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Antitumour Activity of Substituted 9-Anilinoacridines—Comparison of *In Vivo* and *In Vitro* Testing Systems*

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Abstract—*The search for derivatives of* 9-*anilinoacridine by Cain and co-workers* [1] *using murine* L1210 *leukemia as a test system, culminated in the synthesis of* 4′-(9-*acridinylamino*)*methanesulphon*-m-*anisidide* (m-*AMSA*, *NSC* 249,992), *which is now undergoing clinical trial. The steps on the development of this and related compounds have been retraced using* L1210 *cells in culture. The results obtained with the* in vitro *system have indicated* m-*AMSA to have an appropriate choice of substituents for optimal activity, in agreement with results of the corresponding* in vivo *test system.* m-*AMSA causes a* 50% *inhibition of* L1210 *cell growth in culture under standard conditions* (3 *day cultures*) *at* 3.5×10^{-8} M. *Three compounds in which the substituent on the anilino ring contains a second benzene ring are highly active in culture, one being* 100-*fold more active than* m-*AMSA.*

INTRODUCTION

MANY antitumour drugs currently in clinical use or under clinical trial are known to bind to DNA. These include the anthracycline antibiotics such as Adriamycin [2], actinomycin D [3] derivatives of the plant product ellipticine [4] and the synthetic acridine derivative *m*-AMSA [5]. Each compound entering the clinic is usually selected from a large series of analogues which have been compared using transplantable rodent neoplasms. Selection of a particular agent for clinical trial must be made on the basis of a combination of such results with toxicological trials in other animals, and is dependent on the availability of a stable, soluble form of the drug for pharmaceutical use.

In the case of *m*-AMSA, the selection which led eventually to clinical trial was made from among many other derivatives of 9-anilinoacridine on the basis of *in vivo* testing with the murine L1210 leukemia [1, 5, 6], at that time the primary experimental tumour screen acceptable to the National Cancer Institute, U.S.A. It is pertinent to ask whether the congener chosen as optimal in the mouse leukemia system will also be optimal for the treatment of human tumours. Although it is obviously impractical to undertake clinical trials for comparing the activity of such a large series, comparisons could readily be made using cultured human tumour cells. As a necessary step towards testing the relevance of results obtained in cell culture to those obtained *in vivo*, we have compared the activity of a group of anilinoacridine derivatives towards cultured L1210 cells. The group of 68 derivatives includes those from which *m*-AMSA was finally chosen. The results show that, with the exclusion of two subclasses of agents comprising five compounds, the inhibitory activity in culture of 9-anilinoacridines mirrors their capacity to extend the lifetime of leukaemic mice. The activity of these compounds is also related to their association constants for double-stranded DNA, the probable target for their action.

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MATERIALS AND METHODS

Culture methods

The growth medium was RPMI 1640 (Gibco, Grand Island, U.S.A.), supplemented

with 50 μM mercaptoethanol and 10% foetal calf serum (Laboratory Services Ltd., New Zealand, non heat-deactivated). L1210 cells were initially obtained from Dr. I. Wodinsky, Arthur D. Little Inc., Boston, U.S.A., under the auspices of the National Cancer Institute. The cells were the same as those used for *in vivo* testing [6]. They were transferred to New Zealand in carrier DBA/2 mice, passaged in the same mice, and cell stocks were then frozen in liquid nitrogen using standard techniques. A fresh aliquot was thawed for use every 3 months. After thawing, the cells were injected intraperitoneally into DBA/2 mice. Cells were removed from mice in 2 ml of sterile medium 3 days after inoculation of 10^6 cells; approximately 4×10^7 cells were recovered and very few red blood cells were present. The cells were diluted to 10 ml with prewarmed medium and centrifuged (900 ***g***, 5 min, 20°C). They were then resuspended in prewarmed medium, diluted to 3×10^4, 1×10^5 and 3×10^5 cells/ml, and transferred in 4 ml portions to McCartney bottles. The cultures were incubated at 37°C in a humidified incubator with an atmosphere of 5% CO_2 in air. The cell densities of these initial cultures were measured every 24 hr in a Coulter model ZF electronic cell counter. Providing that 2-mercaptoethanol was present, that red cells were virtually absent, and that the cells were not allowed to cool for long periods during the manipulations, almost no lag in growth occurred, and the cells grew with a doubling time of 15–18 hr.

