Growth Inhibition of Drug-Resistant Species of *Plasmodium Falciparum* by Domain Structured N^1,N^2-Derivatized Hydrazines: Denticity Effects, Redox Switches, and Reductant-Driven Redox-Cycling

S. Sarel[1,*], E.N. Iheanacho[2] and S. Avramovici-Grisaru[1]

[1]*Department of Medicinal Chemistry, The Hebrew University of Jerusalem,* [2]*The Kuvin Center for the Study of Infectious and Tropical Diseaeses, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel*

Abstract: Six analogs of bidentate 1-[pyridoxylidene]-2-phenyl]hydrazine, twelve analogs of N_2O-tridentate 1-[pyridoxylidene]-2-[heteroaryl]hydrazine, and four O_2N-tridentate analogs of 1-[pyridoxylidene]-2-[heteroaroyl] hydrazines were synthesized and characterized. Their solutions in water and DMSO were assayed *in vitro* for activity against a chloroquine-resistant species of *P. falciparum* obtained from Hadassah Hospital Blood Bank in Jerusalem. The O_2N-tridentate group was essentially inactive, whereas the bidentate group, with N and O liganding atoms, exhibited slight activity against late-stage trophozoites and schizonts of *P. falciparum.* The N_2O-tridentate group, by contrast, was remarkably active against resistant *P. falciparum*, highlighting the importance of the Denticity Effect in this system. It is assumed that the pyridoxal-based chelator acts as an iron redox mediator, controlling the first coordination sphere and, therefore, the immediate chemical environment of the iron.Chelation of iron-(II) presumably facilitates its oxidation..The Fe(II) → Fe(III) intra-electron transfer, may be viewed as a switch ("redox switch"), controlling the thermodynamic stability and kinetic lability of the coordination shell. The redox-switch is accompanied by the appearance of a carbon-based Fe-(III)-chelate radical, capable of donating its free electron to the parasite-DNA, thus causing death. The antimalarial N_2O-tridentate Fe(III)-chelates appear to be prone to redox-switch, and tend to be converted into their Fe(II) species, whereas the inactive O_2N-tridentate analogs apparently cannot do so.

Key Words: Growth-Inhibition, Drug-Resistant *Plasmodium falciparum*, Pyridoxal-based Chelators, Parasitemia by ^{3}H-Hypoxantin Incorporation, Electronic Effects, Denticity Effects, Redox-Cycling.

INTRODUCTION

The increase in drug resistant strains of the malarial parasite *Plasmodium falciparum* emphasizes the urgent need for new chemotherapeutic strategies against malaria.

Iron chelation may form the basis of a new class of antimalarials. Laboratory and field observations lend support to this possibility [1- 4]. The binding of iron to redox-active chelators may give rise to opposite biological outcomes, namely, either to an increase in free radical damage [5], or inhibition of such an effect. Mechanistically, chelators may operate by several distinctly different modes. One mode may involve mediation in the transfer of the transition metal-ion to a cell receptor, causing a damaging effect on the latter. Another mode could involve modification of the electro-chemical potential of the metal-ion, consequently affecting an essential redox process in the cell. The chelator might act as a catalyst for converting essential cell metabolites into harmful free radicals by promoting single electron transfer (SET).

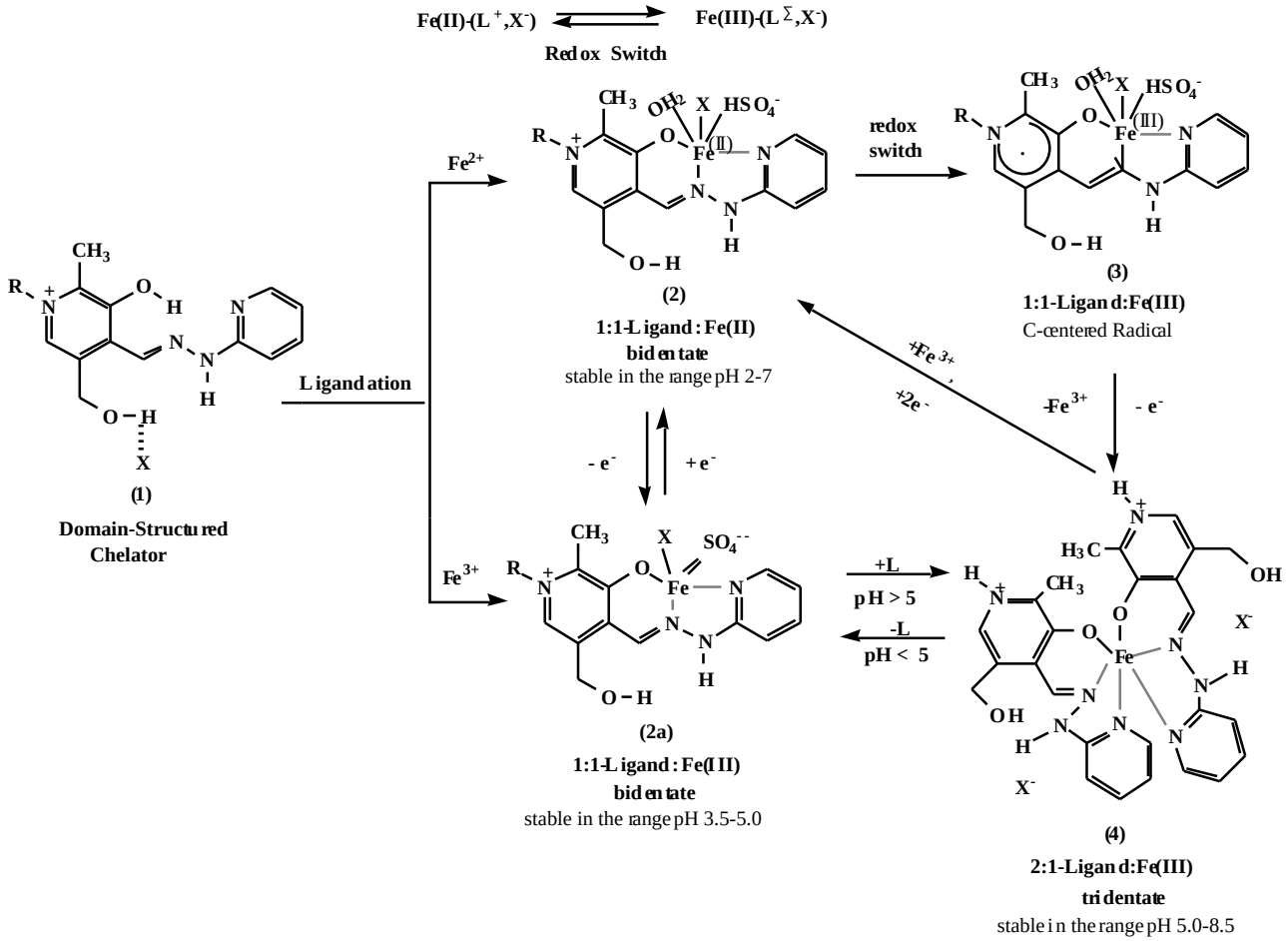
Free radical attack on DNA has a serious effect on living organisms by causing strand fissions in the DNA [6, 7]. The tendency of the DNA to function as an electron-transfer oxidant is well documented [8, 9]. Using isolated DNA it was shown [10] that HO_2 generates a hydroxyl radical through the Fenton reaction in the vicinity of the DNA and that the amount of OH-radical produced correlates with the percentage of DNA strand fissions.

Previously [1] we reported that the pyridoxal-based chelator 1-[N-ethoxycarbonylmethylpryidoxylidenium]-[2'-pyridyl]hydrazine bromide (L^+,X^-) [11] **1 (**see Scheme **1)** was non-toxic to late-stage trophozoites and schizonts of a *Plasmodium falciparum* species (FCR-3) [12] in the absence of iron in the media. But in presence of iron, chelator **1** (Scheme **1**) is converted into a highly toxic species capable of killing the malaria parasite at a concentration of 20 μM within 2 hrs. The inhibitory effect of 1 was reversed [13] by adding an equimolar concentration of desferrioxamine B methane sulphonate (DFO), suggesting that only the combination of 1 with iron leads to the antimalarial outcome. Furthermore, it was shown that, depending on the oxidation-state of the iron, the chelation of 1 with iron leads to two distinctly different iron-chelates: (i) with Fe^{2+} ions and 1:1-Fe(II):(L+,X-) 2, and (ii) with Fe^{3+} ions.and 1:2-Fe(III): (L+,X-) 4 (see Scheme **1**).

