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Synthesis of New 2-Amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-aryl Nicotine Nitrile in Eco-Friendly Media and their Antimicrobials and DPPH Radical Scavenging Activities

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A simple, green, efficient and economical procedure for the synthesis of, 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(aryl) nicotine nitrile (**4a-g**) by two routes carried in same conditions, have been reported. The new compounds **4a-g** were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR and elemental analysis. The synthesized compounds were screened for their antibacterial activities against Gram-positive bacterial strains (*Micrococcus luteus* LB14110, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19117), Gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa*). The coumarin derivative **4a** and 3-acetyl-4-hydroxycouamrin (**1**) were the most active against two bacteria *Staphylococcus aureus* ATCC 6538 and *Candida albicans* respectively. The best minimum inhibitory concentration values were obtained for the compound **4a** against *Candida albicans* (0.0195 g/cm^3). In addition, compounds **4a-g** were investigated for their antioxidant activities by DPPH (2,2-diphenyl-1-picrylhydrazyl) in which most of them displayed significant antioxidant activities.

Keywords: 3-Acetyl-4-hydroxycoumarin, Multicomponent reactions, Biological activities.

INTRODUCTION

Coumarins and their derivatives have attracted considerable attention due to their extensive biological activities, including antioxidant and anti-inflammatory [1], antiviral [2,3], anticoagulant [4], antimicrobial [5,6] and anticancer properties [7,8]. On the other hand, multicomponent reactions have been successfully employed to generate highly diverse combinatorial libraries for high-throughput screening of biological and pharmacological activities [9,10]. This type of reaction becomes increasingly important in organic and medicinal chemistry because it allows to obtain highly sophisticated polyfunctional molecules through simple one-pot procedures [11,12]. Multicomponent reaction protocol with environmentally benign solvents and catalytic systems is one of the most suitable strategies, which meets the requirements of green aspects of chemistry for developing libraries of medicinal scaffolds. In addition, water has emerged as a versatile solvent for organic reactions in the last two decades since it is readily available, inexpensive, environmentally benign, neutral and a natural solvent [13,14]. For these reasons, water has been used for multicomponent reaction (MCRs) as well [15,16]. Multicomponent reaction in water are of outstanding value in organic synthesis and green chemistry. In the course of our continuing interest on the synthesis of condensed coumarin derivatives with anti-inflammatory and antioxidant activities [17,18] we have extended our research to the synthesis, characterization and biological evaluation of new bioactive coumarins derivatives **4a-g** *via* an environmentally friendly reaction condition. Here, we report: (i) the synthesis of novel 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(aryl) nicotine nitrile (**4a-g**) which were obtained by two different routes in the pursuit of finding bio-

logically active compounds that can be further investigated for their antimicrobial and antioxidant activities, (ii) the structures of these new coumarins derivatives were characterized by spectroscopic methods (^{1}H and ^{13}C NMR, FT-IR and elemental analysis, (iii). The obtained compounds were screened for their ability to inhibit the growth of a number of Gram-positive and Gram-negative and fungi strains, which have not been studied in the past. In addition, their antioxidant activities were evaluated.

EXPERIMENTAL

All manipulations were performed using standard Schlenck techniques under argon atmosphere. Chemicals were purchased from Sigma Aldrich and used without further purification. All the solvents were purified and dried by MBraun SPS 800 solvent purification system. Column chromatography was performed using silica gel 60 (70-230 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Chemical shifts, δ, are reported in ppm relative to the internal standard TMS for both ^{1}H and ^{13}C NMR. The NMR studies were carried out in high-quality 5 mm NMR tubes. Signals are quoted in parts per million as δ down-field from tetramethylsilane (δ = 0.00) as an internal standard. IR spectra were recorded on a 398 spectrophotometer (Perkin-Elmer, King Saud University, Ryadh, Saudi Arabia). Elemental microanalysis was performed on an Elementar Vario El III Carlo Erba 1108 elemental analyzer (Rennes, France) and the values found were within ± 0.3 % of the theoretical values. Melting points were determined with Kofler bench at Isste of Borjcedria (Hammam Lif, University of Carthage, Borj Cedria, Tunisia).

General procedure for synthesis of 3-acetyl-4-hydroxycoumarin (1): In a round bottom flask, 3 g (18.6 mmol) of 4-hydroxycoumarin, 16 mL of acetic acid (27.9 mmol) (solvent and reagent at a time) and 5.6 mL (60 mmol) of phosphorus oxychloride are added. The reaction was refluxed under continuous stirring for 1 h. Control of the evolution of the reaction by thin layer chromatography (TLC) (eluent: hexane-ethanol 80:20) revealed the formation of a single compound. At the end of the reaction, the product was filtered, recrystallized from ethanol, dried and recovered (**Scheme-I**).

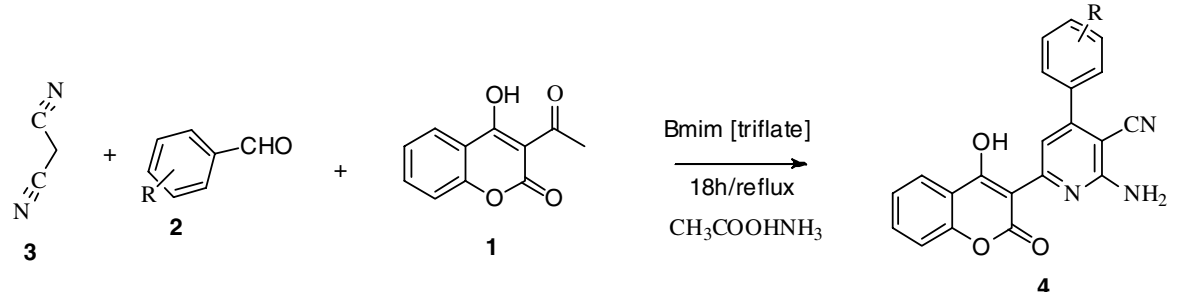
Yield: 92 %; m.p.: 225 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3185 (O-H); 1700 (CO lactone); ^{1}H NMR (400 MHz, DMSO-d_6) ppm δ: 2.45 (s, 3H, CO-CH_3); 7.15-7.92 (m, 4H, H_{arom}), 17.72 (s, OH). ^{13}C NMR (100 MHz, DMSO-d_6) in ppm δ: 32.36 (H_3C-CO); 102.32 (aryl-C=C-OH); 115.91, 122.98, 125.66 (C_{arom}) 132.95 (O-C_{arom}); 157.54 (CO-O); 162.92 (C=C-OH); 176.70 (H_3C-CO). Elemental analysis % calcd. (found) for $C_{11}H_8O_4$: C, 64.707 (64.60); H, 3.949 (4.09); N, 8.123 (8.07).

