Dyes and Pigments 173 (2020) 107917

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: http://www.elsevier.com/locate/dyepig

Synthesis and photophysical properties of isocoumarin-based D-π-A systems

Valentina Pirovano [a], Marialaura Marchetti [b], Jessica Carbonaro [a], Elisa Brambilla [a], Elisabetta Rossi [a], Luca Ronda [b,c], Giorgio Abbiati [a,*]

[a] Dipartimento di Scienze Farmaceutiche, Sezione di Chimica Generale e Organica "A. Marchesini", Università degli Studi di Milano, Via Venezian, 21, 20133, Milano, Italy

[b] Biopharmanet -TEC, Università degli Studi di Parma, Parco Area delle Scienze, 23/A, 43124, Parma, Italy

[c] Dipartimento di Medicina e Chirurgia, Università degli Studi di Parma, Parco Area delle Scienze, 23/A, 43124, Parma, Italy

ARTICLE INFO

Keywords:
Isocoumarins
Polarity sensitive
Push-pull system
Silver catalysis
Solvatochromism
Fluorescence

ABSTRACT

We prepared a small library of polarity-sensitive fluorescent dyes characterized by an isocoumarin core properly functionalized with a conjugated push-pull system. The key step of the synthesis is based on a regio-selective silver(I)/*p*-TSA co-catalyzed cyclization of 2-alkynylbenzoates recently optimized in our laboratory. The photophysical properties of isocoumarin-based D-π-A systems have been investigated and a rationale was proposed based on their dipole moments and Hammett constants of the ED and EW groups involved.

1. Introduction

Environment-sensitive dyes are molecules able to change their spectroscopic properties in response to a variation of the chemical-physical properties of their surroundings. Polarity-sensitive dyes [1] are a subclass that displays different emission maximum as a function of the polarity of the medium (*i.e.,* solvent or environment). This feature makes these molecules interesting potential probes to monitor the local properties of particular cell districts and biological structures [2] and for application in advanced functional materials [3]. Among the two main classes of polarity-sensitive dyes (*i.e.* single-band and two-band solvatochromic dyes) [4,5], the former met more success because the latter displays some problems of photostability [6]. Single-band polarity-sensitive molecules are characterized by a quite rigid (hetero)aromatic backbone end-capped with conjugated electron-donating and electron-withdrawing groups (D-π-A or push-pull systems) [7]. In these molecules, D–A interaction, (so-called intramolecular charge-transfer - ICT), accounts for environment-sensitive photophysical properties. The electrons leaning in a low energy molecular orbital can be easily excited by UV/Vis light and an intramolecular charge transfer occurs, with an increase of the dipole moment. Depending on the polarity of the environment, the excited state of the molecule relaxes differently and dissipates part of the absorbed energy in a non-radiative way. This phenomenon allows using D-π-A molecules as sensitive probes of the polarity of the environment. If their excited states are stabilized by polar interactions with the surrounding medium, these molecules display a bathochromic effect in the emission spectra proportional to the degree of the polarity of the environment (positive solvatochromism). The optimal spectroscopic requirements of a polarity-sensitive dye are a strong solvatochromism, a large Stokes shift, an absorption close to the visible range, high extinction coefficient, quantum yield, and photostability.

A few years ago, we brought our contribution in this field synthesizing a small library of polarity-sensitive fluorescent dyes - nicknamed MediaChrom [8] (Fig. 1) - characterized by a heterocyclic pyrimidoindolone backbone, which displayed interesting photophysical profiles. Within our enduring interest in the development of new strategies for the synthesis of functionalized heterocycles starting from alkynes [9], we recently reported a selective and high yielding approach to isocoumarin nucleus [10]. Throughout this study, we observed that some of the obtained isocoumarins displayed significant fluorescence properties [11], in agreement with the well-known fluorescence features of the isomeric coumarins, documented since the pivotal work of Ronald L. Atkins in 1978 [12]. Based on these premises, we were interested to apply our synthetic approach to isocoumarin nucleus for the synthesis of a small library of D-π-A isocoumarins (Fig. 1) as polarity-sensitive fluorophores with enhanced photophysical properties, which can be interesting for biological application as environment-sensitive probes

* Corresponding author.
E-mail address: giorgio.abbiati@unimi.it (G. Abbiati).

https://doi.org/10.1016/j.dyepig.2019.107917
Received 26 June 2019; Received in revised form 5 September 2019; Accepted 21 September 2019
Available online 21 September 2019

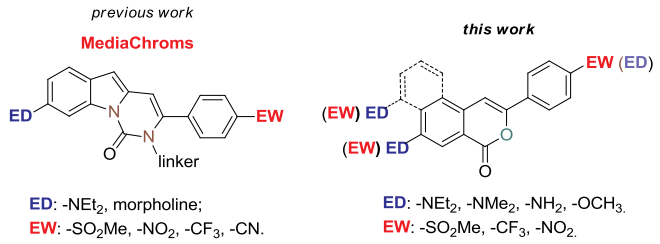


Fig. 1. The two class of heterocycle-based polarity-sensitive fluorescent dye.

and in advanced functional materials applications. The ED and EW groups of the push-pull system were selected taking into account the recent review of Filip Bures [7], together with our previous experience [8]. The photophysical properties of the D-π-A isocoumarins synthesized were evaluated and a rationale on the effect of the substituents on the solvatochromic shift was delineated. In this paper, we describe our results.

2. RESULTS and DISCUSSION

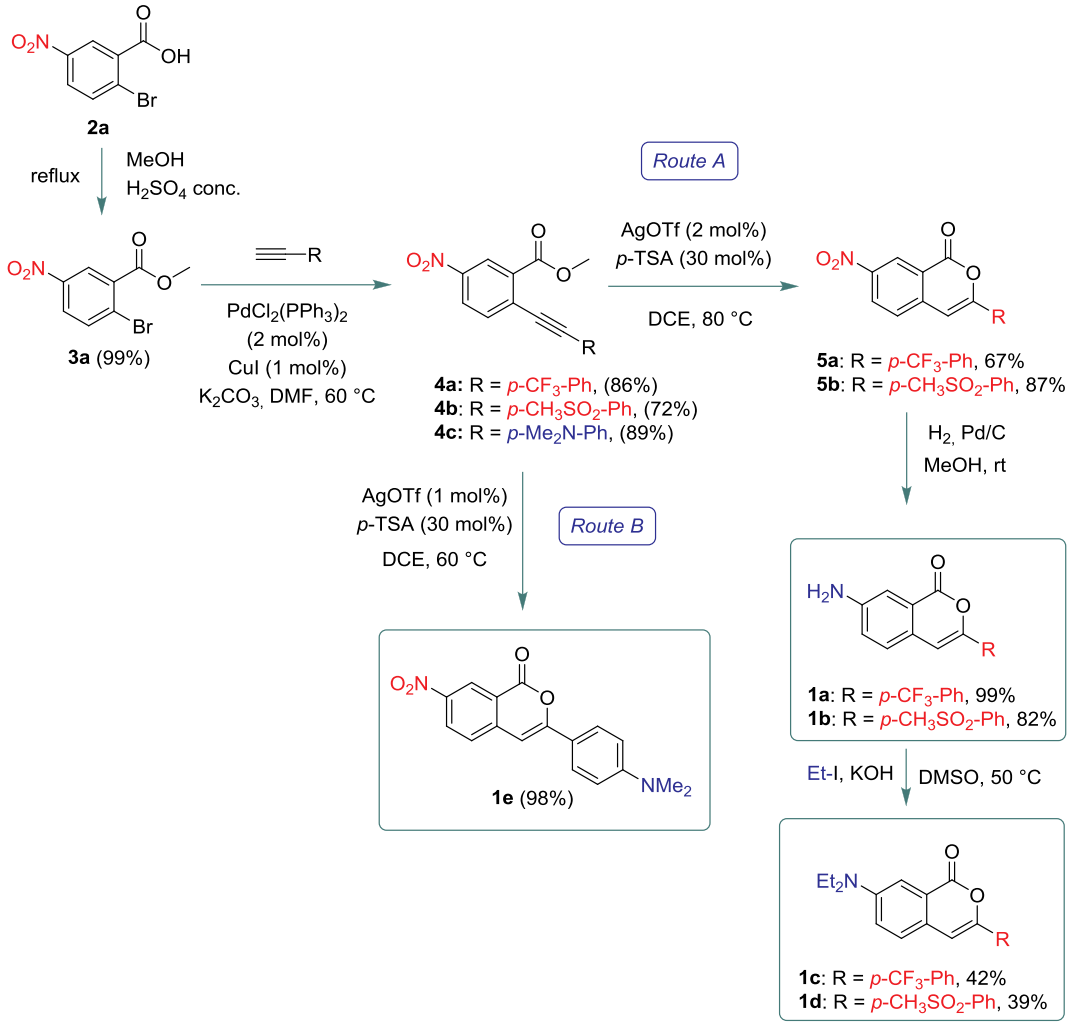
2.1. Synthesis of the isocoumarin-based library

Although the key step for the synthesis of all compounds **1a-g** and **11c,d,f** was the AgOTf/*p*-TSA co-catalyzed cyclization of the corresponding properly substituted 2-alkynyl benzoates, the preparation of suitable starting synthons demonstrated to be not always trivial.

To obtain isocoumarins **1a-d**, characterized by the presence of an amino substituent as ED group in position 5 of the 2-alkynyl benzoate ring, we planned the synthetic strategies depicted in Scheme 1. The ability of amino substituent to improve the fluorescence properties of isocoumarin derivatives has been already observed by Satoh and Miura [11b]. Regarding the choice of the EW groups, we privileged those groups that display more interesting features in the MediaChrom [8] series, and in particular the trifluoromethyl group, a substituent hardly investigated in this context [13].

Thus, commercially available 2-bromo-5-nitrobenzoic acid **2a** was easily converted into the corresponding methyl ester **3a** [14] and alkynylated under standard Sonogashira conditions [15] to give 2-alkynylbenzoates **4a,b** in very good yields.

The direct AgOTf/*p*-TSA co-catalyzed cyclization of intermediates **4a,b**, characterized by the presence of EWGs on the alkyne moiety was successful (Scheme 1, Route A) and gave the 7-nitroisocoumarins **5a,b** in very good yields, although to speed up the reaction a slight increase of the catalyst loading (2 mol%) and the reaction temperature (80 °C) was necessary. Compounds **5a,b** were then converted into the corresponding 7-aminoisocoumarins **1a-b** by catalytic hydrogenation without any undesired reduction of the C3–C4 double bond [16]. Finally, **1a-b** were converted in fair yields into the corresponding desired 7-diethylaminoisocoumarins **1c-d**, by *N,N-bis*-alkylation under basic condition with ethyl iodide at 50 °C [17]. Some solvatochromic fluorescent dyes endowed with a nitro group as EWG are described in the literature, such



Scheme 1. Synthesis of D-π-A isocoumarins **1a-e**.

as for example nitrobenzoxadiazole [18] (NBD). Having in hand freshly prepared the 2-bromo-5-nitrobenzoic methyl ester **3a** we prepared a new D-π-A isocoumarin with EDG and EWG in reverse positions (Scheme 1, Route B). This was achieved by the Pd-catalyzed alkynylation of **3a** with commercially available 4-ethynyl-*N,N*-dimethylaniline to give **4c** in 89% yield, followed by AgOTf/*p*-TSA co-catalyzed cyclization to obtain the desired isocoumarin **1e** in a satisfactory 98% yield (Scheme 1, Route B).

It has been reported that the nature of the electron-donating group in the D-π-A system is critical for the spectroscopic performance of the dye [7]. Alkylated amine seems to have the better features, and in particular dialkylamino group longer than dimethylamino can enhance some photophysical properties of the fluorophore [19]. Moreover, weaker EDG gave lower sensitivity in probing the polarity of the environment [20]. To verify if these statements were also applicable to our isocoumarin-based system, we planned to prepare two new isocoumarins **1f** and **1g** characterized by the presence of an oxygen-based EDG (Scheme 2).

Commercially available 2-bromo-5-methoxybenzaldehyde was oxidized into the corresponding benzoic acid [21] **2b** and then converted in the methyl benzoic ester [14] **3b** by standard methods. The alkynylation of electron-rich 2-bromobenzoate **3b** was a challenging task. Only by modifying the standard Sonogashira reaction condition by addition of a higher amount of a different palladium catalyst and a more electron-rich phosphine ligand, we were able to obtain the desired 2-alkynyl esters **6a** and **6b** in good yields. Finally, the cyclization gave the desired products **1f,g** in fair yields, beside a small amount of the corresponding regioisomeric 3-ethylidene-6-methoxyisobenzofuran-1-ones **7a**,**b** (Scheme 2).

