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Original article

6-Amino-2,4,5-trimethylpyridin-3-ols: A new general synthetic route and antiangiogenic activity

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ABSTRACT

A new synthetic strategy for preparation of a wide range of 6-amino-2,4,5-trimethylpyridin-3-ols from pyridoxine·HCl *via* a six-step sequence has been developed. This approach features an introduction of various amino groups to *C*(6)-position of 3-benzyloxy-6-bromo-2,4,5-trimethylpyridine (**13**), a key intermediate, by a Buchwald–Hartwig amination reaction using palladium(0) transition metal, which certainly renders an expanded scope of amino substituents. Some analogs prepared using the methods described here showed high level of antiangiogenic and antitumor activities in chick chorioallantoic membrane (CAM) assay, demonstrating the potential of these new aminopyridinols as antiangiogenic agents.

1. Introduction

6-Aminopyridin-3-ol (**1**) was first proposed by Porter and colleagues in 2003 as a novel scaffold for antioxidants with improved air-stability [1]. Since then, several types of analogs, including **2** and **3**, have been designed and prepared as potent and air-stable antioxidants [2–16]. Some lipophilic analogs were found to have better antioxidant properties, in many respects, than α-tocopherol (**4**), the most effective natural lipophilic antioxidant. Not only did they show the better antioxidant activities measured by peroxyl radical trapping rate constant (k_{inh}) but also much higher binding affinity to tocopherol transfer protein (TPP) than α-tocopherol, protection of endogenous α-tocopherol from oxidative consumption, and no participation in tocopherol-mediated peroxidation [4,5]. Therefore, interest in 6-aminopyridin-3-ols is growing, however, only limited areas in analog space were explored.

6-Amino-2,4,5-trimethylpyridin-3-ols (**2**, $R^1 = R^2 = R^3 = Me$), first reported by some of us and colleagues in 2010, were developed as a novel class of monocyclic 6-aminopyridin-3-ol antioxidants [8,11]. In addition to a highly practical synthetic method starting from pyridoxine HCl (**5**), a component of vitamin B_6, some derivatives in this class also have potent radical-scavenging activities. A lipophilic analog with *N*-palmityl chain (i.e., $R^1 = R^2 = R^3 = Me$, $R^4 = n\text{-}C_{16}H_{33}$, $R^5 = H$ in **2**) showed greater capacity for prevention of lipid peroxidation in a model membrane system using 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphatidylcholine (PLPC) liposomes, and higher efficacy for protection of cells from oxidative injury than α-tocopherol [8,11]. On the other hand, a hydrophilic, water-soluble analog, 6-amino-2,4,5-trimethylpyridin-3-ol (i.e., $R^1 = R^2 = R^3 = Me$, $R^4 = R^5 = H$ in **2**), was found to act as a co-antioxidant in heterogeneous system, which may serve to prolong the half-life of α-tocopherol [11].

Antioxidant activities against reactive oxygen species (ROS) have mainly been studied as the primary effects of aminopyridinols on biological systems while only a few studies on their pharmacological actions against disease-related systems have been reported [14–16] (Fig. 1).

Angiogenesis is a biological process of generating new blood vessels from preexisting vasculature [17–21]. This process involves the growth, migration, and differentiation of endothelial cells, which line the inside wall of blood vessels. It is one of the normal physiological functions playing important roles in reproduction, development, and wound repair [20,21]. However, at an

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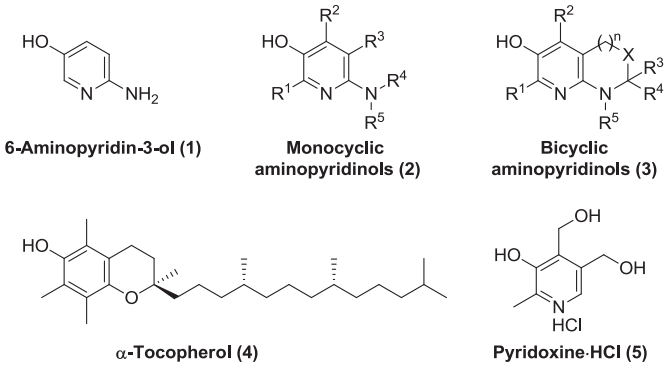


Fig. 1. Aminopyridinol antioxidants.

abnormally high rate, it is deeply implicated in the pathogenesis of many diseases, including cancer, age-related macular degeneration, diabetic retinopathy, and so on [22,23]. Solid tumors, in particular, desperately require new blood vessels for a sufficient supply of nutrients and oxygen, which are essential for growth over a few mm^3 in size. In addition, new blood vessels enable cancer cells to invade adjacent tissue and to migrate throughout the body, which is known as metastasis [24]. Therefore, suppression of such pathological angiogenesis has been one of the most promising approaches in prevention and treatment of cancer [22,23]. Considerable effort has been made in development of angiogenesis inhibitors that block tumor angiogenesis. Synthetic antiangiogenic drugs such as sorafenib and sunitinib were approved by FDA for treatment of cancer [25,26] and various types of angiogenesis inhibitors are currently being tested in clinical trials [27].

Based on the notion that ROS are related to angiogenesis, many natural and synthetic antioxidants including vitamin E and propolis constituents were examined as antiangiogenic agents. For example, vitamin E constituents such as α-tocopherol and δ-tocotrienol and components from propolis including artepillin C and quercetin were found to possess a certain level of antiangiogenic activity [28–32].

We set forth to expand the scope of the analogs of this attractive aminopyridinol scaffold and to explore their biological activities in more physiologically and pathologically relevant systems. Here, we report our data on the feasibility of novel series of 6-amino-2,4,5-trimethylpyridin-3-ol analogs as antiangiogenic agents. A general synthetic strategy for preparation of the analogs was developed and their antiangiogenic and antitumor–formation activities were examined using chick chorioallantoic membrane (CAM) assay as an *in vivo* model [33,34].

2. Results and discussion

2.1. New general route for synthesis of 6-amino-2,4,5-trimethylpyridin-3-ol analogs

A general description of the synthesis of 6-dialkylamino- and 6-monoalkylamino-2,4,5-trimethylpyridin-3-ol derivatives (**7** and **9**) starting from pyridoxine hydrochloride (**5**), which was previously reported by us and colleagues is shown in Fig. 2 [11]. The key intermediate, 6-amino-2,4,5-trimethylpyridin-3-ol (**6**), was prepared in high yield from **5** through a four-step sequence. Then, the attachment of alkyl group(s) to the *C*(6)-NH_2 was achieved using two different methods. The dialkylamino moiety in **7** was successfully attached by a reductive alkylation with various aldehydes. For synthesis of 6-monoalkylaminopyridin-3-ols (**9**), for which the reductive alkylation was not very effective, a three-step strategy

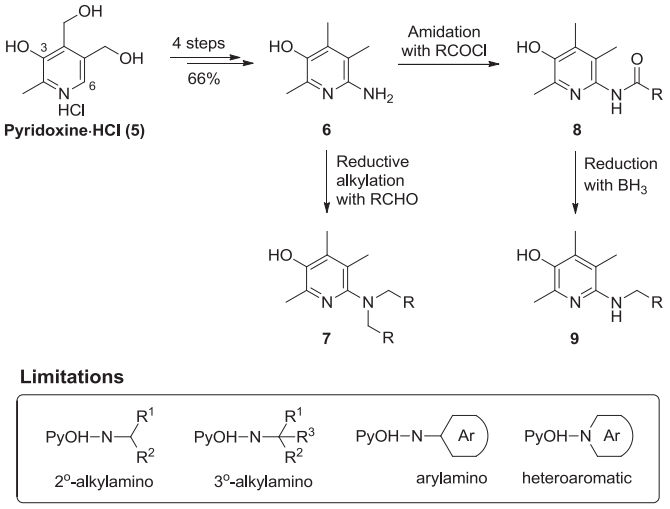


Fig. 2. The previous synthetic strategy for 6-aminopyridin-3-ols from pyridoxine·HCl (**5**) and its limitations on the scope of derivatization.

consisting of 1) *N*,*O*-diacylation of **6**, 2) selective hydrolysis of an *O*-acyl group to afford **8**, and 3) borane reduction of the amide carbonyl group was used.

Although this synthetic strategy is highly practical and efficient in preparation of 6-amino-2,4,5-trimethylpyridin-3-ols in quantity, it has a limitation on the scope of alkyl groups to be attached to *C*(6)-nitrogen. This route involves the formation of either an iminium intermediate for *N*,*N*-dialkyl derivatives **7** or an amide intermediate **8** for *N*-monoalkyl analogs **9**, in both cases, intermediates are to be reduced in order to afford the final product. As a result, the newly introduced alkyl moieties in the final products (**7** and **9**) always tether two hydrogen atoms at α-position to the *C*(6)-nitrogen. Therefore, secondary and tertiary alkyl groups, aryl groups which hold one or no hydrogen, and heterocyclic groups which include *C*(6)-nitrogen cannot be introduced to the *C*(6)-nitrogen under these conditions (Fig. 2). Here, we report a new synthetic route by which those refractory substituents are successfully attached.

For synthesis of a series of 6-amino-2,4,5-trimethylpyridin-3-ols that cannot be prepared *via* the aforementioned route shown in Fig. 2, direct introduction of various amines to the *C*(6)-position of 3-alkoxy-6-halopyridine was attempted (Fig. 3).

Among a number of synthetic methods for formation of a σ-bond between aryl carbon and a nitrogen atom, we chose the Buchwald–Hartwig amination, which features the palladium-catalyzed cross-coupling of amines with aryl halides [35–43]. Accordingly, a new key intermediate for this strategy, which is equipped with a halide leaving group at the *C*(6)-position and a proper protective group on the *C*(3)-OH was required.

Scheme 1 shows the synthesis of the key intermediate, 3-benzyloxy-6-bromo-2,4,5-trimethylpyridine (**13**). Two benzylic hydroxy groups of pyridoxine·HCl (**5**) were chlorinated with excess amounts of thionyl chloride and catalytic amount of DMF to give 4′,5′-dichloropyridoxine **10** as an HCl salt in 93% yield. A solvent

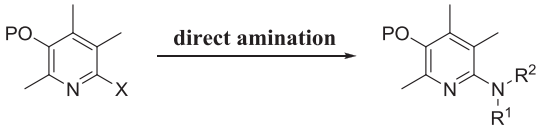
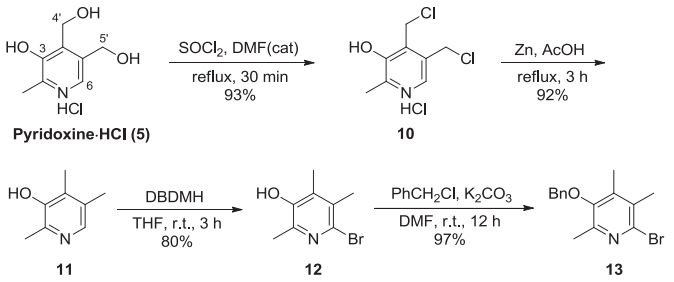


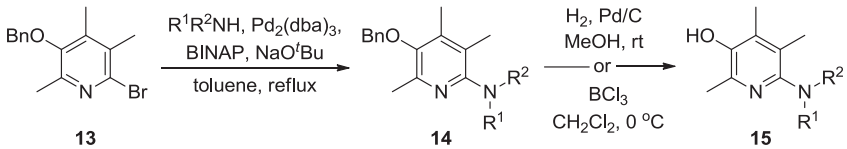
Fig. 3. A new synthetic strategy for aminopyridinols.

