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Synthesis, Antimicrobial, and Antiviral Activities of Some New 5-Sulphonamido-8-hydroxyquinoline Derivatives

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A series of fused pyranopyrazole and pyranoimidazole, namely 5-(3,6-diamino-4-aryl-5-carbonitrile-pyrano(2,3-c)pyrazol-2-yl)sulphonyl-8-hydroxyquinolines (**5a-e**), 5-(6-amino-4-aryl-5-carbonitrile-pyrano(2,3-c)pyrazol-3-yl)sulphonamido-8-hydroxyquinolines (**6a-e**), 5-(2-thioxo-4-aryl-5-carbonitrile-6-amino-pyrano(2,3-d)imidazol-2-yl)sulphonyl-8-hydroxyquinolines (**10a-e**), and 5-(2-oxo-4-aryl-5-carbonitrile-6-amino-pyrano(2,3-d)imidazol-2-yl) sulphonyl-8-hydroxyquinolines (**11a-e**), have been prepared via condensation of some arylidine malononitriles with 5-sulphonamido-8-hydroxyquinoline derivatives **3**, **4**, **8** and **9**. All the synthesized compounds were screened for their antimicrobial activities, and most of the tested compounds showed potent inhibition growth activity towards *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative bacteria). Furthermore, six selected compounds were tested for their antiviral activity against avian paramyxovirus type1 (APMV-1) and laryngotracheitis virus (LTV), and the results showed that a concentration range of 3-4 μg per mL of compounds **2**, **3**, and **4** showed marked viral inhibitory activity for APMV-1 of 5000 tissue culture infected dose fifty ($TCID_{50}$) and LTV of 500 $TCID_{50}$ in Vero cell cultures based on their cytopathic effect. Chicken embryo experiments show that compounds **2**, **3**, and **4** possess high antiviral activity *in vitro* with an inhibitory concentration fifty (IC_{50}) range of 3-4 μg per egg against avian APMV-1 and LTV and their toxic concentration fifty (CC_{50}) of 200-300 μg per egg.

Key words: 8-Hydroxyquinoline-5-sulphonyl chloride, Pyrano(2,3-c)pyrazole, Pyrano(2,3-d) imidazole, Antimicrobial, Antiviral activity

INTRODUCTION

Heterocyclic systems with quinoline nuclei represent privileged moieties in medicinal chemistry and are ubiquitous sub-structures associated with biologically active natural products. Quinoline derivatives have been shown to display a wide spectrum of biological activities such as antibacterial (Hoemann et al., 2002; Hussein et al., 2009; Lilienkampf et al., 2009), antifungal (Vargas et al., 2003; Meléndez Gómez et al., 2008), anti-parasitic (Kouznetsov et al., 2007) and antiviral activities (Chen et al., 2009; Jia et al., 2009). Due to their broad range of biological activities, quinoline compounds have been considered good starting materials for the search for novel antimicrobial and anti-viral agents. Accordingly, the present work is aimed at synthesis of new quinoline derivatives via cyclization of 8-hydroxyquinoline-5-sulphonyl chloride. Moreover, the study includes testing of the target compounds for their expected antimicrobial and antiviral activities.

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MATERIALS AND METHODS

Melting points were determined in open capillary tubes on an Electrothermal 9100 digital melting point

apparatus (Büchi) and are uncorrected. Elemental analyses were performed on a Perkin-Elmer 2400 analyzer and were found to be within ± 0.4% of the theoretical values (Table I). IR spectra were recorded on a Perkin-Elmer 1600 FTIR in KBr discks. ^{1}H-NMR spectra were measured with JEOL 270 MHz (JEOL) and the analysis carried out at Micro-analytical Unit, National Research Center, Cairo, Egypt as well as with Bruker 300 MHz (Bruker) and the analysis carried out at Micro-analytical Center, Faculty of Science, Cairo University, Egypt. The samples were measured in DMSO-d_6 and chemical shifts were recorded in δ ppm relative to TMS as an internal standard. Mass spectra (EI) were run at 70 eV with a Jeol-JMS-AX500 mass spectrometer. The spectral data of the newly synthesized compounds are listed in (Table II). 8-Hydroxyquinoline-5-sulphonyl chloride, 2-cyanoacetic acid hydrazide, 3-amino-5-pyrazolone, 2'-acetyl-2-cyanoacetohydrazide and arylidene malononitrile were prepared as reported (Corson and Stoughton, 1928; Graham et al., 1949; Heibron, 1965; Bankovskis et al., 1979; Callejo et al., 1990).

Preparation of:

5-(2-Acetyl-3-amino-5-oxo-1,2-dihydropyrazol-4-yl) sulphonyl-8-hydroxyquinoline (2)

A mixture of 8-hydroxyquinoline-5-sulphonyl chloride (**1**) (2.4 g, 0.01 mol) and 2'-acetyl-2-cyanoaceto-hydrazide (1.3 g, 0.01 mol) in dioxane (20 mL) containing triethylamine (1 mL) was refluxed for 3 h. After cooling, the formed precipitate was filtered off, washed with water, air dried, and recrystalized from aqueous ethanol.

5-(3-Amino-5-oxo-1,2-dihydropyrazol-1-yl)sulphonyl-8-hydroxyquinoline (3)

A mixture of 8-hydroxyquinoline-5-sulphonyl chloride (**1**) (2.4 g, 0.01 mol) and 2-cyanoacetic acid hydrazide (0.99 g, 0.01 mol) in dioxane (20 mL) containing triethylamine (1 mL) was refluxed for 2 h. The solid that formed on hot was filtered off, washed with water, air dried, and recrystalized from absolute ethanol.