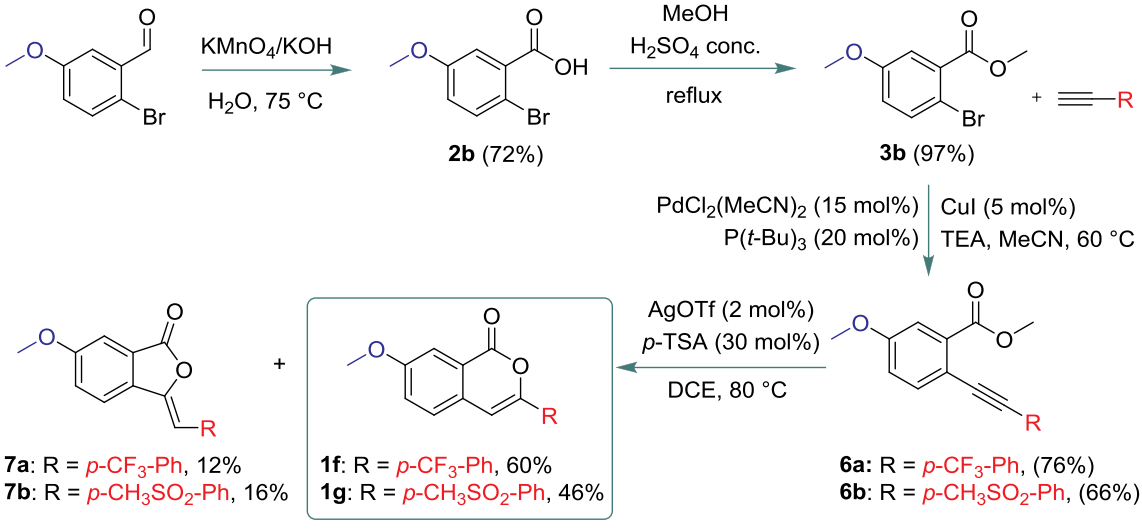
Finally, we decide to test the effect of enhancing the conjugation of the rigid push-pull system without a significant increase in the distance between the ED and the EW groups. Thus, we planned the synthesis of the tricyclic benzofused isocoumarins **11c,d,f** characterized by an angular shape. These molecules were designed with the purpose to achieve a redshift of the excitation wavelength (thanks to the extended conjugation of the D-π-A system) preserving the same ICT (thanks to a shorter distance between ED and EW groups, with respect to hypothetical isomeric linear benzofused isocoumarins). This should bring to an increase of the dipole moment upon light excitation and a greater slope in the Lippert plot relationship, resulting in a larger bathochromic shift as a function of orientational polarizability.

The synthetic strategy is described in Scheme 3. Commercially available 1-bromo-2-naphthoic acid was almost quantitatively transformed in the corresponding methyl ester **9** under the previously described Fisher esterification conditions [14]. The selective nitration on C-5 of the naphthalene ring was a very challenging task, and although a number of different literature methods have been tried, the desired methyl 1-bromo-5-nitro-2-naphthoate **9a** was always obtained in low yield beside the 1-bromo-8-nitro-2-naphthoate **9b** isomer and traces of other nitrated products. Best results (30% yield of **9a**) were obtained by treatment with nitronium tetrafluoborate in acetonitrile/dichloromethane [22]. Fortunately, the subsequent alkynylation was successful, and the 1-alkynyl 5-nitro-2-naphthoate **10a** ready for the cyclization was obtained in nearly quantitative yield. Silver/*p*-TSA co-catalyzed cyclization was in this case very slow, and only after 2-days heating and a slight increase of catalysts loading (i.e., AgOTf 4 mol% and *p*-TSA 50 mol%) the desired 7-nitro benzo[*f*]isocoumarin **11a** was obtained in excellent yield. Finally, 7-dimethylamino benzo[*f*]isocoumarin **11c** and 7-diethylamino benzo[*f*]isocoumarin **11d** were obtained by catalytic reduction of nitro group of **11a** to give the 7-amino benzo[*f*]isocoumarin **11b**, followed by dialkylation of the amino group with alkyl iodide and potassium carbonate in DMSO at 65 °C [23] (Scheme 3). The reaction with ethyl iodide resulted slower and beside the desired product **11d**, a small amount of the monoethylated derivative **11e** was isolated. In analogy to what previously done on bicyclic substrates (see above), by applying our standard alkynylation/cyclization two-step strategy on 1-bromo-5-nitro-2-naphthoate **9a**, we were able to prepare the 7-nitro benzo[*f*]isocoumarin **11f**, characterized by the presence of the ED and EW groups in reverse positions, in very good yields (Scheme 3).

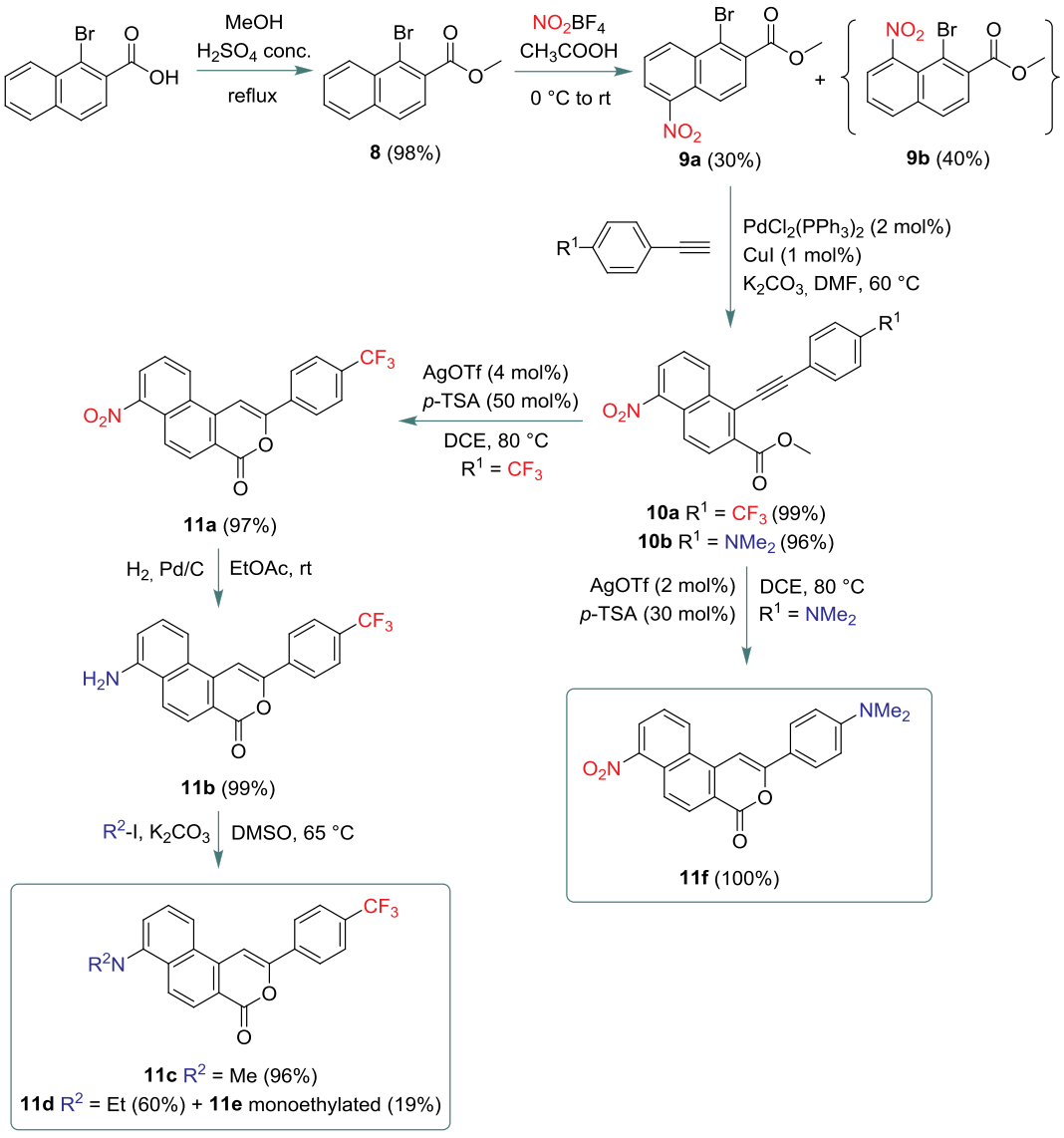
All new compounds were fully characterized by ^{1}H, ^{13}C NMR spectroscopies, and mass spectrometry. Then, photophysical properties were evaluated.

2.2. Photophysical evaluation

As a first step in the characterization of the synthesized molecules, we recorded their absorption spectra in different solvents characterized by different polarities (methanol, ethanol, 1-propanol, 1-butanol, 1-octanol, DMF, acetone, ethyl acetate, chloroform and hexane) to determine their solubility and their capability to absorb light (Table 1). Most of the molecules synthesized are well soluble in the explored solvents, except for 1-octanol, where only **1b** and **1f** demonstrated to be soluble and **1e** (characterized with the EDG and EWG in reverse positions) and **1g**, which are insoluble in almost half of the tested solvents. The absorption peaks for most isocoumarins analysed are localized in the near UV region, ranging (in ethanol) from 324 nm of **1f** to 376 nm for **1d**. Only isocoumarin **1e** has the maximum absorption peak in the visible range (i.e. 444 nm in methanol). When the compounds were



Scheme 2. Synthesis of D-π-A isocoumarins **1f-g**.



Scheme 3. Synthesis of D-π-A benzo[*f*]isocoumarin **11 c,d,f**.

Table 1
Absorption peak wavelength (λ_{exc}) and fluorescent emission peak wavelength (λ_{em}) of isocoumarins **1a-g** solubilized in different solvents.

Solvent	1a		1b		1c		1d		1e		1f		1g	
	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)
DMF	356	448	367	479	370	462	379	499	456	605[d]	328	411	333	410
Methanol	344	461	358 (24400)[c]	473	364	475	376	489	444	605[d]	324	413	330	410
Ethanol	346 (21800)[b]	460	n.s.	n.s.	366 (20500) [b]	471	376 (9900) [b]	485	n.s.	n.s.	324 (16000) [b]	412	331	410
1-Propanol	348	459	366	471	365	470	378	480	n.s.	n.s.	325	410	n.s.	n.s.
1-Butanol	348	460	366	471	365	468	378	476	448	559	326	411	n.s.	n.s.
1-Octanol	n.s.	n.s.	366	461	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	327	410	n.s.	n.s.
Acetone	347	439	358	461	366	454	375	487	446	682	[a]	[a]	[a]	[a]
Ethyl Acetate	347	436	356	446	365	443	374	465	442	621	326	404	331	404
Chloroform	336	438	351	439	368	450	383	456	455	652	327	409	334	408
Hexane	335	418	n.s.	n.s.	362	418	373	419	n.s.	n.s.	326	397	n.s.	n.s.
Δ λ_{em}	–	**43**	-	**40**	-	**57**	-	**80**	-	**123**	-	**16**	-	**6**

[a] absorption peaks are below 320 nm, where acetone absorption precludes reliable absorption spectra acquisition.
[b] numbers under parenthesis are molar extinction coefficients expressed as $M^{-1}\cdot cm^{-1}$ determined in ethanol.
[c] numbers under parenthesis are molar extinction coefficients expressed as $M^{-1}\cdot cm^{-1}$ determined in methanol.
[d] poorly fluorescent.

excited at a wavelength corresponding to their absorption maxima, fluorescent emission peaks appeared in all solvents with good intensities except for **1e** whose fluorescence is poor. The most significant solvatochromic shifts were observed for compounds **1c-e**. A bathochromic effect of the emission peak was observed in relationship with the increasing of the polarity of the medium for compounds **1a-d,f,g**, while **1e** displayed an interesting reversed hypsochromic shift.

As expected [7] isocoumarins **1f** and **1g**, characterized by the presence of a weak methoxy group as EDG display the less pronounced solvatochromism. Also compounds **1a** and **1b**, with primary amines as EDG have a modest solvatochromism. It is worth noting that the "electronically upset" isocoumarin **1e** displayed two interesting features: absorption maximum and fluorescence emission in the visible range and a strong solvatochromic effect ($\Delta\ \lambda_{em} = 123$ nm). Unfortunately, this compound suffers from severe solubility problems and poor fluorescence intensity. So, taking into account all the important features that an ideal environmental sensitive dye should have (i.e. solubility, strong solvatochromism, fluorescence intensity, absorption close to the visible range, large Stokes shift), compound **1d** seems to be the best solvatochromic isocoumarin obtained. The superimposed absorption and emission spectra in all solvents for **1d** are depicted in Fig. 2.

2.3. Quantitative description of solvatochromism by a modified Lippert-Mataga equation

A quantitative description of the solvatochromic behavior of fluorescent dye is commonly carried out by using the Lippert-Mataga equation [24], (eq. (1)):

$$\overline{\nu_a} - \overline{\nu_f} = \frac{2}{hc}\left(\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}\right)\frac{(\mu^* - \mu)^2}{a^3} + const \quad (1)$$

where $\overline{\nu_a}$ and $\overline{\nu_f}$ are the wavenumbers in cm^{-1} of absorption and emission peaks, h is Planck's constant, c is the speed of light, a is the Onsager cavity radius, and μ* and μ are the dipole moment of the molecule in the excited and the ground state, respectively. In this equation, a spherical shape of the chromophore is assumed (a is the radius of the sphere).

Before its application to our isocoumarins, we made a consideration about their shape and we realized that their elongated, planar D-π-A system is more properly described by a spheroid prolate. For other elongated-shaped chromophores such as Prodan and Laurdan, similar considerations have already been made for a correct calculation of the dipole moment [25]. Hence, we applied a modified Lippert-Mataga equation in which the three axes of the spheroid prolate are considered [26] (eq. (2)):

$$\overline{\nu_a} - \overline{\nu_f} = \frac{3}{hc}\left(\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}\right)\frac{(\mu^* - \mu)^2}{abd} + const \quad (2)$$

where a, b and d are the spheroid prolate axes, with a > b = d.

The approach was restricted to the isocoumarins which displayed the more interesting photophysical properties, i.e. compounds **1a-d**. Once isocoumarins **1a-d** have been structurally minimized and fitted into a spheroid prolate to determine a, b and d, the dipole moments were calculated (Table 2).

Isocoumarin **1d**, display the more interesting properties for potential uses as a biological probe due to its excitation peak localized more close to visible light (see Table 1), and the highest dipole moment (μ*-μ) upon light excitation (Table 2). The Lippert plot for **1d** is reported in Fig. 3.

The results obtained from this analysis showed a relevant change in the dipole moment upon excitation, for compound **1d** having the higher bathochromic shift as a function of solvent polarity, and quantitatively demonstrated by the calculated μ*-μ. The linear dependence of Stokes shifts on orientational polarizability in both aprotic and protic solvents suggest a non-specific interaction between **1d** and the investigated solvents.

