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# SYNTHESIS AND PHOTOPHYSICS
# OF ACRIDINE DERIVATIVES

Highly fluorescent acridine derivatives were prepared by a multistep synthesis starting from 2-chlorobenzoic acid and appropriate (aminophenyl)alkanoic acid by means of a modified Ullmann–Jourdan reaction followed by cyclodehydration step, and by amination in the case of aminoacridine analogues. The obtained derivatives were subjected to photophysical studies (absorption and fluorescence). The compounds displayed interesting absorption behavior and high quantum yield of fluorescence. The acridine analogues bearing free carboxylic group can serve as effective fluorescent probes in conformation analysis of peptides.

Keywords: acridone, 9-aminoacridine, fluorescence, photophysics.

## INTRODUCTION

Acridine and its derivatives belong to heterocyclic analogues of antracene, containing nitrogen instead of carbon atom central ring. Derivatives of acridine due to their specific construction (large, planar structure of tricycle, acceptor/donor properties of nitrogen atom) can interact with nucleic acids (RNA and DNA) which means that they can be potentially used as a base for anticancer and anti-inflammatory drug design [1–5]. Additionally, acridine and some of its derivatives have interesting photophysical properties, i.e. high fluorescence quantum yield, and high molar absorption coefficients [6–9]. Moreover, for some of acridine derivatives shift of absorption spectra towards longer wavelengths ("red shift") is observed which allows one to extend range of excitation wavelengths and as such to avoid interference with other chromophores. Such photophysical profile of the compounds allows one to apply them as effective chromophores, especially as energy donors, in conformational studies of biopolymers (peptides, nucleic acids) by means of estimation of energy transfer between donor and acceptor that leads to estimation of interchromophoric distance [10], and in studies of enzymes (kinetics and mechanism of action and inhibition) [11].

There is a need in our laboratory for new, effective, and easy to make chromophores possible to be applied in conformational and enzymatic studies. Therefore we decided to evaluate acridine derivatives as potential chromophores. To perform our studies we prepared from substituted anthranilic acids **1a-d** acridine derivatives bearing carboxyl group, which allowed us to use them as acylating agent for peptide amino groups (compounds **2a-d,** Scheme 1). Starting from appropriate acridones compounds **3a-d** containing amino group were synthesized and used as amino components to be acylated by carboxy groups of peptides (Scheme 2).

## RESULTS AND DISCUSSION

Syntheses of acridine derivatives, both acridone analogues such as 9,10-dihydro-9-oxoacridine-2-carboxylic acid (**2a**), -2-acetic acid (**2b**), 3-(9,10-dihydro-9-oxoacridine-2-yl)propionic acid (**2c**), 9,10-dihydro-9-oxoacridine-4-carboxylic acid (**2d**) and aminoacridine analogues, *viz.* 9-amino-2-carboamoylacridine hydrochloride (**3a**), 9-amino-2-(carbamoylmethyl)acridine hydrochloride (**3b**), (2-carboxyamide)ethyl-9-amino-2-(2-carbamoylethyl)-acridine hydrochloride (**3c**), 4-carboxyamide-9-amino-4-carbamoylacridine hydrochloride (**3d**) were performed as they are outlined at Schemes 1 and 2.