The cells were subcultured when they reached a concentration of no more than 10^6 cells/ml. They were routinely diluted to 1.5×10^4 cells/ml twice, and to 2×10^5 cells/ml once, during one week, i.e., they were subcultured three times per week. The doubling time decreased over the first three weeks to 12–14 hr and thereafter remained essentially constant. Assays for drug inhibition were conducted between 3 weeks and 3 months after the establishment of each new culture.

Tests for growth inhibition were conducted in 24 well culture trays (Nunc or Linbro). Stock cultures containing no more than 10^6 cells/ml were diluted to 3×10^4 cells/ml with prewarmed medium and 1 ml portions were added to the wells. The trays were then placed in the CO_2 incubator for 1 hr while drug solutions and dilutions were prepared. These solutions were made in 50% aqueous ethanol for ease of drug dissolution. Serial 2-fold dilutions in 50% ethanol were prepared immediately before drug addition to minimise drug loss by adsorption. Trays were removed from the incubator and the drug solutions were added (2 μl with an S.M.I. Micropettor) to the centre surface of each culture. Five drug concentrations, each in duplicate, were set up for each agent, together with two control cultures containing 2 μl of 50% ethanol. After drug addition, the cells were immediately returned to the incubator, and the cell number of each well was assayed after 70 hr. The cells were suspended and 0.5 ml of the suspension was diluted with 9.5 ml of 0.15 M NaCl. The presence of 0.1% ethanol in the cultures did not affect growth significantly (0.5% ethanol was found to reduce cell numbers by 10–20% after 3 days).

Agents

All anilinoacridine derivatives were synthesised at the Cancer Research Laboratory, University of Auckland, Medical School, Auckland, and provided by Professor B. F. Cain. Purity was checked by thin layer chromatography on silica gel plates using either chloroform:methanol (11:1) or the upper phase of *n*-butanol:acetic acid:water (5:1:4) as developing solvent. Only one ultraviolet light (365 nm wavelength) absorbing spot was observed in all cases. A fluorescent second spot corresponding to 9 (10H) acridanone was also observed in many cases, and was a result of the very slow spontaneous hydrolysis of the anilinoacridines. However, this was only a minor species, and the degree of contamination with acridone did not correlate with activity. 9-Aminoacridine hydrochloride, ethidium bromide and poly (dA-dT) were from Sigma, U.S.A.

DNA binding assay

Values of C_{50}, defined as the concentration of drug required to halve the observed fluorescence due to DNA-bound ethidium, were determined as previously described [7], in 0.01 M buffer, pH 5 (9.4 mM NaCl, 2 mM sodium acetate buffer, pH 5, 0.1 mM EDTA, 1 μM poly (dA-dT) and 1.26 μM ethidium). Association constants were derived from C_{50} values by assuming competitive intercalative binding between drug and ethidium, and a binding constant of ethidium for poly (dA-dT) of $9.5 \times 10^6\ M^{-1}$ [8]. A correction was made (in a separate assay) for drug-induced quenching of the fluorescence of bound ethidium [9]. This correction varied according to the magnitude of quenching observed, and reduced the estimate of the association constant by up to 4-fold. A full account of the

methods and calculations involved is published elsewhere [9].

RESULTS

Culture conditions

A detailed description of the methods used has been given, since acceptable reproducibility of data depends on the maintenance of optimal conditions. 2-Mercaptoethanol is not essential but in its absence the cell growth rate can decrease unpredictably. Control of pH was achieved by using a CO_2 incubation system rather than a closed system [10]. The culture volume (1 ml) was chosen for economy as well as for good oxygen diffusion into the culture. Rapid subculture and drug addition procedures were used to minimise temperature and pH fluctuations.

The standard deviation on the number of cells counted per control culture in a single set of assays (typically 20 samples) was $\pm 7\%$. Reproducibility in the presence of a drug concentration causing 50% inhibition was $\pm 9\%$. The errors arise mainly from pipetting of cells and drug solutions. Because of the relationship between inhibition and drug concentration, these errors resulted in the standard deviation for an ID_{50} determination in a single test (duplicate samples) being $\pm 15\%$. Over a number of repeat determinations during 3 months, the standard deviation of measured ID_{50} values was $\pm 23\%$. Most of the compounds listed in Tables 1–3 were tested on at least two separate occasions over a three month period, and the reproducibility was within these limits. Typical results are shown in Fig. 1.

