Bioorganic & Medicinal Chemistry Letters 19 (2009) 40–46

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification and synthesis of 2,7-diamino-thiazolo[5,4-*d*]pyrimidine derivatives as TRPV1 antagonists

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ARTICLE INFO

Article history:
Received 26 September 2008
Revised 5 November 2008
Accepted 10 November 2008
Available online 13 November 2008

Keywords:
TRPV1
Antagonists
Thiazolo-pyrimidine

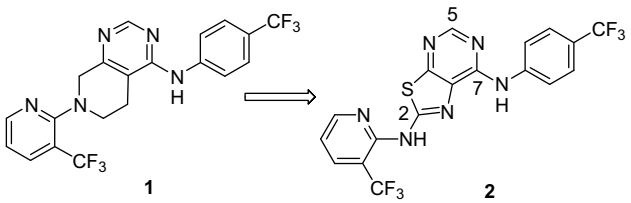
ABSTRACT

We have identified and synthesized a series of 2,7-diamino-thiazolo[5,4-*d*]pyrimidines as TRPV1 antagonists. An exploration of the structure–activity relationships at the 2-, 5-, and 7-positions of the thiazolo[5,4-*d*]pyrimidine led to the identification of several potent TRPV1 antagonists, including **3**, **29**, **51**, and **57**. Compound **3** was orally bioavailable and afforded a significant reversal of carrageenan-induced thermal hyperalgesia with an ED_{50} = 0.5 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]

In our work toward the discovery of novel TRPV1 antagonists, we synthesized 5,6,7,8-tetrahydro-pyrido[3,4-*d*]pyrimidine as exemplified by **1** (Fig. 1).[7,8] In an effort to broaden the structure–activity relationships of compound **1**, we were interested in replacing the 5,6,7,8-tetrahydro-pyrido[3,4-*d*]-pyrimidine core with thiazolo[5,4-*d*]pyrimidine core (e.g., compound **2**) given its convenient preparation from commercially available materials in a single step[9] and ability to functionalize the 2- and 7-positions with the requisite groups (Fig. 1). For these reasons we initially prepared 2,7-diamino-thiazolo[5,4-*d*]pyrimidines **2** and **3** (Table 1). While compound **2** with its 3-trifluoromethyl-pyridin-2-yl substituent provided a direct comparison to compound **1**, we also investigated the introduction of 2,6-dichlorophenyl as a replacement for 3-trifluoromethyl-pyridin-2-yl in compound **3**.[10]

Compounds were evaluated for their ability to inhibit capsaicin-induced influx of Ca^{2+} in cells (HEK293) expressing human and rat TRPV1.[11] 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. While compound **2** was essentially inactive at human



hTRPV1 IC_{50} = 15 ± 1 nM
rTRPV1 IC_{50} = 71 ± 17 nM

Figure 1. 5,6,7,8-Tetrahydro-pyrido[3,4-*d*]pyrimidine **1** and thiazolo[5,4-*d*]pyrimidine **2**.

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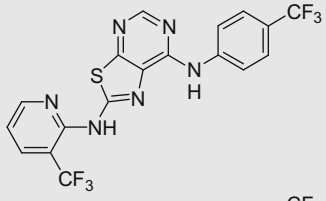
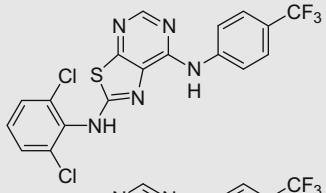
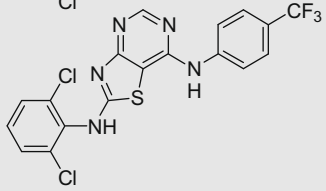
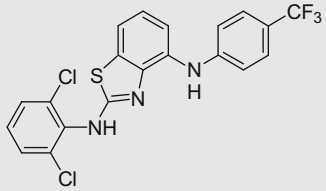
doi:10.1016/j.bmcl.2008.11.024

Table 1
TRPV1 activity for 2,7-diamino-thiazolo[5,4-*d*]pyrimidines **2**–**3**, thiazolo[4,5-*d*]pyrimidine **4**, and 2,4-diamino-benzothiazole **5**

Compound	Structure	hTRPV1 IC_{50} ± SEM (nM)	rTRPV1 IC_{50} ± SEM (nM)
2		>5000	>5000
3		12 ± 3	3 ± 1
4		680 ± 279	864 ± 259
5		>5000	>5000

and rat TRPV1, the potency of compound **3** was similar at human TRPV1 and the potency increased 24-fold at rat TRPV1 when compared with reference compound **1** (Table 1).