Scheme 1



**1a**  X = OH, n = 0
**1b**  X = OH, n = 1
**1c**  X = NH₂, n = 2

**1d**  n = 0

**2a**  n = 0
**2b**  n = 1
**2c**  n = 2

**2d**
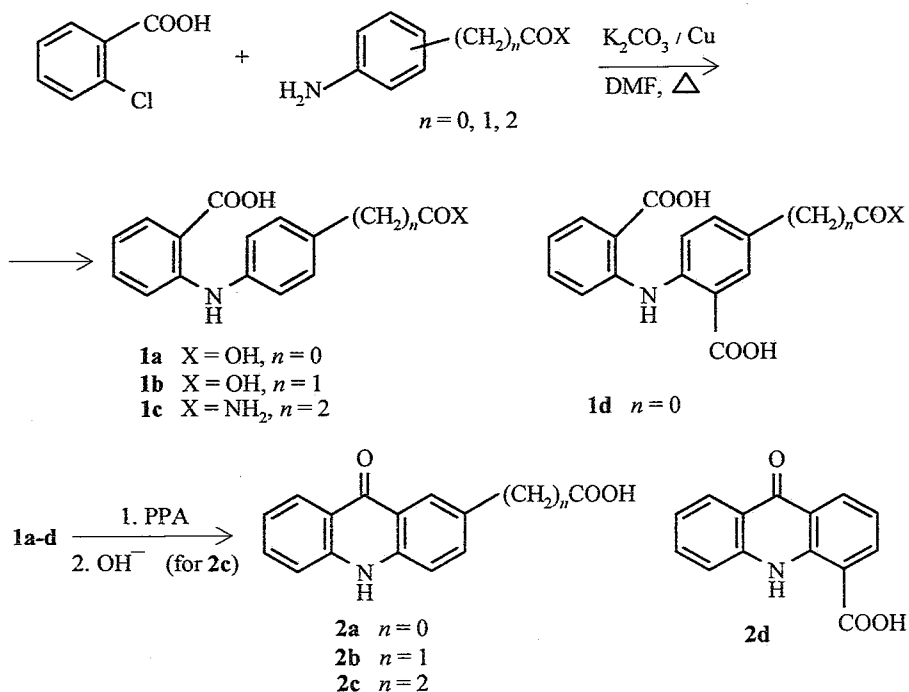
General procedures for preparation of the compounds are included in Experimental part, whereas the parameters of the syntheses and final products are presented in Tables 1–3. Starting compounds for the acridine derivatives (substituted anthranilic acids **1a-d**, Scheme 1) were prepared by a modified Ullmann–Jourdan reaction [12]. Cyclodehydration process was accomplished by heating with polyphosphoric acid. Synthesis of aminoacridine derivatives from appropriate acridones was achieved by reaction with thionyl chloride followed by action of phenol and ammonia. Such obtained free bases were finally converted into hydrochloride salts by treatment with hydrogen chloride. The yields are rather moderate mostly due to the fact that they are multistep processes and some of the steps required many recrystalizations and/or column chromatography. Our attempt to hydrolyze amide moiety in aminoacridines **3a-d** failed because during that process also 9-amino group was hydrolyzed.

Characteristics of acridone derivatives 2a–d

| Compound (formula) | Yield[a], % | M.p., °C (ref. m.p.) | MS $(M+1)^+$ | IR data, $cm^{-1}$ | $^1H$ NMR data, ppm (in $CD_3OD$) |
|---|---|---|---|---|---|
| 2a $C_{14}H_9NO_3$ | 48.4 | >340 dec. (>360 [1]) | 240 | 3262 (NH), 1678 (COOH), 1632 (CO) | 7.35–8.42 (m, 7H, arom.) |
| 2b $C_{15}H_{11}NO_3$ | 45.6 | 303–306 dec. (299–301 [1]) | 254 | 3296 (NH), 1697 (COOH), 1637 (CO) | 3.77 (s, 2H, $CH_2$); 7.32–8.45 (m, 7H, arom.) |
| 2c $C_{16}H_{13}NO_3$ | 45.9 | 301–304 dec. | 268 | 3274 (NH), 1687 (COOH), 1630 (CO) | 2.61 (t, 2H, $\underline{CH_2}CH_2CO$, J=7.8 Hz); 3.05 (t, 2H, $CH_2\underline{CH_2}CO$, J=7.8 Hz); 7.28-8.34 (m, 7H, arom.) |
| 2d $C_{14}H_9NO_3$ | 92.7 | 330–334 dec. (324–325 [14]) | 240 | 3226 (NH), 1694 (COOH), 1620 (CO) | 7.43–8.48 (m, 7H, arom.) |

