Synthesis, structure elucidation, and chemiluminescent activity of new 9-substituted 10-(ω-(succinimidyloxycarbonyl)alkyl) acridinium esters

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Abstract

Several new acridinium esters 2–9 having their central acridinium ring bearing a 9-(2,5-dimethylphenoxycarbonyl), 9-(2,6-bis(trifluoromethyl)phenoxycarbonyl) or 9-(2,6-dinitrophenoxycarbonyl) group, and a 10-methyl, 10-(3-(succinimidyloxycarbonyl) propyl), 10-(5-(succinimidyloxycarbonyl)pentyl), or 10-(10-(succinimidyloxycarbonyl) decyl) group, have been synthesized and their chemiluminescent properties have been tested. The 2,5-dimethylphenyl acridinium esters emit light slowly (glow) when treated with alkaline hydrogen peroxide, while the 2,6-dinitrophenyl and 2,6-bis(trifluoromethyl) phenyl esters emit light rapidly (flash). The substituent at the 10 position affects the hydrolytic stabilities of the compounds.

KEYWORDS
acridinium ester, chemiluminescence kinetics, hydrolytic stability, synthesis

1 | INTRODUCTION

9-(4-(2-(Succinimidyloxycarbonyl)ethyl)phenoxycarbonyl)-10-methylacridinium triflate was the first useful acridinium ester label to be synthesized, in 1983.[1] It has been used in labelling antibodies in immunoassay,[2–5] and labelling oligonucleotides,[6–8] due to its sensitivity for detection of analytes. Various modifications have been made to the first acridinium label in order to improve its hydrolytic stability, chemiluminescent efficiency, and solubility in water, and to alter its chemiluminescent kinetics to allow multi-target analysis.[9–16] Recently, several chemiluminescent compounds have been developed and used in diagnostic technology.[17–22] For example, acridinium esters have been used in the identification of Toxoplasma gondii[19] and determination of specific proteins in human serum.[20]

We have previously reported the synthesis and chemiluminescent properties of a number of phenyl and 2,6-dibromophenyl acridinium-9-carboxylate esters (1, Figure 1),[23] having a variety of substituents, some with a ω-(N-hydroxysuccinimidyl)alkyl group bonded to the 10-nitrogen atom. Such acridinium esters showed reasonable efficien-cies, but different stabilities and luminescent kinetics. Earlier reported work had suggested that substituents at the 10-position had no effect on the stability, efficiency, or luminescent kinetics of acridinium esters.[24–26] By contrast, our data showed that stability was affected significantly by the nature of the substituent at the 10-position. How-ever, only two types of aryl esters had been studied. Therefore, it was of interest to study the properties of other acridinium aryl esters where the N-hydroxysuccinimidyl ester (NHS) linker group was still attached via an alkylene spacer group to the nitrogen atom at the

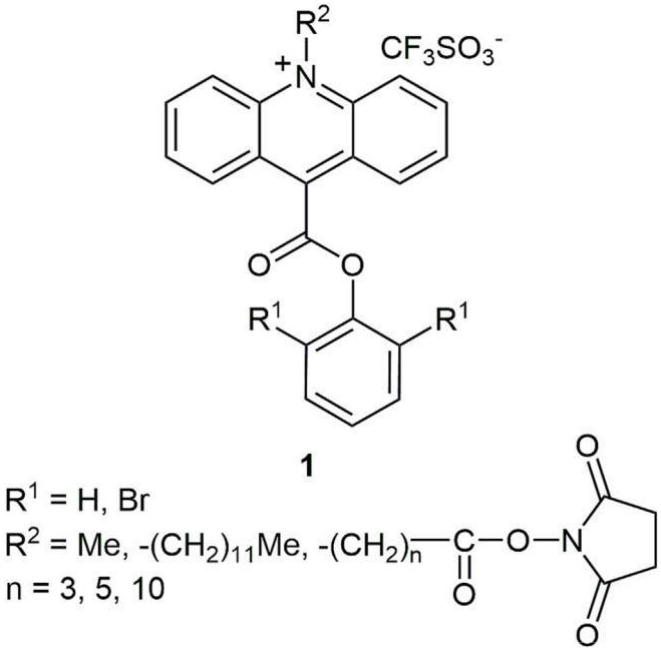


FIGURE 1 Several reported acridinium labels 1.[23]

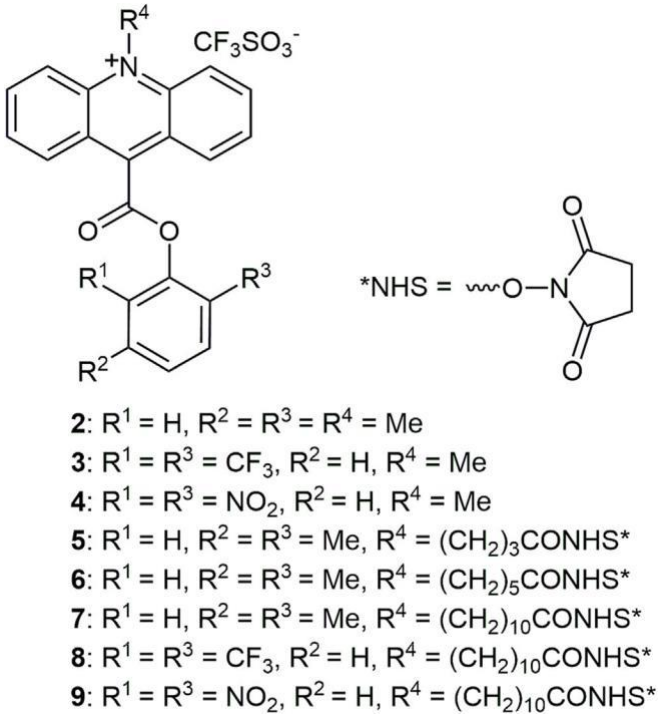


FIGURE 2 Target acridinium esters 2–9.

10-position, but with a wider range of aryl substituents, including ones with a greater range of electronic and steric effects.

Now, we report on the synthesis of novel acridinium labels 2–9 containing different substituents at the 9- and 10-positions (Figure 2). In order to influence the kinetics of the chemiluminescent process, the aryloxy leaving group was varied. The 2,5-dimethylphenoxy group was chosen to achieve slower luminescence kinetics, while the 2,6-bis (trifluoromethyl)phenyl and 2,6-dinitrophenyl moieties were expected to achieve quicker chemiluminescent kinetics than compounds 1 under comparable conditions or to enable reasonable luminescence kinetics at lower pH, which might be consistent with maintaining the integrity of biological samples.