To gain insight into the ability of the thiazolo[5,4-*d*]pyrimidine core to replace the 5,6,7,8-tetrahydro-pyrido[3,4-*d*]pyrimidine core, and also to understand the difference in potency between the 3-trifluoromethyl-pyridin-2-yl substituent in compound **2** and the 2,6-dichlorophenyl substituent in compound **3**, we carried out molecular alignments of compounds **1**, **2**, and **3**. Initially a flexible molecular alignment algorithm in MOE was used to identify pairs of conformers of **1** and **2** and of **1** and **3** with good overall alignments (Figs. 2 and 3).[12] Both pairs of conformers appear quite reasonable, with good alignment between key features of the thiazolo[5,4-*d*]pyrimidine and corresponding features of the 5,6,7,8-tetrahydro-pyrido[3,4-*d*]pyrimidine. Unfortunately, while such alignments rationalize the activity of **3** relative to **1**, they do not explain the inactivity of **2**, which bears the same 3-trifluoromethyl-pyridin-2-yl group shown to be active in **1**.

To understand the difference between **2** and **3** in more detail, conformation searches for each of these compounds was performed

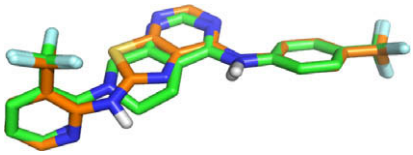


Figure 2. Molecular alignment of compounds **1** (carbon atoms in green) and **2** (carbon atoms in orange).

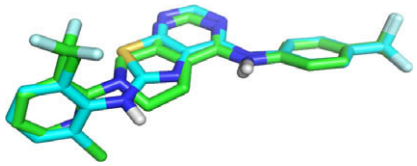


Figure 3. Molecular alignment of compounds **1** (carbon atoms in green) and **3** (carbon atoms in blue).

using the MCMM method in MacroModel with an upper energy limit of 12 kcal/mol and 10,000 conformer generation steps.[13] Each resulting geometry was energy-minimized in Jaguar[14] with the B3LYP hybrid density-functional and the 6-31G* basis set. Each of the conformers of **2** and **3** shown in Figures 2 and 3, respectively, were then used to search the corresponding set of energy-minimized structures obtained from the quantum calculations using ROCS with the ComboScore option to account for similarities in overall shape as well as in pharmacophore features.[15]

Similar conformers to the ones shown in Figures 2 and 3 were identified for each of the compounds **2** and **3**. For compound **3**, the energy of the corresponding minimized conformer was 0.2 kcal/mol relative to the lowest-energy conformer (hereafter referred to as the quantum mechanical reference conformer of compound **3**). However, for compound **2** the energy of the most similar conformer to the one shown in Figure 2 was 10.3 kcal/mol above the lowest-energy conformer. In fact, in the lowest-energy conformer of **2** the 3-trifluoromethyl-pyridin-2-yl group is coplanar with the thiazole ring (Fig. 4). In the lowest-energy conformer of **3**, on the other hand, the 2,6-dichlorophenyl ring is orthogonal to the thiazole ring (Fig. 4). The preferred orientation of the N^2-aromatic ring thus provides a reasonable hypothesis as to the lack of TRPV1 potency observed for **2** versus **3**, the former being unable to attain the proper orthogonal orientation for affinity.

Having discovered compound **3** as a potent TRPV1 antagonist, we turned our attention to determining the importance of the heterocyclic nitrogens in the thiazole and pyrimidine rings while holding the 2,6-dichlorophenyl and 4-trifluoromethyl-phenyl amine substituents constant. To that end, we prepared thiazolo[4,5-*d*]pyrimidine **4** and benzothiazole **5** (Table 1). A substantial

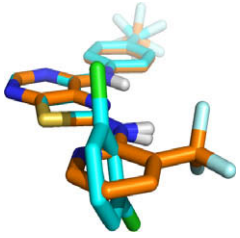


Figure 4. Molecular alignment of the lowest-energy conformers of compounds **2** (carbon atoms in orange) and **3** (carbon atoms in blue).

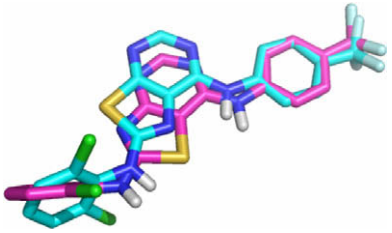


Figure 5. Molecular alignment of compounds **3** (carbon atoms in blue) and **4** (carbon atoms in pink).

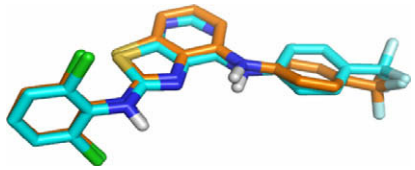


Figure 6. Molecular alignment of compounds **3** (carbon atoms in blue) and **5** (carbon atoms in orange).

loss in potency at TRPV1 was observed for both thiazolo[4,5-*d*]pyrimidine **4** and benzothiazole **5** when compared to thiazolo[5,4-d]pyrimidine **3** (Table 1).

