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Structure-Activity Relationships for Thiolytic Cleavage Rates of Antitumor Drugs in the 4'-(9-Acridinylamino)methanesulfonanilide Series

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SUMMARY

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4'-(9-Acridinylamino)methanesulfon-*m*-anisidide (NSC 156303; *m*-AMSA) is an experimental broad-spectrum antitumor agent also displaying immunosuppressive and antiviral activity. In pharmacological studies this compound had a short half-life *in vivo* as a result of rapid chemical reaction with proteins. Interaction of *m*-AMSA with thiols and other nucleophiles has been studied and the reaction products characterized. The reaction of greatest biological relevance is nucleophilic attack at the acridine C-9 position by thiols. The relationships among structure, thiolysis rates, and antileukemic activity of over 50 substituted acridines have been investigated. Rapid reaction with thiols is not a prerequisite for antileukemic (L1210) activity with these agents.

INTRODUCTION

Acridine derivatives have long been known to exhibit activity in a large number of biological systems (1). Their practical importance in the treatment of malaria is well established (2), and the demonstration of antitumor, antiviral, and immunosuppressive activity of acridine derivatives (1, 3-5) suggests that in the future their practical use may be extended. The demonstration by Cain *et al.* (6, 7) of antitumor activity in a large number of 9-anilinoacridine derivatives has led to the formulation of certain structure-activity relationships in terms both of lipophilic-hydrophilic balance (7) and of certain electronic and steric factors which influence activity toward L1210 leukemia (8). An example of these agents, 4'-(9-acridinylamino)methanesulfon-*m*-anisidide (NSC 156303), has been selected by Drug Research Development, Division of Cancer Treatment, National Cancer Institute, United States Public Health Service, as a candidate for clinical trial.

We observed in pharmacological studies that ^{3}H-labeled *m*-AMSA[1] had a short biological half-life because of a rapid reaction with proteins *in vivo*.[2] This study describes the reaction of *m*-AMSA and related agents with model compounds containing

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[1] The abbreviations used are: AMSA, 4'-(9-acridinylamino)methanesulfonanilide; *m*-AMSA, 4'-(9-acridinylamino)methanesulfon-*m*-anisidide.

[2] W. R. Wilson, B. F. Cain, and B. C. Baguley, manuscript in preparation.

functional groups normally encountered in proteins. Effects of agent substituents on thiolysis reaction rates are quantitated, and possible correspondence between chemical reactivity and antileukemic (L1210) activity is examined.

MATERIALS AND METHODS

Agents. All AMSA congeners were prepared in this laboratory. Synthetic details and physical constants for the compounds either have been described (3, 6–8), are in the process of publication, or will shortly be published. The numbering system employed for these agents is shown in Fig. 1.

Thin-layer chromatography. Aluminum-backed sheets spread with 0.25 mm SiO_2 F_{254} (Merck) were employed. Compounds were located by ultraviolet scanning; 9-anilinoacridines and 4-aminomethanesulfonanilides quenched the fluorescence of the absorbent, and acridine 9-thioethers, 9 (10*H*)-acridones, and 9-aminoacridines were highly fluorescent.

Determination of rates of thiolysis. Spectrophotometric rate assays were carried out with a Shimadzu UV200 double-beam spectrophotometer having jacketed cell holders. Temperature was controlled at 37°. The pH 7.3 phosphate buffer was prepared by mixing solutions of 0.067 M Na_2HPO_4 (765 ml) and 0.067 M KH_2PO_4 (235 ml). Reactants (thiols, glycine, methylamine hydrochloride, etc) were dissolved in the smallest possible volume of the above buffer, and the pH of the solution was adjusted to 7.3 by the addition of 0.067 M KH_2PO_4 as necessary. Solutions were then adjusted to the necessary volume by addition of the pH 7.3 phosphate buffer, and the pH was rechecked. Dilution with an equal volume of methanol provided buffered solvent reactant mixtures for use, in which all the compounds were sufficiently soluble.

