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Discovery and biological profile of 4-(1-aryltriazol-4-yl)-tetrahydropyridines as an orally active new class of metabotropic glutamate receptor 1 antagonist

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ABSTRACT

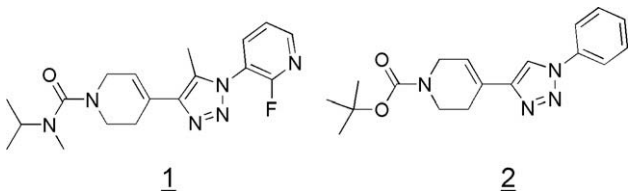
We describe here the discovery and the structure–activity relationship (SAR) of a series of 4-(1-Aryltriazol-4-yl)-tetrahydropyridines as novel mGluR1 antagonists. Our extensive chemical modification of lead compound **2** successfully led to fluoropyridine analogs **7j** and **1** with improved in vivo antagonistic activities. Among the evaluated compounds, chemically stable urea analog **1** showed oral antagonistic activity at dose ranges of 10–30 mg/kg in an animal model.

1. Introduction

Glutamate is one of the major excitatory neurotransmitters in the central nervous system (CNS) and it acts on ionotropic glutamate receptors including NMDA and non-NMDA receptors and on G-protein coupled metabotropic glutamate receptors (mGluRs). The mGluRs are classified into eight subtypes (three subclasses) based on sequence homology, coupling mechanisms to G-protein and pharmacological properties. mGluRs are considered to be drug targets in order to modulate glutamate transmission in the treatment of various neurological and psychiatric diseases including pain, epilepsy, Parkinson's disease, cognitive disorders, drug abuse, anxiety and schizophrenia.[1–4]

Since CPCCOEt[5,6] was identified as the first noncompetitive mGluR1 antagonist with moderate affinity, a number of quite potent and selective ligands have been developed. In general, noncompetitive mGluR1 antagonists act at the seven-transmembrane domain, not at the glutamate binding N-terminal extra cellular domain, and their structural features are very different from glutamate. For example, thiazolo[3,2,*a*]benzimidazole amide derivatives,[7] pyrrole derivatives,[8,9] quinoline derivatives,[10] triazafluorenones[11,12] and other derivatives[13–15] were reported to be low nanomolar mGluR1 antagonists. These structurally diverse agents have facilitated the elucidation of the mechanism of action of noncompetitive mGluR1 antagonists in vitro and also revealed their therapeutic potential by using established animal models. Thus the development of brain penetrable and also orally available mGluR1 antagonists with structural difference would be desired to carry out proof-of-concept studies for elucidation of the functions of mGluR1 in human.

In order to obtain structurally novel lead compound(s) suitable for developing into an orally available mGluR1 antagonist, we primarily screened our chemical library using human mGluR1a expressed in CHO cells, with antagonistic activity measured by FLIPR assay. Among the hit compounds, non-amino acid-like compound **2**[16] (Fig. 1) showed good antagonistic activity on human mGluR1 receptor with IC_{50} value of 9.8 nM but was a highly lipophilic and slightly water-soluble compound. Further profiling also revealed that this compound had only moderate in vitro antagonistic activity on mouse mGluR1a expressed in CHO cells. This level of activity was not sufficient for addressing the precise role of mGluR1 antagonists in vivo. In this paper, we describe the process and result of our chemical modification of this lead compound in order to identify a potent, selective and orally active mGluR1



1 2

Figure 1. Structures of mGluR1 antagonists.

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antagonist in which the potency and physicochemical properties are appropriately balanced.

2. Chemistry

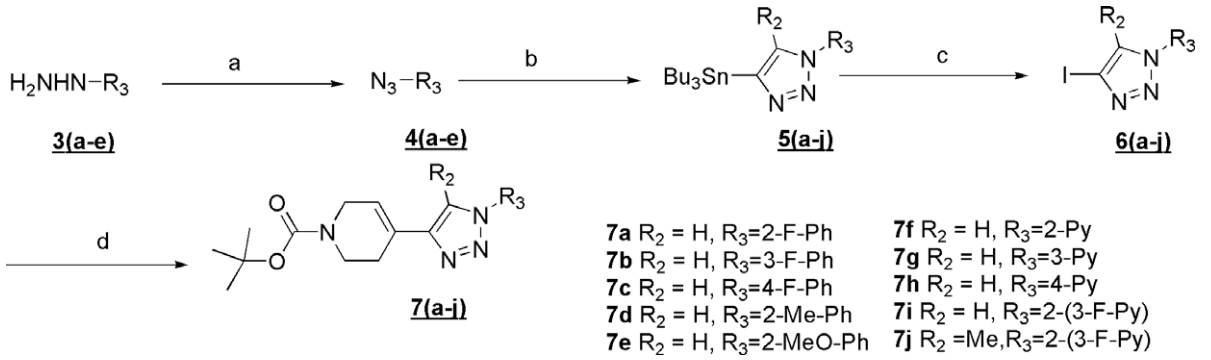
All of target compounds were prepared from arylazides (Scheme 1). Substituted phenylhydrazines were converted to corresponding azides by treating sodium nitrite in acidic condition. Each pyridylazides were prepared with known synthetic manners (Scheme 2).[17,18] These azides were regio-selectively cyclized with alkynyl tributyltin to give triazole **5**, according to the literature.[19] Tributylstannyl groups were replaced immediately by adding iodine at ambient temperature. Iodo-triazoles **6** were coupled with commercially available boronic ester in the presence of Pd catalyst followed by deprotection of the Boc group under an acidic condition, providing the corresponding amines.[20] Some target compounds and intermediates were prepared by treatment of acylchlorides or isocyanates with amine base (Scheme 3). Finally, representative compound **1** (FTIDC)[21] was obtained by methylation of the corresponding urea compound with methyl iodide and sodium hydride in DMF.

3. Results and discussion

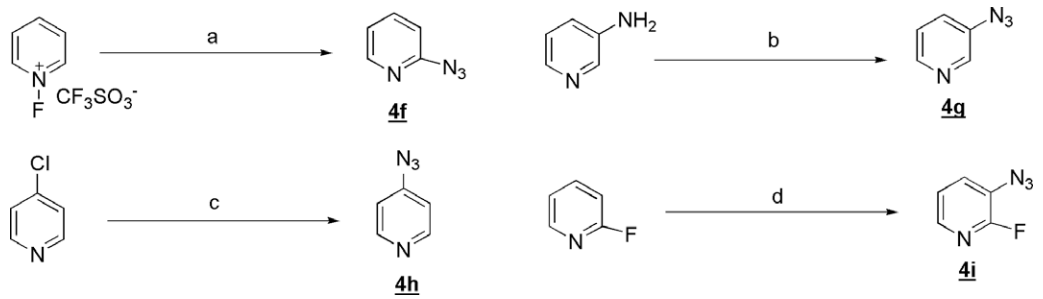
The compounds described here were tested for antagonistic activity on human and mouse mGluR1a CHO cells by measuring $[Ca^{2+}]$ with a FLIPR. Group I mGluRs include mGluR1 and mGluR5, thus synthesized compounds were also evaluated on human mGluR5 to confirm their subtype selectivity. The results are summarized in Table 1. Initially we started to explore the SAR of the right hand part of the molecule by putting a variety of substitutions on the benzene ring. Introduction of a fluorine group into the benzene *ortho* position slightly improved the antagonistic activity for human mGluR1 but the other positions were not affected positively (**7a**, **7b**, **7c**). Corresponding ortho-substituted methyl and methoxy analogs did not improve the activity compared to fluorinated compound. Replacement of the benzene ring itself with pyridine rings also decreased their activities (**7f, 7g, 7h**). Regarding to the R1, minimal alternation of acid labile *tert*-butyl carbamate into chemically stable *tert*-butyl urea and *tert*-butyl acetate also resulted in significant loss of antagonistic activity for human mGluR1 (**8, 9**). Unfortunately, we could not identify a potent antagonist with appropriate physicochemical properties through modifications of the benzene ring and N-Boc moiety.

Oral bioavailability of the compounds is greatly affected by their physicochemical properties. It is now widely recognized that these properties need to be carefully considered in the early drug discovery process, particularly with respect to their effects on the ADME (absorption, distribution, metabolism and excretion) of the compounds in animal and human systems.[22–27] Among the properties, lipophilicity is a fundamental property of compounds and is a major contributor to solubility, permeability and protein binding. Thus, we thought that lipophilicity was one of the important parameters for developing orally available and brain penetrable compounds in this lead class.

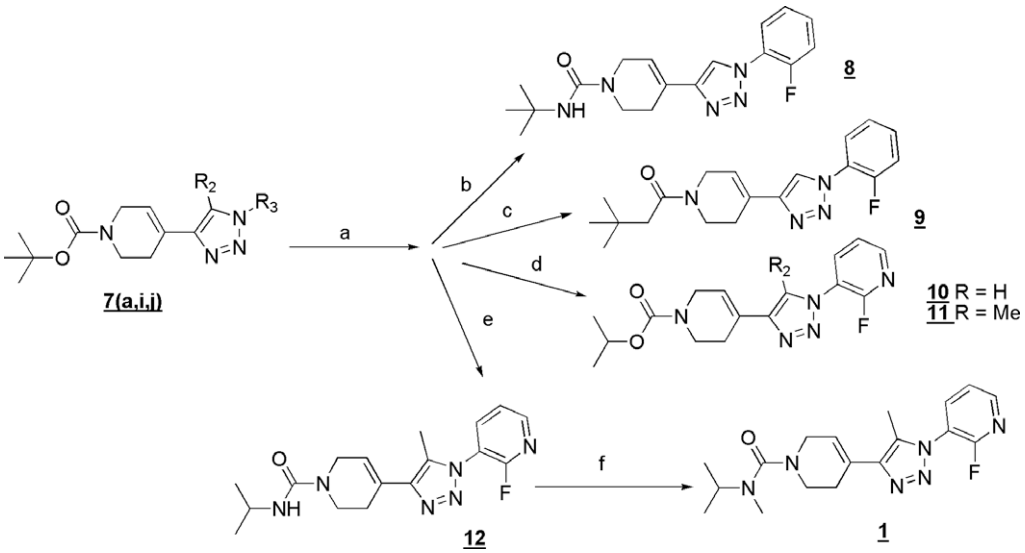
To reiterate, our lead compound **2** had an extremely lipophilic and a poorly aqueous soluble nature. Among the hydrophilic compounds we prepared in the initial SAR study, pyridine compound **7g** alone has both a moderate activity and an appropriate Log$D_{7.4}$ number. The range of optimum Log$D_{7.4}$ values in orally active CNS drug is reported to be 1–3 and our pyridine compound **7g** shows 3.0.[28] Although this Log$D$ number seemed to be right on the upper limit, we thought there was a slight amount of room to introduce limited hydrophobic substitutions into the molecule. In addition, the basicity of the pyridine ring was considered to be a negative factor for maintaining mGluR1 antagonistic activity.



Scheme 1. Synthesis of tetrahydropyridines. Reagents and conditions: (a) $NaNO_2$, aq HCl, Et_2O, (b) tributyl(1-propynyl)tin or tributyl(1-ethynyl)tin, toluene, reflux, (c) I_2, toluene, d) *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_2CO_3, DMF, 90 °C.

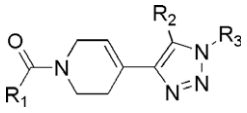


Scheme 2. Synthesis of azidopyridines. Reagents and conditions: (a) NaN_3, MeOH, −78 to −40 °C, (b) $NaNO_2$ then NaN_3, 10% HCl, 0 °C, rt, (c) NaN_3, EtOH, 110 °C, (d) LDA, dodecylbenzenesulfoneazide, THF, −78 to −40 °C.



