Regular Article

Discovery and Synthesis of Heterocyclic Carboxamide Derivatives as Potent Anti-norovirus Agents

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There is an urgent need for structurally novel anti-norovirus agents. In this study, we describe the synthesis, anti-norovirus activity, and structure–activity relationship (SAR) of a series of heterocyclic carboxamide derivatives. Heterocyclic carboxamide 1 (50% effective concentration (EC_{50})=37 μM) was identified by our screening campaign using the cytopathic effect reduction assay. Initial SAR studies suggested the importance of halogen substituents on the heterocyclic scaffold and identified 3,5-*di*-boromo-thiophene derivative 2j (EC_{50}=24 μM) and 4,6-*di*-fluoro-benzothiazole derivative 3j (EC_{50}=5.6 μM) as more potent inhibitors than 1. Moreover, their hybrid compound, 3,5-*di*-bromo-thiophen-4,6-*di*-fluoro-benzothiazole 4b, showed the most potent anti-norovirus activity with a EC_{50} value of 0.53 μM (70-fold more potent than 1). Further investigation suggested that 4b might inhibit intracellular viral replication or the late stage of viral infection.

Key words norovirus; heterocyclic carboxamide; antiviral activity; murine norovirus

Human norovirus causes acute nonbacterial gastroenteritis. Although the gastroenteritis symptoms are generally self-limiting, the elderly, infants, and immunocompromised individuals have a higher risk of mortality. Moreover, norovirus has a low infectious dose and spreads very easily from infected persons, contaminated food, water, or a contaminated environment.[1)]

Noroviruses are single-stranded, positive-sense RNA viruses that belong to the family *Caliciviridae* and genus *Norovirus*, and species *Norwalk virus*. The norovirus genome contains three open reading frames (ORFs): ORF1, ORF2, and ORF3. ORF1 encodes six or seven nonstructural proteins, including RNA-dependent RNA polymerase (RdRp) and 3C-like cysteine protease (3CLpro), responsible for viral replication. ORF2 and ORF3 encode the major and minor structural proteins, respectively.[1)]

Establishment of an efficient cell culture system for human norovirus is still challenging.[2,3)] However, murine norovirus (MNV) can replicate efficiently in the murine macrophage cell line RAW264.7. MNV belongs to the genus *Norovirus* and shares common biological and molecular properties with human norovirus.[4,5)] Therefore, it is frequently used for identifying antiviral compounds based on the cell culture assay.[6–12)]

Several anti-norovirus agents have been reported that target viral attachment/entry, viral protein translation, or viral replication.[7,8)] The RdRp inhibitor used as an anti-hepatitis C virus agent 2′-*C*-methylcytidine (2′-CMC)[9,10)] and anti-influenza agent favipiravir[11)] also have anti-MNV activity, and the broad spectrum 3CLpro inhibitor, dipeptidyl inhibitor GC376 also inhibits MNV protease activity *in vitro*.[12)] Currently, there are no vaccines or drugs for clinical use, and there is an urgent need for new anti-norovirus compounds.

We describe here the discovery and synthesis of anti-norovirus thienyl benzothiazolyl carboxamide compounds bearing multiple halogen substituents, and discuss their structure–activity relationships (SARs) and biological activity.

Results

Screening and Discovery of Anti-norovirus Compounds More than 2000 compounds, which were selected from our in-house library, were screened with a qualitative antiviral activity assay. Each compound was mixed with MNV and the mixture was exposed to RAW264.7 cells. We used RdRp inhibitor 2′-CMC as a positive control and tested if it showed anti-MNV activity. We found that heterocyclic carboxamide derivative **1** had anti-norovirus activity. Compound **1** consisted of a 5-bromo-thiophene ring and 6-fluoro-benzothiazole ring linked by a central amide bond (Fig. 1). However, compounds **1a** and **b**, which had a phenyl ring instead of 5-bromo-thiophene ring, and **1c**, which had an ethylene linker between the 5-bromo-thiophene ring and the amide bond, did not show antiviral activity. Therefore, we identified the thiophene-benzothiazole carboxamide as a key core structure and developed analogs of **1** to find a more potent agent.

Chemistry We focused on the halogen substituents on both the thiophene and benzothiazole rings in **1**, and synthesized various halogenated analogs and other related derivatives (Chart 1). The coupling reagent 1-ethyl-3-(3-

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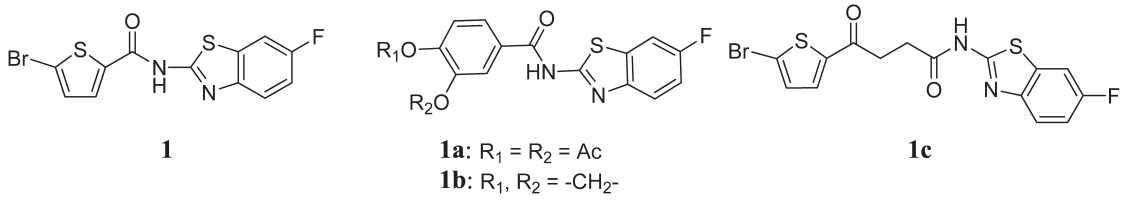
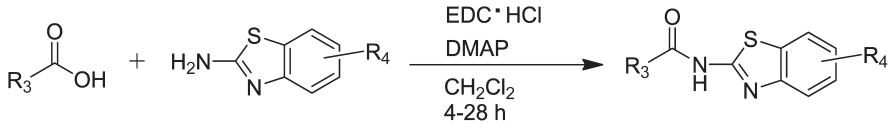


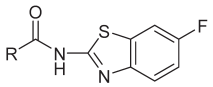
Fig. 1. Structures of Hit Compound **1** and Inactive Analogs **1a–c**



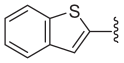
R_3: Tables 1, 3. R_4: Tables 2, 3.

Chart 1. Synthesis of Heterocyclic Carboxamide Derivatives

Table 1. Antiviral Activity and Cytotoxicity of 6-Fluoro-benzothiazole Analogs



1, 2a-w

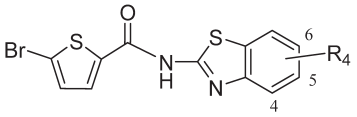
Compound	R	Substituent	EC_{50} (μM)[a]	CC_{50} (μM)[b]
2′-CMC			56	12
1		5-Br	37	>100
2a		None	>100	>100
2b		5-Cl	30	>100
2c		5-F	>100	1.9
2d		5-*t*-Bu	>100	>100
2e		4-Cl	>100	52
2f		4-Br	>100	>100
2g		3-F	>100	>100
2h		3-Cl	>100	65
2i		3-Br	>100	77
2j		3,5-Br	24	7.9
2k		3,5-Cl	6.6	>100
2l		4,5-Br	>100	>100
2m		4,5-Cl	24	22
2n		3,4,5-Cl	89	>100
2o		None	>100	>100
2p		5-Cl	>100	74
2q		5-Br	>100	>100
2r		None	>100	15
2s		None	65	>100
2t		2-Br	>100	>100
2u		4-Br	>100	>100
2v		5-Br	>100	>100
2w		2,5-Br	>100	>100

a) EC_{50} was evaluated by the CPE reduction assay. 280 $TCID_{50}$/50 μL of MNV and a dilution series of each compound were incubated for 30 min. The mixture was exposed to RAW264.7 cells for 1 h (in duplicate). *b*) Cytotoxicity was evaluated by the WST-8 assay. RAW264.7 cells were treated with dilution series of each compound (in triplicate) for 72 h.

dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl) and catalyst 4-dimethylaminopyridine (DMAP) were used to form the amide bond linking the heterocycles.

SAR The anti-norovirus activities of the analogs were evaluated by MNV-induced cytopathic effect (CPE) reduction assay. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) of the analogs are summarized in Tables 1–3.

Table 2. Antiviral Activity and Cytotoxicity of 5-Bromo-thiophene Analogs



3a-x

Compound	R_4	EC_{50} (μM)[a]	CC_{50} (μM)[b]	Compound	R_4	EC_{50} (μM)[a]	CC_{50} (μM)[b]
3a	None	>100	>100	**3m**	5,6-F	>100	25
3b	6-Cl	56	>100	**3n**	6-Me	>100	>100
3c	6-Br	>100	>100	**3o**	4-Me	>100	>100
3d	5-F	>100	>100	**3p**	5,6-Me	>100	>100
3e	5-Cl	>100	>100	**3q**	6-OMe	>100	>100
3f	5-Br	>100	>100	**3r**	4-OMe	>100	>100
3g	4-F	>100	5.1	**3s**	6-OEt	>100	>100
3h	4-Cl	>100	>100	**3t**	6-NO_2	>100	17
3i	4-Br	>100	>100	**3u**	6-CF_3	>100	11
3j	4,6-F	5.6	>100	**3v**	6-OCF_3	>100	22
3k	4,6-Cl	>100	>100	**3w**	6-CO_2Et	>100	>100
3l	4-Br,6-F	20	>100	**3x**	6-*t*-Bu	>100	6.0

a) EC_{50} was evaluated by the CPE reduction assay. 280 $TCID_{50}$/50 μL of MNV and a dilution series of each compound were incubated for 30 min. The mixture was exposed to RAW264.7 cells for 1 h (in duplicate). *b*) Cytotoxicity was evaluated by the WST-8 assay. RAW264.7 cells were treated with dilution series of each compound (in triplicate) for 72 h.

