ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1991, p. 2318–2321
0066-4804/91/112318-04$02.00/0

Vol. 35, No. 11

Petite Mutagenesis in *Saccharomyces cerevisiae* by a Series of *bis*-Cationic Trypanocidal Drugs

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Received 29 April 1991/Accepted 6 August 1991

A group of *bis*-cationic imidazo[1,2-*a*]pyridinium salts and related compounds, some of which exhibit in vivo trypanocidal activity, have been investigated for induction of petite mutagenesis in *Saccharomyces cerevisiae*. All of the compounds which are active trypanocides induce mutagenesis. There appears to be a correlation between trypanocidal activity and mutagenic activity which may have its structural origin in the spatial separation of the cationic centers.

A series of imidazo[1,2-*a*]pyridinium and related heteroaromatic salts was recently reported to have good activity against the bloodstream form of *Trypanosoma rhodesiense* in mice (22). The structures of these compounds resemble those of various *bis*-cationic antitumor compounds such as diamidines, phthalanilides, and *bis*-quaternary ammonium heterocycles (1), which are generally believed to interact with DNA by binding in the minor groove (6, 11–13). Related *bis*-cationic trypanocides such as berenil, stilbamidine, and phthalanilides are known to induce petite mutants in *Saccharomyces cerevisiae* (8) in an assay which provides an indication of antimitochondrial activity (9). In order to compare the new imidazo[1,2-*a*]pyridinium series with the existing *bis*-cationic trypanocides, we have examined selected examples in the petite mutagenesis assays. The structures of the compounds examined in this study are shown in Fig. 1. The structures of several other *bis*-cationic trypanocides are shown in Fig. 2.

MATERIALS AND METHODS

Chemicals. The compounds illustrated in Fig. 1 have been previously reported (22). Compounds were stored as solids at −10°C. Solutions were made up for testing in 50% ethanol and used immediately.

***S. cerevisiae*.** The diploid strain D_5 (24) was kindly provided by B. S. Cox (Botany School, University of Oxford). A single colony isolate was inoculated into liquid yeast complete medium or YC (4) and grown to stationary phase for 24 h. Dimethyl sulfoxide was added to 10%, and 1-ml aliquots were frozen to −70°C and stored at this temperature before use. For all experiments, the 1-ml sample was thawed, added to 10 ml of fresh medium, and grown for exactly 2 h before use.

Microtiter assay for petite mutagenesis. The microtiter assay has been described in detail (7). Briefly, a log-phase culture was washed and then diluted into fresh growth medium. A 96-well microtiter tray (A/S Nunc, Roskilde, Denmark) was inoculated with (usually) 100-μl aliquots of the diluted yeast culture, and drugs were added at various dilutions to the wells to a maximum of 200 μg/ml. Drugs were dissolved in 50% ethanol, and dilutions were made so that there was no more than 1% ethanol in each well. Trays were incubated for 24 h at 30°C, an appropriate dilution was made from each well into saline, and 100 μl was plated onto each of three YC plates. Cell numbers were calculated so that the dilutions at this point were at least $1:10^4$, thereby effectively washing drugs from cells by dilution (7).

In some experiments, cells were tested in nongrowing conditions by diluting the cultures into 0.87% saline rather than complete medium. For a limited number of the compounds, samples were taken at various intervals in order to establish the rate of petite formation.

Plates were incubated at 30°C for 3 days, and then all colonies were counted with an Artek model 880 counter. Scores of some plates were checked by manual counting. Colonies were then overlaid with tetrazolium in order to score petite colonies (16). Following a preliminary experiment to establish the correct concentration range, dose response of all drugs was tested at least twice and the data were compared for reproducibility. Maximum frequencies of petites were recorded. The dose to reduce numbers of yeast colonies to 50% of the value for the untreated control and the dose to convert 50% of the colonies to petite were both estimated by linear regression analysis. The presented data have been averaged.

RESULTS

Table 1 gives the 50% lethal dose and 50% petite mutant conversion dose measured for the test compounds. Comparative data for berenil, stilbamidine, and the phthalanilides structures shown in Fig. 2 have been reported previously (8). The table also includes two indicators of in vivo trypanocidal activity. The indicator of curative effectiveness represents the fraction of test animals cured over 11 doses ranging from 0.4 to 424 mg/kg of body weight (22). Low fractional values indicate low effectiveness or high toxicity or both. The 50% cytotoxic dose is the lowest drug dose for which at least 50% of the animals survived for 30 days longer than controls.

Most of the compounds are potent inducers of petite mutants. The effect is seen under both growing and nongrowing conditions. The time course for petite development was examined for two of the compounds, 1b and 5a. The results for 1b are shown in Fig. 3 and indicate that the process is complete within 4 h. There is a brief period of reversibility but not to the extent observed for ethidium bromide (9, 10, 20). Instead the compounds are qualitatively similar to berenil (14, 15) and to *bis*-quaternary ammonium heterocycles (8).

