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Synthesis and Structure Elucidation for N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide and Assessment Antibacterial Characteristics

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Abstract: Coumarins are structurally motivating molecules for synthesizing pharmaceutical agents such as anticancer, anticoagulants, and antimicrobial. The structure of the synthesized coumarin derivative (N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide) was elucidated by spectroscopically techniques. The bacterial inhibition efficiency of N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide against selected types of bacteria, namely "*Staphyloccocus aureus*, as Gram-positive bacteria and Gram-negative bacteria *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas*, and *Klebsiella pneumoniae* was determined utilizing a disc diffusion method. The scavenger effectiveness of the N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide was also evaluated through the superoxide-radical method. The N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide has significant inhibitive efficiency against all studied microorganism. The N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide molecules have considerable activity as antioxidant compounds against vitamin C.

Keywords: anticoagulents; superoxide; coumarin; antimicrobial; microorganisim.

1. Introduction

Coumarin derivatives, as natural and/or synthetic, have considerable interest due to their wide medicinal performances. Published studies demonstrate that coumarins are the most significant heterocyclic compounds with broad pharmacological activity spectra such as antitumor, anticoagulant, antimicrobial, scavengers, and anti-inflammatory [1-6]. The search for antibiotics has, for many years, created the field of intensive research in the field of drug chemistry due to the resistance that the microorganisms have created against known antibacterial or antifungal agents [6-9]. Antibacterial and antifungal agents are some of the most important factors in fighting microorganisms. It is known that bacteria and fungi cause infection and disease in humans [10]. Coumarins have a wide range of structural modifications [11-13] and can serve as molecular blocks for drug development. Coumarin derivatives are also considered potential antibacterial and antifungal agents, apart from antioxidants [14-17]. Continuing our investigations on the newly synthesized coumarin derivatives [18-41], we found it is interesting to synthesis a Schiff base compound derived from coumarin, namely "N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide" as an efficient antimicrobial

agent. The scavenging activity of the newly synthesized compound was evaluated using the superoxide-radical technique.

2. Materials and Methods

2.1. General.

Starting materials and solvents were purchased from Merck and Fluka. No further purifications for the purchased chemicals. Fourier-transform infrared (FTIR) spectra were conducted by Spectrophotometer-8300 Shimadzu-FTIR. UV-Vis spectra were performed on spectrophotometer-160A Shimadzu-UV-VIS. Nuclear Magnetic Resonance (NMR) spectra were performed on Bruker-spectrometer-DPX 300MHz.Tetramethylsilane (YMS) was used as an internal standard. Carbon/Hydrogen/Nitrogen analyzers (CHN analysis) was achieved on a Carlo CHN-5500, Erba analyzer.

2.2. Synthesis of N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide.

A dry benzene solution which has equimolar quantities of *N'-benzylidene-2-coumarin-4-oxyacetohydrazide* and 2-mercaptoethanoic acid was refluxed for 20 h. Water was used to wash the filtered yield. The dichloromethane was used to recrystallized the dried product. Yield 70 percentage and the melting point was 261.0 celsius; Proton-Nuclear magnetic resonance spectroscopy (Chloroform-d):δ 7.320, δ 7.441, δ 7.92 (s, 1H) for aromatic protons; δ 5.82 and 4.77 (s, 1H) for vinyl proton; δ 5.110 (s, 2H) for methylene oxide proton; δ 8.98 (s, 1H) for amino proton, δ 5.40 (s, 1H) for S-CH, and δ 3.83 2(H) for methylene protons, δ (s, 2H). ^{13}C-Nuclear magnetic resonance spectroscopy: 33.2, 65.4, 77.6, 101.4, 110.1, 112.9, 114.4, 117.1, 120.1, 124.3,126.1, 127.8, 128.2, 159.8,154.1, 167.8,169.0 and 171. Fourier-transform is infrared in cm^{-1}: 1689 and 1667 for carbonyl of the amide group, 1731 carbonyl of lactone group, 2921 aliphatic hydrogen, 3087.7 aromatic hydrogens, 3198.7 amino group, 1632.8 cm^{-1} (aromatic carbon-carbon bonds; Calculated/ Theoretical CHN analysis for $C_{20}H_{16}N_2O_5S$: C 60.41/60.60%, H 3.91/4.07%, N 6.88/7.07%.

