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Antiarrhythmic Activity of Some *N*-Alkylbispidinebenzamides

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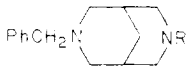
A series of aromatic ring substituted bispidinebenzamides, **2**–**10**, was prepared by condensation of *N*-methyl- or *N*-*n*-butylbispidine with the appropriate acid chlorides. These compounds were initially evaluated in mice for acute toxicity and for their ability to protect against chloroform-induced ventricular fibrillation. All compounds had significant activity, which was optimized in **2**, **3**, and **5**. These last compounds had potencies and LD_{50}/ED_{50} ratios comparable to those of a standard antiarrhythmic, disopyramide. However, their potencies in increasing the effective refactory period in isolated rabbit atria were considerably less than that of disopyramide.

Since the discovery of quinidine as an effective drug for maintaining normal heart rhythm,[1] a structurally diverse group of compounds has been used clinically in management of cardiac arrhythmias.[2] One of the more important structural categories of antiarrhythmic agents is the "amide-type",[3] which includes procainamide, lidocaine, disopyramide, and a large number of experimental compounds.[2,4] Agents in this category are characterized chemically by the presence of amide and amine functional groups.

In a previous paper, we described a series of N,N′-disubstituted bispidines, of which **1a**,**b** are representative.

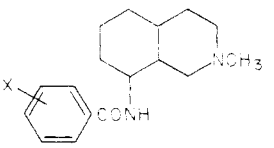


1a, R = CH_3
b, R = *n*-C_4H_9

These compounds were reasonably potent, but were, in general, quite toxic, with LD_{50}/ED_{50} ratios of less than 2.0.[5] In a preliminary attempt to increase potency and reduce toxicity, we evaluated the effect of replacement of the benzyl group of **1b** with a benzoyl group. The resulting "amide-type" analogue retained antiarrhythmic activity, and its toxicity was decreased more than twofold compared to that shown by **1b**. We wanted to determine the effect of a similar replacement in **1a** and to determine the effect of substitution in the aromatic ring of each of these benzamides. In other series of related antiarrhythmics, increases in potency, as well as the therapeutic index, have been observed as a result of the introduction of appropriate benzene-ring substituents.[6] Thus, we describe here the synthesis and evaluation of antiarrhythmic potencies and acute toxicities of **2**–**10**. Ring-substituted compounds were chosen in accordance with a previously reported operational scheme for finding the optimum substitution on a benzene ring in an active lead compound for maximization of pharmacologic activity.[7]

Results and Discussion

Compounds **2**–**10** were initially evaluated in mice for acute toxicity and for their ability to protect against chloroform-induced ventricular fibrillation. Disopyramide, one of the newer "amide-type" antiarrhythmics, was adopted as a standard. Results are shown in Table I. All of the compounds showed significant antiarrhythmic activity, which ranged over about one order of magnitude. Since the acute toxicities of the compounds did not vary greatly, differences in LD_{50}/ED_{50} ratios were primarily a reflection of differences in potency. In the *N*-methyl compounds **2**–**6**, activity was maximized in the 4-chloro compound **5**, with activity approximating that of disopyramide seen in the 4-methoxy compound **3** and the unsubstituted compound **2**. Multiply substituted compounds **4** and **6** were less active. The activity of **2**, **3**, and **5** was greater than that of their respective *N*-*n*-butyl counterparts. As in the *N*-methyl compounds, activity in the *N*-*n*-butyl compounds was maximized by monosubstitution. This finding is in contrast to results of similar studies of 8-substituted decahydroisoquinolines (11) and



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6-substituted analogues as well. In these compounds, antiarrhythmic activity was maximal in the 3,4-dichloro- and 3,4,5-trimethoxybenzamides, respectively. Successive removal of substituents resulted in decreases in potencies.[8]

The favorable potencies of **2**, **3**, and **5** in comparison to that of disopyramide in protection against ventricular fibrillation in the mouse led us to compare these compounds in a test for atrial antiarrhythmic potency. Thus, the compounds were evaluated in isolated rabbit atria for

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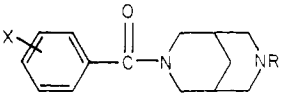
Table I. Antiarrhythmic Potencies and Toxicities of *N*-Alkylbispidinebenzamides



compd	X	R	potency N^a	potency ED_{50},[b] μmol/kg ip	toxicity N^a	toxicity LD_{50},[b] μmol/kg ip	LD_{50}/ED_{50}
2	H	CH_3	12	85 (77-95)	6	621 (578-668)	7.29
3	4-OCH_3	CH_3	8	78 (72-85)	7	463 (422-508)	5.93
4	3,4,5-$(OCH_3)_3$	CH_3	10	137 (121-155)	10	535 (479-598)	3.91
5	4-Cl	CH_3	12	49 (47-52)	8	535 (519-552)	10.89
6	3,4-$(Cl)_2$	CH_3	12	470 (443-499)	8	492 (454-534)	1.05
7	H	*n*-C_4H_9	13	242 (216-271)	7	500 (459-544)	2.07
8	4-OCH_3	*n*-C_4H_9	8	106 (102-110)	6	488 (433-542)	4.60
9	4-Cl	*n*-C_4H_9	8	134 (125-145)	8	463 (424-504)	3.45
10	3,4-$(Cl)_2$	*n*-C_4H_9	7	223 (202-247)	10	605 (541-676)	2.71
disopyramide			9	90 (85-95)	9	517 (460-556)	5.77

[a] Number of mice used. [b] 95% confidence limits are in parentheses.

