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Synthesis and Evaluation of a Series of 1,4-Diarylbutadienes for Anticoccidial Activity

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Abstract—During the course of a collaborative screening program, a set of 1-phenyl-4-pyridyl-butadienes was found to exhibit in vitro activity against *Eimeria tenella* in a cell-based assay. Activity was dependent on the chain length and degree of unsaturation of the linker between the two aryl groups as well as substitution of the pyridine moiety. Structure–activity relationship studies were subsequently conducted over a larger range of 1,4-diarylbutadienes in order to determine the scope of active compounds, to identify structural patterns governing activity and to enhance in vitro potency against *E. tenella*. In addition, the efficacy of many compounds for treating coccidiosis in chickens was measured by testing the ability of the compound to prevent or reduce intestinal and cecal lesions when administered by oral gavage. A few compounds in the series were identified that exhibited a moderate degree of in vitro and in vivo activity.

Introduction

Cryptosporidiosis ('crypto') is caused by *Cryptosporidium parvum*, a coccidian protozoan, and has been reported to be one of the most common causes of illnesses transmitted by water sources in the US, Crypto is a serious illness that can be fatal in children or those with compromised immune systems. There is currently no treatment for cryptosporidiosis except for supportive therapy for the diarrhea and dehydration that accompanies this disease. *Eimeria* sp. is also a coccidian parasite and is the causative agent of coccidiosis in poultry. The anticoccidial market for poultry is currently dominated by members of the polyether class of antibiotics,[1,2] but the possibility for emergence of resistance to these agents always looms as a potential threat to their continued effectiveness.[3] Despite continuous reports in the literature describing the discovery and evaluation of newer agents exhibiting in vitro activity against species of *Eimeria*,[4–7] successful demonstration of in vivo efficacy against coccidiosis usually remains elusive.[8] Consequently, the search for efficacious new anticoccidial agents has proven to be a very challenging goal for both human and animal health research programs.

During the course of a collaborative screening program designed to identify new antiparasitic agents, a series of diarylbutadienes, provided by Idemitsu Kosan Company,[9] was found to exhibit moderate anticoccidial activity in a cell-based in vitro assay system. Since such a series of compounds had not been previously reported to possess anticoccidial activity, the series was further explored through in vivo testing and structure–activity relationship studies.

Results and Discussion

Representative structures from the initial set of compounds[9] synthesized at Idemitsu Kosan that were screened for biological activity against *E. tenella* are illustrated in Figure 1. The in vitro anticoccidial activities of these compounds are listed in Table 1. Compounds were tested in vitro at an initial concentration of 10 μg/mL, and if active at that concentration, were then tested at 5 and 2.5 μg/mL. Analysis of the screening

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results revealed several intriguing relationships between structures and activities. All of the compounds that exhibited activity contained the 3-ethylpyridine ring whereas all compounds lacking the 3-ethyl substituent on the pyridine ring were inactive at the screening concentration. However, the presence of the 3-ethyl substituent was not sufficient by itself to confer activity, as evidenced by compounds **4b** and **5**. To exhibit activity, the nature and size of the linker that connected the 3-ethylpyridine ring to the phenyl ring was also critical. This linker could be either a dienyl moiety (compound **1**, screening sample) or an alkyl moiety of a certain length (compounds **2**, **3a**, **3b** and **4a**). Compounds did not exhibit activity if they either lacked the fully unsaturated butadienyl moiety (compounds **5**–**11**) or possessed a smaller than optimal alkyl linker between the two aryl groups (compounds **8**–**11**) with the exception of compound **3b**. These relatively unusual and unexpected relationships prompted further critical examination of the series, both by (1) synthesis and testing of additional compounds to more fully assess the parameters governing in vitro activity, and (2) in vivo evaluation of the active compounds for the treatment of coccidiosis in chickens.

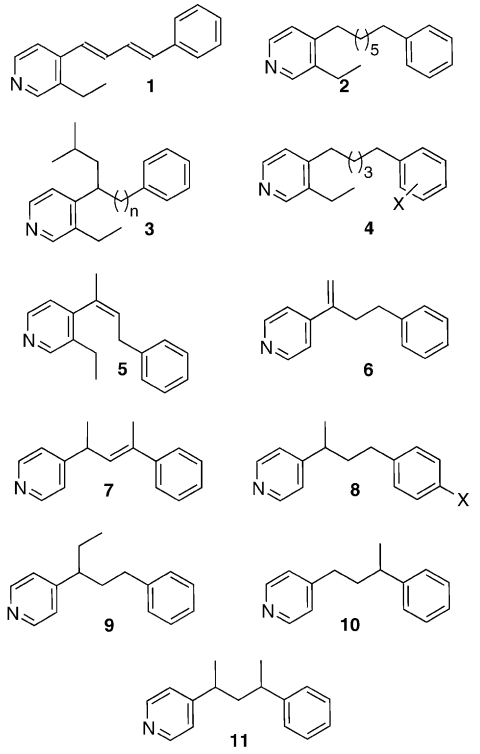


Figure 1.

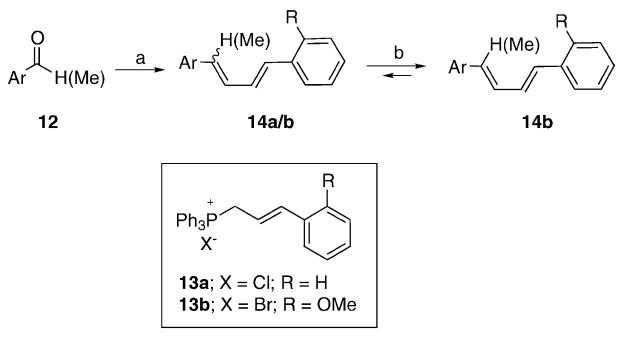
Table 1. In vitro anticoccidial activity of compounds in Figure 1

No.	MEC[a] (μg/mL) in vitro
1 (screening sample)	2.5, 10
2	2.5 (cytotoxic)
3a ($n=3$)	10
3b ($n=2$)	2.5 (cytotoxic)
4a (X = 3-Cl)	2.5 (cytotoxic)
4b (X = 4-Cl)	> 10
5	> 10
6	> 10
7	> 10
8a (X = Cl)	> 10
8b (X = Me)	> 10
8c (X = OMe)	> 10
9	> 10
10	> 10
11	> 10

[a]MEC = Minimum effective concentration.

