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N-Alkyl-4-piperidinyl-2,3-diarylpyrrole derivatives with heterocyclic substitutions as potent and broad spectrum anticoccidial agents

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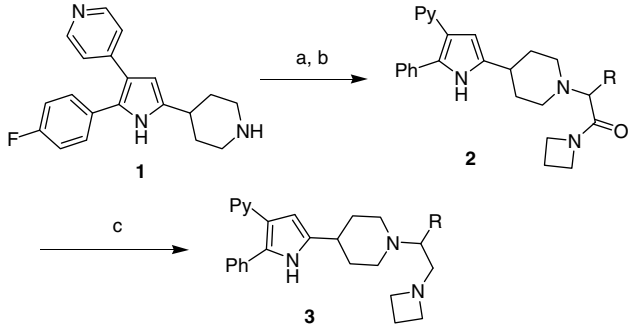
Abstract—Diaryl-(4-piperidinyl)-pyrrole derivatives bearing cyclic amine substituents have been synthesized and evaluated as anticoccidial agents. Improvements in potency of Et-PKG inhibition, such as azetidine derivative **3a**, and broad spectrum anticoccidial activities in feed, such as morpholine derivative **8c**, have been achieved.

Recently, we have reported the discovery of *N*-alkyl-4-piperidinyl-2,3-diarylpyrroles,[1] especially those bearing hydroxyl groups,[2] as potent and broad spectrum anticoccidial agents through inhibition of a novel cGMP-dependent protein kinase (PKG).[3,4] It has been more than two decades since the introduction of the last new commercial anticoccidial agent. Prophylactic uses of coccidiostats over time by all major poultry operations have caused the development of resistance to current coccidiostats among existing strings of parasites.[5] Aside from lacking new biochemical targets to control the disease, 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.[6,7] It is critical that for a new anticoccidial agent to be successful commercially, very low efficacious use level, usually <100 ppm in feed, has to be achieved. Thus, very thorough and careful SAR studies are necessary to discover highly potent compounds, both in vitro and in vivo.

Following the results of our earlier SAR studies,[2,8] we continued our medicinal chemistry project by incorporating nitrogen containing heterocycles into the lead compounds.[9] These compounds were first evaluated in an enzyme inhibition assay against PKG from the native *Eimeria*, and followed by an anticoccidial assay to evaluate their in vivo efficacy in oocyst reduction against two major subtypes of Protozoa parasites: *Eimeria tenella* (E.t.) and *Eimeria acervulina* (E.a.).[10]

First, azetidine moiety was introduced to the lead compound by a reaction sequence shown in Scheme 1. The



Scheme 1. Reagents: (a) $BrCHRCO_2Me$, Et_3N; (b) azetidine, heat; (c) BH_3 THF or $LiAlH_4$.

Keywords: Coccidiosis; PKG; PKG inhibitor.
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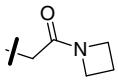
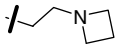
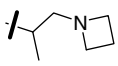
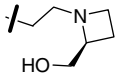
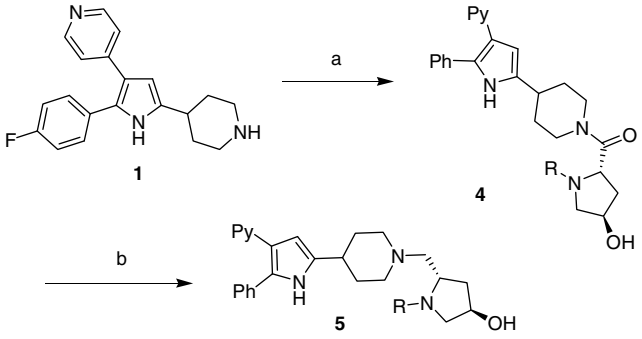
Table 1. Et-PKG inhibition and anticoccidial activities of diaryl-(4-piperidinyl)-pyrrole derivatives **2** and **3**

Compound	R	Et-PKG inhibition IC_{50}[a] (nM)	Anticoccidial activity at 100 ppm in feed[10]	
			E.t.	E.a.
2		1.01	3	3
3a[1]		0.08	3	3
3b		0.4	3	3
3c		1.7	3	3
3d		0.28	3	3

[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. 1.



