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Discovery of GSK2798745: A Clinical Candidate for the Inhibition of Transient Receptor Potential Vanilloid 4 (TRPV4)

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GlaxoSmithKline, Heart Failure Discovery Performance Unit, Metabolic Pathways and Cardiovascular Therapeutic Area, 1250 S Collegeville Road, Collegeville, Pennsylvania, 19426, United States.

KEYWORDS GSK2798745, TRPV4, congestive heart failure, conformational bias, volume of distribution

ABSTRACT: GSK2798745, a clinical candidate was identified as an inhibitor of the Transient Receptor Potential Vanilloid 4 (TRPV4) ion channel for the treatment of pulmonary edema associated with congestive heart failure. We discuss the lead optimization of this novel spirocarbamate series specifically focusing on our strategies and solutions for achieving desirable potency, rat pharmacokinetics and physicochemical properties. We highlight the use of conformational bias to deliver potency and optimization of volume of distribution and unbound clearance to enable desirable *in vivo* mean residence times.

Transient receptor potential vanilloid 4 (TRPV4) is a cation channel that mediates the influx of Ca^{2+} across plasma membranes.[1] The channel can be activated by heat, hypotonicity, physical stress and small molecules.[2-5] TRPV4 activation caused by heightened pulmonary vascular pressure increases permeability of the alveolar septal barrier that leads to pulmonary edema.[6-7]

In pre-clinical congestive heart failure models, increased pressure in the pulmonary vascular system causes the development of pulmonary edema, which is reduced with TRPV4 channel inhibition. Therefore, a TRPV4 inhibitor is hypothesized to prevent channel activation and reduce pulmonary edema in heart failure patients.[8-9]

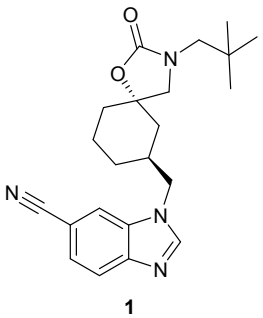
GSK previoulsy reported TRPV4 anatagonists series, quinolines and benzimidazoles, failed to deliver clinical drug candidates necessitating the need to identify an alternative series.[10,19]

Herein we report the discovery of GSK2798745 (**30**) a clinical candidate for the inhibition of TRPV4. GSK2798745 (**30**) arose from a lead optimization campaign aimed at identifying a small molecule oral drug candidate capable of inhibiting the TRPV4 ion channel at or above unbound IC_{50} for 24 hours per day from a low once a day oral dose.

To achieve this target profile, we optimized the molecule for both potency and pharmacokinetics (PK). TRPV4 inhibition was measured via a FLIPR HEK cellular assay with a targeted $IC_{50} \leq 10$ nM required for successful candidates. PK optimization was performed in Sprague Dawley rats.[10] We focused our attention on identification of compounds with robust oral bioavailability (>30 %) and mean residence time (MRT). An extended MRT is highly desirable as it minimizes the peak to trough concentrations during a dose which improves therapeutic index and enables a lower overall dose. A minimum MRT of two hours in rat was projected to scale to enable once a day dosing in human.[11, 12]

In addition to the potency and PK targets we sought to identify a compound with desirable physicochemical properties to mitigate selectivity and attrition liabilities.[13-14] Thus, we targeted compounds exhibiting a cLogP of <3.5 and measured chromatographic logD at pH 7.4 (ChromLogD) of <4.0.[13]

Figure 1. Spirocarbamate lead



1

hTRPV4 IC_{50}[a] = 16 ± 2.5 nM
rTRPV4 IC_{50}[a] = 5.5 ± 1.5 nM

ChromLogD = 4.5
cLogP = 4.2

Rat PK[b,c]
MRT_{iv} = 0.7 h
CL_{iv} = 64 mL/min/kg
$Vdss_{iv}$ = 2.6 L/Kg
F_{po} = 63%
rPPB f_u = 32%
rCL_u = 200 mL/min/kg

[a]TRPV4 IC_{50} are an average of at least two measurements. [b]DMPK properties are an average of measurements taken in at

