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Synthesis of *N*-benzyl-*N*-phenylthiophene-2-carboxamide Analogues as a Novel Class of Enterovirus 71 Inhibitors

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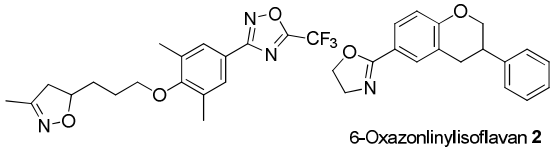
A series of novel human enterovirus 71 inhibitors, *N*-benzyl-*N*-phenylthiophene-2-carboxamide analogues were synthesized and their antiviral activities were evaluated in vitro. Most derivatives of this structure against EV71 with a low micromolar range in the RD (Rhabdomyosarcoma) cell lines. The most potent compound 5a, *N*-(4-bromobenzyl)-*N*-(4-fluorophenyl)thiophene-2-carboxamide, showed low micromolar activity against EV71 (EC_{50} = 1.42 μM) compared to the reference anti-EV71 drug Enviroxime (EC_{50} = 0.15 μM). Preliminary SAR studies revealed that thiophene-2-carboxamide core is crucial for maintaining antiviral activity, and *N*-substituent phenyl groups largely influenced anti-EV71 efficacy of this new class of potent antiviral agents.

Introduction

Human enterovirus 71 (EV71) was first isolated from the feces of infants suffering from encephalitis in 1969, which belongs to the genus enterovirus of the Picornaviridae family.[1, 2] EV71 is one of the major causative agents of hand, foot, and mouth disease (HFMD) that may progress to fatal encephalitis in infants and kids[3]. Mature EV71 virion consists of a single strand of positive-sense RNA and symmetry icosahedral virus capsid with four coat proteins VP1, VP2, VP3, and VP4. Most of the attachment and neutralization sites are densely clustered on VP1[4], and variations of capsid proteins VP1 to VP3 are responsible for the antigenic diversity among those types of enteroviruses.[5-7] Mostly, the symptoms of EV71 infection are similar extremely with Coxsackie A16 (CA16), another main infection factor of HFMD, showing herpangina infection and mild fever. Furthermore, EV71 infection always associate with severe fatal central nervous system diseases, such as acute encephalitis, polio-like acute flaccid paralysis and neurogenic cardiopulmonary failure.[8-10] Generally, children and infants are considered to be relatively immunodeficient, hence, prone to infect EV71 that can be lethal and life threatening, with high risk for morbidity and mortality.

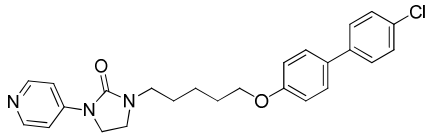
Since April 1998, a major outbreak of EV71 infection caused many severe cases and almost 80 fatalities in Taiwan[11] that concerns a serious public health and highlights the urgency and significance for developing novel anti-enteroviral agents. Because of the genetic heterogeneity and prevalent multiple serotypes, progress of antiviral agents against regional enterovirus infection is therefore very urgent.[12] So far, there are no effective anti-EV71 agents available for preventing or treating enterovirus infection[13, 14] including pleconaril (Figure 1, **1**), a candidate drug for inhibiting viral infection potently showed broad spectrum activity against enterovirus which was developed by Viropharma Inc in 1966.[15] Unfortunately, it has limited activity against EV71 that could not neutralize the cytopathic effect (CPE) in vitro assay.[16, 17] Therefore, using the skeletons of pleconaril (Figure 1), termed WIN compounds,[12] as a template to rational design, synthesis, modification and structure activity relationship (SAR) studies led to the development of many novel classes of significant anti-enteroviral compounds. For all of those, there are only three kinds of distinguished small molecules, including pleconaril that possess anti-EV71 activity, as shown in Figure 1 (compounds **1**-**3**).[18-20]

Figure 1. The anti-enterovirus 71 compounds



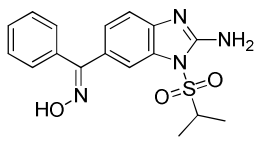
Pleconaril **1**

6-Oxazonlinylisoflavan **2**
EC_{50} = 4.65 μM

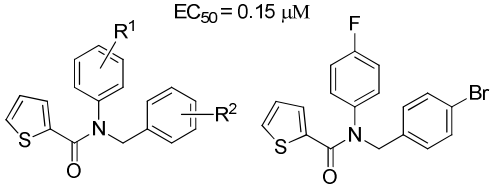


Imidazolidinone derivatives **3**
EC_{50} = 0.038 μM

Figure 2. General structure of Envrioxime **4** and thiophenecarboxamide analogues



Envrioxime **4**
EC_{50} = 0.15 μM



Thiophene-carboxamides **5** Lead Compound **5a**

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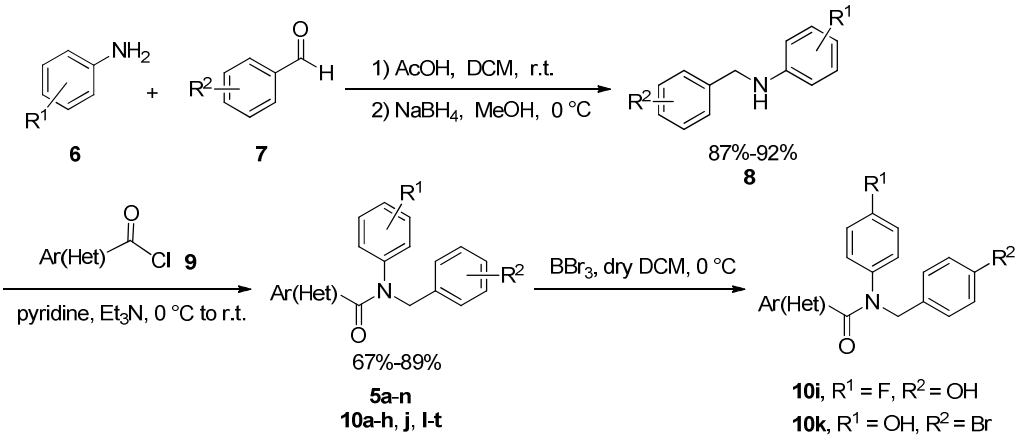
As a part of our continuous efforts to develop anti-virus agents[21-24], herein, a series of novel small molecules within thiophene-carboxamide core, different from the classical core structures of anti-EV71 compounds (As shown in Figure 1)[18-20, 25, 26], were developed and assayed for antiviral activity against EV71. Preliminary screening showed that lead compound **5a** has considerable potency relative to the drug compound Enviroxime **4**, a broad spectrum of activity against both human rhinoviruses and enteroviruses,[27-31] which were tested in low micromolar range (Figure 2). Our SAR studies revealed that aryl substituent analogues at the *para* position, such as compound **5a**, **5b**, and **5f** in generally exhibited excellent efficacy against lethal EV71. These encouraging results prompt us to further ascertain this class of novel compounds and details of investigation described herein.

