Synthesis and Anti-influenza Virus Activity of Novel bis(4*H*-chromene-3-carbonitrile) Derivatives

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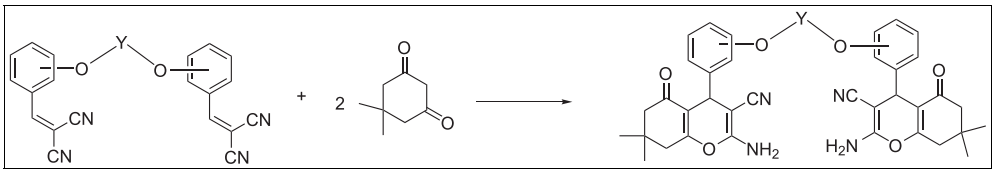

An efficient and convenient method for the synthesis of bis(4*H*-chromene-3-carbonitrile) derivatives by one-pot, multicomponent reaction of bis-aldehydes, malononitrile, and dimedone in the presence of a catalytic amount of piperidine is reported. Bis(2-benzylidene-1*H*-indene)-1,3-(2*H*)-dione derivatives were obtained as the main products as a result of reaction of the bis(arylidenemalononitriles) with indandione. The anti-influenza H5N1 virus activities of the newly prepared bis-chromene derivatives are also investigated.

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INTRODUCTION

The Michael addition reactions of α,β-unsaturated nitriles with β-diketones is an interesting route for the synthesis of chromene and fused chromene derivatives [1–10]. Chromene derivatives are an important class of compounds of considerable interests, which have been the subject of extensive research, especially in the pharmaceuticals, including anticancer [11–15], antiviral [16–18] antimicrobial [19,20], and anti-inflammatory activities [21]. Some chromene derivatives were found to have potential ability in the enhancement of cognitive functions, and thus they are used in the treatment of neurodegenerative diseases [15]. 2-Amino-4*H*-chromene derivatives bearing nitrile functionality showed potential application in the treatment of human inflammatory TNFα-mediated diseases, such as rheumatoid and psoriatic arthritis [22,23].

Chromene derivatives play also an important role in the production of highly effective fluorescent dyes for daylight fluorescent pigments and synthetic fibers [24]. In addition, bis-heterocyclic compounds with a suitable spacer are reported to exhibit various pharmacological activities including, antitumor [25] and antimicrobial activities [26]. Moreover, recent reports showed that among libraries of derivatized heterocycles, the most active library compounds had a bis-heterocyclic structure [27,28].

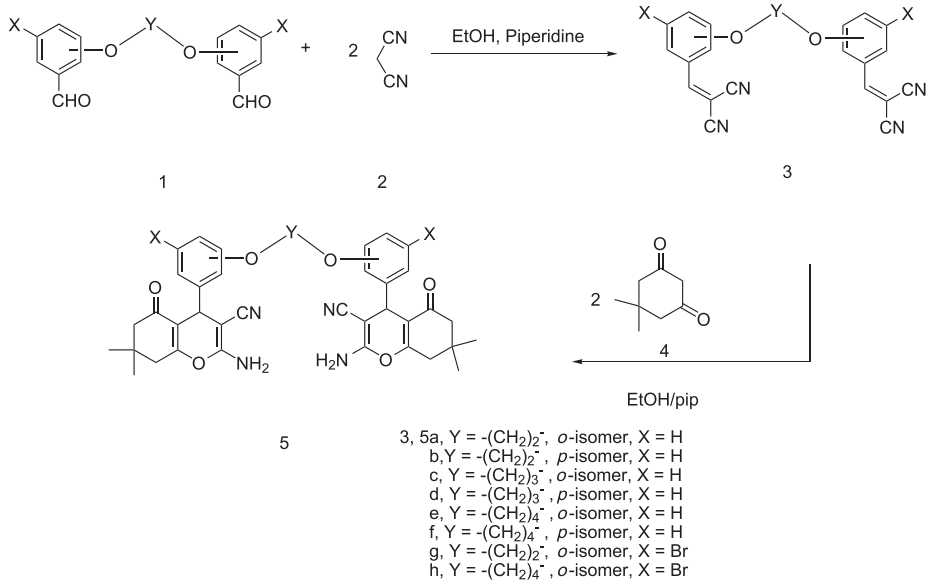
Seasonal human influenza is one of the most common respiratory infection viruses, affecting millions of people worldwide every year [29]. In recent years, the highly pathogenic avian influenza H5N1 virus crossed the species barrier and transmitted to human that has been causing global concern as a potential pandemic threat. Two classes of antiviral agents are recommended as drugs for treatment of influenza virus infection in humans: adamantanes (M2 blockers) [30] and neuraminidase inhibitors [31]. Some evidences of antiviral drug resistance have been recorded in H5N1 viruses isolated from some human cases [32,33]. So the screening and developing of new classes of antiviral drugs is a significant and an urgent task.

As a part of an ongoing research program on Michael addition reactions [34–42] and bis-heterocyclic compounds with a suitable spacer [43–52], and encouraged by the potency of the clinically useful chromene derivatives in treatment of cancer and inflammation and other activities, we report the results of our investigations concerning the synthesis of novel bis-4*H*-chromene-3-carbonitrile derivatives aiming at exploring of their anti-influenza virus activities.