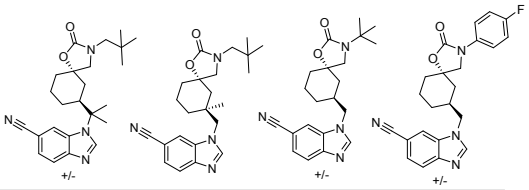
least two Sprague-Dawley rats. [c]Rat PPB f_u are an average of at least two measurements

The spirocarbamate lead **1** (Figure 1) was derived from an HTS screening hit molecule from the GSK screening collection.[15] Compound **1** demonstrated robust potency against TRPV4 (IC_{50} = 16 nM) and an attractive physicochemical properties starting point, with a ChromLogD = 4.5 and LLE = 3.4 (LLE = pIC_{50} – ChromLogD), especially compared to earlier reported TRPV4 inhibitors.[10,16-19]

The initial focus of our lead optimization effort was to improve the rat PK of **1** which demonstrated a short MRT (0.7 h). MRT is the quotient of volume of distribution (V_{dss}) and clearance (CL).[12,20] It can thus be extended by either increased V_{dss} or reduced clearance. Our initial strategy was to address the high *in vivo* clearance (64 mL/min/kg) of **1** by removing metabolic liabilities.

Oxidation adjacent to nitrogen is a well-documented pathway of metabolism and was observed in an *in-vitro* metabolism ID study.[21] To address potential sites of metabolism in **1**, we targeted four analogs (**2-5**) specifically aimed at sterically blocking oxidative metabolism (Table 1).[22]

Table 1. Initial analogs to address clearance



Cmpd	2	3	4	5
hTRPV4 IC_{50} ± SEM (nM)[a]	>25,000 ±0	0.5 ±0.03	5,200 ±710	1,900 ±610
Ch.LogD	4.6	4.6	4.0	4.5
cLogP	4.9	4.7	3.6	3.7
Rat PK[b]				
MRT_{iv} (h)	0.3	0.5	0.5	3.1
CL_{iv} (mL/min/kg)	70	63	55	23
Vd_{iv} (L/kg)	1.1	2.1	1.8	4.0
F_{po}	8%	32%	44%	100%
rPPB f_u [c]	--	14%	--	6.5 %
CL_u (mL/min/kg)	--	450	--	350

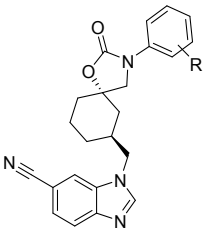
[a]TRPV4 IC_{50} are an average of at least two measurements. . [b]DMPK properties are an average of measurements taken in at least two Sprague-Dawley rats. [c]Rat PPB f_u are an average of at least two measurements

Installing a gemdimethyl group adjacent to the benzimidazole nitrogen (**2**) had no benefit on MRT (0.3 h) and reduced TRPV4 activity significantly (IC_{50} > 25,000 nM). An alternative approach to sterically block metabolism at the methylene center through methyl introduction onto the cyclohexane ring (**3**) similarly had no benefit on MRT (0.5 h). Interestingly, we noted a profound 32-fold TRPV4 potency increase resulting from this change (IC_{50} = 0.5 nM) over compound **1**. The enhanced potency offered by the methyl group (~2 kcal/mol) is beyond that of lipophilic binding energy, as indicated by LLE increase (**1** = 3.4, **3** = 4.7), and is suggestive of a conformational effect, perhaps reducing the conformational flexibility of the cyclohexane or benzimdazole.[23,24]

Compound **4** was designed to address potential oxidation near the carbamate nitrogen through substitution of the neopentyl group with *tert*-butyl but had no benefit on MRT (0.5 h) and demonstrated weak TRPV4 potency (IC_{50} = 5,200 nM). In contrast, *N*-Aryl substitution (**5**) provided a significant increase in both MRT (3.1 h) and oral bioavailability (100%).

