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Original article

Synthesis and antiviral activity of substituted bisaryl amide compounds as novel influenza virus inhibitors

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ABSTRACT

The influenza virus is a persistent cause of mortality and morbidity on an annual basis and thus presents itself as an important target for pharmaceutical investigation. In this work, substituted bisaryl amide compounds were found to be a new class of potential anti-influenza agents, and a series of substituted bisaryl amide compounds were synthesised and evaluated for their anti-influenza virus activities. The analysis of the results produced a preliminary structure–activity relationship study (SAR). Compounds **1a**, **1g**, **1h**, **1j**, **1l** and **1n** exhibited clear antiviral activities against the influenza A (A/Guangdong Luohu/219/2006, H1N1) virus with 50% inhibitory concentrations (IC_{50}) for virus growth ranging from 12.5 to 59.0 μM. Specifically, compound **1j** also possessed antiviral activity against both oseltamivir-resistant influenza (A/Jinnan/15/2009) virus and influenza B (B/Jifang/13/97) virus with IC_{50} values of 9.2 μM and 21.4 μM, respectively. Compound **1j** is thus worth further investigation as an anti-influenza virus candidate.

1. Introduction

Influenza is a type of acute respiratory disease that is caused by the influenza virus. The typical clinical symptoms include acute fever, body aches, significant fatigue and mild respiratory symptoms. The influenza virus mutates easily and often causes flu pandemics [1]. For example, the influenza pandemic that occurred in 2009 in Mexico swept around the globe and caused severe mortality and morbidity [2,3].

Despite the extensive efforts that are made to control the infection of the influenza virus, only two classes of drugs are currently available for clinical use: antagonists of the viral M2 protein, such as amantadine and its derivative rimantadine, and viral neuraminidase inhibitors, such as zanamivir and oseltamivir [4–6]. However, both of these classes are limited in their efficacy due to drug resistance and side effects [7–10]. Recently, Hama et al. found that oseltamivir could acerbate the illness and occasionally even lead to death [11]. Therefore, it is necessary to develop novel, effective antiviral agents against the influenza virus.

In our previous study, a novel class of bisaryl amide compounds were confirmed as APOBEC3G (human apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G, hA3G) stabilizers [12,13]. The hA3G protein was initially identified as a host cellular restriction factor of HIV-1 by Sheehy et al. in 2002 [14], and it was also found to be active against other reverse transcription viruses, such as SIV, MLV and HBV [13,15]. Although there has been no report revealing the relationship between hA3G and the influenza virus until now, we tested some hA3G-stabilizing compounds that were available in our laboratory for whether they exhibited any anti-influenza virus activity. Surprisingly, small molecule **LH-905** (4-methoxy-N-phenyl-3-propionamidobenzamide, Fig. 1) was found to be active against the influenza virus *in vitro*. However, **LH-905** exhibited only modest antiviral activity with an IC_{50} of 43.0 μM. To discover more active candidates for use as anti-influenza virus agents, we synthesised a series of substituted bisaryl amide analogues of **LH-905** in this study. The preliminary SAR was also discussed here.

2. Results and discussion

2.1. Chemistry

The analogues mainly differed in their chemical structures at the following positions of **LH-905**: 1) replacement of substituents and ring structure of benzene ring B; 2) the amide linker between the

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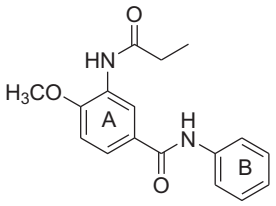


Fig. 1. The chemical structure of **LH-905**.

two benzene rings; and 3) substituents on benzene ring A, especially the amide side-chain at the C3-position.

LH-905 was synthesised by condensing 3-propionamide-4-methoxybenzoic acid (**1-M$_1$**) with aniline using diisopropylcarbodiimide (DIC) as a coupling reagent and N-hydroxybenzotriazole (HOBt) as an activating reagent [16], as shown in Scheme 1. The intermediates **1-M$_1$**–**1-M$_4$**, **2-M** and **3-M** were obtained from the acylation of the amino groups or hydroxy group of starting materials **1**, **2** and **3**, respectively, with different chlorides. Next, these intermediates were condensed with diverse primary amines to yield products **1a**–**1k**, **10** and **11** (Scheme 1a). The intermediates **1-M$_5$** and **4-M**–**7-M** were produced by the direct condensation of starting materials **1** and **4**–**7** with aniline, respectively, and their amino groups at the C3-position were then acylated with cyclopentane carboxylic acid or α-fluoropropionic acid to yield the desired products **1l**, **1m** and **13**–**16** (Scheme 1b). Following the first step of Scheme 1b, compound **12** was directly synthesised from 4-methoxybenzoic acid (**8**) as the starting material.

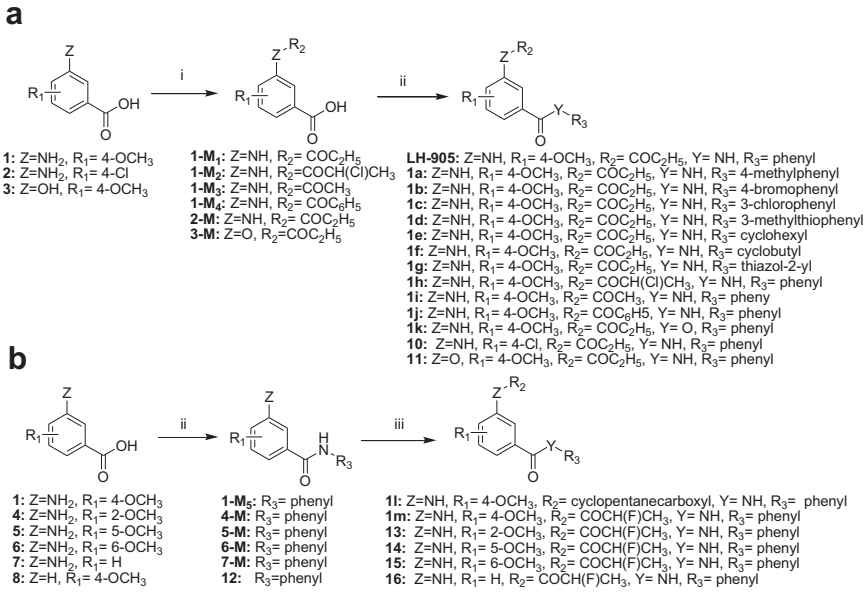
The coupling reagent failed to yield the desired substituted bisaryl compounds when substituted benzenesulphonic acid was used as a starting material or when the weakly nucleophilic arylamine was used with benzoic acid for the condensation. The conjunction of the two benzene rings in these cases was achieved through sulphonyl chloride or carbonyl chloride intermediates. Starting materials **1** and **9** were first reacted with propionyl chloride and isobutyryl chloride, respectively, to yield intermediates **1-M$_1$**, **1-M$_6$** and **9-M$_1$**, which were then converted into the corresponding benzoyl chlorides (**1-M$_7$** and **1-M$_8$**) and benzosulphonyl chloride (**9-M$_2$**). These chlorides were reacted with various arylamines to yield the desired products **1n**–**q** and **17** (Scheme 2).

Compound **21** was synthesised using the route shown in Scheme 3. First, starting material **18** was reacted with propionyl chloride, using triethylamine (TEA) as a base, to yield intermediate **19**, and the nitro group in intermediate **19** was subsequently reduced to an amino group to yield intermediate **20**. Then, intermediate **20** was reacted with benzoyl chloride to afford final product **21**.

