Letter - spectral assignment

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Separation and identification of the mixture of 2-(3,4-dimethoxyphenyl)-1-*n*-propyl or (4-chlorobenzyl)-5 and (6)-1*H*-benzimidazolecarbonitriles

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Introduction

Benzimidazoles are very useful intermediates/subunits for the development of molecules of pharmaceutical or biological interest. Appropriately substituted benzimidazole derivatives have found diverse therapeutic applications such as antimicrobial, antiprotozoal, antibacterial, antiallergic, HIV inhibitors, antiviral, antiparasitic, antitumoral, antihypertensive, cardiotonic, antiulcer, anti-inflammatory, analgesic, antioxidant, antidiabetic, diuretic, androgen receptor antagonist, anticonvulsant and anticoagulant.[1–3] Optimization of benzimidazole-based structures has resulted in various drugs such as Mebendazole as antihelmentic, Clemizole and Astemizole as antihistaminic, Droperidol as antipsychotic, Omeprazole and Lansoprazole as antiulcer, Bendamustin as anti-tumoral and Candesartan as antihypertensive medications.

Benzimidazole with unsubstituted *N*H groups exhibit fast prototropic tautomerism which leads to equilibrium mixtures of asymmetrically substituted compounds. The 1,3-tautomerism associated with benzimidazoles is a very popular topic, and the existence of this tautomerism has been proved by several approaches, including NMR spectroscopy.[4] This 1,3-migration is not found when the imidazole hydrogen is replaced by other substituents such as an alkyl group or under special circumstances where the hydrogen migration is affected by inter- and/or intramolecular hydrogen bonding.[5]

In our previous studies,[6,7] we have also reported synthesis of some regioisomers of benzimidazoles. At that old time, their structural elucidation has been achieved by obtaining only one regiosomer with selective synthesis. Similarly, Willis and coworkers[8] reported a regio-controlled synthesis for 1-benzyl-2-phenyl-1*H*-benzimidazole-6-carbonitrile. As a further contribution to this field, now we report the synthesis of a series of 2-phenyl-5(6)-cyanosubstituted-1*H*-benzimidazoles containing a propyl and 4-chlorobenzyl groups on the N^1-position and their tautomeric behaviours. After separating the regiosomers from each others, for their structural elucidation, Nuclear Overhauser Effect Spectroscopy (NOESY) 2D experiments were used.

Experimental

Uncorrected melting points were measured on an Büchi B-540 (Buchi, Labortechnik, Flawil, Switzerland) capillary melting point apparatus. All NMR experiments were carried out by using VARIAN (AGILENT) MERCURY 400 MHz (Varian, Palo Alto, CA) at a proton resonance frequency of 400.1779 and 100.6243 MHz for carbon, equipped with a 5-mm broadband-observed probe head. The NMR spectrum optimization was conducted by using Agilent VnmrJ version 3.2 revision A software and all parameters were set in it. The samples (6–20 mg) were prepared in 0.7 ml of DMSO-d_6, $CDCl_3$ and CD_2Cl_2. The ^{1}H NMR experiments were traditionally carried out with TMS as an internal standart and its chemical shift set at $\delta = 0$ ppm, at room temperature (Only for compound **4a**, 40 °C). Pulse programme for ^{1}H spectra was relax. delay 1 s; pulse 45.0°; 8 or 16 repetitions; acquisition time 2.559 s; width 6402.0 Hz. Pulse programme for ^{13}C spectra was relax. delay 1 s; pulse 45.0°; 2000 repetitions; acquisition time 1.304 s; width 21 141.6 Hz. The DEPT pulse programme for carbon was relax. delay 1 s; pulse 90.0°; acquisition time 1.304 s; width 21 141.6 Hz; 64 repetitions. The HMBC pulse programme for proton-carbon was relax. delay 1 s; acquisition time 0.15 s; width 6402.0 Hz; 2D width 21 633.3 Hz; 8 repetitions; 2 × 256 increments. The HSQC pulse programme for proton-carbon was relax. delay 1 s; acquisition time 0.15 s; width 6402.0 Hz; 2D width 17 105.0 Hz; 8 repetitions; 2 × 256 increments. The NOESY pulse programme for proton was relax. delay 1 s; acquisition time 0.15 s; width 4046.9 Hz; 2D width 4046.9 Hz; 8 repetitions; 2 × 200 increments. The COSY pulse programme for proton was relax. delay 1 s; acquisition time 0.15 s; width 4046.9 Hz; 2D width 4046.9 Hz; 4 repetitions; 128 increments.

