Synthesis of certain new fused pyranopyrazole and pyranoimidazole incorporated into 8-hydroxyquinoline through a sulfonyl bridge at position 5 with evaluation of their in-vitro antimicrobial and antiviral activities

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Background and objectives
Heterocyclic systems with a quinoline nucleus display a wide spectrum of biological activities such as antimicrobial and antiviral activities. The aim of the present study was the synthesis of new fused pyranopyrazoles, **5a-e** and **6a-e**, and pyranoimidazoles, **10a-e** and **11a-e**, incorporated to 8-hydroxyquinoline through a sulfonyl bridge at position 5 and evaluation of their antimicrobial and antiviral activities.
Methods
The synthesis of the titled quinoline derivatives was achieved through cyclization of 8-hydroxyquinoline-5-sulfonyl chloride (**1**) with 2′-acetyl-2-cyanoacetohydrazide, 2-cyanoacetic acid hydrazide, and 3-amino-5-pyrazolone to afford **2**, **3**, and **4**, respectively. Moreover, reaction of **1** with glycine gives **7**, which on heterocyclization with ammonium thiocyanate yielded the 2-thioxoimidazolidin-2-one derivative **8**. Cyclocondensation reaction of **3**, **4**, **8**, and **9** with different arylidene malononitriles afforded fused systems, **5a-e**, **6a-e**, **10a-e**, and **11a-e**, respectively. The synthesized compounds were evaluated for their in-vitro antimicrobial activity using the disc diffusion method. In addition, they were evaluated for their in-vitro antiviral activity against avian paramyxovirus type 1 (APMV-1) and laryngotracheitis virus (LTV).
Results and conclusion
In-vitro antimicrobial activity of the newly synthesized compounds included an inhibitory effect toward the growth of *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria). Furthermore, of the six selected compounds (**2**, **3**, **4**, **7**, **8** and **9**) tested for their antiviral activity, compounds **2**, **3**, and **4** at a concentration range of 3–4 μg/ml 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 on the basis of their cytopathic effect. Chicken embryo experiments show that compounds **2**, **3**, and **4** possess high antiviral activity *in vitro*, with inhibitory concentration fifty (IC_{50}) ranging from 3 to 4 μg/egg against avian APMV-1 and LTV and toxic concentration fifty (CC_{50}) ranging from 200 to 300 μg/egg.

Keywords:
antimicrobial, antiviral activities, 8-hydroxyquinoline-5-sulfonyl chloride, pyrano[2,3-*c*] pyrazole, pyrano[2,3-*d*]imidazole

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Introduction

Heterocyclic systems with a quinoline nucleus represent privileged moieties in medicinal chemistry and are ubiquitous substructures associated with biologically active natural products. Quinoline derivatives have been shown to display a wide spectrum of biological activities such as antibacterial [1–3], antifungal [4,5], antiparasitic [6], and antiviral activities [7,8]. Because of their wide range of biological activities, quinoline compounds have been considered to be good starting materials for the search of novel antimicrobial and antiviral agents. Accordingly, the aim of the present work was the synthesis of new fused pyranopyrazoles, **5a-e** and **6a-e**, and pyranoimidazoles, **10a-e** and **11a-e**, incorporated into 8-hydroxyquinoline through a sulfonyl bridge at position 5. Moreover, the study includes testing of the target compounds for their expected antimicrobial and antiviral activities.

Subject and methods

Chemistry

Melting points were determined in open capillary tubes, on an Electrothermal 9100 digital melting point apparatus (Büchi, Mount Holly, New Jersey, USA), and were reported

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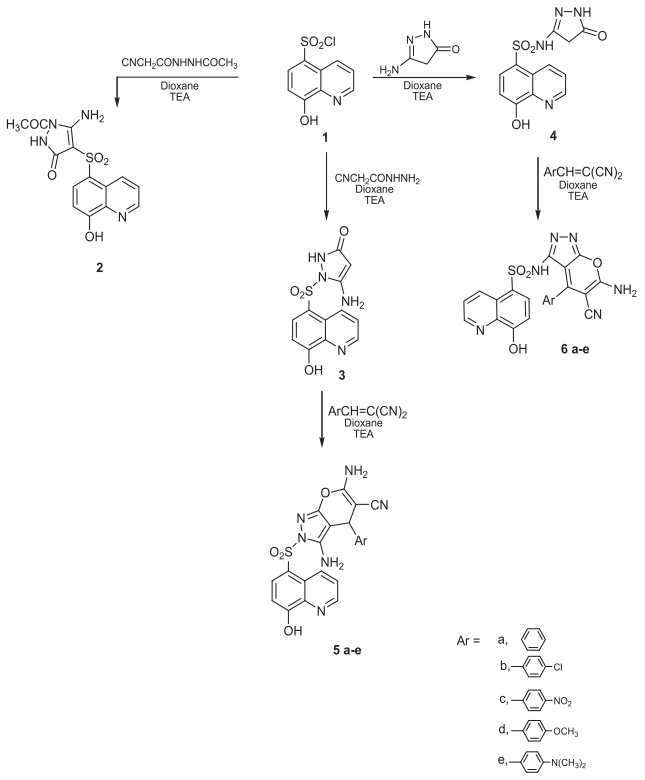
uncorrected. Elemental analyses were carried out using the Perkin-Elmer 2400 analyzer (Norwalk, Connecticut, USA) and the results were found to be within ± 0.4% of the theoretical values (Table 1). Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 Fourier transform infrared spectroscope against KBr discs. ^{1}H NMR spectra were measured on a JEOL 270 MHz spectrometer (JEOL, Tokyo, Japan) in dimethyl sulfoxide-d_6 and chemical shifts were recorded in δ ppm relative to tetramethylsilane as an internal standard. Mass spectra (EI) were measured at 70 eV using a JEOL-JMS-AX500 mass spectrometer (JEOL). 8-Hydroxyquinoline-5-sulfonyl chloride [9], 2-cyanoacetic acid hydrazide [10], 3-amino-5-pyrazolone [11], 2′-acetyl-2-cyanoacetohydrazide [12], and arylidene malononitrile [13] were prepared as reported.

