MUTAGENICITY OF SYNTHETIC ACRIDINONES AND THIOACRIDINES IN DIRECT AMES' *SALMONELLA* MUTAGENICITY ASSAY

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Abstract: Newly synthesized antitrypanosomal and antiplasmid acridinones, thio-substituted acridines and thioacridinones were tested for a direct genotoxic effect on TA97a and TA100 tester strains by Ames' *Salmonella* mutagenicity assay. Of acridinones, only 9-(hydrazinothiazolo[5,4-a])acridinone 1 showed strong mutagenic activity on TA97a strain. However, mutagenic activity was manifested invariably by all three acridinones on TA100 strain. Of thioacridines, 2,7-dimethoxy-9-thioacridinone 4, 2,7-dimethoxy-9-(3'-aminopropyl) 5 and 2,7-dihydroxy-9-acetamidothioacridine 6 were explicitly mutagenic on TA97a, but on TA100 they were just weakly mutagenic.

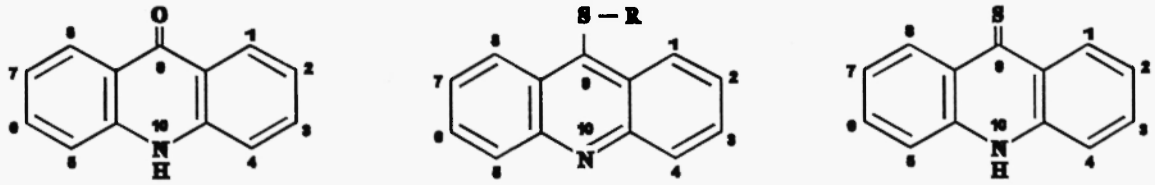
Introduction

Acridine compounds are known to have a broad spectrum of biologic activities such as antibacterial (1, 2, 3), antiparasitic (4, 5, 6, 7), mutagenic (8) and antitumor ones (9, 10, 11, 12). The effects of acridines on bacteria were studied for their antibacterial and antiplasmid actions. Their activities on bacteria seem to depend on the substitution on the tricyclic skeleton (1). Acridinones have been proved to be trypanocidal by being capable of intercalation into the DNA of the *Trypanosoma cruzi* (4, 6). The trypanocidal activity was also shown against the *Trypanosoma brucei* strains with very low concentration of substituted acridines with simultaneous brief irradiation (7) and without irradiation (13).

The antitumor activity of acridine derivatives attributes to their ability of intercalation to DNA, stabilizing the DNA-topoisomerase II intermediate complex. This cleavable complex appears to be toxic to cancerous cells (9). The DNA-topoisomerase II inhibiting acridines are primarily chromosomal mutagens in mammalian cells, although they are frameshift mutagens in bacterial and bacteriophage systems (8).

Mutagenicity of various acridine compounds have been studied by Ames' *Salmonella* mutagenicity assay. Acridines are planar polycyclic aromatic molecules which bind noncovalently and reversibly to DNA by intercalation. Simple acridines are frameshift mutagens. However, benzacridines are rather base-pair substitution mutagens by interacting covalently with DNA following microsomal activation (14). Acridine mustards such as the ICR compounds, acting as carriers to target alkylating agents to DNA, are frameshift as well as base-pair substitution mutants. Nitroacridines may act as either simple acridines or alkylating agents, depending on the position of the nitro group (8).

