

## Investigations on the synthesis and chemiluminescence of novel 2-coumaranones - II

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### Abstract

The optimized one-pot synthesis of 16 new 2-coumaranones containing a carbamate side-chain is described. Furthermore, the quantum yields for the base-activated chemiluminescence in the presence of oxygen was determined for all compounds, with a maximum of  $5.9 \pm 0.1 \times 10^{-2}$  E mol<sup>-1</sup>, and the application of these compounds as substitutes for usual bioluminescent and chemiluminescent reagents in bioassays is anticipated. A preliminary mechanistic pathway is also suggested, making use of recent developments regarding aspects of organic chemiluminescence, in agreement with both experimental and theoretical data available on the induced 1,2-dioxetanone decomposition as well as with the high efficiency of this reaction.

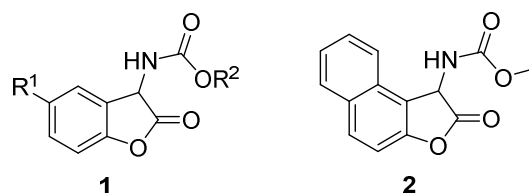
**Keywords:** 2-Coumaranone, chemiluminescence, chemiexcitation, 1,2-dioxetanones,  $\alpha$ -peroxylactones, CIEEL, firefly bioluminescence

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### Introduction

Firefly luciferine and coelenterazine are very important chemiluminescent compounds originally found in living organisms (respectively in *Photinus pyralis* and in several marine organisms across seven *phyla*); they are used extensively in several bioanalytical methods with as yet

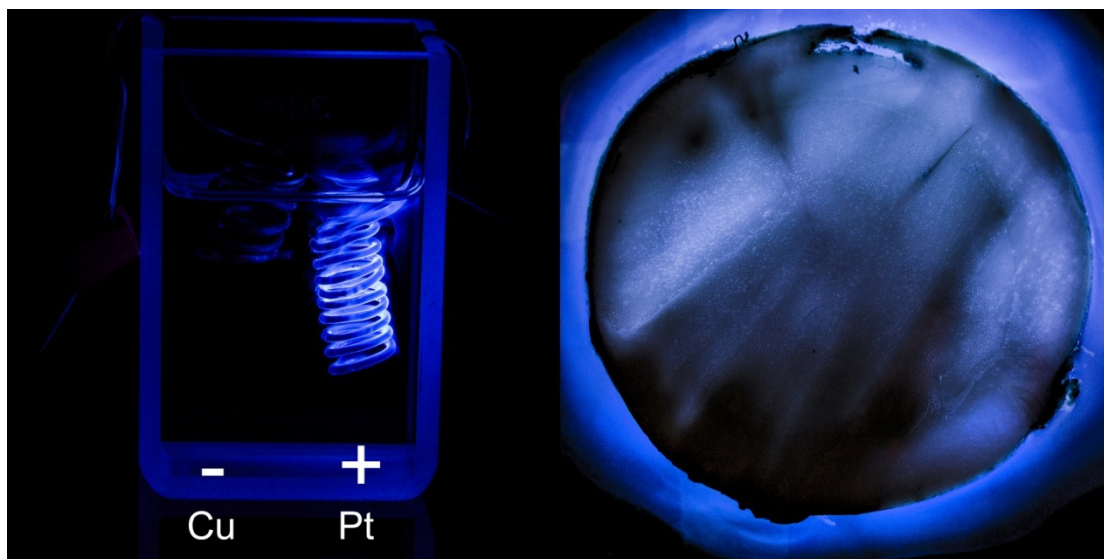
unmatched sensitivity.<sup>1</sup> Despite the significance and large-scale utilization of those compounds, their synthesis is very laborious and expensive, resulting in limited availability of the compounds. We have suggested the substitution of luciferine and coelenterazine in bioanalytical methods by alkyl- and aryl-substituted 2-coumaranones (**1**), which are also very efficient chemiluminescent substrates, in recent publications.<sup>2,3,4,5</sup> This substitution would be a solution to lower the costs of several efficient methods with no loss of sensitivity.



<b>1</b>	<b>R<sup>1</sup></b>	<b>R<sup>2</sup></b>	<b>1</b>	<b>R<sup>1</sup></b>	<b>R<sup>2</sup></b>
a	F	Me	i	F	(CH <sub>2</sub> ) <sub>6</sub> N <sub>3</sub>
b	Cl	Me	j	F	4-FC <sub>6</sub> H <sub>4</sub>
c	Br	Me	k	F	2-BrC <sub>6</sub> H <sub>4</sub>
d	F	Et	l	F	2,4,6-Me <sub>3</sub> C <sub>6</sub> H <sub>2</sub>
e	Cl	Et	m	F	4-MeOC <sub>6</sub> H <sub>4</sub>
f	Br	Et	n	F	4-AcNHC <sub>6</sub> H <sub>4</sub>
g	F	CH <sub>2</sub> C(CH <sub>3</sub> ) <sub>3</sub>	o	F	4-NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub>
h	F	Propargyl			

**Figure 1.** Structure of chemiluminescent alkyl- (**1a–i**), aryl-substituted (**1j–o**) and naphthyl (**2**) 2-coumaranones synthesized in this work.

After the discovery of the chemiluminescence of 2-coumaranones by Lofthouse et al.<sup>6</sup> in 1979, the reaction disappeared from scientific coverage for a long time, despite its simplicity and high chemiluminescence quantum yields. 2-Coumaranones display substantial chemiluminescent emission in polar aprotic solvents upon the addition of strong bases, such as DBU, *t*-BuOK and others. Current research is being carried out to optimize the emission in solvent/water mixture and in water triggered by enzymes, such as peroxidases (e.g. from horseradish, HRP) or ureases, or upon electrochemical reduction in a process known as electrochemiluminescence.