Comparison of in vitro *activity, single substituents*

The structures, biological and DNA binding data for a range of monosubstituted anilinoacridines are shown in Table 1. The *in vivo* data has been published previously [1, 6, 9]. The ID_{50} values in L1210 cultures varied from 0.3 to 17,000 nM, as compared to 2600 nM for 9-aminoacridine. Drug precipitation did not occur, even at the highest concentrations. With the exception of compounds containing an additional benzene ring in the substituent, the most active agent was AMSA [4′-(9-acridinylamino)methanesulphonanilide, compound 10]. Substitution at the 1′-position with benzamido or benzenesulphonamide substituents provided highly active compounds, the ID_{50} value of the *p*-aminobenzenesulphonamide derivative (compound 13) being 100-fold lower than that of compound 10. Compound 13 *in vivo* shows

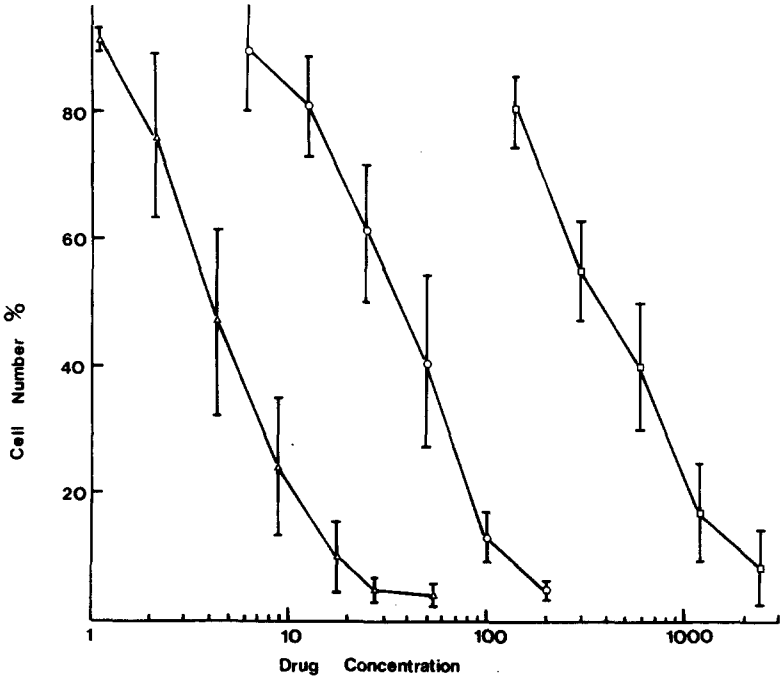
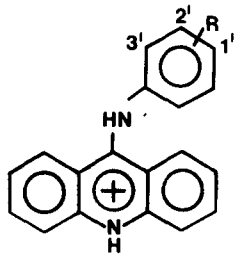


Fig. 1. Relationship between final L1210 *cell density and drug concentration for* m-*AMSA (compound* 36, *Table* 2) *averaged over* 16 *determinations during the course of* 3 *months* (○). *Results for compounds* 4(□) *and* 12(△), *averaged over six determinations, are also shown (see Table* 1). *Cultures were grown in the presence of each drug for* 3 days, *and cell densities are expressed as a percentage of the control cell density (approximately* 10^6 cells/ml). *The standard deviations of the determinations are shown by the vertical lines about each point.*

Table 1. *Antitumour and DNA binding data for monosubstituted* 9*-anilinoacridines*