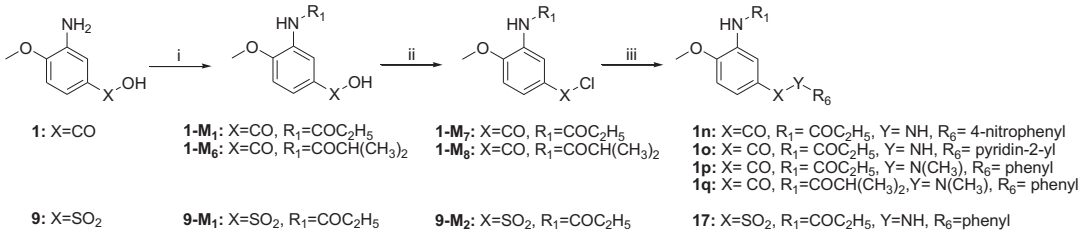
2.2. Evaluation of anti-influenza viral activity of substituted bisaryl amide compounds

The antiviral activities of substituted bisaryl amide compounds against influenza A/Guangdong Luohu/219/2006 (H_1N_1) were initially evaluated by cytopathic effects (CPE) using MDCK cells with Tamiflu and Ribavirin (RBV) as positive drugs. Among the 25 novel synthesized compounds summarized in Table 1, twenty compounds showed antiviral activity against influenza virus A. Although all active compounds showed less activity than positive control drugs Tamiflu (IC_{50} = 2.0 μM) and RBV (IC_{50} = 8.8 μM), compounds **1a**, **1g**, **1h**, **1j**, **1l** and **1n** exhibited considerable or even higher antiviral activities when compared with **LH-905** (IC_{50} = 43.0 μM). The IC_{50} for virus growth of these compounds was in range of 12.5–59.0 μM. Compounds **1h** (IC_{50} = 17.3 μM) and **1j** (IC_{50} = 12.5 μM) which have distinct substituents from other compounds at C3-position of ring A showed the highest activity. The substituent was α-chloropropionamide and benzamide for **1h** and **1j**, respectively.

Nine compounds with selectivity indices (SI = TC_{50}/IC_{50}) > 10 against influenza virus A (A/Guangdong Luohu/219/2006 strain) were further evaluated for their potential antiviral activity against influenza B and oseltamivir resistant influenza A virus strain. Tamiflu and RBV were used as control drugs, and the CPE method was used in these assays. As summarized in Table 2, compounds **1g**, **1j** and **1n** exhibited antiviral activity against influenza B (B/Jifang/13/97) virus. Compound **1j** (IC_{50} = 21.4 μM) possessed of higher activity comparing with positive control RBV against influenza B



Scheme 1. The synthetic route to compounds **1a**–**m** and **10**–**16**. Reagents and conditions: (i) acyl chloride, TEA, CH_2Cl_2; (ii) arylamine or cycloalkylamine, DIC, HOBt, CH_2Cl_2; (iii) cyclopentane carboxylic acid or α-fluoropropionic acid, DIC, HOBt, CH_2Cl_2



Scheme 2. The synthetic route to compounds **1n**–**q** and **17**. Reagents and conditions: (i) acyl chloride, TEA, CH_2Cl_2; (ii) $SOCl_2$; (iii) arylamine, TEA, CH_2Cl_2.

virus. Moreover, compounds **1g** and **1j** also displayed antiviral activity against influenza A oseltamivir resistant strain. Especially, the most effective compound **1j** exhibited several folds higher activity than positive control drug RBV, the IC_{50} and SI values of **1j** for oseltamivir resistant influenza (A/Jinnan/15/2009 strain) virus were 9.2 μM and 21.0, respectively.

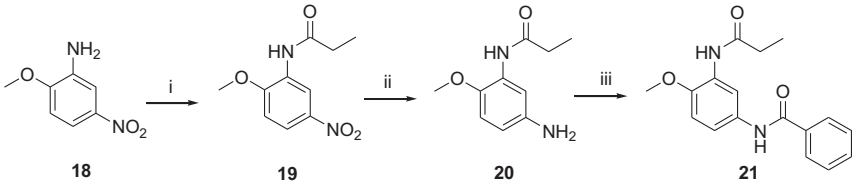
2.3. Structure–antiviral activity relationships

The replacement of propionamido side-chain at C3-position in ring A with acetamido (**1i**), α-fluoropropionamido (**1m**) or propionyloxy group (**11**) led to several folds decreased antiviral activity compared with **LH-905**. However, the most effective antiviral activity of corresponding compounds was exhibited when propionamido (**LH-905**, $IC_{50} = 43.0$ μM)) group was replaced with α-chloropropionamido (**1h**, $IC_{50} = 17.3$ μM) or benzamido group (**1j**, $IC_{50} = 12.5$ μM). The removal of this propionamido group at the C3-position in ring A (**12**) resulted in loss of antiviral activity. Therefore, the results indicated that substitution at the C3-position of ring A is required for activity. And from the stronger activity of compounds **1h** and **1j** than other compounds, we estimated that more lipophilic substituents at the C3-position of ring A led to higher biological activity. Replacement of methoxy group at C4-position of ring A with chloro atom (**10**) led to the significantly decrease of inhibitory activity against influenza virus comparing with **LH-905**. The removal of methoxyl from C4-position (**16**, $IC_{50} = 143.5$ μM) of ring A showed a slightly increased activity than similar compound **1m** ($IC_{50} = 210.8$ μM). The antiviral activities of compounds **1m**, **13** and **15** indicated that altering the position of methoxy group from C4-position to other position in ring A may decrease inhibitory activity against influenza (A/Luohu/219/2006, H1N1) virus. Thus, the substituent at the C4-position of ring A seems be important to antiviral activity. However, we can't conclude whether the methoxyl was the best substituent at C4-position of ring A or not, based on current results. The compounds containing various substituted B benzene rings indicated no specific rules between structure and antiviral activity. The two more effective compounds (IC_{50} is 23.7 μM and 26.3 μM for **1a** and **1n**, respectively) possessed of opposite chemical structure characteristics of substituent in ring B. Replacement of benzene ring B with saturated cycle (**1e**, **1f**) caused decreased antiviral activity of corresponding compounds. The amide linker between ring A and B was methylated (**1p**, $IC_{50} = 100.8$ μM) or replaced with ester (**1k**, $IC_{50} = 222.9$ μM) or sulfonamide linker (**17**, $IC_{50} = 48.4$ μM) led to decreasing of antiviral activity. Noted that sulfonamide linker between two aryl rings (**17**) only induced a weakly decrease of antiviral activity comparing with carbonyl amide linker (**LH-905**). The interchange of CO and NH of amide linker (**21**, $IC_{50} = 74.5$ μM) weakened the anti-influenza viral activity compared with **LH-905**. So the NH at C1-position of ring B and aromatic ring B was also beneficial for anti-influenza virus activity.

Although we still are uncertainty about the exact action mechanism, some classical drug targets of influenza virus, for example viral haemagglutinin, neuraminidase and M2 protein, have been ruled out for target discovery in our studies. The results indicated that these compounds have no inhibitory effect on viral haemagglutinin, influenza neuraminidase and M2 protein at the tested concentrations (over 58 μM). The best compound **1j** definitely showed activity against oseltamivir resistant influenza virus strain even if it exhibited weaker activity comparing with control drugs Tamiflu and RBV against influenza A virus, and the activity of **1j** ($IC_{50} = 9.2$ μM) against oseltamivir resistant virus strain was much higher than that of RBV ($IC_{50} = 35.3$ μM). Hence, we would like to conclude that the action mechanism of these substituted bisaryl amides is different from that of market available drugs.

