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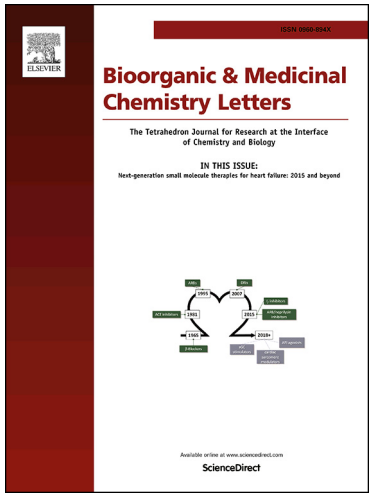

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Towards a TREK-1/2 (TWIK-Related K+ Channel 1 and 2) dual activator tool compound:multi-dimensional optimization of BL-1249

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ABSTRACT

This letter describes a focused, multi-dimensional optimization campaign around BL-1249, a fenamate class non-steroidal anti-inflammatory and a known activator of the K_{2P} potassium channels TREK-1 (K_{2P}2.1) and TREK-2 (K_{2P}10.1). While BL-1249 has been widely profiled *in vitro* as a dual TREK-1/2 activator, poor physicochemical and DMPK properties have precluded a deeper understanding of the therapeutic potential of these key K_{2P} channels across a broad spectrum of peripheral and central human disease. Here, we report multi-dimensional SAR that led to a novel TREK-1/2 dual activator chemotype, exemplified by ONO-2960632/VU6011992, with improved DMPK properties, representing a new lead for further optimization towards robust *in vivo* tool compounds.

The two-pore domain (K_{2P}) family of potassium channels (*KCNK*), or 'leak channels' are responsible for regulating cell excitability and stabilizing cell membrane potential. Since their discovery in 1996, 15 K_{2P} subtypes have been identified that fall into 6 distinct subfamilies with the larger voltage-gated ion channel superfamily[1]. Our interest was in the TREK (TWIK-Related K+ Channel) K_{2P} subfamily, which consists of TREK-1 (K_{2P}2.1), TREK-2 (K_{2P}10.1) and TRAAK (K_{2P}4.1). TREK-1 is widely expressed in the mammalian CNS, as well as the periphery[2], and TREK-1 channel modulation has been reported to have potential therapeutic utility in pain, depression, migraine, ischemia, and arrhythmia among others[1, 3-7]. However, a sentiment routinely echoed in the field is that the lack of selective activator and inhibitor probes for TREK-1 have hindered a deeper understanding of the physiology of the TREK K_{2P} family of channels, and have precluded target validation and realizing therapeutic promise. Several reports have disclosed ligands **1**-**7** capable of activating the TREK K_{2P} family[8-13] (**Fig. 1**), but the majority lack potency, ion channel selectivity, or are thought to be electrophilic,

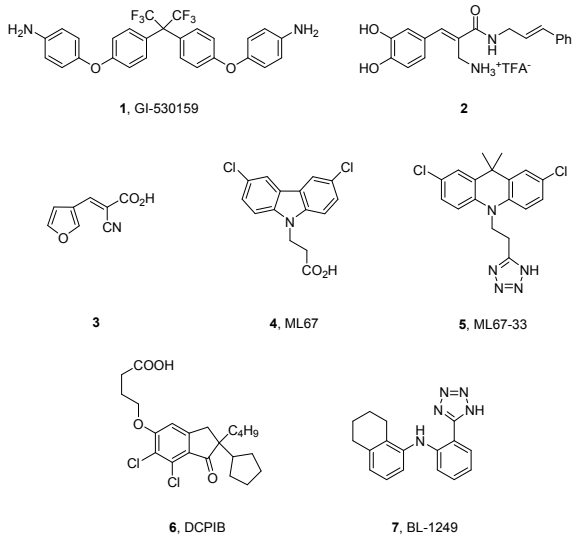


Figure 1. Structures of reported TREK-1 and TREK-2 activators **1**-**7**. To date, these TREK-1/2 ligands lack selectivity and desired potency and/or possess poor physicochemical/drug-like properties coupled with poor DMPK profiles.

and/or possess poor DMPK properties. The most utilized ligand is BL-1249 (**7**), a fenamate class non-steroidal anti-inflammatory, which has served as an *in vitro* and electrophysiology tool as an activator of TREK-1, TREK-2 and TRAAK[14-17]; however, a poor DMPK profile ($t_{1/2}$ = 0.25 hours in rat 0.05 mg/kg i.v. cassette and *vide infra*) has prohibited its use as an *in vivo* probe. Thus, while awaiting hits from a TREK-1 high-throughput screen (HTS), we decided to pursue a limited optimization campaign around **7**, in hopes of delivering an improved TREK K_{2P} family tool compound to aid the field.

