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Optimization and biological evaluation of imidazopyridine derivatives as a novel scaffold for γ-secretase modulators with oral efficacy against cognitive deficits in Alzheimer's disease model mice

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ABSTRACT

Gamma-secretase modulators (GSMs) selectively lower amyloid-β42 (Aβ42) and are therefore potential disease-modifying drugs for Alzheimer's disease (AD). Here, we report the discovery of imidazopyridine derivatives as GSMs with oral activity on not only Aβ42 levels but also cognitive function. Structural optimization of the biphenyl group and pyridine-2-amide moiety of compound **1a** greatly improved GSM activity and rat microsomal stability, respectively. 5-{8-[(3,4′-Difluoro[1,1′-*biphenyl*]-4-yl)methoxy]-2-methylimidazo[1,2-*a*]pyridin-3-yl}-*N*-methylpyridine-2-carboxamide (**1o**) showed high *in vitro* potency and brain exposure, induced a robust reduction in brain Aβ42 levels, and exhibited undetectable inhibition of cytochrome p450 enzymes. Moreover, compound **1o** showed excellent efficacy against cognitive deficits in AD model mice. These findings suggest that compound **1o** is a promising candidate for AD therapeutics.

1. Introduction

Alzheimer's disease (AD) is a severe neurodegenerative disorder and the most common form of dementia. Several drugs which inhibit the hydrolysis of acetylcholine or have mild antagonistic effects on the glutamate receptor are currently used as AD therapies.[1] However, the therapeutic benefits of these drugs are limited because they provide only symptomatic cognitive improvement. Therefore, disease-modifying drugs for AD have long been sought.[2]

Brain accumulation of insoluble amyloid-β (Aβ) fibrils and/or soluble Aβ oligomers are hypothesized to induce synaptic damage and neuronal cell death, ultimately leading to the cognitive impairment observed in AD.[3] Aβ peptides are produced by sequential processing of the amyloid precursor protein (APP) by β-secretase (BACE1) followed by γ-secretase. Gamma-secretase cleaves APP at multiple sites to release Aβ peptides of 37–43 amino acids in length.[4] Among these diverse Aβ peptides, Aβ42 is the most prone to aggregation and is implicated in having a critical role in the initiation of AD pathogenesis.[5] Therefore, lowering the levels of brain Aβ42 by inhibiting γ-secretase activity represents a rational strategy for developing disease-modifying drugs for AD.

Clinical trials of γ-secretase inhibitors (GSIs) have revealed that these drugs cause serious side effects, including skin cancer or worsening cognition, via inhibition of Notch-processing[6] and/or accumulation of the β-carboxy-terminal fragment (β-CTF) of APP.[7] In contrast, γ-secretase modulators (GSMs) lower pathogenic Aβ42 levels by inducing cleavage shifts without affecting Notch-processing or β-CTF.[8] GSMs are therefore promising therapeutic agents for AD.

We previously identified novel GSMs containing an original scaffold through high-throughput screening (HTS).[9] Our structure-activity relationship (SAR) study revealed that the carbonyl group of the A-B system had a significant effect on GSM activity, and that the planar structure formed by 5-membered ring cyclization (including a pseudo 5-membered ring), which fixed the direction of the carbonyl, was important for increasing activity.[9] Most recently, we reported a series of 5-{8-[([1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-*a*]pyridin-3-yl}-*N*-ethylpyridine-2-carboxamide hydrogen chloride (**1a**) derivatives as orally active GSMs (Fig. 1).[9b] Compound **1a** has a metabolically stable *N*-ethylpyridine-2-carboxamide-structured A-B ring system and a biphenyl D ring to improve PK, and showed high *in vitro* GSM activity comparable to that of representative GSM **2** (E2012).[10] Consequently, it has high brain exposure and significantly reduced brain Aβ42 levels in mice without *in vitro* CYP3A4 inhibition.[9b] However, we hypothesized that, from a clinical perspective, **1a** has further room for improvement

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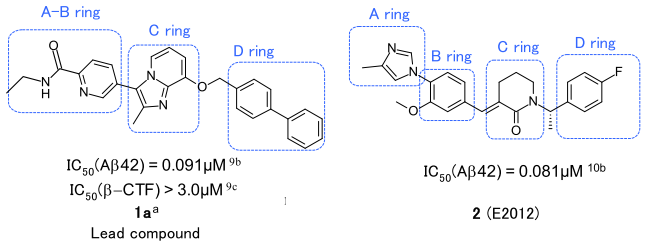


Fig. 1. Structure of the lead compound (**1a**) and representative GSM, E2012 (2). [a]Hydrochloride salt.

in terms of microsomal clearance (CL_{int} in rat = 835 mL/min/kg) and *in vitro* GSM potency because it had limited ability to reduce brain Aβ42 levels in rats at 30 mg/kg po (Table 1).

Here, we report further optimization of lead compound **1a** to improve both metabolic stability and *in vitro* GSM activity. In this process, we discovered a novel imidazopyridine derivative as an orally active GSM that induced a robust reduction in brain Aβ42 levels and showed excellent efficacy against cognitive deficits in AD model mice in the Y-maze test.[11]

1.1. Molecular design

The purpose of this study was to generate highly optimized GSMs that reduced brain Aβ42 levels to a greater extent than **1a** by oral administration and ameliorated cognitive dysfunction in AD model mice. We attempted to optimize two moieties of **1a**, the D ring and the A-B ring system (Fig. 2), because they are clearly important for *in vitro* GSM activity and metabolic stability, as shown by our previous work[9] and a number of studies on non-nonsteroidal anti-inflammatory drug (non-NSAID)-derived heterocyclic GSMs.[12] The strategy for D ring optimization is shown in Fig. 2. We previously reported that *in vitro* activity correlated with the lipophilicity of the D ring.[9b] Therefore, to maintain the lipophilicity and block the putative metabolic site, we introduced various substituents containing fluorine, because these are lipophilic and often improve metabolic stability. The strategy for A-B ring optimization is also shown in Fig. 2. The planar structure of the pyridine-2-amide moiety, which forms a pseudo five-membered ring through intramolecular hydrogen bonding and fixes the direction of the carbonyl group, is important for favorable interaction with γ-secretase.[9] Therefore, we retained the planarity of the moiety during the optimization and only synthesized 5-membered bicyclic derivatives because these were previously found to be acceptable, while incorporation of a 6-membered ring was not.[9b]

We hypothesized that balancing the physicochemical properties by the appropriate combination of these two moieties would lead to the desired final compounds.

2. Chemistry

Synthesis of the fluoroated biphenyl D ring is shown in Scheme 1. Alkylation of **3**[9b] with benzyl halide derivatives produced **4** and **5**, and Suzuki cross-coupling of mono-fluorophenyl boronic acid derivatives or bromo trifluoromethyl benzene derivatives with **4** or **5** produced **1b–d** and **1e–g**, respectively.

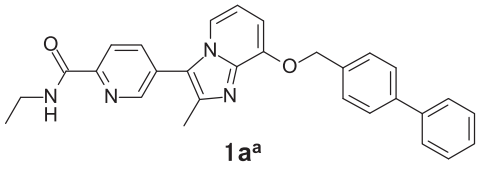
Synthesis of an alternative A-B ring system is outlined in Scheme 2. Debenzylation of **6**[9] under hydrogenation conditions produced **7**. Mitsunobu reaction using (tributylphosphoranylidene)acetonitrile[13] with (4′-fluoro[1,1′-*biphenyl*]-4-yl)methanol and **7** or **8** produced **1 h** and **9**. Ethylation of **10** produced **11**. Ipso-reactions of fluorinated **12** with methanethiolate followed by cyclization[14] with sulfuryl dichloride produced **13**. Diazotization of **14** and sequential cyclization and acylation[15] produced dihydroindazole derivative **15**, and subsequent ethylation produced **16**.[16] A Heck-type reaction with imidazopyridine derivative **9** and corresponding aryl bromide in the presence of Pd $(OAc)_2$ produced **1i**, **1j**, and **17**. Hydrolysis of **17** produced **1k**, and subsequent methylation produced **1l**.

Substituent conversion, namely substitution with a methyl instead of ethyl group, of the A-B ring system is shown in Scheme 3. Condensation of **18**[9b] with methyl amine produced **19**, and subsequent debenzylation under hydrogenation conditions produced **20**. Alkylation with 4-(bromomethyl)-4′-fluoro-1,1′-biphenyl or Mitsunobu reaction using (tributylphosphoranylidene)acetonitrile[13] with (3,4′-difluoro [1,1′-*biphenyl*]-4-yl)methanol produced **1 m** or **1o**. Methylation of **10**, and subsequent Heck-type reaction with imidazopyridine derivative **9** produced **1n**.

3. Results and discussion

Optimization of the D ring is shown in Table 2. *In vitro* activity correlated positively with the lipophilicity (ACD/LogP[17]) of the D ring,[9b] as comprehensively tested using the effect of various substituents containing fluorine, which is lipophilic, on metabolic stability.

Table 1
In vivo Aβ42-lowering effects and plasma and brain concentration of **1a** in mice and rats.



	CL_{int}(mL/min/kg)	Plasma conc. [b], [c](μM)	Brain conc. [b], [d](nmol/g)	Kp, brain [e]	Brain conc./IC_{50}(nmol/g)/(μM)	Reduction in Aβ42[f] in brain/plasma (%)
Mice	227	10[g]	4.6[g]	0.45	51	36[g] ***/66[g] ***
Rats	835	6.8	2.1	0.30	23	13/28*

[a]Hydrochloride salt.
[b] 3 h after oral administration at 30 mg/kg (n = 3).
[c] Plasma concentration of the compounds.
[d] Brain concentration of the compounds.
[e] The brain-to-plasma concentration ratio at steady state.
[f] 3 or 4 h after oral administration at 30 mg/kg (n = 4 or 5).
[g] Previously reported data.[9b]
* $p < 0.05$ vs. vehicle group by Student's *t*-test (n = 4 or 5).
*** $p < 0.001$ vs. vehicle group by Student's *t*-test (n = 4 or 5).

