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Hydroxylated *N*-alkyl-4-piperidinyl-2,3-diarylpyrrole derivatives as potent broad-spectrum anticoccidial agents

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Abstract—Diaryl-(4-piperidinyl)-pyrrole derivatives bearing hydroxylated *N*-alkyl substituents have been synthesized and evaluated as anticoccidial agents. High potency in Et-PKG inhibition and broad-spectrum anticoccidial activities have been observed on compounds, such as **4b** and **5h**, which are fully efficacious in vivo at 50 ppm in feed.

Coccidiosis, a parasitic disease of chickens, is the major cause of morbidity and mortality in the poultry industry worldwide and the economic losses can be devastating. All major poultry operations use anticoccidial agents prophylactically. Resistance to current coccidiostats is becoming widespread and new broad-spectrum drugs directed at novel biochemical targets are needed. Coccidiosis is caused by the invasion of protozoan parasites of the genus *Eimeria* into the avian intestinal lining.[1] It has been recently reported that inhibition of a novel cGMP-dependent protein kinase (PKG), isolated from these parasites, stops the parasite proliferation by blocking parasite invasion.[2,3] High throughput screening of known kinase inhibitors[4] and medicinal chemistry studies resulted in the discovery of *N*-alkyl-4-piperidinyl-2,3-diarylpyrroles as potent PKG inhibitors and broad-spectrum anticoccidial agents.[5] Herein, we report the synthesis, evaluation, and optimization of hydroxylated *N*-alkyl-4-piperidinyl-2,3-diarylpyrroles with improved PKG inhibition potencies and in vivo anticoccidial activities.

To evaluate these compounds as anticoccidial agents, an enzyme inhibition assay against the native *Eimeria tenella* PKG (Et-PKG) was used as the initial screen, and an in vivo anticoccidial assay was carried out to evaluate their in vivo efficacy in oocyst reduction against two major subtypes of protozoa parasites: *E. tenella* (*E.t.*) and *Eimeria acervulina* (*E.a.*). Details of these procedures and rules of scoring were published earlier.[5] Early SAR studies on lead compound **1** have established the fact that modification of either aromatic rings is not well-tolerated, usually resulting in a sharp decrease in both potency of Et-PKG inhibition and in vivo efficacy.[5] On the other hand, alkylation of the piperidine nitrogen (easily accomplished by treating piperidine **1** with sodium hydride and an alkyl halide in DMF at room temperature overnight) resulted in improvement of both Et-PKG inhibition and in vivo broad-spectrum activities,[5] especially for those containing a hydroxyl group (Table 1).[6]

Compared to their alkyl analogs,[5] hydroxylated derivatives **2a**–**d** are more potent PKG inhibitors. More importantly, all of these compounds have shown excellent in vivo activities in anticoccidial assay against both *E.t.* and *E.a.*. Acylation (**2e**) or elimination (**2f**) of the hydroxyl group resulted in a decrease in potency of PKG inhibition and showed no activity in anticoccidial assay at 100 ppm in feed. It has been demonstrated that inhibition of PKG stops the life cycle of these parasites.[2,3] However, in vivo efficacy after oral dosing in feed is also dependent on bioavailability and tissue dis-

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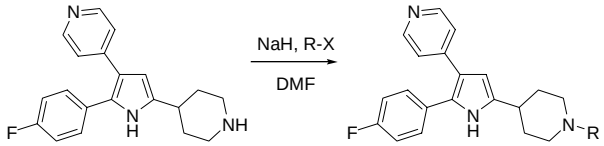
Table 1. Et-PKG inhibition and anticoccidial activities of diaryl-(4-piperidinyl)-pyrrole derivatives **2**



1 **2a-f**

Compounds	R	Et-PKG inhibition IC_{50} (nM[a])	Anticoccidial Activity at 100 ppm in feed[5]	
			E.t.	*E.a.*
2a	$(CH_2)_2OH$	0.71	3	3
2b	$(CH_2)_3OH$	0.53	2	3
2c	$(CH_2)_4OH$	1.34	3	3
2d	$(CH_2)_5OH$	1.46	3	3
2e	$(CH_2)_2OAc$	2.08	0	0
2f	$(CH_2)_2CH_3$	4.2	0	0

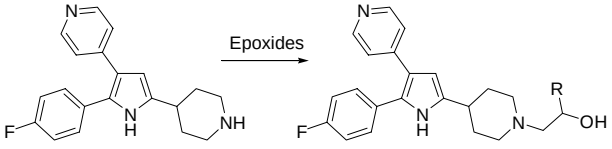
[a] Values are means of three experiments. The *Z* score for the assay is typically 0.9 and the same for data in other tables. For details, see Ref. 5.

tribution of these compounds in chicken, and/or their ability to penetrate into the parasites. This may explain the disproportional decline of in vivo activity among this class of anticoccidial agents. Based on these results, a more detailed evaluation of β-hydroxy derivatives was carried out. Reaction of **1** with various epoxides in refluxing methanol overnight yielded, after silica gel (column or preparative thin layer) chromatography, the desired β-hydroxy derivatives **3a**–**g**. Their anticoccidial activity evaluation is given in Table 2.

