Structural elucidation of a series of 6-methyl-3-carboxamidocoumarins

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Introduction

The original concept of privileged structure introduced by Evans in 1988 has evolved from the original meaning.[1] Nowadays, privileged structures can be defined as molecular frameworks that are able to provide potent and selective ligands for more than one type of biological targets through judicious structural modifications.[2] Benzopyrone based systems, namely chromones and coumarins, are actually recognized as privileged structures[3] and used as templates for the design of new chemical libraries for drug discovery programs.[4]

Simple coumarins are considered an unparalleled template to perform structural modifications allowing the synthesis of an array of compounds with a remarkable pharmacological profile exhibiting diverse activities such as anticancer, antiviral, anti-inflammatory and antimicrobial.[5] Due to the synthetic accessibility and substitution variability these heterocyclic compounds play an important role not only in the Organic Chemistry but also in the Medicinal Chemistry field.

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The application of coumarins in the development of therapeutic solutions for aging related diseases is still an emerging field even though the data acquired so far point out their importance in the development of novel drug candidates for targets ascribed to Alzheimer's and Parkinson's diseases. In the past years our group has screened a large library of benzopyrane based derivatives towards monoaminoxidase isoforms (MAO-A and MAO-B). MAO-B is an important target in Parkinson's disease as it plays a key role in the metabolism of dopamine, a neurotransmitter critical for the maintenance of cognitive function.[6] Within this framework some coumarin-based MAO-B inhibitors have been discovered.[5] Such knowledge led to an ongoing effort to improve their potency and selectivity, and as a result a small library of 6-methyl-3-carboxamidocoumarin derivatives (Table 1) was designed and synthesized. Along this framework, a complete structural elucidation by one- (1D) and two-dimensional (2D) NMR techniques of the heterocyclic compounds is found to be relevant. Therefore, in this work some coumarins of our library containing different electron donor groups (EDG) in diverse positions of the exocyclic aromatic ring (Scheme 1, Table 1) were selected for complete structural analysis. Finally, to complete the study, single crystal X-ray crystallographic analysis of compound **3** (*N*-(4'-hydroxyphenyl)-6-methyl-3-carboxamidocoumarin) was carried out.

Experimental

General procedures

^{1}H and ^{13}C NMR were recorded on a Bruker Avance III 400 NMR spectrometer operating at 400.14 MHz and 100.62 MHz, respectively. For the ^{1}H NMR experiments, the relaxation delay was 90° pulse, spectral width of 8012 Hz and 65K data points. In the case of the ^{13}C NMR experiments the corresponding parameters were 30° pulse, 24038 Hz and 65K respectively and 2.0s relaxation delay. For the Distortionless Enhancement by Polarization Transfer (DEPT) sequence the width of the 90◦ pulse for ^{13}C was 7.7 μs, and the 90º pulse for ^{1}H was 9.8 μs; the delay 2JC, H was set to 2.0 ms. For correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) the data points were set to 2 K × 256 (t2 × t1) with a relaxation delay D_1 of 1.5s. The heteronuclear multiple bond connectivity (HMBC) was acquired with data points set to 4 K × 256 (t2 × t1) and relaxation delay D_1 of 1s. Furthermore, the long-range coupling time for HMBC was set to 71ms. The data were processed using quadratic sine-bell weighting functions in both dimensions. The spectra were recorded at room temperature in 5 mm outer-diameter tubes. Samples were prepared in

deuterated dimethyl sulfoxide (DMSO-d_6) for compounds **1**-**3** and deuterated chloroform ($CDCl_3$) for compounds **4**-**6**. Tetramethylsilane (TMS) was used as internal standard, and chemical shifts (δ) were expressed in parts per million (ppm) and coupling constants (*J)* in Hertz. The ^{1}H and ^{13}C chemical shifts of DMSO-d_6 were 2.5 and 40.0 ppm and of $CDCl_3$ 7.3 and 77.0 ppm.

