Synthesis, Molecular Characterization, Biological and Computational Studies of New Molecule Contain 1,2,4-Triazole, and Coumarin Bearing 6,8-Dimethyl

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Abstract: Synthesis 4-(((4-ethyl-5-(thiophen-2-yl)-4H-1,2,4-triazol-3-yl)thio)methyl)-6,8-dimethyl-coumarin and spectral analysis is carried out using the FT-IR and NMR with the help of quantum chemical calculation by DFT/6-311(d,p). The molecular electrostatic potentials and frontier molecular orbitals of the title compound were carried out at the B3LYP/6-311G(d,p) level of theory. Antimicrobial, antioxidant activity, and In vitro cytotoxic for cell lines were observed. The result shows that the theoretical vibrational frequencies, 1H-NMR and 13C-NMR chemical shift, agree with experimental data. In vitro studies showed that antimicrobial activity was weak, particularly against bacteria such as E. coli, S. aureus, P. aeruginosa, and B. cereus. The test compound's oxidative stress index (OSI) has appeared as 0.079 ± 0.214 in antioxidant and oxidant capacity studies. The compound did not cause a harmful cytotoxic effect on healthy cell lines and showed no potential for anticancer activity on cancerous cell lines such as MCF-7 and MKN-45.

Keywords: coumarin; triazole; Gaussian; molecular modeling; cytotoxic; antioxidant.

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1. Introduction

Natural products are an essential resource for drug development and design [1]. They have been used to treat diseases for thousands of years [2]. Benzene and coumarin derivatives containing pyrone rings are natural products found in high concentrations in fruits, seeds, leaves, and roots [3-7]. These structures can do hydrogen bonding, hydrophobic bonding, electrostatic interaction, metal coordination, and van der Waals force with proteins and enzymes [8, 9]. Thus, it can exhibit various biological activities such as antimicrobial, anti-inflammatory, antioxidant, and anticancer [10]. Because of their synthetic usefulness and a wide variety of biological activities, the chemistry of 1,2,4-triazoles has gained considerable attention [11]. Many research studies have shown that 1,2,4-triazoles have powerful biological characteristics, including antibacterial [12], antimicrobial [13], antifungal [14, 15], anticancer [16], antitubercular [17], antimycotic activity [18, 19], antinoceceptive, [20], antioxidant [21, 22], anticonvulsants [23], antimycobacterial, antiviral [24], anti-inflammatory and analgesic [25].
Since coumarins have an effective biological activity, they have found wide use in medicinal chemistry. Today, it is known that the design and synthesis of new coumarin derivatives prepare new drugs in many laboratories.

In recent years, density functional theory (DFT) has become one of the commonly used theories in theoretical modeling. Through better functions of exchange-correlation, several molecular properties that have accuracy comparable to historically correlated ab initio methods can be measured, all of which could be achieved with more favorable computational costs [26, 27]. During the literature review, it was found that in the replication of the experimental values in geometry, dipole moment, vibrational frequency, etc., the exact precision of the DFT [28-32].

In this study, a new coumarin derivative 4 - (((4-Ethyl-5- (thiophen-2-yl) -4H-1,2,4-triazol-3-yl) thio) methyl) - 6,8-dimethyl-coumarin synthesized. Theoretical calculations were made after the structure of the title compound was determined. Also, biological effects such as antimicrobial, antioxidant, and cytotoxic were investigated.

2. Materials and Methods

2.1. Experimental

All chemical materials were received from Merck without 4-(chloromethyl)-7-methylcoumarin, which was received from an organic lab worker at Firat University. The Infrared spectra were measured with a Perkin–Emler Spectrum one FT-IR spectrophotometer. The $^1$H- and $^{13}$C-NMR spectra were recorded on Bruker AC-400 NMR spectrometer operating at 400 MHz for $^1$H-NMR, 100 MHz for $^{13}$C-NMR. Compounds were dissolved in DMSO (dimethyl sulfoxide), and chemical shifts were referenced to TMS (Tetramethylsilane) for both $^1$H- and $^{13}$C-NMR. Melting points were determined on the Thomas Hoover melting point apparatus. Chemicals were purchased from Aldrich or Merck.

