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Strong green chemiluminescence from naphthalene analogues of luminol†

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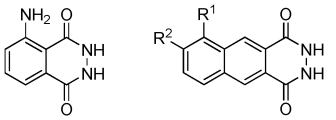
Naphthalene analogues of luminol with several types of substituents were prepared. All molecules exhibit a strong green chemiluminescence in aqueous solution, which is catalysed by iron, pointing to the possibility of increasing the sensitivity of analytical methods currently based on luminol.

The chemiluminescence of luminol, first reported by Albrecht in 1928,[1] is extensively used in analytical and biochemical applications, namely to monitor the production of reactive oxygen species in live cells,[2] and in forensics for the detection of bloodstains invisible to the naked eye.[3–5] In aqueous solution, and when mixed with an oxidizing agent, luminol exhibits a striking blue chemiluminescence, which is catalyzed by iron.[3,4] In this way, a standard forensic test for the visual detection of bloodstains consists of spraying a luminol aqueous solution in a darkened environment. Selective chemiluminescence from the sprayed bloodstains is then observed due to the enhancing (catalytic) effect of the iron-containing heme group.[5] However, the human eye has maximal sensitivity in the green spectral region, a wavelength region where less interference from background emission is also expected. For this reason, luminol analogues with green chemiluminescence in aqueous solution are of great interest.

Here we report the preparation of new luminol analogues with green chemiluminescence in aqueous solution. The emission color shifts from luminol's standard blue and was obtained by both extending the aromaticity, as a result of replacing the benzene (luminol) with a naphthalene core, and by changing the position of the amine group (position 5 or 6 in the naphthalene structure, Chart 1).



Luminol

1 - R^1=NH_2; R^2=H
2 - R^1=NH(CH_2Ph); R^2=H
3 - R^1=N(CH_2Ph)$_2$; R^2=H
4 - R^1=H; R^2=NH(CH_2Ph)
5 - R^1=H; R^2=N(CH_2Ph)$_2$

Chart 1 Chemical structures of luminol and analogues **1–5**, with extended aromaticity and different substituents in position 5 or 6 of the naphthalene ring.

The incorporation of benzyl groups as substituents in the amine also tunes the chemiluminescence emission of the new luminol analogues (Chart 1).

The synthetic strategy for the preparation of analogues **1–3** (amine substituents in position 5 of the naphthalene ring), is presented in Scheme 1. For the synthesis of **1**, we started with the cyclization of 2,3-naphthalenedicarboxylic acid with hydrazine in acetic acid, affording pyridazinedione **6**.[6] The subsequent step was the nitration of **6**, an electrophilic substitution which occurs at electron rich positions. In the case of naphthalene and according to the frontier orbital theory, the electron density is highest at position 1. In our case, because we have substitutions in the naphthalene ring, positions 1 and 5 can be considered for an electrophilic attack. However, because of the pyridazinedione in positions 2 and 3, position 5 is more electron rich than position 1 and with less steric hindrance. In our first attempts for the nitration of compound **6**, we used nitric acid but we could not control the selectivity. The multi-nitration products obtained using nitric acid led us to use nitronium tetrafluoroborate, a mild nitration agent.[7] The nitration occurs with high selectivity, but with low yields, as already reported for this type of reaction.[8,9] The selective nitration at position 5 was followed by an efficient soft reduction with metallic iron and ammonium chloride[10] yielding analogue **1**. The direct preparation of analogues **2** and **3** from analogue **1** (mono- and di-benzyl amine protection) was not possible (interference with the protons in the pyridazine group).

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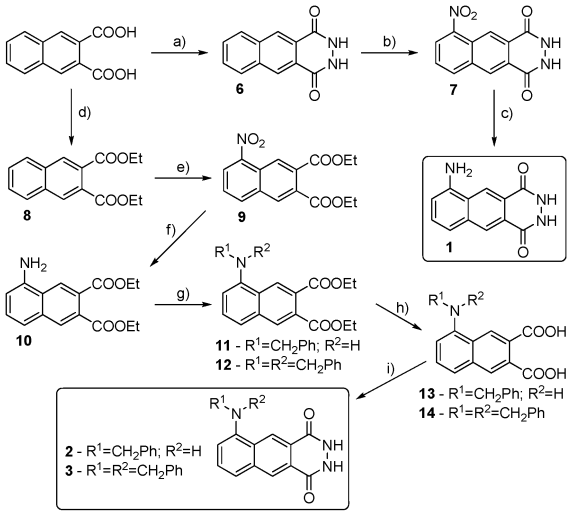
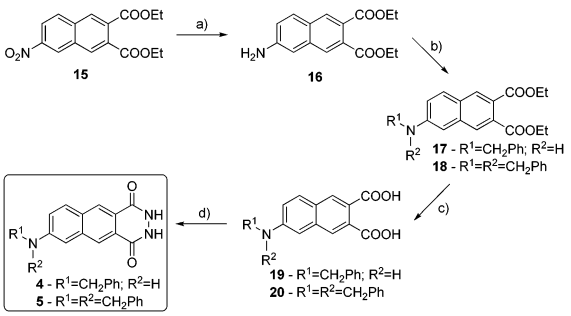