2 | EXPERIMENTAL

2.1 | General

A Griffin capillary melting point apparatus was used to record melting points (mp), which were not corrected. Nuclear magnetic resonance (NMR) spectra [proton (^{1}H) at 400 MHz and carbon-13 (^{13}C) at 100 MHz] were recorded on a Bruker AV 400 instrument. Chemical shifts (ppm) are reported in δ units related to tetramethylsilane as internal standard. A Perkin Elmer spectrometer 1 was used to record infrared (IR) spectra [potassium bromide (KBr) disc method]. Only strong signals (e.g., C=O) are reported. Electron impact (EI), chemical ionization (CI), and electrospray ionization (ESI) mass spectroscopy (MS) were measured using a Micromass Quattro II instrument. Thin layer chromatography (TLC) utilized Whatman plates, visualized by ultraviolet (UV). Column chromatography was performed using Fisher silica gel 60 (35–70 μm). Commercial 2,6-dinitrophenol (20% water) was dried by dissolution in dichloromethane (DCM) and treatment with anhydrous $MgSO_4$.

2.2 | Syntheses

2.2.1 | Preparation of acridine-9-carboxylic acid chloride[27,28]

Acridine-9-carboxylic acid (424 mg, 1.90 mmol) in thionyl chloride (10 ml) was refluxed for 1 h and excess thionyl chloride was then evaporated under reduced pressure to leave acridine-9-carboxylic acid chloride, which was used in subsequent experiments without further purification.

2.2.2 | Preparation of 2,5-dimethylphenyl acridine-9-carboxylate (2b)[27–29]

Acridine-9-carboxylic acid chloride was heated to 50 C in pyridine (5 ml) and when the solid had dissolved completely, the solution was cooled and 2,5-dimethylphenol (267 mg, 2.18 mmol) was introduced. After stirring overnight, the pyridine was removed under reduced pressure. The brown residue was stirred with DCM (10 ml), filtered, and the filtrate was evaporated. Chromatography (toluene: ethyl ace-tate, 4:1 by volume) gave 2b (pale yellow solid, 424 mg, 68%), mp 187 C. ^{1}H NMR ($CDCl_3$) δ: 8.26, (d, 8 Hz, 2H), 8.22 (d, 8 Hz, 2H), 7.79 (dd, 8, 7 Hz, 2H), 7.62 (dd, 8, 7 Hz, 2H), 7.20 (s, 1H), 7.18 (d, 8 Hz,

1H), 7.03 (d, 8 Hz, 1H), 2.39 (s, 3H), 2.29 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 166.3, 149.4, 149.1, 137.8, 136.5, 131.8, 130.8, 130.6, 128.0, 127.9, 127.1, 125.3, 122.9, 122.7, 21.4, 16.9. EI-MS m/z: 327 (M^+, 5%), 206 (100), 178 (82), 77 (78). CI-MS m/z: 328 ($[MH]^+$, 52), 206 (18), 180 (100). IR υ_{max}: 1748 (C=O).

2.2.3 | Preparation of 9-(2,5-dimethylphenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (2)[26–29]

To compound 2b (140 mg, 0.43 mmol), in dry DCM (2 ml), under nitrogen, was added methyl trifluoromethanesulfonate (methyl triflate, 130 µl, 188.5 mg, 1.15 mmol) and the mixture was stirred for 3 h. Filtration and washing of the precipitate with DCM/ethyl acetate (3:1 by volume, 4 ml 5) gave 2 (yellow solid, 144 mg, 68%), mp ≥ 221 C (decomposed). ^{1}H NMR ($CDCl_3$) δ: 8.73 (d, 9 Hz, 2H), 8.35 (dd, 9, 7 Hz, 2H), 8.32 (d, 8 Hz, 2H), 7.91 (dd, 8, 7 Hz, 2H), 7.11 (s, 1H), 7.08 (d, 8 Hz, 1H), 6.97 (d, 8 Hz, 1H), 4.99 (s, 3H), 2.30 (s, 3H), 2.16 (s, 3H). ^{13}C NMR ($CDCl_3$) δ: 165.1, 150.1, 149.1, 144.0, 141.3, 131.4, 129.4, 120.9, 139.6, 133.3129.9, 123.9, 128.4, 124.6, 41.2, 21.5, 17.2. ES$^+$-MS m/z: 396 (9%), 342 ($[M – CF_3SO_3]^+$, 100), 252 (21), 220 (21), 193 (68). ES -MS m/z: 149 ($[CF_3SO_3]$, 100). IR υ_{max}: 1747 (C=O).

2.2.4 | Preparation of 2-(trifluoromethyl)phenol tetrahydropyranyl ether (3d)[29,30]

2-(Trifluoromethyl)phenol (1.00 g, 6.17 mmol), 3,4-dihydropyran (2 ml), and a few crystals of toluenesulfonic acid monohydrate, in diethyl ether (25 ml) were refluxed for 19 h. The mixture was poured into sodium hydrogen carbonate ($NaHCO_3$) solution (20 ml) and extracted with diethyl ether (3 25 ml), then the combined extracts were washed [saturated sodium chloride (NaCl)], dried ($MgSO_4$), and evaporated. Chromatography [ethyl acetate (5%), hexane (94.5%), triethylamine (0.5%)] gave 3d (colourless oil, 1.35 g, 89%). ^{1}H NMR ($CDCl_3$) δ: 7.50 (d, 8 Hz, 1H), 7.38 (app. t, 8 Hz 1H), 7.18 (d, 8 Hz, 1H), 6.94 (app. t, 8 Hz, 1H), 5.48 (dd, 5, 2 Hz, 1H), 3.77 (app. dt, 10, 3 Hz, 1H), 3.55 (m, 1H), 2.01–1.46 (m, 6H). ^{13}C NMR ($CDCl_3$) δ: 155.5, 133.8, 127.5, 127.4, 121.2, 120.1, 116.1, 96.6, 62.2, 30.7, 25.8, 20.4.