RESULTS AND DISCUSSION

The synthetic strategy used in the synthesis of the target bis-4*H*-chromene-3-carbonitrile molecules is outlined in scheme 1. Thus, a variety of new bis-arylidenemalononitrile derivatives **3** were obtained in 80–90% yields *via* Knoevenagel condensation of one mole of bis-aldehyde derivatives **1** with two moles of malononitrile **2** in ethanol and in the presence of piperidine as a basic catalyst. In the next step, one mole of the isolated bis-arylidenemalononitrile derivatives **3** was reacted with two moles of dimedone **4**, in the

Scheme 1. Synthesis of bis(4*H*-chromene-3-carbonitrile) derivatives by a step-wise approach.



presence of piperidine *via* Michael addition reaction to yield **5** in good yields (Scheme 1).

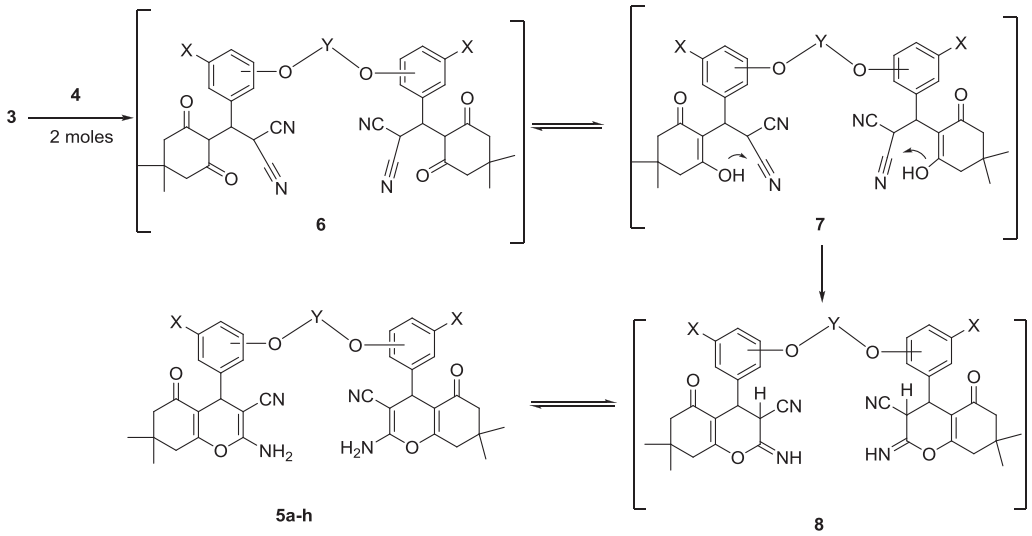
The reaction proceeds most likely *via* initial Michael addition of dimedone **4** to the C=C bond in **3**, followed by cyclization to give the final isolable products **5** through intermediacy of **6–8** (Scheme 2).

Compounds **5a–h** could also be alternatively obtained in a multicomponent reaction of one mole of bis-aldehydes **1** with two moles of both malononitrile and dimedone in EtOH/piperidine (Scheme 3).

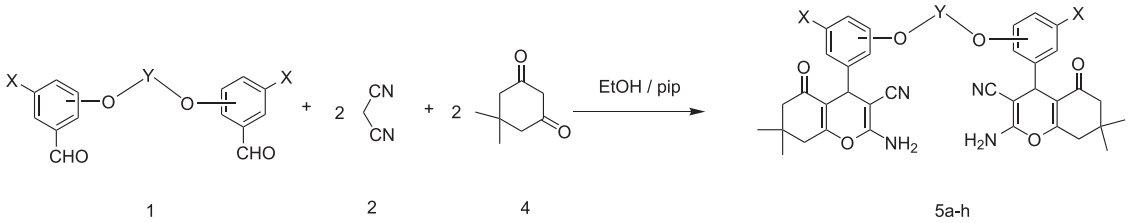
On the other hand, attempts to synthesize bis(4*H*-chromene-3-carbonitrile) derivatives **5** by bis-alkylation of 2-amino-7,7-dimethyl-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile **9** with the appropriate dihalo compounds **10** in basic medium were unfortunately unsuccessful (Scheme 4). The reaction gave instead a mixture of products that were not easily separated and have not been characterized yet. The monopodal 4*H*-chromene-3-carbonitrile **9** was synthesized *via* multicomponent reaction of the appropriate hydroxybenzaldehyde, malononitrile, and dimedone in EtOH in the presence of piperidine as a catalyst [53,54].

The constitutions of compounds **5** were established spectroscopically based on the elemental analysis and spectral data. The IR spectra of compound **5a** as a representive example indicated the presence of amino

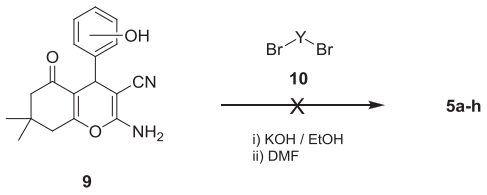
Scheme 2. Proposed mechanism for the formation of bis(4*H*-chromene-3-carbonitrile) derivatives.



Scheme 3. Synthesis of bis(4*H*-chromene-3-carbonitrile) derivatives by multicomponent reactions.



