Acta Pharmaceutica Sinica B 2015;5(3):201–209

HOSTED BY

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

ORIGINAL ARTICLE

Synthesis and antiviral activity of a series of novel *N*-phenylbenzamide and *N*-phenylacetophenone compounds as anti-HCV and anti-EV71 agents

Zhi Jiang, Huiqiang Wang, Yanping Li, Zonggen Peng, Yuhuan Li*, Zhuorong Li**

Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

Received 25 December 2014; received in revised form 28 January 2015; accepted 9 March 2015

KEY WORDS

Synthesis;
N-phenylbenzamide;
N-phenylacetophenone;
Anti-HCV;
Anti-EV71;
Agents

Abstract A series of novel *N*-phenylbenzamide and *N*-phenylacetophenone compounds were synthesized and evaluated for their antiviral activity against HCV and EV71 (strain SZ-98). The biological results showed that three compounds (**23**, **25** and **41**) exhibited considerable anti-HCV activity (IC_{50}=0.57–7.12 μmol/L) and several compounds (**23**, **28**, **29**, **30**, **31** and **42**) displayed potent activity against EV71 with the IC_{50} values lower than 5.00 μmol/L. The potency of compound **23** (IC_{50}=0.57 μmol/L) was superior to that of reported compounds IMB-1f (IC_{50}=1.90 μmol/L) and IMB-1g (IC_{50}=1.00 μmol/L) as anti-HCV agents, and compound **29** possessed the highest anti-EV71 activity, comparable to the comparator drug pirodavir. The efficacy *in vivo* and antiviral mechanism of these compounds warrant further investigations.

*Corresponding author. Tel.: +86 10 6301 0984; fax: +86 10 6301 7302.
**Corresponding author. Tel.: +86 10 8315 2017; fax: +86 10 6301 7302.
E-mail addresses: yuhuanlibj@126.com (Yuhuan Li), l-z-r@263.net (Zhuorong Li).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2015.03.013

1. Introduction

Viral infectious diseases have caused constant threat to human health and the prevention and treatment of viral diseases are a global public health challenge. Hepatitis C virus (HCV) is a single-stranded, positive-sense RNA virus. There was an estimated 170 million people worldwide infected with HCV, and many of them died from HCV-related liver diseases[1]. In the early years, the combination of PEG-interferon and ribavirin was the only treatment for HCV-infected patients with 40%–50% effectiveness[2]. Direct-acting antiviral agents (DAA) such as HCV NS3/4A protease inhibitors telaprevir and boceprevir have significantly increased the efficacy in combination with PEG-interferon and ribavirin[3]. Recently, a new generation of DAAs has become available and currently the interferon-free combination therapy of sofosbuvir (NS5B polymerase inhibitor)[4], ledipasvir (NS5A replication complex inhibitor)[5] and ribavirin has resulted in a cure rate up to 100% among patients infected with HCV. All of the four marketing drugs, telaprevir, boceprevir, sofosbuvir and ledipasvir were targeting on HCV proteins. However, resistant strains may eventually arise[6].

The single-stranded RNA virus EV71 belongs to the Picornaviridae family and usually causes a mild syndrome such as hand-foot-mouth disease (HFMD) and herpangina[7,8]. However, some young children infected by EV71 have developed fatal neurological complications such as aseptic meningitis, encephalitis, poliomyelitis-like paralysis and even death[9]. EV71 infection has been reported worldwide, especially in the Asia-Pacific region[10]. Currently, there was no effective approved antiviral available for the prevention or treatment of EV71 infection. Supportive therapy is the primary treatment option for severe cases[11–13].

Given the limited efficiency, side effects and drug resistance of these antiviral treatments, continued research is required to develop antiviral drugs with new mechanisms of action. Recently, cellular factors have emerged as useful targets for antiviral therapy and strengthening cellular defense mechanism may be an approach to develop broad-spectrum antivirals. APOBEC3G (Human apolipoprotein B mRNA–editing enzyme catalytic polypeptide-like 3G, hA3G) was initially identified as a host cellular restriction factor of HIV-1 by Sheehy et al.[14]. In addition, Peng[15] has demonstrated that the introduction of external hA3G into HCV-infected Huh7.5 cells effectively inhibited HCV replication. The small molecule compound IMB-26 (3, 4, 5-trimethoxyphenyl-3-(2-bromopropionamido)-4-methoxybenzamide) (Fig. 1) was identified as a stabilizer of hA3G and it was effective against HCV replication *in vitro* ($IC_{50}=1.46\pm0.62$ μmol/L, $CC_{50}=11.84\pm2.87$ μmol/L). Our previous work has described a series of substituted *N*-aryl benzamide compounds such as IMB-1f and IMB-1g (Fig. 1) as anti-HCV agents with the IC_{50} values ranging from 1.00 μmol/L to 2.00 μmol/L[16]. In addition, hA3G was also found to be active against other viruses, such as HBV and influenza virus[17,18]. Although there has been no report about the relationship between hA3G and EV71, in our laboratory, a series of novel *N*-phenylbenzamide derivatives were evaluated for their anti-EV71 activity ($IC_{50}=5.7$–12.0 μmol/L)[19]. The results showed that IMB-1e (Fig. 1) was the most active compound against EV71.

These results prompted us to further optimize the *N*-phenylbenzamide scaffold to discover more potent antiviral agents. The optimization began with the alkylation of the amino group by a methyl group at the C3-positon on the benzene ring A, and then lipophilic substituents were introduced to the benzene ring B. Meanwhile, we replaced methoxy group at the C4-positon on the benzene ring A with hydroxyl, methyl groups or a hydrogen atom. In addition, to identify the importance of the amide linker between the two aromatic rings A and B, a series of *N*-phenylacetophenone compounds were also studied. The antiviral activity of the synthesized compounds against HCV and EV71 was evaluated.

2. Results and discussion

2.1. Chemistry

To discover more potent hA3G antiviral agents based on the *N*-phenylbenzamide scaffold, we designed a series of novel *N*-phenylbenzamide derivatives. Our previous work has demonstrated that the amino group at the C3-position on the benzene ring A was crucial for antiviral activity. However, due to the cytotoxicity associated with the amino group, the alkylation products of the amino group at the C3-position on the benzene ring A were designed. Meanwhile, our previous results indicated that lipophilic substituents on the benzene ring B would exhibit strong biological activity. Thus, we introduced some lipophilic substituents into the benzene ring B. The amide linker between the two aromatic rings was unstable *in vivo*, and the hydrolysis of the amide-liker resulted in a loss of antiviral activity. Hence, we inserted a CH_2 group between the CO and NH groups to increase the stability of the amide linker. Additionally, we replaced methoxy group with hydroxyl, methyl groups or a hydrogen atom to investigate the influence of the methoxy group at the C4-positon on the benzene ring A for antiviral activity.

