Eur J Cancer Clin Oncol, Vol. 19, No. 11, pp. 1575–1583, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00

Induction of *Petite* Mutants in Yeast by Non-intercalative DNA-binding Antitumour Agents*

LYNNETTE R. FERGUSON and BRUCE C. BAGULEY†
Cancer Research Laboratory, University of Auckland Medical School, Private Bag, Auckland, New Zealand

Abstract—*A series of 17 bis-charged non-intercalative DNA-binding antitumour agents and 7 related inactive compounds have been tested for the induction of respiratory deficient* (petite) *mutants in* Saccharomyces cerevisiae *D5. Many compounds were strong inducers of* petite *mutants at concentrations which were not toxic to the growth of the yeast cells. Mutagenicity is only weakly correlated with* in vitro *inhibition of L1210 cell growth; however, mutagenicity, yeast toxicity and* in vitro *and* in vivo *antitumour activity are all correlated with selective binding to polydeoxy(adenylic–thymidylic) acid rather than polydeoxy(guanylic–cytidylic) acid, as measured by an ethidium competition assay. It is concluded that A–T-rich DNA may be a target for all the biological effects measured in this study. Furthermore, the possibility that the target for antitumour action may be tumour cell mitochondrial DNA is supported by these results.*

INTRODUCTION

SEVERAL antibiotics and a number of synthetic compounds appear to bind reversibly in the minor groove of the DNA double helix [1]. Many of these compounds show experimental antitumour activity; of one class of these agents, termed the phthalanilides [2], some have been used in clinical trials [3]. Of a large group known as bisquaternary ammonium heterocycles [4], one compound, NSC 176 319, was selected as a candidate for trial [5] but has not been tested clinically. A second group of aromatic bisguanylhydrazones includes one compound, DDUG (4,4′-diacetyldiphenylureabisguanylhydrazone), which has been tested extensively as a candidate for clinical trial [6].

For a large series of bisquaternary ammonium heterocycles, antitumour activity was found to be related to the sequence selectivity of DNA binding (adenine–thymine vs guanine–cytosine) as well as to lipophilic character [7]. Studies using closed circular duplex DNA [8] confirmed earlier predictions based on model building [4] that these compounds bind strongly to DNA but do not intercalate. More recent studies using high field nuclear magnetic resonance techniques [9] indicate that the DNA minor groove is occupied by these molecules.

Few studies have been carried out on the mode of action of these compounds. Many are antiprotozoal agents [1], and many inhibit the development of bacteriophage lambda following its induction in *Escherichia coli* [10]. An action on kinetoplast DNA has been suggested as a mode of action of antiprotozoal compounds [11]. Pine and Di Paolo have reported that one member of the phthalanilide series inhibits mitochondrial function [12]. These studies suggest that closed circular DNA could be a target for the action of these compounds.

A variety of compounds are known to induce a state of respiration deficiency in yeast, characterised by the growth of *petite* colonies [13, 14]. Structural requirements for the induction of *petite* mutants have been established for DNA intercalating agents such as the phenanthridines, aminoacridines and anilinoacridine antitumour drugs [15,16]. Berenil, a non-intercalative DNA binder with antitrypanosomal activity but no antitumour activity, has also been shown to induce *petite* mutants in *Saccharomyces cerevisiae* [17].

In this communication, this observation has been extended to a range of di-charged non-intercalative DNA-binding antitumour agents,

Accepted 3 May 1983.
*This work was supported by the Auckland Division of the Cancer Society of New Zealand (Inc.) and in part by the Medical Research Council of New Zealand.
†To whom requests for reprints should be addressed.

including the bisquaternary ammonium heterocycles discussed above.

MATERIALS AND METHODS

Saccharomyces cerevisiae

The diploid yeast strain, *S. cerevisiae* D5 [18], was kindly provided by Dr B. S. Cox, Botany School, University of Oxford (U.K.). After the strain was received, it was inoculated into 100 ml of liquid yeast complete (YC) medium [17] and grown to stationary phase (24 hr). Ten per cent DMSO was added, and aliquots (1 ml) were frozen to -70°C and stored at this temperature until use.

