Preparation of a novel group of chemiluminescent *N*-substituted acridinium esters

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Abstract

Several novel *N*-substituted acridinium esters **7–16** containing a 10-methyl, 10-dodecyl or 10-(ω-succinimidyloxycarbonylalkyl) group have been synthesized and their chemiluminescent properties have been tested. Their chemiluminescent efficiencies and hydrolytic stabilities have been found to be affected by the character of the group on the nitrogen atom. Dibromo-substituted leaving groups slightly accelerate the chemiluminescence process.

KEYWORDS

acridinium ester, chemiluminescence immunoassay, quantum yield, synthesis

1 INTRODUCTION

Acridinium esters (AEs) have been recognised as chemiluminescent molecules since lucigenin was reported in 1935 to give off light when it reacted with alkaline peroxide.[1] Even after coupling to other molecules, AEs still provide high quantum yields. No catalyst is needed to trigger the chemiluminescent reaction, and they exhibit low background and high sensitivity. AEs as chemiluminescent labels exhibit sensitivities that are greater than ^{3}H isotope labels and similar to ^{125}I isotope labels,[2] but do not carry the inherent difficulties associated with handling radioactive materials. Consequently, many AEs have been synthesised and their applications have been investigated.[3–18]

The AE label **1** (Fig. 1), containing a succinimidyl ester (NHS ester), was developed in 1983.[19] Much effort has been made to modify the structure of **1** in order to improve its performance. AE labels **2–5** (Fig. 1) were designed to influence the chemiluminescence properties by varying the leaving group. The IgG conjugates of labels **2–5** showed higher stabilities compared to that of label **1**,[20] because *ortho*-substitution protects IgG conjugates from hydrolysis. The 2,6-dimethoxy compound showed slightly higher chemiluminescent efficiency than that of **1**, while 2,6-dimethyl, 2,6-dibromo, and 2-methoxy AEs were somewhat less efficient.[20] Label **6** (Fig. 1) is more stable than its analogue that lacks the 2- and 6-methyl groups on the phenyl ring.[21] Also, its IgG conjugate shows three times the light emitting efficiency of the non-methylated analogue.[21]

We have shown that *ortho*- and *meta*-linked phenyl AEs containing different substituents on the phenyl group exhibit chemiluminescence influenced by steric and electronic factors.[22] In addition, we have investigated the synthesis and applications of a number of other AE labels.[23–25] Here we report preparation of novel acridinium labels **7–16** (Fig. 2). Some of these AEs contain an NHS linker bonded to nitrogen by a carbonylalkyl spacer group. When such AEs are attached to an analyte *via* this linker group, the luminescence emanates from the analyte conjugate, so energy transfer to a quencher also associated with the analyte should be more efficient than with labels that separate from the analyte during the chemiluminescent process. This could provide both lower background and more efficient emission. However, changing the *N*-alkyl substituent could itself influence the chemiluminescent properties and therefore the chemiluminescent properties of 10-

dodecylacridinium compounds (without NHS group) have been compared with those of the corresponding 10-(10-succinimidyloxycarbonyldecyl) and 10-methyl derivatives. Also, several 10-(ω-succinimidyloxycarbonylalkyl) compounds with different alkyl spacer lengths have been prepared to assess the effect of the spacer length (Fig. 2; n = 3, 5, 10) on the chemiluminescent properties. The assessment of such chemiluminescent properties should help clarify the significance of these effects.

2 EXPERIMENTAL

2.1 General methods

A Griffin instrument was used to measure melting points (mp). KBr disks and a Perkin Elmer Spectrometer 1 were used to record IR spectra. ^{1}H (400 MHz), ^{13}C (100 MHz) and ^{19}F (282.2 Hz) NMR spectra (chemical shifts (δ) in ppm, coupling constants (J) in Hz) were recorded on a Bruker AV 400 instrument with tetramethylsilane as the internal standard. A Micromass Quattro II Mass Spectrometer was used to record mass spectra (MS). Silica gel 60 (35-70 μ, Fisher Chemicals) was the column chromatography stationary phase and purity was investigated by TLC (Whatman silica gel plates), visualised by UV at 254 nm. The chemiluminescence (relative light units, RLU) was measured using a Ciba-Corning Magic Lite Analyzer luminometer. Reagents 1 and 2 (MLT Research Ltd, Cardiff) were used to initiate chemiluminescence. Reactions were typically conducted in appropriately sized round bottom flasks, stirred with a magnetic follower, with a condenser and/or calcium chloride guard tube when necessary.

2.2 Syntheses of AEs 7–16

The synthetic routes for AEs **7** and **8**, **9** and **10**, and **11–16** are given in Schemes 1, 2 and 3, respectively. The spectroscopic data used to confirm the structures of the products are recorded in the electronic supplementary information document.

2.2.1 Phenyl acridine-9-carboxylate (7b)

Pyridine (15 mL) was used to dissolve acridine-9-carboxylic acid chloride[19] (290 mg, 1.20 mmol) at 50 °C, then the solution was cooled and stirred vigorously for 16 h with phenol (**7a**;

141 mg, 1.50 mmol). The brown solid remaining after removal of pyridine by evaporation was dissolved in dichloromethane (DCM; 10 mL), washed (H_2O, 3 × 50 mL) and dried ($MgSO_4$). Chromatography (PhMe/EtOAc, 4/1 by volume) gave **7b** (pale yellow solid), 226 mg (63%), mp 192–193 °C (lit.[23] mp 180–181 °C; lit.[26] mp 189–190 °C).

2.2.2 10-Methyl-9-(phenoxycarbonyl)acridinium trifluoromethanesulfonate (7)

Compound **7b** (65 mg, 0.22 mmol), anhydrous DCM (2 mL) and CF_3SO_3Me (90 µL, 0.80 mmol) were stirred for 3 h at 20 °C. The filtered solid was washed (DCM, 1 mL; EtOAC, 1 mL; Et_2O, 1 mL) to give **7** (yellow solid), 83 mg (50%), mp 234 °C.