A series of *N*-phenylbenzamide analogs were prepared according to the procedures outlined in Schemes 1 and 2. Compounds **21–34** were synthesized using commercially available 4-methoxy-3-nitrobenzoic acid **1** as the starting material. It was converted into the corresponding benzoyl chloride **2**, which was condensed with a

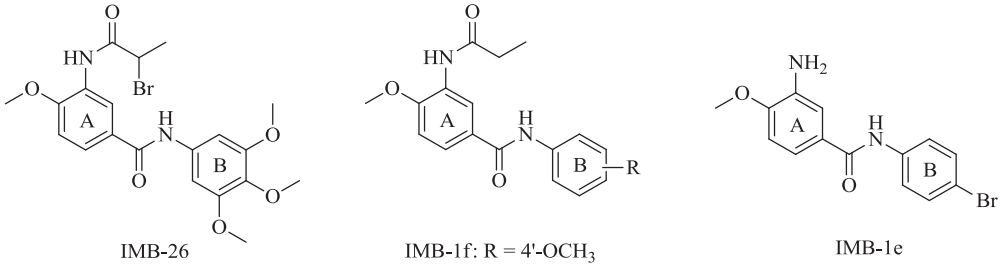
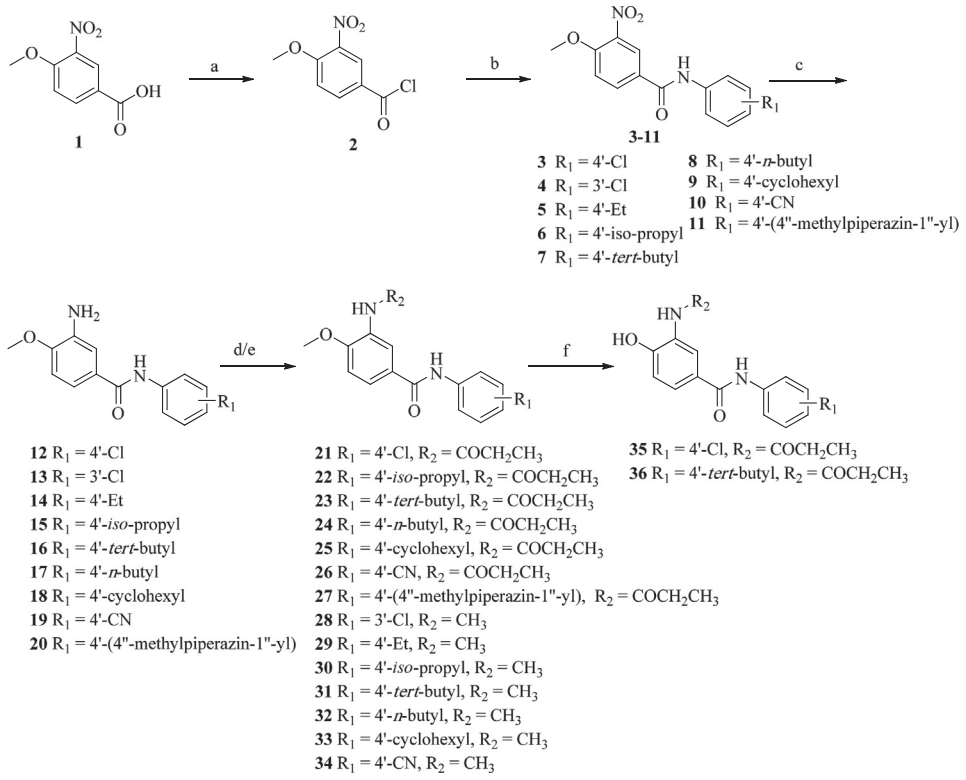
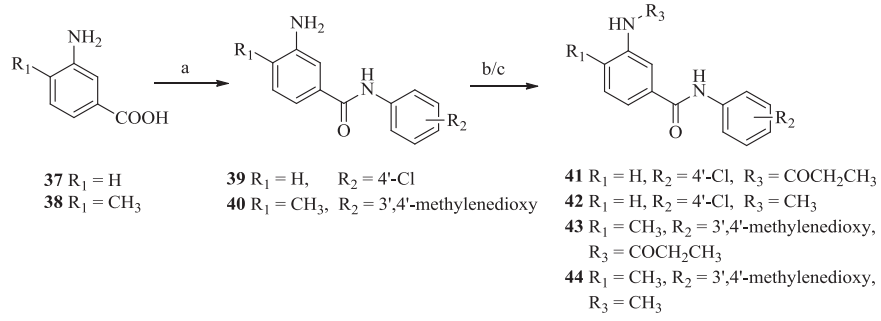


Figure 1 Chemical structures of the antiviral active compounds.



Scheme 1 Synthetic route for target compounds **22**–**36**. Reagents and conditions: (a) $SOCl_2$, reflux; (b) anilines, TEA, r.t.; (c) H_2, 10% dry Pd/C, r.t.; (d) CH_3CH_2COCl, TEA, r.t.; (e) 40% HCHO, r.t.; (f) BBr_3, −78 °C.



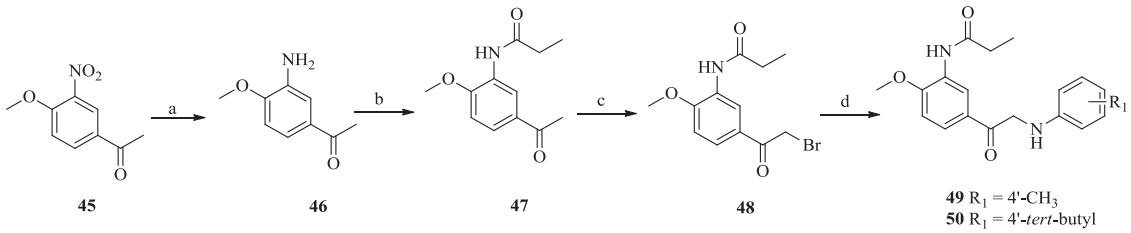
Scheme 2 Synthetic route for target compounds **41**–**44**. Reagents and conditions: (a) anilines, DIC, HOBt, r.t.; (b) CH_3CH_2COCl, TEA, r.t.; (c) CH_3I, K_2CO_3, 50 °C.

variety of anilines using trimethylamine (TEA) as the base to yield the intermediate compounds **3**–**11**. After the nitro groups of the intermediates **3**–**11** were reduced by hydrogen over 10% dry Pd/C, the amino groups at the C3-positon on the benzene ring A were then acylated by propionyl chloride to afford the 3-propionamido compounds **21**–**27** or condensed with formaldehyde to afford the 3-methylamido compounds **28**–**34**.

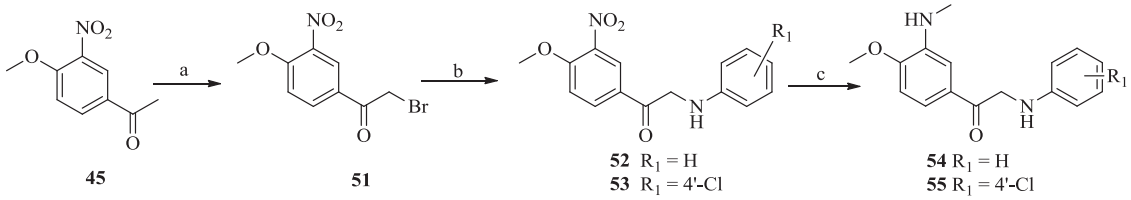
To convert the methoxy group to the hydroxyl group at the C4-position on the benzene ring A, compound **21** or **23** was demethylated using BBr_3 at −78 °C to afford the 4-hydroxyl compounds **35** and **36**.

To alter the methoxy group to the methyl group or a hydrogen atom at the C4-position on the benzene ring A, compounds **41**–**44** were prepared using 3-aminobenzoic acid **37** or 3-amino-4-methylbenzoic acid **38** as the starting materials, which was condensed with anilines using diisopropylcarbodiimide (DIC) as a coupling reagent and *N*-hydroxybenzotriazole (HOBt) as an activating reagent to yield the intermediates **39** or **40**. The amino groups were then acylated by propionyl chloride to afford the desired compounds **41** and **43**, or alkylated by iodomethane (CH_3I) to afford the desired compounds **42** and **44**.