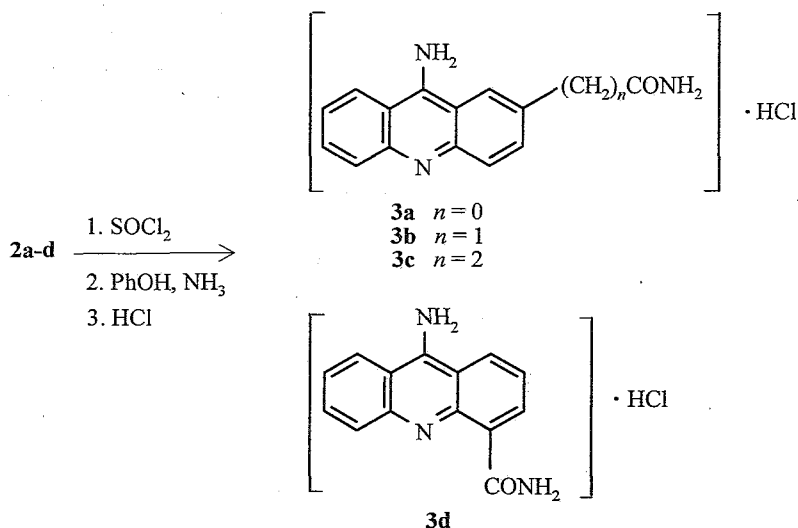[a] From 4(or 2)-(2-carboxyphenylamino)benzenealkanoic acid 1.

Characteristics of 9-aminoacridine derivatives 3a–d

| Compound (formula) | Yield[a], % | M.p., °C | MS $(M+1)^+$ | IR data, $cm^{-1}$ | $^1H$ NMR data, ppm (in $CD_3OD$) |
|---|---|---|---|---|---|
| 3a $C_{14}H_{11}ClN_3O$ | 24.6 | >340 dec. | 238 | 3384 (NH), 3106 (NH), 1669 (amide 1, CO), 1634 (amide 2, NH) | 7.55–8.52 (m, 7H, arom.) |
| 3b $C_{15}H_{13}ClN_3O$ | 45.2 | >340 part. dec. | 252 | 3365 (NH), 3096 (NH), 1669 (amide 1, CO), 1634 (amide 2, NH) | 3.92 (s, 2H, $CH_2$) 7.62–8.64 (m, 7H, arom.) |
| 3c $C_{16}H_{15}ClN_3O$ | 43.,9 | >240 dec. | 266 | 3380 (NH), 3135 (NH), 1661(amide 1, CO), 1638 (amide 2, NH) | 2.92 (t, 2H, $\underline{CH_2}CH_2CO$, J=7.9 Hz), 3.21 (t, 2H, $CH_2\underline{CH_2}CO$, J=7.9 Hz), 7.65–8.55 (m, 7H, arom.) |
| 3d $C_{14}H_{11}ClN_3O$ | 27.2 | 224-227 part.dec. | 238 | 3360 (NH), 3124 (NH), 1675 (amide 1, CO), 1626 (amide 2, NH) | 7.63–8.65 (m, 7H, arom.) |

[a] From compounds 2a–d.

Scheme 2



**3a** $n = 0$
**3b** $n = 1$
**3c** $n = 2$

2a-d  
1. SOCl$_2$  
→  
2. PhOH, NH$_3$  
3. HCl

**3d**

Photophysical parameters of the compounds are presented in Table 3 and in Figs. 1 and 2 (absorption) and Figs. 3 and 4 (fluorescence). As it was expected, in a case of absorption, carboxy and carbamoyl substituents at position 2 of the acridine system caused small batochromic shift. An influence of alkyl spacer was observed only for acridone analogues but there was no significant difference between methylene and ethylene groups. On the other hand the substituents at position 4 resulted in stronger batochromic shift (Figs. 1, 2).

