Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2479–2483

Synthesis and SAR studies of very potent imidazopyridine antiprotozoal agents

Tesfaye Biftu,[a,*] Dennis Feng,[a] Michael Fisher,[a] Gui-Bai Liang,[a] Xiaoxia Qian,[a] Andrew Scribner,[c] Richard Dennis,[c] Shuliang Lee,[c] Paul A. Liberator,[b] Chris Brown,[b] Anne Gurnett,[b] Penny S. Leavitt,[b] Donald Thompson,[b] John Mathew,[b] Andrew Misura,[b] Samantha Samaras,[b] Tamas Tamas,[b] Joseph F. Sina,[d] Kathleen A. McNulty,[d] Crystal G. McKnight,[d] Dennis M. Schmatz[b] and Matthew Wyvratt[a]

[a]*Merck Research Laboratories, Department of Medicinal Chemistry, Merck and Co., Inc, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Human and Animal Infectious Disease Research, Merck and Co., Inc, PO Box 2000, Rahway, NJ 07065, USA*
[c]*Scynexis, Inc, PO Box 12878, Research Triangle Park, NC 27709-2878, USA*
[d]*Merck Research Laboratories, Department of Genetic and Cellular Toxicology, West Point, PA 19486, USA*

Received 29 December 2005; revised 20 January 2006; accepted 20 January 2006
Available online 7 February 2006

Abstract—Compounds **10a** (IC_{50} 110 pM) and **21** (IC_{50} 40 pM) are the most potent inhibitors of *Eimeria tenella* cGMP-dependent protein kinase activity reported to date and are efficacious in the in vivo antiparasitic assay when administered to chickens at 12.5 and 6.25 ppm levels in the feed. However, both compounds are positive in the Ames microbial mutagenesis assay which precludes them from further development as antiprotozoal agents in the absence of negative lifetime rodent carcinogenicity studies.

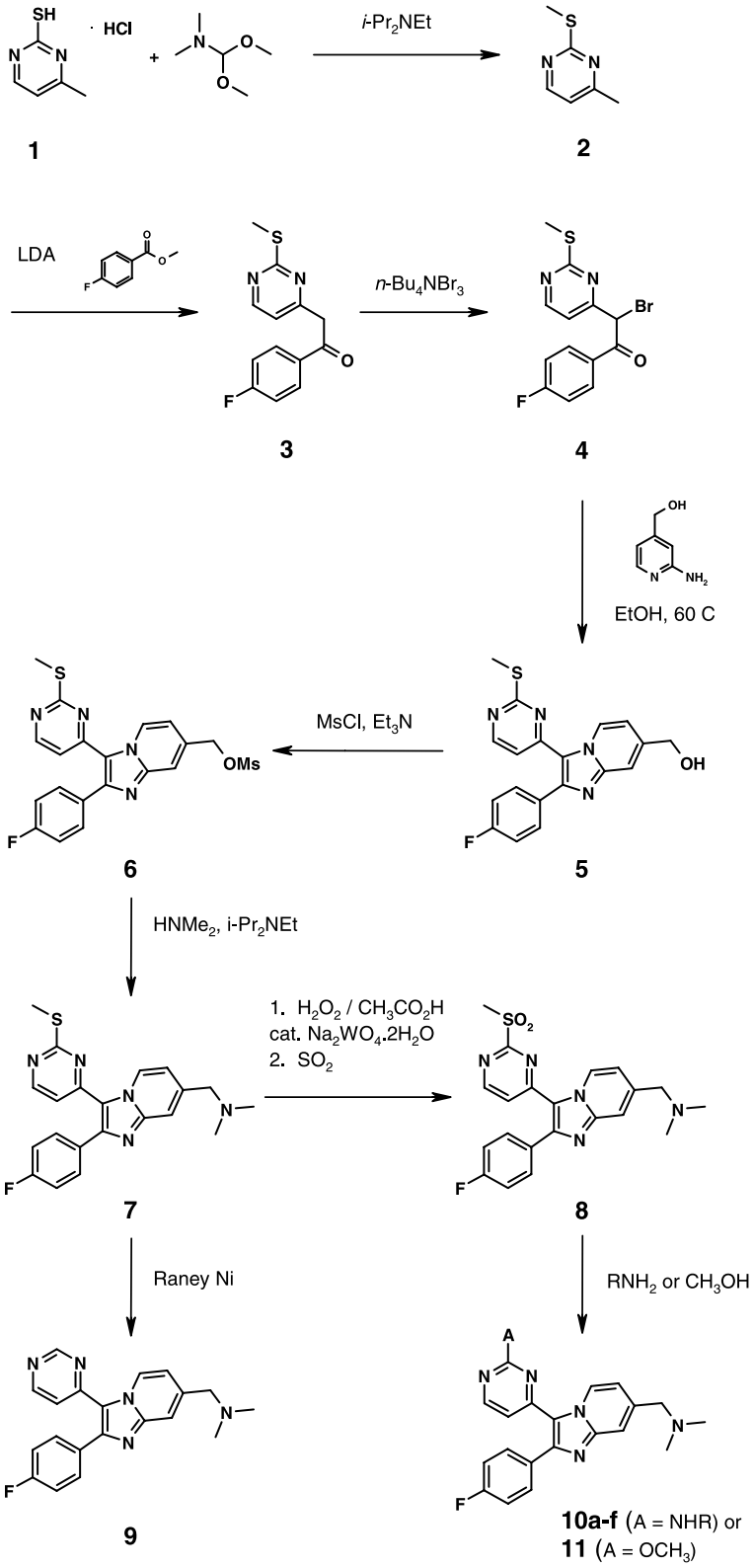
Human diseases such as malaria and toxoplasmosis are caused by the Apicomplexan parasites *Plasmodium falciparum* and *Toxoplasma gondii*, respectively. Coccidiosis is caused by the related Apicomplexans, the *Eimeria* species of parasites.[1] The intestinal pathology due to Eimeria infections in chickens is of major importance to the poultry industry, leading to considerable economic losses through morbidity and mortality. Polyether ionophore anticoccidials, discovered over thirty years ago, have been successfully used as prophylactic agents to combat coccidiosis. However, resistance to these and other existing anticoccidials has been observed and new therapeutic agents with novel mechanisms of action are needed. Recently, we reported on a novel anticoccidial agent[2] with potent in vitro and in vivo activity against *Eimeria* parasites. It was determined that inhibition of parasite growth by these compounds was due to the inhibition of parasite specific cGMP-dependent protein kinase (PKG), a serine and/or threonine protein kinase.[3,4]

