Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1161–1164

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Benzamide Derivatives as Blockers of Kv1.3 Ion Channel

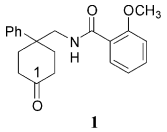
Shouwu Miao,[a,*] Jianming Bao,[a,*] Maria L. Garcia,[b] Joung L. Goulet,[a] Xingfang J. Hong,[a] Gregory J. Kaczorowski,[b] Frank Kayser,[a] Gloria C. Koo,[c] Andrew Kotliar,[a] William A. Schmalhofer,[b] Kashmira Shah,[c] Peter J. Sinclair,[a] Robert S. Slaughter,[b] Marty S. Springer,[c] Mary Jo Staruch,[c] Nancy N. Tsou,[a] Frederick Wong,[a] William H. Parsons[a] and Kathleen M. Rupprecht[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Ion Channels, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[c]*Department of Immunology Research, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*

Received 22 July 2002; accepted 10 December 2002

Abstract—The voltage-gated potassium channel, Kv1.3, is present in human T-lymphocytes. Blockade of Kv1.3 results in T-cell depolarization, inhibition of T-cell activation, and attenuation of immune responses in vivo. A class of benzamide Kv1.3 channel inhibitors has been identified. The structure–activity relationship within this class of compounds in two functional assays, Rb_Kv and T-cell proliferation, is presented. In in vitro assays, *trans* isomers display moderate selectivity for binding to Kv1.3 over other Kv1.x channels present in human brain.

Kv1.3 is a voltage-gated potassium channel that sets the resting potential of human T-cells.[1–3] Blockade of the channel causes membrane depolarization,[4] which attenuates intracellular Ca^{2+} levels required for lymphocyte activation upon T-cell stimulation,[5] and inhibits immune responses in vivo. These findings suggest that Kv1.3 represents a novel target for the development of immunosuppressants. Researchers in the pharmaceutical industry and academia have been seeking potent and selective inhibitors of the Kv1.3 channel that will have efficacy in organ transplantation and other immunologic illnesses.[6–12]



1

Rb_Kv IC_{50} = 200 nM
T-cell (anti CD3) IC_{50} = 1200 nM

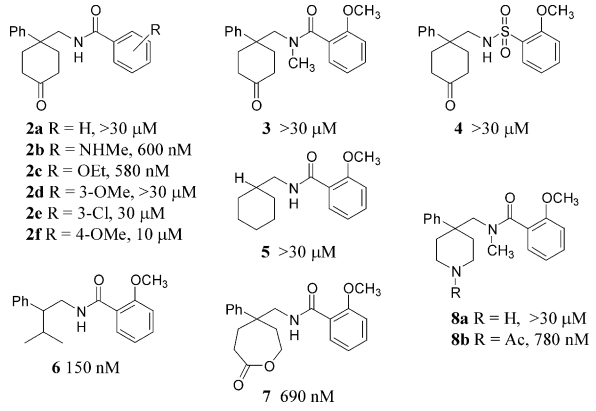
Previously, we have reported that benzamide **1** was a potent Kv1.3 channel inhibitor.[10] SARs at the 2-methoxyphenyl ring were explored and no improvements were found. A few carbamates of C1 alcohol of **1** were also reported. Some of them have improved Kv1.3 activities over **1**. These benzamides are remarkably simple and are selective against the Kv1 family of potassium channels. More interestingly, the *trans* C1 carbamates displayed a modest selectivity between the Kv1.3 and the Kv1.x channels in human brain based on the rate of C-type inactivation of these channels.[10] These promising results have led us to the further exploration of this type of structure. Now, we wish to give a more extensive account of this work.

A survey of SAR around the lead **1** was carried out. A few selected compounds are presented in Scheme 1. While the Kv1.3 activity can be maintained by changes at the 2 position of the 2-methoxy phenyl ring (**2b** and **2c**), substitutions at the 3 or 4 position only resulted in loss of activity (**2d**–**2f**). Methylation of the amide NH or replacement of the amide carbonyl with a sulfonyl group significantly reduced the Kv1.3 activity as exemplified by the amide **3** and the sulfonamide **4**. Removal of the phenyl group also eliminated the activity (**5**). However, the cyclohexyl ring can be modified without a loss of Kv1.3 activity (**6** and **7**). With proper N1 substitutions, a piperidine ring is also tolerated (**8a** vs **8b**). Given these results, efforts were later focused on the C1

*Corresponding author. Fax: +1-732-594-5350; e-mail: bao_jianming@merck.com

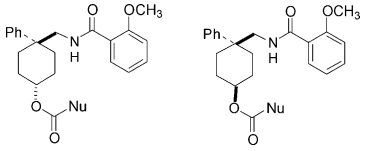
0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00014-3

position of the cyclohexanone ring for SAR studies. A modification at this position would be conformationally distinct and the position of new groups would be defined by the relative stereochemistry at C1. Analogues at the C1 position would also have a plane of symmetry.