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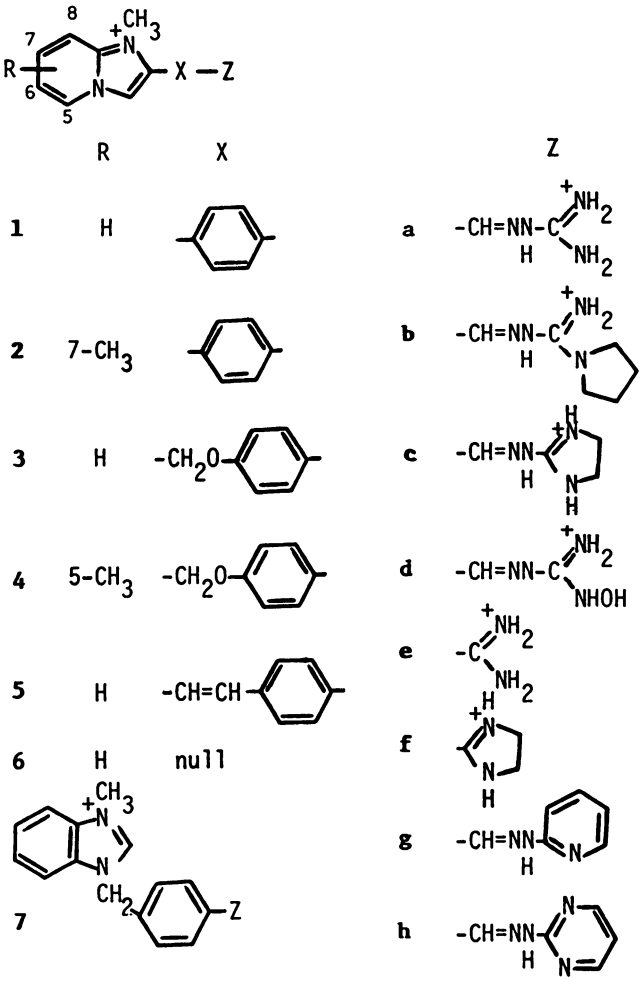

FIG. 1. Structures of the compounds examined.

TABLE 1. Activity in petite mutagenesis assays and as trypanocides[a]

Compound	P_{50} (μM)	D_{50} (μM)	P_{max}	I.C.E.	CD_{50} (mg/kg)
1a	5.3	146	99	0.48	26
1b	5.1	160	100	0.64	6.6
1c	14.0	150	100	0.56	13
1d	3	144	95	0.70	0.8
1e	79	>200	59	0.21	26
1f	NA	>200	<5	0.00	
1g	70	>200	91	0.18	212
1h	9.4	162	100	0.26	6.6
2b	18.9	137	98	0.47	13
2d	1.6	112	99	0.71	0.2
3a	4.5	144	100	0.47	0.1
3b	7.6	89	100	0.36	0.8
3c	7.3	103	100	0.60	0.8
4a	1.6	160	100	0.49	0.8
4b	2.4	118	100	0.75	0.4
4c	3.4	131	99	0.64	0.8
5a	1.6	120	100	0.89	0.1
5b	92	124	55	0.74	0.8
5c	5.7	>200	100	0.79	0.2
5d	6.4	>200	100	0.72	0.4
6a	NA	>200	<5	0.24	106
6b	NA	>200	<5	0.06	106
6c	NA	>200		0.01	
6d	NA	>200	100	0.01	
7a	10.6	>200	99	0.19	6.6
7b	5.8	>200	99	0.23	6.6
7c	30	>200	99	0.19	3.3
Berenil	0.45[b]	8.5[b]		0.9[c]	1.5[c]
Stilbamidine	0.19[b]	7.7[b]		0.8[c]	1.5[c]
Phthalanilide-5	1.9[b]	4.6[b]			0.2[d]
Phthalanilide-6	0.25[b]	33[b]			0.5[d]

[a] P_{50}, drug concentration required to convert 50% of surviving cells to petite mutants; D_{50}, drug concentration required to decrease by 50% the total number of viable yeast cells after drug exposure for 20 h; P_{max}, maximal frequency of induction of petite mutants expressed as a percentage of total cells; ICE, fraction of all animals cured in the dose range from 0.4 to 424 mg/kg, as a relative indicator of curative effectiveness (data are from reference 22); CD_{50}, lowest dose effecting 30-day cures of at least 50% of the test animals (data are from reference 22).

[b] From reference 8.

[c] Estimated from data on *T. rhodesiensis* (5, 21).

[d] Estimated from data on *T. brucei* (17).

