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One-pot, simple and efficient synthesis of novel bioactive 4-aryl-1,2-dihydro-6-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxopyridin-3-carbonitriles via multi-component approach

Ichraf Slimani [a], Salwa Hamzaoui [a], Lamjed Mansour [b,c], Abdel Halim Harrath [b], Naceur Hamdi [a,*]

[a] Research Laboratory of Environmental Sciences and Technologies (LR16ES09), Higher Institute of Environmental Sciences and Technology, University of Carthage, Hammam-Lif, Tunisia
[b] Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[c] Laboratoiry of Biodiversity, Parasitology and Ecology of Aquatic Systems, Departement of Biology, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia

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ABSTRACT

Quantitative multicomponent cyclocondensation of 3-acetyl-4-hydroxycoumarine with ethyl cyanoacetate and aryldehydes has been carried out in ammonium acetate, at reflux. The optimization details of the developed novel protocol are recorded. The structure of newly synthesized compounds have been characterized by spectroscopic data (elemental analysis, IR and ^{1}H NMR spectroscopic studied). Antimicrobial properties of the novel coumarins derivatives **3** are investigated. The acetylcholinesterase inhibition activity (AChEI) was tested for all compounds, and the **3b** shows the highest AChEI activity with 48.25% of inhibition.

1. Introduction

4H- hydroxycoumarins derivatives have a wide range of biological applications (Doshi et al., 2006; Kemnitzer et al., 2007; Kumar et al., 2007; Gao et al., 2010). In this connexion coumarins derivatives are important in organic synthesis studies (Thompson, 2000; Akbarzadeh et al., 2012; Wang et al., 2000). On the other multicomponent reaction protocol with environmentally solvents and catalytic systems is one of the most suitable strategies, for developing libraries of medicinal scaffolds (Saeedi et al., 2013; Dömling, 2002). One pot, multi-component synthetic protocol is more significant than the step wise approach in terms of efficiency, minimal waste production, energy or cost-effectiveness, and operational simplicity (Li and Chan, 1997; Boubakri et al., 2016; Medyouni et al., 2016). As part of our ongoing studies for the development of an environmentally friendly reaction condition to produce heterocyclic compounds (Hamdi et al., 2017; Al-Ayed and Hamdi, 2014; Hamdi et al., 2011), we now report herein an efficient and clean synthesis method of a novel coumarins derivatives carbonitriles **3**. These compounds were subsequently evaluated for their biological activities.

Abbreviations: $POCl_3$, phosphorus oxychloride; DMC, dimethylcarbonate; DEC, diethylcarbonate; DCM, dichloromethane; NH_4OAc, ammonium acetate.

* Corresponding author at: Research Laboratory of Environmental Sciences and Technologies (LR16ES09), Higher Institute of Environmental Sciences and Technology, University of Carthage, Hammam-Lif, Tunisia.
E-mail addresses: lmansour@ksu.edu.sa, lamjed.mansour@gmail.com (L. Mansour), naceur.hamdi@isste.rnu.tn (N. Hamdi).

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2. Experimental section

2.1. General procedures

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian VXR 300 instrument at 293 K in $CDCl_3$ or DMSO- d_6. Spectra were internally referenced to TMS. Peaks are reported in ppm downfield of TMS. The melting points of compounds were determined in open glass capillaries in a paraffin bath and are uncorrected. Elemental microanalysis was obtained by the

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microanalytical laboratory services, university of Rennes 1 in France.

3-Acetyl-4-hydroxycoumarin (1) was synthesized using our previous work (Al-Ayed and Hamdi, 2014).

Preparation of coumarins derivatives 3 Fig. 1 (Supplementary material, SS1).

2.2. Antibacterial activity tests

Antibacterial activities were tested against selected food-borne pathogens and clinical Gram-positive and Gram-negative bacteria strains obtained from local culture collection and International Culture Collections (ATCC). We have selected for this study *Staphylococcus aureus (S. aureus)* ATCC 6538, *Micrococcus luteus* (*M. luteus*) LB14110, *Listeria monocytogenes (L. monocytogenes)* ATCC 19117 *Salmonella typhimurium (S. typhimurium)* ATCC 14028 and *Agrobacterium tumifaciens* (*A. tumifaciens*).