2.2.5 | Preparation of 2-(trifluoromethyl)-6-iodophenol (3e)[28,30]

To 3d (1.52 g, 6.18 mmol) in tetrahydrofuran (THF, 8.5 ml), under nitrogen, at 78 C, was added n-butyllithium (n-BuLi, 3 ml, 2.5 M in hexane, 7.5 mmol). After stirring at 78 C for 30 min, iodine (2.20 g, 8.67 mmol) in THF (8.5 ml) was introduced. After stirring for 10 min more, then warming to room temperature, the mixture was poured into sodium sulfite (Na_2SO_3) solution (20%). Extraction with diethyl ether (3 25 ml), washing of the extracts with saturated NaCl, drying ($MgSO_4$), and evaporation left a yellow-red oil, which was shown by its ^{1}H NMR spectrum to be 2-(trifluoromethyl)-6-iodophenol tetrahydropyranyl ether. Short-path distillation caused decomposition of the tetrahydropyranyl ether to give 3e (1.67 g, 94% if pure, but actually contaminated with c. 15% of dihydropyran hydrate). ^{1}H NMR ($CDCl_3$) δ: 7.88 (dd, 8, 1 Hz, 1H), 7.46 (dd, 8, 1 Hz, 1H), 6.80 (app. t, 8 Hz, 1H).

2.2.6 | Preparation of 2,6-bis(trifluoromethyl) phenol (3a)[29,30]

To cadmium (Cd) powder (3.057 g, 27.2 mmol) in dimethylformamide (DMF, 10 ml), under nitrogen at 0 C, dibromodifluoromethane (CBr_2F_2, 2.50 ml, 27.2 mmol) was added slowly in three portions. After warming to room temperature and stirring for 30 min, hexam-ethylphosphoramide (HMPA, 10 ml) was added, the mixture was cooled to 0 C, then copper bromide (CuBr, 1.80 g, 12.6 mmol) was added and stirring was maintained for 10 min. Compound 3e (1.102 g of 85% pure material, equivalent to 937 mg of pure 3e, 3.25 mmol) was added and the mixture was stirred at 65 C for 2 h. After cooling, it was poured into a mixture of hydrochloric acid (HCl, 30 ml, 3 mol L ^{1}) and diethyl ether (30 ml), and the solid was filtered off. The aqueous phase was extracted with ether (5 25 ml) and the extract was washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. Short-path distillation gave 3a (colourless oil, 283.4 mg, 38%), boiling point (bp) 80 C (0.7 Torr). ^{1}H NMR ($CDCl_3$) δ: 7.69 (d, 8 Hz, 2H), 7.06 (t, 8 Hz, 1H), OH not observed. ^{13}C NMR ($CDCl_3$) δ 153.0, 131.0, 123.8 (q, CF_3), 120.3, 120.0. EI-MS m/z 230 ($[M]^+$, 18%), 213 (28), 179 (23), 163 (30), 135 (100).

2.2.7 | Preparation of 2,6-bis(trifluoromethyl)phenyl acridine-9-carboxylate (3b)

Acridine-9-carboxylic acid chloride (131.6 mg, 0.544 mmol), 3a (130 mg, 0.565 mmol) and pyridine (3 ml) were stirred vigorously at 40 C for 24 h and then evaporated. The residue was extracted with DCM and chromatography (toluene/ethyl acetate, 4:1 by volume) gave 3b (orange solid, 137.5 mg, 58%), mp 171 C. ^{1}H NMR ($CDCl_3$) δ: 8.51 (d, 9 Hz, 2H), 8.32 (d, 9 Hz, 2H), 7.96 (d, 8 Hz, 2H), 7.79 (dd, 9, 7 Hz, 2H), 7.61 (dd, 9, 7 Hz, 2H), 7.56 (t, 8 Hz 1H). ^{13}C NMR ($CDCl_3$) δ: 164.6, 148.6, 146.3, 131.9, 131.1, 130.0, 128.5, 127.8, 126.5, 126.2, 125.8, 123.8, 122.8 (q, CF_3). EI-MS m/z: 435 (M^+, 10%), 206 (82), 178 (100). MS-CI m/z: 436 ($[MH]^+$, 39), 180 (100). IR υ_{max}: 1758 (C=O).

2.2.8 | Preparation of 9-(2,6-bis(trifluoromethyl) phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (3)

Compound 3b (110 mg, 0.252 mmol), dry DCM (3 ml) and methyl trifluoromethanesulfonate (160 µl, 1.42 mmol) were stirred together at

room temperature for 2 h, then at 30 C overnight. The solid was fil-tered and washed with ether. Addition of diethyl ether to the filtrate gave a further solid. The combined solids were washed with diethyl ether and pumped to give 3 (yellow solid, 67.8 mg, 46%), mp 231– 232 C. ^{1}H NMR (CD_3CN) δ: 8.78 (d, 9 Hz, 2H), 8.76 (d, 9 Hz, 2H), 8.53 (dd, 9, 7 Hz, 2H), 8.28 (d, 8 Hz, 2H), 8.15 (dd, 9, 7 Hz, 2H), 7.91 (t, 8 Hz, 1H), 4.95 (s, 3H). ^{13}C NMR (CD_3CN) δ: 161.7, 144.1, 144.0, 142.2, 138.9, 132.2, 129.4, 129.2, 127.0, 124.5 (two lines), 123.7, 119.0, 40.1. ES$^+$-MS m/z: 450 ([M – CF_3SO_3]$^+$, 100%), 252 (22), 224 (17), 193 (15). ES -MS m/z: 149 ([CF_3SO_3] , 100). IR υ_{max}: 1769 (C=O).

2.2.9 | Preparation of 2,6-dinitrophenyl acridine-9-carboxylate (4b)[27,31]

To acridine-9-carboxylic acid chloride (262 mg, 1.08 mmol) and anhydrous 2,6-dinitrophenol (235 mg, 1.28 mmol) in pyridine (1 ml), under nitrogen, was added dicyclohexyl carbodiimide (DCC, 250 mg, 1.23 mmol) in anhydrous THF (3 ml), and the mixture was stirred for 48 h. Removal of solids by filtration, evaporation of the filtrate, and chro-matography (silica, DCM) provided 4b (yellow solid, 93 mg, 22%), mp 203–204 C. ^{1}H NMR ($CDCl_3$) δ: 8.47 (d, 9 Hz 2H), 8.31 (d, 8 Hz, 2H), 8.26 (d, 9 Hz, 2H), 7.79 (dd, 9, 7 Hz, 2H), 7.61–7.65 (m, 3H). ^{13}C NMR ($CDCl_3$) δ: 163.9, 149.1, 144.6, 138.2, 131.8, 130.8, 130.6, 130.4, 128.6, 127.9, 125.7, 123.5. EI-MS m/z: 389 ([M]$^+$, 17%), 206 (96), 178 (100). MS-CI m/z: 390 ([MH]$^+$, 11), 180 (100). IR υ_{max}: 1775 (C=O).

