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DRUG SYNTHESIS

SYNTHESIS OF SOME NEW CARBONITRILES AND PYRAZOLE COUMARIN DERIVATIVES WITH POTENT ANTITUMOR AND ANTIMICROBIAL ACTIVITIES

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Abstract: 3-Acetyl-4-hydroxycoumarin (**2**) was reacted with some aldehydes (4-chlorobenzaldehyde, 4-bromobenzaldehyde, 5-methylfurfural) to afford the chalcones (**3a–c**). Cyclization of these chalcones with malononitrile in the presence of ammonium acetate afforded pyridine carbonitriles (**4a–c**), while the cyclization reaction of chalcones (**3a–c**) with ethyl cyanoacetate afforded the oxopyridine carbonitriles (**5a–c**). On the other hand, the chalcones (**3a–c**) reacted with hydrazine hydrate in alcohol to yield pyrazoles (**6a–c**), but when the same reaction is carried out in the presence of acetic acid, the acetyl pyrazole derivatives (**7a–c**) were obtained. Finally, the reaction of the chalcones (**3a–c**) with phenylhydrazine afforded phenylpyrazole derivatives (**8a–c**). The structures of synthesized compounds were confirmed by their micro analysis and spectral data (IR, NMR and MS). Twelve samples were evaluated for the human breast adenocarcinoma cytotoxicity, three of them showed moderate activity, the rest of the samples showed weak cytotoxic activity (very high IC_{50}), but for the hepatocarcinoma cell lines four samples showed weak cytotoxic effect, while the rest of the compounds showed very weak effect. For antimicrobial study, three compounds proved to be the most promising against tested bacterial organisms.

Keywords: coumarin, chalcones, carbonitriles, pyrazoles, antitumor, antimicrobial

Cancer represents one of the most severe health problems worldwide and the development of new anticancer drugs and more effective strategies are the areas of the utmost importance in drug discoveries and clinical therapy. Much of the research in these areas is currently focused in cancer specific mechanism and the corresponding molecular targets (1).

Coumarins are classified as a members of benzopyrone family. All of them consist of a benzene ring joined to a pyrone ring. Coumarins are of great interest due to their pharmacological properties, in particular, their antitumor and antimicrobial activity made these compounds attractive for further derivatization and screening as novel therapeutic agents. The literature investigation revealed cytotoxic activity of coumarin against several human tumor cell lines (2). From the literature, we can conclude that the chalcones have been reported to possess biological property as antitumor activity (3–6) and antimicrobial activity (7).

It was found that pyrazoles present an interest group of compounds, many of which possess widespread pharmacological properties as antitumor activities (8–11) and antimicrobial activity (12). In addition, we can say that the anticancer activity of many compounds is due to the presence of nitrogen heterocyclic ring (13).

Our goal in this work was to prepare some carbonitriles and pyrazolyl 4-hydroxycoumarins with potent antitumor and antimicrobial activities.

EXPERIMENTAL

Materials and methods

Melting points were determined on Electrothermal IA 9000 apparatus and were uncorrected. Elemental microanalyses were performed on

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Elementar, Vario EL, at the microanalytical center. The infrared (IR) spectra were recorded on Nexus 670 FT-IR FT-Raman spectrometer in potassium bromide discs, the proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on Varian Mercury 500 MHz spectrometer, using tetramethylsilane (TMS) as an internal standard. The mass spectra (MS) were performed on Jeol JMS-AX500 mass spectrometer. All spectral data were carried out at the National Research Center, Cairo, Egypt. The reactions were followed by TLC (silica gel, aluminum sheets 60 F 254, Merck) using benzene : ethyl acetate (8 : 2, v/v) as eluent and visualized with iodine-potassium iodide reagent.

Synthesis of 3-acetyl-4-hydroxy-2H-chromen-2-one (2)

3-Acetyl-4-hydroxycoumarin has been synthesized by boiling 4-hydroxycoumarin (**1**) (1 g, 6 mmol) with phosphorus oxychloride (2 mL) in glacial acetic acid (5 mL). The solution was cooled and water was added to precipitate the desired yellowish brown solid of 3-acetyl-4-hydroxycoumarin (0.90 g, 73% yield) that was recrystallized from ethyl alcohol – water to yield the desired compound with m.p. 132–134^{o}C (as reported in (14)).

