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Design and Optimization of Sulfone Pyrrolidine Sulfonamide Antagonists of Transient Receptor Potential Vanilloid-4 (TRPV4) with In Vivo Activity in a Pulmonary Edema Model

Joseph E. Pero[*,ϕ], Jay M. Matthews[†], David J. Behm[†], Edward J. Brnardic[†], Carl Brooks[†], Brian W. Budzik[ϕ], Melissa H. Costell[†], Carla A. Donatelli[†], Stephen H. Eisennagel[†], Karl Erhard[ϕ], Michael C. Fischer[I], Dennis A. Holt[†], Larry J. Jolivette[†], Huijie Li[ϕ], Peng Li[ϕ], John J. McAtee[†], Brent W. McCleland[ϕ], Israil Pendrak[ϕ], Lorraine M. Posobiec[I], Katrina L.K. Rivera[†], Ralph A. Rivero[ϕ], Theresa J. Roethke[†], Matthew R. Sender[ϕ], Arthur Shu[ϕ], Lamont R. Terrell[†], Kalindi Vaidya[I], Xiaoping Xu[†], Brian G. Lawhorn[†]*

[ϕ]Flexible Discovery Unit, [†]Heart Failure Discovery Performance Unit and [I]Platform Technology and Sciences

GlaxoSmithKline, 1250 Collegeville Road, Collegeville, Pennsylvania, 19426, United States

ABSTRACT:

Pulmonary edema is a common ailment of heart failure patients and has remained an unmet medical need due to dose-limiting side effects associated with current treatments. Preclinical studies in rodents have suggested that inhibition of TRPV4 cation channels may offer an alternative - and potentially superior - therapy. Efforts directed toward small molecule antagonists of the TRPV4 receptor have led to the discovery of a novel sulfone pyrrolidine sulfonamide chemotype exemplified by lead compound **6**. Design elements toward the optimization of TRPV4 activity, selectivity and pharmacokinetic properties are

described. Activity of leading exemplars **19** and **27** in an *in vivo* model suggestive of therapeutic potential is highlighted herein.

■ INTRODUCTION:

Despite numerous medical advancements and increased awareness of risk factors, heart disease remains one of the leading causes of death.[1] Specifically, approximately 2% of adults in developed countries have experienced congestive heart failure (HF), with this number rising to 6-10% for those exceeding 65 years of age.[2] Over a 12-month period post-diagnosis, the risk of death is approximately 35%, which is comparable to that of severe cancers.[3] HF and its related side effects also pose a significant economic burden on society, with total associated costs in the United States alone projected to increase from $31 billion in 2012 to $70 billion in 2030.[4]

HF is caused by the inability of an impaired left ventricle to pump sufficient blood into peripheral circulation. This results in elevated pulmonary venous pressure (PVP) and disrupts the septal barrier, which separates the circulatory aqueous environment from the alveolar airspaces.[5,6] With an increased flow of fluid from pulmonary circulation into the alveolar space, HF patients frequently suffer from pulmonary edema, which significantly compromises their quality of life and can prove fatal if left untreated.[7]

TRPV4 receptors are members of the Transient Receptor Potential (TRP) superfamily of cation channels.[8] These tetrameric voltage-gated channels are highly expressed in lung endothelial cells[9,10] and are up-regulated in HF patients.[11] Their direct association with pulmonary edema has been implicated by studying the effects of selective TRPV4 antagonist GSK2193874 (Compound **1**, Figure 1a) in rodents.[11] In both acute and chronic preclinical models, pre-treatment with GSK2193874 inhibited HF-induced pulmonary edema and increased arterial oxygenation.[11] In addition, GSK2193874 was effective at reversing HF-induced pulmonary edema in mice subjected to myocardial infarction (MI) for one week prior to treatment.[11]

Importantly, strong evidence indicates that these effects may be relevant to humans. GSK2193874 was found to be potent in recombinant hTRVP4 channels (IC_{50} = 50 nM), and prevented agonist-driven cellular contraction in human umbilical vein endothelial cells (HUVEC's).[11]

These experimental findings suggest that TRPV4 blockade has the potential to lead to a novel treatment for HF-induced pulmonary edema that could prove more effective than current standards-of-care (i.e. diuretics, nitroglycerin, angiotensin antagonists), which have been associated with unfortunate side effects including hypotension and deteriorating renal function.[12,13] As such, a number of selective TRPV4 antagonists have been disclosed in the recent literature with superior drug-like properties (i.e. reduced molecular weight, lower lipophilicity) to GSK2193874. Examples from GlaxoSmithKline include 1-(4-piperidinyl)-benzimidazoles[14] and spirocarbamates (**2**, Figure 1b),[15,16] while Pfizer has disclosed azetidine sulfonamides (**3**, Figure 1c) derived from a high-throughput screen.[17]

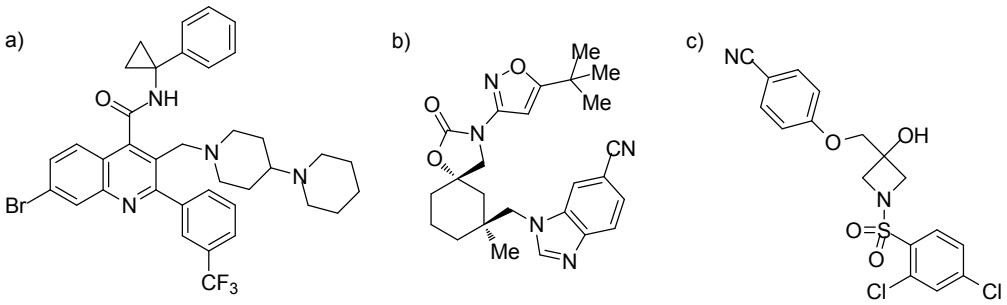


Figure 1. Previously-reported TRPV4 antagonists from GSK (a, b) and Pfizer (c).

With its promising ligand efficiency (LE)[18] and lipophilic ligand efficiency (LLE)[19], **3** was previously utilized as a starting point in efforts directed toward discovering novel TRPV4 antagonists.[20] This led to the discovery of pyrrolidine sulfonamide lead **4**, which displayed encouraging *in vitro* activity (IC_{50} = 32 nM) at recombinant hTRPV4 channels[21] with comparable LE (0.40) and LLE (3.9) relative to

3 (Figure 2). Subsequent scaffold optimization[20] led to diol **5**, which featured excellent LLE (5.3) due to enhanced TRPV4 activity (IC_{50} = 5.0 nM) in conjunction with reduced lipophilicity (cLogP[22] = 3.0).

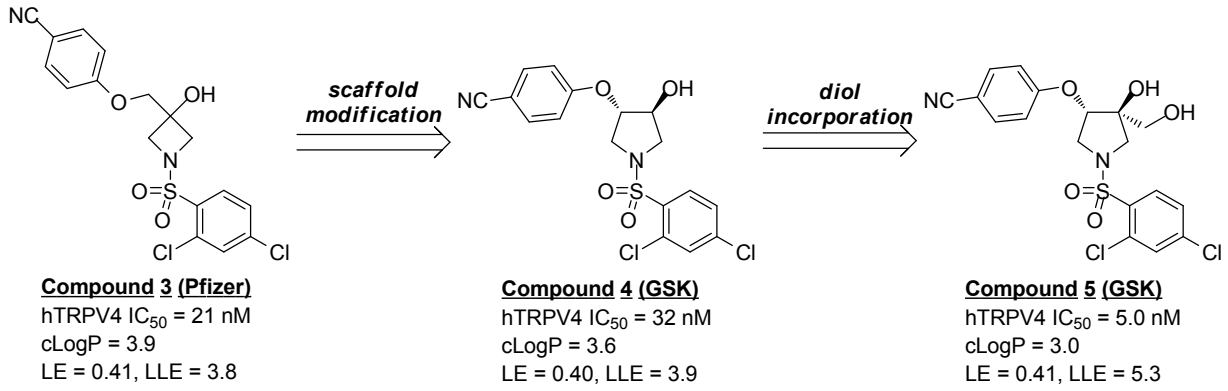


Figure 2. Scaffold modification of **3** to give pyrrolidines **4** and **5**.

The present work describes an analogous structural class exemplified by sulfone **6**, which was viewed as a lead compound superior to ether **4** due to reduced lipophilicity (cLogP = 2.7) and improved LLE (4.9) (Figure 3a). Design elements toward an optimal balance of TRPV4 activity, selectivity and pharmacokinetic (PK) properties are detailed. Evaluation of leading compounds in an *in vivo* rat pulmonary edema assay indicative of therapeutic potential is also provided.

■ RESULTS AND DISCUSSION:

At the outset of our optimization efforts, the potency-enhancing diol of the ether structural class[20] was incorporated to afford Compound **7**, which displayed improvements in both TRPV4 activity (IC_{50} = 7.9 nM) and LLE (5.2) with only a slight increase in lipophilicity (cLogP = 2.9). Notably, the stereoisomers of **7** were also explored (Figure 3b, Compounds **8**-**10**), with each exhibiting considerably weaker activity in the primary FLIPR[21] assay.

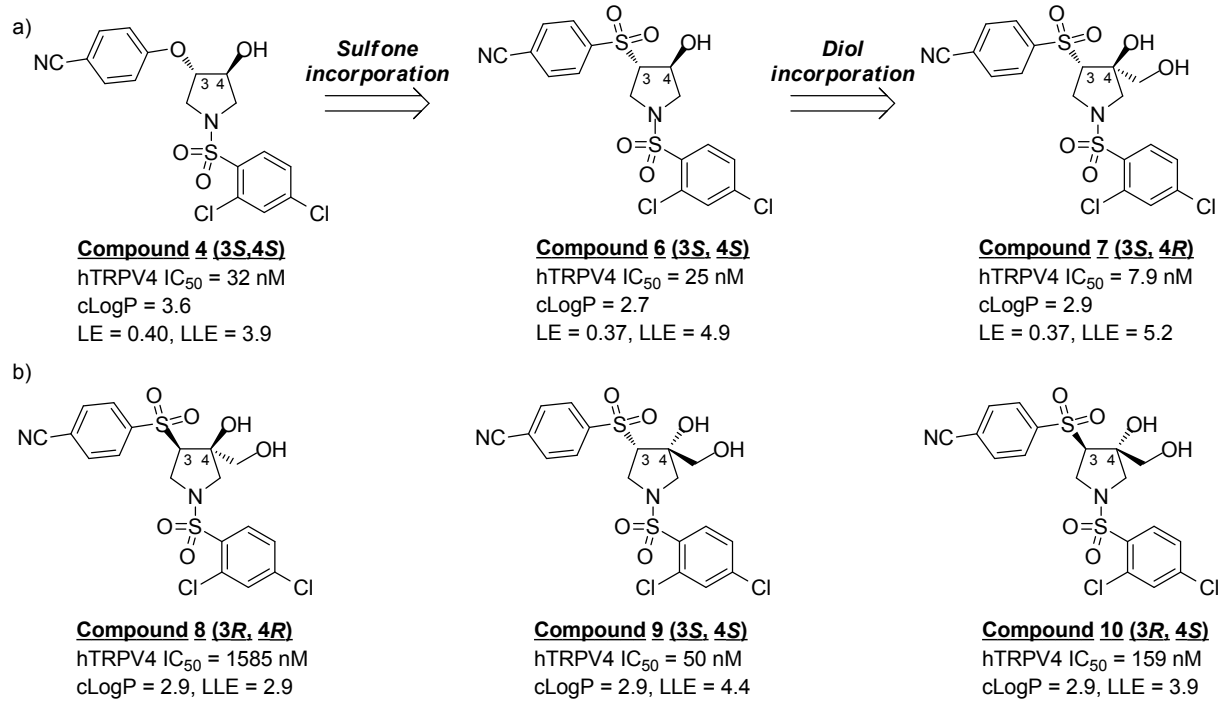


Figure 3. (a) Replacement of phenoxy ether functionality with sulfone, leading to **7**. (b) Additional stereoisomers of sulfone pyrrolidine diol **7**.

