NJC

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

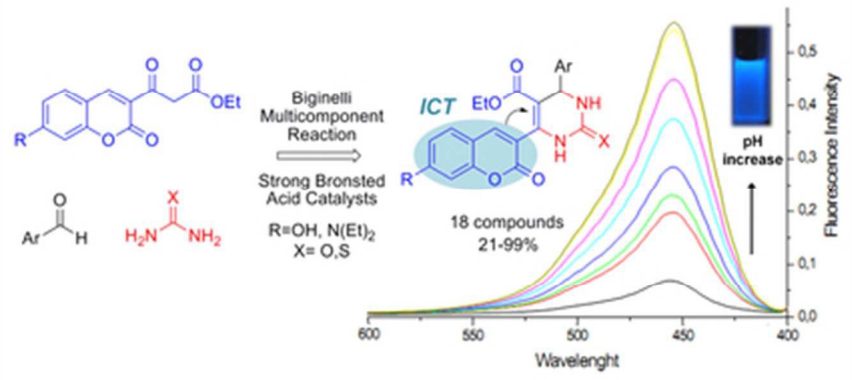
Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc



36x16mm (300 x 300 DPI)

New Journal of Chemistry RSCPublishing

ARTICLE

Synthesis and mechanism of novel fluorescent coumarin-dihydropyrimidinone dyads obtained by Biginelli multicomponent reaction.

Cite this: DOI: 10.1039/x0xx00000x

Felipe Vitório[a,b], Thiago Moreira Pereira[a], Rosane Nora Castro[b], Guilherme Pereira Guedes[b], Cedric Stephan Graebin[a,b] and Arthur Eugen Kümmerle[*,a,b].

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

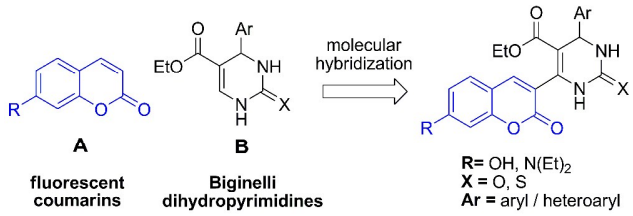
The Optimization of a Biginelli Multicomponent Reaction (MCR) protocol for obtaining a collection of 3,4-dihydropyrimidin-2(1*H*)-one/thione, with UV absortion and blue fluorescent properties, from synthetic coumarin beta-ketoester derivatives is described. This is the first report of Biginelli adducts bearing a coumarin nucleus in the *β*-ketoester moiety and their MCR mechanism seems to pass through a Knoevenagel intermediate, which was considered as unlikely before. A chemical library was obtained and the dihydropyrimidin-2(1*H*)-one nucleus formation confirmed by X-ray diffraction. Photophysical analyses for representative compounds in different solvents shown good Stokes shifts in water that associated to a postulated ICT process and pKa determination make these compounds a good start point for new chemical and biological probes as well as useful pH indicators.

Introduction

Coumarins are lactones of hydroxycinnamic acid (2*H*-1-benzopyran-2-one) widely distributed in plants and that can also be found in fungi and bacteria. About 1.300 coumarins have been isolated from these natural sources as a single nucleus or combined with sugars or acids. This class of compounds presents many pharmacological applications as anti-inflammatory, antispasmodic, antioxidant and antitumor activities, depending on its substitution patterns.[1] The intrinsic photophysical characteristics of coumarins and derivatives enables their uses as biological and chemical probes,[2] which was explored in the detection of proteins,[3] metals,[4] DNA/RNA,[5] tumour cells,[6] besides being useful as pH indicators.[7]

The Biginelli Multicomponent Reaction (MCR) is quite versatile, since it can be performed with many chemical variations in all three major components (*i.e.* β-ketoester, aldehyde and urea or thiorea) leading to a myriad of dihydropyrimidinones/thiones. These reactions can be performed under a variety of experimental conditions and numerous improvements have been reported in recent years.[8,9] Usually the 3,4-dihydropyrimidin-2(1*H*)-one/thione derivatives (Biginelli adducts) are obtained in good to excellent yields with good structural complexity, which have attracted attention to their applications as useful scaffold for the development of new chemical entities with therapeutic and biological activities, such as anticancer,[10] antihypertensive,[11] anti-inflammatory[12] and potassium channel blocker.[13]

Accordingly, the objective of this work is establish a Biginelli MCR synthetic protocol for obtaining novel collection of coumarin-3,4-dihydropyrimidin-2(1*H*)-one/thione dyads with fluorescent properties from synthetic coumarin beta-ketoester derivatives (Scheme 1). As far as we know coumarins were already used before as constituent of MCR,[14-16] however this is the first relate of Biginelli adducts bearing the coumarin nucleus in the β-ketoester moiety.

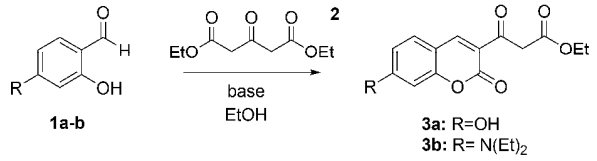


Scheme 1 Planned fluorescent coumarin-dihydropyrimidinone dyads.

Results and discussion

Synthesis of coumarin dihydropyrimidinones

The main focus of our work was based on exploring coumarin *β* -ketoesters **3a-b** as precursors for Biginelli multicomponent reactions. These building blocks were synthesised by using 4-OH-salicylaldehyde **1a** or 4-N(Et)$_2$-salicylaldehyde **1b** and diethyl 3-oxopentanedioate **2**, by means of a reaction involving a two-step mechanism: Knoevenagel condensation followed by a transesterification (Scheme 2 and Table 1). Despite the synthesis of coumarin derivatives **3** has been described in the literature,[17] in our case the described protocols did not work well. Furthermore, only a few reactions used **3a-b** as reagents in synthesis before. Initially we carried out the reaction at reflux temperature and using piperidine as catalyst as previous described for similar systems.[18]



Scheme 2 Synthesis of coumarin beta-ketoester building blocks.

However, despite presenting a good yield, **3a** (79%) and **3b** (65%) were difficult to purify, possibly because of by-products coming from the dimerization of coumarins with salicylaldehydes.[19] Aiming at to optimize this synthesis, we first proceeded the reaction using the same base (piperidine) but at lower temperatures (25 and 5°C), which eventually led to higher yields, *i.e.* 85% for **3a** and 66% for **3b**, after 24h at 25°C. Despite the increase in time reaction, almost no impurities were detected at this condition. Also, other amines were tested as catalysts. Another cyclic secondary amine, morpholine, also showed good yield of 76% for **3a**. However, the use of aliphatic amines led to low yields of 20% for triethylamine and no product formation for diethylamine.

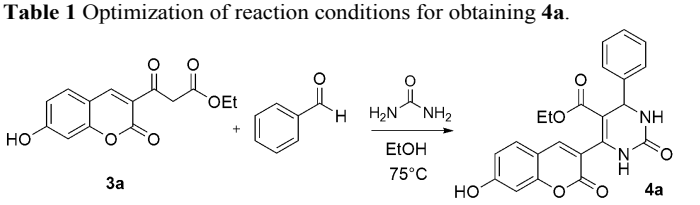
The coumarin beta-ketoesters **3a-b** were confirmed by analysis of ^{1}H and ^{13}C NMR where we could observe the presence of a singlet signal, ranging from 8.56 (**3a**) to 8.71 (**3b**) ppm related to the benzylidene hydrogen from chromenone subunit, which is absent in starting materials and is indicative of coumarin formation.

With the *β*-ketoesters in hand, the first reaction for the multicomponent Biginelli reaction with benzaldehyde and urea was made as classically described in literature, by using concentrated HCl (one drop) as catalyst and ethanol as solvent at 75°C.[20,21] However, the appearance of an intense red coloration was observed, indicating degradation of the coumarin ring, which is sensitive to the concentration and strength of acids and bases.[22] In fact, although TLC observation has indicated the total consummation of reagents and formation of an expected fluorescent product, a mixture too complex to isolate appeared. Based on that result, we decided to investigate the best reaction conditions for obtaining the desired Biginelli product **4a** by using different natures of catalysts, as well as sometimes changing their concentrations (Table 1).

As expected, synthesis of **4a** is totally dependent on presence of a catalyst (Table 1, entry 1).[23] However, if in the literature a wide variety of catalysts can be used for this purpose, in our case the nature and concentration of these catalysts changed drastically the outcome and/or yields of Biginelli product **4a**.

