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Fe (II)-CHELATES BASED ON REDOX-ACTIVE PYRIDOXAL-BETAINES AS C-CENTERED RADICALS CAUSING SINGLE- AND DOUBLE-STRAND SCISSIONS TO DNA

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The ability of 1-[N-Ethoxycarbonylmethylpridoxylidenium]-2-[2′-pyridyl]hydrazine bromide code name - [L2-9 = L^+,X^-]-FE(II) chelate {L2-9-Fe(II)} to induce breaks both in the 43kb linear double-strand λ phage DNA, and in the 4363 base pair supercoiled pBR322 plasmid DNA is herein described. Neither the free ligand nor FE(II) alone demonstrated any effect on the DNA. The cleaving ability is shown to occur instantaneously under strictly anaerobic conditions, either in the presence or absence of the enzyme catalase. It is also shown to be dose dependent. Thus, at λ DNA:L2-9-Fe(II) molar ratio of 3.7:1.0, the linear DNA is randomly cleaved into fragments ranging from 23.1kb to 4.3kb, whereas at approximately 1:1 molar ratio, the range extends down to 2.5kb fragments. By contrast, at 1:2.7 [plasmid DNA]: chelate-Fe(II) molar ratio, a single-strand nick was observed, and a double strand break was noted at a 1:50 ratio ([plasmid DNA] : chelate-Fe(II). A multi-stage redox cycling involving a carbon-centered ($L^{\cdot},X^-$)-Fe(III) radical capable of transferring an electron to the DNA to form high unstable $[DNA]^{\cdot-}$ anion-radical is invoked to explain the degradation of the chain macromolecule. Possible modes for regeneration of the chelate-Fe(III) radical both at the cell-free and at the cell levels are proposed.

KEY WORDS: Iron chelates, free radicals, DNA breakage.

INTRODUCTION

The increase in drug resistant strains of the malarial parasite — *P. falciparum* underscores an urgent need for the development of new chemotherapeutic strategies against malaria. Though immunopreventive and immunocurative strategies are still being experimented upon, the prospect for the development of an effective vaccine looks bleak.[1] Chemotherapy therefore remains the major weapon for reducing malaria morbidity and mortality. Elucidation of the mechanism of resistance and the mode of action of chemotherapeutic agents are important in the development of new chemotherapeutic strategies.

Iron chelation may form the basis of a new class of antimalarials. Laboratory and field observations lend support to this possibility.[2–5] The binding of iron to redox

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active sequestering agents may give rise to opposite biological effects, namely, either to the enhancement of an oxidative (free radical) damage,[6] or inversely, to an inhibition of such effect. Mechanistically, chelators can operate by several distinctly different modes. One mode may involve mediation in transferring of the transition metal-ion to a cell receptor, invoking a damaging process to the latter. Another mode may involve modification of the electrochemical potential of the metal-ion and as a consequence to affect essential redox processes in the cell. Alternatively, the chelator may act as a catalyst for converting essential cell metabolites into harmful free radicals by promoting single electron transfer (SET) processes.

Free radical attack on DNA has been shown to have a serious effect on living organisms by causing strand breaks in the DNA.[7,8] The tendency of the DNA macromolecule to function as an electron-transfer oxidant is well documented.[9,10] Cochrane *et al.*[11] have shown in studies with isolated DNA that hydrogen peroxide (H_2O_2) generates hydroxyl radicals (·OH), following Fenton reaction at the vicinity of the DNA and that the amount of the ·OH formed correlates with the percentage of DNA strand breaks.

