Pyridine C3-arylation of nicotinic acids accessible via a multicomponent reaction: an entry to all-substituted-3,4-diarylated pyridines†

Sankar K. Guchhait,*a Neha Hura,a Kanchan Sinhab and Dulal Pandab

An efficient route for the synthesis of penta-substituted/functionalized-3,4-diarylated pyridines, biologically important templates, via pyridine C3-arylation of nicotinic acids has been developed. The poly-substituted nicotinic acid precursors were prepared by an established multicomponent condensation approach. This route shows an excellent opportunity for introducing versatile (hetero)aryls and other substituents/functionalities into the pyridine ring. Several of the synthesized compounds exhibited significant anti-proliferative properties.

Introduction

Poly-substituted/functionalized pyridines are omnipresent in natural products, bioactive compounds, and functional materials.1 In particular, aryl or poly-aryl substituted pyridine derivatives have attracted considerable synthesis attention because they are often found as a valuable structural motif in a wide range of pharmaceutically active compounds (Fig. 1). Etoricoxib that contains the 2,3-diarylpypyridine motif is a COX-2 selective inhibitor.2,3 2,4,6-Triarylated pyridine compounds exhibit topoisomerase inhibition (Fig. 1).4 Perampanel is a 1,3,5-triarylated pyridine derivative that acts as an AMPA-receptor antagonist.5 5,6-Diheteroarylpyridin-2-carboxamide derivative is a selective A2B adenosine receptor antagonist.6 2-Amino-4,6-diarylpypyridines have been found to be potent ligands for estrogen receptor.7 There are numerous bioactive natural products that contain polyarylated pyridines. Nosiheptide8 has 1,5,6-triarylated 3-hydroxypyridine and promothiocin A9 has 5,6-diarylated pyridine-1-carboxamide and both are potent antibiotics. 2,6-Diarylpyridine-carboxylic ester is useful as a fluorescent chemosensor.10

In recent times, as a part of current momentum of exploring new useful organic functional/bioactive materials, the synthesis of compounds that possess not only important heterocyclic scaffold(s) but also structurally resembles in whole molecular skeleton to drugs/bioactive agents has become a valuable research area to organic chemists.11 In this direction, late stage functionalization of drugs has also attracted significant attention.12 The incorporation of trimethoxyphenyl moiety in target organic compounds is valuable, since it plays role as an important pharmacophoric motif in exhibiting biological properties13 and is present in natural products.14 Keeping these aspects in mind, we were interested15 in the synthesis of 3,4-diarylated pyridines which contain structural features, 3,4,5-trimethoxyphenyl as an aryl motif, and resemblance to a nifedipine drug16 and biologically important nicotinic acid.17

Over the years, numerous synthetic strategies have been developed to access poly-functionalized pyridines.18,19 They involve majorly a pyridine-forming step of appropriately functionalized precursors20 or the sequential introduction of substituents on the preformed pyridine ring.21 The first strategy is limited to accessibility of particularly-functionalized precursor, while the later strategy includes mainly aromatic substitutions,22 direct metallation, or metal halogen-exchange reactions.23 Here we report a novel route for synthesis of all-substituted/functionalized pyridines containing 3,4-diaryl moieties via one-pot pyridine-3-arylation of nicotinic acid skeleton that is easily accessible by an established multicomponent reaction approach.
Results and discussion

At the outset, we investigated for preparation of nicotinic acid precursor (5a) by a Hantzsch multicomponent condensation of aryl aldehyde (1a), ethyl acetooacetate (2a) and ammonium acetate to construct dihydropyridine (3a), its dehydrogenative aromatization to product 4a, and mono-hydrolysis of di-ester (Scheme 1). Hantzsch reaction by a reported method was good yielding.24 Dehydrogenative aromatization reactions utilizing different conditions25 were performed (see ESI, Table 1†). Highest yield was obtained using Mn(OAc)₃ and AcOH at room temperature. Initially, the mono-hydrolysis of pyridine-3,5-diesters 4a was found to be difficult. Both the ester functionalities underwent simultaneously the hydrolysis. A survey of various conditions revealed that 2 equiv. NaOH in 2 mL of EtOH–H₂O (3 : 1) was most effective for promoting mono-hydrolysis to produce nicotinic acid 5a (see ESI, Table 2†).

