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Synthesis and structure-activity relationship study of new biaryl amide derivatives and their inhibitory effects against hepatitis C virus

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ABSTRACT

A series of novel biaryl amide derivatives were synthesized and evaluated for anti-HCV virus activity. Some significant SARs were uncovered. The intensive structural modifications led to fifteen novel compounds with more potent inhibitory activity compared to the hit compounds **IMB 26** and **IMB1f**. Among them, compound **80** was the most active, with EC_{50} values almost equivalent to the clinical drug telaprevir ($EC_{50} = 15$ nM). Furthermore, it also had a good safety and in vitro and oral pharmacokinetic (oral bioavailability in rats: 34%) profile, suggesting a highly drug-like nature. Compound **80** represents a more promising scaffold for anti-HCV virus activity for further study.

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1. Introduction

Hepatitis C virus (HCV) infection seriously threatens global health, with approximately 170 million individuals infected and causing up to 350,000 related deaths per year worldwide [1,2]. HCV infection is an insidious disease, and the early stages of infection are largely asymptomatic such that most are unaware of their infection. HCV-infected people are at high risk for developing chronic liver disease, cirrhosis, and hepatocellular carcinoma [3]. As many as 50−80% of patients newly infected with HCV develop chronic infection; of those chronically infected individuals, approximately 30% progress to liver cirrhosis, and up to 4% will go on to develop life-threatening hepatocellular carcinoma and end-stage liver disease [2,4]. The combination of pegylatedinterferon (PEG-IFN) with ribavirin is the conventional treatment for HCV infection, which requires 24−48 weeks and causes frequent, sometimes severe, side effects. Moreover, the regimen is only effective in the range of one half to two-thirds of persons treated, depending on clinical stage and genotype [5]. The recent approval of several small-molecule direct-acting antiviral (DAA) therapies for HCV infection has dramatically improved the standard of care for HCV. These drugs target viral proteins (NS3/4 A protease, NS5B polymerase, and NS5A) involved in the replication stage of HCV infection [6]. For example, Epclusa: a combination of sofosbuvir (a nucleotide analog inhibitor of HCV NS5B polymerase) with velpatasvir (NS5A inhibitor), is the backbone of the first oral, pangenotype, single-tablet regimen for the treatment of adults with genotype 1−6 chronic HCV infection [7]. Two newly approved combination hepatitis C drugs (Zepatier: elbasvir + grazoprevir and Viekira and PaK: ombitasvir + paritaprevir + ritonavir + dasabuvir) have demonstrated improved safety and efficacy for treating genotype 1 or 4 HCV-infected persons [8]. Although these treatments offer renewed hope toward curing HCV infection, the issues of drug resistance, narrow genotype specificity, lack of vaccines, and high cost remain [9−12]. It is still imperative to develop new anti-HCV agents, especially those with novel mechanisms of action (MOAs) and new molecular structures.

Many compounds with biaryl amide moieties have been studied continuously because of their diverse roles in biological functions and diseases, such as viral infection [13], bacterial infection [14], diabetes [15], spinal muscular atrophy [16], human African

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trypanosomiasis [17] and cancers [18—22]. As shown in Fig. 1, ML336 was found to inhibit potently several Venezuelan equine encephalitis viruses in the low nanomolar range without cytotoxicity [13]. Compound **2** was found to have antibiofilm activity as an adjuvant that enhances the susceptibility of drug-resistant strains of bacteria, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa,* to meropenem [14]. Compound **3** showed potent activity in ex *vivo* diabetic retinopathy models as a new class of selective Rho kinase inhibitors [15]. **SCYX-7158** was used to treat human African trypanosomiasis(HAT) and has begun human clinical trials [17]. Compounds **5—9** show effective anticancer activities. Compound **5**inhibited autotoxin-dependent invasion of A2058 human melanoma cells *in vitro* and reduced B16 melanoma metastasis *in vivo* [18]. Compound **8** was found to be an efficacious RAF protein inhibitor targeting RAS mutant cancer [21]. Compound **9** (Ponatinib) is an orally active multitargeted kinase inhibitor and has been approved to treat chronic myeloid leukemia by the FDA [22].

Human APOBEC3G (apolipoprotein B messenger RNA [mRNA]-editing enzyme catalytic polypeptide-like 3G, hA3G) is a cytidine deaminase and belongs to the APOBEC superfamily. Accumulated evidence shows that hA3G in human T lymphocytes represents an innate immunity factor that displays broad-spectrum antiviral activity, including inhibiting human immunodeficiency virus type 1(HIV-1) [23—26], hepatitis B virus (HBV) [27], HCV [28,29], paramyxovirus [30], enterovirus 71(EV71) [31,32], and T-cell leukemia virus type 1(HTLV-1) [33]. In continuation of our research on antiviral drugs, some biaryl amid ederivatives were found to display significant anti-HIV-1, anti-HCV, and anti-EV17 activities (Fig. 2). An antiviral mechanism study demonstrated that **IMB-26**, as an hA3G stabilizer, directly binds to the hA3G protein and infectively protects hA3G from Vif-mediated degradation and inhibits HIV-1 viral replication [23]. **IMB-1f**, as an analog of **IMB-26**, inhibited hepatitis C virus replication [29,31]. **IMB-Z** was found to increase hA3G encapsidation into EV17 progeny virion particles and to inhibit EV17 replication [32]. In addition, Young et al. reported that biaryl amide derivative **10**, as a small molecule inhibitor of microRNA miR-122, can reduce HCV RNA levels [34]. Since these biaryl amide derivatives target host innate components (hA3G is an innate immunity factor, and microRNA miR-122 is a human liver-specific miRNA), the virus will most likely not be able to develop resistance to these molecules. Therefore, biaryl amide derivatives could be a new class of broad spectrum antiviral agents that merit exploration.

Here, we synthesized a series of new *N*-aryl benzamide analogs by changing R₁, R₂, and R₃(Fig. 2) and evaluated their ability to inhibit hepatitis C virus replication in acutely infected Huh7.5 cells. The medicinal chemistry effort led to the discovery of more potent new lead compounds of anti-HCV **68**, **78**, and **80**, which exhibited strong anti-HCV activity comparable to the clinical drug VX950 ($EC_{50} = 0.015-0.083$ μM). More importantly, a novel pharmacophore of *N*-aryl-(3-nitro-4-alkoxy)benzamide against HCV infection was revealed by structure-activity relationship (SAR) analysis. The physicochemical and ADME properties of compound **80** were evaluated. The primary study of some compounds inhibiting Vif-mediated hA3G degradation progressed.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **13** (**IMB-1f**) and **14** was performed according to a previously reported method (Scheme 1) [31]. Hydrogenation of the nitro group followed by amide coupling reaction with propanoyl chloride furnished compounds**13** and **14**, respectively. Compounds **16—20** were obtained by amide coupling reaction between various substituted anilines and benzoic acid derivative **15**, which was obtained by a selective amide coupling reaction between 4-methoxy-3-aminobenzoic acid and propanoyl chloride.

Compounds **22—35** were obtained as depicted in Scheme 2. 4-hydroxy-3-nitrobenzoic acid acted as a starting compound through an amide coupling reaction with 4-methoxy aniline to afford intermediate **21**, which was reacted with various desired alkyl bromides by nucleophilic substitution to afford corresponding nitro intermediates **22—25**. Hydrogenation of the nitro group offered corresponding amino derivatives **29—32**, which were then reacted with propanoyl chloride to give final products **36—39**. Compound **25** was reacted with various secondary amines in the presence of potassium carbonate to afford corresponding nitro
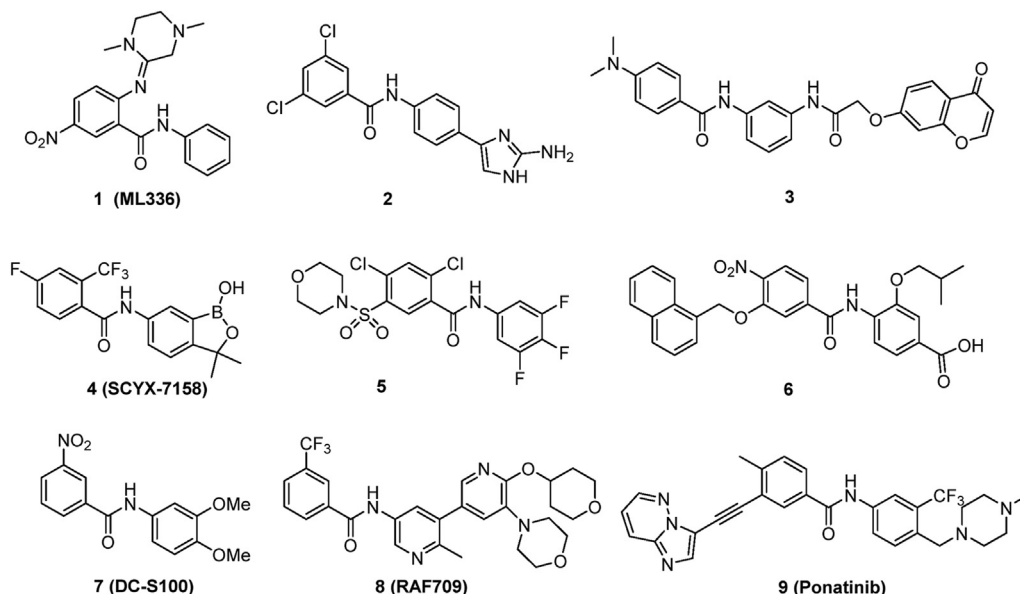

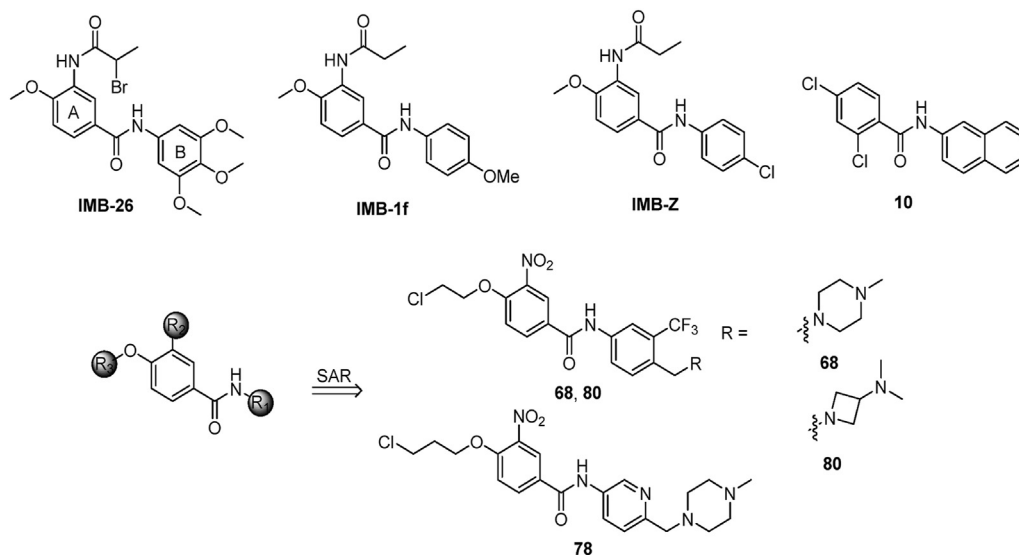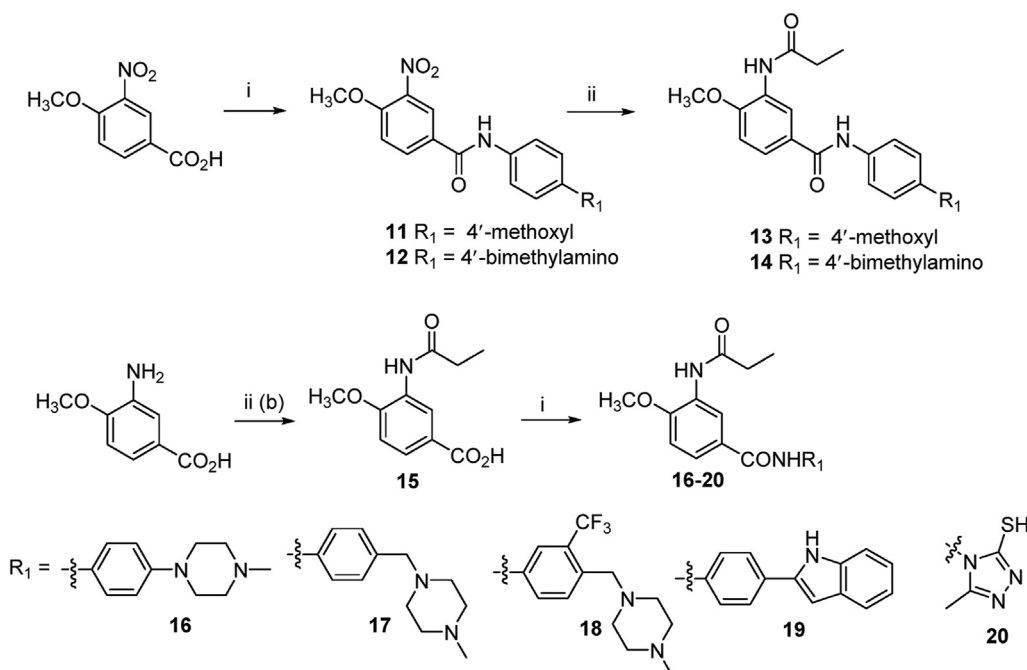**Fig. 1.** Representative biaryl amide derivatives.

Fig. 2. Indicated structural modifications of IMB-26.

Scheme 1. Synthesis of compounds 14 and 16−20. Reagents and conditions: (i) various substituted anilines, EDCI, DMAP, CH$_2$Cl$_2$, rt, 8 h; (ii) a. H$_2$ (30 psi), Pd/C, MeOH/EtOAc (1:1), rt, 2 h; b. propanoyl chloride, Et$_3$N,CH$_2$Cl$_2$, 0 °C−rt, 3−5 h.

compounds 26−28. Hydrogenation of the nitro group offered corresponding amino intermediates 33−35, which were reacted with propanoyl chloride to give final products 40−42.

Compound 45 was synthesized through a 4-step reaction, including two amide coupling reactions, an intramolecular nucleophilic substitution, and a hydrolysis reaction. Using methyl 3,4-dihydro-2H-benzo [1,4]oxazine-6-carboxylate as the starting compound, compound 48 was synthesized through hydrolysis reactions and an amide coupling reaction (Scheme 3).

Compounds 50−57 were obtained as depicted in Scheme 4. Starting from methyl 4-hydroxy-3-nitrobenzoate, a nucleophilic substitution reaction with 2-bromo propane and subsequent hydrolysis reaction under basic conditions afforded 49, which was

coupled with substituted anilines to afford corresponding nitro compounds 50 and 51. Reduction of the nitro group with palladium-catalyzed hydrogenation offered corresponding amino derivatives 52 and 53. Compounds 52 and 53 were reacted with propanoyl chloride or 2-bromo propanoyl chloride in the presence of Et$_3$N to give products 54, 56, 55, and 57, respectively.

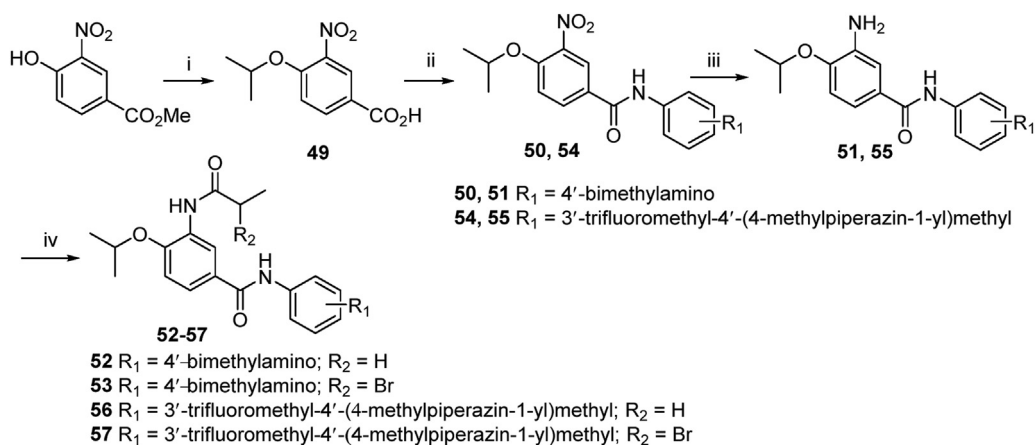Compounds 60−70 were obtained according to the synthetic route depicted in Scheme 5. Starting from various R$_2$-substituted methyl 4-hydroxy benzoate analogs 58a−e, a nucleophilic substituted reaction with 1-bromo-3-chloropropane and subsequent hydrolysis reaction yielded the corresponding benzoic acid derivatives 59a−e. Compounds 59a−e were coupled with 3-trifluoromethyl-4-(4-methylpiperazin-1-yl)-aniline in the
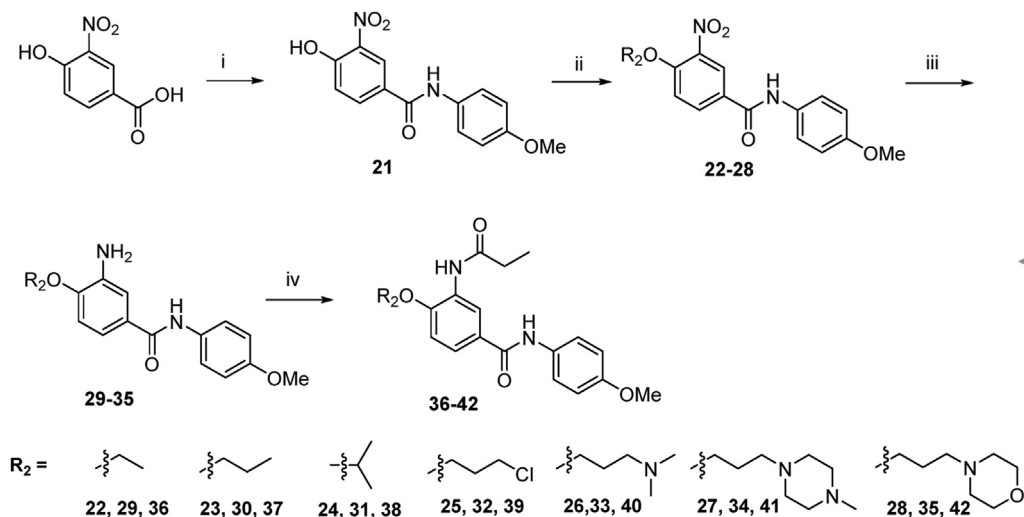
Scheme 2. Synthesis of compounds **36−42**. Reagents and conditions: (i) a. 4-methoxy anilines, EDCI, DMAP, CH$_2$Cl$_2$, rt, 6 h. (ii) a. various alkyl alcohols, PPh$_3$, DEAD,THF,0 °C−rt, 10 h (afforded corresponding compounds **22−25**); b. secondary amines, K$_2$CO$_3$, DMF, 60 °C, 4 h (afforded corresponding compounds **26−28** from compound **25**). (iii) H$_2$ (30 psi), Pd/C, MeOH/EtOAc (1:1), rt, 1.5−3 h(iv)propanoyl chloride, Et$_3$N,CH$_2$Cl$_2$, 0 °C−rt, 3 h.
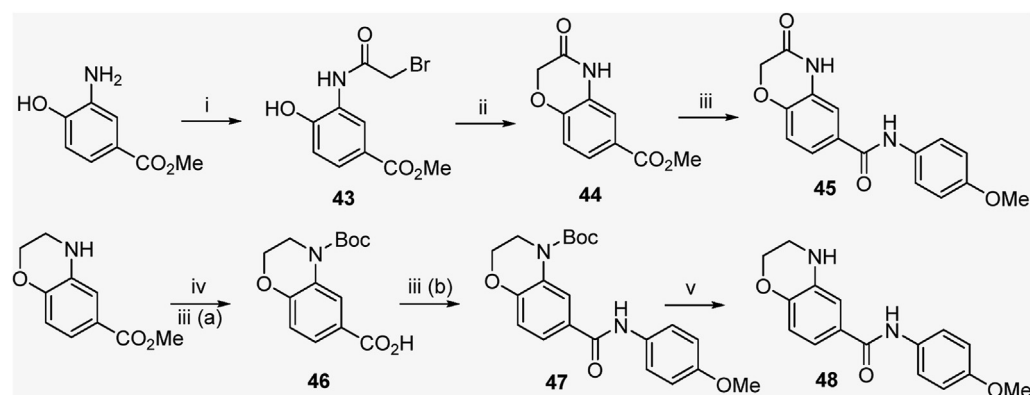
Scheme 3. Synthesis of compounds **45** and **48**. Reagents and conditions: (i) 2-bromoacetyl bromide, NaHCO$_3$, EtOAc/H$_2$O (1:1), rt, 12 h. (ii) K$_2$CO$_3$, DMF, 80 °C, 3 h (iii) a. NaOH, H$_2$O/MeOH (1:2), reflux, 3 h; b. 4-methoxyaniline, EDCI, DMAP, CH$_2$Cl$_2$, rt, 6 h. (iv) Boc$_2$O, DMAP, CH$_3$CN, rt. (v) 30% CF$_3$CO$_2$H in CH$_2$Cl$_2$, 0 °C−rt, 3 h.

