RESEARCH ARTICLE

Synthesis and chemiluminescent characteristics of two new acridinium esters

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Abstract

Two new acridinium esters with a 2-(succinimidyloxycarbonyl)ethyl side arm, namely, 9-(2,6-dibromophenoxycarbonyl)-10-methyl-2-(2-(succinimidyloxycarbonyl)ethyl)acridinium trifluoromethanesulfonate and 9-(4-(2-(succinimidyloxycarbonyl)ethyl)phenoxycarbonyl)-2,7-dimethoxy-10-methylacridinium triflate, have been produced and characterized. The chemiluminescent properties and hydrolytic stabilities of the new acridinium esters have been investigated.

KEYWORDS
acridinium ester, chemiluminescence, hydrolysis, linker arm, side arm, stability

1 | INTRODUCTION

The chemiluminescence properties of acridinium esters (AEs) have been investigated ever since lucigenin was found to be chemiluminescent [1]. Following the development of compound **1** (Figure 1) as a useful biological probe [2] there has been even greater interest [3]. For example, in recent years, various chemiluminescent compounds have been synthesized for use in diagnostic technology [4–9], including for the determination of serum protein in humans [8], and in the identification of protozoan pathogens such as *Toxoplasma gondii* [9]. There is also current interest in the effect of supramolecular constraints on luminescence properties [10].

It is the cation of **1** that generates the luminescence (Figure 2) and it consists of four important components (Figure 1):

i. a group (methyl in compound **1**) to form the quaternary nitrogen, which promotes a rapid chemiluminescent reaction with hydrogen peroxide;
ii. the acridine unit, which gives rise to the excited state *N*-methylacridone luminescent emitter;
iii. an aryloxy-leaving group that is expelled during the reaction with hydrogen peroxide to give rise to a key intermediate in the chemiluminescent reaction;
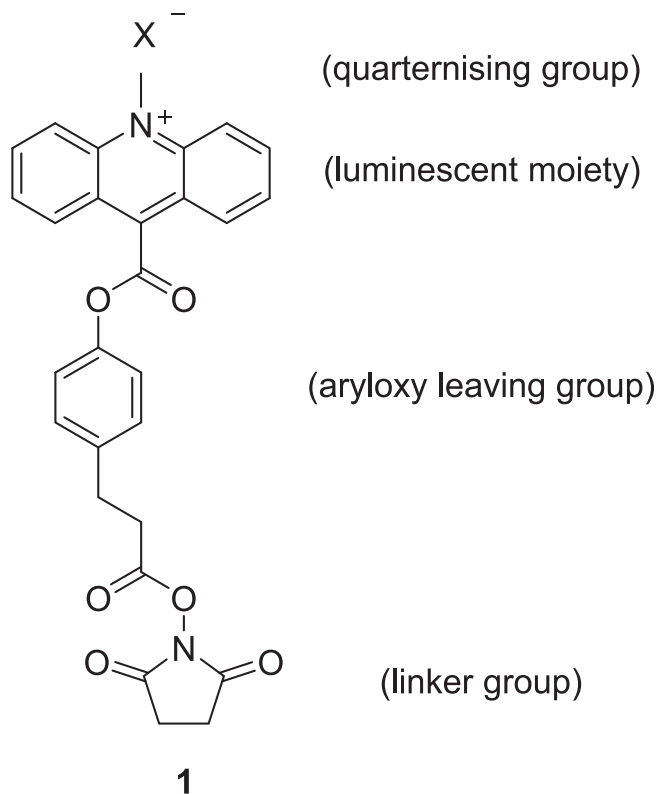
FIGURE 1 The first acridinium ester **1** for useful labelling of biological targets.
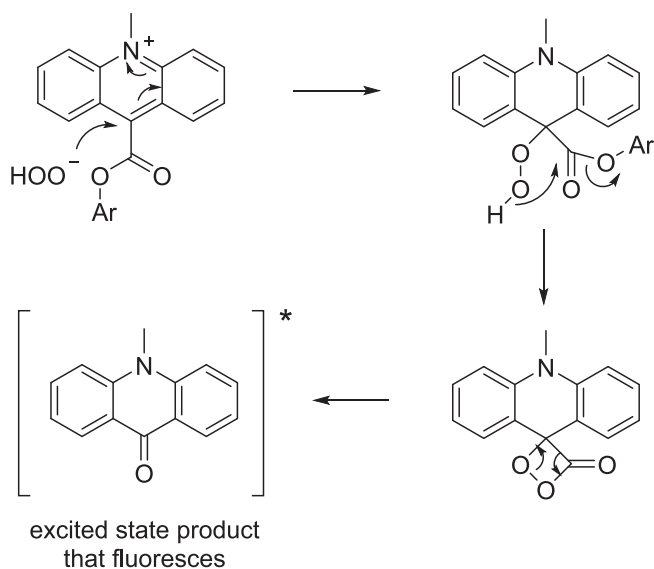

