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Evidence of an effective antiarrhythmic compound by study of *in vivo* metabolisation

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antiarrhythmic compound / metabolites 2 and 3

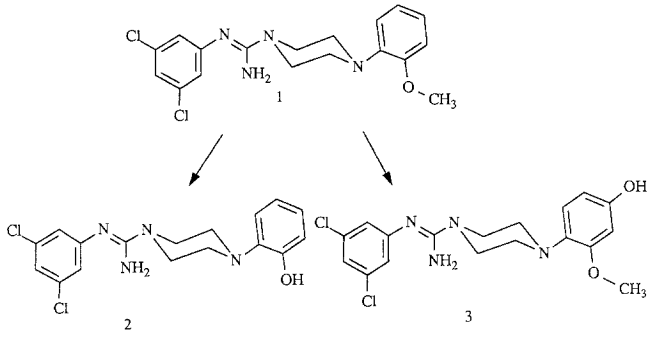
Introduction

In a previous paper, we reported [1] that a substituted piperazine carboximidamide **1** was selected for detailed cardiovascular evaluation and metabolism study. Its efficacy against ventricular arrhythmia induced by left coronary ligation in the dog was demonstrated after oral administration. Nevertheless, this lead compound which shows excellent oral activity, displayed poor acute activity by intravenous bolus, but a moderate effect by intravenous slow infusion in the same animal model [3].

Its lack of activity intravenously and its long-lasting effect orally suggested that the antiarrhythmic potential of **1** was due to an active metabolite. It was therefore essential to determine the chemical structures and the related pharmacological effects of the metabolites of **1**.

Results and Discussion

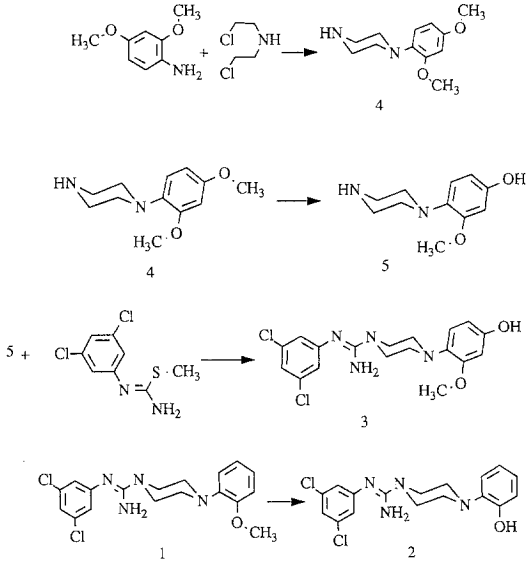
The two metabolites (**2, 3**) found in dog urine had the same chemical structures as those synthesized (scheme 1). Their ^{1}H NMR and mass spectra together with their HPLC retention time were identical. The minor metabolite **2** is likely to be formed by a demethylation process. The major metabolite **3**, was detected in dog plasma and urine after oral administration. It is pro-duced by hydroxylation in position 4 of the parent compound **1**.



Scheme 1.

Synthesis

Condensation of 2,4-dimethoxy-aniline with bis(2-chloro-ethyl) amine in *n*-butoxy ethanol gave the 1-(2,4-di-methoxyphenyl) piperazine which is demethylated by heating in aqueous HBr to afford the corresponding 4-hydroxy derivative. Reaction of this with methyl *N*-(3,5-dichloro-phenyl) carbamamido-thioate [1] in boiling propanol-2 led to compound **3**. Metabolite **2** was prepared by demethylation of compound **1** in aqueous HBr (scheme 2).



Scheme 2.

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Pharmacology

The pharmacological results are summarized in figure 1 and table I. The compounds were evaluated both by intravenous and oral routes and the results are expressed by the inhibition of arrhythmia incidence before the drug administration. In coronary artery ligation (fig 1) **1** markedly protected against the spontaneous arrhythmia after oral administration (30 mg/kg), but was not significantly effective by intravenous route (10 mg/kg). **2** was poorly active both by oral and intravenous route (30 and 10 mg/kg, respectively). Demethylated derivative **2** did not improve significantly intravenous activity of **1** but was devoid of oral activity. The major metabolite **3** exhibited a protective effect both by oral route (30 and 15 mg/kg) and intravenous route (10 and 3 mg/kg) with a long duration (more than 6 h). Quinidine was less potent than **3** by oral (60 mg/kg) and intravenous route (10 mg/kg). Flecainide was equipotent (15 and 3 mg/kg) and disopyramide showed the same potency (30 and 10 mg/kg).

