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Design, Synthesis of Some New Scaffolds based on Pyrrolyl-Pyridines as Potential Anticancer Agents

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Pyrrolyl-pyridine heterocyclic compounds have received considerable attention because of its unique bioisosteric properties and an unusually wide spectrum of biological activities. Thus, it is a perfect framework for the synthesis of novel C–N, C–C bond formations like 5-substituted-1-benzyl-1*H*-pyrrolo[2,3-*b*]pyridines (**3**), 2-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)phenol (**5**) and screened for their anticancer activity. The synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, mass spectral techniques and elemental analysis. The outcomes of these compounds 2,6-difluorobenzyl-pyrrolidin-1*H*-pyrrolo[2,3-*b*]pyridine, 2,6-difluorobenzyl-*N*,*N*-dimethyl-1*H*-pyrrolo[2,3-*b*]-pyridin-5-amine had a signicant anticancer activity against human cervical cancer cell line (Hela) with IC_{50} 4.8, 9.7 µg/mL and whereas pyrrolo[2,3-*b*]pyridin-5-phenol, pyrrolo[2,3-*b*]pyridin-5-vinylphenol are active against human breast cancer cell line (MCF-7) with IC_{50} 8.1, 3.2 µg/mL, respectively.

Keywords: Azaindoles, Anticancer activity, C–C formation, C–N formation, Docking studies.

INTRODUCTION

Approximately 9 million deaths worldwide each year are attributed to cancer, which is defined as the uncontrolled, rapid, and abnormal growth of aberrant cells [1,2]. Despite significant advances in the discovery of anticancer medications, there are still numerous challenges to cancer treatment, such as high toxicity, low efficacy and drug resistance, all of which have had a significant impact on patients' normal lives [3]. As a result, current cancer research is focused on finding strong, safe anti-cancer agents with great selectivity [4]. The indole skeleton is one of the most attractive anti-tumor structures and is widely seen in active molecules and natural chemicals such as koumine, vinblastine, vincristine and topsentine [5].

Azaindoles, which have a pyrrole ring fused to a pyridine ring, are frequently employed as bioisoteres of indoles to increase anticancer drug discovery activities. The azaindole core, rather than other bicyclic fused heterocycles, can be employed to modify and finely adjust Lipinski's rule of five, solubility, pK_a and lipophilicity, target binding and ADME-toxic characteristics [6-9]. Azaindoles have been identified as favoured structures in the control of biological processes, medicinal chemistry and drug discovery programmes [10-12].

Improvements in C–C bond and C–heteroatom (C–N, C–O and C–P) condensed and coupling reactions could be a hot topic in modern organic and medicinal chemistry [13]. Agrochemicals, prescription medications and compounds of interest in material sciences are all synthesized *via* these processes. The pyrrolo-pyridine nucleus is an important class of organic chemistry that encompasses a number of pharmacologically active chemicals that can be generated in the lab or obtained naturally [14]. Pyridine ring is present in many natural products including vitamins such as niacins and vitamin B_6, alkaloids *e.g.* trigonelline and coenzymes *e.g.* nicotinamide adenine dinucleotide [15]. The pyrrolo-pyridines are present in the molecular structure of various biologically active compounds such as antalarmin,vemurafenib, peficitinib, pexidartinib, plexxikon, genentech famitinib, *etc*. [16]. They are displaying interesting biological profiles such as antitumor [17,18], antibacterial [19], antifungal [20], anti-TB [21], anti-inflammatory [22], antiproliferative [23], anti-Parkinson's [24], antiviral [25], muscarinic antagonist [26]. Considering this panorama, herein

we described the optimization of the synthetic route to obtain 1*H*-pyrrolo[2,3-*b*]pyridin-5-yl derivatives under microwave assisted synthesis as well conventional technique and evaluation of anticancer activity.