General procedure for the synthesis of 1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-3-aryl-2-propen-1-ones (3a–c)

A solution of 3-acetyl-4-hydroxycoumarin (**2**) (1 g, 5 mmol) in ethyl alcohol (10 mL) and the selected aldehyde, namely: 4-bromobenzaldehyde, 4-chlorobenzaldehyde, and 5-methylfurfural (5 mmol) in the presence of piperidine (1 mL) was refluxed for 5–7 h. The solution was cooled and water was added to precipitate the desired chalcone compound.

Compounds **3a** and **3b** were prepared according to Zavrsink et al. (15).

4-Hydroxy-3-[(E)-3-(5-methylfuran-2-yl)acryloyl]-2H-chromen-2-one (3c)

M.p. 218–221^{o}C; yield 84%. IR (ν, cm^{-1}): 3434 (OH, brs), 2974 (-CH aliphatic stretching), 1725 (C=O, α-pyrone), 1611 (C=O), 1527 (C=C). ^{1}H NMR ($CDCl_3$, δ, ppm): 2.2 (3H, s, CH_3), 6.2–6.8 (2H, d, acryl-H), 7.2–8.1 (6H, m, Ar-H), 9.1 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A. %): (M^+) 296 (15%), 279 (32%), 254 (20%), 135 (3%), 64 (100%).

General procedure for the synthesis of 4-aryl-2-amino-6-(4-hydroxy-2-oxo-2H-chromen-3-yl)-pyridine-3-carbonitriles (4a–c)

Method A: To a mixture of 3-acetyl-4-hydroxycoumarin (1 g, 5 mmol) and the appropriate aldehyde, namely: 4-bromobenzaldehyde, 4-chlorobenzaldehyde or 5-methylfurfural (5 mmol) in ethyl alcohol (20 mL), malononitrile (0.33 g, 5 mmol) and ammonium acetate (0.75 g, 10 mmol) were added. The reaction mixture was refluxed for 5–6 h. The obtained solid was filtered off, washed with absolute ethyl alcohol and recrystallized from methyl alcohol to give the desired compounds.

Method B: An ethanolic mixture of the selected chalcone (**3a–c**) (1.25 mmol), and malononitrile (0.06 g, 1.25 mmol) in the presence of ammonium acetate (0.2 g, 2.5 mmol) was refluxed for 4–5 h. After cooling, the obtained solid was filtered off, washed with ethyl alcohol and recrystallized from methyl alcohol to give the title compounds.

Compounds **4b** and **4c** were prepared according to Mohamed et al. (16).

2-Amino-4-(4-bromophenyl)-6-(4-hydroxy-2-oxo-2H-chromen-3-yl)pyridine-3-carbonitrile (4a)

M.p. 295–295^{o}C; yield: 89%. IR (ν, cm^{-1}): 3472 (OH, brs), 3372 (NH_2), 2213 (C=N), 1698 (C=O, α-pyrone), 1595 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 4.33 (2H, s, NH_2, D_2O exchangeable), 7.55–8.5 (8H, m, Ar-H) and 7.33 (1H, s, pyridine proton), 8.92 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A. %): (M^+ + 2) 436 (35%), 434 (100%), 408 (13%), 313 (22%), 179 (13%), 121 (93%).

General procedure for the synthesis of 4-aryl-1,2-dihydro-6-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxopyridine-3-carbonitriles (5a–c)

An ethanolic mixture of chalcone (**3a–c**) (1.6 mmol), and ethyl cyanoacetate (0.19 mL, 1.6 mmol) in the presence of ammonium acetate (0.23 g, 3.33 mmol) was refluxed for 12 h. After cooling, the obtained solid was filtered off, washed with ethyl alcohol and recrystallized from methyl alcohol to give the title compounds.

4-(4-Chlorophenyl)-1,2-dihydro-6-(4-hydroxy-2-oxo-2H-chromen-3-yl)-2-oxopyridine-3-carbonitrile (5b)

M.p. 171–173^{o}C; yield 80%. IR (ν, cm^{-1}): 3420 (OH, brs), 3100 (NH), 2700 (C=N), 1680 (C=O, α-pyrone), 1612 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.9 (1H, s, NH, D_2O exchangeable), 7.2–7.8 (8H, m, Ar-H) and 6.8 (1H, s, pyridine carbonitrile proton), 14.6 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A. %): (M^+) 390 (22%), 364 (36%), 312 (65%), 229 (8%), 163 (13%).

