SUMMARY OF DATA FOR CHEMICAL SELECTION

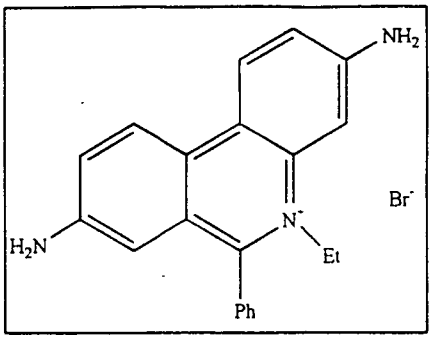
CHEMICAL IDENTIFICATION

CAS Registry Number: 1239-45-8

Chemical Abstract Name: Phenanthridinium, 3,8-diamino-5-ethyl-6-phenyl-, bromide (8CI, 9CI)

Synonyms and Trade Names: Ethidium bromide; Dromilac; homidium bromide; 2,7-diamino-10-ethyl-9-phenanthridinium bromide; 2,7-diamino-9-phenylphenanthridium ethobromide; EB

Structure, Molecular Formula and Molecular Weight:



$C_{21}H_{20}N_3.Br$ Mol.Wt.:394.32

Chemical and Physical Properties

Description: Bitter tasting dark red crystals from alcohol (Budavari, 1989)

Melting Point: 238-240°C (Budavari, 1989); decomposition at 260-262°C (Anon., 1994a; Anon., 1994b)

Solubility: Moderately soluble in water (20 mg/ml) and ethylene glycol monomethyl ether (20 mg/ml); slightly soluble in chloroform and ethanol (Budavari, 1989; Green, 1990)

Stability: Stable under normal temperatures and pressures. Hazardous thermal decomposition may release toxic oxides of carbon and nitrogen, and corrosive hydrogen bromide gas (Anon., 1994b)

UV/Visible Spectral Data: Max. (in water): 210, 285, 316, 343, 506 nm (Budavari, 1989) (Anon., 1994a)

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Technical Products and Impurities: Ethidium bromide is commercially available in both powder (95-96% pure) and aqueous solution forms. Sigma Chemical Company also provides molecular biology reagent grade aqueous solutions at concentrations of 10 mg/ml and 500 µg/ml and tablets at 100 mg per tablet (Anon, 1993).

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EXPOSURE INFORMATION

Commercial Availability

Production and Producers: EB was reported by Watkins (1952) to be prepared from 3.8-dintro-6-phenyl phenanthridine with excess ethyl toluene-p-sulfonate followed by treatment with ammonium bromide. No producers of EB were reported in EPA's 1983 TSCAPP database (CIS, 1994). No listing of large, commercial scale production was found in any of the major chemical industry directories; but it is available from numerous chemical suppliers in research quantities—typically 1, 5, 10, 25, or 250 grams. Ethidium bromide is listed by numerous catalog suppliers, including Sigma Chemical Company; Aldrich Catalog/Handbook of Fine Chemicals; American Tokyo Kasei, Inc.; Atomergic Chemetals Corporation; Calbiochem; Mallinckrodt, Inc.; Lancaster Synthesis Ltd.; Eastman Kodak Company; Janssen Chimica; Fluka Chemical Corp.; J.T. Baker Inc.; Chem Service, Inc. (DIALOG, 1994)

EB is listed on the EPA TSCA Inventory (STN, 1994).

Use Pattern: EB is a widely used red cationic fluorescent dye and nucleic acid visualizing agent which binds to both RNA and DNA (Lunn & Sansone, 1987). As both a DNA-dependent intercalating agent and a DNA-independent protein inhibitor, it is useful as a simple and general indicator in many biochemical and biomedical laboratory procedures (Lai & Herr, 1992). There are many variations on EB's use in biochemical, molecular biology, enzymology and other types of laboratories described in the available literature. Numerous techniques employ EB, including gel and capillary electrophoresis, fluorometry, spectrophotometry, flow cytometry, polymerase chain reaction (PCR) amplification, etc. Some representative examples are summarized as follows:

- as a molecular probe for staining nucleic acids in fluorescent microscopy studies of multidrug resistance (Neyfakh, 1988)
- as a DNA probe for various studies including characterizing and quantifying DNA (Green, 1990).