Scheme 3. Replacement of the N-Boc Moiety. Reagents: (a) TFA, $CHCl_3$, (b) *tert*-butyl isocyanate, THF, (c) *tert*-butyl acetylchloride, THF, (d) *iso*-propyl chlroloformate, TEA, THF, (e) *iso*-propyl isocyanate, THF, (f) MeI, NaH, DMF.

Table 1
In vitro antagonistic activity of tetrahydropyridine derivatives



No	R_1	R_2	R_3	[a]IC_{50} ± SEM (nM)			
				[b]hmGluR$_1$	[c]mmGluR$_1$	hmGluR$_5$	[d]Log $D_{7.4}$
2	^{t}BuO	H	Ph	9.8 ± 0.88	24 ± 2.7	820 ± 170	>4.0
7a	^{t}BuO	H	2-F-Ph	6.3 ± 1.3	16 ± 1.9	1000 ± 140	>4.0
7b	^{t}BuO	H	3-F-Ph	18 ± 4.1	39 ± 5.9	1100 ± 200	>4.0
7c	^{t}BuO	H	4-F-Ph	16 ± 1.1	31 ± 3,0	1100 ± 210	>4.0
7d	^{t}BuO	H	2-Me-Ph	11 ± 0.78	58 ± 10	880 ± 56	>4.0
7e	^{t}BuO	H	2-MeO-Ph	590 ± 220	1600 ± 360	4000 ± 410	NT
8	^{t}BuNH	H	2-F-Ph	57 ± 13	290 ± 39	8600 ± 670	3.2
9	^{t}BuCH$_2$	H	2-F-Ph	120 ± 71	160 ± 68	>10,000	NT
7f	^{t}BuO	H	2-Py	49 ± 18	150 + 22	8700 ± 480	>4.0
7g	^{t}BuO	H	3-Py	55 ± 9.1	180 ± 20	8300 ± 1700	3.0
7h	^{t}BuO	H	4-Py	220 ± 56	1000 + 110	>10,000	NT
7i	^{t}BuO	H	2-(3-F-Py)	2.6 ± 0,54	12 ± 2,2	4300 ± 1600	3.1
7j	^{t}BuO	Me	2-(3-F-Py)	7.0 ± 1,2	1.5 ± 0.15	170 ± 17	2.9

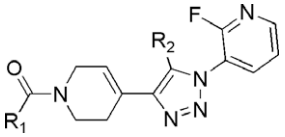
[a] The IC_{50} value is the mean of multiple results (at least three independent determinations performed in duplicate) with standard error of the means.
[b] Human mGluR1.
[c] Mouse mGluR1.
[d] The Log $D_{7.4}$ value is actually measured using reported method.[32]

Thus we designed non-basic fluoropyridine derivative **7i** based on the initial SAR of benzene analogs. As a result, this attempt worked well and drastic enhancement of mGluR1 antagonistic activity against the human receptor was observed. The Log$D_{7.4}$ number also remained very close to the acceptable range, that is, 3.1. However, the antagonistic activity against the corresponding mouse receptor tuned out to be still unsatisfactory (IC_{50} = 12 nM).

During the course of successive modifications of this class, an enhancement of antagonistic activity on mouse mGluR1 was observed when a methyl group was incorporated into a triazole ring (**7j**). Compound **7j** showed single digit in vitro potencies against human and mouse mGluR1a, and had an acceptable physicochemical property in terms of lipophilicity. Further characterization, however, revealed that compound **7j** also enhanced antagonistic activity against hmGluR5. The amount of this activity was 170 nM and the selectivity was no more than 24-folds, which was a potentiality to show unexpected in vivo behavioral changes related to mGluR5 receptor. Moreover, this compound still had an acid labile N-Boc group in the molecule, which also had a potentiality to reduce oral efficacy by degradation in the acidic gastrointestinal system. To identify a more adequate compound to elicit in vivo oral efficacy, we next concentrated our effort on removing the Boc substructure in methyl triazoles.

In the course of successive modification, the presence of a methyl group on the triazole ring turned out to have an unexpected additional benefit for maintaining low nanomolar potency even when the Boc group was replaced with other substitutions such as isopropyloxy carbonyl group (**11**), which was never tolerated when the triazole ring was non-methylated. (Table 2) For example, the Boc analog **7i** showed 2.6 nM for human mGluR1 in contrast to 39 nM for the isopropyl analog **10**. The corresponding methylated triazols, however, showed comparable affinity at human mGluR1, that is, 7.0 nM and 7.2 nM, respectively (**7j, 11**). Although, compound **11** itself had insufficient selectivity ratio mGluR1/mGluR5, these methylated triazoles gave us a great opportunity to obtain better mGluR1 antagonists by replacing

Table 2
In vitro antagonistic activity of fluoropyridine derivatives



No	R_1	R_2	[a]IC_{50} ± SEM (nM)			
			[b]hmGluR$_1$	[c]mmGluR$_1$	hmGluR$_5$	[d]Log $D_{7.4}$
7i	^{t}BuO	H	2.6 ± 0.54	12 ± 2.2	4300 ± 1600	3.1
7j	^{t}BuO	Me	7.0 ± 1.2	1.5 ± 0.15	170 ± 17	2.9
10	^{i}Pr0	H	39 ± 5.7	180 ± 21	3900 ± 490	2.8
11	^{i}PrO	Me	7.2 ± 1.4	3.3 ± 0.64	40 ± 5.9	2.8
1	^{i}PrNMe	Me	5.8 ± 0.49	3.1 ± 0.27	6200 ± 520	2.1

[a] The IC_{50} value is the mean of multiple results (at least three independent determinations performed in duplicate) with standard error of the means.
[b] Human mGluR1.
[c] Mouse mGluR1.
[d] The Log $D_{7.4}$ value is actually measured using reported method.[32]

the Boc group with appropriate, chemically stable substitutions. Among the compounds, we then prepared, good selectivity ratio mGluR1/mGluR5 was observed in urea analog **1**.

The effect of the methyl group on the triazole group is still uncertain, but we hypothesized that this group might affect a conformational change in our lead class. In order to support this hypothesis, we calculated a series of quantum mechanical (QM) calculations for compounds **7i** and **7j** using Gaussian 03. The minimum conformations of compounds **7i** and **7j** are shown in Figure 2. The QM calculation suggested that there are large differences in the dihedral angle of rings A (fluoropyridine) and B (triazole), and rings B and C (tetrahydropyridine). In addition, the orientation of N-Boc group in each compound was quite different. Thus this conformational difference should affect the tolerable substructures at teterahydropyridine nitrogen part for mGluR1 antagonistic activity.

Compound **1** showed low nano molar antagonistic activities against both human and mouse mGluR1a, and showed satisfactory selectivity (greater than 1000-fold) on mGluR5. Regarding the residual mGluR subclasses (groups II and III), this developed compound at 10 μM did not exhibit any agonistic or antagonistic activity against mGluR2, mGluR4, mGluR6, mGluR7 and mGluR8 in [^{35}S]GTPγS binding assay systems.[21] Furthermore, the selectivity of 10 μM compound **1** was tested against 77 target molecule such as an enzyme, neurotransmitter receptors, transporters, and ion channels, including ionotropic glutamate receptors, α-amino-3-hydroxy-5-methly-4-isoxazolepropinonic acid, NMDA, and kainate (MDS Pharma, Bothell, WA). The IC_{50} values of compound **1** were higher than 10 μM against all 77 targets (data not shown). Compound **1** did not displace [^{3}H]L-quisqualate binding to human mGluR1, indicating the allosteric mGluR1 antagonist.[21] These results suggested that compound **1** was a highly selective allosteric mGluR1 antagonist. From the viewpoint of physicochemical properties, this compound had an appropriate Log *D* value ($\text{Log}\,D_{7.4} = 2.1$) and also had a good aqueous solubility (>170 μM, H_2O) in this class. In addition, in vitro metabolic stability was significantly improved in compounds **7j** and **1** (Table 3). Compound **1** was a well-balanced compound, and was expected to also show good in vivo behavior. Thus we next tested some representative compounds similar to **1** to evaluate if they had an improved profile in terms of in vivo antagonistic activity.

Table 3
Metabolic stability in liver microsomes

No	[a]In vitro metabolic stability (remaining%)			[b]Log $D_{7.4}$	[c]Water solubility (μM)
	Human	Rat	Mouse		
2	57	16	[d]NT	>4.0	2.4
7j	90	70	2	2.9	155
1	85	61	27	2.1	>170

[a] In vitro metabolic stability expressed as a percentage of converted parent compound (1 μM) after 30 min.
[b] The Log $D_{7.4}$ value is actually measured using reported method[32]
[c] Water solubility is actually measured using reported method.[33]
[d] Not tested.

In vivo antagonistic activity was evaluated in the reported mouse animal model in which a selective mGluR1 antagonist reversed the behavioral change elicited by the group I metabotropic glutamate receptor agonist (*S*)-3,5-dihydrophenylglycine(DHPG).[29,30]

(*S*)-3,5-DHPG produced a face washing (facial grooming) behavior in mice when this was administered i.c.v. This behavior was reversed by selective mGluR1 antagonists, (*RS*)-1-aminoindan-1,5-dicarboxylic acid (AIDA) and (*S*)-4-carboxyphenylglycine (*S*-4CPG), but not mGluR5 antagonists. Thus (*S*)-3,5-DHPG-induced face washing behavior is thought to be induced by mGluR1 activation and to be a behavioral outcome useful for evaluation of the in vivo potency of mGluR1 antagonist. Using this system, the following three compounds with different in vitro profiles and structural features were evaluated. The first one was lead compound **2**, which had moderate in vitro potency against mouse mGluR1a and an extremely high lipophilic and a poorly aqueous soluble nature.

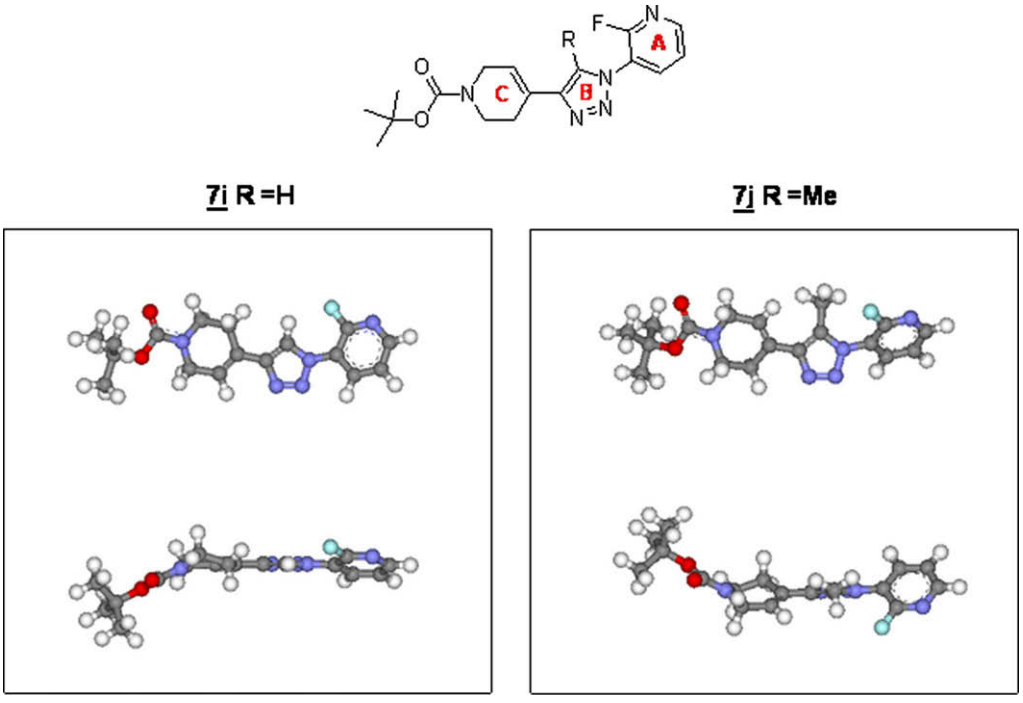


Figure 2. Most stable conformations of non-methylated triazole **7i** (left) and methylated triazole **7j** (right). Final geometries obtained for B3LYP/6-31+G(d,p).