2.3. Antibacterial activity.

The synthesized compound N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide was screened *in vitro* for its antimicrobial efficiency against gram-positive bacteria (*Staphylococcus aureus)* and gram-negative bacteria *(Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa)* by disc diffusion techniques [42]. The method of disc diffusion was utilized due to its Comfortable, worth, and inexpensive. The N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide in dimethyl-formamide as a stock solution has been prepared. The stock solution was diluted by dimethylformamide to prepare various concentrations (50-500 µg/ml). For each dilution, dimethylformamide was utilized as control. The nutrient agar was used for subcultured the bacteria. Chloramphenicol (positive control) was used as a standard antimicrobial drug and compared with the synthesized compound. Whatman filter papers (no. 4) discs of 6 mm diameter have been sterilized and immersed in the desired concentration of the N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide. The socked discs were placed in Petri-dishes, having the nutrient agar with the selected types of bacteria individually and incubated for 24 h., at 37°C. The antibacterial efficiencies were evaluated as mean ± SD.

2.4. Antioxidant activity.

The antioxidant efficiency measurements were conducted by the superoxide anion technique, according to Chang 2002 [43]. Nitro blue tetrazolium solution (1 mL) which was prepared by dissolve Nitroblue tetrazolium in phosphate buffer and various concentrations (50, 100, 150, 200, 300, 500 µg/ml) of N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide. Added 100 µl to the above solution. The incubation was conducted for 5 min., at 25°C, and the absorbance was measure at 560 nm. The comparing of N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide has been done with the standard (vitamin C).

2.5. Statistical analysis.

ANOVA technique was used to evaluate the statistical analysis. $P < 0.05$ was the significant value, and $P < 0.001$ the excellent one.

3. Results and Discussion

3.1. Chemistry.

N'-benzylidene-2-coumarin-4-oxyacetohydrazide was refluxed with 2-mercaptoethanoic acid to synthesis the target N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide as demonstrated in Figure 1. The spectrum of FTIR for the synthesized compound N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide exhibited significant bands at 1689 and 1667 for carbonyl of amide group and 1731 carbonyl of lactone group. The NHM spectrum showed the singlate for single protons at 5.82 ppm and 4.77 ppm for the vinyl group and also siglate at 5.110 ppm for two protons of methylene oxide group.