2.4. Evaluation of the substituent effect of the ED and EW groups of the D-π-A isocoumarin system

While we demonstrated that the synthesized isocoumarin have spectroscopic properties in terms of absorption, fluorescent emission and solvatochromism that make them potentially useful as environment-sensitive probes, we would like to systematically evaluate the effect of the substituents on the spectroscopic properties. The effect of substituents on push-pull system in relation to their mesomeric effects has already been investigated, and the possibility of a fine-tuning of spectroscopic features based on the accurate choice of ED and EW substituent D-π-A has been claimed [7]. Hence, we rationalize our results correlating the absorption peak (in ethanol) and the fluorescent

Table 2
Increase of the dipole moment (μ*- μ) upon light excitation for compound **1a-d** obtained from fitting to the modified Lippert-Mataga equation (eq. (2)).

Compound	μ*- μ protic solvents (Debye)	μ*- μ aprotic solvents (Debye)
1a	7.47 ± 0.94	-[a]
1b	11.37 ± 0.73	7.21 ± 1.35
1c	10.24 ± 1.37	8.99 ± 0.70
1d	13.84 ± 1.51	12.92 ± 0.82

[a] Solvatochromic shift as a function of orientational polarizability in aprotic solvents showed a scattered dependence and did not allow a reliable fitting.

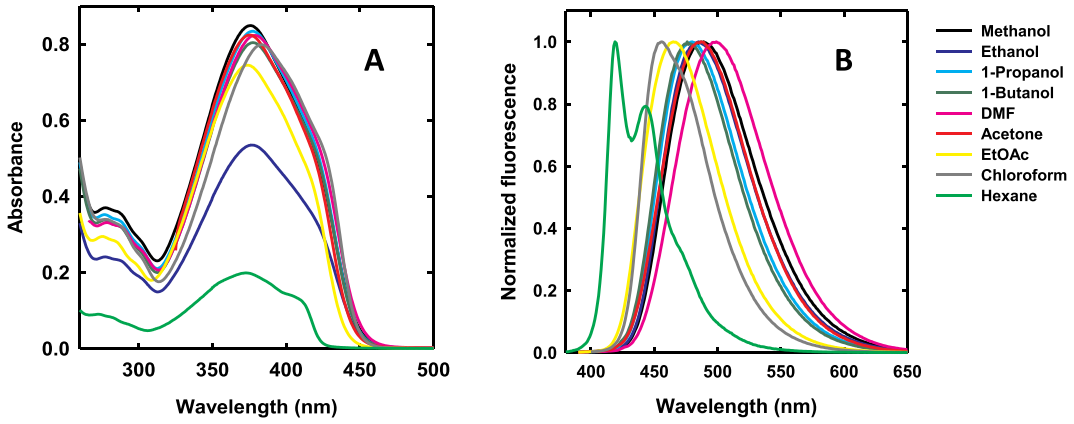


Fig. 2. Absorption spectra of **1d** in different solvents at 53.84 μM at 20 °C. B. Fluorescent emission spectra of **1d** in the same solvent explored for the absorption spectra at 20 °C upon dilution to keep absorbance below 0.1 OD to avoid inner filter effect.

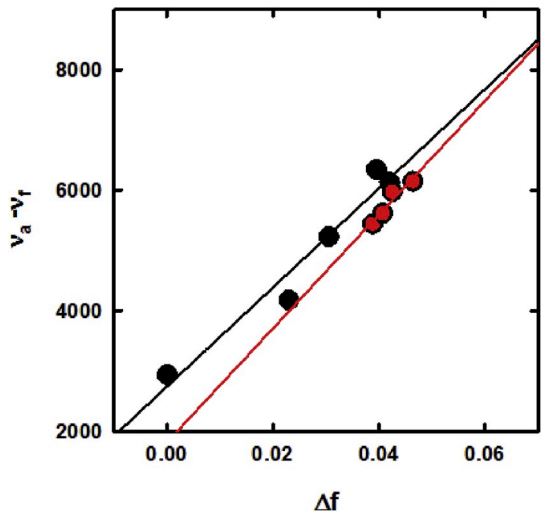


Fig. 3. Lippert plot of **1d**. Black circles, aprotic solvent; red circles, protic solvents. Data points for aprotic and protic solvent are separately fitted to the modified Lippert-Mataga equation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

emission peak with the difference between the Hammett constant (σ_P) for the ED and EW groups (Fig. 4 A and B).

The direct correlation between $\Delta\sigma_P$ of ED and EW groups and solvatochromic shift give a quantitative measure of the ICT. From this analysis, we can observe that the redshift of the emission peak is particularly marked in the more polar solvent, with a maximum for DMF. Moreover, the higher sensitivity towards solvent polarity and the larger bathochromic shift observed in the molecules with greater ICT well agree with the previous observation that a redshift is observed increasing the ED and EW capability of substituents [7] (Fig. 4, A). Finally, we can also observe a well-defined correlation between σ_P ED and σ_P EW difference and the absorption peak of isocoumarins (Fig. 4, B). This result confirms that, also for these series of isocoumarin dyes, an increased degree of ICT, obtained with proper substituents, brings to a red shifting of the excitation wavelength together with a larger solvatochromism of the fluorophore, both valuable properties for the use of these molecules for environment-sensitive imaging purposes and biological applications as probes or dyes.

2.5. Spectroscopic effects of the extension of the conjugated push-pull system

We finally compared the absorption and emission properties of compounds **1a-g** with of those of compounds **11c,d,f**, possessing an extended conjugated push-pull system based on a tricyclic benzofused isocoumarin core (Table 3).

With respect to the former, the elongation of the nucleus brought to a general decrease of the solubility of the compounds, which are almost completely soluble at the stock concentration (5 mg/ml). The uncertainty in solubilisation made the stock concentration poorly reliable and hence molar extinction coefficients for this series was not calculated. Compound **11d** is the most soluble whereas **11f**, as already observed for the analogous bicyclic compound **1e**, is in general poorly soluble and completely insoluble in half of the explored solvents. Moreover, in analogy with **1e**, it displayed a reversed hypsochromic shift, but the fluorescence is poor (Table 3).

As expected, a larger conjugation resulted in a red shifting of the absorption, so falling in the visible range for all compounds examined. Emission as a function of solvent polarity significantly shifted for **11c** and **11d**. In particular, **11d,** the most soluble, displayed the larger solvatochromic effect ranging from 469 nm in hexane to 613 nm in DMF ($\Delta\lambda_{em} = 144$). This latter showed a higher Stokes shift and a larger increase

Table 3
Absorption peak wavelength (λ_{exc}) and fluorescent emission peak wavelength (λ_{em}) of isocoumarins **11c,d,f** solubilized in different solvents.

Solvent	**11c**		**11d**		**11f**	
	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)	λ_{exc} (nm)	λ_{em} (nm)
DMF	395	614	394	613	444	546
Methanol	391	612	391	604	439	548[a]
Ethanol	393	602	392	599	n.s.	n.s.
1-Propanol	393	591	391	587	n.s.	n.s.
1-Butanol	394	586	392	578	n.s.	n.s.
1-Octanol	395	585	393	561	n.s.	n.s.
Acetone	392	611	390	604	438	530
Ethyl Acetate	393	578	389	568	435	624
Chloroform	395	538	393	542	446	634
Hexane	n.s.	n.s.	388	469	n.s.	n.s.
$\Delta\lambda_{em}$		76		144		104

[a] Poorly fluorescent.

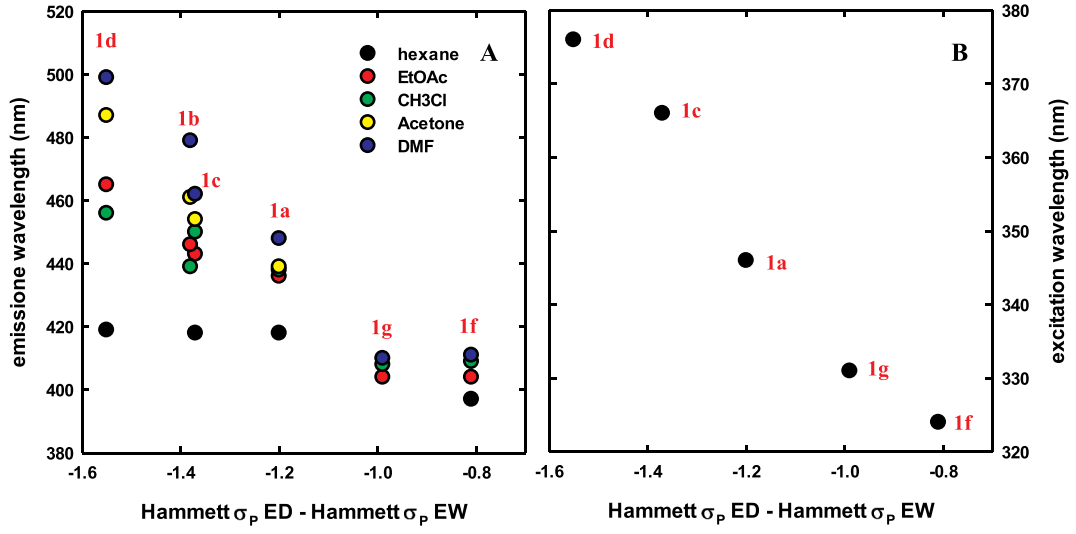


Fig. 4. A: Fluorescent emission peak in different solvents for isocoumarins **1a-d,f,g** as a function of the difference between σ_P of ED and EW groups. **B**: absorption peak in ethanol as a function of the difference between σ_P of ED and EW groups.

in the dipole moment as a function of solvent polarity respect to **1d**, as expected, thanks to the increased size of the polycyclic ring and π-electrons conjugation (Fig. 5). The better physical and spectroscopic properties of **11d**, characterized by the presence of a diethylamino substituent versus **11c**, which has a dimethylamino group, are in agreement with some previous findings [19].

Finally, for most interesting compounds we determined quantum yields (the solvatochromic dye Prodan was used as a reference, QY = 0.71) and fluorescence lifetimes. In particular, the QY of **1c**, **1d**, and **11d** in EtOH resulted to be 0.11, 0.31 and 0.04, respectively, whereas fluorescence lifetime of **1d** and **11d** in the same solvent resulted to be 1.79 and 2.09 ns, respectively (for plots see Supporting Information).

3. Conclusions

We prepared ten polarity-sensitive fluorescent dyes characterized by the presence of an isocoumarin-based nucleus endowed with a conjugated push-pull system. The synthetic approaches involve four/six steps starting from low-cost commercially available materials. The key step of the syntheses involves a highly regioselective silver(I)/*p*-TSA co-catalyzed cyclization of 2-alkynylbenzoates. The photophysical features of the isocoumarins synthesized were investigated. In particular, the effects of the nature of D-π-A system and the dimension of the conjugated system were tentatively rationalized based on dipole moments calculated by Lippert-Mataga equation and the difference between the Hammett constant (σ_P) for the ED and EW groups involved. Isocoumarins **1f** and **1g**, with a methoxy group as EDG, are poorly solvatochromic. Compounds **1e** and **11f**, with a reversed D-π-A system, displayed an interesting reversed solvatochromism in the visible range, but suffer from severe solubility problems and scarce fluorescence. *N,N*-Diethyl derivatives shown in general better features than *N,N*-dimethyl ones. Overall, isocoumarin **1d** and the benzofused isocoumarin **11d** displayed the best physical and spectroscopic features for possible applications in biological and advanced material fields: a wide solvatochromic effect, a good solubility in different solvents, a constant absorption close to the visible range, a quite good fluorescence (**1d**) and a large Stokes shift.

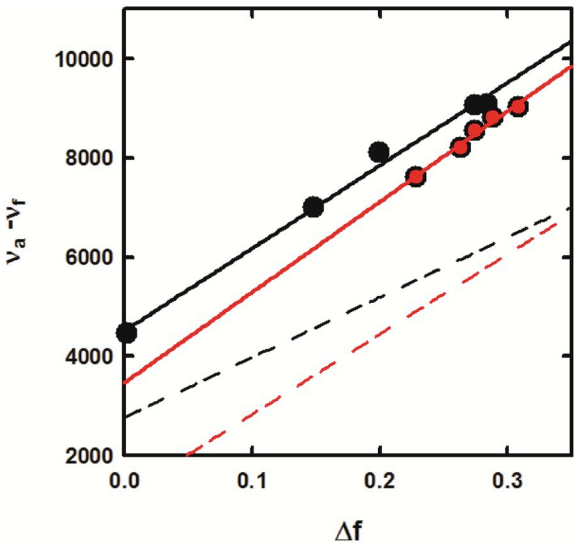


Fig. 5. Lippert plot of **11d**. Black circles, aprotic solvent; red circles, protic solvents. Data points for aprotic and protic solvent are separately fitted to the modified Lippert-Mataga equation (solid lines). Dashed lines are fitting of **1d** as from Fig. 3 for comparison. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Experimental section

4.1. General

Anhydrous solvents are commercially available and stored in a protected atmosphere of nitrogen. All the reactions that involve the use of reagents sensitive to oxygen or hydrolysis, were carried out under nitrogen. The glassware was previously dried in an oven at 110 °C and set with cycles of vacuum and nitrogen. The chromatographic column separations were performed by a flash technique, using silica gel (pore size 60 Å, particle size 230–400 mesh). TLC Alu foils with a fluorescent indicator (254 nm) were used for TLC analysis, and the detection was performed by irradiation with UV light (λ = 254 nm and/or 366 nm). ^{1}H NMR analyses were performed with 300 MHz or 500 MHz spectrometers at rt. Spectra were referenced to residual solvent. The coupling constants (*J*) are expressed in Hertz (Hz), the chemical shifts (*δ*) in ppm. ^{13}C NMR analyses were performed with the same instruments at 50.3 and 75.45 MHz. Attached Proton Test (APT) sequence was used to distinguish the methine and methyl carbon signals from those arising from methylene and quaternary carbon atoms. All ^{13}C NMR spectra were recorded with complete proton decoupling. The absorbance is reported in wavenumbers (cm^{-1}) with values between 4000 and 400 cm^{-1}. Low-resolution MS spectra were recorded with an electron impact source and electrospray/ion trap equipped instrument, using a syringe pump device to directly inject sample solutions. The values are reported as mass-charge ratio and the relative intensities of the most significant peaks are shown in brackets. The melting points of the solid products are uncorrected. UV–visible and fluorescence spectra were collected at 20 °C.

