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Quantum Chemical and Biological Properties of Coumarin Derivative Compound

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ABSTRACT

This work presents the characterization of 8-t-buthyl-4-methyl-2H- chromen 2-one by quantum chemical calculations and spectral techniques. The molecular geometry, vibrational frequencies and gauge including atomic orbital (GIAO) ^{1}H and ^{13}C NMR chemical shift values of title compound in the ground state have been calculated using the density functional method (B3LYP) with the 6-31G(d) basis set. The theoretical vibrational frequencies and chemical shift values show good agreement with experimental values. In addition, DFT calculations of molecular electrostatic potentials and frontier molecular orbitals of the title were carried out at the B3LYP/6-31G(d) level of theory. The title compound was screened for antibacterial, antifungal and antioxidant activities.

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1. Introduction

Coumarins are polyphenols class phytochemicals with some medicinal properties, naturally found in higher plants. Coumarins are members of the benzopyrone class and are a significant resource for the discovery of a novel anticancer agent. In addition, their stable nature, dissolution, and medical applicability properties have fascinated medicinal chemists for the discovery of coumarin derivatives. It has been reported that coumarins exert a wide range of pharmacological properties including antibacterial, antioxidant, antidiabetic, anti-inflammatory, analgesic and anticancer effects. Coumarins are of interest in the clinical field because of their low toxic effects, their low cost, their availability in traditional herbal medicines, and their ease of chemical modification. In recent studies, Maleki et al. reported anticancer effects of coumarin derivative in human cervical cancer cell lines. Likewise, Ipek et al showed that hybrid lonidamine-coumarin derivatives have anticancer activity. In another study, Holiyachi et al. showed anticancer action potential by novel dipyrromethane-coumarin and porphyrin-coumarin derivatives. However, the selectivity of coumarin derivatives for cancer cells remained unclear. For the possible use of coumarins in cancer therapy, a great deal of investigations have been conducted on the design and synthesis of coumarin compounds with highly potent anticancer drug candidates with minimal or no side effects. However, the lack of such drugs to be used in cancer therapy safely encouraged us to work in this field. Moreover, all these developments encouraged us to discover selective, highly effective agents. [1-4].

Density functional theory (DFT) has been one of the widely used theories in theoretical modeling during recent years. By means of the development of better exchange–correlation functionals, it has become possible to calculate many molecular properties which have accuracies that can be comparable to traditionally correlated ab initio methods, all these could be done with more favorable computational costs [5]. It has been figured out during the literature survey that in reproducing the experimental values in geometry, dipole moment, vibrational frequency, etc. DFT has a precise accuracy [6-12]. The aim of this study is to investigate the energetic and structural properties of the 8-t-buthyl-4-methyl-2H-chromen-2-one

(Fig. 1), using density functional theory calculations. In this study, the optimized geometry, vibrational spectra and assignments and statistical energetic parameters, of the title compound have been studied. These calculations are valuable for providing insight into molecular properties of coumarin compounds. Besides the characterization of the title compound, the biological activities of the title compound, such as antibacterial, antifungal and antioxidant activities, were investigated.

2. Material and Method

Chemical assays: The entire set of quantum calculations were performed using the density functional theory (DFT) calculations with B3LYP levels using 6-31G(d) as a basis set using Gaussian 09W [13] program package, invoking gradient geometry optimization [14-16]. To predict the optimized molecular structure and vibrational wavenumber, calculations are carried out using B3LYP method. The optimized geometry corresponding to the minima on the potential has been obtained by solving self-consisting field equation iteratively. The harmonic vibrational frequencies were calculated at the same level of theory for the optimized structure. The incompleteness of the basis set and vibrational anharmonicity consequences the overestimation of the computational frequencies.

Biological assays

1. Examination of antimicrobial properties

Standard broth dilution method was used to measure the Minimum Inhibition Concentration (MIC) values of the in vitro antimicrobial activities of our synthesized substance.

