Substituent Effects on the Hydrolysis of Analogues of Nitracrine {9-[3-(*N,N*-dimethylamino)propylamino]-1-nitroacridine}

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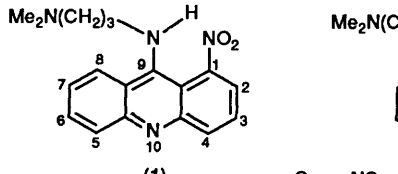
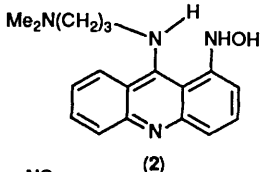
Studies of the hydrolysis of the hypoxia-selective cytotoxic agent 9-[3-(*N,N*-dimethylamino)-propylamino]-1-nitroacridine (nitracrine) and several of its 4-substituted analogues and nitro-positional isomers have been carried out. Examination of the effects of pH and temperature on the hydrolysis of nitracrine itself shows that the reaction is subject to acid catalysis. The value of $\Delta H^{\ddagger}$ increases from 46 to 63 kJ mol^{-1} as the pH falls from 6 to 3, while the value of $\Delta S^{\ddagger}$ increases from -195 to -138 J K^{-1} mol^{-1}. The rate constants for hydrolysis and the acid dissociation constants have been measured at pH 5 and 60 °C. Both the rate constants of hydrolysis, corrected for the substrate–protonation equilibrium, and the substrate–acid association constants are well fitted by the Ehrenson–Brownlee–Taft dual-substituent-parameter σ_R^- relationship. The Swain–Unger–Rosenquist–Swain relationship shows weak correlation but the linear free-energy relationships of Hammett and Yukawa–Tsuno are not fitted. The results are discussed in terms of the resonance interactions of the possible intermediates in the hydrolysis pathways.

Nitroacridine derivatives bearing 9-alkylamino sidechains are very potent cytotoxic agents *in vitro*. 9-[3-(*N,N*-Dimethylamino)propylamino]-1-nitroacridine (nitracrine) (**1**) has been used clinically in Poland for the treatment of mammary, lung, ovarian, and colon tumours.[1,2] Studies of the mode of action suggest the existence of a complex metabolism involving nitro group reduction to provide a DNA-alkylating species.[3,4] Although interest in the use of nitracrine as a classical cytotoxic agent has waned, the discovery[5] of its selective toxicity towards hypoxic mammalian cells in culture has revived interest in this class of compounds. More recently, the 4-methoxy derivative has been shown[6] to be more stable metabolically than nitracrine itself, and has been reported[6] to possess selective activity *in vivo* against hypoxic cells in advanced subcutaneous EMT6 tumours in mice.

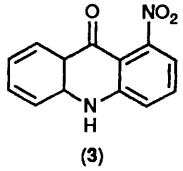
Although the major cellular metabolite of nitracrine under hypoxic conditions has been identified as a reduction product (**2**),[7] it has been suggested[8] that hydrolysis to the 9-acridone derivative (**3**) may be a major detoxication pathway. Thus in the selection of more biologically useful nitracrine analogues, it is important to understand the effect of substitution in the acridine ring on the rate of hydrolysis.



(1)



(2)



(3)

A series of kinetic studies on the hydrolysis of nitracrine (**1**) has been carried out by Skonieczny and co-workers, as part of a detailed investigation of its biochemical and physicochemical properties.[9,10] They found the rate of hydrolysis of 9-amino and 9-chloroacridines in ring-substituted compounds to be dominated by resonance rather than inductive effects. The rate of hydrolysis was also found to vary with pH. Hydrolysis was fastest below pH 4 (*i.e.* in doubly protonated molecules) for 1-nitroacridines. This trend was reversed for 9-aminoacridines not substituted by a nitro group in the 1-position. This work was reviewed in 1980,[11] but studies on nitracrine analogues substituted in the 4-position were not undertaken.

In the present paper we examine the effect of temperature and pH on the hydrolysis of (**1**), the effect of pK_a on the hydrolysis of a series of analogues of nitracrine, and the fit of these data by a variety of linear free-energy or dual-substituent-parameter relationships.

