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# Synthesis and SAR Studies of diarylpyrrole anticoccidial agents

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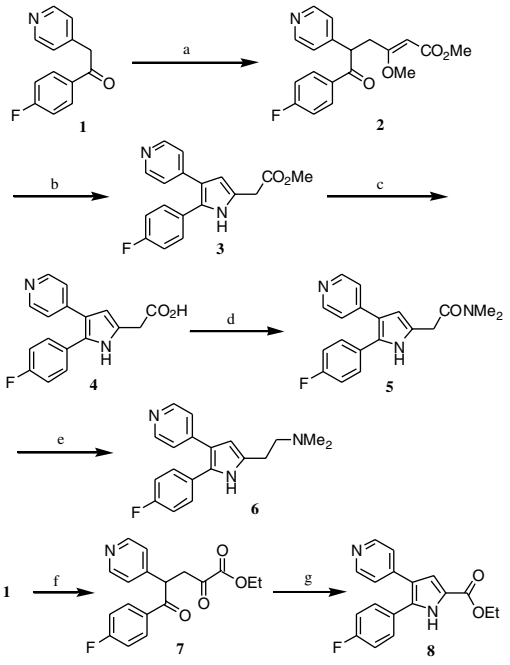
**Abstract**—2-(4-Fluorophenyl)-3-(4-pyridinyl)-5-substituted pyrroles were prepared and evaluated as anticoccidial agents in both in vitro and in vivo assays. Among the compounds evaluated, the dimethylamine-substituted pyrrole **19a** is the most potent inhibitor of *Eimeria tenella* PKG (cGMP-dependent protein kinase). Further SAR studies on the side chain of the 2-pyrrolidine nitrogen did not enhance in vivo anticoccidial activity.


Coccidiosis is a parasitic disease which is the major cause of morbidity and mortality in the poultry industry. Commercial poultry operations would be very costly without the use of effective anticoccidial agents. Resistance to existing coccidiostats is becoming widespread and new broad-spectrum economic drugs directed at novel biochemical targets are needed. Coccidiosis is caused by the invasion of protozoan parasites of the genus *Eimeria* into various sites in the intestine of the bird and causing damage to the intestinal lining.[1] Earlier reports[2,3]on studies of *Eimeria tenella* (Et) demonstrated that inhibition of a cyclic GMP-dependent protein kinase (PKG) stops the life cycle of the parasites, and thus prevents the disease from spreading. Early SAR studies from our laboratories showed that diaryl pyrroles are Et-PKG inhibitors with in vivo anticoccodial activity.[4–6] In this paper, we present the synthesis and SAR studies of 2,3-diaryl pyrroles with various substituents at the 5-position.

To evaluate these compounds as anticoccidial agents, an enzyme inhibition assay against the native *E. tenella* cGMP-dependent protein kinase (Et-PKG) was used for initial in vitro screening. A 7-day in feed model was carried out to evaluate their in vivo efficacy in




**Scheme 1.** Reagents and conditions: (a) 4-chloro-3-methoxy-2-butenate, DBU, $CH_2Cl_2$, −78 °C; (b) $NH_4OAc$, HOAc, 90 °C; (c) $KOH_{aq}$, EtOH, reflux; (d) dimethylamine, EDC, THF; (e) $BH_3$·THF complex, reflux; (f) $BrCH_2COCO_2Et$, DBU, $CH_2Cl_2$, −78 °C; (g) $NH_4OAc$, HOAc, 90 °C.

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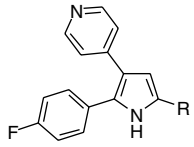
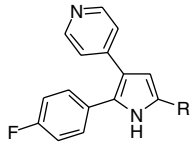
**Table 1.** Et-PKG inhibition and anticoccidial activities of 2-(4-fluorophenyl)-3-(4-pyridinyl)pyrrole analogues



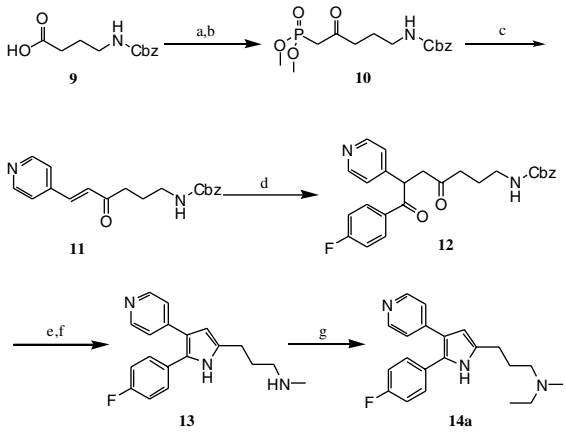
| Compound | R | Et-PKG inhibition $IC_{50}$[a] (nM) | Anticoccidial activity at 100 ppm in feed[4] | |
|---|---|---|---|---|
| | | | *E.t.* | *E.a.* |
| **4** | $-CH_2CO_2H$ | >1000 | 0 | 0 |
| **5** | $-CH_2CONMe_2$ | 4.1 | 0 | 0 |
| **6** | $-(CH_2)_2NMe_2$ | 2.5 | 2 | 2 |
| **8** | $-CO_2Et$ | 430 | 0 | 0 |

[a] Values are means of three experiments and the same for other tables.

