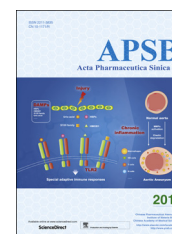


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ORIGINAL ARTICLE

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



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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 ($IC_{50}=0.57\text{--}7.12\ \mu\text{mol/L}$) and several compounds (**23**, **28**, **29**, **30**, **31** and **42**) displayed potent activity against EV71 with the IC_{50} values lower than $5.00\ \mu\text{mol/L}$. The potency of compound **23** ($IC_{50}=0.57\ \mu\text{mol/L}$) was superior to that of reported compounds IMB-1f ($IC_{50}=1.90\ \mu\text{mol/L}$) and IMB-1g ($IC_{50}=1.00\ \mu\text{mol/L}$) 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.

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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 diseases¹. In the early years, the combination of PEG-interferon and ribavirin was the only treatment for HCV-infected patients with 40%–50% effectiveness². 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 ribavirin³. 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 arise⁶.

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 death⁹. EV71 infection has been reported worldwide, especially in the Asia-Pacific region¹⁰. 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, Peng¹⁵ 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* ($IC_{50} = 1.46 \pm 0.62 \mu\text{mol/L}$, $CC_{50} = 11.84 \pm 2.87 \mu\text{mol/L}$). 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 $\mu\text{mol/L}$ to 2.00 $\mu\text{mol/L}$ 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 ($IC_{50} = 5.7\text{--}12.0 \mu\text{mol/L}$)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-position on the benzene ring A, and then lipophilic substituents were introduced to the benzene ring B. Meanwhile, we replaced methoxy group at the C4-position 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-linker 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-position 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

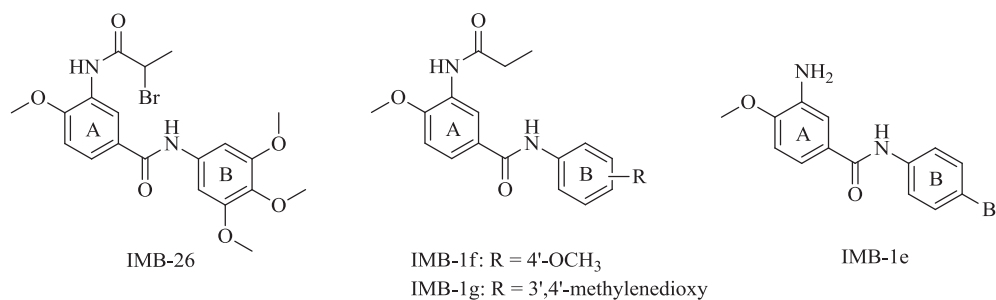
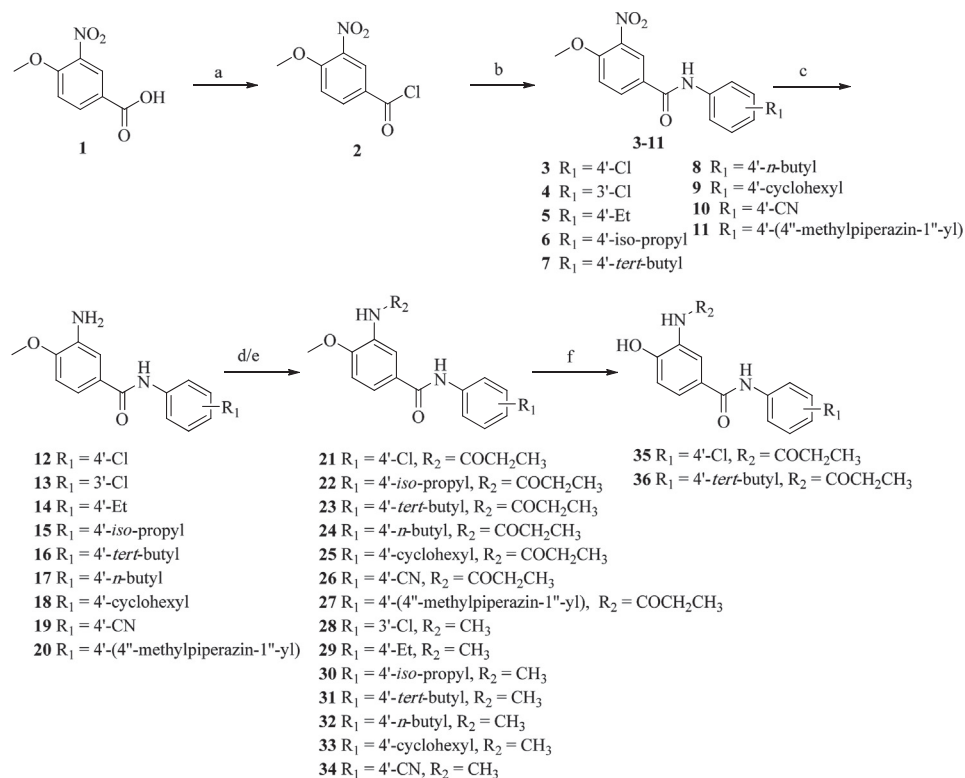
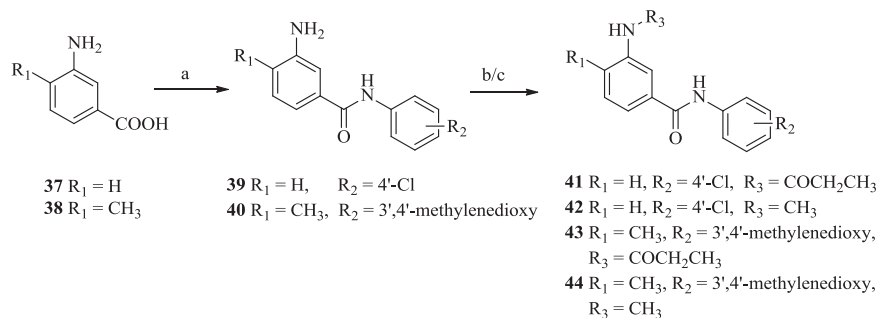


