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Synthesis and SAR studies of potent imidazopyridine anticoccidial agents

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Abstract—Diaryl imidazo[1,2-*a*]pyridine derivatives, such as **6a** and **7i**, have been synthesized and found to be potent inhibitors of parasite PKG activity. The most potent compounds are the 7-isopropylaminomethyl analog **6a** and 2-isopropylamino analog **7i**. These compounds are also fully active in in vivo assay as anticoccidial agents at 25 ppm in feed.

Coccidiosis is the major cause of morbidity and mortality in the poultry industry. Protozoan parasites of the genus *Eimeria*, which invade intestinal epithelial cells, are the causative agents.[1] The standard therapeutic practice used in commercial poultry operations to combat the disease is to include anticoccidial agents in the feed prophylactically. Polyether ionophore anticoccidial agents, discovered over 30 years ago, have been successfully used in this manner. However, this strategy has led to the erosion of efficacy and development of resistance in the field.[2] Accordingly, the need for novel anticoccidial chemotherapeutic options is considerable.

Previous work has demonstrated that small molecule inhibitors of *Eimeria tenella* cGMP-dependent protein kinase (Et-PKG) block parasite motility and host cell invasion in vitro, and prevent parasite infection and associated pathology in a chicken model of infection.[3,4] Analogs in the 2-(4-fluorophenyl)-3-pyrimidin-4-ylimidazo[1,2-*a*]pyridine structural series are among the most potent compounds identified to date.[5,6] In this report, we have extended our medicinal chemistry effort by incorporating various nitrogen containing substituents at the 7-position of the imidazopyridine ring and the 2-position of the pyrimidine ring.

Biological evaluation of compounds includes a measure of the ability to inhibit native Et-PKG enzyme activity (IC_{50}—compound required to inhibit enzyme activity by 50%), as well as their in vivo potency.[7] In the animal model, chickens are infected with either *Eimeria tenella* (Et) or *Eimeria acervulina* (Ea) parasites and treated with a compound (parts per million) mixed in the feed. Antiparasitic efficacy is measured as a function of parasite oocyst burden 7 days following the infection. Treatments resulting in reduction of oocyst burden by >80% are scored a '3', 50–79% reduction is scored a '2', and those treatments with <50% reduction of oocyst burden are scored a '0'.

Synthesis of the previously reported compound **1**[6,8] was scaled up and has served as an advanced intermediate in the synthetic process outlined in Scheme 1. Displacement of the sulfone group of **1** with an amine yielded the 2-aminopyrimidine **2**. The hydroxyl group of compound **2** was either oxidized by manganese (IV) oxide to carboxaldehyde **3**, or converted to mesylate **4** under standard conditions. Carboxaldehyde **3** was then converted to alkylamine derivatives **5** via reductive amination, whereas mesylate **4** was converted to alkylamine derivatives **5** by nucleophilic displacement. In most cases, the overall yields of the final products were comparable. In some cases, however, the reductive amination route works better, and in others, the mesylate displacement route. Because structural variability was introduced at the last step, a big diversified collection

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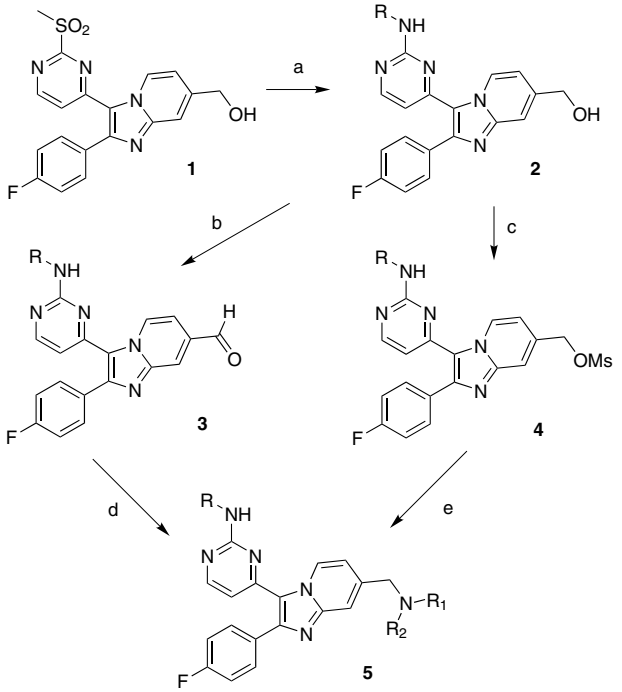