3. Conclusion

Substituted bisaryl amide compounds were found to be a new class of potential anti-influenza agents, and a total of 25 novel bisaryl amide compounds were synthesised and screened for their antiviral activity against influenza A virus in this paper. Moreover, the data were analysed to produce a preliminary SAR. Ten compounds exhibited obvious antiviral activity against tested influenza A virus strain. The nine compounds with SI > 10 were further tested for antiviral activity against influenza B and resistant influenza A virus. Compounds **1g** and **1j** exhibited additional antiviral activity against both influenza B and oseltamivir resistant influenza A virus strain. Worthy of note, compound **1j** with benzamide substituted at C3-position of ring A exhibited the highest antiviral activity against three influenza virus strains among all test compounds, which makes compound **1j** worthy to be exploited as a novel candidate against influenza viruses. Without doubt, the further exploration is needed to produce more potent compounds and to clear the detailed action mechanism of this novel class of biaryl amide compounds against influenza virus. These relative studies are already in progress in our research team.



Scheme 3. The synthetic route to compound **21**. Reagents and conditions: (i) propionyl chloride, TEA, CH_2Cl_2; (ii) H_2, Pd/C; (iii) benzoyl chloride, TEA, CH_2Cl_2.

Table 1
Anti-influenza (A/Guangdong Luohu/219/2006 strain) virus activities and cytotoxicities of the synthesised substituted bisaryl amide compounds.[a]

Compd.	TC_{50} (μM)	IC_{50} (μM)	SI
LH-905	>670.4	43.0	>15.6
1a	162.3	23.7	6.8
1b	90.4	>17.6	–
1c	602.3	>66.9	–
1d	324.4	>63.0	–
1e	510.3	73.0	6.9
1f	>724.2	59.0	12.3
1g	>655.0	35.0	18.7
1h	200.3	17.3	11.6
1i	>703.5	112.7	6.2
1j	192.5	12.5	15.4
1k	463.6	222.9	2.1
1l	458.7	37.9	12.1
1m	>632.3	210.8	3.0
1n	307.1	26.3	11.7
1o	>668.6	222.9	3.0
1p	444.2	100.8	4.4
1q	491.9	116.7	4.2
10	530.3	152.7	3.5
11	518.5	172.9	3.0
12	>880.0	>293.4	–
13	>632.3	>210.8	–
14	490.7	210.8	2.3
15	271.6	>70.2	–
16	578.5	143.5	4.0
17	>598.6	48.4	12.4
21	387.3	74.5	5.2
Tamiflu	3070.2	2.0	1535.1
RBV	4766.8	8.8	541.7

[a] All data were average values from three independent assays.

4. Experimental protocol

4.1. Chemistry

^{1}H NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Varian Inova 400 or 500 MHz spectrometer. Chemical shifts were reported in parts per million relative to tetramethylsilane as the internal standard. The melting points were determined with an X6 microscope melting point apparatus and were uncorrected. Electrospray ionisation (ESI) mass spectra and high-resolution mass spectra (HRMS) were obtained on an MDS SCIEX Q-Trap mass spectrometer. The area normalization purities of the tested compounds were >95% as determined using analytical high-performance liquid chromatography (HPLC method for all compounds: detector wavelength: 260 nm, column temperature: 25 °C, column: ODS (5 μm, 4.6 × 250 mm), mobile phase: methanol:water = 75:25, flow rate = 1 mL/min).

Table 2
Anti-influenza (B/Jifang/13/97 and A/Jinnan/15/2009) virus activities and cytotoxicities of selected synthesised bisaryl amide compounds.[a]

Compd.	Influenza A (strain A/luohu/219/2006)			Influenza B (strain B/jifang/13/97)		Resistant influenza A (strain A/Jinnan/15/2009)	
	TC_{50} (μM)	IC_{50} (μM)	SI	IC_{50} (μM)	SI	IC_{50} (μM)	SI
LH-905	>670.4	43.0	15.5	>223.5	–	>223.5	–
1a	162.3	23.7	6.84	>23.7	–	>23.7	–
1f	>724.2	59.0	12.2	>241.4	–	>241.4	–
1g	>655.0	35.0	18.7	126.1	5.2	93.8	6.9
1h	200.3	17.3	11.7	>22.3	–	>22.3	–
1j	192.5	12.5	15.4	21.4	9.0	9.2	20.9
1l	458.7	37.9	12.1	>65.7	–	>65.7	–
1n	307.1	26.3	11.6	64.8	4.7	>21.6	–
17	>200	16.2	12.4	>66.7	–	>66.7	–
Tamiflu	3070.2	2.0	1535.1	ND	ND	>2436.6	–
RBV	4766.8	8.8	541.7	45.5	103.9	35.3	135.0

ND: not determined.
[a] All data were average values from three independent assays.

4.2. Synthesis

4.2.1. General procedure for the synthesis of **1-M$_1$**–**1-M$_4$**, **1-M$_6$**, **2-M**, **3-M** and **9-M$_1$**

*4.2.1.1. 4-Methoxy-3-propionamidobenzoic acid (**1-M$_1$**).* A total of 2.5 mL (30 mmol) of propionyl chloride was added dropwise to a solution of 5.0 g (30 mmol) of **1** and 4.3 mL (30 mmol) of TEA in dichloromethane at 0 °C. The mixture was then stirred at room temperature until the starting material was completely disappeared. The solvent was evaporated in vacuo to produce a sticky residue that was extracted with dichloromethane and 0.5 N HCl (25 mL). The organic phase was concentrated, and the residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (v:v = 2:1) to yield the desired compound as a white solid (yield: 76%). Mp: 372–373 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.59 (1H, br s, COOH), 9.12 (1H, s, NH), 8.56 (1H, s, PhH), 7.68 (1H, d, J = 8.8 Hz, PhH), 7.11 (1H, d, J = 8.8 Hz, PhH), 3.89 (3H, s, OCH_3), 2.42 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). ESI-MS (m/z): 224 $(M + H)^+$.

*4.2.1.2. 3-(2-Chloropropanamido)-4-methoxybenzoic acid (**1-M$_2$**).* Compound **1-M$_2$** was synthesised using a method similar to that of **1-M$_1$** and was isolated as a white solid (yield: 66%). Mp: 414–416 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.59 (1H, br s, COOH), 9.12 (1H, s, NH), 8.56 (1H, s, PhH), 7.68 (1H, d, J = 8.8 Hz, PhH), 7.11 (1H, d, J = 8.8 Hz, PhH), 4.99 (1H, q, J = 6.4 Hz, CH), 3.90 (3H, s, OCH_3), 1.62 (3H, d, J = 6.4 Hz, CH_3). ESI-MS (m/z): 258 $(M + H)^+$.

*4.2.1.3. 3-Acetamido-4-methoxybenzoic acid (**1-M$_3$**).* Compound **1-M$_3$** was synthesised using a method similar to that of **1-M$_1$** and was isolated as a white solid (yield: 75%). Mp: 331–332 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.59 (1H, br s, COOH), 9.12 (1H, s, NH), 8.56 (1H, s, PhH), 7.68 (1H, d, J = 8.8 Hz, PhH), 7.11 (1H, d, J = 8.8 Hz, PhH), 3.90 (3H, s, OCH_3), 1.96 (3H, s, CH_3CO). ESI-MS (m/z): 210 $(M + H)^+$.