In this paper, we describe the synthesis and structure–activity relationships of very potent imidazopyridine inhibitors of PKG activity, compounds which are more active than our previously reported pyrrole analogs.[2] Inhibition of native *Eimeria tenella* PKG enzyme activity was used as an initial screen for compound evaluation. Second, compounds administered orally in feed were ranked for anticoccidial activity using a seven day in vivo efficacy model. A quantitative measure of *E. tenella* and *Eimeria acervulina* oocyst shedding from infected birds served as a measure of antiparasitic activity. Details of these procedures and rules of scoring have been described.[2]

The synthesis of imidazopyridine analogs with various substituents is outlined in Scheme 1. The ketone **3** was made by coupling the anion generated from 4-methyl-2-methylsulfanylpyrimidine **2** and lithium diisopropylamide with methyl 4-fluorobenzoate in tetrahydrofuran. The bromo ketone **4** was made by brominating ketone **3** with tetra-*n*-butylammonium tribromide in methylene

Keywords: Protein kinase G; Antiprotozoan; Anticoccidial; Imidazopyridine.
* Corresponding author. Tel.: +1 732 594 6514; e-mail: Tesfaye_Biftu@merck.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.01.092

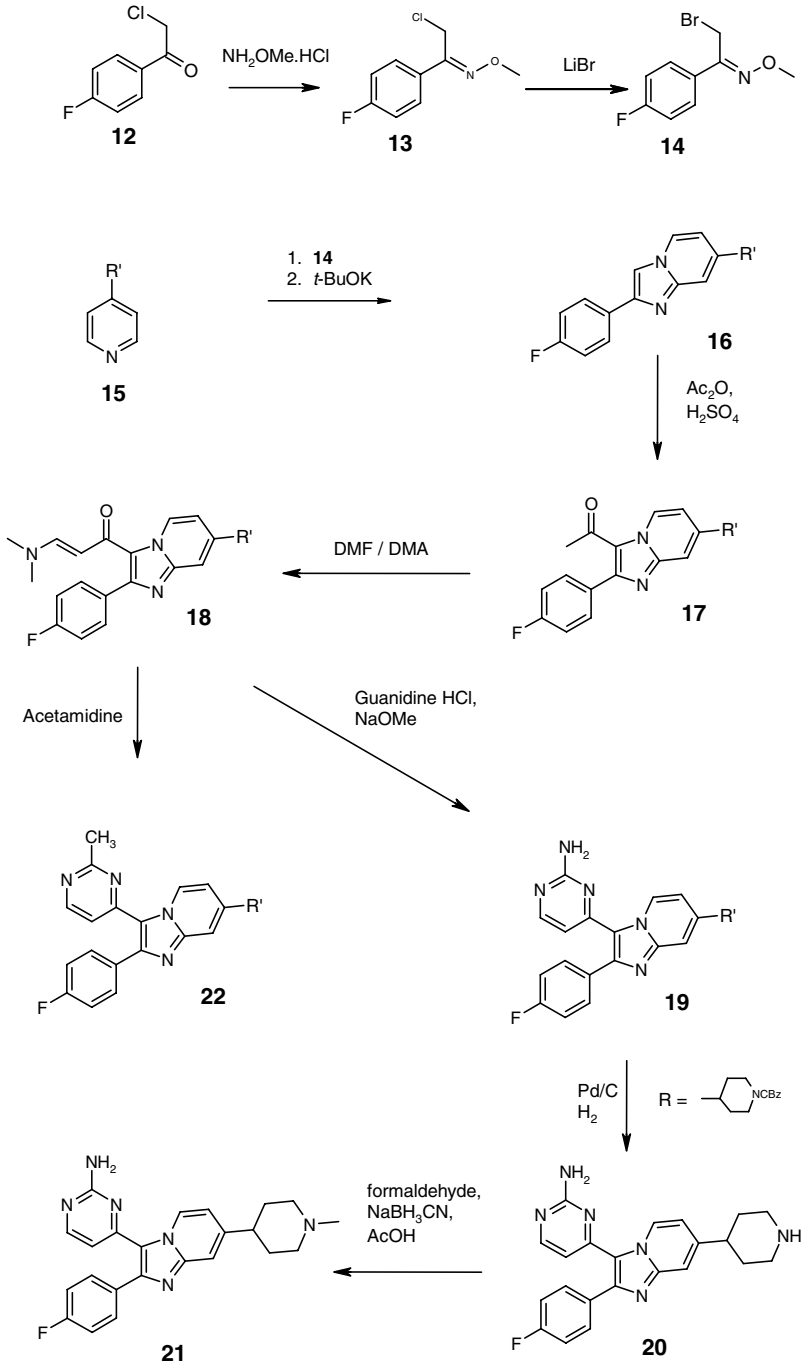


Scheme 1.

chloride. The imidazopyridine **5** was made by cyclization of bromo ketones **4** with 2-amino-4-hydroxymethylpyridine in refluxing ethanol. The primary alcohol **5** was easily converted to the mesylate **6** and displaced with dimethylamine to yield tertiary amine **7**. Removal of the methylthio