2.2.3 2,6-Dibromophenyl acridine-9-carboxylate (8b)

Acridine-9-carboxylic acid chloride[19] (2.036 g, 8.42 mmol), which had been dissolved in anhydrous pyridine (20 mL) at 70 °C then cooled, and 2,6-dibromophenol (**8a**; 2.325 g, 9.23 mmol) were stirred together vigorously for 16 h. Evaporation of pyridine and chromatography (PhMe/EtOAc, 4/1 by volume) gave a solid. A solution of this in DCM was washed with aq. NaOH (0.1 mol/L, 40 mL × 5) to remove any carboxylic acid impurity. Drying ($MgSO_4$) and removal of the solvent gave **8b** (pale yellow powder), 2.08 g, (54%), mp 159–160 °C.

2.2.4 9-(2,6-Dibromophenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (8)

To compound **8b** (89 mg, 0.19 mmol) in dry DCM (2 mL) under N_2 was added CF_3SO_3Me (200 mL, 1.75 mmol). After stirring at 20 °C for 3 h the solid produced was washed (DCM, 1 mL); EtOAC, 1 mL; Et_2O; 1 mL) to give **8** (yellow solid), 83 mg (68%), mp 249–250 °C.

2.2.5 Dodecyl triflate (9a)

A mixture of silver triflate (278 mg, 2.08 mmol), 12-iodododecane (300 mg, 1.01 mmol) and benzene (1 mL) was stirred under N_2 for 18 h at 20 °C. Evaporation of the solvent and chromatography (DCM) gave **9a** (colourless oil), 219 mg (68%); lit.[27] colourless oil.

2.2.6 9-(Phenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (9)

Compounds **9a** (80 mg, 0.25 mmol), **7b** (96 mg, 0.32 mmol) and DCM (2 mL), under N_2, were

refluxed together for 21.5 h. Removal of the solvent then re-dissolution in DCM (0.6 mL) and precipitation with Et_2O (3 mL) (dissolution-precipitation process repeated 6 times) gave **9** (yellow-green solid), 30.3 mg (19.5%), mp 110–111 °C.

2.2.7 9-(2,6-Dibromophenoxycarbonyl)-10-dodecylacridinium trifluoromethanesulfonate (10)

Compounds **9a** (70 mg, 0.22 mmol), **8b** (74.8 mg, 0.16 mmol) and 1,2-dichloroethane (DCE; 2 mL), under N_2, were refluxed together for 31 h. Removal of the solvent then re-dissolution in DCM (0.6 ml) and precipitation with Et_2O (3 ml) (dissolution-precipitation procedure repeated 6 times) gave **10** (yellow-green solid), 11.5 mg (19.%), mp 92–96 °C.

2.2.8 Succinimidyl 4-iodobutanoate (11b)

Application of the published procedure[24] yielded **11b** (yellow solid), 567 mg (84%), mp 86–87 °C; lit.[24] mp 86–87 °C.

2.2.9 Succinimidyl 4-(trifluoromethanesulfonyloxy)butanoate (11c)

CF_3SO_3Ag (163 mg, 0.637 mmol), compound **11b** (145 mg, 0.466 mmol), and dry benzene (2 mL) were stirred under N_2 for 18 h. Chromatography (DCM) gave **11c** (white solid), 73 mg (47%), mp 59–60 °C.

2.2.10 9-(Phenoxycarbonyl)-10-(3-(succinimidyloxycarbonyl)propyl)acridinium trifluoromethanesulfonate (11)

Compound **7b** (78 mg, 0.26 mmol) and freshly prepared **11c** (51 mg, 0.15 mmol) in dry DCE (2 mL) were refluxed for 22.5 h under N_2. The residue following evaporation of the solvent was re-dissolved in DCM (0.6 mL) and re-precipitated with Et_2O (3 mL) 7 times to give **11** (yellow-green solid), 2.6 mg, (2.7%), mp 110–111 °C.

2.2.11 9-(2,6-Dibromophenoxycarbonyl)-10-(3-(succinimidyloxycarbonyl)propyl)acridinium trifluoromethanesulfonate (12)

Freshly prepared **11b** (56.9 mg, 0.17 mmol) and **8b** (61.9 mg, 0.14 mmol) in 1,1,2,2-

tetrachloroethane (TCE; 2 mL) were refluxed for 2 h under N_2. After removal of solvent the product was purified as shown for **11** to give **12** (brown solid), 7.0 mg, (6.3%), mp 114–115 °C.

2.2.12 6-Iodohexanoic acid (13a)

6-Bromohexanoic acid (1.49 g, 7.64 mmol) and dry sodium iodide (3.11 g, 20.7 mmol) in dry acetone (30 mL) were stirred for 4 h then poured into water (15 mL). The organic material was extracted into Et_2O (4 × 20 mL), washed (saturated $Na_2S_2O_3$, 20 mL), dried ($MgSO_4$), and purified by chromatography (hexane/Et_2O/HCO_2H, 1/1/0.1 by volume) to give **13a** (white flakes), 1.48 g (80%), mp 40–41 °C; lit.[28] 43–43.5 °C.

2.2.13 Succinimidyl 6-iodohexanoate (13b)

To a 0 °C solution of **11a** (699.9 mg, 2.89 mmol) in dry tetrahydrofuran (THF; 10 mL), under N_2, was added *N*-hydroxysuccinimide (NHS; 433.8 mg, 3.77 mmol) in THF (2 mL) and *N,N'*-dicyclohexylcarbodiimide (DCC; 680.0 mg, 3.30 mmol). The mixture was stirred at 0 °C for 3 h and at 20 °C for 16 h, followed by evaporation of the solvent. Purification by chromatography (PhMe/EtOAC, 4/1 by volume) followed by recrystallization from hexane/Et_2O gave **13b** (colourless needles), 448 mg (46%), mp 74–75 °C.

2.2.14 Succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate (13c)

CF_3SO_3Ag (166 mg, 0.648 mmol), compound **13b** (109 mg, 0.322 mmol), and dry benzene (2 mL) were stirred under nitrogen for 18 h at 20 °C. Removal of the solvent and purification by chromatography (DCM) gave **13c** (white gum), 54.8 mg (47%).

2.2.15 9-(Phenoxycarbonyl)-10-(5-(succinimidyloxycarbonyl)pentyl)acridinium trifluoromethanesulfonate (13)

Freshly prepared **13c** (88 mg, 0.25 mmol), compound **7b** (77 mg, 0.26 mmol), and dry DCE (2 mL) were refluxed under N_2 for 22.5 h. Removal of the solvent followed by chromatography (gradient from DCM to DCM/MeCN, 3/1 by volume) gave **13** (yellow solid), 15 mg (9%), mp 201–202 °C.

