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Synthesis and biological activity of anticoccidial agents: 2,3-diarylindoles

Andrew Scribner [a,*], Joseph A. Moore III [a], Gilles Ouvry [a], Michael Fisher [b], Matthew Wyvratt [b], Penny Leavitt [c], Paul Liberator [c], Anne Gurnett [c], Chris Brown [c], John Mathew [c], Donald Thompson [c], Dennis Schmatz [c], Tesfaye Biftu [b]

[a] SCYNEXIS, Inc., Discovery Chemistry, PO Box 12878, Research Triangle Park, NC 27709-2878, USA
[b] Merck Research Laboratories, Department of Medicinal Chemistry, Merck & Co., Inc., Rahway, NJ 07065, USA
[c] Merck Research Laboratories, Department of Human and Animal Infectious Disease Research, Merck & Co., Inc., Rahway, NJ 07065, USA

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ABSTRACT

Novel 2,3-diarylindoles bearing an amine substituent at the indole 5- and 6-positions 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 **27**.

Parasitic protozoa are responsible for a wide variety of infectious diseases in both humans and animals. 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] where 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 prophylactic anticoccidial agents. Nevertheless, resistance to current coccidiostats such as polyether ionophores has become widespread,[2] creating the need for new broad spectrum drugs with unprecedented mechanisms of action.

Recently, we have reported on novel anticoccidial agents with potent in vitro and in vivo activity against *Eimeria* parasites. It was found that the reduction of parasite growth by these compounds was due to the inhibition of parasite-specific cGMP-dependent protein kinase (PKG), a serine/threonine protein kinase.[3,4] In particular, we have found that various 2,3-diarylpyrroles[5–8], 2,3-diarylimidazo[1,2-*a*]pyridines[9–13], and 5,6-diarylimidazo[2,1-*b*][1,3]thiazoles[14] show exceptional potency as anticoccidial agents.

In this paper, we present the synthesis and biological activity of a series of 2,3-diarylindoles substituted at the 6-position with various amine sidechains, as we were curious how these compounds would compare with our analogous pyrroles, imidazopyridines, and imidazothiazoles. Within these three series, we found many of our active compounds possessed a 4-fluorophenyl ring, a pyrimidin-4-yl or pyridin-4-yl ring, and a piperidin-4-yl ring at strategic locations across the core heterocycle. We therefore decided to introduce such functionality across an indole template.

Scheme 1 depicts the synthesis of indoles bearing a 2-aminopyrimidin-4-yl or pyrimidin-4-yl ring at the indole 3-position. The synthesis of ketone **1** has been reported previously.[12] Palladium-catalyzed arylation[15] of the α-carbon of ketone **1** with 2,5-dibromonitrobenzene occurred regioselectively with displacement of the bromine ortho rather than meta to the nitro group, yielding α-aryl ketone **2**. Reductive cyclization with iron in acetic acid then rendered indole **3**. The indole nitrogen was subsequently protected to give *N*-tosylindole **4**. The methylsulfanyl group of **4** was then oxidized with *m*-CPBA to yield sulfone **5**, which itself was treated with ammonia to afford 2-aminopyrimidine **6**. Alternatively, the methylsulfanyl group of **4** was reduced with Raney nickel to give 2-H-pyrimidine **7**. Subsequent Negishi coupling[16] of aryl bromides **6** and **7** with 1-Boc-4-iodozincpiperidine[17] gave 6-(1-Boc-piperidin-4-yl)indoles **8** and **9**, respectively. Cleavage of the Boc protecting group followed using trifluoroacetic acid to give NH-piperidines **10** and **11**. Deprotection of the indole nitrogen yielded N–H indoles **12** and **13**, while alkylation by reductive amination with formaldehyde gave *N*-methylpiperi-

* Corresponding author. Tel.: +1 919 544-8610.
E-mail address: andrew.scribner@scynexis.com (A. Scribner).

