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Azacoumarin Derivatives Accessible in Short and Ecofriendly Conditions with High Antifungal Activity

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Abstract: The synthesis of novel class of compounds bearing a coumarin entity was targeted with the aim to propose a rapid and convenient method to access diversity. They are either azacoumarins (iminocoumarins) or characterized by the presence of a pyridone ring fused within the azacoumarin scaffold. They were synthesized through a short, solvent free and eco-friendly method, thanks to the microwave activation. The effectiveness of the method was confirmed by the preparation of two series of compounds. These compounds may find various therapeutic applications. In order to bring a piece of evidence that the described compounds are therapeutically useful, we evaluated the antifungal activity of the newly synthesized compounds against a new fungal strain of *Aspergillus niger*. Most of the compounds have shown inhibition of the investigated fungal strain. The most active compound showed comparable activity with the widely used reference drug, amphotericin B. The conditions and the ease of synthesis prevail high potential of the method for diversity-oriented synthesis (DOS) and generate chemical libraries, very useful within the drug discovery area.

Keywords: Coumarins; Azacoumarins, *Aspergillus niger*, Antifungal activity

1. Introduction

Coumarins are a family of compounds with remarkable therapeutic potential [1]. Being naturally occurring in many plant species, they were and remain the subject of studies for potential therapeutic applications [2]. Moreover, they are used as scaffolds for the design of drug candidates with broad therapeutic applications, targeting major diseases such as, cancer, and infectious diseases [3]. The chemical modifications performed on naturally occurring coumarins have mostly dealt with the modification of existing substituents and functions or the introduction of new substituents on the carbon skeleton [4,5]. The availability of modern and recent synthetic methods have boosted the interest toward coumarin-derived bioactive molecules [6]. In this regard, coumarins bearing pyridine rings have attracted the interest of medicinal chemists [7]. Very recently, coumarins-pyridine hybrids were reported as multitarget drug candidates toward the management of Alzheimer disease [8,9]. In the same vein, coumarins bearing a fused pyridine ring, namely coumarinopyridines were under the spotlight owing to the prevalence of such motifs in naturally occurring alkaloids [10]. Being structurally very close, chromones bearing pyridine rings have been widely investigated for their therapeutic potential in diverse areas [11–20].

The main objective of this work was to set a simple, short and an eco-friendly method to access coumarin-derived compounds bearing nitrogen atoms. The presence of the nitrogen atom may contribute to the biological activity and pharmacokinetic properties of coumarins [21]. Our focus was made on two coumarins series where one or two nitrogen atoms were introduced. The first is azacoumarins (iminocoumarins) in which one of the coumarin oxygen atoms is replaced with a nitrogen and the second is azacoumarins bearing a fused pyridine ring. For both series, we found the activation with microwave in almost solvent-less conditions offers a new and very convenient method. The conditions and the ease of synthesis prevail high potential of the method for diversity-oriented synthesis (DOS) and generate chemical libraries, very useful within the drug discovery area. With the aim to check the input of the generated compounds in one therapeutic area, we focused on aspergillosis infection.

Aspergillosis is an infection caused by *Aspergillus fungus*. Generally, aspergillus are not harmful for most people that are in good health. However, those with lung diseases and generally with weak immune system are more subject to infection with *Aspergillus* and likely to develop health problems, including allergy and lung infections [22]. Among *Aspergillus*, *Aspergillus niger* (*A. niger*) is probably the most common species. It is a known contaminant of foods and can cause a disease namely black mold to fruits. It is well documented that *A.*

niger is frequently involved in mycosis of the ear where it causes local inflammation and mycelial growth on cerumen (ear wax) on the skin of the ear canal [23]. The therapeutic management of aspergillosis calls for the use of antifungals such as azoles, polyenes and echinocandines, in mono or combination therapy [24]. However, despite the availability of effective drugs against *A.niger*, the developed resistance toward the treatment in one hand and the emergence of new strains appeals for constant discovery of novel chemical entities with effective antifungal activities [25]. Recently, it was reported that naturally occurring coumarins and synthetic analogs were of therapeutic potential as anti-aspergillosis [26].

Herewith, in the frame of a project aimed to set novel antifungals especially drug candidates targeting resistant *A.niger* fungus, we studied new coumarin analogs bearing nitrogen atoms. In this study, we were and particularly interested by azacoumarins (iminocoumarin) and azacoumarinopyridones, easily accessible through novel, short and ecofriendly conditions (Figure 1).

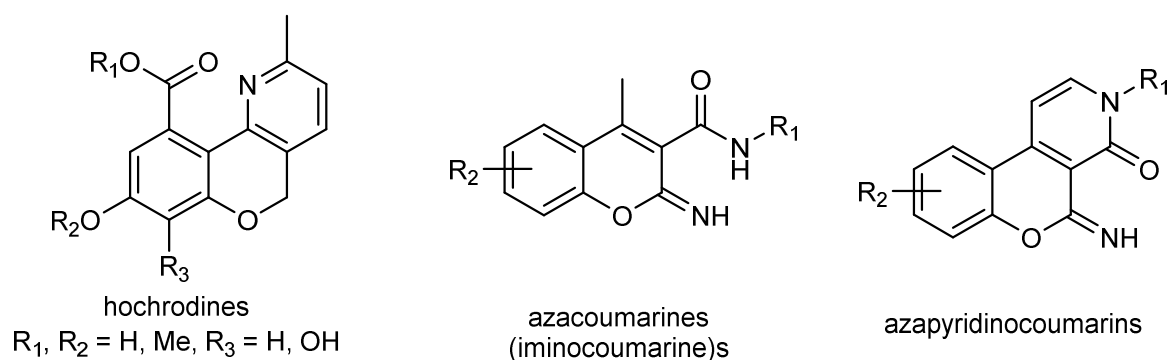


Figure 1. Structures of some naturally occurring coumarinopyridines (hochrodines) and compounds targeted in this study.