By use of a representative of a new class of iron chelators based on pyridoxal-betaine – (1-[N-ethoxycarbonylmethyl-pyridoxylidenium]-2-[2′-pyridyl]hydrazine bromide) – (code named L2-9), we have developed a model for a new chemotherapeutic strategy against the chloroquine resistant malaria parasite – *Plasmodium falciparum* (FCR_{3TC}). Previously, we have shown that the antimalarial action of the new chelator involves a carbon-centered free radical chelate (L2-9FE(II)→[L2-9]·-Fe(III)).[12] In the present study, we show that the free radicals generated by the chelator and Fe^{2+} affects the DNA. Nick translation and electrophoresis of L2-9-FE(II) complex treated DNA showed that the latter suffers both single and double strand fissions only by the chelator-iron complex and not by either the chelator, or the iron (Fe^{2+}) alone.

MATERIALS AND METHODS

Chemicals

The iron chelator – L2-9[13] was synthesized by exposing pyridoxal pyridyl hydrazone[14] to the action of ethyl bromoacetate. Ferric and ferrous sulphates were obtained from Fisher Scientific Co. (Silver Spring, MD, USA); RPMI 1640 from GIBCO (Grand Island, NY, USA); HEPES from Sigma Chemical Co (St Louis, MO, USA); Nick translation System Kit from NEN research Products (Boston. MA, USA); Calf thymus (CT), Lambda (λ) DNA, Plasmid (pBR322), catalase and mineral oil from Sigma Chemical Co. All other chemicals were of analytical grades.

Chelator-iron (II) complex [L2-9-FE(II)] was prepared immediately before use by mixing 1mM L2-9 with 1mM $FeSO_4$ (v/v) to give a 500μM complex. This was diluted to the required working concentrations (0.5–40μM) with RPMI 1640 medium without plasma (washing solution).

Nick translation. The method used as per the instructions in the manufacturer's manual for Nick translation system [^{32}P]. Briefly, the following components were added as follows: [α-^{32}P]dCTP – 5μl; Nick translation buffer – 5μl; cold deoxynucleoside triphosphate mixture (-dCTP) 4μl; either L2-9-Fe(II) treated or untreated CT DNA – 2μl (the final concentration in the reaction mixture was 0.5μg);

translation grade water – 7μl; DNA polymerase 1 (Pol 1, 0.6 units/μl – 2μl. These gave a total reaction mixture of 25μl. This was incubated at 10°C for 2h. The reaction was terminated by the addition of 200μl Nick translation stop buffer. The mixture was then passed through a sephadex G-50 column using a 1ml syringe. The effluent was collected and 10μl sample placed on a Whatman GFC filter paper and radioactivity counted.

Gel electrophoresis (Neutral gel). 2μl of λ DNA (0.5μg/μl) was incubated with 8μl of various concentrations (0.5μM–40μM) of freshly prepared L2-9-Fe(II) complex for 2h. After the 2 h incubation period, 0.5μg of the treated DNA was run in an agarose gel (0.8%) using 50mM Tris-Acetate-EDTA (TAE) pH 7.5 as the running buffer. The plasmid (pBR322) was treated likewise with the L2-9-FE(II) complex and run in 1% agarose gel in TAE buffer pH 7.5. The gel was run at 5V cm^{-1} for 3h.

To measure the time course of DNA cleavage by the chelate, 1μl of plasmid pBR322 (0.5μg/λ) was incubated with 5μl of 40μM freshly prepared L2-9-Fe(II) complex for various lengths of time (0–60 minutes). The reaction was stopped by the addition of 2μl loading buffer containing 1mM EDTA. To mimic the effect of an anoxic condition, a thin layer of mineral oil (heavy white oil) was placed over one of the reaction mixtures and incubated for 60 minutes. To test if hydroxyl radicals (·OH) was involved in L2-9 induced radical damage, catalase (60μg) was added to one of the reaction mixtures and incubated for 60 minutes. The mixtures were run in 0.8% agarose gel in TAE buffer pH 7.5 at 1.5V cm^{-1} for 14 hours (overnight) at room temperature.

