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Discovery of GSK2193874: an Orally Active, Potent and Selective Blocker of Transient Receptor Potential Vanilloid 4

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GlaxoSmithKline, Heart Failure Discovery Performance Unit, Metabolic Pathways and Cardiovascular Therapeutic Area, King of Prussia, Pennsylvania, 19406, United States.

KEYWORDS GSK2193874, TRPV4, TRPV4 antagonist, TRPV4 inhibitor, congestive heart failure, L-type channel inhibition

ABSTRACT: Transient Receptor Potential Vanilloid 4 (TRPV4) is a member of the Transient Receptor Potential (TRP) superfamily of cation channels. TRPV4 is expressed in the vascular endothelium in the lung and regulates the integrity of the alveolar septal barrier. Increased pulmonary vascular pressure evokes TRPV4-dependent pulmonary edema, and therefore, inhibition of TRPV4 represents a novel approach for the treatment of pulmonary edema associated with conditions such as congestive heart failure. Herein we report the discovery of an orally active, potent and selective TRPV4 blocker, 3-(1,4'-bipiperidin-1'-ylmethyl)-7-bromo-*N*-(1-phenylcyclopropyl)-2-[3-(trifluoromethyl)phenyl]-4-quinolinecarboxamide (GSK2193874, **28**) after addressing an unexpected off-target cardiovascular liability observed from *in vivo* studies. GSK2193874 is a selective tool for elucidating TRPV4 biology both *in vitro* and *in vivo*.

TRPV4 is a member of the Transient Receptor Potential (TRP) superfamily of cation channels and is activated by heat, hypotonicity and physical stress.[1-3] TRPV4 is expressed in numerous cell types, including endothelial cells of the lung where it can mediate Ca^{2+} entry.[4] TRPV4 regulates the integrity of the alveolar barrier in the lung and increases barrier permeability when activated, as demonstrated in response to the increased vascular and/or airway pressure.[5] Pharmacological activation of TRPV4 with a small molecule TRPV4 agonist results in contraction of cultured endothelial cells and pulmonary edema associated with cardiovascular collapse *in vivo*.[6,7]

Congestive heart failure patients have a decreased ability of the left ventricle to pump blood into the peripheral circulatory system. This results in left ventricular dilation and a concomitant increase in pulmonary vascular pressure that drives development of pulmonary edema. Therefore, TRPV4 inhibition represents a potential novel approach for the treatment of pulmonary edema associated with congestive heart failure.[8] TRPV4 also has been implicated in many other disease conditions, including neurogenic pulmonary edema, chronic obstructive pulmonary disorder (COPD), acute lung injury,[9] acute respiratory distress syndrome (ARDS), overactive urinary bladder, pain, genetic motor neuron disorders, cardiovascular disease, and bone related disorders.[8]

At the onset of our lead optimization efforts, few small molecule TRPV4 blockers had been reported, and none were suitable for oral dosing. Hydra Biosciences had reported a TRPV4 antagonist HC-067047 with human, rat and mouse *in vitro* TRPV4 IC_{50}s of 48, 133 and 17 nM, respectively (Figure 1).[10] Renovis (Evotec) had reported RN-1734 as a TRPV4 antagonist with modest potency (human, rat and mouse TRPV4 IC_{50}s of 2.3, 3.2 and 5.9 μM, respectively) (Figure 1).[11] Therefore, discovery of a potent and selective TRPV4 blocker of utility for *in vivo* studies was highly desirable to further understand the complexity of TRPV4 biology.[12]

Herein we report the discovery of an orally active, potent and selective TRPV4 blocker, 3-(1,4'-bipiperidin-1'-ylmethyl)-7-bromo-*N*-(1-phenylcyclopropyl)-2-[3-(trifluoromethyl)phenyl]-4-quinolinecarboxamide (GSK2193874, **28**). GSK2193874 is an excellent tool for further understanding the biology of TRPV4 both *in vitro* and *in vivo*.[8] It is worth noting that after our discovery of GSK2193874, several orally bioavailable TRPV4 tools have been reported (Figure 1). These included Pfizer's azetidine-linked compound,[13] GSK's piperidinyl-benzimidazole compound,[14] Renovis' RN-1665 and RN-9893,[15] and Shionogi's aminothiazole compound.[16,17]

