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ARYL CYANOGUANIDINE POTASSIUM CHANNEL OPENERS

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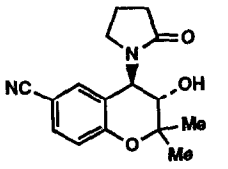
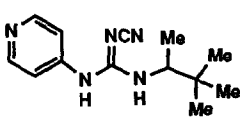
Abstract: To investigate whether potassium channel openers cromakalim (**1**) and pinacidil (**2**) share common pharmacophoric features, the combination compound **6a** was prepared and evaluated for biological activity. The potent vasorelaxant/antihypertensive activity displayed by **6a** and some of its analogs suggest cromakalim (**1**) and pinacidil (**2**) may share common pharmacophoric features.

There is a currently a great deal of interest in modulating the activity of potassium channels in various tissues.[1] The design of novel compounds is hampered by lack of understanding about the three dimensional structure of receptor proteins, which may or may not be part of the potassium channels. Current efforts in medicinal chemistry are focused on the modification of reference agents whose mechanism of action was discovered sometime after their synthesisas potential pharmacological agents.[2] The discovery of potassium channel opening properties of powerful vasodilators such as cromakalim (**1**), pinacidil (**2**), RP 49356 (**3**) and diazoxide (**4**) has attracted the attention of medicinal chemists over the past few years. Those efforts have resulted in a plethora of new analogs, largely related to the most potent of these agents. cromakalim (**1**).[3]



Cromakalim (1)



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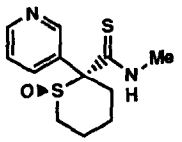
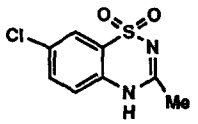
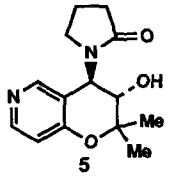


RP 49356 (2)



Diazoxide (4)



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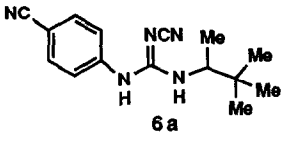
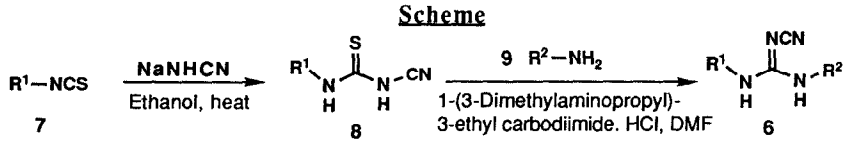


6a

The objective of this study was to determine whether the purported potassium channel openers express their pharmacological effects with similar structural requirements. At the time we began our studies, cromakalim (**1**) and pinacidil (**2**) were the only agents known to have potassium channel opening as their primary mechanism of action.[4] In order to ascertain whether cromakalim (**1**) and pinacidil (**2**) share common pharmacophoric features, we chose to evaluate the combination compounds **5** and **6a**. Since the pyridyl analog **5** of cromakalim (**1**) had been previously reported to be a potent antihypertensive agent,[5] aryl cyanoguanidine **6a**[6] became our synthetic target. In this communication, we report the synthesis and biological activity of **6a** and its additional derivatives. The potent vasorelaxant activity displayed by **6a** and some of its analogs suggest the presence of common pharmacophoric features in cromakalim (**1**) and pinacidil (**2**).

Aryl cyanoguanidines **6** were prepared by treatment of thiourea **8** with appropriate amines **9** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. The starting thioureas **8** were obtained from the commercially available arylisothiocyanates **7** by treatment with sodium cyanamide in an alcoholic solvent. Details of this methodology are published.[7] Vasorelaxant potency was determined by relaxation of the methoxamine contracted rat aorta. The vasorelaxant response to the compounds described herein as well as to the standard potassium channel openers cromakalim (**1**) and pinacidil (**2**), was almost completely reversed by 60mM potassium chloride. This pharmacological behavior is typical of agents known to act *via* a potassium channel opening mechanism.[8] The potassium channel opening mechanism was further confirmed by the ability of glyburide, a known blocker of the ATP-sensitive potassium channels, to inhibit the vasorelaxation caused by selected compounds.[9] Several analogs of **6** were also tested for their ability to relax the spontaneously contracting rat portal vein.[9] Since a similar structure-activity relationship was obtained using the two test systems, vasorelaxant potencies are only reported for the methoxamine contracted art aorta.

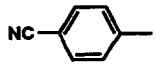
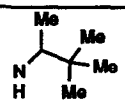
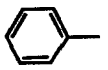
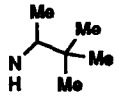
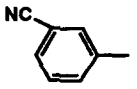
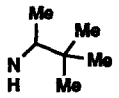
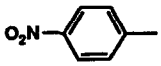
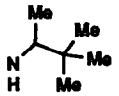
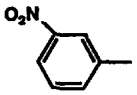
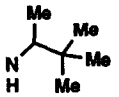
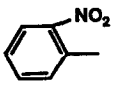
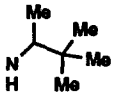
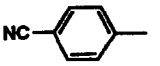
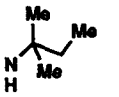
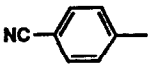
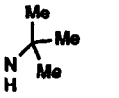
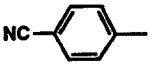
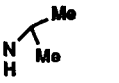
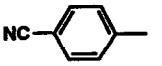
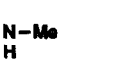
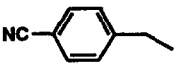
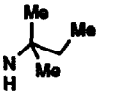
Scheme



