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Lewis acid-promoted transformation of 2-alkoxypyridines into 2-aminopyridines and their antibacterial activity. Part 2: Remarkably facile C–N bond formation

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Abstract—2-Alkoxy-3-cyano-4,6-diarylpyridines **1a**,**b** which were synthesized by condensation of α,β-unsaturated ketones with malononitrils were subjected to Lewis acid-catalyzed nucleophilic displacement reaction with various amines to afford the corresponding 2-aminopyridines **3**–**21**. The potency of the results as antibacterial agents has been evaluated. The structure of the newly prepared compounds was assessed by microanalysis, IR, and NMR spectra. Molecular modeling and QSAR methods are used to study the antibacterial activity of the active compounds by means of the molecular mechanic method.

1. Introduction

The rapid development of resistance in clinically important Gram-positive bacteria represents a serious public health threat.[1] *Staphylococcus aureus* is a common cause of many skin and mucous membrane infections. Many strains have developed resistance and antibiotic treatment options have become more limited.[2,3]

Actually, no new classes of antibiotics have been introduced to the market in the 37 years between the introduction of nalidixic acid in 1962 and linezolid in 2000. All the antibacterials introduced in this period were modifications of existing molecules. Bacteria have exploited this window of opportunity by developing resistance to all commonly used antibiotics, making the need for new antibiotics more pressing.[4] Therefore, the antifungal and antibacterial properties of substituted pyridines have opened up the possibility of their potential use as a novel class of totally synthetic antimicrobial agents.[5] 2-Aminopyridines are promising substituted pyridines which have been shown to be biologically active molecules.[6,7] Additionally, because of their chelating abilities, 2-aminopyridines are commonly used as ligands in inorganic and organometallic chemistry.[8] If substituted with optically active groups, they could potentially serve as chiral auxiliaries or chiral ligands in asymmetric reactions. For these reasons, 2-aminopyridines are valuable synthetic targets. The synthesis of 2-aminopyridine derivatives has been extensively reviewed.[6–13] 2-(Substituted amino)pyridines were previously mostly prepared: (i) by reaction of primary or secondary aliphatic amines with 2-halopyridines or with imidol silyl ethers derived from the corresponding pyrid-2-ones,[14] (ii) by aminolysis of 2-alkoxypyridines,[15] and (iii) by using methodology previously described by Katritzky et al.[16] Of these methods, pathway (i) requires high temperatures and high pressure while pathway (ii) proceeds with alkoxypyridines containing activating groups in the pyridine ring under strong basic condition, high temperatures, and long reaction time. Method (iii) worked reasonably well with simple secondary amines while it was unable to isolate any of the desired 2-aminopyridine products when anilines or primary amines were employed as nucleophiles. Hence, a versatile route for the synthesis of *N*-substituted 2-aminopyridines utilizing primary and secondary amines as nucleophiles under mild condition is highly desirable.

In a previous paper,[5] we reported the synthesis of 4,6-diaryl-3-cyano-2-alkoxypyridines and their reaction

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Keywords: Lewis acid; 2-Alkoxypyridines; 2-Aminopyridines; Antibacterial activity; Molecular modeling.

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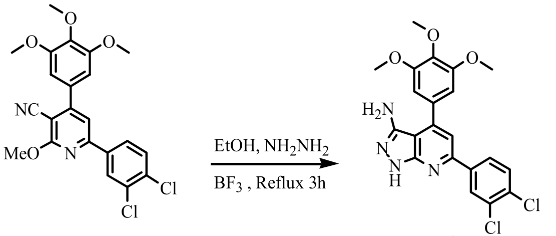
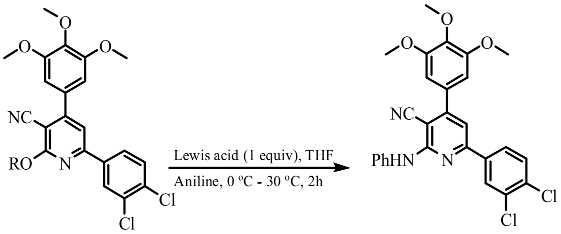


1b **2**

Scheme 1. Lewis acid-promoted reaction of 2-methoxypyridine with anhydrous hydrazine.

with anhydrous hydrazine in the presence of BF_3 to get the corresponding 1*H*-pyrazolo[3,4-*b*]pyridines (Scheme 1). The antimicrobial activity of these products was investigated in which the uncyclized product **1** and the cyclized compound **2** were effective against both *Escherichia coli* and *Candida albicans*. However, our efforts have been directed toward the synthesis of novel 4,6-diaryl-2-aminopyridines that can provide improved Gram-positive antibacterial activity, while retaining the good Gram-negative activity of compound **1**. Gram-positive and Gram-negative bacteria differ remarkably in the features of their cell walls. The cell wall of Gram-positive bacteria is more lipophilic than that of Gram-negative bacteria.

