Eur J Cancer Clin Oncol, Vol. 24, No. 11, pp. 1783–1790, 1988.
Printed in Great Britain

0277–5379/88$3.00 + 0.00
Pergamon Press plc

Cell Line Selectivity and DNA Breakage Properties of the Antitumour Agent *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide: Role of DNA Topoisomerase II*

ERASMUS SCHNEIDER,* SANDRA J. DARKIN,*† PENELOPE A. LAWSON,† LAI-MING CHING, RAYMOND K. RALPH† and BRUCE C. BAGULEY‡

†*Department of Cellular amd Molecular Biology, University of Auckland, Auckland, New Zealand and Cancer Research Laboratory, University of Auckland School of Medicine, Auckland, New Zealand*

Abstract—N-[2-(*Dimethylamino)ethyl*]*acridine-4-carboxamide* (*NSC* 601316) *is a DNA intercalating experimental antitumour agent which is curative against the Lewis lung carcinoma in mice. Its action has been compared with amsacrine, its inactive isomer oAMSA, the solid tumour active derivative CI-*921 (*NSC* 343499), *a C-6 methylene chain-linked bisacridine* (*NSC* 210733), 9-*aminoacridine and quinacrine. All compounds inhibited the unknotting of phage P4 DNA by topoisomerase II in nuclear extracts prepared from L*1210 *cells. NSC* 601316 *inhibited growth of cultured L*1210, *P*388, *P/AMSA* (*P*388 *resistant to amsacrine*) *and P/ACTD* (*resistant to actinomycin D*) *cell lines at concentrations of* 87, 150, 2020 *and* 150 nM *respectively. A* 1 h *drug exposure to* 0.85 μM *NSC* 601316 *killed* 50% *of L*1210 *cells. L*1210 *cells treated for* 1 h *with NSC* 601316 *accumulated DNA breaks and protein–DNA cross-links. There was a good correlation between DNA breakage and cytotoxicity, but the relationship between drug concentration and number of protein–DNA cross-links was non-linear and differed from that of amsacrine and CI-*921. *There was also a positive correlation between the degree of cross-resistance of P/AMSA cells (which have altered topoisomerase II function) and ability to induce DNA breakage or protein–DNA complexes. The results suggest that topoisomerase II is the target of action of NSC* 601316.

INTRODUCTION

ACRIDINECARBOXAMIDE (*N*-[2-(dimethylamino)-ethyl]acridine-4-carboxamide; NSC 601316; see Fig. 1 for structure), was recently synthesized in the Cancer Research Laboratory [1]. It differs from 9-anilinoacridine derivatives such as mAMSA [2] and its derivative CI-921 [3] in several important respects. While mAMSA has only marginal antitumour activity *in vivo* against the Lewis lung carcinoma growing as lung nodules in mice [3], acridinecarboxamide cures approx. 90% of mice when treatment is commenced 5 days after inoculation of the tumour [1]. It is even more active against this tumour than CI-921, which has been selected for clinical trial on the basis of its solid tumour activity.

Acridinecarboxamide is more lipophilic than mAMSA, and in contrast to mAMSA the ionization of its acridine nitrogen is almost completely suppressed [1]. It binds to double-stranded DNA by intercalation with an association constant which is similar to that of CI-921 [1]. The side-chains of acridinecarboxamide and mAMSA project in opposite directions from the acridine nucleus and may thus occupy different grooves when bound to DNA. Kinetic studies using stopped flow techniques have shown that the rate of dissociation of acridinecarboxamide from DNA intercalation sites is much slower than that of mAMSA ([4] and Dr W.A. Denny, personal communication).

As a further step towards understanding the basis for the high activity of acridinecarboxamide towards solid tumours, its mode of action and cell line selectivity have been compared with those of several other acridine derivatives (see Fig. 1 for structures). mAMSA is in clinical use for the treatment of acute

Accepted 11 July 1988.
*Supported by the Auckland Division of the Cancer Society of New Zealand, the Medical Research Council of New Zealand and a Warner-Lambert Laboratory Fellowship. E.S. is a recipient of University Grants Committee and Swiss Cancer League Postdoctoral Fellowships. S.J.D. is a recipient of an Auckland Medical Research Foundation Senior Scholarship.
‡Address correspondence to Dr. Baguley, Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand.