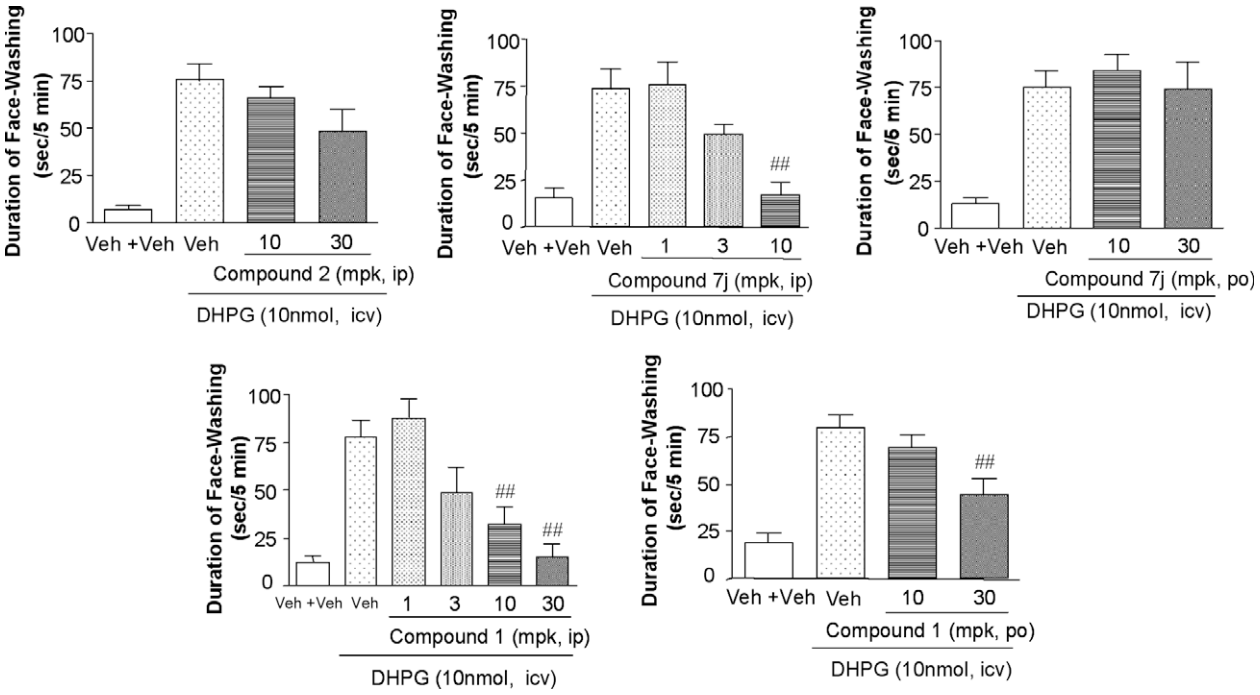


Figure 3. Effects of mGluR1 antagonist on (*S*)-DHPG induced behavioral change in mice. mGluR1 antagonists or its vehicle were administered 30 min prior to (*S*)-DHPG. Data are presented as means ± SEM (n = 5–11 for each treatment group.) $^{\#\#}P < 0.05$ versus (*S*)-DHPG.

The second one was fluoropyridine derivative **7j**, which had good intrinsic potency, a favorable Log*D* value but an acid labile Boc group. The last one was urea analog **1**, which was considered to be the most balanced compound in terms of potency against both human and mouse receptors, selectivity, lipophilicity and aqueous solubility. The result of our in vivo study is summarized in Figure 3[21] and is well consistent with our expectations. The initial lead compound **2** had poor in vivo antagonistic activity, which might result from it having only moderate in vitro potency and/or non-specific binding in brain due to its high lipophilic nature. In contrast, fluoropyridine analog **7j** with improved intrinsic potency and improved physicochemical properties showed good in vivo efficacy by i.p. dosing of at least a dose range of 3–10 mg/kg. The efficacy of this second compound, however, was not observed at all when the compound was administered orally. Possible causes for this ineffectiveness were its acid liable Boc group, which could be degraded in the gastrointestinal tract, and/or extremely poor metabolic stability in mouse microsomes. The optimized compound **1**, on the contrary, showed good in vivo efficacy not only by i.p., but also by p.o. dosing of at least a dose range of 10–30 mg/kg. These results suggest that in vitro potency, metabolic stability, appropriate lipophilicity and water-solubility are important parameters for eliciting oral mGluR1 antagonistic activity in this lead class.

Following oral dosing of 30 mg/kg of compound **1**, the mean (n = 3) plasma and brain concentrations were 0.21 μM and 0.17 nmol/g at 30 min post dose, respectively. The brain level of the compound after oral administration seemed to be good enough to elicit antagonistic activity in vivo given its potent in vitro intrinsic potency. Unfortunately, the compound **1** had a short half life ($t_{1/2}$ = 0.2 h) and a high total clearance (CLp = 57 ml/min/kg) after single intravenous administration at 1 mg/kg in rats. In addition, the oral bioavailability in rats was also moderate, 18%, after a single dose of 3 mg/kg. This result may indicate that the compound **1** has a possibility to have unsatisfactory PK profiles in human. In the preliminary study for the metabolite identification, the major metabolite of this compound in microsomes turned out to be des methylated compound at urea part in all species. We are now concentrating our efforts on identification of the analogs with better PK profile by replacing the other substructures at left hand part in the molecule. In summary, a series of 4-(1-aryltriazol-4-yl)-tetrahydropyridines was identified as a novel allosteric mGluR1 antagonists. Our extensive chemical modifications within this series to improve in vitro antagonistic activity in both human and mice mGluR1s, and to reduce the lipophilic nature from the original lead compound **2**, led to highly selective and orally active antagonist **1**. It is expected that compound **1** will be a good pharmacological tool for elucidating the role of mGluR1 on CNS functions in rodents and humans.[31]

4. Experimental

4.1. Chemistry

Nuclear magnetic resonance spectra were recorded JEOL JNM-AL 400 in the indicated solvents. Infrared absorption spectra (IR) were measured on a SHIMAZU FTIR-8900 spectrometer. Electro ionization (ESI) mass spectra were recorded on a micromass Q-TOF-2 instrument. HPLC analysis was performed on Agilent 1100 using YMC-ProC18 (2.0 × 150 mm, 5 μM particle size) or CAPCEL PAK MGII C18 (2.0 × 150 mm, 5 μM particle size) at 40 °C. Two different conditions were utilized for HPLC analysis: (a) 5:95–90:10 CH_3CN: 0.1% aqueous H_3PO_4, linear gradient over 40 min at 0.2 ml/min on YMC-ProC18, or (b) 5:95–80:20 CH_3CN: 10 mM potassium phosphate buffer, linear gradient over 40 min at 0.2 ml/min on CAPCEL PAK MGII C18. All solvent and reagent were obtained from commercial sources and used without purification. Flash chromatography was carried out using Wako C-300 and FUJI SILSIA Chromatorex NH 100–200 mesh.

4.1.1. *tert*-Butyl-4-(1-phenyl-1H-1,2,3-triazole-4-yl)-3,6-dihydropyridine-1(2H)-carboxylate (2)

Detailed procedure is described in UK Patent Application GB2299581.

4.1.2. 2-Fluorophenylazide (4a)[†]

2-Fluorophenylhydrazine hydrochloride (1.0 g, 6.2 mmol) was added dropwise to a cold (0 °C) solution of concentrated hydrochloric acid (5.0 ml) over a period of 10 min. Diethyl ether (5.0 ml) was then added, followed by the dropwise addition of a solution of sodium nitrite (513 mg, 7.4 mmol) in water (1.0 ml) keeping the temperature below 5 °C. The reaction was then stirred at 0 °C for 3 h and then extracted with ethyl acetate. The organic layer was washed with water, brine, dried over Na_2SO_4 and carefully evaporated in vacuo to give crude title compound as a red oil (420 mg).

4.1.3. 1-(2-Fluorophenyl)-4-(tributylstannyl)-1H-1,2,3-triazole (5a)

A solution of 2-fluorophenylazide (362 mg, 2.67 mmol) and tributyl(ethynyl)tin (1.00 g, 3.17 mmol) in toluene (2.0 ml) was heated to 100 °C for 2 h. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 9:1) to give 947 mg of target compound as yellowish oil.

^{1}H NMR ($CDCl_3$) δ: 0.90 (9H, t, J = 7.3 Hz), 1.06–1.40 (12H, m), 1.48–1.72 (6H, m), 7.28–7.43 (3H, m), 7.96–8.00 (2H, m).; ^{13}C NMR ($CDCl_3$) δ: 10.02 (3C, s), 13.63 (s), 27.20 (3C, s), 28.99 (3C, s), 116.86 (d, J = 20.7 Hz), 125.00 (s), 125.12 (d, J = 3.3 Hz), 125.55 (d, J = 10.8 Hz), 129.55 (d, J = 8.3 Hz), 130.84 (d, J = 8.3 Hz), 144.91 (s), 153.33 (d, J = 249.9 Hz); IR(ATR) 2954, 2922, 1595, 1510, 1464, 1375, 1196, 1020, 864, 754 cm^{-1}; HRMS (ESI+) m/z [M+H]$^+$ 454.1699 (calcd for $C_{20}H_{33}N_3FSn$: 454.1681).

4.1.4. 1-(2-Fluorophenyl)-4-iodo-1H-1,2,3-triazole (6a)

To a solution of 1-(2-fluorophenyl)-4-(tributylstannyl)-1H-triazole (519 mg, 1.12 mmol) in THF (8.0 ml) was added iodine (313 mg, 1.23 mmol), and the reaction mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 8:1), and the resulting solid was washed with hexane to give 127 mg of target compound.

^{1}H NMR ($CDCl_3$) δ: 7.29–7.38 (2H, m), 7.44–7.52 (1H, m), 7.95 (1H, td, J = 7.8, 2.0 Hz), 8.17 (1H, d, J = 2.9 Hz); ^{13}C NMR ($CDCl_3$) δ: 87.69 (s), 117.09 (d, J = 19.9 Hz), 124.59 (d, J = 10.8 Hz), 124.64 (s), 125.36 (d, J = 3.3 Hz), 130.15 (d, J = 9.1 Hz), 130.71 (d, J = 8.3 Hz), 153.31 (d, J = 251.6 Hz); IR(ATR) 3175, 1595, 1508, 1470, 1406, 1271, 1202, 1184, 1033, 816, 795, 750, 642 cm^{-1}; HRMS (ESI+) m/z [M+H]$^+$ 289.9604 (calcd for $C_8H_{63}N_3FI$: 289.9591).

4.1.5. *tert*-Butyl-4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7a)

To a nitrogen flushed flask containing *tert*-Butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (161 mg, 0.52 mmol), K_2CO_3 (119 mg, 0.86 mmol) and 1-(2-fluorophenyl)-4-iodo-1H-1,2,3-triazole (125 mg, 0.43 mmol) was added DMF (3.0 ml) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (10 mol%). The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 2:1), and the resulting solid was washed with hexane to give 51 mg of target compound.

^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.60 (2H, brs), 3.67 (2H, t, J = 5.6 Hz), 4.10–4.15 (2H, m), 6.54 (1H, brs), 7.28–7.47 (3H, m), 7.95–8.00 (2H, m). ^{13}C NMR ($CDCl_3$) δ: 26.41 (s), 28.46 (3C, s), 39.38 (0.5C, s), 40.60 (0.5C, s), 43.35 (s), 79.78 (s), 116.99 (d, J = 19.9 Hz), 119.84 (d, J = 9.1 Hz), 121.90 (s), 124.77 (s), 125.24 (d, J = 9.0 Hz), 125.26 (d, J = 3.3 Hz), 125.75 (s), 130.07 (d, J = 8.3 Hz), 148.29 (s), 153.21 (d, J = 250.8 Hz), 154.87 (s); IR(ATR) 1686, 1506, 1423, 1364, 1231, 1178, 1115, 752 cm^{-1}; HRMS (ESI+) m/z [M+H]$^+$ 345.1729 (calcd for $C_{18}H_{22}N_4O_2F$: 345.1727); HPLC (a) 99.1%; (b) 98.9%.