*4.2.1.4. 3-Benzamido-4-methoxybenzoic acid (**1-M$_4$**).* Compound **1-M$_4$** was synthesised using a method similar to that of **1-M$_1$** and was isolated as a white solid (yield: 69%). Mp: 391–393 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.59 (1H, br s, COOH), 10.07 (1H, s, NH), 9.16 (1H, s, PhH), 7.81 (1H, d, J = 6.0 Hz, PhH), 7.78 (2H, d, J = 7.6 Hz, PhH), 7.33 (2H, m, PhH), 7.20 (1H, d, J = 8.8 Hz, PhH), 7.07 (1H, m, PhH), 3.90 (3H, s, OCH_3). ESI-MS (m/z): 272 $(M + H)^+$.

*4.2.1.5. 3-Isobutyramido-4-methoxybenzoic acid (**1-M$_6$**).* Compound **1-M$_5$** was synthesised using a method similar to that of **1-M$_1$** and was isolated as a white solid (yield: 72%). Mp: 379–381 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.59 (1H, br s, COOH), 9.12 (1H, s, NH), 8.56 (1H, d, J = 8.8 Hz, PhH), 7.68 (1H, m, PhH), 7.11 (1H, d, J = 8.8 Hz, PhH), 3.90 (3H, s, OCH_3), 2.71 (1H, m, CH), 1.04 (6H, d, J = 6.4 Hz, CH_3). ESI-MS (m/z): 238 $(M + H)^+$.

*4.2.1.6. 4-Chloro-3-propionamidobenzoic acid (**2-M**).* Compound **2-M** was synthesised using a method similar to that of **1-M$_1$** and was isolated as a white solid (yield: 70%). Mp: 387–389 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.59 (1H, br s, COOH), 9.12 (1H, s, NH), 8.56 (1H, d, J = 8.8 Hz, PhH), 7.68 (1H, m, PhH), 7.11 (1H, d, J = 8.8 Hz, PhH), 2.42 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). ESI-MS (m/z): 228 $(M + H)^+$.

*4.2.1.7. 4-Methoxy-3-(propionyloxy)benzoic acid (**3-M**).* Compound **3-M** was synthesised using a method similar to that of **1-M$_1$** and was isolated as a white solid (yield: 72%). Mp: 241–243 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.74 (1H, br s, COOH), 8.56 (1H, s, PhH), 7.68 (2H, m, PhH), 3.83 (3H, s, OCH_3), 2.42 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). ESI-MS (m/z): 225 $(M + H)^+$.

*4.2.1.8. 4-Methoxy-3-propionamidobenzenesulphonic acid (**9-M$_1$**).* Compound **9-M$_1$** was synthesised using a method similar to that of **1-M$_1$** and was isolated as a white solid (yield: 47%). Mp: 379–381 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.21 (1H, br s, COOH), 8.93 (1H,s, NH), 8.02 (1H, s, PhH), 7.23 (2H, m, PhH), 3.90 (3H, s, OCH_3), 2.42 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). ESI-MS (m/z): 260 $(M + H)^+$.

*4.2.2. General procedure for the synthesis of **LH-905**, **1a–1k**, **10** and **11***

*4.2.2.1. 4-Methoxy-N-phenyl-3-propionamidobenzamide (**LH-905**).* A total of 0.17 mL (0.18 mmol) of DIC and 0.24 g (0.18 mmol) of HOBt were added to a solution of 0.20 g (0.12 mmol) of **1-M$_1$** in dichloromethane. The reaction mixture was stirred for 0.5 h at room temperature, and 0.13 mL (0.14 mmol) of aniline was then added to the mixture. After 3 h, 0.5 N NaOH was added to the solution. The lower layer was removed, and 0.5 N HCl was added until the aqueous solution was neutral. The dichloromethane layer was washed three times with 10 mL of water, and then $MgSO_4$ was added. After 2 h, the mixture was filtered, and the solvent was removed. The residue was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (v: v = 3:1) to yield the desired compound as a white solid (yield: 72%, HPLC purity: 99.16%). Mp: 178–179 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.07 (1H, s, NH), 9.16 (1H, s, NH), 8.50 (1H, s, PhH), 7.81 (1H, d, J = 6.0 Hz, PhH), 7.78 (2H, d, J = 7.6 Hz, PhH), 7.33 (2H, m, PhH), 7.20 (1H, d, J = 8.8 Hz, PhH), 7.07 (1H, m, PhH), 3.90 (3H, s, OCH_3), 2.43 (2H, q, J = 7.6 Hz, CH_2), 1.16 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{18}N_2O_3$ $(M)^+$: 298.12702; measured: 298.13119.

*4.2.2.2. 4-Methoxy-3-propionamido-N-p-tolylbenzamide (**1a**).* Compound **1a** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 76%, HPLC purity: 99.61%). Mp: 201–203 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.00 (1H, s, NH), 9.16 (1H, s, NH), 8.49 (1H, s, PhH), 7.73 (3H, m, PhH), 7.14 (3H, m, PhH), 3.90 (3H, s, OCH_3), 2.42 (2H, q, J = 8.0 Hz, CH_2), 2.26 (3H, s, Ph-CH_3), 1.07 (3H, t, J = 7.5 Hz, CH_3). HRMS-ESI (m/z): calcd for $C_{18}H_{21}N_2O_3$ $(M + H)^+$: 313.15522; measured: 313.15349.

*4.2.2.3. N-(4-bromophenyl)-4-methoxy-3-propionamidobenzamide (**1b**).* Compound **1b** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 42%, HPLC purity: 96.77%). Mp: 172–174 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.19 (1H, s, NH), 9.17 (1H, s, NH), 8.50 (1H, s, PhH), 7.71–7.74 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, PhH), 7.72 (2H, d, J = 8.88 Hz, PhH), 7.50 (2H, d, J = 8.8 Hz, PhH), 7.14 (1H, d, J = 8.4 Hz, PhH), 3.90 (3H, s, OCH_3), 2.40 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{18}BrN_2O_3$ $(M + H)^+$: 377.05003; measured: 377.05008.

*4.2.2.4. N-(3-chlorophenyl)-4-methoxy-3-propionamidobenzamide (**1c**).* Compound **1c** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 60%, HPLC purity: 97.38%). Mp: 133–134 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.24 (1H, s, NH), 9.18 (1H, s, NH), 8.51 (1H, s, PhH), 7.94 (1H, s, PhH), 7.68–7.74 (2H, m, PhH), 7.36 (1H, t, J = 6.4 Hz, PhH), 7.12–7.17 (2H, m, PhH), 3.91 (3H, s, OCH_3), 2.4 (2H, q, J = 6.0 Hz, CH_2), 1.07 (3H, t, J = 6.0 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{18}ClN_2O_3$ $(M + H)^+$: 333.10200; measured: 333.10059.

*4.2.2.5. 4-Methoxy-N-(3-(methylthio)phenyl)-3-propionamidobenzamide (**1d**).* Compound **1d** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 4:1) as a white solid (yield: 45%, HPLC purity: 97.55%). Mp: 159–161 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.90 (1H, s, PhH), 7.93 (1H, s, NH), 7.83 (1H, s, NH), 7.78 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, PhH), 7.66 (1H, s, PhH), 7.37 (1H, d, J = 8.4 Hz, PhH), 7.25 (1H, t, J = 8.4 Hz, PhH), 7.03 (1H, d, J = 7.6 Hz, PhH), 7.00 (1H, d, J = 8.8 Hz, PhH), 3.96 (3H, s, OCH_3), 2.55 (3H, s, SCH_3), 2.46 (2H, q, J = 7.6 Hz, CH_2), 1.28 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{18}H_{21}N_2O_3S$ $(M + H)^+$: 345.12674; measured: 345.12674.

