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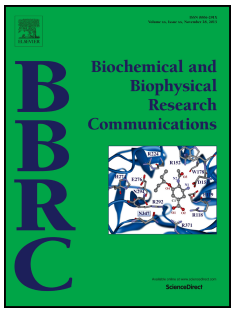

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Terbinafine is a novel and selective activator of the two-pore domain potassium channel TASK3

Paul D Wright[1], Emma L Veale[2], David McCoull[1], David C Tickle[1], Jonathan M Large[1], Emma Ococks[1], Gemma Gothard[1], Catherine Kettleborough[1], Alistair Mathie[2], Jeffrey Jerman[1]

1. LifeArc, Accelerator Building, Open Innovation Campus, Stevenage, SG1 2FX, UK

2. Medway School of Pharmacy, University of Kent**,** Anson Building, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK

Corresponding author: Paul D Wright.

Paul.wright@lifearc.org

Drect: +44 (0)1438 544 095

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Abstract

Two-pore domain potassium channels (K2Ps) are characterised by their four transmembrane domain and two-pore topology. They carry background (or leak) potassium current in a variety of cell types. Despite a number of important roles there is currently a lack of pharmacological tools with which to further probe K2P function. We have developed a cell-based thallium flux assay, using baculovirus delivered TASK3 (TWIK-related acid-sensitive K^+ channel 3, KCNK9, K2P9.1) with the aim of identifying novel, selective TASK3 activators. After screening a library of 1000 compounds, including drug-like and FDA approved molecules, we identified Terbinafine as an activator of TASK3. In a thallium flux assay a pEC50 of 6.2 (+/- 0.12) was observed. When Terbinafine was screened against TASK2, TREK2, THIK1, TWIK1 and TRESK no activation was observed in thallium flux assays. Several analogues of Terbinafine were also purchased and structure activity relationships examined. To confirm Terbinafine's activation of TASK3 whole cell patch clamp electrophysiology was carried out and clear potentiation observed in both the wild type channel and the pathophysiological, Birk-Barel syndrome associated, G236R TASK3 mutant. No activity at TASK1 was observed in electrophysiology studies. In conclusion, we have identified the first selective activator of the two-pore domain potassium channel TASK3.

Keywords: TASK3; Activator; K2P; KCNK9; Terbinafine

Introduction

Two-pore domain potassium channels (K2P), often referred to as 'leak' channels, primarily act to help maintain resting membrane potential in a variety of cell types. The K2P superfamily is formed of

15 channels, each sharing a distinct topology whereby channels comprise four transmembrane segments and two pore loops, forming functional dimers in the membrane. K2P channels are known to be regulated by a diverse set of modulators including physicochemical factors such as temperature, membrane stretch and pH, as well as signalling pathways including GPCRs and kinases and other interacting proteins e.g. 14-3-3 and Calcineurin. They have been implicated in an equally diverse set of pathophysiologies (summarized [1]).

The TASK (TWIK-related acid-sensitive K^+ channel) subfamily is comprised of 3 members TASK1, TASK3 and TASK5. Thus far TASK5 mediated currents have not been demonstrated [2] but TASK1 and TASK3 are functional channels which are highly sensitive to extracellular pH [3],[4]. TASK3 current is known to be reduced by extracellular acidification. TASK3 channels are found throughout the body with expression shown in the brain, heart, kidney, liver, colon, stomach, testis and skeletal muscle [4], [5]. TASK3 KO mice show a number of cognitive impairments, including impaired working memory and altered circadian rhythms [6]. TASK3 has also been shown to function in the migration of cortical pyramidal neurons during development [7], whereby knockdown of TASK3 caused defective migration of late-born cortical excitatory neurons destined to become Layer II/III neurons.

TASK3 has been implicated in a number of disorders including cancer [8], ischemia [9], low renin essential hypertension [10], idiopathic hyperalderosteronism [11] and epilepsy [12]. Of particular note is the observation mutations in TASK3 lead to Birk-Barel syndrome, a mental retardation syndrome characterized by intellectual disability, hypotonia, hyperactivity, and unusual facial features [12]. Birk-Barel syndrome is also referred to as KCNK9 imprinting syndrome as TASK3 is the only K2P to be genetically imprinted – i.e. only the maternal copy of the gene is expressed [13]. The mutated channel, in which the glycine residue at position 236 is replaced by arginine (G236R), has a reduced function, meaning a novel activator may be of therapeutic benefit. Of note are studies

showing the G236R TASK3 mutant channel function causes the previously described defective migration of neurons during development [7].

Like many K2P channels further interrogation of TASK3 function has been limited by the paucity of selective pharmacological tools. A number of non-specific inhibitors have been described including zinc, ruthenium red, anandamide lidocaine, bupiviacine, alphaxolone and Gαq receptor agonists - notably M3 Muscarinic acetylcholine agonists [14]. A thallium-flux screen identified ML308 [15], a TASK3 inhibitor which shows 50-fold selectivity for TASK3 vs TASK1. Halogenated ether, alcohol, alkane anesthetics [16], TNFα [17] and flufenamic acid [18] have been shown to activate TASK3 but there remains an absence of specific TASK3 activators.