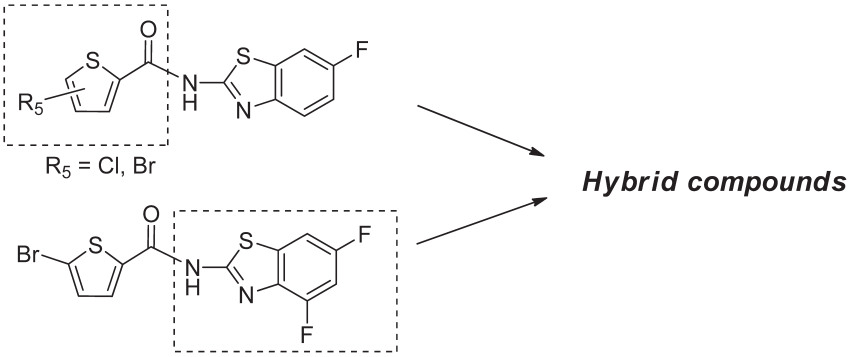


Chart 2. Strategy for Designing Hybrid Compounds

The modified thiophene analogs **2a**–**w** were compared with **1** (Table 1). Because unsubstituted analog **2a** showed no antiviral activity, the bromine in **1** was important for activity. 5-Chloro-thiophene analog **2b** showed similar activity to 5-bromo analog **1** (**2b**, EC_{50}=30 μM; **1**, EC_{50}=37 μM). Moreover, 3,5-*di*-chloro-thiophene analog **2k** had more potent antiviral activity (EC_{50}=6.6 μM) than mono-halogenated analogs (the EC_{50} values was 30 μM or more). However, halogen groups at the other ring positions, such as 3,5-*di*-bromide **2j** or 4,5-*di*-chloride **2m**, did not increase the antiviral activity. The CC_{50} values of these compounds was equal to or less than their EC_{50} values. The other 5-fluorinated, *t*-butylated, 4- or 3-mono-halogenated, 4,5-*di*-brominated, and 3,4,5-*tri*-chlorinated analogs (**2c**–**i**, **l**, and **n**) did not show significant antiviral activity. Furthermore, the alternative heterocyclic analogs (furans **2o**–**q**, thiazole **2r**, and benzothiophene **2s**) and the thiophene linkage regioisomers (**2t**–**w**) had almost no antiviral activity. These results suggest that the halogenated thiophene ring conjugated to the amide bond at the 2-position is essential for anti-norovirus activity, and that the chloro groups at the 3- and 5-positions are the most effective substituents.

Next, the benzothiazole moiety of **1** was modified to elucidate suitable substitutions while retaining the 5-bromo-thiophene ring (Table 2). Because unsubstituted analog **3a** showed no antiviral activity, the fluorine moiety of **1** was important for activity. Although 6-chloro-benzothiazole analog **3b** showed moderate activity of 56 μM, the 4- or 5-mono-halogenated regioisomers **3d**–**i**, and 4- or 6-mono- or 5,6-*di*-substituted analogs **3n**–**x** (Me, MeO, OEt, NO_2, CF_3, OCF_3, CO_2Et, *t*-Bu) did not show antiviral activity. *Di*-halogenated benzothiazole analogs **3j**–**m** were investigated. Only 4,6-*di*-fluoro-benzothiazole analog **3j** showed antiviral activity (EC_{50}=5.6 μM), which was 6-fold higher than that of **1**, and no cytotoxicity at the highest concentration tested. 4-Bromo-6-fluoro analog **3l** showed moderate activity; however, no inhibitory activity was observed in 4,6-*di*-chloro analog **3k** and 5,6-*di*-fluoro analog **3m**. Therefore, 4,6-*di*-fluoro substitution on the benzothiazole ring increases anti-norovirus activity, and is one of most efficient combinations of halogen substituents.

5-Chlorinated thiophene analog **2b**, 3,5- and 4,5-*di*-halogenated thiophene analogs **2j**, **k**, and **m**, and 4,6-*di*-fluorinated benzothiazole analog **3j** showed strong antiviral activity (Tables 1, 2). Thus, *tri*- or *tetra*-halogenated analogs **4a**–**d** were synthesized and evaluated as hybrid derivatives, and were expected to show increased activity because of the multiple halogen groups (Chart 2, Table 3). 3,5-*Di*-bromo-thiophen-4,6-

di-fluoro-benzothiazole analog **4b** had the most potent anti-norovirus activity (EC_{50}=0.53 μM), which was 70-fold greater than that of **1**, and the other hybrid analogs **4a**, **c**, and **d** also showed potent activity (EC_{50}=1.1–2.1 μM). The CC_{50} values of these compounds were much higher than their EC_{50} values.

Antiviral Mechanism

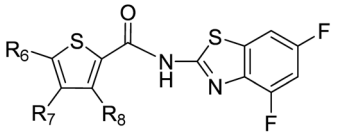
Fifty Percent Tissue Culture Infectious Dose ($TCID_{50}$) Assay with Filtration

To determine the antiviral mechanism, we investigated whether heterocyclic carboxamide derivatives act on viral particles. A mixture of **4b** and virus were filtered to remove **4b**, and then cells were infected with the treated virus. The virus titers were determined by the $TCID_{50}$ 50% tissue culture infectious dose assay. 0.02% (v/v) sodium hypochlorite[13] as a positive control and 2′-CMC and GC376 as negative controls were used. Although 0.02% (v/v) sodium hypochlorite reduced virus infectivity to less than 2.0 ($\log_{10}$ $TCID_{50}$/mL), the virus titer was not reduced by **4b** (4.8 for virus control and 4.7 for virus treated with **4b**) (Table 4). 2′-CMC and GC376 also did not reduce infectivity. This result indicated that **4b** does not act on the viral particle directly.

Time-of-Addition Assay

To identify the mechanism of antiviral activity, **4b** was evaluated by time-of-addition assay. In the simultaneous treatment assay, compounds were mixed with MNV and incubated for 30 min at 37°C, and then cells were exposed to the mixture. After 1 h, the supernatant was replaced with fresh medium. In the post-infection treatment assay, cells were inoculated with MNV for 1 h at 37°C, and then the virus supernatant was replaced with medium containing the compounds and incubated for 72 h (Fig. 2). Similarly to 2′-CMC and GC376, the antiviral activity of **4b** was preserved when it was added after inoculating the cells with the virus. This result suggests that **4b** does not prevent the early stage of viral infection (virus attachment, entry, and uncoating). Moreover, in both assays, **4b** showed more potent antiviral activity than 2′-CMC and GC376, and extending the assay time increased the antiviral activity of **4b** (Table 5).

Table 3. Antiviral Activity and Cytotoxicity of Tetra-halogenated Hybrid Compounds



4a-d

Compound	R_6	R_7	R_8	EC_{50} (μM)[a]	CC_{50} (μM)[b]
4a	Cl	H	H	2.1	>100
4b	Br	H	Br	0.53	>100
4c	Cl	H	Cl	1.1	>100
4d	Cl	Cl	H	1.4	31

a) EC_{50} was evaluated by the CPE reduction assay. 280 $TCID_{50}$/50 μL of MNV and a dilution series of each compound were incubated for 30 min. The mixture was exposed to RAW264.7 cells for 1 h (in duplicate). *b*) Cytotoxicity was evaluated by the WST-8 assay. RAW264.7 cells were treated with dilution series of each compound (in triplicate) for 72 h.

Table 4. Antiviral Activity of **4b** against MNV Particle

	Virus infectivity ($\log_{10}TCID_{50}$/mL)
Control 1[a]	4.8
4b	4.7
2′-CMC	4.8
GC376	4.8
Control 2[b]	4.8
0.02% (v/v) sodium hypochlorite	<2.0

Results from two independent experiments and data are expressed as the mean. Final concentration of compounds was 225 μM and reaction time was 60 min. *a*) Fetal bovine serum-free medium containing 2.3% (v/v) dimethylsulfoxide was used. *b*) Distilled water was used.

Discussion

We used a cell-based screening system to detect antiviral

Table 5. Time-of-Addition Assay Results

Conditions	EC_{50} (μM)		
	4b	2′-CMC	GC376
Simultaneous treatment[a]	0.53	56	>100
Post-infection treatment[b]	0.041	4.6	68

Results from two independent experiments performed in duplicate. Effect of changing the infection order. *a*) Compounds and virus were exposed to cells simultaneously for 1 h. *b*) After viral infection for 1 h, cells were treated with compounds for 72 h.