RESULTS

Using the nick translation method of Kelly *et al.* (1970),[17] the ability of L2-9-Fe(II) complex to induce nicks in DNA was tested. The result (Table) showed that L2-9-Fe(II) complex induced nicks in DNA. CT DNA treated with L2-9-FE(II) complex incorporated [^{32}P]dCTP 3 times as much as the untreated one. CT DNA treated with DNase 1 incorporated [^{32}P]dCTP only twice as much as the L2-9-Fe(II) teated one. L2-9-Fe(II) could therefore be said to have introduced nicks or breaks in the DNA half as effectively as the DNase 1.

TABLE I
Incorporation of [^{32}P]dCTP into L2-9-Fe(II) treated CT DNA

CT DNA treated with:	Counts
RPMI medium (without chelate)	136492 ± 15121
L2-9-FE(II) in RPMI medium	322504 ± 31754
DNase I	619230 ± 60332

Counts are expressed as mean ± SD† of triplicate determinations. CT DNA was suspended in either 40μM L2-9-Fe(II) in RPMI medium or RPMI medium alone for 3h and thereafter used for the nick translation reaction.

†Standard Deviation.

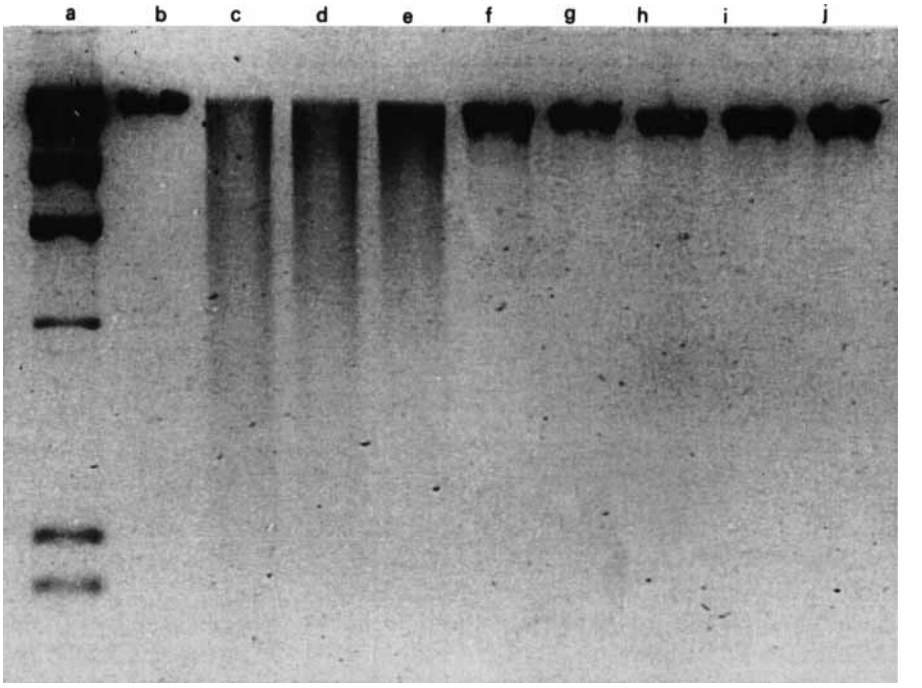


FIGURE 1 Gel electrophoretic pattern of λ phage DNA treated with L2-9-Fe(II) complex. λ phage DNA was suspended for 2h in RPMI medium (without plasma) containing various concentrations of L2-9-Fe(II). The treated DNA was then separated on a 0.8% agarose gel, stained with ethidium bromide and visualized under UV illumination. Lane a, DNA digested with HindIII, lane b, DNA in medium without L2-9-Fe(II), lanes c-h, DNA in medium containing 40, 20, 10, 5, 1, and 0.5μM L2-9-Fe(II) complex respectively. Lanes i, and J, DNA in medium containing 40μM L2-9, and Fe(II) alone, respectively. The final amount of the DNA applied to each well was 0.5μg.