Next, we investigated to explore decarboxylative arylation of nicotinic acid 5a, with the goal of finding a convenient method for synthesis of penta-substituted functionalized pyridines containing 3,4-diaryl moieties (Route A, Scheme 2). Several methods of decarboxylative arylation26 known for other scaffolds were investigated for the reaction of compound 5a. The desired product was obtained, but the yield more than 8% could not be accomplished by the methods as well as variation in reported conditions. We envisaged that protodecarboxylation,27 and subsequent C3–H bond arylation could afford C3-arylated pyridine (Route B, Scheme 2). The protodecarboxylation of compound 5a underwent via Ag-carboxylate on treatment with AgOAc (1 equiv.), K₂CO₃ (30 mol%) in DMA (anhyd.) at 140 °C for 12 h and the product 6a was obtained in 80% yield. Next, the C3–H arylation reactions of compound 6a following the reported pyridine-arylation methods were performed. Ye developed a Pd-catalyzed C3-selective arylation of unsubstituted pyridine with bromoarenes.28 Carrying out C3-arylation of substituted/functionalized pyridine 6a with bromo- or iodosotoluene using Ye’s conditions provided 3-arylated pyridine (8a) in poor yields (8% and 10% yields, respectively). A Pd-catalyzed 3-arylation of pyridine with aryl tosylates was reported by Dai.29 This methodology did not promote the reaction of compound 6a with p-tolyl tosylate. We explored previously a Pd-catalyzed regioselective C6–H arylation of 3-aminoimidazo[1,2-α]pyrazine that underwent via concerted metalation–deprotonation process.30 This method promoted the C3–H arylation of pyridine derivative 6a, but the method as well as the variation of its conditions could not improve the product’s yield more than 20%. Majorly, the substrate remained intact. The poor conversion and yields in the C3-arylation of pyridine 6a by reported methods might be due to significant steric hindrance by 4-aryl moiety to inhibit the substrate to undergo the C3-palladation with arylpalladium complex. With aim of obtaining the C3

![Scheme 1](image1)

**Scheme 1** Synthesis of nicotinic acid derivative 5a.

![Scheme 2](image2)

**Scheme 2** Strategies towards synthesis of polysubstituted 3,4-diarylated pyridines.

Table 1 Evaluation of reagents and conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Variable</th>
<th>Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd[PPh₃]₄</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>Pd(dppf)Cl₂</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>Pd[PPh₃]Cl₂</td>
<td>NR</td>
</tr>
<tr>
<td>4c</td>
<td>Pd(OAc)₃ + PPh₃</td>
<td>NR</td>
</tr>
<tr>
<td>5d</td>
<td>Pd[PPh₃]₄</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>K₂CO₃</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>Cs₂CO₃</td>
<td>55</td>
</tr>
<tr>
<td>8</td>
<td>K₃PO₄</td>
<td>40</td>
</tr>
<tr>
<td>9e</td>
<td>Na₂CO₃</td>
<td>45</td>
</tr>
<tr>
<td>10</td>
<td>Pd[PPh₃]₄</td>
<td>(10 mol%), base (5 equiv.), TBAB (1 equiv.), DMA–H₂O (1 : 1)</td>
</tr>
<tr>
<td>11</td>
<td>DMA</td>
<td>55</td>
</tr>
<tr>
<td>12</td>
<td>EtOH–H₂O (1 : 1)</td>
<td>60</td>
</tr>
</tbody>
</table>