Scheme 2. Reagents: (a) N-protected amino acid, *i*-Pr_2NEt, HOBT, BOP; (b) BH_3 THF or $LiAlH_4$.

piperidine nitrogen of compound **1**[1] was alkylated with bromoacetates. The resulting acetates were directly converted to azetidine amide **2**. Reduction of **2** with either borane or LAH yielded the desired azetidine derivative **3**. These azetidine bearing compounds are highly potent PKG inhibitors, including azetidine amide **2** (Table 1). Reduction of azetidine amide **2** (IC_{50} = 1 nM) to azetidine **3a**[1] (IC_{50} = 0.08 nM) resulted in a 10-time increase in potency, making it one of the most potent PKG inhibitors made on this project. These data suggest the importance of a basic nitrogen atom at this position. Furthermore, all these compounds are fully efficacious in vivo at 100 ppm in feed, but not at lower levels. It has been observed repeatedly on this project that increases in in vitro potencies do not always result in similar increases in in vivo efficacy, probably due to lack of bioavailability, and/or lack of parasite penetration of these compounds.[2]

We have also investigated the derivatives containing hydroxyl pyrrolidine moieties. These compounds were synthesized in a similar reaction sequence through amide formation with hydroxyprolines followed by reduction (Scheme 2), and their biological evaluation data are summarized in Table 2. Hydroxylated derivatives were chosen because of our success in earlier SAR studies.[2] Consistent with the azetidine series, pyrrolidine derivatives are also highly potent PKG inhibitor with IC_{50}'s less than 1 nM. Also, acylation of the pyrrolidine nitrogen of **5a**,[2] (IC_{50} = 0.2 nM) resulted a 10-time decrease in its potency (**5d**,[2] IC_{50} = 2 nM). Although steric effects cannot be ruled out, it is another piece of supporting evidence that a basic nitrogen at this position is favored to boost potency of these compounds. Apart from the azetidine series, however, there is a new trend that these pyrrolidine derivatives are not as efficacious against E.a. as they are against E.t. At 100 ppm in feed, they are only partially efficacious against E.a. with less than 80% oocyst reduction.[10] This led to a speculation that an optimal range of lipophilicity is necessary for broad spectrum in vivo activities of these compounds. Further investigation on this issue is ongoing.

Based on this working hypothesis, we incorporated morpholine moiety into the molecule in an attempt to balance the lipophilicity and reach broad spectrum in vivo activity against both E.t. and E.a. These compounds were made following the reaction sequence outlined in Scheme 3, and N-protected morpholino carboxylic acids were used as starting materials. Their anticoccidial activity evaluation is summarized in Table 3.

Racemic morpholino acid were used as a cost-control measure, and our earlier studies have shown that very little differences in both in vitro and in vivo activities were observed when enantiomerically pure analogs were tested.[2] Similar to the first two series

Table 2. Et-PKG inhibition and anticoccidial activities of diaryl-(4-piperidinyl)-pyrrole derivatives **5**

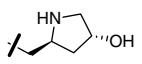
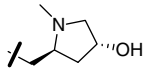
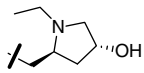
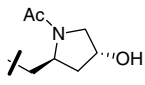
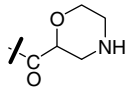
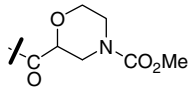
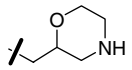
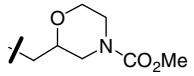
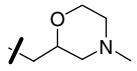
Compound	R	Et-PKG inhibition IC_{50} (nM)	Anticoccidial activity at 100 ppm in feed[10]	
			E.t.	E.a.
5a[2]		0.16	3	2
5b		0.34	3	2
5c		0.36	3	2
5d[2]		2.2	3	2

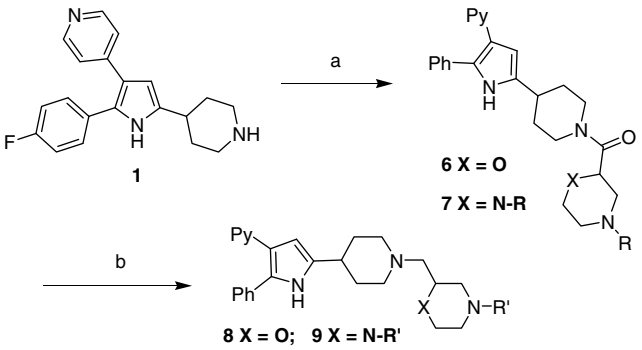
Table 3. Et-PKG inhibition and anticoccidial activities of diaryl-(4-piperidinyl)-pyrrole derivatives **6** and **7**