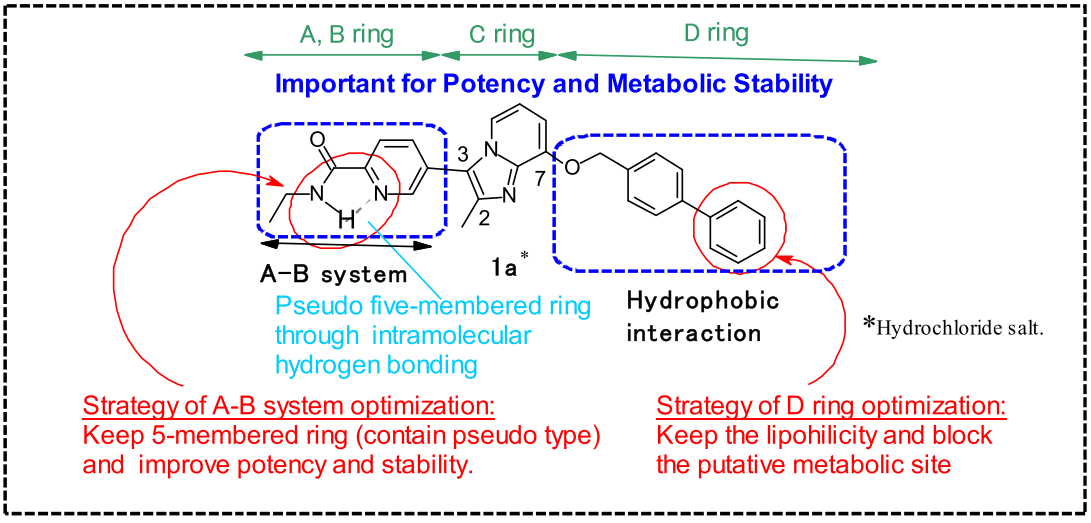
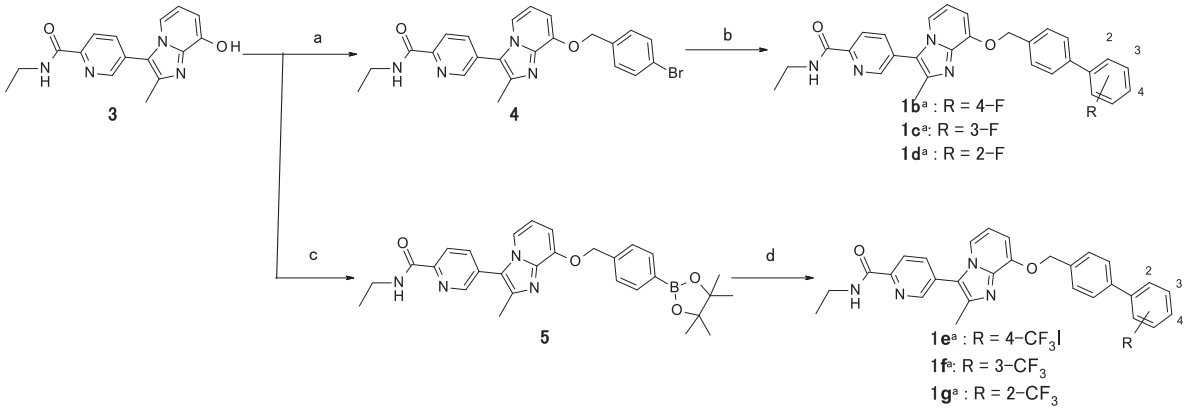


Fig. 2. Strategies for obtaining orally active and effective cognitive-behavioral GSMs.



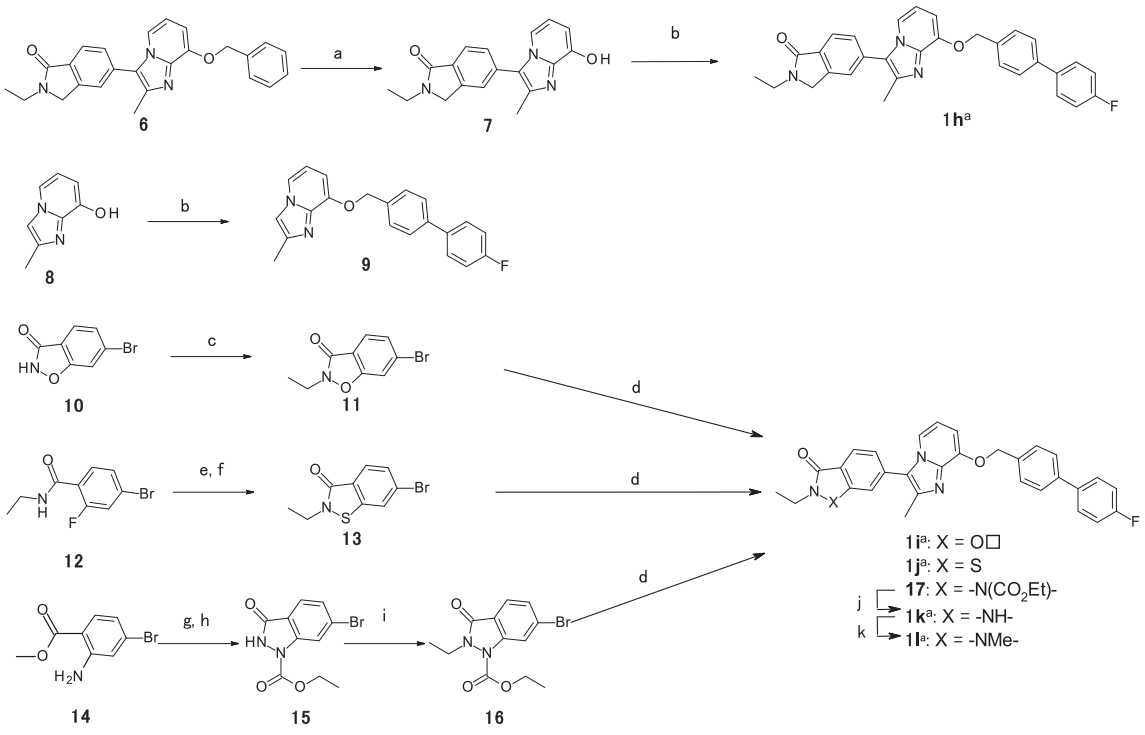
Scheme 1. Reagents and reaction conditions: (a) 1-bromo-4-(bromomethyl)benzene, K_2CO_3, DMF, 50 °C, 64% yield; (b) fluorophenyl boronic acid, $PdCl_2(dppf)\cdot CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, H_2O, 90 °C, 61%[b] yield of **1b**[a], 63%[b] yield of **1c**[a] and 57%[b] yield of **1d**[a]; (c) 2-[4-(chloromethyl)phenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, TBAI, K_2CO_3, DMF, 50 °C, 29% yield; (d) bromo trifluoromethyl benzene derivative, $PdCl_2(dppf)\cdot CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, H_2O, 90 °C, 77%[b] yield of **1e**[a], 55%[b] yield of **1f**[a] and 19%[b] yield of **1g**[a]. [a]Hydrochloride salt. [b] Yield after salification with hydrogen chloride in ethyl acetate.

Some representative compounds are listed in Table 2. The rank order of a series of three fluoro-substituted derivatives (**1b–d**; Table 2) of **1a** in terms of GSM activity and stability was para > meta > ortho, with **1b** showing about 4-fold greater stability than **1a**. As a result, **1b** and **1c** induced about 2-fold greater reduction in brain Aβ42 levels than **1a**. Meanwhile, for a series of trifluoromethyl-substituted derivatives (**1e–g**; Table 2), *ortho*-substituted **1g** was comparably stable, while the meta- and *para*-substituted derivatives were more stable than **1a**. Further, while **1e** and **1f** induced a greater reduction in plasma Aβ42 levels, their effect on reducing brain Aβ42 levels was lower than or similar to that of **1a**. The high molecular weight of the trifluoromethyl group may be responsible for the decline in brain penetration. Indeed, the central nervous system multiparameter optimization (CNS MPO[18]) score for a brain penetration guideline was reduced. Conversions that result in a CNS MPO score below 3.00 may be undesirable for brain penetration.

Optimization of novel A-B systems is shown in Table 3. Isoindolinone derivative **1h** showed activity comparable to and lower stability than **1b**, and induced less than a 2-fold decrease in brain Aβ42 levels. Meanwhile, 1,2-benzisoxazoline-3-one derivative **1i** exhibited a similar profile to that of **1b**, suggesting that this bicyclic structure is promising for the A-B system. Other 5-membered bicyclic derivatives, benzisothiazolinone derivative **1j** and indazolinone derivatives **1k** and **1l**, showed lower GSM potency, with **1j** and **1l** showing an IC_{50} more than 2 μM. These results indicate a lack of space to accommodate not only addition of a methyl group but also a sulfur atom instead of a carbon atom around this A-B ring position when these GSMs compounds bind the pocket of γ-secretase.

Given that the *in vitro* GSM activities of these compounds were sufficient, we subsequently focused on improving both brain penetration and stability. We introduced a methyl instead of ethyl group to reduce the molecular weight, lipophilicity and bond rotation, which was expected to improve the CNS MPO score and possibly the stability. Indeed, methyl-substituted pyridine-2-amide derivative **1m** and 1,2-benzisoxazoline-3-one derivative **1n**, corresponding to **1b** and **1i,** respectively, showed improved stability, and **1m** reduced brain Aβ42 levels by 40%. *In vitro* GSM activity was also improved and **1n** reduced plasma Aβ42 levels by more than 80%. Further minor optimizations were conducted, leading to the discovery of **1o**, which showed the highest GSM potency in this series with an IC_{50} value of 0.029 μM, and significantly reduced brain Aβ42 levels by 23% and 42% at 10 and 30 mg/kg po, respectively, in rats.

