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Complete assignment of the ^{1}H and ^{13}C NMR spectra of antimicrobial 4-arylamino-3-nitrocoumarin derivatives

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Herein, we describe the synthesis and complete assignment of the ^{1}H and ^{13}C NMR chemical shifts of a series of antimicrobial 4-arylamino-3-nitrocoumarin derivatives based on a combination of ^{1}H and ^{13}C NMR, ^{1}H-^{1}H-COSY, NOESY, HSQC and HMBC experiments. Conformational effects upon the chemical shifts of the coumarin moiety arising from the anisotropy of the aryl side group are briefly discussed. This study provides the first complete and fully assigned NMR data for this important group of antimicrobial compounds and bridges the gap existing in the literature with regard to NMR structural data for 4-arylamino-3-nitrocoumarins. Copyright © 2010 John Wiley & Sons, Ltd.

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Keywords: ^{1}H NMR; ^{13}C NMR; 2D NMR; 4-arylamino-3-nitrocoumarins; synthesis; structure elucidation; molecular conformation

Introduction

Coumarins are an important class of naturally occurring benzopyranones with a widespread occurrence in plants. They have been used extensively in traditional medicine and are known to have broad pharmacological functions including antibacterial, antifungal, antitumor, anticoagulant, anti-inflammatory, anti-HIV, vasodilator, sedative, hypnotic, analgesic and hypothermic activities.[1–3] We have previously synthesized a number of coumarin derivatives and tested their *in vitro* antimicrobial and antioxidant activities.[4–9] Several of these compounds, in particular 4-arylamino-3-nitrocoumarin derivatives, showed promising activity in reducing the microbial growth in both fungal and bacterial cells.[8,9] Significant antimicrobial activity of a series of 4-substituted-3-nitrocoumarins was also recently confirmed by a QSAR study.[10] Previous research has shown that 4-amino-3-nitrocoumarins, tested for pharmacological activity in mice and rabbits, had neurotropic activity, generally inhibiting spontaneous locomotor activity, decreasing hyperactivity induced by phenamine, and prolonging thiopental sleep.[11] The same study[11] also pointed out to the most active of the derivatives containing a secondary amino group, 3-nitro-4-phenylamino-chromen-2-one, that inhibited phenamine hyperactivity two- to fivefold and prolonged thiopental sleep twofold. Compounds possessing a 4-amino-3-nitrocoumarin moiety were also interesting due to the previously noted steric inhibition of resonance of the C-3 NO_2 group by the C-4 amino group in 4-morpholinyl-3-nitro-chromen-2-one, evident from their IR and UV spectra.[12] The analysis of crystal structures and gas-phase conformations, inferred from single crystal X-ray and molecular modeling experiments, showed that the changes in π-delocalization of the farmacoactive formal 3-amino-2-nitro-acrylic acid derivatives might explain the observed significant difference of the antimicrobial and antioxidant activities and spectral properties of two 4-arylamino-3-nitro-coumarin derivatives [4-(naphthalen-1-ylamino)-3-nitro-chromen-2-one and 3-nitro-4-phenylamino-chromen-2-one].[13]

The structural elucidation of these coumarin derivatives, which is important in the area of organic and medicinal chemistry, is heavily reliant on ^{1}H and ^{13}C NMR spectroscopy. However, many of the reported studies of these derivatives either do not provide NMR spectral data at all, or provide only limited, and often unassigned, NMR data.[14–17] This situation is further complicated by the fact that full NMR assignments are often not performed due to a partial or complete overlap of the proton signals of the coumarinic moiety and also of the aryl side group. The same situation is found in the literature regarding the assignment of the ^{13}C NMR spectral data for many coumarin derivatives.[18–23]

To address the paucity of fully assigned NMR spectral data for these types of compounds, a series of seven 4-arylamino-3-nitrocoumarins of varying complexity were synthesized and their ^{1}H and ^{13}C NMR spectral data fully assigned based on a combination of 1D and 2D NMR experiments including COSY, NOESY, HSQC, and HMBC.

