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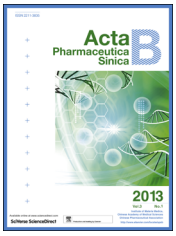

ORIGINAL ARTICLE

Synthesis of novel substituted *N*-aryl benzamides as hA3G stabilizers and their inhibitory activities against hepatitis C virus replication

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KEY WORDS

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Abstract A series of novel amino-substituted *N*-aryl benzamide analogs were synthesized and evaluated for their ability to inhibit hepatitis C virus (HCV) replication in acutely infected Huh7.5 cells. Most of the substituted *N*-aryl benzamide compounds showed convincing anti-HCV activities. Compounds **1f**, **1g** and **4c** exhibited potent anti-replicative activity at low micromolar levels (IC_{50} = 1.0–2.0 μM) with selective indices (SI) greater than 40. Mechanistic analysis indicated that the active compounds increased intracellular hA3G protein levels and inhibited HCV replication in a dose-dependent manner. The results demonstrate that this series of substituted *N*-aryl benzamide compounds warrant further investigation as inhibitors of HCV replication.

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1. Introduction

Over 170 million people worldwide are infected with the hepatitis C virus (HCV), and many of those are at risk of developing liver cirrhosis and hepatocellular carcinoma[1,2]. Moreover, the aging of this cohort will result in HCV-related-mortality increasing for future decades[3]. At present no vaccine is available to prevent HCV infection, and antiviral chemotherapy is an important approach to prevent this projected health burden. Unfortunately, the current classical combination of antiviral therapy with PEG-interferon (PEG-IFN) and ribavirin is effective in only 40–50% of patients who are infected with HCV genotype 1, and causes treatment-limiting side effects[4,5]. Medicinal scientists have made great progress in the development of novel HCV chemotherapy agents. Both long-lasting interferon and ribavirin derivatives are in development to improve efficacy and tolerability of PEG-IFN/ribavirin therapy[6–8]. Moreover, novel direct-acting antiviral agents that specifically target the HCV life cycle have been successfully developed[9–12]. Two HCV NS3/4A protease inhibitors telaprevir and boceprevir (Fig. 1) were approved for treatment of HCV infection by the United States Food and Drug Administration in 2011[13,14]. Addition of telaprevir or boceprevir to PEG-IFN and ribavirin significantly increased the therapeutic efficacy and shortened the duration of therapy in patients with HCV genotype 1. However, this combination was still unsatisfactory for the therapeutic needs of HCV non-1 genotype patients and those patients with contraindications to PEG-IFN or ribavirin therapy. Furthermore, HCV could easily acquire resistance to protease inhibitors through mutation[15,16]. Therefore, new anti-HCV agents with novel mechanisms of action are needed[17–19].

In our previous study, the small compound **IMB-26** (3, 4, 5-trimethoxyphenyl 3-(2-bromopropionamido)-4-methoxybenzamide, Fig. 2) was identified as a stabilizer of human apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G (hA3G). hA3G was a restrictive cellular factor for HCV and HIV-1 replication[20,21]. **IMB-26** was effective against both HCV and HIV-1 *in vitro*[22]. The simple structure of **IMB-26** was distinct from current HCV inhibitors. Its novel antiviral mechanism as an hA3G stabilizer, coupled with the current deficiency of available HCV chemotherapeutics prompted us to seek novel HCV inhibitors using **IMB-26** as starting point. **IMB-26** exhibited cytotoxicity, likely due to the presence of an α-bromocarbonyl group, which resulted in a lower selective index (SI = 7.1). To find more active and less toxic compounds against HCV, 28 substituted *N*-(hetero)arylbenzamide/benzenesulfonamide analogs of **IMB-26** were designed, synthesized and evaluated for their inhibitory activities against HCV replication in cultured Huh7.5 cell lines. Their structure–activity relationships (SAR) and mechanisms of action against HCV replication were investigated in this study.

2. Results and discussion

2.1. Synthesis

In this paper, the structures of the synthesized compounds are summarized as the general formula I (Fig. 2), with alterations in the following fragments of **IMB-26**: (a) substitutions to ring A and B; (b) alterations to the amide linker between two benzene rings; (c) changes to ring B. Compound **4** was commercially available. The detailed synthesis of compounds **4b**, **4c**, **1k** and **3** was previously reported[23]. Briefly, amino or hydroxyl acylation of the starting material 3-amino-4-methoxybenzoic acid (**S1**), 3-hydroxy-4-methoxybenzoic acid (**S2**) or 3-amino-4-chlorobenzoic acid (**S3**) was undertaken to afford intermediates **M1-1**–**M1-3**, **M2** and **M3**, respectively. Products **1a–1l**, **2** and **3** were prepared by coupling various anilines or phenols with above intermediates using diisopropylcarbodiimide (DIC) as a coupling agent and *N*-hydroxybenzotriazole (HOBt) as an activation agent in dried dimethylformamide (DMF) with yields of 38–63%, as shown in Scheme 1. Commercially available compound **4** was mixed with different propionyl chlorides in tetrahydrofuran (THF) to give substituted benzanilide products **4a–4c** (Scheme 1).

N-arylbenzamides **1m–1o** and *N*-heteroarylbenzamides **1p–1r** were synthesized starting from 3-nitro-4-methoxybenzoic acid (**S4**) using the method shown in Scheme 2. 3-Nitro-4-methoxybenzoic acid (**S4**) was first converted into the acid chloride to easily generate nitrated intermediates **M4-1–M4-3**, which were reduced by hydrogenation to form the corresponding aminated intermediates **M4-4–M4-6**. Amino acylation produced the final products **1m–1r** with a total yield greater than 20%. Using a similar method, the nitrated intermediates **M5-1** and **M5-2**, aminated intermediates **M5-3** and **M5-4**, and *N*-(hetero)aryl benzenesulfonamide products **5a–5d** were prepared from nitro-substituted benzosulfonic acid (**S5**) with a total yield of 24–66%. Phenyl benzenesulfonamide (**6**) was also obtained by the same method starting from 3-amino-4-methoxybenzenesulfonic acid (**S6**) working through intermediate **M6**.

2.2. Structure–activity relationship (SAR) analysis

All compounds were tested for antiviral activity using HCV-infected Huh7.5 cells with INF-α as a positive control. Total

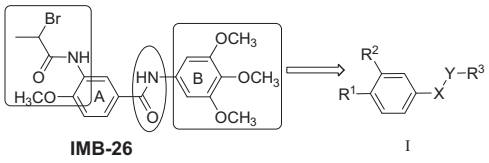


Figure 2 Chemical optimization of **IMB-26**.

