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Regioselective N–alkylation of 2– (3,4–dimethoxyphenyl)imidazo [4,5–b] and [4,5–c]pyridine oxide derivatives : Synthesis and structure elucidation by NMR

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ABSTRACT

Imidazopyridines can exist in several tautomeric forms such as benzimidazole or purine condensed systems. Regioselectivities were determined for *N*-alkylations of 2-(3,4-dimethoxyphenyl)- imidazopyridines and their 4 and 5-oxides (**2**–**4**, **13**, **14**) with *n*-butyl and 4-fluorobenzyl bromides under basic conditions (K_2CO_3 in DMF). It was observed that *N*-4 **(5**–**8)** and *N*-5 **(15**–**17)** regioisomers were mainly formed. Compounds **7** (N^4) and **7a** (N^1) were separated from the mixtures of regioisomers in a 50 : 1 ratio. Their structural assignments were made with the use of two-dimensional ^{1}H-^{1}H NOE (nuclear overhauser effect spectroscopy [NOESY]) enhancements between the *N*-CH_2 and protons on the C-4, 5, 6, and 7 positions of the pyridine moiety. To verify the NOESY data, synthesis of compounds **7a** and **7b** was achieved by the selective method. Complementary structural information was provided by 2D-HMBC spectra of the compounds.

1. Introduction

Imidazopyridines are attractive hetereocycles for medicinal chemists as their derivatives have been shown to exhibit several biological activities. In 2017, a review reported the pharmacological profile of imidazopyridines with their anti-tumoral, anti-microbial, anti-inflammatory, anti-diabetic, anti-hypertensive, and other pharmacological properties [1]. In addition, the use of imidazopyridines in some neurodegenerative disorders [2], as well as their anti-lipidemic [3] and antifungal [4,5] activities, has recently been reported.

Imidazopyridines are comprised of an imidazole ring fused with a pyridine moiety and have various isomeric forms such as imidazo [4,5-*b*]pyridines, imidazo[4,5-*c*]pyridines, imidazo[1,5-*a*] pyridines, and imidazo[1,2-*a*]pyridines. The NH group present in imidazopyridines is strongly acidic and weakly basic. This group exhibits fast prototrophic tautomerism, which leads to equilibrium mixtures. All three possible structures and numbering of imidazopyridines are depicted in Schemes 1 and 4 for compounds **2**–**4**, **13**, and **14**. It was reported that [6], in the gas phase, 3*H* forms are more stable than other forms. Existence of this tautomerism in imidazo[4,5-*b*]pyridines [7,8], imidazo [4,5-*c*]pyridine [9], and imidazo[4,5-*b*]pyridine-4-oxide [10,11] has been shown with spectral data, mainly nuclear magnetic resonance (NMR) spectroscopy. This migration is not observed when the imidazole hydrogen is replaced by other substituents such as an alkyl group or under special circumstances in which hydrogen migration is affected by inter- and/or intramolecular hydrogen bonding [7,9,12].

In our previous studies [13,14], we reported the synthesis of some regioisomers of benzimidazoles and their structural elucidation was achieved by selective synthesis and/or 2D-NMR data. As a further contribution to this field, here we report the synthesis of a series of 2-(3,4-dimethoxyphenyl)imidazopyridines and their oxides with n-butyl and 4-fluorobenzyl groups on the $N^{4,5,3,1}$-positions and their tautomeric behaviours. Following isolation of the pure regiosomers, ^{1}H-^{1}H NOE, nuclear overhauser effect spectroscopy (NOESY), and heteronuclear multiple bond correlation (HMBC) experiments were used for structural elucidations.

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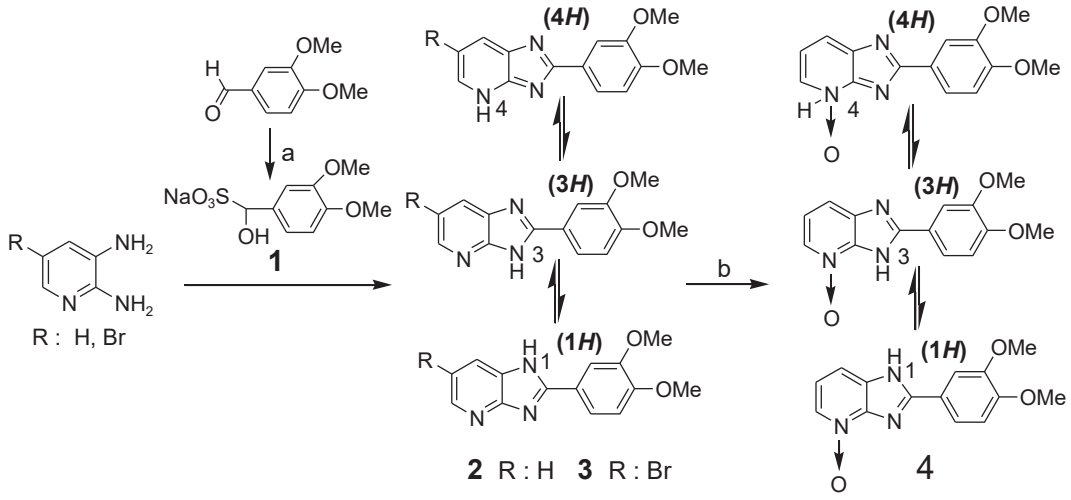


Scheme 1. Synthesis of **2**–**4** and their tautomeric forms.

2. Experimental

Uncorrected melting points were measured using a Büchi B-540 capillary melting point apparatus. All NMR experiments were carried out using VARIAN (Agilent) MERCURY 400 MHz (Varian, Palo Alto, CA, USA) at a proton resonance frequency of 400 and 100 MHz for carbon. The NMR spectrum optimisation was conducted using Agilent VnmrJ version 3.2 revision A software and all parameters were set in it. The samples (5–15 mg) were prepared in 0.7 ml of DMSO-d_6, $CDCl_3$, and CD_3OD. TMS was used as an internal standart. Because of the tautomeric effect of the imidazole ring, NMR spectra of compounds **4** and **14** did not give satisfactory results. To eliminate the tautomeric effect, the related imidazopyridines were dissolved in DMSO-d_6, followed by the addition of a tiny amount of dry NaH and 2–3 drops of D_2O to the NMR tube. This effectively eliminated the tautomeric effect and produced satisfactory results. The liquid chromatography mass spectrometry (LC-MS) spectra were taken on a Waters Micromass ZQ connected with Waters Alliance HPLC (Waters Corporation, Milford, MA, USA), using the ESI(+) method with a C-18 column (XTerra®, 4.6 × 250 mm, 5 μm). Analytical conditions of mass spectrometry were as follows: capillary voltage, 3.11 kV; cone voltage, 29V; source temperature, 100 °C; and desolvation temperature, 300 °C. Compounds **1**–**3**, **13** [15] and **11**–**12** [16], were prepared according to the literature methods. The HCl salt of compounds **5** and **15** were prepared using methanolic HCl.

2.1. 2-(3,4-Dimethoxyphenyl)-1,(3),(4)H-imidazo [4,5-b]pyridine 4-oxide (4)

To a stirred solution of **2** (2 mmol, 0.510 g) in acetic acid (2.5 mL), hydrogen peroxide (35%, 1 mL) was slowly added. The solution was warmed to 70 °C for 2 h and allowed to stir at room temperature (RT) overnight. The reaction mixture was diluted with water and the resulting precipitate was collected by filtration, washed with water, and dried. Crystallisation from MeOH yielded **4**, a white coloured powder, with melting point (mp) > 295 °C and yield 0.325 g (60%). **^{1}H-NMR** (DMSO-d_6) δ ppm: 3.85 (s, 3H, OC$\underline{H}_3$), 3.87 (s, 3H, OC$\underline{H}_3$), 7.15–7.19 (m, 2H), 7.53 (br.s, 1H), 7.79 (br.s, 2H), 8.14 (d, 1H, J = 6Hz); **^{13}C-NMR** (DMSO-d_6): 152.5, 151.2, 148.9, 123.6, 121.1, 120.2, 118.5, 111.9, 110.1, 55.6, and 55.5. **^{1}H-NMR** (DMSO-d_6 + NaH + D_2O) δ ppm: 3.77 (s, 3H, 4′-OC$\underline{H}_3$), 3.84 (s, 3H, 3′-OC$\underline{H}_3$), 6.83 (dd, 1H, J = 7.2 and 6 Hz, H-6), 6.94 (d, 1H, J = 8.4 Hz, H-5′) 7.49 (d, 1H, J = 7.6 and 1.2 Hz, H-7), 7.81 (dd, 1H, J = 6.4 and 0.8 Hz, H-5), 7.86 (d, 1H, J = 2Hz, H-2′), 7.88 (dd, 1H, J = 5.6 and 1.6 Hz, H-5); **COSY**: [H-5': H-6'], [H-6: H-7], [H-6: H-5]; **NOESY:** [H-5': 4′-OCH_3], [H-2': 3′-OCH_3]; **^{13}C-NMR, HSQC**, and **HMBC** (DMSO-d_6 + NaH + D_2O) δ ppm: 162.9 (C-2), 150.7 (C-3a), 149.6 (C-4′), 148.75 (C-3′), 143.6 (C-7a), 128.9 (C-1′), 128.75 (C-5H), 120.4 (C-2′H), 116.8 (C-7H), 113.6 (C-6H), 111.8 (C-5′H), 111.1 (C-6′H), 55.92, and 55.9(O$\underline{C}$H$_3$) (See Supp. Info. pg 1–3); **MS** (ESI+) m/z: 272 (M + H, %100), $C_{14}H_{13}N_3O_3$.

2.2. 4-Butyl-2-(3,4-dimethoxyphenyl)-4H-imidazo[4,5-b]pyridine HCl (5)

Only the *N*-4 regioisomer was obtained. K_2CO_3 (0.1105 g, 0.8 mmol) was added to a suspension of **2** (0.127 g, 0.5 mmol) in DMF (1 mL) and stirred. After 1 h, butyl bromide (0.082 g, 0.6 mmol) was added. After overnight stirring at 35 °C, water was added, CH_2Cl_2 extraction was performed, and solvent was evaporated. The residue was subjected to silica gel column chromatography and eluted with CH_2Cl_2: MeOH (97: 3–5) gradient, oily pure **5** was obtained, which was converted to HCl salt, mp 130–138 °C (bubbling), hygroscopic, yield 0.12 g (41%). **^{1}H-NMR** (CD_3OD) δ ppm: 1.04 (t, 3H), 1.47 (m, 2H), 2.11 (m, 2H), 3.94 (s, 3H, 4′-OC$\underline{H}_3$), 3.97 (s, 3H, 3′-OC$\underline{H}_3$), 4.91 (t, 2H), 7.17 (d, 1H, J = 8.4 Hz, H-5′), 7.68 (dd, 1H, J = 8 and 6.4 Hz, H-6), 7.86 (d, 1H, J = 1.6 Hz, H-2′), 7.95 (dd, 1H, J = 8.4 and 2 Hz, H-6′), 8.5 (dd, 1H, J = 8 and 0.8 Hz, H-7), 8.63 (dd, 1H, J = 6 and 0.8 Hz, H-5); **COSY**: [H-5': H-6'], [H-6: H-7], [H-6: H-5]; [*N*-3 butyl group as expected]; **NOESY**: [H-5': 4′-OCH_3], [H-2': 3′-OCH_3]; [N-C$\underline{H}_2$-: H-5]; **^{13}C-NMR**, **HSQC**, and **HMBC** (CD_3OD) δ ppm: 161.14 (C-2), 155.2 (C-4′), 151.2 (C-3′), 151.14 (C-3a), 138 (C-5H), 134.1 (C-7a), 127.7 (C-7H), 123.9 (C-6′H), 120.8 (C-1′), 119.6 (C-6H), 113.1 (C-5′H), 112.3 (C-2′H), 56.8 and 56.7 (O$\underline{C}$H$_3$), 55.6 (N-$\underline{C}$H$_2$), 33 (N-CH$_2\underline{C}$H$_2$), 20.7 (N-CH$_2$CH$_2\underline{C}$H$_2$), 13.8 (N … -$\underline{C}$H$_3$) (See Supp. Info. pg 4–9); **MS** (ESI+) m/z: 312 (M+H, %100), $C_{18}H_{21}N_3O_2 \cdot HCl$.

