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Anti-Influenza Drug Discovery: Structure−Activity Relationship and Mechanistic Insight into Novel Angelicin Derivatives

Jiann-Yih Yeh,[†,○] Mohane Selvaraj Coumar,[†,○] Jim-Tong Horng,[§,○] Hui-Yi Shiao,[†,‡,○] Fu-Ming Kuo,[†] Hui-Ling Lee,[†] In-Chun Chen,[†] Chun-Wei Chang,[†] Wen-Fang Tang,[§] Sung-Nain Tseng,[∥,⊥] Chi-Jene Chen,[⊥] Shin-Ru Shih,[∥] John T.-A. Hsu,[†,#] Chun-Chen Liao,[‡,▽] Yu-Sheng Chao,[†] and Hsing-Pang Hsieh*[,†]

[†]*Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, 35 Keyan Road, Zhunan Town, Miaoli County 350, Taiwan, ROC,* [‡]*Department of Chemistry, National Tsing Hua University, 101, Section 2, Kuang Fu Road, Hsinchu 300, Taiwan, ROC,* [§]*Department of Biochemistry,* [∥]*Department of Medical Biotechnology and Laboratory Science, and* [⊥]*Research Center for Emerging Viral Infections, Chang Gung University, 259 Wen-Hua first Road, Kweishan, Taoyuan 333, Taiwan, ROC,* [#]*Department of Biological Science and Technology, National Chiao Tung University, 1001 University Road, Hsinchu 300, Taiwan, ROC, and* [▽]*Department of Chemistry, Chung Yuan Christian University, 200 Chung-Pei Road, Chungli 320, Taiwan, ROC.* [○] *These authors contributed equally to this work.*

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By using a cell-based high throughput screening campaign, a novel angelicin derivative **6a** was identified to inhibit influenza A (H1N1) virus induced cytopathic effect in Madin−Darby canine kidney cell culture in low micromolar range. Detailed structure−activity relationship studies of **6a** revealed that the angelicin scaffold is essential for activity in pharmacophore B, while meta-substituted phenyl/2-thiophene rings are optimal in pharmacophore A and C. The optimized lead 4-methyl-9-phenyl-8-(thiophene-2-carbonyl)-furo[2,3-*h*]chromen-2-one (**8g**, IC_{50} = 70 nM) showed 64-fold enhanced activity compared to the high throughput screening (HTS) hit **6a**. Also, **8g** was found effective in case of influenza A (H3N2) and influenza B virus strains similar to approved anti-influenza drug zanamivir (**4**). Preliminary mechanistic studies suggest that these compounds act as anti-influenza agents by inhibiting ribonucleoprotein (RNP) complex associated activity and have the potential to be developed further, which could form the basis for developing additional defense against influenza pandemics.

Introduction

Influenza, usually known as flu, is an infectious disease of birds and mammals caused by ribonucleic acid (RNA[a]) viruses of the family Orthomyxoviridae. It is a contagious, acute, febrile, respiratory disease, which occurs seasonally in epidemic and sometimes in pandemic proportions. On the basis of the serological subtyping, three distinct types A, B, and C are present, of which influenza A and B are of much concern as human pathogens. Further, on the basis of the antigenicity of the two surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), a total of 16 HA (H1−H16) and 9 NA (N1−N9) subtypes are known, resulting in the possibility of distinct strains of influenza virus such as H1N1, H1N2..., H2N1, H2N2..., and so on.[1,2] Influenza epidemic and pandemics in the past century had caused serious impact on global morbidity, mortality, and economy. For example, the "Spanish flu" (H1N1) outbreak of 1918−1919 resulted in the death of at least 20 million people, and the Asian influenza (H2N2) in 1957 had caused the death of 1 million people worldwide. Besides, Hong Kong influenza (H3N2) in 1968 had reached pandemic proportions.[3−5] The recent repeated lethal outbreaks (2007) of the virulent H5N1 avian influenza strains in humans has caused global health threat, as they may adapt to become easily transmissible from human to human, which could cause serious pandemics.[6−8] Currently (2009), swine influenza A (H1N1) outbreak in Mexico and other parts of the world has led to issuances of pandemic alertness by the WHO.[9] As the impact of influenza pandemic is enormous, a renewed drug discovery effort worldwide is essential to counteract the disease more efficiently.

Presently vaccination is the mainstay, along with anti-influenza drugs, to control the spread and treatment of the disease. Because influenza virus continuously evolves to avoid host detection systems by changing its antigenicity through mutation of its surface glycoprotein (NA, HA) genes by a process called "antigenic drift", it could reinfect the same person in the following seasons.[10,11] Another major factor is the reassortment (antigenic shift) of genetic materials between different strains of the virus circulating in humans and avians such as that which occurred during the pandemic of Asian influenza (H2N2) in 1957.[3,10] Because of the higher rate of mutation to the influenza virus, continuous monitoring of viral genetics to identify the circulating strain of the virus every season prior to vaccine development so as to achieve maximum protection for that season is essential.[4,10] To address this issue, WHO experts every season identify the proper combination of antigenic strains for which vaccine is developed.

*To whom correspondence should be addressed. Phone: +886-37-246-166 ext. 35708. Fax: +886-37-586-456. E-mail: hphsieh@nhri.org.tw.

[a] Abbreviations: CPE, cytopathic effect; cRNA, complementary RNA; DMEM, Dulbecco's modified Eagle medium; DNA, deoxyribonucleic acid; dsRNA, double-strand RNA; FBS, fetal bovine serum; Fluc, firefly luciferase; HA, hemagglutinin; HBSS, Hanks' balanced salt solutions; HTS, high throughput screening; MDCK cells, Madin−Darby canine kidney cells; MOI, multiplicity of infection; mRNA, messenger RNA; NA, neuraminidase; NP, nucleoprotein; PFU, plaque forming unit; RBC, red blood cells; Rluc, renilla luciferase; RNA, ribonucleic acid; RNP, ribonucleoprotein; SAR, structure−activity relationship; vRNA, viral RNA; WHO, World Health Organization.

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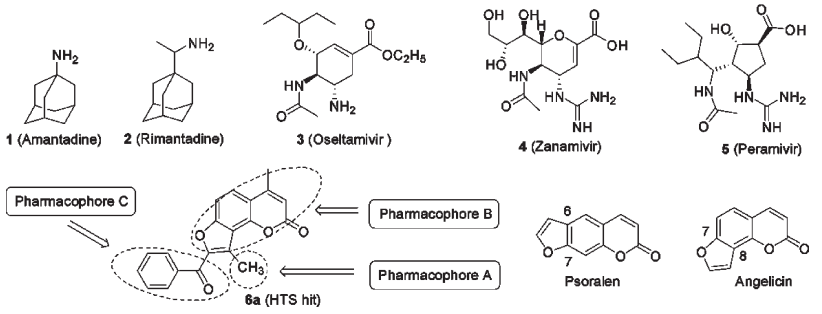
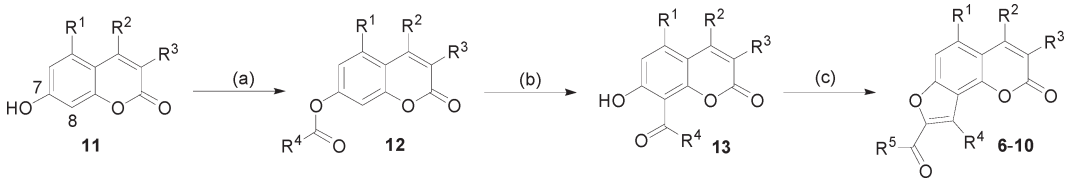


Figure 1. Inhibitors of influenza virus: M2 ion channel inhibitors (**1** and **2**), NA inhibitors (**3**−**5**), and HTS angelicin hit **6a**.

Scheme 1. General Synthetic Method for the Preparation of Angelicin Derivatives **6**−**10**[a]



[a] (a) R^4COCl, Et_3N, THF, rt, 16 h; (b) $AlCl_3$, 170 °C, 2 h; (c) R^5COCH_2Br, K_2CO_3, CH_3CN, reflux, 16 h, 15−44% over three steps.

However, as there is a time gap of 9−12 months from the recommendation to the actual use of vaccine, this could still result in mismatch between the virus and the vaccine, which could result in ineffectiveness.[10,11] Because of these and other complicating factors influencing the development of an all effective flu vaccine, additional weapons in the form of effective anti-influenza agents are a must in our fight against seasonal or pandemic influenza that may arise in the future.[4,8] Adamantane derivatives **1** (amantadine) and **2** (rimantadine) are the first two antiviral drugs used for the treatment of influenza, which target the M2 ion channels (Figure 1). Recently, orally administered **3** (oseltamivir) and inhaled **4** (zanamivir) are approved for use as anti-influenza treatments, while **5** (peramivir) is under clinical investigation; all the three compounds target the neuraminidase (NA).[1−3] However, alternative therapeutics are needed because the development of resistance strains to these drugs targeting NA enzyme, particularly **3**, has been reported as an increasing clinical problem.[12−14] Thus there is an urgent need to identify novel compounds with inhibitory activity against influenza virus, which is the aim of this study.

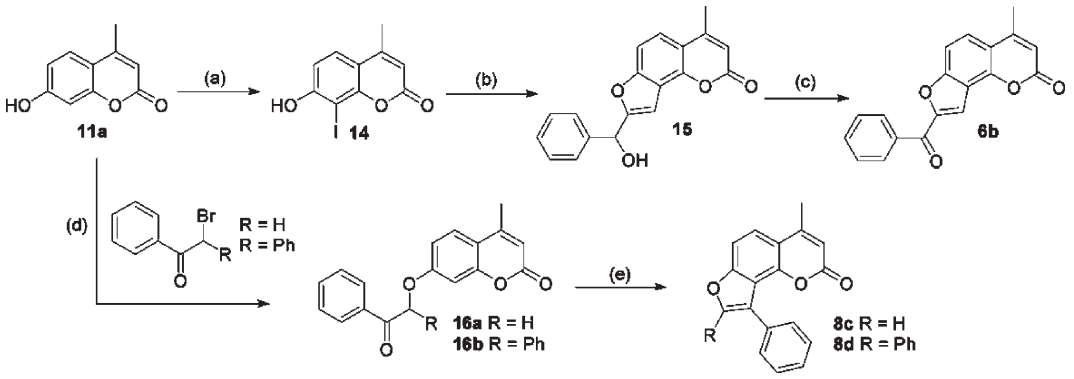
Using a cell-based assay, high throughput screening (HTS) was carried out to identify novel compounds as anti-influenza agents. The HTS assay measured the ability of the test compounds to inhibit the virus-induced cytopathic effect (CPE) on MDCK (Madin−Darby canine kidney) cells and the results expressed as IC_{50} (concentration required to reduce CPE by 50% relative to the virus control). Also, a concurrent assay to determine the general cytotoxicity of the compounds on MDCK cells were performed without virus infection and the results expressed as CC_{50} (concentration required to produce 50% cell death). Cytotoxicity measurement was performed to identify compounds which selectively inhibit virus replication without killing uninfected cells. The ratio between CC_{50} and IC_{50} is an indicator of toxicity potential of a compound and could guide in selecting appropriate compounds for further development. Through the HTS screening of about 20000 compounds, we have discovered a novel angular coumarin derivative **6a** (Figure 1) to inhibit influenza virus, with an IC_{50} of 4.5 μM and a CC_{50} > 25 μM. Literature survey revealed that linear furanocoumarin, in which the furan ring is attached to the 6,7 positions of the coumarin ring, such as psoralen, is highly phototoxic to many organisms due to their ability to cross-link deoxyribonucleic acid (DNA) after photoactivation. An angular furanocoumarin such as angelicin, in which the furan ring is attached to the 7,8 positions of the coumarin ring, which because of their geometry cannot cross-link with DNA, is less phototoxic.[15] To our knowledge, so far angelicin derivatives closely related to **6a** are not reported as antiviral agents, let alone anti-influenza agents. Moreover, only a few reports in the literature report the synthesis of **6a** and related compounds with angelicin scaffold, making the synthetic exploration interesting. On the basis of structural novelty and the necessity to develop anti-influenza agents to combat any future influenza pandemic, we started a MedChem program to investigate the potential of this novel hit **6a**, the results of which are described herein.

Chemistry

For the construction of the most of the desired angelicin derivatives **6**−**10**, appropriately substituted 7-hydroxycoumarin **11** (commercial source or synthesized by Pechmann condensation of *meta*-hydroxy phenol with β-ketoesters under acidic condition as reported by Lee et al.[16,17]) was *O*-acylated with acid chloride (R^4COCl) to give **12**. Fries rearrangement of **12** under acidic condition using anhydrous $AlCl_3$ at elevated temperature gave the key intermediate 8-acyl substituted coumarins **13**. The desired angelicin derivatives **6**−**10** were constructed from **13** and appropriate 2-bromo ketones (R^5COCH_2Br) via a single step *O*-alkylation−cyclization−elimination process under basic condition with an overall yield of 15−44% over three steps (Scheme 1).[18,19]

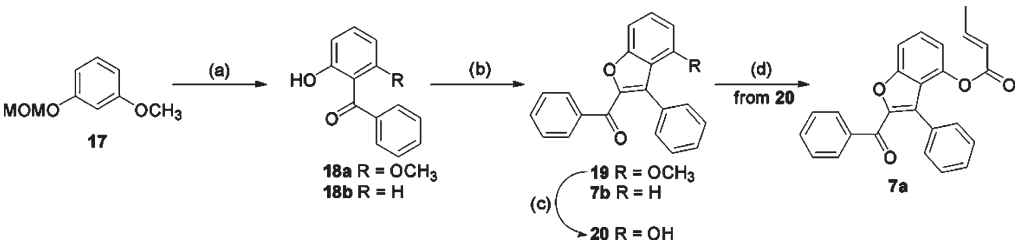
For the preparation of **6b**, coumarin **11a** was first iodiniated[20] and then reacted with 1-phenyl-2-propyn-1-ol using copper mediated ring annulation[21] to construct the furan ring of **15**. Oxidation of the secondary alcohol group of **15** using Jones reagent provided the desired angelicin derivative **6b** in 37% yield over three steps. For the construction of compounds **8c** and **8d** without the ketone group, coumarin **11a**

Scheme 2. Synthesis of Angelicin Derivatives **6b** and **8c**,**d**[a]



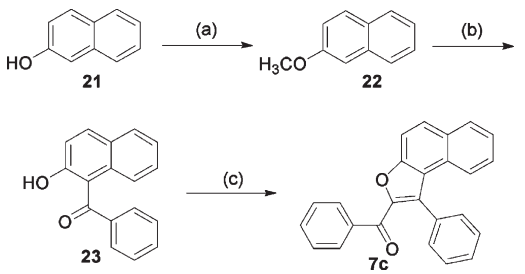
[a] (a) I_2, HIO_4/EtOH, 2 h, rt, 76%; (b) 1-phenyl-2-propyn-1-ol, CuI, Et_3N, DMF, 60 °C, 24 h, 51%; (c) Jones reagent, acetone, 0 °C → rt, 0.5 h, 95%; (d) K_2CO_3, CH_3CN, reflux, 16 h; (e) H_3PO_4, xylene, reflux, 16 h, 18−21% over two steps (**8c** from **16a**; **8d** from **16b**).

Scheme 3. Synthesis of Derivatives **7a**,**b**[a]



[a] (a) (i) *n*-BuLi, PhCOCl, THF, 0 °C → rt, 2 h; (ii) MeOH, HCl, reflux, 0.5 h, 75%. (b) $PhCOCH_2Br$, K_2CO_3, CH_3CN, reflux, 16 h, 72% for **19** from **18a**, 93% for **7b** from **18b**. (c) BBr_3, CH_2Cl_2, 0 °C → rt, 12 h, 80%. (d) Crotonic anhydride, pyridine, imidazole, rt, 2 h, 81%.