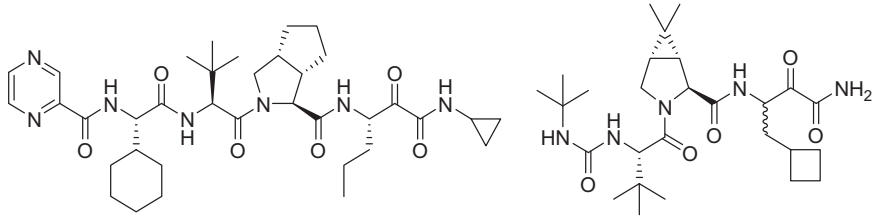
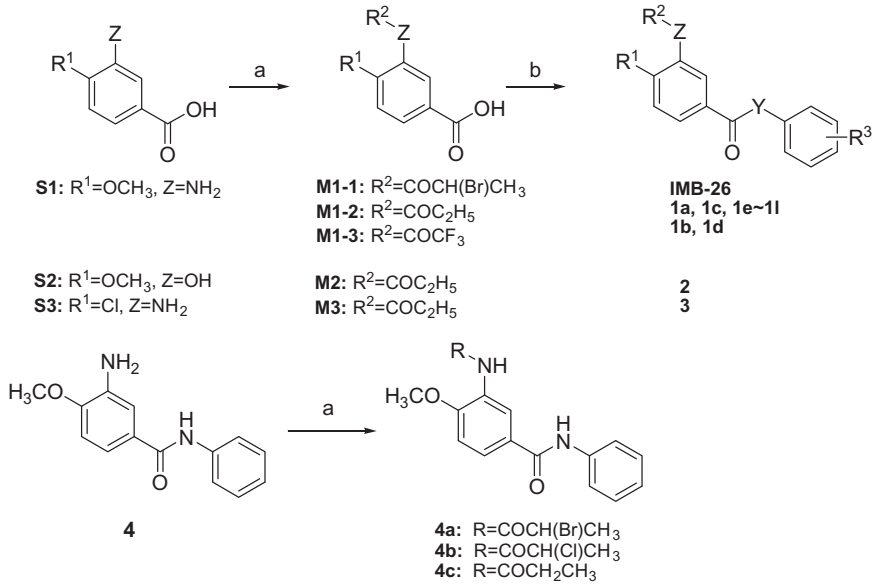
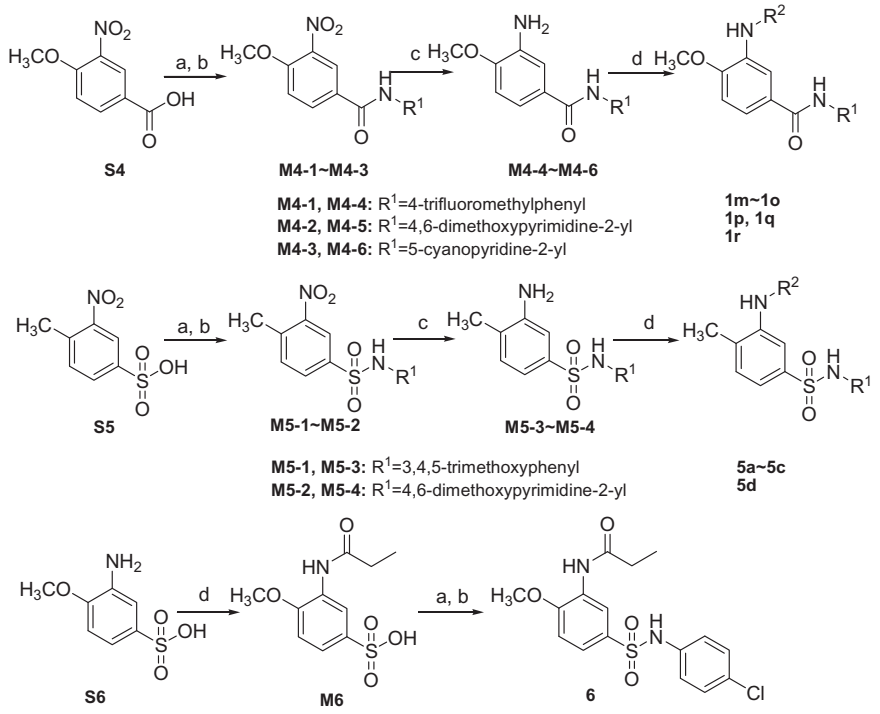


Figure 1 The chemical structures of HCV NS3/4A protease inhibitors telaprevir and boceprevir.



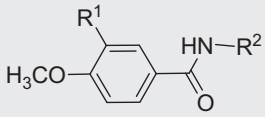
Scheme 1 Synthesis of compounds **1a**–**1l**, **2**, **3** and **4a**–**4c**. Reagents and conditions: (a) acylchloride or anhydride, TEA, THF or DCM; (b) phenylamine or phenol, HOBt, DIC, DMF.



Scheme 2 Synthesis routes of *N*-(hetero)aryl benzamide compounds **1m**–**1r**, *N*-(hetero)aryl benzenesulfonamide compounds **5a**–**5d** and **6**. Reagents and conditions: (a) $SOCl_2$, room temperature or reflux; (b) substituted arylamine, TEA or NaH, THF; (c) H_2, Pd/C, 50 barr; (d) acylchloride or iodomethane, triethylamine, DCM.

intracellular HCV RNA was quantified using a one-step RT-PCR to calculate the IC_{50} of test compounds by dose-response evaluation. In Table 1, **1a, 1b** were firstly synthesized with the expectation that removal of the bromo-substitution on **IMB-26** would decrease cytotoxicity. This resulted in 3–6 fold increases in the selective index ($SI > 20$) due to comparative antiviral activity ($IC_{50} = 3.2$ μM and 6.4 μM for **1a** and **1b**, respectively) and significant lower cytotoxicity ($CC_{50} = 150$ μM and 137 μM respectively) compared to **IMB-26** ($IC_{50} = 2.1$ μM, $CC_{50} = 15$ μM). Substitution of the C3-*N*-propionyl group with C3-*O*-propionyl or C3-*N*-trifluoroacetyl group led to decreased antiviral activity ($6.4\ \mu M < IC_{50} < 71\ \mu M$ for **1b**, **1n** and **2**). The absence of a carbonyl side-chain on the amino group of the A ring (**4**) significantly decreased antiviral activity about 10-fold relative to compounds **4a–4c**, where a carbonyl was present. The relative activity of compounds **4a–4c** also indicated that the C3-*N*-propionyl group was a preferred substituent. As we predicted, all

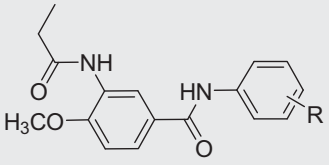
Table 1 Structure–activity relationship for C3 substituent and ring B.



Compd.[a]	R^1	R^2	CC_{50} (μM)	IC_{50} (μM)	SI
IMB-26	–NHCOCH(Br)CH_3	3,4,5-OCH_3-phenyl	15 ± 1.1	2.1 ± 0.40	7.1
1a	–NHCOCH_2CH_3	3,4,5-OCH_3-phenyl	150 ± 50	3.2 ± 0.53	47
1b	–NHCOCF_3	3,4,5-OCH_3-phenyl	137 ± 33	6.4 ± 0.59	21
2	–OCOCH_2CH_3	3,4,5-OCH_3-phenyl	67 ± 7.5	8.1 ± 1.5	8.3
1n	–NHCOCF_3	4-CF_3-phenyl	>492	71 ± 6.2	>6.9
1o	–NHCOCH(Br)CH_3	4-CF_3-phenyl	9.1 ± 1.7	3.7 ± 0.4	2.4
4	–NH_2	phenyl	693 ± 40	41 ± 17	17
4a	–NHCOCH(Br)CH_3	phenyl	11 ± 1.1	4.4 ± 0.73	2.5
4b	–NHCOCH(Cl)CH_3	phenyl	69 ± 10	4.6 ± 0.75	15
4c	–NHCOCH_2CH_3	phenyl	128 ± 17	1.8 ± 0.00	72
1p	–NHCOCH(Br)CH_3	4,6-OCH_3-pyrimidin-2-yl	29 ± 0.50	14 ± 2.7	2.1
1q	–NHCOCH_2CH_3	4,6-OCH_3-pyrimidin-2-yl	416 ± 47	15 ± 0.61	27
1r	–NHCOCH_2CH_3	5-CN-pyridin-2-yl	484 ± 17	19 ± 4.7	24

[a]INF-α showed IC_{50} of 0.31 ± 0.06 μg/mL as the positive control in this test.

Table 2 Substituent effects in ring B.



