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Discovery and synthesis of 6,7,8,9-tetrahydro-5*H*-pyrimido-[4,5-*d*]azepines as novel TRPV1 antagonists

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ABSTRACT

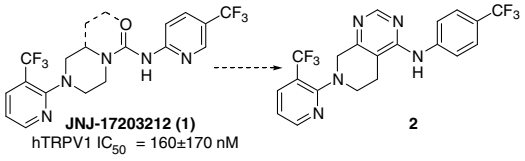
Utilization of a tetrahydro-pyrimdoazepine core as a bioisosteric replacement for a piperazine-urea resulted in the discovery a novel series of potent antagonists of TRPV1. The tetrahydro-pyrimdoazepines have been identified as having good in vitro and in vivo potency and acceptable physical properties.

The transient receptor potential vanilloid subtype I (TRPV1 or VR1) receptor is a ligand-gated cation channel, that is, activated or modulated by a variety of mediators, such as capsaicin, noxious heat, low pH, polyamines and lipids such as endogenous anandamide.[1] In addition, various endogenous substances such as bradykinin, substance P, glutamate, prostaglandins and ATP sensitize TRPV1.[2] As an integrator and mediator of nociceptive and/or inflammatory stimuli, TRPV1 is an attractive therapeutic target for the treatment of various neuro-inflammatory disorders. Recent reviews on the discovery of TRPV1 antagonists highlight efforts to develop novel therapeutics in this area.[3]

We previously characterized a series of piperazine-ureas as potent TRPV1 antagonists.[4] From that series, JNJ-17203212 (hTRPV1 IC_{50} = 160 nM) was identified as a lead compound, showing good in vitro activity, PK properties, and in vivo efficacy. In an effort to develop a new series of compounds as well as improve the in vivo properties of JNJ-17203212, an analogous scaffold to the piperazine-urea series was developed. We envisioned that we could use a fused pyrimidine ring to mimic the urea moiety of JNJ-17203212, leading to a novel series of TRPV1 antagonists. A flexible molecular alignment of the new fused-ring scaffold using a low energy conformation search demonstrated that it overlaid well with JNJ-17203212 (Fig. 1).[5] It was suggested that the newly formed tetrahydro-pyrido-pyrimidine core would serve well as a bioisosteric replacement for the piperazine-urea.[6]

Tetrahydro-pyridopyrimidines were prepared from condensation of commercially available β-ketoesters **4** with various amidines providing pyrimidinol **5** (Scheme 1). Hydrogenolysis of the benzyl group followed by S_N-aryl displacement under microwave conditions with 2-fluoro-3-trifluoromethyl-pyridine provided the pyridinyl-tetrahydro-pyridopyrimidinol **8**. The pyrimidinol was chlorinated using $POCl_3$. The desired tetrahydro-pyridopyrimidines 11–**16** were prepared via S_N-aryl displacement with a variety of aryl amines.

Utilizing the previously disclosed SAR from the piperazine-urea series,[4] the 3-trifluoromethyl-pyridine was chosen as the preferred



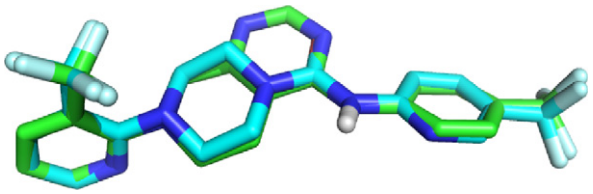


Figure 1. JNJ-17203212-aqua, compound **2**-green.

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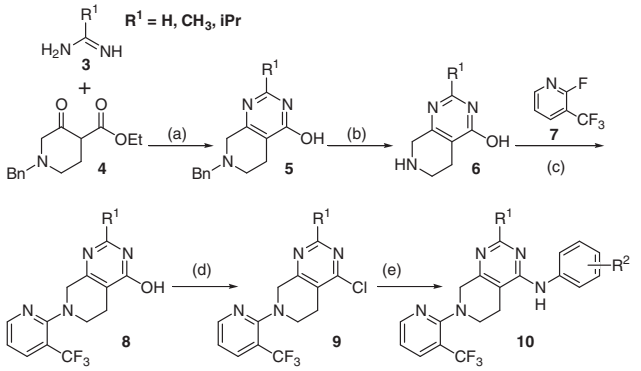


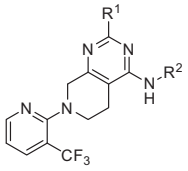
Scheme 1. Reagents and conditions: (a) NaOEt, EtOH, reflux, 75%; (b) $Pd(OH)_2$, 1,4-cyclohexadiene, EtOH, 86%; (c) *t*-amyl-OH, 180 °C μW, 5 h, 60%; (d) $POCl_3$, CH_3CN, reflux, 70%; (e) $ArNH_2$, *n*-BuOH, 120 °C, 2 h, 60–90%.