In ouabain-induced ventricular ectopia (table I) compound **3** was effective by intravenous route (1 and 3 mg/kg) and by oral route (30 and 15 mg/kg). Flecainide and disopyramide were as potent as **3** at the same dose. Quinidine showed a similar activity to **3** only by intravenous administration.

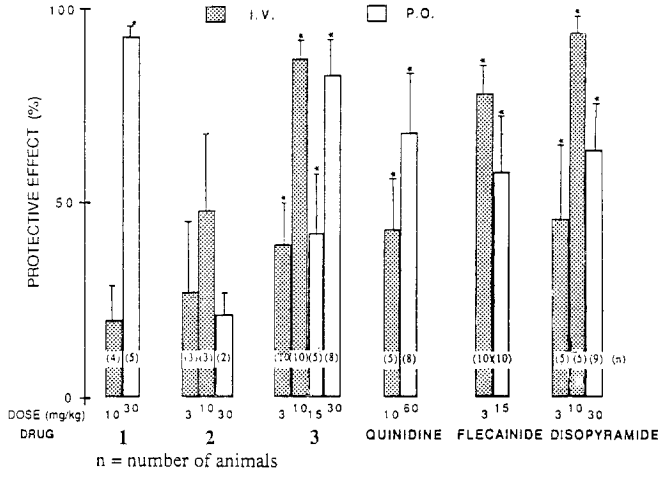

Fig 1. Anti-arrythmic activity compared by oral and venous route in Harris ligated conscious dog.

Table I. Inhibition of ventricular ectopia induced by ouabain perfusion in anesthetized ventilated dog.

Compound	route	dose mg/kg	n	% inhibition[a]
3	intravenous	1	5	46 ± 22
		3	5	100 ± 00*
	oral	15	6	67 ± 18*
		30	6	87 ± 10*
Quinidine	intravenous	3	5	42 ± 23
	oral	55	5	98 ± 15*
Flecainide	intravenous	3	5	100 ± 00*
	oral	30	5	67 ± 21*
Disopyramide	intravenous	3	3	88 ± 11*
	oral	30	5	84 ± 16*

[a]Mean % ± SEM at maximal effect. n=number of animals. *Indicates difference from initial control at $P < 0.05$ using the Student's *t*-test.

Conclusions

Compound **3** is a chemically novel antiarrhythmic agent. This work has demonstrated the metabolisation of **1** into a major active compound **3** after oral administration. In contrast to **1**, compound **3** does not generate any major metabolite. This strongly suggests that **3** is the active metabolite. Compound **3** was, as for the parent compound **1**, orally active but also consistently effective intravenously. The antiarrhythmic activity of **3** was shown in two *in vivo* models both by oral and intravenous administrations. Further toxicology and pharmacology investigations are being pursued with compound **3** selected *via* the *in vivo* metabolic pathways of **1**.

Experimental protocols

Pharmacology

Coronary artery ligation-induced ventricular arrhythmia in conscious dog [3]

This method has been described in a previous paper [1].

Ouabain perfusion-induced ventricular ectopia in the anesthetized dog

We used the classical method described by Alkondon *et al* [4] on the pentobarbitone anesthetized ventilated dog. Ouabain was intravenously injected at 40 μg/kg and the ECG was analysed over 30 min. Administrations of ouabain were repeated by steps of 10 μg/kg every 10 min until ventricular tachycardia was triggered. Arrhythmia was stable at least for 3 h. The compounds were administered by venous route or intragastric route 1 h after triggering ventricular tachycardia. The percentage inhibition was evaluated as the ratio of the number of ectopic complexes after and before treatment, over 3-min periods for 2 h. The data was expressed as the mean ± SEM and analysed using the paired Student's *t*-test in comparison with the control values.

Isolation and identification of metabolites 2 and 3

Following oral administration of compound **1** (60 mg/kg) to dogs, a major metabolite **3** was observed in plasma. After enzymatic hydrolysis of plasma sample by β-glucuronidase another metabolite **2** appeared. Both metabolites were present in dog urine. Urine samples were alcalinized to pH 10 with 1M Na_2CO_3 then extracted with ethyl ether and dichloromethane. The organic extracts were purified by chromatography on silica gel using ethyl acetate/heptane 30/70 as eluting solvent. The metabolite **3** recovered from the richest column fraction was found to be 98% chemically pure by HPLC area normalization. HPLC conditions are: precolumn (CN Guard Pak), column (Radial Pak CN 10 microns, 1000 x 8 mm id), mobile phase (1 M dibutylamine phosphate buffer pH = 2.5/acetonitrile-water 1/35/65), detection wavelength 254 nm; flow rate 3 ml/mn. Its structure assigned by ^{1}H NMR and mass spectra. The structure of metabolite compound **2** was identified by comparison with synthesized material.

