Free Radicals and Antioxidants, 2017; 7(1): 31-35
A multifaceted peer reviewed journal in the field of Free Radicals and Antioxidants
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Original Article

Comparative Molecular Modelling Studies of Coumarin Derivatives as Potential Antioxidant Agents

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ABSTRACT

Background: Coumarin is quite extend in plants including various vegetables, flavors, foods. Coumarin has various significant medicinal activities such as anti-inflammatory, anti-oxidant, anti-viral, anti-microbial, anti-cancer and has been indicated to increase central nervous system activity. **Methods:** The syntheses of tailor-made coumarins, which are highly compelling for medical applications due to their structurally interesting antioxidant activity, were report in this manuscript. Coumarins were synthesized successfully through the modification of 4-hydroxycoumarin by different reaction steps. The molecular structures of the coumarins were characterized by the Fourier transformation infrared and Nuclear magnetic resonance (NMR) spectroscopies. The antioxidant efficiency of the 4-hydroxycoumarins were evaluated by typical spectroscopic method, using radicals DPPH• and H_2O_2. **Results:** The new coumarins synthesized in this work exhibited an excellent antioxidant compared to the free vitamin C. Molecular modelling studies using DFT (Density Functional Theory) calculations showed that there is a high correlation between dipole moment, Ionization potential (IP), Electron affinity (EA), Hardness (η), Softness (S), Electro negativity (μ), energy gap, HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular) energies and antioxidant activity. **Conclusion:** New coumarin derivatives were successfully synthesized using chemical methods. The characterized of these coumarins were done by using different spectroscopic techniques (FT-IR and NMR) and micro-elemental analysis (CHNS). The antioxidant activity of these coumarins were determined by using DPPH and hydrogen peroxide assays. Results indicated that the new coumarins possess higher scavenging activity than vitamin C.

Key words: Antioxidant activity, Coumarin, DPPH, HOMO, LUMO, H_2O_2.

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DOI: 10.5530/fra.2017.1.5

INTRODUCTION

Molecular oxygen is an indispensable element for the life of aerobic organisms because it enables the formation of reactive oxygen species (ROS) which in small quantities are essential for many physiological processes. At high doses, the ROS are very toxic.[1] The main ROS in humans are superoxide anion (O_2 •–), hydrogen peroxide (H_2O_2•), radical hydroxide (OH•–) and the reactive nitrogen species (RNS), and nitric monoxide (NO). These are constantly generated within the cells upon exposure to xenobiotics, pollutants, ultraviolet rays, smoke, and some endogenous metabolites of the redox and respiratory chain during transfer of electrons.[2] Oxidative stress is involved in the pathophysiology of malaria. Plasmodia digest hemoglobin which results in the production of heme. Heme triggers the production of ROS which are implicated in the pathophysiology of malaria[3,4] and can lead to the development of anemia[5,67,8] and apoptosis. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources.[9] As improved antioxidant status helps to minimize the oxidative damage and thus delay or prevent pathological changes. Potential antioxidant therapy should be, therefore, included either as natural free radical scavenging antioxidant enzymes or as an agent which is capable of augmenting the activity of antioxidant enzymes.[9] Free radicals are molecules or atoms (free particles or ions) having no less than one unpaired electron; subsequently, they are very active with an extensive variety of other molecules. They are always created and kept up in balance in biological systems through metabolic processes, furthermore they assume important roles in a variety of typical biochemical capacities, for example, cell signaling, apoptosis, gene expression, ion transport, and pathological processes.[10] Naturally occurring substances having antioxidant property are becoming one of the most appealing modes of modern therapy.[11] Our studies started (Figure 1) from the design of coumarins with enhanced antioxidant

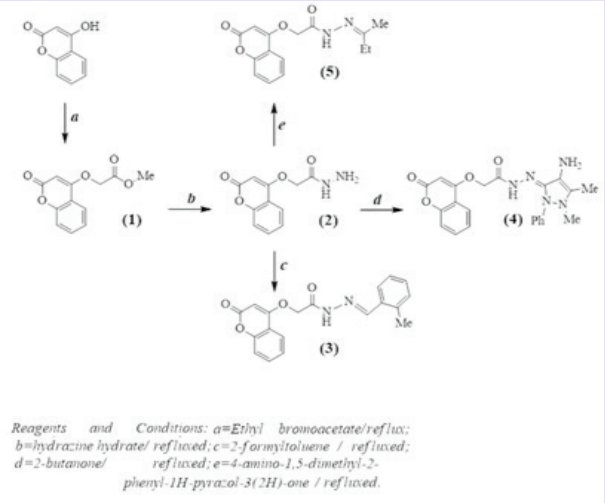

Figure 1: Reaction sequences of the synthesized compounds.

