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Identification of a novel 2-pyridyl-benzensulfonamide derivative, RQ-00203078, as a selective and orally active TRPM8 antagonist

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ABSTRACT

A novel series of 2-pyridyl-benzensulfonamide derivatives have been identified as selective and orally active TRPM8 antagonists via high throughput screening (HTS). Exploration of the structure–activity relationships of compound **1** has led to the identification of **RQ-00203078** (compound **36**) as a highly selective, potent and orally available TRPM8 antagonist.

RQ-00203078 demonstrated excellent in vivo activity in a dose dependent manner with an ED_{50} value of 0.65 mg/kg in the icilin-induced wet-dog shakes model in rats after oral administration and may become an important pharmacological tool for fully assessing the potential therapeutic use of the targets activated by cold stimulation.

The transient receptor potential melastatin 8 (TRPM8) is a member of the TRP melastatin sub-group (TRPM1–8), comprising of seven groups; TRP canonical (TRPC), TRP vanilloid (TRPV), TRP melastatin (TRPM), TRP polycystin (TRPP), TRP mucolipin (TRPML), TRP ankyrin (TRPA) and TRP no mechanoreceptor potential C (TRPN) within the TRP superfamily channels. TRPM8 is a calcium (Ca^{2+})-permeable, nonselective cation channel, expressed on primary sensory neurons (A-delta and C-fibers in the dorsal root ganglia), and is activated by cold temperature in both the innocuous (15–28 °C) and noxious (<15 °C) range, and by chemical agonists such as menthol and icilin.[1] TRPM8 channel is known to play a role in cold hyperalgesia and cold allodynia induced by disease conditions such as chemotherapy-induced peripheral neuropathy (CIPN, using the agents oxaliplatin, paclitaxel, or vincristine), diabetic neuropathy, migraine and overactive bladder (OAB).[2] A number of small molecule TRPM8 antagonists[3] have been disclosed and evaluated in menthol-induced calcium influx assays and the icilin-induced wet-dog shakes (WDS) model (Fig. 1). Studies with these antagonists have confirmed the role of TRPM8 antagonist in various preclinical animal models of cold allodynia and hypersensitivity. Recently, Pfizer has reported that PF-05105679, a selective TRPM8 antagonist, is analgesic in an experimental model of cold pain in humans.[3f] Therefore, small molecule antagonists of the TRPM8 channel are expected to provide important pharmacological tools for fully assessing the therapeutic potential of blocking the TRPM8 channel.

An HTS campaign with in-house library compounds was implemented in order to identify hit compounds. Among the hits, our group focused on sulfonamide **1**, shown in Figure 2. Potential issues associated with compound **1** were a moderate intrinsic

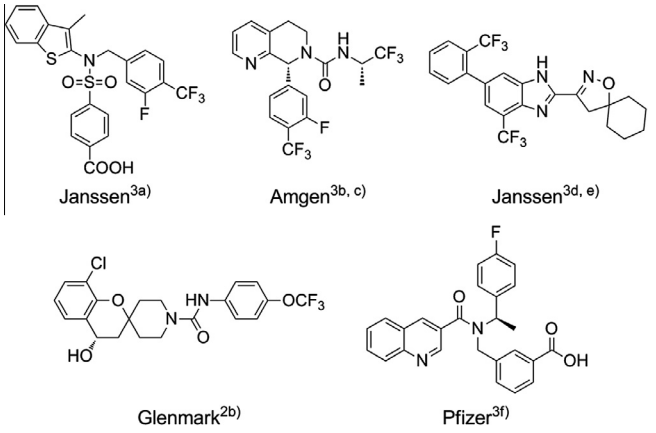

Figure 1. Reported TRPM8 antagonists.