The increased MRT of **5** is a result of both reduced clearance and more importantly increased V_{dss}. The 3-fold clearance reduction can be fully attributed to a 5-fold increase in plasma protein binding for **5** (f_u = 6.5 %) compared to 1 (f_u = 32%). The unbound clearance (CL_u = CL/PPB f_u, **1** = 200, **5** = 350) indicates that there is minimal change in the free drug clearance between the *N*-neopentyl (**1**) and *N*-aryl (**6**). In contrast, the volume of distribution increase is very substantial. The 5-fold increase in PPB f_u between the N-neopentyl (**1**) and *N*-aryl (**5**) would have been expected to elicit a proportional reduction in the V_{dss}.[24] Instead, we observed a 1.5-fold increase in V_{dss}. This indicates that the unbound drug volume of distribution (Vd_{ss}/f_u, **1** = 8.1, **5** = 61) has increased by eight times and is the underlying driver for the increased MRT. The V_{dss} increase cannot be attributed to any change in lipophilicity with ChromLogD remaining unchanged at 4.5. Unfortunately, the aryl substitution producing **5** led to a substantial loss in TRPV4 potency compared to **1** (IC_{50} = 1,900 nM vs 16 nM).

Table 2. *N*-aryl potency SAR on the **5** desmethylcyclohexane core.



Cmpd	R	hTRPV4 IC_{50} ±SEM (nM)[a]	ChromLogD
5	*p*-F (±)	1,900 ± 610	4.5
6	H	820 ± 200	4.6
7	*p*-OEt	140 ± 7.9	4.8
8	*m*-OEt	74 ± 11	5.0
9	*m*-Cl	240 ± 51	5.3
10	*o*-Cl	63 ± 2.9	4.3
11	o-F	540 ± 150	4.2
12	*o*-Me	310 ± 29	4.6
13	*o*-OMe	340 ± 85	4.2

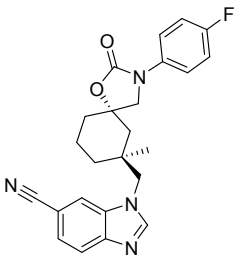
[a]TRPV4 IC_{50} are an average of at least two measurements

Initial attempts to address the potency loss of **5** via *N*-aryl

substitutions led to multiple analogs that improved potency by ~10 fold (**7-10**), but did not meet the 10 nM target.

This led us to incorporate the potency-enhancing methyl observed in **3** into the cyclohexane ring of **5** to give **14.** This modification led to the expected improvement in potency (IC_{50} = 96 nM) and the excellent rat PK was maintained (Figure 2). Based on its improved potency, we were optimistic that **14** could be improved to reach the target potency at TRPV4 (IC_{50} < 10 nM) by leveraging the established *N*-aryl substituent SAR (Table 2).

Figure 2. Early methyl-cyclohexane *N*-aryl analog



14

hTRPV4 IC_{50}[a] = 96 ± 17 nM

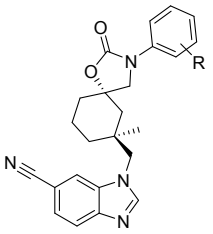
ChromLogD = 4.9
cLogP = 4.1

Rat PK[b]
MRT_{iv} = 2.8 h
CL_{iv} = 39 mL/min/kg
$Vdss_{iv}$ = 6.5 L/kg
F_{po} = 100%

[a]TRPV4 IC_{50} are an average of at least two measurements. [b]DMPK properties are an average of measurements taken in at least two Sprague-Dawley rats.