activities. We approach to increasing of antioxidant activities based on the long conjugated system in the synthesized coumarins. We also carried out DFT calculations in order to associate the antioxidant activity of these coumarins with their electronic surfaces through molecular modelling tools.

MATERIALS AND METHODS

General Information

All chemicals were supplied by Sigma-Aldrich (Selangor, Malaysia). FT-IR spectra were obtained on a Nicolet 6700 FT-IR spectrophotometer (Thermo-Nicolet-Corp., Madison, WI, USA), in cm^{-1}. NMR spectra were recorded using an AVANCE III 600 MHz spectrometer (Bruker, Billerica, MA, USA), using DMSO and expressed in δ ppm. CHN microanalysis was performed on an Elementar Vario El III Carlo Erba 1108 elemental analyzer (Carlo Erba Reagenti SpA, Rodano, Italy).

Synthesis of compounds 1-5

Compounds (1-5) namely methyl 2-(coumarin-4-yloxy)acetate (1); 2-(Coumarin-4-yloxy) acetohydrazide (2); N'-(2-Methylbenzylidene)-2-(coumarin-4-yloxy) acetohydrazide (3); N'-(4-Amino-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-ylidene)-2-(coumarin-4-yloxy)acetohydrazide(4); N'-(Butan-2-ylidene)-2-(coumarin-4-yloxy)acetohydrazide (5) were synthesized according to (Al-Amiery, *et. al*. 2014).

Antioxidant Activity

DPPH free radical scavenging activity

The antioxidant activity of synthesis compounds and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method.[12] The diluted working solutions of the test compound were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:[13]

$$DPPH_{scavenginig\ effect}\% = A° - \frac{A}{A°} \times 100$$

where Ao is the absorbance of the control reaction and *A* is the absorbance in the presence of the samples or standards.

Hydrogen Peroxide Scavenging Activity

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (250, 500, and 1000 µg/mL) of synthesized compounds (or ascorbic acid as control) were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank solution containing phosphate buffer without hydrogen peroxide.[14,15] Hydrogen peroxide percentage scavenging activity was then calculated using the following:

$$H_2O_{2scavenging\ effect}\% = A° - \frac{A}{A°} \times 100$$

where Ao is the absorbance of the control reaction and *A* is the absorbance in the presence of the samples or standards.

Theoretical Calculations

The molecular representation sketch of the reference compound was plotted using Chem Bio Office 2010 software. All the quantum chemical calculations were performed using the Density Functional Theory (DFT) methodology with 3-21 G basis set.[16]

Statistical Analysis

The results were expressed as mean ± standard deviation and the statistical significance of differences were determined utilizing one-way analysis of variance (ANOVA) using the SPSS 17.0 statistical software program. Differences were considered significant at $P<0.05$. The values are presented as mean ± SD (n=3).