Chemistry and Synthesis.

After considerable efforts of anti-EV71 agents discovery by compounds screening, we finally find some typical scaffolds showed potent antiviral activities including these thiophene carboxamide core compounds. Currently, we mainly focused on the modification of aromatic and heterocyclic moieties based on the peptide link to establish the requirements for optimum activity. Several methods were adopted to synthesize analogues in this series depended on the substitution pattern. Preparation of intermediate *N*-benzylaniline **8** is outlined in Scheme 1 by reductive amination process using substituted benzaldehydes **7** with anilines **6**. Further trials find that equivalent acetic acid increasing product yields dramatically.[32] Followed by substitution reaction with aryl(het)carbonyl chlorides **9** under triethylamine and pyridine to provide target compounds **5** and **10** in good to excellent yields (Scheme 1). Demethylation of **10h** and **10j** with BBr_3 under Ar atmosphere to give phenol derivatives **10i** and **10k**, respectively.[33]

Scheme 1. The synthetic route for the target compounds



Results and Discussion

A structure activity study was mainly performed by maintaining the three aromatic rings and varying the substituent groups with bioisostere relationship. The carboxamide derivatives described herein were tested anti-EV71 in a CPE reduction assay (EC_{50}) as well as cytotoxicity evaluation (CC_{50}) in the RD cell lines under a standard procedure. These results are shown in Table 1 and compared to the reference anti-enterovirus drug Enviroxime. As shown in Table 1, these compounds exhibit significant activities against human enterovirus 71. Initially screened compounds **5a** demonstrated a highest level of activity against EV71 (EC_{50} = 1.42 μM) with modest cytotoxicity. Enviroxime tested in this strain as comparison showed EC_{50} value of 0.15 μM. It is very interesting to note that replacement of thiophene in compound **5a**, by furan (**5b**) only get imperceptible changes in potency (EC_{50} = 1.50 μM) (Table 1, entries 1 and 2). However, replacement with benzene ring (**5c**) led to a considerable loss in activity (entry 3). Introducing chloro on 5-position of thiophene ring (**5d** and **5e**) resulted in dramatically reduced or losing activity (entries 4 and 5), probably due to the interaction pocket is not large enough to occupy in the shallow canyon space,[34-36] this observation was also supported by the binding mode of **5a** with rhinoviruses (RV) protease (see **Figure 3** below). These significant results demonstrated that the five-membered aromatic heterocycle seems to be one of the important roles in maintaining the anti-EV71 activity. It is noted that the mono substituent of *ortho* (**5n**), *para* (**10l**)-position on the benzyl ring or 2,4-disubstituted (**5h** and **5m**) also showed absolute no activity, as shown in Table 1 (entries 8, 13, 14 and 26). For example, compound **10e** and **10g** with more electron-withdrawing cyano even nitro group located at the *para*-position were found to be much less of activity (entries 19 and 21). No substituted compound (**5g**) appears to be lower EC_{50} value than the corresponding disubstituted *p*-chloro (**5l**) and *p*-fluoro (**10a**) compounds (entries 7, 12 and 15). Electron-donating group, such as methoxyl group (**10h**) at this position in this series of compounds resulted in a double decrease in activity (EC_{50} = 3.25 μM) than that of lead compound (entry 22). Whereas demethylated product (**10i**) with a more hydrophilic and polar hydroxyl group showed a dramatic decrease in activity against EV71 (entry 23). These observations provide remarkable evidence that the hydrophobic interaction and occupied space largely influence anti-EV71 activity of these novel thiophene-carboxamide compounds. Next, the effect of substitution on phenyl ring with respect to antiviral activity was examined. Most compounds shown that introducing different groups on this ring also exhibited moderate to good potency, however, seemed less effect on activity than the benzyl ring. For example, compound **5f**, **10b** and **10f** with no substituted or *para* bromo or chloro, with EC_{50} values of 1.79 μM, 4.92 μM and 3.27 μM, respectively

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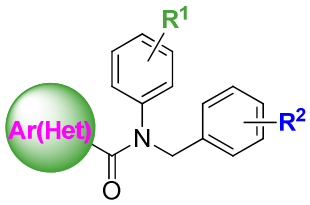
(entries 6, 16 and 20). Replacement of fluoro group by a methyl group at *p*-position resulted in a slightly higher efficacy with **5i** and **5j** in contrast (entries 9 and 10). More electron-withdrawing group such as trifluoromethyl at 3-position (**10d**) resulted in decreased activity against EV71 (EC_{50} = 15 μM) when compared to the methyl analogue (**10c**) at same position (EC_{50} = 5.38 μM) possibly due to the electronic effect (entries 17 and 18). Methoxyl substituted compound (**10j**) exhibited activity against EV71 of EC_{50} value up to 8.3 μM, while demethylation to hydroxyl group (**10k**) resulted in a loss of activity (entries 24 and 25). Therefore, the interaction site appears to be a hydrophobic pocket VP1 that is located under the canyon floor.[37-39] In addition, prolonged linkage to benzyl ring of compound **10m** without loss activity (EC_{50} = 8.54 μM), although almost 5-fold less than that of compound **5f** (entries 6 and 27). This interesting discovery provides more envision to disclosing the binding interaction of virion with this novel class of molecules. Further SAR studies revealed that aryl substituent analogues at the *para* positions, such as compound **5a**, **5b** and **5f** generally exhibited better efficacy against lethal EV71 than that of *ortho/meta* positions compounds **10n-t**. For example, in the case of compound **5a**, when changed fluoro group from *para*-position to *ortho* or *meta* position of phenyl ring (**10n** and **10o**), deceased antiviral activities were observed (entries 1 vs 28 and 29), similar trends were also observed for analogues of compound **5f**, in which the *para* bromo on the phenyl ring of benzyl group was moved to *ortho* or *meta* position (**10p** and **10q**), no obvious antiviral activities were observed (entries 3 vs 30 and 31). For analogues of furan compound **5b**, **10r** and **10s**, which have both *ortho* (or *meta*) positions of benzene ring and benzyl group substituted, the antiviral activities also deceased dramatically (entries 2 vs 32 and 33). No activity was observed for both *ortho* substituted analogue (**10t**) of carboxamide compounds **5a** (entries 1 vs 34). Above all, with thiophene-2-carboxamide core compounds especially **5a** are well qualified to serve as lead compounds for the further development of anti-EV71 agents (entry 1).