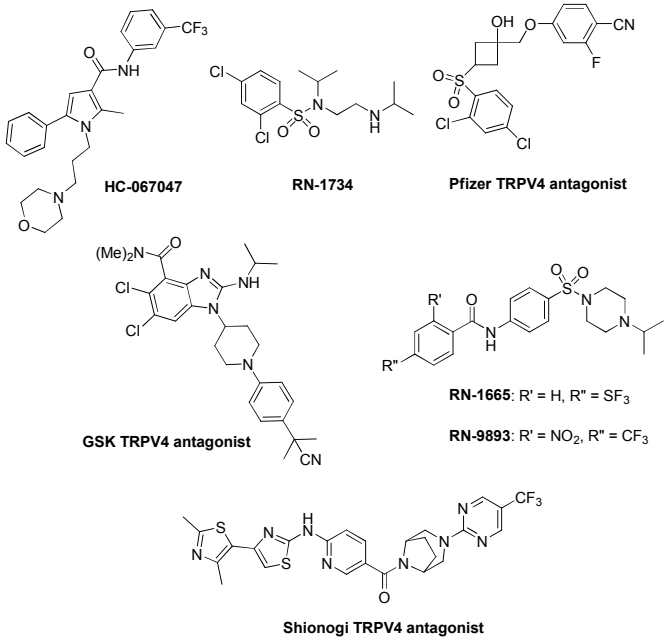


Figure 1. Structures of TRPV4 antagonists

Quinoline hit **1a** was identified as a TRPV4 blocker from high throughput screening (Figure 2). Compound **1a** has modest TRPV4 potency with human and rat TRPV4 IC_{50} of 2 and 0.3 μM, respectively, in a FLIPR assay in HEK293 cells. However, it is a potent neurokinin NK2 and NK3 dual inhibitor with IC_{50} of 100 and 8 nM, respectively, in FLIPR binding assays. Moreover, **1a** has poor pharmacokinetic properties with high clearance (CL = 52 mL/min/kg) in rats. As our primary goal was to validate TRPV4 as a pharmacologically relevant target with a potent and selective TRPV4 inhibitor that could be dosed orally and chronically in rodents, our lead optimization effort was focused on improving TRPV4 potency, removing NK2/3 activity, reducing *in vivo* clearance and improving oral exposure in rats.

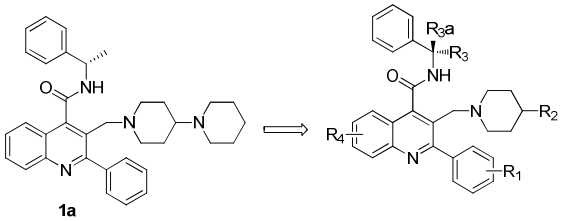
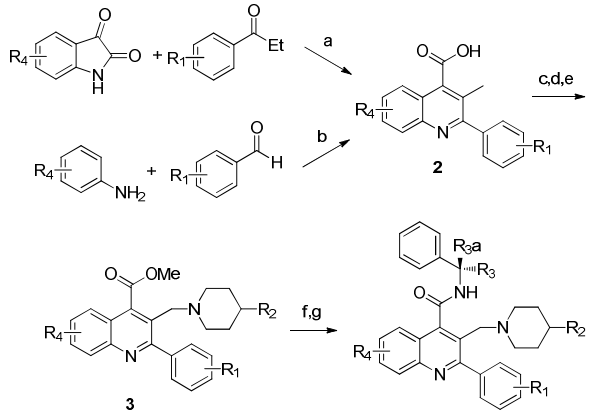


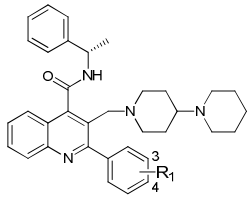
Figure 2. Quinoline hit **1a** and chemistry strategies focused on R1-R4 optimization.