General procedure for the synthesis of 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(aryl) nicotine nitrile (4): A reaction mixture of (5 mmol) of 3-acetyl-4-hydroxycoumarin, (5 mmol) of aromatic aldehyde, malonitrile (5 mmol), with ammonium acetate (10 mmol) and 10 mL of dichloromethane was refluxed for an appropriate time under continuous agitation. The reaction was followed by (TLC). After completion of the reaction, the mixture was cooled to room temperature. The solid obtained was filtered, washed with ice water and purified by recrystallization in 95 % ethanol.

2-Amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-phenyl nicotine nitrile (4a): Yield: 95 %; m.p.: 120 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3505 (O-H); 3402 (NH_2); 1592 (C = C); 1715 (CO lactone), 2215 (CN). ^{1}H NMR (400 MHz, DMSO-d_6) in ppm: δ 7.27-8.18 (m, 12H, 10H_{arom} + aryl-NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) in ppm: δ 104,125.7, 124.6, 123.5, 122.4, 112.9, 119.1, 153.5, 152.1, 143.3, 134, 128.7, 128.4, 127.5 (C_{arom}); 116.2 (aryl-CN); 108.8 (aryl-C=C-OH); 136.6 (O-C_{arom}); 159.5 (C=C-OH); 157.9 (NH_2-C_{arom}, O-CO_{arom}). Elemental analysis % calcd. (found) for $C_{21}H_{13}N_3O_3$: C, 70.980 (70.82); H, 3.687 (3.90); N, 11.825 (11.80).

2-Amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(4-methylphenyl)nicotine nitrile (4b): Yield: 75 %; m.p.: 142 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3502 (O-H); 3410 (NH_2); 1585 (C = C); 1710 (CO lactone), 2215 (CN). ^{1}H NMR (400 MHz, DMSO-d_6) in ppm: δ 2.27 (s, 3H, aryl-CH_3); 7.27-8.19 (m, 11H, 9 H_{arom} + aryl-NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) in ppm: δ 24.5 (aryl-CH_3); 104.1, 125.7, 124.6, 123.5, 116.2, 153.6, 116.2, 104.1, 134.0, 132.8, 129.0, 127.4 (C_{arom}); 136.2 (O-C_{arom}); 119.1 (aryl-CN); 112.9 (aryl-C=C-OH); 157.9 (NH_2-C_{arom}, O-CO_{arom}); 159.5 (C=C-OH). Elemental analysis % calcd. (found) for $C_{22}H_{15}N_3O_3$: C, 71.536 (71.60); H, 4.093 (3.09); N, 11.376 (11.40).

2-Amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(4-N,N′-dimethylaminophenyl)nicotine nitrile (4c): Yield: 80 %; m.p.: 160 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3441 ((O-H); NH_2); 1604 (C = C); 1721 (CO lactone), 2233 (CN). ^{1}H NMR (400 MHz, DMSO-d_6) in ppm: δ 3.10 (s, 6H, aryl-N($CH_{3)2}$); 7.30 (s, 2H, aryl-NH_2); 6.83-8.03 (m, 9H, H_{arom}). ^{13}C NMR (100 MHz, DMSO-d_6) in ppm: δ 58.9, 69.1 (aryl-N(CH_3)$_2$); 105.1, 134.0, 131.3, 128.6, 125.0, 116.2, 104.1, 150.0, 153.2, 116.7, 134.0 (C_{arom}), 112.2 (aryl-C=C-OH); 119.2 (aryl-CN); 152.4 (O-C_{arom}); 159.3 (C=C-OH); 158.3 (O-CO_{arom}), 153.6 (NH_2-C_{arom}).



R = **4a**: H, **4b**: *p*-CH_3, **4c**: *p*-N(CH_3)$_2$, **4d**: *p*-NO_2, **4e**: *m*-Br, **4f**: *m*-OCH_3, **4g**: *m*-OH

Scheme-I: Synthetic protocol of 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl) -4-aryl nicotine nitrile (**4a-g**)

Elemental analysis % calcd. (found) for $C_{23}H_{18}N_4O_3$: C, 69.336 (69.41); H, 4.554 (4.70); N, 14.062 (14.10).

2-Amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(4-nitrophenyl)nicotine nitrile (4d): Yield: 85 %; m.p.: 210 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3507 (O-H); 3405 (NH_2); 1585 (C = C); 1715 (CO lactone), 2215 (CN). ^1H NMR (400 MHz, DMSO-d_6) in ppm: δ 7.28 (s, 2H, aryl-NH_2); 7.62-8.40 (m, 9H, H_{arom}). ^{13}C NMR (100 MHz, DMSO-d_6) in ppm: δ 85.2, 104.1, 134.0, 134.1, 125.6, 124.4, 148.8, 153.2, 143.4, 144.8, 130.1, 125.6 (C_{arom}) 144.8 (O-C_{arom}); 109.9 (C aryl-C=C-OH); 119.2 (aryl-CN); 177.9 (C=C-OH); 161.7 (O-CO_{arom}), 153.6 (NH_2-C_{arom}). Elemental analysis % calcd. (found) for $C_{21}H_{12}N_4O_5$: C, 63.002 (63.10); H, 3.021 (3.20); N, 13.995 (13.90).

2-Amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(3-bromophenyl) nicotine nitrile (4e): Yield: 87 %; m.p.: 160 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3502 (O-H); 3405 (NH_2); 1592 (C = C); 1716 (CO lactone), 2218 (CN). ^1H NMR (400 MHz, DMSO-d_6) in ppm: δ 7.31-8.38 (m, 11H, 9H_{arom} + NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) in ppm: δ 100.9, 134.0, 134.1, 125.6, 124.3, 148.8, 104.1, 132.9, 153.1, 125.7 (C_{arom}), 109.3 (aryl-C=C-OH); 119.2 (aryl-CN); 139.3 (O-C_{arom}). 159.3 (O-CO_{arom}), 178.4 (C=C-OH); 153.6 (Br-C_{arom}); 154.0 (NH_2-C_{arom}). Elemental analysis % calcd. (found) for $C_{21}H_{12}N_3O_3Br$: C, 58.084 (58.10); H, 2.785 (2.90); N, 9.677 (9.80).