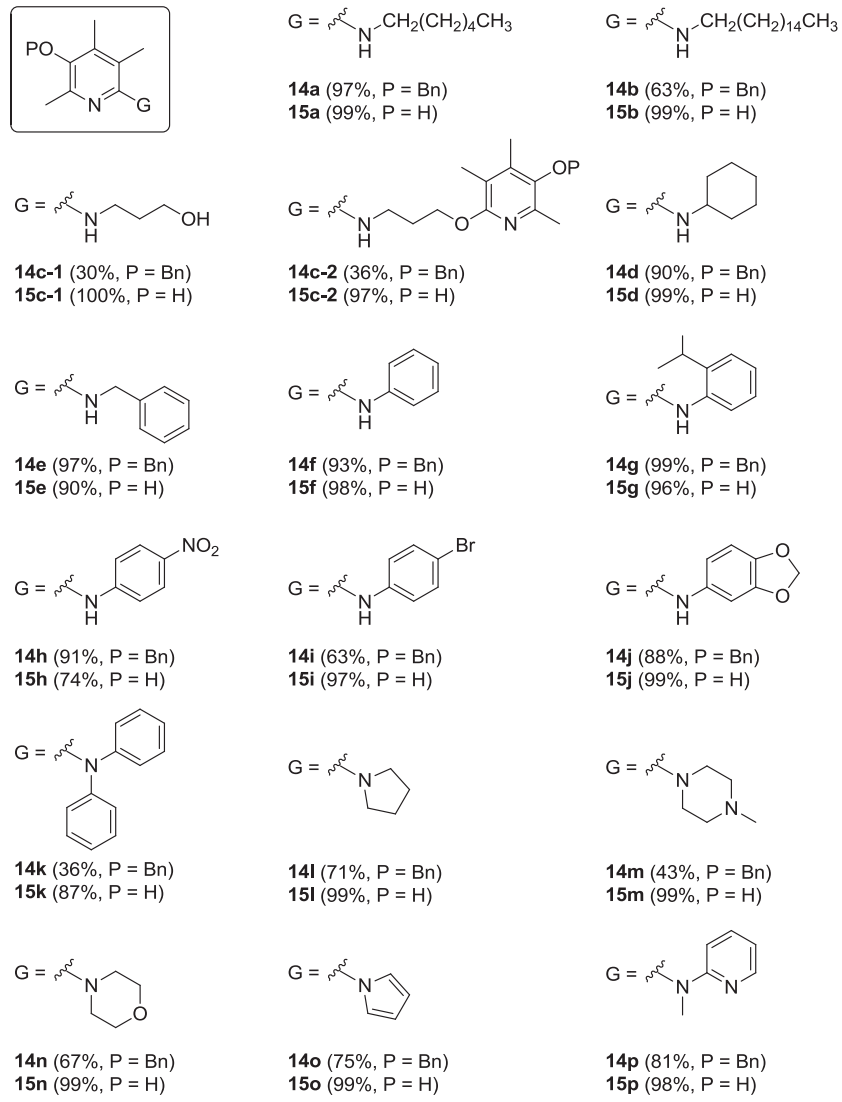


Scheme 1. Synthesis of the key intermediate **13**.

such as 1,2-dichloroethane was often used for better stirring of the reaction mixture. In this case, the color of the product became darker; however, the purity was intact. Two benzylic chloride groups of **10** were then removed with three equivalents of zinc dust in refluxing acetic acid to give the 2,4,5-trimethyl compound **11**. Then, in order to introduce a leaving group at *C*(6)-position, an electrophilic aromatic bromination was performed using 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) to afford the *C*(6)-bromo compound **12** from **10** at an overall yield of approximately 70%. Finally, the phenolic OH of **12** was protected with a benzyl group using benzyl chloride to give the key intermediate **13** in 97% yield.

Coupling of the key intermediate **13** with various amines was performed under one of the typical Buchwald–Hartwig amination reaction conditions with tris(dibenzylideneacetone)dipalladium(0)





Scheme 2. Synthesis of 6-amino-2,4,5-trimethylpyridin-3-ols by the C–N bond formation.

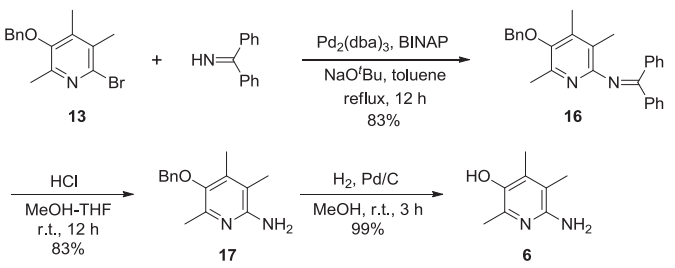
($Pd_2(dba)_3$) and 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) as a ligand in the presence of sodium *tert*-butoxide in refluxing toluene (Scheme 2) [35–43].

A variety of primary and secondary amines were introduced to the *C*(6)-position under these reaction conditions. In particular, secondary alkyl amino- (**14d**) and aryl amino-moieties (**14f–i**) were successfully introduced in the pyridine ring, which was not possible using the previously reported methods [11]. In the case of introduction of 3-aminopropan-1-ol to the pyridine ring, a dimeric by-product, **14c–2** (36% yield) was obtained along with the desired product, **14c–1** (30% yield), in which a hydroxy group of 3-aminopropan-1-ol also participated in the reaction with the bromopyridine **13** under the reaction conditions. The benzyl protecting group of 3-benzyloxy-6-aminopyridin-3-ols **14a–j** was then removed by catalytic hydrogenolysis to afford **15**'s. In the case of compounds such as **14h** and **14i**, which contain functional group(s) that are vulnerable under catalytic hydrogenolysis conditions, BCl_3 was employed as an alternative reducing agent [44]. In fact, reaction of **14i** under catalytic hydrogenolysis conditions afforded the debrominated compound (i.e., **15f** as an HBr salt) in 95% yield. Secondary amines, including diphenyl amine, cyclic amines, pyrrole, and aminopyridine, were also introduced in moderate to high yields. Debenzylation of **14k–p** was performed by catalytic hydrogenolysis to give **15k–p** in high yields.

A slightly different approach was required for installation of an ammonia ($-NH_2$ group). Because ammonia can poison palladium metal during the palladium-catalyzed C–N bond forming reaction, we used a commercially available benzophenone imine as an ammonia equivalent [45]. This strategy was applied successfully to our system; a benzophenone imino-pyridinol **16** was afforded in 83% yield and the subsequent hydrolysis of the imine moiety under acidic condition (**17**, 83%) followed by debenzylation gave the final product **6** (Scheme 3).

2.2. Inhibitory effects of 6-amino-2,4,5-trimethylpyridin-3-ols on VEGF-induced angiogenesis in vivo

Antiangiogenic activity of the compounds was determined using the quantitative chick chorioallantoic membrane (CAM) assay [33,34], one of the most useful *in vivo* assay model of angiogenesis. CAM and rabbit cornea assay are the assay used most widely as an *in vivo* model for angiogenesis study. Of these, CAM provides advantages of being simpler to use, less expensive, reproducible and reliable. CAMs were treated with vascular endothelial growth factor (VEGF), the best characterized pro-angiogenic factor, prior to the compound treatment. As shown in Table 1, treatment of VEGF resulted in significant enhancement of the number of newly formed blood vessel branch points compared to the phosphate buffered saline (PBS)-treated control group. At a fixed dose of 0.01 nmol/CAM, the 6-aminopyridin-3-ol derivatives induced significant suppression of VEGF-induced angiogenesis; compound **6** was



Scheme 3. Introduction of $-NH_2$ group.

the most effective of the derivatives (80.9 ± 2.8% inhibition) and compound **15m** being the least effective.

Because VEGF stimulates tyrosine kinase activity within its receptor [46], and ROS production in downstream signal transduction pathway [47], we also compared the inhibitory effects of the 6-aminopyridin-3-ol derivatives to SU4312, a known receptor tyrosine kinase inhibitor [48]. While all of the test compounds showed considerable activities against angiogenesis, in particular, compounds **6** and **15h** showed the most significant inhibitory activities compared to SU4312. We then examined the 50% inhibitory dose (ID_{50}) of the three compounds (SU4312, **6** and **15h**) for VEGF-induced angiogenesis (Fig. 2). Each compound was prepared in stock solution and diluted with PBS at a concentration of μM; 10 μL was taken from each of the five solutions of different concentrations (0.001, 0.01, 0.1, 1.0 and 10 μM) and each was added directly to the disk on top of CAM. Because we measured the blood vessels in the area under the disk, the drug concentration inhibiting angiogenesis was calculated as 10 μL/CAM × compound concentration. The inhibitory effects of the three compounds were dose-dependent, and ID_{50}'s of SU4312, **6**, and **15h** for VEGF-induced angiogenesis were 10.5 pg/CAM, 3.2 pg/CAM, and 4.4 pg/CAM, respectively.

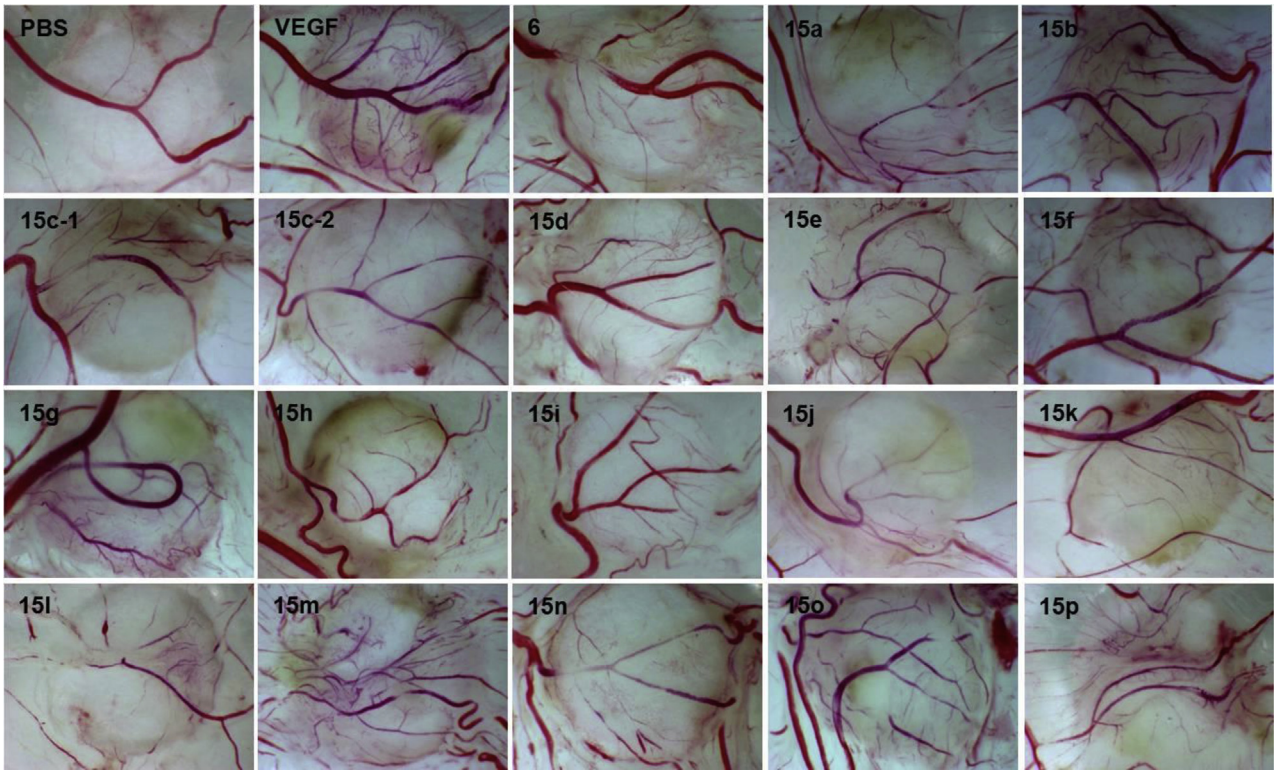
2.3. Antiangiogenic antitumor effects of 6-amino-2,4,5-trimethylpyridin-3-ols on A549 human lung cancer cell-inoculated CAMs

The potential of **6** and **15h** as antiangiogenic antitumor agents was examined using cancer cell-inoculated CAM assay. As shown in Table 2, implanted A549 cancer cells in a mixture of Matrigel onto CAM dramatically induced angiogenesis and corresponding tumor growth. In contrast, after exposure to compounds (once at the time of implantation), dose-dependent inhibition of cancer cell-induced angiogenesis was observed for compounds **6** and **15h**. Similarly, the compounds induced a significant reduction in the size and weight of excised tumors. Results showing proportionate suppression of tumor growth along with the inhibition of angiogenesis suggest that antitumor activity of **6** and **15h** was due to suppression of angiogenesis.

Angiogenesis is a very complex biological process involving a variety of events regarding vascular endothelial cell growth, migration, and differentiation. In this study, we have presented a novel series of 6-amino-2,4,5-trimethylpyridin-3-ols which have significant antiangiogenic activities. Some of them showed greater antiangiogenic activities (2.4- to 3.3-fold) than SU4312, a well-known VEGFR tyrosine kinase inhibitor.

Although some of the phenolic antioxidants such as rosmarinic acid, exhibited antiangiogenic activities [49], structural determinants for antiangiogenic activity have not been clearly defined. In this study, aminopyridinols with a hydrogen and a *p*-nitrophenyl group on the *C*(6)-amino position (**6** and **15h**) showed the best antiangiogenic activity (75–81% inhibition) while relatively electron-rich aminoalkyl derivatives, such as **15b**, **15c–1**, **15d**, **15m**, and **15e**, showed rather weak antiangiogenic activities (30–57% inhibition). In addition, aminopyridinols with aliphatic substituents (**15a**, **15b**, **15c–1**, **15d**, **15m**) were generally weaker than those with aromatic substituents. Initially, we expected significant variation in antiangiogenic activity among various 6-amino-2,4,5-trimethylpyridin-3-ol structures shown in this study, however, the window of their antiangiogenic activities was narrower than expected. Compared to the previously known antioxidant scaffold, it might be possible that aminopyridinols in this study possess similar and high level of antioxidant activities [11], which resulted in a narrow range of antioxidant and anti-angiogenic activity.