Next, we evaluated the drug concentrations of compounds **1m**, **1n** and **1o**, which showed marked *in vivo* Aβ42-lowering effects, in plasma and brain homogenates from rats 3 h after oral administration at a dose of 10 or 30 mg/kg (Table 4). The exposure of *N*-methylpyridine-2-carboxamide derivative **1m** in brain and plasma was markedly improved, at 17 μM and 7.1 nmol/g, compared to **1a**, at 6.8 μM and

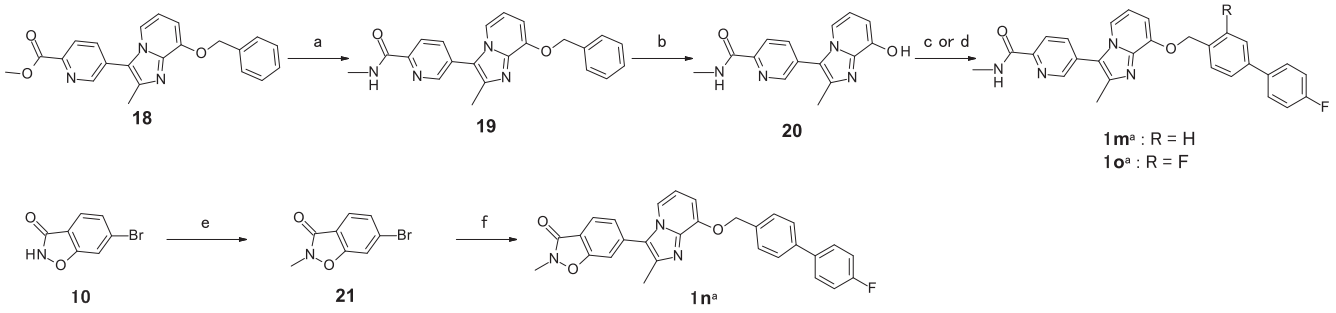


Scheme 2. Reagents and reaction conditions: (a) H_2, Pd/C, EtOH, rt, 66% yield; (b) (4′-fluoro[1,1′-*biphenyl*]-4-yl)methanol, nBu$_3$P = CHCN, toluene, 100 °C, 32% yield of **9** and 60%[b] yield of **1 h**[a]; (c) iodoethane, NaH, DMF, rt, 32% yield; (d) compound **9**, Pd(OAc)$_2$, PPh$_3$, Cs$_2$CO$_3$, 1,4-dioxane, 110 °C or reflux, 94%[b] yield of **1i**[a], 31%[b] yield of **1j**[a] and quant. of **17**; (e) sodium methanethiolate, DMSO, rt; (f) sulfuryl dichloride, toluene, 70–80 °C, 17% yield(2 steps); (g) conc.HCl, sodium nitrite, H_2O, rt, then disodium sulfite, H_2O, rt, then conc.HCl, rt to 80 °C; (h) ethyl carbonochloridate, pyridine, 110 °C, 42% yield(2 steps); (i) iodoethane, KOH, EtOH, 100 °C, 26% yield; (j) KOH, EtOH, reflux, 59%[b] yield; (k) iodomethane, KOH, EtOH, 60 °C, 44%[b] yield. [a] Hydrochloride salt. [b] Yield after salification with hydrogen chloride in ethyl acetate.

2.1 nmol/g, respectively. Further, **1m** showed markedly improved *in vitro* GSM activity, resulting in a significantly higher brain/IC$_{50}$ ratio (193) than **1a** (23). The brain/plasma ratio (Kp, brain = 0.44) was also higher than that of **1a**. Similar results were obtained for the 1,2-benzisoxazoline-3-one derivative **1n**. Although the brain/plasma ratio was slightly lower (Kp, brain = 0.27) than that of **1a**, the plasma concentration of **1n** was the highest in this series (21 μM), resulting in the greatest reduction in plasma Aβ42 levels (81%). Compound **1o**, which had the lowest IC$_{50}$ value (0.029 μM) and robust brain Aβ42-lowering effects (42%, 30 mg/kg) also showed high brain exposure even at 10 mg/kg.

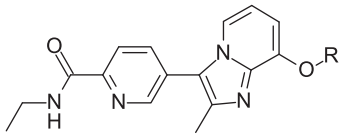
Next, we examined the effects of GSMs on cognitive function in AD model mice. **1o** or vehicle was orally administered once daily for 8 days to Tg2576 mice,[11] which overproduce brain Aβ, and Y-maze tests were performed.[7] The Tg2576 mice model is one of the most well characterized AD model, and is widely used for this study. Overexpressed mutant form of APP (isoform 695) caused by the Swedish mutation (KM670/671NL) and increased Aβ levels ultimately lead to amyloid plaques. At less than 6 months of age, these mice show impaired spatial learning, working memory, and contextual fear conditioning. Vehicle-treated Tg2576 mice demonstrated significantly lower spontaneous alternation rates than wild-type mice in the Y-maze test, indicating deficits in spatial working memory. Treatment with **1o** at 2 and 6 mg/kg completely rescued this memory deficit[20] (Fig. 3).

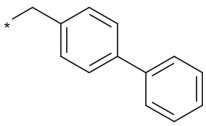
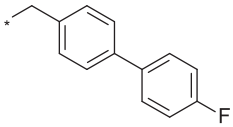
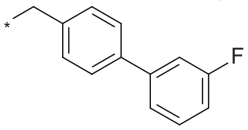
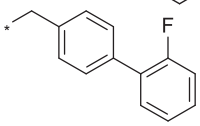
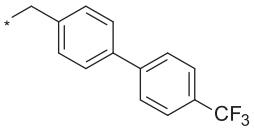
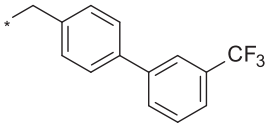
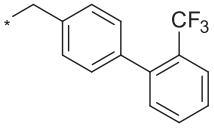
Finally, **1o** did not show any inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4 (Table 5). These results suggest that removing the arylimidazole group is effective in abrogating the inhibition of not only CYP3A4 as previously reported[9] but also 5 types of CYP.



Scheme 3. Reagents and reaction conditions: (a) methyl amine, MeOH, 60 °C, 95% yield; (b) H_2, Pd/C, MeOH, THF, rt, 67% yield; (c) 4-(bromomethyl)-4′-fluoro-1,1′-biphenyl, K$_2$CO$_3$, DMF, 50 °C, 52%[b] yield; (d) (3,4′-difluoro[1,1′-*biphenyl*]-4-yl)methanol, nBu$_3$P = CHCN, toluene, 80 °C, 52%[b] yield; (e) iodomethane, *N*-ethyl-*N*-(propan-2-yl)propan-2-amine, CHCl$_3$, 60 °C, 75% yield; (f) compound **9**, Pd(OAc)$_2$, PPh$_3$, Cs$_2$CO$_3$, 1,4-dioxane, 105 °C, 62%[b] yield. [a]Hydrochloride salt. [b]Yield after salification with hydrogen chloride in ethyl acetate.

Table 2
γ-Secretase modulatory activity of imidazo pyridine derivatives **1a–h.**



Compound	R	Aβ42 IC_{50} (μM)	Rat CL_{int} (mL/min/kg)	ACD/LogP[b]	CNS MPO[c]	Reduction in Aβ42 in brain/plasma[d] (%)
1a[a]		0.091[e]	835	4.78	3.21	13[f]/28[f] *
1b[a]		0.075	221	4.72	3.11	22**/43**
1c[a]		0.099	307	4.80	3.07	22**/42***
1d[a]		0.22	> 1000	5.29	2.97	NT[g]
1e[a]		0.21	No depletion	5.75	2.83	4/40***
1f[a]		0.061	38	5.75	2.83	15/62***
1g[a]		0.18	790	5.75	2.83	NT[g]

[a] Hydrochloride salt.
[b] ACD/LogP values were calculated using ACD/LogP prediction software, ACD/Percepta.
[c] CNS MPO values were calculated using MOE software.[19]
[d] 3 h after oral administration at 30 mg/kg (n = 4 or 5) in rats.
[e] Previously reported data.[9b]
[f] 4 h after oral administration at 30 mg/kg (n = 4 or 5) in rats.
[g] Not tested.
* $p < 0.05$ vs. vehicle group by Student's *t*-test (n = 4 or 5).
** $p < 0.01$ vs. vehicle group by Student's *t*-test (n = 4 or 5).
*** $p < 0.001$ vs. vehicle group by Student's *t*-test (n = 4 or 5).

4. Conclusion

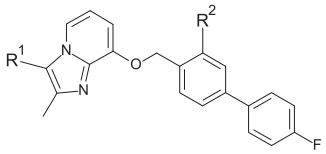
We discovered a series of *N*-methylpyridine-2-carboxamide derivatives as a novel scaffold for GSMs with oral activity on not only brain Aβ42 levels but also cognitive function, and determined their structure-activity relationships. Identification of high *in vitro* GSM activity and careful combination of a metabolically stable A-B system and D ring improved PK and Aβ42 reduction in brain and plasma. We identified compound **1o**, which had the highest *in vitro* GSM activity in this series, undetectable CYP inhibition and high brain exposure. Further, this compound showed robust reduction of brain Aβ42 levels *in vivo* in rats at a dose of 10 mg/kg (po). Further, a low dose of 2 mg/kg (po) of **1o** for 8 days completely rescued cognitive deficits exhibited by AD model mice. These findings suggest that compound **1o** is a promising candidate for AD therapeutics.

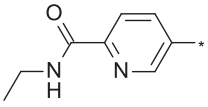
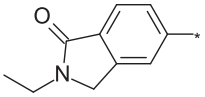
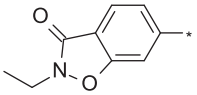
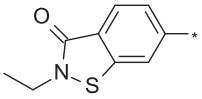
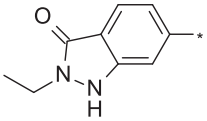
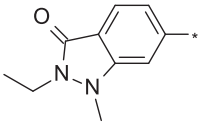
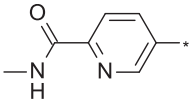
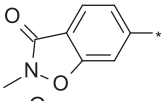
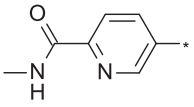
5. Experimental section

5.1. Chemistry

^{1}H NMR spectra were recorded using an Agilent (Varian) 400-MR, Agilent (Varian) VNS 400, Varian Mercury 400, Varian Mercury plus 400 or Bruker AVANCE III HD 500, and chemical shifts are expressed as δ (ppm) values with tetramethylsilane as internal reference (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, and br = broad peak). Mass spectra (MS) were

Table 3
γ-Secretase modulatory activity of imidazo pyridine derivatives **1b** and **1 h–1o.**



Compound	R^1	R^2	IC_{50} (μM)	Rat CL_{int} (mL/min/kg)	CNS MPO[b]	Reduction in Aβ42[c] in brain/plasma (%)
1b[a]		H	0.075	221	3.11	22**/43**
1h[a]		H	0.092[d]	345 [d]	3.06	10/59***
1i[a]		H	0.090	214	3.05	20*/63***
1j[a]		H	> 2	NT[f]	3.00	NT[f]
1k[a]		H	0.39	NT[f]	1.89	NT[f]
1l[a]		H	> 2	NT[f]	3.00	NT[f]
1m[a]		H	0.037	147	3.48	40***/68***
1n[a]		H	0.069	167	3.15	29**/81***
1o[a]		F	0.029	115	3.34	42***/77*** 23[e] **/63[e] ***

[a] Hydrochloride salt.
[b] CNS MPO values were calculated using MOE software.[19]
[c] 3 h after oral administration at 30 mg/kg (n = 4 or 5) in rats.
[d] Evaluated using free form, not hydrochloride salt.
[e] 3 h after oral administration at 10 mg/kg (n = 4 or 5) in rats.
[f] Not tested.
* p < 0.05 vs. vehicle group by Student's *t*-test (n = 4 or 5).
** p < 0.01 vs. vehicle group by Student's *t*-test (n = 4 or 5).
*** p < 0.001 vs. vehicle group by Student's *t*-test (n = 4 or 5).

recorded using Waters ACQUITY SQD, Waters ZQ 2000 or Agilent_G6130A mass spectrometers. Elemental analyses were performed using a Yanaco JM10 or MT-6 (C, H, N), Elementar Vario EL III (C, H, N), and a Dionex ICS-3000 or 5000 (S, halogen) and were within ± 0.4% of theoretical values. Positive electrospray ionization high-resolution mass spectra (HRMS) were acquired using a Thermo Scientific Exactive Plus.