4.1.6. 3-Fluorophenylazide (4b)[†]

3-Fluorophenylhydrazine hydrochloride (2.40 g, 14.8 mmol) was added dropwise to a cold (0 °C) solution of concentrated hydrochloric acid (7.5 ml) over a period of 10 min. Diethyl ether (9.0 ml) was then added, followed by the dropwise addition of a solution of sodium nitrite (1.23 g, 17.8 mmol) in water (3.0 ml) keeping the temperature below 5 °C. The reaction was then stirred at 0 °C for 3 h and then extracted with ethyl acetate. The organic layer was washed with water, brine, dried over Na_2SO_4 and carefully evaporated in vacuo to give crude title compound as a red oil (1.20 g).

4.1.7. 1-(3-Fluorophenyl)-4-(tributylstannyl)-1H-1,2,3-triazole (5b)

A solution of 3-fluorophenylazide (865 mg, 6.31 mmol) and tributyl(ethynyl)tin (2.39 g, 7.57 mmol) in toluene (5.0 ml) was heated to 100 °C for 2 h. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 9:1) to give 2.32 g of target compound as yellowish oil.

^{1}H NMR ($CDCl_3$) δ: 0.90 (9H, t, J = 7.3 Hz), 1.06–1.40 (13H, m), 1.49–1.68 (8H, m), 7.09–7.14 (1H, m), 7.45–7.57 (3H, m), 7.87 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 10.04 (3C, s), 13.64 (3C, s), 27.21 (3C, s), 28.98 (3C, s), 108.30 (d, J = 25.7 Hz), 115.06 (d, J = 20.7 Hz), 115.91 (d, J = 3.3 Hz), 127.75 (s), 131.00 (d, J = 9.1 Hz), 138.43 (d, J = 9.9 Hz), 145.83 (s), 163.07 (d, J = 247.5 Hz); IR(ATR) 2955, 2920, 1612, 1600, 1499, 1377, 1178, 1030, 866, 777, 677 cm^{-1}; HRMS (ESI+) m/z [M+H]$^+$ 454.1699 (calcd for $C_{20}H_{33}N_3FSn$: 454.1681).

4.1.8. 1-(3-Fluorophenyl)-4-iodo-1H-1,2,3-triazole (6b)

To a solution of 1-(3-fluorophenyl)-4-(tributylstannyl)-1H-triazole (986 mg, 2.12 mmol) in THF (15.0 ml) was added iodine (594 mg, 2.34 mmol), and the reaction mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 8:1), and the resulting solid was washed with hexane to give 516 mg of target compound.

^{1}H NMR ($CDCl_3$) δ: 7.19 (1H, tt, J = 7.8, 2.3 Hz), 7.48–7.55 (3H, m), 8.06 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 88.35 (s), 108.37 (d, J = 25.7 Hz), 115.84 (d, J = 3.3 Hz), 116.17 (s), 116.39 (s), 127.21 (s), 131.36 (d, J = 9.1 Hz), 163.06 (d, J = 249.9 Hz); IR(ATR) 3123, 1601, 1499, 1474, 1391, 1265, 1219, 1209, 1196, 1045, 866, 822, 773, 673 cm^{-1}; HRMS (ESI+) m/z [M+H]$^+$ 289.9600 (calcd for $C_8H_{63}N_3FI$: 289.9591).

4.1.9. *tert*-Butyl-4-[1-(3-fuluorophenyl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7b)

To a nitrogen flushed flask containing *tert*-Butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-

[†] Caution! Many phenyl azides are heat- and/or shock sensitive compounds, which have a potential to cause explosive decomposition under severe conditions.

carboxylate (257 mg, 0.83 mmol), K_2CO_3 (190 mg, 1.38 mmol) and 1-(3-fluorophenyl)-4-iodo-1H-1,2,3-triazole (200 mg, 0.69 mmol) was added DMF (4.0 ml) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (10 mol%). The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 2:1), and the resulting solid was washed with hexane to give 83 mg of target compound.

^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.58 (2H, brs), 3.67 (2H, t, J = 5.6 Hz), 4.10–4.14 (2H, m), 6.54 (1H, brs), 7.15 (1H, dq, J = 12.3, 2.9 Hz), 7.49–7.55 (3H, m), 7.85 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 26.40 (s), 28.46 (3C, s), 39.34 (0.5C, s), 40.56 (0.5C, s), 43.27 (s), 79.84 (s), 108.13 (d, J = 26.5 Hz), 115.54 (d, J = 18.0 Hz), 115.66 (s), 116.59 (s), 122.10 (s), 125.63 (s), 131.18 (d, J = 9.1 Hz), 138.14 (d, J = 9.9 Hz), 148.72 (s), 154.84 (s), 163.08 (d, J = 248.3 Hz); IR(ATR) 1676, 1497, 1427, 1366, 1234, 1159, 1113, 868, 777 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 345.1724 (calcd for $C_{18}H_{22}N_4O_2F$: 345.1727); HPLC (a) 99.5%; (b) 94.2%.

4.1.10. 4-Fluorophenylazide (4c)[†]

4-Fluorophenylhydrazine hydrochloride (3.09 g, 19 mmol) was added dropwise to a cold (0 °C) at solution of concentrated hydrochloric acid (20 ml) over a period of 10 min. Diethyl ether (10 ml) was then added, followed by the dropwise addition of a solution of sodium nitrite (1.70 g, 24 mmol) in water (2.0 ml) keeping the temperature below 5 °C. The reaction was then stirred at 0 °C for 3 h and then extracted with ethyl acetate. The organic layer was washed with water, brine, dried over Na_2SO_4 and carefully evaporated in vacuo to give crude title compound as a red oil (1.01 g).

4.1.11. 1-(4-Fluorophenyl)-4-(tributylstannyl)-1H-1,2,3-triazole (5c)

A solution of 4-fluorophenylazide (503 mg, 3.67 mmol) and tributyl(ethynyl)tin (1.27 g, 4.04 mmol) in toluene (4.0 ml) was heated to 100 °C for 3 hours. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 7:1) to give 1.29 g of target compound as yellowish oil.

^{1}H NMR ($CDCl_3$) δ: 0.90 (9H, t, J = 7.1 Hz), 1.06–1.40 (12H, m), 1.49–1.68 (8H, m), 7.18–7.25 (2H, m), 7.70–7.75 (2H, m), 7.83 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 10.01 (3C, s), 13.65 (3C, s), 27.22 (3C, s), 28.99 (3C, s), 116.52 (2C, d, J = 23.2 Hz), 122.56 (2C, d, J = 9.1 Hz), 128.03 (s), 133.58 (s), 145.59 (s), 162.13 (d, J = 248.3 Hz); IR(ATR) 2955, 2922, 1514, 1464, 1375, 1231, 1192, 1032, 835, 619 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 454.1699 (calcd for $C_{20}H_{33}N_3FSn$: 454.1681).

4.1.12. 1-(4-Fluorophenyl)-4-iodo-1H-1,2,3-triazole (6c)

To a solution of 1-(4-fluorophenyl)-4-(tributylstannyl)-1H-triazole (780 mg, 1.68 mmol) in THF (10 ml) was added iodine (469 mg, 1.85 mmol), and the reaction mixture was stirred at room temperature for 1 hour. The resulting mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 8:1), and the resulting solid was washed with hexane to give 461 mg of target compound.

^{1}H NMR ($CDCl_3$) δ: 7.21–7.29 (2H, m), 7.65–7.71 (2H, m), 8.01 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 88.12 (s), 116.89 (2C, d, J = 23.2 Hz), 122.64 (2C, d, J = 8.3 Hz), 127.43 (s), 132.60 (d, J = 3.3 Hz), 162.71 (d, J = 249.9 Hz); IR(ATR) 3128, 1606, 1514, 1477, 1396, 1310, 1211, 1194, 1043, 831, 814, 619 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 289.9600 (calcd for $C_8H_{63}N_3FI$: 289.9591).

4.1.13. *tert*-Butyl-4-[1-(4-fuluorophenyl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7c)

To a nitrogen flushed flask containing *tert*-Butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (179 mg, 0.58 mmol), K_2CO_3 (133 mg, 0.96 mmol) and 1-(4-fluorophenyl)-4-iodo-1H-1,2,3-triazole (140 mg, 0.48 mmol) was added DMF (2.0 ml) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (10 mol%). The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 4:1), and the resulting solid was washed with hexane to give 60.9 mg of target compound.

^{1}H NMR ($CDCl_3$) δ: 1.57 (9H, s), 2.58 (2H, s), 3.67 (2H, t, J = 5.4 Hz), 4.12 (2H, d, J = 2.9 Hz), 6.53 (1H, s), 7.20–7.25 (9H, m), 7.69–7.73 (2H, m), 7.81 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 26.42 (s), 28.46 (3C, s), 39.32 (0.5C, s), 40.53 (0.5C, s), 43.39 (s), 79.82 (s), 116.71 (2C, d, J = 23.2 Hz), 116.94 (s), 121.74 (s), 122.40 (2C, d, J = 9.1 Hz), 125.76 (s), 133.31 (s), 148.65 (s), 154.86 (s), 162.37 (d, J = 249.1 Hz); IR(ATR) 1676, 1518, 1423, 1364, 1177, 814 cm^{-1} ; HRMS (ESI+) m/z $[M+H]^+$ 345.1726 (calcd for $C_{18}H_{22}N_4O_2F$: 345.1727); HPLC (a) 96.5%; (b) 93.9%.

4.1.14. 2-Methylphenylazide (4d)[†]

2-Methylphenylhydrazine hydrochloride (1.00 g, 6.30 mmol) was added dropwise to a cold (0 °C) at solution of concentrated hydrochloric acid (5.0 ml) over a period of 10 min. Diethyl ether (6.0 ml) was then added, followed by the dropwise addition of a solution of sodium nitrite (520 mg, 7.56 mmol) in water (2.0 ml) keeping the temperature below 5 °C. The reaction was then stirred at 0 °C for 3 h and then extracted with ethyl acetate. The organic layer was washed with water, brine, dried over Na_2SO_4 and carefully evaporated in vacuo to give crude title compound as a red oil (670 mg).

4.1.15. 1-(2-Methylphenyl)-4-(tributylstannyl)-1H-1,2,3-triazole (5d)

A solution of 2-Methylphenylazide (500 mg, 3.76 mmol) and tributyl(ethynyl)tin (1.42 g, 4.51 mmol) in toluene (3.0 ml) was heated to 100 °C for 4 h. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 9:1) to give 1.29 g of target compound as yellowish oil.

^{1}H NMR ($CDCl_3$) δ: 0.90 (9H, t, J = 7.3 Hz), 1.15–1.40 (12H, m), 1.56–1.64 (6H, m), 2.19 (3H, s), 7.32–7.39 (4H, m), 7.65 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 9.99 (3C, s), 13.65 (3C, s), 17.85 (s), 27.19 (3C, s), 29.01 (3C, s), 125.97 (s), 126.65 (s), 129.41 (s), 131.29 (s), 131.34 (s), 133.77 (s), 136.86 (s), 144.01 (s); IR(ATR) 2955, 2922, 1501, 1464, 1377, 1188, 1028, 758, 665 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 450.1948 (calcd for $C_{21}H_{36}N_3Sn$: 450.1937).