An investigation of the PK properties of **7** further substantiated the diol chemotype (Table 1). Following intravenous (i.v.) dosing in rat (1 mpk), **7** exhibited reduced clearance adjusted for unbound fraction[23] ($CL/f_u = 9$) relative to parent compound **6** ($CL/f_u = 30$), suggesting that the potency-enhancing diol functionality did not pose an additional metabolic liability. Furthermore, high permeability[24] (450 nm/sec) and 100% oral bioavailability (F, 2 mpk p.o.) were maintained despite an elevated total polar surface area (PSA = 136). This could be rationalized by a reduction in solvent-accessible PSA due to conformational restriction imparted by intramolecular hydrogen bonds, which were confirmed by vibrational circular dichroism (VCD) studies.[25,26,27]. In addition, despite elevated total clearance (25 mL/min/kg), the mean residence time[28] of **7** (MRT = 1.7 h) was nearly equivalent to that of **6** due to its higher volume of distribution (Vdss = 2.3 L/kg).

Table 1. PK properties (rat) of Compounds **5**, **6** and **7**[a]

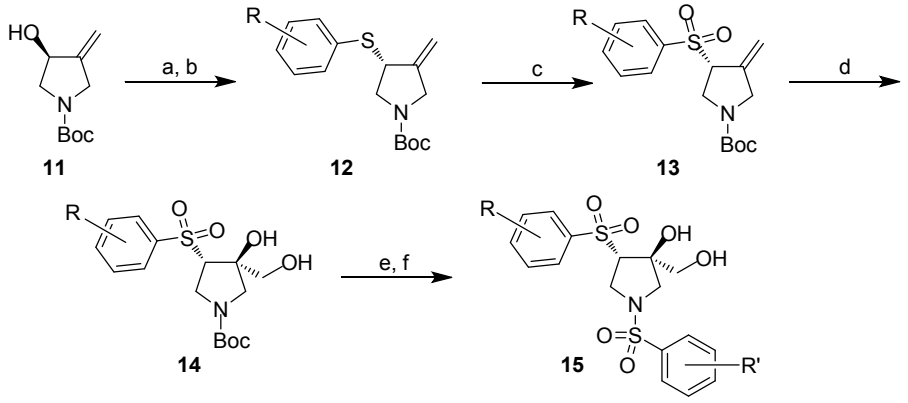
Cmpd	PSA	Perm (nm/sec)	CL (mL/min/kg)	CL/f_u	Vdss (L/kg)	MRT (h)	F (%)
5	111	370	74	15	2.4	0.6	46
6	116	780	9	30	1.1	2.0	100
7	136	450	25	9	2.3	1.7	100

[a]PSA = total polar surface area; Perm = artificial membrane permeability; CL = total clearance; f_u = fraction unbound (rat); Vdss = volume of distribution; MRT = mean resonance time; F = oral bioavailability

A comparison of the PK properties of **7** with corresponding ether **5** provided additional validation of the sulfone structural class. Ether **5** featured higher total clearance (74 mL/min/kg) and clearance adjusted for unbound fraction (CL/f_u = 15) as well as a shorter MRT (0.6 h), potentially due to oxidative metabolism of the phenoxy ether moiety. Furthermore, ether **5** exhibited reduced oral bioavailability (46%) in relation to sulfone **7**.

In order to facilitate analog preparation, a generalized[29] synthetic route toward the sulfone-functionalized pyrrolidine diols was established (Scheme 1). Chiral resolution of known *tert*-butyl-3-hydroxy-4-methylenepyrrolidine-1-carboxylate[30] provided the requisite (*S*)-isomer **11**. Formation of the corresponding mesylate followed by S_N2-displacement with an aryl thiol under basic conditions afforded thioether **12**. Oxidation with *m*-CPBA generated sulfone **13** which then underwent a dihydroxylation to give diol **14** in high diastereomeric fidelity. Establishing the sulfone moiety prior to treatment with osmium tetroxide proved critical, as dihydroxylation of thioether **12** proceeded with significantly lower diastereoselectivity.[31] With advanced intermediate **14** in hand, acid-mediated cleavage of the Boc-carbamate followed by sulfonamide formation under standard conditions generated targeted compounds of generic structure **15**.

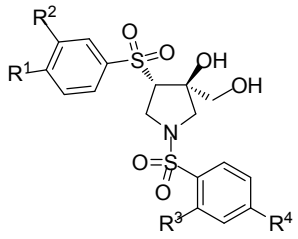
Scheme 1. General synthesis of sulfone pyrrolidine diols[a]



[a]Reagents and conditions: (a) MsCl, TEA, DCM; (b) arylthiol, K_2CO_3, DMF (74-81% over 2 steps); (c) *m*-CPBA, DCM (59-89%); (d) OsO_4, NMO, THF (81-98%, >95:5 dr); (e) HCl/1,4-dioxane or TFA, DCM; (f) arylsulfonyl chloride, $NaHCO_3$, THF (26-85% over 2 steps).

With a superior balance of potency and PK properties being the ultimate goal, the versatility of this synthetic sequence allowed the structure-activity relationships of both the aryl sulfonamide and sulfone moieties to be explored (Table 2). Compound **16** features the installation of a fluoro substituent at R^2 that proved to be a potency enhancer in the aforementioned azetidine[17] and phenoxy ether pyrrolidine[20] structural classes. While Compound **16** indeed demonstrated slight improvements in TRPV4 activity (IC_{50} = 2.0 nM) and LLE (5.6) relative to its des-fluoro counterpart **7**, these benefits were counterbalanced by an erosion in PK properties as exemplified by higher clearance adjusted for unbound fraction (CL/f_u = 14), shorter MRT (0.8 h) and reduced oral bioavailability (51%). One possible rationalization of the higher unbound clearance associated with **16** is that its more electrophilic aryl sulfone functionality was subjected to increased nucleophilic addition by glutathione (GSH), which was suggested by trapping experiments in rat liver microsomes in which only 46% of parent remained after incubation (30 min). Analogous studies with compound **7** showed 89% of parent remaining after incubation.

Table 2. Structure-activity relationships of sulfone pyrrolidine diols[a]



Cmpd	R^1	R^2	R^3	R^4	IC_{50} (nM)	LLE	PSA	CL (mL/min/kg)	CL/f_u	Vdss (L/kg)	MRT (h)	F (%)
7	CN	H	Cl	Cl	7.9	5.2	136	25	9	2.3	1.7	100
16	CN	F	Cl	Cl	2.0	5.6	136	25	14	1.2	0.8	51
17	CN	H	CN	Cl	2.0	6.8	160	82	7	2.6	0.5	25
18	CN	H	Cl	CN	3.2	6.9	160	35	4	2.2	1.0	15
19	Cl	H	Cl	CN	3.2	5.8	136	49	12	4.5	1.6	100

[a]human TRPV4 IC_{50} values measured by a calcium flux FLIPR assay (see Supporting Information for details); LLE = lipophilic ligand efficiency; PSA = total polar surface area; CL = total clearance; f_u = fraction unbound (rat); Vdss = volume of distribution; MRT = mean resonance time; F = oral bioavailability

Replacing the *ortho*-chloro substituent of the aryl sulfonamide with a cyano group (R^3 = CN, Compound **17**) resulted in a 4-fold increase in potency (IC_{50} = 2.0 nM) relative to parent compound **7**. While the LLE improved to 6.8, the PK properties in rat diminished. In particular, the MRT decreased to 0.5 h due to a rise in total clearance (CL = 82 mL/min/kg), and the oral bioavailability was only 25%. Metabolite ID studies in rat hepatocytes suggested that this increased metabolism was largely driven by glutathione-mediated sulfonamide cleavage.

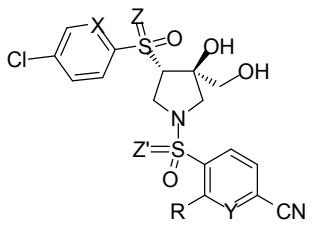
In relation to **17**, Compound **18** featured a transposition of the chloro and cyano substituents on the aryl sulfonamide. This modification preserved the excellent LLE of **17** as well as reduced total clearance (CL = 35 mL/min/kg) and clearance adjusted for unbound fraction (CL/f_u = 4). While the enhanced metabolic stability was reflected in a longer MRT (1.0 h), oral bioavailability remained poor (F

= 15%). Given this result, subsequent targets were aimed at reducing PSA in an effort to improve cell permeability and enhance gastrointestinal absorption. To this end, Compound **19** featured a single-point modification involving replacement of the cyano substituent on the aryl sulfone with a chloride (R^1 = Cl). Relative to parent compound **18**, this maintained potency (IC_{50} = 3.2 nM) while reducing PSA to 136. Despite an increase in both total CL (49 mL/min/kg) and CL/f_u (12), MRT was elongated due to an elevated volume of distribution (V_{dss} = 4.5 L/kg). Gratifyingly, the oral bioavailability improved to 100%, likely driven by enhanced cell permeability (350 nm/sec) and reduced oxidative (first-pass) metabolism of the aryl sulfone functionality.[32]

Our interest in Compound **19** was further buoyed by its clean off-target profile. It exhibited high TRP selectivity (TRPA1, TRPV1, TRPM2, TRPM8, TRPC3-C6 IC_{50} > 30 μM) and weak activity (IC_{50} > 10 μM) against a panel of receptors including CYP's, hERG and $Ca_V1.2$. Furthermore, it was inactive up to 100 μM in an assay measuring time-dependent inhibition (TDI) of CYP3A4.