Figure 1 Chemical structures of the antiviral active compounds.



Scheme 1 Synthetic route for target compounds **22–36**. Reagents and conditions: (a) SOCl₂, reflux; (b) anilines, TEA, r.t.; (c) H₂, 10% dry Pd/C, r.t.; (d) CH₃CH₂COCl, TEA, r.t.; (e) 40% HCHO, r.t.; (f) BBr₃, –78 °C.



Scheme 2 Synthetic route for target compounds **41–44**. Reagents and conditions: (a) anilines, DIC, HOBT, r.t.; (b) CH₃CH₂COCl, TEA, r.t.; (c) CH₃I, K₂CO₃, 50 °C.

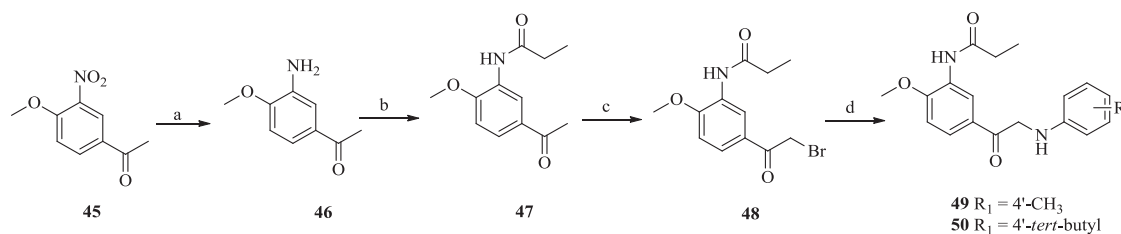
variety of anilines using trimethylamine (TEA) as the base to yield the intermediate compounds **3–11**. After the nitro groups of the intermediates **3–11** were reduced by hydrogen over 10% dry Pd/C, the amino groups at the C3-position on the benzene ring A were then acylated by propionyl chloride 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 BBr₃ at –78 °C to afford the 4-hydroxyl compounds **35** and **36**.

