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Synthesis and SAR of 2-(4-fluorophenyl)-3-pyrimidin-4-ylimidazo[1,2-*a*]pyridine derivatives as anticoccidial agents

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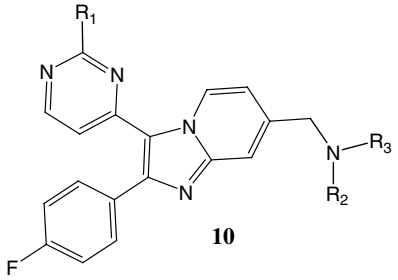
Abstract—Compounds **10a**–**10d** and **10i** are very potent inhibitors of *Eimeria tenella* cGMP-dependent protein kinase (0.081–0.32 nM) and are very efficacious antiparasitic agents in vivo when administered to chickens at 12.5–25 ppm levels in the feed.

Coccidiosis is the major cause of morbidity and mortality in the poultry industry. It is a disease of the avian intestinal lining due to invasion by protozoan parasites of the genus *Eimeria*. Some of the most significant *Eimeria* species in poultry are *E. tenella*, *E. acervulina*, *E. necartrix*, *E. brunetti*, and *E. maxima*. Over 35 billion chickens are raised annually worldwide and all major poultry operations use anticoccidial agents prophylactically. Resistance to current coccidiostats is becoming widespread[1] and new broad spectrum drugs with novel mechanisms are needed.



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The 2-(4-fluorophenyl)-3-pyrimidin-4-ylimidazo[1,2-*a*]-pyridine derivatives (**10**) are potent inhibitors of the *E. tenella* cGMP-dependent protein kinase[2] (Et-PKG). Genetic studies in *Toxoplasma gondii*, a protozoan parasite closely related to *Eimeria*, demonstrate that PKG is essential for survival and represents a desirable therapeutic target.[3] In this paper, the synthesis, SAR studies, and biological data in vitro and in vivo of modified imidazopyridine derivatives which are more potent than our previously reported pyrrole analogs[4] will be discussed.

Synthesis. The imidazopyridine derivatives in Tables 1 and 2[5] were prepared as shown in Scheme 1.[6] Methylation of 4-methyl pyrimidine-2-thiol **1** with DMF-DMA gave 4-methyl-2-(methylthio)pyrimidine **2**. The ketone**3** was made by coupling the anion generated from 4-methyl-2-(methylthio)pyrimidine **2** and lithium diisopropylamide with 4-fluorobenzoate in tetrahydrofuran. The bromo ketone **4** was made by brominating ketone **3** with tetra-*n*-butylammonium tribromide in methylene chloride. The imidazopyridine **5** was made by cyclization of bromo ketones **4** with 2-amino-4-hydroxymethylpyridine **6** by refluxing in ethanol. The primary alcohol **5** was oxidized to the sulfone **7** with oxone and displacement of the sulfone with amines or alcohols gave the pyrimidine **8**. The pyrimidine **8** was easily converted to the mesylate **9** and displaced with different amines to

Keywords: Kinase; Coccidiosis; Parasite; Imidazopyrimidine; PKG inhibitor.
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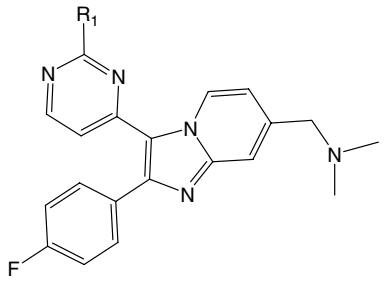
Table 1.