EXPERIMENTAL

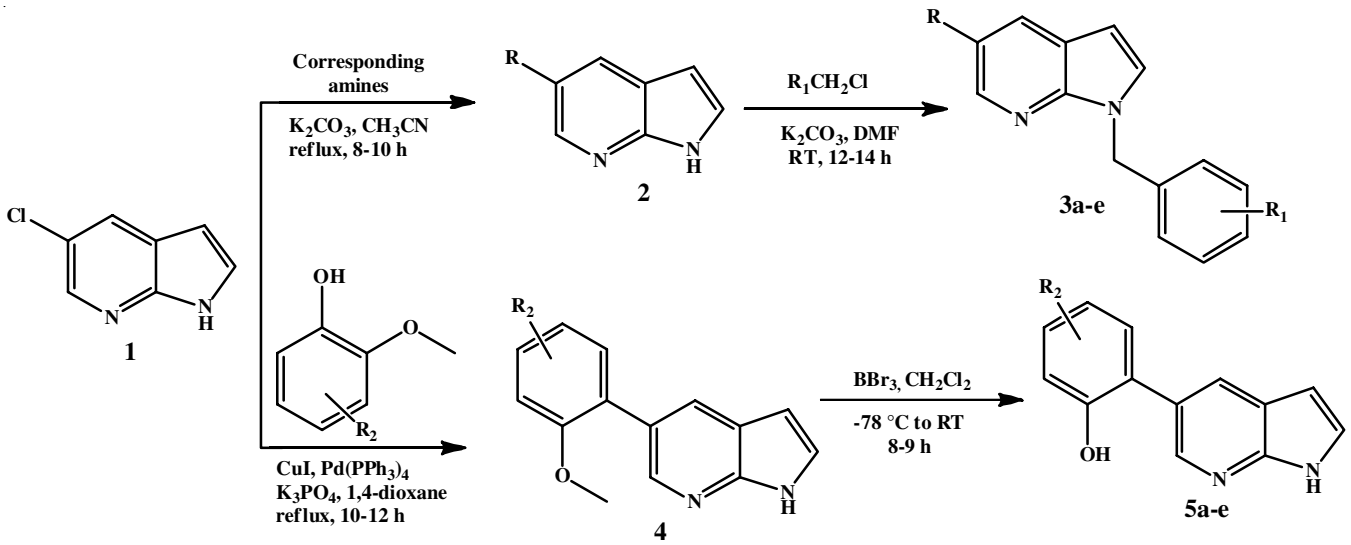
All the reactants, reagents and solvents were obtained from commercially available sources and were of analytical grade. IR (KBr) spectrum was recorded on a Perkin-Elmer 400 FT-IR spectrometer (ν_{max}, cm^{-1}) or a Varian 670-IR in the frequency range of 4000-600 cm^{-1}. Melting points were determined by the open capillary method and are uncorrected. ^1H NMR was recorded by using DMSO-d_6 solvent in the ranging 300-400 MHz and ^{13}C NMR was recorded by using DMSO-d_6 & $CDCl_3$ solvent in the ranging 100-125 MHz and TMS as an internal standard. The mass spectrum was recorded by using VG micro mass 70-70H instrument. The purity of these compounds was checked by TLC on silica gel and using *n*-hexane and ethyl acetate solvents. A monowave 300 mass 24 (Anton par) microwave was used to carry out the microwave reactions in 30 mL microwave process vials with temperature control by the infrared detection temperature sensor.

General procedure for the synthesis of 1-substituted benzyl-1*H*-pyrrolo[2,3-*b*]pyridine (3a-e): Under nitrogen atmosphere, 5-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (**1**) (2 mmol), corresponding amines (2 mmol), K_2CO_3 (0.5 mmol) were added to acetonitrile (20 mL) and the reaction mixture was heated to reflux for 8-10 h. The reaction was carefully monitored using TLC and quenched with ice at 0 ºC and the reaction was then extracted using ethyl acetate as solvent. The organic layer was removed using water followed by brine solution and solvent evaporation, yielding the final azaindole derivatives (**2**). Then compound **2** (2 mmol), substituted halides (2.4 mmol) and K_2CO_3 (1.5 mmol) were added in DMF (10 mL) and then the reaction mixture was stirred at room temperature for 12-14 h. After completion of reaction, it was diluted with water and extracted with ethyl acetate (2 × 25 mL), the crude product **3** was purified by column chromatography petroleum ether:ethyl acetate (9:1).