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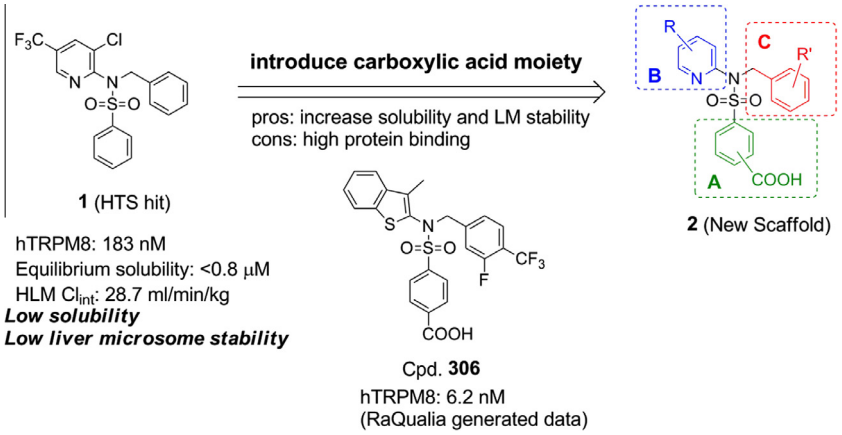
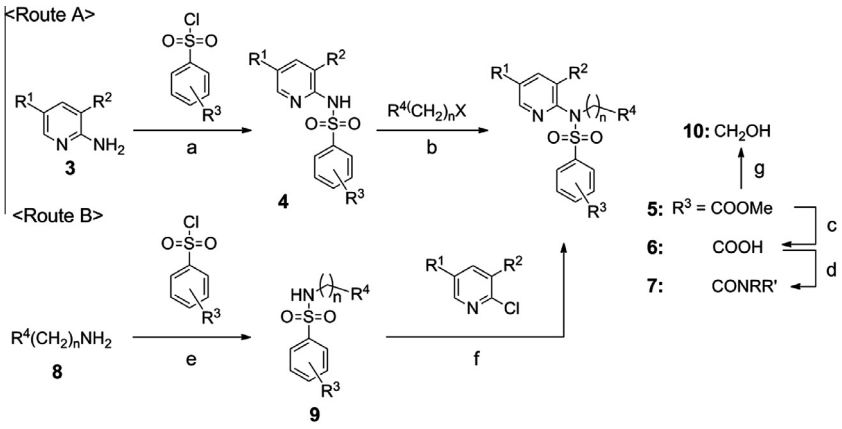


Figure 2. The hit compound in HTS and strategies for improving on several issues.



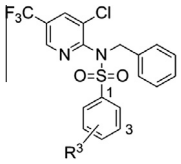
Scheme 1. Reagents and conditions: (a) NaH then sulfonyl chloride, THF, 0 °C—rt; (b) Cs_2CO_3, NaI, DMF, 80 °C; (c) NaOH, H_2O, THF, 50 °C; (d) RR′NH, EDCI, HOBt, Et_3N, CH_2Cl_2, rt; (e) Et_3N, CH_2Cl_2, 0 °C—rt; (f) Cs_2CO_3, MeCN, microwave irradiation, 160 °C, 10 min; (g) $LiAlH_4$, THF, rt.

clearance (Cl_{int} = 28.7 ml/min/kg) in human liver microsomes (HLM) and poor equilibrium solubility (<0.8 μM).[4] However, we were aware that similar TRPM8 antagonists of the sulfonamide derivatives with a carboxylic acid group had been disclosed independently by the Janssen group (e.g., compd **306**). While the introduction of a carboxylic acid group generally increases albumin binding,[5] it also tends to improve the stability in liver microsomes and equilibrium solubility.[6] This report summarizes our structure activity relationship (SAR) pursuit from the original hit compound **1** to the more advanced compound **36**, focusing on the improvements in liver microsome stability and equilibrium solubility through the introduction of a carboxylic acid group.