Scheme 1. Rb_Kv IC_{50}'s of some benzamides.

Table 1. Rb_Kv and T-cell assay results (IC_{50}, nM) for carbamates of C1 alcohols[a]



Compd	Nu	C1	Rb_Kv	T-cell (anti-CD3)
18	NHMe	*trans*	58	195
19	NH*i*Pr	—	75	222
20	NH*n*Pr	—	50	273
21	NHallyl	—	70	361
22	N(Me)allyl	—	320	—
23	NH(2-Me-allyl)	—	90	688
24	NH*n*Bu	—	90	688
25	$NH(CH_2)_2OH$	—	310	1793
26	$NH(CH_2)_2OMe$	—	140	1000
27	$NH(CH_2)_3OMe$	—	145	—
28	NHPh	—	>30 μM	—
29	NHBn	—	1.7 μM	—
30	$N(allyl)_2$	—	840	—
31	NH_2	*cis*	120	519
32	NHMe	—	150	1255
33	NH*i*Pr	—	130	892
34	NH*n*Pr	—	100	—
35	NHallyl	—	130	1099
36	N(Me)allyl	—	145	659
37	NH(2-Me-allyl)	—	120	—
38	NHnBu	—	140	320
39	$NH(CH_2)_2OH$	—	95	—
40	$NH(CH_2)_2OMe$	—	140	—
41	$NH(CH_2)_3OMe$	—	130	—
42	NHPh	—	120	237
43	NHBn	—	245	915
44	$NH(CH_2)_2Ph$	—	420	—
45	$NH(CH_2)_3Ph$	—	535	—
46	$N(allyl)_2$	—	205	—
47	$OCH_2CH=CH_2$	—	230	—

[a]Values are means of three experiments.

Compounds in Tables 1 and 2 were synthesized according to Scheme 2. Ketone **9** was reduced by $NaBH_4$ in THF at low temperature to give exclusively **10** (by ^1H NMR), which was further reduced by LAH to afford **11**.[13] Compound **11** was converted to the *cis* alcohol **12** by a selective *N*-acylation with 2-methoxy benzoyl chloride.

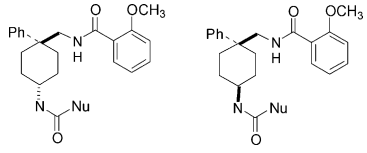
The *trans* alcohol **16** was prepared from **11**. The amino group of **11** was protected with a Boc group to give **14**. The C1 hydroxy group of **14** was inverted under Mitsunobu conditions to give ester **15**. Solvolysis of **15**, removal of the Boc protection group, and selective acylation afforded the alcohol **16**. The relative stereochemistry of **16** was established by X-ray crystallography.

Amines **13** and **17** were prepared from alcohols **12** and **16**, respectively, by a three-step sequence: mesylation of the hydroxy group with MsCl, displacement of the mesylate with azide and reduction of the azide with H_2 and Pd catalyst.

Compounds **12**, **13**, **16**, and **17** were converted to their *p*-nitrophenylcarbonates, which were reacted with different nucleophiles to afford the analogues in Tables 1 and 2.