Materials & methods

Chemistry

Follow up of the reactions and checking the homogeneity of the prepared compounds were ensured by using thin-layer chromatography (TLC). Melting points were recorded on a Griffin melting point apparatus and are uncorrected. The reaction under microwave activation was performed using a monomode reactor Synthewave 402 from Prolabo. IR spectra were recorded using KBr disks using Jasco FT-IR-6300 spectrophotometer. ¹H NMR (250 MHz, Bruker AC 250) or (400 MHz, Bruker AC 400) and ¹³C NMR (62.5 MHz or 150 MHz) spectra were recorded using DMSO-*d*₆ or CDCl₃ as a solvent with TMS as internal standard. Chemical

shifts are reported in parts per million (ppm) and the coupling constants J in Hz. The signals are denoted by the following abbreviations: s: singlet, d: doublet, t: triplet, dd: doublet of doublet, q: quadruplet, m: multiplet. Electrospray ionization (ESI) mass spectra were recorded at the Institut de Chimie Moléculaire de Grenoble (ICMG) on a Waters Xevo G2-S Q TOF instrument (Guyancourt, France) with a nanospray inlet. Liquid Chromatography-Mass Spectrometry (LC-MS) were performed using an LP pmp, a diode-array (DAD) UV detector. Electrospray ionization (ES^+ and ES^-) was used as ionization method.

General procedure for the preparation of 2-cyano-*N*-acetamides (**3**)

In a 50 ml flask fitted were added respectively, a catalytic amount of sodium ethanolate dissolved in 2 mL of ethanol, primary amine (**1**, 1 eq.) and ethyl cyanoacetate (**2**, 1 eq.). The reaction mixture was stirred for 6 hours at room temperature. The solid formed was washed several times with diethyl ether and filtered on a sintered glass filter of porosity 0.4 Å to yield compounds (**3**) as solids.

2-Cyanoacetamide (3a). White solid, yield 85%, m.p. 118-120 °C; 1H NMR (400 MHz, DMSO- d_6) δ : 6.06 (s, 1H), 3.39 (s, 2H); ^{13}C NMR (100.6 MHz, DMSO- d_6) δ : 172.19 (C), 115.05 (C), 25.72 (CH₂); IR (KBr) ν (cm⁻¹): 3276, 2261, 1646, 1351.

2-Cyano-N-ethylacetamide (3b). White solid; yield 57%; m.p. 120-122 °C; 1H NMR (400 MHz, DMSO- d_6) δ : 8.26 (s, 1H), 3.59 (s, 2H), 3.11 (q, $J=8.0$ Hz, 2H), 1.19 (t, $J=8.0$ Hz, 3H); ^{13}C NMR (100.6 MHz, DMSO- d_6) δ : 162.19 (C), 116.75 (C), 34.45 (CH₂), 25.72 (CH₂), 14.84 (CH₃); IR (KBr) ν (cm⁻¹): 3276, 2261, 1646, 1351.

2-Cyano-N-propylacetamide (3c). White solid, yield 67%; m.p. 120-122 °C; 1H NMR (400 MHz, DMSO- d_6) δ : 8.81 (s, 1H), 3.76 (s, 2H), 3.31 (t, $J=8.0$ Hz, 2H), 1.32 (m, 2H), 0.98 (t, $J=8.8$ Hz, 3H); ^{13}C NMR (100.6 MHz, DMSO- d_6) δ : 162.15 (C), 116.15 (C), 42.67 (CH), 22.28 (CH), 20.61 (CH₂), 14.08 (CH₃); IR (KBr) ν (cm⁻¹): 3302, 2255, 1644, 1626.

N-Butyl-2-cyanoacetamide (3d). White solid; yield 59%. m.p. 100-102 °C; 1H NMR (400 MHz, DMSO- d_6) δ : 8.20 (s, 1H), 3.30 (s, 2H), 3.10 (t, $J=5.6$ Hz, 2H), 1.41 (m, 2H), 1.31 (m, 2H), 0.90 (t, $J=5.6$ Hz, 3H); ^{13}C NMR (100.6 MHz, DMSO- d_6) δ : 162.32 (C), 116.75 (C), 39.17 (CH₂), 31.33 (CH₂), 25.70 (CH₂), 19.91 (CH₂), 14.03 (CH₃); IR (KBr) ν (cm⁻¹): 3276, 2263, 1640, 1349.

2-Cyano-N-(cyclohexylmethyl)acetamide (3e). White solid; yield 76%. m.p. 122-124 °C; 1H NMR (400 MHz, DMSO- d_6) δ : 8.13 (s, 1H), 3.58 (s, 2H), 2.01 (m, 10H), 1.76 (quin, $J=5.8$

Hz, 6H), 1.43 (q, $J=5.8$ Hz, 4H); ^{13}C NMR (100.6 MHz, DMSO- d_6) δ 160.87 (C), 116.16 (C), 48.38 (CH₂), 32.03 (CH₂), 28.12 (CH₂), 25.03 (CH₂), 24.84 (CH₂), 24.27 (CH₂), 24.27 (CH₂); IR (KBr) ν (cm⁻¹): 3276, 2261, 1646. EIMS m/z (% relative abundance): 167 (M+H, 52), 85 (100), 83 (56). HRMS (ES-QTOF) Calcd. for C₉H₁₅N₂O (M+H) 167.1184. Found 167.1186.

2-Cyano-N-(furan-3-ylmethyl)acetamide (3f). Orange-yellow solid; yield 85%. m.p. 132-134 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 8.86 (s, 1H), 7.60 (d, $J=4.0$ Hz, 1H), 6.45 (d, $J=4.0$ Hz, 1H), 6.28 (d, $J=4.0$ Hz, 1H), 4.29 (d, $J=4.0$ Hz, 2H), 3.69 (s, 2H); ^{13}C NMR (100.6 MHz, DMSO- d_6) δ 162.58 (C), 151.88 (C), 142.83 (C), 116.61 (C), 110.96 (C), 107.78 (C), 36.35 (CH₂), 25.65 (CH₂); IR (KBr) ν (cm⁻¹): 3276, 2261, 1646, 1351 cm⁻¹.