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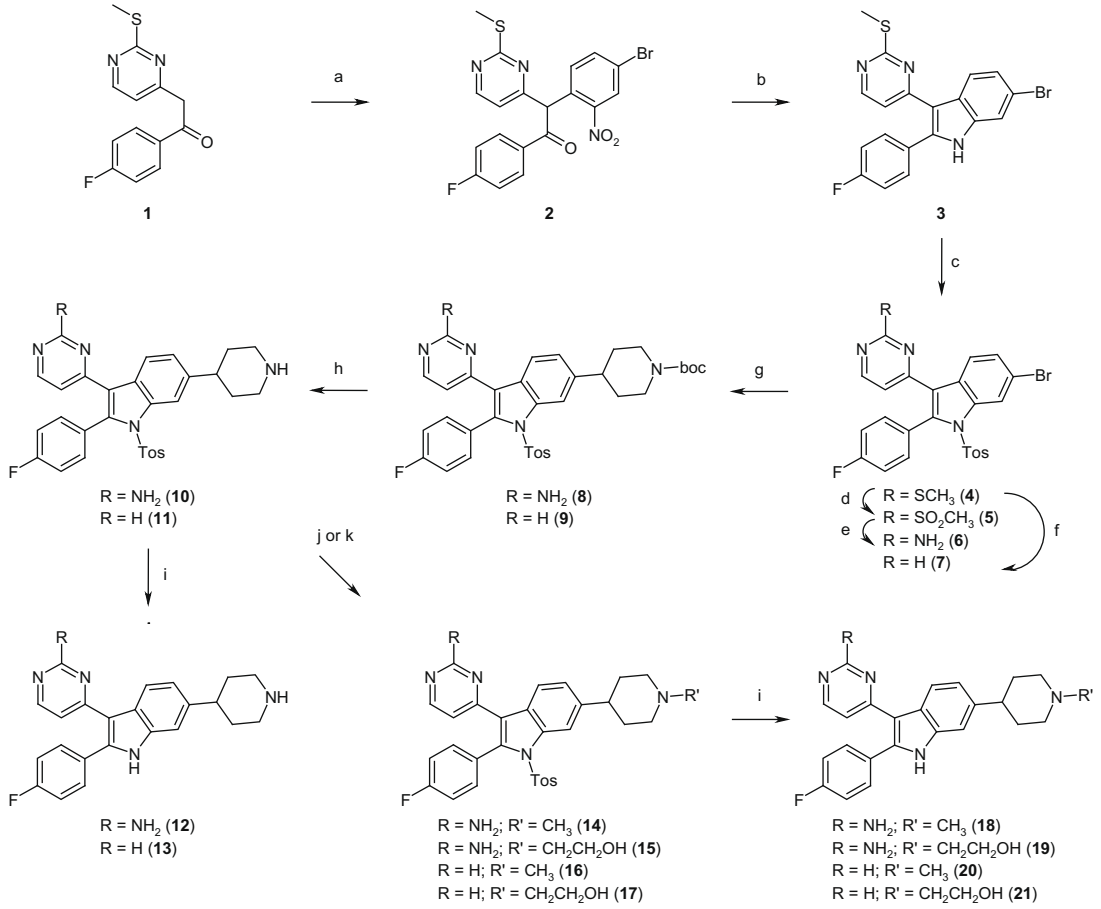
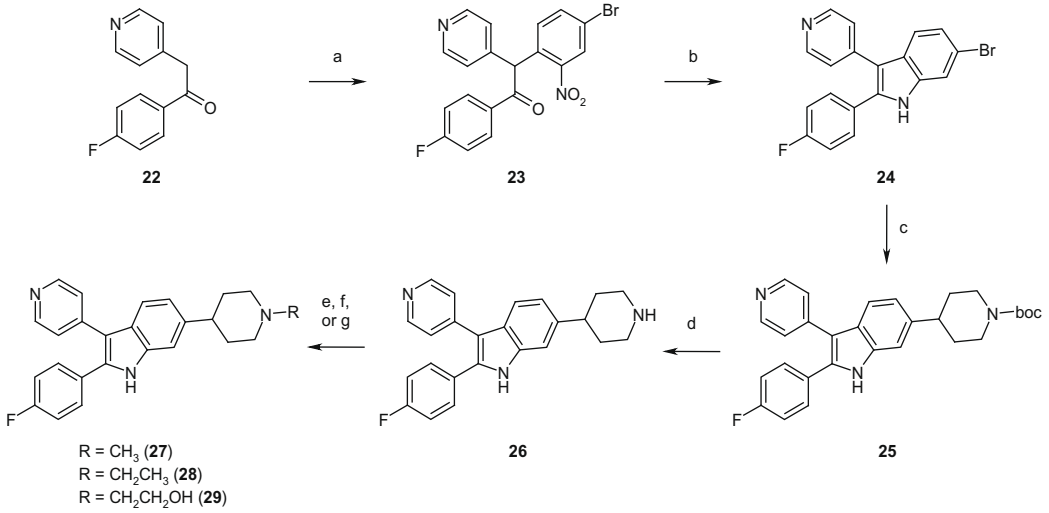