The current study describes the development of a novel thallium flux TASK3 assay and the subsequent identification of Terbinafine as a potent activator of TASK3. Selectivity was assessed against a number of alternative K2P channels and where possible activity confirmed using whole cell patch clamp electrophysiology.

Methods

Thallium Flux assays

U-2 OS cells (ATCC, UK) were maintained in MEM (Sigma, USA) supplemented with 10% FBS (Gibco, USA). TASK3 BacMam solution (SB Drug Discovery, UK) was used to generate transiently expressing cells. Typically, U-2 OS cells from liquid Nitrogen were thawed and re-suspended in media. Cells were centrifuged at 250 x g for 5 minutes before being re-suspended in fresh media containing the

desired concentration (v/v) of TASK3 BacMam solution. Typically for TASK3 BacMam was used at 5% v/v. Cells were plated on black, 384 well clear bottomed TC treated plates (Corning, USA) at 5k cells per well and incubated overnight at 37°C and 5% CO_2. The following day channel activity was measured using a FLIPR TETRA and the FLIPR Potassium assay kit (both Molecular Devices, USA). Compounds were all prepared in 100% DMSO and added to HBSS with 20mM HEPES. Compounds were then pre-incubated with cells for 30 minutes prior to thallium addition (final concentration of Tl^+ 2mM). Pre-addition baselines were established for 14 seconds and channel activity was calculated as rate of fluorescence increase between 14 and 24 seconds.

Selectivity assays

To assess selectivity, U-2 OS cells were transduced with different baculovirus solutions to generate cells expressing TREK2, TASK2, TWIK1, THIK1 or TRESK. Cells were treated as described for TASK3 and transduced with 1% v/v (TASK2, TREK2), 0.05% v/v THIK1, 10% v/v (TWIK1) or 0.5% v/v (TRESK) BacMam solution prior to plating on black, clear bottomed TC treated plates (Corning) at 5k cells per well and incubated overnight at 37°C and 5% CO_2. For TWIK1 the I239A/ I294A mutated variant was used to facilitate membrane expression. TWIK1 cells were also incubated for 48 hours before experiments. All cells were analyzed using the thallium flux assay as previously described for TASK3. To confirm activation, where available, control ligands were prepared in 100% DMSO and added to cells for 30 minutes before measurement.

Whole cell patch clamp

tsA201 cells were grown as described previously [19]. For electrophysiological experiments, the pcDNA3.1 vector was cloned with the gene of interest (hTASK-3 (K2P9.1), hTASK-1 (K2P3.1) and hTASK-3_G236R). These vectors and a similar vector containing GFP were incorporated into the tsA201 cells (0.5 µg per well) using the calcium phosphate method. The cells were incubated for 6 - 12 hours at 37^{o}C in 95% oxygen and 5% carbon dioxide. Cells were then washed using a phosphate buffered saline solution (PBS), and used for experiments after 24 hours.

Currents were recorded using whole cell patch clamp at room temperature using an Axopatch 1D amplifier. Cells were placed in a recording chamber filled with an external medium composed of 145 mM NaCl, 2.5 mM KCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$ and 10 mM HEPES (pH to 7.4, using NaOH). The internal medium used in the glass pipette comprised 150 mM KCl, 3 mM $MgCl_2$, 5 mM (or 0.1 mM) EGTA and 10 mM HEPES (pH to 7.4, using KOH). Terbinafine was applied by bath perfusion.

Currents were recorded and analyzed using pCLAMP 10.2 software and Microsoft Excel. The voltage protocol used for recording current through K2P channels was as previously described [19]. For analysis, we measured the current difference between the -80 mV and - 40 mV steps. Data were expressed as mean ± standard error of the mean (SEM) and 'n' represents the number of cells used for the experiment.

Compounds

Tebinafine Hydrocholoride, Naftifine Hydrocholride and Butenafine Hydrocholride were purchased from Sigma Aldrich (UK). Cis-Terbinafine, N-Desmethyl Terbinafine and compound 6, Table 1 (as HCl salt) were purchased from Toronto Research chemicals Inc (Canada). A-1899 was purchased from Bionet (Camelford, UK). PK-THPP was purchased from Tocris (UK). TASK3 inhibitors, compound 3 (3-CH_3-phenyl) and 52 (4-pyridyl) [20] were purchased from AKos (Germany)

Analysis

For thallium flux assays data are mean +/- standard deviation (n≥3 independent experiments) unless stated. Compound response curves were iteratively fitted to a four parameter logistic model using Graphpad Prism v7.01 (Graphpad, USA). Activity was defined as the rate of fluorescence increase measured using 470-495nM excitation LEDs and 515-575nM emission filter over a pre designated time period. This time period varied for each channel and is described appropriately. Baselines were established for 14 seconds prior to thallium addition. All data was acquired using ScreenWorks 4.0 software (Molecular Devices, USA).