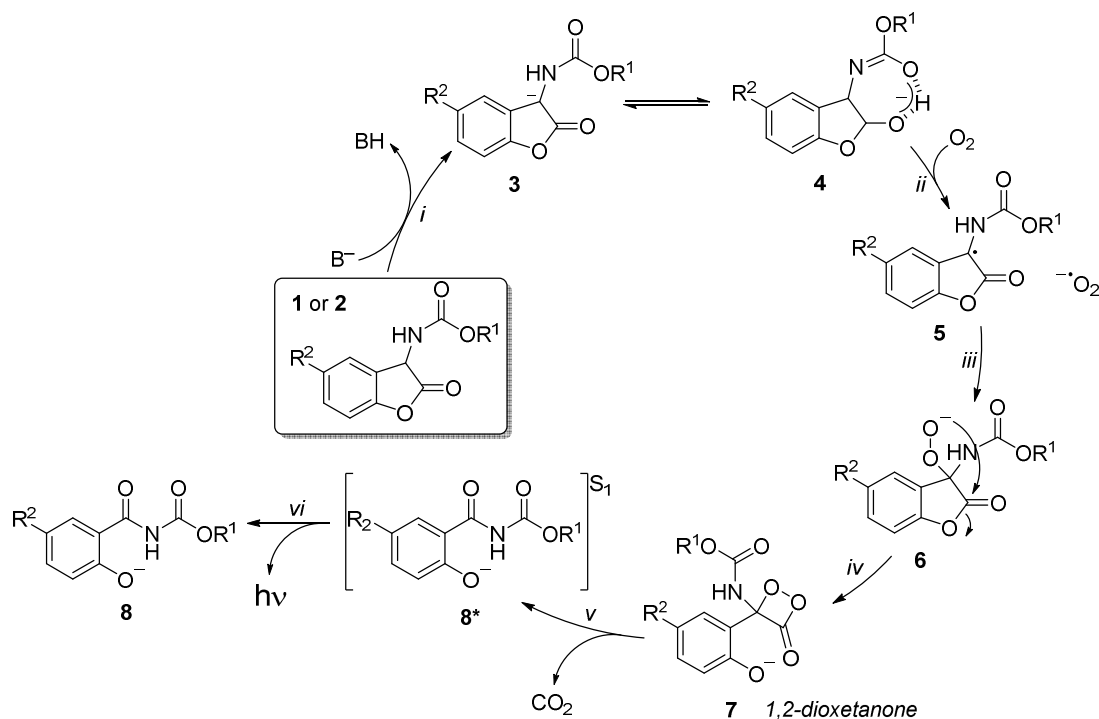
**Picture 1.** Left: electrochemiluminescence of **1d** in DMF; right: acetonitrile solution of **1d** on a freshly-cut slice of horseradish.

The proposed mechanism, able to explain the formation of excited states, is analogous to the one suggested for coelenterazine chemiluminescence,<sup>7,8</sup> and involves several steps, as showed in Scheme 1. The sequential steps can be described as follows: *i*) deprotonation of the  $\alpha$ -carbon of the 2-coumaranone (**1**, **2**) by strong bases, forming a carbanion (**3**) which is stabilized by the formation of a tautomer (**4**); *ii*) the carbanion reacts with dissolved oxygen, supposedly via a single electron transfer (SET) step forming a pair of radical (**5**) and oxygen radical anion (superoxide); *iii*) a fast radical coupling step follows, generating the reactive deprotonated hydroperoxide (**6**); *iv*) this compound suffers a intramolecular nucleophilic addition followed by an elimination, forming the 1,2-dioxetanone (**7**). This is considered to be the essential high-energy intermediate (HEI) of the reaction that, by cleavage and extrusion of CO<sub>2</sub> (step *v*), yields compound (**8**) at the singlet excited state; its decay to the ground state (step *vi*) emits light.

We confirmed in a previous article that the HEI for this reaction is indeed the 1,2-dioxetanone **7**<sup>5</sup> this class of compounds are also proposed intermediates of firefly luciferine and coelenterazine luminescence; some of them have been prepared, isolated and used to produce chemiluminescent emission<sup>9</sup>, validating their ability to generate excited singlet states. For that reason, 2-coumaranones can be used as a reliable model system for generating very unstable 1,2-dioxetanones *in situ*, without the need for difficult, laborious procedures.<sup>10</sup>

Ongoing with the previous work, we want to introduce new derivatives of 2-coumaranones, aiming towards the optimization of the synthesis. The newly established TsE-3CR (Tscherniac-Einhorn three-compound reaction) conditions are fundamental to this optimization, as we obtained better yields with less work and lower costs. Furthermore, the mechanism of the chemiluminescent reaction could be used to rationalize the dependence of the quantum yield

with the new derivatives' chemical structure, for that, we will present the chemiluminescence absolute quantum yields of all new compounds in this article.

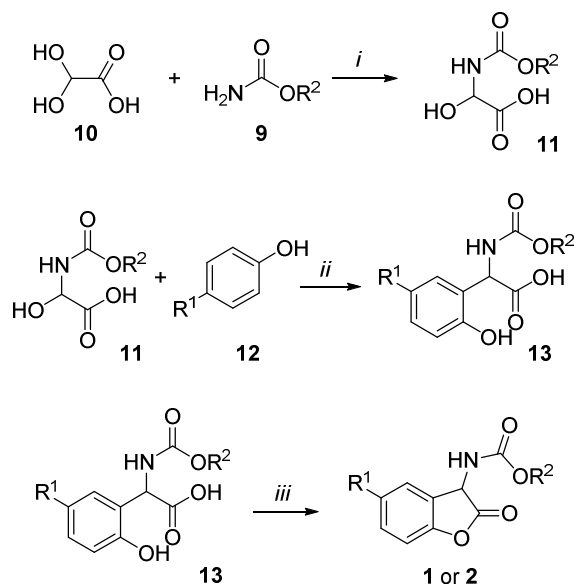


**Scheme 1.** Mechanism for the reaction of 2-coumaranones with a base in the presence of oxygen producing chemiluminescence.

## Results and Discussion

### Synthesis

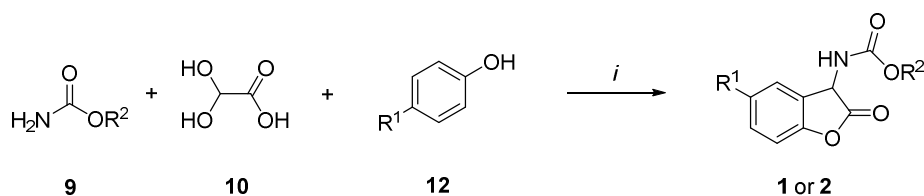
All new compounds were synthesized via a “one-pot” Tscherniac-Einhorn reaction,<sup>11,12</sup> in a considerably optimized way when compared to previous publications.<sup>2,3</sup> Basically, it consists of a three-compound reaction, which is, therefore, referred to as TsE-3CR (Tscherniac-Einhorn three-compound reaction). The major difference to the reaction methodology developed by Matuszczak<sup>13,14,15,16</sup> is that the final cyclization step forming a lactone can be omitted, resulting in a major yield improvement.



