Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1487–1492

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

8-AMINOQUINOLINES AS ANTICOCCIDIALS - II

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Received 10 February 1998; accepted 5 May 1998

Abstract : During a chemistry program aimed at finding a novel analogue of pentaquine with improved *in vivo* activity, a number of hypotheses concerning the way this drug acts in the chicken were investigated. Consideration of the products of monoamine oxidase metabolism of pentaquine suggested that pentaquine aldehyde is the likely active metabolite. Although isolation of this unstable compound was not possible, oxime and cyclic acetal and ketal derivatives were obtained and shown to possess *in vitro* anticoccidial activity
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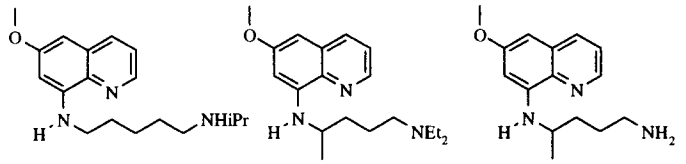
Keywords: *Antiparasitics*

1. Introduction The 8-aminoquinoline antimalarials, primaquine and pamaquine have been reported to possess *in vivo* anticoccidial activity in broilers against the protozoan parasites *Eimeria tenella* and *Eimeria necatrix*[1]. In the first paper of this series[3] we reported *in vivo* studies indicating that pentaquine had improved potency over primaquine and pamaquine in a standard 7 day chick model[2] with activity seen at 12.5 ppm in feed against *Eimeria tenella*. This paper describes how work aimed at understanding the metabolism of 8-aminoquinolines aided our drug discovery program



Pentaquine **Pamaquine** **Primaquine**

2. Results and Discussion There is considerable evidence in the literature that primaquine is a pro-drug[4], i.e. that it requires metabolic activation to show its antimalarial effect. We demonstrated this to be the case for anticoccidial activity by testing primaquine, pamaquine and pentaquine *in vitro* against *E. tenella*. Two assays were used: a) a Madin-Darby bovine kidney cell assay[5], b) a primary chick hepatocyte assay[6]. The latter possessed metabolic capacity, the former did not. We validated this system using the known anticoccidial pro-drug, arprinocid[7] which showed activity in the latter but not the former and is known to require metabolic activation. Pentaquine and primaquine showed the same profile, strongly suggesting metabolic activation. The metabolism of the 8-aminoquinoline antimalarials has been widely studied[8], key studies relating to chick

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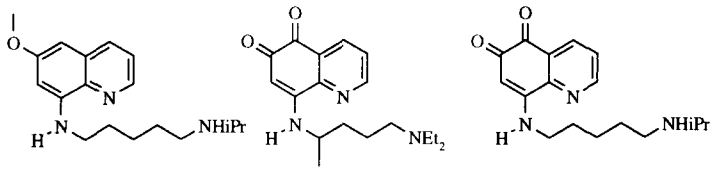
PII: S0960-894X(98)00243-1

metabolism of 8-aminoquinolines reported that the quinone metabolite, **2**, was isolated from chicken faeces when they were treated with pamaquine in feed[9]. By analogy we hypothesised that oxidative metabolism of pentaquine would give the corresponding quinone metabolite, **3.**

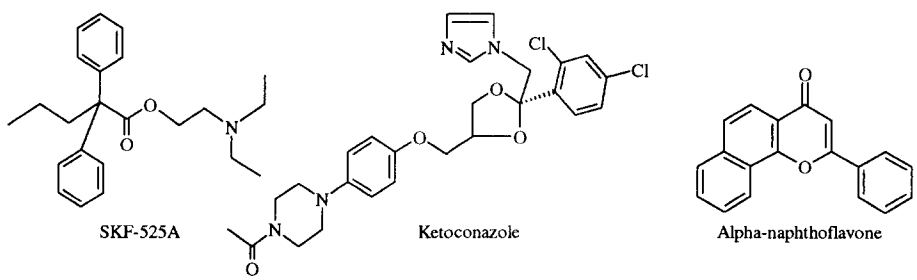


1 **2** **3**

Synthesis of **3** followed by biological testing showed it to be inactive both *in vitro* and *in vivo*[3]. Thus we turned our attention to metabolism of the side-chain attached at the 8-position. A likely metabolic route would involve oxidative deamination, this could be catalysed by either cytochrome P-450 linked oxidases or monoamine oxidase[10]. In order to distinguish between the two we investigated co-administration of an enzyme inhibitor with pentaquine in an *in vitro* anticoccidial assay since if the enzyme were important in metabolic activation, then its inhibition should lead to loss of anticoccidial activity. The results of co-administration of cytochrome P-450 inhibitors is shown in Table 1. (IC_{50} refers to the concentration in ppm of pentaquine required to reduce the number of second generation merozoites that developed by 50%).

