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Arylglycine derivatives as potent transient receptor potential melastatin 8 (TRPM8) antagonists ☆

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A B S T R A C T

A series of arylglycine-based analogs was synthesized and tested for TRPM8 antagonism in a cell-based functional assay. Following structure–activity relationship studies in vitro, a number of compounds were identified as potent TRPM8 antagonists and were subsequently evaluated in an in vivo pharmacodynamic assay of icilin-induced 'wet-dog' shaking in which compound **12** was fully effective. TRPM8 antagonists of the type described here may be useful in treating pain conditions wherein cold hypersensitivity is a dominant feature.

Transient receptor potential melastatin 8 (TRPM8) is a member of the transient receptor potential (TRP) superfamily, comprising a diverse group of non-selective cation channels that are activated by a variety of physical and chemical stimuli. First described in 2002, TRPM8 was shown to be activated by innocuous cool to noxious cold temperatures as well as by chemical agonists, such as menthol and icilin.[1] TRPM8 is expressed on primary nociceptive Aδ and C fibers,[1,2] through which cold responses are transmitted. Studies on TRPM8 knock-out mice have demonstrated decreased sensitivity to cold temperature as well as decreased hypersensitivity to cold after nerve injury or inflammation.[3] Thus, antagonism of TRPM8 provides an attractive approach to the treatment of cold-related painful conditions, such as cold hyperalgesia and cold allodynia, which are commonly associated with certain types of neuropathic and inflammatory pain.[4] A number of small molecule TRPM8 antagonists have been reported in the literature as potential pain therapeutics.[5] Herein, we describe a series of arylglycine-based analogs that are potent TRPM8 antagonists (Fig. 1).

Several synthetic routes have been utilized to prepare the designed analogs. The synthesis of compounds **7** is shown in Scheme 1. Substituted phenylacetic acid **1** was treated with concd HCl in methanol to give its corresponding methyl ester, which was then reacted with *N*-bromosuccinimide (NBS) in the presence of a catalytic amount of aqueous 48% HBr in refluxing CCl_4 to give the

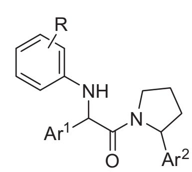
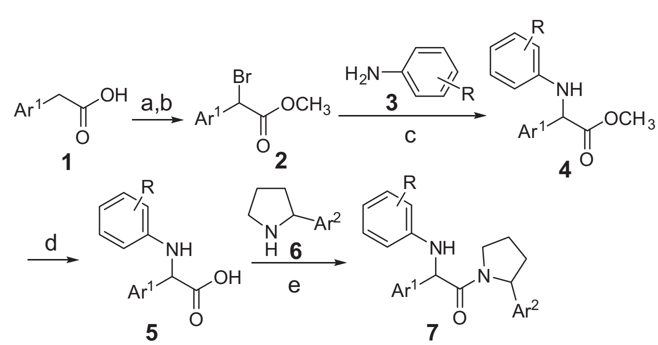


Figure 1. Arylglycine-based TRPM8 antagonists.



Scheme 1. Reagents and conditions: (a) concd HCl, MeOH, rt, 16 h; (b) NBS, cat. aq 48% HBr, CCl_4, reflux, 2 h; (c) CH_3CN, reflux, 16 h; (d) LiOH, H_2O, THF, rt, 3 h; (e) (i) HATU, Et_3N, CH_2Cl_2, rt, 16 h, only for compounds **7q**, **7r**, and **7x**: (ii) LiOH, THF, MeOH, H_2O, rt, 16 h.

