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Biochemical Pharmacology, Vol. 37, No. 24, pp. 4780–4783, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Inhibition of bovine serum amine oxidase activity by aminoalkylaminoanthraquinones

(*Received* 11 *April* 1988; *accepted* 22 *July* 1988)

Anthraquinones, mainly their alkyl derivatives are known to interact with nucleic acids [1]. Amidine derivatives of anthraquinone have been synthesized and their activity against *Entamoeba histolytica* has been demonstrated [2]. More recently bis (substituted amino-alkylamino) anthraquinones have been synthesized [3, 4], at least one of which possessed antineoplastic activity against leukemia and melanoma tumors. The antibacterial activity of 1-amino-4-hydroxy-anthraquinone has also been demonstrated [5]. Previous studies in our laboratory demonstrated that diaminoalkyl-aminoanthraquinones inhibited the growth of leishmanial promostigotes grown *in vitro* [6]. It has been shown that a three carbon diamine derivative is more active than the two or four carbon diamine analogues [6]. It has also been observed that the antiviral activity of aminoalkylaminoanthraquinones can be reduced by adding either putrescine or spermidine to a suspension of T_2 coliphages (unpublished data). These findings suggested that the antiviral activity of the aminoalkylanthraquinone is related to polyamines. Polyamines and diamines are ubiquitous organic cations which are present in all prokaryotic or eukaryotic cells analyzed [7, 8]. Polyamines interact with nucleic acids (including phage DNA) and affect their structure and function [7]. Replacing polyamines by aminoalkylaminoanthraquinones may distort the structure of the nucleic acids and thus interfere with their biological activity.

In this study we tested the effect of various aminoalkylaminoanthraquinones on the activity of polyamine oxidizing enzymes. It will be shown that the oxidation of the polyamines spermidine and spermine is dramatically inhibited by these compounds.

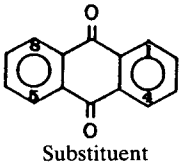
Experimental

Materials. Table 1 shows the structures of the substituted anthraquinones investigated in this study. The preparation of these compounds has been previously described [9, 10].

N-Methyl-2-benzothiazolone hydrazone was obtained from EGA Chemie (Steinheim, F.R.G.) and putrescine, spermidine and spermine hydrochlorides were from Sigma (St. Louis, MO). Pea seedling diamine oxidase was purified by the method of Hill [11] (specific activity 10 units/ml; 100 units/mg; 1 unit catalyses the oxidation of 1 μmol putrescine/hr). Pig kidney diamine oxidase (0.1 unit/mg 10 units/ml) was obtained from Sigma. Bovine serum amine oxidase (SAO) was purified as previously described [12] and its activity assayed [13] using benzylamine as substrate (110 units/ml; 50 units/mg).

Methods. The substituted anthraquinones were tested for their ability to inhibit the oxidation of putrescine by both diamine oxidases and the oxidation of spermidine and spermine by SAO. The oxidation of the amines was quantitated colorimetrically by coupling of the resulting aldehydes with *N*-methyl-2-benzothiazolone hydrazone [14]. The experimental details were as follows. Enzyme solution (100 μl) was added to a mixture of buffer (100 μl of 0.2 M Tris–HCl, pH 7.3), substrate (100 μl of 0.1 M aqueous solution of putrescine, spermidine or spermine) and inhibitor (100 μl of 2–200 μM aqueous solution). The final volume (400 μl) was incubated in a water bath at 37° for 1 hr, after which *N*-methyl-2-benzothiazolone hydrazone (0.5 ml of 0.4% aqueous solution) was added. The reaction was linear over 1 hr of incubation. The solution was kept at room temperature (25°) for 30 min before addition of ferric chloride (2.5 ml of 0.2% aqueous solution). After standing a further 15 min at room temperature, the intensity of the resulting blue colour was measured at 660 nm (Bausch and Lomb Spectronic 1001 spectrophotometer). For the oxidation product of spermidine, $\varepsilon = 6.25 \times 10^3 \mathrm{l \cdot mol^{-1} cm^{-1}}$ and for spermine, $\varepsilon = 12.5 \times 10^3 \mathrm{l \cdot mol^{-1} cm^{-1}}$ [14] was used to quantitate the amount of product formed.