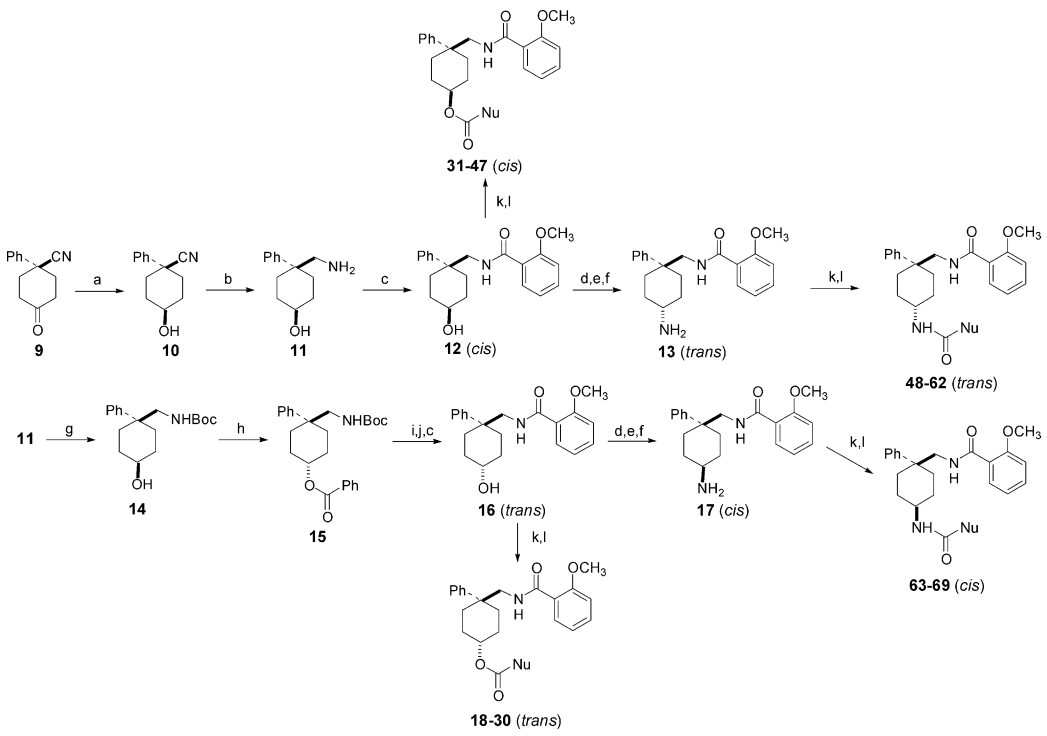
The Kv1.3 activity of these compounds was measured in the Rb_Kv assay, which measures $^{86}Rb^+$ efflux from CHO cells stably transfected with the Kv1.3 channel.[9,10]

Table 2. Rb_Kv and T-cell assay results (IC_{50}, nM) for analogues of C1 amines[a]



Compd	Nu	C1	Rb_Kv	T-cell (anti-CD3)
48	OMe	*trans*	135	1057
49	Oallyl	—	242	539
50	Sme	—	150	618
51	NH(allyl)	—	150	676
52	2-Br–Ph	—	2790	—
53	3-Br–Ph	—	>30 uM	—
54	4-Br–Ph	—	>30 uM	—
55	Me	—	470	—
56	Et	—	210	—
57	*n*Pr	—	200	—
58	$CH=C(Me)_2$	—	470	—
59	CH=CHMe	—	440	—
60	Ph	—	1560	—
61	Bn	—	1045	—
62	Phenethyl	—	570	—
63	Oallyl	*cis*	90	354
64	NH(allyl)	—	140	430
65	N(Me)(allyl)	—	195	—
66	CH=CHMe	—	100	399
67	Ph	—	75	154
68	Bn	—	130	609
69	Phenethyl	—	120	200

[a]Values are means of three experiments.



Scheme 2. (a) $NaBH_4$, THF, −78 °C, 100%; (b) $LiAlH_4$, THF, reflux; (c) 2-methoxybenzoyl chloride, Et_3N, rt, 86% for **12**, two steps; 99% for **16**; (d) MsCl, Et_3N, CH_2Cl_2; (e) NaN_3, DMF; (f) H_2, Pd/C, 87% for **13**; 95% for **17**; three steps; (g) $(Boc)_2O$, Et_3N, 70%; (h) $PhCO_2H$, PPh_3, DEAD, 72%; (i) $NaOCH_3$, CH_3OH/THF, rt; (j) HCl, 98%, two steps; (k) 4-nitrophenyl chloroformate, DMAP, CH_2Cl_2; (l) nucleophile, CH_2Cl_2.

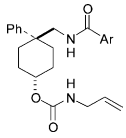
Potent Kv1.3 compounds were evaluated in the human T-cell assay.[11]

