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Synthesis and Evaluation of the Biological Activities of Some Derivatives of 4-Hydroxycoumarin

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Abstract This article presents the synthesis and antioxidant activities of a series of new C-acylated hydroxycoumarins. The compounds were synthesized and characterized using various spectral methods, including Infrared Spectroscopy, Nuclear Magnetic Resonance, and Mass Spectrometry. The biological activities were determined using the DPPH method by assessing the percentage inhibition of the different synthesized compounds. Four new molecules were successfully synthesized: 3-(4-methylbenzoyl)-4-hydroxycoumarin (a), 3-(4-chlorobenzoyl)-4-hydroxycoumarin (b), 3-(4-nitrobenzoyl)-4-hydroxycoumarin (c), and 3-(3,5-dinitrobenzoyl)-4-hydroxycoumarin (d). The percentage of inhibition of the synthesized compounds varies from 52.13% ± 1.04 to 78.13% ± 0.60. Compound (d) is the most active with an inhibition percentage of 78.13% ± 0.60, followed by compound (c) with an inhibition percentage of 72.68% ± 0.37, then compound (b) with an inhibition percentage of 67.38% ± 0.30, and finally compound (a) with an inhibition percentage of 52.13% ± 1.04. These compounds may exhibit interesting biological activities due to their percentage of inhibition. Therefore, these coumarin-derived compounds could be good antioxidant candidates.

Keywords: *4-hydroxycoumarin, C-acylated derivatives, Nuclear Magnetic Resonance, antioxidant activity*

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1. Introduction

Coumarins are a class of bicyclic aromatic compounds derived from coumarinic acid [1]. Found in many plants, they are recognized for their distinctive chemical structure, which combines a benzene ring with a lactone ring [2]. These molecules are widely studied due to their various biological and pharmacological properties, such as antibacterial [3], anticancer [4], anticoagulant [5], antioxidant [6], anti-HIV [7], antimicrobial, and antifungal [8] properties, among others. Structural modification of these compounds through acylation can potentially enhance their pharmacological effectiveness. Acylated derivatives may influence molecular interactions, solubility, and bioavailability of the compounds, making their study relevant for the development of new therapeutic agents. In this work, we propose synthesis methods, characterization, and evaluation of the antioxidant properties using the DPPH method for new derivatives of coumarins. The compounds were synthesized using the method of J. Schnekenburger, improved by A. Saba [9,10], followed by that of Fries [11], starting from 4-hydroxycoumarin and acyl chloride. The structures of the obtained molecules were determined by Infrared Spectroscopy (IR) and Proton Nuclear Magnetic Resonance (NMR) [12].

2. Methodology

2.1. Synthesis

The C-acylated derivatives were synthesized in two steps from 4-hydroxycoumarin.

First step: synthesis of intermediate compounds by O-acylation (**Figure 1**).

Reaction scheme: 4-Hydroxycoumarin reacts with para-substituted benzoyl chlorides (R: para-methyl (p-CH_3), para-tert-butyl (p-$C(CH_3)_3$), para-chloro (p-Cl),

and para-nitro (p-NO_2)) in tetrahydrofuran (THF) in the presence of triethylamine to form O-acylated compounds as shown below.

2.1.1. Procedure First Step

In a round-bottom flask containing 30 ml of THF, one molar equivalent of benzoyl chloride and four molar equivalents of triethylamine are added, respectively. After 15 minutes of moderate stirring, one molar equivalent of 4-hydroxycoumarin is added in small portions over 30 minutes while stirring continuously. After four hours of reflux, the resulting solution is poured into a separating funnel containing about 40 ml of chloroform and then acidified with 10% diluted hydrochloric acid. The organic phase is extracted and washed with distilled water until a neutral pH is reached. After drying and filtration, the solvent is removed under vacuum. The obtained O-acylated derivative is washed with hexane and recrystallized in a chloroform-hexane solvent mixture.

Second step: synthesis of C-acylated compounds by Fries rearrangement (**Figure 2**).