A spectroscopic study (UV, ESR) [13] indicated that unlike the 6 coordinate, low-spin Fe(III) atom in **4**, the 5-coordinate, high-spin Fe(II) atom in **2** tends to switch with the pyridyl-ring π-electron cloud to form a carbon-centered

*Address correspondence to this author at the Department of Medicinal Chemistry, The Hebrew University of Jerusalem, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel; Fax: +972-2-5617734; E-mail: sarel@cc.huji.ac.il

1573-4064/05 $50.00+.00



Scheme 1.

free radical **3** (see Scheme **1**), capable of functioning as an electron-donor in the presence of a suitable electron-acceptor, such as DNA to generate **4** (see Scheme **2**)**:**

$$\mathbf{3 + DNA \rightarrow 4 + \{DNA\}\bullet - -}$$

{DNA}• - - → **DNA**-linearization → **DNA**-fragmentation

Later [14], it was found that the **3** → **4** electron-transfer **(ET)** could be reversed in presence of reducing agents (such as ascorbate, glutathione).

We propose the term "chelatodynamic modality" for a treatment modality that uses a combination of a redox-active chelator with a low-spin Fe(II).

The nick translation method of Kelly *et al*. [15]. was shown [14] to result in nicks or breaks both in λ-phage and in supercoiled pBR322 plasmid DNA. Whereas in the former it gave rise to fragments, ranging from 2.5kb to 23.1kb, in the plasmid DNA it induced single-, and double-strand nicks, giving rise, respectively, to relaxation and linearization of the supercoiled DNA. *Oxygen* was found to be unnecessary for the cleavage to occur, and *catalase* had no effect, indicating the non-involvement of the Fenton reaction (intermediacy of reactive-oxygen-species), and the involvement of Fe^{3+} ions [14]. This modality may, however, be rationalized in terms of the steady-state theory, in which the thermodynamically stable carbon-based Fe(III)-chelate radical (the genuine active species) undergoes redox-switch [16] in the presence of actively metabolizing cells to generate the thermodynamically labile Fe(II)-chelate. The regeneration of free radicals may involve redox stages: **4** → **4a** → **3** → **2** in which ubiquitous cell reductants with a matching electrochemical gradient are involved.

$$\mathbf{4} + \mathbf{e}\ (\text{cell metabolites}) \rightarrow \mathbf{3} + \mathbf{Fe^{3+}}$$

$$\mathbf{3} + \mathbf{e}\ (\text{cell metabolites}) \rightarrow \mathbf{2}$$

Conceivably the novel antimalarial mode of action might be caused by a combination of a non-toxic redox-active ligand and a low oxidation-state of the transition-metal (such as Fe(II)). The emerging toxic Fe(II)-chelate radical presumably causes severe damage to the parasite DNA. The schizonticidal action of the chelator may thus be rationalized in terms of a four-stage redox-cycling process, as delineated by stages i-iv in Scheme **2.**

The purpose of this paper was to gain greater insight into factors governing the growth inhibition of chloroquine-resistant species of *P. falciparum* by pyridoxal-based

Reductant-Driven Redox-Cycling

intra-parasite iron-chelate formation

$[(L^+,X^-) + \text{cell-Fe(II)} \rightarrow \{[\text{parasite}]\text{-Fe(II)}\text{-}(L^+,X^-)$

chelation of cell iron — *parasite-iron-chelate complex*

1 **2**

redox switch

$\text{Fe(II)}\text{-}(L^+,X^-) \leftrightarrow \text{Fe(III)}\text{-}(L^{\cdot},X^-)$

chelate — *chelate radical*

2 **3**

formation of parasite DNA-anion radical

→ **(3)** + parasite-DNA → parasite-$\{DNA\}^{-\cdot}$ + $(L^+.X^-)$-Fe(III)

free radical — *anion-radical* — **4**

$\text{Fe(II)}\text{-}(L^+,X^-) \leftrightarrow \text{Fe(III)}\text{-}(L^{\cdot},X^-)$ ← $+\ \varepsilon^-$

3 — *reducing metabolites*

fragmentation of parasite DNA-anion radical

parasite-$\{DNA\}^{-\cdot}$ → ***DNA****-degradation* → ***parasite death***

anion-radical

Scheme 2.

chelators of structure **3.** To this end we synthesized and tested the antimalarial activity against *P. falciparum* of three different classes of redox-active domain-structured chelators based on pyridoxal (see Scheme **3**). These comprised: i) six bidentate chelators with one O-, and one N-coordination site, ii) 12 tridentate ligands with two N-, and one O-coordination sites, and iii) four tridentate chelators with two O-, and one N-coordination sites. These chelators were designed to : a) unravel denticity effects in the antimalarial activity; b) determine the importance of the nature of the elements constituting the coordination compartment; c) unravel substitution effects: (i) around the hydrophobic site (in N-heteroaromatic rings), (ii) on the C=N-N bridge connecting the two heteroaromatic rings; and d) to emphasize the importance of the H-bonding site.

Of the three classes of chelators : class **I** (**5-8**, Chart **1**) and class **II** (**9-22**, Chart **2**) were prepared by condensation of pyridoxal hydrochloride with the appropriate arylhydrazine, and class **III** (**23-26**, Chart **3**) by condensation of pyridoxal with the appropriate aroylhydrazine. The quaternary salts **9-10,** and **15-22** (Chart **2**)**,** were obtained by exposing the unsubstituted ligand to the action of an excess of the appropriate alkyl halide.

The antimalarial properties of compounds included in this study were assessed in anticipation for possible clinical application. The effect of **5-26** on the growth and development of a chloroquine-resistant strain of *P. falciparum* (FCR-3) [12], was assessed.

RESULTS AND DISCUSSION

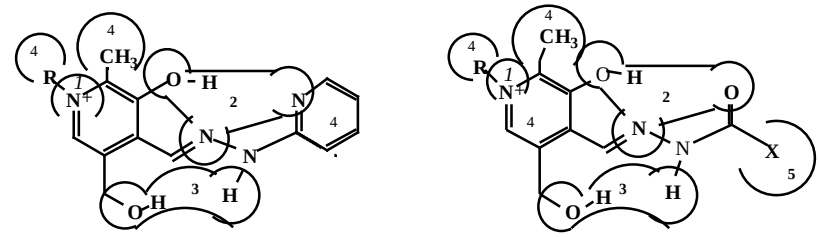
Multi-domain Structures

From Charts **1-3** it appears that, the chelators included in this study are made up of three distinctly different building-blocks:

(a) an α-hydroxy-heteroaromatic aldehyde (pyridoxal), (b) an aromatic ring(substituted phenyl) (see Chart **1**)**,** or