**Table 2.** Et-PKG inhibition and anticoccidial activities of 2-(4-fluorophenyl)-3-(4-pyridinyl)pyrrole analogues



| Compound | R | Et-PKG inhibition $IC_{50}$ (nM) | Anticoccidial activity at 100 ppm in feed[4] | |
|---|---|---|---|---|
| | | | *E.t.* | *E.a.* |
| **14a** | $-(CH_2)_3NMeEt$ | 4.1 | 0 | 0 |
| **6** | $-(CH_2)_2NMe_2$ | 2.5 | 2 | 2 |
| **19a** | $-CH_2NMe_2$ | 0.35 | 0 | 3 |



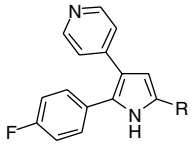

**Scheme 2.** Reagents and conditions: (a) MeI, $K_2CO_3$, DMF; (b) *n*-BuLi, $CH_3P(O)(CH_3)_2$, THF, −78 °C; (c) 4-pyr-CHO, $K_2CO_3$, $CH_3CN$; (d) 4-F-PhCHO, thiazolium iodide, $Et_3N$, EtOH, reflux; (e) $NH_4OAc$, HOAc, 90 °C; (f) $LiAlH_4$, THF, reflux; (g) acetaldehyde, $NaBH(OAc)_3$.

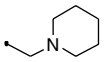
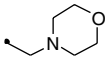
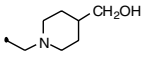
oocyst reduction against two major *Eimeria* species: *E. tenella* (*E.t.*) and *Eimeria acervulina* (*E.a.*). Treatments which provide at least 80% reduction in oocyst production are rated (3), those with 50–79% are rated (2), and those with <50% are rated (0). Details of these procedures and rules of scoring were published in early work.[4]

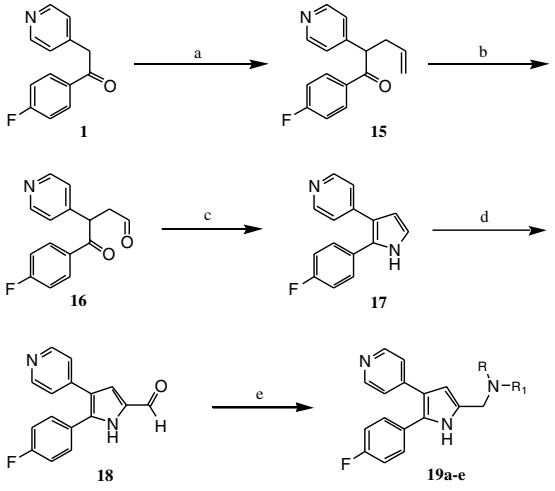
The synthesis of 2,3-diaryl pyrrole analogues with various 5-substituents including carboxylic acid, ester, amide, and amine is outlined in Scheme 1. The aryl ketone **1**[4] was alkylated with 4-chloro-3-methoxy-2-butenate in the presence of DBU to yield keto ester **2**, which was cyclized with ammonium acetate in acetic acid to give pyrrole ester **3**. Hydrolysis of ester **3** afforded acid **4**, which was subsequently converted to dimethyl amide **5** by EDC-mediated coupling. Borane reduction of amide **5** yielded *N*,*N*-dimethyl amine **6**. In a similar manner, ethyl ester **8** was obtained via the formation of diketone **7** by the coupling reaction of aryl ketone **1** with ethyl bromopyruvate in the presence of DBU, followed by cyclization as described above. These analogues were evaluated in both in vitro and in vivo assays, and the results are summarized in Table 1.[7]

When the substituents of the side chain of the pyrrole are carboxylic acid (**4**) or ester (**8**), compounds are inactive both in vitro and in vivo. Although dimethyl amide **5** is a potent Et-PKG inhibitor, it has no in vivo efficacy. Dimethylamine **6**, however, has improved Et-PKG inhibition potency as well as in vivo efficacy. The in vivo anticoccidial activity of the amine side chain is consis-

