Spectral Assignments and Reference Data

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Complete and unambiguous assignments of ^{1}H and ^{13}C chemical shifts of new arylamino derivatives of *ortho*-naphthofuranquinones

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Six new nor-β-lapachones have been synthesized from reaction of 3-bromo-nor-β-lapachone with arylamines. These derivatives have potent anticancer properties against several cell lines. Here, we report complete unambiguous assignments of ^{1}H and ^{13}C chemical shifts of the new compounds. The assignments were made using a combination of one- and two-dimensional NMR techniques (^{1}H, ^{13}C, $^{1}H-^{1}H$ COSY, $^{1}H-^{13}C$ HSQC, and $^{1}H-^{13}C$ HMBC).

Keywords: NMR; ^{1}H NMR; ^{13}C NMR; naphthofuranquinones; nor-β-lapachones

Introduction

Quinones are an important class of synthetic and natural products with much benefits due to several related biological activities.[1] Recently, new quinones have been found as potential drug candidates for antitumor,[2] trypanocidal,[3] molluscicidal,[4] leischmanicidal,[5] anti-inflammatory,[6] and antifungal[7]activities. Among the quinones that deserves mention is lapachol.[8]The quinones promote DNA scission through the redox cycling-based generation of super-oxide anion radicals.[9] β-Lapachone, an isomer of lapachol, is a natural product that can be found in the heartwood of *Tabebuia sp.* of South and Central America. It directly targets Topoisomerase I[10] and II,[11] and has been the subject of investigation for clinical use in cancer therapy.[12]

Among the quinones, the furan derivatives, more specifically, the naphtho[2,3-*b*]furan-4,9-dione derivatives, are widely present in nature and they have several important biological activities, such as anticancer, antibacterial, and anti-inflammatory.[13]

Recently, we described our efforts on the synthesis of new arylamino derivatives of nor-β-lapachone and nor-α-lapachone. The modified arylamino quinones showed anticancer activities on human tumor cell lines indicating their potential as interesting new lead compounds in anticancer drug development.[2]

The information derived from the spectral analysis of naphthoquinones can be used to differentiate between *ortho*- and *para*-substituted isomers and establish the size of the heterocyclic ring attached on the naphthoquinone moieties.[14–16] Although some reports on NMR spectra of *para* and *ortho*-naphthofuranquinones have appeared in the literature in the past,[17–19] some of them have incorrect ^{1}H or ^{13}C assignments,[7,16,17] mainly due to the lack of high-field spectrometers and adequate pulse sequences at the time.

In this article, we report the complete and unambiguous assignments of ^{1}H and ^{13}C chemical shifts of six *ortho*-naphthofuranquinones **2–7**. These results can be used as models for the assignment of compounds belonging to the 2H-naphtho[2,3-*b*]-furan-4-one ring system. The assignments were made by means of a combination of one- and two-dimensional NMR techniques: ^{1}H and ^{13}C NMR, $^{1}H-^{1}H$ COSY, $^{1}H-^{13}C$ HSQC and $^{1}H-^{13}C$ HMBC.

Experimental

Methods

NMR spectra were collected in $CDCl_3$ solution at 25 °C on a Varian Unity Plus spectrometer operating at 299.9 MHz for proton. All one-dimensional and $^{1}H-^{1}H$ COSY spectra were obtained using a 5 mm switchable broadband probe, while $^{1}H-^{13}C$ HSQC and $^{1}H-^{13}C$ HMBC spectra were obtained using a 5 mm indirect detection broadband probe. The sample concentrations were approximately 60 mM for ^{13}C analysis, and 20 mM for the other experiments. Chemical shifts (δ) were reported in ppm and coupling constants (J) in Hz. The chemical-shift standard was internal tetramethylsilane (TMS) for both ^{1}H and ^{13}C.

^{1}H spectra were acquired with a 4000 Hz spectral width, using a 90° pulse of 16.1 μs and a 2 s relaxation delay. The 16-transient free-induction decay (FID) was collected with 16 384 data points, multiplied by a 0.15 Hz Lorentzian function, and zero-filled to 32 768 data points, prior to Fourier transformation (FT).