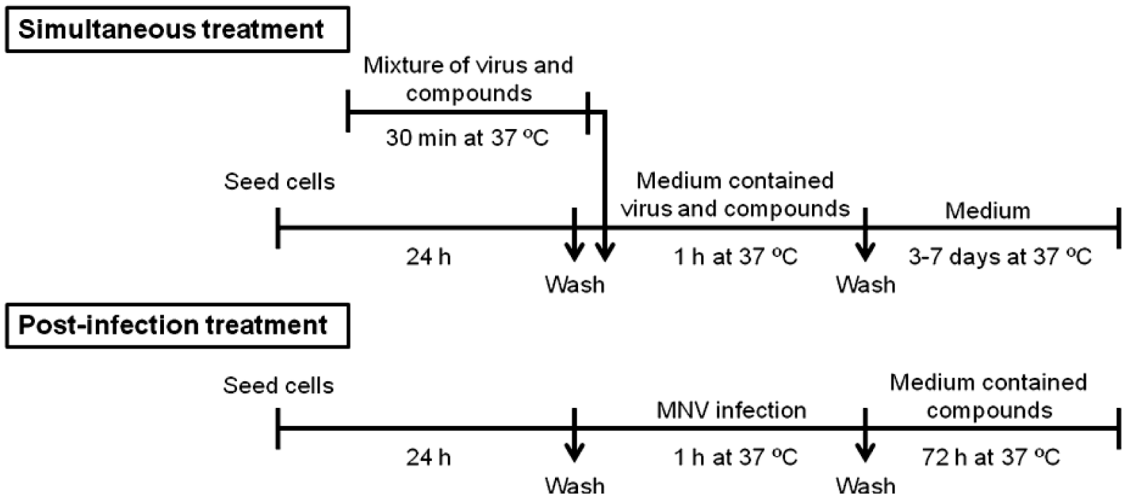


Fig. 2. Protocols for Time-of-Addition Assays

compounds that affect intracellular viral replication and the viral particle directly. We screened approximately 2000 compounds from our chemical library and identified 5-bromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (**1**) as an anti-norovirus agent with an EC_{50} of 37 μM. We conducted SAR studies on thiophene and benzothiazole analogs of **1**, and identified derivatives that showed more potent antiviral activity. For instance, 5-chloro-thiophene **2b** (EC_{50}=30 μM), 3,5-*di*-bromo-thiophene **2j** (EC_{50}=24 μM), 3,5- and 4,5-*di*-chloro-thiophenes **2k** and **m** (EC_{50}=6.6 and 24 μM, respectively), and 4,6-*di*-fluoro-benzothiazole **3j** (EC_{50}=5.6 μM) were potent antiviral agents. Although there were not obvious substituent effects on the thiophene or the benzothiazole core structure, the SAR results showed that the *di*-halogenated analogs were more potent than the mono-halogenated analogues (*e.g.*, **3j**, EC_{50}=5.6 μM *vs.* **1**, EC_{50}=37 μM). We synthesized hybrid compounds **4a**–**d** by combining structural features of **2b**, **j**, **k**, **m**, and **3j**. The combination of bromination at the 3- and 5-positions on the thiophene ring (from **2j**) and fluorination of the 4- and 6-positions on the benzothiazole ring (from **3j**) gave a more potent antiviral agent (**4b**, EC_{50}=0.53 μM) than **4c** (EC_{50}=1.1 μM), synthesized from the combination of **2k** and **3j**, which possessed the highest activities. Physicochemical properties of the optimal balanced thiophene-benzothiazole carboxamide core structure such as hydrophobicity, electron density, and steric effect might be important for potent antiviral activity. We performed a $TCID_{50}$ assay with filtration to investigate whether heterocyclic carboxamide derivatives act directly on the viral particles. The virus infectivity was not reduced by treatment with **4b**, which suggested that **4b** does not act on viral particles directly. Potential therapeutic intervention stages other than direct action on viral particles are: (1) early stages of viral infection (virus attachment, entry, and uncoating); (2) viral replication (RdRp and 3CL pro); and (3) late stages of viral infection (virion assembly and release).[7,8] We performed a time-of-addition assay to identify whether **4b** inhibits the early stage of viral infection. Comparing **4b** with inhibitors of intracellular viral replication, 2′-CMC and GC376, showed that they exerted similar antiviral effects. These results suggest that heterocyclic carboxamide derivatives act on intracellular viral replication or the late stages of viral infection. In addition, **4b** showed antiviral activity at a lower concentration than 2′-CMC and GC376. To investigate viral replication, we evaluated the inhibitory activity of **4b** against MNV protease by using a rabbit reticulocyte lysate *in vitro* translation system.[14] No inhibition was detected (data not shown). The anti-MNV mechanism of **4b** is under further investigation. Because this compound has a low EC_{50} value, it may be a useful tool for identifying target proteins and a good lead compound for developing effective therapeutics and prophylactics for norovirus.

Experimental

General Chemical Procedure Melting points (mp) of the compounds were obtained using Mettler-toledo Excellence MP70 melting-point apparatus. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL AL-400 (at 400 and 100 MHz, respectively) by using dimethylsulfoxide (DMSO)-d_6 with tetramethylsilane (TMS) as the internal standard. The spin multiplicities are indicated by the following symbols s (singlet), d (doublet), dd (doublet of doublets), t (triplet), dt (doublet of triplets), ddd (doublet of doublet of doublets), ddt (doublet of doublet of triplets), q (quartet), m (multiplet). Chemical shift (δ) is expressed in ppm. LC/MS analyses were performed with Waters 2767 sample manager, 2996 photodiode array detector and 600 controller ZQ. Electrospray ionization (ESI) MS spectra were recorded at 60 eV on a Waters ZQ2000. A Cosmosil 5C18-ARII column (2.0×50 mm, Nacalai Tesque) was employed with a linear gradient of CH_3CN containing 0.05% (v/v) trifluoroacetic acid (TFA) at a flow rate of 1 mL/min, and eluting products were detected by UV at 254 nm. The purity of the compounds was determined by HPLC analysis. Reactions were monitored by TLC using E. MERCK silica gel 60 F_{254} glass plate. Preparative layer chromatography was performed using E. MERCK PLC silica gel 60 F_{254}, 2 mm glass plate. Column chromatography was carried out on FUJI SILYSIA CHEMICAL CHROMATOREX NH DM1020 and/or silica gel. 2′-CMC was purchased from Sigma-Aldrich. The commercial bleach (Oyalox), which was contained 6% sodium hypochlorite, was purchased locally. GC376 was synthesized by a reported procedure.[15]

5-Bromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (1) A mixture of 5-bromothiophene-2-carboxylic acid (203 mg, 0.98 mmol), 6-fluorobenzo[*d*]thiazol-2-amine (188 mg, 1.12 mmol), DMAP (133 mg, 1.09 mmol) and EDC·HCl (208 mg, 1.08 mmol) in CH_2Cl_2 (2 mL) was stirred for 4 h at room temperature (rt). The reaction mixture was concentrated and the residue was washed several times with cold MeOH. The solid was crystallized from MeOH to give **1** (67 mg, 19%) as light yellow powder. mp: 257–258°C. ^{1}H-NMR (DMSO-d_6) δ: 7.32 (1H, dt, J=8.8, 2.4 Hz), 7.43 (1H, d, J=4.8 Hz), 7.77 (1H, dd, J=8.8, 4.8 Hz), 7.93 (1H, dd, J=8.8, 2.4 Hz), 8.09 (1H, d, J=3.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.3 (d, J=27.3 Hz), 114.4 (d, J=24.8 Hz), 120.1, 121.1, 132.2, 132.3, 132.5 (d, J=9.9 Hz), 138.9, 144.5, 158.7 (d, J=240.6 Hz), 158.8, 159.8. HPLC purity: >99%, ESI-MS m/z: 357 $[M+H]^+$.

***N*-(6-Fluorobenzo[d]thiazol-2-yl)thiophene-2-carboxamide (2a)** A mixture of thiophene-2-carboxylic acid (143 mg, 1.12 mmol), 6-fluorobenzo[*d*]thiazol-2-amine (207 mg, 1.23 mmol), DMAP (155 mg, 1.27 mmol) and EDC·HCl (230 mg, 1.20 mmol) in CH_2Cl_2 (2 mL) was stirred overnight at rt. The reaction mixture was diluted with CH_2Cl_2 and washed with 2 N HCl, H_2O, and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was washed several times with $CHCl_3$ and MeOH to give **2a** (126 mg, 40%) as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.28–7.35 (2H, m), 7.79 (1H, dd, J=8.8, 4.8 Hz), 7.94 (1H, dd, J=8.8, 2.4 Hz), 8.03 (1H, d, J=4.8 Hz), 8.32 (1H, d, J=3.2 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.2 (d, J=28.1 Hz), 114.3 (d, J=24.0 Hz), 121.4, 128.7, 131.5, 132.7 (d, J=8.2 Hz), 134.2, 136.9, 145.1, 158.5, 158.7 (d, J=240.6 Hz), 160.6. HPLC purity: >99%, ESI-MS m/z 279 $[M+H]^+$.

5-Chloro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2b) A mixture of 5-chlorothiophene-2-carboxylic acid (163 mg, 1.00 mmol), 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), DMAP (183 mg, 1.50 mmol) and EDC·HCl (287 mg, 1.50 mmol) in CH_2Cl_2 (2 mL) was stirred overnight at rt. The reaction mixture was diluted with CH_2Cl_2 and washed with 2 N HCl, H_2O, and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by NH-silica-gel column chromatography (eluent: CH_2Cl_2/MeOH=10 : 1) and the solid was washed several times

with $CHCl_3$ and MeOH to give **2b** (136 mg, 43%) as white powder. mp: 244–246°C. ^{1}H-NMR (DMSO-d_6) δ: 7.30–7.35 (2H, m), 7.77 (1H, dd, J=8.8, 4.8 Hz), 7.93 (1H, dd, J=8.8, 2.4 Hz), 8.15 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 108.3 (d, J=27.3 Hz), 114.4 (d, J=24.8 Hz), 121.3 (d, J=24.8 Hz), 128.8, 131.5, 131.8, 132.6, 136.0, 145.1, 158.7 (d, J=240.5 Hz), 159.0, 159.6. HPLC purity: 98%, ESI-MS m/z 313 $[M+H]^+$.

5-Fluoro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2c) According to the same procedure used for **2a**, starting from 5-fluorothiophene-2-carboxylic acid (146 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2c** (76 mg, 26%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 6.97 (1H, dd, J=4.8, 1.6 Hz), 7.32 (1H, dt, J=8.8, 2.4 Hz), 7.77 (1H, dd, J=8.8, 4.8 Hz), 7.92 (1H, dd, J=8.8, 2.4 Hz), 8.07 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 108.3 (d, J=27.3 Hz), 110.9 (d, J=11.5 Hz), 114.3 (d, J=24.0 Hz), 121.1 (d, J=9.1 Hz), 126.5, 129.8 (d, J=4.1 Hz), 132.6 (d, J=8.3 Hz), 144.9, 158.6, 158.7 (d, J=240.6 Hz), 160.3, 169.1 (d, J=293.5 Hz). HPLC purity: >99%, ESI-MS m/z 297 $[M+H]^+$.

