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Condensation of nicotinaldehydes with acetophenones and NH_4OAc: A convenient synthesis and biological activities of 2',6'-diphenyl-3,4'-bipyridines

Shahnaz Begum[a], B Uma Rajeswari[a], A Zehra[b], A K Tiwari[b], R S Prakasham[a] & B China Raju*[a]

[a] Department of Organic Synthesis & Process Chemistry

and

[b] Centre for Natural Products & Traditional Knowledge,

CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India

E-mail: chinaraju@iict.res.in

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2',6'-Diphenyl-3,4'-bipyridines **3a-t** have been achieved by the three-component, one-pot reaction of nicotinaldehydes **1a-b**, acetophenones **2a-j** and anhydrous ammonium acetate under solvent free conditions at 120°C. All the prepared compounds **3a-t** have been screened for anti-microbial, free-radical scavenging and α-glucosidase inhibitory activities. Compounds **3m-r** have shown anti-bacterial activity and compounds **3m-n** identified as anti-fungal agents. Compounds **3d**, **3h**, **3m** and **3r-s** have shown α-glucosidase inhibitory activity.

Keywords: Nicotinaldehydes, 2',6'-diphenyl-3,4'-bipyridines, anti-microbial activity, α-glucosidase inhibitory activity

Pyridines represent an important class of heterocyclic compounds and structural units present in natural products and pharmaceuticals[1,2]. These are the key intermediates for the preparation of biologically active molecules[2]. Isoniazid (anti-tubercular drug)[3] and Imidacloprid (pesticide molecule)[4] are the potential pyridine derivatives. 2,4,6-Triarylpyridines or 2,6-diaryl-4-heteroarylpyridines are important heterocyclic compounds and the construction of 2,4,6-triarylpyridines have been reported by Chen *et al.*[5] from acetophenone (3 equiv.) and NH_4OAc (nitrogen source) in the presence of $Cu(OTf)_2$ in toluene at 110°C. Preparation of 2,4,6-trisubstituted pyridines was reported by Jiang *et al.* from acetophenones and nicotinal aminescatalyzed by $Cu(OTf)_2/O_2$ with the cleavage of C-N bond of aromatic methylamines[6]. However, to the best of our knowledge there is no method available for the preparation of 2,6-diaryl-4-heteroarylpyridines.

As part our ongoing research on 2-chloronicotinaldehydes, we have reported 2-chloronicotinaldehydes based heterocyclic compounds such as 1,8-naphthyridine-3-carboxylates[7], 2-chloro-5-methylpyridine-3-olefines[8], (*E*)-α,β-unsaturated esters and ketones[9], pyridinyl-1*H*-1,2,3-triazolyldihydroisoxazoles[10]. In continuation of our work on 2-chloronicotinaldehydes, the present manuscript has been designed to prepare 2-chloronicotinaldehydes based 2',6'-diphenyl-3,4'-bipyridines and biological evaluation of anti-microbial, free radical scavenging and α-glucosidase inhibitory activities.

Results and Discussion

In an initial experiment, nicotinaldehyde (**1a**, 1.0 equiv.), acetophenone (**2a**, 1.0 equiv.) and anhydrous NH_4OAc (1.5 equiv.) were heated at 80°C for 6h with catalytic amount of $Cu(OAc)_2$ (30 mol%, Scheme I). The compound was achieved in 54% yield after the column chromatography purification. The reaction was repeated at 120°C with 2.0 equiv of anhydrous ammonium acetate and the product **3a** was obtained with improved yield (78%, Table I). Based on the spectral data, the compound was identified as 2',6'-diphenyl-3,4'-bipyridine **3a**. We have carried out the reaction of **1a** with **2a** with various catalysts such as *p*-TsOH, $Cu(OTf)_2$, $Bi(OTf)_3$, $ZnCl_2$, $Bi(NO_3)_2$, $Zn(OAc)_2$, Indium and I_2. We have studied the reaction of **1a** with **2a** with various ammonium salts such as NH_4F, NH_4Cl, NH_4Br, NH_4NO_3, $(NH_4)_2CO_3$, and $(NH_4)_2SO_4$. We identified NH_4OAc (2 equiv.)

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with catalytic amount of $Cu(OAc)_2$ at 120°C are the better condition to produce the compound **3a** with very good yield. Having optimized conditions in hand, next, we have prepared series of target compounds 2',6'-diphenyl-3,4'-bipyridines **3b-t** by the reaction of nicotinaldehydes **1a-b** with acetophenones **2a-j** (Scheme I, Table I). All the prepared compounds are new and characterized by spectral data.

Biology

The compounds 2',6'-diphenyl-3,4'-bipyridines **3a-t** have been evaluated for their biological activities[11] such as anti-microbial, free radical scavenging (DPPH, $ABTS^{+}$) and α-glucosidase inhibitory and the results are described below.

