-1}

d^{R} – distance between the reaction centres on naringenin and the ultimate chemical carcinogen in the reactants, Å

d^{TS} – distance between the reaction centres on naringenin and the ultimate chemical carcinogen in the transition state, Å

From the values shown in Table 1, it is evident that, for 2-cyanoethylene oxide, β-propiolactone, ethylene oxide, glycidamide, chloroethylene oxide, propylene oxide, styrene oxide, and vinyl carbamate epoxide, there was a small difference between the activation energies in a vacuum for the 6-31G(d) and 6-311++G(d,p) basis sets.

This suggests a similarity of optimised structures obtained with both the lower and higher basis sets. However, for AFB1 exo-8,9-epoxide, a difference in the activation energies in a vacuum was observed between the two basis sets. The activation energy in a vacuum for the 6-31G(d) basis set was 3.97 kcal mol^{-1} higher than for the 6-311++G(d,p) basis set, which included a larger number of wave functions and provided higher accuracy of the results. Therefore, it can be concluded that the activation energy in a vacuum was determined more accurately with the 6-311++G(d,p) basis set, amounting to 27.28 kcal mol^{-1}.

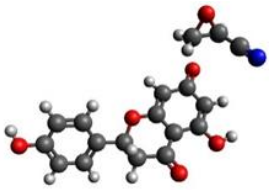
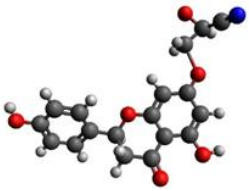
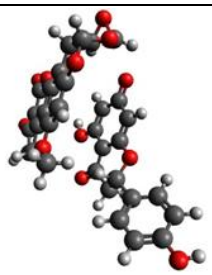
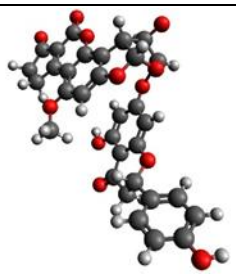
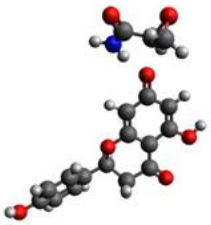
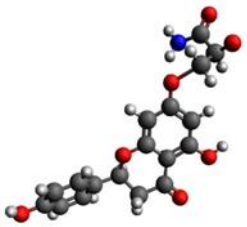
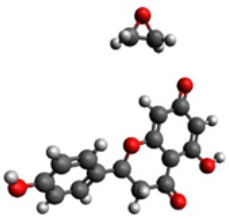
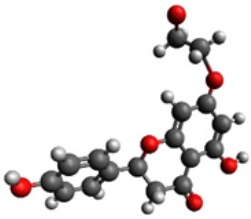
Ultimate chemical carcinogen Karcinogena tvar	Reactant Reaktant	Transition state Aktivirani kompleks
2-Cyanoethylene oxide	 a1)	 b1)
AFB1 exo-8,9-epoxide	 a2)	 b2)
Glycidamide	 a3)	 b3)
Ethylene oxide	 a4)	 b4)

Fig. 3 – Structures of reactants (a1–a4) and transition states (b1–b4) of the alkylation reactions between naringenin and nine chemical carcinogens. The atoms in structures of the reactants and transition states are depicted in different colours: dark grey (C), light grey (H), green (Cl), blue (N) and red (O).

Slika 3 – Strukture reaktanata (a1–a4) i aktiviranih kompleksa (b1–b4) u reakcijama alkilacije naringenina i devet karcinogena. Atomi u strukturama prikazani su različitim bojama: tamno siva (C), svijetlo siva (H), zelena (Cl), plava (N) i crvena (O).

Negative values of relative hydration free energies $\Delta\Delta G_{hydr}^{SCRF}$ for both basis sets, for 2-cyanoethylene oxide, AFB1 exo-8,9-epoxide, β-propiolactone, ethylene oxide, glycidamide, chloroethylene oxide, propylene oxide, and styrene oxide, described the solvation effect, which in this case accelerated the alkylation reaction between naringenin and the ultimate chemical carcinogens. The positive values of relative hydration free energies $\Delta\Delta G_{hydr}^{SCRF}$ for both flexible basis sets in the case of vinyl carbamate epoxide described the solvation effect, which in this case slowed down the alkylation reaction between naringenin and vinyl carbamate epoxide.

From the positive vibrational frequencies of the reactants ω^{R} and precisely one negative or imaginary vibrational frequency for the transition state ω^{TS}, it was confirmed that the reactants and the transition state structures were correctly optimised.