group with Raney-nickel gave the pyrimidine **9**. However, oxidation of **7** with hydrogen peroxide to the sulfone **8** and displacement of the sulfone with ammonia gave the amino pyrimidine **10a**. Compounds **10b–f** and **11**, were made in a similar manner.

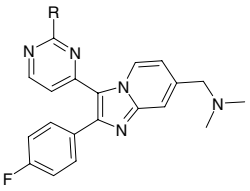
A different method of preparing imidazopyridines is shown in Scheme 2. Oxime **14**, made from α-chloro ketone **12**, was cyclized with a pyridine analog **15** in the presence of potassium *tert*-butoxide to afford imidazopyridine **16.** Acylation at the 3-position of **16** with acetic acid–sulfuric acid yielded **17**, which was treated with DMF–DMA to form enone **18.** Finally **18** was reacted with guanidine and sodium methoxide to afford the core structure **19**. When R′ is 4-CBz protected piperidine, deprotection of the CBZ group by hydrogenolysis gave **20**, which was then alkylated with formaldehyde and sodium cyanoborohydride to give the desired product **21**. Analogs **22**–**24** were made in a similar manner. Conversion of alcohol **24** to the corresponding mesylate and displacement with ammonia or methyl amine gave **25** and **26**, respectively.

The mutagenesis assays were conducted using a plate incorporation protocol.[5,6] The mixture containing bacterial cells (10^8) in 2 mL molten agar, 0.1 mL of test article in dimethylsulfoxide, and 0.5 mL cofactor/with or without S9 mix was poured in triplicate onto plates containing 25 mL of hardened agar. The liver S9 fraction prepared from phenobarbital and β-naphthoflavone-



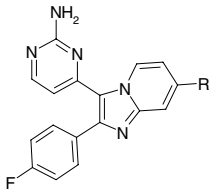
Scheme 2.