Anti-microbial activity

All the compounds **3a-t** were tested for anti-microbial activity against two Gram-positive organisms (*Bacillus subtilis*, *Staphylococcus epidermidis*) and two Gram-negative organism (*Pseudomonas aeruginosa*, *Escherichia coli*) by agar well plate method and the results are presented in Table II. Five compounds **3n-r** have shown moderate anti-bacterial activity against gram positive organism (B. *subtilis*) and compound **3n** have shown significant anti-bacterial activity selectively against gram positive organism (S. *epidermidis*). Compounds **3m** and **3n** have shown significant anti-bacterial activity against gram negative organism E. *coli*. The structure activity relationships of the compounds revealed that the compounds 2-chloro 2',6'-diphenyl-3,4'-bipyridines **3n-r** were obtained with 2-chloronicotinaldehydes have shown the anti-bacterial activity when compared to compounds obtained with nicotinaldehyde. Specifically the compound **3n** have shown activity against S. *epidermidis* and similarly, the compounds **3m** and **3n** have shown activity selectively against E. *coli.*

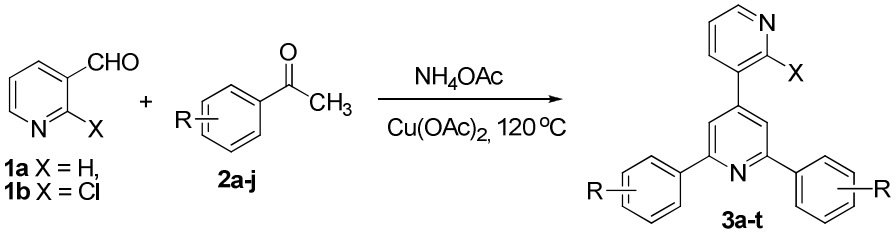
Anti-fungal activities of target the compounds **3a-t** have been tested against *Candida albicans* and fluconazole was used as standard drug (Table II). The compound **3m** have shown significant anti-fungal activity and **3n** shown moderate activity in comparison with the standard drug. The structure activity relationships of the compounds revealed that the products 2-chloro 2',6'-diphenyl-3,4'-bipyridines **3m-n** were obtained with 2-chloronicotinaldehydes have shown the anti-fungal activity when compared to compounds obtained with nicotinaldehyde.

In view of antimicrobial activity of 2',6'-diphenyl-3,4'-bipyridines, compound **3m** were further evaluated for MIC against those culture which revealed better anti-microbial activity. The compound **3m** showed MIC value of 64 $\mu g/mL^{-1}$ with *E. coli* and 32 $\mu g/mL^{-1}$ with *C. albicans* while standard compounds streptomycin depicted 8 $\mu g/mL^{-1}$ and flucanzole depicted 2 mg/mL^{-1} suggesting that **3m** compound is less effective in terms of MIC against the *E.coli.* However, compound **3m** is more effective in terms of MIC than *C. albicans*. This observation is interesting to note that the anti-fungal activity by the above

Table I — Preparation of nicotinaldehyde based 2',6'-diphenyl-3,4'-bipyridines **3a-t**

Entry	Compd	X X	R
1	**3a**	H	H
2	**3b**	H	3-OCH_3
3	**3c**	H	4-OCH_3
4	**3d**	H	3,4-OCH_3
5	**3e**	H	4-CH_3
6	**3f**	H	4-F
7	**3g**	H	3-Cl
8	**3h**	H	4-Cl
9	**3i**	H	3-Br
10	**3j**	H	4-Br
11	**3k**	Cl	H
12	**3l**	Cl	3-OCH_3
13	**3m**	Cl	2,3-OCH_3
14	**3n**	Cl	3,4-OCH_3
15	**3o**	Cl	4-CH_3
16	**3p**	Cl	3-Cl
17	**3q**	Cl	4-Cl
18	**3r**	Cl	2,4-Cl
19	**3s**	Cl	3-Br
20	**3t**	Cl	4-Br

[a] Isolated yields



Scheme I

Table II — Anti-microbial activity profile of compounds **3a-t**

	Anti-bacterial				Anti-fungal
	Gram-positive		Gram-negative		
Compd	*B. subtilis* (MIC)*	*S. epidermidis*	*P. aeruginosa* (MIC)*	*E. coli*	*C. albicans* (MIC)*
3a	–	–	–	–	–
3b	–	–	–	–	–
3c	–	–	–	–	–
3d	–	–	–	–	–
3e	–	–	–	–	–
3f	–	–	–	–	–
3g	–	–	–	–	–
3h	–	–	–	–	–
3i	–	–	–	–	–
3j	–	–	–	–	–
3k	–	–	–	–	–
3l	–	–	–	–	–
3m	–	–	–	12	10
3n	07	10	–	11	07
3o	06	–	–	–	–
3p	06	–	–	–	–
3q	08	–	–	–	–
3r	06	–	–	–	–
3s	–	–	–	–	–
3t	–	–	–	–	–
Control	0	0	0	0	0
Streptomycin	19 (8)	13	13 (8)	16	–
Fluconazole	–	–	–	–	00*

B. subtilis: Bacillus subtilis, *S. epiderimidis*: Staphylococcus epidermidis, *P. aeruginosa*: Pseudomonas aeruginosa, *E. coli*: *Escherichia coli*, *C. albicans*: *Candida albicans*.

