European Journal of Medicinal Chemistry 139 (2017) 232–241

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Clathrodin, hymenidin and oroidin, and their synthetic analogues as inhibitors of the voltage-gated potassium channels

Nace Zidar [a], Aleš Žula [a], Tihomir Tomašič [a], Marc Rogers [b, 1], Robert W. Kirby [b, 1], Jan Tytgat [c], Steve Peigneur [c], Danijel Kikelj [a], Janez Ilaš [a, **], Lucija Peterlin Mašič [a, *]

[a] University of Ljubljana, Faculty of Pharmacy, Aškerčeva cesta 7, 1000 Ljubljana, Slovenia
[b] Xention Limited, Iconix Park, London Road, Pampisford, Cambridge CB22 3EG, UK
[c] University of Leuven (KU Leuven), Toxicology & Pharmacology, O&N2, PO Box 922, Herestraat 49, 3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 18 January 2017
Received in revised form
3 August 2017
Accepted 4 August 2017
Available online 7 August 2017

Keywords:
Voltage-gated potassium channel
K_v1
Modulator
Inhibitor
Clathrodin
Oroidin
Hymenidin

ABSTRACT

We have prepared three alkaloids from the *Agelas* sponges, clathrodin, hymenidin and oroidin, and a series of their synthetic analogues, and evaluated their inhibitory effect against six isoforms of the K_v1 subfamily of voltage-gated potassium channels, $K_v1.1$-$K_v1.6$, expressed in Chinese Hamster ovary (CHO) cells using automated patch clamp electrophysiology assay. The most potent inhibitor was the (*E*)-*N*-(3-(2-amino-1*H*-imidazol-4-yl)allyl)-4,5-dichloro-1*H*-pyrrole-2-carboxamide (**6g**) with IC_{50} values between 1.4 and 6.1 μM against $K_v1.3$, $K_v1.4$, $K_v1.5$ and $K_v1.6$ channels. All compounds tested displayed selectivity against $K_v1.1$ and $K_v1.2$ channels. For confirmation of their activity and selectivity, compounds were additionally evaluated in the second independent system against $K_v1.1$-$K_v1.6$ and $K_v10.1$ channels expressed in *Xenopus laevis* oocytes under voltage clamp conditions where IC_{50} values against $K_v1.3$-$K_v1.6$ channels for the most active analogues (e.g. **6g**) were lower than 1 μM. Because of the observed low sub-micromolar IC_{50} values and fairly low molecular weights, the prepared compounds represent good starting points for further optimisation towards more potent and selective voltage-gated potassium channel inhibitors.

1. Introduction

Voltage-gated potassium (K_v) channels are molecular complexes responsible for initiation and propagation of electrical impulses in excitable cells such as neurons, myocytes and endocrine cells, as well as for the transduction of signals in non-excitable cells such as immune cells. K_v channels are homo- or heterotetrameric proteins composed of four circularly arranged α subunits that form an ion conducting pore and contain voltage-sensing domains, and one or more supplementary β subunits [1,2]. K_v channels are a very large and diverse group of proteins and represent the largest branch of the potassium channel family. In humans, there are currently 40 known genes encoding K_v channel α subunits and based on their sequence homology and function, K_v channels can be divided into 12 major subfamilies (K_v1-12). Additionally, each of the 12 subfamilies is composed of several members that differ in their structures, biophysical profile and expression patterns in different tissue types which facilitate their different biological roles [3]. There are currently eight known pore-forming α subunits of the K_v1 subfamily ($K_v1.1$- $K_v1.8$), among which $K_v1.1$ and $K_v1.2$ channels are mainly expressed in the central nervous system (CNS), $K_v1.3$ channels are typical for T and B lymphocytes and macrophages, the rapidly inactivating $K_v1.4$ channels can be found in CNS, skeletal muscle, heart and pancreatic islets, $K_v1.5$ channels are located in cardiac myocytes, immune cells and vascular smooth muscle, while the $K_v1.6$ channels are expressed in spinal cord, CNS, astrocytes and artery smooth muscle. The high expression of $K_v1.7$ and $K_v1.8$ channels is typical for the heart, skeletal muscle and CNS [2,4].