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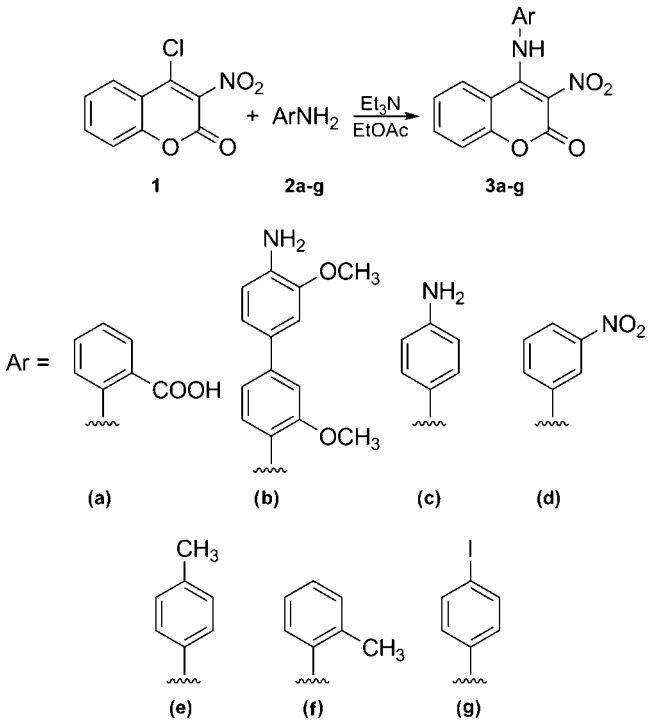
Magnetic Resonance in Chemistry



Scheme 1. Synthesis of 4-arylamino-3-nitrocoumarin derivatives **3a–g**.

Results and Discussion

The reaction of 4-chloro-3-nitrocoumarin (**1**) with a range of differently substituted arylamines (**2a–g**) in a 1:1 molar ratio in ethyl acetate in the presence of triethylamine gave seven 4-arylamino-3-nitrocoumarin derivatives (**3a–g**), in moderate to high yields (65–87%) (Scheme 1). The coumarin derivatives varied in the nature and position of their arylamino substituents including *ortho*-, *meta*-, and *para*-derivatives, along with a mixture of weak and strong electron-donating groups (e.g. methyl, amino) and electron-withdrawing groups (e.g. nitro, carboxylic acid). Five of the derivatives prepared were novel (**3b, 3c, 3d, 3f** and **3g**), while two of the derivatives (**3a** and **3e**) were reported previously.[11,12] However, only limited NMR data are available for the two known compounds (**3a** and **3e**),[11,12] and thus their ^{13}C NMR spectral data, as well as the ^{1}H NMR spectral data for compound **3e**, are reported here for the first time.

The anthranilic acid derivative **3a** was obtained as a yellow crystalline solid. High-resolution electron impact mass spectrometry (HR-EIMS) of **3a** indicated a molecular formula of $C_{16}H_{10}N_2O_6$ ($[M]^+$ at *m/z* 326.0560, $\Delta = +2.1$ mmu). The IR spectrum was found to be identical with that previously described[11] showing characteristic vibrations at 1330 and 1553 cm^{-1} (NO_2), 1703 cm^{-1} (C=O) and 3420 cm^{-1} (O–H, N–H). The ^{1}H NMR spectrum (Table 1) of compound **3a** exhibited eight aromatic methine signals, which appeared as four doublets of doublets and four doublets of triplets. The cross peaks observed in the ^{1}H–^{1}H COSY and NOESY (Fig. 1) spectra for **3a** differentiated two groups of signals, that is, two separate proton spin systems, belonging to rings A and C (Fig. 1). The first group comprised the protons that appear in the ^{1}H NMR spectrum (Table 1) as two sets of doublets of doublets at 7.88 and 7.34 ppm and two sets of doublets of triplets at 7.22 and 7.60 ppm, while the second group consists of two sets of doublets of doublets at 6.86 and 8.11 ppm and two sets of doublet of doublet of doublets at 7.16 and 7.27 ppm, respectively.

From the HMBC spectrum (Fig. 2) it was possible to determine which one of the two groups of aromatic protons mentioned above was bound to which ring. A broad signal at δ 13.78 ppm assigned to the carboxyl proton showed a single interaction with a carbon at 125.9 ppm, attributed to the ipso aryl carbon at C-2′. This carbon (C-2′) in turn showed cross peaks with the protons at 7.16 and 6.86 ppm. The latter was assigned to H-6′ and appeared as a doublet of doublets as expected due to the significantly different coupling constants between H-6′ and H-5′ ($J = 8.0$ Hz) and H-6′ and H-4′ ($J = 1.0$ Hz). The proton signal at 7.16 ppm, which was assigned to H-4′, appeared as a doublet of doublet of doublets as expected due to vicinal coupling with both H-3′ and H-5′ ($J = 8.0$ and 9.0 Hz, respectively) and long-range coupling with H-6′ ($J = 1.0$ Hz).