Scheme 1



Synthesis of compounds **2**, **3**, **4**, **5a-e**, and **6a-e**.

*1-Acetyl-5-amino-4-[(8-hydroxyquinoline-5-yl)sulfonyl]-1,2-dihydro-pyrazol-3-one (**2**)*

A mixture of 8-hydroxyquinoline-5-sulfonyl chloride (**1**; 2.4 g, 0.01 mol) and 2′-acetyl-2-cyanoacetohydrazide (1.3 g, 0.01 mol) in dioxane (20 ml) containing triethylamine (1 ml) was refluxed for 3 h. After cooling, the precipitate formed was filtered off, washed with water, air dried, and recrystallized from aqueous ethanol (Scheme 1, Table 1).

*5-Amino-1-[(8-hydroxyquinoline-5-yl)sulfonyl]-1,2-dihydro-pyrazol-3-one (**3**)*

A mixture of 8-hydroxyquinoline-5-sulfonyl 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 hot solid that formed was filtered off, washed with water, air dried, and recrystallized from absolute ethanol (Scheme 1, Table 1).

*3-[(8-Hydroxyquinoline-5-yl)sulfonamido]-1,2-dihydropyrazol-5(4H) one (**4**)*

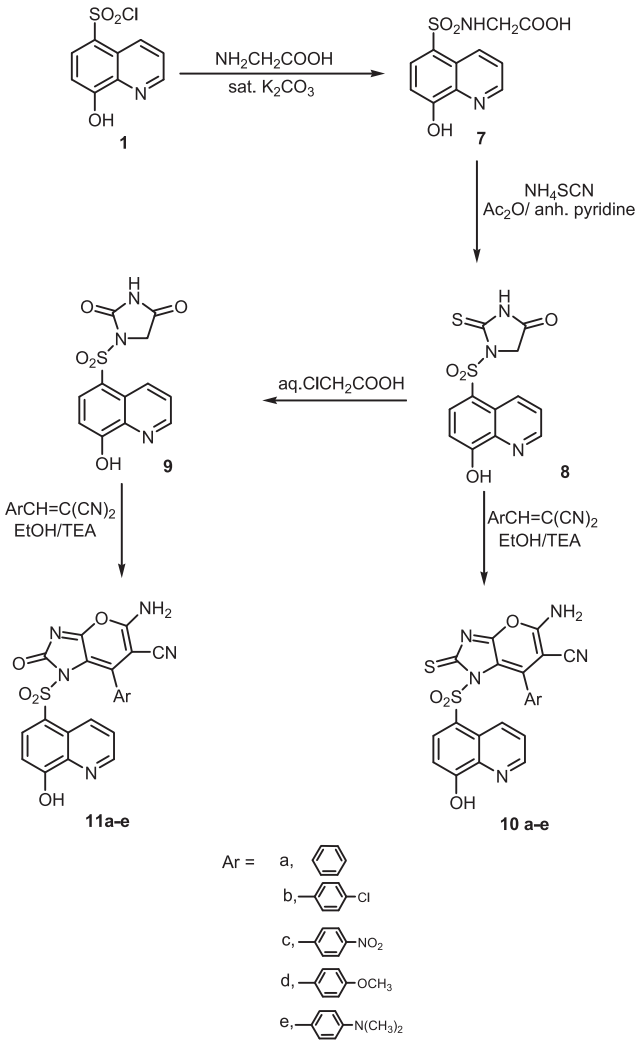
A mixture of 8-hydroxyquinoline-5-sulfonyl chloride (**1**; 2.4 g, 0.01 mol) and 3-amino-5-pyrazolone (0.99 g,