We started our evaluations by using the more traditional catalysts, *i.e.* the Brønsted acids HCl and H_2SO_4 (entries 2 and 3). However, differently from our first related assay, we employed a constant amount of the acid in the reaction medium (25 µL/2 mL). RP-HPLC analysis after 24h indicated the formation of **4a** and the consumption of the *β*-ketoester (**3a**), but with presence of other products (ESI†), Reaction isolations led to **4a** in low yields, 15% (HCl) and 36% (H_2SO_4) (Table 1, entries 2 and 3).

Table 1 Optimization of reaction conditions for obtaining **4a**.



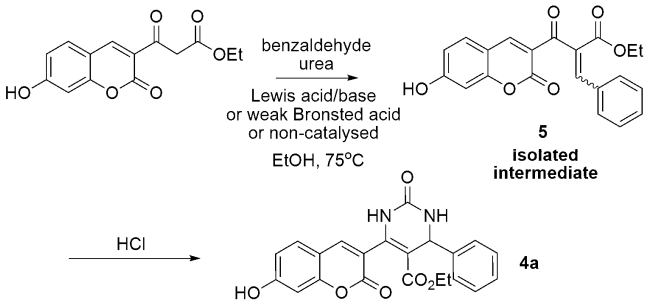
Entry	Catalyst	% Yield[a]
1	no catalyst	trace[b]
2	HCl (25 µL)	15
3	H_2SO_4 (25 µL)	36
4	HCl (12.5 µL)	68
5	H_2SO_4 (12.5 µL)	38
6	AcOH (25 µL)	trace[b]
7	TFA (25 µL)	trace[b]
8	pTsOH (0.1mol%)	59
9	CaF_2 (0.1mol%)	trace[b]
10	$Cu(OAc)_2$ (0.1mol%)	trace[b]
11	$CdCl_2$ (0.1mol%)	trace[b]
12	PPh_3 (0.1mol%)	trace[b]

[a] Isolated yields after 24h reaction; [b] Little amount of Biginelli product formation observed by RP-HPLC chromatograms at 370 nm after 24h reaction, but with presence of other products.

Bearing in mind the chemical sensibility of coumarins, we reduced the amount of acid and the reaction yields were higher, especially for HCl which presented a yield of 68% after chromatography isolation, showing a quite clean reaction (Table 1 entry 4; and ESI†). We also reduced the reaction time, but it seems that 24 h was the best time reaction for complete consumption of **3a** (ESI†). Microwave heating (1h at 90°C) was tried, but the results were similar to conventional heating at the same time reaction (results not shown). Furthermore, weaker Brønsted acids such as acetic and trifluoracetic acid, as well as diferent Lewis acids and base, did not lead to considerable amounts of **4a** (Table 1 entries 6-7 and 9-12).

Interestingly on the course of all Lewis acids/base, weak Brønsted acids and non-catalysed reactions it was observed by TLC a partial consumption of the starting *β*-ketoester and the appearance of a product not detected by TLC during reactions with strong Brønsted acids. This new product formation expends more or less time depending on the reaction conditions. When a catalyst was used, we could observe its appearance and concentration stabilization in the first hours of reaction while for non-catalysed reaction only after 24h. Since we did not observe this product before, we postulated that it could be an intermediate species. In fact, the addition of 12.5 µL HCl on these reactions led to both the disappearance of this new compound and formation of Biginelli product **4a**, corroborating a proposal of a reaction intermediate. In fact, even though this product was not observed by TLC in reactions with strong Brønsted acids, RP-HPLC indicated its presence in small amounts been totally consumed by the end of the reaction time, i.e. 24h (ESI†).

This product was isolated by preparative TLC after the usual work-up procedure. The ^{1}H NMR spectrum of **5** exhibited the presence of a new benzylidene singlet signal at 7.67 ppm, all aromatic hydrogens from coumarin and benzaldehyde and the absence of any signal from the urea moiety, been attributed to a Knoevenagel condensation product (Scheme 3).



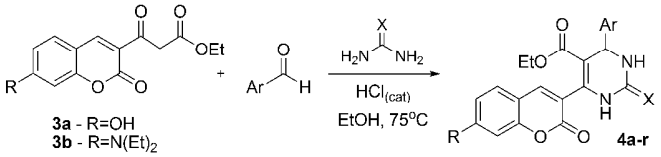
Scheme 3 Isolated intermediate on the course of some reactions and its consumption after addition of HCl.

Knoevenagel adducts were thought to be Biginelli intermediates before Kappe and other authors describe these adducts as unlikely.[24-26] However, the literature explored only the use of aliphatic and aromatic *β*-ketoesters and our reactions use coumarin moieties never described before for this purpose that could pass, at least partially, by a Knoevenagel mechanism. After finding the best reaction conditions we envisioned to study the reaction scope by using the coumarin *β*-ketoesters (**3a** and **3b**); different aldehydes, such as aromatics or heteroaromatics; and urea or thiourea, aiming at the construction of a novel coumarin-Biginelli chemical library (Table 2).

Compounds **4a-r** were obtained in low to excellent yields after chromatographic purification. Normally Biginelli compounds are isolated by simple precipitation/recrystallization, which in our case was not possible due to small residual impurities.. Analysing the reaction scope for **3a**, it is possible to assure that aromatic aldehydes are well tolerated due to yields ranging from 68 to 99% as well as the use of thiourea leading to dihydropyrimidin-2(1*H*)-thione **4o** in 61%. However, the use of heteroaromatics ones seems to be very sensible to its nature where thiophenyl shown an excellent yield of 99% while thiazolyl, pyridyl and 3-Br-2-pyridyl derivatives were obtained with yields around 25%. It is worth noticing that the latter aldehyde has an sp^2 nitrogen that could interfere on the reaction somehow. The same conditions applied to **3b** with three different aromatic aldehydes shown similar results in synthesizing **4p-r** although they have been obtained in subtle lower yields.

The dihydropyrimidinone ring formation of compounds **4a-r** were characterized by ^{1}H NMR and ^{13}C spectra presenting a typical doublet signal in a range of 5.2-5.4 ppm (d, 1H) for the CH originated from the aldehyde carbonyl moiety. The benzylidene hydrogen of chromenones could also be identified as a singlet ranging from 7.8 to 8.0 ppm.

Table 2 Scope and yields of coumarin-dihydropyrimidinone synthesis.



Product	R	Ar	X	% Yield[a]
4a	OH	Ph-	O	68
4b	OH	4-NO_2-Ph-	O	73
4c	OH	4-OH-Ph-	O	77
4d	OH	4-OCH_3-Ph-	O	80
4e	OH	3-OCH_3-Ph-	O	79
4f	OH	3,4-OCH_2O-Ph-	O	81
4g	OH	3,4,5-OCH_3-Ph-	O	75
4h	OH	4-F-Ph-	O	99
4i	OH	4-Cl-Ph-	O	74
4j	OH	4-Br-Ph-	O	90
4k	OH	2-thiophenyl	O	99
4l	OH	2-thiazolyl -	O	25
4m	OH	2-pyridyl-	O	25
4n	OH	3-Br-2-pyridyl-	O	21
4o	OH	Ph-	S	61
4p	$N(Et)_2$	Ph-	O	65
4q	$N(Et)_2$	4-OCH_3-Ph-	O	63
4r	$N(Et)_2$	4-Cl-Ph-	O	58

[a] isolated yields after chromatography purification

Moreover, prismatic colorless single-crystals of derivative **4c** suitable for X-ray diffraction were obtained through slow evaporation of a hexane / ethyl acetate solution at room temperature and confirmed the dihydropirimidinone nucleus formation. Compound **4c** crystallizes in the triclinic P-1 space group and the molecular structure is depicted in Figure 1 Details of crystal data, collection and refinement are summarized in supplementary material (table S1, ESI†).

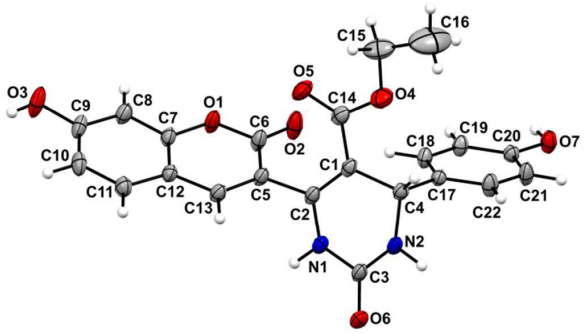


Fig. 1 Molecular structure of compound **4c**. Thermal ellipsoids are drawn at 40 % of probability. Crystallization solvent molecule was omitted for sake of clarity.