Table 3

**Photophysical data of acridone and 9-aminoacridine derivatives**

| Compound (concentration in MeOH) | Absorption | | Fluorescence | |
|---|---|---|---|---|
| | $\lambda_{max}^{a}$ [nm] | log ε | $\lambda_{max}^{a}$ [nm] | QY |
| 2-(COOH)Acridone **2a** ($c = 2.926 \times 10^{-5}$ M) | 317 379 396 | 3.67 3.61 3.63 | 413 435 | 0.925 |
| 2-(CH$_2$COOH)Acridone **2b** ($c = 4.028 \times 10^{-5}$ M) | 383 401 | 3.86 3.87 | 419 441 | 0.714 |
| 2-(CH$_2$CH$_2$COOH)Acridone **2c** ($c = 3.292 \times 10^{-5}$ M) | 384 402 | 3.85 3.86 | 419 441 | 0.702 |
| 4-(COOH)Acridone **2d** ($c = 4.013 \times 10^{-5}$ M) | 391 408 | 3.97 4.06 | 425 449 505 | 0.268 |
| 2-(CONH$_2$)-9-Aminoacridine·HCl **3a** ($c = 3.142 \times 10^{-5}$ M) | 383 402 424 | 3.66 3.86 3.81 | 433 459 485 | 0.732 |
| 2-(CH$_2$CONH$_2$)-9-Aminoacridine·HCl **3b** ($c = 3.267 \times 10^{-5}$ M) | 384 403 424 | 3.65 3.84 3.82 | 435 460 487 | 0.765 |
| 2-(CH$_2$CH$_2$CONH$_2$)-9-Aminoacridine·HCl **3c** ($c = 2.982 \times 10^{-5}$ M) | 384 403 425 | 3.73 3.89 3.80 | 435 460 487 | 0.698 |
| 4-(CONH$_2$)-9-Aminoacridine·HCl **3d** ($c = 3.215 \times 10^{-5}$ M) | 392 410 432 | 3.57 3.79 3.77 | 449 472 | 0.439 |

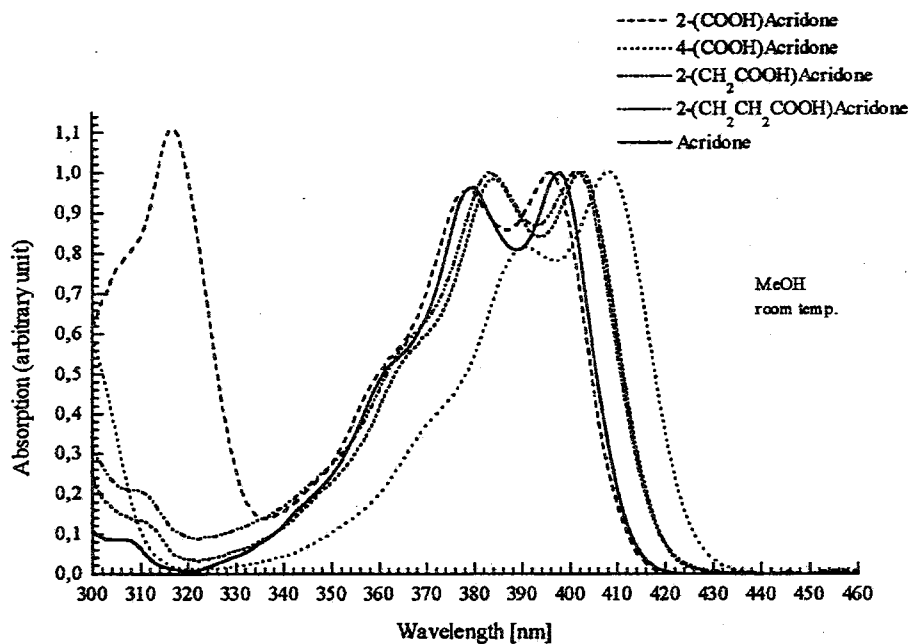a $\lambda_{max}$ denotes position of vibrational bands

