Res Chem Intermed
DOI 10.1007/s11164-013-1045-z

Synthesis and anti-viral activities of some 3-(naphthalen-1-ylmethylene)-5-phenylfuran-2(3*H*)-one candidates

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Received: 30 December 2012 / Accepted: 11 January 2013

Abstract 3-(Naphthalen-1-ylmethylene)-5-phenylfuran-2(3*H*)-one **1** was prepared and converted into a variety of heterocyclic systems of synthetic and biological importance. Benzylamine was reacted with furanone **1** to afford compounds **2** and **3** according to the reaction conditions. Butanamide **2** was reacted with thionyl chloride or thiourea to give derivatives **4** and **5**, respectively. Compound **3** was reacted with ethyl cyanoacetate to give the corresponding pyrrolopyridine derivative **6**. Treatment of **1** with hydrazine hydrate afforded compounds **7** and **8** according to the reaction conditions. Also, compound **1** was reacted with phenyl hydrazine, hydroxyl amine, malononitrile or thiourea to give compounds **9–12**, respectively. Cyclization of **7** with ethoxymethylene-malononitrile, ethyl-(ethoxymethylene)cyanoacetate, carbon disulphide or acetylacetone afforded the corresponding compounds **13–16**, respectively. Condensation of **7** with *p*-nitrobenzaldehyde gave the corresponding hydrazone **17**, which was treated with thioglycolic acid or chloroacetyl chloride to give compounds **18** and **19**, respectively. Also, most of the prepared products were tested for anti-avian influenza virus and revealed promising antiviral activity against H5N1 virus [A/Chicken/Egypt/1/2006 (H5N1)] by determination of both TC_{50} and ED_{50} and confirmed by plaque reduction assay on

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Published online: 24 January 2013

MDCK cells. Compounds **7**, **8**, **11**, **12** and **13** showed the highest effect compared with the other tested compounds.

Keywords Furanone · Acid hydrazide · Anti-avian influenza virus (H5N1)

Introduction

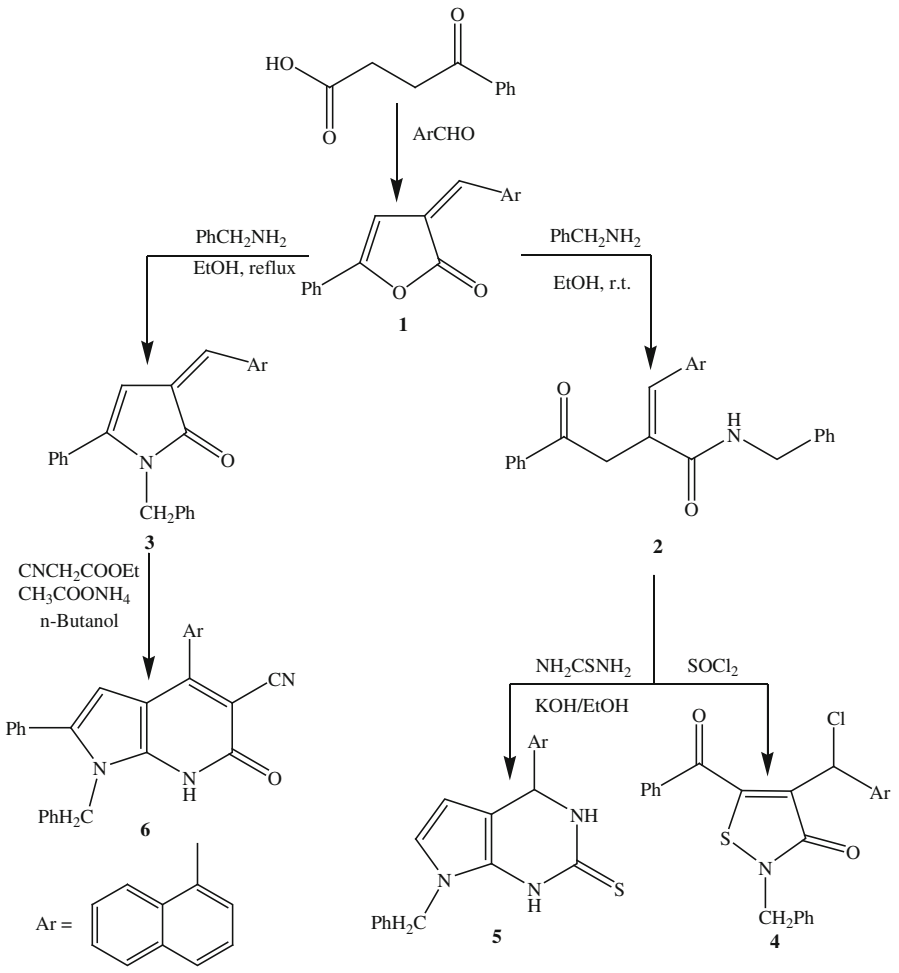
The furanones and other related heterocyclic derivatives exhibit a range of biological activities, such as anti-inflammatory [1, 2], analgesic and ulcerogenic [3] antimicrobial [4, 5], antiviral [6], and antibiotic [7–9]. Pyrimidines and fused pyrimidines, being an integral part of DNA and RNA, play an essential role in several biological processes and have pharmacological importance, particularly, the pyrimidine ring can be found in nucleoside antibiotics, antibacterials, and cardiovascular drugs, as well as in agrochemical and veterinarian products [10–13]. In addition, thiosemicarbazide and triazole derivatives have been found of interest with potent activities including antimicrobial, anti-HIV, analgesic, anticancer and anticonvulsant [14–17]. Also, some of synthetic thiazoles exhibit a range of biological activities, such as antitumor, antibiotic, antibacterial, antifungal, and antiinflammatory [18–20]. In view of these observations, and in continuation of our previous work in heterocyclic chemistry, we synthesized some new furanone and pyrimidine derivatives and tested their anti-HSV-1 activities.

Results and discussion

The reaction of 3-((naphthalen-1-yl)methylene)-5-phenylfuran-2(3*H*)-one **1** with benzylamine in ethanol at room or at refluxing temperature afforded the corresponding open-chain benzylamide **2** and 2(3*H*)-pyrrolone **3**, respectively. Treatment of compound **2** with thionyl chloride or thiourea afforded the corresponding isothiazolone **4** and pyrimidine thione **5**, respectively. The pyrrolone derivative **3** was reacted with ethyl cyanoacetate in the presence of ammonium acetate in *n*-butanol to give the corresponding pyrrolopyridine derivative **6** (Scheme 1).

