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Pyrido[2,3-*b*]pyrazines, discovery of TRPV1 antagonists with reduced potential for the formation of reactive metabolites

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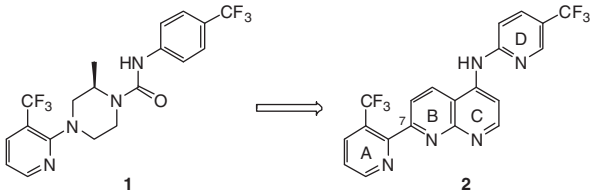
ABSTRACT

The transient receptor potential cation channel, subfamily V, member 1 (TRPV1) is a non-selective cation channel that can be activated by a wide range of noxious stimuli, including capsaicin, acid, and heat. Blockade of TRPV1 activation by selective antagonists is under investigation in an attempt to identify novel agents for pain treatment. During pre-clinical development, the 1,8-naphthyridine **2** demonstrated unacceptably high levels of irreversible covalent binding. Replacement of the 1,8-naphthyridine core by a pyrido[2,3-*b*]pyrazine led to the discovery of compound **26** which was shown to have significantly lower potential for the formation of reactive metabolites. Compound **26** was characterized as an orally bioavailable TRPV1 antagonist with moderate brain penetration. In vivo, **26** significantly attenuated carrageenan-induced thermal hyperalgesia (CITH) and dose-dependently reduced complete Freund's adjuvant (CFA)-induced chronic inflammatory pain after oral administration.

The transient receptor potential cation channel, subfamily V, member 1 (TRPV1) is a non-selective cation channel that can be activated by a wide range of noxious stimuli, including capsaicin, acid, and heat.[1] Blockade of TRPV1 activation by selective antagonists is under investigation in an attempt to identify novel agents for pain treatment.[2] We have previously disclosed the evolution of TRPV1 antagonists from ureas **1**[3] and biarylamides[4] to quinazolines[5] and ultimately 1,8-naphthyridines **2**[6] (Fig. 1). Compound **2** was superior to many of its forerunners in a number of respects including solubility, oral exposure and hERG inhibition. Furthermore, **2** demonstrated efficacy in both acute carrageenan-induced thermal hyperalgesia (CITH) and sub-chronic complete Freund's adjuvant (CFA) models, completely blocking the effects of CFA at 10 mg/kg po in rodents. In addition, **2** did not incorporate the 4-trifluoromethylaniline (TFMA) D-ring, which is known to be both mutagenic and clastogenic in vitro,[7] and was common to many of the more-potent early compounds. In **2**, the TFMA is replaced by the Ames negative 2-amino-5-trifluoromethyl pyridine without a significant loss in potency.[6]

The in vivo formation of reactive metabolites and subsequent formation of covalent adducts with biological macromolecules is recognized as a potential mediator of drug-induced idiosyncratic toxicity.[8] Because these toxicities may not manifest themselves until the later stages of development or after the launch of a drug, it is prudent to minimize the potential for formation of reactive metabolites during the lead optimization stage. During preclinical investigations, compound **2** was [^{14}C]-labeled at the 7-position, incubated with rat and human liver microsomes and afforded levels of irreversible binding of radioactivity (121 and 169 pmol-equiv/mg protein, respectively).[9] These levels exceeded our criteria for a compound to advance into development (<50 pmol/mg).[8a] Presumably, one of the aromatic rings of **2** was oxidized to an intermediate capable of reacting with a nucleophilic species on microsomal proteins, resulting in the formation of covalent adducts. Herein, we report on our efforts to improve the covalent binding profile of this series while maintaining the overall favorable properties exhibited by **2**.



1
hTRPV1 = 17 nM

2
hTRPV1 = 2.2 nM

Figure 1. Evolution of TRPV1 antagonists.