50, 51 R$_1$ = 4′-bimethylamino
54, 55 R$_1$ = 3′-trifluoromethyl-4′-(4-methylpiperazin-1-yl)methyl

52 R$_1$ = 4′-bimethylamino; R$_2$ = H
53 R$_1$ = 4′-bimethylamino; R$_2$ = Br
56 R$_1$ = 3′-trifluoromethyl-4′-(4-methylpiperazin-1-yl)methyl; R$_2$ = H
57 R$_1$ = 3′-trifluoromethyl-4′-(4-methylpiperazin-1-yl)methyl; R$_2$ = Br

Scheme 4. Synthesis of compounds **50−57**. Reagents and conditions: (i) a. 2-Bromo propane, K$_2$CO$_3$, NaI, DMF, 65 °C, 5 h; b. LiOH, MeOH/THF (1:1), 0 °C, 1 h. (ii) substituted anilines, EDCI, DMAP, CH$_2$Cl$_2$, rt, 6 h; (iii) H$_2$ (30 psi), Pd/C, MeOH/EtOAc (1:1), rt, 2 h; (iv) propanoyl chloride or 2-bromopropanoyl chloride, Et$_3$N,CH$_2$Cl$_2$, 0 °C−rt, 3 h.

presence of EDCI and DMAP to afford the corresponding amide derivatives **60−64**. Reduction of the nitro compound **60** via palladium-catalyzed hydrogenation offered the corresponding

amino product **65**, which was reacted with propanoyl chloride to give the desired product **66**. Compound **58a** was reacted with different brominated alkanes and subsequently hydrolyzed to give
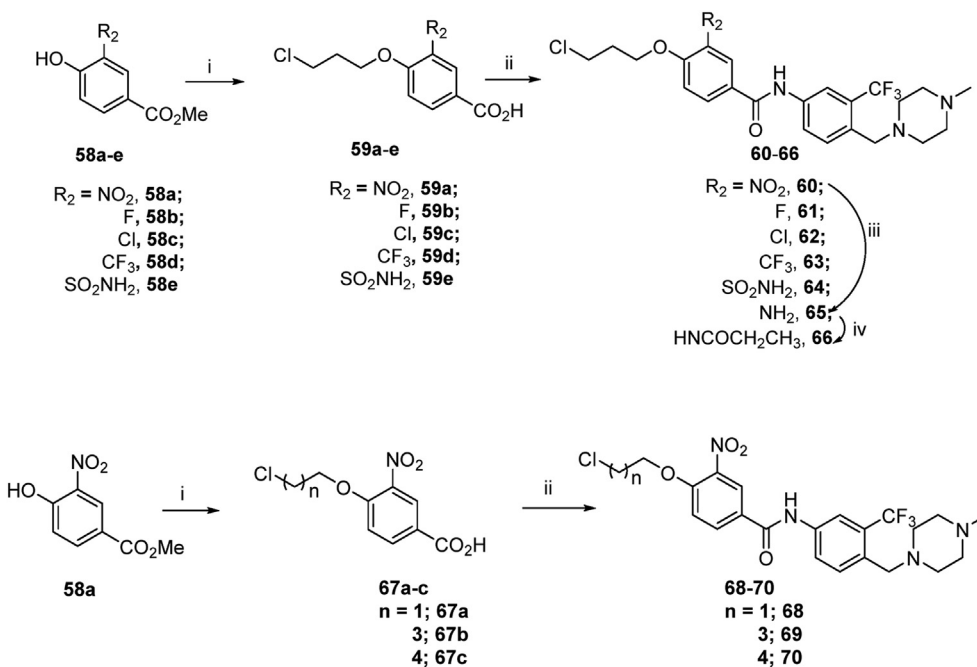
Scheme 5. Synthesis of compounds **60**−**70**. Reagents and conditions: (i) a. 1-bromo-3-chloropropane (1-bromo-2-chloroethane for **67a**;1-bromo-4-chlorobutane for **67b**;1-bromo-5-chloropentane for **67c**), K$_2$CO$_3$, NaI, DMF, 65 °C, 5 h; b. LiOH, MeOH/THF (1:1), 0 °C, 1 h. (ii) 3-trifluoromethyl-4-(4-methylpiperazin-1-yl)-aniline, EDCI, DMAP, CH$_2$Cl$_2$, rt, 6 h (iii) H$_2$ (30 psi), Pd/C, MeOH/EtOAc (1:1), rt, 3 h. (iv) Propanoyl chloride, Et$_3$N,CH$_2$Cl$_2$, 0 °C−rt, 3 h.

intermediates **67a**−**c**, which were coupled with 3-trifluoromethyl-4-(4-methylpiperazin-1-yl)-aniline to give compounds **68**−**70**.

Compounds **71**−**80** were prepared as described above through an amide coupling reaction between compound **59a** or **67a** and various substituted anilines (Scheme 6). The substituted anilines **83a**−**f**, **86**, and **88** that were not commercially available were readily synthesized by a short three-step sequence (Scheme 7). Briefly, the bromination of 2-trifluoromethyl-4-nitrotoluene with *N*-bromosuccinimide followed by a nucleophilic substituted

reaction with various appropriate second amines gave nitro derivatives **82a**−**f**, which were hydrogenated by palladium-catalyzed hydrogenation reduction to afford corresponding amino derivatives **83a**−**f**. Compound **88** was synthesized by a nucleophilic substituted reaction followed by a reduction of the nitro group.

2.2. SAR analysis for anti-HCV activity in vitro

All analogs were screened for inhibition of HCV RNA replication
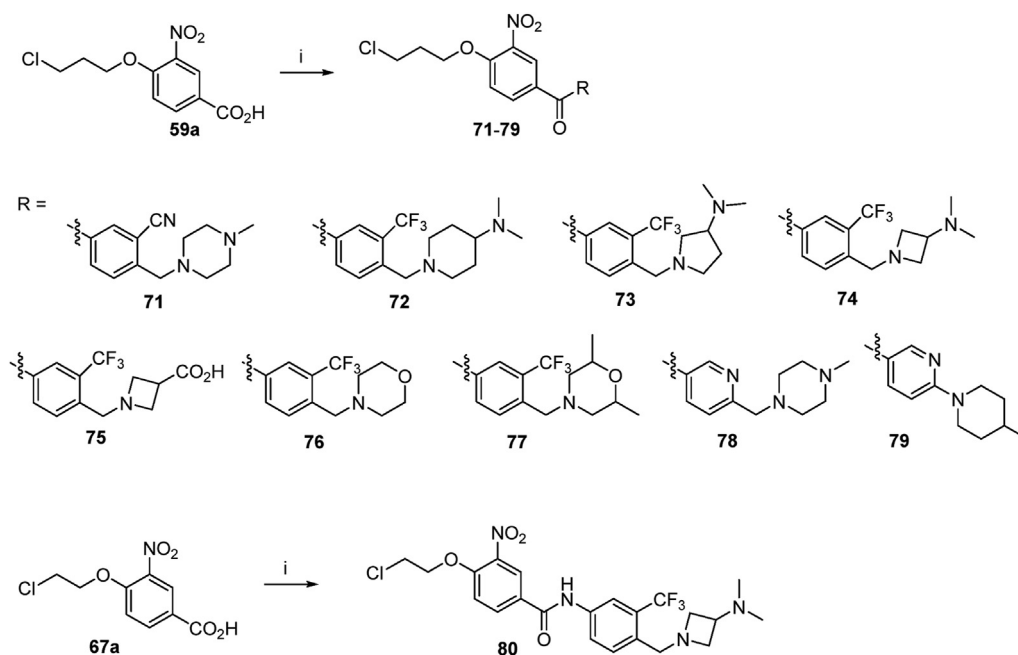
Scheme 6. Synthesis of compounds **71**−**80**. Reagents and conditions: (i) a. various substituted anilines, EDCI, DMAP, CH$_2$Cl$_2$, rt, 6 h; b. LiOH, MeOH/THF, 0 °C−rt, for compound **39**.
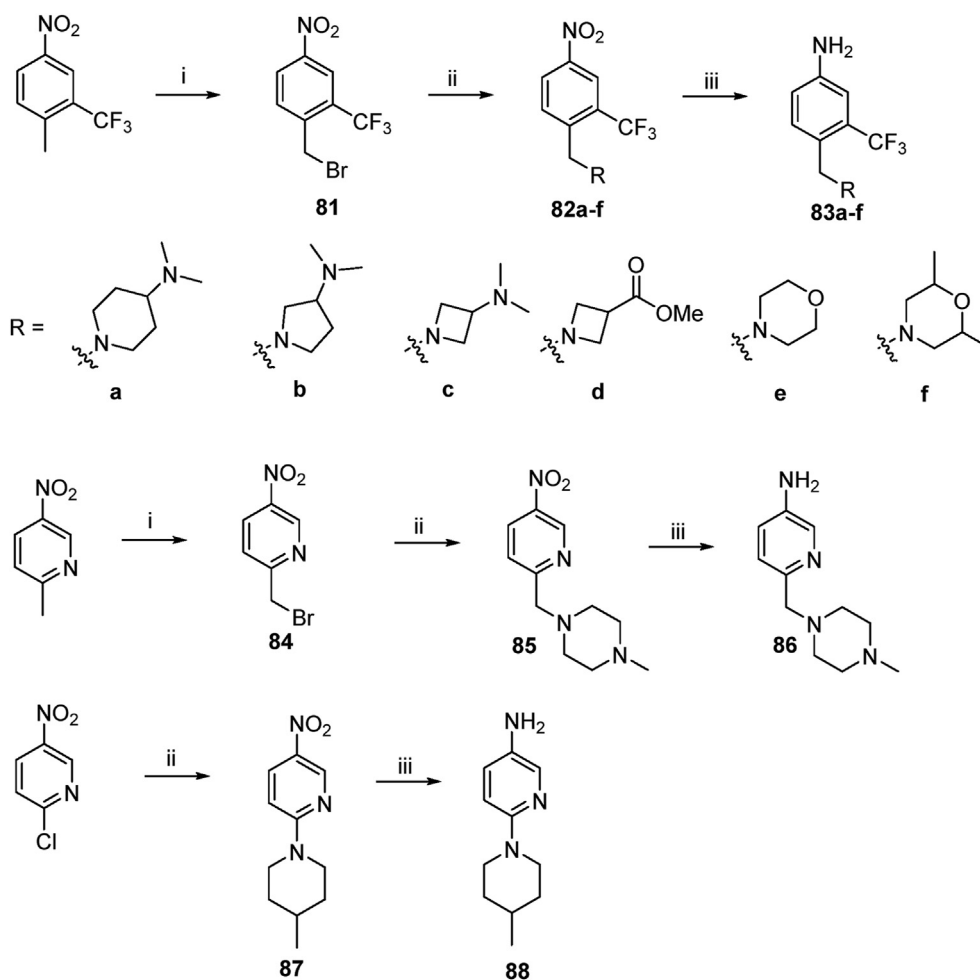
Scheme 7. Synthesis of compounds **83a**−**f**, **86**, and **88**. Reagents and conditions: (i) NBS, AIBN, CH$_2$Cl$_2$, reflux for 12 h; (ii) substituted second amine, K$_2$CO$_3$, NaI, DMF, 65 °C, 5 h; (iii) H$_2$ (14−30 psi), Pd/C, MeOH/EtOAc (1:1), rt, 3−24 h.

in Huh7.5 cells infected with virus J6/JFH/JC-1(recombinant HCV genotype 2a). In the HCVcc (a cell culture system for HCV) system, Huh7.5 cells were infected with HCV vital stock (45 IU/cell) and treated simultaneously with test compounds or the positive control telaprevir (VX-950) and simeprevir (SIM). Total intracellular HCV RNA was extracted and quantified with one-step qRT-PCR. The cytotoxicity was determined using MTT assay. The EC$_{50}$ and CC$_{50}$ values were calculated with the Reed and Muench method.

Among the 43 novel synthesized compounds summarized in Table 1−**4**, twenty-three compounds showed higher potent activity against HCV than **IMB-1f** (IC$_{50}$: <1.31 μM). Six compounds showed strong anti-HCV activities (IC$_{50}$: 0.015−0.083 μM) and high selectivity indices (SI: 113−431). Compound **80** showed the highest activity comparable to the positive control VX-950 (**80**: EC$_{50}$ = 0.015 μM vs. VX-950: EC$_{50}$ = 0.022 μM). Most compounds displayed suitable values of physicochemical parameters, such as the calculated LogP (cLogP < 5) and topological polar surface area (tPSA between 50 and 100 Å), and could have good bioavailability and drug-like features [35,36].

As shown in Fig. 2, the structural elements of R$_1$, R$_2$, and R$_3$ were first investigated during the SAR study. In a previous report, 4-OCH$_3$ in the A ring was considered to be important for antiviral activity, and replacement of the α-bromocarbonyl group with a propionyl group led to lower cytotoxicity [29,31]. Therefore, we first fixed R$_2$ as a propionamino group and R$_3$ as a methyl group and varied the

R$_1$ moiety. Compounds **14** and **16**−**20** were synthesized to probe the effect of R$_1$ moieties on anti-HCV activity. As shown in Table 1, compound **18** with a 4-(4-methylpiperazin-1-yl)-methyl-3-trifluoromethyl-phenyl group, which is an important part of ponatinib, a clinical antitumor drug with multitargeted tyrosine-kinase inhibition, exhibited definitive anti-HCV activity similar to the reported compounds, IMB-26 and 13 (IMB-1f), with selectively index (SI) values higher than 20 and EC$_{50}$ values lower than 1.5 μM. Compounds **14**, **19**, and **20** showed rather modest anti-HCV activity, but IS values were higher than those of IMB-26. Compounds **16** and **17** completely lost their antiviral activity. The SAR data pointed to the choice of the R$_1$ moieties being crucial for high potency, which was not limited to lipophilic or hydrophilic groups but would need suitable volume and polarity distributions.

As compound **13** had a high SI value, we fixed R$_1$ as a 4-methoxy phenyl group and R$_2$ as a propionamino group and varied the R$_3$ moiety to synthesize compounds **36**−**42** (Table 2). Replacing the methyl group in the R$_3$ moiety with ethyl or propanyl groups, as shown in compounds **36** and **37**, slightly decreased anti-HCV activities. Compound **38** with isopropyl substitution displayed higher anti-HCV activity and SI value than IMB-26 and compound **13**. Introduction of the 3-chloro group in compound **37**, as shown in compound **39**, retained partial antiviral activities. Installation of bimethylamino, 4-methyl piperizin-1-yl, or morpholinyl hydrophilic moieties on the head of R$_3$ (40, 41, and 42) resulted in

Table 1
SAR exploration focused on the R_1 moieties.
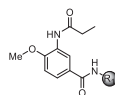
Compd.	Structure of R_1	$CC_{50}(\mu M)$	$EC_{50}(\mu M)$	SI	ClogP	tPSA
IMB 26		15 ± 1.1	2.1 ± 0.40	7	2.72	95.1
13 (IMB-1f)		109 ± 3.5	1.30 ± 0.24	84	2.01	76.7
14		171	2.56 ± 1.24	66	2.13	70.7
16		>200	>22.2	/	1.86	73.9
17		>200	>22.2	/	2.15	73.9
18		35.3 ± 2.4	1.48 ± 0.72	24	3.46	73.9
19		65.6 ± 4.6	7.41 ± 2.03	9	5.38	79.5
20		>200	14.4	>14	−0.32	95.4
VX-950		23.7 ± 7.4	0.022 ± 0.008	1078	2.75	179.5
SIM		38.04 ± 4.02	0.008 ± 0.006	4755	/	/

Table 2
SAR exploration focused on the R_3 Moieties.
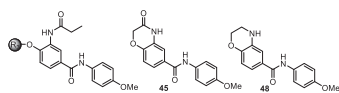
Compd.	Structure of R_3	$CC_{50}(\mu M)$	$EC_{50}(\mu M)$	SI	ClogP	tPSA
36		73.15 ± 4.91	1.47 ± 0.23	48	2.54	76.6
37		106.2	1.55 ± 0.53	68	3.07	76.6
38		112.7 ± 21.4	1.10 ± 0.40	103	2.85	76.6
39		158.2	7.41 ± 1.31	21	3.80	76.6
40		153.78	>22.2	/	2.46	79.9
41		126.5	13.47	9.4	1.51	83.1
42		49.18 ± 7.84	>22.2	/	2.44	89.1
45		77.31 ± 2.10	7.46 ± 1.40	10	1.71	76.7
48		152.8	>22.2		2.53	59.6

significant activity loss. Cyclizing R_2 and R_3 moieties gave compounds **45** and **48**. Compound **45** retained partial antiviral activities, while **48** lost activity. The above results showed that the R_3 position may be lipophilic required for anti-HCV activity, and the isopropyl moiety is the most advantageous group, as shown in Table 2.