Scheme 4. Synthesis of Derivative **7c**[a]



[a] (a) NaH, MeI, THF, 0 °C→ rt, 12 h; (b) $AlCl_3$, CH_2Cl_2, PhCOCl, 16 h; (c) $PhCOCH_2Br$, K_2CO_3, CH_3CN, reflux, 16 h, 8% over three steps.

was alkylated with either 2-bromoacetophenone or 2-bromo-2-phenylacetophenone to give the intermediates **16a** and **16b**, respectively. Cyclization under acidic conditions afforded the desired products **8c**,**d** with a yield of 18−21% over two steps (Scheme 2).

For the preparation of lactone ring opened analogue **7a**, hydrogen abstraction by *n*-BuLi from MOM protected 3-hydroxy anisole[22] (**17**), followed by acylation with benzoyl chloride, and then MOM deprotection in acidic conditions gave **18a**. Condensation of 2-bromoacetophenone with **18a** under basic conditions afforded **19**. Demethylation using BBr_3 to give **20**, followed by acylation with crotonic anhydride, gave the desired **7a**. For the preparation compound **7b** without the lactone ring, commercially available **18b** was condensed with 2-bromoacetophenone to afford **7b** in 93% yield (Scheme 3).

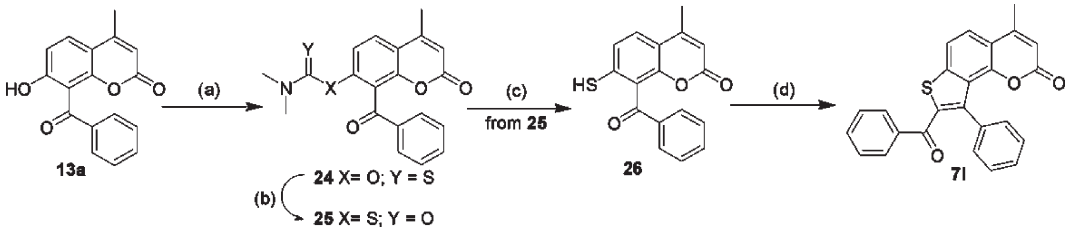
Preparation of **7c** without the lactone ring was carried out as shown in Scheme 4. 2-Naphthol (**21**) was the first methylated using MeI to give **22**, then Fridel−Crafts acylation with benzoyl chloride gave **23**. Without purification, **23** was cyclized with 2-bromoacetophenone in basic conditions to afford **7b** in 8% yield over three steps (Scheme 4). For the preparation of bioisosteric thienocoumarin derivative **7l**, Newman−Kwart thermal rearrangement was utilized to obtain the thiophenol intermediate **26** from phenol **13a**. Condensation of **26** with 2-bromoacetophenone afforded the desired compound **7l**, in an overall yield of 22% in four steps starting from **13a** (Scheme 5).

Reduction of the ketone group of **6f** by sodium borohydride in THF gave the secondary alcohol **8a**. Alternatively, when the reduction process was carried out in the presence of TFA, complete reduction of the keto function to the methylene group was achieved to get the compound **8b** (Scheme 6). The phenol **8u** was obtained from the methoxy derivative **8p** by demethylation using BBr_3. The analogues **9a**−**f** were synthesized by alkylation of the phenol **8u** under basic conditions using the required bromo compound (R^1Br). In the case of compounds **9g**,**h**, TBDMS protected bromoalkanol (R^2Br) was used and deprotection using TBAF in the final step unmasked the OH group (Scheme 7).

Results and Discussion

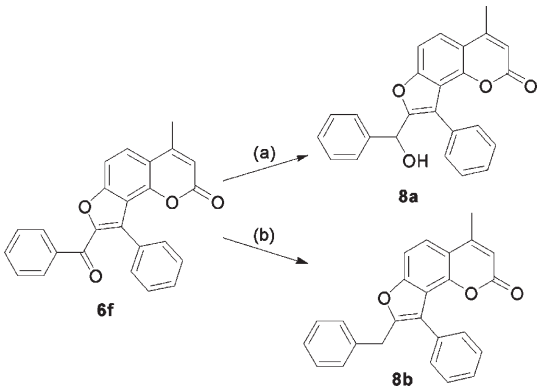
We initiated structure−activity relationship (SAR) studies to explore the pharmacophore (molecular framework responsible for a drug's biological activity) requirements for anti-influenza activity based on the HTS hit **6a**. For convenience of SAR discussion, the lead molecule **6a** is divided into three pharmacophore parts: A (3-methyl group on furan ring), B (angelicin core), and C (2-benzoyl group on furan ring) (Figure 1). In the pharmacophore A (Table 1), removal of the methyl group led to slightly better activity for **6b** than the parent compound **6a**. However, increasing the alkyl chain

Scheme 5. Synthesis of Bioisosteric Thienocoumarin Derivative **7l**[a]



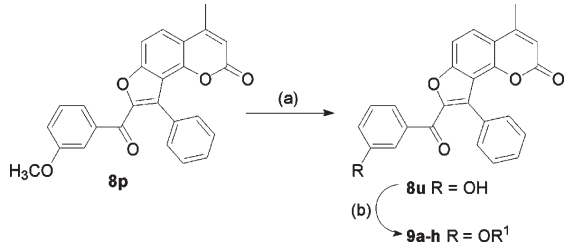
[a] (a) Dimethylthiocarbamoyl chloride, NaH, DMAP, THF, rt, 24 h, 84%; (b) mesitylene, 280 °C, sealed tube, 2 h, 65%; (c) $NaOCH_3$, MeOH, reflux, 1 h; (d) $PhCOCH_2Br$, K_2CO_3, CH_3CN, reflux, 16 h, 40% over two steps.

Scheme 6. Synthesis of Angelicin Derivatives **8a**,**b**[a]



[a] (a) $NaBH_4$, THF, 0 °C, 16 h, 60%; (b) $NaBH_4$, THF, TFA, 0 °C, 16 h, 72%.

Scheme 7. Synthesis of Angelicin Derivatives **8u** and **9a**−**h**[a]

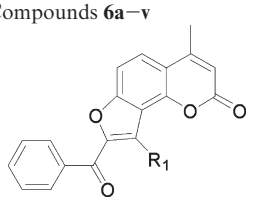


[a] (a) BBr_3, CH_2Cl_2, 2 h, rt, 92%; (b) for **9a**−**f**: R^1Br (R^1 = CH_3-$(CH_2)_n$), or $(CH_3)_2NCH_2CH_2$), K_2CO_3, KI, CH_3CN, reflux, 16 h, 25−38%; for **9g**,**h**: (i) R^2Br (R^2 = $TBDMSO(CH_2)_n$), K_2CO_3, KI, CH_3CN, reflux, 16 h; (ii) TBAF, THF, 0 °C, 1.5 h, 15−19%.

length resulted in significant improvement in activity, with the *n*-propyl (**6c**) and *n*-hexyl (**6d**) analogues showing nanomolar level activity. However, increasing the length further decreased the activity, with the *n*-decyl (**6e**) analogue, showed complete loss of activity. Next we replaced the methyl group of the lead compound **6a** with various aromatic and heteroaromatic rings such as phenyl (**6f**), naphthyl (**6g**,**6h**), thiophene (**6i**), and furan (**6j**). All the five analogues showed improved activity compared to the HTS hit **6a**, showing that aromatic hydrophobic group at this position is preferred. Of particular interest are the phenyl analogue **6f** (IC_{50} = 140 nM) and the bioisosteric equivalent 2-thiophene analogue **6i** (IC_{50} = 150 nM), both of which showed over 30-fold improved activity compared to the HTS hit **6a**.

With the identification of potent compound **6f**, we continued the search for optimal phenyl ring substitution. Initial attempts to introduce electron donating CH_3/OCH_3 (**6k**,**l**) or electron withdrawing NO_2/Cl/Br groups (**6m**−**o**) at the para-position of the phenyl group of **6f**, led to the conclusion that the *para*-Br substitution is better. Furthermore, it was found that when the Br substitution was moved to meta-position in **6p**, the activity improved almost 2-fold of that of the unsubstituted compound **6f**, with the IC_{50} reaching a value of 80 nM. However Br substitution in ortho-position (**6q**) of the phenyl ring led to decreased activity. Moreover, di-Br substituted compound (**6r**) did not show further improvement in activity. Because the *meta*-Br (**6p**) compound showed higher potency than *para*-Br (**6o**) and *ortho*-Br (**6q**) analogues, *meta*-CH_3 (**6s**), *meta*-NO_2 (**6t**), *meta*-Cl (**6u**), and *meta*-CN (**6v**) derivatives were prepared. Even though these meta-substituted analogues (**6s**−**u**) are more potent than their

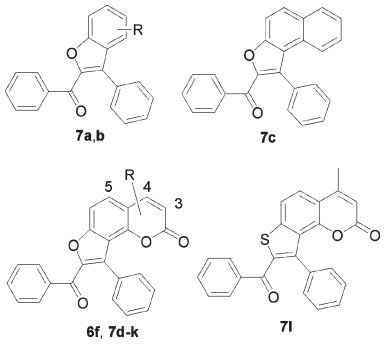
Table 1. Optimization of Pharmacophore A:[a] In Vitro Anti-Influenza A Virus Activity of Compounds **6a**−**v**



compd[b]	R_1	IC_{50}, μM[c]
6a	CH_3	4.51 ± 0.24
6b	H	2.43 ± 0.31
6c	*n*-C_3H_7	0.29 ± 0.03
6d	*n*-C_6H_{13}	0.65 ± 0.02
6e	*n*-$C_{10}H_{21}$	> 25
6f	Ph	0.14 ± 0.01
6g	1-naphthyl	0.88 ± 0.04
6h	2-naphthyl	1.91 ± 0.23
6i	2-thienyl	0.15 ± 0.01
6j	2-furanyl	0.65 ± 0.18
6k	4-CH_3-Ph	0.82 ± 0.10
6l[d]	4-CH_3O-Ph	10.08 ± 0.47
6m	4-NO_2-Ph	0.76 ± 0.12
6n	4-Cl-Ph	3.92 ± 1.70
6o	4-Br-Ph	0.46 ± 0.11
6p	3-Br-Ph	0.08 ± 0.01
6q	2-Br-Ph	0.73 ± 0.03
6r	3,5-diBr-Ph	0.10 ± 0.02
6s	3-CH_3-Ph	0.10 ± 0.03
6t	3-NO_2-Ph	0.25 ± 0.05
6u	3-Cl-Ph	1.41 ± 0.04
6v	3-CN-Ph	0.53 ± 0.02

[a] Using influenza A/WSN/33 (H1N1) strain. [b] All the compounds exhibited CC_{50} > 25 μM. [c] IC_{50}: The concentration required for a test compound to reduce the virus-induced cytopathic effect (CPE) by 50% relative to the virus control. Values are expressed as the mean of at least two independent determinations, each experiments performed in triplicates. [d] Synthesis **6l** was not possible by the standard method A reported, due to demethylation of the methoxy group during Fries rearrangement. Hence an alternative synthetic route for this and related compounds were developed, which will be reported separately.

Table 2. Optimization of Pharmacophore B:[a] In Vitro Anti-Influenza A Virus Activity of Compounds **7a**−**l**



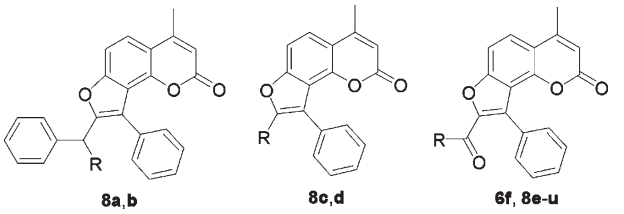
compd[b]	R	IC_{50}[c], μM
6f	4-CH_3	0.14 ± 0.01
7a	OC(O)CH=$CHCH_3$	20.82 ± 1.79
7b	H	> 25
7c		> 25
7d	H	17.6 ± 0.91
7e	3-CH_3	2.79 ± 0.39
7f	5-CH_3	7.76 ± 0.22
7g	4-C_2H_5	0.24 ± 0.04
7h	4-C_3H_7	0.47 ± 0.08
7i	4-Ph	> 25
7j	3-CH_3, 4-CH_3	0.29 ± 0.04
7k	3-$CH_2CH_2CH_2CH_2$-4	> 25
7l		0.72 ± 0.04

[a] Using influenza A/WSN/33 (H1N1) strain. [b] All the compounds exhibited CC_{50} > 25 μM, except **7b** (CC_{50} = 5.27 ± 0.12 μM). [c] The concentration required for a test compound to reduce the virus-induced cytopathic effect (CPE) by 50% relative to the virus control was expressed as IC_{50}. Values are expressed as the mean of at least two independent determinations, each experiments performed in triplicates.

corresponding para analogues (**6k**,**m**,**n**), these compounds are not superior to **6p**. These results suggest that substitution in meta position could help in orienting the phenyl ring at an angle to that of the flat furanocoumarin ring, which could result in better interaction with the active site.

For the pharmacophore B (angelicin core) modification (Table 2), we have used compound **6f** as our template to which all structural modification were compared. When we attempted to either open (**7a**), remove (**7b**), or replace the lactone ring with a phenyl ring (**7c**), complete loss of activity was observed. This shows the critical requirement of the coumarin ring in maintaining the activity. Next, by retaining the coumarin ring intact, we tried to find the effect of substitution at various positions. Removal of the 4-methyl group (**7d**) or shifting it to the 3-position (**7e**) or the 5-position (**7f**) of the coumarin ring led to decreased activity compared to **6f**. Particularly, removal of the 4-position methyl group of **6f** had led to drastic loss of activity in **7d**. It indicates that the methyl substituent at C-4 position has an essential role in maintaining activity. Next, replacing the methyl group with more bulky ethyl (**7g**), propyl (**7h**), and phenyl (**7i**) groups decreased the activity, with the phenyl substituent **7i** being completely inactive, showing that methyl group is optimal for activity at the 4-position of the coumarin ring. By retaining the essential 4-methyl group, we then introduced an additional 3-methyl group (**7j**) in the coumarin ring, which resulted in retention of activity with only 2-fold loss of activity. However, cyclization of 3- and 4-position of coumarin ring (**7k**) through a cyclohexyl ring led to complete loss of activity. Also, we investigated the effect of replacing the furan ring with thiophene ring, which led to 5-fold loss of activity for **7l** compared to that of **6f**. Thus SAR exploration at pharmacophore B clearly shows that the angelicin scaffold is essential and the 4-position methyl group is critical and optimal for activity.

Table 3. Optimization of Pharmacophore C:[a] In Vitro Anti-Influenza A Virus Activity of Compounds **8a**−**u**



compd[b]	R	IC_{50}[c], μM
6f	Ph	0.14 ± 0.01
8a	OH	0.32 ± 0.01
8b	H	0.63 ± 0.04
8c	H	> 25
8d	Ph	> 25
8e	CH_3	> 25
8f	2-furanyl	0.41 ± 0.09
8g	2-thienyl	0.07 ± 0.02
8h	3-thienyl	0.15 ± 0.01
8i	2-pyridyl	0.61 ± 0.02
8j	4-CH_3-Ph	0.87 ± 0.01
8k	4-OCH_3-Ph	1.67 ± 0.03
8l	4-NO_2-Ph	> 6.25
8m	4-Cl-Ph	22.34 ± 1.22
8n	3-CH_3-Ph	0.26 ± 0.10
8o	2-CH_3-Ph	6.62 ± 1.17
8p	3-OCH_3-Ph	0.11 ± 0.03
8q	2-OCH_3-Ph	0.64 ± 0.01
8r	3-NO_2-Ph	1.71 ± 0.56
8s	3-Cl-Ph	0.26 ± 0.01
8t	3-F-Ph	0.29 ± 0.01
8u	3-OH-Ph	0.72 ± 0.11

[a] Using influenza A/WSN/33 (H1N1) strain. [b] All the compounds exhibited CC_{50} > 25 μM, except **8l** (CC_{50} = 3.68 ± 0.09). [c] The concentration required for a test compound to reduce the virus-induced cytopathic effect (CPE) by 50% relative to the virus control was expressed as IC_{50}. Values are expressed as the mean of at least two independent determinations, each experiments performed in triplicates.

