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N,N-DISUBSTITUTED BENZOPYRAN-4-(*N'*-CYANO)CARBOXAMIDINES, CROMAKALIM ANALOGS WITH SELECTIVE ACTIVITY FOR GUINEA PIG TRACHEALIS

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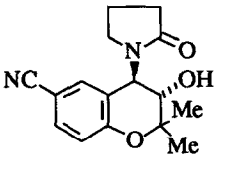
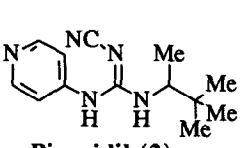
Abstract: *N*-Cyano-2,2-dimethyl-6-nitro-2*H*-1-benzopyran-4-carboxamidines have been synthesized. Some of these compounds exhibited selective activity for guinea pig trachealis.

K^+ channel openers are a new pharmacological class of compounds with potential clinical applications to hypertension, angina pectoris, asthma, irritable bladder syndrome, and alopecia. However, tissue selectivity is probably the key to the successful development of these drugs in fields such as asthma and irritable bladder syndrome.[1]

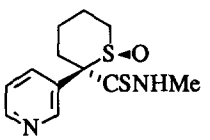
We previously constructed a pharmacophore model of K^+ channel openers.[2] Although this model does not always fully rationalize all of the structure-activity relationships of the chemically diverse and structurally unrelated K^+ channel openers such as cromakalim (**1**), pinacidil (**2**), and RP 49356 (**3**), it gave us many hints to design new prototype compounds. We have designed new K^+ channel openers using this model, some of which were synthesized and found to possess potent vasorelaxant activity.[2] One of these compounds was benzopyran-4-(*N*-cyano)amidine **4**. Since the compound **4** is regarded as a hybrid compound of cromakalim (**1**), pinacidil (**2**), and RP 49356 (**3**), the structure-activity relationship study of **4** may explain structural requirements for K^+ channel openers in more detail. Along these lines, we have synthesized benzopyran-4-(*N*-cyano)amidine derivatives **5** and found that some of the *N,N*-disubstituted derivatives show a selective activity for guinea pig trachealis.



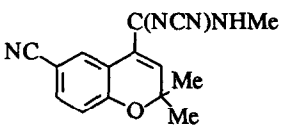
Cromakalim (1)



Pinacidil (2)



RP 49356 (3)



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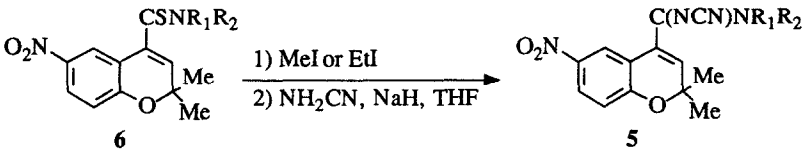
In this paper, we wish to report the synthesis and biological activity of benzopyran-4-(*N*-cyano)carboxamidines **5**.

Benzopyran-4-carbothioamides **6** which were prepared from 3,4-epoxy-3,4-dihydro-2,2-dimethyl-6-nitro-2*H*-1-benzopyran by the method similar to that previously reported,[2] were activated by methyl iodide or ethyl iodide followed by the addition of cyanamide and sodium hydride in tetrahydrofuran to afford the desired cyanamidines **5** (Scheme I and Table I).

Scheme I



6 **5**

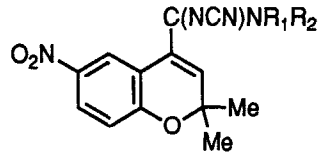
The smooth muscle relaxant activities of compounds were determined by the effects on 30 mM KCl responses in rat isolated aorta and spontaneous tone in guinea pig isolated trachea, and are shown in Table I in comparison with cromakalim (**1**),[1,3] pinacidil (**2**),[1,4] and RP 49356 (**3**).[1,5]

The pharmacophore model previously developed[2] showed that 6-cyano group of compound **4** might work as a hydrogen bond acceptor. Since 6-nitro group, as well as the 6-cyano group, was expected to be a strong hydrogen bond acceptor, 6-nitro compound **5a** of **4** was synthesized. Earlier studies on benzopyran K^+ channel openers revealed that replacement of the 6-cyano function by a nitro group retained high potency.[3] Substitution of the 6-cyano group of **4** for a nitro group to give **5a** enhanced the potency by at least a factor of 10, indicating that the 6-nitro group is more favorable to interact with the receptor than the 6-cyano group.