4.1.16. 4-Iodo-1-(2-methylphenyl)-1H-1,2,3-triazole (6d)

To a solution of 1-(2-Methylophenyl)-4-(tributylstannyl)-1H-triazole (680 mg, 1.51 mmol) in THF (10 ml) was added iodine (422 mg, 1.66 mmol), and the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 8:1 and then Chromatorex NH, Hexane/AcOEt 8:1) to give 344 mg of target compound as colorless amorphous.

^{1}H NMR ($CDCl_3$) δ: 2.22 (3H, s), 7.29–7.46 (4H, m), 7.82 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 17.76 (s), 87.08 (s), 125.89 (s), 126.95 (s), 130.28 (s), 130.41 (s), 131.57 (s), 133.67 (s), 135.77 (s); IR(ATR)

1583, 1501, 1460, 1265, 1186, 1032, 986, 966, 758, 714, 675 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 285.9853 (calcd for $C_9H_9N_3I$: 285.9841).

4.1.17. *tert*-Butyl-4-[1-(2-methylphenyl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7d)

To a nitrogen flushed flask containing *tert*-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (260 mg, 0.84 mmol), K_2CO_3 (193 mg, 1.40 mmol) and 4-Iodo-1-(2-methylphenyl)-1H-1,2,3-triazole (200 mg, 0.70 mmol) was added DMF (4.0 ml) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (10 mol%). The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified silicagel column chromatography (Wako C-300, Hexane/AcOEt 4:1 and then Chromatorex NH, Hexane/AcOEt 4:1) to give 105 mg of target compound.

^1H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.23 (3H, s), 2.60 (2H, brs), 3.68 (2H, t, J = 5.6 Hz), 4.11–4.14 (2H, m), 6.52 (1H, br s), 7.31–7.44 (5H, m), 7.63 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 17.86 (s), 26.43 (s), 28.47 (3C, s), 39.55 (0.5C, s), 40.92 (0.5C, s), 43.46 (s), 79.79 (s), 120.31 (s), 121.29 (s), 125.91 (s), 125.95 (s), 126.82 (s), 129.85 (s), 131.48 (s), 133.72 (s), 136.44 (s), 147.76 (s), 154.79 (s); IR(ATR) 1686, 1501, 1414, 1364, 1238, 1161, 1113, 760 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 341.1980 (calcd for $C_{19}H_{25}N_4O_2$: 341.1978); HPLC (a) 99.0%; (b) 98.8%.

4.1.18. 2-Azidophenyl methyl ether (4e)[†]

2-Methoxyphenylhydrazine hydrochloride (1.08 g, 6.20 mmol) was added to a cold (0 °C) solution of concentrated hydrochloric acid (5.0 ml) over a period of 10 min. Diethyl ether (5.0 ml) was then added, followed by dropwise addition of a solution of Sodium nitrite (511 mg, 7.40 mmol) in Water (2.0 ml) keeping the temperature below 5 °C. The reaction mixture was then stirred at 0 °C for 3 h and then extracted with ethyl acetate. The organic layer was washed with water, brine, dried over Na_2SO_4 and carefully evaporated in vacuo to give crude title compounds as red oil (395 mg).

4.1.19. 1-(2-Methoxyphenyl)-4-(tributylstannyl)-1H-1,2,3-triazole (5e)

A solution of 2-azidophenyl methyl ether (395 mg 2.65 mmol) and tributyl(ethynyl)tin (1.00 g, 3.18 mmol) in toluene (2.0 ml) was heated to 100 °C for 4 h. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 9:1) to give 818 mg of target compound as yellowish oil.

^1H NMR ($CDCl_3$) δ: 0.90 (9H, t, J = 7.3 Hz), 1.06–1.40 (12H, m), 1.50–1.69 (6H, m), 3.41 (3H, s), 7.40 (1H, td, J = 7.9,1.6 Hz)), 7.78 (1H, dd, J = 7.8, 2.0 Hz), 8.01 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 9.99 (3C, s), 13.70 (3C, s), 27.24 (3C, s), 29.04 (3C, s), 55.97 (s) , 111.24 (s), 121.20 (s), 125.66 (s), 126.72 (s), 129.57 (s), 131.85 (s), 143.52 (s), 151.23 (s); IR(ATR) 2954, 2922, 1506, 1464, 1285, 1248, 1024, 748, 673 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 466.1892 (calcd for $C_{21}H_{36}N_3OSn$: 466.1880).

4.1.20. 4-Iodo-1-(2-methoxylphenyl)-1H-1,2,3-triazole (6e)

To a solution of 1-(2-methoxyphenyl)-4-(tributylstannyl)-1H-1,2,3-triazole (529 mg, 1.14 mmol) in THF (8.0 ml) was added iodine (347 mg, 1.34 mmol), and the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 8:1 and then Chromatorex NH, Hexane/AcOEt 8:1) to give 341 mg of target compound as yellow gum.

^1H NMR ($CDCl_3$) δ: 3.91 (3H, s), 7.09–7.14 (2H, m), 7.43–7.47 (1H, m), 7.77 (1H, dd, J = 8.0, 1.7Hz), 8.20 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 56.01 (s), 86.05 (s), 111.24 (s), 121.28 (s), 125.21 (s), 125.64 (s), 130.54 (s), 131.03 (s), 151.02 (s); IR(-ATR) 1601, 1506, 1472, 1245, 1115, 1020, 984, 966, 748, 675 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 301.9794 (calcd for $C_9H_9N_3OI$: 301.9790).

4.1.21. *tert*-Butyl-4-[1-(2-methoxylphenyl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7e)

To a nitrogen flushed flask containing *tert*-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (248 mg, 0.80 mmol), K_2CO_3 (222 mg, 1.60 mmol) and 1-(2-methoxylphenyl)-4-iodo-1H-1,2,3-triazole (290 mg, 0.96 mmol) was added DMF (4.0 ml) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (10 mol%). The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 8:1 and then Chromatorex NH, Hexane/AcOEt 8:1) to give 44.0 mg of target compound as colorless gum.

^1H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.61 (2H, brs), 3.67 (2H, t, J = 5.4 Hz), 3.90 (3H, s), 4.11–4.14 (2H, m), 6.50 (1H, brs), 7.08–7.13 (2H, m), 7.41–7.45 (1H, m), 7.77 (1H, dd, J = 7.8, 2.0 Hz), 7.99 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 26.44 (s), 28.45 (3C, s), 39.55 (0.5C, s), 40.61 (0.5C, s), 43.46 (s), 55.97 (s), 79.70 (s), 112.21 (s), 120.95 (s), 121.21 (2C, s), 125.47 (s), 126.16 (s), 126.27 (s), 130.04 (s), 147.35 (s), 151.08 (s), 154.88 (s); IR(ATR) 1686, 1504, 1414, 1364, 1236, 1161, 1111, 1022, 750 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 357.1915 (calcd for $C_{19}H_{25}N_4O_3$: 357.1927); HPLC (a) 96.9%; (b) 96.9%.

4.1.22. 2-Azidopyridine (4f)[†]

To a solution of sodium azide (389 mg, 5.99 mmol) in MeOH (30 ml) was added *N*-fluoropyridinium triflate (740 mg, 2.99 mmol) in MeOH (6.0 ml) at −78 °C. The mixture was allowed to warm to room temperature and then stirred for 2 h. The mixture was concentrated in vacuo and was added 50 ml Et_2O. The resulting precipitate was removed by filtration and the filtrate was carefully concentrated to give crude target molecule (302 mg).

4.1.23. 2-[4-(Tributylstannyl)-1H-1,2,3-triazole-1-yl]pyridine (5f)

A mixture of 2-azidopyridine (150 mg, 1.25 mmol) and tributyl(ethynyl)tin (473 mg, 1.50 mmol) in toluene (0.5 ml) was heated to 150 °C for 5 h. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 4:1) to give 23.8 mg of target compound as colorless oil.

^1H NMR ($CDCl_3$) δ: 0.90 (9H, t, J = 7.3 Hz), 1.15–1.40 (12H, m), 1.56–1.65 (6H, m), 7.32 (1H, ddd, J = 7.3, 4.9, 1.0 Hz), 7.91 (1H, td, J = 7.9, 1.8 Hz), 8.21 (1H, d, J = 8.3 Hz), 8.22–8.51 (2H, m).; ^{13}C NMR ($CDCl_3$) δ: 9.99 (3H, s), 13.65 (3H, s), 27.23 (3H, s), 28.98 (3H, s), 114.24 (1H, s), 123.09 (1H, s), 127.18 (1H, s), 139.07 (1H, s), 145.33 (1H, s), 148.39 (1H, s), 149.49 (1H, s); IR(ATR) 2922, 2851, 1682, 1593, 1475, 1452, 1377, 1198, 1022, 779, 719, 659 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 437.1739 (calcd for $C_{19}H_{33}N_4Sn$: 437.1727).

4.1.24. 2-(4-Iodo-1H-1,2,3-triazol-1yl)pyridine (6f)

To a solution of 2-[4-(tributylstannyl)-1H-1,2,3-triazole-1-yl]pyridine (21 mg, 0.048 mmol) in THF (1.5 ml) was added iodine (14.7 mg, 0.058 mmol), and the reaction mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned

between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Chromatorex NH, Hexane/AcOEt 6:1) to give 11 mg of target compound as white solid.

^{1}H NMR ($CDCl_3$) δ: 7.38 (1H, ddd, J = 7.8, 4.9, 1.0 Hz), 7.92–7.97 (1H, m), 8.18 (1H, d, J = 8.3 Hz), 8.51 (1H, dt, J = 4.9, 1.0 Hz), 8.67 (1H, s); ^{13}C NMR ($CDCl_3$) δ: 88.22 (s), 113.59 (s), 124.04 (s), 126.71 (s), 139.31 (s), 148.45 (s), 148.63 (s); IR(ATR) 3155, 1595, 1479, 1454, 1267, 1205, 1049, 1003, 777, 719 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 272.9643 (calcd for $C_9H_9N_3I$: 272.9637).

4.1.25. *tert*-Butyl-4-[1-pyridin-2-yl-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7f)

To a nitrogen flushed flask containing *tert*-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate(10.1 mg,0.033 mmol),K_2CO_3(7.5 mg,1.00 mmol)and2-(4-iodo-1H-1,2,3-triazol-1yl)pyridine (7.4 mg, 0.027 mmol) was added DMF (1.5 ml)and $PdCl_2$(dppf)·CH_2Cl_2(10 mol%).The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt3:1)togive4.2 mgoftargetcompound.

^{1}H NMR ($CDCl_3$) δ: 1.43 (9H, s), 2.50–2.54 (2H, m), 3.60 (2H, t, J = 5.6 Hz), 4.02–4.08 (2H, m), 6.51 (1H, brs), 7.28 (1H, ddd, J = 7.3, 4.9, 1.0 Hz), 7.83–7.87 (1H, m), 8.14 (1H, d, J = 7.8 Hz), 8.40 (1H, s), 8.43 (1H, dd, J = 2.9, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ: 26.36 (s), 28.47 (3C, s), 39.34 (0.5C, s), 40.70 (0.5C, s), 43.48 (s), 79.78 (s), 113.78 (s), 115.93 (s), 122.03 (s), 123.46 (s), 125.77 (s), 139.12 (s), 148.11 (s), 148.49 (s), 149.14 (s), 154.84 (s). IR(ATR) 2361, 2341, 1686, 1474, 1412, 1236, 1175, 1117, 768, 723 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 328.1776 (calcd for $C_{17}H_{22}N_5O_2$: 328.1774); HPLC (a) 98.8%; (b) 98.6%.

4.1.26. 3-Azidopyridine (4g)[†]

A solution of sodium nitrite (787 mg, 11.4 mmol) in water (2.0 ml) was added dropwise to over 10 min to a solution of 3-aminopyridine (894 mg, 9.5 mmol) in 10% hydrochloric acid (8.0 ml) with stirring at 0 °C . After stirring for a further 15 min, a solution of sodium azide (649 mg, 10.0 mmol) in water (2.0 ml) was dropwise over 5 min. The reaction mixture was stirred for an additional 45 min at room temperature, then alkaline with Na_2CO_3 and extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and evaporated carefully in vacuo to give crude title compound as colorless oil (304 mg).