Unless otherwise noted, all reagents and solvents obtained from commercial suppliers were used without further purification. The abbreviations used are as follows: DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; EtOH, ethanol; MeOH, methanol; sat. $NaHCO_3$ aq., saturated aqueous $NaHCO_3$ solution; **$PdCl_2$(dppf)· CH_2Cl_2**, [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane; PPh_3, triphenylphosphine; TBAI, tetrabutylammonium iodide; and THF, tetrahydrofuran.

5.1.1. N-Ethyl-5-{8-[(4′-fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}pyridine-2-carboxamide hydrogen chloride (1b)

A mixture of **4** (252 mg, 0.54 mmol), (4-fluorophenyl)boronic acid (97 mg, 0.69 mmol), **$PdCl_2$(dppf)· CH_2Cl_2** (59 mg, 0.072 mmol) and

Table 4
Plasma and brain concentration of **1a**, **1m**, **1n** and **1o** in rats.

Compd.	IC_{50} (μM)	Rat CL_{int} (mL/min/kg)	Dose (mg)	Plasma conc.[b], [c] (μM)	Brain conc. [b], [d] (nmol/g)	Kp,brain [e]	CNS MPO [f]	Plasma conc./ IC_{50}	Brain conc./IC_{50} (nmol/g)/(μM)	Reduction in Aβ42[g] in brain/plasma (%)
1a[a]	0.091[h]	835	30	6.8	2.1	0.30	3.21	75	23	13[i]/28[i] *
1m[a]	0.037	147	30	17	7.1	0.44	3.48	446	193	40***/68***
1n[a]	0.069	167	30	21	5.6	0.27	3.15	304	81	29**/81***
1o[a]	0.029	115	30	16	3.5	0.23	3.34	561	121	42***/77***
			10	5.7	1.5	0.27		196	51	23**/63***

[a] Hydrochloride salt.
[b] 3 h after oral administration at 10 or 30 mg/kg in rats (n = 2 or 3).
[c] Plasma concentration of the compounds.
[d] Brain concentration of the compounds.
[e] Brain-to-plasma concentration ratio at steady state.
[f] CNS MPO values were calculated using MOE software.[19]
[g] 3 h after oral administration at 10 or 30 mg/kg (n = 4 or 5) in rats.
[h] Previously reported data.[9b]
[i] 4 h after oral administration at 30 mg/kg (n = 4 or 5) in rats.
* p < 0.05 vs. vehicle group by Student's *t*-test (n = 4 or 5).
** p < 0.01 vs. vehicle group by Student's *t*-test (n = 4 or 5).
*** p < 0.001 vs. vehicle group by Student's *t*-test (n = 4 or 5).

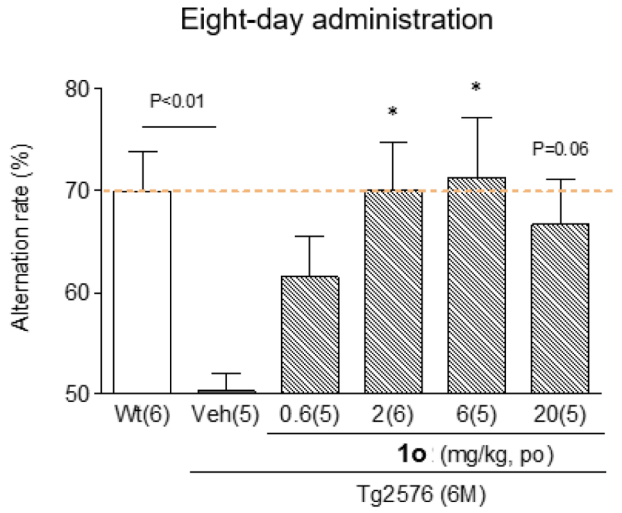


Fig. 3. Effects of **1o** on cognitive deficits in Tg2576 mice in the Y-maze test. Number of mice at each dose is indicated in parentheses. *p < 0.05 vs. vehicle group by Student's *t*-test (n = 5 or 6).

Table 5
Inhibitory activity of compound **1o** on CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.

CYP1A2 IC50 (μM)	CYP2C9 IC50 (μM)	CYP2C19 IC50 (μM)	CYP2D6 IC50 (μM)	CYP3A4* Preincubation time	
				0 min (%)	30 min (%)
> 10	> 10	> 10	> 10	109	104

* Residual activity of human liver microsomes was evaluated using midazolam as a probe substrate.

disodium carbonate (85 mg, 0.80 mmol) in 1,4-dioxane (4 mL) and H_2O (1 mL) was stirred at 90 °C for 20 h and then allowed to cool to room temperature. The mixture was filtered through a Celite pad and $MgSO_4$, and evaporated *in vacuo*. The residue was purified using silica-gel column chromatography (*n*-hexane/EtOAc = 1:1 to 0:1) to give *N*-ethyl-5-{8-[(4′-fluoro[1,1′-*biphenyl*]-4-yl)methoxy]-2-methylimidazo [1,2-*a*]pyridin-3-yl}pyridine-2-carboxamide as a colorless solid (167 mg). Next, 4 M HCl in EtOAc (0.20 mL, 0.80 mmol) was added to a EtOAc (2.0 mL) and EtOH (0.40 mL) solution of this compound. After stirring at room temperature, the precipitate was filtered to give the product as a colorless solid (171 mg, 61% yield). ^{1}H NMR (DMSO-d_6) δ ppm 1.16 (3H, t, *J* = 7.2 Hz), 2.49 (3H, s), 3.33–3.42 (2H, m), 5.55 (2H, s), 7.28–7.39 (3H, m), 7.58 (1H, br d, *J* = 8.0 Hz), 7.67–7.78 (6H, m), 8.23 (1H, d, *J* = 6.7 Hz), 8.25–8.31 (2H, m), 8.87 (1H, dd, *J* = 2.0, 1.2 Hz), 8.96 (1H, t, *J* = 6.1 Hz); MS (ESI) *m/z* $[M + H]^+$ 481. HRMS (ESI) *m/z* calcd for $C_{29}H_{26}FN_4O_2$ ($[M + H]^+$): 481.2034. Found: 481.2037. Anal. calcd for $C_{29}H_{25}FN_4O_2{\cdot}1.0HCl{\cdot}0.3H_2O$: C, 66.68; H, 5.13; N, 10.73; F, 3.64; Cl, 6.79. Found: C, 66.87; H, 5.11; N, 10.60; F, 3.59; Cl, 6.64.

5.1.2. N-Ethyl-5-{8-[(3′-fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}pyridine-2-carboxamide hydrogen chloride (1c)

Compound **1c** was prepared from **4** at 63% yield (2 steps) as a colorless solid using a method similar to that described for **1b**. ^{1}H NMR (DMSO-d_6) δ ppm 1.16 (3H, t, *J* = 7.2 Hz), 2.49 (3H, s), 3.18–3.57 (2H, m), 5.56 (2H, s), 7.20–7.26 (1H, m), 7.35 (1H, t, *J* = 7.3 Hz), 7.50–7.62 (4H, m), 7.69–7.74 (2H, m), 7.79–7.84 (2H, m), 8.23 (1H, d, *J* = 6.7 Hz), 8.25–8.31 (2H, m), 8.87 (1H, dd, *J* = 1.9, 1.1 Hz), 8.96 (1H, t, *J* = 6.1 Hz); MS (ESI) *m/z* $[M + H]^+$ 481. HRMS (ESI) *m/z* calcd for $C_{29}H_{26}FN_4O_2$ ($[M + H]^+$): 481.2034. Found: 481.2037. Anal. calcd for $C_{29}H_{25}FN_4O_2{\cdot}1.0HCl{\cdot}1.8H_2O$: C, 63.40; H, 5.43; N, 10.20; F, 3.46; Cl, 6.45. Found: C, 63.46; H, 5.37; N, 10.27; F, 3.37; Cl, 6.35.

5.1.3. N-Ethyl-5-{8-[(2′-fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}pyridine-2-carboxamide hydrogen chloride (1d)

Compound **1d** was prepared from **4** at 57% yield (2 steps) as a pale yellow solid using a method similar to that described for **1b**. ^{1}H NMR (DMSO-d_6) δ ppm 1.16 (3H, t, *J* = 7.1 Hz), 2.49 (3H, s), 3.34–3.42 (2H, m), 5.57 (2H, s), 7.31–7.39 (3H, m), 7.42–7.49 (1H, m), 7.53–7.63 (2H, m), 7.63–7.67 (2H, m), 7.70–7.75 (2H, m), 8.22–8.32 (3H, m), 8.87 (1H, dd, *J* = 2.0, 1.0 Hz), 8.96 (1H, t, *J* = 6.1 Hz); MS (ESI) *m/z* $[M + H]^+$ 481. HRMS (ESI) *m/z* calcd for $C_{29}H_{26}FN_4O_2$ ($[M + H]^+$): 481.2034. Found: 481.2038. Anal. calcd for $C_{29}H_{25}FN_4O_2{\cdot}1.0HCl{\cdot}2.1H_2O$: C, 62.78; H, 5.49; N, 10.10; F, 3.42; Cl, 6.39. Found: C, 62.70; H, 5.40; N, 10.08; F, 3.38; Cl, 6.39.