5-(5-Oxo-1,2-dihydropyrazol-3-yl)sulphonamido-8-hydroxyquinoline (4)

A mixture of 8-hydroxyquinoline-5-sulphonyl chloride

Table I. Physical and analytical properties of the new prepared compounds

Compd. No.	Formula (M. Wt.)	M. P. (°C)	Yield (%)	Analysis (%) (calculated/found)		
				C	H	N
2	$C_{14}H_{12}N_4O_5S$ (348.33)	222-224	60	48.27/48.33	3.44/3.21	16.09/15.99
3	$C_{12}H_{10}N_4O_4S$ (306.30)	222-224	66	47.05/47.22	3.26/3.34	18.30/18.55
4	$C_{12}H_{10}N_4O_4S$ (306.30)	312-314	85	47.05/47.21	3.26/3.45	18.30/18.55
5a	$C_{22}H_{16}N_6O_4S$ (460.47)	218-220	20	57.38/57.54	3.50/3.67	18.25/18.44
5b	$C_{22}H_{15}ClN_6O_4S$ (494.91)	253-255	18	53.39/53.21	3.05/2.99	16.98/17.01
5c	$C_{22}H_{15}N_7O_6S$ (505.46)	263-265	30	52.28/52.01	2.99/3.03	19.40/19.60
5d	$C_{23}H_{18}N_6O_5S$ (490.49)	310-312	22	56.32/56.43	3.70/3.87	17.13/17.33
5e	$C_{24}H_{21}N_7O_4S$ (503.53)	166-168	20	57.25/57.44	4.20/4.35	19.47/19.44
6a	$C_{22}H_{14}N_6O_4S$ (458.45)	76-78	22	57.64/57.87	3.27/3.47	18.34/18.11
6b	$C_{22}H_{13}ClN_6O_4S$ (492.89)	289-291	20	53.60/53.88	2.63/2.44	17.05/17.21
6c	$C_{22}H_{13}N_7O_6S$ (503.45)	205-207	18	52.84/52.91	2.58/2.76	19.48/19.55
6d	$C_{23}H_{16}N_6O_5S$ (488.48)	199-200	30	56.55/56.76	3.30/3.55	17.20/17.44
6e	$C_{22}H_{19}N_7O_4S$ (501.52)	164-166	34	57.48/57.65	3.79/3.66	19.56/19.77
7	$C_{11}H_{10}N_2O_5S$ (282.27)	215-217	90	46.80/46.91	3.54/3.66	9.92/10.01
8	$C_{12}H_9N_3O_4S_2$ (323.35)	205-207	70	44.58/44.78	2.78/3.00	13.00/13.22
9	$C_{12}H_9N_3O_5S$ (307.28)	290-292	60	46.90/47.01	2.93/3.00	13.68/13.77
10a	$C_{22}H_{13}N_5O_4S_2$ (475.50)	271-273	18	55.57/55.50	2.76/2.88	17.73/17.88
10b	$C_{22}H_{12}ClN_5O_4S_2$ (509.99)	243-245	30	51.82/52.00	2.37/2.55	13.73/13.82
10c	$C_{22}H_{12}N_6O_6S_2$ (520.50)	221-223	22	50.77/50.89	2.32/2.55	16.15/16.32
10d	$C_{23}H_{15}N_5O_5S_2$ (505.53)	305-307	24	54.65/54.77	2.99/3.02	13.85/14.00
10e	$C_{24}H_{18}N_6O_4S_2$ (518.08)	176-178	20	55.59/55.61	3.50/3.52	16.21/16.42
11a	$C_{22}H_{13}N_5O_5S$ (459.43)	207-209	33	57.51/57.68	2.85/3.00	15.24/15.28
11b	$C_{22}H_{12}ClN_5O_5S$ (493.88)	199-201	20	53.50/53.77	2.45/2.65	14.16/14.33
11c	$C_{22}H_{12}N_6O_7S$ (504.43)	350 dec.	18	52.38/52.49	2.40/2.65	16.66/16.82
11d	$C_{23}H_{15}N_5O_6S$ (489.46)	145-147	25	56.44/56.23	3.09/2.99	14.13/14.22
11e	$C_{24}H_{18}N_6O_5S$ (502.50)	279-281	20	57.36/57.43	3.61/3.77	16.72/16.99

