Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 16, pp. 1995-1998, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00275-4

SUBSTITUENT EFFECTS OF BENZOPYRAN-4-(*N*-CYANO)-CARBOXAMIDINE POTASSIUM CHANNEL OPENERS FOR SELECTIVITY TO GUINEA PIG TRACHEALIS

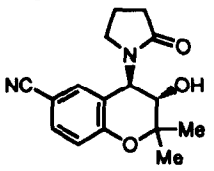
Takenori Ishizawa, Hiroshi Koga,* Haruhiko Sato, Toshihiko Makino, Naoki Taka, Tadakatsu Takahashi, Tsutomu Sato, and Hiroyuki Nabata

Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Company, Ltd., 135, 1-Chome Komakado, Gotemba-shi, Shizuoka, 412, Japan.

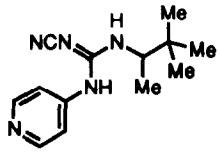
Abstract: 6-Substituted benzopyran-4-(*N*-cyano)amidines **1** and their analogs have been synthesized. Some of these compounds, **1a**, **1e**, and **1f** exhibited selective activity for guinea pig trachealis.

In the last few years, potassium channel openers have become a focus of attention, because they are believed to be potential drugs for diseases such as hypertension, angina pectoris, asthma, and alopecia.[1] It is recognized that these potassium channel openers consist of a chemically diverse type of compounds, represented by cromakalim, pinacidil, and RP49356.[1] Although they differ from each other in their structure, they are thought to open ATP-sensitive potassium channels and their actions are competitively antagonized by glyburide, a selective blocker of ATP-sensitive potassium channels. These, as well as the receptor binding study,[2] seem to imply that the binding sites involved are at least in part the same.[1]

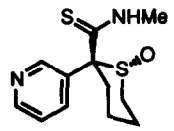
Figure 1.



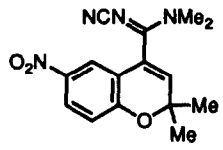
Cromakalim (racemic)



Pinacidil



RP49356 (racemic)



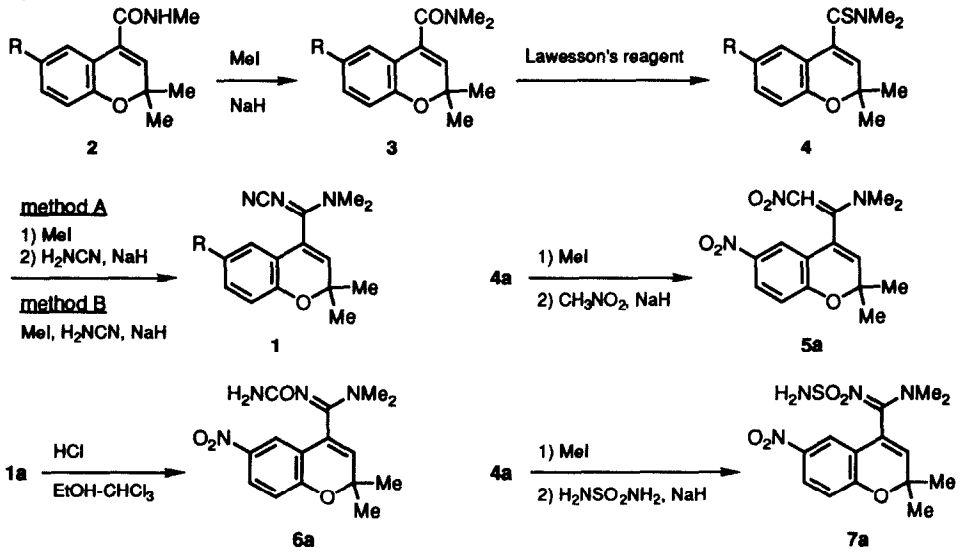
1a

We previously found that *N*-cyano-*N'*,*N'*,2,2-tetramethyl-6-nitro-2*H*-1-benzopyran-4-carboxamidine (**1a**) exhibited selective activity for guinea pig trachealis.[3] The previous paper reported the effect of *N'*-alkyl substituents on selectivity in guinea pig trachealis.[3] Moreover, it is very interesting to explore the effects of the other substituents for the selectivity. This study documents the effects of the 6-substituents and modifications of the cyanoimino group on the selectivity.