The LC/MS spectra were taken on a Waters Micromass ZQ connected with Waters Alliance HPLC (Waters Corporation, Milford, MA), using ESI(+) method, with C-18 column (XTerra®, 4.6 × 250 mm, 5 μm). The analytical condition of mass spectrometry was as follows: capillary voltage: 3.11 kV, cone voltage: 29 V, source temperature: 100 °C: desolvation temperature: 300 °C. For compounds **4a**, **4b** and **5a**, **5b,** the

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mixtures of H_2O: CH_3CN: MeOH : 0.1% HCOOH in CH_3CN (55 : 25 : 10 : 10) and H_2O: CH_3CN: MeOH : 0.1% HCOOH in CH_3CN (45 : 35 : 10 : 10) were selected as mobile phases, with 0.4 and 0.7 ml/min flow rate, respectively. The eluate was monitored by a photo-diode array detector at 254 nm.

3,4-Diaminobenzonitrile 1

4-Amino-3-nitrobenzonitrile (1.0 g, 6.1 mmol) was dissolved in 50-ml ethanol and was subjected to hydrogenation using 35 psi of H_2 and 10% Pd.C until uptake of H_2 ceased. The catalyst was filtered on a bed of Celite, washed with ethanol and concentrated in vacuo. Powder residue was used for the subsequent steps without crystallization. The obtained compound was used without crystallization in further steps. Yield 88% (0.72 g). Mp: 144–146 °C.[9]

Sodium metabisulfite adduct of 3,4-dimethoxybenzaldehyde 2

3,4-Dimethoxybenzaldehyde (1.245 g, 7.5 mmol) was dissolved in EtOH (25 ml), and sodium metabisulfite (0.8 g) (in 5 ml of water) was added in portions. The reaction mixture was stirred vigorously and more EtOH was added. The mixture was kept in a refrigerator for a several hours. The white precipitate was filtered and dried, and used for the further steps without purification and characterization.

2-(3,4-Dimethoxyphenyl)-1*H*-benzimidazole-5(6)-carbonitrile 3

The mixture of **1** (0.133 g, 1 mmol) and **2** (0.270 g, 1 mmol) and in DMF (2 ml) was heated at 120 °C, for 3.5 h. The reaction mixture was cooled, poured into water and made alkaline with dilute K_2CO_3 solution. The resulting precipitate was collected by filtration dried and crystallized from EtOH, mp 205–207 °C, yield 70.4%, 0.19 g. **MS** (ESI+) m/z : 280 (M + H, %100), $C_{16}H_{13}N_3O_2$; **^{1}H-NMR** (400 MHz, DMSO) δ ppm (*J*, Hz) : 3.83(s,3H,5′-OC$\underline{H}_3$), 3.87(s,3H,4′-OC$\underline{H}_3$), 7.14(d,1H,J_o = 8.4 Hz,H-3′), 7.54(d,1H,J_o = 8.4,H-6); **^{1}H-NMR** (400 MHz, DMSO-d_6 + NaH + D_2O) δ ppm (*J*, Hz) : 3.76(s,3H,5′-OC$\underline{H}_3$), 3.82(s,3H,4′-OC$\underline{H}_3$), 6.94(s,1H, J_o = 8.4 Hz,H-3′), 7.07(dd,1H,J_o = 8.4 and J_m = 1.6 Hz, H-6), 7.43(d,1H,J_o = 8.4 Hz,H-7), 7.72(d, 1H,J_m = 1.6 Hz,H-4), 7.81 (dd,1H,J_o = 8.4 and J_m = 1.6 Hz,H-2′), 7.90(d,1H, J_m = 1.6 Hz,H-6′),

Table 1. ^{1}H,^{13}C, COSY, NOESY, DEPT, HSQC and HMBC data of **4a** and **4b**