The change in the position of naringenin relative to the chemical carcinogens can be deduced from the distances between the reactive centres of the flavanone naringenin and the chemical carcinogens.

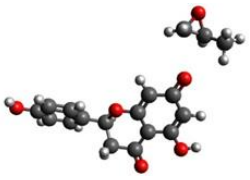
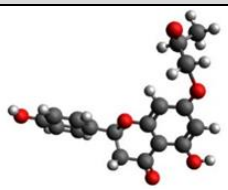
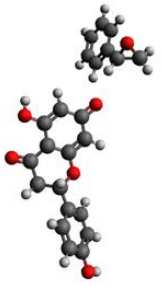
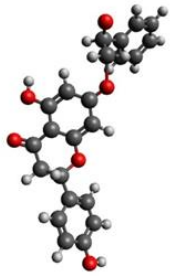
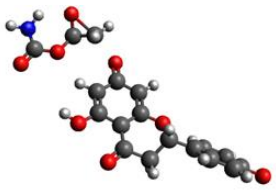
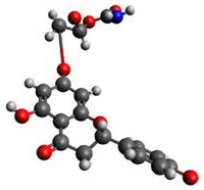
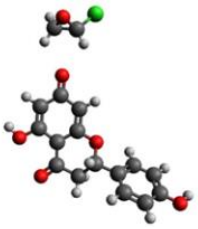
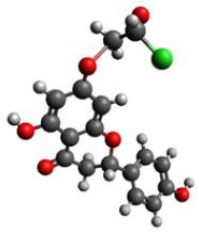
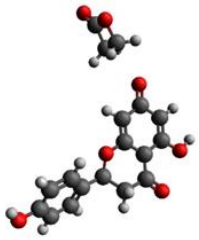
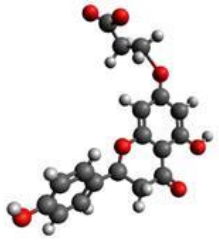
Ultimate chemical carcinogen Odabrana karcinogena tvar	Reactant Reaktant	Transition state Aktivirani kompleks
Propylene oxide	 a5)	 b5)
Styrene oxide	 a6)	 b6)
Vinyl carbamate epoxide	 a7)	 b7)
Chloroethylene oxide	 a8)	 b8)
β-Propiolactone	 a9)	 b9)

Fig. 3 – (continued) Structures of reactants (a5–a9) and transition states (b5–b9) of the alkylation reactions between naringenin and nine chemical carcinogens. The atoms in structures of the reactants and transition states are depicted in different colours: dark grey (C), light grey (H), green (Cl), blue (N) and red (O).

Slika 3 – (nastavak) Strukture reaktanata (a5–a9) i aktiviranih kompleksa (b5–b9) u reakcijama alkilacije naringenina i devet karcinogena. Atomi u strukturama prikazani su različitim bojama: tamno siva (C), svijetlo siva (H), zelena (Cl), plava (N) i crvena (O).

Table 1 – Calculated results for the alkylation reactions of naringenin with the nine ultimate chemical carcinogens
Tablica 1 – Proračunate vrijednosti termodinamičkih parametara alkilacije naringenina s odabranim karcinogenim tvarima