1,2-Dihydro-6-(4-hydroxy-2-oxo-2H-chromen-3-yl)-4-(5-methylfuran-2-yl)-2-oxopyridine-3-carbonitrile (5c)

M.p. 100–102°C; yield 70%. IR (ν, cm^{-1}): 3228 (OH, brs) 3124 (NH), 2272 (C=N), 1746 (C=O, α-pyrone), 1667 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.4 (3H, s, CH_3), 7.25 (1H, s, NH, D_2O exchangeable), 7.3–8.1 (6H, m, Ar-H) and 6.5 (1H, s, pyridine carbonitrile proton), 14.5 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A. %): (M^+) 360 (0.56%), 337 (0.45%), 257 (0.71%), 151 (8%), 109 (21%), 63 (22%).

General procedure for the synthesis of 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromen-2-one derivatives (6a–c)

A mixture of the appropriate chalcone (**3a–c**) (1 mmol) and hydrazine hydrate 99% (2 mmol) in ethyl alcohol (30 mL) was refluxed for one hour. The reaction mixture was cooled and the formed precipitate was filtered off, washed and recrystallized from methyl alcohol to give compounds (**6a–c**).

3-[5-(4-Bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-4-hydroxy-2H-chromen-2-one (6a)

M.p. 229–231°C; yield 84%. IR (ν, cm^{-1}): 3289 (OH, brs), 3167 (NH), 1675 (C=O, α-pyrone), 1608 (C=N). ^{1}H NMR ($CDCl_3$, δ, ppm): 3.5 (1H, dd, H_a), 4.05 (1H, H_c), 4.8 (1H, dd, H_b), 7.0 (1H, s, NH, D_2O exchangeable), 7.2–8.0 (8H, m, Ar-H), 13.37 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A. %): (M^+ + 2) 386 (2%), 384 (7%), 303 (15%), 226 (41%), 78 (9%).

3-[4,5-Dihydro-5-(5-methylfuran-2-yl)-1H-pyrazol-3-yl]-4-hydroxy-2H-chromen-2-one (6c)

M.p. 228–230°C; yield 90%. IR (ν, cm^{-1}): 3746 (OH, brs), 3220 (NH), 1673 (C=O, α-pyrone), 1614 (C=N). ^{1}H NMR ($CDCl_3$, δ, ppm): 2.3 (3H, s, CH_3), 3.9 (1H, dd, H_a), 5.8 (1H, H_c), 6.3 (1H, dd, H_b), 7.2–8.0 (6H, m, Ar-H), 7.0 (1H, s, NH, D_2O exchangeable), 13.37 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A.%): (M^+) 310 (9%), 295 (12%), 229 (5%), 201 (20%), 81 (5%).

General procedure for the synthesis of 3-(5-aryl-1-acetyl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromen-2-ones (7a–c)

A mixture of chalcone (**3a–c**) (1 mmol) and hydrazine hydrate 99% (2 mmol) in glacial acetic acid (30 mL) was refluxed for 4–8 h. The reaction mixture was cooled and diluted with water, the formed precipitate was filtered off, washed and recrystallized from ethyl alcohol to give compounds (**7a–c**).

3-[1-Acetyl-5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-4-hydroxy-2H-chromen-2-one (7a)

M.p. 261–263°C; yield 78%. IR (ν, cm^{-1}): 3424 (OH, brs), 2950 (-CH aliphatic stretching), 1723 (C=O, α-pyrone), 1667 (C=O), 1615 (C=N). ^{1}H NMR ($CDCl_3$, δ, ppm): 2.4 (3H, s, $COCH_3$), 3.6 (1H, dd, H_a), 4.1 (1H, H_c), 5.4 (1H, dd, H_b), 7.1–8.02 (8H, m, Ar-H), 13.37 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A. %): (M^+ + 2) 429 (2%), 427 (5%), 337 (16%), 287 (32%), 234 (51%), 220 (37%), 116 (30%).

3-[1-Acetyl-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-4-hydroxy-2H-chromen-2-one (7b)

M.p. 251–254°C; yield 85%. IR (ν, cm^{-1}): 3425 (OH, brs), 2952 (-CH aliphatic stretching), 1718 (C=O, α- pyrone), 1668 (C=O), 1616 (C=N). ^{1}H NMR ($CDCl_3$, δ, ppm): 2.39 (3H, s, $COCH_3$), 3.6 (1H, dd, H_a), 4.09 (1H, H_c), 5.51 (1H, dd, H_b), 7.1–8.02 (8H, m, Ar-H), 13.37 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A. %): (M^+) 382 (1%), 360 (23%), 277 (27%), 245 (6%), 230 (100%), 171 (8%), 111 (20%).

