Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3296–3301
Bioorganic & Medicinal Chemistry Letters

Synthesis and SAR of 2,3-diarylpyrrole inhibitors of parasite cGMP-dependent protein kinase as novel anticoccidial agents

Tesfaye Biftu,* Dennis Feng, Mitree Ponpipom, Narindar Girotra, Gui-Bai Liang, Xiaoxia Qian, Robert Bugianesi, Joseph Simeone, Linda Chang, Anne Gurnett, Paul Liberator, Paula Dulski, Penny Sue Leavitt, Tami Crumley, Andrew Misura, Terence Murphy, Sandra Rattray, Samantha Samaras, Tamas Tamas, John Mathew, Christine Brown, Don Thompson, Dennis Schmatz, Michael Fisher and Matthew Wyvratt

Merck Research Laboratories, Department of Medicinal Chemistry, Merck and Co., P.O. Box 2000, Rahway, NJ 07065-0900, USA
Merck Research Laboratories, Department of Human and Animal Infectious Disease Research, Merck and Co., P.O. Box 2000, Rahway, NJ 07065-0900, USA

Accepted 19 April 2005
Available online 26 May 2005

Abstract—Several analogs of 2,3-diaryl pyrroles were synthesized and evaluated as inhibitors of *Eimeria tenella* cGMP-dependent protein kinase and in in vivo anticoccidial assays. A 4-fluorophenyl group enhances both in vitro and in vivo activities. The most potent analogs are the 5-(*N*-methyl, *N*-ethyl, and *N*-methylazetidine methyl) piperidyl derivatives **12, 23**, and **34**. These compounds have a broad spectrum of activity. Based on the in vivo efficacy and cost of synthesis, the *N*-ethyl analog **23** was chosen as a novel anticoccidial agent for a field trial.

1. Introduction

Parasitic protozoa are responsible for a wide variety of infections in human and animals. Malaria caused by *Plasmodium* species, myeloencephalitis caused by *Sarcocystis neurona*, trypanosomiasis caused by *Trypanosoma cruzi*, African sleeping sickness caused by *Trypanosoma brucei*, and opportunistic infections in immunocompromised hosts caused by *Pneumocystis carinii*, *Toxoplasma gondii*, and *Cryptosporidium* species are becoming significant in developing and developed countries.

Coccidiosis, a widespread disease of domesticated animals, is caused by a protozoal infection. In the poultry industry, coccidiosis is responsible for high levels of morbidity and mortality in the bird population and may result in extreme economic losses. The infectious agents are protozoa of the genus *Eimeria* such as *E. tenella*, *E. acervulina*, *E. necatrix*, *E. brunetti*, and *E. maxima*. Anticoccidial drugs have profoundly contributed to successful poultry production. In the early 1930s, when the first coccidiostats were discovered, the total annual broiler production in the United States was approximately 34 million.[1] Even though the demand for poultry as a source of protein far outpaced supply, the high incidence of morbidity and mortality kept growth of the industry in check. With the development of safe and effective anticoccidials, the disease was brought under control, and by the year 2000 the annual broiler production in the United States reached eight billion.[2] Since the introduction of monensin to the broiler market in 1971,[3,4] the polyether ionophores have been the leading commercial anticoccidial products. An initial shift in drug sensitivity was observed with this class during the 1980s,[5,6] and a slow process of sensitivity erosion has continued to the present.[7–9] Today, resistant field isolates are fairly common, indicating that the time is approaching when novel new entries in the anticoccidial market will be necessary to maintain current levels of activity and productivity. The most successful anticoccidials have broad-spectrum efficacy, slow resistance selection, and do not retard growth.