Table II. Spectral characterization of the new prepared compounds

Compd. No.	IR (ν_{max} cm^{-1})	^{1}H-NMR (δ, ppm)	Mass (*m/z*, %)
2	3420 (OH), 3200 & 3106 3163 (NH & NH_2), 1702 & 1656 (C=O), 1618 (C=N), 1577 (C=C)	10.45 (s, 1H, OH), 8.01 (s, 1H, NH), 7.20-7.88 (m, 5H, Ar-H), 4.66 (s, 2H, NH_2), 2.99 (3H, s, $COCH_3$)	348 (M^+, 10), 291 (3), 209 (30), 57 (100)
3	3335 (OH), 3209 & 3163 (NH & NH_2), 1686 (C=O), 1651 (C=N), 1606 (C=C), 1385 & 1188 (SO_2N)	10.55 (s, 1H, OH), 9.52 (s, 1H, NH), 8.82 & 9.11 (2d, 2H, H-2 & H-4 quinoline), 6.90 & 7.81 (2d, 2H, H-6 & H-7 quinoline), 7.51 (m, 1H, H-3 quinoline), 5.62 (s, 2H, NH_2), 4.44 ppm (s, 1H, CH-pyrazole)	306 (M^+, 46), 205 (100), 99 (50), 89 (45), 77 (30)
4	3325 (OH), 3218 & 3164 (NH & NH_2), 1687 (C=O), 1651 (C=N), 1601 (C=C), 1368 & 1163 (SO_2N)	10.56 (s, 1H, OH), 9.52 (s, 1H, NH), 7.51-8.65 (m, 5H, Ar-H quinoline), 5.66 (s, 1H, SO_2NH), 4.24 ppm (s, 2H, CH_2-pyrazole)	
5a	3358 (OH), 3200 & 3103 (NH & NH_2), 2230 (CN), 1641 (C=N), 1597 (C=C), 1340 & 1131 (SO_2N)	10.56 (s, 1H, OH), 9.51 (s, 2H, NH_2), 8.42 (s, 1H, H-pyrane), 7.66-8.12 (m, 10H, Ar-H), 4.24 (s, 2H, NH_2)	
5b	3421 (OH), 3200 & 3102 (NH & NH_2), 2223 (CN), 1599 (C=N), 1565 (C=C), 1370 & 1126 (SO_2N)		494 (M^+, 10), 496 (M^++2, 3), 383 (20), 205 (30), 111 (70), 89 (100), 77 (30)
5c	3400 (OH), 3200 & 3103 (NH & NH_2), 2230 (CN), 1641 (C=N), 1597 (C=C), 1346 & 1132 (SO_2N)	9.91 (s, 1H, OH), 8.91 (s, 2H, NH_2), 8.47 (s, 1H, H-pyrane), 7.67-8.19 (m, 9H, Ar-H), 5.66 (s, 2H, NH_2)	
5d	3400 (OH), 3218 & 3100 (NH & NH_2), 2200 (CN), 1620 (C=N), 1587 (C=C), 1346 & 1132 (SO_2N), 1009 (C-O-C)		490 (M^+, 1), 33 (40), 355 (100), 205 (50), 87 (46)
5e	3445 (OH), 3212 & 3135 (NH & NH_2), 2219 (CN), 1601 (C=N), 1529 (C=C), 1369 & 1131 (SO_2N)	11.44 (s, 1H, OH), 8.01 (s, 1H, H-pyrane), 7.88 (s, 2H, NH_2), 6.67-7.61 (m, 9H, Ar-quinoline), 4.14 (s, 2H, NH_2), 2.99 (s, 6H, $2CH_3$)	
6a	4200 (OH), 3320 & 3200 (NH & NH_2), 2215 (CN), 1620 (C=N), 1365 & 1131 (SO_2N), 1009 (C-O-C)	10.56 (s, 1H, OH), 8.91 (s, 1H, NH), 8.56 (s, 2H, NH_2), 7.44-8.32 (m, 5H, Ar-H quinoline), 7.03-7.37 (m, 5H, Ar-H)	
6b	3350 (OH), 3318 & 3191 (NH & NH_2), 2219 (CN), 1618 (C=N), 1365 & 1136 (SO_2N), 1019 (C-O-C), 740 (Cl)		492 (M^+, 12), 494 (M^++2, 2), 396 (100), 330 (30), 206 (45), 111 (40), 87 (50)
6c	3419 (OH), 3354 & 3255 (NH & NH_2), 2223 (CN), 1664 (C=N), 1584 (C=C), 1348 & 1178 (SO_2N), 1040 (C-O-C)	11.24 (s, 1H, OH), 9.91 (s, 1H, NH), 8.87 (s, 2H, NH_2), 7.11-8.37 (m, 9H, Ar-H)	

(**1**) (2.4 g, 0.01 mol) and 3-amino-5-pyrazolone (0.99 g, 0.01 mol) in dioxane (20 mL) containing triethylamine (1 mL) was refluxed for 3 h. After cooling, the formed precipitate was filtered off, washed with water, air dried, and recrystalized from aqueous ethanol.

5-(3,6-Diamino-4-aryl-5-carbonitrile-pyrano(2,3-c) pyrazol-2-yl)sulphonyl-8-hydroxyquinolines (5a-e)

A solution of the appropriate arylidene malononitriles (0.01 mol) and compound **3** (3.06 g, 0.01 mol) in dioxane containing triethylamine (1 mL) was refluxed for 3-6 h. After cooling, the formed precipitate was filtered off, washed with water, air dried, and recrystalized from absolute ethanol.

5-(6-Amino-4-aryl-5-carbonitrile-pyrano(2,3-c)pyrazol-3-yl)sulphonamido-8-hydroxyquinolines (6a-e)

A solution of the appropriate arylidene malononitriles (0.01 mol) and compound **4** (3.06 g, 0.01 mol) in dioxane containing triethylamine (1 mL) was refluxed for 3-6 h. After cooling, the formed precipitate was filtered off, washed with water, air dried, and recrystalized from absolute ethanol.

2-(2-(8-Hydroxyquinolin-5-yl)sulphonamido)acetic acid (7)

A suspension of 8-hydroxyquinoline-5-sulphonyl chloride (**1**) (0.24 g, 0.001 mol) and glycine (0.07 g, 0.001 mol) in a saturated solution of potassium carbonate (5 mL, 1.1 mol/L) was heated at 50°C for 10 min and then at 100°C for 30 min. After cooling, the reaction mixture was neutralized with diluted hydrochloric acid (1:1). The precipitate that formed was filtered off and recrystallized from dioxane.

5-(2-Thioximidazolidinon-1-yl)sulphonyl-8-hydroxy quinoline (8)

A suspension of compound **7** (3.38 g, 0.012 mol), acetic anhydride (6.3 g, 0.067 mol), anhydrous pyridine (15 mL) and ammonium thiocyanate (1.2 g, 0.015 mol) was heated at 110°C for 1 h. The volatiles were removed *in vacuo*, and the residue was suspended in water (100 mL) and stirred for 1 h. The solid formed was filtered off, air dried, and recrystallized from benzene-petroleum ether (60-80°C).