5.1.4. N-Ethyl-5-(2-methyl-8-{[4′-(trifluoromethyl)[1,1′-biphenyl]-4-yl] methoxy}imidazo[1,2-a]pyridin-3-yl)pyridine-2-carboxamide hydrogen chloride (1e)

A mixture of **5** (151 mg, 0.29 mmol), 1-bromo-4-(trifluoromethyl)

benzene (98 mg, 0.43 mmol), **$PdCl_2$(dppf)· CH_2Cl_2** (36 mg, 0.044 mmol) and disodium carbonate (51 mg, 0.48 mmol) in 1,4-dioxane (2 mL) and H_2O (0.5 mL) was stirred at 90 °C for 15 h and then allowed to cool to room temperature. The mixture was filtered through a Celite pad and $MgSO_4$, and evaporated *in vacuo*. The residue was purified using silica-gel column chromatography (*n*-hexane/EtOAc = 1:1 to 0:1) to give *N*-ethyl-5-(2-methyl-8-{[4′-(trifluoromethyl)[1,1′-biphenyl]-4-yl]methoxy}imidazo[1,2-*a*]pyridin-3-yl)pyridine-2-carboxamide as a colorless solid (121 mg). Next, 4 M HCl in EtOAc (0.19 mL, 0.76 mmol) was added to a EtOAc (1.9 mL) and EtOH (0.38 mL) solution of this compound. After stirring at room temperature, the precipitate was filtered to give the product as a pale yellow solid (129 mg, 77% yield). ^{1}H NMR (DMSO-d_6) δ ppm 1.16 (3H, t, *J* = 7.2 Hz), 2.50 (3H, s), 3.34–3.42 (2H, m), 5.59 (2H, s), 7.40 (1H, dd, *J* = 8.1, 6.9 Hz), 7.64 (1H, d, *J* = 7.9 Hz), 7.74–7.79 (2H, m), 7.82–7.88 (4H, m), 7.94 (2H, d, *J* = 7.9 Hz), 8.24–8.32 (3H, m), 8.88 (1H, dd, *J* = 2.1, 1.0 Hz), 8.96 (1H, t, *J* = 6.0 Hz); MS (ESI) *m/z* $[M + H]^+$ 531. HRMS (ESI) *m/z* calcd for $C_{30}H_{26}F_3N_4O_2$ ($[M + H]^+$): 531.2002. Found: 531.2004. Anal. calcd for $C_{30}H_{25}F_3N_4O_2$·1.9HCl·0.5H_2O: C, 59.18; H, 4.62; N, 9.20; F, 9.36; Cl, 11.06. Found: C, 59.31; H, 4.61; N, 9.19; F, 9.20; Cl, 11.23.

5.1.5. N-Ethyl-5-(2-methyl-8-{[3′-(trifluoromethyl)[1,1′-biphenyl]-4-yl]methoxy}imidazo[1,2-a]pyridin-3-yl)pyridine-2-carboxamide hydrogen chloride (1f)

Compound **1f** was prepared from **5** at 55% yield (2 steps) as a colorless solid using a method similar to that described for **1e**. ^{1}H NMR (DMSO-d_6) δ ppm 1.16 (3H, t, *J* = 7.1 Hz), 2.50 (3H, s), 3.34–3.42 (2H, m), 5.59 (2H, s), 7.40 (1H, dd, *J* = 7.8, 6.8 Hz), 7.65 (1H, d, *J* = 8.0 Hz), 7.71–7.79 (4H, m), 7.84–7.89 (2H, m), 7.98–8.06 (2H, m), 8.24–8.32 (3H, m), 8.88 (1H, dd, *J* = 2.0, 1.0 Hz), 8.97 (1H, t, *J* = 6.1 Hz); MS (ESI) *m/z* $[M + H]^+$ 531. HRMS (ESI) *m/z* calcd for $C_{30}H_{26}F_3N_4O_2$ ($[M + H]^+$): 531.2002. Found: 531.2005. Anal. calcd for $C_{30}H_{25}F_3N_4O_2$·1.6HCl·0.7H_2O: C, 59.90; H, 4.69; N, 9.31; F, 9.48; Cl, 9.43. Found: C, 60.07; H, 4.71; N, 9.45; F, 9.21; Cl, 9.17.

5.1.6. N-Ethyl-5-(2-methyl-8-{[2′-(trifluoromethyl)[1,1′-biphenyl]-4-yl]methoxy}imidazo[1,2-a]pyridin-3-yl)pyridine-2-carboxamide hydrogen chloride (1 g)

Compound **1 g** was prepared from **5** at 19% yield (2 steps) as a colorless solid using a method similar to that described for **1e**. ^{1}H NMR (DMSO-d_6) δ ppm 1.16 (3H, t, *J* = 7.1 Hz), 2.49 (3H, s), 3.20–3.68 (2H, m), 5.56 (2H, s), 7.31–7.40 (1H, m), 7.39–7.46 (3H, m), 7.52–7.60 (1H, m), 7.62–7.71 (3H, m), 7.72–7.78 (1H, m), 7.84–7.88 (1H, m), 8.24 (1H, d, *J* = 6.7 Hz), 8.21–8.31 (2H, m), 8.87 (1H, dd, *J* = 2.0, 1.0 Hz), 8.96 (1H, t, *J* = 6.0 Hz); MS (ESI) *m/z* $[M + H]^+$ 531. HRMS (ESI) *m/z* calcd for $C_{30}H_{26}F_3N_4O_2$ ($[M + H]^+$): 531.2002. Found: 531.2008. Anal. calcd for $C_{30}H_{25}F_3N_4O_2$·1.0HCl·0.7H_2O: C, 62.17; H, 4.77; N, 9.67; F, 9.83; Cl, 6.12. Found: C, 62.35; H, 4.68; N, 9.74; F, 9.72; Cl, 6.01.

5.1.7. 2-Ethyl-5-{8-[(4′-fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-2,3-dihydro-1H-isoindol-1-one hydrogen chloride (1 h)

A mixture of **7** (1.0 g, 3.25 mmol), (4′-fluoro[1,1′-*biphenyl*]-4-yl)methanol (0.99 g, 4.88 mmol) and (tributylphosphoranylidene)acetonitrile[15] (1.6 g, 6.51 mmol) in toluene (24 mL) was stirred at 100 °C for 6 h and allowed to cool to room temperature. The mixture was evaporated *in vacuo* and the crude mixture was purified using silica-gel column chromatography ($CHCl_3$/MeOH = 20:1 to 10:1) to give 2-ethyl-5-{8-[(4′-fluoro[1,1′-*biphenyl*]-4-yl)methoxy]-2-methylimidazo[1,2-*a*]pyridin-3-yl}-2,3-dihydro-1*H*-isoindol-1-one as a colorless solid (980 mg, 61% yield). Next, 4 M HCl in EtOAc was added to this compound (50 mg, 0.10 mmol) and to give the product (53 mg, 99% yield). ^{1}H NMR (DMSO-d_6) δ ppm 1.21 (3H, t, *J* = 7.2 Hz), 2.46 (3H, s), 3.61 (2H, q, *J* = 7.2 Hz), 4.60 (2H, s), 5.53 (2H, s), 7.28–7.36 (3H, m), 7.45–7.60 (1H, m), 7.66–7.78 (7H, m), 7.85–7.87 (1H, m), 7.92 (1H, d, *J* = 7.8 Hz), 8.16 (1H, d, *J* = 6.8 Hz); MS (ESI) *m/z* $[M + H]^+$ 492. HRMS (ESI) *m/z* calcd for $C_{31}H_{27}FN_3O_2$ ($[M + H]^+$): 492.2082. Found: 492.2073. Anal. calcd for $C_{31}H_{26}FN_3O_2$·1.0HCl·1.9H_2O: C, 66.22; H, 5.52; N, 7.47; F, 3.38; Cl, 6.31. Found: C, 66.30; H, 5.48; N, 7.52; F, 3.35; Cl, 6.26.

5.1.8. 2-Ethyl-6-{8-[(4′-fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-1,2-benzoxazol-3(2H)-one hydrogen chloride (1i)

A mixture of **11** (100 mg, 0.41 mmol), **9** (150 mg, 0.45 mmol), Pd$(OAc)_2$ (9 mg, 0.04 mmol), PPh_3 (22 mg, 0.08 mmol), and Cs_2CO_3 (202 mg, 0.62 mmol) in 1,4-dioxane (2 mL) was stirred at 110 °C overnight and then allowed to cool to room temperature. The mixture was filtered through a Celite pad and evaporated *in vacuo*. The residue was purified using silica-gel column chromatography ($CHCl_3$/MeOH = 20:1) to give 2-ethyl-6-{8-[(4′-fluoro[1,1′-*biphenyl*]-4-yl)methoxy]-2-methylimidazo[1,2-*a*]pyridin-3-yl}-1,2-benzoxazol-3(2*H*)-one. Next, 4 M HCl in EtOAc (0.20 mL, 0.80 mmol) was added to a 5.0 mL EtOAc solution of this compound. After stirring at room temperature, the mixture was concentrated, the residue was solidified with EtOAc and the precipitate was filtered to give the product as a colorless solid (205 mg, 94% yield). ^{1}H NMR (DMSO-d_6) δ ppm 1.33 (3H, t, *J* = 7.2 Hz), 2.48 (3H, s), 4.09 (2H, q, *J* = 7.2 Hz), 5.54 (2H, s), 7.28–7.38 (3H, m), 7.46–7.64 (2H, m), 7.67–7.72 (2H, m), 7.72–7.78 (4H, m), 7.85–7.88 (1H, m), 8.03–8.07 (1H, m), 8.23 (1H, d, *J* = 6.8 Hz); MS (ESI) *m/z* $[M + H]^+$ 494. HRMS (ESI) *m/z* calcd for $C_{30}H_{25}FN_3O_3$ ($[M + H]^+$): 494.1874. Found: 494.1876. Anal. calcd for $C_{30}H_{24}FN_3O_3$·1.0HCl·2.0H_2O: C, 63.66; H, 5.16; N, 7.42; F, 3.36; Cl, 6.26. Found: C, 63.68; H, 5.16; N, 7.40; F, 3.19; Cl, 6.25

5.1.9. 2-Ethyl-6-{8-[(4′-fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-1,2-benzothiazol-3(2H)-one hydrogen chloride (1j)

Compound **1j** was prepared from **13** and **9** at 31% yield (2 steps) using a method similar to that described for **1i**. ^{1}H NMR (DMSO-d_6) δ ppm 1.29 (3H, t, *J* = 7.2 Hz), 2.49 (3H, s), 3.91 (2H, q, *J* = 7.2 Hz), 5.54 (2H, s), 7.29–7.37 (3H, m), 7.50–7.59 (1H, m), 7.64–7.78 (7H, m), 8.09 (1H, d, *J* = 8.2 Hz), 8.22 (1H, d, *J* = 6.8 Hz), 8.29–8.31 (1H, m); MS (ESI) *m/z* $[M + H]^+$ 510. HRMS (ESI) *m/z* calcd for $C_{30}H_{25}FN_3O_2S$ ($[M + H]^+$): 510.1646. Found: 510.1648. Anal. calcd for $C_{30}H_{24}FN_3O_2S$·1.1HCl·0.1H_2O: C, 65.33; H, 4.62; N, 7.62; S, 5.81; F, 3.44; Cl, 7.07. Found: C, 65.27; H, 4.75; N, 7.61; S, 5.69; F, 3.33; Cl, 6.95.

