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STRUCTURALLY NOVEL ANTIARRHYTHMIC / ANTIOXIDANT QUINAZOLINES

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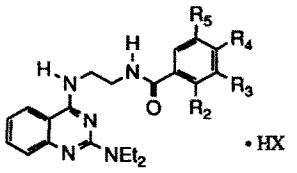
Abstract: We report a structurally novel series of quinazolines with *in vivo* antiarrhythmic and/or *in vitro* iron-dependent lipid peroxidation inhibitory activity. Two analogues, **7** and **12**, were evaluated in a canine model of regional myocardial ischemia and reperfusion.

Through general screening we found that N-[2-[[2-(diethylamino)-4-quinazolinyl]amino]ethyl]-3,4,5-trimethoxybenzamide (compound **1**) exhibited antiarrhythmic activity in mice subjected to either chloroform anesthesia or aconitine infusion. Since this compound appears to be structurally novel and unrelated to known antiarrhythmic agents, we prepared a series of congeners (Table 1) and examined their electrophysiological effects on isolated canine Purkinje fibers. As summarized in Table 2, compound **1** (10 μM) reduced the action potential amplitude (APA), the maximum rate of rise (V_{max}) and shortened the action potential duration (APD). These effects are consistent with potential class I antiarrhythmic activity (Vaughan Williams classification). Structurally related analogues **2** - **6** exhibited similar *in vitro* properties, although only compound **1** had a statistically significant effect on APD_{95}.

Table 1: Compound Numbering and Physical Data



no.	R_2	R_3	R_4	R_5	HX	mp (°C)	formula	analysis
1	H	OMe	OMe	OMe	---	163-164	$C_{24}H_{31}N_5O_4$	C,H,N
2	H	H	OMe	H	oxalate	185-186	$C_{22}H_{27}N_5O_2 \cdot 0.75\ C_2H_2O_4$	C,H,N
3	OMe	H	H	H	---	149-150	$C_{22}H_{27}N_5O_2$	C,H,N
4	H	H	CF_3	H	oxalate	178-179 (dec)	$C_{24}H_{26}N_5O_5F_3$	C,H,N
5	H	H	F	H	oxalate	209-210 (dec)	$C_{23}H_{26}N_5O_5F$	C,H,N
6	H	H	H	H	oxalate	202-204 (dec)	$C_{22}H_{26}N_5O_3 \cdot 0.5\ H_2O$	C,H,N
7	H	OMe	OMe	OMe	MsOH	200-201	$C_{25}H_{35}N_5O_7S$	C,H,N
8	H	OH	OMe	OMe	MsOH	222-224	$C_{24}H_{33}N_5O_7S$	C,H,N
9	H	OH	OMe	OH	MsOH	224-230	$C_{23}H_{31}N_5O_7S$	C,H,N
10	H	OMe	OH	OMe	MsOH	182-185	$C_{24}H_{33}N_5O_7S$	C,H,N
11	H	OH	OH	OMe	MsOH	198-200	$C_{23}H_{31}N_5O_7S$	C,H,N
12	H	OH	OH	OH	MsOH	212-214	$C_{22}H_{29}N_5O_7S$	C,H,N

In the Harris canine arrhythmia model,[1] compound **1** (3 mg/kg, bolus i.v. injection) increased normal sinus rhythm from 17% to >99%, an effect presumably due to a reduction in automaticity and not overdrive suppression since the ventricular vagal escape rate was decreased (Figure 1).

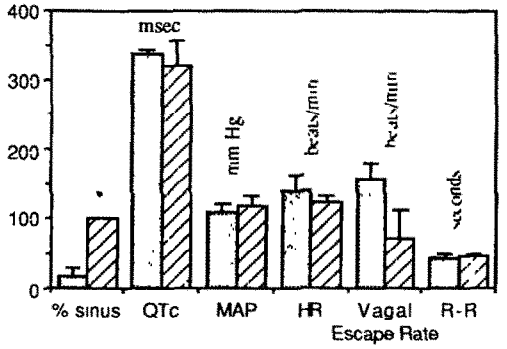


Figure 1. Effect of compound **1** on canine arrhythmias 24 h after occlusion and reperfusion of the left anterior descending artery (Harris model). Each bar represents the mean ± SE (n = 4).