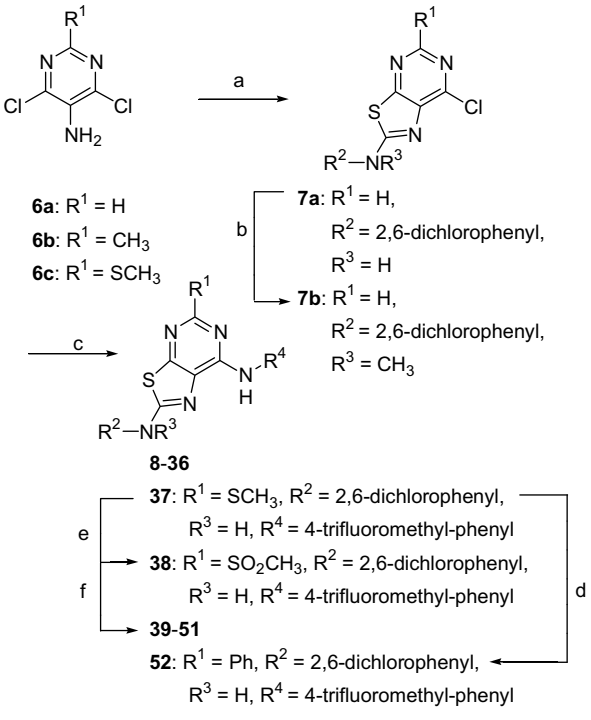
To enhance our understanding of compounds **4** and **5** with respect to compound **3**, we employed molecular alignment methods previously described by using MCMM conformation sampling followed by quantum mechanical calculations and ROCS.[13–15] Figure 5 shows the best alignment of a conformer of **4** and the quantum mechanical reference conformer of compound **3**. Although the conformer of **4** has a low relative energy above its global minimum (0.2 kcal/mol), it afforded poor overlap with the conformer of **3** in the thiazolo-pyrimidine region. This is consistent with the loss of potency at TRPV1.

In contrast to the poor alignment observed between the quantum mechanical reference conformer of compound **3** and the low energy conformer of **4**, good alignment were observed for a low energy conformer of **5** and reference conformer of **3** as shown in Figure 6. The conformer of **5** was 0.4 kcal/mol above its global minimum. Although the comparison of **3** and **5** was not able to provide a structural rationale for the loss of TRPV1 potency in compound **5**, the comparison does underscore the importance of maintaining the pyrimidine ring for optimal TRPV1 potency. Given the findings in Table 1, we focused our synthetic efforts on broadening the structure–activity relationships at the 2-, 5-, and 7-positions of the thiazolo[5,4-*d*]pyrimidine.

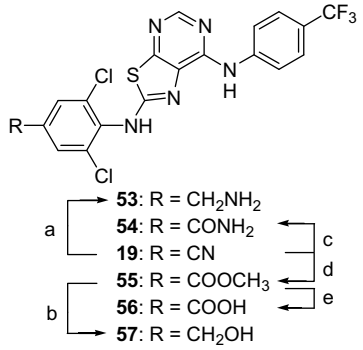
The general synthesis of 2,5,7-substituted-thiazolo[5,4-*d*]-pyrimidines is outlined in Scheme 1.[16] For example, addition of 4,6-dichloro-5-aminopyrimidine **6a** in the presence of 2,6-dichlorophenyl isothiocyanate, cesium carbonate, and CH_3CN at 50 °C afforded the 7-chloro-thiazolo[5,4-*d*]pyrimidine **7a** in 90% yield.[9] N-Methylation of **7a** in the presence of methyl iodide and sodium hydride afforded **7b** in 50% yield.[17] Amination of 7-chloro-thiazolo[5,4-*d*]pyrimidine **7a** in the presence of 4-trifluoromethyl-phenyl amine and *p*-toluene sulfonic acid (TsOH) provided compound **3** in 80% yield. The structure of compound **3** was confirmed by single-crystal X-ray analysis.[18] Installation of amine functionality at the 5-position of the thiazolo[5,4-*d*]pyrimidine was accomplished through a two-step oxidation and displacement sequence from the corresponding 5-methylsulfanyl-thiazolo[5,4-*d*]pyrimidine **37** to provide compounds **39**–**51**. Alternatively, the 5-methylsulfanyl-thiazolo[5,4-*d*]pyrimidine **37** could undergo a thioether-boronic acid cross coupling to afford compound **52** in 24% yield.[19]

Further elaboration of the thiazolo[5,4-*d*]pyrimidine scaffold is summarized in Schemes 2 and 3. Scheme 2 illustrates the synthesis of a variety of compounds through hydrolysis or reduction of nitrile **19** to afford compounds **53**–**57**. Alternative linkages between the thiazolo[5,4-*d*]pyrimidine and the N^7-aryl substituents were prepared from 7-chloro-thiazolo[5,4-*d*]pyrimidine **7a** to provide N-methylated **58**, ether **59**, and thioether **60** (Scheme 3).