Additional structural modifications were investigated to reduce lipophilicity in hopes of further enhancing PK properties. This involved the insertion of heteroatoms into the various subunits of the scaffold (Table 3). Compound **20**, featuring a pyridyl sulfonamide functionality (Y = N), demonstrated reduced total clearance (CL = 22 mL/min/kg), outstanding clearance adjusted for unbound fraction (CL/f_u = 1.2), and nearly a 2-fold improvement in MRT (3 h). Unfortunately, however, the TRPV4 potency of **20** (IC_{50} = 631 nM) was approximately 200-fold weaker than that of parent compound **19**. A significant percentage of this activity could be recovered by the re-introduction of a lipophilic *ortho*-substituent on the aryl sulfonamide moiety. In a representative example, Compound **21** (R = CF_3) demonstrated an *in vitro* activity (IC_{50} = 20 nM) and LLE (6.0) comparable to **19**. However, **21** also featured a startling erosion of PK properties (CL = 310 mL/min/kg, CL/f_u = 66, MRT = 0.2 h, F = 8%), prompting us to shift our attention to other subunits.

Table 3. Incorporation of heteroatoms into the pyrrolidine diol scaffold[a]



Cmpd	X	Y	Z	Z'	R	IC_{50} (nM)	LLE	PSA	CL (mL/min/kg)	CL/f_u	Vdss (L/kg)	MRT (h)	F (%)
19	CH	CH	O	O	Cl	3.2	5.8	136	49	12	4.5	1.6	100
20	CH	N	O	O	H	631	4.9	149	22	1.2	4.1	3.0	62
21	CH	N	O	O	CF_3	20	6.0	149	310	66	3.6	0.2	8
22	N	CH	O	O	Cl	5.0	5.7	149	23	11	2.0	1.4	62
23	CH	CH	NH	O	Cl	20	5.0	143	52	4.8	3.2	1.0	38
24	CH	CH	O	NH	Cl	40	4.2	143	48	8.9	2.7	0.9	80

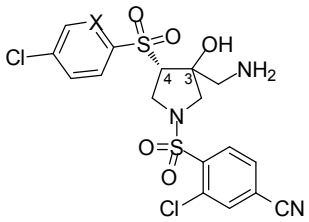
[a]human TRPV4 IC_{50} values measured by a calcium flux FLIPR assay (see Supporting Information for details); LLE = lipophilic ligand efficiency; PSA = total polar surface area; CL = total clearance; f_u = fraction unbound (rat); Vdss = volume of distribution; MRT = mean resonance time; F = oral bioavailability

Installation of a heteroaromatic sulfone proved to be better tolerated in regard to both TRPV4 activity and PK properties. The most encouraging results were attained with a 2-pyridyl sulfone, in which Compound **22** displayed nearly identical TRPV4 potency (IC_{50} = 5 nM), LLE (5.7) and MRT (1.4 h) relative to direct comparator **19**. Notably, oral bioavailability was reduced (F = 62%), possibly due to limited membrane permeability caused by an increase in total polar surface area (PSA = 149).

Structural diversity was further broadened by the exploration of sulfoximine (Compound **23**[33]) and sulfonimidamide (Compound **24**[34]) analogs. While the latter featured excellent oral bioavailability in rat (F = 80%), neither exemplar offered an advantage over parent compound **19** in terms of TRPV4 activity, LLE, or PK properties (CL, MRT, F).

With the inability of Compounds **20**-**24** to improve on the overall profile of Compound **19**, focus was subsequently shifted toward modifications of the diol subunit (Table 4). Specifically, amino alcohol **25**, a direct comparator of **19**, emerged as a benchmark compound with exquisite TRPV4 activity (IC_{50} = 0.3 nM) and exceptional LLE (7.6). Its PK properties in rat were also promising, highlighted by reduced total clearance (CL = 12 mL/min/kg) and CL/f_u (2.7) in addition to an MRT of nearly 6 hours. Compound **26**, the 2-pyridyl sulfone analog of **25**, featured improved oral bioavailability (F = 64%) while maintaining sub-nanomolar potency (IC_{50} = 0.5 nM) and excellent clearance adjusted for unbound fraction (CL/f_u = 3.8). The MRT of Compound **26** (1.9 h), while diminished relative to **25**, remained comparable to that of leading diol **19**. Importantly, Compound **27**, the (3*R*, 4*S*)-diastereomer of **26**, was equipotent in the FLIPR assay (IC_{50} = 0.5 nM), thereby constituting divergent SAR relative to the diol chemotype (Figure 3). Furthermore, it exhibited reduced CL/f_u (1.7) with similar MRT (2.2 h) and oral bioavailability (56%) in comparison to **26**. The encouraging oral bioavailability of **26** and **27** could be due to facilitated gastrointestinal absorption driven by their enhanced aqueous solubility[35] (500 μM), which exceeded that of **25** (280 μM). Along with a potential reduction in first-pass metabolism, this could also explain the improvement relative to diols with similar PSA such as **17** and **18**, which each exhibited lower aqueous solubility (380, 400 μM, respectively).

Table 4. Profiles of amino alcohols **25**, **26** and **27**[a]



Cmpd	X	stereochem.	IC_{50} (nM)	LLE	PSA	CL (mL/min/kg)	CL/f_u	Vdss (L/kg)	MRT (h)	F (%)
25	CH	3*S*, 4*S*	0.3	7.6	142	12	2.7	4.0	5.7	22

26	N	3*S*, 4*S*	0.5	7.4	154	12	3.8	1.3	1.9	64
27	N	3*R*, 4*S*	0.5	7.4	154	1.7	1.7	0.2	2.2	56

[a]human TRPV4 IC_{50} values measured by a calcium flux FLIPR assay (see Supporting Information for details); LLE = lipophilic ligand efficiency; PSA = total polar surface area; CL = total clearance; f_u = fraction unbound (rat); Vdss = volume of distribution; MRT = mean resonance time; F = oral bioavailability

In contrast to the diol exemplars, the off-target profiles of these amino alcohols featured several areas of concern. For one, compound **25** displayed activity in a phenotypic assay measuring phospholipidosis[36] risk (*p*MEC = 4.7, response = 425%)[37], which is not uncommon to lipophilic amines containing aromatic residues (Table 5). Cationic amphiphilic drugs (CAD's) have been reported to cause phospholipidosis in humans and activity in the phenotypic screen has correlated with a CAD "likeness" score exceeding 50.[38] With a CAD score defined as the sum of indicators of lipophilicity (CHI IAM)[39] and basicity (ΔCHI (pH10.5 – pH7.4))[39], design elements to address phospholipidosis risk have been well established. Indeed, amino alcohols **26** and **27**, each featuring lower lipophilicity (CHI IAM = 42, 36, respectively) and basicity (ΔCHI (pH10.5 – pH7.4) = -2.2, 3.7, respectively) relative to **25** (CHI IAM = 49, ΔCHI (pH10.5 – pH7.4) = 9), were gratifyingly inactive in the phenotypic assay (*p*MEC < 4).

Table 5. Off-target profiles of amino alcohols **25**, **26** and **27**[a]

			Phospholipidosis			
Cmpd	hERG IC_{50} (μM)	CAD likeness	*p*MEC	Response[37] (%)	3A4 TDI (100 μM)	3A4 RI IC_{50} (μM)
25	5.3	58	4.7	425	yes	0.1
26	8.0	40	< 4	--	no	0.3
27	8.0	40	< 4	--	no	4.0

[a]Details for hERG, phospholipidosis, 3A4 TDI and 3A4 RI assays described in Supporting Information; *p*MEC = -log(minimum effective concentration)

Compounds **26** and **27** also held additional important advantages over **25**. Whereas the latter exhibited time-dependent inhibition of human CYP3A4 at 100 μM, exemplars **26** and **27** were inactive at the same concentration, potentially a result of their reduced basicity. This may also have contributed to their lower activity (IC_{50} = 8 μM) against the cardiac hERG channel. Ultimately, compound **27** emerged as the most promising amino alcohol within this structural class, with reduced DDI (drug-drug interaction) risk relative to **25** and **26** as demonstrated by its weaker activity in an assay measuring reversible inhibition of CYP3A4 (IC_{50} = 4 μM).

Leading diol **19** and amino alcohol **27** were each investigated in a rodent *in vivo* assay suggestive of therapeutic potential for pulmonary edema (Figure 4). In this model, rats were treated with vehicle or TRPV4 antagonist prior to administration of known TRPV4 agonist, GSK1016790A (100 μg/kg total i.v. dose).[11,40,41] Importantly, previous studies have shown that the effects of GSK1016790A correlate with those induced by myocardial infarction in rodents.[11] In the vehicle-treated group, GSK1016790A demonstrated robust pulmonary edema as quantified by an increase in wet lung weight/body weight ratio relative to naïve animals. Gratifyingly, significant attenuation of agonist effects was observed with diol **19** (2.3 μg/kg/min i.v., $p < 0.01$ vs. vehicle) and amino alcohol **27** (0.2 μg/kg/min i.v., $p < 0.05$ vs. vehicle).[42]

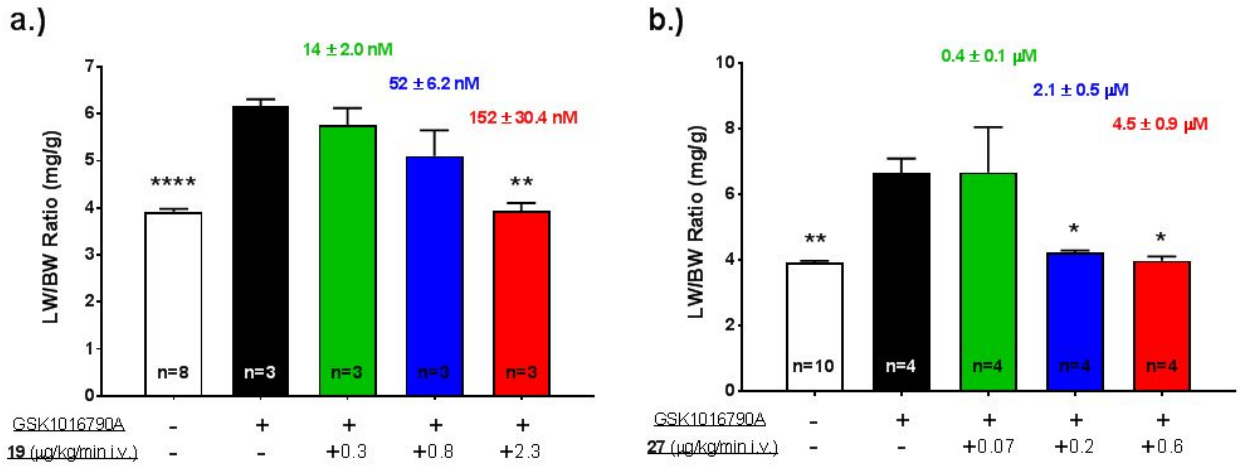


Figure 4. Effects of TRPV4 agonist GSK1016790A (10 μg/kg/min i.v. for 10 min) on lung wet weight/body weight ratio (LW/BW) in vehicle-treated rats and rats pre-treated with TRPV4 antagonists **19** (a) and **27** (b). Full inhibition of agonist-mediated LW/BW response was achieved with the 2.3 μg/kg/min dose of **19** (99% inhibition) and the 0.2 μg/kg/min dose of **27** (88% inhibition). Corresponding steady-state plasma concentrations are indicated above and data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$ vs. vehicle.[43]

Further details regarding the *in vivo* activity of Compounds **19** and **27** are provided in Table 6. Notably, despite slightly weaker *in vitro* potency in rat, Compound **19** featured superior activity (IC_{50} = 33 nM)[44] in the *in vivo* pulmonary edema model in comparison to Compound **27** (IC_{50} = 611 nM). Correcting for rat unbound fraction (rf_u) generated a "free" *in vivo* IC_{50} of 0.3 nM for diol **19**, a significant improvement over that associated with amino alcohol **27** (8.5 nM).

