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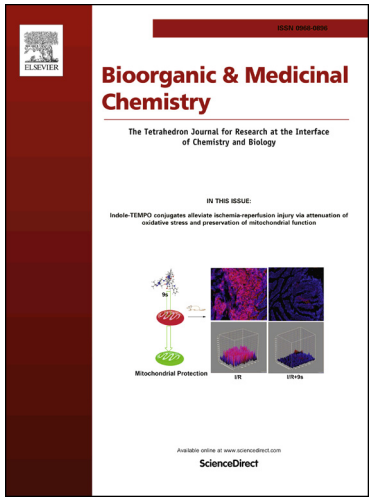

Design, synthesis, and pharmacological evaluation of 4-azolyl-benzamide derivatives as novel GPR52 agonists

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PII: S0968-0896(17)30110-4
DOI: http://dx.doi.org/10.1016/j.bmc.2017.03.064
Reference: BMC 13662

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 18 January 2017
Revised Date: 28 March 2017
Accepted Date: 29 March 2017

Please cite this article as: Tokumaru, K., Ito, Y., Nomura, I., Nakahata, T., Shimizu, Y., Kurimoto, E., Aoyama, K., Aso, K., Design, synthesis, and pharmacological evaluation of 4-azolyl-benzamide derivatives as novel GPR52 agonists, *Bioorganic & Medicinal Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.bmc.2017.03.064

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ABSTRACT

G protein-coupled receptor 52 (GPR52) agonists are expected to improve the symptoms of psychiatric disorders. During exploration for a novel class of GPR52 agonists with good pharmacokinetic profiles, we synthesized 4-(3-(3-fluoro-5-(trifluoromethyl)benzyl)-5-methyl-1*H*-1,2,4-triazol-1-yl)-2-methylbenzamide (**4u**; half maximal effective concentration (EC_{50}) = 75 nM, maximal response (E_{max}) = 122%) starting from a high-throughput screening hit **3** (EC_{50} = 470 nM, E_{max} = 56%). The structural features of a reported GPR52 agonist were applied to **3**, led to design 4-azolylbenzamides as novel GPR52 agonists. A structure–activity relationship study of 4-azolylbenzamide resulted in the design of the 1,2,4-triazole derivative **4u**, which demonstrated excellent bioavailability in rats (F = 53.8%). Oral administration of **4u** (10 mg/kg) significantly suppressed methamphetamine-induced hyperlocomotion in mice. Thus, **4u** is a promising lead compound for drug discovery research of GPR52 agonists.

KEYWORDS

GPCR; GRP52 agonist; Methamphetamine-induced hyperlocomotion; Metabolic stability; 1,2,4-Triazole.

ABBREVIATIONS

SAR, structure–activity relationship; cAMP, 3',5'-cyclic adenosine monophosphate; EC_{50}, half maximal effective concentration; MAP, methamphetamine; NMDA, *N*-methyl-*D*-

aspartate; PK, pharmacokinetic; CHO, Chinese hamster ovary; SEM, standard error of the mean; AUC, area under the curve.

1. INTRODUCTION

G protein-coupled receptors constitute one of the most common therapeutic targets in the central nervous system for treatment of psychiatric disorders[1, 2]. G protein-coupled receptor 52 (GPR52) gene was identified from a high-throughput genome database, and classified as an orphan Gs-coupled G protein-coupled receptor[3]. A detailed histological study of mice tissue has revealed that GPR52 is colocalized with dopamine D2 receptor in the mesolimbic system, and partially with dopamine D1 receptor in the prefrontal cortex[4]. Moreover, GPR52 knockout mice displayed psychosis-related behaviors, whereas GPR52 transgenic mice showed anti-psychiatric behaviors[4]. These results suggest that GPR52 agonists are likely to alleviate the symptoms of psychiatric disorders *via* induction of cAMP accumulation by canceling the dopamine D2 signal and activating the dopamine D1/*N*-methyl-*D*-aspartate (NMDA) signal.

In our previous study, the benzothiophene derivative **1** was developed as a potent and orally active GPR52 agonist[5], as shown in Figure 1. Oral administration of compound **1** (3 mg/kg) significantly suppressed methamphetamine-induced hyperactivity in mice without inducing cataleptogenic effects or extrapyramidal side effects, which may result from dopamine D2 blocking[6]. During exploration for new scaffolds of GPR52 agonists, a scaffold hopping study of compound **1** provided the thiazole derivative **2** (Figure 1), which possessed excellent physicochemical profile and brain penetration[7]. Furthermore, a unique

hit compound **3** was identified by an additional high-throughput screening campaign of our in-house compound library.

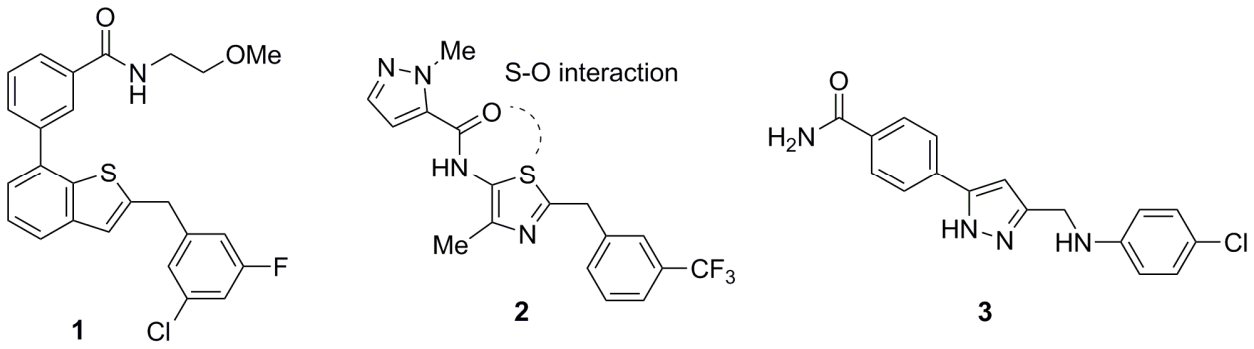


Figure 1. Reported GPR52 agonists **1**–**2** and a HTS hit compound **3**.

2. DESIGN AND SYNTHESIS

2.1. DESIGN

In a previous study, the thiazole derivative **2**[7] was developed by a scaffold hopping design strategy utilizing benzothiophene **1**[5]. The 2-benzyl-4-methylthiazole scaffold in compound **2** was identified as a highly promising structure with GPR52 agonistic activity. Further morphing of this scaffold was considered to provide an attractive series of GPR52 agonists with potent *in vitro* and *in vivo* activities and good pharmacokinetic (PK) profiles.

To understand the functionality of each moiety, various conformations of compounds **2** and **3** were generated by using LowMode MD method in MOE 2015.10[8]. Their superpositions are shown in Figure 2. It is noteworthy that (1) the pyrazole moiety in compound **3** overlapped well with the thiazole moiety in compound **2**; and (2) the benzamide of **3** could replace the pyrazole carboxamide of **2**. These findings led us to combine the azolylbenzamide motif and the 2-benzyl-4-methylthiazole motif, which

resulted in the design of a novel series of azolylbenzamide derivatives (Figure 3). This study describes, in detail, the biological activities of newly designed azolylbenzamides **4a**–**u**.

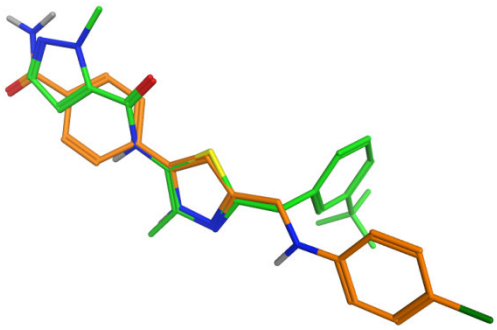


Figure 2. Superposed conformations obtained for compound **2**–**3** by LowMode MD conformational search with the MMFF94x force field. **2** (green), **3** (orange). The picture was generated using MOE 2015.10.

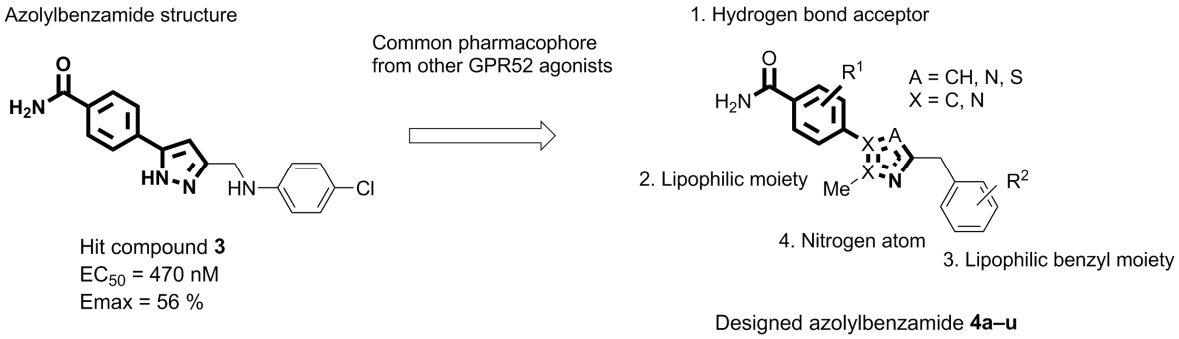
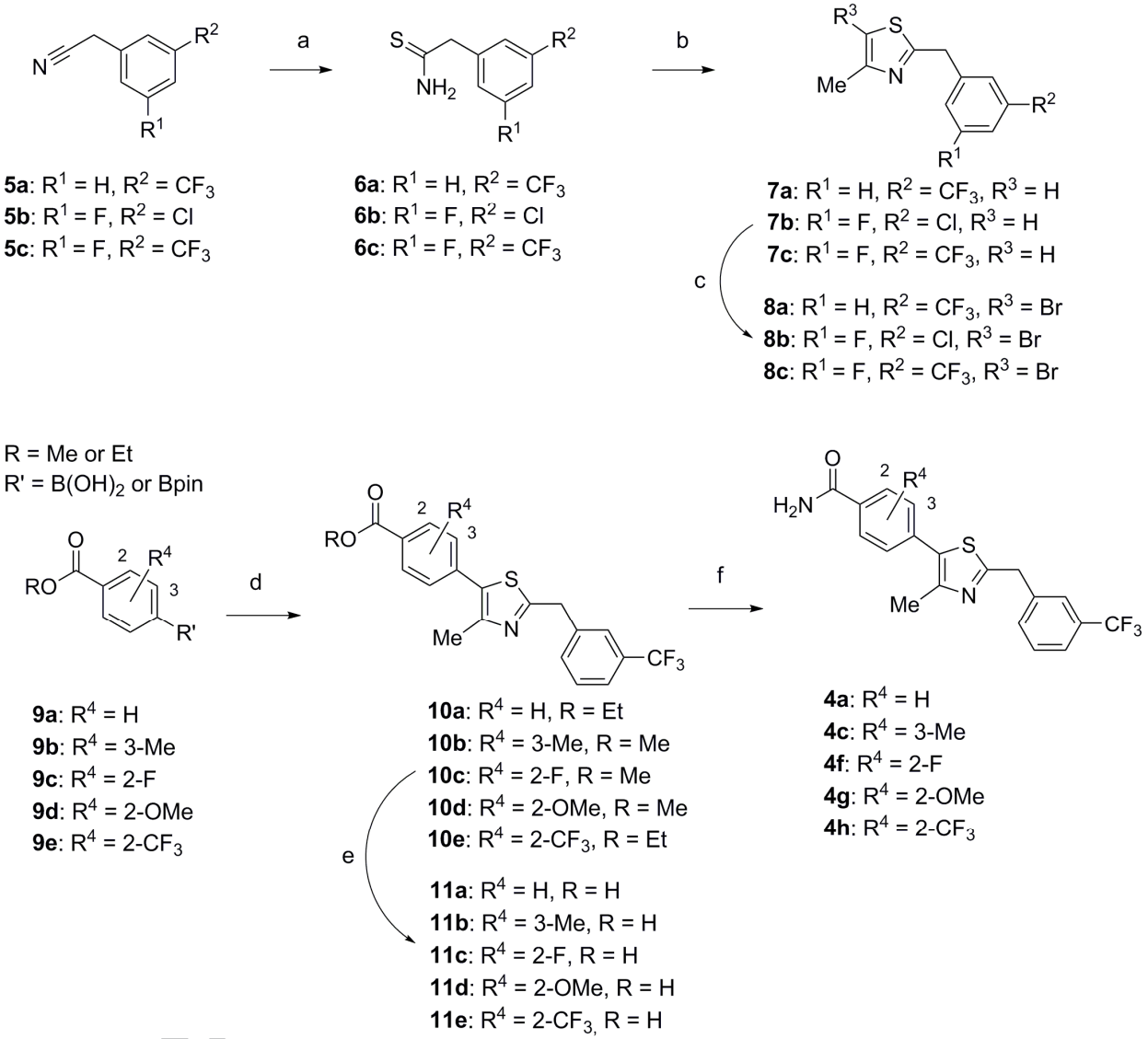


Figure 3. Design of azolylbenzamide **4a**–**u** from the hit compound **3**.

2.2. CHEMISTRY

The synthesis of thiazole analogs (**4a**–**r**), pyrazole analog **4s**, imidazole analog **4t**, and 1,2,4-triazole analog **4u** is described in Schemes 1–5, 6, 7 and 8, respectively. Regarding thiazole compounds (**4a**, **4c**, and **4f**–**h**), their synthesis was achieved by the conversion of carboxylic acids **11a**–**e** as shown in Scheme 1. Furthermore, hydrolysis of nitrile moiety was an important reaction for the preparation of the targets (Scheme 2 for compounds **4b** and **4q**–**r**; Scheme 3 for **4d**–**e**). In Scheme 1, commercially available arylmethyl cyanides **5a**–**c** were allowed to react with diethyl dithiophosphate to afford the thioamides **6a**–**c**. Synthesis of the 5-bromothiazole compounds **8a**–**c** was achieved by formation of thiazole ring *via* reaction of the thioamides **6a**–**c** with bromoacetone followed by bromination with *N*-bromosuccinimide (NBS). The targeted analogs (**4a**–**c**, **4f**–**h**, **4q**, and **4r**) were synthesized from the 5-bromothiazole compounds **8a**–**c** through the following multistep reactions: (A) Suzuki-Miyaura coupling for introduction of benzoic ester, hydrolysis of ester moiety, and condensation with ammonia, as shown in Scheme 1, and (B) Suzuki-Miyaura coupling for introduction of benzonitrile and hydrolysis of nitrile moiety, as shown in Schemes 2 and 3. The preparation of the 5-arylthiazole analogs, which possess an ethyl group (**4d**) or an isopropyl group (**4e**) at the 2-position of the benzene ring is described in Scheme 3. The intermediate **8a** was reacted with arylboronic acid pinacol ester to afford the phenolic analog **13**. Compound **13** was converted to the trifluoromethanesulfonate **14** followed by Suzuki-Miyaura coupling reaction with vinyl or 1-propenylboronic acid pinacol ester, heterogeneous catalytic hydrogenation, and hydrolysis of nitrile to afford the ethyl or isopropyl analogs, **4d** and **4e**.