2.1.1. Synthesis of 4-Ethyl-5-(thiophene-2-yl)-4H-1,2,4-triazole-3-thiol (III).

A mixture of thiophene-2-carboxyhydrazide (I) (0.01 mol), ethyl alcohol (50ml), and ethyl isothiocyanate were refluxed for 3 h. After about 4 h, solid thiosemicarbazide begins to form in the reaction flask. KOH (0.15 mol) was added to the solid, and dissolution started. After 6 h, the reaction was stopped and brought to pH 3-4 with HCl. The residue was poured into crushed ice while stirring. The resulting solid was collected by filtration, dried, and recrystallized from ethyl alcohol.

FT-IR (KBr, cm$^{-1}$, $\nu$): 3072-3107 (Ar-H), 2870-2960 (C-H), 1570 (C=N), 1263 (C=S), 715 (C=S-C); $^1$H NMR (400 MHz, DMSO-d$_6$, $\delta$, ppm): 1.23 t (3H, N-CH$_2$-CH$_3$, $J = 7.2$ Hz), 4.22 q (2H, -N-CH$_2$-CH$_3$, $J = 7.2$ Hz), 7.27 dd (1H, Ar-H, $J = 4.0$, 4.8 Hz), 7.68 d (1H, Ar-H, $J = 3.2$ Hz) 7.86 d (1H, Ar-H, $J = 4.8$ Hz), 13.98 s (1H, SH); $^{13}$C-NMR (100 MHz, DMSO-d$_6$, $\delta$, ppm): 13.7, 39.7, 126.8, 128.9, 129.3, 130.3, 146.3, 167.5.

2.1.2. The synthesis of 4-(((4-ethyl-5-((thiophen-2-yl)-4H-1,2,4-triazol-3-yl)thio)methyl)-6,8-dimethyl-coumarin.

Potassium carbonate (K$_2$CO$_3$) (0.02 mol) was dissolved in 30 ml of dry acetone. The 4-(chloromethyl)-7-methylcoumarin (0.02 mol) was added to this solution. The 4-Ethyl-5-(thiophene-2-yl)-4H-1,2,4-triazole-3-thiol (III) (0.02 mol) was then added dropwise to this solution for 6 h at room temperature. The resulting solid was collected by filtration, dried, and recrystallized.
recrystallized from ethyl alcohol. Synthesis and the structure of the title compound are shown in Figure 1.

IR spectrum, $\nu$, cm$^{-1}$: 2940–3075 cm$^{-1}$ (Ar-H), 1720 (C=O), 1617-1327 cm$^{-1}$ (C=C), 1346-1469 cm$^{-1}$ 522-831 (C-S); $^1$H-NMR (400 MHz, DMSO-d$_6$, $\delta$, ppm): 1.16 (t, 3H, N-CH$_2$CH$_3$), 2.51 s (3H, Ar-CH$_3$), 3.18 s (3H, Ar-CH$_3$), 4.09 q (2H, N-CH$_2$CH$_3$, $J=6.9$ Hz), 4.61 s (2H, S-CH$_2$), 6.35 (s,1H, H-C-C=O), 7.22 s (1H, Ar-H), 7.26 dd (1H, thiophene-H), 7.55 d (1H, thiophene-H), 7.63 s (1H, Ar-H), 7.80 d (1H, thiophene-H); $^{13}$C-NMR (100 MHz, DMSO-d$_6$, $\delta$, ppm): 15.28, 19.04, 20.05, 33.62, 114.3, 115.7, 117.8, 125.6, 127.5, 128.1, 128.7, 129.4, 133.6, 149.2, 150.1, 151.2, 151.9, 160.2.

![Synthesis of the title compound.](image)

2.2. Computational methods.

All theoretical calculations (optimizations, NMR and IR) in this study were computed via Gaussian 09 software [33]. Visualizing of results was made using the GausView5 program. In theoretical computations, the DFT/B3LYP method and 6-311G (d,p) were selected as the basis set [34]. Theoretical NMR shifts ($^1$H-NMR and $^{13}$C-NMR) were computed within the GIAO approach [35]. In NMR calculations, DMSO was selected DMSO as solvent. Experimental infrared frequencies are different from computed infrared frequencies.