Table 6. *In vivo* activity of Compounds **19** and **27** in the rat pulmonary edema model[a]

Cmpd	rTRPV4 IC_{50} (nM)	*in vivo* IC_{50} (nM)	rf_u (%)	"Free" *in vivo* IC_{50} (nM)
19	4.0	33	4.2	0.3

27	1.0	611	1.4	8.5

[a]rat TRPV4 IC_{50} values measured by a calcium flux FLIPR assay (see Supporting Information for details); rf_u = fraction unbound (rat); conc = concentration

Ultimately, with enhanced *in vivo* activity, less off-target promiscuity and dose-proportional oral exposures in both rat and dog (Table 7), diol **19** was selected over amino alcohol **27** for further progression. To our delight, a 7-day rat safety assessment (100/300 mpk) proceeded with no compound-related macro- or microscopic findings. Based on scaling of preclinical PK data, the exposures at 300 mpk provided minimum rat safety margins of 68-fold and 42-fold over the predicted human C_{max} and AUC, respectively, with C_{min} set at the concentration required for full inhibition in the rat pulmonary edema assay (Figure 4a).

Table 7. PK studies with Compound **19** in rat and dog[a]

Species	p.o. dose (mpk)	$AUC_{0\text{-}t}$ (μg·h/mL)	$DNAUC_{0\text{-}t}$ (h·kg/L)
rat	2.4	1.1	0.5
rat	98	40	0.4
dog	0.9	2.5	2.9
dog	30	90	3.0

[a] n = 3 animals; $AUC_{0\text{-}t}$ = area under the plasma concentration time curve to last time point with quantifiable drug; $DNAUC_{0\text{-}t}$ = dose-normalized $AUC_{0\text{-}t}$.

■ CONCLUSIONS:

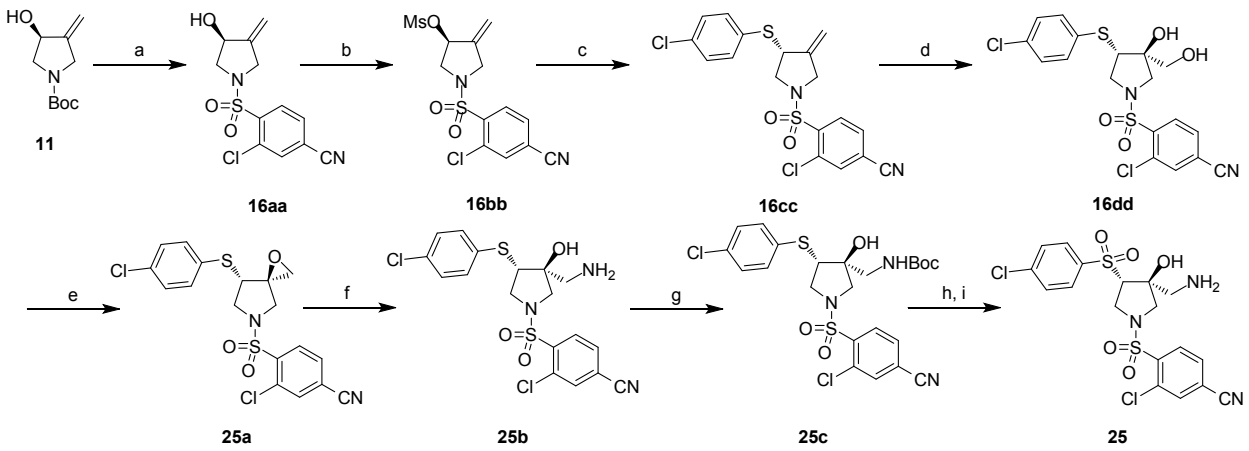
A novel class of sulfone pyrrolidine TRPV4 antagonists[45] has been discovered, with exemplars featuring excellent potency and tractability with respect to physicochemical and PK properties. Structure-activity relationships within a diol chemotype were investigated in order to balance potency with desirable PK properties, whereas efforts within an amino alcohol sub-series leveraged exquisite *in vitro* activity

while addressing off-target concerns. Leading exemplars from both chemotypes (**19** and **27**) demonstrated blockade of TRPV4 agonist effects in an *in vivo* model indicative of therapeutic potential. Of these compounds, **19** emerged as a viable candidate for HF studies which can prove instrumental in assessing the utility of TRPV4 antagonists in the treatment of HF-induced pulmonary edema.

■ EXPERIMENTAL SECTION:

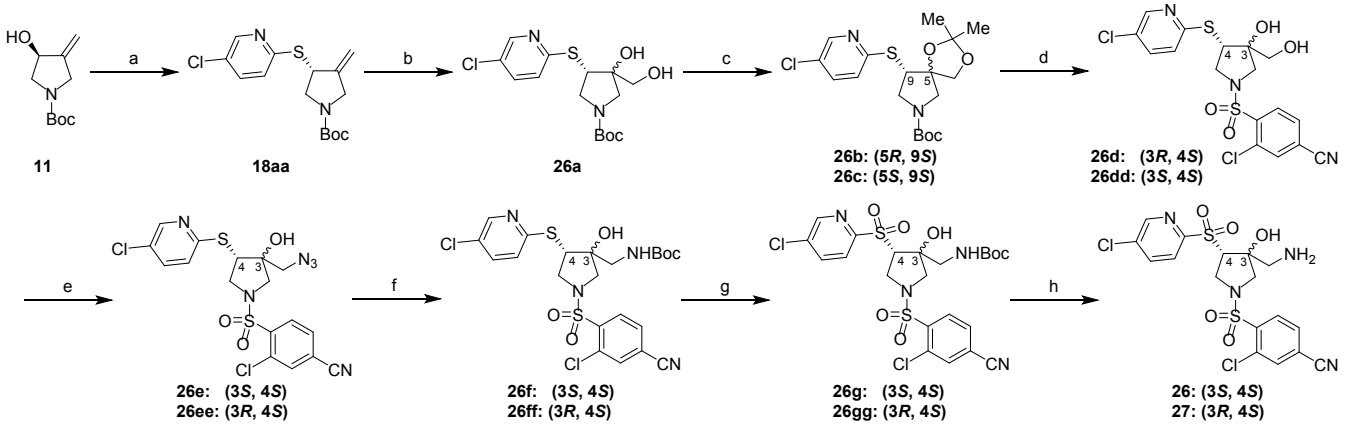
Chemistry. The syntheses of diol **19** and amino alcohols **25-27** are described below. Detailed synthetic protocols and spectroscopic characterizations for Compounds **4**, **6-7**, **16-18** and **20-24** are provided in the Supporting Information (along with procedures for biological assays and pharmacokinetic studies). The purity of each compound was determined to be >95% on a Waters Acquity LCMS equipped with a Acquity™ UPLC BEH C18 1.7mm, 2.1 x 50 mm column using a gradient of 3% to 100% MeCN, 0.1% formic acid at 1 ml/min flow rate with an Acquity PDA detector at 210 and 350 nm. Mass determinations were conducted using a Waters Acquity SQD with positive/negative switching in ESI mode.

Scheme 2. Synthesis of amino alcohol 25[a]



[a]Reagents and conditions: (a) 1. TFA, DCM; 2. 2-chloro-4-cyanobenzene-1-sulfonyl chloride, TEA, DCM (63%); (b) MsCl, TEA, DCM, 0 °C (88%); (c) 4-chlorobenzenethiol, K_2CO_3, DMF, (59%); (d) OsO_4, NMO, THF (37%); (e) MsCl, TEA, K_2CO_3, DCM, MeOH (52%;) (f) NH_3, EtOH, 70 °C, 99%; (g) Boc_2O, TEA, THF (82%); (h) *m*-CPBA, DCM (99%); (i) TFA, DCM (66%).

Scheme 3. Synthesis of amino alcohols 26 and 27[a]



[a]Reagents and conditions: (a) 1. MsCl, TEA, DCM; 2. 5-chloropyridine-2-thiol, K_2CO_3, DMF (91%); (b) OsO_4, NMO, THF (78% (diastereomeric mixture)); (c) propane-2,2-diol, PPTS, DCM (38% (**26b**), 38% (**26c**)); (d) 1. TFA, DCM; 2. $NaHCO_3$, THF, H_2O, 2-chloro-4-cyanobenzene-1-sulfonyl chloride (82% (**26d**), 85% (**26dd**)); (e) 1. MsCl, TEA, DCM; 2. NaN_3, DMF (39% (**26e**), 83% (**26ee**)); (f) 1. PMe_3, THF; 2. Boc_2O, TEA, THF (67% (**26f**), 77% (**26ff**)); (g) *m*-CPBA, DCM (98% (**26g**), 77% (**26gg**)); (h) TFA, DCM (54% (**26**), 50% (**27**)).

(*R*)-*tert*-Butyl 3-((4-chlorophenyl)thio)-4-methylenepyrrolidine-1-carboxylate (12, R = *para*-chloro): To a stirred mixture of (*S*)-*tert*-butyl 3-hydroxy-4 methylenepyrrolidine-1-carboxylate (**11**, 12.33 g, 61.9 mmol, 1 equiv) in DCM (120 mL) was added TEA (12.94 mL, 93 mmol, 1.5 equiv) in one portion. The mixture was subsequently cooled in an ice bath to give an internal temperature of 5 °C. Methanesulfonyl chloride (8.51 g, 74.3 mmol, 1.2 equiv) was added dropwise via a syringe over 40 min such that the internal temperature did not exceed 2 °C. Following the addition, the reaction vessel was removed from the bath and the mixture was allowed to stir at ambient temperature for 45 min. Following this duration, the mixture was diluted with 50 mL of DCM and washed with 40 mL of water. The layers were separated and the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to provide the crude mesylate as a light brown oil (19.9 g), which was immediately carried forward without further purification. To a stirred mixture of the crude mesylate and 4-chlorobenzenethiol (9.40 g, 65.0 mmol) in DMF (125 mL) in an ice bath was added K_2CO_3 (powder form, 12.83 g, 93 mmol) portion-wise. Following the addition, the reaction vessel was removed from the bath and the mixture was allowed to stir at ambient temperature for 1 h. Following this duration, the stirring was stopped and the mixture was allowed to age at RT for 40 min. To this mixture was added 1:1

EtOAc/hexane (400 mL), and ice water (200 mL) sequentially such that the internal temp remained below 20 °C. The resulting mixture was filtered and the filtrate was phase-separated. The organic extract was washed with water (200 mL, then 2 x 150 mL) dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow oil. The crude product was subsequently dissolved in DCM (50 mL), absorbed onto Isolute and concentrated to remove the residual DCM. Purification by silica gel chromatography (0-30% EtOAc:Hexanes) afforded **12** as a clear oil (14.9 g, 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.40 (br. s., 9 H) 3.39 (dd, *J*=11.80, 2.51 Hz, 1 H) 3.69 (dd, *J*=11.80, 6.27 Hz, 1 H) 3.89 (d, *J*=12.05 Hz, 1 H) 4.00 - 4.11 (m, 1 H) 4.47 (br. s., 1 H) 5.01 (br. s., 1 H) 5.11 (s, 1 H) 7.35 - 7.51 (m, 4 H); *m/z* 269.9 $[MH\text{-}t\text{-}Bu]^+$.