Scheme 1. Preparation of 4-arylthiazole analogs 4a, 4c, 4f–h[a]



5a: R^1 = H, R^2 = CF_3
5b: R^1 = F, R^2 = Cl
5c: R^1 = F, R^2 = CF_3

6a: R^1 = H, R^2 = CF_3
6b: R^1 = F, R^2 = Cl
6c: R^1 = F, R^2 = CF_3

7a: R^1 = H, R^2 = CF_3, R^3 = H
7b: R^1 = F, R^2 = Cl, R^3 = H
7c: R^1 = F, R^2 = CF_3, R^3 = H

8a: R^1 = H, R^2 = CF_3, R^3 = Br
8b: R^1 = F, R^2 = Cl, R^3 = Br
8c: R^1 = F, R^2 = CF_3, R^3 = Br

9a: R^4 = H
9b: R^4 = 3-Me
9c: R^4 = 2-F
9d: R^4 = 2-OMe
9e: R^4 = 2-CF_3

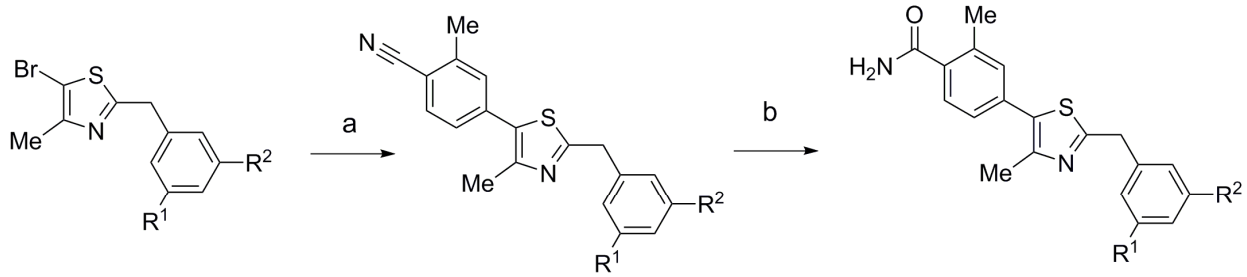
10a: R^4 = H, R = Et
10b: R^4 = 3-Me, R = Me
10c: R^4 = 2-F, R = Me
10d: R^4 = 2-OMe, R = Me
10e: R^4 = 2-CF_3, R = Et

11a: R^4 = H, R = H
11b: R^4 = 3-Me, R = H
11c: R^4 = 2-F, R = H
11d: R^4 = 2-OMe, R = H
11e: R^4 = 2-CF_3, R = H

4a: R^4 = H
4c: R^4 = 3-Me
4f: R^4 = 2-F
4g: R^4 = 2-OMe
4h: R^4 = 2-CF_3

[a]Reagents and conditions: (a) diethyl dithiophosphate, HCl, EtOAc, 0°C to rt; (b) bromoacetone, EtOH, reflux; (c) NBS, MeCN, 0°C to rt; (d) **8a**, $Pd(Ph_3P)_4$, Na_2CO_3, DME–water, 65–80°C; or, **8a**, $Pd_2(dba)_3$, X-Phos, Cs_2CO_3, DME-water, 50°C; or, **8a**, $PdCl_2(Ph_3P)_2$, K_2CO_3, DME-water, 85°C; (e) NaOH aq., EtOH–THF or MeOH–THF, 60–80°C; (f) EDCI, HOBt-NH_3, DMF, rt.

Scheme 2. Preparation of 4-arylthiazole analogs 4b, 4q–r[a]



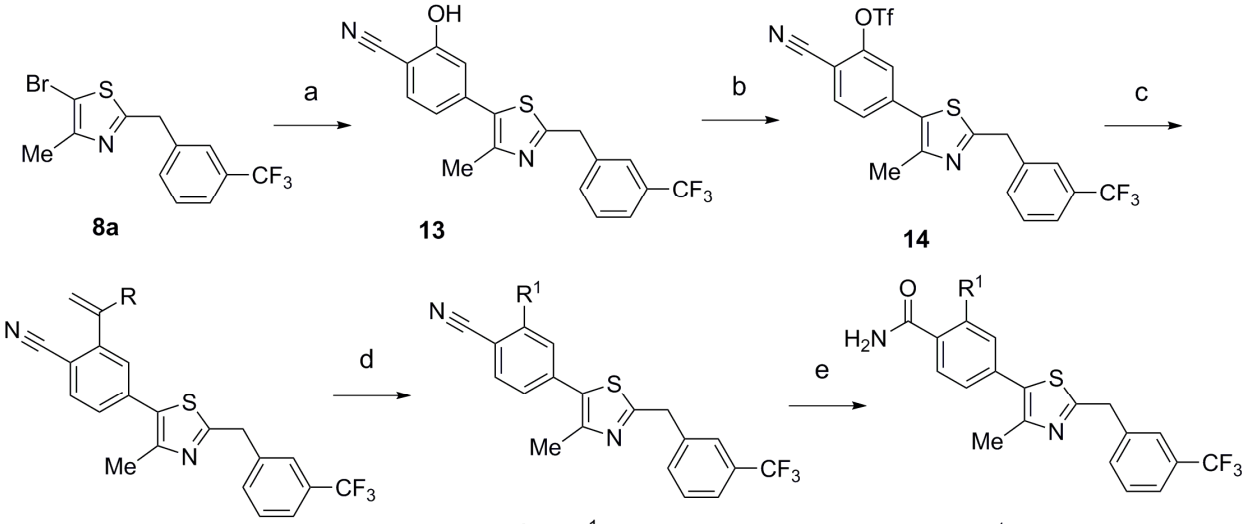
8a: R^1 = H, R^2 = CF_3
8b: R^1 = F, R^2 = Cl
8c: R^1 = F, R^2 = CF_3

12a: R^1 = H, R^2 = CF_3
12b: R^1 = F, R^2 = Cl
12c: R^1 = F, R^2 = CF_3

4b: R^1 = H, R^2 = CF_3
4q: R^1 = F, R^2 = Cl
4r: R^1 = F, R^2 = CF_3

[a]Reagents and conditions: (a) 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile, $Pd_2(dba)_3$, X-Phos, Cs_2CO_3, DME-water, 50°C; (b) H_2O_2 aq., K_2CO_3, DMSO, 0°C to rt; or H_2SO_4, 80°C.

Scheme 3. Preparation of 4-arylthiazole analogs 4d–e[a]



15a: R = H
15b: R = Me

16a: R^1 = Et
16b: R^1 = iPr

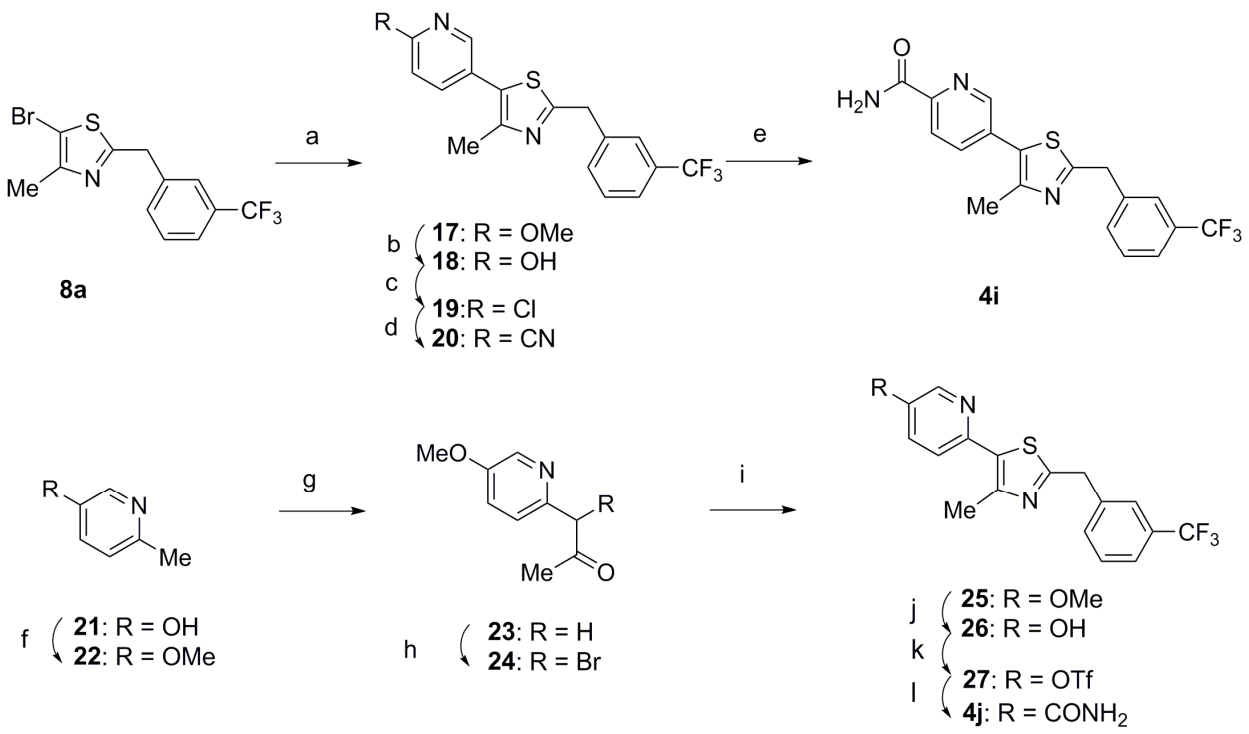
4d: R^1 = Et
4e: R^1 = iPr

[a]Reagents and conditions: (a) 2-hydroxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile, $PdCl_2(Ph_3P)_2$, K_2CO_3, DME–water, 85°C; (b) *N*-phenylbis(trifluoromethanesulfonimide), Cs_2CO_3, DMF, rt; (c) vinylboronic acid pinacol ester for **15a** or isopropenylboronic acid pinacol ester for **15b**, $PdCl_2$(dppf) or $PdCl_2(Ph_3P)_2$,

K_2CO_3, DME–water, 85°C; (d) H_2 (760 Torr), palladium on carbon, THF–MeOH, rt; (e) H_2O_2 aq., K_2CO_3, DMSO, 0°C to rt.

Synthesis of 4-(3-pyridiyl)-thiazole and 4-(2-pyridyl) thiazole analogs is described in Scheme 4. 4-(6-Methoxy-3-pyridiyl)-thiazole **17** was prepared from the intermediate **8a**. Conversion of its methoxy group into a nitrile group afforded compound **20**. This conversion was achieved in three steps; acidic hydrolysis with hydrogen bromide, chlorination with phosphorus oxychloride, and palladium-catalyzed cyanation. Basic hydrolysis of the nitrile in **20** afforded the target **4i**. *O*-Methylation of the hydroxypyridine **21** followed by acylation and bromination at the benzyl position afforded the bromoacetone **24**. Introduction of a thiazole ring in **6a** yielded 4-(5-methoxy-2-pyridiyl) thiazole **25**. Then, its methoxy moiety was converted to the carboxamide **4j** in the same manner as that described for **4i**.

Scheme 4. Preparation of pyridyl thiazole analogs 4i–j[a]

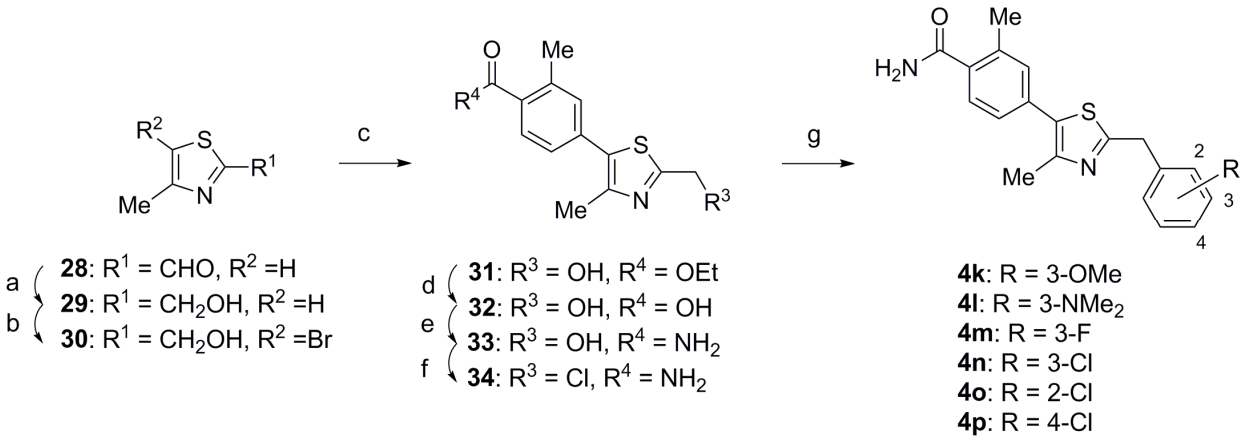


[a]Reagents and conditions: (a) 6-methoxypyridine-3-boronic acid, $Pd_2(dba)_3$, X-Phos, Cs_2CO_3, DME–water, 80°C; (b) hydrogen bromide, acetic acid, 80 °C; (c) phosphorus oxychloride, 100°C; (d) zinc cyanide, $Pd(Ph_3P)_4$, NMP, 100°C; (e) K_2CO_3, H_2O_2 aq., DMSO, rt; (f) iodomethane, potassium *tert*-butoxide, THF, 0°C to rt; (g) *n*-butyl lithium, THF, 0°C; then, dimethylacetamide; (h) NBS, MeCN, 0°C; (i) **6a**, EtOH, 50°C; (j) hydrogen bromide, acetic acid, reflux; (k) trifluoromethanesulfonic anhydride, pyridine, 0°C; (l) zinc cyanide, $Pd(Ph_3P)_4$, NMP, 150°C.

An efficient synthetic route for optimization of the substituents on the benzyl group is developed in Scheme 5. 2-Formyl-4-methylthiazole was allowed to react with sodium borohydride to afford the alcohol **29**, followed by bromination at the 5-position and Suzuki-Miyaura coupling reaction to give the ester analog **31**. The ester moiety of compound **31**

was converted to carboxamide in the same manner described in Scheme 1 to afford compound **33**. Chlorination of **33** provided the key coupling partner **34**. Suzuki-Miyaura coupling reaction of **34** with commercially available arylboronic acids yielded compounds **4k**–**p**.

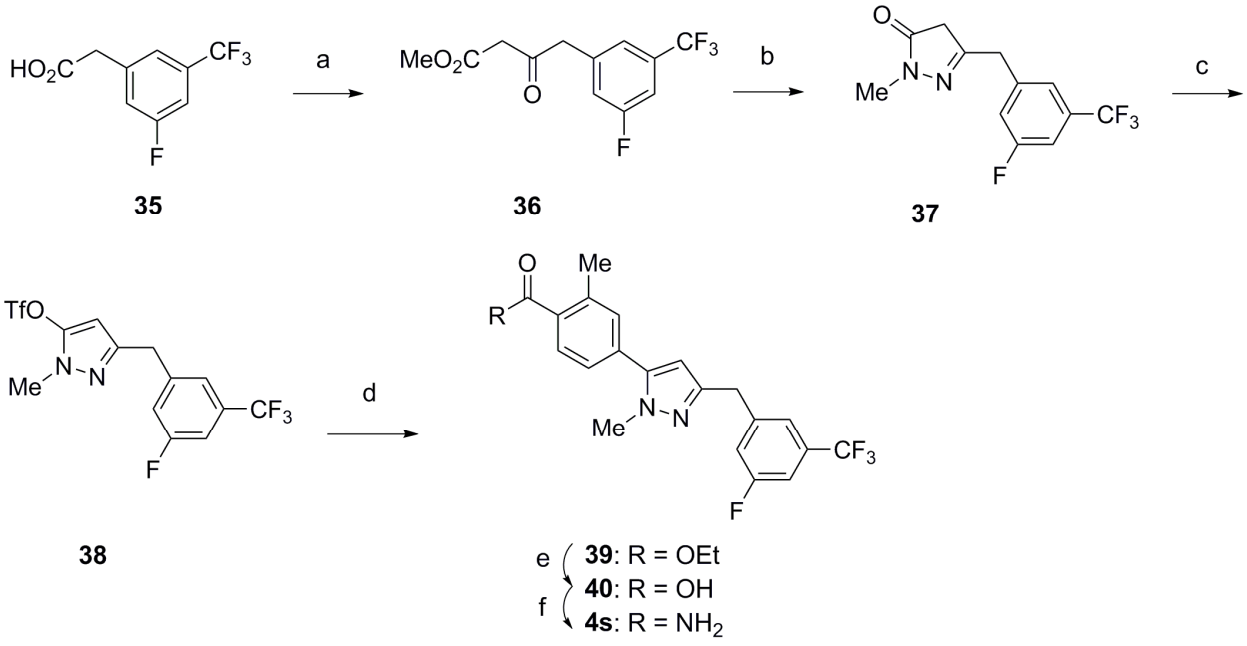
Scheme 5. Introduction of distal aryl groups in the late stage 4k–p[a]



[a]Reagents and conditions: (a) sodium borohydride, MeOH, 0°C; (b) NBS, MeCN, rt; (c) $Pd(Ph_3P)_4$, Na_2CO_3, DME–water, 80°C; (d) NaOH aq., EtOH, reflux; (e) ammonium chloride, EDCI, HOBt, *N*,*N*-diisopropylethylamine, DMF, rt; (f) thionyl chloride, 0°C; (g) arylboronic acid, $PdCl_2$(dppf)-CH_2Cl_2, Cs_2CO_3, THF–water, 150°C, microwave.

