Synthesis of new fluorophores derived from monoazacrown ethers and coumarin nucleus

Ernesto Brunet, Pablo García-Losada, Juan-Carlos Rodríguez-Ubis, and Olga Juanes

Abstract: New ionophores derived from azacrown ethers attached to coumarins have been synthesized and characterized. The alkaline-earth complexes of these new ligands were studied from their UV–vis, NMR, and fluorescence data. Some systems displayed bathochromic shifts and fluorescence decreases upon complexation with Ca²⁺ that may make them useful signaling devices of this metal.

Key words: alkaline earth, emission, molecular modeling.

Résumé : On a synthétisé et caractérisé des nouveaux ionophores dérivés d’éthers aza-couronnes attachés à des coumarines. On a étudié les données de spectroscopie UV–vis, de RMN et de fluorescence des complexes de ces nouveaux ligands avec des métaux alcalino-terreux. Dans quelques systèmes, on observe des déplacements bathochromes et une diminution de la fluorescence lors de la complexation avec du Ca²⁺ ; cette propriété peut les rendre utiles dans les appareils de détection de ce métal.

Mots clés : alcalino-terreux, émission, modelage moléculaire.

Introduction

The coumarin nucleus has been the focus of our recent research concerning the design of photoactive and luminescence probes (1). Coumarins are interesting heterocycles because of their photochemical and photophysical properties (2), leading to numerous industrial and laser dye applications (3). It is well known that coumarins increase their dipolar moment when excited. Albeit the change can be as large as 6 D in some cases, it has been argued that this is too low a variation to support true internal charge transfer (ICT) species in the excited state. Semi-empirical calculations (4) support that it is more likely that the charge separated resonance forms depicted in Fig. 1 have simply a superior contribution in the excited state. Whatever the reason, excitation of coumarin chromophore increases the electron density of its carbonyl groups, this increase being higher when X (Fig. 1) is an effective electron donor as R₂N. Therefore, the interaction of the carbonyl groups of an excited 3-acylcoumarin with metal cations coupled with the presence of an additional cationic receptor might constitute a good signaling device, provided significant selectivity is attained.

In previous work (5), we investigated the behaviour of 3-arylcoumarins 1 (Fig. 2) towards alkaline earth cations. While compound 1b (15-crown-5 derivative) displayed small, similar bathochromic shifts in its UV–vis spectra for the studied cations and, therefore, no selectivity, 1c (18-crown-6 derivative) and especially 1a (ethylenedioxa derivative) (6) suffered quite a large red shift (+48 nm) only when complexed to Mg²⁺.

The conduct of 1a suggested that the carbonyl groups were paramount for the chelation of the metal, the charge–radius ratio of Mg²⁺ being the best fitted within alkaline earth metals, and that only the 15-crown-5 was able to interfere with them. We thus planned the relocation of crowns relative to the 3-acylcoumarin chromophore for them to cooperate, not compete, to obtain different selectivity in cation recognition (7). In this work we report the synthesis of the ligands 2–4 (Fig. 2), in which crown ethers are attached to the 3-position of coumarins through a flexible alkoxy-carbonyl spacer. The alkaline-earth complexes of these new ligands were studied from their UV–vis (Table 1), ¹H NMR (Table 2), and fluorescence data (Table 3).

Results and discussion

Synthesis precious

Preparation of 2–4 were performed in four or five steps from the corresponding 4-substituted salicylaldehyde by means of Knoevenagel condensation with malonic acid as the key step (Scheme 1). This reaction took place with moderate yields except in the case of R = NEt₂, in which it was necessary to use diethyl malonate and hence the additional hydrolysis of the resulting ethyl ester. Subsequent treatment with oxaly chloride led us to the acyl chloride that then re-
acted with the hydroxyethyl azacrown to afford the final compounds in 45–50% yield.

**UV–vis and 1H NMR data**

Ligands 2, 3a, and 3b formed complexes of 1:1 stoichiometry with alkaline earth salts as determined by UV–vis and 1H NMR (CD$_3$CN) titrations. No spectral variations were observed after addition of 1 equiv of metal.