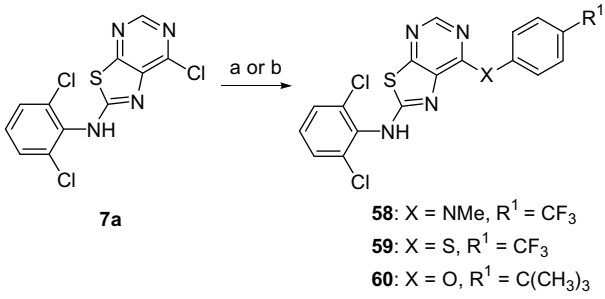
The regioisomeric thiazolo-pyrimidine **4** was synthesized in 5 steps using an improved version of a previously published procedure (Scheme 4).[20] Formation of the 4-amino-thiazole-5-methyl ester **62** was accomplished in two steps from commercially available 2,6-dichlorophenyl isothiocyanate. Exposure of 4-amino-thiazole-



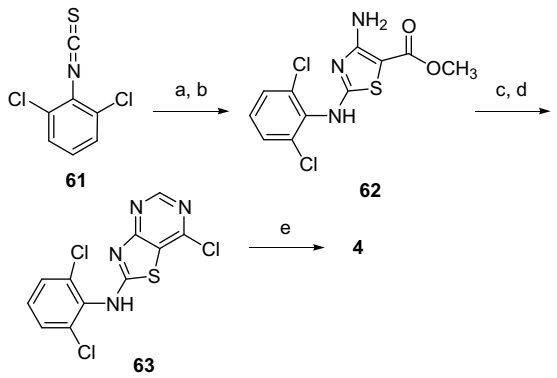
Scheme 1. Reagents and conditions: (a) R^2–N=C=S, Cs_2CO_3, CH_3CN, 50 °C, 12 h (70–95%); (b) 1 equiv MeI, NaH, THF, rt, (50%); (c) 1 equiv H_2N–R^4, TsOH, toluene, 120 °C, sealed tube, 2 h (60–90%); (d) 1.1 equiv phenyl boronic acid, 1.3 equiv copper(I)-thiophene-2-carboxylate, tri-2-furylphosphine (16 mol%), $Pd_2(dba)_3$ (4 mol%), THF, 50 °C, 24 h (24%); (e) 4 equiv Oxone, MeOH–THF–H_2O (1:1:1), rt, 48 h, (90%); (f) 3 equiv amine (R^1), *t*-Amyl-OH, 120 °C, sealed tube, 12 h (50–90%).



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, rt (75%); (b) 3 equiv Dibal, THF, rt (74%); (c) 2 equiv TsOH, toluene, 120 °C, sealed tube, 12 h (40%); (d) MeOH, H_2SO_4, reflux, 48 h (91%); (e) LiOH–H_2O, THF, H_2O, 12 h, (72%).



Scheme 3. Reagents and conditions: (a) 1 equiv methyl-(4-trifluoromethyl-phenyl)-amine, 2.2 equiv HCl in IPA (1.25 M), IPA, reflux, 6 h (90% of **58**); (b) 4-trifluoromethyl-benzenethiol or 4-*tert*-butyl-phenol, K_2CO_3, CH_3CN, 90 °C, 12 h (90% of **59** or 20% of **60**, respectively).

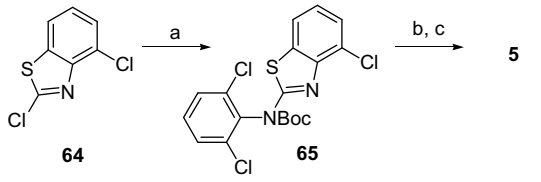


Scheme 4. Reagents and conditions: (a) NH_2CN, sodium methoxide, rt; (b) chloroacetic acid methyl ester, 50 °C, 24 h (77% over two steps); (c) formamide, Ac_2O (cat), 170 °C, sealed tube, 18 h; (d) $POCl_3$, 90 °C, 6 h (26% over two steps); (e) 1 equiv 4-trifluoromethyl-phenyl amine, 2.2 equiv HCl in IPA (1.25 M), IPA, 90 °C, 6 h (40%).

5-methyl ester **62** to formamide at 170 °C followed by chlorination in the presence of $POCl_3$ afforded the 7-chloro-thiazolo[4,5-*d*]-pyrimidine **63** in 26% yield over two steps. Amination of 7-chloro-thiazolo[4,5-*d*]pyrimidine **63** in the presence of HCl and 4-trifluoromethyl-phenyl amine provided compound **4** in 40% yield.