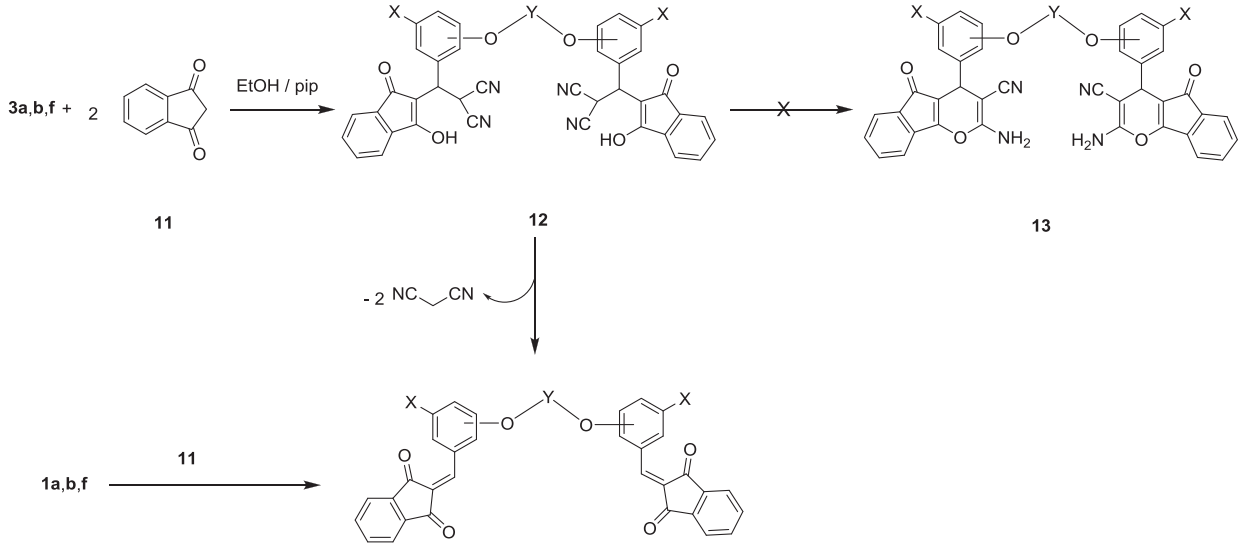
Scheme 4. Attempted synthesis of bis(4*H*-chromene-3-carbonitrile) derivatives by a bis-alkylation strategy.



group at 3435 and 3350. In addition, it revealed the cyano group at 2194 cm^{-1}. The carbonyl group appeared as a broad band at 1668 cm^{-1}. The ^{1}H-NMR spectrum of **5a** indicated the presence of two singlets integrated by 12 protons at δ 0.95 and 1.06 ppm assigned to four CH_3. In addition, it indicated two characteristic doublets of doublet at 2.11 and 2.25 ppm with coupling constant J = 16.1 ppm assigned to H8. The singlet signal at 2.50 ppm is assigned to H6. The pyran-H4 appeared as a singlet signal at δ = 4.50 ppm. Moreover, compound **5a** as well as compounds **5c**,**e**,**g**,**h** also featured the methylene ether linkage OCH_2 as multiplet or two separate doublet signals at 3.98–4.30 ppm, although their precursors **3a**,**c**, **e**,**g**,**h** exhibit singlet or triplet signals for these protons. This suggests that the generated asymmetric center (in the dihydropyran rings) is close enough to this CH_2 group. On the other hand, the two methylene ether linkage OCH_2 resonance appears as a singlet in compounds **5b**,**d**, **f,** which indicate that the asymmetric center is not close enough to effect such splitting.

On the other hand, repeated attempts to prepare bis-dihydroindeno[1,2-*b*]pyran-3-carbonitrile **13**, by the reaction of one mole of bis-aldehydes **1** with two moles of both malononitrile and indanedione (**11**) instead of dimedone under similar reaction conditions were unsuccessful. Instead, the reaction afforded bis(methanylylidene))bis(1*H*-indene-1,3(2*H*)-dione) derivatives **14a–c** as single products in good yield (Scheme 5). The structure of compounds **14a–c** was confirmed by comparison with their physical data with authentic samples synthesized from condensation of one mole of bis-aldehyde derivatives **1** with two moles of

Scheme 5. Attempted synthesis of bis-dihydroindeno[1,2-*b*]pyran-3-carbonitriles by multicomponent reactions.