Electronic impact mass spectroscopy (EI/MS) was carried out on a VG AutoSpec (Fison, Ipswich, United Kingdom) instrument and data was reported as m/z (percentage of relative intensity of the most important fragments).

X-Ray diffraction

A crystal of compound **3** (recrystallized from methanol) was used for X-ray crystallographic analysis. Information about crystal data, experimental data collection conditions and refinement as well as the structural geometric parameters are available as an Electronic Supplementary Publication from the Cambridge Crystallographic Data Centre,[7] (CCDC-1479922) at https://www.ccdc.cam.ac.uk/structures in CIF format. The compound crystallized in space group P-1 with two molecules in the asymmetric unit as a hemisolvate.

Materials

Starting materials and reagents were obtained from commercial suppliers and were used without further purification (Sigma–Aldrich, Portugal). Melting points (mp) are uncorrected and were determined with a Reichert Kofler thermopan or with a Büchi 510 apparatus. Flash chromatography was performed on silica gel (Merck 60, 230–400 mesh) and thin-layer chromatography (TLC) was performed on pre-coated silica gel plates (Merck 60 F254). The spots were visualized under UV detection (254 and 366 nm). Following the workup, the organic solutions were dried over Na_2SO_4. Solvents were evaporated in a Buchi Rotavapor. Organic solutions were dried over anhydrous Na_2SO_4.

Synthesis of coumarin derivatives (1-6)

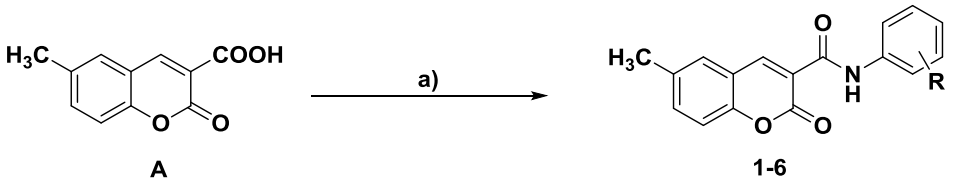
The starting material 6-methylcoumarin-3-carboxylic acid (**A**) was synthesized in a 89% yield according to a previously described method.[8] The structural data (NMR and MS) was in accordance to the literature. [9]

General synthetic method.. Compound **A** (1 mmol) was dissolved in dichloromethane (10 mL) and then 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.1 mmol) and 4-

dimethylaminopyridine (1.1 mmol) were added. The mixture was stirred under a positive argon pressure at 0 °C for five minutes.[10] After this period, the aromatic amine with the appropriated substitution pattern was added. The reaction mixture was stirred for four hours at room temperature. Upon completion, the crude products were filtered and purified by flash chromatography (hexane/ethyl acetate 9:1) or recrystallization (ethanol) to give the desired products (**1**-**6**) in yields ranging from 56% to 82% (see Table 1).[11]

Results and Discussion

A three step synthetic strategy, briefly described in Scheme 1, was used to efficiently obtain the coumarin derivatives (**1**-**6**) efficiently with an overall yield between 56% and 82%.



Scheme 1 - Reagents and conditions: a) EDC, DMAP, DCM, aromatic amine with the appropriate substituent, 0 ºC to r.t., 4h.

All synthesized coumarins (**1**-**6**) are listed in Table 1. To complement compound´s characterization the melting point and mass spectrometry of all the derivatives were also acquired and the data included in Table 1. The unambiguous assignment of all protons and carbons of coumarin derivatives was achieved by means of 1D and 2D resonance techniques. The NMR data are depicted in Tables 2 and 3.