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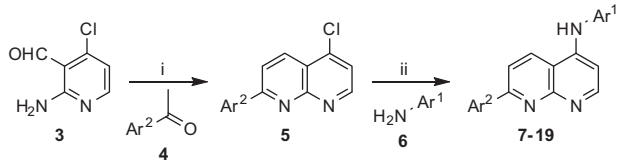
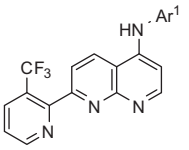
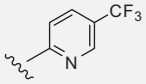
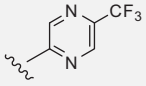
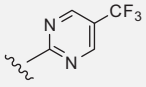
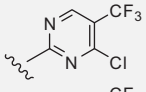
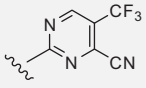
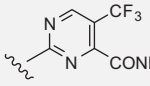
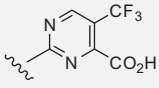
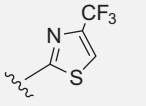


Scheme 1. Synthesis of 1,8-naphthyridines. Reagents and conditions: (i) ^{t}BuOK, THF, −20 °C (65–87%); (ii) Pd_2dba_3, Cs_2CO_3, xantphos, dioxane, 100 °C (43–84%).

Table 1
1,8-Naphthyridine D-ring analogs[a]



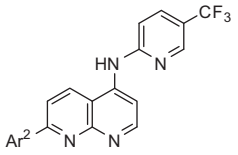
4, 9-16

Compd	Ar_1	hTRPV1-cap[b] IC_{50} (nM)	rTRPV1-pH[c] IC_{50} (nM)
2		2.2	3.2
7		5.5	6.3
8		4.4	4.6
9		81	na
10		25.4	na
11		48.7	na
12		1450	na
13		47	na

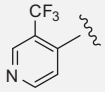
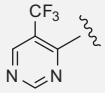
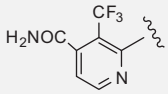
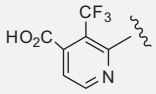
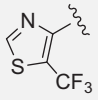
[a] Values are means of ⩾3 experiments.
[b] Human TRPV1 receptor activated by capsaicin.
[c] Rat TRPV1 receptor activated by low pH (5.0–5.5).

As the site of metabolic activation was unknown, we initially chose to keep the 1,8-naphthyridine core constant and target replacement of the A- or D-rings by a more electron deficient surrogate that we hypothesized would be less prone to oxidation. A two-step synthesis of the 1,8-naphthyridines described in this study is outlined in Scheme 1. Friedlander[10] reaction between the aldehyde **3**[6] and the ketone **4** gave the 7-substituted-4-chloro-1,8-naphthyridines **5** in good yields. In the second step, the D-ring was introduced using a palladium-catalyzed coupling reaction[11] between **5** and an aryl-amine **6** to provide compounds **7**–**19**. The compounds were then assayed for their ability to inhibit capsaicin activation of human TRPV1 receptors using fluorometric imaging plate reader (FLIPR) technology as well as their ability to inhibit the rat TRPV1 receptor upon activation by low pH (5.0–5.5).[12]

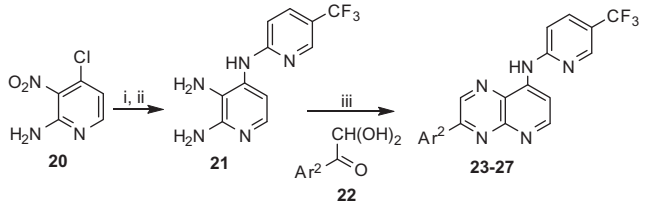
Table 2
1,8-Naphthyridine A-ring analogs[a]



2, 14-19

Compd	Ar_2	hTRPV1-cap[b] IC_{50} (nM)	rTRPV1-pH[c] IC_{50} (nM)
2		2.2	3.2
14		161	na
15		8.4	7.1
16		134	na
17		13	na
18		607	na
19		12	na

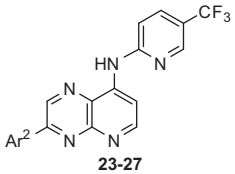
[a] Values are means of ⩾3 experiments.
[b] Human TRPV1 receptor activated by capsaicin.
[c] Rat TRPV1 receptor activated by low pH (5.0–5.5).