Experimental

Nitracrine (**1**) and the 4-substituted analogues and nitro positional isomers were synthesised by published methods.[12,13] For the temperature studies on nitracrine at various pH values, Universal Buffer [containing citric acid (0.025 mol dm^{-3}), potassium dihydrogen phosphate (0.025 mol dm^{-3}), sodium tetraborate (0.025 mol dm^{-3}), tris(hydroxymethyl)aminopropane (0.025 mol dm^{-3}) and either hydrochloric acid or sodium hydroxide (to adjust to the required pH)] was used. For the comparative studies on the 9-aminoacridines at pH 5, formate buffer (0.1 mol dm^{-3}) was used.

The radiometer pH meter 28 and the Ingold 10402 combination electrode were calibrated for each kinetic temperature using accurate standards ('Soloid,' Burroughs Wellcome, buffer solution tablets) for this temperature over the pH range *ca.* 4–9.

Hydrolysis of 9-Aminoacridines.—Buffer (3 cm^3) was placed in a 6Q Spectrosil spectrophotometer cuvette, equilibrated to the desired temperature and deoxygenated by bubbling with nitrogen through a sealed rubber septum for 15 min. The substituted 9-aminoacridine (10 mm^3, 0.14 μmol in CH_3CN)

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Table 1. Effect of pH and temperature on the values of the pseudo first-order rate constant of hydrolysis, k_{obs}, of nitracrine (**1**).[a]

	$k_{obs}/10^{-5}$ s^{-1}					
	T/°C					
pH	25	37	46	56	64	70
3.00			2.50	3.40	8.71	13.2
4.00	0.40	0.84	2.75	4.00	4.86	11.4
5.00	0.41	0.81	2.31	2.99	4.72	9.78
6.00	0.29	0.50	1.23	1.24	3.32	4.40

[a] Determined under anaerobic conditions as described in the Experimental section; λ = 438 nm.

Table 2. Arrhenius parameters for the hydrolysis of nitracrine at various pH values ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ at 25 °C).

pH	ln *A*	$\Delta H^{\ddagger}$/kJ mol^{-1}	ΔS/J K^{-1} mol^{-1}
3.00	13.9	63	−138
4.00	11.0	56	−162
5.00	9.7	48	−175
6.00	7.0	46	−195

was then added and the change in absorbance at the analytical wavelength was monitored on either a Cary 219 or Varian Techtron spectrophotometer. Because of the insolubility of the acridone product, the concentration of substrate was generally limited to micromolar levels, thus producing a maximum full scale deflection of only 0.2 absorbance units. For those compounds whose product acridones were insoluble even at this low concentration, *viz.*, the 2-nitro and 3-nitro isomers of nitracrine, 5 cm pathlength curvettes and still lower substrate concentrations (20% of that for the other acridines) were utilized, but the same magnitude of absorbance at infinite time was maintained. For each reaction condition and substrate at least four determinations of the rate constant of hydrolysis were made, using the cell programming option of the spectrophotometer. The reaction proceeded only very slowly at 37 °C. The hydrolysis of nitracrine was followed within the temperature range 25–75 °C and the pH range 3–6. The appropriate 4-substituted, 1-nitro acridone is the final product.

Determination of pK_a.—The values of pK_a for the various 9-aminoacridines were determined at 60 °C, under conditions where hydrolysis was negligible, by the spectrophotometric method of Albert and Serjeant.[14]

Calculation of Rate Constants.—When the reaction was sufficiently rapid, the pseudo-first-order rate constant of hydrolysis, k_{obs}, was calculated directly from the absorbance data using the Cary Advanced Order Kinetics Calculation Program (Varian 00-997087-00). The observed rate constants for the slower reactions ($t_{\frac{1}{2}} > 4$ h) were calculated by using the method of Guggenheim.[15] The rate data were subjected to statistical analysis for calculation of the standard deviations.