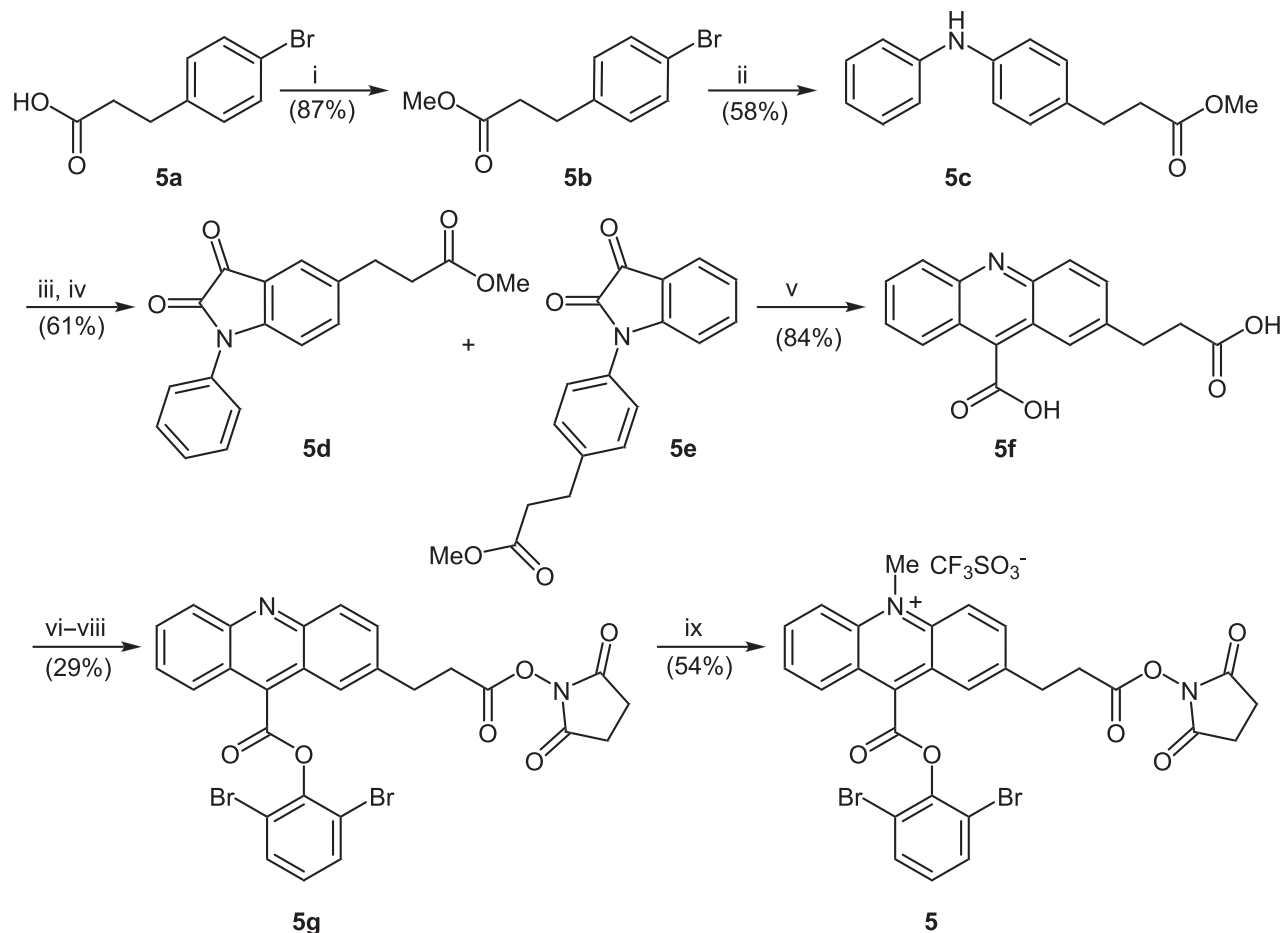**FIGURE 2** The mechanism of a typical acridinium ester chemiluminescent reaction.