*$p < 0.05$

Class I antiarrhythmic agents can interact with phospholipid membranes[2] and representatives from this class reportedly exhibit cardioprotective effects in canine hearts following coronary artery ligation.[3] Certain class I and class III agents also inhibit nonenzymic lipid peroxidation *in vitro*.[4] Lidocaine, a class Ib antiarrhythmic, decreased infarct size in a canine model of regional myocardial ischemia and reperfusion, a salutary effect that was associated with reduced coronary sinus levels of conjugated dienes (a potential marker of lipid peroxidation).[5] Although controversial, toxic oxygen metabolites and lipid peroxidation may play a role in the pathophysiology of postischemic myocardial tissue injury.[6] Propyl gallate (**13**), an antioxidant and weak lipoxygenase inhibitor,[7] blocked iron-dependent lipid peroxidation *in vitro* and reduced ischemic damage in cat hearts following coronary artery occlusion as indexed by creatine kinase accumulation.[8]

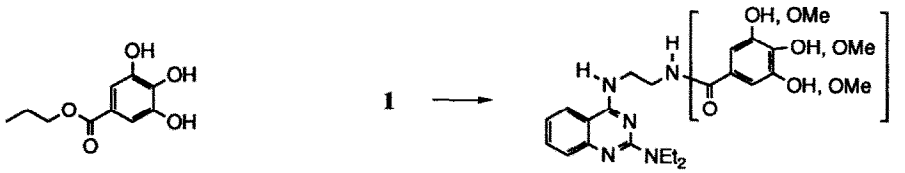
Table 2: Electrophysiological Effects of Compounds **1** - **6**, **12** (10 μM) on Isolated Canine Purkinje Fibers[a]

no.	APA[b]	APD_{50}[c]	APD_{95}	RP[d]	V_{max}[e]	CT[f]
1	-14.4 ± 4†	-46 ± 4**	-24 ± 2**	-4 ± 1†	-40 ± 9†	123 ± 55†
2	-11 ± 3†	-35 ± 9†	-5 ± 11	-6 ± 2†	-30 ± 6†	17 ± 7
3[g]	-31 ± 12†	-24 ± 18*	15 ± 7	-15 ± 12	-71 ± 9†	71 ± 40*
4	-23 ± 7†	-24 ± 12	2 ± 8	-7 ± 4	-62 ± 14†	79 ± 14†
5	-15 ± 13*	-48 ± 7*	1 ± 10	-3 ± 2	-54 ± 10†	72 ± 13†
6	-15 ± 3*	-34 ± 11†	-2 ± 11	-3 ± 2	-43 ± 9†	119 ± 23†
12[h]	0 ± 2	-1 ± 2	1 ± 1	0 ± 0	-2 ± 8	7 ± 6
12[h,i]	-9 ± 4	-13 ± 10	11 ± 6	-4 ± 2	-32 ± 11	11 ± 8

[a]Maximum percent change from base-line values, mean ± SE (n = 5). [b]Action potential amplitude (mV). [c]Action potential duration at 50% of repolarization (msec). [d]Resting potential (mV). [e]Maximal rate of depolarization (V/sec). [f]Conduction time from stimulating to intracellular recording electrode (msec). [g]n = 4. [h]n = 3. [i]Tested at 100 μM. †$p < 0.05$; *$p < 0.01$; **$p < 0.001$.

Since compound **1** contains a substituted benzoyl moiety resembling that of propyl gallate, we targeted a series of antioxidant derivatives within this structural class as potential antiischemic/antiarrhythmic agents. All possible combinations of hydroxyl and methoxyl groups at the C3 - C5 positions of the benzoyl ring were

synthesized to provide hybrid analogues **7** - **12**, which were isolated and characterized as their mesylate salts (Table 1).



13 **7 - 12**

Oxidation potentials for compounds **7** - **12** were measured using cyclic voltammetry with a carbon paste disc electrode[9] against a Ag/AgCl reference electrode (pH 7.4 phosphate buffered saline, 37°C) as previously described (Table 3).[10] The half-wave potential (defined as the potential required for half-maximal oxidation under the experimental conditions) for these compounds are summarized in Table 3. Compound **7** did not oxidize below 600 mV relative to the Ag/AgCl reference electrode indicating that antioxidant activity for congeners **8** - **12** may be attributed to the phenolic hydroxyl group(s) rather than the quinazoline heterocycle. Within this subset of compounds, only **11** exhibited reversible electrochemical behavior under these conditions. Representative voltammograms appear in Figure 2.