Chemical compounds

Bisquaternary ammonium heterocycles were synthesised by Drs B. F. Cain, W. A. Denny and G. J. Atwell (this laboratory). Compounds were pure as judged by thin-layer chromatography. Ethidium bromide and polydeoxy(adenylic–thymidylic) acid[poly(dA–dT)] and polydeoxy (guanylic–cytidylic) acid [poly(dG–dC)] were purchased from the Sigma Chemical Company, U.S.A.

Petite mutagenesis assay

A frozen aliquot of *S. cerevisiae* was quickly thawed, added to 10 ml of fresh YC medium and shaken in a water bath at 30°C for 2 hr. For assays in growth medium, the cell suspension was diluted 100-fold into fresh YC medium before use. For assays under non-growing conditions, cells were centrifuged, washed, resuspended in sterile saline and shaken for a further 2 hr before use.

Drug treatment, plating and scoring of numbers of *petite* and *grande* colonies was as discussed previously [17]. All compounds were tested over a wide dose range, in both growth medium and saline. The rate of *petite* formation was also followed in both media, at a single dose of 50 μg/ml.

Murine L1210 leukaemia cultures

L1210 cells were obtained from leukaemic mice and were from the same source as those used for *in vivo* experiments [19]. They were cultured in Nunc 24-well trays in RPMI 1640 medium (Gibco, Grand Island, U.S.A.) supplemented with 0.05 μg/ml streptomycin, 75 IU/ml penicillin G, 50 μM mercaptoethanol and 10% foetal calf serum (Gibco New Zealand Ltd). Cells were inoculated at 3×10^4 cells/ml and agents were added in 2 μl of 50% aqueous ethanol at appropriate concentrations. Cell densities were measured using a Coulter ZF electronic cell counter. The ID_{50} value was defined as the nanomolar drug concentration required to decrease the cell density after 70 hr by 50% relative to that of the untreated control culture.

RESULTS

Comparison of different classes of non-intercalative DNA binding agents

Table 1 lists the biological data including the effects on *S. cerevisiae* cells, L1210 leukaemia cells in culture and (published) data on L1210 cells growing in mice. DNA binding selectivity is also indicated from the results of studies on the competition between drug and ethidium binding to DNA [7]. Structures are shown in Fig.1.

(*a*) *Diarylamidines.* Berenil (*1*) and stilbamidine (*2*) both induced the formation of *petite* colonies at concentrations of less than 1 μg/ml. Little inhibition of L1210 cell growth was observed, and these compounds are inactive towards L1210 leukaemia *in vivo* [9].

(*b*) *Pthalanilides.* Compounds *3* and *4* are representative of a large class of extended diarylamidines and related compounds termed the phthalanilides. Both are active in *petite* formation and towards L1210 leukaemia.

(*c*) *Bisquanylhydrazones.* Both examples included (*5* and *6*) are active antileukaemic agents, but only DDUG (compound *6*) induces formation of *petite* mutants under these conditions.

(*d*) *Bisquaternary ammonium heterocycles.* Compounds *7–11* cover a range of structures developed under the direction of the late Professor B. F. Cain in this laboratory [4]. As judged by ethidium displacement assays, they cover a range of DNA binding affinities and degrees of sequence selectivity. All are highly active experimental antileukaemia agents and all induce *petite* mutants. Compound 7 is the most active of this group, inducing 50% formation of mutants at 0.025 μg/ml.

(*e*) *Hybrid molecules.* Several compounds (*12–16*) containing a quaternary ammonium centre at one end and either amidine, guanidine, biguanide or methylguanylhydrazone at the other were also tested. These compounds were active in both *petite* mutagenesis and antileukaemia assays.

Comparison of growth medium and saline for, and kinetics of, petite *mutagenesis*

Petite formation occurred in saline even when cultures were starved up to 24 hr before use, with several saline washes. All compounds that formed *petite* mutants induced the conversion of at least 99% of cells. For the most active agents (*7, 17–19*), conversion occurred within 5 min at a drug concentration of 50 μg/ml. All drugs resembled 9-anilinoacridines rather than ethidium in showing a monophasic time course, with no reversibility

Table 1. Petite *forming ability, antitumour and DNA binding data for minor groove binding drugs*