⁠a⁠ Reagents and conditions: PhB(OH)₂ (1.5 equiv.), 100 °C, Ar, 2 h; 0.5 mmol scale. ⁠b⁠ Yield for maximum conversion in optimum time. ⁠c⁠ PPh₃ (40 mol%). ⁠d⁠ Pd[PPh₃]₄ (5 mol%). ⁠e⁠ Na₂CO₃ (2.5 equiv.). ⁠f⁠ Reaction temperature: 80 °C.
arylated product, we then investigated for an alternate approach involving protodecarboxylation, C3-bromination and Suzuki coupling sequentially (Scheme 3). C3-Bromination\(^\text{33}\) of pyridine \(6a\) leading to product \(7a\) was found to be nearly quantitative (95%). Therefore, we were interested to explore one-pot conditions for bromination–Suzuki coupling. A Pd(OAc)\(_2\)-catalyzed method reported by Liu\(^\text{34}\) for Suzuki reaction of \(N\)-heteroaryl halides was followed in the one-pot reaction of pyridine \(6a\) with phenylboronic acid, however, the desired product was obtained in 25% yield only. Christakakou’s\(^\text{35}\) reaction conditions yielded the product \(5a\) in 40% yield only. Gratifyingly, one-pot bromination and Suzuki coupling of pyridine \(6a\) with phenylboronic acid using Pd(PPh\(_3\))\(_4\) catalyst, Na\(_2\)CO\(_3\), TBAB in DMA–H\(_2\)O provided 3,4-diaryl-pyridine \(8a\) in 70% yield (Table 1, entry 1). However, further variation in conditions did not improve yield of the product. Incomplete conversion and inferior yield (15%) were obtained for the reaction carried out in the presence of open air, indicating requirement of non-aerobic conditions for Pd(0)-catalysis. The reaction without TBAB resulted in reduced yield (40%). Other palladium sources were evaluated (Table 1, entries 2–4). Pd(PPh\(_3\))\(_4\) was found to be best. Reducing the Pd-catalyst loading (entry 5) was not beneficial. Among various bases investigated, Na\(_2\)CO\(_3\) provided best result (entries 6–8). Decreasing the equivalence of sodium carbonate below to 5 equiv. resulted in reduced yield (Table 1, entry 9). DMA or EtOH was found effective solvent for bromination, however, they were inferior for promoting the Suzuki coupling in one-pot. Among various solvents and their mixture with water, DMA–H\(_2\)O was found to be most effective for promoting bromination–arylation in one-pot (entries 10–12).

We then investigated protodecarboxylation of nicotinic acid \(5a\), bromination and Suzuki coupling sequentially in one-pot. Amazingly, it worked well in one-pot with overall yield of 55% (Scheme 3). The one-pot multi-reactions synthesis of a target molecule is considered as a useful approach in synthetic organic chemistry.\(^\text{36}\) The present work illustrates an important example of one-pot three-reaction process.

With the optimized procedure, we next investigated to explore its substrate scope and synthesize various substituted pyridines. We were pleased to find that the method was found to be flexible in introducing into pyridine at C3 a variety of (hetero)aryl (Scheme 4). Aryls containing electron-withdrawing or donating functionalities were incorporated. The biologically relevant (hetero)aryl motifs were also introduced easily at C3-position of pyridine derivatives. In the established route, difficulty for pyridine C3-arylation due to steric hindrance by presence of a multi-substituted aryl \((3,4,5\text{-trimethoxyphenyl})\) at C4-position was circumvented.

On survey of literature, we found that Simoni demonstrated an attractive profile of cytotoxicity and apoptosis-inducing activity of 2-(3,4,5-trimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)-pyridine (compound A, Fig. 2).\(^\text{37}\) Zheng documented that 2-(3-hydroxy-4-methoxyphenyl)-6-(3,4,5-trimethoxyphenyl)-pyridine (B) inhibited cell survival and growth, comparable to a clinical agent, combretastatin A-4 (CA-4).\(^\text{38}\) These representative classes of compounds reveal that 3,4-diarylated pyridine derivatives synthesized in the present work have potential of exhibiting versatile bioactivities especially the antiproliferative properties.

We extended our developed method applicable to the synthesis of 3,4-diarylated pyridines with relevant substitutions that are present in CA-4 and the diarylated pyridines synthesized and bio-evaluated by Simoni and Zhang. Ethyl 5-(3-hydroxy-4-methoxyphenyl)-2,6-dimethyl-4-(3,4,5-trimethoxyphenyl)nicotinate (8r) was prepared via an approach (Scheme 5) involving C3-bromination of precursor \(6a\), boronation\(^\text{39}\) of \(7a\) with bispinacolatodiborane, and Suzuki coupling with 5-bromo-2-methoxyphenol. This prompted us to synthesize compound \(8t\) with switch in aryl substitutions of
Several of the synthesized compounds were found to exhibit potential application in important aryl-motifs can be easily introduced in this route. The range of (hetero)aryls, including especially the biologically component condensation and their pyridine C3-arylation were further evaluated for in vitro tubulin polymerization inhibition (see ESI, Table 3). Several of them were found to exhibit considerable antiproliferative activity. Four most potent compounds (pyridines 8b, 8f, 8j, 8p) were further evaluated for in vitro tubulin polymerization inhibition (see ESI, Fig. 1). The non-activity revealed that the antitubulin may not be the pathway for exhibiting cytotoxicity.