2.3. Biological methods.

2.3.1. Antimicrobial activity detection.

The antimicrobial activity of the test compound was determined by the "Microdilution Broth Method" by determining the minimum inhibition concentration (MIC) of plant extracts against microorganisms [36, 37]. Microorganism strains used in the study: Staphylococcus aureus (ATCC 29213), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), Bacillus cereus (ATCC 11778), Candida albicans (ATCC 10231), and Candida tropicalis (DSM 11953). The stock solution was prepared by dissolving the test title compound in 40% Dimethyl sulfoxide (DMSO). Mueller Hinton Broth (Accumix® AM1072) for bacterial strains, Saboraud Dextrose Broth (Himedia ME033) for Candida albicans, and Candida tropicalis were used. A sterile Pasteur pipette added 90 µl (MHB for antibacterial, SDB for antifungal) to the wells in the first row of microliter plates and 50 µL each to the other wells.
The 11 wells row were used as a sterility control, and 100 µL of the medium was added to each (CLSI, 2002; CLSI, 2012). The wells in the 12th row were used as growth control. 10 µL of the stock solution of the test compound was added to the first wells, and the other wells were added by serial dilution. A suspension equivalent to McFarland 0.5 solution was prepared from microorganisms. 50 µL of microorganism suspension was added to each well at 5 x 105 CFU / mL for bacteria and 0.5-2.5 x103 CFU / mL for *Candida albicans* and *Candida tropicalis*. Plates with bacteria added were incubated at 37 °C, plates with *Candida albicans* and *Candida tropicalis* added at 35 °C for 16-24 hours. In the evaluation of the results, the first wells in which the turbidity or appearance of the microorganisms decreased was accepted as the MIC value. The test was repeated 3 times.

2.3.2. Determination of total antioxidant and total oxidant levels.

The total antioxidant level (TAS), total oxidant level (TOS), and oxidative stress index (OSI) of the test compound was determined using commercially available Rel Assay Diagnostic kits with the formulas given below. Trolox standard for TAS analysis, hydrogen peroxide standard for TOS analysis used as reference. Oxidative stress index (OSI (Arbitrary Unit = AU) value is calculated according to the formula below [38].

\[
OSI \text{ (AU)} = \frac{TOS, \mu \text{mol H2O2 equiv./L}}{TAS, \text{mmol Trolox equiv./L X 10}}
\]

2.3.3. Cell cultures study.

In the study, Breast cancer cell lines (MCF-7), Human gastric cancer cell lines (MKN-45), and Human umbilical vein endothelial cells (HUVECs) are used.

2.3.4. Process of growing and reproducing cells.

All cell lines are collected in 25 cm² flasks (Corning-Sigma-Aldrich St. Louis, MO, USA) in an incubator at 37°C with 5% CO₂ content, Dulbecco's modified Eagle's medium (containing high glucose, 2mM L-glutamine and sodium pyruvate). DMEM) and 10% Fetal Bovine Serum (FBS). When the cell’s growth and morphologies were followed, reached 90% density, the transplantation process was started (Nuve MN 120). The 200 µL of the mixture was put into each of 96 wells (5x103 cells in 100 µl / plate space). DMEM, fetal bovine serum (FBS), and sterile phosphate buffer (PBS) were purchased commercially.

2.3.5. MTT experiment.

MTT assay method was used to determine the effects of the test compound in cell cultures. The MTT test (3- [4,5-dimethyl thiazol-2-y 1] -2,5-diphenyl tetrazolium bromide) is a colorimetric analysis method used to evaluate the metabolic activity of the cell. Test compound at different concentrations (1-10-100-1000 mg / mL) was added to the 96-well plates where the cells were cultivated, following the cells' adhesion. After 24 hours, 10 µL of 12 mM MTT solution was added to the wells and incubated for 4 hours at 37 °C in an oven containing 5% CO₂.