Table 1. Antiviral Activity and Cellular Toxicity against EV-71[a]



Entry	Compd	Ar (Het)	R^1	R^2	EC_{50} (μM)[b]	CC_{50} (μM)[c]	SI[d]
1	**5a**	2-thiophene	4-F	4-Br	1.42	11.17	7.9
2	**5b**	2-furan	4-F	4-Br	1.50	57.19	38.1
3	**5c**	phenyl	4-F	4-Br	NA[f]	110.87	--
4	**5d**	5'-Cl-2-thiophene	4-F	4-Br	NA	17.8	0.1
5	**5e**	5'-Cl-2-thiophene	4-F	4-F	NA	130.01	--
6	**5f**	2-thiophene	H	4-Br	1.79	66.35	37.1
7	**5g**	2-thiophene	H	H	2.31	29.76	12.9
8	**5h**	2-thiophene	4-F	2-Br-4-F	NA	22.34	--
9	**5i**	2-thiophene	4-F	4-Cl	2.41	30.65	12.7
10	**5j**	2-thiophene	4-CH_3	4-Cl	2.28	36.27	15.9
11	**5k**	2-thiophene	H	4-Cl	6.77	71.68	10.6
12	**5l**	2-thiophene	4-Cl	4-Cl	4.61	30.92	6.7
13	**5m**	2-thiophene	4-Cl	2-Br-4-F	NA	18.04	--
14	**5n**	2-thiophene	4-Cl	2-Br	NA	114.08	--
15	**10a**	2-thiophene	4-F	4-F	9.84	28.33	2.9
16	**10b**	2-thiophene	4-Br	4-Br	4.92	18.51	3.8
17	**10c**	2-thiophene	3-CH_3	4-Br	5.38	24.67	4.6
18	**10d**	2-thiophene	3-CF_3	4-Br	15.0	18.72	1.3
19	**10e**	2-thiophene	4-F	4-NO_2	18.99	>224	>11.8
20	**10f**	2-thiophene	4-Cl	4-Br	3.27	107.45	32.9
21	**10g**	2-thiophene	4-F	4-CN	4.96	>237	>47.8
22	**10h**	2-thiophene	4-F	4-OCH_3	3.25	50.38	15.5
23	**10i**	2-thiophene	4-F	4-OH	NA	78.50	--
24	**10j**	2-thiophene	4-OCH_3	4-Br	8.30	39.03	4.7
25	**10k**	2-thiophene	4-OH	4-Br	NA	>206	--
26	**10l**	2-thiophene	4-F	3-Br	NA	36.29	--
27	**10m**[e]	2-thiophene	Benzyl	4-Br	8.54	11.442	1.4
28	**10n**	2-thiophene	3-F	4-Br	8.56	68.13	8.0
29	**10o**	2-thiophene	2-F	4-Br	4.23	23.73	5.6
30	**10p**	2-thiophene	H	2-Br	>100	>200	--
31	**10q**	2-thiophene	H	3-Br	>50	88.4	--
32	**10r**	2-furan	2-F	2-Br	>50	59.8	--
33	**10s**	2-furan	3-F	3-Br	>100	>200	--
34	**10t**	2-thiophene	3-F	3-Br	>100	>200	--

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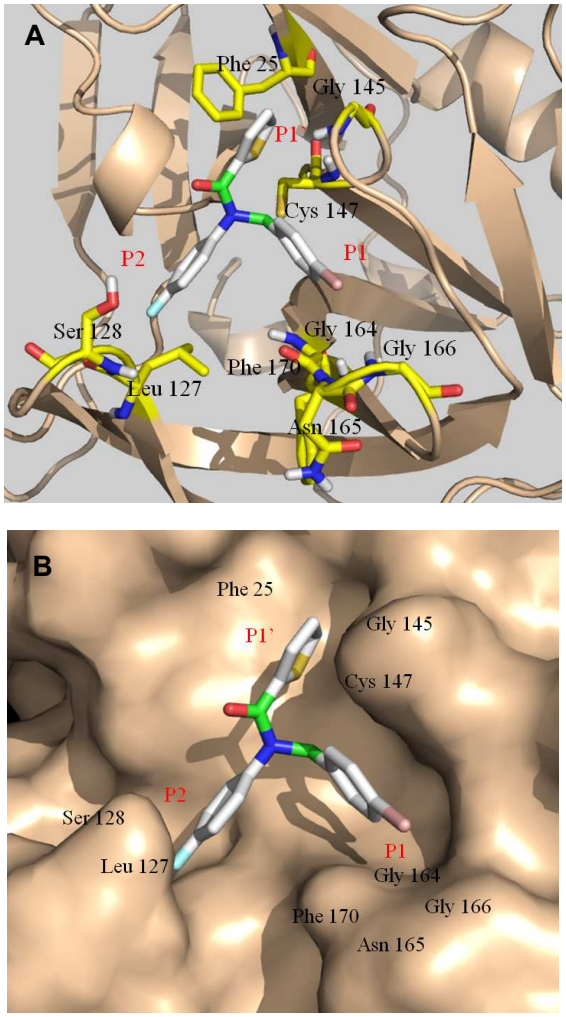
35	**4**	Enviroxime	--	--	0.15	>223.205	>1488

[a] All data are obtained for at least three independent experiments; [b] EC_{50}, concentration which inhibited virus plaque formation by 50%. [c] CC_{50}, concentration which inhibited cell growth by 50% as compared with control cultures. [d] Selectivity Index (SI) was determined for the effective compounds dividing CC_{50} by IC_{50}. [e] Compound **10m** is specially referred as *N*-benzyl-*N*-(4-bromobenzyl)thiophene-2-carboxamide. [f] NA: No Activity (EC_{50} > 100 μM).