Both butadiene **1** and saturated derivatives **2**, **3a**, **3b** and **4a** demonstrated activity in vitro, however compound **1** was selected for structural modification due to the in vitro cellular toxicity observed with most of the latter derivatives. Condensation, Wittig and Horner–Emmons reactions are typical (but not all-inclusive) methods for the synthesis of 1-phenyl-4-pyridyl-butadienes with disconnection at either double bond. We wanted to use methodology that would be applicable to the synthesis of a variety of 4-arylphenylbutadienes. Compounds **1** (freshly synthesized), **15**, **17** and **18** were synthesized via a condensation reaction with 3-ethyl-4-methyl pyridine and the corresponding cinnamaldehyde in the presence of NaOAc in refluxing acetic anhydride in poor yield (5–13%) after extensive purification. Our experimentation with condensation methodology demonstrated that these reactions, in general, were low-yielding and difficult to scale. The Wittig and Horner–Emmons reactions, therefore were appealing. McDonald & Campbell reported the construction of aniline-containing 4-arylphenylbutadienes using convenient and scalable Wittig conditions.[10] We sought to extend this methodology to the synthesis of 4-pyridyl and 4-arylphenylbutadienes.

Scheme 1 outlines the construction of 4-arylphenylbutadienes (**14**) with arylcarboxaldehydes or aryl ketones (**12**) and cinnamyltriphenylphosphonium halides (**13a**, **13b**) with LiOEt in EtOH. Compounds **16**, **19**, **20**, **22**, **24b**, **25b**, **29**, **30**, **34** and **36** (Tables 2 through 5) were synthesized using this methodology. Products from these reactions were often isolated by precipitation. Isomerization of the diastereomeric mixture (**14a**/**b**) with iodine followed by recrystallization gave pure all *trans* products (**14b**) or all *trans* enriched products. Although compounds **21**, **26**–**28**, **31**–**33** and **35** were obtained from an internal compound collection, these diarylbutadienes could also presumably be synthesized by the Wittig methodology. Compound **23** was synthesized by alkylation of 3-ethyl-4-methyl pyridine with



Scheme 1.

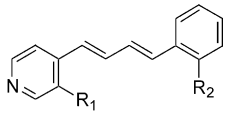
3-bromo-1-phenyl propene in the presence of LDA. Compounds **37**–**40** are either commercially available or have been previously reported in the literature.

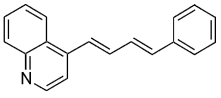
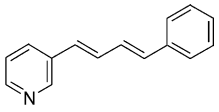
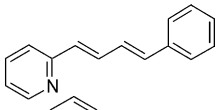
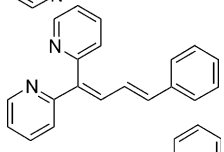
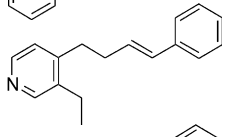
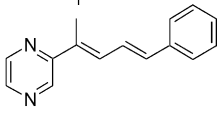
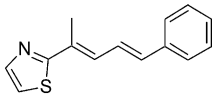
Compounds in these studies all have the *E,E* butadiene configuration where either configuration is possible. This feature holds true for all of the compounds in Tables 2–5, with the exception of compound **36** which is a mixture of *E*,*E* and *Z*,*E* in a 2.5:1 ratio. For those compounds that were tested in vitro more than once, both data points are given. Most compounds tested multiple times in vitro gave numerical results within one dilution factor; two of these, however did not demonstrate consistent activity in the in vitro assay.

Several compounds were tested in vivo in a coccidiosis mixed-infection model in young chicks. The chicks were infected with both *Eimeria acervulina* and *Eimeria tenella* on study day 2, which are known to cause lesions in the intestinal portion of the gastrointestinal tract and the cecum, respectively. On days 1–9, the chicks were dosed once per day with the test compound administered by oral gavage. The dose levels were 33 mg/kg or 67 mg/kg, which approximates the amount of compound that a chick would be estimated to receive from daily consumption of that compound in its feed at a concentration of 100 μg/g or 200 μg/g, respectively. Necropsy occurred on study day 9, at which time the intestine and cecum of each bird were examined for lesions.

Table 2 illustrates the initial group of 1-phenyl-4-pyridyl-butadienes studied. Compound **1** (synthesized at Elanco), which was the most active and least cytotoxic compound in vitro from the first series, was tested in vivo, but unfortunately it did not exhibit activity at the highest concentrations tested. This compound was tested in vitro twice, however neither data point was equivalent to the activity observed with **1** (screening sample). Both compounds were determined to be of high purity, therefore this inconsistency remained unsolved. It was discovered, however that compound **15** having an *ortho*-methoxy substituted phenyl ring demonstrated some in vivo activity against coccidiosis even though it was devoid of in vitro activity. It was therefore suspected that the active species in vivo might be a metabolite of **15**. Consequently, most of the butadienes in Table 2 were tested in vivo in parallel to in vitro testing in order to determine whether this phenomenon was generally true with this class of compounds. This property was not found to be characteristic of the other butadienes tested whose in

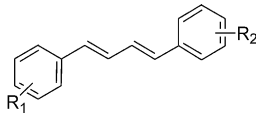
Table 2. Anticoccidial activity of 1-phenyl-4-pyridyl-butadienes and analogues



No.	R_1	R_2	MEC (μg/mL) in vitro	MEC (μg/g)[a] in vivo
1[b]	Et	H	5, >10	>200 (i)[c] >200 (c)
15	Et	OMe	>10, >10	100 (i) 100 (c)
16	H	OMe	>10	>200 (i) >200 (c)
17	Et	NO_2	5, >10	200 (i) 100 (c)
18	H	H	5, 10	200 (i)[d] 200 (c)
19			10	>200 (i)
20			>10	>200 (c) >200 (i)
21			10	>200 (c) 150 (i)
22			NT[e]	>150 (c) >200 (i)
23			>10	>200 (c) >200 (i)
24b			>10	>200 (c) >200 (i)
25b			>10	>200 (c) NT

[a]100 μg/g and 200 μg/g refer to approximate doses if mixed into feed. Compounds were administered via oral gavage at 33 mg/kg or 67 mg/kg, respectively, to approximate these values.
[b]Freshly synthesized.
[c](i) = intestine, (c) = cecum.
[d]Tested as a topdress on feed.
[e]NT = not tested.