Compound 4a							Compound 4b						
No	**^{1}H δ, ^{13}C δ, HSQC**		DEPT*	COSY	NOESY	HMBC	No	^{1}H δ, ^{13}C δ, HSQC		DEPT*	COSY	NOESY	HMBC
2		156.16				H-9,13,16	**2**		156.88				H-9,13,16
3a		142.47				H-7	**3a**		145.8				H-5,7
4	8.01 (d,1H, J_m = 1.6)	124.6	1			H-6	**4**	7.83 (d,1H, J_o = 8.4)	120.53	1	H-5		
5		105.6				H-7	**5**	7.53 (dd,1H, J_o = 8.4, J_m = 1,6)	125.94	1	H-4		H-7
6	7.53 (dd,1H, J_o = 8.4, J_m = 1,6)	125.9	1	H-7		H-4	**6**		105.25				H-4
7	7.44 (d,1H,J_o = 8.4)	110.97	1	H-6	H-16		**7**	7.72 (d,1H, J_o = 1.2)	114.85	1		H-16	H-5
7a		138.46				H-4,6,16	**7a**		135.36				H-4,16
8		121.95				**	**8**		121.82				**
9	7.23 (dd,1H, J_o = 8.4, J_m = 2)	121.86	1	H-10	H-16	H-10,13	**9**	7.23 (dd,1H, J_o = 8.4, J_m = 2)	121.85	1	H-10	H-16	H-10,13
10	7.00 (d,1H, J_o = 8.4)	111.2	1	H-9			**10**	7.00 (d,1H, J_o = 8.4)	111.02	1	H-9		
11		151.05				H-9,10,13,15	**11**		150.96				H-9,10,13,15
12		149.5				H-10,13,14	**12**		149.38				H-10,13,14
13	7.29 (d,1H, J_o = 2)	112.74	1			H-9	**13**	7.29 (d,1H, J_o = 2)	112.49	1			H-9
14	3.95 (s,3H)	56.02	3				**14**	3.95 (s,3H)	56.03	3			
15	3.96 (s,3H)	56.14	3				**15**	3.96 (s,3H)	56.11	3			
16	4.23 (t,2H, J = 7.6)	46.71	2	H-17	H-7,H-9	H-17,18	**16**	4.23 (t,2H, J = 7.6)	46.81	2	H-17	H-7,H-9	H-17,18
17	1.84 (m,2H, J = 7.6)	23.16	2	H-16,18		H-16,18	**17**	1.86 (m,2H,J = 7.6)	23.24	2	H-16,H-18		H-16,18
18	0.89 (t,3H, J = 7.6)	11.13	3	H-17		H-16,17	**18**	0.916 (t,3H,J = 7.6)	11.12	3	H-17		H-16,17
19		119.81				H-4,6	**19**		119.97				H-5,7

δ ppm in $CDCl_3$, *J* in Hz.

* Number in DEPT is the number of attached protons.

** Not observable since overlapped with C-9.

COSY : [H-6 : H-7] and [H-2′ : H-3′], **^{13}C-NMR** (100 MHz, DMSO-d_6 + NaH + D_2O), **HSQC** and **HMBC** δ ppm : 164.3(C-2), 151.3(C-7a), 149.1(C-4′), 148.7(C-5′), 147.11(C-3a), 129.7(C-1′), 123.06(CN), 121.00(C-6H), 120.15(C-4H), 119.90(C-2′H), 116.63 (C-7H), 111.84(C-3′H), 110.97(C-6′H), 97.69(C-5), 55.89(C4′-OMe), 55.76(C5′-OMe).

*Synthesis of the mixture of **4a** and **4b***

A mixture of **3** (0.060 g, 0.215 mmol), n-propylbromide (0.037 g, 0.3 mmol) and sodium hydride (95%) (0.01 g, 0.4 mmol) in *N,N*-dimethylformamide (0.5 ml) was stirred at 60 °C for 5 h. The reaction mixture was cooled, poured into water and made alkaline with dilute K_2CO_3 solution. The resulting precipitate was collected by filtration and dried.

2-(3,4-Dimethoxyphenyl)-1-propyl-1*H*-benzimidazole-5-carbonitrile 4a

Two times crystallization of this precipitate from EtOH afforded pure **4a**. Mp 148–150 °C, yield 30.3%, 0.021 g, **MS** (ESI+) m/z : 322 (M + H, %100), $C_{19}H_{19}N_3O_2$, (NMR data in Table 1).

2-(3,4-Dimethoxyphenyl)-1-propyl-1*H*-benzimidazole-6-carbonitrile 4b

After separation of **4a** the supernatant liquid was evaporated, and purification of this residue by column chromatography (EtOAc 15 : n-hexane 85) gave the pure compound **4b** (from the second fraction), mp 142–144 °C, yield 18.8%, 0.013 g, **MS** (ESI+) m/z : 322 (M + H, %100), $C_{19}H_{19}N_3O_2$, (NMR data in Table 1).