The sulfonamide derivatives **6** were prepared through the sequences A or B as shown in Scheme 1.[7] The sulfonamides **4** were obtained by treatment of the commercially available aniline derivatives **3** with sodium hydride and reaction with the corresponding benzenesulfonyl chloride derivatives. The sulfonamides **4** were then converted to the carboxylic acid derivatives **6** by N-alkylation with an alkyl halide followed by hydrolysis of the ester derivatives **5** (route A). In route B, the sulfonamides **9**, prepared from commercially available amines **8** and sulfonyl chlorides, were converted to the carboxylic acid derivatives **6** by S_NAr reaction with the aryl halide followed by hydrolysis of the ester derivatives **5**. The amide and alcohol derivatives **7** and **10** were prepared by amidation using the coupling reagent (EDCI) and reduction with lithium aluminum hydride ($LiAlH_4$), respectively.

As a result of the introduction of a carboxylic acid group on the A-region, compound **13** bearing a 4-carboxylic acid group was 3 times as potent as compound **1**, while compounds **11** and **12** bearing a 2- or 3-carboxylic acid group were less potent than the nonsubstituted compound **1**, as shown in Table 1. As expected, **12** and **13** exhibited improvements both in liver microsome

Table 1
Initial SAR of phenylsulfonylamide core (A-region)



Compd	R^3	hTRPM8 IC_{50} (nM)[a]	HLM Cl_{int} (ml/min/kg)	Equilibrium Solubility (μM)[b]
1	H	183	28.7	<0.8
11	2-COOH	15,974	N.D.[c]	N.D.
12	3-COOH	406	<7	285
13	4-COOH	61	<7	185
14	4-CH_2OH	42	>168	35
15a	4-$CONH_2$	70	27.2	<2.2
15b	4-CONHMe	57	85.4	20
15c	4-$CONMe_2$	67	>168	37
15d	4-$CONHCH_2CH_2OH$	216	77.7	123

[a] IC_{50} values based on inhibition of menthol (30 μM) induced Ca^{2+} influx in HEK293 cells.
[b] Equilibrium solubility measured in a high throughput automated method.[4]
[c] N.D. = Not determined.

stability and in equilibrium solubility. The amide derivatives (**15a**–**15c**) also had IC_{50} values less than 100 nM in the human TRPM8 calcium influx assays, but the metabolic stability and solubility of these compounds were not improved compared to compound **13**. Similarly, the benzyl alcohol derivative **14**, whilst having good potency with an IC_{50} value of 42 nM, did not show improvements in metabolic stability and solubility. Based on the SAR on the A-region of the molecule (Fig. 2), we selected compound **13** as a lead compound, and shifted our efforts to the SAR exploration on the pyridine (B-region) and benzyl (C-region) moieties.

In order to better understand the role of each region and substituent as pharmacophores, molecular overlay analysis was conducted using the compound **13** and compd **306**. As one can imagine from the similarity in the two-dimensional structures of the molecules, minimized conformers of the two molecules were easily superimposed (Fig. 3). The trifluoromethyl group in compound **13** overlapped well with the benzene moiety of the benzothiophene ring in compd **306**, indicating that these two moieties behave as hydrophobic groups in the pharmacophore. Furthermore, the molecular overlay suggested that there would be space for accommodating substituents on the benzyl moiety of compound **13**. With this hypothesis in mind, we set out to explore the SAR in the B- and C-regions.

As shown in Table 2, the substitution effect of R^1 on the pyridine ring was significant when R^2 was held constant as chlorine. Lipophilic atoms or groups such as chloro (**17**) and phenyl (**20**) were associated with strong antagonistic activity, while the hydrogen (**16**) and cyano (**18**) substituents reduced potency by 160- and 400-fold, respectively, compared with compound **13** bearing a trifluoromethyl group. Methyl derivative **19** showed moderate activity, with a 10-fold drop in potency compared to compound **13**. These results suggested that lipophilicity, rather than electronegativity, plays a key role in the antagonistic activity on TRPM8, which is in good accordance with the pharmacophore hypothesis suggested by the molecular overlay discussed above.