5-(*tert*-Butyl)-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2d) A mixture of 5-(*tert*-butyl)thiophene-2-carboxylic acid (184 mg, 1.00 mmol), 6-fluorobenzo[*d*]thiazol-2-amine (186 mg, 1.10 mmol), DMAP (183 mg, 1.50 mmol) and EDC·HCl (287 mg, 1.50 mmol) in CH_2Cl_2 (2 mL) was stirred overnight at rt. CH_2Cl_2 and 2 N HCl were added, the precipitate was filtered and washed several times with CH_2Cl_2 and MeOH to give **2d** (195 mg, 58%) as white powder. ^{1}H-NMR (DMSO-d_6) δ: 1.39 (9H, s), 7.09 (1H, dd, J=3.6, 0.8 Hz), 7.31 (1H, dt, J=8.8, 2.4 Hz), 7.77 (1H, dd, J=8.8, 4.8 Hz), 7.92 (1H, dd, J=8.8, 2.4 Hz), 8.14 (1H, d, J=3.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 31.8, 34.8, 108.2 (d, J=27.2 Hz), 114.2 (d, J=24.0 Hz), 121.3 (d, J=9.1 Hz), 123.8, 131.6, 132.8 (d, J=9.1 Hz), 133.3, 145.3, 158.7, 158.7 (d, J=239.7 Hz), 160.5, 165.4. HPLC purity: >99%, ESI-MS m/z 335 $[M+H]^+$.

4-Chloro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2e) According to the same procedure used for **2d**, starting from 4-chlorothiophene-2-carboxylic acid (163 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2e** (182 mg, 58%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.33 (1H, dt, J=8.8, 2.4 Hz), 7.79 (1H, dd, J=8.8, 4.8 Hz), 7.94 (1H, dd, J=8.8, 2.4 Hz), 8.06 (1H, s), 8.26 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 108.3 (d, J=26.4 Hz), 114.5 (d, J=24.8 Hz), 121.4 (d, J=10.7 Hz), 124.4, 129.1, 130.7, 132.7, 137.5 (d, J=9.1 Hz), 144.9, 158.5, 158.8 (d, J=240.6 Hz), 160.6. HPLC purity: >99%, ESI-MS m/z 313 $[M+H]^+$.

4-Bromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2f) A mixture of 4-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol), 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), DMAP (184 mg, 1.51 mmol) and EDC·HCl (290 mg, 1.51 mmol) in CH_2Cl_2 (2 mL) was stirred overnight at rt. CH_2Cl_2 and 2 N HCl were added, the precipitate was filtered and washed several times with $CHCl_3$ and MeOH to give **2f** (150 mg, 42%) as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.33 (1H, dt, J=8.8, 2.4 Hz), 7.79 (1H, dd, J=8.8, 4.8 Hz), 7.94 (1H, dd, J=8.8, 2.4 Hz), 8.15 (1H, s), 8.30 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 108.3 (d, J=27.2 Hz), 109.6, 114.4 (d, J=24.0 Hz), 121.4 (d, J=11.5 Hz), 131.6, 132.6 (d, J=9.0 Hz), 133.1, 138.2 (d, J=5.8 Hz), 145.0, 158.5, 158.8 (d, J=239.8 Hz), 159.4. HPLC purity: >99%, ESI-MS m/z 357 $[M+H]^+$.

3-Fluoro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2g) According to the same procedure used for **2b**, starting from 3-fluorothiophene-2-carboxylic acid (147 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2g** (120 mg, 40%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.19 (1H, d, J=4.8 Hz), 7.33 (1H, dt, J=8.8, 2.4 Hz), 7.70 (1H, s), 7.91 (1H, dd, J=8.8, 2.4 Hz), 7.96 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 108.6 (d, J=26.5 Hz), 114.5 (d, J=24.8 Hz), 116.1, 118.7 (d, J=25.6 Hz), 119.9, 131.7, 131.8, 141.7, 156.6, 157.9 (d, J=271.1 Hz), 158.8 (d, J=241.4 Hz), 160.8. HPLC purity: >99%, ESI-MS m/z 297 $[M+H]^+$.

3-Chloro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2h) According to the same procedure used for **2a**, starting from 3-chlorothiophene-2-carboxylic acid (163 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2h** (131 mg, 42%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.25 (1H, d, J=4.8 Hz), 7.33 (1H, dt, J=8.8, 2.4 Hz), 7.66 (1H, s), 7.91 (1H, dd, J=8.8, 2.4 Hz), 7.97 (1H, d, J=4.8 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.8 (d, J=27.3 Hz), 114.6 (d, J=24.8 Hz), 118.9 (d, J=9.0 Hz), 127.7, 128.0, 129.8, 130.0, 131.0, 131.3, 140.4, 158.7 (d, J=239.7 Hz), 161.4. HPLC purity: >99%, ESI-MS m/z 313 $[M+H]^+$.

3-Bromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2i) According to the same procedure used for **2a**, starting from 3-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2i** (41 mg, 11%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.29 (1H, d, J=5.2 Hz), 7.35 (1H, dt, J=8.8, 2.4 Hz), 7.66 (1H, s), 7.91 (1H, dd, J=8.8, 2.4 Hz), 7.95 (1H, d, J=5.2 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.9 (d, J=26.4 Hz), 113.9, 114.6 (d, J=24.8 Hz), 119.2, 130.9, 131.5, 132.0, 132.5, 132.8, 141.7, 158.7 (d, J=239.7 Hz), 163.0. HPLC purity: 99%, ESI-MS m/z 357 $[M+H]^+$.

3,5-Dibromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2j) According to the same procedure used for **2a**, starting from 3,5-dibromothiophene-2-carboxylic acid (286 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2j** (266 mg, 61%) was obtained as light yellow powder. mp: 212–213°C. ^{1}H-NMR (DMSO-d_6) δ: 7.34 (1H, dt, J=8.8, 2.4 Hz), 7.49 (1H, s), 7.56 (1H, s), 7.88 (1H, dd, J=8.8, 2.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 109.2 (d, J=28.9 Hz), 113.6, 114.8 (d, J=29.4 Hz), 116.6 (d, J=11.5 Hz), 118.2, 129.6 (d, J=4.1 Hz), 134.6 (d, J=11.6 Hz), 135.4, 135.7, 158.7 (d, J=248.0 Hz), 164.0, 164.6. HPLC purity: >99%, ESI-MS m/z 435 $[M+H]^+$.

3,5-Dichloro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2k) According to the same procedure used for **2d**, starting from 3,5-dichlorothiophene-2-carboxylic acid (197 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2k** (192 mg, 55%) was obtained as beige powder. mp: 259–261°C. ^{1}H-NMR (DMSO-d_6) δ: 7.29 (1H, dt, J=8.8, 2.4 Hz), 7.73 (1H, dd, J=8.8, 4.8 Hz), 7.85 (1H, dd, J=8.8, 2.4 Hz), 8.17 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 108.4 (dd, J=27.2, 8.0 Hz), 114.5 (dd, J=24.8, 8.0 Hz), 120.7 (dd, J=15.6, 4.1 Hz), 124.1, 130.3, 130.7, 132.3 (d, J=6.6 Hz), 134.6, 135.1 (d, J=4.9 Hz), 142.3, 158.8 (d, J=240.6 Hz), 159.9. HPLC purity: 99%, ESI-MS m/z 347 $[M+H]^+$.

4,5-Dibromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2l) According to the same procedure used

for **2d**, starting from 4,5-dibromothiophene-2-carboxylic acid (287 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2l** (321 mg, 74%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.29 (1H, dt, J=8.8, 2.4 Hz), 7.74 (1H, dd, J=8.8, 4.8 Hz), 7.86 (1H, dd, J=8.8, 2.4 Hz), 8.16 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 108.4 (d, J=27.3 Hz), 114.5 (d, J=24.8 Hz), 114.7, 119.3, 121.4, 129.8, 132.3, 133.2, 138.5, 145.7, 158.8 (d, J=236.4 Hz), 159.1. HPLC purity: 96%, ESI-MS m/z 435 [M+H]$^+$.

4,5-Dichloro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2m) According to the same procedure used for **2d**, starting from 4,5-dichlorothiophene-2-carboxylic acid (197 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2m** (207 mg, 60%) was obtained as white powder. mp: 260–261°C. ^{1}H-NMR (DMSO-d_6) δ: 7.30 (1H, dt, J=8.8, 2.4 Hz), 7.73 (1H, dd, J=8.8, 4.8 Hz), 7.86 (1H, dd, J=8.8, 2.4 Hz), 8.18 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 108.0 (d, J=27.2 Hz), 114.1 (d, J=24.8 Hz), 120.2 (d, J=7.4 Hz), 123.8, 130.1, 130.3, 131.9 (d, J=8.3 Hz), 134.7 (d, J=3.3 Hz), 142.8, 158.6 (d, J=240.6 Hz), 159.5, 159.7. HPLC purity: >99%, ESI-MS m/z 347 [M+H]$^+$.

3,4,5-Trichloro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (2n) According to the same procedure used for **2d**, starting from 3,4,5-trichlorothiophene-2-carboxylic acid (232 mg, 1.00 mmol) and 6-fluorobenzo[*d*]-thiazol-2-amine (185 mg, 1.10 mmol), **2n** (305 mg, 80%) was obtained as white powder. mp: 299–301°C. ^{1}H-NMR (DMSO-d_6) δ: 7.31 (1H, dt, J=8.8, 2.4 Hz), 7.55 (1H, dd, J=8.8, 4.8 Hz), 7.82 (1H, dd, J=8.8, 2.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 109.0 (d, J=27.2 Hz), 114.6 (d, J=24.7 Hz), 115.9 (d, J=9.1 Hz), 116.0, 124.1, 125.0, 128.1, 129.1 (d, J=9.0 Hz), 130.7, 143.9, 156.4, 158.6 (d, J=230.6 Hz). HPLC purity: >99%, ESI-MS m/z 381 [M+H]$^+$.