Scheme 1. Reagents and conditions: (a) 2,5-dibromonitrobenzene, $Pd(OAc)_2$, 2-(di-*tert*-butylphosphino)biphenyl, Cs_2CO_3, DMF, 70 °C; (b) Fe, AcOH, 15 °C–rt; (c) TosCl, NaH, THF; (d) *m*-CPBA, CH_2Cl_2; (e) NH_3, THF, 50 psi; (f) Raney Ni, EtOH, 80 °C; (g) 1-Boc-4-iodozincpiperidine, $PdCl_2(dppf)CH_2Cl_2$, CuI, DMA, 80 °C; (h) CF_3CO_2H, CH_2Cl_2; (i) K_2CO_3, MeOH, 65 °C; (j) $H_2C{=}O$, $NaBH_3CN$, MeOH, AcOH; (k) $BrCH_2CH_2OH$, K_2CO_3, DMF, 65 °C.

dines **14** and **16**, and alkylation with 2-bromoethanol under basic conditions gave *N*-(2-hydroxyethyl)piperidines **15** and **17**. *N*-Tosyl indoles **14–17** were ultimately deprotected to yield NH-indoles **18–21**.

We then prepared the analogous 3-(pyridin-4-yl)indoles, as shown in Scheme 2. The synthesis of ketone **22** has been reported previously.[5] Regioselective arylation of ketone **22** proceeded as in the pyrimidine series to yield α-aryl ketone **23**. At this point, we