Table 1. Lewis acid promoted the reaction of aniline with 2-alkoxypyridines **1a**,**b** in THF



1 **3**
R = Et (**a**), Me (**b**)

Entry	R	Lewis acid	Yield (%)
1	Et	$ZnCl_2$	16
2	Me	$ZnCl_2$	25
3	Et	$BF_3 \cdot Et_2O$	60
4	Me	$BF_3 \cdot Et_2O$	79
5	Et	Et_2AlCl	71
6[a]	Me	Et_2AlCl	97
7	Et	$AlCl_3$	5
8	Me	$AlCl_3$	11
9	Et	Me_3SiCl	—
10	Me	Me_3SiCl	—
11	Et	$SnCl_4$	2
12	Me	$SnCl_4$	5
13	Et	$TiCl_4$	26
14	Me	$TiCl_4$	37

[a] Reaction trials in solvent other than TFH gave lower yields, such as, ether (66%), CH_2Cl_2 (34%), ethanol (9%) and increased by reflux to 30%, toluene (no reaction), and benzene (no reaction).

2. Results and discussion

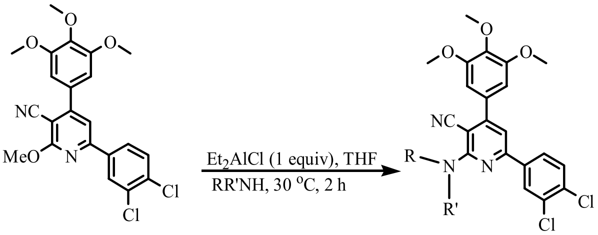
2.1. Chemistry

Considering the above facts, we have focused our attention on the introduction of the novel substituent at the C-2 of the 4,6-diarylpyridines, through selective Lewis acid-catalyzed nucleophilic displacement of the 2-alkoxy moiety with *N*-substituted amino moieties with a variety of lipophilic groups, in order to find potent broad-spectrum antibacterial agents which display strong Gram-positive activity and study their structure–activity relationship. First of all, this group can be readily introduced to the corresponding 4,6-diarylpyridines in a simple manner. Second, it enhances the lipophilicity of these 4,6-diarylpyridines. Our approach depends on the selective amination reaction of 2-alkoxypyridines with appropriate amine catalyzed by Lewis acid without reaction with 3-cyano group to afford new series of 3-cyano-2-aminopyridines. Generally, to the best of our knowledge, no Lewis acid-catalyzed aminations on 2-alkoxypyridines through selective C–N bond formation under mild reaction conditions have been described in the literature yet.[15] The lead compound **1a**,**b** was prepared according to our previous report in which the α,β-unsaturated ketones were condensed with malononitirile in either sodium methoxide/methanol or sodium ethoxide/ethanol to yield the corresponding 2-alkoxypyridines **1a**,**b**[5]

As can be seen in Table 1, We first examined the effect of the Lewis acid catalyst on the treatment of 2-alkoxypyridines **1a**,**b** with aniline. Among the Lewis acid we examined, Et_2AlCl and $BF_3 \cdot Et_2O$ worked most effectively for the conversion of 2-alkoxypyridines **1a**,**b** to 2-phenylaminopyridines **3** (Table 1, entries 3–6). Et_2AlCl was found to be more effective than $BF_3 \cdot Et_2O$ for the above conversion (Table 1, entries 4 and 6). Other Lewis acids such as $ZnCl_2$, $AlCl_3$, $SnCl_4$, and $TiCl_4$ were ineffective for improving the reaction yield and Me_3SiCl was inhibitory (Table 1, entries 9 and 10). It is noteworthy that the observed nucleophilic displacement of the reaction was highly dependent on the nature of Lewis acid, and the tendency of the preferred formation of **3** approximately correlated with the acidity of the conjugated base formed. Moreover, the reaction proceeded smoothly with 2-methoxypyridines rather than 2-ethoxypyridines, which may be due to steric reason (Table 1, entries 3–6). Furthermore, the nature of the solvent also affected the reaction yield. However, the reactions proceeding in the presence of solvents such as, ethanol, diethyl ether, and methylene chloride afforded low yields, while the result of the reaction in THF was completely in contrast with that in toluene and benzene, that is THF gave 2-aminopyridines, exclusively (Table 1, footnote).