2.2.10 | Preparation of 9-(2,6-dinitrophenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonate (4)

To 4b (33 mg, 0.085 mmol) in dry DCM, under nitrogen, was added methyl trifluoromethanesulfonate (30 µl, 0.26 mmol). After stirring for 3 h, diethyl ether (4 ml) was added. After filtration, the precipitate was washed repeatedly with diethyl ether until TLC showed it to be pure. Removal of solvent gave 4 (yellow solid, 21.4 mg, 46% yield), mp 199–200 C. ^{1}H NMR (CD_3CN) δ: 8.83 (d, 9 Hz, 2H), 8.74 (d, 9 Hz, 2H), 8.52–8.56 (m, 4H), 8.14 (app. t, 8 Hz, 2H), 7.94 (t, 8 Hz, 1H), 4.96 (s, 3H). ^{13}C NMR (CD_3CN) δ: 160.4, 143.9, 142.7, 139.2, 135.4, 141.5,

130.4, 129.2, 128.9, 127.5, 118.6, 121.2, 123.0, 42.0. ES$^+$-MS m/z: 404 ([M – CF_3SO_3]$^+$, 100%), 252 (19), 224 (29), 193 (78). ES -MS m/z: 149 ([CF_3SO_3] , 100). IR υ_{max}: 1767 (C=O).

2.2.11 | Preparation of 9-(2,5-dimethylphenoxycarbonyl)-10-(3-(succinimidyloxycarbonyl)propyl)acridinium trifluoromethanesulfonate (5)[32,33]

A mixture of 2b (43.2 mg, 0.13 mmol) and freshly prepared succinimi-dyl 4-(trifluoromethanesulfonyloxy)butanoate[23,34] (30.0 mg, 0.09 mmol) in dichloroethane (DCE, c. 3 ml), under nitrogen, was heated at reflux for 20 h. After removal of solvent, the residue was stirred with DCM (1 ml), filtered, and to the filtrate was added diethyl ether (3 ml). Repeated dissolution in DCM and precipitation with ether gave 5 (yellow solid, 1.6 mg, 2.7%), mp 98–99 C. ^{1}H NMR ($CDCl_3$) δ: 9.27 (d, 9 Hz, 2H), 8.60 (d, 9 Hz, 2H), 8.75 (app. t, 8 Hz, 2H), 8.23 (app. t, 8 Hz, 2H), 7.41 (s, 1H), 7.39 (d, 8 Hz, 1H), 7.27 (d, 8 Hz 1H), 5.83 (t, 8 Hz, 2H), 3.46 (t, 8 Hz, 2H), 3.03 (s, 4H), 2.79 (app. q, 8 Hz, 2H), 2.59 (s, 3H), 2.46 (s, 3H). ES$^+$-MS m/z: 565 (53%), 543 (58), 511 ([M – CF_3SO_3]$^+$, 100), 436 (57), 414 (68). ES -MS m/z:
149 ([CF_3SO_3] , 100). IR υ_{max}: 1737 (C=O).

2.2.12 | Preparation of 9-(2,5-dimethylphenoxycarbonyl)-10-(5-(succinimidyloxycarbonyl)pentyl)acridinium trifluoromethanesulfonate (6)[32,33]

Compound 2b (36.1 mg, 0.11 mmol), freshly prepared succinimidyl 6-(trifluoromethanesulfonyloxy)hexanoate[23,34] (35.6 mg, 0.099 mmol), and dry DCE (c. 3 ml), under nitrogen, were refluxed under nitrogen for 21 h. Removal of the solvent followed by chroma-tography (DCM then DCM/methanol 2:1 by volume) gave 6 (bright yellow solid, 13.4 mg, 20%), mp 87–88 C. ^{1}H NMR ($CDCl_3$) δ: 8.67 (d, 9 Hz, 2H), 8.36 (ddd, 9, 7, 1, 2H), 8.25 (dd, 9, 1 Hz, 2H), 7.89 (dd, 9, 7 Hz, 2H), 7.07 (s, 1H), 7.03 (d, 8 Hz, 1H), 6.90 (d, 8 Hz, 1H), 5.39 (t, 8 Hz, 2H), 2.60 (s, 4H), 2.41–2.47 (t, 7 Hz, 2H), 2.24 (s, 3H), 2.11 (s, 3H), 2.09–2.00 (m, 2H), 1.69 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 169.8, 168.9, 163.2, 149.1, 148.7, 141.7, 140.9, 132.1, 130.2, 128.8, 128.1,
122.1, 120.0, 138.3, 126.6, 123.4, 52.3, 30.9, 29.2, 26.0, 25.5, 24.6, 21.4, 16.8. ES$^+$-MS m/z: 593 (32%), 539 ([M – CF_3SO_3]$^+$, 100), 464 (33), 442 (22), 424 (14). ES -MS m/z: 149 ([CF_3SO_3] , 100). IR υ_{max}: 1737 (C=O).

2.2.13 | Preparation of 9-(2,5-dimethylphenoxycarbonyl)-10-(10-(succinimidyloxycarbonyl)decyl)acridinium trifluoromethanesulfonate (7)[32,33]

Compound 2b (80.0 mg, 0.25 mmol), freshly prepared succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate[23,34] (76.0 mg, 0.18 mmol), and dry DCE (c. 3 ml), under nitrogen, were refluxed for 20 h. Removal of the solvent left a gum, which was dissolved in DCM (1 ml) and then precipitated with diethyl ether (3 ml). The solid was repeatedly (c. six times) re-dissolved in DCM and re-precipitated with diethyl ether until shown pure by TLC, to give 7 (bright yellow oil that solidified on standing, 46 mg, 34%), mp 56–60 C. ^{1}H NMR ($CDCl_3$) δ: 8.80 (d, 9 Hz, 2H), 8.57 (dd, 9, 1 Hz, 2H), 8.47 (ddd, 9, 7, 1 Hz, 2H),
8.12 (dd, 9, 7 Hz, 2H), 7.29 (s, 1H), 7.23 (d, 8 Hz, 1H), 7.11 (d, 8 Hz, 1H), 5.59 (t, 8 Hz, 2H), 2.83 (s, 4H), 2.58 (t, 7 Hz, 2H), 2.44 (s, 3H), 2.31 (s, 3H) 2.26–2.02 (m, 2H), 1.77–1.67 (m, 4H), 1.49–1.25 (m, 10H). ^{13}C NMR ($CDCl_3$) δ: 169.8, 169.1, 163.3, 149.0, 148.7, 141.6, 140.8, 132.1, 130.2, 128.7, 128.2, 122.2, 119.9, 138.3, 126.6, 123.4,

52.6, 31.3, 30.0, 29.6, 29.5, 29.4, 29.2, 29.0, 26.9, 24.9, 26.0, 21.4, 16.8; ES^+-MS m/z: 663 (7%), 609 ($[M - CF_3SO_3]^+$, 100), 512 (9), 481 (10). ES -MS m/z: 149 ($[CF_3SO_3]$, 100). IR υ_{max}: 1734 (C=O).