The purple-colored formazan crystals formed after 4 hours, 100 µL of SDS dissolved in 0.01M HCl was added and left for incubation at 37 °C in an oven containing 5% CO₂. The absorbance of the purple color formed after 4 hours was measured with an Elisa plate at 570
nm. IC50 values of the test compound were calculated according to MTT results obtained using Graphpad prism 6 programs on the computer.

2.3.6. Statistical analysis.

SPSS 22.0 (IBM Corporation, Armonk, New York, United States) program was used to analyze the data. The data were analyzed at 95% confidence level, and if the p-value was less than 0.05, it was considered significant.

3. Results and Discussion

3.1. Molecular geometry.

Figure. 2 presents the theoretical geometric structure and the B3LYP/6-311G(d,p) optimized structure of the title compound.

3.2. Nuclear magnetic resonance (NMR) spectra.

B3LYP method with 6-311G(d,p) basis set was used to calculate GIAO 1H and 13C chemical shift values (concerning TMS), which then compared with the experimental 1H and 13C chemical shift values shows the results Table 1. When the experimental and theoretical 1H-NMR and 13C-NMR spectra of the compound are examined, there are some characteristic peaks in the substituents attached to the triazole and coumarin ring.

The first example of these substituents is the ethyl fragment. While the -CH2 protons of the N-CH2-CH3 in the 3-position were observed to give a quartet peak at 4.09 ppm (J value of this quartet peak is around 6.9 Hz), the same protons were computed as at 4.14 ppm. While the -CH3 protons showed triplet at 1.16 ppm (The J value of this triplet peak is 7.0 Hz), the same protons were calculated at 1.30 ppm. The carbons in the 13C-NMR spectrum of the ethyl group were observed at 15.3 ppm for CH3, at 33.6 ppm for CH2 as experimental, and the same carbons were computed at 16.2 ppm and 35.9 ppm, respectively. Because of the high electronegativity of the nitrogen atom, carbon and hydrogen atoms close to the nitrogen atom are set downfield. Thus, the electron charge density shifts from these atoms toward the nitrogen atom, and these atoms resonance downfield.

The second example of these substituents is the S-CH2 fragment. The protons in the fragment -S-CH2- were appeared as a signal singlet at 4.61 ppm experimentally. These protons were calculated at 4.44 ppm at the B3LYP level. The carbon belonging to the S-CH2 group in the 13C-NMR spectrum appeared at 40.1 ppm experimentally, and the same carbon was
computed at 41.9 ppm. The reason why the carbon and hydrogens attached to the sulfur atom are observed in the low field is the electronegativity of the sulfur atom. Electrons around the proton and carbon atom shift towards the sulfur atom, and thus a low electron density occurs around these atoms, which reduces the shielding effect of carbon and hydrogen, and these atoms resonate in downfield.

The final example of these substituents is CH$_3$ fragments in the coumarin ring. Methyl is attached to the coumarin ring at both the C14 and C16 positions has an electron-donating structure [39]. As this structure donates electrons to the ring, it increases the electron density of the ring, which means that the protons bound to C15 and C17 in the aromatic ring resonance at a higher field and chemical shift values were observed as a singlet at 7.22 and 7.63 ppm experimentally, and as computed at 7.05 and 7.63 ppm, respectively. Chemical structure, $^1$H- and $^{13}$C-NMR spectra of the title compounds are shown in Figures 3–4.

| Atom | Experimental (ppm) DMSO-$d_6$ | Theoretical (ppm) B3LYP/6-311G(d,p) |
|------|-----------------------------|--------------------------------------|
| C1   | 149.2                       | 157.5                                |
| C2   | 151.9                       | 158.1                                |
| C3   | 149.1                       | 141.9                                |
| C4   | 129.4                       | 131.6                                |
| C5   | 128.1                       | 132.7                                |
| C6   | 128.7                       | 139.1                                |
| C7   | 33.6                        | 35.9                                 |
| C8   | 15.3                        | 16.2                                 |
| C9   | 40.1                        | 41.9                                 |
| C10  | 151.2                       | 155.8                                |
| C11  | 114.3                       | 115.8                                |
| C12  | 160.2                       | 165.1                                |
| C13  | 150.1                       | 159.2                                |
| C14  | 125.6                       | 123.2                                |
| C15  | 127.5                       | 130.3                                |
| C16  | 133.6                       | 131.3                                |
| C17  | 117.8                       | 120.5                                |
| C18  | 115.7                       | 121.1                                |
| C19  | 19.1                        | 22.3                                 |
| C20  | 20.1                        | 24.1                                 |
| 3H (thiophene-H) | 7.80, 7.55 and 7.26 | 7.56, 7.44 and 7.33 |
| 2H (N-CH2-CH3) | 4.09 | 4.14* |
| 3H (N-CH2-CH3) | 1.16 | 1.30* |
| 2H (S-CH2) | 4.61 | 4.44* |
| 1H (H-C=O) | 6.35 | 6.25 |
| 6H (Ar-CH3) | 2.51 and 3.18 | 2.16* and 2.35* |
| 2H (Ar-H) | 7.22 and 7.63 | 7.05 and 7.36 |