**Scheme 2.** The unoptimized synthesis of the 2-coumarones **1** developed by Lofthouse. *i*) Acetone, reflux; *ii*) AcOH/H<sub>2</sub>SO<sub>4</sub> 9:1, r.t. (Tscherniac-Einhorn reaction); *iii*) Ac<sub>2</sub>O, reflux.

The first step of the unoptimized synthetic route towards the target compounds (**1** or **2**) is the condensation of the carbamate (**9**) with glyoxylic acid monohydrate (**10**). The condensation product (**11**) is then used together with a *para*-substituted phenol derivative (**12**) as the starting material for the actual Tscherniac-Einhorn reaction. After the recrystallization of the solid product obtained (**13**), it has to be converted into the target compounds (**1** or **2**) by stirring it under reflux in acetic anhydride.

All three separately conducted reactions are condensations; therefore, it seemed to be worthwhile combining the three reactions into a single three-component reaction. In order to do this, all three starting materials (**9**, **10** and **12**) are used together in a Tscherniac-Einhorn reaction approach with slight modifications (**Scheme 3**). The reaction time seems to be the most crucial parameter in this reaction. With enough time (stretching from a few hours to a few days), the target compounds (**1** or **2**) can be produced directly with a considerable increase in the overall reaction yields.



**Scheme 3.** Tscherniac-Einhorn three-compound reaction for the formation of 2-coumarones **1** or **2**. *i*) AcOH/H<sub>2</sub>SO<sub>4</sub> 9:1, r.t.

A chemical equilibrium lies at the center of condensation reactions, and the shift of this equilibrium is determinant for an efficient synthesis. In this case, there is the formal production of three water molecules, whose removal, and therefore, equilibrium shift towards products, is made by the sulfuric acid present in the reaction mixture. Additionally, the products (**1** or **2**) are much less soluble in the mixture than the reactants, which is another factor that shifts the equilibrium favorably.

As a matter of fact, only one reaction mixture, one reaction vessel and one cleanup step are needed for this three-compound reaction synthesis. This means that the workload is reduced by a third compared to the synthesis in consecutive steps; a factor that enhances the productivity when applying this new TsE-3CR approach to prepare several new 2-coumaranones.

**Table 1.** Reaction time, melting point and isolated yield of the 2-coumaranones **1** and **2**, synthesized by the TsE-3CR method

Compound	Reaction time	Melting point (°C)	Isolated yield (%)
<b>1a</b>	24 hours	198	43
<b>1b</b>	6 days	200	36
<b>1c</b>	7 days	208	27
<b>1d</b>	7 days	156	24
<b>1e</b>	5 days	170	20
<b>1f</b>	5 days	182	20
<b>1g</b>	72 hours	142	46
<b>1h</b>	72 hours	171	49
<b>1i</b>	72 hours	91	10
<b>1j</b>	72 hours	250 (dec.)	51
<b>1k</b>	48 hours	220	68
<b>1l</b>	72 hours	245	87
<b>1m</b>	72 hours	156 (dec.)	31
<b>1n</b>	72 hours	208 (dec.)	36
<b>1o</b>	48 hours	210 (dec.)	55
<b>2</b>	72 hours	230	29

### Chemiluminescence

All new compounds carry a carbamate side chain as rest; both alkyl and aryl substituents on (**1**) were prepared. Preliminary experiments showed that 2-coumaranones with a carbamate side chain have a significantly stronger chemiluminescence than the 2-coumaranones with amide substructures described previously.<sup>2,3</sup> Furthermore, the wavelength of the maximum emission intensity shifts hypsochromically compared to the compounds shown previously.<sup>2</sup>

The chemiluminescent emission of the compounds was triggered by the addition of a small quantity of DBU to an air-equilibrated solution of the 2-coumaranone in acetonitrile (MeCN). The intensity of light emission was followed in a spectrofluorimeter at chemiluminescence

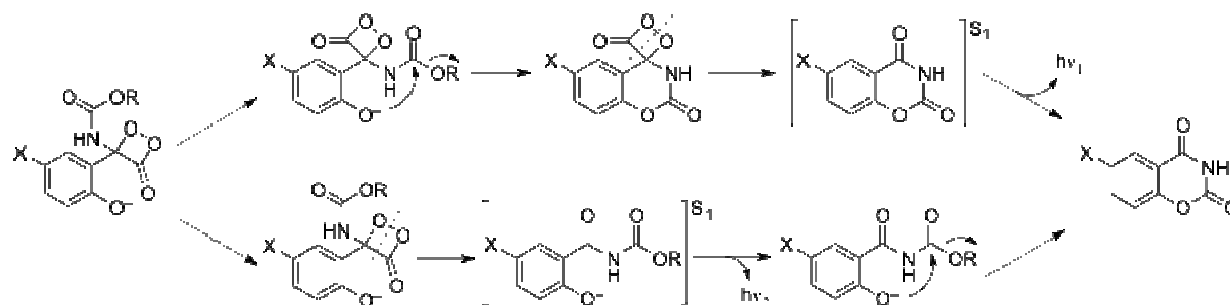
observation conditions, and instantly reaches its maximum, slowly decaying in few minutes in a first-order kinetic profile. The wavelength of the maximum chemiluminescent emission ( $\lambda_{em}$ ) was determined for all compounds, and the wavelength of maximum fluorescence emission ( $\lambda_{fl}$ ) for the spent reaction mixtures. The absolute emission quantum yields were determined with luminol as a secondary standard. All these data are listed in **Table 2**.

**Table 2.** Maximum wavelength for the chemiluminescent emission ( $\lambda_{em}$ ) of the compounds **1** and **2**, maximum wavelength for the fluorescent emission ( $\lambda_{fl}$ ) of the spent reaction mixture, observed rate constant for the decay of light emission ( $k_{obs}$ ) and chemiluminescence quantum yield ( $\Phi_{CL}$ )

