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Article

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Identification of a Selective, Non-Prostanoid EP2 Receptor Agonist for the Treatment of Glaucoma: Omidenepag and its Prodrug Omidenepag Isopropyl

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ABSTRACT: EP2 receptor agonists are expected to be effective ocular hypotensive agents; however, it has been suggested that agonism to other EP receptor subtypes may lead to undesirable effects. Through medicinal chemistry efforts, we identified a scaffold bearing a (pyridin-2-ylamino)acetic acid moiety as a promising EP2-selective receptor agonist. (6-((4-(Pyrazol-1-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic acid **13ax**

(omidenepag, OMD) exerted potent and selective activity toward the human EP2 receptor (h-EP2). Low doses of omidenepag isopropyl (OMDI), a prodrug of **13ax**, lowered intraocular pressure (IOP) in ocular normotensive monkeys. OMDI was selected as a clinical candidate for the treatment of glaucoma.

■ INTRODUCTION

Prostaglandin E_2 (PGE_2) is a highly bioactive molecule and is associated with various biological responses, including inflammation, osteoclastic bone resorption, labor, smooth muscle relaxation, and intraocular pressure (IOP) reduction,[1–3] mediated by signaling via four EP receptor subtypes (EP1, EP2, EP3, and EP4).[4,5] Prostaglandin analogs, such as latanoprost, and their active acid forms are selective FP receptor (prostaglandin $F_{2\alpha}$ receptor) agonists and one of the most potent IOP-lowering agents for the treatment of glaucoma. However, IOP-lowering agents with a different, new mechanism of action (MOA) are desired to be developed because concomitant treatment can further reduce IOP[6,7] and be effective in low/non-responders to FP receptor agonists[8] or when local adverse reactions associated with FP receptor agonists, such as iris/eyelid pigmentation and the deepening of upper eyelid sulcus, affect compliance.[9] Ocular hypotensive agents with new, different MOA from that of FP receptor agonists are expected to overcome problems associated with FP receptor agonists as stated above. EP2 receptor agonists, such as butaprost (in its free acid form), have been reported to be selective for the EP2 receptor among EP receptor subtypes[10,11] and are reported to lower IOP; therefore, they are expected to be beneficial for the treatment of glaucoma[12,13] with better safety profiles than non-selective EP receptor agonists and different safety profiles from FP receptor agonists. Thus, we explored EP2

receptor agonists with potent activity and high selectivity to identify a safe compound that is capable of lowering IOP for the treatment of glaucoma.

CP-533,536[14–16] was selected as the starting compound for the medicinal chemistry efforts because: (1) it has been reported as a selective EP2 receptor agonist,[15]

(2) it was expected to be safe, since a clinical trial studying its effectiveness for fracture healing was under way,[17] and

(3) it was presumed that various structural changes could be more accessible than prostanoid since it has a non-prostanoid structure (Figure 1).

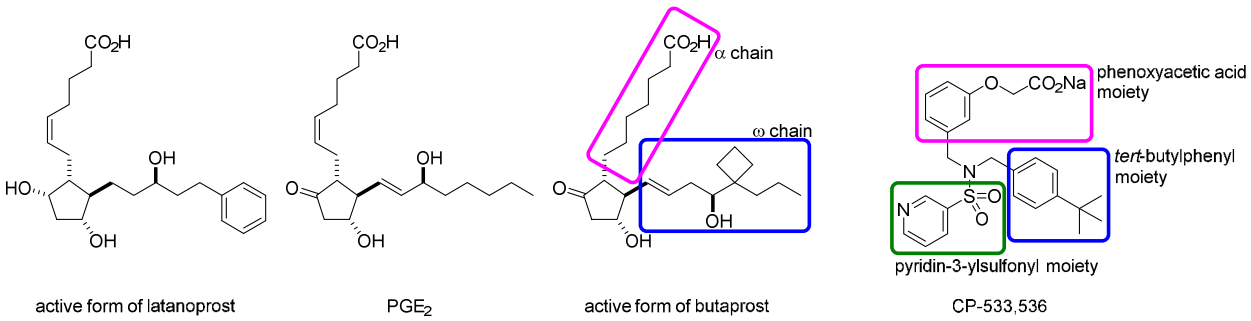


Figure 1. Structures of prostanoids and CP-533,536.

Since CP-533,536 contains a sulfonamide group in the center of the molecule, the structures of the phenoxyacetic acid moiety, the pyridin-3-ylsulfonyl moiety, and the *tert*-butylphenyl moiety can be independently changed. Retrosynthetic analysis indicated that the burden of structural change of the phenoxyacetic acid moiety is greater than that of the pyridin-3-ylsulfonyl and *tert*-butylphenyl moieties (Figure 2). However, the following was considered in terms of activities: In the structural comparison between CP-533,536 and butaprost, the phenoxyacetic acid and *tert*-butylphenyl moieties of CP-533,536 were estimated to correspond to the α and ω chain moieties,

respectively, of prostaglandins (Figure 1). Because the ω chain moiety of prostaglandins has been reported to be derivatized to various structures,[18–23] the *tert*-butylphenyl moiety of CP-533,536 was also predicted to be transformable into various structures. However, we hypothesized that the transformation of the phenoxyacetic acid moiety is likely to exert a large effect on *in vitro* activity because it is a critical substructure containing a carboxy group potentially involved in enthalpy-driven binding.[24,25]

On the basis of these presumptions, our medicinal chemistry strategy involved the following two steps (Figure 2):

STEP 1 Explore chemotypes with activities higher than that of the starting compound by transforming the phenoxyacetic acid moiety of CP-533,536 into a novel substructure.

STEP 2 Transform the pyridin-3-ylsulfonyl and *tert*-butylphenyl moieties using chemotype(s) containing a highly active novel substructure(s) identified in STEP 1, and optimize the structure.

The attainment of STEP 1 objective was a key to this strategy to produce novel compounds with high activities and enabled the *tert*-butylphenyl moiety to be transformed into a wide range of structures. Herein, we describe the synthesis and detailed structure−activity relationship (SAR) leading to the discovery of omidenepag (OMD, **13ax**) and omidenepag isopropyl (OMDI).

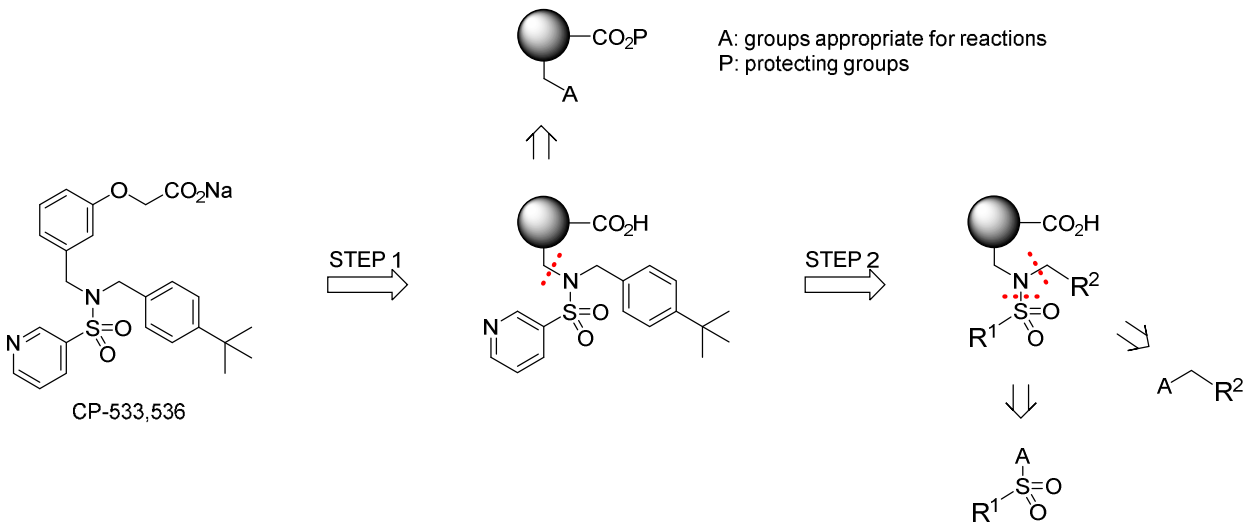
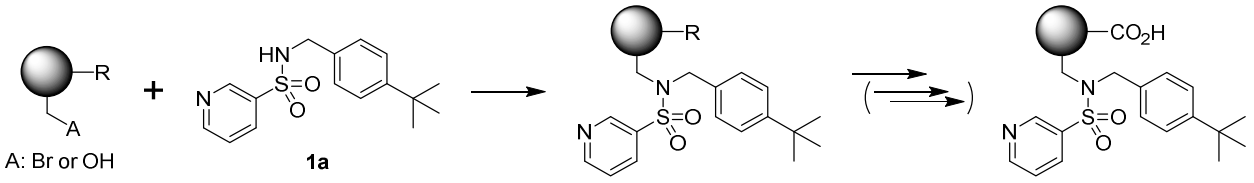


Figure 2. Medicinal chemistry strategy adopted in this study and retrosynthetic analysis.

■ RESULTS AND DISCUSSION

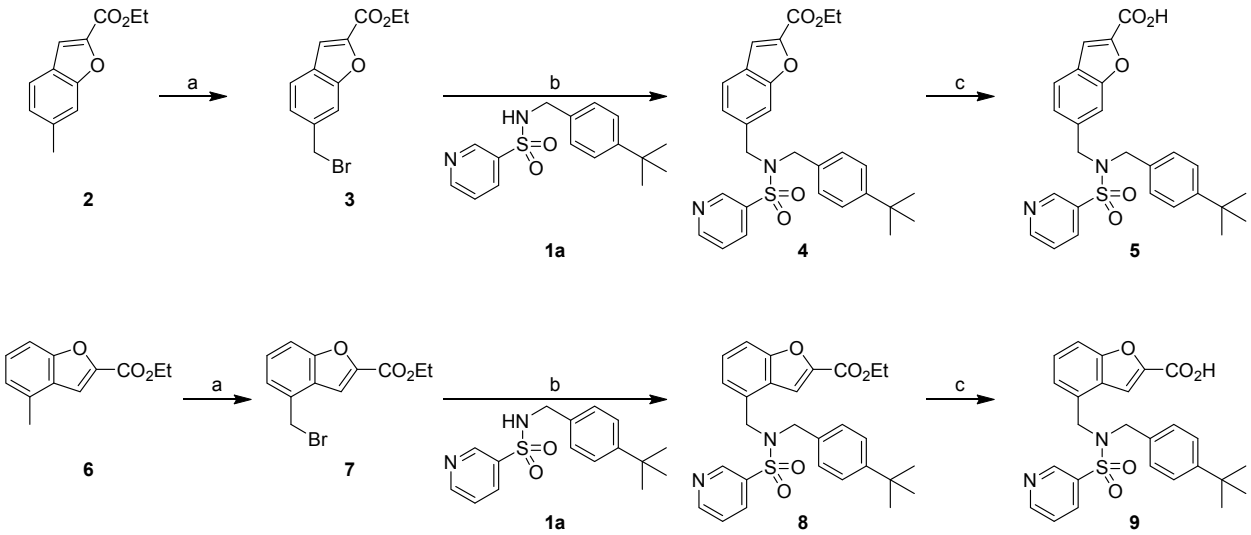
Chemistry. For exploring the phenoxyacetic acid moiety (STEP 1), the synthesis was basically conducted through the route shown in Scheme 1. By reaction of compound **1a**[26] as the common intermediate with the building block of the phenoxyacetic acid moiety corresponding to each compound and inducing the phenoxyacetic acid moiety as appropriate, target compounds were obtained. Specific routes of synthesis for each compound are shown in Scheme 2–11.

Scheme 1. General Synthetic Route to Explore the Phenoxyacetic Acid Moiety (STEP 1)



The benzofuryl derivatives **5** and **9** were obtained through the synthetic routes shown in Scheme 2. Ethyl 6-methylbenzofuran-2-carboxylate (**2**) and ethyl 4-methylbenzofuran-2-carboxylate (**6**) were converted to compounds **3** and **7**, respectively, through the bromination of the benzylic methyl group by a photo-induced radical reaction (step a); compounds **3** and **7** were converted to precursor compounds **4** and **8**, respectively, by reaction with compound **1a** under basic conditions (step b); and compounds **4** and **8** were converted to compounds **5** and **9**, respectively, through basic hydrolysis (step c).

Scheme 2. Synthetic Routes for Benzofuryl Derivatives 5 and 9[a]

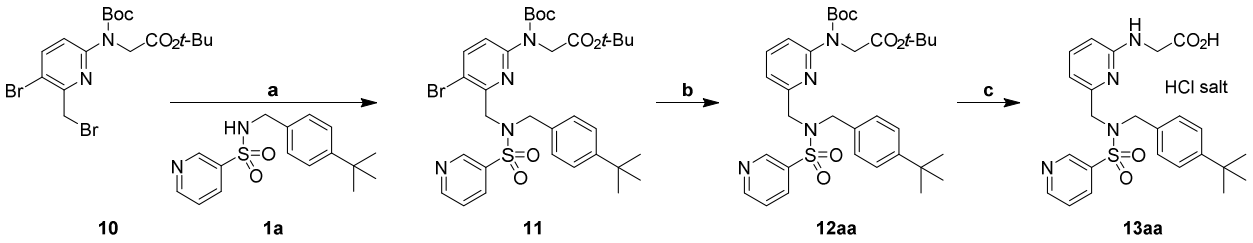


[a]Reagents and conditions: (a) NBS, *hv*, 1,2-dichloroethane, 0.5–1 h, 92% for **3** and 86% for **7**; (b) K_2CO_3, DMF, rt, 5 h to overnight, 83% for **4** and 84% for **8**; (c) NaOH, EtOH/H_2O/THF for **5** and EtOH/H_2O for **9**, rt, 3 h, 58% for **5** and 62% for **9**.

Compound **13aa**, one of the aminopyridyl derivatives, was obtained through the synthetic route shown in Scheme 3.[26] Compound **10**[26–28] was converted by reaction with compound **1a** under basic conditions to compound **11** (step a), which was then converted to the precursor

compound **12aa** by hydrogenolysis (step b). The precursor compound **12aa** was converted to aminopyridyl derivative **13aa** by acidic deprotection (step c).

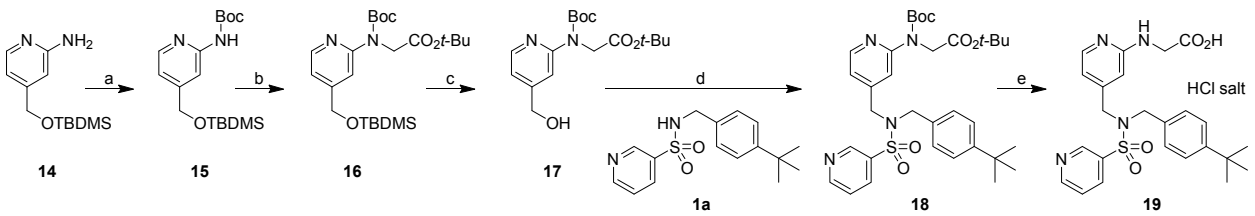
Scheme 3. Synthetic Route for Aminopyridyl Derivative 13aa[a]



[a]Reagents and conditions: (a) K_2CO_3, DMF, rt, 3.5 h, 85%; (b) Pd-C, H_2, Et_3N, EtOH, rt, 1 h, 31%; (c) HCl, CH_2Cl_2/1,4-dioxane, rt, 16 h, quantitative yield.

Compound **19**, a regioisomer of aminopyridyl derivative **13aa,** was obtained through the synthetic route shown in Scheme 4. Compound **15**, which was obtained by protecting the amino group of compound **14** with a *tert*-butoxycarbonyl (Boc) group (step a), was converted by reaction with *tert*-butyl 2-bromoacetate under basic conditions to compound **16** (step b). After the deprotection of the *tert*-butyldimethylsilyl (TBDMS) group (step c), compound **17** was obtained. Compound **17** was converted by reaction with compound **1a** under Mitsunobu reaction conditions to the precursor compound **18** (step d), which was then converted to compound **19** by acidic deprotection (step e).

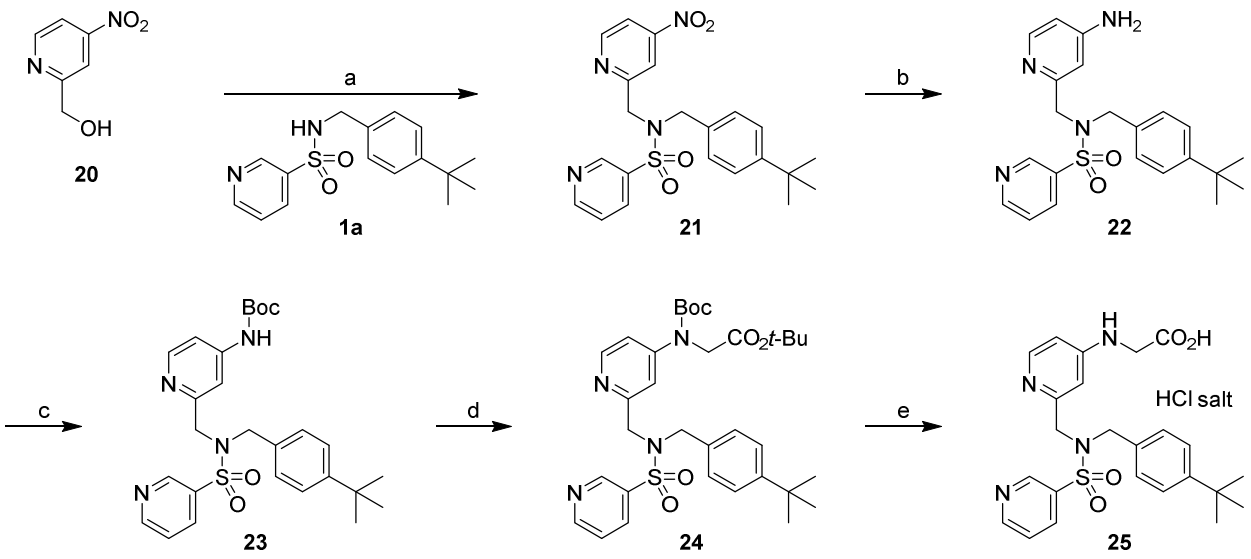
Scheme 4. Synthetic Route for Compound 19[a]



[a]Reagents and conditions: (a) Boc_2O, pyridine/*t*-BuOH/AcOEt, rt, overnight, 62%; (b) (i) **15**, NaH, DMF, rt → 0 °C, 20 min, (ii) $BrCH_2CO_2t$-Bu, 0 °C → rt, 6 h, 87%; (c) TBAF, THF, 0 °C → rt, 4.5 h, 96%; (d) TMAD, P(*n*-Bu)$_3$, THF, rt, overnight, 78%; (e) HCl, CH_2Cl_2/1,4-dioxane, rt, overnight, quantitative yield.

Compound **25**, the other regioisomer of aminopyridyl derivative **13aa**, was obtained through the synthetic route shown in Scheme 5. (4-Nitropyridin-2-yl)methanol (**20**) was converted by reaction with compound **1a** under Mitsunobu reaction conditions to compound **21** (step a). After the subsequent reduction of the nitro group (step b) and the substitution of the amino group with a Boc group and then with an acetic acid *tert*-butyl ester under basic conditions (steps c and d), the precursor compound **24** was obtained, which was converted to compound **25** by acidic deprotection (step e).

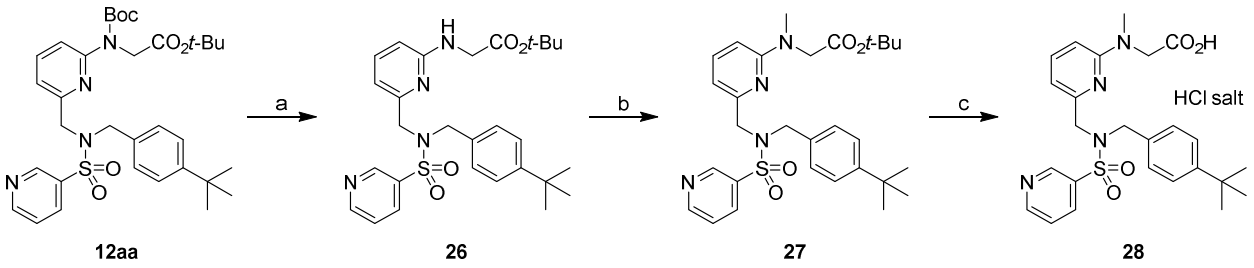
Scheme 5. Synthetic Route for Compound 25[a]



[a]Reagents and conditions: (a) TMAD, P(*n*-Bu)$_3$, THF, rt, 4 h, 94%; (b) Zn dust, CH_3CO_2H, 60 °C, 1 h 20 min, 98%; (c) (i) **22**, NaHMDS, THF, 0 °C, 10 min, (ii) Boc_2O, 0 °C, 1 h 20 min, 28%; (d) (i) **23**, NaH, DMF, 0 °C → rt, 30 min, (ii) $BrCH_2CO_2t$-Bu, 0 °C → rt, 1 h, 76%; (e) HCl, CH_2Cl_2/1,4-dioxane, rt, overnight, 88%.

Compound **28**, with an *N*-methyl-*N*-(pyridin-2-yl)aminoacetic acid substructure corresponding to the compound obtained by methylating the amino nitrogen atom in the aminopyridyl moiety of aminopyridyl derivative **13aa**, was obtained through the synthetic route shown in Scheme 6. Compound **12aa** (the precursor compound of aminopyridyl derivative **13aa**), after the deprotection of the Boc group at the amino nitrogen atom by reaction with *para*-toluenesulfonic acid monohydrate (PTSA•H_2O) in ethanol, formed compound **26** (step a), which was then methylated at the amino nitrogen atom by reaction with methyl iodide under basic conditions to give compound **27** (step b), which was converted to compound **28** by acidic deprotection (step c).

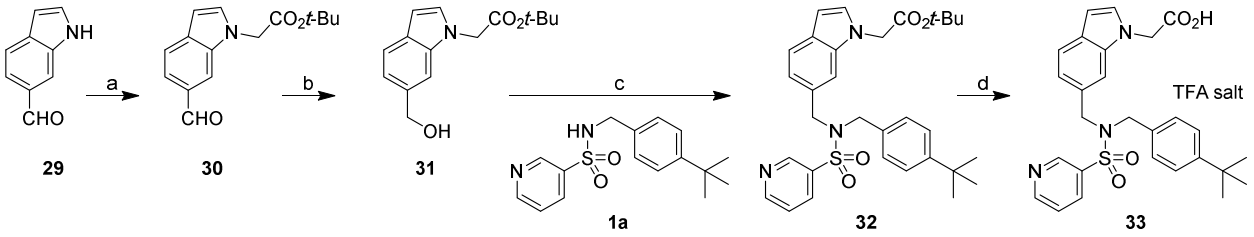
Scheme 6. Synthetic Route for Compound 28[a]



[a]Reagents and conditions: (a) PTSA•H_2O, EtOH, 60 °C → 65 °C, 16.5 h, 82%; (b) (i) **26**, NaH, DMF, 0 °C → rt, 15 min, (ii) MeI, 0 °C → rt, 1 h, 32%; (c) HCl, CH_2Cl_2/1,4-dioxane, rt, overnight, quantitative yield.