Because the lack of the complex crystal structure of protein and carboxamide compound, the binding mode of **5a** was studied by the docking experiments into the EV71 protease. The docking experiments were carried out based on the published crystal structure of rhinoviruses (RV) protease (pdb:1CQQ)[40-41] with AG7088. AutoDock 4.2 was chosen to study the binding modes of **5a** and the results showed good binding score (-6.07 kcal mol^{-1}). The compound **5a** showed a similar binding mode to that of AG7088 in the same protein pocket, with benzyl at P1, thiophene at P1' and benzene at P2, respectively (**Figure 3A**). The surface type in **Figure 3B** can clear show the interaction of **5a** with protein. Especially, the phenyl ring group enters into the Leu127-Ser128 pocket forming a firm interaction. The benzyl group and thiophene enter into the pocket formed by Gly164-Asn165-Gly166-Phe170 and Phe25-Gly145-Cys147, respectively.

Figure 3. (**A**) Computer modeling of the complex structures of EV71 protease with **5a** based on the published structure (PDB:1CQQ) of RV protease with AG7088; (**B**) The surface type between **5a** with EV71 protease (The oxygen atoms are shown in red and nitrogen atoms are shown in blue).



Conclusion

In summary, we have firstly developed an efficient synthesis of thiophene-carboxamides as novel potent anti-EV71 agents. According to the SARs, we successfully explored efficacy of different substituents on the basis of lead compound *N-(4-bromobenzyl)-N-(4-fluorophenyl)thiophene-2-carboxamide* **5a**. Most of these serial compounds showed comparable anti-EV71 activity and low cell cytotoxicity than that of drug Enviroxime. Further mechanistic studies on this new class of antiviral compounds are currently under zealous research and will be reported in a due course.

Experimental Section

Unless otherwise noted, reagents and chemicals were obtained from commercial available suppliers and used without further purification. Dichloromethane (DCM) was distilled and dried over anhydrous $CaCl_2$ and methanol was freshly distilled from Na. All reactions were conducted in common conditions only when specially referred. Reaction progress was monitored by analytical thin-layer chromatography (TLC) and visualized by ultraviolet light (254 nm). ^1H NMR and ^{13}C NMR spectra were detected by a Bruker Biospin AV400 (400 MHz) instrument. The chemical shifts were reported in parts per million (ppm) and were referenced to either tetramethylsilane or the solvent. Residual proton solvent signals (for $CDCl_3$, δ 7.26 ppm, acetone, δ 2.05 ppm, respectively). ^1H NMR spectra were reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad), integration, coupling constant (Hz). ^{13}C NMR spectra were reported as chemical shifts in ppm and multiplicity where appropriate. High Resolution Mass spectra were obtained from Shanghai Mass Spectrometry Center. Melting points were determined by X-4 Beijing Tech melting point apparatus and were uncorrected.

General procedure for reductive amination. 4-Bromo benzaldehyde (100 mg, 1 eq.), 4-fluoroaniline (90.08 mg, 1.5 eq.) and acetic acid (48.68 mg, 1.5 eq.) were placed into a round-bottom flask (50 mL) sequentially, and 20 mL dry DCM were added then stirred at room temperature for 8 h to yield intermediate corresponding imine. Removing the solvent by rotary evaporator under reduced pressure and then dissolved in anhydrous methanol (20 mL), solid $NaBH_4$ (204.5 mg 10 eq.) were carefully added at ice-bath, then allowed to stirred at room temperature until raw material point disappear. After evaporating solvent, 20 mL NaOH (5% M) solution were added to quench the reaction mixture and DCM was used to extract the aqueous layer, then combined organic layers and dried by anhydrous Na_2SO_4, filtered and concentrated in vacuum. The crude product was purified by silica gel column chromatography.

General procedure for the preparation of amides. The formyl chloride (0.45 mmol, 1.5 eq.) was added in one portion to a solution of the amine (0.3 mmol, 1 eq.) with dry dichloromethane (20 mL), then Et_3N (3 mmol, 10 eq.) and pyridine (0.45 mmol, 1.5 eq.) were injected into reaction mixture formed white smoke rapidly. The solution was stirred for overnight at room temperature and evaporate solvent and residual Et_3N, then diluted with H_2O, extracted with DCM, dried by anhydrous Na_2SO_4,

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filtered and concentrated in vacuum, purified with silica gel column chromatography.

***N*-(4-bromobenzyl)-*N*-(4-fluorophenyl)thiophene-2-carboxamide (5a).** White solid (87% yield), mp 116.8-118.9 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.3 Hz, 2H), 7.34 (dd, *J* = 4.4, 1.5 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 2H), 7.07–6.98 (m, 4H), 6.86–6.79 (m, 2H), 4.97 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.55, 162.49, 161.07, 138.17, 138.13, 137.47, 135.94, 132.82, 131.66, 131.28, 130.95, 130.86, 130.79, 126.84, 121.72, 116.87, 116.64, 54.08. HRMS (ESI) calcd for $C_{18}H_{13}BrFNOSH$ $[M+H]^+$ 389.9964, found 389.9955.

***N*-(4-bromobenzyl)-*N*-(4-fluorophenyl)furan-2-carboxamide (5b).** White solid (89% yield),, mp 110.5-112.2 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 1.0 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 2H), 7.07–6.94 (m, 4H), 6.23 (dd, *J* = 3.5, 1.7 Hz, 1H), 5.88 (d, *J* = 2.3 Hz, 1H), 4.96 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.26, 160.79, 159.13, 146.64, 144.77, 138.07, 138.05 135.84, 131.64, 130.81, 130.25, 130.16, 121.73, 117.03, 116.65, 116.42, 111.17, 53.50. HRMS (ESI) calcd for $C_{18}H_{13}BrFNO_2H$ $[M+H]^+$ 374.0192, found, 374.0189.