2.3. General synthesis of compounds 6–8

K_2CO_3 (0.138 g, 1 mmol) was added to a suspension of **2**–**4** (0.8 mmol) in DMF (1 mL) and stirred. After 1 h, 4-fluorobenzyl bromide (0.15 g, 0.8 mmol) was added. After overnight stirring at 35 °C, water was added and the precipitate was filtered; if precipitation did not occur, it was extracted with CH_2Cl_2.

2.4. 2-(3,4-Dimethoxyphenyl)-3-(4-fluorobenzyl)-3H-imidazo[4,5-b]pyridine (6b) and 2-(3,4-Dimethoxyphenyl)-4-(4-fluorobenzyl)-4H-imidazo [4,5-b]pyridine (6) from 2

N-3 and *N-4* regioisomers were obtained. The crude precipitated powder mixture was purified by column chromatography using CH_2Cl_2: MeOH (95:5) as eluant to give the following products in the following order of elution.

Compound 6b (first eluting fraction): mp, 153–155 °C, yield 0.010 g (2.7%). ***Selective synthesis method* for 6b**: The mixture of **12** (0.434 g, 2 mmol) and $Na_2S_2O_5$ adduct of 3,4-dimethoxybenzaldehyde (0.542 g, 2 mmol) in DMF (1.5 mL) was heated at 120 °C for 3 h. The reaction mixture was cooled, water was added, and the resulting precipitate was collected by filtration and crystallised from EtOH: water, mp 154–156 °C, yield 0.47 g (64.5%). **^{1}H-NMR** ($CDCl_3$) δ ppm: 3.76 (s, 3H, 3′-OC*H*3), 3.93 (s, 3H, 4′-OC*H*3), 5.58 (s, 2H, N-CH_2), 6.92 (d, 1H, J = 8 Hz, H-5′), 6.99 (t, 2H, J = 8.8 Hz, H-,3″,5″), 7.1–7.14 (m, 2H, H-2″,6″), 7.2 (d, 1H, J = 1.6 Hz, H-2′), 7.23 (dd, 1H, J = 7.6 and 2 Hz, H-6′), 7.27 (dd, 1H, J = 8.4 and 4.8 Hz, H-6), 8.1 (dd, 1H, J = 8.4 and 1.6 Hz, H-7), 8.54 (dd, 1H, J = 4.8 and 1.2 Hz, H-5); COSY: [H-5': H-6'], [H-6: H-7], [H-6: H-5], [H-2″6'': H-3″,5'']; **NOESY**: [H-5': 4′-OCH_3], [H-2': 3′-OCH_3]; [N-CH_2-: H-2'], [N-CH_2-: H-2″,6'']; **^{13}C-NMR**, **HSQC** and **HMBC** ($CDCl_3$) δ ppm: 162.2 (d, J = 245 Hz, C-4″), 154.8 (C-2), 150.9 (C-4′), 149.2 (C-3′), 148.9 (C-3a), 144.1 (C-5H), 135.0 (C-7a), 132.7 (d, J = 3.2 Hz, C-1″), 128.2 (d, J = 7.7 Hz, C-2″,6″H), 127.1 (C-7H), 122.1 (C-1′), 122.0 (C-6′H), 118.95 (C-6H), 115.8 (d, J = 21 Hz, C-3″,5″H), 112.1 (C-2′H), 111 (C-5′H), 55.98 (4′-OCH3), 55.8 (3′-OCH3), 46.3 (N-CH_2) (See Supp. Info. pg 24–30); **MS** (ESI+) *m/z*: 364 (M+H, %100), $C_{21}H_{18}FN_3O_2$. **6b** prepared by the selective methods was found to be identical in analytical and spectral properties to the product **(6b)** obtained during the synthesis reaction of **6.**

Compound 6 (second eluting fraction): Hygroscopic, yellow coloured powder, mp 80–83 °C, yield 0.15 g (51.7%); **^{1}H-NMR** (DMSO-d_6) δ ppm: 3.84 (s, 3H, 4′-OC*H*$_3$), 3.88 (s, 3H, 3′-OC*H*$_3$), 5.87 (s, 2H, N-CH_2), 7.08 (d, 1H, J = 8.4 Hz, H-5′), 7.15–7.22 (m, 3H, H-3″, 5″, 6 overlapped), 7.64 (m, 2H, H-2″, 6″), 7.94 (d, 1H, J = 1.6 Hz, H-2′), 7.98 (dd, 1H, J = 8.4 and 2 Hz, H-6′), 8.12 (d, 1H, J = 7.6 Hz, H-7), 8.23 (d, 1H, J = 6.4 Hz, H-5); **COSY**: [H-5': H-6'], [H-6: H-7], [H-6: H-5]; **NOESY**: [H-5': 4′-OCH_3], [H-2': 3′-OCH_3]; [N-C*H*$_2$-: H-5], [N-C*H*$_2$-: H-2″, 6'']; **^{13}C-NMR**, **HSQC**, and **HMBC** (DMSO-d_6) δ ppm: 168 (C-2), 162 (d, J = 243 Hz, C-4″), 153.8 (C-3a), 150.4 (C-4′), 148.6 (C-3′), 145.1 (C-7a), 132.1 (d, J = 3.2 Hz, C-1″), 130.7 (d, J = 9 Hz, C-2″, 6″H), 130.4 (C-5H), 127.3 (C-1′), 126.6 (C-7H), 120.8 (C-6′H), 115.5 (d, J = 21 Hz, C-3″, 5″H), 112.8 (C-6H), 111.6 (C-5′H), 110.7 (C-2′H), 55.5 (4′-O$\underline{C}$H$_3$), 55.4 (3′-O$\underline{C}$H$_3$), 54.9 (N-$\underline{C}$H$_2$) (See Supp. Info. pg 10–15); **MS** (ESI+) *m/z*: 364 (M+H, %100), $C_{21}H_{18}FN_3O_2$.

2.5. 2-(3,4-Dimethoxyphenyl)-4-(4-fluorobenzyl)-4H-imidazo [4,5-b]pyridine 4-oxide (7) and 2-(3,4-Dimethoxyphenyl)-1-(4-fluorobenzyl)-1H-imidazo [4,5-b]pyridine 4-oxide (7a) from 4

N-4 and *N-1* regioisomers were obtained. Crystallisation of the resulting precipitate from EtOH: H_2O (50%) gave pure **compound 7**, mp 80–84 °C, yield 0.14 g (73%). **^{1}H-NMR** (DMSO-d_6) δ ppm: 3.85 (s, 3H, 4′-OC*H*$_3$), 3.89 (s, 3H, 3′-OC*H*$_3$), 5.73 (s, 2H, N-CH_2), 7.08 (t, 1H, J = 6.8 Hz, H-6), 7.11 (d, 1H, J = 8.4 Hz, H-5′), 7.23–7.28 (m, 2H, H-3″, 5″), 7.59–7.62 (m, 2H, H-2″, 6″), 7.96 (d, 1H, J = 1.6 Hz, H-2′), 8.01 (dd, 1H, J = 8.4 and 1.6 Hz, H-6′), 8.35 (d, 1H, J = 8Hz, H-7), 8.85 (d, 1H, J = 6.8 Hz, H-5); **COSY**: [H-5': H-6'], [H-3″, 5'': H-2″, 6''], [H-6: H-7], [H-6: H-5]; **NOESY**: [H-5': 4′-OC*H*$_3$], [H-2': 3′-OC*H*$_3$]; [N-C*H*$_2$-: H-5 (weak)], [N-C*H*$_2$-: H-2″, 6'']; **^{13}C-NMR**, **HSQC**, and **HMBC** (DMSO-d_6) δ ppm: 168.4 (C-2), 162.7 (d, J = 245 Hz, C-4″), 150.6 (C-4′), 149.4 (C-3a), 148.6 (C-3′), 148.0 (C-7a), 132.4 (d, J = 9 Hz, C-2″, 6″-H), 129.6 (d, J = 2.6 Hz, C-1″), 128.2 (C-5H), 126.9 (C-1′), 126.2 (C-7H), 121.1 (C-6′H), 115.5 (d, J = 22 Hz, C-3″, 5″H), 112 (C-6H), 111.6 (C-5′H), 110.7 (C-2′H), 79.1 (N-$\underline{C}$H$_2$), 55.5 (4′-O$\underline{C}$H$_3$), 55.4 (3′-O$\underline{C}$H$_3$) (See Supp. Info. pg 31–37); **MS** (ESI+) *m/z*: 380 (M+H, %100), $C_{21}H_{18}FN_3O_3$.