Ultimate chemical carcinogen Odabrana karcinogena tvar	Basis set Osnovni skup	$\Delta G^{\ddagger}$ / kcal mol^{-1}	$\Delta\Delta G^{\ddagger}_{SCRF}$ / kcal mol^{-1}	$\Delta\Delta G^{SCRF}_{hydr}$ / kcal mol^{-1}	ω^{R} / cm^{-1}	ω^{TS} / i cm^{-1}	d^{R} / Å	d^{TS} / Å
2-Cyanoethylene oxide	HF/6-31G(d)	28.37	23.07	−5.30	7.55	−680.35	3.10	1.92
	HF/6-311++G(d,p)	28.36	21.94	−6.42	4.11	−666.60	3.14	1.95
AFB1 exo-8,9-epoxide	HF/6-31G(d)	31.25	25.93	−5.32	10.11	−337.82	3.20	2.67
	HF/6-311++G(d,p)	27.28	22.09	−5.19	5.79	−219.44	3.13	2.06
Glycidamide	HF/6-31G(d)	41.67	34.66	−7.01	2.61	−637.72	4.51	1.85
	HF/6-311++G(d,p)	40.04	32.03	−8.01	3.46	−623.05	4.49	1.89
Ethylene oxide	HF/6-31G(d)	34.53	32.17	−2.36	3.55	−628.29	3.04	1.88
	HF/6-311++G(d,p)	33.07	29.59	−3.48	3.01	−606.85	3.03	1.91
Propylene oxide	HF/6-31G(d)	33.91	31.26	−2.65	4.55	−625.72	3.15	1.87
	HF/6-311++G(d,p)	33.32	28.67	−4.65	3.93	−606.21	4.10	1.91
Styrene oxide	HF/6-31G(d)	33.57	32.37	−1.20	3.79	−644.53	4.11	1.90
	HF/6-311++G(d,p)	33.48	31.01	−2.47	3.68	−626.08	4.11	1.92
Vinyl carbamate epoxide	HF/6-31G(d)	19.48	21.45	1.97	5.06	−629.86	3.08	2.02
	HF/6-311++G(d,p)	20.32	21.90	1.58	1.33	−606.75	3.14	2.03
Chloroethylene oxide	HF/6-31G(d)	23.43	21.21	−2.22	6.38	−674.39	3.10	2.00
	HF/6-311++G(d,p)	23.60	20.89	−2.71	4.71	−642.37	3.14	2.02
β-Propiolactone	HF/6-31G(d)	24.14	20.90	−3.24	5.03	−649.62	2.82	1.99
	HF/6-311++G(d,p)	24.66	20.52	−4.14	4.79	−630.84	2.84	2.01

In the case of the reactants, the distance d^{R} was greater compared to the distance in the transition state d^{TS}, which reflected the proximity of the molecules in the transition state, allowing the formation of a new covalent bond between naringenin and the ultimate chemical carcinogens.

3.2 Comparison of activation free energies obtained with two implicit solvation models

Since reactions in human cells do not occur in a vacuum, solvent effects were incorporated into the calculations by introducing two implicit solvation models. Activation free energy values were obtained using the SCRF method and the LD method, to determine which method would most accurately approximate the known experimental values for reactions of guanine with individual ultimate chemical carcinogens. Comparing these values with experimental values for guanine allowed us to assess the efficacy of naringenin as a scavenger of the nine studied ultimate chemical carcinogens. The values of activation free energies, obtained using the Hartree-Fock method in combination with the 6-311++G(d,p) flexible basis set and the two implicit solvation models mimicking an aqueous solution (ε = 78.36), are shown in Table 2 along with the experimental values:[17]

$\Delta\Delta G^{\ddagger}_{SCRF}$ – activation free energy obtained by the SCRF solvation method, kcal mol^{-1}

$\Delta\Delta G^{\ddagger}_{LD}$ – activation free energy obtained by the LD solvation method, kcal mol^{-1}

$\Delta\Delta G_{exp}$ – experimental values of activation free energies for alkylation reactions between guanine and the nine ultimate chemical carcinogens, kcal mol^{-1}

To determine the optimal combination of method, flexible basis set, and implicit solvation model, and to evaluate how effectively naringenin acts as a scavenger of nine ultimate chemical carcinogens, the activation free energies obtained using two different implicit solvation models were

Table 2 – Activation free energies obtained with two implicit solvation models for the alkylation reactions of naringenin and guanine with the nine studied ultimate chemical carcinogens

Tablica 2 – Energije aktivacije određene implicitnim solvatacijskim modelom za reakcije alkilacije naringenina i gvanina s devet odabranih karcinogenih tvari

Ultimate chemical carcinogen Odabrana karcinogena tvar	Naringenin		Guanine Gvanin		Experimental value for guanine Eksperimentalno određena energija reakcije s gvaninom
	$\Delta G^{\ddagger}_{SCRF}$ / kcal mol^{-1}	$\Delta G^{\ddagger}_{LD}$ / kcal mol^{-1}	$\Delta G^{\ddagger}_{SCRF}$ / kcal mol^{-1}	$\Delta G^{\ddagger}_{LD}$ / kcal mol^{-1}	ΔG_{exp} / kcal mol^{-1}
2-Cyanoethylene oxide	21.94	26.55	28.48 [31]	19.02 [31]	19.2 [31]
AFB1 exo-8,9-epoxide	22.09	15.75	18.9 [32]	14.25 [32]	15.1 [32]
Glycidamide	32.03	26.33	25.78 [33]	23.55 [33]	22.8 [33]
Ethylene oxide	29.59	26.62	21.0 [34]	24.62 [34]	24.7 [34]
Propylene oxide	28.67	25.07	21.26 [35]	25.15 [35]	25.4 [35]
Styrene oxide	31.01	31.52	– [36]	27.65 [36]	26.5 [36]
Vinyl carbamate epoxide	21.90	23.94	22.15 [37]	19.13 [37]	22.4 [37]
Chloroethylene oxide	20.89	19.77	17.26 [38]	22.87 [38]	19.5 [38]
β-Propiolactone	20.52	17.06	22.45 [39]	12.06 [39]	20.8 [39]