Results

Thallium flux assay development and identification of Terbinafine as a novel TASK3 activator

Generation of cell lines stably expressing ion channels can be challenging for a number of reasons including inherent toxicity. Moreover, the ability to identify channel activators can be compromised by systems in which the target is over-expressed at high levels. To avoid these issues we utilized baculovirus ('BacMam') to deliver ion channels into mammalian cells. 'BacMam' confers a number of advantages, including safety and reduced time compared to generating stable cell lines but primarily it allowed the precise titratable expression of the gene of interest. With respect to K2Ps this enabled us to generate cell systems in which we were able to intricately and robustly select a level of TASK3 expression, functionally optimized for the identification of channel activators. We have previously used this approach to successfully identify Cloxyquin as a novel activator of the K2P channel TRESK [21].

Initial experiments sought to demonstrate the 'titratability' of TASK3 expression using the thallium flux system as a measure of channel function. U-2 OS cells transduced with increasing levels of BacMam solution (Figure 1A), gave rise to increasing levels of channel function. As described, K2P channel studies are limited by a lack of specific pharmacological tools, particularly regarding activators; therefore to ensure the thallium signal was representative of TASK3 activity a subset of known TASK3 inhibitors were used (Figure 1C). PK-THPP [22], A-1899 [23] and bis-amide scaffolds [20] although active for TASK1 have also been shown to inhibit TASK3. PK-THPP had a pIC50 of 8.20 (+/- 0.2) in the TASK3 assay and A-1899 a pIC50 of 5.79 (+/- 0.18). Bis-amide analogues tested were

those containing 3-CH_3-phenyl or 4-pyridyl side groups [20]; these had pIC50s of 7.05 (+/- 0.37) and <5 respectively (Figure 1B).

To identify novel activators of TASK3 we screened a library of approximately 1000 structurally diverse compounds. From this a small number of compounds were identified as increasing the rate of thallium flux above baseline, defined as >3 standard deviations of DMSO (no effect) controls. Putative hits were then re-screened against non-transduced cells to remove assay interferers (e.g. compounds which increase fluorescence independent of TASK3). This is another advantage of the BacMam system in that it allows screening against parental cells in the absence of the heterologous expressed ion channel. Terbinafine hydrochloride was identified as a robust activator of TASK3 and shown to have a pEC50 of 6.2 (+/- 0.12) whilst displaying no activity against wild type U-2 OS cells (Figure 1C).

Electrophysiological analysis of Terbinafine confirms it is a subtype specific activator of TASK3

To confirm Terbinafine's activation of TASK3 whole cell electrophysiology experiments were conducted. Using tsA201 cells transiently transfected with wild type TASK3 1 µM Terbinafine was added and current measured using whole cell patch clamp recordings (Figure 2). Outward current was enhanced by 30% (+/- 3, n=5) and the zero current potential (reversal potential) hyperpolarised slightly from -86 mV (+/- 2) to -89 mV (+/- 2). The G236R variant of TASK3, linked to Birk-Barel syndrome has previously been described as having statistically reduced current density [18]. The effect of Terbinafine (1 µM) on G236A TASK3 was also analyzed. A 76% (+/- 4, n=6) enhancement of current was observed and the zero current potential hyperpolarised from -77 mV (+/- 1) to -84 mV (+/- 2). Whilst the lower starting current of the G236A TASK3 cannot be disregarded, it is of interest

that the mutated form is clearly activated to a high degree and indeed this may be as a functional consequence of lower starting activity.

To assess the selectivity of Terbinafine between the TASK channel subtypes whole cell patch clamp recordings were also carried out on cells transiently transfected with TASK1. Experiments showed there was no enhancement of outward current at TASK1. After addition of 1 µM Terbinafine a 0% (+/- 4, n=5) change was observed. Importantly, changing to pH 8.4 markedly enhanced current in the same cells as those which do not respond to Terbinafine, confirming the channel was able to be potentiated by known physiological activators. These data confirm that Terbinafine is able to activate TASK3 and is subtype specific.

Selectivity screening of Terbinafine and initial structure activity relationship analysis

To assess the selectivity of Terbinafine we sought to analyze activity against a subset of alternative K2P channels, using a common thallium flux as a measure of channel activity. Initially, we measured activity at TREK2, TASK2, TWIK1, THIK1 and TRESK, representing at least one member of each of the K2P subfamilies. Selectivity, in terms of activators, is complex to analyze at K2P channels as there is a lack of native ligands with which we can determine a maximal relative efficacy. Additionally, differences in the level of efficacy with different ligands confound yet force comparison of agonist potency, activity relative to baseline and (where available) activity relative to a known activator. It is also important to caveat that these activities are only relevant to the assay system used, in this case thallium flux, but might differ in an alternative assay format. Two of the five channels initially tested have validated activators, 11-deoxy prostaglandin F-2α for TREK2 [24] and phorbol 12-myristate 13-acetate (PMA) for TRESK [25]. For TREK2 activation 11-deoxy prostaglandin F-2α displayed maximal

activity 8.8 (+/- 0.33) fold over baseline, whereas maximal Terbinafine activity was only 1.77 (+/- 0.22) over baseline (Figure 3). Additionally, based on an incomplete curve with a poorly defined maximum asymptote, Terbinafine's pEC50 was <5 at TREK2. For TRESK maximal PMA activation was fold 1.39 (+/- 0.01) fold over baseline with a pEC50 of 8.22 (+/- 0.28) (Figure 3). Terbinafine showed no overt activation of TRESK with pEC50<5. For TASK2 and THIK1 no activity could be observed (Figure 3). Interestingly Terbinafine showed inhibition at TWIK1 with a pIC50 of 5.69 (+/- 0.18) (Figure 3). This data suggests that Terbinafine is a highly selective activator with regards to other K2P channels.