The aromatic methine resonances appearing at 8.11 and 7.27 ppm were attributed to H-3′ (dd, $J = 8.0$, 1.5 Hz) and H-5′ (ddd, $J = 9.0$, 8.0, 1.5 Hz), respectively, based on similar reasoning to that just described. The carbon methine signals (Table 1) of the C ring (i.e. C-3′, C-4′, C-5′, and C-6′) were readily connected to the aforementioned ^{1}H NMR signals according to correlations in the HSQC spectrum, and further corroborated by HMBC data. Thus, the group of protons with chemical shifts at 6.86, 7.16, 7.27, and 8.11 ppm was convincingly allocated to ring C.

The remaining two carbons of the anthranilic side group, C-1′ and the carboxyl carbon atom, were assigned as follows (Table 1): carbon C-1′ (139.2 ppm) interacted through three bonds with the protons H-3′ and H-5′ as seen in the HMBC spectrum. Additionally, the interaction of H-3′ with a carbon appearing at 172.5 ppm in HMBC, along with its distinctive chemical shift, made it possible to assign the carboxyl carbon.

HMBC was also informative in assigning the peaks of the second proton group. A signal at 7.88 ppm, which appeared as a doublets of doublets, had significant cross peaks with quaternary carbons at 146.0 and 152.5 ppm, as well as with a methine carbon at 133.9 ppm, attached to a proton of 7.60 ppm (HSQC). Additionally, the doublet of doublets at 7.34 ppm showed correlations with carbons at 115.1 and 152.5 ppm (quaternary) and with a carbon at 124.1 ppm, having a HSQC interaction with the resonance at 7.22 ppm in the ^{1}H-NMR spectrum. Thus, the proton occupying position 5 is demonstrated to appear at 7.88 ppm, and interacts through three bonds, as seen in the HMBC spectrum (Fig. 2), with the quaternary carbons C-4 (146.0) and C-8a (152.5), while H-8 (7.34) showed interactions with C-4a (115.1, three bonds apart) and a two-bond interaction with C-8a (152.5). This spectrum also contained evidence that a carbon at 133.9 ppm is C-7 by the presence of a cross peak with H-5, which then allowed assignment of H-7 to the peak at 7.60 ppm (HSQC). Analogously, H-8 correlated with a carbon at 124.1 ppm (C-6), and thus the shift of the proton H-6 was deduced to be 7.22 ppm from HSQC. The assignment of carbons C-4a and C-8a was supported through additional HMBC correlations between H-6 and C-4a and H-7 and C-8a. Finally, the last two carbon signals at 155.4 and 118.9 ppm were attributed to the lactone carbonyl carbon (C-2) and nitro-bearing carbon (C-3), respectively, based on their chemical shifts, since no H interactions were observed in any of the 2D spectra and by comparison with the analogous signals in the biphenyl derivative **3b** (discussed below).

Further confirmation of the NMR assignments of **3a** was obtained from the NOESY spectrum. The proton H-5 was shown to interact with both the carboxyl hydrogen and H-6′, indicating the orientation of the aryl substituent bound to the coumarin framework. The abovementioned cross peaks suggest that the C ring is oriented away from the nitro group and that the A and C

Table 1. NMR data[a] of compounds **3a** and **3b** in $CDCl_3$

	Compound							
	3a				**3b**			
Position	δ_H, m (*J*, Hz)	δ_C	NOESY	HMBC[b]	δ_H, m (*J*, Hz)	δ_C	NOESY	HMBC[b]
2	–	155.4	–	–	–	154.2	–	–
3	–	118.9	–	–	–	117.5	–	–
4	–	146.0	–	–	–	152.1	–	–
4a	–	115.1	–	–	–	113.2	–	–
5	7.88, dd (8.5, 1.0)	125.7	6, 6′, COOH	4, 7, 8a	7.55, dd (8.5, 1.5)	126.9	6	4, 7, 8a
6	7.22, td (8.5, 1.0)	124.1	5, 7	4a, 8	7.00, td (8.5, 1.0)	123.6	5, 7	4a, 8
7	7.60, td (8.5, 1.0)	133.9	6, 8	5, 8a	7.56, ddd (8.5, 8.0, 1.5)	134.8	6, 8	5, 8a
8	7.34, dd (8.5, 1.0)	117.7	7	4a, 6, 8a	7.32, dd (8.0, 1.0)	118.1	7	4a, 6, 8a
8a	–	152.5	–	–	–	152.8	–	–
NH	11.20, brs	–	–	–	11.18, brs	–	2′-OCH_3	3, 4a
1′	–	139.2	–	–	–	143.0	–	v
2′	–	125.9	–	–	–	152.8	–	–
3′	8.11, dd (8.0, 1.5)	131.6	4′	1′, 5′, COOH	7.13, d (1.5)	110.2	2′-OCH_3, 6″	1′, 5′, 1″
4′	7.16, ddd (9.0, 8.0, 1.0)	124.5	3′, 5′	2′, 6′	–	125.4	–	–
5′	7.27, ddd (9.0, 8.0, 1.5)	130.2	4′, 6′	1′, 3′	7.15, dd (8.0, 1.5)	119.2	6′, 2″, 6″	1′, 3′, 1″
6′	6.86, dd (8.0, 1.0)	118.8	5′, 5	2′, 4′	7.19, d (8.0)	125.3	5′	2′, 4′
COOH	13.78, brs	172.5	5	2′	–	–	–	–
2′-OCH_3	–	–	–	–	3.84, s	55.8	3′, NH	2′
1″	–	–	–	–	–	130.0	–	–
2″	–	–	–	–	7.01, d (2.0)	109.1	3″- OCH_3, 5′	4′, 4″, 6″
3″	–	–	–	–	–	147.5	–	–
4″	–	–	–	–	–	136.7	–	–
5″	–	–	–	–	6.80, d (8.0)	114.9	6″	1″, 3″
6″	–	–	–	–	7.07, dd (8.0, 2.0)	120.0	3′, 5′, 5″	4′, 2″, 4″
3″-OCH_3	–	–	–	–	3.95, m[c]	55.7	2″	3″
4″-NH_2	–	–	–	–	3.95, m[c]	–	–	–