Racemic epoxides were used in most cases since cost-control is a pressing issue in anticoccidial research and development, due to the very low cost and low profit margin of the poultry industry.[7,8] However, when enantiomerically pure propylene oxides were used (**3a**–**b**), very little differences in both in vitro and in vivo activities were observed. Thus, no further efforts were directed at separating enantiomers and diastereomers unless pure forms were commercially available. Similar to the ω-hydroxy derivative **2a**–**d**, these β-hydroxy derivatives are highly potent Et-PKG inhibitors. Their in vivo activities decrease as the substituent gets larger and more lipophilic, although the effects on PKG inhibition are relatively small. These results are consistent with our working hypothesis that optimal size and/or lipophilicity are necessary for in vivo activity. In an effort to decrease the lipophilicity of the substituent, glycidyl ether derivatives (**4a**–**i**) were synthesized and evaluated (Table 3).

Consistent with our prediction that insertion of an oxygen atom into the alkyl chain would not improve PKG inhibition, but would have positive effects on the compounds' in vivo activities, both methyl glycidyl ether derivatives **4a**–**b** are sub-nanomolar inhibitors of PKG, and both have improved efficacious levels in feed. In the case of the (*S*)-isomer **4b**, complete oocyst reduction (80–100%) can be achieved at 50 ppm against both *E.t* and *E.a.*, slightly better than the (*R*)-isomer **4a**, which showed partial oocyst reduction (60–79%) against *E.t.* at the 50 ppm level. Comparison between **4a**–**b** and **3 d**, and also between **4c** and **3e** seems to suggest that lipophilicity has a larger effect on in vivo activity than steric bulk. These pairs of compounds have similar sizes

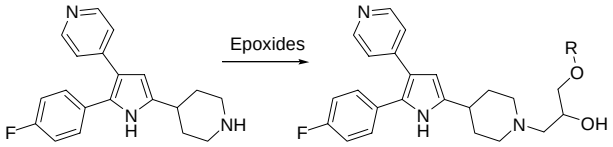
Table 2. Et-PKG inhibition and anticoccidial activities of diaryl-(4-piperidinyl)-pyrrole derivatives **3**



1 **3a-g**

Compounds	R	Et-PKG inhibition IC_{50} (nM)	Anticoccidial activity 100 ppm in feed[5]	
			E.t.	*E.a.*
2a	H	0.71	3	3
3a	(*R*)-CH_3	1.20	3	3
3b	(*S*)-CH_3	0.48	3	3
3c	CH_2CH_3	0.71	3	3
3d	$(CH_2)_2CH_3$	0.38	0	0
3e	$(CH_2)_3CH_3$	0.41	0	0
3f	$(CH_2)_5CH_3$	1.01	0	0
3g	Benzyl	1.28	0	3

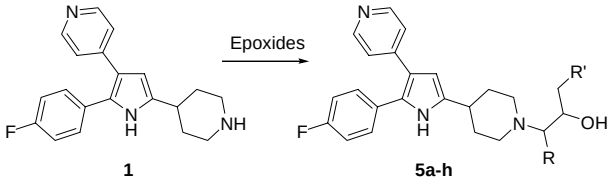
Table 3. Et-PKG inhibition and anticoccidial activities of diaryl-(4-piperidinyl)-pyrrole derivatives **4**



1 **4a-i**

Compounds	R	Et-PKG inhibition IC_{50} (nM)	Anticoccidial activity 100 ppm in feed[5]	
			E.t.	*E.a.*
4a	(*R*)-CH_3	0.69	3	3
4b	(*S*)-CH_3	0.56	3	3
4c	CH_2CH_3	0.59	2	3
4d	*iso*-Propyl	0.69	0	0
4e	*tert*-Butyl	0.98	0	0
4f	Allyl	0.80	0	0
4g	Phenyl	0.66	0	0
4h	Benzyl	0.47	0	0
4i	Furfuryl	0.82	0	0