compared with experimentally determined values for guanine. As shown by the underlined values in Table 2, for β-propiolactone, chloroethylene oxide, and vinyl carbamate epoxide, the activation free energies obtained were compared using the SCRF method with the experimental values for reactions with guanine.

For 2-cyanoethylene oxide, AFB1 exo-8,9-epoxide, ethylene oxide, glycidamide, propylene oxide, and styrene oxide, the activation free energies obtained using the LD method were compared. If the activation free energies for the alkylation reactions between naringenin and the nine studied ultimate chemical carcinogens are lower or comparable with the experimentally determined values for guanine, it can be asserted that naringenin is an effective scavenger of these chemical carcinogens.

The analysis of the results showed that naringenin has significant potential to effectively protect DNA from damage caused by six of the nine ultimate chemical carcinogens, particularly β-propiolactone, vinyl carbamate epoxide, and propylene oxide. In these cases, the activation free energy values for the reactions of these carcinogens with naringenin were lower than the experimentally determined values for guanine. For the alkylation reactions of naringenin with chloroethylene oxide, ethylene oxide, and AFB1 exo-8,9-epoxide, the activation free energy values were comparable to the experimental values for guanine, indicating that naringenin has potential to protect against these carcinogens as well. It is important to highlight the low value of the activation free energy for the reaction of naringenin with AFB1 exo-8,9-epoxide (15.75 kcal mol^{-1}), as it is the most genotoxic among the nine studied ultimate chemical carcinogens. Although its activation free energy value was not lower than the experimentally determined value for guanine, the difference between the values fell within the acceptable computational error of 1 kcal mol^{-1}, further supporting the potential of naringenin to protect DNA against AFB1 exo-8,9-epoxide. Higher activation free energy values were observed for the alkylation reactions between naringenin and 2-cyanoethylene oxide, glycidamide, and styrene oxide. From the results in Table 2, it can be concluded that naringenin would be least effective as a scavenger for these three ultimate chemical carcinogens, as the calculated activation free energy values were 3 to 7 kcal mol^{-1} higher than the experimental values for guanine. Beside the kinetic aspect, the extent of these reactions between the ultimate chemical carcinogens and naringenin, measured by the reaction free energy, is another important factor that may affect the carcinogen-scavenging activity of both naringenin and guanine. To confirm naringenin's efficacy as a scavenger of the nine ultimate chemical carcinogens, an IRC calculation following the identification of the transition state structures was performed. This calculation provided the corresponding reaction products, from which the reaction free energies could be calculated. The values of Gibbs free energies $\Delta G° < 0$ indicated the thermodynamic favourability of the reactions studied.

4 Conclusion

The results indicate that naringenin, a ubiquitous flavanone, has promising potential for protection against the studied ultimate chemical carcinogens. Previous research has already demonstrated naringenin's diverse biological activities, notably its anti-carcinogenic properties. In this study, it was found that naringenin also has the potential to act as a scavenger of nine ultimate chemical carcinogens, thereby helping to prevent cancer initiation. Additionally, a relevant insight to the molecular mechanisms of naringenin as a scavenger of ultimate chemical carcinogens was provided, which, to the best of our knowledge, has not been previously explored in scientific literature. This study is expected to have a significant impact on future computational and experimental research on the anti-carcinogenic effects of naringenin.

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List of symbols and abbreviations
Popis kratica i simbola

d^{TS} – distance between the reactive centres on naringenin and the ultimate chemical carcinogen in transition state, Å
– udaljenost reaktivnog centra naringenina i aktiviranog kompleksa odabrane karcinogene tvari, Å

d^{R} – distance between the reactive centres on naringenin and the ultimate chemical carcinogen in the reactants, Å
– udaljenost reaktivnog centra naringenina i odabranih karcinogenih tvari u početnom stanju, Å

$\Delta G^{\ddagger}$ – activation energy in a vacuum, kcal mol^{-1}
– energija aktivacije u vakuumu, kcal mol^{-1}