4.2. Synthesis of 2-bromo-5-methoxybenzoic acid (2b)

Commercially available 2-bromo-5-methoxybenzaldehyde (4.65 mmol) was dissolved in 20 mL of water. $KMnO_4$ (6.51 mmol, 1.4 eq.) was dissolved in 15 mL of water and added dropwise to the reaction mixture. The reaction mixture was stirred at 75 °C for 2 h, then a solution of KOH at 20% was added to obtain a strongly basic pH. The reaction mixture was filtered through a pleated filter, the filter washed with hot water and the aqueous filtrates were cooled to rt and filtered again to remove aldehyde residues. When the aqueous filtrates were acidified with HCl 37% the 2-bromo-5-methoxybenzoic acid **2b** precipitated as white solid that was collected by filtration under reduced pressure (Yield: 72%, 773 mg). No further purification was necessary. **^{1}H NMR** (300 MHz, $CDCl_3$): *δ* = 7.58 (d, *J* = 8.8 Hz, 1H, arom.), 7.52 (d, *J* = 3.1 Hz, 1H, arom.), 6.95 (dd, *J* = 8.8, 3.1 Hz, 1H, arom.), 3.84 (s, 3H, –CH_3). These data are in good agreement with literature values [27].

4.3. General procedure for the synthesis of methyl 2-haloarylcarboxylates 3a, 3b and 8

The proper 2-haloarylcarboxylic acid (12 mmol) was dissolved in 60 mL of methanol and to this solution 8 mL (12 equiv.) of concentrated sulfuric acid were then added dropwise. Then the reaction mixture was stirred at reflux until no more starting product was detected by TLC analysis. The reaction mixture was then cooled to rt and concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (3 × 30 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was removed at reduced pressure to yield the corresponding methyl 2-haloarylcarboxylates.

4.3.1. Methyl 2-bromo-5-nitrobenzoate (3a)

Reaction time: 3 h. White solid. Yield: 99% (3.12 g). Mp 79–81 °C (lit. 82 °C). **^{1}H NMR** (300 MHz, $CDCl_3$): *δ* = 8.67 (d, *J* = 2.7 Hz, 1H, arom.), 8.19 (dd, *J* = 8.8, 2.8 Hz, 1H, arom.), 7.89 (d, *J* = 8.8 Hz, 1H, arom.), 4.01 (s, 3H, –$COOCH_3$). **^{13}C NMR** (75 MHz, $CDCl_3$): *δ* = 164.5 (C═O), 146.8 (C, arom.), 135.7 (CH, arom.) 133.2 (C, arom.), 129.2 (C,

arom.), 126.6 (CH, arom.), 126.3 (CH, arom.), 53.1 (CH_3). **MS** EI (+): *m/z* (%) = 228 [M(^{79}Br) – CH_3O]$^+$ (100), 230 [M(^{81}Br) – CH_3O]$^+$ (100), 259 [M(^{79}Br)]$^+$ (55), 261 [M(^{81}Br)]$^+$ (55); $C_8H_6BrNO_4$ [260.04]. These data are in good agreement with literature values [28].

4.3.2. Methyl 2-bromo-5-methoxybenzoate (3b)

Reaction time: 4 h. Yellow oil. Yield: 565 mg, 97% (2.85 g). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.53 (d, J = 8.8 Hz, 1H, arom.), 7.31 (d, J = 3.1 Hz, 1H, arom.), 6.89 (dd, J = 8.8, 3.1 Hz, 1H, arom.), 3.93 (s, 3H, –OCH_3), 3.82 (s, 3H, –$COOCH_3$). These data are in good agreement with literature values [29].

4.3.3. Methyl 1-bromo-2-naphthoate (8)

Reaction time: 5 h. Pale yellow wax. Yield: 98% (3.12 g). **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 8.46 (m, 1H, arom.), 7.84 (m, 2H, arom.), 7.70–7.55 (m, J = 8.3 3H, arom.), 4.00 (s, 3H, –$COOCH_3$). These data are in good agreement with literature values [30].

4.4. General procedure for the synthesis of methyl 2-alkynylarylcarboxylates 4a-c, 6a,b and 10a,b: Method A

To a stirred solution of the proper methyl 2-haloarylcarboxylate (**3a** or **9a)** (0.5 mmol) in anhydrous DMF (2 mL), K_2CO_3 (2.5 mmol), the appropriate alkyne (0.6 mmol, 1.2 equiv) and $(PPh_3)_2PdCl_2$ (2 mol%) were added under nitrogen. The reaction was stirred at rt for 10 min, then CuI (1 mol%) was added. The reaction mixture was stirred at 60 °C until no more starting product was detected by TLC analysis. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (3 × 15 mL). The organic layers were united, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude material was purified by flash column chromatography over silica gel. **Method B:** Under a nitrogen atmosphere, methyl 2-bromo-5-methoxybenzoate **3b** (0.408 mmol) was dissolved in 0.5 mL of anhydrous TEA and 1.5 mL of anhydrous CH_3CN. To the stirred mixture the appropriate alkyne (0.489 mmol, 1.2 eq.), bis(acetonitrile)dichloropalladium(II) (14 mol%) and tri-*tert*-butylphosphine (20 mol %) were added. The reaction was stirred at rt for 15 min, then CuI (5 mol%) was added. The stirred reaction was heated at 70 °C until no more starting product was detected by TLC analysis, then filtered on a thin Celite pad. The pad was washed with CH_2Cl_2, and then the united organic layers were evaporated under reduced pressure. The crude material was purified by flash chromatography over silica gel to yield the desired products.

4.4.1. Methyl 5-nitro-2-((4-(trifluoromethyl)phenyl)ethynyl)benzoate (4a)

Method A. Reaction time: 3 h. Eluent for chromatography: hexane/EtOAc 9:1. Orange solid. Yield 86% (175 mg); mp 114–116 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 8.88 (d, J = 2.4 Hz, 1H, arom.), 8.38 (dd, J = 8.6, 2.4 Hz, 1H, arom.), 7.84 (d, J = 8.6 Hz, 1H, arom.), 7.74 (d, J = 8.3 Hz, 2H, arom.), 7.68 (d, J = 8.3 Hz, 2H, arom.), 4.04 (s, 3H, CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 164.3 (C═O), 146.8 (C, arom.), 135.1 (CH, arom.), 133.1 (C, arom.), 132.3 (CH, arom.), 131.1 (q, $^2J(C,F)$ = 32 Hz, 1C, C-CF_3), 129.5 (C, arom.), 126.2 (CH, arom.), 126.0 (C, arom.), 125.9 (CH, arom.), 125.5 (q, $^3J(C,F)$ = 4 Hz, CH-C-CF_3) 123.8 (q, $^1J(C,F)$ = 272 Hz, 1C, CF_3), 98.2 (C sp), 88.8 (C sp), 52.9 (CH_3). **MS** ESI (+): *m/z* (%) = 350 [M+1]$^+$ (100), 372 [M+Na]$^+$ (15); $C_{17}H_{10}F_3NO_4$ [349.26]. Calcd for $C_{17}H_{10}F_3NO_4$: C, 58.46; H, 2.89; N, 4.01; found: C, 58.59; H, 2.68; N, 4.23.

4.4.2. Methyl 2-((4-(methylsulfonyl)phenyl)ethynyl)-5-nitrobenzoate (4b)

Method A. Reaction time: 2.5 h. Eluent for chromatography: hexane/EtOAc 7:3. Orange solid. Yield 72% (129 mg); mp 162–164 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 8.87 (d, J = 2.3 Hz, 1H, arom.), 8.37 (dd, J = 8.6, 2.4 Hz, 1H, arom.), 7.97 (d, J = 8.6 Hz, 2H, arom.), 7.83 (d, J = 8.5 Hz, 1H, arom.), 7.79 (d, J = 8.6 Hz, 2H, arom.), 4.02 (s, 3H, –$COOCH_3$), 3.08 (s, 3H, –SO_2CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 164.1 (C═O), 146.9 (C, arom.), 140.8 (C, arom.), 135.1 (CH, arom.), 133.1 (C, arom.), 132.7 (CH, arom.), 129.2 (C, arom.), 128.0 (C, arom.), 127.6 (CH, arom.), 126.2 (CH, arom.), 125.9 (CH, arom.), 97.5 (C sp), 90.1 (C sp), 52.9 (-COOCH$_3$), 44.4 (-SO_2CH$_3$). **MS** ESI (+): *m/z* (%) = 360 [M+1]$^+$ (100), 382 [M+Na]$^+$ (10); $C_{17}H_{13}NO_6S$ [359.35]. Calcd for $C_{17}H_{13}NO_6S$: C, 56.82; H, 3.65; N, 3.90; found: C, 57.02; H, 3.74; N, 4.05.

4.4.3. Methyl 2-((4-(dimethylamino)phenyl)ethynyl)-5-nitrobenzoate (4c)

Method A. Reaction time: 4 h. Eluent for chromatography: hexane/EtOAc 8:2. Bordeaux solid. Yield 89% (144 mg); mp146.1–147.2 °C. **^{1}H NMR** (300 MHz, DMSO): δ = 8.59 (d, J = 2.5 Hz, 1H, arom.), 8.35 (dd, J = 8.6, 2.5 Hz, 1H, arom.), 7.82 (d, J = 8.6 Hz, 1H, arom.), 7.41 (d, J = 8.9 Hz, 2H, arom.), 6.74 (d, J = 9.0 Hz, 2H, arom.), 3.94 (s, 3H, –OCH_3), 2.98 (s, 6H, –$N(CH_3)_2$). **^{13}C NMR** (75 MHz, DMSO): δ = 164.9 (C═O), 151.4 (C, arom.), 145.7 (C, arom.), 134.7 (CH, arom.), 133.8 (CH, arom.), 131.8 (C, arom.), 130.4 (C, arom.), 126.9 (CH, arom.), 125.6 (CH, arom.), 112.3 (CH, arom.), 107.7 (C, arom.), 103.5 (C, arom.), 86.9 (C, arom.), 53.1 (-OCH_3), 39.8 (-$N(CH_3)_2$). **MS** ESI (+): *m/z* (%) = 325.14 [M+1]$^+$ (100); $C_{18}H_{16}N_2O_4$ [324.33]. Calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64; found: C, 66.74; H, 5.06; N, 8.60.

4.4.4. Methyl 5-methoxy-2-((4-(trifluoromethyl)phenyl)ethynyl)benzoate (6a)

Method B. Reaction time: 1.75 h. Eluent for chromatography: hexane/EtOAc 95:5. Brown solid. Yield 76% (104 mg); mp 50-21 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.69–7.55 (m, 5H arom.), 7.52 (d, J = 2.7 Hz, 1H, arom.), 7.06 (dd, J = 8.6, 2.7 Hz, 1H, arom.), 3.98 (s, 3H, –OCH_3), 3.88 (s, 3H, –OCH_3). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 166.3 (C═O), 159.5 (C, arom.), 135.5 (CH, arom.), 133.4 (C, arom.), 131.7 (CH, arom.), 129.7 (q, $^2J(C,F)$ = 33 Hz, 1C, C-CF_3), 127.6 (C, arom.), 125.3 (q, $^3J(C,F)$ = 4 Hz, CH-C-CF_3), 124.0 (q, $^1J(C,F)$ = 272 Hz, 1C, CF_3), 118.3 (CH, arom.), 115.3 (CH, arom.), 115.1 (C, arom.), 91.1 (C, sp), 90.7 (C, sp), 55.6 (-OCH_3), 52.3 (-$COOCH_3$). **MS** ESI (+): *m/z* (%) = 335 [M+1]$^+$ (100); $C_{18}H_{13}F_3O_3$ [334.29]. Calcd for $C_{18}H_{13}F_3O_3$: C, 64.67; H, 3.92; found: C, 64.42; H, 4.01.

4.4.5. Methyl 5-methoxy-2-((4-(methylsulfonyl)phenyl)ethynyl)benzoate (6b)

Method B. Reaction time: 1 h. Eluent for chromatography: hexane/EtOAc 6:4. Red solid. Yield 66% (93 mg); mp 102.3–103.7 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.92 (d, J = 8.6 Hz, 2H, arom.), 7.72 (d, J = 8.7 Hz, 2H, arom.), 7.59 (d, J = 8.6 Hz, 1H, arom.), 7.52 (d, J = 2.7 Hz, 1H, arom.), 7.07 (dd, J = 8.6, 2.8 Hz, 1H, arom.), 3.97 (s, 3H, –OCH_3), 3.89 (s, 3H, –OCH_3), 3.07 (s, 3H–SO_2CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 166.1 (C═O), 159.8 (C, arom.), 139.4 (C, arom.), 135.6 (CH, arom.), 133.5 (C, arom.), 132.2 (CH, arom.), 129.7 (C, arom.), 127.4 (CH, arom.), 118.3 (CH, arom.), 115.5 (CH, arom.), 114.8 (C, arom.), 92.4 (C, sp), 90.8 (C, sp), 55.6 (-OCH_3), 52.4(-OCH_3), 44.5 (-SO_2CH_3). **MS** ESI (+): *m/z* (%) = 345 [M+1]$^+$ (100), 367 [M+Na]$^+$ (38); $C_{18}H_{16}O_5S$ [344.38]. Calcd for $C_{18}H_{16}O_5S$: C, 62.78; H, 4.68; found: C, 62.92; H, 4.57.

