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Synthesis and anti-arrhythmic activity of aminoguanidine derivatives

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Summary — A series of new aminoguanidine derivatives were synthesized and tested for anti-arrhythmic activity. The compounds selected were investigated on other test models. Finally, compound **13** 1-(2,6-dimethylphenyl)-4,4-dimethyl-aminoguanidine-hydrochloride (B-GYKI-38 233) was chosen for detailed study.

aminoguanidines / anti-arrhythmic activity

Introduction

Despite the fact that reports on several new compounds with anti-arrhythmic activity are published yearly, and some of them have also become commercially available [1–3], the search for new anti-arrhythmic agents continues worldwide.

According to their biological action, the anti-arrhythmic drugs can be divided into 4 categories [4]. Chemically, they represent quite different structures, but most of them possess one general feature as depicted in figure 1. The members of this class of compounds can be characterized by the following properties: they have a linear structure; a lipophilic (LP) group is present at one terminal of the molecule; a hydrophilic (HP) group stands at the other terminal of the molecule; the aromatic moiety (LP) is joined to the basic moiety (HP) by an 'inter-chain' (IC) consisting of carbon and heteroatoms; and in some of the compounds, this spacer is approximately 5.82–5.95 Å long.

The best known representatives of anti-arrhythmic agents having a linear structure are lidocaine **1a**, tocainide **1b** and their analogues [5–12] and mexiletine **2** (scheme 1).

In the course of our study, we investigated *N*(4)-disubstituted aminoguanidines **3** because: 1) reports on such compounds have not been published so far; 2) it is obvious from figure 1 that their structure meets the requirement that the distance between the phenyl group and the terminal nitrogen is nearly 5.9 Å; 3) to date, the anti-arrhythmic effect of aminoguanidines has not been examined.

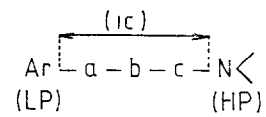
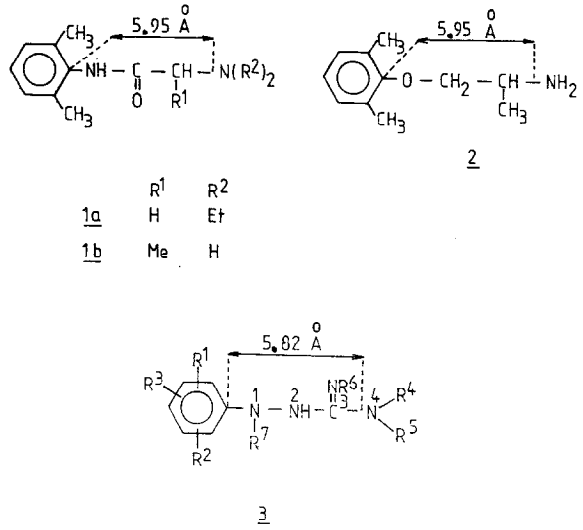


Fig 1. General feature of anti-arrhythmic drugs.



Scheme 1.

Many aminoguanidines are known in the literature. Among these, 1-aryloxyalkyl-aminoguanidines show an adrenergic neuron-blocking effect [13]; the 1,1-dialkyl-aminoguanidines are pesticides [14]; 1-phenyl-alkyl-aminoguanidines [15, 16], 4-phenyl-aminoguanidines [17] and 1-phenyl-aminoguanidines [18] exert

a hypotensive action. However 4,4-disubstituted aminoguanidines (type **3** compounds) have not yet been described. Therefore, we decided to synthesize them and examine their biological activities.