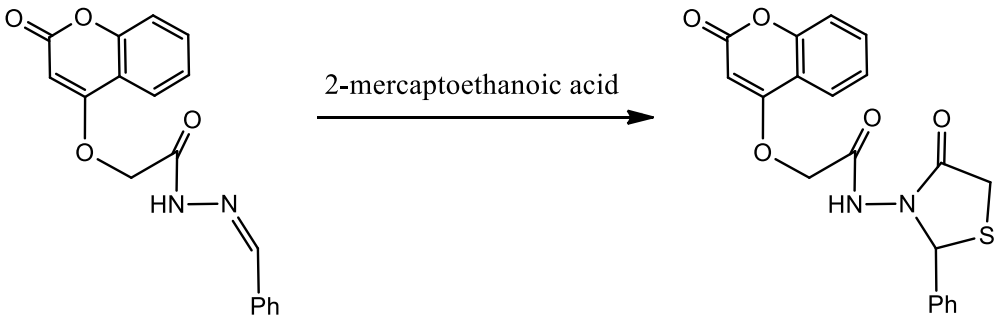
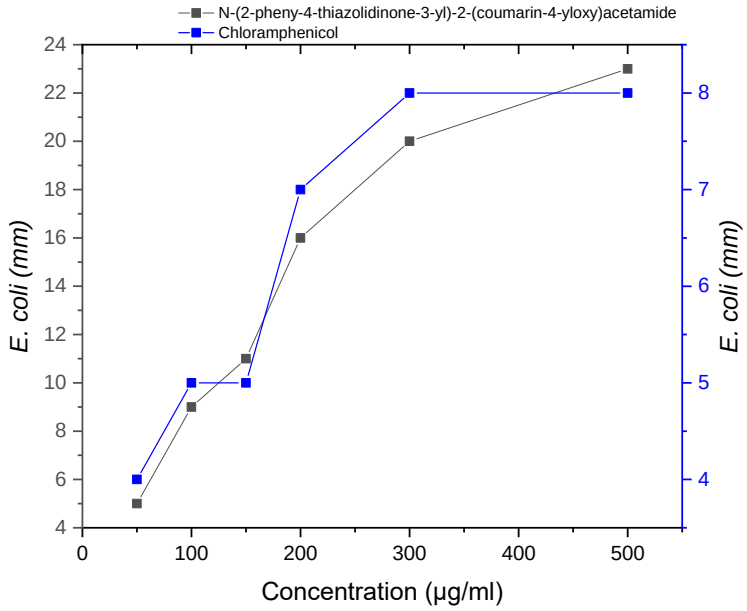
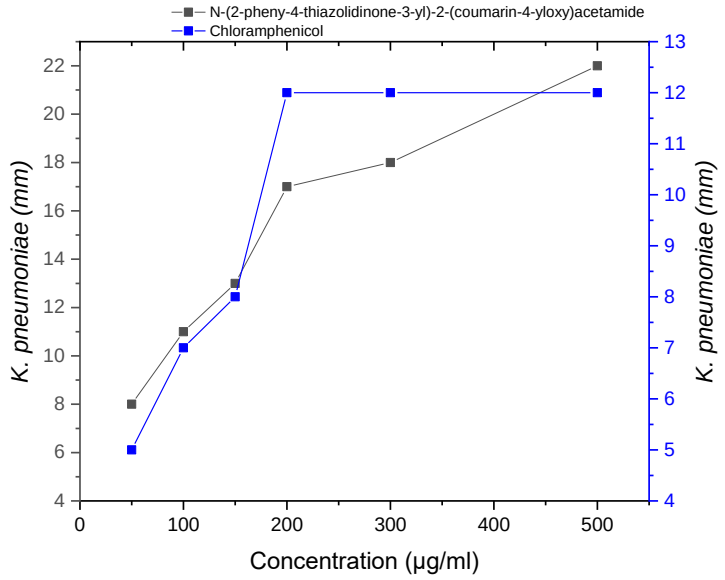


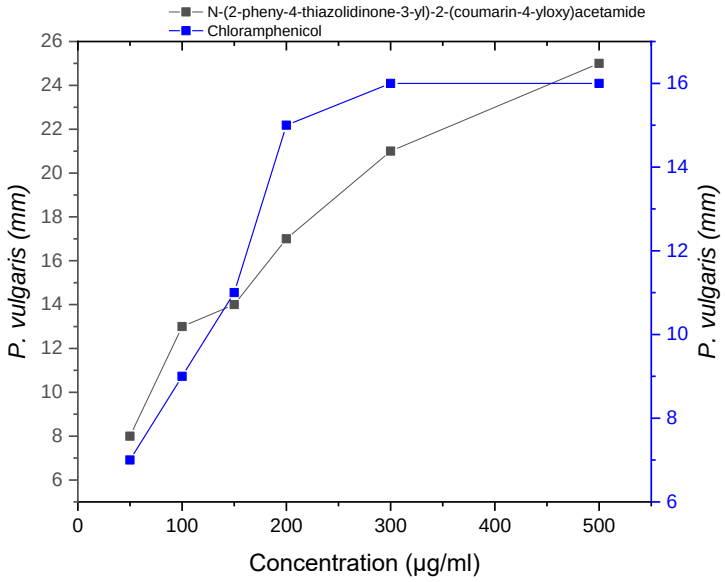
Figure 1. Synthesis of N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide.

3.2. Pharmacology: in vitro antibacterial studies.

The results of the disk diffusion method are shown in Figure 2. The experiments were repeated three times, and the standard error was ±0.1 mm. Dimethylformamide was the negative control and the solvent for the *N*-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide, which was used in this research, while chloramphenicol was the positive standard which was used in all the experiments. The results of these experiments reveal that N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide has significant efficiency against the human pathogenic tested gram-positive bacteria and human pathogenic tested gram-negative bacteria, as shown in Figure 2.