Domain-Structured Chelators Based on Pyridoxal

Tridentate Chelators of the N_2O-Type **Tridentate Chelators of the O_2N-Type**

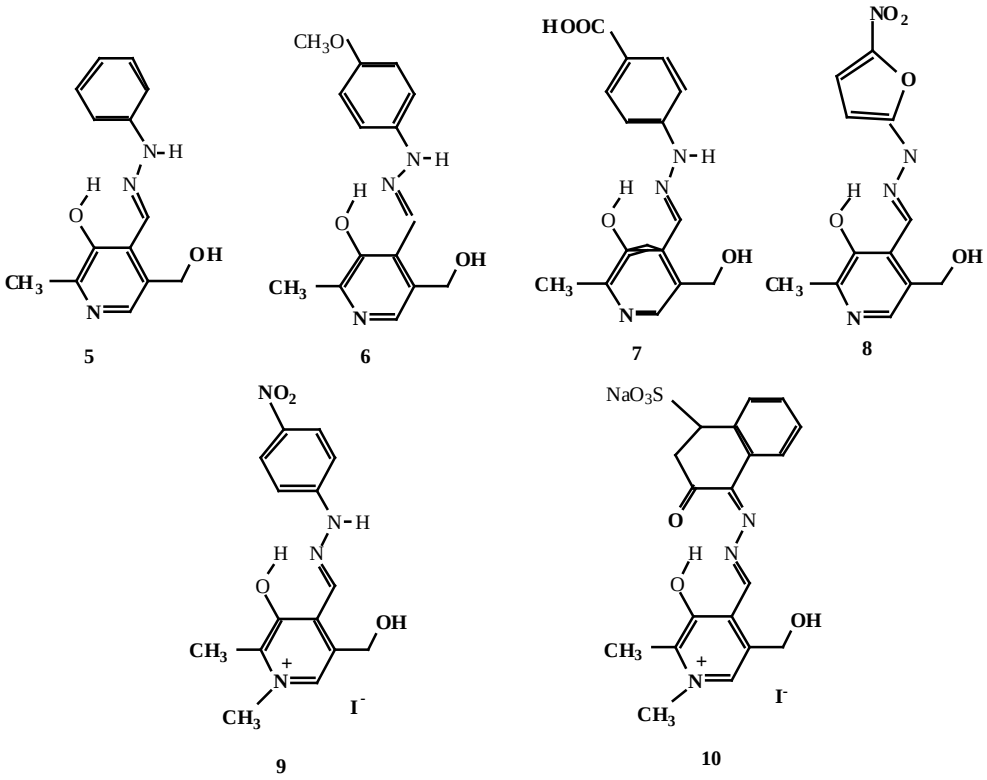


1. Electrophoric Center; 2. Metal Coordination Compartment; 3. Hydrogen Bonding Site

4. Hydrophobic Site *5. Heteroaromatic Ring*

Scheme 3.

Class I . *Bidentate chelators with one O-, and one N-coordination sites*



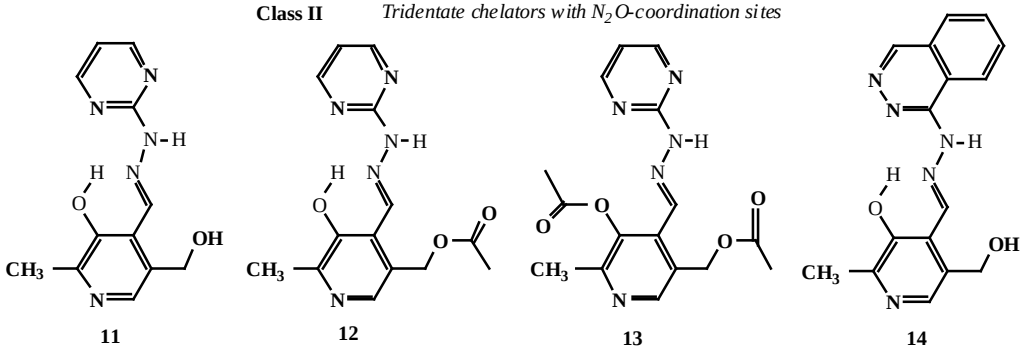
5 **6** **7** **8**

9 **10**

Chart 1.

Pyridoxal Heteroaryl Hydrazones

Class II *Tridentate chelators with N_2O-coordination sites*



11 **12** **13** **14**

(Chart 2. Contd….)

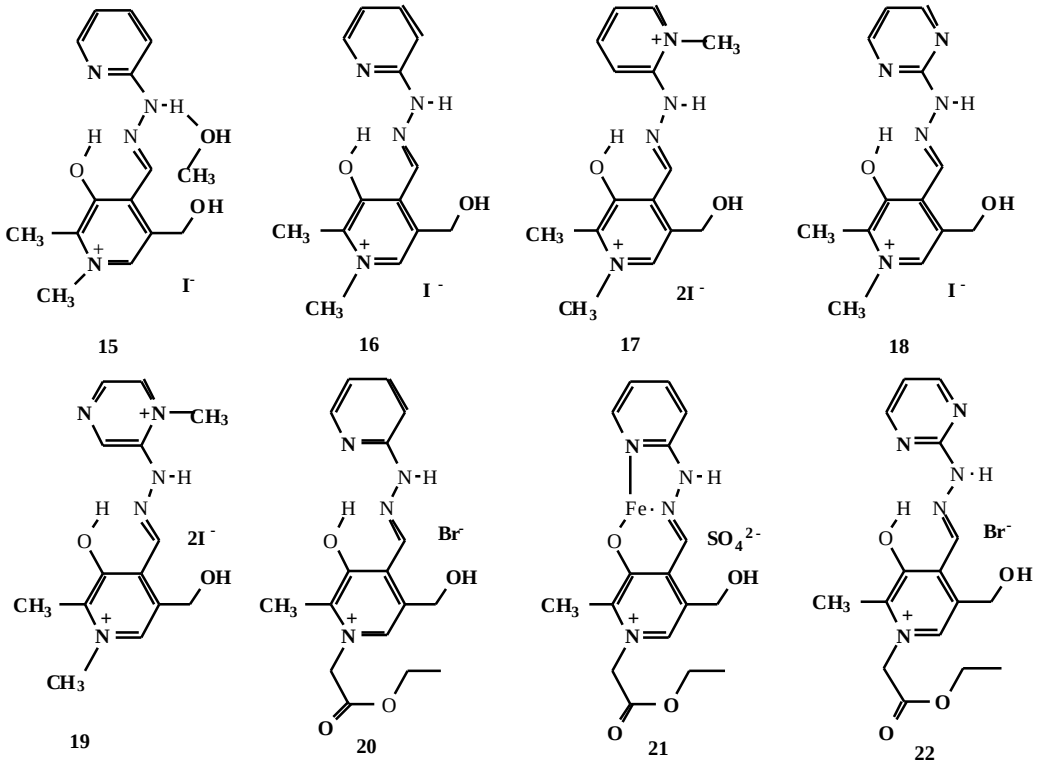


Chart 2.

Pyridoxal Heteroaroyl Hydrazones

Class III. Tridentate chelators with O_2N-coordination site

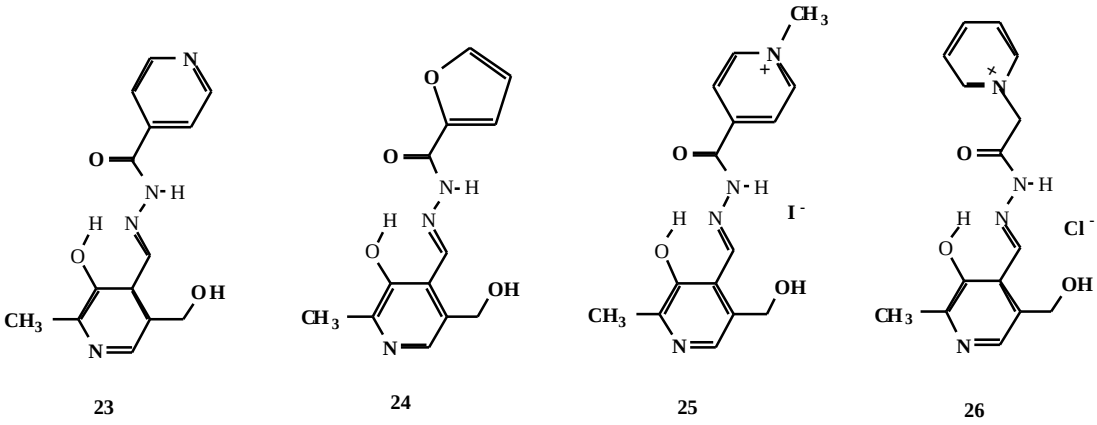


Chart 3.

heteroaromatic ring (pyridine, pyrimidine, pyrazine and phthalazine) (Chart **2**), or heteroaroyl group (isonicotinoyl, α-furoyl) (Chart **3**). The corresponding arylhydrazones or aroyl-hydrazones, contain an inter-ring π-bridge of 4-atom chains, composed of hydrazinic nitrogenes of the CH=N-NH-, or -CH=N-NH-CO-type, are capable of transmitting conjugative effects through the π-system. The metal complexes presented here constitute three different classes based on the number and the electro-negativity of the coordination atoms. Class **I** (Chart **1**) contains bidentate ligands with one O and one N coordination site (**5-10**); Class **II** (Chart **2**) contains tridentate ligands with N_2O coordination sites (**11-22**), and Class **III** includes tridentate ligands with O_2N coordination sites (**23-26)** [see**, 17**]**.** In addition there are three distinctly different sub-classes based on pyridoxal :**(i)** (**5-8, 11-14, 23-26**), (ii) derivatives of pyridoxal-betaine (**9-10, 15-22**), and (iii) N-substituted heteroaroyl building-blocks (**17, 19, 25).**

Denticity Effects

Classes **I-III** represent domain-structured ligands comprising four domains: (1) a metal coordination site (ON, N_2O and O_2N), (2) a hydrophobic moiety (see Scheme **3**), (3)

hydrogen-bonding sites (see Scheme **3**), and (4) an electrophoric center. They may be considered a type of bichromophoric system, **D-Br-Ac,** (integrating a donor, **D**, an acceptor, **Ac**, and a bridge -**Br**), which allows transmission of electronic effects. The D-site is located in the metal-coordination domain [17], whereas the acceptor A resides on the pyridoxal ring-N. The electrophoric site is distinctive in its ability to confer electron-sink properties upon the system. The hydrophobic envelope endows the system with facile transmembrane mobility [18]. Previously [19] it was shown that chelator **20** indeed functions as a reductant-driven redox cycler of iron (Scheme **1**).