After precipitation of **7**, the supernatant liquid was evaporated and the residue was purified by column chromatography (CH_2Cl_2: MeOH, 93:7) gave the pure **compound 7a** (0.006 g). ***Selective synthesis method for 7a:*** To a stirred solution of **6a** (0.19 g, 0.5 mmol) in acetic acid (1 mL), hydrogen peroxide (35%, 0.1 mL) was slowly added. The solution was warmed to 40 °C by stirring for 36 h. The reaction mixture was diluted with water, partly neutralised with dilute K_2CO_3 solution, extracted with CH_2Cl_2, and evaporated. The residue was subjected to silica gel column chromatography. First, unreacted starting material was removed by eluting with EtOAc: MeOH (100:2), then desired **compound 7a** was obtained by the mixture of dichloromethane: MeOH (93:7) as eluant, mp 169–172 °C, yield 0.095 g (47.3%). **^{1}H-NMR** (DMSO-d_6) δ ppm: 3.71 (s, 3H, 3′-OC*H*$_3$), 3.83 (s, 3H, 4′-OC*H*$_3$), 5.65 (s, 2H, N-CH_2), 7.09–7.18 (m, 5H, H-5′,3″,5″,2″,6″, overlapped), 7.2 (dd, 1H, J = 6.4 and 8 Hz, H-6), 7.28 (d, 1H, J = 2 Hz, H-2′), 8.01 (dd, 1H, J = 8.4 and 2 Hz, H-6′), 7.55 (d, 1H, J = 8 Hz, H-7), 8.2 (d, 1H, J = 6.4 Hz, H-5); **COSY**: [H-5': H-6'], [H-6: H-7], [H-6: H-5]; **NOESY**: [H-5': 4′-OCH_3], [H-2': 3′-OCH_3]; [N-C*H*$_2$-: H-7], [N-C*H*$_2$-: H-2'], [N-C*H*$_2$-: H-2″,6'']; **^{13}C-NMR**, **HSQC**, and **HMBC** (DMSO-d_6) δ ppm: 161.4 (d, J = 242 Hz, C-4″), 153.9 (C-2), 150.8 (C-4′), 148.7 (C-3′), 146.0 (C-3a), 133.4 (C-5H), 132.7 (C-7a), 132.3 (d, J = 3.2 Hz, C-1″), 128.2 (d, J = 8.3 Hz, C-2″,6″H), 122.1 (C-6′H), 120.6 (C-1′), 118.9 (C-6H), 115.7 (d, J = 21 Hz, C-3″,5″H), 112.3 (C-2′H), 111.8 (C-5′H), 109.3 (C-7H), 55.6 (4′-O$\underline{C}$H$_3$), 55.4 (3′-O$\underline{C}$H$_3$), 47.7 (N-$\underline{C}$H$_2$) (See Supp. Info. pg 38–44); **MS** (ESI+) *m/z*: 380 (M+H, %100), $C_{21}H_{18}FN_3O_3$. **7a** prepared by the selective method was found to be identical in analytical and spectral properties to the product **(7a)** obtained during the synthesis reaction of **7.**

2.6. 3-(4-Fluorobenzylidene)amino]pyridin-2-amine (9)

To a suspension of 2,3-diaminopyridine (0.36 g, 3.3 mmol) in THF (33 mL), dried molecular sieves (2 g, 4A°) and 4-fluorobenzaldehyde (0.5 g, 4 mmol) were added. After refluxing for 4 h, the mixture was stirred overnight. The reaction mixture was filtered and concentrated, the residue was purified by silica gel column chromatography, and eluted with n-hexane: EtOAc (60 : 40–100) gradient, mp 128–132 °C, yield 0.30 g (50%). **^{1}H-NMR** ($CDCl_3$) δ ppm: 5.23 (br s, 2H, N$\underline{H}_2$), 6.66 (dd, 1H, J = 5.6 and 5.2 Hz, H-5), 7.18 (t, 2H, J = 8 Hz, H-3′,5′), 7.23 (d, 1H, J = 8 Hz, H-4), 7.88–7.94 (m, 3H, H-2′,6′,6), 8.48 (s, 1H, N = CH); **NOESY**: [N = C*H*:H-4] [N = C*H*:H-2′,6']; **^{13}C-NMR** ($CDCl_3$) δ ppm: 164.9 (d, J = 251 Hz), 157.9, 154.7, 145.7, 132.3 (d, J = 3.1 Hz), 132.1, 130.7 (d, J = 9 Hz), 123.5, 116.0 (d, J = 22 Hz), 113.9 (See Supp. Info. pg 64–68); **MS** (ESI+) *m/z*: 216 (M+H, %100), $C_{12}H_{10}FN_3$.

2.7. N^3-(4-Fluorobenzyl)pyridine-2,3-diamine (10)

Compound **9** (0.27 g, 1.26 mmol) was dissolved in ethanol (15 mL) and treated with sodium borohydride (0.3 g, 7.93 mmol). After refluxing for 8 h, water was added, and a light yellow coloured powder was filtered, dried and used for the next step without crystallisation; yield 0.25 g (91.6%). **^{1}H-NMR** ($CDCl_3$) δ ppm: 3.58 (br.t, 1H, N*H*-CH_2), 4.24–4.26 (m, 4H, NH-C*H*$_2$ and 2-N*H*$_2$), 6.66 (dd, 1H, J = 7.6 and 5.2 Hz, H-5), 6.78 (dd, 1H, J = 7.6 and 1.2 Hz, H-4), 7.04 (t, 2H, *J* = 8.4 Hz, H-3′,5′), 7.26–7.35 (m, 2H, J = 8.4 and 5.6 Hz, H-2′,6′), 7.61 (dd, 1H, J = 5.2 and 1.6 Hz, H-6), The values are consistent with literature [17]; **NOESY**: [NH-C*H*$_2$:H-4] [NH-C*H*$_2$:H-2′,6′]; **^{13}C-NMR** ($CDCl_3$) δ ppm: 162.17 (d, *J* = 244 Hz), 148.8, 137.12, 134.1 (d, *J* = 2.6 Hz), 131.7, 129.2 (d, *J* = 8.4 Hz), 117.6, 115.9, 115.5 (d, *J* = 21 Hz), 47.5 (See Supp. Info. pg 69–74); **MS** (ESI+) *m*/*z*: 218 (M+H, %100), $C_{12}H_{12}FN_3$.

2.8. 2-(3,4-Dimethoxyphenyl)-1-(4-fluorobenzyl)-1H-imidazo[4,5-b]pyridine (6a)

A mixture of **10** (0.218 g, 1 mmol) and the $Na_2S_2O_5$ adduct of 3,4-dimethoxybenzaldehyde (0.27 g, 1 mmol) in DMF (1 mL) was heated at 120 °C for 2.5 h. The solvent was evaporated and the oily residue was purified with column chromatography using EtOAc-MeOH (100:1.5) as eluant, mp 151–153 °C, yield 0.21 g (57.8%). **^{1}H-NMR** (benzene-d_6, 15 °C) δ ppm: 3.24 (s, 3H, 3′-OC*H*$_3$), 3.28 (s, 3H, 4′-OC*H*$_3$), 4.7 (s, 2H, N-CH_2), 6.34 (d, 1H, *J* = 8 Hz, H-5′), 6.43–6.47 (m, 2H, H-2″,6″), 6.65 (t, 2H, H-3″,5″), 6.68 (dd, 1H, J = 4.8 and 8 Hz, H-6), 6.76 (dd, 1H, J = 1.6 and 8 Hz, H-7), 7.17 (dd, 1H, J = 8.4 and 1.6 Hz, H-6′), 7.34 (d, 1H, *J* = 1.6 Hz, H-2′), 8.64 (dd, 1H, J = 4.8 and 1.6 Hz, H-5); **COSY**: [H-5':H-6'], [H-6:H-7], [H-6:H-5]; **NOESY**: [H-5':4′-OC*H*$_3$], [H-2':3′-OC*H*$_3$]; [N-C*H*$_2$-:H-7], [N-C*H*$_2$-:H-6'], [N-C*H*$_2$-:H-2″,6"]; **^{13}C-NMR**, **HSQC**, and **HMBC** (benzene-d_6, 15 °C) δ ppm: 162.3 (d, *J* = 244 Hz, C-4″), 157.6 (C-3a), 156.6 (C-2), 152.1 (C-4′), 150.5 (C-3′), 145.9 (C-5H), 132.8 (d, *J* = 3.2 Hz, C-1″), 129.35 (C-7a), 127.9 (d, *J* = 8.3 Hz, C-2″,6″H), 122.8 (C-1′), 122.2 (C-6′H), 118.1 (C-6H), 117.8 (C-7H), 116.5 (d, *J* = 21.8 Hz, C-3″,5″H), 113.5 (C-2′H), 111.8 (C-5′H), 55.77 (4′-O$\underline{C}$H$_3$), 55.56 (3′-O$\underline{C}$H$_3$), 48.0(N-$\underline{C}$H$_2$) (See Supp. Info. pg 16–23); **MS** (ESI+) *m*/*z*: 364 (M+H, %100), $C_{21}H_{18}FN_3O_2$.

2.9. 2-(3,4-Dimethoxyphenyl)-3-(4-fluorobenzyl)-3H-imidazo[4,5-b]pyridine 4-oxide (7b)

To a stirred solution of **6b** (0.19 g, 0.52 mmol) in acetic acid (1 mL), hydrogen peroxide (35%, 0.3 mL) was slowly added. The solution was warmed to 40 °C by stirring overnight. The reaction mixture was diluted with water, extracted with CH_2Cl_2, and concentrated. The residue was purified by column chromatography with CH_2Cl_2: MeOH (100:2–5 gradient), mp 105–110 °C, yield 0.08 g (40%). **^{1}H-NMR** (DMSO-d_6) δ ppm: 3.68 (s, 3H, 3′-OC*H*$_3$), 3.83 (s, 3H, 4′-OC*H*$_3$), 6.1 (s, 2H, *N*-CH_2), 6.98–7.02 (m, 2H, H-2″,6″), 7.045–7.18 (m, 3H, H-5′,3″,5″), 7.15 (d, 1H, *J* = 1.6 Hz, H-2′), 7.25 (dd, 1H, J = 8.4 and 2 Hz, H-6′), 7.4 (dd, 1H, J = 6.4 and 6.8 Hz, H-6), 7.76 (d, 1H, *J* = 7.2 Hz, H-7), 8.21 (d, 1H, *J* = 6.4 Hz, H-5); **COSY**: [H-5':H-6'], [H-6:H-7], [H-6:H-5]; **NOESY**: [H-5':4′-OC*H*$_3$], [H-2':3′-OC*H*$_3$], [N-C*H*$_2$-:H-2'], [N-C*H*$_2$-:H-2″,6"]; **^{13}C-NMR**, **HSQC**, and **HMBC** (DMSO-d_6) δ ppm: 161.2 (d, *J* = 243 Hz, C-4″), 155.01 (C-2), 150.7 (C-4′), 148.6 (C-3′), 139.3 (C-7a), 137.6 (C-3a), 134.6 (C-5H), 134.19 (d, *J* = 3.2 Hz,C-1″), 128.4 (d, *J* = 8.3 Hz, C-2″,6″H), 122.4 (C-6′H), 120.8 (C-1′), 119.4 (C-6H), 118-3 (C-7H), 115.3 (d, *J* = 23 Hz, C-3″,5″H), 112.5 (C-2′H), 111.7 (C-5′H), 55.6 (4′-O$\underline{C}$H$_3$), 55.4 (3′-O$\underline{C}$H$_3$), 48.4 (N-$\underline{C}$H$_2$) (See Supp. Info. pg 45–51); **MS** (ESI+) *m*/*z*: 380 (M+H, %100), $C_{21}H_{18}FN_3O_3$.

2.10. 6-Bromo-2-(3,4-dimethoxyphenyl)-3-(4-fluorobenzyl)-3H-imidazo[4,5-b]pyridine (8a) and 6-bromo-2-(3,4-dimethoxyphenyl)-4-(4-fluorobenzyl)-4H-imidazo [4,5-b]pyridine (8) from 3

N^3 and N^4 regioisomers were obtained. Following the addition of water to the reaction medium, the aqueous solution was extracted with CH_2Cl_2 and concentrated. The crude mixture was subjected to chromatography (CH_2Cl_2: MeOH, 96:4) to give the following products in the following order of elution.