**Table 3.** Et-PKG inhibition and anticoccidial activities of 2-(4-fluorophenyl)-3-(4-pyridinyl)pyrrole analogues



| Compound | R | Et-PKG inhibition $IC_{50}$ (nM) | Anticoccidial activity 100 ppm in feed[4] | |
|---|---|---|---|---|
| | | | *E.t.* | *E.a.* |
| **19a** |  | 0.35 | 0 | 3 |
| **14b** |  | 0.71 | 2 | 3 |
| **24** |  | 1.0 | 0 | 3 |
| **19b** |  | 1.3 | 0 | 0 |
| **14c** |  | 2.7 | 0 | 0 |
| **19c** |  | 3.7 | 0 | 0 |
| **19d** |  | 7.6 | 0 | 0 |
| **19e** |  | 13 | 0 | 0 |



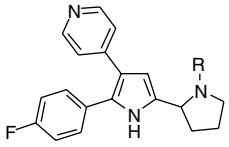
**Scheme 3.** Reagents and conditions: (a) NaHMDS, allyl bromide, DMSO; (b) $O_3$, MeOH, 0 °C, then $(CH_3)_2S$; (c) $NH_4OAc$, HOAc, 90 °C; (d) DMF, $POCl_3$, 90 °C then aq NaOAc, 90 °C; (e) amines, $BH_3$·Pyr, EtOH.

tent with early observations from other studies in our laboratories[4–6] that a basic amine is essential at this position. These results led us to explore the length of the side chain and the effect of different substituents on the nitrogen of the amine group.

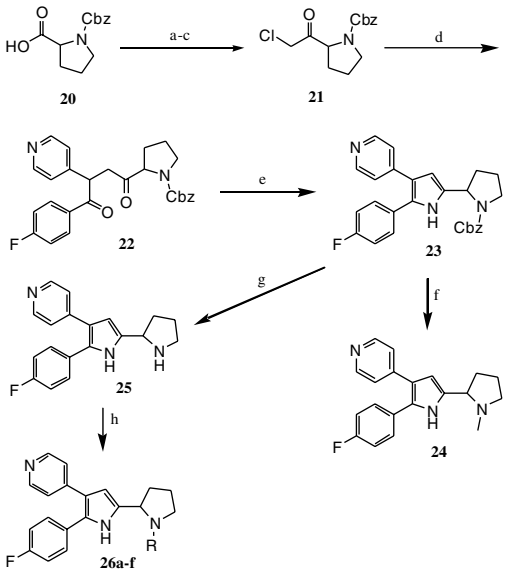
The synthesis of the γ-alkylamine analogue **14a** (in Table 2) was carried out as shown in Scheme 2. Methylation of *N*-Cbz-γ-amino butyric acid **9** followed by treatment of the resulting methyl ester with the anion of methyl phosphonate yielded the intermediate **10**. Horner–Emmons reaction of **10** with 4-pyridine aldehyde yielded ketone **11**. Stetter reaction of **11** using 4-fluorophenyl aldehyde and thiazolium salt afforded the key intermediate diketone **12**. Cyclization and lithium aluminum hydride reduction provided amine **13**, which was reductively alkylated with acetylaldehyde to give **14a**. The same method with *N*-Cbz-α-amino-3-methylbutyric acid and methyl 1-methylpipecolinate as starting materials provided compounds **14b** and **14c**, respectively (in Table 3).

The analogues with an α-alkylamine group side chain **19a–e** shown in Table 3 were prepared by reductive amination of aldehyde **18** with a variety of amines. The synthesis of these compounds is outlined in Scheme 3. Alkylation of aryl ketone **1** with allyl bromide and sodium bis(trimethylsilyl)amide followed by ozonolysis yielded **16**. Formation of pyrrole **17** was accomplished

**Table 4.** Et-PKG inhibition and anticoccidial activities of 2-(4-fluorophenyl)-3-(4-pyridyl)-5-pyrrolidinyl pyrrole analogues



| Compound | R | Et-PKG inhibition $IC_{50}$ (nM) | Anticoccidial activity 100 ppm in feed[4] | |
|---|---|---|---|---|
| | | | *E.t.* | *E.a.* |
| **25** | H | 3.1 | 2 | 0 |
| **24** | $CH_3$ | 1.0 | 0 | 3 |
| **26a** | $–CH_2CH_2OH$ | 2.1 | 0 | 0 |
| **26b** | (*S*)-$CH_2CHOHCH_3$ | 2.8 | 3 | 0 |
| **26c** | (*R*)-$CH_2CHOHCH_3$ | 3.3 | 0 | 0 |
| **26d** | $–CONHCH_3$ | 2.3 | 0 | 0 |
| **26e** | $–CO_2CH_2Ph$ | 2.7 | 0 | 0 |
| **26f** | $–SO_2$(4-F-Ph) | 1.5 | 0 | 0 |



