dialdehyde levels in fresh and frozen myocardium. *Basic Res Cardiol* **79**: 513–518, 1984.
7. Habig WH and Jakoby WB, Assays for differentiation of glutathione *S*-transferases. *Methods Enzymol* 77: 398–405, 1981.
8. Mannervik B, Axelsson K and Larson K, Thioltransferase. *Methods Enzymol* **77**: 281–285, 1981.
9. Lawrence RA and Burk RF, Glutathione peroxidase activity in selenium deficient rat liver. *Biochem Biophys Res Commun* **71**: 952–958, 1976.
10. Carlberg I and Mannervik B, Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J Biol Chem* **250**: 5475–5480, 1975.
11. Ellman CL, Tissue sulphydril groups. *Arch Biochem Biophys* **82**: 70–77, 1959.
12. Klotzsch H and Bergmeyer HU, Glutathione. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), pp. 363–366. Academic Press, New York, 1965.
13. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. *Anal Biochem* **72**: 248–254, 1976.
14. Walker MJ, Curtis MJ, Hearse DJ, Campbell RWF, Janse MJ, Yellon DM, Cobbe SM, Coker SJ, Harness JB, Harron DWG, Higgins AJ, Julian DG, Lab MJ, Manning AS, Northover BJ, Parratt JR, Riemersma RA, Riva E, Russel DC, Sheridan DJ, Winslow E, Woodward B, The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. *Cardiovasc Res* **22**: 447–455, 1988.
15. Julicher RH, Tijburg LBM, Sterrenberg L, Bast A, Koomen JM and Noordhoek J, Decreased defence against free radicals in rat heart during normal reperfusion after hypoxic, ischemic and calcium-free perfusion. *Life Sci* **35**: 1281–1288, 1984.
16. Ferrari R, Ceconi C, Curello S, Guarnieri C, Caldarera CM, Albertini A and Visioli O, Oxygen-mediated myocardial damage during ischemia and reperfusion: role of the cellular defences against oxygen toxicity. *J Mol Cell Cardiol* **17**: 937–945, 1985.
17. Arduini A, Mezzetti A, Lapenna D, Porreca E, DeJulia J, Mancinelli G, De Cesare M, Scarinci A and Cuccurullo F, Glutathione and glutathione related enzymes in ischemic and reperfused rat heart. *Med Sci Res* **15**: 391–392, 1987.
18. Xia Y, Hill KE and Burk R, Effect of selenium deficiency on hydroperoxide-induced glutathione release from the isolated perfused rat heart. *J Nutr* **115**: 733–742, 1985.
19. Ishikawa T and Sies H, Cardiac transport of glutathione disulfide and S-conjugate. *J Biol Chem* **259**: 3838–3843, 1984.

Biochemical Pharmacology, Vol. 39, No. 10, pp. 1620–1623, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Antimalarial activity of substituted anthraquinones

(*Received* 14 *April* 1989; *accepted* 22 *December* 1989)

Most antimalarial agents can be divided into two classes based on their speed of action [1]: (a) cinchona alkaloids, aminoquinoline, 9-aminoacridine derivatives, and (b) sulfa compounds of pyrimidine and biguanine derivatives which interfere with the conversion of dihydrofolic acid (FH_2) to tetrahydrofolic acid (FH_4), and thus inhibit the synthesis of purines and pyrimidines.

It has been suggested that in addition to these two classes, substantial evidence clearly indicates that malaria parasites may impair oxidant defence and repair functions of host erythrocytes through a number of mechanisms [2–7]. According to this view it seems likely that oxidant agents or alternatively agents that generate reactive oxygen, could damage parasitized red cells. This phenomenon seems to be related to the interference of malaria parasites with cellular oxidant repair mechanisms [2–7].