*: Average values
3.3. Fourier-transform infrared spectroscopy (FT-IR).

In the first step of this work, when the FT-IR spectra of the synthesized 4-Ethyl-5-(thiophene-2-yl)-4H-1,2,4-triazole-3-thiol (III) were examined, it was found that the C=O peak in the carboxylic acid hydrazides between 1640-1670 cm\(^{-1}\) was disappeared. Instead of this peak, N-C=S peaks (amide bands) at 1574, 1267, 1065, and 995 cm\(^{-1}\) appeared. In the title compound (V) obtained in the second step of the work, the most characteristic peaks are CO, CH, and CH3 vibrations.

3.3.1. CO vibrations.

The title Compound was observed at two types of CO stretching vibrations; the first one was C–O (O1-C12 and C13-O1), and the second one was C=O (C12=O2) stretching vibrations. The C-O stretching frequency was shown in the range of 1250-850 cm\(^{-1}\) [40]. The title compound C-O stretching vibrations (O1-C12 and C13-O1) appeared at 940 cm\(^{-1}\) and 1270 cm\(^{-1}\) experimentally and calculated at 915 cm\(^{-1}\) and 1240 cm\(^{-1}\) B3LYP/6-311G(d,p) level, respectively. The C=O stretching vibrations were observed in the range of 1650-1850 cm\(^{-1}\). The title compound C=O stretching vibration was appeared at 1720 cm\(^{-1}\) experimentally and calculated at 1755 cm\(^{-1}\) for B3LYP/6-311G(d,p) level. The appearance of C-O and C=O stretching vibrations in the title compound is an important indicator of the presence of the coumarin ring.

3.3.2. CH and CH3 vibrations.

CH stretching vibrations in the aromatic ring are seen in the frequency range of 3100-3000 cm\(^{-1}\) (in the form of multiple bands) [41]. The title compound CH stretching vibrations were appeared between 2937-3081 cm\(^{-1}\) experimentally and calculated at 2983-3144 cm\(^{-1}\) for B3LYP/6-311G(d,p) level. CH stressing vibrations are mostly observed in-plane bending (scissoring and rocking) and out-of-plane bending (wagging and twisting) vibrations. In-plane bending vibrations are seen between 1400-1050 cm\(^{-1}\), and out-of-plane bending vibrations are seen between 1000-675 cm\(^{-1}\) [42-44]. In the synthesized title compound, the in-plane bending vibrations were shown in 1350-1045 cm\(^{-1}\) experimentally and calculated 1341-1028 cm\(^{-1}\) for B3LYP. The out-of-plane bending vibrations were appeared in 850-723 cm\(^{-1}\) experimentally and calculated 828-695 cm\(^{-1}\) for B3LYP.