Reaction scheme:

The O-acylated compound obtained after the first step undergoes rearrangement in DMF in the presence of a Lewis acid ($AlCl_3$) to yield the C-acylated compound after hydrolysis. The reaction scheme is as follows:

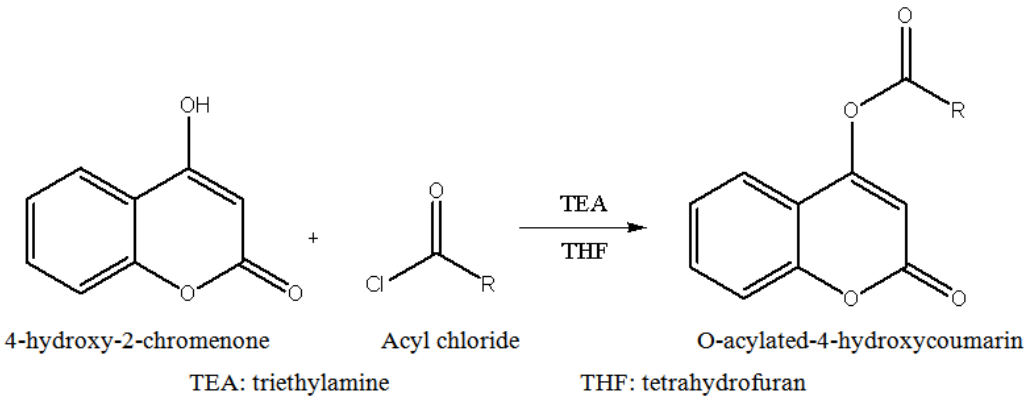


Figure 1. Obtaining coumarin-4-yl benzoate

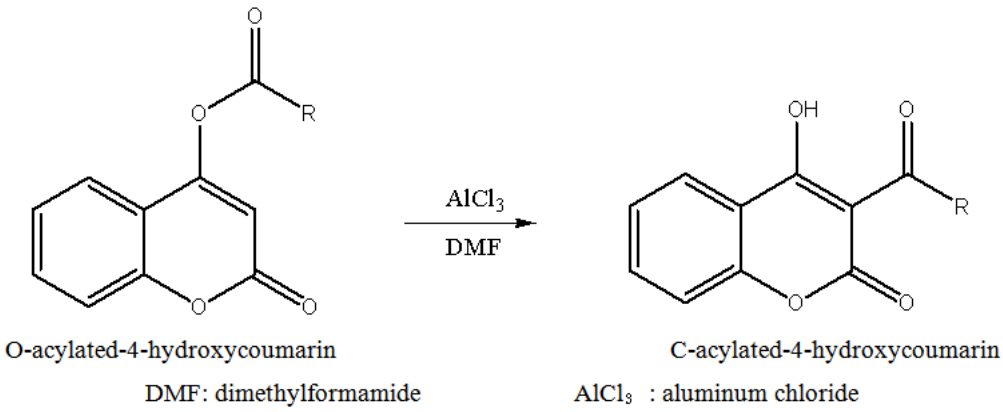


Figure 2. Obtaining 3-benzoyl-4-hydroxycoumarin

2.1.2. Procedure Second Step

Add 2.24 mmol of the synthesized coumarin-3-yl carboxylate to a round-bottom flask containing 30 ml of DMF; under gentle stirring, add 0.45 g (1.5 molar equivalents) of aluminum chloride. The reaction mixture is heated to reflux for 3 hours. The resulting solution is poured into a separating funnel containing 40 ml of chloroform and washed three times with distilled water. The organic phase is extracted and dried with $MgSO_4$. After filtration, the solvent is removed under vacuum. The crude product obtained is washed with hexane and recrystallized in a chloroform-hexane solvent mixture (1/3, V/V).