Preparation of the pyrazole analog **4s** is described in Scheme 6. The trifluoromethanesulfonate **38** was prepared *via* Knoevenagel reaction of the arylacetic acid **35** with Meldrum's acid, followed by cyclization reaction with methylhydrazine and triflation with triflic anhydride. A subsequent three-step reaction afforded the target compound **4s** as described in thiazole synthesis.

Scheme 6. Preparation of pyrazole analog 4s[a]

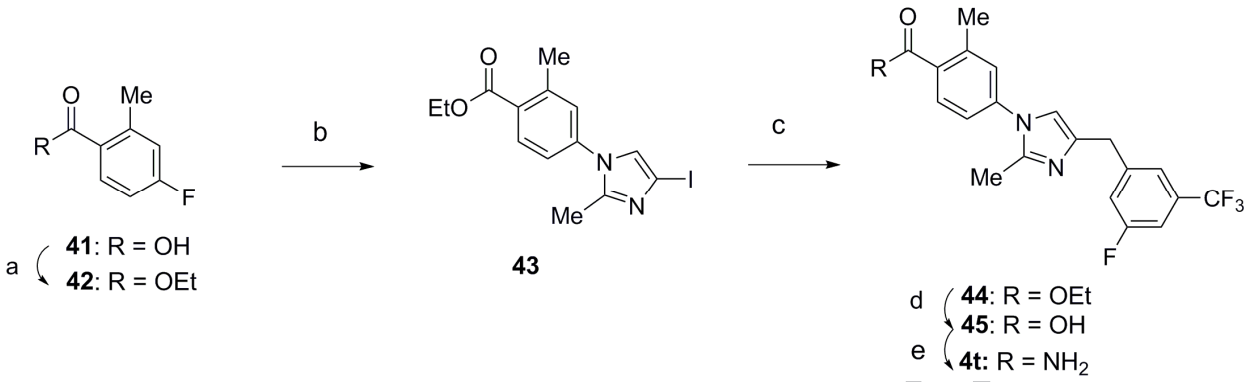


35 36 37 38

[a]Reagents and conditions: (a) Meldrum's acid, EDCI, *N*,*N*-dimethyl-4-aminopyridine, rt; then, MeOH, reflux; (b) methylhydrazine, MeOH, rt; (c) trifluoromethanesulfonic anhydride, pyridine, −20°C to rt; (d) ethyl 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate, $Pd(Ph_3P)_4$, Na_2CO_3, DME, 90°C; (e) NaOH aq., EtOH, 80°C; (f) WSC, HOBt-NH_3, DMF, rt.

The imidazole analog **4t** was synthesized according to Scheme 7. The benzoic acid analog **41** was converted to the ester **42**, and then the regioselective S_NAr reaction with 4-iodo-2-methyl-1*H*-imidazole gave the iodoimidazole analog **43**. Introduction of a benzyl group at the 4-position was achieved *via* Negishi coupling reaction to yield compound **44**. Using compound **44**, the target imidazole analog **4t** was prepared in the same manner as previously described.

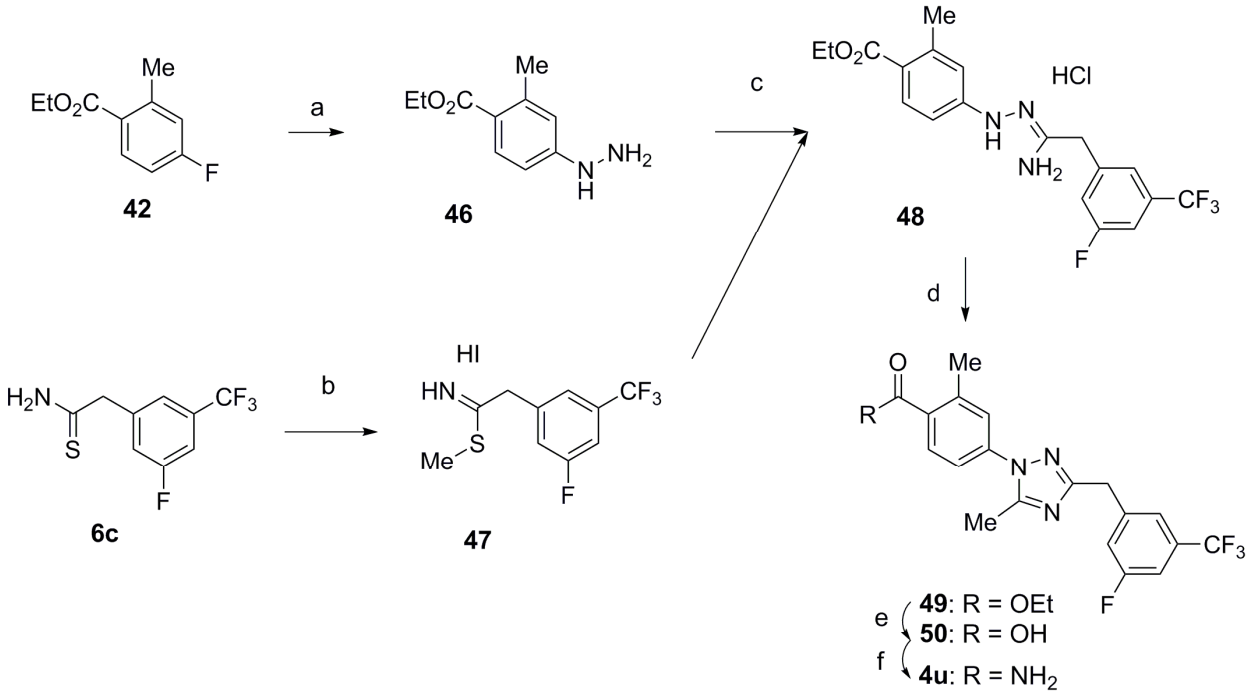
Scheme 7. Preparation of imidazole analog 4t[a]



[a]Reagents and conditions: (a) EtOH, thionyl chloride, 80°C; (b) 4-iodo-2-methyl-1*H*-imidazole, sodium hydride, 18-crown-6, NMP, 110°C; (c) 1-(bromomethyl)-3-fluoro-5-(trifluoromethyl)benzene, zinc, 1,2-dibromobutane, chlorotrimethylsilane, THF, palladium(II) acetate, 2,2'-bis-(diphenylphosphino)-1,1'-binaphthyl, THF, 80°C; (d) NaOH aq., EtOH, rt; (e) EDCI, HOBt-NH_3, DMF, rt.

Preparation of the 1,2,4-triazole analog is described in Scheme 8. *S*-Methylation of the thioamide **6c** with iodomethane, and the successive treatment with the arylhydrazine **46**, which was prepared from the fluorobenzene **42** and hydrazine, afforded the hydrazone analog **48**. Construction of the 1,2,4-triazole using the hydrazone **48** and orthoester, followed by conversion of the ester moiety to carboxamide yielded the target 1,2,4-triazole analog **4u**.

Scheme 8. Preparation of 1,2,4-triazole analog 4u[a]



[a]Reagents and conditions: (a) hydrazine monohydrate, DMSO, 120°C; (b) iodomethane, acetone, 50°C; (c) EtOH, 0°C; (d) 1,1,1-trimethoxyethane, acetic acid, 100°C; (e) NaOH aq., EtOH, 80°C; (f) EDCI, HOBt-NH_3, DMF, rt.

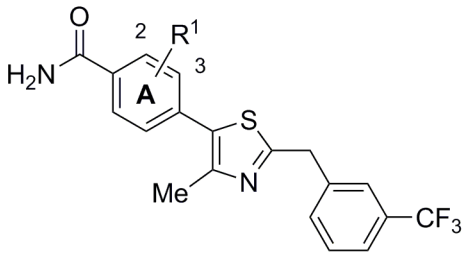
3. BIOLOGICAL RESULTS AND DISCUSSION

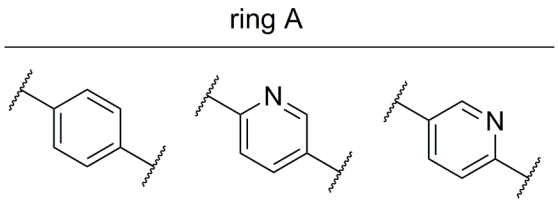
We measured the intracellular levels of cAMP in human GPR52-expressing CHO cells at various concentrations of the compounds. The half maximal effective concentration (EC_{50}) was defined as the concentration of the compound that results in cAMP production equal to half of its E_{max} value, and the cAMP level produced by the reference compound (*N*-(2-hydroxyethyl)-3-(2-(3-(trifluoromethyl)benzyl)-1-benzofuran-4-yl)benzamide)[5] was defined as 100%.

The thiazole derivative **4a** was first prepared to validate our design hypothesis that combined the privileged structure and the azolylbenzamide (**3**). Compound **4a** showed a significantly higher potency (EC_{50} = 220 nM, E_{max} = 93%) than compound **3** (EC_{50} = 470 nM, E_{max} = 56%) as shown in Table 1. Therefore, **4a** was selected as a new lead for further modification. Table 1 shows the modifications of ring A. Introduction of a methyl group on ring A at the C2 position (**4b**) was tolerated with an EC_{50} value of 73 nM, whereas methylation at the C3 position (**4c**) diminished its GPR52 agonistic activity (EC_{50} = 1400 nM). In general, introduction of a substituent at the *ortho*-position of a biaryl structure induces a dihedral angle at the biaryl[10]. This can be interpreted that the planar biaryl structure in this azolylbenzamide series was essential for the potency, similar to the amide structure with S-O interaction in **2**. Further exploration of the structure–activity relationship (SAR) at the C2 position showed that the ethyl (**4d**), methoxy (**4g**), and trifluoromethyl (**4h**) analogs exhibited similar GPR52 agonistic activities with EC_{50} values of 83, 170, and 110 nM, respectively. The results demonstrated that the electrostatic potential of the substituents at the C2 position did not affect the potency. However, the isopropyl derivative **4e** showed weaker agonistic activity (EC_{50} = 370 nM, E_{max} = 63%). Introduction of fluorine (**4f**) and 2-pyridine (**4i**) clearly lowered the potency (EC_{50} = 380 nM, E_{max} = 84% for **4f**; EC_{50} > 1000 nM, E_{max} = 11% for **4i** at 1μM) compared with the unsubstituted benzene (**4a**, EC_{50} = 220 nM), which indicated that intramolecular hydrogen bonding between the amide and fluorine or pyridine could disrupt the interaction between the ligand and GPR52. The 3-pyridyl derivative **4j**, exhibited an EC_{50} value of 400 nM with 70% efficacy, which was weaker than that of the benzene derivative (**4a**; EC_{50} = 220 nM, E_{max} = 93%). Thus, it was

concluded that the 2-methyl-benzamide structure is functionally suitable for GPR52 agonists.

Table 1. Effects of substituents at the ring A on GPR52 agonistic activity.





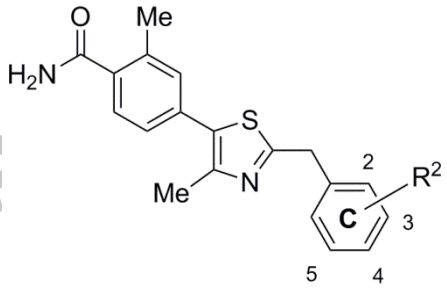
			human GPR52[a]	
Compound	ring A	R^1	EC_{50} (nM)	E_{max} (%)
4a	Ph	H	220 (180–270)	93 (89–97)
4b	Ph	2-Me	73 (56–96)	86 (82–91)
4c	Ph	3-Me	1400 (1200–1700)	87 (83–91)
4d	Ph	2-Et	83 (64–107)	84 (79–89)
4e	Ph	2-iPr	370 (240–570)	63 (54–72)
4f	Ph	2-F	380 (340–430)	84 (82–86)
4g	Ph	2-OMe	170 (140–210)	88 (85–91)
4h	Ph	2-CF_3	110 (100–120)	92 (91–94)
4i	2-Py	H	>1000	11%@1μM
4j	3-Py	H	400 (350–450)	70 (69–72)

[a] EC_{50} values are derived from the mean curves of the experiments (n = 2–4). Numbers in parentheses represent the 95% confidence interval.

According to the previously described SAR of compounds **1** and **2**, substituents on ring C affected the agonistic activity, in particular, substituents at the C3 position had great impact on the potency. Detailed study of the effects of C3-substituents in compound **4** was

conducted for further improvement of the GPR52 agonistic activity (Table 2). The methoxy (**4k**), *N*,*N*-dimethylamine (**4l**), fluorine (**4m**), and chlorine (**4n**) derivatives showed high potency with EC_{50} values of 160, 690, 260, and 130 nM, respectively. Therefore, it was concluded that the electronic nature of substituents at the C3 position did not affect the potency. Chlorine scan, which is a common drug design strategy, was then performed to identify the most promising spot. The GPR52 agonistic activity and efficacy of the *ortho*-substituted (**4o**) and *para*-substituted (**4p**) derivatives (EC_{50} = 3900 and 210 nM, respectively; E_{max} = 51% and 60%, respectively) were lower than those of the *meta*-substituted derivative (**4n**; EC_{50} = 130 nM, E_{max} = 75%). Thus, *meta*-substitution was considered advantageous with respect to both the potency and efficacy. These results led us to introduce multiple substituents at the *meta*-position, such as 3-chrolo-5-fluoro (**4q**) and 3-fluoro-5-trifluoromethyl (**4r**). Compounds **4q** and **4r** were found to be potent GPR52 agonists with EC_{50} values of 25 and 11 nM, respectively. Thus, substituents at the *meta*-position on ring C greatly affected the functional GPR52 activity in a similar manner to that previously reported with compounds **1** and **2**.

Table 2. Effects of substituents at the ring C on GPR52 agonistic activity.



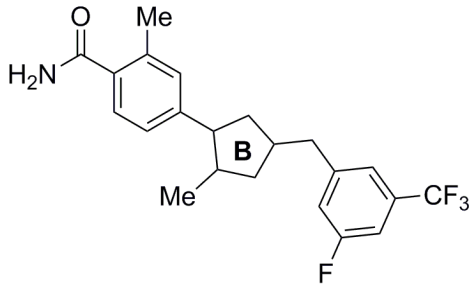
		human GPR52[a]	
Compound	R^2	EC_{50} (nM)	E_{max} (%)

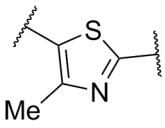
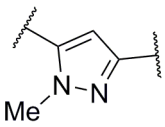
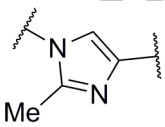
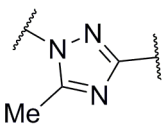
4k	3-OMe	160 (120–220)	85 (79–90)
4l	3-NMe_2	690 (550–860)	101 (95–107)
4m	3-F	260 (180–370)	75 (70–81)
4n	3-Cl	130 (95–190)	75 (70–80)
4o	2-Cl	3900 (1700–8700)	51 (42–60)
4p	4-Cl	210 (150–290)	60 (55–64)
4q	3-Cl-5-F	25 (18–35)	88 (84–92)
4r	3-F-5-CF_3	11 (9.2–14)	93 (90–96)

[a]EC_{50} values are derived from the mean curves of the experiments (n = 2–4). Numbers in parentheses represent the 95% confidence interval.