(*R*)-*tert*-Butyl 3-((4-chlorophenyl)sulfonyl)-4-methylenepyrrolidine-1-carboxylate (13, R = *para*-chloro): To a cooled solution of (*R*)-*tert*-butyl 3-((4-chlorophenyl)thio)-4-methylenepyrrolidine-1-carboxylate (14.57 g, 44.7 mmol, 1 equiv) in 250 mL of DCM was added *m*-CPBA (25 g, 112 mmol, 2.5 equiv) portion-wise over 7 min such that the internal temperature stayed ≤ -5 °C. After 1 h, to the cold mixture was added 10% wt aqueous $Na_2S_2O_3$ solution (140 mL) portion-wise over 40 min such that the internal temperature remained ≤ -2 °C. To the cooled mixture was added 325 mL of saturated aqueous $NaHCO_3$ over 30 min such that the internal temperature remained ≤ 1 °C. The pH of the resulting mixture was 9. The cold mixture was phase-separated. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to approximately 50 mL total volume. The solution was adsorbed onto Isolute and concentrated to remove residual DCM. Purification by silica gel chromatography (0-70% EtOAc:Hexanes) afforded **13 (R = *para*-chloro)** as a white foam (15.9 g, 89% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.41 (s, 4 H) 1.36 (s, 5 H) 3.53 - 3.72 (m, 2 H) 3.72 - 3.85 (m, 1 H) 3.86 - 3.99 (m, 1 H) 4.67 (d, *J*=7.28 Hz, 1 H) 5.18 (br.s., 1 H) 5.39 (br. s., 1 H) 7.76 (d, *J*=8.28 Hz, 2 H) 7.85 (d, *J*=7.53 Hz, 2 H); *m/z* 380.0 $[M+Na]^+$.

(3*R*,4*S*)-*tert*-Butyl-4-((4-chlorophenyl)sulfonyl)-3-hydroxy-3-(hydroxymethyl)pyrrolidine-1-carboxylate (14, R = *para*-chloro): To a stirred, cooled mixture of (*R*)-*tert*-butyl 3-((4-

chlorophenyl)sulfonyl)-4-methylenepyrrolidine-1-carboxylate (15.9 g, 44.4 mmol) and NMO (10.41 g, 89 mmol, 2 equiv) in 300 mL of THF was added OsO_4 solution in *t*-butanol (25 mL, 2.5% wt, 1.99 mmol, 0.045 equiv) via syringe over 4 min such that the internal temperature did not exceed 15 °C. After completion of addition, the internal temperature was allowed to gradually rise to 22-23 °C within 45 min and remained at 23 °C. After 3 h, an additional 2.8 mL of OsO_4 solution (total 0.05 equiv added) was added. After 5 h, stirring was stopped and the mixture was aged at RT over 14 h. The yellow solution was subsequently chilled in an ice bath and saturated aqueous Na_2SO_3 (100 mL) was added over 10 min (keeping internal temperature at approximately 9 °C), bringing the pH of the reaction mixture to 9-10. The mixture was stirred in the ice bath for 1 h. Following this duration, the ice bath was removed. To the cold mixture was added 100 mL of water and the resulting suspension was filtered through Celite. The cake was washed with 300 mL of EtOAc. The filtrate was phase-separated. The organic layer was washed with brine (30 mL), dried over anhydrous Na_2SO_4 and DARCO (activated charcoal), filtered, and concentrated under reduced pressure. The wet residue was taken up in 200 mL of DCM and washed with 20 mL of brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford an off-white foamy residue. The residue was re-dissolved in DCM and purified by silica gel chromatography (5-70% (3:1 EtOAc:EtOH):Hexanes) to provide **14 (R = *para*-chloro)** as an off-white solid (14.1 g, 81% yield). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 7.91 (d, *J*=8.5 Hz, 2H), 7.77 (d, *J*=8.3 Hz, 2H), 5.53 (d, *J*=9.0 Hz, 1H), 4.85 (d, *J*=4.3 Hz, 1H), 3.98 (d, *J*=4.5 Hz, 1H), 3.82 - 3.42 (m, 5H), 3.28 - 3.16 (m, 1H), 1.40 (s, 9H); *m/z* 414.1 $[M+Na]^+$.

(3*R*,4*S*)-4-((4-Chlorophenyl)sulfonyl)-3-(hydroxymethyl)pyrrolidin-3-ol, hydrochloride: (3*R*,4*S*)-*tert*-Butyl-4-((4-chlorophenyl)sulfonyl)-3-hydroxy-3-(hydroxymethyl)pyrrolidine-1-carboxylate (**14**, R = *para*-chloro, 14.0 g, 35.7 mmol) was dissolved in 4N HCl/1,4-dioxane solution (200 mL, 800 mmol) and the resulting reaction mixture was stirred vigorously for 1 h. The slurry was diluted with TBME (50 mL), filtered, and the solid was washed with TBME (50 mL). The solid was subsequently dried under vacuum to afford 10.8 g (92% yield) of (3*R*,4*S*)-4-((4-chlorophenyl)sulfonyl)-3-

(hydroxymethyl)pyrrolidin-3-ol, hydrochloride as a white solid. ^{1}H NMR (400MHz, Methanol-d_4) δ ppm 8.00 (d, *J*=8.5 Hz, 2H), 7.75 (d, *J*=8.5 Hz, 2H), 4.18 - 4.11 (m, 1H), 4.10 - 3.96 (m, 2H), 3.81 (dd, *J*=8.0, 13.6 Hz, 1H), 3.70 - 3.55 (m, 3H), 3.44 (d, *J*=11.8 Hz, 1H); *m/z* 292.0 $[MH]^+$.

(*S*)-3-Chloro-4-((3-hydroxy-4-methylenepyrrolidin-1-yl)sulfonyl)benzonitrile (16aa): To a solution of (*S*)-*tert*-butyl 3-hydroxy-4-methylenepyrrolidine-1-carboxylate (**11**, 10 g, 50.2 mmol) in DCM (40 mL) was added TFA (58.0 mL, 753 mmol) and the resulting reaction mixture was stirred at RT for 1 h. Following this duration, the reaction contents were concentrated under reduced pressure to give a tan oil, which was suspended in DCM (80 mL) and cooled in an ice bath. To this reaction mixture was added TEA (35.0 mL, 251 mmol) cautiously, followed by 2-chloro-4-cyanobenzene-1-sulfonyl chloride (11.85 g, 50.2 mmol). The reaction mixture was subsequently warmed to RT and stirred at 23 °C for 1 h. The mixture was then diluted with water (80 mL) and extracted with DCM (80 mL). The combined organics were washed with brine and concentrated. Purification by silica gel chromatography (0-60% EtOAc:Hexane) provided **16aa** as a pale yellow solid (9.5 g, 63% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.12 (dd, *J*=9.91, 5.65 Hz, 1 H) 3.64 (dd, *J*=10.04, 6.27 Hz, 1 H) 4.04 - 4.10 (m, 1 H) 4.49 (d, *J*=5.27 Hz, 1 H) 5.11 (d, *J*=1.51 Hz, 1 H) 5.18 (d, *J*=1.76 Hz, 1 H) 5.56 (d, *J*=5.02 Hz, 1 H) 8.03 - 8.07 (m, 1 H) 8.11 - 8.14 (m, 1 H) 8.36 (d, *J*=1.26 Hz, 1 H); *m/z* 280.8 $[MH\text{-}H_2O^+]$.

(*S*)-1-((2-Chloro-4-cyanophenyl)sulfonyl)-4-methylenepyrrolidin-3-yl methanesulfonate (16bb): To a solution of (*S*)-3-chloro-4-((3-hydroxy-4-methylenepyrrolidin-1-yl)sulfonyl)benzonitrile (**16aa**, 9.50 g, 31.8 mmol) in DCM (80 mL) in an ice bath was added TEA (7.80 mL, 56.0 mmol) followed by methanesulfonyl chloride (3.10 mL, 39.7 mmol). The resulting reaction mixture was stirred at RT for 1 h. Following this duration, water (80 mL) was added and the resulting layers were separated. The aqueous layer was extracted with DCM (1 x 80 mL). The combined organics were washed with water (2 x), dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give an off-white solid. Trituration with 15% EtOAc in hexane, filtration and concentration under reduced pressure afforded **16bb** as a white solid (10.6 g, 88% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.24 (s, 3 H)

3.66 - 3.81 (m, 2 H) 4.02 (d, *J*=14.56 Hz, 1 H) 4.20 (d, *J*=14.31 Hz, 1 H) 5.45 (s, 1 H) 5.51 (d, *J*=1.76 Hz, 2 H) 7.99 - 8.09 (m, 1 H) 8.11 - 8.20 (m, 1 H) 8.37 (d, *J*=1.51 Hz, 1 H); *m/z* 280.8 $[MH\text{-}H_2O]^+$.

(*R*)-3-Chloro-4-((3-chlorophenyl)thio)-4-methylenepyrrolidin-1-yl)sulfonyl)benzonitrile (16cc): To a solution of (*S*)-1-((2-chloro-4-cyanophenyl)sulfonyl)-4-methylenepyrrolidin-3-yl methanesulfonate (**16bb**, 2.5 g, 6.63 mmol) in chloroform (100 mL) at RT was added 4-chlorobenzenethiol (1.151 g, 7.96 mmol). Triethylamine (2.312 mL, 16.59 mmol) was then added and the resulting mixture was allowed to stir at RT for 18 h. Following this duration, the reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography to give **16cc** as a white solid (1.7 g, 59%). ^{1}H NMR (400MHz, CHLOROFORM-*d*) δ ppm 8.21 (d, *J*=8.0 Hz, 1H), 7.85 (s, 1H), 7.71 (d, *J*=8.3 Hz, 1H), 7.36 - 7.26 (m, 4H), 5.11 (d, *J*=7.3 Hz, 2H), 4.30 - 4.21 (m, 1H), 4.12 (t, *J*=14.2 Hz, 2H), 3.89 (dd, *J*=6.3, 11.0 Hz, 1H), 3.56 (dd, *J*=4.0, 11.0 Hz, 1H); *m/z* 425.0 $[MH]^+$.