The combination of *N*-aryl substituents with the methylcyclohexane core led to many highly potent (<10 nM) compounds (Table 3) but also highlighted that highly divergent SAR existed between the desmethylcyclohexane and methylcyclohexane cores. *Para*- and *meta*-ethoxy substitution (**16** and **17**) demonstrated excellent potency (IC_{50} = 5.2 and 1.6 nM) which can be attributed to the potency of corresponding desmethylcyclohexane analogs **7** & **8** (IC_{50} = 140 & 74 nM) which is supplemented by the expected ~30-fold potency increase from the methylcyclohexane core. *Meta*-chloro substitution (**18**) demonstrated 50 nM potency and the minimal and unexpected 5-fold potency increase over desmethylcyclohexane **6** revealed that divergent SAR existed between the two structurally similar cores. The disparate SAR was most apparent in compounds bearing *ortho*-aryl substituents (**11-14**). *Ortho*-substituted compounds on the desmethylcyclohexane core (**10-13**) typically had minimal effects on potency. However, installing the methylcyclohexane core on these *ortho*-substituted analogs produced activity increases of up to 280x to give compounds such as **22** with TRPV4 IC_{50} = 1.2 nM. We noted that the efficiency for the methylcyclohexane core introduction with *ortho*-substituents also correlated with increased electron donating capacity of the substituent. (σ_{para} Cl = +0.23, F = +0.06, Me = -0.17, OMe = -0.27). We hypothesized that electron donating *ortho*-substituents elicit a strong conformational effect on the *N*-aryl rotation which is highly complementary to the conformational effects elicited by the methylcyclohexane core.[21,23-24]. Of interest, the compound containing a *meta*-Cl (**18**) substituent exhibited a much higher measured lipophilicity (ChromLogD = 5.7) compared to the *ortho*-Cl (**19**) analog (ChromLogd = 4.8), which may arise from the proposed *N*-aryl conformational effects caused by the *ortho*-substituents.

Table 3. *N*-aryl methylcyclohexane TRPV4 potency SAR

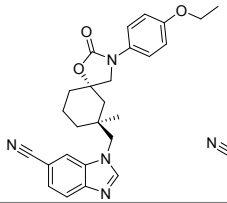


Cmpd	R	hTRPV4 IC_{50} ±SEM (nM)[a]	Chrom LogD[b]	LLE	Me Effect[c]
14	*p*-F	96 ± 17	4.9	2.1	20x
15	H	49 ± 5.9	5.0	2.3	17x
16	*p*-OEt	5.2 ± 0.3	5.4	2.9	26x
17	*m*-OEt	1.6 ± 0.2	5.3	3.5	45x
18	*m*-Cl	48 ± 7.5	5.7	1.6	5x
19	*o*-Cl	2.5 ± 0.3	4.8	3.8	25x
20	o-F	5.2 ± 1.0	4.9	3.4	100x
21	*o*-Me	1.7 ± 0.2	4.8	4.0	200x
22	*o*-OMe	1.2 ± 0.2	4.6	4.3	280x

[a]TRPV4 IC_{50} are an average of at least two measurements. [b]LLE = TRPV4 pIC_{50} – ChromLogD. [c]Methyl effect is the fold change in hTRPV4 IC_{50} for the corresponding analogs in table 2.

Unfortunately, the introduction of the potency enhancing alkoxy groups led to reduced MRT's by differing mechanisms as exemplified by **16** and **22** (Table 4). The reduced MRT of **22** (1.0 h) is the result of reduced volume of distribution (1.4 L/kg) with clearance (26 mL/min/kg) remaining similar to *para*-fluoro **14** (39 mL/min/kg). Apparently, the potency enhancing conformational effect of the *ortho*-methoxy substituent (**22**) led to reduced tissue affinity versus **14**, which would be difficult to correct while maintaining potency. In contrast, the reduced MRT of **16** (0.9 h) is a function of increased clearance (140 mL/min/kg) with volume of distribution remaining unchanged (7.2 L/kg) relative to *para*-fluoro **14** (6.5 L/kg). The *para*-ethoxy group presumably introduced a metabolic liability, but without changing the underlying volume of distribution advantage observed with the early *N*-aryls **5** and **14**.