Modification of the ring B was then conducted to explore the effect of other azoles. The position and number of nitrogen atoms with sp^2-hybridization affected the electrostatics of the ring, which, in turn, affected the basicity, lipophilicity, physicochemical properties, and PK profiles of a rat cassette-dosing test. The GPR52 agonistic activity and PK profiles of **4r–u** are highlighted in Table 3. The pyrazole analog **4s** showed a GPR52 agonistic activity with an EC_{50} value of 23 nM, which was comparable to that of the thiazole analog **4r**. Furthermore, the imidazole analog **4t** and the 1,2,4-triazole analog **4u** exhibited an agonistic activity at a double-digit nanomolar concentrations (EC_{50} = 59 and 75 nM, respectively). A previous series of the thiazole analog **2** considered only the thiazole ring as the core motif for potent GPR52 agonistic activity. However, this series of the azolylbenzamide (**4**) derivatives accepted various five-membered azoles as the core motif. These results can validate the design concept of **4**, which featured the comparability between the benzene in **4** and the amide with S-O interaction in **2**.

Table 3. GPR52 agonistic activity and PK parameters in rat of azolyl benzamide **4r–u**.



Compound	ring B	human GPR52[a] EC50 (nM)	Emax (%)	F[b,c] (%)	AUCpo[b,d] (ng*h/mL)	Cltotal[b,e] (mL/h/kg)	log D[f]
4r		11 (9.2–14)	93 (90–96)	14.0	60.5	2369	5.3
4s		23 (19–29)	123 (118–129)	30.5	155.4	1975	4.2
4t		59 (49–71)	107 (103–110)	28.4	267.9	1129	4.7
4u		75 (60–95)	122 (115–130)	53.8	383.9	1409	3.8

[a]EC_{50} values are derived from the mean curves of the experiments (n = 2–4). Numbers in parentheses represent the 95% confidence interval. [b]Cassette-dosing. Male SD rats (n = 3). Dose: i.v. at 0.1 mg/kg; p.o. at 1 mg/kg. [c]Bioavailability. [d]Area under the curve from 0 to 8 hours. [e]Clearance. [f] logD at pH 7.4 was calculated using ACD Labs version 12[11].

Among the compounds shown in Table 3, the 1,2,4-triazole analog **4u** possessed better bioavailability (F = 53.8%) than compounds **4r**–**t**. Furthermore, compound **4u** showed the highest value of area under the curve $(AUC)_{po}$ (383.9 ng*h/mL) and a moderate total clearance (Cl = 1409 mL/h/kg). Regarding the modification of ring B, reduction of lipophilicity was closely associated with lower clearance rate, resulting in improvement of AUC_{po} and bioavailability. Moreover, compound **4u** showed good metabolic stability against microsomes of several species (metabolic clearance: 17 μL/min/mg for humans, 9 μL/min/mg for rats, 24 μL/min/mg for mice). Therefore, we concluded that low lipophilicity resulted in high bioavailability owing to slow clearance as the result of high metabolic stability. In addition, compound **4u** did not inhibited off-targets such as receptors of dopamine D_1, D_{2L}, 5-HT_2, AMPA, NMDA, and phosphodiesterases with the values of under 50% inhibition at 10 μM concentration[12]. Based on these results, compound **4u**, which showed potent GPR52 activity, high metabolic stability in several species, and excellent PK in rats, was selected for further biological *in vivo* evaluation in mice.

Detailed PK profile of 1,2,4-triazole derivative **4u** (Table 4) was determined in ICR mice using both plasma and brain tissues. The concentrations of **4u** in plasma and brain were measured after an oral administration of 10 or 30 mg/kg (n = 3 in each). Compound **4u** showed good permeability through the blood-brain barrier (BBB) with brain/plasma ratio of 1.53–1.68. It successfully demonstrates that $AUC_{0\text{-}24h}$ values were increased with a dose escalation in both plasma and brain.

Table 4. PK parameters of compound **4u**[a] in mice.

Dosing	10 mg/kg		30 mg/kg	
Sample tissue	Plasma	Brain	Plasma	Brain
C_{max} (μg/mL or μg/g)	0.688	1.018	1.209	2.330
T_{max} (h)	0.50	0.50	1.00	1.00
$AUC_{0\text{-}24h}$ (μg*h/mL or μg*h/g)	2.433	3.731	6.200	10.414
Brain/Plasma ratio[b]	1.53		1.68	
MRT (h)	2.29	2.06	3.54	3.25

[a] ICR mice (n = 3 in each). [b] The values were calculated with $AUC_{0\text{-}24h}$(brain)/$AUC_{0\text{-}24h}$(plasma).

In addition to its excellent PK profile in rodents, it was revealed that compound **4u** possessed mice GPR52 potency (EC_{50} = 71 nM, E_{max} = 114%) with a plasma protein binding value of 80.1% in mice at 1 μM concentration. Therefore, the effects of compound **4u** were investigated in animal models of psychiatric disorders. The dopamine agonist, methamphetamine (MAP), induces some effects that mimic the positive symptoms of schizophrenia. In a MAP-induced (2 mg/kg, s.c) hyperlocomotion activity assay using male ICR mice, compound **4u** showed efficacy in the prevention of the hyperlomotion after oral administration at a dose of 10 mg/kg, as shown in Figure 4. Compound **4u** did not show cataleptogenic effects (unpublished data).

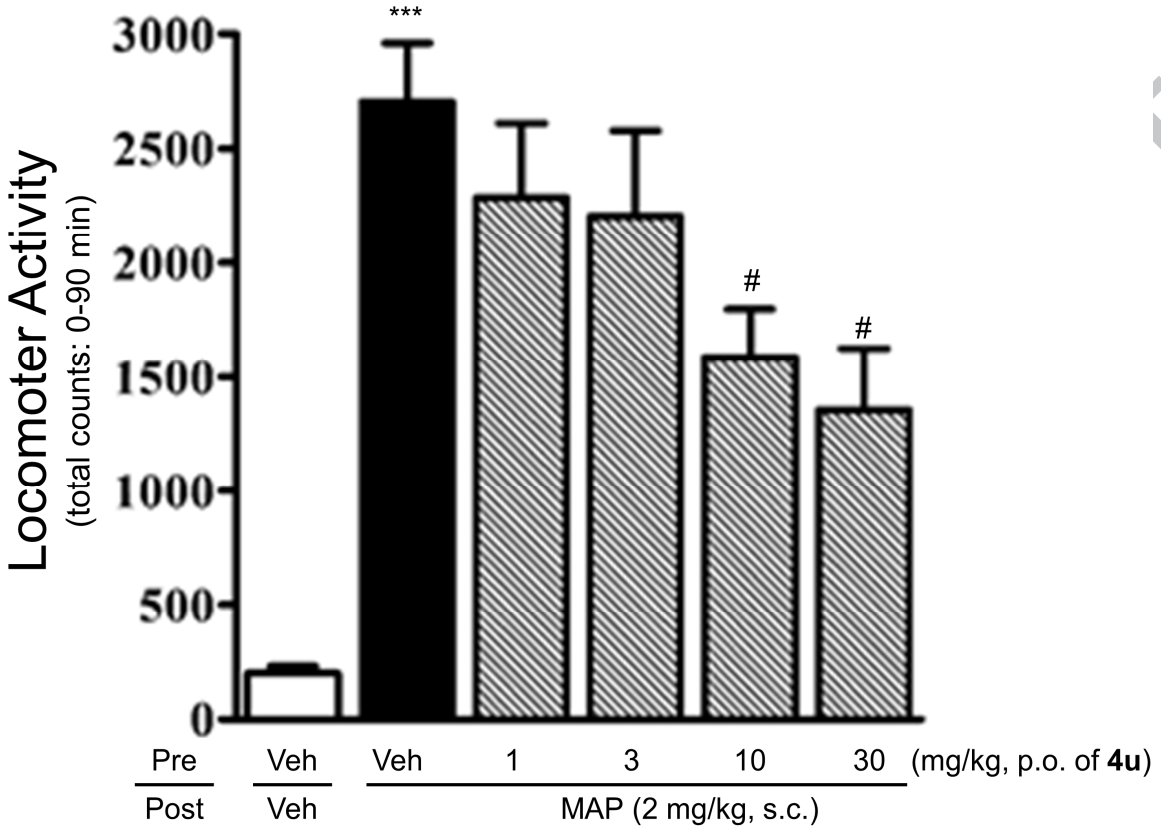


Figure 4. Suppression of MAP-induced hyperactivity by compound **4u** in mice. (Male ICR mice, mean ± SEM, ***$p < 0.01$ by *t*-test, $^{\#}p < 0.025$ by Williams test).

4. CONCLUSION

Azolylbenzamide **4a**–**u** was designed from the high-throughput screening hit **3**, based on the previously reported GPR52 agonist motif, particularly the 2-benzyl-4-methylthiazole. In the SAR study of **4**, it was clear that (1) the ring A benzene motif in **4** could replace the amide motif in **2**; and (2) a planar biaryl system exhibited higher potency over the twisted biaryl structure based on the analysis of dihedral angles. Moreover, introduction of a methyl group at the C2 position of ring A and halogen atoms or trifluoromethyl group at

the *meta*-position of ring C enhanced the GPR52 agonistic activity. Additionally, the ring B tolerated various five-membered azoles, resulting in the identification of potent GPR52 agonists, such as the thiazole (**4r**), pyrazole (**4s**), imidazole (**4t**), and 1,2,4-triazole (**4u**) analogs. Among them, the 1,2,4-triazole analog **4u** showed a good PK profile and BBB penetration. Moreover, MAP-induced hyperlocomotion was significantly suppressed by oral administration of **4u** at a dose of 10 mg/kg. Detailed biological evaluation will be reported in the near future.

5. EXPERIMENTAL SECTION

5.1. CHEMISTRY

The proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Bruker AVANCE II (300 MHz) or Varian INOVA-400 (400 MHz) instruments. Chemical shifts for ^{1}H-NMR were reported in parts per million (ppm) downfield from tetramethylsilane (δ) as the internal standard in deuterated solvent and coupling constants (J) are in Hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad. Elemental analyses were carried out by Takeda Analytical Research Laboratories, Ltd. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck) or NH TLC plate (Fuji Silysia Chemical Ltd.). Column chromatography was carried out on a silica gel column (Chromatorex® NH-DM1020, 100-200 mesh, Fuji Silysia chemical) or on Purif-Pack (SI • 60 μM or NH • 60 μM, Fuji Silysia Chemical, Ltd.). Liquid chromatography-mass spectrometry (LC/MS) analysis was performed on the following methods; (A) an Agilent 1200, equipped with a L-column2 ODS (3.0 x50 mm I.D., 3 μm-particle size, CERI, Japan), eluting with 5 mM

ammonium acetate ($AcONH_4$) in ultrapure water/acetonitrile=90/10 (Mobile phase A), and 5 mM $AcONH_4$ in ultrapure water/acetonitrile=10/90 (Mobile phase B), using the following elution gradient of 5% B to 90% B over 0.9 min followed by 90% B isocratic over 1.1 min at a flow rate of 1.5 mL/min (detection at 220 nm or 254 nm). MS spectra were recorded using an Agilent 6130 with electrospray ionization. (B) a Shimadzu LC-20AD, equipped with a L-column2 ODS (3.0 x50 mm I.D., 3 μm-particle size, CERI, Japan), eluting with 5 mM $AcONH_4$ in ultrapure water/acetonitrile = 90/10 (Mobile phase A), and 5 mM $AcONH_4$ in ultrapure water/acetonitrile = 10/90 (Mobile phase B), using the following elution gradient of 5% B to 90% B over 0.9 min followed by 90% B isocratic over 1.1 min at a flow rate of 1.5 mL/min (detection at 220 nm or 254 nm). MS spectra were recorded using a Shimadzu LCMS-2020 with electrospray ionization. (C) a Shimadzu LC-20AD, equipped with a L-column2 ODS (3.0 x50 mm I.D., 3 μm-particle size, CERI, Japan), eluting with 0.05% TFA in ultrapure water (Mobile phase A), and 0.05% TFA in acetonitrile (Mobile phase B), using the following elution gradient of 5% B to 90% B over 0.9 min followed by 90% B isocratic over 1.1 min at a flow rate of 1.5 mL/min (detection at 220 nm). MS spectra were recorded using a Shimadzu LCMS-2020 with electrospray ionization. (D) a Waters 2795, equipped with a L-column2 ODS (3.0 x50 mm I.D., 3μm-particle size, CERI, Japan), eluting with 0.05% TFA in ultrapure water (Mobile phase A), and 0.05% TFA in acetonitrile (Mobile phase B), using the following elution gradient of 5% B to 90% B over 2 min followed by 90% B isocratic over 1.5 min at a flow rate of 1.2 mL/min (detection at 220 nm or 254 nm). MS spectra were recorded using a Waters ZQ2000 with electrospray ionization. Preparative LC was performed on a Waters 2525

separations module (L-column2 ODS (20 x150 mm I.D., CERI, Japan); 0.1% TFA in distilled water/acetonitrile gradient; MS spectra were recorded using a Waters ZQ2000 with electrospray ionization. The purities of all compounds tested in biological systems were assessed as being >95% using analytical high-performance liquid chromatography (HPLC). Purity data were collected by a HPLC with Corona CAD (Charged Aerosol Detector) or photo diode array detector. The column was a Capcell Pak C18AQ (50 mm x 3.0 mm I.D., Shiseido, Japan) or L-column 2 ODS (30 mm x 2.0 mm I.D., CERI, Japan) with a temperature of 50 °C and a flow rate of 0.5 mL/min. Mobile phase A and B under a neutral condition were a mixture of 50 mmol/L $AcONH_4$, water and acetonitrile (1:8:1, v/v/v) and a mixture of 50 mmol/L $AcONH_4$ and acetonitrile (1:9, v/v), respectively. The ratio of mobile phase B was increased linearly from 5% to 95% over 3 min, 95% over the next 1 min. Mobile phase A and B under an acidic condition were a mixture of 0.2% formic acid in 10 mmol/L ammonium formate and 0.2% formic acid in acetonitrile, respectively. The ratio of mobile phase B was increased linearly from 14% to 86% over 3 min, 86% over the next 1 min. All commercially available solvents and reagents were used without further purification. Yields were not optimized. Abbreviations: B_2pin_2, bis(pinacolato) diboron; $Pd_2(dba)_2$, tris(dibenzylideneacetone) dipalladium(0); $PdCl_2(Ph_3P)_2$, dichloro bis(triphenylphosphine) palladium(II); $PdCl_2(dppf)$-CH_2Cl_2, dichloro [1,1'-bis(diphenylphosphino)ferrocene] palladium(II) CH_2Cl_2 adduct; X-Phos, 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl; Na_2CO_3, sodium carbonate; $NaHCO_3$, sodium hydrogen carbonate; NaOH, sodium hydroxide; K_2CO_3, potassium carbonate; Cs_2CO_3, cesium carbonate; $MgSO_4$, magnesium sulfate; NH_4Cl, ammonium chloride; EDCI,

1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; NBS, *N*-bromosuccinimide; H_2O_2, hydrogen peroxide; HCl, hydrochloric acid; DME, 1,2-dimethoxyethane; DMF, *N*,*N*-dimethylformamide; NMP, *N*-methylpyrrolidone; EtOAc, ethyl acetate; MeOH, methyl alcohol; EtOH, ethyl alcohol; THF, tetrahydrofuran.