Conclusions

In conclusion, we have developed an efficient route for synthesis of all-substituted=functionalized pyridines containing 3,4-dialyl moieties. It involves the preparation of nicotinic acids via multi-component condensation and their pyridine C3-arylation via one-pot protodecarboxylation–bromination–Suzuki coupling. A wide range of (hetero)aryls, including especially the biologically important aryl-motifs can be easily introduced in this route. The structural features of the products, structural resemblance to pharmacologically important agent as well as drug and the presence of a biologically important motif 3,4,5-trimethoxyphenyl as one aryl ring, indicate that these functionalized 3,4-diarylpyridines have potential application in finding of versatile bioactive agents. Several of the synthesized compounds were found to exhibit significant anti-proliferative activity.

Experimental Chemistry

General information. ATR & IR (KB) Microscope spectrometer was used to record Infrared (IR (KB)) spectra. 1H NMR spectra were taken on a 400 MHz spectrometer. Data were reported in sequence of chemical shifts in ppm from tetramethylsilane as an internal standard in CDCl3/CD3OD, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dt = doublet of triplet, dd = doublet of doublet, br = broad), and coupling constants (Hz). 13C NMR spectra were recorded on a 100 MHz spectrometer with protons-decoupling. High-resolution mass spectra (HRMS) were recorded on a high-resolution LCMS/MS instrument with “QTOF” mass analyzer. Thin-layer chromatography (TLC) analysis was done using commercially received pre-coated TLC plates (silica gel 60 GF434, 0.43 mm). Column chromatography silica gel 100–200 (silica gel 100–200 mesh, neutral, spherical) was used for purification of products. The starting materials and solvents were used as received from commercial sources without further purification.

Representative experimental procedure for the synthesis of diethyl 2,6-dimethyl-4-(3,4,5-trimethoxyphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Scheme 1, 4a).25a Diethyl 1,4-dihydro-2,6-dimethyl-4-(3,4,5-trimethoxyphenyl)pyridine-3,5-dicarboxylate (3a) (1 mmol, 253 mg), manganese triacetate (2 mmol, 260 mg, 2 eq.), NH4OAc (2 mmol, 154 mg, 2 eq.), and PhB(OH)2 (0.1 mmol, 12 mg, 0.1 eq.) were taken in a round bottom flask and refluxed in EtOH (2 mL) for 5 h. The reaction mixture was poured into ice-cold H2O. It was then extracted with EtOAc (2 × 10 mL). The organic solution was washed with brine, dried over Na2SO4, and concentrated under vacuum. The crude product was purified by recrystallization from EtOH, which provided 1,4-dihydropyridine 3a in 70% yield.