2-Amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(3-methoxyphenyl) nicotine nitrile (4f): Yield: 87 %; m.p.: 160 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3510 (O-H); 3437 (NH_2); 1604 (C = C); 1710 (CO lactone), 2360 (CN). ^1H NMR (400 MHz, DMSO-d_6) in ppm: δ 3.72 (s, 3H, aryl-OCH_3); 7.40 (s, 2H, aryl-NH_2); 6.80-7.89 (m, 9H, H_{arom}). ^{13}C NMR (100 MHz, DMSO-d_6) in ppm: δ 55.4 (aryl-CH_3); 112.4, 133.3, 130.1, 125.1, 122.9, 120.1, 119.6, 125.7, 117, 97.9, 145.3 (C_{arom}); 97.9 (aryl-C=C-OH); 104.3 (aryl-CN); 160 (C=C-OH); 159.7 (O-CO_{arom}), 158.4 (NH_2-C_{arom}); 153.9 (O-C_{arom}); 152.6 (H_3C-OC_{arom}). Elemental analysis % calcd. (found) for $C_{22}H_{15}N_3O_4$: C, 68.566 (68.61); H, 3.923 (3.90); N 10.904 (10.82).

2-Amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(3-hydroxyphenyl) nicotine nitrile (4g): Yield: 95 %; m.p.: 180 °C; FT-IR (KBr, ν_{max}, cm^{-1}): 3501 (O-H); 3408 (NH_2); 1588 (C = C); 1731 (CO lactone), 2233 (CN). ^1H NMR (400 MHz, DMSO-d_6) in ppm: δ 7.02-8.51 (m, 12 H, 9H_{arom} + aryl-NH_2 + aryl-OH). ^{13}C NMR (100 MHz, DMSO-d_6) in ppm: δ 85.3, 116.6, 120.8, 119.8, 117.7, 104.1, 130.5, 116.6, 117.7 (C_{arom}); 92 (aryl-C=C-OH); 115.2 (aryl-CN); 134.3 (O-C_{arom}); 148.8 (NH_2-C_{arom}); 177.7 (C=C-OH); 158.0 (O-CO_{arom}); 153.2 (HO-C_{arom}). Elemental analysis % calcd. (found) for $C_{21}H_{13}N_3O_4$: C, 67.922 (67.81); H, 3.53 (3.60); N 11.316 (11.42).

Biological analysis was performed according to previous study [19].

Bacterial strains, media and growth conditions: Bacteria strains used as indicator microorganisms for the antibacterial activity assays were: *Micrococcus luteus* LB14110, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19117 *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 49189 were obtained from international culture collections (ATCC) and local culture collection of the Laboratory of Microorganisms and Biomolecules of the Centre of Biotechnology of Sfax, Tunisia. These bacterial strains were grown overnight in Luria-Bertani (LB) agar medium (g/L): peptone 10; yeast extract 5 and NaCl 5 at pH 7.2 under aerobic conditions and constant agitation (200 rpm) at 30 °C for *M. luteus* LB14110 and *L. monocytogenes* ATCC 19117 and at 37 °C for *S. aureus* ATCC 6538, *S. typhimurium* ATCC 14028 and *P. aeruginosa* ATCC 49189 and then diluted 1:100 in LB media and incubated for 5 h under constant agitation (200 rpm) at the appropriate temperature.

Agar well diffusion method: Agar well diffusion method was employed for the determination of the antimicrobials activity of the synthesized compounds according to Güven [20] with some modifications. Briefly, the synthesized compounds are allowed to diffuse out into the appropriate agar medium (LB agar medium) and interact in a plate freshly seeded with a suspension of the indicators microorganisms (0.1 mL of 10^8 cells per mL). The plate was incubated at the appropriate temperature after staying at 4 °C for 2 h. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The antibacterial activity was assayed by measuring in millimeters the diameter of the inhibition zone formed around the well. All tests are assayed in triplicate and expressed as the average ± standard deviation of the measurements.

Minimum inhibitory concentration (MIC) were done regarding our previous study [19].

The minimum inhibitory concentration (MIC) of the synthesized compounds was determined in accordance with NCCLS guideline M7-A6 and M38-P [21]. The test was performed in sterile 96-well microplates with a final volume in each microplate well of 100 mL. The synthesized compounds (20 mg/mL) were properly prepared in solution of dimethylsulfoxide/water (1/9; v/v). The inhibitory activity of each synthesized compound was transferred to each well in order to obtain a twofold serial dilution of the original sample and to produce the concentration range of 0.0048-20 mg/mL. To each test well 10 mL of cell suspension were added to final inoculum concentrations of 10^6 CFU/mL for each microorganisms. Positive growth control wells consisted of microorganisms only in their adequate medium. Cells suspension at the same concentration supplemented with ampicillin was used as control. The plates were then covered with the sterile plate covers and incubated at the appropriate temperature of each microorganism. The MIC was defined as the lowest concentration of the synthesized compound at which the microorganism does not demonstrate visible growth after incubation. As an indicator of microorganism growth, 25 mL of thiazolyl blue tetrazolium bromide (MTT), indicator solution (0.5 mg/mL) dissolved in sterile water were added to the wells and incubated at room temperature for 30 min. This determination was done in triplicate and obtained results were very similar. The reported value is the average of the three tests.

Antioxidant activity: DPPH radical scavenging activity were done regarding our previous study [19].

Scavenging of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH_assay) is the simplest and most widely reported method for screening antioxidant activity. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm. This assay determines the scavenging of

stable radical species according to the method of Kirby and Schmidt [22] with slight modifications. Briefly, synthesized compounds were dissolved in dimethylsulfoxide (DMSO)/ water (1/9; v/v) and diluted with ultrapure water at different concentrations (1, 0.5, 0.250, 0.125, 0.0625, 0.03125 mg/mL). Then, 500 mL of a 4 % (w/v) solution of DPPH radical in ethanol was mixed with 500 mL of samples. The mixture was incubated for 30 min in the dark at room temperature. The scavenging capacity was determined spectrophotometrically by monitoring the decrease in absorbance at 517 nm against a blank. The percentage of antiradical activity (% ArA) had been calculated as follows:

$$\text{ArA (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

All tests are assayed in triplicate and expressed as the average ± standard deviation of the measurements.

