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THE DISCOVERY OF NOVEL OPENERS OF Ca^{2+}-DEPENDENT LARGE-CONDUCTANCE POTASSIUM CHANNELS: PHARMACOPHORE SEARCH AND PHYSIOLOGICAL EVALUATION OF FLAVONOIDS

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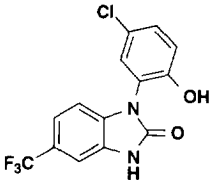
Abstract. Three-dimensional database searching based on the pharmacophore model of the known maxi-K opener NS-004 (**1**) and electrophysiological evaluation led to the discovery of flavonoids (**3c** and **3d**) as openers of the cloned maxi-K channel *m*Slo expressed in *Xenopus laevis* oocytes. The cyclic constrained flavonoids were found to be more efficacious than the acyclic phloretin. © 1997 Bristol-Myers Squibb. Published by Elsevier Science Ltd.

Ca^{2+}-Dependent, large conductance potassium channels (also called maxi-K or BK channels) are distributed in many cell types including neurons and muscle cells, and are thought to play important roles in cellular excitation and function.[4,5] These channels, acted upon by a specific modulator, are potential therapeutic targets for a number of disease states.[6,7] The recent discovery that the benzimidazolone derivative NS004 (**1**)[8-11] is a maxi-K opener has prompted a search for more efficacious and selective openers for maxi-K channels. Since then, several groups have reported new classes of maxi-K openers,[12-16] which have been reviewed.[6,7] We report here the successful identification of novel maxi-K openers using a 3-D pharmacophore model and the results of subsequent electrophysiological evaluation.



1, NS-004

2

Pharmacophore Model and Database Search

On the basis of NS-004 and the structure-activity relationships revealed previously by Meanwell and coworkers,[12] the phenolic OH and carbonyl oxygen were thought to be a surrogate of carboxylic acid. The fact that **1** and **2** are equally effective indicates some flexibility of these bioisosteres.[12] Alternatively, the weakly acidic amide hydrogen and the amide carbonyl oxygen were postulated to surrogate a carboxylic acid and were critical for the maxi-K activity. This seems to be reasonable since imidazolone has been suggested to mimic a phosphate.[17-19] Furthermore, electron-withdrawing groups attached to the heterocyclic nucleus in **1** and **2**,

which increase the acidity of the amide hydrogen, were found to be essential for maxi-K opening properties.[12] Thus, a 3-D query shown in Figure 1 was constructed using pharmacophore involving a carbonyl oxygen as H-bond acceptor, an H-bond donor, and an aromatic group located with a ranging distance to the amide carbonyl oxygen. The orientation of the H-bond donor and acceptor was further constrained by setting the torsional angle HX···C=O to be ±30°. Structural searches were carried out using the 3-D Cambridge Structural Database System (CSDS) which contained more than 120,000 X-ray crystallographically determined structures.[20]

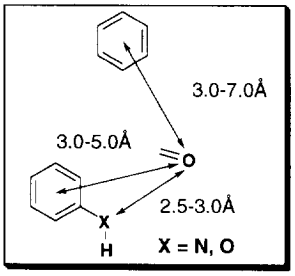


Figure 1. 3-D query used in the 3-D CSDS database search.

Results and Discussion

For electrophysiological evaluation of maxi-K opening properties, compounds were tested using two-electrode voltage clamp recording from *Xenopus laevis* oocytes injected with *m*Slo[21] mRNA, as previously described.[22] Voltage-clamp protocols ranged from a holding potential of -60 mV to a maximal potential of +140 mV with +20 mV increments. The maxi-K current, defined as the iberiotoxin-sensitive component of total outward current, was measured in the absence or in the presence 20 μM of drug. The increase in outward maxi-K current in the presence of drug is reported as percent of drug free control, for a voltage step to +140 mV, and the data are an average of experiments conducted in at least 5 different oocytes.