*Synthesis of the mixture of **5a** and **5b***

A mixture of **3** (0.060 g, 0.215 mmol), 4-chlorobenzyl chloride (0.048 g, 0.3 mmol) and sodium hydride (95%), (0.01 g, 0.4 mmol) in *N,N*-dimethylformamide (0.5 ml) was stirred at 60 °C for 5 h. The reaction mixture was cooled, poured into water and made alkaline with dilute K_2CO_3 solution. The resulting precipitate was collected by filtration and dried.

1-(4-Chlorobenzyl)-2-(3,4-dimethoxyphenyl)-1*H*-benzimidazole-5-carbonitrile 5a

Purification of the above residue by column chromatography (EtOAc 35 : n-hexane 65) gave the pure compound **5a** from the first

Table 2. ^{1}H, ^{13}C, COSY, NOESY, DEPT, HSQC and HMBC data of **5a** and **5b**

Compound 5a							Compound 5b						
No	^{1}H δ, ^{13}C δ, HSQC		DEPT*	COSY	NOESY	HMBC	No	^{1}H δ, ^{13}C δ, HSQC		DEPT*	COSY	NOESY	HMBC
2		156.57				H-9,13,16	**2**		157.67				H-9,13,16
3a		142.47				H-7	**3a**		146.37				H-5,7
4	8.13 (d,1H, J_m = 1.6)	124.45	1			H-6	**4**	7.86 (dd,1H, J_o = 8.4, J_p = 0.8)	120.83	1	H-5		
5		105.82				H-7	**5**	7.56 (dd,1H, J_o = 8.4, J_m = 1,6)	115.49	1	H-4		H-7
6	7.53 (dd,1H, J_o = 8.4, J_m = 1,6)	126.16	1	H-7		H-4,7	**6**		106.05				H-4
7	7.30 (d,1H, J_o = 8.4)	111.22	1	H-6	H-16	H-6	**7**	7.55 (d,1H, J_o = 1.2)	126.73	1		H-16	H-5
7a		138.46				H-4,6,16	**7a**		136.20				H-4,16
8		121.95				H-10	**8**		121.5				H-10
9	7.19 (dd,1H, J_o = 8.4, J_m = 2)	121.88	1	H-10	H-16	H-13	**9**	7.22 (dd,1H, J_o = 8.4, J_m = 2)	122.43	1	H-10	H-16	H-13
10	6.94 (d,1H, J_o = 8.4)	111.25	1	H-9	H-15		**10**	6.95 (d,1H, J_o = 8.4)	111.74	1	H-9	H-15	
11		151.05				H-9,13,15	**11**		151.88				H-9,10,13,15
12		149.5				H-10,13,14	**12**		149.91				H-10,13,14
13	7.21 (d,1H, J_o = 2)	112.36	1		H-14,16	H-9	**13**	7.23 (d,1H, J_o = 2)	112.87	1		H-14,16	H-9
14	3.75 (s,3H)	55.71	3				**14**	3.75 (s,3H)	56.20	3			
15	3.89 (s,3H)	55.85	3				**15**	3.89 (s,3H)	56.32	3			
16	5.48 (s,2H)	48.07	2		**H-7,9,13 H-18(weak)		**16**	5.47 (s,2H)	48.65	2		***H-7,9, 13 H-18(weak)	H-18
17		134.49				H-16,19	**17**		134.74				H-16,19
18	7.05 (d,2H, J_o = 8.4)	127.38	1	H-19	H-16(weak)	H-16	**18**	7.05 (d,2H, J_o = 8.4)	127.80	1	H-19	H-16(weak)	H-16
19	7.35 (d,2H, J_o = 8.4)	129.28	1	H-18			**19**	7.35 (d,2H, J_o = 8.4)	129.81	1	H-18		
20		133.78				H-18	**20**		134.34				H-18
21		119.81				H-4,6	**21**		120.10				H-5,7

δ ppm in CD_2Cl_2, *J* in Hz.

* Number in DEPT is the number of attached protons.

** When the spectrum is run in CD_3CN, H-16 interaction with H-9 and H-13 are observed separately.

*** When the spectrum is run in $CDCl_3$, H-16 interaction with H-9 and H-13 are observed separately.

coming out fraction, which is crystallized from EtOH, mp 196–199 °C, yield 24%, 0.021 g, **MS** (ESI+) m/z : 404(M + H, %100), 406 (M + H + 2, %34), $C_{23}H_{18}ClN_3O_2$, (NMR data in Table 2).