No.	Compound	P_{50}* (mM)	D_{50}† (mM)	C_{50} values‡ (dA–dT) (μM)	(dG–dC) (μM)	Ratio§	ID_{50}‖ (μM)	Antitumour activity¶
1.	Berenil	0.45	8.5	0.9	5.7	6.3	10.4	-
2.	Stilbamidine	0.19	7.7	1.8	8.9	4.9	4.8	-
3.	6664	0.25	3.3	2.2	11	5.0	0.15	+
4.	6665	1.9	4.6	0.86	1.4	1.63	0.78	+
5.	DDUG	—**	9.9	0.43	1.3	3.0	0.11	+
6.	20440	5.0	113	2.9	7.6	2.62	1.95	+
7.	5754	0.20	1.4	0.4	0.7	1.75	0.08	+
8.	4094	22	4.9	1.5	2.1	1.4	0.9	+
9.	6999	.008	0.28	0.45	3.0	18.8	0.02	+
10.	6053	.008	0.46	0.14	1.8	12.9	0.092	+
11.	16814	17	30	5.3	11	2.08	0.08	+
12.	6135	0.074	0.74	0.77	1.71	2.2	0.2	+
13.	6134	2.2	1.1	0.30	0.52	1.73	0.025	+
14.	6136	0.05	0.52	0.91	2.1	2.31	0.13	+
15.	13628	0.49	63	0.22	0.34	1.5	0.060	+
16.	13538	1.1	67	0.38	0.87	2.29	0.11	+
17.	7000	0.009	0.34	0.19	3.0	16.3	0.04	+
18.	7002	0.010	0.62	0.20	3.1	15.0	0.044	+
19.	16041	0.009	0.50	0.20	3.0	14.7	0.08	-
20.	8147	0.42	59	0.22	0.55	2.5	0.27	+
21.	9592	3.8	27	4.5	15	3.3	0.25	-
22.	8518	3.4	72	6.0	10	1.67	1.5	-
23.	8305	7.9	110	2.5	6.5	2.6	1.5	-
24.	19881	—	240	6.4	8.4	1.3	0.56	-

*P_{50}: millimolar drug concentration (μg/ml) required to convert 50% surviving cells to *petite* mutants.

†D_{50}: millimolar drug concentration required to decrease by 50% the total number of viable yeast cells after drug exposure for 24 hr.

‡C_{50} values for poly(dA–dT) and poly(dG–dC) determined at an ionic strength of 0.01, pH 7.0: micromolar drug concentration which reduces by 50% the fluorescence of DNA-bound ethidium [7].

§Ratio: C_{50} for poly(dG–dC) divided by C_{50} for poly(dA–dT).

‖ID_{50}: micromolar concentration of drug required to reduce the growth of cultured L1210 cells by 50% after growth for 3 days.

¶Activity towards L1210 leukaemia in life extension assays. + = significantly active. Data from [4, 7 and 22].

**—, no *petites* formed at any drug concentration.

over 24 hr (data not shown). The reversibility of *petite* formation was tested by reinoculating treated cultures into fresh medium and regrowing for 24 hr before plating. All compounds resembled ethidium and berenil [20] in showing approximately 25% reversal of *petite* formation.

Relationship between petite *mutagenicity and lipophilic character*

Compounds *7*, *17*, *18* and *19* span a range of lipophilic character and vary from highly active to inactive in L1210 leukaemia tests *in vivo* [7]. All were highly potent inducers of *petite* formation, indicating that lipophilic character plays little part in determining effectiveness.

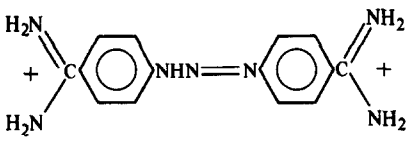
Relationship between petite *mutagenicity and DNA binding*

A table of correlation coefficients between the logarithms of measurements for biological activity and DNA binding parameters is shown in Table 2. As discussed previously [1], ethidium displacement measurements are not necessarily proportional to the inverse of DNA binding constants, since the size of the binding site is not constant. Nevertheless, it is apparent that *petite*

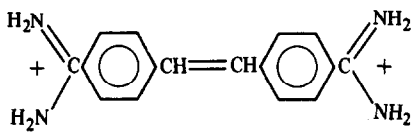
Table 2. *Correlation matrix for dependence of biological activity on DNA binding parameters*

Biological activity	Correlation coefficient log C_{50} poly(dA–dT)	log C_{50} poly(dG–dC)	Log ratio
log P_{50}	0.77	0.20	0.79
log D_{50}	0.61	0.23	0.61
log ID_{50}	0.60	0.46	0.29

