Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3397–3402

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Biologically Selective Potassium Channel Openers Having 1,1-Diethylpropyl Group

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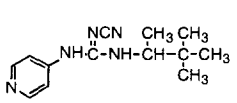
Received 2 September 1998; accepted 14 October 1998

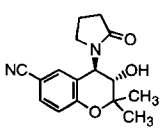
Abstract. To find out selective potassium channel openers (PCOs), we synthesized several 3,5-disubstituted phenylcyanoguanidine derivatives and investigated their structure-activity relationships (SAR). As a result, we discovered selective PCOs having 1,1-diethylpropyl group toward antihypertensive activity.

Potassium channels play a central role in the regulation of cellular excitability, which are involved in setting the membrane potential and controlling the frequency and shape of actions.[1] In addition, recent advanced studies indicate that there are numerous types of potassium channels, each has specific distribution and function in the body.[2] It is expected that PCOs could be useful to treat various diseases, e.g., hypertension, asthma, urinary incontinence, and hypertrichosis so on,[3] in fact, a large number of therapeutic targets are being investigated with PCOs. However, one of drawbacks in well-known PCOs such as pinacidil[4] and cromakalim[5] is their lack of tissue selectivity,[6] which limits their clinical utilities. This disadvantage of first generation PCOs prompted medicinal chemists to find out tissue selective ones. Although a few compounds have been reported as cardiac-selective[7a] or urinary bladder-selective PCOs,[7b] the selective PCOs toward antihypertensive activity have not been found out yet. Therefore, the discovery of vascular-selective PCOs has been exceedingly desired.

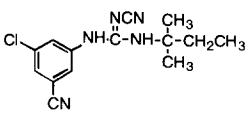
By the way, it has been reported that pinacidil and cromakalim are attractive antihypertensive agents based on the opening of potassium channels.[8] We focused on the modification of pinacidil and have recently reported that compound **1** (KB-R5608) had stronger and more lasting antihypertensive activity than that of pinacidil.[9] However, both pinacidil and KB-R5608 still have broad pharmacological profiles such as relaxation of urinary bladder and trachea, in addition to the antihypertensive activity.



pinacidil



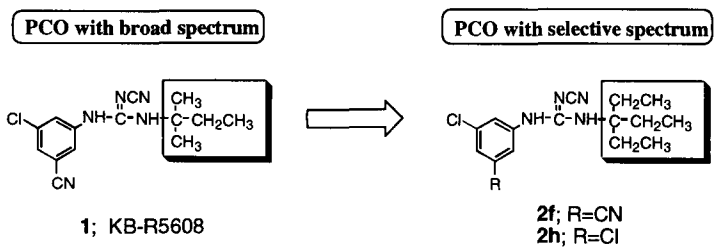
cromakalim



1; KB-R5608

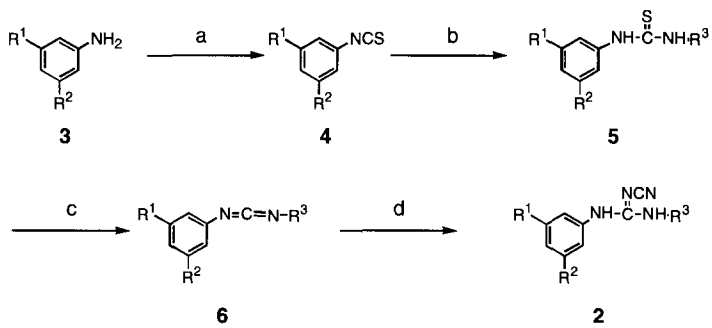
0960-894X/98/$ - see front matter
PII: S0960-894X(98)00625-8

To find out the tissue selective PCOs, we investigated the SAR of compound **1**, and successfully found the selective PCOs (**2f, 2h**) toward antihypertensive activity.



Chemistry Novel phenylcyanoguanidines **2a-h**[10] listed in Table 1 were synthesized from the corresponding 3,5-disubstituted anilines **3** via phenylthioureas **5** (Scheme 1). Among the 3,5-disubstituted anilines **3** used in this study , 3-bromo-5-fluoroaniline **3a**[11] was prepared from 4-fluoro-2-nitroaniline **7** in three steps (Scheme 2). The others were commercially available and/or were synthesized according to literatures. The 3,5-disubstituted anilines **3** were treated with thiophosgene to afford the corresponding phenylisothiocyanates **4**, which were converted into phenylthioureas **5** by reaction with alkylamines $H_2N\text{-}R^3$. Among $H_2N\text{-}R^3$, 1-methyl-1-ethylpropylamine ($H_2NC(CH_3)(CH_2CH_3)_2$) and 1,1-diethylpropylamine ($H_2NC(CH_2CH_3)_3$) were synthesized from the corresponding alcohols **10** by Ritter reaction (Scheme 3). The removal of hydrogen sulfide from **5** by treatment with triphenylphosphine, carbon tetrachloride, and triethyamine gave carbodiimides **6**. The desired phenylcyanoguanidines **2** were prepared by the addition of cyanamide to carbodiimides **6.**