Reagents and conditions: a) NH_2NH_2 (35% in H_2O), AcOH, reflux, 4h, 63%. b) BF_4NO_2, dry CH_3CN, 4Å MS, 0ºC, 30 min., 20%. c) Fe, NH_4Cl, EtOH/H_2O, reflux, 1h, 95%. d) $SOCl_2$, EtOH, reflux, 12h, 98%. e) BF_4NO_2, dry CH_3CN, 4Å MS, 0ºC, 30 min., 39%. f) Fe, NH_4Cl, EtOH/H_2O, reflux, 1h, 92%. g) $PhCH_2Br$, dry DMF, K_2CO_3 or NaH, rt, 2h, 62% for **11** and 78% for **12**. h) MeOH, 40% KOH (aq), reflux, 5h, 81% for **13** and 70% for **14**. i) NH_2NH_2 (35% in H_2O), AcOH, reflux, 4h, 80% for **2** and 82% for **3**.

Scheme 1 Synthesis of analogues **1–3**.

A different pathway was delineated for analogues **2** and **3**, which started with the quantitative esterification of 2,3-naphthalenedicarboxylic acid (compound **8**),[11] followed by nitration at position 5 (compound **9**, confirmed by ^1H–^1H and ^1H–^{13}C COSY NMR correlation spectra-ESI†) and consequent quantitative reduction with iron (compound **10**), as used in analogue **1** synthesis. The mono- and di-benzylation of the amine group was selectively achieved using potassium carbonate (compound **11**) and sodium hydride (compound **12**), respectively.[12] The ester hydrolysis of the 5-((mono or di)benzylamino) diethyl naphthalene-2,3-dicarboxylate (**11** and **12**) was performed under basic conditions,[13] and the cyclization of 5-((mono or di)benzylamino) naphthalene-2,3-dicarboxylic acid (**13** and **14**) with hydrazine in acetic acid afforded analogues **2** and **3** in high yields. We also attempted the hydrolysis (using H_2 with Pd/C) of the benzylamino groups of analogue **2** and **3** to obtain analogue **1**, but without success.

The preparation of analogues **4** and **5** (amine substituents in position 6 of the naphthalene ring) is presented in Scheme 2. The synthetic strategy followed for these compounds was based on ref. 12, aiming at using 2,3-naphthalimide derivatives as probes for monitoring protein–protein interactions. Compound **15**, diethyl 6-nitro-2,3-naphthalenedicarboxylate, was an intermediate of the final products in the study, and we use it as the starting point for the synthesis of analogues **4** and **5**. Compound **15** is in fact a structural isomer of compound **9** (Scheme 1), and the synthetic strategy employed was the same: reduction of the nitro group to an amine (compound **16**), followed by mono- and di-benzylation of the amine (compounds **17** and **18**), basic hydrolysis of the esters groups (compounds **19** and **20**) and finally the cyclization with hydrazine in acetic acid, obtaining analogues **4** and **5** in good yields. We also tried to obtain an unsubstituted amine analogue through the hydrolysis (H_2 with Pd/C) of the benzylamino



Reagents and conditions: a) Fe, NH_4Cl, EtOH/H_2O, reflux, 1h, 96%. b) $PhCH_2Br$, dry DMF, K_2CO_3 or NaH, rt, 2h, 78% for **17** and 80% for **18**. c) MeOH, 40% KOH (aq), reflux, 5h, 75% for **19** and 72% for **20**. d) NH_2NH_2 (35% in H_2O), AcOH, reflux, 4h, 84% for **4** and 85% for **5**.