N-Benzyl-2-cyanoacetamide (3g). White solid; yield 90%. m.p. 119-121 °C; ^1H NMR (250 MHz, CDCl₃) δ 8.81 (t, $J=4.9$ Hz, 1H), 7.27-7.41 (m, 5H), 4.35 (d, $J=5.8$ Hz, 2H), 3.76 (s, 2H); ^{13}C NMR (100.6 MHz, CDCl₃) δ 162.15 (CO), 116.15 (CN), 138.5 (5×C_{arom}) 42.67 (CH₂Ph), 25.28 (CH₂CN); MS. m/z : 167 (M+H); IR (KBr) ν (cm⁻¹): 3302, 2255, 1644, 1626; HRMS (ES-QTOF) Calcd for C₁₀H₁₁N₂O (M+H) 175.0871. Found 175.0873.

General procedure for the preparation of aryl-2-Imino-4-methyl-2H-chromene-3-carboxylic acid (aryl)-amide (5). 2-Hydroxyacetophenone (**4**, 1 eq.) 2-cyano-*N*-ethylacetamide **3a-g** (1g, 8.92 mmol, 1 eq) in the presence of two drops of piperidine were heated under microwaves activation (2450 MHz, 120 W) for 1 min. After cooling, the diethyl ether was added and the mixture was stirred for 15 min. The precipitate obtained is directly filtered and then washed several times with diethyl ether and recrystallized with ethanol to yield iminocoumarins (**5a-g**). The purity of compounds (**5a-g**) was assessed by NMR and LCMS.

2-Imino-4-methyl-2H-chromene-3-carboxylic acid amide (5a). Mustard yellow solid; yield: 56%. m.p. 118-120 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 8.01 (s, 2H), 7.54 (d, $J=6.6$ Hz, 1H), 7.30 (m, $J=6.6$ Hz, 1H), 7.25 (dd, $J=8.1$ Hz, $J=3.0$ Hz, 1H), 6.61 (dd, $J=8.1$ Hz, $J=3.0$ Hz, 1H), 6.16 (s, 1H), 1.03 (s, 3H); ^{13}C NMR (100.6 MHz, DMSO- d_6) δ 175.3 (C), 165.5 (C), 162.0 (C), 154.2 (C), 152.1 (CH), 134.7 (CH), 125.3 (C), 125.1 (CH), 121.3 (CH), 117.2 (C), 17.8 (CH), IR (KBr) ν (cm⁻¹): 3502, 3191, 1650, 1724 ; MS m/z : 203 (M+1).

LCMS: R_t = 4.12 min, ES+ = 203.2

2-Imino-4-methyl-2H-chromene-3-carboxylic acid ethylamide (5b). Brown solid; yield: 36%. m.p. 195-197 °C; ^1H NMR (400 MHz, CDCl₃) δ 7.57 (dd, $J=14.4$ Hz, $J=3.2$ Hz, 1H), 7.26 (dd, $J=8.0$ Hz, $J=6.8$ Hz, 1H), 6.15 (s, 1H), 6.16 (s, 1H), 3.23 (d, $J=2.8$ Hz, 2H), 2.63 (s, 3H),

1,6 (t, 3H); ^{13}C NMR (100.6 MHz, CDCl_3) δ 175.3 (C), 165.5 (C), 162.04 (C), 154.2 (C), 152.1 (CH), 134.79 (CH), 125.6 (C), 125.0 (CH), 121.3 (CH), 117.2 (C), 36.9 (CH_2), 18.5 (CH_3), 16.8 (CH_3); IR (KBr) ν (cm^{-1}): 3295, 2260, 1661, 1554, 1724. MS m/z : 231 (M+H).

LCMS: R_t = 3.98 min, ES^+ = 231.5

2-Imino-4-methyl-2H-chromene-3-carboxylic acid propylamide (5c). Brown solid. yield: 31%. m.p. 200-202 °C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.04 (s, 1H), 7.97 (d, $J=8.0$ Hz, 1H), 7.78 (d, $J=7.6$ Hz, 1H), 7.47 (dd, $J=8.0$ Hz, $J=3.0$ Hz, 1H), 6.16 (s, 1H), 3.56 (s, 1H), 3.04 (t, $J=7.2$ Hz, 2H), 2.71 (s, 3H), 1.39 (q, 2H), 0.83 (t, 3H); ^{13}C NMR (100.6 MHz, $\text{DMSO-}d_6$) δ 166.5 (C), 164.5 (C), 155.2 (C), 152.1 (C), 127.5 (CH), 126.5 (CH), 122.3 (C), 125.17 (CH), 121.3 (CH), 114.7 (C), 45.1 (CH_2), 24.2 (CH_2), 18.0 (CH_3), 11.4 (CH_3); IR (KBr) ν (cm^{-1}): 3542, 3297, 2971, 1675, 1721, 1075. MS m/z : 245 (M+H).

LCMS: R_t = 3.81 min, ES^+ = 245.1

2-Imino-4-methyl-2H-chromene-3-carboxylic acid butylamide (5d). Green solid; yield: 27%. m.p. 210-212 °C; ^1H NMR (400 MHz, CDCl_3) δ 8.04 (s, 1H), 6.98-6.89 (m, 1H), 7.12 (dd, $J=8.1$ Hz, $J=4.5$ Hz, 1H), 6.66 (dd, $J=6.6$ Hz, $J=3.0$ Hz, 1H), 6.16 (s, 1H), 3.17 (q, $J=6.1$ Hz, 2H), 1.59 (q, $J=6.0$ Hz, 2H), 1.35 (q, $J=5.9$ Hz, 2H), 1.69 (t, $J=7.2$ Hz, 3H), 0.98 (q, $J=6.3$ Hz, 3H); ^{13}C NMR (100.6 MHz, CDCl_3) δ 166.5 (C), 164.5 (C), 155.2 (C), 152.1 (C), 127.5 (CH), 126.5 (CH), 122.3 (C), 125.17 (CH), 121.3 (CH), 114.7 (C), 43.1 (CH_2), 33.4 (CH_2), 20.2 (CH_2), 18.01 (CH_3), 13.4 (CH_3); IR (KBr) ν (cm^{-1}): 3375, 3291, 2197, 2998, 1675, 1563; MS m/z : 259 (M+1).