The compounds prepared in this study are listed in Table I, and their synthetic routes are outlined in Scheme I. The known amides **2**[4] were converted by *N*-methylation to *N*,*N*-dimethyl derivatives **3** (MeI, NaH, THF, reflux, 1 h). The thioamides **4** obtained by thiation of **3** (Lawesson's reagent, benzene, reflux, 1 h), were converted under the two conditions to **1** (method A : MeI, THF, reflux, 1 h then H_2NCN, NaH, THF, reflux,

2 h; method B : MeI, H_2NCN, NaH, THF, *room temp.*, 15 h). Compound **4a** was also converted to **5a** (MeI, THF, reflux, 1 h then CH_3NO_2, NaH, THF, reflux, 0.5 h, 53%) and **7a** (MeI, THF, reflux, 1 h then $H_2NSO_2NH_2$, NaH, THF, reflux, 2.5 h, 48%). Compound **6a** was obtained by hydrolysis of **1a** (HCl, EtOH-$CHCl_3$, 4 °C, 10 days, 98%).

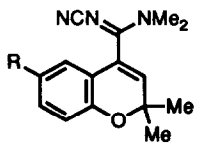
Scheme I



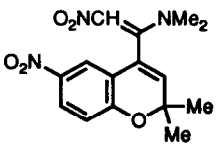
The smooth muscle relaxant activities of compounds were determined by the effects on 30 mM KCl responses in rat aorta and spontaneous tone in guinea pig trachealis, and are listed in Table I in comparison with cromakalim,[5] pinacidil,[6] and RP49356.[7]

The *N*-methylcarboxamide **2a**, *N*,*N*-dimethylcarboxamide **3a**, and *N*,*N*-dimethylcarbothioamide **4a** showed no selectivity for guinea pig trachealis. Replacement of the cyanoimino group of **1a** to nitromethylene (**5a**), aminocarbonylimino (**6a**), and sulfamoylimino (**7a**) groups also gave disappointing results. That is, these compounds showed no significant selectivity for the trachea, or were devoid of activity for both tissues. These results suggest that the cyanoimino group of **1a** is mandatory for the selectivity to the trachea. Among the 6-substituted amidines, compound **1a** as well as the 6-trifluoromethyl and 6-trifluoromethoxy compounds **1e** and **1f**, exhibited selective activity, with somewhat lower activity of the latter. The 6-cyano and 6-benzenesulfonyl compounds **1b** and **1c** showed no the selectivity with weak activity for both tissues. Other compounds **1d** and **1g**-**1j** were devoid of activity for both tissues. Thus, among tested compounds, **1a** possessed the most potent and significant selective activity for the guinea pig trachealis. On the other hand, none of the reference compounds cromakalim, pinacidil, and RP49356 showed the selectivity (Table I). To our knowledge, there is no report of potassium channel opener with selectivity in vitro to trachea, such as **1a**.

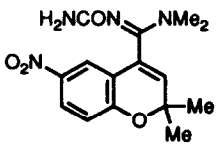
Table I. Smooth Muscle Relaxant Activities of 6-Substituted *N*-Cyano-*N'*,*N'*,2,2-tetramethyl-2*H*-1-benzopyran-4-carboxamidines **1** and the Derivatives **2a-7a**



