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Synthesis of polyhalo acridones as pH-sensitive fluorescence probes

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ABSTRACT

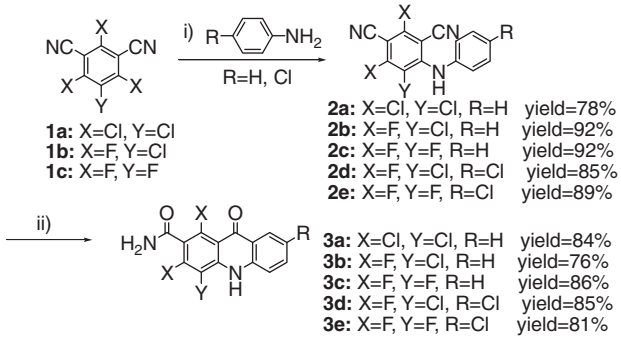
Polyhalo isophthalonitriles were reacted with substituted anilines and subsequently cyclocondensed in the presence of sulfuric acid to give polyhalo acridones. These polyhalo acridones were proven to be useful as pH-sensitive fluorescent probes for a wide range of acidic and basic conditions.

Fluorescent probes that are pH-sensitive have been widely used in analytical chemistry, bioanalytical chemistry, cellular biology (particularly for measuring intracellular pH) and medicine,[1] presumably due to their preferential properties, including nondestructive character, high sensitivity and specificity, and the availability of a wide range of indicator dyes.[2] To date, a variety of pH-sensitive probes have been reported,[3] most of which can be used in either acidic or basic conditions.[4] In fact, new probes with specific fluorescence over a broad pH range under both acidic and basic conditions are continuously being developed.

Acridone derivatives have received much attention over recent years because of their perfect fluorescent properties, such as high quantum yields and excellent photostability,[5] and their potential pharmaceutical applications, possibly being used as antitumor agents,[6] antivirals,[7] antimalarials,[8] and antibacterials, etc.[9] Acridone derivatives can be used as fluorescent probes based on their unique interactions with biomolecules such as DNA and enzymes. Many documents[5a–c,10] regarding the ionization of acridones including their ammonium salts and neutral molecules in a low pH medium are widely available. To date, the ionization of acridones in a high pH medium has not been reported. This is probably because the higher pK_a value of acridones limits their ionic dissociation. Therefore, it is crucial to improve the acidity of acridone derivatives in order to develop pH fluorescent probes that may be applied to a broad pH range. In this context, the acidity of acridone derivatives **3a–e** (Scheme 1) could be enhanced by introducing an acyl group at site 6 of the acridone enatic structure and a halogen atom at the C-1 position. In this manner, it is envisioned that compounds **3** could act as potential acid–base dual-purpose pH fluorescent probes.

Although acridones with 1 or 2 substituents have been synthesized,[7a,b,9d] access to highly functionalized acridones is still limited, probably because introducing multiple substituents to the acridone ring often requires multistep reactions and complicated preparation procedures. Therefore, the development of efficient and concise approaches for producing acridones that tolerate a wide variety of functional groups is desirable.

Polyhalo isophthalonitriles, especially polyfluoro isophthalonitriles, have been widely used as reagents/intermediates in organic synthesis.[11] Halogens and amido groups can be readily derived and thus provide opportunities for constructing molecule libraries for screening biological activity. This communication reports the



Scheme 1. Synthesis of polyhalo acridones. Reagents and conditions: (i) DMF, K_2CO_3, rt; (ii) H_2SO_4, 90 °C.

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synthesis of acid–base dual-purpose pH fluorescent probes with specific fluorescence based on a new class of polyhalo acridones.