*4.2.2.6. N-cyclohexyl-4-methoxy-3-propionamidobenzamide (**1e**).* Compound **1e** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 67%, HPLC purity: 98.36%). Mp: 192–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.08 (1H, s, NH), 8.31 (1H, s, NH), 8.00 (1H, d, J = 8.0 Hz, PhH), 7.59 (1H, m, PhH), 7.05 (1H, d, J = 8.4 Hz, PhH), 3.85 (3H, s, OCH_3), 3.73 (1H, m, CH), 2.38 (2H, q, J = 7.6 Hz, CH_2), 1.72 (4H, m, CH_2), 1.26 (4H, m, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3), 0.99 (m, 2H, CH_2). HRMS-ESI (m/z): calculated for $C_{17}H_{25}N_2O_3$ $(M + H)^+$: 305.18593; measured: 305.18597.

*4.2.2.7. N-Cyclobutyl-4-methoxy-3-propionamidobenzamide (**1f**).* Compound **1f** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 63%, HPLC purity: 99.42%). Mp: 183–185 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.08 (1H, s, NH), 8.42 (1H, d, J = 7.6 Hz, PhH), 8.33 (1H, s, NH), 7.60 (1H, d, J = 8.4 Hz, PhH), 7.06 (1H, d, J = 7.6 Hz, PhH), 4.37 (1H, m), 3.85 (3H, s, OCH_3), 2.36 (2H, q, J = 7.6 Hz, CH_2), 2.17 (2H, m), 2.04 (2H, m), 1.63 (2H, m), 1.06 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{15}H_{19}N_2O_3$ $(M - H)^-$: 275.13983; measured: 275.13902.

*4.2.2.8. 4-Methoxy-3-propionamido-N-(thiazol-2-yl)benzamide (**1g**).* Compound **1g** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 76%, HPLC purity: 99.33%). Mp: 183–185 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.45 (1H, s, NH), 9.18 (1H, s, NH), 8.65 (1H, s, PhH), 7.92 (1H, m, PhH), 7.52 (1H, d, J = 7.6 Hz, PhH), 7.24 (1H, d, J = 7.6 Hz, PhH), 7.16 (1H, d, J = 8.4 Hz, PhH), 3.91 (3H, s, OCH_3), 2.43 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{14}H_{15}N_3O_3S$ $(M + H)^+$: 306.08988; measured: 306.09069.

*4.2.2.9. 3-(2-Chloropropanamido)-4-methoxy-N-phenylbenzamide (**1h**).* Compound **1h** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 69%, HPLC purity: 97.98%). Mp: 161–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.07 (1H, s, NH), 9.16 (1H, s, NH), 8.50 (1H, s, PhH), 7.81 (1H, d, J = 6.0 Hz, PhH), 7.78 (2H, d, J = 7.6 Hz, PhH), 7.33 (2H, m, PhH), 7.20 (1H, d, J = 8.8 Hz, PhH), 7.07 (1H, m, PhH), 4.99 (1H, q, J = 6.4 Hz, CH), 3.90 (3H, s, OCH_3), 1.62 (3H, d, J = 6.4 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{16}N_2O_3Cl$ $(M - H)^-$: 331.08477; measured: 331.08440.

*4.2.2.10. 3-Acetamido-4-methoxy-N-phenylbenzamide (**1i**).* Compound **1i** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 75%, HPLC purity: 98.25%). Mp: 171–173 °C. ^{1}H

NMR (400 MHz, DMSO-d_6) δ (ppm): 10.07 (1H, s, NH), 9.16 (1H, s, NH), 8.50 (1H, s, PhH), 7.81 (1H, d, J = 6.0 Hz, PhH), 7.78 (2H, d, J = 7.6 Hz, PhH), 7.33 (2H, m, PhH), 7.20 (1H, d, J = 8.8 Hz, PhH), 7.07 (1H, m, PhH), 3.90 (3H, s, OCH_3), 2.09 (3H, s, CH_3). HRMS-ESI (m/z): calculated for $C_{16}H_{15}N_2O_3$ $(M - H)^-$: 283.10811; measured: 283.10772.

*4.2.2.11. 3-Benzamido-4-methoxy-N-phenylbenzamide (**1j**).* Compound **1j** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 68%, HPLC purity: 99.72%). Mp: 196–197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.13 (1H, s, NH), 9.62 (1H, s, NH), 8.35 (1H, s, PhH), 7.99 (2H, d, J = 6.8 Hz, PhH), 7.87 (1H, d, J = 6.4 Hz, PhH), 7.77 (2H, d, J = 8.0 Hz, PhH), 7.60 (3H, m, PhH), 7.33 (2H, m, PhH), 7.21 (1H, d, J = 8.8 Hz, PhH), 7.09 (1H,m, PhH), 3.90 (3H, s, OCH_3). HRMS-ESI (m/z): calculated for $C_{21}H_{17}N_2O_3$ $(M - H)^-$: 345.12320; measured: 345.12337.

*4.2.2.12. Phenyl 4-methoxy-3-propionamidobenzoate (**1k**).* Compound **1k** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 53%, HPLC purity: 98.02%). Mp: 147–149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.07 (1H, s, NH), 9.16 (1H, s, PhH), 7.81 (1H, d, J = 6.0 Hz, PhH), 7.78 (2H, d, J = 7.6 Hz, PhH), 7.33 (2H,m, PhH), 7.20 (1H, d, J = 8.8 Hz, PhH), 7.07 (1H, m, PhH), 4.00 (3H, s, OCH_3), 2.43 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{18}NO_4$ $(M + H)^+$: 300.12358; measured: 300.12299.

*4.2.2.13. 4-Chloro-N-phenyl-3-propionamidobenzamide (**10**).* Compound **10** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 4:1) as a white solid (yield: 53%, HPLC purity: 98.21%). Mp: 147–149 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.87 (1H, s, NH), 9.16 (1H, s, NH), 8.50 (1H, s, PhH), 7.81 (1H, d, J = 6.0 Hz, PhH), 7.78 (2H, d, J = 7.6 Hz, PhH), 7.33 (2H, m, PhH), 7.20 (1H, d, J = 8.8 Hz, PhH), 7.07 (1H, m, PhH), 2.43 (2H, q, J = 7.6 Hz, CH_2), 1.16 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{16}H_{16}ClN_2O_2$ $(M + H)^+$: 303.08950; measured: 303.08948.

*4.2.2.14. 2-Methoxy-5-(phenylcarbamoyl)phenyl propionate (**11**).* Compound **11** was synthesised using a method similar to that of **LH-905** and was isolated (eluent: petroleum ether:ethyl acetate = 4:1) as a white solid (yield: 56%, HPLC purity: 99.31%). Mp: 173–175 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.10 (1H, s, NH), 7.93 (1H, d, J = 6.4 Hz, PhH), 7.75 (3H, m, PhH), 7.35 (2H, m, PhH), 7.27 (1H, d, J = 8.4 Hz, PhH), 7.09 (1H, m, PhH), 3.85 (3H, s, OCH_3), 2.28 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{18}NO_4$ $(M + H)^+$: 300.12303; measured: 300.12303.

*4.2.3. General procedure for the synthesis of **1-M$_7$**, **1-M$_8$** and **9-M$_2$***

*4.2.3.1. 4-Methoxy-3-propionamidobenzoyl chloride (**1-M$_7$**).* $SOCl_2$ was added to a flask containing 5.0 g (22 mmol) of **1-M$_1$** until the white solid was completely dissolved. After the starting material had completely disappeared, the excess $SOCl_2$ was removed, producing a brown oil that was used in the next step without further purification.