*MIC (μg/mL): Minimum inhibitory concentration.

The values represent the zone of inhibition in millimeter (mm) on agar plate against the represented microbial strains. The value in parentheses represents the MIC value (μg/mL).

*Selected test strain (Fluconazole) has shown resistant against the standard drug.

compound is greater than the standard which could be the alternative anti-fungal compound in the future.

Free radicals scavenging activity

The DPPH and $ABTS^{\cdot+}$ free radical scavenging activity of compounds **3a-t** are presented in Table III along with the standard drugs Ascorbic acid and Trolox (SC_{50} values). These compounds could not show any free radical scavenging activity.

α-Glucosidase inhibitory activity

α-Glucosidase inhibitory activity of compounds **3a-t** and their IC_{50} values presented in Table III along with the standard drug Acarbose. Five compounds **3d**, **3h, 3m, 3r** & **3s** have shown α-glucosidase inhibitory activity in the present series of compounds. The structure activity relationships of the compounds revealed that the compound **3d, 3m** (dimethoxy substitution, IC_{50} 7.65, 8.05 μg/mL) and **3r-s** (chloro, bromo substitution, IC_{50} 8.21, 15.26 μg/mL) has shown moderate α-glucosidase inhibitory activity. Compound **3h** (chloro substitution, IC_{50} 3.47 μg/mL) has shown significant α-glucosidase inhibitory activity.

Experimental Section

The chemicals were procured from Sigma-Aldrich and local suppliers. All the reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} (mesh); spots were visualized under UV light. Merck silica gel (100-200 mesh) was used for column chromatography. Melting points were determined in open glass capillary tubes on a Stuart melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on an Avance 300, 400 and 500 MHz spectrometer in $CDCl_3$ using

Table III — DPPH, ABTS.+, α-glucosidase inhibitory (α-GI) activity profile of compounds 3a-t

Compd	DPPH % Inhibition 25 µg/ml (SC_{50} µg /ml)	$ABTS^{\cdot+}$ % Inhibition 20 µg /ml (SC_{50} µg /ml)	α-GI % Inhibition 20 µg/ml (IC_{50} µg /ml)
3a	ND	ND	ND
3b	ND	ND	9.92 ± 0.00
3c	ND	ND	13.40 ± 0.64
3d	ND	ND	69.04 ± 0.32 (7.65)
3e	ND	ND	17.49 ± 0.21
3f	ND	ND	14.16 ± 0.00
3g	ND	ND	ND
3h	ND	ND	83.12 ± 0.11 (3.47)
3i	ND	ND	ND
3j	ND	3.02 ± 0.47	48.30 ± 0.11
3k	ND	ND	12.64 ± 1.28
3l	ND	ND	2.95 ± 0.00
3m	ND	ND	68.36 ± 0.43 (8.05)
3n	ND	27.63 ± 1.74	37.02 ± 0.21
3o	ND	ND	4.77 ± 0.00
3p	ND	ND	40.65 ± 0.00
3q	ND	ND	45.27 ± 1.61
3r	ND	ND	68.05 ± 0.00 (8.21)
3s	ND	ND	61.01 ± 0.11 (15.26)
3t	ND	ND	17.03 ± 0.00
Ascorbic acid	84.08 ± 0.12	–	–
Trolox	–	98.88 ± 0.00	–
Acarbose	–	–	89.17 ± 0.11 (2.79)

TMS as internal standard. IR spectra were recorded on a Nicolet 740 FT-IR spectrometer. Mass spectra were obtained on Agilent LCMS instrument. ESI-MS obtained on Quarto micro spectrometer.

General procedure for the preparation of 2',6'-diphenyl-3,4'-bipyridines, 3a-t

$Cu(OAc)_2$ (30 mol%) was added to a stirred reaction mixture of nicotinaldehyde (**1a**, 1.0 equiv.) acetophenone **(2a**, 2.0 equiv.) and anhydrous ammonium acetate (2.0 equiv.). The contents were heated at 120°C. The reaction was monitored by TLC and after completion of the reaction (6 h), the mixture was purified by column chromatography using silica gel (hexane:ethyl acetate) and afforded 2',6'-diphenyl-3,4'-bipyridine **3a** in 70% yield. Similarly, the compounds **3b-t** were prepared by the reaction of nicotinaldehyde **1a** and 2-chloronicotinaldehyde **2b** with substituted acetophenones **2a-i** under our optimized reaction conditions. All the prepared compounds are unknown and characterized by spectral data.