- as a derivatizing analytical reagent in clinical settings for continuous monitoring of levels of anticancer drugs in biological fluids, including blood, serum, and urine by measurement of dose-critical levels of DNA-binding (Miller & Hirschfeld, 1992).

EB has also been used as a drug or drug precursor, for example as a parasitotoxic, antiprotozoal drug for the treatment of Leishmaniasis, in combination therapeutic regimens using DNA reactive agents, and in the development of anionic and hydrophobic derivatives as trypanocides (Pavelic *et al.*, 1985; Budavari, 1989; Anon., 1992). It was one of a number of phenanthridine trypanocides developed for use in cattle in the early 50's (Watkins, 1952). Studies have been conducted in animals to evaluate EB as a potential antitumorigenic chemotherapeutic agent (Kramer & Grunberg, 1973).

Human Exposure: Laboratory personnel are potentially exposed to EB during its use as a biological stain and laboratory reagent. Concern for contact with highly mutagenic EB in laboratory reagent solutions and spills has been expressed in several citations. In spite of routine and widespread use of EB in biochemical and biomedical laboratories, no validated methods of destruction have been established, according to Lunn and Samsone (1987). Safe handling of EB in laboratories to avoid human exposures to mutagenic solutions containing EB has been addressed by Lunn and Sansone (1987) and Quillardet and Hofnung (1988). Armour (1993) has concluded that EB should be handled as a carcinogen in terms of identifying methods of safe waste disposal.

The National Occupational Exposure Survey (NOES) conducted by NIOSH from 1980 to 1983 indicated that a total of 21 employees in 3 occupations employed in 7 facilities of 1 industry were potentially exposed to EB. The estimate of numbers of workers is based on a survey of U.S. companies and did not involve measurements of actual exposures (NOES, 1990; NLM/RTECS, 1994).

Environmental Occurrence: EB is not known to occur naturally. No information was found in the available literature on detection of EB in environmental media. Several spill clean-up and

disposal methods have been recommended in the available literature for EB. They are based on careful removal to achieve elimination of mutagenicity of solutions by decontamination and degradation. Published methods include treatment with potassium permanganate/hydrochloric acid or hypophorous acid/sodium nitrite, adsorption on activated charcoal, and incineration at high temperatures (Quillardet & Hofnung, 1988).

Regulatory Status: No standards or guidelines have been set for occupational exposures to or environmental levels of EB. The American Conference of Governmental Industrial Hygienists (ACGIH) has not adopted a time-weighted average/threshold limit value (TLV/TWA) for this compound. EB is categorized as an acute hazard under SARA sections 311/312 (40 CFR 370.21) (Anon., 1994b).

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EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No positive or negative epidemiological studies or case reports associating EB either positvely or negatively with a cancer risk in humans were found in the available literature. However, the Calbiochem Catalog (Anon. 1994c) 1994/95 contains a warning that EB may be carcinogenic/teratogenic; and several other sources link the term "carcinogen" with this chemical.

Animal Data: No 2-year bioassay studies associating EB either positively or negatively with a carcinogenic effect in animals were found in the available literature.

Catalog supplier, Mallinckrodt, warns that EB is toxic with an LD_{50} of <50 mg/kg (Anon., 1989). In addition, the following acute toxicity levels were reported (Anon., 1994b):

mouse, intraperitoneal LD_{LO}: 20 mg/kg
mouse, subcutaneous, LD_{50}: 110 mg/kg

There is some suggestion in references such as catalog cited that above EB may be carcinogenic in animals, but they appear not to be substantiated by adequate studies but rather to reflect inference based on mutagenic and DNA-damaging activity. [N.B. Fritzenschaf *et al.* (1993) reported that EB was determined to be carcinogenic *in vivo* in a 1970 study attributed to Boller, K. and Schmid, W. and published in *Humangenetik*, 11:35-54: attempts to locate and retrieve this citation were unsuccessful.] On the other hand, several early '70s studies reported that EB demonstrated antitumorigenic effects. Balda and Birkmayer (1973) reported EB to be potentially therapeutic based on specific action against hamster melanoma A Mel 3 cells (a model for human malignant melanoma) which prolonged survival time in experimental animals. The mechanism of action was thought to be based on strong inhibition of RNA-directed DNA synthesis.