We next examined and checked the generality of this reaction with various amines using Et_2AlCl as Lewis acid at 0–30 °C for 2 h as shown in Table 2. Generally, high yield was observed when less hindered primary amines were used as nucleophiles which decreased when secondary amines were investigated (Table 2, entries 8, 11, 12, 15, and 16). Apparently, structural variation in the aryl amine on *o*-position with steric bulky group

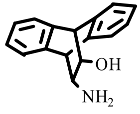
Table 2. Et_2AlCl promoted the reaction of substituted amines with 2-methoxypyridine **1b**



1b **4-21**

Entry	Compound No.	R	R′	Yield (%)
1	**4**	4-Nitrophenyl	H	75
2	**5**	4-Chlorophenyl	H	85
3	**6**	2-Chlorophenyl	H	79
4	**7**	3-Triflouromethylphenyl	H	77
5	**8**	4-Methylphenyl	H	87
6	**9**	2,6-Dimethylphenyl	H	67
7	**10**	2,6-Diisopropylphenyl	H	60
8	**11**	Benzyl	H	93
9	**12**	(*S*)-α-Methylbenzyl	H	89
10	**13**	(*R*)-α-Methylbenzyl	H	90
11	**14**	Phenethyl	H	94
12	**15**	Ethyl	H	96
13	**16**	*tert*-Butyl	H	65
14	**17**	1-Adamantyl	H	61
15	**18**	Methyl	Methyl	74
16	**19**	Methyl	Benzyl	77
17	**20**	(+)-aminoalcohol[a]	H	51
18	**21**	(−)-aminoalcohol[b]	H	49

[a] (2*R*,3*S*)-3-aminodibenzo[*e*,*h*]bicyclo[2.2.2]octa-5,7-diene-2-ol.



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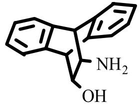
[b] (2*S*,3*R*)-3-aminodibenzo[*e*,*h*]bicyclo[2.2.2]octa-5,7-diene-2-ol.



(-)

affecting the yields of the product, such as aniline (Table 1, entry 6) and *p*-methylaniline, gave higher yield than *o*,*o*-dimethylaniline (Table 2, entries 5 and 6) and increasing the bulkiness of *o*-position from 2,6-dimethyl to 2,6-diisopropylaniline may induce a tremendous effect on the reaction product (Table 2, entry 7). It is clear that the *o*-substituents could significantly affect the chelate structures which might play a crucial role in substitution step, although we have no evidence at present.[17] The same pattern has been observed by increasing steric congestion of amine whether as primary amines, such as *t*-butylamine and 1-adamantylamine (Table 2, entries 13 and 14), or as secondary amines, such as dimethylamine and methylbenzylamine (Table 2, entries 15 and 16), compared with ethylamine, benzylamine, and phenethylamine (Table 2, entries 8, 11, and 12). Moreover, the presence of electron-withdrawing group on arylamine, such as nitro group, leads to decrease in the percentage yield, which may be attributable to its poor nucleophilic character (Table 1, entry 6 and Table 2, entry 1). The synthesis of chiral 2-aminopyridines has been achieved when optically pure cyclic constrained amino alcohol (+)-(2*R*,3*S*)-3-aminodibenzo[*e*,*h*]bicyclo[2.2.2]octa-5,7-diene-2-ol or (−)-(2*S*,3*R*)-3-aminodibenzo[*e*,*h*]bicyclo[2.2.2]octa-5,7-diene-2-ol[18] was treated with Et_2AlCl (1 equiv) and 2-methoxypyridine, the 2-aminopyridine product was isolated in low yield after column chromatography (Table 2, entries 17 and 18). This low yield is most likely due to steric hindrance from the dihydroanthracene moiety. Conversely, when non-sterically demanding (+)-(*R*)-α-methylbenzylamine or (−)-(*S*)-α-methylbenzylamine (Table 2, entries 9 and 10) was used in the same manner, the chiral 2-aminopyridine product was obtained in 90% yield.