Novel *Eimeria tenella* and *Toxoplasma gondii* cGMP-dependent protein kinases (PKGs), the polynucleotide sequences encoding these PKGs, and a facile specific

* Corresponding author. E-mail: tesfaye_biftu@merck.com

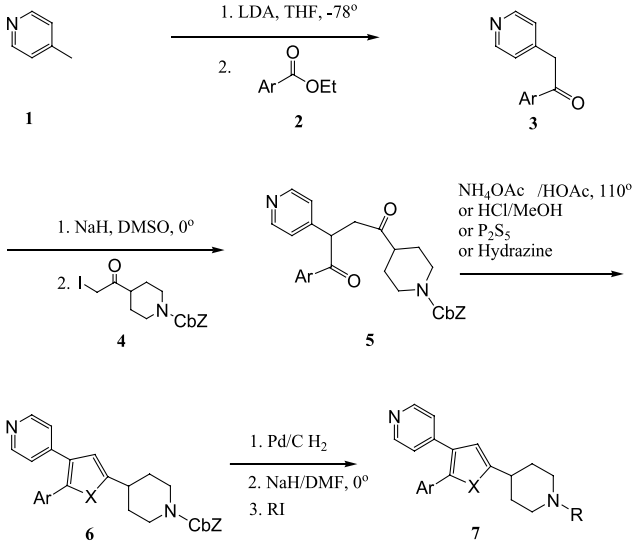
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.04.060

assay to screen compounds as PKG inhibitors have been reported recently.[10,11] The native *Eimeria tenella* PKG (Et-PKG) is a protein of about 120 kDa with 1003 amino acids. In this article, we describe the in vitro activities of several diaryl pyrroles as Et-PKG inhibitors and the in vivo activities of these compounds as anticoccidial agents in chickens.

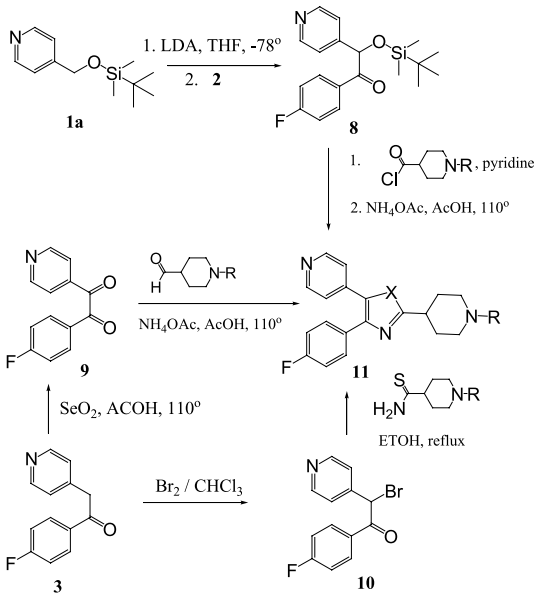
2. Synthesis

The pyrroles, furans, thiophenes, and pyridazines described here are prepared as shown in Scheme 1. 4-Picoline **1** was treated with LDA in THF, and the anion generated was reacted with the esters **2** to form ketones **3**. The latter was then treated with sodium hydride in dimethyl sulfoxide followed by iodoketone **4** to form 1,4-diketones **5,** which were conveniently cyclized to pyrroles **6** by heating with ammonium acetate in acetic acid. Deprotection of **6** followed by alkylation with sodium hydride/alkyl halides gave the desired pyrroles. Furans, thiophenes, and pyridazines were made from the diketone **5** in a similar manner by treatment with hydrochloric acid in methanol, phosphorous pentasulfide, or hydrazine, respectively.

As shown in Scheme 2, oxidation of the ketone **3** with selenium dioxide gave the diketone **9**. The *t*-butyldimethylsilyl (TBDMS) protect hydroxyketone **8** was made by acylation of **1a**, as described above in Scheme 1. Bromination of **3** gave the bromoketone **10**. The oxazole, thiazole, and imidazole inhibitors described in this manuscript were made from TBDMS protected hydroxyketone **8**, diketone **9** or bromoketone **10** by coupling with 4-piperidyl acid chloride, aldehyde or thioamide, respectively.