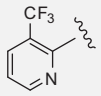
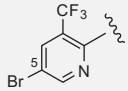
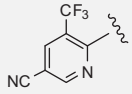
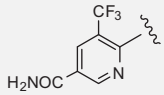
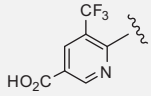
Scheme 2. Synthesis of pyrido[2,3-*b*]pyrazines **23**–**27**. Reagents and conditions: (i) 2-amino-5-trifluoromethyl pyridine·HCl, MeCN (48%); (ii) Pd/C, H_2 (50 psi), MeOH, (87%); (iii) $NaHCO_3$, EtOH (43–63%).

Changes to the D-ring were investigated first and the introduction of a second nitrogen atom to the ring gave pyrazine **7** and pyrimidine **8** and only a small loss in potency relative to **2** (Table 1). The chloro-pyrimidine **9** was less active, but provided a handle to introduce other electron withdrawing groups. However, the nitrile **10**, amide **11** and acid **12** were less potent than **2**. A number of five-membered D-ring analogs were also prepared, for example the thiazole **13,** but a loss in potency was generally observed. The pyrimidine **8** was evaluated in the rat CITH model[13] of acute inflammatory pain and gave 78% inhibition at 3 mg/kg po. On the basis of this encouraging in vivo efficacy, the covalent binding liability of [^{14}C]-labeled **8**[14] in human liver microsomes was assessed.

Table 3
Pyrido[2,3-*b*]pyrazine analogs[a]



23-27

Compd	Ar^2	hTRPV1-cap[b] IC_{50} (nM)	rTRPV1-pH[c] IC_{50} (nM)
23		0.2	na
24		30	na
25		15	na
26		0.8	1.3
27		27	na

[a] Values are means of ⩾3 experiments.
[b] Human TRPV1 receptor activated by capsaicin.
[c] Rat TRPV1 receptor activated by low pH (5.0–5.5).

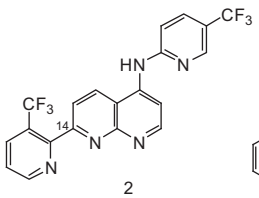
However, the incubation afforded higher levels of irreversible binding of radioactivity (204 pmol equiv/mg protein) than **2** and the pyrimidine analog **8** was not pursued further.

A-ring modifications are compiled in Table 2. The pyridyl isomer **14** was significantly less potent than **2**, the pyridazine **15** was fourfold less active while the pyrimidine **16** was of similar activity as **14**. Introduction of electron withdrawing groups to the A-ring as in amide **17** or acid **18,** led to a loss in potency. A limited number of 2-trifluoromethyl substituted five-membered heterocycles were evaluated and the thiazole **19** had encouraging potency (hTRPV1 12 nM). The [^{14}C]-labeled thiazole **19**[15] was incubated with rat and human liver microsomes but afforded unacceptably high levels of irreversible binding of radioactivity (340 and 356 pmol equiv/mg protein, respectively).

Replacement of the 1,8-naphthyridine by a more electron deficient pyrido[2,3-*b*]pyrazine was investigated next and the synthesis is outlined in Scheme 2. 4-Chloro-pyridine **20**[16] was treated with 2-amino-5-trifluoromethyl pyridine and hydrogenation of the nitro group yielded the diaminopyridine **21**. Cyclization between **21** and **22**[17] was affected by treatment with $NaHCO_3$ in ethanol affording moderate yields of the desired pyrido[2,3-*b*]pyrazines **23**–**27**.

The parent compound **23** (Table 3) had excellent in vitro potency and in in vivo fully inhibited CITH in rats following a 3 mg/kg oral dose, with a significant effect at the 0.3 mg/kg dose. [^{14}C]-Labeled **23** was incubated in human liver microsomes and encouragingly, afforded no irreversible binding of radioactivity. In rat microsomes, however, 54 pmol equiv/mg protein of radioactivity was observed, indicating that although the pyrido-pyrazine core was a significant improvement over the 1,8-naphthyridine core (compare **2** and **23**, Table 4) there was still some risk of reactive metabolite formation. We postulated that the residual covalent binding might result from bioactivation of the A-ring and chose to investigate electron-withdrawing groups at the 5-position of the pyridine, a position that had not been investigated in the 1,8-naphthyridine series. The bromo-**24** and cyano-**25** analogs were several orders of magnitude less potent than **23**. The amide **26,** however, was well tolerated (hTRPV1 0.8 nM and rTRPV1 1.3 nM) while the corresponding acid was less active (hTRPV1 14 nM). [^{14}C]-Labeled amide **26** was incubated with rat and human liver microsomes and afforded acceptable levels of irreversible binding of radioactivity (19 and 8 pmol equiv/mg protein), respectively. In an in vivo study in rats, **26** (8.1 mg/kg po) demonstrated low covalent binding to liver (<10 pmol equiv/mg protein at 2, 6 and 24 h) and plasma proteins (<10 pmol equiv/mg protein at 2, 6 and 24 h).