Table 1
Inhibitory effects of 6-amino-2,4,5-trimethylpyridin-3-ols (**6** and **15a**–**p**) on the VEGF-induced angiogenesis *in vivo*.[a]



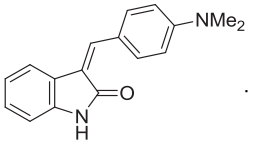
Treatment	Compound	Inhibition (%)[b]
VEGF (20 ng/CAM)+	**SU4312**[c]	70.5 ± 6.5#
	6	80.9 ± 2.8#
	15a	53.7 ± 10.3#
	15b	30.1 ± 20.0
	15c–1	46.3 ± 12.0#
	15c–2	67.3 ± 13.9#
	15d	36.8 ± 10.7
	15e	56.7 ± 16.8#
	15f	69.4 ± 5.7#
	15g	55.3 ± 20.3
	15h	75.0 ± 15.8#
	15i	61.7 ± 7.7#
	15j	62.4 ± 11.6
	15k	65.4 ± 14.6#
	15l	69.5 ± 12.7#
	15m	24.7 ± 21.3
	15n	59.0 ± 11.0#
	15o	44.0 ± 17.2
	15p	42.2 ± 21.7

#$P < 0.05$ compared to the VEGF-treated CAM sample.

[a] Quantitation of new branches formed from existing blood vessels was performed. Photographs were imported into an Image software program, to measure the new branch points. The data is expressed as the mean ± S.E.M. of at least six chick embryos. Dose was 0.01 nmol/CAM for both test compounds and SU4312, which is equivalent to 2–3 ng/CAM depending on the molecular weights.

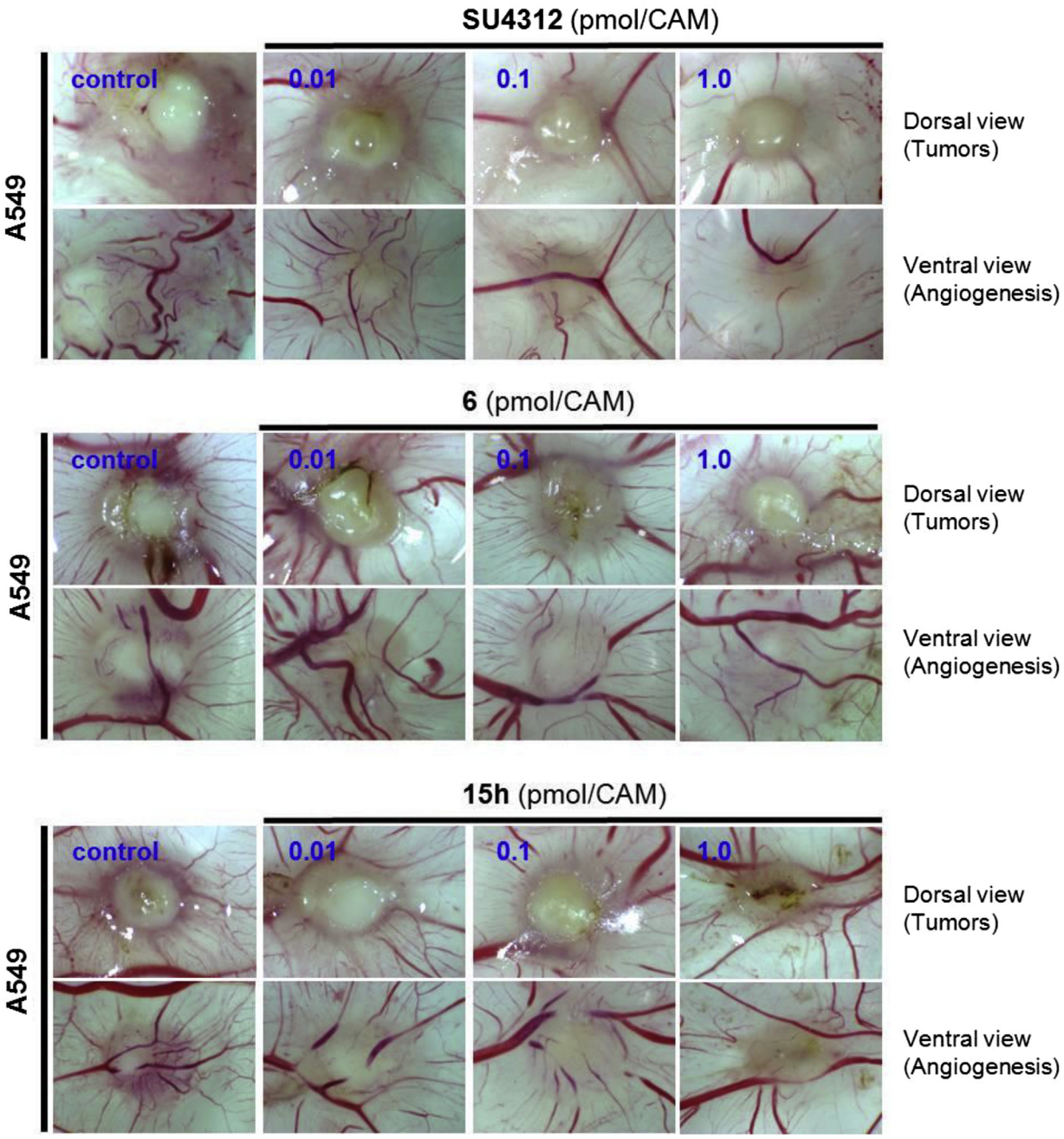
[b] Inhibition (%) = [(the number of vessel branch points induced by VEGF)–(the number of vessel branch points induced by test compound and VEGF)/(the number of vessel branch points induced by VEGF)] × 100.

[c] **SU4312** is known as a potent and selective inhibitor of VEGF receptor.



SU4312

Table 2
Antiangiogenic antitumor effects of the compounds **6** and **15h** on A549 human lung cancer cell-inoculated CAMs.[a].



Treatment (pmol/CAM)		Angiogenesis inhibition (%)	Tumor inhibition (%)
SU4312	0.01	20.8 ± 1.4#	16.2 ± 20.4
	0.1	25.1 ± 2.8#	23.4 ± 9.3
	1.0	36.2 ± 1.9#	26.8 ± 4.9
6	0.01	16.6 ± 3.2#	20.5 ± 5.1
	0.1	18.0 ± 7.8#	25.2 ± 8.0
	1.0	20.7 ± 3.6#	27.4 ± 7.8#
15h	0.01	19.9 ± 1.9	25.3 ± 5.7#
	0.1	25.6 ± 4.5#	44.4 ± 6.0#
	1.0	34.5 ± 2.8#	50.1 ± 5.6#

#$P < 0.05$ compared to the vehicle-treated group.
[a] A549 human lung cancer cells (2×10^6 cells/CAM) were inoculated on top of CAM, and different concentrations of SU4312, **6**, and **15h** were introduced. The number of new vessel branches was quantitated the same method as described in Table 1. Tumor mass grown on top of CAM was isolated and weighed. The data represent the means ± S.E.M. of at least six chick embryos.

There are several important stimulators that are known for angiogenesis, including fibroblast growth factor (FGF), VEGF, angiopoietin (Ang) I and II, and matrix metalloproteinase (MMP) etc. Of these, VEGF has received special attention in the context of tumorigenic condition. Cancer cells utilize angiogenic cues, including VEGF, to promote sprouting of new blood vessels out of preexisting vascular system. In this study, we report another novel finding that the aminopyridinols, **6** and **15h**, which exhibited the best antiangiogenic activities in VEGF-stimulated system, strongly inhibited angiogenesis which was stimulated by inoculated A549 human lung cancer cells (Table 2). The inhibition was dose-dependent, in line with their antiangiogenic and antitumor activities.

Without identifying a specific target protein or enzyme with which our aminopyridinols interact to exert the antiangiogenic activity, it still remains quite difficult to conduct a target-based optimization or rational drug design. However, we believe that the novel antiangiogenic activity of the aminopyridinols deserves future study, including extensive medicinal chemistry work, in order to identify structural determinants for the activity and a lead structure for further optimization.

3. Conclusion

A new synthetic strategy for preparation of a wider range of 6-amino-2,4,5-trimethylpyridin-3-ols from pyridoxine·HCl was developed. This route features the introduction of various amino groups to the *C*(6)-position of 3-benzyloxy-6-bromo-2,4,5-trimethylpyridine using the Buchwald–Hartwig amination reaction using palladium(0) transition metal. It was shown that 6-amino-2,4,5-trimethylpyridin-3-ols have significant antiangiogenic activity. In particular, **6** and **15h** showed greater antiangiogenic activities than SU4312, a well-known VEGFR tyrosine kinase inhibitor. In addition, **6** and **15h** exhibited strong antiangiogenic antitumor activities, and the inhibitory level of **15h** against tumor growth was even higher than that of SU4312. Study of other pharmacological activities, including antioxidant activities, of the novel compounds prepared through this strategy is underway.

4. Experimental section

4.1. General

Materials were purchased from commercial supplier and used without further purification. Progress of the reaction was monitored by thin-layer-chromatography (TLC) using silica gel F_{254} plates. Purification of the products was performed using a Biotage 'Isolera One' with indicated solvents. Melting points were determined using a Fischer–Jones melting point apparatus and are not corrected. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz for ^{1}H NMR and 62.5 MHz for ^{13}C NMR and are reported as ppm from the internal standard tetramethylsilane (TMS). IR spectra were recorded on a Perkin Elmer Spectrum GX FT-IR spectrometer. Low-resolution mass spectra (LRMS) were recorded on an Agilent Technologies Quadruple 6130 LC/MS. High-resolution mass spectra (HRMS) were obtained using a Thermo Scientific LTQ Orbitrap XL mass spectrometer, and recorded in a positive ion mode using an electrospray (ESI) source.

4.2. Synthesis of 6-amino-2,4,5-trimethylpyridin-3-ols

4.2.1. 4,5-Bis(chloromethyl)-2-methylpyridin-3-ol hydrochloride (**10**) [CAS no. 39984-50-4]

DMF (0.2 mL, 2.583 mmol) was added to a suspension pyridoxine·HCl (**5**) (5 g, 24.31 mmol) in thionyl chloride (30 mL). The mixture was stirred for 3 h at 80 °C. Ethyl ether (70 mL) was added, followed by stirring for 1 h in an iced bath. The mixture was filtered and the filter cake was washed with ethyl ether. The filter cake was dried to give **10** (5.5 g, 93%) as a white solid.

4.2.2. 2,4,5-Trimethylpyridin-3-ol (**11**) [CAS no. 5622-78-6]

Zinc dust (8.08 g, 123.69 mmol) was added in small portions to a suspension of **10** (10 g, 41.23 mmol) in acetic acid (50 mL). The solution was refluxed for 3 h, and then cooled to room temperature. The solution was filtered and washed with acetic acid. The pH of the filtrate was adjusted to 6 with 10 M NaOH and saturated with NaCl. The aqueous layer was extracted with EtOAc (100 mL × 6). The combined organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 20:1) to give **11** (5.2 g, 92%) as a white solid.

4.2.3. 6-Bromo-2,4,5-trimethylpyridin-3-ol (**12**)

1,3-Dibromo-5,5-dimethylhydantoin (DBDMH, 2.5 g, 9.11 mmol) was added to a suspension of **11** (2.5 g, 18.22 mmol) in THF (30 mL) and the resulting mixture was stirred for 3 h at room temperature. The mixture was concentrated and the residue was diluted with EtOAc and water. The aqueous layer was extracted with EtOAc. The combined organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:4) to give **12** (3.22 g, 80%) as a yellow solid. mp 140 °C; ^{1}H NMR ($CHCl_3$-*d*) δ 5.56 (br s, 1H), 2.42 (s, 3H), 2.31 (s, 3H), 2.25 (s, 3H) ppm.; ^{13}C NMR ($CHCl_3$-*d*) δ 148.7, 142.7, 135.3, 133.1, 131.8, 18.7, 18.3, 13.3 ppm.; IR (KBr) ν 3419, 2926, 2709, 2595, 2515, 1707, 1582, 1550, 1444, 1404, 1335, 1267, 1246, 1218, 1099, 1011, 954, 920, 768, 729, 689, 613, 541, 519, 475 cm^{-1}; MS(ES-API) [M + H] 216 (^{79}Br).

4.2.4. 3-Benzyloxy-6-bromo-2,4,5-trimethylpyridine (**13**)

K_2CO_3 (20.78 g, 150.04 mmol), benzyl chloride (5.2 mL, 45.12 mmol) was added to a solution of **12** (6.5 g, 30.08 mmol) in DMF (15 mL) and the resulting mixture was stirred for 12 h at room temperature. The mixture was diluted with EtOAc (700 mL) and water. The organic layer was washed with water (20 mL × 10). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:20) to give **13** (8.9 g, 97%) as a white solid. m.p. 50 °C; ^{1}H NMR ($CHCl_3$-*d*) δ 7.38–7.43 (m, 5H), 4.77 (s, 2H), 2.46 (s, 3H), 2.32 (s, 3H), 2.24 (s, 3H) ppm.; ^{13}C NMR ($CHCl_3$-*d*) δ 151.8, 150.7, 142.0, 138.4, 136.8, 132.3, 129.0, 128.8, 128.3, 75.4, 19.5, 19.2, 14.2 ppm.; IR (KBr) ν 3444, 3067, 3036, 2999, 2954, 2913, 2869, 1945, 1885, 1808, 1748, 1578, 1541, 1497, 1454, 1436, 1417, 1399, 1357, 1260, 1240, 1225, 1212, 1096, 1028, 993, 981, 942, 905, 841, 766, 748, 703, 687, 626, 585, 547, 524, 487, 419 cm^{-1}; MS (ES-API) [M + H]$^+$ 306 (^{79}Br).