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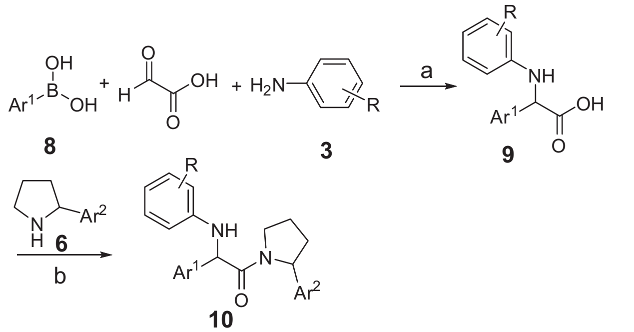
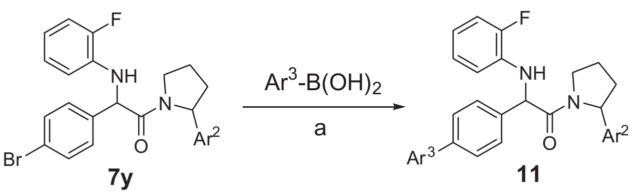
α-bromo compound **2**. Replacement of the α-bromo group with substituted aniline **3** was achieved by using an excess amount of **3** at elevated temperature. The ester group of compound **4** was hydrolyzed under basic conditions (LiOH, H_2O/THF), and the resulting carboxylic acid **5** was coupled with aryl-substituted pyrrolidine **6** under standard amide bond formation conditions [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), triethylamine, CH_2Cl_2] to give the final products **7**.

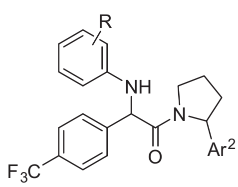


Scheme 2. Reagents and conditions: (a) CH_3CN, reflux, 3 h; (b) (i) HATU, Et_3N, CH_2Cl_2, rt, 16 h, only for compounds **10d** and **10e**: (ii) LiOH, THF, MeOH, H_2O, rt.



Scheme 3. Reagents and conditions: (a) (i) $Pd(dppf)Cl_2$, K_2CO_3, EtOH, H_2O, microwave, 130 °C, only for compound **11d**: (ii) LiOH, THF, MeOH, H_2O, rt.

Table 1
Effect of substituents R and Ar^2 on icilin-induced in vitro canine TRPM8 functional activity

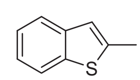


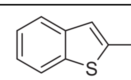
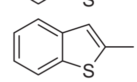
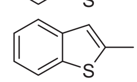
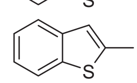
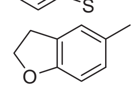
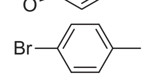
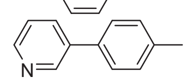
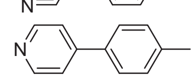
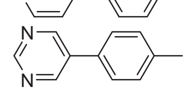
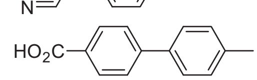
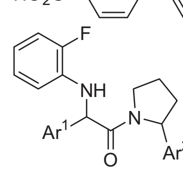
Compd	R	Ar^2	IC_{50} (μM)
7a	H	Phenyl	0.060
7b	H	2-F-phenyl	0.030
7c	H	2-OMe-phenyl	0.033
7d	H	(*S*)-(2-Cl-phenyl)	0.041
7e	H	(*R*)-(2-Cl-phenyl)	0.168
7f	2-F	Phenyl	0.005
7g	2-F	2-F-phenyl	0.007
7h	2-F	3-F-phenyl	0.009
7i	2-F	4-F-phenyl	0.037
7j	2-F	2-Cl-phenyl	0.011
7k	3-F	2-Cl-phenyl	0.138
7l	4-F	2-Cl-phenyl	0.073
7m	2-F	2-OMe-phenyl	0.012
7n	2-F	2-Pyridyl	0.025
7o	2-F	3-Pyridyl	0.007
7p	2-F	4-Pyridyl	0.019
7q	2-F	3-CO_2H-phenyl	0.040
7r	2-F	4-CO_2H-phenyl	0.066
7s	2-F	(*S*)-Phenyl	0.005
7t	2-F	(*R*)-Phenyl	0.043
7u	2-F	(*S*)-(2-F-phenyl)	0.008
7v	2-F	(*R*)-(2-F-phenyl)	0.044
7w	2-F	(*S*)-(2-Cl-phenyl)	0.017
7x	2-F	(*S*)-(3-CO_2H-phenyl)	0.033

