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Synthesis and biological activity of anticoccidial agents: 5,6-Diarylimidazo[2,1-*b*][1,3]thiazoles

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ABSTRACT

Novel 5,6-diarylimidazo[2,1-*b*][1,3]thiazoles bearing an amine substituent at the imidazothiazole 2-position have been synthesized and evaluated as anticoccidial agents in both in vitro and in vivo assays. Both subnanomolar in vitro activity and broad spectrum in vivo potency were detected for several compounds, particularly compound **10**.

Coccidiosis is a parasitic disease that 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 Apicomplexan protozoan parasites of the genus *Eimeria*.[1] The most significant *Eimeria* species in poultry include *E. tenella*, *E. acervulina*, *E. mitis*, and *E. maxima*. Over 35 billion chickens are raised annually worldwide, and all major poultry operations use anticoccidial agents as prophylactics, such as polyether ionophores. Nevertheless, resistance to current coccidiostats has become widespread,[2] creating the need for new broad spectrum drugs with novel mechanisms of action.

Recently, we have reported on novel anticoccidial agents with potent in vitro and in vivo activity against *Eimeria* parasites. Reduction of parasite growth by these compounds was found to be due to the inhibition of parasite-specific cGMP-dependent protein kinase (PKG), a serine and/or threonine protein kinase.[3,4] In particular, we have found that various 2,3-diarylpyrroles[5–8] and 2,3-diarylimidazopyridines[9–13] show exceptional potency as anticoccidial agents.

In this paper, we present the synthesis and biological activity of a series of 5,6-diarylimidazo[2,1-*b*][1,3]thiazoles to see how such compounds compared with the pyrroles and imidazopyridines. Optimal substituents from these two series included a 4-fluorophenyl ring at the core heterocycle 2-position, a pyrimidin-4-yl, 2-aminopyrimidin-4-yl, or pyridin-4-yl ring at the core heterocycle 3-position, and an amine-bearing side chain at the pyrrole 5-position or imidazopyridine 7-position. We therefore used this functionality to explore the imidazo[2,1-*b*][1,3]thiazole template.

Scheme 1 depicts the synthesis of imidazothiazoles bearing a 2-aminopyrimidin-4-yl or pyrimidin-4-yl ring at the imidazothiazole 5-position. Treatment of 4-fluorophenacyl bromide with methyl 2-aminothiazole-5-carboxylate yielded imidazothiazole **1**. Subsequent treatment of **1** with DIBAL reduced the ester completely to the corresponding alcohol **2**, which was then refluxed in acetic anhydride with catalytic sulfuric acid to afford diacylated product **3**. DMFDMA then selectively reacted with the ketone and not the ester of **3** to give *N,N*-dimethylaminoenone **4**. Treatment of **4** with either guanidine–HCl or formamidine–HCl under basic conditions yielded 5-(2-aminopyrimidin-4-yl)imidazothiazole **5** and 5-(pyrimidin-4-yl)imidazothiazole **6**, respectively. Oxidation with manganese(IV) oxide then gave aldehydes **7** and **8**. Reductive amination of the 5-(2-aminopyrimidin-4-yl) substrate with either dimethylamine or 1-methylpiperazine gave the corresponding amine products **9** and **10**, while reductive amination of the pyrimidin-4-yl substrate was carried out with 1-methylpiperazine only to give benzylic piperazine **11**.

Synthesis of the corresponding 5-(pyridin-4-yl)imidazothiazoles is shown in Scheme 2. Introduction of the amine side chain in this case worked best when taking place prior to installation of the 5-(pyridin-4-yl) ring. An alternative approach for converting a benzylic alcohol to an amine was implemented here, entailing mesylation of alcohol **2** to mesylate **12**, followed by nucleophilic