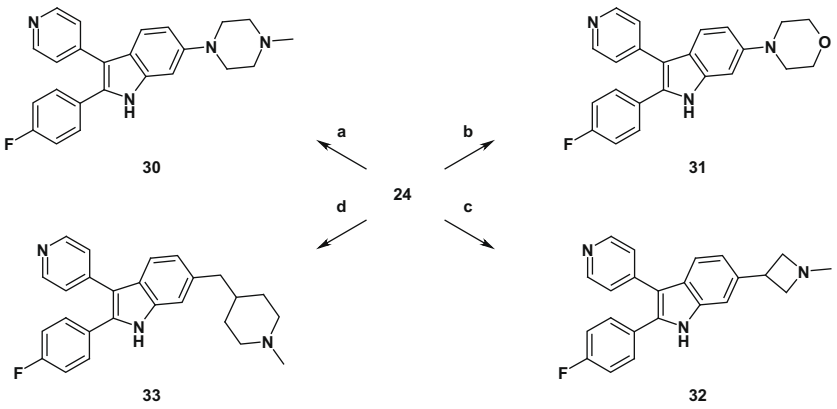


Scheme 2. Reagents and conditions: (a) 2,5-dibromonitrobenzene, $Pd(OAc)_2$, 2-(di-*tert*-butylphosphino)biphenyl, Cs_2CO_3, DMF, 60 °C; (b) $TiCl_3$, NH_4OAc, 10:1 EtOH:EtOAc; (c) 1-Boc-4-iodozincpiperidine, $PdCl_2(dppf)CH_2Cl_2$, $Pd_2(dba)_3$, CuI, XANTPHOS, RuPHOS, CuSCN, NMP, DMA, 100 °C; (d) CF_3CO_2H, CH_2Cl_2; (e) $H_2C{=}O$, $NaBH_3CN$, MeOH, AcOH; (f) $H_3CC(=O)H$, $NaBH_3CN$, MeOH, AcOH; (g) glycolaldehyde, $NaBH_3CN$, MeOH, AcOH.

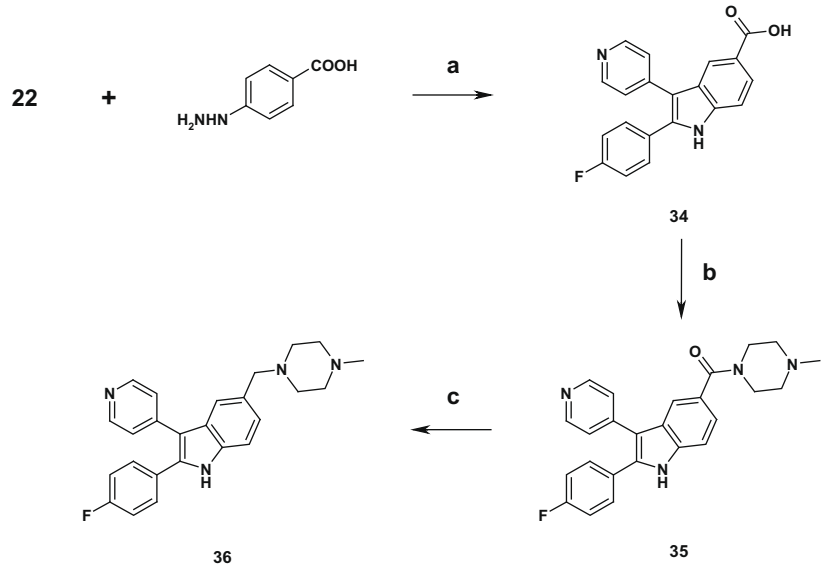
found $TiCl_3$ to be an optimal reagent for carrying out the cyclereduction[18] to give indole **24**, as this procedure did not yield the corresponding *N*-hydroxyindole byproduct that we often encountered previously when using Fe/AcOH. We were then able to proceed through the remaining steps of this synthesis without protecting the indole nitrogen, unlike the 3-(pyrimidin-4-yl)indole synthesis in Scheme 1 which entailed a sulfide oxidation to sulfone, first necessitating indole nitrogen protection. Negishi coupling[16] of the aryl bromide with 1-Boc-4-iodozincpiperidine[17] gave 6-(1-Boc-piperidin-4-yl)indole **25**, which was followed by Boc cleavage to give NH-piperidine **26**. Reductive amination with formaldehyde, acetaldehyde, and glycolaldehyde yielded *N*-methylpiperidine **27**, *N*-ethylpiperidine **28**, and *N*-(2-hydroxyethyl)piperidine **29**, respectively.

We then elaborated the 3-(pyridin-4-yl)indole series further by introducing different heterocycles at the indole 6-position via palladium catalyzed chemistry, as shown in Scheme 3. 6-(4-Methylpiperazin-1-yl)indole **30** and 6-(morpholin-4-yl)indole **31** were prepared by microwave treatment of 6-bromoindole **24** with $Pd(OAc)_2$, 2-(di-*tert*-butylphosphino)biphenyl, NaO-*t*-Bu, and *N*-methylpiperazine or morpholine, respectively. 6-(1-Methylazetidin-3-yl)indole **32** was prepared via Negishi coupling[16] of bromide **24** with 1-Boc-3-iodozincazetidine[19] using similar conditions described previously for the synthesis of the 6-(1-methylpiperidin-4-yl)indoles, followed by Boc cleavage and reductive amination with formaldehyde. 6-Methylene(1-methylpiperidin-4-yl)indole **33** was synthesized via β-alkyl Suzuki–Miyaura coupling[20] of bromide **24** with 1-Boc-4-methylenepiperidine[21] using 9-BBN, $Pd(PPh_3)_4$, and Na_2CO_3, followed by Boc cleavage and reductive amination with formaldehyde.