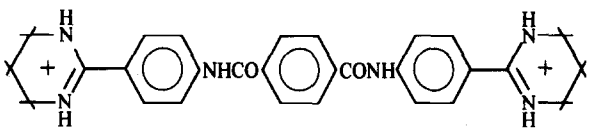
1



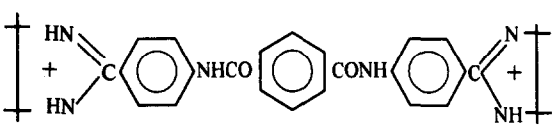
2



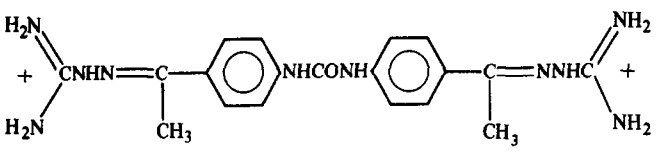
3



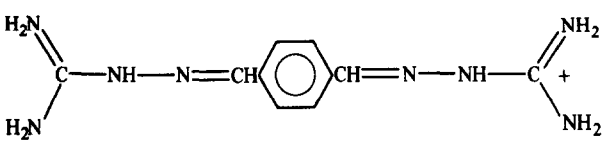
4



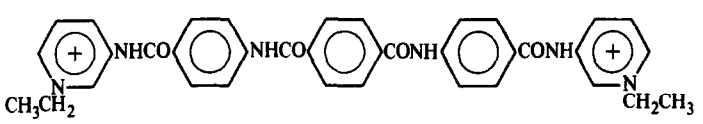
5



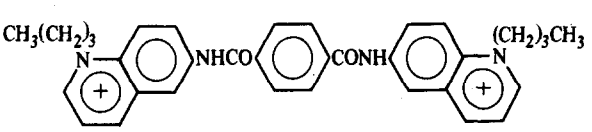
6



7



8



9

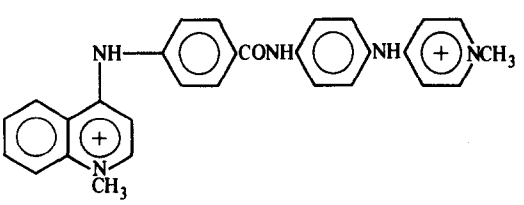


Fig. 1.