Firstly, the NMR data of compound **1** was acquired. From ^{1}H and ^{13}C spectra thirteen protons and seventeen carbons were observed. In the ^{1}H NMR spectrum (Table 2) the signal at 8.98 ppm was set up as H-4, a characteristic singlet of coumarin nucleus. The protons of the methyl substituent were readily assigned from the spectrum and are found to resonate at 2.41 ppm. Additionally, the protons at 7.83, 7.61 and 7.46 ppm were found to correlate with each other. Taking into account COSY data and proton multiplicity pattern it was concluded that these protons take part of the coumarin core: a *meta* coupling of 1.5 Hz at 7.83 ppm consistent with H-5, an *ortho* and *meta* coupling of 8.5 and 1.5 Hz at 7.61 ppm, and an *ortho*

coupling of 8.5 Hz at 7.46 ppm, that are consistent with H-7 and H-8, respectively, were observed. From HSQC data it was possible to assign the carbons bonded to the protons of the coumarin nucleus. Accordingly, the peaks located at 148.7, 130.4, 135.9, and 116.5 ppm were assigned as C-4, C-5, C-7, and C-8, respectively. As COSY indicates the correlation with coupled protons it was concluded that the four protons located at 6.83, 6.92, 6.96 and 8.39 ppm correlate to each other. Based on their multiplicity pattern and HMBC data they were unequivocally assigned to the protons H-5', H-3', H-4' and H-6´, respectively. The exocyclic aromatic ring protons have been found to be bonded to carbons resonating at 119.6, 115.1, 124.8 and 120.4 respectively. By a linear combination of DEPT-135 and proton decoupled ^{13}C spectra eight quaternary carbons at 118.8, 119.1, 127.0, 135.1, 147.1, 152.7, 159.4 and 161.6 ppm were observed. From 2D spectra analysis it was concluded that the signals at 7.61 ppm (H-7) and at 7.46 ppm (H-8) have a long range correlation with the carbons at 152.7 ppm and 118.8 ppm, respectively. Based on their dissimilar chemical environment the signals were assigned to carbons C-8a and C-4a, respectively. The signals at 161.6 and at 159.4 ppm were assigned to C-2 and C-9, respectively, as they exhibit long range interactions with H-4 and NH/H-4, respectively (Figure 1A). Moreover, a long range interaction was observed between the signal of the proton H-4 and the signal at 119.1 ppm that was assigned as C-3. However, the unequivocal assignment of the quaternary carbons C-1' and C-2' was not possible to be performed from analysis of the HMBC spectrum. For that reason, C-1' was assigned at 127.0 ppm and C-2' at 147.1 ppm using the increment system to estimate carbon chemical shifts of substituted benzene.[12] Conversely, the long range couplings that occur between the proton peak at 10.22 ppm and three carbon peaks (C1', C2' and C3') observed in HMBC (Figure 1B) allowed the unequivocal assign the proton of the hydroxyl function. The chemical shifts of OH and NH protons have been strongly affected by the presence of the two intramolecular hydrogen bonds.