3-Chloro-4-(((3*R*,4*S*)-4-((4-chlorophenyl)thio)-3-hydroxy-3-(hydroxymethyl)pyrrolidin-3-yl)sulfonyl)benzonitrile (16dd): To a solution of (*R*)-3-chloro-4-((3-((4-chlorophenyl)thio)-4-methylenepyrrolidin-1-yl)sulfonyl)benzonitrile (**16cc**, 1.66 g, 3.90 mmol) in tetrahydrofuran (13.0 mL) was added NMO (0.503 g, 4.29 mmol) and osmium tetroxide (2.5% in *t*-BuOH, 2.45 mL, 0.195 mmol) sequentially to give a bright yellow solution. After 30 min, an additional 270 mg (0.6 equiv) of NMO was added. After 30 min, an additional 150 mg (0.3 equiv) of NMO was added. After 10 min, saturated aqueous $NaHSO_3$ was added (40 mL) followed by DCM (100 mL). The layers were separated and the organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. Purification by silica gel chromatography (0-50% EtOAc:Hexanes) afforded **16dd** as a white solid (660 mg, 37% yield). ^{1}H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 1.63 (br. s., 1 H) 2.03 (br. s., 1 H) 3.09 (s, 1 H) 3.50 (d, *J*=10.79 Hz, 1 H) 3.59 (dd, *J*=10.54, 2.76 Hz, 1 H) 3.70 - 3.77 (m, 2 H) 3.97 (d, *J*=11.29 Hz, 1 H) 4.03 (dd, *J*=10.54, 6.02 Hz, 1 H) 7.27 - 7.39 (m, 4 H) 7.69 (d, *J*=8.03 Hz, 1 H) 7.84 (s, 1 H) 8.19 (d, *J*=8.28 Hz, 1 H); *m/z* 441.0 $[M\text{-}OH]^+$.

(*R*)-*tert*-Butyl 3-((5-chloropyridin-2-yl)thio)-4-methylenepyrrolidine-1-carboxylate (18aa):

To a solution of (*S*)-*tert*-butyl 3-hydroxy-4-methylenepyrrolidine-1-carboxylate (**11**, 2.0 g, 10.04 mmol) in DCM (30 mL) was added TEA (2.1 mL, 15.07 mmol), followed by mesyl chloride (0.94 mL, 12.06 mmol) dropwise. The resulting reaction mixture was stirred at RT for 30 min. Following this duration, the reaction mixture was diluted with water and extracted with dichloromethane. The organic extract was washed with water, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure to give the desired mesylate. The intermediate was immediately dissolved in *N*,*N*-dimethylformamide (100 mL). 5-Chloropyridine-2-thiol (1.46 g, 10.04 mmol) was then added, followed by potassium carbonate (2.08 g, 15.06 mmol). The reaction mixture was stirred for 1 h at RT, diluted with water and extracted with ethyl acetate. The organic extract was washed with water, dried over anhydrous magnesium sulfate and filtered. The filtrate was subsequently concentrated under reduced pressure and purified by silica gel chromatography (0-15% EtOAc:Hexanes) to give **18aa** as a clear, colorless oil (3.0 g, 91% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.39 - 1.41 (m, 9 H) 3.53 - 3.59 (m, 1 H) 3.86 - 4.00 (m, 2 H) 4.13 - 4.32 (m, 2 H) 4.84 - 4.97 (m, 1 H) 5.01 - 5.13 (m, 1 H) 7.41 - 7.45 (m, 1 H) 7.71 - 7.76 (m, 1 H) 8.46 - 8.49 (m, 1 H); *m/z* 327.0 $[MH]^+$.

3-Chloro-4-(((3*R*,4*S*)-4-((4-chlorophenyl)sulfonyl)-3-hydroxy-3-(hydroxymethyl)pyrrolidin-1-yl)sulfonyl)benzonitrile (19): To a solution of (3*R*,4*S*)-4-((4-chlorophenyl)sulfonyl)-3-(hydroxymethyl)pyrrolidin-3-ol, hydrochloride (9.66 g, 29.4 mmol) in THF (100 mL) was added saturated aqueous $NaHCO_3$ (150 mL) and the resulting reaction mixture was stirred for 5 min, to which was added a suspension of 2-chloro-4-cyanobenzene-1-sulfonyl chloride (7.02 g, 28.8 mmol) in THF (150 mL) portion-wise at 10 °C. The resulting reaction mixture was stirred for 1 h, at which an additional 2-chloro-4-cyanobenzene-1-sulfonyl chloride (0.7 g) was added and the reaction mixture was stirred for an additional 1 h. Following this duration, the reaction mixture was diluted with EtOAc and H_2O, the layers separated, and the aqueous phase extracted with EtOAc. The combined organics were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude oil was purified in two batches, using flash column chromatography eluting with a 5% EtOAc-hexanes to 100% EtOAc gradient. The pure

product-containing fractions were combined and the solvent evaporated under reduced pressure to afford an off-white foam (12.7 g). The foam was triturated with heptane (50 mL) and DCM (5 mL) and the solvent was evaporated under reduced pressure and dried under vacuum to afford 11.6 g (80% yield) of **19** as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.38 (d, *J*=10.04 Hz, 1 H) 3.59 (dd, *J*=11.29, 3.01 Hz, 1 H) 3.63 - 3.72 (m, 2 H) 3.74 - 3.87 (m, 2 H) 4.14 (dd, *J*=7.28, 3.01 Hz, 1 H) 4.94 (t, *J*=5.40 Hz, 1 H) 5.76 (s, 1 H) 7.68 - 7.79 (m, 2 H) 7.82 - 7.90 (m, 2 H) 8.01 - 8.08 (m, 1 H) 8.14 (s, 1 H) 8.36 (s, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 48.3, 56.8, 63.4, 68.6, 81.8, 116.9, 117.1, 130.3, 130.4, 131.9, 132.3, 132.4, 136.2, 137.8, 139.9, 140.9; LRMS: *m/z* 491.1 $[MH]^+$. HRMS: *m/z* 602.96736 (observed), 602.96717 (calculated) (TFA-adduct). Chiral purity was determined to be 100% by chiral HPLC (CHIRALPAK IC, 5 μ, 30 x 250 mm, 30:70 Heptane:EtOH). $[\alpha]^{D}_{20}$ = +53° (c = 0.2, CH_3OH).

3-Chloro-4-(((3*S*,7*S*)-7-((4-chlorophenyl)thio)-1-oxa-5-azaspiro[2.4]heptan-5-yl)sulfonyl-benzonitrile (25a): To a solution of **16dd** (0.700 g, 1.52 mmol) in DCM (20.0 mL) was added TEA (0.380 mL, 2.73 mmol) followed by mesyl chloride (0.140 mL, 1.79 mmol). The resulting reaction mixture was stirred at RT for 0.5 h. Following this duration, the reaction mixture was concentrated under reduced pressure to afford a white solid. The solid was dissolved in MeOH (20 mL) to which was added K_2CO_3 (0.316 g, 2.29 mmol). The resulting reaction mixture was stirred at RT for 3 h, diluted with H_2O and extracted with EtOAc. The combined organics were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (0-35% EtOAc:Hexanes) to afford **25a** as a white solid (350 mg, 52% yield); *m/z* 441.0 $[MH]^+$.

4-(((3*S*,4*S*)-3-(Aminomethyl)-4-((4-chlorophenyl)thio)-3-hydroxypyrrolidin-1-yl)sulfonyl-3-chloro benzonitrile (25b): A suspension of **25a** (0.100 g, 0.227 mmol) in 2 M NH_3 / EtOH (2.0 mL, 4.00 mmol) was irradiated under microwave irradiation at 70 °C for 0.5 h. The reaction mixture was cooled to ambient temperature and the solvent was evaporated under reduced pressure. The residue was dissolved in MeOH and loaded onto a SCX cartridge (2.0 g), eluting with MeOH, followed by 2 M ammonia in

MeOH solution. The ammonia solution was collected and concentrated under reduced pressure to afford **25b** as an oil (100 mg, 99% yield); *m/z* 458.0 $[MH]^+$.

***tert*-Butyl(((3*S*,4*S*)-1-((2-chloro-4-cyanophenyl)sulfonyl)-4-((4-chlorophenyl)thio)-3-hydroxypyrrolidin-3-yl)methyl)carbamate (25c)** To a solution of **25b** (0.100 g, 0.218 mmol) in THF (3.0 mL) was added TEA (0.061 mL, 0.436 mmol), followed by Boc_2O (0.061 g, 0.262 mmol) and the resulting reaction mixture was stirred at RT for 1 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organics were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (0-35% EtOAc:Hexanes) to afford **25b** as a colorless semi-solid (105 mg, 82% yield); *m/z* 558.2 $[MH]^+$.

4-(((3*S*,4*S*)-3-(Aminomethyl)-4-((4-chlorophenyl)sulfonyl)-3-hydroxypyrrolidin-1-yl)sulfonyl)-3-chlorobenzonitrile (25): To a solution of **25c** (0.105 g, 0.188 mmol) in DCM (3.0 mL) was added *m*-CPBA (0.065 mL, 0.376 mmol), and the reaction mixture was stirred at ambient temperature for 24 h. The reaction mixture was diluted with H_2O and extracted with DCM. The combined organics were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (0-40% EtOAc:Hexanes) to afford 110 mg (99% yield) of the corresponding sulfone as a semi-solid (*m/z* 490.0 $[MH]^+$). To a solution of the sulfone (110 mg, 0.186 mmol) in DCM (1 mL) was added TFA (0.2 mL, 2.60 mmol) and the reaction mixture was stirred at RT for 0.5 h. Following this duration, the reaction mixture was concentrated under reduced pressure, diluted with H_2O, neutralized with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The combined organics were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0-5% MeOH:DCM) and further purified by semi-prep HPLC, eluting with a 10% MeCN-H_2O to 90% MeCN-H_2O gradient. The pure product-containing fractions were combined and concentrated under reduced pressure to afford **25** as a white solid (64 mg, 66% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.42 - 3.48 (m, 1 H) 3.56 - 3.62 (m, 1 H) 3.66 - 3.74 (m, 1 H) 3.81 - 3.94 (m, 1

H) 4.21 - 4.30 (m, 1 H) 6.31 - 6.59 (m, 1 H) 7.17 - 7.36 (m, 1 H) 7.83 (s, 4 H) 8.12 (s, 2 H) 8.39 (s, 1 H); *m/z* 489.9 $[MH]^+$.