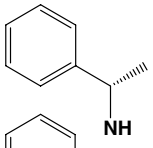
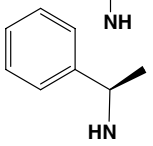
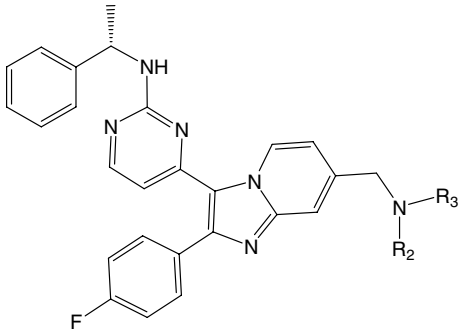
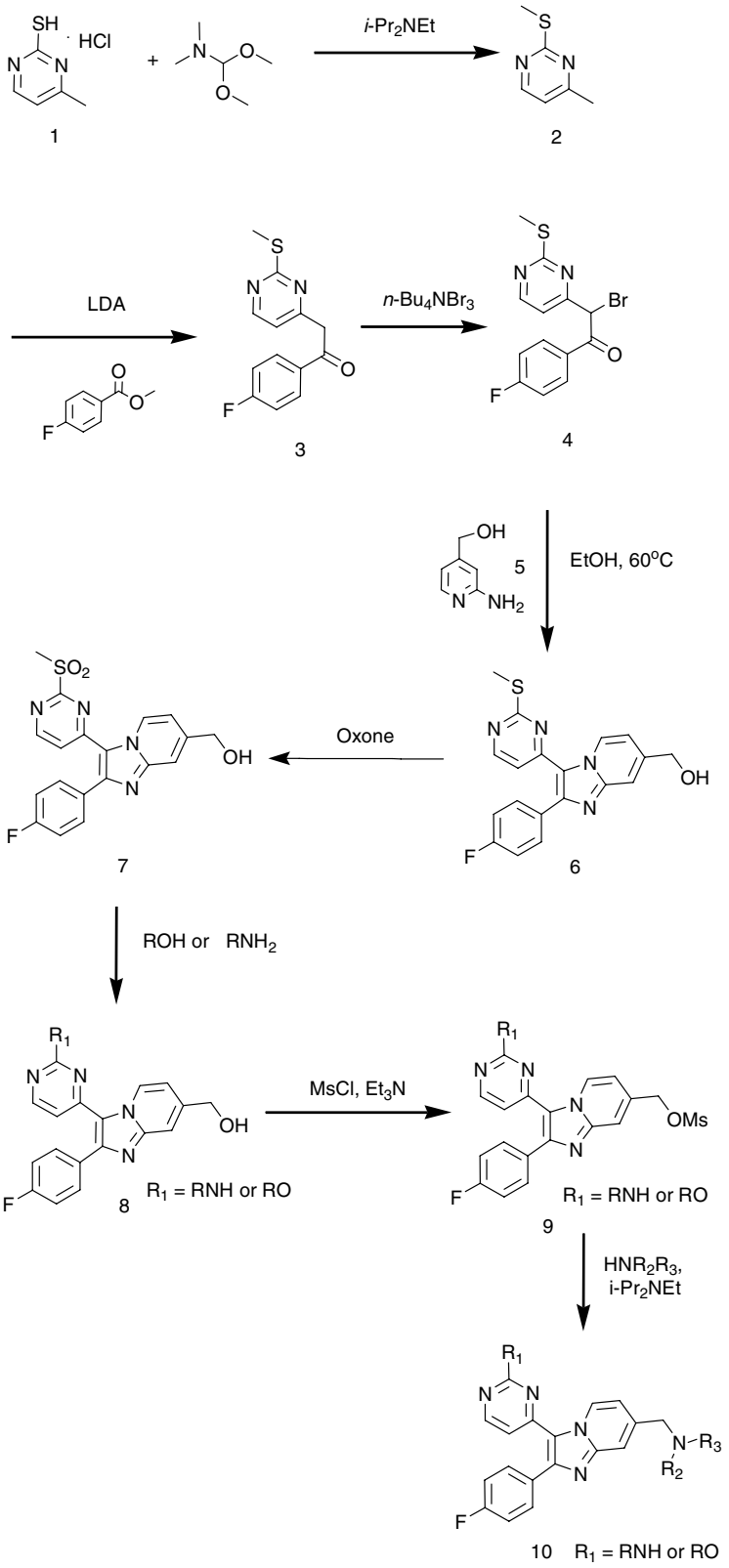
Compound	R_1	Ten_K IC_{50}(nM)	ppm	Et	Ea
10a	NH–Bn	0.081	25	3	3
10b	NH_2	0.11	12.5	3	3
10c	NHMe	0.17	25	3	3
10d		0.32	25	3	3
10e		0.47	25	3	0
10f	N(Me)–Bn	3.0	25	0	2
10g	OBn	0.27	50	0	0
10h	OMe	2.6	25	0	0

Table 2.