***N*-(6-Fluorobenzo[*d*]thiazol-2-yl)furan-2-carboxamide (2o)** According to the same procedure used for **2a**, starting from furan-2-carboxylic acid (112 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (186 mg, 1.11 mmol), **2o** (167 mg, 64%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 6.78 (1H, dd, J=3.6, 1.6 Hz), 7.32 (1H, dt, J=8.8, 2.4 Hz), 7.75 (1H, d, J=3.6 Hz), 7.78 (1H, dd, J=8.8, 4.8 Hz), 7.93 (1H, dd, J=8.8, 2.4 Hz), 8.07 (1H, d, J=1.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.2 (d, J=27.2 Hz), 112.4, 114.3 (d, J=24.0 Hz), 117.2, 121.4 (d, J=8.2 Hz), 132.7 (d, J=13.3 Hz), 145.1 (d, J=5.0 Hz), 145.4, 147.7, 156.4, 158.2, 158.8 (d, J=249.7 Hz). HPLC purity: 98%, ESI-MS m/z 263 [M+H]$^+$.

5-Chloro-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)furan-2-carboxamide (2p) According to the same procedure used for **2a**, starting from 5-chlorofuran-2-carboxylic acid (147 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2p** (97 mg, 33%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 6.82–6.84 (1H, m), 7.32 (1H, ddt, J=8.8, 1.6 Hz), 7.76–7.79 (2H, m), 7.93 (1H, dt, J=8.8, 2.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.2 (d, J=27.3 Hz), 109.9, 114.3 (d, J=24.8 Hz), 119.3, 121.3 (d, J=9.5 Hz), 132.6, 132.7, 140.0, 145.1, 155.6, 158.3, 158.7 (d, J=239.8 Hz). HPLC purity: >99%, ESI-MS m/z 297 [M+H]$^+$.

5-Bromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)furan-2-carboxamide (2q) According to the same procedure used for **2a**, starting from 5-bromofuran-2-carboxylic acid (191 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2q** (78 mg, 23%) was obtained as beige powder. ^{1}H-NMR (DMSO-d_6) δ: 6.92 (1H, d, J=4.0 Hz), 7.32 (1H, dt, J=8.8, 2.4 Hz), 7.79–7.75 (2H, m), 7.93 (1H, dd, J=8.8, 2.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.2 (d, J=27.3 Hz), 114.4 (d, J=24.8 Hz), 114.6, 119.4, 121.4 (d, J=8.3 Hz), 127.8, 132.7 (d, J=8.3 Hz), 145.4, 147.3, 155.5, 158.2, 158.7 (d, J=239.8 Hz). HPLC purity: 97%, ESI-MS m/z 341 [M+H]$^+$.

***N*-(6-Fluorobenzo[*d*]thiazol-2-yl)thiazole-2-carboxamide (2r)** According to the same procedure used for **2a**, starting from thiazole-2-carboxylic acid (129 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (186 mg, 1.11 mmol), **2r** (46 mg, 17%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.34 (1H, dt, J=8.8, 2.4 Hz), 7.82 (1H, dd, J=8.8, 4.8 Hz), 7.96 (1H, dd, J=8.8, 2.4 Hz), 8.19 (1H, d, J=2.8 Hz), 8.25 (1H, d, J=2.8 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.3 (d, J=27.3 Hz), 114.5 (d, J=24.8 Hz), 121.6, 127.8, 132.7 (d, J=8.3 Hz), 132.9, 144.7, 157.9, 158.9 (d, J=240.5 Hz), 159.3, 160.9. HPLC purity: >99%, ESI-MS m/z 280 [M+H]$^+$.

***N*-(6-Fluorobenzo[*d*]thiazol-2-yl)benzo[*b*]thiophene-2-carboxamide (2s)** According to the same procedure used for **2a**, starting from benzo[*b*]thiophene-2-carboxylic acid (179 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (186 mg, 1.11 mmol), **2s** (139 mg, 42%) was obtained as white powder. mp: 235–236°C. ^{1}H-NMR (DMSO-d_6) δ: 7.34 (1H, dt, J=8.8, 2.4 Hz), 7.48–7.57 (2H, m), 7.81 (1H, dd, J=8.8, 4.8 Hz), 7.95 (1H, dd, J=8.8, 2.4 Hz), 8.03 (1H, d, J=7.2 Hz), 8.10 (1H, d, J=8.8 Hz), 8.64 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 108.3 (d, J=26.4 Hz), 114.4 (d, J=24.8 Hz), 121.3 (d, J=10.7 Hz), 122.9, 125.3, 126.0, 127.2, 128.5, 132.6 (d, J=9.1 Hz), 137.1 (d, J=4.9 Hz), 139.0, 141.1, 144.9, 158.7 (d, J=239.8 Hz), 158.8, 161.6. HPLC purity: >99%, ESI-MS m/z 329 [M+H]$^+$.

2-Bromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-3-carboxamide (2t) A mixture of 2-bromothiophene-3-carboxylic acid (207 mg, 1.10 mmol), 6-fluorobenzo[*d*]thiazol-2-amine (186 mg, 1.11 mmol), DMAP (183 mg, 1.50 mmol) and EDC·HCl (288 mg, 1.50 mmol) in CH_2Cl_2 (2 mL) was stirred overnight at rt. The reaction mixture was diluted with CH_2Cl_2 and washed with 2 N HCl, H_2O, and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was washed several times with CH_2Cl_2 and MeOH to give **2t** (72 mg, 20%) as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.33 (1H, ddt, J=8.8, 2.4, 0.8 Hz), 7.61 (1H, dd, J=6.0, 1.2 Hz), 7.75 (1H, dd, J=6.0, 1.2 Hz), 7.80 (1H, dd, J=8.8, 4.8 Hz), 7.94 (1H, dd, J=8.8, 2.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.2 (d, J=27.2 Hz), 114.4 (d, J=24.8 Hz), 117.5, 121.6, 128.0, 128.1, 132.7 (d, J=10.7 Hz), 133.0, 145.1 (d, J=10.7 Hz), 158.2, 158.8 (d, J=239.8 Hz), 161.3. HPLC purity: >99%, ESI-MS m/z 357 [M+H]$^+$.

4-Bromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-3-carboxamide (2u) According to the same procedure used for **2t**, starting from 4-bromothiophene-3-carboxylic acid (207 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2u** (134 mg, 37%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.33 (1H, dt, J=8.8, 2.4 Hz), 7.80 (1H, dd, J=8.8, 4.8 Hz), 7.86 (1H, dd, J=3.6, 1.2 Hz), 7.94 (1H, dd, J=8.8, 2.4 Hz), 8.48 (1H, dd, J=3.6, 1.2 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.2 (d, J=26.4 Hz), 109.0, 114.3 (d, J=24.8 Hz), 121.6 (d, J=9.1 Hz), 126.5, 132.7 (d, J=10.7 Hz), 132.8, 132.9, 145.1, 158.2, 158.7 (d, J=239.7 Hz), 161.2. HPLC purity: >99%, ESI-MS m/z 357 [M+H]$^+$.

5-Bromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-3-carboxamide (2v) According to the same procedure used

for **2d**, starting from 5-bromothiophene-3-carboxylic acid (207 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (186 mg, 1.11 mmol), **2v** (278 mg, 78%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.32 (1H, dt, J=8.8, 2.4 Hz), 7.79 (1H, dd, J=8.8, 4.8 Hz), 7.87 (1h, d, J=1.6 Hz), 7.93 (1H, dd, J=8.8, 2.4 Hz), 8.64 (1H, d, J=1.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.2 (d, J=27.2 Hz), 112.8, 114.3 (d, J=24.8 Hz), 121.5 (d, J=9.1 Hz), 129.8, 132.8 (d, J=11.5 Hz), 134.6, 135.1, 145.2, 158.4, 158.7 (d, J=239.8 Hz), 159.9. HPLC purity: >99%, ESI-MS m/z 357 $[M+H]^+$.

2,5-Dibromo-*N*-(6-fluorobenzo[*d*]thiazol-2-yl)thiophene-3-carboxamide (2w) According to the same procedure used for **2d**, starting from 2,5-dibromothiophene-3-carboxylic acid (286 mg, 1.00 mmol) and 6-fluorobenzo[*d*]thiazol-2-amine (185 mg, 1.10 mmol), **2w** (313 mg, 72%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.33 (1H, dt, J=8.8, 2.4 Hz), 7.78–7.82 (2H, m), 7.94 (1H, dd, J=8.8, 2.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.2 (d, J=27.2 Hz), 111.3, 114.4 (d, J=24.8 Hz), 117.0, 121.6 (d, J=9.8 Hz), 131.1, 132.7 (d, J=11.5 Hz), 134.2, 144.7, 158.1, 158.8 (d, J=239.7 Hz), 160.2. HPLC purity: >99%, ESI-MS m/z 435 $[M+H]^+$.