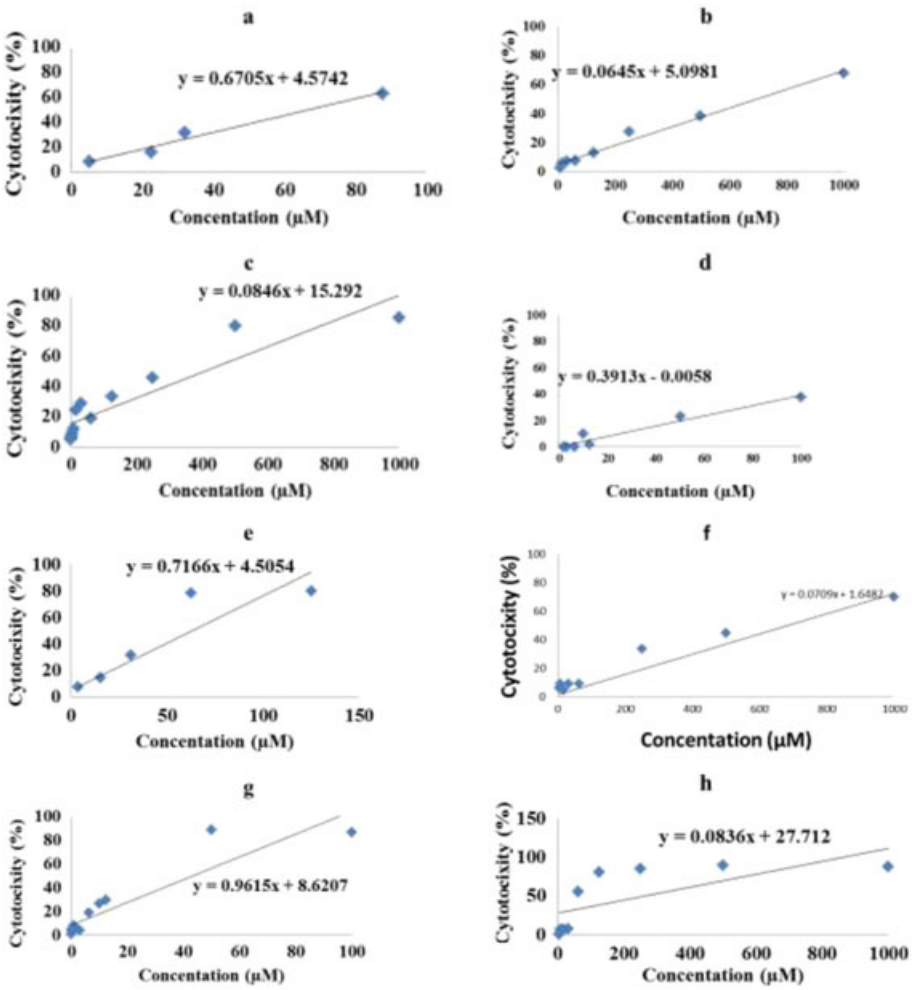


Figure 1. TC50 of synthetic compounds. [Color figure can be viewed at wileyonlinelibrary.com.]

indanedione **11** in EtOH in the presence of piperidine as a basic catalyst. The formation of **14** is assumed to proceed *via* initial formation of the adduct **12**, which then decompose to give **14** *via* elimination of malononitrile. A similar sequence has been reported [34,55–60].

The constitutions of compounds **14** were established spectroscopically. For example, the ^{1}H-NMR spectrum of compound **14a** revealed singlet signal integrated by 4H at δ 4.60 ppm assigned the two –OCH_2 groups. The ylidene H-atoms appeared as singlet signal at 8.25 ppm. In addition, it revealed the aromatic protons as multiplets in their expected positions.

Antiviral Evaluation. The *in vitro* antiviral efficacy of the newly synthesized bis(4*H*-chromene-3-carbonitrile) derivatives against highly pathogenic avian influenza H5N1 virus was screened in MDCK cells. The concentrations of the tested compounds, which exhibited 50% cytotoxicity TC50, were determined (Fig. 1 and Table 1). The obtained results of plaque reduction assay at two safe concentrations (<TC50) of each compound showed that most of tested compounds produced moderate antiviral activity (50% inhibition) against tested influenza virus as shown in Table 2. It has been noticed that the best antiviral potency among tested compounds was obtained by compounds **5b** and **5g** compounds that showed 76.1% and 77.2% antiviral inhibition, respectively.

Table 1

TC50 of synthetic compounds

Compound	TC50 Conc. (µM/µl)
5a	67.7
5b	696.15
5c	410.2
5d	132.1
5e	63.48
5f	638.508
5g	43.5
5h	26.674

Table 2

Antiviral activity of the tested compounds against H5N1 virus

Compound	Concentration (μM)	% of virus inhibition
5a	16.9373	43.9
	8.46864	0
5b	174.038	76.1
	87.0192	65
5c	205.13	54.5
	102.565	51.5
5d	132.104	62.5
	66.06	0
5e	15.8717	52.7
	7.93584	13.8
5f	159.627	68.4
	79.8135	47.3
5g	32.625	77.27
	21.75	65.21
5h	13.3373	45.4
	6.66866	0

CONCLUSIONS

We have developed an efficient synthesis of novel bis-4*H*-chromene-3-carbonitrile derivatives *via* the Michael addition reaction of dimedone towards bis-arylidenemalononitrile derivatives. The new synthesized compounds offer an advantage of their easy synthesis in a simple one-pot or stepwise procedure from inexpensive starting materials. Our current studies are directed to extend the scope of this method to cover additional bis-heterocyclic compounds. The anti-influenza activities of the newly synthesized bis-heterocyclic compounds were investigated, and the best antiviral potency among the tested compounds was obtained by compounds **5b** and **5g**.

EXPERIMENTAL

General. Melting points were measured with a Stuart melting point apparatus and are uncorrected. The IR spectra were recorded using a FTIR Bruker–vector 22 spectrophotometer as KBr pellets. The ^{1}H-NMR spectra were recorded in DMSO–d_6 as solvent on Varian Gemini NMR spectrometer at 300 MHz using TMS as internal standard. Chemical shifts are reported as δ values in ppm. Mass spectra were recorded with a GCMS–QP–1000 EX mass spectrometer (Shimadzu, Japan) in EI (70 eV) model. The elemental analyses were performed at the Microanalytical Center, Cairo University.

General Procedure for the Synthesis of Compounds 3a–h. To a mixture of bis-aldehydes **1a–h** (1 mmol) and malononitrile **2** (2.2 mmol) in ethanol (20 mL) piperidine (0.2 mL) was added. The mixture was heated under reflux for 3 h. The crude solid was isolated and recrystallized from the proper solvent.