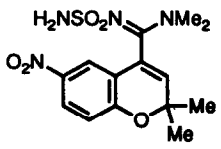
1



5a



6a



7a

					rat aorta			guinea pig trachealis		
No.	R	method	Yield (%)	mp (°C)	pEC_{50} [a]	IA (%) [b]	n [c]	pEC_{50} [d]	IA (%) [b]	n [c]
1a [e]	NO_2				4.5>	-	3	7.29 ± 0.04	90.6 ± 1.1	9
2a [f, g]	NO_2				7.97 ± 0.04	61.0 ± 5.1	4	7.48 ± 0.08	89.6 ± 3.0	8
3a [f]	NO_2		92	115 - 118	6.89 ± 0.13	79.9 ± 7.7	3	6.40 ± 0.08	78.3 ± 9.4	3
4a [f]	NO_2		84	139 - 141	6.02 ± 0.09	71.5 ± 3.3	3	6.31 ± 0.04	77.4 ±10.8	3
5a			53	217 - 219	5.19 ± 0.07	83.1 + 2.6	3	5.95 + 0.21	84.8 + 6.8	4
6a			98	157 - 159	4.5>	-	1	4.5>	-	4
7a			48	212 - 214	4.5>	-	2	4.5>	-	2
1b	CN	A	56	219 - 221	5.30 ± 0.04	73.7 ± 8.6	3	5.67 ± 0.19	85.6 ± 4.1	3
1c	$PhSO_2$	A	22	200 - 201	5.48 ± 0.10	73.8 ± 0.5	3	5.44 ± 0.11	91.1 ± 3.9	3
1d	$MeSO_2$	A	43	246 - 247	4.5>	-	2	4.5>	-	2
1e	CF_3	A	17	132 - 133	4.90 ± 0.09	75.2 ± 8.8	3	6.75 ± 0.15	74.1 ± 8.0	3
1f	CF_3O	B	58	123 - 124	5.0>	-	2	6.39 ± 0.04	56.1 ± 8.3	3
1g	Br	A	98	164 - 165	4.5>	-	2	4.5>	-	2
1h	Cl	B	56	167 - 168	4.5>	-	2	4.5>	-	2
1i	H	B	66	77 - 78	4.5>	-	2	4.5>	-	2
1j	MeO	B	61	111 - 112	4.5>	-	2	4.5>	-	2
Cromakalim					6.77 ± 0.03	74.7 ± 2.1	25	6.07 ± 0.08	85.1 ± 4.1	7
Pinacidil					6.14 ± 0.03	91.9 ± 2.5	5	5.80 ± 0.07	97.3 ± 0.7	6
RP49356					6.28 ± 0.04	79.7 ± 2.2	6	5.39 ± 0.04	91.9 ± 2.3	8

[a] Negative logarithms of the molar concentration required to relax rat aorta precontracted with 30 mM KCl by 50% of IA, with ± SEM. See footnote 8 for experimental details. [b] Intrinsic activity ± SEM (%). [c] Number of determination. [d] Negative logarithms of the molar concentration required to inhibit spontaneous tone in guinea pig trachealis by 50% of IA, with ± SEM. See footnote 9 for experimental details. [e] See reference 3. [f] See scheme I for the structures. [g] See reference 4b.

Part of the interesting pharmacological properties of compound **1a** (KC-128) as an ATP-sensitive potassium channel opener have been presented elsewhere.[10] Further biochemical and pharmacological studies of compound **1a** (KC-128) are in progress.

Compound **1a** (KC-128) will be widely applicable in biochemical and pharmacological studies of potassium channels.

