HETEROCYCLES, Vol. 57, No. 7, 2002, pp. 1319 - 1326, Received, 22nd April, 2002

SYNTHESIS AND ANTICOCCIDIAL ACTIVITY OF SOME AZACYCLO ORGANOBORINATES

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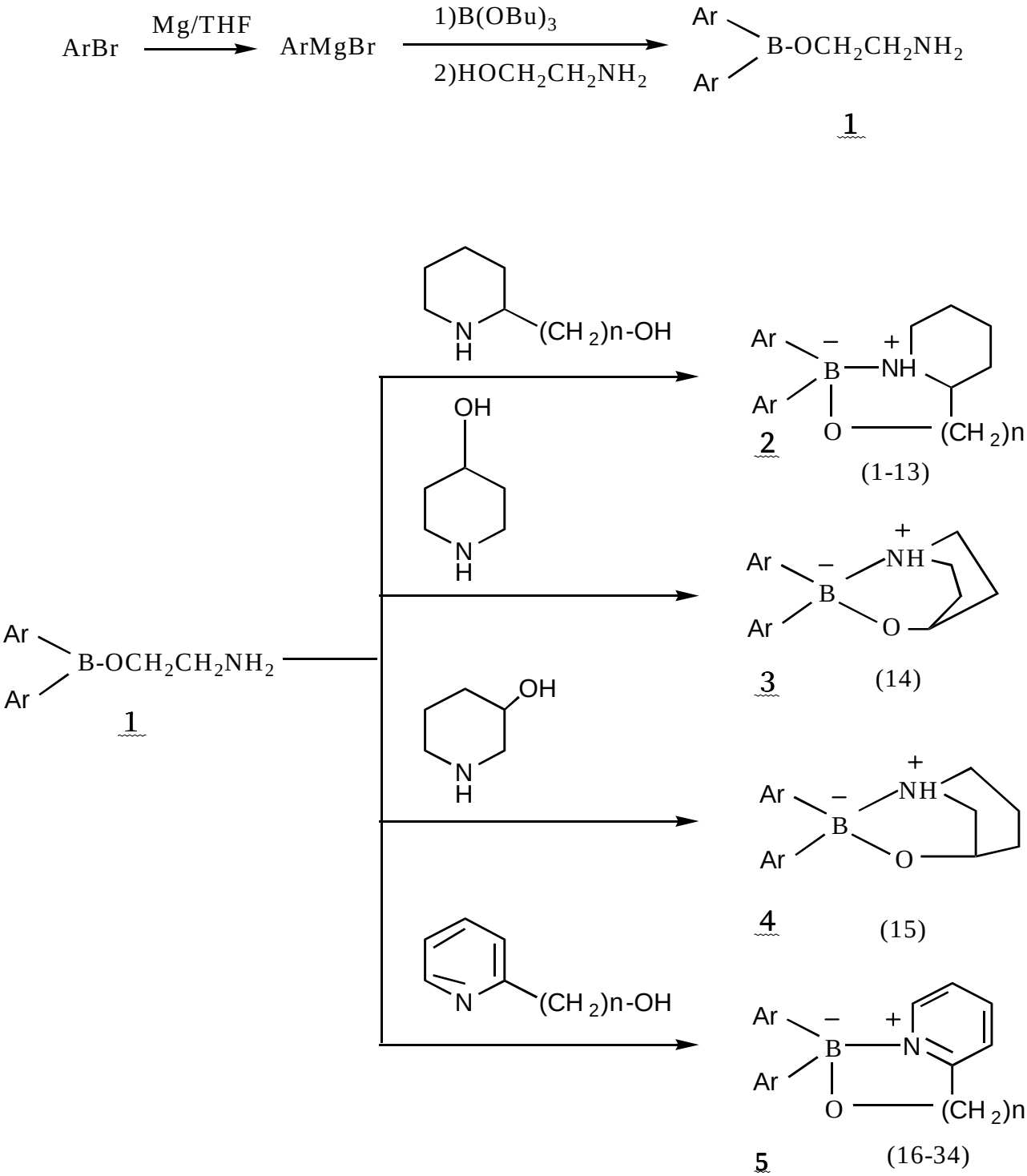
Abstract—A series of azacyclo organoborinates derivatives of piperidinyl and pyridinyl alcohols were prepared and their anticoccidial activity was tested *in vitro* assay system. Among them, di(4-chlorophenyl)(2-piperidinylmethoxy-O,N)-boron, di(3-trifluoromethylphenyl)(2-piperidinylmethoxy-O,N)-boron, di(3-trifluoromethylphenyl)(2-pyridinylmethoxy-O,N)-boron, and di(3-trifluoromethylphenyl)-(2-pyridinylethoxy-O,N)boron, showed moderate anticoccidial activity against *Eimeria tenella.*

