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8-AMINOQUINOLINES AS ANTICOCCIDIALS - PART III

Richard E. Armer, Jacqueline S. Barlow, Narinder Chopra, Christopher J. Dutton*, David H. J. Greenway, Sean D.W. Greenwood, Nita Lad, Jonothan Shaw, Adrian P. Thompson, Kam-Wah Thong and Ivan Tommasini.

Animal Health Discovery, Pfizer Central Research, Ramsgate Road, Sandwich, Kent, CT13 9NJ, U.K.

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Abstract :

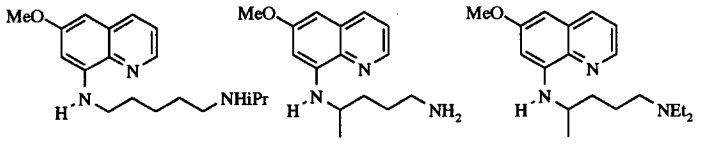
Analogues of the antimalarial pentaquine, **1**, in which the nature of the side-chain on the 8-amino position was varied, were prepared and evaluated for anticoccidial activity both *in vitro* and *in vivo*. Specifically, both the inter-nitrogen distance and the nature of the terminal amino group were investigated. Novel analogues of equal or improved efficacy *in vitro* and *in vivo* to pentaquine were discovered.

Introduction

Coccidiosis is a devastating disease which causes severe economic losses in the poultry industry. Indeed, commercial poultry farming would not be possible without the use of effective anticoccidial agents such as Aviax™ and Coxistac™. Existing anticoccidials are becoming less effective as resistance develops to many of the agents in commercial use. Hence, there is a need to discover and develop new classes of coccidiostats which are not cross-resistant with existing agents and this is a significant challenge to the Animal Health industry. We have reported the anticoccidial activity of the antimalarial agent pentaquine, **1**, in a 7 day chick model of coccidiosis,[1] as well as studies aimed at elucidating the active species *in vivo*.[2] Although the nature of the intracellular target is not known, pentaquine aldehyde is thought to be the active species.[3] We now present the results of studies in which the structure-activity relationships within the 8-(aminoalkylamino) side-chain are explored. These may be related to the affinity of the compounds for monoamine oxidase.

Results and Discussion

With the discovery of the anticoccidial activity of pentaquine, **1**,[1] the preparation of a range of analogues, in which the side-chain inter-nitrogen distance and terminal amino group substitution was varied, was undertaken. Differences in the anticoccidial activity of pentaquine, primaquine, **2**, and pamaquine, **3**, in this model gave encouragement to further explore the side-chain SAR.



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* E-mail: christopher_dutton@Pfizer.sandwich.com

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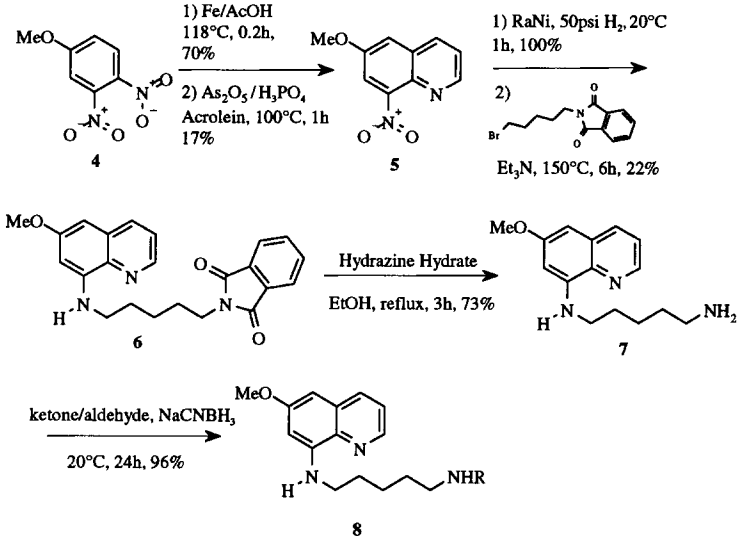
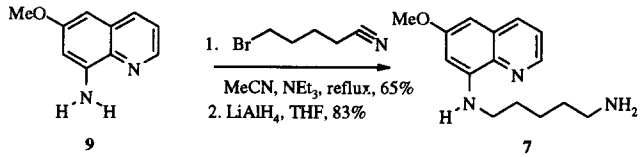
The syntheses of these analogues were carried out as shown below (Scheme 1). Reduction of one of the nitro groups in 1,2-dinitro-4-methoxy benzene **4** followed by a Skraup quinoline synthesis gave the 8-nitro quinoline **5**. Hydrogenation of **5** yielded the corresponding 8-amino quinoline which was then alkylated with n-pentylbromophthalimide to give **6**. Reaction with n-ethyl-, n-propyl-, n-butyl- and n-pentylbromophthalimide (shown) was used to access the C2 to C5 side-chain homologues. Deprotection of **6** with hydrazine hydrate[4] gave the primary amine **7** which was reductively aminated[5] with the appropriate ketone or aldehyde to give **8** (Scheme 1).