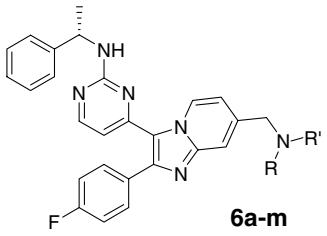
Scheme 1. Reagents and conditions: (a) R–NH_2; (b) MnO_2; (c) MsCl, Et_3N; (d) R_1R_2NH, $NaBH(OAc)_3$, HOAc; (e) R_1R_2NH.

of diaryl imidazo[1,2-*a*]pyridine derivatives was built rather efficiently for the SAR study.[9]

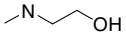
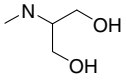
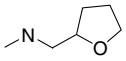
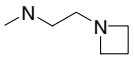
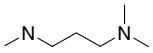
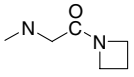
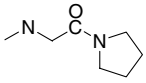
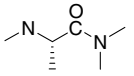
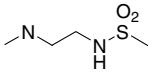
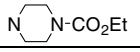
Table 1 summarized the SAR of various amino substituents on the 7 position of the imidazopyridine ring (compounds **6a**–**m**). The (*S*)-methyl benzylamino group was chosen as the fixed substitution at the 2 position of the pyrimidine ring based on results from earlier studies.[6] All but one compound (**6m**) showed subnanomolar IC_{50}'s for the inhibition of Et-PKG enzyme activity. In this group, compounds **6a** and **6h** are particularly potent with IC_{50} values approaching picomolar range. Compounds with small alkylamino substitutions are efficacious against both parasites in the chicken model of infection. Introduction of hydroxyl groups (**6d** and **6e**) had little effect on in vitro inhibition of Et-PKG enzyme activity, but reduced in vivo efficacy against *E. acervulina*. Similar observations were made on compounds with amino (**6g** and **6h**) or amido (**6i**–**l**) functionalities—in vitro potencies are comparable to simple alkylamino substituents, but in vivo activities against one or both parasitic species are compromised. Broad spectrum activity is a necessary requirement for the development of new anticoccidial agents, but historically has been a difficult objective to achieve.[2] The data here highlight an SAR trend that polar substituents, such as a hydroxyl group, tend to decrease efficacy against *E. acervulina*, whereas nonpolar substituents are more likely to impact efficacy against *E. tenella*.

Small alkylamino groups at the 7-position of the imidazopyridine ring are optimal for balanced in vitro and in vivo activities. Listed in Table 2 are compounds with other small alkylamino substituents at this position, as well as some modifications to the substituent

Table 1. Inhibition of Et-PKG enzyme activity and in vivo anticoccidial activity of diaryl imidazo[1,2-*a*]pyridine derivatives **6**