Ethanethiol and 2-mercaptoethanol in buffer–methanol mixtures absorbed oxygen quite rapidly from the air, and, as gauged by changing thiolysis rates, active thiol concentration could drop by two-thirds within 2 weeks. Accordingly, samples of thiols were fractionally distilled in an N_2 atmosphere and used to prepare buffer–methanol solutions. These solutions were immediately dispensed (5-ml quantities) into ampoules, which were evacuated and sealed. With each new batch of prepared thiol the half-life ($t_{1/2}$) of *m*-AMSA was determined as a control measure. In the assays of reaction rate the solvent cell (1-cm path) contained 2 ml of solvent–buffer and 2 ml of 0.2 M thiol solution from a freshly opened, temperature-equilibrated ampoule. The sample cuvette contained 2 ml of thiol solution from the same ampoule in addition to 2 ml of a 40 μM solution of acridine derivative in solvent–buffer. The spectrum was scanned over the interval 350–500 nm at appropriate time intervals. Values of the pseudo-first-order rate constants (k) for the rate of drug disappearance were obtained from the slope of plots of log A at the appropriate wavelength against time. $t_{1/2}$ was calculated from $0.69/k$.

Identification of thiolysis products. A solution of *m*-AMSA (0.02 M) and sodium acetate (0.02 M) in 65% ethanol–water (125 ml) at 10° was treated with ethanethiol (0.04 M). After 24 hr at 10°, solvents were removed under vacuum at 10°, and the residue was shaken with light petroleum (200 ml) and water (100 ml). A solid residue, which thin-layer chromatography showed to consist predominantly of 9(10*H*)-acridone (*5*, Fig. 1), was removed by filtration and the phases were separated. After drying (Na_2SO_4) and concentration, the petroleum base provided 9-ethylthioacridine (1.83 g; 38% crude yield) (*2*; R = C_2H_5, Fig. 1). Further crystallization from light petroleum provided a homogeneous compound which could not be separated from 9-ethylthioacridine by thin-layer chromatography; the melting point of 63.5–64.5° was not depressed on admixture with authentic material, and the ultraviolet spectra of both samples were superimposable. Concentration of the aqueous phase from solvent partition and crystallization of the residue from small volumes of boiling water provided 3-methoxy-4-aminomethanesulfonanilide (2.62 g; 62% yield) as identified by melting point, mixed melting point, thin-layer chromatography, and ultraviolet spectra. If an