Analysis of the activities of compounds against these selected bacteria was conducted using the Agar well diffusion method according to Guven et al. (2005) with some modification as reported by Al-Ayed and Hamdi (2014). Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the tested compound for which the bacteria did not growth in the optimal condition of incubation. The procedures for MIC tests of the newly synthesized products were conducted in accordance with NCCLS guideline M7-A6 and M38-P (National Committee for Clinical Laboratory Standard, 2003) and detailed in Al-Ayed and Hamdi (2014).

2.3. Acetylcholinesterase inhibitory potential (AChEI)

AChEI was performed following the spectrophotometric procedures previous reported by Ellman et al. (1961). All obtained compounds were tested at concentration of 100 mg/ml. We have followed the same protocol reported in Al-Ayed and Hamdi (2014).

For all biological teste we have expressed the measurements in average ± standard deviation after triplicate assays.

3. Results and discussions

3.1. Chemistry

Reaction between 4-hydroxycoumarin and phosphorus oxychloride ($POCl_3$) in glacial acetic acid, under reflux for 24 h afforded 3-acetyl-4-hydroxycoumarine **1** in excellent yield (90%) (Hamdi et al., 2011).

The IR spectra of compound **1** revealed a strong band at 3185 cm^{-1} confirming the presence of OH group and showed band in the region of 1700 cm^{-1} which is the characteristic for C=O of coumarin. The ^1H NMR data of compound **1** revealed signal at 2.72 ppm attributed to the methylic protons, the aromatic protons resoned between 7.1 and 7.98 pp. While hydroxylic proton (OH) resonated at δ 17.69 ppm.

The reaction of 3-acetyl-4-hydroxycoumarine **1** with ethyl cyanoacetate and benzaldehyde in was explored using different solvents (DMC, DEC, ethanol, H_2O, tetrahydrofuran (THF), dichloromethane and toluene). (Table 1, entries 1–7) in the presence of a catalytic amount of ammonium acetate. The results are summarized in Table 1.

From the obtained results we can say that solvents affected the yields (Table 1, entries 1–7), and the best result was obtained in excellent yield and high purity with DMC.

Then, we performed the reaction with different amounts of catalyst at ambient temperature (5 mol%, 10 mol%, 15 mol%, and 20 mol%) in order to optimize the ammonium acetate loading. The results are given in Table 2.

When we increase the percentage of the catalyst the obtained yields were also increased up, but after 20 mol% there is no improvement of the yield of the reaction.

In order to explore more this method, the cyclocondensation reaction with different series of substituted aromatic aldehydes was achieved. The results are given in (Table 3).

The scope of the method was assayed with a series of substituted aromatic aldehydes after optimization of the reaction conditions. The results are summarized in Table 3. As shown in Table 3, the aromatic aldehydes bearing both the electron withdrawing (inputs 2,3) and electron donor groups (1,5) were subjected to successful condensation with ethyl cyanoacetate and 4-hydroxycoumarin. In the presence of a catalytic amount of ammonium acetate in the refluxing DMC to give the corresponding products in convenient yields. It appears that the electronic effects and the nature of the substituents on the arylaldehyde ring have a small effect on the reaction yield and on the time required to complete the reaction. Electron donor groups somewhat increased the reactivity and provided higher yields compared to the electron-withdrawing groups. Remarkably, the reactions were clean, and all products were obtained after only filtration and simple washing with water and ethanol. Thus, a simple treatment gives the title products without need of chromatographic purification.