***N*-(Benzo[*d*]thiazol-2-yl)-5-bromothiophene-2-carboxamide (3a)** A mixture of 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol), benzo[*d*]thiazol-2-amine (166 mg, 1.10 mmol), DMAP (183 mg, 1.50 mmol) and EDC·HCl (288 mg, 1.50 mmol) in CH_2Cl_2 (2 mL) was stirred overnight at rt. CH_2Cl_2 and 2 N HCl were added, the precipitate was filtered and washed several times with CH_2Cl_2 and MeOH. The residue was purified by NH-silica-gel column chromatography (eluent: CH_2Cl_2/MeOH=4 : 1 to 10 : 3) to give **3a** (106 mg, 31%) as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.34 (1H, dt, J=8.4, 1.6 Hz), 7.43 (1H, d, J=3.6 Hz), 7.47 (1H, dt, J=8.4, 1.6 Hz), 7.74 (1H, d, J=8.0 Hz), 8.00 (1H, d, J=8.0 Hz), 8.06 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 119.9, 121.9, 123.7, 126.4, 130.8, 131.2, 132.1, 132.2, 139.5, 152.3, 160.3, 160.7. HPLC purity: >99%, ESI-MS m/z 339 $[M+H]^+$.

5-Bromo-*N*-(6-chlorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3b) According to the same procedure used for **1**, starting from 5-bromothiophene-2-carboxylic acid (205 mg, 0.99 mmol) and 6-chlorobenzo[*d*]thiazol-2-amine (202 mg, 1.10 mmol), **3b** (143 mg, 38%) was obtained as light yellow powder. mp: 254–256°C. ^{1}H-NMR (DMSO-d_6) δ: 7.43 (1H, d, J=3.6 Hz), 7.48 (1H, dd, J=8.4, 1.6 Hz), 7.74 (1H, d, J=8.4 Hz), 8.09 (1H, d, J=3.6 Hz), 8.15 (1H, d, J=1.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 120.3, 121.1, 121.5, 126.6, 127.8, 132.3, 132.4, 132.9, 138.9, 150.4, 158.4, 159.9. HPLC purity: >99%, ESI-MS m/z 373 $[M+H]^+$.

5-Bromo-*N*-(6-bromobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3c) According to the same procedure used for **2a**, starting from 5-bromothiophene-2-carboxylic acid (209 mg, 1.01 mmol) and 6-bromobenzo[*d*]thiazol-2-amine (256 mg, 1.10 mmol), **3c** (40 mg, 10%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.43 (1H, d, J=3.6 Hz), 7.60 (1H, dd, J=8.4, 1.6 Hz), 7.69 (1H, d, J=8.0 Hz), 8.09 (1H, s), 8.28 (1H, d, J=1.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 115.7, 120.3, 121.6, 121.8, 124.3, 129.3, 129.6, 132.3, 133.4, 138.9, 147.8, 159.8. HPLC purity: 97%, ESI-MS m/z 417 $[M+H]^+$.

5-Bromo-*N*-(5-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3d) According to the same procedure used for **2a**, starting from 5-bromothiophene-2-carboxylic acid (206 mg, 1.00 mmol) and 5-fluorobenzo[*d*]thiazol-2-amine (191 mg, 1.10 mmol), **3d** (30 mg, 8%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.23 (1H, dt, J=8.8, 2.4 Hz), 7.44 (1H, d, J=3.6 Hz), 7.59 (1H, d, J=9.6 Hz), 8.04 (1H, dd, J=8.8, 5.6 Hz), 8.11 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 106.3, 111.8 (d, J=24.0 Hz), 120.3, 123.2 (d, J=9.9 Hz), 127.1 (d, J=4.1 Hz), 132.3, 132.6, 138.9 (d, J=6.6 Hz), 148.7, 159.4, 161.0, 161.4 (d, J=239.7 Hz). HPLC purity: >99%, ESI-MS m/z 357 $[M+H]^+$.

5-Bromo-*N*-(5-chlorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3e) According to the same procedure used for **2d**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 5-chlorobenzo[*d*]thiazol-2-amine (203 mg, 1.10 mmol), **3e** (214 mg, 57%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.37 (1H, dd, J=8.8, 1.6 Hz), 7.43 (1H, d, J=3.6 Hz), 7.81 (1H, s), 8.04 (1H, d, J=8.8 Hz), 8.10 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 119.5, 120.3, 123.4, 123.7, 130.1, 130.9, 132.2, 132.3, 138.8, 149.0, 159.8, 160.8. HPLC purity: >99%, ESI-MS m/z 373 $[M+H]^+$.

5-Bromo-*N*-(5-bromobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3f) According to the same procedure used for **2f**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 5-bromobenzo[*d*]thiazol-2-amine (252 mg, 1.10 mmol), **3f** (220 mg, 53%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.43 (1H, d, J=3.6 Hz), 7.49 (1H, ddd, J=8.4, 1.6, 0.8 Hz), 7.95 (1H, s), 7.98 (1H, d, J=8.4 Hz), 8.10 (1H, d, J=3.6 Hz). ^{13}C-NMR (DMSO-d_6) δ: 119.0, 120.4, 122.4, 123.7, 126.4, 130.5, 132.3, 132.4, 138.8, 149.4, 159.9, 160.3. HPLC purity: >99%, ESI-MS m/z 417 $[M+H]^+$.

5-Bromo-*N*-(4-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3g) According to the same procedure used for **2a**, starting from 5-bromothiophene-2-carboxylic acid (212 mg, 1.02 mmol) and 4-fluorobenzo[*d*]thiazol-2-amine (186 mg, 1.11 mmol), **3g** (52.4 mg, 15%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.39 (1H, d, J=3.6 Hz), 7.53 (1H, m), 7.73 (1H, dd, J=8.4, 1.6 Hz), 7.78 (1H, t, J=8.4 Hz), 7.88 (1H, d, J=3.6 Hz), 10.42 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 111.3, 118.3, 118.5 (d, J=2.5 Hz), 122.2 (d, J=8.3 Hz), 126.6 (d, J=12.4 Hz), 126.9 (d, J=4.2 Hz), 128.0, 130.8, 131.9, 140.3, 155.1 (d, J=252.1 Hz), 158.9. HPLC purity: >99%, ESI-MS m/z 357 $[M+H]^+$.

5-Bromo-*N*-(4-chlorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3h) According to the same procedure used for **2d**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 4-chlorobenzo[*d*]thiazol-2-amine (204 mg, 1.10 mmol), **3h** (132 mg, 35%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.33 (1H, dt, J=8.4, 0.4 Hz), 7.44 (1H, dd, J=4.0, 0.4 Hz), 7.55 (1H, d, J=8.0 Hz), 8.00 (1H, d, J=8.0 Hz), 8.21 (1H, d, J=4.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 120.6, 120.8, 124.4, 124.6, 129.3, 132.4, 132.6, 133.3, 138.4, 145.4, 159.3, 159.4. HPLC purity: >99%, ESI-MS m/z 373 $[M+H]^+$.

5-Bromo-*N*-(4-bromobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3i) According to the same procedure used for **2t**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 4-bromobenzo[*d*]thiazol-2-amine (253 mg, 1.10 mmol), **3i** (257 mg, 61%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.26 (1H, t, J=8.4 Hz), 7.44 (1H, d, J=4.0 Hz), 7.70 (1H, d, J=8.0 Hz), 8.03 (1H, d, J=8.0 Hz), 8.22 (1H, d, J=4.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 113.6, 120.6, 121.3, 125.0, 129.4, 132.4, 132.6, 132.7, 138.4,

146.7, 159.1, 159.5. HPLC purity: >99%, ESI-MS *m/z* 417 $[M+H]^+$.

5-Bromo-*N*-(4,6-difluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3j) According to the same procedure used for **2a**, starting from 5-bromothiophene-2-carboxylic acid (212 mg, 1.02 mmol) and 4,6-difluorobenzo[*d*]thiazol-2-amine (205 mg, 1.11 mmol), **3j** (95 mg, 25%) was obtained as white powder. mp: 252–254°C. ^{1}H-NMR (DMSO-d_6) δ: 7.31 (1H, dt, J=10.4, 2.4 Hz), 7.40 (1H, d, J=4.0 Hz), 7.76 (1H, dd, J=8.4, 1.6 Hz), 8.11 (1H, d, J=4.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 101.6 (dd, J=28.9, 22.4 Hz), 104.0 (dd, J=26.5, 4.2 Hz), 120.0 (d, J=5.8 Hz), 131.9, 132.2, 133.6 (d, J=8.2 Hz), 134.7 (dd, J=11.9, 6.6 Hz), 138.1 (d, J=9.9 Hz), 153.1 (dd, J=254.2, 12.8 Hz), 157.9 (dd, J=243.2, 10.7 Hz), 158.4, 158.5. HPLC purity: >99%, ESI-MS *m/z* 375 $[M+H]^+$.

5-Bromo-*N*-(4,6-dichlorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3k) According to the same procedure used for **2d**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 4,6-dichlorobenzo[*d*]thiazol-2-amine (241 mg, 1.10 mmol), **3k** (276 mg, 68%) was obtained as light yellow powder. ^{1}H-NMR (DMSO-d_6) δ: 7.44 (1H, d, J=4.0 Hz), 7.67 (1H, d, J=1.6 Hz), 8.16 (1H, d, J=1.6 Hz), 8.19 (1H, d, J=4.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 120.6, 120.8, 125.0, 126.1, 127.7, 132.4, 132.7, 134.3, 138.3, 144.6, 159.6, 160.2. HPLC purity: >99%, ESI-MS *m/z* 407 $[M+H]^+$.

5-Bromo-*N*-(4-bromo-6-fluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3l) According to the same procedure used for **3a**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 4-bromo-6-fluoro benzo[*d*]thiazol-2-amine (272 mg, 1.10 mmol), **3l** (303 mg, 69%) was obtained as light yellow powder. mp: 231°C. ^{1}H-NMR (DMSO-d_6) δ: 7.44 (1H, d, J=4.0 Hz), 7.69 (1H, dd, J=8.8, 2.4 Hz), 8.00 (1H, dd, J=8.8, 2.4 Hz), 8.20 (1H, d, J=4.0 Hz), 8.32 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 107.9 (d, J=26.4 Hz), 113.5 (d, J=10.7 Hz), 117.7 (d, J=28.1 Hz), 120.6, 132.3, 132.6, 133.2 (d, J=11.5 Hz), 138.3, 143.8, 158.0 (d, J=243.9 Hz), 159.0, 159.5. HPLC purity: >99%, ESI-MS *m/z* 435 $[M+H]^+$.