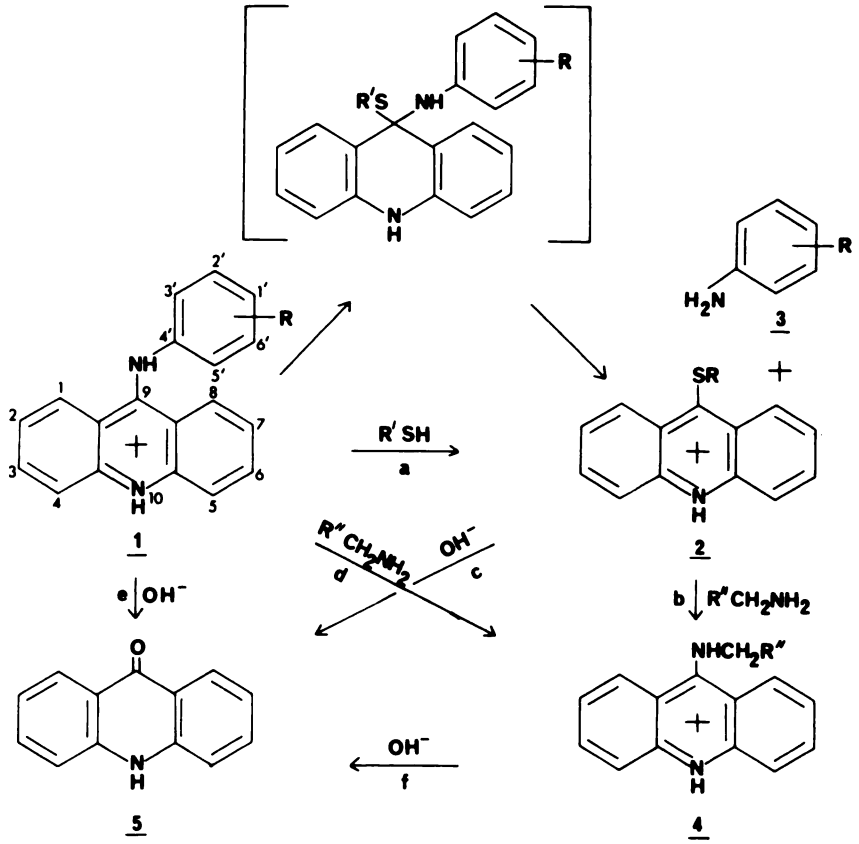


FIG. 1. *Component pathways in reaction of anilinoacridines with nucleophiles*

acid-binding agent, conveniently sodium acetate, was not included in the reaction mixture, the yield of thioether dropped markedly and the proportion of 9(10*H*)-acridone increased. If reaction mixtures identical in composition with that used above were heated to the boiling point of the solvent mixture, no thioether could be recovered and instead 9(10*H*)-acridone (92% of theory) resulted.

Synthesis of reference compounds for product identification. 9-Ethylthioacridine was prepared by addition of 9-chloroacridine (0.1 M) at room temperature to a solution of sodium ethylmercaptide prepared by adding ethanethiol (0.2 M) to a solution of sodium methoxide (0.2 M) in anhydrous methanol (100 ml). NaCl started to separate shortly after the addition of the 9-chloroacridine. After stirring for 1 hr at room temperature, thin-layer chromatography showed that no unchanged chloro compound remained. Following removal of solvent under vacuum at 10°, water (50 ml) was added, and the product was extracted into benzene (50 ml). The separated benzene layer was dried (Na_2SO_4) and evaporated to provide crude thioether. Multiple crystallizations from small volumes of light petroleum (60–80°) removed traces of contaminating acridone and gave chromatographically homogeneous 9-ethylthioacridine as pale yellow crystals, m.p. 63.5–64.5° [reported (9), 65°].

9-Methylaminoacridine was prepared by reaction of 9-phenoxyacridine with methylamine hydrochloride according to a published procedure (10). The hydrochloride salt had a melting point of 302–304° [reported (10), 302–303°], while the free base had a melting point of 173.5–174.5° [reported (10), 173–174°].

Authentic 9-hydroxycarbonylmethylaminoacridine (*4*; R = COOH, Fig. 1) was

prepared from glycine and 9-chloroacridine in a phenol melt. The product melted at 235° (with decomposition with the loss of CO_2) [reported (10), 236–238° with decomposition].

9-Ethylthioacridine proved as effective an intermediate for the preparation of 9-methylaminoacridine and 9-hydroxycarbonylmethylaminoacridine as either 9-phenoxyacridine or 9-chloroacridine. Conveniently ethanol could be employed as solvent. On heating an ethanol–water solution of the reactants, ethanethiol was distilled from the mixture; by displacement of equilibria in this fashion, there was a rapid conversion of the thioacridine to the substituted amine. The product amines were identical with the authentic samples described above as shown by melting point, mixed melting point, thin-layer chromatography, and ultraviolet spectra.

RESULTS AND DISCUSSION

Reaction of m-AMSA with nucleophiles. When *m*-AMSA, tritium-labeled in the acridine moiety, was incubated with freshly isolated mouse blood, radioactive adducts with proteins were formed, and the anilino side chain was liberated as 4-amino-3-methoxymethanesulfonanilide.[2] Clearly, a reaction between some functional group(s) of proteins and agent resulted in displacement of the 9-anilino side chain and covalent attachment of the acridine nucleus to the proteins. Trial reactions were therefore carried out with *m*-AMSA and model compounds containing functional groups commonly encountered in protein side chains.

