Bioorganic & Medicinal Chemistry Letters, Vol.3, No.4, pp. 625-631, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00
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DESIGN OF POTENT K^+ CHANNEL OPENERS BY PHARMACOPHORE MODEL

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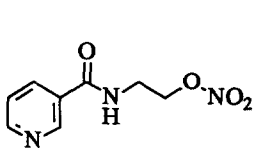
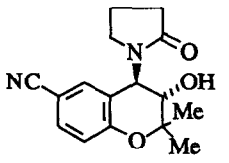
(*Received in USA* 8 *December* 1992)

Abstract: A pharmacophore model which explains rationally structure-activity relationships of chemically diverse potent K^+ channel openers, has been constructed. Potent benzopyran derivatives with thioamide, amide, and (*N*-cyano)amidine groups at the 4-position have been designed using the model.

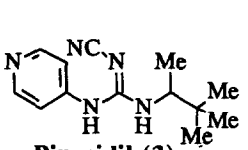
Recently, markedly increased attention has been focused on K^+ channel openers, because of their potential value in the treatment of diseases involving smooth muscle contraction, such as hypertension, angina pectoris, asthma, and urinary incontinence.[1] These K^+ channel openers constitute a chemically diverse and structurally unrelated group of compounds including nicorandil (**1**), cromakalim (**2**), pinacidil (**3**), RP 49356 (**4**), diazoxide (**5**), and minoxidil sulfate (**6**).[1]



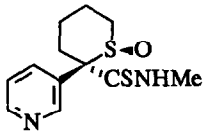
Nicorandil (1)



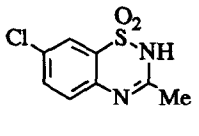
Cromakalim (2)
Lemakalim (3S,4R) (2a)



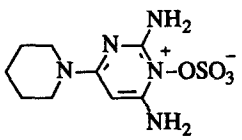
Pinacidil (3)



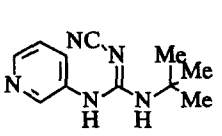
RP 49356 (4)
RP 52891 (1R,2R) (4a)



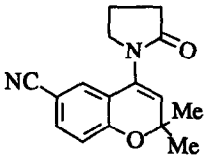
Diazoxide (5)



Minoxidil sulfate (6)



P 1060 (7)



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The antianginal agent, nicorandil (**1**), developed in our laboratories, was the first molecule shown to possess K^+ channel opening properties.[1,2] However, it has been revealed that nicorandil (**1**) exerts its vasodilatory effect through two cellular mechanisms: enhancement of the plasma membrane K^+ conductance and an increase in cyclic GMP levels. These findings have stimulated our renewed interest in discovering compounds with a specific action on K^+ channels.

Recently, it has been demonstrated that three vasodilators, cromakalim (**2**), pinacidil (**3**), and RP 49356 (**4**) are more specific and potent K^+ channel openers than nicorandil (**1**).[1] Investigation of the mode of action have shown that the effects on smooth muscle of these compounds **2-4** are competitively antagonized by glibenclamide, a potent and selective blocker of ATP-sensitive K^+ channels.[1,3] This implies that these compounds may act via K^+ channels. It therefore seems reasonable to assume that the binding sites for each of these compounds are at least in part the same.

In this paper, we attempt to define a pharmacophore model of the K^+ channel openers and describe the design of new K^+ channel openers, based on computer-assisted analysis of our model, together with their synthesis and pharmacological actions.

Active 3S,4R isomer of cromakalim (**2**) (lemakalim, **2a**),[1,4] active 1R,2R isomer of RP 49356 (**4**) (RP 52891, **4a**),[1,5] and P 1060 (**7**)[1,6] were utilized for molecular modeling analysis. We used P 1060 (**7**) instead of pinacidil (**3**), because it is one of the most active of pinacidil analogs and it's activity is more active than **3** (see Table I) and it has been reported that the activity is associated with the opening of K^+ channels and blocked by glibenclamide, like **3**.[1,6]