The synthetic pathway is depicted in Scheme 1. Polyhalo isophthalonitrile **1** was reacted with the substituted aniline in combination with potassium carbonate in *N*,*N*-dimethylformamide at room temperature to produce compound **2**, which easily run intra-cyclization and sequential hydrolysis in the presence of sulfuric acid at 90 °C for 1 h to give the target product polyhalo acridone **3** with an excellent yield. All compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and HRMS.[12,13]

Then, the polyhalo acridones **3a–e** were analyzed by UV spectroscopy at a fixed concentration and at various phosphate buffer pH values. The UV spectrum of the polyhalo acridone was found to be dependent on pH. The UV absorption of compounds **3a–e** were red-shift and strengthened with increasing of pH values in acid environments. While the UV absorption were blue-shift and weakened with increasing of pH values in basic conditions (Fig. 1A and B). At neutral condition, the maximum absorption peaks of compounds **3a–e** were 277, 266, 264, 267 and 266 nm, respectively (see the Supplementary data data). The absorption spectra of **3a** and **3b** are shown in Figure 1A and B.

Next, the pH-dependent fluorescence response of compounds **3** in different buffer solutions was investigated. At pH <4.6, the excitation and emission maxima of protonated **3aH$^+$** were found to be 277 and 456 nm, respectively. The emission wavelength of **3a** was 480 nm. The fluorescence quantum yield was tested according to

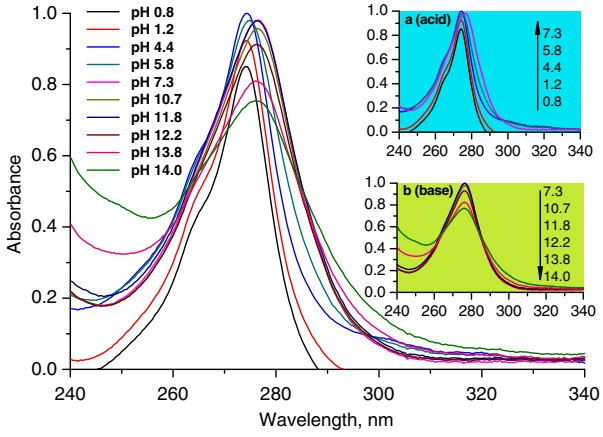


Figure 1A. UV spectrum of **3a** under different pH values (ethanol/buffers) 1:4. (a) UV spectrum of **3a** in acidic conditions. (b) UV spectrum of **3a** in basic conditions.

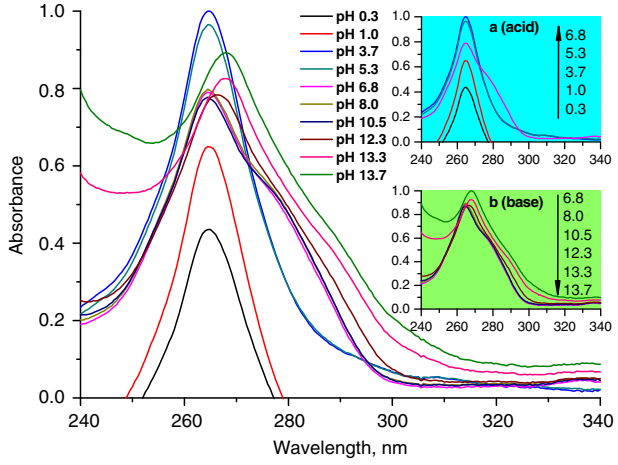


Figure 1B. UV spectrum of **3b** under different pH values (ethanol/buffers = 1:4). (a) UV spectrum of **3b** in acidic conditions. (b) UV spectrum of **3b** in basic conditions.

the published method[14] and was found to be 0.73 (Fig. 2). Compounds **3a–e** had different fluorescence at different pH values in a solvent of H_2O/DMSO = 1:1 (10^{-5} M) (Fig. 3).

The emission maxima of **3aH$^+$** was lower than that of the neutral **3a**, presumably due to the electronic effects of the protonated amino group in **3aH$^+$**. Under acidic conditions, **3a** was reversibly converted to **3aH$^+$**, as shown in the ionization equilibrium between **3a** and **3aH$^+$** (Scheme 2).