Compound 8a (first eluting fraction): mp 192–194 °C, yield 0.022 g (6.2%). **^{1}H-NMR** ($CDCl_3$) δ ppm: 3.77 (s, 3H, 3′-OC*H*$_3$), 3.94 (s, 3H, 4′-OC*H*$_3$), 5.54 (s, 2H, N-CH_2), 6.93 (d, 1H, *J* = 8 Hz, H-5′), 6.99 (t, 2H, H-3″,5″), 7.08–7.12 (m, 2H, H-2″,6″), 7.19 (d, 1H, H-2′), 7.21 (dd, 1H, J = 7.2 and 1.6 Hz, H-6′), 8.2 (d, 1H, *J* = 2 Hz, H-7), 8.42 (d, 1H, *J* = 2 Hz, H-5); **COSY**: [H-5':H-6'], [H-3″,5'': H-2″,6"], [H-5:H-7 (secondary)]; **NOESY:** [H-5':4′-OC*H*$_3$], [H-2':3′-OC*H*$_3$]; [N-C*H*$_2$-: H-2'], [N-C*H*$_2$-:H-2″,6"]; **^{13}C-NMR**, **HSQC**, and **HMBC** ($CDCl_3$) δ ppm: 162.3 (d, *J* = 245 Hz, C-4″), 156 (C-2), 151.2 (C-4′), 149.3 (C-3′), 147.6 (C-3a), 144.7 (C-5H), 136.2 (C-7a), 132.3 (d, *J* = 3.2 Hz, C-1″), 129.4 (C-7H), 128.1 (d, *J* = 7.7 Hz, C-2″,6″H), 122.1 (C-6′H), 121.7 (C-1′), 115.9 (d, *J* = 21 Hz, C-3″,5″H), 114.4 (C-6), 112 (C-2′H), 111 (C-5′H), 56 (4′-O$\underline{C}$H$_3$), 55.8 (3′-O$\underline{C}$H$_3$), 46.5 (N-$\underline{C}$H$_2$) (See Supp. Info. pg 58–63); **MS** (ESI+) *m*/*z*: 442 (M+H, %100), 444 (M + H + 2, %100) $C_{21}H_{17}BrFN_3O_2$.

Compound 8 (second eluting fraction): mp 172–174 °C, yield 0.092 g (26%), light yellow coloured powder. **^{1}H-NMR** (DMSO-d_6) δ ppm: 3.84 (s, 3H, 4′-OC*H*$_3$), 3.87 (s, 3H, 3′-OC*H*$_3$), 5.83 (s, 2H, N-CH_2), 7.0 (d, 1H, *J* = 8 Hz, H-5′), 7.2 (t, 2H, H-3″,5″), 7.67–7.71 (m, 2H, H-2″,6″), 7.92 (d, 1H, H-2′), 7.99 (dd, 1H, J = 8 and 1.6 Hz, H-6′), 8.3 (d, 1H, *J* = 1.6 Hz, H-7), 8.42 (d, 1H, *J* = 1.6 Hz, H-5); **COSY**: [H-5':H-6'], [H-3″,5'':H-2″,6"], [H-5:H-7 (secondary)]; **NOESY**: [H-5':4′-OC*H*$_3$], [H-2':3′-OC*H*$_3$]; [N-C*H*$_2$-:H-5], [N-C*H*$_2$-:H-2″,6"]; **^{13}C-NMR**, **HSQC**, and **HMBC** (DMSO-d_6) δ ppm: 169.8 (C-2), 162.1 (d, *J* = 243 Hz, C-4″), 153.3 (C-3a), 150.8 (C-4′), 148.7 (C-3′), 146.1 (C-7a), 131.7 (d, *J* = 3 Hz, C-1″), 131 (d, *J* = 9.2 Hz, C-2″,6″H), 130.1 (C-5H), 128.6 (C-7H), 126.7 (C-1′H), 121.3 (C-6′H), 115.5 (d, *J* = 21 Hz, C-3″,5″H), 111.6 (C-5′H), 110.8 (C-2′H), 105.1 (C-6), 55.6 (4′-O$\underline{C}$H$_3$), 55.5 (3′-O$\underline{C}$H$_3$), 55.3 (N-$\underline{C}$H$_2$) (See Supp. Info. pg 52–57); **MS** (ESI+) *m*/*z*: 442 (M + H, %100), 444 (M + H + 2, %100) $C_{21}H_{17}BrFN_3O_2$.

2.11. 2-(3,4-Dimethoxyphenyl)-1,(3),(5)H-imidazo[4,5-c]pyridine 5-oxide (14)

This compound was prepared as described in **4,** starting from **13** (2 mmol, 0.510 g). The powder precipitate was purified by silica gel column chromatography. First, unreacted **13** was collected by a mixture of CH_2Cl_2: EtOH: NH_3 (100:20:0.5), and pure compound **14** was obtained by a mixture of CH_2Cl_2: EtOH: NH_3 (100:20:1) as eluant, mp > 295 °C, yield 0.17 g (31%). **^{1}H-NMR** (DMSO-d_6) δ ppm: 3.83 (s, 3H, O$\underline{C}$H$_3$), 3.86 (s, 3H, O$\underline{C}$H$_3$), 7.15 (d, 1H, *J* = 8.4 Hz, H-5′), 7.54 (br.d, 1H), 7.7–7.75 (m, 2H), 7.99 (dd, 1H, J = 6.8 and 1.2 Hz), 8.6 (br.s, 1H); **^{13}C-NMR** (DMSO-d_6): 151.11, 148.9, 133.9, 121.2, 120.0, 111.9, 110.0, 55.6, and 55.59. **^{1}H-NMR** (DMSO-d_6 +NaH+D_2O) δ ppm: 3.81 (s, 3H, 4′-OC*H*$_3$), 3.86 (s, 3H, 3′-OC*H*$_3$), 7.0 (d, 1H, *J* = 8.4 Hz, H-5′), 7.36 (d, 1H, *J* = 8.4 Hz, H-7), 7.71 (dd, 1H, J = 6.4 and 1.6 Hz, 1H,H-6), 7.82 (dd, 1H, J = 8.4 and 2 Hz, 1H, H-6′), 7.89 (d, 1H, *J* = 2 Hz, H-2′), 8.36 (d, 1H, *J* = 2 Hz, H-4); **COSY**: [H-5':H-6'], [H-6:H-7], [H-6:H-4 (secondary)]; **NOESY**: [H-5':4′-OC*H*$_3$], [H-2':3′-OC*H*$_3$];

^{13}C-NMR, **HSQC**, and **HMBC** (DMSO-d_6 + NaH + D_2O) δ ppm: 166.6 (C-2), 149.5 (C-4′), 148.8 (C-3′), 147.4 (C-7a), 144.9 (C-3a), 129.2 (C-1′), 128.8 (C-6H), 126.9 (C-4H), 120.2 (C-6′H), 111.9 (C-5′H), 111.5 (C-7H), 111.1 (C-2′H), 55.92 (4′-OCH_3), 55.8 (3′-OCH_3) (See Supp. Info. pg 75–77); **MS** (ESI+) *m/z*: 272 (M + H, %100), $C_{14}H_{13}N_3O_3$.

2.12. 5-Butyl-2-(3,4-dimethoxyphenyl)-5H-imidazo[4,5-c]pyridine HCl (15)

Only the *N*-5 regioisomer was obtained. K_2CO_3 (0.1105 g, 0.8 mmol) was added to a suspension of **13** (0.127 g, 0.5 mmol) in DMF (0.5 mL) and stirred. After 1 h, butyl bromide (0.082 g, 0.6 mmol) was added. After overnight stirring at 35 °C, water was added, crystallisation of the precipitate from MeOH: H_2O gave pure **15**, mp 95–97 °C, yield 0.1 g (64.5%), mp of HCl salt, 200–205 °C (bubbling): **^{1}H-NMR** (CD_3OD) δ ppm: 1.03 (t, 3H), 1.46 (m, 2H), 2.06 (m, 2H), 3.94 (s, 3H, 4′-OCH_3), 3.97 (s, 3H, 3′-OCH_3), 4.69 (t, 2H), 7.18 (d, 1H, *J* = 8.4 Hz, H-5′), 7.84 (d, 1H, *J* = 1.6 Hz, H-2′), 7.87 (dd, 1H, J = 8.4 and 2 Hz, H-6′), 8.08 (d, 1H, *J* = 6.8 Hz, H-7), 8.66 (d, 1H, *J* = 6.8 Hz, H-6), 9.34 (s, 1H, H-4); **COSY**: [H-5':H-6'], [H-6:H-7], [H-6:H-4 (secondary)]; [*N*-5 butyl group as expected]; **NOESY**: [H-5':4′-OCH_3], [H-2':3′-OCH_3]; [N-CH_2-:H-4 and 6]; **^{13}C-NMR**, **HSQC**, and **HMBC** (CD_3OD) δ ppm: 161.9 (C-2), 155 (C-4′), 151.2 (C-3′), 147.6 (C-7a), 140.1 (C-3a), 138.6 (C-6), 135.3 (C-4H), 123.5 (C-6′H), 120.2 (C-1′), 113.1 (C-5′H), 112.5 (C-7H), 112.2 (C-2′H), 62.4 (N-CH_2), 56.9 and 56.8 (OCH_3), 34.9 (N-CH_2CH_2), 20.6 (N-$CH_2CH_2CH_2$), 13.95 (N …-CH_3) (See Supp. Info. pg 78–83); **MS** (ESI+) *m/z*: 312 (M + H, % 100), $C_{18}H_{21}N_3O_2 \cdot HCl$.

2.13. 2-(3,4-Dimethoxyphenyl)-5-(4-fluorobenzyl)-5H-imidazo [4,5-c]pyridine (16)

Only the N^5 regioisomer was obtained. K_2CO_3 (0.1105 g, 0.8 mmol) was added to a suspension of **13** (0.127 g, 0.5 mmol) in DMF (0.5 mL) and stirred. After 1 h, p-fluorobenzyl bromide (0.6 mmol) was added. After overnight stirring at 35 °C, water was added, and the crude powder was purified by crystallisation with EtOH: H_2O (50%) to give **16**, mp 77–80 °C (bubbling), hygroscopic, yield 0.14 g (77.7%). **^{1}H-NMR** (DMSO-d_6) δ ppm: 3.82 (s, 3H, 4′-OCH_3), 3.86 (s, 3H, 3′-OCH_3), 5.63 (s, 2H, N-CH_2), 7.05 (d, 1H, *J* = 9.2 Hz, H-5′), 7.25 (t, 2H, *J* = 8.8 Hz, H-3″,5″), 7.535–7.57 (m, 2H, H-2″,6″), 7.68 (d, 1H, *J* = 6.8 Hz, H-7), 7.94–7.96 (m, 2H, H-2′,6′, overlapped), 8.16 (dd, 1H, J = 7.2 and 1.6 Hz, H-6), 9.02 (d, 1H, *J* = 1.6 Hz, H-4); **COSY**: [H-5':H-6'], [H-3″,5":H-2″,6"], [H-6:H-7], [H-6: H-4 (secondary)]; **NOESY**: [H-5':4′-OCH_3], [H-2':3′-OCH_3]; [N-CH_2-:H-4], [N-CH_2-:H-6], [N-CH_2-:H-2″,6"]; **^{13}C-NMR**, **HSQC**, and **HMBC** (DMSO-d_6) δ ppm: 171.44 (C-2), 162.3 (d, *J* = 243 Hz, C-4″), 155.9 (C-7a), 150.0 (C-4′), 148.5 (C-3′), 145.6 (C-3a), 132.8 (d, *J* = 2.6 Hz, C-1″), 130.7 (C-6H), 130.45 (C-4H), 130.4 (d, *J* = 8.4 Hz, C-2″,6″H), 127.8 (C-1′), 120.6 (C-6′H), 115.8 (d, *J* = 21 Hz, C-3″,5″H), 111.8 (C-7H), 111.5 (C-5′H), 110.7 (C-2′H), 60.2 (N-CH_2), 55.5 (4′-OCH_3), 55.3 (3′-OCH_3) (See Supp. Info. pg 84–89); **MS** (ESI+) *m/z*: 364 (M + H, %100), $C_{21}H_{18}FN_3O_2$.