Next, we turned our attention to pharmacophore C (2-benzoyl group on furan ring) modifications (Table 3). To examine the role of ketone group of lead **6f**, the ketone group was reduced to a secondary alcohol (**8a**) or to methylene (**8b**) group, which led to 2−4-fold loss of activity. However, either removal of 2-benzoyl group completely (**8c**), attaching the phenyl group directly to the furan ring (**8d**), or replacement of phenyl group with methyl group (**8e**), led to complete loss of activity. Compounds **8a**−**b**, which had the phenyl group attached to the furan ring through one-carbon linker showed only a maximum of 2−4-fold loss of activity compared to **6f**; while compounds **8c**−**e**, either without the phenyl group or with the phenyl group at different spatial orientation than lead **6f** showed complete loss of activity. This clearly shows the importance of 2-benzoyl group attached to the angelicin scaffold for activity.

Further, we attempted to replace the phenyl group of **6f** on pharmacophore C with different heteroaromatic groups such as furan (**8f**), thiophene (**8g**,**8h**), and pyridine (**8i**). 2-Thiophene analogue **8g** (IC_{50} = 70 nM) is of particular interest, which showed a 2-fold improvement in activity than the phenyl analogue **6f**. However, substitution on the thiophene

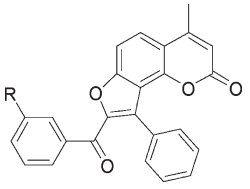
ring of **8g** did not further improve the activity (data not shown). Hence, substitution effect on the phenyl ring of pharmacophore C was explored in order to identify optimal substituent in this region. Introduction of electron donating CH_3/OCH_3 (**8j**,**k**) groups in the para-position led to decrease in activity, while introduction of electron withdrawing NO_2/Cl (**8l**,**m**) groups in the para-position of the phenyl group led to drastic loss of activity compared to the unsubstituted **6f**. When the electron donating CH_3/OCH_3 groups were moved from the para-position to either the ortho- or meta-position (**8n**−**q**), it led to the identification of a potent *meta*-OCH_3 compound **8p** with similar activity to that of unsubstituted **6f**. Because moving the substitution from para- to meta-position improved the activity for CH_3/OCH_3 groups, NO_2 (**8r**), Cl (**8s**), F (**8t**), and OH (**8u**) groups were also introduced at the meta-position of the phenyl ring. Particularly, the *meta*-Cl (**8s**) and *meta*-F (**8t**) substitution led only to a 2-fold loss of activity compared to **6f**, demonstrating that the meta-position could tolerate both electron donating and withdrawing groups much better than the ortho- or para-position of the phenyl ring in pharmacophore C.

With the identification of analogue **8p**, further SAR exploration was carried out by increasing the *O*-alkyl chain length of **8p** from one carbon to eight carbons (**9a**−**9e**) to identify additional binding elements in this region (Table 4). Up to a five-carbon *O*-alkyl chain the activity level was retained; particularly, the *n*-butyl analogue **9c** (IC_{50} = 80 nM) showed a 2-fold improved activity over the lead **6f**. Further increasing the chain length to *n*-octyl (**9e**) led to drastic loss of activity. However, introduction of hydrophilic groups at the end of the alkyl chain, such as the $N(CH_3)_2$ (**9f**) or OH (**9g**,**h**) groups, led to loss of activity. This SAR trend indicates the presence of a tunnel-like hydrophobic cavity with steric limitation at the active site to which pharmacophore C interacts.

Individual optimization of pharmacophores A−C has identified key structural features essential for optimal anti-influenza activity in that region. On the basis of this information, we next synthesized two compounds by bringing together these optimized structural features into a single molecule (Table 5). Thus, the 3-bromo phenyl (**6p**) was chosen as the pharmacophore A optimized structure, and 3-methoxy phenyl (**8p**) and 2-thiophene rings (**8g**) were chosen as the pharmacophore C optimized structures. Results showed that **10a** and **10b** displayed good anti-influenza activity, with the thiophene derivative **10b** being the most potent compound identified in this study. Thus, an overall improvement of ~75-fold anti-influenza activity was achieved for **10b** (IC_{50} = 60 nM) through systematic SAR study on the HTS hit **6a** (IC_{50} = 4.51 μM).

Having identified potent anti-influenza angelicin derivatives, we investigated in detail the potential of a representative

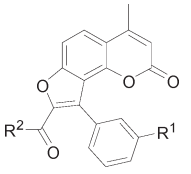
Table 4. Optimization of Pharmacophore C:[a] In Vitro Anti-Influenza A Virus Activity of **9a**−**h**



compd[b]	R	IC_{50}, μM[c]
6f	H	0.14 ± 0.01
8p	OCH_3	0.11 ± 0.03
9a	OCH_2CH_3	0.12 ± 0.02
9b	$O(CH_2)_2CH_3$	0.15 ± 0.01
9c	$O(CH_2)_3CH_3$	0.08 ± 0.01
9d	$O(CH_2)_4CH_3$	0.14 ± 0.01
9e	$O(CH_2)_7CH_3$	7.97 ± 2.96
9f	$O(CH_2)_2N(CH_3)_2$	5.28 ± 0.03
9g	$O(CH_2)_2OH$	6.09 ± 0.67
9h	$O(CH_2)_3OH$	2.65 ± 0.39

[a] Using influenza A/WSN/33 (H1N1) strain. [b] All the compounds exhibited CC_{50} > 25 μM, except **9f** (CC_{50} = 17.65 ± 2.08). [c] The concentration required for a test compound to reduce the virus-induced cytopathic effect (CPE) by 50% relative to the virus control was expressed as IC_{50}. Values are expressed as the mean of at least two independent determinations.

Table 5. Optimization of Pharmacophore A and C Together:[a] In Vitro Anti-Influenza A Virus Activity of Compounds **10a**,**b**



compd[b]	R^1	R^2	IC_{50}, μM[c]
6p	Br	Ph	0.08 ± 0.01
8p	H	3-OCH_3-Ph	0.11 ± 0.03
8g	H	2-thiophene	0.07 ± 0.02
10a	Br	3-OCH_3-Ph	0.09 ± 0.01
10b	Br	2-thiophene	0.06 ± 0.02
4 (Zanamivir)			0.06 ± 0.02

[a] Using influenza A/WSN/33 (H1N1) strain. [b] All the compounds exhibited CC_{50} > 25 μM. [c] The concentration required for a test compound to reduce the virus-induced cytopathic effect (CPE) by 50% relative to the virus control was expressed as IC_{50}. Values are expressed as the mean of at least two independent determinations, each experiments performed in triplicates.

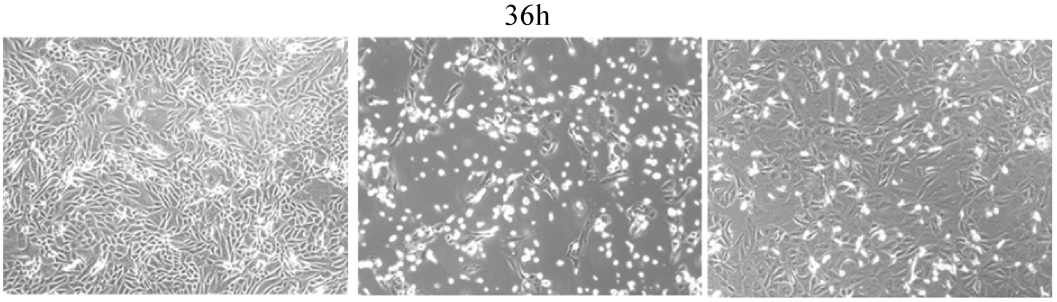


Figure 2. Cytopathic effect (CPE) produced by influenza A/WSN/33 virus strain in MDCK cells and its abrogation on treatment with **8g** (1 μM).

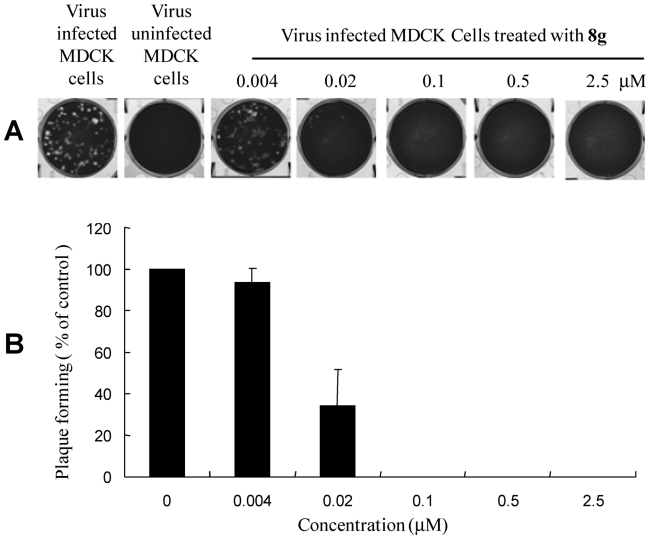


Figure 3. Reduction of plaque formation by influenza A/WSN/33 in MDCK cells upon treatment with **8g**. MDCK cells were infected with influenza virus A at approximate 125 plaque-forming unit (PFU)/well. Different concentrations of compound were added at the stages of viral adsorption and post infection. (A) The effect of **8g** on viral plaque formation. (B) Quantification of viral plaque formation following treatment with serial dilutions of compound.

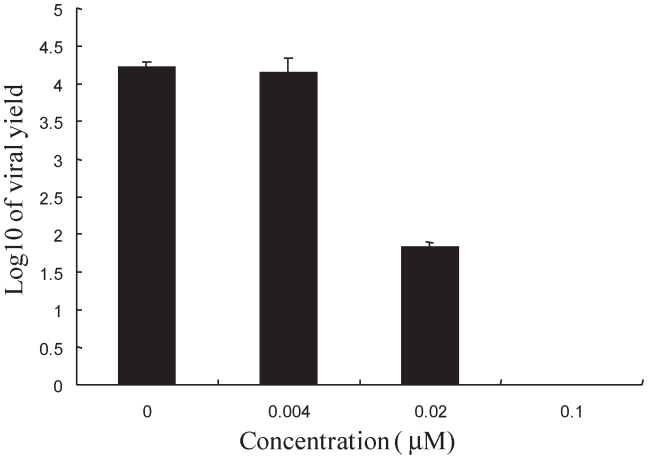


Figure 4. Reduction of influenza A/WSN/33 viral yields by compound **8g**. MDCK cells were infected by 0.0001 multiplicity of infection (MOI) of A/WSN/33 virus, and the culture supernatants were collected at 24 h post infection. Various concentrations of **8g** were added to cells when viral adsorption occurred. Viral yields were measured by plaque assay.

compound **8g**. The cytopathic effect produced by influenza virus A (WSN/33) on MDCK cells and its abrogation by treatment with **8g** is shown in Figure 2. In addition to the standard neutralization assay discussed above, to confirm the anti-influenza activity of **8g**, we carried out plaque reduction assay, which measured the ability of the compound in reducing the number of plaques formed by virus infected MDCK cells. In the plaque reduction assay, **8g** showed over 50% reduction of plaque formation at a concentration of 20 nM and complete abrogation of plaque formation at a concentration of 100 nM (Figure 3). Further, the anti-influenza activity of **8g** was confirmed by determining the viral yield; a reduction in virus yield, a direct indicator of the ability of the compound to decrease the production of next generation influenza (progeny), was observed upon treatment with **8g**. Treatment

Table 6. In Vitro Anti-Influenza Activity of **8g** against Various Strains

virus strain	IC_{50} (μM ± SD)[a]	
	8g	**4** (Zanamivir)
influenza A/WSN/33 (H1N1)	0.07 ± 0.01	0.06 ± 0.02
influenza A/TW/141/04 (H1N1)	0.09 ± 0.01	0.35 ± 0.14
influenza A/TW/3446/04 (H3N2)	0.15 ± 0.01	0.03 ± 0.01
influenza B/TW/710/05	0.09 ± 0.01	0.03 ± 0.02
influenza B/TW/70325/05	0.09 ± 0.01	0.02 ± 0.01
influenza B/TW/99/07	0.04 ± 0.01	0.10 ± 0.01

[a] The concentration required for a test compound to reduce the virus-induced cytopathic effect (CPE) by 50% relative to the virus control was expressed as IC_{50}. Values are expressed as the mean of at least two independent determinations, each experiments performed in triplicates.

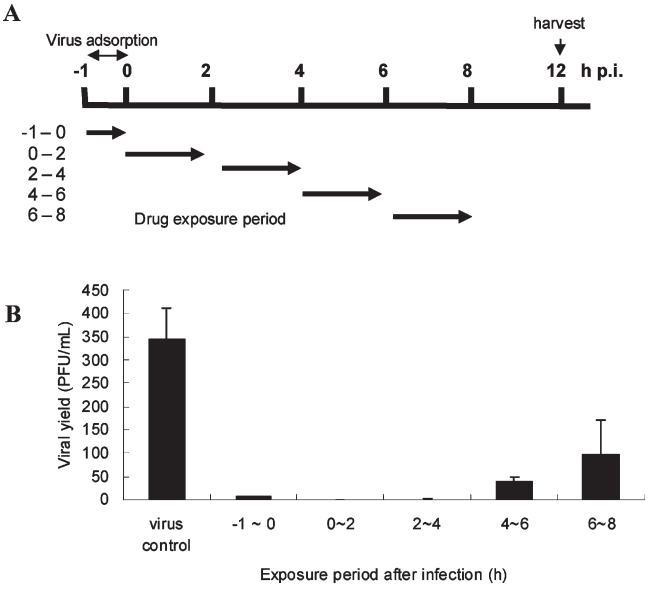


Figure 5. Time-of-addition effect of **8g** on influenza A/WSN/33 infection in MDCK cells. (A) MDCK cells were inoculated with influenza A virus at MOI = 0.001. Compound was added at the indicated times. Viral infection was performed between −1 and 0 h. After each incubation period, the test medium containing 1 μM of compound was removed and the cells were incubated with fresh medium until 12 h post infection (pi). The supernatant was collected and viral yield was determined by plaque assay. (B) Viral yields obtained with respect to different exposure period of **8g** to the culture medium during influenza infection.

with 100 nM of **8g**, during viral adsorption, completely abolished progeny production, as indicated by the viral yield (Figure 4). With the confirmation of the anti-influenza virus A (WSN/33) activity, we were interested to investigate its potential in other strains of the virus. For this purpose, we selected three strains each of influenza A (two H1N1 and one H3N2 strain) and B (Table 6). Influenza inhibition results showed that **8g** effectively inhibits all the influenza virus strains tested similar to clinically used anti-influenza medication zanamivir (**4**), with an IC_{50} in the range of 40−150 nM. This result suggests that **8g** is a broad spectrum anti-influenza agent similar to zanamivir (**4**) and could provide effective coverage against infection over different strains of influenza.

With the confirmation of the anti-influenza activity, we were interested to identify the molecular target for the novel angelicin derivatives. Initially, we carried out time-of-addition study (Figure 5) to determine the effectiveness of **8g** in decreasing the viral yield with regard to the time at which compound was added to the culture medium during the course

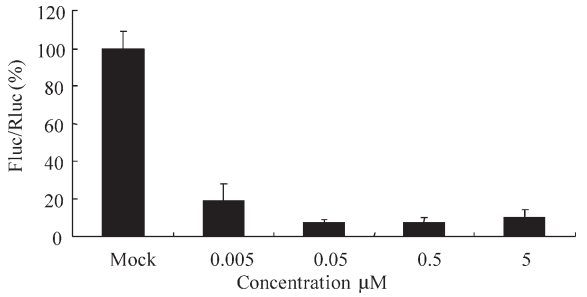


Figure 6. Inhibition of influenza RNP activity by **8g** using reporter assay. Different concentrations of compound **8g** were incubated with 293 (human embryonal kidney) cells previously transfected with pPOLI-Fluc and pHW2000 expression vectors for influenza virus PB1, PB2, PA, and NP proteins. Treatment with **8g** decreased the ratio of Fluc (firefly luciferase) to Rluc (renilla luciferase) activity, indicating that **8g** inhibited RNP complex mediated transcription of pPOLI-Fluc, resulting in decreased Fluc activity without affecting the Rluc activity much. DMSO was included as the mock control, and Rluc expression was used as transfection control.