The redox-active agents, **14, 16**, and **20**, appearing in Chart **2** (see Table **2**), proved to be highly potent growth-inhibitors of resistant species of *P. falciparum*. Compound 14 is the most prominent one. It contains a phthalzyl building-block, and is capable of killing 95% of resistant *P. falciparum* species within 2 hrs., comparable in its antimalarial activity to artemisin. After 48 hrs., **11**, **16** and **18-20**, all reached 100% parasite killing at 20 mmol (see Table **4**). Most significantly, they all were only 10% less active than Artemisin at the same concentration after 48 hrs. (see Table **4**).

Substitution Effects

Of particular interest are the substitution effects described here. Thus, replacement of the pyridyl ring (pKa= 5.2) in **16** by a 2-pyrimidyl radical (pKa= 2.3) (**16** → **18**) *decreased* the antimalarial activity by a factor of 1.6, and replacement of the pyridyl ring in **17** by a pyrazyl radical (**17** → **19**) decreased the activity by a factor of 1.3. In contrast, replacement of 2-pyrimidyl by a 1-phthalazyl radical (**11** → **14**) *increased* the activity by a factor of 1.32.

Iron Affinity

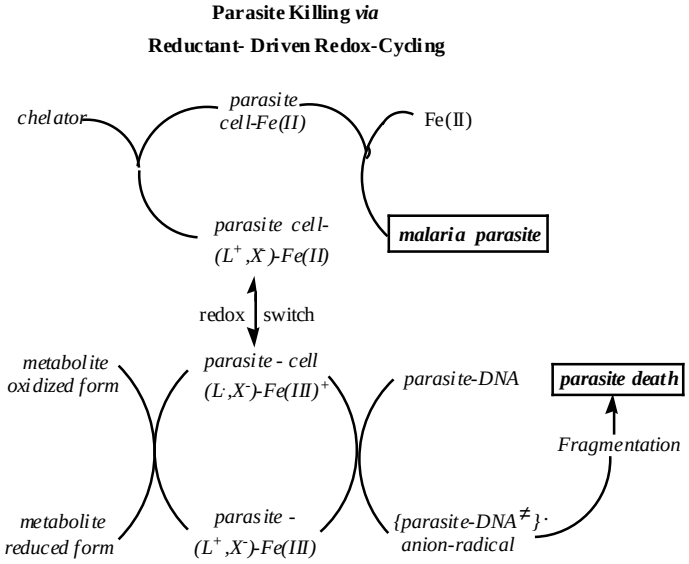
The data in Tables **1-4**, relate to growth inhibition of *P. falciparum* schizonts (from a continuous line maintained in human A^+ erythrocytes), by 22 chelators described in the text. Of these, **5-10** were bidentates, with one O and one N coordination atom with *low affinity for iron* and, with the exception of **6**, also weak antimalarial activity (Table **1**). The four O_2N-tridentates, **23-26** (Table **1**)**,** with *high affinity for iron*, did not show any activity. However, the N_2O-tridentate chelators (Table **2**) with *moderate affinity for iron* showed both the *highest and the quickest schizonticidal activity* (up to 95% in 2 hrs. in the case of **14**).

Electronic Effect

Although the antimalarial activity of Class **I** ligands is *low*, the electronic effect of substitution provides an insight into the electron density-activity relationship in the phenyl ring of **5**. Thus, placing an electron-donating methoxy group at the *para*-position in the phenyl ring (**5** → **6**) enhanced the antimalarial activity, whereas its replacement by an electron-withdrawing substituent such as a carboxyl group (**5** → **7**) resulted in total loss of activity. A similar effect was noted upon putting a positive charge on the pyridoxal-ring N. Thus, the negative influence of the nitro group on activity is canceled out upon quaternization of the pyridoxal ring N (compare **9** with **5**). Similarly, replacement of the phenyl group in the system by a π-excessive furan ring cancels the negative effect of the nitro substituent on the antimalarial activity (compare **8** with **7**).

The conclusions may be drawn: i) that the diarylhydrazone system in Class I is apt to transmit conjugative effects, and ii) electron-donating groups either on pyridoxal ring-N, or on the aryl ring, markedly improve rather remarkably the antiparasitic properties of the system.

Although the activity profile of ligands composing Class III is disappointing, the changes following insertion of a CO group into the hydrazinic π-bridge is of significance in view of drug development. In sharp distinction to the activity-profile of ligands composing Class I chelators (containing



Scheme 4.

Table 1. Growth Inhibition of Plasmodium falciparum (FCR-3) After 2 hrs. Exposure to 40 μmol of Chelators.

Chelator No.	Formula	Mol. wt.	% P A R A S I T E M I A	
			Microscopic counts	^{3}[H]-Hypoxanthine Incorporation
			% Inhibition	% Inhibition
Control			**0**	**0**
Bidentate Chelators with O, N coordination sites				
5	$C_{14}H_{15}N_3O_2$	257	-	33
6	$C_{15}H_{17}N_3O_3$	287	-	61
7	$C_{15}H_{15}N_3O_4$	301	0	0
8	$C_{12}H_{12}N_4O_5$	292	31	33
9	$C_{14}H_{17}N_4O_4I$	432	16	28
10	$C_{19}H_{18}N_3O_6N_aS$	439	50	

% Parasitemia determined by two methods: by microscopic counting, and by ^{3}[H] -hypoxanthine incorporation.

Table 2. Growth Inhibition of *Plasmodium falciparum* (FCR-3) After 2 hrs. Exposure to 40 μmol of Chelators.

			% P A R A S I T E M I A	
Chelator No.	Formula	Mol. wt.	Microscopic counts	^{3}[H]-Hypoxanthine incorporation
			% Inhibition	% Inhibition
Control			**0**	**0**
Tridentate Chelators with N_2O coordination sites				
11	$C_{12}H_{13}N_5O_2$	259	82	72
12	$C_{14}H_{15}N_5O_3$	301	9	24
13	$C_{16}H_{17}N_5O_4$	343	21	28
14	$C_{16}H_{15}N_5O_2$	309	-	95
15	$C_{15}H_{21}N_4O_3I$	432	61	88
16	$C_{14}H_{17}N_4O_2I$	400	80	88
17	$C_{15}H_{21}N_4O_2I_2$	543	68	74
18	$C_{13}H_{16}N_5O_2I$	401	56	55
19	$C_{14}H_{19}N_5O_2I_2$	543	66	58
20	$C_{17}H_{21}N_4O_4Br$	425	94	91
21	$C_{17}H_{20}N_4O_8SFe$	496	7	24
22	$C_{16}H_{20}N_5O_4Br$	426	35	51

% Parasitemia determined by microscopic counts, and ^{3}[H] -hypoxanthine incorporation.

Table 3. Growth Inhibition of *Plasmodium falciparum* (FCR-3) After 2 hrs. Exposure to 40 μmol of Chelators.

