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Photooxidation products of primaquine

Structure, antimalarial activity and hemolytic effects

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Photooxidation of primaquine (**1**) and 5-hydroxyprimaquine (**5**) afforded a blue dye for which *o*-quinone structure **4** was elaborated. Similar oxidation of *N*-ethoxyacetylprimaquine (**10**) afforded *o*-quinone **11**. Tissue schizontocidal activity of **4** and **11**, and bisquinolylmethine **3** prepared earlier, showed that none of them had noteworthy antimalarial activity, but all three produced methemoglobin.

Primaquine; Antimalarial activity; Hemolysis; Photooxidation

1. INTRODUCTION

Hemolytic and antimalarial effects of 8-aminoquinoline antimalarials appear to be intimately related, and suggest that both depend on the formation of reversible redox intermediates formed during biotransformation of the drug in the host [1]. Demethylprimaquine is more potent than the parent compound in vitro against erythrocytic forms of *P. gallinaceum*, and potency is further increased by the addition of another hydroxy group present in 5-hydroxydemethylprimaquine (**5**) [2]. An *o*-quinone obtained by metabolic conversion of the chemically related antimalarial pamaquine was isolated and found to be several times more potent than the parent drug in vitro [3]. Side chain degradation of primaquine (**1**), on the other hand, a major metabolic pathway, afforded a desamino carboxylic acid [4] found to be inactive as a tissue schizontocide [5]. *N*-acylated primaquines are also inactive tissue schizontocides in vitro which supports the view that the terminal amino group in the side chain of primaquine may be necessary for antimalarial activity [5]. Further elucidation of antimalarial and possibly associated hemolytic effects present in oxidation products of primaquine [6] suggested a study of several compounds obtained from **1** and its *N*-acylated analogs by photochemical oxidation. This included a blue dye for which Strother et al. [6] proposed the tricyclic quinonimine structure **2**, now revised to that of *o*-quinone **4**, readily obtained from 5-hydroxydemethylprimaquine (**5**) as described here. Also included in this study was the quinoline-5,6-dione **11**, obtained by photooxidation of the ethoxyacetamide **10**, an analog of the microbial metabolite *N*-acetylprimaquine [7]. A third compound also investigated was the diquinolinylmethine blue dye **3**, prepared earlier from **10** by photooxidation in chloroform solution [8].

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2. MATERIALS AND METHODS

2.1. *Chemistry* (figs 1,2)

Blue compound **4** was prepared as follows: 5-hydroxy-6-demethylprimaquine trihydrobromide (**5** · 3HBr) [9] (500 mg) was dissolved in water (10 ml), made alkaline with concentrated aqueous ammonia (pH 9), and extracted 3 times with methylene chloride in an argon atmosphere. The combined organic extracts were dried (Na_2SO_4), diluted with methylene chloride to 150 ml, placed in a 500 ml Erlenmeyer flask and exposed to direct sunlight for 2 h with occasional stirring. The solution turned from yellow to deep blue. The solution was then evaporated to dryness and chromatographed on an SiO_2 column using 1% methanol in methylene chloride as an eluent to afford 120 mg (47%) of a deep blue amorphous solid **4**: $C_{14}H_{15}N_3O_2$ (257.14) - Calcd. C, 65.33; H, 5.88; N, 16.34. Found C, 64.76; H, 5.93; N, 15.70 (the microanalysis was performed by Dr F. Scheidl, Hoffmann-La Roche, Inc., Nutley, NJ). CI-MS, *m/e* 258 ($M^+ + 1$), EI-MS 257; UV (MeOH) max 239, 270, 310, 360 (sh) and 589 nm; IR ($CHCl_3$) 3300, 2930, 2850, 1690, 1610, 1585, 1540, 1500, 1435, 1360, 1310, 1180 and 1095 cm^{-1}. The ^{13}C-NMR data of this compound as described by Strother et al. [6] were essentially confirmed and are listed in table 1.