5-(2,4-Dioxo-imidazolidin-1-yl)sulphonyl-8-hydroxy quinoline (9)

A suspension of compound **8** (1.77 g, 0.0055 mol), chloroacetic acid (10 g, 0.1 mol) and water (3 mL) was heated at 120°C for 12 h in a sand bath. The reaction mixture was then diluted with water (50 mL) and set aside in a refrigerator at 5°C. The solid formed was filtered off, air dried, and recrystallized from benzene-petroleum ether (60-80°C).

5-(2-Thioxo-4-aryl-5-carbonitrile-6-amino-pyrano (2,3-d)imidazol-2-yl)sulphonyl)-8-hydroxyquinolines (10a-e)

A solution of the appropriate arylidene malononitriles (0.01 mol) and compound **8** (3.23 g, 0.01 mol) in dioxane containing triethylamine (1 mL) was refluxed for 3-6 h. After cooling, the formed precipitate was filtered off, air dried, and recrystalized from absolute ethanol.

5-(2-Oxo-4-aryl-5-carbonitrile-6-amino-pyrano(2,3-d)imidazol-2-yl) sulphonyl-8-hydroxyquinolines (11a-e)

A solution of the appropriate arylidene malononitriles (0.01 mol) and compound **9** (3.07 g, 0.01 mol) in dioxane containing triethylamine (1 mL) was refluxed for 3-6 h. After cooling, the formed precipitate was filtered off, air dried, and recrystalized from absolute ethanol.

Biological assay

Antimicrobial evaluation

The antimicrobial activity of the synthesized compounds was determined *in vitro* using the disc diffusion method (Barry and Thornsberry, 1980) against a variety of pathogenic microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative bacteria), *Staphylococcus aureus*, *Bacillus cereus* (Gram-positive bacteria), and one strain of fungi (*Candida albicans*). They were isolated from clinical samples and identified to the species level according to different API 20E systems (Analytab Products) (bioMerieux). The antimicrobial activities of the tested compounds were estimated by placing presterilized filter paper discs (6 mm in diameter) impregnated with different doses of the tested compounds (100, 50 and 25 μg per disc) on Nutrient and MacConky agar media for bacteria and on Sabouraud dextrose agar for the fungi. Dimethyl formamide (DMF) was used as a solvent for impregnation. The inhibition zones (IZ) of the tested compounds were measured after 24-48 h incubation at 37°C for bacteria and after 5 days incubation at 28°C for fungi. Cefotaxime (CTX) [a standardized 30 μg CTX disc (BBL; Lot 104026, assayed content of 30 μg per disc, was used in the disc diffusion test, Hoechst-Roussel Pharmaceuticals)] and Pipracillin (Pipracillin (PIP) 100 μg per disc manufactured by Bristol-Myers Squibb) were used as reference drugs for the bacteria, whereas Nystatin (30 unit per disc) manufactured by Bristol-Myers Squibb, Giza, Egypt, [European unit = 0.04 μg per disc] was used as the reference drug for the fungi (*C. albicans*).

Antiviral evaluation

Viruses

Live Avian paramyxovirus type1 (APMV-1) and Laryngotracheitis virus (LTV) were obtained from the Strains Bank of Central Laboratory for Evaluation of Veterinary Biologics (CLVB), Cairo, Egypt.

Cell line

Vero (Normal, African green monkey kidney) cell culture was kindly obtained from Veterinary Vaccines and Serum Research Institute, Cairo, Egypt. Cells were cultured in sterile growth medium RPMI-1640 (Sigma-Aldrich) and supplemented with 10% heat activated new born calf serum (Sigma-Aldrich, USA origin) and antibiotics (1000 I.U. per mL Penicillin, 100 μg per mL Streptomycin and 25 μg per mL Amphotericin B, Gibco). The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2, and they were subcultured twice a week. The virus was propagated in Vero cells, and the infective titer of the stock solution was $10^{-7}TCID_{50}$ per mL (50% tissue culture infective dose). Viruses were adapted on Vero cells throughout seven successive passages by which the virus showed distant cytopathic effects (degener-

ation and floatation of the infected cells) on the 3rd day after infection.

Specific pathogen free egg

Specific pathogen free (SPF) embryonated chicken eggs were obtained from Nile SPF Farm, Koam Oshiem, Fayoum, Egypt.

***In vitro* cytotoxicity screening**

Cytotoxicity of the tested compounds was determined using the MTT [3-(4,5-dimethylthiazoyl-2-yl)2,5-diphenyltetrazolium bromide] assay (Mosmann, 1983). The sub-confluent cell cultures were trypsinized and collected. The cells at a concentration of 3×10^3 cells per ml in 100 µL RPM1-1640 culture medium were incubated for 3 h at 37°C and 5% CO_2. The seed cells were incubated in 96-well microplates (3×10^3 cells per well) at 37°C and a 5% CO_2 incubator for 24 h. After 24 h, when the cells became confluent, the supernatant was flicked off and previously diluted media of 100 µL of different concentrations of test compounds was added to the microplates and kept for incubation at 37°C in a 5% CO_2 incubator for 72 h. The cells were periodically checked for granularity, shrinkage, and swelling. After 72 h, the sample solution in the wells was flicked off and 100 µL of MTT (0.5 mg/mL) was added to each well. The plates were gently shaken and incubated for 4 h at 37°C in a 5% CO_2 incubator. The purple crystals that developed were dissolved in 100 µL DMSO, and absorbance was measured using an ELISA Microplate Reader (Bio-Rad Laboratories) at a wavelength of 570 nm.

