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1,2-Diamino-ethane-substituted-6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*d*]azepines as TRPV1 antagonists with improved properties

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ABSTRACT

Based upon a previously reported lead compound **1**, a series of 1,2-diamino-ethane-substituted-6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*d*]azepines were synthesized and evaluated for improved physiochemical and pharmacokinetic properties while maintaining TRPV1 antagonist activity. Structure–activity relationship studies directed toward improving the aqueous solubility (pH 2 and fasted-state simulated intestinal fluid (SIF)) and rat pharmacokinetics led to the discovery of compound **13**. Aqueous solubility of compound **13** (pH 2 = >237 μg/mL and SIF = 11 μg/mL) was significantly improved over compound **1** (pH 2 = 5 μg/mL and SIF = 0.5 μg/mL). In addition, compound **13** afforded improved rat pharmacokinetics (CL = 0.7 L/kg/h) compared to compound **1** (CL = 3.1 L/kg/h). Compound **13** was orally bioavailable and afforded a significant reversal of carrageenan-induced thermal hyperalgesia at 5 and 30 mg/kg in rats.

The vanilloid receptor 1 (VR1 or TRPV1) is the best characterized member of the transient receptor potential family of ion channels.[1,2] TRPV1 is a ligand-gated, non-selective cation channel that is primarily expressed in nociceptive C- and Aδ fibers. The TRPV1 receptor is activated by a wide range of stimuli including heat (>43 °C), low pH, vanilloid ligands such as capsaicin and resiniferatoxin (RTX), and a wide range of endogenous mediators such as bradykinin and anandamide.[3] In general, activation of the TRPV1 channel results in depolarization, neuronal hyper-excitability, and ultimately the sensation of pain.[4] TRPV1 knockout mice demonstrate an impaired ability to develop inflammatory thermal hyperalgesia, suggesting that TRPV1 has an important role in transmitting inflammatory pain signals.[5] For this reason, efforts to discover small molecule TRPV1 antagonists have received considerable attention from many pain research groups.[6] Despite the abundance of structurally distinct TRPV1 antagonist scaffolds, two of the challenges facing a number of molecules reported are their relatively high lipophilicity and correspondingly poor aqueous solubility.[6] Recently, several approaches toward improving the aqueous solubility of TRPV1 antagonists have been reported.[7] Herein, we report our efforts to improve the solubility of a promising TRPV1 antagonist scaffold.

In a previous report, we described a series of 6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*d*]azepines that led to the identification of compound **1** as a potent TRPV1 antagonist.[8] During the course of that report, the structure–activity relationships for the 6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*d*]azepine series were elucidated. These studies suggested that in order to maintain TRPV1 potency, the 3-trifluoromethyl-pyridin-2-yl and 4-trifluoromethylphenyl amine substituents were preferred, whereas a variety of substituents at the C2-position of the pyrimidine were tolerated. Unfortunately, further profiling of compound **1** revealed low aqueous solubility (pH 2 = 5 μg/mL and fasted-state simulated intestinal fluid (SIF; pH 6.8) = 0.5 μg/mL) and high clearance in rats (CL = 3.1 L/h/kg).[8] To gain insight into the clearance issue, we conducted in vitro metabolism studies in the presence of both rat and human liver microsomes. These studies demonstrated that the morpholine ring in compound **1** was oxidized and could potentially contribute to the high clearance observed in rats. Therefore, our strategy was to replace the morpholine ring with substituents that could enhance aqueous solubility and rat pharmacokinetics while maintaining TRPV1 potency. To that end, our efforts were directed toward improving aqueous solubility through the incorporation of 1,2-diamino-ethane analogues at the C2-position of the pyrimidine (e.g., compound **2**) (Fig. 1). We reasoned that incorporation of 1,2-diamino-ethane analogues could provide an additional group for ionization and enhanced polarity. In addition to the potential improvements in aqueous solubility, we also hoped these efforts would afford opportunities for salt formation providing greater ease of development.