Table 1 Physical and analytical properties of the newly synthesized compounds

				Analysis (%; calculated/found)		
Compd number	Formula (M_w)	MP (°C)	Yield (%)	C	H	N
2	$C_{14}H_{12}N_4O_5S$ (348.33)	222–4	60	48.27/48.33	3.44/3.21	16.09/15.99
3	$C_{12}H_{10}N_4O_4S$ (306.30)	222–4	66	47.05/47.22	3.26/3.34	18.30/18.55
4	$C_{12}H_{10}N_4O_4S$ (306.30)	312–4	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)	253–5	18	53.39/53.21	3.05/2.99	16.98/17.01
5c	$C_{22}H_{15}N_7O_6S$ (505.46)	263–5	30	52.28/52.01	2.99/3.03	19.40/19.60
5d	$C_{23}H_{18}N_6O_5S$ (490.49)	310–2	22	56.32/56.43	3.70/3.87	17.13/17.33
5e	$C_{24}H_{21}N_7O_4S$ (503.53)	166–8	20	57.25/57.44	4.20/4.35	19.47/19.44
6a	$C_{22}H_{14}N_6O_4S$ (458.45)	76–8	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)	205–7	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)	164–6	34	57.48/57.65	3.79/3.66	19.56/19.77
7	$C_{11}H_{10}N_2O_5S$ (282.27)	215–7	90	46.80/46.91	3.54/3.66	9.92/10.01
8	$C_{12}H_9N_3O_4S_2$ (323.35)	205–7	70	44.58/44.78	2.78/3.00	13.00/13.22
9	$C_{12}H_9N_3O_5S$ (307.28)	290–2	60	46.90/47.01	2.93/3.00	13.68/13.77
10a	$C_{22}H_{13}N_5O_4S_2$ (475.50)	271–3	18	55.57/55.50	2.76/2.88	17.73/17.88
10b	$C_{22}H_{12}ClN_5O_4S_2$(509.99)	243–5	30	51.82/52.00	2.37/2.55	13.73/13.82
10c	$C_{22}H_{12}N_6O_6S_2$ (520.50)	221–3	22	50.77/50.89	2.32/2.55	16.15/16.32
10d	$C_{23}H_{15}N_5O_5S_2$ (505.53)	305–7	24	54.65/54.77	2.99/3.02	13.85/14.00
10e	$C_{24}H_{18}N_6O_4S_2$ (518.08)	176–8	20	55.59/55.61	3.50/3.52	16.21/16.42
11a	$C_{22}H_{13}N_5O_5S$ (459.43)	207–9	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)	145–7	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

dec., decomposition; MP, melting point; M_w, molecular weight.

Scheme 2



Synthesis of compounds **7**, **8**, **9**, **10a-e** and **11a-e**.

0.01 mol) in dioxane (20 ml) containing triethylamine (1 ml) was refluxed for 3 h. After cooling, the precipitate formed was filtered off, washed with water, air dried, and recrystallized from aqueous ethanol (Scheme 1, Table 1).

*General procedure for the synthesis of 4-aryl-3,6-diamino-2,4-dihydro-2-[(8-hydroxyquinoline-5-yl)sulfonyl]pyrano[2,3-c] pyrazole-5-carbonitriles (**5a-e**)*

A solution of the appropriate arylidene malononitriles (0.01 mol) and compound **3** (3.06 g, 0.01 mol) in dioxane (20 ml) containing triethylamine (1 ml) was refluxed for 3–6 h. After cooling, the precipitate formed was filtered off, washed with water, air dried, and recrystallized from absolute ethanol (Scheme 1, Table 1).

*General procedure for the synthesis of 6-amino-4-aryl-3-[(8-hydroxyquinoline-5-yl)sulfonamido]pyrano[2,3-c]pyrazole-5-carbonitriles (**6a-e**)*

A solution of the appropriate arylidene malononitriles (0.01 mol) and compound **4** (3.06 g, 0.01 mol) in dioxane (20 ml) containing triethylamine (1 ml) was refluxed for 3–6 h. After cooling, the precipitate formed was filtered off, washed with water, air dried and recrystallized from absolute ethanol (Scheme 1, Table 1).

*2-(2-(8-Hydroxyquinoline-5-yl)sulfonamido)acetic acid (**7**)*

A suspension of 8-hydroxyquinoline-5-sulfonyl 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 stirred and 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 formed was filtered off and recrystallized from dioxane (Scheme 2, Table 1).

*1-[(8-Hydroxyquinoline-5-yl)sulfonyl]-2-thioxoimidazolidin-4-one (**8**)*

A suspension of 2-(2-(8-hydroxyquinoline-5-yl)sulfonamido)acetic acid (**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; Scheme 2, Table 1).

*1-[(8-Hydroxyquinoline-5-yl)sulfonyl]imidazolidin-2,4-dione (**9**)*

A suspension of 1-[(8-hydroxyquinoline-5-yl)sulfonyl]-2-thioxo-imidazolidin-4-one (**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 on 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; Scheme 2, Table 1).

*General procedure for the synthesis of 5-amino-7-aryl-1,2-dihydro-1-[(8-hydroxyquinoline-5-yl)sulfonyl]-2-thioxopyrano[3,2-d]imidazole-6-carbonitriles (**10a-e**)*

A solution of the appropriate arylidene malononitriles (0.01 mol) and 1-[(8-hydroxyquinoline-5-yl)sulfonyl]-2-thioxoimidazolidin-4-one (**8**; 3.23 g, 0.01 mol) in dioxane (20 ml) containing triethylamine (1 ml) was refluxed for 3–6 h. After cooling, the precipitate formed was filtered off, air dried, and recrystallized from absolute ethanol (Scheme 2, Table 1).