The effect of R^2 on the pyridine ring was then investigated with R^1 group being held constant as a trifluoromethyl group. The fluoro **21** and methyl **22** derivatives were 4- and 10-fold less potent, respectively, than the chloro-substituted compound **13**. R^2 groups such as cyano **23** and hydrogen **24** led to a drop in potency, with a 40-fold increase of the IC_{50} compared to compound **13**. Compound **25**, where the R^1 and R^2 groups were switched compared to compound **13**, had moderate activity, which suggested that there may be some restriction to the amount of lipophilicity and steric bulk that can be introduced at the R^2 position.

Taken altogether, we concluded that the combination of $R^1 = CF_3$ or Cl and $R^2 = Cl$ are optimum substituents for the B-region, and shifted to the SAR exploration of the benzyl moiety (C-region).

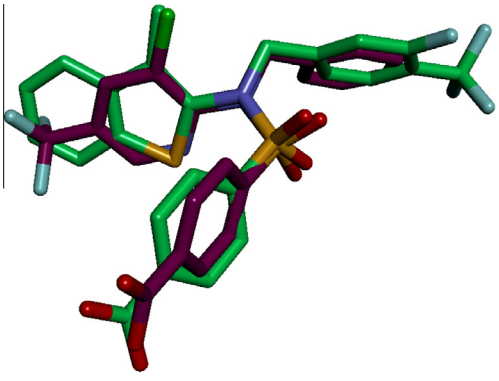
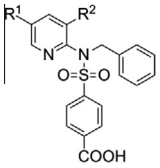


Figure 3. Molecular overlay of **13** (brown) and compd **306** (green).[8]

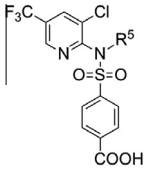
Table 2
Initial SAR of the pyridine core (B-region)

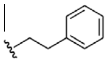
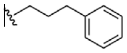
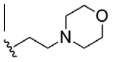


Compd	R^1	R^2	hTRPM8 IC_{50} (nM)[a]	HLM Cl_{int} (ml/min/kg)
13	CF_3	Cl	61	<7
16	H	Cl	9859	N.D.[b]
17	Cl	Cl	201	<7
18	CN	Cl	23,782	N.D.
19	Me	Cl	707	>168
20	Ph	Cl	122	85
21	CF_3	F	213	<7
22	CF_3	Me	736	N.D.
23	CF_3	CN	2472	N.D.
24	CF_3	H	2983	N.D.
25	Cl	CF_3	1562	N.D.

[a] IC_{50} values based on inhibition of menthol (30 μM) induced Ca^{2+} influx in HEK293 cells.
[b] N.D. = Not determined.

Table 3
Initial SAR of benzyl core (C-region)



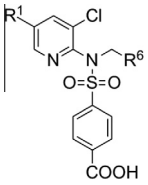
Compd	R^5	hTRPM8 IC_{50} (nM)[a]	HLM Cl_{int} (ml/min/kg)
13		61	<7
26		762	N.D.[b]
27		49	81
28		104	>168
29		8419	<7
30		3720	<7

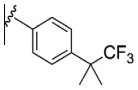
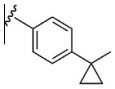
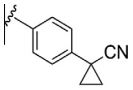
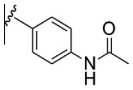
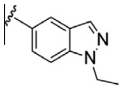
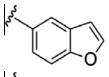
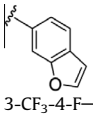
[a] IC_{50} values based on inhibition of menthol (30 μM) induced Ca^{2+} influx in HEK293 cells.
[b] N.D. = Not determined.