2,2'-(((Ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis (methanylylidene))-dimalononitrile (3a). Pale yellow crystals (dioxane), Mp = 243–245°C.[45] ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.6 (s, 4H, OCH_2), 7.16–8.05 (m, 8H, Ar-H), 8.43 (s, 2H, vinyl-H).

2,2'-(((Ethane-1,2-diylbis(oxy))bis(4,1-phenylene))bis (methanylylidene))-dimalononitrile (3b). Pale yellow crystals (dioxane), Mp = 162–164°C.[61] ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.51 (s, 4H, $2OCH_2$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 66.7, 77.1, 113.8, 114.7, 124.4, 133.3, 160.3, 163.2.

2,2'-(((Propane-1,3-diylbis(oxy))bis(2,1-phenylene))bis (methanylylidene))-dimalononitrile (3c). Pale yellow crystals (dioxane / ethanol), Mp = 214–216°C.[45] ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.35 (quintet, 2H, OCH_2), 4.36 (t, 4H, OCH_2), 7.13–8.03 (m, 8H, ArH's), 8.52 (s, 2H, vinyl-H)

2,2'-(((Propane-1,3-diylbis(oxy))bis(4,1-phenylene))bis (methanylylidene))-dimalononitrile (3d). Pale yellow crystals (dioxane/ethanol), Mp = 170–172°C.[54] ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.25 (q, 2H, J = 6 Hz, OCH_2CH_2), 4.29 (t, 4H, J = 6 Hz, $2OCH_2$), 7.20 (d, 4H, J = 9 Hz, Ar-H), 7.96 (d, 4H, J = 9 Hz, Ar-H), 8.37 (s, 2H, vinyl-H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 28.1, 64.9, 76.9, 113.8, 114.7, 124.1, 133.3, 160.3, 163.5.

2,2'-(((Butane-1,4-diylbis(oxy))bis(2,1-phenylene))bis (methanylylidene))-dimalononitrile (3e). Pale yellow crystals (dioxane), Mp = 210–212°C, IR (KBr): ν = 2190 (CN) cm^{-1}.^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.90 (s, 4H, 2 CH_2), 4.22 (s, 4H, 2-OCH_2), 7.11–7.99 (m, 8H, Ar-H), 8.41 (s, 2H, vinyl-H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 24.9, 67.9, 76.7, 113.9, 114.8, 115.6, 120.5, 124.0, 133.3, 160.4, 163.7. MS (EI, 70 eV): *m/z* (%) = 394 [M$^+$], *Anal*. Calcd for $C_{24}H_{18}N_4O_2$: C, 73.08; H, 4.60; N, 14.20. Found: C, 73.19; H, 4.67; N, 14.31.

2,2'-(((Butane-1,4-diylbis(oxy))bis(4,1-phenylene))bis (methanylylidene))-dimalononitrile (3f). Pale yellow crystals (82%), (dioxane/ethanol), Mp = 205–207°C, IR (KBr): ν = 2191 (CN) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.91 (br, 4H, 2 CH_2), 4.19 (br, 4H, 2-OCH_2), 7.19 (d, 4H, Ar-H, J = 9 Hz), 7.97 (d, 4H, Ar-H, J = 9 Hz), 8.37 (s, 2H, vinyl-H). ^{13}C-NMR (75 MHz, DMSO-d6): δ = 24.9, 66.8, 76.7, 113.8, 114.7, 124.0, 133.3, 160.4, 163.7. MS (EI, 70 eV): *m/z* (%) = 394 [M$^+$], *Anal*. Calcd for $C_{24}H_{18}N_4O_2$: C, 73.08; H, 4.60; N, 14.20. Found: C, 73.29; H, 4.72; N, 14.23.

2,2'-(((Ethane-1,2-diylbis(oxy))bis(5-bromo-2,1-phenylene)) bis-(methanylylidene))dimalononitrile (3g). Pale yellow crystals (87%), (dioxane/ethanol), mp 258–260°C, IR (KBr): ν = 2195 (CN) cm^{-1}, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.56 (s, 4H, $2OCH_2$), 7.31 (d, 2H, Ar-H, J = 8.7 Hz), 7.82 (d, 2H, Ar-H, J = 8.7 Hz), 8.09 (s, 2H, Ar-H), 8.42 (s, 2H, vinyl-H). MS (EI, 70 eV): *m/z* (%) = 524 [M$^+$], *Anal*. Calcd for $C_{22}H_{12}Br_2N_4O_2$ C, 50.41; H,

2.31; Br, 30.49; N, 10.69. Found: C, 50.59; H, 2.43; Br, 30.39; N, 10.54.

2,2'-(((Butane-1,4-diylbis(oxy))bis(5-bromo-2,1-phenylene)) bis-(methanylylidene))dimalononitrile (3h). Pale yellow crystals (83%), (dioxane/ethanol), mp 268–270°C, IR (KBr): ν = 2188 (CN) cm^{-1}, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.96 (s, 4H, $2CH_2$), 4.21 (s, 4H, $2OCH_2$), 7.20–8.07 (m, 6H, Ar-H), 8.35 (s, 2H, vinyl-H). MS (EI, 70 eV): *m/z* (%) = 549 [M^+], *Anal*. Calcd for $C_{24}H_{16}Br_2N_4O_2$ C, 52.20; H, 2.92; Br, 28.94; N, 10.15. Found: C, 52.16; H, 2.81; Br, 28.83; N, 10.28.

General Procedure for the Synthesis of Compounds 5a–h.