***In vitro* antiviral assay**

Different nontoxic concentrations of test compounds, *i.e.* lower than CTC_{50}, were checked for antiviral property by a cytopathic effect (CPE) assay against a challenge dose of 10 $TCID_{50}$. Cells were seeded in 96-well microtitre plates with populations of 10,000 cells per well and incubated at 37°C in a 5% CO_2 atmosphere for a period of 48 h. The plates were washed with fresh RPMI-1640 medium and changed into maintenance medium containing virus (10 $TCID_{50}$) and incubated at 37°C for 90 min for adsorption of the virus. Then, the cultures were treated with different dilutions of the test compounds in fresh maintenance medium and incubated at 37°C for five days. Every 24 h, observations were made and cytopathic effects were recorded. Anti-APMV-1and anti-ILTV activity was determined by the inhibition of the cytopathic effect compared with the control, *i.e.* the protection offered by the test samples to the cells scored (Meyyanathan et al., 2006).

In Vero cell cultures

These assays were performed in nine tissue culture plates of 24-wells, according to Cox et al. (Cox et al., 1996). Confluent monolayers of Vero cells were infected with 5000 tissue culture infected dose fifty ($TCID_{50}$) per 0.2 mL per well of APMV-1 or 500 $TCID_{50}$ of ILTV and incubated for 2 h (for virus adsorption); then inoculum was decanted, followed by the addition of different ten-fold concentrations to each test sample separately (from 3-5 µg per mL per well of each concentration). The virus infectivity control and each test compound cytotoxicity control were prepared separately. Test plates were incubated at 37°C and 5% CO_2 for 3 days. Cytotoxicity concentration fifty (CC_{50}) of each test compound was determined as the concentration of compounds that induced any deviation of the morphology than the normal control cells in 50% of the VERO cells' monolayers. Antiviral inhibitory concentration fifty (IC_{50}) of test compounds was assayed as the concentration of compounds that fully inhibited the virus-cytopathic effect (100 TCID) in 50% of monolayers. Also, the therapeutic index (TI) of samples was expressed as CC_{50}/IC_{50} (Reed and Muench, 1938).

In embryonated chicken eggs

Groups of 9-11 days old specific pathogen free (SPF) embryonated chicken eggs (ECE_s) were inoculated with 500 embryo infective dose fifty (EID_{50}) per 0.2 mL per egg of APMV-1 or 50 EID_{50} of ILTV, immediately followed by injection of different concentration of each compound (2-500 mg per 0.2 mL per egg) separately. The virus infectivity control and test sample toxicity controls were inoculated via the chorioallantoic cavity. Test eggs were incubated for 3-4 days at 37°C and 80% humidity. The CC_{50}, IC_{50}, and TI values were determined as mentioned above. APMV-1 infectivity in ECE was detected by haemagglutinating activity of the allantoic fluids of the inoculated eggs as measured by the microtechnique of the haemagglutination (HA) test (Takatsy, 1956), while ILTV infectivity was determined by the criterion of distension of the abdominal region, mottled necrotic or hemorrhagic liver, and mortality scores in embryos. The CC_{50} and IC_{50} were calculated by the method of Reed and Muench (1938).

RESULTS AND DISCUSSION

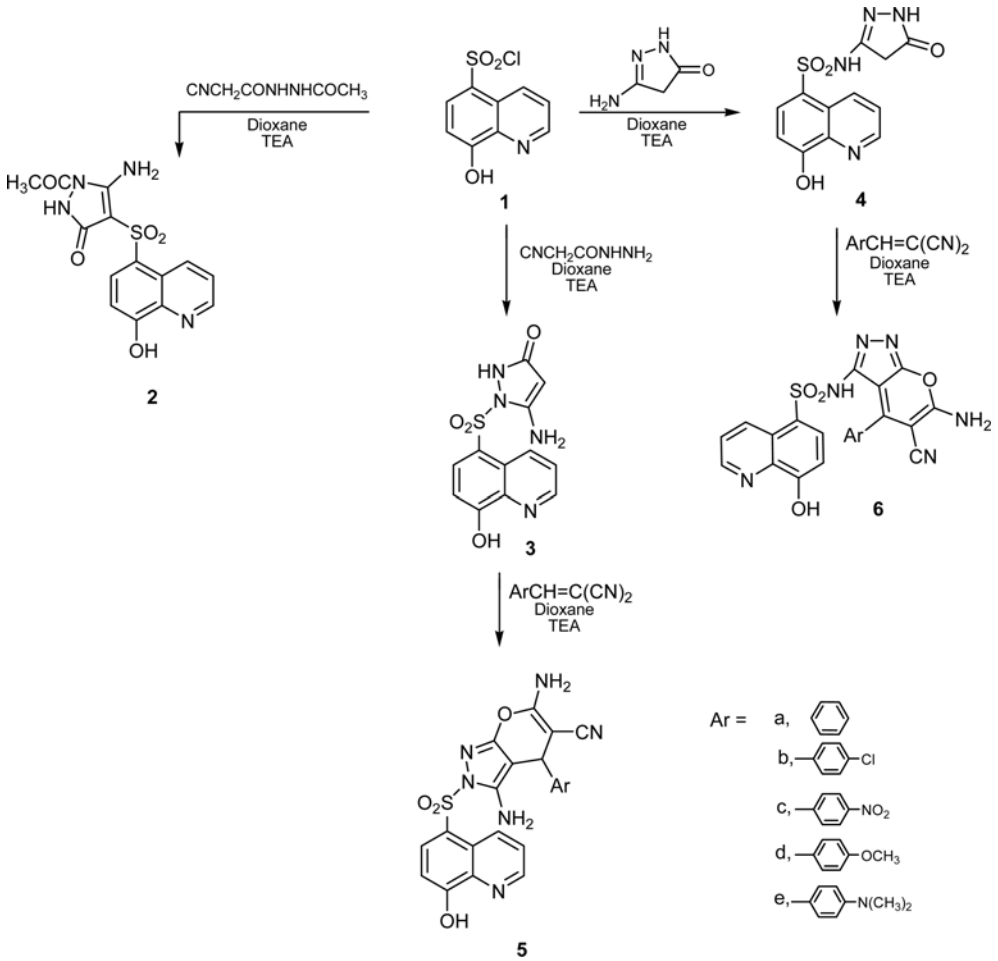
The reaction routes for the synthesis of the title compounds are described in Schemes 1 and 2. A series of 5-substituted sulphonyl-8-hydroxyquinoline derivatives were prepared. Condensation of 8-hydroxyquinoline-5-sulphonyl chloride (**1**) with 2'-acetyl-2-cyanoacetohydrazide in refluxing dioxane in the presence of tri-