Compound	$\lambda_{em}$ (nm)	$\lambda_{fl}$ (nm) <sup>a</sup>	$k_{obs}$ (s <sup>-1</sup> )	$\Phi_{CL}$ (E mol <sup>-1</sup> ) x 10 <sup>2</sup>
<b>1a</b>	436	437	0.013 ± 0.005	4.3 ± 0.1
<b>1b</b>	427	428	0.0065 ± 0.0001	2.95 ± 0.05
<b>1c</b>	427	428	0.0050 ± 0.0002	0.74 ± 0.01
<b>1d</b>	433	434	0.0098 ± 0.0003	3.7 ± 0.2
<b>1e</b>	427	428	0.0071 ± 0.0001	1.60 ± 0.05
<b>1f</b>	428	426	0.006 ± 0.001	0.72 ± 0.01
<b>1g</b>	435	436	0.0120 ± 0.0002	4.92 ± 0.06
<b>1h</b>	437	435	0.0068 ± 0.0001	4.8 ± 0.1
<b>1i</b>	434	433	0.0120 ± 0.0002	3.9 ± 0.1
<b>1j</b>	445	446	0.0121 ± 0.0001	0.90 ± 0.05
<b>1k</b>	432	438	0.062 ± 0.002	0.42 ± 0.01
<b>1l</b>	442	444	0.0058 ± 0.0002	5.9 ± 0.1
<b>1m</b>	444	408	0.012 ± 0.001	3.60 ± 0.05
<b>1n</b>	448	438	0.007 ± 0.001	0.70 ± 0.05
<b>1o</b>	472	478	0.007 ± 0.001	0.01 ± 0.01
<b>2</b>	429	424	0.206 ± 0.001	0.110 ± 0.002

<sup>a</sup>Excitation at 360 nm.

The maximum wavelength for the chemiluminescent emission of all compounds seems to be limited within the range 425–450 nm, right in the blue region of the spectrum, except for the nitro-substituted derivative **1o**. In addition, it can be noted that the emission maxima for all compounds but **1n** and **1m** do not differ significantly from chemiluminescence to fluorescence, which is an indication that the high-energy intermediate directly forms the species that is responsible for the emission without complex bimolecular excited states or other unknown phenomena. The reason for the wavelength shift of **1n** and **1m** will be addressed in further publications.



**Scheme 4.** Two possible pathways for the formation of structurally different emissive species.

The decay from the electronically excited state to the ground state, *i.e.* fluorescence, has a very fast rate constant ( $k_F$ ) of *ca.*  $10^9 \text{ s}^{-1}$  for spin-allowed transitions; this value is much faster than any competing chemical process, therefore, it can be postulated that the emission wavelength and overall emission profile is directly correlated to the emitting species, before it undergoes any chemical transformation, such as the cyclization showed in **Scheme 4**, which depicts two possible pathways for the formation of emissive species. The upper path displays a 1,2-dioxetandione that is stable enough to withstand as the phenolate attacks the nearby carbamate carbonyl group, forming a cyclic compound attached in a *spiro* fashion to the 1,2-dioxetanone, which subsequently cleaves, producing a cyclic compound at the excited state, as the species responsible for the emission at a wavelength corresponding to an energy  $h\nu_1$ . The other possibility is that the 1,2-dioxetanone cleaves faster than the nucleophilic attack of the phenolate, producing a different excited state, with an emission at wavelengths corresponding to an energy  $h\nu_2$ ; the phenolate then produces a cyclization forming the same product as the first reaction. This final cyclic product was isolated and identified via NMR and MS. To differentiate between the two pathways, the emission wavelength has to be correlated to each species produced.

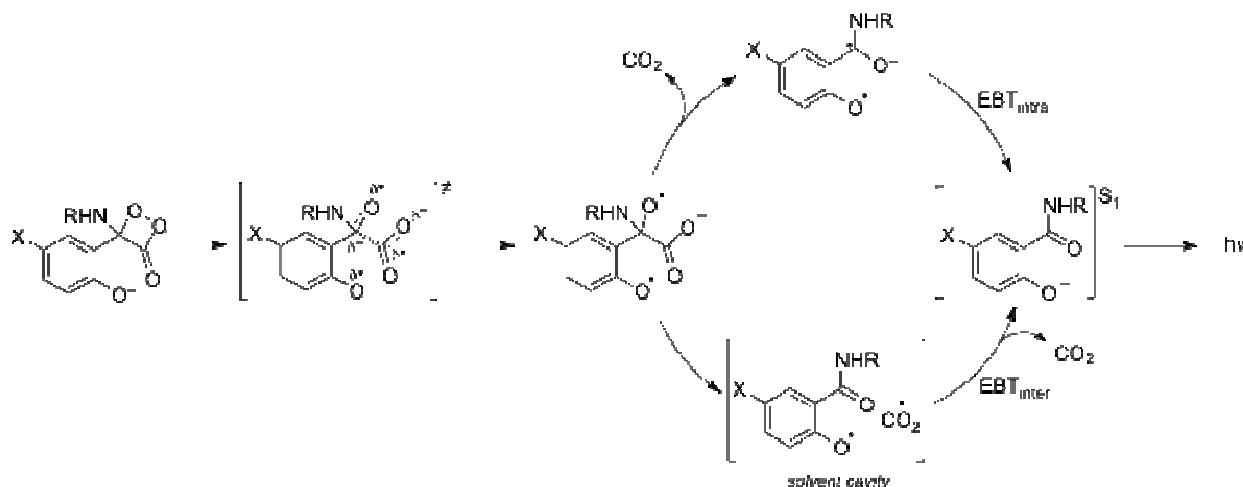
It is important to emphasize, for the following discussion, that any analysis regarding the reaction rate is restricted to effects on the transition state at the chemiexcitation step, since it is the rate-determining step. In a recent article,<sup>5</sup> we demonstrated that the emission rate constant is indeed the decomposition rate constant; the rate constant for the emission was determined with and without activator, and since we reported that this rate constant increases linearly with the activator concentration, we could affirm that the activator is accelerating (*i.e.*, catalyzing) the rate-determining step - the chemiluminescent decomposition of the 1,2-dioxetanone following the CIEEL mechanism. The effects on the emission quantum yield can be related to the whole process, since there is a cumulative formation of several species and the individual formation yield of each species will add to the combined yield of the excited states, which are, in turn, solely responsible for the light emission.

The reaction rate for the chemiluminescent emission follows a clear first-order decay, as expected, since the mechanism involves the formation of an intermediate 1,2-dioxetanone which



cleaves in a unimolecular, rate-determining step, emitting light. Therefore, the emission rates observed are directly correlated to the stability of the intermediate 1,2-dioxetanone.

It is clear, observing the series **1a-f** and **1g-o**, that fluorine has the capacity of producing high chemiexcitation yields, compared to chlorine and bromine; the reaction rates also follow the same tendency, being higher for fluorine. Since the cleavage of the peroxidic intermediate **7** is the rate-determining step, any effect on the reaction rates should be connected to an original effect on the chemiluminescent decomposition of **7** itself. It has been demonstrated that electron donating from a phenolate located at the *ortho* position of a 1,2-dioxetanone can enhance its decomposition enormously, at the same time lowering the emission quantum yield,<sup>17</sup> but we are not sure if this is a suitable explanation for the observed behaviour; additionally, the measured reaction rates are very similar, and therefore do not yield any straightforward mechanistical interpretation. We are currently working on substitution patterns that are able to change the reaction rate in at least one order of magnitude, so we will left a more incisive explanation to a upcoming paper, since we gather all relevant data.