Because the throughput of electrophysiological evaluation is relatively low, we had to limit ourselves to only a few select compounds. A search using the query shown in Figure 1 resulted in some 300 hits, represented by a fewer number of chemotypes. Intuitively, the hits, shown in Figure 2 with corresponding refcode, were found to be intriguing, manifested by an overlay of KAMJUD01 with **1** (NS-004). It is known that ketone-phenol moiety of the flavone quercetin, as noted by Kubo and coworkers, could mimic the carboxylic acid of L-DOPA.[23] Therefore, several analogous compounds available from our compound library were selected for electrophysiological evaluation.

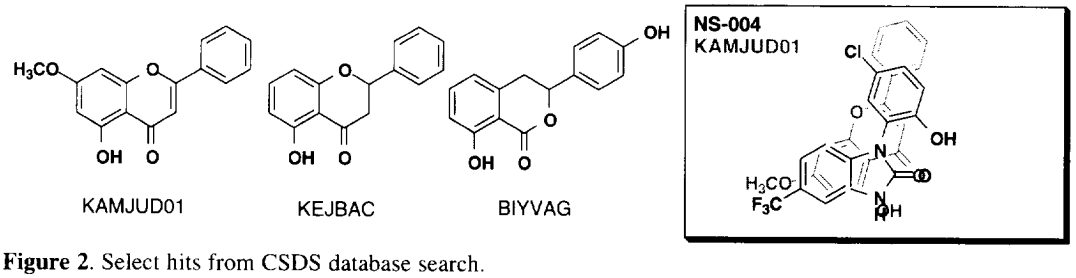
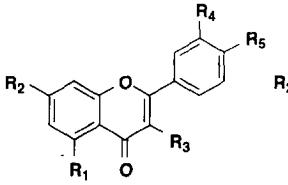
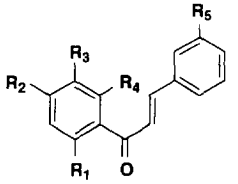


Figure 2. Select hits from CSDS database search.

Table
Structure and cloned maxi-K channel opening properties of flavonoids



3



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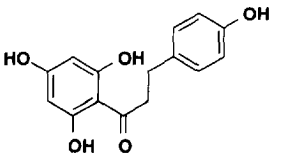

5

Cmpd. #	**R1**	**R2**	**R3**	**R4**	**R5**	Outward current in the presence of test compound (20 μM) as % of control current
1 (NS-004)						131.8 ± 12.8
3a	H	H	H	H	H	102.7 ± 4.1
3b	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	94.6 ± 2.5
3c	OH	OH	H	H	OH	158.7 ± 6.0
3d	OH	OH	OH	H	OH	167.4 ± 10.1
4a	H	H	H	H	H	82.4 ± 4.0 *
4b	OH	H	NHAc	H	H	81.5 ± 2.4 *
4c	OH	H	H	H	NO_2	74.4 ± 2.3 *
5						119.9 ± 4.1

*See reference 24.

Compounds **3c-d** were found to be effective maxi-K openers, with the outward current increase 159% and 167%, respectively, of control current. For a comparison, NS-004 increases the outward maxi-K current by 132%. The fact that without an OH group at the R_1 position compounds **3a-b** and **4a** are inactive is a reminiscent of our pharmacophore hypothesis. The acyclic **4b-c**, however, are all also inactive. During the course of our studies, phloretin **5** was reported[13] to be an opener of axonal Ca^{2+}-activated K channels in amphibian peripheral nerve. We confirmed that **5** is indeed a maxi-K opener, but somewhat less efficacious than the cyclic flavonoids. It is apparent from **4b-c** and **5** that meta-OH substitution at the R_2 position is important. In contrast to the benzimidazolone series, π-electron-donating OH substitution at R_2/R_4 is not devoid of the maxi-K opening activity. Presumably, this is due to the fact that the OH substituent effect is predominated by its *meta*-σ inductive effect, and the fact that OH of R_1 is more acidic than an amide NH.

In summary, we demonstrated the use of a simple pharmacophore model and 3-D database search of the maxi-K channel openers. In spite of their promiscuous pharmacological effects, several flavonoids were identified to be effective openers of maxi-K channels.

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