Table 1 and Fig. 3 show that the larger bathochromic shifts occurred for the Et$_2$N derivatives 4 as expected (vide supra, Fig. 1) but, interestingly, with Ca$^{2+}$ (to a lower extent with Ba$^{2+}$) and not with Mg$^{2+}$, in contrast to compounds 1 and other related compounds reported in the literature (8). This sizable red shift indicates that the metal cation closely interacts with the chromophore.

The relatively large deshielding of crown ether NMR proton signals (cf. Table 2) displayed by 4a when titrated with Ca$^{2+}$, demonstrates the cation–crown interaction. Besides, the protons of the coumarin ring of 4a also bear a similar deshielding with Ca$^{2+}$ to that suffered by 1 with Mg$^{2+}$, where the latter can only interact with the carbonyl groups of the 3-acylcoumarin. This fact and UV–vis data strongly suggest the carbonyl–crown cooperation in Ca$^{2+}$ binding. Molecular modeling at the ZINDO/1 semi-empirical level predicts relatively stable structures where both the chromen carbonyl and ester groups may participate in the chelation to Ca$^{2+}$ (Fig. 4).

**Emission spectra**

Only compounds 3 and 4 (a, b) were fluorescent. Table 3 summarizes the emission parameters of the studied compounds. Compounds 3a and 3b showed a slight or moderated increase of fluorescence. The case of complexes 4a and 4b with calcium is noteworthy, as they displayed extinction of fluorescence (up to 54%). In general, the presence of the metals should provide new pathways for collisional or static quenching of emission, which might be enhanced if the chromophore and the crown are close and 4b gives the best host–guest fit.

**Table 1. Wavelength of the absorption maxima (nm) of ligands and alkaline earth complexes.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ligand</th>
<th>Complexes</th>
</tr>
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<tbody>
<tr>
<td>2a</td>
<td></td>
<td>Mg$^{2+}$</td>
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<tr>
<td>3a</td>
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<td>+1</td>
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<tr>
<td>4a</td>
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<td>2b</td>
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<tr>
<td>4b</td>
<td>418</td>
<td>+4</td>
</tr>
</tbody>
</table>

**Scheme 1. Reaction pathway.**

The variation of Stokes’ shifts (SS) upon complexation is even more interesting. This is a manifold phenomenon that has been largely discussed in related compounds (9). After excitation, the molecule relaxes in a lapse of ps to the lowest vibrational level of the first excited electronic state (S$_1$). This relaxation is so fast that it usually occurs before measurements can be made on the system. (10) The lowest S$_1$ state generally has a longer lifetime that allows changes in the local environment, which may even involve ample conformational movements. Therefore, if excitation increases the molecular dipole moment, as largely occurs in coumarins (11), both reorganization of the solvent and a conformational change may be involved to stabilize the excited state, hence making SS larger (12). These facts are summarized in Fig. 5.

Ligands 3 displayed larger SS as compared to 4. Excitation should lead to less stable S$_1$ species in the case of 3 because the charge-separated resonance forms of Fig. 1 must be less stable when the positive charge develops on an OMe group rather than on a NEt$_2$ group. This increases the need for solvent relaxation by excited states have the lowest requirement of extra stabilization either by solvation or conformational changes. This is compatible with the proposed cooperation between the crown and the coumarin, in which the metal plays part of the stabilization role to be performed by the solvent, interacting with the negative end of the charge-separated species. The cooperation between the crown and the coumarin imposes a certain rigidity impeding large conformational changes. The stronger the crown-mediated interaction between the coumarin and the metal, the higher the stabilization of the excited state, and hence, the smaller SS. This occurs for the 4b·Ca$^{2+}$ complex whose spectroscopic data and molecular modeling suggested the strongest coumarin–metal interaction of the studied compounds.
Experimental

General

$^1$H NMR and $^{13}$C NMR: Bruker AC-200 (Departamento de Química Orgánica, DCO) and AMX-300 (Servicio Interdepartamental de Invesigación, SIdI). Mass spectra: VG Autospec spectrometer (SIdI) in FAB mode (L-SIMS$^+$ or EI$^+$). Absorption spectra: Lambda 6 PerkinElmer spectrophotometer (DCO). Excitation and emission spectra: LS50 PerkinElmer spectrofluorometer (DCO). The excitation spectra were automatically corrected, and the emission spectra were corrected according to the instrument guidebook. Elemental analyses (C, H) of compounds 2–4 (Perkin-Elmer CHN 2400 automatic analyzers, SIdI) were correct within

Table 2. $^1$H NMR chemical shifts (δ, ppm) of compounds 1 and 4a and Δδ induced by Mg$^{2+}$ and Ca$^{2+}$.