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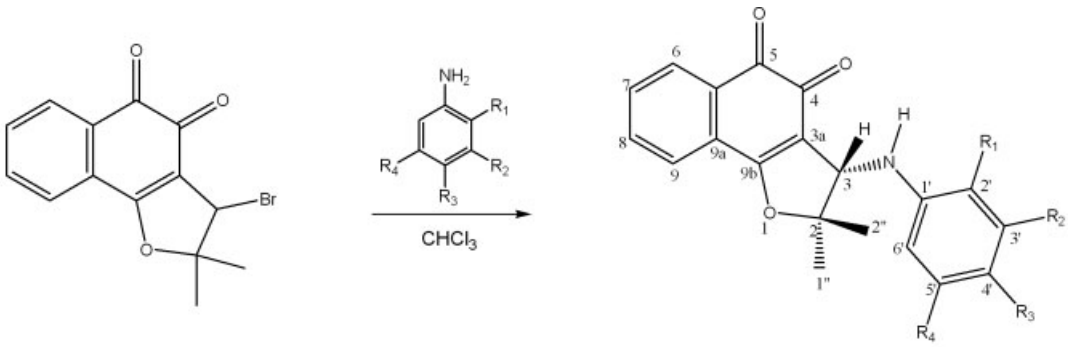
^{13}C spectra were acquired with a 19502.7 Hz spectral width, using a 30° pulse of 4.6 μs and a 0.4 s relaxation delay. The 1868 transient FID was collected with 71 680 data points, multiplied by a 1.0 Hz Lorentzian function, and zero-filled to 143 360 data points, prior to FT.

The $^{1}H-^{1}H$ COSY spectra were obtained as a matrix of 256 × 1024 ($t1 \times t2$) data points, with eight transients per increment, a 1 s relaxation delay, and a spectral width of 4000 Hz in both dimensions. The $t2$ and $t1$ domains were multiplied by a squared sine bell function, and zero-filled to 2048 data points, prior to 2D FT.

The multiplicity edited $^{1}H-^{13}C$ HSQC spectra were obtained as matrices of 256 × 2048 ($t1 \times t2$) data points, with 12 transients per increment and a 1 s relaxation delay. The spectral width was 4000 Hz in F2 and 10 778.8 Hz in F1 with the transmitter for Globally optimized Alternating phase Rectangular Pulse (GARP) decoupling



1 **2-7**

Derivative	R_1	R_2	R_3	R_4	yield (%)
2	Me	H	H	Me	50
3	H	H	F	H	75
4	H	Cl	H	H	70
5	H	Br	H	H	70
6	H	NO_2	H	H	95
7	H	F	H	H	70

*40 mmol of arylamine was added to a solution of 1 mmol of **1** in 25 mL of chloroform. The mixture was stirred for 30 min. at room temperature. The crude product was purified by column chromatography.

Scheme 1. Syntheses of naphthofuranquinones **2**–**7** from 3-bromo-nor-β-lapachone.

Table 1. ^{1}H NMR chemical shifts (δ, ppm), multiplicities and coupling constants (*J*, Hz) for compounds **2**–**7**

	Naphthofuranquinones					
H	**2**	**3**	**4**	**5**	**6**	**7**
3	4.85 (s)	4.72 (s)	4.78 (s)	4.78 (s)	4.84 (s)	4.78 (d, 6.9)
6[a]	8.14 (ddd, 7.7,1.3, 0.5)	8.10 (ddd, 7.7,1.2, 0.4)	8.12 (ddd, 7.7,1.3, 0.5)	8.10 (ddd, 7.7,1.2, 0.4)	8.01 (ddd, 7.7,1.2, 0.4)	8.10 (ddd, 7.7,1.3, 0.5)
7[a]	7.65 (ddd, 7.7, 7.6, 1.2)	7.64 (ddd, 7.7, 7.6, 1.1)	7.65 (ddd, 7.7, 7.6, 1.2)	7.65 (ddd, 7.7, 7.6, 1.2)	7.64 (ddd, 7.7, 7.7, 1.1)	7.64 (ddd, 7.7, 7.6, 1.2)
8[a]	7.71 (ddd, 7.6, 7.6, 1.3)	7.71 (ddd, 7.6, 7.6, 1.2)	7.70 (ddd, 7.6, 7.6, 1.3)	7.70 (ddd, 7.6, 7.6, 1.2)	7.71 (ddd, 7.7, 7.6, 1.2)	7.70 (ddd, 7.6, 7.6, 1.3)
9[a]	7.75 (ddd, 7.6, 1.2, 0.5)	7.75 (ddd, 7.6, 1.1, 0.4)	7.73 (ddd, 7.6, 1.2, 0.5)	7.73 (ddd, 7.6, 1.2, 0.4)	7.75 (ddd, 7.6, 1.1, 0.4)	7.73 (ddd, 7.6, 1.2, 0.5)
2′	–	6.52 (dd, 9.2, 4.5[b])	6.56 (t, 2.2)	6.72 (t, 1.5)	7.38 (t, 1.9)	6.27 (dt, 11.3[b], 2.3)
3′	6.95 (d, 7.5)	6.89 (t, 9.2[c])	–	–	–	–
4′	6.53 (d, 7.5)	–	6.72 (ddd, 8.0, 2.2, 0.7)	6.86 (dd, 7.9, 1.5)	7.49 (dd, 8.0, 1.9)	6.43 (ddd, 8.6[b], 8.2, 2.3)
5′	–	6.89 (t, 9.2[c])	7.09 (t, 8.0)	7.03 (t, 7.9)	7.21 (t, 8.0)	7.10 (ddd, 8.2, 8.2, 6.7[b])
6′	6.34 (s)	6.52 (dd, 9.2, 4.5[b])	6.46 (ddd, 8.0, 2.2, 0.7)	6.50 (dd, 7.9, 1.5)	6.85 (dd, 8.0, 1.9)	6.35 (dd, 8.2, 2.3)
1″	1.58 (s)	1.58 (s)	1.58 (s)	1.58 (s)	1.59 (s)	1.58 (s)
2″	1.72(s)	1.66 (s)	1.68 (s)	1.68 (s)	1.73 (s)	1.68 (s)
R_1	2.08 (s)	–	–	–	–	–
R_4	2.30 (s)	–	–	–	–	–