DISCUSSION

There is a good general agreement between the ability for petite mutagenesis and trypanocidal activity. The compounds with an indicator of curative effectiveness of >4 and/or a 50% cytotoxic dose of <1 are highly active trypanocides (22), and nearly all of the compounds meeting these criteria had a P_{max} of >95 and a 50% petite mutant conversion dose of <10 μM (Table 1). The sole exception was 5b, which, while a potent trypanocide, was only moderately active in the petite mutagenesis assay. The benzimidazoles of series 7, which are moderately active trypanocides with

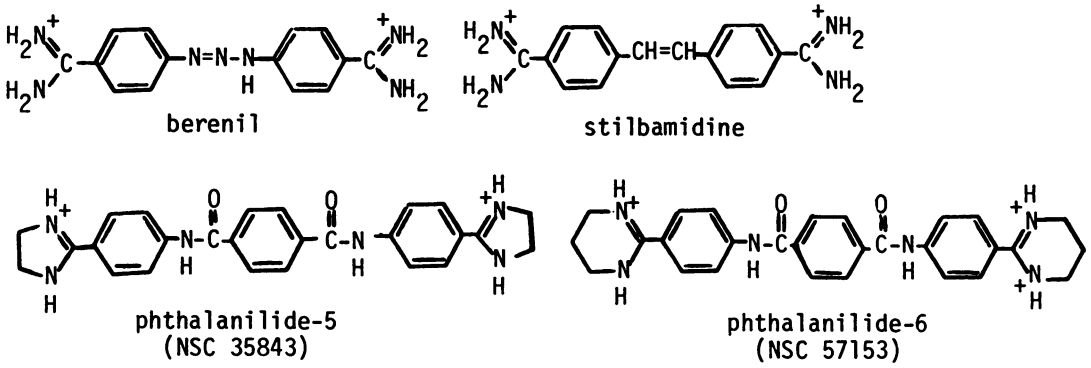

FIG. 2. Structures of various *bis*-cationic trypanocides.

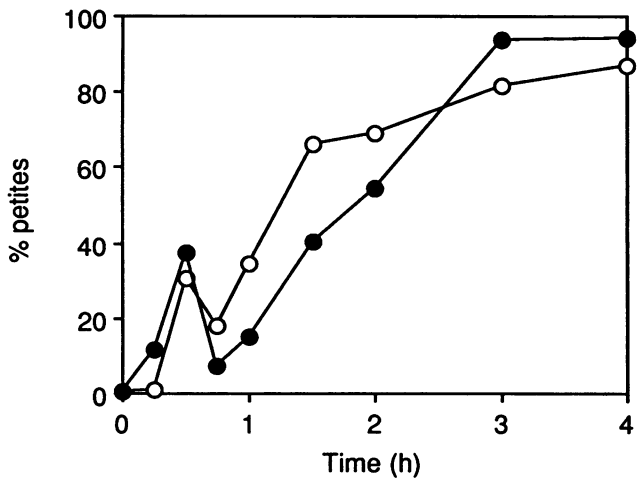

FIG. 3. The rate of formation of petite mutants during a 24-h exposure of *S. cerevisiae* D_5 to 50 μg of compound 1b per ml. Cells were exposed to drug in YC medium (●) or in saline (○) for 24 h. During this time, samples were removed and plated in the absence of drug in order to measure the percentage of petite mutants.

indicators of curative effectiveness of 0.2 to 0.4, also exhibit strong activity in the petite assay.

Structural series 6 shows little trypanocidal activity, and only 6d shows strong mutagenesis. This may indicate that the *N*-hydroxyguanylhydrazone functionality present in 6d has mutagenic activity independent of the general *bis*-cationic structure. The inactivity of 1f and the relatively weak activity of 1e are also noteworthy. These compounds contain, respectively, the imidazole and amidine functionalities which are present in active compounds such as berenil and the phthalanilides. It seems likely that the low activities of 1e, 1f, and 6a to c both in the petite mutagenesis assay and as trypanocides are associated with the distance separating the cationic centers in these molecules. It has been suggested that separations on the order of 0.8 to 0.9 or 1.25 to 1.4 nm are optimum for the binding of *bis*-cationic drugs in the minor groove of DNA (2). The separations in 1e and 1f and in 6a to c are 0.68 and 0.55 nm, respectively, by using the imidazolium N-1 as the point of measurement. The corresponding distance in the more active 1a to d series is 0.87 nm. In the 5a to d series this distance is about 1.2 nm.

Thus, by the criteria of petite mutagenesis, the imidazo[1,2-*a*]pyridinium salts of structural series 1 through 5 are similar to those of other types of *bis*-cationic trypanocides such as berenil, stilbamidine, the phthalanilides, and the *bis*-quaternary ammonium heterocycles (8). As a group, these compounds are considered to be DNA minor groove binders, and structural information supporting this conclusion is available in some cases (3, 18, 23). This class of drugs has been suggested to have the mitochondria as a potential target of activity (9). The sensitivity of kinetoplast DNA (the mitochondrial DNA in trypanosomes) to DNA-binding drugs may be the basis of the trypanocidal action of *bis*-cationic compounds (19).

ACKNOWLEDGMENTS

We thank Hamish Pogai for technical assistance.

This work was funded by the Auckland Division of the Cancer Society of New Zealand.

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