Method A: A mixture of bis-arylidenemalononitrile derivatives **3a–h** (1 mmol) and dimedone **4** (2.2 mmol) in absolute ethanol (15 mL) was heated at reflux in the presence of piperidine (0.2 mL) for 3 h. The crude solid was isolated and recrystallized from the proper solvent.

Method B: To a mixture of bisaldehydes **1a–i** (1 mmol), malononitrile **2** (2.2 mmol), and dimedone **4** (2.2 mmol) in absolute ethanol (15 mL), piperidine (0.2 mL) was added, and the reaction mixture was heated at reflux for 3 h. The crude solid was isolated and recrystallized from the proper solvent.

4,4'-((Ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(2-amino-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile) (5a). Pale yellow crystals (Method A, 84%; Method B, 87%), (dioxane / ethanol), Mp = 277–279°C, IR (KBr): ν = 3435, 3350 (NH_2), 2194 (CN), 1668 (CO) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.95 (s, 6H, $2CH_3$), 1.06 (s, 6H, $2CH_3$), 2.11 (d, 2H, H8, *J* = 16.2 Hz), 2.25 (d, 2H, H8, *J* = 16.2 Hz), 2.50 (s, 4H, H6), 4.27–4.30 (m, 4H, $2OCH_2$), 4.50 (s, 2H, pyran H-4) 6.86–7.22 (m, 12H, ArH + $2NH_2$). ^{13}C-NMR (75 MHz, DMSO-d6): δ = 26.6, 28.4, 31.8, 38.9, 50.1, 57.1, 66.3, 111.7, 112.7, 119.9, 120.7, 127.9, 129.2, 132.1, 156.1, 159.0, 162.8, 195.6. MS (EI, 70 eV): *m/z* (%) = 646 [M^+], *Anal*. Calcd for $C_{38}H_{38}N_4O_6$: C, 70.57; H, 5.92; N, 8.66. Found: C, 70.63; H, 5.87; N, 8.83.

4,4'-((Ethane-1,2-diylbis(oxy))bis(4,1-phenylene))bis(2-amino-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile) (5b). Pale yellow crystals (Method A, 81%, Method B, 83%), (dioxane/ethanol), Mp = 163-165°C, IR (KBr): ν = 3386, 3327 (NH_2), 2191 (CN), 1679 (CO) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.94 (s, 6H, $2CH_3$), 1.03 (s, 6H, $2CH_3$), 2.06 (d, 2H, H8, *J* = 15.9 Hz), 2.26 (d, 2H, H8, *J* = 15.9 Hz), 2.50 (s, 4H, H6), 4.12 (s, 2H, pyran H-4), 4.24 (s, 4H, $2OCH_2$), 6.86 (s, 4H, 2 NH_2), 6.90 (d, 4H, Ar-H, *J* = 8.7 Hz), 7.07 (d, 4H, Ar-H, *J* = 8.7 Hz); ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 26.8, 28.4, 31.8, 34.8, 50.0, 58.5, 66.3, 112.9, 114.2, 119.8, 128.3, 137.1, 157.0, 158.4, 162.1, 195.6. MS (EI, 70 eV): *m/z* (%) = 646 [M^+], *Anal*. Calcd for $C_{38}H_{38}N_4O_6$: C, 70.57; H, 5.92; N, 8.66. Found: C, 70.62; H, 5.89; N, 8.80.

4,4'-((Propane-1,3-diylbis(oxy))bis(2,1-phenylene))bis(2-amino-7,7 dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile) (5c). Pale yellow crystals (Method A, 80%, Method B, 84%), (dioxane / ethanol), Mp = 240–242°C, IR (KBr): ν = 3396, 3305 (NH_2), 2186 (CN), 1654 (CO) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.93 (s, 6H, $2CH_3$), 1.04 (s, 6H, $2CH_3$), 2.0–2.25 (m, 6H, H8+ $OCH_2CH_2CH_2O$), 2.50 (s, 4H, H6), 4.03–4.21 (m, 4H, $2OCH_2$), 4.46 (s, 2H, pyran H-4) 6.81–7.13 (m, 12H, ArH + $2NH_2$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 26.4, 28.5, 28.9, 31.4, 31.7, 50.1, 57.1, 64.8, 111.9, 119.9, 120.0, 120.6, 127.9, 129.3, 131.5, 156.3, 158.9, 162.7, 195.5. MS (EI, 70 eV): *m/z* (%) = 660 [M^+]. *Anal*. Calcd for $C_{39}H_{40}N_4O_6$: C, 70.89; H, 6.10; N, 8.48. Found: C, 70.93; H, 6.02; N, 8.51.

4,4'-((Propane-1,3-diylbis(oxy))bis(4,1-phenylene))bis(2-amino-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile) (5d). Pale yellow crystals (Method A, 81%, Method B, 79%), (dioxane/ethanol), Mp = 140–142°C, IR (KBr): ν =3331 (NH_2), 2191 (CN), 1679 (CO) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.94 (s, 6H, $2CH_3$), 1.03 (s, 6H, $2CH_3$), 1.98–2.26 (m, 6H, H8+ $OCH_2CH_2CH_2O$), 2.50 (s, 4H, H6), 4.02–4.11 (m, 6H, $2OCH_2$+ pyran H-4) 6.84–7.05 (m, 12H, ArH + $2NH_2$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 26.7, 28.6, 31.7, 34.7, 50.0, 58.6, 64.2, 112.9, 114.2, 119.7, 128.2, 136.8, 157.2, 158.4, 162.1, 195.6. MS (EI, 70 eV): *m/z* (%) = 660 [M^+]. *Anal*. Calcd for $C_{39}H_{40}N_4O_6$: C, 70.89; H, 6.10; N, 8.48. Found: C, 70.95; H, 6.13; N, 8.58.