Next, we investigated the importance of the R_2 moiety for anti-HCV activity. As shown in Table 3, because intermediates **12** and **24**−**26** with nitro groups in the R_2 position showed modest inhibitory activity against HCV with EC_{50} values in the range of 2.39−8.96 μM, we synthesized two series of compounds (**50**−**53** and **54**−**57**) with four different R_2 moieties and intensively

investigated the relationship between R_2 moieties and anti-HCV activity. In the two series of compounds, compounds **50** and **54** with nitro groups displayed the most dominant anti-HCV activity (EC_{50} = 0.095 and 3.10 μM, respectively) in the four kinds of substituent derivatives, and the corresponding reduction products **51** and **55** showed the weakest activity. Compounds **52**, **53**, **56**, and **57** with amide groups in the R_2 moiety showed moderate inhibitory activity. Compounds **53** and **57** with α-bromo propionyl groups displayed higher cytotoxicity than compounds **52** and **56** with propionyl groups, which was consistent with previous results [29]. Notably, compounds **54**−**57** exhibited higher potent activity than IMB 26 and compound **13** with EC_{50} values in the range of 0.09−1.32 μM, which means that the 4-(4-methylpiperazin-1-yl)-methyl-3-trifluoromethyl-phenyl group may be more dominant than the 4-methoxy phenyl group in the anti-HCV activity. Compound **25** with a 3-chloropropyl group at the R_3 position exhibited similar antiviral activity to compound **24** with an isopropyl group but possessed lower cytotoxicity. To obtain analogs with various structures and good solubility, compound **60** was synthesized. Compound **60** not only unexpectedly displayed the highest inhibitory activity (EC_{50} = 0.044 μM) but also exhibited the highest SI value (SI = 154) among the above target compounds. Replacing the nitro group (**60**) with a fluoro (**61**), chloro (**62**) trifluoromethyl (**63**) or sulfamoyl group (**64**) deteriorated the anti-HCV activity. Replacing the nitro group (**60**) with an amino or amido group (**65** and **66**) decreased the activity, which is consistent with the above two series of compounds **50**−**53** and **54**−**57**. These data indicate that the nitro group in the R_2 fragment would be an advantage in the anti-HCV activity of these compounds. Decreasing the tether length by one −CH_3 group in the R_3 fragment (**68**) retained similar activity as compound **60**. Increasing the tether length by one or two −CH_3 groups in the R_3 fragment (**69** and **70**, respectively) did not improve the potency but rather the cell toxicity.

Because of the dominance of the 4-(4-methylpiperazin-1-yl)-methyl-3-trifluoromethyl-phenyl group in the anti-HCV activity, novel analogs **71**−**79** were synthesized to explore further the structure-activity relationship (Table 4). Replacing the

Table 3
SAR exploration focused on the R_1, R_2, and R_3 moieties.

Compd.	Structure R_1	R_2	R_3	CC_{50} (μM)	EC_{50} (μM)	SI	ClogP	tPSA
12	(structure)	NO_2	Me	88.2 ± 1.6	4.07 ± 1.63	22	2.82	93.4
24	(structure, OMe)		(structure, isopropyl)	23.4 ± 2.8	2.86 ± 1.80	8	3.44	99.4
25			(structure, Cl-propyl)	39.7 ± 7.4	2.39 ± 2.08	17	3.52	99.4
26			(structure, dimethylamino)	126.5 ± 9.6	8.96 ± 3.57	14	3.05	102.6
50	(structure)		(structure, isopropyl)	50.0 ± 18.4	3.10 ± 0.17	16	3.66	93.4
51		NH_2		72.1 ± 9.2	7.41 ± 2.23	10	2.99	67.6
52		$NHCOCH_2CH_3$		80.6 ± 20.7	6.03 ± 1.74	13	2.96	70.7
53		$NHCOCHBrCH_3$		7.9 ± 0.9	4.34 ± 0.86	2	3.72	70.7
54	(structure, CF_3)	NO_2		3.5 ± 0.3	0.095 ± 0.021	39	4.70	96.6
55		NH_2		21.6 ± 2.3	1.32 ± 0.22	16	4.32	70.8
56		$NHCOCH_2CH_3$		15.3 ± 0.7	0.30 ± 0.04	51	4.30	73.9
57		$NHCOCHBrCH_3$		8.7 ± 1.2	0.32 ± 0.04	27	5.05	73.9
60		NO_2	(structure, Cl-propyl)	**6.8 ± 0.07**	**0.044 ± 0.014**	**154**	4.91	96.6
61		F		8.8 ± 1.2	0.49 ± 0.25	18.9	5.33	44.8
62		Cl		4.1 ± 1.3	0.58 ± 0.35	7.0	5.89	44.8
63		CF_3		13.4 ± 0.97	0.13 ± 0.02	99.6	6.28	44.8
64		SO_2NH_2		5.47 ± 2.92	1.14 ± 0.56	6.2	3.31	104.9
65		NH_2		12.9 ± 1.75	1.18 ± 0.53	10.9	4.52	70.8
66		$NHCOCH_2CH_3$		7.87 ± 0.54	0.22 ± 0.18	36.3	4.38	73.9
68		NO_2	(structure, Cl-ethyl)	**9.04 ± 2.02**	**0.044 ± 0.002**	**204**	4.58	96.6
69			(structure, Cl-butyl)	3.74 ± 0.61	0.100 ± 0.095	37	5.28	96.6
70			(structure, Cl-pentyl)	2.14 ± 0.65	0.060 ± 0.011	36	5.81	96.6

trifluoromethyl group in the B ring with a cyano group afforded less potent activity, as shown in compounds **60** and **71**. Replacing 4-methylpiperazin-1-yl with 4-(dimethylamino)piperidinyl afforded a less potent analog, as shown in compounds **60** and **72**. However, the introduction of smaller substitutions, such as 4-(dimethylamino)cyclopentylamino and 3-(dimethylamino)azetidin-1-yl groups, as shown in compounds **73** and **74**, restored and even increased anti-HCV activity. Replacing the *N,N*-dimethyl group in compound **74** with a carboxy group resulted in a distinct loss of activity (**75**). Introduction of morpholinyl and dimethyl-substituted morpholinyl groups, as in **76** and **77**, compared to compound **60**, led to 3−6-fold decreased activity. Introduction of 4-(4-methylpiperazin-1-yl)-methyl-pyridin-3-yl group, as shown in compound **78**, led to dropped potency of only 2-fold (compared **78** to **60**, Table 4), showing that the pyridine-3-yl moiety was tolerated in the place of 3-trifluoromethyl-phenyl. Replacing the (4-methylpiperazin-1-yl)-methyl group (**78**) with 4-methylpiperidinyl (**79**) led to 8-fold decreased potency. Compound **80** with a 3-(dimethylamino)azetidin-1-yl group in the R_1 fragment and a 2-chloroethyl group in the R_3 fragment displayed the highest activity (EC_{50} = 0.015 μM) and SI value (SI = 431) among all synthesized novel target compounds.

2.3. Safety assessment of compound 80

Because compounds **60** and **80** showed strong anti-HCV activities (EC_{50}: 0.044 and 0.015 μM) and high selectivity indices (SI: 154 and 431), we chose compounds **60** and **80** to investigate their safety profiles. Acute toxicity tests of compounds **60** and **80** were performed in KunMing mice. Each compound was given intraperitoneally in a single-dosing experiment at 50, 100, 150, or 200 mg/kg (n = 6 per group). The mice were closely monitored for 7 days. Compound **60** displayed low safety profiles with median lethal dose (LD_{50}) values lower than 100 mg/kg. Compound **80** demonstrated modest safety profiles with LD_{50} values higher than 150 mg/kg. The results suggested that compound **80** was relatively safe *in vivo*.

2.4. In vitro pharmacokinetic property assessments of compound 80

Compound **80** showed the highest activity among all synthesized novel compounds and low toxicity. While compound **80** has poor solubility in water (<5 μg/mL), the aqueous solubility of the corresponding hydrochloride salt was improved to 7.8 mg/mL (at pH 7.0). Thus, compound **80** was further profiled in four assays to assess *in vitro* drug-like properties: logD, microsomal stability, cell permeability and plasma stability (Table 5). Compound **80** showed decent plasma stability ($t_{1/2, \text{ rat}}$ = 16.9 h and $t_{1/2, \text{ human}}$ = 19.9 h), which could ensure that a high concentration of the compound reached the bloodstream. Compound **80** showed moderate permeability (0.5 < P_{app} < 2.5 (× 10^{-6} cm/s)) and was likely an efflux transporter substrate based on Caco-2 assays. In data from HLM/RLM, it appeared that compound **80** had low to medium metabolic stability based on liver microsome assays.

2.5. In vivo pharmacokinetic property assessments of compound 80

Given its favorable *in vitro* ADME profile, the *in vivo* pharmacokinetics of compound **80** were evaluated in a rat (Sprague-Dawley) model after a single dose of 2 mg/kg through the

Table 4
SAR exploration focused on the R$_1$ fragments.

Compd.	Structure R$_1$	R$_2$	R$_3$	CC$_{50}$ (μM)	EC$_{50}$ (μM)	SI	cLogP	tPSA
71	(structure, CN / N-methylpiperazine)	NO$_2$	(Cl-alkyl)	15.5 ± 11.1	0.65 ± 0.057	23	3.74	120.4
72	(structure, CF$_3$ / dimethylaminopiperidine)	NO$_2$	(Cl-alkyl)	5.5 ± 2.5	0.38 ± 0.13	14	4.32	96.6
73	(structure, CF$_3$ / dimethylaminopyrrolidine)	NO$_2$	(Cl-alkyl)	**5.8 ± 0.77**	**0.051 ± 0.042**	**113**	4.76	96.6
74	(structure, CF$_3$ / dimethylaminoazetidine)	NO$_2$	(Cl-alkyl)	**7.8 ± 0.57**	**0.021 ± 0.005**	**371**	5.20	96.6
75	(structure, CF$_3$ / azetidine-carboxylic acid)	NO$_2$	(Cl-alkyl)	>200	4.72 ± 2.49	>42	2.12	124.7
76	(structure, CF$_3$ / morpholine)	NO$_2$	(Cl-alkyl)	6.1 ± 1.7	0.315 ± 0.380	19.3	4.34	102.6
77	(structure, CF$_3$ / dimethylmorpholine)	NO$_2$	(Cl-alkyl)	2.3 ± 0.54	0.174 ± 0.115	13.3	5.38	102.6
78	(structure, pyridine / N-methylpiperazine)	NO$_2$	(Cl-alkyl)	**12.6 ± 1.2**	**0.083 ± 0.029**	**151.4**	2.90	103
79	(structure, pyridine / methylpiperidine)	NO$_2$	(Cl-alkyl)	9.2 ± 1.1	0.64 ± 0.377	14.4	4.50	99.75
80	(structure, CF$_3$ / dimethylaminoazetidine)	NO$_2$	(Cl-alkyl)	**6.47 ± 1.05**	**0.015 ± 0.005**	**431**	4.87	96.6

Table 5
Solubility data and ADME for Compound 80.

Compd	logD7.4[a]	Solubility of hydrochloride (mg/mL)	Caco2 AB[b]	Caco2 ER[c]	T$_{1/2}$ (h)[d] rat	T$_{1/2}$ (h)[d] human	HLM/RLM (μL/min)/mg
80	3.50	7.8	1.25	20.73	16.9	19.9	65.5/81.5

[e]HLM/RLM: Human liver microsome intrinsic clearance, in (μL/min)/mg protein (high stability, <6.5; low stability, >35); Rat liver microsome intrinsic clearance, in (μL/min)/mg protein (high stability, <15; low stability, >90).
[a] 1-octanol/buffer 7.4.
[b] Permeability coefficient, in 10^{-6} cm/s; low permeability: Papp ≤0.5 (× 10-6 cm/s); moderate permeability: 0.5 < P$_{app}$ < 2.5 (× 10^{-6} cm/s); high permeability: Papp ≥2.5 (× 10-6 cm/s).
[c] Ratio of BA/AB permeability coefficients.
[d] Half-life in rat and human plasma.

intravenous (i.v.) route and 10 mg/kg via the oral route of administration (Fig. 3). The plasma profiles obtained from the pharmacokinetic experiments are shown in Table 6 and Fig. 3. The results indicated that compound **80** has satisfying PK properties with an oral total exposure (AUC) of 1502 ng h/mL, medium in *vivo* clearance (38.3 mL/min/kg), C$_{max}$ of 452 ng/mL, and moderate bioavailability of 34%. Considering that sustained exposure to PK *in vivo* should exceed at least several times the *in vitro* EC$_{50}$ expected to be useful in human efficacy studies, we used 100 ng/mL, equating to 10-fold above the EC$_{50}$ for HCV, as a minimum requirement efficacy concentration. At the 10 mg/kg dose, plasma concentrations remained above 100 ng/mL for over 4 h, indicating a modest stability to metabolism *in vivo* of this kind of compound.

2.6. Some compounds protecting hA3G from Vif-mediated degradation

Twenty-two compounds were subjected to a preliminary screening test to identify their inhibition of Vif-mediated hA3G degradation using our previously reported assay [23]. Briefly, 293T cells were cotransfected with the expression vectors for hA3G and Vif and then treated with 20 μM test compounds and MG132, a well-known proteasome inhibitor, as a positive control. The results in Fig. 4 show that compared with that in the cells treated with DMSO, seven compounds (**12**, **13**, **18**, **19**, **20**, **40**, and **41**) were effective in inhibiting Vif-mediated hA3G degradation in this assay (>50%). Four compounds (**17**, **36**, **37**, and **45**) displayed modest
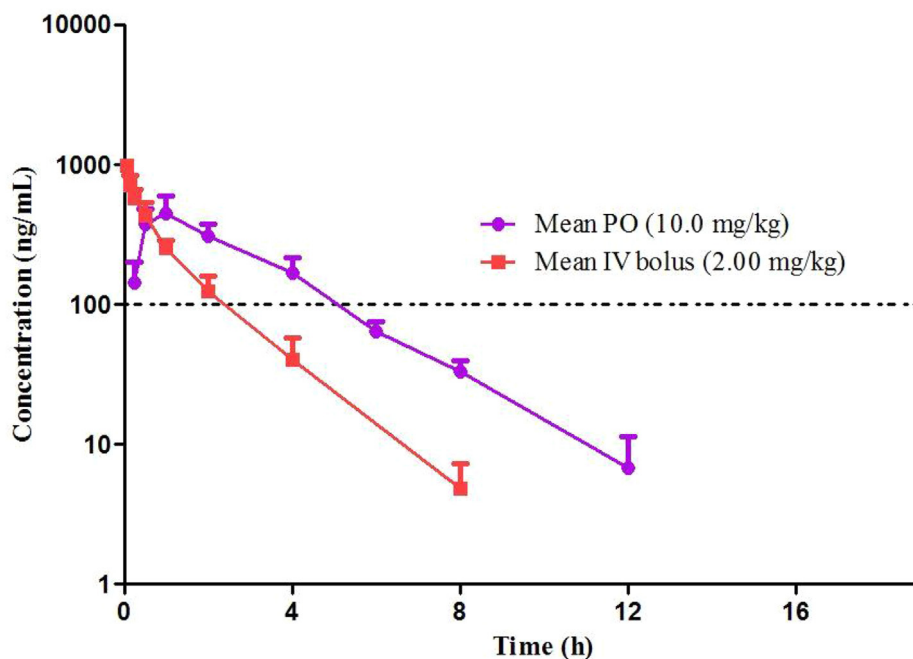
Fig. 3. Exposure curves for compound **80** following oral and *i. v.* dosing in rat.

Table 6
Pharmacokinetic parameters of compound **80** in rat plasma after i.v. and p.o. administration.[a].

parameter	unit	i.v.[b]	p.o.[c]
AUC_{0-last}	ng·h/mL	889 ± 179	1502 ± 342
AUC_{0-inf}	ng·h/mL	898 ± 184	1525 ± 360
MRT_{0-last}	h	1.36 ± 0.182	2.95 ± 0.276
MRT_{0-inf}	h	1.45 ± 0.211	3.10 ± 0.290
C_{max}	ng/mL	/	452 ± 149
$T_{1/2}$	h	1.24 ± 0.101	1.90 ± 0.492
T_{last}	h	8.00	12.0
T_{max}	h	/	1.00
Vd_{ss}	L/kg	3.26 ± 0.426	
Cl	mL/min/kg	38.3 ± 8.89	
F[d]	%	34	

[a] PK parameters (mean ± SD, n = 6).
[b] Dosed intravenously at 2 mg/kg.
[c] Dosed orally at 10 mg/kg.
[d] Bioavailability (%) was calculated using $100 \times (AUC_{0-inf}$ (p.o.) $\times$ 2 mg/kg/AUC_{0-inf} (iv) $\times$ 10 mg/kg).

activity (25%–50%). Eleven compounds showed weak activity (<25%). According to the structure-action relationship, the amido group in the R_2 moiety was superior in inhibiting Vif-mediated hA3G degradation, while nitro and amino groups were adverse (except compound **12**). R_1 and R_3 moieties would be versatile and tolerant to hydrophobic or hydrophilic groups. Compounds **13** and **18** with propionyl moieties in the R_2 moiety had good anti-HCV activity and simultaneously displayed potent inhibition of Vif-mediated hA3G degradation. Compound **54** with a nitro group displayed excellent anti-HCV activity but poor inhibition of Vif-mediated hA3G degradation. Since most nitro compounds displayed poor inhibition of Vif-mediated hA3G degradation, subsequent synthesized compounds were not evaluated for activity. The antiviral mechanism of these nitro compounds is still in process.

3. Conclusion

A series of novel biaryl amide derivatives were synthesized and assayed for anti-HCV activity *in vitro*. Intensive structural modifications led to fifteen novel compounds with higher potent inhibitory activity than IMB 26, especially compound **80**, with EC_{50} values almost equivalent to those of the clinical drug telaprevir. Additionally, some significant SARs were uncovered. Among the structures of the anti-HCV compounds, R_1 moieties are apt to be hydrophobic moieties (for example, an aromatic nucleus) through a methylene linked to hydrophilic moieties (for example, cyclic amine), R_2 moieties should be a hydrogen bond acceptor (for example, a nitro group) and R_3 moieties prefer to be hydrophobic moieties as requirements for anti-HCV activity. Such SARs provided valuable implications for further lead optimizations. Compound **18** showed comparable inhibitory activity against HCV to IMB-26 and moreover displayed effective inhibitory activity against Vif-mediated hA3G degradation, although it possessed obviously different structures at the R_1 position. Most compounds with nitro groups, however, displayed poor inhibition of Vif-mediated hA3G degradation. Compound **80** displayed the highest anti-HCV activity and SI value and possessed good physicochemical properties, making it a more promising scaffold for further study.