To examine both antibacterial and antifungal activities, the synthesized compound and the control group were dissolved in DMSO (dimethylsulfoxide). Moreover, dilution series were prepared for microorganisms at the specified concentrations with decreasing density of 1024, 512, 256, 128, 64, 32, 16, 8, 4 μg ml^{-1}. Stock solutions were prepared in DMSO and it was determined that DMSO did not have any effect on the microorganisms in the concentration. The antimicrobial activities of the compounds were determined by the National Clinical Laboratory Standards Committee (CLSI) according to the broth dilution method. Each bacterial strain, which was in stock the night before, was inoculated with a loop into Nutrient broth (PH: 7.4) liquid medium under sterile conditions and incubated at 37 °C for 24 hours. Yeast strains were sown with a loop in Nutrient Broth (PH: 7.4) liquid medium and incubated at 25 °C for 24 hours. Thus, the density of bacterial and yeast strains in the media was adjusted to 105 CFU ml^{-1}. Test compounds dissolved in DMSO were first prepared at a concentration of 1024 μg ml^{-1}, and a series of dilutions were prepared at concentrations decreasing to 4 μg ml^{-1} by adding medium. In addition, a series of control groups were prepared. Bacterial cultures prepared the day before were inoculated into the prepared dilution tubes and incubated at 37 °C for 24 hours. The yeast-like fungus cultures that we had prepared the day before were inoculated into dilution tubes and then incubated at 25 °C for 48 hours. Minimal Inhibition Concentration (MIC) values were calculated by turbidity determination method after incubation. Experiments were performed in two parallels.

2.Examination of antioxidant properties

DPPH radical reduction method was used as the first method for antioxidant properties. In the study, the radical reduction effect of the compound was determined according to the method of Liyana-Pathiranan and Shahidi. 4 ml of the solution prepared at 25 mg/L DPPH in methanol was taken and mixed with the test substances dissolved in DMSO at various concentrations. In order for the reaction to occur, this mixture was kept in the dark at room temperature for 30 minutes. It was waited and then the absorbance of the mixture was read on a spectrophotometer at 517 nm using 1 μM quercetin as a reference.

Xanthine, Xanthine oxidase method was used as the second method for antioxidant activity. For this, after adding the compound to the mixture of xanthine, xanthine oxidase and nitroblue tetra zolium, the mixture was kept at 37 ◦C for 30 minutes for the reaction to occur. has been kept waiting. The reaction was terminated by the addition of glacial acetic acid. The amount of formazone formed was measured with a spectrophotometer at 560 nm. In another non-enzymatic experiment, the hydroxyl radical was formed in a mixture of deoxyribose, ferrous sulfate, sodium ascorbate and hydrogen peroxide, and the reaction compounds were incubated at 37 ◦C for 90 minutes at various concentrations. Mixtures without added compounds were accepted as the control group. The amount of MDA in control and compound-added samples was determined spectrophotometrically and chromatographically. As the fourth method for antioxidant activity, the solutions of the substance whose activity will be measured were added to the samples containing 106 cells per 1 ml of SAC-SER (Sacromises cerevisia) cells, with a final concentration of 50 and 100 μM. MDA amounts were measured by HPLC after 24 and 48 hours of substance addition. For MDA analysis, 250 μL of 15% trichloroacetic acid and 750 μL of 0.5 M HCIO4 were added to the cells treated with chemicals, shaken and thus broken into small pieces. After the lysate was centrifuged

at 4500 rpm for 5 minutes, the clear part was taken and analyzed by HPLC.