[a] Measured at 500 (^1H) and 125 MHz (^{13}C).
[b] HMBC interactions of the hydrogen from the column Position with carbons from the column HMBC.
[c] Overlapping signals.

rings do not lie in the same plane, but rather are at a specific angle to one another. Simultaneous interaction of H-5 with H-6′ and the carboxyl hydrogen is unlikely to occur in a single conformation of the molecule, but may result from single-bond rotation around the C1′–N bond giving rise to different conformations that are close in energy (this was confirmed by energy minimization calculations using MM2 force field from the ChemDraw 11.0 software package).

The biphenyl derivative **3b** was obtained as a brown crystalline solid. The molecular formula ($C_{23}H_{19}N_3O_6$) of **3b** was deduced from the HR-EIMS of the M^+ ion at *m/z* 433.1285 ($\Delta = +1.1$ mmu). N–H and Ar–H absorptions in the range of 3039–3376 cm^{-1} and a strong band at 1702 cm^{-1} corresponding to the C=O group were observed in the IR spectrum of the synthesized compound. Evidence for the presence of the NO_2 group was provided by the IR absorptions that appeared at 1344 and 1537 cm^{-1}.

The ^1H NMR spectrum of compound **3b** contained ten aromatic methine resonances, four of which (7.00, 7.32, 7.55, and 7.56 ppm) were readily grouped together based on their mutual interactions in the NOESY spectrum (Fig. 3). These four aromatic protons were attributed to ring A, due to their splitting patterns (two dds and two ddds) and ^1H–^1H COSY interactions. The chemical shift of the carbon atoms to which these protons were bonded to was subsequently determined from the HSQC spectrum. In the HMBC spectrum (Fig. 4), the proton resonance at 7.55 ppm was correlated with a protonated carbon at 134.8 ppm, which in turn showed an HSQC correlation to the proton signal at 7.56 ppm. Further HMBC

Magnetic Resonance in Chemistry

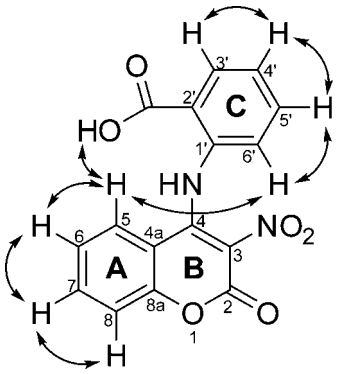


Figure 1. The NOESY correlations of compound **3a**.

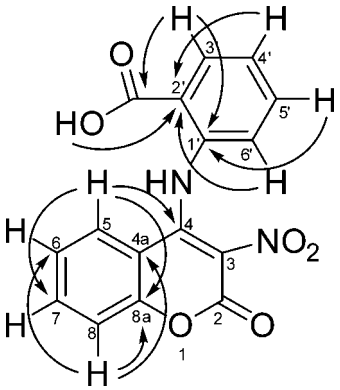


Figure 2. The HMBC correlations of compound **3a**.