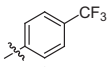
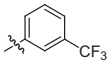
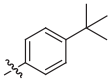
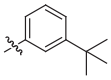
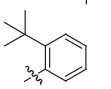
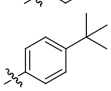
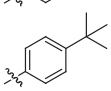
substituent on the piperidine ring nitrogen. The tetrahydro-pyridopyrimidine SAR paralleled the SAR from the piperazine-urea series in showing a preference for *para*-substitution on the aryl amine portion (R^2). Additionally, it was demonstrated that substitution at the 2-position on the pyrimidine **16–17** was tolerated (Table 1), which added an additional point of diversity as compared to the urea series.[7]

Movement of the nitrogen in the piperidine ring from the 7-position to the 6-position resulted in a loss of potency. The lack of potency suggested a conformational distinction between the two regio-isomers. Molecular modeling provided a rationale for this hypothesis by showing poor overlap of **18** with piperazine-urea JNJ-17203212 (Fig. 2).

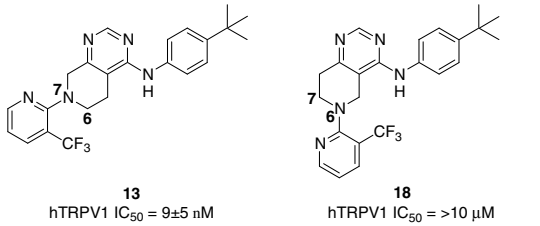
We were concerned that the tetrahydro-pyridopyrimidine core could undergo oxidation, resulting in potentially toxic pyridinium

Table 1
In vitro FLIPR data for recombinant human TRPV1 activated by capsaicin[8]



Compound	R^1	R^2	hTRPV1 IC_{50} (nM)[a]
11	H		15 ± 09
12	H		1450 ± 605
13	H		9 ± 5
14	H		520 ± 355
15	H		>20,000
16	CH_3		80 ± 28
17	*i*-Pr		18 ± 4

[a] Values are means of three experiments ± SEM.



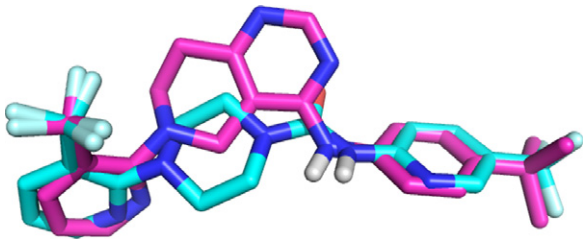
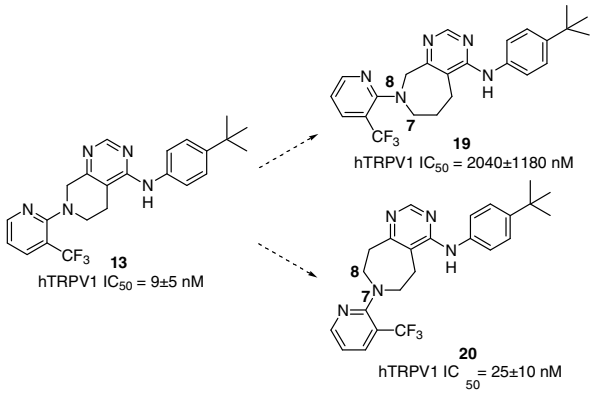


Figure 2. Compound **18**-magenta, JNJ-17203212-aqua.



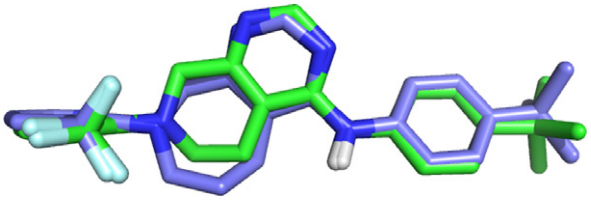


Figure 3. Compound **13**-green, compound **19**-blue.