**Scheme 5.** The CIEEL mechanism applied to the induced decomposition of 1,2-dioxetanones.

It has been observed<sup>18</sup> that the CIEEL mechanism, at least applied to 1,2-dioxetanes conjugated to an electron donor, can undergo two main chemiexcitation pathways, which are represented in Scheme 5. The electron donation from the phenolate to the peroxide induces the homolytic cleavage of the O–O bond, forming, via a transition state TS1, a biradical that can follow two paths upon C–C bond breakage: one is the formation of a biradical anion, which undergoes an electron back-transfer intramolecularly ( $\text{EBT}_{\text{intra}}$ ), forming an emissive excited species; the other is the formation of a separate pair of a radical and carbon dioxide radical anion, which can transfer the electron back to the phenol moiety ( $\text{EBT}_{\text{inter}}$ ) before it diffuses away from the solvent cavity. Since the intramolecular back-transfer has higher yields, the difference in the quantum yields between the 2-coumaranones can be explained by the selectivity of the electron back-transfer pathway: the more the mechanism is shifted to an intramolecular reaction, the

higher the quantum yields. We are, however, not convinced that the electron-donating has to occur via a resonance pathway, since electron-donation over the space has been observed with high emission quantum yields,<sup>22</sup> and may be a better suitable explanation for the efficient emission of coumaranones. However, it seems that the CIEEL mechanism is a good model for the chemiluminescent emission of those compounds.

However, since the chemiluminescence quantum yield ( $\Phi_{CL}$ ) is determined from the product of the singlet quantum yield and the fluorescence quantum yield ( $\Phi_{CL} = \Phi_S \times \Phi_F$ ), the singlet quantum yields should be the basis for this discussion. However, assuming that the fluorescence quantum yield remains almost constant, the analysis above should be regarded as valid.

One important result is that 2-coumaranones containing a propargyl (**1h**) and an azide (**1i**) moiety have very good emission quantum yields,  $0.048 \pm 0.001$  and  $0.039 \pm 0.001 \text{ E mol}^{-1}$ , respectively, which are higher than luminol ( $0.0114 \pm 0.0006 \text{ E mol}^{-1}$ ). Those compounds have a potential application in bioanalytical assays, since they can be easily coupled to several modified biomolecules via a 1,3-cycloaddition reaction and be used as a kind of tag, easily revealed by base addition or different enzymes. Current research is taking place to evaluate the applicability of these compounds in aqueous media.

## Conclusions

A series of 16 new 2-coumaranones has been synthesized by an optimized one-pot Tscherniac-Einhorn procedure, and the chemiluminescence emission of those compounds was examined in detail. As an outstanding result we were able to present new chemiluminescence substrates in the shape of carbamate substituted 2-coumaranones. Due to their easy synthesis and in addition, to their high emission quantum yields they are suitable substitutes for applications in bioassays. Also, some mechanistical aspects have been addressed, confirming a pathway able to produce high yields of excited states upon formal oxidation of the 2-coumaranones in the presence of base and oxygen.

## Experimental Section

**General.** All reagents were obtained from commercial sources (Sigma Aldrich, TCI or Acros Organics) and used as received. The solvents were obtained with the synthesis quality and used as received; when marked as "dry", the solvent was dried with the usual method and distilled under nitrogen atmosphere. The progress of the syntheses was followed by thin layer chromatography (TLC, Merck, silicagel 60 F<sub>254</sub>) analyzed under UV light (254 and 366 nm).

**UV-Vis, fluorescence and chemiluminescence spectrophotometry.** UV-vis, fluorescence and chemiluminescence spectra were obtained on a UV-Vis spectrophotometer or spectrofluorimeter with a cell holder thermostated at 20°C by a water-circulating bath.

**Kinetic chemiluminescence assays.** All measurements were made in a quartz cuvette, with final volume 2.0 mL and concentration of  $1 \times 10^{-5}$  mol L<sup>-1</sup> of **1** and **2** in acetonitrile, in a spectrofluorimeter fitted with a cell holder thermostated at 25 °C by a water-circulating bath. The reaction was started by addition of 30 μL of DBU ( $67 \times 10^{-3}$  mol L<sup>-1</sup>), and the chemiluminescence light intensity decay was followed for at least three half-lives. The light emission intensity was registered in arbitrary units (a.u. s<sup>-1</sup>) and was transformed into absolute light units (E s<sup>-1</sup>) by using the luminol standard. Light emission time profiles were fitted by means of a first-order exponential decay equation, resulting in the determination of the observed rate constants ( $k_{\text{obs}}$ ) in s<sup>-1</sup>.

**Chemiluminescence quantum yield ( $\Phi_{\text{CL}}$ ).** The chemiluminescence quantum yields ( $\Phi_{\text{CL}}$ ) for the reaction of **1** or **2** with DBU in acetonitrile were determined accordingly to a known procedure,<sup>19</sup> using luminol as secondary standard<sup>20, 21</sup> ( $\Phi_{\text{lum}} = (1.14 \pm 0.06) 10^{-2}$  E mol<sup>-1</sup>, independent of the initial luminol concentration). The wavelength sensibility factor ( $f_{\text{PMT}}$ ) was 1 since the wavelength of emission is near to the luminol emission wavelength (428 nm).

### General synthesis procedure

In a round-bottom 250 mL flask fitted with magnetic stirring and a drying tube, 0.030 moles of the appropriate carbamate and 0.035 moles of glyoxylic acid monohydrate were dissolved in 100 mL of a 9:1 mixture of acetic and sulfuric acids. After *ca.* 5 minutes, 0.030 moles of the phenol were added, and the stirring was kept at room temperature and the reaction progress was followed by TLC until completion, which may take from one to several days. After this period, the reaction mixture was poured in 400 mL of distilled water, with precipitation of the reaction product, which was promptly filtered and washed until tested negative for acid. The mother liquor was extracted with chloroform or ethyl acetate, the organic layer was separated, dried with sodium sulfate and dried in vacuum. The two fractions containing the product were combined and recrystallized from ethanol.