Scheme 1. Synthesis of pyrrole, thiophene, and furan **7**.



Scheme 2. Synthesis of imidazole, oxazole, and thiazole **10**.

3. Biological assays

3.1. Enzyme assay and data analysis

Purification of the Et-PKG enzyme was performed as described in a previous communication.[10] Drug solutions at 50 times the desired test concentration in 100% DMSO were prepared and 1:2 serial dilutions into 100% DMSO were made for eight data points. Drug dilutions (2 μL) were added to each well of a Wallac Isoplate (Catalog No. 1450-514) followed by 50 μL of the substrate solution [50 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 3 μM ATP, 4 μM peptide substrate (Gly-Arg-Thr-Gly-Arg-Arg-Asn-Ser-Ile biotinylated at the amino terminal end through a linker region amino-hexanoic acid), 0.2 mg/mL bovine serum albumin, 20 μM cGMP, and 3 μCi/mL [^{33}P]ATP (NEG/602 H Perkin-Elmer Life Sciences (NEN) at 3000 Ci/mmol)]. Then, 50 μL of the enzyme solution was added to substrate (1 μL/mL of partially purified *E. tenella* PKG that does not contain any cAMP-stimulated activity). The mixture was incubated at room temperature for 4 h. At the end, 100 μL of stop solution was added to each well containing substrate; drug, enzyme, and beads were allowed to settle overnight and counted on the Wallac microbeta. The stop solution was prepared by adding 500 mg Streptavidin SPA beads (Amersham, Catalog No. NIF-1077) to 10 mL PBS, mixing by gentle swirling, and then adding 1 mL SPA bead/PBS mixture to 4 mL of 75 mM phosphoric acid. After subtracting the appropriate background for each assay point, titrations were fit to the following modified Hill equation using Kaleidagraph (Synergy Software): $V_A = V_0 + (V_{max} - V_0)/(1 + (K_A/[A])^h)$.

3.2. In vivo anticoccidial assay

One-day-old White Leghorn chickens are obtained from a commercial hatchery and acclimated in a holding

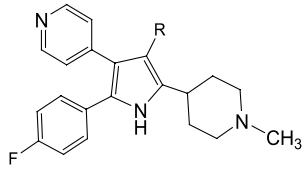
room. At 3 days of age, the test animals are selected by weight, wingbanded, and randomly placed on medicated or control diets for the duration of the experiment. One or two replicates of two birds are utilized per treatment. Following 24 h premedication, in each replicate one bird is infected with *E. acervulina*, while the other bird is infected with *E. tenella*. Both strains of *Eimeria* are sensitive to all anticoccidial products and have been maintained under laboratory conditions for over 25 years. The inocula consist of sporulated oocysts in tap water suspensions, administered at a dose volume of 0.25 mL per bird. The inocula levels are selected by previous dose titrations to provide a low-to-moderate level of infection. The *E. acervulina* portion of the experiment is terminated on Day 5 and that of the *E. tenella* on Day 6 post-infection. The measured parameters are weight gain, feed consumption, and oocyst production. *E. tenella* lesion scores are also recorded for background information. Treatments that 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).

4. Results and discussion

Pyrrole **12** and other heterocycles have been reported as kinase inhibitors.[12] The in vitro Et-PKG and the in vivo anticoccidial activities of 2-aryl substituted 2,3-diaryl heterocycles are shown in Tables 1–5. As shown in Table 1, substitution of the 4-position of pyrrole with groups such as Cl (**13**), Br (**14**) or methyl (**15**) resulted in compounds that maintained in vitro Et-PKG activity; however, these more lipophilic analogs lack significant in vivo activity. The unsubstituted analog **12** is fully active in both *E. tenella* and *E. acervulina* at 100 ppm in feed.