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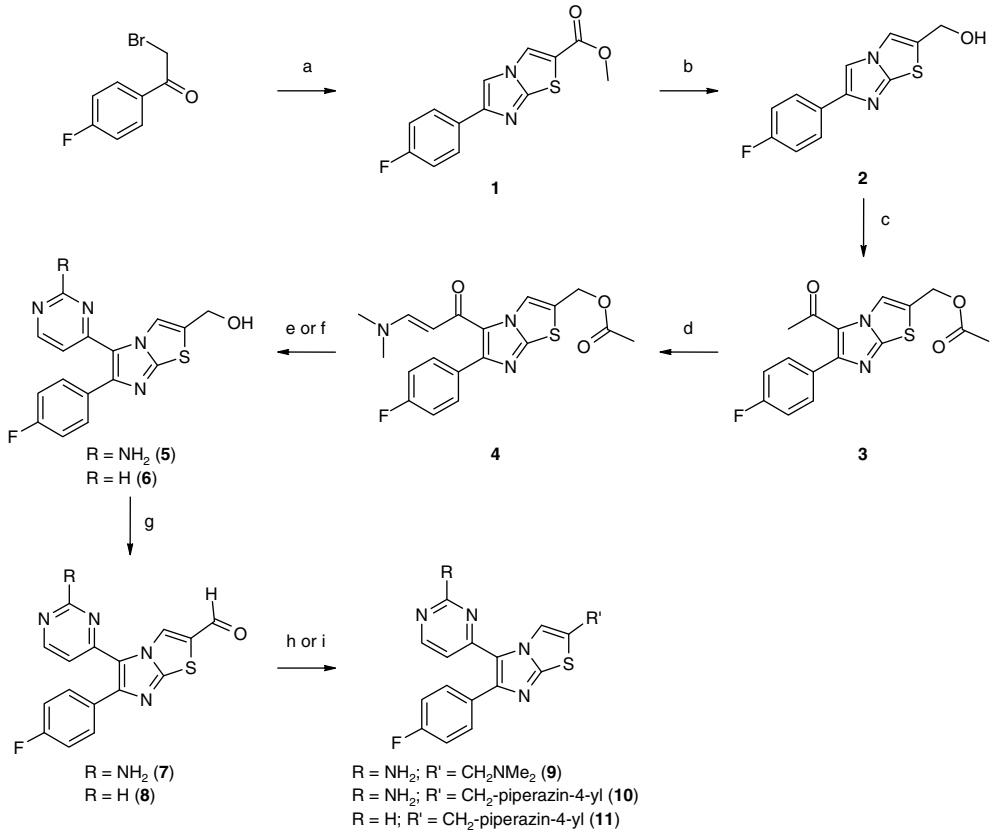
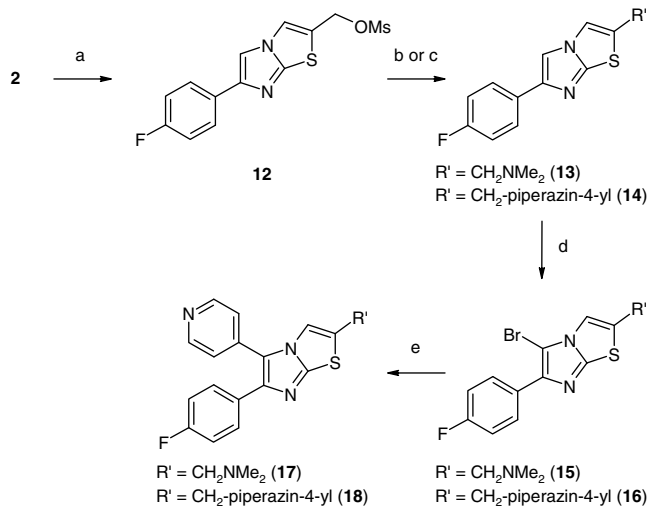


Scheme 1. Reagents and conditions: (a) methyl 2-aminothiazole-5-carboxylate, EtOH, 60 °C; (b) DIBAL, CH_2Cl_2, 0 °C to rt; (c) Ac_2O, H_2SO_4, reflux; (d) DMFDMA, reflux; (e) guanidine–HCl, NaOMe, 1-propanol, reflux; (f) formamidine–HCl, NaOMe, 1-propanol, reflux; (g) MnO_2, CH_2Cl_2; (h) $HNMe_2$, $NaB(OAc)_3H$, CH_2Cl_2; (i) 1-Me-piperazine, $NaB(OAc)_3H$, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) MsCl, Et_3N, THF; (b) $HNMe_2$, THF; (c) 1-Me-piperazine, THF; (d) NBS, CH_2Cl_2; (e) pyridine-4-boronic acid, $Pd(PPh_3)_4$, XANTPHOS, Na_2CO_3, 2:1 1,4-dioxane:water, 90 °C.