Coccidiosis in chickens, turkeys, geese, and ducks is a widespread disease caused by a group of parasitic protozoa, although many polyether ionophores such as monensin,[1] lasalocid,[2] salinomycin,[3] and narasin[4] have been used successfully as anticoccidial agents and as growth promoters. However, polyether-resistant *Eimeria* spp. are emerging with increasing frequency.[5] In our program directed toward developing new anticoccidial agents against polyether-resistant *Eimeria* spp., we have now synthesized a series of diphenyl or dinaphthyl substituted organoborate derivatives of piperidinyl and pyridinyl alcohols were prepared (Scheme 1) and their anticoccidial activity was tested *in vitro.* Many compounds with the antibacterial, fungal, and anti-fouling activities of azacyclo organoborinates have been reported in the literature.[6] We found that diphenyl (2-piperidinyl- methoxyO,N)boron causes high anticoccidial activity against the important parasites, *E. tenella* and *E. acervulina* ,[10] and is easily prepared *via* a three-step procedure.

Based on the high anticoccidial potencies of 5, we have now prepared pyridinylorganoborinates (**16-34**) in order to explore their structure-activity relationship. The main objective of this investigation is to research a new agent with potential activity against coccidiosis and a less cyto-pathogenic effect on cells.

Scheme 1



CHEMISTRY

Studies on the synthesis and characterization of organic boron compounds possessing both N→B and O→B coordination bonds are well documented in the literature.[7-9, 11-20] The compounds in Tables 1 and 2 were prepared by known procedures.[7,11,20] The synthesis of the appropriately substituted azacyclo diarylborinates derivatives was initiated by the preparation of 2-aminoethoxydiarylborane (**1**) from the appropriate Grignard reagent with tributyl borate and ethanolamine using literature procedures.[7,8] The obtained (**1**) was treated with the requisite piperidinyl or pyridinyl alcohols to yield corresponding azacyclo diarylborinates derivatives (**1-34**) (Scheme 1, Tables 1, 2). The general synthetic procedure is described below.

*Step 1 : the preparation of 2-aminoethoxydiarylborane (**1**)*

A solution of arylmagnesium bromide in tetrahydrofuran was prepared according to a conventional method using 0.53 mol of aryl bromide, 12.9 g(0.53 mol) of magnesium, and 250 mL of tetrahydrofuran. Subsequently, 58.5 g(0.26 mol) of tributyl borate ester and 200 mL of ether were mixed in a flask, and the flask was cooled to –60℃. A solution of arylmagnesium bromide (Grignard reagent) in tetrahydrofuran was added dropwise to the mixture while maintaining a temperature of －60℃. After the addition, the reaction mixture was stirred for 10 h at rt and hydrolyzed by the addition of 150 mL of 10% hydrochloric acid, and the organic layer was isolated. 31.1 g(0.51 mol) of ethanolamine and 50 mL of ethanol were added to the organic layer, and the mixture was stirred for 2 h at rt. The precipitated product was collected by filtration, recrystallized from ethanol/water=1/1, and dried to afford 2-aminoethoxydiarylborane (**1**).