The ability of the chelator-iron complex to induce nicks in DNA was also tested by gel electrophoretic analysis of both λ phage and pBR322 plasmid DNA. Figure 1 shows the gel electrophoretic pattern of λ phage DNA treated with the chelator-Fe(II) complex. The cutting ability of the complex is dose dependent. 40μM of the complex formed fragments ranging from 2.5kb to 23.1kb. The 20 and 10μM complexes formed less number of fragments, while the 5 and 1μM complexes formed only fragments of 9.4 and 23.1kb (using λ DNA cut with restriction endonuclease HindIII as the marker). Introducing nicks in a supercoiled plasmid would result in either its relaxation (for single strand nicks) or its linearization (for double strand nicks). When pBR322 plasmid was incubated with the complex for 2h, both single and double strand breakers were induced by 40, 20, and 10μM complexes; while the 5, 1, and 0.5μM complexes induced only single strand breaks (Figure 2). Neither L2-9 nor Fe(II) alone had any effect on the DNA.

The time course of DNA cleavage by the chelate was followed by incubating 0.5μg pBR322 with 40μM complex for times 0–60 minutes. The result (Figure 3) showed that the DNA cleavage was rapid, occurring in less than 5 minutes. Oxygen was not necessary for the cleavage to occur, and catalase had no effect on it, indicating the non-participitation of hydroxyl radicals in the L2-9 induced radical damage.

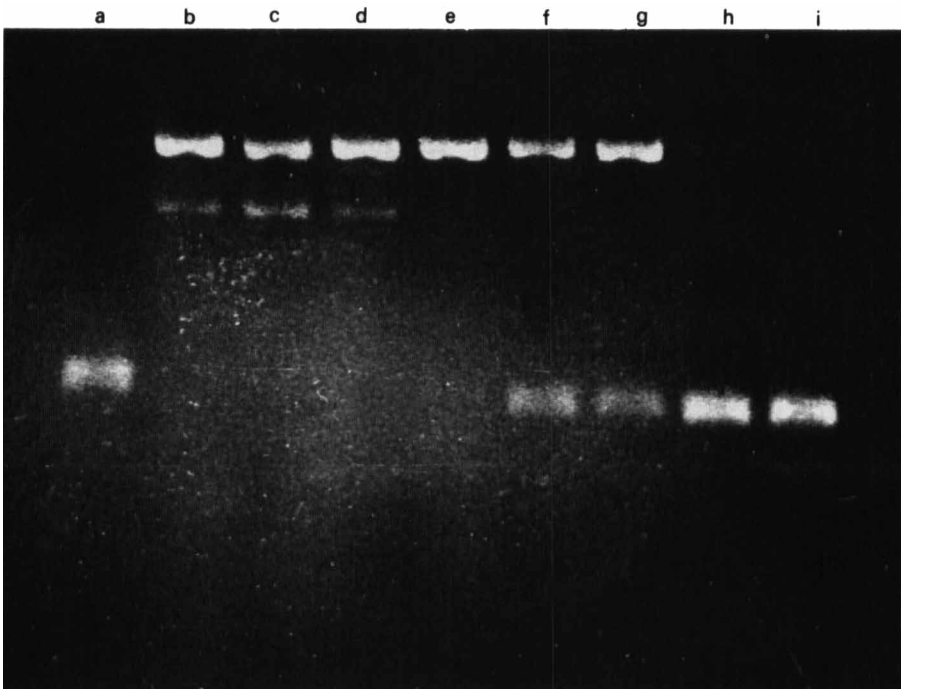


FIGURE 2 Gel electrophoretic pattern of plasmid pBR322 treated with L2-9-Fe(II) complex. Plasmid pBR322 was suspended for 2h in RPMI medium (without plasma) containing various concentrations of L2-9-Fe(II). It was separated on a 0.8% agarose gel, stained with ethidium bromide and visualized under UV illumination. Lane a, plasmid in medium without iron chelate, lanes b–g, plasmid in medium containing 40, 20, 10, 5, 1, and 0.5μM L2-9-Fe(II) complex respectively. Lanes h, and i, plasmid in medium containing 40μM of L2-9 and Fe(II) alone respectively. The amount of DNA in each well was 0.5μg.