2.2. Antibacterial activity

The antibacterial screening of all the synthesized compounds was performed against the Gram-positive *S. aureus* and Gram-negative *E. coli*. Minimum inhibitory concentration (MIC)[19] was determined for each compound along with ciprofloxacin[20] as standard control; results are shown in Table 3. The compounds in the current series showed that the 2-(substituted amino)pyridines **3**–**21** were more active than the 2-methoxy analogue **1b** against Gram-positive and Gram-negative organisms as indicated by their MIC values. Amongst all the compounds tested, **9** and **10** demonstrated the most potent antibacterial activity against both *S. aureus* and *E. coli*. It is obvious from the structure–activity profile of 2-aminopyridines that substituents with an aryl group greatly influence the antibacterial activity (Table 3). Apparently, a small structural variation in the 2-aminopyridines may induce a tremendous effect on antibacterial activity. It is noteworthy that the observed

Table 3. In vitro antibacterial activity in terms of MIC[a] (μg/mL)[b]

Compound No.	*S. aureus*	*E. coli*
1b	>250	6.1
2	>250	5.4
3	20	8.1
4	100	15.9
5	15	5.8
6	14	5.5
7	13	4.7
8	20	7.3
9	11	4.1
10	7	3.2
11	100	22
12	92	27
13	70	20
14	115	29
15	100	23
16	50	15
17	27	14
18	>250	>250
19	>250	>250
20	121	>250
21	113	>250
Ciprofloxacin	2.3	4

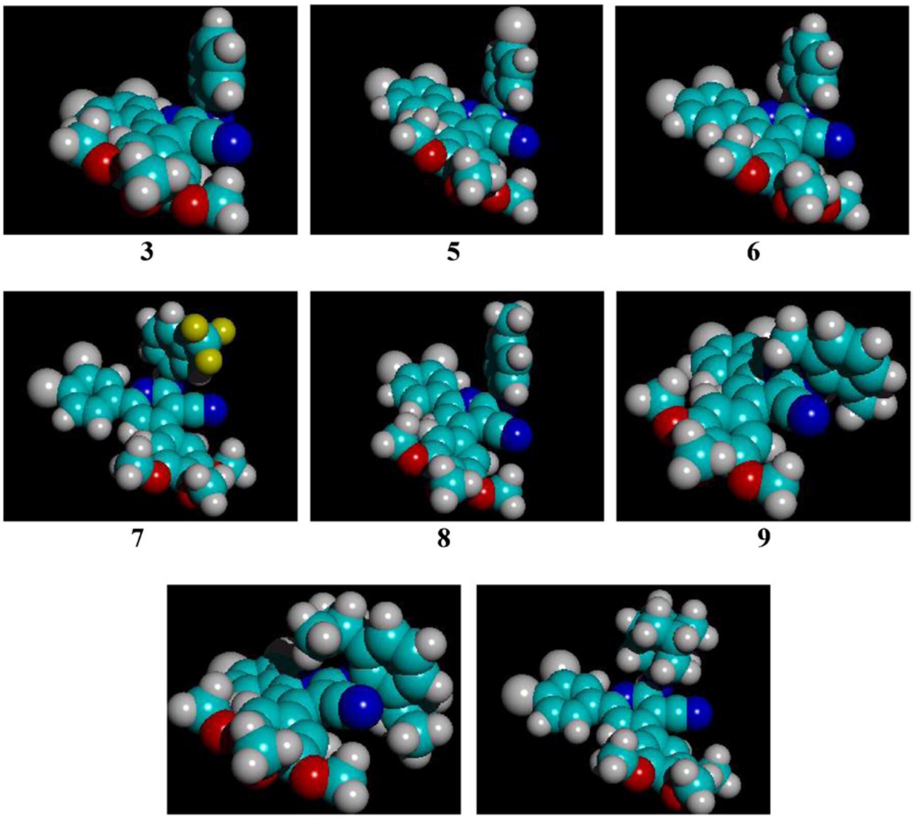
[a] MIC, minimum inhibitory concentration.

[b] The tests were performed in duplicate and repeated twice.

antibacterial activity was highly dependent on the bulkiness of the *N*-arylamino substituents on the pyridine core, in which *ortho*-disubstituents played an important role in achieving an excellent level of antibacterial activity indicating that the steric bulk of the substituents played a critical role in the antibacterial-enhancing activity. The 2,6-diisopropylphenyl and 2,6-dimethylphenyl groups represent the *N*-substituents of choice for expressing significant activity, such as compounds **9** and **10**, which were three times more active than phenylaminopyridine **3** and 2-fold more active than the lead **1b** regarding *S. aureus* and *E. coli*, respectively. Moreover, the current series of conformationally restricted analogues **20** and **21** showed a significant decrease in biological activity against *S. aureus* and none of them were active against *E. coli* when compared with conformationally unrestricted analogues **3**–**17**. Introduction of an electron-donating group on the arylamino moiety such as a methyl group did not affect the activity of **3**, **8** while an electron-withdrawing group such as a nitro group diminished the activity of **4**. Moreover, the introduction of methyl or ethyl spacer group between aryl and amino groups, such as compounds **11** and **14** respectively, resulted in the appearance of only weak activity as represented by their MIC values (Table 3; **11**, **14** vs **3**). Interestingly, compounds **18** and **19** did not display antibacterial activity indicating the importance of the free proton of the amino group for the antibacterial activity.