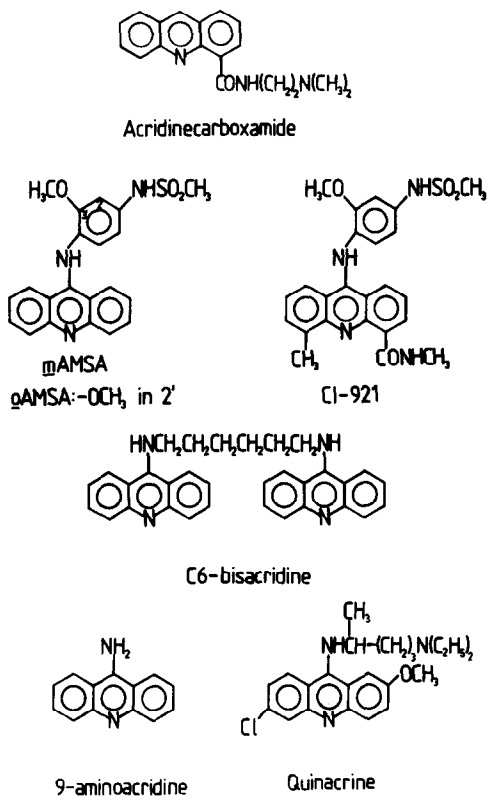

Fig. 1. Structure of acridinecarboxamide (free base) and of other acridine derivatives referred to in this study.

leukaemia [5] and is a potent and specific inhibitor of the enzyme DNA topoisomerase II [6]. oAMSA, an isomer of mAMSA, lacks antitumour activity and is correspondingly less active *in vitro* against topoisomerase II [6] while CI-921 is a potent inhibitor of this enzyme [7]. C-6 bisacridine was selected as a bifunctional intercalator with experimental antileukaemia activity [8]. 9-aminoacridine (an antibacterial) and quinacrine (an antimalarial) were also included for comparison.

MATERIALS AND METHODS

Materials

Methyl[^{3}H]thymidine (70–90 Ci/mmol) was obtained from Amersham and [^{32}P]dATP (800 Ci/mmol) from New England Nuclear. Phage P4 was a generous gift from Dr. R. Calendar, Department of Molecular Biology, Berkeley, California. The restriction enzyme EcoRI was obtained from Promega Biotec and DNA Pol I Klenow fragment from Boehringer Mannheim. mAMSA (isethionate salt) was provided by the Warner-Lambert Company, and 9-aminoacridine hydrochloride and quinacrine hydrochloride were obtained from the Sigma Chemical Co. oAMSA methanesulfonate, CI-921 isethionate, and C-6 bisacridine dihydrochloride were synthesized in the Cancer Research Laboratory, Auckland. Drug solutions for DNA studies were stored as 1 mM stock solutions in water at −20°C, and working dilutions were made immediately prior to use. Dextran grade B (MW 150–200 K) was obtained from BDH, proteinase K from Sigma Chemical Co., and Hoechst 33258 from Calbiochem Biochemicals.

Cell lines

P388 mouse leukaemia cells were from the National Cancer Institute, U.S.A. P/ACTD (resistant to actinomycin D) cells were from Mason Research Inc., Worcester, U.S.A. The P/AMSA line originated in Dr. R. Johnson's laboratory [9] and was provided by Dr. R.C. Jackson, Warner-Lambert Co., Michigan, U.S.A. L1210 cells were from Arthur D. Little Inc., U.S.A. Lines were stored in liquid nitrogen and propagated in carrier DBA/2J mice before establishing in culture.

Growth inhibition assays

These were performed in 24-well trays. Leukaemia cells were grown in RPMI 1640 medium supplemented with 50 μM 2-mercaptoethanol, FBS (10%) and antibiotics (streptomycin 100 μg/ml; penicillin 100 U/ml). Drugs were added after 2 h, the cultures harvested after a further 3 days [10]. In experiments with mAMSA and CI-921, the RPMI 1640 medium was supplemented with 50 μM ascorbate to prevent aerial oxidation of the drug [10]. Other compounds were resistant to oxidation. The IC_{50} was defined as the drug concentration required to reduce the number of cells in a culture by 50% with respect to control cultures.