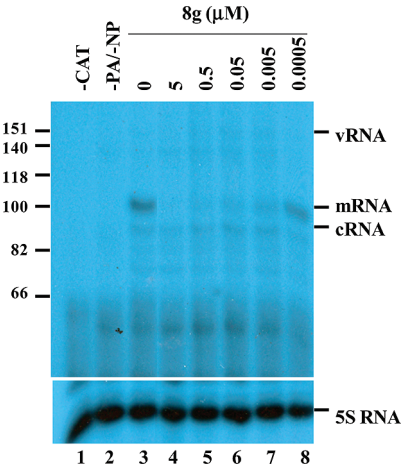


Figure 7. Effect of **8g** on the production of viral-like RNA synthesis. Different concentrations of compound **8g** were incubated with 293 cells previously transfected with pHW2000-PB2, PB1, PA, NP and pPOLI-CAT-RT (lanes 3−8). Total RNA was isolated for primer extension assay and then analyzed by polyacrylamide electrophoresis and autoradiography. The RNA species were marked on the right. No CAT (lane 1) or no PA/NP (lane 2) was served as a control. 5S RNA is a loading control.

of the infection. It was found that **8g** was able to prevent the progeny formation when added up to 4 h from the time of infection, however, less effective in preventing the formation of progeny after this time, as evidenced by increased production of progeny at 4−8 time period post infection. These results suggest that the compound could be acting at early stages of viral replication cycle, including viral adsorption and/or early replication phase. This findings further prompted us to investigate the potential of **8g** to acts as hemagglutinin (HA) inhibitor; HA is a surface glycoprotein of influenza virus involved in viral attachment to the host cell and hence essential for viral absorption on to the cells. Ability of **8g** to inhibit HA was investigated using hemagglutinination inhibition assay, where the ability of the compound to prevent the attachment of virus to guinea pig red blood cells (RBCs) was measured (Figure 1s, Supporting Information). Compound **8g** did not inhibit HA even at a high concentration of 25 μM, as evidenced by the lack of inhibition of virus attachment to RBC, ruling out HA as a target for the anti-influenza activity of this novel angelicin derivatives.

Next, we turned our attention to see if **8g** is acting at the level of viral replication by determining its effect on RNP (ribonucleoprotein) activity through a plasmid-based reverse genetics using luciferase reporter assay.[23,24] Reporter assay was performed in human 293 (human embryonal kidney) cells transfected with pPOLI-Fluc plasmids (contains the firefly luciferase open reading frame in minus sense) and pHW2000 expression vectors for influenza virus PB1, PB2, PA, and NP proteins (these viral proteins are essential to amplify and transcribe the influenza virus-like RNA expressed by pPOLI-Fluc into mRNA, resulting in the detection of firefly luciferase reporter activity), along with pRL-TK as a transfection control. Transfected cells were then treated with different concentrations of **8g** for 20 h, and the firefly (Fluc) and renilla (Rluc) luciferase activity measured and the ratio between Fluc to Rluc activity shown in Figure 6. Treatment with **8g** decreased the amount of Fluc activity without affecting Rluc activity much (Figure 2s, Supporting Information), indicating that the **8g** treatment decreased the viral-like RNA transcription by inhibiting the viral RNP complex, which also drives the reporter gene expression.

In addition to the reporter assay, we carried out primer extension assay to see the effect of **8g** on viral-like mRNA synthesis in 293 cells previously transfected with pcDNA3-PB2, PB1, PA, NP, and pPOLI-CAT-RT. Transfected cells were treated with different concentration of **8g** and the total RNA isolated and analyzed through primer extension assay specific for vRNA (viral RNA) and mRNA through reverse transcription reaction; the results are shown in Figure 7. Treatment with **8g** decreased the amount of mRNA production, indicating that the compound targets viral RNP complex, which drives the reporter gene expression; further, we have already shown that another potent compound from this series, **6f**, act through interference of viral RNP activity.[25] Further, we also evaluated the ability of the potent compound **8g** side-by-side with the inactive compound **7d** (as negative control) in plaque formation, viral yield reduction, and viral-like RNA synthesis assay to show that the anti-influenza inhibition is restricted to only potent compound **8g** (Figures 3s, 4s, and 5s, Supporting Information).

Finally, we have also carried out immunofluorescence microscopic analysis of MDCK cells infected by influenza virus using anti-dsRNA (double strand RNA), anti-NP, and anti-M1 antibodies to visualize the viral RNA and proteins in the infected MDCK cells. Presence of dsRNA, NP (nucleoprotein), and M1 (matrix protein) is an indicator of ongoing viral replication. Treatment of **8g** resulted in decreased amounts of dsRNA, NP, and M1 protein (Figures 6s, 7s, and 8s, Supporting Information), as evidenced by the decreased amount of immunofluorescence signals compared to the control. Thus the preliminary mechanistic studies using **8g** and **6f**[25] suggest that they act through interference of viral RNA synthesis by targeting RNP complex. It should be noted that there are other agents such as ribavirin, viramidine, and T-705 reported in the literature to possess anti-influenza activity, which act as RNA dependent RNA polymerase inhibitors.[3,26,27] Moreover, the novel angelicin derivative **6f** also possesses activity against oseltamivir (**3**) resistant influenza and other RNA viruses such as enterovirus 71, coxsackie B, and human rhinovirus; however, the mechanism for broad antiviral activity needs to be investigated further.[25] These results suggest that the angelicin derivatives described here

are novel anti-influenza agents with mode of action different from that of the approved influenza therapies in the market.

Conclusion

The threat of a pandemic influenza is real and could be a global disaster in waiting. Considering the multifaceted problem of developing vaccines for millions of people, particularly with the genetics of influenza shifting/drifting, there is a global trend to stockpile effective anti-influenza medication. However, emergence of resistant strains to this medication necessitates the development of newer therapeutic alternatives. A novel series of angelicin derivatives identified from the HTS campaign is optimized through detailed SAR exploration. The results suggest that pharmacophore B consisting of the angelicin scaffold is very sensitive to structural modification and is essential for maintaining the activity, while pharmacophores A and C are more amenable for structural modification, with the meta-substituted phenyl/2-thiophene rings being optimal for activity in this region. The optimized compound **8g** inhibits the activity of both influenza A and B strains in vitro, suggesting that it could act as a lead compound for further anti-influenza drug development. As preliminary mechanistic studies point toward viral RNP as a probable molecular target for these agents, combination therapy with existing anti-influenza drugs which act through the NA target could be used to avoid the development of resistant influenza strains. Moreover, as angelicin derivatives are rarely reported as antiviral agents, the leads identified here could open up new opportunities for antiviral drug discovery in general, which need to be explored further to realize the full potential of this novel series. Detailed mechanistic studies and further potential for development are under active pursuit in our laboratories, and the findings will be revealed in due course.

Experimental Section

General Methods. All commercial chemicals and solvents are reagent grade and were used without further treatment unless otherwise noted. All reactions were carried out under an atmosphere of dry nitrogen. Reactions were monitored by TLC using Merck 60 F_{254} silica gel glass backed plates (5 × 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80 °C. Flash column chromatography was done using silica gel (Merck Kieselgel 60, no. 9385, 230−400 mesh ASTM). ^{1}H and ^{13}C NMR spectra were obtained with a Varian Mercury-300 or Varian Mercury-400 or Bruker DMX-600 spectrometers. Chemical shifts were recorded in parts per million (ppm, δ) and were reported relative to the solvent peak or TMS. High-resolution mass spectra (HRMS) were measured with a Finnigan (MAT-95XL) electron impact (EI) or using Finnigan/Thermo Quest MAT 95XL FAB mass spectrometer. LCMS data were measured on an Agilent MSD-1100 ESI-MS/MS system. Purity of the final compounds were determined using a Hitachi 2000 series HPLC system using a C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm, 4.6 mm × 150 mm) or a phenyl column (Waters XBridge 5 μm, 4.6 mm × 150 mm) and were found to be >95% unless otherwise stated.

General Synthetic Method for Compounds 6−10 (Method A; Scheme 1). Appropriately substituted 7-hydroxy-coumarin **11** (1.0 equiv) was dissolved in THF (40 mL), triethylamine (1.1 equiv) was added, and then the appropriate acyl chloride (R^4COCl; 1.2 equiv) and stirred at room temperature for 16 h. The solid product (**12**) formed was filtered, washed with EtOAc, and the product obtained was used for next step without further purification.

The above obtained **12** (1.0 equiv) was mixed well with finely powdered anhydrous aluminum chloride (3.0 equiv) and heated at 170 °C for 2 h. After cooling, ice and diluted hydrochloric acid (20 mL) were added and extracted with EtOAc (30 mL × 3). The EtOAc layer was washed with diluted hydrochloric acid (20 mL × 2) and then with water (20 mL × 2), followed by satd $NaHCO_3$(aq) (30 mL × 2). The combined organic layers were evaporated to give **13** as a solid product, which was used for next step without purification.

A solution of **13** (1.0 equiv), 2-bromo ketone (R^5COCH_2Br) (1.2 equiv), and K_2CO_3 (3.0 equiv) in CH_3CN (5 mL) was refluxed for 16 h. The reaction mixture was filtered, the solvents from the filtrate were removed under vacuum, and the crude product was purified by silica gel column chromatography (hexane/EtOAc: 3/1 → 1/1) to give the desired products **6−10**, with 15−44% yield over three steps.

8-Benzoyl-4,9-dimethyl-furo[2,3-*h*]chromen-2-one 6a. Method A. Yield 31% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06−8.01 (m, 2H), 7.67 (d, J = 8.7 Hz, 1H), 7.64−7.60 (m, 1H), 7.56−7.51 (m, 3H), 7.45 (d, J = 8.7 Hz, 1H), 6.29 (d, J = 0.9 Hz, 1H), 2.91 (s, 3H), 2.51 (d, J = 0.9 Hz, 3H). LCMS $[M + 1]^+$: 319.1.

8-Benzoyl-4-methyl-furo[2,3-*h*]chromen-2-one 6b (Scheme 2). To a solution of 7-hydroxy-4-methylcoumarin **11a** (2.00 g, 11.30 mmol) in ethanol (5 mL), was added I_2 (2.32 g, 9.10 mmol) and periodic acid (0.53 g, 2.3 mmol) and stirred at room temperature for 2 h. The reaction mixture was diluted with water, and the precipitate formed was collected and recrystallized from ethanol to give light-yellow colored needles of 7-hydroxy-8-iodo-4-methylcoumarin (**14**, 2.10 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.77 (s, 1H), 7.43 (d, J = 8.7 Hz, 1H), 6.94 (d, J = 8.7 Hz, 1H), 6.08 (d, J = 1.2 Hz, 1H), 2.41 (d, J = 1.2 Hz, 1H). LCMS $[M + 1]^+$: 302.9.

A solution of **14** (1.01 g, 3.30 mmol), 1-phenyl-2-propyn-1-ol (0.6 mL), CuI (0.01 g, 0.05 mmol), and Et_3N (1 mL) in DMF (5 mL) was stirred at 60 °C for 24 h. The reaction mixture was quenched by addition of water and extracted with EtOAc (50 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered, and evaporated to give the crude product, which was purified by column chromatography over silica gel (hexane/EtOAc: 3/1 → 1/1) to furnish 8-(hydroxy-phenyl-methyl)-4-methyl-furo[2,3-*h*]chromen-2-one (**15**, 0.52 g, 51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50−7.19 (m, 6H), 6.81 (s, 1H), 6.16 (d, J = 0.9 Hz, 1H), 5.93 (s, 1H), 3.70 (br, 1H), 2.40 (d, J = 0.9 Hz, 3H). LCMS $[M + 1]^+$: 307.1.

To a solution of **15** (0.03 g, 0.10 mmol) in acetone (5 mL) was added Jones reagent at 0 °C and then stirred at room temperature for 0.5 h. The reaction mixture was evaporated, and the crude product was purified by column chromatography over silica gel (hexane/EtOAc: 3/1 →1/1) to give **6b** (0.03 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05−8.01 (m, 2H), 7.83 (s, 1H), 7.83−7.68 (m, 2H), 7.66−7.28 (m, 3H), 6.32 (d, J = 0.9 Hz, 1H), 2.53 (s, 3H). LCMS $[M + 1]^+$: 305.0.

8-Benzoyl-4-methyl-9-propyl-furo[2,3-*h*]chromen-2-one 6c. Method A. Yield 32% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06−7.44 (m, 7H), 6.32 (d, J = 1.2 Hz, 1H), 3.37 (t, J = 5.4 Hz, 2H), 2.52 (d, J = 1.2 Hz, 3H), 1.87 (m, 2H), 1.07 (t, J = 7.2 Hz, 3H). LCMS $[M + 1]^+$: 347.2.

8-Benzoyl-9-hexyl-4-methyl-furo[2,3-*h*]chromen-2-one 6d. Method A. Yield 35% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06−7.52 (m, 7H), 6.31 (d, J = 1.2 Hz, 1H), 3.38 (t, J = 7.5 Hz, 2H), 2.52 (d, J = 1.2 Hz, 3H), 1.80 (quintet, J = 7.8 Hz, 2H), 1.62−1.26 (m, 6H), 0.87 (t, J = 6.9 Hz, 3H). LCMS $[M + 1]^+$: 389.2. HPLC purity: 91.9%.

8-Benzoyl-9-decyl-4-methyl-furo[2,3-*h*]chromen-2-one 6e. Method A. Yield 39% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06−7.44 (m, 7H), 6.31 (s, 1H), 3.38 (t, J = 7.5 Hz, 2H), 2.51 (s, 3H), 1.80 (quintet, J = 7.5 Hz, 2H), 1.63 (br, 2H), 1.48 (quintet, J = 7.2 Hz, 2H), 1.43 (br, 10H), 0.86 (t, J = 6.3 Hz, 3H). LCMS $[M + 1]^+$: 445.2.

8-Benzoyl-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 6f. Method A. Yield 35% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79−7.72 (m, 3H), 7.57 (d, J = 9 Hz, 1H), 7.49−7.42 (m, 3H), 7.43−7.27 (m, 5H), 6.25 (d, J = 1.2 Hz, 1H), 2.50 (d, J = 1.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.5, 159.5, 156.5, 152.8, 150.0, 148.1, 136.6, 132.9, 130.6, 129.7, 129.6, 128.8, 128.1, 127.7, 124.2, 116.4, 115.5, 108.9, 19.6. HRMS (M^+): calcd for $C_{25}H_{16}O_4$ 380.1049, found 380.1041.

8-Benzoyl-4-methyl-9-naphthalen-1-yl-furo[2,3-*h*]chromen-2-one 6g. Method A. Yield 32% over three steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84−7.73 (m, 3H), 7.65−7.62 (m, 4H), 7.46−7.25 (m, 5H), 7.07−7.03 (m, 2H), 6.11 (d, J = 0.9 Hz, 1H), 2.44 (d, J = 0.6 Hz, 3H). LCMS $[M + 1]^+$: 431.1. HPLC purity: 90.4%.

8-Benzoyl-4-methyl-9-naphthalen-2-yl-furo[2,3-*h*]chromen-2-one 6h. Method A. Yield 33% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89−7.21 (m, 14H), 6.22 (s, 1H), 2.47 (s, 3H). LCMS $[M + 1]^+$: 431.1.

8-Benzoyl-4-methyl-9-thiophen-2-yl-furo[2,3-*h*]chromen-2-one 6i. Method A. Yield 26% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83−7.29 (m, 10H), 6.26 (d, J = 0.6 Hz, 1H), 2.48 (d, J = 0.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.2, 159.5, 156.3, 152.9, 149.7, 148.5, 136.4, 133.1, 131.3, 129.5, 129.2, 128.3, 128.2, 126.8, 124.3, 120.9, 116.0, 115.4, 113.5, 108.9, 19.5. LCMS $[M + 1]^+$: 387.0. HPLC purity: 94.5%.