Chelator No.	Formula	Mol. wt.	% P A R A S I T E M I A	
			Microscopic counts	^{3}[H]-Hypoxanthine incorporation
			% Inhibition	% Inhibition
Control			**0**	**0**
Tridentate Chelators with O_2N coordination sites				
23	$C_{14}H_{14}N_4O_3$	286	-	21
24	$C_{13}H_{13}N_3O_4$	275	-	10
25	$C_{15}H_{17}N_4O_3I$	428	22	7
26	$C_{12}H_{17}N_4O_3Cl$	336.5	0	0

% Parasitemia determined by microscopic counts, and ^{3}[H] -hypoxanthine incorporation.

Table 4. Effect of Concentration (μmol), and Time Exposure (hrs) on Antimalarial Activity of Chelators Against a Drug-Resistant Species of *P. falciparum*.

Chelator No.	Concentration Mol x 10-6	% I N H I B I T I O N		
		24 hrs	48 hrs	72 hrs
Control		**0**	**0**	**0**
Bidentate chelators				
5	5 10 20	10 - 41	20 28 31	10 21 37
6	5 10 20	14 28 50	3 9 44	8 32 45
10	5 10 20	23 41 50	22 45 56	33 60 79
Tridentate chelators with N_2O coordination sites				
11	2 5 10 20	64 64 71 76	79 83 87 100	98 99 99 100
16	2 5 10 20	63 74 71 74	83 83 91 100	98 98 100 100
18	2 5 10 20	3 32 58 57	33 68 87 91	45 96 98 99

(Table 4. Contd….)

Chelator No.	Concentration Mol x 10-6	% INHIBITION		
		24 hrs	48 hrs	72 hrs
Control		**0**	**0**	**0**
19	2	21	40	52
	5	28	33	96
	10	48	89	100
	20	57	89	100
20	2	34	40	40
	5	58	75	97
	10	68	87	98
	20	76	100	100

% Parasitemia determined by microscopic counts.

phenyl ring by the π-excessive furan ring in Class III (Chart **3**) (containing two O atoms for metal coordination), results in a *decrease* in the antimalarial activity (compare **24** with **5,** and **23** with **24**). This can be rationalized in terms of drug 's mechanism of action.

Drug's Mechanism of Action

The most active iron-chelators included in this study killed, rather than weakened the growth of the drug-resistant species of *Plasmodium falciparum*, within a short period of time. The killing conceivably could arise from two alternative routes. One, damaging synthesis of the parasite-DNA *by* depriving its metalloenzyme, ribonucleoside diphosphate reductase (RdR) of its essential iron co-factor. The other, damaging the molecular structure of the bio-polymer - the parasite DNA, and causing its rapid death. The latter could result from the drug's shooting electrons into the bio-polymer, rendering it a highly unstable anion-radical, that tends to fragment as soon as formed.

The first alternative does not seem plausible. We found i) *no correlation* between the antimalarial activity and the iron-mobilizing capability of the compounds tested here, but ii) we did find a *reciprocal correlation* between the antimalarial activity and the body's iron-mobilizing capacity. Thus, compounds **15-16** and **20**, which are weak iron-mobilizers [24] display high schizonticidal activity (see Table **2**), whereas the body's strong body iron sequestering agents [25], **23-25**, are essentially inactive (see Table **3**). Cumulatively, these lead to the conclusion that the killing of the parasite is not due to removal of iron from the parasite RdR.

The second alternative appears more likely. Parasite killing by the pyridoxal-based chelator probably occurs, as follows (see Scheme **4**):

i) Both the iron ions (with the aid of iron-receptors on the cell membrane) and the lipophilic chelator, readily cross the parasite's outer membrane and penetrate the cell plasma [26] to form the corresponding ligand-iron complex (L^{+},X^{-})-Fe(II), which *via* redox-switch evolves as a carbon-centered radical chelate -$(L^{\cdot},X^{-})$-Fe(III).

ii) The radical-chelate appears to be an electron-donor, capable of donating its excess electron to the DNA, which functions as an electron acceptor, to yield an unstable DNA-radical-anion {DNA}˙ and (L^{+},X^{-})-Fe(III). The DNA radical-anion fragments as soon as it is formed.

iii) The (L^{+},X^{-})-Fe(III) presumably readily undergoes reduction by ubiquitous reducing metabolites (glutathion, ascorbate, etc.) to form a new radical-chelate $(L^{\cdot},X^{-})$-Fe(III), which can start a new cycle of events leading to DNA damage of other malaria parasites.

In short, the radical-complex $(L^{\cdot},X^{-})$-Fe(III), which is formed within the parasite cell can be viewed as a cycler that drives electrons from cell-reductants, delivering them to its DNA, and thus leading to its immediate death.

The parasite's death could conceivably be caused alternatively by another route, by way of destructive interaction between the C-centered radical-chelate $(L^{\bullet},X\text{-})$-Fe(III) and the O-centered radical of RdR [27]. This alternative can, however, ruled out since no correlation was found between the inhibition of the RdR enzyme by **11-13**, **17-18**, **20**, and **23-25** [reported in ref. 19] (see Table **5**) and their antimalarial activity (Tables **1-4**) reported here.

The drop in the antimalarial activity on moving from Class II (Table **2**) (containing one O-coordination atom) to Class III (Table **3**) (containing two O-coordination atoms) underlines the importance of the electron-negativity and electron-density in the coordination sphere upon the biological properties. of the chelators.

The drop in antimalarial activity, by 67% and 61%, respectively, accompanying the **11** → **12** → **13** acetylations, stresses the importance of H-bonding in the schizonticidal process.

SUMMARY

Three classes of lipophilic chelators based on pyridoxal were synthesized and tested against chloroquine-resistant species of *Plasmodium falciparum.* Their four structured domains: (i) an electrophoric center, (ii) a metal coordination sphere, (iii) hydrogen-bonding, and iv) hydrophobic sites,

Table 5. Growth Inhibition of P. falciparum versus Inhibition of Ribonucleotide Reductase.

Chelator No.	Growth Inhibition of P. *falciparum* after 24 hrs. exposure		Inhibition of Ribonucleotide Reductase		
	Concentration Mol x 10^{-6}	% Inhibition	Concentration Mol x 10^{-6}	%Inhibition	
				Chelator	Hydroxyurea
11	10	71	1.0	78	73
12	40	24	1.0	89	73
13	40	28	1.0	79	73
17	40	74	0.01	0	15
18	10	58	0.01	0	15
20	40	94	0.01	0	15
22	40	51	0.01	79	15
23	40	21	0.01	0	15
25	40	7	0.01	0	15

proved to play a pivotal role in killing malaria parasites. Six highly active chelators, capable of killing the parasite *in vitro* within 2 hrs. at 40 mmol were identified. They are all N_2O-tridentates, of moderate affinity for iron and capable of H-bonding. O_2N-tridentates with enhanced affinity for iron, proved to be essentially inactive. The mechanism of the antimalarial action is discussed in terms of reductant-driven redox-cycling involving the mediation of a carbon-centered chelate-radical.

MATERIALS AND METHODS

Chemicals

All the reagents and solvents were of "analytical" or "chemically pure" grade. *Pyridoxal hydrochloride*, mp 173°C (dec.), was purchased from Merck. The following chemicals were purchased from Fluka, and used without further purification: *hydralazine* (mp 172-173°C); *phenylhydrazine* $_D^{20}$1.6083); *4-hydrazinobenzoic acid* (mp 215°C); *4-methoxy-phenylhydrazine hydrochloride* (mp 160°C); *4-nitrophenyl-hydrazine (*mp 158-161°C); *5-nitro-2-furylaldehyde diacetate* (mp 89-91°C); *5-nitro-2-furylaldehyde* (mp 32-35°C); *2-furoylhydrazide* (mp 77-80°C); *sodium 3.4-dihydro-3.4-dioxo-1-naphthalene sulfonate; 1-pyridoxylidene-2-[2'-pyrimidyl]-hydrazine* (mp 298°C)[6] (**11**); *1-[pyridoxylidene-O^2-acetate]-2-[2'-pyrimidyl]hydrazine* (mp177-8°C)[6](**12**); *1-(Pyridoxy-lidene-O^1,O^2-diacetate)-2-(2-'pyrimidyl)hydrazine* (mp 177-178°C) *(***13***)*. *1-pyridoxylidene-2-[2'-pyridyl]hydrazine* (mp 298°C)[2](**16**); *1-[N-methylpyridoxylidenium]-2-[2'-N-methyl-pyridinium]hydrazine iodide* (mp 276-277°C) (**17**)); *1-[N-methyl-pyridoxylidenium]-2-[2'-pyrimidyl]hydrazine iodide* (mp 252-3°C) (**18**); *1-[N-ethoxycarbonylmethyl-pyridoxy-lidenium]-2-[2'-pyridyl]hydrazine bromide* 204°C) (**20**); *1-[N-ethoxycarbonylmethylpyridoxylidenium]-2-[2'-pyrimii-dyl]hydrazine bromide* (mp 188°C)(**22**); *pyridoxal isonico-tinoyl hydrazone (PIH),* (mp 288°C) (**23**); *1-pyridoxyliden]-2-[N-methyl-isonicotinoyl]hydrazine* iodide (mp 250°C) (**25**); were prepared as described. Anhydrous hydrazine, 2-hydrazinopyrazine, were purchased from Sigma Chem, Co (USA), RPMI 1640 from GIBCO (Grand Island, NY); HEPES from Sigma Chem. Co. All other chemicals were of analytical grade.