The indolyl derivative **33** with a (1*H*-indol-1-yl)acetic acid substructure was obtained through the synthetic route shown in Scheme 7. 1*H*-indole-6-carbaldehyde (**29**) was converted by reaction with *tert*-butyl 2-bromoacetate under basic conditions to compound **30** (step a), which was then reduced at the aldehyde group by reaction with $NaBH_4$ to give compound **31** (step b). Compound **31** was converted to the indolyl derivative **33** (steps c and d) according to the procedures similar to those of steps d and e in Scheme 4.

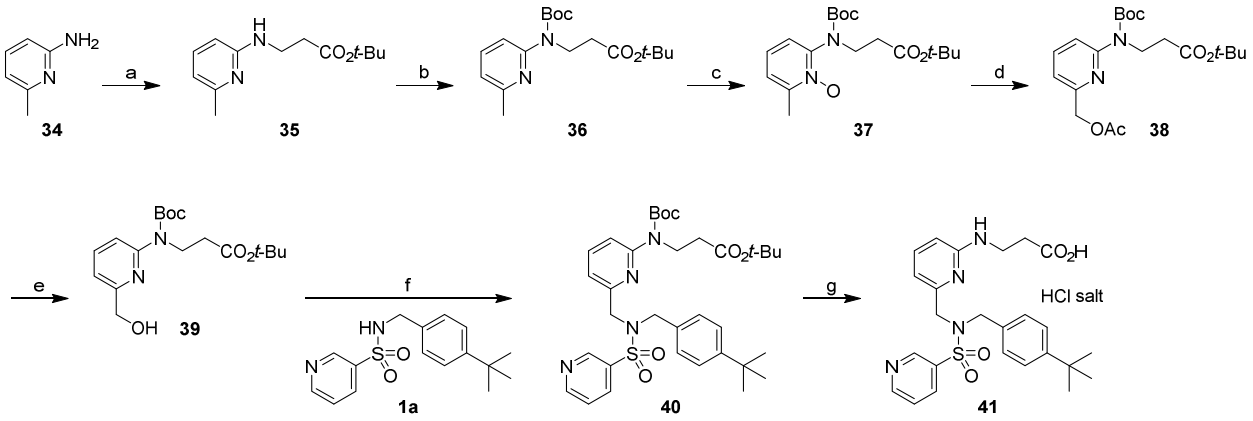
Scheme 7. Synthetic Route for Indolyl Derivative 33[a]



[a]Reagents and conditions: (a) (i) **29**, NaH, DMF, 0 °C, (ii) $BrCH_2CO_2t$-Bu, 0 °C → rt, overnight, 91%; (b) $NaBH_4$, EtOH, rt, 1 h, 85%; (c) TMAD, P(*n*-Bu)$_3$, THF, rt, overnight, 78%; (d) HCl, CH_2Cl_2/1,4-dioxane, rt, overnight, 9.9%.

Compound **41**, with a 3-(pyridin-2-ylamino)propionic acid substructure having one more carbon atom in its carbon chain than the aminopyridyl derivative **13aa**, was obtained through the synthetic route shown in Scheme 8. The amino nitrogen of 6-methylpyridin-2-amine (**34**) was substituted with a propionic acid *tert*-butyl ester and then with a Boc group to give compound **36** (steps a and b). Compound **36** was oxidized at the pyridyl nitrogen atom by reaction with *meta*-chloroperoxybenzoic acid (*m*-CPBA) to give compound **37** (step c), which was converted to compound **38** through a rearrangement reaction (step d) and then converted to compound **39** through the hydrolysis of the acetoxy group (step e). Compound **39** was converted to compound **41** (steps f and g) according to the procedures similar to those of steps d and e in Scheme 4.

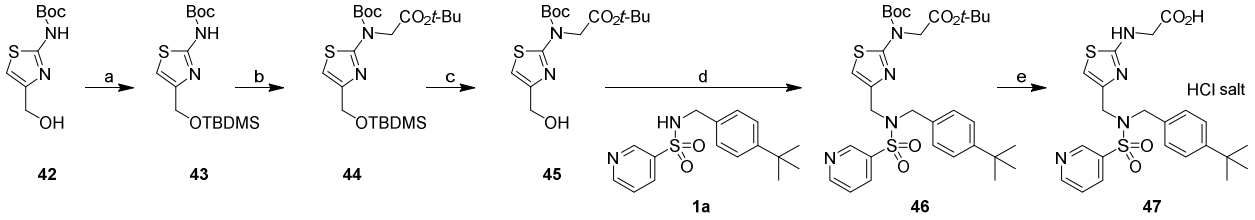
Scheme 8. Synthetic Route for Compound 41[a]



[a]Reagents and conditions: (a) CH_2=$CHCO_2t$-Bu, 2,5-di-*tert*-butylbenzene-1,4-diol, 100 °C, 2 d, 11%; (b) Boc_2O, DMAP, *N*,*N*-diisopropylethylamine, *t*-BuOH, rt, overnight, 50%; (c) *m*-CPBA, CH_2Cl_2, rt, 4.5 h, quantitative yield; (d) Ac_2O, 110 °C, 1 h, 66%; (e) LiOH•H_2O, THF/H_2O, 60 °C, 13.5 h, 85%; (f) TMAD, P(*n*-Bu)$_3$, THF, rt, 2 h, 86%; (g) HCl, CH_2Cl_2/1,4-dioxane, rt, overnight, 75%.

Compound **47** was obtained according to the procedure similar to that for compound **19** described in Scheme 4.

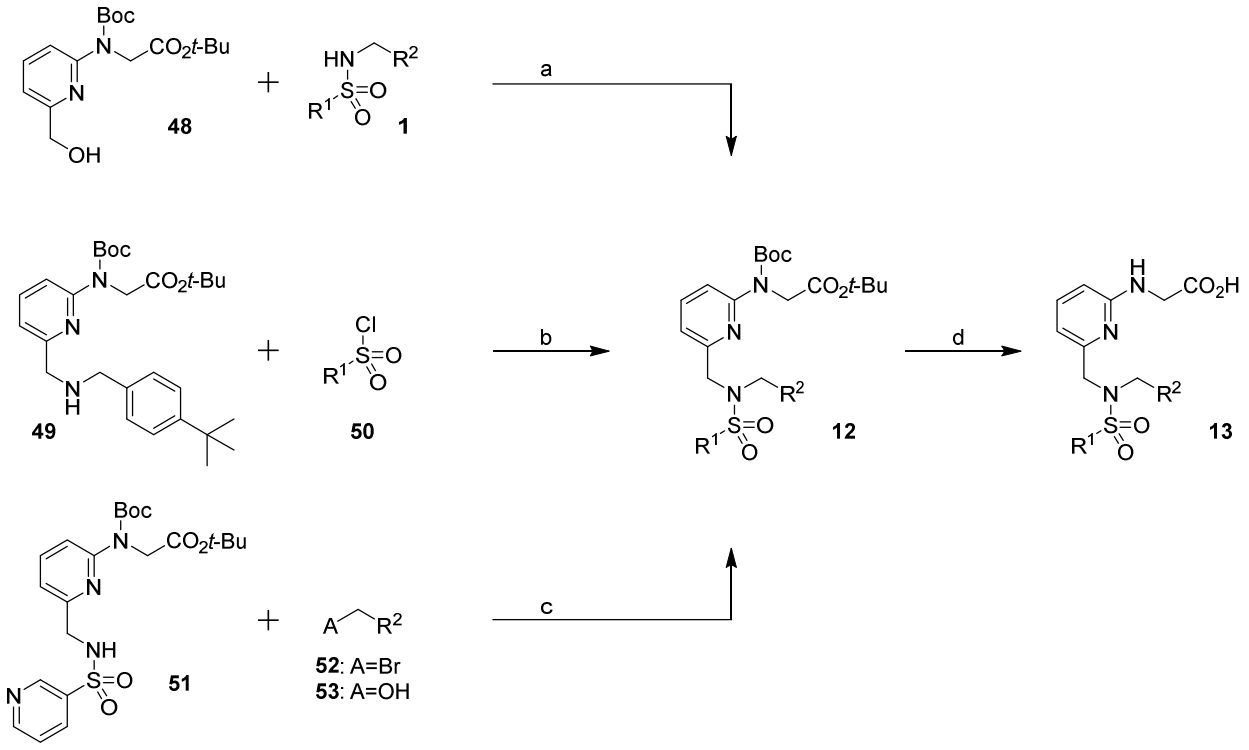
Scheme 9. Synthetic Route for Compound 47[a]



[a]Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt, overnight, 99%; (b) $BrCH_2CO_2t$-Bu, K_2CO_3, DMF, rt, 1 h, 86%; (c) TBAF, THF, 0 °C → rt, 4 h, 96%; (d) TMAD, P(*n*-Bu)$_3$, THF, rt, 2 h, 81%; (e) HCl, CH_2Cl_2/1,4-dioxane, rt, overnight, 90%.

For the exploration of the pyridin-3-ylsulfonyl and *tert*-butylphenyl moieties, the synthesis of aminopyridyl derivatives represented by general formula **13** were efficiently conducted through the routes shown in Scheme 10 and 11 using key intermediates, such as *tert*-butyl (*tert*-butoxycarbonyl(6-(hydroxymethyl)pyridin-2-yl)amino)acetate (**48**), *tert*-butyl (*tert*-butoxycarbonyl(6-((4-*tert*-butylbenzyl)aminomethyl)pyridin-2-yl)amino)acetate (**49**), and *tert*-butyl (*tert*-butoxycarbonyl(6-((pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (**51**).[26–28] Precursor compounds **12** obtained through synthetic steps a, b, or c were converted to aminopyridyl derivatives **13** by acidic deprotection (step d). Compound **48** was converted to precursor compound **12** by reaction with compound **1** under Mitsunobu reaction conditions (step a). Compound **49** was converted to precursor compound **12** by reaction with sulfonyl chloride **50** under basic conditions (step b). Compound **51** was converted to precursor compound **12** by reaction with compound **52** under basic conditions or with compound **53** under Mitsunobu reaction conditions (step c). The synthetic steps to produce each aminopyridyl derivative are shown in Table 1.

Scheme 10. General Synthetic Routes for Aminopyridyl Derivatives 13[a]



[a]Reagents and conditions: (a) TMAD, P(*n*-Bu)$_3$, THF, rt; (b) Et_3N, CH_2Cl_2, rt; (c with **52**) (i) **51**, NaH, DMF, 0 °C → rt, (ii) **52**, 0 °C → rt; (c with **53**) TMAD, P(*n*-Bu)$_3$, THF, rt; (d) HCl, CH_2Cl_2/1,4-dioxane, rt.

Table 1. Synthetic Routes for Aminopyridyl Derivatives 13ab–ba

compound	R^1	R^2	form	synthetic route[a]
13ab	methyl	4-*tert*-butylphenyl	HCl salt	a, d
13ac	phenyl	4-*tert*-butylphenyl	HCl salt	a, d
13ad	pyridin-2-yl	4-*tert*-butylphenyl	HCl salt	a, d
13ae	2-fluorophenyl	4-*tert*-butylphenyl	HCl salt	b, d

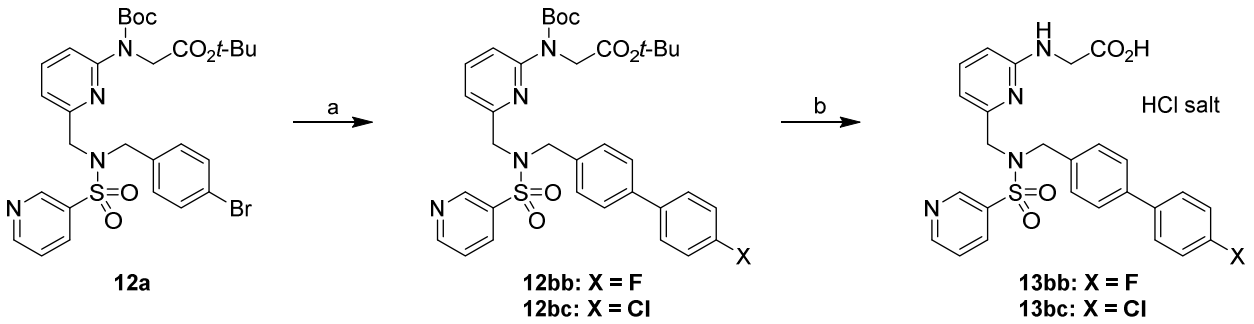
13af	2-chlorophenyl	4-*tert*-butylphenyl	HCl salt	b, d
13ag	3-fluorophenyl	4-*tert*-butylphenyl	HCl salt	b, d
13ah	3-chlorophenyl	4-*tert*-butylphenyl	HCl salt	b, d
13ai	4-fluorophenyl	4-*tert*-butylphenyl	HCl salt	b, d
13aj	4-chlorophenyl	4-*tert*-butylphenyl	HCl salt	b, d
13ak	4-methoxyphenyl	4-*tert*-butylphenyl	HCl salt	b, d
13al	pyridin-3-yl	3-*tert*-butylphenyl	HCl salt	a, d
13am	pyridin-3-yl	4-*tert*-butylcyclohexyl	HCl salt	a, d
13an	pyridin-3-yl	4-trifluoromethylphenyl	HCl salt	c, d
13ao	pyridin-3-yl	4-(1-methylcyclopropyl)phenyl	HCl salt	a, d
13ap	pyridin-3-yl	4-(1-ethylcyclopropyl)phenyl	free form	c, d
13aq	pyridin-3-yl	4-(1-isopropylcyclopropyl)phenyl	free form	c, d
13ar	pyridin-3-yl	4-(1-ethylcyclobutyl)phenyl	free form	c, d
13as	pyridin-3-yl	biphenyl-4-yl	HCl salt	a, d
13at	pyridin-3-yl	4-phenylbutyl	HCl salt	a, d
13au	pyridin-3-yl	4-cyclohexylphenyl	free form	c, d
13av	pyridin-3-yl	4-(pyridin-2-yl)phenyl	free form	c, d
13aw	pyridin-3-yl	4-(pyridazin-4-yl)phenyl	HCl salt	c, d
13ax, OMD	pyridin-3-yl	4-(pyrazol-1-yl)phenyl	free form	c, d
13ay	pyridin-3-yl	4-(1,2,4-triazol-1-yl)phenyl	free form	c, d
13az	pyridin-3-yl	benzofuran-2-yl	HCl salt	a, d
13ba	pyridin-3-yl	benzo[*b*]thiophen-2-yl	HCl salt	c, d

[a]Synthetic route involves synthetic steps for each aminopyridyl derivative in Scheme 10.

Compounds **13bb** and **13bc**, belonging to aminopyridyl derivatives, were obtained through the synthetic route shown in Scheme 11.[27,28] Compound **12a**[27,28] was synthesized using compounds

48 and **1**, as shown in Scheme 10, under the conditions same to step a in Scheme 10. Compound **12a** was converted to the precursor compounds **12bb** and **12bc** by reaction with appropriate boronic acids under Suzuki–Miyaura reaction conditions (step a), which were then converted to compounds **13bb** and **13bc**, respectively, by acidic deprotection (step b).

Scheme 11. Synthetic Route for Aminopyridyl Derivatives 13bb and 13bc[a]



12a **12bb: X = F** **12bc: X = Cl** **13bb: X = F** **13bc: X = Cl**

[a]Reagents and conditions: (a) boronic acid, $Pd(OAc)_2$, tricyclohexylphosphine, K_3PO_4, toluene/H_2O, 100 °C, 94% for **12bb** and 84% for **12bc**; (b) HCl, CH_2Cl_2/1,4-dioxane, rt, 89% for **13bb** and 93% for **13bc**.

Structure−Activity Relationship Study. Exploration of the Phenoxyacetic Acid Moiety. We explored the phenoxyacetic acid moiety of CP-533,536, STEP 1 of the medicinal chemistry strategy described above, using a chemotype with a (4-*tert*-butylbenzyl)(pyridin-3-ylsulfonyl)amino moiety. Our initial design aimed at enhancing the *in vitro* activity of the compound by fixing the conformation of the phenoxyacetic acid moiety. Statistical analysis using the Cambridge Structural Database (CSD) suggested that the following characteristics are associated with the conformation of the phenoxyacetic acid moiety[29–32] (Figure 3 and S2):

1. The distribution of torsional angle 1 in the phenoxyacetic acid moiety has a clear preference for approximately 0° or 180°. In contrast, the *ortho*-unsubstituted phenethyl

moiety has a different distribution, with a maximum at approximately 90° (Figure S1 in the Supporting Information), owing to steric constraints. These analysis results suggest that the phenoxy oxygen atom has sp^2 orbital characteristics and adopts a planar orientation of torsional angle 1 owning to resonance stabilization.

2. The preferred conformations include one in which the phenoxy and carboxy groups are contained in the same plane (planar geometry; torsional angle 1 approximately 0° or 180°, torsional angle 2 approximately 180°) and one in which the carboxy group extrudes from the plane of the phenoxy group (skewed geometry; torsional angle 1 approximately 0° or 180°, torsional angle 2 approximately 90°); among these two, the former conformation is preferred.

We hypothesized that the phenoxyacetic acid moiety bound to the h-EP2 receptor has the conformation equivalent to either of the planar or skewed geometry; consequently, we designed new compounds mimicking these two preferred conformers throughout the exploration of the phenoxyacetic acid moiety.

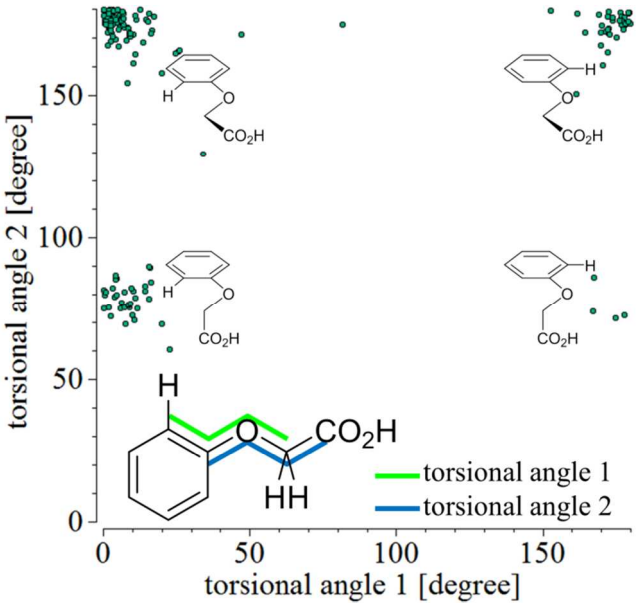


Figure 3. Preferred conformations of the phenoxyacetic acid moiety based on statistical analysis using CSD. The torsional angle of C–O–CH_2–CO_2H (torsional angle 2) is plotted against that of CH=C–O–CH_2 (torsional angle 1). The two islands plotted in the upper graph region represent the conformers with planar geometry and those in the middle graph region represent the conformers with skewed geometry.

We designed a structural mimetic of the planar geometry (the estimated most preferred conformation of the phenoxyacetic acid moiety) by transforming the phenoxyacetic acid moiety into benzofuran-2-carboxylic acid, and fixed the conformation (Figure 4). The planar geometry of the phenoxyacetic acid moiety includes two different conformations with a torsional angle 1 of approximately 0° or 180°, corresponding to two possible regioisomers (**5** and **9**) of the benzofuran-2-carboxylic acid moiety. The binding affinity of the regioisomer **9** for the h-EP2 receptor was comparable to that of CP-533,536 (Table 2) and higher than that of the other regioisomer **5**. The binding affinity of **9** encouraged us to further optimize the benzofuryl derivative, and the *tert*-butylphenyl moiety of **9** was derivatized, resulting in more potent compounds (data not shown). However, chemotypes containing benzofuran-2-carboxylic acid generally appeared to be highly crystalline with limited solubility in solvents, including water. Therefore, we explored the phenoxyacetic acid moiety to identify chemotypes with enhanced *in vitro* activities and without solubility limitations before advancing to STEP 2. The SAR of **5** and **9** suggested that the phenoxyacetic acid moiety of the bound conformer was equivalent to **9** in terms of the geometry of torsional angle 1 and was applied to the subsequent medicinal chemistry efforts.

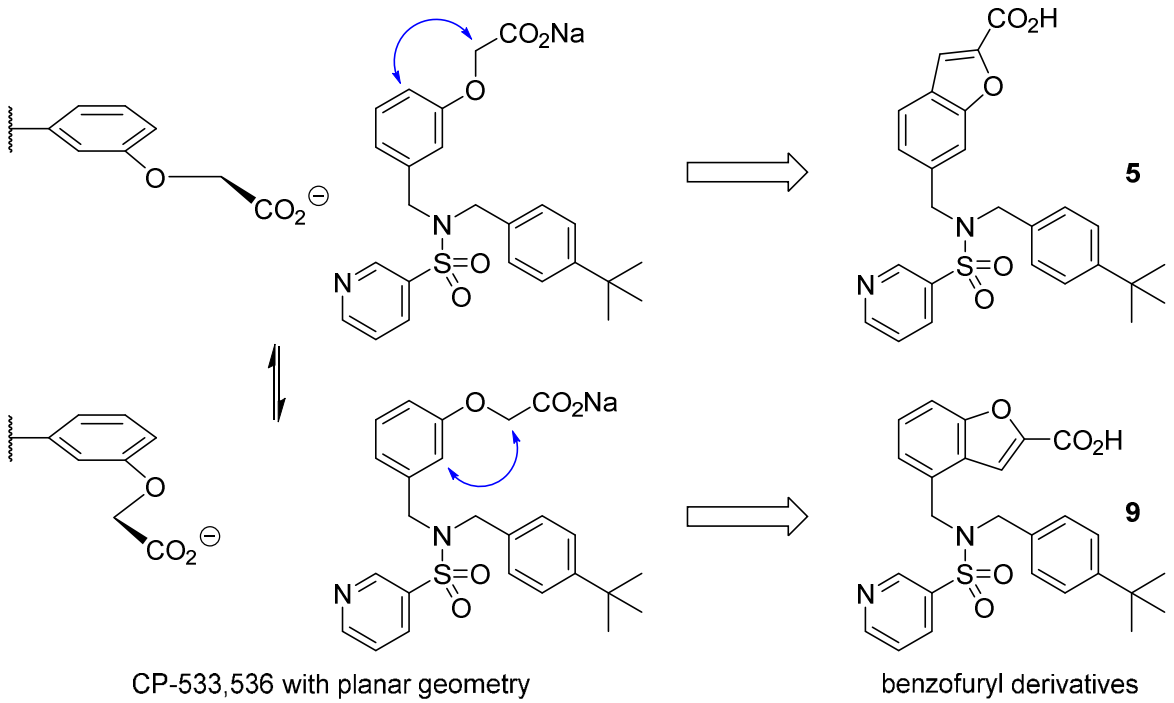


Figure 4. Design of benzofuryl derivatives: structural mimetics of the planar geometry (the estimated most preferred conformation of the phenoxyacetic acid moiety) and fixing the conformation.