General procedure for the synthesis of 3-(5-aryl-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-4-hydroxy-2H-chromen-2-one derivatives (8a–c)

A mixture of the selected chalcone (**3a–c**) (1 mmol) and phenylhydrazine (0.1 mL, 1 mmol) in ethyl alcohol (30 mL) was refluxed for 1–2 h. The reaction mixture was cooled and the formed precipitate was filtered off, washed and recrystallized from methyl alcohol to give the desired compounds (**8a–c**).

3-(5-(4-Bromophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-3-yl)-4-hydroxy-2H-chromen-2-one (8a)

M.p. 227–229°C; yield 87%. IR (ν, cm^{-1}): 3444 (OH, brs), 1715 (C=O, α-pyrone), 1616 (C=N). ^{1}H NMR ($CDCl_3$, δ, ppm): 3.5 (1H, dd, H_a), 4.2 (1H, H_c), 5.17 (1H, dd, H_b), 6.8–8.04 (13H, m, Ar-H), 13.9 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A. %): (M^+ + 2) 463 (5%), 461 (3%), 443 (3%), 383 (5%), 305 (8%), 213 (10%), 168 (64%).

3-[4,5-Dihydro-5-(5-methylfuran-2-yl)-1-phenyl-1H-pyrazol-3-yl]-4-hydroxy-2H-chromen-2-one (8c)

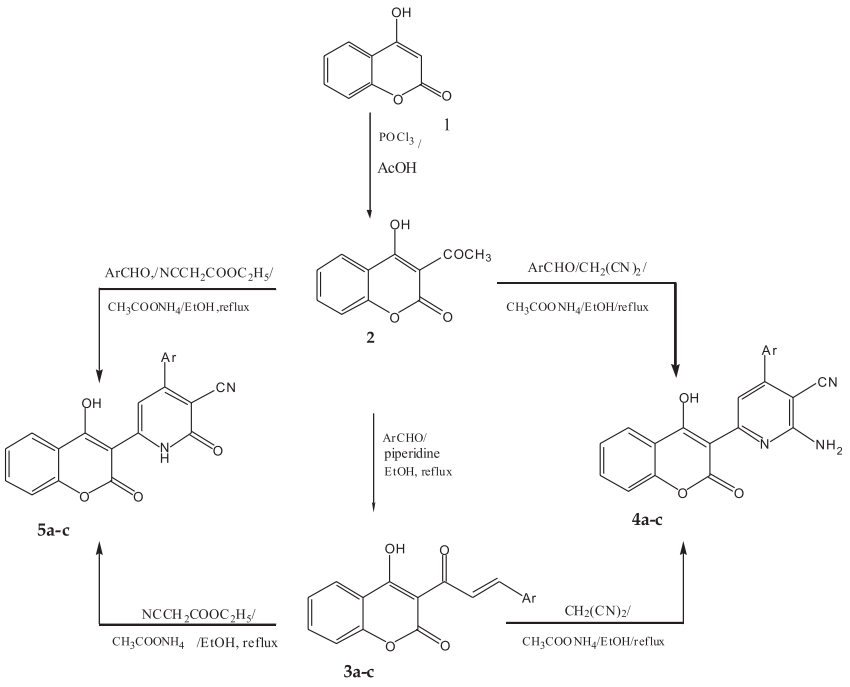
M.p. 201–203°C; yield 92%. IR (ν, cm^{-1}): 3421 (OH, brs), 1710 (C=O, α- pyrone), 1593 (C=N). ^{1}H NMR ($CDCl_3$, δ, ppm): 2.2 (3H, s, CH_3), 3.83 (1H,

dd, H_a), 4.1 (1H, H_c), 5.1 (1H, dd, H_b), 5.8–8.03 (11H, m, Ar-H), 13.9 (1H, s, OH, D_2O exchangeable). MS: m/z (R.A. %): (M^+) 386 (3%), 369 (10%), 309 (5%), 285 (7%), 161 (13%), 77 (81%).