Condensation of furanone **1** with hydrazine hydrate in ethanol at room temperature or at refluxing temperature afforded the corresponding hydrazide **7** and pyridazinone **8**, respectively. Treatment of **1** with phenyl hydrazine or hydroxylamine hydrochloride gave the corresponding compounds **9** and **10**, respectively. The reaction of **1** with malononitrile in glacial acetic acid and sodium acetate afforded the pyran derivative **11.** Also, pyrimidinethione **12** was prepared by reacting of compound **1** with thiourea in the presence of sodium ethoxide (Scheme 2).

Cyclization of the hydrazide derivative **7** with ethoxymethylenemalononitrile or ethyl(ethoxy methylene)cyanoacetate in refluxing ethanol afforded the corresponding substituted pyrazole derivatives **13** and **14**, respectively. Also, hydrazide **7** was reacted with carbon disulphide or acetylacetone to give the corresponding oxadiazolyl butenone **15** and pyrazole **16** derivatives, respectively. Reaction of hydrazide **7** with *p*-nitrobenzaldeyde afforded Schiff base **17**, which was cyclized with thioglycolic

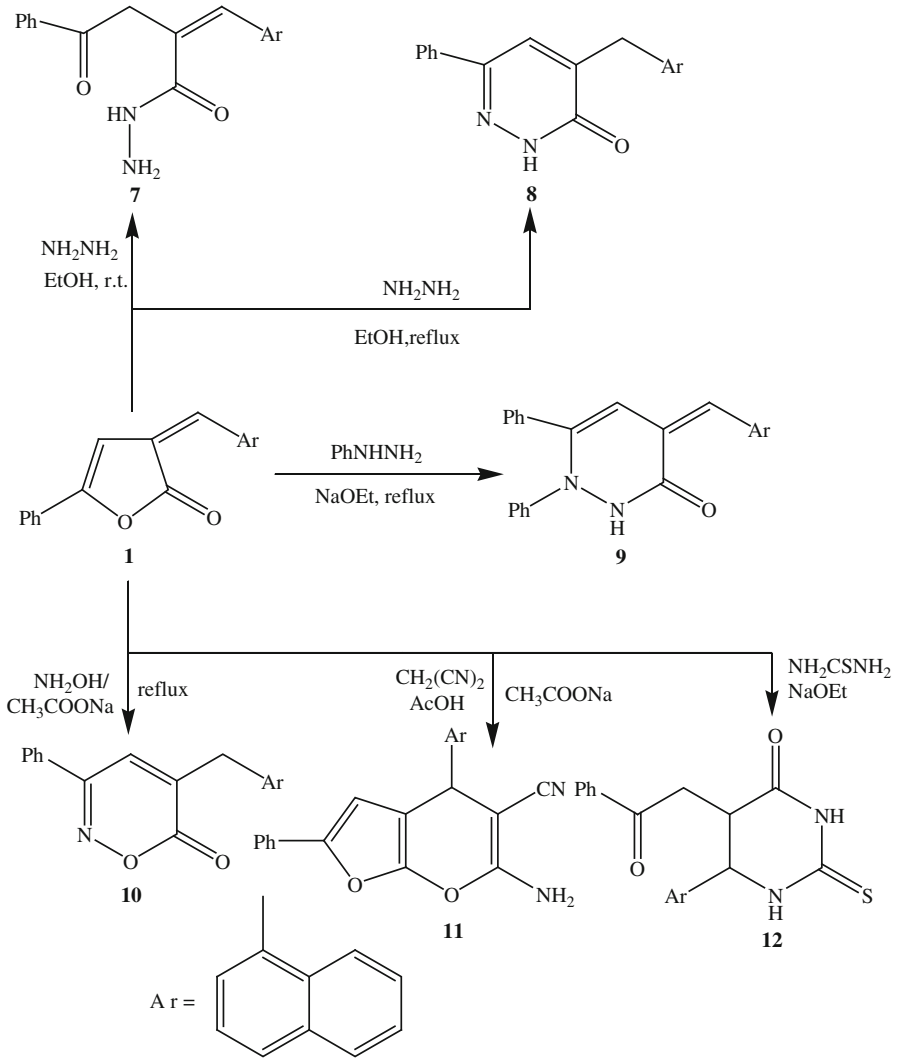


Scheme 1 Synthetic pathway for compounds **2–6**

acid or chloroacetyl chloride to give the corresponding compounds **18** and **19**, respectively (Scheme 3).