2.14. 2-(3,4-Dimethoxyphenyl)-5-(4-fluorobenzyl)-5H-imidazo [4,5-c]pyridine 5-oxide (17)

This compound was prepared as described in **16**, starting from **14** (0.135 g, 0.5 mmol), mp 165–167 °C, yield 0.155g, 81%. **^{1}H-NMR** (DMSO-d_6) δ ppm: 3.82 (s,3H, 4′-OCH_3), 3.86 (s,3H,3′-OCH_3), 5.54 (s, 2H, N-CH_2), 7.05 (d, 1H,*J* = 8.4Hz,H-5′), 7.26 (t, 2H, *J* = 8.8 Hz, H-3″,5″), 7.56–7.6 (m, 2H, H-2″,6″), 7.65 (d, 1H, *J* = 6.8 Hz, H-7), 7.93–7.96 (m,2H, H-2′,6′, overlapped), 8.3 (dd, 1H, J = 7.2 and 2.4 Hz, H-6), 9.16 (d,1H,*J* = 2 Hz, H-4); **COSY**: [H-5': H-6'], [H-3″,5": H-2″,6"], [H-6: H-7], [H-6: H-4(secondary)]; **NOESY:** [H-5': 4′-OCH_3], [H-2': 3′-OCH_3]; [N-CH_2-: H-4], [N-CH_2-: H-6], [N-CH_2-: H-2″,6"]; **^{13}C-NMR, HSQC** and **HMBC** (DMSO-d_6) δ ppm: 172.3 (C-2), 165.4 (d, *J* = 244Hz, C-4″), 155.45 (C-7a), 150.2 (C-4′), 148.5 (C-3′), 144.6 (C-3a), 132.4 (d, *J* = 9 Hz, C-2″,6″H), 129.1 (d, *J* = 2.6Hz, C-1″), 127.8 (C-1′), 127.6 (C-6H), 127.3 (C-4H), 120.6 (C-6′H), 115.6 (d, *J* = 21 Hz, C-3″,5″H), 111.5(C-5′H), 110.7(C-2′H), 110.5(C-7H), 81.4(N-CH_2), 55.5 (4′-OCH_3), 55.3(3′-OCH_3) (See Supp. Info. pg 90–95); **MS** (ESI+) *m/z*: 380 (M+H, %100), $C_{21}H_{18}FN_3O_3$.

2.15. 2-(3,4-Dimethoxyphenyl)-5-(4-fluorobenzyl)(4-^{2}H)-5H-imidazo[4,5-c]pyridine 5-oxide (17a)

0.015g of **17** was kept in room temperature in 0.8 ml of CD_3OD for 24 h and solvent evaporated. **^{1}H-NMR** (CD_3OD) δ ppm: 3.89 (s, 3H, 4′-OCH_3), 3.94 (s, 3H, 3′-OCH_3), 5.5 2 (s, 2H, N-CH_2), 7.06 (d,1H,*J* = 8.8 Hz, H-5′), 7.13 (t,2H,*J* = 8.8Hz,H-3″,5″), 7.45–7.49 (m,2H,H-2″,6″), 7.64 (d, 1H, *J* = 7.2 Hz, H-7), 7.89–7.93 (m, 2H, H-2′,6′, overlapped), 8.3 (d, 1H, *J* = 7.2 Hz, H-6); **COSY**: [H-5': H-6'], [H-3″,5": H-2″,6"], [H-6: H-7]; **NOESY**: [H-5': 4′-OCH_3], [H-2': 3′-OCH_3]; [N-CH_2-: H-6], [N-CH_2-: H-2″,6"]; **^{13}C-NMR**, **HSQC** and **HMBC** (CD_3OD) δ ppm: 173.8 (C-2), 165.4 (d, *J* = 246 Hz, C-4″), 156.4 (C-7a), 152.7 (C-4′), 150.6 (C-3′), 144.95 (C-3a), 133.7(d, *J* = 9 Hz, C-2″,6″H), 130.2 (C-6H), 130.1 (d, *J* = 3.2Hz, C-1″), 128.6 (t, *J* = 29 Hz,C-4D), 127.6 (C-1′H), 122.9 (C-6′H), 116.9 (d, *J* = 22Hz, C-3″,5″H), 112.8 (C-2′H), 112.1 (C-5′H), 83.7 (C-7H), 83.9 (N-CH_2), 56.55 (4′-OCH_3), 56.53 (3′-OCH_3) (See Supp. Info. pg 96–100); **MS** (ESI+) *m/z*: 381(M+H, %100), $C_{21}H_{17}DFN_3O_3$.

3. Results and discussion

Compounds **1**–**17** were prepared using the methods outlined in Schemes 1 and 4. Cyclisation of 2,3 or 3,4-diaminopyridines with the sodyum metabisulfite adduct of 3,4-dimethoxybenzaldehyde (**1**) gave the corresponding imidazopyridines **2**, **3**, and **13**. In this condensed system, the nitrogen bears a hydrogen atom ($N^{1,3}$) that behaves as a pyrrole-like *N*-atom; the other ($N^{4,5}$) resembles a pyridine-like *N*-atom. The hydrogen atom attached to the nitrogen in the 1-position readily tautomerise as 1*H*, 3*H*, 4*H*, and 5*H* as in compounds **2**, **3**, and **13** in Schemes 1 and 4. Because of these tautomeric forms, both ^{1}H and ^{13}C NMR spectra of unsubstituted imidazopyridines cannot be clear unless hexamethylphosphoramide-d_{18} (HMPA-d_{18}) [18] is used as the deuterated solvent. The *N*-4 and *N*-5 oxide derivatives (**4** and **14**) were prepared from **2** and **13** using H_2O_2 [10,11].

In this study, the ^{1}H and ^{13}C NMR signals of **4** and **14** were assigned by inspection on the basis of chemical shifts and coupling constants with and without tautomerism. Initially, their NMR spectra were run under normal conditions in DMSO-d_6, followed by the elimination of tautomerism (by adding a tiny amount of dry NaH and 2–3 drops of D_2O). In tautomeric forms, some proton signals appeared as broad peaks in their ^{1}H NMR spectra and some carbon peaks disappeared in their ^{13}C NMR spectra, whereas in non-tautomeric forms, all of the protons signals with expected splitting patterns and carbon atoms including C3a/C7a, C4/C7, and C5/C6 were observed with sharp peaks. Hence, very fine NMR assignments were determined using both 1D and 2D NMR techniques.

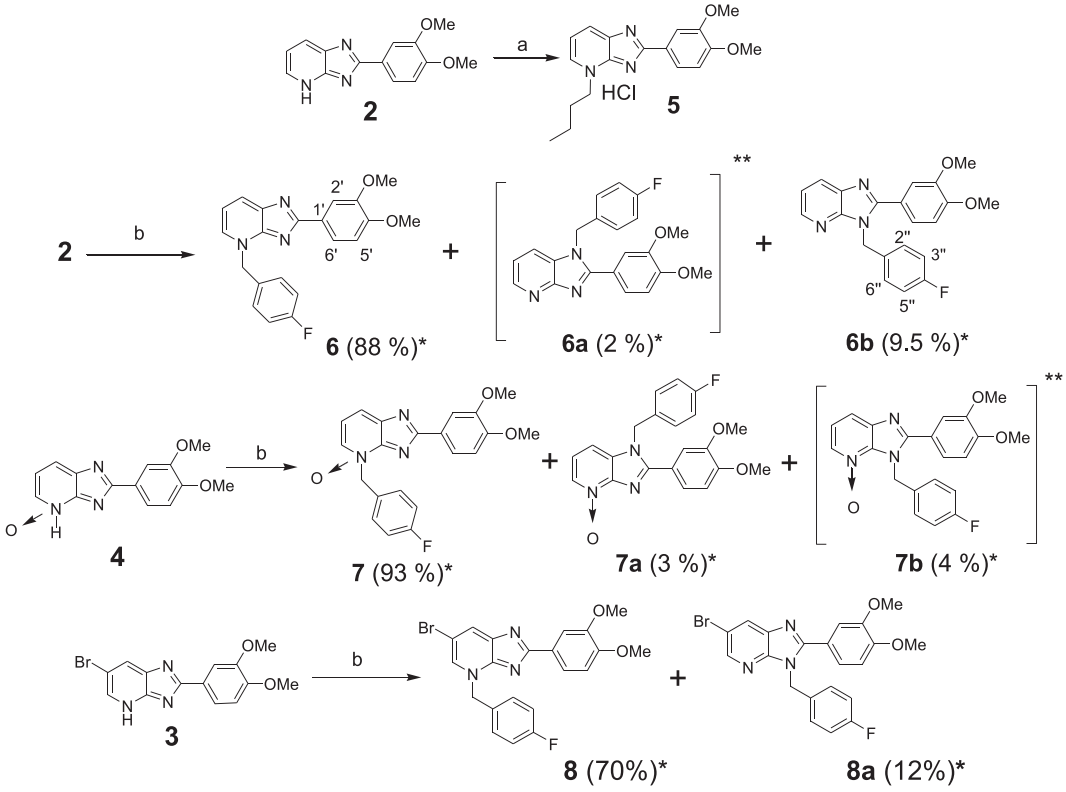
Elimination of the NH proton followed by substitution of this nitrogen atom would prevent rapid tautomerism and lead to a separable mixture of 1*H*, 3*H*, 4*H*, and 5*H* substituted regioisomers

[7–9,11]. Alkylations of imidazopyridines frequently give different yields of both regioisomers. Khanna et al. [7] reported that benzylation of imidazo[4,5-*b*]pyridine with benzylbromide in the presence of NaH afforded all three possible *N*-alkylated products, in a ratio of *N*-1 (1), *N*-3 (3.6) and *N*-4 (1.6) with a 72% yield. Because of the difficulties of this method; e.g., tedious chromatographic separation procedure and lack of large scale preparation, regioselective methods have been suggested for both regioisomers.