Spectroscopy studies

To determine the general photophysical characteristics of coumarin-dihydropirimidinones we selected three representative compounds, **4a** (Ph), **4c** (4-OH-Ph) and **4h** (4-F-Ph), whose had their spectroscopy properties characterized. Initially these compounds were analysed for their UV–vis absorbance, fluorescence emission and quantum yields (Figure 2; Table 3). Initially, spectral characterization was performed in methanol using 7-hydroxy-2*H*-chromen-2-one (umbeliferone - **6**) and 3-acetyl-7-hydroxy-2*H*-chromen-2-one (**7**) as standards. Spectral analyses were also made for the novel coumarin beta-ketoester (**3a**).

The UV-vis spectra demonstrated an interesting structural feature for the coumarin-dihydropirimidinones. Despite presenting a possible conjugation extension exerted by the ethyl cinnamate moiety, compounds **4a**, **4c** and **4h** had quite similar spectra to umbeliferone (**6**) rather than 3-acetyl-7-hydroxy-2*H*-chromen-2-one (**7**) and coumarin *β*-ketoester (**3a**), whose have their coumarin moieties conjugated to a ketone group (Figure 2A). As can be seen, **3a** and **7** presented different double absorption band spectra with maximum absorption at around 370nm and a minor absorption red shifted at 430nm.

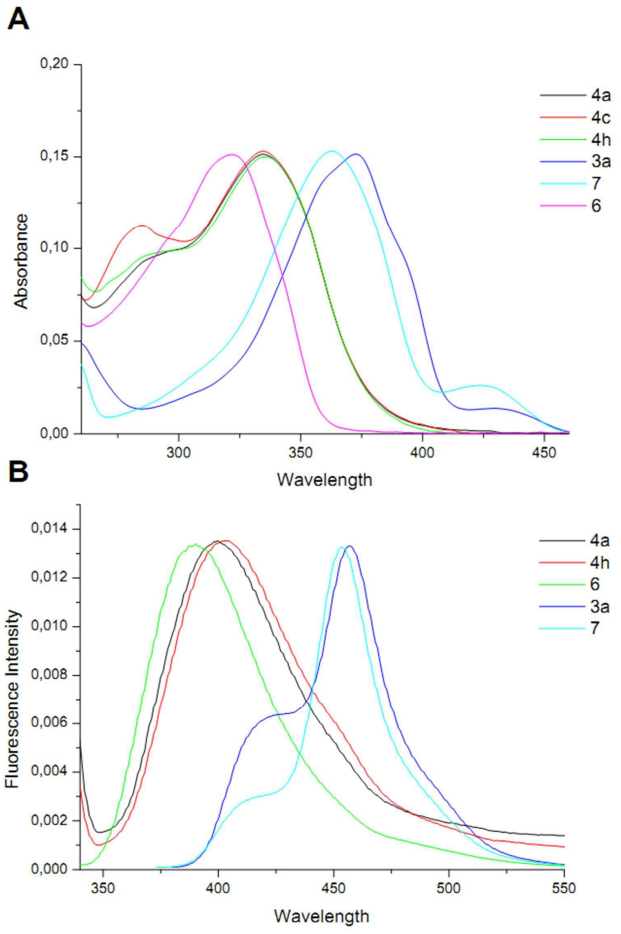


Fig. 2 Spectral analyses for coumarin derived compounds. (A) Normalized UV-vis spectra in MeOH (B) Normalized fluorescence emission spectra in MeOH with excitation on the λ_{max} of each coumpound.

Table 3. Spectroscopy data for coumarin derivatives.

Compound	Solvent	Abs λ_{max} (nm)	Emission λ_{max} (nm)[a]	Φ quantum yield	Stokes shift (nm)
3a	Methanol	372	456	0.73[a]	84
	Water	424	458	0.62[a]	34
	EtOAc	363	424	0.0055[a]	61
4a	Methanol	335	398	0.011[b]	63
	Water	337	452	0.055[b]	115
	EtOAc	327	399	0.013[b]	72
4c	Methanol	335	412	0.0044[b]	77
	Water	337	452	0.032[b]	115
	EtOAc	327	406	0.012[b]	79
4h	Methanol	335	404	0.015[b]	69
	Water	337	451	0.11[b]	114
	EtOAc	327	404	0.022[b]	77
6	Methanol	324	392	0.23[c]	68
	Water	337	451	0.79[c]	114
	EtOAc	327	382	0.018[c]	55
7	Methanol	361	457	0.43[c]	96
	Water	424	452	0.79[c]	28
	EtOAc	363	408	<0.001[c]	51

[a] determined using 3-acetyl-7-hydroxy-2*H*-chromen-2-one (**7**) as a standard[27]; [b] determined using umbeliferone (**6**) as a standard[27]; [c] values obtained from reference 27.

In fact, X-ray diffraction analysis had shown a twisted conformation between the coumarin moiety and ethyl cinnamate one, which hampers the π-electron conjugation and that maybe is maintained in solution. The slight batochromic shift (14nm) observed for Biginelli compounds compared to umbeliferone indicated that an internal charge transfer (ICT) between the chromenone ring and the cinnamic moiety could be occurring.

Fluorescence emission spectra of coumarin-dihydropirimidinones also demonstrated a closed relationship to umbeliferone (**6**) instead of the conjugated ketones **3a** and **7** that possess a two emission wavelengths. Again, a batochromic shift related to **6** and reduction in quantum yield (Table 3) reinforced the ICT process for our compounds. Interestingly, although the aromatic ring from dihydropirimidinone nucleus does not present a direct conjugation with the cinnamic moiety, it influences the fluorescence somehow once the most emissive compound was **4h**, with an electron withdrawing group, while **4b**, with an electron releasing group, was the lesser one (Table 3).

An increase in solvent polarity associated to bathochromic shifts in absorption maxima (abs λ_{max}) means that these main bands corresponding to an electronic π–π* transition of compounds. ICT processes are known to be stabilized in solvents with a greater dielectric constant leading to a red shift on both absorbance λ_{max} and emission λ_{max}.[28] In fact, in water the coumarin-dihydropirimidinones compounds (**4a**, **4c** and **4h**)

presented the biggest bathochromic shifts, resulting from this ICT transfer. Surprisingly, although methanol is a more polar solvent than EtOAc, ε=32.7 and ε=6.02 respectively, it seams that only water (ε=80.1) was able to well stabilize the charge transfer process, since spectroscopy properties associated to fluorescence are not changed when comparing MeOH and EtOAc (Table 3). The ICT stabilization in coumarins has been correlated to the ability of strong polar solvents in stabilizing their excited singlet state.[29,30]

The measurement of the dihydropirimidinone compounds quantum yield (ΦF) indicated a reduction when compared to umbeliferone (**6**) due to the ICT. Again, ΦF for **4a**, **4c** and **4h** did not present any significant difference between methanol and EtOAc. However, unexpectedly, in water dihydropirimidinone compounds showed an increase in ΦF of until five times. We expected a reduced ΦF in a more polar solvent that could better stabilize the ICT. This inverse phenomenon could be explained by a reduction of twisting of the single bonds involved in the charge transfer in the excited state for some specific solvents, as described for other coumarins.[28]

The increase in quantum yields in water summed to a good Stokes shift of about 115nm for our coumarin-dihydropirimidinones, in this medium, and the ICT process makes these compounds a good start point for new chemical and biological probes.

As known, hydroxy-coumarins can also act as pH indicators. Thus we determined the pKa of compound **4a** by means of a spectrophotometric titration. It was observed that increasing in pH led to a slight hypsochromic effect in the band centred at 335nm while hyperchromic effect was observed for the band centred at 390nm forming an isosbestic point at approximately 356nm.

From these spectral variations a graph of pH versus log $((A-A_f) / (A_0-A))$ was plotted and the pKa value of **4a** determined as 7.49 (ESI†). Additionally, decreasing in pH did not lead to any change in UV-vis absorption spectra (ESI†).