[a] The chemical shifts and the coupling constants were extracted by simulation.
[b] J_{HF}.
[c] $J_{HH} = J_{HF}$.

set to the center of F1. A one-bond coupling constant value of 140 Hz was used to select direct correlations. The *t*2 domain was multiplied by a Gaussian function and zero-filled to 2048 data points. In *t*1, forward linear prediction was used to predict 1024 data points, prior to multiplication by a Gaussian function, zero filling to 4096 data points and 2D FT.

The $^{1}H-^{13}C$ HMBC spectra were obtained as matrices of 256 × 2048 (*t*1 × *t*2) data points each with 84 transients and a 1 s relaxation delay. The spectral width was 4000 Hz in F2 and 15 083.0 Hz in F1. Long-range correlations were optimized for a 10 Hz coupling, and a value of 140 Hz was used to suppress direct correlations. The *t*2 domain was multiplied by a shifted Gaussian function and zero-filled to 2048 data points. In *t*1, forward linear prediction was used to predict 1024 data points, prior to multiplication by a sine bell function, zero filling to 4096 data points and 2D FT.

All spectra were processed and analyzed using the MestReC 4.6 software (Mestrelab Research SL, Spain). The spin-systems simulation was performed using SpinWorks 2.5 software (Dr Kirk Marat, Canada).

Materials

The syntheses for compounds **2–7** have been published elsewhere.[2] The naphthofuranquinones **2–7** were obtained, as a racemic mixture, in good yields by nucleophilic substitution of the bromide group of 3-bromo-nor-β-lapachone (**1**) by arylamines and the compounds were purified by column chromatography in silica gel, eluted with an increasing polarity gradient mixture of hexane and ethyl acetate (9/1–7/3) (Scheme 1). The purity of the compounds was observed by high-resolution mass spectra.

Table 2. ^{13}C NMR chemical shifts (δ, ppm) multiplicities and coupling constants (*J*, Hz) for compounds **2–7**