*4.2.3.2. 3-Isobutyramido-4-methoxybenzoyl chloride (**1-M$_8$**).* Compound **1-M$_8$** was synthesised using a method similar to that of **1-M$_7$** and was isolated as a brown oil (yield: 47%).

*4.2.3.3. 4-Methoxy-3-propionamidobenzene-1-sulphonyl chloride (**9-M$_2$**).* Compound **9-M$_2$** was synthesised using a method similar to that of **1-M$_7$** and was isolated as a brown oil (yield: 47%).

*4.2.4. General procedure for the synthesis of **1n–1q** and **17***

*4.2.4.1. 4-Methoxy-3-propionamido-N-(pyridin-2-yl)benzamide (**1o**).* A total of 0.50 g (2.2 mmol) of **1-M$_7$** was added to a dichloromethane solution of 0.21 g (2.2 mmol) pyridin-2-amine and 0.30 mL (2.2 mmol) of TEA at 0 °C. The mixture was reacted at room temperature until the starting material was completely disappeared. The solvent was then evaporated in vacuo to produce a sticky residue that was then extracted with dichloromethane and 0.5 N HCl (25 mL). The organic phase was concentrated, and the solid was purified by column chromatography on silica gel (eluent: petroleum ether:ethyl acetate = 2:1) to yield the desired compound as a white solid (yield: 51%, HPLC purity: 99.54%). Mp: 184–186 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.45 (1H, s, NH), 9.18 (1H, s, NH), 8.65 (1H, s, PhH), 7.92 (1H,m, PhH), 7.53 (1H, d, J = 7.6 Hz, PhH)), 7.24 (1H, d, J = 7.6 Hz, PhH), 7.16 (1H, d, J = 8.4 Hz, PhH), 6.91 (1H, m, PhH), 6.53 (1H, d, J = 7.6 Hz, PhH), 3.90 (3H, s, OCH_3), 2.41 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{16}H_{18}N_3O_3$ $(M + H)^+$: 300.13425; measured: 300.13427.

*4.2.4.2. 4-Methoxy-N-(4-nitrophenyl)-3-propionamidobenzamide (**1n**).* Compound **1n** was synthesised using a method similar to that of **1o** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 53%, HPLC purity: 98.18%). Mp: 154–156 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 9.99 (1H, s, NH), 9.16 (1H, s, NH), 8.49 (1H, s, PhH), 8.25 (2H, m, PhH), 8.05 (2H, m, PhH), 7.78 (1H, m, PhH), 7.19(1H, d, J = 8.5 Hz, PhH), 3.90 (3H, s, OCH_3), 2.42 (2H, q, J = 8.0 Hz, CH_2), 1.07 (3H, t, J = 7.5 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{18}N_3O_5$ $(M + H)^+$: 344.12403; measured: 344.12410.

*4.2.4.3. 4-Methoxy-N-methyl-N-phenyl-3-propionamidobenzamide (**1p**).* Compound **1p** was synthesised using a method similar to that of **1o** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 54%, HPLC purity: 96.09%). Mp: 143–145 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.93 (1H, s, NH), 8.07 (1H, s, PhH), 7.27 (2H, m, PhH), 7.15 (3H, m, PhH), 6.84 (2H, m, PhH), 3.90 (3H, s, OCH_3), 3.30 (3H, s, NCH_3), 2.34 (2H, q, J = 8.0 Hz, CH_2), 1.03 (3H, t, J = 8.0 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{18}H_{21}N_2O_3$ $(M + H)^+$: 313.15461; measured: 313.15467.

*4.2.4.4. 3-Isobutyramido-4-methoxy-N-methyl-N-phenylbenzamide (**1q**).* Compound **1q** was synthesised using a method similar to that of **1o** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 56%, HPLC purity: 99.15%). Mp: 150–151 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.89 (1H, s, NH), 8.05 (1H, s, PhH), 7.27 (2H, m, PhH), 7.15 (3H, m, PhH), 6.79 (2H, m, PhH), 3.90 (3H, s, OCH_3), 3.30 (3H, s, NCH_3), 2.71 (1H, m, CH), 1.04 (6H, d, J = 6.4 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{19}H_{23}N_2O_3$ $(M + H)^+$: 327.16943; measured: 327.17032.

*4.2.4.5. N-(2-methoxy-5-(N-phenylsulphamoyl)phenyl)propionamide (**17**).* Compound **17** was synthesised using a method similar to that of **1o** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 47%, HPLC purity: 97.97%). Mp: 195–197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.07 (1H, s, NH), 8.53 (1H, s, NH), 8.17 (1H, s, PhH), 7.78 (2H, d, J = 7.6 Hz, PhH), 7.33 (2H, m, PhH), 7.81 (2H, m, PhH), 7.20 (1H, d, J = 8.8 Hz, PhH), 3.90 (3H, s, OCH_3), 2.44 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{16}H_{18}N_2O_4S$ $(M)^+$: 334.09956; measured: 334.09873.

*4.2.5. General procedure for the synthesis of **1-M$_5$**, **4-M–7-M** and **12***

*4.2.5.1. 3-Amino-4-methoxy-N-phenylbenzamide (**1-M$_5$**).* A total of 0.17 mL (0.18 mmol) of DIC and 0.24 g (0.18 mmol) of HOBt were

added to a dichloromethane solution of 0.20 g (0.12 mmol) of **1** at room temperature, and the reaction mixture was stirred for 0.5 h. Then, 0.13 mL (0.14 mmol) of aniline was added to the mixture. After approximately 3 h, 0.5 N NaOH was added to the solution. The lower layer was isolated, and 0.5 N HCl was added until the aqueous solution was neutral. The dichloromethane layer was washed three times with 10 mL of water, and then $MgSO_4$ was added. After 2 h, the solution was filtered, and the solvent was removed. The residue was purified by column chromatography on silica gel (eluent: petroleum ether:ethyl acetate = 3:1) to yield the desired compound as a white solid (yield: 76%). Mp: 197–198 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.92 (1H, s, NH), 7.74 (2H, d, J = 9.0 Hz, PhH), 7.32 (2H, m, PhH), 7.21 (2H, d, J = 8.0 Hz, PhH), 7.06 (1H, m, PhH), 6.88 (1H, d, J = 8.0 Hz, PhH), 4.90 (2H, br s, NH_2), 3.83 (3H, s, OCH_3). ESI-MS (m/z): 243 $(M + H)^+$.

*4.2.5.2. 3-Amino-2-methoxy-N-phenylbenzamide (**4-M**).* Compound **4-M** was synthesised using a method similar to that of **1-M$_5$** and was isolated as a white solid (yield: 59%). Mp: 205–206 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.33 (1H, s, NH), 8.00 (1H, d, J = 6.0 Hz, PhH), 7.72 (2H, d, J = 6.4 Hz, PhH), 7.33 (3H, m, PhH), 7.22 (1H, d, J = 6.4 Hz, PhH), 7.07 (1H, t, J = 5.6 Hz, PhH), 5.54 (2H, br s, NH_2), 3.87 (3H, s, OCH_3). ESI-MS (m/z): 243 $(M + H)^+$.

*4.2.5.3. 3-Amino-5-methoxy-N-phenylbenzamide (**5-M**).* Compound **5-M** was synthesised using a method similar to that of **1-M$_5$** and was isolated as a white solid (yield: 54%). Mp: 207–208 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.23 (1H, s, NH), 7.78 (1H, s, PhH), 7.48 (1H, d, J = 8.0 Hz, PhH), 7.55 (1H, s, PhH), 7.36 (3H, m, PhH), 7.23 (1H,s, PhH), 7.11 (1H, m, PhH), 5.54 (2H, br s, NH_2), 3.82 (3H, s, OCH_3). ESI-MS (m/z): 243 $(M + H)^+$.