No. R	ID_{50}* (nM)	MTD† (mg/kg/day)	ILS%‡ obs	ILS%‡ pred	$10^6 \times K$§ (M^{-1})	MR‖	Rm¶
1 H	2600	200	<25‡‡	(17)	0.71	1.03	0.43
2 1'-OH	250	100	58**	58	1.9	2.85	0.32
3 1'-OCH_3	500	110	<25**	(46)	1.3	7.87	0.52
4 1'-NH_2	350	33	72**	52	2.0	5.42	−0.08
5 1'-$NHCH_3$	180	40	51**	64	3.5	10.3	0.24
6 1'-$N(CH_3)_2$	600	60	43**	43	3.2	15.6	0.66
7 1'-$NHCOCH_3$	100	20	53**	75	2.0	14.9	0.26
8 1'-$N(CH_3)COCH_3$	300	50	42**	55	0.33	19.4	0.38
9 1'-$NHCOC_6H_5$	20	67	41**	(103)	1.5	34.6	0.64
10 1'-$NHSO_2CH_3$	35	45	107**	93	1.4	18.2	0.00
11 1'-$N(CH_3)SO_2CH_3$	300	120	58††	55	0.78	22.8	0.12
12 1'-$NHSO_2C_6H_5$	3.5	37	97**	(134)	1.6	37.9	0.45
13 1'-$NHSO_2C_6H_4NH_2$	0.3	4.5	115††	(178)	2.0	40.0	−0.04
14 1'-$NHCOOCH_3$	60	50	62**	84	2.3	17.0	0.47
15 1'-$NHCONHCH_3$	100	60	112‡‡	75	1.7	18.0	0.26
16 1'-CH_3	1000	125	18‡‡	34	1.6	5.65	0.69
17 1'-Cl	12,000	100	<25‡‡	(0)	0.98	6.03	0.60
18 1'-Br	7000	300	<25‡‡	(0)	1.05	8.9	0.60
19 1'-$COCH_3$	300	>500	<25‡‡	(55)	0.74	11.2	0.35
20 1'-$CONH_2$	6000	>500	<25**	(2)	0.68	9.8	−0.25
21 1'-CN	17,000	200	<25‡‡	(0)	0.50	6.3	0.24
22 1'-SO_2NH_2	5000	>500	<25**	(5)	0.91	12.3	−0.47
23 1'-NO_2	17,000	>500	<25‡‡	(0)	0.30	7.4	0.27
24 2'-OH	1600	270	<25**	(25)	1.0	2.85	0.38
25 2'-NH_2	340	40	46**	53	1.3	5.42	0.09
26 2'-$NHCH_3$	1000	55	<25**	(34)	1.3	10.3	0.49
27 2'-$N(CH_3)_2$	2900	75	<25**	(14)	0.95	15.6	0.63
28 2'-$NHCOCH_3$	650	33	33**	41	0.79	14.9	0.35
29 2'-$NHSO_2CH_3$	100	330	93**	75	0.54	18.2	0.02
30 2'-$NHCOOCH_3$	2200	50	<25**	(20)	1.30	17.0	0.49
31 3'-OH	2300	>500	<25**	(19)	0.23	2.85	0.54
32 3'-NH_2	400	85	<25**	(50)	0.58	5.42	0.15

*ID_{50}, nanomolar concentration of drug required to reduce the growth of cultures L1210 cells by 50% after 3 days.

†MTD, maximum tolerated drug dose in L1210 tests in mice. The indicated was given intraperitoneally on days 1–5 after inoculation (day 0) of 10^5 L1210 cells.

‡Percentage increase in lifespan of drug treated animals as compared to control groups receiving 10^5 L1210 cells intraperitoneally. obs = values from indicated sources; pred = values predicted from a regression equation in $\log(1/ID_{50})$. The values in parentheses were not used for the development of this equation. See text.

§Association constant for DNA [poly (dA-dT)] determined at 0.01 M ionic strength [9].

‖Molar refractivity values taken from the compilation of Hansch *et al.* [20], or extrapolated from these values by addition of a value of 4.5 for each added methylene group.

¶*Rm* values $\lceil = \log(1/R_f - 1)\rceil$. Measure of agent lipophilic–hydrophilic balance [12].

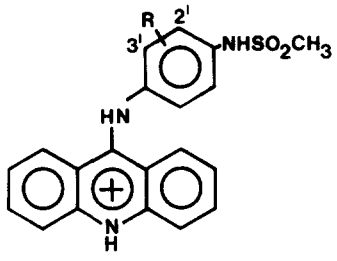
**Ref. [5].

††Ref. [6].

‡‡Ref. [9].

§§Taken from data supplied by Professor B. F. Cain. c.f. Computer-smoothed ILS maximum values which have been published [21].