We also sought to define preliminary structural activity relationships (SAR) for Terbinafine activity at TASK3. As previously described Terbinafine (entry 1, Table 1) is an orally active antimycotic allylamine and a specific inhibitor of squalene epoxidase [26], which is used orally or topically to treat fungal infections. The commercially available analogues, entries 2 – 6 (Table 1), represent related allylamine antimycotics or, in the case of entry 6, an impurity isolated during the preparation of Terbinafine itself. These compounds were chosen to explore the effects of simple structural changes on TASK3 activation. These involved replacement of the triple bond with a phenyl group (in 2 and 3), switching the stereochemistry of the double bond (in 4), demethylation of the basic centre (in 5) and methylation of the naphthalene ring (in 6). Of the analogues tested only one, entry 6, retained the ability to activate TASK3 and this displayed near equipotent activity compared to Terbinafine itself. Of additional note is the observation that all of the Terbinafine analogues described in Table 1 showed near equipotent inhibition of TWIK1 (data not shown). This does not correlate with the SAR seen at TASK3 and is suggestive of divergent SAR for Terbinafine at these two K2P channels.

Discussion

Despite being associated with a number of human pathophysiologies, small molecule K2P channel modulators are not apparent and have yet to deliver therapeutics. Moreover, this lack of pharmacological tools has hindered understandings of the physiological role of K2P channels in native tissues. In this study we aimed to identify novel activators of the TASK3 channel. We identified Terbinafine as a potent activator of TASK3 and in our assays it showed no significant activation of any other K2P channels tested. Activity of Terbinafine was confirmed using whole cell patch clamp electrophysiology and with potential relevance to the human developmental disorder, Birk-Barel syndrome, showed an increased level of activation of the G236R TASK3 variant compared to the wild type channel.

As described Terbinafine, which is sold under the brand name Lamisil, is an antifungal treatment typically used for Dermatophytosis (ringworm/ tinea), pityriasis versicolor and Onychomycosis (fungal infection of the nail). As such it is generally thought of as being most effective on Dermatophyte fungi (*Microsporum*, *Epidermophyton* and *Trichophyton)*. First approved in 1991 in Europe, it is on the World Health Organization's List of Essential Medicines. Although it's primary mode of action is via inhibition of squalene epoxidase and thus synthesis of ergosterol [26], it is interesting to speculate as to whether activity at fungal K2P channels may play a role in Terbinafine's antifungal activity. Yeast express a variant of the K2P channel, TOK1 and like human K2P channels this channel has also been shown to be activated by volatile anaesthetics [27], suggesting some shared pharmacology with human K2P channels. The activity of Terbinafine at TOK1 is not known but it represents a potentially interesting antifungal target given evidence has suggested TOK1 plays a role in modulating killing of *Candida albicans* by the toxin Hst5 [28].

The usefulness of Terbinafine in treating Birk-Barel is likely precluded by its unfavorable side-effect profile, particularly liver toxicity. It is also unlikely to cross the blood brain barrier (BBB) [29]. However, TASK3 activators have been shown to be efficacious in humans with this disorder. Mefanamic acid (MFA) has been reported as improving development and responsiveness [30]. The close analogue flufenamic acid (FFA) shows comparable activity to Terbinafine in whole cell patch clamp electrophysiology experiments at TASK3 and the G236R variant [18], albeit at much higher concentrations than used here for Terbinafine – 100 µM compared to 1 µM. Looking forward, it may be possible to develop Terbinafine analogues which do penetrate the BBB and with reduced side effect limitations.

In conclusion, we have identified Terbinafine as a novel activator of the K2P channel TASK3 and it is hoped this activator will allow further studies into the physiological role of TASK3. We also describe a novel assay for identifying TASK3 activators and work is on-going to develop novel chemotypes which may be of therapeutic benefit in conditions where TASK3 activation is a requirement. It is hoped that the identification of a specific TASK3 activator may allow further interrogation of a target class currently lacking specific pharmacological tools.