With primary amines there was slow conversion of *m*-AMSA to a 9-substituted aminoacridine (cf. *4*, Fig. 1). Even hydroxide ion, at the low levels provided by the buffer solutions, was able to hydrolyze agents slowly to provide a 9(10*H*)-acridone (*5*, Fig. 1). There was quite rapid reaction with thiols, ethanethiol providing 9-ethylthioacridine (*2*; R = C_2H_5, Fig. 1) and 4-amino-3-methoxymethanesulfonanilide (*3*; Fig. 1). The further reaction of 9-alkylthioacridines with amines proved to be more rapid than that of *m*-AMSA with the amines alone (Table 1). Thiols could therefore act catalytically in this reaction and accelerate amine alkylation. Hydrolytic cleavage of the acridine thioether was also more rapid than that of the parent compound (Table 1); thiol also functioned catalytically in this case, accelerating the over-all rate of drug cleavage to acridone.

TABLE 1

Half-lives for component pathways of Fig. 1, where R = 1'-NHSO$_2$CH$_3$

Assays were performed at 37° in a 0.067 M sodium potassium phosphate buffer (pH 7.3)–methanol mixture (1:1). Initial concentrations of drug components were 20 μM, while those of displacing agents (except OH^-) were 0.1 M. Hydrolytic assays (OH^-; path *c*, *e*, and *f*) were measured in buffer mixture alone. Since rates of drug disappearance were measured, the $t_{1/2}$ figures using thiol and amine reactants are composite and contain a component due to the concurrent slower hydrolysis step to 9(10*H*)-acridone (paths *c* and *e*).

Pathway	Reactant	$t_{1/2}$
		hr
a	Ethanethiol	0.99
	2-Mercaptoethanol	0.92
b	Methylamine (R″=H)	3.9
	Glycine (R″=COOH)	4.7
c	Buffer medium	67.5
d	Methylamine (R″=H)	27.5
	Glycine (R″=COOH)	30.2
e	Buffer medium	108
f	Buffer medium	>1000

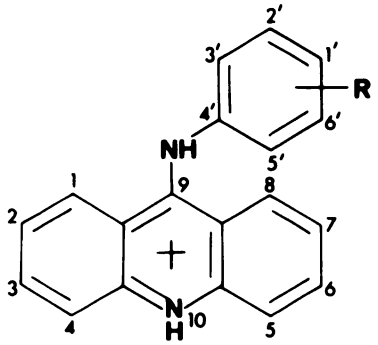
Common purines and pyrimidines which may undergo alkylation by antitumor agents (uracil, cytosine, adenine, guanine) did not appear to react with *m*-AMSA or 9-ethylthioacridine under the assay conditions used.

Determination of substituent effects on thiolysis reaction rate. To clarify the relationships between antileukemic activity and alkylative capacity, the effects of drug substituents on the thiolysis reaction were determined and compared with effects on biological activity (Table 2).

Drug decay in the presence of thiol was monitored by spectrophotometry, using the long-wavelength absorption of the acridinylsulfonanilides. There is also a decay rate component due to concurrent drug hydrolysis to 9(10*H*)-acridone (path *b*, Fig. 1).