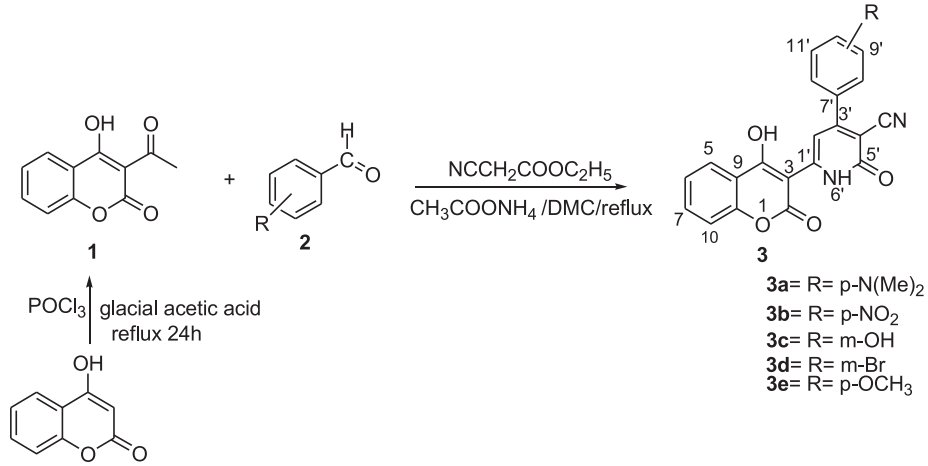

Fig. 1. Synthesis of coumarins derivatives carbonitriles **3** catalyzed by NH_4OAc.

Table 1
Synthesis of coumarins derivatives carbonitriles **3**.

Entry	Solvent	Temp (°C)	Time (min)	Yield (%)[b]
1	DMC	90	30	85
2	DEC	128	30	80
3	ethanol	78	120	72
4	THF	66	120	62
5	H_2O	100	30	83
6	DCM	40	120	60
7	Toluene	110	120	60

[a]Reaction conditions: 3-acetyl-4-hyddroxycoumarine (1 mmol), ethyl cyanoacetate (1 mmol), solvent (5 mL), reflux.
[b]Isolated yield of product.

Table 2
Condensation of benzaldehyde, ethyl cyanoacetate, and 3-acetyl-4-hydroxycoumarine in DMC.[a]

Experience	catalyst	Mol (%)	Time (mn)	Yield (%)[b]
1	NH_4OAc	5	5	60
2	NH_4OAc	10	10	85
3	NH_4OAc	15	3	82
4	NH_4OAc	20	3	95
5	NH_4OAc	25	3	92
6	NH_4OAc	30	620	88
7	No catalyst	–	620	10

[a] Reaction conditions: 3-acetyl-4-hydroxycoumarine (1 mmol), ethyl cyanoacetate (1 mmol), DMC (5 mL), reflux.

Table 3
Synthesis of compounds **3** catalyzed by NH_4OAc.

entry	1	2	3	4	5
Product	**3a**	**3b**	**3c**	**3d**	**3e**
Time (min)	60	50	45	80	75
Yieldb (%)	95	92	90	–	88

[a]3-acetyl-4-hydroxycoumarine (1 mmol), arylaldehydes (1 mmol), ethyl cyanoacetate (1 mmol), DMC (5 mL), NH_4OAc (20%), reflux.
[b]Isolated yield of product.

The structure of the new compounds **3** was identified by spectroscopic data and either by their elemental analysis. ^{13}C NMR showed the amide (NH–CO) signal at δ 177.8 ppm, the $C_{1'}$ signal with a chemical shift of δ = 61.9 ppm, while the C_4 signal was assigned further upfield at δ = 96.47 ppm. However, the C_2 signal was further upfield, δ = 180.13 ppm, than was the $C_{4'}$ signal was assigned as δ = 134.48 ppm.

The proposed mechanism of compound **3** is given by the reaction sequence in Fig. 2. First, condensation of ammonium acetate with ethyl cyanoacetate is proposed to give intermediate (i), then 3-acetyl-4-hydroxycoumarine react with arylaldehydes to give intermediate (ii). The Michael addition of intermediate (i) with intermediate (ii) occurs to provide the intermediate (iii) which undergoes aromatization to form the target coumarins carbonitriles **3**.

3.2. Biological activities

3.2.1. Antibacterial activity

The coumarins derivatives were screened for their antibacterial as reported by our previous work (Boubakri et al., 2016). The antibacterial activity of the compounds against human pathogenic Gram positive and Gram-negative bacteria of derivatives **3** was estimated by measuring the zone of inhibition in disc diffusion method. The antimicrobial activities of synthesized coumarins are given in Table 4.