	Naphthofuranquinones					
C	**2**	**3**	**4**	**5**	**6**	**7**
2	96.8	96.7	96.7	96.7	96.6	96.6
3	61.8	62.4	61.4	61.3	61.1	61.6
3a	115.3	115.0	114.7	114.7	114.3	114.8
4	175.3	175.4	175.3	175.3	175.2	175.3
5	180.9	180.8	180.8	180.8	180.7	180.8
5a	131.2	131.1	131.1	131.1	130.9	131.2
6	129.5	129.5	129.5	129.5	129.3	129.5
7	132.5	132.5	132.6	132.5	132.6	132.6
8	134.6	134.6	134.6	134.6	134.5	134.6
9	125.1	125.1	125.1	125.1	125.1	125.1
9a	127.5	127.3	127.2	127.2	127.0	127.3
9b	169.4	169.6	169.7	169.8	170.2	169.6
1′	145.2	143.5	148.3	148.4	148.0	149.0 (d, 10.6)[a]
2′	119.5	114.1 (d, 7.7)[a]	112.8	115.7	106.6	99.9 (d, 25.4)[a]
3′	130.2	115.7 (d, 22.6)[a]	135.0	123.2	148.9	164.0 (d, 241.4)[a]
4′	118.4	155.0 (d, 234.8)[a]	118.0	120.9	112.2	104.6 (d, 21.3)[a]
5′	136.5	115.7 (d, 22.6)[a]	130.3	130.6	129.5	130.4 (d,10.0)[a]
6′	111.1	114.1 (d, 7.7)[a]	111.3	111.7	118.6	108.9 (d,1.7)[a]
1″	21.6	21.7	21.7	21.7	21.6	21.7
2″	27.3	27.4	27.3	27.4	27.3	27.3
R_1	17.0	–	–	–	–	–
R_4	21.7	–	–	–	–	–

[a] J_{CF}.

Results and Discussion

The ^{1}H and ^{13}C NMR spectra of the compounds **2–7** were well resolved and chemical-shift assignments were based on the analysis of the multiplicity patterns of proton resonances and also on the use of homonuclear $^{1}H-^{1}H$ COSY and heteronuclear $^{1}H-^{13}C$ HSQC and $^{1}H-^{13}C$ HMBC spectra. The nuclear Overhauser effect (NOE) experiment helped to establish the relative configuration between H-3 and the C-2″ (methyl group). The full characterization of compounds **2–7** is presented in Tables 1 and 2.

^{1}H multiplets from the naphthoquinone rings exhibit inconvenient second-order effects at 7.05 T. In this case, a computer-aided spectral analysis was performed to make the extraction of chemical shifts and coupling constants easier. Figure 1 shows the partial simulated spectra of an ABCD spin system formed by the hydrogens of the naphthoquinone ring.

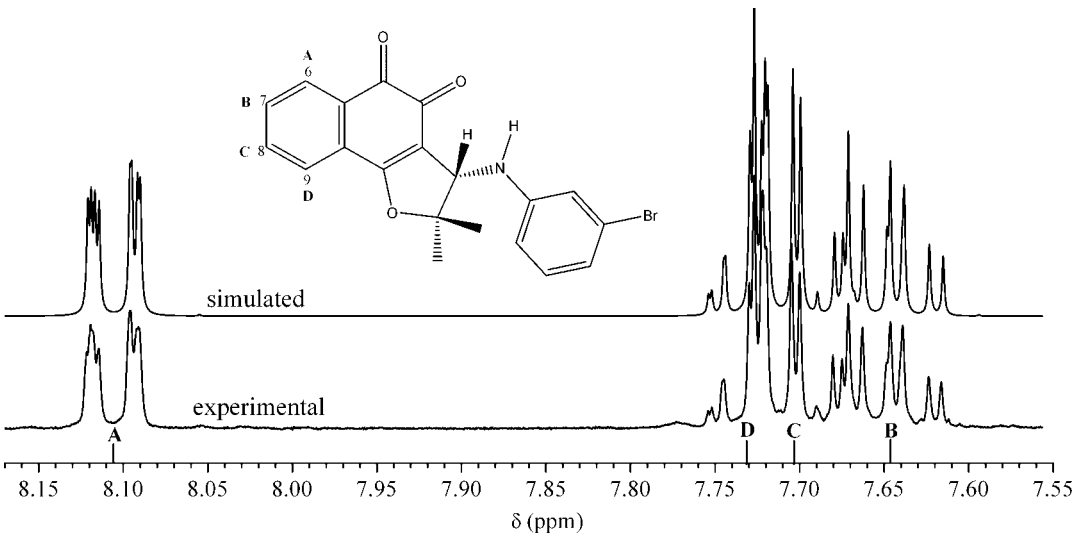


Figure 1. Partial simulated and experimental spectra showing the ABCD spin system formed by the hydrogen naphthoquinone ring from compound **5**.