N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide
Chloramphenicol
E. coli (mm)
E. coli (mm)
Concentration (µg/ml)


N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide
Chloramphenicol
K. pneumoniae (mm)
K. pneumoniae (mm)
Concentration (µg/ml)


N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide
Chloramphenicol
P. vulgaris (mm)
P. vulgaris (mm)
Concentration (µg/ml)

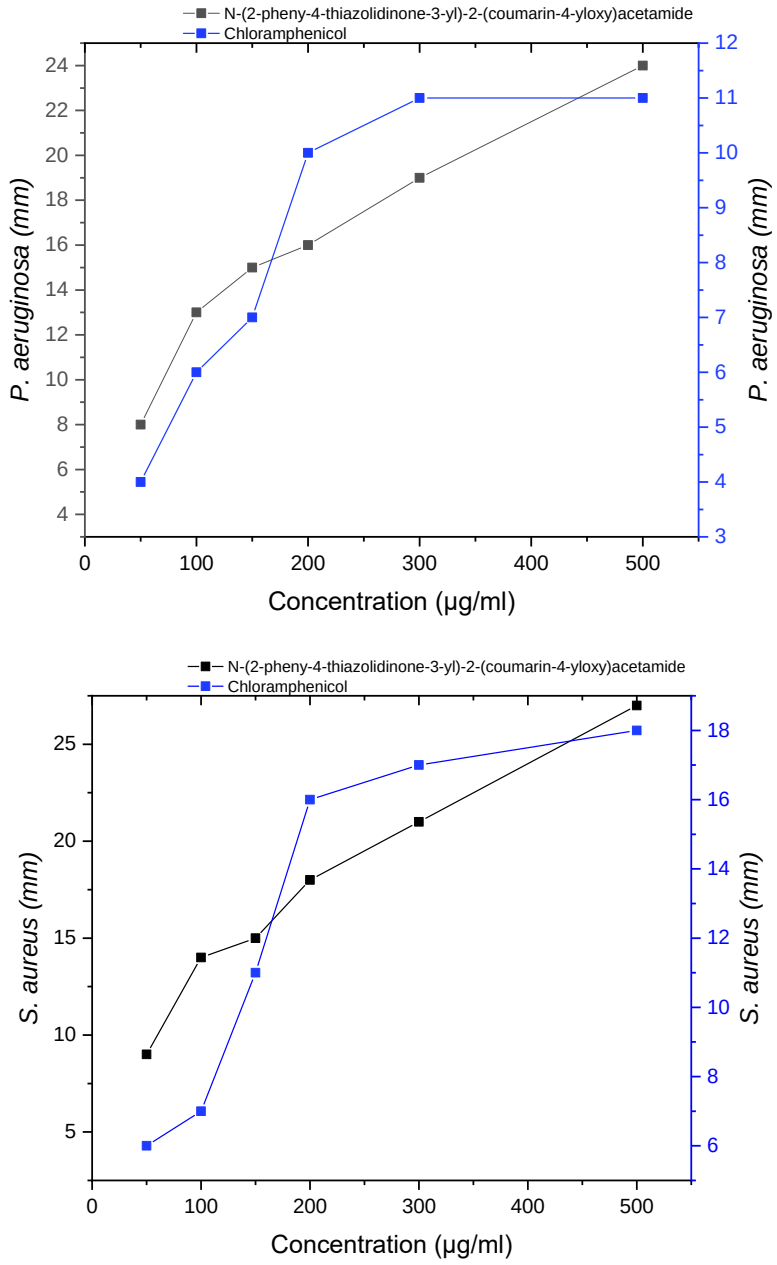


Figure 2. Antibacterial efficiencies of different concentrations N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide against chloramphenicol.

According to inhibition zone findings, the N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide demonstrates significant efficiency versus gram-positive *Staphylococcus aureus* than the efficiency against gram-negative. The efficiency has increased with an increase in the concentration of N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide.

The nitrogen atoms were correlated with an increased inhibition efficiency for *Staphylococcus aureus*. N-(2-pheny-4-thiazolidinone-3-yl) -2-(coumarin-4-yloxy) acetamide demonstrated significant antimicrobial performance against *Proteus vulgaris*. N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide as the investigated compound was a lower efficiency than the control chloramphenicol.