*General procedure for the synthesis of 5-amino-7-aryl-1,2-dihydro-1-[(8-hydroxyquinoline-5-yl)sulfonyl]-2-oxo-pyrano[3,2-d]imidazole-6-carbonitriles (**11a-e**)*

A solution of the appropriate arylidene malononitriles (0.01 mol) and 1-[(8-hydroxyquinoline-5-yl)sulfonyl]imidazolidin-2,4-dione (**9**; 3.07 g, 0.01 mol) in dioxane (20 ml) containing triethylamine (1 ml) was refluxed for 3–6 h. After cooling, the precipitate formed was filtered off, air dried, and recrystallized from absolute ethanol (Scheme 2, Table 1).

Biological assay

Antimicrobial evaluation

The antimicrobial activities of the test compounds **2**, **3**, **4**, **5a-e**, **6a-e**, **8**, **9**, **10a-e**, and **11a-e** against a variety of pathogenic microorganisms such as *Escherichia coli*,

Pseudomonas aeruginosa (Gram-negative bacteria), *Staphylococcus aureus*, *Bacillus cereus* (Gram-positive bacteria), and one strain of fungi (*Candida albicans*) were determined *in vitro* using the disc diffusion method [14]. They were isolated from clinical samples and identified to the species level according to different API 20E systems (Analytab Products Inc., New York, USA). 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/disc) on Nutrient and MacConky agar media for bacteria and on Sabouraud dextrose agar for the fungus. Dimethyl formamide was used as a solvent for impregnation. The inhibition zones of the tested compounds were measured after 24–48 h of incubation at 37°C for bacteria and after 5 days of incubation at 28°C for fungi. Cefotaxime [a standardized 30 μg cefotaxime disc (BBL, Lot 104026; assayed content of 30 μg/disc) was used in the disc diffusion test; Hoechst-Roussel Pharmaceuticals Inc., Somerville, New Jersey, USA] and Pipracillin (Pipracillin, 100 μg/disc; Bristol-Myers Squibb, Giza, Egypt) were used as reference drugs for bacteria, whereas nystatin (30 U/disc; Bristol-Myers Squibb; European unit = 0.04 μg/disc) was used as the reference drug for the fungus (*C. albicans*).

Antiviral evaluation

Viruses

Live avian paramyxovirus type1 (APMV-1) and laryngotracheitis virus (LTV) were obtained from the Strain Bank of Central Laboratory for Evaluation of Veterinary Biologics, Cairo, Egypt.

Cell line

Vero (normal, African green monkey kidney) cell culture was obtained from Veterinary Vaccines and Serum Research Institute, Cairo, Egypt. Cells were cultured in sterile growth medium (RPMI-1640; Sigma-Aldrich, Germany) supplemented with 10% of heat activated new born calf serum (Sigma-Aldrich Chemie GmeH, Taufkirchen, Germany) and antibiotics (1000 IU/ml penicillin, 100 μg/ml streptomycin, and 25 μg/ml amphotericinB; Gibco, Rockville, Maryland, USA). The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured twice a week. The virus was propagated in Vero cells and the infective titer of the stock solution was 10^{-7}tissue culture infected dose fifty ($TCID_{50}$) per ml (50% tissue culture infective dose). Viruses were adapted on Vero cells throughout seven successive passages, by which the viruses showed a distant cytopathic effect (degeneration and floatation of the infected cells) on the third 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 3-(4,5-dimethylthiazoyl-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay [15]. The subconfluent cell cultures were trypsinized and collected. The cells at a concentration of 3×10^3 cells/ml in 100 μl RPM1-1640 culture medium were incubated for 3 h at 37°C in a 5% CO_2 incubator. The seed cells were incubated in the 96-well microplates (3×10^3 cells/well) at 37°C in a 5% CO_2 incubator for 24 h. After 24 h, when the cells became confluent, the supernatant was flicked off and added previously diluted with media of 100 μl of different concentrations of test compounds in 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 dimethyl sulfoxide and absorbance was measured using an ELISA microplate reader (Bio-Rad Laboratories, Hercules, California, USA) at a wavelength of 570 nm.

In-vitro antiviral assay

Different nontoxic concentrations of test compounds, that is lower than the CTC_{50} (concentration required to reduce viability by 50%), were checked for antiviral property using the cytopathic effect assay against a challenge dose of 10 $TCID_{50}$. Cells were seeded in 96-well microtitre plates at a population of 10 000 cells/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 then with maintenance medium containing the virus (10 $TCID_{50}$); thereafter, they were incubated at 37°C for 90 min for adsorption of the virus. After this, the cultures were treated with different dilutions of the test compounds in fresh maintenance medium and incubated at 37°C for 5 days. Observations were made every 24 h and cytopathic effects were recorded. Anti-APMV-1 and anti-LTV activities were determined by the inhibition of the cytopathic effect compared with control – that is the protection offered by the test samples to the cells scored [16].