General procedure for the synthesis of substituted 2-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)phenol (5a-e): Compound 5-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (**1**, 1 mmol) was reacted with 2-substituted phenol (1 mmol) using Pd(*tetrakis*)triphenyl phosphene (0.2 mmol) as catalyst in the presence of Cu(I) and K_3PO_4 in 1,4-dioxane (10 mL) under refluxing conditions in seal-tube for 10-12 h to get 5-(2-methoxyphenyl)-1*H*-pyrrolo-[2,3-*b*]pyridine (**4**). Key intermediate **4** (10 mmol), tribromoborane (5 mmol) was dissolved in dichloromethane (15 mL) was added to the magnetically stirred solution at –78 ºC to room temperature for 8-9 h (**Scheme-I**). The progress of the reaction was monitored by TLC and after completion of the reaction, the mixture was poured into crushed ice and extracted with DCM (2 × 50 mL). Finally, 5-substituted azaindoles was purified by column chromatography using eluent petroleum ether:ethyl acetate (9:1).

Microwave irradiation: A mixture of substituted azaindoles (1 mmol) (**2**), aryl halides (1.2 mmol) were taken in 10 mL seamless pressure vial in a Microwave 300 operating system and heated at 100-120 ºC for 15-20 min. After completion of reaction, it was diluted with water and extracted with ethyl acetate (2 × 25 mL), the crude product was purified by column chromatography by using petroleum ether:ethyl acetate (9:1).

4-(1-(2,6-Difluorobenzyl)-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)morpholine (3a): Pale brown solid, m.p.: 176-178 ºC. ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 3.55 (t, 4H, *J* = 7.3 Hz, morpholine CH_2), 3.72 (t, 4H, *J* = 7.3 Hz, morpholine CH_2), 4.76 (s, 2H, NCH_2), 6.18 (d, 1H, *J* = 7.0 Hz, ArH), 7.04 (s, 1H, ArH), 7.23 (d, 2H, *J* = 7.0 Hz, ArH), 7.50 (d, 1H, *J* = 7.0 Hz, ArH), 7.85 (t, 1H, *J* = 7.5 Hz, ArH), 9.69 (s, 1H, pyridine-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 42.60 (NCH_2), 49.70 (NCH_2), 61.79 (OCH_2), 113.10 (pyrrole C), 114.08 (ArC), 118.88 (ArC), 119.10 (ArC), 119.62 (ArC), 119.77 (pyrrole C), 127.05 (ArC), 127.14 (pyridine C), 131.37 (pyridine C), 133.75 (pyridine C), 143.01 (ArC), 168.55 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3077.76 (=CH), 2918.48, 2847.19 (CH), 1564.24



Scheme-I: Designed strategy of azaindole derivatives

(C=C), 1443.22 (C=N), 1350.44 (C-N), 1105.27 (COC), 967.26 (CF). ESI (*m/z*) calcd. for $C_{18}H_{17}F_2N_3O$ $[M + 1]^+$: 330.21, found: 330.14.

4-(1-(4-Methoxybenzyl)-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)morpholine (3b): White crystalline solid, m.p.: 195-197 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.53 (t, 4H, *J* = 7.3 Hz, morpholine CH_2), 3.71 (t, 4H, *J* = 7.3 Hz, morpholine CH_2), 3.74 (s, 3H, OCH_3), 4.61 (s, 2H, NCH_2), 7.53 (d, 2H, *J* = 7.1 Hz, ArH), 7.64 d (2H, *J* = 7.2 Hz, ArH), 7.81 d (1H, *J* = 7.2 Hz, ArH), 7.92 (d, 1H, *J* = 7.1 Hz, ArH), 8.52 (s, 1H, ArH), 10.23 (s, 1H, pyridine-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 42.63 (morpholine C), 49.77 (OCH_3), 52.23 (NCH_2), 63.86 (morpholine C), 114.33 (pyrrole C), 115.02 (ArC), 121.10 (ArC), 122.98 (pyridine C), 124.10 (pyridine C), 126.27 (pyrrole C), 128.67 (ArC), 131.95 (ArC), 132.96 (pyridine C), 137.72 (pyridine C), 144.98 (ArC), 157.67 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3092.94 (=CH), 2923.62, 2856.06 (CH), 1563.05 (C=C), 1419.96 (C=N), 1250.19 (C-N), 1162.78 (COC), 1105.60 (COC). ESI (*m/z*) calcd. for $C_{19}H_{21}N_3O_2$ $[M + 1]^+$: 324.21, found: 324.06.

