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3-Alkyl- and 3-aryl-7*H*-furo[3,2-*g*]chromen-7-ones as blockers of the voltage-gated potassium channel Kv1.3

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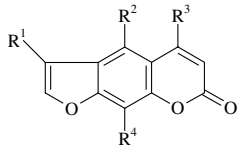
3-Alkyl- and 3-aryl-7*H*-furo[3,2-g]chromen-7-ones were synthesised and studied for their effects on voltage-gated K^+ and Na^+ channels of the neuroblastoma cell-line N1E-115 and K^+ channels of L-929 mouse-fibroblasts, stably transfected with *m*Kv1.3. All furocoumarins tested showed Kv channel blocking activities, the most potent one in a half-blocking concentration of 0.7 μM.

There is increasing evidence that psoriasis, a chronic, hyperproliferative and inflammatory skin disorder, has a T cell-driven immunopathogenesis. Advances in psoriasis treatment are based on the key role of T cells in disease pathogenesis (Griffiths 2003; Prinz 2003).
7*H*-Furo[3,2-*g*]chromen-7-ones (psoralens, furocoumarins), like 5-methoxypsoralen (5-MOP, 4-methoxy-7*H*-furo[3,2-*g*]chromen-7-one) and 8-methoxypsoralen (8-MOP, 9-methoxy-7*H*-furo[3,2-*g*]chromen-7-one), are successfully used in PUVA therapy (psoralen plus UVA-irradiation) as photoreactive drugs in the treatment of various skin diseases such as psoriasis, mycosis fungoides, vitiligo (Averbeck 1989) and in photopheresis, an immunomodulatory therapy effectively used to treat several T cell-mediated diseases (Edelson 1989). The biological activity of furocoumarins has been correlated to their ability to photoreact with DNA (Averbeck 1989). This DNA photodamage is the reason for some of the desired antiproliferative effects but also for the undesired side-effects such as carcinogenicity, genotoxicity and the formation of skin erythemas (Averbeck 1989). It has been reported that furocoumarins also react with proteins (Schmitt et al. 1995) and unsaturated fatty acids (Caffieri et al. 1994), either by direct photoaddition or by generation of singlet oxygen (Dall'Acqua et al. 1991). They also show a biological activity without the usage of UVA light, so-called dark effects (Midden 1988; Wulff et al. 1998; Körner 2003).
4-Alkoxy-7*H*-furo[3,2-*g*]chromen-7-ones were shown to be blockers of a number of voltage-gated K^+ channels, which play an important role in the regulation of physiological functions in excitable as well as non-excitable cells (Wulff et al. 1998). Those tested compounds had high affinities for the *Shaker*-related K^+ channel Kv1.3, which is present mainly in B-cells, macrophages, platelets, osteoclasta, fibroblasts, and T-lymphocytes. The Kv1.3 channel is involved in the control of membrane potential, production of lymphokines, and proliferation of human T-lymphocytes (de Coursey et al. 1984; Lewis et al. 1995). Therefore blockers of the Kv1.3 channel have the potential to be immunosuppressive agents, which can be used in transplantation, autoimmune disease treatment, and inflammation therapy (Slaughter et al. 1996).
We previously reported the synthesis of a series of 3-alkyl- and 3-aryl-7*H*-furo[3,2-*g*]chromen-7-ones, which were investigated for dark- and photobinding (crosslink formation) with DNA, fluorescent properties, lipophilicity and photobleaching capacities (Körner 2002). In the present study, using the whole-cell configuration of the patch-clamp technique, we investigated some of these furocoumarins on

Table: Substitution pattern of furocoumarins, block of peak K^+ currents of L-929 and N1E-115 cells