*Step 2 : the preparation of **azacyclo diarylborinates** derivatives (**1-34**)*

0.012 mol of 2-aminoethoxydiarylborane (**1**), 30 mL of ether and 30 mL of 10% hydrochloric acid contained in a separatory funnel were shaken for about 15 min, and the layers were separated. The organic layer was washed once with water and transferred to a pear-shaped flask to which 0.018 mol of requisite piperidinyl alcohols (**1-13**) or 1.8 g(0.018 mol) of 4-hydroxypiperidine (**14**) or 1.8 g(0.018 mol) of 3-hydroxypiperidine (**15**) or pyridinyl alcohols (**16-34**) and 7 mL of ethanol were added, and the mixture was stirred for 2h at rt. The precipitated product was collected by filtration, recrystallized from a ethanol/water=1/1, and dried to afford azacyclo diarylborinates derivatives(**1-34**)

Table 1. Yeild and mp of diaryl(2-piperidinylalkoxy-O,N)borons

Fomula No.	Compd No.	n	Ar	Yield (%)	mp (℃)
	1	1	Ph	83	169-171
	2	1	2-CH_3-C_6H_4	55	126-128
	3	1	3-CH_3-C_6H_4	67	203-205
	4	1	4-CH_3-C_6H_4	88	134-136
	5	1	3-Cl-C_6H_4	58	182-184
	6	1	4-Cl-C_6H_4	80	150-155
2	**7**	1	4-F-C_6H_4	68	134-136
	8	1	3-CF_3-C_6H_4	55	95-97
	9	1	4-OCH_3-C_6H_4	37	148-151
	10	1	α-Naph	47	176-178
	11	2	3-Cl-C_6H_4	74	188-190
	12	2	4-Cl-C_6H_4	77	86-89
	13	2	3-CF_3-C_6H_4	68	89-92
3	**14**		4-Cl-C_6H_4	31	204-208
4	**15**		3-CF_3-C_6H_4	37	174-178

Table 2. Yeild and mp of diaryl(2-pyridinylalkoxy-O,N)borons

Fomula No.	Compd No.	n	Ar	Yield (%)	mp (℃)
	16	1	Ph	61	150-151
	17	1	2-CH_3-C_6H_4	68	188-192
	18	1	3-CH_3-C_6H_4	57	117-119
	19	1	4-CH_3-C_6H_4	57	147-149
	20	1	3-Cl-C_6H_4	81	105-107
	21	1	4-Cl-C_6H_4	71	133-134
	22	1	4-F-C_6H_4	53	137-139
	23	1	3-CF_3-C_6H_4	63	92-94
	24	1	2-OCH_3-C_6H_4	37	163-165
5	**25**	1	4-OCH_3-C_6H_4	83	129-131
	26	1	α-Naph	46	233-236
	27	2	Ph	58	176-178
	28	2	2-CH_3-C_6H_4	87	208-211
	29	2	3-Cl-C_6H_4	78	137-139
	30	2	4-Cl-C_6H_4	74	140-142
	31	2	4-F-C_6H_4	83	118-120
	32	2	3-CF_3-C_6H_4	65	140-142
	33	2	4-OCH_3-C_6H_4	80	131-133
	34	2	α-Naph	52	215-218

BIOLOGICAL PROPERTIES

Materials and method

Cells：Kidneys were obtained from 3- to 7- day-old chickens under sterile conditions. After removing the tapeta, nerves and blood vessels, the kidneys were crushed through a 5 mL syringe in phosphate-buffered saline(PBS) at 4℃, washed several times with PBS and trypsinized with 0.125 % trypsin solution. The cells (CK cell) were filtered through a wire mesh, the cell number counted with a haemocytometer and suspended in growth medium (medium 199 with 5 % fetal bovine serum penicillin:100 IU/mL and streptomycin:100 IU/mL, pH7.2) at $3\sim5\times10^5$/mL. This cell suspension (100 μL) was transferred to 96 flat-bottom-well plates (Sumitomo Co.) to undergo monolayer cell incubation for 72 h at 37℃ under 5 %CO_2/95 % air conditions.