Very limited and cursory SAR has been reported for **7** with respect to TREK K_{2P} family activation; therefore, we elected to pursue a multi-dimensional optimization strategy exploring four regions of **7** (**Fig. 2**). Initial attention was devoted to identifying a more polar linker replacement for the aniline linker, as **7** displayed a high cLogP of 4.5 and little fraction unbound in rat and human plasma (f_us ~0.001).

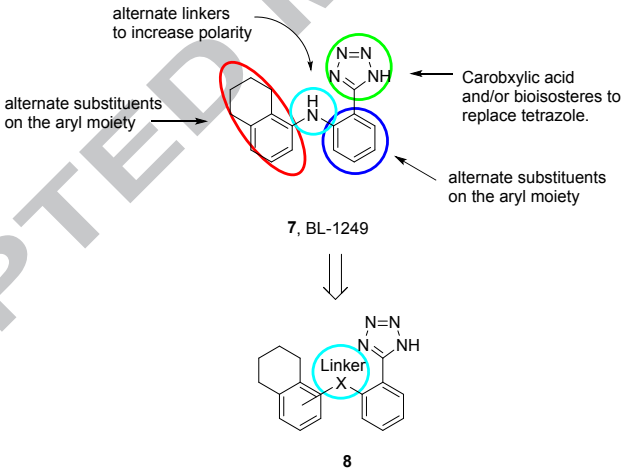
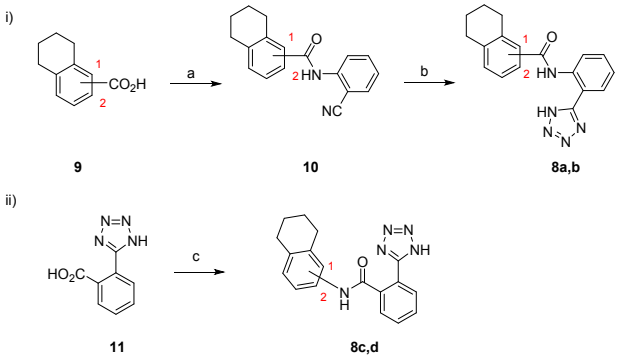


Figure 2. Initial optimization plan for **7** to explore four domains to enhance physicochemical and DMPK properties towards a TREK-1/2 dual activator tool, beginning with surveying replacements for the aniline linker (analogs **8**).

We initially focused on holding the eastern and western regions constant and surveying amide and reverse amide linkers, varying the connectivity to the 1,2,3,4-tetrahydronaphthalene (surveying both C1 and C2) to account for topology changes of the expanded amide linker. The amide congeners **8a** and **8b**, were readily accessible in two steps (**Scheme 1**). Commercial acids **9** were coupled to 2-aminobenzonitrile to afford **10**, followed by a [3+2] cycloaddition reaction with sodium azide to deliver tetrazoles **8a** and **8b** in acceptable overall yields. The reverse amides **8c** and **8d** were prepared in a single step, by a PyClU-mediated coupling of acid **11** and the corresponding tetrahydronaphthalene amines.

Scheme 1. Synthesis of analogs **8**.[a]



[a]Reagents and conditions: (a) $SOCl_2$, PhMe, 90-110 °C, 1.5-2.5 h and then 2-aminobenzonitrile, Et_3N, CH_2Cl_2, 0 °C to r.t., 15-19 h, 24-38%; (b) NaN_3, NH_4Cl, DMF, 130 °C, 5 h, 83%; (c) $ArNH_2$, PyClU, DIPEA, CH_2Cl_2, 0 °C to r.t., 2 h, 28-78%.