Aldehydes [8], diketones [9, 10], nitro compounds [11, 12] and apparently also substituted aminoalkylamino anthraquinones may generate reactive oxygen and thus inactivate malaria parasites (*Plasmodium falciparum, P. vinckei* and *P. berghei*). It has already been demonstrated that aminoalkylamino-anthraquinones exhibit a broad spectrum of activities such as anticancer [13–17], antiviral [18], antibacterial [13], antileishmania [19] and antiamebic [20] activity, together with their inhibitory effect on bovine serum amine oxidase [21]. Aminoalkylamino-anthraquinones intercalate [22–24] with DNA or form free radicals [25–28]. In addition the products formed by oxidizing naturally occurring polyamines by bovine serum amine oxidase [29, 30] or the purified aminoaldehyde products exhibit antimalaria activity [31].

Thus it is most likely that anthraquinone skeletons composed of aminoalkylamino or polyamino side chains should represent promising antimalarial model compounds.

In the light of the growing problem of drug resistant malarial parasites, the development of new drugs is of primary importance. In the present study we tested the effect of various anthraquinone derivatives on the growth and DNA synthesis of the malarial parasite (*P. falciparum*).

Materials and methods

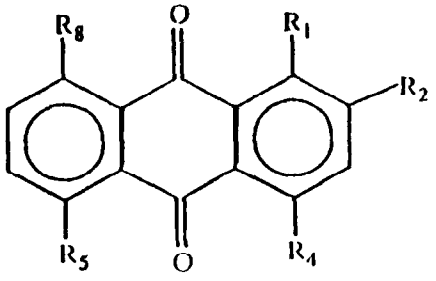
Table 1 shows the structure of the substituted anthraquinones investigated in this study. The preparation of these compounds has been described elsewhere [18].

Parasites. Plasmodium falciparum (FCR 3TC) was cultured according to the candle jar method [32]. Parasites were synchronized by the combination of sorbitol lysis [33] and gelatin sedimentation [34]. Parasitemias and stage distribution were estimated from Giemsa stained smears by counting 5×10^3 erythrocytes and 5×10^2 infected cells, respectively.

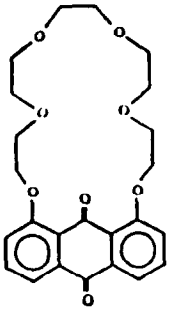
Hypoxantine incorporation. Cultures of *P. falciparum* with an initial parasitemia of 10–12% ring form stage, were distributed into 96 microculture trays (100 μL/well) incubated with the drugs dissolved in RPMI-1640 medium (GIBCO) at final concentrations of 1, 10 and 100 μM. After 22–24 hr [34] hypoxanthine was added to each well (1 μCi per well sp. radioactivity 10 Ci/μmol, New England Nuclear, Boston, MA) and incubation continued for another 7 hr. Thereafter, cultures were harvested by a Microtiter Dynateck Autowash cell Harvester, using 934-H glass filters. Filters were dried and radioactivity was determined by liquid scintillation counting.

Distribution of different stages. P. falciparum parasites were grown as above with an initial parasitemia of 1.5–2.0%. When cultures reached the mature trophozoite stage, drugs at final concentrations of 10 and 100 μM were added to each microwell. After 24 hr, the distribution of different

Table 1. Structure of substituted anthraquinones studied



Anthraquinone



15.