Table 2. Antitumour and DNA binding data for anilino-substituted derivatives of AMSA (compound 10)



No. R	ID_{50}* (nM)	MTD† (mg/kg/day)	ILS%‡ obs	pred	$10^6 \times K$§ (M^{-1})	Rm¶
10 H	35	45	107**	93	1.6	0.00
33 3′-NH_2	1200	45	106††	(30)	1.9	−0.29
34 3′-OH	4000	25	88††	(9)	0.34	−0.17
35 2′-OCH_3	550	>500	<25††	(44)	1.3	0.08
36 3′-OCH_3	35	6.7	114††	93	0.37	0.18
37 2′-CH_3	1100	100	<25††	(32)	1.4	0.22
38 3′-CH_3	120	97	106††	71	0.55	0.25
39 2′-F	1200	>500	<25††	(30)	0.64	0.07
40 3′-F	800	>500	<25††	(38)	0.45	0.08
41 2′-Cl	1300	>500	<25††	(28)	1.5	0.23
42 3′-Cl	1300	>500	<25††	(28)	0.28	0.24
43 2′-NO_2	5500	>500	<25††	(3)	0.87	0.11
44 3′-NO_2	9000	>500	<25††	(0)	0.41	0.03
58 2′-aza	140	110	118††	69	0.37	−0.13
46 2′-$NHSO_2CH_3$	1000	250	53**	34	0.19	−0.18
47 3′-$NHSO_2CH_3$	5600	220	<25††	(3)	0.34	−0.17

See Table 1 for explanation of symbols.

comparable activity to that of AMSA in terms of maximal life extension, but it is considerably more dose-potent [6].

Choice of a second anilino substituent

In the initial development of anilinoacridine antitumour drugs, AMSA (compound 10, Table 1) provided the lead molecule for further synthetic studies [1]. Table 2 lists biological data for available derivatives of this compound having a second anilino substituent. A large range of ID_{50} values was obtained, but the most active molecule was *m*-AMSA (compound 36). In contrast, compounds 33 and 34, which showed high activity in the *in vivo* test, did not show correspondingly low ID_{50} values in cell culture. *m*-AMSA bound less well than AMSA to DNA, both in the ethidium displacement assay and in direct binding studies with calf thymus DNA [11].

Variation in alkyl chain substitution

Lipophilic–hydrophilic balance plays a major role in drug pharmacodynamics [12] and can conveniently be varied in the AMSA series by synthesising the homologous 1′-alkanesulphonamides. Table 3 lists the biological properties of such series based on *m*-AMSA and AMSA, and also compares them with those for 1′-alkanamino- and 1′-alkanamido series. The compounds most active in culture were the methyl and ethyl homologues of AMSA and *m*-AMSA. Although in general, activity as measured by ID_{50} values decreased with increasing chain length, the decrease was not uniform. Furthermore, in the case of the alkanamides (52–58, Table 3) branching of the alkyl chains gave compounds which were more active than their corresponding *n*-alkyl isomers.

DISCUSSION

The results in Table 1 show that substitution of the anilino- ring of 9-anilinoacridine provides a series of compounds which span 60,000-fold in toxicity towards cultured murine leukaemia L1210 cells. The range is much larger than the 19-fold range in DNA binding constants determined for the same series. Of the 68 compounds, 28 have ID_{50} values in culture of less than 200 nanomolar. All 28 are active in *iv vivo* assays for antitumour activity, and with the exception of three compounds containing substituents with an additional benzene ring, the most active

Table 3. Antitumour and DNA binding data for alkyl-substituted series of 9-anilinoacridines