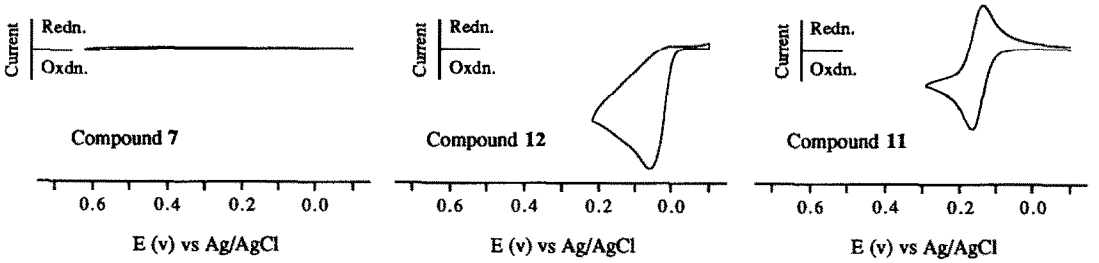


Figure 2. Representative cyclic voltammograms. Measurements were conducted using a carbon paste disc electrode against a Ag/AgCl reference electrode in pH 7.4 phosphate buffered saline at 37°C. The initial potential was -0.1 volts with a scan rate of 10 mV/second in a positive direction. A new electrode surface was used for each compound.

Table 3: Activity of Quinazolines **7** - **12**

no.	clogP[a]	Cyclic voltammetry[b] $E_{1/2}$ (mV)	Lipid Perox.[c] MIC (μM)	Neutrophil[d] EC_{50} (μM)
7	4.88	>600	>100	Not active[e]
8	4.15	440	>100	----[f]
9	3.29	320	>100	9.5 ± 3.3
10	3.87	340	>100	3.3 ± 1.0
11	3.57	130 (reversible)	10	0.9 ± 0.2
12	3.14	15	3	0.3 ± 0.02

[a]Calculated for corresponding free-base. [b]Carbon paste disc electrode against a Ag/AgCl reference electrode. [c]Inhibition of iron-dependent peroxidation of rabbit brain vesicular membrane lipids. [d]Inhibition of C5a-induced luminol dependent chemiluminescence of human neutrophils. [e]No effect at 10 μM. [f]Not sufficiently soluble in saline for testing.

Inhibition of iron-dependent lipid peroxidation was determined using rabbit brain vesicular membrane lipids as described previously (Figure 3).[11] Under these conditions, only compounds **11** and **12** were very effective with MIC[12] values of 10 and 3 μM, respectively (Table 3). These two derivatives also exhibited the lowest oxidation potential of the series. Thus, for this series oxidation potential rather than lipophilicity may represent the primary determinant of activity in this system since clogP[13] and efficacy are inversely related.

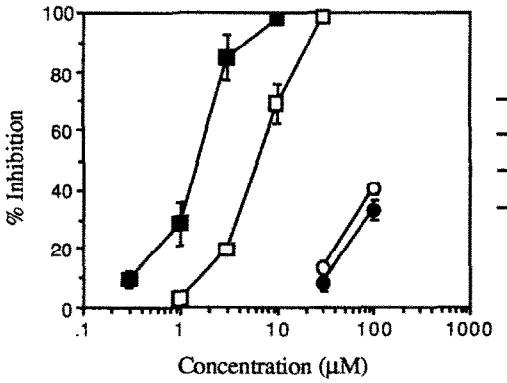


Figure 3. Effect of quinazolines on iron-dependent peroxidation or rabbit brain vesicular membrane lipids in vitro. Formation of lipid peroxide decomposition products was measured by the TBAR method. Each point represents the mean ± SEM (n = 3).

Peroxyl radical scavenging activity in homogeneous aqueous solution was evaluated using an assay that measures 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) induced decay of R-phycoerythrin (RPE) fluorescence emission under conditions where peroxyl radical formation is rate limiting.[14] In contrast to the lipid peroxidation results, compounds **8** - **12** (1 μM) were all active and approximately equipotent to one another (Figure 4). Compound **7**, on the other hand, was ineffective and further underscores the importance of a free phenolic hydroxyl group for antioxidant activity.