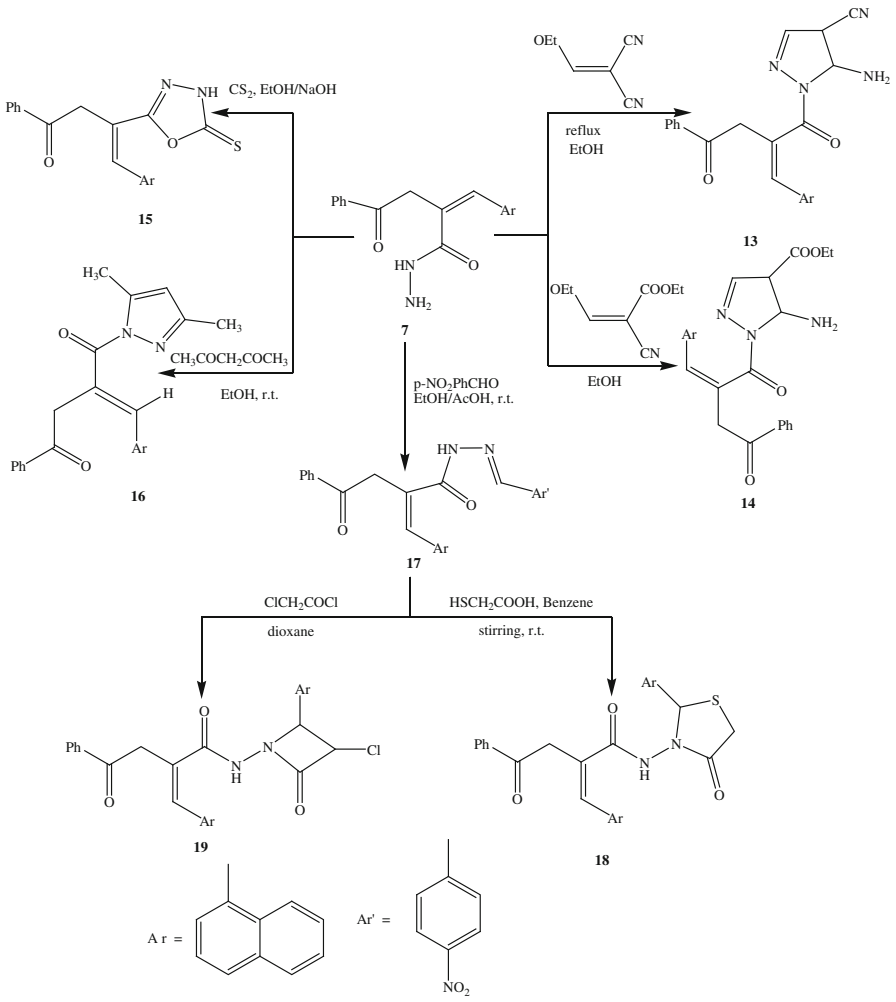
Antiviral activity

Compounds were dissolved in 10 % DMSO and examined for its cytotoxic effect on MDCK cell lines using MTT bioassay. Applying a range of concentrations of 5, 10, 20, 40, 80 μg/100 μL (applied medium volume on each well of 96-well cell culture plate) from each compound in order to determine safe concentrations on cell line. Results were plotted as O.D versus conc. in order to calculate value of TC_{50} of each compound (Table 1).



Scheme 2 Synthetic pathway for compounds **7–12**

The results of the MTT bioassay indicated that all examined compounds showed accepted cytotoxic effects, where the cells start being affected by doses higher than 80 μg/100 μL, except for compounds **2, 10, 12, 13, 14** that showed lower safe concentrations. According to these results, it was found that a concentration lower than 40 μg/10^5 cell/mL could be applied for all compounds under examination through plaque reduction assay, which was applied to determine the percentage of virus count reduction as a result of its treatment with compounds under examination. From the results in Table 2, the percentage reduction of viral counts resulted from virus treatment by 3 concentrations of 10, 20, and 40 μg/10^5 cell



Scheme 3 Synthetic pathway for compounds **13–19**

count/mL treatment of each compound individually in triplicate for each concentration.

Compounds result in a reduction in viral count of ≥80 % are considered a promising ground for emerging a new antiviral drug, from the results, compound 1**2** showed a reduction in viral count of 88 % at a concentration of 40 μg/10^5 cell count/ml with a statistically high significance (Tables 2, 3; Fig. 1).

From Table (2), we indicated that compounds number **7**, **8**, **11**, and **13** showed a percentage of reduction of more than 50 % when applied in their concentration of 40 μg/10^5 cell count/mL (Figs. 2, 3).

Table 1 Values of TC_{50} of each compound using MTT bioassay

Comp. No.	TC_{50} (μg/mL)
1	1050
2	743.2
3	1118.1
4	1473.3
5	903.1
6	2450
7	983.7
8	1819.4
10	658.3
11	1511.4
12	284.7
13	103.5
14	724.3
15	1774.9
17	659.8
19	1393.6

Value of TC_{50} derived from the equation was multiplied by 10 in order to get concentration of TC_{50} effect in 1 mL

Conclusion

There is a increasing work on drug discovery of novel antiviral compounds against H5N1 virus for its recurrent pandemics, especially after the events of the last two decades. In the present study, we examined the effect of 16 compounds for their antiviral activity against rH5N1 (2006) Egyptian strain. Compound **12** showed a reduction of viral activity by 88 % compared to an untreated viral activity, which is considered to be a promising effect leading to a focus on such a compound for further investigation of its potential contribution to drug development. Regarding compounds **7**, **8**, **11**, and **13**, all showed a mild promising effect—if applied singly—on virus activity that may be of pharmaceutical and clinical interest if incorporated in drug combinations or subjected to some chemical modifications through further investigation in order to increase their effect.

Experimental

Melting points were measured using an Electrothermal 9100 digital melting point apparatus (Büchi, Switzerland) and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1600 FTIR (Perkin–Elmer, USA) in KBr discs. ^{1}H NMR spectra were measured on a Jeol 270 MHz spectrometer (Jeol, Japan) and a Bruker Avance spectrometer (300 MHz) (Bruker, Germany) in DMSO-d_6, and chemical shifts were recorded in δ ppm relative to the internal standard TMS. The mass spectra were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermo-electron, Madison, WI, USA) using EI and the values of *m*/*z* are indicated in Dalton. Elemental analyses were performed on a Perkin–Elmer 2400 analyzer (Perkin–Elmer) and were found

Table 2 Antiviral activity of compounds applied in 10, 20, and 40 μg/mL showing the resulted percentage of reduction

Comp. No.	Conc. μg/mL	Intial viral count/mL	PFU/mL ($\times 10^5$) Mean ± SD	% reduction
1	10	6.5×10^5	4.87500 ± 0.12	25.00
	20	6.5×10^5	4.55000 ± 0.200	30.00
	40	6.5×10^5	3.90000 ± 0.180	40.00
2	10	6.5×10^5	5.85100 ± 0.118	10.00
	20	6.5×10^5	5.57186 ± 0.019	14.28
	40	6.5×10^5	4.82885 ± 0.015	25.71
3	10	6.5×10^5	4.64295 ± 0.017	28.57
	20	6.5×10^5	4.48500 ± 0.019	31.00
	40	6.5×10^5	4.27180 ± 0.019	34.28
4	10	6.5×10^5	5.20000 ± 0.170	20.00
	20	6.5×10^5	5.13500 ± 0.180	21.00
	40	6.5×10^5	4.98550 ± 0.110	23.30
5	10	6.5×10^5	3.61400 ± 0.100	44.40
	20	6.5×10^5	3.51650 ± 0.900	45.90
	40	6.5×10^5	3.43200 ± 0.710	47.20
6	10	6.5×10^5	4.61500 ± 0.120	29.00
	20	6.5×10^5	4.33550 ± 0.140	33.30
	40	6.5×10^5	3.97800 ± 0.090	38.80
7	10	6.5×10^5	4.26595 ± 0.090	34.37
	20	6.5×10^5	3.859375 ± 0.100	40.625
	40	6.5×10^5	3.05500 ± 0.130	53.00
8	10	6.5×10^5	4.87500 ± 0.190	25.00
	20	6.5×10^5	4.06250 ± 0.190	37.50
	40	6.5×10^5	3.04720 ± 0.070	53.12
10	10	6.5×10^5	3.82200 ± 0.190	41.20
	20	6.5×10^5	3.68940 ± 0.100	43.24
	40	6.5×10^5	3.51390 ± 0.198	45.94
11	10	6.5×10^5	4.55000 ± 0.090	30.00
	20	6.5×10^5	3.68550 ± 0.080	43.30
	40	6.5×10^5	3.00950 ± 0.100	53.70
12	10	6.5×10^5	2.08000 ± 0.070	68.00
	20	6.5×10^5	1.82000 ± 0.090	72.00
	40	6.5×10^5	0.78000 ± 0.100	88.00
13	10	6.5×10^5	3.50350 ± 0.110	46.10
	20	6.5×10^5	3.52885 ± 0.070	48.71
	40	6.5×10^5	3.00040 ± 0.130	53.84
14	10	6.5×10^5	4.68000 ± 0.080	28.00
	20	6.5×10^5	4.42000 ± 0.070	32.00
	40	6.5×10^5	3.90000 ± 0.116	40.00