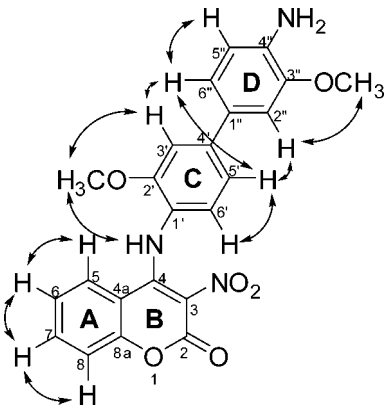


Figure 3. The NOESY correlations of compound **3b**.

correlations for the peak at 7.55 ppm were observed with two quaternary carbons at 152.1 and 152.8 ppm. The proton signal at 7.32 ppm showed HMBC cross peaks with a carbon signal at 123.6 ppm (to which a proton with a signal at 7.00 ppm was bonded), and to two quaternary carbon signals at 113.2 and 152.8 ppm.

On the basis of the data described above, it can be concluded that the proton resonance at 7.55 ppm (dd) occupies position 5 on the A ring of compound **3b**. H-5 showed through space interactions with C-7 (134.8 ppm, thus also making possible the assignment of H-7, 7.56 ppm), C-4 (152.1 ppm), and C-8a (152.8 ppm). H-5 also showed vicinal coupling with H-6 (which must then appear at 7.00 ppm, in accordance with the NOESY data) and long-range

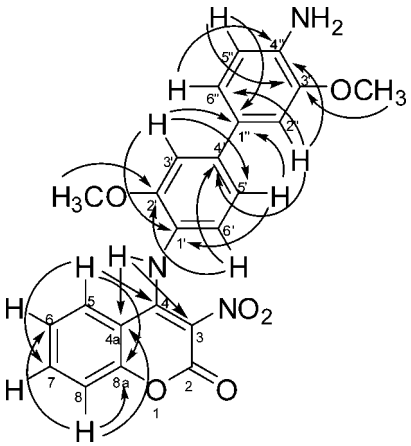


Figure 4. The HMBC correlations of compound **3b**.

coupling with H-7 of 8.5 and 1.5 Hz, respectively. The remaining doublet of doublets from this group at 7.32 ppm was thereby assigned to H-8, and confirmed by HMBC, which showed a three-bond correlation with the C-6 (123.6 ppm) and C-4a (113.2 ppm) signals, and through two bonds with C-8a at 152.8 ppm.

In addition to the aromatic protons, integration of the ^{1}H NMR spectrum of **3b** shows an additional nine protons: one at 11.18 ppm, five at 3.95 ppm, and three at 3.84 ppm. The signal at 11.18 ppm was readily assigned to the proton of the secondary amino group connecting the coumarin and dianisidine parts of the molecule based on its chemical shift. The two protons of the primary amino group and the three protons of one of the methoxy groups were found to overlap as a multiplet at 3.95 ppm, while the other methoxy protons appeared as a singlet at 3.84 ppm. A NOESY interaction between the signals at 11.18 ppm and 3.84 ppm allowed the assignment of the latter signal to the methoxy group on the C ring and demonstrated that these two groups are close in space to one another. A further NOESY cross peak between the methoxy protons with a signal at 7.13 ppm was used to assign the H-3′ proton, and accordingly the C-3′ carbon (110.2 ppm, HSQC). The remaining protons of the C ring were determined from HMBC data (Fig. 4). The three-bond coupling of the H-3′ proton to the carbon atom at 119.2 ppm, which was in turn correlated to the proton at 7.15 ppm (by HSQC), allowed the assignment of these two resonances as C-5′ and H-5′, respectively. The proton H-6′ was resolved by its interaction with H-5′ in the NOESY spectrum, and C-6′ assigned from HSQC data. The remaining carbons on the C ring were identified on the basis of HMBC interactions with the assigned protons.

The assignment of the D ring carbons and protons was performed starting from the three-bond interaction of H-3′ and H-5′ with a carbon at 130.0 ppm in the HMBC spectrum. Since this carbon atom does not fit into the C ring, it was obvious that its position should be C-1″. This was also confirmed by a three-bond correlation with a proton at 6.80 ppm identified as H-5″. Further interactions observed for H-5″ aided the assignment of C-3″, H-6″, and C-6″ from the corresponding spectra. The carbon resonance for C-3″ at 147.5 ppm is also correlated with the D ring methoxy protons (3.95 ppm) through three bonds. The remaining aromatic proton (H-2″) was assigned to the signal at 7.01 ppm, which was confirmed by its interaction with C-6″ (HMBC). Both H-2″ and H-6″ showed a three-bond correlation with a C signal at 136.7 ppm, assigned to the carbon atom bonded to the NH_2 group (C-4″).