Nishiwaki *et al.* (1974) also reported EB to be antitumorigenic based on inhibition of RNA-dependent DNA polymerase activity in experimental tumor systems. They attributed increased life spans in mice with 6C3HED-OG (200%) and mice with L5178Y (83%) to the antileukemic activity of EB.

In Vitro Tests (or Short-Term Tests): Numerous studies have been conducted to evaluate mutagenic and DNA-damaging effects of EB in a variety of test systems. A tabulation of results in studies reported in the available literature is presented in Table 1. The studies represent a battery of short-term tests which contribute information on the genetic toxicity of EB.

Table 1. Summary of Mutagenicity Test Results for Ethidium Bromide

Test System	Strain	Result	Activation	Reference
Ames	TA98 TA1538	+ +	S9 S9	MacGregor and Johnson (1977)
Ames	TA1538 TA1535	+ -	S9 with and without S9	Mattern (1976)
Ames	TA98 TA1538	+ +	S9 S9	McCann (1975)
Ames	TA98 TA1537	+ +	S9 S9	NCI (1994) *(Hard copy not provided)*
Mouse Lymphoma	L5178Y (TK^+/TK^-)	+	with and without S9	NCI (1994) *(Hard copy not provided)*
SOS	TA1535/ pSK1002	+	S9	Nakamura (1987)
SOS Chromotest	*E. coli*	-	S9	Ohta *et al.* (1984)
SOS Chromotest	*E. coli*	+/-	S9	Mamber *et al.* (1986)
Micronucleus (*in vitro*)	Syrian hamster embryo cells	+	NA	Fritzenschaf (1993)

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Test System	Strain	Result	Activation	Reference
Micronucleus (*in vivo*)	Newt larvae	+	NA	Fernandez (1989)
Sex-linked recessive lethal assay	Drosophila	+	NA	Lopez de Sepulveda (1981)
Intrachromosomal mitotic recombination	Drosophila	+/-	NA	Vogel and Nivard (1993)
In vivo exposure followed by *in vitro* assay for SCE	Female C57Bl/6N Mice	+	NA	Shubber (1985)
SCE (*in vitro*)	V79	+/-	NA	Nishi (1984)
6-Thioguanine-resistance (*in vitro*)	V79	-	NA	Nishi (1984)
Yeast (argivine mutation)	Streptomyces lividans TK64	+	-	Chou and Chen (1992)
DNA binding	Cyanobacterium nostoc sp.	+/-	-	Tripathi and Kumar (1986)
CHO/HGPRT (*in vitro*)	CHO	-	with and without S9	Oberly (1990)
DNA repair	Rat hepatocytes	+	-	Williams (1989)
DNA-synthesis inhibition (*in vitro*)	HeLa (Human?)	+	-	Painter and Howard (1982)

+ = positive result; +/- = weak positive; - = negative; NA = not applicable

The preponderance of the results have been reported as positive or weakly positive, usually with metabolic activation, in both *in vitro* and *in vivo* assays supporting the conclusion that EB is genotoxic. Moreover, test results in Ames assays, such as those reported by Mattern (1976), in which EB induced frameshift mutations in strain TA1538 and only after metabolic activation but did not induce mutations in TA1535, a strain characterized by base-pair substitution mutation, demonstrate that EB is a frameshift mutagen.