Scheme 1. Synthesis of pentaquine analogues

The side-chain was also prepared *via* reaction of the 8-aminoquinoline **9** (derived from Raney nickel reduction of 8-nitroquinoline, **5**) with the corresponding 1-bromo-(n-alkylnitrile),[6] followed by lithium aluminium hydride reduction to the primary amine, e.g. **7** in the case of 1-bromo-4-cyanobutane (Scheme 2).



Scheme 2. Alternative side-chain preparation.

Firstly, we decided to investigate the inter-nitrogen spacer distance of the 8-(aminoalkylamino) side chain. Thus, a range of C-2 to C-5 analogues, in which the distal nitrogen substituent was *i*-propyl (as in

pentaquine) were prepared. These were evaluated both *in vitro* (chicken hepatocyte *Eimeria tenella* and *Eimeria acervulina* ELISA assay)[7] and *in vivo* (chick 7-day coccidiosis model).[1] The results are shown in Table 1.

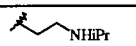
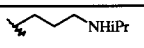
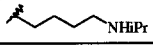
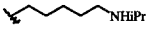
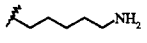
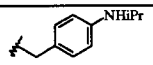
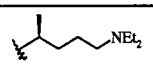
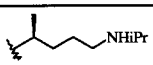
		In vitro (IC_{50} μg/ml)		*In vivo* (ED_{95} ppm in feed)	
Entry	Side chain	*E. tenella*	*E. acervulina*	*E. tenella*	*E. acervulina*
10		>1	1-5	>25	>25
11		0.2-0.04	0.2-0.04	>25	>25
12		0.2-0.04	0.2-0.04	6.25-12.5	6.25-25
1		0.2-0.04	0.2-0.04	12.5	12.5-25
7		>1	1-5	100	100
14		>1	1-5	>>12.5	>>12.5
15	Diclazuril™	0.008-0.04	0.008-0.04	1	1
3		0.2-1	>1	>50	>50
16		>1	1-5	>50	>50

Table 1. Activity vs. inter-nitrogen spacer

There is a sharp drop in both *in vitro* and *in vivo* activity when the N-N spacer distance is ethyl, **10**, indicating a spatial requirement between the lipophilic aryl core and the terminal nitrogen. It could also indicate that both nitrogens, the 8-amino quinoline and the terminal 2° amine are involved in binding to the target active site, and that this cannot be achieved with the smaller N-N distance. C-3 to C-5 (**11**, **12** and **1**) show similar *in vitro* potencies, within the limits of the assay, although there are differences in the *in vivo* activities. Most notably, C-3 shows lower *in vivo* activity (>25ppm), with C-4 and C-5 being nearly equipotent. The reasons for this difference in C-3 *in vivo* activity, in spite of a near identical *in vitro* profile to C-4 and C-5, are not clear. Many factors, such as absorption, distribution and clearance, could be affecting the *in vivo* efficacy, thereby complicating the translation. That aside, the fact that the *in vivo* activity is lower for **11**, indicates that C-3 is poorer than C-4 and C-5, and this guided the synthesis of further analogues.

The primary amine version of pentaquine, **5** shows a marked loss of both *in vitro* and *in vivo* activity, indicating the need for a lipophilic group on nitrogen. This is also the case for the butylamino sidechain analogue (data not shown). **14** in Table 1 shows the effect of a more rigid, bulkier group on activity. The benzylic spacer, with iso-propyl as the nitrogen substituent, is approximately equal to a C-5 alkyl spacer in terms of inter-nitrogen distance, yet activity is sharply diminished. This may indicate a conformational and/or

steric difference, with the phenyl ring directing the terminal nitrogen into a different area of the receptor/active site. Also, the basicity of the terminal nitrogen is greatly reduced (est. pK_a 5) compared to pentaquine (est. pK_a 10.5), and thus could affect binding if it were functioning as an H-bond acceptor. Also, the lower efficacy for the benzylic spacer may indicate an unfavourable steric interaction with the receptor/active site, giving us some information on the nature of the interaction in this region. This is supported by the unfavourable effect of introducing α-branching into the C-4 side chain (**16** *vs* **12**). Activity is greatly diminished both *in vitro* and *in vivo*, indicating either a steric or a conformationally poor interaction by introducing a methyl group.