Methylation of **4** with ethereal diazomethane in methanol afforded *O*-methyl ether **6** as a yellow substance and separated from other products by preparative TLC (SiO_2, 1% MeOH in $CHCl_3$): CI-MS, *m/e* 272 ($M^+ + 1$); UV (MeOH) max 252, 315 and 410 nm; IR ($CHCl_3$) 3300, 2980, 2940, 1620, 1590, 1555, 1500, 1430, 1365, 1335, 1310, 1290, 1150 and 1090 cm^{-1}. Absence of the carbonyl signal present at 1690 cm^{-1} in **4** confirmed its structure.

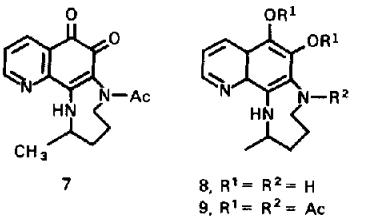

Fig.2.

Acetylation of **4** with acetic anhydride afforded the *N*-acetyl derivative **7** as an orange, amorphous solid: CI-MS, *m/e* 300 ($M^+ + 1$); UV (MeOH) max 233, 276, 297 and 448 nm; IR ($CHCl_3$) 3280, 2975, 2920, 1700, 1645, 1595, 1555, 1515, 1380, 1345, 1315 and 1290 cm^{-1}. Reduction of **4** over a Pt catalyst in methanol afforded hydroquinone **8** not isolated since the yellow solution of **8** rapidly turned blue when exposed to air. Reduction of **4** over a Pt catalyst in acetic anhydride afforded yellow triacetate **9**: CI-MS, *m/e* 386 ($M^+ + 1$); UV

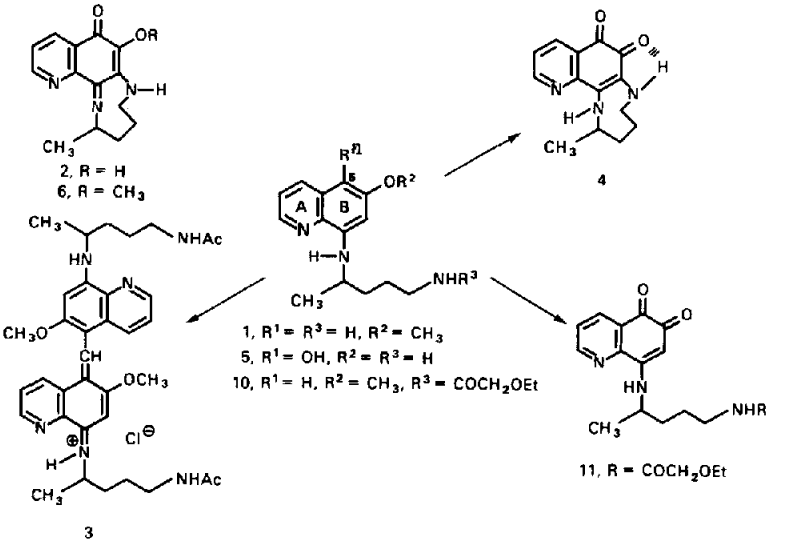

Fig.1.

Table 1

^{13}C-NMR assignments for blue dye **4**[a]

Carbon no.	Chemical shift
2	151.7 (1)
3	124.9 (1)
4	134.5 (1)
4a	126.0 (0)[b]
5	180.6 (0)
6	172.0 (0)
7	124.2 (0)[c]
8	145.7 (0)[d]
8a	148.5 (0)[e]
1′	48.6 (1)
2′	39.4 (2)
3′	23.5 (2)
4′	50.2 (2)
5′	22.4 (3)

[a] Assignments were confirmed by APT, DEPTGL, COSY and HETCOR experiments and agree with those reported [6] except for quaternary signals C-4a, C-8a, C-7 and C-8. The data were generated in $CDCl_3$ on a Varian VXR 300 NMR spectrometer. Assignments for C-4a, C-8a, C-7 and C-8 were established by observing the fully coupled carbon NMR spectrum and by conducting low-power selective long-range decoupling experiments (LSPD)

[b] C-4a is three bond coupled to H-3; irradiation (LSPD) at H-3 (7.37) collapses the doublet to a singlet

[c] C-7 appears as a double doublet in the coupled spectrum being coupled to H-4′ and the NH; irradiation (LSPD) at H-4′ (3.15) and NH (7.49) collapsed the signal to a doublet