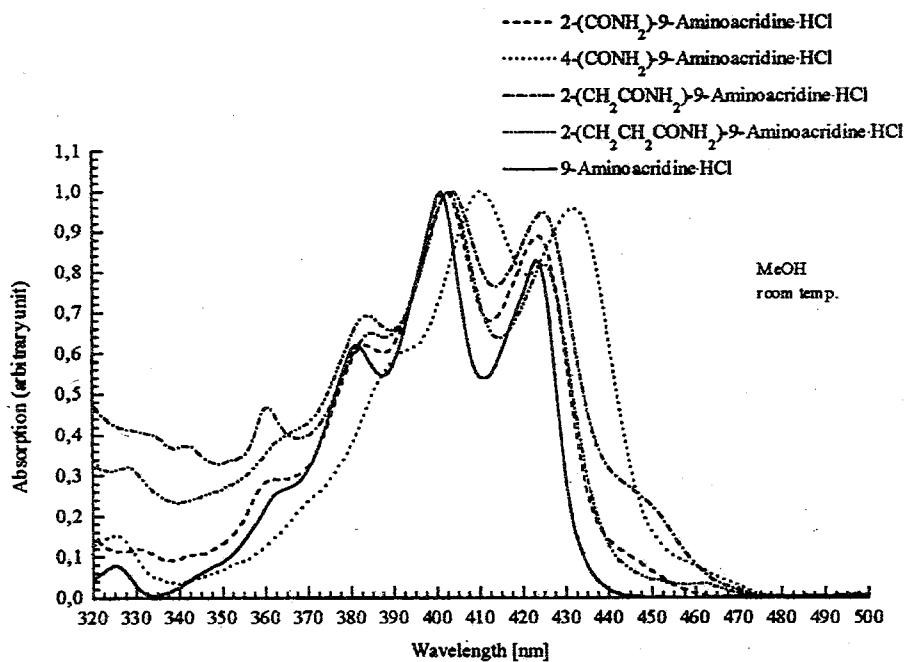Fig. 1. Normalised absorption spectra of acridone derivatives **2a-d** (MeOH, room temp.).



Fig. 2. Normalised absorption spectra of 9-aminoacridine derivatives **3a-d** (MeOH, room temp.).
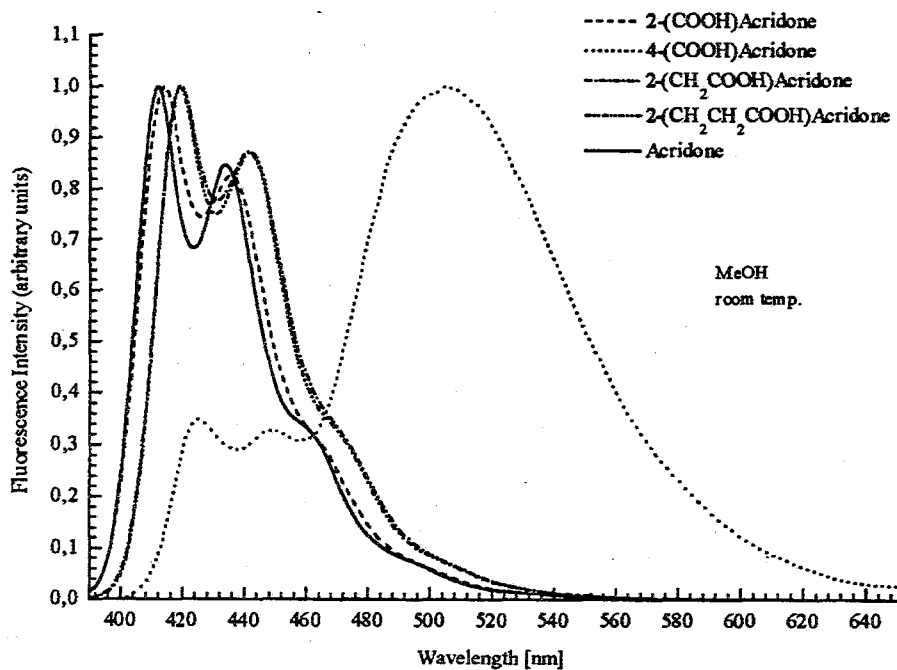
*Fig. 3.* Normalised fluorescence spectra of acridone derivatives **2a-d** (MeOH, room temp.).
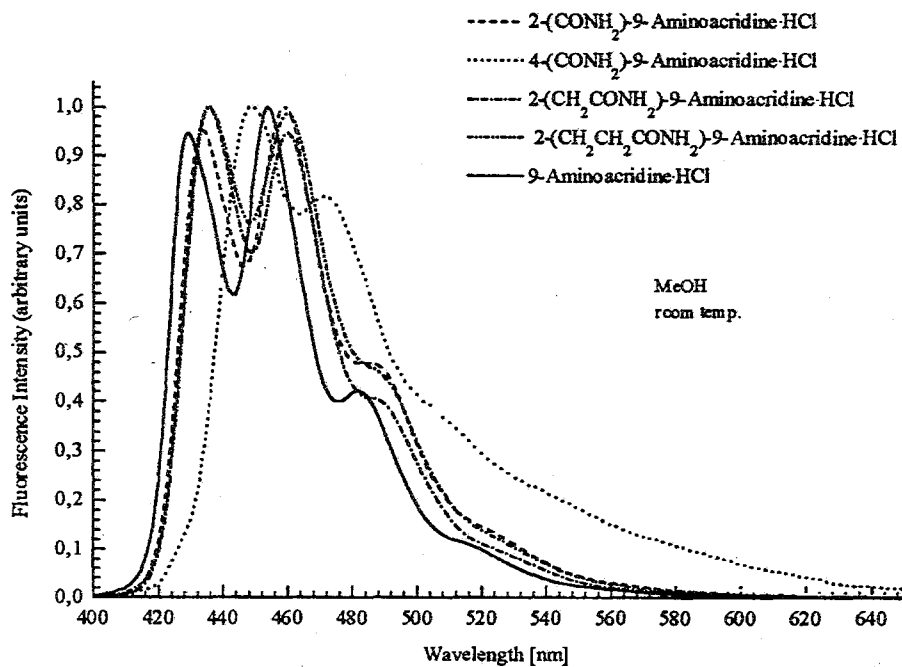