TABLE 2

Comparison of thiolysis half-lives and antileukemic activities



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Substituents in *1*	$t_{1/2}$[a]	Antileukemic activity	
		Increase in life span[b]	Optimum dose[c]
	hr	%	*mg/kg/day*
6. R=1'-$NHSO_2CH_3$	0.92	107	45
7. R=1'-$NHSO_2CH_3$, 1-CH_3	6.5	34	200
8. R=1'-$NHSO_2CH_3$, 2-CH_3	3.2	—[d]	>500
9. R=1'-$NHSO_2CH_3$, 3-CH_3	1.7	94	25
10. R=1'-$NHSO_2CH_3$, 4-CH_3	0.75	90 (1)[e]	50
11. R=1'-$NHSO_2CH_3$, 1-OCH_3	2.6	—[d]	150
12. R=1'-$NHSO_2CH_3$, 2-OCH_3	10.8	—[d]	200
13. R=1'-$NHSO_2CH_3$, 3-OCH_3	2.0	81	35
14. R=1'-$NHSO_2CH_3$, 4-OCH_3	1.8	81	75
15. R=1'-$NHSO_2CH_3$, 1-NO_2	2.4	—[d]	75
16. R=1'-$NHSO_2CH_3$, 2-NO_2	2.8	27	250
17. R=1'-$NHSO_2CH_3$, 3-NO_2	0.82	123 (2)	25
18. R=1'-$NHSO_2CH_3$, 4-NO_2	1.4	—[d]	>500
19. R=1'-$NHSO_2CH_3$, 2-NH_2	26	78	25
20. R=1'-$NHSO_2CH_3$, 3-NH_2	8.8	81 (1)	2.5
21. R=1'-$NHSO_2CH_3$, 4-NH_2	9.8	87	125
22. R=1'-$NHSO_2CH_3$, 2-Cl	1.9	—[d]	200
23. R=1'-$NHSO_2CH_3$, 3-Cl	0.98	103	75
24. R=1'-$NHSO_2CH_3$, 4-Cl	1.5	—[d]	250
25. R=1'-$NHSO_2CH_3$, 2-NHAc	2.7	47	375
26. R=1'-$NHSO_2CH_3$, 3-NHAc	2.6	126 (2)	35
27. R=1'-$NHSO_2CH_3$, 4-NHAc	14.5	—[d]	>500
28. R=1'-$NHSO_2CH_3$, 3-SO_2CH_3	0.73	—[d]	>500
29. R=1'-$NHSO_2CH_3$, 2'-OCH_3	1.1	—[d]	>500
30. R=1'-$NHSO_2CH_3$, 3'-OCH_3	1.8	114 (2)	6.7
31. R=1'-$NHSO_2CH_3$, 3'-CH_3	54	106	97
32. R=1'-$NHSO_2CH_3$, 3,3'-$(CH_3)_2$	196	111	22
33. R=1'-$NHSO_2CH_3$, 3'-F	18.2	—[d]	>500
34. R=1'-$NHSO_2CH_3$, 3'-NH_2	51	106	45
35. R=1'-$NHSO_2CH_3$, α-Cl	0.50	49	110
36. R=H, 10-CH_3	0.48	—[d]	>500
37. R=1'-$NHSO_2CH_3$, 10-CH_3	0.40	83	110
38. R=1'-$NHSO_2CH_3$, 2-NH_2, 10-CH_3	1.35	68	5
39. R=1'-$NHSO_2CH_3$, 2-NHAc, 10-CH_3	0.50	35	15
40. R=1'-$NHSO_2CH_3$, 3-NH_2, 10-CH_3	3.8	74	1.5

continued

TABLE 2 (*continued*)

Substituents in *1*	$t_{1/2}$[a]	Antileukemic activity	
		Increase in life span	Optimum dose[c]
	hr	*%*	*mg/kg/day*
41. R=1′-SO_2NH_2	12	—[d]	>500
42. R=1′-$CONH_2$	5.6	—[d]	>500
43. R=1′-Cl	2.4	—[d]	>500
44. R=H	0.95	—[d]	>500
45. R=2′-NH_2	0.92	46	40
46. R=1′-OCH_3	0.48	—[d]	400
47. R=1′-OH	0.32	58	100
48. R=1′-NH_2	0.20	72	33
Mepacrine	19		
9-Aminoacridine	>1000[f]		
9-Methylaminoacridine	>1000		
Chloroquin	>1000		

[a] Half-life of drug disappearance at 37° in 0.067 M sodium potassium phosphate buffer–methanol (1:1) 0.1 M in 2-mercaptoethanol and with an initial drug concentration of 20 μM. Each value is the mean of duplicate determinations except for that of *30*, which is the mean from 13 experiments (standard deviation, ±0.16).

[b] Maximum percentage increase in life span observed in L1210 tests; inoculum size, 10^5 cells intraperitoneally, with daily intraperitoneal treatment on days 1–5 (3).

[c] The optimum dose which provided the maximum increase in L1210 leukemia tests when doses were spaced at 0.18 log dose intervals; alternatively, for agents inactive against L1210, the maximum dose which did not provide toxic deaths before those of leukemic control animals.