This determined pKa allows our compounds to be useful as probes in physiologic medium (pH = 7.4) since fluorescent pH indicators like coumarins are employed in a pH range around their ground state pKa. In fact, at physiologic pH compound **4a** presented an increase of about 6 times in fluorescence when compared to its less ionized form (pH ≅ 5), reaching its maximum fluorescent at a pH of about 9, where **4a** presented a fluorescent intensity 11 times higher (Figure 4). Although pH increase has led to a change in UV-vis absorption spectra, no modification was observed in the fluorescence one. This is a typical behaviour of fluorophores that undergo photoinduced proton transfer without electron transfer.[31] It could be explain because our coumarin-dihydropirimidinones, as other coumarins, are much more acidic (lower pKa) in the excited state compared to their ground states. By this way, when compound **4a** was assayed in a pH around the ground state pKa, the emitting form was almost exclusively the ionized form due to deprotonation of the acidic form in the excited-state. The fluorescence spectrum is thus unchanged, in contrast to the excitation spectrum.[31]

Finally, as coumarins are widely described as metal complexing probes[32,33] and metals are present in biological media as cells compartments, some of the most important metals (Co^{2+}, Cu^{2+}, Mg^{2+}, Na^{+1}, Zn^{2+} and Ca^{2+}) were tested as possible interfering. Figure 5 shows that no significant changing in UV-vis absorption spectra were observed when 5 equivalents of each metal were added to a water solution of compound **4a**, indicating that our coumarin-dihydropirimidinones have no complexation sites for those metals.

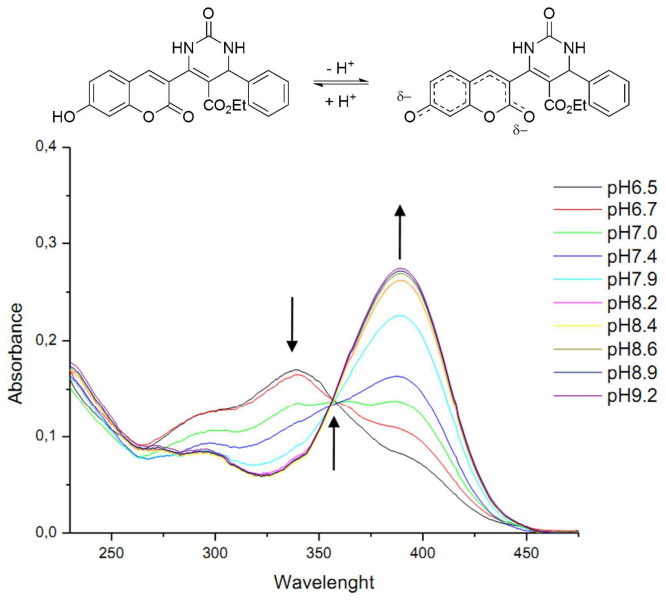


Fig. 3 Spectrophotometric titration of compound **4a** (6 μM) in water.

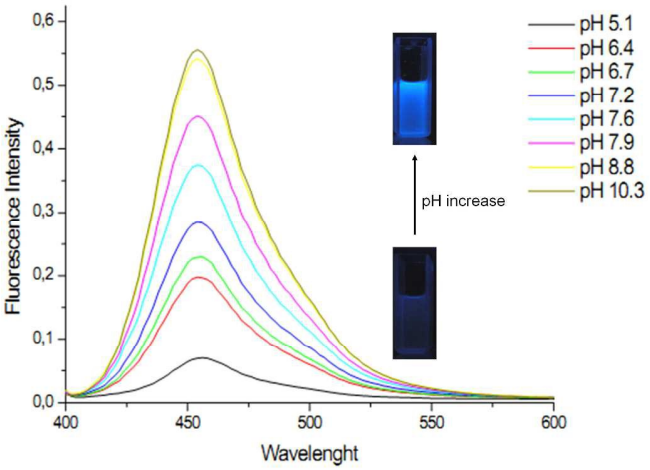


Fig. 4 Fluorescence spectra of **4a** (6 μM) in water upon increasing of medium pH with an excitation wavelength of 337 nm.

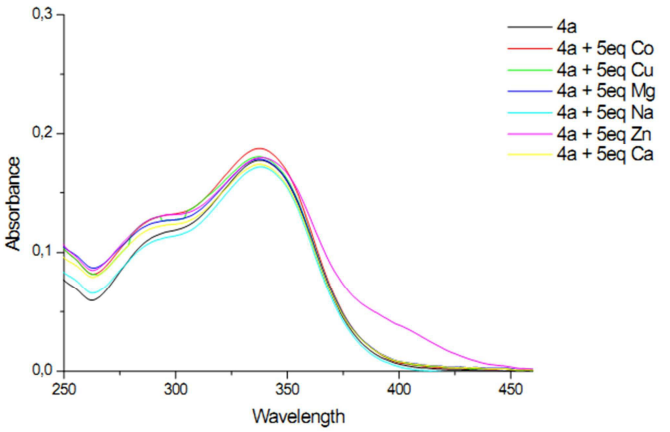


Fig. 5 UV-vis spectra of **4a** (6 μM) and **4a** (6 μM) upon addition of 5 equiv of Co^{2+}, Cu^{2+}, Mg^{2+}, Na^{+1}, Zn^{2+} and Ca^{2+}.

Conclusions

Herein we optimized the synthesis of a Biginelli Multicomponent Reaction (MCR) protocol for obtaining novel 3,4-dihydropyrimidin-2(1*H*)-one/thiones. This is the first description of coumarin beta-ketoester been used for this purpose. Despite the best protocol using the classical Bronsted acid catalyst, HCl, we needed to find the best catalyst concentration due to the coumarin instability in these conditions. Furthermore, other weak Bronsted acid as well as Lewis base/acids catalysts did not lead to the desired coumarin-dihydropyrimidinones, different from many other examples described in the literature. Since the beginning of all synthesis process, we could observe a considerable amount of a product coming from Knoevenagel condensation, which in Bronsted acid catalyzed reactions was totally converted to the dihydropyrimidinones. We proposed this Knovenagel product as an important intermediate in our reactions, conversely of previous descriptions for the Biginelli synthesis. Finally, the evaluation of the fluorescent properties belonging to coumarin-dihydropyrimidinones made their synthesis an opportunity of easily designing new pH probes based on the ICT process that could be useful in chemical and biological process.

Acknowledgements

The authors would like to thank CNPq (BR), FAPERJ (BR) and CAPES (BR) for fellowship and financial support and Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio) for the mass spectrometry analysis.

Experimental

General. The melting points recorded are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were acquired in a 400 MHz or 500 MHz Brucker Avance spectrometer (AC-400 or AC-500), using DMSO-d_6 or $CDCl_3$ and TMS as internal standard. The coupling constants (*J* values) are given in Hz. Mass spectrometry was obtained by negative ionization at Esquire 6000-ESI Ion Trap MS^n System Bruker Daltonics and data analyzed in Compass 1.3.SR2 software. Elemental analyzes were carried out on a Thermo Scientific Flash EA 1112 Series CHN-Analyzer. Infrared spectra (IR) were obtained on a Perkin-Elmer Spectrometer Model 1600. The samples were examined in pellets form of potassium bromide (KBr). The absorption values were pulsed at wave number, using as a unit the reciprocal centimeters (cm^{-1}). The spectra in the UV-visible (UV-Vis) region were obtained on Shimadzu Mine 1240 spectrophotometer with a quartz cell of 1 cm optical path, the emission and excitation spectra were performed on Jasco spectrofluorometer spectrometry using rectangular cells of four polished faces and optical path of 1 cm.

General Procedure for the β-dicarbonyl compounds 3a-b. To a stirred solution of 2,4-dihydroxybenzaldehyde **1a** or 4-(diethylamino)-2-hydroxybenzaldehyde **1b** (3.10 mmol) and diethyl 3-oxopentanedioate **2** (3.10 mmol) in ethanol (10 mL) was added piperidine (0.77 mmol) dropwise. The reaction was stirred at room temperature for 24 h. The precipitated formed was filtrated out and washed with cold ethanol. The residual solution was evaporated to 20% of initial volume under reduced pressure, put at 0 °C for 20h and the solid formed, filtered.