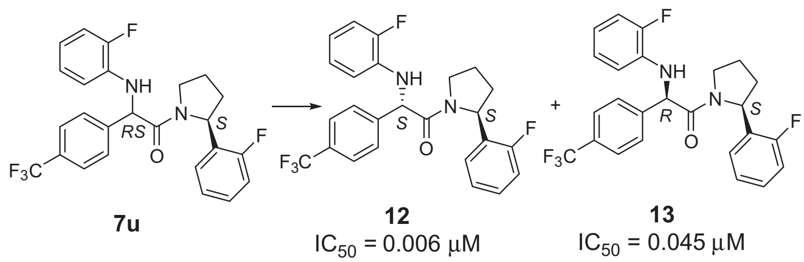
Scheme 2 depicts a more efficient route to the designed compounds via a boronic acid Mannich reaction.[6] The three-component condensation of boronic acid **8**, substituted aniline **3** and glyoxylic acid in refluxing acetonitrile led to carboxylic acid **9**. Coupling of **9** with aryl-substituted pyrrolidine **6** gave the desired final products **10**. Further functionalization at the Ar^1 group was achieved through palladium-catalyzed coupling reactions between 4-bromo-phenyl analog **7y** and aryl/heteroaryl boronic acids (Scheme 3).

The functional activity of the prepared analogs was determined in the canine TRPM8 Ca^{2+} flux assay, in which icilin-induced changes of intracellular calcium concentration in HEK293 cells stably expressing canine TRPM8 channels were measured using a Ca^{2+}-sensitive fluorescent dye.[7,8] Initially, the Ar^1 region was kept constant as a *para*-CF_3-phenyl group to expedite structure–activity relationship (SAR) studies of the R and Ar^2 groups. As illustrated in Table 1, analogs containing a 2-F-aniline (R = 2-F, **7f**, **7g**, **7m**) are more potent TRPM8 antagonists than those with an unsubstituted aniline (R = H, **7a–c**). The position of the fluorine group on the aniline is critical, as 3- or 4-substitution (R = 3-F or R = 4-F) led to much less potent compounds (**7j** vs **7k**, **7l**). Exploring SAR of the Ar^2 group indicated that both phenyl (**7f**) and pyridyl groups (**7n–7p**) afforded relatively high potency. Less polar substituents, such as halogen (**7g–7j**) and methoxy (**7m**), were tolerated on the phenyl group, whereas more polar groups, such as carboxylic acid (**7q**, **7r**), led to slightly reduced potency. The stereochemistry

Table 2
Effect of substituents Ar^1 and Ar^2 on icilin-induced in vitro canine TRPM8 functional activity



Compd	Ar^1	Ar^2	IC_{50} (μM)
10a		(*S*)-(2-F-phenyl)	0.003
10b		2-Pyridyl	0.008
10c		3-Pyridyl	0.025
10d		3-CO_2H-phenyl	0.078
10e		4-CO_2H-phenyl	0.041
10f		(*S*)-(2-F-phenyl)	0.014
7y		(*S*)-(2-F-phenyl)	0.009
11a		(*S*)-(2-F-phenyl)	0.007
11b		2-F-phenyl	0.025
11c		2-F-phenyl	0.010
11d		(*S*)-(2-F-phenyl)	0.056



Scheme 4. Separation of enantiomers **12** and **13**.

Table 3
Inhibition of icilin-induced WDS in rats

Compound	% Inhibition
7s	56 ± 19
7u	63 ± 15
7w	79 ± 13
11a	−13 ± 31
12	99 ± 1

of the pyrrolidine carbon to which Ar^2 is attached plays an important role. The *S* configuration is preferred over the *R* configuration (**7d** vs **7e**, **7s** vs **7t**, and **7u** vs **7v**).

Once the optimal R and Ar^2 groups had been identified through the initial screening, the scope of the Ar^1 substituent was expanded to include a variety of substituted phenyl groups and heteroaromatics (Table 2). The goal was to reduce lipophilicity and improve physiochemical properties of the resulting analogs. Fused bicyclic Ar^1 groups, such as benzothiophene (**10a**–**10e**) and 2,3-dihydrobenzofuran (**10f**), were investigated. Benzothiophene shared similar SAR with *para*-CF_3-phenyl as the Ar^1 group. In particular, benzothiophene analogs exhibited relatively high potency when Ar^2 was a 2-F-phenyl (**10a**) or a pyridyl group (**10b**, **10c**), whereas reduced potency was observed when the Ar^2 group was carboxyphenyl (**10d**, **10e**). For biaryl/heteroaryl Ar^1 groups, substitution of the phenyl ring with a pyridyl or pyrimidyl group was well tolerated, and the corresponding analogs (**11a**–**11c**) achieved potency equivalent to that of the *para*-CF_3-phenyl analogs. On the other hand, introduction of a carboxyphenyl substituent led to a slightly less potent analog **11d**.