Table 3. Anticoccidial activity of 1-phenyl-4-(nitrophenyl)-butadienes

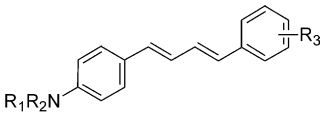


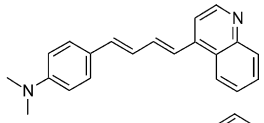
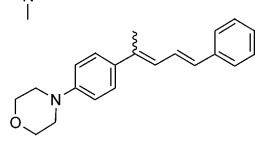
No.	R_1	R_2	MEC (μg/mL) in vitro	MEC (μg/g) in vivo
26	*p*-NO_2	*o*-OMe	>10	>200 (i) >200 (c)
27	*o*-NO_2	*o*-OMe	>10	>200 (i) >200 (c)
28	*p*-NO_2	*p*-NO_2	>10	>200 (i) >200 (c)
29	*p*-NO_2	H	>10	>200 (i) >200 (c)
30	*m*-NO_2	H	>10	>200 (i) >200 (c)

vivo activity generally paralleled in vitro activity, and the behavior of compound **15** remained an anomaly.

Although compound **15** demonstrated in vivo anticoccidial activity, compound **16** which lacks the 3-ethyl substituent on the pyridine moiety was devoid of both in vitro and in vivo activity. This relationship between 3-ethylpyridyl and pyridyl butadienes did not prove to be generally true, however. For instance, compound **1** demonstrated in vitro activity, but no in vivo activity, while its nor-ethyl counterpart, **18**, had both in vitro and in vivo activity (against both intestinal and cecal lesions at 200 μg/g; **18** was tested as a topdress on feed). Benzo-fused (quinoline) analogue **19** demonstrated in vitro anticoccidial activity (and cellular toxicity), but lacked in vivo activity against coccidiosis. The *ortho*-nitrophenyl compound **17** showed modest activity both in vitro and in vivo. The location of the nitrogen in the pyridine ring was also examined. It was found that *meta*-substituted derivative **20** was not active in vitro or in vivo at the highest concentration tested while the *ortho*-substituted derivative **21** was active both in vitro and in vivo (against intestinal lesions at 150 μg/g). This may indicate that the relationship between the nitrogen location in the ring and the anticoccidial activity is an electronic effect. Substitution of a second *ortho*-substituted pyridine moiety at the 4-butadiene position (**22**) resulted in a loss of anticoccidial activity in vivo. Saturation of the double bond conjugated to the pyridine, compound **23**, resulted in a total loss of activity. Substitution of the pyridine heterocycle with a more nitrogen-rich pyrazine (**24b**) or a π-excessive thiazole (**25b**) provided derivatives devoid of activity. However, it may also be argued that the methyl substitution at C-4 in both of these compounds is responsible for the lack of activity. Branching with a single carbon at this position has not produced any active compounds, as observed with compounds **5**, **6**, **8**, **11** and **36** although since the unsubstituted compounds were not synthesized and tested, no direct comparison of substituted versus unsubstituted derivatives can be made.

Table 4. Anticoccidial activity of 1-phenyl-4-(*N,N*-di-substituted-*p*-aniline)-butadienes

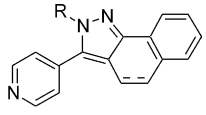


No.	R_1	R_2	R_3	MEC (μg/mL) in vitro	MEC (μg/g) in vivo
31	Me	Me	*o*-CN	>10	>200 (i) >200 (c)
32	Me	Me	*p*-CN	>10	>200 (i) >200 (c)
33	Me	Me	*p*-CO_2H	>10	>200 (i) >200 (c)
34	Me	Me	*o*-OMe	>10	>200 (i) >200 (c)
35				>10	NT
36				>10	>200 (i) >200 (c)

para-Nitrobenzene has generally been accepted as a nonclassical bioisostere of pyridine,[11] therefore several *p*-nitrophenyl butadienes were synthesized (Table 3). *para*-Nitrophenyl butadienes **26**, **28**, and **29** with *o*-methoxy, *p*-nitro and H substitution on the 1-phenyl ring, respectively, showed no activity either in vitro or in vivo. Analogues of these compounds, **27** and **30**, with the nitro group positioned at the *ortho* and *meta* positions, respectively, were also inactive in vitro and in vivo. It is readily apparent by the lack of anticoccidial activity that the nitrophenyl group is not a bioisostere for pyridine in this chemical series.

A series of *N,N*-disubstituted-aniline conjugated butadienes was also examined (Table 4). Compounds **31**–**34** containing *N,N*-dimethylaniline combined with cyano, carboxy, and methoxy substitution on the phenyl ring of the butadiene were synthesized and tested. Unfortunately, these compounds proved to be inactive. The 4-(*N,N*-dimethylamino)phenyl-1-quinoline-butadiene **35** was also found to be inactive in vitro. Compound **36**, containing a *para*-morpholino substituent with a methyl substituent at C-1 in the butadiene linker, was devoid of biological activity.

Table 5. Anticoccidial activity of benz[*g*]indazoles



No.	R	C_4–C_5 Bond type	MEC (μg/mL) in vitro	MEC (μg/g) in vivo
37	H	Unsatd; CH_3SO_3H salt	~5	>200 (i) >200 (c)
38	H	Unsatd	~1	NT
39	H	Satd; CH_3SO_3H salt	10, 10	>200 (i) >200 (c)
40	Bn	Satd; HBr salt	10	NT

In an effort to hold the butadiene linker in a conformationally restricted topology and render the butadiene chain aromatic (in two cases), several 2*H*-benz[*g*]indazoles were examined (Table 5). In each of these compounds, the 4-pyridyl substituent was kept constant while the fused ring system was altered. Compounds with unsaturation at C-4/C-5: **37** (CH_3SO_3H salt) and **38** (free base) had in vitro anticoccidial activity at 5 and 1 μg/mL, respectively. However, no in vivo anticoccidial activity was noted for compound **37**. One derivative having the aromaticity in the central ring of its fused tricyclic system obliterated, compound **39**, gave consistent in vitro activity at 10 μg/mL. *N*-benzyl substituted analogue **40** demonstrated in vitro activity at 10 μg/mL as well. In summary, it was discovered that all of the benz[*g*]indazoles tested demonstrated activity in vitro, but these compounds lacked activity in vivo.

Conclusions

Moderate bioactivity was demonstrated with several 1-phenyl-4-pyridyl-butadienes, but the in vitro and in vivo anticoccidial activity was not always correlated (Table 2). In one case, in vivo anticoccidial activity was observed in the absence of in vitro activity (i.e., compound **15**); however, this feature was not consistently demonstrated with all members of this chemical series. Substitution of the pyridine with a pyrazine or a thiazole (**24b** and **25b**) did not result in anticoccidial activity. Nitrobenzene and *N,N*-disubstituted-aniline did not prove to be bioisosteres for the pyridine ring as demonstrated with several derivatives reported in Tables 3 and 4. Benz[*g*]indazoles **37**–**40** showed anticoccidial activity in vitro, but these compounds were not active in vivo. Some interesting, albeit modest, anticoccidial activity was observed with several of the diaryl butadienes studied, however due to the absence of a definitive lead compound and a clearly defined direction for further optimization, the anticoccidial studies with this series of compounds were discontinued.