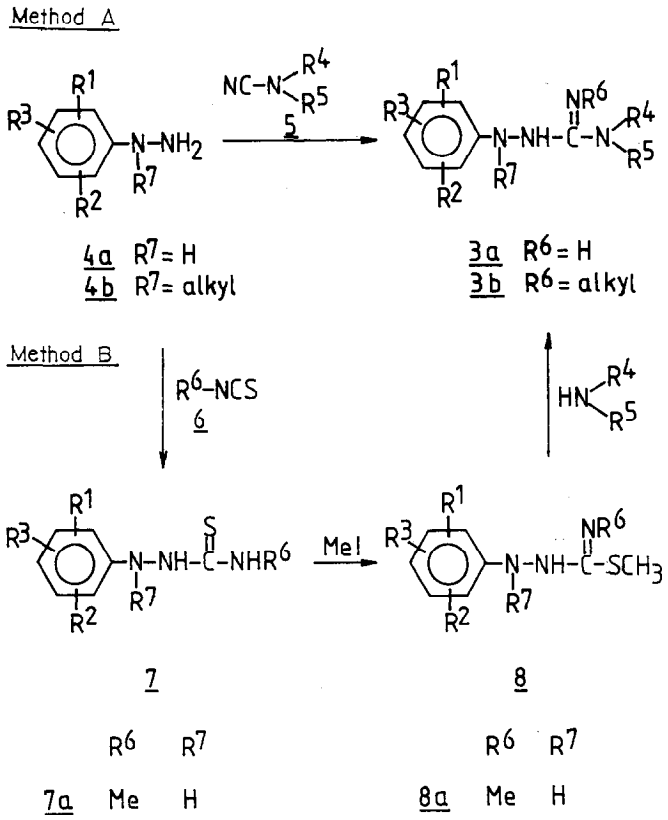
Chemistry

In our compound series, the lipophilic moiety is a phenyl group carrying a wide variety of substituents; the substituents of the guanidine moiety are alkyl groups or form a cycle.

The new compounds **3** were constructed using the following simple methods (scheme 2).

To prepare compounds in which R^6 is a hydrogen, the phenylhydrazines **4** or their hydrochlorides were reacted with *N,N*-disubstituted cyanamides **5** *(Method A)*. These nucleophilic addition reactions were carried out in anhydrous aliphatic alcohols in solutions of high concentrations at 100–110°C. The reaction usually proceeded within 3–5 h. In most cases, the aminoguanidine **3a** obtained was separated as a hydrochloride, which was then purified by recrystallization.

The compounds containing an alkyl group as R^6 were prepared by reacting the phenylhydrazines **4** with the alkylisothiocyanates **6** to yield 1-phenyl-4-alkylthiosemicarbazides **7** [19]. Reaction of the latter with iodomethane resulted in the formation of 1-phenyl-3-(*S*-methyl)-4-R^6-isothiosemicarbazides **8**, which, when reacted with an HNR^4R^5 amine, yielded the aminoguanidines **3b** *(Method B)*.



Scheme 2.

The phenylhydrazines **4a** (R^7 = H) used as starting materials as well as their preparative methods are known [20]. The phenylhydrazines **4b** (R^7 = alkyl) are also known [21, 22].

Some of the *N,N*-disubstituted cyanamides **5** are known in the literature [23, 24]. The new cyanamide derivatives were prepared in the manner described for the synthesis of dimethylcyanamide, *ie* by reacting a secondary amine with cyanogen bromide in an aqueous ethereal system, in the presence of base [23].

The compounds together with the data related to the synthesis are shown in table I. These compounds are not known in the literature, except those published in our own patent specification [25].

Results and discussion

Structure–activity relations

The influence of the substituents (R^1–R^7) on the anti-arrhythmic activity can be briefly summarized as follows.

A precondition of the favourable activity is that both R^6 and R^7 should be hydrogen. When R^6 was Me (**61**, **62**), the activity after ip injection was weak or toxicity was high; and compounds containing an alkyl or alkenyl as R^7 (**40**, **41**, **42**, **48**) were inactive. The strongest activity, after oral or ip administration, was shown by substances containing a 2'-Me group as R^1 (**9**, **47**) or 2 Me groups or 2 chlorine atoms as R^1 and R^2 in the 2'- and 6'-positions of the phenyl substituent (**10**, **13**, **19**). The activity decreased when the corresponding alkyl chains were lengthened (in *eg* **29** containing 2'-Me as R^1 and 6'-Et as R^2). Following ip injection, the activity of **14**, which contains 2'-Cl as R^1, was weaker than that of **9**, which contains 2'-Me as R^1, and **14** was inactive when administered orally. Administered ip, the activity of **24** which contains 2'-Me as R^1 and 6'-Cl as R^2, was low. Compound **17** with 3'-Cl as R^3 was highly active when injected ip but inactive when taken orally. Compound **22**, which contains 2'-CF_3 as R^1 was active after both oral and ip administrations. Introduction of a third substituent on the phenyl group (see **33** containing R^1 = R^2 = R^3 = Me in 2'-, 4'- and 6'-positions) resulted in the loss of activity.