Antitumor activity

Cell culture

Human hepatocarcinoma cell line (HepG2) and human breast adenocarcinoma cell line (MCF-7) purchased from ATCC (American Type Culture Collection), were used to evaluate the cytotoxic effect of the tested samples. Cells were routinely cultured in DMEM (Dulbecco's modified Eagle's medium), which was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/mL of penicillin G sodium, 100 units/mL of streptomycin sulfate, and 250 ng/mL of amphotericin B. Cells were maintained at sub-confluency at 37°C in humidified air containing 5% CO_2. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested samples (20 μL) were dissolved in dimethyl sulfoxide (DMSO), and then diluted serially in the assay to begin with the mentioned concentration. All cell culture material was obtained from Cambrex BioScience (Copenhagen, Denmark). All chemicals were from Sigma/Aldrich, USA, except mentioned otherwise. All experiments were repeated three times, unless mentioned otherwise. Cytotoxicity of tested samples was measured against HepG2 cells using the MTT Cell Viability Assay. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals, which are largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm (17).



Ar, a = C_6H_4-4-Br,
b = C_6H_4-4-Cl,
c = 5-methyl, 2- furyl

Scheme 1. Synthesis of 4-hydroxycoumarin-3-yl chalcones (**3a**–**c**) and 4-hydroxycoumarin-3-ylpyridine carbonitrile derivatives (**4a**–**c**) and (**5a**–**c**)

Table 1. Microanalysis of the synthesized derivatives.

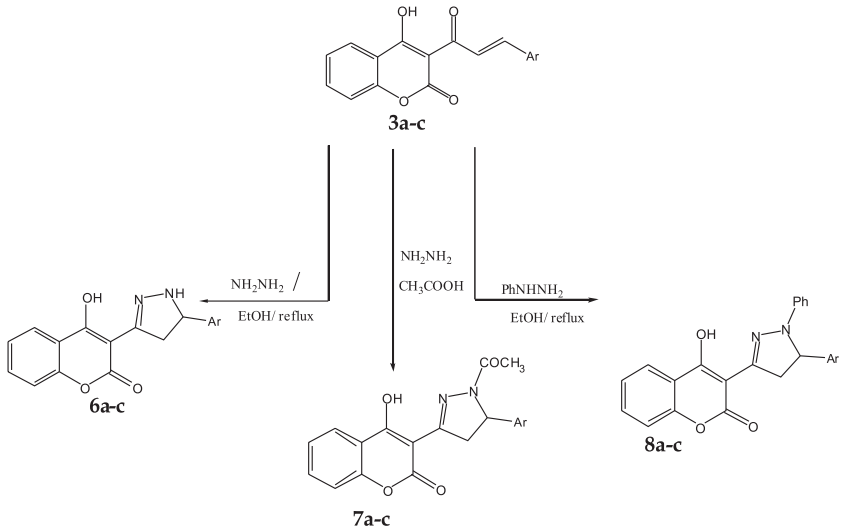
Comp. no.	Molecular formula	Molecular weight	Microanalysis %	
			Calcd.	Found
3c	$C_{17}H_{12}O_5$	296	C 68.92 H 4.08	C 68.89 H 4.1
4a	$C_{21}H_{12}BrN_3O_3$	434.24	C 58.08 H 2.79 N 9.68	C 58.00 H 2.75 N 9.63
5a	$C_{21}H_{11}BrN_2O_4$	435.2	C 57.59 H 2.55 N 6.44	C 57.56 H 2.57 N 6.33
5b	$C_{20}H_{12}ClN_2O_4$	390.7	C 64.54 H 2.84 N 7.17	C 64.49 H 2.80 N 7.09
5c	$C_{20}H_{12}N_2O_5$	360.32	C 66.67 H 3.36 N 7.77	C 66.69 H 3.29 N 7.70
6a	$C_{18}H_{13}Br\ N_2O_3$	384	C,57.59 H,2.55 N,6.44	C 57.61 H 2.49 N 6.41
6b	$C_{18}H_{13}Cl\ N_2O_3$	340.7	C 63.44 H 3.85 N 8.22	C 63.40 H 3.81 N 8.25
6c	$C_{17}H_{14}N_2O_4$	310.3	C 65.80 H 4.55 N 9.03	C 65.78 H 4.51 N 8.97
7a	$C_{20}H_{15}Br\ N_2O_4$	427	C 56.22 H 3.54 N 6.56	C 56.19 H 3.57 N 6.51
7b	$C_{20}H_{15}Cl\ N_2O_4$	382.8	C 62.75 H 3.95 N 7.32	C 62.71 H 3.97 N 7.29
7c	$C_{19}H_{16}N_2O_5$	352.3	C 64.77 H 4.58 N 7.95	C 64.72 H 4.62 N 7.90
8a	$C_{24}H_{17}Br\ N_2O_3$	461.3	C 62.49 H 3.71 N 6.07	C 62.51 H 3.69 N 6.00
8b	$C_{24}H_{17}Cl\ N_2O_3$	416	C 69.15 H 4.11 N 6.72	C 69.11 H 4.09 N 6.69
8c	$C_{23}H_{18}N_2O_4$	386.4	C 71.49 H 4.70 N 7.25	C 71.45 H 4.68 N 7.27

Reagents preparation

MTT solution: 5 mg/mL of MTT in 0.9% NaCl. Acidified isopropanol: 0.04 M HCl in absolute isopropanol.