***N*-(4-bromobenzyl)-*N*-(4-fluorophenyl)benzamide (5c)**. White solid (89% yield), mp 119.8-121.8 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.1 Hz, 2H), 7.32-7.14 (m, 7H), 6.84 (d, *J* = 6.3 Hz, 4H), 5.04 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.59, 162.20, 159.74, 139.16, 139.14, 136.24, 135.47, 131.69, 130.39, 129.89, 129.51, 129.42, 128.65, 127.90, 121.59, 116.22, 116.00, 53.33. HRMS (ESI) calcd for $C_{20}H_{15}BrFNOSH$ $[M+H]^+$ 384.0399, found, 384.0397.

***N*-(4-bromobenzyl)-5-chloro-*N*-(4-fluorophenyl)thiophene-2-carboxamide (5d).** Yellow solid (75% yield), mp 79.7-82.5 ºC.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.6 Hz, 2H), 7.19–6.95 (m, 6H), 6.69 (d, *J* = 12.5 Hz, 2H), 4.93 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.80, 161.31, 137.41, 137.38, 136.63, 135.65, 135.43, 132.75, 131.71, 131.14, 131.05, 130.81, 126.19, 121.86, 117.14, 116.91, 54.13. HRMS (ESI) calcd for $C_{18}H_{12}BrClFNOSH$ $[M+H]^+$ 423.9574, found, 423.9565.

***N*-(4-fluorobenzyl)-5-chloro-*N*-(4-fluorophenyl)thiophene-2-carboxamide (5e).** Yellow solid (79% yield), mp 78.6-82.7 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.10–7.03 (m, 2H), 7.02-6.94 (m, 4H), 6.72-6.65 (m, 2H), 4.95 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.30, 161.25, 137.41, 137.37, 136.55, 135.57, 132.68, 132.47, 132.44, 131.21, 131.12, 130.92, 130.84, 126.17, 117.07, 116.85, 115.55, 115.34, 53.97. HRMS (ESI) calcd for $C_{18}H_{12}ClF_2NOSH$ $[M+H]^+$ 364.0371, found, 364.0366.

***N*-(4-bromobenzyl)-*N*-phenylthiophene-2-carboxamide (5f).** White solid (83% yield). mp 122.2-124.5 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.4 Hz, 2H), 7.38–7.30 (m, 4H), 7.17 (d, *J* = 8.3 Hz, 2H), 7.08-7.03 (m, 2H), 6.78 (dd, *J* = 4.9, 4.0 Hz, 1H), 6.72 (dd, *J* = 3.8, 1.1 Hz, 1H), 5.00 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.52, 142.29, 137.94, 136.19, 132.55, 131.56, 131.06, 130.75, 129.73, 129.05, 128.55, 126.78, 121.55, 54.09. HRMS (ESI) calcd for $C_{18}H_{14}BrNOSH$ $[M+H]^+$ 372.0058, found 372.0049.

***N*-benzyl-*N*-phenylthiophene-2-carboxamide (5g).** White solid (86% yield), mp 101.8-104.7 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.23 (m, 9H), 7.09-7.04 (m, 2H), 6.78 (dd, *J* = 4.9, 3.9 Hz, 1H), 6.72 (dd, *J* = 3.8, 0.9 Hz, 1H), 5.06 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.46, 142.52, 138.26, 137.18, 132.42, 130.89, 129.60, 129.11, 128.94, 128.44, 128.39, 127.50, 126.74, 54.67. HRMS (ESI) calcd for $C_{18}H_{15}NOSH$ $[M+H]^+$ 294.0953, found 294.0943.

***N*-(2-bromo-4-fluorobenzyl)-*N*-(4-fluorophenyl)thiophene-2-carboxamide (5h).** White solid (67% yield), mp 87.1-88.7 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 8.6, 6.1 Hz, 1H), 7.36 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.23 (dd, *J* = 8.2, 2.6 Hz, 1H), 7.10–6.97 (m, 5H), 6.91–6.81 (m, 2H), 5.17 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.64, 162.88, 162.63, 161.16, 160.38, 137.83, 137.80, 137.29, 133.00, 132.11, 132.07, 131.80, 131.71, 131.42, 130.96, 130.87, 126.88, 124.14, 124.04, 120.06, 119.82, 116.82, 116.59, 115.12, 114.91, 53.11. HRMS (ESI) calcd for $C_{18}H_{12}BrF_2NOSH$ $[M+H]^+$ 407.9869, found 407.9850.

***N*-(4-chlorobenzyl)-*N*-(4-fluorophenyl)thiophene-2-carboxamide (5i).** White solid (80% yield), mp 108.0-109.2 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (dd, *J* = 4.2, 1.2 Hz, 1H), 7.30–7.18 (m, 4H), 7.07-6.99 (m, 4H), 6.87–6.79 (m, 2H), 4.98 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.55, 162.49, 161.07, 138.17, 138.14, 137.49, 135.43, 133.56, 132.81, 131.26, 130.96, 130.87, 130.45, 128.70, 126.82, 116.85, 116.62, 54.01. HRMS (ESI) calcd for $C_{18}H_{13}ClFNOSH$ $[M+H]^+$ 346.0469, found 346.0461.

***N*-(4-chlorobenzyl)-*N*-(p-tolyl)thiophene-2-carboxamide (5j).** White solid (83% yield), mp 111.6-114.2 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (dd, *J* = 4.9, 1.1 Hz, 1H), 7.26-7.21 (m, 4H), 7.14 (d, *J* = 8.0 Hz, 2H), 6.93 (d, *J* = 8.2 Hz, 2H), 6.82-6.76 (m, 2H), 4.98 (s, 2H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.54, 139.55, 138.66, 137.98, 135.79, 133.31, 132.65, 131.06, 130.45, 130.36, 128.81, 128.57, 126.77, 54.07, 21.26. HRMS (ESI) calcd for $C_{19}H_{16}ClFNOSH$ $[M+H]^+$ 342.0719, found 342.0719.

***N*-(4-chlorobenzyl)-*N*-phenylthiophene-2-carboxamide (5k).** White solid (88% yield), mp 87.4-89.5 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.30 (m, 4H), 7.24 (d, *J* = 2.7 Hz, 3H), 7.20 (dd, *J* = 4.9, 3.9 Hz, 1H), 7.09–7.02 (m, 2H), 6.78 (dd, *J* = 4.9, 3.9 Hz, 1H), 6.73 (dd, *J* = 3.8, 1.1 Hz, 1H), 5.01 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.59, 142.23, 137.86, 135.63, 134.92, 133.91, 133.39, 132.67, 131.15, 130.43, 129.74, 129.07, 128.61, 128.09, 126.80, 54.06. HRMS (ESI) calcd for $C_{18}H_{14}ClNOSH$ $[M+H]^+$ 328.0563, found 328.0557.