4.4.6. Methyl 5-nitro-1-((4-(trifluoromethyl)phenyl)ethynyl)-2-naphthoate (10a)

Method A. Reaction time: 4 h. Eluent for chromatography: hexane/EtOAc 9:1. Yellow solid. Yield 99% (182 mg); mp 111.4–112.5 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 8.98 (dt, J = 8.5, 1.1 Hz, 1H, arom.), 8.58 (dd, J = 9.2, 0.9 Hz, 1H, arom.), 8.35 (dd, J = 7.6, 1.2 Hz, 1H, arom.), 8.22 (d, J = 9.2 Hz, 1H, arom.), 7.83–7.68 (m, 5H, arom.), 4.05 (s, 3H, –OCH_3). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 166.0 (C═O), 147.0 (C, arom.), 134.2 (C, arom.), 133.7 (CH, arom.), 132.0 (CH, arom.), 130.91 (q, $^2J(C,F)$ = 32 Hz, 1C, C-CF_3), 129.1 (CH, arom.), 126.5 (C, arom.), 125.90 (CH, arom.), 125.8 (CH, arom.), 125.5 (q, $^3J(C,F)$ = 4 Hz, CH-C-CF_3), 123.8 (q, $^1J(C,F)$ = 272 Hz, 1C, CF_3) 123.5 (CH, arom.), 122.3 (C, arom.), 100.4 (C, sp), 87.2 (C, sp), 52.6 (-OCH_3) one quaternary carbon

obscured. **MS** EI (+): *m/z* (%) = 399 $[M]^+$ (100); $C_{21}H_{12}F_3NO_4$ [399.33]. Calcd for $C_{21}H_{12}F_3NO_4$: C, 63.16; H, 3.03; N, 14.27; found: C, 63.38; H, 3.22; N, 14.36.

4.4.7. Methyl 1-((4-(dimethylamino)phenyl)ethynyl)-5-nitro-2-naphthoate (10b)

Method A. Reaction time: 15 h. Eluent for chromatography: hexane/EtOAc 85:15. Bordeaux solid. Yield 94% (180 mg); mp 165.3–166.7 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 9.03 (dt, *J* = 8.5, 1.0 Hz, 1H), 8.44 (dd, *J* = 9.2, 0.8 Hz, 1H), 8.30 (dd, *J* = 7.6, 1.2 Hz, 1H), 8.16 (d, *J* = 9.2 Hz, 1H), 7.69 (dd, *J* = 8.4, 7.7 Hz, 1H), 7.64–7.51 (dt, *J* = 9.0 Hz, 2H), 6.71 (d, *J* = 9.0 Hz, 2H), 4.05 (s, 3H, –OCH_3), 3.04 (s, 6H, –$N(CH_3)_2$). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 166.6 (C═O), 150.8 (C, arom.), 146.9 (C, arom.), 134.3 (CH, arom.), 134.3 (C, arom.), 133.2 (CH, arom.), 130.5 (C, arom.), 129.3 (CH, arom.), 126.6 (C, arom.), 125.6 (CH, arom.), 125.3 (CH, arom.), 124.2 (C, arom.), 121.6 (CH, arom.), 111.8 (CH, arom.), 109.2 (C, arom.), 104.9 (C, sp), 84.(C, sp), 52.4 (-OCH_3), 40.10 (-$N(CH_3)_2$). **MS** ESI (+): *m/z* (%) = 376 $[M+1]^+$ (100); $C_{22}H_{18}N_2O_4$ [374.39]. Calcd for $C_{22}H_{18}N_2O_4$: C, 70.58; H, 4.85; N, 7.48; found: C, 70.40; H, 4.73; N, 7.56.

4.5. General procedure for the cyclization of the o-alkynylbenzoates 4a-c, 6a-b, 10a-b

Under a nitrogen atmosphere, to a solution of the appropriate methyl 2-alkynylarylcarboxylate (0.4 mmol) in anhydrous DCE (1.6 mL), AgOTf (1–4 mol%, see below) and *p*-TSA·H_2O (30–50 mol%, see below) were added. The reaction was stirred at 60–80 °C (see below) until no more starting product was detected by TLC. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc (15 mL) and washed with saturated aqueous solution of $NaHCO_3$ (2 × 15 mL) and brine (2 × 15 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was removed at reduced pressure. The crude material was purified by direct crystallization or by flash column chromatography over silica gel.

4.5.1. 7-Nitro-3-(4-(trifluoromethyl)phenyl)-1H-isochromen-1-one (5a)

Reaction conditions: AgOTf 2 mol%; *p*-TSA 30 mol%; reaction temperature 80 °C; reaction time: 24 h. Recrystallized from CH_3CN. Pale green solid. Yield 67% (90 mg); mp 223–225 °C. **^{1}H NMR** (300 MHz, DMSO-d_6): δ = 8.78 (d, *J* = 2.3 Hz, 1H, arom.), 8.62 (dd, *J* = 8.7, 2.4 Hz, 1H, arom.), 8.14 (d, *J* = 8.2 Hz, 2H, arom.), 7.93 (t, *J* = 8.3 Hz, 3H, arom.), 7.85 (s, 1H, arom.). **^{13}C NMR** (75 MHz, DMSO-d_6): δ = 160.3 (C═O), 154.2 (C, arom.), 147.2 (C, arom.), 142.4 (C, arom.), 135.2 (C, arom.), 131.0 (q, $^2J(C,F)$ = 32 Hz, 1C, C-CF_3), 129.8 (CH, arom.), 129.2 (CH, arom.), 126.6 (CH, arom.), 126.5 (q, $^3J(C,F)$ = 4 Hz, CH-C-CF_3), 124.7 (CH, arom.), 124.3 (q, $^1J(C,F)$ = 272 Hz, 1C, CF_3), 121.3 (C, arom.), 103.8 (CH, arom.). **MS** ESI (+): *m/z* (%) = 331 [M-CO + Na]$^+$ (100), 359 $[M+Na]^+$ (90), 381 $[M+2Na]^+$ (45); $C_{16}H_8F_3NO_4$ [335.23]. Calcd for $C_{16}H_8F_3NO_4$: C, 57.33; H, 2.41; N, 4.18; found: C, 57.51; H, 2.55; N, 4.13.

4.5.2. 3-(4-(methylsulfonyl)phenyl)-7-nitro-1H-isochromen-1-one (5b)

Reaction conditions: AgOTf 2 mol%; *p*-TSA 30 mol%; reaction temperature 80 °C; reaction time: 24 h. Recrystallized from CH_3CN. Green solid. Yield 87% (120 mg); mp > 300 °C. **^{1}H NMR** (300 MHz, DMSO-d_6): δ = 8.81 (d, *J* = 2.5 Hz, 1H, arom.), 8.64 (dd, *J* = 8.7, 2.5 Hz, 1H, arom.), 8.26–8.15 (m, 2H, arom.), 8.15–8.06 (m, 2H, arom.), 7.96 (d, *J* = 8.7 Hz, 1H, arom.), 7.92 (s, 1H, arom.), 3.29 (s, 3H, –CH_3). **^{13}C NMR** (75 MHz, DMSO-d_6): δ = 160.3 (C═O), 154.1 (C, arom.), 147.3 (C, arom.), 142.7 (C, arom.), 142.4 (C, arom.), 136.0 (C, arom.), 129.8 (CH, arom.), 129.2 (CH, arom.), 128.3 (CH, arom.), 126.7 (CH, arom.), 124.8 (CH, arom.), 121.4 (C, arom.), 104.3 (CH, arom.), 43.8 (-CH_3). **MS** ESI (+): *m/z* (%) = 376 $[M + EtOH]^+$ (100), 345 $[M]^+$ (40); $C_{16}H_{11}NO_6S$ [345.33]. Calcd for $C_{16}H_{11}NO_6S$: C, 55.65; H, 3.21; N, 4.06; found: C, 55.81; H, 3.21; N, 4.10.

4.5.3. 3-(4-(dimethylamino)phenyl)-7-nitro-1H-isochromen-1-one (1e)

Reaction conditions: AgOTf 1 mol%; *p*-TSA 30 mol%; reaction temperature 60 °C; reaction time: 50 h. Recrystallized from EtOAc. Dark red solid. Yield 98% (123 mg); mp > 300 °C. **^{1}H NMR** (300 MHz, DMSO): δ = 8.74 (d, *J* = 2.5 Hz, 1H, arom.), 8.51 (dd, *J* = 8.8, 2.5 Hz, 1H, arom.), 7.79 (d, *J* = 8.9 Hz, 3H, arom.), 7.41 (s, 1H, arom.), 6.82 (d, *J* = 9.1 Hz, 2H, arom.), 3.01 (s, 6H, $N(CH_3)_2$). **^{13}C NMR** (126 MHz, DMSO): δ = 160.9 (C, arom.), 157.7 (C, arom.), 152.5 (C, arom.), 145.7 (C, arom.), 144.1 (C, arom.), 129.6 (CH, arom.), 127.9 (CH, arom.), 127.4 (CH, arom.), 125.0 (CH, arom.), 119.1 (C, arom.), 117.7 (C, arom.), 112.3 (CH, arom.), 98.0 (CH, arom.), 40.3 ($N(CH_3)_2$). **MS** ESI (+): *m/z* (%) = 311.1 $[M+1]^+$ (100); $C_{17}H_{14}N_2O_4$ [310.30]. Calcd for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03; found: C, 65.97; H, 4.65; N, 8.89.

4.5.4. 7-Methoxy-3-(4-(trifluoromethyl)phenyl)-1H-isochromen-1-one (1f) + 6-methoxy-3-(4-(trifluoromethyl) benzylidene)isobenzofuran-1(3H)-one (7a)

Reaction conditions: AgOTf 2 mol%; *p*-TSA 30 mol%; reaction temperature 80 °C; reaction time: 45 h. Eluent for chromatography: toluene. **1f:** White solid. Yield 72% (92 mg); mp 160.1–161.5 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.96 (d, *J* = 8.3 Hz, 2H, arom.), 7.72 (m, 3H, arom.), 7.47 (d, *J* = 8.6 Hz, 1H, arom.), 7.34 (dd, *J* = 8.6, 2.7 Hz, 1H, arom.), 7.01 (s, 1H, arom.), 3.94 (s, 3H, –OCH_3). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 161.9 (C═O), 160.1 (C, arom.), 150.0 (C, arom.), 135.4 (C, arom.), 131.1 (q, $^2J(C,F)$ = 32 Hz, 1C, C-CF_3), 130.5 (C, arom.), 127.9 (CH, arom.), 127.5 (q, $^1J(C,F)$ = 272 Hz, 1C, CF_3), 125.7 (q, $^3J(C,F)$ = 4 Hz, CH-C-CF_3), 125.1 (CH, arom.), 124.7 (CH, arom.), 122.1 (C, arom.), 110.3 (CH, arom.), 103.2 (CH, arom.), 55.8 (-OCH_3). **MS** ESI (+): *m/z* (%) = 343 $[M+Na]^+$ (100); $C_{17}H_{11}F_3O_3$ [320.26]. Calcd for $C_{17}H_{11}F_3O_3$: C, 63.76; H, 3.46; N, 14.99; found: C, 63.85; H, 3.38; N, 15.08.

7a: White solid. Yield 12% (15 mg); mp 86.2–87.5 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.92 (d, *J* = 8.2 Hz, 2H, arom.), 7.68 (d, *J* = 8.6 Hz, 1H, arom.), 7.63 (d, *J* = 8.3 Hz, 2H, arom.), 7.36 (d, *J* = 2.2 Hz, 1H, arom.), 7.31 (dd, *J* = 8.5, 2.4 Hz, 2H, arom.), 6.31 (s, 1H, C═CH), 3.93 (s, 3H, –OCH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 161.9 (C═O), 133.1 (C, arom.), 129.8 (CH, arom.), 128.0 (C, arom.), 127.6 (C, arom.), 125.5 (CH, arom.), 124.1 (CH, arom.), 122.9 (C, arom.), 121.4 (CH, arom.), 107.1 (CH, arom.), 103.8 (CH, arom.), 56.0 (-OCH_3). [In this ^{13}C NMR spectrum some signals are missing, including the signals of the C-CF_3 and CF_3, because the spectrum was recorded on only a few milligrams of **7a**]. **MS** EI (+): *m/z* (%) = 343 $[M+Na]^+$ (100); $C_{17}H_{11}F_3O_3$ [320.26]. Calcd for $C_{17}H_{11}F_3O_3$: C, 63.76; H, 3.46; N, 14.99; found: C, 63.62; H, 3.31; N, 15.20.