Table 2. Binding Affinity of CP-533,536 and Benzofuryl Derivatives for h-EP2 Receptor

compound	K_i [nM][a]
CP-533,536	16
5	433
9	15

[a]The binding assay was carried out in compliance with the method of Abramovitz *et al.*[11] The IC_{50} value, the concentration of test compound required to replace 50% of the [^{3}H]prostaglandin E_2 bound to h-EP2 receptor expressed on a membrane fraction of HEK293 cells (ES-562-M, Euroscreen), was calculated on the basis of the results from two or four data points of a single experiment, performed in duplicate, and the inhibition constant (K_i value) was determined.

The limited solubility of chemotypes containing benzofuran-2-carboxylic acid may be attributed in part to their rigid bicyclic structures introduced to reduce rotatable bonds and fix their conformation. Therefore, we tried to transform the phenoxyacetic acid moiety within the scope of the monocyclic structures without increasing the rigidity of the molecule. To enhance *in vitro* activity, we applied nitrogen-scan (N-scan) and basicity enhancement strategies in which we transformed the phenoxy group in the phenoxyacetic acid moiety into an aminopyridyl group.[33,34] The phenyl group was transformed into a pyridyl group to yield different regioisomers and the phenoxy oxygen atom was transformed into –NH– to enhance a basicity of a pyridyl nitrogen atom; that is, the N-scan was performed on the pyridyl nitrogen atom of compounds having a pyridylaminoacetic acid substructure (Figure 5).[35] **13aa**, a compound with a (pyridin-2-ylamino)acetic acid substructure substituted at the 6-position of the pyridyl group, showed higher h-EP2 receptor agonist activity than CP-533,536, while **19** and **25** (regioisomers around the pyridyl nitrogen atom) showed substantially lower *in vitro* activity, demonstrating that the location of the pyridyl nitrogen atom is highly involved in h-EP2 receptor agonist activity (Table 3).

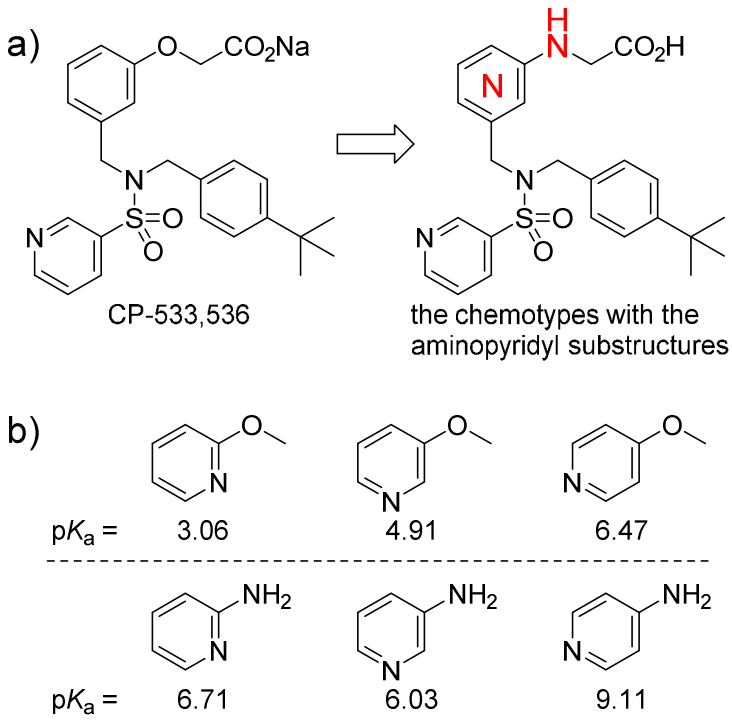
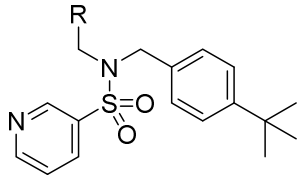
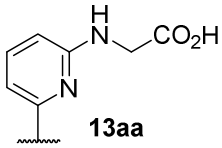


Figure 5. (a) Design of the chemotypes with aminopyridyl substructures based on the N-scan and basicity enhancement strategies. The phenyl group was transformed into a pyridyl group for the N-scan and the phenoxy oxygen atom was transformed into –NH– to enhance a basicity of the pyridyl nitrogen atom. (b) Comparison of the pK_a values between methoxypyridines and aminopyridines in water at 25 °C.[35] Replacement of the –OMe in each methoxypyridine with a –NH_2 enhances the basicity of the pyridyl nitrogen atom.

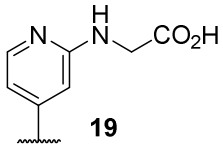
Table 3. *In Vitro* h-EP2 Receptor Agonist Activity of Compounds with Aminopyridyl, Indolyl and Aminothiazolyl Substructures



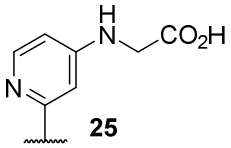
R =



13aa

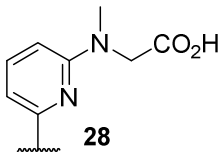


19

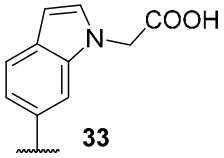


25

regioisomers based on N-scan strategy

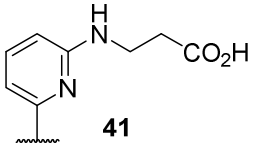


28



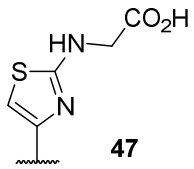
33

skewed geometry



41

one-carbon-elongation



47

isosteric replacement

compound	EC_{50} [nM][a]
CP-533,536	17
13aa	2.8
19	>1000
25	>1000
28	283
33	>1000
41	83
47	360

[a]Measurement of *in vitro* h-EP2 receptor agonist activity was carried out in compliance with the method of Wilson *et al.*[36] The concentration of test compound required to increase the amount of cAMP to 50% of the maximum increase (EC_{50} value) in HEK293 cells expressing h-EP2 receptor (ES-562-C, Euroscreen) was calculated on the basis of the results from at least seven data points of a single experiment, performed in duplicate.

Statistical analysis using the CSD indicated that the following characteristics were associated with the conformation of the (pyridin-2-ylamino)acetic acid moiety (Figure 6 and S3):

1. The amino nitrogen atom in the aminopyridyl moiety could also have sp^2 orbital characteristics, and could adopt a planar orientation of torsional angle 1, as with the phenoxy oxygen atom.
2. The preferred conformations include one in which the aminopyridyl and carboxy groups are contained in the same plane (planar geometry; torsional angle 1 approximately 0° or 180°, torsional angle 2 approximately 180°) and one in which the carboxy group extrudes from the plane of the aminopyridyl group (skewed geometry; torsional angle 1 approximately 0° or 180°, torsional angle 2 approximately 90°).

Although it exhibits a conformation similar to that seen in the phenoxyacetic acid moiety, statistics obtained from the CSD indicated a difference in abundance between the planar and skewed geometries; the former geometry is preferred in the phenoxyacetic acid, while the abundance of the two geometries is comparable in the (pyridin-2-ylamino)acetic acid. Because it was speculated that the enhanced activity of **13aa** resulted from the contribution of the conformer with skewed geometry, we substituted the amino group in the aminopyridyl moiety with a methyl group to increase the proportion of conformers with skewed geometry. The substitution of the amino group with a methyl group leads to a preference for skewed geometry due to pseudo 1,2-strain between the carboxy and methyl groups and pseudo 1,3-strain between the carboxy group and the pyridyl nitrogen atom or CH (Figure 6). **28**, a compound obtained by substituting the amino group in the aminopyridyl moiety with a methyl group, was found to have reduced h-EP2 receptor agonist activity. Furthermore, **33**, a compound in which the 2-(methylamino)pyridyl group was replaced with an indolyl group to fix the conformation so that the skewed geometry with the same torsional angle 1 to that in **9** is dominant, was found to have further reduced h-EP2 receptor agonist activity (Table 3). On the basis of the above SAR and

CSD analysis, the bound conformer was suggested to be in its planar geometry with regard to the (pyridin-2-ylamino)acetic acid moiety.

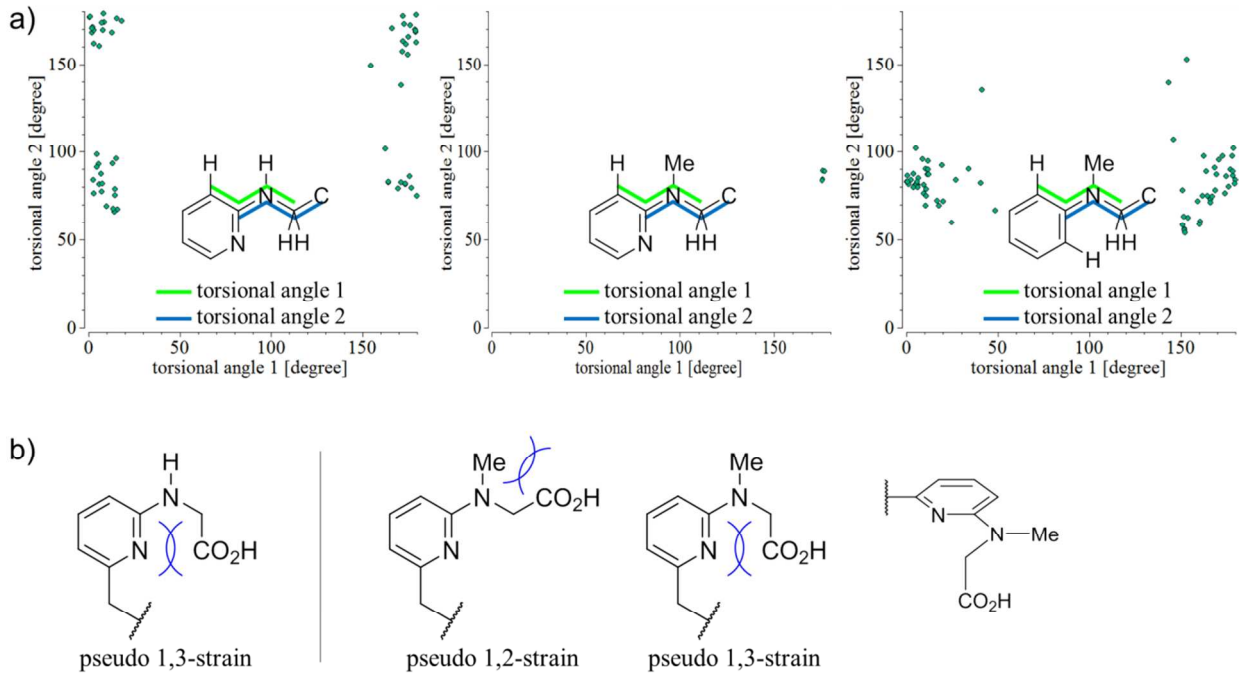


Figure 6. Preferred conformations of the (pyridin-2-ylamino)acetic acid moiety based on the statistical analysis using CSD. (a) Scatterplot for (pyridin-2-ylamino)acetic acid, (*N*-methyl-*N*-(pyridin-2-yl)amino)acetic acid, and (*N*-methyl-*N*-phenylamino)acetic acid substructures in the CSD. The analysis for a (*N*-methyl-*N*-phenylamino)acetic acid substructure was appended as reference because of insufficient data for the (*N*-methyl-*N*-(pyridin-2-yl)amino)acetic acid substructure. The torsional angle of C–N(H or Me)–CH_2–C (torsional angle 2) is plotted against that of CH=C–N(H or Me)–CH_2 (torsional angle 1). The islands plotted in the upper graph region represent the conformers with planar geometry and those in the middle graph region represent the conformers with skewed geometry. (b) Pseudo 1,2-strain and pseudo 1,3-strain in the *N*-unsubstituted/substituted (pyridin-2-ylamino)acetic acid moiety.

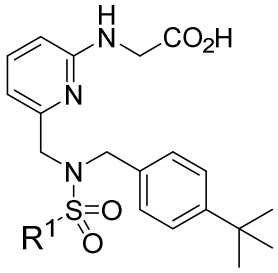
Further transformations were conducted at the (pyridin-2-ylamino)acetic acid moiety of **13aa** to improve *in vitro* activity as follows: elongation of the acetic acid moiety to adjust the position of the carboxy group and the isosteric replacement of the pyridyl group. However, **41** (with a one-carbon-longer alkyl spacer to adjust the location of the carboxy group) and **47** (containing (thiazol-2-ylamino)acetic acid, an estimated isostere of (pyridin-2-ylamino)acetic acid) were found to have weaker h-EP2 receptor agonist activities than **13aa** (Table 3).

These findings suggested that in terms of the exploration of the phenoxyacetic acid moiety of CP-533,536, the conformational preference for planar geometry (the putative bound conformer) and the introduction of a nitrogen atom at a specific site would effectively enhance h-EP2 receptor agonist activity, and the chemotype with a (pyridin-2-ylamino)acetic acid substructure would be a particularly effective EP2 receptor agonist. The higher h-EP2 receptor agonist activity of **13aa** may be due to the physicochemical properties of the pyridyl nitrogen atom (typically, the characteristics of a hydrogen-bonding acceptor) and also to the tendency that a 2-aminopyridyl group has a high level of sp^2 hybridization at the amino nitrogen atom (i.e., a high level of planarity). We subsequently advanced to STEP 2 of the medicinal chemistry strategy and sequentially transformed the structure of the pyridin-3-ylsulfonyl and *tert*-butylphenyl moieties using the chemotype containing a (pyridin-2-ylamino)acetic acid substructure to further enhance *in vitro* activity.

Exploration of the pyridin-3-ylsulfonyl moiety. We attempted to transform the pyridin-3-ylsulfonyl moiety to enhance *in vitro* h-EP2 receptor agonist activity using a chemotype with a (6-((4-*tert*-butylbenzyl)aminomethyl)pyridin-2-ylamino)acetic acid moiety (Table 4). While **13ac** with the phenyl group and **13ad** with the pyridin-2-yl group as R^1 showed slightly reduced

and equivalent agonist activities as compared with **13aa**, respectively; **13ab** with the methyl group as R^1 showed a reduced agonist activity as compared with **13aa**. Thus, the transformation of R^1 was focused on the optionally substituted aromatic groups. Different substituents were introduced into the phenylsulfonyl group of **13ac**, and the steric environment was assessed. The results showed that substitution at the *ortho*- or *meta*-position relative to the sulfonyl group with F or Cl led to a reduction in agonist activity, suggesting a small steric environment around the *ortho*- and *meta*-positions (**13ae–ah**). **13ai** and **13aj**, compounds in which the *para*-position relative to the sulfonyl group was substituted with F and Cl, respectively, were found to be stronger than and equipotent to **13ac**, respectively, in terms of agonist activity, but substitution with a methoxy group (a bulkier group) led to a reduction in agonist activity (**13ak**). Thus, the steric environment was not estimated to be extensive around the *para*-position either. The transformation of the pyridin-3-ylsulfonyl group to a pyridin-2-ylsulfonyl or a 4-fluorophenylsulfonyl group was found to maintain potent *in vitro* h-EP2 receptor agonist activity, as shown in Table 4. Because pyridin-3-ylsulfonyl group, the substructure in CP-533,536 that was under clinical development at the time, was expected to pose a limited safety risk and because no safety data were available for compounds containing a pyridin-2-ylsulfonyl or a 4-fluorophenylsulfonyl group in humans, we selected a pyridin-3-ylsulfonyl group from among these three functional groups, with comparable agonist activity, for further structural optimization.

Table 4. *In Vitro* h-EP2 Receptor Agonist Activity of Aminopyridyl Derivatives 13aa–ak; Optimization of the Pyridin-3-ylsulfonyl Moiety



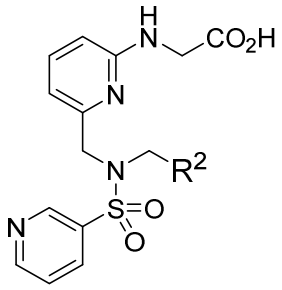
compound	R^1	EC_{50} [nM][a]
13aa	pyridin-3-yl	2.8
13ab	methyl	91
13ac	phenyl	4.3
13ad	pyridin-2-yl	2.7
13ae	2-fluorophenyl	45
13af	2-chlorophenyl	>1000
13ag	3-fluorophenyl	6.1
13ah	3-chlorophenyl	620
13ai	4-fluorophenyl	2.7
13aj	4-chlorophenyl	4.4
13ak	4-methoxyphenyl	19

[a]Measurement of *in vitro* h-EP2 receptor agonist activity was carried out in compliance with the method of Wilson *et al.*[36] The concentration of test compound required to increase the amount of cAMP to 50% of the maximum increase (EC_{50} value) in HEK293 cells expressing h-EP2 receptor (ES-562-C, Euroscreen) was calculated on the basis of the results from at least seven data points of a single experiment, performed in duplicate.

Exploration of the *tert*-butylphenyl moiety. The optimization of the structure regarding *in vitro* h-EP2 receptor agonist activity was attempted by transforming the *tert*-butylphenyl moiety using a chemotype with a (6-((pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic acid moiety, which has both a (pyridin-2-ylamino)acetic acid substructure (as selected in the

"Exploration of the phenoxyacetic acid moiety" section) and a pyridin-3-ylsulfonyl group (as selected in the "Exploration of the pyridin-3-ylsulfonyl moiety" section) (Table 5). The *tert*-butylphenyl moiety could be transformed into various structures as expected. It was confirmed that a *para*-position on the phenylene group was found to be favorable for substitution on the basis of the agonist activity of **13al**. The assessment of the transformation of the phenylene moiety showed that compounds retaining the original 1,4-phenylene group exhibited more potent agonist activity than those with 1,4-cyclohexylene and *n*-butylene groups (**13aa** vs. **13am** and **13as** vs. **13at**). Some derivatives mimicking a *tert*-butyl group showed agonist activity comparable to **13aa**, but none of them were superior (**13an–ar**), whereas the agonist activity of **13as** (in which the *tert*-butyl group is transformed into a phenyl group) tended to be more potent than **13aa**. Therefore, we then focused on the transformation of the *tert*-butylphenyl moiety into an aromatic system, such as optionally substituted biaryl or fused bicyclic aromatic groups. The agonist activity of **13bb** and **13bc**, compounds in which the 4'-position of the biphenyl group of **13as** was substituted with F and Cl, respectively, was reduced with increasing substituent size, and a reduction in agonist activity was also observed in **13au** with a 4-cyclohexylphenyl group, suggesting the constrained steric environment around this moiety. The transformation of the *tert*-butylphenyl moiety into an aromatic system containing a hetero aryl group worked as expected to obtain compounds with potent agonist activity (**13av–ba**). Especially, **13ax** (OMD), which contains a pyrazol-1-yl group, was found to exhibit the most potent and more than 10 times higher h-EP2 receptor agonist activity than CP-533,536. **13ax** was expected to be effective at low concentrations because of its potent EP2 receptor agonist activity and underwent further assessments as described below.

Table 5. *In Vitro* h-EP2 Receptor Agonist Activity of Aminopyridyl Derivatives 13al–bc; Optimization of the *tert*-Butylphenyl Moiety



compound	R^2	EC_{50} [nM][a]
13aa	4-*tert*-butylphenyl	2.8
13al	3-*tert*-butylphenyl	109
13am	4-*tert*-butylcyclohexyl	649
13an	4-trifluoromethylphenyl	119
13ao	4-(1-methylcyclopropyl)phenyl	2.6
13ap	4-(1-ethylcyclopropyl)phenyl	4.5
13aq	4-(1-isopropylcyclopropyl)phenyl	18
13ar	4-(1-ethylcyclobutyl)phenyl	13
13as	biphenyl-4-yl	2.0
13at	4-phenylbutyl	118
13bb	4'-fluoro-[1,1'-biphenyl]-4-yl	35
13bc	4'-chloro-[1,1'-biphenyl]-4-yl	75
13au	4-cyclohexylphenyl	29
13av	4-(pyridin-2-yl)phenyl	1.6
13aw	4-(pyridazin-4-yl)phenyl	307
13ax, OMD	4-(pyrazol-1-yl)phenyl	1.1
13ay	4-(1,2,4-triazol-1-yl)phenyl	43

13az	benzofuran-2-yl	5.6
13ba	benzo[*b*]thiophen-2-yl	3.4

[a]Measurement of *in vitro* h-EP2 receptor agonist activity was carried out in compliance with the method of Wilson *et al.*[36] The concentration of test compound required to increase the amount of cAMP to 50% of the maximum increase (EC_{50} value) in HEK293 cells expressing h-EP2 receptor (ES-562-C, Euroscreen) was calculated on the basis of the results from at least seven data points of a single experiment, performed in duplicate.

Improvement in the membrane permeability of 13ax (OMD) and further biological evaluation. Through the medicinal chemistry efforts described above, **13ax**, a compound with potent h-EP2 receptor agonist activity, was selected as the most promising active form. An evaluation of the binding affinity for h-EP receptor subtypes, namely h-EP1, h-EP2, h-EP3, and h-EP4, revealed that **13ax** binds strongly and with a high selectivity to the h-EP2 receptor (Table 6), based on which it was assumed that **13ax** has better safety profiles than non-selective EP receptor agonists. The *in vitro* ADMET properties of **13ax** are summarized in Tables 7, 8, S1, S3, and S4. **13ax** showed slow metabolic rate in human liver microsome and was expected to pose a low risk of drug-drug interaction as shown by the cytochrome P450 (CYP) inhibition and induction assay. Meanwhile, the cell membrane permeability of **13ax** was presumed to be insufficient: the Caco-2 cell permeability rate of **13ax** was in the order of 1×10^{-7} cm/s at pH 7.4 and 6.5 apically, and the permeability rate measured with parallel artificial membrane permeability assay (PAMPA) for **13ax** was 0.9×10^{-6} cm/s. The lipophilic corneal epithelium represents a major barrier to the absorption of IOP-lowering eye drops into the eyes.[37] Therefore, improvements to cell membrane permeability are considered necessary to facilitate effective IOP-lowering activity following ocular administration. Generally, cell membrane permeability can be enhanced by increasing the lipophilicity of a compound, and the corneal epithelium is negatively charged under physiological conditions.[38] Therefore, a prodrug approach to masking

the carboxy group (an anionic polar group) of **13ax** was investigated. A prodrug with a masked carboxy group should balance susceptibility to enzymatic hydrolysis and chemical stability in an ophthalmic solution. An isopropyl ester prodrug was found to be useful on the basis of reports describing prodrug forms of prostaglandins.[39,40] For example, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) isopropyl ester prodrug was rapidly hydrolyzed in the corneal epithelium[41]; ocular administration of the $PGF_{2\alpha}$ isopropyl ester increased the uptake of $PGF_{2\alpha}$ free acid in the cornea compared with administration of the $PGF_{2\alpha}$ free acid itself.[42] The isopropyl ester form was expected to be stable in ophthalmic solutions because several commercially available ophthalmic drugs including latanoprost, tafluprost, travoprost and unoprostone isopropyl ester, include isopropyl ester forms of prostaglandins as active ingredients. On the basis of these considerations, an isopropyl ester form of **13ax** was synthesized, leading to the discovery of OMDI, which exhibited an improved parallel artificial membrane permeability rate of 2.8×10^{-5} cm/s. OMDI showed a low binding affinity to h-EP receptor subtypes, indicating that it is a prodrug form of **13ax** (Table 6). **13ax** and OMDI showed excellent IOP-lowering activities following ocular administration in ocular normotensive monkeys. In particular, OMDI lowered IOP to an extent comparable to that by **13ax**, but at a dose lower than 1/10 of that of **13ax** (Figure 7). In addition, the IOP-lowering effect of OMDI observed in ocular normotensive monkeys was dose-dependent (Figure 8). On the basis of these results, OMDI represents a promising IOP-lowering drug with an MOA different from those of existing drugs via potent and selective EP2 agonism[43]; therefore, it was selected as a clinical candidate for the treatment of glaucoma.