No.	R	ID_{50}* (nM)	*MTD*† (mg/kg/day)	ILS%‡ obs	ILS%‡ pred	$10^6 \times K$§ (M^{-1})	*MR*‖	*Rm*¶
(a) 1'-NHR derivatives								
5	CH_3	180	40	51**	64	3.5	10.3	0.24
48	CH_2CH_3	180	100	42**	64	2.8	15.0	0.51
49	$(CH_2)_2CH_3$	200	45	<25‡‡	(62)	2.2	19.0	0.74
50	$(CH_2)_3CH_3$	350	70	<25‡‡	(52)	2.8	24.3	0.86
51	$(CH_2)_5CH_3$	400	90	<25‡‡	(50)	2.7	33.3	1.03
(b) 1'-NHCOR derivatives								
7	CH_3	100	20	53**	75	2.0	14.9	0.26
52	CH_2CH_3	170	50	52**	66	1.4	19.4	0.54
53	$(CH_2)_2CH_3$	180	67	64**	65	1.1	23.9	0.62
54	$CH(CH_3)_2$	100	120	66**	75	0.9	23.9	0.69
55	$(CH_2)_3CH_3$	140	40	58**	69	1.4	28.4	0.77
56	$(CH_2)_2CH(CH_3)_2$	100	80	69**	75	1.6	28.4	0.75
57	$(CH_2)_4CH_3$	330	80	32**	53	1.0	32.9	0.80
58	$(CH_2)_3CH(CH_3)_2$	110	50	73**	74	1.1	32.9	0.80
(c) 1'-$NHSO_2R$ derivatives								
10	CH_3	35	45	107**	93	1.5	18.2	0.00
59	CH_2CH_3	35	150	98§§	93	1.7	22.7	0.25
60	$(CH_2)_2CH_3$	50	225	66§§	87	1.5	27.3	0.43
61	$(CH_2)_3CH_3$	100	200	63§§	75	2.0	21.7	0.56
62	$(CH_2)_4CH_3$	270	55	50§§	57	1.8	36.3	0.66
63	$(CH_2)_5CH_3$	500	110	35§§	46	2.0	40.7	0.75
(d) 1'$NHSO_2R$, 3'-OCH_3 derivatives								
49	CH_3	35	9.0	114††	93	0.37	18	0.18
64	CH_2CH_3	35	13.4	94§§	93	0.37	22.7	0.38
65	$(CH_2)_2CH_3$	40	25	88§§	91	0.30	27.3	0.53
66	$(CH_2)_3CH_3$	60	62.5	88§§	84	0.46	21.7	0.62
67	$(CH_2)_4CH_3$	75	16.6	67§§	80	0.27	36.3	0.68
68	$(CH_2)_5CH_3$	75	50	70§§	80	0.36	40.7	0.75

See Table 1 for explanation of symbols.

compounds are AMSA (compound 10), *m*-AMSA (compound 36, Table 2) and the two ethyl homologues of these agents (compounds 59 and 64, Table 3). Perusal of the antitumour data indicates that the culture system in most cases provides results in substantial agreement with those obtained *in vivo* for this series.

Nevertheless, certain discrepancies exist between the *in vitro* and *in vivo* data. The three compounds with an additional aromatic ring in the substituent (9, 12, 13, Table 1) range from highly active to poorly active in *in vivo* assays, but show high activity in culture. Poor solubility of highly lipophilic compounds such as 9 may lead to problems *in vivo*, where, at least for *m*-AMSA and AMSA [13], the cytotoxic effect depends on a transient high drug concentration. Previous results have shown that even the choice of anion used for the formulation of AMSA derivatives can affect their dose potency [1], presumably by affecting drug solubility. In cultures, drug solubility has not been a problem, and a low drug concentration is probably maintained over a long period (our data with *m*-AMSA indicate that a growth inhibitory effect is maintained over at least three days of cell culture).

Two compounds (33 and 34), the 3'-amino and 3'-hydroxy derivatives of AMSA, show good *in vivo* antitumour activity but poor activity in culture. One possible reason for this behaviour is that the amino and hydroxy groups are rapidly oxidised in culture. Apart from these compounds, all 18 derivatives which have ID_{50} values of greater than 1000 nM or above are inactive in the *in vivo* assay system.

With the exclusion of the five compounds mentioned above, a highly significant regression equation can be developed for increase in lifespan (ILS, expressed as a percentage at the optimal dose) in terms of $\log_{10}(1/ID_{50})$, where the units of $1/ID_{50}$ are M^{-1}:

$$ILS = 41\ (\pm 14)\ \log\ (1/ID_{50}) - 212$$
$$r = 0.69,\ n = 36,\ s = 18,\ P < 0.001.$$

The figure in brackets indicates the 95% confidence limits on the slope, and s is the standard error of the regression. The ILS

values predicted with this equation, based on the 36 *in vivo* active compounds, are listed in Tables 1–3. The equation also predicts 14 of the 27 inactive compounds in Tables 1–3 to be not significantly active, and predicts low activity for the remainder of this group.