Experimental

In vitro assay

The cell culture medium used was Dulbecco's Modified Essential Medium (DMEM) containing 10% fetal bovine serum (FBS). Bovine turbinate (BT) cells were used for cell culture of *Eimeria tenella*. Lilly strain 65, a polyether-tolerant strain of *E. tenella* was used as the test organism. Freshly excysted, purified sporozoites were obtained as previously described.[12] For purposes of compound evaluation, 2×10^4 sporozoites were added per well to confluent cultures of BT cells maintained in 96-well tissue culture dishes which were incubated at 37 °C. All compounds were initially solubilized in DMSO at 10 mg/mL. These solutions were diluted in cell culture medium to a final concentration of 10 μg/mL, which was added to each treated well concurrent with the introduction of sporozoites. All culture dishes were then incubated for an additional 48 h. Following incubation, all culture dishes were fixed with 5% acetic acid–methanol for 15 min, and then washed and stained with toluidine blue O for 30 min. After an additional wash, each well was examined microscopically for the presence of developing parasites (schizonts). Compounds were designated active (significant reduction of schizogony), inactive (no reduction) or cytotoxic (destruction of host BT cells). Compounds that were cytotoxic at 10 μg/mL were then tested at lower concentrations.

In vivo test

In each experiment, four chickens were used for each compound and each dosage. 7 Day old male Hubbard X Hubbard chickens were used for these studies. On study day 2, the chickens were infected with a mixed culture of 150,000 sporulated oocysts of *Eimeria acervulina* and 50,000 sporulated oocysts of *E. tenella*, which are known to cause lesions in the intestinal portion of the gastrointestinal tract and the cecum, respectively. On study days 1 through 9, the compounds were individually administered once daily to the infected chickens by oral gavage as a solution or suspension in DMSO/aqeuous Tween carrier (10%) [Aqeuous Tween carrier is 0.5% methylcellulose/2% Tween 80/H_2O]. Solutions/suspensions were prepared fresh daily. The dose levels were 33 mg/kg or 67 mg/kg, which approximates administration of a compound in feed at a concentration of 100 μg/g or 200 μg/g, respectively. Administration of the compound mixed in feed more closely mimics potential commercial applications, although at the early experimental stages of research, administration of the compound by oral gavage provides a greater opportunity to elicit some degree of in vivo efficacy. Necropsy occurred on study day 9. The intestine and cecum of each bird were examined for lesions. A lesion scoring system of 0–4 was used.[13] A test compound was considered active if it prevented any lesions from occurring at both sites of the gastrointestinal tract in all four chickens, or at least, if only very minimal or occasional lesions were present. Lesions in birds that received test compound were compared and scored against results observed for infected controls. Any mortality during the test period was also recorded.

Synthesis of compounds

General methods. The original set of samples of this series, compounds **1** (screening sample), **2**–**11**, were synthesized at Idemitsu Kosan Company by previously described procedures.[9] Samples of other previously known compounds obtained from internal compound libraries were analyzed by ^{1}H NMR and HRMS. ^{1}H NMR spectra were obtained on either a GE-300 or a Varian 400 spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard defined as 0.0 ppm. 2-D NMR spectra were obtained on either a Bruker DRX-500 or a Bruker Avance 500. Mass spectral data was obtained on a VG 70 SE (EI), a Sciex API 100 (ESI), or a Micromass LCT oa-TOF-MS (ESI).

Compounds **18**,[14,15] **19**,[16] and **20**–**21**[15] have been reported in the literature. Compounds **26**–**27**,[17] **28**,[18] **29**,[18,19] **30**,[20,21] **32**[22] and the methyl ester of **33**[23] have been previously reported. Compounds **35**[24,25] **38**,[26] and **39**[27] have also been reported in the literature. Compound **39** is commercially available as the free base and **40** is also commercially available.

3-Ethyl-4-[(*E,E*)-4-phenyl-1,3-butadienyl] pyridine (1). Compound **1** was synthesized from 3-ethyl-4-methyl pyridine and *trans*-cinnamaldehyde by the condensation method described for compound **17** below. It was purified by chromatography (40–50% EtOAc–hexanes), then rotary chromatography (50–75% EtOAc–hexanes with trace MeOH), then recrystallized from Et_2O–pentane to give brown crystals (5% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, $J=7.5$, 3H), 2.75 (dd, $J=7.7$, 15, 2H), 6.80 (t, $J=15$, 2H), 6.96–7.11 (m, 2H), 7.25–7.30 (m, 2H), 7.33–7.40 (m, 2H), 7.47 (d, $J=7.7$,

2H), 8.40 (d, $J=3.3$, 2H). MS (ESI+) m/z +236 $(M+H)^+$. Anal. calcd for $C_{17}H_{17}N$: C, 86.77; H, 7.28; N, 5.95. Found: C, 86.53; H, 7.14; N, 5.95.

3-Ethyl-4-[(*E,E*)-4-(2-methoxyphenyl)-1,3-butadienyl] pyridine (15). Compound **15** was synthesized from 3-ethyl-4-methyl pyridine and *trans*-2-methoxy cinnamaldehyde by the condensation method described for compound **17** below. It was purified by chromatography (30–55% EtOAc–hexanes) to give an orange oil (9% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (t, $J=7.5$, 3H), 2.65 (q, $J=7.5$, 2H), 3.79 (s, 3H), 6.69–7.18 (m, 7H), 7.16 (d, $J=5.2$, 1H), 7.47 (dd, $J=1.6$, 7.7, 1H), 8.31–8.32 (m, 2H). HRMS (ES+) m/z 266.1552 (calcd for $C_{18}H_{19}NO+H$ 266.1545). A second lot of **15** used for the second in vitro test (>10 μg/mL) gave identical spectral data and HRMS obsd 266.1555.