The stereochemistry at C1 is important for the Rb_Kv activity. Among carbamates prepared from the alcohols **12** and **16** (Table 1),[14] Kv1.3 activity of the *cis* derivatives (**31**–**47**) is insensitive to the change of the C1 side chain, and is in a narrow range. In contrast, there is a well-defined structure–activity relationship in the *trans* series (**18**–**30**). A wide range of Rb_Kv activities was observed in these compounds. An increase in the side chain bulkiness causes a progressive decrease in the inhibitory activity of these *trans*-isomers. Compounds prepared from small primary amines potently block the Kv1.3 channel (**18**–**21** and **24**). Carbamates from amines with aromatic groups have reduced Rb_Kv activities (**28** and **29**). Side chains with an oxygen atom lead to compounds with good Rb_Kv activities, but reduced T-cell potencies (**25** and **26**). The discrepancy between the Rb_Kv values and the corresponding T-cell values may be explained by the fact that T-cell proliferation is a distal effect of the consequence of blocking Kv1.3 channels. The T-cell activity of a compound is defined as the effect of the compound on the Ca-dependent pathway of T-cell activation. Therefore, the T-cell potency of a blocker depends on the magnitude of the stimulus, the donor variability, the effect of the compound on other T-cell activation pathways, as well as, its potency on Kv1.3. Major improvements in both the Rb_Kv and the T-cell activities were observed in compounds with small alkyl side chains. Compounds **18**–**21** are among the most potent analogues in both the Rb_Kv and the T-cell proliferation assays.

Compounds prepared from amines **13** and **17** are presented in Table 2. Like compounds in Table 1, the stereochemistry at the C1 position dictates the SAR of these compounds. Analogues from the *cis* amine **17** are generally potent and insensitive to the steric bulkiness of the side chain, while the *trans* compounds display an SAR that correlates with the size of the side chains. Compound **67** is the most potent compound in inhibiting T-cell proliferation and it is 8-fold more potent than the lead (**1**) in the T-cell assay.

With SARs of the C1 position in hand, an effort was also devoted to modification of the 2-methoxyphenyl ring to gain additional potency (Table 3). Introduction of an N atom in the ring resulted in a decrease in the Kv1.3 activity (**70**). Adding a Cl *ortho* to the methoxy caused a sharp decrease in the potency (**71**). However,

Table 3. Modifications at benzylamide



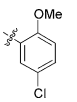
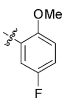
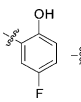
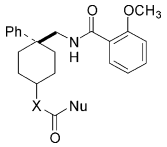
Compd	**70**	**71**	**72**	**73**	**74**	**75**	**76**
Ar							
Rb_Kv IC_{50} (nM)	325	8380	7200	230	45	140	3730

Table 4. Inhibition of binding of DiTc to Kv1.3 and Kv1.x channels in human brain membranes (IC_{50}, nM)[a]



Compd	X	Nu	Cl	Kv1.3	Kv1.x
18	O	NHMe	*trans*	5	33
20	—	NH*n*Pr	—	5	30
21	—	NHallyl	—	18	112
32	—	NHMe	*cis*	99	104
34	—	NH*n*Pr	—	79	76
35	—	NHallyl	—	140	170
51	NH	NHallyl	*trans*	29	120
63	—	Oallyl	*cis*	72	68
64	—	NHallyl	—	80	125

[a]Values are means of three experiments.

addition of either a Cl or an F atom *para* to the methoxy group (**73** and **74**), or demethylation of the 2-methoxy group (**75**), has a minimal effect on the activity. All of these modifications decreased the Rb_Kv activity except **74**, where a small increase in potency was observed.

To test the selectivity of these benzamides against other channels in the Kv1 family, the effect of these compounds on diTC (C20–C29, ditritio-correolide) binding to membranes derived either from cells expressing the homomultimeric Kv1.3 channel or from human brain tissue (Kv1.x, primarily heteromultimeric Kv1.1/Kv1.2 channels) was determined (Table 4).[9] The data indicate that the *cis* derivatives do not display selectivity as inhibitors of diTC binding to Kv1.x channels. However, the *trans* isomers are more potent inhibitors of diTC binding to Kv1.3 than to the heteromultimeric Kv1.x channels in brain. This modest selectivity may be due to conformations unique to Kv1.3, which appear to be related to the C-type inactivation property of the Kv1.3 channel.[10,15] C-type inactivation is a process that involves conformational changes at the outer mouth of the channel. These conformational changes constrict the pore and cause a blockade of the ion conduction pathway.[16] This unique interaction of the *trans* benzamides with conformations of the Kv1.3 channel related to C-type of interaction may allow development of selective Kv1.3 blockers as immunosuppressants.

In summary, starting from the lead **1**, a series of benzamide Kv1.3 channel inhibitors has been prepared. Some of these compounds show improved potencies in both the Rb_Kv and the T-cell assays. Compounds that are 8-fold more potent in the T-cell assay and up to 4-fold more potent in the Rb_Kv assay, respectively, than lead **1** were identified. While the activity of the *cis* analogues is not sensitive to the nature of the C1 substituents, the corresponding *trans*-isomers display a wide range of activities. The *trans*-isomers also display moderate selectivity towards Kv1.3 over other Kv1.x channels in the in vitro binding assays. These data suggest that the selective interaction of the *trans*-isomers with Kv1.3 could be exploited for the development of inhibitors of this channel for the treatment of immunological disorders with a limited side effect profile.