4,4'-((Butane-1,4-diylbis(oxy))bis(2,1-phenylene))bis(2-amino-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile) (5e). Pale yellow crystals (Method A, 80%, Method B, 83%), (dioxane/ethanol), Mp = 215–217°C. IR (KBr): ν = 3326 (NH_2), 2191 (CN), 1660 (CO) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.96 (s, 6H, $2CH_3$), 1.06 (s, 6H, $2CH_3$), 1.94–2.45 (m, 8H, H8 + $OCH_2CH_2CH_2CH_2O$), 2.50 (s, 4H, H6), 3.98–4.05 (m, 4H, $2OCH_2$) 4.49 (s, 2H, pyran H-4), 6.77–7.17 (m, 12H, ArH + $2NH_2$). MS (EI, 70 eV): *m/z* (%) = 674 [M^+]. *Anal*. Calcd for $C_{40}H_{42}N_4O_6$: C, 71.20; H, 6.27; N, 8.30. Found: C, 71.31; H, 6.36; N, 8.27.

4,4'-((Butane-1,4-diylbis(oxy))bis(4,1-phenylene))bis(2-amino-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile) (5f). Pale yellow crystals (Method A, 74%, Method B, 77%), (dioxane/ethanol), Mp = 187–190°C, IR (KBr): ν = 3400 3327 (NH_2), 2193 (CN), 1662 (CO) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.95 (s, 6H, $2CH_3$), 1.03 (s, 6H, $2CH_3$), 1.83 (s, 4H, $OCH_2CH_2CH_2CH_2O$), 2.06 (d, 2H, H8, *J* = 15.9 Hz), 2.27 (d, 2H, H8, *J* = 15.9 Hz), 2.50 (s, 4H, H6), 3.98 (s, 4H, $2OCH_2$) 4.11 (s, 2H, pyran H-4), 6.82–7.05 (m, 12H, ArH + $2NH_2$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 25.4, 26.7, 28.3, 31.7, 34.7, 49.9, 58.6, 67.0, 112.9, 114.2, 119.7, 128.1, 136.7, 157.3, 158.4, 162.0, 195.5. MS (EI, 70 eV): *m/z*

(%) = 674 [M$^+$]. *Anal*. Calcd for $C_{40}H_{42}N_4O_6$: C, 71.20; H, 6.27; N, 8.30. Found: C, 71.29; H, 6.38; N, 8.29.

4,4'-((Ethane-1,2-diylbis(oxy))bis(5-bromo-2,1-phenylene))bis (2-amino-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile) (5g). Pale yellow crystals (dioxane/ethanol), mp 265–268°C, IR (KBr): ν = 3436, 3350 (NH_2), 2195 (CN), 1682 (CO) cm^{-1}, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.95 (s, 6H, 2CH_3), 1.08 (s, 6H, 2CH_3), 2.04–2.42 (m, 4H, H8), 2.50 (s, 4H, H6), 4.24–4.35 (m, 4H, 2OCH_2), 4.50 (s, 2H, pyran H-4), 6.78–7.22 (m, 10H, ArH + 2NH_2). ^{13}C-NMR (75 MHz, DMSO-d6): δ = 26.6, 28.3, 31.2, 31.7, 50.1, 57.1, 66.3, 111.7, 112.7, 119.9, 120.7, 127.9, 129.2, 131.9, 156.1, 159.0, 162.8, 195.6. MS (EI, 70 eV): *m/z* (%) = 804 [M$^+$]. *Anal*. Calcd for $C_{38}H_{36}Br_2N_4O_6$: C, 56.73; H, 4.51; Br, 19.86; N, 6.96. Found: C, 56.67; H, 4.59; Br, 19.78; N, 6.78.

4,4'-((Butane-1,4-diylbis(oxy))bis(5-bromo-2,1-phenylene))bis (2-amino-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile) (5h). Pale yellow crystals (dioxane / ethanol), mp 238–240°C, IR (KBr): ν = 3428, 3316 (NH_2), 2188 (CN), 1682 (CO) cm^{-1}, ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 0.93 (s, 6H, 2CH_3), 1.04 (s, 6H, 2CH_3), 1.89–2.28 (m, 8H, H8+ 2 CH_2), 2.50 (s, 4H, H6), 3.96–4.02 (m, 4H, 2OCH_2), 4.45 (s, 2H, pyran H-4), 6.94–7.35 (m, 10H, ArH + 2NH_2). ^{13}C-NMR (75 MHz, DMSO-d6): δ = 25.6, 26.2, 28.5, 31.3, 31.7, 49.9, 56.5, 68.0, 111.5, 114.5, 119.6, 120.0, 130.3, 131.5, 132.7, 155.8, 158.9, 162.2, 195.7. MS (EI, 70 eV): *m/z* (%) = 832 [M$^+$], *Anal*. Calcd for $C_{40}H_{40}Br_2rN_4O_6$: C, 57.70; H, 4.84; Br, 19.19; N, 6.73. Found: C, 57.83; H, 4.72; Br, 19.28; N, 6.78.

General Procedure for the Synthesis of Compounds 14a–c.

