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Synthesis of *N*-acridinyl-*N*′-alkylguanidines: Dramatic influence of amine to guanidine replacement on the physicochemical properties

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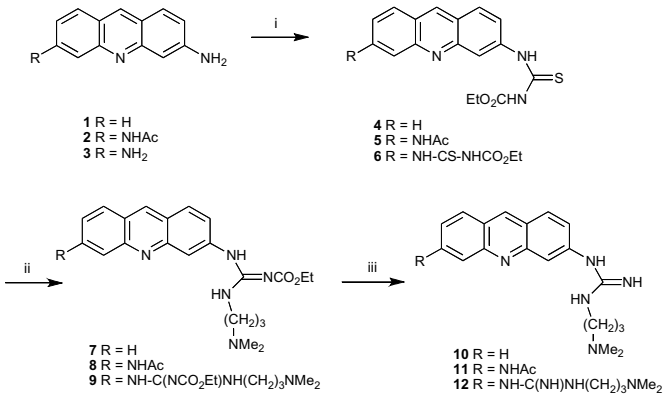
ABSTRACT

Transformation of aminoacridines into *N*-acridinyl-*N*′-alkylguanidines is described. The chosen procedure allows introduction of pendent substituents (exemplified by *N*,*N*-dimethylaminopropyl chain) into key acridinyl thioureas, thus opening the way to structural diversity. Spectroscopic study and pK_a determination show that the presence of the strongly basic guanidine has a dramatic influence on the ionization of the acridine nucleus by lowering the pk_a value down to 4.49.

Acridines, especially amino-substituted acridines, have a long story in drug design. A large number of derivatives, both from natural and synthetic sources, have been described, and their biological properties evaluated. Aminoacridines show activities in the treatment of various pathologies, such as cancer, protozoal, or viral infections and, more recently, amyloid related diseases such as Kreutzfeld–Jakob's (prion) and Alzheimer's diseases.[1–4] In our group, we have developed a family of highly cytotoxic *ortho*-hydroxymethyl aminoacridines. In order to modulate their biological properties, we are exploring innovative structural modifications. It appeared to us that replacement of the amino substituents by guanidines would fit our needs. Indeed, guanidine functionalities are found in a large number of natural compounds.[5] In most cases, the guanidine group is either found in the form of five- or six-membered cycles, or as terminal group of pendent substituents. Considering the diversity of the chemical, physicochemical, and biological properties of guanidine-containing molecules, their synthesis has attracted much attention, and numerous synthetic methodologies have been reported in the literature.[6] However, it is worth noting that, until very recently, guanidinylation has not been developed as a strategy for drug modulation of aromatic or heteroaromatic amines of biological interest. When this present work was under investigation, different groups[7–9] reported on the interest of replacing amino substituent by the corresponding guanidine in drug design; however, they did not introduce other substituents on the guanidine itself. In our strategy, the stepwise construction of the guanidine group will allow introduction of pendent alkyl or aryl substituents, thus increasing the structural diversity. We also anticipated that replacing the amino substituent by the strongly basic guanidine would have an impact on the physical properties, and, in particular, on the ionization and charge distribution. To our best knowledge, *N*-acridinyl-*N*′-alkylguanidines are not known in the literature. We therefore prepared *N*′-alkylguanidino acridines from the corresponding simple 3- and 3,6-aminoacridines, and studied their physicochemical properties.



Scheme 1. Reagents and conditions: (i) EtOCONCS, DMF, rt; (ii) Me$_2$N-(CH$_2$)$_3$-NH$_2$, EDCI, DBU, DMF, 60 °C; (iii) Me$_3$SiBr, DMF, 80 °C or aq 0.4 N NaOH, THF, 60 °C.

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The low reactivity of aminoacridines, and more generally of amines carried by π-deficient nitrogen-containing heterocycles, is a limiting point to their transformation into guanidines. Among the numerous methods described in the literature,[6] we chose the strategy reported by Atwal,[10] and later adapted by Hamilton[11] to the synthesis of *N*-arylguanidines. In this methodology, the aromatic amine (i.e., the less reactive amine) first reacts with N-protected isothiocyanate to give the N′-protected *N*-arylthiourea, which is then coupled to various amines in the presence of carbodiimide.

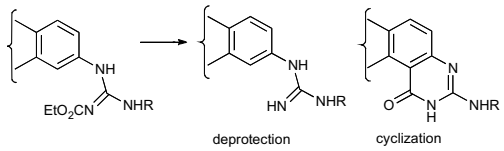

Figure 1. Competitive reactions.