Table 2 continued

Comp. No.	Conc. μg/mL	Intial viral count/mL	PFU/mL ($\times 10^5$) Mean ± SD	% reduction
15	10	6.5×10^5	6.27250 ± 0.600	3.50
	20	6.5×10^5	5.80450 ± 0.900	10.70
	40	6.5×10^5	4.17885 ± 0.150	35.71
17	10	6.5×10^5	6.31150 ± 0.080	2.90
	20	6.5×10^5	6.12300 ± 0.100	5.80
	40	6.5×10^5	5.92800 ± 0.100	8.80
19	10	6.5×10^5	5.72000 ± 0.080	12.00
	20	6.5×10^5	4.74500 ± 0.140	27.00
	40	6.5×10^5	4.55000 ± 0.125	30.00

Results interpreted by *t* test, $p < 0.001$

PFU Plaque-forming unit, *SD* standard deviation

Table 3 TC_{50} and ED_{50} values for promising compounds **7**, **8**, **11**, **12**, and **13**

Comp. No.	TC_{50} (μg/mL)	ED_{50} (μg/mL)	TI
7	983.7	23.95	41.07
8	1819.4	35.85	50.75
11	1511.4	22.27	67.86
12	284.7	7.5	37.96
13	103.5	26.15	3.95

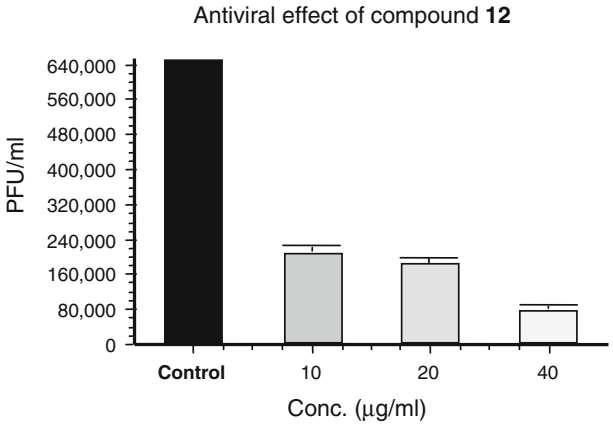


Fig. 1 Antiviral effect of compound **12** (reduction in plaque count compared to untreated control of M.O.I of 0.001)

within ±0.4 % of the theoretical values (Table 1). Follow-up of the reactions and checking the purity of the compounds was made by TLC on silica gel-precoated aluminum sheets (type 60 F_{254}; Merck, Germany). All solvents and chemical reagents were purchased from Aldrich (Germany).

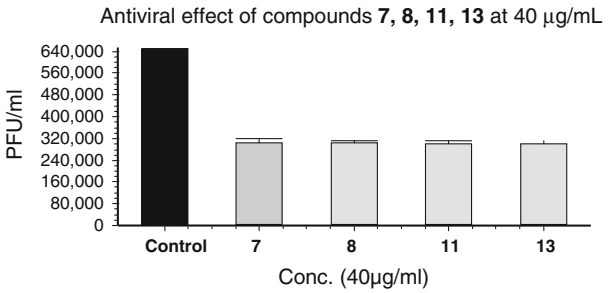


Fig. 2 Antiviral effect of compounds **7**, **8**, **11**, and **13** on viral count when used at 40 µg/mL (reduction in plaque count compared to untreated control of M.O.I of 0.001)

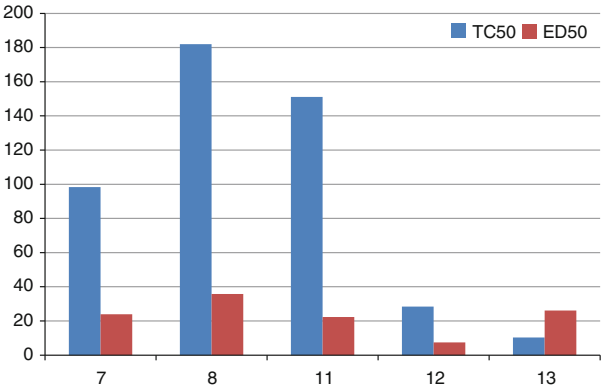


Fig. 3 Values of TC_{50} and ED_{50} of promising compounds **7**, **8**, **11**, **12**, and **13**

3-(Naphthalen-1-ylmethylene)-5-phenylfuran-2(3*H*)-one (**1**)

A mixture of 3-(benzoyl)propionic acid (1.78 g, 10 mmol) and 1-naphthaldehyde (1.56 g, 10 mmol) in acetic anhydride (3 mL) was fused for 5 min then a few drops of triethylamine were added. The reaction mixture was refluxed for 20 min. The obtained solid was crystallized from methanol/chloroform (1:1) to give compound **1**. Yield 62 %; m.p. 148–150 °C; IR (KBr, *v*, cm^{-1}): 1765 (C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 6.8 (s, 1H, furanone ring), 7.49–8.42 (m, 13H, Ar–H+1H, =CH); MS, *m*/*z* (%): 298 (M^+, 100). Analysis for $C_{21}H_{14}O_2$ (298.33): calcd. C, 84.54; H, 4.73; found C, 84.49; H, 4.66.