[d] C-8 appears as a singlet in the coupled spectrum and is unaffected by irradiation (LSPD) at H-2 (8.60), H-3 (7.37), H-4 (8.16), H-1′ (5.35), H-4′ (3.15), NH (7.49) and NH (3.74)

[e] C-8a appears a complex multiplet in the coupled spectrum; irradiation (LSPD) at H-2, H-4 and NH (7.49) simplifies the pattern

(MeOH) max 264, 343 and 368 nm. Blue dye **4** is further oxidized by air, turning the blue color on TLC plates into purple. The reactions mentioned above fully support *o*-quinone structure **4** for the blue dye. This material is quite unpolar and moves on TLC plates faster than **1** or **7**, indicating, together with the strong absorption band at 3300 cm^{-1} (IR), strong hydrogen bonding between the amino group and the adjacent carbonyl group. Photooxidation of amide **10** deposited on a silica gel surface and exposed to sunlight for several hours in the presence of air afforded the orange quinone **11** which served as an important reference substance: CI-MS, *m/e* 346 ($M^+ + 1$); UV (MeOH) max 233, 275, 296 and 446 nm and identical to that of compound **7**.

Table 2

ID_{50} and ID_{90} values of primaquine diphosphate and oxidation products **3**, **4** and **11**

Compound	ID_{50} ($mg \cdot l^{-1}$)	ID_{90} ($mg \cdot l^{-1}$)
Primaquine	0.015	0.2
3	3	12
4	3	22
11	5.8	25

2.2. *Biological testing*

Biological materials and techniques used for cultivating hepatocytes and performing chemotherapy tests were the same as described [5] with the compounds dissolved in absolute alcohol.

The effect of the test compounds on the in vitro formation of methemoglobin was studied using a modification of the procedure of Fraser and Vesell [10]. Whole blood was collected from New Zealand rabbits using heparin as the anticoagulant, the erythrocytes were washed twice with 0.8% NaCl in 0.01 M phosphate buffer (pH 7.4), and then reconstituted as a 50% dispersion in saline phosphate buffer. The incubations were conducted for 1 h at 37°C in a shaker bath using 0.5 ml washed erythrocytes and 0.5 ml test compound (4×10^{-4} M) dissolved in 0.04 M glucose, 0.01 M phosphate buffer (pH 7.4). After incubation, 0.1 ml erythrocyte suspension was hemolyzed

Table 3

Cell toxicity of primaquine diphosphate and oxidation products **3**, **4** and **11**

Compound	1	5	10	20	50	100
	($mg \cdot l^{-1}$)					
Primaquine	HT	HT	HT			
3	0	0	V	HT		
4	0	0	0	0	HT	
11	0	0	0	0	NT	

HT, high toxicity; 0, no toxicity; V, dead cells but intracytoplasmic vacuoles; NT, not tested

Table 4

Effect of primaquine analogs 3, 4 and 11 on the in vitro formation of methemoglobin in erythrocyte suspensions

Compound		% methemoglobin[a]
	Control	0.49 ± 0.43[b]
3	2×10^{-4} M	6.45 ± 1.18
4	2×10^{-4} M	3.51 ± 1.07
11	2×10^{-4} M	3.14 ± 0.90

[a] Methemoglobin as % of total hemoglobins
[b] SD of 3 incubations

in 5.0 ml water, 5.0 ml of 0.05 M collidine buffer (pH 7.0) was added, and the methemoglobin was determined spectrophotometrically [11].

3. DISCUSSION

Compound 3 was found to be both more active and more cytotoxic than the other two compounds, with vacuolation of hepatocytes appearing at 10 $mg \cdot l^{-1}$ and dead cells at 20 $mg \cdot l^{-1}$. Compound 4 was not tested at concentrations between 20 and 50 $mg \cdot l^{-1}$ and quinone 11 at concentrations between 20 and 100 $mg \cdot l^{-1}$ respectively. At 20 $mg \cdot l^{-1}$ the hepatocyte monolayers were intact in 4 and 11, producing dead cells with 4 at 50 $mg \cdot l^{-1}$, and 100 $mg \cdot l^{-1}$ for 11 respectively (fig.3).