[1] P. Enyedi, G. Czirjak, Molecular background of leak K+ currents: two-pore domain potassium channels, Physiol Rev 90 (2010) 559-605.
[2] D. Kim, C. Gnatenco, TASK-5, a new member of the tandem-pore K(+) channel family, Biochem Biophys Res Commun 284 (2001) 923-930.
[3] F. Duprat, F. Lesage, M. Fink, R. Reyes, C. Heurteaux, M. Lazdunski, TASK, a human background K+ channel to sense external pH variations near physiological pH, EMBO J 16 (1997) 5464-5471.
[4] Y. Kim, H. Bang, D. Kim, TASK-3, a new member of the tandem pore K(+) channel family, J Biol Chem 275 (2000) 9340-9347.
[5] S. Rajan, E. Wischmeyer, G. Xin Liu, R. Preisig-Muller, J. Daut, A. Karschin, C. Derst, TASK-3, a novel tandem pore domain acid-sensitive K+ channel. An extracellular histiding as pH sensor, J Biol Chem 275 (2000) 16650-16657.
[6] A.M. Linden, C. Sandu, M.I. Aller, O.Y. Vekovischeva, P.H. Rosenberg, W. Wisden, E.R. Korpi, TASK-3 knockout mice exhibit exaggerated nocturnal activity, impairments in cognitive functions, and reduced sensitivity to inhalation anesthetics, J Pharmacol Exp Ther 323 (2007) 924-934.
[7] Y. Bando, T. Hirano, Y. Tagawa, Dysfunction of KCNK potassium channels impairs neuronal migration in the developing mouse cerebral cortex, Cereb Cortex 24 (2014) 1017-1029.
[8] D. Mu, L. Chen, X. Zhang, L.H. See, C.M. Koch, C. Yen, J.J. Tong, L. Spiegel, K.C. Nguyen, A. Servoss, Y. Peng, L. Pei, J.R. Marks, S. Lowe, T. Hoey, L.Y. Jan, W.R. McCombie, M.H. Wigler, S. Powers, Genomic amplification and oncogenic properties of the KCNK9 potassium channel gene, Cancer Cell 3 (2003) 297-302.
[9] P. Ehling, S. Bittner, N. Bobak, T. Schwarz, H. Wiendl, T. Budde, C. Kleinschnitz, S.G. Meuth, Two pore domain potassium channels in cerebral ischemia: a focus on K2P9.1 (TASK3, KCNK9), Exp Transl Stroke Med 2 (2010) 14.
[10] N.A. Guagliardo, J. Yao, C. Hu, E.M. Schertz, D.A. Tyson, R.M. Carey, D.A. Bayliss, P.Q. Barrett, TASK-3 channel deletion in mice recapitulates low-renin essential hypertension, Hypertension 59 (2012) 999-1005.
[11] L.A. Davies, C. Hu, N.A. Guagliardo, N. Sen, X. Chen, E.M. Talley, R.M. Carey, D.A. Bayliss, P.Q. Barrett, TASK channel deletion in mice causes primary hyperaldosteronism, Proc Natl Acad Sci U S A 105 (2008) 2203-2208.
[12] C. Kananura, T. Sander, S. Rajan, R. Preisig-Muller, K.H. Grzeschik, J. Daut, C. Derst, O.K. Steinlein, Tandem pore domain K(+)-channel TASK-3 (KCNK9) and idiopathic absence epilepsies, Am J Med Genet 114 (2002) 227-229.
[13] P.P. Luedi, F.S. Dietrich, J.R. Weidman, J.M. Bosko, R.L. Jirtle, A.J. Hartemink, Computational and experimental identification of novel human imprinted genes, Genome Res 17 (2007) 1723-1730.
[14] E.L. Veale, L.E. Kennard, G.L. Sutton, G. MacKenzie, C. Sandu, A. Mathie, G(alpha)q-mediated regulation of TASK3 two-pore domain potassium channels: the role of protein kinase C, Mol Pharmacol 71 (2007) 1666-1675.
[15] M.R. Miller, B. Zou, J. Shi, D.P. Flaherty, D.S. Simpson, T. Yao, B.E. Maki, V.W. Day, J.T. Douglas, M. Wu, O.B. McManus, J.E. Golden, J. Aube, M. Li, Development of a Selective Chemical Inhibitor for the Two-Pore Potassium Channel, KCNK9, Probe Reports from the NIH Molecular Libraries Program, Bethesda (MD), 2010.
[16] A. Luethy, J.D. Boghosian, R. Srikantha, J.F. Cotten, Halogenated Ether, Alcohol, and Alkane Anesthetics Activate TASK-3 Tandem Pore Potassium Channels Likely through a Common Mechanism, Mol Pharmacol 91 (2017) 620-629.
[17] M.F. El Hachmane, K.A. Rees, E.L. Veale, V.V. Sumbayev, A. Mathie, Enhancement of TWIK-related acid-sensitive potassium channel 3 (TASK3) two-pore domain potassium channel activity by tumor necrosis factor alpha, J Biol Chem 289 (2014) 1388-1401.

[18] E.L. Veale, M. Hassan, Y. Walsh, E. Al-Moubarak, A. Mathie, Recovery of current through mutated TASK3 potassium channels underlying Birk Barel syndrome, Mol Pharmacol 85 (2014) 397-407.

[19] E.L. Veale, A. Mathie, Aristolochic acid, a plant extract used in the treatment of pain and linked to Balkan endemic nephropathy, is a regulator of K2P channels, Br J Pharmacol 173 (2016) 1639-1652.