Table 2. ^{1}H NMR chemical shifts (δ, ppm), multiplicity and coupling constants (J, Hz) of coumarin derivatives **3c–g** (500 MHz, $CDCl_3$)

	Compound				
Position	**3c**	**3d**	**3e**	**3f**	**3g**
H-5	7.38, dd (8.5, 1.5)	7.41, dd (8.5, 1.5)	7.36, dd (8.5, 1.5)	7.70, dd (8.5, 1.5)	7.31, dd (8.5, 1.5)
H-6	6.96, td (8.5, 1.5)	7.06, ddd (8.5, 7.5, 1.5)	6.99, td (8.5, 1.0)	7.08, td (8.5, 1.5)	7.04, td (8.5, 1.0)
H-7	7.54, td (8.5, 1.5)	7.64, ddd (8.5, 7.5, 1.5)	7.56, ddd (8.5, 8.0, 1.5)	7.61, td (8.5, 1.5)	7.60, td (8.5, 1.5)
H-8	7.30, dd (8.5, 1.5)	7.28, dd (8.5, 1.5)	7.29, dd (8.0, 1.0)	7.32, dd (8.5, 1.5)	7.35, dd (8.5, 1.0)
NH	11.54, brs	10.71, brs	11.28, brs	11.42, brs	11.04, brs
H-2′	7.03, d (8.5)	8.11, dd (2.5, 2.0)	7.15, d (8.0)	–	6.99, d (8.5)
H-3′	6.72, d (8.5)	–	7.25, d (8.0)	7.14, dd (8.0, 1.0)	7.77, d (8.5)
H-4′	–	8.22, ddd (8.0, 2.0, 1.0)	–	7.23, ddd (8.0, 7.0, 1.0)	–
H-5′	6.72, d (8.5)	7.63, t (8.0)	7.25, d (8.5)	7.34, ddd (8.5, 7.0, 1.0)	7.77, d (8.5)
H-6′	7.03, d (8.5)	7.53, ddd (8.0, 2.5, 1.0)	7.15, d (8.5)	7.32, dd (8.5, 1.0)	6.99, d (8.5)
C-2′- $C\underline{H}_3$	–	–	–	2.37, s	–
C-4′-$C\underline{H}_3$	–	–	2.42, s	–	–
NH_2	3.91, s	–	–	–	

The HMBC spectrum of the biphenyl derivative **3b** possessed an important piece of information regarding the connection of the coumarin and dianisidine moieties. The proton resonance for the secondary amino group (N–H) showed a three-bond correlation with both C-4a from the juncture of rings A and B and with the carbon at 117.5 ppm assigned to C-3. This, coupled with NOESY data for the NH signal, confirms the existence of the amino bridge. The only remaining carbon to be assigned was C-2, the lactone carbon, which was thereby attributed to the signal appearing at 154.2 ppm in the ^{13}C spectrum. The mutual spatial relation between the C and D rings can be ascertained from proton NOESY interactions. For example, interactions between both H-3′ and H-6″ and H-5′ and H-6″ in the NOESY spectrum suggest that the C and D rings are not in the same plane but rather are at an angle to one another, and/or able to adopt different low-energy conformations through rotation about the single bond connecting C-4′ and C-1″.

The ^{1}H and ^{13}C NMR spectral data for the five other coumarin derivatives (**3c–g**) were fully assigned using similar reasoning to that described above, with the results presented in Tables 2 and 3. In compounds having *ortho*-substituents on the aryl side groups (**3a, 3b,** and **3f**), the chemical shift of H-5 and H-6 is increased in value compared to the analogous peaks in the compounds without *ortho*-substituted aryl groups. This may be a consequence of a different spatial arrangement resulting in differing anisotropic influence of the aryl groups on these protons.

Table 3. ^{13}C NMR chemical shifts (δ, ppm) of coumarin derivatives **3c–g** (125 MHz, $CDCl_3$)

	Compound				
Position	**3c**	**3d**	**3e**	**3f**	**3g**
C-2	154.7	153.4	154.1	154.5	153.8
C-3	117.4	115.3	117.3	116.6	110.1
C-4	152.2	151.3	151.6	150.7	151.3
C-4a	112.3	114.5	112.3	112.8	111.8
C-5	128.4	127.3	128.0	126.7	128.0
C-6	123.5	124.0	123.6	124.0	123.8
C-7	134.8	135.4	134.9	134.6	135.3
C-8	118.2	118.5	118.0	117.7	118.4
C-8a	153.2	151.8	153.1	152.4	153.6
C-1′	128.8	143.4	135.6	136.4	138.5
C-2′	126.3	119.2	124.6	136.4	126.4
C-3′	116.0	148.8	130.7	125.7	139.4
C-4′	147.0	122.0	138.5	127.1	92.8
C-5′	116.0	131.1	130.7	131.4	139.4
C-6′	126.3	129.7	124.6	128.5	126.4
C-2′-$C\underline{H}_3$	–	–	–	18.0	–
C-4′-$C\underline{H}_3$	–	–	21.1	–	–