2.2. Evaluation of Antioxidant Activity

The antiradical activity of the obtained C-acylated compounds was evaluated using the DPPH free radical method (DPPH) described in the literature [13] and adapted for microplates. DPPH radicals have a maximum absorption at 515 nm, which decreases upon reduction by an antioxidant compound. A solution of DPPH radical in methanol (6 x 10^{-5} M) was prepared just before use by dissolving 2.36 mg of DPPH in 100 mL of methanol. A range of solutions prepared from the synthetic compounds and standards with concentrations ranging from 1 mg/mL to 0.0079 mg/mL was prepared. To 100 µL of methanolic solution of each sample solution, 100 µL of DPPH radical solution was added. The samples were incubated for 20 minutes at room temperature, and then the decrease in absorbance at 515 nm was measured (AE). All measurements were performed in triplicate. Trolox was used as a reference. The inhibition rate of the radical was calculated using the following formula:

$$\% \text{ DPPH inhibition} = [(AB - AE) / AB] \times 100$$

3. Results and Discussion

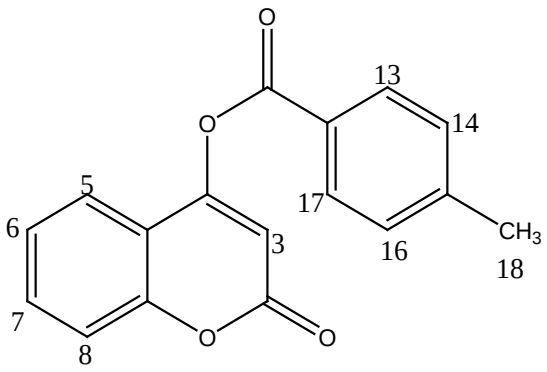
3.1. Characterization of the Synthesized Compounds

The IR spectrum of the reagent (4-hydroxycoumarin) shows all the absorption bands due to the coumarin core and the alcohol function. We primarily observe the C=O and C-O bands of the lactone function at 1684 and 1116 cm^{-1}, respectively. The alcohol function is represented by the absorption band around 3350 cm^{-1}, while the absorption bands observed at 1600 cm^{-1} are attributed to the C=C double bond, and those observed at 3040 cm^{-1} are attributed to the aromatic C-H bond.

In the NMR analysis, we observe a strongly deshielded proton at 12.47 ppm, which we attribute to the proton of the alcohol function of 4-hydroxycoumarin. A singlet is also observed at 6.84 ppm, characteristic of the proton of the alcohol function.

The transition from 4-hydroxycoumarin to the O-acylated intermediate products is reflected in the IR spectrum by the disappearance of the O-H bond at 3350 cm^{-1} and, consequently, the disappearance of the characteristic signal at 12.47 ppm in the proton NMR spectra. These changes are observed in all the spectra of our intermediate products.

The structures of the obtained C-acylated compounds were determined by infrared spectroscopy and by proton and carbon nuclear magnetic resonance (NMR ^{1}H and ^{13}C) [12,14]



➢ **3-(4-methylbenzoyl)-4-hydroxycoumarin (a):**
Yellow solid, yield: 83%, m.p. 191-194°C.

IR (cm^{-1}): 3373 (O-H alcohol) 1154; (C-O alcohol); 1681 (C=O lactone); 1115 (C-O lactone); 1652 (C=O ketone); 1601 (C=C aromatic) 3035 (C-H aromatic); 2901 (C-H aliphatic).

13C-NMR (400 MHz, CDCl3): 27.51 (C-18) 99.59 (C-3); 110.6 (C-10); 114.30 (C-6); 116.72 (C-8); 125.28 (C-5); 121.02 (C-7); 126.51 (C-14 et C-16); 128.63 (C-13 et C-17) ; 137.24 (C-12); 145.15 (C-15); 153.69 (C-9); 158.93 (C-2); 169.96 (C-4); 189.16 (C-11**).**

DEPT 135°: 27.51 (C-18); 114.30 (C-6); 116.72 (C-8); 125.28 (C-5); 121.02 (C-7); 126.51 (C-14 et C-16); 128.63 (C-13 et C-17)**.**

1H-NMR (400 MHz, CDCl3): 7.65 (d, 1H, J = 6.4 Hz, H-5); 7.46 (m, 2H, H-6 et H-8); 7.58 (t, 1H, J = 6.4 Hz, H-7); 7.79 (d, 2H, J = 9.6 Hz H-13 et H-17); 7.71 (d, 2H, J = 9.6 Hz, H-14 et H-16); 2.36 (s, 1H, H-18); 14.02 (s, 1H, H).