Table 4
Covalent binding to rat and human liver microsomal proteins obtained by incubation of [^{14}C]-compounds[9]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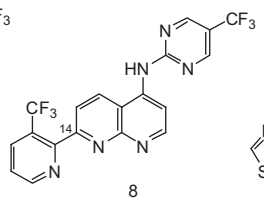
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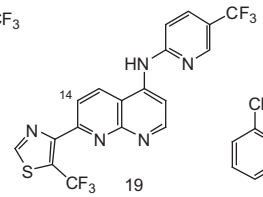
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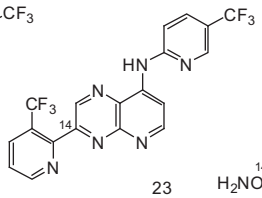
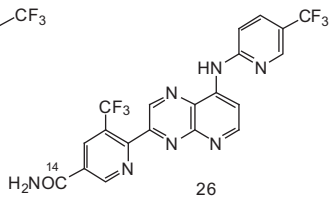

23


26

Compd	hTRPV1-cap[a] IC_{50} (nM)	rTRPV1-pH[b] $2IC_{50}$ (nM)	Rat liver µsomes[c]	Rat µsomes covalent binding[d]	Human µsomes covalent binding[d]	CITH %inhib (mpk, po)[e]	Plasma concn (µM)[f]
2	2.2	3.2	100	121 ± 13	169 ± 4	66 @ 1 mpk 115 @ 3 mpk	0.30 ± 0.14 1.40 ± 0.69
8	4.4	4.6	100	20 ± 3	204 ± 15	78% @ 3 mpk	na
19	12	na	100	340 ± 42	356 ± 8	na	na
23	0.2	na	100	54 ± 9	<5	85 @ 0.3 mpk 134 @ 3 mpk	na
26	0.8	1.3	100	19 ± 2	8 ± 1	49 @ 1 mpk 74 @ 3 mpk	0.24 ± 0.07 1.09 ± 0.06

[a] Human TRPV1 receptors activated by capsaicin.
[b] Rat TRPV1 receptors activated by low pH (5.0–5.5).
[c] Percentage of compound remaining after 10 min in rat liver microsomes.
[d] pmol equiv/mg protein at 1 h of incubation.
[e] Dosed as a suspension in 0.5% methocel.
[f] Plasma samples taken immediately after CITH experiment.

Table 5
Plasma PK data for **26**

	Rat	Dog	Rhesus monkey
Dose IV[a] (mg/kg)	1	1	1
$T_{1/2}$ (h)	2.3	1.5	4.4
Cl (ml/min/kg)	25 ± 3	7.4 ± 1.3	2.8 ± 0.7
Vd (l/kg)	4.3 ± 0.1	1.3 ± 0.1	1.0 ± 0.2
Dose PO[b] (mg/kg)	1	1	1
F (%)	103 ± 15	57 ± 11	38 ± 11
C_{max} (μM)	0.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.3
AUC_{0-24h} (μM h)	1.4 ± 0.1	2.7 ± 0.2	4.9 ± 0.8
Dose PO[b] (mg/kg)	10	10	na
C_{max} (μM)	2.1 ± 0.2	5.2 ± 0.7	
AUC_{0-24h} (μM h)	29.9 ± 3.1	68 ± 6.9	
Dose PO[b] (mg/kg)	100	na	na
C_{max} (μM)	15 ± 4.3		
AUC_{0-24h} (μM h)	268 ± 93		

[a] Dosed as a solution in 3:1 PEG300:water.
[b] Dosed as a suspension in 0.5% methocel.