5.1.10. 2-Ethyl-6-{8-[(4′-fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-1,2-dihydro-3H-indazol-3-one hydrogen chloride (1k)

A mixture of **17** (250 mg, 0.44 mmol) and KOH (99 mg, 1.77 mmol) in EtOH (1.5 mL) was refluxed for 4 h with stirring and allowed to cool to room temperature. Acetic acid (133 mg, 2.21 mmol) was added to the mixture in an ice-cooled water bath and the mixture was evaporated *in vacuo*. The residue was purified using silica-gel column chromatography ($CHCl_3$/MeOH = 19:1) to give 2-ethyl-6-{8-[(4′-fluoro[1,1′-*biphenyl*]-4-yl)methoxy]-2-methylimidazo[1,2-*a*]pyridin-3-yl}-1,2-dihydro-3*H*-indazol-3-one (192 mg, 88% yield). Next, 4 M HCl in EtOAc (0.12 mL, 0.48 mmol) was added to this compound (77 mg, 0.16 mmol) in a solution of EtOAc (3.0 mL), $CHCl_3$ (1.0 mL) and MeOH (1.0 mL). After stirring at room temperature, the mixture was concentrated *in vacuo*, and the residue was solidified with EtOAc and the precipitate was filtered to give the product (55 mg, 67% yield). ^{1}H NMR (DMSO-d_6) δ ppm 1.28 (3H, t, *J* = 7.2 Hz), 2.47 (3H, s), 3.89 (2H, q, *J* = 7.2 Hz), 5.55 (2H, s), 7.25–7.39 (4H, m), 7.51–7.53 (1H, m), 7.55–7.62 (1H, m), 7.67–7.79 (6H, m), 7.89 (1H, dd, *J* = 7.9, 0.7 Hz), 8.16 (1H, d, *J* = 6.8 Hz), 10.71 (1H, br s); MS (ESI) *m/z* $[M + H]^+$ 493. HRMS (ESI) *m/z* calcd for $C_{30}H_{26}FN_4O_2$ ($[M + H]^+$): 493.2034. Found: 493.2038.

Anal. calcd for $C_{30}H_{25}FN_4O_2 \cdot 1.7HCl \cdot 2.4H_2O$: C, 60.28; H, 5.31; N, 9.37; F, 3.18; Cl, 10.08. Found: C, 60.17; H, 5.20; N, 9.55; F, 3.07; Cl, 10.06

5.1.11. 2-Ethyl-6-{8-[(4′-fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-1-methyl-1,2-dihydro-3H-indazol-3-one hydrogen chloride (1 l)

A mixture of 2-ethyl-6-{8-[(4′-fluoro[1,1′-*biphenyl*]-4-yl)methoxy]-2-methylimidazo[1,2-*a*]pyridin-3-yl}-1,2-dihydro-3*H*-indazol-3-one (119 mg, 0.24 mmol) and KOH (17 mg, 0.30 mmol) in EtOH (2.0 mL) was stirred at room temperature for 0.5 h. Iodomethane (86 mg, 0.60 mmol) was added, and the mixture was stirred at 60 °C overnight before being allowed to cool to room temperature and evaporated *in vacuo*. The residue was purified using silica-gel column chromatography ($CHCl_3$/MeOH = 19:1) to give 2-ethyl-6-{8-[(4′-fluoro[1,1′-*biphenyl*]-4-yl)methoxy]-2-methylimidazo[1,2-*a*]pyridin-3-yl}-1-methyl-1,2-dihydro-3*H*-indazol-3-one (63 mg, 51% yield). Next, 4 M HCl in EtOAc (0.10 mL, 0.40 mmol) was added to a EtOAc (2.5 mL) solution of this compound (63 mg, 0.12 mmol) and stirring at room temperature to give the product (59 mg, 87% yield). ^{1}H NMR (DMSO-d_6) δ ppm 1.16 (3H, t, J = 7.1 Hz), 2.50 (3H, s), 3.37 (3H, s), 3.95 (2H, q, J = 7.1 Hz), 5.56 (2H, s), 7.29–7.41 (4H, m), 7.62 (1H, d, J = 8.2 Hz), 7.68–7.78 (6H, m), 7.84–7.86 (1H, m), 7.90–7.95 (1H, m), 8.23 (1H, d, J = 6.6 Hz); MS (ESI) m/z $[M + H]^+$ 507. HRMS (ESI) m/z calcd for $C_{31}H_{28}FN_4O_2$ ($[M + H]^+$): 507.2191. Found: 507.2193. Anal. calcd for $C_{31}H_{27}FN_4O_2 \cdot 1.6HCl \cdot 0.8H_2O$: C, 64.27; H, 5.25; N, 9.67; F, 3.28; Cl, 9.79. Found: C, 64.09; H, 5.17; N, 9.67; F, 3.19; Cl, 9.79.

5.1.12. 5-{8-[(4′-Fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-N-methylpyridine-2-carboxamide hydrogen chloride (1 m)

A mixture of **20** (88 mg, 0.31 mmol), 4-(bromomethyl)-4′-fluoro-1,1′-biphenyl (85 mg, 0.32 mmol) and dipotassium carbonate (70 mg, 0.51 mmol) in DMF (2.0 mL) was stirred at 50 °C for 2 h. After cooling to room temperature, the mixture was diluted with water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The crude mixture was purified using silica-gel column chromatography (*n*-hexane/EtOAc = 1:1 to 0:1) to give 5-{8-[(4′-fluoro[1,1′-*biphenyl*]-4-yl)methoxy]-2-methylimidazo[1,2-*a*]pyridin-3-yl}-*N*-methylpyridine-2-carboxamide as a colorless solid (98 mg). Next, 4 M HCl in EtOAc (0.10 mL, 0.40 mmol) was added to a solution of this compound in 2-propanol (1.0 mL). After stirring at room temperature, the precipitate was filtered to give the product as a colorless solid (82 mg, 52% yield). ^{1}H NMR (DMSO-d_6) δ ppm 2.48 (3H, s), 2.88 (3H, d, J = 4.9 Hz), 5.55 (2H, s), 7.28–7.38 (3H, m), 7.53–7.59 (1H, m), 7.67–7.78 (6H, m), 8.22 (1H, d, J = 6.6 Hz), 8.24–8.31 (2H, m), 8.87 (1H, dd, J = 1.9, 1.0 Hz), 8.84–8.95 (1H, m); MS (ESI) m/z $[M + H]^+$ 467. HRMS (ESI) m/z calcd for $C_{28}H_{24}FN_4O_2$ ($[M + H]^+$): 467.1878. Found: 467.1881. Anal. calcd for $C_{28}H_{23}FN_4O_2 \cdot 1.0HCl \cdot 0.1H_2O$: C, 66.62; H, 4.83; N, 11.10; F, 3.76; Cl, 7.02. Found: C, 66.51; H, 4.87; N, 11.12; F, 3.76; Cl, 7.06.

5.1.13. 6-{8-[(4′-Fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-2-methyl-1,2-benzoxazol-3(2H)-one hydrogen chloride (1n)

Compound **1n** was prepared from **21** and **9** at 62% yield (2 steps) as a colorless solid using a method similar to that described for **1i**. ^{1}H NMR (DMSO-d_6) δ ppm 2.49 (3H, s), 3.66 (3H, s), 5.55 (2H, s), 7.28–7.38 (3H, m), 7.55–7.61 (2H, m), 7.68–7.78 (6H, m), 7.85–7.87 (1H, m), 8.03–8.06 (1H, m), 8.22 (1H, d, J = 6.6 Hz); MS (ESI) m/z $[M + H]^+$ 480. HRMS (ESI) m/z calcd for $C_{29}H_{23}FN_3O_3$ ($[M + H]^+$): 480.1718. Found: 480.1722. Anal. calcd for $C_{29}H_{22}FN_3O_3 \cdot 1.2HCl \cdot 1.0H_2O$: C, 64.35; H, 4.69; N, 7.76; F, 3.51; Cl, 7.86. Found: C, 64.25; H, 4.92; N, 7.57; F, 3.39; Cl, 7.78.

5.1.14. 5-{8-[(3,4′-Difluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-N-methylpyridine-2-carboxamide hydrogen chloride (1o)

Compound **1o** was prepared from **20** and (3,4′-difluoro[1,1′-*biphenyl*]-4-yl)methanol at 52% yield (2 steps) as a colorless solid using a method similar to that described for **1 h**. ^{1}H NMR (DMSO-d_6) δ ppm 2.46 (3H, s), 2.88 (3H, d, J = 4.9 Hz), 5.57 (2H, s), 7.31–7.39 (3H, m), 7.59–7.70 (3H, m), 7.78–7.86 (3H, m), 8.22–8.31 (3H, m), 8.86 (1H, dd, J = 2.0, 1.1 Hz), 8.88–8.94 (1H, m); MS (ESI) m/z $[M + H]^+$ 485. HRMS (ESI) m/z calcd for $C_{28}H_{23}F_2N_4O_2$ ($[M + H]^+$): 485.1784. Found: 485.1786. Anal. calcd for $C_{28}H_{22}F_2N_4O_2 \cdot 1.0HCl \cdot 0.1H_2O$: C, 64.33; H, 4.47; N, 10.72; F, 7.27; Cl, 6.78. Found: C, 64.52; H, 4.65; N, 10.67; F, 7.02; Cl, 6.64.

5.1.15. 5-{8-[(4-Bromophenyl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-N-ethylpyridine-2-carboxamide (4)

A mixture of **3** (499 mg, 1.7 mmol), 1-bromo-4-(bromomethyl) benzene (454 mg, 1.8 mmol) and dipotassium carbonate (344 mg, 2.5 mmol) in DMF (5.0 mL) was stirred at 50 °C for 1.5 h. After cooling to room temperature, the mixture was diluted with water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The crude mixture was purified using silica-gel column chromatography (*n*-hexane/EtOAc = 6:4 to 0:1) to give the product as a white solid (500 mg, 64% yield). ^{1}H NMR ($CDCl_3$) δ ppm 1.31 (3H, t, J = 7.3 Hz), 2.53 (3H, s), 3.52–3.61 (2H, m), 5.33 (2H, s), 6.45–6.50 (1H, m), 6.60–6.66 (1H, m), 7.36–7.41 (2H, m), 7.49–7.53 (2H, m), 7.70 (1H, dd, J = 6.8, 0.8 Hz), 7.95 (1H, dd, J = 8.2, 2.2 Hz), 7.98–8.06 (1H, m), 8.36 (1H, dd, J = 8.0, 0.8 Hz), 8.66 (1H, dd, J = 2.2, 0.8 Hz); MS (ESI) m/z $[M + H]^+$ 467.