2.2.14 | Preparation of 9-(2,6-bis(trifluoromethyl) phenoxycarbonyl)-10-(10-(succinimidyloxycarbonyl) decyl)acridinium trifluoromethanesulfonate (8)

Compound 3b (74.9 mg, 0.172 mmol), succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (74.8 mg, 0.174 mmol) and dry DCE (1.6 ml) were refluxed for 21 h. Removal of the solvent and chromatography (DCM/acetonitrile 3:1 by volume) gave 8 (yellow solid, 15.9 mg, 11%), mp 164–165 C. ^{1}H NMR ($CDCl_3$) δ: 8.78 (d, 9 Hz, 2H), 8.71 (d, 9 Hz, 2H), 8.49 (dd, 9, 7 Hz, 2H), 8.06 (d, 8 Hz, 2H), 7.98 (dd, 9, 7 Hz, 2H), 7.12 (t, 8 Hz, 1H), 5.60 (t, 8 Hz, 2H), 2.76 (s, 4H), 2.51 (t, 7 Hz, 2H), 2.29–2.18 (m, 2H), 1.84–1.69 (m, 4H), 1.51– 1.26 (m, 10H). ^{13}C NMR ($CDCl_3$) δ 169.7, 169.1, 161.8, 145.0, 144.9, 141.7, 140.6, 132.4, 130.0, 129.0, 128.5, 125.9 (visibly split by long-range coupling to F), 124.3, 119.8, 53.3, 26.0, 31.3, 30.1, 29.6, 29.5, 29.4, 29.3, 29.0, 26.9, 24.9 (signal-to-noise ratio too low for CF_3 sig-nals to be seen). ES^+-MS m/z: 717 ($[M - CF_3SO_3]^+$, 100%), 519 (17),

505 (8), 491 (9). ES -MS m/z 149 ($[CF_3SO_3]$, 100); high-resolution MS calculated for $C_{37}H_{35}F_6N_2O_6$ ($[M - CF_3SO_3]^+$): 717.2394; found: 717.2390. IR υ_{max}: 1733 (C=O).

2.2.15 | Preparation of 9-(2,6-dinitrophenoxycarbonyl)-10-(10-(succinimidyloxycarbonyl)decyl)acridinium trifluoromethanesulfonate (9)

Compound 4b (52.6 mg, 0.13 mmol), freshly prepared succinimidyl 11-(trifluoromethanesulfonyloxy)undecanoate (57 mg, 0.13 mmol), and DCE (2 ml), were refluxed under nitrogen for 20 h. Removal of solvent and chromatography (DCM then DCM/acetonitrile 3:1 by vol-ume) gave 9 (yellow oil, 15.8 mg, 14%). ^{1}H NMR ($CDCl_3$) δ: 8.65 (m, 4H), 8.41 (dd, 8, 7 Hz, 2H), 8.34 (d, 8 Hz, 2H), 7.92 (dd, 9, 7 Hz, 2H), 7.78 (t, 8 Hz, 1H), 5.48 (t, 8 Hz, 2H), 2.69 (s, 4H); 2.45 (t, 7 Hz, 2H);

2.08 (m, 2H), 1.68–1.53 (m, 4H), 1.35–1.10 (m, 10H). ^{13}C NMR ($CDCl_3$) δ: 169.7, 169.1, 161.1, 144.8, 143.9, 141.6, 136.6, 140.7, 131.1, 130.2, 129.7, 128.9, 119.6, 124.1, 53.1, 31.3, 30.1, 29.6, 29.5, 29.4, 29.2, 29.0, 26.9, 24.9, 26.0. ES^+-MS m/z: 671 ($[M - CF_3SO_3]^+$,

100%), 519 (15), 505 (23), 491 (42), 361 (77), 246 (44). ES -MS m/z: 149 ($[CF_3SO_3]$, 100), 80 (41). IR υ_{max}: 1735 (C=O).

2.3 | Chemiluminescence measurements

2.3.1 | Kinetics measurements

Each of the acridinium esters was tested for chemiluminescence in a Magic Light Analyser luminometer, using standard reagents supplied by Molecular Light Technology Research Ltd. The kinetic mode was employed. The period over which chemiluminescence was measured was controlled manually, maximum intensity was set at 88 relative light units (RLU), and output in RLU was recorded at intervals of one-tenth of the period of significant chemiluminescent output. The results were presented in graphical form and the 'B-spline' option within Origin software was used to generate the trend lines.

2.3.2 | Measurement of chemiluminescent efficiency and hydrolytic stability

The hydrolytic stabilities of acridinium esters were investigated by methods previously utilized in our laboratories.[27] Solutions of the acridinium esters in acetonitrile were first diluted to 1.00 10 ^{4} mg ml ^{1} with additional acetonitrile. Further repeated dilutions were then carried out with phosphate buffers of pH 6.0, 7.0 and 8.0 to yield ready-to-use solutions of concentration 1.00 nmol L ^{1} at each of the pH values. Each of these solutions was investigated at 8, 24 and 37 C. Those incubated at 37 C were monitored for 6 days; those at 8 and 24 C were monitored for 16 days. All were conducted under identical conditions with the luminescence measurement period fixed at 15 s. The results were depicted graphically and the trend lines were generated using the 'B-spline' option within Origin software.