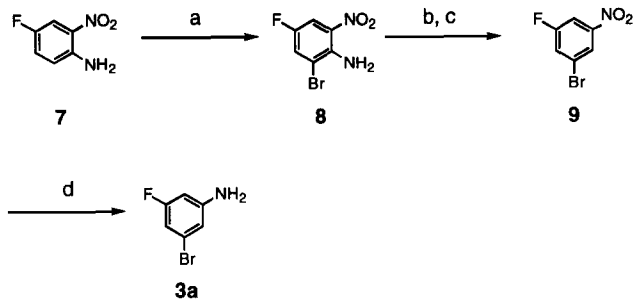
Scheme 1



Reagents: (a) $CSCl_2$; (b) $H_2N\text{-}R^3$; (c) PPh_3, CCl_4, Et_3N / CH_2Cl_2; (d) H_2NCN, *i*-Pr_2EtN

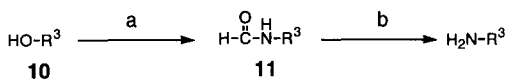
Results and Discussion We have already reported that the relaxant activity toward taenia caecum could be a good index for the estimation of potassium channel opening activity.[9] As shown in Table 1, pinacidil, cromakalim, KB-R5608 (**1**), and compounds **2a-h** exhibited the taenia caecum relaxant activity, especially the activities of compounds **2a-h** were 10-80 fold more potent than that of pinacidil. Namely, it was found that compounds **2a-h** possessed the potent potassium channel

Scheme 2



Reagents: (a) Br_2, $FeCl_3$; (b) H_2SO_4, $NaNO_2$; (c) EtOH, $FeSO_4 \cdot 7H_2O$; (d) Fe, NH_4Cl

Scheme 3



Reagents: (a) NaCN, H_2SO_4; (b) NaOH

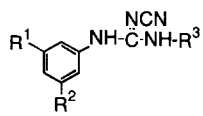
opening activity compared to pinacidil and/or cromakalim. Against all of the compounds, we evaluated the urinary bladder relaxant activity for urinary incontinence, the trachea relaxant activity for asthma, and the antihypertensive activity using dogs for hypertension to investigate the SAR regarding the selectivity of pharmacological profile.

At first, we fixed the alkyl group on the cyanoguanidine unit of compound **1** to the *tert*-pentyl group and estimated the urinary bladder and trachea relaxant activities of compounds **2a-c**. As a result, compounds **2a-c** showed almost the same urinary bladder and trachea relaxant activities as **1**. This result indicates that two substituents on the benzene ring do not affect the selectivity at all.

Next, we fixed two substituents on the benzene ring to the chloro and cyano groups, and investigated the SAR of compounds **2d-f**. Among the compounds with the 3-chloro-5-cyanophenyl moiety, compounds **2d** and **2e** exhibited the potent urinary bladder and trachea relaxant activities (ED_{50} values; 0.19 μM and 0.69 μM for **2d**, 0.016 μM and 0.19 μM for **2e**, respectively). Surprisingly, it was to note that compound **2f** with 1,1-diethylpropyl group, $C(C_2H_5)_3$, revealed much weaker activities toward urinary bladder and trachea than **1** with *tert*-pentyl group, **2d** with 1,1,2-trimethylpropyl group, **2e** with 1-ethyl-1-methylpropyl group, and pinacidil with 1,2,2-trimethylpropyl group. Interestingly, compound **2f** still has more potent taenia caecum relaxant and antihypertensive activities compared to pinacidil. Although a successful selectivity of **2f** toward vasorelaxation may depend on the structure of the alkyl group, there is very few information to conclude so. In order to clarify this point, we synthesized the 3,5-dichlorophenylcyanoguanidine derivatives **2g** and **2h**, and evaluated their pharmacological profile. As shown in Table 1, compound **2h** with the 1,1-diethylpropyl group showed the potent relaxant activity toward taenia caecum (ED_{50} = 0.18 μM), but the weak activities toward urinary bladder (< 5% at 1 μM) and trachea