2',6'-Diphenyl-3,4'-bipyridine, 3a: Yield 70%. Colorless solid. m.p. 164-166°C. FT-IR (KBr): 3447, 3063, 2923, 1601, 1573, 1481, 1386, 1251, 1189, 1025, 774, 690 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 9.01 (s, 1H, aromatic), 8.73 (s, 1H, aromatic), 8.21 (d, *J* = 7.3 Hz, 4H, aromatic), 8.04 (d, *J* = 7.8 Hz, 1H, aromatic), 7.88 (s, 2H, aromatic), 7.53 (t, *J* = 7.5 Hz, 4H, aromatic), 7.47 (d, *J* = 7.2 Hz, 3H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.12, 150.14, 148.34, 147.17, 139.33, 134.75, 134.55, 129.34, 128.83, 127.17, 123.85, 116.90; ESI-MS: *m/z* 309 $[M+H]^+$.

2',6'-Bis(3-methoxyphenyl)-3,4'-bipyridine, 3b: Yield 61%. Pale yellow solid. m.p. 128-130°C. FT-IR (KBr): 3877, 3682, 3023, 2407, 1429, 1215, 739, 671 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.00 (d, *J* = 1.7 Hz, 1H, aromatic), 8.73 (d, *J* = 3.5 Hz, 1H, aromatic), 8.20-8.00 (m, 1H, aromatic), 7.86 (s, 2H, aromatic), 7.82-7.78 (m, 2H, aromatic), 7.75 (dd, *J* = 7.7, 0.8 Hz, 2H, aromatic), 7.48-7.41 (m, 3H, aromatic), 7.04-7.00 (m, 2H, aromatic), 3.92 (s, 6H, $2OCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 160.12, 157.54, 150.09, 148.27, 146.98, 140.66, 134.61, 129.81, 123.88, 119.55, 117.18, 114.96, 112.74, 55.44; ESI-MS: *m/z* 369 $[M+H]^+$.

2',6'-Bis(4-methoxyphenyl)-3,4'-bipyridine, 3c: Yield 77%. Pale yellow solid. m.p. 134-136°C. FT-IR (KBr): 3424, 3037, 2837, 1605, 1513, 1428, 1246, 1170, 1028, 834, 760 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.00 (s, 1H, aromatic), 8.72 (s, 1H, aromatic), 8.16 (d, *J* = 8.8 Hz, 4H, aromatic), 8.02 (d, *J* = 7.8 Hz, 1H, aromatic), 7.75 (s, 2H, aromatic), 7.58-7.41 (m, 1H, aromatic), 7.04 (d, *J* = 8.8 Hz, 4H, aromatic), 3.89 (s, 6H, aromatic, $2OCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.71, 157.29, 149.92, 148.27, 146.76, 134.55, 131.95, 128.41, 123.84, 123.75, 115.41, 114.62, 114.13, 55.42; ESI-MS: *m/z* 369 $[M+H]^+$.

2',6'-Bis(3,4-dimethoxyphenyl)-3,4'-bipyridine, 3d: Yield 73%. Pale yellow solid. m.p. 136-138°C. FT-IR (KBr): 3442, 3078, 2928, 2831, 1603, 1512, 1459, 1397, 1262, 1175, 1023, 825, 793 cm^{-1};

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.01 (s, 1H, aromatic), 8.73 (s, 1H, aromatic), 8.04 (d, *J* = 7.9 Hz, 1H, aromatic), 7.88 (d, *J* = 2.0 Hz, 2H, aromatic), 7.83-7.70 (m, 4H, aromatic), 7.48 (dd, *J* = 7.7, 5.0 Hz, 1H, aromatic), 7.01 (d, *J* = 8.4 Hz, 2H, aromatic), 4.03 (s, 6H, aromatic, 2 OCH_3), 3.97 (s, 6H, 2 OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.20, 150.30, 149.26, 148.21, 147.06, 146.84, 134.68, 132.18, 123.89, 119.68, 115.74, 111.15, 110.21, 56.05, 55.96; ESI-MS: *m/z* 429 $[M+H]^+$.

2',6'-Di-*p*-tolyl-3,4'-bipyridine 3e: Yield 70%. Colorless solid. m.p. 160-162°C. FT-IR (KBr): 3448, 3027, 2918, 1600, 1541, 1247, 1179, 1020, 808, 708 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.99 (d, *J* = 1.9 Hz, 1H, aromatic), 8.71 (dd, *J* = 4.8, 1.3 Hz, 1H, aromatic), 8.10 (d, *J* = 8.2 Hz, 4H, aromatic), 8.04-7.95 (m, 1H, aromatic), 7.81 (s, 2H, aromatic), 7.45 (dd, *J* = 7.8, 4.9 Hz, 1H, aromatic), 7.32 (d, *J* = 8.0 Hz, 4H, aromatic), 2.48 (s, 6H, $2CH_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.72, 149.99, 148.32, 146.79, 139.30, 136.53, 134.92, 134.55, 129.52, 127.03, 123.81, 116.24, 21.39; ESI-MS: *m/z* 336 $[M+H]^+$.