Method A: To a mixture of bisaldehydes **1a–c** (1 mmol), malononitrile (2.2 mmol) and indanedione **11** (2.2 mmol) in absolute ethanol (15 mL) was added piperidine (0.2 mL) and the mixture was heated at reflux for 2 h. The crude solid was isolated and recrystallized from the proper solvent.

Method B: To a solution of bisaldehydes **1a–c** (1 mmol) in absolute ethanol (15 mL), indanedione **11** (2.2 mmol) and piperidine (0.2 mL) was added and the mixture was heated at reflux for 1 h. The crude solid was isolated and recrystallized from the proper solvent.

2,2'-(((Ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis (methanylylidene))-bis(1H-indene-1,3(2H)-dione) (14a). Yellow crystals (dioxane) (Method A, 88%, Method B, 90%), Mp 248–250°C. IR (KBr): ν = 1667 (CO) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 4.60 (s, 4H, 2OCH_2) 7.1–7.87 (m, 14H, ArH), 8.25 (s, 2H, vinyl-H), 8.79 (d, 2H, Ar-H). MS (EI, 70 eV): *m/z* (%) = 526 [M$^+$]. *Anal*. Calcd for $C_{34}H_{22}O_6$: C, 77.56; H, 4.21. Found: C, 77.63; H, 4.16.

2,2'-(((Butane-1,4-diylbis(oxy))bis(2,1-phenylene))bis (methanylylidene))-bis(1H-indene-1,3(2H)-dione) (14b). Yellow crystals (dioxane) (Method A, 85%, Method B, 88%), Mp 202–205°C. IR (KBr): ν = 1666 (CO) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.05 (s, 4H, 2CH_2), 4.27 (s, 4H, 2OCH_2) 6.99–7.96 (m, 14H, ArH), 8.27 (s, 2H, vinyl-H), 8.75 (d, 2H, Ar-H). MS (EI, 70 eV): *m/z* (%) = 554 [M$^+$]. *Anal*. Calcd for $C_{36}H_{26}O_6$: C, 77.97; H, 4.73. Found: C, 77.67; H, 4.78.

2,2'-(((Butane-1,4-diylbis(oxy))bis(4,1-phenylene))bis(methanylylidene))bis(1H-indene-1,3(2H)-dione) (14c). Yellow crystals (dioxane) (Method A, 84%, Method B, 87%), Mp > 300°C. IR (KBr): ν = 1676 (CO) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.95 (s, 4H, 2CH_2), 4.06 (s, 4H, 2OCH_2) 6.74–8.62 (m, 18H, ArH + vinyl-H). MS (EI, 70 eV): *m/z* (%) = 554 [M$^+$]. *Anal*. Calcd for $C_{36}H_{26}O_6$: C, 77.97; H, 4.73. Found: C, 77.86; H, 4.82.

Biology. Stock solutions of the test compounds were dissolved in dimethyl sulfoxide (DMSO) (Sigma). Synthetic compounds were 10-fold serially diluted with Dulbecco's Modified Eagle's Medium. The cytotoxic effect of each compounds was tested individually in Madin-Darby Canine kidney (MDCK) cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Lonza, Wokingham, UK) with minor modification as previously described.[62] Briefly, MDCK cells were seeded in 96 well culture plates and incubated for 24 h at 37°C in 5% CO_2. After 24 h post culture, the cells were treated with desired concentrations of the tested compounds in triplicates. After further 24 h, the medium was aspirated, and cell monolayer was washed with phosphate buffer saline three times then MTT solution (20 µl of 5 mg/ml stock solution) was added to each well and incubated at 37°C for 4 h. A volume of 200 µl of DMSO was added to each well to dissolve formazan crystals. Absorbance of formazan solutions was measured at λ_{max} 540 nm. The percentage of cytotoxicity of each compound was determined with the following equation.

$$\%\,\text{Cytotoxicity} = \frac{(\text{Absorbance of cell without treatment - Absorbance of cell with treatment})}{\text{Absorbance of cell without treatment}} \times 100$$

The plot of % cytotoxicity *versus* concentration of tested compounds was used to calculate the concentration, which exhibited 50% cytotoxicity (TC50).

Plaque reduction assay. Antiviral activities of tested compounds were carried out using plaque reduction assay as previously described [63]. Briefly, MDCK cells were seeded in six well culture plates (10^5 cells/ml) and incubated for 24 h at 37°C in 5% CO_2. Previously titrated A/duck/Egypt/Q5569D/2012(H5N1) virus was diluted to optimal virus dilution that gave countable plaques and mixed with the safe concentration of each tested compounds then incubated for 1 h at 37°C before being added to the cells. Growth medium was removed from the 6-well cell culture plates, and virus-compound mixtures were inoculated in duplicates, After 1 h contact time for virus adsorption, 3 ml of Dulbecco's Modified Eagle's Medium supplemented with 2% agarose, 1% antibiotic antimycotic mixture, and 4% bovine serum albumin (Sigma) was added onto the cell monolayer, plates were left to solidify and incubated at 37°C till formation of viral plaques (3 days). Formalin (10%) was added to each well for 1 h then over layer was removed. Fixed cells were stained with 0.1% crystal violet in distilled water. Control untreated virus was included in each plate. Finally, plaques were counted and percentage of reduction in virus count was calculated as follows:

$$\%\,\text{inhibition} = \frac{\text{viral count (untreated)} - \text{viral count (treated)}}{\text{viral count (untreated)}} \times 100$$

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