[20] D.P. Flaherty, D.S. Simpson, M. Miller, B.E. Maki, B. Zou, J. Shi, M. Wu, O.B. McManus, J. Aube, M. Li, J.E. Golden, Potent and selective inhibitors of the TASK-1 potassium channel through chemical optimization of a bis-amide scaffold, Bioorg Med Chem Lett 24 (2014) 3968-3973.

[21] P.D. Wright, G. Weir, J. Cartland, D. Tickle, C. Kettleborough, M.Z. Cader, J. Jerman, Cloxyquin (5-chloroquinolin-8-ol) is an activator of the two-pore domain potassium channel TRESK, Biochem Biophys Res Commun 441 (2013) 463-468.

[22] C.A. Coburn, Y. Luo, M. Cui, J. Wang, R. Soll, J. Dong, B. Hu, M.A. Lyon, V.P. Santarelli, R.L. Kraus, Y. Gregan, Y. Wang, S.V. Fox, J. Binns, S.M. Doran, D.R. Reiss, P.L. Tannenbaum, A.L. Gotter, P.T. Meinke, J.J. Renger, Discovery of a pharmacologically active antagonist of the two-pore-domain potassium channel K2P9.1 (TASK-3), ChemMedChem 7 (2012) 123-133.

[23] A.K. Streit, M.F. Netter, F. Kempf, M. Walecki, S. Rinne, M.K. Bollepalli, R. Preisig-Muller, V. Renigunta, J. Daut, T. Baukrowitz, M.S. Sansom, P.J. Stansfeld, N. Decher, A specific two-pore domain potassium channel blocker defines the structure of the TASK-1 open pore, J Biol Chem 286 (2011) 13977-13984.

[24] P.K. Dadi, N.C. Vierra, E. Days, M.T. Dickerson, P.N. Vinson, C.D. Weaver, D.A. Jacobson, Selective Small Molecule Activators of TREK-2 Channels Stimulate Dorsal Root Ganglion c-Fiber Nociceptor Two-Pore-Domain Potassium Channel Currents and Limit Calcium Influx, ACS Chem Neurosci 8 (2017) 558-568.

[25] A.K. Rahm, J. Gierten, J. Kisselbach, I. Staudacher, K. Staudacher, P.A. Schweizer, R. Becker, H.A. Katus, D. Thomas, PKC-dependent activation of human K(2P) 18.1 K(+) channels, Br J Pharmacol 166 (2012) 764-773.

[26] G. Petranyi, N.S. Ryder, A. Stutz, Allylamine derivatives: new class of synthetic antifungal agents inhibiting fungal squalene epoxidase, Science 224 (1984) 1239-1241.

[27] A.T. Gray, B.D. Winegar, D.J. Leonoudakis, J.R. Forsayeth, C.S. Yost, TOK1 is a volatile anesthetic stimulated K+ channel, Anesthesiology 88 (1998) 1076-1084.

[28] D. Baev, A. Rivetta, X.S. Li, S. Vylkova, E. Bashi, C.L. Slayman, M. Edgerton, Killing of Candida albicans by human salivary histatin 5 is modulated, but not determined, by the potassium channel TOK1, Infect Immun 71 (2003) 3251-3260.

[29] K.N. Sorensen, R.A. Sobel, K.V. Clemons, L. Calderon, K.J. Howell, P.R. Irani, D. Pappagianis, P.L. Williams, D.A. Stevens, Comparative efficacies of terbinafine and fluconazole in treatment of experimental coccidioidal meningitis in a rabbit model, Antimicrob Agents Chemother 44 (2000) 3087-3091.

[30] J.M. Graham, Jr., N. Zadeh, M. Kelley, E.S. Tan, W. Liew, V. Tan, M.A. Deardorff, G.N. Wilson, L. Sagi-Dain, S.A. Shalev, KCNK9 imprinting syndrome-further delineation of a possible treatable disorder, Am J Med Genet A 170 (2016) 2632-2637.

Figure / Table Legends

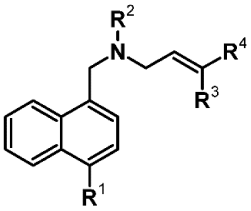
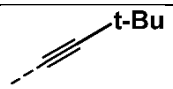
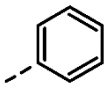
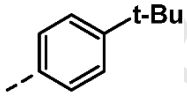
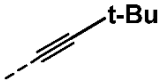
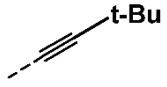
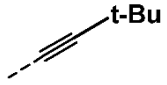
Table 1 – SAR summary of Terbinafine analogues and activity at TASK3. TASK3 pEC50 determined using thallium flux assay.