Table 6
Plasma concentrations of compound **26** for the CFA model (Fig. 1)

Dose of **26** (mg/kg)[a]	Plasma concentration 2 h post dose (μM)		
	Day 1	Day 2	Day 3
1	0.27 ± 0.01	0.36 ± 0.02	0.35 ± 0.04
3	1.20 ± 0.40	1.10 ± 0.11	1.28 ± 0.09
10	2.43 ± 0.80	3.12 ± 0.52	4.24 ± 0.56

[a] Dosed as a suspension in 0.5% methocel.

Compound **26** had acceptable PK parameters in three species; rat, dog and rhesus monkey (Table 5) including excellent oral bioavailability (38–103%) across all three species. At higher doses of **26** excellent exposures were also achieved in both rat (10 and 100 mg/kg po) and dog (10 mg/kg po). At the 10 mg/kg dose in both species the exposure was ~20-fold higher than those at 1 mg/kg while at 100 mg/kg in rat a ~10-fold increase was achieved. In vitro, **26** demonstrated high permeability in MDCK cells (P_{app} = 34 × 10^{-6} cm/s) and a Pgp efflux ratio ((B→A)/(A→B)) of 3.1, indicating moderate Pgp efflux liability. However, at the 10 mg/kg po dose in rat, analysis of brain samples indicated a significant resistance to crossing the blood–brain barrier; AUC_{0-24h} 3.4 μM h, C_{max} 0.3 μM and brain-to-plasma ratio ~0.1.

The amide **26** was profiled in the CITH model and gave 49% and 74% inhibition at 1 and 3 mg/kg po with associated plasma concentrations of 0.24 and 1.09 μM, respectively, and was advanced into the CFA model. Compound **26** reversed mechanical hyperalgesia, in this rat model of persistent inflammation. Rats received an intraplanar injection of complete Freund's adjuvant (CFA, 200 μL) into the left hind paw. The next day the rats were dosed orally once a day for three consecutive days with either **26** (1, 3, or 10 mg/kg), vehicle or naproxen (20 mg/kg, positive control). Mechanical hypersensitivity was recorded as a differential weight-bearing response, measured 0, 1, 2, 4 and 24 h post-dosing. In a separate cohort, plasma samples were taken each day, 2 h after dosing **26** (Table 6). Within experimental errors, the plasma levels showed a close to linear relationship with increasing dose, and at the 10 mg/kg dose plasma concentrations reached 4.24 μM. Figure 2 shows the difference in weight bearing between ipsilateral (CFA-injected) and contralateral hind paws over the course of the study. A single dose of **26** (1, 3 and 10 mg/kg po) produced a dose-dependent inhibition on day 1 of 39%, 65% and 85% at 2 h with plasma concentrations of 0.27 μM, 1.2 μM and 2.4 μM, respectively. Similar levels of inhibition were observed following dosing on days 2 and 3 with an overall increase in the magnitude of inhibition at 24 h post dose. On day 3 this equated to 42%, 64% and 70% inhibition at the three doses tested. The level of inhibition demonstrated by **26** at 10 mg/kg was equivalent to that observed for rats treated with naproxen (20 mg/kg) in the same study. It has been suggested in the literature that CNS penetration is an important attribute for a TRPV1 antagonist in order to achieve maximum efficacy in pain models that incorporate a component of central sensitization (e.g., in the CFA-induced mechanical allodynia model).[18] In our hands, robust efficacy is achieved in the CFA weight-bearing model when **26** is administered at 10 mg/kg po. This corresponds to a C_{max} in the brain of ~300 nM, or about 100-fold over the rat IC_{50} (rTRPV1pH 3.2 nM).