Clonogenicity assays

L1210 cells were cultured at 5×10^4 cells/ml in αMEM supplemented with FBS (10%) and 50 μM 2-mercaptoethanol. The cells were used after 24 h of culture, when the density was $1.5–2.0 \times 10^5$ cells/ml. Cells were incubated with drug in growth medium (10 ml) at 37°C for 1 h. Cells were then centrifuged, washed once, counted and plated in 1% methylcellulose in growth medium. Colony growth was determined after incubation at 37°C for 5 days [11]. The D_{50} was defined as the drug concentration required to reduce the number of surviving clonogenic cells in a culture by 50% with respect to control cells.

Phage P4 DNA unknotting assay

Logarithmic-phase L1210 cells were used for the preparation of nuclei essentially as previously described [12] but using 0.1% Triton X for cell lysis and omitting Ca^{2+} from all nuclei isolation buffers. Topoisomerase II activity was recovered from the nuclei after destabilization of the scaffold with 2-mercaptoethanol and EDTA, sonication, precipitation of nucleic acids with polyethylene glycol and centrifugation at 30,000 ***g*** for 30 min

[12]. Knotted phage P4 DNA was prepared as described [13]. Assay mixtures (20 μl) contained 50 mM Tris–HCl, pH 7.5; 10 mM $MgCl_2$; 0.5 mM EDTA; 0.5 mM dithiothreitol; 30 μg/ml bovine serum albumin; 1 mM ATP; 0.24 μg P4 DNA and varying amounts of drugs. After addition of 4 μl of nuclear extract (approx. 1 unit of topoisomerase activity, 20–40 ng protein and 120 mM KCl), tubes were incubated for 30 min at 37°C. To remove proteins, 1 μl 10% sodium dodecyl sulphate (SDS) and 1 μl 10 mg/ml proteinase K were added and the mixture was incubated for a further 30 min at 37°C. Samples were electrophoresed in 0.7% agarose gels in 89 mM Tris–borate, pH 7.5; 89 mM boric acid; 2 mM EDTA. Gels were stained with ethidium bromide and photographed under u.v. illumination. Photographic negatives were analysed by scanning on a microdensitometer (Joyce, Loebl and Co.). One unit of topoisomerase II activity was defined as the amount of extract that completely unknotted 0.24 μg phage P4 DNA in the absence of drug. Protein concentrations were determined according to Bradford [14].

Quantitation of mAMSA-stimulated covalent protein–DNA complex formation

The SDS/K^+ precipitation assay [15, 16] was used to measure drug-induced formation of protein–DNA complexes in whole cells and nuclear extracts. For studies with whole cells, log-phase L1210 cells were labelled with 0.5 μCi/ml [methyl-^{3}H]thymidine (70–90 Ci/mmol) overnight, collected by centrifugation and washed once with PBS (137 mM NaCl; 2.6 mM KCl; 8 mM Na_2HPO_4; 1.4 mM KH_2PO_4). Cells were resuspended in fresh growth medium at 1×10^5 cells/ml, distributed in 1 ml aliquots in 24-well multiwell plates and incubated for 1 h at 37°C. They were then centrifuged at 1200 ***g*** for 10 min at room temperature, the supernatants discarded, the cells lysed with SDS and protein–DNA complexes collected as previously described [12]. Protein–DNA complexes were resuspended in 1 ml ice-cold 10 mM Tris–HCl, pH 7.5; 100 mM KCl; 2 mM EDTA and collected on Whatman GF/C filters under gravity. Filters were washed five times with wash solution under vacuum, dried and the retained radioactivity measured in a liquid scintillation spectrometer. The total acid-precipitable radioactivity per assay (10^5 cells) was routinely 5–7 × 10^4 cpm.