4.2.5. 5-Benzyloxy-N-hexyl-3,4,6-trimethylpyridin-2-amine (**14a**)

Hexylamine (0.97 mL, 7.35 mmol) was added to a mixture of **13** (1.5 g, 4.90 mmol), NaO^{t}Bu (680 mg, 6.86 mmol), tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$, 101 mg, 0.10 mmol), 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP, 125 mg, 0.20 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 2 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:15) to give **14a** (1.58 g, 97%) as a yellow solid. m.p. 45 °C; ^{1}H NMR ($CHCl_3$-*d*) δ 7.34–7.48 (m, 5H), 4.69 (s, 2H), 3.47 (t, *J* = 6.8 Hz, 2H), 2.43 (s, 3H), 2.19 (s, 3H), 1.99 (s, 3H), 1.57–1.65 (m, 2H), 1.25–1.35 (m, 6H), 0.87–0.92 (m, 3H) ppm; ^{13}C NMR ($CHCl_3$-*d*) δ 152.9, 144.2, 137.8, 128.9, 128.4,

128.3, 75.5, 42.7, 32.0, 30.4, 30.1, 27.2, 23.0, 14.4, 13.2, 13.0 ppm; IR (KBr) ν 3404, 3061, 3031, 2922, 2855, 1590, 1488, 1456, 1402, 1362, 1335, 1212, 1154, 1096, 985, 920, 754, 714, 694, 544, 465 cm^{-1}; MS (ES-API) $[M + H]^+$ 327.

4.2.6. 6-Hexylamino-2,4,5-trimethylpyridin-3-ol (**15a**)

To a solution of **14a** (100 mg, 0.306 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15a** (72 mg, 99%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 7.47 (s, 1H), 5.10 (s, 1H), 3.21–3.26 (t, J = 6.8 Hz, 2H), 2.21 (s, 3H), 2.05 (s, 3H), 1.92 (s, 3H), 1.50–1.52 (m, 2H), 1.27 (m, 6H), 0.83–0.86 (m, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 150.1, 140.1, 138.4, 134.8, 112.7, 41.4, 31.2, 29.2, 26.4, 22.1, 19.1, 13.9, 12.5, 12.4 ppm; IR (KBr) ν 2926, 2851, 1747, 1731, 1714, 1696, 1681, 1668, 1650, 1633, 1614, 1538, 1518, 1505, 1487, 1471, 1455, 1434, 1416, 1394, 1218, 1094, 667, 417 cm^{-1}; MS (ES-API) $[M + H]^+$ 237; HRMS calcd for $C_{14}H_{25}N_2O$ $[M + H]^+$ 237.1967, found 237.1961.

4.2.7. 5-Benzyloxy-N-hexadecyl-3,4,6-trimethylpyridin-2-amine (**14b**)

1-Hexadecylamine (1.96 g, 7.35 mmol) was added to a mixture of **13** (1.5 g, 4.90 mmol), NaO^{t}Bu (680 mg, 6.86 mmol), $Pd_2(dba)_3$ (101 mg, 0.10 mmol), BINAP (125 mg, 0.20 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 2 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 9:1) to give **14b** (1.42 g, 63%) as a yellow solid. m.p. 59 °C; ^{1}H NMR ($CHCl_3$-d) δ 7.24–7.49 (m, 5H), 4.69 (s, 2H), 3.44 (t, J = 7.0 Hz, 2H), 2.41 (s, 3H), 2.24 (s, 3H), 2.18 (s, 3H), 1.56–1.65 (m, 2H), 1.13–1.36 (m, 26H), 0.85–0.91 (m, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 153.2, 146.0, 144.3, 138.9, 138.0, 128.9, 128.3, 128.3, 113.1, 75.4, 42.5, 32.3, 30.5, 30.1, 30.0, 29.9, 29.7, 27.6, 23.1, 19.6, 14.5, 13.1, 12.9 ppm; IR (KBr) ν 3405, 2915, 2848, 1592, 1489, 1470, 1370, 1219, 1154, 1091, 984, 751, 715, 693, 506, 455 cm^{-1}; MS (ES-API) $[M + H]^+$ 467.

4.2.8. 6-Hexadecylamino-2,4,5-trimethylpyridin-3-ol (**15b**)

To a solution of **14b** (100 mg, 0.214 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15b** (73 mg, 90%) as a brown solid. m.p. 80 °C; ^{1}H NMR (DMSO-d_6) δ 7.32 (s, 1H), 4.87 (s, 1H), 3.20–3.37 (m, 2H), 2.18 (s, 3H), 2.03 (s, 3H), 1.88 (s, 3H), 1.21–1.49 (m, 28H), 0.83 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 150.2, 140.1, 139.2, 133.9, 112.3, 41.4, 31.3, 29.3, 29.0, 28.7, 26.7, 22.1, 19.5, 13.2, 12.4 ppm; IR (KBr) ν 3902, 3838, 3734, 3689, 3421, 2917, 2848, 1716, 1698, 1683, 1646, 1636, 1558, 1540, 1507, 1456, 1219, 1092, 669, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 377; HRMS calcd for $C_{24}H_{44}N_2O$ $[M + H]^+$ 377.3532, found 377.3539.

4.2.9. 3-[(5-Benzyloxy-3,4,6-trimethylpyridin-2-yl)amino]propan-1-ol (**14c–1**) & 5-Benzyloxy-N-[3-{(5-benzyloxy-3,4,6-trimethylpyridin-2-yl)oxy}propyl]-3,4,6-trimethylpyridin-2-amine (**14c–2**)

3-Amino-1-propanol (0.6 mL, 7.83 mmol) was added to a mixture of **13** (2 g, 6.53 mmol), NaO^{t}Bu (906 mg, 9.14 mmol), $Pd_2(dba)_3$ (135 mg, 0.13 mmol), BINAP (166 mg, 0.26 mmol) in toluene (30 mL) and the resulting mixture was refluxed for 4 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:4) to give **14c–1** (550 mg, 30%) as a yellow solid, and **14c–2** (630 mg, 36%) as a yellow solid. **14c–1**: m.p. 130 °C; ^{1}H NMR (DMSO-d_6) δ 7.31–7.48 (m, 5H), 5.47 (t, J = 5.5 Hz, 1H), 4.64 (s, 3H), 3.41–3.49 (m, 2H), 3.30–3.40 (m, 2H), 2.24 (s, 3H), 2.11 (s, 3H), 1.93 (s, 3H), 1.64–1.74 (m, 2H) ppm; ^{13}C NMR (DMSO-d_6) δ 152.7, 143.9, 142.7, 137.9, 137.5, 128.2, 127.9, 127.7, 112.7, 74.3, 58.8, 38.4, 32.6, 18.9, 12.3, 12.3 ppm; IR (KBr) ν 3389, 2929, 1603, 1520, 1455, 1397, 1371, 1252, 1229, 1160, 1096, 1068, 990, 935, 862, 752, 712, 695, 545, 492, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 301. **14c–2**: m.p. 90 °C; ^{1}H NMR (DMSO-d_6) δ 7.30–7.47 (m, 10H), 5.49 (t, J = 5.4 Hz, 1H), 4.70 (s, 2H), 4.63 (s, 2H), 4.29 (t, J = 6.2 Hz, 2H), 3.44–3.51 (m, 2H), 2.29 (s, 3H), 2.24 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.00–2.05 (m, 2H), 1.94 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 156.4, 152.5, 145.9, 144.1, 143.9, 142.8, 140.7, 137.7, 137.5, 137.1, 128.3, 128.2, 127.9, 127.8, 127.7, 116.1, 112.7, 79.1, 74.3, 63.5, 59.6, 38.4, 29.1, 19.0, 18.7, 12.4, 12.2, 11.4 ppm; IR (KBr) ν 3429, 2948, 1733, 1716, 1698, 1592, 1558, 1540, 1507, 1488, 1455, 1364, 1332, 1228, 1130, 1091, 991, 745, 717, 697, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 526.

4.2.10. 6-[(3-Hydroxypropyl)amino]-2,4,5-trimethylpyridin-3-ol (**15c–1**)

To a solution of **14c–1** (100 mg, 0.333 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15c–1** (70 mg, 100%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 7.42 (s, 1H), 5.16 (s, 1H), 3.45 (t, J = 6.0 Hz, 2H), 3.31 (t, J = 6.4 Hz, 2H), 2.19 (s, 3H), 2.05 (s, 3H), 1.91 (s, 3H), 1.60–1.70 (m, 2H) ppm; ^{13}C NMR (DMSO-d_6) δ 150.6, 140.2, 138.8, 134.6, 112.6, 58.8, 38.5, 32.8, 19.3, 12.5 ppm; IR (KBr) ν 3913, 3897, 3878, 3860, 3849, 3833, 3812, 3798, 3775, 3764, 3741, 3730, 3719, 3707, 3685, 3666, 3644, 3625, 3605, 3584, 3389, 2938, 1865, 1841, 1789, 1746, 1730, 1714, 1694, 1681, 1667, 1650, 1644, 1633, 1614, 1573, 1556, 1537, 1514, 1504, 1470, 1455, 1372, 1232, 1072, 1032, 941, 666 cm^{-1}; MS (ES-API) $[M + H]^+$ 211; HRMS calcd for $C_{11}H_{19}N_2O_2$ $[M + H]^+$ 211.1447, found 211.1443.

4.2.11. 6-[3-{(5-Hydroxy-3,4,6-trimethylpyridin-2-yl)amino}propoxy]-2,4,5-trimethylpyridin-3-ol (**15c–2**)

To a solution of **14c–2** (100 mg, 0.190 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15c–2** (64 mg, 97%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 7.87 (br s, 1H), 7.43 (br s, 1H), 4.23 (t, J = 6.3 Hz, 2H), 3.40 (t, J = 6.3 Hz, 2H), 2.25 (s, 3H), 2.21 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.85 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 154.0, 150.0, 143.4, 140.3, 138.2, 136.1, 115.3, 112.8, 68.1, 63.3, 48.5, 29.2, 21.6, 19.1, 12.4, 12.4, 11.4 ppm; IR (KBr) ν 3897, 3860, 3849, 3834, 3812, 3798, 3764, 3741, 3730, 3707, 3685, 3666, 3644, 3625, 3443, 1865, 1841, 1789, 1730, 1714, 1694, 1681, 1659, 1650, 1644, 1633, 1573, 1556, 1537, 1514, 1504, 1486, 1470, 1455, 1416, 1372, 1228, 1093, 1054, 1032, 666 cm^{-1}; MS (ES-API) $[M + H]^+$ 346; HRMS calcd for $C_{19}H_{28}N_3O_3$ $[M + H]^+$ 346.2131, found 346.2129.

4.2.12. 5-Benzyloxy-N-cyclohexyl-3,4,6-trimethylpyridin-2-amine (**14d**)

Cyclohexylamine (0.81 g, 7.10 mmol) was added to a mixture of **13** (1.8 g, 5.91 mmol), NaO^{t}Bu (820 mg, 8.27 mmol), $Pd_2(dba)_3$

(122 mg, 0.12 mmol), BINAP (150 mg, 0.23 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 2 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:19) to give **14d** (1.73 g, 90%) as a yellow solid. m.p. 78 °C; ^{1}H NMR ($CHCl_3$-*d*) δ 7.29–7.48 (m, 5H), 4.68 (s, 2H), 3.92–4.01 (m, 1H), 2.38 (s, 3H), 2.16 (s, 3H), 2.04–2.11 (m, 2H), 1.95 (s, 3H), 1.09–1.77 (m, 8H) ppm; ^{13}C NMR ($CHCl_3$-*d*) δ 152.3, 145.9, 144.0, 138.6, 137.9, 128.6, 128.1, 112.7, 75.2, 49.7, 34.1, 26.3, 25.3, 19.6, 12.9, 12.7 ppm; IR (KBr) ν 3421, 2928, 2844, 1592, 1558, 1540, 1507, 1489, 1456, 1396, 1362, 1340, 1212, 1151, 1100, 1014, 884, 739, 713, 694, 669, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 325.