1-(2,6-Difluorobenzyl)-5-(pyrrolidin-1-yl)-1*H*-pyrrolo-[2,3-*b*]pyridine (3c): Pale brown solid, m.p.: 174-176 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.75 (t, 4H, *J* = 7.5 Hz, CH_2), 3.09 (t, 4H, *J* = 7.3 Hz, CH_2), 4.65 (s, 2H, NCH_2), 6.51 (d, 1H, *J* = 6.8 Hz, ArH), 7.08 (d, 1H, *J* = 7.5 Hz, ArH), 7.40 (d, 1H, *J* = 7.5 Hz, ArH), 7.68 (s, 1H, ArH), 7.75 (t, 1H, *J* = 7.0 Hz, ArH), 8.10 (d, 1H, *J* = 7.0 Hz, ArH), 8.70 (s, 1H, pyridine-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 23.56 (pyrrolidine C), 50.31 (NCH_2), 61.67 (pyrrolidine C), 114.27 (pyrrole C), 115.81 (ArC), 118.40 (ArC), 121.60 (ArC), 122.71 (pyridine C), 124.49 (pyridine C), 125.30 (pyrrole C), 134.37 (ArC), 138.37 (pyridine C), 154.63 (pyridine C), 161.64 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3074.24 (=CH), 2978.43, 2844.92 (CH), 1535.80 (C=C), 1448.23 (C=N), 1312.82 (C-N), 966.68 (CF). ESI (*m/z*) calcd. for $C_{18}H_{17}F_2N_3$ $[M + 1]^+$: 314.18, found: 314.21.

1-(4-Methoxybenzyl)-5-(pyrrolidin-1-yl)-1*H*-pyrrolo-[2,3-*b*]pyridine (3d): White crystalline solid, m.p.: 155-157 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.61 (t, 4H, *J* = 7.5 Hz, CH_2), 3.01 (t, 4H, *J* = 7.3 Hz, CH_2), 3.46 (s, 3H, OCH_3), 4.74 (s, 2H, NCH_2), 6.74 (d, 1H, *J* = 7.0 Hz, ArH), 7.16 (d, 2H, *J* = 7.2 Hz, ArH), 7.46 (d, 2H, *J* = 7.2 Hz, ArH), 7.73 (s, 1H, ArH), 7.75 (d, 2H, *J* = 7.2 Hz, ArH), 8.09 (d, 1H, *J* = 7.0 Hz, ArH), 8.75 (s, 1H, pyridine-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 23.56 (pyrrolidine C), 50.96 (pyrrolidine C), 52.80 (OCH_3), 60.51 (NCH_2), 115.02 (pyrrole C), 119.94 (ArC), 120.07 (pyridine C), 121.59 (ArC), 122.11 (pyridine C), 125.96 (ArC), 125.04 (ArC), 135.17 (pyridine C), 139.29 (pyridine C), 156.83 (pyridine C), 162.47 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3052.34 (=CH), 2961.10, 2897.49 (CH), 1571.08 (C=C), 1438.46 (C=N), 1318.31 (C-N), 957.69 (CF). ESI (*m/z*) calcd. for $C_{19}H_{21}N_3O$ $[M + 1]^+$: 308.16, found: 308.09.

1-(2,6-Difluorobenzyl)-*N*,*N*-dimethyl-1*H*-pyrrolo[2,3-*b*]-pyridin-5-amine (3e): Pale brown solid, m.p.: 130-132 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.78 (s, 6H, 2 × CH_3), 4.61 (s, 2H, CH_2), 6.77 (d, 1H, *J* = 7.2 Hz, ArH), 7.47 (s, 1H, ArH), 7.59 (m, 2H, ArH), 7.72 (s, 1H, ArH), 8.04 (d, 1H, *J* = 7.2 Hz, ArH), 8.49 (s, 1H, pyridine-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 43.45 (NCH_3), 50.82 (NCH_2), 114.70 (pyrrole C), 115.13 (ArC), 120.19 (ArC), 123.35 (pyridine C), 125.30 (ArC), 125.37 (pyrrole C), 127.37 (pyridine C), 130.18 (ArC), 136.05 (pyridine C), 138.94 (pyridine C), 148.03 (pyridine C), 162.40 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3045.41 (=CH), 2964.70 (CH), 1555.37 (C=C), 1430.18 (C=N), 1267.34 (C-N), 972.63 (CF). ESI (*m/z*) calcd. for $C_{16}H_{15}F_2N_3$ $[M + 1]^+$: 288.17, found: 288.07.