Representative experimental procedure for the synthesis of diethyl 2,6-dimethyl-4-(3,4,5-trimethoxyphenyl)pyridine-3,5-dicarboxylate (Scheme 1, 4a).25a Aqueous solution of KOH (2 mmol, 112 mg, 2 eq.) in 0.5 mL water was added to a solution of compound 4a (1 mmol, 417 mg) in ethanol (1.5 mL). The mixture was refluxed (80 °C) till completion of the reaction as indicated by TLC (7 h). The solvent was evaporated and the crude mass obtained was redissolved in methanol. It was then neutralised to pH 7 with dropwise addition of ice-cold methanolic HCl. The organic solution was concentrated under reduced pressure. The column chromatographic purification of crude mass on silica gel eluting with MeOH–EtOAc (1:9) provided the product 5a in 67% yield.
Representative experimental procedure for synthesis of 2,6-dimethyl-3-(ethoxycarbonyl)-5-phenyl-4-(3,4,5-trimethoxyphenyl)-pyridine (Scheme 4, entry 1) (8a). Monoadic 5a (1 mmol, 389 mg), AgOAc (1 mmol, 167 mg, 1 eq.) and K₂CO₃ (0.30 mmol, 41.5 mg, 0.3 eq.) were taken under nitrogen in an oven-dried sealed tube equipped with a rubber septum and magnetic bar. DMA (anhyd., 2 mL) was added under nitrogen. The tube was then sealed. The mixture was stirred at 140 °C. Upon completion of reaction as indicated by TLC (12 h), N-bromosuccinimide (1.1 mmol, 196 mg, 1.1 equiv.), p-TsOH (0.1 mmol, 17 mg, 0.1 eq.) and water (2 mL) were added in the reaction tube. After completion of the bromination reaction after 30 min, sodium carbonate (5 mmol, 530 mg, 5 eq.), tetrabutyl ammonium bromide (1 mmol, 322 mg, 1 eq.), phenylboronic acid (1.5 mmol, 182 mg, 1.5 eq.) and Pd(PPh₃)₄ (0.1 mmol, 115 mg, 1 eq.) were added to the reaction tube under nitrogen. The tube was then sealed. The mixture was stirred at 140 °C till the completion of reaction monitored by TLC (2 h). Then, resultant mixture was allowed to cool to room temperature, diluted with ethyl acetate (20 mL). The organic layer was washed with aqueous solution of ammonia (3 x 5 mL) and brine (5 mL), dried over Na₂SO₄ and the organic layer was concentrated under reduced pressure. The column chromatographic purification of crude mass on silica gel eluting with EtOAc–hexane (1:3) provided 2,6-dimethyl-3-(ethoxycarbonyl)-5-phenyl-4-(3,4,5-trimethoxyphenyl)pyridine 8a in 52% overall yield.

Products (8b–8t) were also prepared following this representative procedure.

**Diethyl 2,6-dimethyl-4-(3,4,5-trimethoxyphenyl)-1,4-dihydro-pyridine-3,5-dicarboxylate (3a).** Yellow solid; 293 mg, 70%; mp 140 °C; ¹H NMR (400 MHz, CDCl₃): δ 6.52 (s, 2H), 5.77 (s, 1 NH), 4.98 (s, 1H), 4.17–4.08 (m, 4H), 3.79 (s, 9H), 2.34 (s, 6H), 1.25 (t, J = 7.1 Hz, 3H) ppm; ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 167.7, 152.6, 143.9, 143.4, 105.0, 103.9, 60.8, 59.8, 55.9, 19.6, 14.4 ppm; IR (KBr): v max 3425, 2925, 1730, 1650, 1232, 1123, 1006 cm⁻¹.

**Diethyl 3,5-dicarboxylate (4a).** White solid; 375 mg, 90%; mp 130 °C; ¹H NMR (400 MHz, CDCl₃): δ 6.51 (s, 2H), 4.08 (q, J = 7.1 Hz, 4H), 3.86 (s, 3H), 3.83 (s, 6H), 2.60 (s, 6H), 0.99 (t, J = 7.1 Hz, 3H) ppm; ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 168.0, 155.4, 152.9, 145.7, 138.1, 131.9, 126.8, 105.5, 61.5, 50.9, 56.2, 22.8, 13.7 ppm; IR (KBr): v max 2987, 1719, 1585, 1232, 1123, 1006 cm⁻¹; HRMS (ESI) m/z: calc.d for C₂₃H₂₃NO₂ [M + H]⁺ 398.1866, found: 398.1858.