LCMS: R_t = 3.77 min, ES^+ = 259.7

2-Imino-4-methyl-2H-chromene-3-carboxylic acid cyclohexylamide (5e). Orange-yellow solid; yield: 53%. m.p. 208-210 °C; ^1H NMR (400 MHz, CDCl_3) δ 7.58 (dd, $J=2.1$ Hz, $J=7.6$ Hz, 1H), 7.25 (dd, $J=2.1$ Hz, $J=7.6$ Hz, 1H), 6.16 (s, 1H), 3.49 (m, 1H), 3.42 (q, $J=4.0$ Hz, 2H), 3.25 (q, $J=4.0$ Hz, 2H), 3.26 (m, 2H), 1.50 (s, 3H); ^{13}C NMR (100.6 MHz, CDCl_3) δ 165.5 (C), 164.5 (C), 154.2 (C), 152.1 (C), 135.2 (CH), 129.1 (CH), 128.5 (C), 126.0 (CH), 125.5 (CH), 111.7 (C), 46.9 (CH), 41.5 (CH_2), 41.5 (CH_2), 26.2 (CH_2), 22.3 (CH_2), 22.3 (CH_2), 18.3 (CH_3); IR (KBr) ν (cm^{-1}): 3405, 2269, 1675, 1724; MS m/z : 285 (M+1).

LCMS: R_t = 4.02 min, ES^+ = 285.3

2-Imino-4-methyl-2H-chromene-3-carboxylic acid (furan-2-ylmethyl)-amide (5f). Orange-yellow solid; yield: 37%. m.p. 150-152 °C; ^1H NMR (400 MHz, CDCl_3) δ 9.89 (large s, 1H), 7.63 (d, $J=8.0$ Hz, 1H), 7.58 (d, $J=7.2$ Hz, 1H), 7.31 (d, $J=9.6$ Hz, $J=2.0$ Hz, 1H), 7.22 (d, $J=9.6$ Hz, $J=2.0$ Hz, 1H), 7.13 (d, $J=8.0$ Hz, 1H), 6.26 (s, 1H), 6.21 (s, 1H), 4.37 (d, $J=5.6$ Hz, 2H), 1.45 (s, 3H); ^{13}C NMR (100.6 MHz, CDCl_3) δ 166.05 (C), 164.2 (C), 156.4 (C),

152.1 (C), 151.4 (C), 143.9 (CH), 129.2 (CH), 127.1 (CH), 122.2 (C), 121.5 (CH), 115.4 (CH), 112.1 (C), 109.7 (CH), 102.1 (CH), 46.5 (CH₂), 19.0 (CH₃); IR (KBr) ν (cm⁻¹): 3574, 3291, 2257, 1675, 1724; MS m/z : 283 (M+1).

LCMS: R_t = 2.96 min, ES+ = 283.6

2-Imino-4-methyl-2H-chromene-3-carboxylic acid benzylamide (5g). Light yellow solid; yield: 72%. m.p. 129-131 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.75 (s, 1H), 8.02 (d, J =6.8 Hz, 1H), 7.66 (dd, J =6.7 Hz, J =2.2 Hz, 1H), 7.32 (dd, J =6.9 Hz, J =2.1 Hz, 4H), 6.61 (dd, J =6.6 Hz, J =3.0 Hz, 1H), 7.41 (m, 1H), 6.16 (s, 1H), 4.84 (d, J =4.8 Hz, 2H), 1.03 (s, 3H); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 165.5 (C), 162.1 (C), 155.0 (C), 151.2 (C), 138.5 (C), 130.7 (CH), 128.1 (CH), 128.3 (CH), 127.3 (CH), 125.3 (CH), 125.2 (CH), 122.5 (C), 121.7 (C), 117.2 (CH), 115.3 (CH), 112.1 (C), 48.5 (CH₂), 24.8 (CH₃); IR (KBr) ν (cm⁻¹): 3642, 3291, 1675, 1724; MS m/z : 293 (M+1).

LCMS: R_t = 3.21 min, ES+ = 293.1

General Procedure for the Preparation of aryl-2-ylmethyl-aryl-3H-chromeno[3,4-c]pyridine-4,5-dione from imino-coumarins (7). The azacoumarin (**5**, 1eq) and dimethyl formamide dimethyl acetal (**6**, 1eq) were introduced into a 25 ml flask equipped with a magnetic stirrer. The mixture is stirred at room temperature and without solvent for 24 hours or under microwave irradiation for 2 to 7 min. The solution becomes darker. The obtained solid was washed several times with diethyl ether and a few drops of ethanol then proceed to purification with chromatography column. The purified compounds were recrystallized with ethanol.

4-(2-Dimethylamino-vinyl)-2-imino-2H-chromene-3-carboxylic acid amide (7). Solid brown, yield: 47%. m.p. 153-155 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.50 (d, J =5.6 Hz, 1H), 8.19 (d, J =7.2 Hz, 1H), 7.97 (dd, J =7.6 Hz, 1H), 7.69 (d, J =7.6 Hz, J =2.5 Hz, 1H), 7.49 (d, J =7.6 Hz, 1H), 6.92 (s, 2H), 5.75 (d, J =5.6 Hz, 1H), 5.74 (d, J =5.6 Hz, 1H), 3.14 (s, 6H); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 168.3 (C), 165.5 (C), 155.0 (C), 155.18 (C), 128.7 (CH), 127.9 (CH), 125.3 (CH), 122.3 (C), 121.3 (CH), 113.2 (CH), 115.2 (C), 104.5 (CH), 42.5 (CH), 42.5 (2x CH₃); IR (KBr) ν (cm⁻¹): 3291, 2200, 1719, 1675, 1613, 1250. MS m/z : 258 (M+1).