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<tr>
<th>Compound</th>
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<th>H-8</th>
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Table 3. Wavelengths of the excitation and of the emission maxima (nm).

<table>
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<th>Compound</th>
<th>Ligand</th>
<th>Complexes</th>
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<td></td>
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<td></td>
<td>λ$_{emission}$</td>
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<td>λ$_{emission}$</td>
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<td>Stokes’ shift (1 × 10$^3$, cm$^{-1}$)</td>
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<tr>
<td></td>
<td>Stokes’ shift (1 × 10$^3$, cm$^{-1}$)</td>
<td>2.2</td>
</tr>
</tbody>
</table>

$^a$ΔInt = 100 × (Int$_{comp}$ – Int$_{lig}$)/Int$_{lig}$ (Int = intensity of emission band).

Fig. 3. Spectrophotometric titrations of 4b with Ca$^{2+}$ and Ba$^{2+}$. 
experimental error. All solvents were purified prior to their use. Alkaline earth perchlorates were purchased from Aldrich and used as received. IUPAC names were obtained from ChemWeb: http://cwgen.chemweb.com/autonom/autonomsearch.html.

Synthesis of alkali earth complexes and absorption and emission measurements

The complexes were formed by addition of equimolecular amounts of the corresponding alkaline earth perchlorate salt in acetonitrile (1 × 10⁻² M) to the coumarin solutions (3.2 × 10⁻⁵ M for absorption and 3.2 × 10⁻⁷ M for emission). Absorption–emission parameters were analyzed from the same spectroscopic grade solvent.

General methods

Synthesis of the 3-carboxycoumarin derivatives

The corresponding commercial salicylaldehyde (0.25 mmol) and malonic acid (0.25 mmol) were dissolved in 3 mL of ethanol. Aniline (3 drops) was added, and the mixture was stirred at room temperature (r.t.) for 48 h. Filtration of the cooled mixture yielded a solid which was triturated with water and then refluxed in 6 N HCl (5 mL) for 30 min. After cooling, a white solid formed which was filtered and washed with water yielding the 3-carboxycoumarin as analytically pure crystals. 3-Carboxycoumarines, precursors of 1 and 2, were previously reported (13).

Synthesis of the 3-carbonyl chloride coumarin derivatives

To a mixture of the corresponding 3-carboxycoumarin derivative (1 mmol) and two drops of pyridine in dry toluene under argon was added oxalyl chloride (6 mmol). The reaction compounds were stirred for 48–72 h. The solvent was vacuum evaporated and the residue was triturated with diethyl ether to eliminate the oxalic acid. The solid was filtered off and the filtrate evaporated to yield the 3-carbonyl chloride derivative which was used immediately.

Synthesis of azacrown ethanol derivatives

A solution of 500 mg of the commercial azacrown (15/5 or 18/6) in 25 mL of ethanol, was cooled in an ice bath and 2 equiv of ethylene oxide were bubbled through. The resulting solution was heated to 80°C in an autoclave for 8 h. After vacuum solvent evaporation, the final product was obtained as an analytically pure oil.

Synthesis of the azacrown esters 2–4

To a solution of the azacrown derivative (1 mmol) in dry THF (25 mL) heated under reflux, was added dropwise a solution of the 3-carbonyl chloride (1.5 mmol) during a period of 5 h. The mixture was stirred at r.t. for 1 h. After vacuum solvent evaporation, the final product was isolated in the way indicated in each case.