EB induces mutations in *S. typhimurium* strains TA1538 and TA98 in the presence of S9, and is positive in the L5178Y mouse lymphoma assay. Micronuclei are induced in newt larvae and Syrian hamster embryo cells; EB is positive in the SOS Chromotest in *S. typhimurium* TA1535/pSK1002 cells in the presence of S9, but is negative in *E. coli*. Eye and recessive lethal mutations are induced in Drosophila by EB. EB induced SCEs in female C57BL/6N mice but not in V79 cells. 6-Thioguanine resistance was not induced in V79 cells. EB induced arjJ mutations in *Streptomyces lividans* but was only weakly mutagenic to cyanobacterium. A weakly positive result was obtained in the CHO/HGPRT mutation assay. EB induced DNA repair in rat hepatocytes, but did not inhibit DNA synthesis in HeLa cells.

Some supplementary data not presented in Table 1 has been reported in additional studies in the literature. Some of them are summarized as follows.

- In the presence of S9, the growth of DNA repair deficient strains of *Bacillus subtilis* and *Escherichia coli* was inhibited at EB concentrations 10-times lower than in wild-type bacteria (Suter and Jaeger 1982).

- Exposure of human lymphocytes *in vitro* to EB results in chromosome elongation and antagonizes the chromosome shortening action of the spindle inhibitors colcemid and colchicine(Andersen and Ronne, 1986).

- EB induced chromosomal recombinations in the yeast *Saccharomyces cerevisiae* (Schiestl 1989). Recombinations in yeast strain RS112 include a nine-fold increase in deletion of the 6 kb plasmid pRS6 giving rise to HIS^+ mutations, and a five-fold increase in intrachromosomal recombinations leading to ADE^+ mutations. Cell survival at 250 μg/ml, the highest concentration tested, was 75%. EB has been reported to be mutagenic to yeast mitochondrial DNA (Slonimski 1968) but has been previously determined to be negative in yeast chromosomal mutation and recombination systems (Zimmermann *et al.* 1984).

- Cell division ceased in chick embryo fibroblasts incubated with EB at 10 μg/ml for 24 hours, 1 or 5 μg/ml for 48 hours, or 0.25 μg/ml for 170 hours (Heinen, 1974). After 3 to 5 hours the nucleoli of cells treated with 1 to 10 μg/ml EB become smaller and denser; after 24 hours the nucleoli increase in size, become heterogeneous, and large chromatin blocks appear in some cells. The nucleoli remain normal for several days in cells treated with 0.25 μg/ml, but

mitochondrial swelling and intramitochondrial granules are apparent. Similar results were obtained with mouse Erlich tumor cells.

Metabolism: EB undergoes N-acylation *in vitro* by rat hepatic cytochrome P448 and a soluble protein to at least one metabolite mutagenic to *Salmonella typhimurium* (Lecointe, 1981). Sprague-Dawley rats administered 15 mg/kg EB iv were monitored for biliary metabolites. Biliary excretion totalled approximately 14-15% of the administered dose after 7 hours and the major metabolites were: 8-acetyl EB, unchanged EB and 8-acetylhydroxy EB. Radiolabelled material was not used (Fraire 1981).

Pharmacokinetic determinations of radiolabelled EB administered IM to rabbits and calves were carried out by Gilbert and Newton (1982). Approximately one-third of a 1 mg/kg dose was excreted in rabbit urine and two-thirds in the faeces; maximum concentrations corresponding to 180 ng/ml were achieved in blood and 50 ng/ml in tissue fluid approximately 1 hour after EB administration. The administration of 10 mg/kg resulted in a 2- to 3-fold increase in blood and tissue fluids and did not alter the proportions excreted in the urine and faeces. Two to three percent of the drug remained in the tissues after 9 days, primarily in the liver and kidney.

In calves administered 1 mg/kg, blood and tissue concentrations reached approximately 120-170 ng/ml within 1 hour of administration. These levels fell rapidly during the next 24 hours, and more slowly over the next 8 days to 15 ng/ml. Twenty percent of the administered dose was excreted in the urine and 50% in the faeces within 2 days of treatment. Ten days after EB administration 4% of the administered dose was present in the tissues, primarily in the liver and kidneys.

The tissue distribution of free EB and an EB-DNA complex was determined in male rats four hours after iv injection. Results showed that free EB accumulated preferentially in rat kidneys, adrenals, thyroid, and heart (Ellens, 1978). When administered complexed with DNA, less EB

was taken up by the heart, skeletal muscle, thyroid, adrenals, and intestine, while more was associated with the spleen and blood cells. The authors suggest that administering EB as the DNA complex may result in less toxicity associated with inhibition of heart mitochondria when EB is used as a drug.