3. Examination of antitumor properties

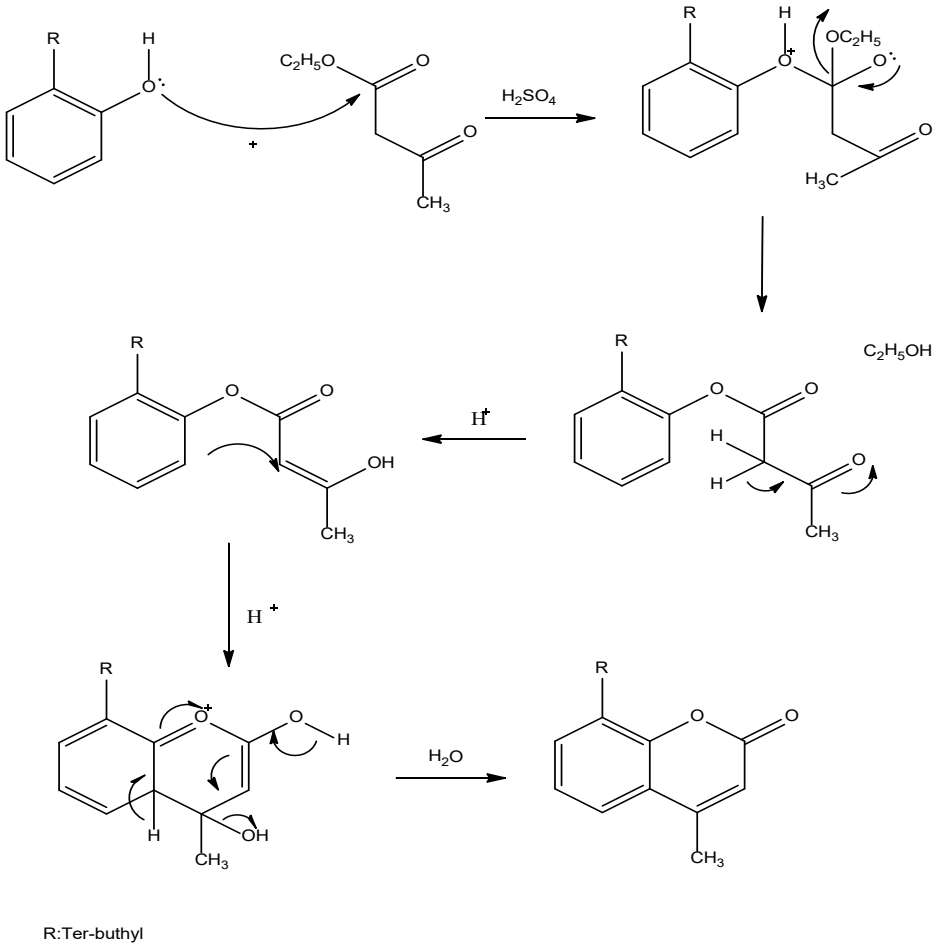
The antitumor properties of the compound have also been investigated in vitro. For this, one ml of the MCF-7 cell suspension was transferred to test tubes and the agent to be tested (the compound we synthesized) was added at concentrations of 7.5, 15, 30, 60, 100 μM. The same amount of DMSO was prepared in Vehicle tubes, but not more than 1%. After 24 hours, the tubes were removed from the incubator and triturated, and the cell suspension was mixed with 0.4% tryphan blue at a ratio of 1:1 (v/v) and 100 randomly selected cells were counted on a hemocytometer. Cell viability rate is expressed as a percentage, the same process was repeated after 48 hours. This process was repeated 3 times in different weeks.

3. Results and Discussions

When the infrared and NMR spectra of the obtained the title compound was examined, In the infrared spectra of the compound, the C-O stretching band was observed at 1179 cm-1, 1185 cm-1, the aromatic C=C stretching band was observed at 1625 cm-1 and the sharp lactone carbonyl peak of coumarin was observed at 1715 cm-1. In the 1H-NMR spectra of the title compounds. The most characteristic peaks of title compounds synthesized which is (CH3) (C-4) hydrogens, (CH) (C-3) hydrogen, hydrogens in the aromatic ring and the proton of the substituted phenol (OH). These peaks are given in more detail below.