*4.2.5.4. 5-Amino-2-methoxy-N-phenylbenzamide (**6-M**).* Compound **6-M** was synthesised using a method similar to that of **1-M$_5$** and was isolated as a white solid (yield: 61%). Mp: 201–202 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.75 (1H, s, NH), 7.73 (2H, d, J = 6.4 Hz, PhH), 7.44 (1H, d, J = 6.8 Hz, PhH), 7.30 (3H, m, PhH), 7.02 (1H, m, PhH), 6.45 (1H, d, J = 6.8 Hz, PhH), 5.54 (2H, br s, NH_2), 3.90 (3H, s, OCH_3). ESI-MS (m/z): 243 $(M + H)^+$.

*4.2.5.5. 3-Amino-N-phenylbenzamide (**7-M**).* Compound **7-M** was synthesised using a method similar to that of **1-M$_5$** and was isolated as a white solid (yield: 65%). Mp: 173–175 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.24 (1H, s, NH), 8.18 (1H, s, PhH), 7.88 (1H, d, J = 8.0 Hz, PhH), 7.76 (2H, d, J = 8.0 Hz, PhH), 7.67 (1H, d, J = 8.0 Hz, PhH), 7.49 (1H, m, PhH), 7.36 (2H, m, PhH), 7.11 (1H, m, PhH), 5.54 (2H, br s, NH_2). ESI-MS (m/z): 213 $(M + H)^+$.

*4.2.5.6. 4-Methoxy-N-phenylbenzamide (**12**).* Compound **12** was synthesised using a method similar to that of **1-M$_5$** and was isolated (eluent: petroleum ether:ethyl acetate = 3:1) as a white solid (yield: 63%, HPLC purity: 98.75%.). Mp: 152–153 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.05 (1H, s, NH), 7.95 (2H, d, J = 7.6 Hz, PhH), 7.75 (2H, d, J = 7.6 Hz, PhH), 7.34 (2H, m, PhH), 7.08 (3H, m, PhH), 3.83 (3H, s, OCH_3). HRMS-ESI (m/z): calculated for $C_{14}H_{14}NO_2$ $(M + H)^+$: 228.10169; measured: 228.10191.

*4.2.6. General procedure for the synthesis of **1l**, **1m**, and **13–16***

4.2.6.1. 2-Fluoropropanoic acid. A total of 6.6 mL (11.0 mmol) of a 1.0 g/20 mL NaOH solution was added to a solution of 1.0 mL (10.0 mmol) of ethyl 2-fluoropropanoate in tetrahydrofuran (THF), and the mixture was reacted under backstreaming for 2 h. After the solution reached room temperature, the pH was adjusted to 6.0, and dichloromethane was added until a total volume of 100 mL was achieved.

*4.2.6.2. 3-(2-Fluoropropanamido)-4-methoxy-N-phenylbenzamide (**1m**).* A total of 0.17 mL (0.18 mmol) of DIC and 0.24 g (0.18 mmol) of HOBt were added to a dichloromethane solution of 14.0 mL (0.12 mmol) of 2-fluoropropanoic acid and THF at room temperature, which was stirred for 0.5 h. Then, 0.35 g (0.14 mmol) of **1-M$_5$** was added to the mixture, and after approximately 3 h, 0.5 N NaOH was added to the solution. The lower layer was isolated, and 0.5 N HCl was added until the aqueous solution was neutral. The dichloromethane layer was washed three times with 10 mL of water, and $MgSO_4$ was then added. After 2 h, the solution was filtered, and the solvent was removed. The residue was purified by column chromatography on silica gel (eluent: petroleum ether:-ethyl acetate = 4:1) to yield the desired compound as a white solid (yield: 67%, HPLC purity: 99.40%). Mp: 182–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.12 (1H, s, NH), 9.22 (1H, s, NH), 8.49 (1H, s, PhH), 7.81 (1H, d, J = 6.0 Hz, PhH), 7.78 (2H,d, J = 7.6 Hz, PhH), 7.33 (2H, t, J = 7.6 Hz, PhH), 7.20 (1H, d, J = 8.8 Hz, PhH), 7.07 (1H, m, PhH), 5.34 (1H, qq, J_{H-H} = 6.4 Hz, J_{H-F} = 48.8 Hz, CH), 3.93 (3H, s, OCH_3), 1.55 (3H, dd, J_{H-H} = 6.4 Hz, J_{H-F} = 20.8 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{17}N_2O_3F$ $(M)^+$: 316.11803; measured: 316.12177.

*4.2.6.3. 3-(Cyclopentanecarboxamido)-4-methoxy-N-phenylbenzamide (**1l**).* Compound **1l** was synthesised using a method similar to that of **1m** and was isolated (eluent: petroleum ether:ethyl acetate = 4:1) as a white solid (yield: 63%, HPLC purity: 96.42%). Mp: 192–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.07 (1H, s, NH), 9.16 (1H, s, NH), 8.50 (1H, s, PhH), 7.81 (1H, d, J = 6.0 Hz, PhH), 7.78 (2H, d, J = 7.6 Hz, PhH), 7.33 (2H, m, PhH), 7.20 (1H, d, J = 8.8 Hz, PhH), 7.07 (1H, m, PhH), 3.90 (3H, s, OCH_3), 2.96 (1H, m), 1.83 (2H, m), 1.64 (4H, m), 1.55 (2H, m). HRMS-ESI (m/z): calculated for $C_{20}H_{23}N_2O_3$ $(M + H)^+$: 339.17056; measured: 339.17032.

*4.2.6.4. 3-(2-Fluoropropanamido)-2-methoxy-N-phenylbenzamide (**13**).* Compound **13** was synthesised using a method similar to that of **1m** and was isolated (eluent: petroleum ether:ethyl acetate = 4:1) as a white solid (yield: 65%, HPLC purity: 98.76%). Mp: 177–179 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.33 (1H, s, NH), 9.43 (1H, s, NH), 8.00 (1H, d, J = 7.5 Hz, PhH), 7.72 (2H, d, J = 9.0 Hz, PhH), 7.33 (3H, m, PhH), 7.22 (1H, d, J = 8.0 Hz, PhH), 7.07 (1H, t, J = 8.0 Hz, PhH), 5.34 (1H, qq, J_{H-H} = 6.5 Hz, J_{H-F} = 49 Hz, CH), 3.87 (3H, s, OCH_3), 1.55 (3H, dd, J_{H-H} = 6.5 Hz, J_{H-F} = 24.5 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{18}N_2O_3F$ $(M + H)^+$: 317.12956; measured: 317.12960.

*4.2.6.5. 3-(2-Fluoropropanamido)-5-methoxy-N-phenylbenzamide (**14**).* Compound **14** was synthesised using a method similar to that of **1m** and was isolated (eluent: petroleum ether:ethyl acetate = 4:1) as a white solid (yield: 65%, HPLC purity: 98.64%). Mp: 186–187 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.23 (1H, s, NH), 10.21 (1H, s, NH), 7.78 (1H, s, PhH), 7.55 (1H, s, PhH), 7.48 (1H, d, J = 8.0 Hz, PhH), 7.36 (2H, q, J = 8.0 Hz, PhH), 7.23 (1H, s, PhH), 7.11 (2H, m, PhH), 5.13 (1H, qq, J_{H-H} = 6.5 Hz, J_{H-F} = 49 Hz, CH), 3.82 (3H, s, OCH_3), 1.55 (3H, dd, J_{H-H} = 6.5 Hz, J_{H-F} = 24.5 Hz, CH_3). HRMS-ESI (m/z): calculated for $C_{17}H_{18}N_2O_3F$ $(M + H)^+$: 317.12955; measured: 317.12960.