Synthesis of precursors of compounds 2–4

2-(1,4,7,10-Tetraoxa-13-aza-cyclopentadec-13-yl)-ethanol

It was synthesized following the general method starting from commercial aza-15-crown-5 (2.28 mmol) and ethylene oxide (4.56 mmol). Yield: >98%. It was obtained as an analytically pure yellow oil. ¹H NMR (CDCl₃) δ: 3.6–3.7 (m, 12H, OCH₂CH₂N), 3.55 (t, 4H, J = 7.2 Hz, OCH₂CH₂O), 3.50 (t, 2H, J = 7.2 Hz, CH₂OH), 2.75 (t, 4H, J = 7.2 Hz, OCH₂CH₂N), 2.65 (t, 2H, J = 7.2 Hz, NCH₂CH₂OH). ¹³C NMR (CDCl₃) δ: 70.6, 70.3, 70.1, 69.9, 69.7 (OCH₂CH₂O).

Fig. 4. Most stable conformations predicted by semiempirical calculations (ZINDO/1) of the indicated complexes showing cooperation or lack thereof between carbonyls.

Fig. 5. Qualitative absorption–emission diagram.
59.3 (CH$_2$OH), 57.9 (NCH$_2$CH$_2$OH), 55.5 (OCH$_2$CH$_2$N). MS (L-SIMS+): 308.2 ([M + H]$^+$, 52%).

2-(1,4,7,10,13-Pentaoxa-16-aza-cyclooctadec-16-yl)-ethanol

It was synthesized following the general method starting from commercial aza-18-crown-6 (2.28 mmol) and ethylene oxide (4.56 mmol). Yield: >98%. It was obtained as an analytically pure light brown oil. $^1$H NMR (CDCl$_3$): 6.35-3.45 (m, 16H, OCH$_2$CH$_2$N), 2.95 (t, 2H, J = 7.2 Hz, OCH$_2$CH$_2$N), 2.65 (t, 2H, J = 7.2 Hz, NCH$_3$CH$_2$OH). $^{13}$C NMR (CDCl$_3$): δ: 164.2 (C-3), 164.2 (C-2), 158.4 (C-9), 158.2 (C-7), 149.1 (C-4), 140.1 (C-5), 109.4 (C-6), 108.9 (C-3), 107.6 (C-10), 96.6 (C-8), 61.1 (CO$_2$CH$_3$CH$_2$), 45.0 (NCH$_2$CH$_3$), 14.3 (CO$_2$CH$_3$), 12.3 (NCH$_2$CH$_3$). MS (L-SIMS+): 290.2 ([M + H]$^+$, 24%).

7-Diethylamino-2-oxo-2H-chromene-3-carboxylic acid ethyl ester

The previous compound (0.23 mmol) was dissolved in water and 0.23 mL of 1 M KOH solution was added. The resulting mixture was stirred for 24 h. After addition of 10% HCl until pH = 2, the solid was filtered and washed with water, yielding the analytically pure hydroxyoxymazocoumarin derivative. Yield: 65%; mp 214 to 215°C. $^1$H NMR (CDCl$_3$): δ: 8.60 (s, 1H, H-4), 7.45 (d, 2H, J = 8.9 Hz, H-5), 6.70 (dd, 1H, J = 2.3, 8.9 Hz, H-6), 6.50 (d, 1H, J = 2.3 Hz, H-8), 3.50 (q, 4H, J = 7.0 Hz, NCH$_2$CH$_3$), 1.25 (t, 3H, J = 7.0 Hz, NCH$_2$CH$_3$). $^{13}$C NMR (CDCl$_3$): δ: 165.2 (C-7), 164.6 (CO), 157.8 (C-2), 153.7 (C-9), 150.0 (C-4), 131.7 (C-5), 110.7 (C-6), 108.2 (C-3), 104.3 (C-10), 96.2 (C-8), 44.9 (NCH$_2$CH$_3$), 12.3 (NCH$_2$CH$_3$). MS (L-SIMS+): 261.2 ([M + H]$^+$, 31%). Anal. calc'd. for C$_9$H$_9$NO$_2$: C 64.36, H 5.79, N 5.36; found: C 64.32, H 5.78, N 5.40.