(*E*)-*o*-Methoxy-cinnamyltriphenyl phosphonium bromide (13b). Triphenylphosphine (44.0 g, 167.7 mmol) was dissolved in benzene (100 mL), then *trans*-1-(3-bromo-1-propenyl)-2-methoxy benzene[28] (35.5 g, 156.3 mmol) was added as a solution in benzene (150 mL). The mixture was stirred at room temperature for 16 h, then the resulting white solid was collected by vacuum filtration, rinsed with benzene–petroleum ether, and dried in a vacuum oven to give 60.3 g of white solid (79% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.38 (s, 3H), 3.71 (s, H_2O), 4.84 (dd, $J=7.6$, 16.8, 2H), 6.12 (app dq, $J=7.4$, 13.6, 15.8, 1H), 6.88 (dd, $J=7.6$, 10.8, 1H), 6.88 (d, $J=16$, 1H), 6.98 (d, $J=8$, 1H), 7.26 (t, $J=7.2$, 1H), 7.32 (d, $J=7.2$, 1H), 7.37 (s, benzene), 7.78–7.84 (m, 15H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.21, 26.69, 55.36, 111.25, 115.34, 115.44, 117.64, 118.48, 120.38, 123.89, 123.92, 126.85, 128.07, 129.40, 129.87, 130.00, 133.53, 133.63, 134.71, 134.74, 156.02.

4-[(*E,E*)-4-(2-Methoxyphenyl)-1,3-butadienyl] pyridine (16). Compound **16** was synthesized from pyridine 4-carboxaldehyde and **13b** by the general Wittig method described for compound **19**. It was isolated by extraction, then isomerized with I_2 in Et_2O and recrystallized from hot DMF–EtOH–H_2O to give dark orange crystals (31% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (s, 3H), 6.55 (d, $J=15.6$, 1H), 6.90 (d, $J=8.4$, 1H), 6.97 (dd, $J=8.2$, 15.2, 1H), 7.01 (d, $J=16.4$, 1H), 7.14 (d, $J=15.6$, 1H), 7.17 (dd, $J=10.4$, 15.6, 1H), 7.24–7.28 (m, 3H), 7.52 (dd, $J=1.6$, 7.6, 1H), 8.53 (d, $J=1.4$, 1H), 8.54 (d, $J=1.4$, 1H). MS (EI+) m/z 238 (M^+). Anal. calcd for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90. Found: C, 80.57 (difference of 0.414%); H, 6.36; N, 6.12.

3-Ethyl-4-[(*E,E*)-4-(2-nitrophenyl)-1,3-butadienyl] pyridine (17). An oven-dried round bottom flask was charged with Ac_2O (60 mL) and NaOAc (5.6 g, 68 mmol) to form a suspension. 3-Ethyl-4-methyl pyridine (3.6 mL, 34 mmol) and *trans*-2-nitro cinnamaldehyde (6.0 g, 34 mmol) were added and the mixture was refluxed for 21 h. The mixture was cooled to room temperature and concd in vacuo to a slurry. The crude reaction mixture was diluted with CH_2Cl_2, washed with H_2O, 5% HCl, water, then satd aqueous $NaHCO_3$. After drying over $MgSO_4$ and filtering, the solution was concd in vacuo. The crude material was dissolved in 25% EtOAc–hexanes and filtered through silica in a 350 mL glass frit. The desired material eluted with 75% EtOAc–hexanes. This crude product was chromatographed (50% EtOAc–hexanes; rotary chromatography), then crystallized from Et_2O–pentane to obtain dark red crystals (13% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, $J=7.5$, 3H), 2.75 (q, $J=7.5$, 2H), 6.93 (d, $J=15.2$, 1H), 7.00 (dd, $J=10.6$, 14.8, 1H), 7.11 (dd, $J=10.8$, 15.2, 1H), 7.25–7.29 (m, 1H), 7.40–7.44 (m, 2H), 7.58–7.62 (m, 1H), 7.74 (dd, $J=1.2$, 8.0, 1H), 7.96 (dd, $J=1.4$, 8.0, 1H), 8.42–8.44 (m, 2H). MS (ESI+) m/z 281 $(M+H)^+$. Anal. calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.54; H, 5.82; N, 9.68.

4-[(*E,E*)-4-Phenyl-1,3-butadienyl] pyridine (18). Compound **18** was synthesized from 4-picoline and *trans*-cinnamaldehyde by the condensation method described for compound **17** above. It was purified by chromatography (25–75% EtOAc–hexanes) then recrystallized from Et_2O–pentane to give an orange solid (12% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.58 (d, $J=15.5$, 1H), 6.78 (d, $J=15.4$, 1H), 6.96 (dd, $J=10.4$, 15.4, 1H), 7.13 (dd, $J=10.4$, 15.4, 1H), 7.26–7.29 (m, 3H), 7.36 (dd, $J=7.2$, 2H), 7.47 (d, $J=7.0$, 2H), 8.54 (d, $J=5.5$, 2H). MS (ESI+) m/z 208 $(M+H)^+$. Anal. calcd for $C_{15}H_{13}N$: C, 86.92; H, 6.32; N, 6.76. Found: C, 86.63; H, 6.17; N, 6.58. This lot was tested for in vitro anticoccidial activity (Table 2) and gave a value of 5 μg/mL. A second lot of **18** was tested for in vitro and in vivo activity and gave values of 10 μg/mL, 200 μg/g (i) and 200 μg/g (c), respectively. The second lot of **18** gave identical spectral data and the following analysis; Found: C, 87.01; H, 6.36; N, 6.79.

4-[(*E,E*) 4-Phenyl-1,3-butadienyl] quinoline (19). Previously published Wittig conditions were used.[10] To absolute ethanol (50 mL) was added **13a** (7.0 g, 17 mmol) and 4-quinoline carboxaldehyde (2.36 g, 15 mmol) and stirred until all the starting materials dissolved. LiOEt (1.0 M in EtOH, 17 mL, 17 mmol) was then added dropwise via syringe. Upon addition of LiOEt, the reaction immediately turned dark brown. The reaction was stirred at room temperature for 16 h then diluted with H_2O and EtOAc and the layers were separated. The organic layer was extracted with 10% HCl, and the aqueous extract was washed with EtOAc. The aqueous layer was made basic, then extracted into EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concd in vacuo. The crude product was dissolved in Et_2O–toluene and stirred with catalytic I_2 for several days. A yellow solid was isolated after concentration in vacuo. The crude product was recrystallized from hot DMF–H_2O to obtain 2.3 g of a light yellow solid (60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (d, $J=15.0$, 1H), 7.11 (dd, $J=10.3$, 15.0, 1H), 7.20 (dd, $J=10.6$, 15.3, 1H), 7.26–7.31 (m, 1H), 7.36–7.40 (m, 3H), 7.49–7.60 (m, 4H), 7.70–7.75 (m, 1H), 8.13 (dd, $J=0.7$, 8.4, 1H), 8.17 (d, $J=8.4$, 1H), 8.87 (d, $J=4.8$, 1H). MS (EI+) m/z 258 (M^+). Anal. calcd for $C_{19}H_{15}N$: C, 88.68; H, 5.88; N, 5.44. Found: C, 88.29; H, 5.86; N, 5.52.