Table 1. Structure of substituted anthraquinones studied



Compound No.	Substituent Position	Substituent
1	1	$-NHC_2H_4NH_2$
2	1	$-NHC_3H_6NH_2$
3	1	$-NHC_4H_8NH_2$
4	2	$-NHC_2H_4NH_2$
5	2	$-NHC_3H_6NH_2$
6	1 and 5	$-NHC_2H_4NH_2$
7	1 and 5	$-NHC_3H_6NH_2$
8	1 and 8	$-NHC_2H_4NH_2$
9	1 and 8	$-NHC_3H_6NH_2$
10	1 and 8	$-NHC_4H_8NH_2$
11	1	$-NHC_2H_4N(CH_3)_2$
12	1	$-NHC_3H_6N(CH_3)_2$
13	1 and 5	$-NHC_2H_4N(CH_3)_2$
14	1 and 5	$-NHC_3H_6N(CH_3)_2$
15	1 and 8	$-NHC_2H_4N(CH_3)_2$
16	1 and 8	$1\text{-}NHC_2H_4N(CH_3)_2\text{-}8\text{-}Cl$
17	1	$-NHC_2H_4NHC_2H_4NH_2$
18	1	$-NHC_2H_4NHC_3H_6NH_2$
19	1	$-NHC_3H_6NHC_3H_6NH_2$

Results and discussion

The substituted anthraquinones studied here (Table 1) inhibit the oxidation of both spermidine and spermine by SAO when present in the incubation mixture at levels above 1 μM. Figure 1A shows the effect of the inhibitors on the oxidation of spermidine by SAO. A wide range in inhibitor activity was observed. The most active inhibitors were found to be compounds 17–19 in which the substituent side chains closely resemble polyamine groups. Of the other inhibitors, compound 3 (substituent $1\text{-}NH(CH_2)_4NH_2$) and a number of disubstituted compounds (Nos. 6–8) also exhibited high activity. Further study of compounds 3, 7 and 17 (Fig. 1A) show 50% inhibition at 8 μM, 4 μM and 2 μM respectively. The activity of the substituted amino-alkyl-aminoanthraquinones as inhibitors of SAO tended, in general, to increase as the length of the intervenient carbon chain increased from 2 carbon atoms to 4 carbon atoms. With regard to the position of the substituents, position 2 produced weak inhibitors. Bis-substitution at positions 1,5 produced a stronger inhibitory effect and at positions 1,8 a weaker effect although compound 8 is a marked exception. In the case of the compounds with the tertiary amine end-group, the 1,5 bis-substitution exhibited weaker inhibition than the mono-substituted analogues. Methylation of the primary amine end-group resulted in a slight enhancement in the inhibitory activity of the mono-substituted compounds, while compound 14, the analogue of bis-substituted compound 7, had considerably less activity.

Figure 1B shows the inhibition effect on SAO activity by the substituted anthraquinones when spermine was used as substrate. The presence of a second aminopropyl group in the substrate generally reduced the extent of inhibition observed. The most active inhibitors were the same compounds as for spermidine. The concentrations of compounds 3, 7 and 17 required for 50% inhibition increased to 68 μM, 15 μM and 6 μM respectively.

These substituted anthraquinones, at the concentrations shown in Fig. 1, failed to exhibit any major inhibitory effect on the oxidation of putrescine by either pig kidney or pea seedling diamine oxidase. This result can be compared to the minor inhibition previously noted with *N*-alkyl-putrescines, which are weak inhibitors of SAO activity [15].

The nature of the inhibition of SAO activity by these compounds was further investigated. Figures 2A and 2B show the Lineweaver–Burk reciprocal plots for both spermidine and spermine substrates with compound 3 as inhibitor and Figs 3A and 3B show the plots for compound 7 as inhibitor. The inhibition appears to be of mixed-type [16] with K_i for spermidine substrate 2 μM for compound 3 and 3 μM for compound 7, while for spermine substrate the values were 13 μM and 5 μM respectively. The larger values with spermine are indicative of the weaker inhibition observed with this substrate.