As shown in **Figure 3**, only amide **8b**, with an alternate C2 connectivity to the tetrahydronaphthalene core, displayed dual TREK-1/2 activation pharmacology (TREK-1: 24%@10 μM, TREK-2: 21%@10 μM). While **8b** was weaker (TREK-1 EC_{50} = 13.1 μM (46%@30 μM), TREK-2 EC_{50} = ND (Not determined) (77%@30 μM)) than **7** (TREK-1 EC_{50} = 5.2 ± 1.1 μM (109 ± 14%@30 μM), TREK-2 EC_{50} = 7.7 ± 1.9 μM (133 ± 23 %@30 μM)), the $cLogD_{7.4}$ (calculated logD by ADMET Predictor™ for pH7.4) was lowered (**8b**, $cLogD_{7.4}$ = 1.5 versus **7**, $cLogD_{7.4}$ = 2.1). In our experience, employing tetrazoles as a bioisostere of carboxylic acids can offer certain advantages,[18] but we prefer the carboxylic acid moiety, as it is the carboxylate anion at physiological pH and generally shows improvements in fraction unbound in plasma compared to tetrazoles.[19-20] Therefore, we elected to employ the amide linker motif of **8b**, while surveying TREK-1/2 activity in the context of a carboxylic acid moiety, either directly attached to the phenyl ring, or with a methylene spacer. Furthermore, we wanted to replace the metabolically labile tetrahydronaphthalene core (with two benzylic sites) with other lipophilic groups, such as CF_3 and SCF_3 (**Fig. 4**).

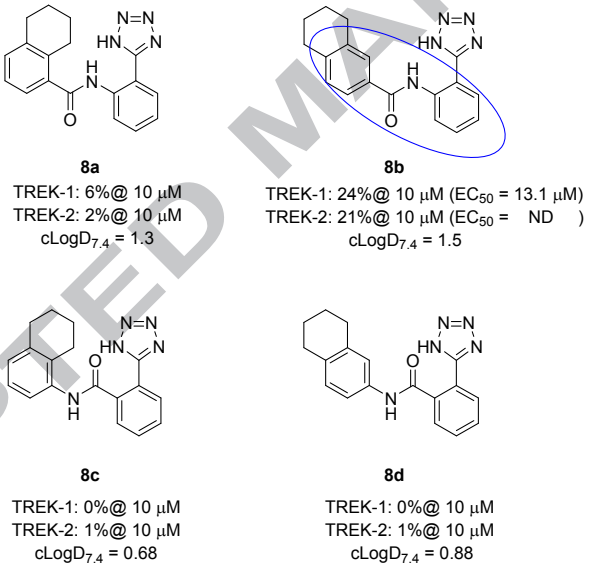


Figure 3. SAR of alternate amide linkers **8a-d** in place of simple amine linkers in **7**. While **8b** lost TREK-1/2 activator potency relative to **7**, $cLogD_{7.4}$ was improved significantly (from 2.1 to 1.5), and future SAR would maintain this linker. ND, not determined. Thallium flux mobilization human TREK-1 and TREK-2 assays were performed n = 1 independent times in triplicate. %activation values are standardized based on the thallium influx of 10μM of BL-1249 as 100% and 0.3% DMSO as 0%, respectively.

As shown in **Figure 4**, a library of analogs **12** was prepared wherein the western phenyl ring harbored CF_3 and SCF_3 groups in the *ortho*-, *meta*- and *para*-positions, in combination with carboxylic acid moieties on the eastern phenyl ring as shown. In order to quickly evaluate the library, we screened the analogs in the human TREK-1 and TREK-2 thallium flux assay at a set, single-point concentration of 30 μM and noted percent activation. SAR was steep, with only a few analogs, including **13** and **14**, displaying TREK-1 and TREK-2 activator pharmacology (See supporting information). Of these, **14** was the most active (TREK-1, 50%@30 μM, TREK-2, 19%@30 μM), and future efforts maintained the 4-SCF_3 moiety.

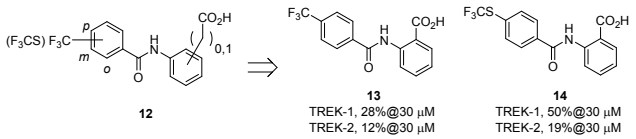
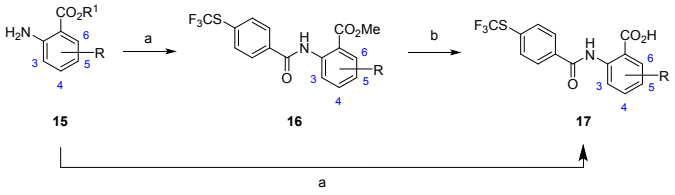


Figure 4. Multi-dimensional SAR exploring lipophilic substituents on the Western phenyl ring in combination with carboxylic acid moieties on Eastern aryl in varying positions and differing tether lengths. Only a few analogs including **13** and **14**, displayed dual TREK-1/2 activation at 30 μM. Thallium flux mobilization human TREK-1 and TREK-2 assays were performed n = 1 independent times in triplicate. %activation values are standardized based on the thallium influx of 10μM of BL-1249 as 100% and 0.3% DMSO as 0%, respectively.