Correction of k_{obs} *for the Equilibrium Constant,* K_{eq}.—For a reaction in which there is a prior equilibrium between an acid, HA, and substrate, S, followed by a rate determining reaction with another reagent, R, then:

$$S + HA \underset{}{\overset{K_{eq}}{\rightleftharpoons}} SH^+ + A^- \quad (1)$$

$$SH^+ + R \xrightarrow{k_2} \text{products} \quad (2)$$

If

$$\text{Rate} = k_{obs}([S] + [SH^+]) \quad (3)$$

and we define,

$$f_{SH^+} = \frac{[SH^+]}{[SH^+] + [S]} = \frac{1}{1 + [S]/[SH^+]} = \frac{1}{1 + K_a/[H_3O^+]} \quad (4)$$

then,

$$\frac{[SH^+]}{[S] + [SH^+]} = \frac{[H_3O^+]}{[H_3O^+] + K_a} \quad (5)$$

$$[SH^+] = \frac{[H_3O^+]}{[H_3O^+] + K_a} \cdot ([S] + [SH^+]) \quad (6)$$

which corresponds to

$$[S] + [SH^+] = \frac{[SH^+]}{f_{SH^+}} \quad (7)$$

If k_2 is the rate determining step then

$$\text{Rate} = k_2[SH^+][R] \quad (8)$$

Therefore, $k_{obs}([S] + [SH^+]) = k_2[SH^+][R]$;

$$k_{obs}\frac{[SH^+]}{f_{SH^+}} = k_2[SH^+][R] \quad k_2 = \frac{k_{obs}}{f_{SH^+}[R]} \quad (9)$$

where R = H_2O and [R] = 55.5 mol dm^{-3}. If $K_a \gg [H_3O^+]$ then

$$f_{SH^+} = \frac{[H_3O^+]}{K_a} \quad (10)$$

Values of k_2 were calculated for the hydrolysis reactions of the 4-substituted 9-aminoacridines using equation (9). The hydrolysis of the 2-, 3-, and 4-nitro isomers does not show similar dependence of rate upon pH. k_2 was not calculated for these compounds.

Results

Table 1 gives the values of the rate constant of hydrolysis, k_{obs}, of nitracrine (**1**) within the temperature range 25–75 °C and pH range 3–6. From plots of ln k_{obs} *vs.* T^{-1}, the values of the Arrhenius parameters, shown in Table 2, were calculated.

Table 3 gives the values of k_{obs} and k_2 (at pH 5), p$K_a^{SH^+}$ and the analytical wavelength, λ, determined for both the 4-substituted analogues of nitracrine and the nitro positional isomers at 60 °C.

The data for hydrolysis of the 4-substituted 1-nitro-9-aminoacridines were subjected to analysis by the Hammett[16] and Yukawa–Tsuno[17] linear free-energy-relationships and the Swain–Unger–Rosenquist–Swain[18] and a modified Ehrenson–Brownlee–Taft[19] dual-substituent-parameter relationships.

The data for both $K_a^{SH^+}$ and k_2 were not fitted by the Hammett σ^-, σ^+, or σ values, nor the Yukawa–Tsuno relationships. The Swain–Unger–Rosenquist–Swain method [equation (11)] produced only weak correlation [equations (12) and (13)]. The poorer correlation is expected because the

Table 3. Pseudo-first-order rate constants of hydrolysis, k_{obs} (pH 5.00; 60 °C), acid dissociation constants, pK_a (60 °C), corrected rate constants, k_2, and analytical wavelengths of nitro-9-aminoacridines.

