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DPPH Antioxidant Assays, Molecular Docking Studies and ADMET Predictions of Some 4-Chloromethyl Substituted Coumarin Compounds

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Abstract

Coumarins and their derivatives, which are secondary metobalites of many plants, are heterocyclic bioactive compounds with various biological properties. Due to these properties, the synthesis of various derivatives and the investigation of their properties are of great interest. 4-(chloromethyl)-7-hydroxy-5-methyl coumarin (**1**), 4-(chloromethyl)-7-hydroxy-8-methyl coumarin (**2**), and 4-(chloromethyl)-7-hydroxy coumarin (**3**) synthesized by Pechmann condensation reaction and characterized by FT-IR, NMR spectral data and elemental analysis data. The antioxidant capacities of the compounds were investigated by difhenyl-2-picrylhydrazyl (DPPH) radical scavenger assay using the UV-Vis spectrophotometric method. The interactions of the compounds with ROS-producing cytochrome P-450, xanthine oxidase, lipooxygenase, monoamine oxidase, and nicotinamide adenine dinucleotide phosphate oxidase enzymes were investigated by molecular docking. All compounds interacted well in the active binding site of most of the enzymes (about 6-8 kcal/mol). The pharmacokinetic and toxicokinetic properties of the compounds, indicating their potential as drug candidates, were analyzed by

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ADMET predictions. All the results obtained showed that the compounds have properties that could be drug candidates.

Keywords: Coumarin; Antioxidant; Molecular docking; ADMET.

Bazı 4-klorometil Substitüe Kumarin Bileşiklerinin DPPH Antioksidan Deneyleri, Moleküler Kenetlenme Çalışmaları ve ADMET Tahminleri

Öz

Birçok bitkinin ikincil metobalitleri olan kumarinler ve türevleri, çeşitli biyolojik özelliklere sahip heterosiklik biyoaktif bileşiklerdir. Bu özelliklerinden dolayı çeşitli türevlerinin sentezi ve özelliklerinin araştırılması oldukça ilgi çekicidir. 4-(klorometil)-7-hidroksi-5-metil kumarin (**1**), 4-(klorometil)-7-histoksi-8-metil kumarin (**2**) ve 4-(klorometil)-7-hidroksi kumarin (**3**) Pechmann kondenzasyon reaksiyonu ile sentezlenmiş ve FT-IR, NMR spektrumları ve elementel analiz ile karakterize edilmiştir. Bileşiklerin antioksidant kapasiteleri UV-Vis spektrofotometrik yöntemi kullanılarak difenil-2-pikrilhidrazil (DPPH) radikal süpürme deneyleri ile araştırılmıştır. Moleküler yerleştirme ile bileşiklerin, ROS üreten enzimler sitokrom P-450, ksantin oksidaz, lipooksijenaz, monoamin oksidaz ve nikotinamid adenin dinükleotit fosfat oksidaz enzimleri ile etkileşimleri incelenmiştir. Tüm bileşikler enzimlerin aktif bölgeleriyle iyi etkileşim göstermiştir (about 6-8 kcal/mol). Bileşiklerin farmakokinetik ve toksikokinetik özellikleri, ilaç adayı olabilme potansiyelleri ADMET tahminleri yapılarak incelenmiştir. Tüm sonuçlar bileşiklerin, ilaç adayı olma potansiyellerine sahip özelliklere sahip bileşikler olduklarını göstermiştir.

Anahtar Kelimeler: Kumarin; Antioksidan; Moleküler yerleştirme; ADMET.

1. Introduction

Coumarins and their derivatives are heterocyclic bioactive compounds that are secondary metabolites of plants [1, 2]. They are among the essential compounds of organic chemistry due to their biological activities. Coumarin and its derivatives have various biological activities such as anticancer, antifungal, antibacterial, antioxidant, anticoagulant, antiviral, anti-inflammatory, etc [3-9]. The coumarin ring can be synthesized by various methods such as Pechmann, Perkin, etc, and its derivatives can be prepared by substituting different groups at multiple positions. New

derivatives with improved biological activity have been obtained by substituting the coumarin ring from various positions with various groups [10-17].

ROS (Reactive oxygen species) play a biological role in cell signaling and defense against structures such as xenobiotics and bacteria. These are unstable and reactive species derived from molecular oxygen. The increase of ROS levels above certain levels in cells causes oxidative stress and damages structures such as lipids, enzymes, and proteins in the body. This causes many diseases, such as cancer, diabetes, etc. Molecules with antioxidant properties prevent the production of ROS by scavenging reactive oxygen species or inhibiting ROS-producing enzymes such as cytochrome P-450, xanthine oxidase, lipooxygenase, cyclooxygenase, monoamine oxidase, nicotinamide adenine dinucleotide phosphate oxidase [18,19].

In this study, three coumarin compounds with chloromethyl group at the C-4 position and hydroxy group at the C-7 position of the coumarin ring were synthesized according to the literature and characterized [20-23]. The antioxidant capacities of the compounds were investigated by DPPH (difhenyl-2-picrylhydrazyl) radical scavenger assay [24]. The synthesized compounds interactions with five enzymes known to produce reactive oxygen species (ROS) in the organism (cytochrome P-450, xanthine oxidase, nicotinamide adenine dinucleotide phosphate oxidase, human myeloperoxidase and lipoxygenase) were investigated by *in silico* molecular docking investigations. Also, the pharmacokinetic and toxicokinetic properties of the synthesized compounds were studied to evaluate their potential as drug candidates.

Antioxidant capacity assays, molecular docking studies, and ADMET predictions of the compounds synthesized in this study were performed and compared for the first time in this study.

2. Materials and methods

2.1. Materials and equipment

4-(chloromethyl)-7-hydroxy-5-methylcoumarin (**1**), 4-(chloromethyl)-7-hydroxy-8-methylcoumarin (**2**) and 4-(chloromethyl)-7-hydroxycoumarin (**3**) were synthesized according to the literature by Pechmann condensation reaction [20, 25, 26].

All chemicals and solvents used were purchased from Sigma-Aldrich. The compounds' purity was checked by TLC (thin layer chromatography) technique. For antioxidant analysis, ultraviolet-visible absorptions of compounds were recorded on Agilent 8453 UV-Vis Spectrophotometer. FT-IR spectra were recorded on Bruker Tensor 27 FTIR Spectrometer. NMR (^{1}H-NMR and ^{13}C-NMR) analysis of all compounds was performed on Bruker NMR 500 MHz

Spectrometer using deutero dimethyl sulfoxide (DMSO) at GUTMAM (Gazi University Basic and Engineering Sciences Central Laboratory Application and Research Center), Ankara, Turkey.