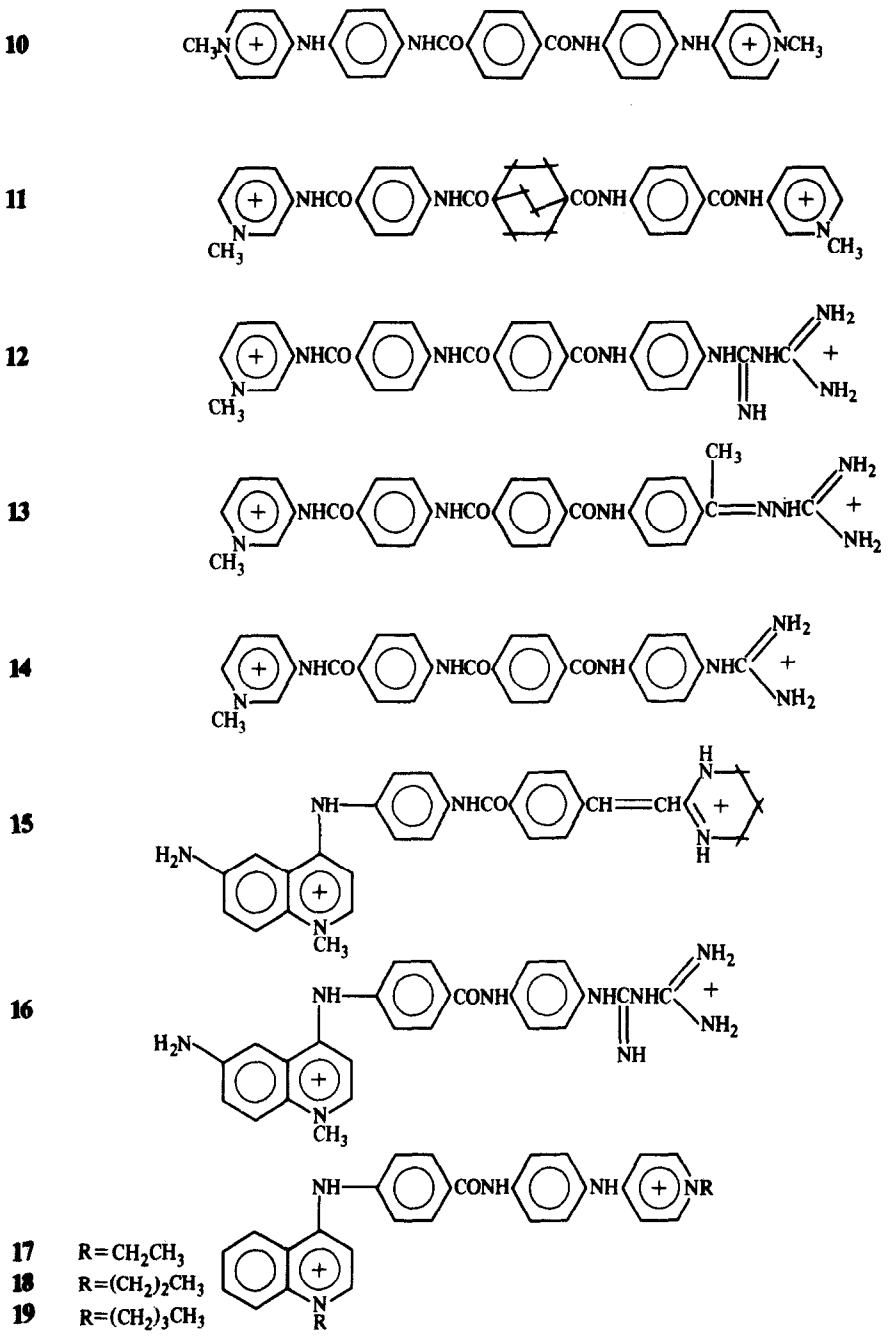
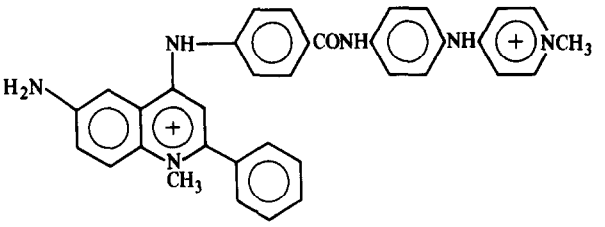
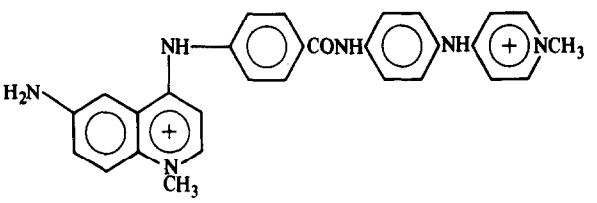


Fig. 1 (*cont.*)

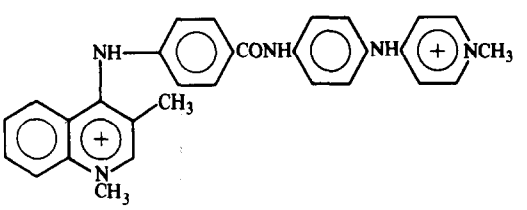
20



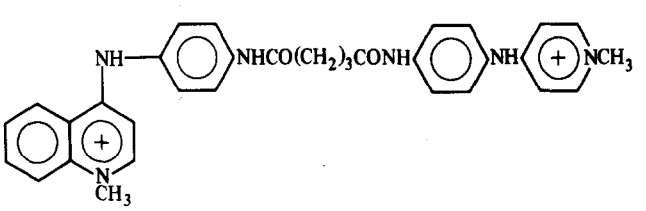
21



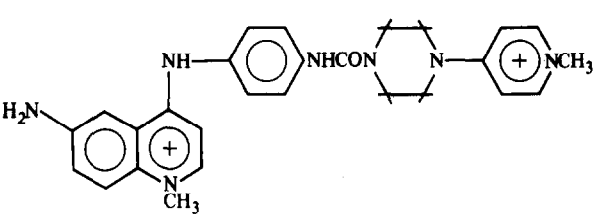
22



23



24



25

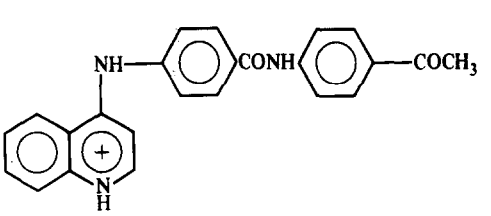


Fig. 1. *Structures of compounds discussed in the text.*

mutagenicity is highly correlated with C_{50} values using the synthetic copolymer poly(dA–dT):