2.2.16 9-(2,6-Dibromophenoxycarbonyl)-10-(5-(succinimidyloxycarbonyl)pentyl) acridinium trifluoromethanesulfonate (14)

Freshly prepared **13c** (70 mg, 0.19 mol), compound **8b** (82.5 mg, 0.18 mol), and DCE (2 mL) were refluxed under N_2 for 21.5 h. Following solvent removal, the residue was repeatedly re-dissolved in DCM (1 mL) and re-precipitated with Et_2O (3 mL) until no starting materials were evident by TLC, resulting in pure **14** (yellow solid), 17 mg (12%), mp 96–98 °C.

2.2.17 11-Iodoundecanoic acid (15a)

11-Bromoundecanoic acid (2.19 g, 8. 27 mmol), dry sodium iodide (3.04 g, 20.3 mmol), and dry acetone (20 mL) were stirred under nitrogen for 4 h at 20 °C, and then filtered. To the filtrate was added further dry NaI (1.50 g, 10.1 mmol), and following stirring for another 4 h, the mixture was added to water (20 mL). The product was extracted into Et_2O (25 mL × 4), washed (saturated $Na_2S_2O_3$, 20 mL), and dried ($MgSO_4$). Removal of the solvent and purification by chromatography (hexane/Et_2O/HCO_2H, 1/1/0.1 by volume) gave **15a** (white flakes), 2.23 g (83%), mp 62–63 °C; lit.[28] mp 64–65 °C.

2.2.18 Succinimidyl 11-iodoundecanoate (15b)

A mixture of **13a** (989 mg, 3.17 mmol) in anhydrous THF (10 mL), *N*-Hydroxysuccinimide (655.5 mg, 5.70 mmol) in dry THF (5 mL) and DCC (760 mg, 3.69 mmol) in THF (5 mL) was stirred under N_2 for 3 h at 0 °C then for 16 h at 20 °C. Filtration, evaporation, purification by chromatography (PhMe/EtOAC, 4/1 by volume), and crystallisation (hexane/Et_2O; 1/1 by volume) gave **15b** (colourless needles), 847 mg (65%), mp 84 °C.

2.2.19 Succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (15c)

CF_3SO_3Ag (266 mg, 1.03 mmol), compound **13b** (147 mg, 0.359 mmol), and dry benzene (2 mL) were stirred under nitrogen for 18 h at 20 °C, then evaporated. Chromatography (DCM) of the residue gave **15c** (white gum), 110.4 mg (71%).

2.2.20 9-(Phenoxycarbonyl)-10-(10-(succinimidyloxycarbonyl)decyl)acridinium trifluoromethanesulfonate (15)

Freshly prepared **13c** (43 mg, 0.10 mmol), compound **7b** (65 mg, 0.22 mmol), and dry DCE (2 mL) were refluxed under N_2 for 21 h and the mixture was then evaporated. Chromatography (DCM then DCM/MeCN, 3/1 by volume) of the residue gave **15** (yellow solid), 30 mg (41%), mp 88–89 °C.

2.2.21 9-(2,6-Dibromophenoxycarbonyl)-10-(10-(succinimidyloxycarbonyl)decyl)acridinium trifluoromethanesulfonate (16)

Freshly prepared **15c** (94.1 mg, 0.22 mmol), compound **8b** (93.2 mg, 0.20 mmol), and dry TCE (2 mL) were heated under N_2 for 4 h at 140 °C. Purification as shown for **15** gave **16** (yellow solid), 28.0 mg (15%), mp 165–166 °C.

3 CHEMILUMINESCENT TESTS

3.1 Kinetics

The chemiluminescence rates for compounds **7–16**, expressed as RLU/s, are represented in Figs. 3–5. The chemiluminescence was automatically measured by the luminometer, but the length of time over which it was monitored was controlled manually. Curves representing the trend lines used the "B-spline" option within *Origin* software.

3.2 Hydrolytic stabilities

The hydrolytic stabilities of acridinium esters are of great significance when they are used for labelling analytes (antibodies or oligonucleotide fragments). To compare the stabilities of the prepared AEs, stock solutions of 1×10^{-4} mg/mL in acetonitrile were first prepared. Further dilution to *ca*. 1 ng/ml was made using buffers with pH of 6, 7 and 8. Each solution was divided into three portions, to be incubated at 37, 24 and 8 °C, respectively. The experimental light intensities for these solutions were adjusted to correspond to 1 nmol/L concentration. Monitoring for solutions incubated at 37 °C was continued for 8 days, but for samples incubated at 24 and 8 °C it was continued for 16 days. Total emission (in RLUs) was measured over a time of 15 seconds at various points during the incubation (see Figs. 6–8). Trend lines were drawn using the "B-spline" option (average smooth, 2 points) within *Origin* software. The 16-day values for sample 10A were unreliable, so the monitoring in that case is reported only

for the first 8 days (Fig. 6).

4 RESULTS AND DISCUSSION

4.1 Syntheses of AEs 7–16

Syntheses of the AEs **7** and **8**, **9** and **10**, and **11–16** are outlined in Schemes 1, 2 and 3, respectively, which are primarily based on modified existing approaches. The crude yields in the final alkylation step for the dodecyl compounds were measured by ^{1}H NMR as **9** (39%) and **10** (31%), but the isolated yields were much lower. As such, the yields for the 10-dodecyl compounds were substantially poorer than those for the corresponding 10-methyl compounds, which can be understood since methyl triflate is a more reactive electrophile than dodecyl triflate on both electronic and steric grounds. The trend is consistent with other reports; for example, dodecylation of a pyridyl ring occurred only to the extent of 40%.[19] Although dodecyl triflate has a molecular mass similar to that of **11b**, the yields for 10-(3-succinimidyloxycarbonylpropyl) compounds were greatly poorer again and the reaction conditions required for bringing about the reaction were harsh. However, the yields for other 10-(ω-succinimidyloxycarbonylalkyl) derivatives were higher and the necessary reaction conditions were milder as the number of CH_2 groups increased, *i.e*., **15** > **13** > **11** and **16** > **14** > **12**. All of these results demonstrate that the proximity of the succinimidyloxycarbonyl group has a deleterious effect on the alkylation reactivity of a substituted alkyl triflate.