(3*R*,4*S*) and (3*S*,4*S*)-*tert*-Butyl 4-((5-chloropyridin-2-yl)thio)-3-hydroxy-3-(hydroxymethyl)pyrrolidine-1-carboxylate (26a): To a solution of **18aa** (3.0 g, 9.18 mmol) in THF (30 mL) was added 50% aqueous NMO (2.85 mL, 13.8 mmol) followed by 2.5% OsO_4 in *t*-BuOH (4.61 mL, 0.367 mmol) and the reaction mixture was stirred at RT for 2 h. Following this duration, the reaction mixture was quenched with saturated aqueous Na_2SO_3 and extracted with EtOAc. The organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0-55% EtOAc:Hexanes) to afford **26a** as a colorless oil (2.58 g, 78% yield, diastereomeric mixture). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.36 - 1.42 (m, 9 H) 3.15 - 3.31 (m, 2 H) 3.85 - 3.98 (m, 1 H) 4.00 - 4.07 (m, 2 H) 4.13 - 4.32 (m, 1 H) 4.86 - 5.09 (m, 1 H) 5.33 - 5.49 (m, 1 H) 7.27 - 7.49 (m, 1 H) 7.66 - 7.86 (m, 1 H) 8.36 - 8.61 (m, 1 H); *m/z* 305.0 $[MH\text{-}Boc]^+$.

(5*R*,9*S*)-*tert*-Butyl 9-((5-chloropyridin-2-yl)thio)-2,2-dimethyl-1,3-dioxa-7-azaspiro[4.4]nonane-7-carboxylate (26b) and **(5*S*,9*S*)-*tert*-butyl 9-((5-chloropyridin-2-yl)thio)-2,2-dimethyl-1,3-dioxa-7-azaspiro[4.4]nonane-7-carboxylate (26c):** To a solution of **26a** (2.58 g, 7.15 mmol) in DCM (20 mL) was added 2,2-dimethoxypropane (2.64 mL, 21.4 mmol) and PPTS (0.136 g, 0.715 mmol) and the resulting reaction mixture was stirred at RT for 0.5 h. Following this duration, the reaction mixture was diluted with H_2O and extracted with DCM. The organics were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0-25% EtOAc:Hexanes). Pure product-containing fractions of the *trans*-isomer (first eluting peak) were combined and concentrated under reduced pressure to afford **26b** as a clear oil (1.1 g, 38% yield). Pure product-containing fractions of the *cis*-isomer (second eluting peak) were combined and concentrated under reduced pressure to afford **26c** as a clear oil (1.1 g, 38% yield). **26b**: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.30 - 1.56 (m, 15 H) 3.39 (d, *J*=12.80 Hz, 1 H) 3.51 (d, *J*=11.04 Hz, 1 H) 3.70

- 3.93 (m, 1 H) 4.00 - 4.12 (m, 3 H) 4.37 (d, *J*=15.31 Hz, 1 H) 7.46 (d, *J*=8.53 Hz, 1 H) 7.83 (d, *J*=8.53 Hz, 1 H) 8.55 (s, 1 H); *m/z* 345.2 [MH-*t*-Bu]$^+$. **26c:** ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.33 - 1.44 (m, 15 H) 3.23 (d, *J*=11.80 Hz, 1 H) 3.45 - 3.61 (m, 2 H) 3.93 - 4.04 (m, 3 H) 4.29 - 4.49 (m, 1 H) 7.44 (d, *J*=8.53 Hz, 1 H) 7.79 (d, *J*=6.53 Hz, 1 H) 8.51 (s, 1 H); *m/z* 345.2 [MH-*t*-Bu]$^+$.

3-Chloro-4-(((3*R*,4*S*)-4-((5-chloropyridin-2-yl)thio)-3-hydroxy-3-(hydroxymethyl)pyrrolidin-1-yl)sulfonyl)benzonitrile (26d): To a solution of **26b** (0.800 g, 1.99 mmol) in DCM (3.0 mL) was added TFA (7.69 mL, 100 mmol) and the resulting reaction mixture was stirred for 0.5 h. Following this duration, the reaction mixture was concentrated and treated with saturated aqueous $NaHCO_3$. The resulting suspension was diluted with THF (10 mL). A mixture of 2-chloro-4-cyanobenzene-1-sulfonyl chloride (0.921 g, 3.90 mmol) in THF (10 mL) was added dropwise and the reaction mixture was allowed to stir at RT for 0.5 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The organics were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0-60% EtOAc:Hexanes) to afford **26d** as a semi-solid (853 mg, 82% yield); *m/z* 460.1 [MH]$^+$.

3-Chloro-4-(((3*S*,4*S*)-4-((5-chloropyridin-2-yl)thio)-3-hydroxy-3-(hydroxymethyl)pyrrolidin-1-yl)sulfonyl)benzonitrile (26dd) was prepared from **26c** analogously to the synthesis of **26d** from **26b** to afford **26dd** as a white solid (390 mg, 85% yield). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.50 (s, 1H), 8.37 (s, 1H), 8.15 (d, *J*=8.0 Hz, 1H), 8.10 - 8.03 (m, 1H), 7.76 (dd, *J*=2.3, 8.5 Hz, 1H), 7.39 (d, *J*=8.5 Hz, 1H), 5.63 (s, 1H), 5.18 - 5.04 (m, 1H), 4.27 (t, *J*=9.2 Hz, 1H), 4.21 - 4.13 (m, 1H), 3.67 (d, *J*=10.3 Hz, 1H), 3.54 - 3.40 (m, 2H), 3.36 (br. s., 2H); *m/z* 460.3 [MH]$^+$.

4-(((3*S*,4*S*)-3-(Azidomethyl)-4-((5-chloropyridin-2-yl)thio)-3-hydroxypyrrolidin-1-yl)sulfonyl)-3-chlorobenzonitrile (26e): To a solution of **26d** (0.280 g, 0.608 mmol) in DCM (8.0 mL) was added TEA (0.153 mL, 1.09 mmol) followed by MsCl (0.057 mL, 0.730 mmol) and the reaction mixture was stirred at RT for 10 min. Following this duration, the reaction mixture was diluted with H_2O and extracted with DCM. The combined organics were dried over anhydrous $MgSO_4$, filtered and concentrated under

reduced pressure. The residue was dissolved in DMF (5.0 mL), to which was added NaN_3 (0.099 g, 1.52 mmol) and the reaction mixture was stirred at 80 °C for 40 min. The reaction mixture was subsequently cooled to ambient temperature, diluted with H_2O and extracted with EtOAc. The combined organics were washed with H_2O, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (0-25% EtOAc:Hexanes) to afford **26e** as a colorless wax (115 mg, 39% yield); *m/z* 485.1 $[MH]^+$.

4-(((3*R*,4*S*)-3-(Azidomethyl)-4-((5-chloropyridin-2-yl)thio)-3-hydroxypyrrolidin-1-yl)sulfonyl)-3-chlorobenzonitrile (26ee) was prepared from **26dd** analogously to synthesis of **26e** from **26d** to afford **26ee** as a colorless wax (343 mg, 83% yield). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.49 (d, *J*=1.8 Hz, 1H), 8.38 (s, 1H), 8.15 (d, *J*=8.3 Hz, 1H), 8.10 - 8.02 (m, 1H), 7.79 (dd, *J*=2.3, 8.5 Hz, 1H), 7.42 (d, *J*=8.5 Hz, 1H), 6.16 (s, 1H), 4.28 (t, *J*=8.8 Hz, 1H), 4.12 (t, *J*=8.5 Hz, 1H), 3.64 - 3.43 (m, 5H); *m/z* 485.3 $[MH]^+$.

***tert*-Butyl (((3*S*,4*S*)-1-((2-chloro-4-cyanophenyl)sulfonyl)-4-((5-chloropyridin-2-yl)thio)-3-hydroxypyrrolidin-3-yl)methyl)carbamate (26f):** To a solution of **26e** (0.115 g, 0.237 mmol) in THF (4.0 mL) was added a 1.0 M solution of PMe_3 in THF (0.474 mL, 0.474 mmol) and the resulting reaction mixture was stirred at RT for 24 h. Following this duration, the reaction mixture was loaded onto a SCX cartridge and flushed with MeOH followed by 2 M NH_3 in MeOH. The filtrate was concentrated under reduced pressure to afford 92 mg (85% yield) of the requisite amine as a colorless wax (*m/z* 459.1 $[MH]^+$). The amine (92 mg, 0.200 mmol) was dissolved in THF (4.0 mL) to which was added Boc_2O (0.060 g, 0.260 mmol) followed by TEA (0.056 mL, 0.401 mmol) and the reaction mixture was stirred at RT for 0.5 h. Following this duration, the reaction mixture was diluted with H_2O and extracted with EtOAc. The combined organics were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0-30% EtOAc:Hexanes) to afford **26f** as a colorless semi-solid (75 mg, 67% yield); *m/z* 559.2 $[MH]^+$.

***tert*-Butyl(((3*R*,4*S*)-1-((2-chloro-4-cyanophenyl)sulfonyl)-4-((5-chloropyridin-2-yl)thio)-3-hydroxy-pyrrolidin-3-yl)methyl)carbamate (26ff)** was prepared from **26ee** analogously to the synthesis of **26f** from **26e** to afford **26ff** as a colorless semi-solid (178 mg, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.38 (d, *J*=13.30 Hz, 9 H) 3.18 (d, *J*=4.77 Hz, 2 H) 3.48 (s, 1 H) 3.65 (d, *J*=10.54 Hz, 1 H) 4.01 - 4.15 (m, 2 H) 4.31 (s, 1 H) 5.35 - 5.61 (m, 1 H) 7.39 (d, *J*=8.53 Hz, 1 H) 7.76 (dd, *J*=8.53, 2.51 Hz, 1 H) 8.01 - 8.09 (m, 1 H) 8.16 (d, *J*=8.03 Hz, 1 H) 8.36 (s, 1 H) 8.49 (s, 1 H); *m/z* 559.5 $[MH]^+$.

***tert*-Butyl (((3*S*,4*S*)-1-((2-chloro-4-cyanophenyl)sulfonyl)-4-((5-chloropyridin-2-yl)sulfonyl)-3-hydroxypyrrolidin-3-yl)methyl)carbamate (26g):** To a solution of **26f** (0.075 g, 0.134 mmol) in DCM (4.0 mL) was added *m*-CPBA (0.077 g, 0.335 mmol) and the reaction mixture was stirred at RT for 3 h. Following this duration, the reaction mixture was quenched with 10% aqueous $Na_2S_2SO_3$ and the layers were separated. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0-35% EtOAc:Hexanes) to afford **26g** as a colorless semi-solid (78 mg, 98% yield); *m/z* 491.1 $[MH\text{-}Boc]^+$.