1-(4-Chlorobenzyl)-2-(3,4-dimethoxyphenyl)-1*H*-benzimidazole-6-carbonitrile 5b

The crystallization of second fraction from EtOH: n-hexane gave the pure compound **5b**. Mp 200–202 °C, Yield 11.5%, 0.010 g, **MS** (ESI+) m/z : 404(M + H, %100), 406(M + H + 2, % 32), $C_{23}H_{18}ClN_3O_2$. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ ppm (*J*, Hz) : 3.82(s,3H,14-OC*H*$_3$), 3.93 (s,3H,15-OC*H*$_3$), 5.48(s,2H,H-16), 6.93(d,1H,J_o = 8 Hz,H-10), 7.04(d,2H, J_o = 8.8 Hz,H-18), 7.19(dd,1H,J*m* = 2 Hz and J_o = 8 Hz,H-9), 7.27(d,1H, J_m = 1.6 Hz,H-13), 7.36(d,2H,J_o = 8.8 Hz,H-19), 7.51(s,1H,H-7), 7.58 (dd,1H, J_m = 1.2 Hz and J_o = 8.4 Hz,H-5), 7.92(d,1H,J_o = 8.4 Hz,H-4). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ ppm (*J*, Hz) : 157.06, 151.48, 149.48, 145.22, 135.48, 134.38, 133.72, 129.67, 127.10, 126.73, 121.97, 120.56, 119.54, 114.93, 112.32, 111.15, 106.08, 56.05, 55.96, 48.32 (One carbon atom is missing, that is why, the NMR spectra of this compound was run in CD_2Cl_2 in Table 2). **COSY** : [H-10 : H-9], [H-18 : H-19], [H-5 : H-4], **NOESY :** [H-16 : H-7, H-9, H-13, H-18], [H-10 : H-15], [H-13 : H-14] (Fig. 5).

Results and Discussion

Compounds **4a**, **4b** and **5a**, **5b** (Tables 1 and 2) were prepared using the methods outlined in Fig. 1. Reduction of 4-amino-3-nitrobenzonitrile with H_2/Pd-C afforded 3,4-diaminobenzonitrile **1**. Cyclization of **1** with sodium metabisulfite adduct of 3,4-dimethoxybenzaldehyde gave benzimidazolecarbonitrile **3**. In benzimidazole ring, the nitrogen bears a hydrogen atom (N^1) that seems like a pyrrole-like N-atom; the other (N^3) resembles a pyridine-like N-atom.

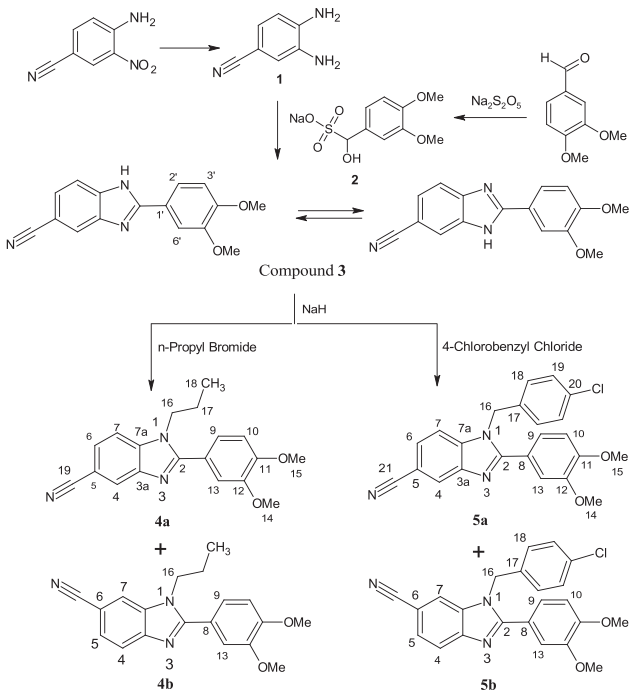

Figure 1. Synthesis of targeted 1*H*-benzimidazoles **4a, 4b** and **5a, 5b**.

Hydrogen atom attached to nitrogen in the 1-position readily tautomerise.[10] This may be depicted as in compound **3** in (Fig. 1). Thus, 1*H*-benzimidazole-5-carbonitrile is a tautomer of 1*H*-benzimidazole-6-carbonitrile, and both structures represent the same compound as 5(6). The NMR spectra of benzimidazoles show the presence of two tautomeric forms, which are rapidly equilibrating on the NMR timescale, and the relations between the tautomer and nontautomer type were reported in different deuterated solvents.[11–13] Figure 2 shows the ^{1}H and ^{13}C NMR spectra of compound **3** under normal conditions. Because of the mixture of tautomers in compound **3**, its ^{1}H-NMR spectra were not clear enough under standard conditions as expected. Similar situation was also observed with the ^{13}C spectra of same compound; some carbon peaks are missing. After elimination of the tautomeric effects by addition of a tiny amount of dry NaH, and two to three drops of D_2O, all the protons with expected splitting patterns and carbon atoms including C3a/C7a, C4/C7 and C5/C6 have been observed as sharp peaks, in its NMR spectra (Fig. 2). Hence, very fine NMR assignments without tautomerism were made by combination of 1D and 2D NMR techniques.