	$k_{obs}/10^{-6}$ s^{-1}	pK_a	$k_2/10^{-7}$ s^{-1}	λ/nm
9-Amino-1-nitroacridines 4-substituent				
CO_2Me	30.3 ± 0.6	4.55 ± 0.04	20.9 ± 0.6	420
Cl	43.7 ± 0.6	4.99 ± 0.12	15.9 ± 0.6	442
F	52.4 ± 0.4	5.10 ± 0.07	16.9 ± 0.4	432
Me	9.4 ± 0.3	6.00 ± 0.04	1.86 ± 0.07	430
NMe_2	20.2 ± 0.9	5.94 ± 0.04	4.1 ± 0.2	438
OMe	38.9 ± 0.8	5.96 ± 0.06	7.8 ± 0.2	426
H	35.0 ± 0.3	5.66 ± 0.07	7.7 ± 0.2	438
Positional isomers of 9-aminonitroacridines				
2-NO_2	125 ± 0.1	6.86 ± 0.06		426
3-NO_2	66.0 ± 0.5	5.98 ± 0.08		442
4-NO_2	441.0 ± 0.06	6.37 ± 0.09		454

nucleus used is acridine rather than benzene. The resonance interactions are derived in equations (11)–(13).

$$\log k_X/k_H = fF + rR + h \quad (11)$$

% resonance is defined as $100r/(f + r)$ for k_2,

$$\log k_X/k_H = (1.00 \pm 0.20)F + (0.19 \pm 0.08)R - (0.26 \pm 0.02) \quad (12)$$

$$n = 7,\ r = 0.85,\ \sigma_n = 0.31,\ f = (SD/RMS) = 0.24$$

Therefore, % resonance = 17, and similarly for $K_a^{SH^+}$:

$$\log K_X/K_H = (1.27 \pm 0.32)F + (0.35 \pm 0.11)R - (0.07 \pm 0.02) \quad (13)$$

$$n = 7,\ r = 0.96,\ \sigma_n = 0.71,\ f = (SD/RMS) = 0.60$$

Therefore, % resonance = 21.5. The relationship of best fit was similar to that of Ehrenson *et al.*,[19] differing only in that an intercept at zero was not enforced.

$$\log k_X/k_H = I^i + R^i = \sigma_I\rho_I^i + \sigma_R^r\rho_R^i + I \quad (14)$$

$$\lambda = \rho_R/\rho_I$$

$$\log k_X/k_H = (1.6 \pm 0.2)\sigma_I + (0.6 \pm 0.1)\sigma_R^- - (0.25 \pm 0.09) \quad (15)$$

$$n = 7,\ r = 0.97,\ \sigma_n = 0.22,\ f(SD/RMS) = 0.17$$

$$\lambda = 0.35$$

$$\log K_X/K_H = (1.43 \pm 0.12)\sigma_I + (2.29 \pm 0.21)\sigma_R^- - (0.05 \pm 0.07) \quad (16)$$

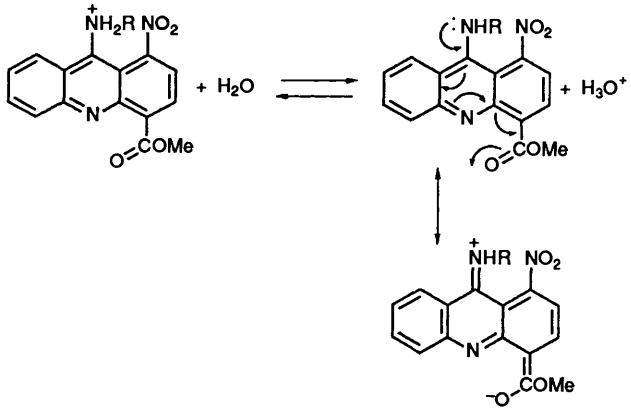
$$n = 7,\ r = 0.996,\ \sigma_n = 0.12,\ f(SD/RMS) = 0.099$$

Therefore, $\lambda = 0.64$. I and R are the polar and resonance effect parameters, respectively. ρ_R and ρ_I are reaction sensitivity coefficients. σ_I and σ_R ($\sigma_R = \sigma_R^-$, σ_R^0, σ_R^{BA}, or σ_R^+) represent measurements of the electronic distribution within the nucleus caused by inductive and resonance effects arising from attachment of a reacting side chain. The parameters of best fit, obtained after substituting σ_R^- into equation (14), for the Ehrenson–Brownlee–Taft dual substituent parameter calculation are given in equations (15) and (16).