The general synthesis of 1,2-diamino-ethane substituted-6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*d*]azepines is outlined in

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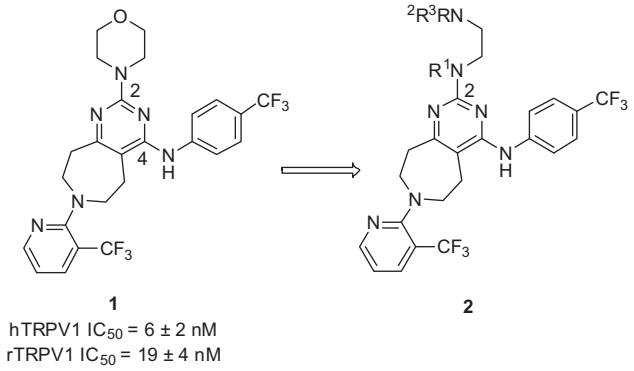
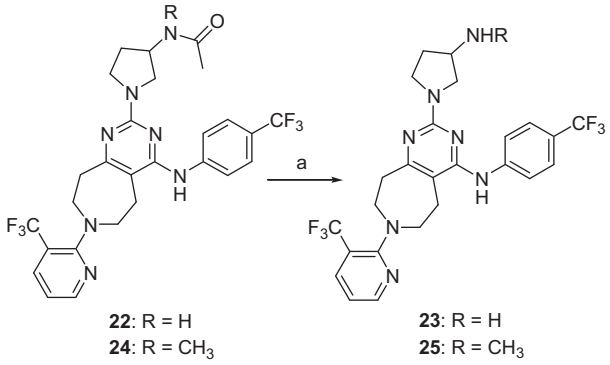
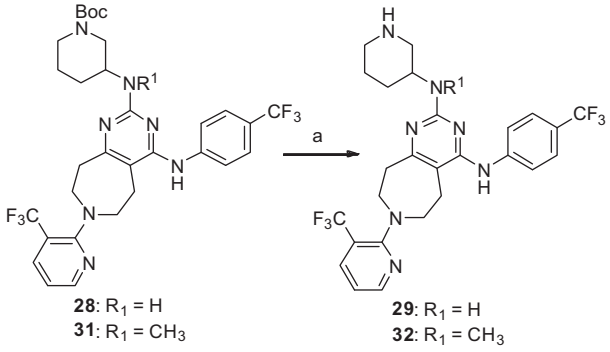


Figure 1. 6,7,8,9-Tetrahydro-5*H*-pyrimido[4,5-*d*]azepines **1** and **2**.



Scheme 2. Reagents and conditions: (a) 2 N HCl (aq), reflux, 12 h, 85–89%.

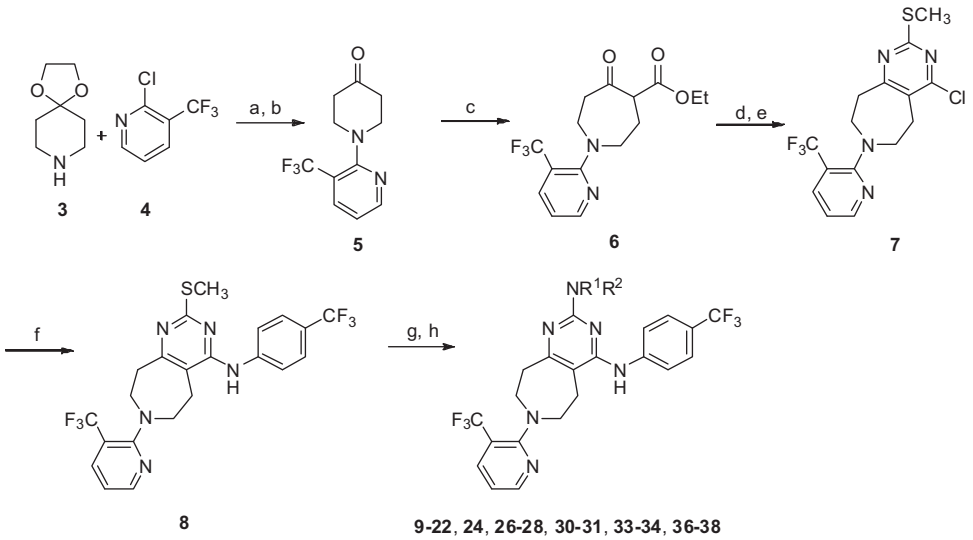


Scheme 3. Reagents and conditions: (a) 4 N HCl in dioxane, rt, 2 h, 38–62%.

Scheme 1.[8] The synthesis began from commercially available 1,4-dioxa-8-aza-spiro[4.5]decane (**3**) and 2-chloro-3-trifluoromethyl-pyridine (**4**) which were transformed to piperidin-4-one **5** in two steps.[9] Ring expansion of piperidin-4-one **5** in the presence of diazo-acetic acid ethyl ester and $BF_3{\cdot}OEt_2$ afforded β-keto ester **6** in 92% yield. Condensation of β-keto ester **6** with thiourea followed by chlorination in the presence of $POCl_3$ provided 4-chloro-2-methylsulfanyl-pyrimidine **7** in 55% yield over two steps. Amination of compound **7** by reaction with 4-trifluoromethylphenyl amine and hydrochloric acid afforded compound **8** in 77% yield. Installation of the 1,2-diamino-ethane analogues at the C2-position of the pyrimidine was accomplished through a two-step oxidation and displacement sequence to furnish 1,2-diamino-ethane substituted-6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*d*]azepines **9**–**22**, **24**, **26**–**28**, **30**, **31**, **33**, **34**, **36**–**38**.