Experimental

General remarks

Melting points were determined on a Kofler hot-plate apparatus and are uncorrected. HRMS(EI) spectra were recorded on a Finnigan-MAT 8230 BE mass spectrometer. The IR measurements (attenuated total reflectance, ATR) were carried out with a Thermo Nicolet 6700 FTIR instrument. For TLC, silica gel plates (Kiesel 60 F_{254}, Merck) were used. Visualization was affected by spraying the plates with 1 : 1 aqueous sulfuric acid and then heating. All the reagents and solvents were obtained from commercial sources (Aldrich, USA; Merck, Germany; Fluka, Germany) and

used as supplied, except the solvents, which were purified by distillation.

NMR spectra

All NMR spectra were recorded at 25 °C in $CDCl_3$ with TMS as an internal standard. Chemical shifts are reported in ppm (δ) and referenced to TMS ($\delta_H = 0$ ppm) in ^{1}H NMR spectra or to residual $CDCl_3$ ($\delta_H = 7.25$ ppm, $\delta_C = 77$ ppm) in heteronuclear 2D spectra. Scalar couplings are reported in Hertz. Typically, 20–30 mg of sample was dissolved in 1 ml of $CDCl_3$, and 0.7 ml of the solution transferred into a 5-mm Norell 507 NMR tube.

The ^{1}H and ^{13}C NMR spectra of compounds **3a–g** were recorded on a Bruker DMX 500 spectrometer operating at 500.26 and 125.8 MHz, respectively, equipped with a 5-mm dual ^{13}C/^{1}H probe head. The ^{1}H spectra were recorded with 16 scans, 1 s relaxation delay, 4 s acquisition time, 0.125 Hz digital FID resolution, 51 280 FID size, with 6410 Hz spectral width, and an overall data point resolution of 0.0003 ppm. The ^{13}C spectra were recorded with Waltz 16^{1}H broadband decoupling, 1024 scans, 0.5 s relaxation delay, 1 s acquisition time, 0.5 Hz digital FID resolution, 65 536 FID size, 31 850 Hz spectral width, and an overall data point resolution of 0.005 ppm.

2D spectra were recorded on a Bruker DMX 500 spectrometer (500.26 MHz for ^{1}H, 125.8 MHz for ^{13}C) equipped with an inverse detection triple resonance 5-mm probe (TXI). Standard pulse sequences were used for 2D spectra. COSY and NOESY spectra were recorded at spectral widths of 5 kHz in both *F*2 and *F*1 domains; 1 K × 512 data points were acquired with 32 scans per increment and the relaxation delays of 2.0 s. The mixing time in NOESY experiments was 1 s. Data processing was performed on a 1K × 1K data matrix. Inverse-detected 2D heteronuclear correlated spectra were measured over 512 complex points in *F*2 and 256 increments in *F*1, collecting 128 (HSQC) or 256 (HMBC) scans per increment with a relaxation delay of 1.0 s. The spectral widths were 5 and 27 kHz in *F*2 and *F*1 dimensions, respectively. The HSQC experiments were optimized for C–H couplings of 145 Hz; the HMBC experiments were optimized for long-range C–H couplings of 10 Hz. Fourier transforms were performed on a 512 × 512 data matrix. $\pi/2$ Shifted sine-squared window functions were used along *F*1 and *F*2 axes for all 2D spectra.

Synthesis of 4-chloro-3-nitrocoumarin (1)

4-Hydroxycoumarin (Merck, Germany) was nitrated using 72% HNO_3 in glacial AcOH according to a published procedure,[12] to afford 4-hydroxy-3-nitrocoumarin. The starting compound **1** was prepared from 4-hydroxy-3-nitrocoumarin following the method of Chechi *et al*.,[24] modified by Kaljaj *et al*.[25] Melting point, IR and ^{1}H NMR spectral data were identical to those described.[24]

General synthesis of 4-arylamino-3-nitrocoumarins (3a–g)

The solution of 4-chloro-3-nitrocoumarin (1 g, 4.4 mmol) and the appropriate arylamine (**2a–g**) (4.4 mmol) in ethyl acetate (10 cm^3) in the presence of triethylamine (1 cm^3, 7.2 mmol) was refluxed for 1–3 h. After cooling, the precipitated solid was filtered off, washed with ethyl acetate and then water. The purity of the synthesized compounds was assessed by TLC.