Compound No.	Substituted	Code
1.	R_1=HN$(CH_2)_2$N$(CH_3)_2$; R_2=R_4=R_5=H; R_8=Cl	1-C_2DM 8Cl
2.	R_1=HN$(CH_2)_2$NH$(CH_2)_2$NH_2; R_2=R_4=R_5=R_8=H	1-C2C2
3.	R_1=HN$(CH_2)_2$NH$(CH_2)_3$NH_2; R_2=R_4=R_5=R_8=H	1-C2C3
4.	R_1=HN$(CH_2)_3$NH$(CH_2)_2$NH_2; R_2=R_4=R_5=R_8=H	1-C3C2
5.	R_1=HN$(CH_2)_3$NH$(CH_2)_3$NH_2; R_2=R_4=R_5=R_8=H	1-C3C3
6.	R_1=HN$(CH_2)_4$NH_2; R_2=R_4=R_5=R_8=H	1-C4
7.	R_1=R_4=HN$(CH_2)_2$N$(CH_3)_2$; R_2=R_5=R_8=H	1,4-C2DM
8.	R_1=R_4=HN$(CH_2)_2$NH$(CH_2)_2$OH; R_2=R_5=R_8=H	1,4-C2NHE
9.	R_1=R_5=HN$(CH_2)_2$(2PYR); R_2=R_4=R_8=H	1,5-C2PYR2
10.	R_1=R_5=HN$(CH_2)_2$N$(CH_3)_2$; R_2=R_4=R_8=H	1,5-C2DM
11.	R_1=R_5=HN$(CH_2)_3$N$(CH_2CH_2OH)_2$; R_2=R_4=R_8=H	1,5-C3NHDE
12.	R_1=R_5=HN$(CH_2)_2$NH$(CH_2)_2$OH; R_2=R_4=R_8=H	C2NHE
13.	R_1=R_8=HN$(CH_2)_2$NH$(CH_2)_2$OH; R_2=R_4=R_5=H	C2NHE
14.	R_2=HN$(CH_2)_2$NH_2; R_1=R_4=R_5=T_8=H	2-C2

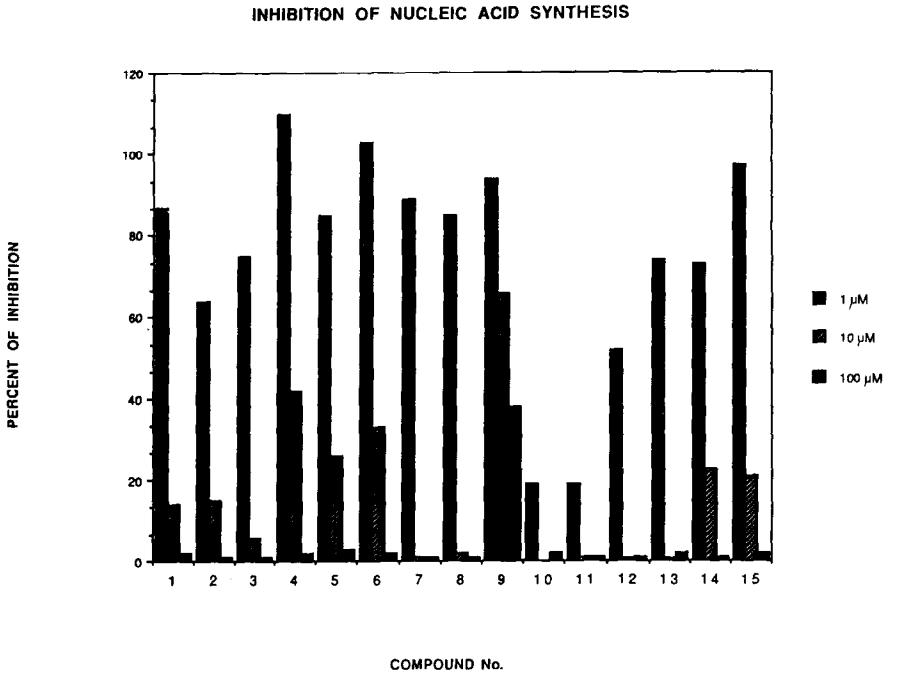


Fig. 1. Effect of substituted anthraquinones on nucleic acid synthesis by *Plasmodium falciparum*. The compounds tested were those listed in Table 1, except that 1,8-pentaethylene glycol was added as No. 15. Results are expressed as percent of [^{3}H]hypoxanthine incorporated into malarial nucleic acids. Value for untreated controls (100%) was 53,000 counts/min/well.