DNA breakage

DNA breakage induced by the acridine drugs in L1210 cells was determined by the fluorimetric assay of DNA unwinding (FADU) method [17]. Cells were drug treated for 60 min, centrifuged and resuspended to 10^6 cells/ml in ice-cold PBS. Aliquots of this suspension were assayed in triplicate for residual double-stranded DNA after adding an equal volume of 0.1 M NaOH and allowing DNA to denature for 30 min at room temperature (group B samples). Three samples were treated similarly under non-denaturing conditions (group A) and three were sonicated for 5 s before denaturation to ensure complete unwinding (group C). Fluorescence intensities were determined using a Shimadzu RF-540 spectrofluorimeter (excitation 351 nm, emission 451 nm). The fraction of residual double-stranded DNA, F, in group B samples after the fixed unwinding period was calculated using the relationship $F = (B-C)/(A-C)$ where A, B and C are the mean relative fluorescence intensities in groups A, B and C respectively.

RESULTS

In vitro *cytotoxicity of acridine derivatives in survival assays*

Growth inhibition tests using a panel of cultured mouse cell lines are shown in Table 1. Acridine carboxamide was considerably less toxic potent than mAMSA and CI-921 against L1210 and P388 lines. P/AMSA cells, were highly cross-resistant to acridinecarboxamide and CI-921, slightly cross-resistant to oAMSA, and slightly collaterally sensitive to C6-bisacridine, 9-aminoacridine and quinacrine. P/ACTD cells were cross resistant to C-

Table 1. In vitro *growth inhibitory activity and cytotoxicity of acridine derivatives*

Compound	Cell Line IC_{50} (nM)				Cell Line D_{50} (nM)
	L1210	P388	P/AMSA	P/ACTD	L1210
Acridinecarboxamide	87	150	2020	150	850
mAMSA	6.8	12.5	890	7.9	46
oAMSA	660	950	2800	210	
CI-921	4.2	4.9	200	5.1	20
C-6 bisacridine	110	340	150	1900	
9-Aminoacridine	1900	4600	1540	2100	
Quinacrine	2400	3300	1200	1800	

6 bisacridine but not to acridine carboxamide, mAMSA, oAMSA or CI-921 (Table 1).

The cytotoxicity of antitumour acridines was also compared following a short term incubation. Logarithmic phase L1210 cells were exposed to drug for 1 h, then washed free of drug and assayed for ability to form colonies *in vitro*. D_{50} values are shown in Table 1. D_{10} values (10% survival) were approx. 3-fold higher than D_{50} values, which were in turn 5–10-fold higher than the IC_{50} values determined for continuous exposure.

Effect of acridinecarboxamide and related compounds on DNA strand breakage

In order to determine the relative capacity of acridine derivatives to cause DNA breakage, a FADU assay was used which has recently been shown to give the same information as alkaline elution techniques [18, 19]. DNA breaks were detected in L1210 cells following a 1 h drug exposure by enhancement of the rate of alkaline denaturation of DNA, using the bisbenzamide fluorophore Hoechst 33258 as a probe for residual double-stranded DNA after a fixed denaturation time. Acridine carboxamide was less potent than mAMSA or CI-921 at high drug concentrations, consistent with the D_{50} results (Fig. 2). However, at low drug concentrations acridinecarboxamide produced almost as much DNA breakage as did mAMSA and CI-921. oAMSA had a slight effect and the other compounds produced little or no DNA breakage.

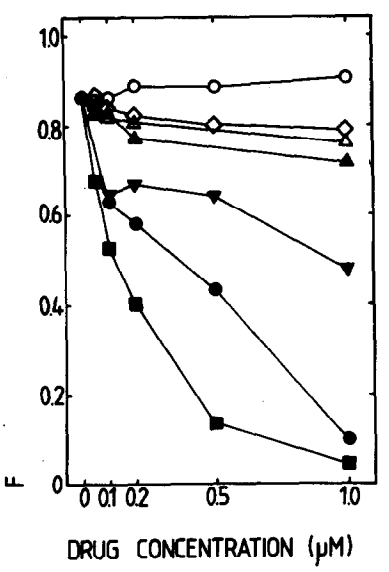

Fig. 2. Fluorescence assay for DNA unwinding after treatment of logarithmic phase L1210 cells (2 × 10^5 cells/ml) with drugs. L1210 cells were diluted with fresh growth medium before drug exposure. F is the fraction of DNA still in the duplex form after an alkaline unwinding time of 30 min. Values given are means from three independent experiments. Acridinecarboxamide (▼—▼); mAMSA (●—●); oAMSA (▲—▲); CI-921 (■—■); C6-bisacridine (△—△); 9-aminoacridine (◊—◊); quinacrine (○—○).