The direct genotoxic effect of newly synthesized three acridinones, three thio-substituted acridines and two thioacridinone (Fig. 1) was investigated in this experiment by the Ames' *Salmonella* mutagenicity assay on



(1) (2) (3)

Figure 1: Molecular formulas and numbering system of acridinone(1), thioacridine(2) and thioacridinone(3)

the well characterized strains of *Salmonella typhimurium*, TA97a and TA100. The direct Ames *Salmonella* mutagenicity assay employs only bacterial metabolic enzymes. The S9 liver microsomal fraction as additional mammalian metabolic enzymes was omitted. TA97a tester strain detects a frameshift mutagen which alters six cytosine sequence in the *hisD6610* gene (15). On the other hand, TA100 strain identifies a base-pair substitution mutagen which affects one of G-C pairs in *hisG46* gene (16) coding for the first enzyme of histidine biosynthesis (17). This study improves the drug design against protozoas by establishing relationships between the types and sites of substitution and the mutagenic properties of acridines. Thereby, harmful mutagenic acridine derivatives should be excluded from further *in vivo* experiments and the data of the substitution-mutagenicity relationships further facilitate the rational drug design.

Materials and Methods

Chemicals: 9-(Hydrazinothiazolo[5,4-a])acridinone 1, 9-(1,4-dioxano[5,6-a])acridinone 2, 2,3-dimethoxy-9-acridinone 3, 2,7-dimethoxy-9-thioacridinone 4, 2,7-dimethoxy-9-(3'-aminopropyl) thioacridine 5, 2,7-dihydroxy-9-acetamidothioacridine 6, 2,7-dihydroxy-9-(3'-aminopropyl)thioacridine 7 and 1,4,7,10,13,16,19-heptaoxanonadecyl-2,7-[(10-methyl)-9-thioacridinone] 8 were synthesized according to the general procedures previously described (18, 19). Compounds were dissolved in DMSO.

Bacterial strains and mutagenicity assay: TA97a and TA100 strains of *Salmonella typhimurium* were obtained from Bruce Ames. The plate incorporation test was carried out as described by Maron and Ames (20). The bacterial strains were cultured in Oxoid nutrient broth No.2 for 10-12 hours for turbidity to reach the optical density of 0.5 at 600nm. 0.5 ml of 0.2 mM sodium phosphate buffer (pH 7.4) was used in place of S9 microsomal fraction for the direct mutagenicity assay. A 20 minute preincubation at 37 degrees in Celsius in the turbulating incubator preceeded addition of top agar. After 48 hours the presence of the background lawn on all plates was confirmed and the number of revertant colonies was counted. The assay was performed in triplicate on two separate occasions.

Results

Back-mutation was significantly increased on 9-aminoacridine-sensitive TA97a strain with 9-(hydrazinothiazolo[5,4-a])acridinone 1, 2,7-dimethoxy-9-thioacridinone 4, 2,7-dimethoxy-9-(3'-aminopropyl)thioacridine 5 and 2,7-dihydroxy-9-acetamidothioacridine 6 and only slightly increased with 9-(1,4-dioxano[5,6-a])acridinone 2. Sodium azide-sensitive TA100 strain showed markedly increased back-mutation with 9-(hydrazinothiazolo[5,4-a])acridinone 1, 9-(1,4-dioxano[5,6-a])acridinone 2 and 2,3-dimethoxy-9-acridinone 3, and slightly increased with 2,7-dimethoxy-9-thioacridinone 4, 2,7-dimethoxy-9-(3'-aminopropyl)thioacridine 5, 2,7-dihydroxy-9-acetamidothioacridine 6 and 2,7-dihydroxy-9-(3'-aminopropyl)thioacridine 7 (Table 1).

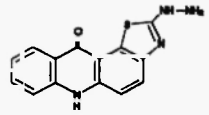
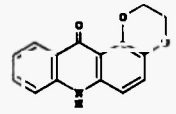
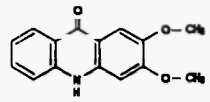
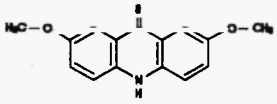
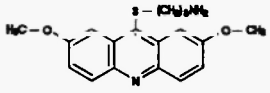
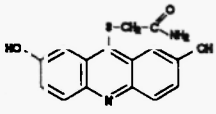
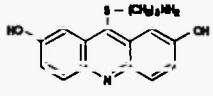
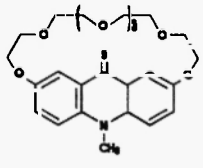
1,4,7,10,13,16,19-heptaoxanonadecyl-2,7-[(10-methyl)-9-thioacridinone] 8 can be recommended for further *in vitro* and *in vivo* studies for antiparasitic drug design because of the absence of its mutagenic activity on both strains. The mutagenic activity of 9-(1,4-dioxano[5,6-a])acridinone 2 and 2,7-dihydroxy-9-(3'-aminopropyl)thioacridine 7 can probably be reduced by well-planned substitution of the tricyclic skeleton.