***N*-(4-chlorobenzyl)-*N*-(4-chlorophenyl)thiophene-2-carboxamide (5l).** White solid (86% yield), mp 79.4-82.3 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.30 (m, 3H), 7.26-7.19 (m, 4H), 7.02–6.95 (m, 2H), 6.86-6.81 (m, 2H), 4.99 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.50, 140.75, 137.38, 135.31, 134.89, 134.43, 133.86, 133.60, 132.86, 131.32, 130.40, 130.34, 129.98, 128.74, 128.08, 126.92, 53.94. HRMS (ESI) calcd for $C_{18}H_{13}Cl_2NOSH$ $[M+H]^+$ 362.0168, found 362.0164.

***N*-(2-bromo-4-fluorobenzyl)-*N*-(4-chlorophenyl)thiophene-2-carboxamide (5m).** Yellow solid (69% yield), 85.8-87.3 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 8.6, 6.0 Hz, 1H), 7.37 (dd, *J* = 4.9, 1.2 Hz, 1H), 7.33–7.27 (m, 2H), 7.24 (dd, *J* = 8.2, 2.6 Hz, 1H), 7.06–6.97 (m, 3H), 6.90-6.83 (m, 2H), 5.16 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.89, 162.62, 160.40, 140.50, 137.26, 134.50, 132.93, 132.02, 131.98, 131.64, 131.55, 131.40, 130.28, 129.90, 126.93, 124.03, 123.94, 120.11, 119.87, 115.13, 114.92, 53.06. HRMS (ESI) calcd for $C_{18}H_{12}BrClFNOSH$ $[M+H]^+$ 423.9568, found 423.9563.

***N*-(2-bromobenzyl)-*N*-(4-chlorophenyl)thiophene-2-carboxamide (5n).** White solid (70% yield), mp 96.5-99.8 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (dd, *J* = 10.7, 8.4 Hz, 2H), 7.37 (d, *J* = 4.8 Hz, 1H), 7.28 (dd, *J* = 7.5, 5.5 Hz, 3H), 7.12 (t, *J* = 7.1 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 2H), 6.92–6.82 (m, 2H), 5.20 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.63, 140.76, 137.41,

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135.88, 134.32, 132.86, 131.29, 130.22, 130.19, 129.82, 129.14, 127.76, 126.91, 123.88, 53.93. HRMS (ESI) calcd for $C_{18}H_{13}BrClNOSH$ $[M+H]^+$ 405.9663, found 405.9659.

***N*-(4-fluorobenzyl)-*N*-(4-fluorophenyl)thiophene-2-carboxamide (10a).** White solid (81% yield), mp 93.5-95.6 ºC.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (dd, J = 4.3, 1.9 Hz, 1H), 7.28–7.20 (m, 2H), 7.07–6.92 (m, 6H), 6.86-6.80 (m, J = 2H), 4.99 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.53, 162.42, 161.09, 161.05, 138.17, 138.13, 137.61, 132.75, 132.72, 131.21, 131.02, 130.94, 130.88, 130.80, 126.82, 116.80, 116.58, 115.49, 115.28, 53.91. HRMS (ESI) calcd for $C_{18}H_{13}F_2NOSH$ $[M+H]^+$ 330.0759, found 330.0755.

***N*-(4-bromobenzyl)-*N*-(4-bromophenyl)thiophene-2-carboxamide (10b).** White solid (77% yield), mp 125.3-127.1 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49–7.44 (m, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.38–7.33 (m, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.95–6.89 (m, 2H), 6.83 (d, J = 3.1 Hz, 2H), 4.97 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.40, 141.33, 137.42, 135.83, 132.97, 132.77, 131.69, 131.27, 130.71, 130.62, 126.92, 122.43, 121.77, 53.94. HRMS (ESI) calcd for $C_{18}H_{13}Br_2NOSH$ $[M+H]^+$ 449.9157, found 449.9151.

***N*-(4-bromobenzyl)-*N*-(m-tolyl)thiophene-2-carboxamide (10c).** White solid (85% yield), mp 92.6-94.8 ºC.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.3 Hz, 2H), 7.31 (d, J = 4.4 Hz, 1H), 7.24–7.14 (m, 4H), 6.91 (s, 1H), 6.86–6.72 (m, 3H), 4.97 (s, 2H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.43, 142.18, 139.87, 137.99, 136.30, 132.59, 131.51, 131.08, 130.72, 129.44, 129.43, 129.34, 126.77, 126.13, 121.47, 54.15, 21.31. HRMS (ESI) calcd for $C_{19}H_{16}BrNOSH$ $[M+H]^+$ 386.0214, found 386.0209.

***N*-(4-bromobenzyl)-*N*-(3-(trifluoromethyl)phenyl)thiophene-2-carboxamide (10d).** White solid (68% yield), mp 122.7-124.8 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 7.7 Hz, 1H), 7.47-7.39 (m, 4H), 7.36-7.34 (m, 1H), 7.20-7.13 (m, 3H), 6.84-6.80 (m, 2H), 5.02 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.49, 142.94, 137.12, 135.62, 132.84, 132.64, 131.76, 131.40, 130.63, 130.30, 126.90, 125.74, 125.71, 125.18, 125.15, 121.88, 54.00. HRMS (ESI) calcd for $C_{19}H_{13}BrF_3NOSH$ $[M+H]^+$ 439.9932, found 439.9927.

***N*-(4-fluorophenyl)-*N*-(4-nitrobenzyl)thiophene-2-carboxamide (10e).** Yellow solid (83% yield), mp 142.5-145.2 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 8.7 Hz, 2H), 7.48 (d, J = 8.7 Hz, 2H), 7.37 (dd, J = 4.9, 1.3 Hz, 1H), 7.06 (d, J = 6.4 Hz, 4H), 6.90–6.83 (m, 2H), 5.11 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.33, 133.13, 131.63, 130.76, 130.67, 129.66, 126.95, 123.84, 117.12, 116.89, 54.21. HRMS (ESI) calcd for $C_{18}H_{13}BrFN_2O_3SH$ $[M+H]^+$ 357.0704, found 357.0698.