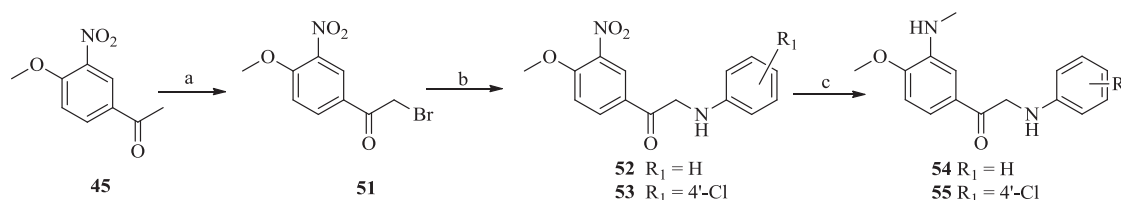
To alter the methoxy group to the methyl group or a hydrogen atom at the C4-position on the benzene ring A, compounds **41–44** were prepared using 3-aminobenzoic acid **37** or 3-amino-4-methylbenzoic acid **38** as the starting materials, which was condensed with anilines using diisopropylcarbodiimide (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 (CH₃I) 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) $\text{CH}_3\text{CH}_2\text{COCl}$, 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 aryls²¹.

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 $\mu\text{mol/L}$ to 7.12 $\mu\text{mol/L}$. Among these three compounds, the anti-HCV activity of compound **23** ($\text{IC}_{50} = 0.57 \pm 0.00 \mu\text{mol/L}$) was superior to that of the reported compounds IMB-26 ($\text{IC}_{50} = 1.46 \pm 0.62 \mu\text{mol/L}$), IMB-1f ($\text{IC}_{50} = 1.90 \pm 0.57 \mu\text{mol/L}$) and IMB-1f ($\text{IC}_{50} = 1.00 \pm 0.05 \mu\text{mol/L}$). 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'-position 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} ($\mu\text{mol/L}$)	CC_{50} ($\mu\text{mol/L}$)	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

^aAll 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 $\mu\text{mol/L}$. Especially, the antiviral activity of **29** ($\text{IC}_{50} = 0.95 \pm 0.11 \mu\text{mol/L}$) was close to that of the comparator drug ($\text{IC}_{50} = 0.16 \mu\text{mol/L}$). 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'-position 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

^aAll data were average values from three independent assays.

activity. When strong electron-withdrawing groups such as cyano group were introduced at the C4'-position on the benzene ring B (**26**), a decreased activity was observed. In addition, altering the propionamido group at the C3-position 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-position 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 CH₂ 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 CH₂ group was inserted between the CO and NH groups of the amide linker, or a methoxy group at the C4-position 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 IC₅₀ 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 IC₅₀ 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

¹H NMR and ¹³C NMR spectra were recorded in CDCl₃ 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 SOCl₂ (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 NH₄Cl (20 mL), extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried over Na₂SO₄ 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 CH₃OH (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 Na₂SO₄ 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. ¹H 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). ¹³C 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 C₂₀H₂₄N₂O₃: 341.1859 [M+H]⁺.

N-(4-*tert*-Butylphenyl)-4-methoxy-3-propionamidobenzamide (**23**): a white solid; yield: 85%. m.p: 165–166 °C. ¹H 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). ¹³C 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 C₂₁H₂₆N₂O₃: 355.2016 [M+H]⁺.

N-(4-Butylphenyl)-4-methoxy-3-propionamidobenzamide (**24**): a white solid; yield: 82%. m.p: 165–168 °C. ¹H 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). ¹³C 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 C₂₁H₂₆N₂O₃: 355.2016 [M+H]⁺.

N-(4-Cyclohexylphenyl)-4-methoxy-3-propionamidobenzamide (**25**): a white solid, yield: 78%. m.p: 167–169 °C. ¹H 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). ¹³C 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 C₂₃H₂₉N₂O₃: 381.2172 [M+H]⁺.

N-(4-Cyanophenyl)-4-methoxy-3-propionamidobenzamide (**26**): a white solid, yield: 75%. m.p: 154–157 °C. ¹H 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). ¹³C 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 C₁₈H₁₇N₃O₃: 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. ¹H 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). ¹³C 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 C₂₂H₂₈N₄O₃: 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 CH₃OH (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. ¹H 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). ¹³C 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 C₁₅H₁₅N₂O₂Cl: 291.0894 [M+H]⁺.