Macdonald et al. [19] reported that protection of the parent imidazo[4,5-*b*] pyridine with 2-methoxyethoxymethyl chloride (MEM-chloride) gave a significant amount (55–69%) of the *N*-3 regioisomer. *N*-3-protection of 6-Br and 7-Cl-imidazo[4,5-*b*]pyridines was also achieved with high *N*-3 regioselectivity (62–92%). In another study [8], the alkylation reactions of 2-aryl-1(3)*H*-imidazo [4,5-*b*]pyridines with alkoxymethyl chlorides and bromoacetonitril was reported and attention was drawn to the fact that *N*-3 alkyl derivatives were produced exclusively in basic (Et_3N/NaH) nonpolar media. It was declared that the formation ratio of the regioisomers could be changed with the reaction conditions and solvent effects were evident with the loss of *N*-3/*N*-1 selectivity when the polar aprotic solvent DMF was used [8]. Zeinyeh et al. [11] stated that benzylation of imidazo[4,5-*b*] pyridine-4-oxide and 2-methylimidazo[4,5-*b*]pyridine-4-oxide with benzyl bromide or benzyl iodide at RT using K_2CO_3 in DMF gave the corresponding regioisomers with only a slight difference in *N*-1/*N*-3 ratios.

There are more samples during the *N*-alkylation of imidazo[4,5-*b*]pyridine derivatives mainly led to *N*-3 regioisomer [7,20,21]. Conversely, some studies have shown that *N*-1 regiosomers were obtained at a higher ratio during *N*-alkylation of imidazopyridines [22]. Moreover, the influence of the electronic nature of the C-2 aryl substituent on the formation of regioisomers has been reported. While this position of imidazopyridines was substituted with a 4-MeO-phenyl group, the *N*-3 ratio was found to be higher [8], whereas when substituted with 4-Cl-phenyl, the *N*-1 ratio was higher [23]. It has been reported that 4-chloro-1*H*-imidazo[4,5-*c*] pyridine alkylated with benzyl bromide or 2-fluorobenzyl bromide in the presence of potassium carbonate gave a mixture of *N*-1 and *N*-3 isomers in almost equal ratio [24], whereas treatment of 2-aryl-imidazo[4,5-*c*]pyridine with benzyl chloride only produced the *N*3 regisiomer [25]. Furthermore, 2-phenyl-1(3)*H*-imidazo[4,5-*c*]pyridines were mainly converted substituted 5-benzyl-2-phenyl-5*H*-imidazo[4,5-*c*] pyridines with alkyl bromides and aqueous NaOH solution in DMF [26,27].

Finally, the most accurate result was published in obtaining 4-benzyl-6-bromo-2-(4-methoxyphenyl)-4*H*-imidazo [4,5b]pyridine under basic conditions by X-ray data [28]. When we attempted alkylation of compounds **2**–**4** with *n*-butyl bromide and 4-fluorobenzyl bromide under basic conditions (K_2CO_3, DMF), alkylation occurred primarily at the *N*-4 position, so compounds **5**–**8** were obtained as the *N*-*4* isomer, consistent with the literature [28]. Benzylation of **2** and **4** also gave mainly **6** and **7** (*N*-4) with a small amount of **6b** (*N*-3) and **7a** (*N*-1) in the same reaction medium (Scheme 2), which were isolated by chance using column chromatography. Both of the third regioisomers **6a** and **7b** were followed in their MS chromatogram (Fig. 1) but could not be isolated since their quantities were too low. In the case of alkylation of **4** with 4-fluorobenzyl bromide, compound **7**, which was present at a higher concentration ($t_R = 4.1$ min) was separated by



Reagents : a) K_2CO_3 / n-butyl bromide b) K_2CO_3 / 4-F-Benzyl bromide. *These values were calculated from MS chromatograms ** Could not be isolated.

Scheme 2. *N* [1,3,4]- Butylation and benzylation of imidazo[4,5-*b*]pyridines.

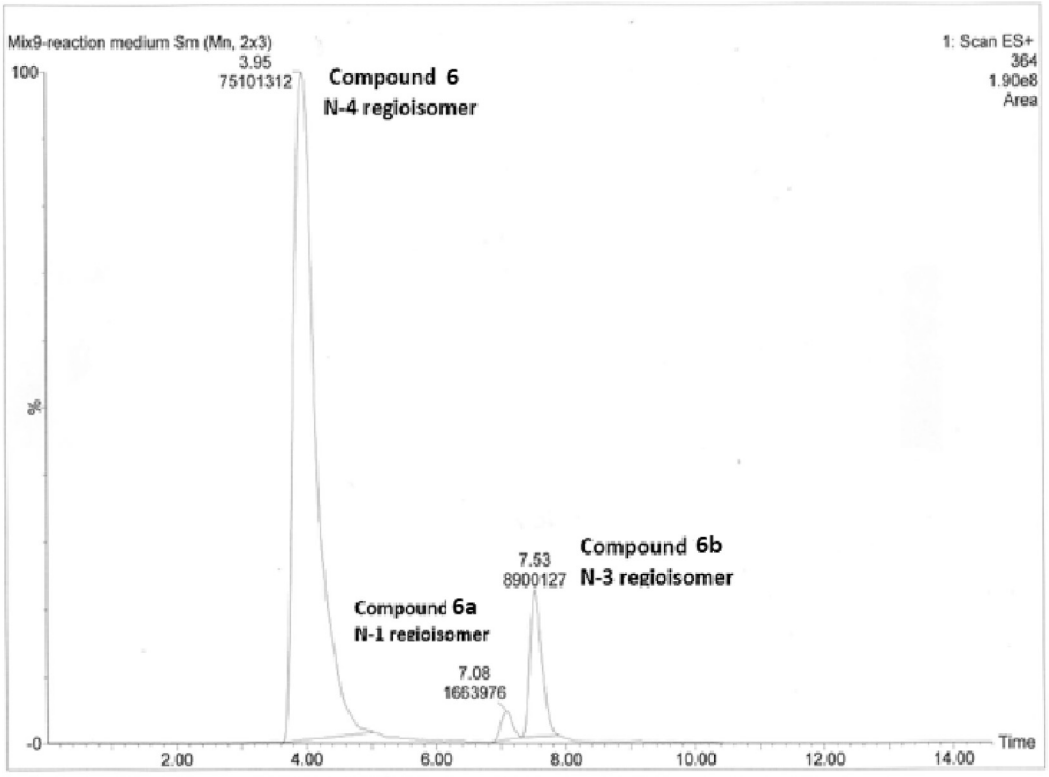


Fig. 1. MS chromatogram (for 364 m/e) for **6**, **6a** & **6b** at the endpoint of synthesis.

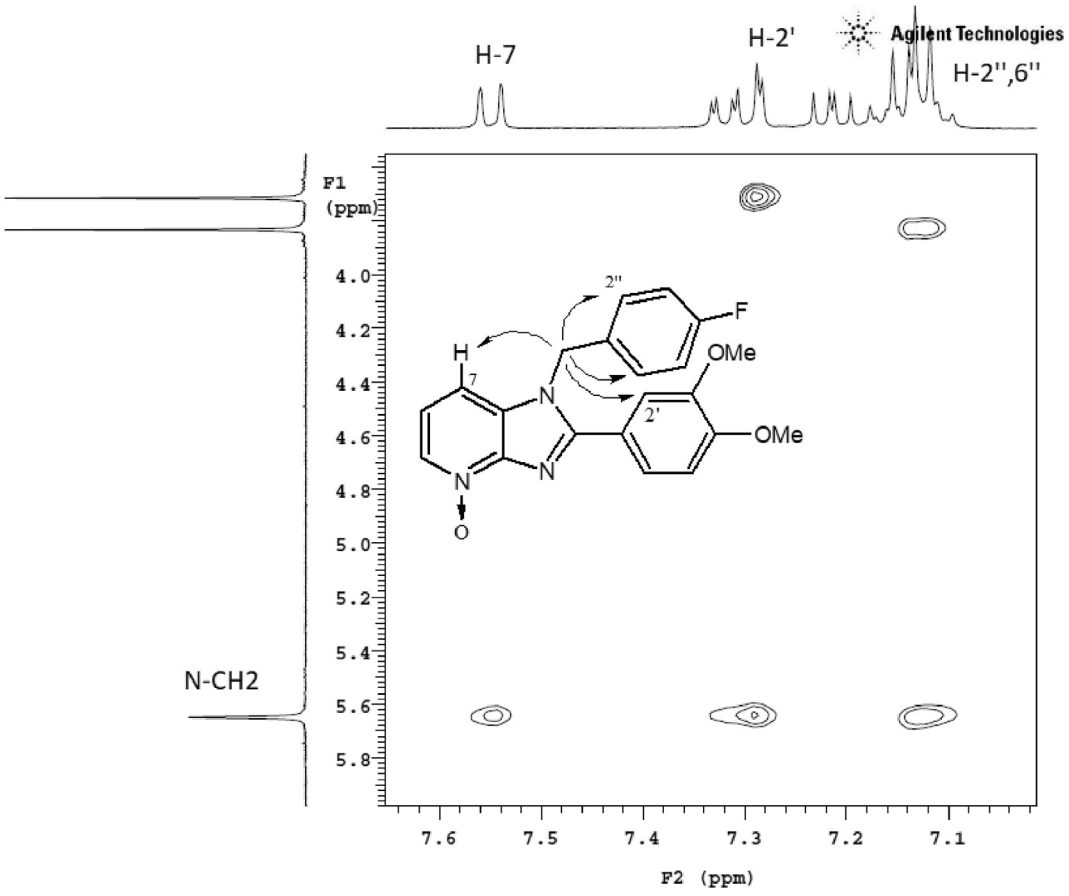


Fig. 2. Partial NOESY spectrum of compound **7a** showing cross. peaks between *N*-CH_2 and H-7, 2′,2″,6'' (in DMSO-d_6).

crystallisation, then compound **7a** ($t_R = 6.3$ min) was isolated by column chromatography from the supernatant liquid after the separation of **7**. Characterisation of the individual isomeric products was determined by observation of 2D-NOESY enhancements between the *N*-CH_2 and H-7,6,5,4 protons. In the NOESY spectra of compounds **6a** and **7a** (Fig. 2), strong correlations were observed between the benzylic and H-7 proton. For compounds **5**, **6**, **7** (Fig. 3), and **8**, NOE cross peaks between the *N*-CH_2 protons and H-5 were observed in the NOESY spectra. In the literature [11], NOESY interaction was reported between the benzylic protons and H-5 for compound 3-benzyl-3*H*-imidazo [4,5-b]pyridine 4-oxide. However, we do not believe this is possible, since the distance is too far for NOE interaction. It is likely they obtained the *N-4* isomer, instead of the *N-3* isomer, as we observed that none of our *N-3* regioisomers gave similar NOE interactions at the same position. *N*-oxide groups could not be accounted as a protection group; this nitrogen atom is easily alkylated as shown in Schemes 2 and 4. In the LC-MS chromatogram of compound **6** (Fig. 1), the R_t values of the *N-1* (7.1 min) and *N-3* (7.5 min) regioisomers are close to one another (i.e., this is why it is difficult to separate them from each other), whereas the *N-4* (3.95 min) regioisomer is far away from the *N-1* and *N-3* peaks and the peak area of *N-4* is greater than the other isomers. Hence, the *N-4* regioisomer could be easily separated from the others, as shown in our experiments. The NOE effect is weaker when the nitrogen atoms have oxides, so a stronger NOE effect was observed in compound **6** compared with its *N*-oxide derivative **7**.