Chemistry

Melting points were determined on a Köfler bank and are uncorrected. The ^{1}H NMR spectra were measured in CD_3OD with tetra-methylsilane (TMS) as the internal standard; the instrument used was a Brücker 300 MHz spectrometer. Elemental analyses (C, H, N) were within ± 0.4% of the theoretical values.

1-(2,4-Dimethoxy-phenyl)-piperazine hydrochloride ***4***
A solution of 148 g (0.97 mol) of 2,4-dimethoxy-aniline, 189.6 g (1.06 mol) of bis-2-chloro-ethylamine hydrochloride and 200 g (1.45 mol) of K_2CO_3 in 1.31 of *n*-butoxy-ethanol were refluxed for 20 h then cooled to 60°C. 1.31 of methanol was added then the mixture was filtered on Celite. The solvent was evaporated off and the residue triturated with 700 ml of acetone. The resulting powder was filtered and dried to give 147 g, yield 56.8%, mp: 224°C. ^{1}H NMR (DMSO–d_6) δ (ppm): 3.35 (s, 6H); 4.05 (m, 4H); 6.78 (d, 2H); 7.10 (s, 1H); 9.75 (m, 2H).

1-(2-Methoxy-4-hydroxy-phenyl)-piperazine dihydrobromine ***5***
The compound **4** 147 g (0.568 mol) was added to 600 ml of HBr solution (37%). The mixture was heated at 100°C overnight. After cooling, crystals were separated and filtered to give 155 g, yield 78%.

The free base was obtained in 95% yield by adding NaOH 10 N to the hydrobromic salt in aqueous solution, mp: 217°C. ^{1}H NMR (DMSO–d_6) δ (ppm): 3.70 (m, 11H); 6.45 (m, 2H); 7.35 (d, 1H); 9.37 (m, 2H); 10.30 (m, 1H).

N-*(3,5-Dichloro-phenyl)-4-(2-methoxy-4-hydroxy-phenyl)-1-piperazine carboximidamide dihydrochloride* ***3***
A mixture of 7.05 g (0.03 mol) of methyl *N*-(3,5-dichloro-phenyl) carbamimidothioate [1], 6.5 g (0.03 mol) of **5** and 0.4 ml of triethylamine in 200 ml of propanol-2 was refluxed for 20 h. After cooling to room temperature, crystals were separated, then recrystallized in a mixture of acetone/water to give 7.6 g, yield 70%, mp: 138°C. Its dihydrochloride salt melted at 230°C. ^{1}H NMR (CD_3OD) δ (ppm): 3.88 (m, 4H); 4 (s, 3H); 4.11 (m, 4H); 6.55 (d, 1H); 6.68 (d, 1H); 7.39 (m, 3H); 7.55 (d, 1H). MS: 394–396 (M^+), 230–232, 216–218, 178–179.

N-*(3,5-Dichloro-phenyl)-4-(2-hydroxy-phenyl)-1-piperazine carboximidamide dihydrochloride* ***2***
N-(3,5-Dichloro-phenyl)-4-(2-methoxy-phenyl)-1-piperazine carboximidamide dihydrochloride 8 g (0.0017 mol) was dissolved in 100 ml of aqueous hydrobromic acid (37%) and heated for 3 h at 80°C. After cooling to room temperature, the solvent was evaporated off. The residue was poured into water, alcalinized with NaOH and extracted with dichloro-methane. After evaporation of the solvent, the residue was triturated in a mixture of ethanol/-hydrochloric acid to produce 6.5 g, yield 84%, mp: 210°C. ^{1}H NMR (CD_3OD) δ (ppm): 4 (m, 4H); 4.2 (m, 4H); 7 (m, 1H); 7.08 (d, 1H); 7.28 (t, 1H); 7.32 (d, 2H); 7.36 (m, 1H); 7.79 (d, 1H). MS: 364–366 (M^+), 216–218, 204–206, 187–189, 161.

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