2-Oxo-2H-chromene-3-carboxylic acid 2-(1,4,7,10-tetraoxa-13-aza-cyclopentadec-13-yl)-ethyl ester (2a)

It was synthesized following the esterification general method starting from the 15-crown-5 derivative (0.325 mmol) and the corresponding 3-carbonyl chloride coumarin (0.48 mmol). The reaction mixture was dissolved in water and extracted with CH$_2$Cl$_2$. The aqueous phase was adjusted to pH 7 with a saturated solution of NaHCO$_3$ and extracted with ethyl acetate. Solvent evaporation of the organic layers yielded 2a as a light brown oil. Yield: 66%. $^1$H NMR (CDCl$_3$): δ: 8.55 (s, 1H, H-4), 7.75–7.65 (m, 2H, H-5, H-7), 7.35–7.25 (m, 2H, H-8, H-6), 4.42 (t, 2H, J = 7.2 Hz, CO$_2$CH$_2$), 3.65–3.45 (m, 16H, OCH$_2$CH$_2$O), 2.95 (t, 2H, J = 7.2 Hz, CO$_2$CH$_2$CH$_2$N), 2.65 (t, 4H, J = 7.2 Hz, OCH$_3$CH$_2$O), 1.35 (t, 6H, J = 7.2 Hz, NCH$_3$CH$_2$O). $^{13}$C NMR (CDCl$_3$): δ: 162.7 (C-2), 156.4 (C-9), 155.0 (CO), 148.7 (C-5), 129.4 (C-10), 124.7 (C-6), 124.3 (C-3), 116.5 (C-8), 70.6–70.1 (CH$_2$O), 63.6 (CH$_2$O), 54.8 (NCH$_2$CH$_3$), 53.3 (OCH$_2$CH$_3$). MS (L-SIMS+): 480.2 ([M + Na]$^+$, 28%), 502.2 ([M + Na]$^+$, 8%).

7-Methoxy-2-oxo-2H-chromene-3-carboxylic acid 2-(1,4,7,10-tetraoxa-13-aza-cyclopentadec-13-yl)-ethyl ester (3a)

It was synthesized following the esterification general method starting from the 15-crown-5 derivative (0.28 mmol) and the corresponding 3-carbonyl chloride coumarin (0.42 mmol). The reaction mixture was dissolved in water and extracted with CH$_2$Cl$_2$. The aqueous phase was adjusted to pH 7 with a saturated solution of NaHCO$_3$ and extracted with ethyl acetate. Solvent evaporation of the organic layers yielded 3a as a light brown oil. Yield: 54%. $^1$H NMR (CDCl$_3$): δ: 8.50 (s, 1H, H-4), 7.50 (d, 2H, J = 8.6 Hz, H-5), 6.85 (dd, 1H, J = 2.5, 8.6 Hz, H-6), 6.75 (d, 1H, J = 2.5 Hz, H-8), 4.40 (t, 2H, J = 7.2 Hz, CO$_2$CH$_2$CH$_2$N), 3.95 (s, 3H, OCH$_3$), 3.70–3.50 (m, 16H, OCH$_2$CH$_2$O), 3.00 (t, 2H, J = 7.2 Hz, COOCH$_2$CH$_2$N), 2.85 (t, 4H, J = 7.2 Hz, OCH$_2$CH$_2$N). $^{13}$C NMR (CDCl$_3$): δ: 162.5 (C-2), 163.3 (C-9), 157.5 (CO), 149.7 (C-5), 149.7 (C-7), 128.4 (C-10), 127.6 (C-6), 124.7 (C-3), 116.5 (C-8), 70.9–70.2 (CH$_2$O), 64.5 (CH$_2$O), 55.9 (CH$_3$O), 54.8 (NCH$_2$CH$_3$), 54.2 (OCH$_2$CH$_3$). MS (L-SIMS+): 466.3 ([M + H]$^+$, 26%), 488.3 ([M + Na]$^+$, 4%), 504.3 ([M + K]$^+$, 3%).