2,6-Dibromophenyl esters gave slightly lower yields than the corresponding phenyl esters, *i.e*., **9** > **10**; **11** > **12**; **13** > **14** and **15** > **16**, because of electron withdrawal by the two bromo substituents, leading to a slight decrease in electron density within the acridine ring.

4.2 Chemiluminescent properties

None of the target AEs reported here have been reported previously, but one of them (compound **12**) differs only in the nature of the counter-anion (trifluoromethanesulfonate instead of iodide) from a previously reported compound.[22] Since the chemiluminescence is generated from the acridinium cation, there will be negligible difference in the chemiluminescent properties between **12** and its iodide anion analogue. The latter compound's properties were compared directly with those of several other AEs, including the

original AE label **1** and its dibromo analogue **3**.[22] Therefore, the results below, which compare the chemiluminescent properties of the compounds reported here, can also be compared to those for more traditional AEs.

4.2.1 Kinetics

Dibromophenyl esters display quicker chemiluminescent kinetics than the corresponding phenyl esters (*i.e.*, **8** < **7**; **10** < **9**; **12** < **11**; **14** < **13** and **16** < **15**, see Figs. 3–5), because of easier expulsion of the dibromophenoxide anion. Typically, maximum intensity and ending of emission for the dibromophenyl esters were reached in about half of the time needed for the corresponding phenyl esters.

4.2.2 Hydrolytic stabilities and chemiluminescent efficiencies

Although the 2,6-dibromophenyl esters exhibited quicker kinetics than the corresponding phenyl esters, they showed better hydrolytic stabilities (*i.e.*, **10** > **9**; **12** > **11**; **14** > **13** and **16** > **15**, see Figs. 6–8), presumably because the bulky bromo substituents shield the ester group. However, most of the dibromophenyl esters showed lower light output efficiency than the corresponding phenyl esters, *i.e.*, **10** < **9**; **12** < **11**; **14** < **13**, while **15** and **16** showed no significant difference in efficiency. All compounds showed better storage stabilities in a pH 6 or 7 buffer (*i.e.*, in a weakly acidic or neutral buffer) than in a pH 8 (weakly basic) buffer, at all temperatures tested (8, 24 and 37 °C). Similar observations have been noted previously in other cases.[15]

The hydrolytic stability studies also showed that otherwise comparable AEs with longer alkyl chains at the 10-position tended to be less stable than those with shorter alkyl chains at the 10-position (*i.e.*, **9** < **7**; **10** < **8**; **15** < **13** < **11**; **16** < **14** < **12**). The reasons for this are not entirely clear, but may depend more on physical factors (such as the nature of the micelles formed within the buffer solutions) than on purely intrinsic chemical phenomena such as electronic and steric effects. The trends do not necessarily imply that 10-(10-(succinimidyloxycarbonyl)decyl) derivatives are less attractive labelling agents than 10-(5-(succinimidyloxycarbonyl)pentyl) or 10-(3-(succinimidyloxycarbonyl)propyl) derivatives, because the former compounds are more easily prepared than the latter. Each type of compound could therefore be a more appropriate choice for a particular application.

5 CONCLUSIONS

Several acridinium labels with different substituents at the 10-position have been successfully synthesized and their chemiluminescent efficiencies, kinetics and the hydrolytic stabilities have been measured. The final alkylation step is more difficult with dodecyl triflate than with methyl triflate and more difficult again with alkyl triflates substituted with a ω-(succinimidyloxycarbonyl) group. The hydrolytic stabilities of the compounds in various buffer solutions tend to be lower with AEs having longer alkyl chains at the 10-position. 2,6-Dibromophenyl esters undergo the chemiluminescent reaction rather more quickly than the corresponding phenyl esters, but in most cases display somewhat lower light generation.

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CONFLICT OF INTEREST

No conflict of interest to declare.

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Figures and Schemes Captions

FIGURE 1 Known AEs **1–6**

FIGURE 2 Target AEs **7–16**

SCHEME 1 Synthesis of AEs **7** and **8**

SCHEME 2 Synthesis of AEs **9** and **10**

SCHEME 3 Synthesis of AEs **11–16**

FIGURE 3 Chemiluminescence for compounds **7–10**

FIGURE 4 Chemiluminescence for compounds **11–14**

FIGURE 5 Chemiluminescence for compounds **15** and **16**

FIGURE 6 Storage stabilities for compounds **7–10** stored at 8 °C (A), 24 °C (B) and 37 °C (C) at pH of 6 (green), 7 (red) and 8 (black)

FIGURE 7 Storage stabilities for compounds **11–14** stored at 8 °C (A), 24 °C (B) and 37 °C (C) at pH of 6 (green), 7 (red) and 8 (black)

FIGURE 8 Storage stabilities for compounds **15** and **16** stored at 8 °C (A), 24 °C (B) and 37 °C (C) at pH of 6 (green), 7 (red) and 8 (black)