N-Benzyl-2-(naphthalen-1-ylmethylene)-4 oxo-4-phenylbutanamide (**2**)

To a solution of **1** (1.5 g, 5 mmol) in ethanol (20 mL), benzylamine (0.54 g, 5 mmol) was added. The reaction mixture was stirred at room temperature for 30 min. The obtained product was filtered off, washed with benzene, dried, and crystallized from benzene to give the amide derivative **2**. Yield 82 %; m.p. 184–186 °C; IR (KBr, *v*, cm^{-1}): 1675 (C=O), 3248 (NH); ^{1}H NMR

(DMSO-d_6, δ ppm): 3.29 (s, 2H, CH_2–CO), 4.2 (s, 2H, NH–CH_2), 6.38–8.44 (m, 18H, Ar–H+1H, =CH), 9.18 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%): 405 (M^+, 40). Analysis for $C_{28}H_{23}NO_2$ (405.49): calcd. C, 82.94; H, 5.72; N, 3.45; found C, 82.87; H, 5.61; N, 3.31.

1-Benzyl-3-(naphthalen-1-ylmethylene)-5-phenyl-1*H*-pyrrol-2 (3*H*)-one (**3**)

To a solution of **1** (1.5 g, 5 mmol) in ethanol (20 mL), benzylamine (0.54 g, 5 mmol) was added. The reaction mixture was heated under reflux for 3 h. After cooling, the formed product was filtered off, washed with ethanol and crystallized from ethanol to give the pyrrolone **3**. Yield 80 %; mp 160–162 °C; IR (KBr, *v*, cm^{-1}): 1672 (C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 4.9 (s, 2H, NH–CH_2), 6.31 (s, 1H, pyrrol), 7.02–8.22 (m, 17H, Ar–H) 8.62 (s, 1H, =CH), MS, *m/z* (%): 387 (M^+, 100). Analysis for $C_{28}H_{21}NO$ (387.47): calcd. C, 86.79; H, 5.46; N, 3.61; found C, 86.70; H, 5.39; N, 3.52.

5-Benzoyl-2-bezyl-4-(chloro(naphthalen-1-yl)methyl)isothiazol-3 (2*H*)-one (**4**)

To a cold (−5 °C) thionyl chloride (10 mL), compound **2** (2.03 g, 5 mmol) was added portion wise with stirring. The reaction mixture was heated for 3 h on a water bath. Chloroform (50 mL) was then added and the reaction mixture was decomposed with ice-cold saturated sodium carbonate solution and separated. The organic layer was washed with water and dried over anhydrous calcium chloride. After evaporation of the solvent, the residue was recrystallized from ethanol to give compound **4**. Yield 72 %; m.p. 150–152 °C; IR (KBr, *v*, cm^{-1}): 1678, 1594 (2 C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 4.04 (s, 2H, CH_2), 5.83 (s, 1H, methine), 7.12–7.47 (m, 17H, Ar–H); MS, *m/z* (%): 369 (M^+, 100). Analysis for $C_{28}H_{20}ClNO_2S$ (469.98): calcd. C, 71.56; H, 4.29; Cl, 7.54; N, 2.98; S, 6.82; found C, 70.36; H, 4.18; Cl, 7.39; N, 2.82; S, 6.76.

7-Bezyl-4-(naphthalen-1-yl)-3,4-dihydro-1*H*-pyrrolo[2,3-d]pyrimidine-2(7*H*)-thione (**5**)

A mixture of **2** (2.03 g, 5 mmol) and thiourea (0.4 g, 5 mmol) in ethanolic potassium hydroxide (15 mL, 10 %) was refluxed for 6 h. The reaction mixture was concentrated under reduced pressure, and the residue was triturated with diluted hydrochloric acid. The solid formed was collected by filtration, washed with water, dried, and crystallized from ethanol to give **5**. Yield 72 %; m.p. 100–102 °C; IR (KBr, *v*, cm^{-1}): 3300, 3220 (2NH), 1252 (C=S); ^{1}H NMR (DMSO-d_6, δ ppm): 4.15 (s, 1H, CH-pyrimidine), 4.39 (2H, methylene), 5.98–6.30 (m, 2H, pyrrole), 7.13–8.14 (m, 12H, Ar–H), 9.20, 10.66 (2s, 2H, 2 NH exchangeable with D_2O); MS, *m/z* (%): 369 (M^+, 100). Analysis for $C_{23}H_{19}N_3S$ (369.48): calcd. C, 74.77; H, 5.18; N, 11.37; S, 8.68; found. 74.76; H, 5.10; N, 11.29; S, 8.56.

1-Bezyl-4-(naphthalen-1-yl)-6-oxo-2-phenyl-6,7-dihydro-1*H*-pyrrolo[2,3-b] pyridine-5-carbo-nitrile (**6**)

A mixture of **3** (0.388 g, 1 mmol), ethyl cyanoacetate (0.113 g, 1 mmol) and ammonium acetate (0.616 g, 8 mmol) in *n*-butanol (5 mL) was refluxed for 10 h. The precipitated solid was filtered off, dried, and crystallized from dioxane to give compound **6**. Yield 30 %; m.p. 212–214 °C; IR (KBr, *v*, cm^{-1}): 3430 (NH), 2222 (CN), 1680 (CO); ^1H NMR (DMSO-d_6, δ ppm): 5.44 (s, 2H, CH_2), 6.00 (s, 1H, pyrrole), 7.60–8.40 (m, 17H, Ar–H), 13.00 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%): 451 (M^+, 100). Analysis for $C_{31}H_{21}N_3O$ (451.52): calcd. C, 82.46; H, 4.69; N, 9.31; found C, 82.41; H, 4.58; N, 9.22.

2-(Naphthalen-1-ylmethylene)-4-oxo-4-phenylbutanehydrazide (**7**)

A mixture of **1** (2.98 g, 10 mmol) and hydrazine hydrate (0.6 mL, 12 mmol) in ethanol (30 mL) was stirred at room temperature for 12 h. The obtained product was filtered off, washed with ethanol and crystallized from ethanol to give acid hydrazide derivative **7**. Yield 75 %; m.p. 88–90 °C; IR (KBr, *v*, cm^{-1}): 1,650, 1,685 (2 C=O), 3300, 3190, 3110 (NH_2+NH); ^1H NMR (DMSO-d_6, δ ppm): 3.22 (s, 2H, CH_2–CO), 4.49 (s, 2H, NH_2, D_2O exchangeable), 6.69 (s, 1H, NH–CO, D_2O exchangeable), 7.30–7.98 (m, 12H, Ar–H), 8.96 (s, 1H, =CH); MS, *m/z* (%): 330 (M^+, 100). Analysis for $C_{21}H_{18}N_2O_2$ (330.38): calcd. C, 76.34; H, 5.49; N, 8.48; found C, 76.30; H, 5.50; N, 8.41.

