CORRELATION OF pK_a AND ACRIDINE SUBSTITUTION

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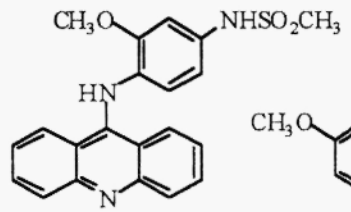
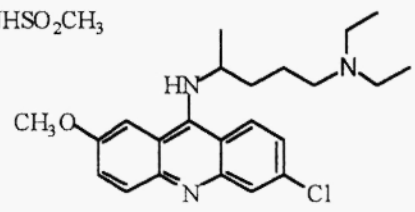
Abstract: The pK_a of a series of substituted acridines was confirmed by uv-vis spectroscopy. A quantitative structure activity relationship (QSAR) between the semi-empirical (AM1) calculated enthalpy of protonation and the pK_a of the acridine was developed.

Introduction. Successful antitumor and antibiotic 9-aminoacridines have been known for some time.[1,2] For example, m-amsacrine (m-AMSA) is a successful antiproliferative agent used in the fight against childhood leukemia.[3] Quinacrine, another molecule in this class, was used in the 1930's and 40's as an antimalarial agent.[4] This class of compounds exhibits biological activity through interaction with DNA in both intercalative and associative modes.



m-AMSA (*m*-amsacrine)



quinacrine

The stronger intercalative mode of interaction has been shown to be facilitated with planar aromatic systems capable of pi stacking, hydrogen bonding, and van der Waals intermolecular forces. The interaction is reinforced by electrostatic forces of attraction between the inserted acridine and negatively charged phosphate backbone of DNA. In fact, it has been noted that the ability of the acridine nucleus to be protonated at physiological pH is a requirement of intercalation.[5] The associative mode of interaction involves hydrogen bonding, van der Waals, and electrostatic forces of attraction. However, the external association of the acridine with the DNA backbone limits the extent of pi stacking and results in a much weaker interaction.

Problems associated with the use of 9-aminoacridines as therapeutic agents are focused on the relative ease of hydrolysis and/or thiolysis of the C9-N bond, resulting in the reduction or loss of biological activity.[6] In an effort to eliminate cleavage of this bond as a viable metabolic pathway, we prepared a series of 9-aminomethyl-acridines.[7] The biological activity of this series of compounds was examined via thermal denaturation studies of genomic calf thymus DNA and via zone of inhibition studies with *S. aureus* and *E. coli*. Lack of any change in the thermal denaturation temperature of the genomic DNA was observed. Moreover, the limited antibiotic effect observed in this series most

likely arose from the toxicity of the compounds. Subsequent analysis of the basicity of these compounds indicated that they were not protonated at physiological pH, thus they were unable to exhibit strong association with DNA via intercalation.

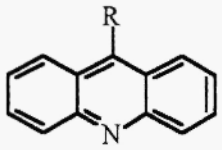
Results and Discussion. A quantitative structure-activity relationship (QSAR) correlating the acridine's pK_a and the semi-empirical calculation of the enthalpy of protonation would save time during future studies. Protonation occurs at the acridine nitrogen, as evidenced by spectroscopic studies.[8] However, the charge on the protonated acridine has been shown to be resonance delocalized and stabilized by substituents on the acridine ring.[9]

Since many of the reported pK_a values for substituted acridines have been measured in 50% ethanol or 20% DMSO solution, a series of 9-substituted acridines were prepared in the laboratory and their pK_a's determined in aqueous solution. The pK_a of each compound at room temperature (25 °C ± 0.5 °C) was obtained by spectroscopic analysis in phosphate buffered solutions that spanned the pH scale (Table I). Further examination of these compounds was accomplished using the SPARTAN computational analysis package. Semi-empirical (AM1) calculations of the enthalpy of protonation, ΔH_{rxn} (in Kcal/mol), showed a strong correlation with the Hammett

Table I. Experimental 9-Substituted Acridine pK_a Values.