RESULTS AND DISCUSSION

The sequence for the preparation of the target coumarins namely; 2-((2-oxo-2H-chromen-4-yl)oxy)acetohydrazide (2), (E)-N'-(2-methylbenzylidene)-2-((2-oxo-2H-chromen-4-yl)oxy)acetohydrazide(3),N'-(4-amino-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-ylidene)-2-((2-oxo-2H-chromen-4-yl)oxy)acetohydrazide (4), N'-(butan-2-ylidene)-2-((2-oxo-2H-chromen-4-yl)oxy)acetohydrazide (5) starting from methyl 2-((2-oxo-2H-chromen-4-yl)oxy)acetate (1) that was prepared from refluxation of 4-hydroxycoumarin with methyl bromoacetate is shown in Figure 1. Compound (1) was synthesized by the reaction of methyl bromoacetate with 4-hydroxycoumarin and anhydrous acetone in the presence of anhydrous potassium carbonate under reflux conditions. The FT-IR spectrum of (1) showed a significant absorption stretching band at 1,723.1 cm^{-1} for esteric carbonyl group. 1H-NMR spectrum for compound (1) exhibited a singlet at δ 3.63 ppm due to methyl protons. Compound 2 was synthesized in good yield by reaction of 1 with hydrazine hydrate. The FT-IR spectrum of (2) showed significant absorption bands for amine groups at 3,233.3 and 3,210.0 cm^{-1}. 1H-NMR spectrum exhibited a singlet at δ 8.21 ppm and a singlet at δ 4.45 ppm due to the secondary amine group and methylene protons respectively. The reaction of (2) with carbonyls namely 2-Formyltoluene, 4-amino-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one and 2-butanone yielded the Schiff bases namely N'-(2-methylbenzylidene)-2-[(coumarin-4-yl)oxy]acetohydrazide (3), N'-[4-amino-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-ylidene]-2-[(coumarin-4-yl)oxy]acetohydrazide (4) and N'-(butan-2-ylidene)-2-[(coumarin-4-yl)oxy]acetohydrazide (5) respectively.

Antioxidant Activity

An antioxidants are molecules that inhibit the oxidation of other compounds by hydrogen or electron donors and neutralizing free radicals.[17,18] A number of tests were developed to conclude the antioxidant activitis depend on various mechanisms, include; Trolox equivalent antioxidant,[19] oxygen radical absorption,[20] ferric reducing power[21] and free radical scavenging.[22] DPPH was the most broadly utilized.[23] The scavenging activity of different organic compounds can be evaluated utilizing DPPH or H2O2 or ABTS+or superoxide anion radical.[24] It was been reported that numerous natural and synthetic organic compounds have an excellent performance as antioxidants, consequently it is important to comprehend the method of activity and productivity of these antioxidants. DPPH method is the commonly way to investigate the antioxidant activities of the tested compounds due to simple, efficient and costly worth, it was developed by Blois in 1958 and used as a reference point.[25,26] The stable free radical DPPH can accept an electron or a hydrogen atom from other molecule to become more stable [27] Free radicals have been a subject of critical interest among researchers in the previous decade. The wide range of free radical effects in biological systems has garnered interest from many specialists. It has been demonstrated that free radicals

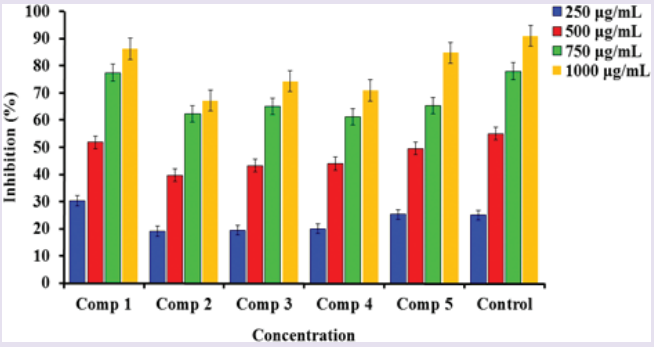

Figure 2: Percentage inhibition of DPPH scavenging activity of synthesized compounds (1-5) in comparison to Vitamin C. n=3. Error bars indicate standard deviation.

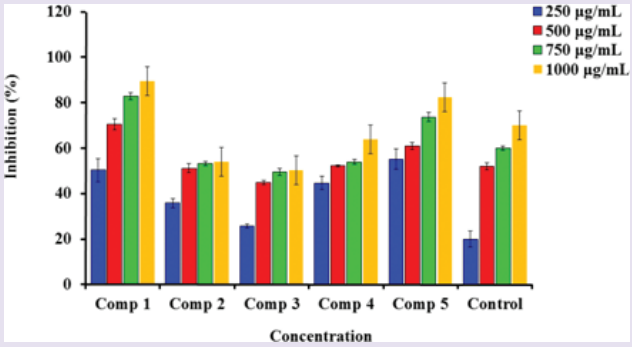

Figure 3: Percentage inhibition of hydrogen peroxide scavenging activity of synthesized compounds (1-5) in comparison to Vitamin C. n=3. Error bars indicate standard deviation.