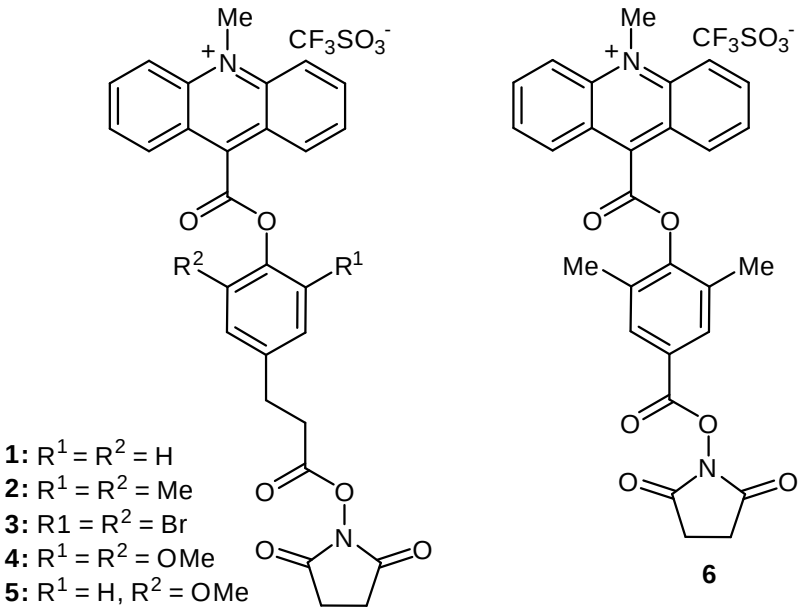


FIGURE 1 known AEs **1–6**

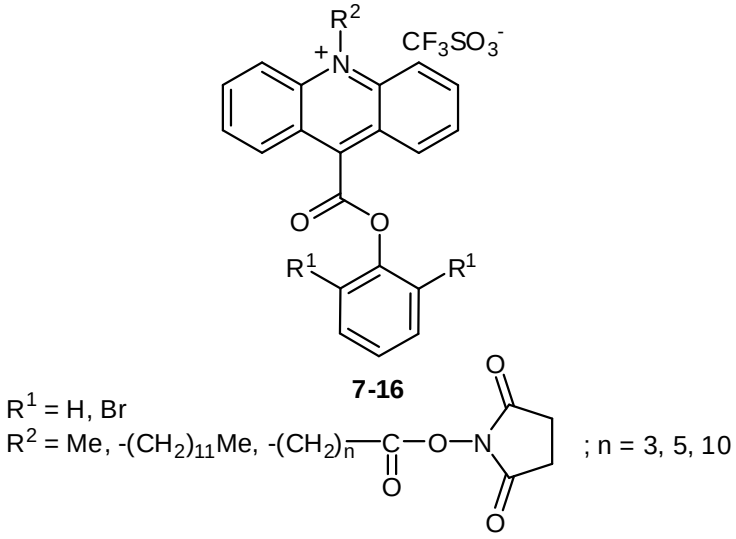
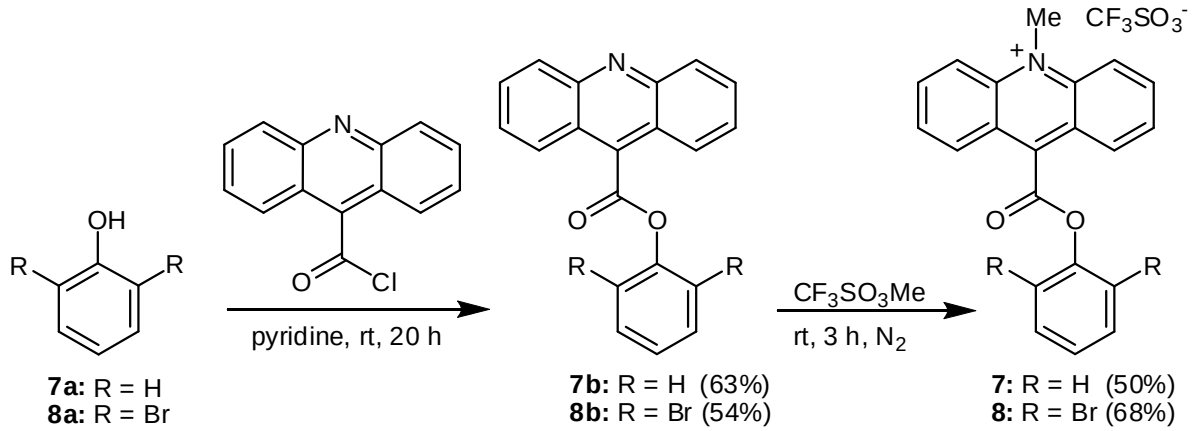