**Methyl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1a).** From methyl carbamate, glyoxylic acid monohydrate and 4-fluorophenol; after 24 hours of stirring, the product starts to fall off, and the total reaction time was 48 hours. White powder, 43%, mp 198 °C. <sup>1</sup>H NMR (250 MHz, DMSO)  $\delta$  8.40 (d, *J* 7.7 Hz, 1H), 7.35 – 6.99 (m, 3H), 5.35 (d, *J* 7.6 Hz, 1H), 3.53 (s, 3H). <sup>13</sup>C NMR (63 MHz, DMSO)  $\delta$  174.25, 161.23, 157.41, 156.60, 149.85, 128.50, 116.49, 112.14, 52.56, 31.11. MS (EI): *m/z* (%) 225 [M<sup>+</sup>] (100), 197 (80), 165 (95), 151 (45), 137 (50), 59 (45). HR-MS: 225.04373 (theo.), 225.04361 (obs.). IR (ATR) cm<sup>-1</sup> 3371, 2970, 2900, 1789, 1697, 1519, 1265, 1070, 1018, 902, 833, 786.

**Methyl (5-chloro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1b).** From methyl carbamate, glyoxylic acid monohydrate and 4-chlorophenol; 6 days reaction time; white powder, 36%, mp 200 °C. <sup>1</sup>H NMR (300 MHz, DMSO)  $\delta$  8.42 (d, *J* 7.6 Hz, 1H), 7.50 – 7.29 (m, 2H), 7.23 (d, *J* 8.5 Hz, 1H), 5.35 (d, *J* 7.5 Hz, 1H), 3.53 (s, 3H). <sup>13</sup>C NMR: (75 MHz, DMSO)  $\delta$  173.80, 156.62, 152.52, 129.78, 128.87, 128.60, 124.49, 112.62, 52.61, 52.31. MS (EI) *m/z* (%)

241 [M<sup>+</sup>] (100), 213 (75), 181 (80), 154 (40), 59 (40). HR-MS: 241.01418 (theo.), 241.01427 (obs.). IR (ATR) cm<sup>-1</sup> 3379, 2970, 2900, 1797, 1705, 1519, 1465, 1219, 1134, 1080, 1018.

**Methyl (5-bromo-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1c).** From methyl carbamate, glyoxylic acid monohydrate and 4-bromophenol; 7 days reaction time; white powder, 27%, mp 208 °C. <sup>1</sup>H NMR (300 MHz, DMSO) δ 8.41 (d, *J* 7.6 Hz, 1H), 7.62 – 7.50 (m, 2H), 7.18 (d, *J* 8.5 Hz, 1H), 5.35 (d, *J* 7.6 Hz, 1H), 3.53 (s, 3H). <sup>13</sup>C NMR (75 MHz, DMSO) δ 173.66, 156.62, 152.97, 132.65, 129.29, 127.26, 116.30, 113.11, 52.62, 52.20. MS (EI) *m/z* (%) 287 [M<sup>+</sup>+2] (100), 285 [M<sup>+</sup>] (95), 259 (80), 63 (85), 59 (85). HR-MS: 284.96367 (theo.), 284.96343 (obs.). IR (ATR) cm<sup>-1</sup> 3356, 1828, 1690, 1527, 1465, 1080, 1018, 894.

**Ethyl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1d).** From ethyl carbamate, glyoxylic acid monohydrate and 4-fluorophenol. 7 days reaction time; white crystals (long needles), 24%, mp 156 °C. <sup>1</sup>H NMR (400 MHz, DMSO) δ 8.35 (d, *J* 7.6 Hz, 1H), 7.28 – 7.12 (m, 3H), 5.34 (d, *J* 7.6 Hz, 1H), 4.08 – 3.89 (m, 2H), 1.14 (t, *J* 7.1 Hz, 3H). <sup>13</sup>C NMR (101 MHz, DMSO) δ 174.29, 160.52, 158.14, 156.19, 149.83, 128.49, 116.15, 112.20, 61.25, 52.63, 14.85. MS (ESI) *m/z* (%) 262.0 [M+Na<sup>+</sup>] (100). HR-MS: 262.04915 ([M+Na<sup>+</sup>], theo.), 262.04907 (obs.). IR (ATR) cm<sup>-1</sup> 3371, 2985, 2924, 1797, 1697, 1519, 1103, 1072, 1026, 887, 825.

**Ethyl (5-chloro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1e).** From ethyl carbamate, glyoxylic acid monohydrate and 4-chlorophenol; 100 hours reaction time; white powder, 20%, mp 170 °C. <sup>1</sup>H NMR (250 MHz, DMSO) δ 8.36 (d, *J* 7.6 Hz, 1H), 7.50 – 7.30 (m, 2H), 7.23 (d, *J* 8.5 Hz, 1H), 5.34 (d, *J* 7.6 Hz, 1H), 4.07 – 3.87 (m, 2H), 1.14 (t, *J* 7.1 Hz, 3H). <sup>13</sup>C NMR (63 MHz, DMSO) δ 173.82, 156.18, 152.50, 129.73, 128.95, 128.58, 124.44, 112.59, 61.28, 52.26, 14.82. MS (EI) *m/z* (%) 255 [M<sup>+</sup>] (80), 227 (50), 182 (60), 167 (45), 155 (100), 127 (30). HR-MS: 255.02983 (theo.), 255.02991 (obs.). IR (ATR) cm<sup>-1</sup> 3332, 2985, 1828, 1697, 1535, 1465, 1064, 1010, 879.

**Ethyl (5-bromo-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1f).** From ethyl carbamate, glyoxylic acid monohydrate and 4-bromophenol; 100 hours reaction time; white powder, 20%, mp 182 °C. <sup>1</sup>H NMR (250 MHz, DMSO) δ 8.35 (d, *J* 7.6 Hz, 1H), 7.62 – 7.38 (m, 2H), 7.17 (d, *J* 8.5 Hz, 1H), 5.33 (d, *J* 7.6 Hz, 1H), 4.16 – 3.70 (m, 2H), 1.13 (t, *J* 7.1 Hz, 3H). <sup>13</sup>C NMR (63 MHz, DMSO) δ 173.68, 156.18, 152.95, 132.61, 129.37, 127.21, 116.27, 113.08, 61.29, 52.15, 14.82. MS (EI) *m/z* (%) 301 [M+2] (70), 299 [M<sup>+</sup>] (80), 273 (60), 271 (45), 228 (55), 226 (70), 201 (80), 199 (100), 75 (40), 63 (55). HR-MS: 298.97932 (theo.), 298.97956 (obs.). IR (ATR) cm<sup>-1</sup> 3348, 2985, 2900, 1828, 1698, 1527, 1465, 1072, 1026, 887, 779.