5.1.16. N-Ethyl-5-(2-methyl-8-{[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methoxy}imidazo[1,2-a]pyridin-3-yl)pyridine-2-carboxamide (5)

A mixture of **3** (500 mg, 1.7 mmol), 2-[4-(chloromethyl)phenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (471 mg, 1.9 mmol), dipotassium carbonate (346 mg, 2.5 mmol) and TBAI (103 mg, 0.28 mmol) in DMF (5.0 mL) was stirred at 50 °C for 3.5 h. After cooling to room temperature, the mixture was diluted with water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The crude mixture was purified using silica-gel column chromatography (*n*-hexane/EtOAc = 6:4 to 0:1) to give the product as a yellow solid (248 mg, 29% yield). ^{1}H NMR ($CDCl_3$) δ ppm 1.31 (3H, t, J = 7.2 Hz), 1.34 (12H, s), 2.53 (3H, s), 3.52–3.60 (2 H, m), 5.42 (2 H, s), 6.42–6.47 (1 H, m), 6.58 (1 H, dd, J = 7.6, 6.8 Hz), 7.47–7.52 (2 H, m), 7.64–7.69 (1 H, m), 7.79–7.83 (2 H, m), 7.95 (1 H, dd, J = 8.1, 2.2 Hz), 7.98–8.04 (1 H, m), 8.35 (1 H, dd, J = 8.0, 0.8 Hz), 8.66 (1 H, dd, J = 2.2, 0.8 Hz); MS (ESI) m/z $[M + H]^+$ 513.

5.1.17. 2-Ethyl-5-(8-hydroxy-2-methylimidazo[1,2-a]pyridin-3-yl)-2,3-dihydro-1H-isoindol-1-one (7)

10% Pd/C, 50% wet (0.49 g, 0.23 mmol) was added to a solution of **6** (9.7 g, 24 mmol) in EtOH (100 mL) and the mixture was stirred in a hydrogen atmosphere at room temperature for 3 h. The catalyst was removed by filtration through a Celite pad and the filtrate was concentrated *in vacuo*. The residue was purified using silica-gel column chromatography ($CHCl_3$/MeOH = 10:1), and the product was triturated with EtOAc and the precipitate was collected and dried to give the product as a pale green solid (4.9 g, 66% yield). ^{1}H NMR (DMSO-d_6) δ ppm 1.20 (3H, t, J = 7.1 Hz), 2.40 (3H, s), 3.59 (2H, q, J = 7.2 Hz), 4.56 (2H, s), 6.54 (1H, dd, J = 7.5, 0.9 Hz), 6.71 (1H, t, J = 7.1 Hz), 7.61 (1H, dd, J = 7.9, 1.5 Hz), 7.73–7.77 (1H, m), 7.80–7.86 (2H, m); MS (ESI) m/z $[M + H]^+$ 308.

5.1.18. 8-[(4′-Fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridine (9)

A mixture of **8** (0.5 g, 3.4 mmol), (4′-fluoro[1,1′-*biphenyl*]-4-yl)

methanol (0.75 g, 3.7 mmol) and (tributylphosphoranylidene)acetonitrile[12] (1.2 g, 5.1 mmol) in toluene (12 mL) was stirred at 100 °C for 6 h and allowed to cool to room temperature. The mixture was evaporated *in vacuo* and the crude mixture was purified using amino-functionalized silica-gel column chromatography (*n*-hexane/AcOEt = 2:1 to 1:1) to give the product as a colorless solid (0.36 g, 32% yield). ^{1}H NMR (DMSO-d_6) δ ppm 2.31 (3H, d, J = 0.8 Hz), 5.31 (2H, s), 6.66–6.73 (2H, m), 7.27–7.33 (2H, m), 7.56–7.60 (2H, m), 7.63–7.66 (1H, m), 7.67–7.76 (4H, m), 8.05 (1H, dd, J = 5.4, 2.2 Hz); MS (ESI) *m/z* $[M + H]^+$ 333.

5.1.19. 6-Bromo-2-ethyl-1,2-benzoxazol-3(2H)-one (11)

Sodium hydride 60% dispersion in mineral oil (157 mg, 3.9 mmol) was added to a solution of **10** (0.7 g, 3.3 mmol) and iodoethane (1.5 g, 9.8 mmol) in DMF (11 mL) while cooling on ice. After stirring at room temperature for 3 h, sat. $NaHCO_3$ aq. was added and the mixture was extracted with EtOAc, washed with water, dried over $MgSO_4$, and evaporated *in vacuo*. The crude mixture was purified using silica-gel column chromatography (*n*-hexane/EtOAc = 3:1) to give the product as a brown solid (0.25 g, 32% yield). ^{1}H NMR (DMSO-d_6) δ ppm 1.28 (3H, t, J = 7.1 Hz), 4.01 (2H, q, J = 7.2 Hz), 7.52 (1H, dd, J = 8.3, 1.5 Hz), 7.71–7.75 (1H, m), 7.90–7.93 (1H, m); MS (ESI) *m/z* $[M + H]^+$ 242.

5.1.20. 6-Bromo-2-ethyl-1,2-benzothiazol-3(2H)-one (13)

Sodium methanethiolate (0.68 g, 9.8 mmol) was added to a solution of **12** (2.0 g, 8.1 mmol) in DMSO (10 mL) while cooling on ice. After stirring at room temperature for 6 h, water was added and the resulting precipitate was filtered to give 4-bromo-*N*-ethyl-2-(methylsulfanyl) benzamide as a pale yellow solid (1.77 g, 79% yield). Sulfuryl dichloride (0.52 g, 3.8 mmol) was added to a solution of this compound (1.0 g, 3.6 mmol) in toluene (3 mL) at room temperature and the mixture was stirred at 70–80 °C for 1 h. The reaction mixture was cooled to room temperature, and the resulting precipitate was filtered and washed with toluene to give the product as a white solid (0.21 g, 22% yield). ^{1}H NMR ($CDCl_3$) δ ppm 1.37 (3H, t, J = 7.2 Hz), 3.93 (2H, q, J = 7.2 Hz), 7.51 (1H, dd, J = 8.2, 1.6 Hz), 7.72 (1H, d, J = 1.6 Hz), 7.88 (1H, d, J = 8.2 Hz); MS (APCI/ESI) *m/z* $[M + H]^+$ 258, 260.

5.1.21. Ethyl 6-bromo-3-oxo-2,3-dihydro-1H-indazole-1-carboxylate (15)

Concentrated hydrochloric acid (1.4 mL, 17 mmol) was added to a suspension of **14** (1.0 g, 4.3 mmol) in H_2O (6.7 mL) while cooling on ice. Sodium nitrite (0.30 g, 4.4 mmol) in H_2O (1.1 mL) was subsequently added to the mixture at the same temperature. After stirring at room temperature for 1 h, disodium sulfite (1.5 g, 12 mmol) in H_2O (6.7 mL) was added and the mixture was stirred at room temperature for 2 h. Concentrated hydrochloric acid (2.2 mL, 27 mmol) was added and the mixture was stirred overnight at room temperature and then for an additional 18 h at 80 °C. After cooling to room temperature, the mixture was neutralized with aqueous NaOH solution and the resulting precipitate was filtered and washed with $CHCl_3$ to give 6-bromo-1,2-dihydro-3*H*-indazol-3-one as a brown solid (0.47 g, 50% yield). Ethyl carbonochloridate (0.46 g, 4.2 mmol) was added to a solution of this compound (0.45 g, 2.1 mmol) in pyridine (1.8 mL) while cooling on ice, and the mixture was stirred at 110 °C for 2 h. The reaction mixture was concentrated under reduced pressure, water was added and the resulting precipitate was filtered and washed with water to give the product as a solid (0.50 g, 83% yield). ^{1}H NMR (DMSO-d_6) δ ppm 1.36 (3H, t, J = 7.1 Hz), 4.42 (2H, q, J = 7.2 Hz), 7.51 (1H, dd, J = 8.5, 1.7 Hz), 7.68–7.72 (1H, m), 8.18–8.21 (1H, m), 12.29 (1H, br s); MS (ESI) *m/z* $[M + H]^+$ 287.

5.1.22. Ethyl 6-bromo-2-ethyl-3-oxo-2,3-dihydro-1H-indazole-1-carboxylate (16)

Potassium hydroxide (0.12 g, 2.1 mmol) in EtOH (1.0 mL) was added to a solution of **15** (0.49 g, 1.7 mmol) in EtOH (2.0 mL) at room temperature. After stirring at the same temperature for 30 min, iodoethane (0.66 g, 4.3 mmol) was added and the mixture was stirred at 100 °C for 6 h. The mixture was evaporated *in vacuo* and the crude mixture was purified using silica-gel column chromatography ($CHCl_3$/EtOAc = 9:1) to give the product (0.14 g, 26% yield, lower Rf value by TLC [SiO_2, CH_2Cl_2- EtOAc])[15] and ethyl 6-bromo-3-ethoxy-1*H*-indazole-1-carboxylate as a regioisomer (0.18 g, 34% yield, higher Rf value by TLC [SiO_2, CH_2Cl_2- EtOAc], data not shown). ^{1}H NMR ($CDCl_3$) δ ppm 1.19 (3H, t, J = 7.1 Hz), 1.48 (3H, t, J = 7.2 Hz), 4.26 (2H, q, J = 7.1 Hz), 4.49 (2H, q, J = 7.1 Hz), 7.46 (1H, dd, J = 8.2, 1.5 Hz), 7.73 (1H, d, J = 8.4 Hz), 8.12 (1H, d, J = 1.3 Hz); MS (ESI) *m/z* $[M + H]^+$ 313.

5.1.23. Ethyl 2-ethyl-6-{8-[(4′-fluoro[1,1′-biphenyl]-4-yl)methoxy]-2-methylimidazo[1,2-a]pyridin-3-yl}-3-oxo-2,3-dihydro-1H-indazole-1-carboxylate (17)

Compound **17** was prepared from **16** at quantitative yield using a method similar to that described for **1i** without salification. ^{1}H NMR ($CDCl_3$) δ ppm 1.24 (3H, t, J = 7.1 Hz), 1.44 (3H, t, J = 7.1 Hz), 3.70 (3H, s), 4.32 (2H, q, J = 7.1 Hz), 4.47 (2H, q, J = 7.1 Hz), 5.42 (2H, s), 6.51–6.55 (1H, m), 6.62 (1H, t, J = 7.0 Hz), 7.09–7.16 (2H, m), 7.35–7.75 (7H, m), 7.82 (1H, dd, J = 6.8, 0.7 Hz), 8.01 (1H, d, J = 8.4 Hz), 8.02–8.04 (1H, m); MS (ESI) *m/z* $[M + H]^+$ 565.