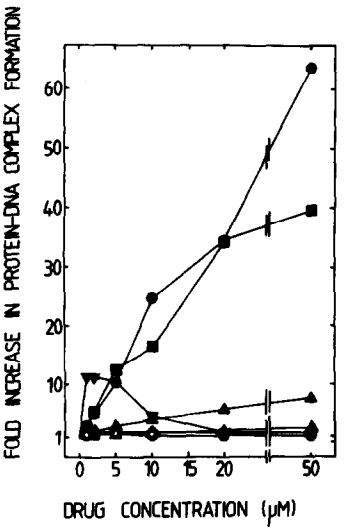

Fig. 3. Drug-stimulated formation of protein–DNA complexes in L1210 cells using [^{3}H]thymidine labelled cellular DNA as a substrate. Protein–DNA complexes formed were precipitated by SDS/K^+. Values given are means from three independent experiments. Acridinecarboxamide (▼—▼); mAMSA (●—●); oAMSA (▲—▲); CI-921 (■—■); C6-bisacridine (△—△); 9-aminoacridine (◊—◊); quinacrine (○—○).

Stimulation of protein–DNA complex formation

Acridine derivatives were compared in the extent to which they induced protein–DNA complex formation in whole L1210 cells in response to varying amounts of drug after a 1 h treatment (Fig. 3). As in the DNA breakage assay, only acridinecarboxamide, mAMSA, CI-921 and to a small extent oAMSA induced dose-dependent protein–DNA complex formation. Acridinecarboxamide was unusual in that it induced a higher rate of protein–DNA complex formation than mAMSA at low drug concentrations (1 μM induced as much complex as 5 μM mAMSA) but there was a gradual decline in complex formation at higher drug concentrations and no stimulation at all at 20 μM. An experiment using nuclear extracts from L1210 cells and [^{32}P]3′-end-labelled pBR322 DNA [12] produced the same overall pattern as that observed in whole cells except that the stimulation of protein–DNA complex formation by acridinecarboxamide occurred at higher drug concentrations and over a wider concentration range than with whole cells (results not shown).

Inhibition of topoisomerase II

To test the effect of the compounds on topoisomerase II activity, drugs were incubated with nuclear extract in a P4 unknotting assay and analysed by gel electrophoresis (Fig. 4). The minimal concentrations of drug inhibiting topoisomerase II P4 unknotting activity were: acridinecarboxamide, 50 μM; mAMSA, 20 μM; CI-921, 20 μM; oAMSA, 100 μM; C6-bisacridine, 20 μM; 9-aminoacridine, 20 μM; quinacrine, 50 μM.

