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THE DISCOVERY OF A NOVEL CALCIUM CHANNEL BLOCKER RELATED TO THE STRUCTURE OF POTASSIUM CHANNEL OPENER CROMAKALIM

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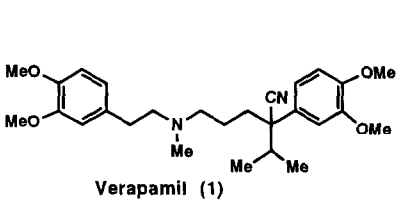
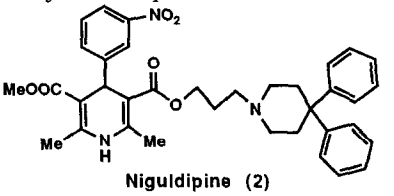
Abstract: During our studies aimed at the identification of novel analogs of the potassium channel opener cromakalim (**3**), we serendipitously observed pyranoquinoline **4** to possess pure calcium channel blocking activity. The results of the studies conducted to confirm the calcium channel blocking mechanism of **4** are reported in this paper.

Ion channels play a major role in cell excitability which regulates various physiological functions in many tissue types. Consequently, modulation of these channels has formed the basis of therapy for a variety of illnesses of smooth muscle, cardiac tissue and the central nervous system. Recent studies using techniques of molecular biology and patch clamping indicate a high degree of structural homology between various cation channels.[1] Thus, it is not surprising for certain cation channel modulators to interact with more than one channel or channel types. For example, the phenylalkyl amine type calcium channel blocker verapamil (**1**) can also block sodium channels[2] and the dihydropyridine calcium channel blocker niguldipine (**2**) has the ability to open large conductance calcium-activated potassium channels.[3] Similarly, the peptidic toxin charybdotoxin, a blocker of the calcium-activated potassium channels, can also block voltage-gated potassium channels.[4]

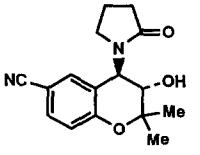
While cross activity among various ion channels or channel types is widely known for ion channel modulators, the abilities of two structurally related molecules to distinguish cleanly between two different cation channels is unprecedented, as far as we know. During our studies aimed at the discovery of novel potassium channel openers related to cromakalim (**3**)[5], we made the serendipitous observation that pyranoquinoline **4** may be acting as a vasodilator *via* a calcium channel blocking mechanism. Therefore, studies were carried out to confirm the calcium channel blocking mechanism of **4** and to probe its pharmacological utility. The results of those studies and the synthesis of pyranoquinoline **4** are the subject of this publication.



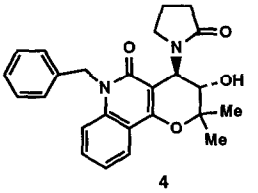
Verapamil (1)



Niguldipine (2)



Cromakalim (3)



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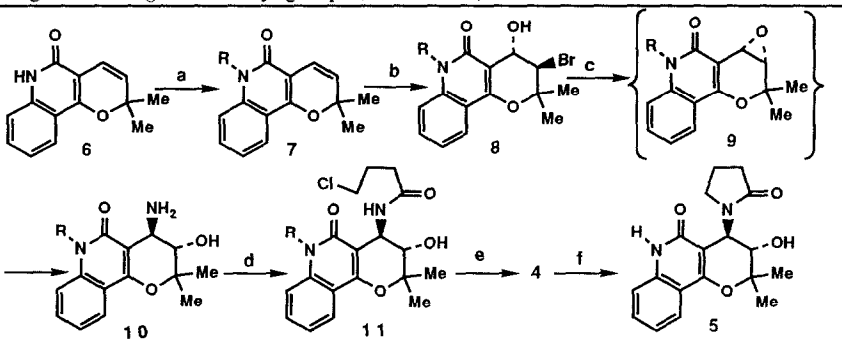
The synthesis of pyranoquinoline **4** and its protio analog **5** from the known olefin **6**[6] is summarized below. The *trans* stereochemistry of bromohydrin **8** is based on a large vicinal coupling (J = 8.3 Hz) observed between the two *trans*-diaxial protons. The formation of the exclusive *trans*-product **10** from **8** can be rationalized by the intermediacy of epoxide **9**, which we were unable to isolate due to its instability under the reaction conditions.