In an initial SAR study of the R^5 substituent, the phenylpropyl and cyclohexylmethyl derivatives (**27** and **28**) proved similar to or more potent than the benzyl derivative **13** in the in vitro assay of human calcium influx, but the phenethyl derivative **26** displayed a 10-fold drop in potency (Table 3). Additionally, the metabolic stability of these compounds decreased, as shown by a high clearance in HLM. Introduction of heteroaryl and heterocyclic moieties such as pyridyl and morpholyl (**29** and **30**) also resulted in a moderate activity, despite the improved metabolic stability. Therefore, as for the optimization of group, we selected the benzyl scaffold for R.[5]

Finally, we proceeded to optimize the substitution on the benzyl moiety (C-region), focusing on the in vitro antagonistic activity,

Table 4
SAR optimization of benzyl core (C-region)



Compd	R^1	R^6	hTRPM8 IC_{50} (nM)[a]	HLM Cl_{int} (ml/min/kg)
Compd 306	—	—	6.2	8.5
13	CF_3	Ph–	61	<7
31		2-CF_3—Ph–	27	<7
32		3-CF_3—Ph–	41	<7
33		4-CF_3—Ph–	8.9	<7
34		2-CF_3O—Ph–	96	<7
35		3-CF_3O—Ph–	57	<7
36		4-CF_3O—Ph–	8.3	<7
37		4-F—Ph–	40	<7
38		3-CF_3-4-F—Ph–	14	<7
39		4-*t*Bu—Ph–	0.96	>168
40			3.3	26.7
41			8.0	>168
42			67	<7
43			18,216	N.D.[b]
44			46	53
45			15	7.5
46			18	8.6
47	Cl	3-CF_3-4-F—Ph–	16	<7
48		4-CF_3—Ph–	11	<7

[a] IC_{50} values based on inhibition of menthol (30 μM) induced Ca^{2+} influx in HEK293 cells.
[b] N.D. = Not determined.

Table 5
Rat wet-dog shakes assay[a]

Compd	ED_{50} (mg/kg)	rTRPM8 IC_{50} (nM)	RLM Cl_{int} (ml/min/kg)	rPPB (%fu)[b]
36	0.65	5.8	<10.4	0.31
37	7.5	43	<10.4	1.4
46	8.8	28	41.1	1.2
48	1.3	9.0	<10.4	1.1

[a] Compounds **36**, **48** (0.3, 1, 3 and 10 mg/kg), compounds **37**, **46** (1, 3 and 10 mg/kg) and vehicle (0.5% methyl cellulose (MC)) were administered to fasted rats 1 h before icilin injection. After intraperitoneal injection of icilin (1 mg/kg), the number of wet-dog shakes (WDS) was counted over 30 min. The number of animals per group was 6.
[b] Rat plasma protein binding (percent fraction unbound).

hoping to achieve a drastic improvement of potency by introducing bulky lipophilic groups at the 4-position on the benzene ring (Table 4). In the event, compound **39** bearing a 4-*tert*-butyl group

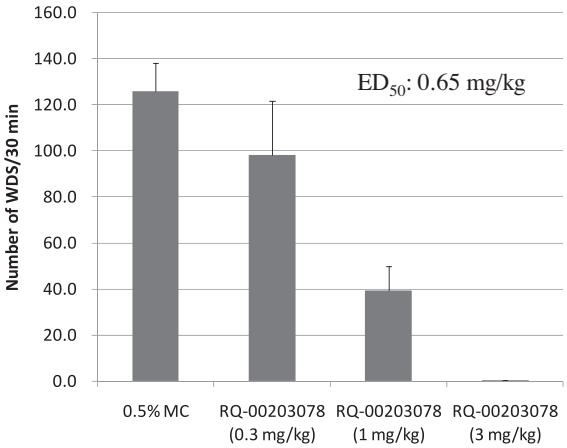
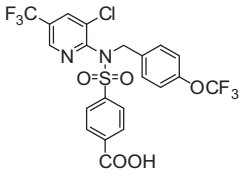


Figure 4. Dose dependent inhibitory effect on wet-dog shakes of compound **36** (**RQ-00203078**) Compounds **36** (**RQ-00203078**) and vehicle (0.5% methyl cellulose (MC)) were administered to fasted rats 1 h before icilin injection. After intraperitoneal injection of icilin (1 mg/kg), the number of wet-dog shakes (WDS) were counted over 30 min. The number of animals per group was 6.