In cases where displacement of the 2-methylsulfonyl substituent with the desired 1,2-diamino-ethane analogue had the potential to afford mixtures of regioisomeric products, incorporation of acetyl or a *tert*-butoxycarbonyl (Boc) group on the distal nitrogen suppressed unwanted regioisomers. Removal of acetyl or Boc protecting groups in compounds **22**, **24**, **28**, and **31** using acidic conditions is summarized in Schemes 2 and 3. Consistent with conditions shown in Scheme 3, the Boc group in compound **34** was removed to provide compound **35** in 56% yield.

Compounds were evaluated for their ability to inhibit capsaicin-induced influx of Ca^{2+} in cells (HEK293) expressing human and rat TRPV1.[10] Inhibition is reported as IC_{50} ± SEM (nM) and the results are the average of at least three independent experiments. In addition, none of the compounds reported herein displayed agonist activity. Aqueous equilibrium solubility of each compound was determined in (SIF; pH 6.8) and pH 2 using 30 mM phosphate buffer.[11]



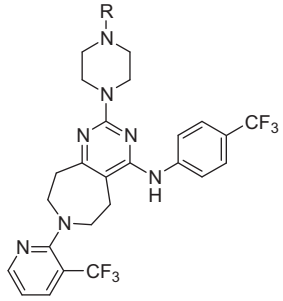
Scheme 1. Reagents and conditions: (a) K_2CO_3, DMSO, 100 °C, 12 h (quant); (b) concd HCl, 24 h (70%); (c) diazo-acetic acid ethyl ester, $BF_3{\cdot}OEt_2$ (1.3 equiv) Et_2O/CH_2Cl_2, 0 °C to rt, 3 h (92%); (d) 1.5 equiv thiourea, 3 equiv sodium ethoxide, EtOH, 90 °C, 12 h, followed by 1.1 equiv MeI at rt, 1 h (98%); (e) 2 equiv $POCl_3$, CH_3CN, 90 °C (55% over two steps); (f) 4-trifluoromethyl-phenylamine, 2.2 equiv HCl, IPA, 90 °C (77%); (g) 2 equiv *m*-CPBA, CH_2Cl_2, rt (93%); (h) HNR_1R_2, *t*-amyl-OH, 120 °C (60–90%).

As part of our effort to improve aqueous solubility while maintaining TRPV1 potency, we first evaluated a variety of piperazines as 1,2-diamino-ethane analogues (Table 1). In general, piperazines **12**–**18** with bulky alkyl substituents provided excellent human TRPV1 potency. On the other hand, piperazines **9**–**11** that contained smaller substituents were at least eightfold less potent at human TRPV1 when compared to compound **13**. The addition of polar functionality to the substituted piperazine in compounds **19** and **20** also significantly decreased TRPV1 potency. Compounds shown in Table 1 improved on both pH 2 solubility (>43-fold) and SIF solubility (>4-fold) when compared to the aqueous solubility of compound **1**. Unfortunately, in the many instances where SIF and pH 2 solubility were dramatically improved, such as in compounds **9**, **10** and **20**, TRPV1 potency of each compound was significantly reduced. A balance was achieved in compounds **12**–**18** where potent human TRPV1 activity was observed while aqueous solubility in both pH 2 and SIF was enhanced compared to compound **1**.

Having identified several piperazines that improved aqueous solubility and retained potency for TRPV1, we turned our attention to the evaluation of other 1,2-diamino-ethane analogues (Table 2). Replacement of a *N*-methylated-piperazine in compound **10** with an *N*,*N*,*N*′-trimethyl-ethane-1,2-diamine in compound **21** significantly reduced TRPV1 potency. This result suggested that a more rigid orientation of the distal nitrogen of the piperazine was important for TRPV1 potency. Overall, the structure–activity trends for compounds **22**–**38** suggested that while aqueous solubility (pH 2 and SIF) could be improved, the TRPV1 potency could not be maintained when compared to compound **1**. For example, compounds **23**, **25**–**27**, **29**, **30**, **32**, and **35**–**37** improved aqueous solubility to a significant extent, but human TRPV1 potency was reduced by as much as 833-fold for compound **29** when compared to compound **1**. In contrast, synthetic intermediates containing *tert*-butoxycarbonyl groups such as compounds **28** and **31** afforded poor aqueous solubility and human TRPV1 IC_{50}'s of 140 and 53 nM, respectively.