Molecular modeling was performed using the SYBYL system.[7,8] The pharmacophore model we have developed is depicted in Figure 1. It shows four common regions; two represent areas of lipophilic interaction (L_1 and L_2) and the other two hydrogen bonding regions (H_1 and H_2) which seem to work as hydrogen bond accepting groups. The L_1 corresponds to area occupied by the aromatic rings and the L_2 to area occupied by 2,2-dimethyl, 4,5,6-trimethylene, and *tert*-butyl groups of lemakalim (**2a**), RP 52891 (**4a**), and P 1060 (**7**), respectively. The H_1 indicates region occupied by an oxygen atom of amide group, a sulfur atom of thioamide group, and a nitrogen atom of *N*-cyano group of lemakalim (**2a**), RP 52891 (**4a**), and P 1060 (**7**), respectively and the H_2 region occupied by nitrogen atoms of cyano substituent of **2a** and pyridine rings of **4a** and **7**. Thus, it is expected that functional groups or atoms of compounds occupying same region in the model may exhibit same function for the receptor. The model also shows that other groups of compounds (e.g., 3-hydroxyl group of lemakalim) may be not always critical for the activity. The dehydrated compound **8** of cromakalim (**2**) has been reported to show almost the same activity as the parent compound cromakalim.[4,9] On the other hand, it is difficult to define the function of the oxygen atom of sulfoxide of RP 52891 (**4a**) to increase the activity[5] from the model, though it is expected to be important for the interaction with the receptor such as hydrogen bonding interaction. The *N*-methyl group of RP 52891 (**4a**) and the methylene group of 4-substituent of lemakalim (**2a**) are thought to contribute to hydrophobic interaction with the receptor.[1,4,5]

The model gives us many hints to design new prototype compounds. For example, it suggests that groups or atoms of lemakalim (**2a**), RP 52891 (**4a**), and P 1060 (**7**) exhibiting same function in the model, may be mutually replaceable (Figure 1). Thus, the pyrrolidinone group of cromakalim (**2**), the thioamide group of RP 49356 (**4**), and the cyanamide group of P 1060 (**7**) may be mutually exchangeable, to lead to new prototype compounds. To confirm this assumption, the study was first undertaken with the synthesis of benzopyran derivatives with thioamide (amide) and (*N*-cyano)amidine groups at the 4-position.

Benzopyran derivatives **9-11** were designed so that they occupy all the four common regions (L_1, L_2, H_1, and H_2) of pharmacophor model (Figure 2).[8]

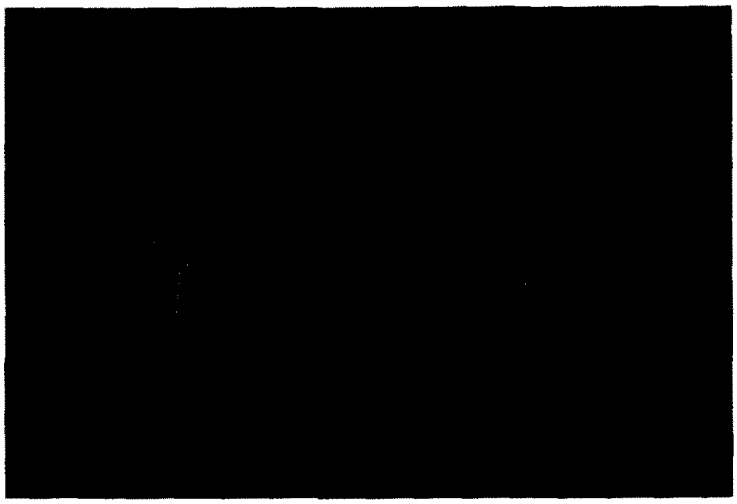


Figure 1. Stereoview of the proposed pharmacophore model of K^+ channel openers. Proposed active conformers of lemakalim (**2a**) (white), RP 52891 (**4a**) (green), and P 1060 (**7**) (magenta) are superimposed (see footnote 8 for experimental details). Hydrogen atoms are omitted for clarity except the hydrogen atom (cyan) of 3-hydroxyl group of lemakalim. Nitrogen, oxygen, and sulfur atoms are shown in blue, red, and yellow, respectively. L_1 and L_2 are areas of potential lipophilic interaction indicated by the arrows on the bottom right and left, respectively. H_1 and H_2 show regions of potential hydrogen bonding indicated by the arrows on the top left and right, respectively.