The ^1H NMR spectrum of **4** showed broadening of most proton signals at room temperature due to the presence of two rotamers around the C4-nitrogen bond. Therefore, to confirm regio- and stereochemistry, ^1H NMR spectrum of **4** was recorded at 120°C in dimethylsulfoxide. The coupling constant of 8.0 Hz between C3 (δ 4.64) and C4 (δ 3.96) protons is consistent with *trans* diequatorial relationship of pyrrolidone ring and the hydroxyl group. This stereochemical relationship could be further confirmed by lack of NOE between C3 and C4 *trans* diaxial protons. The regiochemistry was apparent from the lack of NOE between pyrrolidone protons (δ 3.5, 3.2) adjacent to nitrogen and the *gem*-dimethyl groups (δ 1.56, 1.31).



(a) benzyl bromide, K_2CO_3, DMF, 50%; (b) aq. N-bromosuccinimide, rt, 77%; (c) NH_4OH, THF, ethanol; (d) aq. Na_2CO_3, 4-chlorobutyryl chloride, THF, pH 8.5, 31% from **8**; (e) sodium hydride, THF, 53%; (f) $HCOONH_4$, 10% Pd-C, heat, 45%.

For a comparison of the vasorelaxant potencies of potassium channel openers, we routinely employ methoxamine contracted rat aorta.[7] The relaxation caused by potassium channel openers in this test is reversible by increasing concentration of external potassium.[8] The pyranoquinoline analog **4** of cromakalim (**3**) relaxed the methoxamine contracted rat aorta with an IC_{50} value of 0.44 μM (Table). However, the relaxation induced by **4** was not reversible by increasing external potassium, pharmacological behavior that is typical of calcium channel blockers (diltiazem, IC_{50} = 2.38 μM; nifedipine, IC_{50} = 0.004 μM) in this test.[7] In contrast to **4**, the vasorelaxation caused by the potassium channel opener cromakalim (IC_{50} = 0.055 μM) was almost completely reversed by high external potassium (60 mM). While the ATP-sensitive potassium channel blocker glyburide caused a parallel shift in the dose-response to cromakalim (K_b = 0.039 μM), it had very little effect on the vasorelaxation due to pyranoquinoline **4**. These results indicated that the pyranoquinoline analog **4** of cromakalim (**3**) may be acting *via* a calcium channel blocking mechanism. Interestingly, the protio analog **5** of **4** was devoid of vasorelaxant activity. These preliminary results led us to perform additional experiments to confirm the calcium channel blocking mechanism of **4** and investigate its site of action and pharmacological utility.

Table: Vasorelaxant and antihypertensive potencies of 4, cromakalim, diltiazem and nifedipine

	Vasorelaxant Potency	Antihypertensive Activity[a]
Compound	IC_{50}, μM (95% C. I.)[b]	% Maximum decrease in blood pressure (0-6 hours) (n = 6)
Pyranoquinoline **4**	0.44 (0.17, 1.11)	-[c]
Cromakalim (**3**)	0.055 (0.041, 0.074)	63±6
Nifedipine	0.004 (0.002, 0.007)	33±5
Diltiazem	2.38 (1.09, 5.19)	-[c]

[a]The effect on blood pressure was measured at equimolar (45 μmol/kg) doses of all compounds given orally. [b]IC_{50} is presented as a mean with 95% confidence interval in parenthesis, n = 4 from different animals. [c]No significant effect on blood pressure.

Electrophysiological experiments were carried out to investigate the effect of pyranoquinoline **4** on the outward potassium currents in isolated guinea pig ventricular myocytes.[9] No increase in outward potassium current was observed up to 100 μM of **4** (Figure 1, data for 10 and 50 μM). The potassium channel opener cromakalim (**3**) induced an increase in the outward potassium current in a concentration dependent manner (Figure 1). These studies clearly indicate that pyranoquinoline **4**, compared to cromakalim (**3**), has minimal effect on outward potassium currents. Further studies were directed towards studying the effects of this compound on inward calcium currents. The effects of 10-100 μM pyranoquinoline **4** on inward calcium currents were studied using a step depolarization to ~0 mV in isolated guinea pig ventricular myocytes.[10] As shown in figure 2, pyranoquinoline **4** inhibited inward calcium currents in a concentration dependent manner. The inhibition (30% at 10 μM) of inward calcium by **4** is comparable to that observed for diltiazem: 33% inhibition at 10 μM (data not shown). Thus the electrophysiological evidence clearly supports a calcium channel blocking mechanism for **4**.