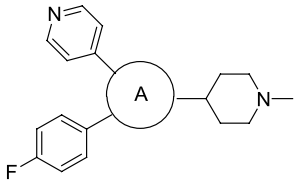
As shown in Table 2, we kept the 4-pyridyl, 4-fluorphenyl, and piperidyl groups fixed and varied the middle heterocyclic group. In general, five-membered heterocycles are more potent than six-membered heterocycles. For example, pyrrole **12** (IC_{50} = 0.7 nM), imidazole **16** (IC_{50} = 1.1 nM), furan **17** (IC_{50} = 2.1 nM), and oxazole

Table 1. Et-PKG and anticoccidial activities of 2-aryl substituted diaryl pyrroles



R	Et-PKG IC_{50} (nM)	Anticoccidial activity at 100 ppm	
		Et	Ea
H **12**	0.70	3	3
Cl **13**	1.02	2	0
Br **14**	1.2	0	0
Me **15**	1.0	0	0

Table 2. Et-PKG and anticoccidial activities of diaryl substituted heterocycles



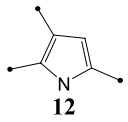
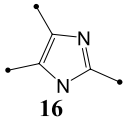
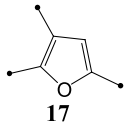
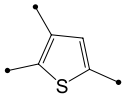
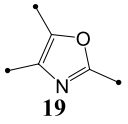
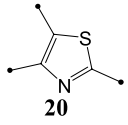
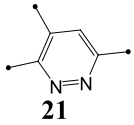
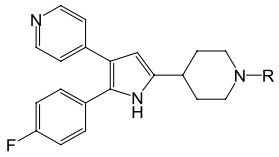
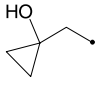
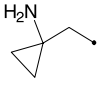
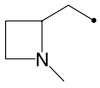
A	Et-PKG IC_{50} (nM)	Anticoccidial activity at 100 ppm	
		Et	Ea
 **12**	0.70	3	3
 **16**	1.1	3	0
 **17**	2.1	0	2
 **18**	4.2	3	3
 **19**	0.97	3	3
 **20**	1.5	0	0 (25 ppm)
 **21**	1000	0	0

Table 3. Et-PKG and anticoccidial activities of N-substituted piperidine diaryl pyrroles



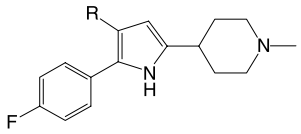
R	Et-PKG IC_{50} (nM)	Anticoccidial activity at 100 ppm	
		Et	Ea
Methyl **12**	0.70	3	3
Ethyl **23**	0.28	3	3
2-Hydroxyethyl **24**	0.71	3	3
2-Aminoethyl **25**	0.28	0	0
n-Propyl **26**	4.2	0	0
Acetone **27**	0.32	0	0
2-Aminopropyl **28**	2.0	3	3
3-Hydroxypropyl **29**	0.53	2	3
3-Aminopropyl **30**	0.046	0	0
 **31**	1.1	3	3
 **32**	0.24	0	0
 **34**	0.28	3	3

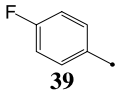
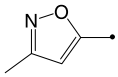
19 (IC_{50} = 0.7 nM) are more potent Et-PKG inhibitors than pyridazine **21** (IC_{50} = 1000 nM). The thiazole **18** and oxazole **19** are active in in vivo assay in both species at 100 ppm. The basic heterocycles such as imidazole **16** and thiazole **20** are not active in both species.

Et-PKG inhibition activity and in vivo anticoccidial activities of alkyl, hydroxyalkyl, and aminoalkyl substituted piperidines are shown in Table 3. The *N*-methyl and *N*-ethyl analogs **12** and **23** (0.28 nM) are potent PKG inhibitors and are active in in vivo assays. The *N*-propyl analog **26** (4.2 nM) has reduced activity. Reducing lipophilicity by introducing hydroxy or amino substituents at the 2- or 3-position of the alkyl side chain maintained Et-PKG activity. Amino ethyl **25** and azetidine **34** substituted piperidine analogs are more potent Et-PKG inhibitors than the methyl substituted analog **12**. Azetidine **34** is also a very potent anticoccidial agent and has in vivo activity even at a 25 ppm level.