Because stereoselective synthetic methods were not utilized, the final products **7**, **10** and **11** were all obtained as mixtures of (*R*) and (*S*) isomers at the carbon to which the aniline is attached. To identify the optimal stereochemistry at this position, the two diastereomers comprising compound **7u** were separated as the (*S*,*S*)-isomer **12** and the (*R*,*S*)-isomer **13**.[9] Compound **12** (IC_{50} = 0.006 μM), having the (*S*)-configuration at the asymmetric center, was more potent than compound **13** (IC_{50} = 0.045 μM), having the (*R*)-configuration (Scheme 4).

Several selected compounds were assessed in a rat ‘wet-dog’ shaking (WDS) assay. Administration of icilin, a potent TRPM8 agonist, causes WDS in rats, mice and other animals.[1a,10] This effect is mediated by TRPM8, because such behaviors are not manifested in TRPM8 knock-out mice.[3b] Treating rats with a TRPM8 antagonist reverses icilin-induced WDS, thereby providing a convenient pharmacodynamic assay to evaluate the test compounds in vivo. Icilin was administered at 3 mg/kg (ip) in 10% solutol/H_2O, and instances of spontaneous WDS were counted over a 10-min interval at 10–20 min post-icilin. Animals that exhibited 10 or more instances of WDS within this 10-min period were randomized into treatment groups and orally administered the test compounds at a dose of 30 mg/kg in 10% solutol/H_2O or the vehicle at a volume of 5 mL/kg. Ongoing WDS was counted again for 10 min at 60–70 min post-drug to assess treatment effects. The results are presented in Table 3 as a percent inhibition of WDS (average ± standard error), which was calculated as [1 − (test compound WDS count/vehicle WDS count)] × 100%.

As indicated in Table 3, compounds **7s**, **7u**, **7w** and **12** exhibited moderate to full efficacy in the reversal of icilin-induced WDS, with the enantiomerically pure compound **12** being the most effective at the dose tested. On the other hand, the pyridyl-substituted analog **11a** showed no efficacy in this model, possibly due to low plasma levels upon oral dosing.

In summary, a series of arylglycine-based analogs have been prepared and evaluated as TRPM8 antagonists. SAR studies led to a number of compounds with potent in vitro activity. Selected compounds also demonstrated robust in vivo efficacy in the icilin-induced WDS assay. In particular, compound **12** exhibited an excellent in vitro and in vivo profile and emerged as a strong candidate for further investigation. Ultimately, it is the goal of this research to identify TRPM8 antagonists that may be useful in treating pain and/or other conditions wherein cold hypersensitivity is a dominant feature.

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8. TRPM8 functional activity was determined by measuring changes in intracellular calcium concentration using a Ca^{2+} sensitive fluorescent dye. The changes in fluorescent signal were monitored by a fluorescence plate reader, either FLIPR (manufactured by Molecular Devices) or FDSS

(manufactured by Hamamatsu). Increases in intracellular Ca^{2+} concentration were readily detected upon activation with icilin. At 24 h prior to the assay, HEK293 cells stably expressing canine TRPM8 were seeded in culture medium in black wall, clear-base poly-D-lysine coated 384-well plates (BD Biosciences, NJ, USA) and grown overnight in 5% CO_2 at 37 °C. On assay day, the growth media was removed and cells were loaded with Calcium 3 Dye (Molecular Devices) for 35 min at 37 °C under 5% CO_2 and then for 25 min at rt. Subsequently, cells were tested for agonist induced increases in intracellular Ca^{2+} levels using FLIPR or FDSS. Cells were then exposed to test compound (at varying concentrations), and intracellular Ca^{2+} was measured for 5 min prior to the addition of icilin to all wells to achieve a final concentration that produces approximately an 80% maximal response. The IC_{50} values were determined from eight-point concentration–response studies. Curves were generated using the average of quadruplicate wells for each data point.

9. The absolute configurations of compounds **12** and **13** were determined by Vibrational Circular Dichroism (VCD).
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