2',6'-Bis(4-fluorophenyl)-3,4'-bipyridine 3f: Yield 79%. Colorless solid. m.p. 232-234°C. FT-IR (KBr): 3442, 3044, 2924, 1719, 1607, 1510, 1215, 1157, 829, 799 cm^{-1} ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.99 (d, *J* = 2.2 Hz, 1H, aromatic), 8.74 (dd, *J* = 4.8, 1.5 Hz, 1H, aromatic), 8.21-8.16 (m, 4H, aromatic), 8.06-7.99 (m, 1H, aromatic), 7.81 (s, 2H, aromatic), 7.48 (dd, *J* = 7.9, 4.8 Hz, 1H, aromatic), 7.24-7.18 (m, 4H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.79, 162.81, 156.86, 150.26, 148.28, 134.53, 128.96, 128.93, 123.87, 116.50, 115.86, 115.69; ESI-MS: *m/z* 345 $[M+H]^+$.

3-(3,3''-dichloro-[1,1':3',1''-terphenyl]-5'-yl)pyridine 3g: Yield 75%. Colorless solid. m.p. 168-170°C. FT-IR (KBr): 3874, 3593, 3309, 3013, 2360, 1689, 1432, 1217, 813, 770, 674 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (s, 2H, aromatic), 8.05 (t, *J* = 6.0 Hz, 3H, aromatic), 7.87 (s, 3H, aromatic), 7.50-7.43 (m, 6H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.58, 150.34, 148.22, 147.54, 140.72, 134.99, 134.53, 130.56, 130.11, 129.47, 127.29, 125.39, 125.24, 117.65, 117.51; ESI-MS: *m/z* 377 $[M+H]^+$.

2',6'-Bis(4-chlorophenyl)-3,4'-bipyridine, 3h: Yield 72%. Colorless solid. m.p. 228-230°C. FT-IR (KBr): 3442, 3073, 3035, 1901, 1601, 1546, 1494, 1382, 1089, 831, 799 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.99 (s, 1H, aromatic), 8.75 (s, 1H, aromatic), 8.13 (d, *J* = 8.6 Hz, 4H, aromatic), 8.02 (d, *J* = 7.8 Hz, 1H, aromatic), 7.85 (d, *J* = 7.0 Hz, 2H, aromatic), 7.50 (d, *J* = 8.6 Hz, 5H, aromatic); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.68, 150.28, 148.22, 147.38, 137.37, 135.61, 134.51, 129.03, 128.37, 116.86; ESI-MS: *m/z* 327 $[M+H]^+$.

6'-Bis(3-bromophenyl)-3,4'-bipyridine, 3i: Yield 71%. Pale brownish solid. m.p. 152-154°C. FT-IR (KBr): 3875, 3595, 3319, 1598, 1484, 1386, 1218, 771, 687 cm^{-1} ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.33 (s, 2H, aromatic), 8.21-8.02 (m, 4H, aromatic), 7.86 (d, *J* = 10.7 Hz, 2H, aromatic), 7.70-7.53 (m, 4H, aromatic), 7.41 (t, *J* = 7.8 Hz, 2H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.51, 140.97, 134.70, 132.41, 130.40, 130.21, 128.89, 127.18, 125.75, 123.16, 117.56; ESI-MS: *m/z* 467 $[M+H]^+$.

2',6'-Bis(4-bromophenyl)-3,4'-bipyridine 3j: Yield 64%. Pale brownish solid. m.p. 237-239°C. FT-IR (KBr): 3422, 3034, 2923, 2851, 1906, 1604, 1577, 1547, 1483, 1378, 1071, 829, 800 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.99 (s, 1H, aromatic), 8.75 (s, 1H, aromatic), 8.04 (dd, *J* = 18.5, 8.2 Hz, 5H, aromatic), 7.85 (s, 2H, aromatic), 7.66 (d, *J* = 8.5 Hz, 4H, aromatic), 7.48 (dd, *J* = 7.6, 4.9 Hz, 1H, aromatic); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.81, 156.68, 150.34, 148.26, 147.46, 137.84, 134.54, 132.01, 128.67, 123.99, 116.94; ESI-MS: *m/z* 467 $[M+H]^+$.

2-Chloro-2',6'-diphenyl-3,4'-bipyridine 3k: Yield 82%. Colorless solid. m.p. 133-135°C. FT-IR (KBr): 3448, 3060, 2104, 1599, 1557, 1416, 1381, 1241, 1122, 776, 738 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (dd, *J* = 4.8, 1.9 Hz, 1H, aromatic), 8.20-8.17 (m, 4H, aromatic), 7.84-7.76 (m, 3H, aromatic), 7.54-7.49 (m, 4H, aromatic), 7.48-7.43 (m, 2H, aromatic), 7.40 (dd, *J* = 7.5, 4.8 Hz, 1H, aromatic); ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.27, 149.55, 146.76, 139.38, 139.08, 135.12, 129.36, 128.82, 127.16, 122.80, 119.08; ESI-MS: *m/z* 343 $[M+H]^+$.