Table 2. Effect of substituted anthraquinones on the cell cycle of cultured *Plasmodium falciparum* parasite

		Relative distribution of parasite stage in presence of anthraquinone					
		100 μM			10 μM		
		Ring	Tropozoite	Schizont	Ring	Tropozoite	Schizont
1.	1-C2DM-8Cl	0	0	0	0	6	4
2.	1-C2C2	0	0	0	26	0	5
3.	1-C2C3	0	6	0	0	10	0
4.	1-C3C2	0	0	0	0	0	10
5.	1-C3C3	0	0	0	0	5	5
6.	1-C4	27	—	3	30	0	4
8.	1,4-C2NHE	0	0	0	0	0	0
9.	1,5-C2PYR2	0	0	0	33	0	3
14.	2-C2	0	0	0	0	6	4
15.	1,8 pentaethylen glycol	0	0	0	12	0	8
Control		36	0	4	37	0	4

stages was determined by examining Giemsa-stained smears. Results are expressed as number of parasites per field (average of five fields, approximately 400 erythrocytes per field).

Results and discussion

Hypoxanthine is a purine precursor and is incorporated into DNA and RNA of malarial parasites. The exposure of parasites to the drugs at a concentration of 100 μM for 7 hr led to a significant reduction in nucleic acid synthesis (Fig. 1). The most active compounds were compounds Nos 10, 11 and 12 which caused a significant inhibition at a 10 μM concentration and a moderate effect even at 1 μM. It should be noted that all of these compounds lack a primary amino group. All of them are substituted at positions 1 and 5 and are either amino-alcohols or dimethylamino derivatives. It is also of interest that substituent as 2-*N*-dimethyl-amino-ethylamino groups at positions 1 and 5 caused a better inhibition of nucleic acid synthesis than in positions 1 and 4 (compare compounds 10 and 7).

Of the monosubstituted anthraquinones compound No. 3 was most active. It appears that the asymmetrical derivative containing an *N*-aminopropyl group (compound No. 3) was more active than the symmetrical derivative (compound No. 2) which consisted of a 2-*N*-aminoethyl,1,2 diaminoethane derivative. The addition of a chlorine atom at position 8 (compound No. 1) did not result in a significant antimalarial activity.

In the host, *P. falciparum* multiplies rapidly, producing up to 32 merozoites within 48 hr. The cell cycle of the malarial parasites can be divided into three major stages. The first, the ring (R) stage is followed by the second trophozoite (T) stage at the end of which DNA synthesis is initiated. The final stage in the cell cycle is the schizont (S) stage. At the end of this stage the infected erythrocyte bursts and the released merozoites infect other erythrocytes.

If a drug inhibits DNA synthesis, then the development of the parasites should be arrested at the end of the trophozoite or at the schizont stage and the number of new ring form should be reduced. Table 2 shows that in the untreated culture most of the parasites reached the ring stage. A similar process took place when compounds No. 6 (1-C4) or 9 (1,5-C2PYR2) were added to the cultures. Data presented in Fig. 1 also show that compound No. 9 only slightly inhibited the synthesis of malarial nucleic acids.

The most active compound was compound No. 8 which inhibited the growth of the parasites even at a concentration of 10 μM. It should be noted that this compound was not the best inhibitor of the synthesis of nucleic acid (cf. Fig. 1). This suggests that the antimalarial activity of this compound is not exclusively due to inhibition of nucleic acid synthesis.

Of the anthraquinones substituted at position 1, compounds Nos 1, 3, 4 and 5 gave similar results. None of the parasites reached the ring stage and all of them were arrested at the trophozoite or schizont stage. These compounds also inhibited nucleic acid synthesis (cf. Fig. 1). On the other hand compound Nos 2 and 6, which inhibited nucleic acid synthesis failed to block the development of the parasites and ring forms appeared as in the untreated controls (Table 2).

Compound Nos 2 and 3, which inhibit the synthesis of malarial nucleic acids (Fig. 1) are not substrates of serum amine oxidases [21] and therefore do not yield toxic oxidation products. It thus appears that the antimalarial activity of the anthraquinone derivatives resides in their ability to intercalate DNA. It is conceivable that the aminoalkyl side chains which resemble polyamines and diamines in their structure, facilitate a better interaction between the anthraquinone moiety and the bihelical DNA.

It should be stressed that many of the antiparasitic drugs such as chloroquine, quinacrine, imidocarb, berenil and pentamidine resemble polyamines in their structure [35] but contain tertiary amines. The addition of aminoalkyl groups to the anthraquinone moiety may provide a new line of antiparasitic drugs.