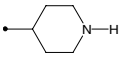
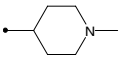
Table 1.



Compound[7]	R	Ten_K IC_{50} (nM)	Dose (ppm)	Et score	Ea score
9	H	1.9	25	0	0
10a	NH_2		12.5	3	3
		0.11	6.25	2	2
10b	NHMe	0.17	25	3	3
10c	NMe_2	2.5	25	0	0
10d	NH–*n*Pr	0.13	12.5	3	3
10e	NH–neopentyl	0.15	12.5	3	3
10f	NH–Bn	0.081	25	3	3
11	OMe	2.6	25	0	0

Table 2.



Compound[7]	R	Ten_K IC_{50} (nM)	Dose (ppm)	Et score	Ea score
20		0.05	25	0	0
			6.25	3	3
21		0.04	2.5	2	2
22	Cl	2.7	25	0	0
23	CN	11.3	25	0	0
24	CH_2OH	2.7	25	0	0
25	CH_2NH_2	0.18	25	0	0
26	CH_2NHMe	0.15	25	0	0

induced male Sprague–Dawley rats was present at 50 μL/plate. The plates were incubated at 37 °C for 48 h prior to counting colonies in an automated colony counter. The number of revertant colonies is given as mean ± standard deviation ($n = 3$).

The data presented in Table 1 summarize the effect of substituents in the 2-position of the pyrimidine ring on PKG activity and in vivo efficacy. Inhibition of PKG enzyme activity (the Ten_K assay) is reported as the amount of compound required to inhibit activity by 50% (IC_{50}—nM). The antiparasitic activity of compounds administered to chickens (parts per million in feed) against *E. tenella* (Et) and *E. acervulina* (Ea) is graded based on percent oocyst reduction. Treatments which provide ⩾80% reduction in oocyst production are scored with a '3', 50–79% reduction are scored a '2', and those treatments with <50% reduction are rated '0'. In general, primary amines **10a** and secondary amines **10b** in this position retain PKG activity (110 and 170 pM). By way of comparison, the tertiary amine **10c** is an order of magnitude less potent. Since the neopentyl **10e** and benzyl **10f** amines retain activity, steric interaction is not very important in this position. However, hydrogen bonding could be quite important since the hydrogen analog **9** and the methoxy analog **11** are over an order of a magnitude less active as PKG inhibitors than the primary and secondary amines. As shown in Table 2, a basic substituent in the 7-position of the imidazopyridine is very important for PKG activity.

Table 3.

Compound	μg/plate	Number of revertants/plate *Salmonella typhimurium*				*E. coli* WP2 uvrA pKM101
		TA100	TA1535	TA97a	TA98	
10a	0	188.0 ± 19.7	19.2 ± 4.1	315.8 ± 29.5	50.2 ± 8.2	224.0 ± 19.2
	30	223.3 ± 18.6	18.7 ± 3.5	350.7 ± 11.7	**124.7 ± 9.3**	233.3 ± 12.6
	100	241.7 ± 33.5	19.7 ± 5.1	401.7 ± 10.5	**204.3 ± 4.0**	265.3 ± 23.7
	300	362.3 ± 25.1	18.7 ± 2.9	455.3 ± 8.7	**353.0 ± 42.0**	254.3 ± 23.5
	1000	**473.7 ± 34.4**	18.3 ± 5.1	495.0 ± 17.4	**573.7 ± 42.9**	241.7 ± 12.9
2-Aminoanthracene	1	**634.3 ± 53.3**	**86.7 ± 7.1**	**750.7 ± 40.3**	**364.0 ± 65.8**	**449.3 ± 7.4**
	2	**1428.0 ± 78.0**	**188.0 ± 7.2**	**1435.0 ± 51.3**	**1068.7 ± 108.2**	**1450.0 ± 78.6**
21	0	172.3 ± 14.4	26.0 ± 5.5	252.1 ± 27.9	37.4 ± 6.6	152.8 ± 12.0
	30	215.0 ± 22.3	23.0 ± 2.0	294.7 ± 7.4	54.0 ± 6.0	156.7 ± 4.6
	100	235.0 ± 11.8	21.7 ± 10.6	289.3 ± 14.3	61.3 ± 20.5	168.7 ± 26.0
	300	**363.0 ± 33.8**	23.7 ± 3.5	298.0 ± 19.7	67.7 ± 7.8	162.7 ± 10.6
	1000	**562.0 ± 42.8**	23.0 ± 4.0	333.0 ± 38.2	**141.7 ± 38.1**	145.0 ± 10.6
2-Aminoanthracene	1	**383.7 ± 11.6**	**62.0 ± 6.6**	**528.0 ± 53.2**	**278.0 ± 27.7**	**411.0 ± 51.2**
	2	**732.7 ± 42.3**	**112.0 ± 20.1**	**827.7 ± 27.5**	**563.7 ± 43.7**	**1122.7 ± 37.9**