Table II. Effects of *N*-Methylbispidinebenzamides on the Isolated Rabbit Atrium

compd	N^a	ED_{25},[b] μM FAC	ED_{25},[b] μM MFF	ratio
2	10	>400	400[c]	
3	6	>400	500[c]	
5	12	400[c]	110	3.64
disopyramide	10	56	18	3.11

[a] Number of atria used. [b] Average bath concentrations required to reduce force of atrial contraction (FAC) and maximal following frequency (MFF) by 25% with respect to control values. [c] Estimated by extrapolation.

their ability to reduce maximum following frequency (MFF), a measure of antiarrhythmic activity, and for their ability to reduce the force of atrial contraction (FAC), an undesirable effect often exhibited by antiarrhythmic compounds. Results are summarized in Table II. Compound **5** had equivalent MFF- and FAC-lowering activities to those of disopyramide at respective six- and sevenfold greater concentrations. The other benzamides were essentially inactive with respect to disopyramide in these assays.

This study has revealed several compounds which compare favorably with disopyramide, in particular with regard to their ventricular antiarrhythmic effects. Compound **5** has been selected for further pharmacologic studies.

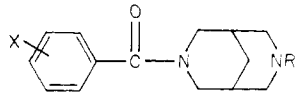
Experimental Section

Uncorrected melting points were obtained using a Thomas-Hoover capillary melting point apparatus. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. *N*-Methyl- and *N*-*n*-butylbispidine were obtained as described previously.[9] Acid chlorides were obtained from Aldrich Chemical Co., Milwaukee, Wis.

General Procedure for Synthesis of *N*-Alkylbispidinebenzamides. The compounds shown in Table II were prepared as follows. To a cold (5 °C) stirred solution of 5 mmol of the *N*-alkylbispidine in 10 mL of methylene chloride was added dropwise a solution of 5.5 mmol of the appropriate acid chloride in 10 mL of methylene chloride. After completion of addition, the solution was allowed to warm to room temperature and, after stirring for 2 h, the mixture was concentrated in vacuo. The residue was shaken with 20 mL of ether and 20 mL of water, and the upper phase was discarded. The aqueous layer was washed with 20 mL of ether and made basic by the addition of ca. 1 g of anhydrous sodium carbonate. The resulting suspension was extracted with two 25-mL portions of ether. The combined ethereal extracts were dried (anhydrous sodium sulfate), filtered, and concentrated in vacuo. Residual moisture was removed azeotropically with benzene, in vacuo. The resulting free base was dissolved in about 10 mL of anhydrous ether and treated with excess ethereal hydrogen chloride to precipitate the hydrochloride salt. Alternatively, the ethereal solution of the base was treated with an equimolar amount of fumaric acid dissolved in a minimum volume of ethanol, in the event that the hydrochloride salt proved to be hygroscopic. The precipitated salt was recrystallized from the appropriate solvent (Table III).

Pharmacologic Evaluation. Prevention of chloroform-induced ventricular fibrillation in adult CF_1 mice (female) was used for initial estimation of antiarrhythmic activities of **2**–**10** and disopyramide, as previously described.[5] Acute toxicities of these compounds were also determined in adult CF_1 mice as previously described.[5] Further evaluation of antiarrhythmic activity was obtained by a modification of the maximum following frequency assay of Dawes[10] as follows. Left atria of New Zealand white male

Table III. *N*-Alkylbispidinebenzamides



compd	X	R	formula[a]	yield, %	mp, °C	recrystn solvent
2	H	CH_3	$C_{15}H_{20}N_2O \cdot C_4H_4O_4$	86	171-175	ethanol-ether
3	4-OCH_3	CH_3	$C_{16}H_{22}N_2O_2 \cdot C_4H_4O_4$	85	154-156	acetone
4	3,4,5-$(OCH_3)_3$	CH_3	$C_{18}H_{26}N_2O_4 \cdot C_4H_4O_4$	86	161.5-163	ethanol-ether
5	4-Cl	CH_3	$C_{15}H_{19}ClN_2O \cdot C_4H_4O_4$	93	201.5-204[b]	ethanol
6	3,4-$(Cl)_2$	CH_3	$C_{15}H_{18}Cl_2N_2O \cdot C_4H_4O_4$	88	201-203[b]	ethanol
7	H	*n*-C_4H_9	$C_{18}H_{26}N_2O \cdot HCl$	57	187-188[b]	chloroform-ethyl acetate
8	4-OCH_3	*n*-C_4H_9	$C_{19}H_{28}N_2O_2 \cdot 1.25C_4H_4O_4$	94	133-135	ether
9	4-Cl	*n*-C_4H_9	$C_{18}H_{25}ClN_2O \cdot C_4H_4O_4$	62	140-141.5[b]	benzene
10	3,4-$(Cl)_2$	*n*-C_4H_9	$C_{18}H_{24}Cl_2N_2O \cdot C_4H_4O_4$	97	150.5-152	ethanol