As terminal groups R^4 and R^5, Me has its strongest effect (**9**, **13**); the activity was decreased in compounds **18** which have Et groups as R^4 and R^5. The efficiency

Table I. Substituents, physical data and yields of compounds **3** tested.

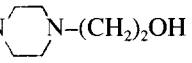
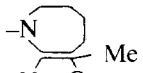
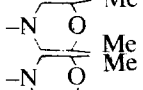
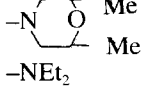
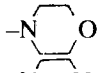
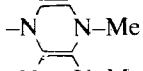
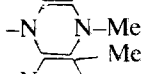
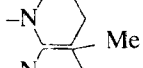
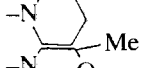
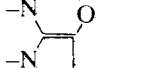
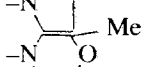
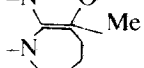
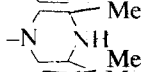
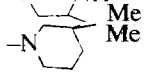
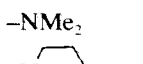
Compd	R^1	R^2	R^3	$-N\begin{matrix}R^4\\R^5\end{matrix}$	R^6	R^7	*Method*	*mp (°C)*	*Cryst solvent*	*Yield (%)*
9	2-Me	H	H	$-NMe_2$	H	H	A	219–221	EtOH/hexane	58
10	2-Cl	6-Cl	H	$-NMe_2$	H	H	A	255–257	EtOH/hexane	62
11	2-Cl	H	H	$-NEt_2$	H	H	A	216–217	EtOH/acetone	39
12	2-Me	H	H	$-NEt_2$	H	H	A	174–176	EtOH/Et_2O	37
13	2-Me	6-Me	H	$-NMe_2$	H	H	A	258–260	EtOH/hexane	61
14	2-Cl	H	H	$-NMe_2$	H	H	A	252–253	EtOH/hexane	69
15	2-Me	H	H		H	H	A	258–260	EtOH/hexane	47
16	2-Cl	H	H		H	H	A	212–213	Acetone/hexane	45
17	H	H	3-Cl	$-NMe_2$	H	H	A	171–174	EtOH/Et_2O	39
18	2-Me	6-Me	H	$-NEt_2$	H	H	A	212–215	EtOH/Et_2O	42
19	2-Me	6-Me	H		H	H	A	272–275	EtOH/Et_2O	44
20	2-Me	6-Me	H		H	H	A	233–237	EtOH/Et_2O	23
21	2-CF_3	H	H	$-NMe_2$	H	H	A	238–242	EtOH/Et_2O	71
22	2-CF_3	H	H	$-NEt_2$	H	H	A	202-206	Acetone/Et_2O	60
23	2-Cl	5-Cl	H	$-NMe_2$	H	H	A	257-258	EtOH/hexane	63
24	2-Me	6-Cl	H	$-NMe_2$	H	H	A	256-258	EtOH/Et_2O	40
25	2-Me	H	3-Me	$-NMe_2$	H	H	A	239–242	EtOH/hexane	42
26	H	H	H	$-NMe_2$	H	H	A	162–164	EtOH/Et_2O	36
27	H	H	4-Cl	$-NMe_2$	H	H	A	192–200	EtOH/Et_2O	58
28	2-Me	H	H		H	H	A	245–247[a]	EtOH/Et_2O	75
29	2-Me	6-Et	H	$-NMe_2$	H	H	A	253–256	EtOH/Et_2O	53
30	2-Me	H	H		H	H	A	160–163	EtOH/acetone	51
31	2-Me	H	H		H	H	A	204–205	Acetone	39
32	2-Me	H	3-Cl	$-NMe_2$	H	H	A	260–264	EtOH/Et_2O	50
33	2-Me	6-Me	4-Me	$-NMe_2$	H	H	A	248–251	EtOH/Et_2O	16
34	H	5-MeO	4-MeO		H	H	A[b]	206–207	Acetone	31
35	H	H	4-NO_2	$-NMe_2$	H	H	A	258–260	EtOH/Et_2O	68
36	2-MeO	H	H	$-NMe_2$	H	H	A	95–97	Acetone/Et_2O	39

Table I. (continued).