4.1.27. 3-[4-(Tributylstannyl)-1H-1,2,3-triazole-1-yl]pyridine (5g)

A solution of 3-azidopyridine (266 mg, 2.21 mmol) and tributyl(ethynyl)tin (835 mg, 2.65 mmol) in toluene (1.5 ml) was heated to 100 °C for 4 h. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 4:1) to give 896 mg of target compound as yellowish oil.

^{1}H NMR ($CDCl_3$) δ: 0.91 (9H, t, J = 7.3 Hz), 1.10–1.41 (12H, m), 1.51–1.68 (6H, m), 7.47–7.52 (1H, m), 8.14–8.18 (1H, m), 8.68 (1H, dd, J = 4.9, 1.5 Hz), 9.02 (1H, d, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ: 10.07 (3C, s), 13.65 (3C, s), 27.22 (3C, s), 28.98 (3C, s), 124.19 (s), 127.71 (s), 128.18 (s), 133.87 (s), 141.62 (s), 146.25 (s), 149.46 (s); IR(ATR) 2955, 2916, 1585, 1493, 1462, 1429, 1375, 1190, 1032, 808, 700, 662 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 437.1743 (calcd for $C_{19}H_{33}N_4Sn$: 437.1727).

4.1.28. 3-(4-Iodo-1H-1,2,3-triazol-1yl)pyridine (6g)

To a solution of 3-[4-(tributylstannyl)-1H-1,2,3-triazole-1-yl]pyridine (654 mg, 1.51 mmol) in THF (5.0 ml) was added iodine (382 mg, 1.51 mmol), and the reaction mixture was stirred at room temperature for 30 min. The resulting mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 1:1), and the resulting solid was washed with diisopropylether to give 333 mg of target compound.

^{1}H NMR ($CDCl_3$) δ: 7.52–7.55 (1H, m), 8.11–8.15 (2H, m), 8.75 (1H, dd, J = 4.6, 1.2 Hz), 8.98 (1H, d, J = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ: 88.64 (s), 124.35 (s), 127.23 (s), 128.18 (s), 132.99 (s), 141.54 (s), 150.49 (s); IR(ATR) 3126, 1582, 1491, 1456, 1394, 1279, 1204, 1032, 804, 700, 689, 615 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 272.9637 (calcd for $C_7H_6N_4I$: 272.9637).

4.1.29. *tert*-Butyl-4-[1-pyridin-3-yl-1 H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7g)

To a nitrogen flushed flask containing *tert*-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (186 mg, 0.60 mmol), K_2CO_3 (138 mg, 1.00 mmol) and 3-(4-iodo-1H-1,2,3-triazol-1yl)pyridine (136 mg, 0.50 mmol) was added DMF (2.0 ml) and $PdCl_2$(dppf)·CH_2Cl_2 (10 mol%). The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 1:1), and the resulting solid was washed with diisopropylether to give 54.0 mg of target compound.

^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.60 (2H, brs), 3.68 (2H, t, J = 5.4 Hz), 4.12–4.14 (2H, m), 6.57 (1H, brs), 7.52 (1H, td, J = 5.4, 2.8 Hz), 7.91 (1H, s), 8.15–8.19 (1H, m), 8.71 (1H, dd, J = 4.9, 1.5 Hz), 9.01 (1H, d, J = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ: 26.41 (s), 28.44 (3C, s), 38.28 (0.5C, s), 39.75 (0.5C, s), 43.55 (s), 79.86 (s), 116.53 (s), 122.65 (s), 124.26 (s), 125.52 (s), 127.96 (s), 133.62 (s), 141.36 (s), 149.03 (s), 149.86 (s), 154.83 (s); IR(ATR) 1682, 1497, 1425, 1238, 1173, 1117, 793, 700 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 328.1768 (calcd for $C_{17}H_{22}N_5O_2$: 328.1774); HPLC (a) 99.6%; (b) 99.4%.

4.1.30. 4-Azidopyridine (4h)[†]

To a solution of 4-chloropyridine hydrochloride (780 mg, 5.2 mmol) in 1 N-NaOH (2.6 ml) and EtOH (3 ml) was added sodium azide. The mixture was stirred at 110 °C for 4 h. After being cooled to room temperature, the resulting mixture was partitioned between Et_2O and water. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated carefully in vacuo to give crude title compound (290 mg).

4.1.31. 4-[4-(Tributylstannyl)-1H-1,2,3-triazole-1-yl]pyridine (5h)

A solution of 2-azidopyridine (230 mg, 1.91 mmol) and tributyl(ethynyl)tin (724 mg, 2.30 mmol) in toluene (1.0 ml) was heated to 120 °C for 12 h. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 4:1) to give 32.2 mg of target compound as a colorless oil.

^{1}H NMR ($CDCl_3$) δ: 0.90 (9H, t, J = 7.3 Hz), 1.14–1.40 (12H, m), 1.53–1.68 (6H, m), 7.75 (2H, dd, J = 4.4, 1.5 Hz), 7.98 (1H, s), 8.76 (2H, dd, J = 4.6, 1.7 Hz); ^{13}C NMR ($CDCl_3$) δ: 10.09 (3C, s), 13.64 (3C, s), 27.22 (3C, s), 28.98 (3C, s), 113.87 (2C, s), 127.06 (s), 143.21 (s), 146.63 (s), 151.60 (2C, s); IR(ATR) 2955, 2920, 1589,

1508, 1464, 1422, 1198, 1022, 826, 694, 667 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 437.1741 (calcd for $C_{19}H_{33}N_4Sn$: 437.1727).

4.1.32. 4-(4-Iodo-1H-1,2,3-triazol-1yl)pyridine (6h)

To a solution of 4-[4-(tributylstannyl)-1H-1,2,3-triazole-1-yl]pyridine (28 mg, 0.06 mmol) in THF (2.0 ml) was added iodine (19.6 mg, 0.07 mmol), and the reaction mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was washed with *n*-hexane to give 11 mg of target compound as a white solid.

^1H NMR ($CDCl_3$) δ: 7.71 (2H, dd, J = 4.4, 1.5 Hz), 8.18 (1H, s), 8.82 (2H, dd, J = 4.6, 1.7 Hz) IR(ATR) 2359, 2339, 1591, 1580, 1425, 1398, 1203, 1034, 997, 820, 793, 698, 690 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 272.9632 ($C_7H_6N_4I$: 272.9637).

4.1.33. *tert*-Butyl-4-[1-pyridin-4-yl-1 H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7h)

To a nitrogen flushed flask containing *tert*-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (15 mg, 0.05 mmol), K_2CO_3 (11 mg, 0. 08 mmol) and 3-(4-iodo-1H-1,2,3-triazol-1yl)pyridine (11 mg, 0.04 mmol) was added DMF (1.0 ml) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (10 mol%). The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 1:1), and the resulting solid was washed with diisopropylether to give 4.8 mg of target compound.

^1H NMR ($CDCl_3$): 1.50 (9H, s), 2.58 (2H, br s), 3.68 (2H, t, J = 5.6 Hz), 4.12–4.16 (2H, m), 6.60 (1H, br s), 7.73 (2H, dd, J = 4.6, 1.7 Hz), 7.95 (1H, s), 8.79 (2H, dd, J = 4.6, 1.7 Hz). ^{13}C NMR ($CDCl_3$) δ: 26.39 (s), 28.46 (3C, s), 39.51 (0.5C, s), 40.98 (0.5C, s), 43.67 (s), 79.89 (s), 113.50 (2C, s), 115.69 (s), 125.33 (s), 142.96 (s), 149.19 (s), 151.71 (2C, s), 154.87 (s); IR(ATR)2359, 2341, 1674, 1589, 1404, 1242, 1171, 1119, 1036, 826, 795, 702 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 328.1760 (calcd for $C_{17}H_{22}N_5O_2$: 328.1774); HPLC (a) 95.1%; (b) 97.1%.

4.1.34. 3-Azido-2-fluorophenylazide (4i)[†]

To a solution of diisopropylamine (9.2 ml, 66 mmol) in THF (100 ml) was added 1.58 M *n*-BuLi in hexane (41 ml, 66 mmol) at −78 °C. The solution was allowed to warm to 0 °C, stirred for 5 min and then re-cooled to −78 °C. To the resulting solution was added a solution of 2-fluoropyridien(6.4 g, 66 mmol) in THF (10 ml). After stirring 10 min at −78 °C, a solution of dodecylbenzenesulfone azide (11.58 g, 33 mmol) in THF (10 ml) was added to the mixture at −78 °C. The resulting mixture was allowed to warm to −60 °C, then partitioned between AcOEt and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then carefully concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 8:1) to give crude target compound (3.99 g).

4.1.35. 2-Fluoro-3-[4-(tributylstannyl)-1H-1,2,3-triazole-1-yl]pyridine (5i)

A solution of 3-azido-2-fluoropyridine (400 mg, 2.9 mmol) and tributyl(ethynyl)tin (945 mg, 3.0 mmol) in toluene (1.0 ml) was heated to 100 °C for 1 h. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 10:1) to give 269 mg of target compound as yellow oil.

^1H NMR ($CDCl_3$) δ: 0.90 (9H, t, J = 7.3 Hz), 1.12–1.40 (12H, m), 1.53–1.64 (6H, m), 7.42 (1H, ddd, J = 7.8, 4.9, 1.5 Hz), 8.08 (1H, d, J = 2.9 Hz), 8.26–8.28 (1H, m), 8.50–8.54 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 10.07 (3C, s), 13.63 (3C, s), 27.19 (3C, s), 28.96 (3C, s), 121.07 (d, J = 26.5 Hz), 122.62 (d, J = 5.0 Hz), 130.25 (s), 134.30 (s), 146.88 (s), 146.41 (d, J = 14.9 Hz), 153.35 (d, J = 237.5 Hz); IR(ATR) 2955, 2922, 1607, 1583, 1487, 1447, 1377, 1231, 1188, 1018, 964, 800, 745, 658 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 455.1649 (calcd for $C_{19}H_{32}N_4FSn$: 455.1633).

4.1.36. 2-Fluoro-3-(4-iodo-1H-1,2,3-triazol-1-yl)-pyridine (6i)

To a solution of 2-fluoro-3-[4-(tributylstannyl)-1H-1,2,3-triazole-1-yl]pyridine (210 mg, 0.46 mmol) in THF (4.0 ml) was added iodine (140 mg, 0.55 mmol), and the reaction mixture was stirred at room temperature for 1 h. The resulting mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 8:1), and the resulting solid was washed with Hexane to give 98 mg of target compound.

^1H NMR ($CDCl_3$) δ: 7.46 (1H, ddd, J = 7.9, 4.8, 1.3 Hz), 8.27 (1H, d, J = 2.9 Hz), 8.27–8.35 (1H, m), 8.47–8.52 (1H, m); ^{13}C NMR ($CDCl_3$) δ: 88.34 (s), 120.11 (d, J = 26.5 Hz), 122.80 (d, J = 5.0 Hz), 129.73 (d, J = 9.9 Hz), 134.14 (d, J = 1.7 Hz), 147.62 (d, J = 14.1 Hz), 153.25 (d, J = 238.3 Hz); IR(ATR) 3123, 1611, 1583, 1493, 1447, 1198, 1115, 1028, 986, 808, 741, 642, 679 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 290.9559 (calcd for $C_7H_5N_4FI$: 290.9543).

4.1.37. *tert*-Butyl-4-[1-(2-fuluoropyridine-3-yl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7i)

To a nitrogen flushed flask containing *tert*-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (102 mg, 0.33 mmol), K_2CO_3 (77.3 mg, 0.56 mmol) and 2-fluoro-3-(4-iodo-1H-1,2,3-triazol-1-yl)-pyridine (80.0 mg, 0.28 mmol) was added DMF (2.0 ml) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (24.5 mg, 0.03 mmol). The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 2:1) and the resulting solid was washed with Hexane to give 32 mg of target compound as white solid.