Parasites

P. falciparum (FCR-3) was obtained from a continuous line maintained in human A^+ erythrocytes in RPMI 1640 medium containing 10% plasma. Red blood cells were obtained from healthy donors who had no prior contact with malaria. Serum and plasma were obtained from the Hadassah Hospital Blood Bank. Radiolabelled [^{3}H]hypoxanthine specific activity 10 Ci/mM, was purchased from the NEN Corp. (Boston, MA USA).

Parasites were grown in 9 cm Petri dishes using the candle jar method [20]. They were cultured in group A^+or O red blood cells in RPMI 1640 medium containing 25 mM HEPES buffer, 2 g/litre $NaHCO_3$, 100 mg/ml gentamicin (Sigma) and 10% human serum or type A plasma. The parasites were synchronized by sorbitol treatment [21] and gelatine sedimentation [22] or by a combination of the two Before use, the parasites were washed twice with RMPI 1640 without plasma and once with HEPES/$NaHCO_3$, buffer (pH 7.2), and resuspended in HEPES/$NaHCO_3$ buffer to a concentration of 10^9 cells/ml. Parasitemia was determined by microscopical counts of the number of parasites per 1000 cells and [^{3}H]Hypoxanthine incorporation [23]. The cells were harvested with a MASH II automatic cell harvester and transfered to glass fiber filters and their radioactivity was determined.

All experiments were begun with highly synchronized parasite cultures of either schizonts or ring stages. Since the inoculum varied from experiment to experiment, parasite

growth depended on the inoculum and [^{3}H]Hypoxanthine incorporation and growth curves were comparable only within a given experimental set and not between different experiments. All experiments were repeated at least three times. All the microscopic and radioactive counts are averages of at least triplicate determinations.

Because of low solubility in water, chelators **5, 8, 11, 12, 13, 20, 23,** and **25** were first dissolved in 1 molar aqueous DMSO. They were then diluted with fresh RPMI to the required concentration. The first concentration of DMSO in the test solutions was 0.01 mol. The positive control was normal RPMI washing solution, namely, without DMSO. The results are presented in Tables **1**-**3**.

Spectroscopic Measurements

IR measurements were made using a *Perkin-Elmer* Model 457 Grating infrared spectrophotometer. **^{1}H and ^{13}C *NMR*** spectra were made using a *Varian VXR 300 Spectrometer*, with TMS *as an internal standard and DMSO-d6 as solvent. UV measurements were made using a Kontron Uvikon 810 spectrophotometer. MS were recorded with a LKB 90 spectrometer.*

Elemental analyses were performed by the Microanalytical Unit of the Hebrew University, Dept. of Organic Chemistry, Givat Ram Campus, Jerusalem.

EXPERIMENTAL

1-Pyridoxylidene-2-[phenyl]hydrazine] (5)

(a) Pyridoxal hydrochloride (5 g, 2.46 mmol) in 50 mL H_2O was neutralized with anhydrous sodium acetate (5 g) and then allowed to react with phenyl hydrazinehydrochloride (3.5 g) to which sodium acetate (5 g) in 50 mL H_2O was added. This resulted in the spontaneous separation of a yellow precipitate, which was collected, washed (H_2O), and recrystallized from ethanol, yielding yellow crystals (95%) of **5**, mp 217-8^{o}C. (b) The desired chelator, **5.** was obtained in 95% yield, also by adding dropwise phenyl hydrazine (2.4 mL, 2.65 g, 2.46 mmol) into a solution of pyridoxal hydrochloride (5 g) in 50 mL H_2O, followed by the addition of sodium acetate (5 g) in H_2O (50 mL). *Anal.* Found: C, 65.07; H, 5.91; N, 16.00. $C_{14}H_{15}N_3O_2$ requires: C, 65.37; H, 5.84; N, 16.34.

1-Pyridoxylidene-2-[4'-methoxyphenyl]hydrazine hydrochloride] (5)

An aqueous solution of pyridoxal hydrochloride (4.07 g, 20 mmol, in 50 mL H_2O) was initially neutralized with anhydrous sodium acetate (1.64 g) and then heated and allowed to react with 4-methoxyphenyl hydrazine hydrochloride (3.05 g, 2.2 mmol), the resulting reaction mixture was neutralized with an aqueous solution of sodium acetate (51.64 g in 50 mL H_2O), yielding an orange precipitate (5.7 g, quantitative yield). Recrystallization from MeOH-H_2O afforded yellow-orange crystals, mp > 300^{o}C.

1-Pyridoxylidene-2-[4'-methoxyphenyl]hydrazine Hydrochloride (6)

An aqueous solution of pyridoxal hydrochloride (4.07 g, 20 mmol, in 50 mL H_2O) was initially neutralized with anhydrous sodium acetate (1.64 g) and then heated and allowed to react with 4-methoxyphenyl hydrazine hydrochloride (3.05 g, 2.2 mmol). The resulting reaction mixture was neutralized by an aqueous solution of sodium acetate (51.64 g in 50 mL H_2O), yielding an orange precipitate (5.7 g, quantitative yield). Recrystallization from MeOH-H_2O afforded yellow-orange crystals, mp > 300^{o}C. *Anal.* Found: C, 56.07; H, 5.48; N, 12.9. $C_{15}H_{18}N_3O_3Cl$ requires : C, 55.62; H, 5.56; N, 12.98.

1-Pyridoxylidene-2-[4'-carboxyphenyl]hydrazine Hydrochloride (7)

A warm aqueous solution (60^{o}C) of pyridoxal hydrochloride (4.07 g, 20 mmol, in 50 mL H_2O) was initially neutralized with sodium acetate (1.64 g) in H_2O (50 mL) and then allowed to react with 4-hydrazinobenzoic acid (3.05 g, 2 mmol) in 50:100-H_2O:MeOH (150 mL). The expected intensed yellow phototropic product (6 g, 100% yield) emerged spontaneously from the reaction mixture. The methanol-soluble product was readily recrystalized from MeOH:H_2O, mp> 300^{o}C. Anal. Found, 53.35; H, 4.93; N, 12.49; $C_{15}H_{16}N_3O_4Cl$ requires C, 53.29; H, 4.74; N, 12.43.

1-Pyridoxylidene-2-[5'-nitrofurfurylidene]hydrazine (8)

The desired chelator (7) was synthetized by two alternative two-stage processes : (a) and (b). In route (a) the known pyridoxal mono-hydrazone (PH) is formed, and then condensed with 5-nitro-2-furylaldehyde diacetate to form **8.** In route (b) the known 5-nitro-2-furylaldehyde (NFH) is formed, and then condensed with free-base pyridoxal. Route a. A hot solution of of pyridoxal mono-hydrazone (PH) (0.36 g, 2 mmol) in MeOH (50 mL) was added to a hot solution of 5-nitro-2-furfuraldehyde dicetate (0.48 g, 2 mmol) in MeOH (50 mL) containing 0.4 mL conc. HCl. The reaction mixture was then refluxed for 5 minutes, and allowed to stand overnight at R.T. A total crop of 0.4 g (67%) of mustard-yellow product was collected following the addition of ether to the reaction mixture, mp 244-5^{o}C. *UV* (MeOH) λ_{max} 3531Å, 3395, 2801, 2099Å Route b**.**

(i) *Formation of 5-nitro-2-furfualdehyde mono-hydrazone (NFH)* {British Patent PB16886 (1959)}. A mixture of 5-nitrofurylaldehyde diacetate (12 g, 40 mmol), and a solution of anhydrous hydrazine (1.5 mL, 48 mmol) in ethanol (70 mL) was refluxed for 30 minutes, yielding orange crystals of NFH, mp 164^{o}C.