CH3 stretching (symmetrical and asymmetrical) vibrations are seen in the frequency range of 2850-3000 cm\(^{-1}\) [45, 46]. CH3 stretching vibrations in title compound appeared at 2937 cm\(^{-1}\) (Vs, triazole), 3051 (Vs coumarin, C19), 3009 cm\(^{-1}\) (Vs coumarin, C20) experimentally, and calculated 2928 cm\(^{-1}\) (Vs, triazole), 3020 (Vs coumarin, C19), 2981 cm\(^{-1}\)
(Vs coumarin, C20) respectively. Some other important peaks as C=C, C-S and C=N vibrations are seen in the frequency range of 1650-1200 cm$^{-1}$, 600-772 cm$^{-1}$, 1675-1480 cm$^{-1}$, respectively according to the literature [47], while for our compound obtained between 1655--1340 cm$^{-1}$, 550-760 cm$^{-1}$, 1472-1429 cm$^{-1}$, experimentally, respectively and calculated between 1617--1327 cm$^{-1}$, 522-831 cm$^{-1}$, 1346-1469 cm$^{-1}$, respectively. Also, Table 2 is shown at other levels of calculations.

According to these results, it is seen that there are some differences between experimental and theoretical calculated values. The first reason for these differences is that the experimental results were taken in the solid phase and the gas phase's theoretical results.

Secondly, in Gaussian infrared calculations belong to harmonic frequencies. However, in reality, there are anharmonic oscillations in molecules. Finally, experimental calculations are found in the presence of intermolecular interactions, but theoretical calculations are made on a single molecule.

Table 2. The Observed and Calculated Vibrational Spectra of the Title Compound.

| Assignments | Unscaled Frequencies (B3LYP/6-311(d,p)) | Experimental (FT-IR(cm$^{-1}$)) |
|--------------|----------------------------------------|---------------------------------|
| sCC17H       | 3090                                   |                                 |
| sasCC15H     | 3060                                   |                                 |
| rCC11H       | 3110                                   |                                 |
| ssCC6H       | 3147                                   |                                 |
| sasCC5H      | 3107                                   |                                 |
| rsCC4H       | 3128                                   |                                 |
| rCC19H       | 3022                                   | 3051                            |
| rCC20H       | 2981                                   | 3009                            |
| rCC9H        | 3019                                   |                                 |
| rsCC7H       | 3028,2968,3007                         |                                 |
| rsCC8H       | 3028,2928,3007                         | 2937                            |
| rCC1202      | 1755                                   | 1720                            |
| rCC10C11     | 1617,1567                              | 1655                            |
| rCC2C1       | 1346,1415                              | 1429                            |
| rCC1C2       | 1469,1415                              | 1472                            |
| rCC5C6       | 1472,1415,1327                         |                                 |
| rCC3C4       | 1552,1415,1327                         | 1340                            |
| rCC1N3       | 1371,1346                              |                                 |
| rCC13O1      | 1240,1095                              | 1270                            |
| rCC12O1      | 1134,915                               | 940                             |
| rCCN1N2      | 1064                                   | 1084                            |
| rCC3C7       | 1203,676                               |                                 |
| rCC2C6       | 831,726                                | 760                             |
| rCC1C1       | 546,522                                | 550                             |
| rCC1C9       | 762,607                                |                                 |
| rCC9C10C11   | 1363,1134,806                          | 1348                            |
| rCC3C1N2     | 1415,1203                              | 1392                            |
| rCC13C14C15  | 1240                                   |                                 |
| rCC2C6H      | 1327,1116                              |                                 |
| rCC1C9H      | 1159,906                               |                                 |
| rCC12O2      | 806,573,568                            | 1392                            |
| rCC13C14     | 915                                    |                                 |
| rCC2N1C2     | 1337,922                               |                                 |
| rCC5C6H      | 1341                                   | 1350                            |
| rCC5C9H      | 1077                                   | 1084                            |
| rCC9H        | 1028                                   | 1045                            |
| rCC10C18C17H | 886,857                                |                                 |
| rO1C12C11H   | 910,857,828                            | 897                             |
| rCC1S1C9H    | 1226,1159,910                          |                                 |
| rCC3C7C8H    | 1457,1121,1068                         |                                 |
| rCC3C1N1N2   | 704,664                                |                                 |
| rCC2C6C5C4   | 833,546                                |                                 |
| rO2C11O1C12  | 828,695                                | 850                             |
| rCC3N3N1C2   | 704                                    | 723                             |

$\nu$, stretching; $\beta$: bending; $\omega$: in-plane bending; $\delta$: out of plane bending; $\alpha$: torsional; $s$: symmetric; as asymmetric.