The fluorescent properties of compounds **3a–e** can be seen in Figure 4A–E. Equimolar solutions of the compounds were made up in phosphate buffers over a broad pH range of ca. 0.5–14.6. The relative fluorescent intensities of the probes were greatly reduced as the buffers became more basic. Accordingly, the fluorescence intensity of **3a** was quickly reduced at 4.6 < pH < 7.0, whereas there is a little change in the range of pH >7.0, indicating that the optimum pH value for **3a** as a fluorescence indicator would be between pH 4.6 and 7.0. The fluorescence intensity of **3a** was almost unchanged within 0.8 < pH < 5.6. The emission peak position was only affected by pH (Fig. 4A). These results suggest that there were two kinds of molecular morphologies present as luminescence species: the neutral molecule **3a** and the acidic salt **3aH$^+$**.

All of the polyhalo-substituted derivatives **3a–e**, compound **3b** gave the highest fluorescence quantum yield (up to 0.98). The order of the maximum excitation wavelength was **3a** > **3d** > **3e** = **3b** > **3c**. The emission maxima of protonated **3aH$^+$**, **3bH$^+$**, **3cH$^+$**, **3dH$^+$**, and **3eH$^+$** were 456 nm, 463 nm, 462 nm, 450 nm and 470 nm, respectively. The emission maxima of the neutral molecules **3a–e** were 480 nm, 490 nm, 487 nm, 496 nm and 492 nm, respectively (Figs. 2 and 4A–E). Accordingly, the emission maxima of protonated **3aH$^+$–3eH$^+$** were lower than those of neutral **3a–e** because of the strong electron-withdrawing properties of the protonated nitrogen of the amino group in **3aH$^+$–3eH$^+$**. Similarly, compounds **3b–e** and **3bH$^+$–3eH$^+$** reached ionization equilibrium under acidic conditions as depicted in Scheme 2.

Figure 5A and B illustrates the pH responses of probes **3a–e** as a function of I/I_{max} versus pH, where I is the measured fluorescent emission and I_{max} is the maximum output of the probe. Figure 5A indicates that the most suitable condition for **3b** as a fluorescence indictor was 2.9 < pH < 8.0, while the working pH of **3c–e** was lower than that of **3b**. Unlike **3a**, compounds **3b–e** reached ionization equilibrium under basic conditions. For example, the peak profile of **3b** changed at pH >6.8, becoming higher toward the right than the left with increasing pH, thus showing the ionization equilibrium of **3b** and **3b$^-$** (Scheme 2). At pH >10.5, the fluorescence intensity rapidly decreased with increasing pH, indicating that **3b** is a suitable fluorescence indicator for 10.5 < pH < 14.1. (Fig. 5A). In short, **3a** can only be used as a pH fluorescence probe in acidic conditions, while **3b–e** can be applied to either acidic or basic environ-

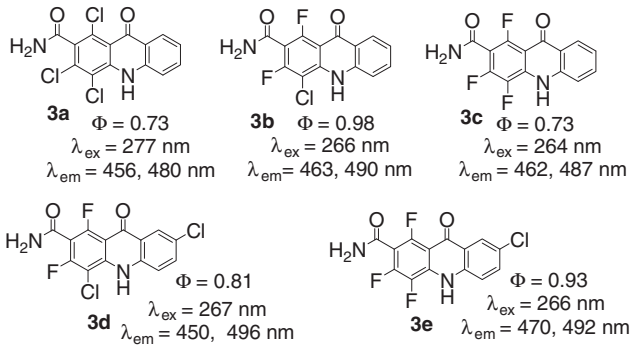


Figure 2. Polyhalo acridones and their fluorescence quantum yield (Φ), and excitation and emission maxima in H_2O/DMSO = 1:1 (10^{-5} M).