Table 4. Potent *N*-aryl compounds with reduced MRTs



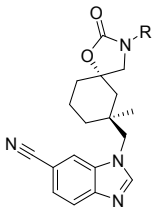
Cmpd	16	22

hTRPV4 IC_{50} ± SEM (nM)[a]	5.2 ± 0.3	1.2 ± 0.2
ChromLogD	5.4	4.6
cLogP	4.5	4.0
Rat PK[b]		
MRT_{iv} (h)	0.9	1.0
CL_{iv} (mL/min/kg)	140	26
Vd_{iv} (L/kg)	7.2	1.4
F_{po}	29 %	100 %
rPPB f_u [c]	--	3.6 %
CL_u (mL/min/kg)	--	720

[a]TRPV4 IC_{50} are an average of at least two measurements. [b]DMPK properties are an average of measurements taken in two Sprague-Dawley rats. [c]Rat PPB f_u are an average of at least two measurements.

Consistent with this conclusion, we could reduce clearance and restore the MRT of **16** via the introduction of heterocycles (**23-25**) which are thought to attenuate oxidative metabolism of the aryl ring (Table 5).[26]

Table 5. Rat PK SAR for pyridyl and diazine analogs

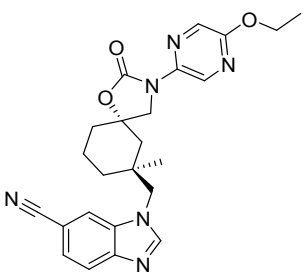


Cmpd	R	hTRPV4 IC_{50} +SEM (nM)[a]	rCL$_{iv}$ (mL/min/kg)[b]	PPB f_u (%)[c]	MRT (h)[b]
16		5.2 ± 0.3	140	--	0.9
23		3.1 ± 0.5	24	3.1	1.6
24		60 + 15	14	--	1.9
25		4.2 ± 0.5	17	3.7	5.2

[a]TRPV4 IC_{50} are an average of at least two measurements. [b]DMPK properties are an average of measurements taken in two Sprague-Dawley rats. [c]Rat PPB f_u are an average of at least two measurements.

The 2-ethoxy pyridyl (**23**) improved potency (IC_{50} = 3.1 nM), lowered clearance (24 mL/min/kg) and enhanced MRT (1.6 h) versus **16**, prompting efforts to incorporate an additional nitrogen to produce alkoxy diazines. Ethoxy pyridazine (**24**) led to a further reduction in clearance (14 mL/min/kg) and increase in MRT (1.9 h), but also reduced the TRPV4 potency (IC_{50} = 60 nM). In contrast, the ethoxy pyrazine (**25**) demonstrated robust TRPV4 potency (IC_{50} = 4.2 nM) and a long MRT (5.2 h) but with similar clearance (17 mL/min/kg) to **23** and **24**. Overall our conclusion is that *para*-ethoxy substitution (**16**) enhanced or introduced arene oxidation and that this can be attenuated or eliminated by pyridyl nitrogen insertion. We hypothesized that the nitrogen containing heterocycles may be reducing metabolism either through reduced cytochrome P450 binding affinity or by reducing aryl oxidative capacity.[26]

Figured 3. A potent TRPV4 inhibitor with desirable rat PK



25

hTRPV4 IC_{50}[a] = 4.2 ± 0.5 nM

ChromLogD = 5.5
cLogP = 3.1

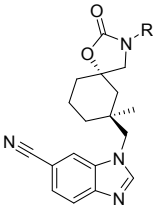
Rat PK[b,c]
MRT_{iv} = 5.2 h
CL_{iv} = 17 mL/min/kg
$Vdss_{iv}$ = 5.4 L/kg
F_{po} = 100%
rPPB f_u = 3.7%
rCL_u = 460 mL/min/kg

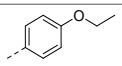
[a]TRPV4 IC_{50} are an average of at least two measurements. [b]DMPK properties are an average of measurements taken in two Sprague-Dawley rats. [c]Rat PPB f_u are an average of at least two measurements.

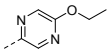
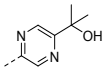
Having established our target TRPV4 potency and rat PK in compounds such as **25** (Figure 3) we also noted that the replacement of phenyl (**16**) with pyrazine (**25**) dramatically lowered cLogP from 4.5 to 3.1. Therefore, we progressed **25** to candidate selection, but it was subsequently surpassed by a superior compound, which resulted from lowering measured lipophilicity.