4.5.5. 7-Methoxy-3-(4-(methylsulfonyl)phenyl)-1H-isochromen-1-one (1g) + 6-methoxy-3-(4-(methylsulfonyl) benzylidene)isobenzofuran-1(3H)-one (7b)

Reaction conditions: AgOTf 2 mol%; *p*-TSA 30 mol%; reaction temperature 80 °C; reaction time: 20 h. Eluent for chromatography: hexane/CH_2Cl_2/EtOAc 5:4:1. **1g:** White solid. Yield 46% (61 mg); mp 238.2–239.7 °C. **^{1}H NMR** (300 MHz, CD_2Cl_2): δ = 8.07 (d, *J* = 8.8, 2H, arom.), 8.02 (d, *J* = 8.9, 2H, arom.), 7.75 (d, *J* = 2.7 Hz, 1H, arom.), 7.55 (d, *J* = 8.7 Hz, 1H, arom.), 7.38 (dd, *J* = 8.7, 2.7 Hz, 1H, arom.), 7.14 (s, 1H sp^2), 3.96 (s, 3H, –OCH_3), 3.09 (s, 3H, –SO_2CH_3). **^{13}C NMR** (75 MHz, CD_2Cl_2): δ = 161.5 (C═O), 160.4 (C, arom.), 149.4 (C, arom.), 141.0 (C, arom.), 137.2 (C, arom.), 130.2 (C, arom.), 128.1 (CH, arom.), 127.9 (CH, arom.), 125.4 (CH, arom.), 124.3 (CH, arom.), 122.4 (C, arom.), 110.5 (CH, arom.), 104.1 (CH, arom.), 55.8 (-OCH_3), 44.3 (-SO_2CH_3). **MS** ESI (+): *m/z* (%) = 331 $[M+1]^+$ (100); $C_{17}H_{14}O_5S$ [330.36]. Calcd for $C_{17}H_{14}O_5S$: C, 61.81; H, 4.27; found: C, 61.74; H, 4.32.

7b: White solid. Yield 16% (21 mg); mp 212.6–213.8 °C. **^{1}H NMR** (300 MHz, CD_2Cl_2): δ = 8.03 (d, *J* = 8.6 Hz, 2H, arom.), 7.97 (d, *J* = 8.6 Hz, 2H, arom.), 7.77 (d, *J* = 8.5 Hz, 1H, arom.), 7.41–7.36 (m, 2H, arom.), 6.41 (s, 1H, C═CH), 3.97 (s, 3H, –OCH_3), 3.09 (s, 3H,

–SO_2CH_3). **^{13}C NMR** (75 MHz, CD_2Cl_2): $\delta = 166.2$ (C═O), 162.2 (C, arom.), 147.2 (C, arom.), 139.1 (C, arom.), 139.0 (C, arom.), 132.7 (C, arom.), 130.1 (CH, arom.), 127.7 (CH, arom.), 125.4 (C, arom.), 123.9 (CH, arom.), 121.7 (CH, arom.), 107.3 (CH, arom.), 102.9 (CH, arom.), 56.1 (-OCH_3), 44.4 (-SO_2CH_3). **MS** ESI (+): *m/z* (%) = 353 $[M+Na]^+$ (100); $C_{17}H_{14}O_5S$ [330.36]. Calcd for $C_{17}H_{14}O_5S$: C, 61.81; H, 4.27; found: C, 61.69; H, 4.22.

*4.5.6. 7-Nitro-2-(4-(trifluoromethyl)phenyl)-4H-benzo[f]isochromen-4-one (**11a**)*

Reaction conditions: AgOTf 4 mol%; *p*-TSA 50 mol%; reaction temperature 80 °C; reaction time: 72 h. Eluent for chromatography: hexane/EtOAc 9:1. Yellow solid. Yield 97% (149 mg); mp 213–215 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): $\delta = 8.75$ (d, $J = 8.5$ Hz, 1H, arom.), 8.52 (d, $J = 9.3$ Hz, 1H, arom.), 8.43 (d, $J = 9.3$ Hz, 1H, arom.), 8.38 (dd, $J = 7.7$, 1.0 Hz, 1H, arom.), 8.11 (d, $J = 8.2$ Hz, 2H, arom.), 7.90-7.77 (m, 4H, arom.). **^{13}C-NMR** (75 MHz, $CDCl_3$): $\delta = 160.9$ (C═O), 154.5 (C, arom.), 147.7 (C, arom.), 135.9 (C, arom.), 134.8 (C, arom.), 132.4 (q, $^2J(C,F) = 33$ Hz, 1C, C-CF_3) 129.4 (CH, arom.), 129.0 (C, arom.), 127.9 (C, arom.), 127.6 (CH, arom.), 126.4 (CH, arom.), 126.1 (CH, arom.), 126.0 (CH, arom.), 125.9 (q, $^3J(C,F) = 4$ Hz, CH-C-CF_3), 123.5 (q, $^1J(C,F) = 272$ Hz, 1C, CF_3) 123.3 (CH arom.), 118.8 (C arom.), 98.3 (CH arom.). **MS** EI: *m/z* (%) = 385 [M] (100); $C_{20}H_{10}F_3NO_4$ [385,29]. Calcd for $C_{20}H_{10}F_3NO_4$: C, 62.35; H, 2.62; N, 3.64; found: C, 62.49; H, 2.64; N, 3.67.

*4.5.7. 2-(4-(dimethylamino)phenyl)-7-nitro-4H-benzo[f]isochromen-4-one (**11f**)*

Reaction conditions: AgOTf 2 mol%; *p*-TSA 30 mol%; reaction temperature 80 °C; reaction time: 15 h. Crystallized from diisopropyl ether. Bordeaux solid. Yield 100% (144 mg); mp 261–262 °C. **^{1}H NMR** (300 MHz, DMSO): $\delta = 9.32$ (d, $J = 8.5$ Hz, 1H, arom.), 8.48 (d, $J = 7.7$ Hz, 1H, arom.), 8.26 (d, $J = 9.2$ Hz, 1H, arom.), 8.18 (d, $J = 9.2$ Hz, 1H, arom.), 8.09 (s, 1H, arom.), 7.98 (d, $J = 8.7$ Hz, 2H, arom.), 7.91 (t, $J = 8.0$ Hz, 1H, arom.), 6.84 (d, $J = 8.9$ Hz, 2H, arom.) 3.02 (s, 6H, $N(CH_3)_2$). **^{13}C-NMR** (75 MHz, DMSO): $\delta = 161.8$ (C═O), 157.1 (C, arom.), 152.1 (C, arom.), 147.5 (C, arom.), 138.7 (C, arom.), 132.0 (CH, arom.) 129.2 (C, arom.), 127.8 (CH, arom.), 127.5 (C, arom.), 127.4 (CH, arom.), 127.0 (CH, arom.), 126.7 (CH, arom.), 120.9 (CH, arom.), 118.6 (C, arom.), 116.5 (C, arom.), 112.3 (CH, arom.), 94.7 (CH, arom.), 40.3 ($N(CH_3)_2$). **MS** EI (+): *m/z* (%) = 361.04 [M+1] (100); $C_{21}H_{16}N_2O_4$ [360.36]. Calcd for $C_{21}H_{16}N_2O_4$: C, 69.99; H, 4.48; N, 7.77; found: C, 70.21; H, 4.38; N, 7.65.

4.6. General procedure for the synthesis of 7-amino-3-arylisocoumarins 1a,b

To a suspension of 3-aryl-7-nitroisocoumarin **5a,b** (0.3 mmol) in 6 mL of methanol, Pd/C 10% (5% w/w) was added. The mixture was charged with hydrogen and stirred at rt until no more starting material was detected by TLC analysis. The reaction mixture was then filtered on a thin Celite pad and the pad was washed with acetone. The organic filtrate was freed from solvents under reduced pressure yielding the corresponding 7-amino-3-arylisocoumarins **1a,b** sufficiently pure to do not need further purification steps.

*4.6.1. 7-Amino-3-(4-(trifluoromethyl)phenyl)-1H-isochromen-1-one (**1a**)*

Reaction time: 1.5 h. Yellow solid. Quantitative yield 99% (92 mg); mp 219–221 °C. **^{1}H NMR** (300 MHz, DMSO-d_6): $\delta = 8.01$ (d, $J = 8.2$ Hz, 2H, arom.), 7.81 (d, $J = 8.4$ Hz, 2H, arom.), 7.50 (s, 1H, arom.), 7.44 (d, $J = 8.5$ Hz, 1H, arom.), 7.32 (d, $J = 2.3$ Hz, 1H, arom.), 7.11 (dd, $J = 8.4$, 2.4 Hz, 1H, arom.), 3.80 (bs, 2H, –NH_2). **^{13}C NMR** (75 MHz, DMSO-d_6): $\delta = 161.8$ (C═O), 150.3 (C, arom.), 146.7 (C, arom.), 136.5 (C, arom.), 128.9 (q, $^2J(C,F) = 32$ Hz, 1C, C-CF_3), 128.7 (CH, arom.), 126.3 (q, $^3J(C,F) = 4$ Hz, CH-C-CF_3), 125.9 (C, arom.), 125.7 (q, $^1J(C,F) = 272$ Hz, 1C, CF_3), 125.0 (CH, arom.), 122.8 (CH, arom.), 122.1 (C, arom.), 110.8 (CH, arom.), 105.2 (CH, arom.). **MS** ESI (+): *m/z* (%) = 306 $[M+1]^+$ (100), 328 $[M+Na]^+$ (30); $C_{16}H_{10}F_3NO_2$ [305.25]. Calcd for $C_{16}H_{10}F_3NO_2$: C, 62.96; H, 3.30; N, 4.59; found: C, 63.05; H, 3.18; N, 4.68.

*4.6.2. 7-Amino-3-(4-(methylsulfonyl)phenyl)-1H-isochromen-1-one (**1b**)*

Reaction time: 20 h. Yellow solid. Yield 82% (78 mg); mp 247 °C (dec.). **^{1}H NMR** (300 MHz, DMSO-d_6): $\delta = 8.05$ (d, $J = 8.9$ Hz, 2H, arom.), 7.99 (d, $J = 8.8$ Hz, 2H, arom.), 7.54 (s, 1H, arom.), 7.43 (d, $J = 8.5$ Hz, 1H, arom.), 7.30 (d, $J = 2.4$ Hz, 1H, arom.), 7.09 (dd, $J = 8.4$, 2.4 Hz, 1H, arom.), 6.01 (s, 2H, –NH_2), 3.24 (s, 3H, –CH_3). **^{13}C NMR** (75 MHz, DMSO-d_6): $\delta = 161.8$ (C═O), 150.8 (C, arom.), 146.4 (C, arom.), 140.6 (C, arom.), 137.3 (C, arom.), 128.8 (CH, arom.), 128.1 (CH, arom), 125.5 (C, arom.), 124.9 (CH, arom.), 122.6 (CH, arom.), 122.2 (C, arom.), 110.6 (CH, arom.), 105.8 (CH, arom.), 44.0 (-CH_3). **MS** ESI (+): *m/z* (%) = 316 $[M+1]^+$ (100); $C_{16}H_{13}NO_4S$ [315.34]. Calcd for $C_{16}H_{13}NO_4S$: C, 60.94; H, 4.16; N, 4.44; found: C, 61.02; H, 4.15; N, 4.49.

4.7. General procedure for the synthesis of 7-(diethylamino)-3-arylisocoumarins 1c,d

Under a nitrogen atmosphere, the appropriate 7-amino-3-arylisocoumarin **1a,b** (0.3 mmol) was dissolved in 3 mL of dry DMSO. To the reaction mixture, KOH (0.6 mmol, 2 equiv) and iodoethane (1.2 mmol, 4 equiv) were added. The reaction was stirred at 50 °C for 4 h, then further 2 equivalents of iodoethane were added. The reaction was then stirred overnight until no more starting product was detected by TLC. Then 3 mL of aqueous saturated solution of $Na_2S_2O_3$ were added to the reaction mixture and the mixture was stirred for 10 min. The solution was poured in 100 mL of distilled water and extracted with EtOAc (3 × 25 mL). The organic phases were united, dried over Na_2SO_4 and filtered; the solvent was then removed at reduced pressure. The crude material was purified by flash column chromatography over silica gel to yield the corresponding 7-(diethylamino)-3-arylisocoumarin **1c,d**.

*4.7.1. 7-(diethylamino)-3-(4-(trifluoromethyl)phenyl)-1H-isochromen-1-one (**1c**)*

Reaction time: 24 h. Eluent for chromatography: hexane/EtOAc/TEA 9:1:0.2. Yellow solid. Yield 42% (46 mg); mp 189–192 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): $\delta = 7.94$ (d, $J = 8.3$ Hz, 2H, arom.), 7.67 (d, $J = 8.3$ Hz, 2H, arom.), 7.48 (d, $J = 2.6$ Hz, 1H, arom.), 7.39 (d, $J = 8.8$ Hz, 1H, arom.), 7.10 (d, $J = 6.6$ Hz, 1H, arom.), 6.98 (s, 1H, arom.), 3.47 (q, $J = 7.1$ Hz, 4H, -CH_2-CH_3), 1.23 (t, $J = 7.1$ Hz, 6H, –CH_2-CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$): $\delta = 162.7$ (C═O), 149.3 (C, arom.), 148.1 (C, arom.), 147.6 (C, arom.), 135.9 (C, arom.), 130.3 (q, $^2J(C,F) = 32$ Hz, 1C, C-CF_3), 127.7 (CH, arom.), 127.6 (CH, arom.), 125.6 (q, $^1J(C,F) = 272$ Hz, 1C, CF_3), 125.0 (CH, arom.), 124.5 (CH, arom.), 124.1 (q, $^3J(C,F) = 4$ Hz, CH-C-CF_3), 122.4 (C, arom.), 103.7 (CH, arom.), 103.0, 44.6 (-CH_2-CH_3), 12.4 (–CH_2–CH_3). **MS** ESI (+): *m/z* (%) = 362 $[M+1]^+$ (100), 384 $[M+Na]^+$ (15); $C_{20}H_{18}F_3NO_2$ [361.36]. Calcd for $C_{20}H_{18}F_3NO_2$: C, 66.48; H, 5.02; N, 3.88; found: C, 66.29; H, 4.92; N, 3.99.