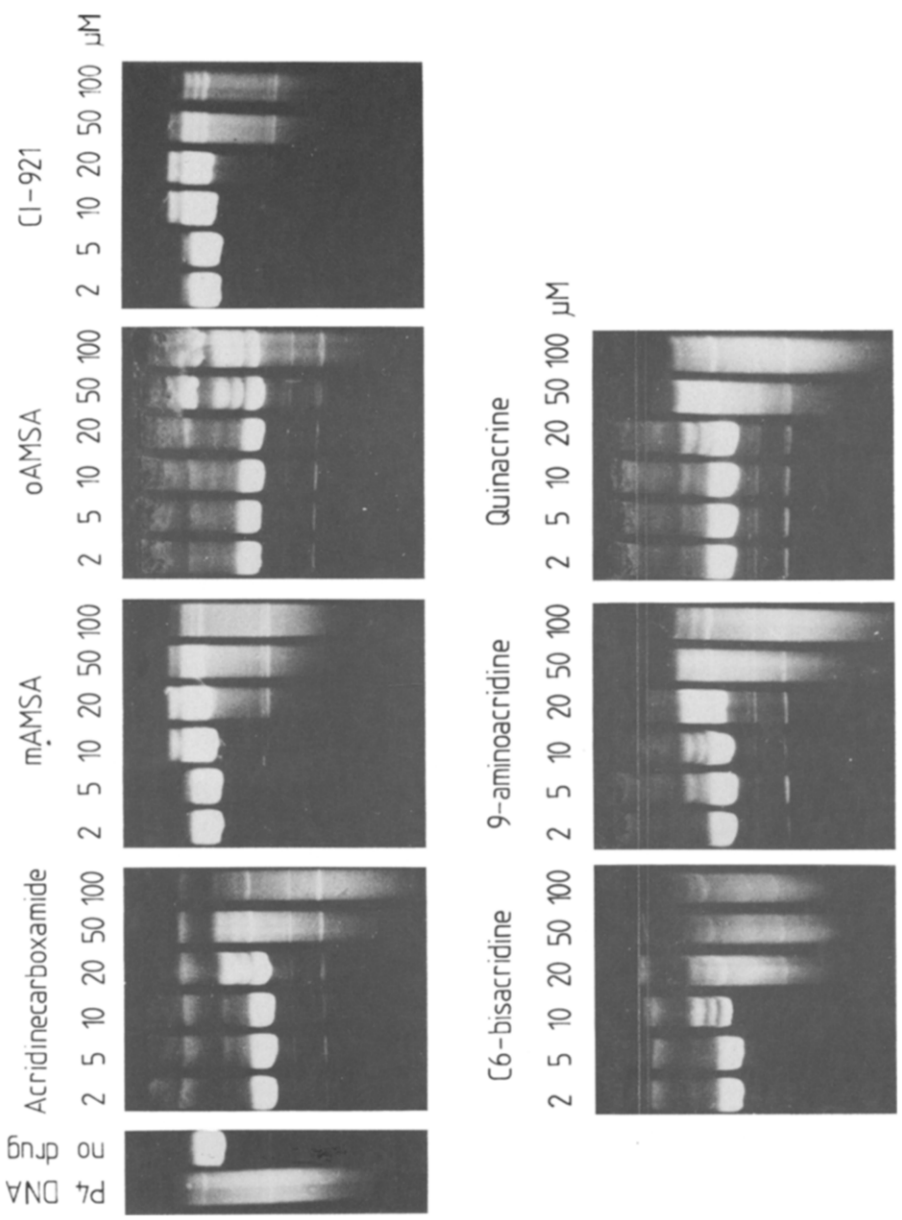

Fig. 4. Drug effects on the P4 unknotting activity in nuclear extracts. The minimal amount of nuclear extract from L1210 cells required for complete unknotting of 0.24 µgh P4 DNA (1 unit) was used in an unknotting assay with varying amounts of drug (0–100 µM) added as indicated. Reaction products were separated on a 0.7% agarose gel, stained with 0.5 µg/ml ethidium bromide and photographed under u.v. illumination.

DISCUSSION

The effects of acridinecarboxamide on DNA breakage (Fig. 2) and on the formation of protein–DNA complexes (Fig. 3), together with the cross-resistance of acridinecarboxamide to P/AMSA (Table 1), a cell line with altered topoisomerase II activity [9], support the proposal that topoisomerase II is the likely cytotoxic target of acridinecarboxamide. A clear relationship is apparent for the acridine derivatives in Fig. 1 between the degree of cross-resistance to the P/AMSA cell line (as shown by the ratio of IC_{50} values) and the induction of DNA breakage as measured by the FADU assay. Acridinecarboxamide, mAMSA and CI-921 show good activity and oAMSA shows a small effect while C6-bisacridine, 9-aminoacridine and quinacrine are inactive. In contrast to the P/AMSA results, acridinecarboxamide shows no cross-resistance to P/ACTD which is cross-resistant to vincristine and has the characteristics of a multidrug resistant line [20]. It is possible this property, in addition to factors such as drug distribution [10], explains the high activity of acridinecarboxamide against solid tumours.

There is an obvious difference between acridinecarboxamide and mAMSA in the dose dependence of formation of DNA–protein complexes (Fig. 3). The D_{50} concentration (0.85 μM) of acridinecarboxamide induces an 11-fold increase in complex formation, while equitoxic concentrations of mAMSA (0.046 μM) or CI-921 have no significant effect. In contrast, these concentrations cause similar DNA breakage (30–40% reduction in fluorescence) for all three drugs. The reason for the inhibition of drug-stimulated formation of protein–DNA complexes by high concentrations of acridinecarboxamide (Fig. 3), which is similar to that reported for 2-methyl-9-hydroxyellipticine [21], has not been elucidated.