**2,6-Dimethyl-5-(ethoxycarbonyl)-4-(3,4,5-trimethoxyphenyl)nicotinic acid (5a).** Off white solid; 260 mg, 67%; mp charred at 225 °C; ¹H NMR (400 MHz, CDCl₃): δ 7.03 (s, 1H), 6.58 (s, 2H), 4.14 (q, J = 7.2 Hz, 2H), 3.88 (s, 3H), 3.87 (s, 6H), 2.60 (s, 3H), 2.59 (s, 3H), 1.06 (t, J = 7.2 Hz, 3H) ppm; ¹³C{¹H} NMR (100 MHz, CDCl₃): δ 169.2, 158.7, 155.0, 153.3, 148.2, 138.2, 134.3, 125.8, 120.8, 105.1, 61.4, 60.9, 56.2, 24.5, 22.8, 13.8 ppm; IR (KBr): v max 3423, 2970, 1718, 1582, 1249, 1122, 1010 cm⁻¹; HRMS (ESI) m/z: calc.d for C₂₆H₂₆NO₃ [M + H]⁺ 410.1933, found: 410.1957.
2.6-Dimethyl-3-(ethoxycarbonyl)-4-fluorophenyl-4-(3,4,5-trimethoxyphenyl)pyridine (8g). White solid; 250 mg, 55%; mp 130 °C; 1H NMR (400 MHz, CDCl3): δ 7.38–7.36 (m, 4H), 7.34 (s, 1H), 7.07 (d, J = 8 Hz, 2H), 6.71 (d, J = 7.3 Hz, 2H), 6.65 (s, 1H), 6.41 (s, 1H), 4.19–4.12 (m, 2H), 3.95 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.61 (s, 3H), 2.58 (s, 3H), 1.17 (t, J = 7.3 Hz, 3H) ppm; 13C(C)NMR (100 MHz, CDCl3): δ 167.8, 157.7, 155.3, 153.0, 147.1, 145.3, 143.1, 133.6, 128.7, 117.8, 117.7, 116.2, 116.1, 56.1, 24.2, 23.9, 13.3 ppm; IR (KBr): v max 3057, 2927, 2852, 1726, 1577, 1533, 1530, 1497, 1423, 137.4, 133.6, 133.1, 128.6 (q, J C-F = 32 Hz), 126.2, 124.2, 124.9, 124.2 (q, J C-F = 270 Hz), 122.4, 108.4, 61.1, 61.0, 56.1, 24.2, 23.1, 13.8 ppm; HRMS (EI) m/z: calcd for C28H26FNO4 [M + H]^+ 490.1841, found: 490.1841.

2.6-Dimethyl-3-(ethoxycarbonyl)-5-(3-hydroxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyridine (9.). White solid; 199 mg, 43%; mp 129 °C; 1H NMR (400 MHz, CDCl3): δ 7.84 (d, J = 6.9 Hz, 7H), 7.32 (dd, J = 7.7 Hz, J = 6.7 Hz 1H), 6.58 (s, 1H), 6.43 (s, 1H), 4.19–4.14 (m, 2H), 3.95 (s, 3H), 3.85 (s, 3H), 3.60 (s, 3H), 2.58 (s, 3H), 2.57 (s, 3H), 2.27 (s, 3H), 1.05 (t, J = 7.1 Hz, 3H) ppm; 13C(C)NMR (100 MHz, CDCl3): δ 198.0, 168.7, 157.8, 155.3, 152.8, 151.6, 148.0, 143.3, 141.1, 135.3, 133.5, 130.9, 127.7, 126.5, 126.3, 122.5, 108.4, 61.3, 61.1, 61.0, 56.1, 26.6, 24.3, 23.1, 13.8 ppm; IR (KBr): v max 2924, 2853, 1725, 1683, 1586, 1462, 1262, 1136, 1085, 1005 cm⁻¹; HRMS (EI) m/z: calcd for C27H28NO6 [M + H]^+ 464.2073, found: 464.2082.