4.2.13. 6-Cyclohexylamino-2,4,5-trimethylpyridin-3-ol (**15d**)

To a solution of **14d** (100 mg, 0.308 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15d** (71 mg, 98%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 7.41 (s, 1H), 4.51 (s, 1H), 3.77 (s, 1H), 2.19 (s, 3H), 2.04 (s, 3H), 1.90 (s, 3H), 1.10–1.66 (m, 10H) ppm; ^{13}C NMR (DMSO-d_6) δ 149.5, 140.1, 139.2, 134.1, 112.4, 49.0, 33.1, 25.8, 25.1, 19.7, 12.5, 12.5 ppm; IR (KBr) ν 2926, 2844, 1735, 1596, 1494, 1450, 1358, 1298, 1239, 1210, 1137, 1098, 1051, 1032, 921, 889, 757, 655, 546 cm^{-1}; MS (ES-API) $[M + H]^+$ 235; HRMS calcd for $C_{14}H_{23}N_2O$ $[M + H]^+$ 235.1810, found 235.1815.

4.2.14. N-Benzyl-5-benzyloxy-3,4,6-trimethylpyridin-2-amine (**14e**)

Benzylamine (0.8 mL, 7.35 mmol) was added to a mixture of **13** (1.5 g, 4.90 mmol), NaO^{t}Bu (680 mg, 6.86 mmol), $Pd_2(dba)_3$ (101 mg, 0.10 mmol), BINAP (125 mg, 0.20 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 4 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:15) to give **14e** (1.6 g, 97%) as a yellow solid. m.p. 85 °C; ^{1}H NMR ($CHCl_3$-*d*) δ 7.24–7.52 (m, 10H), 4.74 (s, 2H), 4.70 (s, 2H), 4.18 (s, 1H), 2.46 (s, 3H), 2.22 (s, 3H), 2.00 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-*d*) δ 152.7, 146.1, 144.8, 141.2, 139.1, 138.0, 129.0, 128.9, 128.8, 128.4, 128.3, 128.3, 127.4, 113.3, 75.4, 46.7, 19.7, 13.1, 12.9 ppm; IR (KBr) ν 3450, 3028, 2905, 2866, 1591, 1505, 1452, 1396, 1356, 1272, 1210, 1152, 1096, 1027, 993, 911, 855, 755, 730, 710, 695, 604, 451, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 333.

4.2.15. 6-Benzylamino-2,4,5-trimethylpyridin-3-ol (**15e**)

To a solution of **14e** (100 mg, 0.301 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15e** (66 mg, 90%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 7.15–7.34 (m, 5H), 5.69 (t, J = 5.6 Hz, 1H), 4.50 (d, J = 5.3 Hz, 2H), 2.17 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 149.8, 142.2, 140.5, 139.1, 134.3, 127.8, 127.3, 126.0, 112.6, 44.5, 19.6, 12.5, 12.4 ppm; IR (KBr) ν 3913, 3897, 3878, 3860, 3849, 3833, 3812, 3798, 3775, 3764, 3741, 3730, 3707, 3685, 3666, 3644, 3625, 3605, 3584, 3562, 3361, 1865, 1841, 1789, 1746, 1730, 1714, 1694, 1681, 1667, 1650, 1644, 1633, 1614, 1573, 1556, 1537, 1514, 1504, 1494, 1486, 1470, 1454, 1433, 1416, 1228, 1096, 1032, 699 cm^{-1}; MS (ES-API) $[M + H]^+$ 243; HRMS calcd for $C_{15}H_{19}N_2O$ $[M + H]^+$ 243.1497, found 243.1501.

4.2.16. 5-Benzyloxy-3,4,6-trimethyl-N-phenylpyridin-2-amine (**14f**)

Aniline (0.71 mL, 7.838 mmol) was added to a mixture of **13** (2 g, 6.53 mmol), NaO^{t}Bu (906 mg, 9.14 mmol), $Pd_2(dba)_3$ (135 mg, 0.13 mmol), BINAP (166 mg, 0.26 mmol) in toluene (30 mL) and the resulting mixture was refluxed for 2 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:19) to give **14f** (1.94 g, 93%) as a yellow solid. m.p. 95 °C; ^{1}H NMR ($CHCl_3$-*d*) δ 7.25–7.51 (m, 9H), 6.93–6.99 (m, 1H), 6.38 (s, 1H), 4.79 (s, 2H), 2.49 (s, 3H), 2.27 (s, 3H), 2.11 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-*d*) δ 149.0, 147.0, 146.3, 142.5, 141.6, 137.5, 129.2, 129.0, 128.6, 128.3, 121.5, 118.5, 118.1, 75.5, 19.2, 14.1, 13.5 ppm; IR (KBr) ν 3399, 3025, 3005, 2918, 2884, 2844, 1924, 1869, 1809, 1749, 1597, 1583, 1517, 1497, 1441, 1413, 1392, 1364, 1299, 1256, 1213, 1180, 1116, 1088, 1028, 1014, 996, 925, 889, 877, 828, 743, 715, 695, 665, 620, 552, 494, 473 cm^{-1}; MS (ES-API) $[M + H]^+$ 319.

4.2.17. 2,4,5-Trimethyl-6-(phenylamino)pyridin-3-ol (**15f**)

To a solution of **14f** (100 mg, 0.314 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15f** (70 mg, 98%) as a yellow solid. m.p. 172 °C; ^{1}H NMR (DMSO-d_6) δ 8.07 (br s, 1H), 7.53 (s, 1H), 7.25–7.28 (m, 2H), 7.10–7.16 (m, 2H), 6.68–6.73 (m, 1H), 2.27 (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 145.5, 144.2, 143.9, 139.9, 134.5, 128.2, 118.4, 118.4, 116.4, 19.4, 13.5, 12.6 ppm; IR (KBr) ν 3385, 2890, 2495, 1603, 1558, 1540, 1499, 1436, 1375, 1296, 1211, 1090, 744, 266, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 229; HRMS calcd for $C_{14}H_{17}N_2O$ $[M + H]^+$ 229.1341, found 229.1345.

4.2.18. 5-Benzyloxy-N-(2-isopropylphenyl)-3,4,6-trimethylpyridin-2-amine (**14g**)

2-Isopropylaniline (1.03 mL, 7.35 mmol) was added to a mixture of **13** (1.5 g, 4.90 mmol), NaO^{t}Bu (680 mg, 6.86 mmol), $Pd_2(dba)_3$ (101 mg, 0.10 mmol), BINAP (125 mg, 0.20 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 2 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 99:1) to give **14g** (1.78 g, 99%) as a brown solid. m.p. 70 °C; ^{1}H NMR ($CHCl_3$-*d*) δ 7.25–7.49 (m, 7H), 7.06–7.13 (m, 1H), 6.94–7.01 (m, 1H), 5.92 (s, 1H), 4.77 (s, 2H), 3.13–3.24 (m, 1H), 2.42 (s, 3H), 2.23 (s, 3H), 2.03 (s, 3H), 1.32 (s, 3H), 1.29 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-*d*) δ 149.9, 147.1, 147.0, 140.0, 139.9, 137.6, 137.5, 128.7, 128.3, 128.1, 126.2, 125.6, 122.1, 119.6, 118.3, 75.2, 27.9, 22.9, 19.3, 14.1, 13.2 ppm; IR (KBr) ν 3483, 3031, 2963, 1749, 1716, 1698, 1683, 1602, 1521, 1472, 1455, 1418, 1395, 1363, 1294, 1218, 1091, 1019, 750, 730, 693, 444, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 361.

4.2.19. 6-[(2-Isopropylphenyl)amino]-2,4,5-trimethylpyridin-3-ol (**15g**)

To a solution of **14g** (100 mg, 0.277 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite

pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15g** (72 mg, 96%) as a yellow solid. m.p. 140 °C; ^{1}H NMR (DMSO-d_6) δ 7.19 (d, J = 7.4 Hz, 1H), 6.77–7.01 (m, 4H), 3.11–3.22 (m, 1H), 2.20 (s, 3H), 2.13 (s, 3H), 2.02 (s, 3H), 1.17 (s, 3H), 1.14 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 147.1, 144.0, 141.2, 140.4, 138.6, 134.5, 125.5, 125.1, 121.1, 119.8, 118.6, 26.7, 22.9, 22.3, 19.2, 13.6, 12.6 ppm; IR (KBr) ν 3734, 3401, 2960, 2877, 1698, 1602, 1558, 1540, 1507, 1473, 1456, 1418, 1338, 1243, 1205, 1094, 1022, 754, 457, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 271; HRMS calcd for $C_{17}H_{23}N_2O$ $[M + H]^+$ 271.1810, found 271.1819.

4.2.20. 5-Benzyloxy-3,4,6-trimethyl-N-(4-nitrophenyl)pyridin-2-amine (**14h**)

4-Nitroaniline (1.03 mL, 7.35 mmol) was added to a mixture of **13** (2.18 g, 7.12 mmol), NaO^{t}Bu (987 mg, 9.96 mmol), $Pd_2(dba)_3$ (147 mg, 0.14 mmol), BINAP (177 mg, 0.28 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 6 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$ only) to give **14h** (2.36 g, 91%) as a yellow solid. m.p. 171 °C; ^{1}H NMR ($CHCl_3$-d) δ 8.12 (dd, J = 7.2, 1.9 Hz, 2H), 7.35–7.46 (m, 7H), 6.50 (s, 1H), 4.77 (s, 2H), 2.46 (s, 3H), 2.24 (s, 3H), 2.15 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 148.7, 148.3, 147.6, 146.8, 141.1, 140.6, 137.1, 128.8, 128.5, 128.1, 125.7, 119.3, 116.0, 75.3, 19.5, 13.7, 13.3 ppm; IR (KBr) ν 3396, 1593, 1525, 1504, 1475, 1455, 1405, 1374, 1319, 1300, 1268, 1240, 1215, 1184, 1109, 1086, 990, 845, 748, 708, 696, 575, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 364.

4.2.21. 2,4,5-Trimethyl-6-[(4-nitrophenyl)amino]pyridin-3-ol (**15h**)

To a solution of **14h** (100 mg, 0.277 mmol) in CH_2Cl_2 (3 mL) was added 1 M BCl_3 (0.55 mL, 0.55 mmol) in an iced bath. The mixture was stirred for 30 min in an iced bath. A solution of chloroform and methanol (volume ratio of 9:1, 1 mL) was added to the reaction solution and stirred for 1 h at room temperature. The reaction solution was concentrated and the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 9:1) to give **15h** (56 mg, 74%) as a yellow solid. m.p. 223 °C; ^{1}H NMR (DMSO-d_6) δ 8.86 (s, 1H), 8.44 (s, 1H), 8.03 (d, J = 9.1 Hz, 2H), 7.21 (d, J = 9.1 Hz, 2H), 2.31 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 151.2, 146.1, 142.7, 140.9, 137.5, 134.7, 125.3, 121.6, 114.1, 19.2, 13.2, 12.5 ppm; IR 3392, 2923, 1597, 1498, 1479, 1319, 1277, 1197, 1113, 841, 810, 751, 467 cm^{-1}; MS (ES-API) $[M + H]^+$ 274; HRMS calcd for $C_{14}H_{16}N_3O_3$ $[M + H]^+$ 274.1192, found 274.1196.

4.2.22. 5-Benzyloxy-N-(4-bromophenyl)-3,4,6-trimethylpyridin-2-amine (**14i**)

4-Bromoaniline (869 mg, 4.90 mmol) was added to a mixture of **13** (1.5 g, 4.90 mmol), NaO^{t}Bu (680 mg, 6.86 mmol), $Pd_2(dba)_3$ (101 mg, 0.10 mmol), BINAP (125 mg, 0.20 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 6 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:9) to give **14i** (1.23 g, 63%) as a yellow solid. m.p. 150 °C; ^{1}H NMR ($CHCl_3$-d) δ 7.25–7.48 (m, 9H), 6.02 (s, 1H), 4.74 (s, 2H), 2.42 (s, 3H), 2.22 (s, 3H), 2.09 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 148.4, 146.9, 146.8, 141.5, 140.4, 137.3, 131.8, 128.8, 128.3, 128.1, 119.6, 117.2, 112.8, 75.2, 19.4, 13.6, 13.2 ppm; IR (KBr) ν 3444, 3032, 2912, 1596, 1570, 1540, 1508, 1486, 1455, 1402, 1372, 1299, 1254, 1213, 1177, 1082, 1071, 1031, 989, 911, 822, 808, 749, 713, 703, 694, 591, 498, 444 cm^{-1}; MS (ES-API) $[M + H]^+$ 397.

4.2.23. 6-[(4-Bromophenyl)amino]-2,4,5-trimethylpyridin-3-ol (**15i**)

To a solution of **14i** (100 mg, 0.251 mmol) in CH_2Cl_2 (3 mL) was added 1 M BCl_3 (0.55 mL, 0.55 mmol) in an iced bath. The mixture was stirred for 30 min in an iced bath. A solution of chloroform and methanol (volume ratio of 9:1, 1 mL) was added to the reaction solution and stirred for 1 h at room temperature. The reaction solution was concentrated and the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 9:1) to give **15i** (75 mg, 97%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 8.44 (br s, 1H), 7.99 (s, 1H), 7.30 (d, J = 8.9 Hz, 2H), 7.19 (d, J = 8.9 Hz, 2H), 2.30 (s, 3H), 2.16 (s, 3H), 2.09 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 144.6, 143.2, 139.1, 131.0, 129.2, 118.4, 115.1, 109.8, 48.5, 18.3, 13.4, 12.8 ppm; IR (KBr) ν 1592, 1573, 1556, 1537, 1513, 1504, 1487, 1462, 1454, 1415, 1352, 1224, 1088, 1072, 1004, 815, 666, 416 cm^{-1}; MS (ES-API) $[M + H]^+$ 307; HRMS calcd for $C_{14}H_{16}BrN_2O$ $[M + H]^+$ 307.0446, found 307.0456.