Having established that an n-alkyl, C-4/C-5 inter-nitrogen distance was optimal for both *in vitro* and *in vivo* activity, we then prepared a range of C-5 analogues in which the nature of the terminal nitrogen substituent was varied. These were prepared to probe lipophilicity, nitrogen basicity and steric requirements for activity. The results are shown in Table 2. **1** is pentaquine.

		In vitro (IC_{50} μg/ml)		*In vivo* (ED_{50} gms in feed)	
Entry	N-substituent	*E. tenella*	*E. acervulina*	*E. tenella*	*E. acervulina*
17	ethyl	0.2-0.04	0.2-1	6.25-50	>50
1	*iso*-propyl	0.2-0.04	0.2-0.04	12.5	12.5-25
18		0.2-0.04	0.2-0.04	>>12.5	>>12.5
19		0.2-0.04	0.2-1	6.25-25	>25
20		0.2-1	0.2-1	>25	>25

Table 2. Activity vs. Nitrogen substituent

Entry	N-substituent	*E. tenella*	*E. acervulina*	*E. tenella*	*E. acervulina*
21		0.2-1	0.2-1	>25	>25
22		0.2-1	1-5	12.5-25	12.5-50
23		0.2-0.04	0.2-0.04	6.25	12.5
24		0.2-1	1-5	>50	>50
25		>1	>5	>25	>25

Table 2. Activity vs. Nitrogen substituent

It is clear from Table 2 that only one novel analogue **23** matches the potency of pentaquine **1** both *in vitro* and *in vivo* although compounds **17, 18,** and **19** have similar *in vitro* potency. Further increase in lipophilicity to cyclopentyl **20** reduces activity against all parasites. Benzyl **21** is no better, but there is a small improvement in *in vivo* potency with 2-tetrahydrofuranyl **22**, perhaps indicating that the oxygen is participating in a hydrogen bonding interaction.

Building on this, the 4-pyranyl **23** was prepared, and this was found to be as good, if not marginally better, than pentaquine **1**. This activity was lost in the thiopyranyl moiety **24** and it's S-oxide **25**, indicating that the oxygen is participating in an H-bonding interaction that neither the thiopyran or the S-oxide can. In fact, it is likely that **24** is being oxidised to the inactive S-oxide **25** both *in vitro* and *in vivo*. As there are a number of N-groups that give reasonable *in vitro* activity (**18, 19** and **23**), another explanation is that the pyran moiety is simply mimicking the steric environment that the *iso*-propyl group creates, and that the remainder of this group is in an insensitive area of the active site. Further analogues failed to shed light on this hypothesis.

Although the nature of the intracellular target is not known, pentaquine aldehyde is thought to be the active species.[3] Thus the *in vitro* SAR described above may be related to the affinity of the compounds for monoamine oxidase. There is considerable evidence for this: the poor activity of **7** could be explained by the fact that the related primary amine, primaquine **2**, is claimed to have only moderate affinity for human monoamine oxidase.[6] Similarly, the lack of potency of **10** could be due to the fact that the ethylene diamine unit is a common motif in inhibitors of monoamine oxidase.[8] Pentylamine has been shown to be a selective substrate of monoamine oxidase B[9] so it is perhaps not surprising that the C-5 side-chain provides the most potent analogues.

Conclusion

Variation of the inter-nitrogen spacer distance and nature has revealed an optimal C-5 distance, expressed as an n-pentyl group (as in pentaquine). Other analogues were less potent *in vitro* or *in vivo* or both. Also, an exploration of the terminal nitrogen substitution has revealed the tetrahydropyran group to be optimal, perhaps through contribution the of an additional H-bond acceptor in the form of the tetrahydropyran oxygen atom. These investigations have led to the discovery of a novel, pentaquine-like anticoccidial **23**, albeit closely related to the starting lead. The results are entirely consistent with the hypothesis that pentaquine aldehyde is the active metabolite and the *in vitro* SAR described above may be related to the affinity of the compounds for monoamine oxidase. Further pharmacological studies would be required to confirm this.

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References and Notes

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