2.2. General synthesis of coumarin compounds

Ethyl 4-chloro-3-oxobutanoate (6.1 mmol) and a resorcinol compound (6 mmol) (5-methylresorcinol for **1**; 2-methylresorcinol for **2** and resorcinol for **3**) were dissolved in 10 mL of concentrated sulfuric acid. The mixture was stirred at 0-5 ^{o}C for 5 hours and then poured into cold water. The crude product was filtered and washed with water until acidity was removed. The solid products obtained were purified by crystallization from organic solvents.

2.2.1. 4-(chloromethyl)-7-hydroxy-5-methylcoumarin (1)

White solid. Yield 91% (1.15 g). MP: 163-166 ^{o}C. FTIR (ATR), (υ_{max}, cm^{-1}): 3102 (-OH); 3006 (ar. CH); 2959-2892 (alip. CH); 1676 (lactone C=O); 1607 (C-O); 1569 (C=C). ^{1}H NMR (DMSO, 500 MHz, ppm): 10.87, brd s, 1H (phenolic OH); 6.59, brd s, 2H (ar. CH); 6.37, s, 1H (lactone C=CH); 5.04, s, 2H (CH_2-Cl); 2.26, s, 3H (CH_3). ^{13}C NMR (DMSO, 500 MHz, ppm): 160.18 (C=O); 156.06-104.73 (C=C); 45.46 (CH_2-Cl); 21.58 (CH_3). Anal. Calc.: C, %58.81 ; H %4.04 Found: C, %58.79 ; H %4.02.

2.2.2. 4-(chloromethyl)-7-hydroxy-8-methylcoumarin (2)

White solid. Yield 89% (1.20 g). MP: 282-284 ^{o}C. FTIR (ATR), (υ_{max}, cm^{-1}): 3263 (-OH); 3066 (ar. CH); 2975-2832 (alip. CH); 1681 (lactone C=O); 1599 (C-O); 1573 (C=C). ^{1}H NMR (DMSO, 500 MHz, ppm): 10.50, brd s, 1H (phenolic OH); 7.49, d, J≈8.7 Hz, 1H (ar. CH); 6.68, d, J≈8.7 Hz, 1H (ar. CH); 6.38, s, 1H (lactone C=CH); 4.90, s, 2H (CH_2-Cl); 2.15, s, 3H (CH_3). ^{13}C NMR (DMSO, 500 MHz, ppm): 160.72 (C=O); 159.68-109.75 (C=C); 41.91 (CH_2-Cl); 8.43 (CH_3). Anal. Calc.: C, %58.81 ; H %4.04 Found: C, %58.80 ; H %4.03.

2.2.3. 4-(chloromethyl)-7-hydroxycoumarin (3)

White solid. Yield 92% (1.24 g). MP: 180-183 ^{o}C. FTIR (ATR), (υ_{max}, cm^{-1}): 3246 (-OH); 3097 (ar. CH); 2943-2821 (alip. CH); 1683 (lactone C=O); 1604 (C-O); 1563 (C=C). ^{1}H NMR (DMSO, 500 MHz, ppm): 10.65, brd s, 1H (phenolic OH); 7.68, d, J≈8.7 Hz, 1H (ar. CH); 6.85, dd, J≈8.7-2.2 Hz, 1H (ar. CH); 6.76, d, J≈2.2 Hz, 1H (ar. CH); 6.42, s, 1H (lactone C=CH); 4.96, s, 2H (CH_2-Cl). ^{13}C NMR (DMSO, 500 MHz, ppm): 161.94 (C=O); 160.61-102.99 (C=C); 40.02 (CH_2-Cl). Anal. Calc.: C, %57.03 ; H %3.35 Found: C, %57.02; H %3.32.

2.3. DPPH antioxidant assay

The antioxidant activity of compounds (**1-3**) was determined with DPPH (difhenyl-2-picrylhydrazyl) radical scavenger assay. Solutions of coumarin compounds at three different concentrations (0.25-0.50-1 mg/mL) and DPPH (25 μg/mL) solution in ethanol were prepared. The compound solution at various concentrations (100 μL) was added to the DPPH solution (3 mL). The solutions were incubated for 30 minutes in the dark at room temperature. A solution containing ethanol instead of the sample was prepared as a control solution. Absorbances of compounds were measured at 517 nm, and DPPH radical percent inhibition was calculated using % Inhibition= [(Absorbance$_{control}$-Absorbance$_{compound}$)/Absorbance$_{control}$]x 100 equation.

2.4. Molecular docking

For molecular docking calculations, Autodock Vina software [27] was used to calculate the binding affinity for coumarin compounds (**1-3**). The X-ray crystal structure of target proteins (human cytochrome P-450 (PDB:1OG5) [28], xanthine oxidase (PDB: 3NRZ) [29], nicotinamide adenine dinucleotide phosphate oxidase (PDB:2CDU) [30], human myeloperoxidase (PDB: 1DNU) [31] and lipoxygenase (PDB: 1N8Q) [32] were obtained from the RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data Bank (http://www.rcsb.org/) [33]. The water molecules in proteins have been removed. The missing polar hydrogens were added. Also, Kollman charges were added. The root of the ligand (synthesized compounds **1-3**) was detected automatically, and torsions were selected. The torsions of the ligands (synthesized compounds **1-3**) were allowed to rotate, and then selected residues were tested. The ligands (synthesized compounds **1-3**) were docked randomly to see where they would preferentially bind. Lamarckian Genetic Algorithm was used as a docking engine and all docking parameters were set as default [34]. The amino acid residues in the active site of target proteins were investigated using the BIOVA Discovery Studio Visualizer 2021 [35].

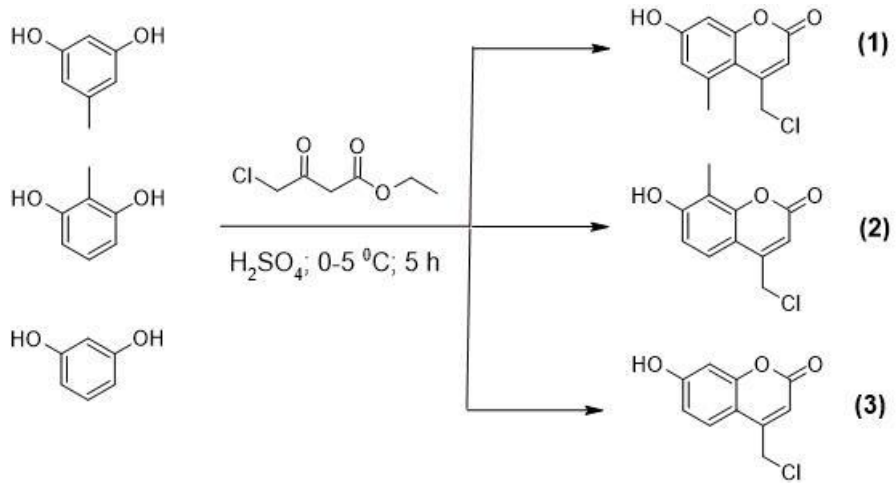
2.5. ADMET predictions

Absorbtion (A), distribution (D), metabolism (M), excretion (E) and toxicity (T) parameters (ADMET) define the properties that a drug molecule should possess. In drug design, investigating these toxicokinetic and pharmacokinetic properties of potential drug candidate molecules saves time and cost by preventing excessive experimentation and increases the success rate. Two free online databases, the SwissADME [36] and PRoTox-II [37] were used to predict the properties (ADMET) of the synthesized coumarin compounds (**1-3**).