The mechanism of action of these inhibitors of SAO activity can only be surmised. Bovine SAO is a dimer with

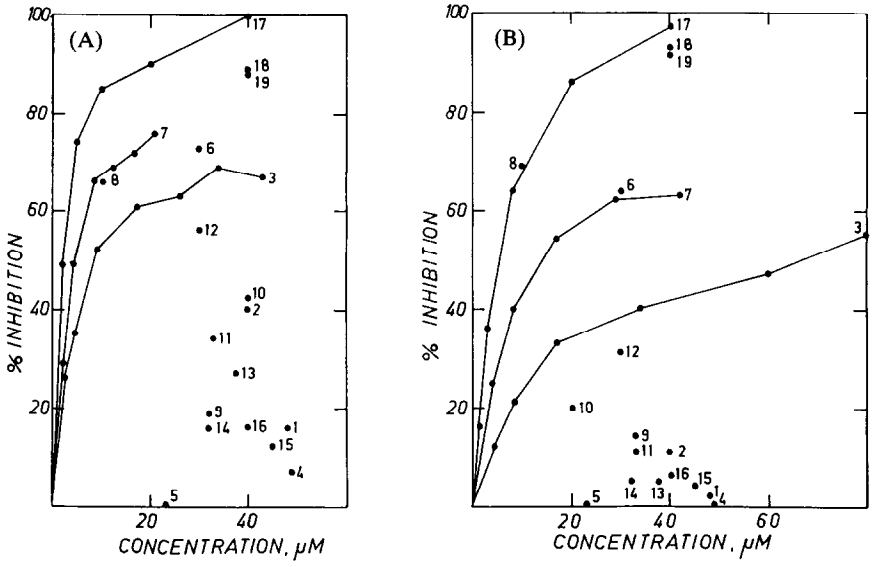


Fig. 1. Inhibition of spermidine (A) and spermine (B) oxidation by substituted anthraquinones. (Compounds numbered according to Table 1. Concentration of polyamine substrate in the incubation mixture was 25 mM.)

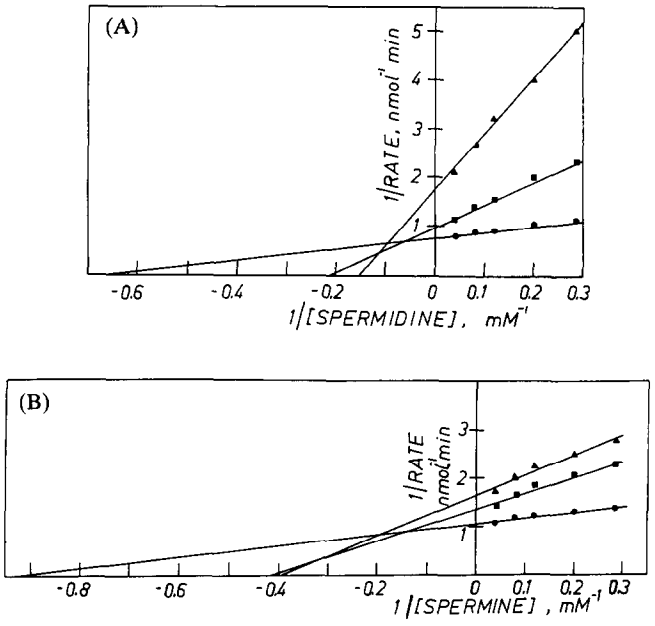


Fig. 2. Kinetics of inhibition of serum amine oxidase by compound 3 with spermidine (A) and spermine (B) as substrates. (A) ●, control; ■, 4.3 μM; ▲, 22 μM compound 3; (B) ●, control; ■, 17 μM; ▲, 43 μM compound 3.