Furthermore, elimination of NH proton then substitution of this nitrogen atom would prevent rapid tautomerism and lead to a separable equimolar mixture of 1,5- and 1,6-substituted products.[14] When we have attempted alkylation of compound **3,** with *n*-propyl bromide and 4-chlorobenzyl chloride under strong basic conditions (NaH 95%, DMF), because of the tautomerism of the imidazole moiety, alkylation occurred at both N^1 and N^3 positions and two regioisomers: **4a**, **4b** and **5a**, **5b** were formed as a solid mixture as expected, respectively.

Alkylations of benzimidazoles give frequently similiar yields of both regioisomers;[15–17] sometimes they give a different ratio.[6,18–20] It has been reported that, when 5(6)- or 4(7)-substituted benzimidazoles are alkylated, the product ratios depend on the resonance electronic effects as well as position of the substituent.[21] In our experiments, propylation and benzylation of compound **3** (Fig. 1) gave the compounds **4a**, **5a** (1,5-isomers) and **4b**, **5b** (1,6-isomers) with the ratio of 70 : 30 and 72 : 28, according to their LC chromatograms, respectively (Fig. 3). We were able to separate regioisomers from each other by crystallization or column chromatography as reported in the experimental part. Because the ratio of **4a** is much higher than **4b**, it was possible to obtain **4a** only

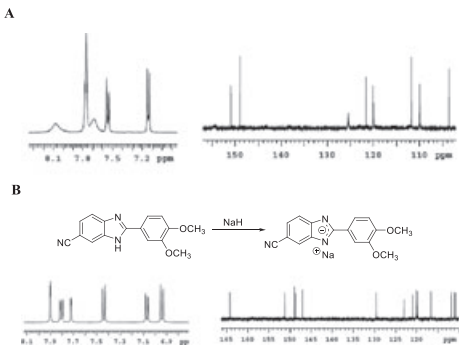

Figure 2. (A) Aromatic region of ^{1}H and ^{13}C-NMR spectra of compound **3** under normal conditions. (B) Aromatic region of ^{1}H and ^{13}C-NMR spectra of compound **3** after elimination tautomerism.