3. Results and Discussion

3.1. Synthesis and Characterization

The coumarin compounds (**1-3**) were prepared by Pechmann condensation (Scheme 1). Reaction according to the literature. Synthesized compound's characterization was performed by spectroscopic methods FTIR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis.



Scheme 1: The synthetic route of coumarin compounds (**1-3**).

In the FTIR spectrum of compounds 1-3, phenolic –OH peaks appeared at 3102, 3263 and 3246 cm^{-1}, respectively. Aromatic –CH peaks appeared at 3006, 3066 and 3097 cm^{-1}; aliphatic peaks appeared at 2959-2892, 2975-2832 and 2943-2821 cm^{-1}; lactone C=O peaks appeared at 1676, 1681 and 1683 cm^{-1}; ester O=C-O peaks appeared at 1607, 1599 and 1604 cm-1; aromatic C=C peaks appeared at 1569, 1573 and 1563 cm^{-1}, respectively. The results of FTIR data of compounds (1-3) support synthesized structures. FTIR spectrum of compounds is given in Figures 1, 2, and 3.

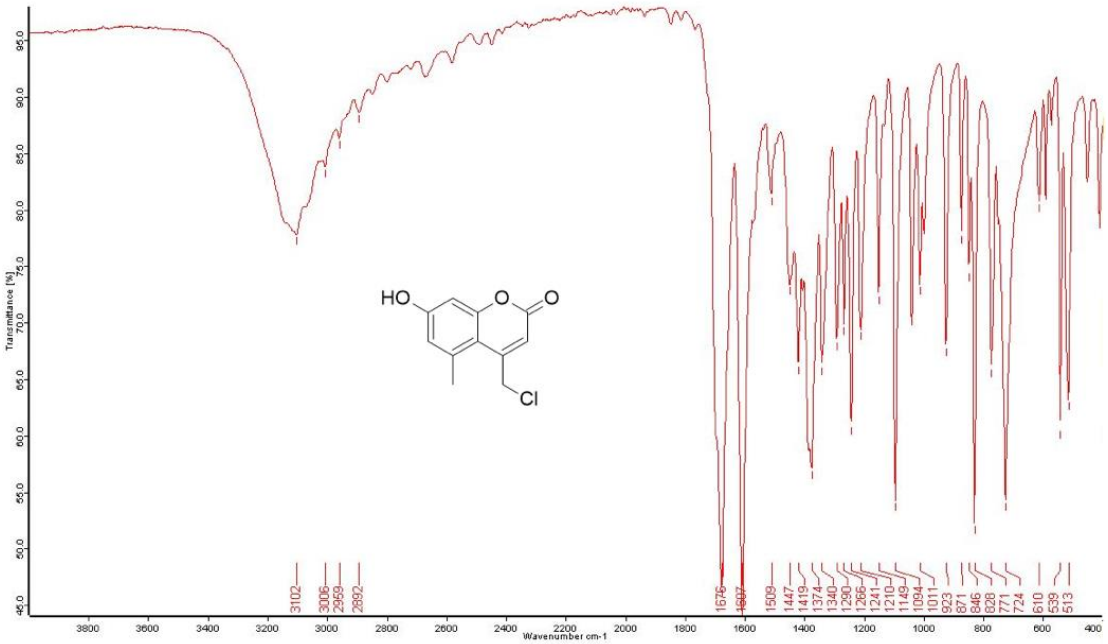


Figure 1: FT-IR spectrum of compound **1.**

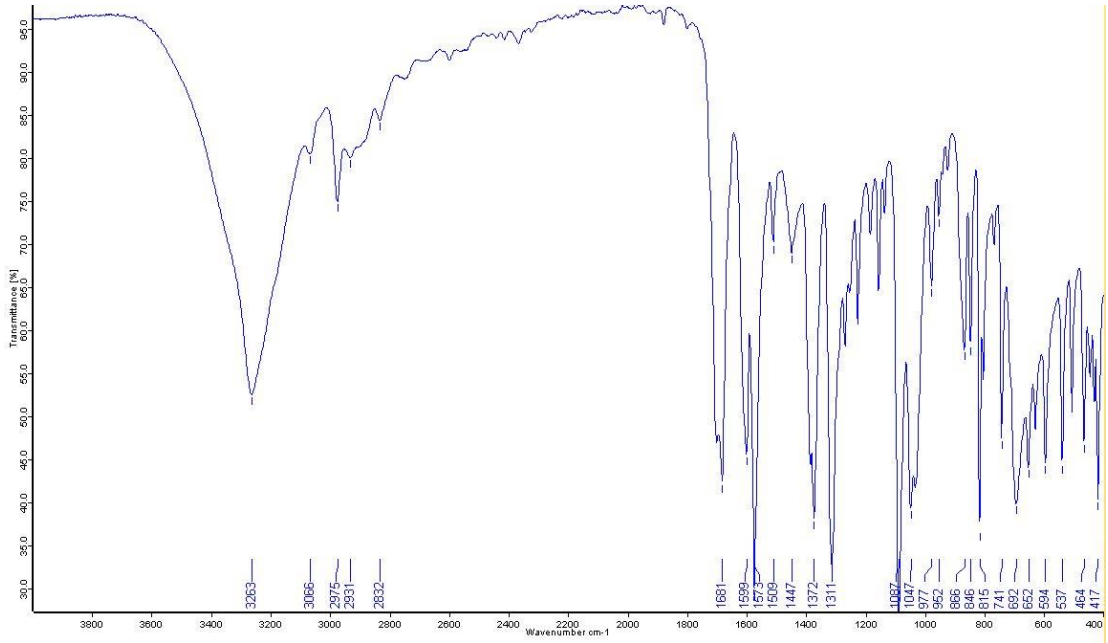


Figure 2: FT-IR spectrum of compound **2.**

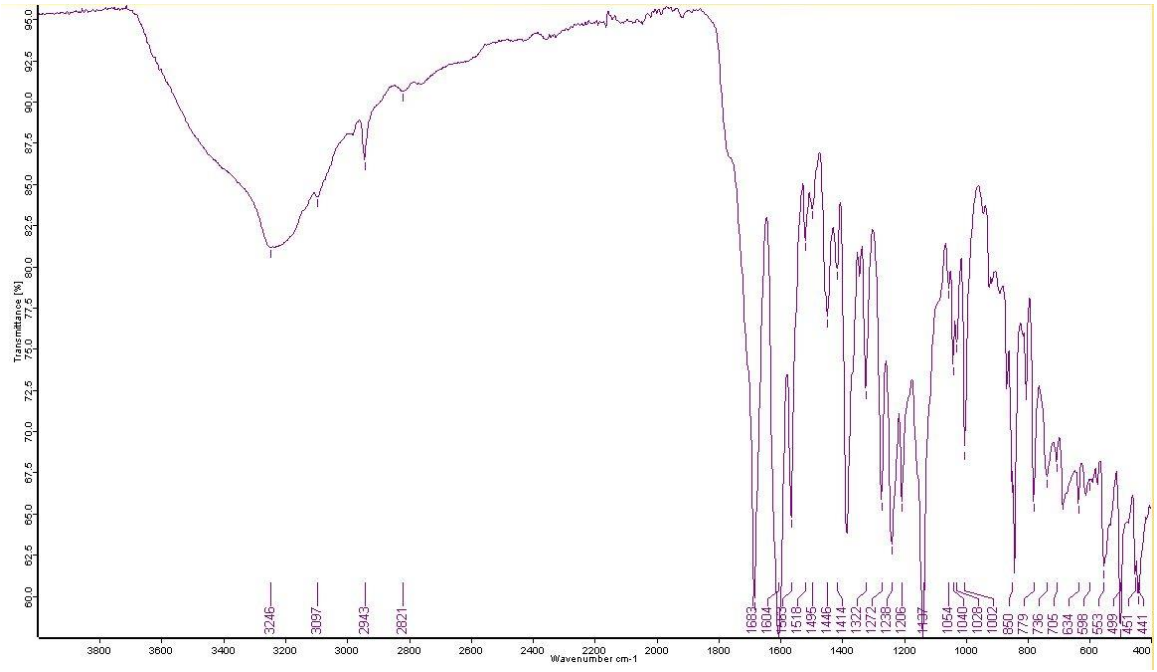


Figure 3: FT-IR spectrum of compound **3.**