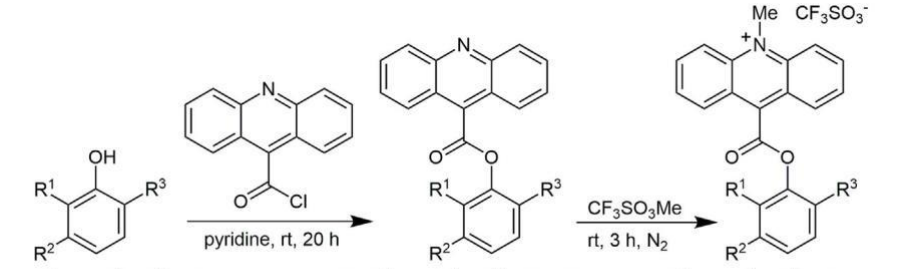
3 | RESULTS AND DISCUSSION

3.1 | Syntheses

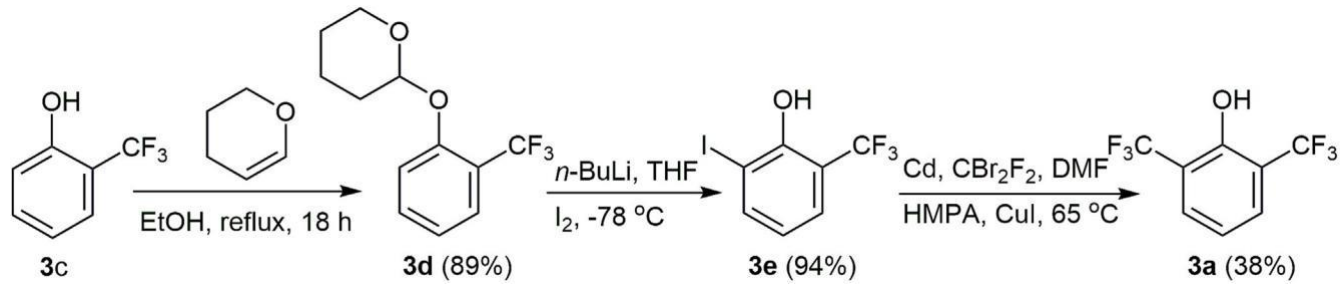
The synthetic routes, illustrated in Schemes 1–3, were similar to ones we had adopted for other acridinium esters in previous research studies.[15–17,24,28–30,32] The syntheses of 9-(substituted phenoxycarbonyl)-10-methylacridinium triflates 2–4 are represented in Scheme 1. The complete routes for syntheses of 2b (R^1 = H, R^2 = R^3 = Me) and 4b (R^1 = R^3 = NO_2, R^2 = H) are outlined in Scheme 1, but additional steps were required for synthesis of the precursor, 2,6-bis(trifluoro-methyl)phenol (3a, Scheme 2), in the case of compound 3b. The syn-thetic route used for the production of a series of 9-(substituted phenoxycarbonyl)-10-(ω-(succinimidyloxycarbonyl)alkyl)acridinium esters 5–9 is shown in Scheme 3. The yields and detailed characterizations of all compounds are recorded in the experimental sections and no further discussion is necessary.

3.2 | Chemiluminescent kinetics

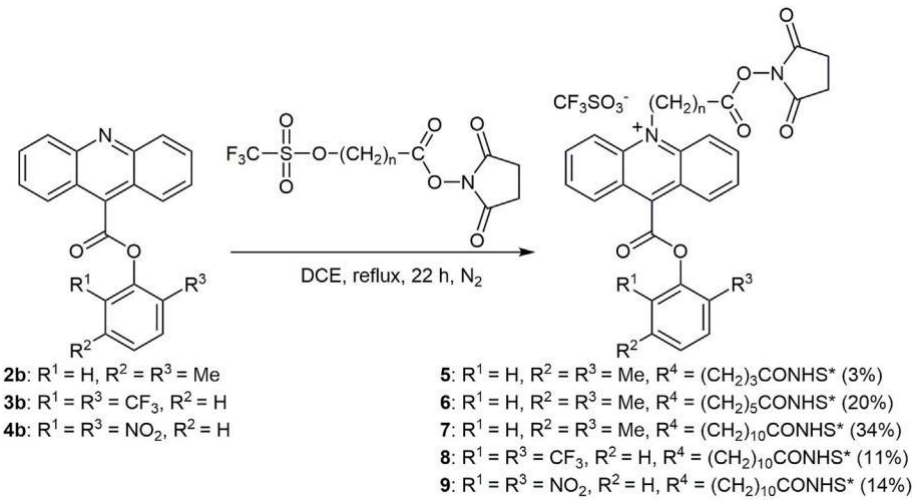
The results of the chemiluminescent kinetics experiments are recorded in Figure 3. From Figure 3, the chemiluminescence of 2,5-dimethyl compounds 2 and 5–7 reached maximum intensity at around 1 s; then the luminescence decayed slowly, so that even at 3 s, the intensities were still around 20–60% of the maximum. For compounds 3, 4 and 8, the chemiluminescence reached maximum



SCHEME 1 Preparation of 10-methyl acridinium esters 2–4.



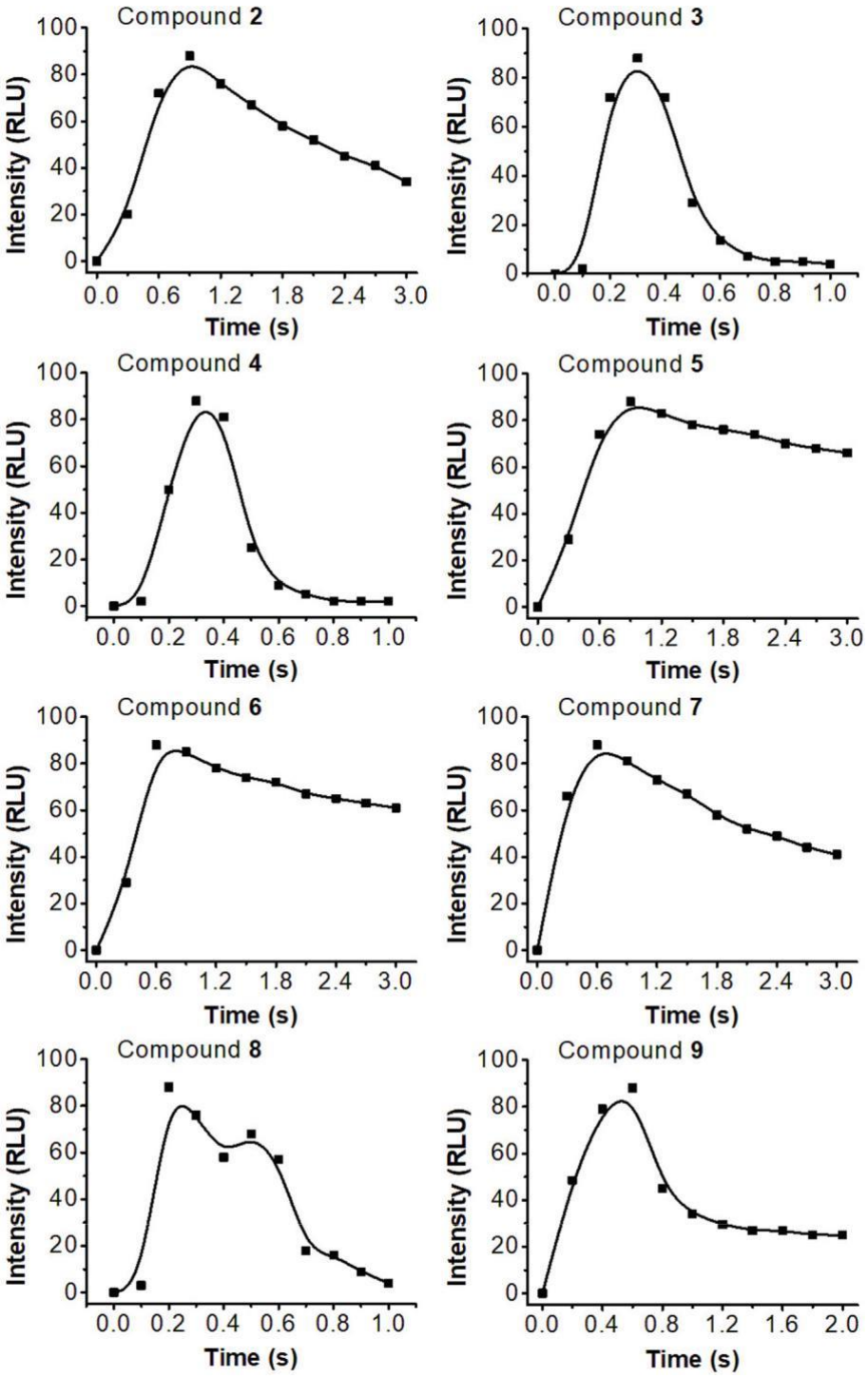
SCHEME 2 Preparation of 2,6-bis(trifluoromethyl)phenol (3a).