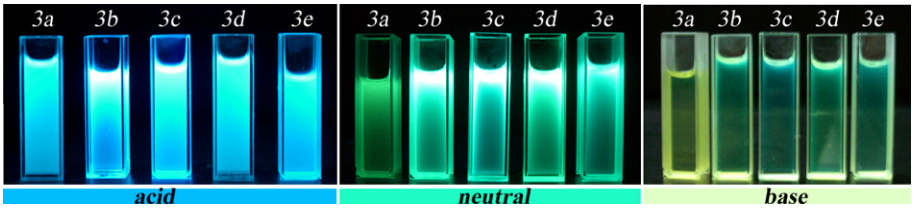
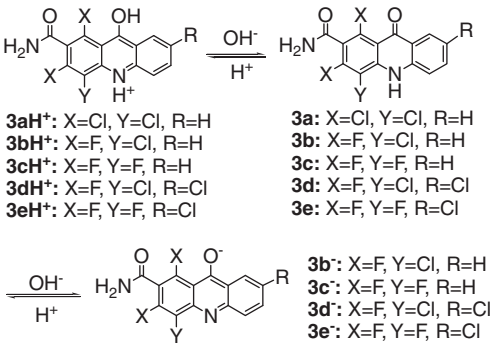


Figure 3. Photo of fluorescence of **3a**–**3e** (from left to right) taken under a hand-held UV (365 nm) lamp.



Scheme 2. Ionization equilibrium of **3** and **3H**$^+$.

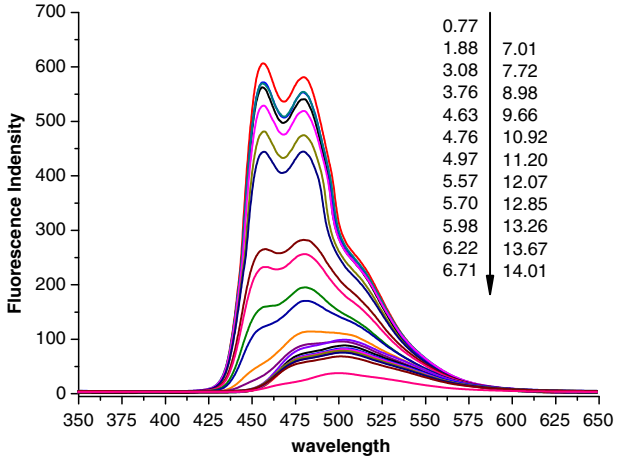


Figure 4A. Fluorescence characteristics of **3a** under different pH values (ethanol/buffers = 1:4).

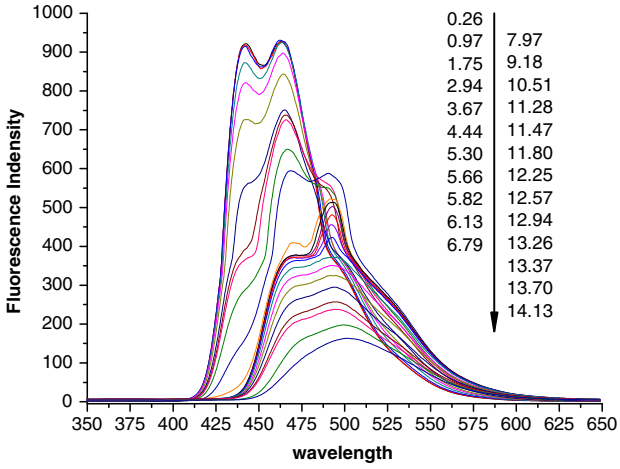


Figure 4B. Fluorescence characteristics of **3b** under different pH values (ethanol/buffers = 1:4).