4. Experimental section

4.1. Chemistry

All reagents and solvents were purchased from commercial sources and used as obtained. All reactions were carried out in flame-dried glassware and monitored by thin layer chromatography using aluminum TLC plate 60F254D (Merck Millipore) and visualized under UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker 400 or a Varian Inova 500 or 600 NMR spectrometer. Chemical shifts are reported in parts per million (ppm) and are referenced to the residual solvent peak. The following notations are used: singlet (s); doublet (d); triplet (t); quartet (q); multiplet (m); broad (br). Data are reported in the following manner: chemical shift (multiplicity, coupling constant if appropriate, integration). Signals are quoted as δ values in ppm and coupling constants (J) are reported in Hertz. Using
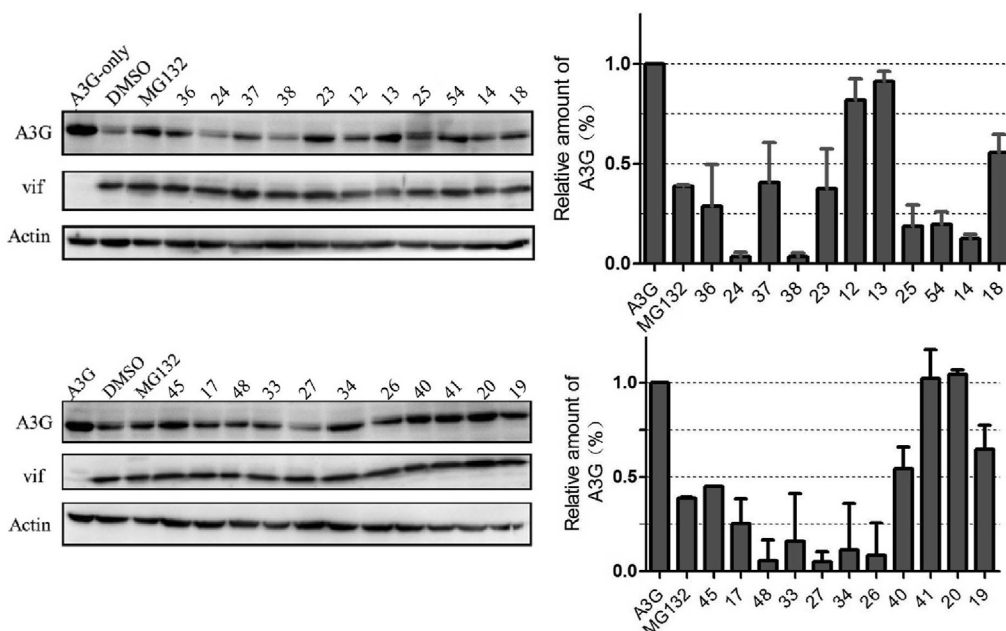
Fig. 4. Compounds targeting the interface of the hA3G/Vif interaction.

residual protonated solvent signals as internal standard ([1]H: δ(CHCl$_3$) = 7.26 ppm, δ((CH$_3$)$_2$SO) = 2.50 ppm, δ(CH$_3$OH) = 3.31 ppm, δ(H$_2$O) = 4.67 ppm; and [13]C: δ(CHCl$_3$) = 77.16 ppm, δ((CH$_3$)$_2$SO) = 39.52 ppm, δ(CH$_3$OH) = 49.00 ppm). Mass spectra were recorded on Micromass Q-ToF (ESI) spectrometer. HRMS data were measured using a Thermo LTQ Orbitrap XL mass spectrometer. Flash column chromatography was conducted using silica gel (Silicycle 40−64 μM).

General Procedure A: Coupling of 4-substituted-3-nitrobenzoic acid and various substituted anilines fragment. To a mixture of 4-substituted-3-nitrobenzoic acid, substituted anilines (1.0−1.2 equiv), DMAP (0.1 equiv) in CH$_2$Cl$_2$ (1.5−4 mL, ca. 0.05 M) was added N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.0 equiv). The reaction was stirred at room temperature for 16 h. The reaction mixture was extracted with CH$_2$Cl$_2$, washed with water and dried with anhydrous Na$_2$SO$_4$. The solvent was removed under vacuum, and the residue was purified by silica gel flash chromatography (eluents: 10−50% THF in petroleum ether) to offer the coupled nitro product in 78−93% yield.

General Procedure B: Hydrogenation of nitro compounds. The nitro compound (0.5 mmol) was dissolved in ethyl acetate (5 mL), and methanol (5 mL) and Pa/C (0.05 g, 10%) was added. The resulting mixture was hydrogenated at hydrogen gas pressure of 14−35 psi for 2.5−24 h. The catalyst was removed by filtration and the filtrate was concentrated under *vacuo* to give amino derivatives, which was used in the next step without further purification.

General procedure C: Coupling of various substituted anilines and acyl chloride. Propionyl chloride (1.0 eq) was added dropwise to a solution of substituted aniline (1.0 mmol) and TEA (1.5 mmol) in dichloromethane at 0 °C. The mixture was then stirred at room temperature until the starting material was completely disappeared. The reaction was quenched with water and extracted with dichloromethane. The organic layer was washed with brine, dried over Na$_2$SO$_4$, concentrated in *vacuo*, and further purified by flash chromatography on silica gel (eluents: 1−10% MeOH in dichloromethane with 0.2% NH$_4$OH$_{(aq)}$) or C-18 functionalized silica chromatography (eluents: 5−90% MeOH in deionized water with 0.5% NH$_4$OH$_{(aq)}$) to give the product.

N-(4-bimethylamino-phenyl)-3-nitro-4-methoxy-benzamide (12) 4-methoxy-3-nitrobenzoic acid (0.197 g, 1.0 mmoL) and 4-dimethylamino-aniline (0.136 g, 1.0 mmoL) were reacted according to general procedure A to afford 12 (0.280 g, yield 89%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.32 (s, 1H), 8.11 (d, J = 8.4, 1H), 7.8 (br s, 1H), 7.44 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 9.0 Hz, 1H), 6.71 (d, J = 9.0 Hz, 2H), 4.01 (s, 3H), 2.93 (s, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 162.8, 155.1, 148.3, 138.9, 133.4, 127.3, 126.9, 124.2, 122.3, 113.5, 112.8, 56.8, 40.7. ESI-HRMS calcd for C$_{16}$H$_{18}$N$_3$O$_4$ [M + H]$^+$ 316.1292; found 316.1284.

4.1.1. N-(4-bimethylaminophenyl)-3-propionamido-4-methoxy-benzamide (14)

Compound 12 (0.157 g, 0.5 mmoL) was reduced according to general procedure B to afford N-(4-bimethylamino-phenyl)-3-amino-4-methoxy-benzamide, which was reacted with propionyl chloride (44 μL, 0.5 mmoL) and TEA (0.105 mL, 0.75 mmoL) according to general procedure C to afford compound 14 (0.136 g, yield 80%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.87 (s, 1H), 7.80 (m, 3H), 7.49 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 8.8 Hz, 1H), 6.78 (m, 2H), 3.95 (s, 3H), 2.94 (s, 6H), 2.48 (q, J = 7.6 Hz, 2H), 1.28 (t, J = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.4, 164.9, 150.1, 148.1, 128.0, 127.8, 127.4, 124.5, 122.2, 116.9, 113.1, 110.0, 56.0, 41.0, 31.0, 9.6. ESI-HRMS calcd for C$_{19}$H$_{24}$N$_3$O$_3$ [M + H]$^+$ 342.1812; found 342.1806.

4.1.2. 3-Propionamido-4-methoxy-benzoic acid (15)

4-methoxy-3-aminobenzoic acid (6.0 mmoL), propionyl chloride (0.58 mL, 6.6 mmoL) and TEA (1.26 mL, 9.0 mmoL) were reacted according to general procedure C to afford compound 15 (1.02 g, yield 76%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (br s, 1H), 9.13 (s, 1H), 8.57 (s, 1H), 7.68 (dd, J = 8.8, 2.0 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 3.89 (s, 3H), 2.41 (q, J = 7.6 Hz, 2H), 1.06 (t, J = 7.6 Hz, 3H). LC/MS (ESI, m/z): 222.1 [M − H]$^+$.

4.1.3. N-((4-(4-methylpiperazin-1-yl)phenyl)-3-propionamido-4-methoxy-benzamide (16)

Compound 15 (0.223 g, 1.0 mmoL) and 4-(4-methylpiperazin-1-yl)aniline (0.191 g, 1.0 mmoL) were reacted according to general

procedure A to afford compound 16 (0.278 g, yield 70%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.87 (s, 1H), 7.89 (br s, 1H), 7.82 (br s, 1H), 7.78 (dd, J = 8.4, 1.6, Hz, 1H), 7.52 (d, J = 7.2 Hz, 2H), 6.96 (d, J = 8.8 Hz, 1H), 6.93 (d, J = 7.6 Hz, 2H), 3.95 (s, 3H), 3.22 (m, 4H), 2.63 (m, 4H), 2.49 (q, J = 7.6 Hz, 2H), 2.38 (s, 3H), 1.27 (t, J = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.2, 164.8, 150.0, 148.1, 130.5, 127.2, 124.5, 121.7, 116.8, 116.5, 109.5, 55.9, 55.0, 49.3, 46.0, 30.9, 9.5. LC/MS (ESI, *m/z*): 397.2 [M + H]$^+$.

4.1.4. N-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-3-propionamido-4-methoxy-benzamide (17)

Compound 15 (0.223 g, 1.0 mmoL) and 4-((4-methylpiperazin-1-yl)methyl)aniline (0.205 g, 1.0 mmoL) were reacted according to general procedure A to afford compound 17 (0.298 g, yield 73%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.89 (s, 1H), 7.94 (br s, 1H), 7.82 (br s, 1H), 7.78 (dd, J = 6.8, 2.0 Hz, 1H), 7.59 (dd, J = 6.8, 1.6 Hz, 2H), 7.30 (d, J = 6.4 Hz, 2H), 6.98 (d, J = 6.8 Hz, 1H), 3.95 (s, 3H), 3.48 (s, 2H), 2.47 (m, 10H), 2.29 (s, 3H), 1.27 (t, J = 6.0 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.4, 165.1, 150.3, 137.1, 134.3, 127.5, 124.7, 120.2, 117.0, 110.1, 62.6, 56.1, 55.2, 53.2, 46.1, 31.1, 9.6. ESI-HRMS calcd for C$_{23}$H$_{31}$N$_4$O$_3$ [M + H]$^+$ 411.2391; found 411.2397.

4.1.5. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl)phenyl)-3-propionamido-4-methoxy-benzamide (18)

Compound 15 (0.223 g, 1.0 mmoL) and 4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)aniline (0.273 g, 1.0 mmoL) were reacted according to general procedure A to afford compound 18 (0.358 g, yield 75%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.89 (br s, 1H), 8.19 (br s, 1H), 7.91 (d, J = 2.0 Hz, 1H), 7.85 (m, 2H), 7.82 (dd, J = 8.4, 2.4 Hz, 1H), 8.00 (s, 1H), 7.73 (d, J = 8.8 Hz, 1H), 6.99 (d, J = 8.8 Hz, 1H), 3.96 (s, 3H), 3.63 (s, 2H), 2.49 (m, 10H), 2.32 (s, 3H), 1.28 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.5, 165.3, 150.6, 137.1, 131.3, 129.3 (q, J = 20.0 Hz), 127.4, 127.0, 125.2, 125.0, 123.4, 123.3, 117.8 (q, J = 3.9 Hz), 117.1, 110.3, 57.9, 56.1, 55.3, 53.2, 46.1, 31.1, 9.6. ESI-HRMS calcd for C$_{23}$H$_{28}$N$_4$O$_3$F$_3$ [M + H]$^+$ 479.2265; found 479.2267.

4.1.6. N-(4-(1H-indol-2-yl)phenyl)-3-propionamido-4-methoxy-benzamide (19)

Compound 15 (0.223 g, 1.0 mmoL) and 4-(1H-indol-2-yl)aniline (0.208 g, 1.0 mmoL) were reacted according to general procedure A to afford compound 19 (0.338 g, yield 82%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.46 (br s, 1H), 10.21 (br s, 1H), 9.19 (br s, 1H), 8.56 (br s, 1H), 7.86 (m, 4H), 7.78 (d, J = 6.4 Hz, 1H), 7.51 (d, J = 6.0 Hz, 1H), 7.39 (d, J = 6.4 Hz, 1H), 7.17 (d, J = 6.8 Hz, 1H), 7.08 (t, J = 6.0 Hz, 1H), 6.99 (t, J = 6.0 Hz, 1H), 6.84 (s, 1H), 3.92 (s, 3H), 2.44 (d, J = 5.4 Hz, 2H), 1.09 (t, J = 5.4 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 163.1, 156.8, 154.8, 139.0, 133.3, 130.5, 126.6, 124.2, 122.4, 114.3, 114.2, 71.4, 55.4, 22.2, 10.3. ESI-HRMS calcd for C$_{25}$H$_{24}$N$_3$O$_3$ [M + H]$^+$ 414.1812; found 414.1805.

4.1.7. N-(3-mercapto-5-methyl-4H-1,2,4-triazol-4-yl)-3-propionamido-4-methoxy-benzamide (20)

Compound 15 (0.223 g, 1.0 mmoL) and 4-amino-5-methyl-4H-1,2,4-triazole-3-thiol (0.130 g, 1.0 mmoL) were reacted according to general procedure A to afford compound 20 (0.216 g, yield 65%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.97 (br s, 1H), 7.74 (s, 2H), 6.96 (d, J = 7.5 Hz, 1H), 4.64 (br s, 2H), 3.97 (s, 3H), 2.45 (s, 3H), 2.43 (d, J = 6.0 Hz, 2H), 1.24 (t, J = 6.0 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.0, 169.6, 165.4, 151.9, 149.5, 128.0, 127.4, 124.2, 122.5, 109.7, 56.2, 31.1, 10.9, 9.6. ESI-HRMS calcd for C$_{14}$H$_{17}$N$_5$O$_3$SNa [M + Na]$^+$ 358.0944; found 358.0936.

4.1.8. N-(4-methoxyphenyl)-3-nitro-4-hydroxy-benzamide (21)

4-hydroxy-3-nitrobenzoic acid (5.00 g, 27.3 mmoL) and 4-

methoxylaniline (4.03 g, 32.8 mmoL) were reacted according to general procedure A to afford 21 (4.08 g, yield 52%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 10.81 (s, 1H), 8.62 (s, 1H), 8.15 (d, J = 7.6, 1H), 7.52 (d, J = 8.4, 2H), 7.27 (m, 1H), 6.92 (d, J = 8.8 Hz, 2H), 3.82 (s, 3H). LC/MS (ESI, *m/z*): 289.1 [M + H]$^+$.

4.1.9. N-(4-methoxyphenyl)-3-nitro-4-ethoxy-benzamide (22)

To a solution of compound 21 (0.289 g, 1.0 mmoL) in tetrahydrofuran (8.0 mL) was added ethanol (67 μL, 1.2 mmoL) and triphenylphosphine (0.520 g, 2.0 mmoL). The mixture was cooled at 0 °C and Diethyl azodicarboxylate in toluene (40%, 0.77 mL, 1.7 mmoL) was added dropwise. The mixture was then stirred at room temperature until the starting material was completely disappeared. The reaction was quenched with water and extracted with dichloromethane. The organic layer was washed with brine, dried over Na$_2$SO$_4$, concentrated *in vacuo*, and further purified by flash chromatography on silica gel to give 22 (0.240 g, 76%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.31 (s, 1H), 8.10 (d, J = 4.8 Hz, 1H), 7.72 (br s, 1H), 7.51 (d, J = 7.0 Hz, 2H), 7.15 (d, J = 6.8 Hz, 1H), 6.91 (d, J = 7.0 Hz, 2H), 4.27 (q, J = 5.2 Hz, 2H), 3.82 (s, 3H), 1.51 (t, J = 4.8 Hz, 3H); ^{13}C NMR (101 MHz, CDCl$_4$) δ 162.9, 156.9, 154.7, 139.3, 133.2, 130.4, 126.7, 124.1, 122.3, 114.3, 65.8, 55.5, 14.4. LC/MS (ESI, *m/z*): 317.1 [M + H]$^+$.

4.1.10. N-(4-methoxyphenyl)-3-propionamido-4-ethoxy-benzamide (36)

Compound 22 (0.5 mmoL) was reduced according to general procedure B to afford 29, which was reacted with propionyl chloride (44 μL, 0.5 mmoL) and TEA (0.105 mL, 0.75 mmoL) according to general procedure C to afford compound 36 (0.145 g, yield 85%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.91 (d, J = 2.0 Hz, 1H), 7.85 (br s, 1H), 7.78 (br s, 1H), 7.76 (dd, J = 8.8, 2.0 Hz, 1H), 7.54 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.8 Hz, 1H), 6.90 (d, J = 9.2 Hz, 2H), 4.20 (q, J = 14.0, 7.2 Hz, 2H), 3.81 (s, 3H), 2.49 (q, J = 15.2, 7.6 Hz, 2H), 1.50 (t, J = 7.2 Hz, 3H), 1.28 (t, J = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.3, 165.1, 156.5, 149.6, 131.3, 127.3, 124.7, 122.3, 116.9, 114.2, 110.9, 64.6, 55.5, 31.1, 14.8, 9.6. ESI-HRMS calcd for C$_{19}$H$_{23}$N$_2$O$_4$ [M + H]$^+$ 343.1652; found 343.1650.

4.1.11. N-(4-methoxyphenyl)-3-nitro-4-propoxy-benzamide (23)

Compound 21 was reacted with n-propanol (90 μL, 1.2 mmoL) according to a method similar to that of compound 22 to afford 23 (0.280 g, 85%). ^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ 8.30 (d, J = 1.6 Hz, 1H), 8.06 (dd, J = 9.0, 1.6 Hz, 1H), 8.00 (s, 1H), 7.50 (d, J = 9.0 Hz, 2H), 7.10 (d, J = 9.0 Hz, 1H), 6.86 (d, J = 9.0 Hz, 2H), 4.11 (t, J = 6.0 Hz, 2H), 3.79 (s, 3H), 1.88 (m, 2H), 1.07 (t, J = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-*d*$_6$) δ 163.1, 156.8, 154.8, 139.0, 133.3, 130.5, 126.6, 124.2, 122.4, 114.3, 114.2, 71.4, 55.4, 22.2, 10.3. LC/MS (ESI, *m/z*): 331.1 [M + H]$^+$.

4.1.12. N-(4-methoxyphenyl)-3-propionamido-4-propoxy-benzamide (37)

Compound 23 (0.165 g, 0.5 mmoL) was reduced according to general procedure B to afford 30, which was reacted with propionyl chloride (44 μL, 0.5 mmoL) and TEA (0.105 mL, 0.75 mmoL) according to general procedure C to afford compound 37 (0.145 g, yield 85%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.88 (s, 1H), 7.88 (br s, 1H), 7.86 (s, 1H), 7.78 (dd, J = 8.8, 2.0 Hz, 1H), 7.54 (dd, J = 6.8, 2.0 Hz, 2H), 6.96 (d, J = 8.4 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 6.90 (dd, J = 6.8, 2.0 Hz, 2H), 4.08 (t, J = 6.4 Hz, 2H), 3.81 (s, 3H), 2.49 (q, J = 7.6 Hz, 2H), 1.91 (m, 2H), 1.28 (t, J = 7.6 Hz, 3H), 1.09 (t, J = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.1, 164.9, 156.4, 149.5, 131.2, 127.2, 124.5, 122.1, 116.7, 114.0, 110.8, 70.3, 55.4, 31.0, 22.3, 10.4, 9.5. ESI-HRMS calcd for C$_{20}$H$_{25}$N$_2$O$_4$ [M + H]$^+$ 357.1809; found 357.1795.

4.1.13. N-(4-methoxyphenyl)-3-nitro-4-(isopropoxy)-benzamide (24)

Compound 21 was reacted with isopropanol (93 μL, 1.2 mmoL) according to a method similar to that of compound 22 to afford 24 (0.264 g, 80%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.27 (s, 1H), 8.06 (d, J = 2.0 Hz, 1H), 7.77 (s, 1H), 7.51 (d, J = 8.8 Hz, 2H), 7.15 (d, J = 8.8 Hz, 2H), 6.89 (dd, J = 8.8, 2.0 Hz, 2H), 4.77 (m, 1H), 3.81 (s, 3H), 1.44 (d, J = 7.0 Hz, 6H). LC/MS (ESI, m/z): 331.1 [M + H]$^+$.