***N*-(4-bromobenzyl)-*N*-(4-chlorophenyl)thiophene-2-carboxamide (10f).** White solid (82% yield), mp 103.6-105.3 ºC.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.3 Hz, 2H), 7.36–7.27 (m, 3H), 7.15 (d, J = 8.3 Hz, 2H), 7.02–6.95 (m, 2H), 6.85-6.80 (m, 2H), 4.97 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.43, 140.79, 137.45, 135.87, 134.40, 132.77, 131.69, 131.27, 130.73, 130.33, 129.98, 126.92, 121.76, 53.98. HRMS (ESI) calcd for $C_{18}H_{13}BrClNOSH$ $[M+H]^+$ 405.9663, found 405.9651.

***N*-(4-cyanobenzyl)-*N*-(4-fluorophenyl)thiophene-2-carboxamide (10g).** White solid (73% yield), mp 132.6-138.8 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 8.2 Hz, 2H), 7.42 (d, J = 8.2 Hz, 2H), 7.36 (dd, J = 4.8, 1.3 Hz, 1H), 7.09-7.01 (m, 4H), 6.89–6.81 (m, 2H), 5.07 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.59, 162.66, 161.11, 142.34, 138.14, 138.11, 137.06, 133.05, 132.40, 131.57, 130.76, 130.68, 129.50, 126.95, 118.69, 117.06, 116.83, 111.56, 54.46. HRMS (ESI) calcd for $C_{19}H_{13}FN_2OSH$ $[M+H]^+$ 337.0806, found 337.0801.

***N*-(4-fluorophenyl)-*N*-(4-methoxybenzyl)thiophene-2-carboxamide (10h).** White solid (71% yield), mp 69.8-75.1 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.29 (m, 1H), 7.18 (d, J = 8.6 Hz, 2H), 7.05–6.96 (m, 4H), 6.85–6.77 (m, 4H), 4.96 (s, 2H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.47, 162.30, 159.07, 138.29, 138.26, 137.93, 132.58, 131.12, 131.03, 131.01, 130.46, 129.05, 126.76, 116.66, 116.43, 113.81, 55.23, 54.02. $C_{19}H_{16}FNO_2SH$ $[M+H]^+$ 342.0964, found 342.0957.

***N*-(4-fluorophenyl)-*N*-(4-hydroxybenzyl)thiophene-2-carboxamide (10i).** White solid (89% yield), mp 172.7-175.3 ºC.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (dd, J = 4.9, 1.3 Hz, 1H), 7.12 (d, J = 8.5 Hz, 2H), 7.05–6.98 (m, 4H), 6.85-6.80 (m, 2H), 6.75 (d, J = 8.5 Hz, 2H), 5.63 (s, 1H), 4.95 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.53, 162.50, 155.46, 138.20, 137.65, 132.80, 131.19, 131.10, 131.01, 130.57, 128.77, 126.81, 116.71, 116.48, 115.42, 54.18. HRMS (ESI) calcd for $C_{18}H_{14}FNO_2SH$ $[M+H]^+$ 328.0803, found 328.0799.

***N*-(4-bromobenzyl)-*N*-(4-methoxyphenyl)thiophene-2-carboxamide (10j).** Yellow solid (76% yield), mp 101.8-104.6 ºC ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.4 Hz, 2H), 7.32 (dd, J = 4.9, 1.2 Hz, 1H), 7.17 (d, J = 8.4 Hz, 2H), 6.97–6.92 (m, 2H), 6.87-6.80 (m, 4H), 4.95 (s, 2H), 3.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.56, 159.57, 137.78, 136.30, 134.74, 132.84, 131.54, 131.21, 130.88, 130.31, 126.76, 121.52, 114.81, 55.48, 54.17. HRMS (ESI) calcd for $C_{19}H_{16}BrNO_2SH$ $[M+H]^+$ 402.0163, found 402.0158.

***N*-(4-bromobenzyl)-*N*-(4-hydroxyphenyl)thiophene-2-carboxamide (10k).** White solid (88% yield), mp 203.1-205.7 ºC.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.4 Hz, 2H), 7.33 (dd, J = 5.0, 1.2 Hz, 1H), 7.16 (d, J = 8.4 Hz, 2H), 6.93–6.86 (m, 3H), 6.85–6.77 (m, 3H), 5.44 (s, 1H), 4.95 (s, 2H). ^{13}C NMR (100 MHz, Acetone) δ 163.37, 159.02, 139.88, 138.77, 135.38, 133.56, 132.72, 132.52, 132.29, 131.79, 128.08, 122.10, 117.56, 54.91. HRMS (ESI) calcd for $C_{18}H_{14}BrNO_2SH$ $[M+H]^+$ 388.0001, found 387.9997.

***N*-(3-bromobenzyl)-*N*-(4-fluorophenyl)thiophene-2-carboxamide (10l).** White solid (89% yield), mp 111.4-113.4 ºC.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.37 (m, 2H), 7.35 (dd, J = 4.8, 1.3 Hz, 1H), 7.23-7.14 (m, 2H), 7.05 (d, J = 6.5 Hz, 4H), 6.86-6.81 (m, 2H), 4.99 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.58, 162.50, 161.10, 139.23, 138.17, 138.14, 137.40, 132.90, 131.84, 131.33, 130.94, 130.84, 130.11, 127.58, 126.85, 122.55, 116.89, 116.66, 54.11. HRMS (ESI) calcd for $C_{18}H_{13}BrFNOSH$ $[M+H]^+$ 389.9964, found 389.9960.

***N*-benzyl-N-(4-bromobenzyl)thiophene-2-carboxamide (10m).** Colorless viscous oil (70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.44 (m, 3H), 7.40-7.24 (m, 6H), 7.15 (d, J = 7.9 Hz, 2H), 6.96 (dd, J = 5.2, 4.0 Hz, 1H), 4.71 (s, 2H), 4.65 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.12, 137.41, 136.28, 135.68, 131.98, 129.72, 129.01, 128.76, 127.81, 127.67, 127.08, 121.59, 110.00. HRMS (ESI) calcd for $C_{19}H_{16}BrNOSH$ $[M+H]^+$ 386.0214, found 386.0206.