Ethyl 3-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-3-oxopropanoate (3a). Yield 740 mg, 86%; mp 142-144 °C; IR υ_{max}(KBr)/cm^{-1} 3485, 1712, 1602; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (1H, s), 8.71 (1H, s), 7.84 (1H, d, *J* 8.5 Hz), 6.88 (1H, d, J 8.2 Hz), 6.78 (1H, s), 4.12 (2H, q), 4.00 (2H, s), 1.18 (3H, t); ^{13}C NMR (125 MHz, DMSO-d_6) δ 190.3, 168.1, 165.2, 159.7, 157.9, 149.3, 133.6, 188.4, 115.00, 111.3, 102.3, 91.5, 60.9, 48.7, 14.5; MS (ESI-) m/z; 275.0, 229.0, 185.0, 157.0; MS/MS (m/z 275.0) 275.0, 228.9, 185.0; elemental analysis calcd (%) for $C_{14}H_{12}O_6$: C 60.87, H 4.38; found: C 60.56, H 4.42.

Ethyl 3-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)-3-oxopropanoate (3b). Yield 677 mg, 66%; mp 108-110 °C; IR υ_{max}(KBr)/cm^{-1} 3442, 1741, 1716; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 (1H, s), 7.79 (1H, d, *J* 8.8 Hz), 6.82 (1H, d, *J* 9.1 Hz), 6.59 (1H, d), 4.09 (2H, q), 3.94 (2H, s), 3.51 (4H, q) 1.16 (9H, m); ^{13}C NMR (125 MHz, DMSO-d_6) δ 189.8, 168.4, 160.5, 158.9, 153.8, 148.7, 133.2, 114.0, 110.9, 108.1, 96.3, 60.8, 48.8, 45.0, 14.5, 12.8; MS (ESI-) m/z; 330.1, 275.0, 229.0, 185.0; MS/MS (m/z 330.0) 330.1, 284.1, 262.1, 216.1, 188.1, 145; elemental analysis calcd (%) for $C_{18}H_{21}NO_5$: C 65.24, H 6.39, N 4.23; found: C 64.98, H 6.41, N 4.22.

General Procedure for Biginelli compounds 6a-o. To a stirred solution of **3a** or **3b** (0.3 mmol), (thio)urea (0.35 mmol) and the corresponding aldehyde (0.5 mmol) in ethanol (2 mL) was added HCl 37% (12.5 μL) at room temperature. The reaction was stirred for 24h at 75°C. The crude reaction mixture was aggregated to silica gel through reduced pressure evaporation and it was purified by silica gel column chromatography (ethyl acetate : hexane mixture, gradient elution).

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a). Yield 80 mg, 68%; mp 218-220 °C; IR υ_{max}(KBr)/cm^{-1} 3381, 1695, 1612, 1224; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (1H, s), 9.37 (1H, s), 7.97 (1H, s), 7.87 (1H, s), 7,60 (1H, d, *J* 8.5 Hz), 7.42 (5H, m), 6.84 (1H, d, *J* 8.3 Hz), 6.79 (1H, d), 5.25 (1H, d, *J* 3.2 Hz), 3.84 (2H, q), 0.86 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 162.1, 159.1, 155.7, 152.4, 143.7, 142.3, 130.6, 129.0, 127.1, 113.9, 111.5, 102.4, 59.9, 54.6, 14.1; MS (ESI-) m/z; 405.1, 362.1, 325.2; MS/MS (m/z 405.0) 406.1, 362.1; elemental analysis calcd (%) for $C_{22}H_{18}N_2O_6$: C 65.02, H 4.46, N 6.89; found: C 65.14, H 4.47, N 6.62.

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-4-(4-nitro-phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4b). Yield 95.8 mg, 73%; mp 123-125 °C; IR υ_{max}(KBr)/cm^{-1} 3263, 1699, 1610, 1220; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (1H, s), 9.57 (1H, s), 8.27 (2H, d, *J* 8.8 Hz), 8.07 (1H, s), 8.00 (1H, s), 7.70 (2H, d, *J* 8.8 Hz), 7.58 (1H, d, *J* 8.5 Hz), 6.83 (1H, d, *J* 8.5 Hz), 6.78 (1H, d), 5.37 (1H, d, *J* 3.2 Hz), 3.82 (2H, q), 0.85 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.7, 162.3, 159.1, 155.7, 152.2, 151.6, 147.4, 144.5, 142.3, 130.6, 128.4, 124.4, 119.1, 114.0, 111.4, 102.5, 60.2, 54.1, 14.1; MS (ESI-) m/z; 451.1, 411.1, 405.1, 339.2, 325.2, 311.2; MS/MS (m/z 405.0) 450.1, 407.1, 360.1; elemental analysis calcd (%) for $C_{22}H_{17}N_3O_8$: C 58.54, H 3.80, N 9.31; found: C 58.70, H 3.98, N 9.27.

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-4-(4-hydroxy-phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4c). Yield 94.6 mg, 77%; mp 265-267 °C; IR υ_{max}(KBr)/cm^{-1} 3222, 1683, 1593, 1226; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (1H, s), 9.41 (1H, s), 9.33 (1H, d), 7.96 (1H, s), 7.79 (1H, s), 7.60 (1H, d, *J* 8.5 Hz), 7.23 (2H, d, *J* 8.5 Hz), 6.85 (1H, d, J 8.5 Hz), 6.79 (1H, d), 6.75 (2H, d, J 8.5 Hz), 5.15 (1H, d, *J* 3.2 Hz), 3.84 (2H, q), 0.87 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 162.1, 159.2, 157.2, 155.7, 152.5, 143.2, 142.2, 135.1, 130.5, 128.2, 119.7, 115.5, 113.9, 111.5, 102.4, 59.9, 54.1, 14.1; MS (ESI-) m/z;421.1, 379.1, 349.0 275.0; MS/MS (m/z 421.0) 422.1, 378.1; elemental analysis calcd (%) for $C_{22}H_{18}N_2O_7$: C 62.56, H 4.30, N 6.63; found: C 62.43, H 4.42, N 6.67.

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-4-(4-methoxy-phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4d). Yield 101.3 mg, 80%; mp 140-142 °C; IR υ_{max}(KBr)/cm^{-1} 3417.75, 1693, 1610, 1222; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (1H, s), 9.37 (1H, d), 7.97 (1H, s), 7.84 (1H, s), 7.60 (1H, d, *J* 8.5 Hz), 7.36 (2H, d, *J* 8.5 Hz), 6.94 (2H, d, *J* 8.8 Hz), 6.84 (1H, d, *J* 8.5 Hz), 6.79 (1H, s, *J* 8.5 Hz), 5.20 (1H, d, J 3.2 Hz), 3.84 (2H, q), 3.76 (3H, s), 0.87 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 162.1, 159.2, 159.1, 155.7, 152.5, 143.4, 142.3, 135.7, 130.6, 128.2, 119.6, 114.3, 113.9, 111.5, 102.4, 59.9, 55.6, 53.9, 14.1; MS (ESI-) m/z; 435.1, 421.1, 393.1, 275.0; MS/MS (m/z 435.0) 436.1, 392.1; elemental analysis calcd (%) for $C_{23}H_{20}N_2O_7$: C 63.30, H 4.62, N 6.42; found: C 62.18, H 4.87, N 6.39.

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-4-(3-methoxy-phenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4e). Yield 100 mg, 79%; mp 245-247 °C; IR υ_{max}(KBr)/cm^{-1} 3338, 1685, 1606; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.72 (1H, s), 9.40 (1H, s), 7.97 (1H, s), 7.89 (1H, s), 7.60 (1H, d, *J* 8.5 Hz), 7.30 (1H, d, *J* 8.0), 7.03 (2H, d, *J* 6.6 Hz), 6.86 (1H, d, *J* 6.9 Hz), 6.84 (1H, d, *J* 8.5), 6.78 (1H, d), 5.21 (1H, d, *J* 3.2 Hz), 3.84 (2H, q), 3.77 (3H, s), 0.86 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 162.3, 159.9, 159.2, 155.7, 152.5, 145.9, 143.8, 142.3, 130.6, 130.1, 119.5, 119.2, 114.0, 113.3, 111.4, 102.4, 59.9, 55.5, 54.4, 14.1; MS (ESI-) m/z; 435.1, 392.1, 363.1, 275.0, 229.0, 184.9; MS/MS (m/z 435.0) 436.1, 392.1; elemental analysis calcd (%) for $C_{23}H_{20}N_2O_7$: C 63.30, H 4.62, N 6.42; found: C 62.22, H 4.69, N 6.41.