4.1.14. N-(4-methoxyphenyl)-3-propionamido-4-isopropoxy-benzamide (38)

Compound 24 (0.165 g, 0.5 mmoL) was reduced according to general procedure B to afford 31, which was reacted with propionyl chloride (44 μL, 0.5 mmoL) and TEA (0.105 mL, 0.75 mmoL) according to general procedure C to afford compound 38 (0.128 g, yield 72%).^{1}H NMR (400 MHz, CDCl$_3$) δ 8.89 (s, 1H), 7.87 (br s, 2H), 7.76 (dd, J = 8.4, 2.4 Hz, 1H), 7.55 (dd, J = 6.8, 2.0 Hz, 2H), 6.97 (d, J = 8.8 Hz, 1H), 6.89 (dd, J = 6.8, 2.0 Hz, 2H), 4.70 (m, 1H), 3.80 (s, 3H), 2.48 (q, J = 7.6 Hz, 2H), 1.41 (d, J = 6.0 Hz, 6H), 1.28 (t, J = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.1, 165.0, 156.3, 148.4, 131.2, 127.0, 124.4, 122.2, 116.9, 114.0, 112.0, 71.5, 55.4, 30.9, 22.0, 9.5. ESI-HRMS calcd for C$_{20}$H$_{25}$N$_2$O$_4$ [M + H]$^+$ 357.1809; found 357.1809.

4.1.15. N-(4-methoxyphenyl)-3-nitro-4-(3-chloropropoxy)-benzamide (25)

Compound 21 was reacted with 3-chloropropanol (0.10 mL, 1.2 mmoL) according to a method similar to that of compound 22 to afford 25 (0.316 g, 87%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.36 (s, 1H), 8.22 (s, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.0 Hz, 1H), 6.86 (d, J = 8.5 Hz, 2H), 4.32 (t, J = 5.0 Hz, 2H), 3.79 (s, 5H), 2.29 (m, 2H). LC/MS (ESI, m/z): 365.1 [M + H]$^+$.

4.1.16. N-(4-methoxyphenyl)-4-(3-chloropropoxy)-3-propionamidobenzamide (39)

Compound 25 (0.183 g, 0.5 mmoL) was reduced according to general procedure B to afford 32, which was reacted with propionyl chloride (44 μL, 0.5 mmoL) and TEA (0.105 mL, 0.75 mmoL) according to general procedure C to afford compound 39 (0.116 g, yield 65%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.90 (s, 1H), 7.80 (m, 3H), 7.53 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.5 Hz, 1H), 6.90 (d, J = 8.5 Hz, 2H), 4.30 (m, 2H), 3.81 (s, 3H), 3.75 (m, 2H), 2.47 (m, 2H), 2.35 (t, J = 5.5 Hz, 2H), 1.28 (t, J = 7.5 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 172.4, 165.0, 156.6, 131.3, 127.6, 122.4, 117.3, 114.3, 111.3, 66.2, 55.6, 41.5, 31.9, 31.2, 9.7. ESI-HRMS calcd for C$_{20}$H$_{24}$N$_2$O$_4$Cl [M + H]$^+$ 391.1419; found 391.1411.

4.1.17. N-(4-methoxyphenyl)-3-nitro-4-(3-bimethylaminopropoxy)-benzamide (26)

To a solution of compound 25 (0.300 g, 0.82 mmoL) in DMF (5.0 mL) was added sodiumiodide (0.246 g, 1.64 mmoL) and dimethylamine (0.82 mL, 2.0 M in THF). The mixture was stirred at 70 °C for 4 h. After cooling to rt, the solvent was removed, and the residue was purified by C-18 functionalized silica chromatography (eluents 5−90% MeOH in deionized water with 0.5% NH$_4$OH$_{(aq)}$ to afford compound 26 (0.178 g, 58%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.36 (s, 1H), 8.08 (d, J = 8.8 Hz, 1H), 7.87 (br s, 1H), 7.55 (d, J = 8.8 Hz, 2H), 7.17 (d, J = 8.8 Hz, 1H), 6.92 (dd, J = 8.8, 2.0 Hz, 2H), 4.31 (q, J = 6.4 Hz, 2H), 3.81 (s, 3H), 2.69 (m, 2H), 2.41 (s, 6H), 2.13 (m, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 157.0, 155.0, 139.3, 133.3, 130.6, 126.9, 124.3, 122.4, 114.7, 114.4, 68.3, 55.8, 55.6, 45.6, 29.8, 27.1. ESI-HRMS calcd for C$_{19}$H$_{24}$N$_3$O$_5$ [M + H]$^+$ 374.1710; found 374.1713.

4.1.18. N-(4-methoxyphenyl)-3-amino-4-(3-(dimethylamino)propoxy)benzamide (33)

Compound 26 (0.183 g, 0.5 mmoL) was reduced according to

general procedure B to afford 33 (0.163 g, 95%). ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 9.83 (s, 1H), 7.67 (m, 3H), 7.25 (s, 1H), 7.20 (s, 1H), 6.90 (m, 3H), 4.91 (m, 2H), 4.07 (t, J = 5.5 Hz, 2H), 3.76 (s, 3H), 2.43 (m, 2H), 2.18 (s, 6H), 1.91 (t, J = 5.5 Hz, 2H); ^{13}C NMR (150 MHz, CD$_3$OD) δ 168.9, 158.1, 150.9, 138.1, 132.9, 128.7, 124.1, 119.1, 114.9, 111.7, 67.6, 57.3, 55.8, 45.4, 28.1. LC/MS (ESI, m/z): 344.2 [M + H]$^+$.

4.1.19. N-(4-methoxyphenyl)-4-(3-(dimethylamino)propoxy)-3-propionamidobenzamide (40)

Compound 33 (0.150 g, 0.43 mmol) was reacted with propionyl chloride (44 μL, 0.5 mmol) and TEA (0.105 mL, 0.75 mmol) according to general procedure C to afford compound 40 (0.92 g, yield 54%). ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.32 (s, 1H), 7.75 (d, J = 7.0, 1H), 7.54 (d, J = 7.5, 2H), 7.13 (d, J = 7.5 Hz, 1H), 6.92 (d, J = 7.5 Hz, 2H), 4.22 (t, J = 5.0 Hz, 2H), 3.79 (s, 3H), 3.05 (t, J = 6.5 Hz, 2H), 2.68 (s, 6H), 2.50 (m, 2H), 2.20 (m, 2H), 1.24 (t, J = 6.5 Hz, 3H); ^{13}C NMR (150 MHz, CD$_3$OD) δ 175.6, 167.9, 158.2, 154.2, 132.8, 128.6, 127.8, 126.7, 124.2, 124.0, 114.9, 112.7, 67.3, 56.7, 55.8, 44.2, 30.8, 26.3, 10.3. ESI-HRMS calcd for C$_{22}$H$_{30}$N$_3$O$_4$ [M + H]$^+$ 400.2231; found 400.2227.

4.1.20. N-(4-methoxyphenyl)-4-(3-(4-methylpiperazin-1-yl)propoxy)-3-nitrobenzamide (27)

To a solution of compound 25 (0.530 g, 1.46 mmoL) in DMF (5.0 mL) was added 1-methylpiperazine (0.23 mL, 2.12 mmoL) according to a method similar to that of compound 26 to afford 27 (0.490 g, 80%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.31 (d, J = 1.8 Hz), 1H), 8.18 (s, 1H), 8.07 (dd, J = 8.4, 1.8 Hz, 1H), 7.50 (d, J = 8.4, 2H), 7.12 (d, J = 9.0 Hz, 1H), 6.85 (d, J = 8.5 Hz, 2H), 4.21 (t, J = 6.0 Hz, 2H), 3.78 (s, 3H), 2.54 (t, J = 6.6 Hz, 2H), 2.48 (m, 8H), 2.28 (s, 3H), 2.02 (m, 2H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 163.3, 156.9, 154.8, 139.1, 133.5, 130.7, 126.9, 124.4, 122.6, 114.5, 114.3, 68.3, 55.5, 55.1, 54.4, 53.1, 46.0, 29.8, 26.2. LC/MS (ESI, m/z): 429.2 [M + H]$^+$.

4.1.21. N-(4-methoxyphenyl)-3-amino-4-(3-(4-methylpiperazin-1-yl)propoxy)benzamide (34)

Compound 27 (0.214 g, 0.5 mmoL) was reduced according to general procedure B to afford compound 34. ^{1}H NMR (600 MHz, CD$_3$OD) δ 7.52 (d, J = 9.0 Hz, 1H), 7.30 (s, 1H), 7.28 (d, J = 8.4 Hz, 1H), 6.90 (m, 3H), 4.12 (t, J = 6.0 Hz, 2H), 3.79 (s, 3H), 2.57 (t, J = 7.2 Hz, 2H), 2.29 (s, 6H), 2.03 (m, 2H); ^{13}C NMR (150 MHz, CD$_3$OD) δ 168.9, 158.0, 150.9, 138.2, 132.9, 128.7, 124.1, 119.0, 115.1, 114.9, 111.8, 67.7, 56.1, 55.8, 55.6, 53.6, 45.9, 27.4. LC/MS (ESI, m/z): 399.2 [M + H]$^+$.

4.1.22. N-(4-methoxyphenyl)-3-propionamido-4-(3-bimethylaminopropoxy)-benzamide (41)

Compound 34 (0.189 g, 0.5 mmol) was reacted with propionyl chloride (44 μL, 0.5 mmol) and TEA (0.105 mL, 0.75 mmol) according to general procedure C to afford compound 41 (0.111 g, yield 49%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.84 (s, 1H), 8.00 (s, 1H), 7.86 (s, 1H), 7.73 (dd, J = 8.4, 1.8 Hz, 1H), 7.53 (d, J = 9.0 Hz, 2H), 6.54 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 9.0 Hz, 2H), 4.14 (t, J = 6.0 Hz, 2H), 3.79 (s, 3H), 2.60 (m, 10H), 2.47 (m, 2H), 2.40 (s, 3H), 2.06 (m, 2H), 2.04 (m, 2H), 1.25 (m, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 173.5, 165.1, 156.6, 149.7, 131.4, 127.6, 127.5, 124.7, 122.4, 117.4, 114.3, 111.1, 67.1, 55.6, 54.7, 54.5, 45.4, 31.1, 26.4, 9.7. LC/MS (ESI, m/z): 455.2 [M + H]$^+$.

4.1.23. N-(4-methoxyphenyl)-4-(3-morpholinopropoxy)-3-nitrobenzamide (28)

To a solution of compound 25 (0.200 g, 0.54 mmoL) in DMF (3.0 mL) was added morpholine (0.12 mL, 0.81 mmoL) according to a method similar to that of compound 26 to afford 28 (0.133 g, 59%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.33 (d, J = 2.4 Hz, 1H), 8.10 (dd, J = 8.8, 2.0 Hz, 1H), 7.77 (br s, 1H), 7.52 (d, J = 8.8 Hz, 2H), 7.21 (d, J = 8.8, 1H), 6.91 (d, J = 9.2 Hz, 2H), 4.27 (t, J = 6.0 Hz, 2H), 3.82 (s, 3H), 3.74

(m, 4H), 2.61 (m, 2H), 2.51 (m, 4H), 2.07 (m, 2H). LC/MS (ESI, *m/z*): 416.2 [M + H]⁺.

4.1.24. N-(4-methoxyphenyl)-3-amino-4-(3-morpholinopropoxy) benzamide (35)

Compound 28 (0.214 g, 0.5 mmoL) was reduced according to general procedure B to afford 35. ¹H NMR (400 MHz, CD₃OD) δ 7.63 (d, *J* = 7.2 Hz, 2H), 7.21 (s, 1H), 7.17 (d, *J* = 6.4 Hz, 1H), 6.87 (m, 3H), 4.04 (m, 2H), 3.72 (s, 3H), 3.60 (m, 4H), 2.49 (m, 2H), 2.35 (m, 4H), 1.94 (m, 2H); ¹³C NMR (100 MHz, DMSO-*d₆*) δ 165.2, 155.2, 147.9, 137.5, 132.5, 127.6, 121.7, 115.9, 113.1, 110.6, 66.0, 61.3, 60.5, 55.0, 54.5, 53.0, 30.5. LC/MS (ESI, *m/z*): 386.2 [M + H]⁺.

4.1.25. N-(4-methoxyphenyl)-4-(3-morpholinopropoxy)-3-propionamidobenzamide (42)

Compound 35 (0.194 g, 0.5 mmol) was reacted with propionyl chloride (44 μL, 0.5 mmol) and TEA (0.105 mL, 0.75 mmol) according to general procedure C to afford compound 42 (0.100 g, yield 45%). ¹H NMR (400 MHz, CDCl₃) δ 7.91 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.70 (s, 1H), 7.61 (s, 1H), 7.50 (d, *J* = 9.2 Hz, 2H), 7.04 (d, *J* = 8.4 Hz, 1H), 6.90 (d, *J* = 9.2 Hz, 2H), 4.09 (m, 2H), 3.81 (s, 3H), 3.75 (m, 4H), 2.62 (m, 2H), 2.53 (m, 6H), 1.95 (m, 2H), 1.11 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 176.8, 163.7, 156.6, 156.3, 131.4, 130.1, 129.0, 127.7, 127.2, 121.9, 113.9, 112.3, 66.6, 66.5, 55.4, 54.9, 53.5, 31.4, 25.7, 8.9. LC/MS (ESI, *m/z*): 442.2 [M + H]⁺.

4.1.26. Methyl 3-oxo-3,4-dihydro-2H-benzo[b] [1,4]oxazine-6-carboxylate (44)

2-bromoacetyl bromide (0.48 mL, 5.0 mmol) was added dropwise to a solution of methyl 3-amino-4-hydroxybenzoate (0.84 g, 5.0 mmol) and NaHCO₃ (0.69 g, 8.25 mmol) in EtOAc/H₂O (40 mL, 1:1) at 0 °C. The mixture was then stirred at room temperature until the starting material was completely disappeared. The reaction was extracted with EtOAc (50 mL). The organic layer was orderly washed with 10% HCl, water, and brine, dried over Na₂SO₄, and concentrated *in vacuo* to give methyl 3-(2-bromoacetamido)-4-hydroxybenzoate (43, 1.07 g). The compound was dissolved in DMF (5.0 mL) and K₂CO₃ (0.56 g, 5.25 mmol) was added the above mixture. The mixture was heated to 80 °C and stirred for 3 h until the starting material was completely disappeared. After cooling to room temperature, the solvent was removed, and the residue was extracted with ethyl acetate, washed with water and brine in turn, and dried over anhydrous Na₂SO₄. After filtration and concentration, compound 44 was obtained. ¹H NMR (400 MHz, CDCl₃) δ 8.71 (s, 1H), 7.70 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.56 (d, *J* = 2.0 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 2H), 4.69 (s, 2H), 3.91 (s, 3H).

4.1.27. N-(4-methoxyphenyl)-3-oxo-3,4-dihydro-2H-benzo[b] [1,4]oxazine-6-carboxamide (45)

To a solution of compound 44 (0.72 g, 3.48 mmoL) in MeOH/H₂O (12 mL, 2:1) was added sodium hydroxide (0.28 g, 7.0 mmol). The mixture was stirred at 70 °C for 3 h to give substituted benzoic acid, which was reacted 4-methoxy aniline according to general procedure A to give the target compound 45 (0.593 g, 82%). ¹H NMR (400 MHz, DMSO-*d₆*) δ 10.9 (s, 1H), 10.03 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.57 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.46 (d, *J* = 2.0 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 2H), 4.66 (s, 2H), 3.74 (s, 3H). LC/MS (ESI, *m/z*): 299.1 [M + H]⁺.

4.1.28. tert-butyl 6-((4-methoxyphenyl)carbamoyl)-2,3-dihydro-4H-benzo[b] [1,4]oxazine-4-carboxylate (47)

To the solution of methyl 3,4-dihydro-2*H*-benzo[b] [1,4]oxazine-6-carboxylate (0.20 g, 1.03 mmol) in acetonitrile (2.5 mL) was added DMAP (0.025 g, 0.2 mmol) and Ditertbutyl dicarbonate (0.23 g, 1.14 mmol). The mixture was stirred at room temperature

for 3 h until the starting material was completely disappeared. The solvent was removed, and the residue was extracted with ethyl acetate, washed with water and brine in turn, and dried over anhydrous Na₂SO₄. After filtration and concentration, the residual material was purified by flash column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (20:1) to yield *N*-Boc substituted product. The compound was dissolved in MeOH/H₂O (5 mL, 2:1) and added sodium hydroxide (0.054 g, 1.36 mmol). The mixture was stirred at 70 °C for 3 h to give substituted benzoic acid (46). ¹H NMR (400 MHz, CDCl₃) δ 8.54 (s, 1H), 7.74 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 4.31 (t, *J* = 4.4 Hz, 2H), 3.88 (t, *J* = 4.4 Hz, 2H), 1.57 (s, 9H). Compound 46 was reacted with 4-methoxy aniline according to general procedure A to give the compound 47 (0.341 g, 89%). ¹H NMR (400 MHz, CDCl₃) δ 8.38 (s, 1H), 7.69 (s, 1H), 7.52 (m,3H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 2H), 4.30 (t, *J* = 4.4 Hz, 2H), 3.89 (t, *J* = 4.4 Hz, 2H), 3.81 (s, 3H), 1.57 (s, 9H). LC/MS (ESI, *m/z*): 385.2 [M + H]⁺.

4.1.29. N-(4-methoxyphenyl)-3,4-dihydro-2H-benzo[b] [1,4]oxazine-6-carboxamide (48)

To a solution of compound 47 (0.21 g, 0.52 mmol) in dichloromethane (4.0 mL) was added trifluoroacetic acid (2.0 mL) at °C and the mixture was stirred for 3 h until the starting material was completely disappeared. The reaction was extracted with dichloromethane (20 mL) and the organic layer was orderly washing with 0.5 M sodium hydroxide solution, water and brine, dried with Na₂SO₄. After filtration and concentration, compound 48 was obtained. ¹H NMR (400 MHz, CDCl₃) δ 7.67 (s, 1H), 7.50 (d, *J* = 9.2 Hz, 1H), 7.24 (d, *J* = 2.0 Hz, 1H), 7.11 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.4 Hz, 1H), 5.29 (s, 1H), 4.30 (t, *J* = 4.4 Hz, 2H), 3.81 (s, 3H), 3.45 (t, *J* = 4.4 Hz, 2H). LC/MS (ESI, *m/z*): 285.1 [M + H]⁺.

4.1.30. 4-Isopropoxy-3-nitrobenzoic acid (49)

To a solution of methyl 4-hydroxy-3-nitrobenzoate (1.50 g, 7.6 mmol) in DMF (15.0 mL) was added 2-bromopropane (1.10 mL, 11.4 mmol) and potassium carbonate (1.57 g, 11.4 mmol). The mixture was stirred at 60 °C for 12 h. After cooling to rt, the solvent was removed, and the residue was extracted with ethyl acetate, washed with water and brine in turn, and dried over anhydrous Na₂SO₄. After filtration and concentration, the obtained residue was dissolved in methanol and THF (10 mL, 1:1) and 1 N NaOH (10 mL) was added. The mixture was stirred at 60 °C for 1.5 h. After cooling to rt, the solvent was removed, and the residue was extracted with ethyl acetate, washed with 1 N HCl, water, and brine in turns, and dried over anhydrous Na₂SO₄. The mixture was filtered, and the solvent was removed to give compound 49 (1.10 g, 64%). LC/MS (ESI, *m/z*): 224.0 [M−H]⁺.