In Vero cell cultures

These assays were performed in nine 24-well tissue culture plates according to the procedure described by Cox *et al.* [17]. Confluent monolayer's of Vero cells were infected with 5000 $TCID_{50}$/0.2 ml/well of APMV-1 or 500 $TCID_{50}$ of LTV and incubated for 2 h (for virus adsorption); thereafter, inoculum was decanted, followed by addition of different 10-fold concentrations of each test sample separately (from 3 to 5 μg/ml/well of each concentration). Virus infectivity and cytotoxicity of each test compound were controlled. Test plates were incubated at 37°C in a 5% CO_2 incubator for 3 days. Cytotoxicity concentration fifty (CC_{50}) of each test compound was defined as the concentration of compounds that induced any deviation in the morphology from that of the normal control cells in 50% of Vero cell monolayers. Antiviral inhibitory concentration fifty (IC_{50}) of test compounds was defined as the concentration of compounds that fully inhibited the cytopathic effect of viruses (100 TCID) in 50% of

monolayers. In addition, the therapeutic index of samples was expressed as CC_{50}/IC_{50} [18].

In embryonated chicken eggs

Groups of 9–11-day-old SPF embryonated chicken eggs were inoculated with 500 embryo infective dose fifty (EID_{50}) per 0.2 ml per egg of APMV-1 or 50 EID_{50} of LTV, immediately followed by injection of different concentrations of each compound (from 2 to 500 µg/0.2 ml/egg) separately. The virus infectivity control and test sample toxicity control were inoculated through the chorioallantoic cavity. Test eggs were incubated for 3–4 days at 37°C and 80% humidity. The CC_{50}, IC_{50}, and therapeutic index values were determined as mentioned before. APMV-1 infectivity in embryonated chicken eggs was detected by haemagglutinating activity of the allantoic fluids of the inoculated eggs, as measured by a microtechnique of the haemagglutination test [19], whereas LTV infectivity was determined on the basis of distension of the abdominal region, mottled necrotic or hemorrhagic liver, and mortality scores in embryos. CC_{50} and IC_{50} were calculated by the reported method [18].

Results and discussions

Chemistry

The reaction routes for the synthesis of the title compounds are described in Schemes 1 and 2. Condensation of 8-hydroxyquinoline-5-sulfonyl chloride (**1**) with 2′-acetyl-2-cyanoacetohydrazide in refluxing dioxane in the presence of triethylamine led to the formation of 1-acetyl-5-amino-4-[(8-hydroxyquinoline-5-yl)sulfonyl]-1,2-dihydropyrazol-3-one (**2**), Scheme 1. The reaction may be preceded by reaction of the chlorine atom of **1** with the active methylene group of 2′-acetyl-2-cyanoacetohydrazide, followed by intramolecular cyclization to give **2**. The structure of **2** was confirmed by its correct elemental analysis, Table 1 as well as its IR, ^{1}H NMR, and MS spectra (Table 2). ^{1}H NMR of **2** revealed two singlet signals at 10.45 and 8.01 ppm for the OH and NH group, respectively, multiple signals at 7.20–7.88 ppm for five aromatic protons, and two singlet signals at 4.66 and 2.99 ppm for the amino (NH_2) and acetyl ($COCH_3$) group, respectively (Table 2).

In contrast, reaction of **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 5-amino-1-[(8-hydroxyquinoline-5-yl)sulfonyl]-1,2-dihydropyrazol-3-one (**3**) and 3-[(8-hydroxyquinoline-5-yl)sulfonamido]-1,2-dihydropyrazol-5(4H) one (**4**) in 66 and 85% yield, respectively (Scheme 1). The characteristic features of **3** are the absence of the absorption bands for the Cl atom in the IR spectrum and the presence of absorption bands at 3209, 3163, 1686, 1385 and 1188/cm for NH_2, NH, C = O, and SO_2, respectively. The ^{1}H NMR spectrum 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 2).

Cyclocondensation reaction of compounds **3** and **4** with some arylidene malononitriles such as 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 4-aryl-3,6-diamino-2,4-dihydro-2-[(8-hydroxyquinoline-5-yl)sulfonyl]-pyrano [2,3-*c*]pyrazole-5-carbonitriles (**5a-e**) and 6-amino-4-aryl-3-[(8-hydroxyquinoline-5-yl)sulfonamido]pyrano[2,3-*c*]pyrazole-5-carbonitriles (**6a-e**), respectively, in 18–34% yields (Scheme 1; Table 1).

Moreover, reaction of 8-hydroxyquinoline-5-sulfonyl chloride (**1**) with glycine in the presence of saturated potassium carbonate solution led to the formation of 2-(2-(8-hydroxyquinoline-5-yl)sulfonamido)acetic acid (**7**; Scheme 2).

Heterocyclization of the latter compound through its reaction with ammonium thiocyanate in acetic anhydride in the presence of anhydrous pyridine gave 1-[(8-hydroxyquinoline-5-yl)sulfonyl]-2-thioxoimidazolidin-4-one (**8**; Scheme 2). The IR spectrum of **8** showed absorption bands at 1240/cm for C = S besides those for the sulfonamido group at 1371 and 1136/cm. Its ^{1}H NMR spectrum revealed a singlet signal at 8.76 ppm for NH and at 4.24 ppm for CH_2 of the imidazole moiety besides other signals that were located at those positions (Table 2).