DISCUSSION

From Figures 1 and 2, it is evident that L2-9-Fe(II) [= (L^+,X^-)-Fe(II)] is able to induce breaks both in the high molecular λ phage DNA (26.57×10^6 mol. wt., from *E.coli* strain C600), a 43000 base pair linear double strand DNA, and in a lower molecular pBR322 plasmid DNA (2.7×10^6 mol. wt., from *E.coli* strain RPI), a 4363 base pair supercoiled DNA. Neither the free ligand (L2-9) nor Fe(II) alone exhibited any effect on the DNA. The cleaving ability of (L^+,X^-)-Fe(II) is shown to be both instantaneous and dose-dependent. At molar ratio 3.7 : 1.0 of λ DNA : (L^+,X^-)-Fe(II), the macromolecule is randomly cut into fragments ranging from 23100 to 4360 base pairs, and at aproximately 1:1 – molar ratio, the range extends down to 2500 base pair fragments.

In contrast to the observed chain breaking yield (DNA/Fe-chelate $\times$ 100) of 370% in λ DNA, the yield of macromolecular scission in the supercoiled DNA plasmid is considerably lower (37–4%). Thus, at [pBR322 plasmid DNA] : [(L^+,X^-)-Fe(II)] molar ratio of 1 : 2.7, the supercoiled DNA undergoes a single strand nick leading to its relaxation, whereas at 1 : 50 molar ratio, a double-strand scission takes place giving rise to its linearization. From Figure 3 it can be seen that these DNA chain cuts occur

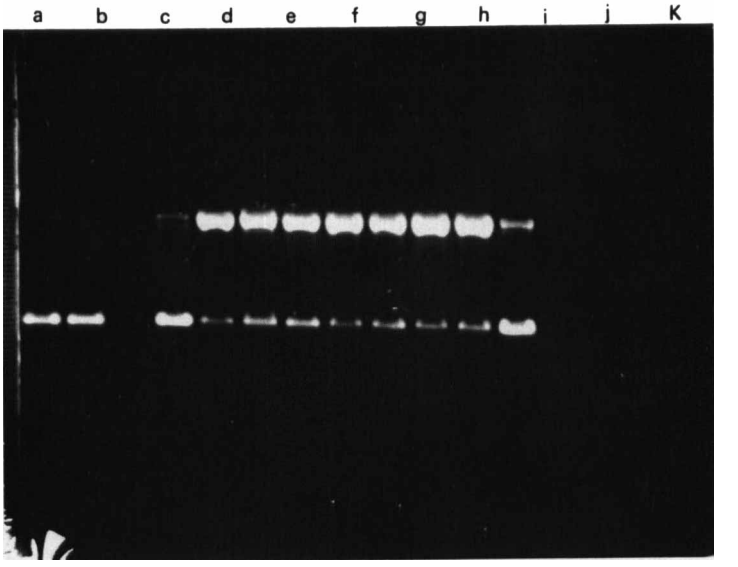
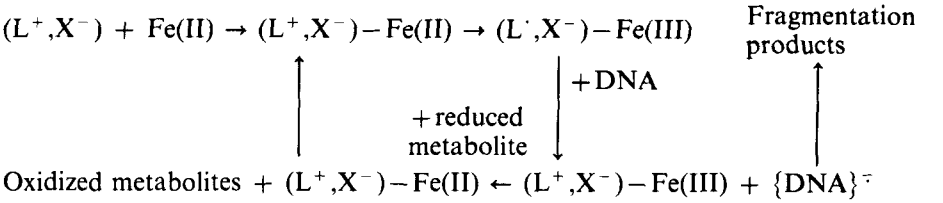