4-(Naphthalen-1-ylmethylene)-6- phenyl pyridazin-3-(2H)-one (**8**)

To a solution of **1** (0.298 g, 1 mmol) in ethanol (10 mL), hydrazine hydrate ($\sim$0.06 mL, 1.2 mmol) was added. The reaction mixture was refluxed for 3 h, after cooling, the obtained product was filtered off, washed with cold ethanol, dried, and crystallized from ethanol to give pyridazinon derivative **8**. Yield 86 %; m.p. 164–166 °C; IR (KBr, *v*, cm^{-1}): 1675 (C=O), 3280 (NH); ^1H NMR (DMSO-d_6, δ ppm): 3.22 (s, 2H, CH_2),5.18 (s, 1H, pyridazinone), 6.72–8.11 (m, 12H, Ar–H), 8.4 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%): 312 (M^+, 100). Analysis for $C_{21}H_{16}N_2O$ (312.36): calcd. C, 80.75; H, 5.16; N, 8.97; found C, 80.70; H, 5.10; N, 8.79.

4-(Naphthalen-1-ylmethylene)-1, 6- diphenyl-1,2-dihydropyridazin-3(4*H*)-one (**9**)

A mixture of **1** (0.298 g, 1 mmol) and phenylhydrazine (0.108 g, 1 mmol) in sodium ethoxide (10 mL, 1 %) was refluxed for 3 h. The reaction mixture was poured into water, the obtained solid was filtered off, washed with water, dried, and crystallized from benzene/petroleum ether (60–80) to give compound **9**. Yield 80 %; m.p. 120–122 °C; IR (KBr, *v*, cm^{-1}): 1657 (C=O), 3226 (NH); ^1H NMR (DMSO-d_6, δ ppm): 6.1 (s, 1H, H pyridazinone), 6.35–8.4 (m, 15H, Ar–H+ethylene–H), 10.2 (br., 1H, NHCO–, D_2O exchangeable), MS, *m/z* (%):388 (M^+, 100). Analysis for $C_{27}H_{20}N_2O$ (388.46): calcd. C, 83.48; H, 5.19; N, 7.21; found C, 83.36; H, 5.00; N, 7.01.

5-(Naphthalen-1-ylmethyl)-3-phenyl-6*H*-1, 2-oxazin-6-one (**10**)

A mixture of **1** (0.298 g, 1 mmol), hydroxylamine hydrochloride (0.69 g, 1 mmol) and sodium acetate (0.2 g, ~2 mmol) in ethanol (10 mL) was refluxed for 5 h. The reaction mixture was evaporated under reduced pressure, the obtained residue was crystallized from ethanol to give compound **10**. Yield 62 %; m.p. 78–80 °C; IR (KBr, *v*, cm^{-1}): 1761 (C=O), 1618 (ArC=C); ^{1}H NMR (DMSO-d_6, δ ppm): 4.37 (s, 2H, CH_2 methylene), 7.48–8.14 (m, 13H, Ar–H+1H, =CH); MS, *m/z* (%): 313 (M^+, 100). Analysis for $C_{21}H_{15}NO_2$ (313.35): calcd. C, 80.49; H, 4.82; N, 4.47; found C, 80.20; H, 4.80; N, 4.33.

6-Amino-4-(naphthalen-1-yl)-2-phenyl-4*H*-furo[2,3-b]pyran-5-carbonitrile (**11**)

A mixture **1** (0.298 g, 1 mmol), malononitrile (~0.1 g, ~1 mmol) and sodium acetate (0.2 g, ~2 mmol) in glacial acetic acid (5 mL) was heated under reflux for 6 h. After cooling, the obtained product was filtered off, washed with water, dried, and crystallized from dioxane to give compound **11**. Yield 60 %; m.p. 158–160 °C; IR (KBr, *v*, cm^{-1}): 2210 (CN), 3290–3410 (NH_2); ^{1}H NMR (DMSO-d_6, δ ppm): 5.22 (s, 1H, pyran), 6.90–8.06 (m, 13H, Ar–H+1H, furan), 9.18 (s, 2H, NH_2, D_2O exchangeable), MS, *m/z* (%): 364 (M^+, 100). Analysis for $C_{24}H_{16}N_2O_2$ (364.40): calcd. C, 79.11; H, 4.43; N, 7.69; found C, 79.14; H, 4.40; N, 7.55.

6-(Naphthalen-1-yl)-5-(2-oxo-2-phenylethyl)-2-thioxotetrahydro-pyrimidin-4(1*H*)-one (**12**)

A solution of **1** (0.298 g, 1 mmol) and thiourea (0.1 g, 1 mmol) in sodium ethoxide (10 mL, 1 %) was heated under reflux for 5 h. The reaction mixture was cooled, poured into cold water and neutralized with HCl (pH ~7). The precipitated solid was filtered off, washed with water, dried, and crystallized from ethanol to give compound **12**. Yield 80 %; m.p. 118–120 °C; IR (KBr, *v*, cm^{-1}): 1250 (C=S), 1650, 1710 (2 C=O), 3200, 3350 (2 NH); ^{1}H NMR (DMSO-d_6, δ ppm): 3.6 (m, 1H, CH– pyrmidinone),4.26 (d, 1H, CH– pyrmidinone), 5.10 (m, 2H, CH_2), 6.96–8.03 (m, 12H, Ar–H), 11.10, 12.00 (2s, 2H, 2NH, D_2O exchangeable); MS, *m/z* (%):374 (M^+, 100). Analysis for $C_{22}H_{18}N_2O_2S$ (374.46): calcd. C, 70.57; H, 4.85; N, 7.48; S, 8.56 found C, 70.51; H, 4.73; N, 7.39; 8.40.

Synthesis of compounds **13** and **14**

To a solution of compound **7** (0.33 g, 1 mmol) in absolute ethanol (20 mL), ethoxymethylene-malononitrile or ethyl-(ethoxymethylene)cyanoacetate (1 mmol) was added. The reaction mixture was refluxed for 1 h, after cooling, the obtained product was collected by filtration, dried, and crystallized from ethanol to give compounds **13** and **14**, respectively.