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3b as a light brown oil. Yield: 52%. 1H NMR (CDCl₃): δ: 8.50 (s, 1H, H-4), 7.50 (d, 2H, J = 8.6 Hz, H-5), 6.85 (dd, 1H, J = 2.5, 8.6 Hz, H-6), 6.75 (d, 1H, J = 2.5 Hz, H-8), 4.40 (t, 2H, J = 7.2 Hz, CO₂CH₂CH₂N), 3.85 (s, 3H, OCH₃), 3.70–3.50 (m, 20H, OCH₂O), 2.95 (t, 2H, J = 7.2 Hz, COOCH₂CH₂N), 2.85 (t, 4H, J = 7.2 Hz, OCH₂CH₂N). 13C NMR (CDCl₃) δ: 165.2 (C-2), 155.8 (C-9), 155.0 (CO), 148.7 (C-4), 134.2 (C-7), 129.4 (C-5), 127.2 (C-10), 104.3 (C-1), 103.4 (C-6), 70.9–70.1 (CH₂O), 63.5 (CH₂CO₂), 56.0 (CH₃O), 54.9 (NCH₂), 54.0 (OCH₂CH₂N), MS (L-SIMS+): 510.4 ([M + H]+, 30%), 532.4 ([M + Na]+, 6%), 548.3 ([M + K]+, 8%).

7-Diethylamino-2-oxo-2H-chromene-3-carboxylic acid 2-(1,4,7,10-tetraaza-13-aza-cyclopentadec-16-yl)-ethyl ester (4a)

It was synthesized following the esterification general method starting from the 15-crown-5 derivative (0.32 mmol) and the corresponding 3-carboxyl chloride coumarin (0.49 mmol). The reaction mixture was dissolved in water and extracted with CH₂Cl₂. The aqueous phase was adjusted to pH 7 with a saturated solution of NaHCO₃ and extracted with ethyl acetate. The organic layer was washed with 10% HCl, 20% NaHCO₃, and water. Solvent evaporation gave 4a as a dark brown oil. Yield: 45%. 1H NMR (CDCl₃) δ: 8.40 (s, 1H, H-4), 7.30 (d, 2H, J = 8.9 Hz, H-5), 6.55 (dd, 1H, J = 2.5, 8.9 Hz, H-6), 6.40 (d, 1H, J = 2.5 Hz, H-8), 4.40 (t, 2H, J = 7.2 Hz, CO₂CH₂CH₂N), 3.74–3.51 (m, 16H, OCH₂O), 3.40 (q, 4H, J = 7.1 Hz, CH₂CH₃), 2.92 (t, 2H, J = 7.2 Hz, COOCH₂CH₂N), 2.85 (t, 4H, J = 7.2 Hz, OCH₂CH₂N), 1.30 (t, 6H, J = 7.1 Hz, NCH₂CH₃). 13C NMR (CDCl₃) δ: 164.0 (C-7), 158.4 (C-2), 158.1 (CO), 152.8 (C-9), 149.3 (C-4), 131.1 (C-5), 111.6 (C-6), 109.4 (C-3), 104.3 (C-10), 96.8 (C-8), 70.3–70.0 (CH₂O), 63.5 (CH₂CO₂), 55.3 (NCH₂CH₂), 54.1 (OCH₂CH₂N), 44.9 (NCH₂CH₃), 12.3 (NCH₂CH₃). MS (L-SIMS+): 507.1 ([M + H]+, 20%), 529.1 ([M + Na]+, 8%).

Conclusion

Several new ionophores derived from coumarins bearing azacrown ethers attached to the 3-position by means of an ethylenocarbonyl spacer have been synthesized. We observed that the systems with strong electron donors at the 7-position displayed the largest bathochromic shifts upon complexation with Ca²⁺ but they were insensitive to Mg²⁺. NMR data, molecular modeling, and SS of emission strongly suggest cooperation between the chromon nucleus and the crowns in their binding to Ca²⁺ that was accompanied with a relatively large extinction of fluorescence. These facts may make the 4 compounds as leads for useful signaling devices of Ca²⁺ without interference from Mg²⁺.

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References