Table 6. Selectivity of 13ax (OMD), OMDI, and PGE_2 to the h-EP2 Receptor among EP Receptor Subtypes

	binding affinity; IC_{50} [nM][a]			
	h-EP1	h-EP2	h-EP3	h-EP4
13ax, OMD	>10,000	10	>10,000	5,480
OMDI	>10,000	4,180	>10,000	>10,000
PGE_2	<30	4	<3	5

[a]The inhibitory effect of test compounds on [^{3}H]prostaglandin E_2 binding to each EP receptor subtype (h-EP1, h-EP2, h-EP3, and h-EP4) was determined. For h-EP2 and h-EP4, concentrations causing 50% inhibition of the binding of the radioligand to the receptor (IC_{50} values) were calculated on the basis of the results from at least four and three data points, respectively, and each data point involved duplicate measurements. For h-EP1 and h-EP3, assays were performed at a single concentration for each compound, in duplicate.

Table 7. Cell Membrane Permeability and Metabolic Stability of 13ax (OMD)[c]

permeability rate[a]			metabolic rate in human liver microsome [pmol/min/mg protein][b]
Caco-2 [cm/s]		PAMPA [cm/s]	
pH 7.4/7.4	pH 6.5/7.4		
2.8×10^{-7}	5.9×10^{-7}	0.9×10^{-6}	57

[a]The cell membrane permeability rate of the test compound was determined using Caco-2 cells and parallel artificial membrane (Corning Gentest Pre-coated PAMPA Plate System). For Caco-2 cell permeability, the experiments were performed in the apical to basolateral (AP–BL) direction under non-gradient pH conditions (pH 7.4 on both sides of the membrane; iso-pH 7.4) and pH-gradient conditions (pH 6.5 apically; pH 7.4 basolaterally). The Caco-2 cell permeability rate was calculated on the basis of the results from four data points of a single experiment, performed in triplicate. The parallel artificial membrane permeability rate was calculated on the basis of the results from one data point of a single experiment, performed in triplicate. [b]The experiments were performed in human liver microsomes at 37 °C and the metabolic rate per unit amount of protein was calculated on the basis of the results from six data points of a single experiment. [c]The detailed experimental procedures and the data for the positive control compounds are described in the Supporting Information.

Table 8. CYP Inhibition and Induction of 13ax (OMD)[c]

CYP inhibition[a]	CYP induction[b]

IC_{50} [μM]					h-CYP3A4	h-CYP1A2
h-CYP3A4	h-CYP2D6	h-CYP2C9	h-CYP2C19	h-CYP1A2		
>20	>20	>20	>20	>20	no induction up to 100 μM	

[a]The inhibitory effect of the test compound on each recombinant human CYP (h-CYP3A4, h-CYP2D6, h-CYP2C9, h-CYP2C19, and h-CYP1A2) was determined. The IC_{50} value, the concentration of test compound causing 50% inhibition, was calculated on the basis of the results from eight data points of a single experiment, performed in triplicate. [b]The inductive effect of test compound on each human CYP (h-CYP3A4 and h-CYP1A2) was determined using Fa2N-4 cells on the basis of the results from five data points of a single experiment, performed in triplicate. [c]The detailed experimental procedures and the data for the positive control compounds are described in the Supporting Information.

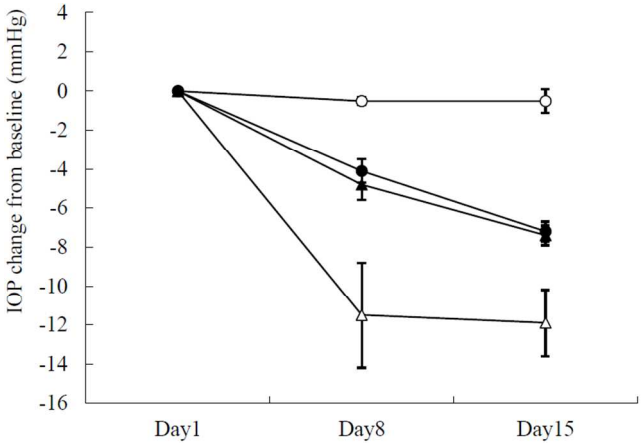


Figure 7. IOP-lowering effects of **13ax** (OMD) and OMDI in ocular normotensive monkeys. Data represent the mean ± S.E. of four eyes. Each symbol represents as follows: Open circle: vehicle, closed circle: 0.3% **13ax**, open triangle: 1% **13ax**, and closed triangle: 0.01% OMDI.

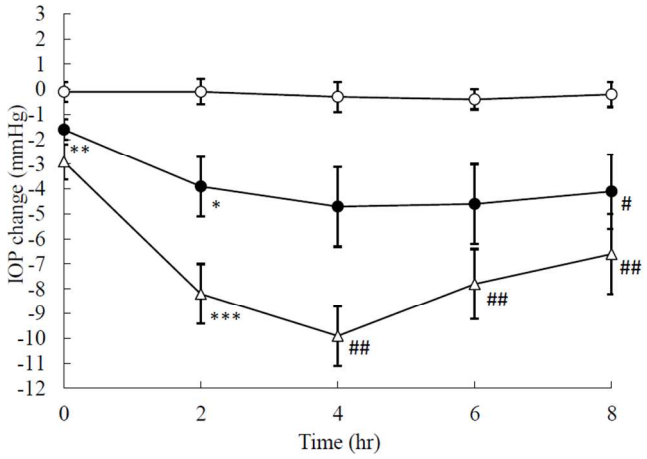


Figure 8. Dose dependence of IOP-lowering effects of OMDI in ocular normotensive monkeys at Day 7. Data represent the mean ± S.E. of eight eyes. *, **, *** $p < 0.05$, 0.01, 0.001 vs Vehicle by Dunnett's test. #, ## $p < 0.05$, 0.01 vs Vehicle by Steel test. Each symbol represents as follows: Open circle: vehicle, closed circle: 0.0006% OMDI, and open triangle: 0.003% OMDI.

■ CONCLUSION

Our detailed medicinal chemistry efforts using CP-533,536 as the starting point were based on a two-steps strategy and led to the discovery of isopropyl (6-((4-(pyrazol-1-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetate (OMDI) as a clinical candidate for the treatment of glaucoma. We explored the phenoxyacetic acid moiety and identified the chemotype with a (pyridin-2-ylamino)acetic acid substructure. **13aa** showed more potent agonist activity than CP-533,536 and SAR suggested that the (pyridin-2-ylamino)acetic acid substructure would be particularly effective to exert potent *in vitro* agonist activity to the h-EP2 receptor. On the basis of these findings, we further optimized the *tert*-butylphenyl moiety and identified (6-((4-(pyrazol-1-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic acid **13ax**

(OMD) as a potent and selective h-EP2 receptor agonist (h-EP2 receptor agonist activity (EC_{50}) 1.1 nM; binding affinities (IC_{50}) >10,000 nM (h-EP1), 10 nM (h-EP2), >10,000 nM (h-EP3), 5,480 nM (h-EP4)). OMDI, a prodrug of **13ax**, exerted favorable IOP-lowering profiles in ocular normotensive monkeys, lowered IOP at low concentrations (significantly at 0.0006% concentration) in a dose-dependent manner. These results suggested that OMDI could be an effective ocular hypotensive agent like FP receptor agonists, based on the new, different MOA, and is useful for the treatment of glaucoma.

■ EXPERIMENTAL SECTION

General Procedures. All solvents and reagents were used as purchased without further purification. The progress of reactions was monitored with thin-layer chromatography (TLC) and/or high-performance liquid chromatography (HPLC). Proton and carbon nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR with proton-decoupled manner) were taken on a Bruker Biospin Avance500 (500 MHz), a JEOL EX-400WB (400 MHz), or a JEOL AL-300 (300 MHz) using deuterated solvent: deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), or deuterated dimethyl sulfoxide (DMSO-d_6). The chemical shifts (δ) and coupling constants (J) were reported in parts per million (ppm) and in hertz (Hz), respectively. Mass spectra were measured with some ionization methods: electron impact (EI, Hitachi M80B), chemical ionization (CI, Hitachi M80B), fast atom bombardment (FAB, JEOL JMS-700QQ), and atmospheric pressure chemical ionization (APCI, JEOL JMS-T100LC AccuTOF). IR spectra were recorded on a BIO·RAD FTS-65A, a Digilab FTS7000e, or a Varian 3100. Elemental analysis was performed on a J-Science Micro Corder JM10. Final compounds were determined their purity with HPLC and indicated 95% or higher, except for the compounds **13ad**, **13bc**, **25**, **33**, and **41**.

Ethyl 6-(Bromomethyl)benzofuran-2-carboxylate (3). To 13 mL of a 1,2-dichloroethane solution containing 494 mg (2.42 mmol) of ethyl 6-methylbenzofuran-2-carboxylate (**2**) was added 457 mg (2.57 mmol) of *N*-bromosuccinimide (NBS). The mixture was stirred at ambient temperature and irradiated by a mercury lamp under argon atmosphere for 1 h. The reaction mixture was concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 20/1→5/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 631 mg of the title compound (92% yield) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63-7.69 (m, 1H), 7.59-7.63 (m, 1H), 7.51 (d, J = 1.0 Hz, 1H), 7.36 (dd, J = 8.1, 1.4 Hz, 1H), 4.63 (s, 2H), 4.45 (q, J = 7.1 Hz, 2H), 1.43 (t, J = 7.1 Hz, 3H). MS (CI+) *m/z* 283, 285 $(M + H)^+$.

Ethyl 6-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)benzofuran-2-carboxylate (4). To 2.5 mL of a *N*,*N*-dimethylformamide (DMF) solution containing 173 mg (0.611 mmol) of **3** and 165 mg (0.542 mmol) of *N*-(4-*tert*-butylbenzyl)pyridine-3-sulfonamide (**1a**) was added 146 mg (1.06 mmol) of K_2CO_3, and the mixture was stirred at room temperature overnight. After completion of the reaction, the reaction mixture was diluted with ethyl acetate and washed with H_2O. The separated organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 3/1→0/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 257 mg of the title compound (83% yield) as a pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.05 (dd, J = 2.4, 0.8 Hz, 1H), 8.79 (dd, J = 4.8, 1.7 Hz, 1H), 8.04 (ddd, J = 8.0, 2.4, 1.7 Hz, 1H), 7.55 (d, J = 8.1 Hz, 1H), 7.47 (d, J = 0.9 Hz, 1H), 7.40 (ddd, J = 8.0, 4.8, 0.8 Hz, 1H), 7.29-7.31 (m, 1H), 7.18-

7.22 (m, 2H), 7.15 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.94-6.98 (m, 2H), 4.49 (s, 2H), 4.45 (q, *J* = 7.2 Hz, 2H), 4.35 (s, 2H), 1.43 (t, *J* = 7.2 Hz, 3H), 1.25 (s, 9H). MS (FAB+) *m/z* 507 $(M + H)^+$.

6-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)benzofuran-2-carboxylic Acid (5). To a solution consisting of 4 mL of tetrahydrofuran (THF) and 4 mL of ethanol, containing 201 mg (0.397 mmol) of **4** was added 500 μL (0.500 mmol) of aqueous 1 M NaOH, and the mixture was stirred at room temperature for 3 h. The reaction mixture was adjusted pH to 5.0 with aqueous 1 M HCl and extracted with 10 mL of $CHCl_3$. The organic layer was washed with 10 mL of H_2O and 10 mL of a saturated aqueous NaCl solution in turn, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to obtain 111 mg of the title compound (58% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.01 (d, *J* = 1.7 Hz, 1H), 8.84 (dd, *J* = 4.8, 0.7 Hz, 1H), 8.24 (ddd, *J* = 8.0, 2.4, 1.7 Hz, 1H), 7.61 (ddd, *J* = 8.0, 4.8, 0.7 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.25 (s, 1H), 7.13-7.19 (m, 2H), 6.99-7.09 (m, 3H), 4.49 (s, 2H), 4.36 (s, 2H), 3.51 (s, 1H), 1.17 (s, 9H). MS (CI+) *m/z* 479 $(M + H)^+$.

Ethyl 4-(Bromomethyl)benzofuran-2-carboxylate (7). 193 mg of the title compound (86% yield) was obtained as a white solid according to the procedure similar to that for **3**, using 4-methylbenzofuran-2-carboxylate (**6**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 0.9 Hz, 1H), 7.55-7.58 (m, 1H), 7.40 (dd, *J* = 8.3, 7.4 Hz, 1H), 7.30-7.32 (m, 1H), 4.73 (s, 2H), 4.47 (q, *J* = 7.2 Hz, 2H), 1.45 (t, *J* = 7.2 Hz, 3H). MS (CI+) *m/z* 283, 285 $(M + H)^+$.

Ethyl 4-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)benzofuran-2-carboxylate (8). 232 mg of the title compound (84% yield) was obtained as an orange yellow oil according to the procedure similar to that for **4**, using **7** and **1a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.05 (dd, *J* = 2.4, 0.9 Hz, 1H), 8.79 (dd, *J* = 4.9, 1.6 Hz, 1H), 8.01 (ddd, *J* = 8.0, 2.4, 1.6 Hz, 1H), 7.52 (d, *J* = 0.9 Hz, 1H), 7.44-7.47 (m, 1H), 7.40 (ddd, *J* = 8.0, 4.9, 0.9 Hz, 1H), 7.29 (dd, *J* =

8.3, 7.4 Hz, 1H), 7.09-7.12 (m, 2H), 7.05 (dd, *J* = 7.4, 0.5 Hz, 1H), 6.85-6.88 (m, 2H), 4.63 (s, 2H), 4.45 (q, *J* = 7.1 Hz, 2H), 4.34 (s, 2H), 1.44 (t, *J* = 7.1 Hz, 3H), 1.24 (s, 9H). MS (FAB+) *m/z* 507 $(M + H)^+$.

4-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfon)aminomethyl)benzofuran-2-carboxylic Acid (9). 102 mg of the title compound (62% yield) was obtained as a white solid according to the procedure similar to that for **5**, using **8**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.55 (br s, 0.7H), 9.03 (d, *J* = 2.0 Hz, 1H), 8.86 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.26 (ddd, *J* = 8.3, 1.8, 1.8 Hz, 1H), 7.61-7.67 (m, 2H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.27-7.34 (m, 1H), 7.13 (d, *J* = 7.4 Hz, 1H), 6.95-7.01 (m, 2H), 6.82-6.88 (m, 2H), 4.68 (s, 2H), 4.33 (s, 2H), 1.14 (s, 9H). MS (CI+) *m/z* 479 $(M + H)^+$.

***tert*-Butyl ((5-Bromo-6-((4-*tert*-butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)(*tert*-butoxycarbonyl)amino)acetate (11)**. To 0.2 mL of a DMF solution containing 11.9 mg (0.0391 mmol) of **1a** were added 27.1 mg (0.0564 mmol) of *tert*-butyl ((5-bromo-6-(bromomethyl)pyridin-2-yl)(*tert*-butoxycarbonyl)amino)acetate (**10**) and 11.1 mg (0.0803 mmol) of K_2CO_3, and the mixture was stirred at room temperature for 3.5 h. H_2O was added to the reaction mixture and the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 1/0→1/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 23.4 mg of the title compound (85% yield) as a pale yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.99 (dd, *J* = 2.4, 0.9 Hz, 1H), 8.71 (dd, *J* = 4.9, 1.7 Hz, 1H), 7.93 (ddd, *J* = 8.1, 2.4, 1.7 Hz, 1H), 7.70 (d, *J* = 8.9 Hz, 1H), 7.62 (d, *J* = 8.9 Hz, 1H), 7.32 (ddd, *J* = 8.1, 4.9, 0.9 Hz,

1H), 7.28-7.22 (m, 2H), 7.06-7.00 (m, 2H), 4.57 (s, 4H), 4.42 (s, 2H), 1.53 (s, 9H), 1.47 (s, 9H), 1.28 (s, 9H). MS (CI+) *m/z* 703, 705 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12aa)**. To 0.6 mL of an ethanol solution containing 21.1 mg (0.0300 mmol) of **11** were added 4.0 mg of 10% palladium on carbon (Pd-C, 50% hydrate) and 0.021 mL (0.15 mmol) of triethylamine, and the mixture was stirred at room temperature for 1 h under hydrogen atmosphere at 1 atm. The insoluble material was filtered off and the filtrate was concentrated under reduced pressure. The obtained residue was subjected to reverse phase C18 column chromatography (eluent: H_2O/CH_3CN = 1/0→0/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 5.8 mg of the title compound (31% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94 (dd, J = 2.2, 0.6 Hz, 1H), 8.69 (dd, J = 4.9, 1.7 Hz, 1H), 7.84 (ddd, J = 8.1, 2.2, 1.7 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H), 7.51 (dd, J = 8.3, 7.5 Hz, 1H), 7.31-7.25 (m, 3H), 7.15-7.11 (m, 2H), 6.86 (d, J = 7.5 Hz, 1H), 4.53 (s, 2H), 4.39 (s, 2H), 4.39 (s, 2H), 1.53 (s, 9H), 1.43 (s, 9H), 1.29 (s, 9H). MS (FAB+) *m/z* 625 $(M + H)^+$.

General Procedures for the Synthesis of Precursor Compounds 12ab–ba. 12ab–ba, the precursor compounds of **13ab–ba**, respectively, were synthesized in compliance with the synthetic routes in Scheme 10 and the adopted synthetic steps for each **12** are described in Table 1. The general procedures for the synthetic steps in Scheme 10 are described below. Moreover, the specific procedures for each **12** have been detailed in ref. 26–28.

General Procedure for Step a in Scheme 10. To a THF solution containing compound **1**[26–28] (1.0 equiv.) were added *tert*-butyl (*tert*-butoxycarbonyl(6-(hydroxymethyl)pyridin-2-yl)amino)acetate (**48**)[26–28] (1.0 equiv.), tri-*n*-butylphosphine ($P(n\text{-}Bu)_3$, 1.5 equiv.) and *N*,*N*,*N*′,*N*′-

tetramethylazodicarboxamide (TMAD, 1.5 equiv.), and the mixture was stirred at room temperature. After completion of the reaction, H_2O was added to the reaction mixture and the mixture was extracted with ethyl acetate. The organic layer was washed with a saturated aqueous NaCl solution, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate), and the fractions containing the desired compound were concentrated under reduced pressure to obtain the precursor compound **12**.

General Procedure for Step b in Scheme 10. To a CH_2Cl_2 solution containing *tert*-butyl (*tert*-butoxycarbonyl(6-((4-*tert*-butylbenzyl)aminomethyl)pyridin-2-yl)amino)acetate (**49**)[26] (1.0 equiv.) were added sulfonyl chloride **50** (1.0 equiv.) and triethylamine (3.2 equiv.), and the mixture was stirred at room temperature. After completion of the reaction, the reaction mixture was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate), and the fractions containing the desired compound were concentrated under reduced pressure to obtain the precursor compound **12**.

General Procedure for Step c with 52 in Scheme 10. To a DMF solution containing *tert*-butyl (*tert*-butoxycarbonyl(6-((pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (**51**)[26–28] (1.2 equiv.) was added NaH (1.9 equiv.) at 0 °C. After the mixture was raised to room temperature, the mixture was again cooled to 0 °C and a DMF solution containing compound **52** (1.0 equiv.) was added. The mixture was raised to room temperature and stirred. After completion of the reaction, a saturated aqueous NH_4Cl solution was added to the reaction mixture and the mixture was extracted with ethyl acetate. The organic layer was washed with a saturated aqueous NaCl solution, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-

hexane/ethyl acetate), and the fractions containing the desired compound were concentrated under reduced pressure to obtain the precursor compound **12**.

General Procedure for Step c with 53 in Scheme 10. To a THF solution containing compound **51**[26–28] (1.0 equiv.) were added compound **53** (1.1 equiv.), P(*n*-Bu)$_3$ (2.0 equiv.) and TMAD (1.6 equiv.), and the mixture was stirred at room temperature. After completion of the reaction, a saturated aqueous NaCl solution was added to the reaction mixture and the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate), and the fractions containing the desired compound were concentrated under reduced pressure to obtain the precursor compound **12**.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(methylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ab).** 108 mg of the title compound (93% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, J = 8.1 Hz, 1H), 7.62 (dd, J = 8.1, 7.5 Hz, 1H), 7.37-7.34 (m, 2H), 7.28-7.25 (m, 2H), 6.96 (dd, J = 7.5, 0.6 Hz, 1H), 4.64 (s, 2H), 4.43 (s, 2H), 4.34 (s, 2H), 2.79 (s, 3H), 1.54 (s, 9H), 1.46 (s, 9H), 1.31 (s, 9H). MS (FAB+) *m/z* 562 $(M + H)^+$.