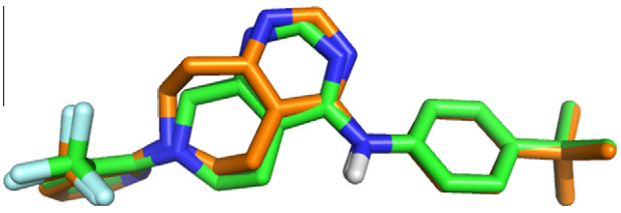
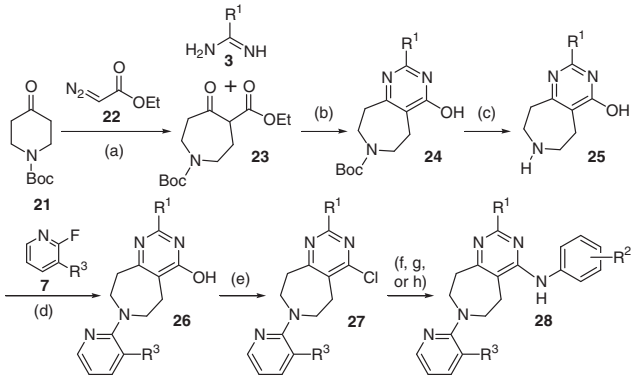
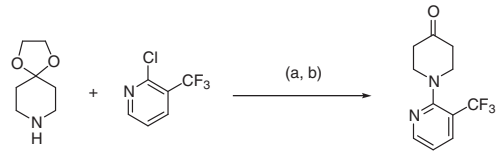


Figure 4. Compound **13**-green, compound **20**-orange.

formation.[9] To circumvent this issue, we chose to evaluate the tetrahydro-pyrimidoazepine scaffold as well. In the tetrahydro-pyridopyrimidine scaffold, a loss of activity was observed when the position of the piperidine nitrogen was moved from the 7-position **13** to the 6-position **18**. It was unclear which azepine, the 8-position **19** or the 7-position **20**, would be most analogous to the 7-position tetrahydro-pyridopyrimidine **13**; therefore, both were synthesized. Based on the data from pyridopyrimidines **13** and **18** our expectation was that the 8-position azepine would be the more potent isomer. Unexpectedly, in vitro data showed that the

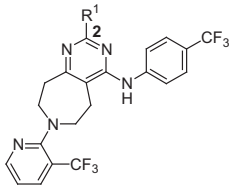


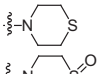
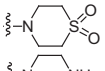
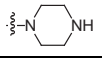
Scheme 2. Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, ether, 0 °C, 1 h 58%; (b) NaOEt, EtOH, reflux, 24 h, 70%; (c) HCl/dioxane, CH_2Cl_2, RT, 12 h then PL-HCO_3 MP-resin, MeOH, RT, 30 min., quant.; (d) *t*-amyl-OH, 180 °C μW, 3 h, 40–60%; (e) $POCl_3$, CH_3CN, reflux, 70%; (f) R^2NH_2, *n*-BuOH, 120 °C μW, 30 min., 60–90%; (g) R^2NH_2, TsOH, toluene, 130 °C, 1 h, 50–80%; (h) R^2NH_2, $Pd(OAc)_2$, DCPB, NaOt-Bu, toluene, 130 °C μW, 30 min., 70–85%.



Scheme 3. Reagents and conditions: (a) K_2CO_3, DMSO, 100 °C; (b) conc. HCl, 70% over two-steps.

Table 2
In vitro FLIPR data for recombinant human TRPV1activated by capsaicin



Compound	R^1	hTRPV1 IC_{50} (nM)[a]
32	H	188 ± 76
33	CH_3	97 ± 69
34		31 ± 13
35		51 ± 12
36		37 ± 24
37	NH_2	1058 ± 480
38		56 ± 37
39		87 ± 58
40		61 ± 59
41		6 ± 2
42		19 ± 7
43		>20,000
44		3350 ± 351

[a] Values are means of three experiments ± SEM.

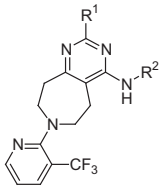
7-position azepine **20** was the more potent isomer. This result was rationalized via molecular alignment. Molecular alignments of **13** together with each of the azepines are shown in Figures 3 and 4. While the 7-position azepine **20** shows good overlap with **13**, the 8-position azepine **19** shows significant deviation. In particular, the azepine ring is somewhat bulkier than the analogous piperazine ring, and in **19** it occupies a region of space thought be sensitive to steric bulk, based on the SAR described in the piperazine-urea series.[4]

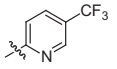
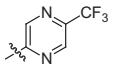
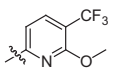
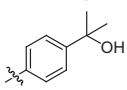
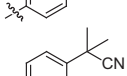
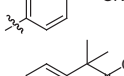
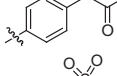
The tetrahydro-pyrimidoazepine were synthesized in a straightforward fashion (Scheme 2).[10] Ring expansion of 4-*N*-Boc-piperidone **21** using ethyl diazoacetate and $BF_3{\cdot}OEt_2$ followed by condensation using various amidines **3** afforded the pyrimidinol **24**. Deprotection of the azepine nitrogen and S_NAr displacement under microwave conditions with a variety of 2-chloropyridines resulted in the appropriate N-substituted azepine **26**. Chlorination of the pyrimidinol with $POCl_3$ provided the chloropyrimidine **27**, which could be displaced with a variety of aryl amines via S_NAr displacement or with heteroaryl amines using palladium-mediated coupling to provide the desired tetrahydro-pyrimidoazepine.