2.3. Molecular modeling study

An attempt to gain a better insight into the molecular structures of the active compounds **3**, **5**–**10**, and **17**, conformational analysis of the target compounds has been performed by use of the MM+[21] force-field (calculations in vacuo, bond dipole option for electrostatics, Polak–Ribiere algorithm, RMS gradient of 0.1 kcal/Å mol) as implemented in HyperChem 5.1.[22] It is clear from the calculation (Fig. 1), that compounds **3**, **5**–**10**, and**17** exhibit strong structural similarity as indicated by their molecular parameters (Table 4). The results show that the lowest energy minimized structures exhibited the same arrangement of the aryl groups (rings **A**, **B**, and **C**, Fig. 2) around the pyridine core in which the dichlorophenyl group (**C**-ring) was coplanar with pyridine ring while the 3,4,5-trimethoxyphenyl group (**B**-ring) was deviated from the coplanar structure by 30° (Fig. 1). Moreover, the trimethoxy groups; whose rotations are highly free, were arranged in spatial manner in which the terminal dimethoxy groups arranged itself approximately coplanar and parallel with aryl edges.[5,23] On the contrary, the middle methoxy group arranged itself perpendicular to the plane of the phenyl ring and syn-position with arylamino group. Similarly, the arylamino groups (**A**-ring) arranged itself perpendicular to the plane of the pyridine rings such as compounds **3**, **5**–**8** which was deviated by 45° in case of compounds **9** and **10** (Fig. 1). The occurrence of such


3 **5** **6**

7 **8** **9**

10 **17**

Figure 1. Lowest energy conformers of the most active compounds **3**, **5**–**10**, and **17** with CPK rendering.

Table 4. Molecular parameters of the active compounds

Compound No.	Clog$P$[a]	R[b]	V[c]	SA[d]	D[e]	d_1[f]	d_2[g]	d_3[h]
1b	5.7	115.3	1158.2	670.2	6.2	—	—	—
3	7.5	137.3	1311.3	752.1	7.1	7.7	7.8	8.3
5	8.3	142.1	1359.2	764.6	7.3	7.6	7.8	8.3
6	8.3	142.1	1344.2	756.7	8.0	7.7	7.8	8.4
7	8.5	143.3	1386.2	782.3	8.2	7.7	7.8	8.3
8	8.1	142.4	1364.0	774.3	7.1	7.7	7.8	8.3
9	8.5	147.4	1400.5	789.5	7.5	8.4	7.8	8.2
10	10.4	165.7	1559.5	853.8	7.8	8.5	7.8	8.2
17	8.2	154.7	1456.9	801.6	7.4	7.9	7.8	8.4

[a] Ref. 25.
[b] Refractometry (Å^3).
[c] Molecular volume (Å^3).
[d] Surface area; grid (Å^2).
[e] Dipole moment (Debye).
[f] Nonbonded distance between the centroid of aromatic rings **A** and **B**.
[g] Nonbonded distance between the centroid of aromatic rings **B** and **C**.
[h] Nonbonded distance between the centroid of aromatic rings **A** and **C**.

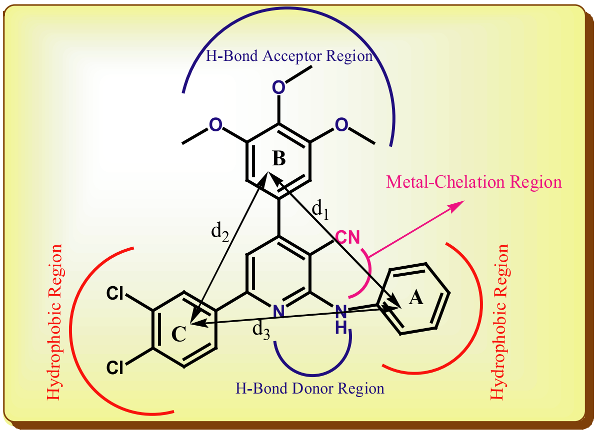


Figure 2. Deduced pyridine pharmacophore for broad-spectrum antibacterial activity.