RESULTS AND DISCUSSION

The first efficient one-pot synthesis of 4-aryl-1,2-dihydro-6-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxopyridin-3-carbonitriles has been developed from malonitrile, aromatic aldehyde and 3-acetyl-4-hydroxy coumarin, the approach is outlined in **Scheme-I**.

3-Acetyl-4-hydroxycoumarin (**1**) was synthesized by acetylation of the 4-hydroxycoumarin, by the method of Dholakia *et al.* [23] using glacial acetic acid as acetylating agent in the presence of $POCl_3$. The reaction was rapid, without involving any kind of competition from the intramolecular condensation of 4-hydroxycoumarin [24].

This compound **1** was characterized by IR, ^{1}H and ^{13}C NMR. 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 ^{1}H NMR shows that aromatic proton arrow as a multiplet between 7.15 and 7.92 ppm. A singlet at 2.45 ppm was assigned to methylic proton whilst the OH signal appeared at 17.72 ppm. This very high value of the chemical shift might be explained by only an intermolecular hydrogen bond [25].

In order to seek optimal solvent and optimal amounts of catalyst, we run a model reaction by stirring equimolecular amounts of 3-acetyl-4-hydroxycoumarin (**1**) with malonitrile and benzaldehyde in the presence of ammonium acetate. The model reaction was explored using different solvents such as Bmim[triflate], DCM, DCE, ethanol, tetrahydrofuran and toluene in the presence of ammonium acetate. The results are summarized in Table-1.

It was found that polarity of solvent and the presence of ammonium acetate play an important role for the success of the reaction. The results indicated that solvents were also affected on the yield of of the products (Table-1). In the organic solvents such as dichloromethane, THF, ethanol, or toluene, the yield of **4** were lower and longer reaction times were required.

It was observed that among all solvents and media, the best result was obtained when Bmim[triflate] was chosen in the presence of ammonium acetate at 90 °C. The desired product was obtained in excellent yield and high purity.

TABLE-1
SYNTHESIS OF 4-PHENYL-1,2-DIHYDRO-6-(4-HYDROXY-2-OXO-2*H*-CHROMEN-3-YL)-2-OXOPYRIDIN-3-CARBONITRILE (**4a-g**) IN THE PRESENCE OF DIFFERENT SOLVENTS

Entry	Solvent	Temp. (°C)	Time (min)	Yield (%)[a]
1	DCM	90	30	83
2	DCE	128	30	80
3	Ethanol	78	120	72
4	THF	66	120	62
5	Bmim [triflate]	100	30	95
6	DCM	40	120	60
7	Toluene	110	120	62

Reaction conditions: 3-acetyl-4-hydroxycoumarin (5 mmol), malonitrile (5 mmol), benzaldehyde (5 mmol), solvent (15 mL), reflux, ammonium acetate (10 mmol).
[a]Isolated yield of product.

Also, in order to optimize the ammonium acetate loading, the model reaction was performed with different amounts of catalyst at ambient temperature. The results are summarized in Table-2.

TABLE-2
EFFECT OF CATALYST AMOUNT ON THE CONDENSATION OF BENZALDEHYDE, ETHYL CYANOACETATE AND 3-ACETYL-4-HYDROXYCOUMARINE (**1**) IN Bmim[triflate][a]

Entry	Catalyst	Mol (%)	Time (h)	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	10 h 20 min	88
7	No catalyst	-	10 h 20 min	10

[a]Reaction conditions: 3-acetyl-4-hydroxycoumarin (5 mmol), malonitrile (5 mmol), benzaldehyde (5 mmol), solvent (15 mL), reflux.
[b]Isolated yield of product.

It was found that, when the reaction was carried out in the presence of 5 mol % of catalyst, 60 % of yield was obtained. As we increase the percentage of the catalyst to 10, 15 and 20 mol %, the yields were also found to be increased up to 85, 82 and 95 %, respectively, but beyond 20 mol % there is no significant improvement of the rate as well as yield of the reaction and further increase in the quantity of catalyst did not show appreciable improvement in the yield of product. Thus, 20 mol % of catalyst was chosen as maximum quantity of the catalyst for the reaction.

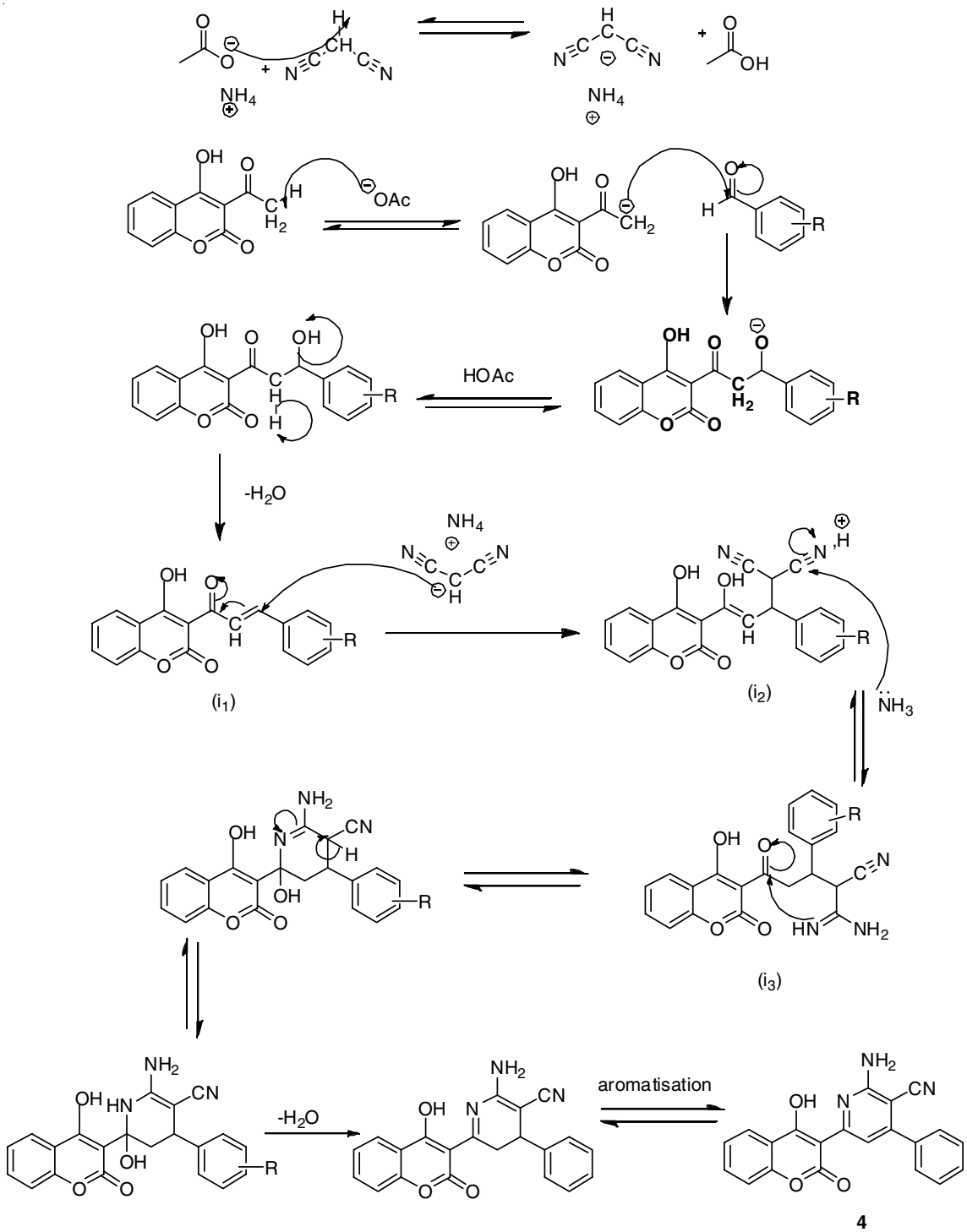
To explore the scope and generality of this multicomponent reaction (MCR), we tried to construct a library of 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-aryl nicotine nitrile (**4a-g**) derivatives using the optimized reaction conditions, the scope of the method was investigated with a series of substituted aromatic aldehydes (**Scheme-I**).