Alternatively, the hydroxy-pyrimidine can be accessed by installing the pyridine onto the piperidone prior to ring expansion and condensation (Scheme 3).[11]

Utilizing the SAR from our previous work, the 4-CF_3-aniline was chosen as the optimal substituent. Maintaining the 4-CF_3-aniline constant, the 2-position of the pyrimidine was evaluated. It was shown that the 2-position tolerated a variety of groups, some showing a substantial increase in potency when compared to the unsubstituted analog **32** (Table 2). Compounds **38–43** suggested that a lipophilic basic amine at the 2-position of the pyrimidine

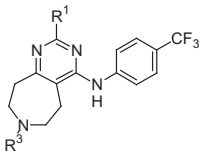
Table 3
In vitro FLIPR data for recombinant human TRPV1 activated by capsaicin

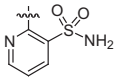
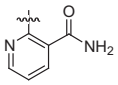
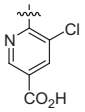
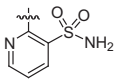
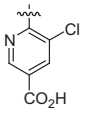
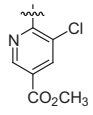


R^2	R^1 = *i*-Pr compound hTRPV1 IC_{50} (nM)[a]	R^1 = R^1 =  Compound hTRPV1 IC_{50} (nM)[a]	R^1 = R^1 = Compound hTRPV1 IC_{50} (nM)[a]
	**45** 130 ± 48	**46** 43 ± 13	**47** 38 ± 2
	**48** 70 ± 5	**49** 21 ± 4	ND
	**50** 2460 ± 780	**51** >6670	ND
	**52** 180 ± 110	**53** 361 ± 391	**54** 56 ± 10
	**55** 580 ± 76	**56** 18 ± 7	**57** 37 ± 27
	**58** 1450 ± 920	**59** 58 ± 31	**60** 230 ± 170
	**61** 2520 ± 2550	**62** 25 ± 5	**63** 52 ± 27

[a] Values are means of three experiments ± SEM. ND, not determined.

Table 4
In vitro FLIPR data for recombinant human TRPV1 activated by capsaicin



Compound	R^1	R^3	hTRPV1 IC_{50} (nM)[a]
64	H		6160 ± 622
32	H		190 ± 76
65	H		907 ± 507
66	H		2330 ± 887
67	H		2780 ± 2390
68	H		2360 ± 2150
69	*i*-Pr		1940 ± 1180
70	*i*-Pr		4220 ± 1630
71			449 ± 89
72			462 ± 65
73			176 ± 163

[a] Values are means of three experiments ± SEM.

Table 5
In vitro data for **41**[8]

	Human TRPV1 (nM)	Rat TRPV1 (nM)
RTX binding K_i	14 ± 2	180 ± 80
Capsaicin IC_{50}	6 ± 2	19 ± 4
pH IC_{50}	6 ± 7	ND

Table 6
Pharmacokinetic profile for **41** (1 mg/kg iv and 5 mg/kg p.o) in fasted Sprague–Dawley rats

	$T_{1/2}$ (h)	Cl (L/h/kg)	V_{ss} (L/kg)	AUC_{inf} (h ng/mL)	C_{max} (μM)	%*F*
41	1.1	3.1	4.7	322 (iv) 236 (p.o.)	0.160	14

provided improved activity. Additionally, simple alkyl substituents **33–36** also showed an increase in activity.