The aminopropyl **30** and cyclopropylamine **32** are potent PKG inhibitors with IC_{50}s of 0.046 and 0.24 nM, respectively, but they both lack in vivo activity against

Table 4. Et-PKG and anticoccidial activities of diaryl substituted heterocycles

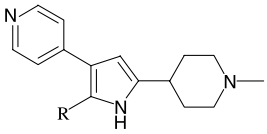


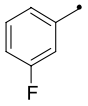
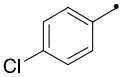
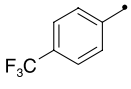
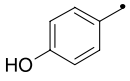
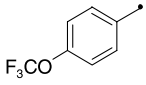
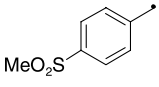
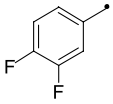
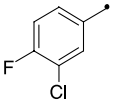
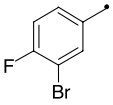
R	Et-PKG IC_{50} (nM)	Anticoccidial activity at 100 ppm	
		Et	Ea
 **12**	0.70	3	3
 **35**	1.7	0	0
 **36**	2.8	0	0
 **37**	11.7	0	2
 **38**	23.6	0	0
 **39**	15.6	0	0
 **40**	>1000	0	0

Et and Ea parasites. This could be due to the inability of the compounds to penetrate the appropriate host tissue or to penetrate the parasite. Lipophilicity plays a major role in the in vivo activity and spectrum of activity of these compounds.

As shown in Table 4, replacement of the 4-pyridyl group with 3-pyridyl **37**, 2-pyridyl **38**, 4-fluorophenyl **39**, or isooxazole **40** resulted in over an order of magnitude loss in Et-PKG activity. However, a methyl substituent on the 4-pyridyl group, **35** and **36**, reduced activity by

Table 5. Et-PKG and anticoccidial activities of diaryl substituted pyrroles **1a**–**1i**



Compound	R	Et-PKG IC_{50} (nM)	Anticoccidial activity at 100 ppm	
			Et	Ea
41		3.1	NA[a]	NA[a]
42		7.4	0	0
43		1.0	0	3
44		11.1	0	0
45		11.9	0	0
46		26.3	0	0
47		39.7	0	0
48		55	0	0
49		50	0	0
50		46	3	0
51		42	3	0
52		2.0	3	0
53		0.44	3	2
54		0.15	0	0

[a] Data are not available.

two- to fourfold only. Compounds **35**–**40** were not significantly active in in vivo assays.

As shown in Table 5, the 4-fluoro phenyl group plays a very important role in the Et-PKG activities of these classes of compounds. The unsubstituted phenyl **41** and the 3-fluoro phenyl **42** are weaker inhibitors of Et-PKG. Replacement of the 4-fluoro group with trifluoromethyl **48**, hydroxyl **49**, trifluoromethoxy **50** or methyl sulfone **51** significantly reduced Et-PKG activity compared to **12**. Replacement of 4-fluorophenyl group with other heterocycles such as furan (**44** and **46**), thiophene (**45**) or thiazole (**47**) also resulted in over two orders of magnitude loss in Et-PKG activity. However, when the 4-fluoro group is kept and a second halogen is added in the meta position, the resulting analogs **52**, **53**, and **54** are very active in the enzyme assays with a tendency toward improved potency for the least electronegative substituent.