5.1.1. 2-(3-(Trifluoromethyl)phenyl)ethanethioamide (**6a**)

A mixture of (3-(trifluoromethyl)phenyl)-acetonitrile (45.2 g, 244 mmol) and diethyl dithiophosphate (50.0 g, 269 mmol) in 4 N HCl solution in EtOAc (500 mL, 2000 mmol) was stirred at room temperature overnight. The mixture was washed with water, 1 N aqueous NaOH solution, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was triturated with hexane with ice-bath cooling. The precipitate was collected by filtration followed by washing with hexane to give 35.0 g (65%) of **6a** as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 4.14 (2H,s), 6.66 (1H, brs), 7.38–7.88 (5H, m).

5.1.2. 2-(3-Chloro-5-fluorophenyl)ethanethioamide (**6b**)

Compound **6b** was obtained as pale yellow solid in 68% yield by a method similar to that described for **6a**. ^{1}H NMR (300 MHz, $CDCl_3$) 4.03 (2H, s), 6.78 (1H, brs), 6.96 (1H, dd, *J* = 8.9, 1.7 Hz), 7.03–7.14 (2H, m), 7.81 (1H, brs).

5.1.3. 2-(3-Fluoro-5-(trifluoromethyl)phenyl)ethanethioamide (**6c**)

Compound **6c** was obtained as colorless solid in 83% yield by a method similar to that described for **6a**. ^{1}H NMR (300 MHz, $CDCl_3$) 4.10 (2H, s), 6.69 (1H, brs), 7.21–7.41 (3H, m), 7.56 (1H, brs).

5.1.4. 4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazole (**7a**)

A mixture of **6a** (4.38 g, 20 mmol) and 80% bromoacetone (2.3 mL, 22 mmol) in EtOH (20 mL) was refluxed for 2 h. After cooling, the mixture was concentrated *in vacuo*. The mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with EtOAc in hexane) to give 4.97 g (97%) of **7a** as colorless oil. ^{1}H NMR (300MHz, $CDCl_3$) 2.44 (3H, s), 4.35 (2H, s), 6.77 (1H, s), 7.33–7.67 (4H, m).

5.1.5. 2-(3-Chloro-5-fluorobenzyl)-4-methyl-1,3-thiazole (**7b**).

Compound **7b** was obtained as pale brown oil in quantitative yield by a method similar to that described for **7a**. ^{1}H NMR (300 MHz, $CDCl_3$) 2.44 (3H, s), 4.25 (2H, s), 6.78 (1H, s), 6.89–7.03 (2H, m), 7.10 (1H, s).

5.1.6. 2-(3-Fluoro-5-(trifluoromethyl)benzyl)-4-methyl-1,3-thiazole (**7c**)

Compound **7c** was obtained as pale yellow oil in 95% yield by a method similar to that described for **7a**. ^{1}H NMR (300 MHz, $CDCl_3$) 2.45 (3H, d, J = 0.8 Hz), 4.34 (2H, s), 6.80 (1H, q, J = 1.1 Hz), 7.22 (2H, d, J = 9.0 Hz), 7.38 (1H, s).

5.1.7. 5-Bromo-4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazole (**8a**)

To a solution of **7a** (1.29 g, 5.01 mmol) in MeCN (10 mL) was added NBS (0.979 g, 5.50 mmol) with several portions at 0 °C. The mixture was stirred at room temperature overnight. The mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel,

eluted with 1%–10% EtOAc in hexane) to give 0.907 g (54%) of **8a** as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.38 (3H, s), 4.28 (2H, s), 7.42–7.60 (4H, m).

5.1.8. 5-Bromo-2-(3-chloro-5-fluorobenzyl)-4-methyl-1,3-thiazole (**8b**)

Compound **8b** was obtained as pale oil in 82% yield by a method similar to that described for **8a**. ^{1}H NMR (300 MHz, $CDCl_3$) 2.38 (3H, s), 4.18 (2H, s), 6.88–6.94 (1H, m), 6.98–7.04 (1H, m), 7.08 (1H, s).

5.1.9. 5-Bromo-2-(3-fluoro-5-(trifluoromethyl)benzyl)-4-methyl-1,3-thiazole (**8c**)

Compound **8c** was obtained as pale brown oil in 99% yield by a method similar to that described for **8a**. ^{1}H NMR (300 MHz, $CDCl_3$) 2.39 (3H, s), 4.28 (2H, s), 7.16–7.38 (3H, m).

5.1.10. Ethyl 4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzoate (**10a**)

A mixture of **8a** (1.35 g, 4.00 mmol), (4-(ethoxy-carbonyl)phenyl)boronic acid (**9a**; 853 mg, 4.40 mmol), $Pd(Ph_3P)_4$ (139 mg, 0.12 mmol), 2 M aqueous Na_2CO_3 solution (4.4 mL, 8.8 mmol), and DME (18 mL) was stirred at 80°C overnight. The mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 5%–25% EtOAc in hexane) to give 1.53 g (94%) of **10a** as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 1.40 (3H, t, J = 7.2 Hz), 2.51 (3H, s), 4.32–4.44 (4H, m), 7.41–7.65 (6H, m), 8.02–8.10 (2H, m).

5.1.11. Methyl 3-methyl-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)thiazol-5-yl)benzoate (**10b**)

A mixture of **8a** (338 mg, 1.00 mmol), methyl 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (**9b**; 331 mg, 1.2 mmol), $Pd(Ph_3P)_4$ (35 mg, 0.030 mmol), 2 M

aqueous Na_2CO_3 solution (1.2 mL, 2.4 mmol) in DME (3.6 mL) was stirred at 65 °C overnight. The mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 5%–25% EtOAc in hexane) to give 280 mg (69%) of **10b** as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.22 (3H, s), 2.25 (3H, s), 3.93 (3H, s), 4.37 (2H, s), 7.29 (1H, d, *J* = 7.9 Hz), 7.44–7.65 (4H, m), 7.86 (1H, dd, *J* = 7.9, 1.9 Hz), 7.93–7.96 (1H, m).

5.1.12. Methyl 2-fluoro-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzoate (**10c**)

A mixture of **8a** (335 mg, 1.00 mmol), (3-fluoro-4-(methoxycarbonyl)phenyl)boronic acid (**9c**; 297 mg, 1.50 mmol), $Pd_2(dba)_2$ (46 mg, 0.050 mmol), X-Phos (48 mg, 0.10 mmol), Cs_2CO_3 (652 mg, 2.0 mmol), DME (2 mL), and water (0.5 mL) was stirred at 50°C for 3 h. The mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 3%–30% EtOAc in hexane) to give 258 mg (63%) of **10c** as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.53 (3H, s), 3.94 (3H, s), 4.35 (2H, s), 7.15–7.25 (2H, m), 7.42–7.65 (4H, m), 7.96 (1H, t, *J* = 7.9 Hz).

5.1.13. Methyl 2-methoxy-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzoate (**10d**)

A mixture of **8a** (335 mg, 1.0 mmol), (3-methoxy-4-(methoxycarbonyl)phenyl)boronic acid (**9d**; 315 mg, 1.5 mmol), $Pd_2(dba)_2$ (46 mg, 0.05 mmol), X-Phos (48 mg, 0.10 mmol), Cs_2CO_3 (652 mg, 2.0 mmol) in DME (2 mL) and water (0.5 mL) was stirred at 50°C for 3 h. The mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, and

concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 5%–50% EtOAc in hexane) to give 241 mg (57%) of **10d** as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.52 (3H, s), 3.90 (3H, s), 3.92 (3H, s), 4.36 (2H, s), 6.96 (1H, d, *J* = 1.5 Hz), 7.01 (1H, dd, *J* = 7.9, 1.5 Hz), 7.44–7.65 (4H, m), 7.82 (1H, d, *J* = 7.9 Hz).

5.1.14. Ethyl 4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)-2-(trifluoromethyl)benzoate (**10e**)

A mixture of **8a** (661 mg, 1.97 mmol), the crude **9e** (reference: the Supporting Information), $PdCl_2(Ph_3P)_2$ (69 mg, 0.099 mmol), and potassium carbonate (817 mg, 5.91 mmol) in DME (23 mL) and water (7 mL) was stirred under Ar atmosphere at 85°C for 4 h. The mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 5%–20% EtOAc in hexane) and preparative HPLC to give 542 mg (58%) of **10e** as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) 1.39 (3H, t, *J* = 7.2 Hz), 2.50 (3H, s), 4.36 (2H, s), 4.41 (2H, q, *J* = 7.2 Hz), 7.44–7.65 (5H, m), 7.73–7.76 (1H, m), 7.83 (1H, d, *J* = 8.0 Hz).

5.1.15. 4-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzoic acid (**11a**)

To a solution of **10a** (405 mg, 1.0 mmol) in EtOH (6 mL) was added 1 N aqueous NaOH solution (3.0 mL, 3.0 mmol) at room temperature. After being stirred at 80 °C for 3 h, the mixture was neutralized with 1 N aqueous HCl solution at room temperature and extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give 356 mg (94%) of **11a** as colorless solid. ^{1}H NMR (300 MHz,

$CDCl_3$) 2.54 (3H, s), 4.38 (2H, s), 7.44–7.66 (6H, m), 8.13 (2H, d, *J* = 8.4 Hz), COOH peak was not observed.

5.1.16. 3-Methyl-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzoic acid (**11b**)

To a solution of **10b** (270 mg, 0.67 mmol) in MeOH (4 mL) was added 1 N aqueous NaOH solution (2 mL, 2 mmol) at room temperature. After being stirred at 50°C for 3 h, the mixture was neutralized with 1 N aqueous HCl solution in water and extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give 260 mg (quant.) of **11b** as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.27 (6H, s), 4.44 (2H, s), 7.32 (1H, d, *J* = 8.0 Hz), 7.41–7.70 (4H, m), 7.87–8.07 (2H, m), 10.60 (1H, brs).

5.1.17. 2-Fluoro-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzoic acid (**11c**)

The compound **11c** were prepared from methyl ester (**10c**) by a method similar to that described for **11b**. Yield quant., as colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) 2.45 (3H, s), 4.46 (2H, s), 7.33–7.46 (2H, m), 7.55–7.81 (4H, m), 7.90 (1H, t, *J* = 7.9 Hz), 13.33 (1H, brs).

5.1.18. 2-Methoxy-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzoic acid (**11d**)

To a solution of **10d** (241 mg, 0.57 mmol) in MeOH (3.4 mL) was added 1 N aqueous NaOH solution (1.7 mL, 1.7 mmol) at room temperature. After being stirred at 50 °C for 3 h, the mixture was neutralized with 1 N aqueous HCl solution in water and extracted with

EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give 232 mg (quant.) of **11d** as colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) 2.45 (3H, s), 3.84 (3H, s), 4.45 (2H, s), 7.05 (1H, dd, *J* = 7.9, 1.9 Hz), 7.10 (1H, d, *J* = 1.1 Hz), 7.57–7.80 (5H, m), 12.69 (1H, brs).

5.1.19. 4-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)-2-(trifluoromethyl)benzoic acid (**11e**)

To a solution of **10e** (542 mg, 1.1 mmol) in THF (9 mL) and MeOH (3 mL) was added 1 N aqueous NaOH solution (3.0 mL, 3.0 mmol) at room temperature. After being stirred at 60°C for 1 h, the mixture was neutralized with 1 N aqueous HCl solution and extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from hexane–EtOAc to give 479 mg (94%) of **11e** as pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.53 (3H, s), 4.40 (2H, s), 7.44–7.69 (5H, m), 7.79 (1H, s), 7.99 (1H, d, *J* = 7.9 Hz), COOH peak was not observed.

5.1.20. 4-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzamide (**4a**)

A mixture of **11a** (180 mg, 0.48 mmol), EDCI (138 mg, 0.72 mmol) and HOBt-NH_3 (109 mg, 0.72 mmol) in DMF (2 mL) was stirred at room temperature for 3 h. The mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ solution, water, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from hexane–EtOAc to give 140 mg (78%) of **4a** as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.51 (3H, s), 4.35 (2H, s), 6.05 (2H, brs), 7.42–7.64 (6H, m), 7.80–7.87 (2H, m). MS (ESI+) *m/z* = 377.2 $[M+H]^+$. Anal. Calcd for $C_{19}H_{15}F_3N_2OS$: C, 60.63; H, 4.02; N, 7.44. Found: C, 60.40; H, 4.05; N, 7.34.

The following compounds (**4c**, **4f**, **4g**, **4h**) were prepared from corresponding carbonic acid (**11b**–**e**) by a method similar to that described for **4a**.

5.1.21. 3-Methyl-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzamide (**4c**)

Yield 61%, as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.22 (3H, s), 2.25 (3H, s), 4.37 (2H, s), 5.76 (1H, brs), 6.09 (1H, brs), 7.30 (1H, d, *J* = 7.9 Hz), 7.43–7.66 (5H, m), 7.72–7.78 (1H, m). MS (ESI+) *m/z* = 391.1 $[M+H]^+$. Anal Calcd for $C_{20}H_{17}F_3N_2OS$: C, 61.53; H, 4.39; N, 7.18. Found: C, 61.15; H, 4.47; N, 7.12.

5.1.22. 2-Fluoro-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzamide (**4f**)

Yield 68%, as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.53 (3H, s), 4.36 (2H, s), 5.83 (1H, brs), 6.65 (1H, brs), 7.18 (1H, dd, *J* = 13.0, 1.7 Hz), 7.30 (1H, dd, *J* = 8.1, 1.7 Hz), 7.44–7.65 (4H, m), 8.15 (1H, t, *J* = 8.3 Hz). MS (ESI+) *m/z* = 395.0 $[M+H]^+$. Anal. Calcd for $C_{19}H_{14}F_4N_2OS$: C, 57.86; H, 3.58; N, 7.10. Found: C, 57.82; H, 3.61; N, 7.10.

5.1.23. 2-Methoxy-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzamide (**4g**)

Yield 64%, as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.53 (3H, s), 3.99 (3H, s), 4.36 (2H, s), 5.79 (1H, brs), 6.97 (1H, d, *J* = 1.5 Hz), 7.12 (1H, dd, *J* = 8.3, 1.5 Hz), 7.43–7.75 (5H, m), 8.23 (1H, d, *J* = 8.3 Hz). MS (ESI+) *m/z* = 407.0 $[M+H]^+$. Anal. Calcd for $C_{20}H_{17}F_3N_2O_2S$: C, 59.10; H, 4.22; N, 6.89. Found: C, 59.03; H, 4.32; N, 6.76.

5.1.24. 4-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)-2-(trifluoromethyl)benzamide (**4h**)

Yield 62%, as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.49 (3H, s), 4.36 (2H, s), 5.72–5.99 (2H, m), 7.43–7.74 (7H, m). MS (ESI+) *m/z* = 445.2 $[M+H]^+$. Anal Calcd for $C_{20}H_{14}F_6N_2OS$: C, 54.05; H, 3.18; N, 6.30. Found: C, 53.09; H, 3.17; N, 6.31.

5.1.25. 2-Methyl-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzonitrile (**12a**)

To a solution of **8a** (670 mg, 1.99 mmol), 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (583 mg, 2.40 mmol), $Pd_2(dba)_2$ (92 mg, 0.10 mmol), and X-Phos (95 mg, 0.20 mmol) in DME (4 mL) and water (1 mL) was added Cs_2CO_3 (1.30 g, 3.99 mmol). The mixture was stirred at 50°C under N_2 atmosphere for 5 h. The mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with EtOAc in hexane) and to give 700 mg (94%) of **12a** as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.50 (3H, s), 2.57 (3H, s), 4.35 (2H, s), 7.27–7.35 (2H, m), 7.41–7.65 (5H, m).