Our results extend those of Rowe *et al.* [16] who showed that the capacity of mAMSA and other acridine-derived topoisomerase II directed anticancer drugs to cause DNA breakage was related to cytotoxicity as measured by IC_{50} assays. It is clear that the correlation between cytotoxicity and the capacity to introduce DNA breaks is not a universal feature of acridine derivatives, since C-6 bisacridine has been reported to have biological activity [8]. Markovits *et al.* [22] have suggested a different mechanism of action for the anticancer agent ditercalinium, which is also a bifunctional intercalator, and this mechanism may also apply to C-6 bisacridine.

In agreement with others [6] we find no correlation between the drug concentrations needed to inhibit DNA strand-passing activity (Fig. 4) and IC_{50} or D_{50} values. For instance, 9-aminoacridine has a much lower cytotoxic activity but shows the same inhibition of topoisomerase II as mAMSA in the phage P4 unknotting assay. A qualitatively similar result has also been obtained using a catenation assay for the measurement of topoisomerase II activity (A.M. Hutchins and R.K. Ralph, unpublished). Formation and stabilization of the cleavable complex by topoisomerase II, rather than inhibition of its formation, appears to be related to DNA strand breakage and cell death.

In conclusion, although acridinecarboxamide has been demonstrated to target the enzyme topoisomerase II, it differs from 9-anilinoacridine derivatives typified by CI-921 and mAMSA in being less potent both *in vitro* (Table 1) and *in vivo* [1]. This is surprising since acridinecarboxamide has a similar DNA binding constant to that of CI-921 [1, 3] and slightly higher potency than CI-921 or mAMSA in stimulating the formation of protein–DNA complexes (Fig. 3). It is notable that acridinecarboxamide, like its 9-amino derivative [4], lacks the presence of easily and reversibly oxidizable functions such as the phenolic or anilino groups which are found in mAMSA, CI-921 [23, 24], the anthracyclines, ellipticines, epipodophyllotoxins and anthracenediones. It is possible that the chemical reactivity of these topoisomerase II-directed cytotoxic drugs contributes an additional mechanism of toxicity, not shown by acridinecarboxamide, by which DNA breakage is induced at drug concentrations which cause only low amounts of protein–DNA complex formation. If this is the case, then acridine carboxamide, by not having this second mechanism, may belong to a distinct class of topoisomerase II-directed cytotoxic agent.

Acknowledgements—The authors are grateful to Linley Fray for IC_{50} determinations, to Margaret Snow for help with the manuscript.

REFERENCES

1. Atwell GJ, Rewcastle GW, Baguley BC, Denny WA. Potential antitumor agents. 50. *In vivo* solid tumour activity of derivatives of *N*-[2-(dimethylamino)ethyl]-acridine-4-carboxamide. *J Med Chem* 1987, **30**, 664–669.
2. Cain BF, Atwell GJ. The experimental antitumour properties of three congeners of the acridinyl methanesulphonanilide (AMSA) series. *Eur J Cancer* 1974, **10**, 539–549.
3. Baguley BC, Denny WA, Atwell GJ *et al.* Synthesis, antitumor activity and DNA binding properties of a new derivative of amsacrine *N*-5-dimethyl-9-((2-methoxy-4-methylsulfonylamino)phenylamino)4-acridinecarboxamide. *Cancer Res* 1984, **44**, 3245–3251.