References and Footnotes

1. a) Robertson, D. W.; Steinberg, M. I. *J. Med. Chem.* **1990**, *33*, 1529. b) Edwards, G.; Weston, A. H. *Trends Pharmacol. Sci.* **1990**, *11*, 417. c) Evans, J. M.; Longman, S. D. *Ann. Rep. Med. Chem.* **1991**, *26*, 73.
2. Bray, K. M.; Quast, U. *J. Biol. Chem.* **1992**, *267*, 11689.
3. Koga, H.; Sato, H; Ishizawa, T.; Kuromaru, K.; T.; Nabata, H.; Imagawa, J.; Yoshida, S.; Sugo, I. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1111.
4. a) Koga, H.; Ohta, M.; Sato, H.; Ishizawa, T.; Nabata, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 625. b) Koga, H.; Sato, H; Ishizawa, T.; Kuromaru, K.; Makino, T.; Taka, N.; Takahashi, T.; Sato, T.; Nabata, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1115. c) Ishizawa, T.; Koga, H.; Sato, H; Makino, T.; Kuromaru, K.; Taka, N.; Takahashi, T.; Sato, T.; Nabata, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1659. d) Sato, H.; Koga, H; Ishizawa, T.; Kuromaru, K.; Makino, T.; Taka, N.; Takahashi, T.; Sato, T.; Nabata, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2627.
5. Ashwood, V. A.; Buckingham, R. E.; Cassidy, F.; Evans, J. M.; Faruk, E. A.; Hamilton, T. C.; Nash, D. J.; Stemp, G.; Willcocks, K. *J. Med. Chem.* **1986**, *29*, 2194.
6. Peterson, H. J.; Nielsen, C. K.; Arrigoni-Martelli, E. *J. Med. Chem.* **1978**, *21*, 773.
7. Aloup, J. C.; Farge, D.; James, C.; Mondot, S.; Cavero, I. *Drugs Fut.* **1990**, *15*, 1097.
8. Rats (Sprague-Dawley, male 400-700 g) were killed by decapitation. The thoracic aorta was dissected out, immersed in cold Krebs-Henseleit (K-H) solution, and cleaned of surrounding connective tissues. The artery was cut into 2-3 mm long ring segments. Each ring was mounted under a resting tension of 2 g in a 10 ml organ bath containing a modified K-H solution of the following composition (mM): NaCl, 119; KCl, 4.8; $CaCl_2$, 2.53; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 24.8; glucose 10. The solution was equilibrated with a gas mixture containing 95% O_2 and 5% CO_2. One side of the ring preparation was fixed to the bottom of the bath and the other end was connected by a hook at the level of a force-displacement transducer (Nihon Kohden, TB611T). Before the initiation of the experiments, all preparations were allowed to equilibrate for at least 1.5 h at 37 °C. The artery rings were contracted by displacement of normal K-H solution to the K-H solution containing 30 mM KCl (high K^+ K-H solution). After the increased force of contraction had reached a plateau, test compounds were added in a cumulative way to construct concentration-relaxation curves. Relaxation responses were calculated as percentage of reduction of 30 mM KCl contraction. The intrinsic activity (IA) for each compound was calculated as a percentage of its maximum reduction of 30 mM KCl contraction. Only one concentration-relaxation curve was obtained from each preparation.
9. Guinea pigs (Hartley, male 600-1000 g) were killed by blow the head and bleeding. The trachea was dissected out, immersed in ambient Krebs-Henseleit (K-H) solution, and cleaned of surrounding connective tissues. The trachea was cut into 2-3 mm long ring segments. Each ring was cut diametrically opposite the tracheal muscle, opened out, and mounted in a 10 ml organ bath containing a modified K-H solution of the composition described in footnote 9. Resting tension was set at 1 g under exposure to 1 mM aminophylline. When aminophylline was washed out, each preparation contracted spontaneously. After the increased force of contraction had reached a plateau, test compounds were added in a cumulative way to construct concentration-relaxation curves. Only one concentration-relaxation curve was obtained from each preparation. Drug-induced relaxation of tone was expressed as a percentage of maximum relaxation to 1 mM aminophylline given at the end of each experiment. The intrinsic activity (IA) for each compound was calculated as a percentage of its maximum relaxant activity to the maximum response to aminophylline (1mM).
10. Kamei, K.; Imagawa, J.; Nabata, H.; Kuriyama, H. XXXII Congress of the International of Physiological Sciences, August 1-6, 1993, Glasgow: *Abstract of papers*; p210.

(*Received in USA* 4 *April* 1994; *accepted* 7 *July* 1994)