The correlation coefficient is a derivative which provides at best a non-linear acceptability scale with good and bad correlations often crowded within the range 0.9–1.0. For this reason, a second test of correlation, f = SD/RMS, has also been used. SD is the standard deviation (root mean square of the deviations) and RMS the root mean square of the data, ($\log k_X/k_H$). The value of f should be less than 0.1 for a good correlation to exist.[19]

Discussion

The acid dissociation constants at 60 °C for the 4-substituted nitracrines, recorded in Table 3, are generally 0.3–0.7 pK_a units lower than those previously recorded[12] for the same set of compounds at 20 °C, but the overall rankings are very similar. In the previous report[12] it was noted that there was an approximate correlation between pK_a and substituent electronic properties, but no quantitative relationship was derived, although equations relating pK_a to substituent σ values have been published for acridine-substituted 9-anilinoacridines.[20] We have analysed the present K_a data by dual-substituent-parameter analysis, and find it is best fitted by the Ehrenson–Brownlee–Taft method. The data obtained by using the resonance parameter σ_R^- gave good results for K_a (r = 0.996, Figure 1). This suggests that the ionization process for 9-aminoacridines, as with anilines and phenols, involves direct resonance interaction between the reaction centre and the resonance electron withdrawing group (Scheme 1).



Scheme 1.

In a similar analysis of the hydrolysis data, we find the values of k_2 are also correlated best with σ_R^- [r = 0.97; equation (15) and Figure 2]. The value of f = 0.17 is rather too large for k_2, *i.e.* $f > 0.1$, but this result is so much better than the results obtained by using the other σ_R parameters that it seems reasonable to accept this relationship as the one of best fit. The rate constant, k_2, may be composite and represent more than one step in the hydrolysis pathway in any case. As before, direct resonance interaction with the reaction centre is possible in at least one place in the hydrolysis pathway; see, for example, Scheme 2 for a possible rate-limiting step. The rate of hydrolysis increases with decreasing pH. This result is similar to that of Skonieczny and Ledochowski[10] who found increases in the rate of hydrolysis of nitracrine at 80 °C as the pH fell from a value of 6 to 3. Nitracrine is present in solution as two tautomers and they attributed the change in rate to a change in concentration of the tautomers with changing pH. Below pH 3 the acridine is essentially completely protonated and no further changes in rate are expected.

The low values of $\lambda = \rho_R/\rho_I$ for both K_a (0.64) and k_2 (0.35)

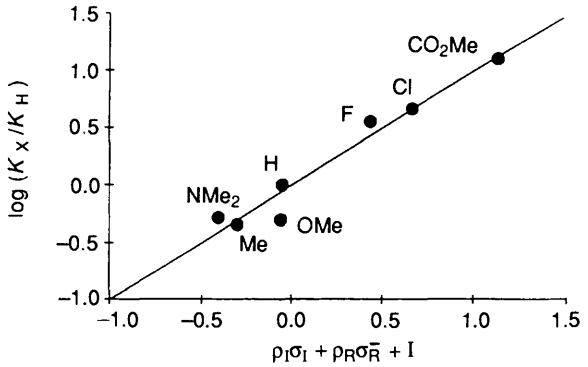


Figure 1. Analysis of the acid association constants, K_a, of the 4-substituted-9-amino-1-nitroacridines by the modified Ehrenson–Brownlee–Taft dual substituent parameter σ_R^- relationship.

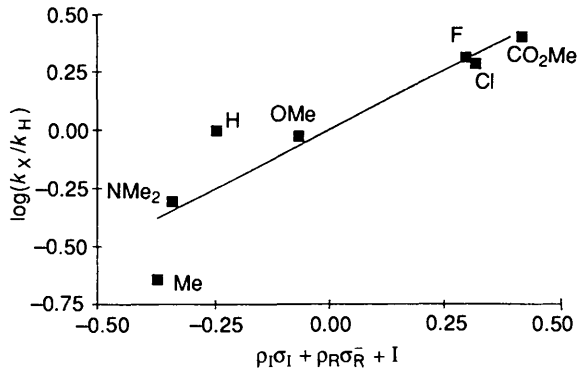
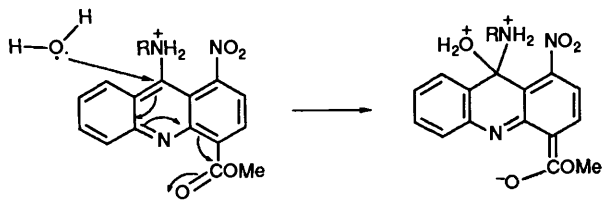