(CH3) (C-4) hydrogens are singlet at 2.50 ppm; (CH) (C-3) hydrogen is singlet at 6.30 ppm, and hydrogens in the aromatic ring are observed as a multiplet in the range of 7,20-7.80 ppm, and the proton of the substituted phenol (OH) used in the starting material are not observed at 5.35 ppm. In the 13C-NMR spectrum of the compounds, the coumarin-determining lactone peak was observed at 163 ppm. The reaction sequences employed for the synthesis of the title compounds are shown in Figure 1.

Reaction analysis of the title compounds: The reaction to form the coumarin ring took place via the von Pechmann condensation mechanism. In this condensation, 2-t-Butylphenol was reacted with ethyl acetoacetate under sulfuric acid catalysis to obtain 8-tert- 4-methylcoumarin. The reaction mechanisms of synthesized compounds are shown in Figure1



R:Ter-buthyl

Figure 1 The reaction mechanisms of 8-tert- 4-methylcoumarin

Frontier molecular orbitals (FMO) and Electronic features. Molecule boundary orbitals are used to explain chemical reactions between molecules. The boundary orbitals are the highest energy occupied molecular orbital (HOMO) and the lowest energy vacant molecular orbital (LUMO). Since most of the chemical reactions take place by gaining or losing electrons, HOMO and LUMO have a first-order effect on the chemical behavior of the molecule. The lower the energy of the lowest energy vacant molecular orbital (LUMO), which is the orbit in which the electron to be taken will be placed, the easier it is to take the electron. Since the electron will be given from the highest energy filled molecular orbital (HOMO), the higher the energy of this orbital, the greater the tendency to donate electrons. Electrons in HOMO are the first to be removed during ionization. If the infinity energy of the electron is zero and it is assumed that there is not much new arrangement at the orbital level after ionization in the molecule, it is seen that ionization energy = –HOMO and electron affinity = –LUMO. The HOMO and LUMO energy values of the compounds were calculated on the 6-31G(d,p) basis set of the B3LYP method. As seen in the figure 2, the energy band gap between the ground state and first excited states of the molecules is 4.648 eV.

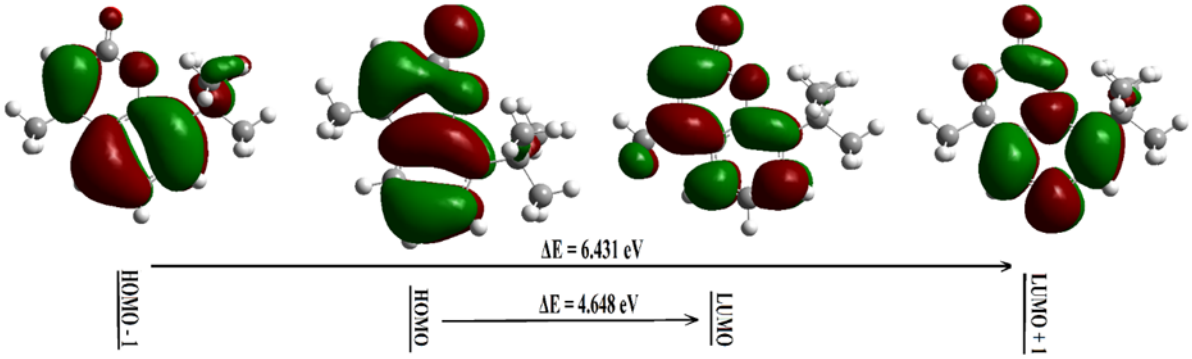


Figure 2 Molecular orbital surfaces and energy levels given in parentheses for the HOMO, HOMO -1, LUMO and LUMO + 1 of the title compound computed at B3LYP/6-31G(d) level

1.Antibacterial and antifungal activities of Test Compound:

Table 1 Minimum inhibition concentration values indicating the antimicrobial activity of the test compound

Microorganisms	*B. subtilis* ATCC 6633	*S.aureus* ATCC 6538 P	*E. coli* ATCC 25922	*S.typhimurium* NRRL B 4420	*C. globrata* ATCC 66032	*C.tropicalis* ATCC 13803
Test Compound	6.25	6.25	12.5	6.25	6.25	6.25
Ampilisin (Standard)	3.12	1.56	6.25	3.12	0	0
Fuconazole (Standard)	0	0	0	0	3.12	6.25
DMSO (Control)	0	0	0	0	0	0

According to the results, the test compound was found to have moderate activity against all fungal and bacterial strains compared to standards.