References and Notes

1. Kaczorowski, G. J.; Koo, G. C. *Perspect. Drug Discov. Des.* **1994**, *2*, 233.
2. Lewis, R. S.; Cahalan, M. D. *Annu. Rev. Immunol.* **1995**, *13*, 623.
3. Cahalan, M. D.; Chandy, K. G. *Curr. Opin. Biotechnol.* **1997**, *8*, 749.
4. Leonard, R. J.; Garcia, M. L.; Slaughter, R. S.; Reuben, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10094.
5. Lin, C. S.; Boltz, R. C.; Blake, J. T.; Nguyen, M.; Talento, A.; Fischer, P. A.; Springer, M. S.; Sigal, N. H.; Slaughter, R. S.; Garcia, M. L.; Kaczorowski, G. J.; Koo, G. C. *J. Exp. Med.* **1993**, *177*, 637.
6. Hill, R. J.; Grant, A. M.; Volberg, W.; Faltynek, C.; Miller, D.; Pagani, K.; Baizman, E.; Wang, S.; Guiles, J. W.; Krafte, D. S. *Mol. Pharm.* **1995**, *48*, 98.
7. Nguyen, A.; Kath, J. C.; Hanson, D. C.; Biggers, M. S.; Canniff, P. C.; Donovan, C. B.; Mather, R. J.; Rauer, H.; Aiyar, J.; Lepple-Wienhues, A.; Gutman, G. A.; Grissmer, S.; Cahalan, M. D.; Candy, K. G. *Mol. Pharm.* **1996**, *50*, 1672.
8. Burgess, L. E.; Koch, K.; Cooper, K.; Biggers, M. S.; Ramchandani, M.; Smitrovich, J. H.; Gilbert, E. J.; Bruns, M. J.; Mather, R. J.; Donovan, C. B.; Hanson, D. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1047.
9. Felix, J. P.; Bugianesi, R. M.; Schmalhofer, Borris, Robert. B.; Goetz, M. A.; Hensens, O. D.; Bao, J.; Kayser, F.; Parsons, W. H.; Rupprecht, K.; Garcia, M. L.; Kaczorowski, G. J.; Slaughter, R. S. *Biochemistry* **1999**, *38*, 4922.
10. Schmalhofer, W. A.; Bao, J.; McManus, O. B.; Green, B.; Matyskiela, M.; Wunderler, D.; Bugianesi, R. M.; Felix, J. P.; Hanner, M.; Linde-Arias, A.; Ponte, C. G.; Velasco, L.; Koo, G.; Staruch, M. J.; Miao, S.; Parsons, W.; Rupprecht, K.; Slaughter, R. S.; Kaczorowski, G. J.; Garcia, M. L. *Biochemistry* **2002**, *41*, 7781.
11. Koo, G. C.; Blake, J. T.; Shah, K.; Staruch, M. J.; Dumont, F.; Wunderler, D.; Sanchez, M.; McManus, O. B.; Fischer, P.; Boltz, R. C.; Goetz, M. A.; Baker, R.; Bao, J.; Kayser, F.; Rupprecht, K. M.; Parsons, W. H.; Tong, X. C.; Ita, I. E.; Pivnichny, J.; Vincent, S.; Cunningham, P.; Hora, D., Jr.; Kaczorowski, G. J.; Springer, M. S. *Cell. Immunol.* **1999**, *197*, 99.
12. Lew, A.; Chamberlin, A. R. *Bioorg. Med. Chem. Lett.* **1999**, *23*, 3267.
13. Ketone **9** is commercially available from Acros. When ketone **9** was directly reduced to **11** by LAH in THF at reflux, a ratio of 11 to 1 was obtained.
14. Compounds **18**, **20–21**, **25–26**, **28–29**, **30–32**, **34–35**, **39–40**, **42–43**, and **46** have been previously disclosed in ref 10.
15. Vianna-Jorge, R.; Oliveira, C. F.; Garcia, M. L.; Kaczorowski, G. J.; Suarez-Kurtz, G. *Br. J. Pharmacol.* **2000**, *131*, 772.
16. Liu, Y.; Jurman, M. E.; Yellen, G. *Neuron* **1996**, *16*, 859.