Table 1
Reactivity with halomethylsilanes

Compound	Reagents	Conditions[a] (equiv)	% Deprotection	% Cyclization[b]
7	Me_3SiCl	5	0	100
7	Me_3SiBr	5	100	0
7	Me_3SiI	10	20	80
8	Me_3SiCl	5	99	<1
8	Me_3SiCl	15	80	20
8	Me_3SiBr	5	100	0
8	$MeSiCl_3$	10	<1	99
9	Me_3SiCl	10	<3	97
9	Me_3SiBr	10	100	0

[a] The reactions were performed in DMF. The solutions were heated at 80 °C, and the reactions were monitored by HPLC.
[b] The cyclized compounds will be described elsewhere. Their spectral characterizations are given in the Supplementary material.

This methodology was applied to the synthesis of guanidinoacridines **10**–**12** (Scheme 1). For solubility reasons, the polar dimethylaminopropyl chain was introduced as *N′*-alkylguanidine substituent. The ethoxycarbonyl-protected guanidines were obtained in two steps and in reasonable yields.

Unlike what was reported in the literature,[12] removal of the ethoxycarbonyl group could be achieved quantitatively in basic conditions (THF-aqueous 0.4 N NaOH, 60 °C, 24–36 h) as indicated by HPLC analysis. However, tedious extraction and purification procedures, due to the high polarity of the final guanidines **10** and **12**, lower the yields in isolated products (48–50%). Another drawback is that these conditions are not compatible with the presence of alkaline sensitive acetamido group of **11**. We therefore turned our attention to acidic conditions. Manimala and Anslyn[12] have previously mentioned a deprotection procedure of carbamoyl-protected *N*-alkylguanidines using bromotrimethylsilane. However, we have recently shown that treatment of *N*-aryl ethoxycarbonylguanidines with chlorotrimethylsilane quantitatively yielded the corresponding quinazolinones following an intramolecular Friedel–Crafts type reaction.[13] In an effort to understand these seemingly contradictory data, we examined the effect of a

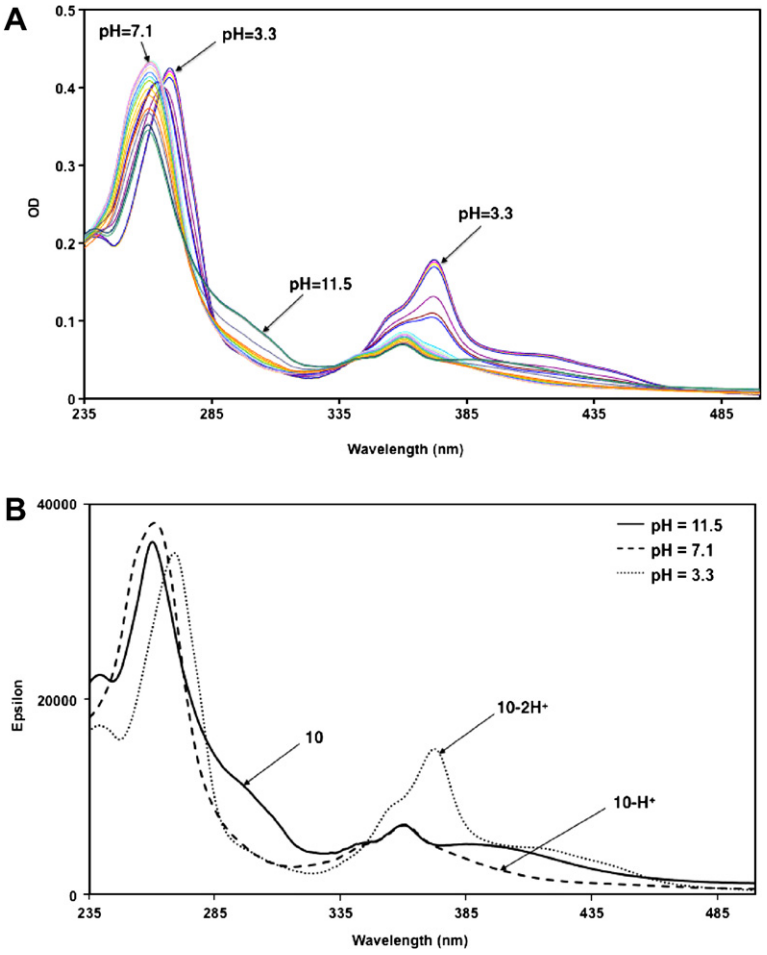

Figure 2. (A) Evolution of the spectroscopic properties of compound **10** as a function of pH. (B) Selected spectra corresponding to each form (**10**, **10**-H^+, and **10**-$2H^+$).

series of acids on the reactivity of compounds **7**–**9**. The reactions were followed by HPLC. The results are collected in Table 1 and Figure 1.