Parasites：The NIAH strain (standard strain) of *E. tenella* was supplied by the National Institute of Animal Health. The oocysts were sterilized with 10% sodium hypochloride which was then removed by centrifugation, and the oocysts washed three times with sterilized PBS. The oocysts were re-suspended in PBS (pH8.0) and stored at 4℃. Before the assay, they were crushed by shaking with glass-beads (No.8). Sporozoites were excysted from sporocysts by shaking for 2 h at 40℃ in a water bath.

Excystion fluid : 0.25 % trypsin(1:250 Difco Lab) and 10 % chicken bile in PBS.

The sporozoite suspension was washed twice with sterilized PBS, and whole oocysts and oocyst wall debris were removed by centrifugation. The pure sporozoite pellet was resuspended in maintenance medium (medium 199 with 1 % FBS, 1 % tryptose phosphate broth and 1 % yeast extract, pH 7.2). The final sporozoite concentration was about $2.0\sim3.0\text{x}10^5$/mL.

Method：The solution of the compounds in methanol (1000μg/mL) was diluted with the final sporozoite suspension ($2.0\sim3.0\times10^5$/mL) at a concentration of 10, 1, 0.1 μg/mL. The solution was then added to the CK monolayer cells, and incubated at 40℃ in 5 % CO_2/95 % air for 48 h. CK cells stained with 2 % Giemsa solution were observed microscopically.

Judgment： Anticoccidial activity was judged by the presence of immature and mature 1st-schizonts grown in the CK cell.

Anticoccidial activity

(+) ： 1st-schizonts not found

(±)： A few 1st-schizonts found (>80% inhibition)

Cytopathogenic effect (CPE)

(+++) ：death of whole cells (the protozoan was invisible)

(++) ：severe,

(±～+) ：poor,

(－) ：no effect

RESULTS AND DISCUSSION

In vitro anticoccidial activity was judged by microscopic observation of the formation of 1st schizonts against the important parasite, *E. tenella* in the cells, and the biological data are summarized in Tables 3 and 4. Of all the compounds (**1**-**34**) tested, compounds (**6**, **8**, **14**) of the piperidine type, and compounds (**23, 32**) of the pyridine type exhibited good activity.

Table 3. Anticoccidial activity of diaryl(piperidinylalkoxy-O,N)borons

	Dosage (μg/mL)		
Compd No.	10 (CPE)	1 (CPE)	0.1 (CPE)
1	- (-)	- (-)	- (-)
2	- (-)	- (-)	- (-)
3	- (-)	- (-)	- (-)
4	- (-)	- (-)	- (-)
5	(+++)	- (-)	- (-)
6	+ (+)	- (-)	- (-)
7	- (-)	- (-)	- (-)
8	+ (+)	- (-)	- (-)
9	- (-)	- (-)	- (-)
10	± (-)	- (-)	- (-)
11	(+++)	- (-)	- (-)
12	(+++)	± (-)	- (-)
13	(+++)	- (-)	- (-)
14	(+++)	+ (-)	- (-)
15	(+++)	- (-)	- (-)

Table 4. Anticoccidial activity of diaryl(pyridinylalkoxy-O,N)borons

Compd No.	Dosage (μg/mL) 10 (CPE)	1 (CPE)	0.1 (CPE)
16	- (-)	- (-)	- (-)
17	- (-)	- (-)	- (-)
18	± (-)	- (-)	- (-)
19	- (-)	- (-)	- (-)
20	(+++)	- (-)	- (-)
21	- (-)	- (-)	- (-)
22	- (-)	- (-)	- (-)
23	+ (+)	- (-)	- (-)
24	- (-)	- (-)	- (-)
25	- (-)	- (-)	- (-)
26	± (-)	- (-)	- (-)
27	- (-)	- (-)	- (-)
28	- (-)	- (-)	- (-)
29	(+++)	- (-)	- (-)
30	- (-)	- (-)	- (-)
31	- (-)	- (-)	- (-)
32	+ (+)	- (-)	- (-)
33	- (-)	- (-)	- (-)
34	± (-)	- (-)	- (-)