2. Antioxidant activities of the synthesized compound

Test	DPPH scavenging activity (%)				Xanthine oxidase activity (%)		Hydroxyl Radical Scavenging Activity		*MDA levels of Saccharomyces cerevisiae samples*	
Compound	100μM	250μM	500 μM	1000 μM	50 μM	100 μM	50 μM	100 μM	50 μM	100 μM
	54.2 ±0.2	57.4±0.3	72.9±0.3	87.4 ± 0.1	45.6 ±0.1	47.3 ± 0.2	35,15± 0.2	42,46± 0.2	0.32 ± 0.01	0.43 ± 0.01

Table 2 In vitro antioxidant activities of the synthesized compounds.

In experiments conducted with different antioxidant methods, it was determined that the test compound had potential antioxidant activity. It has also been found that there is a dose-dependent increase in activity

3. Antitumor activity of the synthesized compound

	Viability of MCF-7 cells in % depending on dose (24 hours)					Viability of MCF-7 cells in % depending on dose (48 hours)				
Test Compound	7.5 μM	15 μM	30 μM	60 μM	100 μM	7.5μM	15 μM	30 μM	60 μM	100 μM
	40.2±0.1	36.3±0.1	33.9±0.1	32.3±0.3	28.1 ± 0.2	39.2±0.1	36,45± 0.1	32,13± 0.1	30.56 ± 0. 1	29.2 ± 0.1

Table 3 The time- and dose-dependent viability of the in vitro antitumor activity of the synthesized compound in MCF-7 cells.

When the results obtained were examined, it was found that the test compound reduced cancer cell viability more at the highest dose applied. Accordingly, it can be said that the test compound has potential antitumor activity at high doses.

4. Discussion

In this investigation, when the 8-t-buthyl-4-methyl-2H-chromen-2-one were evaluated according to their in silico physicochemical properties, they showed full agreement.

A complete structural, thermodynamic, vibrational and electronic investigations of 8-t-buthyl-4-methyl-2H-chromen-2-one have been carried out with FT-IR, and NMR spectroscopic technique along with DFT/B3LY P method with 6-31G(d) basis sets. The molecular geometry parameters such as bond length, bond angle and vibrational frequencies calculated using B3LYP method using 6-31G(d) basis set. The energies of HOMO & LUMO and their orbital energy gaps are calculated using B3LYP/6-31G(d) method which provide the nature of reactivity, structural and physical properties of molecules. ^{1}H and ^{13}C NMR spectra were recorded and its isotropic chemical shifts were calculated theoretically at B3LYP/6-31G(d) level. The frontier orbital energy gap (EHOMO-ELUMO) is found to be 4.648 eV.

The vibrational frequencies and ^{1}H and ^{13}C NMR chemical shift values are precisely assigned to its molecular structure with the aid of the theoretical calculations at B3LYP/6−31G(d) level, in which the experimental and theoretical results support each other. It was noted here that the experimental results belong to solid phase and theoretical calculations belong to gaseous phase. In the solid state, the existence of the crystal field along with the intermolecular interactions have connected the molecules together, which result in the differences of FT-IR and NMR parameters between the calculated and experimental values.

On the other hand, when biological results are evaluated, it can be revealed that the test compound has a moderate antimicrobial effect and, at high doses, a good potential antioxidant and antitumor activity.

Competing interests

The authors declare that they have no competing interests.

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