Table 6
In vitro activities for TRP channels of compound **36** (**RQ-00203078**)



Compound	**36**
hTRPM8 (menthol)	8.3 nM
rTRPM8 (menthol)	5.8 nM
h/rTRPA1[a]	>10 μM/>10 μM
hTRPV4[b]	>3 μM
h/rTRPV1[c]	>10 μM/>3 μM

[a] IC_{50} values based on inhibition of allyl isothiocyanate (AITC) (100 μM) induced Ca^{2+} influx in HEK293 cells.
[b] IC_{50} values based on inhibition of hypotonic solution (90 mMD-mannitol + 1.26 mM $CaCl_2$) induced Ca^{2+} influx in HEK293 cells.
[c] IC_{50} values based on inhibition of capsaicin (*h*/*r*:600 nM/300 nM) induced Ca^{2+} influx in HEK293 cells.

demonstrated excellent potency with an IC_{50} value of 0.96 nM, a 60-fold improvement in antagonistic activity compared to that of compound **13** (IC_{50} 61 nM), but its stability in HLM was much decreased with an intrinsic Cl_{int} of more than 168 ml/min/kg. Accordingly, we decided to replace the *tert*-butyl group by trifluoromethyl-isopropyl and methylcyclopropyl moieties,[9] which are considered to be bioisosteres of the *tert*-butyl group. Compounds **40** and **41** also showed strong activity with IC_{50} values of 3.3 nM and 8.0 nM, respectively, but the metabolic stability of the compounds was still unsatisfactory with HLM clearances of 26.7 ml/min/kg and >168 ml/min/kg, respectively. Bicyclic fused ring systems such as indazole and benzofuran derivatives (**44**–**46**) demonstrated a 1.5- to 4-fold improvement in potency, compared to the compound **13**, with good to moderate metabolic stability. Furthermore, the derivatives **47** and **48**, where the R^1 substituent is now chlorine, also showed a dramatic increase of activity by the introduction of trifluoromethyl group at the 4-position of the C-region, compared to the corresponding analog **17** (IC_{50} = 201 nM). Having thus optimized in vitro activity, liver microsome stability and equilibrium aqueous solubility, we selected compounds **36**, **37**, **46** and **48** for in vivo evaluation in the wet-dog shakes (WDS) model in rats.

The 4 compounds were evaluated in the icilin-induced wet-dog shakes model in rats (Table 5),[10] and all 4 compounds attenuated the shaking behavior with ED_{50}'s of less than 10 mg/kg (p.o.), thus demonstrating that these sulfonamide derivatives are orally active TRPM8 antagonists. In particular, compound **36** showed significant efficacy with an ED_{50} of 0.65 mg/kg in a dose dependent manner (Fig. 4). In addition, excellent oral exposure of compound **36** was confirmed independently in rat PK studies at 3 mg/kg (p.o.) administration, with a C_{max} value of 2300 ng/mL and 86% bioavailability. Taken altogether, compound **36** was selected as an advanced compound with the code name **RQ-00203078**.

Finally, compound **36** was highly selective over other TRP channels (Table 6).

In summary, structure–activity relationship studies initiating from hit compound **1** have led to the identification of an advanced compound **36** (code name: **RQ-00203078**). **RQ-00203078** is a novel and highly potent TRPM8 antagonist with human and rat IC_{50} values of 8.3 nM and 5.8 nM, respectively, in the menthol-induced calcium influx assay, and demonstrated excellent activity in vivo in a dose dependent manner with an ED_{50} value of 0.65 mg/kg in the icilin-induced wet-dog shakes model in rats after oral administration. Pharmacological studies using **RQ-00203078** are now underway in several pharmacological models other than the pain model,[11] and the results will be reported in the near future.

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