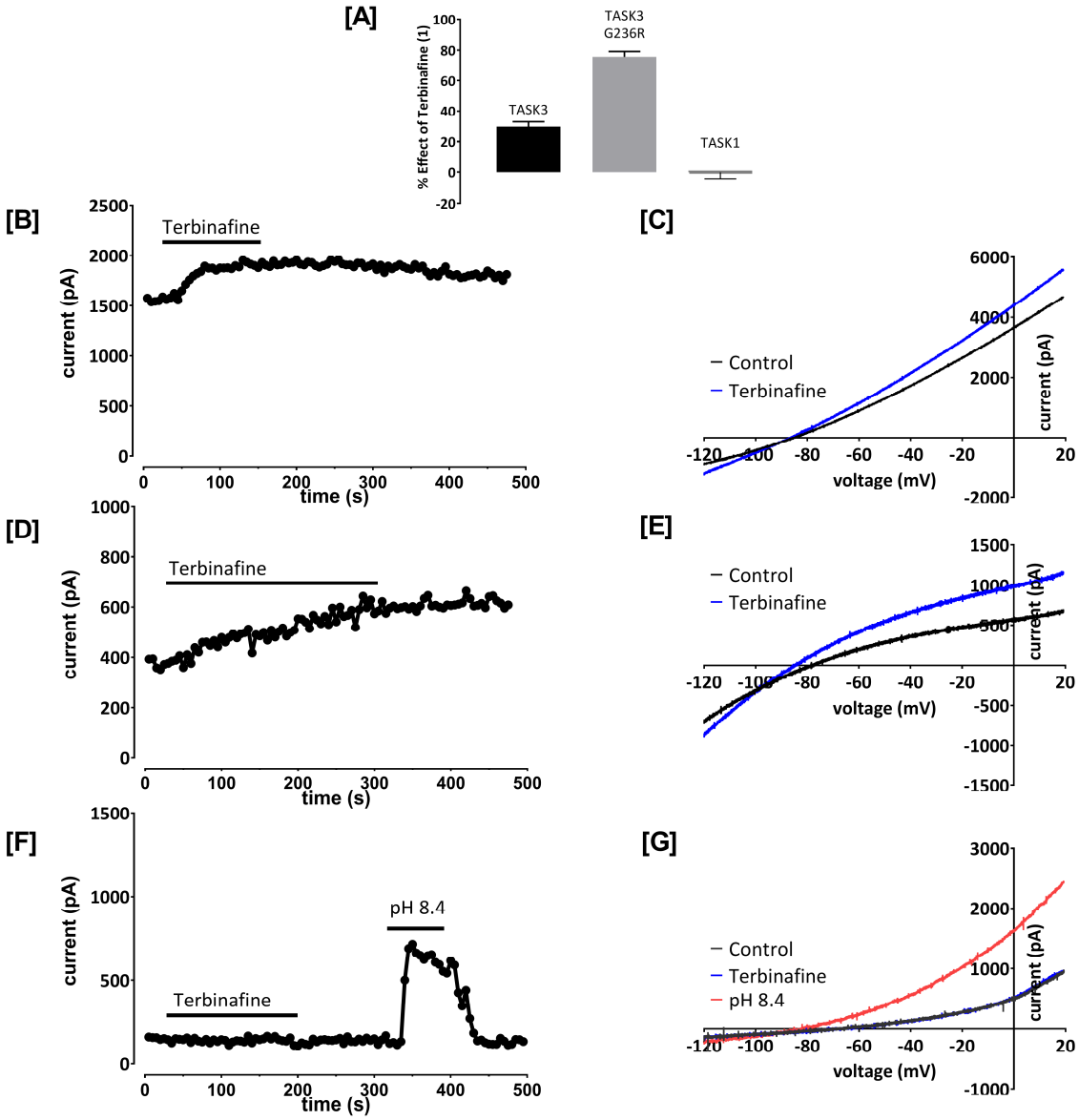
Figure 1 – (A) Assay development shows titration of BacMam correlates with TASK3 activity. Increasing amounts of BacMam (v/v) cause an increase in the rate of thallium flux into cells indicative of channel (TASK3) activity. (B) Pharmacology of TASK3 inhibitors. Exemplar concentration-response curves for A-1899 (circles), 3-CH3 phenyl variant of bis-amide scaffold (squares), 4-pyridyl variant of bis-amide scaffold (triangles) and PK-THPP (diamonds). (C) Terbinafine is an activator of TASK3. Exemplar compound response curves shows activity of Terbinafine at non-transduced U-2 OS cells (circles) or U-2 OS cells transduced with 5% (v/v) TASK3 (squares). All graphs show rate of fluorescence increase between 14-24 seconds. Baseline established for 14 seconds before addition of 2mM Tl^+. Error bars show standard deviation.