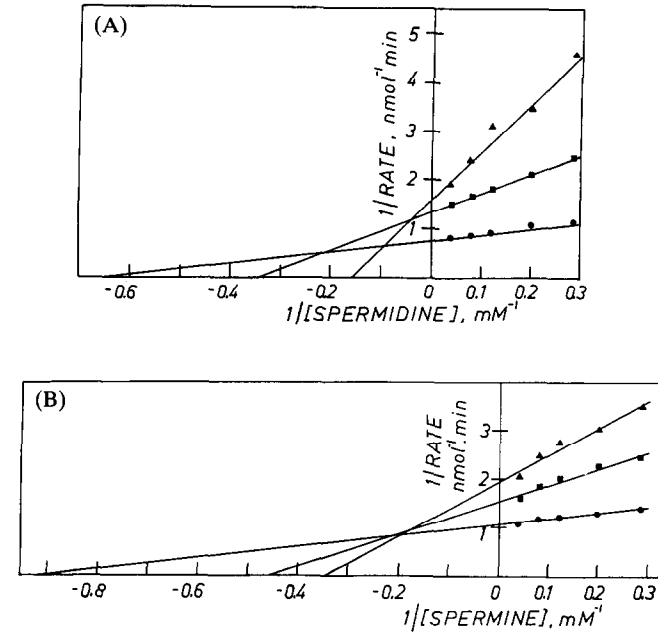


Fig. 3. Kinetics of inhibition of serum amine oxidase by compound 7 with spermidine (A) and spermine (B) as substrates. (A) ●, control; ■, 8.4 μM; ▲, 14.7 μM compound 7; (B) ●, control; ■, 8.5 μM; ▲, 17 μM compound 7.

two identical polypeptide subunits and contains two non-equivalent copper (II) ions [17] and a carbonyl cofactor recently identified as a pyrroloquinoline quinone [18, 19]. It has also recently been shown that the presence of copper (II) is essential for the reoxidation of the carbonyl cofactor, which is prevented by the loss of one of the copper atoms [20]. Stereochemical considerations [20] and NMR studies [21] exclude the close proximity of the copper atoms to the carbonyl group. The mode of action of the amino-substituted anthraquinone inhibitors can either be as substrate analogues or as copper chelators [5]. In this latter respect, the substituted anthraquinone can act as a bidentate ligand either via the amine groups on the side chain or via the amine at position 1 and the quinone oxygen (position

9). The possible involvement of this latter chelation mechanism is indicated by the decrease in inhibitor activity observed when this chelation is prevented by the shift in the substituent from position 1 in the ring system to position 2. Chelation by the amine groups of the side chain appears to be an unlikely mechanism since compound 3, which has high activity, would give rise to a sterically unfavourable 7-membered chelate ring as compared to compounds 1 and 2 which are of lower activity, but which would yield favourable 5- or 6-membered rings.

Acknowledgements—The financial assistance of the South African Medical Research Council and the Israel National Council for Research and Development to one of us (GSS) is gratefully acknowledged.

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Biochemical Pharmacology, Vol. 37, No. 24, pp. 4783–4786, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

δ-Aminolaevulinate synthase: mechanism of its response to malathion

(*Received* 20 *April* 1987; *accepted* 21 *June* 1988)

Aminolaevulinate (ALA)-synthase is the first and rate-limiting enzyme of hepatic heme biosynthesis [1]. ALA-synthase is induced by several drugs and steroids in the liver [1–4]. This experimentally induced porphyria resembles the human acute hepatic porphyrias in its biochemistry [5]. 2-Allyl-2-isopropylacetamide (AIA) and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) also have been studied as potent inducers of ALA-synthase [6, 7]. In animals treated with these drugs a considerable amount of the enzyme accumulates in mitochondria, as well as cytosol, of the liver [7]. The enzyme is synthesized on free polyribosomes as a cytosolic precursor which is processed during incorporation into mitochondria to give the mature form [8–10].

Heme regulates the activity of ALA-synthase in the liver [1]. Ferrochelatase catalyzes the insertion of ferrous iron