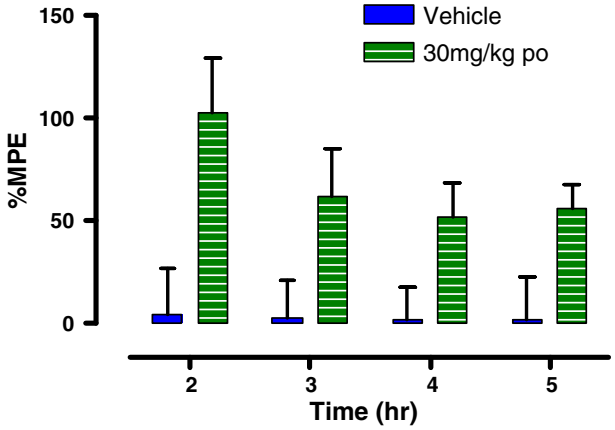


Figure 5. Representative data showing the effect of **41** on carrageenan-induced thermal hyperalgesia in rats (dosed p.o. in 5% pharmasolve/20% RH-40 cremophor/75% dextrose (5%) in water, administered 1 h prior to carrageenan injection). % Maximal possible effect (%MPE).[14]

Further exploration of the aniline substituent yielded a limited set of groups that maintained good activity (Table 3). Initially, good activity was seen when R^1 was an isopropyl group. However, these compounds had limited oral absorption in vivo due to poor aqueous solubility. Introduction of an amine in the 2-position of the pyrimidine not only resulted in an increase in potency but also provided an opportunity for improvement in developability via salt formation.

Analysis of the SAR suggested that substitution in the 3-position of the pendant pyridine on the azepine was required for activity, preferably 3-CF_3 **32**. It was proposed that a substituent in the 3-position imparts an orthogonal arrangement between the pyridine and the azepine core. This hypothesis may be confirmed with the lack of activity observed with compound **64** in which a co-planar arrangement is predicted. A variety of compounds containing pyridyl groups with substituents in the 3-position that might be expected to give an orthogonal arrangement were prepared (Table 4). However, most of these substituents resulted in decreased activity with respect to compound **32**, indicating that orthogonal geometry was not the only requirement for activity. Additionally, a combination of substituents in the 2-position of the pyrimidine as well as di-substitution in the 3- and 5-positions of the pyridine led to active compounds **72**, **73**. Introduction of polar substituents in the 5-position of the pyridine also resulted in compounds with improved solubility (**72**, SIF[12] = 218 μg/mL) at the expense of hTRPV1 activity.

Upon identifying a novel series of functional antagonists of TRPV1, compound **41** was chosen for further profiling both in vitro and in vivo (Table 5).

The rat pharmacokinetic profile of **41** displayed moderate clearance and volume of distribution (Table 6). Limited absorption due to poor solubility (pH 2 = 5 μg/mL and SIF = 0.5 μg/mL) was observed for **41**. Although **41** did not exhibit ideal PK properties, it was examined in an in vivo model of inflammatory pain, carrageenan-induced thermal hyperalgesia[13] of the paw. Compound **41** significantly attenuated thermal hyperalgesia, expressed as % maximal possible effect (% MPE), when dosed orally at 30 mg/kg (Fig. 5). Despite the low oral bioavailability, the compound concentrations were adequate in order to achieve efficacy in vivo. Terminal plasma concentrations, which were taken approximately 5 h post-carrageenan injection, were determined to be 146 ± 35 nM for compound **41**.

In conclusion, utilizing a pyrimidine-azepine core as a bioisosteric replacement for the piperazine-ureas resulted in the discovery a novel series of potent antagonists of TRPV1. The tetrahydro-pyrimdoazepines have been identified as having good in vitro activity

across species and acceptable physical properties for evaluation in vivo. The examination of **41** aids in the further evaluation of the therapeutic potential of TRPV1 antagonists.[15]

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12. Aqueous equilibrium solubility determined in fasted-state simulated intestinal fluid. The solubility assay was conducted in a 96-well format using DMSO stock solutions (10 mM of compound). DMSO was evaporated and residual solids were re-suspended in fasted-state simulated intestinal fluid (SIF; pH 6.8) or pH 2.0 for 3 days. The resulting mixtures were filtered and analyzed by HPLC against external standards.
13. Under anesthesia, 100 μL of 1% carrageenan (Sigma) in saline was injected subcutaneously into the plantar surface of the hind paw. For methods and references on the thermal hyperalgesia testing see Ref. 4a.
14. The paw withdrawal latency (PWL), in seconds, for each group is expressed as the mean ± standard error of the mean (SEM) at each time point. PWL of the injected paw is compared between the vehicle and the treated group. A % MPE value was generated by taking the area under curve (AUC) of PWL in seconds over the time course of the experiment in hours for the drug-treated group and normalized against the vehicle-treated group. The 100% MPE mark was defined as the pre-test latencies of each animal multiplied by the duration of the experiment and the baseline 0% MPE mark was defined as the mean vehicle AUC. Statistical analysis was performed using two-way ANOVA with Bonferroni's multiple comparisons with a significance level of $p < 0.05$.
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