**Scheme 4.** Reagents and conditions: (a) $(COCl)_2$, DMF; (b) $TMSCHN_2$, THF; (c) HCl, ether; (d) ketone 1, DBU, $CH_2Cl_2$, −78 °C; (e) $NH_4OAc$, HOAc, 90 °C; (f) LiALH, THF, reflux; (g) $H_2$, 20% Pd/C, HOAc, MeOH; (h) epoxides or $CH_3NCO$, or Cbz-Cl, or 4-F-phenyl sulfonyl chloride.

as previously described. Reaction of **17** with phosphorus oxychloride in the presence of dimethylformamide and subsequent heating with aqueous sodium acetate gave the aldehyde **18**.

The substituted 2-pyrrolidine analogues **24**, **25**, and **26a–f** shown in Table 4 were prepared in a similar manner (Scheme 4). After conversion of *N*-Cbz proline **20** to the acid chloride, the resulting compound was treated with trimethylsilyl diazomethane followed by aqueous work-up and displacement with hydrogen chloride to yield the chloromethyl ketone **21**. The pyrrolidine **23** was obtained as described earlier. Lithium aluminum hydride reduction of **23** gave *N*-methyl compound **24**, while hydrogenolysis of the Cbz group provided **25**, which was further modified at the pyrrolidine nitrogen by acylation, sulfonylation, and alkylation to provide analogues **26a–f** (in Table 4).

The data presented in Table 2 compare the activities of analogues with the side chain from C-1 to C-3. The γ-alkylamine **14a** is a potent Et-PKG inhibitor ($IC_{50}$ = 4.1 nM), but has no in vivo anticoccidial activity at 100 ppm against *E.t.* and *E.a.*, while the β-alkylamine **6**, Et-PKG ($IC_{50}$ = 2.5 nM), caused partial reduction of oocyst count (50–79%) at 100 ppm against both *E.t.* and *E.a.* in feed. The shorter α-alkylamine side chain **19a** ($IC_{50}$ = 0.35 nM) is one of the most potent Et-PKG inhibitors and has full in vivo anticoccidial activity (80–100%) at 100 ppm against *E.t.*

For active Et-PKG inhibitors, the in vivo efficacy is determined by many factors, such as absorption in both host and parasites. Changes in the basicity and lipophilicity of the compound with various substituents on the amine group might improve the bioavailability of these compounds, and thus improve the in vivo anticoccidial activity. Therefore, we prepared a series of α-alkylamine analogues with different ring sizes (**19b–e**) and α-alkyl branches (**14b–c**, **24**). Their anticoccidial activities are summarized in Table 3.

All the cyclic amine derivatives (**19b–e**, **24**, and **14c**) are very potent Et-PKG inhibitors with $IC_{50}$ in the low nanomolar range. As the ring size gets larger from azetidine (**19b**) to piperidine (**19c**), their potencies drop slightly. Introduction of oxygen or a hydroxyl group on the piperidine ring (**19d–e**) resulted in decreased in vitro activities. These results indicate neither the more lipophilic groups nor the polar groups are tolerated at this position. On the other hand, introducing an α-alkyl branch generated mixed results. Although compound **14b** showed no improvement in Et-PKG activity, it has in vivo anticoccidial activity for both *E.t.* and *E.a.* And *N*-methyl pyrrolidine **24** shows a slight drop in

potency, but it retains the full in vivo anticoccidial activity for *E.a.*, while the slightly less potent piperidine **14c** has lost in vivo activity.

Modification on the nitrogen of 2-pyrrolidine **25** gave analogues **26a**–**f** shown in Table 4. In general, the substituents have little effects on Et-PKG inhibition. Most derivatives have similar in vitro activities. For example, the enantiomeric isopropanol **26b** and **26c** have similar Et-PKG activity, but only **26b** shows anticoccidial activity for *E.t.* at 100 ppm in feed. These results are different from those observed for 4-piperidine pyrrole containing a hydroxyl group[4,6], while the compounds here do not have a broad-spectrum of in vivo activity.

In summary, modification of 2-(4-fluorophenyl)-3-(4-pyridinyl) pyrrole by introduction of various basic amines at the 5-position has been examined. Most analogues have good Et-PKG potency. Substitution of large lipophilic groups or polar groups on the carbon α to the amine nitrogen is not tolerated. Compounds **19a** and **14b** are subnanomolar inhibitors of Et-PKG. However, they did not display a broad-spectrum of anticoccidial activity.

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7. All new compounds were characterized by $^{1}$H NMR and LC–MS prior to submission for biological evaluation.