**Neopentyl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1g).** From 2,2-dimethylpropanyl carbamate, glyoxylic acid monohydrate and 4-fluorophenol; 72 hours reaction time; white powder, 46%, mp 142 °C. <sup>1</sup>H NMR (250 MHz, DMSO) δ 8.38 (d, *J* = 7.7 Hz, 1H), 7.34 – 7.04 (m, 3H), 5.34 (d, *J* = 7.7 Hz, 1H), 3.75 – 3.57 (m, 2H), 0.86 (s, 7H), 0.57 (s, 2H). <sup>13</sup>C NMR (63 MHz, DMSO) δ 174.26, 161.23, 156.43, 149.86, 128.65, 116.41, 112.03, 111.63, 74.09, 52.64, 31.79, 26.52. MS (ESI) *m/z* (%) 304.0 [M+Na<sup>+</sup>] (100). HR-MS: 304.09610 ([M+Na<sup>+</sup>], theo.), 304.09468 (obs.). IR (ATR) cm<sup>-1</sup> 3441, 2958, 2870, 1816, 1693, 1512, 1477, 1261, 1211, 1103, 1060, 1010, 894, 806, 779.

X-ray crystallography data available at the supporting information.

**Prop-2-yn-1-yl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1h).** From propargyl carbamate,<sup>22</sup> glyoxylic acid monohydrate and 4-fluorophenol. 72 hours reaction time; white powder, 49%, mp 171 °C (recryst. from MeOH). <sup>1</sup>H NMR (250 MHz, DMSO) δ 8.58 (d, *J* 7.6 Hz, 1H), 7.38 – 7.11 (m, 3H), 5.41 (d, *J* 7.6 Hz, 1H), 4.63 (s, 2H), 3.51 (s, 1H). <sup>13</sup>C NMR (63 MHz, DMSO) δ 174.02, 161.25, 157.43, 155.32, 149.86, 128.25, 128.10, 116.62, 112.20, 78.14, 52.92, 52.67. MS (ESI) *m/z* (%) 272.0 [M+Na<sup>+</sup>] (100). HR-MS: 272.03350 ([M+Na<sup>+</sup>], theo.), 272.03447 (obs.). IR (ATR) cm<sup>-1</sup> 3271, 3066, 2125, 1809, 1693, 1539, 1477, 1273, 1107, 1076, 1013, 875, 783, 678.

**6-Azidohexyl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1i).** From 6-azidohexyl carbamate (prepared according to [12] using Et<sub>2</sub>O as solvent), glyoxylic acid monohydrate and 4-fluorophenol. 72 hours reaction time; purification of crude product by column chromatography (silica gel, hexane/AcOEt 2:1 to 1:4) and subsequent crystallization from methanol; white powder, 10%, mp 91 °C. <sup>1</sup>H NMR (250 MHz, DMSO) δ 8.34 (d, *J* 7.7 Hz, 1H), 7.33 – 7.09 (m, 3H), 5.33 (d, *J* 7.5 Hz, 1H), 3.92 (t, *J* 6.2 Hz, 2H), 1.66 – 1.10 (m, 10H). <sup>13</sup>C NMR (63 MHz, DMSO) δ 174.24, 156.26, 154.18, 149.83, 116.42, 116.04, 112.21, 112.07, 65.09, 52.62, 50.96, 28.75, 28.55, 26.17, 25.23. MS (ESI) *m/z* (%) 259.0 [M+Na<sup>+</sup>] (100). HR-MS: 359.11315 ([M+Na<sup>+</sup>], theo.), 359.11373 (obs.). IR (ATR) cm<sup>-1</sup> 3672, 3371, 2939, 2090, 1797, 1697, 1519, 1481, 1219, 1107, 1076, 1037, 1010, 902, 821, 783.

**4-Fluorophenyl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1j).** From 4-fluorophenyl carbamate, glyoxylic acid monohydrate and 4-fluorophenol. 72 hours reaction time; white powder, 71% crude, 51% recryst. from ethanol, mp 250 °C (dec.). <sup>1</sup>H NMR (300 MHz, DMSO) δ 8.86 (dd, *J* 7.6 Hz, 1H), 7.26 (m, 7H), 5.55 (d, *J* 7.6 Hz, 1H). <sup>13</sup>C NMR (126 MHz, DMSO) δ 173.97, 160.69, 154.33, 149.79, 147.28, 127.84, 124.56, 123.25, 117.12, 115.81, 112.99, 111.66, 52.26. MS (EI) *m/z* (%) 305 [M<sup>+</sup>] (20), 212 (10), 169 (10), 166 (15), 151 (20), 139 (10), 122 (10), 112 (100), 95 (40), 83 (30), 75 (10), 64 (5), 57 (10), 45 (5), 43 (5), 28 (5). HR-MS: 305.04996 (theo.), 305.05020 (obs.). IR (ATR) cm<sup>-1</sup> 3456, 3317, 3016 (b), 1745, 1435, 1365, 1211, 1095, 1010, 833, 763, 640.

**2-Bromophenyl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1k).** From 2-bromophenyl carbamate, glyoxylic acid monohydrate and 4-fluorophenol. 48 hours reaction time; white powder, 68%, mp 220 °C. <sup>1</sup>H NMR (300 MHz, DMSO) δ 9.21 (d, *J* 7.6 Hz, 1H), 7.67 (d, *J* 7.5 Hz, 1H), 7.40 (t, *J* 7.6 Hz, 1H), 7.33 – 7.11 (m, 5H), 5.56 (d, *J* 7.5 Hz, 1H). <sup>13</sup>C NMR (126 MHz, DMSO) δ 173.72, 160.40, 157.08, 153.55, 149.86, 133.00, 132.81, 130.02, 128.69, 127.90, 125.50, 124.27, 116.47, 112.97, 52.27. MS (EI) *m/z* (%) 368 [M+2] (30), 366 [M<sup>+</sup>] (35), 340 (5), 174 (100), 172 (95), 166 (25), 151 (30), 137 (5), 123 (5), 122 (5), 95 (5), 75 (5), 63 (5). HR-MS: 364.96989 (theo.), 364.96954 (obs.). IR (ATR) cm<sup>-1</sup> 3456, 3340, 3016 (b), 1743, 1519, 1442, 1355, 1211, 1041, 1002.