8-Benzoyl-9-furan-2-yl-4-methyl-furo[2,3-*h*]chromen-2-one 6j. Method A. Yield 40% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84−7.24 (m, 9H), 6.48−6.46 (m, 1H), 6.28 (s, 1H), 2.49 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.5, 159.6, 156.4, 153.0, 149.3, 148.0, 143.3, 142. 8, 136.8, 133.0, 129.2, 128.3, 124.1, 116.9, 115.4, 114.6, 114.4, 113.3, 111.9, 108.9, 19.5. LCMS $[M + 1]^+$: 371.0. HPLC purity: 90.5%.

8-Benzoyl-4-methyl-9-*p*-tolyl-furo[2,3-*h*]chromen-2-one 6k. Method A. Yield 26% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78−7.68 (m, 3H), 7.53−7.45 (m, 2H), 7.42−7.27 (m, 4H), 7.13−7.10 (m, 2H), 6.22 (s, 1H), 2.46 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.4, 159.5, 156.3, 152.9, 149.8, 147.9, 138.4, 136.5, 132.7, 130.5, 129.6, 128.7, 128.4, 128.0, 127.8, 126.4, 124.1, 116.2, 115.2, 113.3, 108.8, 21.3, 19.4. LCMS $[M + 1]^+$: 395.2.

8-Benzoyl-9-(4-methoxyphenyl)-4-methyl-2*H*-furo[2,3-*h*]chromen-2-one 6l. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75−7.69 (m, 3H), 7.54 (d, J = 8.8 Hz, 1H), 7.46−7.34 (m, 3H), 7.30−7.26 (m, 2H), 6.84−6.80 (m, 2H), 6.24 (q, J = 1.2 Hz, 1H), 3.78 (s, 3H), 2.48 (d, J = 1.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.6, 160.0, 159.6, 156.5, 152.9, 150.1, 148.0, 136.7, 132.8, 132.1, 129.7, 128.7, 128.1, 124.1, 121.6, 116.4, 115.3, 113.5, 113.3, 109.0, 55.2, 19.6. HRMS calcd for $C_{26}H_{18}O_5$ 410.1154, found 410.1153.

8-Benzoyl-4-methyl-9-(4-nitro-phenyl)-furo[2,3-*h*]chromen-2-one 6m. Method A. Yield 28% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.27−8.24 (m, 2H), 7.90−7.80 (m, 2H), 7.77−7.70 (m, 3H), 7.70−7.53 (m, 2H), 7.44−7.27 (m, 2H), 6.28 (d, J = 0.9 Hz, 1H), 2.52 (d, J = 0.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.6, 159.1, 156.2, 152.8, 147.9, 136.9, 136.3, 133.5, 131.5, 130.2, 129.8, 129.5, 128.4, 128.2, 126.4, 124.7, 123.0, 115.7, 113.9, 109.1, 19.5. LCMS $[M + 1]^+$: 426.1. HPLC purity: 91.3%.

8-Benzoyl-9-(4-chloro-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one 6n. Method A. Yield 35% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82−7.78 (m, 2H), 7.75 (d, J = 9.3 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H), 7.54−7.49 (m, 1H), 7.45−7.42 (m, 2H), 7.38−7.30 (m, 4H), 6.27 (d, J = 1.2 Hz, 1H), 2.51 (d, J = 0.9 Hz, 3H). LCMS $[M + 1]^+$: 415.1.

8-Benzoyl-9-(4-bromo-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one 6o. Method A. Yield 23% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82−7.36 (m, 11H), 6.28 (d, J = 1.2 Hz, 1H), 2.51 (d, J = 1.2 Hz, 3H). HRMS (M^+): calcd for $C_{25}H_{15}BrO_4$ 458.0154, found 458.0148.

8-Benzoyl-9-(3-bromo-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one 6p. Method A. Yield 37% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81−7.74 (m, 3H), 7.60−7.44 (m, 5H), 7.38−7.33 (m, 2H), 7.28−7.22 (m, 1H), 6.23 (d, J = 1.2 Hz, 1H), 2.46 (d, J = 1.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.1, 159.2, 156.3, 152.8, 149.7, 148.3, 136.4, 132.6, 133.1, 131.4, 129.5, 129.2, 129.2, 128.2, 127.14, 124.4, 121.6, 116.0, 115.4, 113.7, 108.9, 19.5. HRMS (M^+): calcd for $C_{25}H_{15}BrO_4$ 458.0154, found 458.0144.

8-Benzoyl-9-(2-bromo-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one 6q. Method A. Yield 22% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81−7.74 (m, 3H), 7.59−7.44 (m, 5H), 7.38−7.33 (m, 2H), 7.28−7.22 (m, 1H), 6.27 (d, J = 1.2 Hz, 1H), 2.51 (d, J = 1.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.1, 159.2, 156.3, 152.8, 149.7, 148.3, 136.4, 133.6, 133.1, 131.7, 129.5, 129.2, 129.2, 128.2, 127.1, 124.4, 121.6, 116.0, 115.4, 113.7, 108.9, 19.5. LCMS $[M + 1]^+$: 459.0.

8-Benzoyl-9-(3,5-dibromo-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one 6r. Method A. Yield 34% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, J = 6.9 Hz, 2H), 7.76 (d, J = 9.0 Hz, 1H), 7.64 (t, J = 1.8 Hz, 1H), 7.53−7.59 (m, 4H), 7.43 (t, J = 6.0 Hz, 2H), 6.29 (d, J = 1.5 Hz, 1H), 2.52 (d, J = 1.5 Hz, 3H). LCMS $[M + 1]^+$: 539.9. HPLC purity: 90.7%.

8-Benzoyl-9-(3-methyl-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one 6s. Method A. Yield 26% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76−7.72 (m, 3H), 7.57 (d, J = 9.0 Hz, 1H), 7.46−7.41 (m, 1H), 7.33−7.20 (m, 5H), 7.10 (d, J = 7.5 Hz, 1H), 6.25 (d, J = 0.9 Hz, 1H), 2.50 (d, J = 0.9 Hz, 3H), 2.25 (s, 3H). HRMS (M^+): calcd for $C_{26}H_{18}O_4$ 394.1205, found 394.1207.

8-Benzoyl-9-(3-nitro-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one 6t. Method A. Yield 42% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25−7.61 (m, 11H), 6.21 (s, 1H), 2.51 (d, J = 1.2 Hz, 3H). LCMS $[M + 1]^+$: 426.0.

8-Benzoyl-9-(3-chloro-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one 6u. Method A. Yield 33% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, J = 7.2 Hz, 2H), 7.76 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 9.0 Hz, 1H), 7.56−7.53 (m, 3H), 7.48−7.37 (m, 4H), 6.28 (s, 1H), 2.52 (s, 3H). LCMS $[M + 1]^+$: 415.0.

8-Benzoyl-9-(3-cyano-phenyl)-4-methyl-furo[2,3-*h*]chromen-2-one 6v. Method A. Yield 26% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88−7.76 (m, 5H), 7.75−7.51 (m, 4H), 7.43−7.38 (m, 2H), 6.27 (d, J = 1.2 Hz, 1H), 2.51 (d, J = 1.2 Hz, 3H). LCMS $[M + 1]^+$: 406.1.

(*E*)-But-2-enoic Acid 2-Benzoyl-3-phenyl-benzofuran-4-yl Ester 7a (Scheme 3). To a solution of 1-methoxy-3-methoxymethoxy-benzene[22] (**17**, 0.20 g, 1.19 mmol) in dry THF (10 mL) at 0 °C was added *n*BuLi (0.96 mL, 1.6 mol in hexane) dropwise. After addition was completed, the mixture was stirred at same temperature for 10 min. Benzoyl chloride (0.17 mL, 1.45 mmol) was then added dropwise to the solution. The mixture was stirred for 2 h at room temperature. The reaction was quenched by addition of water, extracted with EtOAc, dried over $MgSO_4$, filtered, and concentrated. The residue was diluted with MeOH (15 mL), and 2 drops of 13N HCl were added and then heated at reflux temperature for 0.5 h. Then the solvent was removed under reduced pressure, and water was added to the residue and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure and the residue obtained was purified by column chromatography on silica gel (hexane/EtOAc: 10/1) to give (2-hydroxy-6-methoxy-phenyl)-phenyl-methanone (**18a**, 0.20 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.72 (s, 1H), 7.61−7.59 (m, 2H), 7.51−7.46 (m, 1H), 7.41−7.35 (m, 3H), 6.65 (dd, J = 0.8, 8.4 Hz, 1H), 6.40 (d, J = 8.4 Hz, 1H), 3.49 (s, 3H). HRMS (FAB) calcd for $C_{14}H_{12}O_3$ 228.0786, found 228.0782.

A mixture of **18a** (0.30 g, 1.31 mmol), potassium carbonate (0.40 g, 2.89 mmol), 2-bromoacetophenone (0.31 g, 1.57 mmol), and 20 mL of acetonitrile was heated at reflux temperature for 16 h. The solution was cooled to room temperature, acetonitrile removed under reduced pressure, water added to the residue, and extracted with EtOAc (20 mL × 3). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the residue by column chromatography

on silica gel (hexane/EtOAc: 10/1) furnished (4-methoxy-3-phenyl-1-benzofuran-2-yl)(phenyl)methanone (**19**, 0.31 g, 72%) as a yellow viscous liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79−7.56 (m, 2H), 7.47−7.39 (m, 4H), 7.31−7.23 (m, 6H), 6.70 (d, J = 10.4 Hz, 1H), 3.74 (s, 3H). HRMS calcd for $C_{22}H_{16}O_3$ 328.1099, found 328.1097.

To a solution of **19** (0.25 g, 0.75 mmol) in dry dichloromethane (15 mL) at 0 °C was added BBr_3 (2.25 mL, 1.0 mol in dichloromethane) dropwise. After addition was completed, the mixture was then brought to room temperature and stirred for 12 h. The reaction was quenched by addition of water, extracted with dichloromethane, dried over $MgSO_4$, filtered, and concentrated. Purification of the residue by column chromatography on silica gel (hexane/EtOAc: 5/1) furnished (4-hydroxy-3-phenyl-1-benzofuran-2-yl)(phenyl)methanone (**20**, 0.19 g, 80%) as a yellow viscous liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87−7.85 (m, 2H), 7.51−7.31 (m, 9H), 7.19 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 7.8 Hz, 1H), 5.31 (br, 1H). HRMS calcd for $C_{21}H_{14}O_3$ 314.0943, found 314.0943.

To a solution of **20** (0.05 g, 0.16 mmol) in dry pyridine (5 mL) was added crotonic anhydride (0.03 g, 0.20 mmol) and imidazole (2 mg, 0.03 mmol). The mixture was stirred at room temperature for 2 h. The reaction was quenched by addition of water, extracted with EtOAc, dried over $MgSO_4$, filtered, and concentrated. Purification of the residue by column chromatography on silica gel, (hexane/EtOAc: 8/1) furnished **7a** (0.05 g, 81% yield) as a yellow viscous liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88−7.86 (m, 2H), 7.56−7.46 (m, 3H), 7.38−7.33 (m, 5H), 7.28−7.24 (m, 2H), 7.07 (dd, J = 1.6, 7.2 Hz, 1H), 6.63 (dq, J = 14.0, 7.0 Hz, 1H), 5.48 (qd, J = 1.8, 14.0 Hz, 1H), 1.74 (dd, J = 1.8, 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.2, 164.1, 155.3, 147.6, 146.7, 145.6, 136.9, 132.7, 130.9, 130.1, 130.0, 129.7, 128.3, 128.0, 127.7, 127.5, 121.2, 117.2, 110.2, 17.9. HRMS calcd for $C_{25}H_{18}O_4$ 382.1205, found 382.1196. HPLC purity: 94.6%.

Phenyl-(3-phenyl-benzofuran-2-yl)-methanone 7b (Scheme 3). To a solution of (2-hydroxy-phenyl)-phenyl-methanone (**18b**) (0.30 g, 1.51 mmol) in CH_3CN (20 mL) was added K_2CO_3 (0.52 g, 3.80 mmol) and 2-bromo acetophenone (0.36 g, 1.81 mmol) and the mixture refluxed for 16 h. The solvents were removed under reduced pressure and the residue dissolved in EtOAc and washed with water. The organic layer was dried over $MgSO_4$, concentrated, and the residue purified by column chromatography on silica gel (hexane/EtOAc: 20/1) to give **7b** (0.42 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87−7.85 (m, 2H), 7.38−7.31 (m, 6H), 7.71−7.69 (m, 1H), 7.65−7.62 (m, 1H), 7.54−7.44 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.6, 154.5, 147.0, 137.1, 132.6, 130.8, 129.9, 129.7, 129.3, 128.24, 128.20, 128.1, 128.0, 123.8, 122.3, 112.3. HRMS (M^+): calcd for $C_{21}H_{14}O_2$ 298.0994, found 298.0976.

Phenyl-(1-phenyl-naphtho[2,1-*b*]furan-2-yl)-methanone 7c (Scheme 4). To a solution of 2-naphthanol (**21**) (0.50 g, 3.47 mmol) in dry THF at 0 °C was added NaH (0.18 g, 4.40 mmol) and MeI (0.26 mL, 4.20 mmol). The mixture was stirred at room temperature for 12 h. The reaction was quenched by addition of water, extracted with EtOAc, dried over $MgSO_4$, filtered, and concentrated to give 2-methoxy-naphthalene (**22**). This residue was diluted with dry CH_2Cl_2 (50 mL) and added $AlCl_3$ (1.38 g, 10.40 mmol) and benzoyl chloride (0.5 mL, 4.26 mmol). After stirring for 16 h, the reaction was quenched by addition of water, extracted with CH_2Cl_2, dried over $MgSO_4$, filtered, and concentrated to give (2-hydroxy-naphthalen-1-yl)-phenyl-methanone (**23**). This residue was diluted with CH_3CN (50 mL) and added K_2CO_3 (0.97 g, 7.00 mmol) and 2-bromo acetophenone (0.72 g, 3.60 mmol). The reaction mixture was refluxed for 16 h and then the solvents were removed under reduced pressure. The residue obtained was dissolved in EtOAc and washed with water. The organic layer was dried over $MgSO_4$, concentrated, and the residue purified by column chromatography on silica gel (hexane/EtOAc: 30/1) to give **7c** (0.10 g, 8%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.95−7.91 (m, 4H), 7.74 (dd, J = 1.2, 9.0 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.50−7.44 (m, 7H), 7.39−7.32 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 184.8, 153.0, 147.6, 137.4, 132.8, 132.4, 131.5, 131.0, 130.3, 129.8, 129.7, 129.2, 128.7, 128.5, 128.3, 128.0, 127.0, 125.2, 123.2, 122.0, 112.7. HRMS (M^+): calcd for $C_{25}H_{16}O_2$ 348.3933, found 348.3947.

8-Benzoyl-9-phenyl-furo[2,3-*h*]chromen-2-one 7d. Method A. Yield 35% over three steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78−7.74 (m, 3H), 7.58−7.41 (m, 5H), 7.32−7.26 (m, 5H), 6.35 (d, J = 9.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.4, 159.4, 156.6, 150.5, 148.2, 144.0, 136.6, 132.9, 130.6, 129.7, 129.5, 128.8, 128.4, 128.1, 127.8, 127.5, 116.4, 114.9, 114.3, 109.4. HRMS (M^+): calcd for $C_{24}H_{14}O_4$ 366.0892, found 366.0876.

8-Benzoyl-3-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 7e. Method A. Yield 40% over three steps. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76−7.75 (m, 2H), 7.57 (d, J = 1.1 Hz, 1H), 7.51−7.41 (m, 5H), 7.31−7.26 (m, 5H), 2.15 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 185.5, 160.9, 155.8, 149.1, 147.9, 139.7, 136.6, 132.8, 130.6, 129.6, 128.7, 128.4, 128.0, 127.7, 126.8, 124.1, 116.1, 114.9, 109.1, 17.7. HRMS (M^+): calcd for $C_{25}H_{16}O_4$ 380.1049, found 380.1039.

8-Benzoyl-5-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 7f. Method A. Yield 41% over three steps. ^{1}H NMR (400 MHz, $CDCl_3$): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 10.0 Hz, 1H), 7.77−7.75 (m, 2H), 7.74−7.38 (m, 4H), 7.32−7.26 (m, 5H), 6.38 (d, J = 10.0 Hz, 1H), 2.66 (s, 3H). LCMS $[M + 1]^+$: 381.7 HPLC purity: 94.2%.