Discussion

The biological activities of some newly synthesized acridine compounds have been shown by *in vitro* studies. Thioacridine derivatives are cytotoxic against K562 and Raji cell lines, and also inhibitory to nucleic acid synthesis in K562 (10). Anti-amebic activity of thioacridanones was also found to be against *Naegleria* and *Acanthamoeba* species (6). Furthermore, acridines bearing aromatic rings inhibited the mitogen-induced blast transformation of human-peripheral lymphocytes, showing their immunomodulatory activity, possibly by acridine-mitogen complex formation (21). A correlation between the biological activity of tricyclic compounds such as benz[*c*]acridines and some molecular orbital parameters was suggested by quantum mechanical analysis (22). But the fourth ring attached to acridines does not favor the antiplasmid action. Antiplasmid and mutagenic compounds appear to have basically different molecular orbitals responsible for their actions to DNA analysis (22).

The effective dose against *Trypanosoma* was shown to be very low in the range of 0.1 μM (7) and the minimal inhibitory concentration (MIC) was determined on *E.coli* to be some 200-280 μg/ml (23), indicating a possibly large therapeutic window. At near MIC concentration, however, some acridinones and thioacridines tested in this experiment were capable to induce both frameshift and base-pair substitution mutation; the others caused either of them or even none. The nature of mutagenicity of the related acridinones and thioacridines appears to depend mostly on the substitution on the tricyclic rings. The characterization of biological activities of acridine derivatives are important because of their considerable potential for antibacterial, antiprotozoal, immunoregulatory and even antitumor drugs.

Table 1: The Mutagenicity of Acridinones, Thioacridines and Thioacridinones

	test chemicals	dose (μg/sample)	colony numbers TA97a	colony numbers TA100	mutagenicity index[a] TA97a	mutagenicity index[a] TA100
1		200	132	-	1.4	-
		20	296	88	3.1	2.8
2		200	23	41	0.2	1.3
		20	30	23	0.3	0.7
3		200	5	32	0.1	1.0
		20	0	26	0.0	0.8
4		200	-	-	-	-
		20	118	12	1.3	0.4
5		200	-	-	-	-
		20	122	19	1.3	0.6
6		200	85	7	0.9	0.2
		20	0	18	0.0	0.6
7		200	0	4	0.0	0.1
		20	0	16	0.0	0.5
8		200	3	5	0.0	0.2
		20	14	0	0.1	0.0
	positive control	-	94	31	1.0	1.0

Note: **1.** 9-(hydrazinothiazolo[5,4-a])acridinone **2.** 9-(1,4-dioxano[5,6-a])acridinone **3.** 2,3-dimethoxy-9-acridinone **4.** 2,7-dimethoxy-9-thioacridinone **5.** 2,7-dimethoxy-9-(3'-aminopropyl)thioacridine **6.** 2,7-dihydroxy-9-acetamidothioacridine **7.** 2,7-dihydroxy-9-(3'-aminopropyl)thioacridine **8.** 1,4,7,10,13,16,19-heptaoxanonadecyl-2,7-[(10-methyl)-9-thioacridinone] a: mutagenicity index = colony numbers of test chemical / colony numbers of positive control × 100. Negative control values were subtracted. 9-Aminoacridine(15μg/sample) was used as a positive control for TA97a strain; sodium azide (0.4μg/sample) for TA100 strain. -: inhibition due to toxicity.

Acknowledgments

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