4.2.24. 2,4,5-Trimethyl-6-(phenylamino)pyridin-3-ol hydrobromide (**15f**–**HBr**)

To a solution of **14i** (100 mg, 0.251 mmol) in methanol–THF (1:1, 2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15f**–**HBr** (76 mg, 98%) as a yellow solid. m.p. 226 °C; ^{1}H NMR (DMSO-d_6) δ 9.63 (br s, 1H), 8.82 (br s, 1H), 7.25–7.31 (m, 2H), 6.92–7.02 (m, 3H), 2.44 (s, 3H), 2.32 (s, 3H), 2.17 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 146.2, 145.6, 142.3, 141.2, 135.7, 129.4, 125.4, 121.6, 117.1, 14.8, 14.1, 13.8 ppm; IR (KBr) ν 3734, 3419, 3254, 2980, 2791, 1749, 1683, 1646, 1635, 1594, 1558, 1540, 1507, 1473, 1456, 1375, 1350, 1198, 1096, 1026, 903, 745, 693, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 229.

4.2.25. N-(Benzo[d][1,3]dioxol-5-yl)-5-benzyloxy-3,4,6-trimethylpyridin-2-amine (**14j**)

3,4-(Methylenedioxy)aniline (1.01 g, 7.35 mmol) was added to a mixture of **13** (1.5 g, 4.90 mmol), NaO^{t}Bu (680 mg, 6.86 mmol), $Pd_2(dba)_3$ (101 mg, 0.10 mmol), BINAP (125 mg, 0.20 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 2 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:9) to give **14j** (1.57 g, 88%) as a brown solid. m.p. 130 °C; ^{1}H NMR ($CHCl_3$-d) δ 7.33–7.47 (m, 5H), 7.17 (d, J = 2.0 Hz, 1H), 6.61–6.72 (m, 2H), 5.89 (s, 3H), 4.73 (s, 2H), 2.41 (s, 3H), 2.20 (s, 3H), 2.07 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 149.4, 147.9, 146.6, 146.5, 142.1, 140.1, 137.5, 137.3, 128.7, 128.3, 128.1, 116.4, 111.2, 108.2, 101.7, 101.0, 75.2, 19.4, 13.5, 13.1 ppm; IR (KBr) ν 3413, 2874, 1635, 1592, 1558, 1503, 1489, 1455, 1416, 1395, 1365, 1328, 1281, 1258, 1216, 1192, 1142, 1109, 1098, 1080, 1042, 1021, 935, 844, 815, 805, 789, 754, 736, 720, 695, 667, 606, 568, 458, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 363.

4.2.26. 6-(Benzo[d][1,3]dioxol-5-ylamino)-2,4,5-trimethylpyridin-3-ol (**15j**)

To a solution of **14j** (100 mg, 0.276 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15j** (75 mg, 100%) as a brown solid. m.p. 168 °C; ^{1}H NMR (DMSO-d_6) δ 7.33 (s, 1H), 7.11 (s, 1H), 6.71 (s, 2H), 5.87 (s, 2H), 2.24(s, 3H), 2.10 (s, 3H), 2.05 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 146.8, 146.1, 143.3, 139.8, 139.6, 138.9,

134.6, 117.0, 109.3, 107.7, 100.2, 99.8, 19.4, 13.3, 12.6 ppm; IR (KBr) ν 3838, 3731, 3648, 3568, 3565, 3393, 2890, 1749, 1683, 1646, 1635, 1558, 1540, 1507, 1488, 1456, 1201, 1038, 928, 786, 669, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 273; HRMS calcd for $C_{15}H_{17}N_2O_3$ $[M + H]^+$ 273.1239, found 273.1246.

4.2.27. 5-Benzyloxy-3,4,6-trimethyl-N,N-diphenylpyridin-2-amine (**14k**)

Diphenylamine (829 mg, 4.90 mmol) was added to a mixture of **13** (1.5 g, 4.90 mmol), NaO^{t}Bu (680 mg, 6.86 mmol), $Pd_2(dba)_3$ (101 mg, 0.10 mmol), BINAP (125 mg, 0.20 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 2 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:9) to give **14k** (695 mg, 36%) as a brown oil. ^{1}H NMR ($CHCl_3$-*d*) δ 7.35–7.49 (m, 5H), 7.17–7.23 (m, 5H), 6.89–6.96 (m, 5H), 4.83 (s, 2H), 2.42 (s, 3H), 2.21 (s, 3H), 1.94 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-*d*) δ 151.8, 150.4, 149.7, 146.8, 141.9, 137.1, 129.1, 128.8, 128.7, 128.4, 128.0, 121.8, 121.7, 77.4, 74.9, 19.6, 14.6, 13.3 ppm; IR (KBr) ν 3913, 3897, 3878, 3860, 3849, 3833, 3812, 3798, 3775, 3764, 3741, 3730, 3719, 3707, 3697, 3685, 3666, 3645, 3625, 3605, 3584, 3562, 3541, 3028, 1865, 1841, 1789, 1746, 1730, 1714, 1694, 1681, 1667, 1650, 1644, 1633, 1587, 1573, 1556, 1537, 1515, 1504, 1494, 1486, 1470, 1454, 1433, 1416, 1367, 1216, 1089, 750, 694 cm^{-1}; MS (ES-API) $[M + H]^+$ 395.

4.2.28. 6-(Diphenylamino)-2,4,5-trimethylpyridin-3-ol (**15k**)

To a solution of **14k** (100 mg, 0.253 mmol) in methanol–THF (1:1, 2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 5 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15k** (68 mg, 87%) as a yellow solid. m.p. 227 °C; ^{1}H NMR (DMSO-d_6) δ 7.16–7.22 (m, 4H), 6.79–6.98 (m, 6H), 2.28 (s, 3H), 2.13 (s, 3H), 1.91 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 148.1, 146.9, 146.3, 143.2, 134.6, 128.9, 127.7, 120.9, 120.3, 19.4, 13.8, 12.5 ppm; IR (KBr) ν 3418, 2922, 2619, 1739, 1590, 1493, 1416, 1380, 1293, 1273, 1260, 1217, 1177, 1093, 1029, 933, 748, 693, 586, 527, 510 cm^{-1}; MS (ES-API) $[M + H]^+$ 305; HRMS calcd for $C_{20}H_{21}N_2O$ $[M + H]^+$ 305.1654, found 305.1660.

4.2.29. 3-Benzyloxy-2,4,5-trimethyl-6-(pyrrolidin-1-yl)pyridine (**14l**)

Pyrrolidine (0.40 mL, 4.90 mmol) was added to a mixture of **13** (1.5 g, 4.90 mmol), NaO^{t}Bu (680 mg, 6.86 mmol), $Pd_2(dba)_3$ (101 mg, 0.10 mmol), BINAP (125 mg, 0.20 mmol) in toluene (25 mL) and the resulting mixture was refluxed for 4 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:9) to give **14l** (1.04 g, 71%) as a yellow oil. ^{1}H NMR ($CHCl_3$-*d*) δ 7.33–7.48 (m, 5H), 4.71 (s, 2H), 3.36 (t, *J* = 6.7 Hz, 4H), 2.41 (s, 3H), 2.18 (s, 3H), 2.14 (s, 3H), 1.85–1.93 (m, 4H) ppm; ^{13}C NMR ($CHCl_3$-*d*) δ 156.3, 146.3, 145.2, 140.2, 137.7, 128.7, 128.1, 128.0, 118.9, 75.0, 50.4, 25.5, 19.5, 15.7, 13.2 ppm; IR (KBr) ν 3913, 3897, 3878, 3860, 3849, 3833, 3812, 3797, 3764, 3741, 3730, 3707, 3685, 3666, 3644, 3625, 3605, 3584, 3562, 2959, 2867, 1865, 1841, 1789, 1768, 1746, 1730, 1714, 1694, 1681, 1667, 1650, 1644, 1633, 1574, 1556, 1537, 1514, 1495, 1454, 1406, 1368, 1345, 1218, 1146, 1087, 1013, 914, 734, 716, 696, 622 cm^{-1}; MS (ES-API) $[M + H]^+$ 297.

4.2.30. 2,4,5-Trimethyl-6-(pyrrolidin-1-yl)pyridin-3-ol (**15l**)

To a solution of **14l** (100 mg, 0.337 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 10 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15l** (70 mg, 100%) as a pink oil. ^{1}H NMR (DMSO-d_6) δ 8.04 (br s, 1H), 3.23 (t, *J* = 6.5 Hz, 4H), 2.27 (s, 3H), 2.10 (s, 6H), 1.78–1.84 (m, 4H) ppm; ^{13}C NMR (DMSO-d_6) δ 151.4, 143.9, 138.4, 136.0, 119.4, 50.2, 24.4, 18.8, 14.8, 12.7 ppm; IR (KBr) ν 3913, 3897, 3878, 3860, 3850, 3834, 3812, 3798, 3764, 3741, 3730, 3707, 3685, 3666, 3644, 3625, 3442, 1865, 1841, 1789, 1746, 1730, 1714, 1694, 1681, 1659, 1650, 1644, 1633, 1573, 1556, 1537, 1518, 1504, 1494, 1486, 1470, 1454, 1415, 1346, 1216, 1087, 1032, 666 cm^{-1}; MS (ES-API) [$[M + H]^+$ 207; HRMS calcd for $C_{12}H_{19}N_2O$ $[M + H]^+$ 207.1497, found 207.1494.

4.2.31. 1-(5-Benzyloxy-3,4,6-trimethylpyridin-2-yl)-4-methylpiperazine (**14m**)

N-Methylpiperazine (0.73 mL, 6.53 mmol) was added to a mixture of **13** (2 g, 6.53 mmol), NaO^{t}Bu (906 mg, 9.14 mmol), $Pd_2(dba)_3$ (135 mg, 0.13 mmol), BINAP (166 mg, 0.26 mmol) in toluene (30 mL) and the resulting mixture was refluxed for 6 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 20:1) to give **14m** (918 mg, 43%) as a yellow solid. m.p. 98 °C; ^{1}H NMR ($CHCl_3$-*d*) δ 7.32–7.47 (m, 5H), 4.71 (s, 2H), 3.07 (t, *J* = 4.7 Hz, 4H), 2.55 (br s, 4H), 2.41 (s, 3H), 2.33 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-*d*) δ 157.3, 147.0, 146.4, 140.5, 137.5, 128.7, 128.2, 128.0, 122.2, 74.9, 55.7, 50.4, 46.5, 19.5, 14.8, 13.2 ppm; IR (KBr) ν 3838, 3734, 3446, 2965, 2932, 2851, 2789, 1576, 1507, 1497, 1455, 1404, 1376, 1364, 1284, 1265, 1229, 1212, 1161, 1146, 1088, 1009, 972, 914, 845, 796, 750, 718, 691, 619, 591, 508, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 326.

4.2.32. 2,4,5-Trimethyl-6-(4-methylpiperazin-1-yl)pyridin-3-ol (**15m**)

To a solution of **14m** (100 mg, 0.307 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 2 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15m** (72 mg, 99%) as a yellow solid. m.p. 149 °C; ^{1}H NMR (DMSO-d_6) δ 2.87 (t, *J* = 4.7 Hz, 4H), 2.50 (t, *J* = 1.9 Hz, 4H), 2.27 (s, 3H), 2.25 (s, 3H), 2.08 (s, 6H) ppm; ^{13}C NMR (DMSO-d_6) δ 153.3, 145.2, 139.9, 134.4, 121.6, 54.8, 49.9, 45.5, 19.4, 13.7, 12.5 ppm; IR (KBr) ν 3735, 3348, 2967, 2922, 2860, 1735, 1587, 1455, 1413, 1368, 1307, 1270, 1229, 1105, 1065, 1032, 918, 901, 843, 697, 614 cm^{-1}; MS (ES-API) $[M + H]^+$ 236; HRMS calcd for $C_{13}H_{22}N_3O$ $[M + H]^+$ 236.1763, found 236.1769.