It is interesting to note that at ID_{90} concentrations the remaining schizonts were different in cultures treated with 3 and 4 from those treated with primaquine diphosphate. Treatment with primaquine gave schizonts which had the same seize and morphology as control cultures, whereas 3 and 4 produced schizonts which were either trophozoites or three and four nucleated schizonts, indistinguishable from very young and normal (18 hold) parasites.

The ability of 4 to promote the formation of methemoglobin in an in vitro erythrocyte model system was found to be similar to that previously described [6]. The present study showed that 3, 4, and 11 were all fairly active in the erythrocyte system, an in vitro model in which primaquine does not show activity [6]. The dimeric compound 3, which has also been shown to block ATP production by inhibition of mitochondria F_0F_1-ATPase [12], was found to be the most active in promoting the formation of methemoglobin in the present study. It would appear that the intact and possibly protonated amine side chain is not needed to express activity in the in vitro erythrocyte model or in the F_0F_1-ATPase model, but the amino group seems to be necessary for hepatocyte toxicity or antimalarial activity.

Formation of 4 from primaquine and 5-hydroxydemethylprimaquine (5) requires oxygen and light, and is greatly accelerated if one starts with 5. This suggests that primaquine is first hydroxylated at C(5) and then demethylated at

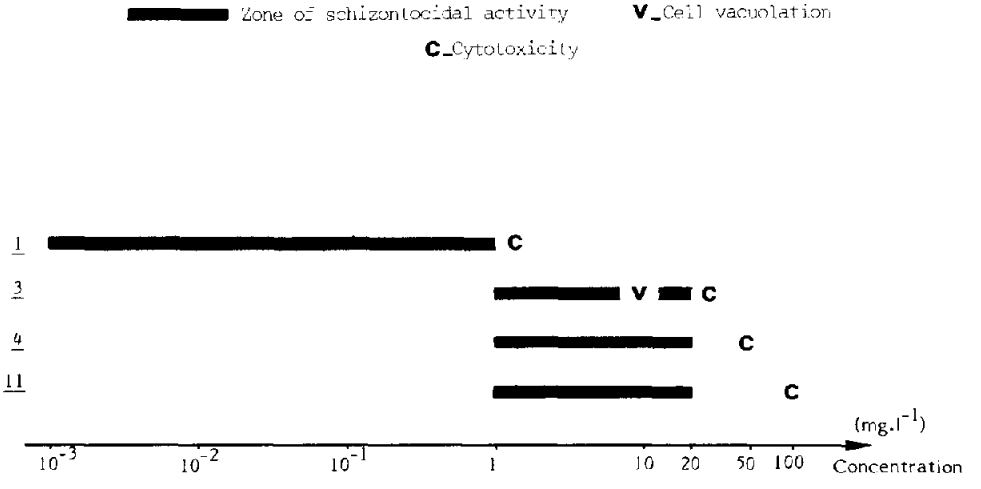

Fig.3. Zones of activity and cytotoxicity of three primaquine derivatives.

C(6), or vice versa, before compound **4** is formed. Blue dye **4** is strongly hydrogen bonded (−NH....O=) with the blue color disappearing on *O*-methylation or *N*-acetylation, and its structure is somewhat reminescent to that of 'indanthrone'. Photooxidation of *N*-acylated primaquines provides a simple entry into quinoline-5,6-diones, so far difficult to obtain.

4. CONCLUSIONS

It would appear that the types of structures seen in **4** and **11** or in the dimeric structure **3** are not associated with the schizontocidal activity of primaquine given the low activity of the three model compounds compared to primaquine. Considering the low schizontocidal activity of *N*-acylated primaquine (ID_{50} = 3 mg/l [5]), however, the lack of an amide side chain in **3**, **4**, and **11** may preclude observation of antimalarial activity.

The toxicity of primaquine towards hepatocytes was found to be fairly high while those of **3**, **4**, and **11** were fairly low and comparable to that previously reported for *N*-acylated primaquine [7]. Thus, it would appear that quinone or dimeric heterocyclic structures alone are not sufficient for the expression of hepatocyte toxicity and antimalarial activity.

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