Acid hydrolysis of compound **8** using aqueous monochloroacetic acid yielded the corresponding imidazolidin-2,4-dione derivative **9** (Scheme 2). The IR spectrum of **9** showed the absence of the absorption bands of C = S and the presence of absorption bands at 1705 and 1715/cm for C = O groups (Table 2).

In a manner similar to that used to obtain compounds **5a-e** and **6a-e**, 1-[(8-hydroxyquinoline-5-yl)sulfonyl]-2-thioxoimidazolidin-4-one (**8**) and 1-[(8-hydroxyquinoline-5-yl)sulfonyl]- imidazolidin-2,4-one (**9**) were condensed with the previous arylidine malononitriles to yield the fused systems 5-amino-7-aryl-1,2-dihydro-1-[(8-hydroxyquinoline-5-yl)sulfonyl]-2-thioxopyrano[3,2-d]imidazole-6-carbonitriles (**10a-e**) and 5-amino-7-aryl-1,2-dihydro-1-[(8-hydroxyquinoline-5-yl)sulfonyl]-2-oxo-pyrano[3,2-*d*]imidazole-6-carbonitriles (**11a-e**), respectively, in 18–33 yields (Scheme 2; Table 1). The ^{1}H NMR spectra of compounds **10a**,**c**,**e** and **11a**,**c**,**e** lack the presence of the CH_2 proton of imidazole and revealed new singlet signals for NH_2 at 8.99, 9.15, 6.76, 8.87, 8.81 and 8.57 ppm, respectively (Table 2).

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), *S. aureus*, *B. cereus* (Gram-positive bacteria), and one strain of fungi (*Candida albicans*), at different doses of the tested compounds (100, 50, and 25 µg/disc) (Table 3). The results showed that compounds **3**, **4**, **5c**, **8**, and **9** were the most active of all test compounds with growth inhibition of 28, 27, 22, 22, and 20 mm, respectively, at 100 µg/disc against *E. coli* when compared with the reference drug cefatoxime (32 mm) at 30 µg/disc.