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As a result, although there are some differences between experimental and theoretical values, it is seen that the results are in great agreement.

3.4. Frontier molecular orbitals and global reactivity descriptors.

Frontier molecular orbital theory applies molecular orbital theory describing HOMO-LUMO interactions [48-50]. Global reactivity descriptors are given softness, hardness, and electronegativity in the literature [51-53]. As seen in Figure 5 the HOMO-1 electrons are delocalized on the coumarin ring; the HOMO electrons are delocalized on the triazole and thiophene ring, the LUMO electrons are delocalized coumarin ring, the LUMO+1 electrons are delocalized on the triazole and thiophene ring. The value of the energy separation between the HOMO and LUMO is 4.068 eV. This shows that the energy gap reflects the chemical activity of the molecule. For a molecule, by using HOMO and LUMO energy values can calculate the following parameters:

Ionization potential is the minimum amount of energy required to remove an electron from the atom or molecule in the gaseous state. Electron affinity is defined as the amount of energy released when an electron is added to a molecule in the gaseous state. Electronegativity is the tendency of an atom to attract electrons. Chemical hardness is a measure of the prevention of weight transfer in molecules. The molecules with higher chemical hardness values have little or no weight transfer [54]. Electronic structure parameter values calculated by the B3LYP method using 6-311G(d,p) are showed in Table 3.

![Energy levels of HOMO, HOMO-1, LUMO and LUMO-1 of the title compound (V) computed at B3LYP/6-311 G(d,p) level.](image)

**Figure 5.** Energy levels of HOMO, HOMO-1, LUMO and LUMO-1 of the title compound (V) computed at B3LYP/6-311 G(d,p) level.

| Parameters       | Gas phase |
|------------------|-----------|
| $E_{\text{HOMO}}$ | -6.040    |
| $E_{\text{LUMO}}$ | -1.972    |
| $\chi$           | 4.006     |
| $\eta$           | 2.034     |
| $S$              | 0.245     |
| $E=E_{\text{HOMO-LUMO}}$ | 4.068 |

Table 3. Global Reactivity Descriptors for the Title Compound (V).

Consideration of only the HOMO and LUMO may not yield a realistic description of the frontier orbitals because in the boundary region, neighboring orbitals may show quasi degenerate energy levels. For this reason, the density of states (DOS) was calculated both the gas phase by using the Gauss Sum 3.0 software [55]. Figure 6 is shown the density of states diagram for the title compound.

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3.5. Molecular electrostatic potential (MEP).

Molecular Electrostatic Potential [56] is related to dipole moment, electronegativity, partial charges, and the molecule's chemical reactivity region. It provides a visual method to understand the relative polarity of the molecule. While the negative electrostatic potential is the region where the electron density is higher than the nucleus over the entire molecule (colored in red tones on the ESP surface), the positive electrostatic potential is the region where the low electron density is high. (Colored in blue tones on the ESP surface) [57,58]. The MEP map of the compound is given in Figure 7.

According to the figure, the negative regions in the molecule are located on the Nitrogen N1 (−0.018 a.u.), N2 (−0.013 a.u.) atoms of the triazole ring, and Oxygen O1 (−0.021 a.u.), O2 (−0.041 a.u.) atoms of the coumarin ring. It can be said that these regions are the most suitable regions for the electrophilic attack. For the positive regions, it is seen that the ethyl group attached to the triazole ring with a value of (+0.027 a.u.) is located around the hydrogens, which can be said to be the region most susceptible to nucleophilic attack.

3.6. Antimicrobial activity.

The antimicrobial activity of the test compound dissolved in DMSO is shown in Table 4. The test title compound is known to be significant when the MIC value is 0.1 mg / mL or less, moderately effective in the range 0.1 <MIC ≤ 0.625 mg / mL, and weakly effective when greater than 0.625 mg / mL [59]. In this study, it is seen that, in general, the test compound has moderate antimicrobial activities on some of the 6 different microorganism strains tested. The test title compound does not have a strong effect on the microorganisms tested. In general, it can be said that the test title compound is more effective on B. cereus, P. aeruginosa, and fungi.
Table 4. Antimicrobial Activities of the title Compound (Mg/Ml).