Compounds **10a** and **21** are positive in two of the bacterial strains (TA98 and TA100) that comprise the Ames microbial mutagenesis assay. A twofold or greater increase in the number of revertants relative to the solvent control is considered positive (positive data points are highlighted in bold font). All assays described in this table were conducted with an S9 protein fraction. Experimental compound **10a** (as well as the S9-dependent positive control 2-aminoanthracene) is not positive in the assay without metabolic activation provided by the S9 fraction, while compound **21** did give a 2.0- to 2.1-fold increase in the number of revertants at 10–1000 mg/plate in the TA98 strain without S9 (data not included). Both compounds have antibacterial activity at levels of 3000 and 6000 μg/plate (data not included).

Substituents such as chlorine **22**, cyano **23**, or alcohol **24** are much less active as inhibitors of PKG activity than the amines **20**, **21**, **25**, and **26**.

For active PKG inhibitors, the 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**,**b**,**d**–**f**, and **21** are active in in vivo assays when administered to birds in feed. The most potent analogs are **10a** and **21**. These compounds are active in feed at a level of 12.5 and 6.25 ppm, respectively. Commercial coccidiostats such as salinomycin and amprol are active at 66 and 130 ppm, respectively. These imidazopyridines are also active against other protozoan parasites, including the causative agents of malaria and toxoplasmosis (manuscripts in preparation). Due to their potent in vivo anticoccidial activity, both **10a** and **21** have been advanced for further evaluation. Unfortunately both compounds are positive in the Ames microbial mutagenesis assay (Table 3). A twofold or greater increase in the number of revertants is reproducibly seen for both compounds in two (TA98 and TA100) of the five standard bacterial strains that comprise the full Ames assay. Of note, both compounds are positive after metabolic activation, whereas compound **21** was weakly positive without S9 activation in the TA98 strain (data not included). Due to the potential for genotoxicity predicted by the Ames assay, these compounds will not be developed for human or animal use in the absence of negative lifetime rodent carcinogenicity studies.

Novel imidazopyridine analogs were found to be potent inhibitors of parasite PKG activity. The most potent compounds are the dimethyl amine **10a** and the *N*-methyl piperidine **21** analogs. These compounds were also fully active in in vivo assays as anticoccidial agents at 6.25 to 12.5 ppm level in feed. However, the potential genotoxicity of these compounds precludes them from further development.

Acknowledgment

The authors thank Dr. Phil Eskola, Dr. Gerard Kieczykowshi, Ms. Amanda Makarewicz, Mr. Bob Frankshun, and Mr. Glen Reynolds for scale-up of key intermediates, Drs. Ann Weber, Sheila Galloway, and Vijay Reddy for review of the manuscript, and Merck Research Laboratory Basic Chemistry Analytical Support Group for open access service on LC/MS.

References and notes

1. Williams, R. B. *Int. J. Parasitol.* **1999**, *29*, 1209.
2. Biftu, T.; Feng, D.; Liang, G.-B.; Qian, X.; Gurnett, A. M.; Liberator, P. A.; Dulski, P. M.; Donald, R. G. K.; Diaz, C. A.; Darkin-Rattray, S. J.; Nare, B.; Crumley, T.; Blum, P. S.; Misura, A. S.; Tamas, T.; Wyvratt, M.; Fisher, M. H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3296.
3. 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.
4. Donald, R. G. K.; Liberator, P. A. *Mol. Biochem. Parasitol.* **2002**, *180*, 136.
5. Matshushima, T.; Takamoto, Y.; Shirai, A.; Sawamura, M.; Sugimura, T. *Prog. Mutat. Res.* **1981**, *1*, 387.
6. Maron, D. M.; Ames, B. N. *Mutat. Res.* **1983**, *113*, 173.
7. All new compounds were characterized by ^{1}H NMR and LC–MS prior to submission for biological evaluation.