To study the importance of the amide linker between the two aromatic rings A and B, we designed a series of *N*-phenylacetophenone compounds according to the procedures outlined in Schemes 3 and 4. The starting material 1-(4-methoxy-3-nitrophenyl) ethanone **45** was reduced by hydrogen over 10% dry Pd/C and then acylated by propionyl chloride to yield the intermediate **47**, which was converted to bromo compound **48** by NBS. The bromo compound was then reacted with anilines to afford the *N*-phenylacetophenone products **49** and **50**. The synthesis of the alkylation products of the amino group was conducted according to the procedure shown in Scheme 4.



Scheme 3 Synthetic route for target compounds **49** and **50**. Reagents and conditions: (a) H_2, 10% dry Pd/C, r.t.; (b) CH_3CH_2COCl, TEA, r.t.; (c) NBS, *p*-TSA, reflux; (d) anilines, $NaHCO_3$, r.t.



Scheme 4 Synthetic route for target compounds **54** and **55**. Reagents and conditions: (a) NBS, *p*-TSA, reflux; (b) anilines, $NaHCO_3$, r.t.; (c) H_2, 10% dry Pd/C, 40% HCHO, r.t.

Firstly, the starting material **45** was converted into the bromo compound **51**, which was then coupled with anilines to yield the intermediates **52** and **53**[20]. The desired compounds **54** and **55** were obtained using a one-pot procedure from the corresponding nitro aryls[21].

2.2. *Evaluation of anti-HCV activity*

The antiviral activity of the synthesized compounds against HCV was tested in infected Huh7.5 cells using the RT-PCR method, and VX-950 (telaprevir), a NS3/4A protease inhibitor, was used as a positive control. As shown in Table 1, three compounds **23**, **25** and **41** showed relatively potent anti-HCV activity with the IC_{50} values ranging from 0.57 μmol/L to 7.12 μmol/L. Among these three compounds, the anti-HCV activity of compound **23** (IC_{50} = 0.57 ± 0.00 μmol/L) was superior to that of the reported compounds IMB-26 (IC_{50} = 1.46 ± 0.62 μmol/L), IMB-1f (IC_{50} = 1.90 ± 0.57 μmol/L) and IMB-1f (IC_{50} = 1.00 ± 0.05 μmol/L). The cytotoxicity was lower, and especially the selective index (SI) of compound **23** was 53.4. But the potency and selectivity of all the tested compounds against HCV were much lower than that of the control drug telaprevir.

From the results above, we can observe that compounds **23**, **25** and **41** possessing the same substituent (propionamido group) at the C3-position on the benzene ring A showed higher anti-HCV activity than those with the methylamino group (**31**, **33** and **42**). Meanwhile, the substituents of these three compounds at the C4′-position on the benzene B were lipophilic (*tert*-butyl, cyclohexyl, and chloro groups). Thus, these indicated that the propionylamino group at the C3-position on the benzene ring A and lipophilic groups at the C4′-positon on the benzene ring B were the preferred substituents for the activity against HCV. Among these compounds with the propionamido group at the C3-position on the benzene ring A, compound **41** without a substituent has decreased antiviral activity compared with those with a methoxy substituent (**23** and **25**).

Table 1 Anti-HCV activity and cytotoxicity of the synthesized compounds.[a]

Compd.	IC_{50} (μmol/L)	CC_{50} (μmol/L)	SI
23	0.57 ± 0.00	30.65 ± 1.25	53.4
25	2.36 ± 0.12	122.24 ± 2.60	51.8
31	32.00 ± 2.14	56.63 ± 4.20	1.8
33	34.60 ± 3.10	39.96 ± 7.25	1.20
41	7.12 ± 2.48	> 200	> 28
42	19.51 ± 7.18	163.73 ± 9.84	8.00
VX-950	0.01 ± 0.00	21.27 ± 4.70	2331

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

2.3. *Evaluation of anti-EV71 activity*

The antiviral activity of the synthesized compounds against EV71 (strain SZ-98) were evaluated in Vero cells using the CPE method and a broad-spectrum picornavirus inhibitor, pirodavir, was used as a positive control. The results of antiviral activity are summarized in Table 2. The IC_{50} values of several *N*-phenylbenzamide derivatives (**23**, **28**, **29**, **30**, **31** and **42**) were lower than 5.00 μmol/L. Especially, the antiviral activity of **29** (IC_{50} = 0.95 ± 0.11 μmol/L) was close to that of the comparator drug (IC_{50} = 0.16 μmol/L). Moreover, the SI values of compound **28** and **29** were much larger than 20. Unfortunately, the *N*-phenylacetophenone compounds **49, 50**, **54** and **55** were inactive against EV71 in this experiment. The result might indicate that the amide linker between the two aromatic rings A and B was essential for anti-EV71 activity.

Based on the results of anti-EV71 activity above, the preliminary structure-activity relationships (SAR) have been established. The replacement of chlorine at the C4′-positon on the benzene ring B with other lipophilic substituents, such as isopropyl, *tert*-butyl, and *n*-butyl group (**22–24**) increased the anti-EV71 activity. However, the *N*-methyl piperazine derivative (**27**) did not display antiviral activity against EV71. So we concluded that lipophilic substituents on the benzene ring B was crucial to display anti-EV71

Table 2 Anti-EV71 (strain SZ-98) activity and cytotoxicity of the synthesized compounds.[a]

Compd.	IC_{50} (μmol/L)	TC_{50} (μmol/L)	SI
22	8.41±5.94	>65.35	>7.77
23	3.53±0.73	36.24±0.00	10.31
24	14.18±2.94	36.24±0.00	2.56
26	>68.79	297.67	–
27	>168.36	291.60±0.00	–
28	4.93±0.00	110.50±0.00	22.41
29	0.95±0.11	>26.09	>27.44
30	3.56±1.74	>8.29	>2.33
31	4.01±0.83	34.23±0.00	8.58
32	6.89±5.00	41.12±0.00	5.97
34	16.26±2.88	>79.07	>4.86
35	11.73±2.45	100.79±0.00	8.59
36	9.10±2.06	86.08±0.00	–
41	14.17±0.00	106.12±0.00	7.49
42	4.07±0.00	>28.5	>6.99
43	98.74±20.47	196.10±0.00	>2.00
44	19.63±7.42	322.65±0.00	16.45
49	>22.73	98.31	–
50	>20.14	>20.14	–
54	82.30±0.00	167.11±34.70	2.03
55	>24.38	73.10±0.00	–
Pirodavir	0.16±0.00	49.78±3.96	306.17

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

activity. When strong electron-withdrawing groups such as cyano group were introduced at the C4′-positon on the benzene ring B (**26**), a decreased activity was observed. In addition, altering the propionamido group at the C3-postion on the benzene ring A to the methylamino group (**28**–**34**) led to an increase in antiviral activity. In particular, the activity of the 4′-ethyl derivative (**29**) was close to that of the comparator drug pirodavir. The replacement of the methoxy group at the C4-positon on the benzene ring A with a hydroxy group (**35** and **36**), or a hydrogen atom (**42**) increased the antiviral activity. However, when a methyl group was placed at the C4-position on the benzene ring A (**43** and **44**), the antiviral activity was poor.

Because the amide linker was metabolically unstable *in vivo*, we inserted a CH_2 group between the CO and NH groups of the amide linker and synthesized four *N*-phenylacetophenone compounds (**49**, **50**, **54**, **55**). But the antiviral activity against EV71 was much lower than the corresponding *N*-phenylbenzamide compounds. The result highlighted the importance of the amide linker in anti-EV71 activity.