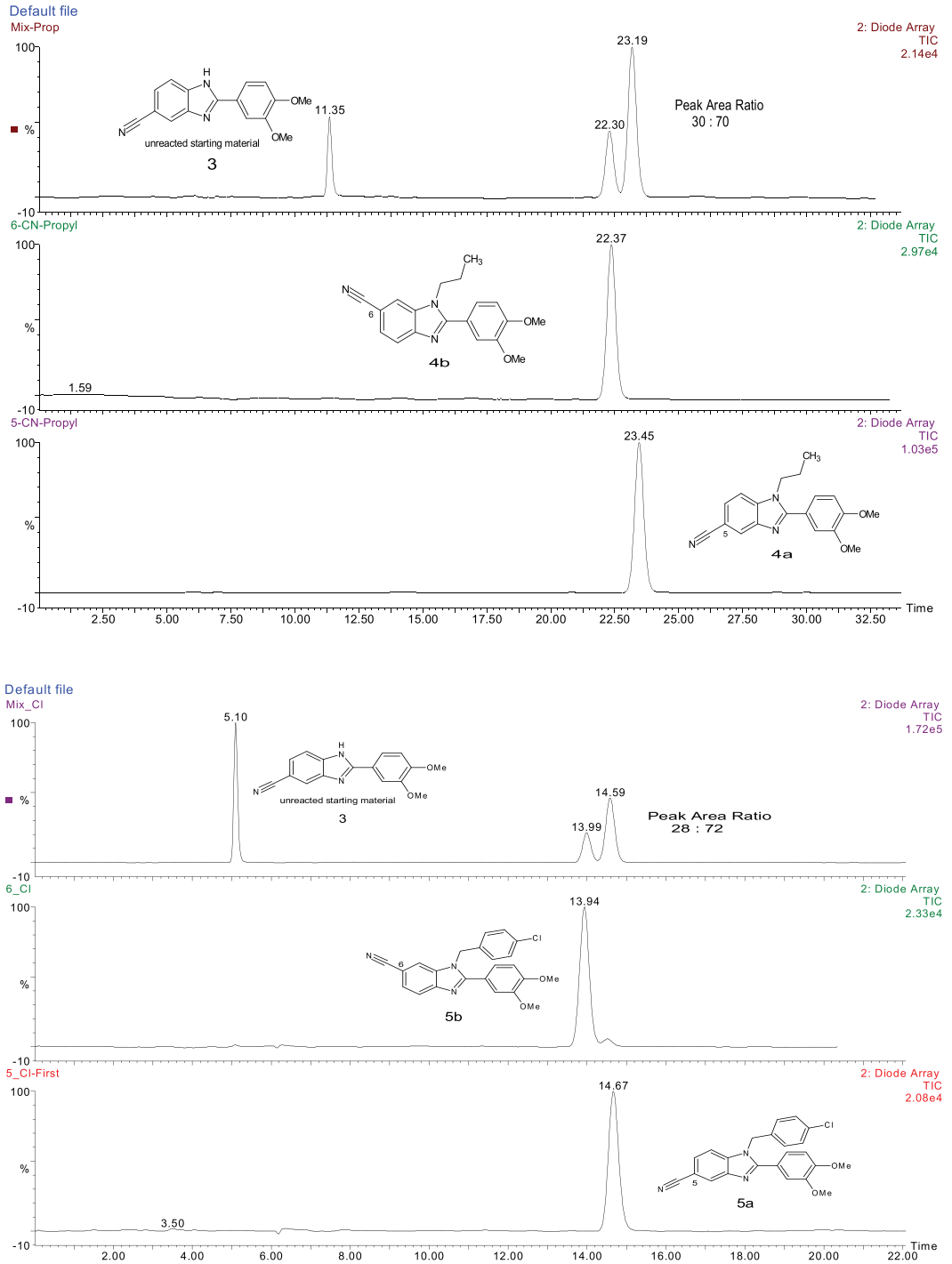

Figure 3. HPLC chromatogram of compounds **4a–4b** and **5a–5b**.

by crystallization. Characterization of the individual isomeric products was determined by observation of ^{1}H-NMR NOESY enhancements between methylene protons H-16 and H-7, allowing the H-7 proton to be characterized and identified for each particular nitrile substituted isomers on the basis of its ^{1}H-NMR splitting pattern. Actually, in NOESY spectrum (Fig. 4) of compound **4a**, very strong correlation has been observed between the H-16 and aromatic proton H-7. Because this best correlated benzene proton (H-7) having orto coupling constant ($J = 8.4$ Hz), it means that this structure of the regioisomer must be the 1,5-isomer without any doubt. Weaker NOE occurs between the methylene protons and 2-phenyl aromatic H-9 and H-13 protons. In contrast, in NOESY spectrum of compound **4b**, this time best correlated benzene proton with H-16 give the *m*eta-coupling constant ($J = 1.2$ Hz), so the CN group must be substituted at C-6 position. Similar observation was received in the NOESY spectra of the compound **5a** and **5b.** Because some of the aromatic protons of