ethylamine led to the formation of 5-(2-acetyl-2-amino-5-oxo-1,2-dihydropyrazol-4-yl)sulphonyl-8-hydroxyquinoline (**2**) (Scheme 1). The reaction may be preceded via reaction of a chlorine atom of **1** with an active methylene group of **2** followed by intramolecular cyclization to give **3**. ^{1}H-NMR of **2** revealed signals at 10.45 (s, 1H, OH), 8.01 (s, 1H, NH), 7.20-7.88 (m, 5H, Ar-H), 4.66 (s, 2H, NH_2), and 2.99 ppm (3H, s, $COCH_3$) (Table II).

Furthermore, the reaction of compound **1** with the amino group of 2-cyanoacetic acid hydrazide and its cyclic form 3-amino-5-pyrazolone in refluxing dioxane in the presence of triethylamine gave the corresponding 5-(3-amino-5-oxo-1,2-dihydropyrazol-1-yl)sulphonyl-8-hydroxy-quinoline (**3**) and 5-(5-oxo-1,2-dihydropyrazol-3-yl)sulphonamido-8-hydroxyquinoline (**4**), respectively (Scheme 1). The characteristic features of **3** showed the absence of the absorption bands for the Cl atom in the IR spectrum and showed absorption bands at 3209, 3163, 1686, 1385 and 1188 cm^{-1} for NH_2, NH, C=O and SO_2, respectively. The ^{1}H-NMR of **3** revealed signals at 10.55 (s, 1H, OH), 9.52 (s, 1H, NH), 9.11 and 8.82 (2d, 2H, H-2 and H-4 quinoline), 7.81 and 6.90 (2d, 2H, H-6 and H-7 quinoline), 7.51 (m, 1H, H-3 quinoline), 5.62 (s, 2H, NH_2) and 4.44 ppm (s, 1H, CH-pyrazole) (Table I)

The cyclocondensation reactions of compounds **3** and **4** with some arylidenemalononitriles, namely benzylidenemalononitrile, *p*-chlorobenzylidenemalononitrile, *p*-nitrobenzylidenemalononitrile, *p*-methoxybenzylidenemalononitrile, and *p*-(*N,N*-dimethylamino) benzylidenemalononitrile, in refluxing dioxane in the presence of triethylamine as a catalyst led to the formation of fused systems 5-(3,6-diamino-4-aryl-5-carbonitrile-pyrano(2,3-c)pyrazol-2-yl)sulphonyl-8-hydroxylquinolines (**5a-e**) and 5-(6-amino-4-aryl-5-carbonitrile-pyrano(2,3-c)pyrazol-3-yl)sulphonamido-8-hydroxyquinolines (**6a-e**), respectively (Scheme 1).

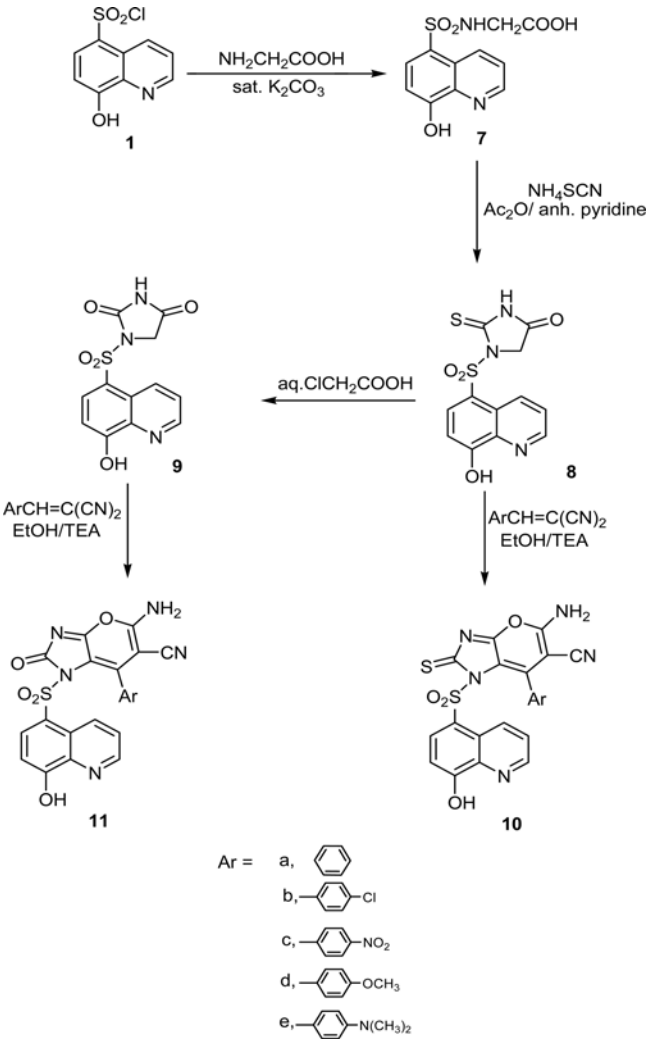
Moreover, the reaction of 8-hydroxyquinoline-5-sulphonyl chloride (**1**) with glycine in the presence of saturated potassium carbonate solution led to the formation of 2-(2-(8-hydroxyquinolin-5-yl)sulphonamido)acetic