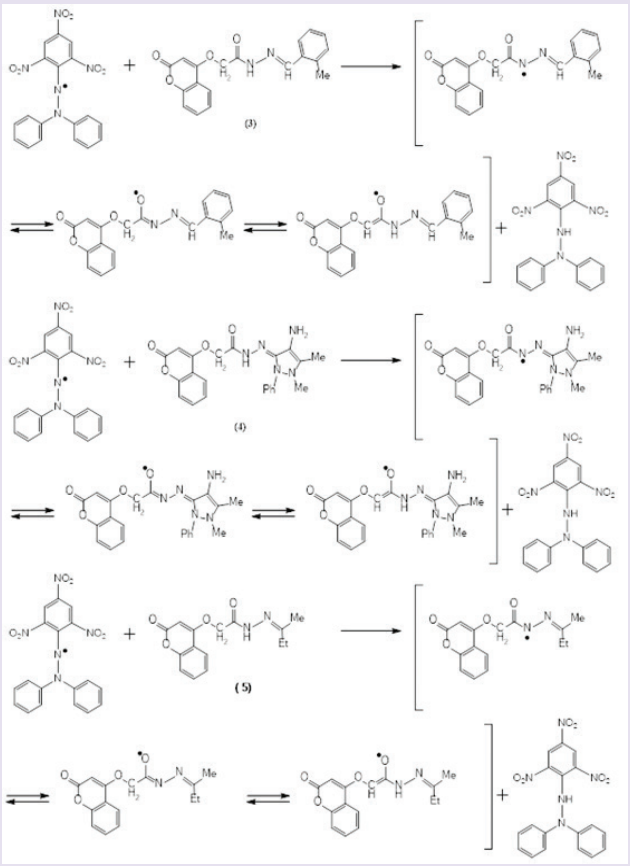

Figure 4: The reaction scheme between DPPH free radicals and compounds 3-5.

assume an important role in the pathogenesis of specific diseases and aging.[28,29] Numerous synthetic cancer prevention agents have presented toxic and/or mutagenic effects; thus, naturally occurring antioxidants have been considered.[30] Synthesized coumarins 1-5 were screened for *in vitro* scavenging activity utilizing DPPH and hydrogen peroxide. These tested coumarins showed high scavenging activity (Figure 2 and 3). Figure 2 showed that the five synthesized compounds (1-5) demonstrated a strong scavenging activity against DPPH. At a very low concentration of 250 µg/mL we observed a concentration dependent decrease in DPPH activity. A very weak inhibitory activity was found in compounds 2 and 4 (19.1 ± 2.5 and 2.0 ± 1.5). The highest concentration was found at 1000 µg/mL (Figure 2). The best percentage scavenging activity was shown by compound 1 (86.2 ± 0.50), followed by compound 5 and 3 (84.8 ± 1.00; and 74.3 ± 2.5). Vitamin C was used as standard drugs with percentage inhibition of 90.00 ± 1.5. The hydrogen-donating activity, measured utilizing DPPH as the hydrogen acceptor, demonstrated that a strong association could be found between the concentration of the coumarin molecule and the rate of inhibition.[31] Using the hydrogen peroxide test, coumarins 1-5 demonstrated their ability to diminish the stable radical.

H_2O_2 scavenging assay

Hydrogen peroxide can be highly reactive when crossing cell membrane and form hydroxyl radical. Figure 3 showed that compounds (1-5) demonstrated a strong scavenging activity against hydrogen peroxide at a very low concentration of 250 µg/mL we observed a concentration dependent decrease in DPPH activity. A very weak inhibitory activity was found in compounds 3 (27.5 ± 1.00). The highest concentration was found at 1000 µg/mL (Figure 3). The best percentage scavenging activity was shown by compound 1 (89.4 ± 1.50), followed by compound 5 (82.4 ± 2.00). Vitamin C was used as standard drugs with percentage inhibition of 70.00 ± 2.5.