Figure 2. Analysis of the corrected rate of hydrolysis, k_2, of 4-substituted-9-amino-1-nitroacridines by the modified Ehrenson–Brownlee–Taft dual substituent parameter σ_R^- relationship.

are suggestive of the expected diminished effective transmittance of π electrons.

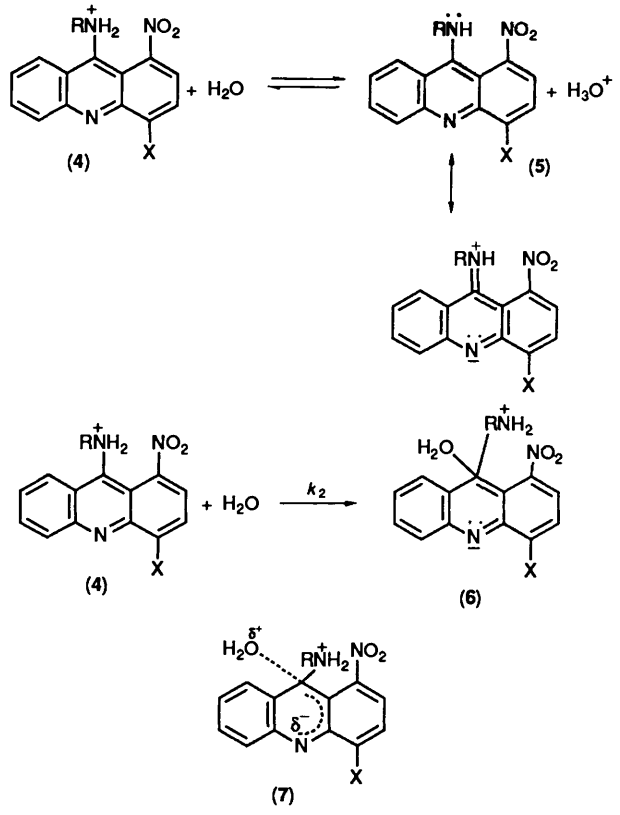
Analysis of the temperature effect on the reaction rate shows that nitracrine hydrolysis follows the simple Arrhenius



Scheme 2.

equation. $\Delta H^{\ddagger}$ increases from 46 to 63 kJ mol^{-1} and $\Delta S^{\ddagger}$ increases from −195 to −138 J K^{-1} mol^{-1} with increasing pH. K_a varies with temperature which makes the determination of k_2 difficult. $\Delta H^{\ddagger}_{obs}$ is a function of the temperature coefficients of the k_2 step, pH, and the pre-equilibrium. The energy parameters, unsurprisingly, vary with pH. The value of $\Delta H^{\ddagger}$ is somewhat lower than the one found by Skonieczny[10] ($\Delta H^{\ddagger}$ at 25 °C = 81.2 kJ mol^{-1}) but this is probably attributable to a difference in solvent.

The value of k_{obs} varies between 9.4×10^{-6} s^{-1} and 52.4×10^{-6} s^{-1} at pH 5 and 60 °C over the range of substituents tested. The mechanism of hydrolysis probably involves normal nucleophilic aromatic substitution on the protonated substrate. In the K_a equilibrium (**4**) ⇌ (**5**) (Scheme 3), the substituent in



Scheme 3.

the 4-position will feel the development of a full negative charge on the heterocyclic nitrogen in (**5**). The values of $\rho_I = 1.4$ and $\rho_R^- = 2.3$ indicate that the 4-substituent delocalizes the negative charge by electron withdrawal. The k_2 process probably involves normal nucleophilic aromatic substitution on the protonated substrate, (**4**) ⟶ (**6**). A full negative charge is developed in the product adduct (**6**) but it will only be partially developed in the transition state (**7**). Thus the 4-substituent has less charge to act on than in the K_a equilibrium and hence the rates will be less sensitive to the identity of the 4-substituent and ρ_I and ρ_R^- (1.6 and 0.6, respectively) will be smaller, as observed.