4.1.31. N-(4-dimethylaminophenyl)-3-nitro-4-isopropoxy-benzamide (50)

Compound 49 (0.450 g, 2.0 mmol) and 4-dimethylamino-aniline (0.272 g, 2.0mmol) were reacted according to general procedure A to afford 50 (0.597 g, yield 87%). ¹H NMR (500 MHz, CDCl₃) δ 8.26 (s, 1H), 8.06 (d, *J* = 9.5 Hz, 1H), 7.70 (s, 1H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 9.0 Hz, 1H), 6.72 (d, *J* = 9.5 Hz, 2H), 4.76 (m, 1H), 2.94 (s, 6H), 1.42 (d, *J* = 6.0 Hz, 6H); ¹³C NMR (125 MHz, CDCl₃) δ 163.1, 153.7, 148.4, 140.2, 133.2, 127.2, 127.0, 124.2, 122.5, 115.6, 113.0, 73.1, 40.9, 21.9. ESI-HRMS calcd for C₁₈H₂₂N₃O₄ [M + H]⁺ 344.1605; found 344.1601.

4.1.32. N-(4-dimethylaminophenyl)-3-amino-4-isopropoxy-benzamide (51)

Compound 50 (0.514 g, 1.5 mmol) was reduced according to general procedure B to afford 51. ¹H NMR (500 MHz, CDCl₃) δ 7.59 (s,

1H), 7.45 (d, *J* = 9.5 Hz, 2H), 7.26 (s, 1H), 7.18 (d, *J* = 9.0 Hz, 1H), 6.80 (d, *J* = 9.0 Hz, 1H), 6.73 (d, *J* = 9.0 Hz, 2H), 4.61 (m, 1H), 3.91 (br s, 2H), 2.93 (s, 6H), 1.38 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 165.5, 147.9, 137.1, 128.1, 127.7, 121.9, 117.1, 113.9, 113.1, 112.0, 70.6, 40.9, 22.1. LC/MS (ESI, *m/z*): 314.2 [M + H]$^+$.

4.1.33. N-(4-dimethylaminophenyl)-3-propionamido-4-isopropoxy-benzamide (52)

Compound 51 (0.156 g, 0.5 mmol) was reacted with propionyl chloride (44 µL, 0.5 mmol) and TEA (0.105 mL, 0.75 mmol) according to general procedure C to afford compound 52 (0.127 g, 69%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.88 (s, 1H), 7.86 (s, 1H), 7.82 (s, 1H), 7.76 (d, *J* = 9.5 Hz, 1H), 7.48 (q, *J* = 9.5 Hz, 2H), 6.96 (d, *J* = 9.0 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 2H), 4.70 (m, 1H), 2.93 (s, 6H), 2.48 (d, *J* = 7.5 Hz, 2H), 1.42 (d, *J* = 5.5 Hz, 6H), 1.28 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 172.2, 165.0, 148.4, 128.0, 127.5, 124.6, 122.2, 117.1, 113.3, 112.2, 71.6, 41.1, 31.1, 22.1, 9.6. ESI-HRMS calcd for C$_{21}$H$_{28}$O$_3$N$_3$ [M + H]$^+$ 370.2125; found 370.2117.

4.1.34. N-(4-dimethylaminophenyl)-3-(2-bromopropionamido)-4-isopropoxy-benzamide (53)

Compound 51 (0.156 g, 0.5 mmol) was reacted with 2-bromopropionyl chloride (50 µL, 0.5 mmol) and TEA (0.105 mL, 0.75 mmol) according to general procedure C to afford compound 53 (0.134 g, yield 60%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.94 (s, 1H), 8.79 (s, 1H), 7.78 (s, 1H), 7.78 (d, *J* = 9.5 Hz, 1H), 7.47 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 8.5 Hz, 1H), 6.73 (d, *J* = 8.5 Hz, 2H), 4.70 (m, 1H), 4.60 (q, *J* = 6.0 Hz, 1H), 2.91 (s, 6H), 1.98 (d, *J* = 6.0 Hz, 3H), 1.42 (d, *J* = 5.5 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 167.3, 164.9, 149.1, 147.8, 128.2, 127.5, 127.4, 125.1, 117.2, 113.2, 112.4, 72.0, 45.7, 41.0, 23.1, 22.1. ESI-HRMS calcd for C$_{21}$H$_{27}$O$_3$N$_3$Br [M + H]$^+$ 448.1230; found 448.1235.

4.1.35. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-nitro-4-isopropoxy-benzamide (54)

Compound 49 (0.450 g, 2.0 mmol) and 3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) aniline (0.546 g, 2.0 mmol) were reacted according to general procedure A to afford 54 (0.538 g, yield 56%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.32 (m, 3H), 8.10 (d, *J* = 8.5 Hz, 1H), 7.87 (s, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.75 (m, 1H), 7.15 (dd, *J* = 8.5, 3.0 Hz, 1H), 4.78 (m, 1H), 3.62 (s, 2H), 2.52 (m, 8H), 2.32 (m, 3H), 1.44 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 163.5, 154.0, 139.9, 136.4, 133.7, 133.4, 131.4, 129.2 (q, *J* = 30.1 Hz), 125.9, 124.5, 124.1 (q, *J* = 260.1 Hz), 123.7, 118.1 (d, *J* = 6.0 Hz), 115.5, 73.1, 57.7, 55.1, 52.8, 45.8, 21.6. ESI-HRMS calcd for C$_{23}$H$_{28}$O$_4$N$_4$F$_3$ [M + H]$^+$ 481.2057; found 481.2043.

4.1.36. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-amino-4-isopropoxy-benzamide (55)

Compound 54 (0.481 g, 1.0 mmol) was reduced according to general procedure B to afford compound 55. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.84 (m, 3H), 7.73 (d, *J* = 8.5 Hz, 1H), 7.19 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 4.63 (m, 1H), 3.94 (br s, 2H), 3.62 (s, 2H), 2.52 (m, 8H), 2.31 (m, 3H), 1.39 (d, *J* = 6.0 Hz, 6H).

4.1.37. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-propionamido-4-isopropoxy-benzamide (56)

Compound 55 (0.225 g, 0.5 mmol) was reacted with propionyl chloride (44 µL, 0.5 mmol) and TEA (0.105 mL, 0.75 mmol) according to general procedure C to afford compound 56 (0.131 g, yield 52%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.91 (s, 1H), 8.22 (s, 1H), 7.91 (s, 1H), 7.86 (m, 2H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.64 (d, *J* = 8.5 Hz, 1H), 6.98 (d, *J* = 8.5 Hz, 1H), 4.71 (m, 1H), 3.66 (s, 2H), 2.74 (m, 8H), 2.47 (m, 5H), 1.41 (d, *J* = 7.5 Hz, 6H), 1.27 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 172.2, 165.6, 149.0, 137.7, 132.1, 131.3, 129.2 (q, *J* = 30.6 Hz),127.7, 126.5, 124.9, 124.2(q, *J* = 272.6 Hz), 123.5, 118.1 (d,

J = 6.3 Hz), 117.7, 112.1, 71.7, 54.5, 51.4, 45.9, 44.8, 31.0, 9.6. ESI-HRMS calcd for C$_{26}$H$_{34}$O$_3$N$_4$F$_3$ [M + H]$^+$ 507.2578; found 507.2577.

4.1.38. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-(2-bromopropionamido)-4-isopropoxy-benzamide (57)

Compound 55 (0.225 g, 0.5 mmol) was reacted with 2-bromopropionyl chloride (50 µL, 0.5 mmol) and TEA (0.105 mL, 0.75 mmol) according to general procedure C to afford compound 57 (0.134 g, yield 46%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.94 (s, 1H), 8.81 (s, 1H), 8.16 (s, 1H), 7.88 (s, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.79 (d, *J* = 8.5 Hz, 1H), 7.74 (m, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 4.72 (m, 1H), 4.60 (m, 1H), 3.61 (s, 2H), 2.49 (m, 8H), 2.28 (m, 3H), 1.99 (d, *J* = 6.0 Hz, 3H), 1.43 (d, *J* = 5.0 Hz, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 167.5, 165.2, 149.6, 136.9, 133.4, 131.4, 129.4, 127.7, 126.6, 125.4, 123.4, 118.4, 117.9, 117.0, 112.5, 72.2, 60.5, 59.7, 57.9, 55.3, 53.0, 46.0, 45.7, 42.2, 23.2, 22.1, 14.3. ESI-HRMS calcd for C$_{26}$H$_{33}$BrF$_3$N$_4$O$_3$ [M + H]$^+$ 585.1683; found 585.1684.

4.1.39. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-nitro-4-(3-chloropropoxy)-benzamide (60)

To a solution of methyl 4-hydroxy-3-nitrobenzoate (58a, 1.50 g, 7.6 mmol) in DMF (15.0 mL) was added 1-bromo-3-chloropropane (1.12 mL, 11.4 mmol) and potassium carbonate (1.56 g, 11.4 mmol). The mixture was stirred at 60 °C for 12 h. After cooling to rt, the solvent was removed, and the residue was extracted with ethyl acetate, washed with water and brine in turn, and dried over anhydrous Na$_2$SO$_4$. After filtration and concentration, the obtained residue was purified by flash column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (V = 5:1) to yield methyl 3-chloropropoxy-3-nitrobenzoate (1.95 g, 94%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.52 (s, 1H), 8.23 (s, 1H), 7.14 (s, 1H), 4.33 (m, 2H), 3.93 (s, 3H), 3.66 (m, 2H), 2.31 (m, 2H). The compound (1.16 g, 4.25 mmol) was dissolved in tetrahydrofunan (10 mL) and methanol (10 mL) and lithium hydroxide (0.117 g, 5.10 mmol) was added. The mixture was stirred at room temperature for about 3.0 h. The solvent was removed, and the residue was extracted with ethyl acetate, washed with 1 N HCl, water, and brine in turns, and dried over anhydrous Na$_2$SO$_4$. After 2 h, the mixture was filtered, and the solvent was removed to give 4-(3-chloropropoxyl)-3-nitrobenzoic acid (59a, 1.10 g, 98%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.58 (s, 1H), 8.27 (d, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 7.5 Hz, 1H), 4.37 (t, *J* = 5.0 Hz, 2H), 3.81 (t, *J* = 5.5 Hz, 2H), 2.33 (t, *J* = 6.0 Hz, 2H). Compound 59a (0.518 g, 2.0 mmol) and 3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl)aniline (0.546 g, 2.0 mmol) were reacted according to general procedure A to afford 60 (0.894 g, yield 87%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.45 (s, 1H), 8.23 (m, 2H), 7.92 (m, 2H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.27 (d, *J* = 8.5 Hz, 1H), 4.41 (t, *J* = 5.0 Hz, 2H), 3.86 (t, *J* = 6.0 Hz, 2H), 3.68 (s, 2H), 2.58 (m, 8H), 2.37 (m, 5H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 163.5, 154.8, 149.7, 139.0, 136.5, 133.9, 129.3 (q, *J* = 30.6 Hz),126.7, 124.8, 124.2 (q, *J* = 273.9 Hz), 123.7, 118.1 (d, *J* = 4.5 Hz), 114.6, 66.3, 57.8, 55.2, 52.9, 45.9, 41.0, 31.7. ESI-HRMS calcd for C$_{23}$H$_{27}$O$_4$N$_4$ClF$_3$ [M + H]$^+$ 515.1667; found 515.1667.

4.1.40. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-fluoro-4-(3-chloropropoxy)-benzamide (61)

Compound 61 was synthesized using a method similar to that of 60. Methyl 4-hydroxy-3-fluorobenzoate (58b, 0.204 g, 1.20 mmol) and 1-bromo-3-chloropropane (0.14 mL, 1.44 mmol) was using the starting materials to yield the intermediate methyl 4-isopropoxy-3-fluorobenzoate (0.250 g, 85%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.73 (d, *J* = 7.5 Hz, 1H), 7.67 (d, *J* = 11.5 Hz, 1H), 6.94 (t, *J* = 8.0 Hz, 1H), 4.19 (t, *J* = 5.5 Hz, 2H), 3.84 (s, 3H), 3.72 (t, *J* = 6.0 Hz, 1.4H), 3.57 (t, *J* = 6.0 Hz, 0.6H), 2.32 (t, *J* = 5.5 Hz, 0.6H), 2.24 (t, *J* = 5.5 Hz, 1.4H). The intermediate was hydrolyzed to yield 4-(3-chloropropoxyl)-3-fluorobenzoic acid (59b, 0.150 g, 65%). ^{1}H NMR (500 MHz, CDCl$_3$)

δ 7.89 (d, *J* = 7.5 Hz, 1H), 7.81 (d, *J* = 11.5 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 4.27 (m, 2H), 3.78 (t, *J* = 6.0 Hz, 1.47H), 3.63 (t, *J* = 6.0 Hz, 0.62H), 2.39 (t, *J* = 6.0 Hz, 0.62H), 2.32 (t, *J* = 6.0 Hz, 1.47H). Compound 59b (0.140 g, 0.60 mmol) and 3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl)aniline (0.160 g, 0.60 mmol) were reacted according to general procedure A to afford 61 (0.260 g, yield 89%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.22 (br s, 1H), 7.88 (s, 1H), 7.83 (m, 2H), 7.74 (m, 2H), 6.96 (d, *J* = 8.5 Hz, 1H), 4.22 (t, *J* = 5.0 Hz, 2H), 3.79 (t, *J* = 6.0 Hz, 2H), 3.60 (s, 2H), 2.48 (m, 8H), 2.28 (m, 5H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 164.5, 157.2, 136.7, 133.9, 131.4, 131.3, 129.4, 129.1, 127.6, 127.4, 124.1 (q, *J* = 273.3 Hz), 123.5, 123.3, 117.8 (d, *J* = 6.1 Hz), 112.7, 65.6, 57.9, 55.3, 53.2, 46.1, 41.2, 32.0. ESI-HRMS calcd for C$_{23}$H$_{27}$O$_2$N$_3$ClF$_4$ [M + H]$^+$ 488.1722; found 488.1716.

4.1.41. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-chloro-4-(3-chloropropoxy)-benzamide (62)

Compound 62 was synthesized using a method similar to that of 60. Methyl 4-hydroxy-3-chlorobenzoate (58c, 0.204 g, 1.07 mmol) and 1-bromo-3-chloropropane (0.16 mL, 1.60 mmol) was using the starting materials to yield the intermediate methyl 4-isopropoxy-3-chlorobenzoate (0.249 g, 89%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.05 (s, 1H), 7.92 (d, *J* = 7.5 Hz, 1H), 6.95 (d, *J* = 7.5 Hz, 1H), 4.24 (s, 2H), 3.89 (s, 3H), 3.80 (m, 1.4H), 3.66 (m, 0.6H), 2.39 (m, 0.6H), 2.31 (m, 1.4H). The intermediate was hydrolyzed to yield 4-(3-chloropropoxyl)-3-chlorobenzoic acid (59c, 0.250 g, 99%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.12 (s, 1H), 8.00 (d, *J* = 7.5 Hz, 1H), 6.99 (d, *J* = 7.5 Hz, 1H), 4.27 (m, 2H), 3.81 (t, *J* = 6.0 Hz, 1.32H), 3.67 (t, *J* = 6.0 Hz, 0.61H), 2.41 (t, *J* = 6.0 Hz, 0.67H), 2.32 (t, *J* = 6.0 Hz, 1.37H). Compound 59c (0.250 g, 1.08 mmol) and 3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl)-aniline (0.273 g, 1.00 mmol) were reacted according to general procedure A to afford 62 (0.468 g, yield 93%).^{1}H NMR (500 MHz, CDCl$_3$) δ 8.60 (s, 1H), 7.88 (s, 1H), 7.85 (m, 2H), 7.75 (d, *J* = 8.5 Hz, 1H), 7.70 (d, *J* = 8.5 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 4.20 (t, *J* = 5.0 Hz, 2H), 3.78 (t, *J* = 6.0 Hz, 2H), 3.58 (s, 2H), 2.46 (m, 8H), 2.28 (m, 5H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 164.7, 157.1, 136.8, 133.7, 131.3, 131.3, 129.5, 129.1 (q, *J* = 30.4 Hz), 127.6, 127.5, 124.1 (q, *J* = 272.6 Hz), 123.6, 123.2, 117.9 (d, *J* = 5.5 Hz), 112.5, 65.5, 57.8, 55.3, 53.1, 46.1, 41.2, 32.0. ESI-HRMS calcd for C$_{23}$H$_{27}$O$_2$N$_3$Cl$_2$F$_3$ [M + H]$^+$ 504.1427; found 504.1415.

4.1.42. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-trifluoromethyl-4-(3-chloropropoxy)-benzamide (63)

Compound 63 was synthesized using a method similar to that of 60. Methyl 4-hydroxy-3-(trifluoromethyl)benzoate (58d, 0.204 g, 0.91 mmol) and 1-bromo-3-chloropropane (0.13 mL, 1.36 mmol) was using as the starting materials to yield the intermediate methyl 4-(3-chloropropoxyl-3-(trifluoromethyl)benzoate (0.253 g, 94%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.27 (s, 1H), 8.20 (d, *J* = 8.5 Hz, 1H), 7.05 (d, *J* = 9.0 Hz, 1H), 4.28 (t, *J* = 5.0 Hz, 2H), 3.91 (s, 3H), 3.77 (t, *J* = 6.0 Hz, 1.63H), 3.63 (t, *J* = 6.0 Hz, 0.37H), 2.37 (t, *J* = 5.0 Hz, 0.37H), 2.30 (t,*J* = 6.0 Hz, 1.65H). The intermediate was hydrolyzed to yield 4-(3-chloropropoxyl-3-(trifluoromethyl)benzoic acid (59d, 0.223 g, 96%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.34 (s, 1H), 8.26 (d, *J* = 9.0 Hz, 1H), 7.08 (d,*J* = 8.5 Hz, 1H), 4.31 (t,*J* = 5.5 Hz, 2H), 3.78 (t, *J* = 6.0 Hz, 1.65H), 3.64 (t, *J* = 6.0 Hz, 0.37H), 2.39 (t, *J* = 6.0 Hz, 0.40H), 2.31 (t, *J* = 6.0 Hz, 1.66H). Compound 59d (0.100 g, 0.35 mmol) and 3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl) methyl)aniline (0.097 g, 0.35 mmol) were reacted according to general procedure A to afford 63 (0.101 g, yield 53%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.48 (s, 1H), 8.06 (s, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.83 (d, *J* = 7.0 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 4.24 (t, *J* = 5.0 Hz, 2H), 3.75 (t, *J* = 6.0 Hz, 2H), 3.58 (s, 2H), 2.47 (m, 8H), 2.28 (m, 5H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 164.6, 159.4, 136.6, 134.0, 133.0, 131.3, 129.2 (q, *J* = 30.5 Hz), 126.5, 126.3, 124.1 (q, *J* = 272.6 Hz), 123.7, 123.1 (q, *J* = 272.6 Hz), 119.0 (q, *J* = 30.6 Hz),

118.0 (d, *J* = 5.2 Hz), 112.7, 65.3, 57.8, 55.3, 53.2, 46.1, 41.0, 31.9, 30.4. ESI-HRMS calcd for C$_{24}$H$_{27}$O$_2$N$_3$ClF$_6$ [M + H]$^+$ 538.1690; found 538.1681.