(ii) *Formation of 1-Pyridoxylidene-2-[5'-nitrofurfurylidene] hydrazine (8). A* hot solution of NFH (0.5 g, 1.67 mmol) in MeOH (35 mL) was allowed to react with 3 millimolar free-base pyridoxal {formed *in situ* by neutralizing methanolic solutions of pyridoxal hydrochloride (0.66 g) with 3 millimolar (0.24 g) of sodium acetate}, mp 244-5^{o}C. *^{1}H-NMR* (300 MHz, Me_2SO-d_6) Å 11.78 (s, 1H), 9.25 (d, 1H), 8.95 (d, 1H), 8.02 (s, 1H), 7.86 - 7.84 (q, 1H), 5.47 (m, 1H), 4.69 (dt, 2H), 3.35 - 3.34 (d, 4H), 2.44 (s, 3H).

Anal. Found: C, 51.56;H, 3.97; N, 18.30.

$C_{13}H_{12}N_4O_5$

requires : C, 51.32; H, 3.95; N, 18.42

1-[N-Methylpyridoxylidenium-2-[4'-nitrophenyl]hydrazine Iodide (9). ***1-Pyridoxylidene-2-[4'-nitrophenyl]hydrazine*** **(9a)**

A solution of 4-nitrophenyl hydrazine (4 g, 27 mmol) in hot ethanol was added to a solution of *free-base-pyridoxal* (prepared *in situ* by neutralizing an aqueous solution of 5 g of pyridoxal hydrochloride [20 mmol in 50 mL H_2O]. The expected hydrazone (7 g, 94% yield) crystallized spontaneously. Recrystallization from ethanol afforded light brown crystals, mp > 300°C. *Anal*. Found :C, 56.73; H, 4.73' N, 18.70. $C_{14}H_{14}N_4O_4$ requires : C, 55.52; H, 4.64; N, 18.54.

1-[N–Methylpyridoxylidenium-2-[4'-nitrophenyl]hydrazine Iodide (9)

A mixture of 1-pyridoxylidene-2-[4'-nitrophenyl]hydrazine (3g, 10 mmol, in 200 mL methanol)), and methyl iodide (2 mL, 16 mmol) was refluxed for 72 hrs. The unreacted starting material (1.5 g, 50%) was removed by filtration. The filtrate was concentrated to a volume of 50 mL and the emerging solid, light-brown product (0.8 g, 36% yield computed on the basis of starting material consumed) was collected, and recrystallised from MeOH, mp 253°C. *UV* (MeOH, c. $5x10^{-6}$ M) λ_{max} 5000 Å, 4087, 3410, 3397, 2189Å. *^{1}H NMR* (300 MHz, Me_2SO-d_6) δ 12.2 (s, 0.5H), 12.1 (s, 0.5H), 8.54 (d, 1H J= 1Hz), 8.48 (s, 1H), 8.26 (d, 2H J=0.5 Hz), 8.23 (d, 1H J=o.5 Hz), 7.22 (d, 1H), 7.20-7.12 (sextet, 4H), 485 (s, 4H), 4.25 (s,2H), 2.67 (s, 3H),. Anal. Found : C, 38.73; H, 3.60; N, 12.16; I, 26.21. $C_{15}H_{17}N_4O_4$ IxH_2O requires C, 38.96; H, 4.11; N, 12.12; I, 27.49.

1-Pyridoxylidene-2-[2'-oxo-3'-sulfonic acid naphthylidene]hydra zine (10)

A solution of naphthoquinone-4-sulfonic acid sodium salt (0.52 g, 2 mmol) in water (20 mL) was added dropwise with stirring to a cooled (0 - 5°C) solution of pyridoxal hydrazone (PH) (0.36 g, 2 mmol) in water (20 mL), containing 0.1 mL of concentrated HCl (32%), The desired chelator (10) quickly emerged from the reaction mixture as a grey precipitate. It was collected, washed with boiling ethanol, yielding white-grey powder (0.5 g, 60%), mp > 300°C. UV (MeOH, c. 1x10-3M) λmax 4399 Å (A=0.296), 3410 (A=0.325), 3397 (A=0.346), 2836 (A=0.725), 2134 (A=1.206), 2107 (A= 1.196). *Anal*. Found: C, 48.24; H, 4.10; N, 9.75; S, 6.17. $C_{18}H_{14}N_3O_6Na$ x 1.5 H_2O requires: C, 48.00; H, 3.77; N, 9.33; S, 7.11.

1-Pyridoxylidene-2-[1'-phthalazyl]hydrazine Dihydrochloride (15)

A hot solution of pyridoxal hydrochloride (0.41 g, 2 mmol) in 1:1-H_2O:MeOH (15 mL) was added with stirring over 10-15 min., to a hot solution of 1-hydrazinophthalazine hydrochloride (0.4 g, 2 mmol) in 3:1-H_2O:MeOH (30 mL), giving rise to orange-red precipitate (0.85 g, quantitative yield). The red product was collected and subjected to fractional recrystallization from ethanol-water. Analysis of *first crop* (0.58 g, 75%), mp 283°C, revealed it to be a compound composed of the expected product (**15**), containing one-half mole of water of crystallization. *Anal.* Found : C, 49.53; H, 4.56; N, 17.74; Cl, 18.32. $C_{16}H_{18}N_5O_{2.5}Cl_2$ requires: C, 49.11; H, 4.60; N, 17.90; Cl, 17.90.

The second crop **(15a)** (15%), was made up of red crystals, mp 265°C, composed of the free base plus 1.25 moles of HCl and 0.3 moles of H_2O. *Anal.* Found: C, 53.50; H, 4.75; N, 19.09; Cl, 12.28. $C_{16}H_{17}N_5O_{2.3}Cl_{1.25}$ requires : C, 53.24; H, 4.60; N, 19.41; Cl, 12.38.

1-[N-Methylpyridoxylidenium-2-[4'-methyl-2'-pyrazyl] hydrazine Diiodide(19). ***Parent compound*****: 1-Pyridoxylidene-2-[2'-pyrazylyl]hydrazine (19a)**

A solution of pyridoxal hydrochloride (2 g, 10 mmol) in 50:50-H_2O:EtOH (15 mL), followed by 5 mL of an aqueous solution of 0.2 M NaOH was added dropwise with stirring to a solution of 2-hydrazinopyrazine (1.32 g, 12 mmol) in ethanol (15 mL). The emerging yellow, solid product (2.06 g, 80%) was collected, washed with 50:50-H_2O:EtOH, and recrystallized from ethanol, mp 288-290°C. *MS (EI)* at m/z : 259 (molecular ion-peak). *Anal.* Found : C, 30.38; H, 3.08; N, 12.72; I, 47.17. $C_{14}H_{19}N_5O_{2.}I_2$ requires :C, 30.94; H, 3.50; N, 12.89; I, 46.78.

N^1,N^4-Dimethylation of Parent Compound (19a → 19)

Methyl iodide (2.6 mL, 20 mmol), was added to a solution of parent compound(**19a**) (1.3 g, 5 mmol) in ethanol (100 mL) was added and then refluxed with stirring for 48 hrs. The mustard-orange solid product (2 g, 74%) (*major crop*) was filtered, washed with EtOH and ether, and recrystallized from methanol, mp 250°C. *Anal.* Found : C, 30.38; H, 3.08; N, 12.72; I, 47.17. $C_{14}H_{19}N_5O_{2.}I_2$ requires :C, 30.94; H, 3.50; N, 12.89; I, 46.78. *Minor crop - semi-hydrated form of 19***.** The filtrate and washings were combined, the solvent removed, and the residue (0.3 g, 11%) recrystallized from methanol-ether, mp 240°C. *Anal.* Found : C, 30.42; H, 2.90; N, 12.17; I, 45.53. $C_{14}H_{19}N_5O_{2.}I_2$x0.5H_2O requires : C, 30.43; H, 3.62; N, 12.69; I, 46.02.