Suggested mechanism for compounds 3-5 as an antioxidant

The postulated antioxidant mechanism for synthesized coumarin 3, 4 and 5, as shown in Figure 4, relies on the amide hydrogen atom, which is under the influence of inductive effect. The inductive effect of the carbonyl facilitates the release of hydrogen, resulting in stability of the molecule.[32] Note that coumarins 3-5 have scavenging activitiesdue to the stability of the free radical intermediates of these compounds.[33] An abstraction of a hydrogen atom from amide group may occur easily.[34] The introduction of electron-donating groups, such as amine group, increases the antioxidant activity. In addition, the presence of carbonyl groups, enhances the antioxidant activity of amide. The steric hindrance of amide by a neighboring inert group, nitrogen and carbonyl group, enhances the antioxidant activity of amide.[35,36]

Molecular Modelling Studies

In a pursuit of electronic level understanding of the antioxidant activities for compounds 3-5, the HOMO and the LUMO studies have been carried out using density functional theory (DFT) based quantum chemical descriptors. The calculated HOMO and LUMO energies as in Figure 5, show that charge transfer occurs within the molecules. Figure 5 shows the HOMO and LUMO in electron volt values. A better antioxidant

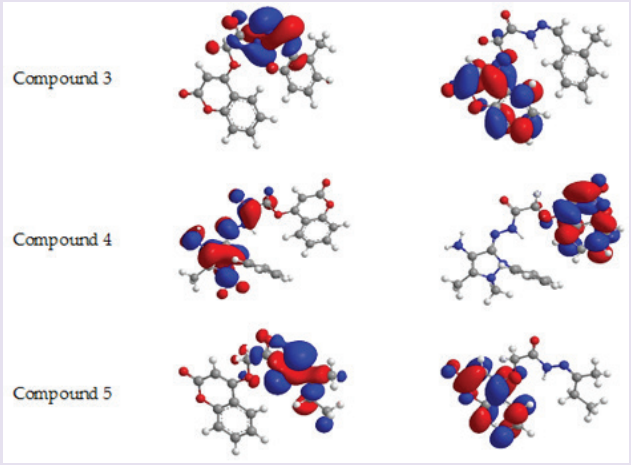

Figure 5: Highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital of compounds 3-5.

Table 1: Electronic Properties of synthesized coumarins

Parameters	Compound 3	Compound 4	Compound 5	Ascorbic acid
Dipole moment Depy	8.7365	11.497	7.397	6.312
Ionization potential (IP) eV	9.526	5.116	10.091	8.669
Electron affinity (EA) eV	3.889	3.11	3.778	0.095
Hardness (η)	2.8185	1.003	3.1565	4.287
Softness (S)	0.177	0.489	0.158	0.116
Electro negativity(μ)	6.7075	2.613	6.9345	4.3775
Electrophilic index (ω)	7.891	3.403	7.617	2.234
E_{HOMO}	-9.526	-5.116	-10.091	-8.669
E_{LUMO}	-3.889	-3.11	-3.778	-0.095
Band gap=E_{HOMO}-E_{LUMO}	5.637	2.006	6. 313	8.564

activity can be established on the basis of these values. Our experimental studies using the DPPH method demonstrate that the scavenging activity of compounds 1 and 5 are higher than that of ascorbic acid because of the electron withdrawing of carbonyl and free radical structure of the former. Theoretical studies demonstrate that HOMO energy is a good indicator of scavenging activity. However, scavenging activity does not depend on LUMO energy. The difference in the antioxidant activity of compounds 3-5, as reflected in the calculated and experimental HOMO values, is often attributed to p-electron delocalization, which leads to the stabilization of radicals obtained after H-abstraction. Therefore, the p-electron delocalization present in compounds 3-5 also exists in the corresponding radical. The electron distribution in HOMO should be examined to understand the relationship between the electron delocalization and the reactivity of the radicals.[37]