4.1.43. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-sulfamoyl-4-(3-chloropropoxy)-benzamide (64)

Compound 64 was synthesized using a method similar to that of 60. Methyl 4-hydroxy-3-(sulfamoyl)benzoate (58e, 0.231 g, 1.00 mmol) and 1-bromo-3-chloropropane (0.13 mL, 1.36 mmol) was using as the starting materials to yield the intermediate methyl 4-(3-chloropropoxyl)-3-(trifluoromethyl)benzoate. The intermediate was hydrolyzed to yield 4-(3-chloropropoxy)-3-(trifluoromethyl) benzoic acid (59e, 0.223 g, 76%). ^{1}H NMR (500 MHz, DMSO-*d$_6$*) δ 8.30 (s, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 8.04 (s, 1H), 7.29 (d, *J* = 8.5 Hz, 1H), 7.10 (s, 2H), 4.30 (s, 2H), 3.85 (s, 2H), 2.28 (s, 2H). Compound 59e (0.100 g, 0.34 mmol) and 3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) aniline (0.097 g, 0.35 mmol) were reacted according to general procedure A to afford 64 (0.093 g, yield 50%). ^{1}H NMR (500 MHz, DMSO-*d$_6$*) δ 10.65 (s, 1H), 8.43 (s, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.21 (s, 1H), 8.10 (d, *J* = 7.5 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 7.5 Hz, 1H), 7.20 (s, 2H), 4.35 (s, 2H), 3.87 (s, 2H), 3.63 (s, 2H), 2.93 (br s, 4H), 2.57 (br s, 4H), 2.30 (s, 2H); ^{13}C NMR (125 MHz, DMSO-*d$_6$*) δ 164.1, 157.8, 138.5, 133.3, 131.3, 127.9, 127.6, 125.6, 123.6, 117.0, 113.1, 65.9, 56.8, 53.2, 50.2, 42.1, 40.0, 31.2. ESI-HRMS calcd for C$_{23}$H$_{29}$O$_4$N$_4$F$_3$SCl [M + H]$^+$ 549.1545; found 549.1550.

4.1.44. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-amino-4-(3-chloropropoxy)-benzamide (65)

Compound 60 was hydrogenated according to general procedure B to afford 65. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.87 (m, 3H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.27 (d, *J* = 8.5 Hz, 1H), 7.22 (d, *J* = 8.5 Hz, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 4.23 (t, *J* = 5.0 Hz, 2H), 3.95 (br s, 2H), 3.743 (t, *J* = 6.0 Hz, 2H), 3.62 (s, 2H), 2.52 (m, 8H), 2.31 (m, 5H). ESI-HRMS calcd for C$_{23}$H$_{29}$O$_2$N$_4$ClF$_3$ [M + H]$^+$ 485.1927; found 485.1923.

4.1.45. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-propionamido-4-(3-chloropropoxy)-benzamide (66)

Acylation of compound 65 afforded 66 according to general procedure C. ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.83 (s, 1H), 8.53 (s, 1H), 7.91 (s, 1H), 7.85 (m, 2H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 1H), 4.27 (t, *J* = 5.0 Hz, 2H), 3.73 (t, *J* = 6.0 Hz, 2H), 3.61 (s, 2H), 2.52 (m, 8H), 2.42 (m, 2H), 2.33 (m, 5H), 1.24 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 172.5, 165.4, 149.8, 137.3, 133.0, 131.3, 129.2 (q, *J* = 30.6 Hz),127.4, 127.3, 125.1, 124.2(q, *J* = 271.0 Hz), 123.4, 123.3, 117.9 (d, *J* = 4.5 Hz), 117.7, 111.2, 66.1, 57.8, 55.0, 52.7, 45.7, 41.4, 31.8, 31.0, 9.6. ESI-HRMS calcd for C$_{26}$H$_{33}$O$_3$N$_4$ClF$_3$ [M + H]$^+$ 541.2188; found 541.2174.

Compounds 68−70 were synthesized using a method similar to that of 60.

4.1.46. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-nitro-4-(4-chloroethoxy)-benzamide (68)

Compound 58a (0.20 g, 1.0 mmol)was reacted with 1-bromo-2-chloroethane (0.125 mL, 1.5 mmol) and potassium carbonate (0.28 g, 2.0 mmol) to give methyl 2-chloroethoxy-3-nitrobenzoate (0.181 g, 70%). The compound was hydrolyzed to give 4-(2-chloroethoxy)-3-nitrobenzoic acid (67a, 0.168 g, 98%). ^{1}H NMR (500 MHz, DMSO-*d$_6$*) δ 13.35 (br s, 1H), 8.34 (s, 1H), 8.16 (d, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 7.5 Hz, 1H), 4.54 (m, 2H), 3.98 (m, 2H). Compound 67a (0.15 g, 0.61 mmol) and 3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl)aniline (0.167 g, 0.61 mmol) were reacted according to general procedure A to afford 68 (0.265 g, yield 87%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.36 (d, *J* = 1.0 Hz, 1H), 8.13 (d, *J* = 8.5 Hz, 1H), 7.85 (s, 1H), 7.84 (d,*J* = 9.0 Hz, 1H), 7.76 (d,*J* = 8.5 Hz,

1H), 7.15 (d, *J* = 9.0 Hz, 1H), 4.42 (t, *J* = 6.0 Hz, 2H), 3.87 (t, *J* = 6.0 Hz, 2H), 3.61 (s, 2H), 2.49 (m, 8H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.3, 154.1, 139.4, 136.3, 134.4, 133.8, 131.5, 129.3 (q, *J* = 30.6 Hz), 127.3, 124.7, 124.2 (q, *J* = 273.9 Hz), 123.7, 118.1 (d, *J* = 4.5 Hz), 115.0, 69.9, 57.8, 55.2, 53.2, 46.2, 41.0, 30.4, 29.8. ESI-HRMS calcd for C$_{22}$H$_{25}$O$_4$N$_4$ClF$_3$ [M + H]$^+$ 501.1511; found 501.1503.

4.1.47. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-nitro-4-(4-chlorobutoxy)-benzamide (69)

^{1}H NMR (500 MHz, CDCl$_3$) δ 8.37 (s, 1H), 8.13 (d, *J* = 8.5 Hz, 1H), 8.05 (s, 1H), 7.87 (s, 1H), 7.85 (d, *J* = 9.0 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.17 (d, *J* = 8.5 Hz, 1H), 4.24 (s, 2H), 3.63 (s, 4H), 2.51 (m, 8H), 2.30 (s, 3H), 2.04 (m, 4H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 163.4, 155.1, 139.3, 136.4, 134.4, 133.8, 131.6, 129.4 (q, *J* = 30.3 Hz), 126.6, 124.6, 124.2 (q, *J* = 273.9 Hz), 123.7, 118.0 (d, *J* = 6.0 Hz), 114.7, 69.4, 57.9, 55.3, 53.2, 46.2, 44.6, 29.0, 26.4. ESI-HRMS calcd for C$_{24}$H$_{29}$O$_4$N$_4$ClF$_3$ [M + H]$^+$ 529.1824; found 529.1827.

4.1.48. N-(3-(trifluoromethyl)-4-((4-methylpiperazin-1-yl)methyl) phenyl)-3-nitro-4-((5-chloropentyl)oxy)-benzamide (70)

^{1}H NMR (500 MHz, CDCl$_3$) δ 8.37 (s, 1H), 8.12 (m, 2H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.78 (d, *J* = 8.5 Hz, 1H), 7.17 (d, *J* = 8.5 Hz, 1H), 4.20 (t, *J* = 6.0 Hz, 2H), 3.64 (s, 2H), 3.58 (t, *J* = 6.0 Hz, 2H), 2.53 (m, 8H), 2.32 (s, 3H), 1.88 (m, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.1, 155.1, 139.1, 136.2, 133.8, 131.4, 126.2, 124.4, 123.4, 117.8, 114.5, 69.8, 57.7, 55.1, 52.9, 45.9, 44.7, 32.0, 28.1, 23.2. ESI-HRMS calcd for C$_{25}$H$_{31}$O$_4$N$_4$ClF$_3$ [M + H]$^+$ 543.1980; found 543.1981.

4.1.49. N-(3-cyano-4-((4-methylpiperazin-1-yl)methyl)phenyl)-3-nitro-4-(3-chloropropoxy)-benzamide (71)

Compound 59a (0.259 g, 1.00 mmol) and 3-cyano-4-((4-methylpiperazin-1-yl)methyl)aniline (0.230 g, 1.00 mmol) were reacted according to general procedure A to afford 71 (0.315 g, yield 67%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.50 (s, 1H), 8.44 (d, *J* = 2.4 Hz, 1H), 8.17 (d, *J* = 8.8 Hz, 1H), 8.02 (s, 1H), 7.87 (d, *J* = 8.4 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.21 (d, *J* = 8.8 Hz, 1H), 4.35 (t, *J* = 5.6 Hz, 2H), 3.80 (t, *J* = 6.4 Hz, 2H), 3.67 (s, 2H), 2.56 (m, 8H), 2.32 (m, 5H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.5, 155.0, 139.1, 138.6, 137.4, 134.1, 131.0, 126.4, 124.9, 124.7, 124.5, 117.6, 114.7, 113.5, 66.3, 60.0, 55.1, 52.7, 45.9, 41.1, 31.8. ESI-HRMS calcd for C$_{23}$H$_{27}$O$_4$N$_5$Cl [M + H]$^+$ 472.1746; found 472.1741.

4.1.50. N-(3-(trifluoromethyl)-4-((4-(dimethylamino)piperidin-1-yl)methyl))phenyl)-3-nitro-4-(3-chloropropoxy)-benzamide (72)

Compound 59a (0.259 g, 1.00 mmol) and 3-(trifluoromethyl)-4-((4-(dimethylamino)piperidin-1-yl)methyl)aniline (83a, 0.302 g, 1.00 mmol) were reacted according to general procedure A to afford 72 (0.411 g, yield 76%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.42 (s, 1H), 8.36 (s, 1H), 8.16 (d, *J* = 8.8 Hz, 1H), 7.92 (s, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 1H), 4.34 (m, 2H), 3.80 (t, *J* = 6.0 Hz, 2H), 3.59 (s, 2H), 2.87 (m, 2H), 2.30 (m, 8H), 2.18 (m, 1H), 2.04 (m, 2H), 1.79 (m, 2H), 1.54 (m, 2H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.4, 154.9, 139.1, 136.3, 134.8, 133.9, 131.3, 129.2 (q, *J* = 30.1 Hz), 126.8, 124.8, 124.2 (q, *J* = 272.7 Hz), 123.8, 118.0 (q, *J* = 5.3 Hz), 114.7, 66.3, 62.4, 57.8, 53.2, 41.8, 41.1, 31.8, 28.6. ESI-HRMS calcd for C$_{25}$H$_{31}$O$_4$N$_4$ClF$_3$ [M + H]$^+$ 543.1980; found 543.1965.

4.1.51. N-(3-(trifluoromethyl)-4-((3-dimethylaminopyrrolidin-1-yl) methyl)phenyl)-3-nitro-4-(3-chloropropoxy)-benzamide (73)

Compound 59a (0.259 g, 1.00 mmol) and 3-(trifluoromethyl)-4-(3-dimethylamino)-pyrrolidin-1-yl)methylaniline (83b, 0.288 g, 1.00 mmol) were reacted according to general procedure A to afford 73 (0.205 g, yield 39%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.12 (br s, 1H), 8.39 (s, 1H), 8.12 (d, *J* = 9.0 Hz, 1H), 7.88 (s, 1H), 7.82 (d,

J = 8.0 Hz, 1H), 7.67 (d, *J* = 9.5 Hz, 1H), 7.10 (d, *J* = 9.5 Hz, 1H), 4.27 (t, *J* = 5.5 Hz, 2H), 3.75 (m, 2H), 3.68 (m, 2H), 2.73 (m, 2H), 2.64 (dd, *J* = 14.5, 7.5 Hz, 1H), 2.54 (dd, *J* = 14.5, 7.5 Hz, 1H), 2.41 (t, *J* = 7.5 Hz, 1H), 2.25 (m, 2H), 2.17 (s, 6H), 1.95 (m, 1H), 1.71 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.8, 154.7, 148.9, 138.8, 136.5, 134.6, 133.9, 131.0, 128.4 (q, *J* = 37.7 Hz), 126.7, 124.1 (q, *J* = 272.7 Hz), 123.9, 118.1 (d, *J* = 6.0 Hz), 114.3, 106.8, 66.1, 65.5, 58.4, 55.5, 53.5, 43.8, 41.0, 31.7, 29.3. ESI-HRMS calcd for C$_{24}$H$_{29}$O$_4$N$_4$ClF$_3$ [M + H]$^+$ 529.1824; found 529.1812.

4.1.52. N-(3-(trifluoromethyl)-4-((3-dimethylaminoazetidin-1-yl) methyl)phenyl)-3-nitro-4-(3-chloropropoxy)-benzamide (74)

Compound 59a (0.145 g, 0.56 mmoL) and 3-(trifluoromethyl)-4-(3-dimethylamino)azetidin-1-yl)methylaniline (83c, 0.140 g, 0.51 mmoL) were reacted according to general procedure A to afford 74 (0.107 g, yield 41%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.39 (d, *J* = 1.5 Hz, 1H), 8.17 (s, 1H), 8.14 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.89 (s, 1H), 7.84 (d, *J* = 7.0 Hz, 1H), 7.65 (d, *J* = 7.0 Hz, 1H), 7.20 (d, *J* = 7.0 Hz, 1H), 4.35 (t, *J* = 4.5 Hz, 2H), 3.80 (m, 3H), 3.55 (t, *J* = 5.0 Hz, 2H), 2.96 (t, *J* = 5.5 Hz, 2H), 2.91 (m, 2H), 2.32 (t, *J* = 5.0 Hz, 2H), 2.12 (s, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 163.2, 154.9, 139.1, 136.3, 133.9, 133.8, 130.6, 128.7, 126.7, 124.7, 123.6, 118.0, 114.7, 66.3, 59.9, 57.0, 42.2, 41.1, 31.8. ESI-HRMS calcd for C$_{23}$H$_{27}$O$_4$N$_4$ClF$_3$ [M + H]$^+$ 515.1667; found 515.1665.

4.1.53. 1-(4-(4-(3-chloropropoxy)-3-nitrobenzamido)-2-(trifluoromethyl)benzyl)azetidine-3-carboxylic acid (75)

Compound 59a (0.259 g, 1.00 mmoL) and methyl 1-(4-amino-2-(trifluoromethyl)benzyl)azetidine-3-carboxylate (83d, 0.288 g, 1.00 mmoL) were reacted according to general procedure A to afford methyl ester intermediate (0.238 g, yield 45%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.77 (s, 1H), 8.39 (s, 1H), 8.13 (d, *J* = 7.5 Hz, 1H), 7.88 (s, 1H), 7.80 (d, *J* = 7.0 Hz, 1H), 7.56 (d, *J* = 7.0 Hz, 1H), 7.14 (d, *J* = 7.5 Hz, 1H), 4.31 (s, 2H), 3.78 (s, 2H), 3.71 (m, 5H), 3.53 (m, 2H), 3.35 (m, 3H), 2.28 (m, 2H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 173.8, 163.6, 154.9, 138.8, 136.5, 134.1, 133.1, 130.3, 128.5, (q, *J* = 29.4 Hz), 126.5, 124.8, 124.0 (q, *J* = 272.5 Hz), 123.8, 118.2, 66.2, 58.5, 57.1, 52.2, 41.1, 34.2, 31.7. To a solution of the intermediate (0.200 g, 0.38 mmoL) in MeOH/THF (4 mL, 1:1) was added lithium hydroxide (18 mg, 0.76 mmoL). The mixture was stirred for 4 h, the reaction was neutralized by diluted hydrochloric acid to give white solid, then filtering and drying derived product 75 (0.185 g, 96%). ^{1}H NMR (500 MHz, MeOD) δ 8.51 (s, 1H), 8.27 (s, 1H), 8.24 (d, *J* = 8.5 Hz, 1H), 8.08 (d, *J* = 9.0 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.46 (d, *J* = 9.0 Hz, 1H), 4.41 (m, 2H), 4.37 (s, 2H), 4.09 (m, 5H), 3.81 (m, 1H), 3.41 (m, 1H), 2.37 (m, 1H), 2.30 (m, 1H); ^{13}C NMR (125 MHz, MeOD) δ 166.1, 155.9, 141.2, 140.9, 134.8, 133.0, 130.5, 126.1, 125.1, 119.6, 118.6, 115.8, 68.7, 67.7, 59.0, 56.9, 41.9, 35.9, 33.0, 30.1. ESI-HRMS calcd for C$_{22}$H$_{22}$O$_6$N$_3$ClF$_3$ [M + H]$^+$ 516.1144; found 516.1139.

4.1.54. N-(3-(trifluoromethyl)-4-(morpholinmethyl)phenyl)-3-nitro-4-(3-chloropropoxy)-benzamide (76)

Compound 59a (0.164 g, 0.63 mmoL) and 3-(trifluoromethyl)-4-(morpholinmethyl)aniline (83e, 0.151 g, 0.58 mmoL) were reacted according to general procedure A to afford 76 (0.210 g, yield 72%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.38 (d, *J* = 2.0 Hz, 1H), 8.15 (dd, *J* = 7.5, 1.5 Hz, 1H), 8.02 (s, 1H), 7.87 (s, 1H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.80 (d, *J* = 6.0 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 1H), 4.36 (t, *J* = 5.0 Hz, 2H), 3.81 (t, *J* = 5.0 Hz, 2H), 3.72 (m, 4H), 2.48 (m, 4H), 2.33 (m, 2H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 163.2, 155.0, 139.2, 136.4, 133.9, 133.8, 131.6, 129.6 (q, *J* = 30.4 Hz), 126.7, 124.6, 124.1 (q, *J* = 272.1 Hz), 123.6, 118.0 (d, *J* = 6.3 Hz), 114.8, 67.2, 66.3, 58.3, 53.7, 41.1, 31.8. ESI-HRMS calcd for C$_{22}$H$_{24}$O$_5$N$_3$ClF$_3$ [M + H]$^+$ 502.1351; found 502.1348.

4.1.55. N-(3-(trifluoromethyl)-4-((3,5-dimethylmorpholin)methyl) phenyl)-3-nitro-4-(3-chloropropoxy)-benzamide (77)

Compound 59a (0.164 g, 0.63 mmoL) and 3-(trifluoromethyl)-4-((3,5-dimethylmorpholin)methyl)aniline (83f, 0.185 g, 0.58 mmoL) were reacted according to general procedure A to afford 77 (0.241 g, yield 79%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.38 (d, J = 1.8 Hz, 1H), 8.15 (dd, J = 8.0, 1.8 Hz, 1H), 8.03 (s, 1H), 7.86 (m, 2H), 7.85 (d, J = 7.2 Hz, 1H), 7.23 (d, J = 7.2 Hz, 1H), 4.36 (t, J = 6.0 Hz, 2H), 3.81 (t, J = 6.0 Hz, 2H), 3.69 (m, 2H), 3.69 (s, 2H), 2.68 (s, 1H), 2.66 (s, 1H), 2.33 (t, J = 6.0 Hz, 2H), 1.84 (t, J = 9.0 Hz, 2H), 1.15 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 163.2, 155.0, 139.2, 136.3, 134.1, 133.8, 131.6, 129.5 (q, J = 30.4 Hz), 126.7, 124.6, 124.1 (q, J = 273.7 Hz), 123.6, 118.0 (d, J = 6.0 Hz), 114.8, 71.9, 66.3, 59.5, 57.9, 41.0, 31.8, 19.2. ESI-HRMS calcd for C$_{24}$H$_{28}$O$_5$N$_3$ClF$_3$ [M + H]$^+$ 530.1664; found 530.1662.