*Fig.4.* Normalised fluorescence spectra of 9-aminoacridine derivatives **3a-d** (MeOH, room temp.).

Fluorescence spectra of all studied compounds were recorded with excitation at $\lambda = 380$ nm and they are presented at Figs. 3 and 4.

Apart from 4-substituted derivatives, all investigated acridine analogues displayed high quantum yield of fluorescence (Table 3.) The much lower quantum yield of fluorescence found for the 4-substituted acridine analogues is due to a possibility of excited state proton transfer between acridine nitrogen atom and carboxylate moiety [13]. Additional proof of the proton transfer process came from a decrease of short-wave fluorescence and observation of an extra fluorescence band with maximum at 510 nm for the acridone analogue. Maxima of the fluorescence spectra are shifted towards long-wave region (420 – 480 nm) in comparison with acridine spectrum, which can be useful for conformational studies of peptides and proteins by means of fluorescence methods. Quantum yield of fluorescence of the appropriate pair of acridone and aminoacridine was found to be generally similar. From the point of view of application in conformational studies of peptides and proteins aminoacridines are more suitable than acridines because their solubility is higher. At the same time, aminoacridines are less stable since they form acridones upon hydrolysis.

Prepared acridine analogues because of their interesting photophysical properties can be applied in many aspects of studies of biopolymers. Especially, their high fluorescence quantum yields and possibility of direct incorporation into peptide chain by either carboxyl or amino group are worth to mention. Moreover, the syntheses of the compounds are quite straightforward, which make them additionally useful as chromophoric markers.

## EXPERIMENTAL

^{1}H NMR spectra were recorded on Varian Mercury 400 Hz apparatus in $CD_3OD$ with TMS as internal standard. IR spectra were obtained with Brukner IFS 66 spectrophotometer in KBr pellets. Recording of absorption spectra was performed using Perkin Elmer Lambda 18 spectrophotometer. Fluorescence spectra were recorded on Perkin Elmer LS-50 spectrophotometer with 2.5 nm spectral width. Quantum yield was calculated relative to quinine sulfate in 1M $H_2SO_4$ as a standard ($QY = 0.55$). Mass spectra were obtained using VG Masslab Trio-3 spectrometer. The homogeneity of compounds was accessed by TLC (aluminium sheets precoated with silicagel 60 F-254, Merck) and by HPLC analysis on Kromasil column 4,6×250 mm, C-8, 5μm, using gradient 0 – 100 % B (B = 80 % $CH_3CN$ + 0,08 % TFA, A = $H_2O$ + 0,1 % TFA). Melting points are given uncorrected.