Other Biological Effects: EB has been shown to be a teratogen, causing severe malformations in a Frog Embryo Teratogenesis Assay: Xenopus (FETAX). Three-week-old South African frog (*Xenpous laevis*) larvae exposed to near toxic EB concentrations developed gross malformations of all major organ systems, including spinal curvature, anencephaly, microcephaly, and microphthalmia . The LC_{50} for EB in this system was 0.05 mg/ml while the EC_{50} for malformation was 0.035 mg/ml (Courchesne and Bantle, 1985).

The principle uses of EB are based on its role as a nucleic acid intercalating agent. EB is further known as an inhibitor of RNA, DNA and protein synthesis and is, thereby, considered to be an antimetabolite.

Sea urchin (*Paracentrotus lividus and Arbacia lixula*) eggs exposed to sea water containing 20 µg/ml or more EB failed to undergo normal division (Vacquier and Brachet, 1969). The authors attributed this effect to a stabilization of DNA by binding with EB preventing replication; this effect was most pronounced in prophase.

Treatment of CHO cells with 10 µg/ml EB for 22 hours followed by EB removal resulted in the loss of the ability by many cells to form normal, bipolar mitotic spindles (McGill, 1976). Thirty-nine percent of mitotic cells exhibited multipolar spindles or polyploidy. Mitotic abnormalities appeared in succeeding generations suggesting that EB treatment affected procentriole formation.

Nass (1972) indicated that the growth of mouse fibroblasts and baby hamster kidney cells was completely inhibited by concentrations of 0.1 to 5 μg/ml. Cell growth resumed following the removal of EB after a lag period dependent on the length of exposure to EB. Mitochondrial, but not nuclear, DNA synthesis was inhibited by exposure to EB.

The induction of the tumor suppressor protein p53, a G_1 cell cycle regulator and apoptosis inducer, was studied in the human ML-1 myeloid leukemia and LNCaP prostatic adenocarcinoma cell lines (Nelson and Kaston, 1994). Results indicated that EB did not stimulate significant p53 induction. The authors determined that DNA breaks are required to stimulate p53 production, and compounds that cause other forms of DNA damage do not induce production of p53.

Escherichia coli expression of mammalian cytochrome b5 was reduced by 50% in the presence of 35 μg EB/ml (Hoare, 1993); expression was nearly eliminated at 200 μg/ml EB. Cell growth was unaffected at EB concentrations below 6.25 μg/ml, while concentrations up to 200 μg/ml reduced almost all cell growth.

Bleiomycin and EB were studied to determine interactive affects for use in cancer therapy. Treatment of mouse laukemia cells *in vitro* with EB first and then bleiomycin resulted in synergistic effects on the inductin of the release of nuclear chromatin 8X over additivity) (Agostino, 1984).

Mattern and Painter (1978) reported that in CHO cells made permeable with 1% Tween 80, semiconservative DNA synthesis was stimulated at low EB concentrations and inhibited at higher concentrations. Maximum ^{3}HdTMP incorporation was observed at 2 μg/ml EB, the concentration corresponding to minimum chromosomal supercoiling.

Yajima and Suzuki (1979) reported diffuse status spongiosis in the subpial areas of the ventro-lateral brain stem of rats receiving a single 2.5 μg intracisternal (intracranial) EB injection.

These lesions were characterized by oligodendrocyte degeneration and the formation of intra-myelinic vacuoles. EB did not cross the blood brain barrier in mice (Cesarini 1985) following the IV injection of 0.1 ml of a 0.1% EB solution. EB concentration was observed in the nuclei and nucleoli of certain nerve cells outside the blood brain barrier.

Finally, possible mechanims for EB's action in biological systems have been investigated, as in the following two studies.