Compound	R	Et-PKG inhibition IC_{50} (nM)	Anticoccidial activity at 100 ppm in feed[10]	
			E.t.	E.a.
6a		0.27	3	2
6b		4.70	2	2
8a		0.17	3	2
8b		1.03	3	2
8c		0.67	3	3

of compounds, morpholine containing analogs **6** and **8** are highly potent Et-PKG inhibitors. Also, a 10-time decrease in potency was observed when the basic morpholine nitrogen was acylated (from **8a**, IC_{50} = 0.17 nM to **8b**, IC_{50} = 1.03 nM). The potency was further reduced when the basic piperidine nitrogen was also acylated (**6b**, IC_{50} = 4.70 nM). These results are consistent with the trend we mentioned earlier that basic nitrogen atoms in that part of the molecule are essential for in vitro activity. However, incorporation of morpholine moiety does not provide the kind of balanced in vivo efficacies required for broad spectrum anticoccidial agents. Most compounds showed greater efficacy against E.t. than those against E.a. Only compound **8c** in the series stands out with balanced activities and reaching full reduction of oocyst burden in both E.t. and E.a. at 50 ppm in feed.

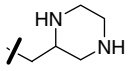
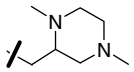
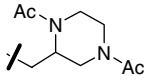
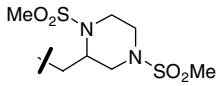
To take the notion one step further that basic nitrogen atoms are important for the intrinsic potency of these compounds against PKG, we replaced the oxygen atom in the morpholine ring with a nitrogen atom, and synthesized a series of piperazine containing analogs (**9a–d**), using the same reaction sequence shown in Scheme 3. Their anticoccidial activity evaluation is summarized in Table 4.

Indeed, the piperazino analog **9a** is an extremely potent PKG inhibitor with an IC_{50} of 70 pM, a 2–3 time improvement over its morpholin analog **8a** (IC_{50} = 0.17 nM). Methylation (**9b**, IC_{50} = 1.08 nM), acylation (**9c**, IC_{50} = 2.2 nM), and sulfonylation (**9d**, IC_{50} = 1.41 nM) all resulted in loss of potency by more than 10 times. However, these piperazine analogs, although as potent as any other analogs, do not have the broad spectrum activities, presumably because of their less than optimal lipophilicity. No further development of these analogs is warranted.



Scheme 3. Reagents: (a) N-protected morpholino or piperazino carboxylic acid, *i*-Pr_2NEt, HOBT, BOP; (b) BH_3THF or $LiAlH_4$.

Table 4. Et-PKG inhibition and anticoccidial activities of diaryl-(4-piperidinyl)-pyrrole derivatives **9**

Compound	R	Et-PKG inhibition IC_{50} (nM)	Anticoccidial activity at 100 ppm in feed[10]	
			E.t.	E.a.
9a		0.07	3	2
9b		1.08	2	2
9c		2.2	n/a	n/a
9d		1.41	n/a	n/a

In summary, our continued SAR study on diaryl-(4-piperidinyl)-pyrrole derivatives revealed that nitrogen containing heterocyclic substitution on the piperidine nitrogen improves the inhibition potency against Et-PKG with IC_{50}'s lower than 100 pM (**3a** and **9a**). On the other hand, improvement of in vivo anticoccidial activities was only observed with morpholino analog **8c**, which has broad spectrum activities and has reached full reduction of oocyst burden in two of the major *Eimeria* subtypes (E.t. and E.a.) at 50 ppm level in feed. These results have indicated that other than its potency which can be improved by incorporating basic nitrogen atoms, many other factors, such as lipophilicity, are also of fundamental importance in determining the in vivo efficacy of these compounds.

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Analytical Support Group for open access service on LC/MS.

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9. All new compounds were characterized by ^{1}H NMR and MS.
10. Three-day-old chicks are randomly placed on medicated or control diets for the duration of the experiment. Following 24 h premedication, in each replicate one bird is infected with *Eimeria acervulina*, the other bird is infected with *E. tenella*. The *E. acervulina* portion of the experiment is terminated on Day 5, the *E. tenella* on Day 6 post infection. Treatments which provide at least 80% reduction in oocyst production are rated (3), those with 50–79% are rated (2), and those with <50% are rated (0). For a detailed description, please see Ref. 1.