2-Chlorobenzoic acid, 4-aminophenylacetic acid and polyphosphoric acids were purchased from Lancaster, anthranilic acid and 4-aminobenzoic acid were purchased from Aldrich. All used compounds were p.a. grade.

**Preparation of 4-(2-carboxyphenylamino)benzenealkanoic acids (1a–c)** (according to optimized procedure [1]). A mixture of 2-chlorobenzoic acid (1 eq.), 4-aminophenylalkanoic acid (1.1 eq.), anhydrous potassium carbonate (3 eq.) and freshly prepared copper powder (0.2 eq.) in dimethylformamide was heated with stirring at 140–145 °C for 6–10 h under a stream of argon. Resulting mixture was then cooled to 60 °C and reaction was quenched with ice-water. The dark solution was decolorized with activated carbon, filtered and carefully neutralized with 6 M HCl (in ice bath). Formed precipitate was filtered off or centrifuged, washed with water and dried in desiccator over $P_2O_5$. The crude product was recrystallized from aqueous ethanol or dimethylformamide / water. In the case of compound **Ic** the 4-aminophenylpropionic acid amide was used instead of the free acid. Final 4-(2-carboxyphenylamino)phenylpropionic acid amide was purified by multiple crystallization from EtOH/$H_2O$.

**N-(2-Carboxyphenyl)anthranilic acid (1d )** was prepared by reaction of 2-chlorobenzoic acid with anthranilic acid using procedure described for **1a-c.**

**Preparation of 9,10-dihydro-9-oxoacridine-2(or 4)-alkanoic acids (2a-d)** (cf. optimized literature procedure [1]). Polyphosphoric acid (10 ml/g of substrate) was heated with stirring to 80–90°C and the 4-(2-carboxyphenylamino)benzenealkanoic acid was added during 10–15 min. Reaction mixture was stirred at this temperature for an additional 1 to 1.5 h (in case of 9,10-dihydro-9-oxoacridine-2-propionic carboxamide for 0.5 h), then cooled to room temperature and poured slowly into ice-water. The resulting suspension was boiled for 5 min, chilled to room temperature and finally filtered. The crude product was recrystallized from glacial acetic acid. In the case of compound **2c** the crude product was purified by column chromatography on silicagel using $CH_2Cl_2$: MeOH (10:1) as an eluent to give pure 9,10-dihydro-9-oxoacridine-2-propionic acid amide. This compound was next hydrolyzed to the acid derivative with boiling 1M NaOH. Characteristics of resulting products **2a-d** are presented in Table 1.

**Preparation of 2(or 4)-carbamoylalkyl-9-aminoacridine hydrochloride salts (3a-d)** (general procedure). A suspension of the appropriate acridone derivative in thionyl chloride (1 g / 3 ml $SOCl_2$), containing a catalytic amount of DMF was refluxed with stirring for 1–2 h. The solution was evaporated to dryness *in vacuo*. Removal of traces of $SOCl_2$ was accomplished by additional evaporation with toluene (3 times). Thus obtained 9-chloro derivative was dissolved in phenol and heated slowly to 100 °C for 15 min. The resulting mixture was then cooled to 50 °C and stream of dry ammonia was passed through the solution while temperature was raised to 110-120 °C and held for 0.5–1 h. The mixture was then cooled and diluted with of 5 M NaOH. Resulting solid was collected and crystallized from aqueous EtOH. The free base was then dissolved in MeOH, treated with HCl / dioxane (2 eq.), evaporated to dryness and finally crystallized from MeOH / $Et_2O$. Characteristics of products **3a-d** are presented in Table 2.

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