FIGURE 3 Gel electrophoretic pattern of plasmid pBR322 treated with 40μM L2-9-Fe(II) complex for times ranging from 0-60 minutes. 0.5μg of plasmid pBR322 was incubated with 5μl of 40μM freshly prepared L2-9-Fe(II) complex for various lengths of time (0–60 minutes). The reaction was stopped by the addition of 2μl loading buffer containing 1mM EDTA. It was separated on a 0.8% agarose gel, stained with ethidium bromide and visualized under UV illumination. Lane a, untreated plasmid; lane b, untreated plasmid under anerobic condition; lane c. plasmid treated with 100μM L2-9 alone, lane d, treated wtih 40μM chelate for 60 minutes; lane e, treated with 40μM chelate + 60μg catalase for 60 minutes; lane f, treated with 40μM chelate under anaerobic condition for 60 minutes; lanes g-k, treated with 40μM chelated and incubated for 30, 20, 10, 5, and 0 minutes respectively. The amount of DNA in each well was 0.5μg. The gel was run at 1.5V cm^{-1} for 14 hours (overnight) at room temperature.

instantaneously under anaerobic conditions, and that they are unaffected by the presence of the enzyme catalase, implying the uninvolvement of reactive oxygen species (ROS) in the chain breakings.

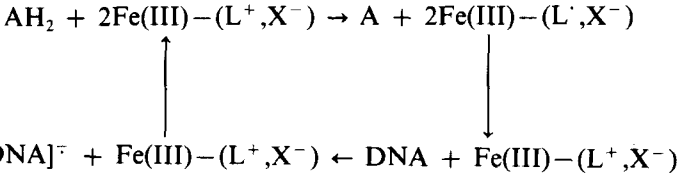
ESR study[12] has shown that (L^+,X^-)-Fe(II) exist as a transient carbon-centered free radical presumably of $L^{\cdot},X^-)$-Fe(III) structure, and its X-ray crystal analysis[14,19] indicated that the ligand (L^+,X^-) utilizes all available hydrogen acceptors and donors for intra- and inter-molecular hydrogen contacts. On the basis of data now available, it is permissible to invoke a multi-stage process for the macromolecular chain degradation initiated by (i) binding (L^+,X^-)-Fe(II) to DNA chain, followed by (ii) single-electron-transfers (SET): (L^+,X^-)-Fe(II) $\rightarrow$ $(L^{\cdot},X^-)$-Fe(III) + DNA $\rightarrow$ (L^+,X^-)-Fe(III) + [DNA]$^{\dot{-}}$, and (iii) degradation of the anion — radical [DNA]$^{\dot{-}}$ into [base pair fragments]$^{\dot{-}}$, then finally by (iv) redox-cycling: [base pair fragment]$^{\dot{-}}$ + (L^+, X^-)-Fe(III) $\rightarrow$ DNA-fragments + $(L^{\cdot},X^-)$-Fe(III), to start all over again. The discrepancy in the chain breaking yields underlined above could be rationalized in terms of redox gradients, being higher for the linear [DNA]$^{\dot{-}}$ than for the circular/relaxed [DNA]$^{\dot{-}}$ species.



The mechanistic scheme portrayed above could reasonably be invoked to explain the *in vitro* cytocidal effect of L2-9 on the drug resistant malarial parasite – *Plasmodium falciparum*. It implies that the ligand sequesters first the endogenous cellular Fe^{2+} to form the respective Fe(II)-complex, since pre-treatment of the parasite culture with a stronger iron chelator – desferrioxamine – obliterates the antimalarial properties of L2-9.[2]