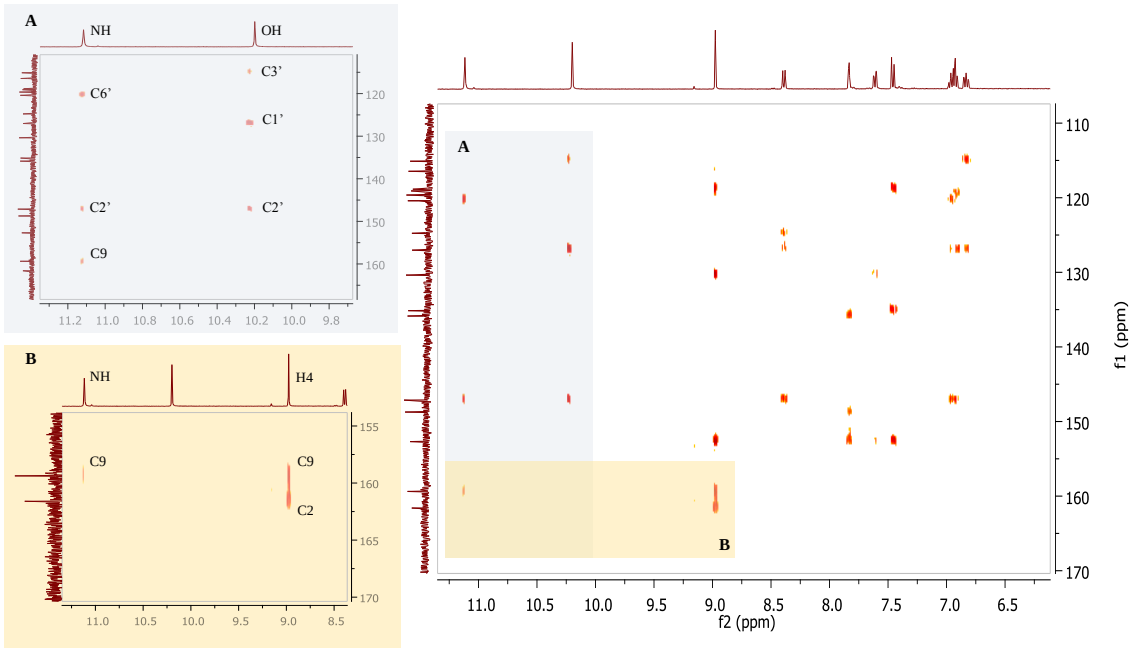


Figure 1 –HMBC spectrum of compound **1**. Sections of the long-range couplings of proton peaks (A) at 10.22 ppm (-OH) and 11.12 ppm (NH) and (B) at 8.98 (H4) and at 11.12 ppm (NH) with structural and carbonyl carbons peaks, respectively, are shown.

As all the compounds herein reported (compounds **1**-**6**, Table 1) own the same coumarin nucleus substitution pattern (3-carboxamide-6-methylcoumarin) as compound **1,** the unambiguous assignment of the sub substructure was performed directly from their 1D and 2D NMR spectra (Tables 2 and 3). However, as they have different substitution patterns on the exocyclic aromatic substituent a thorough spectral analysis was done for compound **2**, which has a hydroxyl group in the *meta* position, and compound **3**, which has an hydroxyl group in *para* position. A correlation analysis similar to that performed compound **1** was tracked.

For compound **2**, H-2', H-4', H-5' and H-6' protons were assigned taking into account the multiplicity pattern of each signal in the ^{1}H NMR spectrum (Table 2), COSY and HMBC correlation data (Figure 2). The peak at 7.31 ppm was assigned as H-2' since it shows a multiplicity pattern of *doublet of doublets* with a constant of 2.1 Hz, owed to the coupling of two *meta* protons. H-4' was attributed to the signal at 6.56 ppm while the peaks at 7.16 and 7.03 ppm were identified as H-5' and H-6', respectively, based on their multiplicity patterns. The corresponding carbons were ascribed based on the HSQC data. Thus, the carbon directly attached to proton H was assigned as 107.3 ppm and C-4' resonate at 111.9 ppm. The quaternary carbons of the exocyclic aromatic ring, namely C-1' and C-3', were assigned

taking into account DEPT-135 and HMBC data. The peak at 139.4 ppm was labelled as C-1'as it has a long range interaction with the proton of the hydroxyl group. The signal at 158.3 ppm was attributed to C-3'.

Regarding compound **3**, the ^{1}H NMR spectrum showed the presence of equivalent protons with the signals at 7.51 and 6.77 ppm. Bearing in mind that the proton of the amide group shows a long range interaction with two carbons at 122.0 ppm while the proton of the hydroxyl group possess a long range interaction with other two carbons at 115.3 ppm, C-2' and C-6' were then assigned at 122.0 ppm. As a result, C-3' and C-5' were found to resonate at 115.3 ppm. From HSQC data the peak at 7.51 ppm was attributed to both H-2'and H-6', whereas H-3' and H-5' were assigned with the signal at 6.77 ppm. In both cases a difference of NH and OH proton shifts were observed, when compared with compound **1,** due to the absence of one of the intramolecular hydrogen bonds.