Aktipis and Panayotatos (1976) indicated that the inhibition of RNA polymerase by EB results from a modification of template sites thereby preventing productive binding of the enzyme. This modification limits the number of RNA polymerase molecules able to form initial, or I, complexes but does not affect the rate of transformation of the I complex to the "readily-starting" (RS) complex responsible for the rapid initiation of RNA chains.

Pena, et al. (1977) reported that EB is actively accumulated by isolated rat liver mitochondria resulting in the inhibition of the coupled oxidation of glutamate and succinate. In mitochondria exposed to concentrations greater than 80 μM, however, EB enhanced the State 4 respiratory rate with a succinate substrate. The differences between the effect of EB on glutamate and succinate oxidation were attributed to the inhibition of the NADH-ubiquinone segment of the respiratory chain.

Structure/Activity Relationships: A screening of literature sources relevant to carcinogenicity or mutagenicity was carried out for relevant information on the 15 structural analogs of EB presented in Appendix A. No SAR information at all was found for a number of the compounds [2,3,4,7,8,10,11]. Studies with information on the other compounds are summarized in the following paragraphs, with reference tags [#] to the appropriate numbered compounds in Appendix A.

Lecointe et al (1981) carried out a structure-mutagenic activity study of 11 phenanthridine derivatives of EB, including compound [9], in an Ames/*Salmonella* assay. They reported that the mutagenicity of EB realtive to [9] in strains TA98, TA1538 and TA1537 was 400/29, 300/33 and 3/20 revertants per nmole per plate respectively. Both compounds were reported negative in strains TA100 and TA1535.

The mutagenicity of the EB azide analogs, ethidium diazide (EAdi) [13] and 3-amino-8-azido-ethidium (EAmomo) [15], was studied in *Salmonella typhimurium* strain TA1538 (Yielding 1979). The azide analogs are photoactivated mutagens acting without S9. EAdi is the less toxic and mutagenic of the two analogs inducing 70-90 mutations above background per 5 x 10^7 cells compared to 300 to 600 mutations per 5 x 10^7 cells for EAmono. Toxicity and mutagenicity were enhanced by co-incubation of the cells with EB.

Yielding and Firth (1980) reported on the toxicity/mutagenciity of nine EB analogs in *S. typhimurium* strains TA1537, 1538, 1977, and 1978. The purpose of the experiment was to identify structure-function for EB photoaffinity labellling. Results indicated that some of the compounds without azido substitrents [12] were mutagenic. The most mutagenic compounds were analogs 14 and 15, and, to a lesser extent, analog 13.

EB and several analogs in the presence of S9 induced mutations in *S. typhimurium* TA1538 (Fukunga, 1984). The analogs included the ethidium chloride (EC) derivatives 3,8-diazido EC [13]; 3-azido EC [14]; 8-azido EC [15]; 3,8-diamino-5-(diethylmethylaminopropyl)-6-phenylphenanthridinium diiodide; and 3-amino-8-azido-5-diethylmethylaminopropyl)-6-phenylphenanthridinium chloride. None of the compounds tested induced a significant increase in mutation frequency in the DNA-proficient analog strain TA1978.

Creech and Bono (1971) tested nine DNA reactive drugs, including compound [1] for *in vitro* *E. coli* RNA polymerase inhibition. Compound [1] caused 50% inhibition at a concentration of 100 μM and was named in the category "least effective."

Dougherty (1982) studied comparative DNA polynucleotide binding affinities for EB and compound [5]. He reported that both compounds had similar binding affinities and apparantly acted by a single mode.

The inhibition of macromolecular synthesis by EB and several analogs was studied in *E. coli*, L5178Y cells, and mitochondria (Bosmann and MacGregor, 1971) the EB analogs employed included 3.8-diamino-6-*p*-aminophenyl-5-methylphenanthridinium chloride (150C47) [12]; and the drug prothidium (3-amino-8-(2-amino-6-methylpyrimidin-4-yl-amino)-6-*p*-aminophenyl-5, 1'-dimethylphenanthridinium bromide). Acylated derivatives of these analogs were also studied. All compounds except prothidium inhibited RNA synthesis in *E. coli* by about 50% at 175 μM concentration; EB and 150C47 [12] were equally effective in L5178Y cells while prothidium and carbidium were much less effective. DNA synthesis in *E. coli* was only moderately inhibited by any of the compounds, with carbidium reducing synthesis by 40% and EB by about 25% at 500 μM. At 500 μM in L5178Y cells, DNA synthesis was almost completely inhibited by EB, reduced approximately 75% by carbidium, reduced 50% by prothidium, and reduced 25% by 150C47. EB at 175μM reduced DNA synthesis by about 25%.