$$\log P_{50} = 1.69 \log C_{50}(\text{dA–dT}) + 2.85$$

($r = 0.77$, $n = 22$, $P < 0.001$). The equation is expressed in molar units and indicates that tight DNA binding is associated with high *petite* mutagenicity. In contrast, no significant correlation was found with C_{50} values for poly(dG–dC), suggesting that sequence-selective drug binding is required for *petite* mutagenicity. A highly significant correlation is also found in the ratio of C_{50} values for the two DNA copolymers:

$$\log P_{50} = 2.34 [\log C_{50}(\text{dA–dT}) - \log C_{50}(\text{dG–dC})] - 2.12$$

($r = 0.79$, $n = 22$, $P < 0.001$). The ratios of C_{50} values provide values which are largely independent of the size of the DNA binding site, and may be closely related to the ratios of true binding constants [7]. High biological activity is associated with high A–T selectivity.

Drug toxicity (D_{50} values) towards yeast cells is also correlated with C_{50} values for poly(dA–dT) and for ratios of C_{50} values (as above), but not with C_{50} values for poly(dG–dC). Toxicity is also correlated with *petite* mutagenicity, suggesting that at least part of the drug toxicity may be a result of effects on mitochondria:

$$\log P_{50} = 0.94 \log D_{50} - 1.14$$

($r = 0.76$, $n = 22$, $P < 0.001$).

Relationship between antitumour activity, petite *mutagenicity and DNA binding*

The *in vitro* toxicity of the drugs listed in Table 1, as measured by ID_{50} values, is only poorly correlated with either *petite* mutagenicity ($r = 0.47$, $P < 0.05$) or toxicity ($r = 0.51$, $P < 0.05$) in yeast. However, it correlates better with C_{50} values for poly(dA–dT):

$$\log ID_{50} = 0.79 \log C_{50}(\text{dA–dT}) - 0.55$$

($r = 0.60$, $n = 24$, $P < 0.01$). This result compares with the *in vivo* correlation between *in vivo* antitumour activity (measured using life extension assays with L1210 leukaemia) and C_{50} values found with a large series of bisquaternary salts [7]. In the latter case, the best correlation was found for the ratio of C_{50} values for poly(dA–dT) and poly(dG–dC), with antitumour activity related to high A–T selectivity. This was not observed for the series of compounds in Table 1. However, for the subset of bisquaternary ammonium heterocycles in this table, activity was well correlated with the C_{50} ratio:

$$\log ID_{50} = 1.02 [\log C_{50}(\text{dA–dT}) - \log C_{50}(\text{dG–dC})] - 6.11$$

($r = 0.74$, $n = 12$, $P < 0.005$).

It is possible that other factors are involved in determining toxicity towards L1210 cells in culture, including lipophilic character [7] and the size of the DNA binding site [4, 23]. The limited size of the series in Table 1 precludes a comprehensive analysis of all the variables involved. However, the similarity of dependence of *petite* mutagenicity, yeast toxicity, *in vitro* inhibition of L1210 cell growth and *in vivo* antileukaemia L1210 activity on A–T selective DNA binding suggests similar modes of action.

DISCUSSION

A broad range of bis-charged compounds which are thought to bind to DNA by a non-intercalative mechanism induce the formation of respiratory-deficient *petite* mutants of yeast. This range includes the phthalanilides, aromatic bisguanylhydrazones and bisquaternary salts, which have shown experimental, and in some cases clinical, antitumour activity. The agent DDUG (compound *5*) did not show an effect in the particular yeast strain used in these experiments, but did form *petites* in another strain (Ferguson, unpublished results).

The range of compounds active as *petite* mutant inducers includes many that do not have demonstrable antileukaemic activity *in vivo* (Table 1). Many of these examples were chosen to provide reduced DNA binding through the introduction of flexible or non-planar linkage groups [1]. However, it is notable that those variants still show measurable DNA affinity and are also inhibitory to the growth of cultured L1210 leukaemia cells. It is likely that the generally low solubility of the bisquaternary ammonium heterocyclic compounds at physiological salt concentrations limits their *in vivo* antitumour activity.