The activity of compound **5** was also greatly dependent on the *N*-substituent. Replacement of the *N*-methyl group of **5a** by a longer alkyl chain, ethyl group to afford **5b** led to lower potency for both tissues. On the other hand, *N,N*-dimethyl compound **5c** was devoid of the vasorelaxant activity with retaining the activity for the trachea. This seems to be the first example to show significant selectivity for the trachea in cromakalim analogs.[1] Conversion of the *N,N*-dimethyl of **5c** to *N*-ethyl-*N*-methyl to give **5d** diminished the potency with retaining the selectivity for the trachea. However, compounds with more bulky alkyl group (e.g., ethyl, propyl, butyl, etc.) as the R_1 and R_2 of **5** were devoid of the activities for both tissues (data not shown except for *N,N*-diethyl compound **5e**). These results show that introduction of dialkyl group as the R_1 and R_2 of **5** was detrimental to the vasorelaxant activity, while it is favorable for the selectivity for the trachea though there was some limitation as to the size of the alkyl group.

Subsequent mechanistic study of **5c** revealed that the relaxant activity in guinea pig trachealis was non-competitively inhibited by glibenclamide, a potent and selective blocker of ATP-sensitive K^+ channels,[1] in contrast with cromakalim (**1**), pinacidil (**2**), and RP 49356 (**3**), the relaxant activity for the trachea of which were competitively blocked by glibenclamide with the pA_2 values of 7.27-7.40 (data not shown), suggesting that the binding site of **5c** to relax the contraction of guinea pig tracheal tissue may be different from these K^+ channel openers.

Table I. Physical Properties and Smooth Muscle Relaxant Activities of Benzopyrans 5



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					rat aorta			guinea pig trachealis		
compd	R_1	R_2	yield(%)	mp,°C	pEC_{50}[a]	IA(%)[b]	n[c]	pEC_{50}[d]	IA(%)[b]	n[c]
5a	H	Me	42	240-243	7.82±0.11	79.9±0.1	3	7.63±0.06	86.5±5.5	5
5b	H	Et	38	198-200	7.05±0.04	75.1±2.8	3	6.77±0.24	85.3±9.6	4
5c	Me	Me	94	209-211	<4.5[e]		3	7.29±0.04	90.6±1.1	9
5d	Me	Et	81	151-152	<4.5		3	6.75±0.18	77.9±5.2	3
5e	Et	Et	88	159-161	<4.5		3	<4.5		3
4[f]					6.47±0.02	73.0±2.3	3	6.19±0.08	86.0±4.8	3
cromakalim (**1**)					6.77±0.03	74.7±2.1	25	6.07±0.08	85.1±4.1	7
pinacidil (**2**)					6.14±0.03	91.9±2.5	5	5.80±0.07	97.3±0.7	6
RP 49356 (**3**)					6.28±0.04	79.7±2.2	6	5.39±0.04	91.9±2.3	8

[a] Negative logarithm of the molar concentration required to relax rat aorta precontracted with 30 mM KCl by 50% of IA, with ± SEM. See footnote 6 for experimental details. [b] Intrinsic activity ± SEM (%). [c] Number of determinations. [d] Negative logarithm of the molar concentration required to inhibit spontaneous tone in guinea pig trachealis by 50% of IA, with ± SEM. See footnote 7 for experimental details. [e] The pEC_{50} for guinea pig aorta was also less than 4.5. [f] See reference 2.

References and Footnotes

1. (a) Potassium Channels: Structure, Classification, Function, and Therapeutic Potential; Cook, N. S., Ed.; Ellis Horwood Limited: Chichester, 1990. (b) Robertson, D. W.; Steinberg, M. I. *J. Med. Chem.* **1990**, *33*, 1529-1541. (c) Edwards, G.; Weston, A. H. *Trends Pharmacol. Sci.* **1990**, *11*, 417-422. (d) Evans, J. M.; Longman, S. D. *Ann. Rep. Med. Chem.* **1991**, *26*, 73-82.

2. Koga, H.; Ohta, M.; Sato, H.; Ishizawa, T.; Nabata, H. *Bioorg. Med. Chem. Lett.*, accepted for publication.

3. 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*, 2194-2201.

4. Petersen, H. J.; Nielsen, C. K.; Arrigoni-Martelli, E. *J. Med. Chem.* **1978**, *21*, 773-781.

5. Aloup, J. C.; Farge, D.; James, C.; Mondot, S.; Cavero, I. *Drugs Fut.* **1990**, *15*, 1097-1108.

6. 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 hr 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 reductions of the 30 mM KCl contraction. The intrinsic activity (IA) for each compound was calculated as a percentage of its maximum reduction of the 30 mM KCl contraction. Only one concentration-relaxation curve was obtained from each preparation.

7. Guinea pigs (Hartley, male 500-1000 g) were killed by a blow to the head and bleeding. The trachea was dissected out, immersed in warmed (37 °C) Krebs-Henseleit (K-H) solution, and cleaned of sorrounding 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 6. Resting tention 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. For the assessment of antagonism by glibenclamide, the preparations had been exposed to glibenclamide immediately after the wash-out of aminophylline. Only one concentration-relaxation curve was obtained from each preparation. Drug-induced relaxation of tone was expressed as a percentage of the 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 (1 mM).