iv. an active group (an *N*-hydroxysuccinimide ester in compound **1**) capable of linking to biological molecules such as proteins or nucleic acids.

Many variations of the basic structure have been introduced in attempts to provide probes with advantageous properties for particular applications. Although there have been reports of some different active linker groups [11], most reports continue to recommend the *N*-hydroxysuccinimide ester. However, the other three components have been extensively varied. In particular, some of the variants [12–15] involve modification of the ester leaving group part of the molecule, which particularly affects the kinetics of the luminescence process and the chemical stability of an AE in aqueous conditions. Variants involving modification of the substituent on the nitrogen atom of the acridinium system usually have little effect on the chemiluminescence properties [14–18], but can be used to introduce groups capable of influencing other properties, such as the solubility of the compound. A particularly interesting type of variation in the nitrogen substituent involves incorporating the linker into that moiety rather than into the aryloxy-leaving group [15, 18–21]. This results in the luminescence moiety remaining attached to the biological target following the chemiluminescence reaction, which can be useful for certain types of processes for target monitoring [22, 23]. The greatest effect on luminescence properties, such as the wavelength of the emission, comes with modification on the acridine ring [7, 14, 23–26]. However, comparatively, modifications on the acridinium ring generally require longer multistep syntheses and, in some cases, the introduction of substituents onto the acridine ring is unselective, and the separation step may be tedious and not very efficient. Therefore, the range of modifications reported has not been very wide.

For example, some time ago, our research group introduced AEs **2–4** (Figure 3) [12, 22], involving modifications of **1** in which an analogous linker group [a ω-(succinimidyloxycarbonyl)alkyl group] was placed at the 2- or 3-position of the aryloxy ring instead of at the 4-position, or on the nitrogen atom of the acridinium ring rather than on the leaving group, but we have not previously reported a similar modification in which the linker group was placed on one of the carbocyclic rings of the acridinium component.

We now report on the preparation of AE **5** (Figure 4), which has the *N*-hydroxysuccinimide (NHS) ester linker group attached via an alkylene spacer to one of the carbocyclic acridinium rings, rather than on the leaving group (aryloxy group) or bonded with the nitrogen atom of the acridinium ring. We also report the synthesis of AE **6**. We have previously reported the preparation of a compound with a 2,7-dimethoxyacridinium ring [23], as part of the development of a dual analyte measurement method, but the compound utilized had the linker group attached at the nitrogen atom and a dibromophenoxy leaving group. In that paper, we mentioned a precursor to **6**, with a benzyloxycarbonylethyl group instead of the succinimidyloxycarbonylethyl group, but its synthesis was not detailed. Therefore, we now report its synthesis in detail, along with its conversion into **6**. We also report on the chemiluminescence properties of AEs **5** and **6**.