[d] L1210-inactive. Increase in life span was less than 25% at the doses employed; the maximum dose used was 500 mg/kg/day.

[e] Figures in parentheses provide the number of 50-day survivors (from an initial group of six) that resulted when L1210 leukemic animals were treated with drug at the optimum dose.

[f] Drug breakdown could not be detected under the standard assay conditions when the thiolysis half-life exceeded 1000 hr.

This latter component was negligible in relation to thiolytic cleavage (cf. routes *a* and *b*, Fig. 1 and Table 1), and the observed reaction rates were considered to approximate those for the thiolysis reactions. Results are quoted as $t_{1/2}$ values for rate of drug disappearance. Under assay conditions using large excesses of thiol, the kinetics of drug disappearance was pseudo-first-order.

Structure-activity relationships: 9-anilino ring substituents. There appeared to be an excellent correspondence between the effect of anilino ring substituents on antileukemic (L1210) activity and rates of thiolysis. In earlier publications we have shown that the lipophilic-hydrophilic balance of agents is a dominant factor controlling biological activity; increasing lipophilic character with respect to the parent AMSA agent reduces or abolishes antileukemic activity (7). In addition there is a requirement for electron donor substituents at position 1′, 2′, or both (6, 8). Of the agents with different 1′-substituents in Table 2 (*6*, *41–44*, *46–48*), only those in which the substituent is hydrophilic and electron-donating are active in L1210 antileukemic assays. Electron donor substituents also markedly increase rates of thiolysis. There is an excellent correlation between log $t_{1/2}$ and Hammett's σ_p (11) (Fig. 2).[3]

These findings suggested that a prerequisite for antileukemic activity might be an ability to alkylate a critical host or tumor cell function. However, further investigation has shown the thiolytic cleav-

[3] Alteration of the 1′-anilino substituents has been found to change the pK_a of the acridinium nitrogen, with a dependence on σ_p of the substituent (G. J. Atwell, unpublished results). Alteration of pK_a may account for the changes in the half-life of thiolysis in this series.

age to be very sensitive to steric hindrance. A 3′-sulfonanilide ring substituent increases the half-life of thiolysis sharply without corresponding reduction of antileukemic activity. For example, a 3′-CH_3 (*31*) provides a 59-fold increase. Combination in one molecule of two substituents which independently augment $t_{1/2}$ [as in the 3,3′-$(CH_3)_2$ variant (*32*)] provides a molecule in which thiolysis proceeds at a negligible rate while full biological activity is retained.

The pronounced steric effect of a 3′ function appears anomalous when the spatial separation of that function from the presumed point of thiol attack at C-9 is considered. Molecular models show that a marked steric interaction between the 9-methanesulfonanilide ring and the 1,8-positions of the acridine could be expected. X-ray crystallographic analysis has shown that in crystals of AMSA (*6*, Table 2) the $C_9-N-C_{4'}$ bond angle is widened from usually observed values and the ring plane of the anilino function lies orthogonally to that of the acridine (12). Models of such a structure make it appear remarkable that a single 3′-CH_3 group can produce a 59-fold increase in $t_{1/2}$. However, examination of models of the transition state complex resulting from thiol addition to C-9, this atom now having tetrahedrally oriented substituents, provides a satisfactory explanation. Assuming that the thiol approaches the unhindered acridine face, i.e., from the opposite face to that obtended by the 3′-CH_3 group, the steric interactions between the adjacent 1,8-acridine positions and the 9-anilino ring must force the 3′ group against the acridine ring as a tetrahedral configuration is attained (Fig. 3). The steric inhibition of thiolysis observed could then be a result of a buttressing effect of the substituent, making the formation of a tetrahedral C-9 atom less favorable.