Compd	R^1	R^2	R^3	$-N\begin{matrix}R^4\\R^5\end{matrix}$	R^6	R^7	*Method*	*mp (°C)*	*Cryst solvent*	*Yield (%)*
37	2-Me	5-Me	H	$-NMe_2$	H	H	A	238–240	$EtOH/Et_{187}O$	41
38	2-Me	H	4-Me	$-NMe_2$	H	H	A	219–222	EtOH/hexane	52
39	H	H	4-Me	$-NMe_2$	H	H	A	176–179	$EtOH/Et_2O$	12
40	H	H	H	$-NMe_2$	H	Me	A	196–200	$EtOH/Et_2O$	46
41	H	H	H	$-NMe_2$	H	*i*-Pr	A	98–105	$EtOH/Et_2O$	58
42	H	H	H	$-NMe_2$	H	Allyl	A	161–163	$EtOH/Et_2O$	41
43	2-Me	H	4-Cl	$-NMe_2$	H	H	A	252–256	$EtOH/Et_2O$	45
44	2-Me	6-Me	H		H	H	A	260–265	EtOH/acetone	26
45	2-Me	H	H		H	H	A	195–198	EtOH/acetone	35
46	2-Me	6-Me	H		H	H	A	276–281	EtOH/acetone	40
47	2-Me	H	H		H	H	A	236–240	EtOH/acetone	54
48	H	H	H	$-NEt_2$	H	Me	A	198–200	$EtOH/acetone/Et_2O$	32
49	2-Me	6-Me	H		H	H	A	229–231	$EtOH/acetone/Et_2O$	52
50	2-Me	6-Me	H		H	H	A	205–211	EtOH/acetone	54
51	2-Me	H	H		H	H	A	196–198	EtOH/acetone	12
52	2-Me	6-Me	H		H	H	A	216–219	EtOH/acetone	24
53	2-Me	H	H		H	H	A	201–205	EtOH/acetone	10
54	2-Me	6-Me	H		H	H	A	209–211	$EtOH/Et_2O$	12
55	3-Cl	H	H		H	H	A	222–226	EtOH/acetone	28
56	3-Cl	H	H		H	H	A	228–230	EtOH/acetone	27
57	2-Cl	H	H		H	H	A	219–221	$EtOH/Et_2O$	42
58	2-Cl	6-Cl	H		H	H	A	277–279	$EtOH/E_2O$	39
59	2-Me	6-Me	H		H	H	A	286–287[a]	EtOH/acetone	18
60	2-Cl	H	H		H	H	A	224–226	$EtOH/Et_2O$	35
61	2-Me	6-Me	H	$-NMe_2$	Me	H	B	191–192[c]	Acetone/hexane	40
62	2-Me	6-Me	H		Me	H	B	199–200[c]	Acetone/hexane	45
63	2-Cl	6-Cl	H	$-NMe_3$	H	H	[d]	214–216[e]	$Acetone/Et_2O$	78

[a] x 2HCl; [b] reaction in 5 times dimethyl-acetamide (DMA); [c] hydroiodide; [d] prepared by methylation of **10** with MeI (in MeOH, 12 h at 25°C); [e] iodide.

was also sharply decreased by the introduction of a quaternary ammonium group (N^+Me_3 in **63**). Among the 5–7-membered saturated heterocyclic NR^4R^5 groups, 1-pyrrolidinyl (**19**) and 2,6-dimethyl-4-morpholinyl **47** have the highest activities.

In conclusion, in those compounds which exhibit the most favorable activity, the phenyl ring is mono- or disubstituted in the 2'- and 6'-positions by Me groups or chlorine atoms, and the 4-N atom is simultaneously disubstituted, carrying Me groups or incorporated into a pyrrolidine ring.

Screening system, results and selection

Compounds were first screened with the aconitine arrhythmia test in mice, with the control group treated simultaneously with each compound. The test compounds were administered intraperitoneally. Compounds exerting an anti-arrhythmic activity comparable to those of the reference substances (mexiletine and quinidine) were then given at different ip and oral doses. Table II shows representative test data of compounds **3**.

Based on the data obtained, the criteria for selection of a compound for further investigation was high activity after ip and *po* administrations. Five of the compounds tested, *ie* **9**, **10**, **13**, **19** and **47** met these requirements. Based on the better LD_{50} values, 2 of these, **13** and **19** were further studied. Compounds **13** and **19**, evaluated in the fibrillation threshold model in open-chest cats after intraduodenal administration, were comparable to quinidine and mexiletine (table III). In this test, compound **13** proved to be more active than compound **19**. Compound **13** (B-GYKI-38 233) and quinidine effects on the total and ectopic number of beats were measured in Harris dogs (fig 2).