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REFERENCES

1. Roche EB and Kier LB, Parasite chemotherapy. In: *Principles of Medicinal Chemistry* (Ed. Foye WO), pp. 793–807. Lea and Febiger, Philadelphia, 1981.
2. Stocker R, Hunt NH, Buffinton GD, Weidemann MJ, Lewis-Hughes PH and Clark IA, Oxidative stress and protective mechanisms in erythrocytes in relation to *Plasmodium vinckei* load. *Proc Natl Acad Sci USA* **82**: 548–551, 1985.

3. Eckman JR and Eaton JW, Dependence of plasmodial glutathione metabolism on the host cell. *Nature* **278**: 754–756, 1979.
4. Allison AC and Eugui EM, The role of cell mediated immune responses in resistance to malaria with special reference to oxidant stress. *Annu Rev Immunol* **1**: 361–392, 1983.
5. Cox FEG, Oxidant willing of the malaria parasites. *Nature* **302**: 19, 1983.
6. Eaton JW and Wood PA, Malaria and red cell. In: *Progress in Clinical and Biological Research* (Ed. Brewer G-J), Vol. 165, pp. 395–410. Alan R Liss, New York, 1984.
7. Vennerstrom JL and Eaton JW, Oxidants, oxidant drugs and malaria. *J Med Chem* **31**: 1269–1277, 1988.
8. Royer RE, Deck LM, Campos NM, Hunsaker LA and Vander Jagert DL, Biologically active derivatives of gassypoli synthesis and antimalarial activities of peri-acylated gossylic nitrites. *J Med Chem* **29**: 1799–1801, 1986.
9. Fitch CD, Dutta P, Kanjananggulpan P and Chevli R, *Malaria and Red Cell*, pp. 119–130. Alan R Liss, New York, 1984.
10. Cohen G and Hochstein P, Generation of hydrogen peroxide in erythrocytes by hemolytic agents. *Biochemistry* **3**: 895–900, 1964.
11. Kolling VH, Haberkorn A and Herbold B, Untersuchungen an malariawirksamen omega-Aminoacyl-Verbindungen. *Arzneim-Forsch* **36**: 230–233, 1986.
12. Cabantchik ZI, Kutner S, Krugliak M and Ginsburg H, Amino transport inhibitors as suppressors of *Plasmodium falciparum* growth *in vitro* cultures. *Mol Pharmacol* **23**: 92–99, 1983.
13. Katzhendler J, Gean KF, Bar-Ad G, Tashma Z, Ben-Shoshan R, Ringel I, Bachrach U and Ramu A, Synthesis of amino-anthraquinone derivatives and their *in vitro* evaluation as potential anticancer drugs. *Eur J Med Chem* **24**: 23–30, 1989.
14. Zee-Cheng RKY, Podnebarac EG, Menon CS and Chen CC, Structural modification study of bis-(substituted aminoalkylamino)anthraquinones. An evaluation of the relationship of the ±2-±2-hydroxyethyl)-aminoijethylijaminos side chain with antineoplastic activity. *J Med Chem* **22**: 501–505, 1979.
15. Cheng CC and Zee-Cheng RK, The design, synthesis and development of a new class of potent antineoplastic anthraquinones. *Prog Med Chem* **20**: 83–118, 1983.
16. Zee-Cheng RK, Mathew AE, Zu PL, Northcutt RV and Cheng CC, Structural modification study of mitoxantrone (DHAQ) chloro-substituted mono and bis [(aminoalkyl)amino]anthraquinones. *J Med Chem* **30**: 1682–1689, 1987.
17. Murdock KC, Child RG, Lin YI, Warren JD, Fabio PF, Lee VJ, Izzo PT, Lang SA, Angier RB, Citarella RV, Wallace RE and Durr FE, Antitumor agents 2. bisguanylhydrazones of anthracene-9,10-dicarboxaldehydes. *J Med Chem* **25**: 505–518, 1982.