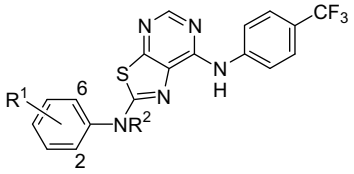
Scheme 5 describes the preparation of 2,4-diamino-benzothiazole **5** through a 3 step sequence. Treatment of 2,6-dichlorophenyl amine with sodium hydride followed by addition of 2,4-dichloro-benzothiazole **64** and di-*tert*-butyl dicarbonate (Boc_2O) provided 2-amino-4-chloro-benzothiazole **65** in 90% yield. Palladium mediated amination of **65** followed by removal of the *tert*-butoxycarbonyl group gave 2,4-diamino-benzothiazole **5**.

To firmly establish what type of phenyl substitution at the N^2-position of compound **3** was required for optimal TRPV1 potency, compounds **8**–**19** and **53**–**57** were evaluated (Table 2). To gain insight into the importance of a hydrogen bond donor at the N^2-position, we replaced the hydrogen at the R^2-position with a methyl group to provide compound **8**. Compound **8** led to a >42-fold loss in TRPV1 potency when compared with compound **3**. Holding the hydrogen at the R^2-position constant, we turned our attention to the N^2-phenyl ring. In general, 2,6-disubstitution was preferred over 2-substitution on the N^2-phenyl ring. For example, 2,6-dichlorophenyl **3** was >5-fold more potent in human and rat TRPV1 than 2-chlorophenyl **11**. From the structure–activity trends in compounds **15**–**18**, it was apparent that 2-substituted phenyl rings were required for potency. For example, placement of a trifluoromethyl group at the 3 or 4 position of N^2-phenyl ring in analogues **16** and **17** led to a complete loss of human TRPV1 potency whereas 2-trifluoromethyl analogue **15** afforded a TRPV1 IC_{50} less than 100 nM. In addition, compound **18** with no substitution on the phenyl ring lacked significant TRPV1 potency. Interestingly, a combination of substitution at the 2-, 4-, and 6-positions of the N^2-phenyl ring resulted in hydroxymethyl analogue **57**, a compound with improved TRPV1 potency as compared to compound **3**.



Scheme 5. Reagents and conditions: (a) 2,6-dichloro-phenylamine, NaH, DMF, rt, 30 min, followed by Boc_2O, 12 h (90%); (b) 1.1 equiv 4-trifluoromethyl-phenyl amine, $Pd(OAc)_2$ (10 mol%), 2-di-*tert*-butylphosphino-2'-(*N*,*N*-dimethyl-amino)biphenyl (30 mol%), 1.4 equiv K_3PO_4, toluene, 90 °C, 12 h (20%); (c) 4 N HCl in dioxane, rt, 6 h (95%).

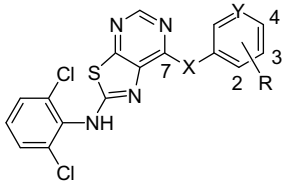
Table 2
TRPV1 activity for N^2-phenyl ring substituted thiazolo[5,4-*d*]pyrimidines **3**, **8**–**19**, and **53**–**57**



Compound	R^1	R^2	hTRPV1 IC_{50} ± SEM (nM)	rTRPV1 IC_{50} ± SEM (nM)
3	2,6-Dichloro	H	12 ± 3	3 ± 1
8	2,6-Dichloro	CH_3	502 ± 257	540 ± 132
9	2,6-Dimethyl	H	23 ± 11	6 ± 2
10	2-Chloro-6-Methyl	H	9 ± 5	1.6 ± 0.4
11	2-Cl	H	64 ± 27	32 ± 5
12	2-SCH_3	H	43 ± 31	26 ± 17
13	2-SO_2CH_3	H	234 ± 31	221 ± 29
14	2-CH_3	H	51 ± 23	14 ± 3.0
15	2-CF_3	H	74 ± 26	37 ± 10
16	3-CF_3	H	>5000	>5000
17	4-CF_3	H	>5000	1700 ± 320
18	H	H	>5000	2680 ± 690
19	2,6-Dichloro-4-CN	H	95 ± 58	53 ± 19
53	2,6-Dichloro-4-CH_2NH_2	H	125 ± 50	54 ± 24
54	2,6-Dichloro-4-$CONH_2$	H	69 ± 27	57 ± 13
55	2,6-Dichloro-4-$COOCH_3$	H	820 ± 300	3190 ± 2130
56	2,6-Dichloro-4-COOH	H	>5000	>5000
57	2,6-Dichloro-4-CH_2OH	H	1.0 ± 0.4	0.5 ± 0.2