The synthesis of AEs has been reviewed in a recent publication [27] and generally involves the initial preparation of the corresponding acridine carboxylic acid and a phenol needed

FIGURE 3 Acridinium esters (AEs) **2–4**, previously reported by our research group, that are analogues of **1** [12, 22].

FIGURE 4 Structures of target acridinium esters (AEs) **5** and **6**.

for its esterification; the final step in the syntheses is usually quaternization of the acridine nitrogen. This is the approach we adopted.

2 | EXPERIMENTAL

2.1 | Materials and methods

The general experimental details are reported in the Supporting information.

2.2 | Synthesis

The synthesis routes for AEs **5** and **6** are illustrated in Schemes 1 and 2, respectively. The details of the syntheses of precursors **5b–e** and **6b–e** are shown in the Supporting information.

2.2.1 | Preparation of 9-(2,6-dibromophenoxycarbonyl)-2-(2-[succinimidyloxycarbonyl]ethyl)-10-methylacridinium trifluoromethanesulfonate (**5**)

Compound **5 g** (47 mg, 0.075 mmol), dry DCM (3 ml) and methyl triflate (CF$_3$SO$_3$Me; 110 μl, 0.97 mmol) were stirred under argon for 23 h. Removal of the solvent and chromatography (DCM:acetonitrile [MeCN], 4:1 then 3:1 by volume) gave a brown-yellow oil (**5**, 32 mg, 54%), which solidified on standing, mp 86–87°C. ^{1}H NMR (CD$_3$COCD$_3$) δ 8.98–8.90 (m, 2H), 8.59–8.53 (m, 2H), 8.49 (app. t, 8 Hz 1H), 8.42 (d, 8 Hz, 1H), 8.14 (app. t, 8 Hz, 1H), 7.52 (d, 8 Hz, 2H), 7.03 (t, 8 Hz, 1H), 5.08 (s, 3H), 3.44 (t, 7 Hz, 2H), 3.33 (t, 7 Hz, 2H), 3.07 (s, 4H). ^{13}C NMR (CD$_3$COCD$_3$) δ 170.2, 169.0, 161.8, 146.5, 143.7, 143.1, 142.4, 142.2, 142.0, 139.9, 133.0, 130.7, 129.4, 127.2, 125.5, 124.1, 124.0, 120.4, 120.3, 117.8, 40.4, 33.4, 30.3, 26.3. IR υ$_{max}$ (KBr) 1738; ES$^+$-MS m/z 641 ([M−CF$_3$SO$_3$]$^+$, 100%), 558 (76), 544 (78), 530 (48), 420 (29), 344 (48), 248 (31), 209 (42), 196 (49). ES$^-$-MS m/z 149 ([CF$_3$SO$_3$]$^-$, 100).

2.2.2 | Preparation of 9-(4-[2-(succinimidyloxycarbonyl)ethyl] phenoxycarbonyl)-2,7-dimethoxy- 10-methylacridinium trifluoromethanesulfonate (**6**)

Compound **6f** (51.9 mg, 0.095 mmol), dry DCM (2.5 ml) and CF$_3$SO$_3$Me (200 μl, 1.77 mmol) were stirred overnight under N$_2$. After removal of the volatiles and chromatography (DCM:MeCN, 3:1 by volume), **6** was obtained as an orange solid (31.2 mg, 46%), mp 94–96°C. ^{1}H NMR (DMSO-d$_6$) δ 8.86 (d, 10 Hz, 2H), 8.15 (dd, 3, 10 Hz, 2H), 7.63 (d, 8.5 Hz, 2H), 7.57 (d, 8.5 Hz, 2H), 7.51 (d, 3 Hz, 2H), 4.94 (s, 3H), 4.13 (s, 6H), 3.16–3.02 (m, 4H), 2.83 (s, 4H). ^{13}C NMR (CD$_3$COCD$_3$) δ 170.0, 168.6, 164.2, 159.8, 149.1, 142.0, 139.5, 137.9, 132.3, 130.7, 125.7, 122.1, 121.9, 102.5, 56.6, 40.5, 32.4, 29.3, 25.8. IR υ$_{max}$ (KBr) 1732 (C=O). ES$^+$-MS m/z 543 ([M−CF$_3$SO$_3$]$^+$, 100%), 446 (10), 253 (6). ES$^-$-MS m/z 149 ([CF$_3$SO$_3$]$^-$, 100).