4.1.56. N-(2-((4-methylpiperazin-1-yl)methyl)pyridin-3-yl)-3-nitro-4-(3-chloropropoxy)-benzamide (78)

Compound 59a (0.260 g, 1.00 mmoL) and 5-amine- 2-((4-methylpiperazin-1-yl)methylpyridine (86, 0.200 g, 1.00 mmoL) were reacted according to general procedure A to afford 78 (0.205 g, yield 46%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.65 (d, J = 2.4 Hz, 1H), 8.42 (d, J = 2.4 Hz, 1H), 8.33 (s, 1H), 8.21 (dd, J = 8.4, 2.4 Hz, 1H), 8.16 (dd, J = 8.4, 2.4 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.20 (d, J = 9.0 Hz, 1H), 4.35 (d, J = 6.0 Hz, 2H), 3.79 (d, J = 6.0 Hz, 2H), 3.63 (s, 2H), 2.52–2.45 (m, 8H), 2.31 (m, 2H), 2.27 (s, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 163.6, 154.9, 141.3, 139.1, 133.9, 133.2, 128.6, 126.5, 124.8, 123.6, 114.6, 66.3, 64.1, 55.1, 53.4, 46.1, 41.1, 31.7. ESI-HRMS calcd for C$_{21}$H$_{27}$O$_4$N$_5$Cl [M + H]$^+$ 448.1746; found 448.1744.

4.1.57. N-(2-(4-methylpiperidin-1-yl)pyridin-5-yl)-3-nitro-4-(3-chloropropoxy)-benzamide (79)

Compound 59a (0.380 g, 1.48 mmoL) and 5-amine- 2-(4-methylpiperidin-1-yl)pyridine (88, 0.260 g, 1.35 mmoL) were reacted according to general procedure A to afford 79 (0.250 g, yield 43%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.35 (s, 1H), 8.21 (s, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.95 (s, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 6.66 (d, J = 8.0 Hz, 1H), 4.33 (q, J = 5.4 Hz, 2H), 4.20 (d, J = 13.2 Hz, 2H), 3.80 (t, J = 6.0 Hz, 1.5H), 3.66 (t, J = 6.0 Hz, 0.5H), 2.81 (m, 2H), 2.38 (m, 0.5H), 2.31 (m, 1.5H), 1.72 (s, 1H), 1.70 (s, 1H), 1.66 (s, 1H), 1.59 (m, 1H), 1.20 (m, 3H), 0.95 (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 163.4, 157.6, 154.6, 141.2, 139.4, 133.5, 132.0, 127.2, 124.6, 124.2, 114.6, 107.1, 67.4, 66.4, 46.1, 41.1, 33.9, 31.9, 31.2, 29.5, 22.0. ESI-HRMS calcd for C$_{21}$H$_{26}$O$_4$N$_4$Cl [M + H]$^+$ 433.1643; found 433.1625.

4.1.58. N-(3-(trifluoromethyl)-4-((3-dimethylaminoazetidin-1-yl) methyl)phenyl)-3-nitro-4-(2-chloroethoxy)-benzamide (80)

4-(2-chloroethoxyl)-3-nitrobenzoic acid (67a, 0.245 g, 1.00 mmoL) and 3-(trifluoromethyl)-4-(3-dimethylamino)azetidin-1-yl)methylaniline (83c, 0.140 g, 1.00 mmoL) were reacted according to general procedure A to afford 80 (0.335 g, yield 67%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.57 (s, 1H), 8.54 (d, J = 1.8 Hz, 1H), 8.28 (dd, J = 9.0, 1.8 Hz, 1H), 8.16 (s, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 5.75 (s, 1H), 4.56 (t, J = 4.8 Hz, 2H), 4.99 (t, J = 4.8 Hz, 2H), 3.69 (s, 2H), 3.41 (s, 2H), 2.82 (t, J = 4.8 Hz, 2H), 2.00 (s, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.1, 153.1, 139.0, 137.8, 133.8, 132.1, 130.5, 126.7, 124.6, 123.6, 117.3, 115.3, 69.9, 58.9, 58.5, 56.3, 54.9, 42.4, 41.7. ESI-HRMS calcd for C$_{22}$H$_{25}$O$_4$N$_4$ClF$_3$ [M + H]$^+$ 501.1511; found 501.1521.

Hydrochloric acid salt of compound 80.

To the solution of compound 80 (0.25 g, 0.5 mmol) in anhydrous methanol (2.0 mL) was added HCl/ethyl acetate (2.0 M, 0.52 mL) at 0 °C and the mixture was stirred for 30 min. The reaction solution was then evaporated *in vacuo* to provide white solid (0.286 g, 100%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.30 (br s, 1H), 11.76 (br s, 1H), 10.85 (s, 1H), 8.58 (s, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.32 (s, 1H), 8.18 (d, J = 8.0 Hz, 1H), 7.86 (br s, 1H), 7.58 (d, J = 9.0 Hz, 1H), 4.57 (t, J = 5.0 Hz, 2H), 4.60–4.25 (m, 7H), 3.99 (t, J = 5.0 Hz, 2H), 2.73 (s, 6H).

Synthesis of compound 83a-f.

Taking 83a as an example: To a solution of 1-(bromomethyl)-4-nitro-2-(trifluoromethyl)benzene (81, 0.65 g, 2.3 mmoL) in anhydrous acetronitrile (6.0 mL) was added 4-(dimethylamino)piperidine (0.27 mL, 2.3 mmoL) and potassium carbonate (0.31 g, 2.3 mmoL). The mixture was stirred at 60 °C for 4 h. After cooling to room temperature, the solvent was removed, and the residue was extracted with ethyl acetate, washed with water and brine in turns, and dried over anhydrous Na$_2$SO$_4$. After filtration and concentration, the obtained residue was purified by flash column chromatography ((eluents 1–5% MeOH in dichloromethane with 0.2% NH$_4$OH$_{(aq)}$)) to yield 82a (0.53 g, 70%). The intermediate was hydrogenated according to general procedure B to afford 83a (0.451 g, yield 95%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.49 (d, J = 7.5 Hz, 1H), 6.90 (s, 1H), 6.79 (d, J = 7.5 Hz, 1H), 3.75 (s, 2H), 3.49 (s, 2H), 2.88 (d, J = 10 Hz, 2H), 2.34 (m, 6H), 2.18 (m, 1H), 1.97 (m, 2H), 1.78 (m, 2H), 1.54 (m, 2H).

Synthesis of 83b was similar to that of compound 83a: compound 81 (0.50 g, 1.77 mmoL) and 3-dimethylaminopyrrolidine (0.26 mL, 2.12 mmoL) and potassium carbonate (0.24 g, 1.77 mmoL) were reacted to yield 82b (0.431 g, 77%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.53 (s, 1H), 8.40 (d, J = 9.0 Hz, 1H), 8.10 (d, J = 9.0 Hz, 1H), 3.89 (m, 2H), 2.86 (m, 1H), 2.74 (m, 3H), 2.55 (m, 1H), 2.25 (s, 6H), 2.04 (m, 1H), 1.83 (m, 1H). The product was hydrogenated according to general procedure B to afford 83b (0.351 g, yield 92%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.42 (d, J = 9.0 Hz, 1H), 6.88 (s, 1H), 6.76 (d, J = 9.0 Hz, 1H), 3.62 (m, 2H), 2.85 (m, 1H), 2.71 (m, 1H), 2.58 (m, 2H), 2.45 (m, 1H), 2.22 (s, 6H), 1.96 (m, 1H), 1.74 (m, 1H).

Synthesis of 83c was similar to that of compound 83a: compound 81 (1.55 g, 5.47 mmoL) and 3-(dimethylamino)azetidine dihydrochloride (0.95 g, 5.47 mmoL) were reacted to afford 3-(trifluoromethyl)-4-(3-dimethylamino)azetidin-1-yl)methyl-nitrobenzene (82c, 1.00 g, 60%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.44 (s, 1H), 8.32 (d, J = 7.0 Hz, 1H), 7.94 (d, J = 7.0 Hz, 1H), 3.88 (s, 2H), 3.55 (t, J = 6.0 Hz, 2H), 2.99 (t, J = 6.0 Hz, 2H), 2.93 (m, 1H), 2.11 (s, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 146.4, 145.5, 130.8, 129.3 (q, J = 26.6 Hz), 126.5, 123.1 (q, J = 227.3 Hz), 121.4 (q, J = 5.0 Hz), 59.9, 59.0, 56.9, 42.1. The product was hydrogenated according to general procedure B at hydrogen gas pressure of 14 psi for 24 h to afford 83c (0.576 g, yield 65%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.32 (d, J = 6.0 Hz, 1H), 6.85 (s, 1H), 6.72 (d, J = 6.0 Hz, 1H), 3.79 (br s, 2H), 3.64 (s, 2H), 3.47 (t, J = 5.0 Hz, 1H), 2.85 (m, 3H), 2.06 (s, 6H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 145.2, 130.9, 128.8 (q, J = 24.7 Hz), 126.3, 124.3 (q, J = 227.1 Hz), 117.7, 112.2 (q, J = 4.9 Hz), 59.6, 58.9, 56.9, 42.0. ESI-HRMS calcd for C$_{13}$H$_{19}$N$_3$F$_3$ [M + H]$^+$ 274.1526; found 274.1525.

Synthesis of compound 83d was similar to that of 83a: compound 81 (0.50 g, 1.76 mmoL) and methyl azetidine-3-carboxylate hydrochloride (0.27 g, 1.76 mmoL) were reacted to afford methyl 1-(4-nitro-2-(trifluoromethyl)benzyl)azetidine-3-carboxylate (82e, 0.274 g, 49%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.51 (s, 1H), 8.37 (d, J = 8.4 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 3.74 (s, 6H), 2.51 (s, 4H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 146.7, 145.4, 131.7, 130.2, 129.4 (q, J = 32.0 Hz), 126.6, 123.1 (q, J = 272.7 Hz), 121.4 (q, J = 7.2 Hz), 58.6, 57.3, 52.2, 34.1. The product was hydrogenated according to general procedure B to afford 83e (0.158 g, yield 64%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.44 (d, J = 7.0 Hz, 1H), 6.93 (d, J = 1.5 Hz, 1H), 6.83 (d, J = 6.5 Hz, 1H), 3.88 (s, 2H), 3.79 (t, J = 7.0 Hz, 2H), 3.71 (s, 3H), 3.58 (t, J = 6.5 Hz, 2H), 3.45 (m, 1H).

Synthesis of compound 83e was similar to that of 83a: compound 81 (0.30 g, 1.16 mmoL) and morpholine (0.11 mL, 1.27 mmoL)

were reacted to afford 3-(trifluoromethyl)-4-(morpholinmethyl)-nitrobenzene (82e, 0.260 g, 77%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.51 (s, 1H), 8.37 (d, J = 8.4 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 3.74 (s, 6H), 2.51 (s, 4H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 146.7, 145.4, 131.7, 130.1 (q, J = 31.8 Hz), 126.5, 123.2 (q, J = 272.7 Hz), 121.6 (q, J = 6.0 Hz), 67.1, 58.3, 53.8. The product was hydrogenated according to general procedure B to afford 83e (0.150 g, yield 64%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.48 (d, J = 8.4 Hz, 1H), 6.92 (s, 1H), 6.78 (dd, J = 8.4, 1.8 Hz, 1H), 3.77 (s, 2H), 3.69 (s, 4H), 3.52 (s, 2H), 2.44 (s, 4H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 145.3, 132.1, 129.7 (q, J = 29.7 Hz), 126.4, 124.5 (q, J = 272.4 Hz), 121.6 (q, J = 6.0 Hz), 67.2, 58.4, 53.7. ESI-HRMS calcd for C$_{12}$H$_{16}$N$_2$F$_3$ [M + H]$^+$ 261.1209; found 261.1209.

Synthesis of compound 83f was similar to that of 83a: compound 81 (0.30 g, 1.16 mmoL) and 2,6-dimethylmorpholine (0.14 mL, 1.16 mmoL) were reacted to afford 3-(trifluoromethyl)-4-(3,5-dimethylmorpholin)-nitrobenzene (82f, 0.270 g, 73%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.46 (s, 1H), 8.34 (d, J = 8.4 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 3.71 (m, 4H), 2.62 (d, J = 11.4 Hz, 2H), 1.88 (t, J = 10.4 Hz, 2H), 1.12 (d, J = 6.6 Hz, 6H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 146.6, 145.6, 131.7, 129.9 (q, J = 31.9 Hz), 126.5, 123.2 (q, J = 272.7 Hz), 121.4 (q, J = 6.0 Hz), 71.8, 59.5, 57.8, 19.1. The product was hydrogenated according to general procedure B to afford 83f. ESI-HRMS calcd for C$_{14}$H$_{20}$ON$_2$F$_3$ [M + H]$^+$ 289.1522; found 289.1522.

Synthesis of compound 86 was similar to that of 83a: 2-(bromomethyl)-5-nitropyridine (84, 0.43 g, 2.0 mmoL) and 4-methylpiperazine (0.22 mL, 2.0 mmoL) were reacted to afford 5-nitro-2-((4-methylpiperazin-1-yl)methylpyridine (85, 0.315 g, 67%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.48 (d, J = 2.4 Hz, 1H), 8.37 (dd, J = 8.4, 1.8 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 3.77 (s, 2H), 2.55 (m, 4H), 2.45 (m, 4H), 2.28 (s, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 150.0, 146.9, 130.9, 128.1, 127.2, 115.6, 114.2, 60.0, 55.0, 53.2, 46.0. The product was hydrogenated according to general procedure B to afford 86 which was directly used without further purification.

Synthesis of compound 88 was similar to that of 83a: 2-chloro-5-nitropyridine (0.50 g, 3.16 mmoL) and 4-methylpiperidine (0.37 mL, 3.16 mmoL) were reacted to afford 5-nitro-2-((4-methylpiperidin-1-yl)pyridine (87, 0.607 g, 87%). ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.03 (s, 1H), 8.17 (dd, J = 9.5, 2.5 Hz, 1H), 6.55 (d, J = 9.5 Hz, 1H), 4.49 (d, J = 10.0 Hz, 2H), 3.00 (t, J = 12.5 Hz, 2H), 1.78 (m, 2H), 1.20 (m, 3H), 0.98 (d, J = 6.5 Hz, 3H). The product was hydrogenated according to general procedure B to afford 88 which was directly used without further purification. ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.77 (s, 1H), 6.95 (d, J = 9.0 Hz, 1H), 6.57 (d, J = 9.5 Hz, 1H), 3.99 (d, J = 12.0 Hz, 2H), 2.69 (t, J = 11.5 Hz, 2H), 1.70 (m, 2H), 1.52 (s, 1H), 1.25 (m, 2H), 0.93 (d, J = 6.0 Hz, 3H).

4.2. Biological assay

4.2.1. Cell culture and HCV infection
Human liver cell line Huh7.5 cells and the plasmid pFL-J6/JFH/JC1 containing the full-length chimeric HCV complementary DNA (cDNA) were kindly provided by Vertex Pharmaceuticals Inc. (Boston, USA) Huh7.5 cells were cultured in Dulbecco's modified eagle medium (DMEM, Invitrogen, CA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen) and 1% penicillin–streptomycin (Invitrogen). Cells were digested with 0.05% trypsin-ethylene diamine tetraacetic acid (EDTA) and split twice a week. HCV virus stock was prepared and used to infect native Huh7.5 cells at an infective dose of 45 IU/cell as described previously [29].

4.2.2. Agents
Telaprevir (HY-10235, VX-950) was purchased from the MedChemExpress (Princeton, NJ). The pAbs to glyceraldehyde 3-

phosphate dehydrogenase (GAPDH) (10494-1-AP) were from Protein Tech Inc. All test compounds were dissolved supplied in DMSO at 10 mM and then diluted in Dulbecco's modified Eagle's medium culture medium. For EC$_{50}$ and CC$_{50}$ determinations, test compounds were serially diluted in eight steps of 1:5 dilutions in 96-well plates.

4.2.3. Anti-HCV activity assay in vitro [37]
Huh7.5 cells were seeded into 96-well or 6-well plates (Costar) at a density of 3 × 10^4 cells/cm^2. After 24 h of incubation, the cells were infected with HCV viral stock (recombination virus strain J6/JFH/JC, 45 IU/cell) and simultaneously treated with different concentration of compounds or solvent control. The culture medium was removed after 72 h of incubation, and the intracellular total RNA (in 96-well plates) was extracted with RNeasy Mini Kit (Qiagen) and quantified with qRT-PCR. It was performed on a 7500 Fast Real-Time PCR system (Applied Biosystems, Singapore) using an AgPath-ID One-Step RT-PCR Kit (Applied Biosystems, Foster, CA, USA) according to the manufacturer's instructions. All quantifications were normalized to the level of the internal control gene, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), the levels of HCV RNA were analyzed with the 2$^{-\triangle\triangle CT}$ method, and a value of half maximal effective concentration (EC$_{50}$) was calculated with the Reed-Muench Method.

4.2.4. Cytotoxicity assay
Huh7.5 cells were seeded into 96-well plates (Costar) at a density of 3.0 × 10^4 cells/cm^2. After 24 h of incubation, fresh culture medium containing test compounds at various concentrations were added. Cytotoxicity was evaluated with the tetrazolium-based MTT assay at 96 h.

4.2.5. Aqueous solubility determination of compound 80 and its hydrochloride salt
Hydrochloride salts of compound 80 were added to distilled water (1.0 mL). After shaking for 1.0 h at 25 °C and then centrifuging at 3000 rpm for 10 min, the saturated supernatants were measured the volume and then lyophilized to determine the concentration dissolved in water. For compound 80, the saturated supernatants were transferred to other vials for analysis by HPLC-UV. Each sample was performed in triplicate. For quantification, a model 1200 HPLC-UV (Agilent) system was used with an Agilent TC-C18 column (250 × 4.6 mm, 5 μm) and elution of 2 mM HCO$_2$NH$_4$/methanol-water (95:5). The flow rate was 1.0 mL/min and injection volume was 10 μL with the detection wavelength at 254 nm. Aqueous concentration was determined by comparison of the peak area of the saturated solution with a standard curve plotted peak area versus known concentrations, which were prepared by solutions of test compound in methanol at 135.0, 45.0, 15.0, 5.0, and 2.5 μg/mL.

In vivo toxicity, *In vitro* and *In vivo* pharmacokinetic, and Compounds protecting hA3G from Vif-mediated degradation assessment methods. See supporting information. All *in vivo* studies were in accordance with the Animal Care and Use Committee of People's Republic of China.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.114033.

Abbreviations Used

EDCI *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride
DMAP 4-dimethylaminopyridine
TEA triethylamine
THF tetrahydrofuran
DEAD diethylazodicarboxylate
PPh₃ triphenylphosphine
MeOH methanol
DMF dimethyl formamide
rt room temperature
EtOAc ethyl acetate
NBS *N*-bromosuccinimide
AIBN azobisisobutyronitrile
qRT-PCR real-time quantitative reverse-transcription polymerase chain reaction
MTT 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium
ip intraperitoneal
NMR nuclear magnetic resonance
TMS trimethylsilance
HRMS high resolution mass spectrometry
min minutes
h hours

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