3. Conclusions

Our previous work has demonstrated that *N*-phenylbenzamide compounds were a class of potential broad-spectrum antiviral agents. In this paper, a series of novel *N*-phenylbenzamide and *N*-phenylacetophenone compounds with a methylamino group on the benzene ring A were synthesized. Lipophilic substituents were introduced at the C4′-position on the benzene ring B, or a CH_2 group was inserted between the CO and NH groups of the amide linker, or a methoxy group at the C4-positon on the benzene ring A was replaced with a hydroxy, a methyl group or a hydrogen atom. Totally 23 novel analogs were synthesized and evaluated for their antiviral activity against HCV and EV71 (strain SZ-98). Compounds **23**, **25** and **41** exhibited considerable anti-HCV activity with the IC_{50} values ranging from 0.57 to 7.12 μmol/L, and several compounds (**23**, **28**, **29**, **30**, **31** and **42**) exhibited potent activity against EV71 with IC_{50} values lower than 5.00 μmol/L. Particularly, the potency of compound **23** was superior to that of the reported compound IMB-26, IMB-1f and IMB-1g for anti-HCV activity, and compound **29** displayed the highest anti-EV71 activity almost comparable to the comparator drug pirodavir. Their efficacy *in vivo* and antiviral mechanism will be further investigated in the future.

4. Experimental

4.1. Synthesis and characterization

^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker BioSpin GmbH 400, 500 or 600 spectrometer. Chemical shifts were reported in parts per million relative to tetramethylsilane as an internal standard. High-resolution mass spectra (HRMS) were obtained on an MDS SCIEX Q-Trap mass spectrometer. Melting points were determined with an X6 microscope melting point apparatus and uncorrected. All reagents and solvents were purchased from J&K and Alfa Aesar Chemicals without purification.

*4.1.1. General procedure for the synthesis of compounds **12–20***

A solution of **1** (2.5 mmol) in $SOCl_2$ (5 mL) was refluxed for 3 h. The mixture was cooled to ambient temperature and then the solvent was removed under reduced pressure. Aniline (2.5 mmol) and TEA (2.5 mmol) were dissolved in dichloromethane (DCM) (20 mL) and a solution of the acyl chloride above in DCM (5 mL) was added to the reaction. After the mixture was stirred at room temperature for 5 h, the reaction was quenched with saturated aqueous NH_4Cl (20 mL), extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA = 10:1, *v/v*) to give **3**–**11**, which was then dissolved in CH_3OH (20 mL) followed by the addition of 10% dry Pd/C (0.01 mmol). The mixture was stirred under hydrogen for 4 h. The catalyst was filtered off and the combined organic solution was concentrated under reduced pressure to afford the intermediates **12**–**20**.

*4.1.2. General procedure for the synthesis of compounds **21–27***

To a solution of **12** or **15**–**20** (2.0 mmol) in DCM (20 mL) was added TEA (2.0 mmol), and then a solution of propionyl chloride (3.0 mmol) in DCM (5 mL) was added slowly. After the mixture was stirred at room temperature for 3 h, the reaction was extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried under Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA = 10:1, *v/v*) to give **21**–**27**.

*N-(4-Iso-propylphenyl)-4-methoxy-3-propionamidobenzamide (**22**)*: a white solid; yield: 89%. m.p: 171–173 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, 1H), 9.18 (s, 1H), 8.51 (s, 1H), 7.75 (dd, J = 8.6, 2.0 Hz, 1H), 7.68–7.64 (m, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.15 (d, J = 8.7 Hz, 1H), 3.91 (s, 3H), 2.88–2.85 (m, 1H), 2.42 (q, J = 7.5 Hz, 2H), 1.20 (d, J = 6.1 Hz, 6H), 1.09 (*t*, J = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.72, 165.34, 152.80, 143.92, 137.55, 127.50, 127.36, 126.70, 124.60, 122.65, 120.93, 110.85, 56.45, 33.37, 29.63, 24.45, 10.20.

HR-MS (ESI$^+$): 341.1857, Calcd. for $C_{20}H_{24}N_2O_3$: 341.1859 $[M+H]^+$.

N-(4-tert-Butylphenyl)-4-methoxy-3-propionamidobenzamide ***(23)***: a white solid; yield: 85%. m.p: 165–166 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, 1H), 9.18 (s, 1H), 8.51 (s, 1H), 7.74 (dd, J=8.6, 2.2 Hz, 1H), 7.68–7.66 (m, 2H), 7.37–7.34 (m, 2H), 7.15 (d, J=8.7 Hz, 1H), 3.92 (s, 3H), 2.42 (q, J=7.5 Hz, 2H), 1.29 (s, 9H), 1.09 (*t*, J=7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.73, 165.36, 152.80, 146.16, 137.23, 127.50, 127.36, 125.60, 124.60, 122.66, 120.59, 110.84, 56.45, 34.50, 31.69, 29.63, 10.20. HR-MS (ESI$^+$): 355.2014, Calcd. for $C_{21}H_{26}N_2O_3$: 355.2016 $[M+H]^+$.

N-(4-Butylphenyl)-4-methoxy-3-propionamidobenzamide ***(24)***: a white solid; yield: 82%. m.p: 165–168 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H), 9.18 (s, 1H), 8.50 (s, 1H), 7.74 (dd, J=8.6, 2.2 Hz, 1H), 7.66–7.63 (m, 2H), 7.15 (d, J=8.6 Hz, 3H), 3.91 (s, 3H), 2.55 (*t*, J=8.0 Hz, 2H), 2.42 (q, J=8.0 Hz, 2H), 1.59 − 1.51 (m, 2H), 1.31 (m, 2H), 1.09 (*t*, J=8.0 Hz, 3H), 0.91 (*t*, J=8.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.52, 165.35, 151.76, 137.80, 137.46, 128.73, 127.50, 127.39, 124.57, 122.63, 120.85, 110.86, 56.45, 34.73, 33.69, 29.63, 22.16, 14.26, 10.19. HR-MS (ESI$^+$): 355.2014, Calcd. for $C_{21}H_{26}N_2O_3$: 355.2016 $[M+H]^+$.

N-(4-Cyclohexylphenyl)-4-methoxy-3-propionamidobenzamide ***(25)***: a white solid, yield: 78%. m.p: 167–169 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.01 (s, 1H), 9.18 (s, 1H), 8.50 (s, 1H), 7.73 (dd, J=8.6, 2.2 Hz, 1H), 7.66–7.64 (m, 2H), 7.19–7.14 (m, 3H), 3.91 (s, 3H), 2.42 (m, 3H), 1.79 (d, J=10.5 Hz, 4H), 1.71 (m, 1H), 1.4 –1.37 (m, 4H), 1.28 − 1.25 (m, 1H), 1.09 (t, J=7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.72, 165.35, 143.18, 137.56, 127.51, 127.39, 127.06, 124.58, 122.63, 120.90, 110.84, 56.45, 43.71, 34.54, 29.63, 26.85, 26.09, 10.19. HR-MS (ESI$^+$): 381.2171, Calcd. for $C_{23}H_{29}N_2O_3$: 381.2172 $[M+H]^+$.

N-(4-Cyanophenyl)-4-methoxy-3-propionamidobenzamide ***(26)***: a white solid, yield: 75%. m.p: 154–157 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.51 (s, 1H), 9.22 (s, 1H), 8.56 (s, 1H), 8.01 − 7.98 (m, 2H), 7.83 − 7.81 (m, 2H), 7.78 (dd, J=8.6, 2.2 Hz, 1H), 7.20 (d, J=8.7 Hz, 1H), 3.94 (s, 3H), 2.44 (q, J=7.5 Hz, 2H), 1.08 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.82, 166.11, 153.20, 144.23, 133.51, 127.68, 126.56, 125.01, 122.64, 120.62, 119.62, 110.95, 105.45, 56.53, 29.64, 10.17. HR-MS (ESI$^+$): 324.1340, Calcd. for $C_{18}H_{17}N_3O_3$: 324.1342 $[M+H]^+$.