For structural elucidation of the regioisomers, in addition to NOESY data, selective magnetisation transfer experiments, namely the INAPT method [8], have been used for determining the correlation of C-3a,7a,2 with *N*-CH_2 protons. Since this application is known today as HMBC (Heteronuclear Multiple Bond Correlation), we also carried out these experiments for all of our synthesised compounds and obtained good profiles with expected correlations in the HMBC spectra. It is possible to determine the chemical shift value (δ ppm) of C-3a and C-7a by their correlations with pyridine hydrogens in their HMBC spectra. Thus, it should be possible to determine other possible correlations (whether *N*-CH_2: C-3a or *N*-CH_2: C-7a) for the separation of regioisomers, as observed in the gHMBC spectrum of **6b** (Fig. 4).

Finally, to verify both NOESY and gHMBC data, we designed a regioselective synthesis method for **6a**, **6b**, **7a**, and **7b** (Scheme 3). Reaction between 2,3-diaminopyridine and 4-fluorobenzaldehyde gave the selective formation of a Schiff base **9**. Reduction of **9** with $NaBH_4$ formed **10**. Cyclisation of **10** with the $Na_2S_2O_5$ adduct of 3,4-dimethoxybenzaldehyde gave **6a** with a good yield. Oxidising **6a** with H_2O_2 gave **7a**. For the *N-3* regioisomer, compounds **11** and **12** were prepared according to the literature [16]. Cyclisation of **12** with the $Na_2S_2O_5$ adduct of 3,4-dimethoxybenzaldehyde gave **6b** in good yield. Finally, **6b** was converted to **7b** with H_2O_2. The R_T values of **6a** and **7b** exactly correspond with the third unknown peaks obtained in the LC-MS chromatograms of **6** and **7** (at the end point of the reaction).

Butylation and 4-fluorobenzylation of **13** and **14** (Scheme 4) in the same conditions gave mainly 5*H* isomers (**15**–**17**) as recently reported in the literature [26,27], hence very strong NOE interactions have been demonstrated between *N*-CH_2 and H-4,6 in the NOESY spectra of **15**, **16** (Fig. 5), and **17**.

Actually, there are no important differences in the ^{1}H NMR

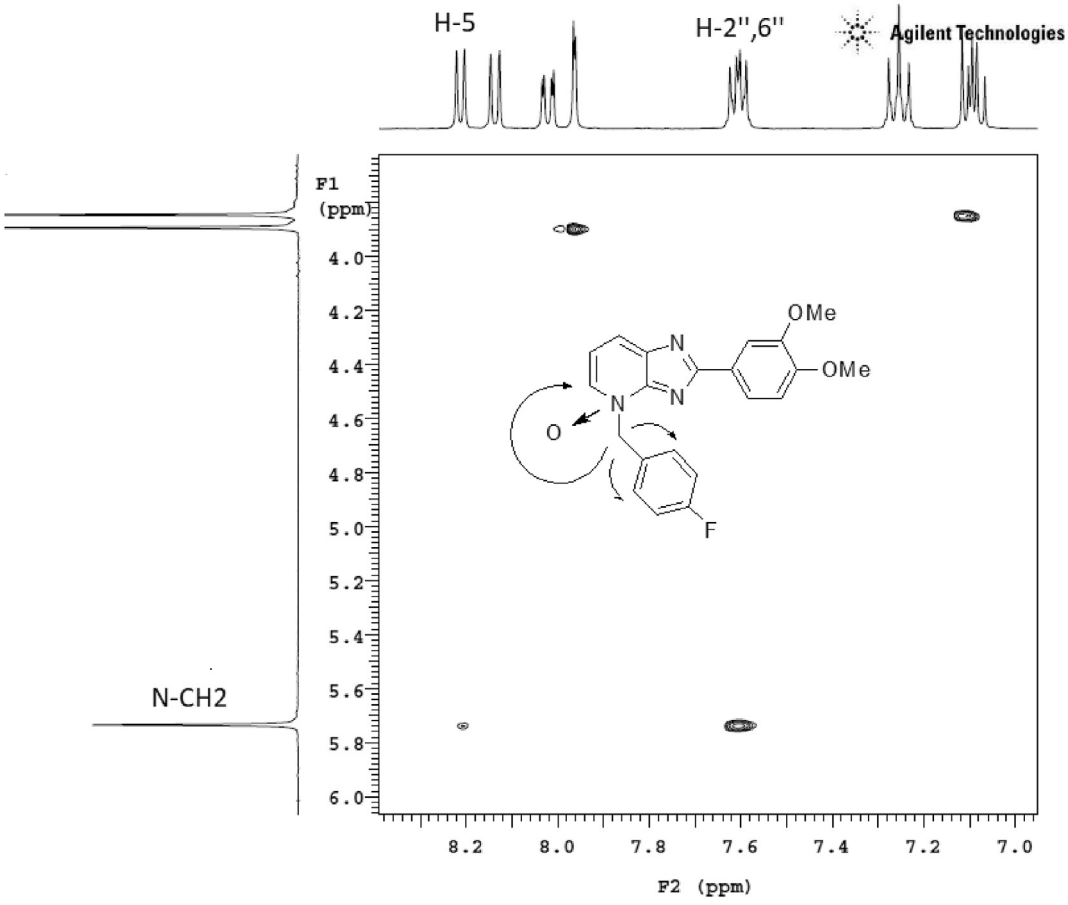


Fig. 3. Partial NOESY spectrum of compound **7** showing cross peaks between. *N*-CH_2 and H-5, 2″,6” (in DMSO-d_6).

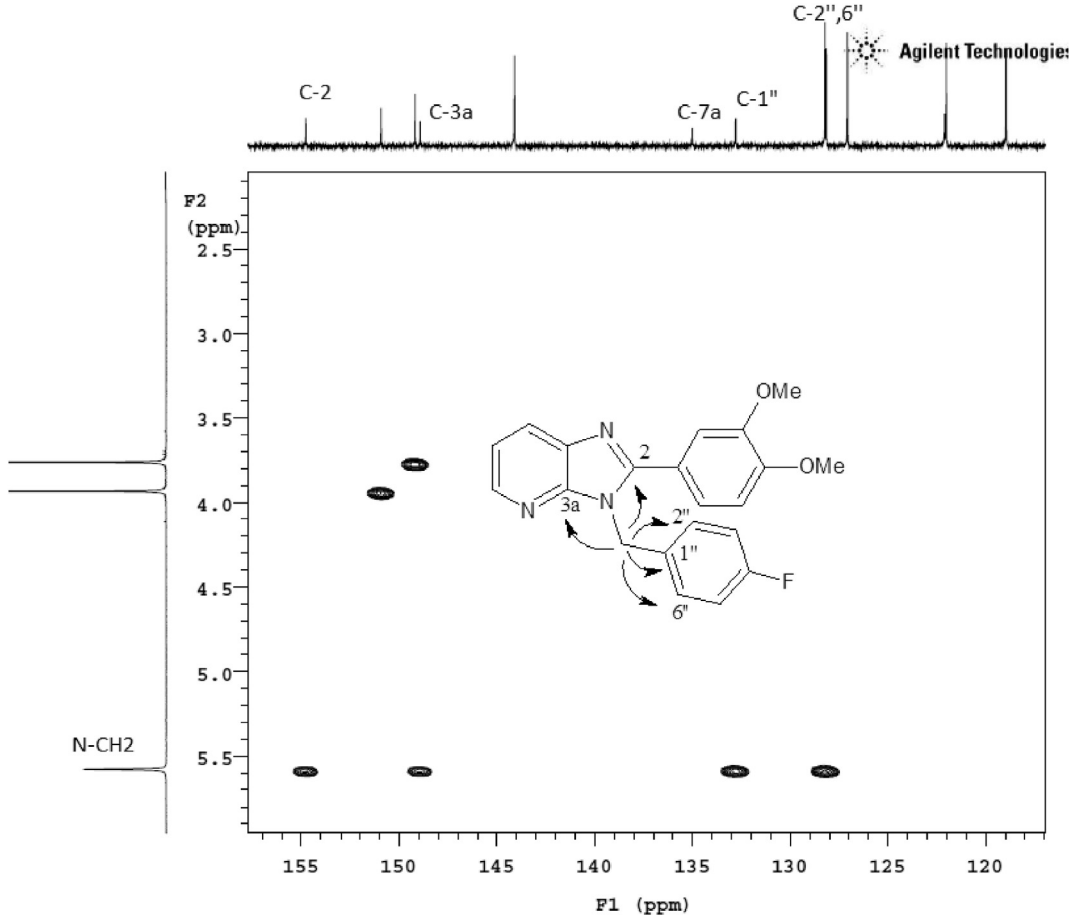
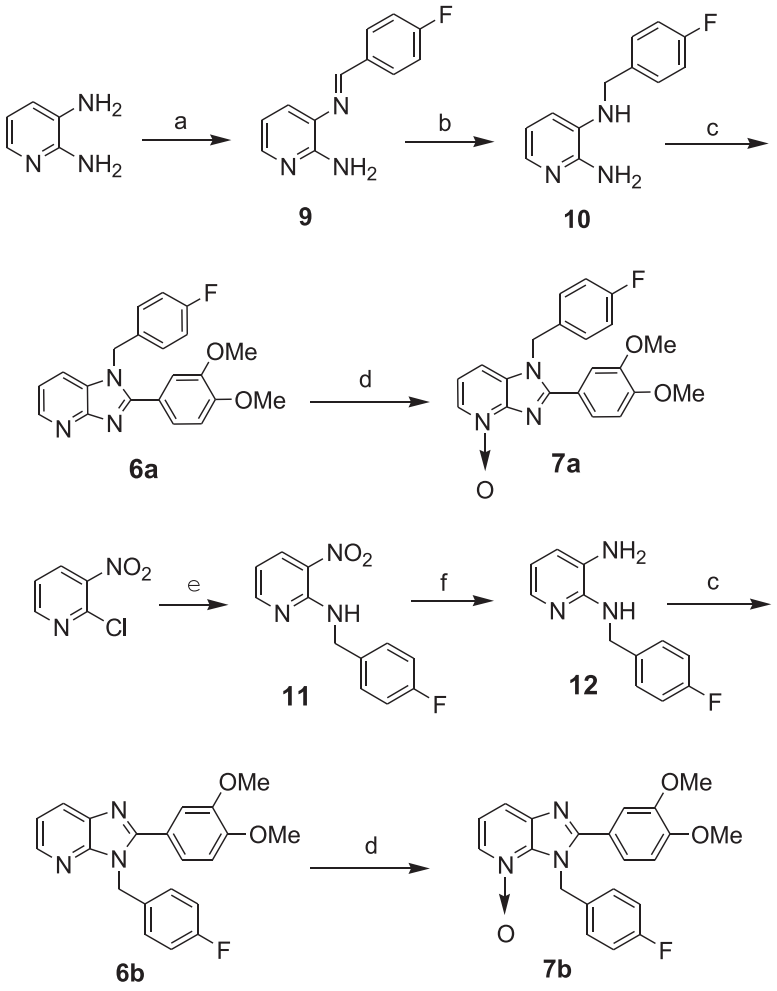
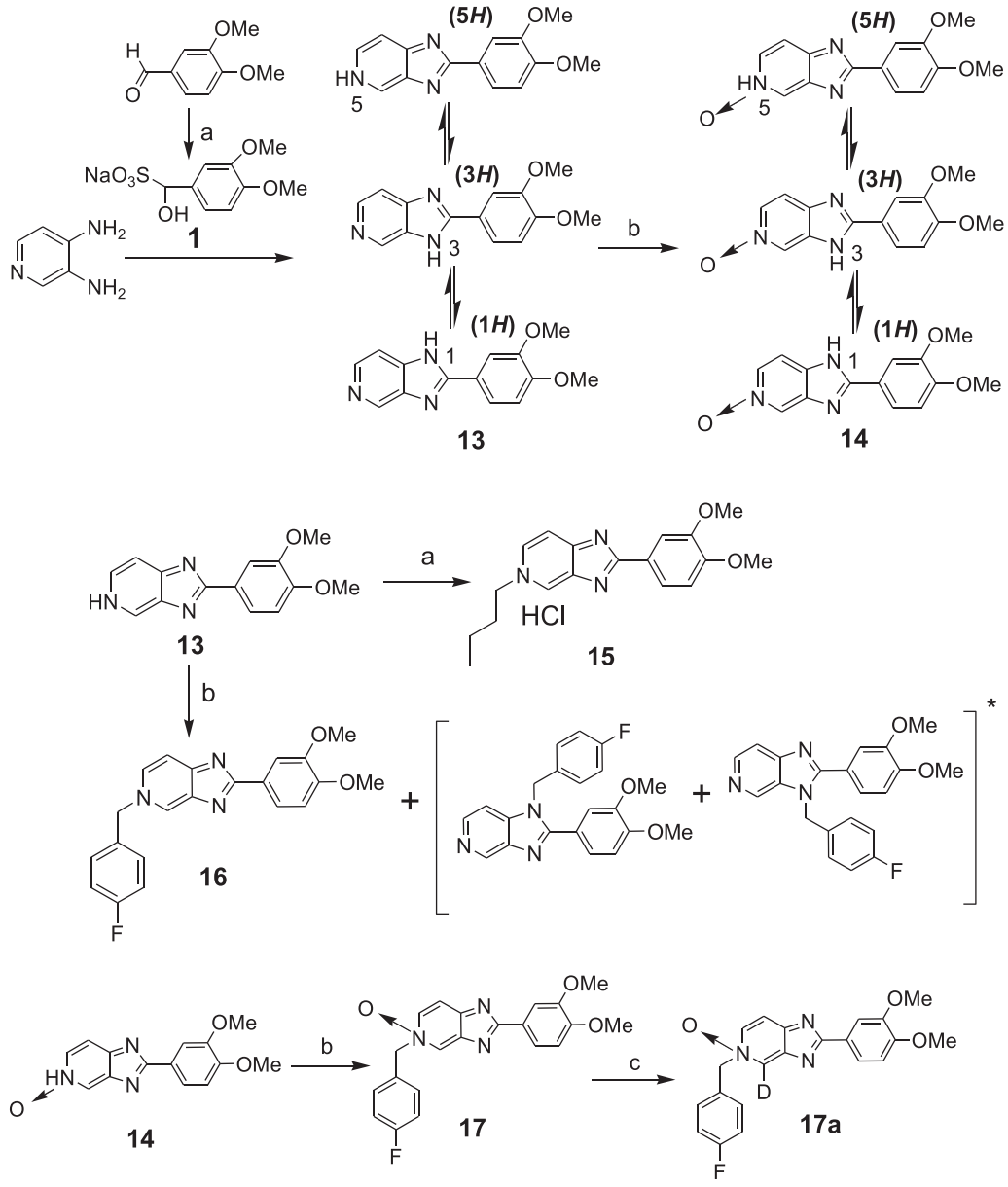