➢ **3-(4-chlorobenzoyl)-4-hydroxycoumarin (b):**
Yellow solid, yield: 72%, **m.p.** 235-238°C**.**

IR (cm^{-1}): 3371 (O-H alcohol) 1155; (C-O alcohol); 1684 (C=O lactone); 1116 (C-O lactone); 1652 (C=O ketone); 1602 (C=C aromatic) 3032 (C-H aromatic); 716 (C-Cl).

13C-NMR (400 MHz, CDCl3): 98.40 (C-3); 109.10 (C-10); 114.35 (C-6); 116.72 (C-8); 125.27 (C-7); 126.53 (C-5); 128.63 (C-14 et C-16); 131.74 (C-13 et C-17); 136.89 (C-12); 139.38 (C-15); 149.43 (C-9); 159.20 (C-2); 171.48 (C-4); 190.11 (C-11**).**

DEPT 135° : 114.35 (C-6); 116.72 (C-8); 125.27 (C-7); 126.53 (C-5); 128.63 (C-14 et C-16); 131.74 (C-13 et C-17)**.**

1H-NMR (400 MHz, CDCl3) : 7.82 (d, 1H, J = 4 Hz, H-5); 7.35 (m, 2H, H-6 et H-8); 7.64 (t, J = 4Hz, 1H, H-7); 8.09 (d, 2H, J = 8 Hz H-13 et H-17); 7.94 (d, 2H, J = 10 Hz, H-14 et H-16); 14.51 (s, 1H, H).

➢ **3-(4-nitrobenzoyl)-4-hydroxycoumarin (c):**
Yellow solid, yield: 80%, **m.p.** 263-265°C**.**

IR (cm^{-1}): 3372 (O-H alcohol) 1155; (C-O alcohol); 1684 (C=O lactone); 1115 (C-O lactone); 1652 (C=O ketone); 1601 (C=C aromatic) 3035 (C-H aromatic); 1349 ($C-NO_2$); 1524 (N-O).

13C-NMR (400 MHz, CDCl3) : 99.31(C-3); 110.33 (C-10); 114.31 (C-6); 116.73 (C-8); 123.75 (C-7); 125.28 (C-5); 126.84 (C-14 et C-16); 128.98 (C-13 et C-17); 137.80 (C-12); 151.28 (C-15); 146.05 (C-9); 156.19 (C-2); 169.95 (C-4); 191.02 (C-11**).**

DEPT 135° : 114.31 (C-6); 116.73 (C-8); 123.75 (C-7); 125.28 (C-5); 126.84 (C-14 et C-16); 128.98 (C-13 et C-17)**.**

1H-NMR (400 MHz, CDCl3) : 7.91 (d, 1H, J = 6.4 Hz, H-5); 7.40 (m, 1H, H-6 et H-8); 7.70 (t, 1H, J = 6.4 Hz, H-7); 8.42 (d, 2H, J = 6.4 Hz H-13 et H-17); 8.29 (d, 2H, J = 6.4 Hz, H-14 et H-16); 14.62 (s, 1H, H).

➢ **3-(3,5-dinitrobenzoyl)-4-hydroxycoumarin (d):**
Yellow solid, yield: 78%, **m.p.** 286-289°C

IR (cm^{-1}): 3370 (O-H alcohol) 1155; (C-O alcohol); 1689 (C=O lactone); 1155 (C-O lactone); 1652 (C=O ketone); 1602 (C=C aromatic) 3035 (C-H aromatic); 1349 ($C-NO_2$); 1525 (N-O).

13C-NMR (400 MHz, CDCl3): 99,04 (C-3); 109,43 (C-10); 114,60 (C-6); 125,27 (C-8); 126,84 (C-7); 128,63 (C-5); 147,91 (C-14 et C-16); 131,75 (C-13 et C-17); 138,76 (C-12); 116,72 (C-15); 149,43 (C-9); 154,65 (C-2); 168,98 (C-4); 191.30 (C-11).