Table 1

Inhibitor of cytochrome P-450 linked oxidase	**IC_{50} (n=2) vs. *E.tenella* of pentaquine in ppm (chick hepatocyte assay)**	
	+ Inhibitor 5μg/ml	No Inhibitor
α-naphthoflavone[11]	0.2-1	0.2-1
ketoconazole[12]	0.2-1	0.2-1
SKF525A[13]	0.2-1	0.2-1

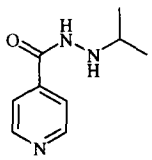


SKF-525A Ketoconazole Alpha-naphthoflavone

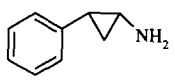
None of the inhibitors had any effect on pentaquine potency indicating that cytochrome P-450 linked oxidases are not important in the metabolic activation of pentaquine. In contrast several inhibitors of monoamine oxidase reduced the activity of pentaquine as shown in Table 2. For comparison, the effect of MAO inhibition on several commercial anticoccidial agents which are not pro-drugs is shown, as expected there was no change in anticoccidial potency of these compounds when either inhibitor was added. (IC_{50} refers to the concentration in ppm of drug required to reduce the number of second generation merozoites that developed by 50%).

Table 2

Compound	IC_{50} (n=2) vs. *E.tenella* in ppm (chick hepatocyte assay)		
	No inhibitor	+Tranylcypromine[14]	+Iproniazid[15]
Pentaquine	0.2-1	1-5	1-5
Salinomycin	0.08-0.016	0.08-0.016	0.08-0.016
Decoquinate	0.004-0.0008	0.004-0.0008	0.004-0.0008
Diclazuril	0.004-0.0008	0.004-0.0008	0.004-0.0008



Iproniazid

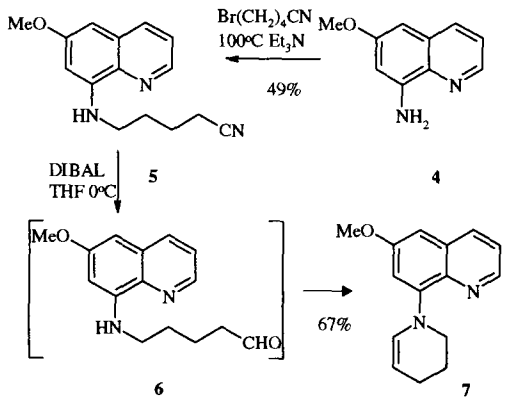


Tranylcypromine

Having identified the type of enzyme involved in the activation we could now look at the products of metabolism, i.e. what is the nature of the active metabolite? The general catalytic reaction of MAO is shown below:

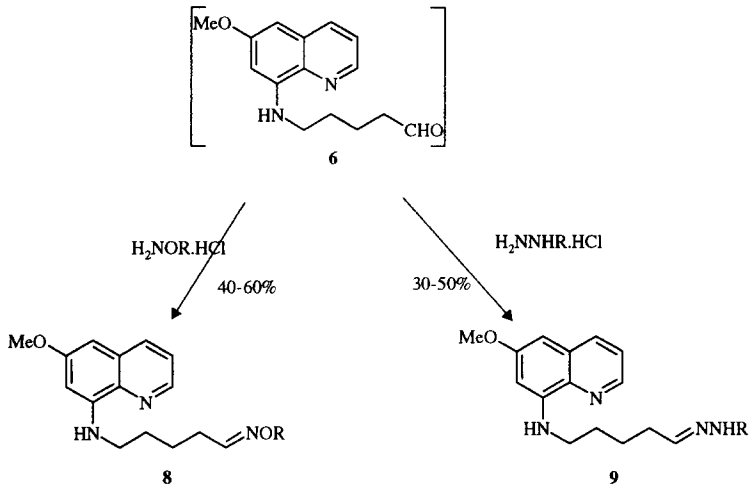
$$RCH_2NR_1R_2+O_2+H_2O \rightarrow RCHO+H_2O_2+HNR_1R_2$$

One possibility is that hydrogen peroxide, produced as a by-product of amine oxidation is responsible for killing the parasites[16]. Alternatively, since the initial product of MAO metabolism is an aldehyde and there is evidence that primaquine aldehyde is the active metabolite of primaquine[4], we surmised that pentaquine aldehyde could be the active metabolite of pentaquine and we set about synthesising it.