Compd.	R	CC_{50} (μM)	IC_{50} (μM)	SI
1a	3,4,5-OCH_3	150 ± 50	3.2 ± 0.53	47
1f	4-OCH_3	208 ± 16	1.9 ± 0.57	108
1g	3-OCH_2CH_2O-4	45 ± 5.4	1.0 ± 0.05	44
1h	4-CH_3	75 ± 2.2	2.2 ± 0.19	33
1i	4-Cl	134 ± 3.6	4.6 ± 0.21	29
1j	4-F	134 ± 24	11 ± 1.8	12
1l	2,4-Cl	197 ± 15	16 ± 3.0	11
1m	4-CF_3	431 ± 16	17 ± 0.02	25
4c	H	128 ± 17	1.8 ± 0.00	72

synthesized compounds (**1o**, **1p** and **4a**) with an α-bromocarbonyl group at the C3-position of ring A exhibited no antiviral selectivity (SI < 5) due to their cytotoxicity in Huh7.5 cells. Meanwhile, when ring B was changed from substituted phenyl into heteroaryl group, the antiviral activity was decreased several fold (14 μM < IC_{50} < 19 μM for **1p**, **1q**, **1r**).

To investigate the importance of trimethoxyl substitution on the ring B, compounds with a single methoxyl substitution at the C4-position of ring B (**1f**) were synthesized and are shown in Table 2. Compound **1f** exhibited slightly higher antiviral activity (IC_{50} = 1.9 μM) than trimethoxyl substituted compound **1a**, and had an SI value of 108. Compounds with diverse substitutions on ring A (**1g**–**1m**) were synthesized and showed clear antiviral activities (Table 2). Compounds with an electron-withdrawing substitution (**1i**, **1j**, **1l** and **1m**) showed weaker anti-HCV activity than compounds with an electron-donating substitution (**1f**, **1g**, **1h**). Among those compounds, compound **1g** with a dioxyethylene group substitution exhibited the most potent inhibitory activity (IC_{50} = 1.0 μM, SI = 44). Compound **4c** without a substitution in ring B had a comparable antiviral ability to compound **1g** with a higher SI (IC_{50} = 1.8 μM, SI = 72).

In Table 3, replacing the amide linker between ring A and B of compounds **1a**, **1b**, **1f** and **4c** with an ester linker or *N*-CH_3 amide linker led to **1c**–**1e** and **1k** with 3–16-fold decreases in antiviral potency. The replacement of amide linker between ring A and B with sulfonamide linker **6** (IC_{50} = 18 μM) also resulted in about 4-fold lower HCV inhibition compared to corresponding compound **1i** (IC_{50} = 4.6 μM). When the methoxyl group at the C4-position of ring A in the benzenesulfonamide compounds was simultaneously replaced with methyl group, almost complete loss of antiviral activity was observed for compounds **5a**–**5d**. Taken these together, these results indicated that the NH of the amide linker and the C4–OCH_3 group made contributions to the antiviral activity of these compounds, while a hydrophilic sulfonamide linker was unfavorable to antiviral activity. Moreover, loss activity of compound **3** with a chloro instead of methoxyl substitution (**4c**) at C4-position of ring A also confirmed the importance of the methoxyl group.

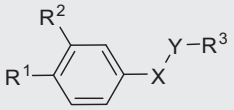
2.3. *Effects of compound **1g** on intracellular hA3G expression and HCV NS3 synthesis*

The most active compound **1g** was chosen to explore the mechanism of action of these inhibitors by immunoblot measurement. The expression of intracellular hA3G protein and HCV NS3 protein were measured in the Huh7.5 cells with or without treatment of the test compound. Compound **1g** treatment produced higher intracellular hA3G protein levels (Fig. 3A) and lower HCV NS3 protein levels (Fig. 3B). These effects were dose-dependent.

3. Conclusions

The present study identified several novel substituted *N*-aryl benzamide compounds that displayed potent anti-HCV activity at low micromolar levels. The pharmacophore of *N*-aryl-(3-amide-4-methoxy)benzamide

Table 3 SAR for C4 substituent and linker.



Compd.	R^1	R^2	X	Y	R^3	CC_{50} (μM)	IC_{50} (μM)	SI
1a	OCH_3	$–NHCOCH_2CH_3$	C(O)	NH	3,4,5-OCH_3-phenyl	150±50	3.2±0.53	47
1b	OCH_3	$–NHCOCF_3$	C(O)	NH	3,4,5-OCH_3-phenyl	137±33	6.4±0.59	21
1c	OCH_3	$–NHCOCH_2CH_3$	C(O)	O	3,4,5-OCH_3-phenyl	194±12	11±0.02	17
1d	OCH_3	$–NHCOCF_3$	C(O)	O	3,4,5-OCH_3-phenyl	133±11	19±0.34	7.0
1e	OCH_3	$–NHCOCH_2CH_3$	C(O)	O	4-OCH_3-phenyl	279±3.9	18±4.2	15
1f	OCH_3	$–NHCOCH_2CH_3$	C(O)	NH	4-OCH_3-phenyl	208±16	1.9±0.57	108
1k	OCH_3	$–NHCOCH_2CH_3$	C(O)	$N(CH_3)$	phenyl	>320	29	>11
3	Cl	$–NHCOCH_2CH_3$	C(O)	NH	phenyl	49	NA	/
4c	OCH_3	$–NHCOCH_2CH_3$	C(O)	NH	phenyl	128±17	1.8±0.00	72
5a	CH_3	$–NHCH_3$	$–S(O)_2–$	NH	3,4,5-OCH_3-phenyl	91±19	NA	/
5b	CH_3	$–NHCOCH_3$	$–S(O)_2–$	NH	3,4,5-OCH_3-phenyl	94±12	120±32	/
5c	CH_3	$–NHCOCH_2CH_3$	$–S(O)_2–$	NH	3,4,5-OCH_3-phenyl	172±34	NA	/
5d	CH_3	$–NHCOCH_2CH_3$	$–S(O)_2–$	NH	4,6-OCH_3-pyrimidin-2-yl	>525	NA	/
1i	OCH_3	$–NHCOCH_2CH_3$	C(O)	NH	4-Cl-phenyl	134±3.6	4.6±0.21	29
6	OCH_3	$–NHCOCH_2CH_3$	$–S(O)_2–$	NH	4-Cl-phenyl	101±8.9	18±9.7	5.6

NA: not active.

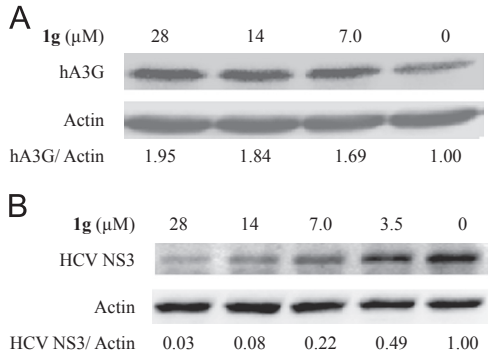


Figure 3 Effects of compound **1g** on the HCV replication. (A) Intracellular hA3G protein level elevated dose-dependently after 24 h treatment of **1g** in naive Huh7.5 cells. (B) HCV NS3 protein synthesis dose-dependently decreased after 72 h treatment of **1g** in HCV-infected Huh7.5 cells.

was revealed by SAR analysis. An increase in intracellular hA3G levels and a decrease in HCV NS3 protein levels was observed after treatment with active compounds. Although the antiviral activity was not comparable to that of current protease inhibitors, this series of substituted *N*-aryl benzamides deserves further research as potentially therapeutic HCV inhibitors because of their novel mechanism of action and possible lower susceptibility to developed drug-resistance. Further optimization should produce more potent compounds; we anticipate that hA3G regulators could provide a new approach to HCV chemotherapy.

4. Experimental section

4.1. Chemistry

Melting points (Mp) were determined using an X6 microscope melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Varian Mercury-400 or 500 MHz spectrometer in $CDCl_3$ or DMSO-d_6 solutions at room temperature with TMS as an internal standard, and HRMS (high resolution mass spectra) were recorded on an Autospec Ultima-TOF mass spectrometer. Start materials **S1**, **S2**, **S3**, **S4**, **S5 S6** and compound **4** were purchased from J&K Chemicals. Compounds **4b**, **4c**, **1k** and **3** were previously reported[23]. HPLC analysis was performed on a SHIMADZU LC-10ATvp instrument equipped with a UV detector. Detector wavelength: 260 nm. Column: PARTISIL# ODS 5 μm, 250 mm × 4.6 mm (Dikma Technologies), Mobile phase: acetonitrile/water = 65/35. The flow rate was 1.0 mL/min. HPLC purity of all product compounds was >95%.