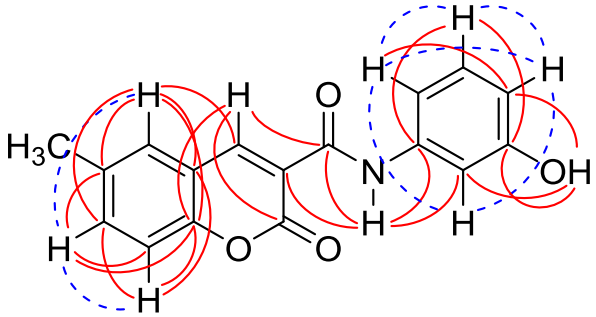


Figure 2 – Correlations observed in COSY (blue dotted lines) and HMBC (red solid lines) spectra of compound **2**.

The NMR signal assignments of compounds **4**, **5** and **6** were performed by a correlation with the data acquired for compounds **1**, **2** and **3** (Tables 2 and 3). However, due to solubility constrains the spectra were recorded using $CDCl_3$ as solvent instead of DMSO-d_6. So, a slight alteration on chemical shifts values was observed. The main changes were found in the ^{1}H NMR multiplicity signals of the coumarin nucleus. In the ^{1}H NMR spectra of compounds **4**, **5** and **6** the H-5 and H-7 appeared as multiplets. In the case of compound **5** it was found a similar multiplet that was assigned as H-5, H-7 and H-2', as it integrates for 3 protons. From the HSQC spectra (compounds **4** and **6**) we can observe that H-7 is more downshielded than H-5. In the case of compound 5 proton H-2' is upshielded in relation to H-5 and H-7.

X-ray diffraction

Compound **3** provided suitable crystals for X-ray analysis. The thermal ellipsoid plot for compound **3** together with the adopted numbering scheme is shown in Figure 3. There are two molecules in the asymmetric unit connected by hydrogen bonding *via* a methanol solvent molecule. The configuration of the molecule is given by the C-N rotamer of the amide which defines the position of the aromatic rings with respect to one other: both molecule displays an *anti* configuration, with respect to carbonyl of the coumarin (Table 1), thus allowing for the establishment of an N–H···O intramolecular H bond (geometric parameters are given in Table 4). In addition, there is a weak hydrogen bond linking the exocyclic aromatic ring, namely between H-2' and the carbonyl of the amide group. Both intramolecular bonds form pseudo S6 rings interactions in each molecule. The two molecules are linked by one molecule of methanol solvent *via* hydrogen bonding where the methanol acts as a bridge since its oxygen atom acts as acceptor of the 4'-hydroxyl group of one molecule of **3** and as a donor to the amide carbonyl oxygen atom in the other molecule. An inspection of the bond lengths shows that there is a slightly asymmetry of the electronic distribution around the pyrone ring: the C23–C24 and C13–C14 bond (Figure 3), correspondent to the pyrone double bond of each molecule, distances have values of 1.350(3) and 1.352(2) Å, respectively. The shorter distance values, which are lower than the expected for a C*ar*–C*ar* bond, indicate the existence of a higher electronic density area in pyrone ring. The values for distances of C13–C131 and C23-C231 bonds connecting the coumarin to the amide spacer are typical of a C_{sp3}–C_{sp3} bond conferring freedom for the rotation of the phenylamide substituent around it. The dihedral angles between the mean planes of the coumarin, OCN spacer and exocyclic aromatic ring (Table 5), indicate that the molecules are essentially planar.

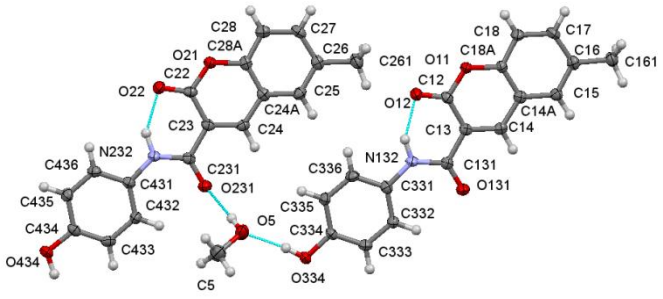


Figure 3 – Structure and the adopted numbering scheme of compound **3**.