**Mesityl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1l).** From mesityl carbamate, glyoxylic acid monohydrate and 4-fluorophenol. 72 hours reaction time; white powder, 87%, mp 245 °C. <sup>1</sup>H NMR (300 MHz, DMSO) δ 8.99 (d, *J* 7.4 Hz, 1H), 7.24 (m, 3H),

6.85 (m, 2H), 5.50 (d, *J* 7.4 Hz, 1H), 2.19 (s, 3H), 2.02 (s, 6H). <sup>13</sup>C NMR (126 MHz, DMSO) δ 174.07, 163.13, 160.84, 152.88, 140.61, 138.19, 134.89, 132.00, 130.30, 125.16, 117.36, 112.70, 69.31, 23.53, 15.57. MS (EI) *m/z* (%) 329 [M<sup>+</sup>] (15), 151 (5), 136 (100), 121 (20), 91 (5), 77 (2). HR-MS: 329.10633 (theo.), 329.10613 (obs.). IR (ATR) cm<sup>-1</sup> 3456, 3325, 3016 (b), 1739, 1519, 1442, 1365, 1211, 1126, 1072, 894, 779.

**4-Methoxyphenyl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1m).** From 4-methoxyphenyl carbamate, glyoxylic acid monohydrate and 4-fluorophenol. 72 hours reaction time; white powder, 31%, mp 156 °C (dec.). <sup>1</sup>H NMR (300 MHz, DMSO) δ 8.56 (d, *J* 6.0 Hz, 1H), 7.08 – 6.89 (m, 7H), 5.53 (s, 1H), 3.76 (s, 3H). <sup>13</sup>C NMR (126 MHz, DMSO) δ 172.41, 156.97, 154.72, 150.83, 146.19, 144.62, 143.44, 133.59, 123.69, 122.42, 115.38, 114.07, 56.48, 55.25. MS (EI) *m/z* (%) 317 [M<sup>+</sup>] (15), 124 (100), 109 (25), 95 (5), 81 (5). HR-MS: 317.06995 (theo.), 317.07015 (obs.). IR (ATR) cm<sup>-1</sup> 3271, 3008, 2947, 2839, 1746, 1489, 1365, 1200, 1026, 825, 779.

**4-Acetamido (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1n).** From 4-acetamidophenyl carbamate, glyoxylic acid monohydrate and 4-fluorophenol. 72 hours reaction time; pale yellow powder, 36%, mp 208 °C (dec.). <sup>1</sup>H NMR (300 MHz, DMSO) δ 9.97 (s, 1H), 8.94 (d, *J* 7.8 Hz, 1H), 7.54 (d, *J* 8.9 Hz, 2H), 7.40 – 7.17 (m, 3H), 7.02 (d, *J* 8.9 Hz, 2H), 5.52 (d, *J* 7.7 Hz, 1H), 2.02 (s, 3H). <sup>13</sup>C NMR (126 MHz, DMSO) δ 172.24, 168.61, 154.89, 151.85, 146.58, 136.89, 122.95, 121.64, 120.83, 119.59, 117.35, 116.01, 51.87, 23.98. MS (EI) *m/z* (%) 345 [M<sup>+</sup>] (20), 344 [M<sup>+</sup>] (10), 302 (10), 194 (5), 151 (75), 109 (100), 80 (10), 60 (5), 44 (20). HR-MS: 344.08085 (theo.), 344.08097 (obs.). IR (ATR) cm<sup>-1</sup> 3456, 3363, 3317, 3016 (b), 1745, 1666, 1496, 1365, 1211, 1149, 1103, 1018, 817, 763, 640.

**4-Nitrophenyl (5-fluoro-2-oxo-2,3-dihydrobenzofuran-3-yl)carbamate (1o).** From 4-nitrophenyl carbamate, glyoxylic acid monohydrate and 4-fluorophenol; 48 hours reaction time; pale yellow powder, 55%, mp 210 °C (dec.). <sup>1</sup>H NMR (500 MHz, DMSO) δ 9.26 (d, *J* 7.9 Hz, 1H), 8.29 (m, 3H), 7.45 (d, 4H), 5.63 (d, *J* 7.8 Hz, 1H). <sup>13</sup>C NMR (126 MHz, DMSO) δ 173.77, 160.35, 158.46, 155.88, 153.43, 149.86, 145.03, 127.63, 125.71, 122.98, 116.82, 112.28, 52.78. MS (EI) *m/z* (%) 332 [M<sup>+</sup>] (80), 193 (100), 165 (100), 151 (90), 139 (95), 137 (60), 123 (50), 109 (55), 95 (40), 81 (15), 75 (20), 65 (35), 50 (10), 39 (15), 28 (20). HR-MS: 332.04446 (theo.), 332.04418 (obs.). IR (ATR) cm<sup>-1</sup> 3456, 3317, 3016, 1730, 1442, 1365, 1211, 1010, 902, 871, 817, 779, 609.

**Ethyl (2-oxo-1,2-dihydronaphtho[2,1-*b*]furan-1-yl)carbamate (2).** From ethyl carbamate, glyoxylic acid monohydrate and β-Naphthol. 72 hours reaction time; white powder, 29% (rekrst. EtOH), mp 230 °C. <sup>1</sup>H NMR (400 MHz, DMSO) δ 8.63 (d, *J* 7.2 Hz, 1H), 8.01 (dd, *J* 8.5, 2.9 Hz, 2H), 7.87 (d, *J* 8.3 Hz, 1H), 7.62 (t, *J* 11.1, 4.0 Hz, 1H), 7.48 (d+s, *J* 7.8, 6.3 Hz, 2H), 5.74 (d, *J* 7.6 Hz, 1H), 3.53 (s, 3H). <sup>13</sup>C NMR (101 MHz, DMSO) δ 174.68, 156.48, 151.73, 131.03, 130.91, 129.68, 129.31, 128.21, 125.26, 122.72, 118.57, 112.01, 52.54. MS EI: 258 [M+1]<sup>+</sup> (20), 257 [M<sup>+</sup>] (100), 229 (30), 214 (25), 198 (80), 183 (20), 169 (50), 152 (5), 140 (5), 127 (10), 115 (30), 113 (5), 89 (5), 63 (3), 59 (5). HR-MS: 280.05857 ([M+Na], theo.), 280.05866 (obs.).

IR (ATR)  $\text{cm}^{-1}$  3356, 1805, 1689, 1635, 1577, 1519, 1450, 1327, 1273, 1242, 1211, 1111, 1056, 1014, 991, 810, 748, 628.

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