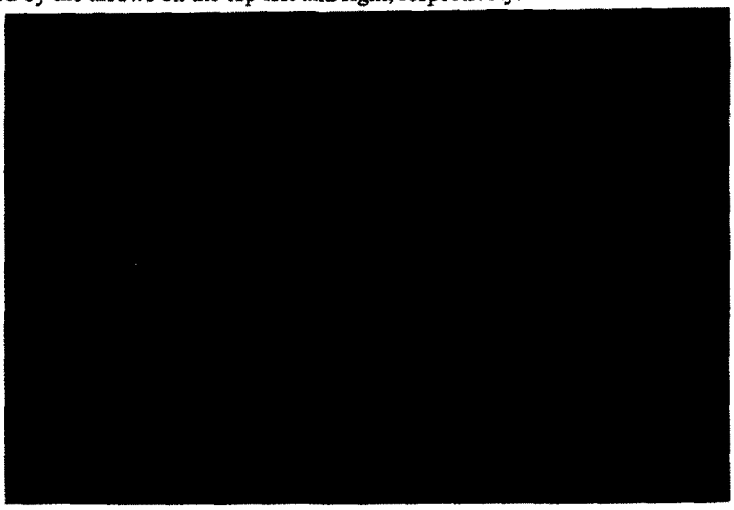


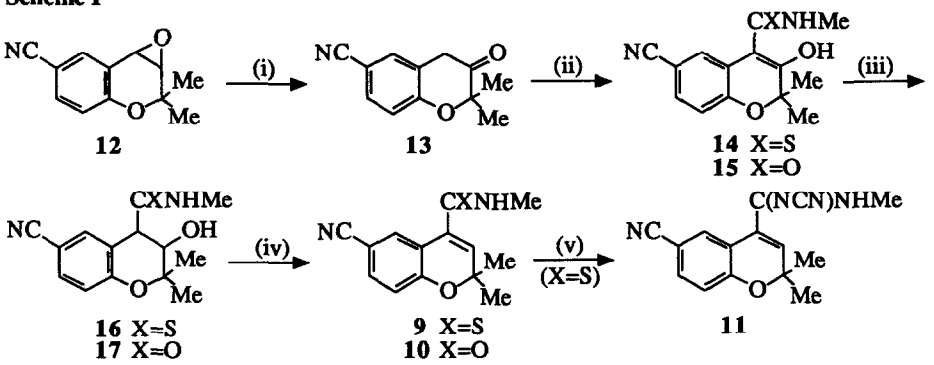
Figure 2. Stereoview of the superposition of the proposed active conformers of lemakalim (**2a**) (white), **9** (green), **10** (orange), and **11** (magenta) (see footnote 8 for experimental details). Hydrogen atoms are omitted for clarity except the hydrogen atom (cyan) of 3-hydroxyl group. Nitrogen, oxygen, and sulfur atoms are shown in blue, red, and yellow, respectively. L_1 and L_2 are shown by the arrows on the bottom right and left, respectively. H1 and H2 are shown by the arrows on the top left and right, respectively.

Convenient starting material for the synthesis of the desired benzopyran-4-carbothioamide **9**, -carboxamide **10**, and -(*N*-cyano)carboxamidine **11** (Scheme I and Table I) is 6-cyano-3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**12**), the synthetic method of which has been described before.[4] Thus, treatment of the epoxide **12** with *p*-toluenesulfonic acid gave the benzopyran-3-one **13** (mp 114-115 °C, 71%). Enolization of **13** and the subsequent acylation with methyl isothiocyanate or methyl isocyanate afforded the thioamide **14** (mp 189-190 °C, 66%) or amide **15** (mp 200-201 °C, 58%). Reduction of the β-keto thioamide **14** and amide **15** with sodium borohydride in methanol gave the β-

hydroxy thioamide **16** (63%) and amide **17** (33%) as a mixture of cis and trans isomers which could be separated by chromatography on silica. The mixtures **16** and **17** were dehydrated by heating with *p*-toluenesulfonic acid in toluene to provide the corresponding thioamide **9** (mp 141-142 °C, 44%) and amide **10** (mp 198-199 °C, 44%). The thioamide **9** was activated by *p*-toluenesulfonyl chloride followed by the addition of cyanamide and sodium hydride in tetrahydrofuran to afford the corresponding (*N*-cyano)amidine **11** (mp 261-263 °C, 15%).