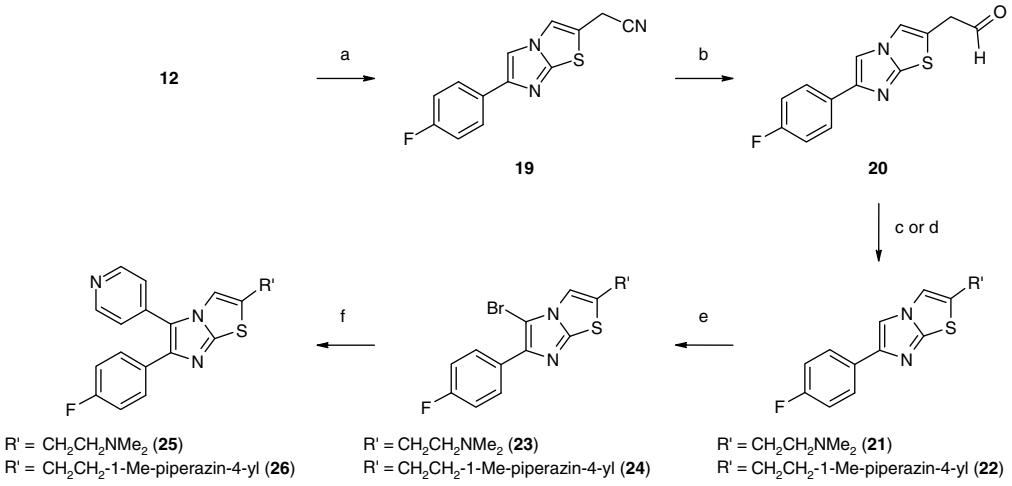
displacement by either dimethylamine or 1-methylpiperazine to give amine products **13** and **14**, respectively. Regioselective bromination at the imidazothiazole 5-position proceeded with *N*-bromosuccinimide to give bromides **15** and **16**, which were ultimately subjected to Suzuki coupling conditions with pyridine-4-boronic acid to give 5-(pyridin-4-yl)imidazothiazoles **17** and **18**.

The 5-(pyridin-4-yl) series was expanded to include amine side chains homologated relative to compounds **17** and **18**, shown in Scheme 3. Treatment of mesylate **12** with tetra-*n*-butylammonium cyanide gave nitrile **19**, which was reduced with DIBAL to yield aldehyde **20**. Subsequent reductive amination with either dimethylamine or 1-methylpiperazine afforded amine products **21** and **22**, respectively. Subsequent bromination and Suzuki coupling as described before gave bromides **23** and **24**, and then 5-(pyridin-4-yl)imidazothiazoles **17** and **18**.

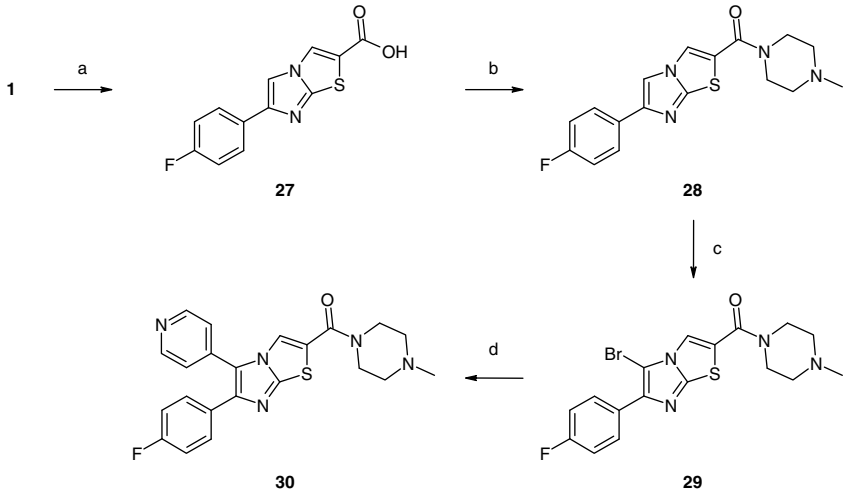
Schemes 4 and 5 show the synthesis of analogs of compound **18** bearing modification to the imidazothiazole 2- and 3-positions, respectively. Synthesis of the benzamide analog of **18** is shown in Scheme 4. Hydrolysis of ester **1** under basic conditions yielded carboxylic acid **27**, which was then coupled with 1-methylpiperazine to afford amide **28**. Subsequent bromination and Suzuki coupling as described before gave bromide **29**, and then 5-(pyridin-4-yl)imidazothiazole **30**.

Synthesis of the 3-methylimidazothiazole analog of **18** is shown in Scheme 5. Treatment of 4-fluorophenacyl bromide with ethyl 2-amino-4-methylthiazole-5-carboxylate yielded imidazothiazole **31**. DIBAL reduction afforded alcohol **32**, which was then oxidized with manganese(IV) oxide to give aldehyde **33**. Reductive amination with 1-methylpiperazine gave amine target **34**, which was treated with *N*-bromosuccinimide to give bromide **35**. Suzuki coupling with pyridine-4-boronic acid ultimately gave 5-(pyridin-4-yl)imidazothiazole **36**.