Ethyl 4-(benzo[d][1,3]dioxol-5-yl)-6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4f). Yield 108 mg, 81%; mp 248-250 °C; IR υ_{max}(KBr)/cm^{-1} 3398, 1689, 1660, 1232; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.72 (1H, s), 9.40 (1H, s), 7.99 (1H, s), 7.85 (1H, s), 7.60 (1H, d, *J* 8.5 Hz), 6.98 (1H, s), 6.90 (2H, s), 6.84 (1H, d, *J* 8.5 Hz), 6.79 (1H, d), 6.02 (2H, s) 5.17 (1H, d, *J* 2.8 Hz), 3.84 (2H, q), 0.86 (3H, t); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.9, 162.2, 159.2, 155.7, 152.4, 147.8, 147.0, 143.6, 142.3, 138.4, 130.6, 120.4, 119.5, 113.9, 111.5, 108.6, 107.4, 102.4, 101.4, 59.6, 54.2, 14.1; MS (ESI-) m/z; 449.1, 407.1, 349.0; MS/MS (m/z 449.0) 450.1, 406.1; elemental analysis calcd (%) for $C_{23}H_{18}N_2O_8$: C 61.33, H 4.03, N 6.22; found: C 61.20, H 4.12, N 6.11.

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-4-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4g). Yield 110 mg, 75%; mp 290-292 °C; IR υ_{max}(KBr)/cm^{-1} 3313, 1720, 1130; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.72 (1H, s), 9.42 (1H, s), 7.99 (1H, s), 7.87 (1H, s), 7.60 (1H, d, J 8.5 Hz), 6.85 (2H, d), 6.82 (1H, d, J 8.2), 6.77 (1H, d), 5.18 (1H, d, J 3.1 Hz), 3.86 (2H, q), 3.78 (6H, s), 3.65 (3H, s), 0.87 (3H, t); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.9, 162.2, 159.5, 155.6, 153.3, 152.4, 143.9, 142.2, 137.1, 130.7, 122.4, 119.7, 114.0, 111.4, 107.2, 104.2, 102.5, 60.4, 59.9, 56.5, 56.2, 54.3, 14.2; MS (ESI-) m/z; 495.1, 451.1; MS/MS (m/z 495.0) 496.1, 452.1; elemental analysis calcd (%) for $C_{25}H_{24}N_2O_9$: C 60.48, H 4.87, N 5.64; found: C 62.37, H 4.92, N 5.61.

Ethyl 4-(4-fluorophenyl)-6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4h). Yield 118 mg, 99%; mp 130-132 °C; IR υ_{max}(KBr)/cm^{-1} 3386, 1693, 1230; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.72 (1H, s), 9.44 (1H, s), 7.99 (1H, s), 7.93 (1H, s), 7.59 (1H, d, *J* 8.5 Hz), 7.46 (2H, m), 7.21 (2H, m, *J* 8.5), 6.85 (1H, d, *J* 8.2 Hz), 6.79 (1H, s), 5.24 (1H, d, *J* 2.8 Hz), 3.83 (2H, q), 0.85 (3H, t); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.8, 162.9, 162.3, 161.0, 160.1, 159.2, 155.7, 152.3, 143.8, 142.4, 140.7, 130.6, 129.0,

119.4, 115.6, 114.0, 111.4, 102.5, 60.0, 53.9, 14.1; MS (ESI-) m/z; 423.1, 380.1; MS/MS (m/z 423.0) 424.1, 380.0; elemental analysis calcd (%) for $C_{22}H_{17}FN_2O_6$: C 62.26, H 4.04, N 6.60; found: C 62.05, H 4.06, N 6.59.

Ethyl 4-(4-chlorophenyl)-6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4i). Yield 95.8 mg, 74%; mp 180-182 °C; IR υ_{max}(KBr)/cm^{-1} 3392, 1697, 1660, 1228; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (1H, s), 9.47 (1H, s), 7.99 (1H, s), 7.95 (1H, s), 7.60 (1H, d, *J* 8.3 Hz), 7.46 (4H, s), 6.85 (1H, d, *J* 8.5 Hz), 6.80 (1H, d), 5.25 (1H, d, *J* 3.2 Hz), 3.84 (2H, q), 0.87 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 162.2, 159.1, 155.7, 152.3, 143.9, 143.4, 142.4, 132.6, 130.6, 129.0, 119.4, 114.0, 111.4, 102.5, 60.0, 54.0, 14.1; MS (ESI-) m/z; 439.1, 397.1; MS/MS (m/z 439.0) 441.1, 396.1, 275.1; elemental analysis calcd (%) for $C_{22}H_{17}ClN_2O_6$: C 59.94, H 3.89, N 6.35; found: C 60.03, H 4.01, N 6.28.

Ethyl 4-(4-bromophenyl)-6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4j). Yield 127 mg, 90%; mp 128-130 °C; IR υ_{max}(KBr)/cm^{-1} 3433, 1660, 1616, 1228; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.46 (1H, s), 7.96 (1H, s), 7.96 (1H, s), 7.58 (3H, m), 7.39 (2H, d, *J* 8.5 Hz), 6.83 (1H, d, *J* 8.5 Hz), 6.77 (1H, s), 5.22 (1H, d, *J* 3.1 Hz), 3.83 (2H, q), 0.86 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 162.2, 159.1, 155.7, 152.3, 143.9, 142.4, 132.6, 130.6, 129.0, 119.4, 114.0, 111.4, 102.5, 60.0, 54.0, 14.1; MS (ESI-) m/z; 485.0, 406.2, 275.1, 229.0, 185.0; MS/MS (m/z 485.0) 483.1, 442.0; elemental analysis calcd (%) for $C_{22}H_{17}BrN_2O_6$: C 54.45, H 3.53, N 5.77; found: C 54.21, H 3.52, N 5.82.

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-4-(thiophen-2-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4k). Yield 118 mg, 99%; mp 143-145 °C; IR υ_{max}(KBr)/cm^{-1} 3384, 1689, 1606, 1211; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.50 (1H, s), 8.05 (1H, d), 789 (1H, s), 7.58 (1H, d, *J* 8.5 Hz), 7.45 (1H, d, *J* 6.0 Hz), 7.08 (1H, d), 7.00 (1H, m), 6.81 (1H, d, *J* 8.8 Hz), 6.74 (1H, s), 5.48 (1H, d, *J* 3.5 Hz), 3.89 (2H, q), 0.91 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.7, 163.2, 160.1, 159.1, 155.9, 152.6, 148.3, 143.9, 142.6, 130.6, 127.2, 125.7, 124.6, 118.6, 114.3, 111.0, 60.1, 49.9, 14.2; MS (ESI-) m/z; 411.1, 369.1, 275.0, 229.0; MS/MS (m/z 411.0) 412.0, 368.0; elemental analysis calcd (%) for $C_{20}H_{16}N_2O_6S$: C 58.25, H 3.91, N 6.79; found: C 58.21, H 3.94, N 6.75.

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-4-(thiazol-2-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4l). Yield 30 mg, 25%; mp 160-162 °C; IR υ_{max}(KBr)/cm^{-1} 3390, 1701, 1610, 1220; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (1H, s), 9.56 (1H, s), 8.13 (1H, s), 7.89 (1H, s), 7.78 (1H, d), 7.70 (1H, d), 7.61 (1H, d, *J* 8.0 Hz), 6.83 (1H, d, *J* 6.5 Hz), 6.77 (1H, d), 5.58 (1H, d, *J* 3.2 Hz), 3.90 (2H, q), 0.90 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 164.5, 162.3, 158.8, 155.8, 152.5, 144.8, 143.0, 142.6, 130.7, 121.1, 118.9, 114.0, 111.3, 102.4, 60.2, 52.3, 14.2; MS (ESI-) m/z; 412.1, 369.1, 275.0, 229.0; MS/MS (m/z 412.0) 413.0, 369.0, 336.1, 323.0, 160.9; elemental analysis calcd (%) for $C_{19}H_{15}N_3O_6S$: C 55.20, H 3.66, N 10.16; found: C 55.01 H 3.87, N 10.18.