After our study was almost completed,[10] other workers have reported on the synthesis and biological evaluation of thioamide derivative **9**.[11]

Scheme I [a]



[a] Reagent: (i) *p*-TSA, PhMe, reflux; (ii) KO*t*-Bu or NaH, MeNCX (X=S, O), DMF, 0 °C; (iii) $NaBH_4$, MeOH; (iv) *p* -TSA, PhMe, reflux; (v) *p*-TsCl, Et_3N/NH_2CN, NaH, THF

Table I. Smooth Muscle Relaxant Activity

	rat aorta		
Compd.	pEC_{50} [a]	IA (%) [b]	n [c]
9	7.61±0.07	89.0±2.5	4
10	6.58±0.03	66.6±5.5	3
11	6.47±0.02	73.0±2.3	3
cromakalim (**2**)	6.77±0.03	74.7±2.1	25
pinacidil (**3**)	6.14±0.03	91.9±2.5	5
RP 49356 (**4**)	6.28±0.04	79.7±2.2	6
P 1060 (**7**)	7.04±0.02	88.0±2.9	3

[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 15 for experimental details. [b] Intrinsic activity ± SEM (%). [c] Number of determinations.

The smooth muscle relaxant activities of compounds were determined by the effects on 30 mM KCl responses in rat isolated aorta and are listed in Table I in comparison with **2-4** and **7**.

Evaluation of **9** confirmed that it is highly active (Table I). Compound **9** also showed higher activity when compared with **2, 4,** and **7** which were used to construct the pharmacophore model (Table I). These may suggest that **9** is more favorable to interact with the receptor than these K^+ channel openers **2, 4,** and **7** and that the pharmacophore model is very useful for designing new K^+ channel opener.

Replacement of the 4-thioamide group of compound **9** by an amide group to give compound **10** reduced potency. This situation may be explained by the difference in the interaction at the H_1 site with the receptor between sulfur and oxygen atoms, possibly due to the difference in the van der Waals volumes of these atoms since the conformations of these compounds were almost identical (Figure 2).[8,12]

Benzopyran-4-(*N*-cyano)carboxamidine **11** is regarded as a hybrid compound of cromakalim (**2**), RP 49356 (**4**), and P 1060 (**7**) (Figure 2).[13] Biological evaluation of **11** showed that it possesses a similar smooth muscle relaxant activity to those of **2, 4, 7,** and the corresponding amide **10** and is significantly less active than the corresponding thioamide **9** (Table I). This is in contrast with the result obtained in pinacidil derivatives in which cyanoguanidines were generally more potent than the corresponding thioureas and ureas.[6,14] The difference in the activity between **11** and **9** may be also explained by the difference in the interaction at the H_1 site between the cyano or imino nitrogen of cyanoimino group and the sulfur atom (Figure 2).[12]

In summary, we have constructed a first pharmacophore model which explains rationally structure-activity relationships of chemically diverse potent K^+ channel openers, and designed potent benzopyran derivatives with thioamide (amide) and (*N*-cyano)amidine groups at the 4-position using the pharmacophore model. Thus, the pharmacophore model we have developed seems to be very useful for design of new K^+ channel opener.

References and Footnotes

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7. SYBYL Molecular Modeling System; Tripos Associates: St. Louis.
8. The molecular modeling was carried out on an Evans & Sutherland ESV 3/33 graphic workstation with SYBYL version 5.5.[7] The crystallographic structure of lemakalim [Cassidy, F.; Evans, J. M.; Smith, D. M.; Stemp, G.; Edge, C.: Williams, D. J. *J. Chem. Soc. Chem. Commun.* **1989**, 377-378] was used as the initial conformation and optimized by using an AMl program (MOPAC version 6.0) [Stewart, J. P. *J. Comp.-Aided Mol. Design* **1990**, *4*, 1-103] deviced within Insight II molecular modeling system