3-[(*E,E*)-4-Phenyl-1,3-butadienyl] pyridine (20). Compound **20** was synthesized from 3-pyridine carboxaldehyde and **13a** by the general Wittig method described for compound **19**. The product was isolated by extractive workup (H_2O/EtOAc), then recrystallized twice from DMF–H_2O to give compound **20** as a pale beige powder (38% yield). ^{1}H NMR (300 MHz; DMSO-d_6) δ 6.75 (d, J=15.0, 1H), 6.79 (d, J=15.0, 1H), 7.11 (dd, J=10.6, 15.0, 1H), 7.18–7.29 (m, 2H), 7.33–7.38 (m, 3H), 7.53 (d, J=7.3, 2H), 7.94 (dt, J=1.8, 8.1, 1H), 8.43 (dd, J=1.5, 4.8, 1H), 8.69 (d, J=1.8, 1H); MS (ES+) m/z 208 (M+1). Anal. calcd for $C_{15}H_{13}N$: C, 86.92; H, 6.32; N, 6.76. Found: C, 85.94; H, 6.18; N, 6.74.

2-[(*E,E*)-4-Phenyl-1,3-butadienyl] pyridine (21). The synthesis of compound **21** has been reported by Piechucki.[15] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.40 (d, J=11.4, 1H), 6.53 (t, J=11.4, 1H), 6.74 (d, J=15.4, 1H), 7.11 (ddd, J=0.888, 4.8, 7.5, 1H), 7.23–7.26 (m, 2H), 7.32 (t, J=7.5, 2H), 7.48 (dd, J=1.4, 7.7, 2H), 7.63 (dt, J=1.8, 7.5, 1H), 8.26 (dd, J=11.0, 15.8, 1H), 8.67 (dd, J=0.88, 4.8, 1H). HRMS (ES+) m/z 208.1145 (calcd for $C_{15}H_{13}N$+H 208.1127).

2-[(3*E*)-4-Phenyl-1-(2-pyridyl)-1,3-butadienyl] pyridine (22). Compound **22** was synthesized from di-2-pyridyl ketone and **13a** by the general Wittig method described for compound **19**. After stirring at room temp, H_2O was added and stirred for 48 h; the ppt was collected by vacuum filtration and dried to yield a light yellow powder (45% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (m, 2H), 7.05 (dt, J=7.6, 0.8, 1H), 7.14 (ddd, J=7.6, 4.8, 0.8, 1H), 7.21 (m, 1H), 7.27–7.35 (m, 5H), 7.39 (dt, J=8.0, 1.2, 1H), 7.57 (dd, J=8.0, 2.0, 1H), 7.67 (ddd, J=15.6, 12.0, 1H), 7.80 (td, J=8.0, 2.0, 1H), 8.63 (ddd, J=4.8, 1.6, 0.8, 1H), 8.79 (ddd, J=4.8, 1.6, 0.8, 1H). MS (ESI+) m/z 285 $(M+H)^+$. Anal. calcd for $C_{20}H_{16}N_2$: C, 84.48; H, 5.67; N, 9.85. Found: C, 84.18; H, 5.56; N, 9.41.

3-Ethyl-4-[(3*E*)-4-phenyl-3-butenyl] pyridine (23). An oven-dried flask was charged with anhydrous THF (6 mL) and diisopropylamine (1.3 mL, 9.5 mmol) and cooled to ~−50 °C. *n*BuLi (2.5 M in hexanes, 3.8 mL, 9.5 mmol) was added dropwise and the reaction was stirred for 10 min. 3-Ethyl-4-methyl pyridine (1.0 mL, 9.4 mmol) was added, then the mixture was stirred for 40 min at −50 °C and 45 min at −10 °C. The mixture was then cooled again to −50 °C and a solution of 3-bromo-1-phenyl propene (2.05 g, 9.4 mmol) in THF was added dropwise. The reaction mixture was allowed to warm slowly to room temperature and quenched with H_2O after stirring for a total of 3 h. After dilution with EtOAc, the organic layer was washed with H_2O, then brine, then dried over $MgSO_4$, vacuum filtered through a layer of silica and Celite and concd in vacuo. The crude product was chromatographed (50–75% EtOAc–hexanes; rotary chromatography) to give 991 mg of a brown oil (45% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, J=7.5, 3H), 2.50 (q, J=7.4, 2H), 2.68 (dd, J=7.5, 15.2, 2H), 2.75–2.81 (m, 2H), 6.23 (dt, J=6.8, 15.8, 1H), 6.42 (d, J=15.9, 1H), 7.08 (d, J=5.1, 1H), 7.18–7.35 (m, 5H), 8.35 (d, J=5.1, 1H), 8.39 (s, 1H). HRMS (ES+) m/z 238.1607 (calcd for $C_{17}H_{19}N$+H 238.1597).

2-[(1*E*,3*E*)-1-Methyl-4-phenyl-1,3-butadienyl] pyrazine (24b). Compound **24** was synthesized from 2-acetyl pyrazine and **13a** by the general Wittig method described for compound **19**. The product was isolated by extractive workup (H_2O/hexanes). Purification by Biotage flash chromatography (0–30% EtOAc–CH_2Cl_2) yielded two lots: **24a** as an orange oil (41% yield), which solidified upon standing, and **24b** as an orange solid (42% yield). **24a** was spectroscopically determined (COSY, difference NOE) to be the (*Z,E*)-isomer, but it isomerized in solution to ~1:1 mixture of the (*Z,E*)- and (*E,E*)-isomers. **24b** was spectroscopically determined to be the (*E,E*)-isomer. **24b** ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (3H, s), 6.85 (d, J=14.4, 1H), 7.21–7.37 (m, 5H), 7.50 (dd, J=7.2, 1.6, 2H), 8.39 (d, J=2.8, 1H), 8.53 (dd, J=2.4, 1.6, 1H), 8.81 (d, J=1.6, 1H). MS (ESI+) m/z 223 $(M+H)^+$. Anal. calcd for $C_{15}H_{14}N_2$: C, 81.05; H, 6.35; N, 12.60. Found: C, 80.72; H, 6.36; N, 12.63.