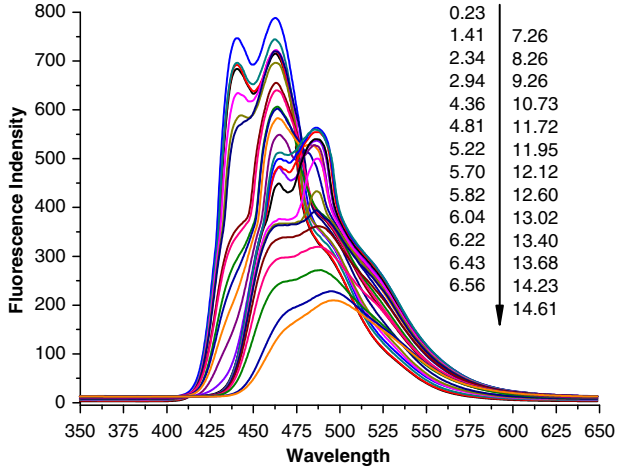


Figure 4C. Fluorescence characteristics of **3c** under different pH values (ethanol/buffers = 1:4).

ments (Fig. 5B). As such, compounds **3b**–**e** have high potential for application as pH sensors for acidic and basic conditions compared with the current commercially available pH-sensitive fluorescent probes. The presence of fluorine atom is crucial to the fluorescence properties of acridones, as the electron-withdrawing effects of the fluorine atom. However, compound **3a** showed different fluorescence characteristics and only can use as a base to accept proton to produce **3aH**$^+$ in acidic environments. The presence of fluorine atom would be crucial to the fluorescence properties of acridones, as the electron-withdrawing effects of the fluorine atom could account for higher acidic properties in fluorine-containing acridones **3b**–**e**. Therefore, **3b**–**e** could easily release proton and thus form **3b**$^-$**–3e**$^-$ under basic conditions. However, compound **3a** showed different fluorescence characteristics, and only can be used as a base to accept hydrogen proton to produce **3aH**$^+$ in acidic environments.

In conclusion, a novel type of pH fluorescent probe was prepared and proven to have excellent fluorescent properties, such as broad pH application range, intense fluorescence and high fluorescence quantum yield. The preliminary results for biological activity using the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay[15] showed that some of the title compounds **3a**–**e** possessed moderate anti-cancer activities against the K562, HL60, A431, HepG2 and Skov-3 cell lines (see Supplementary data). Consequently, this simple process pre-

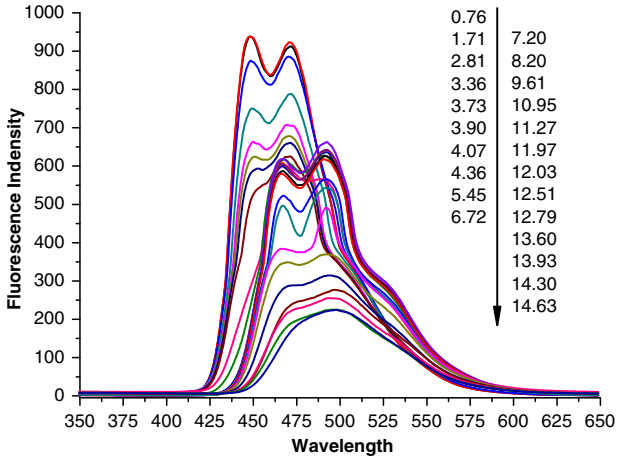


Figure 4D. Fluorescence characteristics of **3d** under different pH values (ethanol/buffers = 1:4).

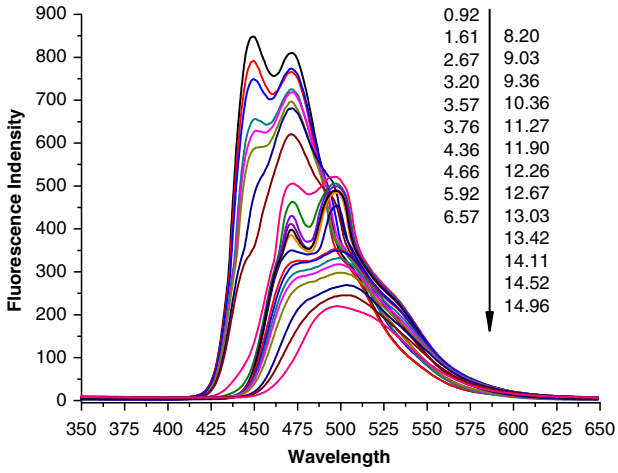


Figure 4E. Fluorescence characteristics of **3e** under different pH values (ethanol/buffers = 1:4).