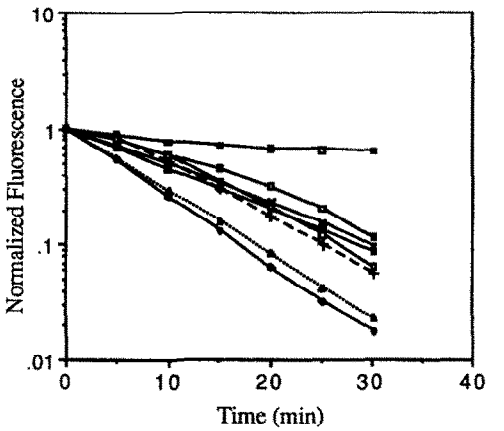


Figure 4. Effect of compounds 7 - 12 (1 μM) on 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) induced decay of R-phycoerythrin (R-PE) fluorescence emission in phosphate buffer (pH 7.0) at 37°C. The control line represents the fluorescence of R-PE in the absence of drug and AAPH. The AAPH line represents R-PE fluorescence in the presence of AAPH without drug. Each point represents the mean of 4 determinations.

Since oxidants produced by activated neutrophils in response to complement activation may extend irreversible tissue injury in the postischemic myocardium,[15] the ability of these compounds to block luminol dependent chemiluminescence of human neutrophils induced by C5a was determined *in vitro* (Figure 5).[16]

Consistent with an antioxidant mechanism of inhibition, compound **12** under these conditions exhibited the greatest efficacy (EC_{50} = 0.3 μM), whereas compound **7** was totally ineffective (EC_{50} > 10 μM).

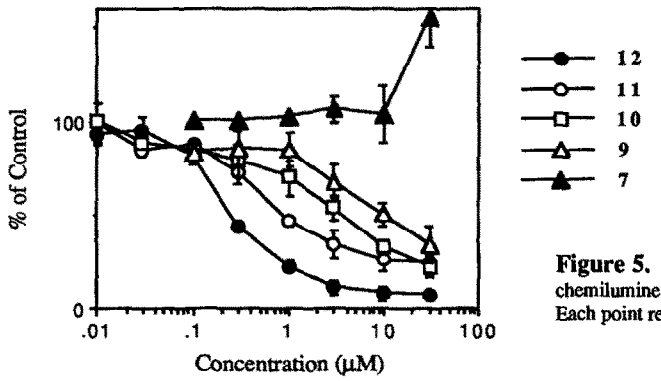


Figure 5. Effect of quinazolines on luminol dependent chemiluminescence of human neutrophils induced by C5a. Each point represents the mean ± SEM (n = 3 - 5).

Thus, based upon a variety of *in vitro* measurements compound **12** was identified as the most potent antioxidant analogue within this series of quinazolines. However, in contrast to its progenitor, compound **1**, derivative **12** at concentrations as high as 100 μM did not significantly alter measured electrophysiological parameters using isolated canine Purkinje fibers (Table 2). Consequently, potential antiarrhythmic activity appears to be highly structure dependent for this series of quinazolines and may be related to molecular lipophilicity.

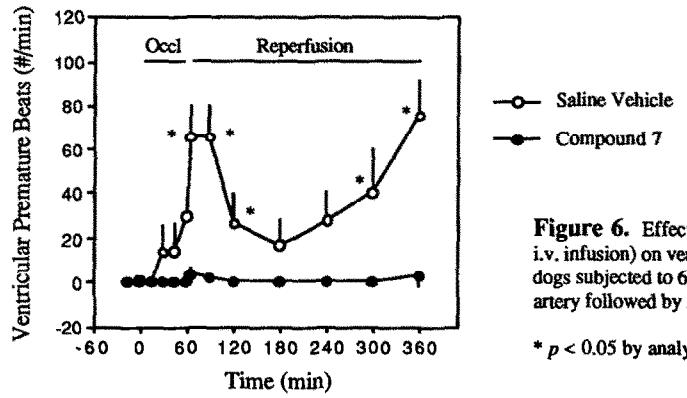


Figure 6. Effect of compound **7** (3 mg/kg/h continuous i.v. infusion) on ventricular premature beats in anesthetized dogs subjected to 60 minutes occlusion of the left circumflex artery followed by 5 hours of reperfusion.