deviation may be attributed to the strong CH/π[24] interaction of the methyl and isopropyl groups in compounds **9** and **10** with pyridine ring, respectively. It is evident from the experimental data, that the structural features (pharmacophore) essential for the antibacterial activity against Gram-positive and Gram-negative organisms of this series are as follows (Fig. 2): (1) the presence of aromatic **B**-ring and **C**-ring, at the 4-position and 6-position of pyridine is necessary for the activity, (2) the two phenyl groups should reside in certain distance (d_2), about 7.8 Å, (3) introduction of arylamino **A**-ring at the 2-position of pyridine greatly enhanced the activity against Gram-positive organism, about 7.6–8.5 Å (d_1) and 8.3 Å (d_3) from **B**-ring and **C**-ring, respectively, and (4) the presence of one free proton located on the 2-amino group is necessary as hydrogen bond donor region and for tautomerization of such amino group which could be considered as metal chelation region (Fig. 2 and Table 4). Further evidence for identical biological activity comes from QSAR data that were derived from the QSAR module of HyperChem using the conformations depicted in Figure 1. As a result, the QSAR displays calculated data for Clog$P$ (calculated log$P$; the hydrophobic parameters),[25] refractivity (steric and polarizability parameters), molecular volume (steric and polarizability parameters), grid surface area, and dipole moment (Table 4).[26] Despite a variation of the molecular shape of the active compounds, measurements of global molecular parameters (surface area, volume, and refractivity) reflect their similarity. As can be seen, Clog$P$ values of compound **3**, **5**–**9**, and **17** are well in this range of 7.5–8.5 while compound **10** is of somewhat higher value 10.4, so the optimum hydrophobicity of the active compounds is close to 7.5–10.4. However, direct correlation could be established between the Clog$P$ and antibacterial activity of these series and lead compound **1b** as indicated by their MIC values (Tables 3 and 4), such as the Clog$P$ values of these series (7.5–10.4) and **1b** (5.7). It becomes apparent that the criteria relating to favorable Clog$P$ value range may partially be the sole predicting factor for antimicrobial activity. It is noteworthy to say that the structural similarity among these series was responsible for similar biological activity as is evident from the experimental data (Table 3, Fig. 2). The mechanism of antibacterial activity of these compounds is not known at the moment and work is in progress to elucidate in detail the mechanism of antibacterial action, as well as for the design of more effective compounds.

3. Conclusion

We successfully explore an efficient new methodology for synthesis of *N*-substituted 2-aminopyridines from the parent 2-alkoxypyridines by their reaction with appropriate amine under mild condition using Lewis acid, Et_2AlCl, as a catalyst. Using this method, we have successfully synthesized enantiomerically pure 2-aminopyridines, which can serve as potential ligands in asymmetric reactions. The novel compounds showed promising antibacterial activity against Gram-positive and Gram-negative bacteria which were more active than the parent 2-alkoxypyridine derivatives. Conformational analysis of the most active molecules **3**, **5**–**10**, and **17** was performed using MM+ calculations. The reported results should serve as an element to understand pharmacophore requirements for broad-spectrum antibacterial agents, the identification of which may lead to rational design of new antibiotics.

4. Experimental

Melting points were determined with a Fisher-Johns apparatus (°C, uncorrected). IR spectra (KBr) were measured with a Mattson 5000 FT-IR Spectrophotometer (v in cm^{-1}). ^{1}H NMR spectra recorded on Varian EM-390 (200 MHz) and JEOL ALPHA 500 (500 MHz) spectrometers using TMS as internal standard (chemical shift, δ ppm). Microanalytical data (C, H, and N) agreed with the proposed structures within ±0.4% of the theoretical values. Column chromatography was performed using 60 (70–230 mesh, Merck). The following standard organisms used in the antimicrobial screening were obtained from IFO (Institute Fermentation of Osaka); *S. aureus* IFO 3060 and *E. coli* IFO 3301.

4.1. General method for preparation of substituted 2-amino-3-cyano-4,6-diaryl pyridines 3–21

The Lewis acid Et_2AlCl (1 equiv) was stirred with 2-alkoxypyridines **1a** or **1b** (1.1 equiv) in dry THF (0.01 mol/L) for 10 min at 0 °C under nitrogen atmosphere, and then an appropriate amine (1.1 equiv) was added over a period of 15 min. After being stirred for 2 h at 30 °C, the mixture was quenched by adding ammonium chloride solution (5 mL), extracted with ethylacetate, washed with brine, and dried over sodium sulfate. The solid product obtained after evaporation was crystallized from appropriate solvent to get the crystals of the desired product.