18. Katzhendler J, Bar-Ad G, Haran M, Gean KF, Tashma Z, Ringel I, Ramu A and Bachrach U, The effect of substituted aminoalkylaminoanthraquinones on eukaryotic cells. *Durg Design Deliv* **4**: 289–294, 1989.
19. Schnur L, Bar-Ad G, Haran M, Tashma Z, Talmi M, Katzhendler J and Bachrach U, The effect of diaminoalkyl-anthraquinone derivatives on the growth of the promastigotes of *Leishmania tropica minor, L.t. major. L. donovani and L. aethiopica. Biochem Pharmacol* **32**: 1729–1732, 1983.
20. Fabio PF, Fields TL, Lin Y-I, Burden EJ, Carvajal S, Murdock KC and Lang Jr SA, Bisamidines of 2,6-diaminoanthraquinone as antiamebic agents. *J Med Chem* **21**: 273–276, 1978.
21. Shepard GS, Katzhendler J, Gean KF, Kreisel M and Bachrach U, Inhibition of bovine serum amine oxidase activity by aminoalkyl-aminoanthraquinones. *Biochem Pharmacol* **37**: 4780–4783, 1989.
22. Zunino F, DiMarco A, Zaccara A and Gambetta RA, The interaction of daunorubicin and doxorubicin with DNA and chromatin. *Biochim Biophys Acta* **607**: 206–214, 1980.
23. Plumbridge TW and Brown JR, The interaction of adriamycin and adriamycin analogues with nucleic acids in the B and A conformations. *Biochim Biophys Acta* **563**: 181–192, 1979.
24. Crooke ST, Duvernay VH and Mong S, *Molecular Actions and Targets for Cancer and Chemotherapeutic Agents*. Academic Press, New York, 1981.
25. Bachur NR, Gee MV and Friedman RD, Unclear catalyzed antibiotic free radical formation. *Cancer Res* **42**: 1078–1081, 1982.
26. Karaschi ED and Novak RF, Ring current effects in adriamycin-flavin mononucleotide complexation as observed by ^{1}HFT, NMR spectroscopy. *Biochem Biophys Res Commun* **92**: 1320–1326, 1980.
27. Kharash ED and Novak RF, The molecular basis for complexation of adriamycin with flavin mononucleotide and flavin adenine dinucleotide. *Arch Biochem Biophys* **212**: 20–36, 1981.
28. Land EJ, Mukherjee T, Swallow AJ and Bruce JM, One electron reduction of adriamycin: properties of the semiquinone. *Arch Biochem Biophys* **225**: 116–121, 1983.
29. Bachrach U, Metabolism and function of spermine and related polyamines. *Annu Rev Microbiol* **24**: 109–134, 1970.
30. Bachrach U, Oxidation of polyamines and diamines. In: *Polyamines in Biology and Medicine* (Eds. Morris DR and Marton LJ), pp. 152–168. Marcel Dekker, New York, 1981.
31. Morgan DM, Bachrach U, Assaraf YG, Harari E and Golenser J, The effect of purified aminoaldehydes produced by polyamine oxidation on the development *in vitro* of *Plasmodium falciparum* in normal and glucose-6-phosphate dehydrogenase deficient erythrocytes. *Biochem J* **236**: 97–101, 1986.
32. Trager W and Jensen JB, Human malaria parasites in continuous culture. *Science* **193**: 673–675, 1976.
33. Lambros C and Vanderberg JP, Synchronization of *Plasmodium falciparum* erythrocytes stage in culture. *J Parasitol* **65**: 418–420, 1979.
34. Jensen JB, Concentration from continuous culture of erythrocytes infected with trophozoites and schizonts of *Plasmodium falciparum*. *Am J Trop Med* **27**: 1274–1276, 1978.
35. Bacchi CJ, Nathan HC, Hutner SH, Duch DC and Nichol CA, Prevention by polyamines of the curative effects of amicarbolide and imidocarb for Trypanosoma brucei infection in mice. *Mol Pharmacol* **30**: 883–886, 1981.