The loss- and gain-of-function mutations - the channelopathies - of the K_v1 channel subfamily have been associated with various pathological phenotypes which highlight the potential of K_v1 channels as promising drug targets [3,4]. Modulators of $K_v1.1$ and $K_v1.2$ channels have been described as potential targets for the

* Corresponding author.
** Corresponding author.
E-mail addresses: Janez.Ilas@ffa.uni-lj.si (J. Ilaš), Lucija.PeterlinMasic@ffa.uni-lj.si (L.P. Mašič).
[1] Current address: Metrion Biosciences Limited, Babraham Research Campus, Cambridge, CB22 3AT, UK.

http://dx.doi.org/10.1016/j.ejmech.2017.08.015
0223-5234/

treatment of epilepsy and neuropathic pain [5,6], inhibitors of $K_v1.3$ channels exert immunosuppressive effects and have potential for the treatment of various autoimmune diseases [7–9], blockers of $K_v1.5$ channels are in development as antiarrhythmics [10–12], while $K_v1.4$, $K_v1.6$, $K_v1.7$ and $K_v1.8$ channels have so far a less well defined therapeutic relevance.

Among the K_v1 subfamily, in recent years, the $K_v1.3$ channel has arguably received the most scientific attention as a drug target. Efflux of K^+ ions through $K_v1.3$ channels is necessary to repolarise the membrane potential in immune cells. A negative membrane potential is required to allow activation of calcium release-activated channels (CRAC) channels upon a new depolarization. As such, Kv1.3 channels indirectly create a driving force for the entry of Ca^{2+} ions through CRAC channels. The resultant influx of Ca^{2+} is necessary for the translocation of nuclear factors which results in T cell activation, proliferation and inflammatory cytokine secretion. Thus, selective $K_v1.3$ inhibition could be regarded as a novel approach for the treatment of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and psoriasis [3,4,13].

There are two main binding sites for small-molecules on K_v1 channels reported in the literature: the inner-pore site and a side-pocket cavity [14]. Since the sequence of the inner pore is well conserved among the K_v1 family, the majority of blockers that bind to this site are not very selective, e.g. 4-aminopyridine (4-AP, Fig. 1) and tetrabutylammonium (TBA, Fig. 1). Reasonable $K_v1.3$ selectivity has been reported for correolide (Fig. 1) [15], and for PAP-1 (Fig. 1) [16], a member of the phenoxyalkoxypsoralen family, which displays a 2 nM IC_{50} value against $K_v1.3$ and a 4- to 20-fold selectivity against $K_v1.1$, $K_v1.2$, $K_v1.4$, $K_v1.5$ and $K_v1.6$ channels.

Despite tremendous research efforts in the K_v channel drug discovery field the therapeutic potential of K_v channels remains underexploited. The main problems are (*i*) the lack of structural channel data and the information on the exact binding site of compounds, (*ii*) low structural diversity of leads, (*iii*) poor gene family selectivity and/or (*iv*) unfavourable physicochemical properties of compounds, which result in non-optimal efficacy and toxicity issues. However, recent advances in crystallisation of K_v channels [17–19] and new *in silico* studies [14] are increasing our knowledge of their structure and function. Additionally, over the last few years development of new reliable high- and medium throughput screening methods such as the Sophion QPatch 48 automated patch-clamp electrophysiology system have helped accelerate the drug discovery process for ion channel modulators [4].

Marine organisms, such as sponges, fish, cone snails, cnidarians and sea anemones, are important sources of various bioactive compounds that are used in their native environment to capture pray or function as defence against predators [20]. Alkaloids isolated from marine sponges *Agelas* sp., clathrodin, hymenidin and oroidin (Fig. 2), and their synthetic analogues, have been shown to possess modulatory activities on voltage-gated sodium (Na_v) channels [21–23], but so far their activity on K_v channels has not been reported. However, complex marine compounds isolated from other species of marine sponges (acredinones and crambescins) have recently shown inhibitory activity against K_v channels [24,25]. Because of their relatively simple structures and lead-like properties, clathrodin, hymenidin and oroidin represent interesting starting compounds for medicinal chemistry optimisation of their pharmacological properties.