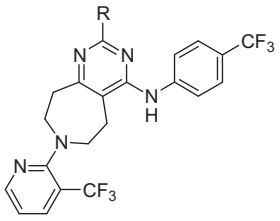
Compounds **12**, **13**, and **15** were selected for pharmacokinetic assessment in Sprague–Dawley rats based on their excellent TRPV1

Table 1
TRPV1 activity and solubility for compounds **9**–**20**



Compound	R	hTRPV1 IC_{50} ± SEM (nM)	rTRPV1 IC_{50} ± SEM (nM)	pH 2 solubility (μg/mL)	SIF solubility (μg/mL)
9	–H	3350 ± 490	>5000	215	158
10	–Me	367 ± 102	506 ± 81	>221	193
11	–Et	132 ± 92	506 ± 295	>226	6
12	–*iso*-Propyl	91 ± 37	386 ± 279	>232	2
13	$-CH_2CH(CH_3)_2$	11 ± 6	89 ± 43	>237	11
14	$-CH(CH_2CH_3)_2$	41 ± 22	185 ± 120	243	109
15	–Cyclopropyl	11 ± 5	45 ± 11	230	9
16	–Cyclobutyl	24 ± 13	161 ± 81	>237	2
17	–Cyclopentyl	15 ± 7	123 ± 13	221	11
18	$-CH_2CH_2OCH_3$	65 ± 45	207 ± 107	>238	6
19	$-CH_2CH_2OH$	498 ± 265	1161 ± 343	>233	4
20	$-CH_2CH_2N(CH_3)_2$	2000 ± 740	2600 ± 1650	>243	>243

Table 2
TRPV1 activity and solubility for compounds **21**–**38**



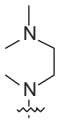
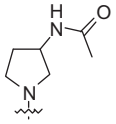
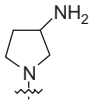
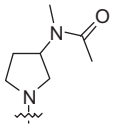
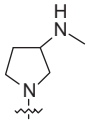
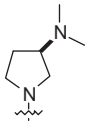
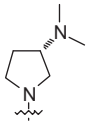
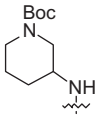
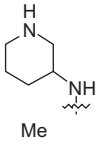
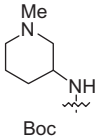
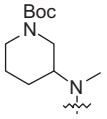
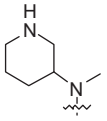
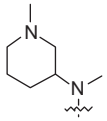
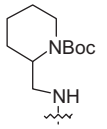
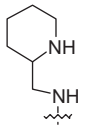
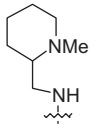
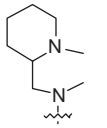
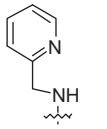
Compound[a]	R	hTRPV1 IC_{50} ± SEM (nM)	rTRPV1 IC_{50} ± SEM (nM)	pH 2 solubility (μg/mL)	SIF solubility (μg/mL)
21		2667 ± 367	4449 ± 1444	>221	210
22		150 ± 62	410 ± 397	202	19
23		3700 ± 234	>5000	>215	>215
24		62 ± 20	460 ± 341	21	2
25		2790 ± 35	>5000	>220	233
(*R*)-**26**		243 ± 124	1042 ± 402	>226	57
(*S*)-**27**		185 ± 104	586 ± 231	>226	57
28		140 ± 99	239 ± 15	1	14
29		>5000	>5000	>235	203
30		3080 ± 370	>5000	204	226
31		53 ± 39	97 ± 9	1	4
32		3600 ± 480	1540 ± 470	>241	211

Table 2 (*continued*)

Compound[a]	R	hTRPV1 IC_{50} ± SEM (nM)	rTRPV1 IC_{50} ± SEM (nM)	pH 2 solubility (μg/mL)	SIF solubility (μg/mL)
33		>5000	>5000	>232	>232
34		250 ± 80	230 ± 180	3	3
35		100 ± 34	>5000	>272	237
36		1756 ± 650	2277 ± 415	>277	254
37		3330 ± 1424	>5000	>283	>283
38		476 ± 271	1342 ± 779	224	1

[a] Compounds **22**–**25** and **28**–**37** described in Table 2 were racemates.