Table 1 presents both in vitro and in vivo biological data of each compound tested.[14] In vitro activity was assessed by measuring compound inhibition of native *E. tenella* (E_t) PKG enzyme activity, and is reported as an IC_{50}. In vivo activity was determined by administering each compound orally in feed, and then ranking each for anticoccidial activity using a 7 day efficacy model. A quantitative measure of *E. tenella* (E_t), *E. acervulina*



Scheme 3. Reagents and conditions: (a) n-Bu_4NCN, CH_2Cl_2; (b) DIBAL, CH_2Cl_2; (c) $HNMe_2$, $NaB(OAc)_3H$, CH_2Cl_2; (d) 1-Me-piperazine, $NaB(OAc)_3H$, CH_2Cl_2; (e) NBS, CH_2Cl_2; (f) pyridine-4-boronic acid, $Pd(PPh_3)_4$, XANTPHOS, Na_2CO_3, 2:1 1,4-dioxane:water, 90 °C.



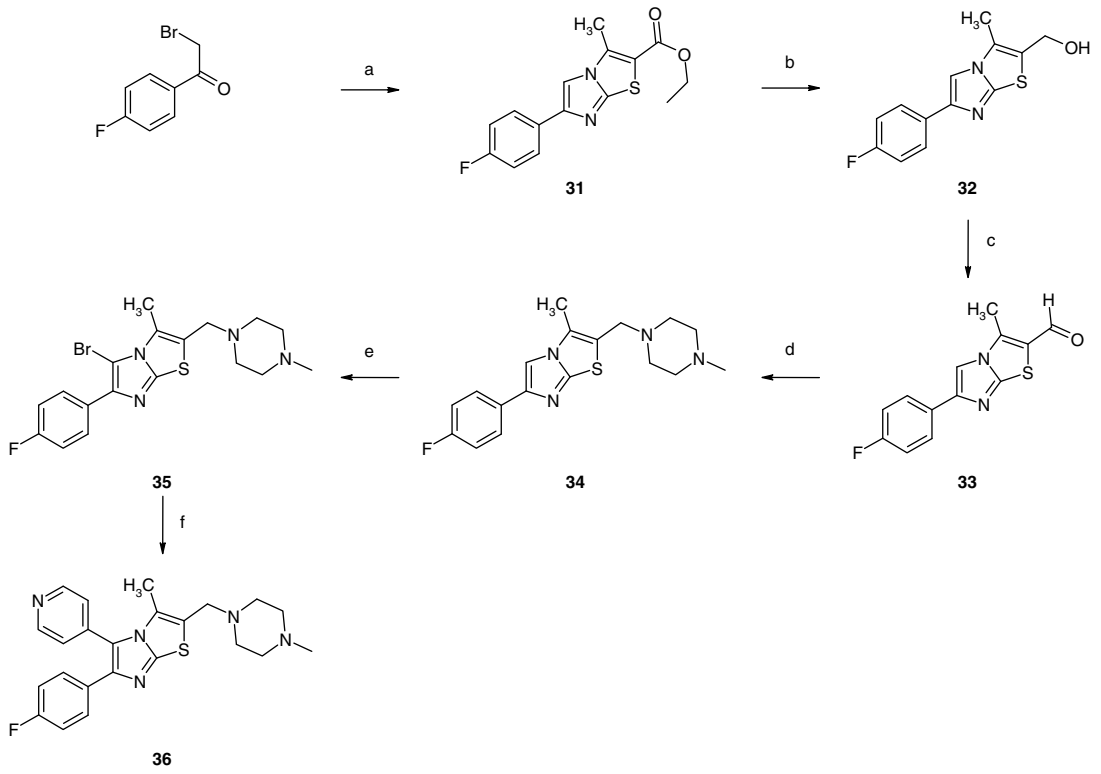
Scheme 4. Reagents and conditions: (a) NaOH, CH_3OH, 60 °C; (b) 1-Me-piperazine, EDC–HCl, Et_3N, CH_2Cl_2; (c) NBS, CH_2Cl_2; (d) pyridine-4-boronic acid, $Pd(PPh_3)_4$, XANTPHOS, Na_2CO_3, 2:1 1,4-dioxane:water, 90 °C.