2-Chloro-2',6'-bis(3-methoxyphenyl)-3,4'-bipyridine 3l: Yield 81 %. Pale yellow solid. m.p. 141-143°C. FT-IR (KBr): 3422, 3085, 3109, 3000, 2904, 2832, 1912, 1748, 1667, 1595, 1044, 866, 687 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.50 (dd, *J* = 4.8, 1.9 Hz, 1H, aromatic), 8.07-7.57 (m, 7H, aromatic), 7.47-7.37 (m, 3H, aromatic), 7.01 (m, 2H, aromatic), 3.92 (s, 6H, $2OCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 160.12, 156.97, 149.56, 146.60, 140.52, 139.38, 135.16, 129.81, 122.80, 119.55, 119.37, 115.00, 112.71, 55.43; ESI-MS: *m/z* 403 $[M+H]^+$.

2-Chloro-2',6'-bis(2,4-dimethoxyphenyl)-3,4'-bipyridine, 3m: Yield 72%. Pale yellow solid. m.p. 130-132°C. FT-IR (KBr): 3420, 2926, 2842, 1649, 1615, 1592, 1395, 1256, 1213, 1016, 825, 795 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.39 (dd, *J* = 4.7, 1.8 Hz, 2H, aromatic), 7.99 (dd, *J* = 7.7, 1.7 Hz, 2H, aromatic), 7.91 (s, 1H, aromatic), 7.79 (s, 1H, aromatic), 7.51 (s, 1H, aromatic), 7.29 (dd, *J* = 7.6, 4.7 Hz, 2H, aromatic), 6.60-6.58 (m, 1H, aromatic), 6.50 (s, 1H, aromatic), 3.91 (s, 6H, 2 OCH_3), 3.88 (s, 6H, 2 OCH_3); ^{13}C NMR: (101 MHz, $CDCl_3$): δ 189.44, 164.69, 160.62, 151.62, 150.00, 136.09, 135.98, 133.31, 131.55, 130.78, 122.78, 121.66, 105.47, 98.63, 55.81, 55.66; ESI-MS: *m/z* 463 $[M+H]^+$.

2-Chloro-2',6'-bis(3,4-dimethoxyphenyl)-3,4'-bipyridine, 3n: Yield 67%. Pale yellow solid. m.p. 124-126°C. FT-IR (KBr): 3877, 3594, 2925, 2856, 1659, 1593, 1518, 1412, 1271, 1214, 1162, 1024, 771, 691 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.43 (d, *J* = 2.9 Hz, 1H, aromatic), 8.04 (dd, *J* = 6.5, 4.7 Hz, 2H, aromatic), 7.64 (d, *J* = 11.8 Hz, 3H, aromatic), 7.49 (s, 1H, aromatic), 7.33 (dd, *J* = 7.6, 4.7 Hz, 2H, aromatic), 6.94 (d, *J* = 8.4 Hz, 2H, aromatic), 3.98 (s, 12H, 4 OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 188.04, 153.70, 151.71, 150.36, 149.43, 138.13, 136.18, 126.57, 123.39, 122.82, 110.88, 110.04, 56.18, 56.12; ESI-MS: *m/z* 462 $[M+H]^+$.

2-Chloro-2',6'-di-p-tolyl-3,4'-bipyridine, 3o: Yield 79%. Colorless solid. m.p. 180-182°C. FT-IR (KBr): 3872, 3593, 3308, 3024, 2923, 2736, 2356, 1915, 1773, 1606, 1551, 1391, 1217, 1120, 1069, 819, 764 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.49 (dd, *J* = 4.8, 1.9 Hz, 1H, aromatic), 8.08 (d, *J* = 8.2 Hz, 4H, aromatic), 7.78 (dd, *J* = 7.5, 1.9 Hz, 1H, aromatic), 7.71 (s, 2H, aromatic), 7.39 (dd, *J* = 7.5, 4.8 Hz, 1H, aromatic), 7.31 (d, *J* = 7.9 Hz, 4H, aromatic), 2.43 (s, 6H, $2CH_3$).^{13}C NMR (125 MHz, $CDCl_3$): δ 157.17, 149.45, 149.43, 146.56, 139.38, 139.32, 136.40, 135.21, 129.51, 127.01, 122.75, 118.44, 21.38; ESI-MS: *m/z* 371 $[M+H]^+$.

2-Chloro-2',6'-bis(3-chlorophenyl)-3,4'-bipyridine 3p: Yield 73%. Colorless solid. m.p. 184-186°C. FT-IR (KBr): 3873, 3592, 3378, 3068, 1599, 1556, 1388, 1216, 1083, 879, 769, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.52 (dd, *J* = 4.7, 1.8 Hz, 1H, aromatic), 8.16 (s, 2H, aromatic), 8.12-8.01 (m, 2H, aromatic), 7.89-7.72 (m, 3H, aromatic), 7.57-7.37 (m, 5H, aromatic); ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.05, 149.81, 140.59, 139.32, 134.99, 130.12, 129.50, 127.29, 125.25, 122.84, 119.72; ESI-MS: *m/z* 413 $[M+H]^+$.