Table 4. Et-PKG inhibition and anticoccidial activities of diaryl-(4-piperidinyl)-pyrrole derivatives **5**



1 **5a-h**

Compounds	R	R′	Et-PKG inhibition IC_{50} (nM)	Anticoccidial activity 100 ppm in feed[5]	
				E.t.	*E.a.*
5a	(*R*)-H	OH	0.51	3	0
5b	(*S*)-H	OH	0.71	3	3
5c	(*S*,*S*)-CH_2OH	H	0.82	3	3
5d	(*R*,*R*)-CH_2OH	H	1.01	3	3
5e	H	CH_2OH	1.27	3	0
5f	H	$CH(CH_3)OH$	0.40	3	3
5g	(*S*,*R*)-CH_3	H	0.86	3	3
5h	(*R*,*S*)-CH_3	H	0.94	3	3

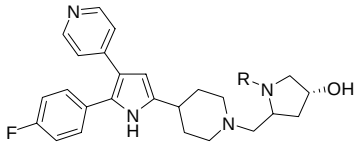
but somewhat different lipophilicity when the methylene group is replaced by an oxygen atom. As a result, their Et-PKG inhibitions are very similar. In both cases, however, glycidal ether derivatives showed better in vivo activities. Not surprisingly, a decline in in vivo efficacy was observed as the steric bulk and lipophilicity of the substituent further increased (**4d**–**i**).

Between these boundary conditions, we were able to synthesize additional hydroxyl group bearing derivatives in search for improved in vivo activities. By limiting the number of carbon atoms on the side chain to not more than four, we achieved a higher success rate in making active agents in vivo, although there was no noticeable improvement in Et-PKG inhibition (Table 4). This is consistent with the speculation that other factors, such as lipophilicity mentioned above, may be playing important roles in affecting bioavailability and/or parasite penetration, which are essential for in vivo efficacy. Quite interestingly, when a second hydroxyl group was added to further reduce its lipophilicity, we began to observe a decline in activities against *E.a.* as compounds (**5a** and **5e**) are only active against *E.t.*, although **5b** remains active against both species at 100 ppm. Since broad-spectrum activity is critical for the development of novel coccidiostats, these diols are not suitable for further development. It is worth mentioning that **5g** and **5h**, which are isomers of **3c** (not active at 50 ppm in feed), showed improved in vivo activities. Compound **5h** showed complete control of both *E.t.* and *E.a.* oocysts at the 50 ppm level in feed, whereas **5g** only controlled *E.t.* oocysts at 50 ppm.

Our data on in vitro Et-PKG inhibition suggest that this enzyme is not very discriminating with respect to the substituent on the piperidine nitrogen as long as its overall size is modest. Importantly, however, small changes, especially in polarity of the substituent, often resulted in changes in the compound's in vivo efficacy. Thus, it is conceivable that further improvement can be made by

modification of the aforementioned hydroxylated *N*-alkyl groups and fine-tuning their lipophilicity.

Based on early SAR studies, we realize that amino group can also improve the potency and anticoccidial activity of our lead.[5] To elaborate further on hydroxyl bearing substituents on the piperidine nitrogen, we have synthesized hydroxyl pyrrolidine derivative **6**, and we were delighted to find that compound **6** is one of the most potent inhibitors against Et-PKG (IC_{50} 0.16 nM) and also showed broad-spectrum anticoccidial activities in vivo. It is interesting to point out that possibly the basic amino group is contributing to improved potency, because acylation of the pyrrolidine nitrogen (compound **7**, IC_{50} 2.2 nM) resulted in a decrease of Et-PKG inhibition by an order of magnitude. These preliminary results have prompted us to continue our investigation in other heterocycles as substituents on the piperidine nitrogen.



6 R = H 7 R = Ac

In summary, our SAR study on diaryl-(4-piperidinyl)-pyrrole derivatives has revealed that hydroxylated alkyl substituents on the piperidine nitrogen improve the inhibition against Et-PKG with IC_{50}s less than 1 nM. On the other hand, improvement in in vivo anticoccidial properties was only observed with small alkyl groups. These results have suggested that lipophilicity might be one of the important factors in determining the in vivo efficacy of these compounds. In the case of (*S*)-methyl glycidyl ether derivative **4b**, and (*R*)-methyl β-(*S*)-hydroxy derivative **5h**, two of the major *Eimeria* subtypes (*E.t.* and *E.a*) are effectively controlled at 50 ppm level in feed. Further investigation to improve anticoccidial efficacy is ongoing.

Acknowledgments

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