Interestingly, the pyrazine had no effect on measured lipophilicity as ChromLogD was nearly identical for **16** (5.4) and **25** (5.5). We sought to reduce the measured lipophilicity recognizing that this value is more relevant for positively impacting off-target activity and solubility.[14] Specifically for this series we had noted that the unbound clearance of **25** was inferior to **1** and **5** both of which demonstrated lower ChromLogD. We hypothesized that by decreasing lipophilicity we could reduce unbound clearance, presumably through attenuating binding affinity to metabolizing enzymes. An improvement in unbound clearance while maintaining potency and MRT is highly desirable as it leads to increased free drug concentration that lowers the overall clinical dose.[27]

Table 6. Aryl substituent lipophilicity SAR



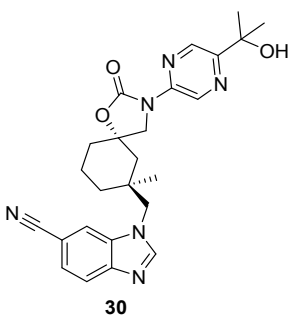
Cmpd	R	hTRPV4 IC_{50} ± SEM(nM)[a]	Ch.LogD	rCL_u[b,c]
16		5.2 ± 0.3	5.4	--

25		4.2 ± 0.5	5.5	460
26		14 ± 1.6	4.8	400
27		60 ± 1.8	4.2	--
28		1.5 ± 0.2	5.3	340
29		1.2 ± 0.3	6.3	--
30		1.8 ± 0.2	4.0	79

[a]TRPV4 IC_{50} are an average of at least two measurements. . [b]DMPK properties are an average of measurements taken in two Sprague-Dawley rats. [c]Rat PPB f_u are an average of at least two measurements. CL_u = CL x PPB *f*u

We modulated lipophilicity by varying the pyrazine substituent of **25** (Table 6). Contraction from ethoxy (**25**) to methoxy (**26**) reduced ChromLogD (5.5 to 4.8) and CL_u (460 to 400) but at the expense of potency (IC_{50} = 14 nM). Exchange of ethoxy for a methyl substituent (**27**) reduced ChromLogD to 4.2 but again led to modest TRPV4 potency (IC_{50} = 60 nM). Target potency could be increased through larger alkyl groups such as *cyclo*-propyl (**28**) and *tert*-butyl (**29**) to 1.5 nM and 1.2 nM respectively, with the former demonstrating slightly improved CL_u relative to **25**. The alkyl substituent data (**27**-**29**) illustrated the importance of lipophilicity for obtaining excellent TRPV4 potency, but also demonstrated that a potency/lipophilicity plateau had been reached since **29** gave no activity increase over **28**. The latter observation led us to replace one of *tert*-butyl methyls in **29** with a hydroxy group to give isopropyl alcohol (**30**) which demonstrated excellent TRPV4 potency (IC_{50} = 1.8 nM) along with significantly reduced ChromLogD (4.0). The reduced lipophilicity led to a six fold improvement in CL_u compared to **25**. We hypothesize that the hydroxy may be internally hydrogen-bonded to the adjacent pyrazine nitrogen when bound to TRPV4, thereby masking some its polarity leading to the excellent potency. However, the polarity of the hydroxy group is apparently dynamically exposed in aqueous solvent leading to its reduced ChromLogD and unbound clearance.[28]

Figure 4. GSK2798745 TRPV4 oral candidate



30

hTRPV4 IC_{50}[a] = 1.8 ± 0.2 nM
rTRPV4 IC_{50}[a] = 1.6 ± 0.2 nM

ChromLogD = 4.0
cLogP = 1.3

Rat PK[b,c]
MRT_{iv} = 4.9 h
CL_{iv} = 6.5 mL/min/kg
$Vdss_{iv}$ = 1.9 L/kg
F_{po} = 59%
rPPB f_u = 8.2%
rCL_u = 79 mL/min/kg