In order to explore SAR around **14** and examine the impact of additional substituents on the eastern benzoic acid moiety, a rapid 1- to 2-step sequence was developed (**Scheme 2**). In cases where the desired amino-benzoic acid **15** (R_1 = H) was readily available, standard acylation with 4-((trifluoromethyl)thio)benzoyl chloride afforded the desired analogs **17**. For benzoate analogs **15** (R_1 = Me), the standard acylation followed by saponification afforded analogs **17**.

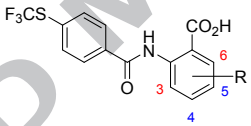
Scheme 2. Synthesis of analogs **17**.[a]



[a]Reagents and conditions: (a) 4-((trifluoromethyl)thio)benzoyl chloride, Et_3N, THF or CH_2Cl_2, 0 °C to r.t. or r.t., 2h to overnight; (b) NaOH-*aq*, DME/MeOH or MeOH/THF, 0 °C to r.t. or r.t., 2h to overnight, 2-65% for two steps.

Structures and TREK-1 and TREK-2 activities for selected analogs **17** are highlighted in **Table 1**. For reference, when full concentration response curves (CRCs) were performed with the unsubstituted congener **14**, it proved to be more potent, but less efficacious for TREK-2 (TREK-1 EC_{50} = 12.8 μM (52%@30 μM), TREK-2 EC_{50} = 5.0 μM (17%@30 μM)), than **7**. A variety of electron withdrawing groups were tolerated in terms of TREK-1/2 potency (e.g., **17b-d and i**), but efficacy was diminished relative to **7**. Electron donating groups proved detrimental to both potency and efficacy (e.g., **17g**, **h**), possibly indicating a key role of the acidity of the carboxylic acid moiety. From this, a di-substituted derivative, **17f**, emerged, harboring a 4-F,5-Br-functionalization, which was equipotent and equi-efficacious (TREK-1 EC_{50} = 6.1 μM (101%@30 μM), TREK-2 EC_{50} = 5.5 μM (84%@30 μM)) with **7**, yet structurally distinct.

Table 1. Structure and TREK-1/2 activities of analogs **17**.



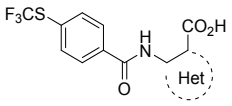
17

Cmpd	R	TREK-1 EC_{50} (μM)[a] (% activation@30 μM)	TREK-2 EC_{50} (μM)[a] (% activation@30 μM)
7	-	5.2 ± 1.1 (109 ± 14%)	7.7 ± 1.9 (133 ± 23%)
14	H	12.8 (52%)	5.0 (17%)
17a	5-F	9.5 (54%)	>30 (43%)
17b	4-Cl	4.4 (66%)	7.5 (73%)
17c	5-Cl	3.8 (69%)	6.8 (76%)
17d	4-Br	4.3 (76%)	5.9 (74%)
17e	5-Br	5.9 (114%)	7.7 (101%)
17f	4-F, 5-Br	6.1 (101%)	5.5 (84%)
17g	4-OMe	16.5 (52%)	28.5 (27%)
17h	5-OMe	20.2 (50%)	>30 (31%)
17i	5-OCF_3	4.6 (57%)	5.6 (60%)
17j	5-NO_2	13.7 (56%)	26.5 (65%)

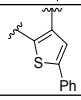
[a]For SAR determination, thallium flux mobilization human TREK-1 and TREK-2 assays were performed n = 1 independent times in triplicate (Mean ± SD for **7**). %activation values are standardized based on the thallium influx of 10 μM of BL-1249 as 100% and 0.3% DMSO as 0%, respectively.

As **17f** and electron deficient ring systems were favored for TREK-1/2 activator pharmacology, we surveyed a variety of electron deficient heterocycles **18** as potential replacements for the phenyl ring of the benzoic acid (**Table 2**). All 6-membered azacines were inactive, including pyridazine (**18a**), pyrazine (**18b**), pyrimidine (**18c**) and pyridine (**18d**). Five-membered heterocycles fared better, with thiophenes showing reasonable potency (e.g., **18g**), but very low efficacy. Thus, they were not progressable as putative tool compounds. Interestingly, it became clear that for this chemotype, as well as the progenitor BL-1249 (**7**), TREK-1 and TREK-2 activator pharmacology could not be separated; thus, a dual TREK-1/2 activator tool became the goal.

Table 2. Structure and TREK-1/2 activities of analogs **18**.



