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Antimalarial activity and inhibition of monoamine oxidases A and B by exo-erythrocytic antimalarials

Optical isomers of primaquine, *N*-acylated congeners, primaquine metabolites and 5-phenoxy-substituted analogues

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When the terminal amino group in the side chain of primaquine was blocked with an ethoxyacetyl group shown in **2**, or eliminated by oxidative deamination to carboxylic acid **3**, the antimalarial effect was markedly reduced in a screening assay which measures tissue schizonticidal activity. The optical isomers **1A** and **1B** of primaquine had similar antimalarial potency to the racemic mixture but **1B** appeared less toxic. The 5-phenoxy-substituted analogue **4**, belonging to a new class of antimalarials, showed similar potency in the assays to either **1A** or **1B** but seemed less cytotoxic than (±)-primaquine. Compounds **1A** and **1B** were found to be competitive inhibitors of human monoamine oxidase (MAO) A and B (K_i range 103–225 μM), but **4** showed 10–30-fold greater competitive inhibition of MAO A ($K_i = 6.8$ μM) and 40–90-fold greater non-competitive inhibition of MAO B ($K_i = 2.3$ μM).

Monoamine oxidase; Primaquine; Antimalarial activity; Enzyme inhibition

1. INTRODUCTION

(±)-Primaquine (**1**), an 8-aminoquinoline, is presently the only tissue schizontocide available for the radical treatment of *Plasmodium vivax* and *P. ovale* infections. Its usefulness is offset by inherent toxicity, resulting from methaemoglobinaemia and haemolysis in patients with glucose 6-phosphate deficiency. Recent research on primaquine conducted by Chemal [1] led to the discovery of two metabolic pathways, one affecting the aromatic ring A, the other the 8-*N*-aminobutyl chain of primaquine. The second pathway, originally discovered in bacteria [2], resulted in the formation of *N*-acetylprimaquine and a desaminocarboxylic acid **2**, later found to represent a major metabolite of primaquine in rat, hamster and monkey [3], as well as humans [4,5].

The testing of tissue schizontocidal activity of antimalarials has been greatly facilitated by a novel in vitro assay, evaluating exo-erythrocytic schizontocides in primary cultures of hepatocytes [6]. Since little is known regarding the mechanism of action of primaquine and its toxicity, we decided to evaluate in vitro schizontocidal activity and cytotoxicity of several key compounds chemically related to primaquine by the new assay method. The conversion of primaquine into metabolite **3**, could in principle be achieved through oxidative deamination by monoamine oxidases A and B (MAO A and B). Therefore, these compounds

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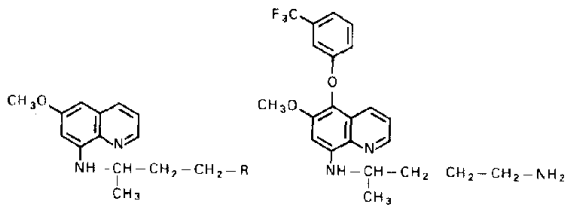

1A R = CH_2-NH_2, (+)-Primaquine
1B R = CH_2-NH_2, (−)-Primaquine
2 R = $CH_2-NH-COCH_2OCH_2CH_3$
3 R = COOH

4

Fig.1. Compounds 2, 3 and 4 are racemic mixtures. Compounds **1A** and **1B** were assayed as diphosphate salts, compound 4 as citrate salt and compound 2 as a monophosphate salt.

were also tested as inhibitors of highly purified human placental MAO A [7] and human liver MAO B [8] as an initial step in assaying the interaction of primaquines with these amine-degrading enzymes.

Chosen for this study were (+)- and (−)-primaquine (**1A** and **1B**), the two optical isomers of primaquine of still unknown configuration [9], (±)-*N*-ethoxyacetylprimaquine **2** [10], an *N*-acylated primaquine resembling the bacterial metabolite (±)-*N*-acetylprimaquine [2], (±)-carboxy-metabolite **3** obtained by total synthesis [11], and (±)-5-(*m*-trifluorophenoxy)primaquine (**4**), a representative of a superior class of primaquine-related antimalarials [12].

2. MATERIALS AND METHODS

(+)- and (−)-primaquine diphosphate (**1A**·$2H_3PO_4$ and **1B**·$2H_3PO_4$) and (±)-5-(*m*-trifluoromethylphenoxy)primaquine citrate (**4**·$C_6H_8O_7$) were obtained from Dr D. Klayman, Walter Reed Institute of Research, Washington, DC. (±)-Desaminocarboxyprimaquine (**3**) was obtained from Dr J. McChesney, School of Pharmacy, University of Mississippi, University, MS, and (±)-*N*-ethoxyacetylprimaquine (**2**) was prepared as in [10].

The strain used in the assay was *P. yoelii* 265 BY, isolated from *Thamnomys rutilans* captured in the Central African Republic in 1969. The vector *Anopheles stephensi* originated from a colony established by Shute and Maryon [13] in England. Cell cultures were primary cultures of laboratory-bred *T. gazellae* hepatocytes, cultivated as discs of 5 mm diameter [6,14]. Tests were performed on petri dishes measuring 35 mm in diameter each, with two discs of hepatocytes. The compounds were dissolved in 1 ml MEM culture medium (Gibco) complemented with $NaHCO_3$ (2.2 mg/ml), bovine albumin (2 mg/ml), insulin (10 mg/ml), fetal calf serum (10%), penicillin (200 IU/ml), streptomycin (0.2 mg/ml) and hydrocortisone (3.5×10^{-5} M), added to cultures 2 h after the addition of sporozoites, and solutions renewed after 24 h. Each drug concentration was tested in 3 petri dishes (6 discs), 3 infected petri dishes kept as untreated controls and 3 others treated with primaquine diphosphate at 0.1 mg/l (LD_{90}) and cultures fixed with methanol after 48 h [6,14]. At that time the size of the schizonts varying between 20 and 40 μm parasites were counted with a 20 × objective. ID_{50} and ID_{90} values were calculated by the graphic method of Trevan [15]. Dead cells were counted after vital staining with trypan blue. Compounds also were tested as inhibitors of highly purified human liver MAO B [8] and human placental MAO A [7] using kynuramine as a substrate.