On the other hand, compounds (**10** and **12**) of the piperidine type, and compounds (**18** and **19**) of the pyridine type showed moderate activity. However, compounds (**1-5**, **7**, **9**, **11**, **13**, **15**, **16**, **17**, **20-22**, **24**, **25**, **27-31**, **33** and **34**) have poor activity. It is noticeable that a comparatively small change in chemical structure leads to a marked increase in biological potency. The structure-activity relationship between the non-aromatic piperidine type and the aromatic pyridine types is interesting. Replacement of the piperidine moiety (compounds (**6** and **12**)) of bis-4-chlorophenyl derivatives with the pyridine moiety (compounds (**21** and **30**)) extinguishes the activity. On the other hand, replacement of the piperidine moiety (compound (**8**)) of bis-3-trifluoromethyl derivatives with the pyridine moiety (compound (**23**)) did not significantly change the activity.

ACKNOWLEDGEMENTS

The skillful technical help of Mr. H. Fujikawa, and the valuable discussion of Dr. T. Nakamura, are gratefully acknowledged.

REFERENCES

1. R. F. Shumard and M. E. Callender, *Antimicrob. Agents Chemother.*, 1968, **1967**, 369.
2. M. Mitrovic and E. G. Schildknecht, *Poult. Sci.*, 1974, **53,** 1448.
3. P. Yvore, J.-P. Paynaud, L, Conan, and M. Naciri, *Poult. Sci.*, 1980, **59**, 2412.
4. D. H. Berg and R. L. Hamill, *J. Antibiotics.*, 1978, **31**, 1.
5. H. Onaga, T. Ishii, and T. Koyama, *Jpn. J. Vet. Sci.*, 1974, **36**, 73.
6. P. Patel, *U.S. Patent 5, 348, 947,* 1994 (*Chem. Abstr.,* **1994**, *121,* 295090).
7. K. Smith, *Organometallic Compounds of Boron,* Chapman and Hall, New York. **1985.**
8. G. N. Chremos, H. Weidmann, and H. K. Zimmerman, *J. Chem. Soc., Chem. Commun.*, 1961, 1683.
9. L. A. Torres, A. Perez, N. Farfan, D. Castillo, and R. L. Santillan, *J. Chem. Thermodynamics.* 1994, **26**, 337.
10. H. Imazaki, M. Kawaguchi, H. Fijikawa, and Y. Hayase, *Patent WO00/044387,* 2000. (*Chem. Abstr.,* **2000**, *133,* 144898)
11. N. Farfan, D. Castillo, J.-N. Pedro, and R. Contreras, *J. Chem. Soc., Perkin Trans.Ⅱ,* 1992, 527.
12. N. Farfan and R. Contreras, *J. Chem. Soc., Perkin Trans.Ⅱ,* 1988, 1787.
13. W. Kliegel, *Tetrahedron Letters,* 1969, 223.
14. W. Kliegel, *Angew. Chem., Internat. Ed. Engl.,* 1968, **7**, 626.
15. F. Umland, *Angew. Chem., Internat. Ed. Engl.,* 1967, **6**, 1082.
16. S. J. Trofimenko, *J. Amer. Chem. Soc.,* 1966, **88**, 1842.
17. F. Umland, *Angew. Chem., Internat. Ed. Engl.*, 1965, **4,** 958.
18. F. Umland, *Angew. Chem., Internat. Ed. Engl.,* 1965, **4,** 432.
19. H. J. Roth and B. Miller, *Arch. Pharm.,* 1964, **9**, 513.
20. H. Hopfl, N. Farfan, D. Castillo, R. Santillan, A. Gutierrez, and J. C. Daran, *J. Organomet. Chem.,* 1998, **553**, 221.