Despite the apparent similarity of DNA binding mode, the compounds in Table 1 are likely to have diverse actions on cells. Trypanocidal and antitumour activity of the phthalanilides are not parallel [4] and it is not unexpected that the yeast cell and L1210 cell growth inhibition data are not well correlated. Nuclear and mitochondrial mechanisms for cytotoxic action have been proposed for both phthalanilides and the aromatic bisguanylhydrazones [1, 12]. Similarities between the action of DDUG (compound *5*) and methylglyoxalbisguanylhydra-

zone have been reported [21]. The latter compound shows only very weak DNA binding [22] and the accepted mechanism for its action involves the inhibition of polyamine metabolism [6]. It is possible that non-intercalative DNA binding agents compete for polyamine binding sites on the DNA, mimicking the effects of polyamine deprivation. In the case of mitochondrial DNA, which exists as a closed circular duplex, the conformation of the DNA could differ from that of nuclear DNA, providing a potential target for chemotherapeutic attack. All of the *petite*-inducing agents in Table 1 have antibacterial activity, and many in addition show a selective inhibitory effect on phage lambda DNA [10, 23]. In the latter case a differential effect on RNA synthesis has been suggested [23]. Ethidium bromide, a classical inducer of *petite* mutants in yeast [16], is known to inhibit selectively the synthesis of RNA in mammalian mitochondria [24]. Inhibition of mitochondrial RNA synthesis is therefore a possible mechanism for the cytostatic or cytotoxic effects of some DNA binding agents. Compounds related to the antitumour agent amsacrine but having two acridine amino groups with a similar orientation to those of proflavine have good experimental antileukaemia activity both *in vitro* [17] and *in vivo* [25], and show similar antimitochondrial effects in yeast to the present drugs [16]. The anthracycline antitumour agent daunorubicin selectively blocks mitochondrial transcription in yeast cells and in isolated rat liver mitochondria [26]. Ellipticine, along with a number of other antitumour agents, has been found to inhibit the respiration of isolated mitochondria [27].

In conclusion, a wide range of non-intercalative DNA binding antitumour agents show antimitochondrial effects in yeast. This could indicate either selective uptake of drugs by mitochondria or a selective effect on DNA function in mitochondria. Only limited studies have been carried out in the mode of cytotoxic action of these agents in the past, and future work should take account of tumour cell mitochondria as a possible site of action.

Acknowledgements—The authors wish to thank Noelene M. Harris and Rosalee Nash for expert technical assistance, and Sally A. Hill for preparing the manuscript.

REFERENCES

1. BAGULEY BC. Nonintercalative DNA-binding antitumour compounds. *Mol Cell Biochem* 1982, **43**, 167–181.
2. YESAIR DW, KESSLER CJ. The phthalanilides. In: BRODIE BB, GILLETTE JR, eds. *Handbook of Experimental Pharmacology: Antineoplastic and Immunosuppressive Agents*. Berlin, Springer Verlag, 1975, Vol. 38, Part 2, 820–828.
3. OETTGEN HF, CLIFFORD P, BURCHENAL JH. Malignant lymphoma involving the jaw in african children: treatment with 2 chloro-4,4″-di-2-imidazolin-2-yl terephthalanilide dihydrochloride. *Cancer Chemother Rep* 1963, **27**, 45–54.
4. CAIN BF, ATWELL GJ, SEELYE RN. Potential antitumour agents. 10. Bisquaternary salts. *J Med Chem* 1969, **12**, 199–206.
5. PLOWMAN J, ADAMSON RH. Initial studies on the disposition of quinolinium dibromide (NSC-176319) in mice and rats. *Pharmacology* 1978, **17**, 61–68.
6. MIHICH E. Bisguanylhydrazones. In: BRODIE BB, GILLETTE JR, eds. *Handbook of Experimental Pharmacology: Antineoplastic and Immunosuppressive Agents*. Berlin, Springer Verlag, 1975, Vol. 38, Part 2, 766–788.
7. DENNY WA, ATWELL GJ, BAGULEY BC, CAIN BF. Potential antitumour agents. 29. Quantitative structure–activity relationships for the antileukemic bisquaternary ammonium heterocycles. *J Med Chem* 1979, **22**, 134–150.
8. BRAITHWAITE AW, BAGULEY BC. Existence of an extended series of antitumour compounds which bind to deoxyribonucleic acid by nonintercalative means. *Biochemistry* 1980, **19**, 1101–1106.
9. FEIGON JL, DENNY WA, LEUPIN W, KEARNS DR. The interaction of antitumour drugs with natural DNA: NMR studies of binding mode and kinetics. Submitted to *Biochemistry*.
10. COUNSILMAN AC, CAIN BF, BAGULEY BC. Selective inhibition of the growth of bacteriophage lambda by experimental antileukaemic bisquaternary Salts. *Eur J Cancer* 1974, **10**, 75–81.
11. BENARD J, RIOU GF. *In vivo* effects of intercalating and non-intercalating drugs on the tertiary structure of kinetoplast deoxyribonucleic acid. *Biochemistry* 1980, **19**, 4197–4201.