Procedure

Cells (0.5×10^5 cells/well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20 µL of different concentrations of the tested sample for 48 h at 37°C, in a humidified 5% CO_2 atmosphere. After incubation, media were removed and 40 µL MTT solution/well were added and incubated for additional 4 h. MTT crystals were solubilized by adding 180 µL of acidified isopropanol/well and plate was shaken at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader. Triplicate repeats were performed for



Ar = a = C_6H_4-4-Br
b = C_6H_4-4-Cl,
c = 5-methyl-2-furyl

Scheme 2. Synthesis of 4-hydroxycoumarin-3-yl-pyrazoline derivatives

Table 2. Antimicrobial activity of the synthesized compounds.

Test organisms →	Diameter of inhibition zone (mm)						
	Bacterial strain			Yeast	Fungal strain		
↓ Comp. no.	*B. subtilis*	*E. coli*	*S. aureus*	*C. albicans*	*F. oxysporum*	*F. solani*	*F. verticillioides*
2	15	20	25	18	12	18	12
3a	16	14	15	11	–	12	–
3b	16	17	15	12	–	–	–
4b	16	–	12	–	–	–	–
4c	11	13	17	10	16	12	14
5a	10	–	14	–	–	–	–
5c	30	26	24	25	12	14	11
8b	17	–	–	–	15	–	–

– = no inhibition for a microbe by the tested compound

each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by < 100% relative viability.

Calculation

Percentage of relative viability was calculated using the following equation: [Absorbance of treated cells/Absorbance of control cells)] × 100, then the half maximal inhibitory concentration (IC_{50}) was calculated from the equation of the dose response curve.

Antimicrobial testing

Media

Czapek-Dox agar (CDA) (20): $NaNO_3$ 2.0 g; K_2HPO_4 1.0 g; KCl 0.5 g; $MgSO_4 \times 7\ H_2O$ 0.5 g;

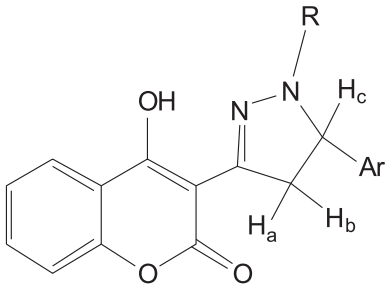


Figure 1. A general model for proton positions in the pyrazole ring of compounds (**6a–c**) – (**8a–c**)

$FeSO_4 \times 7\ H_2O$ 0.001 g; sucrose 30 g; agar 20 g; H_2O 1 L.

Nutrient agar (NA): mass/volume): 0.5% peptone, 0.3% beef extract/yeast extract, 1.5% agar, 0.5% NaCl and 1000 mL distilled water, pH adjusted to neutral (6.8) at 25°C.

Antagonistic effect between compounds and test organisms

The aim of these experiments was to determine the antimicrobial activities of the selected compound against pathogenic fungi (*Fusarium oxysporum*, *Fusarium solani* and *Fusarium verticillioides*), bacteria (*Bacillus subtilis; Escherichia coli* and *Staphylococcus aureus*) and yeast *(Candida albicans*).

All the synthesized compounds were screened for their toxicity against the pathogenic fungi, bacteria and yeast. Two mL spore suspension of 7 days old culture of fungi, bacteria and yeast were inoculated on surface of Petri dish containing CDA medium for fungi and NA medium for bacteria and yeast. Filter paper disk method was applied (18, 19). A sample of 20 µg of the pure toxin was dissolved in the proper solvent ($CHCl_3$, DMSO) and applied to the filter paper disk (5 mm in diameter). The prepared disks were dried and firmly applied to the surface of the inoculated agar plates, then the plates were incubated at 28–30°C for 48–72 h for fungi and for 24 h for bacteria. Diameter of inhibition zone around each disk was measured in mm.

RESULTS AND DISCUSSION

Chemistry

The present work deals with the synthesis of some pyridine carbonitriles and pyrazolyl derivatives derived from the 4-hydroxycoumarin of expected antitumor and antimicrobial activities.