4.2. General procedure for the synthesis of compounds *1a–1l, 2 and 3*

Step 1: To a solution of substituted benzoic acid (2.0 mmol) and TEA (2.5 mmol, 1.25 equiv.) in THF (50 mL) was added acyl chloride or anhydride (3.0 mmol, 1.5 equiv.) while on an ice bath, and the reaction was stirred at room temperature after addition under nitrogen until completion (6–8 h). The reaction was monitored by TLC (10% DCM/methanol). The solvent was evaporated *in vacuo*. The residue was dissolved in DCM which was washed with 0.5 M HCl (25 mL) followed by water. The organic phase was concentrated and dispersed in ether (80 mL), and the insoluble substance was filtered to give the crude intermediate **M1–M3**.

4.2.1. 3-(2-Bromo)propionamido-4-methoxybenzoic acid (*M1-1*)

Reaction of 3-amino-4-methoxybenzoic acid (**S1**) with 2-bromopropionyl chloride afforded intermediate **M1-1** as off-white solid in 77% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.72 (d, J = 6.8 Hz, 3H), 3.91 (s, 3H), 5.04 (q, J = 6.8 Hz, 1H), 7.14 (d, J = 8.8 Hz, 1H), 7.72 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 8.57 (d, J = 2.0 Hz, 1H), 9.657 (s, 1H, NH), 12.670 (br, 1H). MS (ESI): *m/z* 301.1 $[M+H]^+$.

*4.2.2. 3-Propionamido-4-methoxybenzoic acid (**M1-2**)*
Reaction of **S1** with propionyl chloride afforded intermediate **M1-2** as off-white solid in 91% yield. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.06 (t, J=7.5 Hz, 3H), 2.39 (q, 2H, J=7.5 Hz), 3.89 (s, 3H), 7.10 (d, J=8.0 Hz, 1H), 7.67 (d, J=7.5 Hz 1H), 8.56 (s, 1H), 9.12 (s, 1H), 12.59 (br, 1H). MS (ESI): *m/z* 224.0 $[M+H]^+$.

*4.2.3. 3-Trifluoroacetamido-4-methoxybenzoic acid (**M1-3**)*
Reaction of **S1** with trifluoroacetyl anhydride afforded intermediate **M1-3** as off-white solid in 78% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.89 (s, 3H), 7.23 (d, J=8.8 Hz, 1H), 7.90 (dd, J_1=8.8 Hz, J_2=1.6 Hz, 1H), 7.96 (d, J=2.0 Hz, 1H), 10.79 (br, 1H), 12.79 (s, 1H). MS (ESI): *m/z* 286.0 $[M+Na]^+$.

*4.2.4. 4-Methoxy-3-(propionyloxy)benzoic acid (**M2**)*
Reaction of 3-hydroxyl-4-methoxybenzoic acid (**S2**) with propionyl chloride afforded **M2** as white solid in 72% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.07 (t, J=7.6 Hz, 3H), 2.42 (q, J=7.6 Hz, 2H), 3.83 (s, 3H), 7.68 (m, 2H), 8.56 (s, 1H), 12.74 (s, 1H). MS(ESI): *m/z* 225 $[M+H]^+$.

*4.2.5. 4-Chloro-3-propionamidobenzoic acid (**M3**)*
Reaction of 3-amino-4-chlorobenzoic acid (**S3**) with propionyl chloride afforded intermediate **M3** as white solid in 70% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.07 (3H, t, J=7.6 Hz), 2.42 (2H, q, J=7.6 Hz), 7.11 (1H, d, J=8.8 Hz), 7.68 (1H, m), 8.56 (1H, d, J=8.8 Hz), 9.12 (1H, s, NH), 12.59 (1H, s). MS (ESI): *m/z* 228 $[M+H]^+$.

Step 2: To a solution of the above crude intermediates (2.0 mmol) in DMF (10 mL), HOBt (2.3 mmol) and DIC (2.3 mmol) in 10 mL of DMF were added substituted aniline or phenol (2.5 mmol) with stirring at room temperature. The reaction mixture was stirred for 8 h and rinsed with 0.5 M aqueous NaOH solution and water. The organic phase was concentrated and purified by silica gel chromatography and eluted with a solution of DCM/methanol (20/1) to afford the final products as white or off-white solids.

*4.2.6. 3′,4′,5′-Trimethoxyphenyl 3-propionamido-4-methoxybenzamide (**1a**)*
Reaction of **M1-2** with 3,4,5-trimethoxybenzenamine afforded product **1a** as white solid. Yield, 48%. Mp: 81–83 °C. ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.3 (s, J=7.6 Hz, 3H), 2.5 (q, J=7.6 Hz, 2H), 3.82 (s, 3H), 3.9 (s, 6H), 3.95 (s, 3H), 6.99 (s, 2H), 7.01 (d, J=8.8 Hz, 1H), 7.78 (dd, J_1=7.6 Hz, J_2=2.0 Hz, 1H), 7.85 (br, 2H), 8.9 (d, J=1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.48, 164.99, 153.26 (2C), 150.28, 134.64, 134.29, 127.26 (2C), 124.89, 116.61, 110.19, 97.92 (2C), 60.97, 56.41 (2C), 56.03, 31.03, 9.56; HR-MS (ESI): *m/z* calcd. for $C_{20}H_{25}N_2O_6$ 389.17126, found: 389.17327 $[M+H]^+$.

*4.2.7. 3′,4′,5′-Trimethoxyphenyl 3-trifluoroacetamido-4-methoxybenzamide (**1b**)*
Reaction of **M1-3** with 3,4,5-trimethoxybenzenamine afforded product **1b** as white solid. Yield, 25%. Mp: 142-144 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 9H), 4.0 (s, 3H), 6.97 (s, 2H), 7.03 (d, J=8.8 Hz, 1H), 7.86 (d, J=8.8 Hz, 1H), 7.9 (s, 1H), 8.6 (s, 1H), 8.75 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.44, 155.09, 153.31 (2C), 150.86, 134.84, 133.95, 127.61, 126.73 (2C), 124.72, 117.66, 110.55, 97.97(2C), 60.95, 56.36, 56.11 (2C); HR-MS (ESI): *m/z* calcd. for $C_{19}H_{20}F_3N_2O_6$ 429.12735, found: 429.13112 $[M+H]^+$.

*4.2.8. 3′,4′,5′-Trimethoxyphenyl 3-propionamido-4-methoxybenzonate (**1c**)*
Reaction of **M1-2** with 3,4,5-trimethoxyphenol afforded product **1c** as white solid. Yield, 31%. Mp: 71-73 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.3 (s, J=7.6 Hz, 3H), 2.5 (q, J=7.6 Hz, 2H), 3.8 (s, 9H), 3.95 (s, 3H), 6.44 (s, 2H), 6.98 (d, J=8.4 Hz, 1H), 7.76 (br, 1H), 7.9 (dd, J_1=8.4 Hz, J_2=1.6 Hz, 1H), 7.9 (br, 2H), 9.2 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.33, 164.94, 156.46, 150.09, 131.15, 127.46, 127.38, 124.64, 122.25 (2C), 116.71, 114.13 (2C), 110.06, 60.97, 56.41 (2C), 56.03, 31.03, 9.56; HR-MS (ESI): *m/z* calcd. for $C_{20}H_{24}NO_7$ 390.15494, found: 390.15528 $[M+H]^+$.