2-Chloro-2',6'-bis(4-chlorophenyl)-3,4'-bipyridine 3q: Yield 78%. Colorless solid. m.p. 214-216°C. FT-IR (KBr): 3688, 3022, 2408, 2689, 1525, 1427, 1367, 1215, 739, 671 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.52 (d, *J* = 3.2 Hz, 1H, aromatic), 8.11 (d, *J* = 8.6 Hz, 4H, aromatic), 7.82-7.70 (m, 3H, aromatic), 7.49 (d, *J* = 8.6 Hz, 4H, aromatic), 7.42 (dd, *J* = 7.5, 4.8 Hz, 1H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.68, 150.28, 148.22, 147.38, 137.37, 135.61, 134.51, 129.03, 128.37, 116.86; ESI-MS: *m/z* 411 $[M+H]^+$.

2-Chloro-2',6'-bis(2,4-dichlorophenyl)-3,4'-bipyridine 3r: Yield 77%. Colorless solid. m.p. 192-194°C. FT-IR (KBr): 3875, 3593, 3546, 3385, 1798, 1598, 1555, 1481, 1383, 1217, 1102, 1059, 820, 770, 676 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.51 (dd, *J* = 4.8, 1.9 Hz, 1H, aromatic), 8.07-7.70 (m, 5H, aromatic), 7.59-7.35 (m, 5H, aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.94, 149.86, 145.36, 139.56, 137.02, 135.36, 134.16, 133.03, 132.80, 130.07, 129.07, 128.41, 127.60, 123.93, 122.88; ESI-MS: *m/z* 503 $[M+H]^+$.

2',6'-Bis(3-bromophenyl)-2-chloro-3,4'-bipyridine 3s: Yield 82%. Pale brownish solid. m.p. 184-186°C. FT-IR (KBr): 3679, 3023, 2403, 1429, 1358, 1215, 740, 671 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.53

(dd, *J* = 4.8, 1.9 Hz, 1H, aromatic), 8.31 (t, *J* = 1.8 Hz, 2H, aromatic), 8.11-8.06 (m, 2H, aromatic), 7.79-7.75 (m, 3H, aromatic), 7.64-7.56 (m, 2H, aromatic), 7.43 (dd, *J* = 6.1, 3.4 Hz, 2H, aromatic), 7.40 (s, 1H, aromatic); ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.93, 149.80, 147.22, 140.82, 139.32, 132.41, 130.39, 130.18, 130.12, 125.74, 123.15, 122.85, 119.74; ESI-MS: *m/z* 502 $[M+H]^+$.

2',6'-Bis(4-bromophenyl)-2-chloro-3,4'-bipyridine, 3t: Yield 80%. Colorless solid. m.p. 218-220°C. FT-IR (KBr): 3876, 3685, 3593, 3532, 3023, 2405, 1432, 1215, 742, 671 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (dd, *J* = 4.7, 1.7 Hz, 1H, aromatic), 8.08 (dd, *J* = 27.1, 8.6 Hz, 4H, aromatic), 7.82-7.71 (m, 3H, aromatic), 7.64 (d, *J* = 8.6 Hz, 3H, aromatic), 7.60-7.27 (m, 2H, aromatic).^{13}C NMR (101 MHz, $CDCl_3$): δ 156.26, 149.75, 139.31, 137.72, 132.00, 129.04, 128.67, 128.39, 124.02, 122.83, 119.13; ESI-MS: *m/z* 502 $[M+H]^+$.

Anti-bacterial activity

Anti-bacterial activities of prepared compounds were evaluated against two Gram-positive organisms (Bacillus subtilis; Staphylococcus epidermidis) and two Gram-negative organisms (Pseudomonas aeruginosa; *Escherichia coli*) by agar well plate method by using streptomycin as standard[11]. The anti-fungal activities of prepared compounds were evaluated against yeast (*Candida albicans*) and fluconazole was used as standard drug.

Zone of inhibition plate tests

Well plate method is followed for both the anti-bacterial and anti-fungal activities for measuring the zone of inhibitions[11]. For anti-bacterial activity test strains used Gram positive and Gram negative in nutrient agar. For anti-fungal studies test strains used yeast and the medium used is potato dextrose agar. The synthesized compounds were used for activity studies and the concentration of each compound is 1.0 mg/mL along with standard and control. The media, petri dishes were autoclaved at 121 ºC for 15 min. After sterilization the plates were poured with appropriate medium left over for 30 min for solidification, later the plates were inoculated with 60 µl of test inoculum using sterile cotton swabs. An 8 mm width size wells were made with sterile cork borer and in each well exactly 100 µl of sample were loaded. Control and standard also placed in separate wells. The plates were initially incubated for 20-30 min at 4°C to allow the compounds to diffuse into the agar, and then subsequently incubated for 24 h at 37°C for bacteria and 48 h at 28°C for fungi. Zone diameters were expressed in mm using calibrated scale. Experiment was triplicate to minimize the deviations.