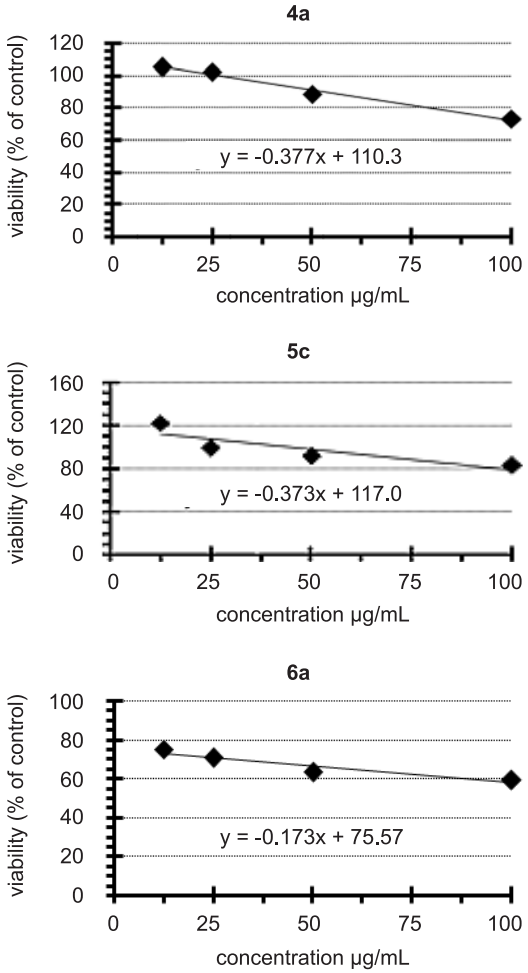


Figure 2. Cytotoxic effect of different samples against MCF-7cells using MTT assay (n = 4); data expressed as the mean value of cell viability (% of control) ± SE

The reflux of 4-hydroxycoumarin (**1**) with acetic acid in the presence of phosphorus oxychloride leads to the formation of 3-acetyl-4-hydroxycoumarin (**2**), which reacted with some aldehydes (4-chlorobenzaldehyde, 4-bromobenzaldehyde, 5-methylfurfural) to yield the chalcones (**3a–c**) (Scheme 1).

The cyclization of the chalcones (**3a–c**) with malononitrile in ethyl alcohol in the presence of ammonium acetate leads to the formation of pyridine carbonitrile, (**4a–c**) (Scheme 1), while the reaction of (**3a–c**) with ethyl cyanoacetate afforded the oxopyridine carbonitrile (**5a–c**) (Scheme 1).

The pyrazolyl derivatives (**6a–c**) (Scheme 2) were formed by the reaction of the chalcones (**3a–c**) with hydrazine hydrate in alcohol, while the acetylpyrazole derivatives (**7a–c**) (Scheme 2) were formed when the same reaction is carried out in the

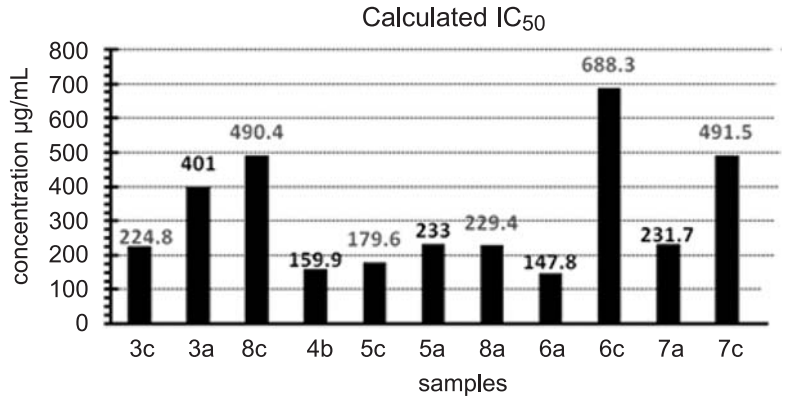


Figure 3. Calculated IC_{50} for the tested samples indicating difference in toxicity between samples. (IC_{50} = dose of the compound which reduces survival by 50%)