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-4-(pyridin-2-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4m). Yield 30 mg, 25%; mp 150-152 °C; IR υ_{max}(KBr)/cm^{-1} 3248, 1697, 1608, 1220; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (1H, s), 9.34 (1H, s), 8.58 (1H, s), 7.91 (1H, s), 7.80 (2H, m), 7.61 (1H, d, *J* 8.5 Hz), 7.46 (1H, d, *J* 7.7 Hz), 7.32 (1H, m), 6.83 (1H, d, *J* 8.5 Hz), 6.78 (1H, d), 5.31 (1H, s, *J* 3.0 Hz), 3.84 (2H, q), 0.86 (3H, t); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 162.2, 159.1, 155.7, 152.7, 149.8, 144.2, 142.3, 137.3, 130.6, 123.3, 121.3, 119.5, 113.9, 102.4, 59.9, 56.3, 14.1; MS (ESI-) m/z; 406.1, 363.1, 275.0, 229.0; MS/MS (m/z 406.0) 407.1, 363.1, 317.0; elemental analysis calcd (%) for $C_{21}H_{17}N_3O_6$: C 61.91, H 4.21, N 10.31; found: C 61.58, H 4.43, N 10.14.

Ethyl 4-(6-bromopyridin-2-yl)-6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4n). Yield 29 mg, 21%; mp 256-258 °C; IR υ_{max}(KBr)/cm^{-1} 3440, 1674, 1608, 1211; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (1H, s), 9.47 (1H, s), 7.94 (2H, s), 7.79 (1H, m), 7.59 (2H, m), 7.52 (1H, d), 6.83 (1H, d, *J* 8.5 Hz), 6.77 (1H, d), 5.26 (1H, s), 3.84 (2H, q), 0.85 (3H, t); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.8, 163.8, 162.2, 159.1, 155.7, 152.3, 144.6, 142.4, 141.5, 140.9, 130.6, 127.6, 120.7, 119.3, 114.0, 111.4, 102.5, 100.7, 60.0, 55.9, 14.1; MS (ESI-) m/z; 486.0, 441.0, 255.2; MS/MS (m/z 486.0) 484.0, 441.0, 396.9, 361.1; elemental analysis calcd (%) for $C_{21}H_{16}BrN_3O_6$: C 51.87, H 3.32, N 8.64; found: C 51.75, H 3.36, N 8.61.

Ethyl 6-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4o). Yield 75 mg, 61%; mp 163-165 °C; IR υ_{max}(KBr)/cm^{-1} 3220, 2981, 1704, 1610, 1223; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.73 (1H, s), 10.55 (1H, s), 9.78 (1H, s), 7.98 (1H, s), 7.60 (1H, d, *J* 8.5 Hz), 7.41 (5H, m), 6.82 (1H, d, *J* 8.5 Hz), 6.78 (1H, d), 5.26 (1H, d, *J* 3.5 Hz), 3.84 (2H, q), 0.85 (3H, t); ^{13}C NMR (125 MHz, DMSO-d_6) δ 184.2, 174.8, 164.7, 162.2, 159.3, 155.7, 143.2, 142.9, 140.6, 130.7, 129.2, 129.0, 128.4, 127.2, 118.6, 114.0, 111.5, 102.4, 60.3, 54.7, 14.0; MS (ESI-) m/z; 421.1, 419.1, 409.1, 405.1, 403.1, 387.1, 379.1; MS/MS (m/z 421.0) 422.0, 419.0; elemental analysis calcd (%) for $C_{22}H_{18}N_2O_5S$: C 62.55, H 4.29, N 6.63; found: C 62.52, H 4.33, N 6.67.

Ethyl 6-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4p). Yield 86 mg, 65%; mp 156-157 °C; IR υ_{max}(KBr)/cm^{-1} 2974, 1699, 1612, 1224; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.35 (1H, s), 7.87 (1H, m), 7.83 (1H, s), 7.49 (1H, d, *J* 8.8 Hz), 7.44 (2H, m, *J* 7.6 Hz), 7.38 (2H, m, *J* 7.6 Hz), 7.30 (1H, m, *J* 7.2 Hz), 6.73 (1H, d, *J* 8.8 Hz), 6.58 (1H, d), 5.22 (1H, d, *J* 3.5 Hz), 3.83 (2H, q), 3.46 (2H, q), 1.14 (6H, t), 0.88 (3H, t); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.1, 159.6, 156.6, 152.7, 151.3, 144.6, 144.2, 142.6, 130.2, 128.9, 127.9, 127.1, 116.1, 109.5, 107.8,

96.8, 59.8, 54.3, 44.6, 14.2, 12.8; MS (ESI-) m/z; 460.3, 432.2, 325.2; MS/MS (m/z 460.0) 461.2, 414.2, 373.2; elemental analysis calcd (%) for $C_{26}H_{27}N_3O_5$: C 67.66, H 5.90, N 9.10; found: C 67.49, H 6.04, N 9.03.

Ethyl 6-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)-4-(4-methoxyphenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4q). Yield 83 mg, 63%; mp 176-178 °C; IR υ_{max}(KBr)/cm^{-1} 2925, 1695, 1610, 1226; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.31 (1H, s), 7.82 (1H, s), 7.80 (1H, s), 7.49 (1H, d, *J* 8.8 Hz), 7.36 (2H, m, *J* 8.8 Hz), 6.93 (2H, d, *J* 8.5 Hz), 6.73 (1H, d, *J* 8.8 Hz), 6.58 (1H, d), 5.17 (1H, d, *J* 3.1 Hz), 3.83 (2H, q), 3.75 (3H, s), 3.46 (4H, q), 1.14 (6H, t), 0.89 (3H, t); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.1, 159.6, 159.0, 156.6, 152.7, 151.2, 143.9, 142.5, 136.7, 130.2, 128.2, 116.2, 114.2, 109.5, 107.8, 96.8, 59.8, 55.6, 53.9, 44.6, 14.2, 12.8; MS (ESI-) m/z; 490.2, 396.8; MS/MS (m/z 490.0) 491.2, 444.2, 403.2; elemental analysis calcd (%) for $C_{27}H_{29}N_3O_6$: C 65.97, H 5.95, N 8.55; found: C 65.88, H 6.01, N 8.58.

Ethyl 4-(4-chlorophenyl)-6-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4r); Yield 70 mg, 58%; mp 95-97 °C; IR υ_{max}(KBr)/cm^{-1} 2976, 1712, 1614, 1510; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.44 (1H, s), 7.93 (1H, s), 7.83 (1H, s), 7.46 (5H, m), 6.73 (1H, d, *J* 8.8 Hz), 6.57 (1H, d), 5.24 (1H, d, *J* 3.1 Hz), 3.84 (2H, q), 3.43 (4H, q), 1.13 (6H, t), 0.88 (3H, t); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.0, 159.9, 156.6, 152.6, 151.3, 144.4, 143.5, 142.7, 132.5, 130.3, 129.0, 115.9, 109.5, 107.7, 96.7, 59.9, 53.9, 44.6, 14.1, 12.7; MS (ESI-) m/z; 494.2, 472.2, 387.1, 382.2, 371.1, 357.2; MS/MS (m/z 494.0) 495.1, 448.1, 407.1; elemental analysis calcd (%) for $C_{26}H_{26}ClN_3O_5$: C 62.97, H 5.28, N 8.47; found: C 62.99, H 5.35, N 8.50.

Synthesis of Biginelli intermediate - ethyl 2-(7-hydroxy-2-oxo-2*H*-chromene-3-carbonyl)-3-phenylacrylate (5). To a stirred solution of **3a** (80 mg, 0.3 mmol), urea **4a** (0.35 mmol) and the corresponding benzaldehyde **5a** (0.5 mmol) in ethanol (2 mL) was added CaF_2 (0.1 mol%) at room temperature. The reaction was maintained under stirring for 24h at 75°C. The reaction solvent was evaporated under reduced pressure. The crude product was solubilised in CH_2Cl_2, applied in a preparative TLC plate and separated using a mixture of ethyl acetate/hexane (1:2) as eluent. Yield 5 mg, 5%; mp 164-168 °C; IR υ_{max}(KBr)/cm^{-1} 3431, 1730, 1616, 1190; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.37 (1H, s), 8.68 (1H, s), 7.90 (1H, d, *J* 6.9 Hz), 7.67 (1H, s), 7.38 (5H, m), 6.91 (1H, d, *J* 6.3 Hz), 6.79 (1H, s), 4.19 (2H, d), 1.14 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 190.4, 165.6, 164.5, 159.3, 158.2, 149.9, 140.3, 134.4, 133.9, 133.5, 130.6, 129.3, 118.7, 115.1, 111.5, 102.5, 61.3, 14.5; MS (ESI-) m/z; 363.1, 275.0, 229.0, 185.0; MS/MS (m/z 363.0) 363.1, 291.0; elemental analysis calcd (%) for $C_{21}H_{16}O_6$: C 69.23, H 4.43; found: C 69.22, H 4.45.