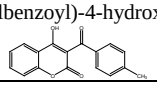
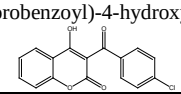
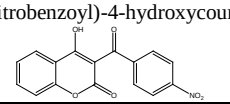
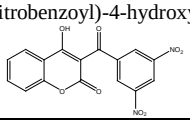
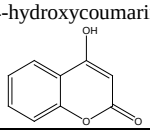
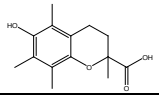
DEPT 135°: 114,60 (C-6); 125,27 (C-8); 126,84 (C-7); 128,63 (C-5); 131,75 (C-13 et C-17).

1H-NMR (400 MHz, CDCl3): 8,06 (d, 1H, J = 9,8 Hz, $H-_5$); 7.45 (t, 1H, J = 9.6Hz, $H-_6$); 7.65 (t, 1H, J = 9,6 Hz, $H-_7$); 9.11 (s, 2H, $H-_{13}$ et $H-_{17}$); 9.22 (s, H, $H-_{15}$); 14.65 (s, 1H, OH).

3.2. Antioxidant Activities by DPPH

The results obtained for each compound, as well as the ratio of the inhibition percentages of 4-hydroxycoumarin compared to the acylated derivatives, are summarized in the following Table 1:

Table 1. Percentage of inhibition of the compounds

Sampl	% inh DPPH	Ratio % inh DPPH subs/sampl	Name Structure
a	52,13 ±1,04	1,9	3-(4-methylbenzoyl)-4-hydroxycoumarin 
b	67,38 ±0,30	1,5	3-(4-chlorobenzoyl)-4-hydroxycoumarin 
c	72,68 ±0,37	1,3	3-(4-nitrobenzoyl)-4-hydroxycoumarin 
d	78,13 ±0,60	1,3	3-(3,5-dinitrobenzoyl)-4-hydroxycoumarin 
Substrate	97,99 ± 0,13	1	4-hydroxycoumarin 
Trolox	98,41 ± 0,15	1	Trolox 

DPPH: 2,2-diphenyl-1-picrylhydrazyl; Subs: Substrate

The percentage of inhibition of the synthesized compounds varies from 52.13% ± 1.04 to 78.13% ± 0.60. Trolox used as a control shows an inhibition percentage of 98.41% ± 0.15. In comparison to Trolox, compound (d) is the most active with an inhibition percentage of 78.13% ± 0.60, followed by compound (c) with an inhibition percentage of 72.68% ± 0.37, then compound (b) with an inhibition percentage of 67.38% ± 0.30, and finally compound (a) with an inhibition percentage of 52.13% ± 1.04.

The percentage of inhibition of 4-hydroxycoumarin was also evaluated, and a value of 97.99% ± 0.13 was obtained, a result very close to that of the Trolox control.

By comparing the percentage of inhibition of 4-hydroxycoumarin with the synthesized compounds through the calculation of the inhibition percentage ratio, it appears that the percentage of inhibition of 4-hydroxycoumarin is 1.3 times that of compounds (d) and (c), about 1.5 times that of compound (b), and 1.9 times that of compound (a). From these analyses, the substrate, which is 4-hydroxycoumarin, is more active than the derived compounds. However, it should be noted that these derivatives also exhibit interesting activities due to their percentage of inhibition. Therefore, these derived compounds could be good antioxidant candidates.

4. Conclusion

The results obtained could contribute to a better understanding of coumarin derivatives and open the way for new synthesis strategies. This study successfully synthesized four C-acylated derivatives of 4-hydroxycoumarin:

3-(4-methylbenzoyl)-4-hydroxycoumarin,

3-(4-chlorobenzoyl)-4-hydroxycoumarin,

3-(4-nitrobenzoyl)-4-hydroxycoumarin, and 3-(3,5-dinitrobenzoyl)-4-hydroxycoumarin. Each compound exhibits distinct structural characteristics. The evaluation of their biological activities revealed encouraging results, highlighting the ability of these compounds to exert significant antioxidant effects.