(E_a), *E. mitis (E_{mi})*, and *E. maxima (E_{ma})* oocyst shedding from infected birds provided an assessment of antiparasitic activity. Treatments resulting in reduction of oocyte burden by 100% are scored a '4', those with 99% reduction are scored a '3+', those with 80–98% reduction are scored a '3', those with 50–79% reduction are scored a '2', and those with <50% reduction are scored a '0'.

Regarding in vitro activity, it is seen that subnanomolar activity was achieved with the 5-(2-aminopyrimidin-4-yl) compounds bearing either a benzylic dimethylamine or 1-methylpiperazine side chain (**9** and **10**, respectively), as well as the 5-(pyridin-4-yl) compound bearing a benzylic 1-methylpiperazine (**18**). Within the 5-(2-aminopyrimidin-4-yl) and 5-(pyridin-4-yl) families, the most tightly binding compounds possess a piperazine ring in the side chain. Compounds bearing an amide at the benzylic carbon (**30**), or a methyl group at the 3-position (**36**), either of which can alter the most stable conformation of the key pharmacophore sites of the molecule, were considerably less potent.

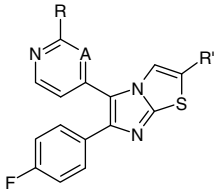
In vivo activity was optimal in compounds bearing a benzylic piperazine ring, suggesting that the most external nitrogen is likely the more important of the two basic nitrogens on the piperazine ring, and that the optimal distance between this amine nitrogen and the imidazothiazole 2-position is five atoms. The limited entropic freedom of this nitrogen, enforced by the piperazine ring, may also be important for in vivo activity. The most potent compounds include benzylic piperazines **10** and **11**, which were strongly active against all four species of *Eimeria* tested, as well as benzylic piperazine **18**, which was strongly active against three out of four species of *Eimeria* tested.

In conclusion, we have prepared several novel 5,6-diaryl-2-substituted imidazo[2,1-*b*][1,3]thiazoles, three of which show subnanomolar potency in vitro against *E. tenella* cGMP-dependent protein kinase (PKG), and three of which show strong in vivo potency against at least three of the four species of *Eimeria* tested. Of these, 5-(2-aminopyrimidin-4-yl)-2-(CH_2-1-methylpiperidin-4-yl)imidazo[2,1-b][1,3]thiazole **10** also showed significant activity versus *E. tenella*, *E. acervulina*, *E. maxima*, and *E. mitis*, respectively, when tested at 25 ppm (4,4,4,4), 12.5 ppm (4,4,4,0), and 6.25 ppm (4,4,4,0). This level of potency compares with that of our most potent imidazopyridine (IC_{50} = 0.044 nM, with 6.0 ppm scores of 4,3,3,4).[13] Hence, the 5,6-diarylimidazo[2,1-*b*][1,3]thiazole family holds significant promise for the treatment of coccidiosis.



Scheme 5. Reagents and conditions: (a) ethyl 2-amino-4-methylthiazole-5-carboxylate, EtOH, 60 °C; (b) DIBAL, CH_2Cl_2, 0 °C to rt; (c) MnO_2, CH_2Cl_2; (d) 1-Me-piperazine, $NaB(OAc)_3H$, CH_2Cl_2; (e) NBS, CH_2Cl_2; (f) pyridine-4-boronic acid, $Pd(PPh_3)_4$, XANTPHOS, Na_2CO_3, 2:1 1,4-dioxane:water, 90 °C.

Table 1
E_t PKG inhibition and in vivo anticoccidial activity of 5,6-diaryl-2-substituted imidazo[2,1-*b*][1,3]thiazoles



Compound	R	A	R′	E_t PKG IC_{50} (nM)	Anticoccidial activity at 50 ppm			
					E_t	E_a	E_{mi}	E_{ma}
9	NH_2	N	CH_2NMe_2	0.9	3+	4	2	0
10	NH_2	N	CH_2-1-Me-piperazin-4-yl	0.08	4	4	4	4
11	H	N	CH_2-1-Me-piperazin-4-yl	1.04	4	3+	4	4
17	H	CH	CH_2NMe_2	22	3	2	0	0
18	H	CH	CH_2-1-Me-piperazin-4-yl	0.7	3+	4	3	0
25	H	CH	$CH_2CH_2NMe_2$	10.1	2	2	3+	0
26	H	CH	CH_2CH_2-1-Me-piperazin-4-yl	2.5	3+	3	0	2
30	H	CH	C(=O)-1-Me-piperazin-4-yl	100	Not tested			
36	H	CH	CH_2-1-Me-piperazin-4-yl*	>100	Not tested			

* Imidazothiazole core bears a methyl substituent at the 3-position.

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