3 | RESULTS AND DISCUSSION

3.1 | Synthesis

The synthesis of AEs **5** and **6** (Schemes 1 and 2) followed routes that were similar to routes we have reported previously [12, 18, 20–22, 26]. The major differences were only in the generation of the appropriate intermediate isatins with the necessary substitution patterns to create the desired substituted acridinium rings. The latter were produced through rearrangement reactions of the isatins, which were in turn derived from the corresponding diarylamines. In principle, diarylamines with two nonidentical aryl rings should give at least two isomers during the formation of isatins, as with the formation of

Reagents: i: SOCl$_2$, MeOH, reflux, 3 h; ii: PhNH$_2$, t-BuOK, 5% Pd(DPPF)Cl$_2$, 15% DPPF, N$_2$, PhMe, reflux, 3 h; iii: (COCl)$_2$, DCM, rt, 0.5 h; iv: AlCl$_3$, reflux, 50 min; v: 10% KOH, H$_2$O, reflux, 48 h; vi: SOCl$_2$, NHS, reflux, 3 h; vii: pyridine, rt, overnight; viii: 4-toluenesulfonyl chloride, 2,6-dibromophenol, rt, 24 h; ix: CF$_3$SO$_3$Me, rt, N$_2$, 23 h

SCHEME 1 Synthesis of compound **5**.

compounds **5d** and **5e** from **5c**. If the *ortho*-positions on either ring of a diarylamine were themselves nonidentical, more than two isomers would be likely.

Formation of the isatins involved oxalyl chloride coupling first to the N atom of the diarylamine, followed by cyclization of the other acid chloride unit of the original oxalyl chloride onto one of the aryl positions *ortho* to the nitrogen atom (intramolecular Friedel-Crafts cyclization). In the processes of cyclization reported here, there were two identical atoms *ortho* to the N atom on each ring and, for the synthesis of compound **6**, the two rings were identical, so only one isatin was formed. However, for synthesis of compound **5**, where the two rings were different, but where the two *ortho*-positions on each ring were identical, just two isatins (**5d** and **5e**) were formed. The two compounds could not easily be separated, but fortunately both compounds led to the same substituted acridinecarboxylic acid, **5f**, in the next step, so the mixture could be used directly. The other steps of the synthesis were entirely standard and proceeded as expected. The details are given in the Experimental

section (for the final products) or the Supplementary information (for the precursors).

3.2 | Chemiluminescence tests

3.2.1 | Chemiluminescence kinetics

Kinetics curves [intensity versus time; relative light unit (RLU) against seconds] for AEs **5** and **6** were plotted automatically by the luminometer, but the length of time over which the chemiluminescence was monitored was controlled manually. The results are displayed in Figure 5.