To help demonstrate that the observed behavioral effects were specific to blockade of TRPV1, compound **26** was tested for off-target activity against a panel of more than 160 receptors, ion channels, transporters and kinases (MDS Pharma Services). Significant effects were only observed at the hERG channel (IC_{50} 5.9 μM) and monoamine oxidase MAO-A (IC_{50} 2.4 μM) and MAO-B (IC_{50} 2.5 μM). Cardiovascular effects were assessed in anaesthetized dogs but no significant effects on cardiovascular parameters (MAP, HR or ECG intervals) were observed at plasma concentrations of up to 24 μM. Additional studies evaluating the inhibition

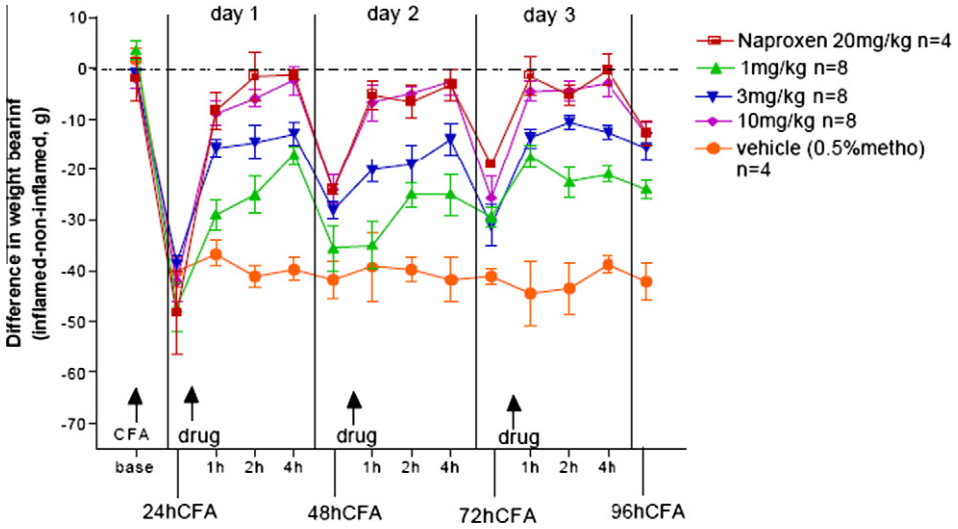


Figure 2. Effect of compound **26** at 1, 3 and 10 mg/kg po in the CFA model of inflammation and spontaneous nociception.

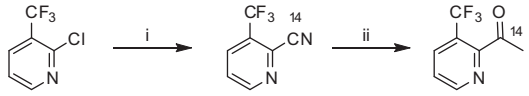
of MAO activity in vivo and the potential for tyramine interaction may be required to fully assess the risk of hypertension from using compound **26** in a clinical setting.

Recent reports have indicated that administration of TRPV1 antagonists causes hyperthermia in rodents[19] and humans.[20] None of the compounds in the current study were assessed in this paradigm, however, we have previously reported that compound **2** in rodents caused a significant increase in initial core body temperature an effect that was tolerated upon repeat dosing.[6] It remains to be established if this undesirable effect can be disconnected from analgesic activity.

In summary, the 1,8-naphthyridine **2** demonstrated levels of irreversible binding of radioactivity that exceeded our criteria for a compound to advance into development. Substitution of either the A- or D-ring of **2** by a more electron deficient surrogate was investigated but no improvement in covalent binding was observed. Replacement of the 1,8-naphthyridine by a pyrido[2,3-*b*]pyrazine core led to the identification of compound **23** and a significant lowering of the potential for reactive metabolite formation. Finally, the incorporation of a primary amide at the 5-position of the A-ring, afforded **26** (hTRPV1 0.8 nM and rTRPV1 1.3 nM) and minimal potential for covalent protein binding both in vitro and in vivo. The amide **26** had excellent oral exposure across three species and at high doses (100 mg/kg) in rat. The pyrido[2,3-*b*]pyrazine **26** displayed a good selectivity profile in an off-target screen of over 160 receptors, ion channels and enzymes although MAO inhibition was identified as a potential issue that would need to be further evaluated in preclinical studies. The overall profile of **26** made it an excellent candidate for additional in vivo studies to assess the pharmacological effects of a TRPV1 antagonist with moderate brain penetration. In rats, **26** significantly attenuated inflammatory pain in both an acute (carrageenan) and a sub-chronic (CFA) model, further supporting the utility of a TRPV1 antagonist for the treatment of inflammatory pain states (e.g., osteo-arthritis).