N-(4-Ethylphenyl)-4-methoxy-3-(methylamino)benzamide (**29**): a white solid, yield: 67%. m.p: 156–157 °C. ¹H 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). ¹³C 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 C₁₇H₂₀N₂O₂: 285.1597 [M+H]⁺.

N-(4-*iso*-propylphenyl)-4-methoxy-3-(methylamino)benzamide (**30**): a white solid, yield: 60%. m.p: 166–167 °C. ¹H 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). ¹³C 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 C₁₈H₂₂N₂O₂: 299.1754 [M+H]⁺.

N-(4-*tert*-Butylphenyl)-4-methoxy-3-(methylamino)benzamide (**31**): a white solid, yield: 69%. m.p: 145–146 °C. ¹H 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). ¹³C 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 C₁₉H₂₄N₂O₂: 313.1910 [M+H]⁺.

N-(4-Butylphenyl)-4-methoxy-3-(methylamino)benzamide (**32**): a white solid, yield: 60%. m.p: 160–162 °C. ¹H 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). ¹³C 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 C₁₉H₂₄N₂O₂: 313.1910 [M+H]⁺.

N-(4-Cyclohexylphenyl)-4-methoxy-3-(methylamino)benzamide (**33**): a white solid, yield: 65%. m.p: 165–167 °C. ¹H 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). ¹³C 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 C₂₁H₂₇N₂O₂: 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). ¹³C 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 C₁₆H₁₅N₃O₂: 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 BBr₃ (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 NH₄Cl, extracted with DCM (20 mL × 3), and washed with brine. The combined organic layers were dried with Na₂SO₄ 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). ¹³C 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 C₁₆H₁₅N₂O₃Cl: 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). ¹³C 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 C₂₀H₂₅N₂O₃: 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 Na₂SO₄ 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 Na₂SO₄ 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). ¹³C 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 C₁₆H₁₅N₂O₂Cl: 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). ¹³C 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 C₁₉H₂₀N₂O₄: 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 K₂CO₃ (3.0 mmol) and CH₃I (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 Na₂SO₄ 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, CDCl₃): 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). ¹³C 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 C₁₄H₁₃N₂OCl: 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). ¹³C 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 C₁₇H₁₈N₂O₃: 299.1390 [M+H]⁺.

N-(5-(2-Bromoacetyl)-2-methoxyphenyl)propionamide (**48**): to a solution of **45** (1.0 mmol) in CH₃OH (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 Na₂SO₄ and concentrated under reduced pressure. The residue **47** (1.5 mmol) was dissolved in CCl₄ (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 \times 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. ^1H 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 $[\text{M}+\text{H}]^+$.

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 \times 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. ^1H NMR (500 MHz, $\text{DMSO}-d_6$): 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, $\text{DMSO}-d_6$): 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 $\text{C}_{19}\text{H}_{22}\text{N}_2\text{O}_3$: 327.1703 $[\text{M}+\text{H}]^+$.

N-(5-(2-(4-(*tert*-Butyl)phenyl)amino)acetyl)-2-methoxyphenyl)propionamide (**50**): yellow solid. yield: 25%. m.p: 130–132 °C. ^1H 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, 2H), 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, $\text{DMSO}-d_6$): 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 $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_3$: 369.2172 $[\text{M}+\text{H}]^+$.

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 \times 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 \times 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 $[\text{M}+\text{H}]^+$.

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

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. ^1H NMR (500 MHz, $\text{DMSO}-d_6$): 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, $\text{DMSO}-d_6$): 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 $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_2$: 271.1441 $[\text{M}+\text{H}]^+$.

2-((4-Chlorophenyl)amino)-1-(4-methoxy-3-(methylamino)phenyl)ethanone (**55**): a yellow solid, yield: 25%. m.p: 140–141 °C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): 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, $\text{DMSO}-d_6$): 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 $\text{C}_{16}\text{H}_{17}\text{N}_2\text{O}_2\text{Cl}$: 305.1051 $[\text{M}+\text{H}]^+$.

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 (CC₅₀) 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 (TC₅₀) 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/cm². 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 (IC₅₀) was calculated with the Reed & Muench method. SI value was calculated as the ratio of CC₅₀/IC₅₀.

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 IC₅₀ was determined by the Reed and Muench method. The SI value was calculated as the ratio of TC₅₀/IC₅₀.

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