5-Bromo-*N*-(5,6-difluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3m) According to the same procedure used for **2d**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.02 mmol) and 5,6-difluorobenzo[*d*]thiazol-2-amine (205 mg, 1.11 mmol), **3m** (210 mg, 56%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.43 (1H, dd, J=4.0, 1.6 Hz), 7.84 (1H, dd, J=11.2, 7.2 Hz), 8.11 (1H, d, J=4.0 Hz), 8.16 (1H, t, J=8.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 108.3 (d, J=13.3 Hz), 110.0 (dd, J=23.0, 5.8 Hz), 120.5, 127.2 (d, J=6.6 Hz), 132.2, 132.3, 132.4, 138.5, 147.0 (dd, J=242.7, 14.5 Hz), 149.2 (dd, J=242.7, 14.5 Hz), 159.4, 160.3. HPLC purity: >99%, ESI-MS *m/z* 375 $[M+H]^+$.

5-Bromo-*N*-(6-methylbenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3n) According to the same procedure used for **3a**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 6-methylbenzo[*d*]thiazol-2-amine (180 mg, 1.10 mmol), **3n** (92 mg, 26%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 2.42 (3H, s), 7.28 (1H, d, J=8.0 Hz), 7.41 (1H, d, J=4.0 Hz), 7.62 (1H, d, J=8.0 Hz), 7.78 (1H, s), 8.04 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 21.0, 119.3, 119.7, 121.5, 127.6, 131.1, 131.9, 132.2, 133.2, 139.6, 139.7, 159.4, 162.6. HPLC purity: >99%, ESI-MS *m/z* 353 $[M+H]^+$.

5-Bromo-*N*-(4-methylbenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3o) According to the same procedure used for **2a**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 4-methylbenzo[*d*]thiazol-2-amine (181 mg, 1.10 mmol), **3o** (159 mg, 45%) was obtained as light yellow powder. ^{1}H-NMR (DMSO-d_6) δ: 2.62 (3H, s), 7.21–7.29 (2H, m), 7.43 (1H, d, J=4.0 Hz), 7.81 (1H, d, J=8.0 Hz), 8.17 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 18.1, 119.1, 120.2, 123.7, 126.8, 129.9, 131.3, 132.1, 132.3, 138.8, 147.7, 157.3, 159.2. HPLC purity: >99%, ESI-MS *m/z* 353 $[M+H]^+$.

5-Bromo-*N*-(5,6-dimethylbenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3p) According to the same procedure used for **3a**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 5,6-dimethylbenzo[*d*]thiazol-2-amine (196 mg, 1.10 mmol), **3p** (121 mg, 33%) was obtained as beige powder. ^{1}H-NMR (DMSO-d_6) δ: 2.32 (3H, s), 2.33 (3H, s), 7.41 (1H, d, J=4.0 Hz), 7.52 (1H, s), 7.72 (1H, s), 8.02 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 19.5, 19.7, 119.6, 121.7, 128.0, 128.2, 131.8, 132.2, 132.6, 135.1, 139.2, 139.7, 159.5, 160.2. HPLC purity: >99%, ESI-MS *m/z* 367 $[M+H]^+$.

5-Bromo-*N*-(6-methoxybenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3q) According to the same procedure used for **1**, starting from 5-bromothiophene-2-carboxylic acid (208 mg, 1.01 mmol) and 6-methoxylbenzo[*d*]thiazol-2-amine (205 mg, 1.14 mmol), **3q** (143 mg, 39%) was obtained as light yellow powder. ^{1}H-NMR (DMSO-d_6) δ: 3.82 (3H, s), 7.06 (1H, dd, J=8.8, 2.4 Hz), 7.42 (1H, d, J=4.0 Hz), 7.60 (1H, d, J=2.4 Hz), 7.64 (1H, d, J=8.8 Hz), 8.06 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 55.6, 104.9, 115.1, 119.8, 120.4, 120.7, 131.9, 132.2, 132.4, 139.4, 156.3, 156.7, 160.9. HPLC purity: >99%, ESI-MS *m/z* 369 $[M+H]^+$.

5-Bromo-*N*-(4-methoxybenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3r) According to the same procedure used for **3a**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 4-methoxylbenzo[*d*]thiazol-2-amine (198 mg, 1.10 mmol), **3r** (170 mg, 46%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 3.93 (3H, s), 7.02 (1H, d, J=8.0 Hz), 7.29 (1H, t, J=8.0 Hz), 7.43 (1H, d, J=4.0 Hz), 7.55 (1H, d, J=8.0 Hz), 8.12 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 55.7, 107.6, 113.4, 120.1, 124.8, 132.0, 132.3, 132.9, 138.4, 138.8, 151.9, 156.6, 159.1. HPLC purity: >99%, ESI-MS *m/z* 369 $[M+H]^+$.

5-Bromo-*N*-(6-ethoxybenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3s) According to the same procedure used for **2b**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 6-ethoxylbenzo[*d*]thiazol-2-amine (214 mg, 1.10 mmol), **3s** (163 mg, 43%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 1.36 (3H, t, J=2.8 Hz), 4.08 (2H, q, J=2.8 Hz), 7.05 (1H, ddd, J=8.4, 2.4, 0.8 Hz), 7.42 (1H, d, J=4.0 Hz), 7.58 (1H, d, J=2.4 Hz), 7.63 (1H, d, J=8.4 Hz), 8.06 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 14.7, 63.6, 105.5, 115.5, 119.8, 120.6, 131.9, 132.2, 132.5, 139.4, 142.4, 155.5, 156.7, 159.5. HPLC purity: >99%, ESI-MS *m/z* 383 $[M+H]^+$.

5-Bromo-*N*-(6-nitrobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3t) According to the same procedure used for **2d**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 6-nitrobenzo[*d*]thiazol-2-amine (215 mg, 1.10 mmol), **3t** (339 mg, 88%) was obtained as yellow powder. ^{1}H-NMR (DMSO-d_6) δ: 7.40 (1H, d, J=4.0 Hz), 7.88 (1H, d, J=8.8 Hz), 8.09 (1H, d, J=4.0 Hz), 8.28 (1H, dd, J=8.8, 2.4 Hz), 8.99 (1H, d, J=2.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 118.6, 119.6, 120.2, 121.5, 131.7, 131.9, 132.5, 138.3, 143.0, 152.1, 160.1, 164.0. HPLC purity: 96%, ESI-MS *m/z* 382 $[M-H]^-$.

5-Bromo-*N*-(6-(trifluoromethyl)benzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3u) A mixture of 5-bromothiophene-2-carboxylic acid (208 mg, 1.00 mmol), 6-(trifluoromethyl)-benzo[*d*]thiazol-2-amine (241 mg, 1.10 mmol), DMAP (184 mg, 1.50 mmol) and EDC·HCl (288 mg, 1.50 mmol) in CH_2Cl_2 (2 mL) was stirred overnight at rt. CH_2Cl_2 and 2 N HCl were added, the precipitate was washed several times with CH_2Cl_2 and MeOH. The residue was purified by preparative layer chromatography (eluent: $CHCl_3$/MeOH=30 : 1) to give **3u** (184 mg, 70%) as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.44 (1H, d, J=4.0 Hz), 7.77 (1H, dd, J=8.0, 1.6 Hz), 7.92 (1H, d, J=8.0 Hz), 8.12 (1H, s), 8.51 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 120.3 (d, J=4.1 Hz), 120.4, 120.6, 123.2 (d, J=4.1 Hz), 123.9 (q, J=32.2 Hz), 125.9, 128.6, 131.9, 132.4, 132.5, 132.7, 138.7, 165.2. HPLC purity: >99%, ESI-MS m/z 407 $[M+H]^+$.

5-Bromo-*N*-(6-(trifluoromethoxy)benzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3v) According to the same procedure used for **2a**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 6-(trifluoromethoxyl)-benzo[*d*]thiazol-2-amine (258 mg, 1.10 mmol), **3v** (22 mg, 5%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 7.43–7.47 (2H, m), 7.84 (1H, dt, J=8.8 Hz), 8.11 (1H, d, J=3.2 Hz), 8.15 (1H, d, J=2.4 Hz). ^{13}C-NMR (DMSO-d_6) δ: 115.1, 120.0, 120.2 (q, J=256.2 Hz), 120.3, 121.1, 132.3, 132.4, 132.5 (d, J=5.7 Hz), 138.8 (d, J=6.6 Hz), 144.2, 144.9, 160.0, 160.3. HPLC purity: >99%, ESI-MS m/z 423 $[M+H]^+$.

Ethyl 2-(5-Bromothiophene-2-carboxamido)benzo[*d*]thiazole-6-carboxylate (3w) According to the same procedure used for **2a**, starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and ethyl 2-aminobenzo[*d*]thiazole-6-carboxylate (244 mg, 1.10 mmol), **3w** (184 mg, 45%) was obtained as white powder. ^{1}H-NMR (DMSO-d_6) δ: 1.36 (3H, t, J=3.2 Hz), 4.35 (2H, q, J=3.2 Hz), 7.43 (1H, d, J=4.0 Hz), 7.82 (1H, d, J=8.0 Hz), 8.03 (1H, dd, J=8.0, 1.6 Hz), 8.10 (1H, s), 8.65 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 14.2, 60.7, 119.7, 120.4, 120.5, 123.9, 125.0, 127.2, 131.3, 132.3, 132.4, 138.7, 138.8, 159.8, 165.4. HPLC purity: >99%, ESI-MS m/z 411 $[M+H]^+$.