R	pK_a	pK_a^{lit}
H	5.36	5.3
CH_3	5.76	5.8
NH_3	10.45	9.99

R	pK_a
C_2H_5	6.00
CHO	4.52
C_6H_5	5.12



pK_a^{lit} : The literature values taken from reference 10.

electronic parameter, σ_p [7]. The positive slope of the linear correlation indicates that electron-withdrawing substituents tend to increase the enthalpy of reaction. Conversely, electron-donating substituents decrease the enthalpy of protonation. This agrees with the previously noted ability of a substituent to stabilize the charge on the protonated acridine.

$$\Delta H_{rxn} = 16.421\ \sigma_p + 138.61$$
$$R^2 = 0.9666, n = 6$$

Plots of the experimental pK_a values versus Hammett's σ_p^+ [11] did, in fact, illustrate a strong correlation, as shown below. While the fit to the data was good, $R^2 > 0.9$, with Hammett's σ_p value, the linearity of the relationship was dramatically improved with the use of the σ_p^+ value. Not surprisingly, this indicates that the ability of a substitent to stabilize the protonated product plays a large role in the resulting pK_a of the acridine. The large negative slope of the correlation indicates that electron-withdrawing substituents have a rather marked effect on the pK_a of the acridine. Electron-donating substituents are indicated to be the best choice for an acridine that would be protonated at physiological pH (pH 7.3). This QSAR agrees well with the previously published [8] QSAR between pK_a values and the Hammett σ_p.

$$pK_a = -3.3315\ \sigma_p^+ + 5.3524$$
$$R^2 = 0.9575, n = 6$$

Use of this equation, however, requires knowledge of the Hammett σ_p^+ value for a particular substituent. In addition, significant differences exist between many of the published Hammett values due to variations in experimental conditions and the relationship from the benzene system to the acridine system. To simplify the estimation of pK_a values for substituted acridines, a QSAR can be developed by combination of the two previous relationships. The resulting QSAR, relates the results of a semi-empirical (AM1) calculation of the enthalpy of protonation versus the experimental pK_a.

$$pK_a = -0.3022\ \Delta H_{rxn} + 47.71$$

$$R^2 = 0.9484, n = 6$$

The resulting QSAR between the pKa and a computationally derived value can be used to determine the requirements for intercalation of the 9-substituted acridines. Specifically, solving the QSAR equation for pKa values above 7.3 gives the molecular requirements for favorable intercalation in aqueous solution. At this pK_a value, the enthalpy of protonation would need to be smaller than 133.72 Kcal/mol. This corresponds to substituent σ_p^+ values less than -0.58. An aqueous solution of such a compound would contain a large component of the protonated species at physiological pH.

Table II. Calculated pK_a Values for 9-Substituted Acridines.

R	pK_a	ΔH_{rxn}
OH	7.84	131.71
Cl	4.26	140.53
COOH	2.49	144.89
OCH_3	6.83	134.20
NO_2	2.41	149.90

R	pK_a	ΔH_{rxn}	pK_a^{lit}
CH_2NH_2	5.72	136.95	5.53[a]
$NHCH_3$	9.82	126.85	>9.35
NHPh	8.21	130.81	8.05
OPh	5.27	138.05	5.28[a]
NH_2	9.93	126.57	9.99

Literature values for pK_a in 20% DMSO are taken from reference 12 and adjusted to aqueous solution. [a]Calculated using equation from reference 12.

For example, calculation of the pK_a for a series of 9-substituted acridines is shown in Table II. As observed, the 9-(aminomethyl)acridines have a pK_a less than 7.3 and are not protonated at physiological pH. Conversely, the substituted 9-aminoacridines have pK_a values indicating complete protonation under physiological conditions. The QSAR equation also allows the calculation of pK_a for molecules that are unstable or do not exist in aqueous solution. 9-Hydroxyacridine, which exists in aqueous solution as the tautomer 9-acridanone, was calculated to have a pK_a of 7.84. This contrasts markedly to the experimentally determined pK_a for 9-acridanone, -0.32.[10]

In another example, 9-chloroacridine has been shown to undergo rapid hydrolysis to 9-acridone in aqueous solution.[13] The rate of hydrolysis is increased dramatically under slightly acidic conditions. Measurement of the pK_a for 9-chloroacridine in aqueous solution is then difficult to perform. However, by semi-empirical calculations of the enthalpy of protonation, its pK_a can be determined. Calculation of its pK_a, 4.26, indicates that it is only marginally protonated at physiological pH.