***tert*-Butyl ((6-((Phenylsulfonyl)(4-*tert*-butylbenzyl)aminomethyl)pyridin-2-yl)(*tert*-butoxycarbonyl)amino)acetate (12ac).** 247 mg of the title compound (81% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74-7.71 (m, 2H), 7.67 (d, J = 8.2 Hz, 1H), 7.54-7.50 (m, 1H), 7.47 (dd, J = 8.2, 7.4 Hz, 1H), 7.45-7.40 (m, 2H), 7.25-7.21 (m, 2H), 7.09-7.05 (m, 2H), 6.85 (d, J = 7.4 Hz, 1H), 4.47 (s, 2H), 4.37 (s, 2H), 4.35 (s, 2H), 1.52 (s, 9H), 1.42 (s, 9H), 1.27 (s, 9H). MS (FAB+) *m/z* 624 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(pyridin-2-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ad).** 311 mg of the title compound (76% yield) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60-8.57 (m, 1H), 7.83-7.79 (m, 1H), 7.75 (ddd, J = 7.7, 7.5, 1.8 Hz, 1H), 7.64 (d, J = 8.3 Hz, 1H), 7.44 (dd, J = 8.3, 7.3 Hz, 1H), 7.37 (ddd, J = 7.5, 4.4, 1.5 Hz, 1H), 7.24-7.19 (m, 2H), 7.15-7.11 (m, 2H), 6.90 (d, J = 7.3 Hz, 1H), 4.64 (s, 2H), 4.49 (s, 2H), 4.47 (s, 2H), 1.52 (s, 9H), 1.43 (s, 9H), 1.27 (s, 9H). MS (FAB+) *m/z* 625 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(2-fluorophenylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ae).** 128 mg of the title compound (74% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (ddd, J = 7.7, 7.0, 1.7 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.51-7.43 (m, 2H), 7.24-7.20 (m, 2H), 7.16 (ddd, J = 7.7, 7.7, 1.1 Hz, 1H), 7.10 (ddd, J = 10.2, 8.3, 1.1 Hz, 1H), 7.08-7.04 (m, 2H), 6.82 (d, J = 7.3 Hz, 1H), 4.57 (s, 2H), 4.48 (s, 2H), 4.45 (s, 2H), 1.52 (s, 9H), 1.44 (s, 9H), 1.27 (s, 9H).

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(2-chlorophenylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12af).** 153 mg of the title compound (quantitative yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (dd, J = 8.0, 1.5 Hz, 1H), 7.65-7.78 (m, 1H), 7.41-7.51 (m, 3H), 7.31 (ddd, J = 8.0, 7.2, 1.5 Hz, 1H), 7.20-7.24 (m, 2H), 6.98-7.02 (m, 2H), 6.83 (d, J = 7.3 Hz, 1H), 4.59 (s, 2H), 4.51 (s, 2H), 4.49 (s, 2H), 1.53 (s, 9H), 1.44 (s, 9H), 1.27 (s, 9H). MS (FAB+) *m/z* 658 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(3-fluorophenylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ag).** 128 mg of the title compound (74% yield) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (d, J = 8.4 Hz, 1H), 7.53-7.45 (m, 2H), 7.41-7.36 (m, 2H), 7.27-7.24 (m, 2H), 7.22-7.17 (m, 1H), 7.12-7.08 (m, 2H),

6.86 (d, *J* = 7.5 Hz, 1H), 4.49 (s, 2H), 4.40 (s, 2H), 4.36 (s, 2H), 1.52 (s, 9H), 1.42 (s, 9H), 1.28 (s, 9H).

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(3-chlorophenylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ah).** 139 mg of the title compound (98% yield) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68-7.72 (m, 1H), 7.65 (t, *J* = 1.8 Hz, 1H), 7.44-7.56 (m, 3H), 7.33 (t, *J* = 7.8 Hz, 1H), 7.25-7.28 (m, 2H), 7.10-7.13 (m, 2H), 6.85-6.89 (m, 1H), 4.50 (s, 2H), 4.40 (s, 2H), 4.36 (s, 2H), 1.52 (s, 9H), 1.42 (s, 9H), 1.28 (s, 9H). MS (FAB+) *m/z* 658 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(4-fluorophenylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ai).** 163 mg of the title compound (93% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72-7.66 (m, 3H), 7.49 (dd, *J* = 8.3, 7.5 Hz, 1H), 7.27-7.24 (m, 2H), 7.12-7.04 (m, 4H), 6.86 (dd, *J* = 7.5, 0.6 Hz, 1H), 4.47 (s, 2H), 4.42 (s, 2H), 4.35 (s, 2H), 1.52 (s, 9H), 1.42 (s, 9H), 1.28 (s, 9H). MS (FAB+) *m/z* 642 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(4-chlorophenylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12aj).** 131 mg of the title compound (96% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.1 Hz, 1H), 7.63-7.59 (m, 2H), 7.50 (dd, *J* = 8.1, 7.4 Hz, 1H), 7.39-7.35 (m, 2H), 7.27-7.23 (m, 2H), 7.11-7.07 (m, 2H), 6.86 (dd, *J* = 7.4, 0.6 Hz, 1H), 4.46 (s, 2H), 4.41 (s, 2H), 4.36 (s, 2H), 1.53 (s, 9H), 1.42 (s, 9H), 1.28 (s, 9H). MS (FAB+) *m/z* 658 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(4-methoxyphenylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ak).** 153 mg of the title compound (92% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68-7.64 (m, 3H), 7.48

(dd, *J* = 8.4, 7.5 Hz, 1H), 7.25-7.21 (m, 2H), 7.09-7.06 (m, 2H), 6.91-6.86 (m, 3H), 4.43 (s, 2H), 4.42 (s, 2H), 4.32 (s, 2H), 3.86 (s, 3H), 1.52 (s, 9H), 1.42 (s, 9H), 1.27 (s, 9H). MS (FAB+) *m/z* 654 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((3-*tert*-butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12al).** 289 mg of the title compound (74% yield) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (dd, *J* = 2.3, 0.8 Hz, 1H), 8.71 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.88 (ddd, *J* = 8.0, 2.3, 1.6 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.52 (dd, *J* = 8.4, 7.5 Hz, 1H), 7.31 (ddd, *J* = 8.0, 4.8, 0.8 Hz, 1H), 7.29-7.26 (m, 1H), 7.22-7.17 (m, 2H), 7.03-7.00 (m, 1H), 6.88 (d, *J* = 7.5 Hz, 1H), 4.56 (s, 2H), 4.39 (s, 2H), 4.38 (s, 2H), 1.52 (s, 9H), 1.42 (s, 9H), 1.25 (s, 9H). MS (FAB+) *m/z* 625 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-(((4-*tert*-butylcyclohexyl)methyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12am).** 114 mg of the title compound (68% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95-8.96 (m, 1H), 8.71-8.75 (m, 1H), 7.91-7.93 (m, 1H), 7.69-7.78 (m, 1H), 7.55-7.60 (m, 1H), 7.33-7.39 (m, 1H), 7.00 (d, *J* = 7.3 Hz, 1H), 4.38-4.46 (m, 4H), 3.11-3.31 (m, 2H), 1.67-1.76 (m, 4H), 1.52 (s, 9H), 1.43 (s, 9H), 0.75-0.94 (m, 15H). MS (FAB+) *m/z* 631 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((pyridin-3-ylsulfonyl)(4-trifluoromethylbenzyl)aminomethyl)pyridin-2-yl)amino)acetate (12an).** 67.8 mg of the title compound (81 % yield) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.94 (dd, *J* = 2.3, 0.7 Hz, 1H), 8.72 (dd, *J* = 4.9, 1.7 Hz, 1H), 7.86 (ddd, *J* = 8.0, 2.3, 1.7 Hz, 1H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.50 (dd, *J* = 8.3, 7.3 Hz, 1H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.32 (ddd, *J* = 8.0, 4.9, 0.7 Hz, 1H), 6.82 (d, *J* = 7.3 Hz, 1H), 4.64 (s, 2H), 4.38 (s, 2H), 4.32 (s, 2H), 1.53 (s, 9H), 1.41 (s, 9H). MS (FAB+) *m/z* 637 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-(1-methylcyclopropyl)benzyl)(pyridin-3-yl-sulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ao).** 868 mg of the title compound (91% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.93 (dd, J = 2.3, 0.8 Hz, 1H), 8.69 (dd, J = 4.8, 1.6 Hz, 1H), 7.84 (ddd, J = 8.0, 2.3, 1.6 Hz, 1H), 7.71 (d, J = 8.3 Hz, 1H), 7.51 (dd, J = 8.3, 7.4 Hz, 1H), 7.29 (ddd, J = 8.0, 4.8, 0.8 Hz, 1H), 7.15-7.09 (m, 4H), 6.85 (d, J = 7.4 Hz, 1H), 4.53 (s, 2H), 4.38 (s, 2H), 4.37 (s, 2H), 1.53 (s, 9H), 1.43 (s, 9H), 1.37 (s, 3H), 0.87-0.68 (m, 4H). MS (FAB+) *m/z* 622 $(M)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-(1-ethylcyclopropyl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ap).** 4.52 g of the title compound (97% yield) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (dd, J = 2.4, 0.7 Hz, 1H), 8.69 (dd, J = 4.9, 1.6 Hz, 1H), 7.84 (ddd, J = 8.1, 2.4, 1.6 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.50 (dd, J = 8.4, 7.4 Hz, 1H), 7.29 (ddd, J = 8.1, 4.9, 0.7 Hz, 1H), 7.20-7.14 (m, 2H), 7.13-7.08 (m, 2H), 6.86 (d, J = 7.4 Hz, 1H), 4.53 (s, 2H), 4.38 (s, 4H), 1.59-1.50 (m, 11H), 1.42 (s, 9H), 0.81 (t, J = 7.4 Hz, 3H), 0.75-0.61 (m, 4H). MS (FAB+) *m/z* 637 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-(1-isopropylcyclopropyl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12aq).** 434 mg of the title compound (53% yield) as a pale yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94 (dd, J = 2.3, 0.9 Hz, 1H), 8.69 (dd, J = 4.9, 1.7 Hz, 1H), 7.84 (ddd, J = 8.1, 2.3, 1.7 Hz, 1H), 7.70 (d, J = 8.1 Hz, 1H), 7.50 (dd, J = 8.1, 7.3 Hz, 1H), 7.29 (ddd, J = 8.1, 4.9, 0.9 Hz, 1H), 7.19-7.14 (m, 2H), 7.12-7.06 (m, 2H), 6.86 (d, J = 7.3 Hz, 1H), 4.53 (s, 2H), 4.39 (s, 4H), 1.52 (s, 9H), 1.42 (s, 9H), 1.23-1.08 (m, 1H), 0.83 (d, J = 6.8 Hz, 6H), 0.68-0.58 (m, 4H). MS (CI+) *m/z* 651 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-(1-ethylcyclobutyl)benzyl)(pyridin-3-yl-sulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ar).** 121 mg of the title compound (97%

yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (dd, J = 2.4, 0.8 Hz, 1H), 8.69 (dd, J = 4.9, 1.7 Hz, 1H), 7.85 (ddd, J = 8.0, 2.4, 1.7 Hz, 1H), 7.71 (d, J = 8.2 Hz, 1H), 7.51 (dd, J = 8.2, 7.2 Hz, 1H), 7.30 (ddd, J = 8.0, 4.9, 0.8 Hz, 1H), 7.15-7.09 (m, 2H), 7.01-6.95 (m, 2H), 6.86 (d, J = 7.2 Hz, 1H), 4.53 (s, 2H), 4.40 (s, 2H), 4.39 (s, 2H), 2.33-2.19 (m, 2H), 2.14-1.96 (m, 3H), 1.86-1.70 (m, 1H), 1.75 (q, J = 7.3 Hz, 2H), 1.52 (s, 9H), 1.43 (s, 9H), 0.60 (t, J = 7.3 Hz, 3H). MS (CI+) *m/z* 651 $(M + H)^+$.

***tert*-Butyl ((6-((Biphenyl-4-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)(*tert*-butoxycarbonyl)amino)acetate (12as).** 934 mg of the title compound (94% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (dd, J = 2.3, 0.7 Hz, 1H), 8.71 (dd, J = 4.9, 1.7 Hz, 1H), 7.87 (ddd, J = 8.0, 2.3, 1.7 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.57-7.54 (m, 2H), 7.52 (dd, J = 8.4, 7.4 Hz, 1H), 7.51-7.48 (m, 2H), 7.46-7.41 (m, 2H), 7.37-7.33 (m, 1H), 7.33-7.28 (m, 3H), 6.87 (d, J = 7.4 Hz, 1H), 4.62 (s, 2H), 4.42 (s, 2H), 4.38 (s, 2H), 1.52 (s, 9H), 1.42 (s, 9H). MS (FAB+) *m/z* 645 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((5-phenylpentyl)(pyridine-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12at).** 219 mg of the title compound (93% yield) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.98 (dd, J = 2.3, 0.7 Hz, 1H), 8.74 (dd, J = 4.8, 1.6 Hz, 1H), 7.96 (ddd, J = 8.1, 2.3, 1.6 Hz, 1H), 7.70-7.78 (m, 1H), 7.59 (dd, J = 8.3, 7.5 Hz, 1H), 7.37 (ddd, J = 8.1, 4.8, 0.7 Hz, 1H), 7.23-7.28 (m, 2H), 7.15-7.20 (m, 1H), 7.10-7.14 (m, 2H), 7.04 (d, J = 7.5 Hz, 1H), 4.40 (s, 2H), 4.39 (s, 2H), 3.24-3.29 (m, 2H), 2.51-2.55 (m, 2H), 1.40-1.57 (m, 22H), 1.17-1.30 (m, 2H). MS (FAB+) *m/z* 625 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-cyclohexylbenzyl)(pyridine-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12au).** 261 mg of the title compound (95% yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94 (dd, J = 2.3, 0.9 Hz, 1H), 8.69

(dd, *J* = 4.9, 1.7 Hz, 1H), 7.84 (ddd, *J* = 8.1, 2.3, 1.7 Hz, 1H), 7.67-7.74 (m, 1H), 7.51 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.29 (ddd, *J* = 8.1, 4.9, 0.9 Hz, 1H), 7.07-7.15 (m, 4H), 6.86 (dd, *J* = 7.4, 0.7 Hz, 1H), 4.53 (s, 2H), 4.38 (s, 4H), 2.32-2.60 (m, 1H), 1.14-1.95 (m, 28H). MS (FAB+) *m/z* 651 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-(pyridin-2-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12av).** 1.76 g (pure content 1.26 g) of the title compound (quantitative yield) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.96 (dd, *J* = 2.3, 0.9 Hz, 1H), 8.73-8.66 (m, 2H), 7.94-7.89 (m, 2H), 7.87 (ddd, *J* = 8.1, 2.3, 1.7 Hz, 1H), 7.80-7.67 (m, 2H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.51 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.38-7.32 (m, 2H), 7.31 (ddd, *J* = 8.1, 4.9, 0.9 Hz, 1H), 7.24 (ddd, *J* = 7.1, 4.8, 1.5 Hz, 1H), 6.86 (dd, *J* = 7.4, 0.6 Hz, 1H), 4.65 (s, 2H), 4.41 (s, 2H), 4.37 (s, 2H), 1.52 (s, 9H), 1.42 (s, 9H). MS (FAB+) *m/z* 646 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-(pyridazin-4-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12aw).** 152 mg of the title compound (73% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.44 (dd, *J* = 2.5, 1.2 Hz, 1H), 9.23 (dd, *J* = 5.4, 1.2 Hz, 1H), 8.96 (dd, *J* = 2.4, 0.8 Hz, 1H), 8.73 (dd, *J* = 4.8, 1.7 Hz, 1H), 7.91 (ddd, *J* = 8.0, 2.4, 1.7 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.63 (dd, *J* = 5.4, 2.5 Hz, 1H), 7.61-7.58 (m, 2H), 7.49 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.45-7.42 (m, 2H), 7.34 (ddd, *J* = 8.0, 4.8, 0.8 Hz, 1H), 6.84 (dd, *J* = 7.3, 0.6 Hz, 1H), 4.65 (s, 2H), 4.41 (s, 2H), 4.34 (s, 2H), 1.53 (s, 9H), 1.42 (s, 9H). MS (FAB+) *m/z* 647 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4-(pyrazol-1-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12ax).** 6.57 g of the title compound (94% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.95 (dd, *J* = 2.3, 0.7 Hz, 1H), 8.71

(dd, J = 4.9, 1.6 Hz, 1H), 7.91 (dd, J = 2.5, 0.6 Hz, 1H), 7.87 (ddd, J = 8.0, 2.3, 1.6 Hz, 1H), 7.72 (dd, J = 1.8, 0.6 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.63-7.60 (m, 2H), 7.51 (dd, J = 8.4, 7.3 Hz, 1H), 7.35-7.30 (m, 3H), 6.85 (d, J = 7.3 Hz, 1H), 6.47 (dd, J = 2.5, 1.8 Hz, 1H), 4.61 (s, 2H), 4.39 (s, 2H), 4.35 (s, 2H), 1.53 (s, 9H), 1.42 (s, 9H). MS (FAB+) *m/z* 635 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((pyridin-3-ylsulfonyl)(4-(1,2,4-triazol-1-yl)-benzyl)aminomethyl)pyridin-2-yl)amino)acetate (12ay).** 938 mg of the title compound (84% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (dd, J = 2.3, 0.7 Hz, 1H), 8.73 (dd, J = 4.9, 1.7 Hz, 1H), 8.56 (s, 1H), 8.10 (s, 1H), 7.90 (ddd, J = 8.0, 2.3, 1.7 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.62-7.57 (m, 2H), 7.49 (dd, J = 8.3, 7.4 Hz, 1H), 7.42-7.38 (m, 2H), 7.34 (ddd, J = 8.0, 4.9, 0.7 Hz, 1H), 6.83 (d, J = 7.4 Hz, 1H), 4.63 (s, 2H), 4.39 (s, 2H), 4.33 (s, 2H), 1.53 (s, 9H), 1.42 (s, 9H). MS (FAB+) *m/z* 636 $(M + H)^+$.

***tert*-Butyl ((6-((Benzofuran-2-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)(*tert*-butoxycarbonyl)amino)acetate (12az).** 397 mg of the title compound (88% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.04 (d, J = 2.0 Hz, 1H), 8.66 (dd, J = 4.9, 1.7 Hz, 1H), 7.96 (ddd, J = 8.1, 2.0, 1.7 Hz, 1H), 7.79 (d, J = 8.3 Hz, 1H), 7.62 (dd, J = 8.3, 7.4 Hz, 1H), 7.48-7.44 (m, 1H), 7.26-7.16 (m, 4H), 7.09 (d, J = 7.4 Hz, 1H), 6.55 (s, 1H), 4.69 (s, 2H), 4.51 (s, 2H), 4.50 (s, 2H), 1.53 (s, 9H), 1.42 (s, 9H). MS (FAB+) *m/z* 609 $(M + H)^+$.

***tert*-Butyl ((6-((Benzo[*b*]thiophen-2-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)(*tert*-butoxycarbonyl)amino)acetate (12ba).** 153 mg of the title compound (93% yield) as a slightly yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.99 (dd, J = 2.3, 0.9 Hz, 1H), 8.69 (dd, J = 4.8, 1.6 Hz, 1H), 7.91 (ddd, J = 8.1, 2.3, 1.6 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.74-7.66 (m, 2H), 7.57 (dd, J = 8.4, 7.4 Hz, 1H), 7.35-7.28 (m, 2H), 7.28 (ddd, J = 8.1, 4.8, 0.9 Hz, 1H), 7.12

(d, J = 0.7 Hz, 1H), 6.96 (dd, J = 7.4, 0.6 Hz, 1H), 4.84 (s, 2H), 4.50 (s, 2H), 4.44 (s, 2H), 1.53 (s, 9H), 1.42 (s, 9H).

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4'-fluorobiphenyl-4-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12bb).** To 2 mL of a toluene solution containing 185 mg (0.285 mmol) of *tert*-butyl ((6-((4-bromobenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)(*tert*-butoxycarbonyl)amino)acetate (**12a**) were added 61.3 mg (0.438 mmol) of 4-fluorophenylboronic acid, 4.9 mg (0.044 mmol) of palladium acetate, 202 mg (0.953 mmol) of K_3PO_4, and 0.2 mL of H_2O, followed by being subjected to argon atmosphere. Then 130 μL (0.088 mmol) of 20% tricyclohexylphosphine in toluene was added to the mixture, and the mixture was stirred at 100 °C for 2.5 h under argon atmosphere. A saturated aqueous NaCl solution was added to the reaction mixture and the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 17/3), and fractions containing the desired compound were concentrated under reduced pressure to obtain 178 mg of the title compound (94% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (dd, J = 2.4, 0.8 Hz, 1H), 8.71 (dd, J = 4.8, 1.7 Hz, 1H), 7.88 (ddd, J = 8.1, 2.4, 1.7 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.53-7.49 (m, 3H), 7.46-7.43 (m, 2H), 7.31 (ddd, J = 8.1, 4.8, 0.8 Hz, 1H), 7.31-7.28 (m, 2H), 7.15-7.10 (m, 2H), 6.86 (dd, J = 7.3, 0.6 Hz, 1H), 4.62 (s, 2H), 4.41 (s, 2H), 4.37 (s, 2H), 1.52 (s, 9H), 1.42 (s, 9H).

***tert*-Butyl (*tert*-Butoxycarbonyl(6-((4'-chlorobiphenyl-4-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (12bc).** 166 mg of the title compound (84% yield) was obtained as a colorless oil according to the procedure similar to that for **12bb**, using **12a** and 4-chlorophenylboronic acid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (dd, J = 2.4, 0.8

Hz, 1H), 8.71 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.88 (ddd, *J* = 8.0, 2.4, 1.6 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.51 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.50-7.44 (m, 4H), 7.42-7.39 (m, 2H), 7.32-7.28 (m, 2H), 7.31 (ddd, *J* = 8.0, 4.8, 0.8 Hz, 1H), 6.86 (dd, *J* = 7.3, 0.6 Hz, 1H), 4.62 (s, 2H), 4.41 (s, 2H), 4.36 (s, 2H), 1.52 (s, 9H), 1.42 (s, 9H).

(6-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13aa). 35.0 mg of the title compound (quantitative yield) was obtained as a white solid according to the procedure similar to the general procedure for step d in Scheme 10, using **12aa**. ^{1}H NMR (500 MHz, CD_3OD) δ 9.12 (dd, *J* = 2.4, 0.8 Hz, 1H), 8.90 (dd, *J* = 5.0, 1.5 Hz, 1H), 8.41 (ddd, *J* = 8.1, 2.4, 1.5 Hz, 1H), 7.75 (ddd, *J* = 8.1, 5.0, 0.8 Hz, 1H), 7.69 (dd, *J* = 9.0, 7.3 Hz, 1H), 7.25-7.21 (m, 2H), 7.16-7.12 (m, 2H), 6.83 (d, *J* = 9.0 Hz, 1H), 6.61-6.56 (m, 1H), 4.53 (s, 2H), 4.45 (s, 2H), 4.15 (s, 2H), 1.23 (s, 9H). MS (FAB+) *m/z* 469 $(M + H)^+$. TLC R_f value 0.45 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

General Procedure for the Synthesis of Aminopyridyl Derivatives 13ab–ba (Step d in Scheme 10). A deprotection of the precursor compounds **12** was achieved under acidic conditions. A typical procedure: to a CH_2Cl_2 solution containing the precursor compound **12** (1.0 equiv.) was added 4 M HCl in 1,4-dioxane, and the mixture was stirred at room temperature. The purification process was conducted in the usual manner and the aminopyridyl derivatives **13** could be obtained as a free form or a salt form with acid (ex. hydrochloride) either. The specific procedures for each **13** have been detailed in ref. 26–28.