A variety of aryl aldehydes containing different substituents (*e.g.* $-NO_2$, –OH, –Br, $-OCH_3$, –OMe, -*p*-$N(CH_3)_2$ reacted in this MCR to provide excellent product yields (75–95 %). The aromatic aldehydes carrying both electron withdrawing (Entries **1-5**) and electron-donating functional groups under-went successful condensation with ethyl cyanoacetate and 4-hydroxycoumarin in the presence of catalytic amount of ammonium acetate in Bmim[triflate] at reflux to afford the

corresponding products **4a-g** in good yields. moreover, the electronic effects of the substituent were not observed.

In IR spectra, bands in the region 3510-3414 cm^{-1} were attributed to the OH group of **4a-g** seriesand bands in the region 1625-1520 cm^{-1} were attributed to symmetric and asymmetric frequency of the NO_2 group of the compound **4d**. Bands at 1731-1710 cm^{-1} obtained from the lactone ring of coumarin CO. 1585-1604 cm^{-1} stretching frequencies correspond to the C=C groups of coumarins derivatives. In ^{1}HNMR spectra, the singlet between δ 7.27–7.40 ppm corresponds to the presence of the NH_2 group of the compounds **4a-g**. The absence of methylic proton signal at δ = 2.45 ppm and the presence of the signal of NH_2 at support the formation of compounds **4a-g**. In ^{13}C NMR, signal at δ 119.2 assigned to CN, signal at δ 168.2–169.2 confirmed lactone carbonyl of **4c** and the signal at δ 3.10 ppm due to $N(\underline{C}H_3)_2$. Other signals are in good agreement with the target compounds in accordance with the literature data for other 4-hydroxycoumarin derivatives [26-33].

The formation of compound **4** could be explained by the reaction sequence in **Scheme-II**. First, condensation of



Scheme-II: A proposed mechanism for the three-component synthesis of 4-aryl-1,2-dihydro-6-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxopyridin-3-carbonitriles (**4a-g**)

3-acetyl-4-hydroxycoumarin (**1**) with arylaldehydes is proposed to give intermediate (i_1), then malonitrile react with intermediate (i_1) to give intermediate (i_2) occurs to provide the intermediate (i_3) which undergoes isomerization to form the target 4-aryl-1,2-dihydro-6-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxopyridin-3-carbonitriles (**4a-g**).

In second path, we tried to synthetize coumarins derivatives **4** with another route. The 3-acetyl-4-hydroxycoumarin (**1**) condensed with arylaldehydsin the presence of piperidine gaves intermediate (i), then this precursor was refluxed with malonitrile and Bmim[triflate] to achieve targeted molecule **4** (**Scheme-III**).

The molecular structures and purity of the newly synthesized compounds were identified by NMR (^{1}H and ^{13}C), FT-IR and elemental analysis (CHN). In FT-IR spectra, absorption bands attributed to symmetric and asymmetric stretching vibrations of amino groups appeared within ν = 3456-3428 and 3326-3281 cm^{-1}, as well as stretching vibrations of nitro groups were recorded within ν = 1543-1514 and 1331-1318 cm^{-1}. The presence of nitrile groups was deduced both from IR bonds at ν = 2228-2206 cm^{-1}.

In summary, both synthetic routes successfully provided a strategy for synthesis of synthesized 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(aryl) nicotinenitrile **4a-g**.