Conclusions

In the present work 6-methyl-3-carboxamidocoumarin derivatives (compounds **1**-**6**), bearing electron-donating substituents in different positions of the exocyclic aromatic ring, were synthesized in good yields and in mild reaction conditions and characterized by homo- and hetero-nuclear NMR techniques. Their unequivocal identification constitutes a valuable database for the accurate identification of the coumarins of our library. In addition, these results can be used as reference for structural elucidation of newer naturally occurring and synthetic coumarins.

Acknowledgments

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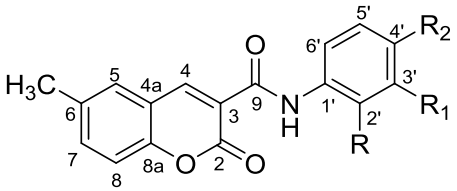
Table 1 – Structure, names, melting points, yields and mass spectroscopy analysis of compounds **1**-**6**.



Compound	R	R_1	R_2	Name	MS/EI (rel. int.)	Melting point / ºC	Yield
1	OH	H	H	*N*-(2'-hydroxyphenyl)- 6-methyl-3-carboxamidocoumarin	295 (M, 46), 187 (100), 115 (12)	274-275	56%
2	H	OH	H	*N*-(3'-hydroxyphenyl)- 6-methyl-3-carboxamidocoumarin	296 (M^+, 40), 295 (M, 99), 187 (100), 115 (64), 103 (51)	217-218	61%
3	H	H	OH	*N*-(4'-hydroxyphenyl)- 6-methyl-3-carboxamidocoumarin	296 (M^+, 26), 295 (M, 92), 187 (100), 115 (25), 103 (18)	230-231	61%
4	OCH_3	H	H	*N*-(2'-methoxyphenyl)- 6-methyl-3-carboxamidocoumarin	310 (M^+, 29), 309 (M, 78), 187 (100), 115 (34), 77 (20).	204-205	82%
5	H	OCH_3	H	*N*-(3'-methoxyphenyl)- 6-methyl-3-carboxamidocoumarin	310 (M^+, 16), 309 (M, 66), 187 (100), 115 (33), 77 (20).	174-175	74%
6	H	H	OCH_3	*N*-(4'-methoxyphenyl)- 6-methyl-3-carboxamidocoumarin	310 (M^+, 70), 309 (M, 96), 187 (100), 115 (62), 77 (44)	199-200	73%

Table 2 – ^{1}H NMR chemical shifts, multiplicity and coupling constants of coumarins **1-6**.

	Compounds					
Position	**1** [a)]	**2** [a)]	**3** [a)]	**4** [b)]	**5** [b)]	**6** [b)]
4	8.98 (s)	8.82 (s)	8.82 (s)	8.94 (s)	8.96 (s)	8.95 (s)
5	7.83 (d, 1.5)	7.78 (d, 1.5)	7.78 (d, 1.6)	*	**	*
				7.46-7.50 (m)	7.46-7.50 (m)	7.46-7.50 (m)
7	7.61 (dd, 1.5,	7.60 (dd, 1.5,	7.58 (dd, 1.6,	*	**	*
	8.5)	8.5)	8.5)			
8	7.46 (d, 8.5)	7.45 (d, 8.5)	7.44 (d, 8.5)	7.32 (d, 8.1)	7.34 (d, 9.1)	7.33 (d, 9.1)
2’	-	7.31 (dd, 2.1,	7.51 (d, 8.7)	-	**	7.65 (d, 9.0)
		2.1)				
3’	6.92 (dd, 1.7,	-	6.77 (d, 8.7)	6.94 (dd, 1.6,	-	6.91 (d, 9.0)
	8.0)			8.0)		
4’	6.94-6.98(m)	6.56 (ddd,	-	7.11 (ddd,	6.72 (ddd,	-
		1.0, 2.1, 8.1)		1.6, 7.8, 8.0)	1.4, 2.5, 7.8)	
5’	6.83 (ddd,	7.16 (dd, 8.1,	6.77 (d, 8.7)	7.00 (ddd,	7.27 (dd, 7.8,	6.91 (d, 9.0)
	1.7, 6.8, 8.0)	8.1)		1.6, 7.8, 8.0)	8.1)	
6’	8.39 (dd, 1.4,	7.01- 7.05	7.51 d, 8.7)	8.54 (dd, 1.6,	7.22 (ddd,	7.65 (d, 9.0)
	8.0)	(m)		8.0)	1.4, 1.6, 8.1)	
CH_3	2.41 (s)	2.40 (s)	2.40 (s)	2.45 (s)	2.46 (s)	2.46 (s)
OH	10.22 (s)	9.54 (s)	9.38 (s)	-	-	-
OCH_3	-	-	-	3.97 (s)	3.84 (s)	3.82 (s)
NH	11.12 (s)	10.58 (s)	10.47 (s)	11.31 (s)	10.87 (s)	10.75 (s)