General procedure for the preparation coumarinopyridones (9). Enamino-iminocoumarin (**7**, 1eq) with primary amine **8** (1 eq) were introduced into a 100 mL bicol flask equipped with a condenser and a thermometer. The reaction mixture was heated under reflux of DMF at 90 °C for 2 h or under microwave irradiation for 2 to 7 min. The solution takes on an increasingly dark brown color. The recovered mixture is decanted three times with dichloromethane and washed several times with distilled water in order to remove the DMF.

The crude was purified by chromatography over silica gel using ethyl acetate/cyclohexane (7/3) as an eluent to provide coumarinopyridones (**9a-d**). The purity of compounds (**9a-d**) was assessed by NMR and LCMS.

3-Amino-5-imino-3,5-dihydro-chromeno[3,4-c]pyridin-4-one (9a). Brown solid; yield: 66%. m.p. 204-206 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.26 (d, *J*=4.5 Hz, 1H), 7.32-6.19 (m, 4H), 7.19 (s, 1H), 5.97 (d, *J*=4.8 Hz, 1H), 4.47 (s, 2H); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 167.4 (C), 160.5 (C), 156.3 (C), 155.0 (C), 127.6 (CH), 122.3 (CH), 121.0 (CH), 120.2 (C), 119.3 (CH), 113.3 (CH), 116.2 (CH), 109.3 (C); IR (KBr) ν (cm⁻¹): 3316, 2240, 1651, 1603, 1542, 1250. MS *m/z*: 228 (M+H).

3-Ethyl-5-imino-3,5-dihydro-chromeno[3,4-c]pyridin-4-one (9b). Orange-yellow solid; yield: 69%. m.p. 210-212 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.22 (d, *J*=5.0 Hz, 1H), 7.30-6.15 (m, 4H), 7.14 (s, 1H), 5.83 (d, *J*=5.0 Hz, 1H), 4.43 (s, 2H), 3.38 (q, *J*=6.6 Hz, 2H), 2.4 (t, *J* = 6.5 Hz, 3H); MS *m/z*: 241 (M+1).

LCMS: R_t = 2.21 min, ES+ = 241.1

3-Furan-2-ylmethyl-5-imino-3,5-dihydro-chromeno[3,4-c]pyridin-4-one (9c). Brown solid; yield: 47%. m.p. 215-217 °C; ¹H NMR (400 MHz, CDCl₃) δ 9.11 (d, *J*=8.4 Hz, 1H), 8.75 (dd, *J*=6.2 Hz, *J*=4.1 Hz, 1H), 7.67 (dd, *J*=12.0 Hz, *J*=4.2 Hz, 1H), 7.54 (dd, *J*=8.1 Hz, *J*=3.2 Hz, 1H), 7.42 (d, *J*=4.1 Hz, 1H), 6.73 (d, *J*=4.1 Hz, 1H), 6.63 (t, *J*=5.8 Hz, 1H); 6.40 (dd, *J*=4.8 Hz, *J*=1.6 Hz, 1H), 4.53 (s, 1H), 4.49 (d, *J*=10.6 Hz, 1H), 3.39 (d, *J*=5.6 Hz, 2H), 1.69-1.35 (m, 2H), 1.08-1.04 (m, 2H); ¹³C NMR (100.6 MHz, CDCl₃) δ 165.5 (C), 159.0 (C), 155.0 (C), 152.1 (C), 146.2 (CH), 129.7 (CH), 127.3 (CH), 124.2 (CH), 122.6 (CH), 119.3 (C), 116.2 (CH), 113.3 (C), 111.2 (CH), 110.1 (CH), 104.3 (CH), 48.0 (CH₂); IR (KBr) ν (cm⁻¹): 3507, 2932, 1601, 1505, 1349, 1028; MS *m/z*: 310 (M+18).

LCMS: R_t = 1.98 min, ES+ = 293.1

3-Benzo[1,3]dioxol-5-ylmethyl-5-imino-3,5-dihydro-chromeno[3,4-c]pyridin-4-one (9d). Brick-red solid; yield: 54%. m.p. 235-236 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.78 (s, 1H), 7.39 (d, *J*=3.2 Hz, 1H), 7.33 (dd, *J*=3.2 Hz, *J*=5.6 Hz, 1H), 7.23 (d, *J*=6.0 Hz, 1H), 7.19 (d, *J*=6.4 Hz, 1H), 6.79 (d, *J*=8.0 Hz, 1H), 6.77 (s, 1H), 6.67 (d, *J*=7.2 Hz, 1H), 5.94 (s, 2H), 4.47 (d, *J*=6.0 Hz, 2H); ¹³C NMR (100.6 MHz, CDCl₃) δ 164.5 (C), 156.3 (C), 155.0 (C), 152.1 (C), 146.2 (C), 145.2 (C), 133.2(C), 129.7 (CH), 127.61 (CH), 124.3 (CH), 121.01 (C), 120.17 (CH), 119.3 (CH), 113.3 (CH), 116.2 (C), 111.3 (CH), 109.3 (CH), 107.45 (CH), 92.1 (CH), 51.2 (CH₂); IR (KBr) ν (cm⁻¹): 3312, 2943, 2199, 1653, 1614; MS *m/z*: 347 (M+1).

LCMS: $R_t = 1.45$ min, $ES^+ = 347.2$

Preparation of dicoumarinopyridones. In a 100 mL two-necked flask equipped with a condenser and a thermometer, were introduced *4-(2-Dimethylamino-vinyl)-2-imino-2H-chromene-3-carboxylic acid amide (7)* (0.5 g, 2 mmol, 2eq); diamine (0.058 g, 1 mmol, 1eq) and DMF (5 mL) as solvent. The reaction mixture was heated under reflux at 90°C for 6 h. The brown solution obtained was cooled and washed several times with distilled water and dichloromethane to remove the DMF. The crude was purified over column chromatography eluted ethyl acetate/cyclohexane (3:7) to provide a pure product after recrystallization in ethanol. The purity of compounds (**12** and **13**) was assessed by NMR and LCMS.