In our study, we have prepared clathrodin, hymenidin and oroidin, and a series of their synthetic analogues, and evaluated their K_v1 channel inhibitory activity against six different human K_v1 channel subtypes expressed in Chinese hamster ovary cells (CHO cells) using automated patch clamp electrophysiology. All of the prepared compounds were evaluated against $K_v1.3$ channels, and active inhibitors also against $K_v1.1$, $K_v1.2$, $K_v1.4$, $K_v1.5$ and $K_v1.6$ channels. In addition to the automated patch clamp electrophysiology, compounds were also studied in the second, independent test system under voltage clamp conditions against $K_v1.1$-$K_v1.6$ and $K_v10.1$ channels expressed in *Xenopus laevis* oocytes. Based on the inhibitory activities, structure-activity relationship (SAR) of this new structural class of voltage-gated potassium channel modulators was studied.

2. Results and discussion

2.1. Design

We have prepared clathrodin, hymenidin and oroidin (Fig. 2), and evaluated their inhibitory effect against $K_v1.1$-$K_v1.6$ channels. With the aim of improving the activities and/or stabilities of the natural alkaloids, we have designed two structural classes of their analogues, (*E*)-*N*-(3-(2-amino-1*H*-imidazol-4-yl)allyl)carboxamides **I** and *N*-(3-(2-amino-1*H*-imidazol-4-yl)propyl)carboxamides **II** (Fig. 2).

In the type **I** series we decided to retain the (*E*)-4-(3-aminoprop-1-en-1-yl)-1*H*-imidazol-2-amide motif on the left-hand side of the molecules and to replace the right-hand side pyrrol-2-yl (**6a**, Table 1), 4-bromopyrrol-2-yl (**6b**, Table 1), and 4,5-dibromopyrrol-2-yl (**6c**, Table 1) groups that are present in natural alkaloids, with other substituted pyrrol-2-yl (**6d-g**, Table 1), indol-2-yl (**6h-k**, Table 1), indolin-2-yl (**6l**, Table 1), pyridin-3-yl (**6m**, Table 1), and piperidin-4-yl (**6n-o**, Table 1) groups. In this way we examined the influence of different size, polarity and/or acid/base properties of the right-hand side groups on the biological activity. Additionally, with piperidine derivatives **6n** and **6o**, we examined if the aromaticity of the right-hand side groups is important for K_v1 inhibitory activity.

In the type **II** series we aimed at increasing the chemical stability of compounds by eliminating the potentially reactive double bond in the central part of the molecules and replacing it with the saturated propylene group. In this way, we also studied the influence of increased flexibility and conformational freedom of molecules on the K_v1 inhibitory activity. Analogously to the type **I** series, in addition to the analogue with naturally occurring 4,5-dibromopyrrol-2-yl group (**14a**, Table 1), we have prepared

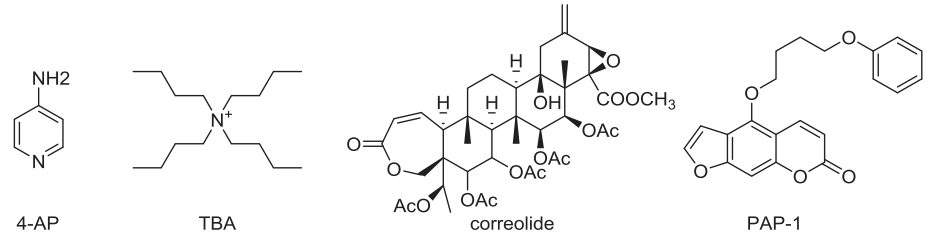

Fig. 1. Structures of the unselective K_v channel inhibitors (4-AP, TBA) and $K_v1.3$ selective inhibitors (correolide, PAP-1).