The following compounds (**12b** and **12c**) were prepared by a method similar to that described for **12a**.

5.1.26. 4-(2-(3-Chloro-5-fluorobenzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzonitrile (**12b**)

Yield 78%, as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.50 (3H, s), 2.57 (3H, s), 4.25 (2H, s), 6.93–7.05 (2H, m), 7.11–7.17 (1H, m), 7.28–7.36 (2H, m), 7.63 (1H, d, *J* = 7.9 Hz).

5.1.27. 4-(2-(3-Fluoro-5-(trifluoromethyl)benzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzonitrile (**12c**)

Yield 97%, as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.51 (3H, s), 2.58 (3H, s), 4.35 (2H, s), 7.21–7.45 (5H, m), 7.63 (1H, d, J = 7.9 Hz).

5.1.28. 2-Methyl-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzamide (**4b**)

To a mixture of **12a** (200 mg, 0.54 mmol) and K_2CO_3 (37 mg, 0.27 mmol) in DMSO (4 mL) was added 30% aqueous H_2O_2 solution (0.4 mL) at 0°C. The mixture was warmed to room temperature and stirred overnight. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 50%–100% EtOAc in hexane) and recrystallized from EtOH–water to give 85 mg (40%) of **4b** as solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.49 (3H, s), 2.52 (3H, s), 4.35 (2H, s), 5.78 (2H, brs), 7.22–7.28 (2H, m), 7.43–7.66 (5H, m). MS (ESI+) *m/z* = 391.1 $[M+H]^+$. Anal. Calcd for $C_{20}H_{17}F_3N_2OS$: C, 61.53; H, 4.39; N, 7.18. Found: C, 61.38; H, 4.38; N, 7.18.

5.1.29. 4-(2-(3-Chloro-5-fluorobenzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**4q**)

A mixture of **12b** (510 mg, 1.43 mmol) and concentrated sulfuric acid (10 mL) was stirred at 80°C for 30 min. The mixture was poured onto iced water, extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (NH silica gel, eluted with EtOAc in hexane) and recrystallized from EtOAc–hexane to give 120 mg (22%) of **4q** as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) 2.39 (3H, s), 2.41 (3H, s), 4.35 (2H, s), 7.23–7.45 (7H, m),

7.76 (1H, brs). MS (ESI+) *m/z* = 374.9 $[M+H]^+$. Anal. Calcd for $C_{19}H_{16}ClFN_2OS$: C, 60.88; H, 4.30; N, 7.47. Found: C, 61.08; H, 4.23; N, 7.44.

5.1.30. 4-(2-(3-Fluoro-5-(trifluoromethyl)benzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**4r**)

Compound **4r** was obtained as solid in 31% yield by a method similar to that described for **4b**. ^{1}H NMR (300 MHz, $CDCl_3$) 2.49 (3H, s), 2.53 (3H, s), 4.34 (2H, s), 5.72 (2H, brs), 7.20–7.32 (4H, m), 7.39–7.55 (2H, m). MS (ESI+) *m/z* = 409.3 $[M+H]^+$. Anal Calcd for $C_{20}H_{16}F_4N_2OS$: C, 58.82; 3.95; N, 6.86. Found: C, 58.83; H, 3.87; N, 6.89.

5.1.31. 2-Hydroxy-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzonitrile (**13**)

To a solution of 4-bromo-2-hydroxybenzonitrile (891 mg, 4.5 mmol), KOAc (1.32 g, 13.5 mmol), B_2pin_2 (1142 mg, 4.5 mmol) in anhydrous DMF (35 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (184 mg, 0.23 mmol). The mixture was stirred at 85 °C under Ar atmosphere overnight. The mixture was poured into water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo* to give crude product of 2-hydroxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile as black oil. To a solution of 2-hydroxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (crude), **8a** (1.01 g, 3.0 mmol), $PdCl_2(Ph_3P)_2$ (106 mg, 0.15 mmol), DME (30 mL) and water (10 mL) was added K_2CO_3 (1.24 g, 9.0 mmol). The mixture was stirred at 85°C under Ar atmosphere for 4 h. The mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by

column chromatography (silica gel, eluted with 10%–50% EtOAc in hexane) and to give 767 mg (68%) of **13** as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.49 (3H, s), 4.35 (2H, s), 6.95–7.04 (2H, m), 7.42–7.62 (6H, m).

5.1.32. 2-Cyano-5-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)phenyl trifluoromethanesulfonate (**14**)

A mixture of **13** (480 mg, 1.28 mmol), McMurry-Hendrickson reagent (549 mg, 1.54 mmol), and Cs_2CO_3 (501 mg, 1.54 mmol) in DMF (5 mL) was stirred at room temperature for 5 h. The mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 10%–33% EtOAc in hexane) and to give 349 mg (54%) of **14** as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.54 (3H, s), 4.37 (2H, s), 7.46–7.64 (6H, m), 7.74–7.80 (1H, m).

5.1.33. 4-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)-2-vinylbenzonitrile (**15a**)

A mixture of **14** (760 mg, 1.5 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (305 μl, 1.8 mmol), $PdCl_2(Ph_3P)_2$ (53 mg, 0.075 mmol), and K_2CO_3 (622 mg, 4.5 mmol) in DME (15 mL) and water (5 mL) was stirred at 85°C under Ar atmosphere overnight. The mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 10%–33% EtOAc in hexane) and to give 493 mg (85%) of **15a** as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.52 (3H, s), 4.36 (2H, s),

5.58 (1H, d, *J* = 10.9 Hz), 5.95 (1H, d, *J* = 17.3 Hz), 7.07 (1H, dd, *J* = 17.5, 11.1 Hz), 7.33–7.40 (1H, m), 7.44–7.69 (6H, m).

5.1.34. 4-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)-2-(prop-1-en-2-yl)benzonitrile (**15b**)

Compound **15b** was obtained as colorless oil in 72% yield by a method similar to that described for **15a**. ^{1}H NMR (300 MHz, $CDCl_3$) 2.19 (3H, dd, *J* = 1.5, 0.8 Hz), 2.51 (3H, s), 4.36 (2H, s), 5.26–5.29 (1H, m), 5.39–5.43 (1H, m), 7.34–7.40 (2H, m), 7.44–7.70 (5H, m).

5.1.35. 2-Ethyl-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzonitrile (**16a**)

A mixture of **15a** (493 mg, 1.3 mmol) and 10% palladium on carbon (50 mg) in THF (10 mL) and MeOH (5 mL) was stirred at room temperature under hydrogen atmosphere for 30 min. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 5%–33% EtOAc in hexane) and to give 461 mg (93%) of **16a** as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) 1.31 (3H, t, *J* = 7.6 Hz), 2.51 (3H, s), 2.89 (2H, q, *J* = 7.6 Hz), 4.36 (2H, s), 7.27–7.36 (2H, m), 7.44–7.66 (5H, m).

5.1.36. 2-Isopropyl-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzonitrile (**16b**)

Compound **16b** was obtained as colorless oil in 98% yield by a method similar to that described for **16a**. ^{1}H NMR (300 MHz, $CDCl_3$) 1.33 (6H, d, *J* = 6.8 Hz), 2.50 (3H, s), 3.32–3.47 (1H, m), 4.36 (2H, s), 7.29 (1H, dd, *J* = 7.9, 1.9 Hz), 7.38 (1H, d, *J* = 1.5 Hz), 7.44–7.64 (5H, m).

5.1.37. 2-Ethyl-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzamide (**4d**)

To a mixture of **16a** (455 mg, 1.2 mmol) and K_2CO_3 (81 mg, 0.59 mmol) in DMSO (8 mL) was added 30% aqueous H_2O_2 solution (0.5 mL) at 0°C. The mixture was warmed to room temperature and stirred for 1 h. The mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 33%–75% EtOAc in hexane) and recrystallized from hexane–EtOAc to give 230 mg (67%) of **4d** as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 1.27 (3H, t, J = 7.6 Hz), 2.49 (3H, s), 2.88 (2H, q, J = 7.6 Hz), 4.35 (2H, s), 5.75 (2H, brs), 7.20–7.32 (2H, m), 7.41–7.64 (5H, m). MS (ESI+) *m/z* = 405.2 $[M+H]^+$. Anal. Calcd for $C_{21}H_{19}F_3N_2OS$: C. 62.36; H. 4.74; N. 6.93: found: C. 62.44; H, 4.80; N. 6.96.

5.1.38. 2-Isopropyl-4-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)benzamide (**4e**)

Compound **4e** was obtained as colorless solid in 29% yield by a method similar to that described for **4d**. ^{1}H NMR (300 MHz, $CDCl_3$) 1.28 (6H, d, J = 6.8 Hz), 2.49 (3H, s), 3.42–3.55 (1H, m), 4.35 (2H, s), 5.71 (2H, brs), 7.19–7.25 (1H, m), 7.36–7.43 (2H, m), 7.44–7.65 (4H, m). MS (ESI+) *m/z* = 419.2 $[M+H]^+$. Anal. Calcd for $C_{22}H_{21}F_3N_2OS$: C, 63.14; H, 5.06; N, 6.69. Found: C, 63.08; H, 5.10; N, 6.72.

5.1.39. 2-Methoxy-5-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)pyridine (**17**)

To a solution of **8a** (335 mg, 1.00 mmol), (6-methoxypyridin-3-yl)boronic acid (230 mg, 1.50 mmol), $Pd_2(dba)_2$ (46 mg, 0.50 mmol), and X-Phos (48 mg, 0.10 mmol) in DME (4 mL) and water (1 mL) was added Cs_2CO_3 (652 mg, 2.00 mmol). The mixture was stirred at

80°C under N_2 atmosphere for 3 h. The mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 3%–30% EtOAc in hexane) and to give 319 mg (87%) of **17** as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.45 (3H, s), 3.96 (3H, s), 4.35 (2H, s), 6.78 (1H, dd, J = 8.7, 0.8 Hz), 7.42–7.65 (5H, m), 8.19 (1H, dd, J = 2.6, 0.8 Hz).

5.1.40. 5-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)pyridin-2-ol (**18**)

A mixture of **17** (150 mg, 0.41 mmol) and 48% aqueous hydrogen bromide solution (2 mL) in acetic acid (2 mL) was stirred at 80°C for 6 h. The mixture was diluted with EtOAc, washed with water, saturated aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4 and concentrated *in vacuo* to give 116 mg (81%) of **18** as yellow amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) 2.41 (3H, s), 4.32 (2H, s), 6.62 (1H, d, J = 9.6 Hz), 7.39–7.62 (6H, m), OH peak was not observed.

5.1.41. 2-Chloro-5-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)pyridine (**19**)

A mixture of **18** (568 mg, 1.62 mmol) and phosphorus oxychloride (3 mL) was stirred at 100°C for 4 h. The mixture was concentrated *in vacuo*. The residue was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 10%–50% EtOAc in hexane) to give 286 mg (48%) of **19** as brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.48 (3H, s), 4.36 (2H, s), 7.37 (1H, dd, J = 8.3, 0.8 Hz), 7.44–7.68 (5H, m), 8.42 (1H, dd, J = 2.6, 0.8 Hz).

5.1.42. 5-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)pyridine-2-carbonitrile

(**20**)

A mixture of **19** (286 mg, 0.780 mmol), zinc cyanide (136 mg, 1.16 mmol) and $Pd(Ph_3P)_4$ (90 mg, 0.078 mmol) in NMP (2 mL) was stirred at 100°C overnight. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 10%–50% EtOAc in hexane) to give 153 mg (55%) of **20** as pale brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.53 (3H s), 4.37 (2H, s), 7.45–7.63 (4H, m), 7.72 (1H, dd, J = 8.3, 0.8 Hz), 7.82 (1H, dd, J = 8.2, 2.2 Hz), 8.75 (1H, dd, J = 2.2, 0.8 Hz).

5.1.43. 5-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)pyridine-2-carboxamide

(**4i**)

To a mixture of **20** (143 mg, 0.40 mmol) and K_2CO_3 (28 mg, 0.20 mmol) in DMSO (2 mL) was added 30% aqueous H_2O_2 solution (0.2 mL). The mixture stirred at room temperature for 2 h. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 30%–100% EtOAc in hexane) and recrystallized from heptane–EtOAc to give 98 mg (65%) of **4i** as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.53 (3H, s), 4.38 (2H, s), 5.67 (1H, brs), 7.45–7.66 (4H, m), 7.80 (1H, brs), 7.86 (1H, dd, J = 8.3, 2.3 Hz), 8.23 (1H, dd, J = 8.3, 0.8 Hz), 8.61 (1H, dd, J = 2.3, 0.8 Hz). MS (ESI+) m/z = 378.2 $[M+H]^+$. Anal. Calcd for $C_{18}H_{14}F_3N_3OS$: C, 57.29; H, 3.74; N, 11.13. Found: C, 57.05; H, 3.82; N, 11.11.

5.1.44. 5-Methoxy-2-methylpyridine (**22**)

To a suspension of 6-methylpyridin-3-ol (25.0 g, 229 mmol) in THF (500 mL) was added potassium *tert*-butoxide (28.2 g, 252 mmol) portionwise at 0°C. After 30 min, iodomethane (15.7 mL, 252 mmol) was added and the mixture was stirred overnight. To the mixture was added water and the solvent was removed *in vacuo*. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 50% EtOAc in hexane) to give 8.20 g (29%) of **22** as pale orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.49 (3H, s), 3.83 (3H, s), 7.02–7.15 (2H, m), 8.20 (1H, d, J = 2.6 Hz).

5.1.45. 1-(5-Methoxypyridin-2-yl)acetone (**23**)

To a solution of **22** (3.69 g, 30.0 mmol) in THF (50 mL) was added 1.6 M *n*-butyl lithium in hexane solution (23 mL, 36 mmol) dropwise at 0°C. After stirring for 30 min, dimethylacetamide (3.3 mL) in THF (10 mL) was added dropwise. The mixture was warmed to room temperature and stirred overnight. The mixture was poured into aqueous NH_4Cl solution and extracted with EtOAc. Organic layer was separated, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 20%–100 % EtOAc in hexane) to give 1.48 g (30%) of **23** as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.21 (3H, s), 3.86 (3H, s), 3.86 (2H, s), 7.10–7.22 (2H, m), 8.26 (1H, dd, J = 3.0, 0.8 Hz).

5.1.46. 1-Bromo-1-(5-methoxypyridin-2-yl)acetone (**24**)

To a solution of **23** (165 mg, 1.00 mmol) in MeCN was added NBS (187 mg, 1.05 mmol) portionwise at 0°C. After stirring for 1 h, the mixture was concentrated *in vacuo*. The

residue was purified by column chromatography (silica gel, eluted with 3%–30 % EtOAc in hexane) to give 179 mg (73%) of **24** as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.40 (3H, s), 3.86 (3H, s), 5.51 (1H, s), 7.22 (1H, dd, *J* = 8.8, 3.0 Hz), 7.48 (1H, d, *J* = 8.5 Hz), 8.23 (1H, d, *J* = 3.0 Hz).

5.1.47. 5-Methoxy-2-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)pyridine (**25**)

A mixture of **24** (179 mg, 0.73 mmol) and **6a** (161 mg, 0.73 mmol) in EtOH (2 mL) was stirred at 50°C for 3 h. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 5%–70% EtOAc in hexane) to give 34 mg (13%) of **25** as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 2.62 (3H, s), 3.88 (3H, s), 4.33 (2H, s), 7.23 (1H, dd, *J* = 8.7, 3.0 Hz), 7.41-7.63 (5H, m), 8.29 (1H, dd, *J* = 3.0, 0.8 Hz).