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Although the NMR experiments and the assignments studies were made for all compounds, the quinone **6** (2,2-dimethyl-3-(3-nitro-phenylamino)-2,3-dihydro-naphtho[1,2-b]furan-4,5-dione) was taken as an example to illustrate the ^{1}H and ^{13}C assignments of the quinones **2**–**7**. We can identify, in ^{1}H NMR spectrum, singlets at $\delta = 1.59$, 1.73, and 4.84 ppm, corresponding to the hydrogens of the two methyl groups and H-3. To confirm the assignments based on the $^{1}H-^{13}C$ HSQC and $^{1}H-^{1}H$ COSY spectra, and to assess more information about the structure of quinone **6**, a $^{1}H-^{13}C$ HMBC spectrum was recorded. From this spectrum we can conclude the following:

The H-3 furan ring hydrogen at $\delta = 4.84$ ppm shows long-range correlation with the carbon resonances for C-3a at $\delta = 114.3$ ppm and C-2 at $\delta = 96.6$ ppm ($^{2}J_{CH}$), C-1′ at $\delta = 148.0$ ppm, C-2″ at $\delta = 27.3$ ppm and C-9b at $\delta = 170.2$ ppm ($^{3}J_{CH}$). The H-1″ signal at $\delta = 1.59$ ppm is correlated with the carbon resonances for C-2 at $\delta = 96.6$ ppm ($^{2}J_{CH}$), for C-3 at $\delta = 61.1$ ppm and C-2″ at $\delta = 27.3$ ppm ($^{3}J_{CH}$). The H-2″ signal at $\delta = 1.73$ ppm is correlated with the carbon resonances for C-2 at $\delta = 96.6$ ppm ($^{2}J_{CH}$), C-3 at $\delta = 61.1$ and C-1″ at $\delta = 21.6$ ppm ($^{3}J_{CH}$) (Fig. 2). The relative configuration between the C-2″ and the hydrogen H-3 of the furan ring was assigned employing the NOE experiment: the H-2″ signal ($\delta = 1.73$ ppm) was enhanced when the H-3 hydrogen ($\delta = 4.84$ ppm) was irradiated.

The H-2′ benzene ring hydrogen at $\delta = 7.38$ ppm shows long-range correlation with the carbon resonances for C-3′ at $\delta = 148.9$ ppm ($^{2}J_{CH}$), C-4′ at $\delta = 112.2$ ppm, and C-6′ at $\delta = 118.6$ ppm ($^{3}J_{CH}$). The H-4′ signal at $\delta = 7.49$ ppm is correlated with the carbon resonances for C-3′ at $\delta = 148.9$ ppm ($^{2}J_{CH}$), C-2′ at $\delta = 106.6$ ppm, and C-6′ at $\delta = 118.6$ ppm ($^{3}J_{CH}$). The H-5′ signal at $\delta = 7.21$ ppm is correlated with the carbon resonances for C-1′ at $\delta = 148.0$ ppm, and C-3′ at $\delta = 148.9$ ppm ($^{3}J_{CH}$). The H-6′ signal at $\delta = 6.85$ ppm is correlated with the carbon resonances for C-2′ at $\delta = 106.6$ ppm, and C-4′ at $\delta = 112.2$ ppm ($^{3}J_{CH}$) (Fig. 3). On the use of homonuclear $^{1}H-^{1}H$ COSY spectrum there were observed correlations between H-4′ and H-5′, H-5′, and H-6′. Additionally, for the compounds **3** and **7,** there were observed the heteronuclear coupling constants, J_{HF} and J_{CF}, for the ^{1}H and ^{13}C signals of the arylamine ring.

The H-6 naphthoquinone ring hydrogen at $\delta = 8.01$ ppm shows long-range correlation with the carbon resonances for C-5 at $\delta = 180.7$ ppm, C-8 at $\delta = 134.5$ ppm, and C-9a at $\delta = 127.0$ ppm ($^{3}J_{CH}$). H-7 signal at $\delta = 7.64$ ppm is correlated with the carbon resonances for C-5a at $\delta = 130.9$ ppm, and C-9 at $\delta = 125.1$ ppm ($^{3}J_{CH}$). The H-8 signal at $\delta = 7.71$ ppm is correlated with the carbon resonances for C-6 at $\delta = 129.3$ ppm ($^{3}J_{CH}$). The H-9 signal is correlated for C-7 at $\delta = 132.6$, and C-9b at $\delta = 170.2$ ppm ($^{3}J_{CH}$) (Fig. 4). The conclusive correlations of the naphthoquinone ring between H-6 and C-5, and H-9 and C-9b can be observed in Fig. 5. On the use of homonuclear $^{1}H-^{1}H$ COSY spectrum there were observed correlations between the hydrogens H-6 and H-7, H-7 and H-8, and H-8 and H-9.