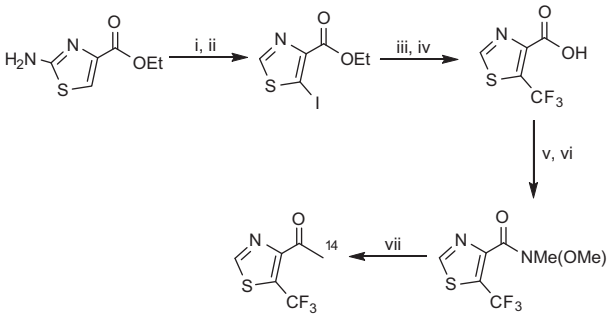
References and notes

1. Szallasi, A.; Cortright, D. N.; Blum, C. A.; Eid, S. R. *Nat. Rev. Drug Disc.* **2007**, *6*, 357.
2. Westaway, S. M. *J. Med. Chem.* **2007**, *50*, 2589; Gharat, L.; Szallasi, A. *Drug Dev. Res.* **2007**, *68*, 477.
3. (a) DeSimone, R. W.; Hodgetts, K.; Krause, J. E.; White, G. PCT WO 02/08221.; (b) Bakthavatchalam, R.; Hutchison, A.; DeSimone, R. W.; Hodgetts, K.; Krause, J. E.; White, G. U.S. Patent 6,723,730, 2004.
4. Zheng, X.; Hodgetts, K. J.; Brielmann, H.; Hutchison, A.; Burkamp, F.; Jones, A. B.; Blurton, P.; Clarkson, R.; Chandrasekhar, J.; Bakthavatchalam, R.; De Lombaert, S.; Crandall, M.; Cortright, D.; Blum, C. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5217.
5. Blum, C. A.; Zheng, X.; Brielmann, H.; Hodgetts, K. J.; Bakthavatchalam, R.; Chandrasekhar, J.; Krause, J. K.; Cortright, D.; Matson, D.; Crandall, M.; Ngo, C. K.; Fung, L.; Day, M.; Kershaw, M.; De Lombaert, S.; Chenard, B. L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4573.
6. Blum, C. A.; Caldwell, T.; Zheng, X.; Bakthavatchalam, R.; Capitosti, S.; Brielmann, H.; De Lombaert, S.; Kershaw, M. T.; Matson, D.; Krause, J. E.; Cortright, D.; Crandall, M.; Jones, A. B.; Martin, W. J.; Murphy, B. A.; Boyce, S.; Mason, G.; Rycroft, W.; Perrett, H.; Conley, R.; Burnaby-Davies, N.; Chenard, B. L.; Hodgetts, K. J. *J. Med. Chem.* **2010**, *53*, 3330.
7. Brent, R. L. *Teratology* **2001**, *63*, 106.
8. (a) Evans, D. C.; Watt, A. P.; Nicoll-Griffith, D. A.; Baillie, T. A. *Chem. Res. Toxicol.* **2004**, *17*, 3; (b) Nassar, A. F.; Lopez, A. A. *Curr. Opin. Drug Disc. Dev.* **2004**, *7*, 126; (c) Kalgutkar, A. S.; Soglia, J. R. *Expert Opin. Drug Metab. Toxicol.* **2005**, *1*, 91; (d) Ju, C.; Uetrecht, J. P. *Curr. Drug Metab.* **2002**, *3*, 367.
9. Samuel, K.; Yin, W.; Stearns, R. A.; Tang, Y. S.; Chaudhary, A. G.; Jewell, J. P.; Lanza, T., Jr.; Lin, L. S.; Hagmann, W. K.; Evans, D. C.; Kumar, S. *J. Mass Spectrom.* **2003**, *38*, 211.
10. Cheng, C.-C.; Yan, S.-J. *Org. React.* **1982**, *28*, 37.
11. Yin, J.; Zhao, M. M.; Huffman, M. A.; McNamara, J. M. *Org. Lett.* **2002**, *4*, 3481.
12. Compounds were tested as previously described: Szallasi, A.; Blumberg, P. M.; Annicelli, L. L.; Krause, J. E.; Cortright, D. N. *Mol. Pharmacol.* **1999**, *56*, 581.
13. Carrageenan-induced thermal hyperalgesia model of acute inflammatory pain as described by: Field, M. J.; Oles, R. J.; Lewis, A. S.; McCleary, S.; Hughes, J.; Singh, L. *Br. J. Pharmacol.* **1997**, *121*, 1513.
14. The brevity of the route to the 1,8-naphthyridine core allowed the introduction of the [^{14}C]-label at a late stage in the synthesis. The synthesis of the [^{14}C]-labeled ketone which was then used in the Friedlander cyclization (as outlined in Scheme 1) is shown below.