(6-((4-*tert*-Butylbenzyl)(methylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13ab). 85.1 mg of the title compound (quantitative yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.67 (dd, *J* = 8.6, 7.3 Hz, 1H), 7.29-7.25 (m, 2H), 7.23-7.19 (m, 2H),

6.81 (d, J = 8.6 Hz, 1H), 6.58 (d, J = 7.3 Hz, 1H), 4.51 (s, 2H), 4.46 (s, 2H), 4.13 (s, 2H), 3.09 (s, 3H), 1.24 (s, 9H). TLC R_f value 0.64 (n-butanol/CH_3CO_2H/H_2O = 3/1/1).

(6-((Phenylsulfonyl)(4-*tert*-butylbenzyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13ac). 161 mg of the title compound (83% yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.98-7.93 (m, 2H), 7.77-7.72 (m, 1H), 7.70-7.61 (m, 3H), 7.24-7.20 (m, 2H), 7.14-7.10 (m, 2H), 6.79 (d, J = 8.8 Hz, 1H), 6.52 (d, J = 7.2 Hz, 1H), 4.42 (s, 2H), 4.38 (s, 2H), 4.13 (s, 2H), 1.22 (s, 9H). MS (FAB+) m/z 468 $(M + H)^+$. TLC R_f value 0.58 (n-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((4-*tert*-Butylbenzyl)(pyridin-2-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13ad). 198 mg of the title compound (76% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.76-8.71 (m, 1H), 8.08 (ddd, J = 7.6, 7.6, 1.8 Hz, 1H), 8.05-8.02 (m, 1H), 7.70-7.64 (m, 2H), 7.25-7.19 (m, 2H), 7.15-7.11 (m, 2H), 6.80 (d, J = 9.3 Hz, 1H), 6.62 (d, J = 7.3 Hz, 1H), 4.71 (s, 2H), 4.48 (s, 2H), 4.14 (s, 2H), 1.23 (s, 9H). MS (FAB+) m/z 469 $(M + H)^+$. TLC R_f value 0.40 (n-butanol/CH_3CO_2H/H_2O = 10/1/1). The purity [%] determined with HPLC: 91.

(6-((4-*tert*-Butylbenzyl)(2-fluorophenylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13ae). 82.0 mg of the title compound (83% yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.97 (ddd, J = 7.9, 7.2, 1.8 Hz, 1H), 7.77-7.72 (m, 1H), 7.67 (dd, J = 9.0, 7.3 Hz, 1H), 7.43-7.37 (m, 2H), 7.24-7.20 (m, 2H), 7.13-7.09 (m, 2H), 6.81 (d, J = 9.0 Hz, 1H), 6.61 (d, J = 7.3 Hz, 1H), 4.58 (s, 2H), 4.47 (s, 2H), 4.14 (s, 2H), 1.23 (s, 9H). MS (FAB+) m/z 486 $(M + H)^+$. TLC R_f value 0.63 (n-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((4-*tert*-Butylbenzyl)(2-chlorophenylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13af). 105 mg of the title compound (90% yield) as a white solid. ^{1}H

NMR (500 MHz, CD_3OD) δ 8.13-8.16 (m, 1H), 7.63-7.71 (m, 3H), 7.52 (ddd, J = 8.1, 6.8, 2.0 Hz, 1H), 7.21-7.24 (m, 2H), 7.07-7.10 (m, 2H), 6.83 (br d, J = 9.0 Hz, 1H), 6.66-6.69 (m, 1H), 4.63 (s, 2H), 4.54 (s, 2H), 4.17 (s, 2H), 1.24 (s, 9H). MS (FAB+) *m/z* 502 $(M + H)^+$.

(6-((4-*tert*-Butylbenzyl)(3-fluorophenylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13ag). 68.0 mg of the title compound (73% yield) as a white solid. ^1H NMR (500 MHz, CD_3OD) δ 7.79-7.74 (m, 1H), 7.72-7.66 (m, 3H), 7.64 (dd, J = 8.8, 7.4 Hz, 1H), 7.52-7.47 (m, 1H), 7.25-7.21 (m, 2H), 7.15-7.12 (m, 2H), 6.78 (d, J = 8.8 Hz, 1H), 6.54 (dd, J = 7.4, 0.7 Hz, 1H), 4.45 (s, 2H), 4.42 (s, 2H), 4.12 (s, 2H), 1.23 (s, 9H). MS (FAB+) *m/z* 486 $(M + H)^+$. TLC R_f value 0.67 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((4-*tert*-Butylbenzyl)(3-chlorophenylsulfonyl)aminomethyl)pyridin-2-ylamino)acetate Hydrochloride (13ah). 95 mg of the title compound (85% yield) as a white solid. ^1H NMR (500 MHz, CD_3OD) δ 7.91-7.93 (m, 1H), 7.85-7.88 (m, 1H), 7.72-7.76 (m, 1H), 7.62-7.67 (m, 2H), 7.22-7.25 (m, 2H), 7.12-7.15 (m, 2H), 6.79 (d, J = 8.8 Hz, 1H), 6.53-6.56 (m, 1H), 4.46 (s, 2H), 4.42 (s, 2H), 4.12 (s, 2H), 1.23 (s, 9H). MS (FAB+) *m/z* 502 $(M + H)^+$.

(6-((4-*tert*-Butylbenzyl)(4-fluorophenylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13ai). 137 mg of the title compound (quantitative yield) as a white solid. ^1H NMR (500 MHz, CD_3OD) δ 8.02-7.96 (m, 2H), 7.64 (dd, J = 8.8, 7.3 Hz, 1H), 7.42-7.35 (m, 2H), 7.25-7.21 (m, 2H), 7.15-7.11 (m, 2H), 6.78 (d, J = 8.8 Hz, 1H), 6.53 (dd, J = 7.3, 0.6 Hz, 1H), 4.42 (s, 2H), 4.40 (s, 2H), 4.12 (s, 2H), 1.23 (s, 9H). MS (FAB+) *m/z* 486 $(M + H)^+$. TLC R_f value 0.67 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((4-*tert*-Butylbenzyl)(4-chlorophenylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13aj). 113 mg of the title compound (quantitative yield) as a white solid. ^1H NMR (500 MHz, CD_3OD) δ 7.92-7.88 (m, 2H), 7.68-7.61 (m, 3H), 7.25-7.21 (m, 2H), 7.15-

7.11 (m, 2H), 6.78 (d, J = 9.0 Hz, 1H), 6.54 (dd, J = 7.3, 0.6 Hz, 1H), 4.43 (s, 2H), 4.40 (s, 2H), 4.11 (s, 2H), 1.23 (s, 9H). MS (FAB+) *m/z* 503 $(M + H)^+$. TLC R_f value 0.72 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((4-*tert*-Butylbenzyl)(4-methoxyphenylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13ak). 119 mg of the title compound (98% yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.86-7.81 (m, 2H), 7.58 (dd, J = 8.9, 7.2 Hz, 1H), 7.25-7.20 (m, 2H), 7.16-7.09 (m, 4H), 6.72 (d, J = 8.9 Hz, 1H), 6.49 (d, J = 7.2 Hz, 1H), 4.37 (s, 2H), 4.35 (s, 2H), 4.09 (s, 2H), 3.90 (s, 3H), 1.23 (s, 9H). MS (FAB+) *m/z* 498 $(M + H)^+$. TLC R_f value 0.55 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((3-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13al). 137 mg of the title compound (89% yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.28 (d, J = 2.1 Hz, 1H), 9.02 (dd, J = 5.3, 1.4 Hz, 1H), 8.72 (ddd, J = 8.2, 2.1, 1.4 Hz, 1H), 8.01 (dd, J = 8.2, 5.3 Hz, 1H), 7.72 (dd, J = 8.9, 7.4 Hz, 1H), 7.27-7.24 (m, 2H), 7.21-7.16 (m, 1H), 7.14-7.10 (m, 1H), 6.86 (d, J = 8.9 Hz, 1H), 6.61 (d, J = 7.4 Hz, 1H), 4.64 (s, 2H), 4.54 (s, 2H), 4.17 (s, 2H), 1.22 (s, 9H). MS (FAB+) *m/z* 469 $(M + H)^+$. TLC R_f value 0.46 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-(((4-*tert*-Butylcyclohexyl)methyl)(pyridine-3-ylsulfonyl)aminomethyl)pyridin-2-amino)acetic Acid Hydrochloride (13am). 82 mg of the title compound (84% yield, diastereomer mixture: cis/trans = 8/92) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.13 (dd, J = 2.2, 0.8 Hz, 1H), 8.91 (dd, J = 5.1, 1.5 Hz, 1H), 8.48 (ddd, J = 8.0, 2.2, 1.5 Hz, 1H), 7.97 (dd, J = 8.9, 7.4 Hz, 1H), 7.82 (ddd, J = 8.0, 5.1, 0.8 Hz, 1H), 7.06-7.09 (m, 1H), 6.99 (dd, J = 7.4, 0.7 Hz, 1H), 4.54 (s, 2H), 4.28-4.30 (m, 2H), 3.13-3.18 (m, 2H), 1.72-1.79 (m, 2H), 1.63-1.72 (m, 2H), 0.82-0.94 (m, 6H), 0.81 (s, 9H). MS (FAB+) *m/z* 475 $(M + H)^+$.

(6-((Pyridin-3-ylsulfonyl)(4-trifluoromethylbenzyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13an). 42.8 mg of the title compound (76% yield) as a pale yellow solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.16 (d, J = 2.2 Hz, 1H), 8.92 (dd, J = 5.0, 1.5 Hz, 1H), 8.46 (ddd, J = 8.1, 2.2, 1.5 Hz, 1H), 7.78 (ddd, J = 8.1, 5.0, 0.6 Hz, 1H), 7.70 (dd, J = 9.0, 7.4 Hz, 1H), 7.52 (d, J = 8.2 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H), 6.85 (d, J = 9.0 Hz, 1H), 6.66 (d, J = 7.4 Hz, 1H), 4.59 (s, 2H), 4.59 (s, 2H), 4.13 (s, 2H). MS (FAB+) *m/z* 481 $(M + H)^+$. TLC R_f value 0.45 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((4-(1-Methylcyclopropyl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13ao). 694 mg of the title compound (quantitative yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.36 (d, J = 1.9 Hz, 1H), 9.06 (dd, J = 5.4, 1.4 Hz, 1H), 8.82 (ddd, J = 8.2, 1.9, 1.4 Hz, 1H), 8.10 (ddd, J = 8.2, 5.4, 0.6 Hz, 1H), 7.73 (dd, J = 8.9, 7.3 Hz, 1H), 7.17-7.13 (m, 2H), 7.09-7.05 (m, 2H), 6.85 (d, J = 8.9 Hz, 1H), 6.68 (d, J = 7.3 Hz, 1H), 4.64 (s, 2H), 4.51 (s, 2H), 4.16 (s, 2H), 1.32 (s, 3H), 0.77-0.69 (m, 4H). MS (FAB+) *m/z* 467 $(M + H)^+$. TLC R_f value 0.45 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((4-(1-Ethylcyclopropyl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid (13ap). 2.47 g of the title compound (74% yield) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.37 (br s, 0.9H), 8.79 (dd, J = 2.4, 0.8 Hz, 1H), 8.70 (dd, J = 4.8, 1.5 Hz, 1H), 7.98 (ddd, J = 8.0, 2.4, 1.5 Hz, 1H), 7.45 (ddd, J = 8.0, 4.8, 0.8 Hz, 1H), 7.23 (dd, J = 8.2, 7.1 Hz, 1H), 7.21-7.16 (m, 4H), 6.75 (t, J = 5.6 Hz, 0.9H), 6.36 (d, J = 8.2 Hz, 1H), 6.30 (d, J = 7.1 Hz, 1H), 4.61 (s, 2H), 4.16 (s, 2H), 3.70 (d, J = 5.6 Hz, 2H), 1.55 (q, J = 7.3 Hz, 2H), 0.77 (t, J = 7.3 Hz, 3H), 0.73-0.64 (m, 4H). ^{13}C NMR (proton-decoupled spectrum, 500 MHz, DMSO-d_6) δ 172.5 (s), 157.6 (s), 152.8 (s), 152.6 (s), 146.9 (s), 144.0 (s), 136.8 (s), 136.6 (s), 134.4 (s), 133.3 (s), 128.2 (s), 128.2 (s), 123.7 (s), 110.7 (s), 107.7 (s), 51.0 (s), 50.9 (s), 42.3 (s),

31.8 (s), 25.9 (s), 12.9 (s), 11.2 (s). MS (FAB+) *m/z* 481 $(M + H)^+$. TLC R_f value 0.48 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((4-(1-Isopropylcyclopropyl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid (13aq). 261 mg of the title compound (82% yield) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 9.06 (dd, J = 2.4, 0.7 Hz, 1H), 8.73 (dd, J = 4.9, 1.6 Hz, 1H), 7.96 (ddd, J = 8.1, 2.4, 1.6 Hz, 1H), 7.37-7.28 (m, 2H), 7.18 (s, 4H), 6.51 (d, J = 7.3 Hz, 1H), 6.29 (d, J = 8.1 Hz, 1H), 4.60 (s, 2H), 4.36 (s, 2H), 3.88 (s, 2H), 1.23-1.08 (m, 1H), 0.83 (d, J = 6.8 Hz, 6H), 0.69-0.58 (m, 4H). MS (FAB+) *m/z* 495 $(M + H)^+$. TLC R_f value 0.49 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((4-(1-Ethylcyclobutyl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid (13ar). 54.2 mg of the title compound (70% yield) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.74 (s, 1H), 7.96 (d, J = 8.3 Hz, 1H), 7.39-7.17 (m, 4H), 7.04-6.96 (m, 2H), 6.47 (d, J = 7.1 Hz, 1H), 6.27 (d, J = 8.3 Hz, 1H), 4.62 (s, 2H), 4.35 (s, 2H), 3.85 (s, 2H), 2.34-2.19 (m, 2H), 2.14-1.96 (m, 3H), 1.86-1.69 (m, 1H), 1.75 (q, J = 7.3 Hz, 2H), 0.60 (t, J = 7.3 Hz, 3H). MS (FAB+) *m/z* 495 $(M + H)^+$. TLC R_f value 0.49 (*n*-butanol/CH_3CO_2H/H_2O = 10/1/1).

(6-((Biphenyl-4-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13as). 760 mg of the title compound (94% yield) as a white solid. ^1H NMR (500 MHz, CD_3OD) δ 9.26 (dd, J = 2.3, 0.8 Hz, 1H), 8.99 (dd, J = 5.2, 1.5 Hz, 1H), 8.65 (ddd, J = 8.1, 2.3, 1.5 Hz, 1H), 7.94 (ddd, J = 8.1, 5.2, 0.8 Hz, 1H), 7.72 (dd, J = 8.9, 7.3 Hz, 1H), 7.54-7.51 (m, 2H), 7.48-7.40 (m, 4H), 7.36-7.29 (m, 3H), 6.80 (d, J = 8.9 Hz, 1H), 6.74 (d, J = 7.3 Hz, 1H), 4.63 (s, 2H), 4.56 (s, 2H), 3.99 (s, 2H). MS (FAB+) *m/z* 489 $(M + H)^+$. TLC R_f value 0.62 (*n*-butanol/CH_3CO_2H/H_2O = 3/1/1).

(6-((5-Phenylpentyl)(pyridine-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13at). 184 mg of the title compound (98% yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.16 (dd, J = 2.3, 0.8 Hz, 1H), 8.93 (dd, J = 5.1, 1.5 Hz, 1H), 8.52 (ddd, J = 8.2, 2.3, 1.5 Hz, 1H), 7.94 (dd, J = 9.0, 7.3 Hz, 1H), 7.85 (ddd, J = 8.2, 5.1, 0.8 Hz, 1H), 7.20-7.24 (m, 2H), 7.04-7.14 (m, 4H), 6.95-6.98 (m, 1H), 4.55 (s, 2H), 4.27 (s, 2H), 3.31-3.35 (m, 2H), 2.52 (t, J = 7.5 Hz, 2H), 1.44-1.56 (m, 4H), 1.18-1.30 (m, 2H). MS (FAB+) *m/z* 469 $(M + H)^+$.

(6-((4-Cyclohexylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid (13au). 186 mg of the title compound (94% yield) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.46 (br s, 0.7H), 8.78-8.80 (m, 1H), 8.70 (dd, J = 4.8, 1.6 Hz, 1H), 7.98 (ddd, J = 8.2, 2.3, 1.6 Hz, 1H), 7.45 (ddd, J = 8.2, 4.8, 0.7 Hz, 1H), 7.23 (dd, J = 8.3, 7.2 Hz, 1H), 7.14-7.21 (m, 4H), 6.65-6.76 (m, 1H), 6.36 (d, J = 8.3 Hz, 1H), 6.30 (d, J = 7.2 Hz, 1H), 4.61 (s, 2H), 4.16 (s, 2H), 3.68 (d, J = 5.3 Hz, 2H), 2.43-2.49 (m, 1H), 1.63-1.87 (m, 5H), 1.15-1.48 (m, 5H). MS (FAB+) *m/z* 495 $(M + H)^+$.

(6-((4-(Pyridin-2-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid (13av). 553 mg of the title compound (58% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (dd, J = 2.2, 0.5 Hz, 1H), 8.79 (dd, J = 4.8, 1.6 Hz, 1H), 8.67 (ddd, J = 4.9, 1.6, 0.9 Hz, 1H), 8.08 (ddd, J = 8.1, 2.2, 1.6 Hz, 1H), 7.81 (ddd, J = 7.9, 7.8, 1.6 Hz, 1H), 7.75-7.69 (m, 2H), 7.64 (ddd, J = 7.9, 1.0, 0.9 Hz, 1H), 7.43 (ddd, J = 8.1, 4.8, 0.5 Hz, 1H), 7.34-7.27 (m, 2H), 7.23-7.17 (m, 2H), 6.58 (d, J = 7.1 Hz, 1H), 6.29 (d, J = 8.3 Hz, 1H), 4.58 (s, 2H), 4.28 (s, 2H), 3.86 (s, 2H). MS (FAB+) *m/z* 490 $(M + H)^+$. TLC R_f value 0.35 (*n*-butanol/CH_3CO_2H/H_2O = 3/1/1).

(6-((4-(Pyridazin-4-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13aw). 137 mg of the title compound (98% yield) as a

slightly yellow solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.91 (dd, J = 2.4, 0.9 Hz, 1H), 9.58 (dd, J = 6.0, 0.9 Hz, 1H), 9.23 (d, J = 2.0 Hz, 1H), 8.98 (dd, J = 5.2, 1.5 Hz, 1H), 8.77 (dd, J = 6.0, 2.4 Hz, 1H), 8.63 (ddd, J = 8.2, 2.0, 1.5 Hz, 1H), 7.99-7.95 (m, 2H), 7.92 (ddd, J = 8.2, 5.2, 0.5 Hz, 1H), 7.71 (dd, J = 8.9, 7.3 Hz, 1H), 7.61-7.57 (m, 2H), 6.83 (d, J = 8.9 Hz, 1H), 6.76 (d, J = 7.3 Hz, 1H), 4.69 (s, 2H), 4.69 (s, 2H), 4.17 (s, 2H). MS (FAB+) *m/z* 491 $(M + H)^+$. TLC R_f value 0.38 (*n*-butanol/CH_3CO_2H/H_2O = 3/1/1).

(6-((4-(Pyrazol-1-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid (13ax, OMD). 4.61 g of the title compound (94% yield) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.41 (br s, 1H), 8.84 (dd, J = 2.3, 0.7 Hz, 1H), 8.72 (dd, J = 4.8, 1.6 Hz, 1H), 8.48 (dd, J = 2.5, 0.4 Hz, 1H), 8.04 (ddd, J = 8.1, 2.3, 1.6 Hz, 1H), 7.81-7.77 (m, 2H), 7.74 (d, J = 1.7 Hz, 1H), 7.48 (ddd, J = 8.1, 4.8, 0.7 Hz, 1H), 7.41-7.37 (m, 2H), 7.24 (dd, J = 8.3, 7.1 Hz, 1H), 6.79 (t, J = 5.9 Hz, 1H), 6.54 (dd, J = 2.5, 1.7 Hz, 1H), 6.37 (d, J = 8.3 Hz, 1H), 6.33 (d, J = 7.1 Hz, 1H), 4.69 (s, 2H), 4.21 (s, 2H), 3.72 (d, J = 5.9 Hz, 2H). ^{13}C NMR (proton-decoupled spectrum, 500 MHz, DMSO-d_6) δ 172.6 (s), 157.7 (s), 152.8 (s), 152.7 (s), 147.1 (s), 140.9 (s), 139.1 (s), 136.9 (s), 136.6 (s), 134.6 (s), 134.0 (s), 129.5 (s), 127.7 (s), 123.9 (s), 118.3 (s), 110.9 (s), 107.8 (assigned for two nonequivalent carbons with identical chemical shift), 51.1 (s), 50.8 (s), 42.4 (s). MS (FAB+) *m/z* 479 $(M + H)^+$. IR wavelength [cm^{-1}] 3372 (N–H), 3200-2500 (O–H), 1721 (C=O), 1603 and 1526 (C=C and C=N), 1337 (SO_2), 1167 (SO_2), 781 (C–H). Elemental analysis [%] (average of three experiments) calculated for $C_{23}H_{22}N_6O_4S$: C 57.73, H 4.63, N 17.56. Found: C 57.71, H 4.33, N 17.60.

(6-((Pyridin-3-ylsulfonyl)(4-(1,2,4-triazol-1-yl)benzyl)aminomethyl)pyridin-2-ylamino)acetic Acid (13ay). 618 mg of the title compound (88% yield) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.27 (s, 1H), 8.85 (dd, J = 2.4, 0.8 Hz, 1H), 8.73 (dd, J = 4.8, 1.7

Hz, 1H), 8.24 (s, 1H), 8.05 (ddd, *J* = 8.1, 2.4, 1.7 Hz, 1H), 7.84-7.79 (m, 2H), 7.49 (ddd, *J* = 8.1, 4.8, 0.8 Hz, 1H), 7.47-7.43 (m, 2H), 7.24 (dd, *J* = 8.3, 7.1 Hz, 1H), 6.75 (t, *J* = 5.6 Hz, 1H), 6.36 (d, *J* = 8.3 Hz, 1H), 6.33 (d, *J* = 7.1 Hz, 1H), 4.71 (s, 2H), 4.21 (s, 2H), 3.69 (d, *J* = 5.6 Hz, 2H). MS (FAB+) *m/z* 480 $(M + H)^+$. TLC R_f value 0.36 (*n*-butanol/CH_3CO_2H/H_2O = 3/1/1).

(6-((Benzofuran-2-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13az). 134 mg of the title compound (77% yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.28 (dd, *J* = 2.3, 0.5 Hz, 1H), 8.89 (dd, *J* = 5.3, 1.4 Hz, 1H), 8.70 (ddd, *J* = 8.2, 2.3, 1.4 Hz, 1H), 7.90 (ddd, *J* = 8.2, 5.3, 0.5 Hz, 1H), 7.78 (dd, *J* = 9.0, 7.2 Hz, 1H), 7.47-7.44 (m, 1H), 7.27-7.22 (m, 2H), 7.21-7.15 (m, 1H), 6.88 (d, *J* = 7.2 Hz, 1H), 6.82 (d, *J* = 9.0 Hz, 1H), 6.73 (s, 1H), 4.77 (s, 2H), 4.76 (s, 2H), 4.12 (s, 2H). MS (FAB+) *m/z* 453 $(M + H)^+$. TLC R_f value 0.59 (*n*-butanol/CH_3CO_2H/H_2O = 3/1/1).