|                  | E. coli ATCC 25922 | S. aureus ATCC 29213 | P. aeruginosa ATCC 27853 | B. cereus ATCC11778 | C. albicans ATCC10231 | C. tropicalis DSM11953 |
|------------------|--------------------|----------------------|--------------------------|---------------------|-----------------------|------------------------|
| The title compound | >5                 | 2.5                  | 2.5                      | 2.5                 | 2.5                   | 1.25                   |

3.7. Antioxidant activity.

The antioxidant potential of the active ingredient or compounds is generally due to their ability to remove or transform the effects of damaging free radicals. The higher antioxidant capacity of the compounds, the better the therapeutic quality [60]. In this study, the antioxidant capacity, oxidant capacity, and oxidative stress indices of the compound are given in Table 5. The title compounds may contain both oxidizing groups and oxidation-inhibiting groups, so it is important to calculate the total oxidative stress index and evaluate the overall antioxidant-oxidant load. According to the experiment results, the antioxidant value of the synthesized compound is 6.198 ± 0.310. When the oxidant load was examined, it was found to be 4.903 ± 0.122. When the oxidative stress index is examined, it is seen that it is 0.079 ± 0.214.

Table 5. TAS, TOS and OSI values of the compound.

| The title compound | TAS (mmol/L) | TOS (µmol/L) | OSI     |
|--------------------|--------------|--------------|---------|
|                    | 6.198±0.310  | 4.903±0.122  | 0.079±0.214 |

3.8. In vitro cytotoxic activity.

Doses of the test compound at varying concentrations of 1, 10, 100, and 1000 µg / mL were administered on three different cell lines and left to incubation for 24 hours. IC50 value showing the test compound's cytotoxic effects on different cell lines is given in Table 6. It has been determined that IC50 values are generally well above the 10 µM dose. Therefore, it can be said that the test compound does not have a significant cytotoxic effect on the cell lines tested. The very weak cytotoxic effect of the test compound on the HUVEC cell line is beneficial for the compound's anticarcinogenic potential. It indicates that the synthesized test compound has no detrimental cytotoxic effect on healthy cells, shown in Table 6. However, the lack of a strong cytotoxic effect of the synthesized test compound on cancerous cell lines such as MCF-7 and MKN-45 indicates that the synthesized compound does not have a drug potential to be effective on these types of cancer.

Table 6. Ic50 Values indicating the Cytotoxic Effects of the Compound in Different Cell Lines.

| The title compound | MCF7 Human breast adenocarcinoma cell line | HUVEC Human umbilical vein endothelial cell line | MKN-45 Human gastric cancer cell line |
|--------------------|--------------------------------------------|-----------------------------------------------|-------------------------------------|
|                    | 981,198                                    | 978,561                                       | 196,475                             |

4. Conclusions

In this study successfully Synthesis 4-((4-ethyl-5- (thiophen-2-yl) -4H-1,2,4-triazol-3-yl thio) methyl) -6,8-dimethyl-coumarin by the reaction of 6, 8-(dimethyl) -4- (chloromethyl) –coumarin and 4-ethyl-5- (thiophen-2-yl) –4H-1,2,4-triazole-3-thiol. Characterization of 6-ethoxy-4-methylcoumarin was confirmed by FT–IR, 1H NMR, and 13C NMR. The spectroscopic and electronic properties of the title compound were investigated both experimentally and theoretically. The title compound's structural data (V) calculated at B3LYP/G-311G(d,p) were in very good correspondence with experimental values. HOMO-LUMO boundry orbitals of the title compound, the energy difference between them, were
calculated. Also, it was determined in which regions the substituents in the compound were localized and whether the molecule had a stable structure. The regions closest to the electrophilic and nucleophilic attack were determined by creating a Molecular Electrostatic Potential (MEP) map. An agreement was determined between the theoretical and experimental results concerning the accurate allocation of the vibrational frequencies to the molecular structure based on the theoretical calculations. The antioxidant, antimicrobial and cytotoxic properties of the title compound were studied.

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Conflicts of Interest

The authors declare no conflict of interest.

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