Figure 2 graphically demonstrates the changes in the ratio of nomotopic to heterotopic stimulus formation after the administration of quinidine or B-GYKI-38 233 to Harris dogs. Both compounds strongly decreased the rate of ectopic beats in relation to the total number of heartbeats. However, B-GYKI-38 233 was more effective than quinidine (from 79.5 to 14.3% and from 83.5 to 57.6%, respectively). Thus, B-GYKI-38 233 is able to transform a dominant ventricular rhythm into a sinus rhythm.

An additional advantage of compound **13** is its lack of undesirable circulatory side effects that commonly appear following administration of known anti-arrhythmic agents; *ie*, it did not induce any pressure drop in the systemic circulation or a pressure increase in the pulmonary circulation in animals with intact chest or in unanesthetized permanently cannulated animals, at a dose range of 0.5–4.0 mg/kg. The anti-arrhythmic effect of compound **13** was not

Table II. The effect of the tested compounds on aconitine-induced arrhythmia in mice.

Compd	*Dose (mg/kg)*	*n*	*Onset of arrhythmia (time in s)*	*ED_{50}[a] (mg/kg, ip)*	*LD_{50} (mg/kg, ip)*
Control[b]	Saline	362	78.2 ± 1.4		
Mexiletine	10, ip	20	90.0 ± 2.3		
	25, ip	20	111.1 ± 4.2[c]	31.6	114
	50, ip	20	152.9 ± 9.5[c]		
	100, ip	20	158.6 ± 10.0[c]		
Quinidine	10, ip	19	98.0 ± 5.7		
	25, ip	19	119.2 ± 6.5[c]	28	
	50, ip	19	178.4 ± 8.4[c]		
	100, ip	18	251.0 ± 8.6		
9	25, ip	18	210.9 ± 12.2[c]		
	50, ip	18	224.2 ± 11.7[c]	9	81
	50, *po*	6	162.0 ± 17.6[c]		
	100, *po*	15	244.1 ± 16.5[c]		
10	25, ip	12	179.8 ± 12.8[c]		
	50, ip	12	216.9 ± 18.7[c]		
	25, ip	6	121.0 ± 11.5[c]		
	50, *po*	12	154.7 ± 11.3[c]		
11	25, ip	3	Toxic		
12	25, ip	3	Toxic		
13	20, ip	13	120.7 ± 8.0[c]		
	25, ip	27	167.5 ± 9.3[c]	18	125.5
	50, ip	28	210.9 ± 11.5[c]		
	50, *po*	29	112.3 ± 5.6[c]		
	75, *po*	18	121.8 ± 10.7[c]		
	100, *po*	26	148.0 ± 9.4[c]		
14	50, ip	6	174.0 ± 20.3[c]		
15	25, ip	6	117.4 ± 13.2[d]		
	50, ip	6	182.4 ± 10.7[c]		
16	25, ip	3	Toxic		
17	50, ip	12	201.0 ± 4.5[c]		
	100, *po*	5	83.4 ± 4.5		
18	50, ip	6	147.2 ± 25.7[d]		
19	25, ip	5	142.0 ± 28.2[d]	20	105

Table II. (continued)

Compd	*Dose (mg/kg)*	*n*	*Onset of arrhythmia (time in s)*	ED_{50}[a] *(mg/kg, ip)*	LD_{50} *(mg/kg, ip)*
20	25, ip	6	163.3 ± 18.8[c]		
	50, ip	3	Toxic		
21	50, ip	5	83.7 ± 3.4		
22	50, ip	6	147.3 ± 13.3[c]		
	100, *po*	6	134.6 ± 23.1[d]		
23	50, ip	6	135.8 ± 11.7[c]		
24	50, ip	5	127.6 ± 32.1		
25	50, ip	6	124.6 ± 9.4[d]		
26	50, ip	7	123.4 ± 14.3[d]		
27	50, ip	6	108.5 ± 6.5[c]		
28	50, ip	9	128.8 ± 14.4[c]		
29	25, ip	6	106.3 ± 12.8[d]		
	50, ip	8	170.5 ± 17.6[c]		
	100, *po*	8	112.7 ± 7.4[d]		
30	25, ip	6	98.2 ± 8.7		
	50, ip	10	152.8 ± 10.6[c]		
31	25, ip	3	Toxic		
32	50, ip	7	112.8 ± 10.3		
33	50, ip	6	89.8 ±5.5		
34	50, ip	9	94.1 ± 6.9		
35	50, ip	5	72.0 ± 7.3		
36	50, ip	5	67.6 ± 3.4		
37	50, ip	6	75.8 ± 7.5		
38	50, ip	8	113.0 ± 9.8[d]		
39	50, ip	14	121.1 ± 13.7[c]		
40	50, ip	6	75.3 ± 2.7		
41	50, ip	6	73.5 ± 4.3		