At 175μM, protein synthesis in *E. coli* was reduced approximately 40% by EB and 20% by carbidium. In L5178Y cells, protein synthesis was largely unaffected by any compound at 75 μM while an approximately 25% reduction occurred at 175 μM EB. At 500 μM, EB almost eliminated protein synthesis in L5178Y cells while the other three compounds reduced synthesis by approximately 30 to 60 %.

None of the compounds significantly reduced DNA synthesis in rat cerebral cortex mitochondria, or DNA or RNA synthesis in rat liver mitochondria at concentrations below 500 μM. RNA synthesis in rat cerebral cortex mitochondria was reduced marginally by all four compounds at 175 μM. Protein synthesis was inhibited approximately 25% in both rat cerebral cortex and liver mitochondria; the order of effectiveness was EB > 150C47 > prothidium > carbidium in the cerebral cortex and prothidium > 150C47 > EB > carbidium in the liver.

In general, the acylated derivatives tested were less inhibitory of cellular RNA synthesis, and similar in the inhibition of DNA and protein synthesis, than the non-acylated compounds. Mono and diacylated EB were less inhibitory of RNA synthesis, and equally inhibitory of DNA and protein synthesis, in *E. coli*. In L5178Y cells, the acylated EB derivatives were less inhibitory of RNA, DNA, and protein synthesis than non-acylated EB.

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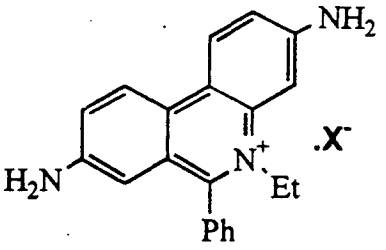
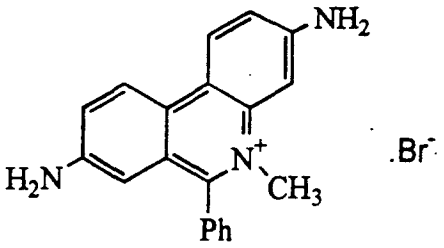
APPENDIX A

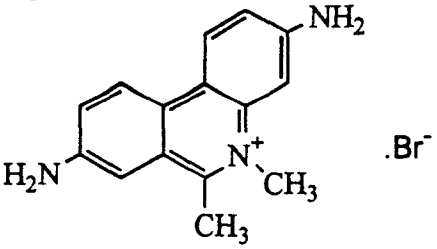
Structural Analogs of EB

1) Ethidium chloride [602-52-8]: X = Cl
2) Ethidium fluoride [112076-54-7]: X = F
3) Ethidium hydroxide [51636-14-7]: X = OH
4) Monoazide ethidium [64295-07-4]: X = -N=N$^+$=N$^-$
5) Dimidium bromide [518-67-2]

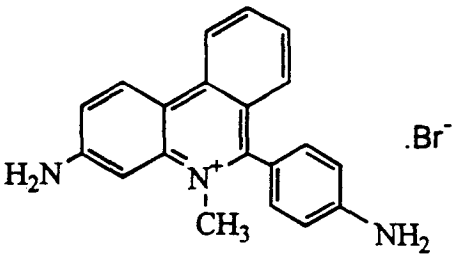




6) Phenanthridinium, 3,8-diamino-5,6-dimethyl-, bromide [32155-21-8]

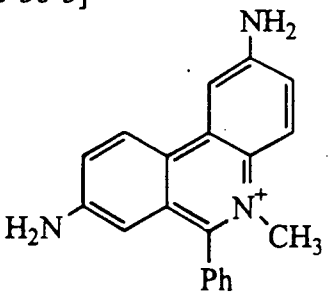


7) 3-Amino-6-(p-aminophenyl)-5-methylphenanthridinium bromide [113090-55-4]

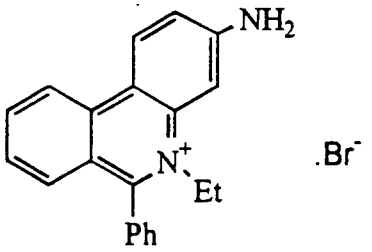


Structural Analogs of EB (cont.)