5-Methyl-2-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)phenol (5a): White crystalline solid, m.p.: 110-112 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.73 (s, 3H, CH_3), 5.64 (s, 1H, OH), 6.65 (d, 1H, *J* = 7.0 Hz, ArH), 7.34 (s, 1H, ArH), 7.60 (d, 1H, *J* = 7.3 Hz, ArH), 7.70 (s, 1H, ArH), 8.06 (d, 1H, *J* = 7.3 Hz, ArH), 8.17 (d, 1H, *J* = 7.0 Hz, ArH), 8.40 (s, 1H, pyridine-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 23.04 ($ArCH_3$), 110.43 (pyrrole C), 114.27 (ArC), 120.15 (pyridine C), 122.31 (pyridine C), 124.36 (ArC), 125.45 (ArC), 126.38 (pyrrole C), 130.45 (pyridine C), 135.05 (ArC), 138.30 (ArC), 149.76 (pyridine C), 160.20 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3420.15 (OH), 3228.35 (NH), 3046.22 (=CH), 2925.17 (CH), 1544.15 (C=C), 1432.79 (C=N). ESI (*m/z*) calcd. for $C_{14}H_{12}N_2O$ $[M + 1]^+$: 225.18, found: 225.10.

5-Ethyl-2-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)phenol (5b): White crystalline solid, m.p.: 115-117 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.73 (t, 3H, *J* = 7.8 Hz, CH_3), 3.10 (q, 2H, *J* = 9.2 Hz, CH_2), 5.10 (s, 2H, CH_2), 6.60 (d, 1H, *J* = 7.0 Hz, ArH), 7.30 (s, 1H, ArH), 7.61 (d, 1H, *J* = 7.2 Hz, ArH), 7.68 (s, 1H, ArH), 8.04 (d, 1H, *J* = 7.2 Hz, ArH), 8.14 (d, 1H, *J* = 7.0 Hz, ArH), 8.41 (s, 1H, pyridine-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 23.04 (CH_3), 27.31 (CH_2), 111.34 (pyrrole C), 114.10 (pyridine C), 120.16 (pyridine C), 122.25 (ArC), 124.37 (ArC), 125.49 (ArC), 126.17 (pyrrole C), 131.33 (ArC), 134.22 (pyridine C), 137.35 (pyridine C), 148.35 (ArC), 160.88 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3410.21 (OH), 3298.16 (NH), 3055.21 (=CH), 2925.30 (CH), 1605.01, 1536.30 (C=C), 1398.22 (C=N). ESI (*m/z*) calcd. for $C_{15}H_{14}N_2O$ $[M + 1]^+$: 239.15, found: 239.19.

2-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)-5-vinylphenol (5c): Light yellow solid, m.p.: 120-122 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.09 (d, 2H, *J* = 7.5 Hz, CH_2), 5.84 (d, 1H, *J* = 7.5 Hz, CH), 6.71 (s, 1H, OH), 7.24 (d, 3H, *J* = 6.5 Hz, ArH), 8.01 (d, 2H, *J* = 8.6 Hz, ArH), 8.32 (s, 1H, ArH), 9.06 (s, 1H, pyridine-H), 10.04 (brs, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 114.70 (pyrrole C), 115.13 (=CH_2), 120.17 (ArC), 120.29 (pyridine C), 121.16 (ArC), 122.15 (ArC), 125.94 (pyrrole C), 126.03 (ArC), 129.52 (ArC), 138.07 (=CH), 145.04 (pyridine C), 146.77 (pyridine C), 155.93 (pyridine C), 160.43 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3383.50 (OH), 3218.24 (NH), 3043.18 (=CH), 2923.54 (CH), 1507.18 (C=C), 1348.57 (C=N). ESI (*m/z*) calcd. for $C_{15}H_{12}N_2O$ $[M + 1]^+$: 237.16, found: 237.09.