Determination of MIC and MBC

Minimum inhibition concentration (MIC) is the lowest concentration of an anti-microbial agent that will inhibit the visible growth of a microorganism. The MIC was determined using the tube dilution method. The compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 0.500 mg/mL (stock solution). The compounds having better anti-microbial activity were selected for the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) studies against all above microbial strains. The concentrations of test samples were serially diluted from 500 to 1.9 µg/mL and one tube without drug serves as control. All the tubes were inoculated with 1 mL of respective cultures having an OD of 0.2 (~ McFarland standard) and the tubes were incubated at 37°C for 16 h. The turbidity of each tube is measured with respect to control tube. MIC values are defined as the lowest concentration of compound at which growth is completely inhibited. After incubation the culture from each tube was spread on nutrient agar plates to evaluate the MBC concentration. The concentration at which the cells are completely dead was defined as MBC.Biological activity.

DPPH scavenging activity

Assay for the scavenging of stable free radical based on DPPH [1,1-diphenyl-2-picrylhydrazyl] was done as reported earlier was performed.[11] Briefly, in a 96-well micro plate, 25 µL of test sample dissolved in DMSO (1 mg/mL), 125 µL of 0.1 M tris-HCl buffer (*p*H 7.4) and 125 µL of 0.5 mM DPPH solution dissolved in absolute ethyl alcohol were added. The reaction mixture was shaken well and incubated in dark condition for 30 min and read at 517 nm spectrophotometrically (Spectra Max plus384, Molecular Devices Corporation, Sunnyvale, CA, USA). Percentage of DPPH scavenging was calculated as (1-B/ A) x 100 where 'A' represents absorbance of control without test samples and 'B' represents absorbance in presence of test samples.

$ABTS^{\cdot+}$ free radical scavenging assay

Scavenging of the $ABTS^+$ [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] was performed as described by Walker and Everette with suitable modifications[11]. Briefly, 100 mL stock solution of $ABTS^+$ (0.5 mM) was prepared by addition of 1 mL potassium persulfate (6.89 mM PBS, *p*H 8.0). The mixture was stored in the dark for 16 h. Test compounds were dissolved in DMSO (5mg/mL). Primary screening was done by mixing 10 µL of test compound in 100 µL of methanol followed by 190 µL of $ABTS^+$ in a 96-well microplate. Absorbance of decolorized $ABTS^+$ was measured at 734 nm after 15 min incubation in the dark on a BioTek ^{synergy4} multi-mode microplate reader. For each test sample a separate blank sample (devoid of $ABTS^+$) was used for background subtraction. The percentage of $ABTS^+$ scavenging was calculated applying following formula:

$$\%\ ABTS^+ \text{ scavenging} = [(Absorbance_{control} - Absorbance_{test})/Absorbance_{control} \times 100]$$

Various serial dilutions of active compounds were prepared and tested for determination of SC_{50} values. Suitable regression analysis was applied for calculation of SC_{50}.

Anti-hyperglycemic activity

α-Glucosidase inhibitory activity was determined as per earlier reported method.[11] Rat intestinal acetone powder in normal saline (100:1; w/v) was sonicated properly and the supernatant was used as a source of crude intestinal α-glucosidase after centrifugation. In brief, 20 µL of test samples (5 mg/mL DMSO solution) were reconstituted in 100 µL of 100 mM-phosphate buffer (*p*H 6.8) in 96-well microplate and incubated with 50 µL of crude intestinal α-glucosidase for 5 min before 50 µL substrate (5 mM, *p*-nitrophenyl- α-D-glucopyranoside prepared in same buffer) was added. Release of *p*-nitrophenol was measured at 405 nm spectrophotometrically (Spectra Max plus 384), Molecular Devices Corporation, Sunnyvale, CA, USA) 5 min after incubation with substrate. Individual blanks for test samples were prepared to correct background absorbance where substrate was replaced with 50 µL of buffer. Control sample contained 10 µL DMSO in place of test samples. Percentage of enzyme inhibition was calculated as (1-B/A) x 100 where 'A' represents absorbance of control without test samples and 'B' represents absorbance in presence of test samples. For calculation of 50% enzyme inhibitory activity (IC50%) more than five dilutions of primary screening concentration (5 mg/mL DMSO solution) of test compounds were prepared. The IC_{50} values were calculated applying logarithmic regression analysis.

Conclusions

In conclusion, series of 2',6'-diphenyl-3,4'-bipyridines **3b-t** have been prepared by the reaction of nicotinaldehydes **1a-b** with acetophenones **2a-j** in the presence of anhydrous NH_4OAc with $Cu(OAc)_2$ (30 mol%) as the catalyst at 120°C. The target compounds were evaluated for anti-microbial, free-radical scavenging and α-glucosidase inhibitory activities. Compounds **3m** and **3n** have shown anti-microbial activity**.** Compound **3h** have shown equi potent α-glucosidase inhibitory activity when compared to standard compound. Compounds **3d**, **3m**, **3r** and **3s** have shown significant α-glucosidase inhibitory activity.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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