Structure-activity relationships: acridine substituents. Cationic drug species would be expected to be more susceptible to nucleophilic attack than the corresponding neutral molecules. In agreement with this, more acid pH values, which provide a greater proportion of cat-

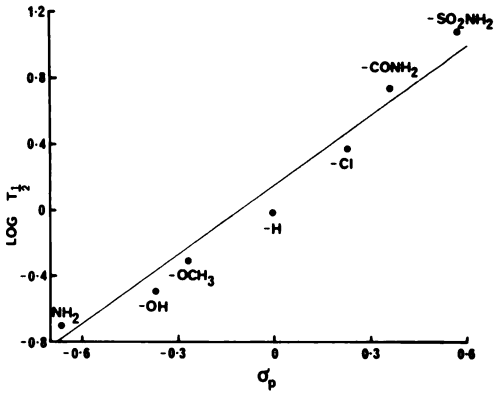

FIG. 2. *Relationship between half-lives ($t_{1/2}$) for 1′-substituted agents in thiolysis assays and Hammett's σ_p values*

The substituents are on position 1′ (*1*, Fig. 1) $t_{1/2}$ values are taken from Table 2 and plotted against Hammett's σ_p values quoted by Tute (11).

ionic drug species,[4] decrease $t_{1/2}$ values (Table 3). At alkaline pH the converse effect is observed.

The rates of thiolysis might also be responsive to acridine nucleus substitution because of (a) effects on pK_a; electron-withdrawing substituents reduce pK_a and thereby provide less of the highly reactive cationic species; (b) a counter-effect, since the neutral molecule is made more susceptible to nucleophilic attack by electron-withdrawing groups; (c) steric inhibition of thiol approach by substituents at positions 1 and/or 8; or (d) resonance effects of substituents on C-9 electron density of the cation and the corresponding neutral species.

In agreement with (a), N-10 quaternization, which increases base strength, provides derivatives which decay at least twice as rapidly as their unquaternized counterparts at pH 7.3[4] (compare the corresponding pairs *6, 37*; *19, 38*; *20, 40*; and *36, 44*). The quaternary analogues are similar to the parent bases in their antileukemic activity.

In conflict with (a), electron donor substituents (CH_3, *7–9*; OCH_3, *11–14*; NH_2, *19–21*), although augmenting base

[4] The pK_a of *m*-AMSA has been determined as 7.34 ± 0.05 (Dr. Robert Engles, Drug Research and Development, National Cancer Institute, personal communication).

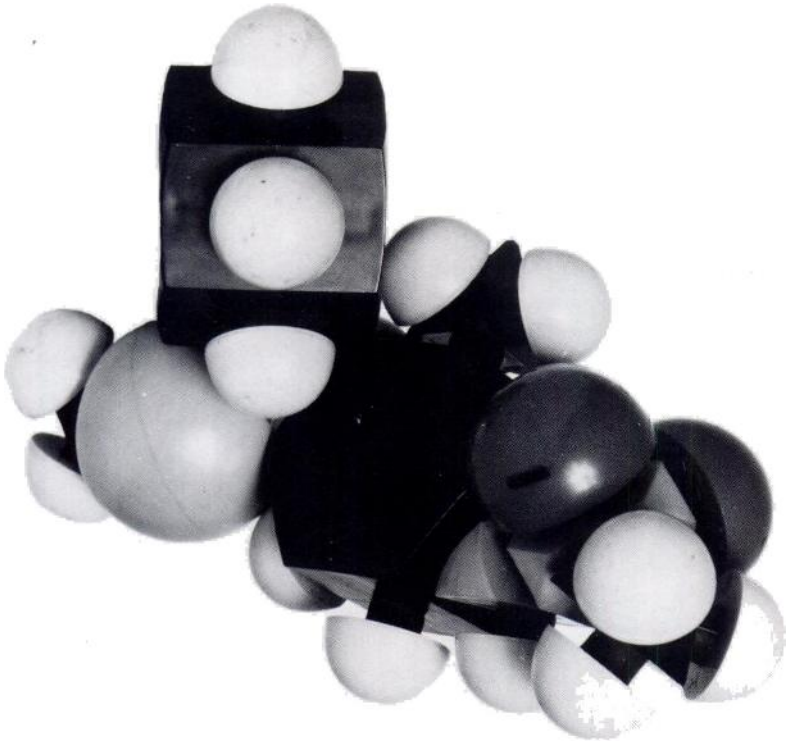

FIG. 3. *Model of proposed tetrahedral intermediate in thiolysis reaction*

The acridine nucleus is seen edge-on at the top of the figure. As the thiol (large sulfur atom) enters from the left, the methanesulfonanilide moiety moves to the right. The 3'-methyl group on this function, seen adjacent to the acridine nucleus on the right, hinders the formation of the tetrahedral intermediate.