We divided quinoline **1a** into four regions for structure activity relationship (SAR) exploration (Figure 2): 2-aryl substitution (R_1), amine modification (R_2), benzyl amide modification (R_3, R_{3a}) and quinoline substitution (R_4). Quinoline derivatives were synthesized using standard conditions as described in Scheme 1.[18,19] 3-Methyl-2-phenyl-4-quinolinecarboxylic acid **2** was prepared by treatment of the appropriately substituted 1H-indole-2,3-dione and ethyl aryl ketone with KOH in refluxing ethanol. Alternatively, compound **2** was prepared by treatment of the appropriately substituted aniline with the appropriately substituted benzaldehyde and 2-oxobutanoic acid. Esterification of acid **2** resulted in the substituted methyl 3-methyl-2-phenyl-4-quinolinecarboxylate which was then treated with *N*-bromosuccinimide and benzoyl peroxide to form an appropriately substituted bromomethyl quinoline. Subsequent displacement of the bromomethyl quinoline with an appropriately substituted piperidine in acetonitrile afforded the corresponding tertiary amine **3**. The methyl ester was then hydrolyzed to acid by treatment with KOH in methanol/water. Coupling of the acid with an appropriately substituted benzylamine under standard conditions, for example, EDC/HOBT or T3P, provided quinoline derivatives.



Scheme 1. General synthesis of quinoline derivatives. a) KOH, H_2O/EtOH; b) 2-oxobutanoic acid, EtOH; c) $(COCl)_2$, DMF, CH_2Cl_2, then MeOH; d) benzoyl peroxide, NBS, CCl_4; e) R_2-substituted piperidine, CH_3CN; f) KOH, MeOH/H_2O; g) R_3, R_{3a}-substituted benzylamine, T_3P, i-Pr_2NEt, CH_2Cl_2.

Table 1. Biochemical activities of substituted phenyl analogs in human and rat TRPV4 FLIPR assay



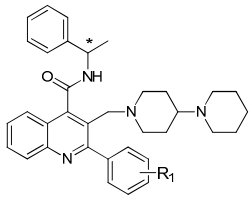
Compound	R_1	hTRPV4 IC_{50} (μM)[a]	rTRPV4 IC_{50} (μM)[a]
1a	H	2	0.32
4	4-Cl	0.32	0.016
5	4-CF_3	0.4	0.016
6	4-Me	1.58	0.1
7	4-OMe	5.01	0.32
8	3-Cl	0.2	0.008
9a	3-CF_3	0.08	0.0025
10	3-Me	0.5	0.013
11	3-OMe	3.98	0.63

[a]IC_{50} values are the mean of at least two independent experiments.

First we focused on the aryl substitution on the 2-position of the quinoline, as this position was known to be sensitive to NK2 and NK3 activity. Substitution of electron-donating groups such as Me and OMe at 4-position (**6**, **7**) or OMe at 3-position (**11**) of the phenyl ring maintained TRPV4 potency (Table 1). Methyl at 3-position (**10**) of the phenyl ring improved human and rat TRPV4 potency over compound **1a** by 4x and 24x, respectively. Interestingly substitution of electron-withdrawing groups such as Cl and CF_3 in both positions (**4**, **5**, **8**, **9a**) improved human and rat TRPV4 potency by up to 25 and 128 fold, respectively, over unsubstituted quinoline hit **1a**. 3-CF_3 compound (**9a**) is the most potent analog with rat TRPV4 IC_{50} of 2.5 nM. Compound **9a** was evaluated for NK2 and NK3 selectivity, and as expected, **9a** has reduced NK2 and NK3 activity with IC_{50}s of 1.3 and 2.5 μM, respectively, representing ≥520 fold selectivity over rat TRPV4 potency.

Next we evaluated whether there was a stereochemistry preference for binding to TRPV4. Both *R*- and *S*- enantiomers for compounds **1** and **9** were synthesized using enantiomerically pure benzyl amines. As shown in Table 2, there was no significant stereo preference when R_1 is H (**1a** vs. **1b**). Surprisingly, when R_1 is 3-CF_3, the (*S*)-enantiomer (**9a**) was 25 and 160 fold more potent than the corresponding (*R*)-enantiomer (**9b**) in human and rat TRPV4 respectively, demonstrating a stereo preference for (*S*)-enantiomer.

Table 2. Stereoselectivity of quinoline analogs



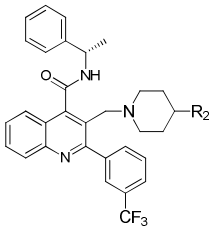
Compound	R_1	Stereochemistry	hTRPV4 IC_{50} (μM)[a]	rTRPV4 IC_{50} (μM)[a]
1a	H	*S*	2	0.32
1b	H	*R*	2	0.63
9a	3-CF_3	*S*	0.08	0.0025
9b	3-CF_3	*R*	2	0.4

[a]IC_{50} values are the mean of at least two independent experiments.