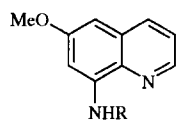
Alkylation of the amine **4** proceeded smoothly[17] however, following reduction of **5** with diisobutyl aluminium hydride (DIBAL) only the cyclised material **7** could be isolated, interestingly this retained activity *in vitro*. Conversely the fully saturated version of **7**, produced by alkylation of **4** with 1,5-dibromopentane, was inactive. Presumably the aldehyde **6** is unstable to the acidic conditions of the work-up. To investigate this we decided to trap the aldehyde as a more stable derivative e.g. an oxime or a hydrazone. This was achieved by adding a large

excess of the appropriate hydroxylamine or hydrazine directly to the reaction mixture following reduction of the nitrile with DIBAL. Interestingly adding isopropylamine to this reaction led to production of pentaquine **1**, presumably by formation of the corresponding imine followed by reduction by excess DIBAL.



A range of oximes **8** and hydrazones **9** were produced. The latter were inactive in our *in vitro* anticoccidial assay but the former showed levels of activity similar to pentaquine itself. All the oximes were active except for the parent **10**, a carboxylic-acid containing oxime **17**, and the nitrobenzyl derivative **18**. It is possible that the first two were too polar to penetrate the hepatocyte cells and reach the parasites. The results are shown in Table 3. (IC_{50} refers to the concentration in ppm of drug required to reduce the number of second generation merozoites that developed by 50%).

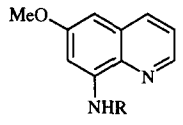
Table 3



10-18

Number	R	IC_{50} (n=2) vs. *E.tenella* in ppm (chick hepatocyte assay)
10	$(CH_2)_4CH{=}NOH$	>1
11	$(CH_2)_4CH{=}NOMe$	0.2-1
12	$(CH_2)_4CH{=}NOEt$	0.2-1
13	$(CH_2)_4CH{=}NOtBu$	0.2-1
14	$(CH_2)_4CH{=}NOPh$	0.2-1
15	$(CH_2)_4CH{=}NOBz$	0.2-1

Table 3 continued

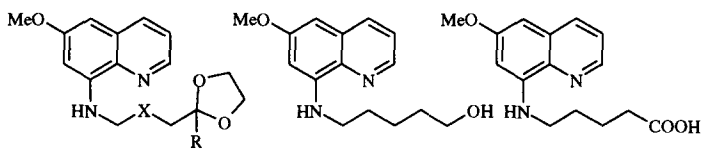


10-18

Number	R	IC_{50} (n=2) vs. *E.tenella* in ppm (chick hepatocyte assay)
16	$(CH_2)_4CH{=}NOallyl$	0.2-1
17	$(CH_2)_4CH{=}NOCH_2COOH$	>1
18	$(CH_2)_4CH{=}NOCH_2C_6H_4NO_2$	>1

An alternative way of producing a masked aldehyde was achieved by using the appropriate cyclic acetal or ketal. These were prepared by the alkylation of **4** using a suitable bromo derivative and a number showed activity in the *in vitro* assay. Interestingly, the methyl ketal **21**, was inactive, unlike the more electrophilic 4-fluorophenyl ketal **22**, suggesting that reactivity towards nucleophiles of the aldehyde or ketone formed is important in producing an anticoccidial effect. The results are shown in Table 4.

Table 4



19-22 **23** **24**

Number	X	R	IC50 vs. *E.tenella* in ppm (chick hepatocyte assay)
19	CH_2CH_2	H	0.2-1
20	CH_2	H	>1
21	CH_2	Me	>1
22	CH_2	4-F-Ph	0.2-1
23	n/a	n/a	>1
24	n/a	n/a	>1

3. Conclusion The results of *in vitro* testing of the 8-aminoquinolines in assays which possessed or lacked metabolic capability indicated that metabolic activation is required for them to demonstrate their activity i.e. that they are pro-drugs. The use of inhibitors of the two major classes of oxidative enzymes likely to be involved in metabolism showed that monoamine oxidase is the key enzyme involved. Consideration of the products of MAO metabolism of pentaquine suggested that pentaquine aldehyde, **6**, is the likely active metabolite. Although isolation of this unstable compound was not possible, enamine, oxime, cyclic acetal and ketal derivatives were obtained and shown to possess *in vitro* anticoccidial activity at a similar level to pentaquine itself. A likely

explanation for this is that these derivatives are themselves pro-drugs and release the active aldehyde or a common active metabolite under the conditions of the assay.

4. Acknowledgement : We would like to thank our colleagues in Pfizer Animal Health Discovery Biology for their useful discussions and for providing the *in vitro* screening of these compounds.

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