In the ^{1}H-NMR spectrum of compounds **1-3** in DMSO, phenolic protons appeared at 10.87, 10.50, and 10.65 ppm as broad singlet peaks, respectively. For compound **1**, aromatic protons appeared as doublet peaks at 6.67 and 6.59 ppm each with 1H integration. C=C-H proton in the lactone ring appeared as a singlet peak at 6.37 ppm with 1H integration, CH_2-Cl protons appeared as a singlet peak at 5.04 ppm with 2H integration, and CH_3 protons appeared as a singlet peak at 2.26 ppm with 3H integration. For compound **2**, aromatic protons appeared as doublet peaks at 7.49 and 6.88 ppm each with 1H integration. C=C-H proton in the lactone ring appeared as a singlet peak at 6.38 ppm with 1H integration, CH_2-Cl protons appeared as a singlet peak at 4.90 ppm with 2H integration, and CH_3 protons appeared as a singlet peak at 2.15 ppm with 3H integration. For compound **3**, aromatic protons appeared as doublet, double doublet, and doublet peaks at 7.68, 6.85, and 6.76 ppm, each with 1H integration. C=C-H proton in the lactone ring appeared as a singlet peak at 6.42 ppm with 1H integration, and CH_2-Cl protons appeared as a singlet peak at 4.96 ppm with 2H integration.

In the ^{13}C-NMR spectrum of **1-3** in DMSO, lactone C=O carbons appeared at 160.18, 160.72 and 161.94 ppm; C=C carbons appeared at between 156.06 and 104.73, 159.68 and 109.75, 160.61 and 102.99; CH_2-Cl carbons appeared at 45.46, 41.91 and 40.02 ppm, respectively. Also, methyl carbon for compound **1** appeared at 21.58, and for compound **2** appeared at 8.43 ppm. NMR spectrums of compounds are given in Figures 4,5 and 6.