*4.2.9. 3′,4′,5′-Trimethoxyphenyl 3-trifluoroacetamido-4-methoxybenzonate (**1d**)*
Reaction of **M1-3** with 3,4,5-trimethoxyphenol afforded product **1d** as white solid. Yield, 28%. Mp: 165–166 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (s, 9H), 4.0 (s, 3H), 6.46 (s, 2H), 7.05 (d, J=8.0 Hz, 1H), 8.07 (dd, J_1=8.4 Hz, J_2=2.0 Hz, 1H), 8.54 (s, 1H), 9.12 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.00, 165.04, 153.40 (2C), 151.76, 147.11, 135.59, 127.60, 126.76, 122.24, 121.24, 109.51, 99.33 (2C), 60.89, 56.10 (2C), 56.07, 30.94, 23.68, 9.55; HR-MS (ESI): *m/z* calcd. for $C_{19}H_{19}F_3NO_7$ 430.11333, found: 430.11136 $[M+H]^+$.

*4.2.10. 4′-Methoxyphenyl 3-propionamido-4-methoxybenzonate (**1e**)*
Reaction of **M1-2** with 4-methoxyphenol afforded product **1e** as white solid. Yield, 18%. Mp: 119–122 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, J=7.6 Hz, 3H), 2.5 (q, J=7.6 Hz, 2H), 3.82 (s, 3H), 3.98 (s, 3H), 6.91 (d, J=8.8 Hz, 2H), 6.96 (d, 1H, J=8.8 Hz), 7.11 (d, J=8.8 Hz, 2H), 7.75 (br, 1H), 7.92 (d, J=8.4 Hz, 1H), 9.21 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.16, 162.43, 153.05, 143.52, 128.84, 128.60, 128.28, 127.57, 124.73, 121.25, 120.40, 117.15, 110.04, 108.53, 56.28 (2C), 30.91, 9.46; HR-MS (ESI): *m/z* calcd. for $C_{18}H_{20}NO_5$ 330.13415, found: 330.13403 $[M+H]^+$.

*4.2.11. 4′-Methoxyphenyl 3-propionamido-4-methoxybenzamide (**1f**)*
Reaction of **M1-2** with 4-methoxybenzenamine afforded product **1f** as white solid. Yield, 25%. Mp: 175–177 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.07 (t, J=10.0 Hz, 3H), 2.4 (q, J=10.0 Hz, 2H), 3.73 (s, 3H), 3.89 (s, 3H), 6.89 (d, J=12.0 Hz, 2H), 7.1 (d, J=12.0 Hz, 1H), 7.6 (d, J=12.0 Hz, 2H), 7.7 (dd, J_1=12.0 Hz, J_2=1.2 Hz, 1H), 8.48 (br, 1H), 9.15 (s, 1H), 10.0 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.33, 164.94, 156.46, 150.09, 131.15, 127.46, 127.38, 124.64, 122.25 (2C), 116.71, 114.13 (2C), 110.06, 60.97, 56.41 (2C), 56.03, 31.03, 9.56; HR-MS (ESI): *m/z* calcd. for $C_{18}H_{21}N_2O_4$ 329.15013, found: 329.15135 $[M+H]^+$.

*4.2.12. 3′,4′-Dioxyethylenephenyl 3-propionamido-4-methoxybenzamide (**1g**)*
Reaction of **M1-2** with 3′,4′-dioxyethylenebenzenamine afforded product **1g** as white solid. Yield, 23%. Mp: 152–154 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.06 (t, J=7.6 Hz, 3H), 2.39 (q, J=7.6 Hz, 2H), 3.89 (s, 3H), 4.21 (q, J=8.8 Hz, 4H), 6.79 (d, J=8.8 Hz,

1H), 7.12 (d, $J=8.8$ Hz, 1H), 7.16 (dd, $J_1=8.8$ Hz, $J_2=2.4$ Hz, 1H), 7.34(d, $J=2.4$ Hz, 1H), 7.69 (dd, $J_1=8.8$ Hz, $J_2=2.4$ Hz), 8.46 (s, 1H), 9.15 (s, 1H), 9.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.31, 164.91, 150.12, 143.46, 140.46, 131.78, 127.48, 127.31, 124.67, 117.11, 116.80, 114.11, 110.30, 110.05, 64.41, 64.29, 56.00, 31.01, 9.57; HR-MS (ESI): *m/z* calcd. for $C_{19}H_{21}N_2O_5$ 357.14505, found: 357.14391 $[M+H]^+$.

*4.2.13. 4′-Methylphenyl 3-propionamido-4-methoxybenzamide (**1h**)*

Reaction of **M1-2** with 4-methoxybenzenamine afforded product **1h** as white solid. Yield, 33%. Mp: 154–156 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.25 (t, $J=7.6$ Hz, 3H), 2.31 (s, 3H), 2. 44 (q, $J=7.6$ Hz, 2H), 3.92 (s, 3H), 6.93 (d, $J=8.2$ Hz, 1H), 7.13 (d, $J=8.0$ Hz, 2H), 7.52 (d, $J=8.0$ Hz, 2H), 7.75 (d, $J=8.2$ Hz, 1H), 7.82 (s, 1H), 8.07 (br, 1H), 8.85 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.28, 165.01, 150.12, 135.53, 133.74, 129.34 (2C), 127.43, 127.27, 124.46, 120.34 (2C), 116.97, 109.90, 55.89, 30.88, 20.81, 9.49; HR-MS (ESI): *m/z* calcd. for $C_{18}H_{21}N_2O_3$ 313.15522, found: 313.15349 $[M+H]^+$.

*4.2.14. 4′-Chlorophenyl 3-propionamido-4-methoxybenzamide (**1i**)*

Reaction of **M1-2** with 4-chlorobenzenamine afforded product **1i** as white solid. Yield, 35%. Mp: 130–132 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (t, $J=7.6$ Hz, 3H), 2.48 (q, $J=7.6$ Hz, 2H), 3.96 (s, 3H), 6.98 (d, $J=8.4$ Hz, 1H), 7.33 (d, $J=8.8$ Hz, 2H), 7.61 (d, $J=8.8$ Hz, 2H), 7.77 (dd, $J_1=8.4$ Hz, $J_2=2.0$ Hz, 1H), 7.82 (s, 1H), 8.09 (s, 1H), 8.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.43, 165.26, 150.38, 136.97, 128.88, 128.73 (2C), 127.09, 126.97, 124.87, 121.52 (2C), 117.31, 110.01, 55.93, 26.85, 9.51; HR-MS (ESI): *m/z* calcd. for $C_{17}H_{17}ClN_2NaO_3$ 355.08254, found: 355.08101 $[M+Na]^+$.

*4.2.15. 4′-Fluorophenyl 3-propionamido-4-methoxybenzamide (**1j**)*

Reaction of **M1-2** with 4-fluorobenzenamine afforded product **1j** as white solid. Yield, 43%. Mp: 167–169 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, $J=7.6$ Hz, 3H), 2. 48 (q, $J=7.6$ Hz, 2H), 3.96 (s, 3H), 6.98 (d, $J=8.4$ Hz, 1H), 7.04 (t, $J=8.4$ Hz, 2H), 7.59 (q, $J_1=8.4$ Hz, $J_2=4.8$ Hz, 2H), 7.80 (dd, $J_1=8.4$ Hz, $J_2=1.6$ Hz, 1H), 7.83 (s, 1H), 7.92 (br, 1H), 8.91 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.39, 165.06, 158.21, 150.24, 134.07, 127.40, 127.14, 124.73, 122.26, 122.19, 116.76, 115.69, 115.47, 110.11, 56.03, 31.02, 9.55; HR-MS (ESI): *m/z* calcd. for $C_{17}H_{18}FN_2O_3$ 317.13015, found: 317.12898 $[M+H]^+$.