8-Benzoyl-4-ethyl-9-phenyl-furo[2,3-*h*]chromen-2-one 7g. Method A. Yield 28% over three steps. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76−7.73 (m, 3H), 7.54 (d, J = 8.9 Hz, 1H), 7.45−7.42 (m, 3H), 7.30−7.27 (m, 5H), 6.23 (q, J = 2.0 Hz, 1H), 2.85 (qd, J = 7.4, 2.0 Hz, 2H), 1.33 (t, J = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 185.5, 159.8, 157.8, 156.3, 150.1, 148.1, 136.6, 132.8, 130.6, 129.6, 128.7, 128.0, 127.7, 123.8, 116.5, 114.6, 111.5, 108.9, 25.4, 12.2. HRMS (M^+): calcd for $C_{26}H_{18}O_4$ 394.1205, found 394.1205.

8-Benzoyl-9-phenyl-4-propyl-furo[2,3-*h*]chromen-2-one 7h. Method A. Yield 38% over three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77−7.72 (m, 3H), 7.53−7.41 (m, 4H), 7.31−7.31 (m, 5H), 6.20 (s, 1H), 2.76 (t, J = 7.6 Hz, 2H), 1.76−1.71 (m, 2H), 1.05 (t, J = 7.6 Hz, 3H). LCMS $[M + 1]^+$: 409.1. HPLC purity: 94.2%.

8-Benzoyl-4,9-diphenyl-furo[2,3-*h*]chromen-2-one 7i. Method A. Yield 18% over three steps. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77−7.75 (m, 2H), 7.58 (d, J = 9.0 Hz, 1H), 7.53−7.52 (m, 3H), 7.50−7.47 (m, 3H), 7.50−7.42 (m, 3H), 7.33−7.32 (m, 3H), 7.29−7.27 (m, 2H), 6.31 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.5, 159.4, 156.6, 156.3, 150.7, 148.2, 136.6, 135.7, 132.9, 130.7, 129.8, 129.7, 128.9, 128.8, 128.3, 128.1, 127.8, 126.7, 116.6, 114.5, 114.2, 113.6, 108.9. HRMS (M^+): calcd for $C_{30}H_{18}O_4$ 442.1205, found 442.1206.

8-Benzoyl-3,4-dimethyl-9-phenyl-furo[2,3-*h*]chromen-2-one 7j. Method A. Yield 38% over three steps. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76−7.72 (m, 2H), 7.73 (d, J = 9.0 Hz, 1H), 7.54 (d, J = 9.0 Hz, 1H), 7.46−7.41 (m, 3H), 7.30−7.26 (m, 5H), 2.46 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 185.6, 160.7, 155.7, 148.1, 148.0, 146.3, 136.7, 132.8, 130.7, 129.8, 129.7, 128.8, 128.7, 128.0, 127.7, 124.2, 120.8, 116.1, 115.9, 108.7, 15.9, 13.4. HRMS (M^+): calcd for $C_{26}H_{18}O_4$ 394.1205, found 394.1194.

16-Benzoyl-15-phenyl-1,2,3,4-tetrahydro-7,17-dioxa-cyclopenta[*a*]phenanthren-6-one 7k. Method A. Yield 25% over three steps. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76 (dd, J = 1.2, 8.4 Hz, 2H), 7.69 (d, J = 8.9 Hz, 1H), 7.53 (d, J = 8.9 Hz, 1H), 7.46−7.41 (m, 3H), 7.30−7.26 (m, 5H), 2.85−2.83 (m, 2H), 2.54−2.52 (m, 2H), 1.88−1.86 (m, 2H), 1.79−1.78 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 185.6, 160.4, 155.7, 147.9, 147.3, 136.7, 132.8, 130.1, 129.9, 129.8, 129.7, 128.8, 128.7, 128.0, 127.7, 123.1, 122.4, 116.2, 115.5, 108.6, 25.9, 24.0, 21.5. HRMS (M^+): calcd for $C_{28}H_{20}O_4$ 420.1263, found 420.1265. HPLC purity: 90.1%.

8-Benzoyl-4-methyl-9-phenyl-2*H*-thieno[2,3-*h*]chromen-2-one 7l (Scheme 5). A mixture of **13a** (0.10 g, 0.35 mmol), dimethylthiocarbamoyl chloride (0.09 g, 0.71 mmol), sodium hydride (0.03 g, 0.73 mmol, 60% in mineral oil), 4-dimethylaminopyridine (2 mg, 0.12 mmol), and dry THF (10 mL) was stirred at room temperature for 24 h. The reaction was quenched with water and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure, and the residue purified by silica gel column chromatography (hexane/EtOAc: 2/1) to give dimethyl-thiocarbamic acid *O*-(8-benzoyl-4-methyl-2-oxo-2*H*-chromen-7-yl) ester (**24**, 0.11 g, 84%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85−7.83 (m, 2H), 7.64 (d, J = 8.8 Hz, 1H), 7.54−7.50 (m, 1H), 7.40−7.36 (m, 2H), 7.22 (d, J = 2.0 Hz, 1H), 6.20 (q, J = 1.2 Hz, 1H), 3.18 (s, 3H), 2.96 (s, 3H), 2.42 (d, J = 1.2 Hz, 3H). HRMS calcd for $C_{20}H_{17}NO_4S$ 367.0878, found 367.0805.

In a sealed tube, **24** (0.11 g, 0.29 mmol) and mesitylene (12 mL) were heated at 280 °C for 2 h. The solvent was evaporated and the residue chromatographed on silica gel (hexane/EtOAc: 1/1) to afford dimethyl-thiocarbamic acid *S*-(8-benzoyl-4-methyl-2-oxo-2*H*-chromen-7-yl) ester (**25**, 0.07 g, 65%) as a light-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79−7.77 (m, 2H), 7.69 (d, J = 8.4 Hz, 1H), 7.57−7.53 (m, 2H), 7.42−7.38 (m, 2H), 6.28 (q, J = 1.2 Hz, 1H), 2.83 (br, 6H), 2.45 (d, J = 1.2 Hz, 3H). HRMS calcd for $C_{20}H_{17}NO_4S$ 367.0878, found 367.0874.

To a solution of **25** (0.14 g, 0.39 mmol) in dry MeOH (20 mL) was added sodium methoxide (0.03 g, 0.57 mmol). The mixture was heated at reflux temperature for 1 h and then cooled to room temperature and the solvent removed under reduced pressure to give 8-benzoyl-7-mercapto-4-methyl-chromen-2-one (**26**). This was dissolved in acetonitrile (20 mL) and added potassium carbonate (0.06 g, 0.40 mmol) and 2-bromoacetophenone (0.15 g, 0.75 mmol) and refluxed for 16 h. The reaction mixture was cooled to room temperature, removed solvent under reduced pressure, water added to the residue, and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure, and the residue purified by silica gel column chromatography (hexane/EtOAc: 4/1) to give **7l**. (0.06 g, 40% yield over 2 steps) as a light-yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78 (d, J = 8.6 Hz, 1H), 7.65 (d, J = 8.6 Hz, 1H), 7.58 (dd, J = 1.0, 7.2 Hz, 2H), 7.34−7.29 (m, 3H), 7.16−7.19 (m, 5H), 6.21 (q, J = 0.8 Hz, 1H), 2.47 (d, J = 0.8 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 191.4, 159.0, 152.6, 151.1, 144.1, 140.4, 139.3, 137.0, 134.2, 132.7, 130.6, 129.5, 128.3, 127.9, 127.4, 126.7, 112.4, 118.5, 116.1, 114.2, 19.5. HRMS calcd for $C_{25}H_{16}O_3S$ 396.0820, found 396.0815.

8-(Hydroxy-phenyl-methyl)-4-methyl-9-phenyl-furo[2,3-*h*]-chromen-2-one 8a (Scheme 6). To a solution of **6f** (0.05 g, 0.13 mmol) in THF (5 mL) cooled to 0 °C, $NaBH_4$ (6 mg, 0.16 mmol) was added, and the reaction mixture was stirred for 16 h. Water was added and extracted with EtOAc (20 mL × 3), and the combined organic layers washed with brine, dried over $MgSO_4$, and concentrated to give a crude product, which was purified over silica gel column chromatography (hexane/EtOAc: 2/1) to give compound **8a** (0.03 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58−7.54 (m, 2H), 7.52−7.44 (m, 6H), 7.42−7.28 (m, 4H), 6.18 (d, J = 1.2 Hz, 1H), 5.96 (d, J = 5.4 Hz, 1H), 2.76 (d, J = 5.4 Hz, 1H), 2.44 (d, J = 1.2 Hz, 3H). LCMS $[M + 1]^+$: 383.0.

8-Benzyl-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8b (Scheme 6). To a solution of **6f** (0.06 g, 0.15 mmol) in CH_2Cl_2 cooled to 0 °C, TFA (0.56 mL, 0.73 mmol) was slowly added with stirring for 5 min. Then $NaBH_4$ (0.03 g, 0.73 mmol) was slowly added to the reaction mixture and stirred at 0 °C for 16 h. Water was added, extracted with EtOAc (20 mL × 3), and the combined organic layers washed with brine, dried over $MgSO_4$, and concentrated to give a crude product, which was purified over silica gel column chromatography (hexane/EtOAc: 2/1) to give compound **8b** (0.04 g, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57−7.23 (m, 12H), 6.19 (d, J = 0.9 Hz, 1H), 4.15 (s, 2H), 2.25 (d, J = 0.9 Hz, 3H). LCMS $[M + 1]^+$: 367.1.

4-Methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8c (Scheme 2). A solution of 7-hydroxy-4-methylcoumarin **11a** (2.00 g, 11.30 mmol), 2-bromo acetophenone (2.93 g, 14.70 mmol), and K_2CO_3 (2.34 g, 16.90 mmol) in CH_3CN (20 mL) was refluxed for 16 h. The reaction mixture was filtered, the solvents from the filtrate were removed under vacuum, and the crude product **16a** (4-methyl-7-(2-oxo-2-phenyl-ethoxy)-chromen-2-one) obtained was used as such for the next step without purification.

A mixture of **16a** (0.10 g, 0.34 mmol) and phosphoric acid (5 mL) in xylene (20 mL) was heated at 140 °C for 16 h. The reaction mixture was cooled to ambient temperature, poured into crushed ice, and extracted with EtOAc (20 mL × 3). The organic layer was pooled, then dried over $MgSO_4$ and evaporated to give crude product, which was purified over silica gel column chromatography (hexane/EtOAc: 1/4) to give compound **8c** in 18% yield for two steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74−7.70 (m, 3H), 7.57 (d, J = 9.0 Hz, 1H), 7.52−7.39 (m, 4H), 6.26 (d, J = 1.2 Hz, 1H), 2.50 (d, J = 0.9 Hz, 3H). LCMS $[M + 1]^+$: 277.1.

4-Methyl-8,9-diphenyl-furo[2,3-*h*]chromen-2-one 8d (Scheme 2). Compound **8d** was prepared in a manner similar to **8c**, except that 7-hydroxy-4-methylcoumarin **11a** and 2-bromo-2-phenyl-acetophenone were used as the staring materials to give **8d** (21% yield for two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65−7.61 (m, 3H), 7.56−7.45 (m, 6H), 7.39−7.29 (m, 3H), 6.25 (d, J = 0.9 Hz, 1H), 2.44 (d, J = 1.2 Hz, 3H). LCMS $[M + 1]^+$: 353.1.

8-Acetyl-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8e. Method A. Yield 38% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73−7.51 (m, 7H), 6.21 (s, 1H), 2.48 (s, 3H), 2.34 (d, J = 0.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 188.7, 159.3, 155.9, 152.7, 150.1, 148.2, 130.3, 130.0, 129.2, 128.4, 128.2, 127.9, 124.6, 117.0, 115.2, 113.5, 108.8, 28.5, 19.4. LCMS $[M + 1]^+$: 319.1. HPLC purity: 91.5%.

8-(Furan-2-carbonyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8f. Method A. Yield 35% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (d, J = 8.7 Hz, 1H), 7.62−7.56 (m, 4H), 7.50−7.44 (m, 4H), 6.55 (dd, J = 1.8, 1.8 Hz, 1H), 6.25 (d, J = 1.2 Hz, 1H), 2.50 (d, J = 1.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2, 170.70, 159.4, 156.2, 152.8, 151.0, 149.9, 147.6, 147.1, 130.4, 129.5, 128.8, 127.8, 124.3, 121.2, 116.5, 115.3, 113.6, 112.4, 108.8, 19.6. LCMS $[M + 1]^+$: 371.0. HPLC purity: 90.9%.

4-Methyl-9-phenyl-8-(thiophene-2-carbonyl)-furo[2,3-*h*]chromen-2-one 8g. Method A. Yield 44% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11−8.10 (m, 1H), 7.76−7.71 (m, 2H), 7.63−7.60 (m, 3H), 7.58−7.26 (m, 3H), 7.16−7.14 (m, 1H), 6.25 (d, J = 1.2 Hz, 1H), 2.50 (d, J = 1.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.2, 161.69, 160.37, 155.09, 149.83, 149.60, 142.78, 135.99, 135.26, 134.98, 134.85, 129.59, 129.17, 128.66, 128.55, 126.48, 126.02, 113.56, 113.46, 113.01, 108.77, 107.84, 25.46. HRMS (M^+): calcd for $C_{23}H_{14}O_4S$ 386.0613, found 386.0609.

4-Methyl-9-phenyl-8-(thiophene-3-carbonyl)-furo[2,3-*h*]chromen-2-one 8h. Method A. Yield 39% for three steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30−8.29 (m, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.63−7.62 (m, 1H), 7.58−7.55 (m, 3H), 7.44−7.42 (m, 3H), 7.27−7.25 (m, 1H), 6.23 (d, J = 1.2 Hz, 1H), 2.48 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.3, 159.4, 156.0, 153.0, 152.8, 149.8, 148.2, 140.3, 134.8, 133.3, 130.4, 129.7, 129.2, 128.8, 128.5, 128.2, 127.9, 127.8, 127.2, 126.8, 126.6, 126.5, 126.4, 125.6, 124.2, 116.5, 115.3, 113.5, 113.1, 108.8, 19.5. LCMS $[M + 1]^+$: 387.0. HPLC purity: 90.5%.

4-Methyl-9-phenyl-8-(pyridine-2-carbonyl)-furo[2,3-*h*]chromen-2-one 8i. Method A. Yield 31% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25−8.24 (m, 1H), 7.80−7.77 (m, 1H), 7.74−7.67 (m, 2H), 7.58 (d, J = 9.0 Hz, 1H), 7.42−7.38 (m, 2H), 7.25−7.20 (m, 4H), 6.24 (d, J = 1.2 Hz, 1H), 2.49 (d, J = 1.2 Hz, 3H). LCMS $[M + 1]^+$: 382.1.

4-Methyl-8-(4-methyl-benzoyl)-9-phenyl-furo[2,3-*h*]chromen-2-one 8j. Method A. Yield 25% for three steps. ^{1}H NMR

(300 MHz, $CDCl_3$): δ 7.73−7.69 (m, 3H), 7.55 (d, J = 8.7 Hz, 1H), 7.51−7.47 (m, 2H), 7.36−7.32 (m, 3H), 7.11 (d, J = 8.1 Hz, 2H), 6.24 (d, J = 0.9 Hz, 1H), 2.49 (d, J = 0.6 Hz, 3H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.1, 159.5, 156.3, 152.8, 149.9, 148.3, 143.9, 133.9, 130.6, 129.9, 129.7, 128.8, 128.7, 128.6, 128.2, 127.7, 124.0, 116.3, 115.3, 113.5, 108.9, 21.6, 19.5. LCMS $[M + 1]^+$: 395.1. HPLC purity: 93.5%.