*4.7.2. 7-(diethylamino)-3-(4-(methylsulfonyl)phenyl)-1H-isochromen-1-one (**1d**)*

Reaction time: 24 h. Eluent for chromatography: hexane/EtOAc/TEA 4:5:0.5. Yellow solid. Yield 39% (43 mg); mp 160 °C (dec.). **^{1}H NMR** (300 MHz, $CDCl_3$): $\delta = 8.02$ (m, 4H), 7.45 (dd, $J = 21.5$, 8.5 Hz, 3H), 7.04 (s, 1H), 3.48 (q, $J = 7.2$ Hz, 4H), 3.09 (s, 3H, SO_2–CH_3), 1.23 (t, $J = 7.1$ Hz, 6H). **^{13}C NMR** (75 MHz, $CDCl_3$): $\delta = 162.5$ (C═O), 140.6 (C, arom.), 139.9 (C, arom.), 137.7 (C, arom.), 137.3 (C, arom.), 129.9 (C, arom.), 128.00 (CH, arom.), 127.95 (CH, arom.), 127.9 (CH, arom.), 125.4 (CH, arom.), 124.9 (CH, arom.), 122.6 (C, arom.), 104.9 (CH, arom.), 44.7 (-CH_2-CH_3), 44.6 (-SO_2CH_3), 12.4 (–CH_2–CH_3). **MS** ESI (+): *m/z* (%) = 372 $[M+1]^+$ (100); $C_{20}H_{21}NO_4S$ [371.45]. Calcd for

$C_{20}H_{21}NO_4S$: C, 64.67; H, 5.70; N, 3.77; found: C, 64.52; H, 5.66; N, 3.81.

4.8. Synthesis of methyl 1-bromo-5-nitro-2-naphthoate (9a)

Nitro naphthoate **9a** and **9b** were obtained by modification of a known procedure [22]. To a N_2-flushed solution of NO_2BF_4 (0.8 g, 6 mmol) in CH_3CN/CH_2Cl_2 (24 + 14 ml), methyl 1-bromo-2-naphthoate **8** (1.0 g, 3.77 mmol), dissolved in the minimum amount of CH_2Cl_2, was added dropwise at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then was quenched with $NaHCO_3$ s.s. (20 ml) and extracted with EtOAc (2 × 20 ml). The combined organic phases were washed with brine (50 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash column chromatography over silica gel (hexane/EtOAc 95:5 to 75:25) to yield progressively **9a** (353 mg, 30%) and the isomeric naphthalene derivative **9b** (472 mg, 40%).

4.8.1. Methyl 1-bromo-5-nitro-2-naphthoate (9a)

Yellow solid. Yield 30% (353 mg); mp 120.8–121.9 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): $\delta = 8.81$ (dt, $J = 8.7$, 1.0 Hz, 1H, arom.), 8.50 (dd, $J = 9.1$, 0.9 Hz, 1H, arom.), 8.29 (dd, $J = 7.6$, 1.1 Hz, 1H, arom.), 7.85 (d, $J = 9.1$ Hz, 1H, arom.), 7.72 (dd, $J = 8.7$, 7.7 Hz, 1H, arom.), 4.02 (s, 3H, OCH_3). **^{13}C-NMR** (75 MHz, $CDCl_3$): $\delta = 166.9$ (C═O), 146.9 (C, arom.), 134.7 (CH, arom.), 133.1 (C, arom.), 133.0 (C, arom.), 128.8 (CH, arom.), 126.9 (C, arom.), 126.3 (CH, arom.), 125.4 (CH, arom.), 122.9 (C, arom.), 122.8 (CH, arom.), 52.9 (-OCH_3). **MS** ESI (+): *m/z* (%) = 332 $[M+Na]^+$ (48), 334 $[M+Na]^+$ (44); $C_{12}H_8BrNO_4$ [310.10]. Calcd for $C_{12}H_8BrNO_4$: C, 46.48; H, 2.60; N, 4.52; found: C, 46.55; H, 2.56; N, 4.44.

4.8.2. Methyl 1-bromo-8-nitro-2-naphthoate (9b)

Yellow solid. Yield 40% (472 mg); mp 138.2–139.0 °C. **^{1}H NMR** (300 MHz, $CDCl_3$): $\delta = 8.06$ (d, $J = 8.2$ Hz, 1H), 7.97 (d, $J = 8.5$ Hz, 1H), 7.83 (dd, $J = 7.4$, 0.8 Hz, 1H), 7.69 (d, $J = 8.4$ Hz, 1H), 7.62 (t, $J = 7.8$ Hz, 1H), 4.02 (s, 3H). **^{13}C-NMR** (75 MHz, $CDCl_3$): $\delta = 167.6$ (C═O), 149.4 (C, arom.), 136.9 (C, arom.), 136.2 (C, arom.), 132.6 (CH, arom.), 128.6 (CH, arom.), 126.9 (CH, arom.), 126.5 (CH, arom.), 124.8 (CH, arom.), 123.3 (C, arom.), 115.5 (C, arom.), 53.1 (-OCH_3). **MS** ESI (−): *m/z* (%) = 352 $[M+Na]^-$ (100); $C_{12}H_8BrNO_4$ [310.10]. Calcd for $C_{12}H_8BrNO_4$: C, 46.48; H, 2.60; N, 4.52; found: C, 46.45; H, 2.63; N, 4.50.

4.9. Synthesis of 7-amino-2-(4-(trifluoromethyl)phenyl)-4H-benzo[f] isochromen-4-one (11b)

To a suspension of **11a** (354 mg, 0.92 mmol) in 14 mL of EtOAc, Pd/C 10% (35.4 mg, 10% w/w) was added. The mixture was charged with hydrogen and stirred at rt for 2 h. The reaction mixture was then filtered on Celite and washed with EtOAc. The resulting solution was concentrated under reduced pressure yielding **11b** sufficiently pure to do not need further purification steps. Orange solid. Yield 99% (325 mg); mp 261.2–262.5 °C. **^{1}H-NMR** (300 MHz, $CDCl_3$): $\delta = 8.24$ (d, $J = 8.9$ Hz, 1H, arom.), 8.11 (d, $J = 8.3$ Hz, 2H, arom.), 7.94 (d, $J = 9.0$ Hz, 1H, arom.), 7.90 (d, $J = 8.5$ Hz, 1H, arom.), 7.77 (m, 3H, arom.), 7.54 (t, $J = 8.0$ Hz, 1H, arom.), 7.05 (d, $J = 7.4$ Hz, 1H, arom.), 4.32 (bs, 2H, NH_2). **^{13}C-NMR** (75 MHz, DMSO): $\delta = 161.9$ (C═O), 152.1 (C, arom.), 146.2 (C, arom.), 136.6 (C, arom.), 136.1 (C, arom.), 130.2 (q, $^2J(C,F) = 32$ Hz, 1C, C-CF_3), 129.6 (C, arom.), 129.0 (CH, arom.), 126.5 (CH, arom.), 126.2 (q, $^3J(C,F) = 3.7$ Hz, CH-C-CF_3), 124.53 (C, arom.), 124.50 (q, $^1J(C,F) = 273$ Hz, 1C, CF_3), 124.45 (CH, arom.), 121.1 (CH, arom.), 117.8 (C, arom.), 112.9 (CH, arom.), 112.2 (CH, arom.), 101.1 (CH, arom.). **MS** EI (+): *m/z* (%) = 355 $[M]^+$ (100); $C_{20}H_{12}F_3NO_2$ [355.08]. Calcd for $C_{20}H_{12}F_3NO_2$: C, 67.61; H, 3.40; N, 3.94; found: C, 67.75; H, 3.32; N, 4.12.

4.10. Synthesis of 7-(dimethylamino)-2-(4-(trifluoromethyl)phenyl)-4H-benzo[f]isochromen-4-one (11c)

Product **11c** was obtained by modification of a known procedure [23]. To a solution of **11b** (100 mg, 0.28 mmol) in anhydrous DMF (1 ml), K_2CO_3 (58 mg, 0.42 mmol) and CH_3I (74 µl, 1.18 mmol) were added and the mixture was stirred for 4 h at 65 °C. Then, the reaction mixture was diluted with water (3 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined organic phases were washed with brine (15 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was crystallized from EtOAc to give pure **11b**. Yellow solid. Yield 96% (103 mg); mp 272.8–273.2 °C. **^{1}H-NMR** (500 MHz, DMSO): $\delta = 8.64$ (d, $J = 8.5$ Hz, 1H, arom.), 8.47 (s, 1H, arom.), 8.40 (d, $J = 8.1$ Hz, 2H, arom.), 8.35 (d, $J = 9.1$ Hz, 1H, arom.), 8.15 (d, $J = 9.0$ Hz, 1H, arom.), 7.95 (d, $J = 8.4$ Hz, 2H, arom.), 7.74 (t, $J = 7.9$ Hz, 1H, arom.), 7.46 (d, $J = 7.6$ Hz, 1H, arom.), 2.87 (s, 6H, 2 CH_3). **^{13}C-NMR** (125 MHz, DMSO): $\delta = 161.9$ (C═O), 152.5 (C, arom.), 151.8 (C, arom.), 137.1 (C, arom.), 136.1 (C, arom.), 130.4 (q, $^2J(C,F) = 30$ Hz, 1C, C-CF_3), 131.1 (C, arom.), 129.9 (C, arom.), 128.4 (CH, arom.), 126.6 (CH, arom.), 126.4 (q, $^3J(C,F) = 4$ Hz, CH-C-CF_3), 125.9 (CH, arom.), 124.5 (q, $^1J(C,F) = 273$ Hz, 1C, CF_3), 123.1 (CH, arom.), 120.0 (CH, arom.), 118.7 (CH, arom.), 117.9 (C, arom.), 101.1 (CH, arom.), 45.4 (-$N(CH_3)_2$). **MS** ESI (+): *m/z* (%) = 384 $[M+H]^+$ (100); $C_{22}H_{16}F_3NO_2$ [383.36]. Calcd for $C_{22}H_{16}F_3NO_2$: C, 68.93; H, 4.21; N, 3.65; found: C, 68.80; H, 4.16; N, 3.76.

4.11. Synthesis of 7-(diethylamino)-2-(4-(trifluoromethyl)phenyl)-4H-benzo[f]isochromen-4-one 11d and 7-(ethylamino)-2-(4-(trifluoromethyl)phenyl)-4H-benzo[f]isochromen-4-one (11e)

Products **11d** and **11e** were obtained by modification of a known procedure [23]. To a solution of **11b** (91 mg, 0.26 mmol) in anhydrous DMF (0.93 ml), K_2CO_3 (53 mg, 0.39 mmol) and CH_3CH_2I (89 µl, 1.10 mmol) were added and the mixture was stirred for 4 h at 65 °C. After that time, an additional amount of CH_3CH_2I (32 µl, 0.39 mmol) was added and the mixture was heated at 100 °C for 4 h. Then, the reaction mixture was diluted with water (3 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined organic phases were washed with brine (15 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash column chromatography over silica gel (hexane/EtOAc 9:1 to 8:2) to yield progressively **11d** (64 mg, 60%) and a minor amount of mono-ethylated derivative **11e** (20 mg, 19%).

4.11.1. 7-(diethylamino)-2-(4-(trifluoromethyl)phenyl)-4H-benzo[f] isochromen-4-one (11d)

Yellow solid. Yield 60% (64 mg); mp 151.4–152.3 °C. **^{1}H-NMR** (300 MHz, DMSO): $\delta = 8.66$ (d, $J = 8.5$ Hz, 1H, arom.), 8.45 (s, 1H, arom.), 8.38 (t, $J = 7.9$ Hz, 3H, arom.), 8.11 (d, $J = 9.0$ Hz, 1H, arom.), 7.92 (d, $J = 8.4$ Hz, 2H, arom.), 7.74 (t, $J = 8.0$ Hz, 1H, arom.), 7.53 (d, $J = 7.5$ Hz, 1H, arom.), 3.17 (q, $J = 7.0$ Hz, 4H, 2 CH_2), 0.98 (t, $J = 7.0$ Hz, 6H, 2 CH_3). **^{13}C-NMR** (75 MHz, DMSO): $\delta = 161.8$ (C═O), 152.4 (C, arom.), 148.6 (C, arom.), 137.0 (C, arom.), 136.1 (C, arom.), 133.5 (C, arom.), 130.3 (q, $^2J(C,F) = 31$ Hz, 1C, C-CF_3), 129.9 (C, arom.), 128.0 (CH, arom.), 126.51 (CH, arom.), 126.3 (q, $^3J(C,F) = 4$ Hz, CH-C-CF_3), 125.7 (CH, arom.), 124.5 (q, $^1J(C,F) = 273$ Hz, 1C, CF_3), 123.2 (CH, arom.), 122.6 (CH, arom.), 120.8 (CH, arom.), 117.9 (C, arom.), 101.0 (CH, arom.), 47.8 (2 –NCH_2), 12.5 (2 CH_3). **MS** ESI (+): *m/z* (%) = 412 $[M+H]^+$ (100); $C_{24}H_{20}F_3NO_2$ [411.12]. Calcd for $C_{24}H_{20}F_3NO_2$: C, 70.06; H, 4.90; N, 3.40; found: C, 70.21; H, 4.79; N, 3.36.