N-(4-(4-Methylpiperazin-1-yl)phenyl)-4-methoxy-3-propionamidobenzamide ***(27)***: a white solid, yield: 65%, m.p: 161–164 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.91 (s, 1H), 9.99 (s, 1H), 9.18 (s, 1H), 8.50 (s, 1H), 7.75 (dd, J=8.6, 2.0 Hz, 1H), 7.65 (d, J=9.0 Hz, 2H), 7.14 (d, J=8.7 Hz, 1H), 6.99 (d, J=9.0 Hz, 2H), 3.91 (s, 3H), 3.34 − 3.26 (m, 8H), 2.80 (s, 3H), 2.42 (q, J=7.4 Hz, 2H), 1.09 (t, J=7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.73, 165.11, 152.75, 146.20, 132.75, 127.47, 127.38, 124.54, 122.64, 121.98, 116.68, 110.84, 56.46, 52.57, 46.41, 42.43, 29.63, 10.20. HR-MS (ESI$^+$): 397.2231, Calcd. for $C_{22}H_{28}N_4O_3$: 397.2234 $[M+H]^+$.

4.1.3. General procedure for the synthesis of compounds ***28–34***

To the intermediates **13–19** (2.0 mmol) in CH_3OH (20 mL) was added 10% dry Pd/C (0.01 mmol) and 40% HCHO solution (3.0 mmol), and the mixture was stirred under hydrogen for 4 h. The catalyst was filtered off and the combined organic solutions were concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA = 10:1, *v/v*) to afford compounds **28–34**.

N-(3-Chlorophenyl)-4-methoxy-3-(methylamino)benzamide ***(28)***: a white solid, yield: 50%. m.p: 143–144 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.11 (s, 1H), 7.96 (t, J=2.0 Hz, 1H), 7.72 (dd, J=8.3, 1.0 Hz, 1H), 7.37 (t, J=8.1 Hz, 1H), 7.26 (dd, J=8.2, 2.1 Hz, 1H), 7.17–7.07 (m, 1H), 7.02 (d, J=2.1 Hz,1H), 6.91 (d, J=8.3 Hz, 1H), 5.25 (s, 1H), 3.86 (s, 3H), 2.79 (d, J=5.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.52, 149.74, 141.51, 139.52, 133.32, 130.66, 127.56, 123.33, 120.08, 119.00, 116.23, 109.01, 107.85, 56.04, 30.19. HR-MS (ESI$^+$): 291.0895, Calcd. for $C_{15}H_{15}N_2O_2Cl$: 291.0894 $[M+H]^+$.

N-(4-Ethylphenyl)-4-methoxy-3-(methylamino)benzamide ***(29)***: a white solid, yield: 67%. m.p: 156–157 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.88 (s, 1H), 7.66 (d, J=8.5 Hz, 2H), 7.26 (dd, J=8.3, 2.1 Hz, 1H), 7.17 (d, J=8.5 Hz, 2H), 7.04 (d, J=2.1 Hz, 1H), 6.89 (d, J=8.3 Hz, 1H), 5.21 (q, J=5.0 Hz, 1H), 3.85 (s, 3H), 2.79 (d, J=5.0 Hz, 3H), 2.58 (q, J=7.6 Hz, 2H), 1.18 (t, J=7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.09, 149.45, 139.43, 139.07, 137.64, 128.15, 128.10, 120.93, 116.04, 108.96, 107.92, 56.00, 30.22, 28.09, 16.21. HR-MS (ESI$^+$): 285.1597, Calcd. for $C_{17}H_{20}N_2O_2$: 285.1597 $[M+H]^+$.

N-(4-iso-propylphenyl)-4-methoxy-3-(methylamino)benzamide ***(30)***: a white solid, yield: 60%. m.p: 166–167 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.88 (s, 1H), 7.71–7.61 (m, 2H), 7.26 (dd, J=8.3, 2.1 Hz, 1H), 7.20 (d, J=8.5 Hz, 2H), 7.03 (d, J=2.1 Hz, 1H), 6.89 (d, J=8.4 Hz, 1H), 5.20 (m, 1H), 3.85 (s, 3H), 2.86 (m, 1H), 2.79 (d, J=5.1 Hz, 3H), 1.20 (d, J=4.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.08, 149.45, 143.74, 139.43, 137.71, 128.09, 126.65, 120.92, 116.03, 108.95, 107.93, 56.00, 33.36, 30.22, 24.46. HR-MS (ESI$^+$): 299.1752, Calcd. for $C_{18}H_{22}N_2O_2$: 299.1754 $[M+H]^+$.

N-(4-tert-Butylphenyl)-4-methoxy-3-(methylamino)benzamide ***(31)***: a white solid, yield: 69%. m.p: 145–146 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.89 (s, 1H), 7.74 − 7.60 (m, 2H), 7.42 − 7.31 (m, 2H), 7.27 (dd, J=8.3, 2.1 Hz, 1H), 7.04 (d, J=2.1 Hz, 1H), 6.89 (d, J=8.4 Hz, 1H), 5.21 (q, J=4.7 Hz, 1H), 3.86 (s, 3H), 2.79 (d, J=5.0 Hz, 3H), 1.29 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.09, 149.46, 145.98, 139.44, 137.39, 128.08, 125.54, 120.58, 116.04, 108.94, 107.94, 56.00, 34.48, 31.70, 30.23. HR-MS (ESI$^+$): 313.1906, Calcd. for $C_{19}H_{24}N_2O_2$: 313.1910 $[M+H]^+$.

N-(4-Butylphenyl)-4-methoxy-3-(methylamino)benzamide ***(32)***: a white solid, yield: 60%. m.p: 160–162 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.89 (s, 1H), 7.65 (d, J=8.5 Hz, 2H), 7.25 (dd, J=8.3, 2.1 Hz, 1H), 7.14 (d, J=8.5 Hz, 2H), 7.02 (d, J=2.1 Hz, 1H), 6.89 (d, J=8.4 Hz, 1H), 5.23 (m, 1H), 3.85 (s, 3H), 2.78 (d, J=5.1 Hz, 3H), 2.55 (t, J=7.6 Hz, 2H), 1.58 − 1.52 (m, 2H), 1.33–1.29 (m, 2H), 0.90 (t, J=7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.09, 149.44, 139.43, 137.62, 128.68, 128.11, 120.87, 116.02, 108.97, 107.91, 56.00, 34.73, 33.70, 30.22, 22.17, 14.27. HR-MS (ESI$^+$): 313.1907, Calcd. for $C_{19}H_{24}N_2O_2$: 313.1910 $[M+H]^+$.

N-(4-Cyclohexylphenyl)-4-methoxy-3-(methylamino)benzamide ***(33)***: a white solid, yield: 65%. m.p: 165–167 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.87 (s, 1H), 7.66 − 7.64 (m, 2H), 7.25 (dd, J=8.3, 2.1 Hz, 1H), 7.17 (d, J=8.5 Hz, 2H), 7.02 (d, J=2.1 Hz, 1H), 6.89 (d, J=8.4 Hz, 1H), 5.21 (m, 1H), 3.85 (s, 3H), 2.78 (d, J=5.1 Hz, 3H), 2.46 (s, 1H), 1.79 (m, 4H), 1.71 (m, 1H), 1.4 − 1.31 (m, 4H), 1.22 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.08, 149.44, 143.01, 139.43, 137.73, 128.11, 127.01, 120.89, 116.03, 108.96, 107.92, 56.00, 43.70, 34.56,

30.22, 26.86, 26.09. HR-MS (ESI^+): 339.2065, Calcd. for $C_{21}H_{27}N_2O_2$: 339.2067 $[M+H]^+$.