4. Denny WA, Roos IAG, Wakelin LPG. Interrelations between anti-tumour activity, DNA breakage, and DNA binding kinetics of 9-aminoacridinecarboxamide anti-tumour agents. *Anti-Cancer Drug Design* 1986, **1**, 141–147.
5. Arlin Z. Current status of amsacrine combination chemotherapy programs in acute leukemia. *Cancer Treat Rep* 1983, **67**, 967–970.
6. Nelson EM, Tewey KM, Liu LF. Mechanism of antitumor drug action: poisoning of mammalian topoisomerae II on DNA by 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide. *Proc Natl Acad Sci USA* 1984, **81**, 1361–1364.
7. Covey JM, Kohn KW, Kerrigan D, Tichen EJ, Pommier Y. Topoisomerase II-mediated DNA damage produced by 4′-(9-acridinylamino)methanesulfon-*m*-anisidide and related acridines in L1210 cells and isolated nuclei: relationship to cytotoxicity. *Cancer Res* 1988, **48**, 860–865.
8. Chen TC, Fico R, Canellakis ES. Diacridines, bifunctional intercalators. Chemistry and antitumor activity. *J Med Chem* 1978, **21**, 868–874.
9. Per SR, Mattern MR, Mirabelli CK, Drake FH, Johnson RK. Characterization of a subline of P388 leukaemia resistant to amsacrine: evidence of altered topoisomerase II function. *Mol Pharmacol* 1987, **32**, 17–25.
10. Baguley BC, Finlay GJ. Derivatives of amsacrine: determinants required for high activity against Lewis lung carcinoma. *J Natl Cancer Inst* 1988, **80**, 195–199.
11. Finlay GJ, Ching L-M, Wilson WR, Baguley BC. Resistance of cultured Lewis lung carcinoma cell lines to tiazofurin. *J Natl Cancer Inst* 1987, **79**, 291–296.
12. Schneider E, Darkin SJ, Robbie MA, Wilson WR, Ralph RK. Mechanism of resistance of non-cycling mammalian cells to 4′-[9-acridinylamino]methanesulphon-*m*-anisidide; role of DNA topoisomerase II in log- and plateau-phase CHO cells. *Biochim Biophys Acta* 1988, **949**, 264–272.
13. Liu LF, Davis JL, Calendar R. Novel topologically knotted DNA from bacteriophage P4 capsids: studies with DNA topoisomerases. *Nucl Acids Res* 1981, **9**, 3979–3989.
14. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976, **72**, 248–254.
15. Liu LF, Rowe TC, Yang L, Tewey KM, Chen GL. Cleavage of DNA by mammalian DNA topoisomerase II. *J Biol Chem* 1983, **258**, 15365–15370.
16. Rowe TC, Chen GL, Hsiang YH, Liu LF. DNA damage by antitumour acridines mediated by mammalian DNA topoisomerase II. *Cancer Res* 1986, **46**, 2021–2026.
17. Kanter PM, Schwartz HS. A fluorescence enhancement assay for cellular DNA damage. *Mol Pharmacol* 1982, **22**, 145–151.
18. Robbie MA, Baguley BC, Denny WA, Gavin JB, Wilson WR. Mechanism of resistance of non-cycling mammalian cells to 4′-(acridinylamino)methanesulfon-*m*-anisidide (mAMSA): comparison of uptake, metabolism and DNA breakage in log- and plateau-phase cell cultures. *Cancer Res* 1988, **48**, 310–319.
19. Taningher M, Bordone R, Russo P, Grilli S, Sauti L, Parodi S. Major discrepancies between results obtained with two different methods for evaluating DNA damage: alkaline elution and alkaline unwinding. Possible explanation. *Anticancer Res* 1987, **7**, 669–680.
20. Rose WC, Trader MW, Laster W, Schabel FM. Comparative antitumor activity of actinomycin analogs in mice bearing Ridgeway osteogenic sarcoma or P388 leukemia. *Cancer Treat Rep* 1978, **62**, 779–789.
21. Pommier Y, Minford JK, Schwartz RE, Zwelling LA, Kohn KW. Effects of the intercalators 4′-(acridinylamino)methanesulfon-*m*-anisidide and 2-Me-9-OH-ellipticine on DNA topoisomerase II mediated DNA strand cleavage and strand passage. *Biochemistry* 1985, **24**, 6410–6415.
22. Markovits J, Pommier Y, Mattern MR *et al.* Effects of the bifunctional antitumour intercalator ditercalinium on DNA in mouse leukemia L1210 cells and DNA topoisomerase II. *Cancer Res* 1986, **46**, 5821–5826.
23. Jurlina JL, Lindsay A, Baguley BC, Denny WA. Redox chemistry of the 9-anilinoacridine class of antitumor agent. *J Med Chem* 1987, **30**, 473–480.
24. Davis LM, Harvey JD, Baguley BC. Electron donor properties of the antitumor drug amsacrine as studied by fluorescence quenching of DNA-bound ethidium. *Chem-Biol Interact* 1987, **62**, 45–58.