2-[(*E,E*)-1-Methyl-4-phenyl-1,3-butadienyl] thiazole (25b). Compound **25b** was synthesized from 2-acetyl thiazole and **13a** by the general Wittig method described for compound **19**. The product was isolated by extractive workup (H_2O/hexanes) and concd in vacuo. It was then purified by Biotage flash chromatography (100% CH_2Cl_2) and yielded two lots: **25a** as a brown oil, which solidified upon standing and **25b** as a brown oil. **25b** was spectroscopically determined (COSY, difference NOE) to be the (*E,E*)-isomer. **25a** isomerized in solution to **25b**; the two lots were pooled to give **25b** as a brown oil (87% yield). **25b** ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39 (s, 3H), 6.81 (m, 1H), 7.14–7.29 (m, 4H), 7.35 (t, J=7.6, 2H), 7.49 (d, J=7.6, 2H), 7.80 (dd, J=3.2, 0.8, 1H). MS (ESI+) m/z 228 $(M+H)^+$.

4-[(*E,E*)-4-(2-Methoxyphenyl)-1,3-butadienyl] nitrobenzene (26). Compound **26** has been reported by Cheng et al.[17] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, minor imp., 0.24H), 3.90 (s, 3H), 6.68 (d, J=15.7, 1H), 6.91 (d, J=8.2, 1H), 6.97 (t, J=7.4, 1H), 7.01–7.05 (m, 1H), 7.13 (d, J=10.6, 1H), 7.17 (d, J=10.2, 1H), 7.25–7.26 (m, 1H), 7.52–7.55 (m, 3H), 8.05 (d, J=8.8, minor imp., 0.14H), 8.19 (d, J=9.0, 2H). ++ HRMS (ES+) m/z 282.1138 (calcd for $C_{17}H_{15}NO_3$+H 282.1131).

2-[(*E,E*)-4-(2-Methoxyphenyl)-1,3-butadienyl] nitrobenzene (27). Compound **27** has been reported by Cheng et al.[17] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H), 6.88 (d, J=8.2, 1H), 6.98 (dd, J=7.4, 14.5, 2H), 7.01–7.15 (m, 3H), 7.22–7.26 (m, 1H), 7.33 (dt, J=1.0, 7.7, 1H), 7.52 (dd, J=1.2, 7.0 1H), 7.55 (d, J=7.4, 1H), 7.71 (d, J=7.8, 1H), 7.89 (dd, J=0.78, 8.2, 1H). HRMS (ES+) m/z 282.1120 (calcd for $C_{17}H_{15}NO_3$+H 282.1131). Anal. calcd for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.38; N, 4.98. Found: C, 72.74; H, 5.43; N, 4.95.

4-[(*E,E*)-4-(4-Nitrophenyl)-1,3-butadienyl] nitrobenzene (28). The synthesis of compound **28** has been reported

by Mitsudo et al.[18] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (dd, J = 3.0, 12.1, 2H), 7.12 (dd, J = 2.9, 12.1, 2H), 7.59 (d, J = 8.6, 4H), 8.22 (d, J = 9.0, 4H). MS (FD+) m/z 296 (M^+). Anal. calcd for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.46. Found: C, 64.69; H, 4.21; N, 9.45.

4-[(*E*,*E*)-4-Phenyl-1,3-butadienyl] nitrobenzene (29). Compound **29** was synthesized from *p*-nitro benzaldehyde and **13a** by the general Wittig method described for compound **19**. After cooling the rxn mixture, the ppt was collected by vacuum filtration and dried to yield a bright yellow solid. The crude product was recrystallized from hot DMF–H_2O–EtOH (25% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (d, J = 15.2, 1H), 6.81 (d, J = 15.2, 1H), 6.98 (dd, J = 10.8, 15.2, 1H), 7.12 (dd, J = 10.8, 15.2, 1H), 7.30 (d, J = 7.2, 1H), 7.37 (t, J = 7.2, 2H), 7.47 (d, J = 7.2, 2H), 7.55 (d, J = 9.2, 2H), 8.20 (d, J = 8.4, 2H). MS (ESI+) m/z 251 (M + H). Anal. calcd for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.22; N, 5.57. Found: C, 76.12; H, 5.13; N, 5.57.

3-[(*E*,*E*)-4-Phenyl-1,3-butadienyl] nitrobenzene (30). Compound **30** was synthesized from 3-nitro benzaldehyde and **13a** by the general Wittig method described for **19**. After cooling the reaction mixture, the ppt was collected by vacuum filtration and dried to give a solid (41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (d, J = 15.2, 1H), 6.78 (d, J = 15.2, 1H), 6.97 (dd, J = 10.4, 15.2, 1H), 7.08 (dd, J = 10.4, 15.2, 1H), 7.29 (d, J = 7.2, 1H), 7.36 (t, J = 7.2, 2H), 7.47 (d, J = 7.2, 2H), 7.51 (d, J = 8.0, 1H), 7.72 (d, J = 7.6, 1H), 8.07 (dd, J = 1.6, 8.0, 1H), 8.29 (s, 1H). MS (EI+) m/z 251 (M^+). Anal. calcd for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.22; N, 5.57. Found: C, 76.12; H, 5.12; N, 5.57.

2-[(*E*,*E*)-4-[4-(Dimethylamino)-phenyl]-1,3-butadienyl] benzonitrile (31). This compound was obtained from an internal compound library. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.00 (s, 6H), 6.67–6.72 (m, 3H), 6.84 (dd, J = 10.2, 15.2, 1H), 6.89 (d, J = 16.0, 1H), 7.10 (dd, J = 10.6, 15.2, 1H), 7.21 (d, J = 7.8, 1H), 7.35 (d, J = 8.6, 2H), 7.50 (t, J = 7.8, 1H), 7.58 (d, J = 7.8, 1H), 7.69 (d, J = 8.2, 1H). HRMS (ES+) m/z 275.1555 (calcd for $C_{19}H_{18}N_2$ + H 275.1550). Anal. calcd for $C_{19}H_{18}N_2$: C, 83.18; H, 6.61; N, 10.21. Found: C, 83.09; H, 6.60; N, 10.09.

4-[(*E*,*E*)-4-[4-(Dimethylamino)-phenyl]-1,3-butadienyl] benzonitrile (32). The synthesis of compound **32** has been reported by Singh et al.[22] ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.99 (s, 6H), 6.52 (d, J = 15.4, 1H), 6.66–6.70 (m, 3H), 6.77 (dd, J = 10.1, 15.4, 1H), 7.03 (dd, J = 10.1, 15.8, 1H), 7.33–7.35 (m, 2H), 7.44 (d, J = 8.4, 2H), 7.56 (dd, J = 1.8, 8.4, 2H). HRMS (ES+) m/z 275.1541 (calcd for $C_{19}H_{18}N_2$ + H 275.1550).