General Procedure for pKa determination. Sample analysis of **4a** (10 x 10^{-6} M, 1% methanol - 99% milli-Q water) where prepared from a stock solution previously prepared in methanol (UV/HPLC, 1 x 10^{-3} M). At the same time, it was prepared a saturated solution of NaOH. Aliquots of 1 µL of the basic solution were added to the previously prepared solutions of the compound **4a** in different 13 beakers followed by measurement of absorbance using spectrometer. UV-Vis scan was performed at a range of 200 nm to 800 nm. As it was observed an increase in the band centred at 390nm and a reduction at 335nm depending on pH, these wavelengths were used as references for pKa calculation. From these spectral variations, a graph of pH versus log ((A-A_f) / (A_0-A)) was plotted to determine the pKa of compound **4a** as 7.45.

General Procedure for quantum yield determination. The concentration of each molecule studied was adjusted to obtaining an UV peak absorption of above 0.1 at the excitation wavelength. All blank measurement spectra used the same cuvettes and solvents. The solvents used in these experiments were methanol and ethyl acetate UV/HPLC grade and milli-Q water.

The emission quantum yields were determined against a standard reference. For this purpose, it was calculated the ratio between the area of the emission spectrum of tested compound and the area of a known compound in the same solvent, as equation 1.

$$\phi_{em} = \frac{S_{em}}{S_{em}^{P}} \frac{\varepsilon^{P} C^{P}}{\varepsilon C} \phi_{em}^{P} \quad \text{equation 1}$$

Where φ_{em} is the quantum yield of the compound studied; S_{em} is the emission band of the sample area; S_{em}^{P} is the emission band of the standard; ε is the molar absorptivity of the sample in the λ of excitation; ε^{P} is the molar absorptivity of the standard; C is the molar concentration of the sample; C^{P} is the molar concentration of the standard; φ_{em}^{P} is the photoluminescence quantum yield of the standard.

For experiments using 3-acetyl-7-hydroxy-2*H*-chromen-2-one as standard, maximum absorbance of 0.27 at 335nm in the UV spectra was used. Emission spectra were obtained from Jasco spectrofluorimeter with a 485 volts lamp power set and an excitation bandwidth of 10 nm. For experiments using umbelliferone as standard, maximum absorbance of 0.19 at 335nm in the UV spectra was used. Emission spectra were obtained from Jasco spectrofluorimeter with a 395 volts lamp power set and an excitation bandwidth of 5 nm.

Notes and references

[a] Laboratório de Diversidade Molecular e Química Medicinal (LaDMol-QM, Molecular Diversity and Medicinal Chemistry Laboratory), Departament of Chemistry, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, 239897-000, Brazil.

[b] Programa de Pós-Gradução em Química (PPGQ), Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, 239897-000, Brazil.

* E-mail: akummerle@ufrrj.br

CCDC-No.1025676 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Electronic Supplementary Information (ESI) available: NMR and mass spectra, HPLC analyses, additional figures and crystallographic data as mentioned in the text. See DOI: xxxxxxx

1 R. F. Rodrigues, A. K. Tashima, R. M. S. Pereira, R. S. Mohameda and F. A. Cabral, *J. Supercrit. Fluids.*, 2008, **43**, 375–382.
2 L. G. Meimetis, J. C. T. Carlson, R. J. Giedt, R. H. Kohler and R. Weissleder, *Angew. Chem.*, 2014, **53**, 7531-7534.
3 Q. Sun, J. Qian, H. Tian, L. Duan and W. Zhang, *Chem. Commun.*, 2014, **50**, 8518.
4 C. Féau, E. Klein, C. Dosche, P. Kerthc and L. Lebeau, *Org. Biomol. Chem.*, 2009, **7**, 5259-5270.
5 S. K. Chattopadhyay, I. Kundua and R. Maitra, *Org. Biomol. Chem.*, 2014, **Advance Article**.
6 Z. Yang, J. Cao, Y. He, J. H. Yang, T. Kim, X. Peng and J. S. Kim, *Soc. Rev.*, 2014, **43**, 4563.
7 P. Pallavicini, V. Amendola, C. Massera, E. Munduma and A. Taglietti, *Chem. Commun.*, 2002, 2452–2453.
8 N. A. Kuznetsova and O. L. Kaliya, *Russ. Chem. Rev.,* 1992, **61**, 683-696.
9 M. M. Heravi, S. Asadi, B. M. Lashkariani. *Mol. Diversity*, 2013, **17**, 389-407.
10 J. G. Sośnicki, L. Struk, M. Kurzawski, M. Perużyńska, G. Maciejewskac and M. Droździk, *Org. Biomol. Chem.*, 2014, **12**, 3427.
11 S. R. Jetti, A. Upadhyaya and S. Jain, *Med. Chem. Res.*, 2014, **23**, 4356–4366.
12 M. S. Mohamed, S. M. Awad, Y. M. Zohny and Z. M. Mohamed, *Pharmacophore*, 2012, **3**, 62-75.
13 J. Lloyd, H. J. Finlay, W. Vacarro, T. Hyunh, A. Kover, R. Bhandaru, L. Yan, K. Atwal, M. L. Conder, T. Jenkins-West, H. Shi, C. Huang, D. Li, H. Sun, P. Levesque, *Bioorg. and Med. Chem. Lett.*, 2010, **20**, 1436-1439.
14 M. N. Khan, S. Pal, S. Karamthulla and L H. Choudhury, *New J. Chem.*, 2014, **38**, 4722-4729.
15 M. Matachea, C. Dobrotaa, N. D. Bogdana, I. Dumitrua, L. L. Rutab, C. C. Paraschivescub, I. C. Farcasanub, I. Baciub and D. P. Funeriu, *Tetrahedron*, 2009, **65**, 5949-5957.
16 H. Valizadeh, A. Fakhari, *Mol. Divers.*, 2011, **15**, 233-237.
17 P. Czerney and H. Hartmann, *J. Prakt. Chem.*, 1982, **324**, 21–28.
18 J. R. Barrio, A. J. Petric, N. Satyamurthy. *PCT Int. Appl.*, 2005, WO 2005040337 A2 20050506.
19 D. P. Specht, P. A. Martic and S. Farid. *Tetrahedron*, 1982, **38**, 1203–1211.
20 H. Sharghi and M. Jokar, *Synth. Commun.*, 2009, **39**, 958.
21 P. G. Biginelli, *Chim. Ital.*, 1893, **23**, 360-416.
22 R. D. H. Murray. Naturally Occurring Plant Coumarins, Springer Vienna, v. 35, 1978, pp. 200-209.
23 K .Folkers and T. B. Johnson, *J. Am. Chem. Soc.*, 1933, **55**, 3784–3791.
24 C. O. Kappe, *Multicomponent Reactions* (eds J. Zhu and H. Bienaymé), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2005, pp.95-120.
25 C. O. Kappe, *J. Org. Chem*. 1997, **62**, 7201-7204.
26 R. O. M. A. De Souza, E. T. da Penha, H. M. S. Milagre, S. J. Garden, P. M. Esteves, M. N. Eberlin and O. A. C. Antunes, *Chem. Eur. J*., 2009, **15**, 9799-9804.
27 K. Azuma, S. Suzuki, S. Uchiyama, T. Kajiro, T. Santa and K. Imai, *Photochem. Photobiol. Sci.*, 2003, **2**, 443–449.
28 M. Cigáň, J. Donovalová, V. Szöcs, J. Gaspar, K. Jakusová and A. Gáplovsky, *J. Phys. Chem. A*, 2013, **117**, 4870 – 4883.
29 P. Dahiya, M. Kumbhakar, T. Mukherjee and H. Pal, *Chem. Phys. Lett.*, 2005, **414**, 148-154.
30 A. S. Satpati, M. Kumbhakar, S. Nath and H. Pal, *Photochem. Photobiol*., 2009, **85**, 119-129.
31 B. Valeur, *Molecular Fluorescence: Principles and Applications*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2001, pp.273-350.
32 H. Li, L. Cai and Z. Chen, *Advances in Chemical Sensors* (ed W. Wang), 2012, pp.121-150.