*4.2.16. 2′,4′-Dichlorophenyl 3-propionamido-4-methoxybenzamide (**1l**)*

Reaction of **M1-2** with 2,4-dichlorobenzenamine afforded product **1l** as white solid. Yield, 33%. Mp: 159–161 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.07 (t, $J=7.6$ Hz, 3H), 2.40 (q, $J=7.6$ Hz, 2H), 3.90 (s, 3H), 7.15 (d, $J=8.8$ Hz, 1H), 7.45 (dd, $J_1=8.4$ Hz, $J_2=2.4$ Hz, 1H), 7.60 (d, $J=8.8$ Hz, 1H), 7.70 (d, $J=2.0$ Hz, 1H), 7.75 (dd, $J_1=8.4$ Hz, $J_2=2.4$ Hz, 1H), 8.54 (br, 1H), 9.17 (s, 1H), 9.91 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.19, 164.75, 150.53 (2C), 133.65, 128.89, 128.66 (2C), 127.85 (2C), 127.79, 126.72, 124.12, 123.84, 122.31, 117.15, 109.99(2C), 56.01 (2C), 31.07, 9.64; HR-MS (ESI): *m/z* calcd. for $C_{17}H_{17}Cl_2N_2O_3$ 367.06162, found: 367.06016 $[M+H]^+$.

*4.2.17. 3′,4′,5′-Trimethoxyphenyl 3-propionyloxy-4-methoxybenzamide (**2**)*

Reaction of **M2** with 3,4,5-trimethoxybenzenamine afforded product **2** as white solid. Yield, 27%. Mp: 155–157 °C. ^{1}H NMR($CDCl_3$) δ: 1.20 (t, $J=7.6$ Hz, 3H) , 2.57 (q, $J=7.6$ Hz, 2H), 3.87 (s, 9H), 3.96 (s, 3H), 6.43 (s, 2H), 6.98 (d, $J=8.2$ Hz, 1H), 7.76 (br, 1H), 7.90 (dd, $J=8.2$, 1.8 Hz, 1H), 9.02 (s, 1H), HR-MS(ESI) calcd. for $C_{20}H_{24}N_3O_7$ $[M+H]^+$, *m/z*=390.15528, found: 390.15739.

*4.3. General procedure for the synthesis of compounds **1m–1o**, **1p–1r**, **5a–5d***

Step 1: 3-nitro-3-methoxybenzoic acid (**S4**) or 3-nitro-substituted benzosulfonic acid (**S5** and **S6**) (5.0 mmol) was dissolved in excess thionyl chloride (5 mL) with a catalytic volume of DMF (0.5 mL) to afford crude acid chloride. To a solution of aniline or heteroarylamine (6.0 mmol, 1.2 equiv.) and TEA (6.0 mmol, 1.2 equiv.) in dried DCM (60 mL) was added dropwise the solution of above acid chloride obtained in dried DCM under ice bath. The subsequent mixture was left at room temperature after addition and stirred for 4–8 h. The reaction was stopped by adding water, and the diluted mixture was washed twice with a 10% NaCl aqueous solution. The organic phase was dried over anhydrous Na_2SO_4 and filtered. The filtrate was purified by silica gel column chromatography to afford the pure nitrated intermediates **M4-1–M4-3**, **M5-1**, **M5-2**. The synthesis step for intermediate **M5-2** was carried out using sodium hydride as an acid-binding agent at −20 °C in dried DMF.

*4.3.1. 4′-Trifluoromethylphenyl 3-nitro-4-methoxybenzamide (**M4-1**)*

Reaction of 3-nitro-4-methoxybenzoic acid (**S4**) with thionyl chloride afforded sticky 3-nitro-4-methoxybenzoyl chloride which was used without further purification for later reaction with 4-trifluoroaniline to afford intermediate **M4-1** as gray solid in yield 43%. MS (ESI): *m/z* 341.1 $[M+H]^+$.

*4.3.2. 4′,6′-Dimethoxypyrimidin-2-yl 3-nitro-4-methoxybenzamide (**M4-2**)*

Reaction of 3-nitro-4-methoxybenzoyl chloride with 2-amino-4,6-dimethoxypyrimidine and NaH as acid binding reagent to afford intermediate **M4-2** as white solid in total yield 21%. ^{1}H NMR (DMSO-d_6): δ 2.57 (s, 3H), 3.74 (s, 6H), 5.80 (s, 1H), 7.73 (d, $J=8.0$ Hz, 1H), 8.13 (dd, $J_2=2.0$ Hz, $J_1=8.0$ Hz, 1H), 8.49 (d, $J=2.0$ Hz, 1H); MS (ESI): *m/z* 355.4 $[M+H]^+$.

*4.3.3. 5′-Cyanopyridin-2-yl 3-nitro-4-methoxybenzamide (**M4-3**)*

Reaction of 3-nitro-4-methoxybenzoyl chloride with 2-amino-5-cyanopyridine afforded intermediate **M4-3** as white solid in total yield 47%. ^{1}H NMR ($CDCl_3$): δ 4.06 (s, 3H), 7.23 (d, $J=8.8$ Hz, 1H), 8.05 (dd, $J_2=2.0$ Hz, $J_1=8.8$ Hz, 1H), 8.21 (d, $J=8.8$ Hz, 1H), 8.49 (d, $J=2.0$ Hz, 1H), 8.56 (d, $J=8.8$ Hz, 1H), 8.61 (d, $J=1.6$ Hz, 1H), 9.14 (br, 1H, –NH).

*4.3.4. 3′,4′,5′-Trimethoxyphenyl 3-nitro-4-methylbenzenesulfonamide (**M5-1**)*

Reaction of 3-nitro-4-methylbenenasulfonic acid (**S5**) with thionyl chloride afforded sticky 3-nitro-4-methylbenzenesulfonyl chloride which was directly used for reaction with 3,4,5-trimethoxyaniline to afford intermediate **M5-1** as white solid in total yield 36%.

^{1}H NMR (DMSO-d_6): δ 2.55 (s, 3H), 3.55 (s, 3H), 3.65 (s, 6H), 6.39 (s, 2H), 7.70 (d, J=8.0 Hz, 1H), 7.94 (dd, J_1=8.0 Hz, J_2=2.0 Hz, 1H), 8.30 (d, J=2.0 Hz, 1H), 10.30 (s, 1H); EI-MS: *m/z* 382 (M). HR-MS (ESI): *m/z* calcd. for $C_{16}H_{19}N_2O_7S$ 383.09130, found: 383.09030 $[M+H]^+$.

*4.3.5. 4′,6′-Dimethoxypyrimidin-2-yl 3-nitro-4-methylbenzenesulfonamide (**M5-2**)*

Reaction of 3-nitro-4-methylbenzenesulfonyl chloride with 2-amino-4,6-dimethoxypyrimidine to afford **M5-2** as white solid in yield 44%. ^{1}H NMR (DMSO-d_6): δ 2.57 (s, 3H), 3.74 (s, 6H), 5.80 (s, 1H), 7.73 (d, J=8.0 Hz, 1H), 8.13 (dd, J_2=2.0 Hz, J_1=8.0 Hz, 1H), 8.49 (d, J=2.0 Hz, 1H); MS (ESI): *m/z* 355.4 $[M+H]^+$.

Step 2: To a solution of the nitrated intermediate (1.0 mmol) from *step 1* in methanol was added a catalytic amount of 10% Pd/C (30 mg) and the resulting mixture was stirred until the reaction was completed (about 3–8 h) with H_2 under a pressure of 40–50 barr. The mixture was filtered and the filtrate was concentrated *in vacuo* to give the intermediates of **M4-4–M4-6**, **M5-3** and **M5-4. M4-6** was used without further purification for next step to synthesize product **1r**.

*4.3.6. 4′-Trifluoromethylphenyl 3-amino-4-methoxybenzamide (**M4-4**)*

^{1}H NMR (DMSO-d_6): δ 3.83 (s, 3H), 4.94 (s, 2H, D_2O exchanged), 6.89 (d, J=8.8 Hz, 1H), 7.23 (m, 3H), 7.67 (d, J=8.8 Hz, 1H), 7.97 (d, J=8.8 Hz, 2H), 10.27 (s, 1H, D_2O exchanged); MS (ESI): *m/z* 311$[M+H]^+$.