4-[(*E*,*E*)-4-[4-(Dimethylamino)-phenyl]-1,3-butadienyl] benzoic acid (33). The methyl ester of compound **33** has been reported by Singh et al.[23] ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.93–2.94 (m, 6H), 6.63–6.72 (m, 4H), 6.86 (dd, J = 10.5, 15.2, 1H), 7.18 (dd, J = 10.2, 15.2, 1H), 7.35 (d, J = 8.6, 2H), 7.55 (d, J = 8.2, 2H), 7.86 (d, J = 8.2, 2H). HRMS (ES+) m/z 294.1498 (calcd for $C_{19}H_{19}NO_2$ + H 294.1494).

***N*,*N*-Dimethyl-4-[(*E*,*E*)-4-(2-methoxyphenyl)-1,3-butadienyl] benzenamine (34).** Compound **34** was synthesized from 4-dimethylamino benzaldehyde and **13b** by the general Wittig method described for compound **19**. The product was isolated by extractive workup (H_2O/EtOAc), then recrystallized from hot DMF–H_2O to give a white powder (45% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.88–2.97 (m, 6H), 3.87 (s, 3H), 6.58 (d, J = 15.6, 1H), 6.69 (dt, J = 2.0, 8.8, 2H), 6.79–7.0 (m, 3H), 6.95 (d, J = 15.6, 1H), 7.16–7.20 (m, 1H), 7.33 (dt, J = 2.0, 8.8, 2H), 7.51 (dd, J = 2.0, 7.6, 1H), 8.01 (s, 1H). HRMS (ES+) m/z 280.1695 (calcd for $C_{19}H_{21}NO$ + H 280.1701).

4-[(*E*,*E*) 4-(4-*N*,*N*-Dimethylaminophenyl)-1,3-butadienyl] quinoline (35). Compound **35** was reported by both Shibata[24] and Pforr.[25] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.01 (s, 6H), 6.71 (d, J = 8.6, 2H), 6.78 (d, J = 15.3, 1H), 6.92–7.00 (m, 1H), 7.20–7.31 (m, 2H), 7.41 (d, J = 9, 2H), 7.54 (d, J = 16, 1H), 7.57 (t, J = 7.4, 1H), 7.71 (t, J = 7.3, 1H), 8.10 (d, J = 8.0, 1H), 8.20 (d, J = 8.22, 1H), 8.85 (d, J = 4.3, 1H). HRMS (ES+) m/z 301.1718 (calcd for $C_{21}H_{20}N_2$ + H 301.1705).

Compound 36. Compound **36** was synthesized from 4-morpholino benzaldehyde and **13a** by the general Wittig method described for compound **19** except that it was refluxed during the reaction time. After cooling to room temperature, H_2O was added and the yellow crystals that resulted were collected by vacuum filtration. The product was recrystallized from hot DMF–H_2O to give ~1:1 mixture of isomers. After isomerization from I_2/Et_2O/toluene with subsequent recrystallization, yellow crystals were obtained as ~2.5:1 mixture of isomers (26% yield). The stereochemistry of the isomers were determined spectroscopically (ROESY, COSY). (*1E, 3E*) is the major isomer (isomer A); (1*Z*,3*E*) is the minor isomer (isomer B). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.6 (s, H_2O), 2.17 (s, 3H, isomer B), 2.25 (s, 3H, isomer A), 3.18–3.23 (m, 4H, A/B), 3.86–3.90 (m, 4H, A/B), 6.27 (d, J = 11.2, 1H, isomer B), 6.53 (d, J = 16, 1H, isomer A), 6.63 (d, J = 15.6, 1H, isomer A), 6.62 (dd, J = 0.8, 11.2, 1H, isomer B), 6.88–6.98 (m, 2H), 7.16–7.35 (m, 6H), 7.43–7.46 (m, 3H). MS (ESI+) m/z 306 $(M + H)^{++}$. Anal. calcd for $C_{21}H_{23}NO$: C, 82.59; H, 7.59; N, 4.59. Found: C, 82.52; H, 7.57; N, 4.76.

3-(4-Pyridinyl)-2*H*-benz[*g*]indazole mono methanesulfonate (37). The synthesis of the free base form of compound **37** has been reported by Coombs et al.[26] ^{1}H NMR (400 MHz, DMSO-d_6) 2.33 (s, 3H), 7.70 (dt, J = 1.3, 7.9, 1H), 7.76 (dt, J = 1.3, 7.9, 1H), 7.81 (d, J = 8.8, 1H), 8.12 (d, J = 7.9, 1H), 8.27 (d, J = 9.2, 1H), 8.54 (d, J = 7.9, 1H), 8.62 (d, J = 6.6, 2H), 8.93 (dt, J = 1.3, 6.6, 2H). HRMS (ES+) m/z 246.1029 (calcd for $C_{16}H_{11}N_3$ + H 246.1033).

3-(4-Pyridinyl)-2*H*-benz[g]indazole (38). The synthesis of compound **38** has been reported by Coombs et al.[26] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63–7.70 (m, 3H), 7.95–8.03 (m, 4H), 8.78 (d, J = 5.9, 2H), 11.04 (br s, 1H). HRMS (ES+) m/z 246.1041 (calcd for $C_{16}H_{11}N$ + H 246.1033).

4,5-Dihydro-3-(4-pyridinyl)-2*H*-benz[*g*]indazole mono methylsulfonate (39). Compound **39** is commercially available as the free base and has been reported by Habeck et al.[27] ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (s, 3H), 3.01 (t, J = 6.8, 2H), 3.09 (t, J = 6.8, 2H), 7.30 (q, J = 6.8, 2H), 7.36 (t, J = 8.0, 1H), 7.72 (d, J = 7.2, 1H), 8.21 (2H), 8.86 (d, J = 6.8, 2H). HRMS (ES+) m/z 248.1191 (calcd for $C_{16}H_{34}N_3$ + H 248.1189).

4,5-Dihydro-2-(phenylmethyl)-3-(4-pyridinyl)-2*H*-benz[*g*]-indazole mono hydrobromide (40). Compound **40** is commercially available. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.99–3.03 (m, 2H), 3.10 (dt, J = 1.8, 8.3, 2H), 5.85 (s, 2H), 7.28–7.32 (m, 2H), 7.36 (t, J = 7.5, 1H), 7.42–7.48 (m, 3H), 7.52–7.54 (m, 2H), 7.70 (dd, J = 0.88, 7.5, 1H), 8.39 (d, J = 6.6, 2H), 9.10 (d, J = 6.6, 2H). HRMS (ES+) m/z 338.1672 (calcd for $C_{23}H_{29}N_3$ + H 338.1659).

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