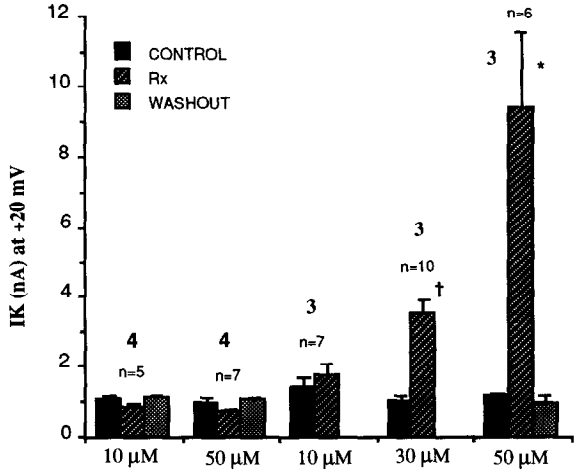


Figure 1. Summary of the effects of 10 and 50 μM pyranoquinoline **4** and 10-50 μM cromakalim (**3**) on outward potassium current (IK) measured at +20 mV in isolated guinea pig ventricular myocytes. Paired student t-test was used for statistical comparison, $^{*}p < 0.05$.

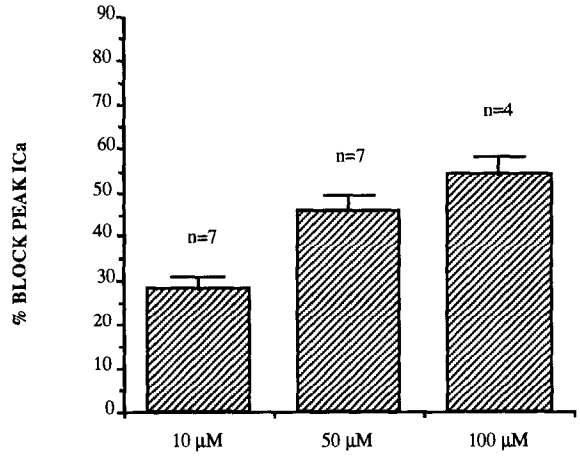


Figure 2. Percent inhibition of inward calcium current (ICa) by 10 - 100 μM of pyranoquinoline **4**. Number of cells in each condition are indicated.

In order to investigate the possible interaction of **4** with dihydropyridine or benzothiazepine receptor site, radioligand binding studies were carried out in skeletal muscle membranes according to the technique described by

Bolger.[11] Pyranoquinoline **4** induced, what appeared to be a concentration-dependent inhibition of [^{3}H]-nitrendipine binding at concentrations higher than 10 μM. However, the maximum inhibition observed at 100 μM was less than 50 percent. Therefore, a true k_d value could not be calculated from the inhibition curve. At the highest concentration tested (100 μM), pyranoquinoline **4** induced only 40% inhibition of [^{3}H]-diltiazem binding. We conclude from these studies that **4** interacts only weakly with dihydropyridine and benzothiazepine receptor sites. The true nature of this interaction could not be assessed due to incomplete inhibition.

To investigate its effect on blood pressure, pyranoquinoline **4** was administered (45 μmol/kg, po) to spontaneously hypertensive rats (SHR). For comparison, potassium channel opener cromakalim and calcium channel blockers diltiazem and nifedipine were also tested at equimolar doses for their effects on blood pressure in the SHR. Whereas cromakalim and nifedipine caused large reductions in blood pressure, both diltiazem and pyranoquinoline **4** were devoid of antihypertensive effects at this dose level (Table). The reasons for the lack of effect of **4** on blood pressure are not clear at the present time but they may be related to its pharmacokinetics or lower potency as a calcium channel blocker.

The above results show that pyranoquinoline **4**, unlike its predecessor cromakalim (**3**), does not open outward potassium currents. We have clearly demonstrated that **4** is an inhibitor of calcium entry through voltage dependent calcium channels, its effects being on the L-type channel.[10] Its potency (IC_{50} = 0.44 μM) as a vasorelaxant agent lies in between those of diltiazem (IC_{50} = 2.38 μM) and nifedipine (IC_{50} = 0.004 μM). The discovery of calcium entry blocking properties of **4** may represent a new chemical class of calcium channel blockers, although identification of its specific site of action must await receptor binding studies using the radiolabelled compound. Considering the lack of effect of cromakalim on the voltage-sensitive calcium channels[12], these results demonstrate that structural modifications of ion channel modulators can have dramatic consequences on the identity of ion channel(s) they interact with. To our knowledge, this is the first calcium channel blocking agent derived from the structure of a potassium channel opener (cromakalim).

Pharmacological Studies: Vasorelaxant potencies were determined by relaxation of the methoxamine contracted rat aorta.[7] For determination of antihypertensive activity, male spontaneously hypertensive rats (SHR) were prepared surgically according to the method of Weeks and Jones.[13] The test compounds were administered as a suspension in agar and blood pressure was recorded using the method described by Laffin et al.[14] Methods for isolation and voltage clamping of guinea pig ventricular myocytes have been described.[9]

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