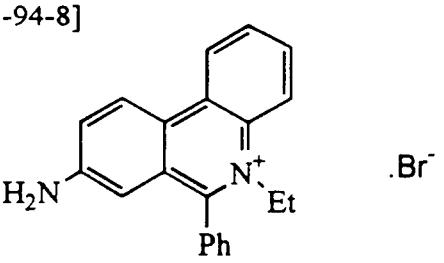
8) 2,8-Diamino-5-methyl-6-phenylphenanthridinium bromide [113090-38-3]



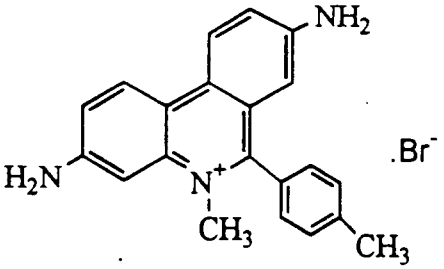
9) Phenanthridinium, 3-amino-5-ethyl-6-phenyl-, bromide [62394-24-5]



10) Phenanthridinium, 8-amino-5-ethyl-6-phenyl-, bromide [61525-94-8]

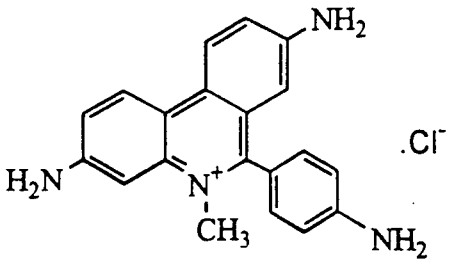


11) 3,8-Diamino-5-methyl-6-p-tolylphenanthridinium bromide [113038-36-1]



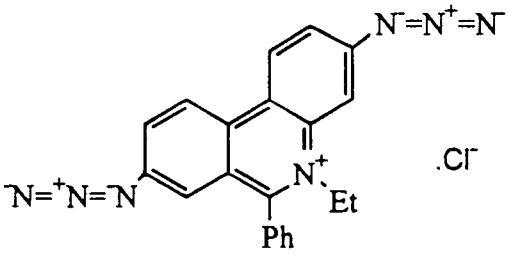
Structural Analogs of EB (cont.)

12) 3,8-.Diamino-6-(p-aminophenyl)-5-methylphenanthridinium chloride



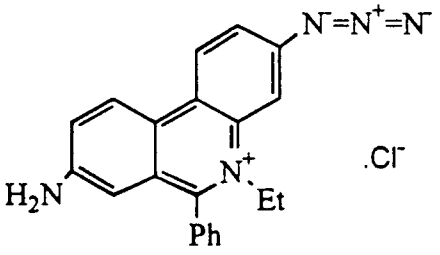
EB1

13) 3,8-Diazide-5-ethyl-6-phenylphenanthridinium chloride



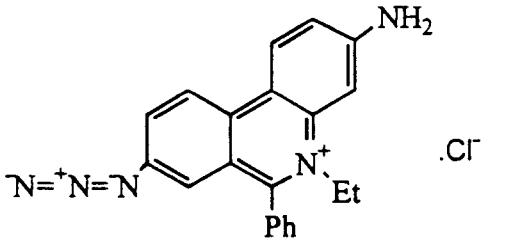
EB2

14) 3-Azido-8-amino-5-ethyl-6-phenylphenanthridinium chloride



EB3

15) 3-Amino-8-azido-5-ethyl-6-phenylphenanthridinium chloride



EB4