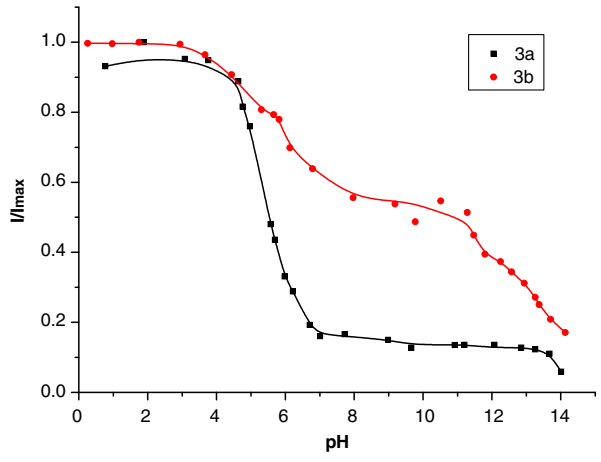


Figure 5A. pH dependence of **3a** and **3b** (I/I_{max}) at 295 K in phosphate buffers of differing pH values (**3a**, λ_{exc} = 277 nm; **3b**, λ_{exc} = 266 nm).

sented herein has great potential for parallel synthesis in drug discovery.

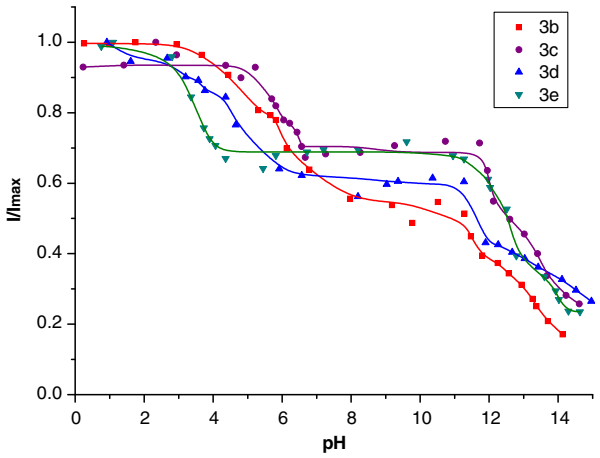


Figure 5B. pH dependence of **3b**, **3c**, **3d** and **3e** (I/I_{max}) at 295 K in phosphate buffers of differing pH values (**3b**, λ_{exc} = 266 nm; **3c**, λ_{exc} = 264 nm; **3d**, λ_{exc} = 267 nm; **3d**, λ_{exc} = 266 nm).

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.101.

