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ANTICOCCIDIAL ACTIVITY OF SOME AZACYCLO ORGANOBORINATES

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Abstract - A series of new azacyclo organoborinates derivatives of piperidinyl and pyridinyl alcohols with anticoccidial activity *in vivo* assay system in battery trials are described. Parameters of anticoccidial efficacy that were evaluated were body weight, lesion scores and number of oocysts. Among them, di(4-chloro-phenyl)(2-piperidinylmethoxy-O,N)boron,and di(3-trifluoromethyl-phenyl)(2-pyridinylethoxy-O,N)boron showed good anticoccidial activity against both of the important parasite, *Eimeria tenella* and *Eimeria acervulina*

Coccidiosis is an infectious disease caused by protozoan belonging to coccidium. Poultry is mainly infected with *E. tenella* and *E. aceruvulina and E. necatrix,* and suffers from various troubles such as bleeding of the gastrointestinal tract, mortality, growth inhibition, and so forth. Poultry includes chickens, turkeys, quails and ducks. Mass outbreak of avian cocciodiosis in a commercial poultry farm imparts an extremely great loss to an owner and has often become a serious problem.[1] Accordingly, the development of an anticoccidial drug, which is effective for prevention, and remedy of cocciodiosis have drawn a keen attention of those concerned in the art. Conventionally, sulfanilamides,[2] nitrofurans,[3] quinolines,[4] antithiamines,[5] and benzamides[6] have been put into practical application as the anticoccidial drug, and polyether-based antibiotics[7] have been used mainly at present. However, these compounds have the drawbacks in that, although their efficacy against coccidiosis is not very high, they are toxic to hosts. Moreover resistant strain

appears in the course of continuous use of these chemicals, and their efficacy drops progressively with time.[8] In view of these circumstance, the development of a novel anticoccidial agent which is effective for the resistant strains and at the same time, hardly imparts resistance to the strains, has been desired earnestly. Many compounds with antibacterial, fungal, and anti-fouling activity of azacyclo organoborinates have been reported in the literature.[9,10] In the previous paper of this series we have described the syntheses and anticoccidial activity (*in vitro* evaluation) of derivatives of piperidinyl and pyridinyl alcohols.[11-12] As further investigation, we studied the anticoccidial activity (*in vivo* evaluation) of these compounds, to reach new active derivatives of piperidinyl and pyridinyl anticoccidial agents. We are developing new anticoccidial agents of derivatives of piperidinyl and pyridinyl alcohols against the important parasite, *Eimeria tenella in vivo* assay.

CHEMISTRY

Azacyclo organoborinate derivatives(**1-34**, Figure 1) were prepared from 2-aminoethoxydiarylborinate with requisite piperidinyl and pyridinyl alcohol as described in our previous paper.[11-12]

BIOLOGICAL PROPERTIES

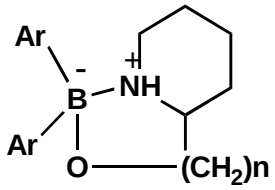
Measurement of preventive/curative effect against cocciodiosis.

Animals: Animals used in this experiment were white Leghorn breed chickens. They were breeds raised free from coccidia in an animal's house at the Aburahi Laboratories. Chickens of both sexes, 10 days old and weighing about 70 g, were divided into groups of two or three chickens each. They were fed chick food (SDL-No.1: Nippei Co., Ltd.) any additional coccidiostate other than the compounds tested. Water was *ad libitum*.

Pathogens: Pathogens used were drug susceptive strains *Eimeria tenella* (NIAH) and *E.acervulina* (NIAH) provided by the National Institute of Animal Health. They were newly passed into chickens, and the collected oocysts were kept in a solution 2 % potassium bichromate. Inoculum size of each coccidium per chicken was 5×10^4 sprouted oocysts of *E.tenella* and 5×10^5 sprouted oocysts of *E.acervulina*

Environmental conditions:The animal room was conditioned by fresh filtered air at 25±2℃ and relative humidity, 40~60 %. Each group of animal placed in a sterilized wire floored metal cage a 25-watt electric lamp.