5-Imino-3-[2-(5-imino-4-oxo-5,6-dihydro-4H-benzo[f]isoquinolin-3-yl)-cyclohexyl]-3,5-dihydro-chromeno[3,4-c]pyridin-4-one (12). Obtained from trans-1,2-diaminocyclohexane. Purple solid; yield: 31%. m.p. 346-348 °C; 1H NMR (400 MHz, DMSO- d_6) δ 11.95 (s, 1H), 8.17 (d, $J=4.8$ Hz, 2H), 7.79-7.32 (m, 8H), 6.49 (s, 2H), 6.36 (d, $J=4.5$ Hz, 2H), 2.18 (s, 1H), 2.23 (s, 1H), 1.13-1.89 (m, 4 \times 2H); ^{13}C NMR (100.6 MHz, DMSO- d_6) δ 165.3 (2C), 159.2 (2C), 155.0 (4C), 128.7 (2CH), 127.9 (2CH), 125.3 (2CH), 122.3 (2C), 121.3 (2CH), 115.2 (2CH), 113.4 (2C), 111.4 (2CH), 46.6 (2CH), 26.4 (2 CH $_2$), 22.4 (2 CH $_2$); IR (KBr) ν (cm $^{-1}$): 3230, 2287, 1665, 1551, 1254; MS m/z 505 (M+H).

LCMS: $R_t = 5.22$ min, $ES^+ = 505.2$

5-Imino-3-[3-(5-imino-4-oxo-5,6-dihydro-4H-benzo[f]isoquinolin-3-ylmethyl)-benzyl]-3,5-dihydro-chromeno[3,4-c]pyridin-4-one (13). Obtained from 1,2-xylenediamine. Green solid; yield: 18%. m.p. 354-356 °C; 1H NMR (400 MHz, DMSO- d_6) δ 8.37-7.97 (d, $J=4.8$ Hz, 2 \times 2H), 7.32-7.79 (m, 4 \times 1H), 6.49 (s, 2 \times 1H), 6.36 (d, $J=8.2$ Hz, 2 \times 1H), 4.45 (d, $J=6.2$ Hz, 2 \times 2H); ^{13}C NMR (100.6 MHz, DMSO- d_6) δ 165.4 (2C), 159.3 (2C), 155.0 (4C), 142.2 (2C), 129.2 (2CH), 128.4 (CH), 125.8 (CH), 127.2 (2CH), 125.2 (2CH), 124.7 (2CH), 122.7 (2C), 121.9 (2CH), 115.3 (2CH), 113.3 (2C), 111.2 (2 \times CH), 56.6 (2 CH $_2$); IR (KBr) ν (cm $^{-1}$): 3134, 2286, 1665, 1554, 1254.2; MS m/z : 527 (M+H).

LCMS: $R_t = 4.67$ min, $ES^+ = 572.3$

Biology

Antifungal activity assessment

Aspergillus niger was chosen for this study, the fungi was obtained from the collection of department of biology of the faculty of science, Béchar University, Algeria. The micro-culture method [27,28] was used to confirm the *Aspergillus* genera. For the species confirmation, single spore method was used to culture *A. niger* species on three different media: malt extract agar at 25 °C, glycerol nitrate agar (G25N) at 25 °C, and Czapek yeast agar at 5 °C and 37 °C. Observation was made after 14 days using Pitt and Hocking's identification keys [29].

The compounds at different doses (2, 4, 8 and 10 mg) were poured into petri dishes after being thoroughly shaken under aseptic conditions. After the agars had cooled, the mold was seeded. At a temperature of 25-27°C, the petri dishes were incubated [30]. The assay was carried out in triplicate with a control sample.

To assess the inhibition of mycelia growth of fungi by hydrosols, the fungi colonies diameters were measured, since the day of incubation and the hydrosol inhibition percentage was calculated using the following formula: $I = ((DT-D)/DT)*100$, where: DT is mean diameter of mycelia growth in control and D is mean diameter of mycelia growth in treatment [31].

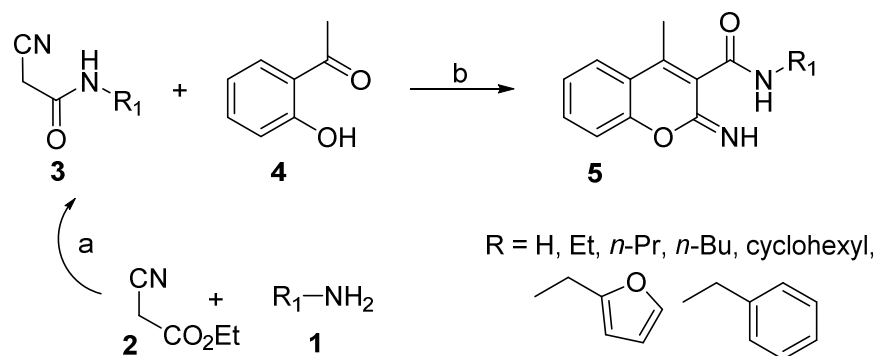
Cytotoxicity

The MTT colorimetric assay was used for the evaluation of the cytotoxicity of the compound **9d**. Cells were seeded into 96-well plates at a density of 1×10^5 cells/well for a total growth medium volume of 100 μ L and incubated overnight. 100 μ L of fresh medium containing compound **9d** (dissolved in DMSO in a 0, 1 and 10 μ M concentration) were added to each well while DMSO control was fixed at 0.5% (v/v). After 72 h incubation at 37 °C, MTT dye in PBS (5 mg/mL) was added and incubation was prolonged for an additional 4 h. After medium removal and drying, the formazan dye crystals were solubilized in DMSO/ethanol (1:1, v/v, 200 μ L). The absorbance was measured at 570 nm and 690 nm as reference wavelength. The effect of **9d** on cell viability was calculated as the difference in absorbance between test wells and medium control wells. For both concentrations, the cell viability was higher than 90%.