Compd.	R^1	R^2	R^3	R^4	$B_K(\%)_{L\text{-}929}$ ± s.e.m. (IC_{50} [μM])[d]	$B_K(\%)_{N1E\text{-}115}$ ± s.e.m. (IC_{50} [μM])[e]	$B_{Na}(\%)_{N1E\text{-}115}$ ± s.e.m. (IC_{50} [μM])[f]	S[g]
1a[a]	Me	H	Me	Me	21 ± 3	18 ± 2	8 ± 2	nd
1b[a]	*tert*-Bu	H	Me	Me	56 ± 6 (4)	39 ± 4 (15)	39 ± 3 (14)	3.8
1c[a]	Ph	H	Me	Me	84 ± 8 (1)	47 ± 5 (6)	31 ± 5 (35)	6
1d[a]	4-MeOPh	H	Me	Me	7 ± 2	16 ± 3	9 ± 3	nd
1e[b]	2,5-$(MeO)_2$Ph	H	Me	Me	91 ± 2 (0.7)	22 ± 5 (7)	13 ± 5 nd	10
2[c]	H	MeO	H	H	(101)	nd	nd	nd
3[c]	–	–	–	–	98 (5)	32 (38)	5 nd	7.6

[a]conc. 5 μM ; [b]conc. 2.5 μM ; [c]conc. 25 μM (Wulff et al. 1998; Butenschön et al. 2001); [d]blockade of K^+ currents of Kv1.3 channels of L-929 cells; [e]blockade of K^+ currents of neuroblastoma cells N1E-115; [f]accompanying blockade of peak N1E-115 Na^+ currents; [g]S, selectivity of L-929 Kv1.3 current blockade over N1E-115 Kv current blockade given by IC_{50} (Kv N1E-115) /IC_{50} (Kv1.3 L-929); **2**, 5-MOP, 5-methoxypsoralen (Wulff et al. 1998); **3**, 8-methoxy-2-(1-methylethyl)-5-methyl-4,5-dihydrofuro[3,2-*c*]quinolin-4-one (Butenschön et al. 2001); nd, not determined; s.e.m., standard error of mean; number of experiments ≥ 3; Me, methyl; Bu, butyl; Ph, phenyl; MeO, methoxy.

L-929 mouse-fibroblasts, stably transfected with *m*Kv1.3. In addition, the compounds **1a–e** were studied for their effects on voltage-gated K^+ and Na^+ channels of the neuroblastoma cell-line N1E-115 to determine potential selectivity for Kv1.3 (see Table). The N1E-115 cells bear at least two voltage controlled K^+ channels (one of them is Kv3.1, Kv1.3 is not existent) and one voltage-gated Na^+ channel. The compounds were tested in a concentration of 5 μM unless otherwise stated. All tested furocoumarins (**1a-e**) showed Kv channel blocking activities. For the most effective compounds we determined the half-blocking concentrations (IC_{50}) for the Kv1.3 of L-929 and Kv channels of N1E-115 cells. Two reference compounds (**2**, **3**) are reported in accordance to the literature (Wulff et al. 1998; Butenschön et al. 2001). The K^+ channel blocking data revealed that the tested furocoumarins **1b**, **1c** and **1e** are the most promising compounds, blocking Kv1.3 currents in the low-micromolar range and with higher potency than the N1E-115 K^+ current. Compound **1e** reveals 10-fold selectivity for Kv1.3 over Kv channels of N1E-115, whereas **1c** and **1b** show 6-fold and 3.8-fold selectivity for Kv1.3. The compounds **1a–c** and **1e** block Kv1.3 currents with higher potency than the N1E-115 Na^+ current. The tested 3-aryl-7*H*-furo[3,2-*g*]chromen-7-one **1d** bearing a 3-(4-methoxyphenyl) group displays weak Kv1.3 channel blocking activities, whereas compounds **1c** and **1e**, lacking a methoxy group in 4 position of the 3-aryl substituent, show stronger blocking effects. Comparing the tested 3-alkyl-7*H*-furo[3,2-*g*]chromen-7-ones **1a** and **1b** it can be seen that the bulky and hydrophobic 3-*tert*-butyl substituent increases the blocking activity of Kv1.3 currents.

According to the data, substitution of the 7*H*-furo[3,2-*g*]chromen-7-one skeleton in position 3 with bulky and hydrophobic substituents resulted in three derivatives **1b**, **1c,** and **1e,** which block Kv1.3 currents in the low-micromolar range and with higher potency than the N1E-115 K^+ current. The most potent compound, 3-(2,5-dimethoxyphenyl)-5,9-dimethyl-7*H*-furo[3,2-*g*]chromen-7-one (**1e**) inhibited Kv1.3 channels with a half-blocking concentration of 0.7 μM. In order to proof selectivity for Kv1.3 it will be necessary to investigate further closely related homomeric Kv channels. The compounds might lack the selectivity and potency required for a therapeutic drug, nevertheless they can serve as templates for the design of Kv 1.3 selective K^+-blockers.