Both compounds exhibited relatively quick chemiluminescence emission, which was over within approximately three-quarters of a second. Somewhat surprisingly, the curve for compound **6** was rather narrower than that for compound **5**, despite the fact that compound **6** had two electron-donating methoxy groups on the
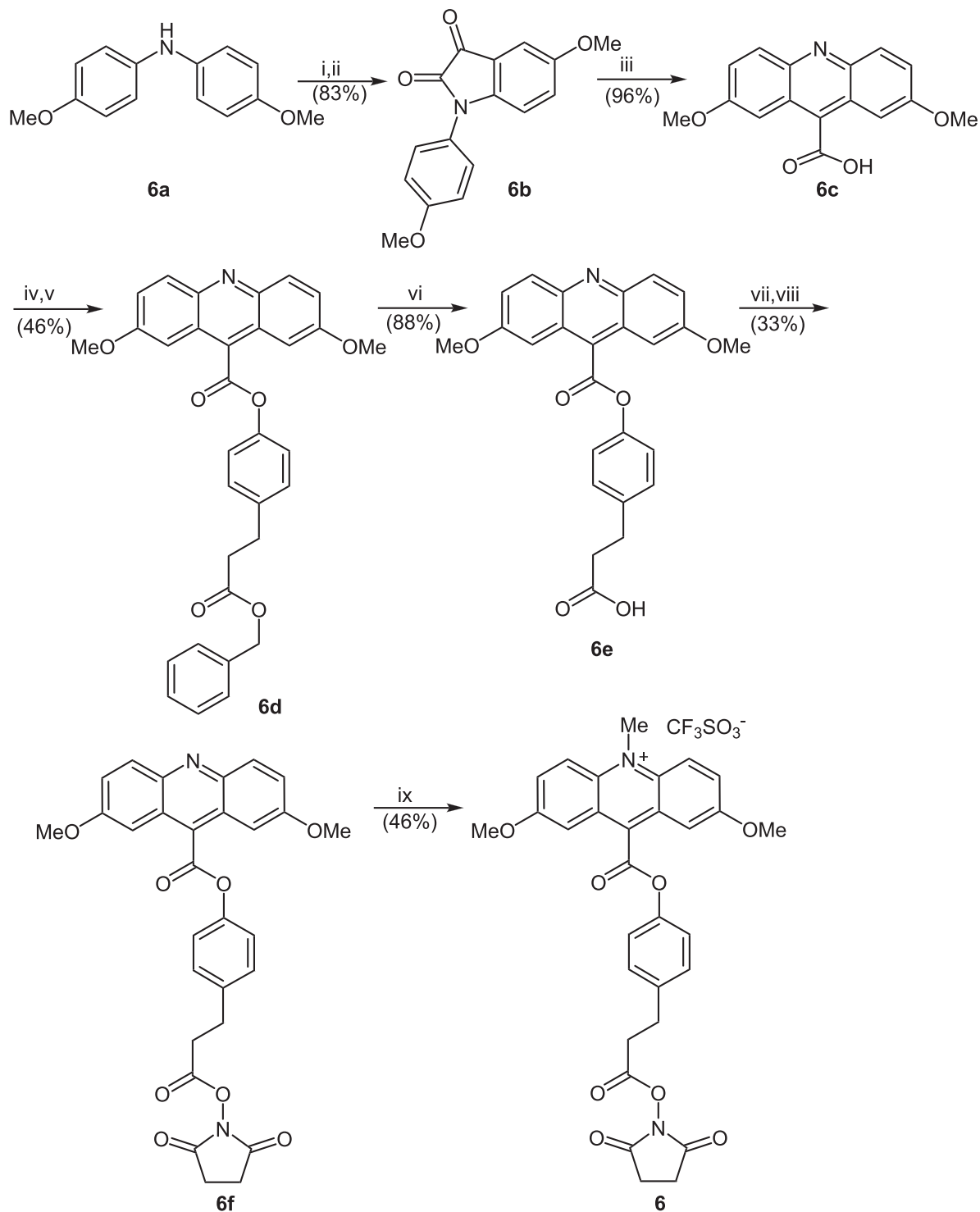
Reagents: i: (COCl)₂, DCM, rt, 0.5 h; ii: AlCl₃, reflux, 1 h; iii: 10% KOH, H₂O, reflux, 48 h; iv: SOCl₂, reflux, 1 h; v: 4-HO-C₆H₄CH₂CH₂CO₂CH₂Ph, 60 °C, (CF₃CO)₂O, 3 h; vi: HBr, HOAc, reflux; vii: SOCl₂, reflux, 1 h; viii: NHS, pyridine, rt, overnight; ix: CF₃SO₃Me, rt, N₂, ovenight