***N*-(4-bromobenzyl)-*N*-(3-fluorophenyl)thiophene-2-carboxamide (10n).** Colorless viscous oil (81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.38 (m, 2H), 7.36–7.33 (m, 1H), 7.32–7.25 (m, 1H), 7.17 (d, J = 8.4 Hz, 2H), 7.10-7.03 (m, 1H), 6.89–6.77 (m, 4H), 4.99 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.18, 162.48, 161.70, 143.85, 143.76, 137.47, 135.86, 132.61,

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131.68, 131.26, 130.89, 130.79, 130.62, 126.92, 124.93, 124.90, 121.73, 116.34, 116.12, 115.74, 115.54, 53.98. MS (ESI) *m / e*, 390.00.

***N*-(4-bromobenzyl)-*N*-(2-fluorophenyl)thiophene-2-carboxamide (10o).** White solid (67% yield), mp 86.8-88.7 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.37 (m, 2H), 7.36-7.31 (m, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.14–7.06 (m, 2H), 7.01 (td, *J* = 8.0, 1.8 Hz, 1H), 6.91 (dd, *J* = 3.8, 0.8 Hz, 1H), 6.82 (dd, *J* = 5.0, 3.8 Hz, 1H), 5.29 (d, *J* = 11.1 Hz, 1H), 4.65 (d, *J* = 11.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.92, 159.74, 137.17, 135.74, 132.14, 131.53, 131.12, 131.02, 130.82, 130.49, 130.41, 126.82, 124.96, 124.92, 121.69, 117.21, 117.01, 109.99, 53.08. MS (ESI) *m / e*, 390.08.

***N*-(2-bromobenzyl)-*N*-phenylthiophene-2-carboxamide (10p).** Colorless viscous oil (77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.33 (m, 2H), 7.22–7.12 (m, 5H), 7.05–7.00 (m, 2H), 6.99-6.95 (m, 1H), 6.71–6.64 (m, 2H), 5.11 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.69, 142.32, 137.94, 136.19, 132.78, 132.68, 131.19, 130.02, 129.68, 128.98, 128.86, 128.55, 127.70, 126.89, 123.77, 54.28. MS (ESI) *m / e*, 372.00.

***N*-(3-bromobenzyl)-*N*-phenylthiophene-2-carboxamide (10q).** White solid (85% yield), mp 96.1-97.7 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (t, *J* = 1.6 Hz, 1H), 7.27–7.19 (m, 4H), 7.19–7.16 (m, 1H), 7.11 (d, *J* = 7.7 Hz, 1H), 7.04-6.99 (m, 1H), 6.98–6.94 (m, 2H), 6.66-6.60 (m, 2H), 4.90 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.49, 142.28, 139.56, 137.93, 132.62, 131.77, 131.18, 130.68, 130.10, 129.81, 129.00, 128.65, 127.54, 126.89, 122.50, 54.12. MS (ESI) *m / e*, 372.00.

***N*-(2-bromobenzyl)-*N*-(2-fluorophenyl)furan-2-carboxamide (10r).** White solid (68% yield), mp 104.8-105.6 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, *J* = 7.7, 1.0 Hz, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.35–7.21 (m, 3H), 7.14–7.00 (m, 4H), 6.24 (d, *J* = 1.8 Hz, 1H), 6.07 (s, 1H), 5.46 (d, *J* = 10.8 Hz, 1H), 4.94 (d, *J* = 10.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.78, 159.50, 157.29, 146.68, 144.81, 135.75, 132.63, 130.67, 130.42, 130.11, 130.03, 129.64, 129.51, 129.11, 127.62, 124.68, 124.64, 123.98, 116.78, 116.58, 116.54, 111.20, 52.53. MS (ESI) *m / e*, 374.08.

***N*-(3-bromobenzyl)-*N*-(3-fluorophenyl)furan-2-carboxamide (10s).** Yellow viscous oil (67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (t, *J* = 1.6 Hz, 1H), 7.41–7.36 (m, 1H), 7.34–7.24 (m, 2H), 7.22-7.12 (m, 2H), 7.05-7.03 (m, 1H), 6.86–6.76 (m, 2H), 6.25 (dd, *J* = 3.6, 1.7 Hz, 1H), 6.07 (d, *J* = 3.6 Hz, 1H), 4.99 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.07, 161.60, 159.09, 146.54, 144.85, 139.02, 131.66, 130.84, 130.67, 130.58, 130.11, 127.36, 124.15, 124.12, 122.55, 117.30, 115.61, 115.41, 115.39, 115.20, 111.27, 53.40. MS (ESI) *m / e*, 374.00.

***N*-(3-bromobenzyl)-*N*-(3-fluorophenyl)thiophene-2-carboxamide (10t).** Colorless viscous oil (71% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (t, *J* = 1.6 Hz, 1H), 7.41–7.36 (m, 1H), 7.34 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.32-7.26 (m, 1H), 7.24-7.20 (m, 1H), 7.18–7.14 (m, 1H), 7.09-7.04 (m, 1H), 6.89–6.78 (m, 4H), 5.00 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.18, 162.52, 161.70, 143.81, 143.72, 139.15, 137.37, 136.10, 135.46, 132.73, 131.68, 131.35, 130.95, 130.86, 130.17, 128.55, 127.40, 126.96, 124.91, 124.87, 122.58, 116.32, 116.10, 115.81, 115.61, 54.00. MS (ESI) *m / e*, 390.08.

Antiviral Activity.

The antiviral activity of the compounds was calculated by an MTS-based CPE reduction assay, which compares the optical density of infected compound- treated cells with uninfected compound-free cells.[19] Briefly, 5x10^5 RD cells (rhabdomyosarcoma) were grown in 96-well plates, then serial dilutions of the compounds and 100 TCID50 of EV71 virus were added. After incubation at 37 ^{o}C for 24-36 h (until complete CPE was observed in the virus infected and compounds free control (VC)), cell viability was measured using the MTS/PMS method (Promega, Leiden, The Netherlands). The absorbance at 498 nm was recorded using a TecanGENiosmicroplate reader (Tecan, Switzerland). CPE values were calculated and the 50% effective concentration (EC_{50}) was defined as the concentration of compound that inhibited virus-induced cytopathic effect formation by 50% and was calculated using the software Calcusyn.[42] Each experiment was repeated at least three times.

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Supplementary Material

Electronic supplementary information (ESI) available. NMR spectra of **5a-n** and **10a-t** in electronic format see DOI: XXXXXX

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