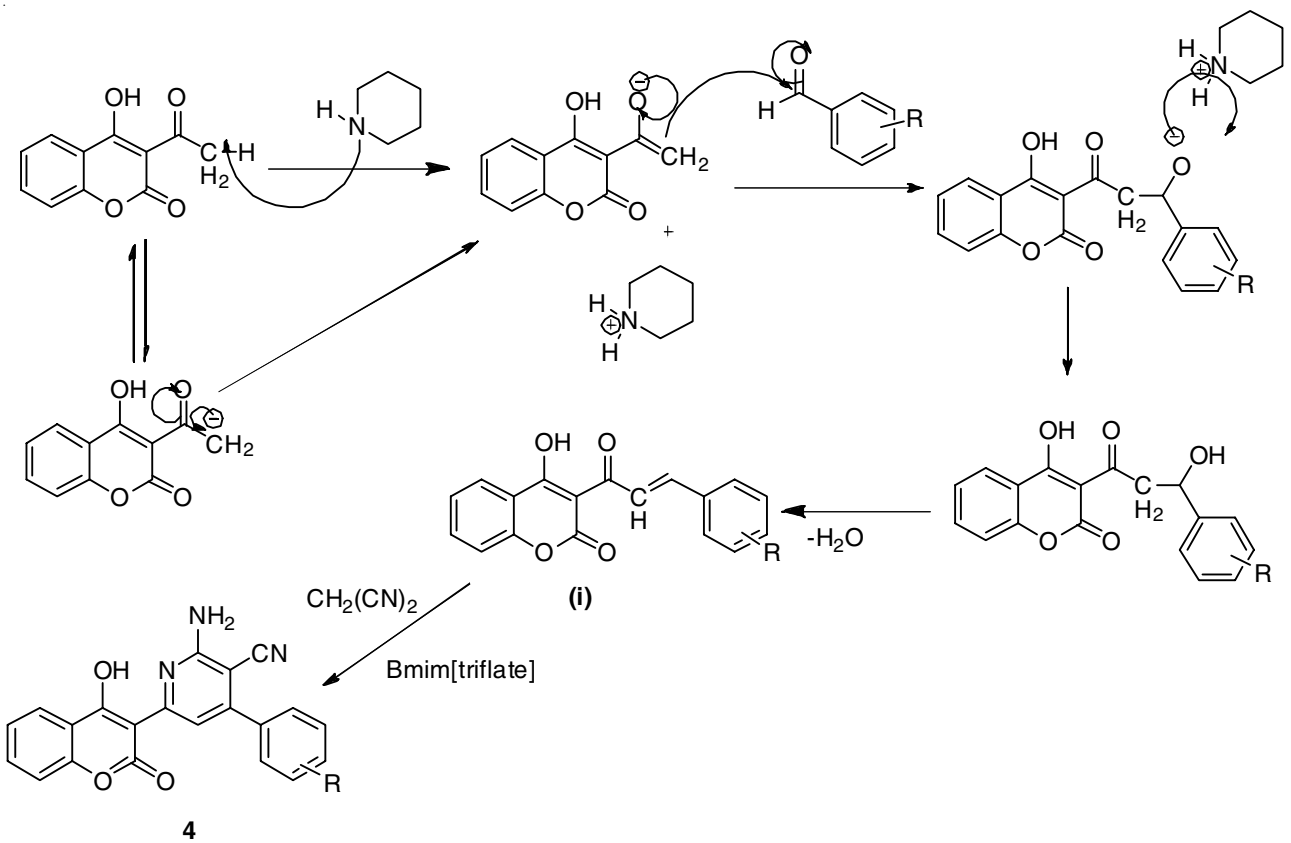
In first route, the 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(aryl) nicotine nitrile **4a-g** is available and the starting material is low-cost and can be obtained easily. This synthetic route not only saves the time but lowers the cost for manufacture. The second route is longer. For these reasons, it is not suitable for large scale preparation. The second route is based on the materials commercially available, the reaction involves 2 steps. The total yield was up to 20 %.

Antioxidant activity of synthesized 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(aryl)nicotinenitrile (4a-g): The *in vitro* antioxidant activities of the coumarins derivatives **4** were screened using the DPPH (1,1-diphenyl-2-picrilhydrazyl). The results obtained from the two approaches were consistent

L-Ascorbic acid and BHT were used as the standard antioxidant. The results are expressed as mg/mL The activity results of the newly synthesized compounds are represented in Fig. 1. We found that most of the compounds showed considerable free radical scavenging activity.

The analysis of the results shows that the profiles of antiradical activity obtained reveal that most of the synthetic products tested have an antiradical activity. The three products **4a, 4d** and **4e** have a medium antioxidant activity, while the product **4f** has a very significant activity and close to the antiradical activity of the two synthetic antioxidants used as references (gallic acid and BHT).

Antimicrobial activity of 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(aryl) nicotine nitrile (4a-g): The *in vitro* antibacterial activity of the samples **4a-g** was assessed against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Micrococcus luteus* and *Candida albicans*, respectively. The different bacterial species used Gram-positive bacteria: *Micrococcus luteus* LB14110, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19117, Gram-negative bacteria: *Escherichia coli*, *Salmonella typhimurium* ATCC