SCHEME 2 Preparation of compound **6**.

acridinium ring, which might be expected to slow down the attack of the hydroperoxide anion on position 9 of the ring, and that compound **5** had two bromine substituents on the leaving group, which might be expected to increase the rate of reaction. It may

be that the bromo-substituted leaving group and the lengthy substituent at position 2 of the acridinium ring in **5** collectively provided some shielding of position 9 from attack by hydroperoxide anions.
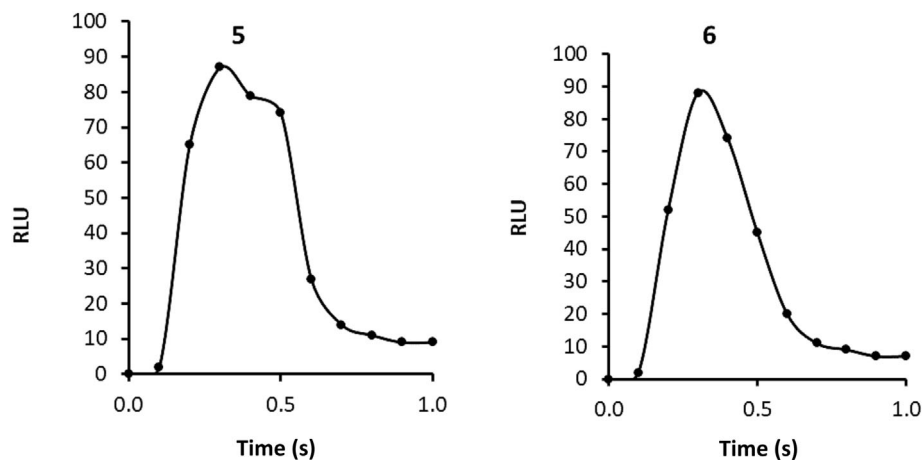
FIGURE 5 Chemiluminescence kinetics of acridinium esters (AEs) **5** and **6**.
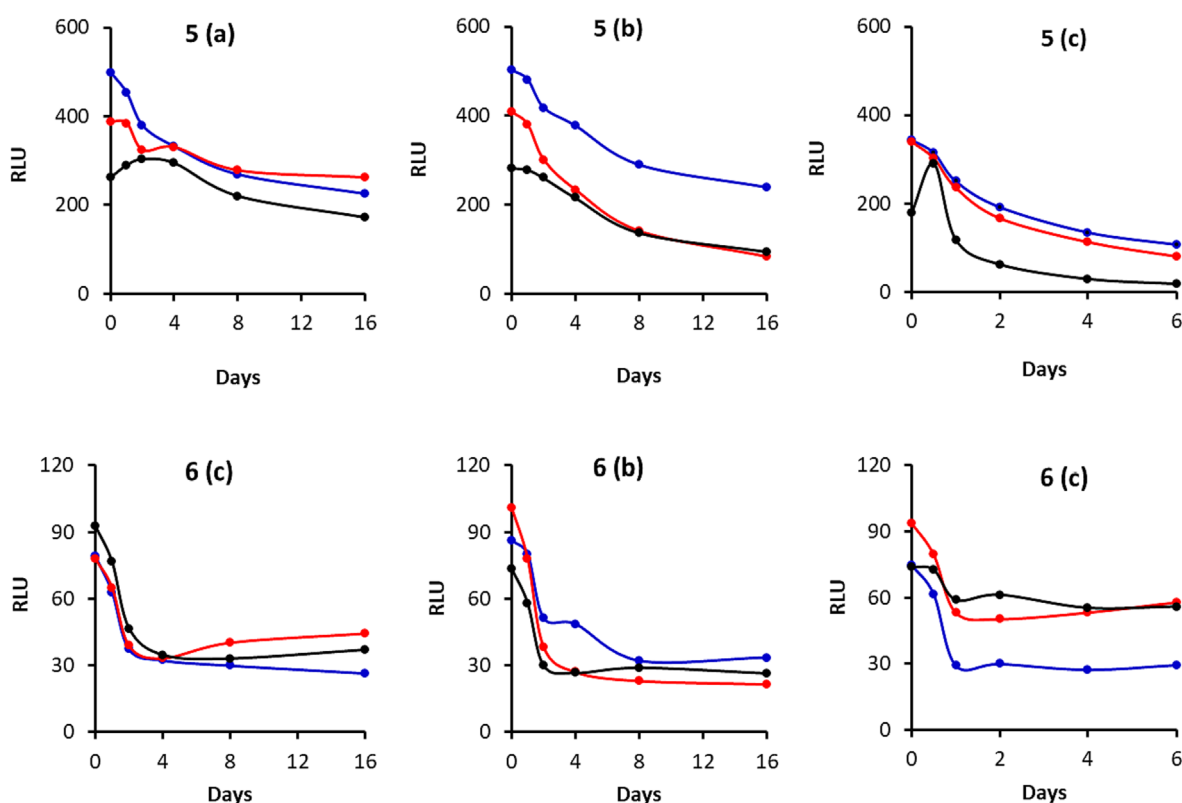
FIGURE 6 Storage stabilities for acridinium esters (AEs) **5** and **6** incubated at pH 6 (blue curves), 7 (red curves), and 8 (black curves) at 8°C (a), 24°C (b), and 37°C (c).