A previous publication [7] suggested a correlation between biological activity of anilinoacridine derivatives *in vivo* and a combination of DNA binding, and molar refractivity of anilino 1′-substituents. This latter parameter is intended to reflect the ability of a substituent to interact with a nearby ion or dipole by induced polarisation [9]. A plot of DNA association constants versus molar refractivity (*MR*) values (Fig. 2) shows that those compounds with the highest *in vitro* activity are clustered in a region where log *K* and *MR* values are high. Regression equations were therefore developed, using log $(1/ID_{50})$ as a measure of biological activity, log *K* as a measure of DNA binding, and *MR* as a measure of the polarisability of the 1′-substituents. Since lipophilic–hydrophilic balance has been found to play a major role in the biological activity of anilinoacridines [1], terms in *Rm* (a chromatographic mobility parameter describing lipophilic–hydrophilic balance) and Rm^2 (since there is a parabolic dependence on *Rm* [1]) were tested as additional terms. The final equation (for the 39 1′-substituted compounds) contained terms in log *k*, *MR* and Rm^2, all of which entered the equation with significance above 99%:

$$\log (1/ID_{50}) = 1.84\ (\pm 1.06) \log K + 0.06\ (\pm 0.01)\ MR - 1.52\ (\pm 0.80)\ Rm^2 - 5.34$$
$$r = 0.82,\ n = 39,\ s = 0.56,\ P < 0.001.$$

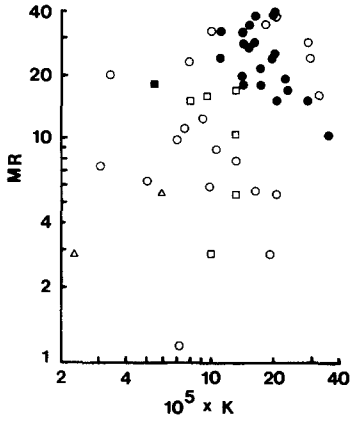


Fig. 2. Relationship between DNA association constant (K), *group molar refractivity values* (MR) *and activity in* L1210 *cell cultures. The circles, squares and triangles represent results for* 1′-, 2′- *and* 3′- *monosubstituted derivatives of* 9-*anilinoacridine, respectively* (*numbering as in Table* 1). *Closed symbols represent compounds with* ID_{50} *values less than* 200 nM.

Despite the overall success in deriving a regression equation for biological activity, this equation is an obvious oversimplification. For example, the capacity of the anilino substituent to form hydrogen bonds with a potential target may be extremely important, and is not considered in the equation. All but one of the 28 most active compounds contain a 1′-substituent containing potentially hydrogen bonding (acceptor or donor) functions. The remaining compound (36) has such a group in the 2′-position, and the stereochemistry of this methanesulphonamide substituent [14] implies that one of the sulphonamide oxygen functions has a similar position to that of one of the oxygens in the 1′-methanesulphonamide (compound 10).

The crystal structure of AMSA [14] indicates that the methanesulphonanilide substituent lies with the plane of the benzene ring almost orthogonal to that of the acridine. In this respect, AMSA resembles ethidium, and since it binds to DNA by intercalation [15], the anilino group may, by analogy with ethidium, project into the minor groove of the DNA double helix [16]. 1′-Substituents, and to a lesser extent 2′-substituents, project away from the DNA.

An attractive hypothesis to explain the wide range of biological activity of these compounds is that the 1′- or 2′-substituents make a secondary contact with a protein, and that this putative protein also binds to DNA. The ternary complex so formed would be expected to have a much greater stability than would the drug–DNA complex alone. Any hydrogen bond formed could increase the binding energy considerably, and dipole-mediated or hydrophobic bonding could allow further increases. Since hydrogen bonds are highly directional, both the chemical nature and the orientation of a substituent would be of prime importance.

There is at present no evidence as to the nature of the protein involved in this hypothetical ternary complex. It could be a chromosomal protein; ethidium is known to have high affinity sites in isolated chromatin [17]. Alternatively, it could be a deoxyribonuclease. Ethidium is known to activate a mitochondrial DNAse [18], and *m*-AMSA causes single-stranded breaks in mammalian DNA [19]. In the search for a potential target site, the availability of a compound which is 100-fold more potent than *m*-AMSA in culture (compound 13, Table 1) could be of considerable importance.

In conclusion, the use of culture techniques

in close conjunction with chemical synthesis can provide a rapid feedback of information to the medicinal chemist. For the group of agents discussed in this study, the information obtained from cell culture is highly relevant to the generation of ideas for new compounds. The outlay of time amounts to less than 2 hr per compound, assuming two assays on separate occasions, and the information gathered is admirably suited to the study of structure–activity relationships. Finally the relationship between *in vitro* and *in vivo* testing data for mice provides support for the concept that at least for congeners in this series, the *in vitro* screening of drugs using human cancer cells grown in culture will be relevant to the development of new agents for clinical use.

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