Compound	R_2	R_3	Ten_K IC_{50} (nM)	ppm	Et	Ea
10i	CH_2CH_2OH	Et	0.1	25	3	3
10j	CH_2CH_2OH	Me	0.11	25	3	0
10k	Et	Me	0.13	50	3	3
10l	Et	H	0.14	N.A.	N.A.	N.A.
10m	Me	H	0.16	25	0	2
10n	*t*-Bu	H	0.16	50	3	3
10o	Et	Et	0.16	25	3	0
10d	Me	Me	0.32	25	3	3
10p	OH	Me	0.81	50	3	0
10q	OMe	Me	0.93	25	2	0
10r	COMe	H	0.99	50	3	0
10s	H	H	1.4	50	3	0

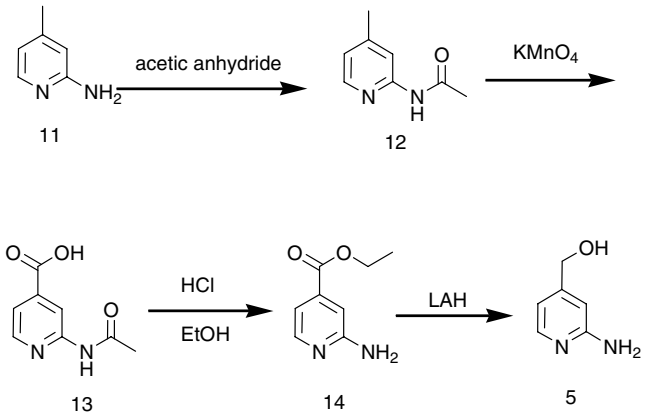
N.A., not available.



Scheme 1.

yield **10**. Compounds in Tables 1 and 2 were made in a similar manner.

Preparation of intermediate **5** is outlined in Scheme 2.[7] 2-Aminopicoline **11** was converted to **12** with acetic anhydride. Oxidation of the **12** by potassium permanganate gave the acid **13**. Bubbling of hydrochloric gas into ethanolic solution of the **13** provided the ester **14** and reduction of the **14** by lithium aluminum hydride yielded the alcohol **5**.



Scheme 2.

Biological assays. To evaluate these compounds as anticoccidial agents, an enzyme inhibition assay against *E. tenella* cGMP-dependent protein kinase (Et-PKG) was used as the initial in vitro screening assay; oocyst reduction against two major *Eimeria* species in poultry (*E. tenella* (E.t.) and *E. acervulina* (E.a.)) was used for in vivo assay. 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 the preceding paper.[4,6]

Results and discussion. The data shown in Table 1 summarize the effect of substituents in the 2-position of the pyrimidine ring on PKG activity and in vivo efficacy. In general, primary amine (**10b**) and secondary amines (**10a**, **10c**, and **10d**) in this position have excellent PKG activity (0.081–0.32 nM). However, a tertiary amine (**10f**) is at least 14-fold less potent. Steric interaction may not be very important in this position, since the 1-phenylethaneamine (**10d**) retains activity. Orientation of methyl group plays a very important role in the PKG activity since the *S* isomer (**10d**) is more potent than the *R* isomer (**10e**). Strong hydrogen bonding reduces the PKG activity as shown for the methoxy (**10h**) and benzyloxy (**10g**) analogs which are less potent than the methylamine (**10c**) and benzylamine (**10a**), respectively. Anticoccidial activity of **10a**–**10d** in vivo is better than that of **10e**–**10h**, and is consistent with PKG activity in vitro.

As shown in Table 2, basicity in the 7-position of imidazopyridine is very important for PKG activity. Substituents such as hydroxyl and methoxy amine (**10p** and **10q**), amide (**10r**), and primary amine (**10s**) are much less potent than the secondary and tertiary amines **10i**–**10o**. For active PKG inhibitors, in vivo efficacy is determined by many factors (including absorption in both host and parasites) that could be affected by the basicity and lipophilicity of the compounds. In general, for compounds listed in Tables 1 and 2, the in vivo activity tracks with the PKG activity fairly well. Potent PKG inhibitors (**10a**–**10d**, **10i**,**10k**, and **10o)** are active in in vivo assays when administered to birds in feed. The most potent analogs are **10a**–**10d** and **10i**. These compounds are active in feed at a level of 12.5–25 ppm. Commercial coccidiostats such as salinomycin and amprol are active at 66 and 130 ppm, respectively.

Conclusion. Novel imidazopyridine analogs were found to be potent inhibitors of parasite PKG activity. The most potent compounds are the tertiary amines **10a**–**10d** and **10i** (PKG activity 0.081–0.32 nM). These compounds were also fully active in in vivo assays as anticoccidial agents at 12.5–25 ppm level in feed.

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