Results and discussion

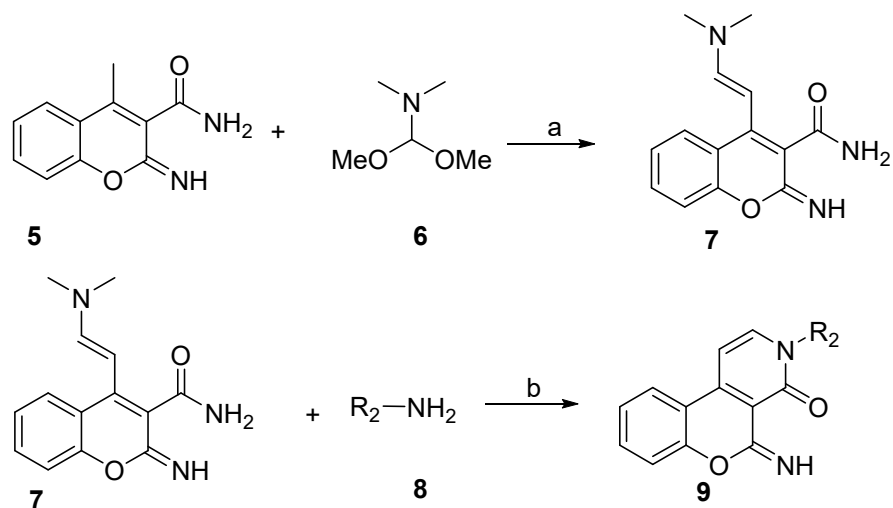
The access to the targeted compounds shown in Figure 1 was conducted according to scheme 1 in one step and solvent free conditions starting from *N*-alkylcyanoacetamids (**3**) and 2'-hydroxyacetophenone (**4**). In the case where *N*-alkylcyanoacetamids (**3**) are not commercially available, we found a novel and rapid method for their synthesis in medium to very good yield (57 to 90%) by the reaction of primary amines (**1**) with ethyl cyanoacetates (**2**) using sodium

in catalytic amounts in methanol. Herewith, the cyanoacetamides (**3**) were engaged in a Knoevenagel reaction with hydroxyacetophenone (**4**) in the presence of piperidine under microwave irradiation to obtain azacoumarins (**5**) in yields ranging from 27 to 72%. It should highlight that the microwave conditions were highly advantageous over classical conditions (6 hours, 90 °C in DMF). Although, we did not observe a significant difference in yields between the two methods, however, the reaction time is dramatically shorter for the MW-assisted method (2 to 7 minutes) and the reaction works in solvent free conditions.



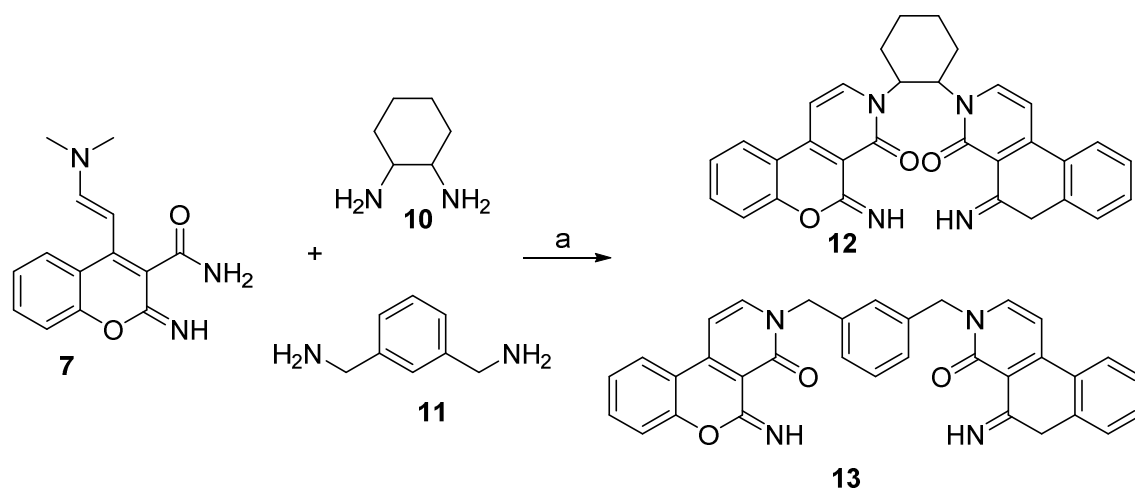
Scheme 1. (a) Na, CH₃OH, room temp.; (b) piperidine, MW, 2 to 7 min.

For the azacoumarinopyridones series, they were obtained in two steps starting from iminocoumarins (**5**), according to scheme 2. The iminocoumarins (**5**) were reacted with dimethylformamide dimethyl acetal (**6**) under microwave irradiation and solvent-free conditions to afford the corresponding enaminoiminocoumarins (**7**) with 47% yield. The microwave conditions were highly advantageous over classical conditions. The last step is the reaction of enaminoiminocoumarins (**7**) with primary amines (**8**) by using microwave irradiation without solvent to afford chromenopyridones (**9**) with yields ranging from 47 to 69%. Here again, the MW irradiation was found to be more effective over conventional heating (DMF at reflux). The structures of synthesized iminocoumarins (**5**) and coumarinopyridones (**9**) are shown in Table 1.



Scheme 2. (a) MW, 2 to 7 min; (b) MW, 2 to 7 min, or DMF, 90 to 120 °C.

In order to increase the generality of the synthesis method and to go for further pharmacomodulation, we synthesized two symmetrical diazocoumarinopyridones linked through diamines (**10**) and (**11**) (Scheme 3) to afford derivatives (**12**) and (**13**) with moderate yields (31 and 18%, respectively). This action was taken in the context of multivalent active compounds that are structures bearing at least two identical or different motifs. Becoming frequent within the field of drug discovery area, they are conceived to gain in synergy in terms of biological activity [32].



Scheme 3.(a) MW, 2 to 7 min, or DMF, 90 to 120 °C.