Fig. 4. gHMBC spectra of compound **6b**, showing cross peaks between *N*-CH_2/C-2, C-3a, C-1″, C-2″,6″correlations (in $CDCl_3$).



Reagents : **a)** 4-F-benzaldehyde **b)** $NaBH_4$ **c)** $Na_2S_2O_5$ adduct of 3,4-dimethoxybenzaldehyde (**1**) **d)** H_2O_2 **e)** 4-F-Benzylamine **f)** H_2 / Pd.C

Scheme 3. Selective synthesis of **6a - 6b** and **7a - 7b**.



Scheme 4. N^5- Butylation and benzylation of imidazo[4,5-*c*]pyridines.

spectra of **6** and **7**, and **16** and **17**. However, in the carbon spectrum of **16**, benzylic carbon was observed at 60.2 δ ppm, whereas in the carbon spectrum of **17,** the corresponding carbon was observed at 81.4 δ ppm because of the paramagnetic effect of *N*-oxides. Similar results were shown for compounds **6** (54.9 δ ppm) and **7** (79 δ ppm). The complete assignments of all synthesised compounds were made using 1D and 2D NMR experiments including COSY, NOESY, gHSQC, and gHMBC techniques.

Compound **17** was totally converted to its C4-D form (**17a**) by keeping it at RT in CD_3OD for 24 h. This was also observed with D_2O in DMSO-d_6 after 20 days. The C6-H signal appeared at 8.3 δ ppm as *dd* ($J_{m,o} = 2.4$ and 7.2 Hz) in the ^{1}H NMR spectrum of **17,** whereas the same proton was observed at the same point, but only *d* ($J_o = 7.2$ Hz) coupling in the ^{1}H NMR spectrum of **17a** as C4-H was transformed to C4-D. Furthermore, in the ^{13}C spectrum of **17a,** the C-4 signal was observed at 128.6 δ ppm as *t* (J = 29 Hz) due to the spin-spin coupling pattern rules [2NI+1]. For this reason, the H-4 atoms of imidazo[4,5-*c*]pyridine 5-oxides are easily converted to chlorine by $POCl_3$ [10,22,29].

Compound **6** was also prepared from **2**, with dry NaH and p-fluorobenzylbromide in DMF, and its LC-MS chromatogram (See Supp. Inf. for LC-MS pg 13) was similiar to our previous data obtained with K_2CO_3. There were no important differences in the formation ratio of regioisomers.

4. Conclusions

It was found that the *N*-4 and *N*-5 regiosomers were formed at

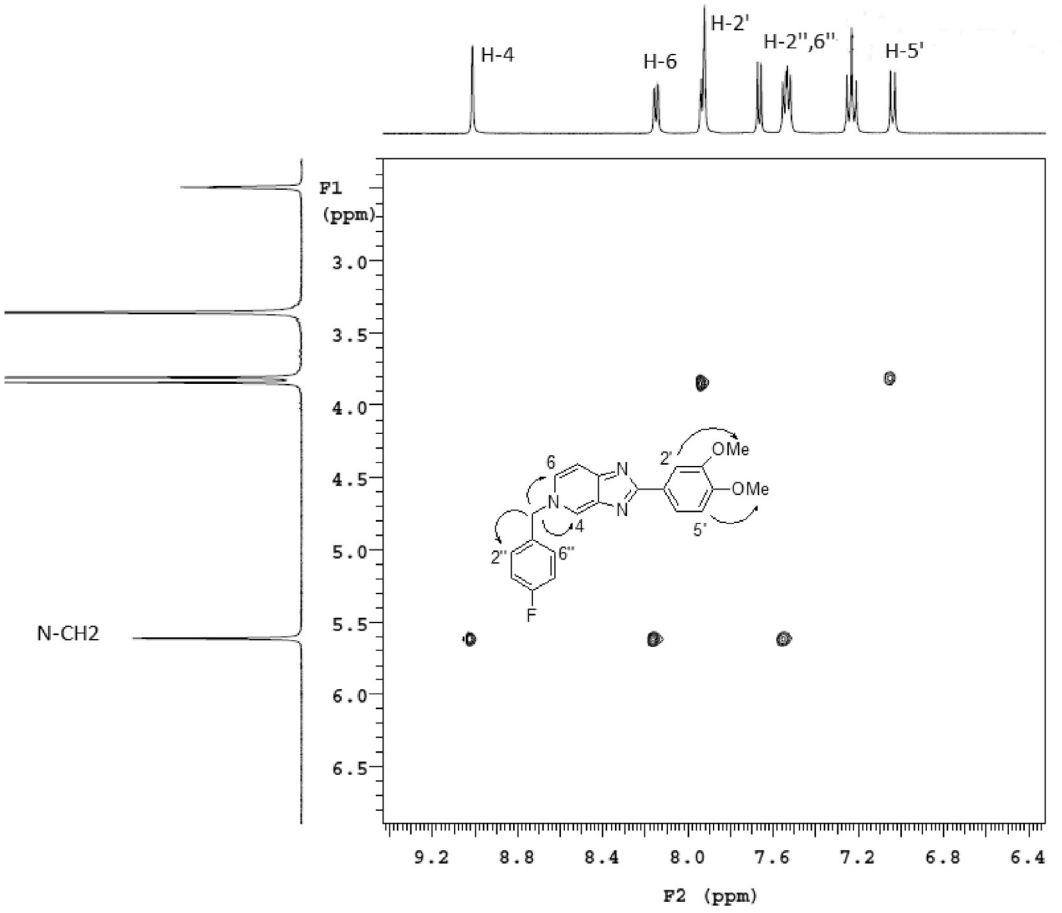


Fig. 5. Partial NOESY spectrum of compound **16** showing cross peaks between. *N*-CH_2 and H-4, 6, 2″,6" (in DMSO-d_6).

higher ratio during the *N*-alkylation of 2-(3,4-dimethoxyphenyl) imidazo[4,5-*b*]pyridine (**2**) and 2-(3,4-dimethoxyphenyl)imidazo [4,5-*c*] pyridine (**13**), respectively. Based on our experience and literature examples that support us, we recognize that the previous tautomeric forms corresponding to these regioisomers are more stable. NOESY was used for the structural elucidation of these regioisomers. The cross peaks for *N*-CH_2 protons (at approximately around 5–6 ppm) and H-4,5,6,7 protons (at approximately 7–8 ppm) confirms their spatial proximity as evidence for *N-1,3,4,5*-substitution. gHMBC analysis could be effective as an alternative way for assignment of the regiosomers.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molstruc.2019.07.058.

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