The synthesized coumarin **3a** show activity against *LB 1411*with three different concentration 0.1, 0.3 and 0.5 mg/ml. Compound **3b** show activity against two bacteria *Micrococcus luteus* LB 14110 and *Agrobacterium tumifaciens* only at concentration of 0.5 mg/ml. Coumarin derivative **3d** show antibacterial activities against all the species of bacteria. The Minimal Inhibitory Concentrations (MICs) values of coumarins derivatives **3** were determined against the same bacteria. The results are given in Table 5.

The Minimal Inhibitory Concentrations (MICs) values of coumarins derivatives **3** were determined. The most active compound was **3c** which presents the highest activity against the Gram-negative bacteria *Salmonella typhimurium* ATCC14028, often greater than the used standard (ampicillin). Low effect of all substances was observed against the Gram-positive bacteria, *Listeria monocytogenes* ATCC 19117. All the compounds show efficient effect against the Gram-positive *Micrococcus luteus* LB 1411

3.2.2. Acetylcholinesterase inhibition

The acetylcholinesterase enzyme (AChE) is hydrolysis of the neurotransmitter acetylcholine allowing the termination of impulse transmission at cholinergic synapses of the central and peripheral nervous systems. Degenerative nervous disease as Alzheimer's disorder has been associated with a deficiency of acetylcholine. Acetylcholinesterase inhibitors (AchEIs) are introduced for symptomatic treatment of Alzheimer disorders (Shah et al., 2008).