*N-(4-Cyanophenyl)-4-methoxy-3-(methylamino)benzamide (**34**)*: a white solid, yield: 73%. m.p: 165–167 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 10.36 (s, 1H), 8.01−7.97 (m, 2H), 7.82−7.79 (m, 2H), 7.28 (dd, J=8.3, 2.2 Hz, 1H), 7.02 (d, J=2.1 Hz, 1H), 6.93 (d, J=8.4 Hz, 1H), 5.28 (m, 1H), 3.87 (s, 3H), 2.78 (t, J=7.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.87, 149.94, 144.38, 139.57, 133.50, 127.35, 120.52, 119.66, 116.44, 109.01, 107.88, 105.26, 56.07, 30.17. HR-MS (ESI^+): 282.1236, Calcd. for $C_{16}H_{15}N_3O_2$: 282.1237 $[M+H]^+$.

*4.1.4. General procedure for the synthesis of compounds **35–36***

To a solution of **21** or **23** (1.0 mmol) in DCM (20 mL) was added BBr_3 (1.2 mmol) in DCM (5 mL) over 15 min at −78 °C. The resulting solution was allowed to warm to 25 °C and stirred for 2 h. The reaction was quenched with saturated aqueous NH_4Cl, extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA=10:1, *v/v*) to give **35–36**.

*N-(4-Chlorophenyl)-4-hydroxy-3-propionamidobenzamide (**35**)*: a white solid, yield: 34%. m.p: 179–181 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 10.55 (s, 1H), 10.15 (s, 1H), 9.31 (s, 1H), 8.36 (d, J=1.2 Hz, 1H), 7.82−7.78 (m, 2H), 7.62 (dd, J=8.4, 2.0 Hz, 1H), 7.41−7.37 (m, 2H), 6.96 (d, J=8.4 Hz, 1H), 2.43 (q, J=7.5 Hz, 2H), 1.10 (t, J=7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 173.17, 165.73, 138.91, 128.90, 127.31, 126.61, 125.76, 125.00, 123.18, 122.20, 115.48, 40.19, 39.77, 39.35, 29.54, 10.21. HR-MS (ESI^+): 319.0842, Calcd. for $C_{16}H_{15}N_2O_3Cl$: 319.0844 $[M+H]^+$.

*N-(4-tert-Butylphenyl)-4-hydroxy-3-propionamidobenzamide (**36**)*: a white solid, yield: 40%. m.p: 182–184 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 10.46 (s, 1H), 9.95 (s, 1H), 9.32 (s, 1H), 8.33 (s, 1H), 7.65−7.61 (m, 3H), 7.35 (d, J=8.6 Hz, 2H), 6.95 (d, J=8.4 Hz, 1H), 2.43 (m, 2H), 1.28 (s, 9H), 1.10 (t, J=7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 173.18, 165.44, 151.60, 146.05, 137.30, 126.50, 126.21, 125.58, 124.92, 123.26, 120.53, 115.48, 34.49, 31.69, 29.52, 10.22. HR-MS (ESI^+): 341.1859, Calcd. for $C_{20}H_{25}N_2O_3$: 341.1859 $[M+H]^+$.

*4.1.5. General procedure for the synthesis of **39, 40***

To a solution of **37** or **38** (3.6 mmol) in DCM (30 mL) and DMF (5 mL) was added DIC (4.3 mmol), HOBt (4.3 mmol) and aniline (3.8 mmol). After the mixture was stirred at room temperature for 24 h, the reaction was extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA=5:1, *v/v*) to give **39** or **40**.

*4.1.6. General procedure for the synthesis of **41** or **43***

To a solution of **39** or **40** (2.0 mmol) in DCM (30 mL) was added TEA (2.0 mmol), and then the solution of propionyl chloride (3.0 mmol) was added slowly. After the mixture was stirred at room temperature for 3 h, the reaction was extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA =10:1, *v/v*) to give **41** or **43**.

*N-(4-Chlorophenyl)-3-propionamidobenzamide (**41**)*: a white solid, yield: 80%. m.p: 160–163 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 10.38 (s, 1H), 10.08 (s, 1H), 8.10 (s, 1H), 7.83 (m, 3H), 7.60 (d, J=7.8 Hz, 1H), 7.44 (m, 3H), 2.35 (q, J=7.5 Hz, 2H), 1.10 (t, J=7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.70, 166.17, 140.02, 138.62, 135.88, 129.21, 129.00, 127.69, 122.52, 122.32, 122.25, 118.95, 29.98, 10.07. HR-MS (ESI^+): 303.0893, Calcd. for $C_{16}H_{15}N_2O_2Cl$: 303.0894 $[M+H]^+$.

*N-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-4-methyl-3-propionamidobenzamide (**43**)*: a white solid, yield: 77%. m.p: 167–168 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H), 9.42 (s, 1H), 7.94 (s, 1H), 7.68 (dd, J=7.9, 1.6 Hz, 1H), 7.38 (d, J=2.4 Hz, 1H), 7.34 (d, J=8.0 Hz, 1H), 7.20 (dd, J=8.8, 2.5 Hz, 1H), 6.82 (d, J=8.7 Hz, 1H), 5.76 (s, 1H), 4.23 (q, J=5.0 Hz, 4H), 2.38 (q, J=7.6 Hz, 2H), 2.26 (s, 3H), 1.12 (t, J=7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 172.60, 165.14, 143.23, 140.01, 137.04, 136.32, 133.33, 133.17, 130.55, 125.19, 124.60, 117.01, 114.14, 109.98, 64.53, 29.35, 18.47, 10.40. HR-MS (ESI^+): 341.1494, Calcd. for $C_{19}H_{20}N_2O_4$: 341.1495 $[M+H]^+$.

*4.1.7. General procedure for the synthesis of compounds **42** or **44***

To a solution of **39** or **40** (2.0 mmol) in acetone (15 mL) was added K_2CO_3 (3.0 mmol) and CH_3I (5.00 mmol), and then the resulting solution was stirred at 50 °C for 10 h. The mixture was cooled to ambient temperature and the solvent was removed under reduced pressure. The residue was dissolved in DCM (35 mL), and washed with brine. The organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA=10:1, *v/v*) to afford **42** or **44**.

*N-(4-Chlorophenyl)-3-(methylamino)benzamide (**42**)*: a white solid, yield: 45%. m.p: 164–167 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 7.60 (d, J=8.8 Hz, 2H), 7.33 (d, J=8.8 Hz, 2H), 7.12 (s, 1H), 7.07 (d, J=7.6 Hz, 1H), 6.78 (dd, J=8.1, 2.1 Hz, 1H), 2.90 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ 166.92, 150.38, 138.79, 136.08, 129.29, 128.92, 127.47, 122.22, 115.31, 115.01, 110.93, 30.17. HR-MS (ESI^+): 261.0789, Calcd. for $C_{14}H_{13}N_2OCl$: 261.0789 $[M+H]^+$.

*N-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-4-methyl-3-(methylamino)benzamide (**44**)*: a white solid, yield: 77%. m.p: 170–173 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 9.85 (s, 1H), 7.39 (d, J=2.4 Hz, 1H), 7.19 (dd, J=8.8, 2.4 Hz, 1H), 7.10 (m, 2H), 6.98 (d, J=1.0 Hz, 1H), 6.81 (d, J=8.7 Hz, 1H), 5.24 (q, J=4.9 Hz, 1H), 4.23 (m, 4H), 2.80 (d, J=4.9 Hz, 3H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 166.31, 147.99, 143.24, 139.86, 134.11, 133.52, 129.63, 125.86, 116.99, 115.17, 114.05, 109.89, 107.73, 64.64, 64.41, 30.52, 23.76. HR-MS (ESI^+): 299.1388, Calcd. for $C_{17}H_{18}N_2O_3$: 299.1390 $[M+H]^+$.