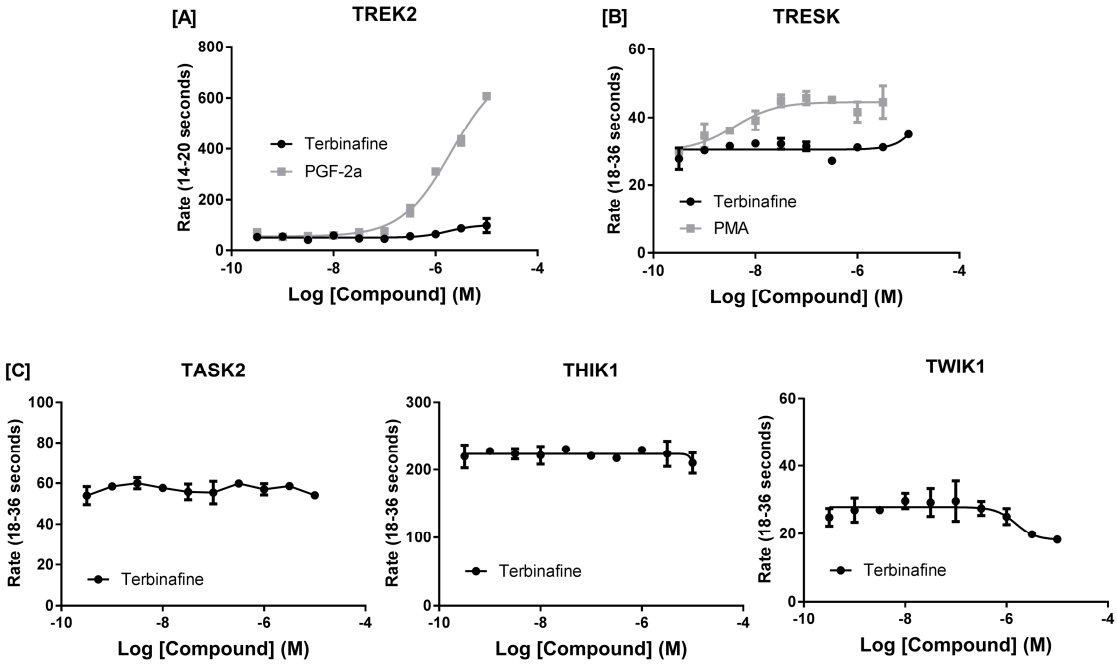
Figure 2 - (A) Enhancement of TASK3, TASK3_G236R and TASK1 channels by terbinafine (1 μM). Time course of enhancement by terbinafine for TASK3 (B), TASK3_G236R (D) and TASK1 (F) channels. Effect of terbinafine on current voltage relationship for TASK3 (C), TASK3_G236R (E) and TASK1 channels (G). The effect of alkaline pH (8.4) on TASK1 channels is also shown (F, G).

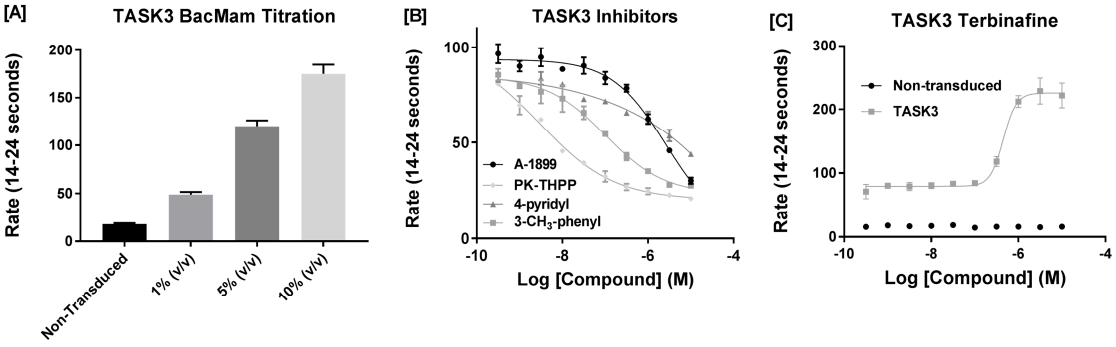
Figure 3 – Activity of Terbinafine at selected K2P channels in thallium flux assays. (A) Terbinafine activity at TREK2. Compound response curves show activity of terbinafine compared to the known TREK2 activator 11-deoxy Prostaglandin F2α (PGF 2a). (B) Terbinafine activity at TRESK. Compound

response curves show activity of Terbinafine compared to the known TRESK activator Phorbol 12-myristate 13-acetate (PMA). (C) Terbinafine activity at TASK2, THIK1 and TWIK1. For these channels no suitable activators were available. All graphs show rate of fluorescence increase between timepoints described on y axis. Baseline established for 14 seconds before addition of 2mM Tl^+. Error bars show standard deviation.

	Entry	Name	R^1	R^2	R^3	R^4	TASK3 pEC50
	1	Terbinafine	H	Me	H		6.2
	2	Naftifine	H	Me	H		<5
	3	Butenafine	H	Me	H		<5
	4	cis-Terbinafine	H	Me		H	<5
	5	N-Desmethyl Terbinafine	H	H	H		<5
	6	n/a	Me	Me	H		6.4


[A]
% Effect of Terbinafine (1)
TASK3
TASK3 G236R
TASK1
[B]
Terbinafine
current (pA)
time (s)
[C]
Control
Terbinafine
current (pA)
voltage (mV)
[D]
Terbinafine
current (pA)
time (s)
[E]
Control
Terbinafine
current (pA)
voltage (mV)
[F]
pH 8.4
Terbinafine
current (pA)
time (s)
[G]
Control
Terbinafine
pH 8.4
current (pA)
voltage (mV)





- Two-pore domain potassium channels (K2Ps) carry background (or leak) potassium current
- Lack of specific, pharmacological tools for K2Ps
- Developed a cell-based assay to identify activators of TASK3
- Terbinafine is a selective activator of TASK3