5-Amino-1-(2-naphthalen-1-ylmethylene)-4-oxo-4-phenylbutanoyl)-4,5-dihydro-1*H*-pyrazole-4-carbonitrile (**13**)

Yield 86 %; m.p. 158–160 °C; IR (KBr, *v*, cm^{-1}): 3408, 3210 (NH_2), 2,218 (CN); ^1H NMR (DMSO-d_6, δ ppm): 3.40 (s, 2H, CH_2), 4.34–5.2 (m, 2H, 2 CH_2), 6.78–8.08 (m, 14h, Ar–H+ethylene–H+pyrazole–H), 13.26 (s, 2H, NH_2, D_2O exchangeable); ^{13}C NMR: 38.87 (CH–CN), 39.71 (CH_2, aliphatic), 40.12 (C-NH_2), 115.59 (C, 1-nitrile), 125.46-128.89 (C–Ar), 134.77 (C, 1-ethylene), 137.16 (CH, 1-ethylene), 160.5, (CH, 1-imine), 165.42 (C, amide), 194 (C, 1-carbonyl); MS, *m/z* (%): 408 (M^+, 42). Analysis for $C_{25}H_{20}N_4O_2$ (408.45): calcd. C, 73.51; H, 4.94; N, 13.72; found C, 73.49; H, 5.01; N, 13.64.

Ethyl-5-amino-1-(2-[naphthalen-1-ylmethylene)-4-oxo-4-phenylbutanoyl]-4,5-dihydro-1*H*-pyrazole-4-carboxylate (**14**)

Yield 80 %; m.p. 114–116 °C; IR (KBr, *v*, cm^{-1}): 3465, 3350 (NH_2), 1,685 (CO, ester); ^1H NMR (DMSO-d_6, δ ppm): 1.37 (t, $J = 6.9$ Hz, 3H, CH_3), 4.34 (*q*, $J = 7.5$ Hz, 2H, CH_2), 4.5–5.4 (m, 2H, 2 CH), 6.70 (s, 1H, pyrazole), 7.3–8.36 (m, 13H, Ar–H+1H ethylene), 10.65 (s, 2H, NH_2, D_2O exchangeable); MS, *m/z* (%): 455 (M^+, 38). Analysis for $C_{27}H_{25}N_3O_4$ (455.51): calcd. C, 71.19; H, 5.53; N, 9.22; found C, 71.20; H, 5.44; N, 9.10.

4-(Naphthalen-1-yl)-1-phenyl-3-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl) but-3-en-1-one (**15**)

To a solution of hydrazide **7** (0.33 g, 1 mmol) in alcoholic sodium hydroxide (15 mL, 1 %), carbon disulphide (3 mL) was added. The reaction mixture was refluxed for 2 h, cooled at room temperature and then poured into ice-cold water, and acidified with HCl. The precipitated solid was filtered off, washed with water, dried, and crystallized from ethanol to give compound **15.** Yield 76 %; mp 124–126 °C; IR (KBr, *v*, cm^{-1}): 3215 (NH), 1665 (CO), 1254 (C=S); ^1H NMR (DMSO-d_6, δ ppm): 4.30 (s, 2H, CH_2), 7.37–8.00 (m, 12H, Ar–H), 8.8 (s, 1H, =CH), 13.27 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%):372 (M^+, 36). Analysis for $C_{22}H_{16}N_2O_2S$ (372.44): calcd. C, 70.95; H, 4.33; N, 7.52; S, 8.61, found 71.00; H, 4.28; N, 7.40; S, 8.56.

1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-(naphthalen-1-ylmethylene)-4-phenylbutane-1,4-dione (**16**)

A mixture of **7** (0.33 g, 1 mmol) and acetylacetone (0.11 g, 1 mmol) in absolute ethanol (10 mL) was stirred for 10 h at room temperature. The solvent was evaporated under reduced pressure to dryness, the formed precipitate was crystallized from ethanol to give compound **16**. Yield 62 %; m.p. 138–140 °C; IR (KBr, *v*, cm^{-1}): 1655,1688 (2 C=O); ^1H NMR (DMSO-d_6, δ ppm): 1.9, 2.4 (2s, 6H, $2CH_3$), 4.3 (s, 2H, CH_2), 6.9 (s, 1H, pyrazole), 7.30–8.29 (m, 13H, Ar–H+1H,

ethylene); MS, *m/z* (%): 394 (M^+, 41). Analysis for $C_{26}H_{22}N_2O_2$ (394.47): calcd. C, 79.16; H, 5.62; N, 7.10, found C, 78.94; H, 5.43; N, 6.98.

2-(Naphthalen-1-ylmethylene)-*N*-(4-nitrobezylidene)-4-oxo-4-phenylbutanehydrazide (**17**)

To a solution of hydrazide **7** (3.3 g, 10 mmol) in absolute ethanol (30 mL) containing a few drops of acetic acid, *p*-nitrobenzaldehyde (1.51 g, 10 mmol) was added. The reaction mixture was stirred at room temperature for 10 h. After cooling, the solid formed was filtered off and crystallized from ethanol/water to give compound **17**. Yield 70 %; m.p. 238–240 °C; IR (KBr, *v*, cm^{-1}): 3350 (NH), 1595(C=N), 1655,1688 (2 C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 3.95 (s, 2H, CH_2), 7.33–8.39(m, 17H, Ar–H+1H, ethylene), 8.68 (s, 1H, CH=N), 10.25 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%): 463 (M^+, 39). Analysis for $C_{28}H_{21}N_3O_4$ (463.48): calcd. C, 72.56; H, 4.57; N, 9.07; found C, 72.57; H, 4.48; N, 8.98.

2-(Naphthalen-1-ylmethylene)-*N*-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl)-4-oxo-4-phenyl butanamide (**18**)

A solution of **17** (1.7 g, 5 mmol) in dry benzene (20 mL), thioglycolic acid (2.5 ml, 5 mmol) was added dropwisely during 10 min, with stirring. The reaction mixture was stirred overnight at room temperature, and then evaporated of the solvent under reduced pressure, the residue was triturated with petroleum ether. The obtained product was filtered off and crystallized from ethanol to give compound **18**. Yield 65 %; m.p. 152–154 °C; IR (KBr, *v*, cm^{-1}): 3400 (NH), 1690 (CO, ester); ^{1}H NMR (DMSO-d_6, δ ppm): 1.86 (s, 1H, CH–N of thiazolidine ring), 3.20 (m, 2H, CH_2, aliphatic), 3.48 (s, 2H, thiazolidine ring), 7.38–8.26 (m, 17H, Ar–H+1H ethylene), 9.17 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%):537 (M^+, 61). Analysis for $C_{30}H_{23}N_3O_5S$ (537.59): calcd. C, 67.03; H, 4.31; N, 7.82; S, 5.96 found C, 67.00; H, 4.32; N, 7.73; S, 5.80.