Table 2 Spectral characterization of the newly synthesized compounds

Compound number	IR (ν_{max},/cm)	^{1}H NMR (δ, ppm)	Mass (*m/z*, %)
2	3420 (OH), 3200 and 3106 3163 (NH and NH_2), 1702 and 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 and 3163 (NH and NH_2), 1686 (C=O), 1651 (C=N), 1606 (C=C), 1385 and 1188 (SO_2N)	10.55 (s, 1H, OH), 9.52 (s, 1H, NH), 8.82 and 9.11 (2d, 2H, H-2 and H-4 quinoline), 6.90 and 7.81 (2d, 2H, H-6 and 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 and 3164 ((NH and NH_2), 1687 (C=O), 1651 (C=N), 1601 (C=C), 1368 and 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 and 3103 (NH and NH_2), 2230 (CN), 1641 (C=N), 1597 (C=C), 1340 and 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 and 3102 (NH and NH_2), 2223 (CN), 1599 (C=N), 1565 (C=C), 1370 and 1126 (SO_2N)		494 (M^+, 10), 496 ($M^+ + 2$, 3), 383 (20), 205 (30), 111 (70), 89 (100), 77 (30)
5c	3400 (OH), 3200 and 3103 (NH and NH_2), 2230 (CN), 1641 (C=N), 1597 (C=C), 1346 and 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 and 3100(NH and NH_2), 2200 (CN), 1620 (C=N), 1587 (C=C), 1346 and 1132 (SO_2N), 1009 (C–O–C)		490 (M^+, 1), 33 (40), 355 (100), 205 (50), 87 (46)
5e	3445 (OH), 3212 and 3135 (NH and NH_2), 2219 (CN), 1601 (C=N), 1529 (C=C), 1369 and 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 and 3200 (NH and NH_2), 2215 (CN), 1620 (C=N), 1365 and 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 and 3191 (NH and NH_2), 2219 (CN), 1618 (C=N), 1365 and 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 and 3255 (NH and NH_2), 2223 (CN), 1664 (C=N), 1584 (C=C), 1348 and 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)	
6d	3228 (OH), 3198 and 3105 (NH and NH_2), 2219 (CN), 1645 (C=N), 1579 (C=C), 1348 and 1137 (SO_2N), 1009 (C–O–C)		488 (M^+, 40), 412 (30), 385 (5), 340 (100), 205 (16), 77 (50)
6e	3423 (OH), 3370 and 3250 (NH and NH_2), 2206 (CN), 1612 (C=N), 1565 (C=C), 1358 and 1174 (SO_2N), 1031 (C–O–C)	10.88 (s, 1H, OH), 9.01 (s, 1H, NH), 8.56 (s, 2H, NH_2), 7.01–8.34 (m, 9H, Ar-H), 3.11 and 3.34 (2s, 6H, CH_3)	
7	3403 (OH), 3178 (NH), 1740 (C=O), 1651 (C=N), 1620 (C=C), 1339 and 1127 (SO_2N)	10.51 and 9.90 (2s, 2H, 2OH), 7.30–7.67 (m, 5H, Ar-H quinoline), 6.66 (s, 1H, NH), 4.24 (s, 2H, CH_2)	
8	3353 (OH), 3138 (NH), 1721 (C=O), 1674 (C=N), 1528 (C=C), 1240 (C=S), 1371 and 1136 (SO_2N)	10.51 (s, 1H, OH), 8.76 (s, 1H, NH), 7.31–7.87 (m, 5H, Ar-H quinoline), 4.24 (s, 2H, CH_2)	
9	3424 (OH), 3164 (NH), 1705 and 1715 (C=O), 1631 (C=N), 1543 (C=C), 1345 and 1154 (SO_2N)	10.51 (s, 1H, OH), 9.99 (s, 1H, NH), 7.10–7.67 (m, 5H, Ar-H quinoline), 4.44 (s, 2H, CH_2)	
10a	4210 (OH), 3220 and 3108 (NH_2), 2219 (CN), 1620 (C=N), 1578 (C=C), 1245 (C=S), 1375 and 1168 (SO_2N), 1009 (C–O–C)	10.52 (s, 1H, OH), 8.99 (s, 2H, NH_2), 7.10–7.37 (m, 5H, Ar-H), 7.31–8.37 (m, 5H, Ar-H quinoline)	
10b	3390 (OH), 3321 and 3218 (NH_2), 2219 (CN), 1620 (C=N), 1558 (C=C), 1245 (C=S), 1336 and 1168 (SO_2N), 1009 (C–O–C), 740 (Cl)		509 (M^+, 30), 511 ($M^+ + 2$, 10), 482 (24), 399 (30), 205 (43), 77 (70), 65 (100)
10c	4350 (OH), 3310 and 3200 (NH_2), 2219 (CN), 1620 (C=N), 1555 (C=C), 1245 (C=S), 1335 and 1173 (SO_2N), 1009 (C–O–C)	10.37 (s, 1H, OH), 9.15 (s, 2H, NH_2), 7.10–8.37 (m, 9H, Ar-H)	
10d	3330 (OH), 3218 and 3108 (NH_2), 2219 (CN), 1620 (C=N), 1568 (C=C), 1245 (C=S), 1375 and 1167 (SO_2N), 1009 (C–O–C)		505 (M^+, 10), 451 (20), 383 (40), 329 (20), 205 (50), 87 (100), 77 (70)
10e	3350 (OH), 3300 and 3208 (NH_2), 2219 (CN), 1620 (C=N), 1567 (C=C), 1245 (C=S), 1375 and 1167 (SO_2N), 1009 (C–O–C)	10.51 (s, 1H, OH), 6.76 (s, 2H, NH_2), 7.10–8.37 (m, 9H, Ar-H), 2.99 and 3.04 (2s, 6H, $2CH_3$)	
11a	3355 (OH), 3320 and 3218 (NH_2), 2219 (CN), 1678 (C=O), 1620 (C=N), 1567 (C=C), 1375 and 1156 (SO_2N), 1009 (C–O–C)	10.57 (s, 1H, OH), 8.87 (s, 2H, NH_2), 7.01–7.23 (m, 5H, Ar-H), 7.30–8.37 (m, 5H, Ar-H quinoline)	
11b	4320 (OH), 3352 and 3210 (NH_2), 2219 (CN), 1645 (C=O), 1620 (C=N), 1567 (C=C), 1375 and 1167 (SO_2N), 1009 (C–O–C), 740 (Cl)		493 (M^+, 10), 495 (1), 383 (100), 358 (30), 330 (45), 205 (10), 111 (12)

Table 2 (*continued*)

Compound number	IR (ν_{max}/cm)	^{1}H NMR (δ, ppm)	Mass (*m/z*, %)
11c	3340 (OH), 3222 and 3108 (NH_2), 2219 (CN), 1670 (C=O), 1620 (C=N), 1567 (C=C), 1375 and 1167 (SO_2N), 1009 (C–O–C)	9.99 (s, 1H, OH), 8.81 (s, 2H, NH_2), 7.01–8.47 (m, 9H, Ar-H)	
11d	4340 (OH), 3320 and 3208 (NH_2), 2219 (CN), 1678 (C=O), 1620 (C=N), 1567 (C=C), 1375 and 1167 (SO_2N), 1009 (C–O–C)		489 (M^+, 20), 382 (10), 330 (34), 206 (20), 77 (70), 65 (100)
11e	3380 (OH), 3320 and 3211 (NH_2), 2219 (CN), 1670 (C=O), 1620 (C=N), 1567 (C=C), 1375 and 1167 (SO_2N), 1009 (C–O–C)	10.51 (s, 1H, OH), 8.57 (s, 2H, NH_2), 7.01–8.27 (m, 9H, Ar-H), 2.66 and 3.04 (2s, 6H, $2CH_3$)	

IR, infrared; NMR, nuclear magnetic resonance.