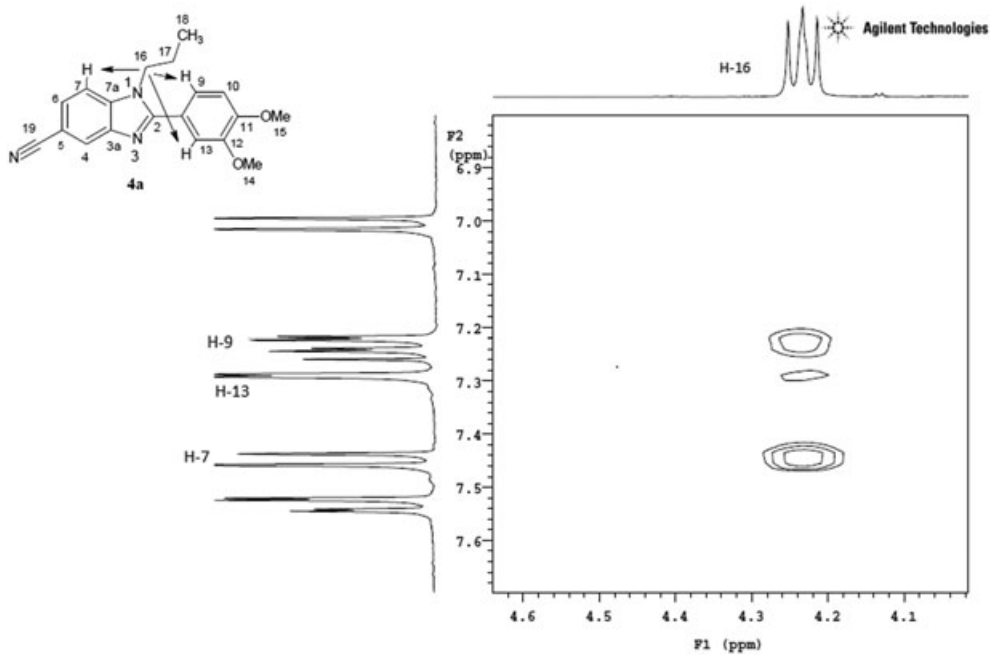

Figure 4. NOESY spectrum of compound **4a** in $CDCl_3$.

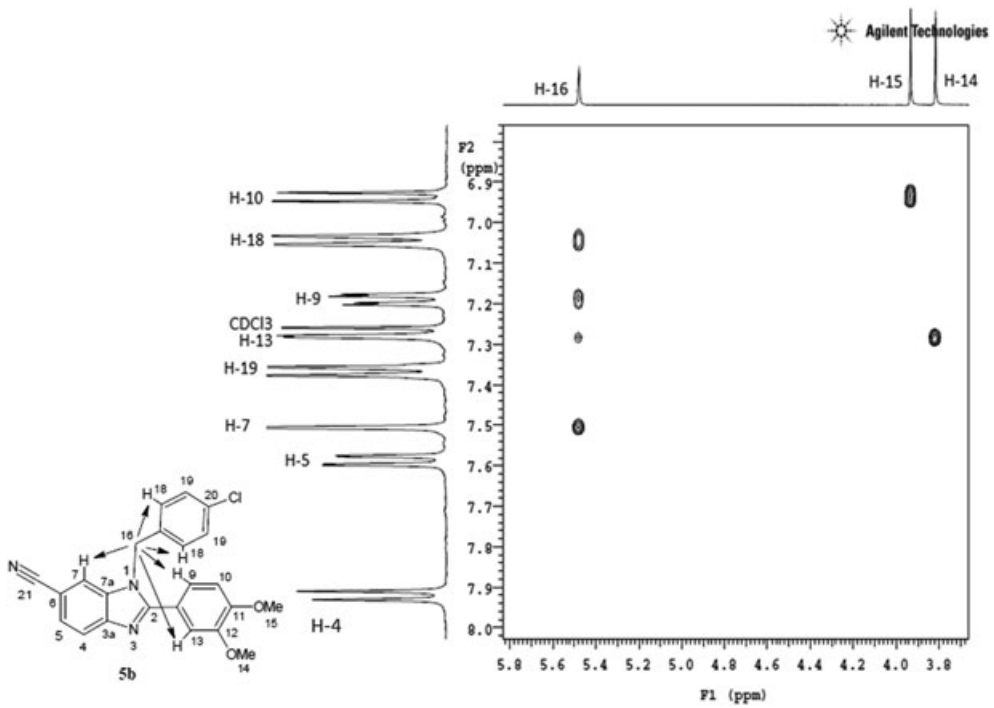

Figure 5. NOESY spectrum of compound **5b** in $CDCl_3$.

compound **5b** in CD_2Cl_2 are observed as overlapped, the spectrum was run in $CDCl_3$ for NOESY experiment (Fig. 5). This time NOESY interaction particularly between H-16 and H-7 has been observed very clearly. However, in the $CDCl_3$ one carbon atom was missing (overlapped), that is why, total NMR characterization was made in CD_2Cl_2 in Table 2. The complete assignments of **4a, 4b** (Table 1) and **5a, 5b** (Table 2) were made using 1D and 2D NMR including full DEPT, COSY, NOESY, HSQC and HMBC techniques in $CDCl_3$ and CD_2Cl_2.

Conclusion

It is well known since 19th century,[22] there is a rapid exchange between the —NH— and =NH— nitrogen atoms in benzimidazole

ring, and two tautomers may be drawn for the compound **3** (Fig. 1). The 5 and 6 positions (as well as 4 and 7 positions) and any group present at that position in the ring system is chemically equivalent. However, tautomerism is no longer possible in *N*-substituted benzimidazoles and two distinct non-equivalent molecules or regioisomers may be isolated and characterized. NOESY experiments is the best available methods for the structural elucidation of these regioisomers.

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