For compounds 3-5, HOMO are delocalized over the entire molecules, which corresponds to the orbital containing the unpaired electron. The spin densities of the radicals formed from compounds 3-5 were compared. The more delocalized the spin density in the radical is, the easier the formation of the radical and the lower the formation of compound 2. The spin population appears to be slightly more delocalized for the radicals issued from Compounds 3 and 5 than from compound 4. The HOMO energies of synthetic coumarins (3-5) and ascorbic acid are computed as -9.526 eV, -5.116 eV, -10.091 eV and -8.669 eV respectively. While the LUMO energies of synthetic coumarins (3-5) and ascorbic acid are calculated as -3.889 eV, -3.11 eV, -3.778 eV and 0.095 eV respectively. The energy gap (5.637 eV, 2.006 eV and 6. 313 eV) of synthesized coumarins 3-5 as compared with ascorbic acid (8.564 eV) might be because of its shifted absorption toward blue spectrum. Charge density delocalization over the amide group in the compounds 3-5 detect measurable differences for HOMO and LUMO of these coumarins. We have compared the antioxidant potential of synthesized coumarins and ascorbic acid with band gaps and It was clear that that highest bang gap was for ascorbic acid (control) which was 8.564 eV and the next value for compound 5, and this is highly compatible with experimentally results as in Figure 2. Charge density, reactivity index and bond properties of synthetic coumarins (3-5) and ascorbic acid were calculated depend on dipole moment. R can be calculated with the help of dipole moment of bonds. The values of dipole moment for synthetic coumarins (3-5) and ascorbic acid indicated that synthetic coumarins (3-5) and ascorbic acid are polar molecules and soluble in polar solvents. Ionization potential gives the perception about energy launch of the electron an electron from the molecules and higher values means that the molecules do not lose the electrons easily[38] and in other word the an inverse relationship between antioxidant and ionization potential. The electron affinity can be expressed as the amount of energy released when an electron absorbed by a neutral molecule. The greater electronic affinity of molecules mean to absorb the electrons easily in other word the positive relationship between antioxidant and electron affinity. The chemical hardness is the resistance to charge transfers[39] and the electronegativity is the capacity to attract electrons in a chemical bond. The electrophilic index utilized to found the electrons affinity and measures maximum electron flow between a donor and an acceptor.[40] Table 1 clearly described the chemical potential values. These can also advocate their good antioxidant potential.

CONCLUSION

New coumarin derivatives were successfully synthesized using chemical methods. The characterized of these coumarins were done by using different spectroscopic techniques (FT-IR and NMR) and micro-elemental analysis (CHNS). The antioxidant activity of these coumarins were determined by using DPPH and hydrogen peroxide assays. Results indicated that the new coumarins possess higher scavenging activity than vitamin C. The comparative study of molecular modelling corroborated pharmacological trials, showing a high correlation between calculated HOMO and LUMO energies and antioxidant activity. The availability of these coumarins would also facilitate further investigations of their pharmacological properties.

ACKNOWLEDGMENTS

This study was supported by the University of Technology, Baghdad, Iraq, and Universiti Kebangsaan Malaysia under the DIP-2012-02 Grant.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

A.B.M. and A.A.H.K conceived and designed the experiments; Y.K.A. and D.L.A.performed the experiments; A.A.A. analyzed the data; A.B.M. and A.A.H.K. contributedreagents/materials/analysis tools; A.A.A. wrote the paper.

ABBREVIATION USED

CHNS: Carbon, Hydrogen, Nitrogen, Sulfur; **NMR:** Nuclear magnetic resonance; **FT-IR:** Fourier transform infrared; **HOMO:** Highest occupied molecular orbital; **LUMO:** Lowest unoccupied molecular orbital; **DPPH:** 1-diphenyl-2-picrylhydrazyl; H_2O_2: Hydrogen peroxide.

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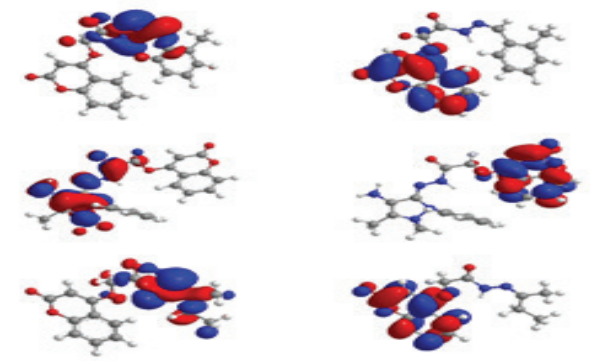
PICTORIAL ABSTRACT



SUMMARY

- Synthesis of coumarin derivatives has been described by simple and efficient protocol.
- Synthesized coumarins were Characterization spectroscopically.
- Synthesized coumarins were screened for antioxidant activities and they showed excellent antimicrobial activity.
- Synthesized antioxidants were studied theoretically by DFT with 3-21 G basis set.
- Theoretical studies for these synthesized antioxidants demonstrated the correlation of scavenging activities and theoretical parameters.