(6-((Benzo[*b*]thiophen-2-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13ba). 96.1 mg of the title compound (74% yield) as a white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 9.27 (dd, *J* = 2.3, 0.7 Hz, 1H), 8.94 (dd, *J* = 5.2, 1.5 Hz, 1H), 8.66 (ddd, *J* = 8.1, 2.3, 1.5 Hz, 1H), 7.89 (ddd, *J* = 8.1, 5.2, 0.7 Hz, 1H), 7.75-7.64 (m, 3H), 7.34-7.28 (m, 2H), 7.17 (s, 1H), 6.85 (dd, *J* = 7.3, 0.7 Hz, 1H), 6.76 (d, *J* = 9.0 Hz, 1H), 4.87 (s, 2H), 4.72 (s, 2H), 4.00 (s, 2H). MS (FAB+) *m/z* 469 $(M + H)^+$. TLC R_f value 0.60 (*n*-butanol/CH_3CO_2H/H_2O = 3/1/1).

(6-((4'-Fluorobiphenyl-4-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13bb). 134 mg of the title compound (89% yield) was obtained as a white solid according to the procedure similar to the general procedure for step d in Scheme 10, using **12bb**. ^{1}H NMR (500 MHz, CD_3OD) δ 9.29 (dd, *J* = 2.2, 0.7 Hz, 1H), 9.01 (dd, *J* = 5.3, 1.5 Hz, 1H), 8.69 (ddd, *J* = 8.2, 2.2, 1.5 Hz, 1H), 7.98 (ddd, *J* = 8.2, 5.3, 0.7 Hz, 1H),

7.72 (dd, J = 9.0, 7.3 Hz, 1H), 7.57-7.53 (m, 2H), 7.46-7.42 (m, 2H), 7.33-7.30 (m, 2H), 7.18-7.13 (m, 2H), 6.82 (d, J = 9.0 Hz, 1H), 6.74 (d, J = 7.3 Hz, 1H), 4.65 (s, 2H), 4.57 (s, 2H), 4.03 (s, 2H). MS (FAB+) *m/z* 507 $(M + H)^+$. TLC R_f value 0.62 (*n*-butanol/CH_3CO_2H/H_2O = 3/1/1).

(6-((4'-Chlorobiphenyl-4-ylmethyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (13bc). 133 mg of the title compound (93% yield) was obtained as a white solid according to the procedure similar to the general procedure for step d in Scheme 10, using **12bc**. ^{1}H NMR (500 MHz, CD_3OD) δ 9.31 (dd, J = 2.2, 0.6 Hz, 1H), 9.02 (dd, J = 5.3, 1.4 Hz, 1H), 8.74 (ddd, J = 8.1, 2.2, 1.4 Hz, 1H), 8.02 (ddd, J = 8.1, 5.3, 0.6 Hz, 1H), 7.72 (dd, J = 9.0, 7.3 Hz, 1H), 7.55-7.51 (m, 2H), 7.48-7.41 (m, 4H), 7.35-7.32 (m, 2H), 6.81 (d, J = 9.0 Hz, 1H), 6.74 (d, J = 7.3 Hz, 1H), 4.66 (s, 2H), 4.58 (s, 2H), 4.03 (s, 2H). MS (FAB+) *m/z* 523 $(M + H)^+$. TLC R_f value 0.64 (*n*-butanol/CH_3CO_2H/H_2O = 3/1/1). The purity [%] determined with HPLC: 86.

***tert*-Butyl (4-(((*tert*-Butyldimethylsilyl)oxy)methyl)pyridin-2-yl)carbamate (15).** To a solution consisting of 6 mL of *tert*-butanol and 1 mL of ethyl acetate, containing 824 mg (3.78 mmol) of di-*tert*-butyl dicarbonate was added 450 mg (1.89 mmol) of 4-(((*tert*-butyldimethylsilyl)oxy)methyl)pyridin-2-amine (**14**) dissolved in 4.7 mL of pyridine at room temperature, and the mixture was stirred at the same temperature overnight. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatograph (eluent: *n*-hexane/ethyl acetate = 4/1→2/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 398 mg of the title compound (62% yield) as a pale brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (dd, J = 5.3, 0.6 Hz, 1H), 7.80-7.84 (m, 1H), 7.46 (s, 1H), 7.01-7.06 (m, 1H),

4.72-4.75 (m, 2H), 1.52 (s, 9H), 0.93-0.97 (m, 9H), 0.08-0.14 (m, 6H). MS (FAB+) *m/z* 339 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(4-(((*tert*-butyldimethylsilyl)oxy)methyl)pyridin-2-yl)amino)acetate (16).** To 6 mL of a DMF solution containing 394 mg (1.16 mmol) of **15** was added 61 mg (1.4 mmol) of NaH (55%, oil coated) portionwise under argon atmosphere with H_2O cooling. After the reaction mixture was stirred at 0 °C for 20 min, 206 μL (1.41mmol) of *tert*-butyl bromoacetate was added dropwise. After the completion of the addition, the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was allowed to cool to -20 °C and 18 g of ice was added, followed by stirring at room temperature, then extracting with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 10/1→0/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 456 mg of the title compound (87% yield) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (dd, J = 5.1, 0.7 Hz, 1H), 7.73 (s, 1H), 7.00-7.05 (m, 1H), 4.72-4.76 (m, 2H), 4.59 (s, 2H), 1.52 (s, 9H), 1.45 (s, 9H), 0.93-0.97 (m, 9H), 0.09-0.12 (m, 6H). MS (FAB+) *m/z* 453 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(4-(hydroxymethyl)pyridin-2-yl)amino)acetate (17).** To 3.3 mL of a THF solution containing 448 mg (0.990 mmol) of **16** was added 1.1 mL (1.1 mmol) of 1 M tetrabutylammonium fluoride (TBAF) in THF at 0 °C under argon atmosphere. The reaction mixture was stirred at 0 °C for 30 min, then at room temperature for 4 h, and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 2/1→0/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 323 mg of the title compound (96% yield) as

a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (dd, J = 5.1, 0.5 Hz, 1H), 7.85 (s, 1H), 6.99-7.03 (m, 1H), 4.72 (d, J = 6.2 Hz, 2H), 4.61 (s, 2H), 1.88 (t, J = 6.2 Hz, 1H), 1.53 (s, 9H), 1.45 (s, 9H). MS (FAB+) *m/z* 339 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(4-((4-*tert*-butylbenzyl)(pyridine-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)acetate (18).** 145 mg of the title compound (78% yield) was obtained as a white foam according to the procedure similar to the general procedure for step a in Scheme 10, using **17** and **1a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.05 (dd, J = 2.3, 0.8 Hz, 1H), 8.78 (dd, J = 4.8, 1.7 Hz, 1H), 8.14 (dd, J = 5.0, 0.7 Hz, 1H), 8.03 (ddd, J = 8.1, 2.3, 1.7 Hz, 1H), 7.67 (s, 1H), 7.40 (ddd, J = 8.1, 4.8, 0.8 Hz, 1H), 7.21-7.25 (m, 2H), 7.00-7.04 (m, 2H), 6.76 (dd, J = 5.0, 1.5 Hz, 1H), 4.57 (s, 2H), 4.38 (s, 2H), 4.36 (s, 2H), 1.53 (s, 9H), 1.46 (s, 9H), 1.27 (s, 9H). MS (FAB+) *m/z* 625 $(M + H)^+$.

(4-((4-*tert*-Butylbenzyl)(pyridine-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetic Acid Hydrochloride (19). 123 mg of the title compound (quantitative yield) was obtained as a white solid according to the procedure similar to the general procedure for step d in Scheme 10, using **18**. ^{1}H NMR (500 MHz, CD_3OD) δ 9.18 (dd, J = 2.3, 0.8 Hz, 1H), 8.93 (dd, J = 5.3, 1.5 Hz, 1H), 8.54 (ddd, J = 8.1, 2.3, 1.5 Hz, 1H), 7.86 (ddd, J = 8.1, 5.3, 0.8 Hz, 1H), 7.62 (d, J = 6.7 Hz, 1H), 7.22-7.25 (m, 2H), 7.13-7.16 (m, 2H), 6.84 (s, 1H), 6.73 (dd, J = 6.7, 1.3 Hz, 1H), 4.50 (s, 2H), 4.45 (s, 2H), 4.11 (s, 2H), 1.24 (s, 9H). MS (FAB+) *m/z* 469 $(M + H)^+$.

***N*-(4-*tert*-Butylbenzyl)-*N*-(4-nitropyridin-2-ylmethyl)pyridine-3-sulfonamide (21).** 483 mg of the title compound (94% yield) was obtained as a pale brown solid according to the procedure similar to the general procedure for step a in Scheme 10, using (4-nitropyridin-2-yl)methanol (**20**) and **1a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.06 (dd, J = 2.4, 0.7 Hz, 1H), 8.87 (dd, J = 4.9, 1.6 Hz, 1H), 8.65 (dd, J = 5.5, 0.7 Hz, 1H), 8.30 (ddd, J = 8.1, 2.4, 1.6 Hz, 1H), 7.85 (dd, J = 5.5,

2.2 Hz, 1H), 7.69-7.72 (m, 1H), 7.65 (ddd, *J* = 8.1, 4.9, 0.7 Hz, 1H), 7.13-7.19 (m, 2H), 7.05-7.11 (m, 2H), 4.68 (s, 2H), 4.49 (s, 2H), 1.16 (s, 9H).

***N*-(4-Aminopyridin-2-ylmethyl)-*N*-(4-*tert*-butylbenzyl)pyridine-3-sulfonamide (22).** To 5.4 mL of an CH_3CO_2H solution containing 480 mg (1.09 mmol) of **21** was added 712 mg (10.9 mmol) of zinc dust portion wise in several times at 60 °C under argon atmosphere, and the mixture was stirred at the same temperature for 80 min. The reaction mixture was cooled to 0 °C and 54 mL of CH_3OH was added. The mixture was filtered through Celite, followed by washing with 54 mL of CH_3OH, and the filtrate was concentrated under reduced pressure. 20 mL of CH_2Cl_2 and 50 mL of a saturated aqueous $NaHCO_3$ solution were added to the obtained residue, followed by filtering and washing with CH_2Cl_2. 50 mL of a saturated aqueous $NaHCO_3$ solution was added to the filtrate and the mixture was extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to obtain 440 mg of the title compound (98% yield) as a pale yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.87 (dd, *J* = 2.4, 0.8 Hz, 1H), 8.75 (dd, *J* = 4.9, 1.5 Hz, 1H), 8.11 (ddd, *J* = 8.0, 2.4, 1.5 Hz, 1H), 7.72 (d, *J* = 5.6 Hz, 1H), 7.51 (ddd, *J* = 8.0, 4.9, 0.8 Hz, 1H), 7.26-7.33 (m, 2H), 7.09-7.15 (m, 2H), 6.39 (d, *J* = 2.2 Hz, 1H), 6.28 (dd, *J* = 5.6, 2.2 Hz, 1H), 5.98-6.03 (m, 2H), 4.45 (s, 2H), 4.21 (s, 2H), 1.25 (s, 9H). MS (FAB+) *m/z* 411 $(M + H)^+$.

***tert*-Butyl (2-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-4-yl)carbamate (23).** To 4 mL of a THF solution containing 335 mg (0.816 mmol) of **22** were added 2 mL (2 mmol) of 1 M sodium bis(trimethylsilyl)amide (NaHMDS) solution in THF dropwise over 10 min and di-*tert*-butyl dicarbonate at 0 °C under argon atmosphere. The reaction mixture was stirred at the same temperature under argon atmosphere for 80 min. To the reaction mixture were added 2 mL of a saturated aqueous NH_4Cl solution and 2 mL of H_2O, and the

mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 1/1→0/1) and the fractions containing the desired compound were concentrated under reduced pressure to obtain 117 mg of the title compound (28% yield) as a yellowish brown foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.76 (s, 1H), 8.90-8.92 (m, 1H), 8.78 (dd, J = 4.9, 1.4 Hz, 1H), 8.17 (ddd, J = 8.1, 2.3, 1.4 Hz, 1H), 8.06 (d, J = 5.6 Hz, 1H), 7.55 (ddd, J = 8.1, 4.9, 0.7 Hz, 1H), 7.32-7.35 (m, 1H), 7.22-7.28 (m, 2H), 7.17-7.21 (m, 1H), 7.09-7.15 (m, 2H), 4.44 (s, 2H), 4.35 (s, 2H), 1.49 (s, 9H), 1.23 (s, 9H). MS (FAB+) *m/z* 511 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(2-((4-*tert*-butylbenzyl)(pyridin-3-sulfonyl)aminomethyl)pyridin-4-yl)amino)acetate (24).** 104 mg of the title compound (76% yield) was obtained as a pale yellow foam according to the procedure similar to that for **16**, using **23** and *tert*-butyl bromoacetate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.99 (dd, J = 2.4, 0.8 Hz, 1H), 8.72 (dd, J = 4.8, 1.7 Hz, 1H), 8.22 (d, J = 5.5 Hz, 1H), 7.99 (ddd, J = 8.1, 2.4, 1.7 Hz, 1H), 7.33 (ddd, J = 8.1, 4.8, 0.8 Hz, 1H), 7.25-7.27 (m, 2H), 7.05-7.17 (m, 4H), 4.48 (s, 2H), 4.47 (s, 2H), 4.15 (s, 2H), 1.52 (s, 9H), 1.51 (s, 9H), 1.28 (s, 9H). MS (FAB+) *m/z* 625 $(M + H)^+$.

(2-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-4-ylamino)acetic Acid Hydrochloride (25). 27 mg of the title compound (88% yield) was obtained as a pale brown solid according to the procedure similar to the general procedure for step d in Scheme 10, using **24**. ^{1}H NMR (500 MHz, CD_3OD) δ 9.15-9.19 (m, 1H), 8.91-8.95 (m, 1H), 8.47-8.51 (m, 1H), 7.70-7.85 (m, 2H), 7.10-7.27 (m, 4H), 6.39-6.80 (m, 2H), 4.40-4.58 (m, 4H), 3.96-4.11 (m, 2H), 1.20-1.26 (m, 9H). MS (FAB+) *m/z* 469 $(M + H)^+$. The purity [%] determined with HPLC: 91.

***tert*-Butyl (6-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetate (26).** To a solution consisting of 2.7 mL of diethyl ether and 8.1 mL of ethanol, containing 334 mg (0.534 mmol) of **12aa** was added 112 mg (0.587 mmol) of PTSA•H_2O, and the mixture was concentrated under reduced pressure. 8 mL of ethanol was added to the obtained residue, and the mixture was heated at 60 °C under argon atmosphere for 13 h. 31 mg (0.16 mmol) of PTSA•H_2O was added to the reaction mixture, followed by concentrating under reduced pressure, and the obtained residue was heated at 65 °C for 3.5 h. 5 mL of a saturated aqueous $NaHCO_3$ solution and 0.5 mL of H_2O were added to the reaction mixture, followed by extracting with ethyl acetate, and the organic layer was concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 2/1→0/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 230 mg of the title compound (82% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95-8.97 (m, 1H), 8.68 (dd, J = 4.9, 1.5 Hz, 1H), 7.89-7.94 (m, 1H), 7.19-7.35 (m, 6H), 6.44 (d, J = 6.8 Hz, 1H), 6.24 (d, J = 8.3 Hz, 1H), 4.71 (t, J = 5.4 Hz, 1H), 4.58 (s, 2H), 4.32 (s, 2H), 3.79 (d, J = 5.4 Hz, 2H), 1.49 (s, 9H), 1.30 (s, 9H). MS (FAB+) *m/z* 525 $(M + H)^+$.

***tert*-Butyl (*N*-(6-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)-*N*-methylamino)acetate (27).** To 2.2 mL of a DMF solution containing 229 mg (0.436 mmol) of **26** was added 23 mg (0.53 mmol) of NaH (55%, oil coated) at 0 °C under argon atmosphere, and the mixture was stirred at room temperature for 15 min. 30 μL (0.48 mmol) of CH_3I was added at 0 °C and the mixture was stirred at room temperature for 1 h. After the reaction mixture was cooled to 0 °C, 4.4 mL of a saturated aqueous $NaHCO_3$ solution and 4.4 mL of H_2O were added to the reaction mixture and the mixture was extracted with ethyl acetate. The organic layer was

dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 3/1→0/1) and reverse phase C18 column chromatography (eluent: H_2O/CH_3CN = 5/5→0/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 77 mg of the title compound (32% yield) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90-8.92 (m, 1H), 8.66 (dd, *J* = 4.9, 2.0 Hz, 1H), 7.80-7.85 (m, 1H), 7.35 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.29-7.32 (m, 2H), 7.21-7.25 (m, 3H), 6.45 (d, *J* = 7.2 Hz, 1H), 6.36 (d, *J* = 8.4 Hz, 1H), 4.63 (s, 2H), 4.32 (s, 2H), 4.05 (s, 2H), 3.00 (s, 3H), 1.40 (s, 9H), 1.31 (s, 9H). MS (FAB+) *m/z* 539 $(M + H)^+$.

(*N*-(6-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)-*N*-methylamino)acetic Acid Hydrochloride (28). 78 mg of the title compound (quantitative yield) was obtained as a pale yellow solid according to the procedure similar to the general procedure for step d in Scheme 10, using **27**. ^{1}H NMR (400 MHz, CD_3OD) δ 9.17-9.19 (m, 1H), 8.94 (dd, *J* = 5.3, 1.5 Hz, 1H), 8.47-8.52 (m, 1H), 7.82 (dd, *J* = 8.1, 5.3 Hz, 1H), 7.70 (dd, *J* = 9.2, 7.2 Hz, 1H), 7.26-7.31 (m, 2H), 7.15-7.20 (m, 2H), 7.04 (d, *J* = 9.2 Hz, 1H), 6.54 (d, *J* = 7.2 Hz, 1H), 4.60 (s, 4H), 4.49 (s, 2H), 1.27 (s, 9H). MS (FAB+) *m/z* 483 $(M + H)^+$.

***tert*-Butyl (6-Formyl-1*H*-indol-1-yl)acetate (30).** 1.57g of the title compound (91% yield) was obtained as a pale yellow oil according to the procedure similar to that for **16**, using 6-formyl-1*H*-indole (**29**) and *tert*-butyl bromoacetate. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.07 (s, 1H), 7.82-7.85 (m, 1H), 7.72-7.76 (m, 1H), 7.65 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.33 (d, *J* = 3.2 Hz, 1H), 6.64 (dd, *J* = 3.2, 1.0 Hz, 1H), 4.83 (s, 2H), 1.46 (s, 9H).

***tert*-Butyl (6-(Hydroxymethyl)-1*H*-indol-1-yl)acetate (31).** To 20 mL of an ethanol solution containing 1.57 g (6.05 mmol) of **30** was added 120 mg (3.17 mmol) of $NaBH_4$ portionwise at

room temperature, and the mixture was stirred at the same temperature for 1 h. Acetone was added and the mixture was stirred at the same temperature for 30 min. The reaction mixture was concentrated under reduced pressure and toluene was added to the obtained residue. The mixture was washed with a saturated aqueous NH_4Cl solution and a saturated aqueous NaCl solution in turn, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 9/1→1/8), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 1.35g of the title compound (85% yield) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.61 (d, J = 8.1 Hz, 1H), 7.29 (d, J = 0.9 Hz, 1H), 7.08-7.15 (m, 2H), 6.54 (dd, J = 3.2, 0.9 Hz, 1H), 4.80 (d, J = 5.8 Hz, 2H), 4.74 (s, 2H), 1.60 (t, J = 5.8 Hz, 1H), 1.45 (s, 9H).

***tert*-Butyl (6-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)-1*H*-indol-1-yl)acetate (32).** 129 mg of the title compound (78% yield) was obtained as a yellow oil according to the procedure similar to the general procedure for step a in Scheme 10, using **31** and **1a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.03 (dd, J = 2.4, 0.8 Hz, 1H), 8.72 (dd, J = 4.8, 1.6 Hz, 1H), 7.91 (ddd, J = 8.0, 2.4, 1.6 Hz, 1H), 7.47 (dd, J = 8.1, 0.6 Hz, 1H), 7.29 (ddd, J = 8.0, 4.8, 0.8 Hz, 1H), 7.23-7.27 (m, 2H), 7.09 (d, J = 3.2 Hz, 1H), 7.03-7.07 (m, 3H), 6.81 (dd, J = 8.1, 1.5 Hz, 1H), 6.50 (dd, J = 3.2, 0.8 Hz, 1H), 4.66 (s, 2H), 4.52 (s, 2H), 4.36 (s, 2H), 1.46 (s, 9H), 1.29 (s, 9H). MS (FAB+) *m/z* 548 $(M + H)^+$.

(6-((4-*tert*-Butylbenzyl)(pyridine-3-sulfonyl)aminomethyl)-1*H*-indol-1-yl)acetic Acid Trifluoroacetate (33). To 0.5 mL of a CH_2Cl_2 solution containing 124 mg (0.226 mmol) of **32** was added 2 mL (8 mmol) of 4 M HCl in 1,4-dioxane at room temperature, and the mixture was stirred at the same temperature overnight. After completion of the reaction, diisopropyl ether was added to the reaction mixture. The resulting solid precipitate was collected by filtration, washed

with a small amount of diisopropyl ether, and dried under reduced pressure. To the obtained solid, ethyl acetate and a saturated aqueous $NaHCO_3$ solution were added, and the mixture was extracted with ethyl acetate. The organic layer was concentrated under reduced pressure and the obtained residue was subjected to reverse phase C18 column chromatography (eluent: 0.1% TFA aq/CH_3CN = 95/5→40/60). The fractions containing the desired compound were concentrated under reduced pressure and diisopropyl ether was added to the obtained residue. The resulting solid precipitate was collected by filtration, washed with diisopropyl ether, and dried under reduced pressure to obtain 13.5 mg of the title compound (9.9% yield) as a beige solid. ^{1}H NMR (500 MHz, CD_3OD) δ 8.92 (d, J = 1.8 Hz, 1H), 8.66-8.71 (m, 1H), 8.07-8.13 (m, 1H), 7.47 (dd, J = 7.9, 5.0 Hz, 1H), 7.39 (d, J = 8.1 Hz, 1H), 7.23-7.29 (m, 2H), 7.17 (d, J = 3.1 Hz, 1H), 7.04-7.10 (m, 2H), 6.96 (s, 1H), 6.79 (dd, J = 8.1, 1.0 Hz, 1H), 6.41 (d, J = 3.1 Hz, 1H), 4.83 (s, 2H), 4.50 (s, 2H), 4.37 (s, 2H), 1.27 (s, 9H). MS (FAB+) *m/z* 492 $(M + H)^+$. The purity [%] determined with HPLC: 92.