Scheme-III: Synthesis of coumarins derivatives **4** by two steps

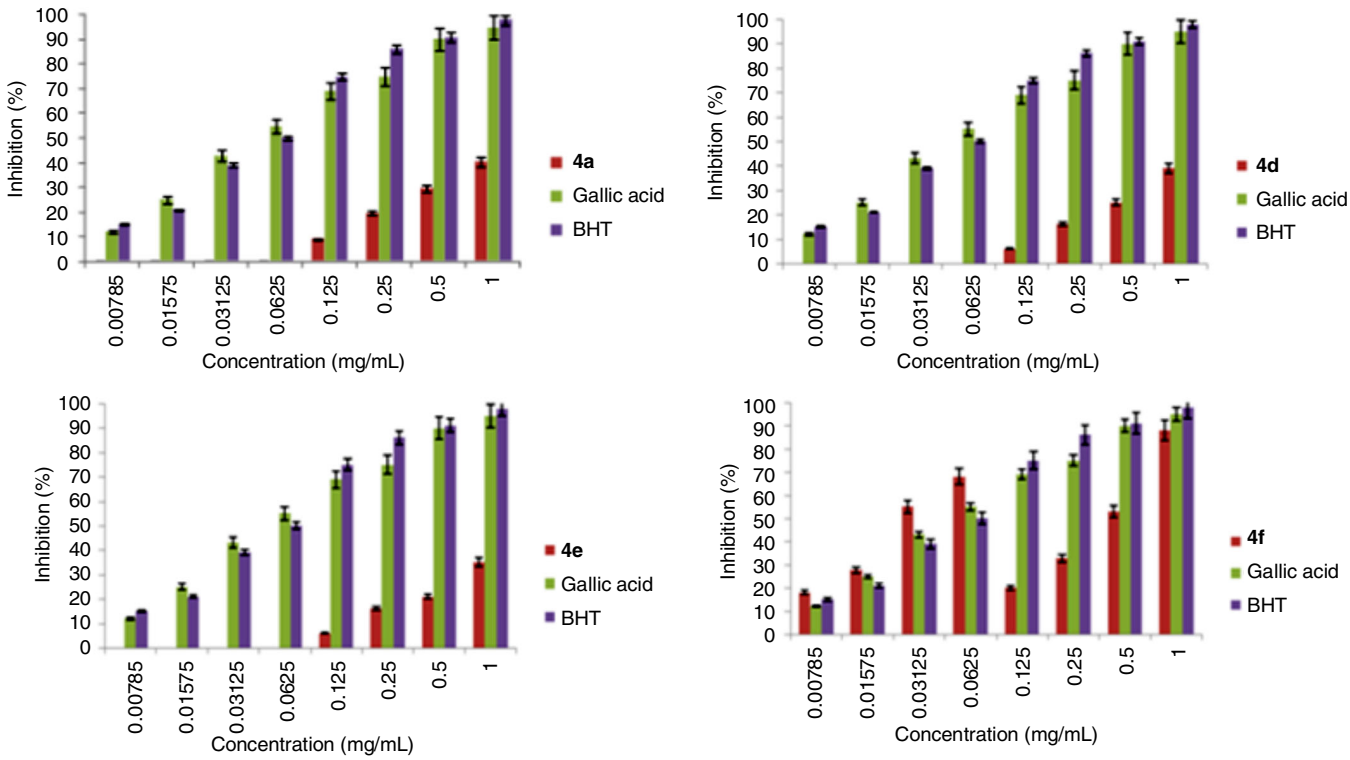


Fig. 1. Scavenging activity of 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(aryl)nicotine nitrile (**4**) on DPPH radical

14028 and *Pseudomonas aeruginosa* and the fungus *Candida albicans* were provided by the Laboratory of Microorganisms and Biomolecules of the Biotechnology Center of Sfax. These species kept on nutrient agar, are reactivated on agar medium 24 h before performing the antimicrobial tests.

As a first approach, we used the traditional method of detecting antimicrobial activities (well method). The synthesized products are prepared with DMSO at an initial concentration of 20 mg/mL. All the results of this study are shown in Fig. 2.

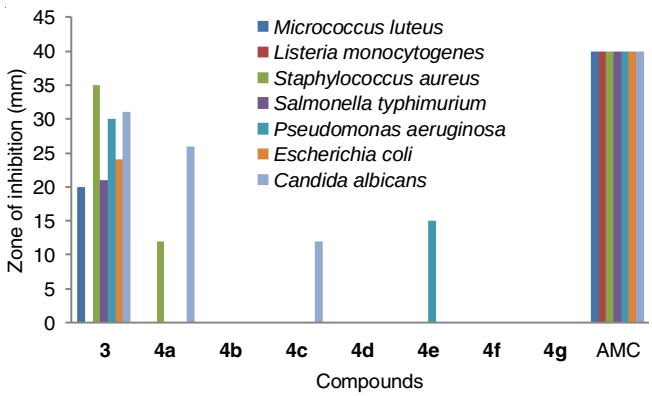


Fig. 2. Antibacterial activity of the prepared compounds **4a-g**

It is found that the activity of the synthesized compounds depends on their concentration and the bacterial strain tested. Gram-positive bacteria were more sensitive to the antimicrobial properties of synthesized compounds than Gram-negative bacteria. This effect can be attributed to the great complexity of the membrane-containing cell envelope in Gram-negative bacteria compared to the unique structure of Gram-positive membrane.

From Fig. 2, compound **4a** was effective against *Candida albicans* and against *Staphylococcus aureus* ATCC 6538, this compound has moderate activity. However, compound **4e** synthesized and tested in this study shows significant activity against *Pseudomonas aeruginosa* ATCC 49189.

Determination of minimum inhibitory concentration (MIC): Minimum inhibitory concentration (MIC) values were determined using the standard broth micro-dilution technique recommended by the Clinical and Laboratory Standard Institute [34,35]. The MIC results showed that all tested coumarin peptide salts (Table-3) had weak antimicrobial effects against *E. coli* and good to moderate effects against *S. aureus*.

TABLE-3
MIC VALUES OF PRODUCTS TESTED
AGAINST TWO BACTERIA AND ONE FUNGUS

Microorganisms	**3**	**4a**	Ampicilline	Fluconazole
S. aureus	0.6250	1.2500	0.04	–
P. aeruginosa	0.3125	1.2500	0.04	–
C. albicans	0,1562	0.0195	–	0.00125

According to the data reported in Table-3, the derivatives were ordered based on the spread of inhibitory properties and the MIC values as follows:

4b > 4e > 4d > 4c

We evaluated the antimicrobial activity of the two synthetic products **3** and **4a** with the highest activity against two bacteria (*S. aureus* and *P. aeruginosa*) and against a fungus (*Candida albicans*) by the determination of the MIC in a liquid

medium. For *S. aureus* ATCC 6538: 0.625 and 1.25 µg/mL respectively for products **3** and **4a** and for *P. aeruginosa* ATCC 49189: 0.3125 and 1.25 µg/mL, respectively for products **3** and **4a**.