Conclusion. 9-Substituted acridines can exhibit intercalative binding with genomic DNA. The requirements of intercalation are such that the acridine must be capable of being protonated at physiological pH. Strong electron-

donating substituents are required to increase the pK_a of the acridine and enhance its basicity. The pK_a's of any 9-substituted acridine can be estimated by conducting semi-empirical (AM1) calculations. These calculations can also be used to confirm the value of Hammett σ_p values for substituents on the acridine ring system.

Experimental. Acridine and 9-aminoacridine were obtained commercially and used after chromatographic purification. Flash chromatography of all materials was performed on silica gel (70-230 mesh) with freshly distilled solvents by the method of Still.[14] The method of Bernthsen [15] was used to prepare 9-methylacridine, 9-ethylacridine, and 9-phenylacridine. 9-Acridine carboxaldehyde was prepared as described previously.[16] For spectroscopic determination of pK_a a series of phosphate buffers covering the pH range of 2 to 10 were prepared. A known mass of substituted acridine was then added to each buffer and its concentration determined. Then, uv-vis spectra were obtained of each solution. To determine the pK_a, the absorbance (normalized for concentration) at a particular wavelength showing maximal deflection throughout the series of spectra was plotted versus pH. The resulting sigmoidal curve was analyzed to determine the pH at which the absorbance was changing most rapidly. This point corresponded to the pK_a value for the compound.

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References and Notes

1. A.C.R. Dean, The Antibacterial Action of Acridines, in R.M. Acheson (Ed), Acridines, Wiley-Interscience, New York, 1973, vol. 9, pp. 789
2. D.B. Clayson, Carcinogenic and Anticarcinogenic Properties of Acridines, in R.M. Acheson (Ed), Acridines, Wiley-Interscience, New York, 1973, vol 9, pp. 822
3. R. Zittoun, *Eur. J. Cancer Clin. Oncol.* **21**, 649 (1985)
4. D.W. Henry, Acridine Antimalarials, in R.M. Acheson (Ed), Acridines, Wiley-Interscience, New York, 1973, vol. 9, pp. 829
5. A. Blake and A.R. Peacocke, *Biopolymers* **6**, 1225 (1968)
6. G.D. Jaycox, G.W. Gribble and M.P. Hacker, *J. Heterocycl. Chem.* **24**, 1405 (1987). W.A. Denny, G.J. Atwell and B.F. Cain, *J. Med Chem.* **22**, 1453 (1979). W.R. Wilson, B.F. Cain and B.C. Baguley, *Chem Biol. Interactions* **18**, 163 (1977)
7. M.D. Mosher and E. Johnson, unpublished results, 1999
8. L.E Orgel, *Chem. Heterocycl Compd.* **9**, 289 (1956)
9. J.L. Irvin and E.M. Irvin, *J. Am. Chem. Soc.* **72**, 2743 (1950)
10. A Albert and R.J. Goldacre, *J. Chem. Soc.* 706 (1946). A. Albert, S.D. Rubbo, R.J. Goldacre, M.E. Davey and J.D. Stone, *Brit. J. Exp. Path.* **26**, 160 (1945). J.M.F. Gagan, *Chem. Heterocycl. Compd.* **9**, 197 (1973)
11. The values were taken from C Hansch, A. Leo and D. Hoekma, Exploring QSAR: Hydrophobic, Electronic, and Steric Constants, American Chemical Society, Washington, DC, 1995
12. W.A. Denny, B.F. Cain, G.J. Atwell, C. Hansch, A. Panthananickal and A. Leo, *J. Med. Chem.* **25**, 276 (1982)
13. S. Skonieczny, *Heterocycles* **14**, 985 (1980)
14. W.C. Still, M Kahn and A Mitra, *J. Org. Chem.* **43**, 2923 (1978)
15. A. Bernthsen, *Liebigs Ann.* **192**, 1 (1878). F.D. Popp, *J. Org. Chem.* **27**, 2658 (1962)
16. M.D. Mosher and N.R Natale, *J. Heterocycl. Chem.* **32**, 779 (1995)

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