2.6-Dimethyl-3-(ethoxycarbonyl)-5-(3-hydroxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyridine (9a). White solid; 217 mg, 48%; mp 119 °C; 1H NMR (400 MHz, CDCl3): δ 7.57 (d, J = 7.8 Hz, 2H), 7.05–7.00 (m, 1H), 6.75 (d, J = 8 Hz, 2H), 6.57 (d, J = 7.3 Hz, 2H), 6.50 (s, 1H), 6.41 (s, 1H), 4.18–4.12 (m, 2H), 3.95 (s, 3H), 3.85 (s, 3H), 3.61 (s, 3H), 2.58 (s, 3H), 2.27 (s, 3H), 1.05 (t, J = 7.1 Hz, 3H) ppm; 13C(C)NMR (100 MHz, CDCl3): δ 168.7, 157.8, 155.3, 152.6, 151.6, 148.0, 142.3, 134.5, 133.5, 132.1, 126.4, 126.3, 122.5, 120.4 (q, J C-F = 255 Hz), 120.1, 108.3, 61.2, 61.1, 56.1, 24.1, 23.1, 13.8 ppm; IR (KBr): v max 2924, 2850, 1725, 1586, 1484, 1251, 1161, 1082, 1005 cm⁻¹; HRMS (EI) m/z: calcd for C26H25FNO5 [M + H]^+ 456.1790, found: 456.1778.
61.1, 61.0, 56.1, 23.8, 22.6, 13.7 ppm; IR (KBr): \( v_{\text{max}} \) 3395, 2936, 2851, 1726, 1592, 1448, 1280, 1133, 1096 cm\(^{-1}\); HRMS (ESI) \( m/z \): 438.196; found: 438.1916.

2.6-Dimethyl-3-(ethoxycarbonyl)-5-(4-hydroxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyridine (8q). White solid; 203 mg, 42%; mp 107 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 8.48 (s, 1H), 7.65 (d, 1H, J = 1.5 Hz, 1H), 7.65 (d, 1H, J = 7.1 Hz, 1H), 7.53 (d, 1H, J = 6.7 Hz, 1H), 7.15 (d, \( J = 7.6 \) Hz, 1H, J = 4.9 Hz, 1H), 6.58 (s, 1H), 6.43 (s, 1H), 4.18–4.09 (m, 2H), 3.96 (s, 3H), 3.86 (s, 3H), 3.64 (s, 3H), 2.57 (s, 3H), 2.30 (s, 3H), 1.03 (t, \( J = 7.1 \) Hz, 3H) ppm; \(^13\)C{\(^1\)H} NMR (100 MHz, CDCl\(_3\)): \( \delta \) 168.8, 157.5, 155.5, 152.6, 151.7, 148.5, 142.2, 139.4, 134.9, 133.6, 130.8, 127.5, 126.4, 122.7, 108.2, 64.9, 61.2, 61.1, 60.9, 56.1, 24.2, 22.9, 13.8 ppm; IR (KBr): \( v_{\text{max}} \) 2978, 2936, 2840, 1725, 1586, 1501, 1255, 1087, 1015 cm\(^{-1}\); HRMS (ESI) \( m/z \): 452.2073 found: 452.2073.

Biology

Cell-based screening assay. Synthesized penta-substituted/functionalized-4,4-diarylated pyridine compounds 8a-8t used in this study were dissolved in 100% DMSO (cell culture grade, Himedia). The compounds were serially diluted in Dulbecco’s Modified Eagle (DMEM) cell culture medium to maintain the DMSO concentration less than 0.1% for analyzing its cell proliferation inhibiting potency in HeLa cell line. Briefly, 5 \times 10^4 cells were seeded in 96 well plate. After 16 h compounds (5 \( \mu \)M) were added and incubated with cells for 24 h. Subsequently, cells were fixed with TCA and processed for sulforhodamine B (SRB) assay.

Effects of diarylated pyridine compounds on in vitro tubulin assembly. Tubulin was purified from goat brain using 1 M glutamate as described earlier. TB tubulin concentration was determined by Bradford method. Purified tubulin (12 \( \mu \)M) in PEM buffer (25 mM PIPES pH 6.8, 3 mM MgCl\(_2\) and 1 mM EGTA) was incubated in the absence and presence of 20 \( \mu \)M of compounds (8b, 8f, 8j and 8p) for 10 min on ice and then, DMSO (final concentration 10%) and 1 mM GTP was added to the reaction mixtures. Subsequently, the assembly kinetics was monitored at 37 °C by 90° light scattering (350 nm) using SpectraMax M2. The extent of inhibition of polymerization was measured after 30 min of assembly. The light scattering data of only compounds (20 \( \mu \)M) were also recorded and subtracted from their respective data set. Three independent set of experiments were performed.

Acknowledgements

We gratefully acknowledge financial support from CSIR, New Delhi for this investigation. NH is thankful to DST, New Delhi for her DST-INSPIRE fellowship. DP is thankful to DAE, Government of India for DAE-SRC fellowship.

Notes and references