Table 1

In vitro schizontocidal activity of compounds related to primaquine

Compound	Schizontocidal activity	
	ID_{50}	ID_{90}
1	0.03	0.1
1A	0.03	0.1
1B	0.2	0.8
4	0.2	0.8
3	30	50
2	3	40

3. RESULTS

The results are presented in the form of zones of activity and cytotoxicity. (+)-Primaquine (**1A**) is about as active as (±)-primaquine (**1**) and only slightly less toxic. (−)-Primaquine (**1B**), however, is probably both less active and less toxic. There does not seem to be any important separation of

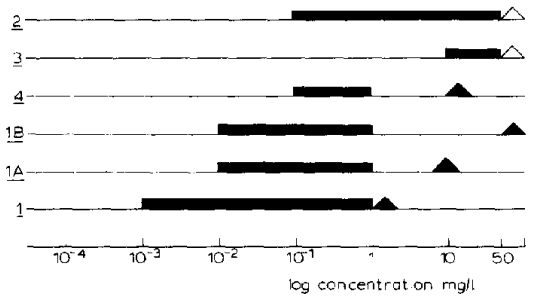

Fig.2. Zones of schizontocidal activity and cytotoxicity of primaquine diphosphate and 5 relatives of primaquine. (▬) Zone of schizontocidal activity; (▲) zone of cell toxicity; (△) cell vacuolation.

toxicity and activity for the two isomers. Compounds **2** and **3** were only active at 50 mg/l. The cytotoxicity of the compounds tested was different: **2** and **3** were cytotoxic near the dose giving full schizontocidal activity, whereas **4** was cytotoxic at doses which were much higher than that required for schizontocidal activity. Table 3 summarizes K_i values and the type of inhibition of compounds against MAO A and MAO B. All compounds tested were found to be competitive inhibitors of MAO A with **4** being the most potent (K_i = 6.8 μM). These analogues demonstrated similar activity against MAO B with the exception of **4**, which gave non-competitive inhibition (K_i = 2.3 μM).

Table 2

In vitro cell toxicity of 5 compounds related to primaquine

Compound	Cell toxicity					
	10^{-3}	10^{-2}	10^{-1}	1	10	50
1		0	0	HT		
1A	0	0	0	V	HT	
1B			0	0	0	HT
4		0	0	0	HT	HT
3				0	0	V
2				0	0	V

0, no toxicity; ST, slight toxicity (few dead cells at the periphery of the disc of hepatocytes); HT, high toxicity (all cells dead); V, no dead cells, but numerous intracytoplasmic vacuoles

Table 3

MAO A and B inhibition by primaquines

Compound	Type of inhibition	
	MAO A	MAO B
1A	competitive (225 μM)	competitive (193 μM)
1B	competitive (125 μM)	competitive (103 μM)
4	competitive (6.8 μM)	non-competitive (2.3 μM)

Human liver MAO B and human placental MAO A were assayed with 6 different concentrations of kynuramine (40–400 μM) in the presence and absence of the primaquines to be tested. Values for $K_{m,app}$ and $V_{max,app}$ were obtained by linear regression analysis of duplicate determinations plotted as $1/V$ vs $1/[S]$. Concentrations of inhibitors ranged from 0 to $5K_i$. Initial reaction rates were obtained by monitoring the increase in absorbance at 314 nm (4-hydroxyquinoline, E_M = 12300 M^{-1} cm^{-1}) using a Beckman DU-7U spectrometer. Reactions were carried out in 0.5 ml of 25 mM sodium phosphate buffer (pH 7.6) at 30°C and started by the addition of substrate

4. DISCUSSION

Compounds **1A**, **1B** and **4** displayed similar activity in vitro to (±)-primaquine tested as the diphosphate salt. Both, **1B** and **4** appeared to be less cytotoxic than (±)-primaquine, with **1B** being the least toxic of the compounds tested. The active concentrations of **2** and **3** were substantially higher than those of (±)-primaquine. The fact that blocking of the primary amino group with an ethoxyacetyl group or its elimination by simultaneous oxidation of the terminal methylene group to a carboxy group was accompanied by a complete loss of antimalarial activity is noteworthy, indicating that the terminal primary amino group in the side chain of 8-aminoquinoline antimalarials is an essential structural feature. The finding of similar and relatively moderate inhibition of MAO A and B in vitro by **1A** and **1B** (K_i ranges 103–225 μM), enhanced 10–90-fold with **4**, does not strongly support the concept that MAO A and B could catalyze their deamination to **3** in vivo at drug concentrations that give schizontocidal activity. Comparison of the optical isomers **1A** and **1B** of

primaquine, favouring here the (−) isomer **1B** as the less toxic compound, is offset by earlier reports showing that the (+) isomer **1A** had a better therapeutic index in rhesus monkeys [16], with both **1A** and **1B** similarly inhibiting drug metabolism [17]. In view of the appearance of new and much improved antimalarials for radical cure and clearing of tissue parasites, such as **4** [12] or its 4-methyl-substituted analogues [18], further development of either (−)-primaquine (**1B**) or its (+) isomer **1A** does in our opinion not seem warranted. However, the question regarding differences in activity and toxicity of optical isomers of the new generation of analogues should be pursued.

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