SCHEME 3 Preparation of 10-(ω-(succinimidyloxycarbonyl)alkyl)acridinium esters 5–9.

intensity by 0.4 s, and mostly decayed completely within 1 s. Compound 9 showed somewhat slower kinetics than compounds 3, 4 and 8, but far quicker kinetics compared to compounds 2 and 5–7. Com-pounds 3, 4, 8, and 9 (with electron-withdrawing groups in the leaving groups) probably give off light more quickly due to easy expulsion of the leaving group to form a dioxetanone ring, which is a key interme-diate in the chemiluminescent process.

The effect of the substituent attached to nitrogen on the chemiluminescence kinetics was much less marked. Within the series of compounds having a dimethylphenyl ester group, i.e., compounds 2, 5, 6, and 7, the shapes of the kinetic curves were very similar, although the two compounds with the longest alkylene spacer groups (6 and 7) appeared to reach maximum intensity marginally quicker than that with a methyl group (2) or that with a short alkylene spacer (5), while those with the ω-(succinimidyloxycarbonyl)alkyl substituents (5, 6, and 7) appeared to reduce in intensity of the emission marginally more slowly than that with the methyl group (2). Of the two dinitrophenyl esters (4 and 9), the one with the ω-(succinimidyloxycarbonyl)decyl substituent (9) showed a somewhat broader kinetic curve than that of the methyl compound (4) and its emission had not fully decayed after 1 s. Similarly, in the series with a bis(trifluoromethyl)phenyl ester group (3 and 8), the one with the ω-(succinimidyloxycarbonyl)decyl substituent (8) again showed a somewhat broader kinetic curve than that of the methyl compound (4). However, none of the kinetic curves within any series having the same leaving group showed dramatic differences and it would be unwise to try to draw significant conclusions from the minor differences



F I G U R E 3 Chemiluminescent kinetics for acridinium esters 2–9.

seen, particularly when there may be other, unrelated factors contribut-ing to the effects (see discussion in Section 3.3).

In summary, all compounds showed reasonable chemilumines-cence kinetics. The initial goal of the study, to obtain acridinium esters containing various substituents, including NHS-substituted alkyl groups at position 10 and a broader range of phenoxycarbonyl sub-stituents at position 9, leading to a broader range of chemilumines-cent kinetics, has therefore been accomplished.

3.3 | Chemiluminescent efficiency and hydrolytic stability of the acridinium esters

The results of the studies of the abilities of the various acridinium esters to luminesce after storage in various buffers are recorded in Figures 4 and 5. It is important when comparing the various diagrams to notice that the horizontal scale is different for the experiments conducted at 37 C (column C) from that used for the experiments

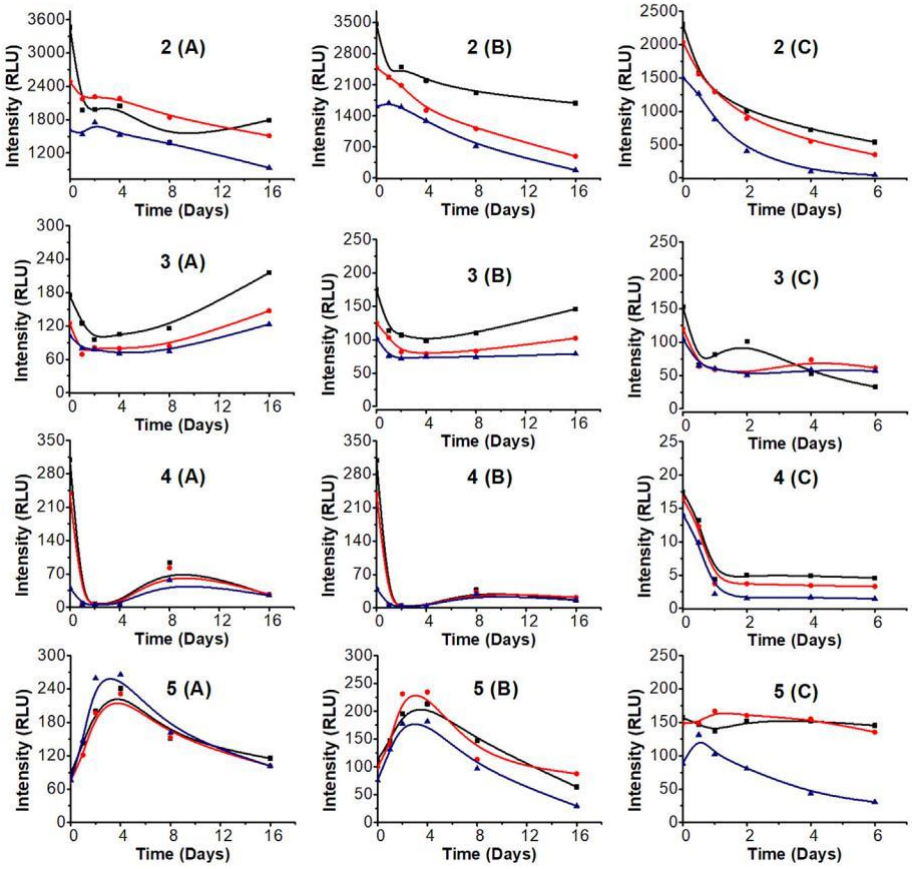


FIGURE 4 Efficiency of luminescence and hydrolytic stability for acridinium esters 2–5 incubated at: 8 C (A); 24 C (B); and 37 C (C). The blue, red, and black colours represent incubations in phosphate buffers in mildly basic (pH 8.0), neutral (pH 7.0), and mildly acidic (pH 6.0) conditions, respectively.

conducted at 8 C (column A) or 24 C (column B), and that the vertical scales differ substantially across the diagrams.