TABLE 3

Variation in decay rate of AMSA (1, Fig. 1; R=1-$NHSO_2CH_3$) with changing pH in thiolysis assays

Assays were carried out as described for Table 1, the thiol being 2-mercaptoethanol. The half-lives are the means of duplicate determinations except for that at pH 7.3, which is the mean of seven determinations (standard deviation, ±0.06).

pH	$t_{1/2}$
	hr
5.6	0.32
6.1	0.35
6.7	0.51
7.3	0.92
8.0	1.40

strength (ref. 1, pp. 437–439), provide analogues with increased half-lives of thiolysis. This is the opposite effect to that encountered with electron donor sulfonanilide ring substituents. Donor substituents at position 3 or 4 (*9, 10*; *13, 14*; *20, 21*) are compatible with anti-L1210 activity. Most functions substituted at position 1 or 2 have proved inactive against L1210 leukemia; the only 2-substituted derivative with appreciable activity is the amine *19*, which is relatively stable to thiolysis.

Electron acceptor functions, by reducing pK_a, decrease levels of the reactive cation. However, no simple relationship between effects of substituents on pK_a in the 9-aminoacridines (ref. 1, p. 438) and measured $t_{1/2}$ values could be found. For example, 2-, 3-, and 4-nitro-9-aminoacridines have the same pK_a (7.5), but rates of thiolysis for the nitro-substituted AMSA agents (*15–18*) are appreciably different. Of analogues with electron-accepting 3-functionality, one (SO_2CH_3, *28*) proved inactive against L1210 leukemia whereas two were active (NO_2, *17*; Cl, *23*). The latter functions proved detrimental to antileukemic activity when attached at the adjacent position 2 (*16, 23*) or 4 (*18, 24*).

The larger $t_{1/2}$ values of the 1-CH_3 (*7*), 1-

OCH_3 (*11*), and 1-NO_2 (*15*) analogues, in comparison with their vinylogous 3-isomers (*9*, *13*, and *17*), might reflect steric effects as in (c), but the differences are slight and examples with more sterically demanding functions need examination to confirm this point. The 1-substituted variants screened have very poor antileukemic properties.

Thiolysis of simple acridines. 9-Aminoacridine and 9-methylaminoacridine did not undergo thiolysis under these conditions, nor did they show anti-L1210 activity. Interestingly, the antimalarial agent mepacrine, as an example of a clinically utilized acridine, underwent thiolytic cleavage (Table 2). It is remarkable, in view of the current intense interest in antimalarial therapy, that this reaction with thiols has escaped attention. Gniazdowski *et al.* (13) have recently reported that the tumor-inhibitory 1-nitro-(9-dialkylaminoalkylamino)acridines react with thiols and have suggested that the 1-nitro group is involved in the reaction. In the light of our results, it would be interesting to measure the amount of C-9 thiolysis occurring in their reactions.

CONCLUSIONS

Substituent effects on antileukemic activity and rates of thiolytic cleavage differ. Electron donor 1′-substituents are necessary for biological activity and also increase reaction rates with thiols. However, the steric inhibition of thiolysis (as in *30* and *31*) with full retention of antileukemic activity suggests that a capacity to react rapidly with a thiol is not a prerequisite for antileukemic activity in this drug series. The acridine ring-substituted variants (Table 2) also display a range of rates of thiolysis, and there is no obvious relationship between these and the life extensions obtained in L1210 leukemia tests. However, from the thiolysis rate data, substituents can be selected which will confer agent stability. Effective modulation of biological half-lives of agents should then be possible. The utilization of thiolysis data in drug design and the possible integration of thiolysis reaction rates into quantitative structure-activity relationships are currently under examination.

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