a) Solvent was DMSO-d_6.
b) Solvent was $CDCl_3$
* H-7 is more downshielded than H-5 (from HSQC data).
** The multiplet integrates for 3 protons. H-2' is upshielded in relation to H-5 and H-7 (from HSQC data).

Table 3 – ^{13}C NMR chemical shifts of compounds **1**-**6**.

	Compounds					
Position	**1** [a)]	**2** [a)]	**3** [a)]	**4** [b)]	**5** [b)]	**6** [b)]
2	161.6	161.2	161.2	159.3	159.5	159.2
3	119.1	120.3	120.2	119.0	118.6	118.6
4	148.7	147.7	147.6	148.5	148.5	148.6
4a	118.8	118.7	118.7	118.5	118.4	118.6
5	130.4	130.2	130.1	129.4	129.5	129.5
6	135.1	135.2	135.1	135.2	135.4	135.4
7	135.9	135.7	135.6	135.3	135.6	135.4
8	116.5	116.5	116.4	116.4	116.4	116.4
8a	152.7	152.5	152.5	152.8	152.7	152.7
9	159.4	160.2	159.6	161.7	162.0	162.0
1’	127.0	139.4	130.0	127.8	138.9	131.0
2’	147.1	107.3	122.0	149.2	106.0	122.1
3’	115.1	158.3	115.3	110.3	160.2	114.2
4’	124.7	111.9	154.6	124.5	111.1	156.7
5’	119.4	130.2	115.3	120.7	129.7	114.2
6’	120.4	111.0	122.0	121.0	112.8	122.1
CH_3	20.7	20.7	20.7	20.8	20.8	20.8
OCH_3	-	-	-	56.0	55.4	55.5

a) Solvent was DMSO-d_6.;
b) Solvent was $CDCl_3$

Table 4 - Hydrogen-bond geometry (distances in Å, angles in º) for compound **3**.

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O5—H5···O231	0.83	1.89	2.719 (2)	176
O334—H334···O5	0.86	1.80	2.655 (2)	173
N132—H132···O12	0.92 (3)	1.83 (3)	2.655 (2)	148 (2)
C332—H332···O131	0.95	2.38	2.972 (2)	120
N232—H232···O22	0.94 (3)	1.87 (3)	2.675 (2)	143 (2)
C432—H432···O231	0.95	2.34	2.936 (2)	120

Table 5 - Selected dihedral angles, ° for compound **3**.

Compound	θ_1°	θ_2°	θ_3°
1	3.28 (11)	3.0 (2)	4.4 (2)
2	2.06 (12)	2.2 (3)	1.1 (3)

θ_1 is the dihedral angle between the mean planes of the coumarin and exocyclic benzene ring . θ_2 is the dihedral angles between the mean planes of the coumarin ring and the plane defined by the OCN amide atoms. θ_3 is the dihedral angle between the mean planes of the exocyclic benzene ring and the plane defined by the OCN amide atoms.