IR (KBr pellet, cm^{-1}): v 3200–3100 (NH), 2220–2210 (CN), and 1640–1590 (C=N). **3**: (97%), mp 188–189 °C (methanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.35 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 6.93–8.25 (m, 12H, ArH, NH). Anal. Calcd for $C_{27}H_{21}Cl_2N_3O_3$: 64.04, 4.18, 8.30. Found: 64.24, 4.33, 8.47. **4**: (75%), mp 177–178 °C (methanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.43 (s, 3H, OCH_3), 3.99 (s, 6H, $2OCH_3$), 6.72–8.11 (m, 11H, ArH, NH). Anal. Calcd for $C_{27}H_{20}Cl_2N_4O_5$: 58.81, 3.66, 10.16. Found: 59.01, 3.43, 10.22. **5**: (85%), mp 235–236 °C (ethanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.41 (s, 3H, OCH_3), 3.89 (s, 6H, $2OCH_3$), 7.01–8.10 (m, 11H, ArH, NH). Anal. Calcd for $C_{27}H_{20}Cl_3N_3O_3$: 59.96, 3.73, 7.77. Found: 60.11, 3.88, 7.97. **6**: (79%), mp 251–252 °C (ethanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.49 (s, 3H, OCH_3), 3.72 (s, 6H, $2OCH_3$), 6.94–8.29 (m, 11H, ArH, NH). Anal. Calcd for $C_{27}H_{20}Cl_3N_3O_3$: 59.96, 3.73, 7.77. Found: 60.31, 3.91, 7.68. **7**: (77%), mp 210–211 °C (ethanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.55 (s, 3H, OCH_3), 4.00 (s, 6H, $2OCH_3$), 7.10–8.33 (m, 11H, ArH, NH). Anal. Calcd for $C_{28}H_{20}Cl_2F_3N_3O_3$: 58.55, 3.51, 7.32. Found: 58.35, 3.77, 7.43. **8**: (87%), mp 165–166 °C (methanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.35 (s, 3H, CH_3), 3.44 (s, 3H, OCH_3), 3.88 (s, 6H, $2OCH_3$), 6.66–7.84 (m, 11H, ArH, NH). Anal. Calcd for $C_{28}H_{23}Cl_2N_3O_3$: 64.62, 4.45, 8.07. Found: 64.86, 4.60, 8.29. **9**: (67%), mp 199–200 °C (ethanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.55 (s, 6H, CH_3), 3.47 (s, 3H, OCH_3), 3.89 (s, 6H, $2OCH_3$), 6.66–7.84 (m, 10H, ArH, NH). Anal. Calcd for $C_{29}H_{25}Cl_2N_3O_3$: 65.17, 4.71, 7.86. Found: 65.30, 4.83, 8.02. **10**: (60%), mp 221–222 °C (DMF). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.12 (d, 12H, $4CH_3$), 3.06 (m, 2H, 2CH), 3.36 (s, 3H, OCH_3), 3.74 (s, 6H, $2OCH_3$), 6.85–7.90 (m, 10H, ArH, NH). Anal. Calcd for $C_{33}H_{33}Cl_2N_3O_3$: 67.12, 5.63, 7.12. Found: 67.25, 5.77, 7.43. **11**: (93%), mp 155–156 °C (methanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.66 (s, 3H, OCH_3), 4.01 (s, 6H, $2OCH_3$), 4.33 (d, 2H, CH_2), 7.11–7.90 (m, 12H, ArH, NH). Anal. Calcd for $C_{28}H_{23}Cl_2N_3O_3$: 64.62, 4.45, 8.07. Found: 64.70, 4.54, 8.33. **12**: (89%), mp 169–170 °C (ethanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.46 (d, 3H, CH_3), 3.33 (s, 3H, OCH_3), 3.95 (s, 6H, $2OCH_3$), 4.11 (m, 1H, CH), 6.99–7.86 (m, 12H, ArH, NH). Anal. Calcd for $C_{29}H_{25}Cl_2N_3O_3$: 65.17, 4.71, 7.86. Found: 65.27, 4.99, 7.91. **13**: (90%), mp 167–169 °C (ethanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.46 (d, 3H, CH_3), 3.33 (s, 3H, OCH_3), 3.95 (s, 6H, $2OCH_3$), 4.11 (m, 1H, CH), 6.99–7.86 (m, 12H, ArH, NH). Anal. Calcd for $C_{29}H_{25}Cl_2N_3O_3$: 65.17, 4.71, 7.86. Found: 65.29, 4.82, 8.01. **14**: (94%), mp 163–164 °C (DMF). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.60 (t, 2H, CH_2), 3.35 (m, 2H, CH_2), 3.46 (s, 3H, OCH_3), 3.87 (s, 6H, $2OCH_3$), 6.65–6.77 (br s, 1H, NH exchange with D_2O), 7.01–8.10 (m, 11H, ArH). Anal. Calcd for $C_{29}H_{25}Cl_2N_3O_3$: 65.17, 4.71, 7.86. Found: 65.30, 4.77, 7.87. **15**: (96%), mp 133–143 °C (DMF). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.21 (t, 3H, CH_3), 3.21 (m, 2H, CH_2), 3.35 (s, 3H, OCH_3), 3.92 (s, 6H, $2OCH_3$), 6.45–6.52 (br s, 1H, NH exchange with D_2O), 7.13–8.24 (m, 6H, ArH). Anal. Calcd for $C_{23}H_{21}Cl_2N_3O_3$: 60.27, 4.62, 9.17. Found: 60.42, 4.84, 9.39. **16**: (65%), mp 194–195 °C (DMF). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 0.91 (s, 9H, $3CH_3$), 3.41 (s, 3H, OCH_3), 4.11 (s, 6H, $2OCH_3$), 6.31–6.36 (br s, 1H, NH exchange with D_2O), 7.07–7.95 (m, 6H, ArH, NH). Anal. Calcd for $C_{25}H_{25}Cl_2N_3O_3$: 61.73, 5.18, 8.64. Found 61.67, 5.24, 8.69. **17**: (61%), mp 246–247 °C (DMF). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.43 (s, 6H), 1.60–1.63 (d, 3H), 1.70–1.72 (d, 3H), 2.00 (s, 3H), 3.56 (s, 3H, OCH_3), 4.01 (s, 6H, $2OCH_3$), 6.28–6.33 (br s, 1H, NH exchange with D_2O), 6.90–7.89 (m, 6H, ArH). Anal. Calcd for $C_{31}H_{31}Cl_2N_3O_3$: 65.96, 5.54, 7.44. Found: 66.07, 5.71, 7.72. **18**: (74%), mp 143–144 °C (DMF). ^{1}H NMR (DMSO-d_6, 200 MHz): 2.65 (s, 6H, $2CH_3$). 3.52 (s, 3H, OCH_3), 3.78 (s, 6H, $2OCH_3$), 6.99–7.79 (m, 6H, ArH). Anal. Calcd for $C_{23}H_{21}Cl_2N_3O_3$: 60.27, 4.62, 9.17. Found: 60.33, 4.55, 9.29. **19**: (77%), mp 181–182 °C (ethanol). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.51 (s, 3H, CH_3), 3.60 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 4.26 (s, 2H, CH_2), 7.05–8.21 (m, 11H, ArH). Anal. Calcd for $C_{29}H_{25}Cl_2N_3O_3$: 65.17, 4.71, 7.86. Found: 65.40, 4.97, 7.91. **20**: (51%), mp >300 °C (acetonitrile). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.23 (br s, 1H, OH), 3.43 (s, 3H, OCH_3), 3.73 (s, 6H, $2OCH_3$), 3.95 (dd, 1H), 4.20