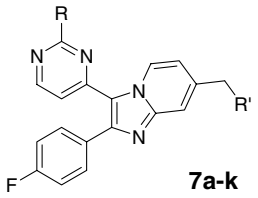


6a-m

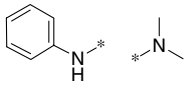
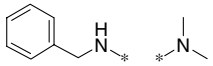
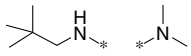
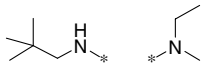
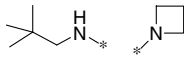
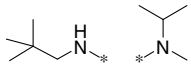
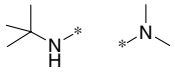
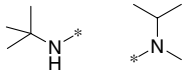
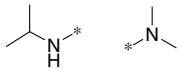
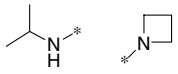
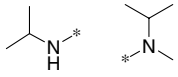
Compound	R–N–R′	Et-PKG IC_{50} (nM)	Dose (ppm)	Et score	Ea score
6a		0.05	25	3	3
6b		0.13	50	3	3
6c		0.11	25	0	3
6d		0.11	25	3	0
6e		0.15	50	3	0
6f		0.27	25	3	0
6g		0.10	50	3	0
6h		0.07	12.5	0	2
6i		0.57	50	0	0
6j		0.39	50	3	0
6k		0.26	25	2	2
6l		0.15	25	3	0
6m		5.6	50	0	0

on the 2-position of the pyrimidine ring. Consistent with our earlier findings,[6] aniline (**7a**) and benzylamino (**7b**) substituents at the 2-position of the pyrimidine ring are preferred in terms of inhibition of Et-PKG activity, with IC_{50} values approaching picomalor range. When these aromatic groups are replaced by alkyl substituents, in vitro potencies as inhibitors of Et-PKG enzyme activity decrease somewhat. Nevertheless, these alkyl analogs are still highly potent Et-PKG inhibitors with most IC_{50} values in the sub-nanomolar range. Only **7h** and **7k** have IC_{50} values >1 nM. Both of these compounds have an isopropylmethylamino substituent at the 7-position of the imidazopyridine ring.

Table 2. Inhibition of Et-PKG enzyme activity and in vivo anticoccidial activities of diaryl imidazo[1,2-*a*]pyridine derivatives **7**



7a-k

Compound	R,R′	Et-PKG IC_{50} (nM)	Dose (ppm)	Et score	Ea score
7a		0.06	12.5	3	0
7b		0.081	25	3	3
7c		0.28	25	3	0
7d		0.29	25	3	0
7e		0.45	25	0	0
7f		0.60	25	0	0
7g		0.31	—	n/a	n/a
7h		2.8	—	n/a	n/a
7i		0.20	25	3	3
7j		0.86	—	n/a	n/a
7k		1.3	—	n/a	n/a

n/a, date not available.

While the majority of these compounds are potent inhibitors of Et-PKG enzyme activity, many showed little to no control of parasite oocyst production in the animal model. In vivo efficacy following oral dosing in the feed is dependent upon many additional factors, including oral bioavailability in chicken, and the ability of the compound to access the intracellular enzyme target in the parasites. One notable exception in Table 2 is compound **7i**. While compound **7i** is a less potent Et-PKG inhibitor than compound **7b** (by about 2.5 times), the two compounds have similar in vivo efficacy in the animal infection model, and reached total control of oocyst production at levels of 25 ppm in feed.

Further evaluation of compounds **6a** and **7i** against other *Eimeria* spp. of parasites has pointed to holes in the spectrum of antiparasitic activity. For example, neither compounds offer good control of oocyst production in *Eimeria maxima* or *Eimeria brunette* infected chickens (data not shown). Broad spectrum activity against seven commercially important species of chicken Eimeria is an absolute requirement for any new anticoccidial agent and remains a significant challenge for medicinal chemistry.[2]

In summary, this SAR study on diaryl imidazo[1,2-*a*]pyridine derivatives demonstrated that simple alkylamino substituents at the 7-position of the imidazopyridine ring with an aromatic group on the 2-aminopyrimidine ring (e.g., **6a** and **7a**) improve the potency of inhibitory activity against Et-PKG, with IC_{50} values approaching picomolar range. Meanwhile improvement of in vivo anticoccidial activities was observed not only with a benzyl group on the 2-aminopyrimidine ring (**6a**

and **7b**) but also with an isopropyl group (**7i**) at this position. Two of the major *Eimeria* species (*E. tenella* and *E. acervulina*) are effectively controlled at the level of 25 ppm in feed. Lack of broader spectrum activity against other *Eimeria* spp. has prevented further development of these compounds.

Acknowledgments

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