*4.3.7. 4′,6′-Dimethoxypyrimidin-2-yl 3-amino-4-methoxybenzamide (**M4-5**)*

^{1}H NMR (DMSO-d_6): δ 3.82 (s, 3H), 3.85 (s, 6H), 4.88 (br, 2H), 5.92 (s, 1H), 6.84 (d, J=9.2 Hz, 1H), 7.19 (d, J=2.0 Hz, 1H), 7.22 (dd, J_1=9.2 Hz, J_2=2.0 Hz, 1H), 10.30 (s, 1H); MS (ESI): *m/z* 325.1$[M+H]^+$.

*4.3.8. 3′,4′,5′-Trimethoxyphenyl 3-amino-4-methylbenzenesulfonamide (**M5-3**)*

^{1}H NMR (DMSO-d_6): δ 2.03 (s, 3H), 3.54 (s, 3H), 3.64 (s, 6H), 5.33 (br, 2H, D_2O exchangable), 6.37 (s, 2H), 6.85 (dd, J_1=8.0 Hz, J_2=2.0 Hz, 1H), 7.02 (d, J=1.6 Hz, 1H), 7.04 (d, J=8.0 Hz, 1H), 9.90 (s, 1H, D_2O exchangable); MS (ESI): *m/z* 353.3 $[M+H]^+$.

*4.3.9. 4′,6′-Dimethoxypyrimidin-2-yl 3-amino-4-methylbenzenesulfonamide (**M5-4**)*

^{1}H NMR($CDCl_3$): δ 2.23 (s, 3H), 3.81 (s, 6H), 5.63 (s, 1H), 7.15 (d, J=7.6 Hz, 1H), 7.40 (s, 1H), 7.44 (d, J=8.0 Hz, 1H); MS (EI): *m/z* 324 [M].

Step 3: To a solution of aminated intermediates (1.0 mmol) from step 2 and TEA (1.2 mmol, 1.2 equiv) in DCM was added acyl chloride or methyl iodide (1.3 mmol, 1.3 equiv). The resulting reaction was stirred at room temperature until completion. Diluted reaction mixture was rinsed with 0.5 M NaOH and subsequently water. The organic phase was purified by silica gel chromatography to afford compounds **1m**–**1r** and **5a**–**5d** as white or off-white solids with total yields of 14–26%.

*4.3.10. 4′-Trifluoromethylphenyl 3-propionamido-4-methoxybenzamide (**1m**)*

Reaction of **M4-4** with propionyl chloride afforded product **1m** as white solid. Yield, 22%. Mp: 148–150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, J=7.6 Hz, 3H), 2. 48 (q, J=7.6 Hz, 2H), 4.03 (s, 3H), 7.04 (d, J=8.4 Hz, 1H), 7.13 (d, J=8.0 Hz, 2H), 7.52 (d, J=8.0 Hz, 2H), 7.80 (br, 1H), 7.99 (dd, J_1=8.4 Hz, J_2=2.0 Hz, 1H), 8.09 (d, J =2.0Hz, 1H), 8.82 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.44, 165.23, 150.48, 141.25, 127.44, 126.81, 126.19, 126.15, 126.04, 124.86 (2C), 119.82 (2C), 116.92, 110.14, 56.03, 31.00, 9.53; HR-MS (ESI): *m/z* calcd. for $C_{18}H_{17}F_3N_2NaO_3$ 389.10890, found: 389.11085 $[M+Na]^+$.

*4.3.11. 4′-Trifluoromethylphenyl 3-trifluoroacetamido-4-methoxybenzamide (**1n**)*

Reaction of **M4-4** with trifluoroacetyl anhydride afforded product **1n** as white solid. Yield, 14%. Mp: 177–179 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.03 (s, 3H), 7.08 (d, J=8.8 Hz, 1H), 7.63 (d, J=8.4 Hz, 2H), 7.79 (d, J=8.4 Hz, 2H), 7.90 (d, J=8.4 Hz, 1H), 7.97 (br, 1H), 8.61 (br, 1H), 8.82 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.57, 156.17, 153.09, 142.83, 128.68, 126.55, 126.20, 125.90, 125.86, 123.19 (2C), 120.16 (2C), 113.86, 117.63, 111.80, 56.43; HR-MS (ESI): *m/z* calcd. for $C_{17}H_{13}F_6N_2O_3$ 407.08304, found: 407.08389 $[M+H]^+$.

*4.3.12. 4′-Trifluoromethylphenyl 3-(2-bromopropionamido)-4-methoxybenzamide (**1o**)*

Reaction of **M4-4** with 2-bromopropionyl chloride afforded product **1o** as white solid. Yield, 18%. Mp:159–161 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.99 (d, J=7.2 Hz, 3H), 4.01 (s, 3H), 4. 48 (q, J=7.2 Hz, 1H), 7.03 (d, J=8.4 Hz, 1H), 7.61 (d, J=8.4 Hz, 2H), 7.79 (d, J=8.4 Hz, 2H), 7.84 (dd, J_2=2.0 Hz, J_1=8.4 Hz, 1H), 8.12 (br, 1H), 8.74 (br, 1H), 8.85 (d, J=1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.62, 165.05, 151.05, 141.12, 126.83, 126.28, 126.24, 126.20, 125.85, 125.56, 122.76, 119.82 (2C), 116.91, 110.30, 56.26, 45.00, 22.84; HR-MS (ESI): *m/z* calcd. for $C_{18}H_{17}BrF_3N_2O_3$ 445.03746, found: 445.04045 $[M+H]^+$.

*4.3.13. 4′,6′-Dimethoxypyrimidin-2-yl 3-(2-bromopropionamido)-4-methoxybenzamide (**1p**)*

Reaction of **M4-5** with 2-bromopropionyl chloride afforded product **1p** as white solid. Yield, 12%. Mp: 77–79 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.73 (d, J=6.8 Hz, 3H), 3.85 (s, 6H), 3.92 (s, 3H), 5.04 (q, J=6.7 Hz, 1H), 5.95 (s, 1H), 7.14 (d, J=8.4 Hz, 1H), 7.75 (dd, J_1=8.4 Hz, J_2=2.0 Hz, 1H), 8.51 (s, J=2.0 Hz, 1H), 9.66 (s, 1H), 10.63 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.84, 167.40 (2C), 164.17, 156.13, 151.30, 126.96, 126.90, 125.83, 117.68, 110.19, 85.29, 56.26, 54.45 (2C), 45.17, 22.95; HR-MS (ESI): *m/z* calcd. for $C_{17}H_{20}BrN_4O_5$ 439.06171, found: 439.06632 $[M+H]^+$.

*4.3.14. 4′,6′-Dimethoxypyrimidin-2-yl 3-propionamido-4-methoxybenzamide (**1q**)*

Reaction of **M4-5** with propionyl chloride afforded product **1q** as white solid. Yield, 26%. Mp: 87–89 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28 (t, J=7.6 Hz, 3H), 2.48 (q, J=7.6 Hz, 2H), 3.97 (s, 3H), 4.08 (s, 6H), 7.00 (d, J=8.4 Hz, 1H), 7.83 (s, 1H), 7.84 (dd, J_2=2.0 Hz, J_1=8.4 Hz, 1H), 8.95 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.22, 171.94 (2C), 164.32, 156.35, 150.61, 127.49, 127.05, 125.12, 117.39, 110.06, 85.44, 56.03,

54.26 (2C), 31.02, 9.57; HR-MS (ESI): *m/z* calcd. for $C_{17}H_{21}N_4O_5$ 361.15119, found: 361.15154 $[M+H]^+$.