N-(3-Chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl)-2-(naphthalen-1-ylmethylene)-4-oxo-4-phenylbutanamide (**19**)

To a solution of **17** (1.7 g, 5 mmol) in dioxane (20 mL) containing of a few drops of triethylamine, chloroacetyl chloride (0.07 g, 5 mmol) was added with stirring at room temperature during 1 h. The reaction mixture was stirred overnight at the same temperature. The formed precipitate was filtered off and crystallized from dioxane to give compound **19**. Yield 68 %; m.p. 206–208 °C; IR (KBr, *v*, cm^{-1}): 3430 (NH), 2920 (CH of alicyclic ring), 1670 (CO), 649 (C–Cl); ^{1}H NMR (DMSO-d_6, δ ppm): 3.18 (m, 2H, CH_2, aliphatic), 3.56 (s, 1H, methane-H), 3.86 (s, 1H, CH–Cl), 6.74 (s, 1H, ethylene), 7.29–8.32 (m, 16H, Ar–H), 9.76 (s, 1H, NH, D_2O exchangeable); MS, *m/z* (%): 539 (M^+, 21). Analysis for $C_{30}H_{22}ClN_3O_5$ (539.97): calcd. C, 66.73; H, 4.11; Cl, 6.57; N, 7.78, found C, 66.69; H, 4.02; Cl, 6.53; N, 7.62.

Antiviral assay

Influenza viruses belong to the family of *Orthomyxoviridae* which is defined by viruses possess a negative sense, single-stranded and segmented RNA genome. It is well known that influenza viruses cause highly contagious respiratory disease with potentially fatal outcomes.

Influenza viruses remain a constant health threat, and major efforts have been directed at discovering effective antivirals over the past several decades. Fortunately, there are now four US Food and Drug Administration (FDA) drugs available for use for human: amantadine, rimantadine, oseltamivir and zanamivir. From the fact that viruses are obligate intracellular parasites, antiviral agents must be capable of selectively inhibiting viral functions without damaging the host [21], and amantadine or rimantadine are useful in the therapy of illness caused by influenza H1N1, H2N2, and H3N2 viruses. And even though the highly pathogenic H5N1 viruses are resistant to such antivirals [22], it is still sensitive to both zanamivir and oseltamivir which are well tolerated in animal models and in human [23, 24], but resistance to the latter antiviral is now being observed clinically [24] that leads to the importance of searching for new antiviral agents.

Materials and methods

Virus and cells

Avian influenza A virus (A/chicken/Egypt/1/2006 (H5N1)), was used to prepare low pathogenic rH5N1 by plasmid-based reverse genetics. The prepared low pathogenic form of H5N1 was tittered on Madin-Darby canine kidney (MDCK) at a multiplicity of infection (M.O.I) of 0.001. The MDCK cell line was kindly provided by Dr. Richard Webby at St. Jude Children's Research Hospital, Memphis, TN, USA.

MDCK cells were routinely passaged in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1 % antibiotic–antimycotic mixture (penicillin–streptomycin–amphotericin B).

Compounds preparation for biological assays

Compounds under examination were dissolved in 10 % dimethyl sulphoxide (DMSO) in ddH_2O to a concentration of 10 mg ml^{-1} stock samples used for further dilutions according to the assay applied.

Cytotoxicity assay (MTT assay)

The cytotoxic activity of the prepared compounds were tested in MDCK cell line by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [25] with minor modification. Briefly, the cells were seeded in 96-well cell culture plates (100 µL/well at a density of 10^5 cells mL^{-1}) and treated with concentrations of 5, 10, 20, 40, and 80 µg/100 µL of the sample solutions. At 24 h,

after washing with sterile phosphate buffer (PBS) three times, MTT solution (20 μL of 5 mg mL^{-1}) was added to each well and incubated at 37 °C for 4 h. After the medium had been aspirated, the formed formazan crystals were dissolved with 200 μL of acidified isopropanol (0.04 M HCl in absolute isopropanol). An absorbance of formazan was detected by a dual wavelength UV spectrometer at 540 nm with 620 nm reference wavelength. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation.

$$\% \text{ cytotoxicity} = \frac{\text{Absorbance of cell without treatment} - \text{Absorbance of cell with treatment}}{\text{Absorbance of cell without treatment}} \times 100$$

And 50 % cytotoxicity (TC_{50}) for each compound was calculated from the equation derived from the plot of its standard curve of percentage of cytotoxicity versus sample concentration.

Antiviral activity and therapeutic index

The antiviral activity of the compounds was determined using plaque reduction assay. In a six-well cell culture plate, confluent MDCK cells were infected with a pre-incubated mixture of: 100 μL of avian influenza H5N1 virus (MOI of 0.001), 100 μL of DMEM [containing 2 % FBS and 1 mg mL^{-1} of L-1-tosyl-amido-2-phenylethyl chloromethyl ketone (TCPK)] and different concentrations of each compound (10, 20, and 40 mg mL^{-1}). Inoculated plates were incubated for 1 h at 37 °C in 5 % CO_2 to allow virus adsorption, followed by the addition of 2 mL of agarose overlayer in 2× DMEM containing 1 % FBS per well. The over-layered cultures were incubated at 37 °C in 5 % CO_2 for 3–4 days of plaque formation monitoring. Upon plaque observation, plates were fixed with 3.7 % formalin in phosphate-buffered saline for 2 h, washed, and stained with 0.1 % crystal violet dye. Plaques were counted manually from triplicate wells for each treatment under examination based on plaque number but not plaque size. Viral counts and percentage of virus reduction were calculated according to Hayden et al. [26].

The effective dose ED_{50} (50 % reduction of viral count) for each compound was calculated from the equation derived from the plot of its standard curve from: percentage of plaque reduction versus sample concentration. The therapeutic index was calculated by dividing TC_{50} by ED_{50}.

Acknowledgments The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-0172.

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