We then evaluated the SAR around piperidine substitution while keeping R1 as 3-CF_3, and R_3 as *S*-Me. Piperidine-piperidine analog (**9a**) and piperidine-morpholine derivative (**14**) had optimal TRPV4 potency (Table 3). The terminal piperidine or morpholine was important for TRPV4 activity as compounds without those moieties have reduced TRPV4 activities (**12**, **13**, **15** and **16**).

Since chirality at the alpha position of benzyl amide (R_3) played an important role in TRPV4 potency (Table 2), we explored additional SAR at that region. As shown in Table 4, increasing size of the R_3 group from methyl to ethyl and isopropyl decreased TRPV4 potency (**9a**, **18**, **19**). Interestingly when R_3 or R_{3a} is CF_3, the potency difference for these two analogs (**20** and **21**) was not as significant as the methyl analogs (**9a** and **9b**). Non chiral benzyl amides were synthesized to understand stereochemistry preference (**17**, **22**, and **23**). The unsubstituted benzyl amide (**17**) and dimethyl benzyl amide (**22**) maintained modest TRPV4 potency. Interestingly the cyclopropyl benzyl amide (**23**) possessed superior TRPV4 potency as **9a** and **20** analogs.

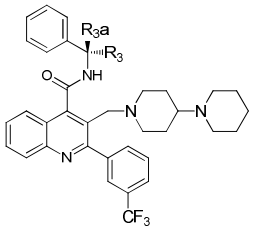
Table 3. Biochemical activities of substituted piperidine analogs in human and rat TRPV4 FLIPR assays



Compound	R_2	hTRPV4 IC_{50} (μM)[a]	rTRPV4 IC_{50} (μM)[a]
9a	piperidine	0.08	0.0025
12	H	>25	>25
13	NMe_2	2.51	0.32
14	morpholine	0.05	0.004
15	piperazine	3.16	0.79
16	cyclohexane	>25	>25

[a]IC_{50} values are the mean of at least two independent experiments.

Table 4. Biochemical activities of substituted amine analogs in human and rat TRPV4 FLIPR assays



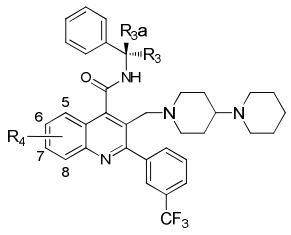
Compound	R_3	R_{3a}	hTRPV4 IC_{50} (μM)[a]	rTRPV4 IC_{50} (μM)[a]
9a	Me	H	0.08	0.0025
17	H	H	0.5	0.05
18	Et	H	0.16	0.006
19	iPr	H	0.13	0.025
20	CF_3	H	0.04	0.0025
21	H	CF_3	0.1	0.008
22	Me	Me	1	0.04
23	cyclopropyl		0.05	0.0025

[a]IC_{50} values are the mean of at least two independent experiments.

Quinoline substitution (R_4) was then examined. A variety of substitutions were generally tolerated. Exemplar data for substitutions at the 6- or 7- position are shown in

Table 5. In general, compounds with 7-ethoxy substitution (**27** and **30**) are more potent in the human TRPV4 assay than the corresponding 7-methoxy analogs (**26** and **29**). Electron-withdrawing groups such as bromo are tolerated at both 6- and 7- positions (**24**, **25**, **28**). Interestingly all substitutions maintained similar rat TRPV4 potency.

Table 5. Biochemical activities of substituted quinolines in human and rat TRPV4 FLIPR assays



Compound	R_3	R_{3a}	R_4	hTRPV4 IC_{50} (μM)[a]	rTRPV4 IC_{50} (μM)[a]
9a	Me	H	H	0.08	0.0025
20	CF_3	H	H	0.04	0.0025
24	CF_3	H	6-Br	0.06	0.0025
25	CF_3	H	7-Br	0.06	0.0032
26	CF_3	H	7-OMe	0.05	0.004
27	CF_3	H	7-OEt	0.01	0.0032
23	cyclopropyl		H	0.05	0.0025
28	cyclopropyl		7-Br	0.04	0.002
29	cyclopropyl		7-OMe	0.25	0.0025
30	cyclopropyl		7-OEt	0.08	0.0016

[a]IC_{50} values are the mean of at least two independent experiments.