4.2.33. 4-(5-Benzyloxy-3,4,6-trimethylpyridin-2-yl)morpholine (**14n**)

Morpholine (0.59 mL, 6.53 mmol) was added to a mixture of **13** (2 g, 6.53 mmol), NaO^{t}Bu (906 mg, 9.14 mmol), $Pd_2(dba)_3$ (135 mg, 0.13 mmol), BINAP (166 mg, 0.26 mmol) in toluene (30 mL) and the resulting mixture was refluxed for 5 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:9) to give **14n** (1.38 g, 67%) as a yellow solid. m.p. 97 °C; ^{1}H NMR ($CHCl_3$-*d*) δ 7.33–7.47 (m, 5H), 4.72 (s, 2H), 3.83

(t, J = 4.6 Hz, 4H), 3.03 (t, J = 4.6 Hz, 4H), 2.42 (s, 3H), 2.17 (s, 3H), 2.16 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 157.0, 148.3, 146.6, 140.7, 137.4, 128.7, 128.3, 128.0, 122.5, 74.9, 67.5, 51.0, 29.8, 19.8, 14.6, 13.2 ppm; IR (KBr) ν 3902, 3838, 3734, 3648, 3566, 3419, 2970, 2915, 2851, 1716, 1698, 1683, 1636, 1575, 1558, 1540, 1521, 1507, 1488, 1456, 1418, 1362, 1266, 1223, 1112, 1092, 981, 920, 836, 748, 720, 699, 617, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 313.

4.2.34. 2,4,5-Trimethyl-6-morpholinopyridin-3-ol (**15n**)

To a solution of **14n** (100 mg, 0.320 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 2 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15n** (71 mg, 99%) as a yellow solid. m.p. 148 °C; ^{1}H NMR (DMSO-d_6) δ 3.69 (t, J = 4.5 Hz, 4H), 2.83 (t, J = 4.6 Hz, 4H), 2.27 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 153.1, 145.4, 140.0, 136.3, 121.7, 66.4, 50.8, 48.2, 19.4, 13.7, 12.5 ppm; IR (KBr) ν 3855, 3839, 3736, 3677, 3650, 3630, 2949, 2828, 2473, 1735, 1637, 1585, 1455, 1365, 1274, 1236, 1215, 1150, 1092, 1051, 1032, 1005, 929, 802, 777, 669, 452, 415 cm^{-1}; MS (ES-API) $[M + H]^+$ 223; HRMS calcd for $C_{12}H_{19}N_2O_2$ $[M + H]^+$ 223.1447, found 223.1451.

4.2.35. 3-Benzyloxy-2,4,5-trimethyl-6-(1H-pyrrol-1-yl)pyridine (**14o**)

Pyrrole (0.45 mL, 6.53 mmol) was added to a mixture of **13** (2 g, 6.53 mmol), NaO^{t}Bu (906 mg, 9.14 mmol), $Pd_2(dba)_3$ (135 mg, 0.13 mmol), BINAP (166 mg, 0.26 mmol) in toluene (30 mL) and the resulting mixture was refluxed for 12 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:9) to give **14o** (1.43 g, 75%) as a yellow solid. m.p. 118 °C; ^{1}H NMR ($CHCl_3$-d) δ 7.36–7.50 (m, 5H), 6.96 (t, J = 2.1 Hz, 2H), 6.30 (t, J = 2.1 Hz, 2H), 4.82 (s, 2H), 2.49 (s, 3H), 2.27 (s, 3H), 2.14 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 151.0, 148.9, 147.0, 141.8, 136.9, 128.8, 128.5, 128.1, 124.8, 121.4, 109.2, 75.1, 19.4, 14.9, 13.4 ppm; IR (KBr) ν 3902, 3838, 3734, 3432, 3083, 2954, 2898, 2855, 1698, 1588, 1540, 1520, 1507, 1497, 1474, 1455, 1430, 1405, 1367, 1312, 1250, 1220, 1105, 1082, 1016, 1001, 926, 903, 835, 746, 728, 697, 670, 647, 590, 458, 418 cm^{-1}; MS (ES-API) $[M + H]^+$ 293.

4.2.36. 2,4,5-Trimethyl-6-(1H-pyrrol-1-yl)pyridin-3-ol (**15o**)

To a solution of **14o** (100 mg, 0.342 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 2 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15o** (69 mg, 99%) as a yellow solid. m.p. 181 °C; ^{1}H NMR (DMSO-d_6) δ 8.79 (s, 1H), 6.90 (t, J = 2.0 Hz, 2H), 6.15 (t, J = 2.0 Hz, 2H), 2.34 (s, 3H), 2.18 (s, 3H), 2.00 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 148.4, 142.7, 141.9, 134.4, 124.1, 121.3, 107.9, 19.2, 14.1, 12.6 ppm; IR (KBr) ν 3734, 2869, 1736, 1595, 1486, 1455, 1373, 1217, 1086, 1056, 1032, 1015, 778, 729, 669, 527, 419 cm^{-1}; MS (ES-API) $[M + H]^+$ 203; HRMS calcd for $C_{12}H_{15}N_2O$ $[M + H]^+$ 203.1184, found 203.1187.

4.2.37. 5-Benzyloxy-N-3,4,6-tetramethyl-N-(pyridin-2-yl)pyridin-2-amine (**14p**)

2-(Methylamino)pyridine (0.68 mL, 6.53 mmol) was added to a mixture of **13** (2 g, 6.53 mmol), NaO^{t}Bu (906 mg, 9.14 mmol), $Pd_2(dba)_3$ (135 mg, 0.13 mmol), BINAP (166 mg, 0.26 mmol) in toluene (30 mL) and the resulting mixture was refluxed for 12 h. The mixture was cooled to room temperature, and then diluted with EtOAc (500 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 50:1) to give **14p** (1.78 g, 81%) as a yellow solid. m.p. 49 °C; ^{1}H NMR ($CHCl_3$-d) δ 8.17–8.20 (m, 1H), 7.30–7.48 (m, 6H), 6.55–6.58 (m, 1H), 6.11 (d, J = 8.5 Hz, 1H), 4.82 (s, 2H), 3.40 (s, 3H), 2.47 (s, 3H), 2.23 (s, 3H), 1.98 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 158.3, 151.5, 150.7, 149.7, 148.1, 141.8, 137.0, 136.9, 128.8, 128.6, 128.4, 128.0, 112.9, 108.1, 77.4, 74.9, 36.5, 19.5, 14.4, 13.4 ppm; IR (KBr) ν 2915, 1598, 1560, 1483, 1422, 1397, 1365, 1293, 1270, 1229, 1191, 1159, 1102, 1054, 985, 914, 839, 764, 754, 730, 720, 697, 613, 589, 522 cm^{-1}; MS (ES-API) $[M + H]^+$ 334.

4.2.38. 2,4,5-Trimethyl-6-[methyl(pyridin-2-yl)amino]pyridin-3-ol (**15p**)

To a solution of **14p** (100 mg, 0.299 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 20 mg). The mixture was stirred with hydrogen balloon at room temperature for 10 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **15p** (72 mg, 98%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 9.48 (br s, 1H), 8.07 (d, J = 4.2 Hz, 1H), 7.56–7.66 (m, 1H), 6.80 (t, J = 6.0 Hz, 1H), 6.40 (d, J = 8.6 Hz, 2H), 3.33 (s, 3H), 2.40 (s, 3H), 2.23 (s, 3H), 1.98 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 155.7, 149.4, 144.1, 142.8, 139.6, 128.8, 113.3, 109.1, 48.5, 37.0, 18.3, 13.5, 13.2 ppm; IR (KBr) ν 3913, 3897, 3878, 3860, 3849, 3834, 3812, 3798, 3775, 3764, 3741, 3730, 3707, 3685, 3666, 3645, 3625, 365, 3584, 3562, 3225, 1865, 1841, 1789, 1746, 1730, 1714, 1694, 1681, 1667, 1644, 1633, 1600, 1566, 1556, 1537, 1515, 1504, 1486, 1470, 1454, 1415, 1216, 1103, 1054, 1032, 770 cm^{-1}; MS (ES-API) $[M + H]^+$ 244; HRMS calcd for $C_{14}H_{18}N_3O$ $[M + H]^+$ 244.1450, found 244.1455.

4.2.39. 5-Benzyloxy-N-diphenylmethylene-3,4,6-trimethylpyridin-2-amine (**16**)

Benzophenone imine (1.73 mL, 9.80 mmol) was added to a mixture of **13** (3 g, 9.80 mmol), NaO^{t}Bu (1.36 g, 13.71 mmol), $Pd_2(dba)_3$ (203 mg, 0.20 mmol), BINAP (249 mg, 0.39 mmol) in toluene (30 mL) and the resulting mixture was refluxed for 12 h. The mixture was cooled to room temperature, and then diluted with EtOAc (700 mL) and water. The organic layer was washed with brine (20 mL × 5). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (EtOAc:Hexanes = 1:4) to give **16** (3.28 g, 83%) as a yellow solid. m.p. 133 °C; ^{1}H NMR ($CHCl_3$-d) δ 7.80 (d, J = 7.1 Hz, 2H), 7.17–7.48 (m, 13H), 4.69 (s, 2H), 2.29 (s, 3H), 2.03 (s, 3H), 1.91 (s, 3H) ppm.; ^{13}C NMR ($CHCl_3$-d) δ 169.6, 157.2, 147.9, 147.2, 140.1, 139.3, 137.2, 137.0, 131.0, 129.7, 129.0, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 127.6, 119.7, 75.1, 29.8, 19.1, 14.0, 12.8 ppm.; IR (KBr) ν 2921, 1737, 1637, 1448, 1398, 1363, 1314, 1225, 1055, 1032, 976, 951, 915, 783, 753, 695, 611, 419 cm^{-1}; MS (ES-API) $[M + H]^+$ 407.

4.2.40. 5-Benzyloxy-3,4,6-trimethylpyridin-2-amine (**17**)

16 (2 g, 4.920 mmol) was dissolved in a mixed solvent of MeOH (50 mL) and THF (5 mL), and 2% methanolic hydrogen chloride (20 mL) was added to the solution. The resulting mixture was stirred for 12 h at room temperature, and then concentrated. The residue was diluted with EtOAc (300 mL) and washed with sat. $NaHCO_3$ solution (20 mL × 4). The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 20:1) to give **17** (992 mg, 83%) as a yellow solid. m.p. 127 °C; ^{1}H NMR ($CHCl_3$-d) δ 7.31–7.45 (m, 5H), 4.68 (s, 2H), 4.25 (br s, 1H), 2.34 (s, 3H), 2.16 (s, 3H), 1.99 (s, 3H) ppm; ^{13}C NMR ($CHCl_3$-d) δ 152.5, 146.1, 145.4, 140.2, 137.4, 128.7,

128.2, 128.1, 113.7, 75.2, 18.8, 13.2, 12.5 ppm; IR (KBr) ν 3676, 3458, 3306, 3169, 3027, 2906, 2859, 1634, 1588, 1447, 1417, 1367, 1273, 1230, 1144, 1083, 1029, 985, 911, 862, 754, 716, 696, 612, 509, 431 cm^{-1}; MS (ES-API) $[M + H]^+$ 243.

4.2.41. 6-Amino-2,4,5-trimethylpyridin-3-ol (**6**) [CAS no. 1245315-08-5]

To a solution of **17** (50 mg, 0.206 mmol) in methanol (2 mL) was added palladium (10% on activated carbon, 10 mg). The mixture was stirred with hydrogen balloon at room temperature for 3 h. The solid in the reaction mixture was filtered through Celite pad and the filtrate was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **6** (31 mg, 100%) as an orange solid.

4.3. CAM assay

4.3.1. Chick chorioallantoic membrane (CAM) model of angiogenesis

Angiogenesis was examined using previously published methods [50]. Fertilized chicken eggs were incubated at 37 °C with 55% relative humidity for ten days. By day 10 of incubation, the ectoderm of the CAM, one of the three comprising layers (ectoderm, mesoderm, and endoderm) contained a fully developed capillary plexus, a network of tiny capillaries connecting the arterial and venous blood vessels. On 11th day, a small hole in the shell concealing the air sac was made using a hypodermic needle. A second hole was made on the broad side of the egg directly over the avascular portion of the embryonic membrane by candling. A false air sac was created beneath the second hole by application of negative pressure through the first hole, causing the CAM to separate from the shell. An approximately 1.0 cm^2 window was made in the shell over the dropped CAM using a small grinding wheel (Dremel, Racine, WI, USA). Sterile disks of No. 1 filter paper (Whatman International) were pretreated with 3 mg/mL of cortisone acetate and air dried under sterile conditions. Each disk was suspended in 10 μL of PBS containing VEGF (20 ng/CAM), as a standard proangiogenic agent, or the control solvent, and the disks were then placed on growing CAMs. Compounds were added to the disk after 30 min. The drug-treated CAMs were incubated for three days. In the tumor angiogenesis experiments, all procedures were the same as above except that A549 human lung cancer cells (2×10^6 cells/CAM) were inoculated onto the CAM instead of VEGF [51,52]. The number of vessel branch points contained in a tumor region was counted by two observers in a double-blind manner.