Table 3 Antimicrobial activity of the newly synthesized compounds

	Inhibition zone (mm)														
	E. coli			*P. aeruginosa*			*S. aureus*			*B. cereus*			*C albicans*		
	Compounds (μg/disc)														
Compound number	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/disc)	32	22	17	22	18	12	31	26	17	26	20	14	–	–	–
Piperacillin (100 μg/disc)	–	–	–	20	15	10	27	18	10	20	15	10	–	–	–
Nystatin (30 U/disc)	–	–	–	–	–	–	–	–	–	–	–	–	40		

In addition, they showed growth inhibition of 18, 18, 16, 14, and 14 mm, respectively, at 50 μg/disc against *E. coli* when compared with the reference drug cefatoxime (22 mm) at 30 μg/disc. In contrast, compounds **3**, **4**, **8**, and **9** were found to be the most active of all the test compounds with growth inhibition of 19, 20, 18, and 18 mm, respectively, at 100 μg/disc against *P. aeruginosa* when compared with the reference drugs cefatoxime (22 mm) at 30 μg/disc and piperacillin (20 mm) at 100 μg/disc. The rest of the tested compounds were inactive 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 the virus cytotoxicity effect inhibitory assay. The results revealed that compounds **2** and **3** as well as **4** were completely inhibited by 5000 $TCID_{50}$ of APMV-1 and 500 $TCID_{50}$ of LTV infectivity at concentrations of 3, 4, 3 μg/ml, respectively (Table 4). Substantial therapeutic indices of 66, 75, and 66 were recorded. A cytotoxicity assay indicated that CC_{50} of **2**, **3**, and **4** were greater than 200, 300, and 200 mg/ml, respectively (Table 4). These results proved that the three compounds possessed antiviral activity in Vero cells with the absence of apparent cytotoxicity.

In chicken embryos

Studies on the activity of the six selected compounds (**2**, **3**, **4**, **7**, **8**, and **9**), as determined by haemagglutinating activity in allantoic fluids and LTV infectivity criterion in embryos, showed that 4, 3, and 4 μg/0.2 ml/egg of compounds **3**, **4**, and **2** were fully reduced by the

Table 4 Cytotoxic effect of test compounds on normal Vero cell lines

	CC_{50}		IC_{50}		TI	
Compound number	APMV-1	LTV	APMV-1	LTV	APMV-1	LTV
2	>500	>500	≤5	≤5	100	100
3	>500	>500	≤4	≤4	125	100
4	>400	>400	≤3	≤4	100	100
7	>300	>300	≤4	≤4	75	75
8	>200	>200	≤3	≤3	66	66
9	>200	>200	≤3	≤3	66	66

Avian paramyxovirus type 1 (APMV-1) = 5000 $TCID_{50}$; laryngotracheitis virus (LTV) = 500 $TCID_{50}$; CC_{50} (μg/ml): toxic concentration fifty; IC_{50} (μg/ml): inhibiting concentration fifty; $TCID_{50}$,tissue culture infected dose fifty; TI: therapeutic index.

Table 5 Cytotoxic effect of test compounds in embryonated chicken specific-pathogen-free eggs

	CC_{50}		IC_{50}		TI	
Compound number	APMV-1	LTV	APMV-1	LTV	APMV-1	LTV
2	>400	>400	≤5	≤4	80	100
3	>400	>400	≤4	≤4	100	100
4	>400	>400	≤4	≤4	100	100
7	>300	>300	≤4	≤3	75	66
8	>200	>200	≤3	≤4	66	50
9	>200	>200	≤4	≤4	50	50

Avian paramyxovirus type 1 (APMV-1) = 500 EID_{50}; laryngotracheitis virus (LTV) = 50 EID_{50}; CC_{50} (μg/ml), toxic concentration fifty; EID_{50}, embryo infective dose fifty; IC_{50} (μg/ml), inhibiting concentration fifty; TI, therapeutic index.

infectivities of 500 EID_{50} of APMV-1 and 50 EID_{50} of LTV, respectively (Table 5). The toxicity assays of compounds **3**, **4**, and **2** in chicken embryos at concentrations of 300, 200, and 200 μg/egg showed 100% survival of the inoculated eggs 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 LTV. In conclusion, chicken embryo experiments showed that compounds **3**, **4**, and **2** had high antiviral activities *in vitro*, with IC_{50} ranging from 3 to 4 μg/egg against avian APMV-1 and LTV and toxic CC_{50} ranging from 200 to 300 μg/egg. The results showed that a concentration range of 3–4 μg/ml of compounds **2**, **3**, and **4** showed marked viral inhibitory activity for APMV-1 of 5000 $TCID_{50}$ and LTV of 500 $TCID_{50}$ in Vero cell cultures on the basis of their cytopathic effect. Chicken embryo experiments show that compounds **2**, **3**, and **4** had high antiviral activity *in vitro*, with IC_{50} ranging from 3 to 4 μg/egg against avian APMV-1 and LTV and toxic CC_{50} ranging from 200 to 300 μg/egg.

Conclusion

A series of 5-substituted sulfonyl-8-hydroxyquinoline derivatives have been prepared. 8-Hydroxyquinolines that were incorporated into rings of pyrazole **2**, **3**, and **4** and imidazole **8** and **9** through a sulfonyl bridge at position 5 showed inhibition growth towards *E. coli* and *P. aeruginosa* (Gram-negative bacteria) and exhibit marked viral inhibitory activity against APMV-1 and LTV.

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Conflicts of interest

There are no conflicts of interest.

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