Table II. (continued)

Compd	*Dose (mg/kg)*	*n*	*Onset of arrhythmia (time in s)*	ED_{50}[a] *(mg/kg, ip)*	LD_{50} *(mg/kg, ip)*
42	50, ip	6	81.8 ± 5.2		
43	50, ip	6	99.0 ± 9.3		
44	50, ip	3	Toxic		
45	25, ip	3	Toxic		
46	25, ip	6	100.2 ± 6.3[c]		
	50, ip	9	197.5 ± 16.3[c]		
47	25, ip	5	131.1 ± 10.8[d]		
	50, ip	13	210.7 ± 7.1[c]	25	
	100, *po*	9	163.2 ± 12.8[c]		
	200, *po*	12	238.8 ± 16.5[c]		
48	50, ip	6	96.8 ± 6.4[d]		
49	50, ip	6	101.5 ± 6.4[d]		
50	50, ip	5	94.8 ± 9.2		
51	50, ip	6	154.8 ± 16.0[c]		
52	25, ip	3	Toxic		
53	25, ip	3	Toxic		
54	50, ip	7	160.6 ± 15.9		
55	50, ip	6	120.5 ± 9.8		
56	50, ip	5	111.6 ± 4.1		
57	50, ip	5	114.0 ± 10.0[d]		
58	50, ip	5	139.2 ± 8.4[d]		
59	50, ip	6	129.8 ± 15.2[d]		
60	12.5, ip	13	172.0 ± 7.3[c]		
	25, ip	5	Toxic		
61	50, ip	7	101.0 ± 16.7		
62	25, ip	3	Toxic		
63	50, ip	6	133.6 ± 14.2[c]		

[a]ED_{50} values were defined as the doses of drugs required to raise the dysrhythmic dose of aconitine by 50%, see [22]; [b]the controls were pooled. Each treatment was performed in parallel with a control group consisting of the same number of animals. The value given here for onset of arrhythmia is an average of 362 measurements; [c]$P < 0.01$; [d]$P < 0.05$.

Table III. Effect of intraduodenal administration of tested compounds on the fibrillation threshold in open-chest cats.

Compd	*Dose (mg/kg)*	*n*	*Change in fibrillation threshold*[a] *(%)*
Quinidine	10	7	+ 39.7 ± 15.1[c]
Mexiletine	20	7	+ 166.0 ± 38.8[b]
13	10	9	+ 150.7 ± 55.4[c]
	20	11	+ 165.2 ± 33.3[b]
19	20	4	+ 154.5 ± 19.4[b]

[a]Peak effects measured 30–60 min after drug administration. [b]$P < 0.01$; [c]$P < 0.05$.

accompanied by any other activity effecting the vegetative nervous system, *ie* the compound had no α- or β-adrenergic blocking, adrenergic neuron blocking or parasympatholytic activities [26].

In addition, compound **13** exhibited significant cardioprotective potency, since its anti-arrhythmic activity was also observed in the ischemic heart. This cardioprotective effect was 3 times as high as that of lidocaine **1a** [27].

During the determination of its LD_{50} value, **13** did not evoke any strong central nervous system excitation; thus, it can be postulated that this compound does not enter the brain, unlike other known class I anti-arrhythmic agents. Further study of this compound is in progress.