5.1.24. 5-[8-(Benzyloxy)-2-methylimidazo[1,2-a]pyridin-3-yl]-N-methylpyridine-2-carboxamide (19)

A mixture of **18** (1.0 g, 2.7 mmol) and methanamine (9.8 mol/L in MeOH; 1.5 mL, 15 mmol) in MeOH (10 mL) was stirred at 60 °C for 15 h and allowed to cool to room temperature. The mixture was evaporated *in vacuo* and the crude mixture was purified using silica-gel column chromatography ($CHCl_3$/MeOH = 1:0 to 24:1) to give the product as a white solid (0.95 g, 95% yield). ^{1}H NMR ($CDCl_3$) δ ppm 2.53 (3H, s), 3.08 (3H, d, J = 5.0 Hz), 5.38 (2H, s), 6.51 (1H, d, J = 7.3 Hz), 6.63 (1H, t, J = 7.2 Hz), 7.30–7.40 (3H, m), 7.49–7.52 (2H, m), 7.68 (1H, dd, J = 6.9, 0.8 Hz), 7.95 (1H, dd, J = 8.0, 2.2 Hz), 7.98–8.05 (1H, m), 8.35 (1H, dd, J = 8.1, 0.8 Hz), 8.65 (1H, dd, J = 2.1, 0.8 Hz); MS (ESI) *m/z* $[M + H]^+$ 373.

5.1.25. 5-(8-Hydroxy-2-methylimidazo[1,2-a]pyridin-3-yl)-N-methylpyridine-2-carboxamide (20)

Compound **20** was prepared from **19** at 67% yield as a pale yellow solid using a method similar to that described for **7**. ^{1}H NMR ($CDCl_3$) δ ppm 2.55 (3H, s), 3.10 (3H, d, J = 5.0 Hz), 6.94–7.01 (2H, m), 7.69 (1H, dd, J = 6.0, 1.5 Hz), 7.96–8.04 (2H, m), 8.40–8.44 (1H, m), 8.66–8.69 (1H, m); MS (ESI) *m/z* $[M + H]^+$ 283.

5.1.26. 6-Bromo-2-methyl-1,2-benzoxazol-3(2H)-one (21)

A mixture of **10** (0.30 g, 1.4 mmol), *N*-ethyl-*N*-(propan-2-yl)propan-2-amine (0.40 g, 3.1 mmol) and iodomethane (0.70 g, 4.9 mmol) in $CHCl_3$ (3.0 mL) was stirred at 60 °C overnight and allowed to cool to room temperature. The mixture was evaporated *in vacuo* and the crude mixture was purified using silica-gel column chromatography (*n*-hexane/EtOAc = 2:1) to give the product as a colorless solid (0.24 g, 75% yield). ^{1}H NMR (DMSO-d_6) δ ppm 3.58 (3H, s), 7.52 (1H, dd, J = 8.2, 1.5 Hz), 7.71–7.74 (1H, m), 7.89–7.91 (1H, m); MS (ESI) *m/z* $[M + H]^+$ 228.

5.2. Biology

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. Furthermore, Astellas Pharma Inc., Tsukuba Research Center has been awarded Accreditation Status by the AAALAC International.

5.2.1. Cellular Aβ assay

The human neuroblastoma cell line SK-*N*-BE (2) was maintained in

RPMI1640 medium supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cells were cultured in 96-well plates overnight and subsequently treated with different concentrations of each drug in 0.5% dimethyl sulfoxide for 6 h. The next day, Aβ1-42 levels in media were measured using a 384-well plate-based sandwich enzyme-linked immunosorbent assay (ELISA) system and the 44A3 anti-Aβ42 monoclonal antibody (IBL, Gunma, Japan), a biotin-labeled 82E1 anti-Aβ *N*-terminal monoclonal antibody (IBL), streptavidin-horseradish peroxidase conjugate (Invitrogen, Carlsbad, CA, USA), and TMB as the chromogen. Aβ1-x (total Aβ) levels in media were measured using an ELISA kit (Wako, Osaka, Japan). Values are shown as the mean of two wells.

5.2.2. Liver microsomal stability in vitro

To estimate the metabolic stability of compounds against rat and mouse hepatic CYPs, the test compound (0.2 μM) was incubated with pooled rat and mouse liver microsomes (0.2 mg protein/mL), NADPH (1 mM) and EDTA (0.1 mM) in pH 7.4 Na^+-K^+ phosphate buffer (100 mM) at 37 °C. Incubations were conducted for 0, 15, 30, and 45 min. The peak area of the compound and internal standard was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and analyzed to calculate CL_{int} (mL/min/kg). Values are shown as the mean of duplicate experiments.

5.2.3. Pharmacokinetic studies using rats

Compound **1a, 1m**, **1n**, or **1o** (10 or 30 mg/kg) suspended in a 0.5% (w/v) methylcellulose solution was orally administered to nonfasted 6-week-old male SD rats (SLC, Shizuoka, Japan, n = 2 or 3). After sample collection, acetonitrile was used to precipitate proteins from plasma and brain homogenate samples, and the samples were subjected to LC-MS/MS. Values are shown as the mean concentration from two or three rats.

5.2.4. Aβ reduction in rats

Compounds **1a–1c**, **1e**, **1f**, **1h**, **1i**, and **1m–1o** (10 or 30 mg/kg) were suspended in 0.5% (w/v) methylcellulose and each orally administered to nonfasted 6-week-old male SD rats (SLC, Shizuoka, Japan, n = 4 or 5). Blood and brains were collected under isoflurane anesthesia 3 h after oral administration, and the hippocampi were isolated and stored at –80 °C. Each frozen hippocampus was homogenized using an ultrasonic homogenizer in a 10-fold volume of TBS (Tris 25 mM, NaCl 137 mM, KCl 2.68 mM; pH 7.4) containing Complete Protease Inhibitor Cocktail on ice, and the mixture was ultracentrifuged at 100,000*g* at 4 °C for 1 h. Aβ levels in the supernatant and in plasma samples were measured using the Aβ x-42 and Aβ x-40 ELISA kits (Wako). Values are shown as the mean. Student's *t*-test was conducted using GraphPad Prism version 7.0 (GraphPad Software).

5.2.5. Y-maze test

Spatial working memory in mice was evaluated by recording spontaneous alternation behavior in the Y-maze test.[11] The maze was made of gray polyvinylchloride, with three arms converging at equal angles. Arm dimensions were length, 40 cm; height, 13 cm; and width at the bottom and top, 3 and 10 cm, respectively. Compound **1o** (0.6, 2.0, 6.0 or 20 mg/kg) was suspended in 0.5% methyl cellulose and orally administered to nonfasted 6-month-old female Tg2576 mice (n = 5 or 6) once daily for 8 days. Three hours after administration on day 8, each animal was placed at the end of one arm and allowed to freely explore the apparatus for 8 min. The sequence and number of all arm entries were recorded for each animal throughout the period. Sequence triads, in which all three arms were represented (ABC, ACB, BAC, BCA, CAB, and CBA), were calculated as successful alternations to evaluate normal cognition and working memory of the last arm entered. Alternation rate was defined as entries into all three arms on consecutive occasions using the following formula: Alternation rate (%) = Number of alternations/(Number of total arm entries –2) × 100. Data were excluded in cases in which the number of total arm entries was < 10.

Table 6
Summary of assay conditions for different CYP isoforms.

CYP isoform	Protein conc. (pmol/well)	Substrate	Substrate conc. (μM)
1A2	0.026	CEC	5
2C9	0.25	MFC	75
2C19	0.19	CEC	25
2D6	0.20	AMMC	1.5

CEC, 3-cyano-7-ethoxycoumarin; MFC, 7-methoxy-4-trifluoromethylcoumarin; AMMC, 3-[2-(*N*,*N*-diethyl-*N*-methyl amino)-ethyl]-7-methoxy-4-methylcoumarin.

5.2.6. Measurement of CYP inhibition

Inhibitory activities of the test compounds against CYP1A2, 2C9, 2C19 and 2D6 were determined using a fluorescence-based assay. Reaction mixtures containing recombinant human CYP protein, cofactors, fluorogenic substrates, a test compound (0.31–20 μM) and potassium phosphate buffer (pH 7.4) were prepared. Fluorogenic substrates and assay conditions for the different CYP isoforms are summarized in Table 6. Reactions were initiated by incubating at 37 °C. Incubation times for each isoform were as follows: 20 min for CYP1A2, 30 min for 2C9, 20 min for 2C19 and 20 min for 2D6. After the incubation, the reactions were terminated by addition of stop solution (20% 0.5 M Tris base [2-amino-2-hydroxymethyl-1,3-propanediol], 80% acetonitrile). Fluorescence was measured to quantify metabolite formation, and the IC_{50} was determined. Values are shown as the mean of duplicate experiments.

Midazolam was used as a probe substrate in the CYP3A4 inhibition assays. The assay cocktail included the probe substrates phenacetin, diclofenac, S-mephenytoin, dextromethorphan, or midazolam. Reaction mixtures containing 0.1 mg protein/mL of human liver microsomes (HLMs), 1 mM NADPH, 0.1 mM EDTA, 100 mM Na^+-K^+ phosphate buffer (pH 7.4) and 5 μM test compound were prepared and pre-incubated for 0 or 30 min at 37 °C. Reactions were initiated by adding 1.5 μM midazolam, followed by incubation for 20 min, and terminated by adding 80% acetonitrile containing the internal standard. The peak area of 1′-hydroxymidazolam and the internal standard was measured using LC-MS/MS. Residual metabolic activity for reversible (Eq. (1)) and time-dependent (Eq. (2)) inhibition was calculated as follows:

$$\text{Residual activity}(\%) = A_{c,0}/A_{v,0} \times 100 \quad (1)$$

$$\text{Residual activity}(\%) = (A_{c,30}/A_{v,30})/(A_{c,0}/A_{v,0}) \times 100 \quad (2)$$

where $A_{c,\ 0}$ denotes the peak area ratio in the presence of a test compound without preincubation, $A_{v,\ 0}$ denotes the peak area ratio in the absence of a test compound without preincubation, $A_{c,\ 30}$ denotes the peak area ratio in the presence of a test compound with preincubation, and $A_{v,\ 30}$ denotes the peak area ratio in the absence of a test compound with preincubation. Values are shown as the mean of duplicate experiments.

Declaration of Competing Interest

None.

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20. Significant and dose-dependent decrease in brain Aβ42 levels was confirmed; Reduction in brain Aβ42 levels is 5%, 21% ** and 35% ** at 2.0, 6.0 and 20 mg/kg po, respectively. Values are shown as the mean. ** $p < 0.01$ vs. vehicle group by Student's t-test ($n = 5$ or 6). Additionally, PK data of 1o in mice, although not measured, is expected to better than that of rats, similarly to PD, because compound 1o is an analogue of compound 1a, which has an excellent PK/PD correlation, as mentioned in this paper and reported previously.9.