(d, 1H), 4.46 (d, 1H), 4.79 (dd, 1H), 6.77 (br s, 1H, NH, exchange with D_2O), 7.11–8.26 (m, 14H, ArH). Anal. Calcd for $C_{37}H_{29}Cl_2N_3O_4$: 68.31, 4.49, 6.46. Found: 68.57, 4.66, 6.74. **21**: (49%), mp >300 °C (acetonitrile). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.23 (br s, 1H, OH), 3.43 (s, 3H, OCH_3), 3.73 (s, 6H, $2OCH_3$), 3.95 (dd, 1H), 4.20 (d, 1H), 4.46 (d, 1H), 4.79 (dd, 1H), 6.77 (br s, 1H, NH, exchange with D_2O), 7.10–8.24 (m, 14H, ArH). Anal. Calcd for $C_{37}H_{29}Cl_2N_3O_4$: 68.31, 4.49, 6.46. Found: 68.43, 4.59, 6.60.

4.2. In vitro antibacterial activity

Minimal inhibitory concentration (MIC) was determined using the broth dilution technique.[26] The following standard organisms used in the antimicrobial screening were obtained from IFO (Institute Fermentation of Osaka); *S. aureus* IFO 3060 and *E. coli* IFO 3301. Ceprofloxacin was used during the test procedure as reference antibiotic.

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