3.2.2 | Storage stabilities

The stock solutions of AEs **5** and **6** were first diluted to 1×10^{-4} mg/ml with MeCN. Further dilution then utilized buffers at pH 6, 7, and 8, to give solutions (∼1 ng/ml) that were each divided into three portions, and which were separately incubated at 8°C, 25°C, and 37°C. The values of measured intensities in RLUs for the 1 ng/ml ready-to-use samples were corrected to values corresponding to a concentration of 1 nmol L^{-1}. The samples stored at 37°C were monitored over 6 days, while those stored at 8°C and 25°C

were monitored over 2 weeks. The results are expressed graphically in Figure 6. Each data point on the graphs represents the total light output (in RLU) measured over 15 sec for the sample stored in the recorded conditions.

Both compounds **5** and **6** showed reasonable chemiluminescent efficiency (total light output from a sample prior to storage), but, interestingly, **5** showed much better efficiency than **6**, and actually exhibited better efficiency than most known AEs. The curve for compound **5** incubated at 24°C (diagram 5[b]) shows the expected pattern, involving steady loss of chemiluminescence intensity over a

period of 16 days, with the rate of loss being greater at the higher pH values. The loss of chemiluminescence over time is thought to be due to hydrolysis of the aryl ester, leading to a decomposition product that is much less prone to chemiluminescence. The results for compound **5** stored at other temperatures (Figure 6 5(a) and 5(c)) were qualitatively similar, but with somewhat less regular shapes. In general, as the temperature was increased, the rate of decomposition also increased, and at 37°C and pH 8, there was almost no chemiluminescence from samples stored for more than 4 days, while at pH 6 or 7, 20%–25% of the original level of light emitted still remained after 6 days. The irregularity of the shapes of the curves was more marked for compound **6** under all conditions studied, and the chemiluminescence did not disappear entirely during the period of monitoring. A possible explanation for such phenomena was discussed in our previous publication [21], but essentially was thought to depend on the level of dispersion of the compound in the aqueous medium, which was affected by the temperature, length of time stirring, and so on. The most significant result for compound **6**, however, is that the level of luminescent emission is significantly lower than it is for compound **5**, even for samples before storage.

4 | CONCLUSION

Two new AEs were prepared and their chemiluminescent properties were tested. The new probe **5** shows better efficiency than most other AEs. Also, it showed reasonable chemiluminescence kinetics and significant retention of chemiluminescent activity even after storage for many days in aqueous conditions at pH values from pH 6 to pH 8.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Data are contained within the article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.