8-(4-Methoxy-benzoyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8k. Method A. Yield 41% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (dd, J = 10.2, 2.4 Hz, 2H), 7.70 (dd, J = 9.0, 1.5 Hz, 1H), 7.56−7.47 (m, 3H), 7.37−7.32 (m, 3H), 6.79 (dd, J = 8.7, 2.7 Hz, 2H), 6.23 (d, J = 0.9 Hz, 1H), 3.81 (s, 3H), 2.48 (d, J = 0.9 Hz, 3H). LCMS $[M + 1]^+$: 411.1.

4-Methyl-8-(4-nitro-benzoyl)-9-phenyl-furo[2,3-*h*]chromen-2-one 8l. Method A. Yield 15% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14−8.09 (m, 2H), 7.89−7.85 (m, 2H), 7.79 (d, J = 9.0 Hz, 1H), 7.59 (d, J = 8.7 Hz, 1H), 7.46−7.43 (m, 2H), 7.36−7.30 (m, 3H), 6.27 (d, J = 0.9 Hz, 1H), 2.52 (d, J = 1.2 Hz, 3H). LCMS $[M + 1]^+$: 426.1.

8-(4-Chloro-benzoyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8m. Method A. Yield 41% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76−7.71 (m, 3H), 7.57 (d, J = 8.7 Hz, 1H), 7.48−7.44 (m, 2H), 7.38−7.32 (m, 3H), 7.29−7.24 (m, 2H), 6.25 (d, J = 1.2 Hz, 1H), 2.50 (d, J = 1.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.1, 159.4, 156.4, 152.8, 150.0, 147.8, 139.2, 134.9, 131.0, 130.6, 129.4, 129.1, 128.9, 128.4, 127.9, 124.4, 116.3, 115.4, 113.6, 108.9, 19.5. LCMS $[M + 1]^+$: 415.1.

4-Methyl-8-(3-methyl-benzoyl)-9-phenyl-furo[2,3-*h*]chromen-2-one 8n. Method A. Yield 28% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75−7.18 (m, 11H), 6.26 (s, 1H), 2.51 (d, J = 0.6 Hz, 3H), 2.25 (s, 3H). LCMS $[M + 1]^+$: 395.2. HPLC purity: 91.9%.

4-Methyl-8-(2-methyl-benzoyl)-9-phenyl-furo[2,3-*h*]chromen-2-one 8o. Method A. Yield 25% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75−6.93 (m, 11H), 6.24 (d, J = 1.2 Hz, 1H), 2.42 (s, 3H), 2.18 (s, 3H). LCMS $[M + 1]^+$: 396.5. HPLC purity: 90.1%.

8-(3-Methoxy-benzoyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8p. Method A. Yield 30% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 8.7 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H), 7.50−7.47 (m, 2H), 7.40−7.28 (m, 5H), 7.20 (t, J = 8.7 Hz, 1H), 7.01−6.97 (m, 1H), 6.24 (d, J = 1.2 Hz, 1H), 3.76 (s, 3H), 2.49 (d, J = 1.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.2, 159.5, 159.2, 156.4, 152.8, 149.9, 148.1, 137.8, 130.6, 129.7, 129.1, 128.8, 127.7, 124.2, 122.5, 119.7, 116.3, 115.3, 113.7, 113.5, 108.9, 55.3, 19.5. HRMS (M^+): calcd for $C_{26}H_{18}O_5$ 410.1154, found 410.1142.

8-(2-Methoxy-benzoyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8q. Method A. Yield 42% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70 (d, J = 9.0 Hz, 1H), 7.55 (d, J = 8.7 Hz, 1H), 7.37 (dd, J = 7.2 Hz, 1.8 Hz, 1H), 7.33−7.29 (m, 2H), 7.26−7.17 (m, 4H), 6.86 (td, J = 7.4 Hz, 0.7 Hz, 1H), 6.52 (d, J = 8.4 Hz, 1H), 6.21 (d, J = 1.2 Hz, 1H), 3.57 (s, 3H), 2.48 (d, J = 1.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.5, 159.5, 157.3, 156.4, 152.9, 150.1, 149.1, 132.8, 130.3, 130.0, 129.3, 128.5, 128.4, 127.9, 127.1, 124.2, 120.2, 116.6, 115.1, 113.3, 110.5, 109.0, 55.2, 19.5. LCMS $[M + 1]^+$: 411.1.

4-Methyl-8-(3-nitro-benzoyl)-9-phenyl-furo[2,3-*h*]chromen-2-one 8r. Method A. Yield 41% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (t, J = 2.0 Hz, 1H), 8.25 (dd, J = 8.1, 1.2 Hz, 1H), 8.08 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 8.7 Hz, 1H), 7.62 (d, J = 9.0 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 7.465−7.42 (m, 2H), 7.31−7.26 (m, 3H), 6.27 (s, 1H), 2.52 (d, J = 0.6 Hz, 3H). LCMS $[M + 1]^+$: 426.0.

8-(3-Chloro-benzoyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8s. Method A. Yield 30% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64−7.37 (m, 11H), 6.23 (d, J = 1.2 Hz, 1H), 2.50 (d, J = 1.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.0, 159.3, 156.5, 152.8, 150.0, 147.6, 138.1, 134.2, 132.7, 130.5, 129.6, 129.5, 129.4, 129.0, 127.8, 127.6, 124.6, 116.3, 115.4, 113.6, 109.0, 19.5. LCMS $[M + 1]^+$: 415.0.

8-(3-fluoro-benzoyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8t. Method A. Yield 35% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (d, J = 8.7 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H), 7.56−7.11 (m, 9H), 6.26 (d, J = 1.2 Hz, 1H), 2.51 (d, J = 1.2 Hz, 3H). LCMS $[M + 1]^+$: 399.0. HPLC purity: 94.0%.

8-(3-Hydroxy-benzoyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 8u (Scheme 7). To a solution of **8p** (1.0 equiv) in DCM (5 mL) BBr_3 (2.0 equiv) was added and stirred at room temperature for 2 h. Ice water was added to the reaction mixture and extracted with EtOAc (20 mL × 3). The combined organic layer was dried over $MgSO_4$ and evaporated to give crude product, which was purified over silica gel column chromatography (hexane/EtOAc: 1/3 → 1/1) to give compound **8u** in 92% yield ^{1}H NMR (400 MHz, $CDCl_3$): δ 7. 77 (d, J = 8.4 Hz, 1H), 7.58 (dd, J = 8.4, 0.4 Hz, 1H), 7.48−7.46 (m, 2H), 7.34 (t, J = 2.4 Hz, 4H), 7.24−7.23 (m, 1H), 7.16 (t, J = 8 Hz, 1H), 6.94 (ddd, J = 8.0, 2.4, 0.8 Hz, 1H), 6.26 (s, 1H), 2.51 (s, 3H). LCMS $[M + 1]^+$: 397.1. HPLC purity: 91.1%.

General Method for the Alkylation of 8u with Alkylbromide (R^1Br) to Give 9a−f (Method B; Scheme 7). A solution of **8u** (1.0 equiv), R^1Br (1.5 equiv), K_2CO_3 (3.0 equiv), and KI (0.2 equiv) in CH_3CN (5 mL) was refluxed for 16 h. Solvent was removed under vacuum, and water was added and extracted with EtOAc (20 mL × 3). The combined organic layer was dried over $MgSO_4$, concentrated under vacuum to give crude product, which was purified over silica gel column (hexane/EtOAc: 3/1 → 1/1) to give compounds **9a−f**.

8-(3-Ethoxy-benzoyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 9a. Method B. Yield 38%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 8.7 Hz, 1H), 7.78 (d, J = 9.0 Hz, 1H), 7.50−7.46 (m, 2H), 7.37−7.27 (m, 5H), 7.18 (dd, J = 8.4, 8.4 Hz, 1H), 7.00−6.96 (m, 1H), 6.25 (d, J = 0.9 Hz, 1H), 3.98 (q, J = 6.9 Hz, 2H), 2.50 (d, J = 1.2 Hz, 3H), 1.40 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.3, 159.5, 158.6, 156.4, 152.9, 149.9, 148.1, 137.7, 130.6, 129.7, 129.1, 128.8. 128.7, 127.7, 124.2, 122.4, 120.1, 116.3, 115.3, 114.3, 113.5, 109.0, 63.6, 19.6, 14.7. LCMS $[M + 1]^+$: 425.1.

4-Methyl-9-phenyl-8-(3-propoxy-benzoyl)-furo[2,3-h]chromen-2-one 9b. Method B. Yield 39%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 8.7 Hz, 1H), 7.77 (d, J = 8.7 Hz, 1H), 7.50−7.46 (m, 2H), 7.37−7.26 (m, 5H), 7.21−7.15 (m, 1H), 7.00−6.96 (m, 1H), 6.26 (d, J = 1.2 Hz, 1H), 3.86 (d, J = 6.6 Hz, 2H), 2.50 (d, J = 1.2 Hz, 3H), 1.81−1.72 (m, 2H), 1.02 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.4, 159.5, 158.8, 156.4, 152.9, 149.9, 148.1, 137.7, 132.3, 130.6, 130.1, 129.7, 129.2, 129.1, 128.7, 128.6, 128.2, 127.9, 127.7, 124.2, 122.3, 120.1, 120.0, 116.3, 115.3, 114.5, 114.4, 113.502, 109.0, 69.6, 22.4, 19.6, 10.5. LCMS $[M + 1]^+$: 439.1.

8-(3-Butoxy-benzoyl)-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one 9c. Method B. Yield 38%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 8.7 Hz, 1H), 7.49−7.46 (m, 2H), 7.37−7.26 (m, 5H), 7.18 (dd, J = 7.8, 7.8 Hz, 1H), 7.00−6.96 (m, 1H), 6.25 (d, J = 1.2 Hz, 1H), 3.90 (t, J = 6.9 Hz, 2H), 2.60 (d, J = 1.2 Hz, 3H), 1.78−1.70 (m, 2H), 1.51−1.43 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.4, 159.5, 158.8, 156.4, 152.9, 149.9, 148.1, 137.7, 130.6, 129.7, 129.1, 128.7, 128.6, 127.7, 124.2, 122.3, 120.1, 116.3, 115.3, 114.4, 113.5, 109.0, 67.8, 31.1, 19.6, 19.2, 13.8. LCMS $[M + 1]^+$: 453.2.

4-Methyl-8-(3-pentyloxy-benzoyl)-9-phenyl-furo[2,3-*h*]chromen-2-one 9d. Method B. Yield 38%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 9.0 Hz, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.49−7.46 (m, 2H), 7.37−7.26 (m, 5H), 7.18 (dd, J = 7.8, 7.8 Hz, 1H), 7.00−6.96 (m, 1H), 6.25 (d, J = 0.9 Hz, 1H), 3.89 (t, J = 6.6 Hz, 2H), 2.50 (s, 3H), 1.78−1.73 (m, 2H), 1.44−1.36 (m, 4H), 0.93 (d, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.4, 159.5, 158.8, 156.4, 152.9, 149.9, 148.1, 137.7, 130.6, 129.6, 129.0, 128.7, 128.6, 127.7, 124.2, 122.3, 120.1, 116.3, 115.3, 114.3, 113.5, 109.0, 68.1, 28.8, 28.1, 22.4, 19.6, 14.0. LCMS $[M + 1]^+$: 467.2.

4-Methyl-8-(3-octyloxy-benzoyl)-9-phenyl-furo[2,3-*h*]chromen-2-one 9e. Method B. Yield 37%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72 (d, J = 8.7 Hz, 1H), 7.56 (d, J = 8.7 Hz, 1H), 7.50−7.27

(m, 7H), 7.18 (t, J = 8.1 Hz, 1H), 6.98 (ddd, J = 8.1, 2.7, 0.9 Hz, 1H), 6.24 (d, J = 0.9 Hz, 1H), 3.89 (t, J = 6.6 Hz, 2H), 2.49 (d, J = 0.9 Hz, 3H), 1.75 (quintet, J = 6.6 Hz, 2H), 1.46−1.29 (m, 10H), 0.87 (t, J = 6.6 Hz, 3H). LCMS $[M + 1]^+$: 509.2. HPLC purity: 94.8%.

8-[3-(2-Dimethylamino-ethoxy)-benzoyl]-4-methyl-9-phenyl-furo-[2,3-*h*]chromen-2-one 9f. Method B. Yield 25%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, J = 9.0 Hz, 1H), 7.50−7.21 (m, 9H), 7.11 (dd, J = 8.4, 2.1 Hz, 1H), 6.26 (s, 1H), 4.47 (t, J = 4.2 Hz, 2H), 3.43 (t, J = 4.2 Hz, 2H), 2.91 (s, 6H), 2.51 (s, 3H). LCMS $[M + 1]^+$: 468.2. HPLC purity: 91.2%.

8-[3-(2-Hydroxy-ethoxy)-benzoyl]-4-methyl-9-phenyl-furo[2,3-*h*]-chromen-2-one 9g (Scheme 7). Compound **8u** (0.05 g, 0.13 mmol) was reacted with (2-bromo-ethoxy)-*tert*-butyl-dimethyl-silane (0.06 g, 0.25 mmol) (R^2Br) using method B as for **9a**−**f** to give the intermediate 8-{3-[2-(*tert*-butyl-dimethyl-silanyloxy)-ethoxy]-benzoyl}-4-methyl-9-phenyl-furo[2,3-*h*]chromen-2-one (45 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 6.6 Hz, 1H), 7.57 (d, J = 6.6 Hz, 1H), 7.49−6.99 (m, 9H), 6.25 (d, J = 0.9 Hz, 1H), 3.99−3.93 (m, 4H), 2.50 (d, J = 0.9 Hz, 3H), 0.90 (s, 9H), 0.09 (s, 6H). LCMS $[M + 1]^+$: 555.3.

The above obtained product (0.035 g, 0.06 mmol) was dissolved in THF (5 mL), cooled to 0 °C, and TBAF (0.08 g, 0.32 mmol) was added and stirred for 1.5 h. Water was added slowly to quench the reaction and extracted with EtOAc (20 mL × 3). The combined organic layer was dried over $MgSO_4$ and concentrated under vacuum to give crude product, which was purified over silica gel column (hexane/EtOAc: 1/2) to give **9g** (7 mg, 24%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 8.7 Hz, 1H), 7.49−7.00 (m, 9H), 6.26 (s, 1H), 4.04 (t, J = 4.5 Hz, 2H), 3.94 (t, J = 3.9 Hz, 2H), 2.50 (s, 3H). LCMS $[M + 1]^+$: 441.1.

8-[3-(3-Hydroxy-propoxy)-benzoyl]-4-methyl-9-phenyl-furo-[2,3-*h*]chromen-2-one 9h (Scheme 7). Compound **8u** (0.05 g, 0.13 mmol) was reacted with (3-bromo-propoxy)-*tert*-butyl-dimethyl-silane (0.06 g, 0.25 mmol) (R^2Br) using method B as for **9a**−**f** to give the intermediate 8-{3-[3-(*tert*-butyl-dimethyl-silanyloxy)-propoxy]-benzoyl}-4-methyl-9-phenyl-furo[2,3-*h*]-chromen-2-one (52 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (d, J = 9.0 Hz, 1H), 7.58 (d, J = 9.0 Hz, 1H), 7.50−6.97 (m, 9H), 6.26 (d, J = 1.2 Hz, 1H), 4.02 (t, J = 6.3 Hz, 2H), 3.78 (t, J = 6.0 Hz, 2H), 2.51 (d, J = 1.2 Hz, 3H), 1.96 (t, J = 6.0 Hz, 2H), 0.88 (s, 9H), 0.04 (s, 6H). LCMS $[M + 1]^+$: 569.3.

The above obtained product (0.04 g, 0.07 mmol) was dissolved in THF (5 mL), cooled to 0 °C, and TBAF (0.09 g, 0.35 mmol) was added and stirred for 1.5 h. Water was added slowly to quench the reaction and extracted with EtOAc (20 mL × 3). The combined organic layer was dried over $MgSO_4$ and concentrated under vacuum to give crude product, which was purified over silica gel column (hexane/EtOAc: 1/2) to give **9h** (8 mg, 26%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 8.7 Hz, 1H), 7.58 (d, J = 8.7 Hz, 1H), 7.49−6.97 (m, 9H), 6.25 (d, J = 1.2 Hz, 1H), 4.06 (t, J = 6.0 Hz, 2H), 3.85 (t, J = 6.0 Hz, 2H), 2.50 (d, J = 1.2 Hz, 3H), 2.06−1.98 (m, 2H). LCMS $[M + 1]^+$: 455.1.