Figure 1 Structure of some azacyclo organoborinates



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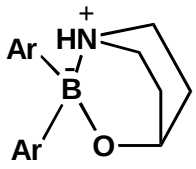

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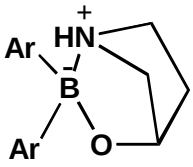

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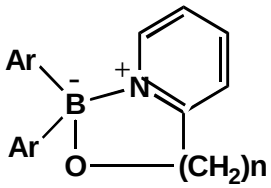

16-34

Compd. No.	n	Ar
1	1	Ph
2	1	2-CH_3-C_6H_4
3	1	3-CH_3-C_6H_4
4	1	4-CH_3-C_6H_4
5	1	3-Cl-C_6H_4
6	1	4-Cl-C_6H_4
7	1	4-CH_3-C_6H_4
8	1	4-F-C_6H_4
9	1	4-CH_3O-C_6H_4
10	1	α-Naph
11	2	3-Cl-C_6H_4
12	2	4-Cl-C_6H_4
13	2	3-CF_3-C_6H_4
14		4-Cl-C_6H_4
15		3-CF_3-C_6H_4

Compd. No.	n	Ar
16	1	Ph
17	1	2-CH_3-C_6H_4
18	1	3-CH_3-C_6H_4
19	1	4-CH_3-C_6H_4
20	1	3-Cl-C_6H_4
21	1	4-Cl-C_6H_4
22	1	4-F-C_6H_4
23	1	3-CF_3-C_6H_4
24	1	2-CH_3O-C_6H_4
25	1	4-CH_3O-C_6H_4
26	1	α-Naph
27	2	Ph
28	2	2-CH_3-C_6H_4
29	2	3-Cl-C_6H_4
30	2	4-Cl-C_6H_4
31	2	4-F-C_6H_4
32	2	3-CF_3-C_6H_4
33	2	4-CH_3O-C_6H_4
34	2	α-Naph

Experimental design: Prophylactic effects of the compounds were tested against NIAH strain. Administration of the compounds mixed in the feed was started from before one days of oral inoculation of the test pathogens and continued for 9 and 7days in the test of *E.tenella* and *E.acervulina* infection respectively.

Evaluation of anticoccidial activity: At the end of the experimental period, the number of surviving chickens in each group, the average body weight gain of surviving chickens of each group were recorded, and the relative weight gain (Rel. wt. gain) of survivors were calculated as following formula.

$$\text{Rel.wt.gain (\%)} = \frac{\text{Weight gain of infected-medicated group}}{\text{Weight gain of uninfected-unmedicated group}} \times 100$$

The degree of bloody discharge was observed in each group during the test period. Animal was autopsied at the end of the experiment and examined for gross cecal lesions. Cecal lesions on day 7 after *E.tenella* infection were scored as follows.
0: no lesion, 1: slight with a pinpoint lesion, 2: moderate slight hemorrhage and numerous lesion, 3: considerable cecal wall thickened with atrophy due to heavy hemorrhage, 4: serious atrophied and shorted with cheesy substance in the contents.
Number of oocysts per gram of feces (OPG) was counted by means of a Fuchs-Rosenthal hemocytometer on day 6 or day 4 after *E.tenella* and *E.acervulina* infection, respectively.