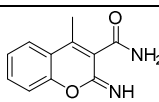
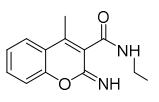
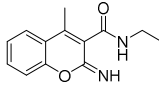
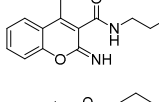
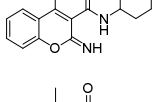
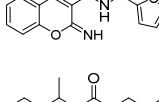
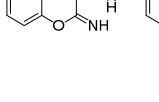
The newly synthesized compounds were tested for *in vitro* antifungal activity against *A. niger*, using a growth radial method in agar medium [33]. The compounds were dissolved in DMSO at different concentrations (2, 4, 8, and 10 mg). The antifungal drug amphotericin B and DMSO were used as a positive and negative control, respectively. To assess the antifungal

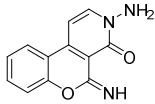
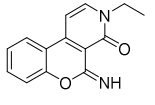
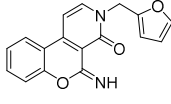
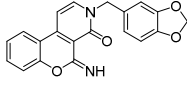
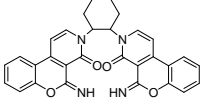
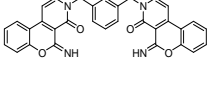
effect, the diameters of mold mycelial growth were measured in millimeters on the day-7 of incubation, and hydrosol inhibition percentages were calculated using the following formula: $I = ((DT-D)/DT) * 100$ where DT is mean diameter of mycelial growth in control and D is mean diameter of mycelial growth in treatment [33].

The obtained results are shown in Table 1. For all tested compounds, the diameters of *A. niger* colony is reduced following the increase of the concentration of tested compounds with clear relation between the structure and the activity of compounds is observed.

Among the azaocoumarins series (**5a-g**), the antifungal activity is influenced by the size and the nature of the group linked to the amide function. Indeed, the optimal chain length is an ethyl group (compound **5b** versus **5c** and **5d**). Moreover, the presence of a bulky or an aryl group at the amide function is synonymous of even higher activity as can be observed with compounds (**5e-g**). The correlation between the size and the shape of the substituent at the amide part can inform about the accommodation of the compound within the active site of the strain of *A. Niger*. Regarding the azacoumarinopyridone series (**9a-d**), the same structure-activity relationship tendency is observed where the presence of an aryl group at the lactam of the pyridine moiety is crucial (compounds **9c** and **9d** versus **9a** and **9b**).

Table 1. Antifungal activity of iminocoumarins and coumarinopyridones

Cmpd.	Structure	Compound concentration (mmol)			
		Growth inhibition (%)			
(5a)		9.7	19.8	39.6	49.4
		26.2±2.2	40.5±2.9	70.0±1.4	88.6±3.7
(5b)		8.5	17.4	34.7	43.4
		79.0±2.2	87.1±0.01	94.7±1.64	100±0.01
(5c)		8.03	16.4	32.7	40.9
		20.0±1.42	40.0±2.8	45.7±0.08	60.0±2.8
(5d)		7.6	15.5	31.0	38.7
		0±0.00	15.2±8.7	36.6±3.6	50.5±2.2
(5e)		6.9	14.0	28.1	35.2
		40.0±0.01	73.8±3.0	93.3±0.82	100±0.01
(5f)		6.9	14.2	28.3	35.4
		69.5±2.2	82.4±3.6	95.7±0.01	100±0.0
(5g)		6.7	13.7	27.4	34.2
		15.2±1.6	89.5±3.6	100±0.01	100±0.01

(9a)		8.6 27.5±5.7	17.6 46.2±2.8	35.2 78.6±1.4	44.0 96.4±0.8
(9b)		8.1 43.3±4.3	16.6 57.1±4.3	33.32 68.5±2.8	41.6 76.4±2.5
(9c)		6.7 75.7±4.5	13.7 100±0.01	27.4 100±0.01	34.2 100±0.00
(9d)		5.6 90.4±4.3	11.5 100±0.01	23.1 100±0.01	28.9 100±0.01
(12)		3.9 90.4±4.3	7.9 100±0.01	15.8 100±0.01	19.8 100±0.01
(13)		3.7 46.2± 3.6	7.6 61.4± 2.8	15.2 85.7± 1.4	19.0 96.2± 0.8
Amphotericin B		2.1 94.3	4.3 100	8.6 100	10.8 100

In order to go forward through the potential development of the most active compound, we evaluated the cellular toxicity of derivative **9d** by using the MTT test on the HEK293 (human embryonic kidney cells). Tested at 10 mM, it showed a high cell viability (> 90%), indicating prevailing a high therapeutic index (high biological activity *versus* low toxicity).

Conclusion

In this work we report a novel, short and eco-friendly synthesis of coumarins derivatives. The method is general and applicable to the synthesis of large chemical libraries. The synthesized compounds can find interest in diverse therapeutic area. Within this work we were interested by the antifungal activity and especially the effect against *A.niger*. The high antifungal activity and the innocuity of the most active compound are two key requirement for further development. The insertion of one or two nitrogen atoms within the coumarin scaffold offers multiple opportunities for pharmacomodulation. One of the pharmacomodulation point would be the imino group which can be easily reduced to primary amine. The latter can undergo alkylation, acylation and reductive-amination to provide diversity. The later diversity-oriented

synthesis is eased by the reported method allows the synthesis of iminocoumarins and coumarinopyridones in large scale.

Future perspectives

Our work report a short and eco-friendly method for the preparation of nitrogen-containing coumarins that could have potential in the medicinal chemistry field. The method allows rapid - generation of chemical libraries of compounds with functional diversity. The therapeutic potential of synthesized compounds was assessed through the evaluation of their activity as antifungals. The synthetic conditions of our method will be useful for the achievement of diversity oriented synthesis (DOS), a valuable tool for compounds screening in the drug design field.

Summary points

- Microwave activation was applied as a green method for the synthesis of imino and coumarins and pyridnocoumarins.
- The method was applied to the synthesis of a series of compounds including dimers-like derivatives.
- The antifungal activity against strains of *Aspergillus niger* was assessed and demonstrated potent effect as compared with a reference drug.
- The reported synthetic method may serve as a rapid and eco-friendly synthesis of nitrogen-containing coumarins for chemical libraries generation.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

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