Lastly, we extended the 3-(pyridin-4-yl)indole series even further to include compounds possessing a 1-methylpiperazin-4-yl group linked by either a carbonyl or methylene group to the indole 5-position (Scheme 4), as we were curious if the distant basic nitrogen would occupy the same region of space as that of a 6-(1-methylpiperidin-4-yl)indole, as suggested by our imidazothiazoles.[14] Fischer indole synthesis of ketone **22** with 4-hydrazinobenzoic acid yielded 5-carboxyindole **34**. Subsequent amide coupling with *N*-methylpiperazine afforded amide **35**, which was then reduced to give 6-(methylene-1-methylpiperazin-4-yl)indole **36**.

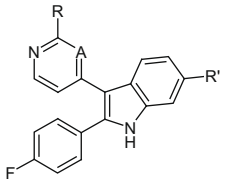


Scheme 3. Reagents and conditions: (a) 1-methylpiperazine, $Pd(OAc)_2$, 2-(di-*tert*-butylphosphino)biphenyl, NaO-*t*-Bu, microwave, 120 °C; (b) morpholine, $Pd(OAc)_2$, 2-(di-*tert*-butylphosphino)biphenyl, NaO-*t*-Bu, microwave, 120 °C; (c) i—1-Boc-3-iodozincazetidine, $PdCl_2(dppf)CH_2Cl_2$, CuI, XANTPHOS, RuPHOS, CuSCN, NMP, DMA, 100 °C; ii—CF_3CO_2H; iii—$H_2C{=}O$, $NaB(OAc)_3H$, MeOH; (d) i—1-Boc-4-methylenepiperidine, 9-BBN, $Pd(PPh_3)_4$, Na_2CO_3, 4:1 DMF:water, 120 °C; ii—CF_3CO_2H, CH_2Cl_2; iii—$H_2C{=}O$, $NaB(OAc)_3H$, MeOH, AcOH.



Scheme 4. Reagents and conditions: (a) i—EtOH, reflux; ii—AcOH, BF_3-OEt_2, 115 °C; (b) HOBt, EDCI, 1-methylpiperazine, 5:2 CH_2Cl_2:DMF; (c) $LiAlH_4$, THF, reflux.

Table 1
Et-PKG inhibition and in vivo anticoccidial activity of 2,3-diaryl-6-substituted indoles



Compound	R	A	R′	Et-PKG IC_{50} (nM)	Anticoccidial Activity at 50 ppm			
					E_t	E_a	E_{mi}	E_{ma}
12	NH_2	N	1-H-Piperidin-4-yl	0.21	0	0	—	0
18	NH_2	N	1-Me-Piperidin-4-yl	0.22	3	0	—	0
19	NH_2	N	1-CH_2CH_2OH-Piperidin-4-yl	0.3	0	0	—	0
13	H	N	1-H-Piperidin-4-yl	0.65	0	0	0	0
20	H	N	1-Me-Piperidin-4-yl	0.4	0	3+	2	0
21	H	N	1-CH_2CH_2OH-Piperidin-4-yl	0.8	3+	2	4	0
26	H	CH	1-H-Piperidin-4-yl	0.15	0	0	0	0
27	H	CH	1-Me-Piperidin-4-yl	0.15	2	3+	3+	2
28	H	CH	1-Et-piperidin-4-yl	0.16	2	0	3	3
29	H	CH	1-CH_2CH_2OH-Piperidin-4-yl	0.1	2	3	3	0
30	H	CH	1-Me-Piperazin-4-yl	—	0	0	0	—
31	H	CH	Morpholin-4-yl	9.2	2	0	0	—
32	H	CH	1-Me-azetidin-3-yl	0.62	2	0	0	—
33	H	CH	1-Me-piperidin-4-CH_2-yl	1.6	0	0	2	—
35	H	CH	1-Me-piperazin-4-(C=O)-yl*	12.7	3	0	NA	—
36	H	CH	1-Me-piperazin-4-CH_2-yl*	8.9	3	0	0	—