As shown in Table I, 4-cyanophenyl analog **6a** relaxed the methoxamine contracted rat aorta with a slightly higher potency (IC_{50} = 0.022μM) than either cromakalim (IC_{50} = 0.055μM) or pinacidil (IC_{50} = 0.070μM). Limited number of analogs of **6a** were prepared to explore the structure-activity relationship for vasorelaxant activity. The corresponding unsubstituted analog **6b** was considerably less active as a vasorelaxing agent. This result taken together with the findings of Peterson,[6] suggest the requirement of an aryl ring substituted with an electron withdrawing group or a pyridine ring for optimal activity. Vasorelaxant potencies of cyano (**6a,c**) and nitro analogs (**6d-f**) indicated no clear preference for the location of the electron withdrawing substituent. A bulky alkyl group R^2 on the guanidine nitrogen, 1, 2, 2-trimethyl-propyl being optimum (compare **6a** with **6g,h**), is required for good vasorelaxant potency. Reduction in its size (compare **6a** with **6i,j**) led to lower potency *in vitro*. As demonstrated by the comparison of **6g** with **6k**, the direct attachment of aryl ring to nitrogen is preferred for biological activity. Selected aryl cyanoguanidine analogs **6** were also tested for antihypertensive activity in spontaneously hypertensive rats (po) and the data comparing **6a** with cromakalim (**1**) and pinacidil (**2**) is shown in Table II. The lead compound **6a** was equipotent to pinacidil (**2**) in lowering blood pressure; this effect lasted for the entire 24 hours of the test. Cromakalim (**1**) was more potent than either **6a** or pinacidil (**2**). For all compounds, the decrease in blood pressure was accompanied by an increase in heart rate, presumably reflexogenic in nature, which normalized after 6 hours (data not shown).

The potent vasorelaxant properties of **6a** and some of its analogs indicate an aryl group having a suitably placed electron withdrawing substituent can effectively mimic the pyridine ring of pinacidil. This result along with the observation that most active analogs of cromakalim contain an electron withdrawing group on the aryl

Table I: Physical properties and vasorelaxant potencies of aryl cyanoguanidine analogs 6.

Compound	R1	R2	% yield[a]	mp°C[b]	IC_{50}, μM (95% C.I.)[c]
6a			-[d]	-[d]	0.022 (0.017, 0.029)
6b			-[d]	-[d]	0.88 (0.61, 1.25)
6c			39	172-173 (A)	0.42 (0.32, 0.53)
6d			41	211-212 (B)	5.15 (3.98, 6.66)
6e			47	183-185 (B)	0.17 (0.11, 0.28)
6f			34	116-118 (C)	0.24 (0.18, 0.32)
6g			40	137-138 (D)	0.12 (0.085, 0.16)
6h			46	184-185 (E)	0.43 (0.29, 0.65)
6i			48	184-185 (F)	19 (14, 25)
6j			61	218-219 (A)	123 (98, 155)
6k			-[d]	-[d]	1.5 (1.1, 2.1)
1 (Cromakalim)					0.055 (0.041, 0.074)
2 (Pinacidil)					0.070 (0.051, 0.096)

[a]Satisfactory microanalysis was obtained for all crystalline compounds. [b]Solvent for crystallization: A, isopropyl ether; B, 2-propanol; C, acetonitrile-isopropyl ether; D, 2-propanol-isopropyl ether; E, acetonitrile; F, dichloromethane-isopropyl ether; acetonitrile-ether. [c]IC_{50} is presented as mean with 95% confidence interval in parenthesis, n ≥ 4 from different animals. [d]see reference 7

ring or a pyridine in the benzopyran portion supports the presence of common pharmacophoric features in cromakalim (**1**) and pinacidil (**2**). It is also of interest to note that analogs of RP 49356 replacing pyridine with an aryl ring having electron withdrawing substituents have been reported to maintain vasorelaxant potency.[10] Taken together, these data are consistent with the requirement of a pyridine or an aryl ring substituted with an electron withdrawing group for potassium channel opening properties of several prototype compounds.

Table II: Antihypertensive activities of 6a, cromakalim (1) and pinacidil (2) in the SHR (po).

	% maximum decrease in blood pressure @45 μmol/kg (n = 6)			
Compound	0-6 hrs	6-12 hrs	12-18 hrs	18-24 hrs
6a	33±1	26±2	26±2	25±5
Cromakalim (**1**)	63±6	35±5	37±8	42±7
Pinacidil (**2**)	40±9	21±6	23±6	14±6

Pharmacological Studies: Rings of thoracic aortae from male Wistar Kyoto rats were denuded of endothelium and individually mounted for isometric force recording in individual chambers containing bicarbonate buffered physiological salt solution and 1μM propranolol at 37°C aerated with 95% O_2/5% CO_2 (pH 7.4). Rings were contracted with 0.3 μM methoxamine, then a cumulative concentration relaxation curve was obtained for the test compound. IC_{50} values were determined from a quadratic fit to the logit transformation of the concentration relaxation curves. Stock solutions of test compounds were prepared daily in water or DMSO as appropriate. For determination of antihypertensive activity, male SHR were prepared surgically according to the method of Weeks and Jones.[11] The test compounds were administered as a suspension in agar and blood pressure was recorded using the method described by Laffin et al.[12]

References And Notes

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