The (L^+,X^-)-Fe(II) complex is prone to an internal electron transfer to form the respective radical species capable of delivering an electron to the DNA after its binding to the macromolecule to yield an unstable $[DNA]^{\cdot-}$ which decomposes as soon as formed (see chart). It is of interest to compare the mode of action of $(L^{\cdot},X^-)$-Fe(III) with that of the iron chelate radical-cation Fe(II)-$Dox^{+\cdot}$,[21] originating from sequestration of the anti-tumour antibiotic doxorubicin (DOX) (adriamycin) with Fe^{3+}, following the sequence: Fe(III)-Dox → Fe(II)-$Dox^{+\cdot}$. Whereas the former does not require molecular oxygen to damage DNA, the latter by contrast, does require O_2 to produce ROS via the reaction: Fe(II)-$Dox^{+\cdot}$ + O_2 → Fe(III)-Dox + $O_2^{\cdot}$ (ROS). The ligand in (L^+,X^-)-Fe(II) functions apparently as an internal electron transfer oxidant, whereas DOX in Fe(III)-Dox functions as an internal electron transfer reductant.[21] The antimalarial action of dialuric/alloxan,[22] divicine,[23] and butyl hydroperoxide,[24] seems also to involve ROS[25,26] via redox cycling.[27] In the latter, electrons appear to emerge from endogenous cellular reductants, AH_2, which in combination with a redox metal (Cu/Fe) complex and oxygen can generate ROS *via* $3AH_2 + 2O_2 \rightarrow 3A + 2H_2O + 2{\cdot}OH$ (net reaction).

Antimalarial chelators based on redox-active pyridoxal-betaine seems to operate quite differently, following a hitherto unknown redox cycle:



Conceptually, the ability of L2-9 to interact with and cut DNAs (λ phage and plasmid) was reasonably extended to explain its antimalarial action *in vitro*. This offers an interesting venue to antimalarial drug design implicating intracellular generation of harmful free radicals by iron-dependent inducers. The rapid spread of multi-drug resistant strains of the malarial parasites prompts the use of new models for drug design to provide more effective antimalarials operating by hitherto unknown mechanisms. It is highly likely that L2-9, or its analogues could demonstrate other antiparasitic and/or antitumor activity of potential therapeutic utility.