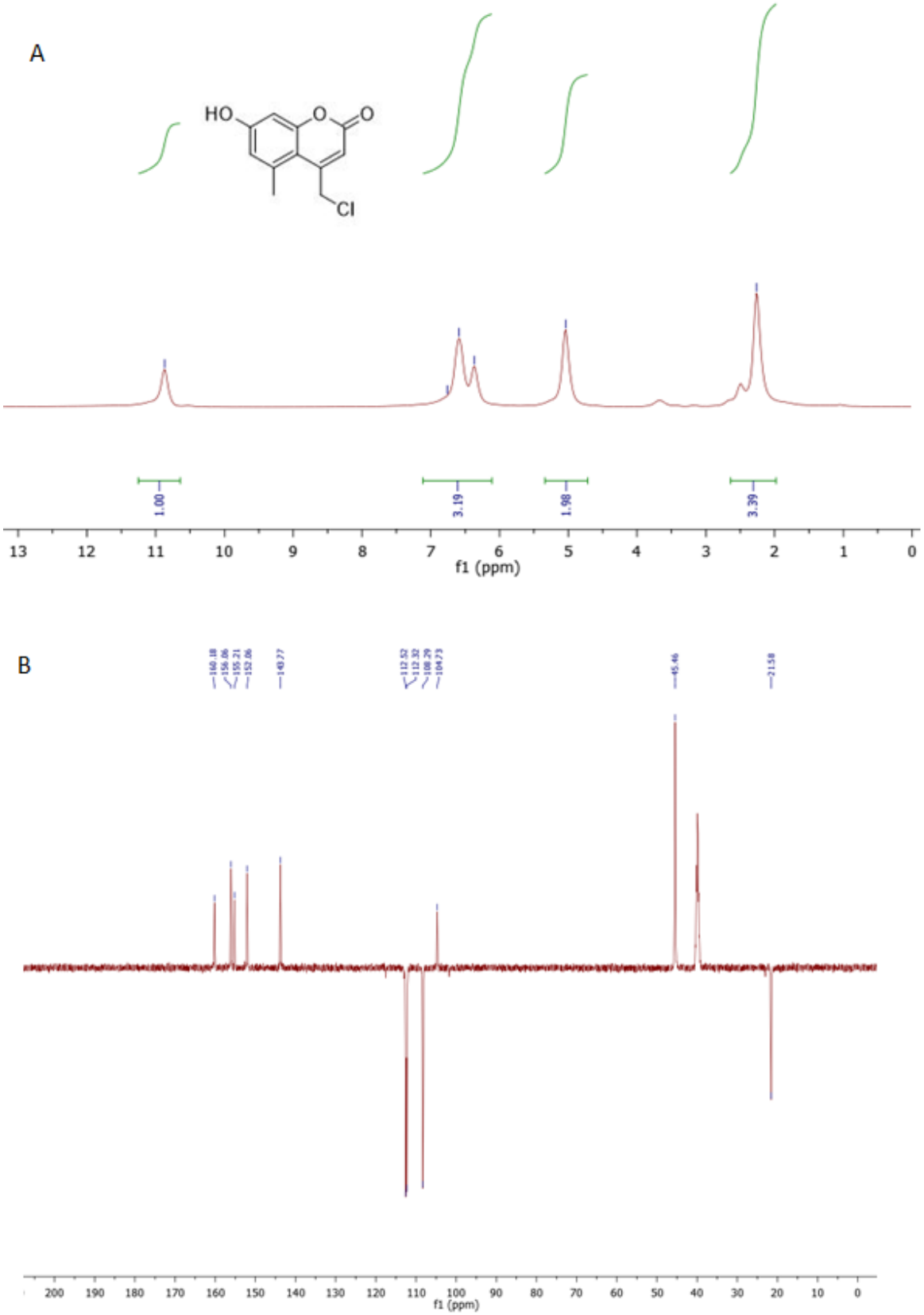


Figure 4: ^{1}H-NMR (A) and ^{13}C-NMR (B) spectrum of compound **1.**

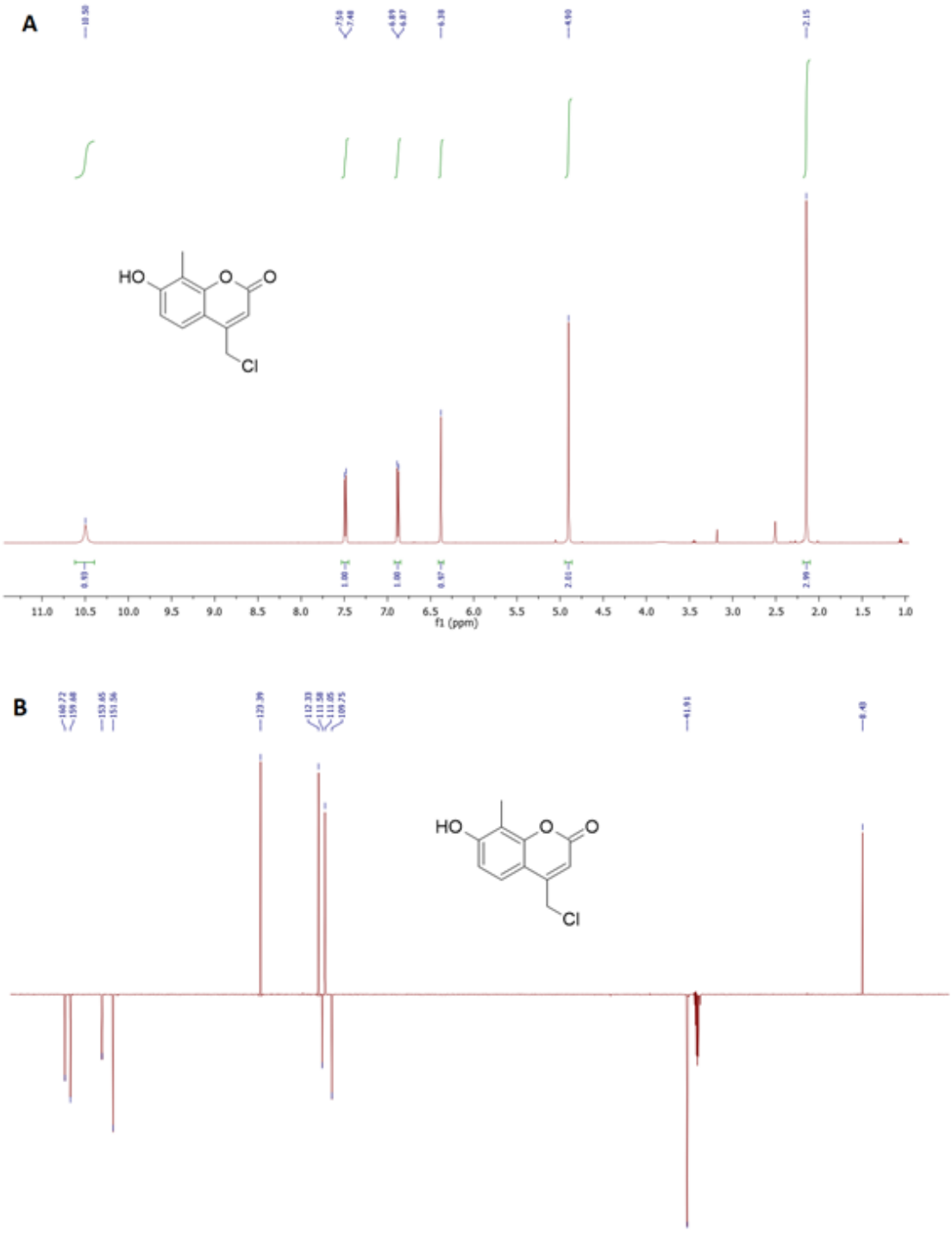


Figure 5: ^{1}H-NMR (A) and ^{13}C-NMR (B) spectrum of compound **2.**

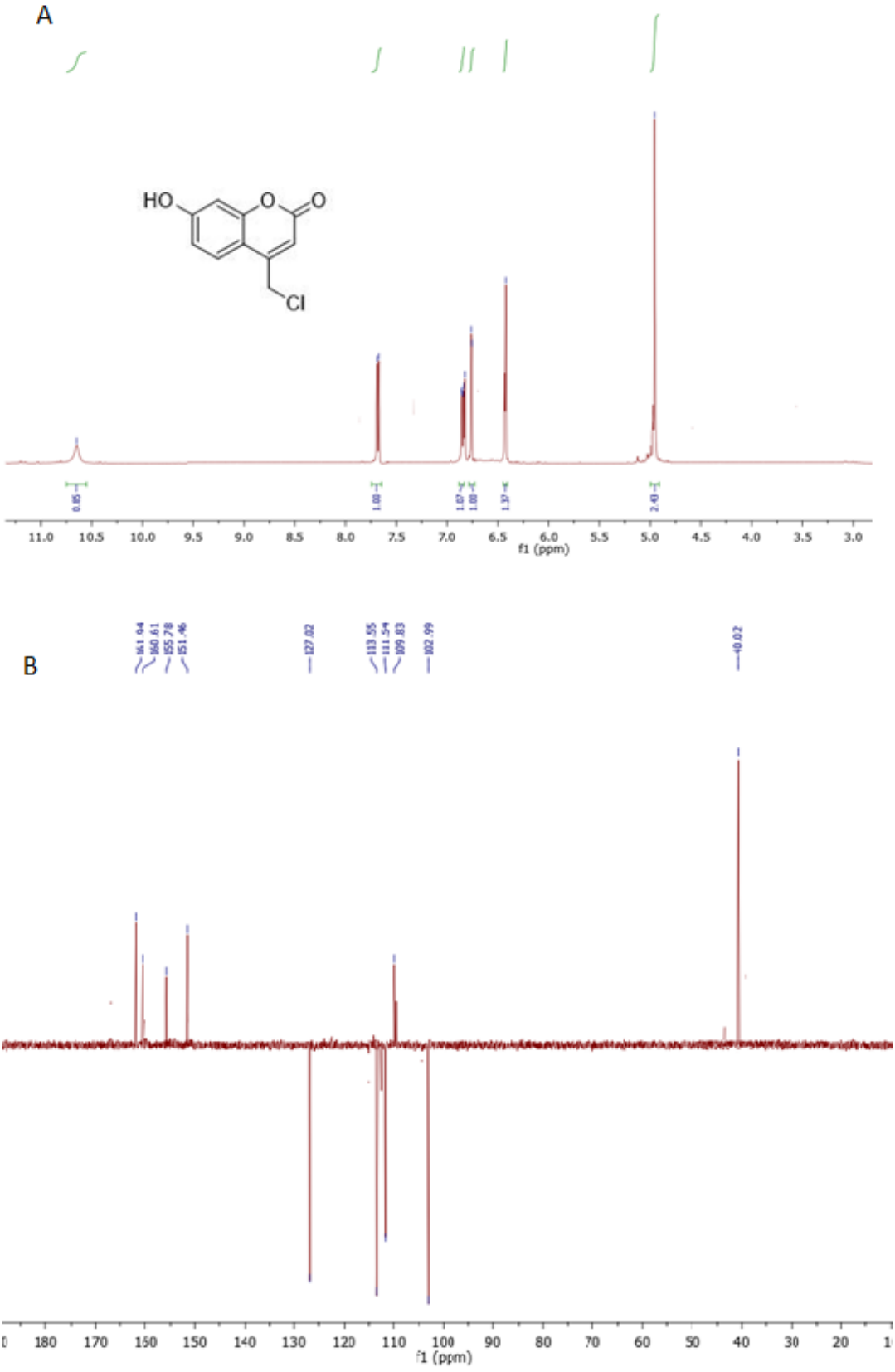


Figure 6: ^{1}H-NMR (A) and ^{13}C-NMR (B) spectrum of compound **3.**

The elemental analysis data results of the synthesized compounds **1-3** were given in the Materials and Methods section. The results of elemental analysis and other spectroscopic data support synthesized coumarin structures.

3.2. Antioxidant activity

DPPH radical scavenging tests were performed to determine the antioxidant activity of the compounds. The percentage of DPPH radical scavenging of the sample solution at three different concentrations for each compound was calculated using the equation given in section 2.3. As a result of DPPH antioxidant assays, it was observed that all three compounds had antioxidant activity at the concentrations studied ($p<0.05$). Furthermore, the results showed that the methyl group substituted at C-5 (compound **1**) and C-8 (compound **2**) positions affected the antioxidant activity. The compound with the highest DPPH radical scavenging activity was determined as compound **1,** containing the methyl group at the C-5 position, and the compound with the lowest activity was determined as compound **2**, containing the methyl group at the C-8 position. The % inhibition values of the compounds are given comparatively in Fig. 7.

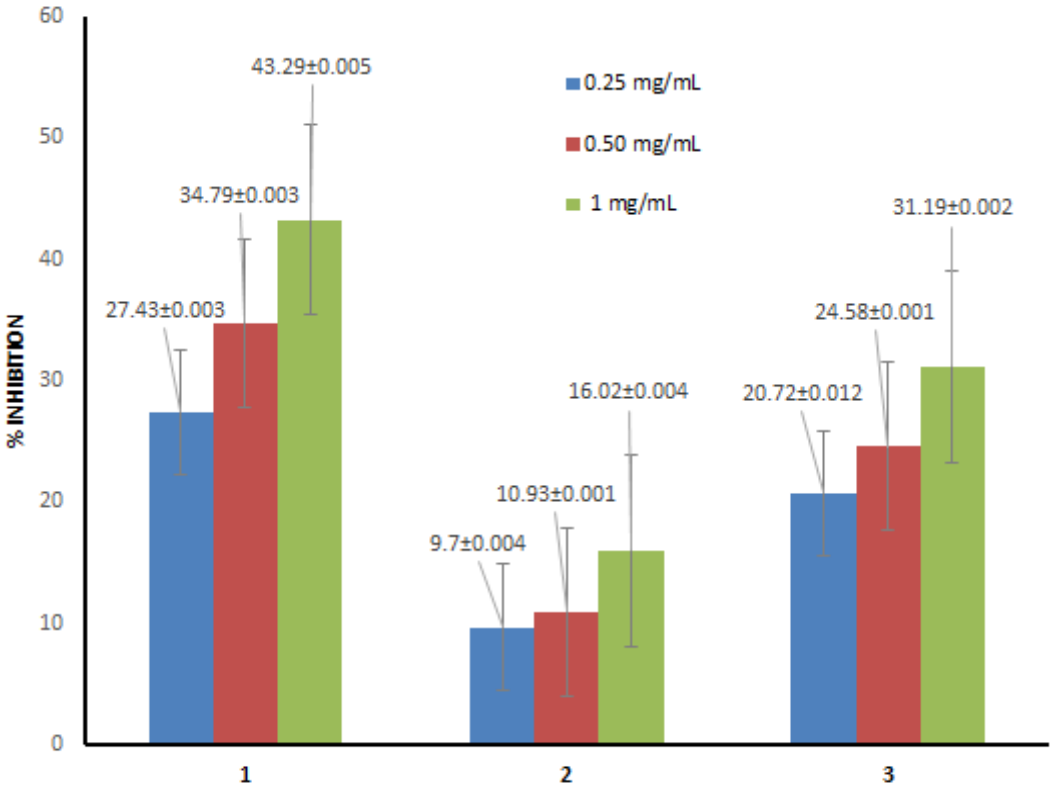
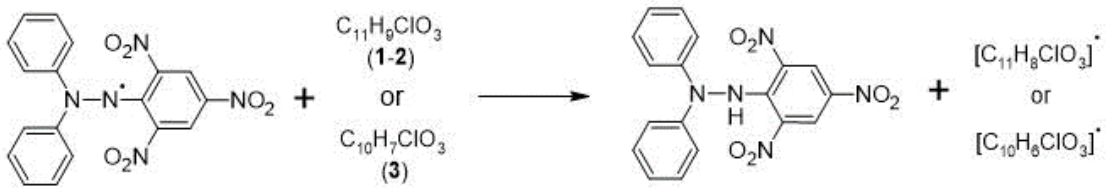


Figure 7: Antioxidant activity results of compounds **1**, **2,** and **3** at three different concentrations (DPPH assay values are mean of triplicate determination (n=3) ± standard deviation; $P<0.005$).

The DPPH radical scavenging ability of the compounds can be attributed to stable radical formation. This ability can be explained by hydrogen transfer from different parts of the compounds to DPPH, as suggested in Scheme 2.