3.3. Antioxidant activity.

There is a growing interest in scavengers, specifically in these supposed to prevent the presumed deleterious outcomes of free radicals in the human physique and to stop the deterioration of fats and other ingredients of food stuffs. In each case, there is a choice for scavengers from herbal rather than from synthetic sources. As expanded scavenger fame helps limit the oxidative damage and delays or prevents pathological changes, a viable antioxidant remedy has to be blanketed both as natural free-radical-scavenging antioxidant enzymes or as an agent that is successful in augmenting the recreation of scavenger enzymes [44].

Superoxide anion radical (O2-) is an important factor in the killing of bacteria- [45]. Indicating the superoxide exhaustion within the reaction mixtures. Figure 3 shows the inhibitive performance of superoxide radical using the concentrations 50, 100, 150, 200, 300, 500 µg/ml of N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide compared with vitamin C. The concentration of the N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide has a significant level ($P < 0.001$) of superoxide radical antioxidant activity as compared with vitamin C.

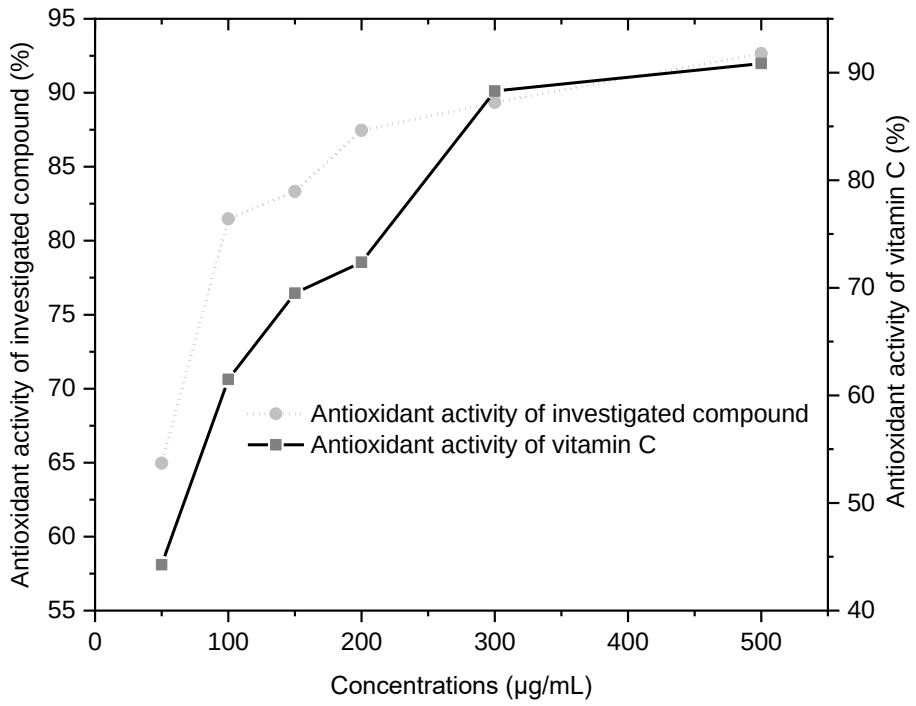


Figure 3. Scavenging efficiencies of different concentrations of *N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide* aginst vitamin C.

4. Conclusions

In conclusion, N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide was synthesized by reacting coumarin-Schiff base with 2-mercaptoethanoic acid. The chemical structure of N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide was confirmed by various spectroscopic techniques, and the antimicrobial and antioxidant activities were assessed. N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide was examined against a slected types of human pathogenic strains of Gram-positive (*Staphyloccocus aureus*) and Gram-negative (E. *coli*, *Proteus vulgaris*, *Pseudomonas*, and *Klebsiella pneumoniae*) bacteria. N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide a significant growth inhibition efficient against selected types of bacteria and

is promising to act as a considerable antibacterial agent as compared with chloramphenicol. Antioxidant results also revealed that N-(2-pheny-4-thiazolidinone-3-yl)-2-(coumarin-4-yloxy)acetamide demonstrates significant efficiency against superoxide.

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Conflicts of Interest

The authors declare no conflict of interest.

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