Table 3
Mean pharmacokinetic parameters for compounds **12**, **13**, and **15** following 0.5 mpk iv and 2 mpk po administration in fasted Sprague–Dawley rats using 5% pharmasolve/20% RH-40 cremophor/75% dextrose (5%) in water as a vehicle

Compound	CL (L/h/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	po C_{max} (μM)	po AUC_{inf} (h ng/mL)	po T_{max} (h)	%F
12	4.8	5.1	1.5	–	–	–	–
13	0.7	0.5	1.8	0.3	598	2.3	21
15	1.3	1.0	1.6	–	–	–	–

Table 4
Additional TRPV1 activity for compound **13**

Compound	hTRPV1(pH) IC_{50} ± SEM (nM)	rTRPV1(pH) IC_{50} ± SEM (nM)	rRTX Binding K_i ± SEM (nM)
13	67 ± 39	63 ± 42	330 ± 160

potency and improved aqueous solubility (Table 3). Although compounds **12** and **15** demonstrated high to moderate rates of clearance (CL = 4.8 and 1.3 L/h/kg, respectively), we were pleased to discover that compound **13** afforded a low rate of clearance (CL = 0.7 L/h/kg) and an oral bioavailability of 21%.

Given the rat pharmacokinetic profiles shown in Table 3, compound **13** was chosen for further evaluation. Since TRPV1 receptors are also activated by endogenous factors such as low pH, we evaluated the ability of compound **13** to inhibit proton-induced TRPV1 activation in HEK293 cells expressing human and rat TRPV1 (Table 4). In addition, we also investigated the ability of compound **13** to displace [^{3}H]-RTX in HEK293 cells expressing rat TRPV1 (Table 4). Compound **13** blocked proton activation to an extent similar to its blockade of capsaicin activation.

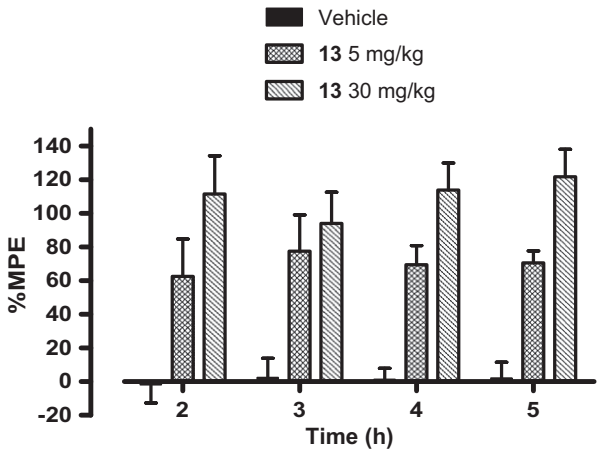


Figure 2. Representative data showing the effect of **13** on carrageenan-induced thermal hyperalgesia in rats, $n = 6$/group (dosed po in 5% pharmasolve/20% RH-40 cremophor/75% dextrose (5%) in water, administered 1 h prior to carrageenan injection).

When dosed orally, compound **13** was efficacious in a carrageenan-induced thermal hyperalgesia model in rats (Fig. 2).[12] Compound **13** significantly prevented the development of thermal hyperalgesia at 5 and 30 mg/kg compared with vehicle ($p < 0.05$). When expressed as % Maximal Possible Effect (% MPE), the maximal degree of inhibition was approximately 100% in rats treated with 30 mg/kg at 3 h post-carrageenan injection. Compound **13** did not affect basal paw withdrawal latency of the contralateral hind paws. Terminal plasma concentrations (approximately 5 h post-carrageenan injection) were determined to be 277 ± 68 nM ($n = 6$) at 5 mg/kg and 1054 ± 442 nM ($n = 4$) at 30 mg/kg for compound **13**.

In conclusion, we have synthesized and evaluated a series of 1,2-diamino-ethane-substituted-6,7,8,9-tetrahydro-5*H*-pyrimido[4,5-*d*]azepines in an effort to improve the aqueous solubility and rat pharmacokinetics. We identified compound **13** as a potent TRPV1 antagonist with improved aqueous solubility (pH 2 = >237 μg/mL and SIF = 11 μg/mL) compared to compound **1** (pH 2 = 5 μg/mL and SIF = 0.5 μg/mL). In addition, compound **13** provided improved rat pharmacokinetics (CL = 0.7 L/h/kg) compared to compound **1** (CL = 3.1 L/h/kg). Compound **13** was orally bioavailable and afforded a significant reversal of carrageenan-induced thermal hyperalgesia at 5 and 30 mg/kg in rats.

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11. 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.
12. 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. 6d.