***tert*-Butyl 3-(6-Methylpyridin-2-ylamino)propanoate (35).** A mixture of 2.00 g (18.5 mmol) of 6-methyl-2-aminopyridine (**34**), 2.60 g (20.3 mmol) of *tert*-butyl acrylate, and 20.8 mg (0.0936 mmol) of 2,5-di-*tert*-butylbenzene-1,4-diol was stirred at 100 °C for 2 days. The mixture was diluted with ethyl acetate, washed with H_2O and a saturated aqueous NaCl solution in turn, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 95/5→50/50), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 458 mg of the title compound (11% yield) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (dd, J = 8.2, 7.3 Hz, 1H), 6.44 (d, J = 7.3 Hz, 1H), 6.21 (d, J =

8.2 Hz, 1H), 4.66-4.84 (m, 1H), 3.54 (q, J = 6.4 Hz, 2H), 2.53 (t, J = 6.4 Hz, 2H), 2.36 (s, 3H), 1.45 (s, 9H). MS (EI+) *m/z* 236 $(M)^+$.

***tert*-Butyl 3-(*tert*-Butoxycarbonyl(6-methylpyridin-2-yl)amino)propanoate (36).** To 3 mL of a *tert*-butanol solution containing 452 mg (1.91mmol) of **35** were added 600 μL (2.6 mmol) of di-*tert*-butyl dicarbonate and 25 mg (0.20 mmol) of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) at room temperature, and the mixture was stirred at the same temperature for 3 h. To the reaction mixture, 350 μL (2.0 mmol) of *N*,*N*-diisopropylethylamine, 600 μL (2.6 mmol) of di-*tert*-butyl dicarbonate and 212 mg (1.74 mmol) of DMAP were added, and the mixture was stirred at room temperature overnight. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The obtained residue was diluted with ethyl acetate and washed with 5% aqueous $KHSO_4$ solution, a saturated aqueous $NaHCO_3$ solution, and a saturated aqueous NaCl solution in turn. The separated organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 10/0→5/5), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 321 mg of the title compound (50% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47-7.54 (m, 1H), 7.30 (d, J = 8.1 Hz, 1H), 6.85-6.91 (m, 1H), 4.14-4.20 (m, 2H), 2.58-2.65 (m, 2H), 2.48 (s, 3H), 1.49 (s, 9H), 1.39 (s, 9H).

2-((3-*tert*-Butoxy-3-oxopropyl)(*tert*-butoxycarbonyl)amino)-6-methylpyridine 1-Oxide (37). To 6 mL of a CH_2Cl_2 solution containing 310 mg (0.921mmmol) of **36** was added 505 mg (2.23 mmol) of *m*-CPBA (77%) at room temperature, and the mixture was stirred at the same temperature for 4.5 h. The reaction mixture was washed with a saturated aqueous $NaHCO_3$ solution, and the separated organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography

(eluent: *n*-hexane/ethyl acetate = 9/1→5/5), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 335 mg of the title compound (quantitative yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16-7.22 (m, 2H), 7.07-7.15 (m, 1H), 3.88 (br s, 2H), 2.50-2.60 (m, 5H), 1.41 (br s, 9H), 1.40 (s, 9H).

***tert*-Butyl 3-((6-(Acetoxymethyl)pyridin-2-yl)(*tert*-butoxycarbonyl)amino)propanoate (38).** 1.5 mL of an acetic anhydride solution containing 310 mg (0.921 mmol) of **37** was stirred at 110 °C for 1 h. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 1/0→5/5), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 242 mg of the title compound (66% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (dd, J = 8.3, 7.1 Hz, 1H), 7.53-7.57 (m, 1H), 7.02-7.06 (m, 1H), 5.15 (s, 2H), 4.14-4.21 (m, 2H), 2.60-2.67 (m, 2H), 2.17 (s, 3H), 1.52 (s, 9H), 1.41 (s, 9H).

***tert*-Butyl 3-(*tert*-Butoxycarbonyl(6-(hydroxymethyl)pyridin-2-yl)amino)propanoate (39).** To 3 mL of a THF solution containing 240 mg (0.608 mmol) of *tert*-butyl 3-((6-(acetoxymethyl)pyridin-2-yl)(*tert*-butoxycarbonyl)amino)propanoate (**38**) were added 28 mg (0.67 mmol) of LiOH•H_2O and 0.3 mL of H_2O at room temperature, and the mixture was stirred at 60 °C for 13.5 h. The reaction mixture was diluted with ethyl acetate, and washed with a saturated aqueous $NaHCO_3$ solution and a saturated aqueous NaCl solution in turn. The separated organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 1/0→5/5), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 183 mg of the title compound (85% yield) as a

colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (dd, J = 8.3, 7.1 Hz, 1H), 7.54-7.59 (m, 1H), 6.91-6.96 (m, 1H), 4.71 (d, J = 5.1 Hz, 2H), 4.18-4.26 (m, 2H), 3.54 (t, J = 5.1 Hz, 1H), 2.61-2.68 (m, 2H), 1.53 (s, 9H), 1.40 (s, 9H).

***tert*-Butyl 3-(*tert*-Butoxycarbonyl(6-((4-*tert*-butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-yl)amino)propanoate (40).** 281 mg of the title compound (86% yield) was obtained as a white foam according to the procedure similar to the general procedure for step a in Scheme 10, using **39** and **1a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (dd, J = 2.4, 0.7 Hz, 1H), 8.68 (dd, J = 4.9, 1.6 Hz, 1H), 7.83 (ddd, J = 7.9, 2.4, 1.6 Hz, 1H), 7.46-7.54 (m, 2H), 7.25-7.30 (m, 3H), 7.16-7.19 (m, 2H), 6.94 (dd, J = 7.2, 1.1 Hz, 1H), 4.59 (s, 2H), 4.43 (s, 2H), 3.92-3.97 (m, 2H), 2.48-2.53 (m, 2H), 1.53 (s, 9H), 1.39 (s, 9H), 1.29 (s, 9H). MS (FAB+) *m/z* 639 $(M + H)^+$.

3-(6-((4-*tert*-Butylbenzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)propionic Acid Hydrochloride (41). 183 mg of the title compound (75% yield) was obtained as a white solid according to the procedure similar to the general procedure for step d in Scheme 10, using **40**. ^{1}H NMR (500 MHz, CD_3OD) δ 9.14 (br s, 1H), 8.91 (br d, J = 4.2 Hz, 1H), 8.43 (ddd, J = 8.1, 2.2, 1.5 Hz, 1H), 7.77 (dd, J = 8.1, 5.1 Hz, 1H), 7.63 (dd, J = 8.9, 7.7 Hz, 1H), 7.22-7.26 (m, 2H), 7.14-7.18 (m, 2H), 6.81 (d, J = 8.9 Hz, 1H), 6.51-6.54 (m, 1H), 4.54 (s, 2H), 4.47 (s, 2H), 3.59 (t, J = 6.1 Hz, 2H), 2.70 (t, J = 6.1 Hz, 2H), 1.24 (s, 9H). MS (FAB+) *m/z* 483 $(M + H)^+$. The purity [%] determined with HPLC: 93.

***tert*-Butyl (4-(((*tert*-Butyldimethylsilyl)oxy)methyl)thiazol-2-yl)carbamate (43).** To 6 mL of a DMF solution containing 450 mg (1.95 mmol) of *tert*-butyl (4-(hydroxymethyl)thiazol-2-yl)carbamate (**42**) and 401 mg (5.89 mmol) of imidazole was added 356 mg (2.36 mmol) of *tert*-butyldimethylchlorosilane (TBDMSCl) at room temperature, and the mixture was stirred at the

same temperature overnight. After completion of the reaction, H_2O was added to the reaction mixture and the mixture was extracted with toluene. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 1/0→2/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 667 mg of the title compound (99% yield) as a pale yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.37 (br s, 0.5H), 6.86 (t, J = 1.2 Hz, 1H), 4.59 (d, J = 1.2 Hz, 2H), 1.47 (s, 9H), 0.87-0.90 (m, 9H), 0.06-0.06 (m, 6H). MS (CI+) *m/z* 345 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(4-(((*tert*-butyldimethylsilyl)oxy)methyl)thiazol-2-yl)amino)acetate (44).** To 10 mL of a DMF solution containing 658 mg (1.91mmol) of **43** were added 340 μL (2.32 mmol) of *tert*-butyl bromoacetate and 530 mg (3.83 mmol) of K_2CO_3, and the mixture was stirred at room temperature for 1 h. H_2O was added to the reaction mixture and the mixture was extracted with toluene. The organic layer was washed with H_2O and a saturated aqueous NaCl solution in turn, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The obtained residue was subjected to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 1/0→5/1), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 756 mg of the title compound (86% yield) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77 (s, 1H), 4.72 (s, 2H), 4.68 (d, J = 1.2 Hz, 2H), 1.54 (s, 9H), 1.44 (s, 9H), 0.91-0.95 (m, 9H), 0.08-0.11 (m, 6H). MS (CI+) *m/z* 459 $(M + H)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(4-(hydroxymethyl)thiazol-2-yl)amino)acetate (45).** 323 mg of the title compound (96% yield) was obtained as a pale yellow oil according to the procedure similar to that for **17**, using **44**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.97 (t, J = 1.1 Hz, 1H), 5.20

(t, J = 5.7 Hz, 1H), 4.68 (br s, 2H), 4.40 (d, J = 5.7 Hz, 2H), 1.49 (s, 9H), 1.41 (s, 9H). MS (EI+) *m/z* 344$(M)^+$.

***tert*-Butyl (*tert*-Butoxycarbonyl(4-((4-*tert*-butylbenzyl)(pyridine-3-ylsulfonyl)aminomethyl)thiazol-2-yl)amino)acetate (46).** 149 mg of the title compound (81% yield) was obtained as a white foam according to the procedure similar to the general procedure for step a in Scheme 10, using **45** and **1a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.01 (br s, 1H), 8.70 (dd, J = 4.9, 1.6 Hz, 1H), 7.91 (ddd, J = 7.9, 2.4, 1.6 Hz, 1H), 7.32-7.36 (m, 3H), 7.23-7.26 (m, 2H), 6.58 (s, 1H), 4.50 (s, 2H), 4.48 (s, 2H), 4.32 (s, 2H), 1.55 (s, 9H), 1.45 (s, 9H), 1.31 (s, 9H). MS (FAB+) *m/z* 631 $(M + H)^+$.

(4-((4-*tert*-Butylbenzyl)(pyridine-3-ylsulfonyl)aminomethyl)thiazol-2-ylamino)acetic Acid Hydrochloride (47). 115 mg of the title compound (90% yield) was obtained as a white solid according to the procedure similar to the general procedure for step d in Scheme 10, using **46**. ^{1}H NMR (500 MHz, CD_3OD) δ 9.16 (dd, J = 2.3, 0.8 Hz, 1H), 8.93 (dd, J = 5.2, 1.5 Hz, 1H), 8.52 (ddd, J = 8.1, 2.3, 1.5 Hz, 1H), 7.85 (ddd, J = 8.1, 5.2, 0.8 Hz, 1H), 7.30-7.32 (m, 2H), 7.16-7.19 (m, 2H), 6.61-6.62 (m, 1H), 4.43 (s, 2H), 4.41 (s, 2H), 4.06 (s, 2H), 1.28 (s, 9H). MS (FAB+) *m/z* 475 $(M + H)^+$.

Isopropyl (6-((4-(Pyrazol-1-yl)benzyl)(pyridin-3-ylsulfonyl)aminomethyl)pyridin-2-ylamino)acetate (OMDI). To 10 mL of an isopropanol solution containing 1.00 g (2.09 mmol,) of **13ax** (OMD) was added 10 mL (40 mmol) of 4 M HCl in 1,4-dioxane, and the mixture was stirred at room temperature. After completion of the reaction, the reaction mixture was evaporated. A saturated aqueous $NaHCO_3$ solution was added to the obtained residue, and the mixture was extracted with toluene and ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained residue was subjected

to silica gel column chromatography (eluent: *n*-hexane/ethyl acetate = 2/1→1/8), and the fractions containing the desired compound were concentrated under reduced pressure to obtain 702 mg of the title compound (88% yield) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.87 (dd, J = 2.4, 0.7 Hz, 1H), 8.75 (dd, J = 4.8, 1.6 Hz, 1H), 8.48 (dd, J = 2.4, 0.5 Hz, 1H), 8.08 (ddd, J = 8.1, 2.4, 1.6 Hz, 1H), 7.80-7.77 (m, 2H), 7.74 (dd, J = 1.8, 0.5 Hz, 1H), 7.51 (ddd, J = 8.1, 4.8, 0.7 Hz, 1H), 7.36-7.33 (m, 2H), 7.26 (dd, J = 8.3, 7.1 Hz, 1H), 6.89 (t, J = 6.1, 1H), 6.54 (dd, J = 2.4, 1.8 Hz, 1H), 6.38 (d, J = 8.3 Hz, 1H), 6.34 (d, J = 7.1 Hz, 1H), 4.87 (sept, J = 6.3 Hz, 1H), 4.62 (s, 2H), 4.21(s, 2H), 3.76 (d, J = 6.1 Hz, 2H), 1.10 (d, J = 6.3 Hz, 6H). ^{13}C NMR (proton-decoupled spectrum, 500 MHz, DMSO-d_6) δ 171.2 (s), 158.1 (s), 153.4 (s), 153.2 (s), 147.6 (s), 141.4 (s), 139.6 (s), 137.5 (s), 137.0 (s), 135.1 (s), 134.4 (s), 129.9 (s), 128.2 (s), 124.4 (s), 118.8 (s), 111.4 (s), 108.3 (assigned for two nonequivalent carbons with identical chemical shift), 68.0 (s), 51.9 (s), 51.2 (s), 43.1 (s), 22.0 (s). MS (CI+) *m/z* 521 $(M + H)^+$. IR wavelength [cm^{-1}] 3437 (N-H), 1736 (C=O), 1608, 1525 and 1511 (C=C and C=N), 1321 (SO_2), 1161 (SO_2). Elemental analysis [%] (average of three experiments) calculated for $C_{26}H_{28}N_6O_4S$: C 59.98, H 5.42, N 16.14. Found: C 59.76, H 5.28, N 16.01. TLC R_f value 0.39 (ethyl acetate).

Biological Evaluation. Binding Affinity for h-EP2 Receptor. Measurement of the binding affinity for h-EP2 receptor was carried out in compliance with the method of Abramovitz *et al*.[11] The test compound dissolved in DMSO and [^{3}H]prostaglandin E_2 (NET-428, PerkinElmer) (final concentration: 10 nM) were added to a buffer solution (10 mM 2-morpholinoethanesulfonic acid (MES)-KOH (pH 6.0), 10 mM $MgCl_2$, 1 mM EDTA) in which was suspended 10 μg of a membrane fraction of HEK293 cells expressing h-EP2 receptor (ES-562-M, Euroscreen) followed by incubating at 30 °C for 60 min. The membrane fraction was recovered on glass fiber filter paper (GF/B, Whatmann) using a cell harvester (M30R, Brandel), and after washing with

buffer solution (10 mM MES-KOH (pH 6.0), 10 mM $MgCl_2$), radioactivity was measured with a liquid scintillation analyzer (2000CA, Packard). The concentration of test compound required to replace 50% of the [^{3}H]prostaglandin E_2 bound to the receptor (IC_{50} value) was calculated using EXSAS (Arm Systex) on the basis of the results from two or four data points of a single experiment, performed in duplicate, and the inhibition constant (K_i value) was determined using the formula indicated below. The dissociation constant (K_d) was calculated by Scatchard analysis.

$K_i = IC_{50}/(1 + ([^3H]\text{prostaglandin } E_2 \text{ concentration}/K_d))$

***In Vitro* h-EP2 Receptor Agonist Activity.** Measurement of EP2 agonist activity was carried out in compliance with the method of Wilson *et al*.[36] HEK293 cells expressing h-EP2 receptor (ES-562-C, Euroscreen, tested for the absence of Mycoplasma sp.) were cultured in Eagle's minimum essential medium (MEM) containing 10% fetal bovine serum and seeded at 2×10^4 cells per well of a 96-well plate. On the following day, the medium was replaced with serum-free MEM containing 3-isobutyl-1-methylxanthine (final concentration: 500 μM) and after culturing at 37 °C for 30 min, the test compound dissolved in DMSO was added. After further incubation at 37 °C for 30 min, the cells were lysed and the amount of cAMP in the cells was measured with cAMP assay kit (cAMP Biotrak EIA System kit, RPN2251, GE Healthcare Sciences or cAMP dynamic 2, 62AM4PEB, Cisbio Bioassays). The concentration of test compound required to increase the amount of cAMP to 50% of the maximum increase (EC_{50} value) was calculated by non-linear regression of the test compound concentration and amount of cAMP using EXSAS on the basis of the results from at least seven data points of a single experiment, performed in duplicate.

Selectivity to h-EP2 Receptor. The selectivity of **13ax** (OMD) and OMDI to the h-EP2 receptor were determined on the basis of the inhibitory effect on radioligand binding to each EP receptor subtype (h-EP1, h-EP2, h-EP3, and h-EP4).

Binding Affinity for h-EP1 Receptor: The test compound dissolved in DMSO (final concentrations: 10 μM **13ax**, 10 μM OMDI, and 30 nM PGE_2) and [^{3}H]prostaglandin E_2 (final concentration: 3 nM) were added to assay buffer solution (50 mM Tris-HCl (pH 7.4), 10 mM $MnCl_2$, 1 mM EDTA) in which was suspended 10 μg of a membrane fraction of Chem-1 cells expressing h-EP1 receptor (HTS099M, Chemicon) followed by incubating at 25 °C for 60 min. The membrane fraction was recovered on glass fiber filter paper (GF/C, Whatmann) using a cell harvester, and after washing with assay buffer solution, radioactivity was measured with a liquid scintillation analyzer (1500, PerkinElmer). The inhibition ratio of the [^{3}H]prostaglandin E_2 bound to the receptor was calculated from the results of a single experiment, performed in duplicate.

Binding Affinity for h-EP2 Receptor: The test compound dissolved in DMSO and [^{3}H]prostaglandin E_2 (final concentration: 2 nM) were added to assay buffer solution (10 mM MES-KOH (pH 6.0), 10 mM $MgCl_2$, 1 mM EDTA) in which was suspended 10 μg of a membrane fraction of HEK293 cells expressing h-EP2 receptor (ES-562-M, PerkinElmer) followed by incubating at 25 °C for 60 min. The membrane fraction was recovered on glass fiber filter paper using a cell harvester, and after washing with assay buffer solution, radioactivity was measured with a liquid scintillation analyzer (1500 and 2500, PerkinElmer). The concentration of test compound required to replace 50% of the [^{3}H]prostaglandin E_2 bound to the receptor (IC_{50} value) was calculated by regression analysis based on the results from at least four data points of a single experiment, performed in duplicate.

Binding Affinity for h-EP3 Receptor: The test compound dissolved in DMSO (final concentrations: 10 μM **13ax**, 10 μM OMDI, and 3 nM PGE_2) and [^{3}H]prostaglandin E_2 (final concentration: 0.9 nM) were added to assay buffer solution (50 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 1 mM EDTA) in which was suspended 10 μg of a membrane fraction of Chem-1 cells expressing h-EP3 receptor (HTS092M, Chemicon) followed by incubating at 25 °C for 60 min. The membrane fraction was recovered on glass fiber filter paper using a cell harvester, and after washing with assay buffer solution, radioactivity was measured with a liquid scintillation analyzer (1500, PerkinElmer). The inhibition ratio of the [^{3}H]prostaglandin E_2 bound to the receptor was calculated from the results of a single experiment, performed in duplicate.

Binding Affinity for h-EP4 Receptor: The test compound dissolved in DMSO and [^{3}H]prostaglandin E_2 (final concentration: 0.8 nM) were added to assay buffer solution (50 mM 2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanesulfonic acid-NaOH (pH 7.4), 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA) in which was suspended 10 μg of a membrane fraction of Chem-1 cells expressing h-EP4 receptor (HTS142M, Chemicon) followed by incubating at 25 °C for 60 min. The membrane fraction was recovered on glass fiber filter paper using a cell harvester, and after washing with assay buffer solution, radioactivity was measured with a liquid scintillation analyzer (3100, PerkinElmer). The concentration of test compound required to replace 50% of the [^{3}H]prostaglandin E_2 bound to the receptor (IC_{50} value) was calculated by regression analysis based on the results from at least three data points of a single experiment, performed in duplicate.

Measurement of IOP-lowering effect in ocular normotensive monkeys. IOP studies were carried out at Santen Pharmaceutical Co., Ltd. as described in previous our study.[43] Cynomolgus ocular normotensive monkeys were used in the IOP studies. All experiments were conducted in

accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research and the internal ethics code for animal study of Santen Pharmaceutical Co., Ltd.

For the comparison of IOP-lowering effect between **13ax** (OMD) and OMDI, **13ax** (0.3%, 1%), OMDI (0.01%) or vehicle was topically administrated to the eyes in each monkey once a day at around 5 pm from Day 1 to Day 14. IOP was measured at 10 am at Day 1 (pre), 8 and 15. IOP change was calculated as the difference from Day 1 value. For the evaluation of dose dependence of IOP-lowering effect of OMDI, OMDI (0.0006%, 0.003%) or vehicle was topically administrated to the eye in each monkey at time 0 of each day (around 9 am) and the fellow eye remained untreated. IOP change was calculated as the difference from the time 0 values at Day 1.

3D Structure Analysis Using the CSD. The 3D structure of the discussed substructure was analyzed using the version 5.39 of the CSD (November 2017, ConQuest version 1.20, Mercury CSD 3.10). The subset was generated through the filters to remove the following entities: disordered structures, structures with errors, polymeric compounds, ions, powder structures, organometallic compounds and structures with an R-factor of less than 10%. The torsional angle was expressed as an absolute value between 0° to 180°.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Conformational analysis of the phenethyl and phenoxy methyl moieties; distributions of torsional angles in the phenoxyacetic acid and (pyridin-2-ylamino)acetic acid moieties; details of Caco-2

cell permeability, PAMPA, metabolic rate in human liver microsome, CYP inhibition assay, and CYP induction assay

Molecular formula strings

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

OMD, omidenepag; OMDI, omidenepag isopropyl; IOP, intraocular pressure; PGE_2, prostaglandin E_2; TMAD, *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide; P(*n*-Bu)$_3$, tri-*n*-butylphosphine; Pd-C, palladium on carbon; Boc, *tert*-butoxycarbonyl; TBDMS, *tert*-butyldimethylsilyl; NaHMDS, sodium bis(trimethylsilyl)amide; PTSA, *para*-toluenesulfonic acid; CSD, cambridge structural database; N-scan, nitrogen-scan; prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$;

APCI, atmospheric pressure chemical ionization; br, broad; C18, octadecylsilyl; MES, 2-morpholinoethanesulfonic acid; MEM, Eagle's minimum essential medium.

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