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12. *General procedure for the synthesis of polyhalo isophthalonitrile* **2**: A 50 mL round-bottomed flask was charged with polyhalo isophthalonitrile **1** (5 mmol), DMF (30 mL), aniline derivatives (6.0 mmol), and potassium carbonate 1.4 g (10 mmol). The solution was stirred for 0.5–18 h at room temperature until the substrate **1** was completely consumed. The mixture was put into a beaker (100 mL) and quenched by the addition of water (30 mL). The reaction mixture was stirred for another 15 min, and then filtered off. The residue was washed with water to give a crude product, which was further recrystallized by ethyl acetate to form the final products **2**.
13. *General procedure for the synthesis of polyhalo acridone* **3**: Polyhalo isophthalonitrile **2** (2 mmol) was suspended in 6 mL of 95–98% sulfuric acid. The reaction mixture was stirred at 90 °C for 1 h. After the reaction, the mixture was cooled to room temperature, put into a beaker (100 mL) with 50 mL iced water, and then neutralized with solid Na_2CO_3 to a pH of 9–10. The solid was filtered off and washed with water to give a crude product that was purified by flash column chromatography, to afford polyhalo acridone **3** in 76–86% yield. *1,3,4-Trichloro-9-oxo-9,10-dihydroacridine-2-carbox-amide* **3a**: yellow solid, mp: 251–252 °C. IR (KBr): 3478, 3416, 1665, 1568, 1398, 1326, 1164, 755, 612 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 8.48–8.18 (m, 3H, PhH), 7.95–7.80 (m, 3H, NH_2, PhH), 7.48 (br, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.7, 152.5, 148.5, 145.5, 132.2, 131.9, 130.7, 130.0, 129.2, 125.4, 124.4, 123.4, 115.0, 108.4. HRMS (TOF ES$^+$): *m/z* calcd for $C_{14}H_8Cl_3N_2O_2$ [(M+H)$^+$], 340.9646; found, 340.9644. *4-Chloro-1,3-difluoro-9-oxo-9,10-dihydroacrid- ine-2-carboxamide* **3b**: yellow solid, mp: >300 °C. IR (KBr): 3486, 3371, 3246, 1679, 1559, 1382, 1260, 834, 759, 601 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 8.54–7.78 (m, 6H, PhH, NH_2), 7.46–7.43 (br, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.3, 156.2 (d, J = 267.5 Hz), 154.8 (d, J = 258.8 Hz), 151.8, 149.5, 145.2, 132.5, 129.1, 123.8, 123.4, 114.1, 111.6, 109.1 (t, J = 27.5 Hz), 101.2 (d, J = 32.5 Hz). ^{19}F NMR (470 MHz, DMSO-d_6): δ −111.3 (d, J = 4.7 Hz, 1F), −111.7 (d, J = 4.7 Hz, 1F). HRMS (TOF ES$^+$): *m/z* calcd for $C_{14}H_8ClF_2N_2O_2$ [(M+H)$^+$], 309.0237; found, 309.0233. *1,3,4-Trifluoro-9-oxo-9,10-dihydroacridine-2-carboxamide* **3c**: yellow solid, mp: >300 °C. IR (KBr): 3526, 3421, 3151, 1685, 1557, 1388, 1265, 967, 758, 602 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 8.53–7.78 (m, 6H, PhH, NH_2), 7.44 (br, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.0, 152.6 (d, J = 261.3 Hz), 151.2, 149.4, 145.3 (d, J = 252.5 Hz), 140.5 (d, J = 248.8 Hz), 140.4, 132.4, 129.1, 123.7, 123.5, 114.1, 108.4 (t, J = 25.0 Hz), 101.2. HRMS (TOF ES$^+$): *m/z* calcd for $C_{14}H_8F_3N_2O_2$ [(M+H)$^+$], 293.0532; found, 293.0529. *4,7-Dichloro-1,3- difluoro-9-oxo-9,10-dihydro-acridine-2-carboxamide* **3d**: yellow solid, mp: >300 °C. IR (KBr): 3433, 3353, 3245, 1650, 1554, 1376, 1251, 1102, 834, 630 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 8.67 (br, 1H, NH), 8.28–7.74 (m, 6H, PhH, NH_2). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.0, 156.0 (d, J = 255.0 Hz), 155.0 (d, J = 247.5 Hz), 151.2, 147.9, 145.4, 132.9, 131.3, 128.2, 122.4, 114.6, 111.8, 109.9 (t, J = 26.3 Hz), 101.2. HRMS (TOF ES$^+$): *m/z* calcd for $C_{14}H_7Cl_2F_2N_2O_2$ [(M+H)$^+$], 342.9847; found, 342.9845. *7-Chloro-1,3,4-trifluoro-9,10-dihydro-9-oxoacridine-2-carboxamide* **3e**: yellow solid, mp: 181–185 °C. ^{19}F NMR (467 MHz, DMSO-d_6): δ −115.4 (d, J = 14.1 Hz, 1F), −139.3 (s, 1F), −155.9 (d, J = 14.1 Hz, 1F). HRMS (TOF ES$^+$): *m/z* calcd for $C_{14}H_7ClF_3N_2O_2$ [(M+H)$^+$], 327.0143; found, 327.0140.

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