*2-[(3-nitro-2-oxo-2H-chromen-4-yl)amino]benzoic acid (**3a**, $C_{16}H_{10}N_2O_6$)*

Yellow crystals, yield 79%, mp 226–228 °C (Lit.[11] 227–228 °C). HRMS(EI): M^+ ($C_{16}H_{10}N_2O_6$) 326.0560, requires 326.0539 (Δ = +2.1 mmu). IR (neat): 3420 (O–H, N–H), 1703 (C═O), 1606 (C═C), 1553 and 1330 (NO_2), 1289, 1032, 823, 782 cm^{-1}. For ^{1}H and ^{13}C NMR spectra, see Table 1. For NOESY, HMBC and HSQC spectra, see Supporting information.

*4-[(4′-amino-3,3′-dimethoxybiphenyl-4-yl)amino]-3-nitro-2H-chromen-2-one (**3b**, $C_{23}H_{19}N_3O_6$)*

Brown crystals, yield 72%, mp 215–218 °C. HRMS(EI): M^+ ($C_{23}H_{19}N_3O_6$) 433.1285, requires 433.1274 (Δ = +1.1 mmu). IR (neat): 3376–3039 (N–H and Ar–H), 2941 (C–H), 1702 (C═O), 1608 (C═C), 1537 and 1344 (NO_2), 1224, 1032, 751, 690 cm^{-1}. For ^{1}H and ^{13}C NMR spectra, see Table 1. For NOESY, HMBC and HSQC spectra, see Supporting information.

*4-[(4-aminophenyl)amino]-3-nitro-2H-chromen-2-one (**3c**, $C_{15}H_{11}N_3O_4$)*

Brown crystals, yield 84%, mp 228–230 °C. HRMS(EI): M^+ ($C_{15}H_{11}N_3O_4$) 297.0743, requires 297.0750 (Δ = −0.7 mmu). IR (neat): 3371–3068 (N–H and Ar–H), 1710 (C═O), 1605 (C═C), 1549 and 1332 (NO_2), 1240, 1056, 945, 782, 756 cm^{-1}. For ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3.

*3-nitro-4-[(3-nitrophenyl)amino]-2H-chromen-2-one (**3d**, $C_{15}H_9N_3O_6$)*

Yellow crystals, yield 72%, mp 253–255 °C. HRMS(EI): M^+ ($C_{15}H_9N_3O_6$) 327.0474, requires 327.0491 (Δ = −1.7 mmu). IR (neat): 3348–3080 (N–H and Ar–H), 1689 (C═O), 1606 (C═C), 1548 and 1354 (NO_2), 1266, 1064, 821, 761 cm^{-1}. For ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3.

*4-[(4-methylphenyl)amino]-3-nitro-2H-chromen-2-one (**3e**, $C_{16}H_{12}N_2O_4$)*

Yellow crystals, yield 70%, mp 192–194 °C (Lit.[12] 193–195 °C). HRMS(EI): M^+ ($C_{16}H_{12}N_2O_4$) 296.0815, requires 296.0797 (Δ = +1.8 mmu). IR (neat), 3276–3072 (N–H and Ar–H), 2919 (CH_3), 1693 (C═O), 1610 (C═C), 1548 and 1322 (NO_2), 1208, 1054, 813, 757 cm^{-1}. For ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3.

*4-[(2-methylphenyl)amino]-3-nitro-2H-chromen-2-one (**3f**, $C_{16}H_{12}N_2O_4$)*

Yellow crystals, yield 81%, mp 180–182 °C. HRMS(EI): M^+ ($C_{16}H_{12}N_2O_4$) 296.0781, requires 296.0797 (Δ = −1.6 mmu). IR (neat): 3120–3036 (N–H and Ar–H), 2947 (CH_3), 1705 (C═O), 1612 (C═C), 1556 and 1323 (NO_2), 1218, 1054, 798, 749 cm^{-1}. For ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3.

*4-[(4-iodophenyl)amino]-3-nitro-2H-chromen-2-one (**3g**, $C_{15}H_9IN_2O_4$)*

Yellow crystals, yield 76%, mp 250–253 °C. HRMS(EI): M^+ ($C_{15}H_9IN_2O_4$) 407.9616, requires 407.9607 (Δ = +0.9 mmu). IR (neat): 3286 (N–H), 1686 (C═O), 1604 (C═C), 1548 and 1334 (NO_2), 1207, 1057, 843, 784 cm^{-1}. For ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3.

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Supporting information

Supporting information may be found in the online version of this article.

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