Scheme 2: Proposed mechanism for antioxidant ability of compounds **1**, **2,** and **3.**

There are many studies in the literature to investigate the antioxidant activity of coumarin compounds. In these studies, the change in antioxidant activity was investigated by adding various substituents to the 7-hydroxy coumarin compound. The importance of the substituents attached to the coumarin ring on antioxidant activity is very important [1, 14, 38]. In this study, the antioxidant activities of 7-hydroxy coumarin compounds containing the 4-chloromethyl group were investigated by the DPPH method. In the literature, studies investigating the antioxidant activities of 7-hydroxy-4-methyl coumarin derivative compounds are found [39, 40]. When compared with the results of the DPPH radical scavenging assay in the literature, it was observed that the % inhibition value of 4-(chloromethyl)-7-hydroxycoumarin compound synthesized in this study was higher than the % inhibition value of the 7-hydroxy-4-methyl coumarin given in the literature [39].

3.3. Molecular docking

In silico radical scavenging properties of the synthesized compounds were studied on five potential target enzymes that can produce free radical species in the organism. The enzymes chosen as targets are xanthine oxidase, human cytochrome P-450, nicotinamide adenine dinucleotide phosphate oxidase (NADPH), human myeloperoxidase, and lipoxygenase. The enzymes and the ligands (compounds **1**, **2,** and **3**) whose interactions with the enzymes were studied are prepared for molecular docking as described in section 2.4. The molecular docking results of the compounds are given in Table 1. The compounds were found to interact with four of the five selected enzymes with high molecular docking scores.

When all docking scores were analyzed, it was observed that compound **1** interacted with xanthine oxidase, NADPH oxidase, and myeloperoxidase; compound **2** with myeloperoxidase and xanthine oxidase; compound **3** with myeloperoxidase and xanthine oxidase receptors with high scores. The best-scoring interaction of all three compounds was observed against the myeloperoxidase enzyme.

Table 1: The molecular docking results of the compounds **1**, **2,** and **3.**

Target enyzme	Molecular Docking Scores of Compounds (kcal/mol)		
	1	**2**	**3**
Cytochrome P-450	-6.6	-7.0	-6.3
Xanthine oxidase	-7.5	-7.3	-7.6
NADPH oxidase	-7.1	-7.0	-6.8
Myeloperoxidase	-8.0	-7.5	-7.7
Lipoxygenase	-2.9	-2.6	-4.0

The compounds synthesized in this study were observed to interact with many residues in the active site of enzymes. Interactions of the compounds with some target enzymes are given in the figure 8.

According to the literature and PDB; important active site residues of cytochrome P-450 are ARG97, ILE99, GLY98, ALA103, PHE100, LEU102, ASN217, PHE114VAL113, , LEU366, SER365, THR364, PHE476 and PRO367 [1,28]. Compound **1** made π-π stacking and π-alkyl interactions with PHE114, PHE100, LEU366, PRO367, ILE99, and ALA103. Compound **2** made hydrogen bond interactions with ARG97, π-σ interaction with VAL113, π-alkyl interaction with LEU366. Compound **3** made hydrogen bond interactions with GLY98 and ASN217 residues. Also, compound **3** made π-alkyl interactions with ALA103, LEU366, and PRO367 residues.

Important active site residues of NADPH oxidase are GLY156, ILE243, GLY158, TYR159, GLY244, ASP179, GLY180, LYS213, HIS181, TYR188, VAL214, and ILE160 [19,30]. Compound **1** made hydrogen bond interactions with GLY180 and LYS213, π-σ interaction and π-cation interaction with LYS213, π-alkyl interactions with ILE243 and VAL214. Compound **2** made hydrogen bond interactions with GLY180 and LYS213, π-cation interaction with LYS213, π-alkyl interactions with ILE243 and VAL214, π-σ interaction with HIS181. Compound **3** made hydrogen bond interactions with GLY180 and LYS213, π-cation interaction with LYS213, π-alkyl interactions with ILE243 and VAL214 residues.

Important active site residues of myeloperoxidase are HIS336, GLN91, PHE170, SER174, ASP94, THR168, ASP 172, ASP96, PHE 99, GLU102, PHE407 and HIS95 [31,41]. Compound **1** made hydrogen bond interaction with HIS95, π-σ interaction with GLN91, π-cation interaction with HIS336 residue. Compound **2** made π-cation interactions with HIS336 and HIS95, π-σ interactions with GLN91. Compound **3** made π-alkyl interactions with HIS95 and HIS336, π-π stacking with GLN91.

Important active site residues of xanthine oxidase are GLN1194, ALA1079, MET1038, ARG912, GLU802, ARG880, ALA910, GLY913, PHE914, PHE1005, SER1008, PHE1009, SER1080, THR1010, LEU1014 and PRO1076 [29,42]. Compound **1** made π-sulphur interaction with MET1038, hydrogen bond interaction with SER1082, π-alkyl interaction with ARG912. Compound **2** made π-alkyl interactions with ARG912 and MET1038. Compound **3** made π-π stacking with PHE1009 and PHE914; hydrogen bond interactions with ARG880 and THR1010; π-alkyl interactions with LEU1014 and ALA1079 residues.

3.4. ADMET predictions

The physicochemical, toxicokinetic, and pharmacokinetic properties of compounds were predicted by using the SwissADME [36] and the ProTox-II online [37] data bases. All properties of the studied compounds are given in Table 2. Estimating these properties of drug candidate molecules is very important for the investigation of drug-likeliness of these compounds.

In this study, Lipinski's rule of 5 (Ro5) was studied to determine the drug likeliness of the coumarin compounds. According to the Ro5, the compound can be used as a drug candidate if it has some suitable properties. These properties are suitable molecular weight (MW) (<500 g/mol), hydrogen bond donors (HBD) (<5), hydrogen bond acceptors (HBA) (<10), lipophilicity (Log P) (<5) and rotatable bonds (RB) (<5) [10, 43]. As can be seen in the table, all of the investigated coumarin compounds comply with Lipinski's rule of 5. Also, according to the other rules for the drug likeliness such as Veber's rule (polar surface area TPSA < 140 Å²), Egan's rule (TPSA 0-132 Å² and Log P -1-6), Muegge's rule (MW 200-600 g/mol, Log P -2-5, TPSA 150 < Å², cyclic rings <7, carbon atoms >4, heteroatoms >1, RB <15, HBA<10 and HBD <5) and Ghose rule (MW 160-480 g/mol, Log O -0.4-5.6, MR 40-130 and atoms 20-70) [44] the compounds comply with all rules. These results showed that the studied compounds meet the suggested properties and could be the drug candidate. When the other ADMET parameters in Table 2 are examined, it is seen that gastrointestinal absorption of the compounds is high; all compounds can cross the blood-brain barrier (BBB) and can not be used as a substrate of P-glycoprotein. In addition, the TPSA values shown in the table indicate that the oral bioavailability of the compounds may be good. As can be seen from the results in Table 2, it was observed that all compounds may show an inhibitory effect by interacting with CYP1A2. Toxicity classes of all compounds are 5. 1 means highly toxic, and 6 means non-toxic on the toxicity scale. Compounds are inactive for cytotoxicity, immunotoxicity, hepatotoxicity, mutagenicity, and mitochondrial membrane potential (MMP). But compounds **1** and **3** are active for carcinogenicity. As a result, the studied compounds are molecules with the potential to be used as drug candidates by evaluating their advantages and disadvantages.