2-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)phenol (5d): Pale brown solid, m.p.: 140-142 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.38 (s, 1H, OH), 6.44 (d, 1H, *J* = 7.0 Hz, ArH), 6.70 (d, 1H, *J* = 7.2 Hz, ArH), 7.22 (dd, 1H, *J* = 7.5, 3.2 Hz, ArH), 7.56 (dd, 1H, *J* = 7.8, 3.0 Hz, ArH), 8.01 (d, 2H, *J* = 8.0 Hz, ArH), 8.25 (s, 1H, ArH), 9.01 (s, 1H, pyridine-H), 9.69 (brs, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 114.37 (pyrrole C), 120.44 (ArC), 121.64 (pyridine C), 122.20 (pyridine C), 123.44 (ArC), 125.99 (pyrrole C), 126.25 (ArC), 129.33 (ArC), 137.10 (ArC), 144.37 (pyridine C), 154.66 (pyridine C), 160.28 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3408.40 (OH), 3326.20 (NH), 2934.24 (CH),

1545.05 (C=C), 1368.28 (C=N). ESI (*m/z*) calcd. for $C_{13}H_{10}N_2O$ $[M + 1]^+$: 211.12, found: 211.04.

4-(1*H*-Pyrrolo[2,3-*b*]pyridin-5-yl)benzene-1,3-diol (5e): Pale brown solid, m.p.: 133-135 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.32 (s, 1H, OH), 6.25 (d, 1H, *J* = 6.8 Hz, ArH), 6.68 (s, 1H, ArH), 7.12 (d, 1H, *J* = 6.8 Hz, ArH), 7.42 (d, 1H, *J* = 6.5 Hz, ArH), 7.57 (d, 1H, *J* = 6.5 Hz, ArH), 8.03 (s, 1H, ArH), 8.68 (s, 1H, ArH), 9.72 (s, 1H, pyridine-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 115.72 (pyrrole C), 119.33 (ArC), 120.14 (ArC), 121.30 (pyridine C), 122.16 (pyridine C), 125.95 (pyrrole C), 128.15 (ArC), 130.40 (ArC), 137.86 (ArC), 146.75 (pyridine C), 148.47 (pyridine C), 160.39 (ArC), 162.43 (ArC). IR (KBr, ν_{max}, cm^{-1}): 3420.14 (OH), 2346.11 (NH), 2925.43 (CH), 1605.21 (C=C), 1376.17 (C=N). ESI (*m/z*) calcd. for $C_{13}H_{10}N_2O_2$ $[M + 1]^+$: 227.24, found 227.12.

Anticancer activity: The anticancer activity of the final compounds was determined using the ATCC procedure [27] and the MTT colorimetric assay. The most common human breast cancer cell line (MCF-7) as well as cervical cancer cell line (Hela), was obtained from the National Centre for Cell Science, Pune, India. The P388 leukaemia cancer cell line was grown in DMEM media with 10% neonatal calf serum (NBCS), 1% non-essential amino acids, 1% sodium pyruvate, 0.2% $NaHCO_3$ and 1% antibiotic combination (10,000 U penicillin and 10 mg streptomycin per mL). The MCF-7 breast cancer cell line was grown in RPMI-1640 media with 10% NBCS, 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mM glutamine. Cell lines were kept at 37 ºC in a humidified 5% CO_2 incubator and processed by first trypsinizing adherent cells and then centrifuging to obtain cell pellets. Fresh media was added to pellets for cell counting on a haemocyto-meter and 100 µL of media was dispensed in a 96-well plate with cells ranging from 5,000 to 6,000 per well. The plate was placed in a CO_2 incubator overnight to allow the cells to adhere and restore their shape. Cells were treated with 25 µM solutions of the test compounds in the medium after 24 h. The cells were cultured for 48 h to see if the test chemicals had any effect on the cell lines. To deduct even more from 48 h data, a zero-hour reading with untreated cells and a control with 1% DMSO were taken. The cells were treated with MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide diluted in PBS (5 mg/mL) and incubated for 3–4 h at 37 ºC after a 48 h incubation period. The formazan crystals were then dissolved in 100 µL of DMSO and the viability was determined using a Spectra Max multimode reader at 540 nm.

RESULTS AND DISCUSSION

A reaction between 5-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (**1**), corresponding amines and K_2CO_3 in acetonitrile were heated for 8-10 h to give 5-(pyrrolidine/morpholine)-1*H*-pyrrolo[2,3-*b*]-pyridines (**2**) in good yields. Further to obtain compound **2**, substituted halides were added in presence of K_2CO_3 in DMF at room temperature for 12-14 h in excellent yields and the derived analogues **3** was purified by column chromatography using petroleum ether:ethyl acetate (9:1) as solvent. Whereas another series of azaindoles have been synthesized by using the reaction between 5-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (**1**) with substituted phenol in the presence of $Pd(PPh_3)_4$, Cu(I) and K_3PO_4 in 1,4-dioxane under refluxing conditions for 10-12 h to get 5-(2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (**4**). Moreover, key intermediate **4** was treated with tribromoborane in dichloromethane as a solvent under room temperature for 8-9 h to desired final 5-substituted azaindoles **5** (**Scheme-I**).