(BIOSYM Technologies, San Diego). This conformation was arbitrarily employed as an active conformation of lemakalim because it was the most stable conformation found in the crystalline state, the minimum-energy conformation by molecular modeling study, and a major conformation in solution [Cassidy, F.; Evans, J. M.; Smith, D. M.; Stemp, G.; Edge, C.; Williams, D. J. *J. Chem. Soc. Chem. Commun.* **1989**, 377-378: Thomas, W. A.; Whitcombe, I. W. A. *J. Chem. Soc. Chem. Commun.* **1990**, 528-529]. The initial molecular models for RP 52891, **9**, **10**, and **11** were built with the SYBYL standard fragment library. Conformations were examined by the RANDOM SEARCH routine (default setting) in the SYBYL system with Tripos force field [Clark, M.; Cramer III, R. D.; Van Opdenbosh, N. *J. Comp. Chem.* **1989**, *10*, 982-1012] and the low energy conformers were optimized by using the AMl program. The initial model for P 1060 was constructed by using the Insight II and the conformational analysis was carried out with GRID search option of Insight II/Discover system with CVFF force field [Dauber-Osguthorpe, P.; Roberts, V. A.; Osguthorpe, D. J.; Wolff, J.; Genest, M.; Hagler, A. T. *Proteins: Struct. Funct. Genetics* **1988**, *4*, 31-47]. All rotatable bonds were rotated with 60° increment and each conformation was optimized. No constraints were used. The minimum-energy conformation was optimized by the AMl method. The minimum-energy conformations for compounds were superposed on the proposed active conformation of lemakalim and the conformers best matched with lemakalim were selected as active conformers. Thus RP 52891 was superposed on lemakalim by matching the 6-cyano nitrogen, the amide oxygen, the 4-carbon, and the 2,2-dimethyl carbons of lemakalim, with the pyridine nitrogen, the thioamide sulfur, the 2-carbon, and the 4,6-carbons of RP 52891, respectively (Figure 1). P 1060 was also superposed on lemakalim by matching the 6-cyano nitrogen, the amide oxygen, the 4-carbon, and the 2-carbon of lemakalim, with the pyridine nitrogen, the *N*-cyano nitrogen, the guanidino carbon, and the *tert*-butyl quaternary carbon of P 1060, respectively (Figure 1). Compounds **9**, **10**, and **11** were superposed on lemakalim by matching the corresponding carbon atoms of benzene rings of each molecule (Figure 2). These selected active conformers were also the lowest energy conformers. In Figure 1, the distances between the amide oxygen of lemakalim and the thioamide sulfur of RP 52891, the amide oxygen of lemakalim and the *N*-cyano nitrogen of P 1060, and the thioamide sulfur of RP 52891 and the *N*-cyano nitrogen of P 1060 are estimated to be 1.3, 0.7, and 1.7 Å, respectively. The distances between the 6-cyano nitrogen of lemakalim and the pyridine nitrogen of RP 52891, the 6-cyano nitrogen of lemakalim and the pyridine nitrogen of P 1060, and the pyridine nitrogen of RP 52891 and that of P 1060 are also estimated to be 2.2, 2.2, and 1.3 Å, respectively.

9. Attwood, M. R.; Jones, P. S.; Kay, P. B.; Paciorek, P. M.; Redshaw, S. *Life Sciences* **1991**, *48*, 803-810.

10. After our study was almost completed (patent publication number: WO 92/02514, priority date: 27 July 1990), similar compounds have appeared in the literature (see reference 11).

11. (a) Patent publication number: WO 90/14346 (publication date: 29 Nov 1990). (b) Arch, J. R. S.; Buckle, D. R.; Carey, C.; Parr-Dobrzanski, H.; Faller, A.; Foster, K. A.; Houge-Frydrych, C. S. V.; Pinto, I. L.; Smith, D. G.; Taylor, S. G. *J. Med. Chem.* **1991**, *34*, 2588-2594.

12. In Figure 2, the distances between the amide oxygen of lemakalim and the thioamide sulfur atom of **9**, the amide oxygen of **10**, the *N*-cyano nitrogen of **11**, and the imino nitrogen of **11** are estimated to be 0.4, 0.7, 2.1, and 0.8 Å, respectively and those between the thioamide sulfur of **9** and the amide oxygen of **10**, the *N*-cyano nitrogen of **11**, and the imino nitrogen of **11** to be 0.5, 2.4, and 0.6 Å, respectively.

13. Recently, benzopyran-4-(*N*-cyano)guanidine derivatives have been designed as hybrid compounds of cromakalim and pinacidil [Atwal, K. S.; Moreland, S.; McCullough, J. R.; Ahmed, S. Z.; Normandin, D. E. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 87-90].

14. Manley, P. W.; Quast, U. *J. Med. Chem.* **1992**, *35*, 2327-2340.

15. 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.