(30 μM). On the other hand, compounds **2g** with the 1-ethyl-1-methylpropyl group showed the potent relaxant activities toward all of three types of smooth muscles. These findings indicate that the urinary bladder and trachea relaxant activities would be comparatively sensitive against the structure of the alkyl group on the cyanoguanidine unit. The relaxant activities of pinacidil and cromakalim toward vascular smooth muscle, urinary bladder, and trachea are known to be based on the opening of ATP-sensitive potassium channels among several types of potassium channels.[3a] It is considered that compounds **2f** and **2h** would open ATP-sensitive potassium channels in vascular smooth muscle, not open ones in urinary bladder and trachea. It is not clear why **2f** and **2h** with the 1,1-diethylpropyl group show the selectivity, but two possibilities can be considered. One is the difference in the delivery of the compounds to potassium channels in each tissue, and the other is the difference in the structure of potassium channels in each tissue.

Table 1 Biological Activities of Pinacidil, Cromakalim, Compounds **1** and **2a-h**



Compound	R^1	R^2	R^3	TCRA[a] (ED_{50}, μM)	UBRA[b] (ED_{50}, μM)	TRA[c] (ED_{50}, μM)	AHA[d] (ΔmmHg)
pinacidil				2.0	0.31	2.3	-11.3±1.1
cromakalim				0.47	0.042	1.2	-37.8±6.2
1(KB-R5608)	Cl	CN	$-C(CH_3)_2CH_2CH_3$	0.075	0.02	0.28	-29.8±5.0
2a	F	Br	$-C(CH_3)_2CH_2CH_3$	0.025	0.016	0.18	-33.4±0.9
2b	Br	CN	$-C(CH_3)_2CH_2CH_3$	0.032	0.011	0.08	NT[e]
2c	F	Cl	$-C(CH_3)_2CH_2CH_3$	0.054	0.032	0.26	NT[e]
2d	Cl	CN	$-C(CH_3)_2CH(CH_3)_2$	0.17	0.19	0.69	-20.3±2.9
2e	Cl	CN	$-C(CH_3)(CH_2CH_3)_2$	0.10	0.016	0.19	-25.2±7.8
2f	Cl	CN	$-C(CH_2CH_3)_3$	0.094	>1μM[f]	5.6	-18.9±2.5
2g	Cl	Cl	$-C(CH_3)(CH_2CH_3)_2$	0.081	0.013	0.44	-26.1±1.8
2h	Cl	Cl	$-C(CH_2CH_3)_3$	0.18	>1μM[g]	30	-15.9±3.4

a) TCRA: taenia caecum relaxant activity. See References and Notes 12. *b)* UBRA:urinary bladder relaxant activity. See References and Notes 13 for details. *c)* TRA: trachea relaxant activity. See References and Notes 14 for details. *d)* AHA:antihypertensive activity in dogs by i.v. injection at a dose 30 μg/kg. Each value represents the mean ± S.E. of a maximum decrease in mean blood pressure. n=20 for pinacidil, n=5 for **1**, n=3 for the others. See References and Notes 15. *e)* not tested. *f)* 22% at 1μM. g) <5% at 1μM.

In conclusion, we investigated the SAR of compound **1** (KB-R5608) for the discovery of the selective PCOs, and found that the modification of the alkyl group of compound **1** (KB-R5608) could give pharmacological selectivity for the potential PCOs. Especially, the two phenylcyanoguanidine derivatives having the 1,1-diethylpropyl group, **2f** and **2h**, were good PCOs with respect of the selectivity toward the antihypertensive activity. These new findings will be useful for the design of new types of PCOs.