*4.3.15. 5′-Cyanopyridin-2-yl 3-propionamido-4-methoxybenzamide (**1r**)*

Reaction of **M4-6** with propionyl chloride afforded product **1r** as white solid. Yield, 11%. Mp: 169–171 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.07 (t, J=7.6 Hz, 3H), 2.39 (q, J=7.6 Hz, 2H), 3.91 (s, 3H), 7.13 (d, J=8.8 Hz, 1H), 7.85 (dd, J_2=2.0 Hz, J_1=8.8 Hz, 1H), 8.26 (dd, J_2=2.0 Hz, J_1=8.8 Hz, 1H), 8.32 (d, J=8.8 Hz, 1H), 8.60 (s, 1H), 8.83 (d, J=2.0 Hz, 1H), 9.17 (s, 1H), 11.12 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.38, 167.11 (2C), 152.89, 127.83, 127.39, 127.22, 126.09, 124.52, 122.90, 122.61, 119.18, 110.61, 109.60, 56.00, 29.24, 9.72; HR-MS(ESI): *m/z* calcd. for $C_{17}H_{17}N_4O_3$ 325.13006, found: 325.12894 $[M+H]^+$.

*4.3.16. 3′,4′,5′-Trimethoxyphenyl 3-methylamino-4-methylbenzenesulfonamide (**5a**)*

Reaction of **M5-3** with methyl iodide afforded product **5a** as white solid. Yield, 17%. Mp: 143–145 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.21 (s, 3H), 3.13 (s, 3H), 3.74 (s, 6H), 3.83 (s, 3H), 6.31 (s, 2H), 6.87 (s, 1H), 6.94 (d, J=7.6 Hz, 1H), 7.13 (d, J=8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.91 (2C), 144.87, 137.44, 137.40, 134.78, 130.48, 127.14, 117.84, 113.61, 104.75 (2C), 60.88, 56.11 (2C), 38.65, 17.46; HR-MS (ESI): *m/z* calcd. for $C_{17}H_{23}N_2O_5S$ 367.13277, found: 367.13261 $[M+H]^+$.

*4.3.17. 3′,4′,5′-Trimethoxyphenyl 3-acetamido-4-methylbenzenesulfonamide (**5b**)*

Reaction of **M5-3** with acetyl chloride afforded product **5b** as white solid. Yield, 18%. Mp: 242–245 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.07 (s, 3H), 2.23 (s, 3H), 3.30 (s, 3H), 3.64 (s, 6H), 5.05 (br, 2H), 6.38 (s, 2H), 7.36 (d, J=8.0 Hz, 1H), 7.43 (dd, J_1=8.0 Hz, J_2=2.0 Hz, 1H), 8.05 (s, 1H), 9.39 (s, 1H), 10.08 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.60, 152.93 (2C), 137.13, 137.03, 135.97, 134.00, 133.64, 131.02, 122.77, 122.46, 97.73 (2C), 60.01, 55.68 (2C), 23.40, 17.96; HR-MS (ESI): *m/z* calcd. for $C_{18}H_{22}N_2NaO_6S$ 417.10963, found: 417.11079 $[M+Na]^+$.

*4.3.18. 3′,4′,5′-Trimethoxyphenyl 3-propionamido-4-methylbenzenesulfonamide (**5c**)*

Reaction of **M5-3** with propionyl chloride afforded product **5c** as white solid. Yield, 21%. Mp: 183–186 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, J=7.6 Hz, 3H), 2.28 (s, 3H), 2.43 (q, J=7.6 Hz, 2H), 3.76 (s, 9H), 6.37 (s, 2H), 6.51 (br, 1H), 6.99 (br, 1H), 7.22 (d, 1H, J=8.0 Hz), 7.41 (d, J=7.6 Hz, 1H), 8.47 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.10, 153.41 (2C), 137.27, 136.45, 132.24 (2C), 130.76 (2C), 123.82, 121.22, 100.02 (2C), 60.88, 56.12 (2C), 30.56, 17.95, 9.62; HR-MS (ESI): *m/z* calcd. for $C_{19}H_{25}N_2O_6S$ 409.14333, found: 409.14419 $[M+H]^+$.

*4.3.19. 4′,6′-Dimethoxypyrimidin-2-yl 3-propionamido-4-methylbenzenesulfonamide (**5d**)*

Reaction of **M5-4** with propionyl chloride afforded product **5d** as white solid. Yield, 24%. Mp: 188–191 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, J=7.2 Hz, 3H), 2.16-2.22 (q, J=7.2 Hz, 2H), 3.99 (s, 6H), 6.10 (s, 1H), 7.03 (br, 2H), 7.37 (d, J=8.0 Hz, 1H), 7.97 (d, J=8.0 Hz, 1H), 8.61 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.83 (2C), 172.28, 155.13, 138.11, 136.27 (2C), 130.89, 126.26, 123.81, 90.86, 55.11 (2C), 29.64, 18.56, 8.20; HR-MS (ESI): *m/z* calcd. for $C_{16}H_{21}N_4O_5S$ 381.12326, found: 381.12495 $[M+H]^+$.

*4.3.20. Phenyl 3-(2-bromopropionamido)-4-methoxybenzamide (**4a**)*

Compound **4a** was synthesized by acylation of the amino group at the C3-position of compound **4** with 2-bromopropionyl chloride (a total yield of 85% was obtained). Mp: 169–172 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.98 (d, J=7.2 Hz, 3H), 3.99 (s, 3H), 4.58 (q, J=7.2 Hz, 1H), 7.01 (d, J=8.4 Hz, 1H), 7.13 (t, J=7.4 Hz, 1H), 7.36 (t, J=8.0 Hz, 2H), 7.64 (d, J=8.4 Hz, 2H), 7.82 (dd, J_1=8.4 Hz, J_2=2.0 Hz, 1H), 7.88 (br, 1H), 8.73 (br, 1H), 8.50 (d, J=2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.54, 164.93, 150.80, 138.00, 128.97 (2C), 127.46, 126.72, 125.43, 124.37, 120.35 (2C), 116.92, 110.23, 56.21, 54.07, 22.87; HR-MS (ESI) *m/z* calcd. for $C_{17}H_{18}BrN_2O_3$ 377.05008, found 377.05141 $[M+H]^+$.

*4.3.21. 4′-Chlorophenyl 3-propionamido-4-methoxybenzenesulfonamide (**6**)*

Compound **6** was synthesized from 3-amino-4-methoxy-benzenesulfonic acid (**S6**). The impure intermediates **M6** were obtained after removal of solvents in vacuum and then were used for later procedures without purification to afford final product **6** with a total yield of 19%. Mp: 190–192 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, J=7.6 Hz, 3H), 2. 44 (q, J=7.6 Hz, 2H), 3.90 (s, 3H), 6.82 (d, J=8.4 Hz, 1H), 7.04 (d, J=8.8 Hz, 2H), 7.18 (d, J=8.8 Hz, 2H), 7.43 (dd, J_1=8.4 Hz, J_2=2.4 Hz, 1H), 7.74 (br, 1H), 8.93 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.22, 150.89, 135.41, 130.92, 130.47, 129.26 (2C), 128.23, 123.92, 122.85 (2C), 118.02, 109.27, 56.14, 30.97,9.52; HR-MS (ESI): *m/z* calcd. for $C_{16}H_{18}ClN_2O_4S$ 369.06758, found: 369.06894 $[M+H]^+$.

4.4. Cells, plasmids and reagents

Huh7.5 human liver cells were kindly provided by Vertex Pharmaceuticals (Boston, MA), 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, MA).

4.5. Biological assays

The Huh7.5 cells were seeded at a density of 3×10^4 cells/cm^2. After 24 h of incubation the cells were infected with an HCV viral stock (about 45 IU per cell) and simultaneously treated with IFN-α, the test compound, or the solvent control. The culture medium was removed 96 h after inoculation, and the total intracellular RNA and total intracellular proteins were extracted with a Qiagen Kit according to kit instructions. The intracellular HCV RNA was quantified using a one-step real time RT-PCR kit (Invitrogen). Cytotoxicity was tested using the MTT assay, and the IC_{50} and CC_{50} were calculated using the Reed & Muench methods. The details for the antiviral assay and immunoblot experiments were similar to those previously described by Peng et al[22].

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