Scheme 2 Synthesis of analogues **4** and **5**.

groups of analogue **4** and **5**, but without success. Other attempts, namely the hydrolysis of the benzylamino groups (H_2 with Pd/C) of derivatives **19** and **20** or the ester hydrolysis of compound **16** (and consequent hydrazine cyclization) under basic and acidic conditions, proved to be unfruitful, as it was not possible to isolate the products because of their zwitterionic nature.

The chemiluminescence of the different analogues was studied in aqueous solutions (5% DMSO, due to the insolubility of analogues **3** and **5** in pure water), using potassium persulfate as an oxidant.[14] This procedure also slows down the reaction rates, allowing the facile recording of the spectra, when compared with the Fe–NaOH system, which has faster kinetics. The chemiluminescence spectra of luminol and those of analogues **1–5** in H_2O/DMSO (5%) after addition of $K_2S_2O_8$ are presented in Fig. 1. Although the intensities are comparable, the analogues exhibit a very large bathochromic shift of the chemiluminescence maxima with respect to that of luminol: the standard luminol chemiluminescence has a maximum at *ca.* 420 nm, whereas the maxima of analogues **1–5** vary between 490 nm and 590 nm, depending on the position of the amine and substituents. Analogues **4** and **5**, with the amine group in position 6 of the naphthalene ring, both have a maximum at 490 nm. When the amine group (either, mono or di-benzyl substituents) is in position 5 of the naphthalene ring (analogues **2** and **3**), the chemiluminescence maximum further shifts to 540–550 nm. This can be explained by an increase of the electron density in position 5, which is already high, with a consequent change in the intramolecular charge distribution of the analogue. In Fig. 1A, as inset, we show color pictures of chemiluminescence of luminol and analogue **2**, with the change from dark blue to green being apparent. The highest shift is obtained with analogue **1** (primary amine in position 5 of the naphthalene ring) whose chemiluminescence maximum occurs at 590 nm. It is clear that the presence of the benzyl substituents in the amine, affects the chemiluminescence, most probably because of the reduction of the electron donor behavior of the amine. The synthesis and chemiluminescence of analogue **1** are described in the literature,[15,16] however a chemiluminescence maximum similar to that of luminol (*ca.* 420 nm) was reported (for the corresponding secondary amine, used for amino acid

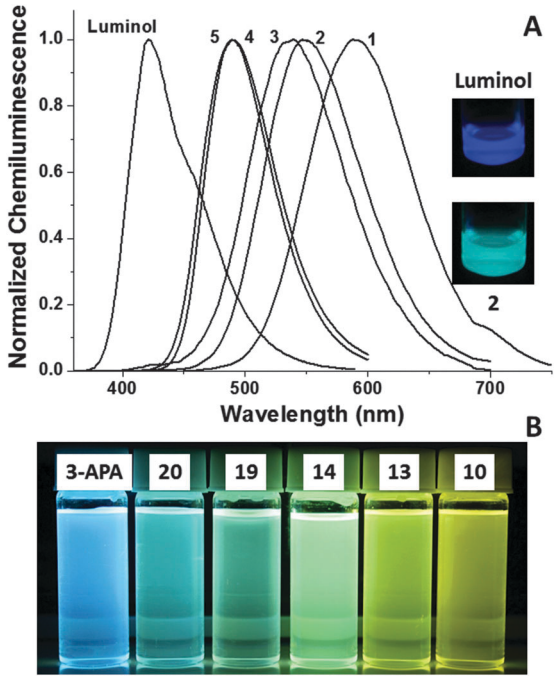


Fig. 1 (A) Normalized chemiluminescence emission spectra of luminol and analogues **1–5** in H_2O/DMSO (5%) with $K_2S_2O_8$. The inset shows color pictures of chemiluminescence from luminol and analogue **2**. (B) Color pictures of the fluorescence of oxidation products in H_2O/DMSO (5%) under excitation at 365 nm.