[a] All compounds analyzed for C, H, and N within ±0.4% of calculated values. [b] The product melted with decomposition.

rabbits were isolated and mounted in baths at 35 °C in Krebs-Henseleit buffer and aerated with 95% O_2–5% CO_2. They were driven with square-wave pulses of 10-ms duration at five times the threshold voltage. The maximum effective frequency of stimulation (maximum following frequency, MFF) was determined by gradually increasing the stimulus rate from 3 Hz (the control or basal rate) to the point where skipped beats were evident and then reducing the frequency to a rate that the atria would just follow. This rate was taken as the end point, and is essentially the reciprocal of the effective refractory period of the atrium. After determination of control MFF, the MFF reduction caused by **2**, **3**, **5**, and disopyramide were determined at concentrations of 1, 3, 13, 33, and 133 μM. The dose–response curves for all atria were plotted, and the ED_{25} values (drug concentration required to reduce MFF to 75% of its control value) were estimated. Since compounds with ability to reduce MFF were seen to reduce the force of atrial contraction (FAC) at 3 Hz, the ED_{25} for this side effect was also calculated.

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Antifungal Agents. 4.[1] Chemical Modification of Antibiotics from *Polyangium cellulosum* var. *fulvum*. Ester and Amide Analogues of Ambruticin

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A series of ester and amide analogues of ambruticin (**1**) was prepared. The analogues were tested against *Histoplasma capsulatum*, *Microsporum fulvum*, *Candida albicans* and *Streptococcus pyogenes*. Structure–activity relationships are described.

We previously described the isolation[3] and characterization[4,5] of two potent antifungal antibiotics elaborated by *Polyangium cellulosum* var. *fulvum*. The compounds were highly active against such medically important systemic pathogens as *Histoplasma capsulatum* and *Coccidioides immitis* in vitro[3] and in vivo[6]. In view of the success of chemical-modification programs in other areas of antimicrobial chemistry and the need for nontoxic agents to combat systemic and dermatophytic fungal disease, we undertook the synthesis of chemically modified analogues.

The goals of the program were to establish the structural features necessary for activity and perhaps point the way to the construction of simpler molecules, which would retain antifungal activity and could be readily synthesized. It was also hoped to uncover molecules with more potent activity or expanded antimicrobial spectra (activity against *Candida albicans* and bacteria). Another phase of interest would be molecules retaining antifungal activity but with reduced serum binding.

In this paper, we describe the chemical modification of ambruticin (**1**)[4] to give esters and amides (Table I) and the resulting effects that were observed in the in vitro antifungal and antibacterial activities.

The analogues were synthesized by the routes shown in Scheme I. Acylation of **1** gave esters **9**–**12**. Treatment of ester **2** with amines gave amides **3**–**7**. The amides were converted to the corresponding diacetates, which exhibited the expected molecular ions in their mass spectra. Treatment of ester **2** with aryl isocyanates gave carbamates **16** and **17**.

The analogues were tested against Gram-positive bacteria, including *Streptococcus pyogenes*, and fungi, including *Histoplasma capsulatum*, *Microsporum fulvum*, and *Candida albicans*. Antimicrobial testing was carried out by a standard broth dilution procedure. The results are summarized in Table I.

Conversion of acid **1** to amides **3**–**7** resulted in increased activity against *S. pyogenes*. Esterification of the hydroxyl groups in acid **1** to give compounds **10**–**12** also resulted in increased activity against this organism. The remaining analogues in Table I showed little or no antibacterial activity.

The compounds (Table I) were all inactive against *C. albicans*. In general, the analogues were active against *H. capsulatum* and *M. fulvum* unless they contained bulky substituents at strategic positions (compounds **8**, **16**, and **17**). Exceptions were the diacetates (**20**, **21**, and **23**), which were inactive. A comparison of the activities of compounds **1**–**7** and molecules in which C_1 is a ketone carbonyl[7] group indicates the absence of any well-defined electronic effect at the C_1 carbonyl group on antifungal activity. The decrease in antifungal activity (especially against *M. fulvum*) in the series **1**–**4** and **8** indicates a strong steric effect at C_1 on antifungal activity. The declining activity in the series **1** and **9**–**12** and **2** and **14**–**17** illustrates the effect of increasing bulk at positions 5 and 6. Once again, antifungal activity against *M. fulvum* appears to be more sensitive to the steric effect at C_5 and C_6, as it was with respect to the steric effect at C_1.

None of the analogues showed any advantage over acid **1** with respect to antifungal activity in the presence of serum.

The polar functions at C_1, C_5, and C_6 are important for antifungal activity, and the incorporation of bulky groups at these positions reduces activity. A differentiation of the factors necessary for maximization of antifungal activity and antibacterial activity was observed in the series **1** and

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