Table 2: The pharmacokinetic and toxicokinetic properties of compound **1**, **2** and **3.**

Properties	Compound		
	1	**2**	**3**
MW (g/mol)	224.64	224.64	210.61
Number of atoms	24	24	21
Number of heavy atoms	15	15	14
Number of bonds	25	25	22
Number of RB	1	1	1
Number of HBA	3	3	3
Number of HBD	1	1	1
Molar Refractivity	59.23	59.23	54.27
TPSA (Å²)	50.44	50.44	50.44
Log $P_{o/w}$	2.26	2.31	2.03
GI absorption	High	High	High
BBB permeant	Yes	Yes	Yes
P-gp substrate	No	No	No
CYP1A2 inhibitor	Yes	Yes	Yes
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No
Skin permeation, Log K_p (cm/s)	-6.35	-6.35	-6.22
Lipinski	Yes, 0 violation	Yes, 0 violation	Yes, 0 violation
Toxicity class*	5	5	5
Predicted LD_{50} (mg/kg)*	3200	3200	3200
Hepatotoxcity*	Inactive	Inactive	Inactive
Carcinogenicity*	Active	Inactive	Active
Immunotoxicity*	Inactive	Inactive	Inactive
Mutagenicity*	Inactive	Inactive	Inactive
Cytotoxicity*	Inactive	Inactive	Inactive
Mitochondrial Membrane Potential (MMP)*	Inactive	Inactive	Inactive

** ProTox-II data. Unlabelled data are SwissADME data.*

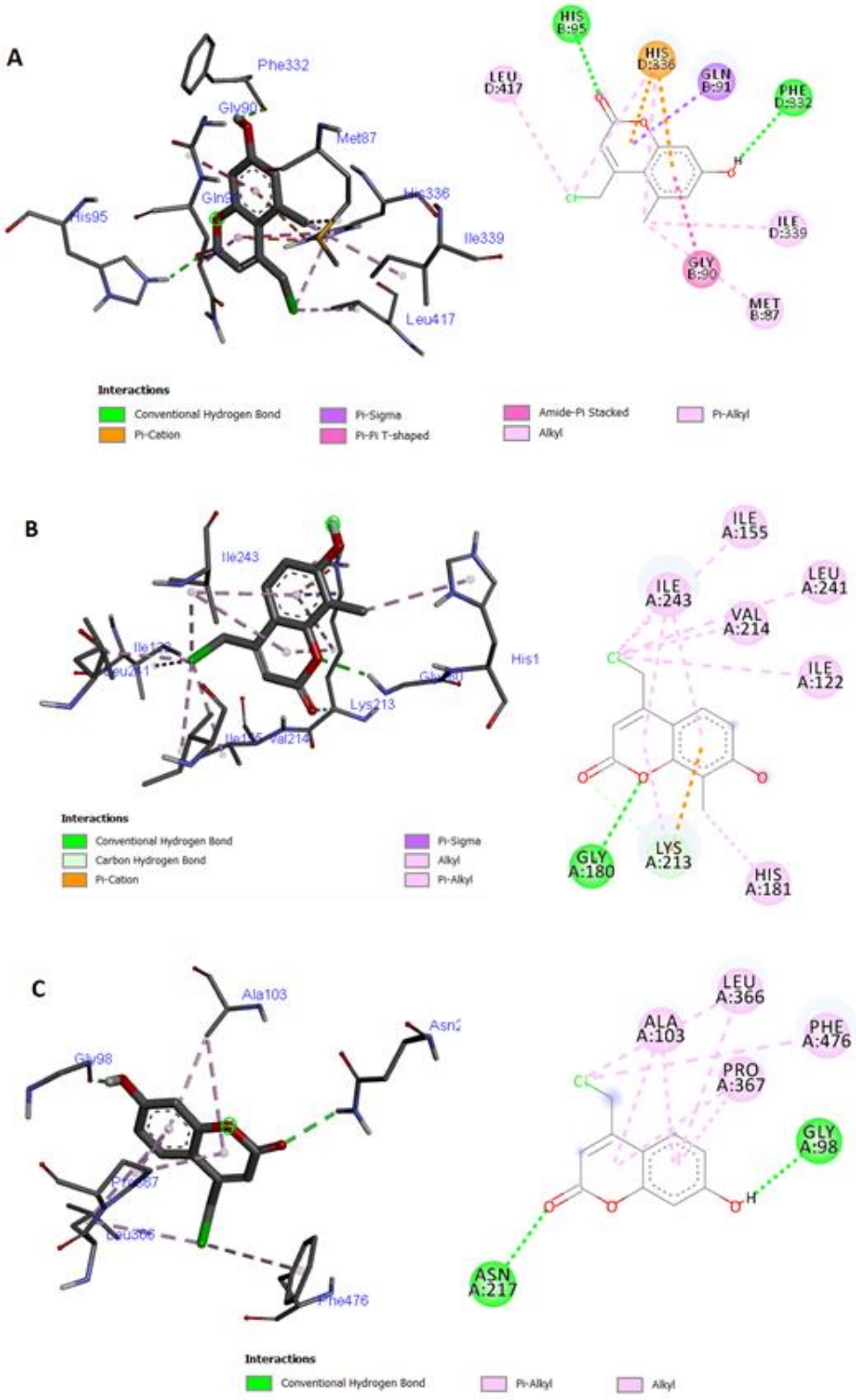


Figure 8: The 2D ligand-enzyme interactions diagrams (**A**: compound **1**-myeloperoxidase; **B**: compound **2**-NADPH oxidase; **C**: compound **3**-cytochrome P-450).

4. Conclusion

In this study, 4-chloromethyl-7-hydroxy substituted coumarin compounds with antioxidant properties were synthesized and characterized. All spectroscopic data and elemental analysis results obtained confirmed the chemical structures of the compounds. The antioxidant capacity determination by the DPPH method showed that all compounds exhibited antioxidant properties at the concentrations studied. The compound with the highest antioxidant activity was determined to be compound **1**. Molecular docking studies showed that the compounds interacted well with four of the five target enzymes studied. ADMET predictions showed that the compounds have the properties to be drug candidates. As a result of all the experimental and theoretical studies, it was concluded that the compounds may be lead compounds for various research due to their antioxidant properties and drug candidate properties.

Declaration of Competing Interest

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Declaration

The study was not funded by any organization or individual.

Data Availability

All data related to the study are given in the manuscript.

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