5-Bromo-*N*-(6-(*tert*-butyl)benzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (3x) According to the same procedure used for **3u** (eluent : $CHCl_3$/MeOH=40 : 1), starting from 5-bromothiophene-2-carboxylic acid (207 mg, 1.00 mmol) and 6-(*tert*-butyl)benzo[*d*]thiazol-2-amine (227 mg, 1.10 mmol), **3x** (225 mg, 57%) was obtained as light yellow powder. ^{1}H-NMR (DMSO-d_6) δ: 1.35 (9H, s), 7.42 (1H, d, J=3.6 Hz), 7.52 (1H, d, J=8.8 Hz), 7.64 (1H, s), 8.00 (1H, s), 8.05 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 31.4, 34.7, 118.0, 119.1, 119.8, 124.1, 131.0, 131.9, 132.2, 139.5, 146.7, 147.0, 159.7, 160.4. HPLC purity: 97%, ESI-MS m/z 395 $[M+H]^+$.

5-Chloro-*N*-(4,6-difluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (4a) According to the same procedure used for **2f**, starting from 5-chlorothiophene-2-carboxylic acid (163 mg, 1.00 mmol) and 4,6-difluorobenzo[*d*]thiazol-2-amine (205 mg, 1.10 mmol), **4a** (192 mg, 58%) was obtained as white powder. mp: 247–249°C. ^{1}H-NMR (DMSO-d_6) δ: 7.35 (1H, d, J=4.0 Hz), 7.40 (1H, dt, J=10.2, 2.4 Hz), 7.83 (1H, dd, J=8.4, 2.4 Hz), 8.21 (1H, d, J=4.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 102.4 (dd, J=28.9, 22.3 Hz), 104.5 (dd, J=26.4, 4.1 Hz), 129.0, 131.8, 133.8, 133.9 (dd, J=13.3, 4.1 Hz), 134.9 (dd, J=13.3, 4.1 Hz), 135.5, 136.5, 153.3 (d, J=250.4, 15.4 Hz), 158.2 (dd, J=242.2, 9.8 Hz), 158.6. HPLC purity: >99%, ESI-MS m/z 331 $[M+H]^+$.

3,5-Dibromo-*N*-(4,6-difluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (4b) According to the same procedure used for **2f**, starting from 3,5-dibromothiophene-2-carboxylic acid (286 mg, 1.00 mmol) and 4,6-difluorobenzo[*d*]-thiazol-2-amine (204 mg, 1.10 mmol), **4b** (270 mg, 60%) was obtained as white powder. mp: 245–246°C. ^{1}H-NMR (DMSO-d_6) δ: 7.43 (1H, dt, J=10.2, 2.0 Hz), 7.56 (1H, s), 7.83 (1H, dd, J=8.4, 2.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 102.2 (dd, J=28.0, 23.1 Hz), 104.7 (dd, J=26.4, 3.3 Hz), 114.3, 118.4, 131.4 (d, J=7.4 Hz), 134.3 (d, J=10.7 Hz), 134.9, 135.2, 152.7 (d, J=241.2, 20.7 Hz), 158.3 (dd, J=242.2, 10.7 Hz), 159.0, 159.7. HPLC purity: >99%, ESI-MS m/z 453 $[M+H]^+$.

3,5-Dichloro-*N*-(4,6-difluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (4c) According to the same procedure used for **3a** (eluent : $CHCl_3$/MeOH=20 : 1 to 4 : 1), starting from 3,5-dichlorothiophene-2-carboxylic acid (197 mg, 1.00 mmol) and 4,6-difluorobenzo[*d*]thiazol-2-amine (205 mg, 1.10 mmol), **4c** (165 mg, 45%) was obtained as white powder. mp: 224–225°C. ^{1}H-NMR (DMSO-d_6) δ: 7.29 (1H, dt, J=10.2, 2.4 Hz), 7.75 (1H, ddd, J=8.8, 2.4, 1.2 Hz), 8.26 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 101.5 (dd, J=28.1, 21.5 Hz), 103.9 (dd, J=26.4, 4.9 Hz), 123.8, 130.4, 130.8, 133.5 (dd, J=12.4, 4.2 Hz), 133.7, 134.7 (dd, J=12.4, 4.2 Hz), 153.0 (dd, J=254.6, 14.1 Hz), 158.0 (dd, J=243.0, 10.7 Hz), 158.3, 158.6. HPLC purity: 96%, ESI-MS m/z 365 $[M+H]^+$.

4,5-Dichloro-*N*-(4,6-difluorobenzo[*d*]thiazol-2-yl)thiophene-2-carboxamide (4d) According to the same procedure used for **2d**, starting from 4,5-dichlorothiophene-2-carboxylic acid (198 mg, 1.00 mmol) and 4,6-difluorobenzo[*d*]-thiazol-2-amine (205 mg, 1.10 mmol), **4d** (268 mg, 73%) was obtained as white powder. mp: 225–226°C. ^{1}H-NMR (DMSO-d_6) δ: 7.29 (1H, t, J=10.2 Hz), 7.75 (1H, d, J=8.0 Hz), 8.26 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 101.6 (dd, J=28.9, 22.3 Hz), 104.0 (dd, J=26.5, 4.9 Hz), 123.8, 130.4, 130.9, 133.4 (dd, J=13.3, 4.9 Hz), 133.6, 134.7 (dd, J=13.3, 4.9 Hz), 153.1 (dd, J=253.7, 13.3 Hz), 158.0 (dd, J=243.0, 10.7 Hz), 158.3, 158.6. HPLC purity: >99%, ESI-MS m/z 365 $[M+H]^+$.

Biological Assay

Material

Stock solutions (10 and 100 mM) of each compound were prepared in DMSO and kept at −20°C. Appropriate dilutions were freshly prepared just prior to each assay. MNV (S7 strain, kindly provided by Prof. Yukinobu Tohya, Department of Veterinary Medicine, Nihon University, Kanagawa, Japan) was propagated in RAW 264.7 cells (ATCC TIB-71; American Type Culture Collection, Manassas, VA, U.S.A.) cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (growth medium) or 2% (maintenance medium) heat-inactivated fetal bovine serum, antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B), 4500 mg/L D-glucose, 4 mM L-glutamine, 25 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES), 1 mM sodium pyruvate, and 15 mg/L phenol red at 37°C in a humidified atmosphere of 5% CO_2. The virus was titrated in RAW264.7 cells with a conventional assay as described previously.[16] After infection for 3 d, the cells were observed for CPE, and the $TCID_{50}$ was calculated by the Kärber formula. Cell debris was removed by centrifugation at 10000×***g*** for 1 h, and the supernatant was stored at −80°C until use. RAW264.7 cells (5.0×10^3 cells/well) were seeded in a 96-well plate in growth medium. After incubation for 24 h, cells were used for antiviral or cytotoxicity assay.

Measurement of Anti-norovirus Activity

Method A: Screening and CPE Reduction Assay

The antiviral activity of the compounds was determined by using a water-soluble tetrazolium salt (WST)-8 CPE reduction assay. 120 μL of the mixture containing 280 $TCID_{50}$ of MNV and a dilution series of compounds (0.0061–100 μM) with fetal bovine serum-free medium was incubated for 30 min. RAW264.7 cells were exposed to the mixture. After incubation for 1 h, the cells were washed, replaced with the maintenance medium and incubated for 3 d (*i.e.*, until complete CPE was observed in infected untreated cells). To quantify cell viability, WST-8 solution was added and the plates were incubated for 1 h. The absorbance was measured at 450 nm. The EC_{50} was defined as the compound concentration that protected 50% of the cells from virus-induced CPE.

Antiviral activity of **4b** was evaluated further at different time points. After infection with MNV for 1 h, RAW264.7 cells were treated with a dilution series of **4b**, 2′-CMC, and GC376 for 72 h.

The methods for screening and measuring the antiviral activity of the compounds were similar. However, for screening, 100 $TCID_{50}$/50 μL of MNV and 25 μM of the compounds were used and CPE was observed by microscope without measuring the absorbance.

Method B: $TCID_{50}$ Assay with Filtration

A total volume of 50 μL of the mixture containing 225 μM compound (**4b**, 2′-CMC, and GC376) in fetal bovine serum-free medium and 3150 $TCID_{50}$ of MNV was incubated at room temperature in a centrifugal filter tube (Amicon Ultra-0.5 (100 K), Merck Millipore). In an experiment of 0.02% (v/v) sodium hypochlorite, commercial breach in distilled water was used instead of compound solution. After 1 h, 450 μL of fetal bovine serum-free medium was added and spun at 20000×$\boldsymbol{g}$ for 1 min to eliminate compound. The wash step was repeated once more. MNV was recovered from the filter according to the manufacturer's instructions and was 5-fold serially diluted with fetal bovine serum-free medium. RAW264.7 cells in a 96-well plate were infected with the diluted virus solution. After 3 d, the cells were observed for CPE, and $TCID_{50}$ was calculated.

Cytotoxicity Assay The cytotoxicity of compounds was evaluated by the WST-8 assay in triplicate. RAW264.7 cells were exposed to 3–100 μM of each compound for 72 h. Control cells were treated with the maximum concentration of DMSO (0.1%) and the same incubation time. Cell viability was evaluated by the WST-8 method described in the previous section. CC_{50} was defined as the compound concentration that reduces the number of viable cells by 50%.

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Conflict of Interest The authors declare no conflict of interest.

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