Iron(II)-chelate of 1-[N-Ethoxycarbonylmethylpyridoxylidenium]-2-[2'-pyridyl] Hydrazine Bromide-Sulphate (21)

5 mL volume of an aqueous $5x10^{-4}$ M solution of $FeSO_4$ x7H_2O (0.14 g, in 5 mL) was added dropwise under N_2 to a stirred solution of 1-[N-ethoxycarbonylmethylpyridoxylidenium]-2-[2'-pyridyl]hydrazine bromide (0.22 g, 0.5 mmol) in water (20 mL), The resulting green solution was first diluted with ethanol (2 mL) and then with ether (25 mL), causing the ferromagnetic dark-green iron(II)-chelate (0.26 g, 87%) to emerge as a crystalline product. *Anal.* Found: C, 34.09; H, 3.53; N, 10.04; S, 5.89; Fe, 10.3. $C_{17}H_{20}N_4O_{4.}BrFexSO_4xH_2O$ requires C, 34.29; H, 3.70; N, 9.41; S, 5.39; Fe, 9.41.

1-Pyridoxylidene-2-[2'-furoyl]hydrazine hydrochloride (24)

A solution of 2-furoylhydrazide (0.13 g, 1 mmol) in H_2O (5 mL). was added to an aqueous solution of pyridoxal hydrochloride (0.2 g, 1 mmol, in 10 mL H_2O). The desired chelator soon crystallized out as a yellow product (95%). Recrystallization from methanol yielded bright-yellow

crystals, mp 219-21°C. *UV* (H_2O, c. $5x10^{-6}$) λ_{max} 3400Å. *Anal.* Found: N, 16.00. $C_{15}H_{13}N_3O_3$ requires: N, 16.21.

1 - Pyridoxylidene - 2 - [N$^{1'}$-methcarboxypyridyl]hydrazine Hydrochloride (26)

An aqueous solution of pyridine-N-acethydrazide chloride (Girard reagent P) (0.19 g, 1 mmol in H_2O). was added to an aqueous solution of pyridoxal hydrochloride (0.2 g, 1 mmol, in 10 mL H_2O) The resulting bright yellow, solid product was recrystallized from ethanol, mp 206-8oC. UV (MeOH, c. 5x10-6M) λ_{max} 3340, 2880Å. IR (KBr, cm^{-1}): 3250, 3100, 3050, 3005, 2650-2850, 2450, 2060, 1700. Anal. Found: C, 53.25; H, 4.90. $C_{15}H_{17}N_4O_3Cl$ requires: C, 53.49; H, 5.05.

ACKNOWLEDGEMENT

This work was carried out in the framework of the US-Israel Cooperative Development Research {CDR} program C-7160, grant no. DPE-5544-G-7021-00.

REFERENCES

[1] Iheanacho, E. N.; Sarel, S.; Samuni, A.; Avramovic-Grisaru, S.; Spira, D. T. *Trans. R. Soc. Trop. Med. Hyg.*, **1990,** *84*, 213-216.
[2] Heppner, D.G.; Hallaway, P.E.; Kontoghiorges, G.J.; Eaton, J.W. *Blood*, **1988**, *72*, 358-361.
[3] Harvey, P.W.J.; Heywood, P.F.; Neshheim, M.C.; Galme, K.; Zegans, M.; Habicht, J-P.; Stephenson, L.S.; Radimer, K.I.; Brabin, B.; Forsyth, K.; Alpers, M.P. *Amer. J. Trop. Med. & Hygiene*, **1989,***40*, 12-1.
[4] Oppenheimer, S.J.; Gibson, F.D.; MacFarlane, S.B.; Moody, J.B.; Harrison, O.; Spencer, A.; Bunari, O. *Trans. R. Soc. Trop. Med. Hyg.*, **1986,** *80, 60*3-612.
[5] Saltman, P. *Seminars in Hematology*, **1989,** *26*, 249-256.
[6] Chapman, D.; Gillespie, C.J. *Adv. in Radiation Biol.*, **1981,** *9*, 143-198.
[7] Marx, J.L. *Science*, **1987,** *235*, 529-531.
[8] Floyd, R.A. *Biochem. & Biophys. Res. Comm*., **1981,** *99*, 1209-215.
[9] Birnboim, H.C.; Kanabus-Kaminska, M. *Proc. Natl. Acad. Sci.* **1985,** *82*, 6820- 6824.
[10] Cochrane, C.G.; Schraufstatter, I.U.; Hyslop, P.; Jackson, J. Cellular and biochemical events in oxidative injury. In Halliwell, B. ed., *Fed. Amer. Soc. Exp. Biol. Proceedings*, **1988,** pp. 49-54
[11] Avramovici-Grisaru, S.; Cohen, S.; Sarel, S. *Heterocycles*, **1990,** *30*, 1079-1090.
[12] Obtained from the Hadassah HospitalBlood Bank, Jerusalem.
[13] Iheanacho, E. N.; Sarel, S.; Samuni, A.; Avramovic-Grisaru, S.; Spira, D. T. *Free Radical Res. Commun.*, **1991,** *15*, 1-10;.
[14] Iheanacho, E. N.; Sarel, S.; Samuni, A.; Avramovic-Grisaru, S.; Spira, D. T. *Free Radical Res. Commun.*, **1991,** *11*, 307-315.
[15] Kelly, R.B.; Cozzarelli, N.R.; Deutscher, M.P.; Lehman, I.R. and Kronberg, A. *J. Biol. Chem.*, **1970,** *245, 39-45*
[16] (a) Albrecht-Gary, A.-M.; Crumbliss, A.L. in Iron *Transport and Storage in Microorganisms, Plant and Animals. Metal Ions in Biological Systems*, Siegel A. and Siegel, H. eds. **1998,** *35*, 239-327; (b) Boukhalfa, H.; Crumbliss, A.L.; Chemn, S.; Sarel, S. *Israel J. Chem.*, **1985,** *25*, 288-292. (c) Sarel, S.; Avramovici-Grisaru, S. and Cohen, S. *Heterocycles*, **1998,** *49*, 393-404.
[18] Ponka, P.; Richardson, D.R.; Edward, J.T.; Chubb, F.L. *Can. J. Physiol. Pharmacol.*, **1994,** *72*, 659-666.
[19] Sarel, S.; Fizames, C.; Lavelle, F.; Avramovici-Grisaru, S. *J. Med. Chem.*, **1999,** *42*, 242-248.
[20] Jensen, J.B.; Trager, W. *J. of Parasitology*, **1977,** *63*, 883-886.
[21] Lambrose, C.; Vanderberg, J.P. *J. Parasitology*, **1979,** *65*, 418-420.
[22] Jensen, J.B. Amer. J. *Tropic Medicine and Hygiene*, **1978,** *27*, 1274-1276.
[23] Golenser, J.; Cassuto, D.; Pollack,Y. *Exper. Parasitology*, **1981,** *52*, 371-377
[24] (a) Sarel, S.;Avramovici-Grisaru, S.; Hershko, C.; Link, G.; Spira, D., *European Patent No. 0315 434 A2, **1989*** [*Chem. Abst.*, **1990,** *112*, P98388^{m}]. (b) Sarel, S.; Cohen, S.;Avramovici-Grisarue, S. *Hetercycles*, **1998,** *49*, 393-404.
[25] Doungdee, P.; Sarel, S.;Wongvisetsirikul, N.; Avramovici-Grisaru, S. *J. Chem. Soc. Perkin Trans.*, **1995**, *2*, 319-323.
[26] Ponka, P.; Grady, RW ; Wilczynska, A.; Schulman, M. *Biochem. et Biophysica Acta*, **1984**, *802* (3), 477-489.
[27] a) Stubbe, J.; vander Donk, W.A. *Chem. Biol.*, **1995,** *34*,141-154; b) Stubbe, J.; Nocera, D.G.; Yee, C.S.; Chang, M.C.Y.; *Chem. Rev*., **2003**, *103*, 2167-2201.

Received: 13 September, 2004 Accepted: 5 November, 2004