FIGURE 2 Target AEs **7–16**

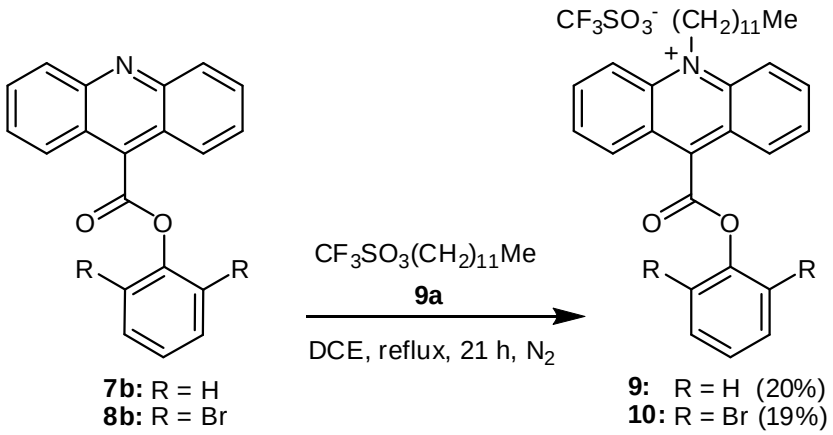


7a: R = H
8a: R = Br

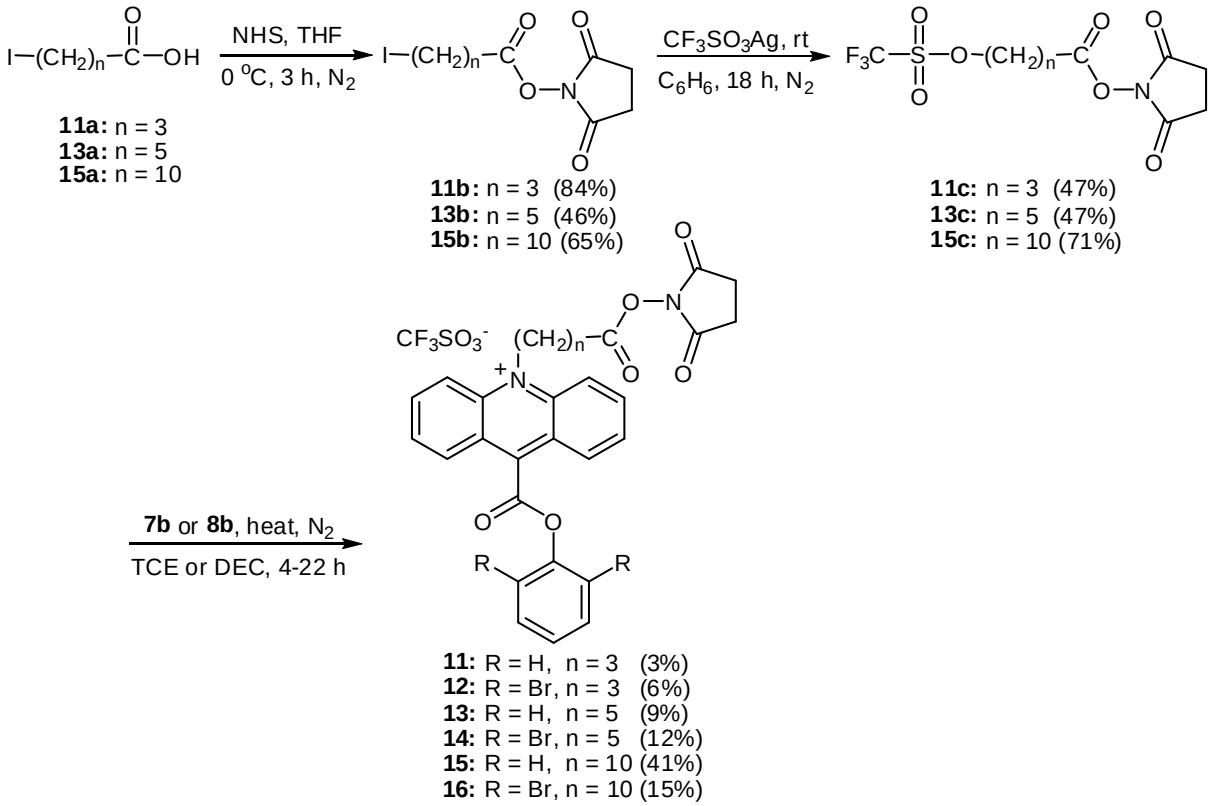
7b: R = H (63%)
8b: R = Br (54%)

7: R = H (50%)
8: R = Br (68%)

SCHEME 1 Synthesis of AEs **7** and **8**



SCHEME 2 Synthesis of AEs **9** and **10**



11a: n = 3
13a: n = 5
15a: n = 10

11b: n = 3 (84%)
13b: n = 5 (46%)
15b: n = 10 (65%)

11c: n = 3 (47%)
13c: n = 5 (47%)
15c: n = 10 (71%)

11: R = H, n = 3 (3%)
12: R = Br, n = 3 (6%)
13: R = H, n = 5 (9%)
14: R = Br, n = 5 (12%)
15: R = H, n = 10 (41%)
16: R = Br, n = 10 (15%)