Conclusion

In conclusion, a series of 2-amino-6-(4-hydroxy-2-oxo-chromen-3-yl)-4-(aryl) nicotine nitrile were designed, synthesized using two routes and evaluated for their antimicrobial and antioxidant activities. The obvious advantages of the methods are: operational simplicity, high atom economy and excellent yields. The preparations of the new products are supported by elemental analyses, IR and ^{1}H NMR and ^{13}C NMR. The compounds **4a-g** were investigated for antioxidant activities by DPPH (2,2-diphenyl-1-picrylhydrazyl). Furthermore, compounds **4a-g** were evaluated for antimicrobial activity by well method and determination of minimum inhibitory concentration (MIC). These compounds may serve as lead compounds for further development into novel antimicrobial and antioxidant agents. In addition the obtained compounds **4** we be used as new materials for hydrogen storage.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. M.A. Musa, V.L. Badisa, L.M. Latinwo, J. Cooperwood, A. Sinclair and A. Abdullah, *Anticancer Res.*, **31**, 2017 (2011).
2. P. Borah, P. Seetham Naidu and P.J. Bhuyan, *Tetrahedron Lett.*, **53**, 5034 (2012); https://doi.org/10.1016/j.tetlet.2012.07.060.
3. S.L. El-Ansary, S.E. Abbas, A.N. Mikhael and H.A. El-Banna, *Egypt. J. Pharm. Sci.*, **33**, 639 (1992).
4. S. Manfredini, D. Simoni, R. Ferroni, R. Bazzanini, S. Vertuani, S. Hatse, J. Balzarini and E. De Clercq, *J. Med. Chem.*, **40**, 3851 (1997); https://doi.org/10.1021/jm9602322.
5. L.W. Wattenberg, K.T. Lam and A.V. Fladmoe, *Cancer Res.*, **39**, 1651 (1979).
6. Y. Kashman, K.R. Gustafson, R.W. Fuller, J.H. Cardellina, J.B. McMahon, M.J. Currens, R.W. Buckheit, S.H. Hughes, G.M. Cragg and M.R. Boyd, *J. Med. Chem.*, **35**, 2735 (1992); https://doi.org/10.1021/jm00093a004.
7. T.C. Mckee, R.W. Fuller, C.D. Covington, J.H. Cardellina, B.L. Krepps, R.J. Gulakowski, J.B. McMahon and M.R. Boyd, *J. Nat. Prod.*, **59**, 754 (1996); https://doi.org/10.1021/np9603784.
8. N.F. Anjum, A. Aleem, N. Nayeem and S.M. Asdaq, *Der Pharm. Chem.*, **3**, 56 (2011).
9. S.M. De Souza, F. Delle Monache and A. Smânia Jr., *Z. Naturforsch. C*, **60**, 693 (2005); https://doi.org/10.1515/znc-2005-9-1006.
10. A. Behrami, *Orient. J. Chem.*, **30**, 1747 (2014); https://doi.org/10.13005/ojc/300433.
11. J. Jung, J. Kim and O. Park, *Synth. Commun.*, **29**, 3587 (1999); https://doi.org/10.1080/00397919908085993.
12. W.M. Barker, M.A. Hermodson and K.P. Link, *J. Med. Chem.*, **14**, 167 (1971); https://doi.org/10.1021/jm00284a022.
13. M. Greaves, *PLoS Med.*, **2**, 342 (2005); https://doi.org/10.1371/journal.pmed.0020342.
14. C. Montagner, S.M. de Souza, C. Groposo, F. Delle Monache, E.F.A. Smânia and A. Smânia Jr., *Z. Naturforsch. C*, **63**, 21 (2008); https://doi.org/10.1515/znc-2008-1-205.
15. R. de Araújo, F. Guerra, E. de O. Lima, C. de Simone, J. Tavares, L. Scotti, M. Scotti, T. de Aquino, R. de Moura, F. Mendonça and J. Barbosa-Filho, *Int. J. Mol. Sci.*, **14**, 1293 (2013); https://doi.org/10.3390/ijms14011293.
16. G. Mazzone, N. Malaj, A. Galano, N. Russo and M. Toscano, *RSC Adv.*, **5**, 565 (2015); https://doi.org/10.1039/C4RA11733F.
17. J. Raboin, M. Beley and G. Kirsch, *Tetrahedron Lett.*, **41**, 1175 (2000); https://doi.org/10.1016/S0040-4039(99)02255-8.
18. N.B. Karatzas, *Hellenic J. Cardiol.*, **55**, 89 (2014).
19. N. Touj, A. Chakchouk-Mtibaa, L. Mansour, A.H. Harrath, J.H. Al-Tamimi, I. Özdemir, L. Mellouli, S. Yasar and N. Hamdi, *J. Organomet. Chem.*, **853**, 49 (2017); https://doi.org/10.1016/j.jorganchem.2017.09.024.
20. K. Güven, E. Yücel and F. Cetintas, *Pharm. Biol.*, **44**, 79 (2006); https://doi.org/10.1080/13880200600591253.
21. National Committee for Clinical Laboratory Standard, Referece method for Broth Dilution Antifungal Susceptibility Testing of Conidium-Forming Filamentous Fungi, Proposed Standard M38-P; National Committee for Clinical Laboratory Standard: Wayne, USA (1998).
22. A.J. Kirby and R. Schmidt, *J. Ethnopharmacol.*, **56**, 103 (1997); https://doi.org/10.1016/S0378-8741(97)01510-9.
23. V.N. Dholakia, M.G. Parekh and N.K. Trivedi, *Aust. J. Chem.*, **21**, 2345 (1968); https://doi.org/10.1071/CH9682345.
24. M. Eggenweiler, M. Gassen, J. Rochus, O. Poeschke and M. Wolf, *Chem. Abstr.*, **134**, 331 (2001).
25. K.C. Fylaktakidou, D.J. Hadjipavlou-Litina, K.E. Litinas and D.N. Nicolaides, *Curr. Pharm.*, **10**, 3813 (2004); https://doi.org/10.2174/1381612043382710.
26. F. Moreau, N. Desroy, J.M. Genevard, V. Vongsouthi, V. Gerusz, G. Le Fralliec, C. Oliveira, S. Floquet, A. Denis, S. Escaich, K. Wolf, M. Busemann and A. Aschenbrenner, *Med. Chem. Lett.*, **18**, 4022 (2008); https://doi.org/10.1016/j.bmcl.2008.05.117.
27. A. Dömling, *Chem. Rev.*, **106**, 17 (2006); https://doi.org/10.1021/cr0505728.
28. J.J. Wang, X. Feng, Z. Xun, D.Q. Shi and Z.B. Huang, *J. Org. Chem.*, **80**, 8435 (2015); https://doi.org/10.1021/acs.joc.5b01314.
29. A. Kumar, M.K. Gupta and M. Kumar, *Tetrahedron Lett.*, **52**, 4521 (2011); https://doi.org/10.1016/j.tetlet.2011.06.040.
30. P.P. Ghosh and A.R. Das, *Tetrahedron Lett.*, **53**, 3140 (2012); https://doi.org/10.1016/j.tetlet.2012.04.033.
31. H.Y. Wang, X.C. Liu, X. Feng, Z.B. Huang and D.Q. Shi, *Green Chem.*, **15**, 3307 (2013); https://doi.org/10.1039/c3gc41799a.
32. S. Khodabakhshi and B. Karami, *Tetrahedron Lett.*, **55**, 7136 (2014); https://doi.org/10.1016/j.tetlet.2014.11.016.
33. S. Yaragorla, A. Pareek and R. Dada, *Tetrahedron Lett.*, **56**, 4770 (2015); https://doi.org/10.1016/j.tetlet.2015.06.049.
34. A. Braca, N. De Tommasi, L. Di Bari, C. Pizza, M. Politi and I. Morelli, *J. Nat. Prod.*, **64**, 892 (2001); https://doi.org/10.1021/np0100845.
35. J.C. Cappuccino and N. Sherman, Microbiology-A Laboratory Manual, Addison Wesley: California, USA, p. 263 (1999).