In summary, this work highlights the potential of C-acylated derivatives of 4-hydroxycoumarin as promising candidates for the development of new therapeutic agents. Further studies, including in vivo trials and investigations into toxicity, will be essential to validate these results and further explore their clinical applications.

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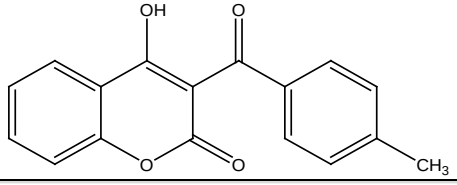
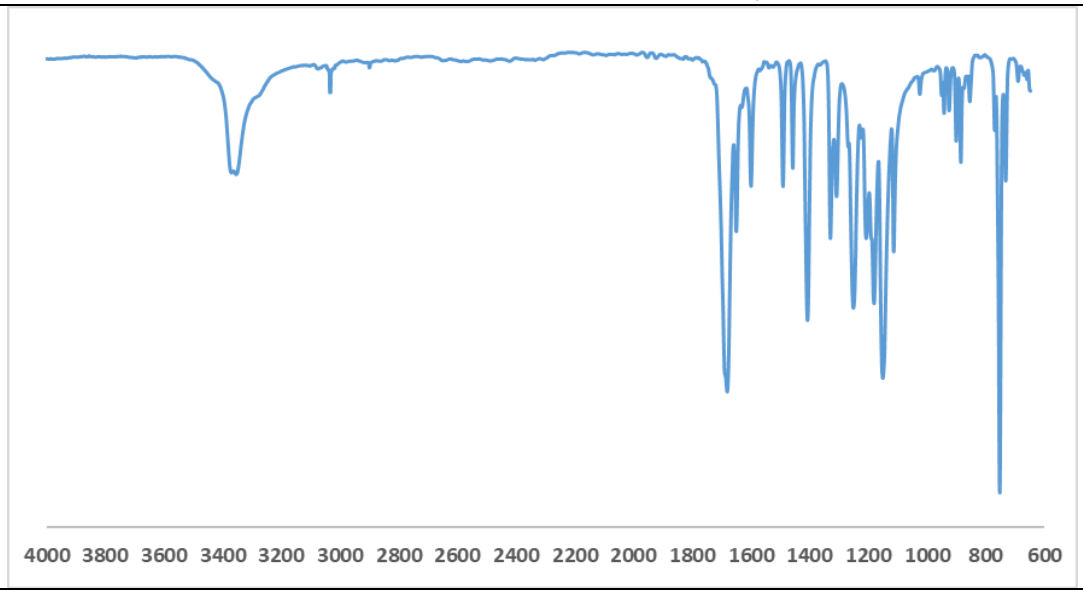
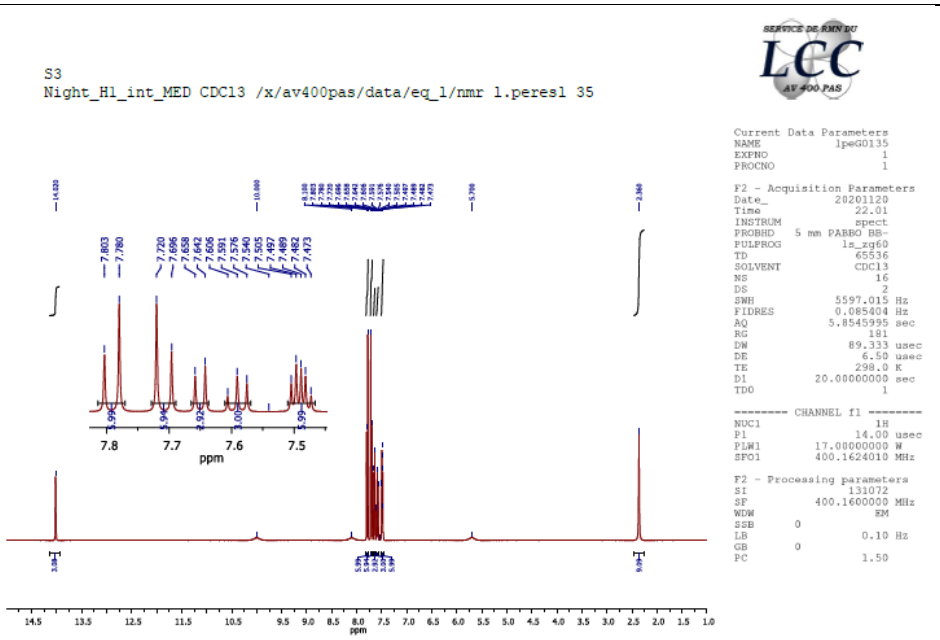
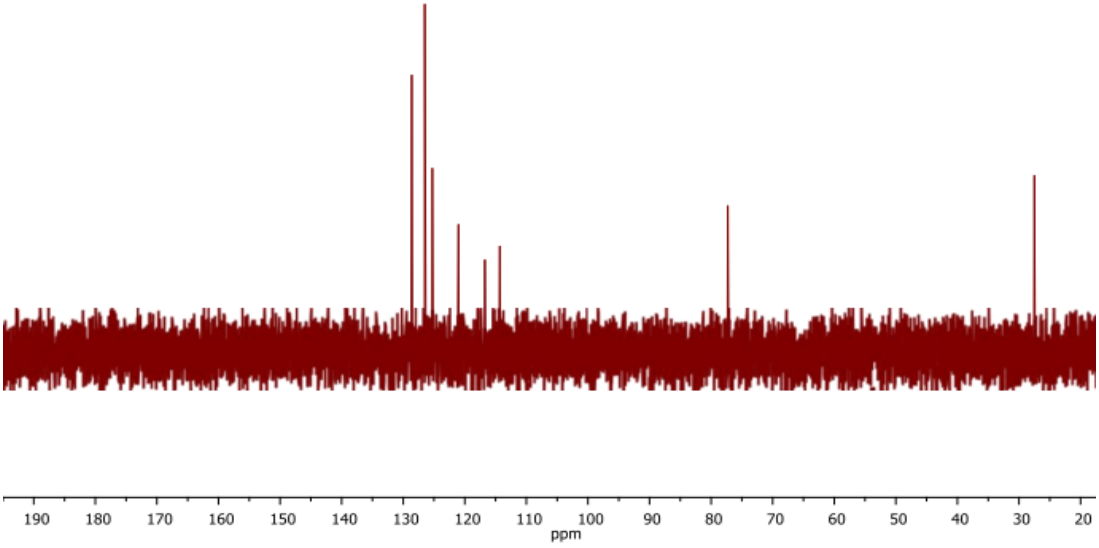
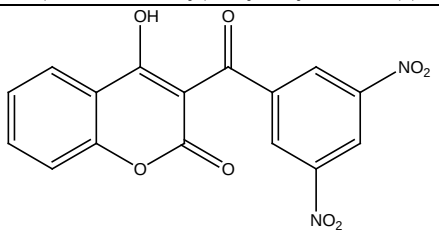
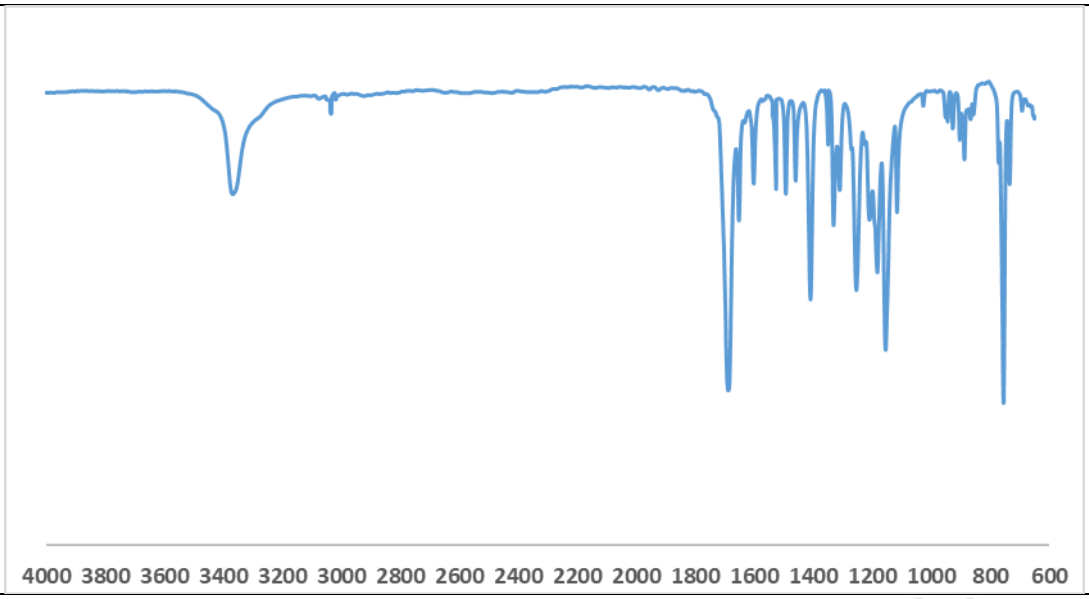
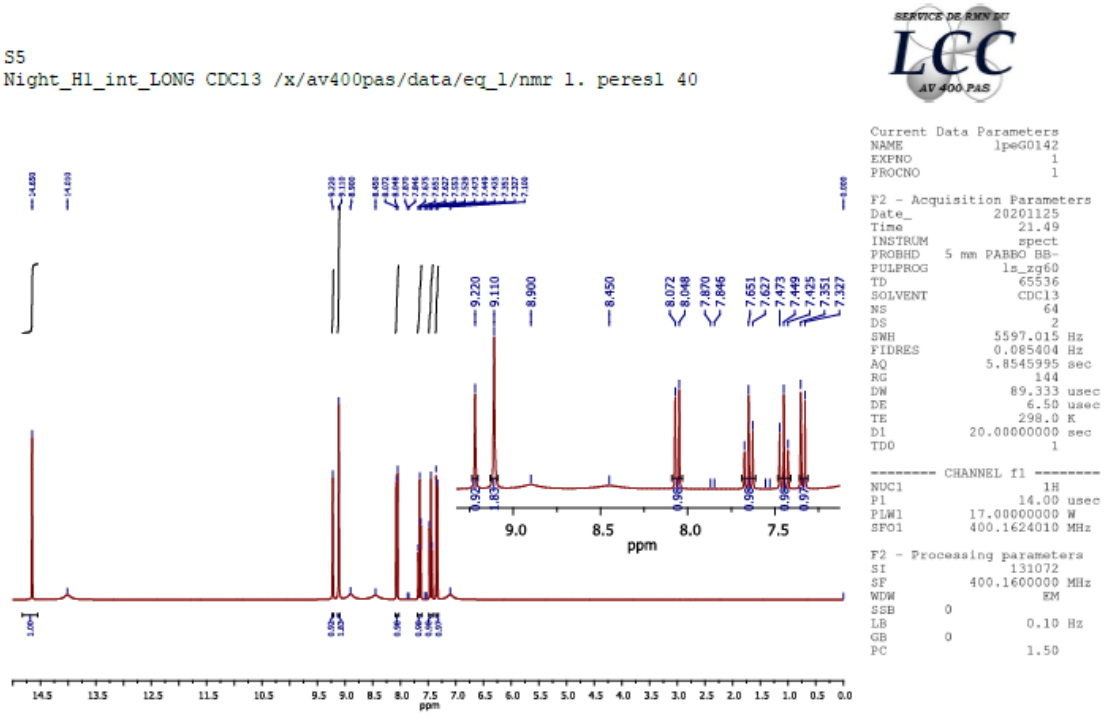
Attachments

You will find the IR and NMR spectra of (a) and (d)

compounds	3-(4-methylbenzoyl)-4-hydroxycoumarin (a)
Structure	
IR spectra	

NMR spectra: ^{1}H	
NMR spectra: ^{13}C DEPT	

compounds	3-(3,5-dinitrobenzoyl)-4-hydroxycoumarin (d)
Structure	

IR spectra	
NMR spectra: ^{1}H	

NMR spectra: ^{13}C DEPT