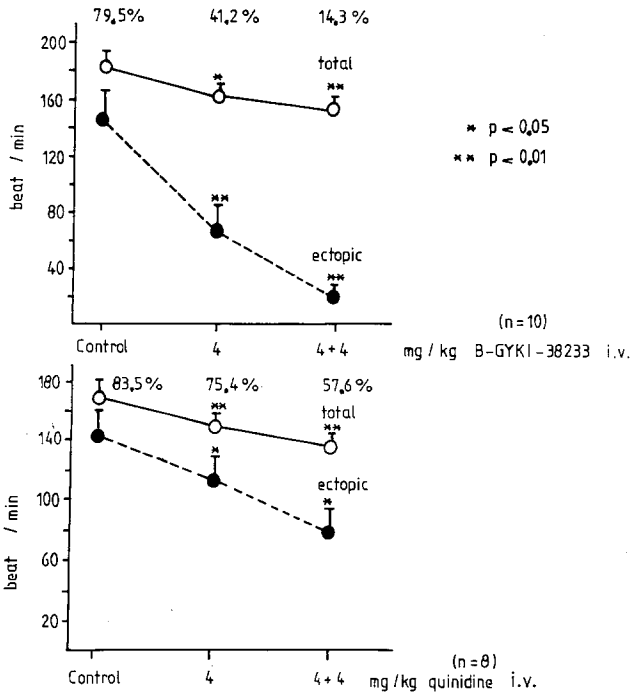


Fig 2. Effect of compound **13** and quinidine on the total and ectopic beat numbers in Harris dogs. On the ordinate, the heart rate expressed as beats/minute and, on the abscissa, the dose of the compounds tested are shown. ○: total heart rate (normal and ectopic); ● : ectopic beats. The percentage values above each curve indicate the ectopic: normal beat ratios. Asterisks denote significant differences from control bars representing ± standard errors.

Experimental protocols

Chemistry

Melting points were determined on a Boetius device and are uncorrected. All new compounds were analyzed for C, H and N, and, when desired, for Cl, *I* and/or S. The values found were within ± 0.4% of the theoretical values. The structures were proven by IR, ^{1}H-NMR and mass spectra. IR spectra were recorded on a Bruker Model JFS 85 spectrometer. ^{1}H-NMR spectra were run on a Varian Model EM 380 instrument with tetramethylsilane as the internal standard, and mass spectra were taken on a Varian MAT Model SM-1 spectrometer.

*Aminoguanidines **3a** (R^6 = H): Method **A***

Step a

*N, N-Disubstituted cyanamides **5**.* Compounds **5** shown in table IV were prepared by reacting a secondary amine with cyanogen bromide as described for dimethylcyanamide **18**.

Step b

*Aminoguanidines **3a** (R^6 = H).* A mixture containing the phenylhydrazine **4** hydrochloride (R^7 = H, alkyl or alkenyl) (0.01 mol), *N,N*-disubstituted cyanamide **5** (0.0125 mol) and 3 ml of anhydrous *n*-propanol was heated at 110°C for 4 h with stirring under nitrogen.

Then the solution (or suspension) was cooled to 20°C and 10 ml of hexane or ether were added. The crystalline precipitate was filtered, washed with a 4:1 mixture of ethanol/hexane or ethanol/ether and dried. The crude product was suspended in 10 ml of anhydrous acetone, stirred at room temperature for 30 min, filtered, washed with acetone and dried. (This operation was omitted for compounds which were recrystallized from acetone.) The product was recrystallized from the solvent mixtures shown in table I to give the compounds **9–60**.

*Aminoguanidines **3b** (R^6 = alkyl): Method B*

Step a

*4-Alkyl-**1**-phenylthiosemicarbazides **7** (R^6 = alkyl).* 1-(2,6-Dimethylphenyl)-4-methyl-thiosemicarbazide **7a**, mp: 199–201°C (from *n*-propanol) was obtained in 66% yield using the method described for preparing 1-(2,4-dimethylphenyl)-4-methyl-thiosemicarbazide [19].

Step b

*3-(S-Methyl)-1-phenyl-4-R^6-isothiosemicarbazides **8** (R^6 = alkyl)* 1-(2,6-Dimethylphenyl)-3-(*S*-methylisothiosemicarbazide hydro-iodide **8a.** A solution containing 2.1 g (0.01 mol) of 1-

Table IV. Substituents, physical data and yields of cyanamides **5**.

Compd	$-N\begin{matrix}R^4\\R^5\end{matrix}$	*bp (°C/mm)*	*Yield (%)*
5a		75–76/0.2	88
5b		93–94/0.4	66
5c		98–101/0.9	80
5d		116–120/0.9[a]	42
5e		168–172/0.3	53
5f		98–101/1.2 100–102/1.5	61

[a] mp: 62–65°C.