5.1.48. 6-(4-methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)pyridin-3-ol (**26**)

Compound **26** was obtained as pale yellow solid in 77% yield by a method similar to that described for **18**. ^{1}H NMR (300 MHz, $CDCl_3$) 2.57 (3H, s), 4.33 (2H, s), 7.18 (1H, dd, *J* = 8.6, 2.9 Hz), 7.31–7.60 (5H, m), 8.20 (1H, d, *J* = 3.0 Hz), OH peak was not observed.

5.1.49. 6-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)pyridin-3-yl trifluoromethanesulfonate (**27**)

To a solution of **26** (700 mg, 2.00 mmol) in pyridine (5 mL) was added trifluoromethanesulfonic anhydride (0.67 mL, 4.0 mmol) dropwise at 0°C. After stirring for 2 h, the mixture was added to saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The extracts were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel,

eluted with 5%–50% EtOAc in hexane) to give 780 mg (81%) of **27** as colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.69 (3H, s), 4.35 (2H, s), 7.42–7.71 (6H, m), 8.54 (1H, d, *J* = 1.9 Hz).

5.1.50. 6-(4-Methyl-2-(3-(trifluoromethyl)benzyl)-1,3-thiazol-5-yl)nicotinamide (**4j**)

A mixture of **27** (600 mg, 1.24 mmol), zinc cyanide (219 mg, 1.87 mmol) and $Pd(Ph_3P)_4$ (143 mg, 0.124 mmol) in NMP (3 mL) was stirred at 150°C overnight. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 10%–50% EtOAc in hexane) and recrystallized from heptane–EtOAc to give 129 mg (28%) of **4j** as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.72 (3H, s), 4.36 (2H, s), 5.96 (2H, brs), 7.40–7.70 (5H, m), 8.19 (1H, dd, *J* = 8.3, 2.3 Hz), 8.95 (1H, dd, *J* = 2.3, 0.8 Hz). MS (ESI+) *m/z* = 378.4 $[M+H]^+$. Anal. Calcd for $C_{18}H_{14}F_3N_3OS\ H_2O$: C, 55.95; H, 3.91; N, 10.88. Found: C, 55.72; H, 4.06; N, 10.73.

5.1.51. (4-Methyl-1,3-thiazol-2-yl)methanol (**29**)

To a solution of 4-methyl-1,3-thiazole-2-carbaldehyde (10.0 g, 79.0 mmol) in MeOH (200 mL) was added sodium borohydride (2.98 g, 79.0 mmol) at 0°C. After being stirred for 1 h at 0°C, the reaction mixture was quenched with aqueous NH_4Cl solution at 0°C and then extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, eluted with 50% EtOAc in hexane) to give 10.0 g (98%) of **29** as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) 2.43 (3H, s), 3.29 (1H, brs), 4.91 (2H, d, *J* = 6.0 Hz), 6.85 (1H, s)

5.1.52. (5-Bromo-4-methyl-1,3-thiazol-2-yl)methanol (**30**)

To a solution of **29** (10.0 g, 77 mmol) in MeCN (387 mL) was added NBS (13.8 g, 77.0 mmol) at room temperature. After being stirred for 2 h at room temperature, the reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc, washed with saturated aqueous NH_4Cl solution, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 33% EtOAc in hexane) to give 12.6 g (78%) of **30** as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) 2.37 (3H, s), 2.74 (1H, brs), 4.85 (2H, s).

5.1.53. Ethyl 4-(2-(hydroxymethyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzoate (**31**)

A mixture of **30** (18.0 g, 87.0 mmol), ethyl 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (25.1 g, 87 mmol), $Pd(Ph_3P)_4$ (2.00 g, 1.73 mmol) and 2 M aqueous Na_2CO_3 solution (87 mL) in DME (433 mL) was stirred for 15 h at 80°C. The reaction mixture was cooled to room temperature and treated with water. The separated organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 50% EtOAc in hexane) to give 17.4 g (69%) of **31** as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) 1.41 (3H, t, J = 7.2 Hz), 2.50 (3H, s), 2.64 (3H, s), 2.75 (1H, t, J = 6.0 Hz), 4.38 (2H, t, J = 7.2 Hz), 4.93 (2H, d, J = 6.0 Hz), 7.30 (1H, s), 7.31 (1H, d, J = 7.6 Hz), 7.97 (1H, d, J = 7.6 Hz).

5.1.54. 4-(2-(Hydroxymethyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzoic acid (**32**)

To a solution of **31** (7.00 g, 24.0 mmol) in EtOH (80 mL) was added 6 N aqueous NaOH solution (8.0 mL, 48 mmol) at 0°C. After being refluxed for 3 h, the mixture was concentrated *in vacuo*. The residue was diluted with water and washed with

dichloromethane. The aqueous layer was acidified with 6 N aqueous HCl solution and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residual solid was suspended in EtOAc and hexane, collected by filtration and rinsed with hexane to give 6.09 g (96%) of **32** as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.43 (3H, s), 2.57 (3H, s), 4.70 (2H, d, J = 4.8 Hz), 6.09 (1H, brs), 7.39–7.40 (2H, m), 7.90 (1H, d, J = 8.0 Hz), 12.9 (1H, brs).

5.1.55. 4-(2-(Hydroxymethyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**33**)

A mixture of **32** (6.09 g, 23.1 mmol), NH_4Cl (3.71 g, 69.4 mmol), EDCI (4.88 g, 25.4 mmol), HOBT (3.90 g, 25.4 mmol) and *N*,*N*-diisopropylethylamine (12.1 mL, 69.4 mmol) in DMF (58 mL) was stirred for 18 h at room temperature. After concentration *in vacuo*, the residue was diluted with saturated aqueous NH_4Cl solution and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by recrystallization from hexane–EtOAc to give 6.07 g (84%) of **33** as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (3H, s), 2.41 (3H, s), 4.69 (2H, d, J = 5.6 Hz), 6.07 (1H, t, J = 6.0 Hz), 7.30–7.33 (2H, m), 7.42 (1H, brs), 7.44 (1H, d, J = 7.6 Hz), 7.77 (1H, brs).

5.1.56. 4-(2-(Chloromethyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**34**)

A mixture of **33** (8.15 g, 31.1 mmol) and thionyl chloride (22.7 mL, 311 mmol) was stirred for 1 h at 0°C. The mixture was concentrated *in vacuo*. The residue was quenched with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by recrystallization from hexane–EtOAc to give 6.46 g (74%) of **34** as

white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (3H, s), 2.55 (3H,s), 4.82 (2H, s), 5.76 (2H, brs), 7.28–7.31 (2H, m), 7.53 (1H, d, J = 8.0 Hz).

5.1.57. 4-(2-(3-Methoxybenzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**4k**)

To a solution of **34** (100 mg, 0.36 mmol), Cs_2CO_3 (174 mg, 0.53 mmol), 3-methoxyphenyl boronic acid (83.0 mg, 0.55 mmol) in THF (4.0 mL) and water (0.80 mL) was added $PdCl_2$(dppf)-CH_2Cl_2 (20.2 mg, 0.02 mmol). The mixture was stirred at 130°C for 30 min under microwave irradiation. The residue was purified by column chromatography (NH silica gel, eluted with EtOAc) then preparative HPLC. The desired fraction was neutralized with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*, then recrystallized from EtOAc–heptane to give 53.5 mg (43%) of **4k** as amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (3H, s), 2.40 (3H, s), 3.75 (3H, s), 4.25 (2H, s), 6.82–6.88 (1H, m), 6.91–6.96 (2H, m), 7.23–7.31 (3H, m), 7.36–7.44 (2H, m), 7.75 (1H, brs). MS (ESI+) m/z = 353.1 $[M+H]^+$.

The following compounds (**4l**–**p**) were prepared from corresponding aryl boronic acids by a method similar to that described for **4k**.

5.1.58. 4-(2-(3-(Dimethylamino)benzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**4l**)

Yield 32%, as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (3H, s), 2.40 (3H, s), 2.89 (6H, s), 4.19 (2H, s), 6.59–6.67 (2H, m), 6.71–6.76 (1H, m), 7.15 (1H, t, J = 7.8 Hz), 7.24–7.30 (2H, m), 7.36–7.44 (2H, m), 7.75 (1H, brs). MS (ESI+) m/z = 366.2 $[M+H]^+$.

5.1.59. 4-(2-(3-Fluorobenzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**4m**)

Yield 34%, as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (3H, s), 2.40 (3H, s), 4.33 (2H, s), 7.07–7.17 (1H, m), 7.19–7.32 (4H, m), 7.35–7.48 (3H, m), 7.75 (1H, s). MS (ESI+) *m/z* = 341.1 $[M+H]^+$.

5.1.60. 4-(2-(3-Chlorobenzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**4n**)

Yield 38%, as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (3H, s), 2.40 (3H, s), 4.32 (2H, s), 7.24–7.51 (8H, m), 7.76 (1H, brs). MS (ESI+) *m/z* = 357.1 $[M+H]^+$.

5.1.61. 4-(2-(2-Chlorobenzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**4o**)

Yield 44%, as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (3H, s), 2.40 (3H, s), 4.41 (2H, s), 7.24–7.30 (2H, m), 7.34–7.44 (4H, m), 7.48–7.56 (2H, m), 7.75 (1H, s). MS (ESI+) *m/z* = 357.1 $[M+H]^+$.

5.1.62. 4-(2-(4-Chlorobenzyl)-4-methyl-1,3-thiazol-5-yl)-2-methylbenzamide (**4p**)

Yield 37%, as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.38 (3H, s), 2.40 (3H, s), 4.30 (2H, s), 7.24–7.30 (2H, m), 7.35–7.47 (6H, m), 7.75 (1H, brs). MS (ESI+) *m/z* = 357.1 $[M+H]^+$. Anal. Calcd for $C_{19}H_{17}N_2OSCl$: C, 63.95; H, 4.80; N, 7.85. Found: C, 63.61; H, 5.00; N, 7.52.

5.1.63. Methyl 4-(3-fluoro-5-(trifluoromethyl)phenyl)-3-oxobutanoate (**36**)

To a solution of 2-(3-fluoro-5-(trifluoromethyl)phenyl)acetic acid (9.96 g, 44.8 mmol), 2,2-dimethyl-1,3-dioxane-4,6-dione (8.40 g, 58.3 mmol) and EDCI (11.2 g, 58.3 mmol) in anhydrous DMF (30 mL) was added *N*,*N*-dimethylaminopyridine (8.76 g, 71.7 mmol) at room temperature. The mixture was stirred for 4 h. The mixture was poured into 1 N aqueous HCl solution and extracted with EtOAc. The organic layer was separated, washed

with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in MeOH (50 mL). The mixture was refluxed overnight. After cooling, the mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 0%–10% EtOAc in hexane) to give 7.7 g (48%) of **36** as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 3.53 (2H, s), 3.76 (3H, s), 3.93 (2H, s), 7.08–7.38 (3H, m).

5.1.64. 5-(3-Fluoro-5-(trifluoromethyl)benzyl)-2-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**37**)

A solution of **36** (5.00 g, 18.0 mmol) and methylhydrazine (1.24 g, 27.0 mmol) in MeOH (30 mL) was stirred at room temperature for 4 h. The mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 0%–90% EtOAc in hexane) to give 3.39 g (69%) of **37** as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) 3.12 (2H, s), 3.31 (3H, s), 3.77 (2H, s), 7.14 (1H, d, J = 8.3 Hz), 7.23–7.31 (2H, m).

5.1.65. 3-(3-Fluoro-5-(trifluoromethyl)benzyl)-1-methyl-1*H*-pyrazol-5-yl trifluoromethanesulfonate (**38**)

To a solution of **37** (3.39 g, 12.4 mmol) in pyridine (50 mL) was added trifluoromethanesulfonic anhydride (2.51 mL, 14.8 mmol) at −20°C. The mixture was allowed to warm to room temperature and stirred at room temperature under N_2 for 1 h. The mixture was azeotroped with toluene. The residue was purified by column chromatography (NH silica gel, eluted with 0%–50% EtOAc in hexane) to give 2.42 g (48%) of **38** as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 3.79 (3H, s), 3.96 (2H, s), 5.92 (1H, s), 7.07–7.35 (3H, m).

5.1.66. Ethyl 4-(3-(3-fluoro-5-(trifluoromethyl)benzyl)-1-methyl-1*H*-pyrazol-5-yl)-2-

methylbenzoate (**39**)

To a solution of **38** (2.42 g, 5.96 mmol), ethyl 2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (2.59 g, 8.93 mmol) and $Pd(Ph_3P)_4$ (0.688 g, 0.60 mmol) in DME (30 ml) was added Na_2CO_3 (5.96 ml, 11.9 mmol) at room temperature. The mixture was stirred at 90°C under N_2 overnight. The mixture was poured into water at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 0%–20% EtOAc in hexane) to give 2.16 g (86%) of **39** as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 1.41 (3H, t, *J* = 7.0 Hz), 2.64 (3H, s), 3.87 (3H, s), 4.04 (2H, s), 4.38 (2H, q, *J* = 7.2 Hz), 6.11 (1H, s), 7.14–7.24 (2H, m), 7.24–7.32 (2H, m), 7.37 (1H, s), 7.98 (1H, d, *J* = 8.3 Hz).

5.1.67. 4-(3-(3-Fluoro-5-(trifluoromethyl)benzyl)-1-methyl-1*H*-pyrazol-5-yl)-2-methylbenzoic acid (**40**)

To a solution of **39** (1.81 g, 4.31 mmol) in EtOH (100 mL) was added 1 N aqueous NaOH solution (10.0 mL, 10.0 mmol) at room temperature. After being stirred at 80°C for 2 h, the mixture was neutralized with 1 N aqueous HCl solution at room temperature and concentrated *in vacuo*. The residue was diluted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was crystallized from EtOAc–hexane to give 1.35 g (80%) of **40** as colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) 2.56 (3H, s), 3.83 (3H, s), 4.03 (2H, s), 6.36 (1H, s), 7.32–7.58 (5H, m), 7.89 (1H, d, *J* = 8.0 Hz), 12.93 (1H, brs).

5.1.68. 4-(3-(3-Fluoro-5-(trifluoromethyl)benzyl)-1-methyl-1*H*-pyrazol-5-yl)-2-

methylbenzamide (**4s**)

A mixture of **40** (4.18 g, 10.7 mmol), EDCI (3.06 g, 16.0 mmol) and HOBt-NH_3 (2.43 g, 16.0 mmol) in DMF (30 mL) was stirred at room temperature for 3 h. The mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ solution, water, and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was recrystallized from heptane–EtOAc to give 3.73 g (89%) of **4s** as colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) 2.41 (3H, s), 3.81 (3H, s), 4.03 (2H, s), 6.30 (1H, s), 7.30–7.59 (7H, m), 7.78 (1H, brs). MS (ESI+) *m/z* = 392.3 $[M+H]^+$. Anal. Calcd for $C_{20}H_{17}F_4N_3O$: C, 61.38; H, 4.38; N, 10.74. Found: C, 61.35; H, 4.44; N, 10.71.

5.1.69. Ethyl 4-fluoro-2-methylbenzoate (**42**)

Thionyl chloride (10 ml, 137 mmol) was added to 4-fluoro-2-methylbenzoic acid (10.0 g, 64.9 mmol) at 0°C. The mixture was stirred at 80°C for 2 h. After cooling, the mixture was concentrated *in vacuo*. To the residue EtOH (20 ml) was added slowly at 0°C. The mixture was stirred at room temperature overnight. The mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 30% EtOAc in hexane) to give 11.0 g (93%) of **42** as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 1.39 (3H, t, *J* = 7.0 Hz), 2.61 (3H, s), 4.35 (2H, q, *J* = 7.1 Hz), 6.86–6.98 (2H, m), 7.90–8.00 (1H, m).