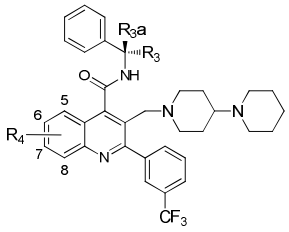
Among all the analogs that were synthesized and evaluated, **9a** represented an exemplar tool compound with excellent rodent TRPV4 activity. Compound **9a** was evaluated in a PK study in rat and found to have reduced clearance [CL = 25 mL/min/kg) and significantly improved oral bioavailability (~100% F) and exposure (DNAUC = 0.8 μg*h/mL/(mg/kg)] in Sprague-Dawley (SD) rats. Next we assessed selectivity of **9a** against a panel of cardiac ion channels. Compound **9a** was evaluated against hERG (PatchXpress), CaV1.2 (PatchXpress) and NaV1.5 (IonWorks) and found to have IC_{50}s of 1.2, 4.5 and 40 μM, respectively, representing ≥480 fold selectivity over rat TRPV4 potency. Based on the potency, selectivity and PK, **9a** was progressed as the first TRPV4 inhibitor for *in vivo* evaluation.

Compound **9a** was evaluated in an anesthetized dog CV study monitoring blood pressure (BP), heart rate (HR), cardiac output and left ventricular (LV) function. Dogs were pretreated with 1 and 3 mg/kg of **9a** dosed intravenously (n=4). QTc prolongation was observed with compound treatment, consistent with hERG inhibition measured *in vitro* (IC_{50} = 1.2 μM). However, **9a** reduced BP, HR, cardiac output and LV function unexpectedly at 3 mg/kg with Cmax of ~20 μM (free fraction of 0.94 μM). No effect was observed at 1 mg/kg with Cmax of ~7 μM (free fraction of ~0.3 μM).

To understand if the depressor effect of **9a** observed in dog was a TRPV4 mechanism-based or an off-target effect, **9a** was administered to TRPV4 KO and WT mice while monitoring BP and HR. Compound **9a** was dosed at 30 mg/kg via an iv infusion reaching a terminal plasma concentration of 10-11 μM. As observed in dog, BP and HR were reduced with **9a** administration. The responses were similar in KO and WT mice, suggesting **9a** possesses off-target activity that contributes to the reduction in BP and HR.

To investigate what off-target activity might contributed to the reduction in BP and HR, we evaluated **9a** in a number of assays including rabbit ventricular wedge, HCN_4 (pace maker channel), $CaV_{1.2}$ (L-type channel), $CaV_{2.2}$ (N-type channel), $CaV_{3.2}$ (T-type channel), and *in vitro* contractility in mouse isolated aorta and right atria assays. Based on the overall results, we concluded that the off-target effect observed with **9a** was likely due to frequency-dependent L-type channel inhibition with an IC_{30} of 1.7 μM at 0.1 Hz, and an IC_{30} of 0.12 μM at 2 Hz. Two Hz (i.e. 120 beats per minute) is considered to be more physiological relevant than the 0.1 Hz.[20] Minimal or no activities were observed with **9a** in all other assays evaluated.

Table 6. L-type channel inhibition activity for TRPV4 analogs



Compound	R_3	R_{3a}	R_4	hTRPV4 IC_{50} (μM)[a]	rTRPV4 IC_{50} (μM)[a]	CaV1.2 IC_{30} (μM) 0.1 (2) Hz
9a	Me	H	H	0.08	0.0025	1.7 (0.12)
27	CF_3	H	7-OEt	0.01	0.0032	10 (12)
28	cyclopropyl		7-Br	0.04	0.002	10.5 (10.5)

[a]IC_{50} values are the mean of at least two independent experiments.

In order to further understand the SAR relating to frequency dependent L-type channel inhibition, a set of structurally diverse quinolines with potent TRPV4 potency were evaluated for L-type channel inhibition using electrophysiology patch assay. Interestingly substitution at the quinoline improves the L-type channel selectivity. A shown in Table 6, **27** and **28** showed significant improvement in L-type selectivity over **9a** and a lack of frequency dependence. Upon further selectivity profiling,

we identified **27** as a PXR activator with EC_{50} of 1.3 μM while **28** is not.