Scheme 1. Synthesis of 5-(3,6-diamino-4-aryl-5-carbonitrile-pyrano(2,3-c)pyrazol-2-yl)sulphonyl-8-hydroxylquinolines and 5-(6-amino-4-aryl-5-carbonitrile-pyrano (2,3-c)pyrazol-3-yl)sulphonamido-8-hydroxyquinolines.

acid (**7**) (Scheme 2). Heterocyclization of the latter compound via its reaction with ammonium thiocyanate in acetic anhydride in the presence of anhydrous pyridine gave 5-(2-thioximidazolidinon-1-yl)sulphonyl-8-hydroxyquinoline (**8**) (Scheme 2). The IR spectrum of **8** showed absorption bands at 1240 cm^{-1} for C=S besides the sulphonamido group at 1371 and 1136 cm^{-1}. In addition, its ^1H NMR spectrum revealed singlet signals at 8.76 ppm for NH and at 4.24 ppm for CH_2 of the imidazole moieties besides the other signals which were located at their positions (Table II).

Acid hydrolysis of compound **8** using aqueous monochloroacetic acid yielded the corresponding imidazolidine-2,4-dione derivatives **9** (Scheme 2). IR spectrum of **9** showed no absorption bands for C=S and showed absorption bands at 1705 & 1715 cm^{-1} for (C=O) groups



Scheme 2. Synthesis of 5-(2-thioxo-4-aryl-5-carbonitrile-6-amino-pyrano(2,3-d)imidazol-2-yl)sulphonyl-8-hydroxyquinolines and 5-(2-oxo-4-aryl-5-carbonitrile-6-amino-pyrano(2,3-d)imidazol-2-yl)sulphonyl-8-hydroxyquinolines.

(Table II).

Condensation of compounds **8** and **9** with some arylidene malononitriles, namely benzylidenemalononitrile, *p*-chlorobenzylidene-malononitrile, *p*-nitrobenzylidenemalononitrile, *p*-methoxybenzylidene- malononitrile, and *p*-(*N,N*-dimethylamino)benzylidenemalononitrile, under reflux in dioxane in the presence of triethylamine as a catalyst led to the formation of the fused systems of pyrano(2,3-d)imidazole derivatives **10a**-**e** and **11a**-**e**, respectively (Scheme 2). The ^1H-NMR of compounds **10a**,**c**,**e** and **11a**,**c**,**e** lack the presence of CH_2 protons of imidazole and revealed new singlet signals at 8.99, 9.15, 6.76, 8.87, 8.81, and 8.57 ppm, respectively, for NH_2 (Table II).

Antimicrobial activity

All the newly synthesized compounds were tested for their antimicrobial activity against a variety of pathogenic microorganisms, *E. coli*, *P. aeruginosa* (Gram-negative bacteria) and *S. aureus*, *B. cereus* (Gram-positive bacteria), and one strain of fungi (*C. albicans*) at different doses of the tested compounds (100, 50 and 25 μg per disc) (Table III). The results showed that compounds **3**, **4**, **5c**, **8** and **9** were the most highly active of all tested compounds with growth inhibition zones 28, 27, 22, 22 and 20 mm, respectively, at 100 μg per disc against *E. coli*, compared to the reference drug cefatoxime (32 mm) at 30 μg per disc. Also, inhibition zones 18, 18, 16, 14 and 14 mm showed growth at 50 μg per disc against *E. coli,* compared to the reference drug cefatoxime (22 mm) at 30 μg per disc. On the other hand, compounds **3**, **4**, **8** and **9** were found to be the most active of all the tested compounds with growth inhibition zones 19, 20, 18 and 18mm at 100 μg per disc against *P. aeruginosa,* compared to the reference drugs cefatoxime (22 mm) at 30 μg per disc and piperacillin (20 mm) at 100 μg per disc. The rest of the tested compounds were non-active against all microorganisms tested.

Antiviral activity

In Vero cell cultures

Six selected compounds were tested for their antiviral activity against avian paramyxovirus type1 (APMV-1) and laryngotracheitis virus (LTV) using a virus cytotoxicity effect inhibitory assay. The results revealed that compounds **2**, **3**, and **4** completely inhibit 5000 $TCID_{50}$ of APMV-1 and 500 $TCID_{50}$ of ILTV infectivity in the range of 3, 4, 3 μg per mL, respectively (Table IV). Substantial therapeutic indices of 66, 75, and 66 were recorded. The cytotoxicity assay indicated that CC_{50} of **2**, **3** and **4** were greater than 200, 300 and 200 mg per mL, respectively (Table IV). These results