As shown in Table 1, the nature of the haloalkylsilane has a striking influence on the course of the reaction. For the compounds **7** and **9**, Me_3SiI or Me_3SiCl gave either a mixture of deprotection and cyclization compounds, or quasi-exclusively, the cyclized derivative. On the other hand, Me_3SiBr only yielded the desired deprotected guanidine with no trace of the cyclized by-product. Surprisingly, with compound **8**, deprotection was observed as major pathway with either Me_3SiCl or Me_3SiBr. Increasing amount of Me_3SiCl (5–15 equiv) or using a stronger Lewis acid ($MeSiCl_3$) changed the course of the reaction in favor of the cyclization process. However, it should be noted that, despite the harsh acidic conditions, the acetamido substituent of **8** remains untouched.

As a conclusion, deprotection of the guanidine substituent can be achieved either by treatment in alkaline solution (THF-aqueous 0.4 N NaOH, 60 °C, 24–36 h), or by using Me_3SiBr (DMF, 80 °C, 3–5 h) as previously proposed by Manimala and Anslyn.[12] The two procedures are complementary, and the choice between them will depend on the presence and nature of other substituents.

To evaluate the effects of the guanidine moiety on the properties of the acridine core, we studied the variations of the UV/vis spectra of compounds **10**–**12** as a function of pH, and calculated their pk_a values. For calculations (using SPECFIT software), we considered that the protonation of the pendent tertiary amine had negligible influence on the spectroscopic properties of the molecules. Therefore, we only measured the ionization constants of the heterocyclic amine (pK_a^a) and of the guanidine (pK_a^g).

For the monoguanidino compounds, pK_a values of 4.85 and 9.61 were found for **10**, and 5.32 and 9.48 for the acetamido analog **11**. For the bisguanidino derivative **12**, three values were calculated at 4.49, 7.68, and 10.25.

As exemplified in Figures 2 and 3, several species were observed for each compound, and were associated with the different ionization states. Examination of the absorption variations clearly indicates that protonation of the guanidine groups occurs above pH 8 as it is usually observed for a similar guanidine (pk_a = 10.5 for 2-guanidinopyridine[14]). Indeed, the protonation of the guanidine substituent of **10**–**12** had hypochromic and hypsochromic effects

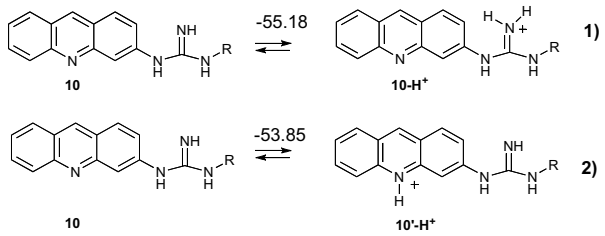

Figure 4. Acid–base equilibrium of **10**. The ΔG values (kJ/mol, vapor phase) are indicated above the arrows.

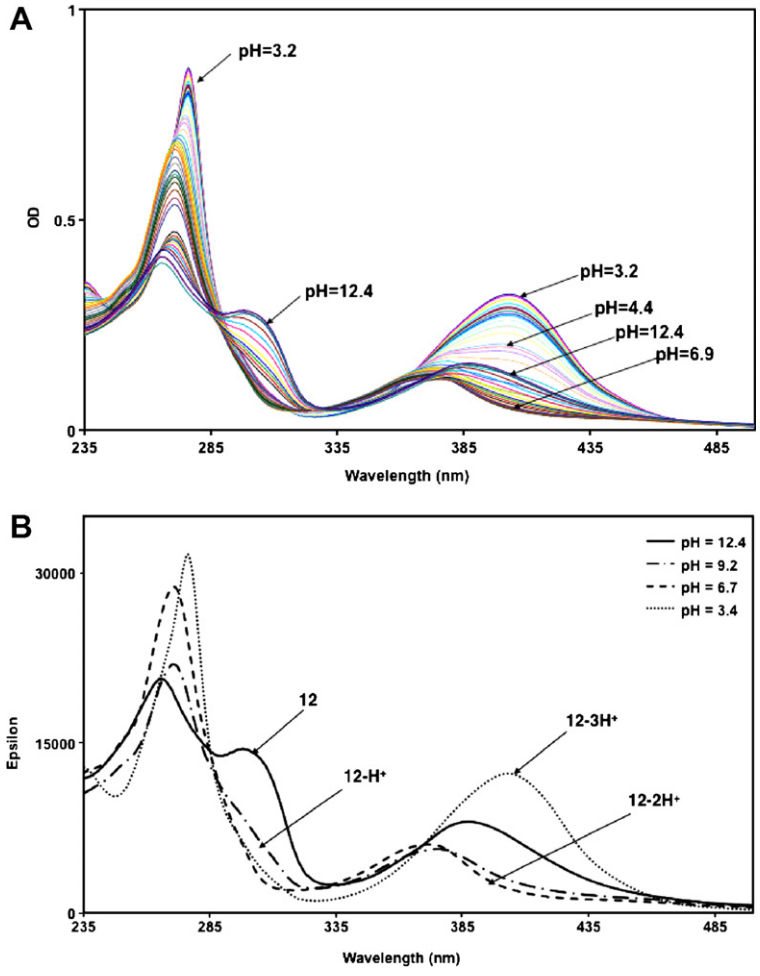

Figure 3. (A) Evolution of the spectroscopic properties of compound **12** as a function of pH. (B) Selected spectra corresponding to each form (**12**, **12**-H^+, **12**-$2H^+$, and **12**-$3H^+$).