SCHEME 3 Synthesis of AEs **11–16**

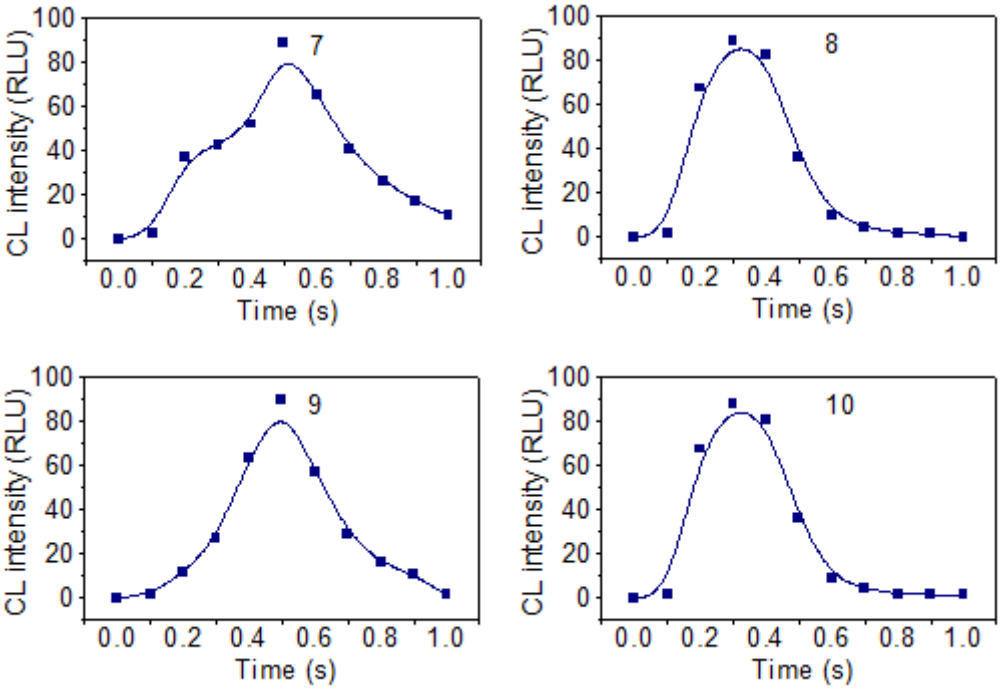


FIGURE 3 Chemiluminescence for compounds **7–10**

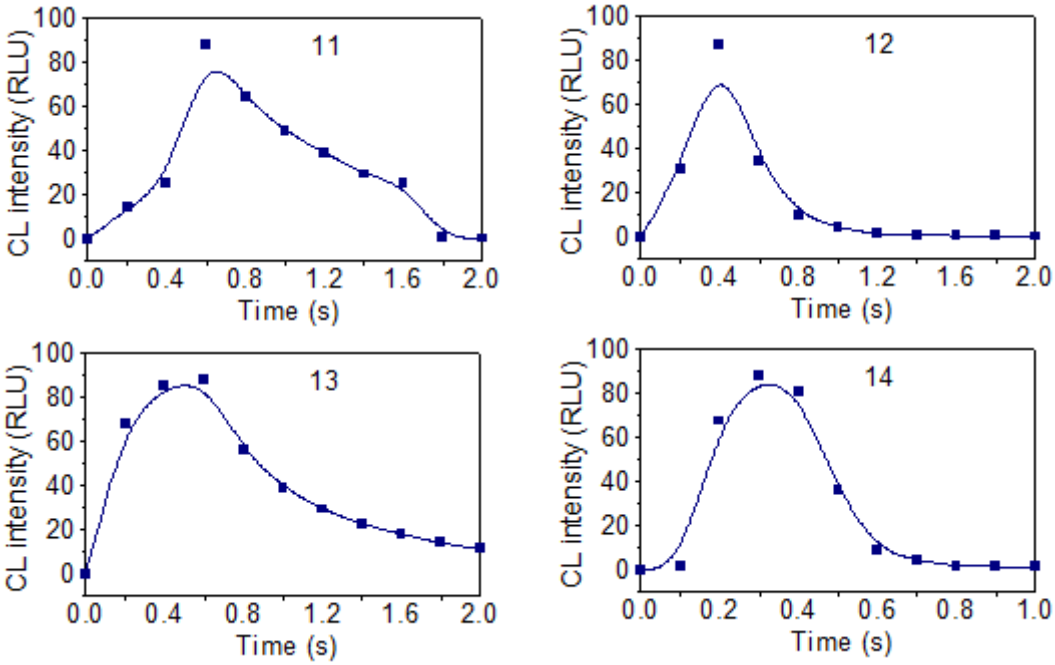


FIGURE 4 Chemiluminescence for compounds **11–14**

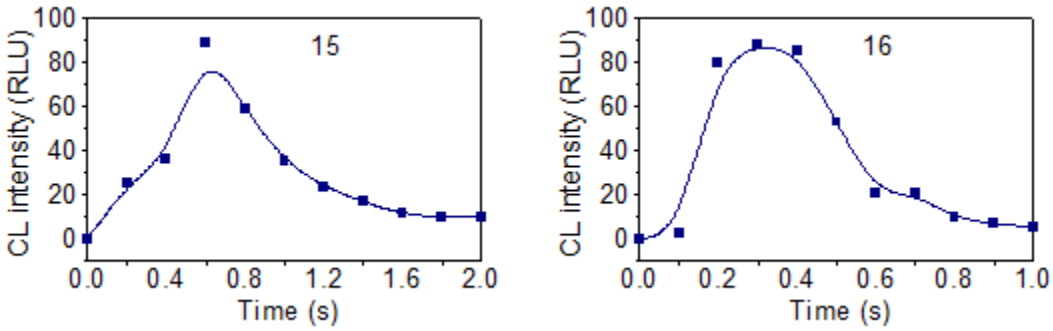


FIGURE 5 Chemiluminescence for compounds **15** and **16**

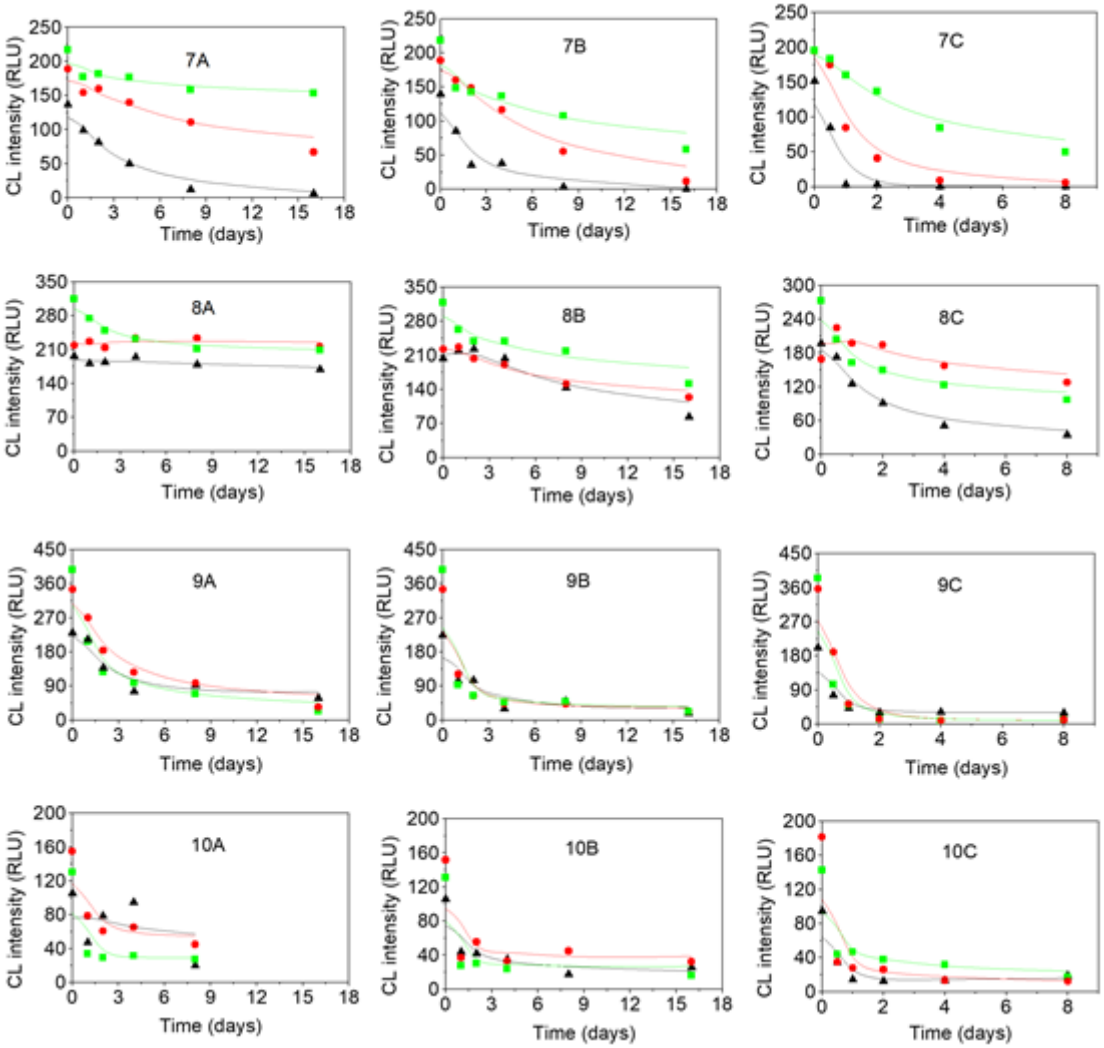


FIGURE 6 Storage stabilities for compounds **7–10** stored at 8 °C (A), 24 °C (B) and 37 °C (C) at pH of 6 (green), 7 (red) and 8 (black)