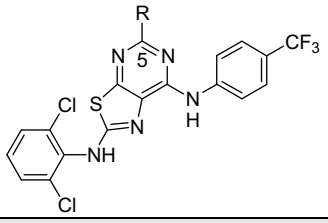
Having discovered the 2,6-dichlorophenyl as one of the optimal substituents at the N^2-position, we held this group constant and turned our attention to the 7-position of the thiazolo[5,4-*d*]pyrimidine (Table 3). Initially, we prepared compounds **58**–**60** to investigate the importance of the amino linker between the

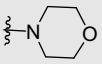
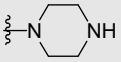
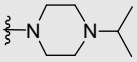
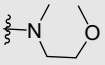
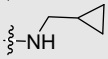
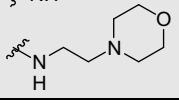
Table 3
TRPV1 activity for N^7-substituted thiazolo[5,4-*d*]pyrimidines **3**, **20**–**35**, and **58**–**60**



Compound	X	Y	R	hTRPV1 IC_{50} ± SEM (nM)	rTRPV1 IC_{50} ± SEM (nM)
3	NH	CH	4-CF_3	12 ± 3	3 ± 1
58	NCH_3	CH	4-CF_3	>5000	>1000
59	S	CH	4-CF_3	>5000	>5000
60	O	CH	4-$C(CH_3)_3$	>5000	>5000
20	NH	CH	3-CF_3	206 ± 146	192 ± 141
21	NH	CH	2-CF_3	>5000	>5000
22	NH	CH	H	176 ± 93	170 ± 97
23	NH	N	4-CF_3	14 ± 3.0	4 ± 1
24	NH	CH	4-CH_3	33 ± 16	32 ± 13
25	NH	CH	4-$CH(CH_3)_2$	10 ± 5.0	3.0 ± 0.6
26	NH	CH	4- $C(CH_3)_3$	11 ± 4.0	1.0 ± 0.3
27	NH	CH	4-$C(CH_3)_2COOH$	31 ± 22	2.0 ± 0.2
28	NH	CH	4-SCH_3	96 ± 66	12 ± 1.0
29	NH	CH	4-SO_2CH_3	5 ± 2	3 ± 2
30	NH	CH	4-COOH	>5000	>5000
31	NH	CH	4-$CONH_2$	>5000	>5000
32	NH	CH	4−CO−N(pyrrolidine)	>5000	>5000
33	NH	CH	4-SO_2NH_2	>5000	590 ± 130
34	NH	CH	4−SO_2−N(pyrrolidine)	1.0 ± 0.3	0.5 ± 0.1
35	NH	CH	3-Cl-4-CF_3	21 ± 2.0	9 ± 2

Table 4
TRPV1 activity for 5-substituted thiazolo[5,4-*d*]pyrimidines **36**–**52**



Compound	R	hTRPV1 IC_{50} ± SEM (nM)	rTRPV1 IC_{50} ± SEM (nM)
3	–H	12 ± 3	3 ± 1
36	$-CH_3$	3 ± 1	2 ± 1
52	–Phenyl	539 ± 379	104 ± 51
37	$-SCH_3$	224 ± 14	113 ± 56
38	$-SO_2CH_3$	274 ± 110	77 ± 23
39		59 ± 32	25 ± 7
40		12 ± 5	36 ± 28
41		63 ± 11	33 ± 13
42		30 ± 8	16 ± 3
43		>5000	>5000
44		184 ± 78	119 ± 68
45		59 ± 36	43 ± 19
46		48 ± 15	34 ± 15
47		41 ± 23	54 ± 38
48		13 ± 6	9 ± 4
49		2 ± 1	1.0 ± 0.3
50		3 ± 2	3 ± 2
51		39 ± 22	11 ± 5.0

thiazolo[5,4-*d*]pyrimidine and the aryl substituent in compound **3**. Introduction of a variety of linkages including N^7-methylated compound **58**, thioether **59**, and ether **60** led to a substantial loss in TRPV1 potency, suggesting possible hydrogen bond donor interactions with the binding site of the receptor. Given the importance of this interaction, we held X as an NH group and explored substitution of the N^7-aryl substituent. In general, 4-substituted aryl groups were favored over their 2- and 3-substituted counterparts. For example, 2-trifluoromethyl-phenyl **21** was essentially inactive and 3-trifluoromethyl-phenyl **20** was >17-fold less potent than 4-trifluoromethyl-phenyl **3**. Although compound **20** lost TRPV1 potency when compared to compound **3**, it did suggest that substituents at the 3-position of N^7-phenyl could be tolerated. An investigation of this effect led to the discovery of 3-chloro-4-trifluoromethyl-phenyl **35** which had TRPV1 potency similar to that of compound **3**. When compared to compound **3**, potency was generally maintained with lipophilic functionality such as 4-alkyl analogues **24**–**26**. Although installation of polar functionality in compounds **30**–**33** led to a marked loss of TRPV1 potency, several exceptions included acid **27**, sulfone **29**, and sulfonamide **34**.