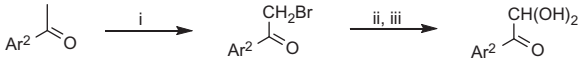
Reagents and conditions: (i) $Zn(^{14}CN)_2$, $Pd_2(dba)_3$, dppf, DMF, 100 °C (87%); (ii) CH_3MgBr, THF, −20 °C, H_3O^+ (78%).

15. The synthesis of the [^{14}C]-labeled thiazole is outlined below.



Reagents and conditions: (i) NIS, DMF, 60 °C (73%); (ii) ^{t}BuONO, THF, 50 °C (49%); (iii) methyl 2,2,-difluoro-2-(fluorosulfonyl)acetate, CuI, DMF, 85 °C (47%); (iv) 1 M NaOH, dioxane, rt (93%); (v) $(COCl)_2$, DMF_{cat}, CH_2Cl_2 (99%); (vi) HN(OMe)Me·HCl, $NEt(^iPr)_2$, CH_2Cl_2 (61%); (vii) $^{14}CH_3MgBr$, THF, −20 °C (84%).

16. Deady, L. W.; Korytsky, O. L.; Rowe, J. E. *Aust. J. Chem.* **1982**, *35*, 2025.
17. The dihydroxy-ketones used in the cyclization to pyrido[2,3-*b*]pyrazines were prepared from the methyl ketone.



Reagents: (i) Br_2, HBr, AcOH (67%); (ii) $AgNO_3$, MeCN (93%); (iii) NaOAc, DMSO (95%).

18. Cui, M.; Honore, P.; Zhong, C.; Gauvin, D.; Mikusa, J.; Hernandez, G.; Chandran, P.; Gomtsyan, A.; Brown, B.; Bayburt, E. K.; Marsh, K.; Bianchi, B.; McDonald, H.; Niforatos, W.; Neelands, T. R.; Moreland, R. B.; Decker, M. W.; Lee, C.-H.; Sullivan, J. P.; Faltynek, C. R. *J. Neurosci.* **2006**, *26*, 9385.
19. (a) Honore, P.; Chandran, P.; Hernandez, G.; Gauvin, D. M.; Mikusa, J. P.; Zhong, C.; Joshi, S. K.; Ghilardi, J. R.; Sevcik, M. A.; Fryer, R. M.; Segreti, J. A.; Banfor, P. N.; Marsh, K.; Neelands, T.; Bayburt, E.; Daanen, J. F.; Gomtsyan, A.; Lee, C. H.; Kort, M. E.; Reilly, R. M.; Surowy, C. S.; Kym, P. R.; Mantyh, P. W.; Sullivan, J. P.; Jarvis, M. F.; Faltynek, C. R. *Pain* **2009**, *142*, 27; (b) Tamayo, N.; Liao, H.; Stec, M. M.; Wang, X.; Chakrabarti, P.; Retz, D.; Doherty, E. M.; Surapaneni, S.; Tamir, R.; Bannon, A. W.; Gavva, N. R.; Norman, M. H. *J. Med. Chem.* **2008**, *51*, 2744; (c) Garami, A.; Shimansky, Y. P.; Pakai, E.; Oliveira, D. L.; Gavva, N. R.; Romanovsky, A. A. *J. Neurosci.* **2010**, *34*, 1435.
20. Gavva, N. R.; Treanor, J. J. S.; Garami, A.; Fang, L.; Surapaneni, S.; Akrami, A.; Alvarez, F.; Bak, A.; Darling, M.; Gore, A.; Jang, G. R.; Kesslak, J. P.; Ni, L.; Norman, M. H.; Palluconi, G.; Rose, M. J.; Salfi, M.; Tan, E.; Romanovsky, A. A.; Banfield, C.; Davar, G. *Pain* **2008**, *136*, 202.