The pharmacokinetic (PK) properties for compound **28** were evaluated in both rat and dog and found to have half-lives and oral exposure suitable for oral dosing in chronic animal models (Rat PK: iv CL = 7.3 mL/min/kg, po t1/2 = 10 h, % F = 31; Dog PK: iv CL = 6.9 mL/min/kg, po t1/2 = 31 h, %F = 53). Compound **28** was profiled against TRP channels and was selective against TRPV1, TRPA1, TRPC3, TRPC6, TRPM8 (IC_{50} >25 μM). In addition, **28** showed no blood pressure or heart rate effect in rats when dose up to 30 mg/kg.[8] GSK2193874 (**28**) is the first-in-class orally bioavailable $TRPV_4$ inhibitor which demonstrated ability to improve pulmonary functions in a number of heart failure models.[8]

In summary, starting from the quinoline screening hit **1a**, a lead optimization effort identified **9a** as a potential *in vivo* tool compound. Unfortunately **9a** exhibited unexpected hemodynamic effect in dogs. By evaluating **9a** in various *in vitro* cardiac channel assays and in *vitro* contractility assays in mouse isolated aorta and right atria, we concluded that the off-target effect observed with **9a** was likely due to frequency-dependent L-type channel inhibition. Further lead optimization resulted in the discovery of GSK2193874 (**28**), a highly potent, selective and orally active inhibitor of the $TRPV_4$ channel. GSK2193874 is an excellent *in vitro* and *in vivo* tool for target validation studies probing the biology of $TRPV_4$. Additional pharmacology data of GSK2193874 including $TRPV_4$ potency across species, potency against different $TRPV_4$ stimuli, selectivity data on CEREP panel and activities in pulmonary edema and heart failure models had been disclosed by Thorneloe et al.[8] Further SAR optimization leading to the identification of the pre-clinical candidates will be reported in due course.

ASSOCIATED CONTENT

Supporting Information. Material and methods for $TRPV_4$ FLIPR assays, ion channel assays (hERG, Cav1.2, Nav1.5) and hemodynamic measurement in anesthetized mice and dogs. Compound synthesis and spectroscopic characterization of **9a** and **28**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

* E-mail: mui.h.cheung@gsk.com

Present Addresses

N/A

Author Contributions

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All animal studies were performed in compliance with the Guide for the Care and Use of Laboratory Animals as published by the US National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of GlaxoSmithKline.

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ABBREVIATIONS

BP, blood pressure; compd, compound; CL, clearance; CV, cardiovascular; DNAUC, dose normalized area under the curve; FLIPR, fluorometric imaging plate reader; HR, heart rate; iv, intravenous; PK, pharmacokinetic; PXR, pregnane X receptor; SAR, structure-activity relationship; TRPV, transient receptor potential vanilloid; KO, knockout; WT, wild type.

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(20) Patch-Clamp recording of L-type Ca2+ current from freshly isolated guinea pig ventricular myocytes with stimulus frequency of either 0.1 Hz or 2 Hz.

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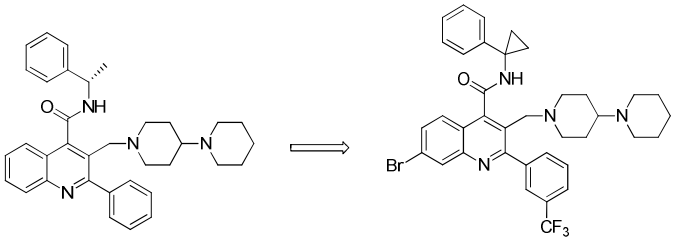
Discovery of GSK2193874: an Orally Active, Potent and Selective Blocker of Transient Receptor Potential Vanilloid 4

Mui Cheung,* Weike Bao, David J. Behm, Carl A. Brooks, Michael J. Bury, Sarah E. Dowdell, Hilary S. Eidam, Ryan M. Fox, Krista B. Goodman, Dennis A. Holt, Dennis Lee, Theresa J. Roethke, Robert N. Willette, Xiaoping Xu, Guosen Ye, Kevin S. Thorneloe



1a GSK2193874 (28)