The microwave irradiation technique has been offering a standard shift in the target synthesis due to their peculiar direct 'in core' heating of the reactions thus decreasing the reaction times [28]. Moreover, it offered myriad advantages such as acceleration of sluggish transformation, higher yields and environmentally greener conditions. Under the non classical reaction rates are faster than classical heating conditions, for example, the synthesis of azaindoles (**5**) were obtained in 12 h by conventional method whereas, under microwave conditions, it was acquired in 15 min. Reaction between 5-substituted azaindoles **2** and aromatic halides, K_2CO_3 in DMF, whereas another series of substituted azaindoles with BBr_3 in DCM were taken in a closed vessel and irradiated under the microwave at 70-120 ºC for 15-20 min. All the reactions were performed in the presence of 80-90 watts of microwave irradiation.

Moreover, we exploited the developed methodology to synthesize a novel series of simplied heterocyclic analogues designed by changing the azaindoles attached substituted rings in order to investigate the stereo-electronic and the lipophilic inuences at this position on the sedative role of pyrrolo-pyridine scaffolds. On the other hand, the exploitation of successive molecular simplications on the structure of sedative prototype (**3a**) led us to the design of the morpholine azaindole **2a** and 2,6-difluorobenzene as shown in Table-1.

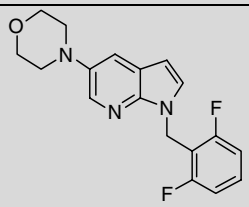
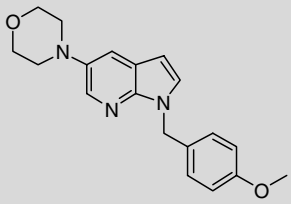
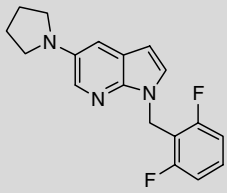
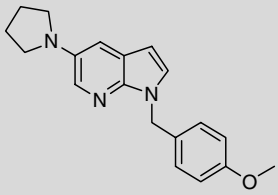
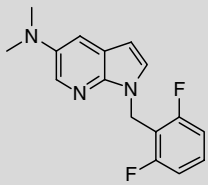
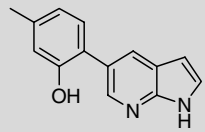
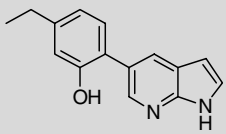
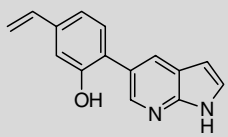
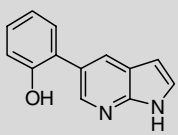
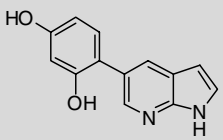
TABLE-1
OPTIMIZATION OF REACTION CONDITIONS FOR THE SYNTHESIS OF AZAINDOLE DERIVATIVE **3a**

Entry	Solvent	Base	Mol (%)	Temp./time	Yield (%)[a]
1	ACN	K_2CO_3	1	RT/11 h	66
2	*t*-BuOH	K_2CO_3	1	RT/12 h	72
3	DMSO	K_2CO_3	1	RT/11 h	67
4	EtOH	K_2CO_3	1	RT/12 h	76
5	DMF	K_2CO_3	1	RT/12 h	84
6	–	K_2CO_3	1	70 °C/15 min	70[b]
7	–	K_2CO_3	1	80 °C/15 min	74[b]
8	–	K_2CO_3	1	90 °C/15 min	82[b]
9	–	K_2CO_3	1	100 °C/15 min	95[b]
10	–	K_2CO_3	1	120 °C/15 min	89[b]

[a]Isolated yield, [b]Under microwave

However, adding 1 mol% K_2CO_3 to the reaction under room temperature conditions in acetonitrile after 11 h yielded the desired product in 66% yield (Table-1, entry 1). Another model reaction produced the required product **3a** in 72% yields, under 1 mol% K_2CO_3, *tert.*-butanol at ambient temperature (Table-1, entry 2). In order to reduce reaction time, an attempt was made to implement the framework reaction conditions in DMSO at room temperature, which resulted in a 67% decrease in product yield (Table-1, entry 3). However, further increment