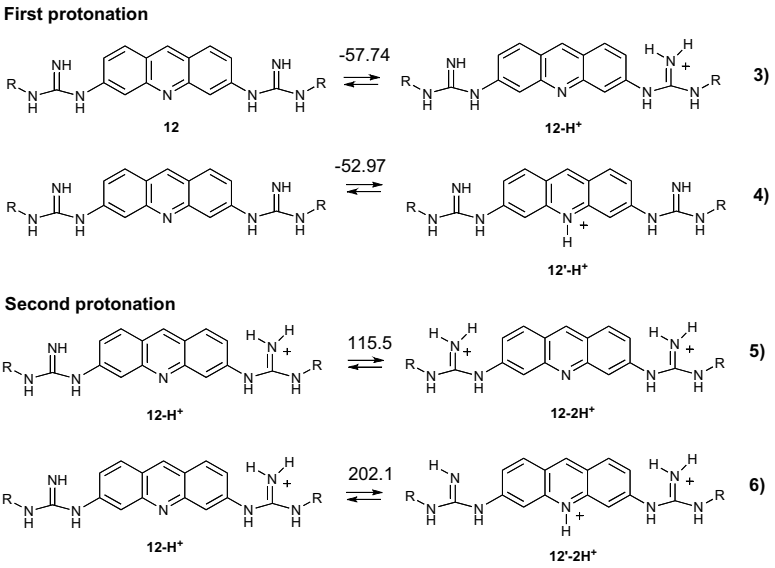

Figure 5. Acid–base equilibrium of **12**. The ΔG values (kJ/mol, vapor phase) are indicated above the arrows.

on the acridine absorption, suggesting a positive charge located on the guanidine group with little delocalization on the acridine chromophore. The strong hyperchromic and bathochromic effects observed in acidic media (pH < 6), are characteristic of the formation of the acridinium cation as it has been described by Albert.[15] Therefore, the species associated to the spectra showing the highest values for λ_{max} and ε were therefore assigned to the acridinium forms **10**-**2H**$^+$ and **12**-**3H**$^+$. Very similar variations of the spectra as a function of the pH were also observed for compound **11**. The pK_a values measured for the acridine nitrogen for the three molecules (pK_a^a 4.49–5.32) are significantly lower than the values observed for amino-substituted acridines (9.7 and 8.4 for 3-amino and 3,6-diaminoacridines, respectively).

The spectroscopic analysis is supported by theoretical ΔG calculations[16] (Figs. 4 and 5). For compounds **10** and **12**, the protonation on the guanidine nitrogens gives more stable (lower energy) species than protonation on the acridine nitrogen. These data are in accordance with the existence of the form **10**-**H**$^+$ (Fig. 4, Eq. 1) for guanidinoacridine **10** at neutral pH.

For the bisguanidinoacridine **12**, form **12**-**H**$^+$ (Fig. 5, Eq. 3) is favored in slightly basic conditions (first protonation). The second protonation occurs on the other guanidine (form **12**-**2H**$^+$, Fig. 5, Eq. 5) below pH 7 (pK_a 7.68).

As a conclusion, new (*N*′-alkylguanidino)acridines, exemplified by compounds **10**–**12**, were prepared in three steps from the corresponding aminoacridines and alkylamines adapting literature procedures. The conversion of aromatic amines into substituted guanidines has major advantages. First of all, changing the nature of the alkylamine reagent allows the easy modulation of the structural diversity and of the lipophilicity/hydrophilicity character of the resulting molecules. The guanidine group has also a major impact on ionization of the acridine ring. For the three guanidinoacridines prepared in this study, at neutral pH, the major cationic species are those protonated on the basic substituents (guanidines and amines), the acridine ring remaining uncharged. Compared to aminoacridines, which are protonated on the heterocyclic nitrogen, this difference would have a critical impact on the interaction with macromolecules such as DNA, both in terms of mode of binding and affinity. This study clearly highlighted the interest of introducing guanidino substituent onto the nitrogen heterocycles in terms of biologically active drug modulation.

Supplementary data

The detailed synthetic procedures and the spectral characterization of the new compounds are given. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.100.

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