5.1.70. Ethyl 4-(4-iodo-2-methyl-1*H*-imidazol-1-yl)-2-methylbenzoate (**43**)

4-Iodo-2-methyl-1*H*-imidazole (5.44 g, 26.14 mmol) was added to a suspension of sodium hydride (1.10 g, 27.4 mmol) and 18-crown-6-ether (0.691 g, 2.61 mmol) in NMP (85 mL) at 0°C. The mixture was stirred at room temperature under N_2 atmosphere for 30 min. The solution of **42** (5.00 g, 27.4 mmol) in NMP (15 mL) was added dropwise to a

solution of the mixture in at 0°C. The mixture was stirred at 110°C under N_2 atmosphere overnight. After cooling, the mixture was poured into saturated aqueous NH_4Cl solution at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 10%–50% EtOAc in hexane) to give 5.57g (58%) of **43** as off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) 1.42 (3H, t, J = 7.2 Hz), 2.38 (3H, s), 2.66 (3H, s), 4.40 (2H, q, J = 7.2 Hz), 7.10 (1H, s), 7.12–7.19 (2H, m), 8.00–8.07 (1H, m).

5.1.71. Ethyl 4-(4-(3-fluoro-5-(trifluoromethyl)benzyl)-2-methyl-1*H*-imidazol-1-yl)-2-methylbenzoate (**44**)

To a suspension of zinc (0.530 g, 8.10 mmol) in anhydrous THF (15.0 mL) was added 1,2-dibromobutane (0.032 mL, 0.27 mmol) at room temperature under Ar atmosphere. The mixture was stirred at 70°C under Ar atmosphere for 30 min. After cooling, chlorotrimethylsilane (0.047 mL, 0.54 mmol) was added to the mixture at room temperature. After being stirred at room temperature for 15 min, 1-(bromomethyl)-3-fluoro-5-(trifluoromethyl)benzene (2.08 g, 8.10 mmol) in anhydrous THF (5.00 mL) was slowly added to the reaction mixture. The mixture was stirred at room temperature under N_2 atmosphere for 5 h. To the mixture was added **43** (2.00 g, 5.40 mmol), palladium(II) acetate (0.121 g, 0.54 mmol), 2,2'-bis-(diphenylphosphino)-1,1'-binaphthyl (0.336 g, 0.54 mmol) at room temperature. The mixture was stirred at 80°C under N_2 atmosphere overnight. The mixture was poured into water at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated *in*

vacuo. The residue was purified by column chromatography (silica gel, eluted with 0%–40% EtOAc in hexane) to give 1.83 g (81%) of **44** as dark yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 1.41 (3H, t, J = 7.2 Hz), 2.38 (3H, s), 2.65 (3H, s), 3.97 (2H, s), 4.39 (2H, q, J = 7.1 Hz), 6.73 (1H, s), 7.13–7.26 (4H, m), 7.38 (1H, s), 7.98–8.06 (1H, m).

5.1.72. 4-(4-(3-Fluoro-5-(trifluoromethyl)benzyl)-2-methyl-1*H*-imidazol-1-yl)-2-methylbenzoic acid (**45**)

1 N aqueous NaOH solution (6.53 mL, 6.53 mmol) was added to a solution of **44** (1.83 g, 4.35 mmol) in EtOH (18 mL) at room temperature. The mixture was stirred at room temperature overnight. 1 N aqueous HCl solution (6 mL, 6 mmol) was added to bring the pH of the solution to 7–8 and extracted with EtOAc. The water layer was separated. 1 N aqueous HCl solution was added to bring the pH of the water layer to 6–7 and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was triturated with IPE to give 1.40 g (82%) of **45** as solid. ^{1}H NMR (300 MHz, DMSO-d_6) 2.30 (3H, s), 2.57 (3H, s), 3.93 (2H, s), 7.18 (1H, s), 7.32–7.58 (5H, m), 7.93 (1H, d, J = 7.9 Hz), COOH peak was not observed.

5.1.73. 4-(4-(3-Fluoro-5-(trifluoromethyl)benzyl)-2-methyl-1*H*-imidazol-1-yl)-2-methylbenzamide (**4t**)

To a solution of **45** (300 mg, 0.76 mmol), HOBt-NH_3 (175 mg, 1.15 mmol) in anhydrous DMF (3 mL) was added EDCI (220 mg, 1.15 mmol) at room temperature. The mixture was stirred at room temperature overnight. The mixture was poured into water at room temperature and extracted with EtOAc. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$ and concentrated *in*

vacuo. The solid was crystallized from EtOAc–heptane to give 220 mg (74%) of **4t** as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) 2.27 (3H, s), 2.41 (3H, s), 3.92 (2H, s), 7.12 (1H, s), 7.25–7.36 (2H, m), 7.41–7.60 (5H, m), 7.82 (1H, brs). MS (ESI+) *m/z* = 392.3 $[M+H]^+$. Anal. Calcd for $C_{20}H_{17}F_4N_3O$: C, 61.38; H, 4.38; N, 10.74. Found: C, 60.22; H, 4.38; N, 10.49.

5.1.74. Ethyl 4-hydrazino-2-methylbenzoate (**46**)

To a solution of **42** (20.0 g, 110 mmol) in DMSO (100 mL) was added hydrazine monohydrate (53.2 mL, 1100 mmol). The mixture was stirred at 120°C for 4 h. The mixture was diluted with EtOAc. The mixture was washed with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give 18.3 g (86%) of **46** as off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) 1.36 (3H, t, *J* = 7.2 Hz), 2.58 (3H, s), 3.61 (2H, s), 4.30 (2H, q, *J* = 7.2 Hz), 5.44 (1H, brs), 6.59–6.64 (2H, m), 7.86–7.92 (1H, m).

5.1.75. Methyl 2-(3-fluoro-5-(trifluoromethyl)phenyl)ethanimidothioate hydroiodide (**47**)

To a solution of **6c** (6.00 g, 25.3 mmol) in acetone (30 mL) was slowly added iodomethane (2.37 mL, 38.1 mmol). The mixture was stirred at room temperature for 5 h. The mixture was concentrated *in vacuo*. The residue was triturated with IPE. The solid was collected by filtration to give 9.20 g (96%) of **47** as off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) 2.65 (3H, s), 4.35 (2H, s), 7.62-7.77 (3H, m), 11.67 (2H, brs).

5.1.76. Ethyl 4-(2-(1-amino-2-(3-fluoro-5-(trifluoromethyl)phenyl)ethylidene)hydrazino)-2-methyl benzoate hydrochloride (**48**)

To a solution of **47** (9.00 g, 23.7 mmol) in EtOH (50 mL) was added **46** (4.61 g, 23.7 mmol) with several portions with ice bath. The mixture was stirred with ice bath for 1 h. The mixture was concentrated *in vacuo*. The residue was diluted with EtOAc, washed with aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was triturated with EtOAc–hexane and the insoluble material was removed by filtration. To the filtrate was added 4 N HCl solution in EtOAc (90 mL, 360 mmol) dropwise with ice bath. The resulting solid was collected by filtration, followed by washing with EtOAc–hexane to give 10.1 g (98%) of **48** as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) 1.29 (3H, t, J = 7.1 Hz), 2.42 (3H, s), 4.12 (2H, s), 4.22 (2H, q, J = 7.1 Hz), 6.49 (1H, d, J = 2.1 Hz), 6.65 (1H, dd, J = 8.7, 2.1 Hz), 7.71–7.82 (2H, m), 7.87–7.99 (2H, m), 9.04 (1H, s), 9.35 (1H, brs), 10.05 (1H, brs), 12.10 (1H, brs).

5.1.77. Ethyl 4-(3-(3-fluoro-5-(trifluoromethyl)benzyl)-5-methyl-1*H*-1,2,4-triazol-1-yl)-2-methylbenzoate (**49**)

To a solution of **48** (9.50 g, 21.9 mmol) in 1,1,1-trimethoxyethane (70 mL, 550 mmol) was added acetic acid (0.251 mL, 4.38 mmol) at room temperature. The mixture was stirred at 100°C for 4 h. After cooling, the mixture was concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 10%–35% EtOAc in hexane). The desired fraction was collected, and concentrated *in vacuo* to give crude product. The crude product was triturated with hexane to give 6.60 g (72%) of **49** as colorless solid. ^{1}H

NMR (300 MHz, $CDCl_3$) 1.42 (3H, t, J = 7.2 Hz), 2.54 (3H, s), 2.68 (3H, s), 4.13 (2H, s), 4.40 (2H, q, J = 7.2 Hz), 7.17–7.23 (1H, m), 7.27–7.46 (4H, m), 8.06 (1H, d, J = 8.3 Hz).

5.1.78. 4-(3-(3-Fluoro-5-(trifluoromethyl)benzyl)-5-methyl-1*H*-1,2,4-triazol-1-yl)-2-methylbenzoic acid (**50**)

To a solution of **49** (6.40 g, 15.2 mmol) in EtOH (23 mL) was added 4 N aqueous NaOH solution (11.4 mL, 55.6 mmol). The mixture was stirred at 80°C for 3 h. To the mixture was neutralized with addition of 1 N aqueous HCl solution and the mixture was diluted with EtOAc–THF. The organic layer was separated and dried over Na_2SO_4, and concentrated *in vacuo*. The residue was triturated with IPE, and the solid was collected by filtration to give 5.64 g (94%) of **50** as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) 2.49 (3H, s), 2.59 (3H, s), 4.17 (2H, s), 7.45–7.65 (5H, m), 7.95 (1H, d, J = 8.3 Hz), COOH peak was not observed.

5.1.79. 4-(3-(3-Fluoro-5-(trifluoromethyl)benzyl)-5-methyl-1*H*-1,2,4-triazol-1-yl)-2-methylbenzamide (**4u**)

A mixture of **50** (5.40 g, 13.7 mmol), EDCI (2.90 g, 15.1 mmol), HOBt-NH_3 (2.30 g, 15.1 mmol) in DMF (50 mL) was stirred at room temperature overnight. The mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was recrystallized from water–EtOH to give 4.70 g (87%) of **4u** as white solid. ^{1}H NMR (300 MHz, $CDCl_3$) 2.52 (3H, s), 2.58 (3H, s), 4.13 (2H, s), 5.84 (1H, brs), 5.92 (1H, brs), 7.16–7.23 (1H, m), 7.27–7.47 (4H, m), 7.60 (1H, d, J = 8.2 Hz). MS (ESI+) *m/z*

= 393.2 $[M+H]^+$. Anal. Calcd for $C_{19}H_{16}F_4N_4O$: C, 58.16; H, 4.11; N, 14.28. Found: C, 58.12; H, 4.15; N, 14.22.

5.2. BIOLOGY

5.2.1. cAMP assay

CHO cells expressing human GPR52 (10,000 cells) were incubated with GPR52 agonists for 30 min at 37°C in 30 µL/well of assay buffer (Hanks' balanced salt solution (HBSS) containing 5 mM HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) (pH7.6), 0.5% BSA (bovine serum albumin), 100 µM IBMX (3-isobutyl-1-methylxanthine), and 100 µM Ro20-1724 (4-(3-Butoxy-4-methoxybenzyl)imidazolidin-2-one). Then, 10 µL/well of 0.1U/ µL anti cAMP acceptor beads (PerkinElmer) and 10 µL/well of 0.1U/µL Biotinylated cAMP donor beads (PerkinElmer) were added and incubated overnight at room temperature. cAMP levels were determined by measuring alphascreen signal with Envision (PerkinElmer).

5.2.2. Metabolic stability assay

Mouse, rat and human hepatic microsomes were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture with a final volume of 50 µL consisted of microsomal protein in 50 mmol/L phosphate buffer (pH 7.4) and 1 µmol/L test compound. The concentration of microsomal protein was 0.2 mg/mL. An NADPH-generating system containing 25 mmol/L $MgCl_2$, 25 mmol/L glucose-6-phosphate, 2.5 mmol/L -NADP+ and 7.5 unit/mL glucose-6-phosphate dehydrogenase was added to the incubation mixture with a 20% volume of the reaction mixture to initiate the enzyme reaction. After the addition of the NADPH-generating system, the mixture was incubated at 37°C for 15 and 30 min. The reaction was

terminated by the addition of acetonitrile equivalent to the volume of the reaction mixture. All incubations were made in duplicate. The test compound in the reaction mixture was measured by LC/MS/MS analysis. For metabolic stability determinations, chromatograms were analyzed for parent compound disappearance from the reaction mixtures.

5.2.3. Plasma protein binding assay

Plasma used in the study was obtained from male ICR mice. The plasma protein binding of compound **4u** was determined by the equilibrium dialysis method with HTDialysis™ Teflon dialysis chambers and cellulose membranes (MWCO 6-8 kDa). The compound was added to the plasma at the final concentration of 1 μmol/L. Dialysis was conducted against phosphate buffered saline (PBS) at room temperature for 24 h. The compound concentrations in both plasma and PBS sides were determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS). The value of plasma protein binding was calculated from the measured concentrations in PBS and plasma after the equilibrium.

5.2.4. Pharmacokinetic analysis in rat cassette dosing

Test compounds were administered intravenously (0.1 mg/kg) or orally (1 mg/kg) by cassette dosing to non-fasted rats. After administration, blood samples were collected and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized followed by centrifugation. The compound concentrations in the supernatant were measured by LC/MS/MS.

5.2.5. Pharmacokinetic analysis in mice

Compound **4u** was suspended in 0.5% methylcellulose solution and orally administered to male ICR mice at a dose of 10 or 30 mg/kg. The blood and brain samples were collected at

0.25, 0.5, 1, 2, 4, 8, 24 h after oral administration (n = 3 in each time-point). The blood samples were centrifuged to obtain the plasma and the brain was homogenized with saline. The plasma and brain homogenate samples were mixed with acetonitrile and centrifuged. The supernatants were analyzed by the high-performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) system composed of HPLC (Shimadzu, Kyoto, Japan) and API5000 (ABSciex, CA).

5.2.6. Psychostimulants-induced hyperlocomotion test

Male ICR mice (7–8 weeks) were used for all experiments. The locomotor activity in mice was measured using locomotor activity monitors, MDC-LT (Brain Science Idea Co., Ltd). Mice were individually placed in transparent polycarbonate cages (30×40×20 cm). After habituation for 60 min **4u** (1 ~ 30 mg/kg, p.o.) was administered. 60 min later, methamphetamine (2 mg/kg, s.c.) was injected. Activity counts were monitored for 150 min after administration of **4u**.

6. ACKNOWLEGMENT

We are grateful to Mr. Eiji Kimura, Dr. Hiroki Takahagi, Dr. Keiji Nishiyama and Dr. Takanobu Kuroita for helpful discussion in the course of preparing this manuscript.

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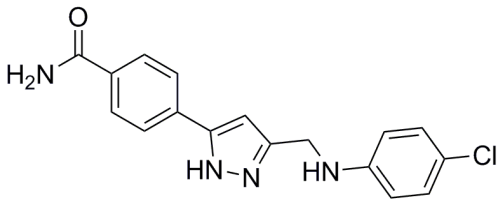

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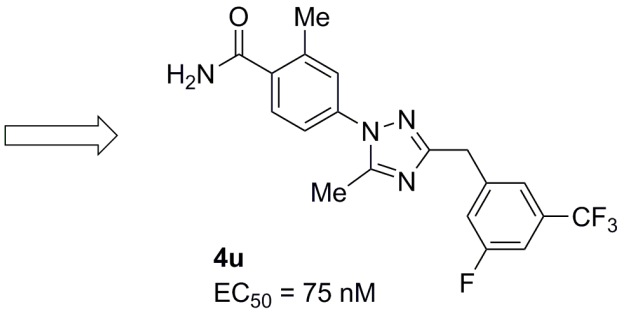
EC_{50} = 470 nM

E_{max} = 56 %



4u

EC_{50} = 75 nM

E_{max} = 122 %