[a]TRPV4 IC_{50} are an average of at least two measurements. [b]DMPK properties are an average of measurements taken in two Sprague-Dawley rats. [c]Rat PPB f_u are an average of at least two measurements

Overall, compound **30** demonstrated excellent TRPV4 potency, owing largely to the methyl-cyclohexane core, and showed exceptional rat PK. The long MRT (4.9 h) and low unbound clearance (81) could be attributed to the metabolic stability of the *N*-aryl pyrazine and low overall lipophilicity of the compound. Compound **30** (designated GSK2798745) demonstrated efficacy in an *in vivo* rat PK/PD model of pulmonary edema, excellent TRP selectivity (M5, A1, C3 and C6 > 25 μM) and an adequate preclinical safety profile that enabled its candidate selection and subsequent progression to clinical trials.[9,29]

The spirocarbamate TRPV4 inhibitors were accessed via the synthetic method described in supplemental data Schemes 1 and 2.[30]

In summary, a lead optimization effort beginning with spirocarbamate **1** identified GSK2798745 (**30**), a clinical candidate for the inhibition of TRPV4. The lead optimization began by successfully addressing the poor rat PK of the lead but resulted in a significant reduction in TRPV4 potency. The TRPV4 potency loss was addressed primarily through the introduction of a highly efficient methyl group. This was then followed by subtle refinement of TRPV4 potency, rat pharmacokinetics and physiochemical properties to achieve a desirable oral candidate profile. GSK2798745 is a clinical candidate that is being used to assess the role of TRPV4 in human diseases.

ASSOCIATED CONTENT

Supporting Information. Compound synthesis and spectroscopic characterization of **1, 16, 25** and **30**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

* E-mail: carl.a.brooks@gsk.com

Present Addresses

1250 S Collegeville Road, Collegeville, PA, 19426

Author Contributions

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NOTES

All studies involving the use of animals were conducted after review by the GlaxoSmithKline (GSK) Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

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ABBREVIATIONS

ChromLogD, chromatographic logD at pH 7.4; cmpd, compound; CL, clearance; CL_u, clearance of unbound drug, FLIPR, fluorometric imaging plate reader; IV, intravenous; F_{po}, oral bioavailability; MRT, mean residence time; PK, pharmacokinetic; PPB F_u, plasma protein binding fraction unbound; SAR, structure-activity relationship; TRPV, transient receptor potential vanilloid; V_{dss}, volume of distribution at steady state;

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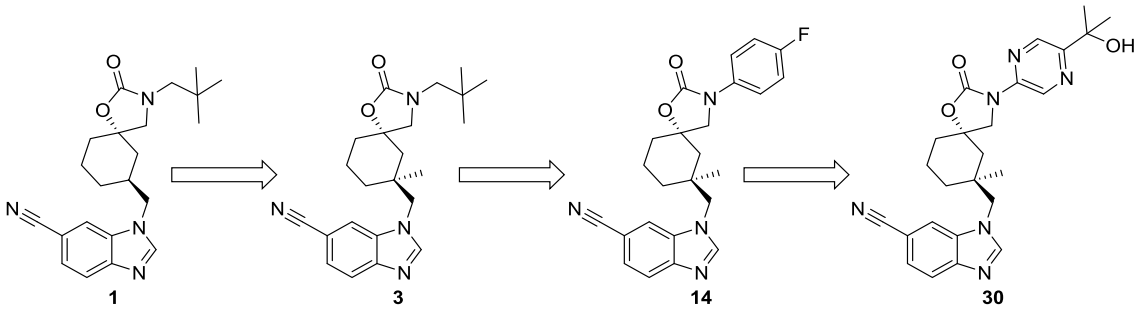

1

hTRPV4 IC_{50} = 16 nM
rMRT = 0.7 h

3

hTRPV4 IC_{50} = 0.5 nM
rMRT = 0.5 h

14

hTRPV4 IC_{50} = 96 nM
rMRT = 2.8 h

30
GSK2798745

hTRPV4 IC_{50} = 1.8 nM
rMRT = 4.9 h