Finally, we explored the influence of substituents at the C5-position of the thiazolo[5,4-*d*]pyrimidine while holding the N^2-2,6-dichlorophenyl and N^7-4-trifluoromethyl-phenyl substituents constant (Table 4). Replacement of the C5 hydrogen in compound **3** with a methyl group in compound **36** resulted in a slight improvement in potency (4-fold) in human TRPV1. However, installation of phenyl, methylsulfanyl, or methylsulfonyl at the C5-position deteriorated human TRPV1 potency by >19-fold for compounds **52**, **37**, **38** when compared to compound **3**. In an effort to improve the aqueous solubility of compound **3** (fasted-state simulated intestinal fluid (SIF) = 2 μg/mL and simulated gastric fluid (SGF) = <1 μg/mL)[21] we introduced amino substituents at the C5-position of the thiazolo[5,4-*d*]pyrimidine. Introduction of a variety of tertiary amines at the C5-position resulted in similar or decreased TRPV1 potency (1- to 5-fold) for compounds **39**–**42** and **45**–**47** and little improvement in solubility when compared to compound **3**. Installation of secondary amines **49** and **50** led to an increase in TRPV1 potency (4- to 6-fold) and little improvement in solubility relative to compound **3**. When compared to compounds **3** and **39**–**50**, compound **51** afforded best balance between TRPV1 potency and improved solubility (SIF = 10 μg/mL and SGF = 116 μg/mL). These results reveal that the 5-position of the thiazolo[5,4-*d*]pyrimidine was tolerant of a variety of amino substituents and the aqueous solubility could be improved through incorporation of amino substituents.

Compounds **3**, **29**, **51**, and **57** were selected for pharmacokinetic assessment in Sprague–Dawley rats since they exhibited excellent TRPV1 potency and each represented a distinct change to the 2-, 5-, or 7-position of the thiazolo[5,4-*d*]pyrimidine (Table 5). Although compounds **29**, **51**, and **57** demonstrated high to moderate rates of clearance (CL = 3.6 L/h/kg, CL = 1.8 L/h/kg, and CL = 1.4 L/h/kg, respectively), we were pleased to discover that compound **3** afforded a low rate of clearance (CL = 0.6 L/h/kg) and was well absorbed with a bioavailability of 43%.

Given the rat pharmacokinetic profiles of compounds **3**, **29**, **51**, and **57**, compound **3** was chosen for further evaluation.[22] Since TRPV1 receptors are also activated by endogenous factors such as low pH, we evaluated the ability of compound **3** to inhibit proton-induced TRPV1 activation in HEK293 cells expressing human and rat TRPV1 (Table 6).[11] In addition, we also investigated the ability of compound **3** to displace [^{3}H]-RTX in HEK293 cells expressing rat TRPV1 (Table 6).[11] Compound **3** blocked proton activation of the channel to a similar extent as its ability to block capsaicin activation and the K_i was consistent with functional activity.

Table 5
Mean pharmacokinetic parameters for compounds **3**, **29**, **51**, and **57** following 0.5 mg/kg iv and 2 mg/kg 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 T_{max} (h)	po AUC_{inf} (h ∗ μg/L)	%F
3[a]	0.6	1.6	2.2	1.1	2	10049	43
29	3.6	2.2	1.2	BLOQ	—	BLOQ	—
51	1.8	2.8	1.8	0.07	2	196	17
57	1.4	2.9	1.8	0.2	4	638	45

[a] 1 mg/kg iv and 5 mg/kg po administration in fasted Sprague–Dawley rats.

Table 6
Additional TRPV1 activity for compound **3**

Compound	hTRPV1 (pH) IC_{50} ± SEM (nM)	rTRPV1 (pH) IC_{50} ± SEM (nM)	rRTX binding K_i ± SEM (nM)
3	19 ± 11	6 ± 4	4 ± 3

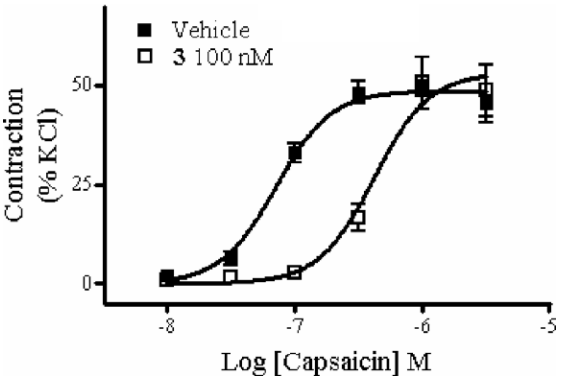


Figure 7. Functional effect of compound **3** ($n = 4$) in isolated rat bladder compared with vehicle ($n = 6$). Tissue contraction is expressed as percentage of KCl (70 mM)-induced tone.