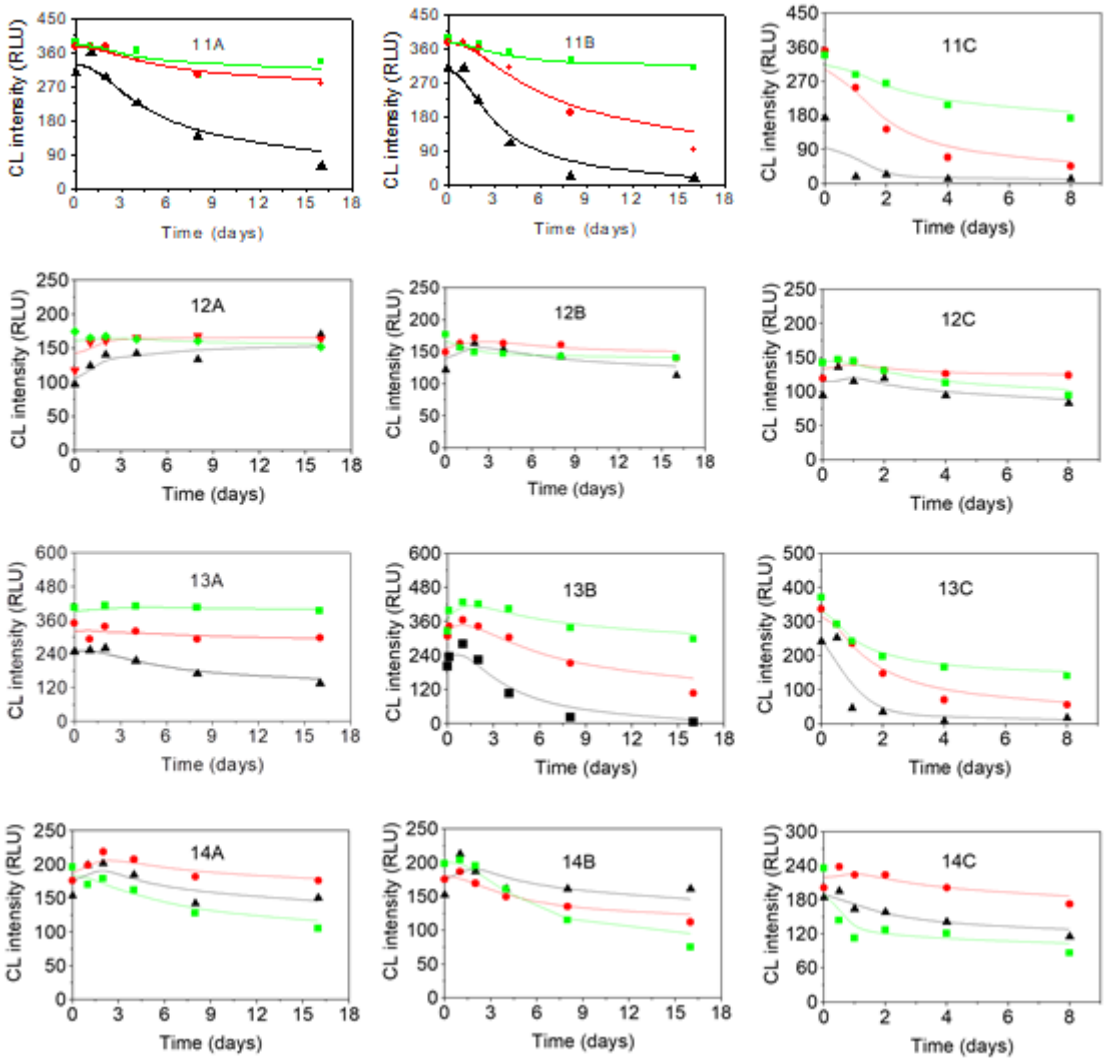


FIGURE 7 Storage stabilities for compounds **11–14** stored at 8 °C (A), 24 °C (B) and 37 °C (C) at pH of 6 (green), 7 (red) and 8 (black)

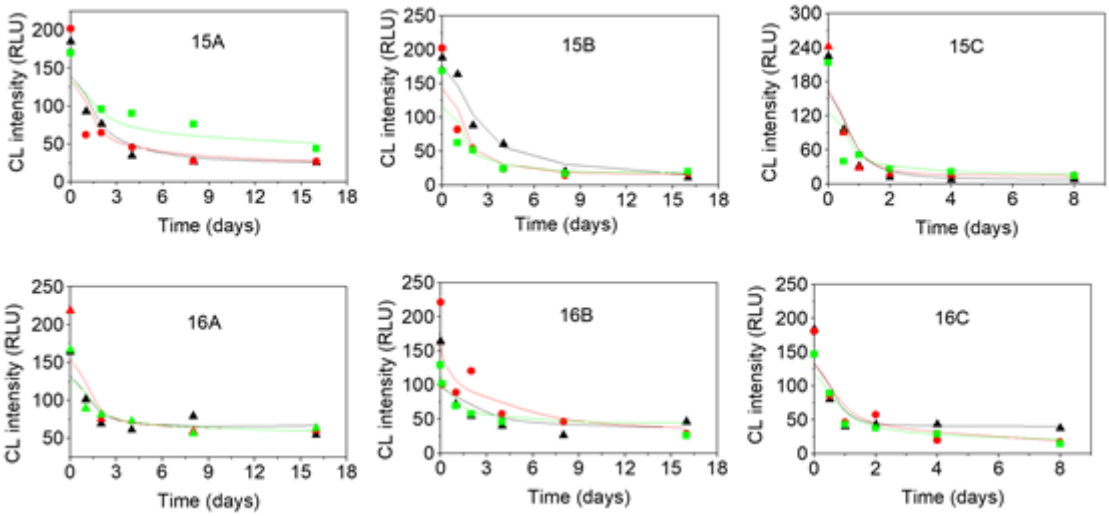


FIGURE 8 Storage stabilities for compounds **15** and **16** stored at 8 °C (A), 24 °C (B) and 37 °C (C) at pH of 6 (green), 7 (red) and 8 (black)

Graphical Abstract

Several novel acridinium esters containing various substituents at the 10-position have been synthesised and their chemiluminescent properties have been tested. The chemiluminescent efficiencies are dependent on both the phenoxy leaving group and the structural character of the 10-substituent. The hydrolytic stabilities of acridinium esters tend to be lower for longer alkyl chains at the 10-position.