18

Cmpd	Het	TREK-1 EC_{50} (μM)[a] (% activation@30 μM)	TREK-2 EC_{50} (μM)[a] (% activation@30 μM)
7	-	5.2 ± 1.1 (109 ± 14%)	7.7 ± 1.9 (133 ± 23%)
17f	-	6.1 (101%)	5.5 (84%)
18a		ND (1%)	ND (1%)
18b		ND (-6%)	ND (0%)
18c		ND (-4%)	ND (0%)
18d		ND (-4%)	ND (1%)
18e		ND (8%)	ND (5%)
18f		>30 (58%)	>30 (28%)
18g		<3.1 (31%)	<3.6 (12%)
18h		22.4 (83%)	>30 (49%)

[a]For SAR determination, thallium flux mobilization human TREK-1 and TREK-2 assays were performed n = 1 independent times in triplicate (Mean ± SD for **7**). ND, not determined. %activation values are standardized based on the thallium influx of 10μM of BL-1249 as 100% and 0.3% DMSO as 0%, respectively.

Attention then returned to **14**, **17e** and **17f**, and a deeper assessment of physicochemical and *in vitro* DMPK properties (**Table 3**). Relative to **7**, all three new analogs displayed lower or comparable $cLogD_{7.4}$, which translated to improved fraction unbound in plasma (f_u plasma), combined with nature of the carboxylic acid generally showing higher f_u plasma. For comparison, **7** was highly protein bound (rat f_u <0.001), whereas **17f** displayed a lower degree of protein binding (rat f_u = 0.041), coupled with moderate predicted hepatic clearance (rat CL_{hep} = 35.5 mL/min/kg). These properties, along with the exceptional potency and efficacy of **17f** relative to the other members of this series of compounds, necessitated further profiling.

Table 3. *In vitro* DMPK profiles of selected TREK-1/2 activators.

Property	**7**	**14**	**17e**	**17f**
MW	291.3	341.3	420.2	438.1
cLogP (protonated)	4.5	5.0	6.0	6.2
$cLogD_{7.4}$	2.1	1.3	2.2	2.3
TPSA	66.5	66.4	66.4	66.4
In vitro PK parameters				
CL_{hep} (mL/min/kg), rat[a]	>50.9	52.2	19.7	35.5
CL_{hep} (mL/min/kg), human[a]	15.5	10.7	9.7	18.5
Rat f_u plasma	0.001	0.04	0.013	0.041
Mouse f_u plasma	ND	0.081	0.029	0.052
Human f_u plasma	<0.001	0.026	0.003	0.003

ND= not determined; [a]predicted from hepatic microsomal CL_{int} assays.

An oral PK study (3 mg/kg) with **17f** (ONO-2960632/VU6011992) in rats demonstrated a favorable elimination half-life ($t_{1/2}$ = 6.3 hours), a plasma C_{max} of 8.8 μM and an AUC of 60.6 μM*hr, indicating the potential *in vivo* utility of **17f** as a new TREK-1/2 dual activator probe. A Eurofins lead profiling screen (% inhibition @ 30 μM), indicated no significant ancillary pharmacology (<50% inhibition @ 30 μM) at a variety of potassium, calcium and sodium ion channels, as well as relatively selective profile over a diverse panel of GPCRs and transporters (10 targets showing >50% inhibition @ 30 μM out of total 72 targets. See supporting information). Moreover, a manual patch clamp selectivity panel (@ 30 μM) against a large group of diverse potassium channels, was generally clean for channel activation, with the exception of TRAAK activation (See supporting information. TRAAK activator activity was also reported for **7**). Overall, **17f** represents a new TREK-1/2 dual activator chemotype with improved *in vitro* DMPK properties, as well as favorable ancillary pharmacology and oral PK in rats.

In summary, we have reported one of the most extensive SAR campaigns to date around TREK-1/2 activator ligands. Significant progress has been made in advancing the field, and **17f** (ONO-2960632/VU6011992) is a major improvement in pharmacokinetic profile over the previous gold standard in the field, BL-1249 (**7**). Work now focuses on further optimization towards additional TREK-1/2 dual activator *in vivo* probes as well as TREK-1 selective and TREK-2 selective activator *in vivo* probes from within this chemotype as well as other scaffolds[21]. Optimization efforts are underway, and additional findings will be reported in due course.

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Highlights

- First detailed SAR account around TREK1/2 activators
- First optimization campaign directed at BL-1249
- Discovery of new in vitro and in vivo tool compound, ONO-2960632/VU6011992