Pyrrole **23** (Et-PKG IC_{50} = 0.28 nM) was selected for spectrum studies against eight of the most common *Eimeria* species in chicken (*E. tenella*, *E. acervulina*, *E. necartrix*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. mivati*, and *E. praecox*). As shown in Table 6, analog **23** has excellent anticoccidial activity at 50–125 ppm. In the same study, the anticoccidial agent salinomycin has

Table 6. Spectrum assays of compound **23** on eight species of *Eimeria* (40,000 parasites per bird)

Treatment	In feed (ppm)	Anticoccidial activity
Normal control	0	3
Infected control	0	0
23	125	3
23	75	3
23	50	3
23	25	2
Salinomycin	66	2

marginal activity at its recommended use level of 66 ppm. Compound **23** has a half-life of 1.2 h and oral bioavailability of 84% in the chicken. In a preliminary study, drug resistance was not observed for the related *N*-methyl analog **12** after 11 cycles of application at 80 ppm against *E. tenella* species (T. Tamas, unpublished).

5. Conclusion

In conclusion, we have discovered 2-(4-fluorophenyl)-3-(4-pyridyl)-5-(piperidyl)pyrroles as very potent inhibitors of parasite PKG, with excellent activity against commercially important strains of *Eimeria* in chickens. Among these, the methyl, ethyl, and the methylazetidine substituted analogs **12, 23**, and **34** are potent and have a broad spectrum of activity. The *N*-ethyl piperidine analog **23** has excellent activity when administered at 50–125 ppm levels in feed in an in vivo spectrum model against eight of the most common *Eimeria* species. Sensitivity to current commercial anticoccidial agents is eroding. Based on these studies and ease of synthesis, analog **23** was chosen for a large-scale field trial as a potential novel anticoccidial agent. Results will be reported elsewhere.

Acknowledgments

The authors thank Dr. Phil Eskola, Dr. Gerard Kieczykowski, Amanda Makarewicz, Bob Frankshun and Glenn Reynolds for scale-up of key compounds, Dr. A. E. Weber for review of the manuscript, and Merck Research Laboratories Drug Metabolism and Analytical support group for pharmacokinetic studies and open access LC/MS.

References and notes

1. Hyatt, D. The U.S. Poultry Industry. Web version by the TAMU Poultry Network Administration Team 1995.
2. Cosgrove, T.; Shane, S. M. *Poultry* **2001**, *9*, 10.
3. Shumard, R. F.; Callender, M. E. *Poult. Sci.* **1969**, *48*, 1870.
4. McDougald, L. R. In *Coccidiosis of Man and Domestic Animals*; Long, P. L. Ed.; Boca Raton, FL, 1990; pp 307–320.
5. Mathis, G. F.; McDougald, L. R.; McMurray, B. *Avian Dis.* **1984**, *28*, 453.
6. Chapman, H. D.; Shirley, M. W. *Res. Vet. Sci.* **1989**, *46*, 114.
7. Jeffers, T.K., 1989 In *Vth International Coccidiosis Conference*; Ed. INRA Publ.; Tours, France, 1989; pp 295–308.
8. Rotibi, A.; McDougald, L. R.; Solis, J. *Avian Dis.* **1989**, *33*, 365.
9. Tamas, T.; Ostlind, D. A. *Poultry Sci.* **1994**, *73*, 15 (Abstr.).
10. Gurnett, A. M.; Liberator, P. A.; Dulski, P. M.; Salowe, S. P.; Donald, R. G. K.; Anderson, J. W.; Wiltsie, J.; Diaz, C. A.; Harris, G.; Chang, B.; Darkin-Rattray, S. J.; Nare, B.; Crumley, T.; Blum, P. S.; Misura, A. S.; Tamas, T.; Sardana, M. K.; Yuan, J.; Biftu, T.; Schmatz, D. M. *J. Biol. Chem* **2002**, *277*, 15913.
11. Wiersmaa, H. I.; Galuskaa, S. E.; Tomleyb, F. M.; Sibleyc, L. D.; Liberator, P. A.; Donald, R. G. K. *Int. J. Parasitol.* **2004**, *34*, 369.
12. de-Laszlo, S.; Visco, D.; Agarwal, L. *Bioorg. Med. Chem. Lett.* **1998**, *8*(19), 2689.