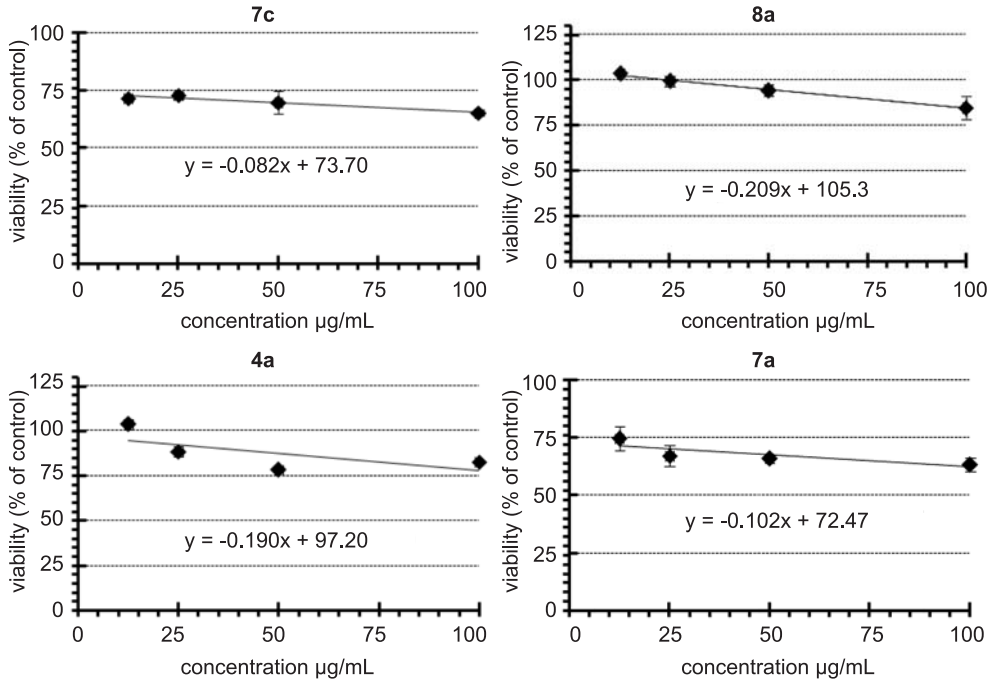


Figure 4. Cytotoxic effect of different samples against Hep-G2 cells using MTT assay (n = 4); data expressed as the mean value of cell viability (% of control) ± SE

presence of acetic acid; on the other hand, the reaction of the chalcones (**3a–c**) with phenylhydrazine resulted in the formation of phenylpyrazole derivatives (**8a–c**) (Scheme 2).

he structures of the new synthesized compounds were confirmed by spectral data (IR, NMR and MS).The physicochemical characteristics are presented in Table 1.

Antitumor activity

Cytotoxic activity for 12 synthesized compounds were tested against human breast adenocarcinoma and the hepatocarcinoma cell lines.

The effect of the samples on the proliferation of MCF-7 cells was studied after 48 h of incubation. The treatment with **4a**, **5c** and **6a** showed almost moderate cytotoxic effect against MCF-7, as con-

cluded from their close IC_{50} values 159.9, 179.6 and 147.8 µg/mL, respectively, as shown in Figure 2, treatment with samples **3c**, **5a**, **8a** and **7a** showed weak cytotoxic effect as they had higher IC_{50} calculated to be 224.8, 233, 229.4 and 231.7 µg/mL, respectively. Samples **3a**, **8c**, **6c** and **7c** showed very weak cytotoxic effect concluded from their very high IC_{50} 401, 490.4, 688.3 and 491.5 µg/mL, respectively. Finally, sample **4c** did not show any cytotoxic effect as it has very high IC_{50} (2654 µg/mL) as shown in Figure 3. For human hepatocarcinoma cell line (HepG2), samples **3c**, **3a**, **8c**, **4c**, **5a**, **6a**, **6c** and **5c** did not show any cytotoxic effect as they increased proliferation of cells and samples **4a**, **8a**, **7a** and **7c** showed weak cytotoxic effect as shown in Figure 4.

Antimicrobial activity

The data in Table 2 show various degrees of antagonism against pathogenic bacteria, yeast and fungi. Three compounds proved to be most promising against tested bacterial organisms i.e., compounds **2, 4c** and **5c**, which were the most active against both Gram negative (*Escherichia coli*) and Gram positive bacteria (*Bacillus subtilis*, and *Staphylococcus aureus*). Compounds **3b** and **3a** proved to be active against bacteria and yeast while no activity was determined with fungi. Compounds **2** and **5c** proved to be most active against *Candida albicans* and all tested fungal organisms. Compounds **4b, 5a** and **8b** showed moderate activity and the rest of the compounds showed low effect against the tested bacterial organisms.

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