(2,6-dimethylphenyl)-4-methylthiosemicarbazide **7a** and 0.62 ml (1.42 g, 0.01 mol) of iodomethane in 20 ml of anhydrous methanol was kept at room temperature overnight, then evaporated. The residue was triturated with a mixture of 15 ml of anhydrous acetone and 45 ml of anhydrous ether, the crystals were collected, washed with a 3:1 mixture of ether/acetone and dried to give 2.58 g of **8a** (74% yield), mp: 181°C (from acetone/ether).

Step c

Aminoguanidines ***3b*** *(R^6 = alkyl).* **8a** (R^6 = Me) (0.01 mol) was stirred with the amine HNR^4R^5 (0.05 mol) in 20 ml ethanol at room temperature for 48 h. Evolution of methyl mercaptan was observed. The excess amine and solvent were evaporated under reduced pressure, the oily residue was dissolved in 50 ml of anhydrous acetone and anhydrous ether was added portionwise. The crystalline precipitate was filtered, washed with a mixture of acetone and ether and dried to give the compounds **61** and **62**.

Pharmacology

Aconitine-induced ventricular arrhythmias in mice

Ventricular arrhythmias were provoked by continuous intravenous *(iv)* infusion of aconitine (5 μg/ml) at an infusion rate of 0.2 ml/min into anesthetized male mice (20–25 g).

The time of the onset of severe ventricular arrhythmias was measured [28]. The drug-induced delay in the appearance of the arrhythmias was determined and compared to the control group of mice pretreated with physiological saline solution only [29].

Drugs were administered either intraperitoneally (ip) or orally *po* 15 or 60 min, respectively, before starting the infusion. For the estimation of the anti-arrhythmic ED_{50} value of a given drug, we determined the dose that could delay the onset of arrhythmias to the same degree as the 2-fold diluted aconitine infusion (2.5 μg/ml) in separate control experiments (118.6 ± 5.9 s; $n = 20$).

The ED_{50} values were obtained from the dose–response curve. The LD_{50} values were determined by using the method of Litchfield and Wilcoxon [30] in mice. (The results are summarized in table II.)

Measurement of the ventricular fibrillation threshold in cats

The method was essentially identical to that of Szekeres and Papp [31]. Cats of both sexes were anesthetized with a mixture of chloralose and urethane (1:5). After starting artificial ventilation, a thoracotomy was performed. The pericardial sac was opened and a bipolar platinum electrode was sutured to the epicardial surface of the right ventricle. The right femoral vessels (artery and vein) were exteriorized, and a polyethylene cannula was inserted in each of them.

Systemic arterial blood pressure was measured through an arterial cannula with the aid of a Statham (P 23 Db) transducer. Drugs were administered intraduodenally. During the entire experiment, blood pressure was continuously registered on a Hellige Multiscriptor. The heart was temporarily stimulated by square wave impulses (20 Hz) of 1 ms duration and the intensity of the electric current was gradually increased. The onset of auricular fibrillation was accompanied by the sudden drop in blood pressure. The ventricular fibrillation threshold was measured by the intensity of stimulatory current applied at that time.

The elevation of fibrillation threshold was considered to be an anti-arrhythmic action (table III).

Harris dog

Mongrel dogs of either sex were anesthetized with pentobarbital (30 mg/kg, iv), intubated and ventilated with room air (Harvard Respiratory Model 607) at a rate of 10 cycles/min and stroke volume of 30 cm^3/kg and 5 cm H_2O positive end-expiratory pressure. Sterile surgical techniques were used. A left lateral thoracotomy was performed at the fifth intercostal space and the pericardium was incised to expose the left anterior descending coronary artery which was ligated by using the 2-stage technique of Harris [32].

The chest was closed and, once spontaneous respiration was restored, the dogs were returned to their cages.

Anti-microbial prophylaxis was provided by administration of penicillin (Retardillin, Biogal 200 000 IU im) prior to and again approximately 6 h after surgery.

Approximately 24 h after coronary artery occlusion, dogs were taken to a quiet laboratory and placed in a restraining sling. After stabilization for 1 h, a control lead II ECG was recorded. The heart rate was determined by counting all ventricular electrocardiographic complexes (normal and ectopic) over a 20-min period.

Arrhythmia was quantified by counting all normal or ectopic complexes and expressing the result as percentage of the total heart rate (% sinus beats; % ectopic beats) (fig 2).

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