RESULT AND DISCUSSION

Of synthesized compounds, di(4-chloro-phenyl)(2-piperidinylmethoxy-O,N)boron (**6**) and di(3-trifluoromethyl-phenyl)(2-pyridinylethoxy-O,N)boron (**32**) exhibited most active activity *in vitro* test against *E. tenella* as described in our previous paper.[12] The result of activity *in vivo* test of compound (**6**) and (**32**) was shown in Tables 1-3 in comparison with a comparative group treated with an untreated infected control group and an untreated non-infected control group. When administered to both *E. tenella* and *E. acervulina* in the form of feed at 100 ppm, compound (**6**) explicitly provided improvements in the clinical conditions, the ratio of relative weight gain, and the cecum lesion index(only *E. tenella),* demonstrating that compound (**6**) exhibits a excellent anticoccidial effect. Particularly, the number of oocysts on the 6th day of the infection was 0 even in the group infected with 200 ppm of the specimen. Compound (**6**) was completely inhibited against *E. tenella* even at 200ppm (see Tabel 1,2) and similar results were obtained against *E. acervulina.* (see Tabal 3). In summary, the compounds of the present studies exhibit strong anticoccidial activities in chicken chicks, such as inhibition of the decrease in the ratio of relative weight gain, inhibition of hemorrhagic stool excretion, decrease in oocysts counts(OPG), improvement of cecum lesion index, and the like. Furthermore , the compounds(**6, 32**) have a low toxicity as demonstrated by the data of mortality. It is of interest that this case a comparatively small change in chemical structure lead to a increase in biological potency. The current findings

with compound (**6**) encourage that further synthetic and biological studies of the azacyclo organoborinate derivatives are being carried out in our laboratories.

Table 1. Efficacy of new azacyclo organoborate against *Eimeria tenella* infection

Compound No.	Dosage(ppm in feed)	Rel. wt. gain (%)	No. of death (/)	Bloody dischage	Oocyst per gram of feces	Cecum lesion score (mean)	Judgement
5	200		3/3	+++	NT	4.0	—
6	400	82.5	0/3	−	NT	0.0	+ *
8	200	25.7	0/3	+++	NT	4.0	—
10	400	66.8	0/3	+++	NT	4.0	—
11	200	58.6	0/3	+++	3.2×10^{6}	4.0	—
12	200	81.9	0/3	++	1.8×10^{5}	2.7	+
14	200	70.0	0/3	+++	1.3×10^{6}	4.0	—
15	200	38.7	0/2	++	2.2×10^{6}	4.0	—
18	400	71.8	1/3	+++	NT	4.0	—
20	200	12.2	0/3	+++	ND	4.0	—
23	400	57.4	0/3	++	NT	3.7	—
26	400	56.5	0/3	+++	NT	4.0	—
29	200	56.2	0/3	+++	1.8×10^{6}	4.0	—
32	400	82.5	0/3	++	NT	3.3	+
34	400	53.8	0/3	+++	NT	4.0	—

NT : not tested ND : not don * : Highly active

Table 2. Anticoccidial activity of 6 against *Eimeria tenella* infection in broiler chickens

Compound No.	Dosage(ppm in feed)	Rel. wt. gain (%)	No. of death (/)	Bloody disch			Oocyst per gram of feces		Cecal lesion score (mean)
				4	5	6d	6	7d	
6	200	70.2	0/3	—	—	—	0	0	0.0
	100	101.0	0/3	+	+	±	1.0×10^{5}	6.3×10^{3}	1.7
	50	54.8	0/3	+	+++	++	2.9×10^{5}	9.8×10^{4}	4.0
Infected cont.	0	49.0	1/3	++	+++	++	1.9×10^{5}	1.3×10^{5}	4.0
Uninfected cont.	0	100.0	0/3	−	−	−	0	0	0.0

Table 3. Anticoccidial activity of 6 against *Eimeria acervulina* infection in broiler chickens

Compound No.	Dosage(ppm in feed)	Rel. wt. gain (%)	No. of death (/)	Mucos feces		Oocyst per gram of feces	
				4	5d	4	5d
6	200	82.3	0/3	—	—	0	0
	100	80.6	0/3	—	++	6.6×10^{6}	2.8×10^{6}
	50	67.8	0/3	++	+++	5.9×10^{6}	1.7×10^{6}
Infected cont.	0	54.9	0/3	++	+++	1.7×10^{7}	7.5×10^{6}
Uninfected cont.	0	100.0	0/3	−	−	0	0

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