***tert*-Butyl(((3*R*,4*S*)-1-((2-chloro-4-cyanophenyl)sulfonyl)-4-((5-chloropyridin-2-yl)sulfonyl)-3-hydroxypyrrolidin-3-yl)methyl)carbamate (26gg)** was prepared from **26ff** analogously to the synthesis of **26g** from **26f** to afford **26gg** as a white solid (150 mg, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.35 (s, 9 H) 3.38 (br. s., 4 H) 3.94 (d, *J*=9.29 Hz, 1 H) 4.11 (br. s., 1 H) 4.37 (br. s., 1 H) 6.94 (br. s., 1 H) 8.01 (d, *J*=8.53 Hz, 1 H) 8.05 (d, *J*=8.28 Hz, 1 H) 8.14 (d, *J*=8.03 Hz, 1 H) 8.25 - 8.31 (m, 1 H) 8.36 (s, 1 H) 8.82 (s, 1 H); *m/z* 491.4 $[MH\text{-}Boc]^+$.

4-(((3*S*,4*S*)-3-(Aminomethyl)-4-((5-chloropyridin-2-yl)sulfonyl)-3-hydroxypyrrolidin-1-yl)sulfonyl)-3-chlorobenzonitrile (26): To a solution of **26g** (0.078 g, 0.132 mmol) in DCM (1.0 mL) was added TFA (0.305 mL, 3.96 mmol) and the resulting reaction mixture was stirred at RT for 0.5 h. Following this duration, the reaction mixture was concentrated under reduced pressure. The residue was diluted with H_2O and the resulting solution was basified with saturated aqueous $NaHCO_3$. The white

precipitate was filtered, washed with H_2O and dried under vacuum to afford **26** as a white solid (37 mg, 54% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.82 - 3.00 (m, 2 H) 3.43 (d, *J*=10.29 Hz, 1 H) 3.58 (d, *J*=10.04 Hz, 1 H) 3.69 (d, *J*=11.54 Hz, 1 H) 3.91 (d, *J*=4.02 Hz, 1 H) 4.29 (d, *J*=6.27 Hz, 1 H) 5.62 - 5.98 (m, 1 H) 8.04 (dd, *J*=13.05, 8.53 Hz, 2 H) 8.10 - 8.17 (m, 1 H) 8.29 - 8.40 (m, 2 H) 8.90 (s, 1 H); *m/z* 491.3 $[MH]^+$.

4-(((3*R*,4*S*)-3-(Aminomethyl)-4-((5-chloropyridin-2-yl)sulfonyl)-3-hydroxypyrrolidin-1-yl)sulfonyl)-3-chlorobenzonitrile (27) was prepared from **26gg** analogously to the synthesis of **26** from **26g** to afford **27** as a white solid (61 mg, 50% yield). ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.84 (s, 1H), 8.36 (s, 1H), 8.28 (d, *J*=8.5 Hz, 1H), 8.14 (d, *J*=8.0 Hz, 1H), 8.03 (dd, *J*=8.3, 19.8 Hz, 2H), 4.49 (t, *J*=7.5 Hz, 1H), 4.16 - 4.02 (m, 1H), 3.88 (d, *J*=9.3 Hz, 1H), 3.51 (d, *J*=10.0 Hz, 1H), 3.40 (br. s., 2H), 2.81 (d, *J*=8.3 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 44.7, 57.6, 64.3, 73.2, 77.9, 116.8, 117.3, 124.3, 132.6, 136.0, 136.2, 139.1, 140.4, 142.7, 145.2, 148.8, 155.7; LRMS: *m/z* 491.3 $[MH]^+$. HRMS: *m/z* 491.0018 (observed), 491.0017 (calculated).

■ ASSOCIATED CONTENT:

The Supporting Information is available free of charge on the ACS Publications website at DOI: ***

Included are experimental details regarding the calcium flux FLIPR, CLND solubility, hERG, $Ca_V1.2$, phospholipidosis, 3A4 TDI, 3A4 RI assays; *in vivo* pharmacokinetics studies; rat pulmonary edema model and *in vivo* IC_{50} calculations; complete experimental procedures and compound characterization data (NMR, MS); VCD analyses of Compounds **7** and **10**; crystal structure of Compound **19** (PDF)

Molecular formula strings and associated biological data (CSV)

■ AUTHOR INFORMATION:

Corresponding Author

*E-mail for J.E.P.: joseph.e.pero@gsk.com. Phone: (484)-923-3836

ORCID[ID]

Joseph E. Pero: 0000-0003-2674-4713

Notes

The authors declare the following competing financial interest(s): Authors affiliated with GlaxoSmithKline have received compensation in the form of salary and stock.

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■ ABBREVIATIONS USED:

AUC, area under the (concentration) curve; CAD, cationic amphiphilic drug; CHI, chromatographic hydrophobicity index; CL, total clearance; CLND, Chemiluminescence Nitrogen Detection; C_{max}, maximum concentration; C_{min}, minimum concentration; Cmpd, compound; CYP, cytochrome P450; DCM, dichloromethane; DDI, drug-drug interaction; DMF, *N*,*N*-dimethylformamide; DNAUC, dose-normalized area under the (concentration) curve; F, oral bioavailability; FLIPR, fluorometric imaging plate reader; f_u, fraction unbound; h, hour(s); GSH, glutathione; HCl, hydrochloric acid; hERG, human Ether-à-go-go-Related Gene; HF, heart failure; HPLC, high pressure liquid chromatography; HUVEC, human umbilical vein endothelial cells; IAM, immobilized artificial membrane; IC_{50}, half-maximal inhibitory concentration; i.v., intravenous administration; LE, ligand efficiency; LLE, lipophilic ligand efficiency; *m*-CPBA, *meta*-chloroperoxybenzoic acid; MEC, minimum effective concentration; MI, myocardial infarction; mpk, milligrams per kilogram; min, minutes; MRT, mean resonance time; MsCl, mesyl chloride; nM, nanomolar; NMO, 4-methylmorpholine *N*-oxide; Perm, permeability; PK, pharmacokinetics; p.o., per os (oral) administration; PSA, polar surface area; PPTS, pyridinium *p*-toluenesulfonate; PVP, pulmonary venous pressure; RI, reversible inhibition; RT, room temperature;

SAR, structure-activity relationships; SEM, standard error of mean; TBME, *tert*-butyl methyl ether; TDI, time-dependent inhibition; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TRPV4, Transient Receptor Potential Vanilloid-4; μM, micromolar; VCD, vibrational circular dichroism; V_{dss}, volume of distribution.

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(18) Ligand efficiency is defined as LE = 1.37*(pIC$_{50}$)/HAC, in which HAC = heavy atom count.

(19) Lipophilic ligand efficiency is defined as LLE = pIC$_{50}$ – cLogP. For more details, see: Ryckmans, T.; Edwards, M.P.; Horne, V.A.; Correia, A.M.; Owen, D.R.; Thompson, L.R.; Tran, I.; Tutt, M.F.; Young, T. Rapid Assessment of a Novel Series of Selective CB(2) Agonists Using Parallel Synthesis Protocols: A Lipophilic Efficiency (LipE) Analysis. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4406-4409.

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two measurements with a SEM ± 0.1 for the pIC$_{50}$. For further details, please see the Supporting Information.

(22) All cLogP values presented herein are calculated from ChemAxon JChem for Excel software package based on the following method: Viswanadhan, V.D.; Ghose, A.K.K.; Revankar, G.R.; Robins, R.K. Atomic Physicochemical Parameters for Three-Dimensional Structure Directed Quantitative Structure-Activity Relationships. *J. Chem. Inf. Comput. Sci.* **1989**, *29*, 163-172.

(23) Clearance adjusted for unbound fraction is defined as CL/f_u in which CL is the total body clearance (mL/min/kg) and f_u is the unbound fraction as a percent (%).

(24) Permeability values were provided by an artificial membrane permeability assay. For details, please see the Supporting Information.

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(27) For VCD analysis of Compounds **7** and **10**, please see the Supporting Information.

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(29) While the synthetic sequence illustrated in Scheme 1 generally proved to be the most reliable and diastereoselective approach toward the desired sulfone pyrrolidine sulfonamides, alternative routes were also utilized for select compounds. Please see the Supporting Information section for further details.

(30) Alcaraz, L; Crindland, A.; Kinchin, E. Novel Conversion of 1,2-Disubstituted *cis*-Epoxides to One-Carbon Homologated Allylic Alcohols Using Dimethylsulfonium Methylide. *Org. Lett*. **2001**, *3*, 4051-4053.

(31) A number of examples were prepared through this less-diastereoselective approach, which was established prior to the highly-diastereoselective sequence depicted in Scheme 1. For further details, please see the Supporting Information.

(32) PK studies in rat liver microsomes suggested that oxidative metabolism (intrinsic clearance) of the aryl sulfone functionality decreases as the electron-withdrawing effect(s) of its substituent(s) decreases.

(33) The sulfoximine functionality of Compound **23** is chiral. For details concerning the elucidation of its stereochemical configuration, please refer to the Supporting Information.

(34) The sulfonimidamide functionality of Compound **24** is chiral. For details concerning the elucidation of its stereochemical configuration, please refer to the Supporting Information.

(35) Kinetic aqueous solubility measurements were measured using Chemiluminescence Nitrogen Detection (CLND) quantification. For further details, please refer to Supporting Information.

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(42) All animal studies were conducted in accordance with the GlaxoSmithKline Policy on the Care, Welfare, and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee at GlaxoSmithKline.

(43) For details on the % inhibition calculations and statistical analyses, please refer to the Supporting Information.

(44) For details on the calculation of the *in vivo* IC_{50} values, please refer to the Supporting Information.

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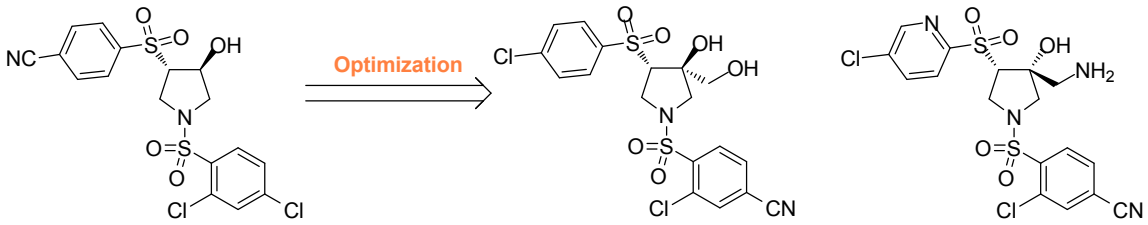
Table of Contents graphic.



Compound 6
hTRPV4 IC_{50} = 25 nM
LLE = 4.9
Sulfone pyrrolidine sulfonamide lead

Compound 19
hTRPV4 IC_{50} = 3.2 nM
LLE = 5.8
oral F = 100%

Compound 27
hTRPV4 IC_{50} = 0.5 nM
LLE = 7.4
oral F = 56%

in vivo efficacy in rat pulmonary edema model