*N-(5-(2-Bromoacetyl)-2-methoxyphenyl)propionamide (**48**)*: to a solution of **45** (1.0 mmol) in CH_3OH (15 mL) was added 10% dry Pd/C (0.01 mmol), the mixture was reacted with hydrogen (40 psi) for 4 h. The catalyst was filtered off and the combined organic solutions were concentrated under reduced pressure. The residue was dissolved in DCM (25 mL) followed by the addition of TEA (1.0 mmol), and then a solution of propionyl chloride (1.5 mmol) was added slowly. After the mixture was stirred at room temperature for 3 h, the reaction mixture was extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue **47** (1.5 mmol) was dissolved in CCl_4 (25 mL) followed by the addition of NBS (1.2 mmol) and *p*-TSA (0.01 mmol). After the mixture was refluxed for 5 h, the

mixture was cooled to ambient temperature and then the reaction was quenched with saturated aqueous NH_4Cl (30 mL), extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA = 10:1, *v/v*) to give **48**: a white solid, yield: 70%. m.p: 101–103 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.37 (s, 1H), 8.22 (d, J = 2.0 Hz, 1H), 7.68 (dd, J = 8.9, 2.0 Hz, 1H), 7.12 (d, J = 8.7 Hz, 1H), 4.72 (s, 2H), 3.92 (s, 3H), 2.71 (q, J = 7.7 Hz, 2H), 1.21 (t, J = 7.9 Hz, 3H). ESI-MS (*m/z*): 300.1 $[M+H]^+$.

4.1.8. General procedure for the synthesis of *49* or *50*

To a solution of **48** (0.5 mmol) in ethanol (15 mL) was added $NaHCO_3$ (1.0 mmol) and aniline (0.55 mmol). After the mixture was stirred at room temperature for 24 h, the solvent was removed under reduced pressure. The residue was extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA = 10:1, *v/v*) to give **49** or **50**.

*N-(2-Methoxy-5-(2-(p-tolylamino)acetyl)phenyl)propionamide (**49**)*: a yellow solid, yield: 34%. m.p: 132–134 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.23 (s, 1H), 8.62 (s, 1H), 7.91 (dd, J = 8.6, 1.8 Hz, 1H), 7.17 (d, J = 8.7 Hz, 1H), 6.90 (d, J = 8.1 Hz, 2H), 6.58 (d, J = 8.3 Hz, 2H), 5.59 (t, J = 5.3 Hz, 1H), 4.55 (d, J = 5.4 Hz, 2H), 3.94 (s, 3H), 2.43 (q, J = 7.5 Hz, 2H), 2.15 (s, 3H), 1.08 (t, J = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 195.62, 172.91, 154.21, 146.33, 129.72, 128.11, 128.04, 125.81, 124.94, 113.04, 111.14, 56.61, 50.17, 29.64, 20.55, 10.13. HR-MS (ESI^+): 327.1700, Calcd. for $C_{19}H_{22}N_2O_3$: 327.1703 $[M+H]^+$.

*N-(5-(2-((4-(tert-Butyl)phenyl)amino)acetyl)-2-methoxyphenyl) propionamide (**50**)*: yellow solid. yield: 25%. m.p: 130–132 °C. ^{1}H NMR (500 MHz, DMSO): δ 9.24 (s, 1H), 8.63 (s, 1H), 7.91 (dd, J = 8.6, 2.2 Hz, 1H), 7.18 (d, J = 8.7 Hz, 1H), 7.12–7.09 (m, 2H), 6.60 − 6.58 (m, 2 H), 5.62 (t, J = 5.4 Hz, 1H), 4.55 (d, J = 5.5 Hz, 2H), 3.94 (s, 3H), 2.43 (d, J = 7.5 Hz, 2H), 1.22 (s, 9H), 1.08 (t, J = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 195.76, 172.91, 154.20, 146.23, 138.80, 128.12, 128.06, 125.87, 125.79, 121.68, 112.67, 111.15, 56.61, 50.12, 33.91, 31.93, 29.65, 10.13. HR-MS (ESI^+): 369.2169, Calcd. for $C_{22}H_{28}N_2O_3$: 369.2172 $[M+H]^+$.

4.1.9. General procedure for the synthesis of *52* or *53*

To a solution of **45** (0.5 mmol) in CCl_4 (15 mL) was added NBS (0.6 mmol) and *p*-TSA (0.01 mmol). After the mixture was refluxed for 5 h, the mixture was cooled to ambient temperature and then the reaction was quenched with saturated aqueous NH_4Cl (30 mL), extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA = 10:1, *v/v*) to give **51**, which was then dissolved in ethanol (25 mL) followed by the addition of $NaHCO_3$ (1.0 mmol) and anilines (0.55 mmol). After the mixture was stirred at room temperature for 24 h, the solvent was removed under reduced pressure. The residue was extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA = 10:1, *v/v*) to give **52** or **53**.

*1-(4-Methoxy-3-nitrophenyl)-2-(phenylamino)ethanone (**52**)*: a yellow solid, yield: 79%. ESI-MS (*m/z*): 287.1 $[M+H]^+$.

*2-((4-Chlorophenyl)amino)-1-(4-methoxy-3-nitrophenyl)ethanone (**53**)*: a yellow solid, yield: 60%. ESI-MS (*m/z*): 321.0 $[M+H]^+$.

4.1.10. General procedure for the synthesis of *54* or *55*

To a solution of **52** or **53** (1.0 mmol) in CH_3OH (15 mL) was added 10% dry Pd/C (0.01 mmol) and 40% HCHO (2.5 mmol). The mixture was stirred under hydrogen for 4 h. The catalyst was filtered off and the combined organic solutions were concentrated under reduced pressure. The residue was purified by column chromatography (PE/EA = 10:1, *v/v*) to afford **54** or **55**.

*1-(4-Methoxy-3-(methylamino)phenyl)-2-(phenylamino)ethanone (**54**)*: a yellow solid, yield: 32%. m.p: 135–137 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.45 (dd, J = 8.3, 1.7 Hz, 1H), 7.08 (t, J = 7.7 Hz, 2H), 7.04 (d, J = 1.5 Hz, 1H), 6.92 (d, J = 8.3 Hz, 1H), 6.68 (d, J = 8.2 Hz, 2H), 6.56 (t, J = 7.2 Hz, 1H), 5.76 (t, J = 5.3 Hz, 1H), 5.32 (q, J = 4.8 Hz, 1H), 4.58 (d, J = 5.4 Hz, 2H), 3.88 (s, 3H), 2.77 (d, J = 5.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 195.94, 151.27, 148.67, 139.79, 129.26, 129.01, 117.81, 116.50, 112.96, 109.09, 106.85, 56.14, 49.72, 30.10. HR-MS (ESI^+): 271.1440, Calcd. for $C_{16}H_{18}N_2O_2$: 271.1441 $[M+H]^+$.

*2-((4-Chlorophenyl)amino)-1-(4-methoxy-3-(methylamino)phenyl)ethanone (**55**)*: a yellow solid, yield: 25%. m.p: 140–141 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.44 (dd, J = 8.3, 2.1 Hz, 1H), 7.12 − 7.08 (m, 2H), 7.03 (d, J = 2.0 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.71 − 6.68 (m, 2H), 6.01 (t, J = 5.4 Hz, 1H), 5.30 (q, J = 5.1 Hz, 1H), 4.58 (d, J = 5.4 Hz, 2H), 3.88 (s, 3H), 2.77 (d, J = 5.1 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 195.61, 151.30, 147.70, 139.79, 128.93, 128.90, 119.68, 117.83, 114.34, 109.08, 106.84, 56.14, 49.73, 30.10. HR-MS (ESI^+): 305.1051, Calcd. for $C_{16}H_{17}N_2O_2Cl$: 305.1051 $[M+H]^+$.