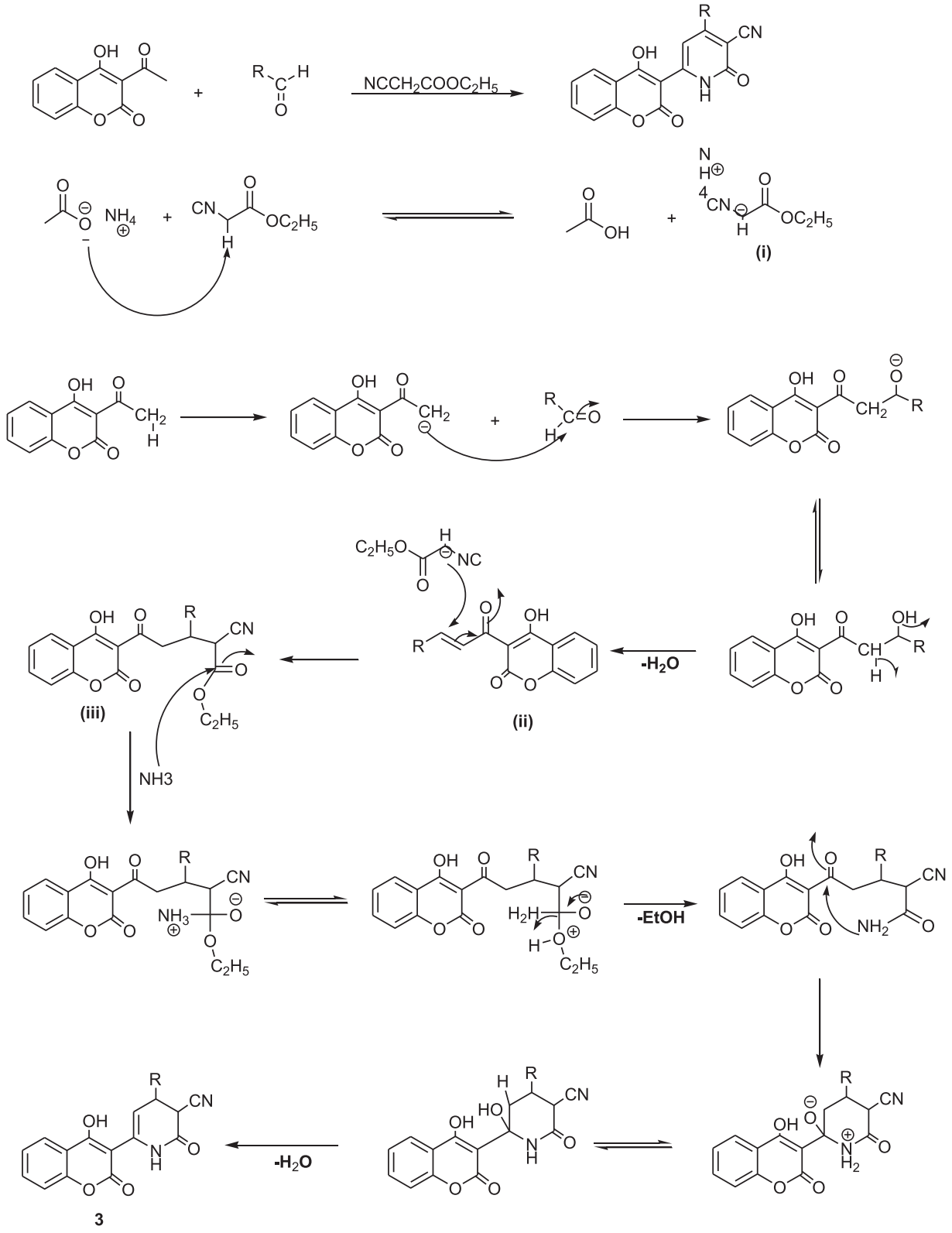

Fig. 2. Proposed mechanism for the synthesis of compound **3.**

The acetylcholinesterase enzyme (AChE) were done as reported work (Kalauni et al., 2002; Atta-ur-Rahman et al., 2004; Ahmad et al., 2003), and they are presented in Table 6.

Three compounds show significant AChEI activity. The compound **3b** possesses the most AChEI effect.

4. Conclusion

In conclusion, we have demonstrated a highly efficient method for the synthesis of coumarins carbonitriles **3** via three component reaction of aromatic aldehydes, ethyl cyanoacetoacetate, and 3-

Table 4
Antibacterial activity of the coumarin derivatives **3** (**3a**, **3b**, **3c**, **3d**, and **3e**) against the growth of some bacteria.

Bacteria	Conc. mg/ml	Inhibition zones diamater (mm)					AMC
		3a	3b	3c	3d	3e	
Micrococcus luteus	0.1	19	0	16	18	19	42
LB 14,110	0.3	23	0	16	15	16	
	0.5	23	24	16	14	15	
Staphylococcus aureus	0.1	24	0	40	16	17	
ATCC 6538	0.3	24	0	36	15	16	
	0.5	33	0	33	15	17	
Listeria monocytogenes	0.1	0	0	0	22	23	
ATCC 19117	0.3	0	0	0	23	18	
	0.5	22	0	0	24	17	
Salmonella Typhimurium	0.1	0	0	0	23	16	
ATCC 14028	0.3	0	0	0	24	15	
	0.5	16	0	22	25	17	
Agrobacterium tumifaciens	0.1	21	21	0	15	18	
	0.3	20	19	16	14	17	
	0.5	21	18	0	16	16	

AMC: Ampicillin.

Table 5
Determination of the Minimum Inhibitory Concentrations (MICs) expressed in mg/mL of compounds **3**.

Microorganism indicator	Compounds	MIC (mg/mL)
Micrococcus luteus LB 1411	Ampicillin	0.0195
	3a	0.04
	3b	0.03
	3c	0.02
	3d	0.035
	3e	0.042
Listeria monocytogenes ATCC 19117	Ampicillin	0.039
	3a	2.5
	3b	1.25
	3c	2.5
	3d	2.4
	3e	2.3
*Salmonella typhimurium*ATCC14028	Ampicillin	0.625
	3a	1.25
	3b	1.26
	3c	0.078
	3d	1.5
	3e	1.6

Table 6
Acetylcholinesterase inhibitory activity (AChEI) (%) of coumarins derivatives **3**.

Compound	(AChEI) (%)
3a	–
3b	48.25
3c	–
3d	37.15
3e	37.80

acetyl 4-hydroxycumarine using cheap and readily available low toxic organocatalyst ammonium acetate. This proposed method has main advantages to be work-up procedures and simple experimental, small amount of catalyst, solvent-free reaction conditions, high yields, short reaction time, and using reusable and non-expensive catalyst. The obtained compounds show efficient antibacterial activity against pathogenic foodborne bacteria belong either to gram-positive and gram-negative. These notable antibacterial effects confirm the necessity for synthesizing new series derived from these compounds. The availability of these compounds will also facilitate further investigations of their pharmacological properties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2019.11.023.

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