4.3.2. Microscopic analysis of CAM sections

After incubation at 37 °C for three days with a 55% relative humidity, the tissue directly beneath each filter disk was resected from the control and the drug-treated CAM samples. Each tissue sample was washed three times with PBS, placed in a 35 mm Petri dish (Nalge Nunc), and examined under a stereomicroscope (Zeiss) at ×50 magnification. Digital images of the CAM beneath the filters were collected using a 3-charge-coupled device color video camera system (Toshiba). The images were then analyzed using Image-Inside software. The number of vessel branch points contained in a circular region (equal to the area of each filter disk) was counted. One image was counted for each CAM preparation, and the findings from 6 to 8 CAM preparations were analyzed for each of the treatment conditions. The resulting angiogenesis index was expressed as the mean ± S.E.M. of the new branch points for each set of samples.

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Appendix A. Supplementary data

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References

[1] M. Wijtmans, D.A. Pratt, L. Valgimigli, G.A. DiLabio, G.F. Pedulli, N.A. Porter, 6-Amino-3-pyridinols: towards diffusion-controlled chain-breaking antioxidants, Angewandte Chemie International Edition 42 (2003) 4370–4373.
[2] M. Wijtmans, D.A. Pratt, J. Brinkhorst, R. Serwa, L. Valgimigli, G.F. Pedulli, N.A. Porter, Synthesis and reactivity of some 6-substituted-2,4-dimethyl-3-pyridinols, A novel class of chain-breaking antioxidants, Journal of Organic Chemistry 69 (2004) 9215–9223.
[3] T.G. Nam, M. Wijtmans, D.A. Pratt, N.A. Porter, Regioselective addition of n-alkyllithiums to α,α-disubstituted-1,8-naphthyridines: synthesis of 6-amino-3-pyridinol analogs of α-tocopherol, Synthesis (2005) 1397–1404.
[4] H.Y. Kim, D.A. Pratt, J.R. Seal, M. Wijtmans, N.A. Porter, Lipid-soluble 3-pyridinol antioxidants spare α-tocopherol and do not efficiently mediate peroxidation of cholesterol esters in human low-density lipoprotein, Journal of Medicinal Chemistry 48 (2005) 6787–6789.
[5] T.G. Nam, C.L. Rector, H.-Y. Kim, A.F.P. Sonnen, R. Meyer, W.M. Nau, J. Atkinson, J. Rintoul, D.A. Pratt, N.A. Porter, Tetrahydro-1,8-naphthyridinol analogues of α-tocopherol as antioxidants in lipid membranes and low-density lipoproteins, Journal of the American Chemical Society 129 (2007) 10211–10219.
[6] S. Kumar, H. Johansson, T. Kanda, L. Engman, T. Müller, M. Jonsson, G.F. Pedulli, S. Petrucci, L. Valgimigli, Catalytic chain-breaking pyridinol antioxidants, Organic Letters 10 (2008) 4895–4898.
[7] S. Kumar, H. Johansson, T. Kanda, L. Engman, T. Müller, H. Bergenudd, M. Jonsson, G.F. Pedulli, R. Amorati, L. Valgimigli, Catalytic chain-breaking pyridinol antioxidants, Journal of Organic Chemistry 75 (2010) 716–725.
[8] Y. Omata, Y. Saito, Y. Yoshida, B.S. Jeong, R. Serwa, T.G. Nam, N.A. Porter, E. Niki, Action of 6-amino-3-pyridinols as novel antioxidants against free radicals and oxidative stress in solution, plasma, and cultured cells, Free Radical Biology & Medicine 48 (2010) 1358–1365.
[9] J. Lu, O.M. Khdour, J.S. Armstrong, S.M. Hecht, Design, synthesis, and evaluation of an α-tocopherol analogue as a mitochondrial antioxidant, Bioorganic & Medicinal Chemistry 18 (2010) 7628–7638.
[10] J. Lu, X. Cai, S.M. Hecht, Concise synthesis of bicyclic pyridinol antioxidants, Organic Letters 12 (2010) 5189–5191.
[11] R. Serwa, T.G. Nam, L. Valgimigli, S. Culbertson, C.L. Rector, B.S. Jeong, D.A. Pratt, N.A. Porter, Preparation and investigation of vitamin B_6-derived aminopyridinol antioxidants, Chemistry – A European Journal 16 (2010) 14106–14114.
[12] T.G. Nam, J.M. Ku, H.G. Park, N.A. Porter, B.S. Jeong, New synthetic route to N-tocopherol derivatives: synthesis of pyrrolopyridinol analogue of α-tocopherol from pyridoxine, Organic & Biomolecular Chemistry 9 (2011) 1749–1755.
[13] T.G. Nam, J.M. Ku, C.L. Rector, H. Choi, N.A. Porter, B.S. Jeong, Pyridoxine-derived bicyclic aminopyridinol antioxidants: synthesis and their antioxidant activities, Organic & Biomolecular Chemistry 9 (2011) 8475–8482.
[14] O.M. Khdour, J. Lu, S.M. Hecht, An acetate prodrug of a pyridinol-based vitamin E analogue, Pharmaceutical Research 28 (2011) 2896–2909.
[15] X. Cai, O.M. Khdour, J. Jaruvangsanti, S.M. Hecht, Simplified bicyclic pyridinol analogues protect mitochondrial function, Bioorganic & Medicinal Chemistry 20 (2012) 3584–3595.
[16] P.M. Arce, R. Goldschmidt, O.M. Khdour, M.M. Madathil, J. Jaruvangsanti, S. Dey, D.M. Fash, J.S. Armstrong, S.M. Hecht, Analysis of the structural and mechanistic factors in antioxidants that preserve mitochondrial function and confer cytoprotection, Bioorganic & Medicinal Chemistry 20 (2012) 5188–5201.
[17] W. Risau, I. Flamme, Vasculogenesis, Annual Review of Cell and Developmental Biology 11 (1995) 73–91.
[18] W. Risau, Mechanisms of angiogenesis, Nature 386 (1997) 671–674.
[19] P. Carmeliet, Mechanisms of angiogenesis and arteriogenesis, Natural Medicines 6 (2000) 389–395.
[20] P. Carmeliet, Angiogenesis in health and disease, Natural Medicines 9 (2003) 653–660.
[21] P. Carmeliet, Angiogenesis in life, disease and medicine, Nature 438 (2005) 932–936.
[22] J. Folkman, Tumor angiogenesis: therapeutic implications, The New England Journal of Medicine 285 (1971) 1182–1186.

[23] J. Folkman, Angiogenesis: an organizing principle for drug discovery? Nature Reviews Drug Discovery 6 (2007) 273–286.
[24] I.J. Fidler, L.M. Ellis, The implications of angiogenesis for the biology and therapy of cancer metastasis, Cell 79 (1994) 185–188.
[25] T. Shin, C. Lindley, Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies, Clinical Therapeutics 28 (2006) 1779–1802.
[26] K.J. Gotink, H.M. Verheul, Anti-angiogenic tyrosine kinase inhibitors: what is their mechanism of action? Angiogenesis 13 (2010) 1–14.
[27] D.W. Siemann, The unique characteristics of tumor vasculature and preclinical evidence for its selective disruption by tumor-vascular disrupting agents, Cancer Treatment Reviews 37 (2011) 63–74.
[28] M.R. Ahn, K. Kunimasa, T. Ohta, S. Kumazawa, M. Kamihira, K. Kaji, Y. Uto, H. Hori, H. Nagasawa, T. Nakayama, Suppression of tumor-induced angiogenesis by Brazilian propolis: major component artepillin C inhibits in vitro tube formation and endothelial cell proliferation, Cancer Letters 252 (2007) 235–243.
[29] K. Nakagawa, A. Shibata, S. Yamashita, T. Tsuzuki, J. Kariya, S. Oikawa, T. Miyazawa, In vivo angiogenesis is suppressed by unsaturated vitamin E, tocotrienol, Journal of Nutrition 137 (2007) 1938–1943.
[30] M.R. Ahn, K. Kunimasa, S. Kumazawa, T. Nakayama, K. Kaji, Y. Uto, H. Hori, H. Nagasawa, T. Ohta, Correlation between antiangiogenic activity and antioxidant activity of various components from propolis, Molecular Nutrition & Food Research 53 (2009) 643–651.
[31] A. Shibata, K. Nakagawa, P. Sookwong, T. Tsuduki, S. Oikawa, T. Miyazawa, δ-Tocotrienol suppresses VEGF induced angiogenesis whereas α-tocopherol does not, Journal of Agricultural and Food Chemistry 57 (2009) 8696–8704.
[32] S.R. Wells, M.H. Jennings, C. Rome, V. Hadjivassiliou, K.A. Papas, J.S. Alexander, α-, γ- and δ-tocopherols reduce inflammatory angiogenesis in human microvascular endothelial cells, Journal of Nutritional Biochemistry 21 (2010) 589–597.
[33] R. Auerbach, R. Lewis, B. Shinners, L. Kubai, N. Akhtar, Angiogenesis assays: a critical overview, Clinical Chemistry 49 (2003) 32–40.
[34] C. Storgard, D. Mikolon, D.G. Stupack, Angiogenesis assays in the chick CAM, Journal of Molecular Biology 294 (2005) 123–136.
[35] J.F. Hartwig, Palladium-catalyzed amination of aryl halides: mechanism and rational catalyst design, Synlett 4 (1997) 329–340.
[36] J.F. Hartwig, Transition metal catalyzed synthesis of arylamines and aryl ethers from aryl halides and triflates: scope and mechanism, Angewandte Chemie International Edition 37 (1998) 2046–2067.
[37] J.P. Wolfe, S. Wagaw, J.F. Marcoux, S.L. Buchwald, Rational development of practical catalysts for aromatic carbon–nitrogen bond formation, Accounts of Chemical Research 31 (1998) 805–818.
[38] J.F. Hartwig, Carbon–heteroatom bond-forming reductive eliminations of amines, ethers, and sulfides, Accounts of Chemical Research 31 (1998) 852–860.
[39] J.F. Hartwig, Approaches to catalyst discovery. New carbon-heteroatom and carbon-carbon bond formation, Pure and Applied Chemistry 71 (1999) 1416–1423.
[40] A.R. Muci, S.L. Buchwald, Practical palladium catalysts for C-N and C-O bond formation, Topics in Current Chemistry 219 (2002) 131–209.
[41] D.S. Surry, S.L. Buchwald, Biaryl phosphane ligands in palladium-catalyzed amination, Angewandte Chemie International Edition 47 (2008) 6338–6361.
[42] J.F. Hartwig, Evolution of a fourth generation catalyst for the amination and thioetherification of aryl halides, Accounts of Chemical Research 41 (2008) 1534–1544.
[43] D.S. Surry, S.L. Buchwald, Dialkylbiaryl phosphines in Pd-catalyzed amination: a user's guide, Chemical Science 2 (2011) 27–50.
[44] K. Okano, K. Okuyama, T. Fukuyama, H. Tokuyama, Mild debenzylation of aryl benzyl ether with BCl_3 in the presence of pentamethylbenzene as a non-Lewis-basic cation scavenger, Synlett 13 (2008) 1977–1980.
[45] J.P. Wolfe, J. Ahman, J.P. Sadighi, R.A. Singer, S.L. Buchwald, An ammonia equivalent for the palladium-catalyzed amination of aryl halides and triflates, Tetrahedron Letters 38 (1997) 6367–6370.
[46] J.P. Borg, O. deLapeyriere, T. Noguchi, R. Rottapel, P. Dubreuil, D. Birnbaum, Biochemical characterization of two isoforms of FLT4, a VEGF receptor-related tyrosine kinase, Oncogene 10 (1995) 973–984.
[47] R. Colavitti, G. Pani, B. Bedogni, R. Anzevino, S. Borrello, J. Waltenberger, T. Galeotti, Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR, Journal of Biological Chemistry 277 (2002) 3101–3108.
[48] L. Sun, N. Tran, F. Tang, H. App, P. Hirth, G. McMahon, C. Tang, Synthesis and biological evaluations of 3-substituted indolin-2-ones: a novel class of tyrosine kinase inhibitors that exhibit selectivity toward particular receptor tyrosine kinases, Journal of Medicinal Chemistry 41 (1998) 2588–2603.
[49] S.S. Huang, R.L. Huang, Rosmarinic acid inhibits angiogenesis and its mechanism of action in vitro, Cancer Letters 239 (2005) 271–280.
[50] R.W. Colman, R.A. Pixley, I.M. Sainz, J.S. Song, I. Isordia-Salas, S.N. Muhamed, J.A. Powell, S.A. Mousa, Inhibition of angiogenesis by antibody blocking the action of proangiogenic high-molecular-weight kininogen, Journal of Thrombosis and Haemostasis 1 (2003) 164–170.
[51] J.A. Kim, K.B. Cho, M.R. Kim, B.C. Park, S.K. Kim, M.Y. Lee, K.W. Kang, Decreased production of vascular endothelial growth factor in adriamycin-resistant breast cancer cells, Cancer Letters 268 (2008) 225–232.
[52] D. Thapa, J.S. Lee, S.W. Heo, Y.R. Lee, K.W. Kang, M.K. Kwak, H.G. Choi, J.A. Kim, Novel hexahydrocannabinol analogs as potential anti-cancer agents inhibit cell proliferation and tumor angiogenesis, European Journal of Pharmacology 650 (2011) 64–71.