Table III. Antimicrobial activity of the newly synthesized compounds

Compd. No.	Inhibition zone (mm)														
	E. coli			*P. aeruginosa*			*S. aureus*			*B. cereus*			*C. albicans*		
	Compounds (μg per disc)														
	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25
2	17	14	-	18	14	-	14	9	-	12	-	-	12	-	-
3	28	18	12	19	14	10	17	10	-	14	9	-	12	-	-
4	27	18	13	20	14	10	17	12	-	12	-	-	12	-	-
5a	19	14	-	16	10	-	12	-	-	12	-	-	10	-	-
5b	19	12	-	16	10	-	12	-	-	10	-	-	10	-	-
5c	22	16	-	16	12	-	12	-	-	10	-	-	10	-	-
5d	17	12	-	14	9	-	12	-	-	10	-	-	10	-	-
5e	17	10	-	14	8	-	10	-	-	10	-	-	10	-	-
6a	17	9	-	12	8	-	10	-	-	9	-	-	10	-	-
6b	17	8	-	12	8	-	12	-	-	10	-	-	10	-	-
6c	17	8	-	12	8	-	12	-	-	12	-	-	10	-	-
6d	17	8	-	12	8	-	12	-	-	12	-	-	10	-	-
6e	17	8	-	12	8	-	12	-	-	12	-	-	10	-	-
8	22	14	9	18	12	9	14	8	-	14	8	-	12	-	-
9	20	14	9	18	12	9	14	9	-	14	8	-	12	-	-
10a	18	12	9	16	10	-	14	8	-	14	8	-	9	-	-
10b	17	10	8	16	10	-	14	8	-	14	8	-	9	-	-
10c	17	10	8	16	10	-	14	8	-	14	8	-	9	-	-
10d	17	10	-	14	-	-	12	8	-	14	8	-	9	-	-
10e	17	10	-	14	-	-	12	8	-	14	8	-	9	-	-
11a	12	9	-	10	-	-	12	8	-	12	-	-	9	-	-
11b	12	9	-	10	-	-	12	8	-	12	-	-	9	-	-
11c	12	9	-	10	-	-	12	8	-	12	-	-	9	-	-
11d	12	9	-	10	-	-	12	8	-	12	-	-	9	-	-
11e	12	9	-	10	-	-	12	8	-	12	-	-	9	-	-
Cefatoxime (30 μg per disc)	32	22	17	22	18	12	31	26	17	26	20	14	-	-	-
Piperacillin (100 μg per disc)	-	-	-	20	15	10	27	18	10	20	15	10	-	-	-
Nystatin (30 unit per disc)	-	-	-	-	-	-	-	-	-	-	-	-	40		

E. coli: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *B. cereus*: *Bacillus cereus*; *C albicans*: *Candida albicans.*

proved that the three compounds possessed antiviral activity in Vero cells with absence of apparent cytotoxicity.

In chicken embryos

The activity of the six selected compounds **2**, **3**, **4**, **7**, **8**, and **9**, as determined by haemagglutinating activity in allantoic fluids and ILTV infectivity criterion in embryos, showed that 4, 3 and 4 μg per 0.2 mL per egg of compounds **3**, **4**, and **2**, respectively, fully reduced the infectivity of 500 EID_{50} of APMV-1 and 50 EID_{50} of ILTV (Table V). The toxicity assays of compounds **3**, **4**, and **2** in chicken embryos with concentrations of 300, 200, and 200 μg per egg, respectively, showed 100% of the inoculated eggs without death on the fifth day after inoculation. Thus, the recorded therapeutic indices of the three compounds were 75, 66, and 50, respectively, in the case of APMV-1 and 66, 50, and 50, respectively, in the case of ILTV. In conclusion, chicken embryo experiments show that compounds **3**, **4**, and **2** had high antiviral activity *in vitro* with inhibitory concentration fifty (IC_{50}) ranges of 3-4 μg per egg against avian APMV-1 and LTV and a toxic concentration fifty (CC_{50}) of 200-300 μg per egg.

The results showed that the concentration range of

Table IV. Cytotoxic effect of test compounds on normal Vero cell lines

Compd. No.	CC_{50}		IC_{50}		TI	
	APMV-1	LTV	APMV-1	LTV	APMV-1	LTV
Z5	>500	>500	≤5	≤5	100	100
Z6	>500	>500	≤4	≤4	125	100
Z7	>400	>400	≤3	≤4	100	100
Z18	>300	>300	≤4	≤4	75	75
Z19	>200	>200	≤3	≤3	66	66
Z27	>200	>200	≤3	≤3	66	66

Avian paramyxovirus type 1 (APMV-1) = 5000 $TCID_{50}$; Laryngotracheitis virus (LTV) = 500 $TCID_{50}$; CC_{50} (μg per mL): toxic concentration fifty; IC_{50} (μg per mL): Inhibiting concentration fifty; TI: Therapeutic index.

Table V. Cytotoxic effect of test compounds in embryonated chicken SPF eggs

Compd. No.	CC_{50}		IC_{50}		TI	
	APMV-1	LTV	APMV-1	LTV	APMV-1	LTV
Z5	>400	>400	≤5	≤4	80	100
Z6	>400	>400	≤4	≤4	100	100
Z7	>400	>400	≤4	≤4	100	100
Z18	>300	>300	≤4	≤3	75	66
Z19	>200	>200	≤3	≤4	66	50
Z27	>200	>200	≤4	≤4	50	50

Avian paramyxovirus type 1 (APMV-1) = 500 EID_{50}; Laryngotracheitis virus (LTV) = 50 EID_{50}; CC_{50} (μg per mL): toxic concentration fifty; IC_{50} (μg per mL): Inhibiting concentration fifty; TI: Therapeutic index.

3-4 μg per mL of compounds **2**, **3**, and **4** showed marked viral inhibitory activity for APMV-1 of 5000 tissue culture infected dose fifty ($TCID_{50}$) and (LTV) of 500 $TCID_{50}$ in Vero cell cultures based on their cytopathic effect. Chicken embryo experiments showed that compounds **2**, **3**, and **4** had high antiviral activity *in vitro* with inhibitory concentration fifty (IC_{50}) ranges of 3-4 μg per egg against avian APMV-1 and LTV and toxic concentration fifty (CC_{50}) of 200-300 μg per egg.

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