9-(3-Bromo-phenyl)-8-(3-methoxy-benzoyl)-4-methyl-furo-[2,3-*h*]chromen-2-one 10a. Method A. Yield 28% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 8.7 Hz, 1H), 7.57−7.54 (m, 2H), 7.49−7.43 (m, 2H), 7.39−7.35 (m, 1H), 7.29−7.20 (m, 3H), 7.04−7.00 (m, 1H), 6.24 (d, J = 1.5 Hz, 1H), 3.78 (s, 3H), 2.48 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.9, 159.3, 159.2, 156.2, 152.8, 149.7, 148.2, 137.6, 133.5, 131.7, 131.6, 129.2, 129.1, 127.1, 124.5, 122.2, 121.5, 119.7, 115.9, 115.4, 113.6, 113.5, 108.9, 55.3, 19.5. LCMS $[M + 1]^+$: 489.0.

9-(3-Bromo-phenyl)-4-methyl-8-(thiophene-2-carbonyl)-furo-[2,3-*h*]chromen-2-one 10b. Method A. Yield 30% for three steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (d, J = 8.7 Hz, 1H), 7.56−7.48 (m, 2H), 7.48−7.43 (m, 2H), 7.34−7.17 (m, 3H), 7.01−6.98 (m, 1H), 6.27 (d, J = 0.9 Hz, 1H), 2.50 (d, J = 1.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.0, 159.2, 155.9, 152.6, 149.7, 147.5, 142.6, 135.3, 134.9, 133.3, 131.9, 131.7, 129.3, 129.0, 128.3, 127.2 124.5, 121.7, 116.4, 115.5, 113.8, 108.8, 19.6. LCMS $[M + 1]^+$: 465.0.

Virus Source. Influenza A/WSN/33 were generated using the 12-plasmid-based reverse genetics system described by Fodor et al.[28] Influenza A/TW/141/02 (H1N1), influenza A/TW/3446/02 (H3N2), influenza B/TW/710/05, influenza B/TW/99/07, and influenza B/TW/70325/05 were obtained from the Clinical Virology Laboratory in Chang Gang Children's Hospital (Taipei, Taiwan).

Inhibition of Virus Induced CPE on MDCK Cells (IC_{50} Determination). This assay measured the ability of a test compound to inhibit the cytopathic effect induced by influenza virus on MDCK cells. The 96-well tissue culture plates were seeded with 200 μL of MDCK cells at a concentration of 1.1×10^5 cells/mL in Dulbecco/Vogt modified Eagle's minimal essential medium (DMEM) with 10% fetal bovine serum (FBS). The plates were incubated for 24−30 h at 37 °C and were used at about 90% confluency. Virus (100 $TCID_{50}$) mixed with different concentrations of test compounds was added to the cells and incubated at 37 °C for 1 h. After adsorption, the infected cell plates were overlaid with 50 μL of serum free DMEM (E_0: DMEM with penicillin [100 U/mL], streptomycin [100 μg/mL], L-glutamine [2 mM], nonessential amino acid mixture [0.1 mM], and trypsin [2.5 μg/mL]) and 0.1% DMSO. The plate was incubated at 37 °C for 72 h. At the end of incubation, the plates were fixed by the addition of 100 μL of 4% formaldehyde for 1 h at room temperature. After the removal of formaldehyde, the plates were stained with 0.1% crystal violet for 15 min at room temperature. The plates were washed and dried, and the density of the well was measured at 570 nm. The concentration required for a test compound to reduce the virus-induced cytopathic effect (CPE) by 50% relative to the virus control was expressed as IC_{50}. All assays were performed in triplicate and at least twice.

Cytotoxicity Assay (CC_{50} Determination). The 96-well tissue culture plates were seeded with 200 μL of MDCK cells at a concentration of 1.1×10^5 cells/mL in DMEM with 10% FBS. The plates were incubated for 24−30 h at 37 °C and were used at about 90% confluency. Test compounds at various concentrations were added to MDCK cells. The plate was incubated at 37 °C for 72 h. At the end of incubation, the plates were fixed by the addition of 100 μL of 4% formaldehyde for 1 h at room temperature. After the removal of formaldehyde, the plates were stained with 0.1% crystal violet for 15 min at room temperature. The plates were washed and dried, and the density of the well was measured at 570 nm. The concentration of a test compound required to reduce cell viability to 50% of the tested control culture was expressed as CC_{50}. All experiments were performed in triplicate and at least twice.

Inhibition of Virus Induced CPE on MDCK Cells by 8g (Figure 2). MDCK cells (2.5×10^5 cells/well) were seeded in a 6 cm tissue culture dish, grown for overnight, and then challenged with virus (multiplicity of infection [MOI] = 0.01). After adsorption of virus for 1 h on ice, the cells were washed with Hanks' balanced salt solutions (HBSS), after which E_0 containing **8g** (1 μM) was added. After incubation for 36 h, the CPE was evaluated under a microscope.

Plaque Reduction Assay. MDCK cells (5.5×10^5 cells/well) were seeded into six-well tissue culture plates and incubated overnight. The cells were incubated with influenza virus at approximately 125 PFU/well with or without different concentrations of the compound. After adsorption of the virus for 1 h at 37 °C, the viral suspension was removed, and the cells were washed with HBSS. The cells were then overlain with E_0 containing 0.3% agarose with indicated compound concentration. After incubation for 48 h at 37 °C under 5% CO_2, the cells were fixed with 10% formaldehyde and then stained with 1% crystal violet. The numbers of plaques were counted and the antiviral activity of the compound was calculated with respect to virus control.

Time of Addition Assay. MDCK cells (2.4×10^5 cells/well) were seeded in six-well tissue culture plates and incubated overnight. The cells were challenged with virus (MOI 0.001) on ice for 1 h. After adsorption of the virus, the viral suspension was removed and the cells were washed with HBSS, then replenished with fresh E_0 medium (pi = 0). The test medium containing 1 μM of **8g** was added during the periods −1 to 0 h (adsorption), 0−2, 2−4, 4−6, and 6−8 h. After each incubation period, the monolayer was washed with HBSS and incubated with fresh medium until 12 h postinfection (pi). The supernatant was collected and the viral yield was determined by plaque assay.

Inhibition of Viral RNP Activity Using Reporter Assay. Reporter assay was performed in 293 cells transfected with reporter plasmids and pHW2000 expression vectors for influenza virus PB1, PB2, PA, and NP proteins. In a 48-well dish, approximately 5×10^4 per well of 293 cells were transfected with pPOLI-Fluc plasmid (0.1 μg), pHW2000-PB1 (0.1 μg), -PB2 (0.1 μg), -PA (0.1 μg), -NP (0.1 μg), and pRL-TK (5 ng, Promega) as a transfection control by using calcium phosphate method. Ten-fold serial dilutions of **8g** were added 8 h post-transfection, and the cells were incubated for further 20 h. Cells were then harvested for luciferase assay using Dual-Luciferase assay system (Promega, Madison, WI) and a VICTOR^3 plate reader (PerkinElmer). Values at each dose were analyzed in triplicate and graphed with standard deviations. This is an average result from three experiments and the *y*-axis is the ratio of Fluc to Rluc controls, normalized to mock treatment (set arbitrarily to 1.0).

Evaluation of Viral-like RNA Synthesis Inhibition Using Primer Extension Assay. The production of viral RNAs was measured by plasmid-based reverse genetics system and primer extension assay.[23] One μg of each pHW2000-PB1, PB2, PA, and NP and pPOLI-CAT-RT were cotransfected into 293 cells in a 6-well plate. Compound **8g** was added at 8 h post transfection. Total cellular RNA was isolated (RNease Mini kit, Qiagen) 20 h post transfection and hybridized with [γ-^{32}P] end-labeled primers at 50 °C for 3 h. This primer extension reaction was carried out using avian myeloblastosis virus reverse transcriptase (primer extension system-AMV reverse transcriptase, Promega) at 42 °C for 2 h. The resulting mixtures were terminated by denaturing at 90 °C for 10 min and analyzed with 8% polyacrylamide gel containing 7 M urea. The expected sizes of mRNA, cRNA (complementary RNA), and vRNA were 98−106, 89, and 158 bp, respectively.

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Supporting Information Available: Hemagglutination inhibition assay protocol and results, reporter assay (Fluc) results, immunofluorescence microscopic analysis (dsRNA, NP, and M1 protein) protocol and results upon **8g** treatment, comparative activity of **8g** and **7d** in plaque formation, viral yield reduction, and viral-like RNA synthesis assay. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) von Itzstein, M. The war against influenza: discovery and development of sialidase inhibitors. *Nat. Rev. Drug Discovery* **2007**, *6*, 967–974.
(2) Hsieh, H. P.; Hsu, J. T. Strategies of development of antiviral agents directed against influenza virus replication. *Curr. Pharm. Des.* **2007**, *13*, 3531–3542.
(3) De Clercq, E. Antiviral agents active against influenza A viruses. *Nat. Rev. Drug Discovery* **2006**, *5*, 1015–1025.
(4) Webby, R. J.; Webster, R. G. Are we ready for pandemic influenza? *Science* **2003**, *302*, 1519–1522.
(5) Kilbourne, E. D. Influenza pandemics of the 20th century. *Emerging Infect. Dis.* **2006**, *12*, 9–14.
(6) Yuen, K. Y.; Wong, S. S. Human infection by avian influenza A H5N1. *Hong Kong Med. J.* **2005**, *11*, 189–199.
(7) Buchy, P.; Mardy, S.; Vong, S.; Toyoda, T.; Aubin, J. T.; Miller, M.; Touch, S.; Sovann, L.; Dufourcq, J. B.; Richner, B.; Tu, P. V.; Tien, N. T.; Lim, W.; Peiris, J. S.; Van der Werf, S. Influenza A/H5N1 virus infection in humans in Cambodia. *J. Clin. Virol.* **2007**, *39*, 164–168.
(8) Michaelis, M.; Doerr, H. W.; Cinatl, J., Jr. Of chickens and men: avian influenza in humans. *Curr. Mol. Med.* **2009**, *9*, 131–151.
(9) Fraser, C.; Donnelly, C. A.; Cauchemez, S.; Hanage, W. P.; Van Kerkhove, M. D.; Hollingsworth, T. D.; Griffin, J.; Baggaley, R. F.; Jenkins, H. E.; Lyons, E. J.; Jombart, T.; Hinsley, W. R.; Grassly, N. C.; Balloux, F.; Ghani, A. C.; Ferguson, N. M.; Rambaut, A.; Pybus, O. G.; Lopez-Gatell, H.; Apluche-Aranda, C. M.; Chapela, I. B.; Zavala, E. P.; Guevara, D. M. E.; Checchi, F.; Garcia, E.; Hugonnet, S.; Roth, C.; The WHO Rapid Epidemic Assessment Collaboration. Pandemic Potential of a Strain of Influenza A (H1N1): Early Findings. *Science* **2009**, *324*, 1557–1561.
(10) Carrat, F.; Flahault, A. Influenza vaccine: the challenge of antigenic drift. *Vaccine* **2007**, *25*, 6852–6862.
(11) Treanor, J. Influenza vaccine—outmaneuvering antigenic shift and drift. *N. Engl. J. Med.* **2004**, *350*, 218–220.
(12) Le, Q. M.; Kiso, M.; Someya, K.; Sakai, Y. T.; Nguyen, T. H.; Nguyen, K. H.; Pham, N. D.; Ngyen, H. H.; Yamada, S.; Muramoto, Y.; Horimoto, T.; Takada, A.; Goto, H.; Suzuki, T.; Suzuki, Y.; Kawaoka, Y. Avian flu: isolation of drug-resistant H5N1 virus. *Nature* **2005**, *437*, 1108.
(13) de Jong, M. D.; Tran, T. T.; Truong, H. K.; Vo, M. H.; Smith, G. J.; Nguyen, V. C.; Bach, V. C.; Phan, T. Q.; Do, Q. H.; Guan, Y.; Peiris, J. S.; Tran, T. H.; Farrar, J. Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N. Engl. J. Med.* **2005**, *353*, 2667–2672.
(14) Moscona, A. Global Transmission of Oseltamivir-Resistant Influenza. *N. Engl. J. Med.* **2009**, *360*, 953–956.
(15) Bethea, D.; Fullmer, B.; Syed, S.; Seltzer, G.; Tiano, J.; Rischko, C.; Gillespie, L.; Brown, D.; Gasparro, F. P. Psoralen photobiology and photochemotherapy: 50 years of science and medicine. *J. Dermatol. Sci.* **1999**, *19*, 78–88.
(16) Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H. Anti-AIDS agents. 37. Synthesis and structure−activity relationships of (3′*R*,4′*R*)-(+)-cis-khellactone derivatives as novel potent anti-HIV agents. *J. Med. Chem.* **1999**, *42*, 2662–2672.
(17) Xie, L.; Takeuchi, Y.; Cosentino, L. M.; McPhail, A. T.; Lee, K. H. Anti-AIDS agents. 42. Synthesis and anti-HIV activity of disubstituted (3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone analogues. *J. Med. Chem.* **2001**, *44*, 664–671.
(18) Babiak, P.; Reymond, J. L. A high-throughput, low-volume enzyme assay on solid support. *Anal. Chem.* **2005**, *77*, 373–377.
(19) Geetanjali, Y.; Rajitha, B.; Rao, M. K. Synthesis of 2-aroyl-3-phenylfuranobenzopyrones. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1983**, *22*, 164–165.
(20) Ahluwalia, V. K.; Bhat, K.; Prakash, C.; Bala, S. Synthesis of Linear Pyranocoumarins: Xanthoxyletin, 4-Methyl-xanthyletin, and 4-Phenylxanthyletin. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1070–1072.
(21) Kundu, N. G.; Pal, M.; Mahanty, J. S.; De, M. Palladium-catalysed heteroannulation with acetylenic compounds: synthesis of benzofurans. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2815–2820.
(22) Moon, B.; Han, S.; Yoon, Y.; Kwon, H. Macrocyclic lactam synthesis via a ring expansion reaction: construction of the cripowellin skeleton. *Org. Lett.* **2005**, *7*, 1031–1034.
(23) Pleschka, S.; Jaskunas, R.; Engelhardt, O. G.; Zurcher, T.; Palese, P.; Garcia-Sastre, A. A plasmid-based reverse genetics system for influenza A virus. *J. Virol.* **1996**, *70*, 4188–4192.
(24) Lutz, A.; Dyall, J.; Olivo, P. D.; Pekosz, A. Virus-inducible reporter genes as a tool for detecting and quantifying influenza A virus replication. *J. Virol. Methods* **2005**, *126*, 13–20.
(25) Shih, S. R.; Horng, J. T.; Poon, L. L.; Chen, T. C.; Yeh, J. Y.; Hsieh, H. P.; Tseng, S. N.; Chiang, C.; Li, W. L.; Chao, Y. S.; Hsu, J. T. BPR2-D2 targeting viral ribonucleoprotein complex-associated function inhibits oseltamivir-resistant influenza viruses. *J. Antimicrob. Chemother.* **2010**, *65*, 63–71.
(26) Beigel, J.; Bray, M. Current and future antiviral therapy of severe seasonal and avian influenza. *Antiviral Res.* **2008**, *78*, 91–102.
(27) Furuta, Y.; Takahashi, K.; Kuno-Maekawa, M.; Sangawa, H.; Uehara, S.; Kozaki, K.; Nomura, N.; Egawa, H.; Shiraki, K. Mechanism of action of T-705 against influenza virus. *Antimicrob. Agents Chemother.* **2005**, *49*, 981–986.
(28) Fodor, E.; Devenish, L.; Engelhardt, O. G.; Palese, P.; Brownlee, G. G.; Garcia-Sastre, A. Rescue of influenza A virus from recombinant DNA. *J. Virol.* **1999**, *73*, 9679–9682.