4.11.2. 7-(ethylamino)-2-(4-(trifluoromethyl)phenyl)-4H-benzo[f] isochromen-4-one (11e)

Orange solid. Yield 19% (20 mg); m.p. 238.7–239.9, dec. **^{1}H-NMR** (300 MHz, DMSO): $\delta = 8.39$–8.30 (m, 4H, arom.), 8.09 (d, $J = 8.3$ Hz,

1H, arom.), 7.97 (d, $J = 9.1$ Hz, 1H, arom.), 7.89 (d, $J = 8.5$ Hz, 2H, arom.), 7.55 (t, $J = 8.1$ Hz, 1H, arom.), 6.79 (d, $J = 7.8$ Hz, 1H, arom.), 6.37 (m, 1H, NH), 3.24 (m, 2H, $-NCH_2$), 1.29 (t, $J = 7.1$ Hz, 3H, CH_3). **^{13}C-NMR** (75 MHz, DMSO): $\delta = 161.9$ (C═O), 152.1 (C, arom.), 145.4 (C, arom.), 136.5 (C, arom.), 136.1 (C, arom.), 136.2 (q, $^2J(C,F) = 31$ Hz, 1C, C-CF_3), 129.5 (C, arom.), 129.3 (CH, arom.), 126.5 (CH, arom.), 126.3 (q, $^3J(C,F) = 3.7$ Hz, CH-C-CF_3), 125.2 (C, arom.), 124.5 (q, $^1J(C,F) = 274$ Hz, 1C, CF_3), 123.9 (CH, arom.), 121.4 (CH, arom.), 117.9 (C, arom.), 112.4 (CH, arom.), 107.4 (CH, arom.), 101.2 (CH, arom.), 38.3 (-NCH_2), 14.4 (CH_3). **MS** ESI (+): *m/z* (%) = 384 $[M+H]^+$ (100); $C_{22}H_{16}F_3NO_2$ [383.37]. Calcd for $C_{22}H_{16}F_3NO_2$: C, 68.93; H, 4.21; N, 3.65; found: C, 69.05; H, 4.14; N, 3.76.

4.12. Absorption spectroscopy

Stock solutions of isocoumarins (5 mg/mL) were prepared by dissolving the lyophilized powders in a compatible solvent (**1a,b,e** and **11c,d,f** in DMSO, **1c,d** in chloroform, and **1f,g** in dichloromethane) and stored protected from light. For each absorption spectrum, 8 μL of stock solutions were vacuum dried and resuspended in 2 mL of different organic solvents (20 μg/mL, final concentration). Absorption spectra were recorded at 300 nm/min at 20 °C with a 1 cm path length quartz cuvette on a spectrophotometer equipped with a thermostated cell-holder.

4.13. Fluorescent spectroscopy

To avoid inner filter effects, dye solutions were diluted to have absorbance at the maximum wavelength lower than 0.1 OD. Fluorescence spectra were collected on a Fluoromax-3 fluorimeter (HORIBA Jobin Yvon) recording the emission signal upon excitation at the maximum absorbance wavelength, with slits set at 2 nm and an integration time of 0.3 s. For measuring fluorescence quantum yield (QY), Prodan was used as a reference (QY 0.71). Fluorescence emission spectra for determining QY were collected in EtOH at 380 nm excitation on 1c, 1d and 11d in comparison with Prodan. Fluorescence lifetime measurements in EtOH were carried out on a FLS1000 photoluminescence spectrometer (Edinburg Instruments) (375 nm excitation) by time-correlated single-photon counting. A LUDOX® suspension was used to measure instrument response function (IRF). Data were fitted to a single exponential function.

4.14. Fluorophore characterization

Molecular weight and molar volume of the fluorophores were obtained by dedicated software (see Supplementary File). Dipole moment changes upon excitation were calculated by the Lippert-Mataga equation, modified for spheroid prolate shaped molecules [25,26]:

$$\overline{\nu_a} - \overline{\nu_f} = \frac{3}{hc}\left(\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}\right)\frac{(\mu^* - \mu)^2}{abd} + const$$

where $\overline{\nu_a}$ and $\overline{\nu_f}$ are the wavenumbers in cm^{-1} of absorption and emission peaks, h is the Planck's constant, c is the speed of light, n is the solvent refractive index, ε is the solvent dielectric constant, μ and μ* are the dipole moment of the molecule in the ground and excited state, respectively, and a,b,d are the molecule dimensions in angstrom, with a > b = d.

Notes

The authors declare no competing financial interest.

Acknowledgements

We would like to thank Donatella Nava for the NMR analysis. We acknowledge Francesca Terenziani and Matteo Masino for technical assistance with fluorescence lifetime measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dyepig.2019.107917.

References

[1] (a) Reichardt C. Solvatochromic dyes as solvent polarity indicators. Chem Rev 1994;94:2319–58.(b) Marini A, Muñoz-Losa A, Biancardi A, Mennucci B. What is Solvatochromism? J Phys Chem B 2010;114:17128–35.

[2] (a) Klymchenko AS. "Solvatochromic fluorescent dyes as universal tools for biological research. Actual Chimique 2012;359:20–6.(b) Klymchenko AS, Mely Y. Fluorescent environment-sensitive dyes as reporters of biomolecular interactions. In: Morris MC, editor. Progress in molecular biology and translational science, vol. 113. Academic Press; 2013. p. 35–58. Cap. 2.

[3] (a) Materials for Nonlinear Optics: Chemical Perspectives, Marder S R, Sohn J E, Stucky G D. ACS symposium series, vol. 455. Washington, DC: American Chemical Society; 1991.(b) Mishra A, Bäuerle P. Small molecule organic semiconductors on the move: promises for future solar energy technology". Angew Chem Int Ed 2012;51:2020–67.

[4] Klymchenko AS, Demchenko AP. Multiparametric probing of intermolecular interactions with fluorescent dye exhibiting excited state intramolecular proton transfer. Phys Chem Chem Phys 2003;5:461–8.

[5] Klymchenko AS, Pivovarenko VG, Ozturk T, Demchenko AP. "Modulation of the solvent-dependent dual emission in 3-hydroxychromones by substituents. New J Chem 2003;27:1336–43.

[6] Chou PT, Martinez ML. "Photooxygenation of 3-hydroxyflavone and molecular design of the radiation-hard scintillator based on the excited-state proton transfer. Radiat Phys Chem 1993;41:373–8.

[7] Bures F. "Fundamental aspects of property tuning in push–pull molecules". RSC Adv 2014;4:58826–51.

[8] Dell'Acqua M, Ronda L, Piano R, Pellegrino S, Clerici F, Rossi E, Mozzarelli A, Gelmi ML, Abbiati G. Mediachrom: exploring a new family of pyrimidoindolone-based polarity-sensitive dyes. J Org Chem 2015;21:10939–54.

[9] Some recent representative examples:. (a) Dell'Acqua M, Pirovano V, Peroni S, Tseberlidis G, Nava D, Rossi E, Abbiati G. Silver catalyzed domino approach to 1,3-dicarbosubstituted isochromenes. Eur J Org Chem 2017:1425–33.(b) Dell'Acqua M, Castano B, Cecchini C, Pedrazzini T, Pirovano V, Rossi E, Caselli A, Abbiati G. Mild regiospecific synthesis of 1-alkoxy-isochromenes catalysed by well-defined [silver(I)(*Pc*-L)] complexes. J Org Chem 2014;79:3494–505.(c) Dell'Acqua M, Pirovano V, Confalonieri G, Arcadi A, Rossi E, Abbiati G. "Synthesis of 3-benzylisoquinolines by domino imination/cycloisomerisation of 2-propargylbenzaldehydes. Org Biomol Chem. 2014;12:8019–30.(d) Dell'Acqua M, Facoetti D, Abbiati G, Rossi E. From domino to multicomponent: synthesis of dihydroisobenzofurans. Tetrahedron 2011;67:1552–6.(e) Dell'Acqua M, Abbiati G, Arcadi A, Rossi E. "Silver-Catalysed intramolecular cyclisation of 2-alkynylacetophenones and 3-Acetyl-2-alkynylpyridines in the presence of ammonia. Org Biomol Chem. 2011;9:7836–48.

[10] Gianni J, Pirovano V, Abbiati G. "Silver triflate/*p*-TSA Co-catalysed synthesis of 3-substituted isocoumarins from 2-alkynylbenzoates. Org Biomol Chem. 2018;16:3213–9.

[11] For some recent representative examples of fluorescent isocoumarins see:. (a) Amemura-Maekawa J, Hayakawa Y, Sugie H, Moribayashi A, Kura F, Chang B, Wada A, Watanabe H. Legioliulin, a new isocoumarin compound responsible for blue-white autofluorescence in Legionella (Fluoribacter) dumoffii under long-wavelength UV light. Biochem Biophys Res Commun 2004;323:954–9.(b) Shimizu M, Hirano K, Satoh T, Miura M. "Waste-Free synthesis of condensed heterocyclic compounds by rhodium-catalyzed oxidative coupling of substituted arene or heteroarene carboxylic acids with alkynes". J Org Chem 2009;74:3478–83.(c) Pathak S, Das D, Kundu A, Maity S, Guchhait N, Pramanik A. "Synthesis of 4-hydroxyindole fused isocoumarin derivatives and their fluorescence 'turn-off' sensing of Cu(II) and Fe(III) ions". RSC Adv 2015;5:17308–18.(d) Asai M, Hattori Y, Makabe H. Synthesis of legioliulin, a fluorescent isocoumarin compound, isolated from Legionella dumoffii using cyclic acylpalladation and Heck reaction. Tetrahedron Lett 2016;57:3942–4.(e) Karuk E, Sukriye N, Dincer ZE, Erturk AS, Bostanci A, Karagoz A, Koca M, Sadi G, Yilmaz I. "A novel fluorescent probe based on isocoumarin for Hg^{2+} and Fe^{3+} ions and its application in live-cell imaging. Spectrochim Acta Part B 2020;224:117402.

[12] Atkins RL, Bliss DE. Substituted coumarins and azacoumarins. Synthesis and fluorescent properties. J Org Chem 1978;43:1975–80.

[13] Jones II G, Jackson WR, Choi CY, Bergmark WR. Solvent effects on emission yield and lifetime for coumarin laser dyes. Requirements for a rotatory decay mechanism. J Phys Chem 1985;89:294–300.

[14] Thiede S, Winterscheid P, Hartmann J, Schnakenburg G, Essig S, Menche D. Regiodivergent iodocyclizations for the highly diastereoselective synthesis of syn- and anti-hydroxyl-isochromanones and -isobenzofuranones: concise synthesis of the isochromanone core of the Ajudazols. Synthesis 2016;48(5):697–709.

[15] (a) Sonogashira K, Tohda Y, Hagihara N. A convenient synthesis of acetylenes: catalytic substitutions of acetyleyic hydrogen with bromoalkenes, iodoarenes, and bromopyridines. Tetrahedron Lett 1975;50:4467–9.(b) Chinchilla R, Nájera C.

"The Sonogashira Reaction: a booming methodology in synthetic organic chemistry". Chem Rev 2007;107:874–922.
[16] Bihel F, Quéléver G, Lelouard H, Petit A, Alvès da Costa C, Pourquié O, Checler F, Thellend A, Pierre P, Kraus JL. "Synthesis of new 3-Alkoxy-7-amino-4-chloro-isocoumarin derivatives as new beta-amyloid peptide production inhibitors and their activities on various classes of protease. Bioorg Med Chem. 2003;11:3141–52.
[17] Zhang L, Peng C, Zhao D, Wang Y, Fu H-J, Shen Q, Li J-X. "Cu(II)-Catalyzed C–H (sp^3) oxidation and C–N cleavage: base-switched methylenation and formylation using tetramethylethylenediamine as a carbon source". Chem Commun 2012;48:5928–30.
[18] Ghosh PB, Whitehouse MW. 7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole: a new fluorigenic reagent for amino acids and other amines. Biochem J. 1968;108:155–6.
[19] Grimm JB, English BP, Chen J, Slaughter JP, Zhang Z, Revyakin A, Patel R, Macklin JJ, Normanno D, Singer RH, Lionnet T, Lavis LD. "A general method to improve fluorophores for live-cell and single-molecule microscopy. Nat Methods 2015;12:244–50.
[20] Catalan J, Perez P, Laynez J, Garcia Blanco F. Analysis of the solvent effect on the photophysics properties of 6-Propionyl-2-(dimethylamino)naphthalene (PRODAN). J Fluoresc. 1991;1:215–23.
[21] Kleppinger R, Lillya CP, Yang C. Discotic liquid crystals through molecular self-assembly. J Am Chem Soc. 1997;119:4097–102.
[22] Tanaka M, Muro E, Ando H, Xu Q, Fujiwara M, Souma Y, Yamaguchi Y. NO_2^+ nitration mechanism of aromatic Compounds: Electrophilic vs charge-transfer process". J Org Chem. 2000;65:2972–8.
[23] Kumar K, Tepper RJ, Zeng J, Zimmt MB. Syntheses of rigid and semirigid molecules for investigations of photoinduced electron transfer reactions. J Org Chem. 1995;60:4051–4.
[24] (a) Lippert E. Spektroskopische Bestimmung des Dipolmomentes aromatischer Verbindungen im ersten angeregten Singulettzustand. Z Elektrochem. 1957;61:962–75.(b) Mataga N, Kaifu Y, Koizumi M. Solvent effects upon fluorescence spectra and the dipolemoments of excited molecules. Bull Chem Soc Jpn. 1956;29:465–70.
[25] Vequi-Suplicy CC, Coutinho K, Lamy MT. "Electric dipole moments of the fluorescent probes prodan and laurdan: experimental and theoretical evaluations. Biophys Rev 2014;6:63–74.
[26] Scholte TG. A contribution to the theory of the dielectric constant of polar liquids. Physica 1949;15:437–49.
[27] Pump E, Poater A, Zirngast M, Torvisco A, Fischer R, Cavallo L, Slugovc L. Impact of electronic modification of the chelating benzylidene ligand in *cis*-dichloro-configured second-generation olefin metathesis catalysts on their activity. Organometallics 2014;33:2806–13.
[28] Klein M, Boche G. Synthesis 1999:1246–50.
[29] Barhate NB, Gajare AS, Wakharkar RD, Bedekar AV. Simple and practical halogenation of arenes, alkenes and alkynes with hydrohalic acid/H_2O_2 (or TBHP). Tetrahedron 1999;55:11127–42.
[30] Kratzschmar F, Kaßel M, Delony D, Breder A. "Selenium-Catalyzed C(sp^3)–H acyloxylation: application in the expedient synthesis of isobenzofuranones. Chem Eur J. 2015;21:7030–4.