^1H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.57–2.62 (2H, m), 3.68 (2H, t, J = 5.6 Hz), 4.11–4.15 (2H, m), 6.57 (1H, brs), 7.44 (1H, ddd, J = 7.8, 4.6, 1.2 Hz), 8.06 (1H, d, J = 2.9 Hz), 8.29–8.31 (1H, m), 8.50–8.55 (1H, m); ^{13}C NMR ($CDCl_3$) δ: 26.38 (s), 28.44 (3C, s), 39.26 (0.5C, s), 40.73 (0.5C, s), 43.95 (s), 79.83 (s), 119.23 (d, J = 9.1 Hz), 120.79 (s), 122.68 (s), 122.73 (d, J = 5.0 Hz), 125.43 (s), 134.12 (d, J = 1.7 Hz), 146.92 (d, J = 14.9 Hz), 148.81 (s), 153.11 (d, J = 238.3 Hz), 154.82 (s); IR(ATR) 1692, 1479, 1458, 1416, 1236, 1167, 1115, 799, 768, 737 cm^{-1}; HRMS (ESI+) *m/z* $[M+H]^+$ 346.1680 (calcd for $C_{17}H_{21}N_5O_2F$: 346.1679); HPLC (a) 99.6%; (b) 99.6%.

4.1.38. 2-Fluoro-3-[5-methyl-4-(tributylstannyl)-1H-1,2,3-triazole-1-yl]pydidine (5j)

A solution of 3-azido-2-fluoropyridine (1.68 g 12 mmol) and tributyl(propynyl)tin (5.0 g, 15 mmol) in toluene (6.0 ml) was heated to 120 °C for 4 hours. After being cooled to room temperature, the mixture was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 3:1) to give 4.2 g of target compound as yellow oil.

^1H NMR ($CDCl_3$) δ: 0.90 (9H, t, J = 7.3 Hz), 1.11–1.41 (12H, m), 1.49–1.68 (6H, m), 2.29 (3H, d, J = 2.0 Hz), 7.43 (1H, ddd, J = 7.8, 4.9, 1.0 Hz), 7.97–8.02 (1H, m), 8.38 (1H, td, J = 3.0, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ: 9.59 (d, J = 4.1 Hz), 9.82 (3C, s), 13.64 (3C, s), 27.23 (3C, s), 29.03 (3C, s), 120.20 (d, J = 28.1 Hz), 122.33 (d,

J = 4.1 Hz), 138.97 (d, J = 1.7 Hz), 141.05 (s), 144.08 (s), 148.58 (d, J = 14.1 Hz), 156.62 (d, J = 242.5 Hz); IR(ATR) 2926, 2955, 1607, 1583, 1479, 1454, 1375, 1248, 1213, 999, 968, 847, 806, 671 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 469.1801 (calcd for $C_{20}H_{34}N_4FSn$: 469.1789).

4.1.39. 2-Fluoro-3-(4-iodo-5-methyl-1H-1,2,3-triazol-1-yl)-pyridine (6j)

To a solution of 2-fluoro-3-[5-methyl-4-(tributylstannyl)-1H-1,2,3-triazole-1-yl]pydidine (2.66 g, 5.69 mmol) in THF (30 ml) was added a solution of iodine (1.59 g, 6.26 mmol) in THF (20 ml) at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was partitioned between AcOEt and saturated aqueous $Na_2S_2O_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 3:1), and the resulting solid was washed with hexane to give 1.63 g of target compound.

^{1}H NMR ($CDCl_3$) δ: 2.32 (3H, d, J = 2.0 Hz), 7.47 (1H, ddd, J = 7.6, 4.9, 1.2 Hz), 7.97–8.02 (1H, m), 8.42–8.53 (1H, m); ^{13}C NMR ($CDCl_3$) δ: 9.60 (d, J = 5.0 Hz), 90.26 (s), 119.37 (d, J = 28.1 Hz), 122.54 (d, J = 5.0 Hz), 138.33 (s), 138.85 (d, J = 1.7 Hz), 149.57 (d, J = 14.1 Hz), 156.39 (d, J = 243.3 Hz); IR(ATR) 1607, 1578, 1479, 1441, 1213, 1080, 966, 749, 806, 822, 841 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 304.9714 (calcd for $C_8H_7N_4FI$: 304.9700).

4.1.40. *tert*-Butyl-4-[1-(2-fuluoropyridine-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (7j)

To a nitrogen flushed flask containing *tert*-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (587 mg, 1.90 mmol), K_2CO_3 (436 mg, 3.16 mmol) and 2-fluoro-3-(4-iodo-5-methyl-1H-1,2,3-triazol-1-yl)-pyridine (480 mg, 1.58 mmol) was added DMF (20 ml) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (129 mg, 0.16 mmol). The mixture was heated to 90 °C and stirred under N_2 overnight. The resulting mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 2:1) and the resulting solid was washed with Hexane to give 352 mg of target compound as white solid.

^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.35 (3H, d, J = 2.0 Hz), 2.72–2.77 (2H, m), 3.68 (2H, t, J = 5.9 Hz), 4.13 (2H, brs), 6.05 (1H, brs), 7.46 (1H, ddd, J = 7.8, 4.9, 1.0 Hz), 7.99–8.04 (1H, m), 8.43 (1H, td, J = 3.2, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ: 9.67 (d, J = 4.1 Hz), 27.20 (s), 28.46 (3C, s), 39.67 (0.5C, s), 43.07 (0.5C, s), 43.37 (s), 79.77 (s), 119.54 (d, J = 28.1 Hz), 122.49 (d, J = 5.0 Hz), 123.12 (s), 127.57 (s), 130.79 (s), 139.07 (d, J = 1.7 Hz), 144.71 (s), 149.11 (d, J = 14.1 Hz), 154.85 (s), 156.59 (d, J = 242.5 Hz); IR(ATR) 1678, 1659, 1456, 1412, 1366, 1248, 1169, 1115, 970, 851, 816, 750 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 360.1832 (calcd for $C_{18}H_{23}N_5O_2F$: 360.1836); HPLC (a) 97.1%; (b) 97.2%.

4.1.41. *N*-(*tert*-Butyl)-4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxamide (8)

To a solution of *tert*-butyl-4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (60 mg, 0.17 mmol) in $CHCl_3$ (2.0 ml) was added trifluoroacetic acid (2.0 ml), and the reaction mixture was stirred at room temperature for 20 min. After removing the solvents in vacuo, the residue was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo to give crude 42 mg of 4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]-1,2,3,6-tetrahydropyridine.

To a solution of 4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]-1,2,3,6-tetrahydropyridine (14.7 mg, 0.06 mmol) in THF (2.0 ml) was added *tert*-butyl isocyanate (12 μl, 0.11 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 30 min. The resulting mixture was partitioned between AcOEt and H_2O and the organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 2:1) to give 14.2 mg of target compound as white solid.

^{1}H NMR ($CDCl_3$) δ: 1.32 (9H, s), 2.55 (2H, td, J = 4.9, 2.6 Hz), 3.57 (2H, t, J = 5.6 Hz), 3.94 (2H, q, J = 2.8 Hz), 4.28 (1H, s), 6.48–6.49 (1H, m), 7.19–7.29 (2H, m), 7.34–7.40 (1H, m), 7.88–7.92 (2H, m); ^{13}C NMR ($CDCl_3$) δ: 26.57 (s), 29.51 (3C, s), 39.47 (s), 43.68 (s), 50.86 (s), 117.00 (d, J = 19.9 Hz), 119.97 (d, J = 8.3 Hz), 120.93 (s), 124.76 (s), 125.24 (s), 125.28 (s), 126.36 (s), 130.11 (d, J = 7.4 Hz), 148.07 (s), 153.21 (d, J = 250.8 Hz), 156.80 (s). ; IR(ATR) 2361, 2341, 1622, 1526, 1506, 1246, 1217, 820 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 344.1882 (calcd for $C_{18}H_{23}N_5OF$: 344.1887); HPLC (a) 99.6%; (b) 99.9%.

4.1.42. 1-(3,3-Dimethylbutanolyl)-4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]-1,2,3,6-tetrahydorohydropyridine (9)

To a solution of 4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]-1,2,3,6-tetrahydropyridine (14.0 mg, 0.057 mmol) and triethylamine (25 μl, 0.18 mmol) in THF (2.0 ml) was added *tert*-butylacetylchloride (19 μl, 0.14 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was partitioned between AcOEt and H_2O and the organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 4:1 and then $CHCl_3$/MeOH 50:1) to give 11.0 mg of target compound as colorless gum.

^{1}H NMR ($CDCl_3$) δ: 1.07 (4.5H, s), 1.09 (4.5H, s), 2.32 (1.5H, s), 2.36 (1.5H, s), 2.54–2.60 (1H, m), 2.65–2.75 (1H, m), 3.76 (1H, t, J = 5.6 Hz), 3.88 (1H, t, J = 5.6 Hz), 4.23 (1H, d, J = 3.4 Hz), 4.30 (1H, d, J = 2.9 Hz), 6.49 (0.5H, br s), 6.60 (0.5H, brs), 7.28–7.36 (2H, m), 7.42–7.47 (1H, m), 7.95–8.01 (2H, m); IR(ATR) 2359, 2341, 1632, 1506, 1466, 1427, 1234, 818, 764 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 343.1930 ($C_{19}H_{24}N_4OF$: 343.1934); HPLC (a) 98.6%; (b) 98.5%.

4.1.43. *iso*-Propyl-4-[1-(2-fuluoropyridine-3-yl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (10)

To a solution of *tert*-butyl-4-[1-(2-fuluoropyridine-3-yl)-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (30.0 mg, 0.08 mmol) in $CHCl_3$ (2.0 ml) was added Trifluoroacetic acid (2.0 ml), and the reaction mixture was stirred at room temperature for 20 min. After removing the solvents in vacuo, the residue was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo to give crude 19.8 mg of 2-fluoro-3-[4-(1,2,3,6-tetrahydropyridin-4-yl)-1H-1,2,3-triazole-1-yl]pydidine.

To a solution of 2-fluoro-3-[4-(1,2,3,6-tetrahydropyridin-4-yl)-1H-1,2,3-triazole-1-yl]pydidine (18.0 mg, 0.073 mmol) and triethylamine (25 μl, 0.18 mmol) in THF (4.0 ml) was added isopropyl chloroformate (10 μl, 0.088 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 45 min. The resulting mixture was partitioned between AcOEt and H_2O and the organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 2:1) to give 16.9 mg of target compound as white solid.

^{1}H NMR ($CDCl_3$) δ: 1.28 (6H, d, J = 6.3 Hz), 2.60 (2H, br s), 3.70–3.74 (2H, m), 4.16–4.20 (2H, m), 4.95–5.01 (1H, m), 6.58 (1H, brs), 7.44 (1H, ddd, J = 7.8, 4.9, 1.0 Hz), 8.07 (1H, d, J = 2.9 Hz), 8.29–8.31 (1H, m), 8.50–8.55 (1H, m); ^{13}C NMR ($CDCl_3$) δ: 22.27 (2C, s), 26.29 (s), 40.01 (s), 43.30 (s), 68.73 (s), 119.26 (d, J = 9.1 Hz), 120.79 (d, J = 25.7 Hz), 122.11 (s),

122.72 (d, J = 5.0 Hz), 125.47 (s), 134.13 (s), 146.95 (d, J = 14.1 Hz), 148.76 (s), 153.12 (d, J = 238.3 Hz), 155.29 (s); IR(ATR) 1684, 1458, 1425, 1285, 1229, 1105, 802 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 332.1524 (calcd for $C_{16}H_{19}N_5O_2F$: 332.1523); HPLC (a) 98.6%; (b) 98.1%.