* $p < 0.05$ by analysis of variance.

To establish if compounds **7** (antiarrhythmic) and **12** (antioxidant) might exhibit cardioprotective effects, we evaluated both derivatives in a canine model of regional myocardial ischemia and reperfusion (60 minute occlusion of the left circumflex artery followed by 5 hours of reperfusion).[17] Compound **7**, when administered according to a pretreatment protocol at an infusion rate of 3 mg/kg/h for the duration of the infarction experiment, reduced the number of ventricular premature beats (Figure 6). However, at this infusion rate there was also attendant hypotension during the latter period of reperfusion and a lack of containment of infarction. Compound **12**, when administered by continuous infusion during the reperfusion period (3 mg/kg/h), did not lower mean arterial pressure or influence the magnitude of myocardial ischemia as judged by electrogram deviation recorded

from ischemic tissue (Figure 7).[18] At this dose it also did not alter the extent of myocardial infarction. Levels of parent compound in plasma samples taken from dogs treated with **12** were monitored by liquid chromatography and found to be in excess of that required for *in vitro* efficacy (see Figure 5) when measured 1 and 5 hours following initiation of reperfusion (240 ± 190 ng/mL at 30 min, 1700 ± 680 ng/mL at 60 min and 1300 ± 620 ng/mL at 300 min, mean ± SE, n = 7). Thus, although these structurally novel quinazolines exhibit *in vivo* antiarrhythmic and/or *in vitro* antioxidant activity, they do not appear to be efficacious as cardioprotective agents in a canine model of regional myocardial ischemia.

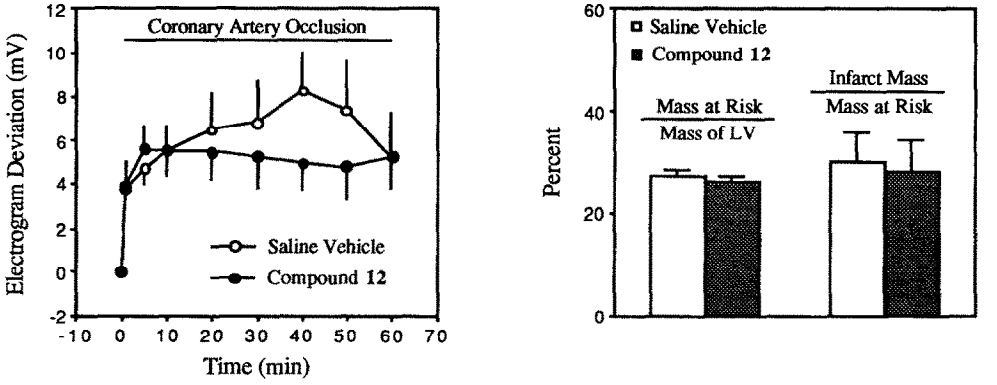


Figure 7. Electrogram deviation recorded from ischemic myocardium in anesthetized dogs subjected to 60 minutes occlusion of the left circumflex artery (left panel). Group mean infarct size after 5 hours of reperfusion (right panel). Compound **12** was administered by continuous i.v. infusion (3 mg/kg/h).

References and Notes

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12. Minimum tested concentration of inhibitor that gave ≥ 50% inhibition.
13. Octanol/water partition coefficients were calculated using the CLOGP program (Pomona College, Medchem Software, v. 3, 1984).
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16. Human neutrophils ($1x10^7$/mL) were stimulated with recombinant C5a (10 nM, pH 7.4) in the presence of luminol (0.3 - 3.0 μM), cytochalasin B (2 μg/mL), Ca^{2+} (1 mM), Mg^{2+} (0.5 mM) and test agent. Peak responses were measured with a Chrono-log lumi-aggregometer and compared with the amount of luminescence produced in the absence of test agent.
17. For a representative experimental protocol, see: Hahn, R. A.; MacDonald, B. R.; Simpson, P. J.; Wang, L.; Towner, R. D.; Ho, P. P. K.; Goodwin, R. M.; Breau, A. P.; Suarez, T.; Mihelich, E. D. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 94.
18. Higher infusion rates reduced mean arterial pressure.