4.2. Cells and virus

Huh7.5 human liver cells were kindly provided by Vertex Pharmaceuticals (Boston, USA), and were cultured in Dulbecco's Modified Eagle's Medium, which was supplemented with 10% inactivated fetal bovine serum and 1% penicillin-streptomycin. The cells were cultured at 37 °C in 5% CO_2, released with 0.05% trypsin-EDTA and split twice a week. The plasmid pFL-J6/JFH/JC1, which contains the full-length chimeric HCV cDNA was kindly provided by Vertex Pharmaceutical (Boston, USA). Vero cells were purchased from the American Type Culture Collection and were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/mL penicillin G and 100 mg/mol streptomycin). EV71 strain SZ-98 was kindly provided by Dr Qi Jin, Institute of Pathogen Biology, Chinese Academy of Medical Sciences and Peking Union Medical School.

4.3. Cytotoxicity assay

The Huh7.5 cells were used in the test; Huh7.5 cells (1×10^4 cells/well) were planted into 96-microwell plates. Six hours later the culture media was replaced with fresh medium containing the tested compounds at various concentrations. Cytotoxicity was evaluated by the MTT assay at 96 h. The 50% cytotoxic

concentration (CC_{50}) was calculated with the Reed & Muench method.

The cytotoxic effect of the target compounds on Vero cells was assayed by the CPE method. Briefly, cells were seeded into 96-well culture plates (3×10^4 cells/well) and were incubated overnight. Then, different concentrations of the test compounds were applied in triplicate. After incubation for 3 d, Median toxic concentration (TC_{50}) was defined as the concentration that inhibits 50% cellular growth in comparison with untreated controls and calculated by the Reed and Muench method.

4.4. Anti-HCV assay

The Huh7.5 cells were seeded into 6-well plates (Costar) at a density of 3×10^4 cells/cm^2. After 6 h incubation, cells were infected with HCV viral stock (45 u/cell) and treated simultaneously with the test compounds or the control. The culture medium was removed after 96 h inoculation and the intracellular total RNA was extracted with RNeasy Mini Kit (Qiagen, Hilden, Germany). The HCV RNA was quantified directly with a one-step RTPCR kit (Invitrogen). The 50% inhibition concentration (IC_{50}) was calculated with the Reed & Muench method. SI value was calculated as the ratio of CC_{50}/IC_{50}.

4.5. Anti-EV71 assay

The anti-EV71 activity of the target compounds was also assayed by the CPE method. Briefly, cells (3×10^4 cells/well) were plated into 96-well culture plates. The cells were infected with EV71 of 100TCID50. Then, various concentrations of the test compounds were supplemented immediately for incubation of another 48 h. The IC_{50} was determined by the Reed and Muench method. The SI value was calculated as the ratio of TC_{50}/IC_{50}.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 81273439 and 81202414) and Student Fund of Innovation Project of Peking Union Medical College (No. 2013-1007-10).

References

1. Davila JA, Morgan RO, Shaib Y, McGlynn KA, EI-Serag HB. HCV and increasing incidence of hepato cellular carcinoma: apopulation-based study. *Gastroenterology* 2004;**127**:1372–80.
2. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon α-2b or α-2a with ribavirinfor treatment of hepatitis infection. *N Engl J Med* 2009;**361**:580–93.
3. Stankiewicz-Drogoń A, Dörner B, Erker T, Boguszewska-Chachulska AM. Synthesis of new acridone derivatives, inhibitors of NS3 helicase, which efficiently and specifically inhibit subgenomic HCV replication. *J Med Chem* 2010;**53**:3117–26.
4. Kayali Z, Schmidt WN. Finally sofosbuvir: an oral anti-HCV drug with wide performance capability. *Pharmgenomice Pers Med* 2014;**7**:387–98.
5. Gentile I, Buonomo AR, Borgia F, Castaldo G, Borgia G. Ledipasvir: a novel synthetic antiviral for the treatment of HCV infection. *Expert Opin Investig Drugs* 2014;**23**:561–71.
6. Lin C, Lin K, Luong YP, Rao BG, Wei YY, Brennan DL, et al. *In vitro* resistance studies of hepatitis C virus serine protease inhibitors, VX-950 and BILN2061: structural analysis indicates different resistance mechanisms. *J Biol Chem* 2004;**279**:17508–14.
7. Schmidt NJ, Lennette EH, Ho HH. An apparently new enterovirus isolated from patients with disease of the central nervous system. *J Infect Dis* 1974;**129**:304–9.
8. Chang LY, Lin TY, Hsu KH, Huang YC, Lin KL, Hsueh C, et al. Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. *Lancet* 1999;**354**:1682–6.
9. Shimizu H, Utama A, Yoshii K, Yoshida H, Yoneyama T, Sinniah M, et al. Enterovirus 71 from fatal and nonfatal cases of hand, foot and mouth disease epidemics in Malaysia, Japan and Taiwan in 1997–1998. *Jpn J Infect Dis* 1999;**52**:12–5.
10. Yang F, Ren L, Xiong Z, Li J, Xiao Y, Zhao R, et al. Enterovirus 71 outbreak in the People's Republic of China in 2008. *J Clin Microbiol* 2009;**47**:2351–2.
11. Wu KX, Ng MM, Chu JJ. Developments towards antiviral therapies against enterovirus 71. *Drug Discov Today* 2010;**15**:1041–51.
12. Shang LQ, Xu MY, Yin Z. Antiviral drug discovery for the treatment of enterovirus 71 infections. *Antivir Res* 2013;**97**:183–94.
13. Kuo RL, Shih SR. Strategies to develop antivirals against enterovirus 71. *Virol J* 2013;**10**:28–35.
14. Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 2002;**418**:646–50.
15. Peng ZG, Zhao ZY, Li YP, Wang YP, Hao LH, Fan B, et al. Host apolipoprotein B messenger RNA–editing enzyme catalytic polypeptide-like 3G is an innate defensive factor and drug target against hepatitis C virus. *Hepatology* 2011;**53**:1980–9.
16. Li YP, Peng ZG, Hao LH, Wu ZY, Zhu YP, Hu LX, et al. Synthesis of novel substituted *N*-aryl benzamides as hA3G stabilizers and their inhibitory activity against hepatitis C virus replication. *Acta Pharm Sin B* 2013;**3**:312–21.
17. Mangeat B, Turelli P, Caron G, Friedli M, Perrin L, Trono D. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* 2003;**424**:99–103.
18. Hao LH, Li YP, He WY, Wang HQ, Shan GZ, Jiang JD, et al. Synthesis and antiviral activity of substituted bisarylamide compounds as novel influenza virus inhibitors. *Eur J Med Chem* 2012;**55**:117–24.
19. Ji XY, Wang HQ, Hao LH, He WY, Gao RM, Li YP, et al. Synthesis and antiviral activity of *N*-phenylbenzamide derivatives, a novel class of enterovirus 71 inhibitors. *Molecules* 2013;**18**:3630–40.
20. Pal MJ, Swamy NK, Hameed SH, Padakanti S, Yeleswarapu KR. A rapid and direct access to symmetrical/unsymmetrical 3, 4-diaryl-maleimides and pyrrolin-2-ones. *Tetrahedron* 2004;**60**:3987–97.
21. Lunde SA, Sydnes MO. One-pot procedures for the formation of secondary aryl amines from nitro aryls. *Synlett* 2013;**24**:2340–4.