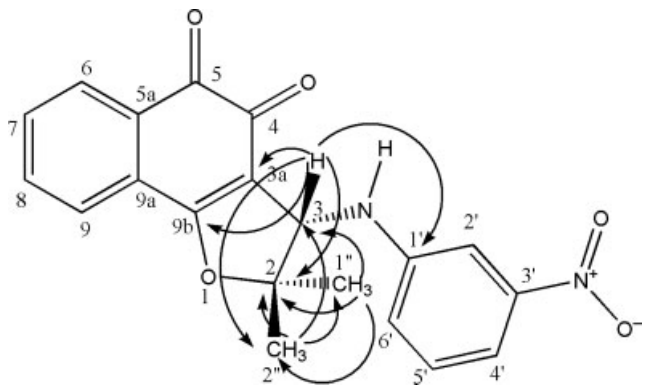

Figure 2. Connectivities found in the compound **6** HMBC spectrum for the furan ring.

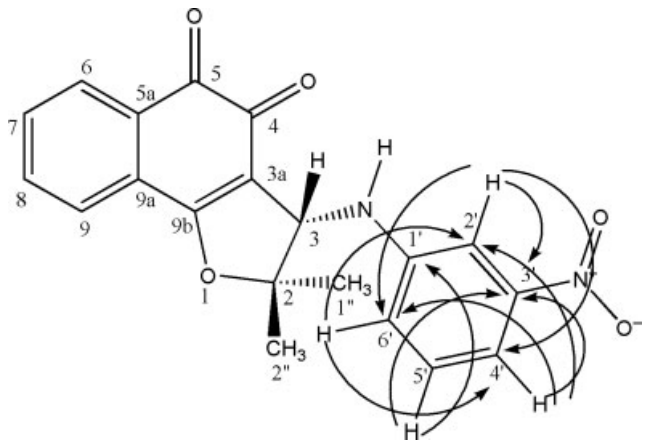

Figure 3. Connectivities found in the compound **6** HMBC spectrum for the benzene ring.

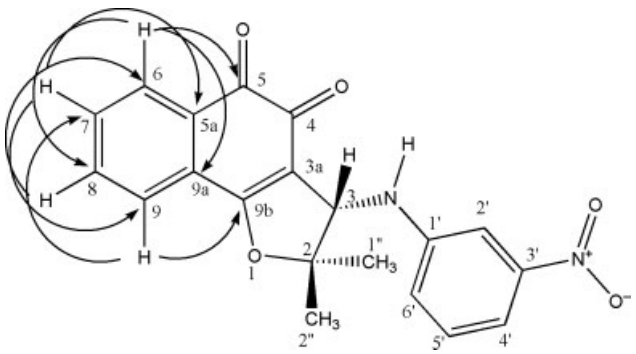

Figure 4. Connectivities found in the compound **6** HMBC spectrum for the naphthoquinone ring.

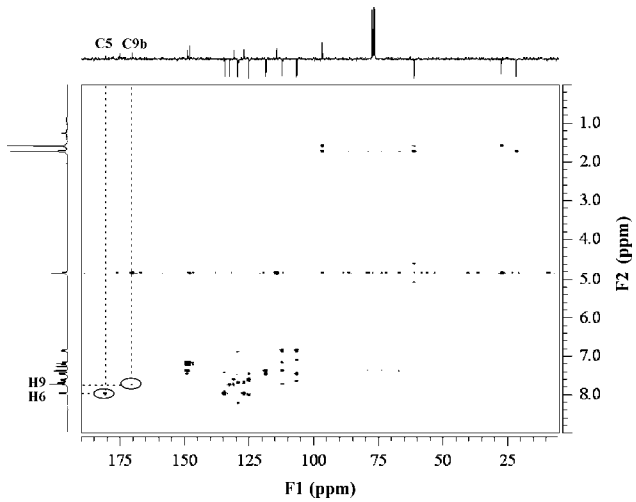

Figure 5. HMBC spectrum of derivative **6**.

In conclusion, we have shown in this article the complete and unambiguous assignments of ^{1}H and ^{13}C chemical shifts of six new *ortho*-naphthofuranquinones **2–7**. We observed that the nature of the substituents on the arylamine ring did not affect significantly the ^{1}H and ^{13}C chemical shifts of the naphthofuranquinone system. Therefore, this work can be used as a model for ^{1}H and ^{13}C assignments of other compounds possessing the 2H-naphtho[2,3-*b*]-furan-4-one system in their structures.

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