12. PINE MJ, DI PAOLO JA. The antimitochondrial action of 2-chloro-4',4''-bis(2-imidazolin-2-yl)terephthalanilide and methylglyoxal bis(guanylhydrazone). *Cancer Res* 1966, **26**, 18–25.
13. EPHRUSSI B, HOTTINGUER H, CHIMENES AM. Action de l'acriflavine sur les levures. 1. La mutation 'petites colonies'. *Ann Inst Pasteur* **76**, 351–367.
14. BERNARD G. The petite mutation in yeast. *Trends Biochem Sci* 1979, September, 1–5.
15. MAHLER HR. Structural requirements for mitochondrial mutagenesis. *J Supramol Struct* 1973, **1**, 449–460.
16. FERGUSON LR, BAGULEY BC. Induction of 'petite' formation in *Saccharomyces cerevisiae* by experimental antitumour agents: structure–activity relationships for 9-anilinoacridines. *Mutat Res* 1981, **90**, 411–423.
17. MAHLER HR, PERLMAN PS. Induction of respiratory deficient mutants in *Saccharomyces cerevisiae* by berenil. 1. Berenil, a novel non-intercalating mutagen. *MGG* 1973, **121**, 285–294.
18. ZIMMERMAN FK. A yeast strain for visual screening for the two reciprocal products of mitotic crossing over. *Mutat Res* 1973, **21**, 263–269.
19. BAGULEY BC, NASH R. Antitumour activity of substituted 9-anilinoacridines—comparison of *in vivo* and *in vitro* testing systems. *Eur J Cancer* 1981, **17**, 671–679.
20. MATTICK JS, NAGLEY P. Comparative studies of the effects of acridines and other petite inducing drugs on the mitochondrial genome of *Saccharomyces cerevisiae*. *MGG* 1977 **152**, 267–276.
21. BYCZKOWSKI JZ, ZYCHLINSKI L, PORTER CW. Potentiation of the antimitochondrial and antiproliferative effects of bis(guanylhydrazones) by phenethylbiguanide. *Cancer Res* 1982, **42**, 3592–3595.
22. DENNY WA, CAIN BF. Potential antitumour agents. 31. Quantitative structure–activity relationships for the antileukemic bis(guanylhydrazones). *J Med Chem* 1979, **22**, 1234–1238.
23. ROBERTSON IGC, BAGULEY BC. A comparison of the requirements for antitumour activity, antibacteriophage lambda activity and DNA binding for a series of bisquaternary ammonium heterocycles. *Eur J Cancer Clin Oncol* 1982, **18**, 271–279.
24. FAN H, PENMAN S. Mitochondrial RNA synthesis during mitosis. *Science* 1970, **168**, 135–138.
25. ROBERTSON IGC, DENNY WA, BAGULEY BC. Inhibition of T4 bacteriophage yield by 9-anilinoacridines; comparison with *in vivo* antitumour activity. *Eur J Cancer* 1980, **16**, 1133–1139.
26. EVANS I, INSTEAD D, RHODES PM, WILKIE D. *Biochim Biophys Acta* 1973, **305**, 323–336.
27. GOSALVEZ M, BLANCO M, HUNTER J, MIKO M, CHANCE B. Effects of anticancer agents on the respiration of isolated mitochondria and tumour cells. *Eur J Cancer* 1974, **10**, 567–574.