4.1.44. *iso*-Propyl-4-[1-(2-fuluoropyridine-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (11)

To a solution of *tert*-butyl-4-[1-(2-fuluoropyridine-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-3,6-dihydropyridine-1(2H)-carboxylate (300 mg, 0.83 mmol) in $CHCl_3$ (8.0 ml) was added Trifluoroacetic acid (8.0 ml), and the reaction mixture was stirred at room temperature for 20 min. After removing the solvents in vacuo, the residue was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo to give crude 138 mg of 2-fluoro-3-[5-methyl-4-(1,2,3,6-tetrahydropyridin-4-yl)-1H-1,2,3-triazole-1-yl]pyridine.

To a solution of 2-fluoro-3-[5-methyl-4-(1,2,3,6-tetrahydropyridin-4-yl)-1H-1,2,3-triazole-1-yl]pyridine (135 mg, 0.52 mmol) and triethylamine (173 μl, 1.24 mmol) in THF (4.0 ml) was added isopropyl chloroformate (70.3 μl, 0.62 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 45 min. The resulting mixture was partitioned between AcOEt and H_2O and the organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was roughly purified by silicagel column chromatography (Wako C-300, Hexane/AcOEt 2:1), and the resulting solid was washed with hexane to give 133 mg of target compound as white solid.

^1H NMR ($CDCl_3$) δ: 1.28 (6H, d, J = 6.3 Hz), 2.35 (3H, d, J = 2.0 Hz), 2.74–2.78 (2H, m), 3.72 (2H, t, J = 5.4 Hz), 4.17 (2H, brs), 4.98 (1H, Sept, J = 6.3 Hz), 6.06 (1H, brs), 7.46 (1H, ddd, J = 7.8, 4.9, 1.0 Hz), 7.99–8.04 (1H, m), 8.42–8.44 (1H, td, J = 3.2, 1.6 Hz). ^{13}C NMR ($CDCl_3$) δ: 9.68 (d, J = 4.1 Hz), 22.29 (2C, s), 27.13 (s), 40.23 (s), 43.39 (s), 68.68 (s), 119.54 (d, J = 28.1 Hz), 122.50 (d, J = 4.1 Hz), 123.09 (s), 127.62 (s), 130.83 (s), 139.08 (d, J = 1.7 Hz), 144.68 (s), 149.14 (d, J = 14.1 Hz), 155.30 (s), 156.60 (d, J = 242.5 Hz); IR(ATR) 1688, 1452, 1433, 1236, 1119, 806, 750 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 346.1684 (calcd for $C_{17}H_{21}N_5O_2F$: 346.1679); HPLC (a) 98.0%; (b) 97.3%.

4.1.45. 4-[1-(2-Fuluoropyridine-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-*N*-isopropyl-3,6-dihydropyridine-1(2H)-carboxamide (12)

To a solution of 2-fluoro-3-[5-methyl-4-(1,2,3,6-tetrahydropyridin-4-yl)-1H-1,2,3-triazole-1-yl]pyridine (85.8 mg, 0.33 mmol) in THF (4.0 ml) was added isopropyl isocyanate (49 μl, 0.50 mmol), and the reaction mixture was stirred at room temperature for 45 minutes. The resulting mixture was then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, AcOEt) to give 68.4 mg of target compound.

^1H NMR ($CDCl_3$) δ: 1.20 (6H, d, J = 6.3 Hz), 2.35 (3H, d, J = 2.0 Hz), 2.78 (2H, brs), 3.64 (2H, t, J = 5.6 Hz), 4.01–4.09 (3H, m), 4.27 (1 H, d, J = 6.3 Hz), 6.09 (1H, brs), 7.47 (1H, dd, J = 7.3, 4.9 Hz), 7.99–8.04 (1 H, m), 8.43 (1H, d, J = 4.9 Hz); ^{13}C NMR ($CDCl_3$) δ: 9.67 (d, J = 4.1 Hz), 23.54 (2C, s), 27.24 (s), 40.19 (s), 42.56 (s), 43.49 (s), 119.51 (d, J = 29.8 Hz), 122.47 (s), 122.53 (s), 127.99 (s), 130.88 (s), 139.10 (s), 144.52 (s), 149.16 (d, J = 14.9 Hz), 156.60 (d, J = 242.0 Hz), 156.92 (s); IR(ATR) 1624, 1524, 1450, 1242, 1115, 1094, 964, 808, 754 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 345.1841 (calcd for $C_{17}H_{21}N_5O_2F$: 345.1839).

4.1.46. 4-[1-(2-Fuluoropyridine-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-*N*-isopropyl-*N*-methyl-3,6-dihydropyridine-1(2H)-carboxamide (1)

To a solution of 4-[1-(2-fuluoropyridine-3-yl)-5-methyl-1H-1,2,3-triazol-4-yl]-*N*-isopropyl-3,6-dihydropyridine-1(2H)-carboxamide (45 mg, 0.13 mmol) in DMF(3.0 ml) was added 60% NaH in oil (52 mg, excess) and then MeI (200 μl, excess) at 0 °C. The reaction mixture was stirred at room temperature for over night. The resulting mixture was partitioned between AcOEt and H_2O and the organic layer was dried over anhydrous Na_2SO_4, filtered and then concentrated in vacuo. The residue was purified by silicagel column chromatography (Wako C-300, AcOEt), and the resulting solid was washed with hexane to give 24.8 mg of target compound as white solid.

^1H NMR ($CDCl_3$) δ: 1.17 (6H, d, J = 5.9 Hz), 2.35 (3H, d, J = 2.0 Hz), 2.75 (3H, s), 2.79–2.83 (2H, m), 3.46 (2H, t, J = 5.6 Hz), 3.93–3.97 (2H, m), 4.07–4.13 (1H, m), 6.11 (1H, br s), 7.46 (1H, ddd, J = 7.8, 4.9, 1.0 Hz), 7.99–8.03 (1H, m), 8.42–8.43 (1H, m); ^{13}C NMR ($CDCl_3$) δ: 9.71 (d, J = 4.1 Hz), 19.59 (2C, s), 27.31 (s), 29.31 (s), 44.28 (s), 46.33 (s), 48.10 (s), 119.55 (d, J = 28.1 Hz), 122.47 (d, J = 4.1 Hz), 123.72 (s), 127.73 (s), 130.76 (s), 139.08 (d, J = 1.7 Hz), 144.71 (s), 149.11 (d, J = 14.1 Hz), 156.62 (d, J = 242.5 Hz), 164.64 (s); IR(ATR)1623, 1603, 1454, 1394, 1364, 1186, 1144, 1090, 1057, 968, 845, 806, 758 cm^{-1}; HRMS (ESI+) m/z $[M+H]^+$ 359.1999 (calcd for $C_{18}H_{24}N_6O_2F$: 359.1996); HPLC (a) 99.6%; (b) 99.8%.

4.1.47. Log *D* and water solubility

Log D and water solubility were actually measured by using reporting methods.[32,33]

4.1.48. Methods in quantum mechanical calculation

Potential energy surface scanning (PES) calculations varying the dihedral angles were performed for fragments of compounds **7i** and **7j**, in which the rest of the system was fixed. The optimizations were carried out for compounds **7i** and **7j** with the initial conformations based on the local minima obtained by PES. B3LYP/6-31+G(d,p) level of theory was used in both PES and optimization.

4.2. Pharmacology

4.2.1. Intracellular Ca^{2+} mobilization assays (FLIPR assay)

CHO cells expressing mGluR1 or mGluR5 receptors were incubated with 4 μM Fluo-3 or Fluo-4 in assay buffer (Hanks'balanced salt solution containing 20 mM Hepes, 2.5 mM probenecid) containing 1% dialyzed FBS for 1 h in the CO_2 incubator.

The extracellular dye was removed by washing four times with assay buffer. Ca^{2+} flux was measured using Fluorometric Imaging Plate Reader (FLIPR; Molecular Devices Corp.). For determination of the potency, the cells were incubated with various concentrations of compound for 5 min before addition of glutamate (final concentrations are 10, 50 and 10 μM at human mGluR1, mouse mGluR1 and human mGluR5, respectively).

4.2.2. Metabolic stability in liver microsomes

Pooled human, rat and mouse liver microsomes were purchased from Xenotech LLC. Nicotinamide adenine dinucleotide phosphate oxidized form (β-$NADP^+$), glucose 6-phosphate (G-6-P) and glucose 6-phosphate dehydrogenase (G-6-PDH) were obtained from Oriental Yeast Co., Ltd., Japan. Phosphate buffer powder was purchased from Sigma–Aldrich, Inc.

The reaction mixture (400 μl) contained 0.1 M potassium phosphate buffer (pH7.4), 3 mM $MgCl_2$, liver microsomes (0.25 mg-protein/ml) and an NADPH-generating system (1 mM β-$NADP^+$, 10 mM G-6-P, 2 U/ml G-6-PDH) was preincubated at 37 °C for

5 min. Compounds were dissolved in 50% acetnitrile to produce 50 μM dosing solution.

The incubations were initiated by the addition of 8.0 μl of dosing solution (final 1 μM). After 0 min and 30 min incubation at 37 °C, reactions were terminated by adding the three volume of ethanol following vigorous mixing. The resultant samples were centrifuged for 10 min at 4 °C. The supernatant was filtered through glass-filter paper (Whatman GF/C) and injected to an LC-MS/MS. All incubations were performed in duplicate.

LC-MS/MS system consisted of Waters Alliance 2790 and Quattro-Ultima tandem quadrupole mass spectrometer equipped with an electrical ion spray (ESI) probe (Waters corporation, Milford, MA).

The chromatographic separation was performed on CAPCELL PAK C18 UG120 cartridge column (2.0 × 10 mm, 5 μm particle size) at room temperature. The mobile phase consisted of 100 mM ammonium acetate/acetnitrile/Distilled Water (10:10:80, solvent A) and 100 mM ammonium acetate/acetnitrile (10:90, solvent B). The mobile phase was delivered at 1.2 ml/min with the following linear gradient elution program: The proportion of solvent B was maintained at 0% until 0.2 min and increased to 100% over 0.8 min. Following the maintenance of % B at 100% until 1.0 min, the proportion of B was reduced to 0% for 0.1 min and maintained until 2.5 min. The column eluent was split through a flow splitter so that 20% of the flow was lead to the mass spectrometer. The flow was diverted to waste before 0.5 min and after 1.5 min in order to prevent endogenous substances from entering the ion source. The mass data capture was performed from 0.5 to 1.5 min. Test compounds were detected in a positive ionization mode and the combinations of the precursor ion and its production for each compound were monitored.

The metabolic stability was determined by comparing the peak areas of the compound at 30 min incubation and that of 0 min with following calculation.

% remaining = (compound peak area at 30 min/compound peak area at 0 min) × 100.

4.2.3. DHPG- induced face washing behavior in mice

The in vivo experiment was approved by Banyu Institutional Animal care and use committee based on adherence to Japanese Pharmacological Society Guidance for Animal Use. Male CD1(ICR) mice (6-weeks-old, Japan SLC, Shizuoka, Japan) were housed in a controlled animal room (room temperature; 23 ± 2 °C, humidity 55±15%) on a 12 h light–dark cycle (light on 7 a.m. to 7 p.m.). (*S*)-3,5-DHPG (10 nM) was dissolved in 0.9% NaCl and administered i.c.v. to awake mice in a volume of 10 μl using a Hamilton syringe. mGluR1 antagonists at dose 1–30 mg/kg were given i.p. and/or p.o. 30 minutes prior to (*S*)-3,5-DHPG administration. Effects of test compounds on face-washing behavior were recorded 5 min after administration of (*S*)-3,5-DHPG administration for 5 min. Statistical analysis was conducted with one-way analysis of variance (ANOVA) followed by post-hoc multiple comparison test (Dunnett's test). A probability level of <0.05 was considered as statistical significance.

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