ΔG_{exp} – experimental values of the activation free energies for the alkylation reactions between guanine and the nine ultimate chemical carcinogens, kcal mol^{-1}
– eksperimentalno određene vrijednosti promjene Gibbsove energije alkilacije gvanina s odabranim karcinogenim tvarima, kcal mol^{-1}

$\Delta\Delta G^{\ddagger}_{LD}$ – activation free energy obtained using the LD solvation method, kcal mol^{-1}
– energija aktivacije određena metodom LD solvatacije, kcal mol^{-1}

$\Delta\Delta G^{\ddagger}_{SCRF}$ – activation free energy obtained using the SCRF solvation method, kcal mol^{-1}
– energija aktivacije određena korištenjem SCRF solvatacijskog modela, kcal mol^{-1}

$\Delta\Delta G^{\ddagger}_{hydr}$ – difference between the hydration free energy of the transition state and the hydration free energy of the reactants, kcal mol^{-1}
– razlika energije hidratacije kompleksa prijelaznog stanja i energije hidratacije reaktanata, kcal mol^{-1}

ω^{TS} – imaginary frequency value corresponding to the transition state, i cm^{-1}
– imaginarna vibracijska frekvencija koja odgovara prijelaznom stanju, i cm^{-1}

ω^{R} – lowest positive frequency of the reactants, cm^{-1}
– najmanja pozitivna vibracijska frekvencija reaktanata, cm^{-1}

Greek symbols
Grčki simboli

ε – dielectric constant of the solvent
– dielektrična permitivnost otapala

Abbreviations
Kratice

DNA – deoxyribonucleic acid
– deoksiribonukleinska kiselina

HF – Hartree-Fock method
– Hartree-Fock metoda

LD – Langevin Dipole Model
– Langevin dipolni model

PCM – Polarisable Continuum Model
– model polarizabilnog kontinuuma

SCRF – Self-Consistent Reaction Field
– samousklađeno reakcijsko polje

PES – potential energy surface
– ploha potencijalne energije

VRANA – parallel computer for accelerating numerical algorithms (slo. vzporedni računalnik za akceleracijo numeričnih algoritmov)

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SAŽETAK

Računalno modeliranje antitumorskih učinaka naringenina

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Naringenin je bioaktivni polifenol dobiven iz citrusa, poznat po brojnim blagotvornim učincima na ljudsko zdravlje. Ovaj rad usredotočen je na istraživanje aktivnosti naringenina kao polifenolnog hvatača devet odabranih karcinogenih tvari: 2-cijanoetilen-oksida, aflatoksina B1 ekso-8,9-epoksida, glicidamida, etilen-oksida, propilen-oksida, stiren-oksida, epoksida vinil-karbamata, kloretilen-oksida i β-propiolaktona. Cilj rada bio je odrediti energije aktivacije i razjasniti molekularne mehanizme alkilacije naringenina i predmetnih genotoksičnih karcinogenih tvari, primjenjujući Hartree-Fock metodu u sprezi s dva osnovna skupa, 6-31G(d) i 6-311++G(d,p). Proračuni Gibbsovih energija aktivacije provedeni su u programskom paketu Gaussian 09 u vakuumu te koristeći dva implicitna modela solvatacije, tzv. „Polarisable Continuum Model" (PCM) i „Langevin Dipoles". Kako bi se procijenila učinkovitost naringenina kao hvatača, energije aktivacije dobivene proračunima pomoću ovih modela solvatacije uspoređene su s eksperimentalnim vrijednostima energija aktivacije između istih konačnih kemijskih karcinogena i najreaktivnije nukleobaze DNK, gvanina. Rezultati ukazuju na učinkovito djelovanje naringenina kao hvatača šest istraživanih karcinogena, osobito β-propiolaktona, epoksida vinil-karbamata i propilen-oksida. Za kloretilen-oksid, aflatoksin B1 ekso-8,9-epoksid i etilen-oksid, naringenin je također pokazao značajan antikarcinogeni potencijal, jer su izračunate energije aktivacije bile usporedive s eksperimentalno određenim vrijednostima za gvanin. Međutim, očekivana zaštitna aktivnost naringenina manja je za 2-cijanoetilen-oksid, glicidamid i stiren-oksid, pri čemu su proračunate energije aktivacije bile znatno veće od eksperimentalnih vrijednosti za gvanin.

Ključne riječi

Naringenin, karcinogene tvari, energija aktivacije, Hartree-Fockova metoda, implicitni solvatacijski model

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