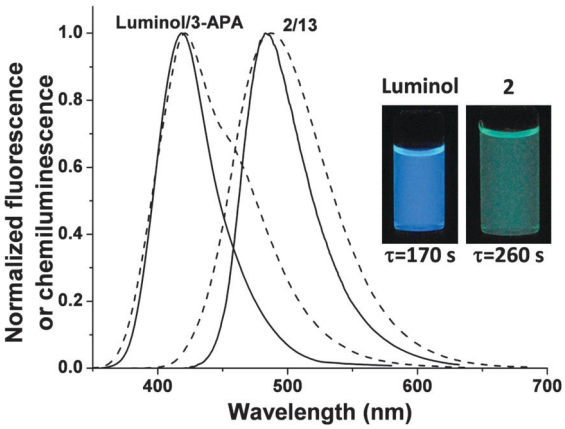


Fig. 2 Chemiluminescence spectra (solid lines, using Fe/NaOH) of luminol and analogue **2**, and fluorescence spectra (dashed lines) of 3-APA and oxidation product **13** in H_2O/NaOH/H_2O_2. The inset shows color pictures of chemiluminescence from luminol and analogue **2**.

labeling, a chemiluminescence maximum wavelength of 480–510 nm is indicated).[15,16]

As mentioned earlier, and as is well known, the chemiluminescence is the result of an oxidation reaction, and the oxidation products are the emitting species.[2,5,17] The oxidation products of our analogues were also synthesized, namely: analogue **5** *vs.* compound **20**; analogue **4** *vs.* compound **19**; analogue **3** *vs.* compound **14**; analogue **2** *vs.* compound **13**; analogue **1** *vs.* compound **10** (nonhydrolyzed ester), and luminol *vs.* 3-aminophthalic acid (3-APA). In Fig. 1, we present color pictures of the fluorescence of oxidation products in H_2O/DMSO (5%) under excitation at 365 nm. It is possible to see that the chemiluminescence of the new analogues cover a wide range of the visible spectrum. The chemiluminescence colors of compounds **13** and **14** (Fig. 1B) are similar to that of analogue **2** (Fig. 1A, inset).

One of the main applications of the chemiluminescence of luminol is analytical, especially in blood analysis and detection, where iron-containing heme is the catalyst, as mentioned above. In Fig. 2 we present the chemiluminescence spectra of luminol and analogue **2**, using iron as a catalyst. Although the chemiluminescence maximum of analogue **2** experiences a significant hypsochromic shift (from 550 nm to 490 nm, compare Fig. 1), a green chemiluminescence is still observed (Fig. 2, inset). The chemiluminescence decay time measured for analogue **2** (260 s), is even higher that that obtained for luminol (170 s) under the same conditions. The similarity of the fluorescence spectra of the oxidation products, 3-APA and compound **13**, with the respective chemiluminescence, can also be observed.

In summary, we successfully prepared new luminol analogues with extended aromaticity and different substituents in the aromatic ring, leading to green chemiluminescence. The new luminol analogues have a naphthalene core (whereas luminol has a benzene core), with the amine group in position 5 or 6 of the polycyclic ring. The increase of aromaticity leads to a bathochromic shift of the chemiluminescence maximum by 70 nm when compared with luminol, if the amine is in position 6 of the naphthalene ring. However, for the analogues with the amine in position 5, the chemiluminescence maximum red shifts by 120–130 nm, when compared with luminol. This is due to the changes in the electron density on the aromatic rings. The presence of substituents in the amine group also influences the chemiluminescence performance. The highest increase in the chemiluminescence maximum was obtained for an analogue without substituents in the amine group (in position 5), with a shift of 170 nm when compared with luminol. Chemiluminescence bathochromic shifts are also observed upon using iron as a catalyst, and this new evidence can open new doors to develop more sensitive chemiluminescence detection methods in blood analysis or for new sensing systems, taking into account possible toxicity issues of the reactants, when relevant.

Experimental

Chemiluminescence procedure: the chemiluminescence experiments were performed in a quartz cuvette (1 × 1 cm). Typically, to a solution (2.5 mL) of luminol or analogues (5×10^{-5} M) in H_2O (with 5% DMSO), a solution of $K_2S_2O_8$ (6×10^{-2} M) in H_2O was added (100 μL). In the case of iron catalyzed chemiluminescence, to a solution (2.5 mL) of luminol or analogues (5×10^{-5} M) in H_2O, a solution of H_2O_2 (1×10^{-2} M) and $K_3Fe(CN)_6$ (2×10^{-3} M) in alkaline H_2O (NaOH, 2.5×10^{-2} M) was added (200 μL). The chemiluminescence spectra were recorded immediately after mixing. The fluorescence spectra of 3-APA and analogue **2** (5×10^{-5} M) in alkaline H_2O (NaOH, 2.5×10^{-2} M) were recorded at room temperature.

Acknowledgements

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