* Substituent at indole 5-position instead of 6-position.

Table 1 presents both in vitro and in vivo biological data of each compound tested.[22] 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 seven day efficacy model. A quantitative measure of *E. tenella* (E_t), *E. acervulina* (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'.

It is seen that the in vitro activity of all compounds was in the subnanomolar range except for morpholine **31**, which lacks a basic nitrogen at the indole 6-position sidechain, as well as piperidine **33** and piperazines **35** and **36**, each of whose most external basic nitrogen is positioned further away from the indole core, and with greater rotational degrees of freedom than those in all other compounds tested. Within each 6-(N-substituted-piperidin-4-yl)indole series, the order of in vitro activity with regard to the heterocycle at the indole 3-position is consistently 3-(pyridin-4-yl) > 3-(2-aminopyrimidin-4-yl) > 3-(pyrimidin-4-yl). Overall, the data suggests that a basic nitrogen entropically fixed 3 to 4 atoms away from the indole 6-position is optimal for in vitro activity.

Several observations can be made regarding in vivo activity. The *N*-alkylpiperidines clearly showed greater potency than the NH-piperidines. Both the 3-(pyridin-4-yl) and 3-(pyrimidin-4-yl) series showed comparable activity. 3-(Pyridin-4-yl)-6-(1-methylpiperidin-4-yl)indole **27** is the only compound tested that showed activity against all four species of *Eimeria* test. All compounds tested that lacked a piperidine ring at the indole 6-position (**30–33**, **35,36**) did not show remarkable in vivo potency.

In conclusion, we have prepared several novel 2,3-diaryl-6-substituted indoles, 11 of which show subnanomolar potency in vitro against *E. tenella* cGMP-dependent protein kinase (PKG), and 4 of which show in vivo potency against at least 3 of the 4 species of *Eimeria* tested. Of these, 3-(pyridin-4-yl)-6-(1-methylpiperidin-4-yl)indole **27** shows potency against all 4 species of *Eimeria*. While the level of potency exhibited in the 2,3-diarylindole series thus far does not match that of our most potent imidazopyridine (compound **69** from reference 13, with an IC_{50} = 0.044 nM and in vivo scores of 4 (E_t), 3 (E_a), 3 (E_{mi}), and 4 (E_{ma}) at 6 ppm), the 2,3-diarylindole class does hold promise in anticoccidial therapy.

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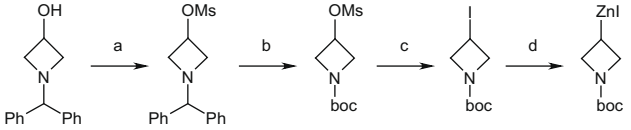

(a) MsCl Et_3N.; (b) i. ACECl, DCE; ii. MeOH; iii; Boc_2O, Et_3N, CH_2Cl_2.; (c) I_2, DMSO, 140 °C.; (d) zinc, chlorotrimethylsilane, 1,2-dibromoethane, DMA.
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21. 1-Boc-4-methylenepiperidine was prepared by treating 1-Boc-piperidone under Wittig conditions with methyltriphenylphosphonium bromide and *n*-butyllithium.
22. All compounds were characterized by ^1H NMR, LC/MS, and HPLC ($\geqslant$90% pure at 254 nm) prior to biological testing.