TABLE-2
ANTICANCER ACTIVITY RESULTS (μg/mL) OF THE SYNTHESIZED PYRROLYL-PYRIDINES

Entry	Products	Hela (IC_{50})	MCF-7 (IC_{50})	Convention time/yield	Microwave time/yield
3a		14.6	3.8	12 h/84%	15 min/95%
3b		ND	23.1	12 h/86%	15 min/97%
3c		4.8	10.7	12.5 h/75%	17 min/86%
3d		19.2	5.0	11.5 h/70%	15 min/81%
3e		9.7	18.3	11 h/65%	15 min/74%
5a		3.8	8.1	9.5 h/80%	17 min/89%
5b		6.9	ND	9 h/82%	20 min/92%
5c		8.0	3.2	10 h/82%	17 min/94%
5d		ND	24.8	9.5 h/70%	16 min/81%
5e		15.7	ND	9 h/75%	15 min/93%
DXN	–	5.0	5.0	–	–

ND = IC_{50} > 50 μg/mL.

of time from 11 h to 12 h led to increasing in yields (76%) in the presence of 1 mol% K_2CO_3, ethanol employing as a solvent (Table-1, entry 4). Despite the superior yield, product **3a** was synthesized by 1 mol% K_2CO_3 in DMF to obtained 84% yield (Table-1, entry 5). In order to minimize the reaction time, further an attempt to carryout out the model reaction in the microwave reactor using sealed vessels under fast conventional heating at 70 ºC was successful, resulting in the desired product **3a** in 70% yield of within 15 min (Table-1, entry 6). Further optimizing of the reaction conditions, it was found that under neat reaction the conditions at 80 ºC the product yield improved up to 74% (Table-1, entry 7). Whereas the reaction was found in the reaction conditions at 100 ºC, the desired compound give excellent yields up to 95% (Table-1, entry 9). However, further increment of temperature from 100 ºC to 120 ºC led to decrease in yield (Table-1, entries 10). Despite the good yield, product **3a** was isolated without using column chromatography purification process as sole product. After completion of the reaction, water, ethyl acetate was added to the reaction mixture and the precipitated solid was filtered and washed with ethanol to give the pure 4-(1-(2,6-difluorobenzyl)-1*H*-pyrrolo[2,3-*b*]-pyridin-5-yl)morpholine (**3a**).

Anticancer activity: The anticancer activity of the synthesized compounds was assessed *in vitro* by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against Hela and MCF-7 cell lines. The percentage of cell death was measured for the novel indole linked to oxadiazoles (**3** and **5**) exhibited higher anticancer activity against at various concentrations along with IC_{50} values (Table-2). Compounds **3c**, **3e**, **5a** and **5c** showed excellent anticancer activity against both Hela, MCF-7 cell lines. From screening results, the synthesized compounds displayed promising activity, whereas **3c**, **3e** exhibited the highest cytotoxicity against Hela cell line with IC_{50} 4.8, 9.7 µg/mL and active against MCF-7 cell line with IC_{50} 10.7, 18.3 µg/mL values respectively. Compounds **3a**, **3d** with difluorobenzyl indole morpholine, methoxybenzyl indole elicited good potent activity against MCF-7 cell line with IC_{50} value 14.6, 19.2 µg/mL, respectively. Compounds **5a** and **5c** displayed excellent anticancer activity against MCF-7 cell line with IC_{50} value of 8.1, 3.2 µg/mL where as these indoles revelas the superiar anticancer activity against Hela cell line along with IC_{50} value of 3.8, 8.0 µg/mL, respectively. Morover, compounds **5b**, **5d** and **5e** show the moderate to good anticancer activity against both the cell lines with IC_{50} values range 6.9-24.8 µg/mL, respectively.

Conclusion

Carbon-carbon, carbon-heteroatom forms the 'spine' of the organic molecule and is essentially the critical transformation in organic synthesis to line up the carbon backbone of organic particles. Final azaindoles **3c**, **3e**, **5a** and **5c** displayed promising anticancer activity against Hela, MCF-7 cell lines and prepared scaffolds are most active against both the cell lines. These synthetic methodologies provide a controlled, modular and facile access to azaindole scaolds with high eciency, broad substrate scope and excellent functional group compatibility.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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