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References

1. J. Cherfas (1990). Malaria vaccines: The failed promise, *Science*, **247**, 402–403.
2. E.N. Iheanacho, A. Samuni, S. Avramovici-Grisaru, S. Sarel, and D.T. Spira (1990). Inhibition of Plasmodium falciparum growth by a synthetic iron chelator. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **84**, 213–216.
3. D.G. Heppner, P.E. Hallaway, G.J. Kontoghiorghes, and J.W. Eaton (1988). Anti-Malarial properties of orally active iron chelators. *Blood*, **72**, 358–361.
4. P.W.J. Harvey, P.F. Heywood, M.C. Neshheim, K. Galme, M. Zegans, J-P. Habicht, L.S. Stephenson, K.L. Radimer, B. Brabin, K. Forsyth, and M.P. Alpers (1989). The effect of iron therapy on malaria infection in Papua New Guinean school children. *American Journal of Tropical Medicine and Hygiene*, **40**, 12–18.
5. S.J. Oppenheimer, F.D. Gibson, S.B. MacFarlane, J.B. Moody, O. Harrison, A. Spencer, O. and Bunari (1986). Iron supplementation increases prevalance and effects of malaria: Clinical studies in Papual New Guinea. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **80**, 603–612.
6. P. Saltman (1989). Oxidative stress: A radical view. *Seminars in Hematology*, **26**, 249–256.
7. D. Chapman, and C.J. Gillespie (1981). Radiation – induced events and their time scale in mammalian cells. *Advances in Radiation Biology*, **9**, 143–198.
8. J.L. Marx (1987). Oxygen free radicals linked to many diseases. *Science*, **235**, 529–531.
9. R.A. Floyd (1981). DNA-ferrous catalyzed hydroxyl free radical formation from hydrogen peroxide. *Biochemical and Biophysical Research Communications*, **99**, 1209–1215.
10. H.C. Birnboim, and M. Kanabus-Kaminska (1985). The production of DNA strand breaks in human leuckoyctes by superoxide anion may involve a metabolic process. *Proceedings of the National Academy of Sciences*, **82**, 6820–6824.
11. C.G. Cochrane, I.U. Schraufstatter, P. Hyslop, and J. Jackson (1988). Cellular and biochemical events in oxidative injury. In: Halliwell, B; ed. Oxygen radicals and tissue injury Symposium (Proceedings). Bethesda: Federation of American Socities for Experiemental Biology. pp. 49–54.
12. E.N. Iheanacho, A. Samuni, S. Avramovici-Grisaru, S. Sarel, and D.T. Spira (1990). Growth inhibition of Plasmodium falciparum involving carbon centered radical ($L \cdot X^-$)-Fe(III) based on pyridoxal-betaine. A novel type of antimalarials active against chloroquine – resistant malaria parasites. *Free Radical Research Communications*. (in press).
13. S. Sarel, S. Avramovici-Grisaru, C. Hershko, G. Link, and D. Spira (1988). Pyridoxal Hydrazone Derivatives: Their production and use and pharmaceutical compositions containing the same. *European Patent Application*. 0 315 343 A2, 2, 11.
14. A. Avramovici-Grisaru, S. Cohen, and S. Sarel (1990). Synthesis and crystal structure of H-bonded long-chain of 1:1-Methanol: 1-[methylpyridoxylidenium]-2-[2′-pyridyl] hydrazine iodide. *Heterocycles*:, **30**, 1079–1090.
15. J. Golenser, D. Casuto, and Y. Pollack (1981). Plasmodium falciparum: *In vitro* induction of resistance to aminopterin. *Experimental Parasitology*, **52**, 371–377.
16. J.B. Jensen (1978). Concentration from continuous culture of erythrocytes infected with trophozoites and schizonts of Plasmodium falciparum. *American Journal of Tropical Medicine and Hygiene*, **27**, 1274–1276.
17. R.B. Kelly, N.R. Cozzarelli, M.P. Deutscher, I.R. Lehman, and A. Kornberg (1970). Enzymatic synthesis of deoxyribonucleic acid. *Journal of Biological Chemistry*, **245**, 39–45.
18. S. Sarel, and S. Avramovici-Grisaru, In: "Trends in Medicinal Chemistry 88", (1989). H. van der Goot, G. Domany, L. Pallos, and H. Timmerman, eds. Elsevier Science Publishers, Amsterdam. pp. 743–755.
19. S. Avramovici-Grisaru, S. Sarel, and S. Cohen (1990). Formation and crystal structure of 1-[N-Methylpyridoxylidenium]-2-[2′-N-methylpyridinium]hydrazine diperchlorate and its Red Tautomer. *Journal of Organic Chemistry*. (in press).
20. N. Farrell (1989). "Transition metal complexes as drugs and chemotherapeutic agents", Kluwer Academic Publication, London, chapter 7.
21. B.B. Hasinoff (1989). Self-reduction of the Iron(III)-Doxorcubicin complex. *Free Radicals Biology and Medicine*, **7**, 583–593.

22. I.A. Clark, and N.H. Hunt (1983). Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria. *Infection and Immunity*, **39**, 1–6.
23. I.A. Clark, W.B. Cowden, .H. Hunt, L.C. Maxwell, and E.J. Mackei (1984) Activity of divicine in Plasmodium vinckei infected mice has implications for treatment of favism and epidemiology of G6PD deficiency. *British Journal of Hematology*, **57**, 479–487.
24. I.A. Clarke, E.J. Mackei, and W.B. Cowden (1986). Injection of free radical generators cause premature onset of tissue damage in malaria infected mice. *Journal of Pathology*, **148**, 301–305.
25. I.B. Afanasev (1989). "Superoxide Ion: Chemistry and Biological implications", Volume I, CRC Press Inc., Boca Raton, Florida, USA.
26. B. Halliwell (1990). How to characterize a biological antioxidant. *Free Radical Research Communications*, **9**, 1–32.
27. J.L. Vennerstrom, and J.W. Eaton (1988). Oxidants, Oxidant Drugs, and Malaria. *Journal of Medicinal Chemistry*, **31**, 1269–1277.

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