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10. mp (℃), solvent for recrystallization, and ^{1}H-NMR (300MHz) data of novel disubstituted phenylcyanoguanidines are as follows :
    - **2a**, 129.0-130.0, benzene-*n*-hexane, ^{1}H-NMR (DMSO-d_6) δ: 0.82(3H, t), 1.28(6H, s), 1.70(2H, q), 6.8-7.3(4H, m), 9.30(1H, bs),
    - **2b**, 186.0-188.0, ethanol, ^{1}H-NMR (DMSO-d_6) δ: 0.83(3H, t), 1.29(6H, s), 1.70(2H, q), 7.29(1H, bs), 7.48(1H, t), 7.57(1H, t), 7.78(1H, t), 9.42(1H, bs),
    - **2c**, 130.0-131.0, isopropanol, ^{1}H-NMR (DMSO-d_6) δ: 0.82(3H, t), 1.29(6H, s), 1.70(2H, q), 6.8-7.1(3H, m), 7.25(1H, bs), 9.31(1H, bs),
    - **2d**, 157.0-159.0, benzene, ^{1}H-NMR (DMSO-d_6) δ: 0.84(6H, d), 1.26(6H, s), 2.3-2.5(1H, m), 7.27(1H, bs), 7.4-7.5(2H, m), 7.6-7.7(1H, m), 9.41(1H, bs),
    - **2e**, 181.0-183.5, ethanol, ^{1}H-NMR (DMSO-d_6) δ: 0.81(6H, t), 1.23(3H, s), 1.5-1.9(4H, m), 7.20(1H, bs), 7.4-7.5(2H, m), 7.68(1H, t), 9.45(1H, bs),
    - **2f**, 192.0-195.0, ethanol, ^{1}H-NMR (DMSO-d_6) δ: 0.77(9H, t), 1.67(6H, q), 7.05(1H, bs), 7.3-7.5(2H, m), 7.6-7.7(1H, m), 9.47(1H, bs),
    - **2g**, 148.0-150.0, benzene, ^{1}H-NMR (DMSO-d_6) δ: 0.81(6H, t), 1.22(3H, s), 1.5-1.9(4H, m), 7.08(2H, d), 7.15(1H, bs), 7.23(1H, t), 9.30(1H, bs),
    - **2h**, 178.5-180.5, benzene, ^{1}H-NMR (DMSO-d_6) δ: 0.77(9H, t), 1.67(6H, q), 7.00(1H, bs), 7.06(2H, d), 7.23(1H, t), 9.32(1H, bs).

11. bp$_7$ 103.0-105.0℃. ^{1}H-NMR (DMSO-d$_6$) δ:5.73(2H, bs), 6.2-6.4(1H, m), 6.4-6.7(2H, m).
12. *n*=20 for pinacidil, *n*=5 for cromakalim, **1**, and **2a-h**. For details, see experimental section in References and Notes 9.
13. Male guinea pigs weighing 300-600 g were killed by stunning and bleeding. Urinary bladder was excised, cleaned of connective tissue, and cut into four pieces of 10-15 mm x 3-5 mm. These specimens were each suspended in an organ bath filled with a solution containing 120.0 mM NaCl, 5.4 mM KCl, 25.0 mM $NaHCO_3$, 2.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.6 mM glucose, pH 7.4. The bathing solution was continuously bubbled with 95% O_2 - 5% CO_2 gas and maintained at 37 ±1℃. Resting tension of each specimen was adjusted to 1 g and the amplitude of spontaneous contraction was recorded isometrically. The specimens were allowed to stabilize before the start of the test. A solution of each test compound in dimethyl sulfoxide was cumulatively added to the bathing solution. Each responses is expressed as a percentage of the amplitude of spontaneous contraction before the addition of compound. The relaxing effect of each test compound was expressed in terms of the dose giving 50% relaxation (ED_{50}) as determined by linear regression analysis. *n*=4 for **2c-2e**, and **2h**, *n*=5 for the others.
14. Male guinea pigs weighing 362-594 g were killed by stunning and bleeding. Trachea were excised, cleaned of connective tissue, opened by cutting longitudinally through the cartilage rings diametrically opposite the trachealis. These specimens were each suspended in an organ bath filled with a 3-(*N*-morpholino) propanesulfonic acid-physiological salt solution (MOPS-PSS) containing 129.7 mM NaCl, 5.9 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgCl_2$, 10.0 mM MOPS and 11.1 mM glucose, pH 7.4. The bathing solution was continuously bubbled with 100% O_2 gas and maintained at 37 ± 1℃. Resting tension of each specimen was adjusted to 1 g and the spontaneous response was recorded isotonically. The specimens were allowed to stabilize before the start of the test. A solution of each test compound in 50% ethanol-50% PEG400 (v/v) was cumulatively added to the bathing solution. Relaxation induced with aminophylline (10^{-3} M) was taken as 100% relaxation. The relaxing effect of each test compound was expressed in terms of the dose giving 50% relaxation (ED_{50}) as determined by linear regression analysis. *n*=5 for all the tested compounds.
15. For details, see : Yoshiizumi, K.; Ikeda, S.; Nishimura, N.; Yoshino, K. *Chem. Pharm. Bull.*, **1997**, *45*, 2005.