*4.2.6.6. 5-(2-Fluoropropanamido)-2-methoxy-N-phenylbenzamide (**15**).* Compound **15** was synthesised using a method similar to that of **1m** and was isolated (eluent: petroleum ether:ethyl acetate = 4:1) as a white solid (yield: 65%, HPLC purity: 99.53%). Mp: 172–174 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 9.75 (1H, s, NH), 9.49 (1H, s, NH), 7.73 (2H, d, J = 7.0 Hz, PhH), 7.44 (1H, d, J = 7.5 Hz, PhH), 7.30 (3H, m, PhH), 7.02 (1H, m, PhH), 6.45 (1H, d, J = 7.5 Hz, PhH), 5.34 (1H, qq, J_{H-H} = 6.5 Hz, J_{H-F} = 49 Hz, CH), 3.84

(3H, s, OCH_3), 1.55 (3H, dd, J_{H-H} = 6.5 Hz, J_{H-F} = 24.5 Hz, CH_3). HRMS-ESI (*m*/*z*): calculated for $C_{17}H_{18}N_2O_3F$ $(M + H)^+$: 317.12957; measured 317.12960.

*4.2.6.7. 3-(2-Fluoropropanamido)-N-phenylbenzamide (**16**).* Compound **16** was synthesised using a method similar to that of **1m** and was isolated (eluent: petroleum ether:ethyl acetate = 4:1) as a white solid (yield: 57%, HPLC purity: 99.09%). Mp: 142–143 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.27 (1H, s, NH), 10.24 (1H, s, NH), 8.18 (1H, s, PhH), 7.88 (1H, d, J = 8.0 Hz, PhH), 7.76 (2H, d, J = 8.0 Hz, PhH), 7.67 (1H, d, J = 8.0 Hz, PhH), 7.49 (1H, m, PhH), 7.36 (2H, m, PhH), 7.11 (1H, m, PhH), 5.34 (1H, qq, J_{H-H} = 6.5 Hz, J_{H-F} = 49 Hz, CH), 1.55 (3H, dd, J_{H-H} = 6.5 Hz, J_{H-F} = 24.5 Hz, CH_3). HRMS-ESI (*m*/*z*): calculated for $C_{16}H_{16}N_2O_2F$ $(M + H)^+$: 287.11901; measured: 287.11903.

*4.2.7. General procedure for the synthesis of **21***

*4.2.7.1. N-(2-methoxy-5-nitrophenyl)propionamide (**19**).* A total of 2.5 mL (30 mmol) of propionyl chloride was added dropwise to a mixture of 5.0 g of 2-methoxy-5-nitroaniline (**18**) and 4.3 mL (30 mmol) of TEA in dichloromethane at 0 °C. The mixture was then stirred at room temperature until the starting material was completely disappeared. The solvent was evaporated in vacuo to produce a sticky residue that was extracted with dichloromethane and 0.5 N HCl (25 mL). The organic phase was concentrated, and the solid was purified by column chromatography on silica gel (eluent: petroleum ether:ethyl acetate = 3:1) to yield the desired compound as a white solid (yield: 74%). Mp: 192–193 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.12 (1H, s, NH), 8.51 (1H, s, PhH), 7.72 (1H, d, J = 7.6 Hz, PhH), 7.07 (1H, d, J = 7.6 Hz, PhH), 3.90 (3H,s, OCH_3), 2.41 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). ESI-MS (*m*/*z*): 225 $(M + H)^+$.

*4.2.7.2. N-(5-amino-2-methoxyphenyl)propionamide (**20**).* A total of 0.50 g (2.2 mmol) of **19** was dissolved in 20 mL of methanol, and 10% Pd/C was added. The mixture was reacted under H_2 at 50 bar for 5 h. Then, the solution was filtered, and the methanol was removed (yield: 57%). Mp: 167–169 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.12 (1H, s, NH), 8.51 (1H, s, PhH), 7.72 (1H, d, J = 7.6 Hz, PhH), 7.07 (1H, d, J = 7.6 Hz, PhH), 5.32 (2H, br s, NH_2), 3.90 (3H, s, OCH_3), 2.41 (2H, q, J = 7.6 Hz, CH_2), 1.07 (3H, t, J = 7.6 Hz, CH_3). ESI-MS (*m*/*z*): 195 $(M + H)^+$.

*4.2.7.3. N-(4-methoxy-3-propionamidophenyl)benzamide (**21**).* A total of 0.35 mL (3.0 mmol) of benzoyl chloride was added dropwise to a mixture of 0.58 g (3.0 mmol) of **20** and 0.25 mL (3.0 mmol) of TEA in dichloromethane at 0 °C. The mixture was then stirred at room temperature until the starting material was completely disappeared. The solvent was evaporated in vacuo to produce a sticky residue that was extracted with dichloromethane and 0.5 N HCl (25 mL). The organic phase was concentrated, and the solid was purified by column chromatography on silica gel (eluent: petroleum ether:ethyl acetate = 4:1) to yield the desired compound as a white solid (yield: 73%, HPLC purity: 98.65%). Mp: 183–185 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.12 (1H, s, NH), 9.00 (1H, s, NH), 8.30 (1H, s, PhH), 7.95 (2H, d, J = 7.0 Hz, PhH), 7.56 (4H, m, PhH), 7.00 (1H, d, J = 9.0 Hz, PhH), 3.81 (3H, s, OCH_3), 2.40 (2H, q, J = 7.5 Hz, CH_2), 1.08 (3H, t, J = 7.5 Hz, CH_3). HRMS-ESI (*m*/*z*): calculated for $C_{17}H_{19}N_2O_3$ $(M + H)^+$: 299.13907; measured: 299.13902.

4.3. Antiviral assays

MDCK cells, influenza A (A/Guangdong Luohu/219/2006, H1N1), influenza A/Tianjin Jinnan/15/2009 (H1N1, oseltamivir-resistant strain) and influenza B (B/Jifang/13/97) were obtained from the Institute of Virology, Chinese Academy of Preventive Medicine.

4.3.1. Cytotoxicity assay

The cytotoxicities of the compounds in the presence of MDCK cells were monitored by CPE method [17]. MDCK cells (2.5×10^4/well) were plated into a 96-well plate. After 24 h, the monolayer cells were incubated in the presence of various concentrations of the test compounds. After 48 h of culture at 37 °C and under a 5% CO_2 atmosphere in a carbon dioxide incubator, the cells were monitored by CPE. Median toxic concentrations (TC_{50}) were calculated by Reed and Muench [18] analyses.

4.3.2. Anti-influenza A assays

Confluent MDCK cells grown in 96-well microplates were infected with 100 median tissue culture infective doses ($100TCID_{50}$) of the test strains. After 2 h of adsorption at 37 °C, the monolayers were washed with phosphate-buffered saline (PBS) and incubated at 37 °C in the maintenance medium with or without various concentrations of the test compounds. The viral CPE was observed when the viral control group reached 4, and the antiviral activities (IC_{50}) of the bisaryl amide derivatives were determined with Reed and Muench analyses. The SI value was calculated with TC_{50}/IC_{50}.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in online version at http://dx.doi.org/10.1016/j.ejmech.2012.07.008.

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