In general, as expected, the amount of light emitted by a particu-lar acridinium ester after storage at higher temperatures reduced more quickly than after storage of the acridinium ester at lower tem-peratures. However, there were exceptions, such as with compound 9, which showed greater emission after storage at 37 C than after storage at lower temperatures. Furthermore, the shapes of the curves were often different for a particular compound stored at different temperatures. These phenomena are not easy to rationalize by refer-ence to a single parameter such as the rate of hydrolysis of the acridi-nium ester under different conditions.

The intrinsic chemiluminescent efficiency of an acridinium ester is indicated by its chemiluminescent intensity immediately after prepara-tion of its solution, prior to storage. By comparison of the chemilumi-nescent intensities of acridinium esters under acidic conditions at 4 C (as a typical set of standard conditions), it can be seen that compound 2 luminesced most efficiently, emitting around 3500 RLU under the specified conditions, while compound 9 luminesced most weakly, emitting only around 2 RLU. By comparing the chemiluminescent effi-ciencies of compounds 2 and 5–7, all of which have a 9-(2,5-dimethylphenoxycarbonyl) group but have different substitu-ents at position 10, it can be seen that the nature of the substituent at the 10-position is a significant factor affecting chemiluminescent efficiency. Compounds 5–7 all showed much lower chemiluminescent efficiency than 2, suggesting that larger groups or groups containing an NHS ester unit at position 10 in some way inhibit the chemilumi-nescent process. The fact that compounds 5 and 6 show similar levels of luminescence, while 7, with a longer alkyl chain and a more remote NHS group, shows a significantly lower level of chemiluminescence, at least when the pH is 6 or 7, suggest that it is the physical size of the group, rather than the presence of an NHS ester substituent, that has the major influence. We have observed related results in other series of acridinium esters.[23] This trend may reflect the effectiveness of the dispersion of the compounds in the aqueous buffer solutions. The bet-ter leaving group properties of the bis(trifluoromethyl)phenoxy group present in compounds 3 and 8, and of the dinitrophenoxy group pre-sent in compounds 4 and 9, may be responsible for some loss of lumi-nescence before the initial reading can be recorded. However, it is also possible that the much lower initial levels of emission, even for compounds 3 and 4, which like compound 2 have a simple methyl group at position 10, is primarily caused by low solubility and poor dis-persion of these compounds in the aqueous media as a result of the increased bulk of the leaving groups compared to that of 2.

In terms of changes in the level of emission over time, the stabili-ties of compounds 2, 3, and 4 were in the order: 2,5-dimethylphenyl ester > 2,6-bis(trifluoromethyl)phenyl ester > 2,6-dinitrophenyl ester. As expected, electron-donating phenoxy substituents confer greater stability than electron-withdrawing groups. Indeed, the very rapid ini-tial decay of the luminescence of 4 indicates just how easily the com-pound undergoes decomposition in the aqueous medium. However, issues associated with the effectiveness of dispersion of the

FIGURE 5 Efficiency of luminescence and hydrolytic stability for acridinium esters 6–9 incubated at 8 C (A); 24 C (B); and 37 C (C). The blue, red, and black colours represent incubations in phosphate buffers in mildly basic (pH 8.0), neutral (pH 7.0), and mildly acidic (pH 6.0) conditions, respectively.

compounds may provide insight into some of the odd subsequent shapes of the curves for the compounds showing low efficiencies of chemiluminescence. Improved dispersion (leading to more efficient emission) over time may counterbalance loss of compound through decomposition, leading either to growth in level of chemilumines-cence prior to longer term decay (e.g., with compound 5 at 8 or 24 C), to retention of luminescence being better at 37 C than at 8 or 24 C (e.g., with compound 9), or indeed to growth in chemiluminescence with longer storage time if the dispersion rate improves over time as aggregates become smaller and therefore present in greater numbers. The latter phenomenon is particularly pronounced with compounds 3 and 8, which contain a bis(trifluoromethyl)phenoxy leaving group, especially at 8 C. This group could itself cause difficulties for disper-sion in an aqueous medium, even when the group at the 10-position is a simple methyl group (compound 3).

The low overall levels of emission from all of the compounds studied, with the exception of compound 2, are probably due to the poor solubility and poor dispersion of the compounds in the aqueous media. This implies that the presence of detergents will be important if such compounds are to be used as labels for biological molecules. The conditions of incubation used in these studies do not reflect the storage stability of the compounds, which can be stored successfully for prolonged periods as dry solids in the absence of solvent and pro-tected from light and moisture, or even stored in dry organic solvents for shorter periods, particularly at low temperature. However, the results show that it will be important to keep the period of exposure to aqueous solution to a minimum, so only the latest dilutions should be conducted with aqueous buffer when preparing solutions for use.

4 | CONCLUSIONS

Several novel acridinium esters, with various substituents at position 10 and variously substituted phenoxy leaving groups have been

synthesized successfully. The kinetics of the chemiluminescent reac-tions of the compounds and their hydrolytic stabilities along with chemiluminescent efficiencies have been investigated. Unsurprisingly, acridinium esters with phenoxy groups having electron-withdrawing substituents emit light more rapidly than those with phenoxy groups having electron-donating substituents. Compound 2, having a 2,5-dimethylphenoxycarbonyl group at position 9 and a methyl group at position 10, was the most efficient light emitter of the ones that were tested. Both the character of the leaving group and the 10-substituent affected the stability, with better stability resulting for acridinium esters with smaller N-substituents and poorer leaving groups. The level of luminescent emission, and also the change in level of emission over time of storage in aqueous buffer, may be affected by the extent of dispersion of these low-solubility compounds in such media.

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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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