Values of pK_a and log k_2 are both correlated with σ_R^-, although the effects are not cumulative, *i.e.* k_{obs} is not correlated. The compounds with the highest values of k_{obs} are those substituted in the 4-position by groups such as CO_2Me. These compounds have also been shown to undergo the most rapid nitro-reduction,[12] and show very poor hypoxia-selectivity *in vivo*.[12] The most interesting nitracrine derivatives from the biological point of view have been shown[6] to be those possessing electron-donating substituents. The present-work demonstrates that such compounds will also be the most resistant to hydrolytic breakdown, since both ρ_R and ρ_I values are positive.

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References

1 W. A. Denny, B. C. Baguley, B. F. Cain, and M. J. Waring, in 'Molecular Aspects of Anticancer Drug Action,' eds. S. Neide and M. J. Waring, MacMillan, London, 1983, p. 1.
2 M. Warwas, B. Narezewska, and W. Dobryszycka, *Arch. Immunol. Ther. Exp.*, 1977, **25**, 235.
3 J. Konopa, J. W. Pawlak, and K. Pawlak, *Chem. Biol. Interact.*, 1983, **43**, 175.
4 K. Pawlak, A. Matuszkiewicz, J. Pawlak, and J. Konopa, *Chem. Biol. Interact.*, 1983, **43**, 131.
5 W. R. Wilson, W. A. Denny, S. A. Twigden, B. C. Baguley, and J. C. Probert, *Br. J. Cancer*, 1984, **49**, 215.
6 W. R. Wilson, L. H. Thompson, R. F. Anderson, and W. A. Denny, *J. Med. Chem.*, 1989, **32**, 31.
7 W. R. Wilson, W. A. Denny, G. M. Stewart, A. Fenn, and J. C. Probert, *Int. J. Radiat. Oncol. Biol. Phys.*, 1986, **12**, 1235.
8 S. Skonieczny, A. Organiak, A. Snarska, A. Kunikowski, S. Nowak, and A. Ledochowski, *Pol. J. Chem.*, 1978, **52**, 2125.
9 A. Ledochowski and E. Zylkiewicz, *Ann. Soc. Chim. Pol.*, 1969, **43**, 291.
10 S. Skonieczny and A. Ledochowski, *Heterocycles*, 1976, **4**, 1693.
11 S. Skonieczny, *Heterocycles*, 1980, **14**, 985.
12 W. R. Wilson, R. F. Anderson, and W. A. Denny, *J. Med. Chem.*, 1989, **32**, 23.
13 A. Ledochowski and B. Stefanska, *Ann. Soc. Chim. Pol.*, 1966, **40**, 301.
14 A. Albert and E. P. Serjeant, in 'The Determination of Ionization Constants. A Laboratory Manual,' Chapman and Hall, New York, 1984, pp. 80–81.
15 E. A. Guggenheim, *Philos. Mag.*, 1926, **2**, 538.
16 L. P. Hammett, *J. Am. Chem. Soc.*, 1937, **59**, 96.
17 Y. Yukawa and Y. Tsuno, *Bull. Chem. Soc. Jpn.*, 1959, **32**, 971.
18 C. G. Swain, S. H. Unger, N. R. Rosenquist, and M. S. Swain, *J. Am. Chem. Soc.*, 1983, **105**, 492.
19 S. Ehrenson, R. T. C. Brownlee, and R. W. Taft, *Prog. Phys. Org. Chem.*, 1973, **10**, 1.
20 W. A. Denny, B. F. Cain, G. J. Atwell, C. Hansch, A. Panthananickal, and A. Leo, *J. Med. Chem.*, 1982, **25**, 276.

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