Experimental

1. Cell culture

Two different cell lines were applied. The L-929 mouse fibroblasts stably transfected with *m*Kv1.3 were a generous gift of Prof. Dr. S. Grissmer, Department of Applied Physiology, University of Ulm. To investigate the selectivity of the Kv1.3 current blockade over other voltage gated ion channels the mouse neuroblastoma cell line N1E-115 was used, which was obtained from Prof. Dr. B. Hamprecht, Department of Physiological Chemistry, University of Tübingen. Cell culturing was performed as described (Butenschön et al. 2001), but the concentration of kanamycin was 25 μg/ml.

2. Electrophysiology

The experiments were carried out in the whole-cell configuration of the patch-clamp technique using an EPC-9 patch-clamp amplifier (HEKA). The holding potential was – 80 mV. Currents were elicited by a pulse with an amplitude of 40 mV for the K^+ channels and an amplitude of 0 mV for the Na^+ channel. The pulse length amounted to 100 ms and 200 ms for the N1E-115 cells and the L-929 cells, respectively. The blockade of steady-state peak K^+ currents (B_K) and Na^+ currents (B_{Na}) was determined. All of the measurements were performed as described (Butenschön et al. 2001), except that a 50% series-resistance compensation was used for the N1E-115 cells.

References

Averbeck D (1989) Recent advances in psoralen phototoxicity mechanism. Photochem Photobiol 50: 859–882.

Butenschön I, Möller K, Hänsel W (2001) Angular methoxy-substituted furo- and pyranoquinolinones as blockers of the voltage-gated potassium channel Kv1.3. J Med Chem 44: 1249–1256.

Caffieri S, Ruzzene M, Guerra B, Frank S, Vedaldi D, Dall'Acqua F (1994) Psoralen-fatty acid cycloadducts activate protein kinase C (PKC) in human platelets. J Photochem Photobiol B 22: 253–256.

Dall'Acqua F, Martelli P (1991) Photosensitizing action of furocoumarins on membrane components and consequent intracellular events. J Photochem Photobiol B 8: 235–254.

De Coursey TE, Chandy KG, Gupta S, Calahan MD (1984) Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis? Nature 307: 465- 468.

Edelson RL (1989) Photopheresis: a new therapeutic concept. Yale J Biol Med 62: 565–577.

Griffiths CE (2003) The immunological basis of psoriasis. J Eur Acad Dermatol Venereol 17 Suppl 2: 1–5.

Körner P (2002) 3-Alkyl- and 3-Aryl-7*H*-furo[3,2-*g*]-1-benzopyran-7-ones: synthesis, photoreactivity and fluorescence properties. Arch Pharm Pharm Med Chem 5: 187–199.

Körner P (2003) 3-Alkyl- and 3-aryl-(7-oxo-7*H*-furo[3,2-*g*]chromen-5-yl)-alkanoic acids as inhibitors of leukotriene B_4 biosynthesis. Arch Pharm Pharm Med Chem 6–7: 273 –284.

Lewis RS, Cahalan MD (1995) Potassium and calcium channels in lymphocytes. Annu Rev Immunol 13: 623–653.

Midden WR (1988) in Gasparro FP (ed.) Psoralen DNA Photobiolgy, Vol. II (Ed.:). CRC Press, Boca Raton 1, p. 49.

Prinz JC (2003) The role of T cells in psoriasis. J Eur Acad Dermatol Venereol 17: 257–270.

Schmitt IM, Chimenti S, Gasparro FP (1995) Psoralen-protein photochemistry- a forgotten field. J Photochem Photobiol B 27: 101–107.

Slaughter RS, Garcia ML, Kaczorowski GJ (1996) Ion channels as targets in the immune system Curr Pharm Des 2: 610–623.

Wulff H, Rauer H, Düring T, Hanselmann C, Ruff K, Wrisch A, Grissmer S, Hänsel W (1998) Alkoxypsoralens, novel nonpeptide blockers of Shaker-type K^+ channels: synthesis and photoreactivity. J Med Chem 41: 4542–4549.