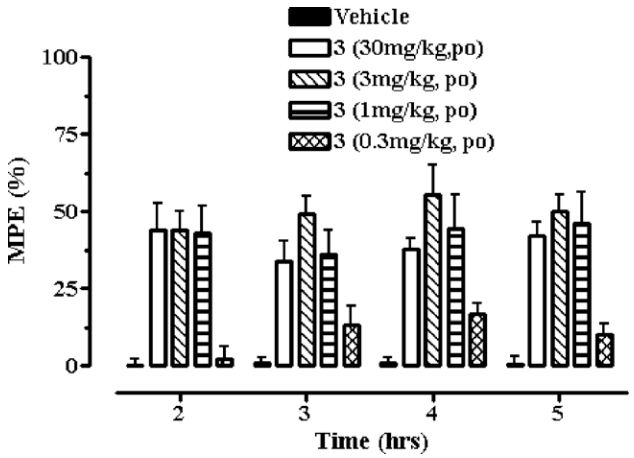


Figure 8. Representative data showing the effect of **3** on carrageenan-induced thermal hyperalgesia in rats, $n = 6$/group (dosed po in 5% pharmasolve/20% RH-40 cremophor/75% dextrose (5%) in water, administered 1h prior to carrageenan injection).

In addition to the in vitro data obtained for compound **3** in recombinant systems, we also examined compound **3** in a native tissue (Fig. 7).[11] Isolated longitudinal strips of rat bladder were used to record capsaicin-induced isometric contractions. The potency (EC_{50}) of capsaicin shifted from 70 to 420 nM (6-fold) in the presence of 100 nM of compound **3**, without suppression of the maximal tissue contractility.

When dosed orally, compound **3** was efficacious in a carrageenan-induced thermal hyperalgesia model in rats (Fig. 8).[23] Compound **3** significantly prevented development of thermal hyperalgesia at 1, 3, and 30 mg/kg compared with vehicle ($p < 0.001$). When expressed as % maximal possible effect (% MPE), the maximal degree of inhibition was approximately 57% (in rats treated with 3 mg/kg at 4 h post-carrageenan injection). The dose required for 50% of the maximum effect achieved by compound **3** in the carrageenan model was approximately 0.5 mg/kg po. Compound **3** did not affect basal paw withdraw latency of the contralateral hind paws. Compound **3** plasma concentrations determined at the end of each experiment (approximately 5 h post-carrageenan injection) were 10 ± 2 nM ($n = 6$), 58 ± 17 nM ($n = 7$), 428 ± 40 nM ($n = 7$), and 3052 ± 444 nM ($n = 7$) in animals dosed orally with 0.3, 1, 3, and 30 mg/kg, respectively.

In summary, we have identified a series of 2,7-diamino-thiazolo[5,4-*d*]pyrimidines as potent TRPV1 antagonists. Initial studies demonstrated the importance of a thiazolo[5,4-*d*]pyrimidine core to maintain TRPV1 potency over related analogues such as thiazolo[4,5-*d*]pyrimidine **4** or a benzothiazole **5**. An exploration of the structure–activity relationships at the 2-, 5-, and 7-positions of the thiazolo[5,4-*d*]pyrimidine led to the identification of several potent TRPV1 antagonists including **3**, **29**, **51**, and **57**. Further evaluation of compound **3** in a rat model of carrageenan-induced thermal hyperalgesia afforded a significant reversal of thermal hyperalgesia with an $ED_{50} = 0.5$ mg/kg.

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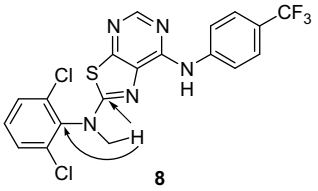
17. Spectroscopic methods including 2D NMR $^{1}H/^{13}C$ HSQC and HMBC were used to confirm the structure of compound **8** and therefore **7b**. In the HMBC, a strong 3-bond coupling was observed between the proton on the N-methyl and the two adjacent quaternary carbons for compound **8**.



8

18. The authors have deposited coordinates for this compound with the Cambridge Crystallographic Data Centre. The coordinates of **3** are CCDC 702151.
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21. 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 simulated gastric fluid (SGF; pH 1.2) for 3 days. The resulting mixtures were filtered and analyzed by HPLC against external standards.
22. When tested at 1 or 10 μM, compound **3** afforded no significant hits in a Cerep panel of 60 targets.
23. 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: Swanson, D. M.; Dubin, A. E.; Shah, C.; Nasser, N.; Chang, L.; Dax, S. L.; Jetter, M.; Breitenbucher, J. G.; Liu, C.; Mazur, C.; Lord, B.; Gonzales, L.; Hoey, K.; Rizzolio, M.; Bogenstaetter, M.; Codd, E. E.; Lee, D. H.; Zhang, S.; Chaplan, S. R.; Carruthers, N. I. *J. Med. Chem.* **2005**, *48*, 1857.