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Trisubstituted pyrimidines as transient receptor potential vanilloid 1 (TRPV1) antagonists with improved solubility

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Abstract—A series of trisubstituted pyrimidines were synthesized to improve aqueous solubility of our first TRPV1 clinical candidate (**1**; AMG 517), while maintaining potent TRPV1 inhibitory activity. Structure–activity and structure–solubility studies led to the identification of compound **26**. The aqueous solubility of **26** ($\geqslant$200 μg/mL, 0.01 HCl; 6.7 μg/mL, phosphate buffered saline (PBS); 150 μg/mL, fasted-state simulated intestinal fluid (SIF)) was significantly improved over **1**. In addition, compound **26** was found to be orally bioavailable (rat F_{oral} = 24%) and had potent TRPV1 antagonist activity (capsaicin IC_{50} = 1.5 nM) comparable to that of **1**.

Vanilloid receptor-1 (VR1 or TRPV1)[1] is a membrane-bound, non-selective ion channel with high permeability for calcium[2] and belongs to a super-family of ion channels known as the transient receptor potential channels or TRPs.[3] TRPV1 is activated or sensitized by several stimuli including heat (>42 °C), protons (low pH),[4] and ligands such as the endocannabinoid anandamide [5] and lipoxygenase metabolites.[6] TRPV1 is also activated by exogenous ligands such as the vanilloid capsaicin[7] and resiniferatoxin (RTX).[8] Recent studies have demonstrated a reduction in thermal hyperalgesia in acute- and sub-acute inflammatory pain models in mice lacking the TRPV1 gene.[9,10] These observations provide evidence for the role of TRPV1 in the perception of pain resulting from inflammation. Consequently, TRPV1 represents a novel target for the treatment of pain.

In our efforts toward the discovery of new analgesic agents, we identified a series of 4,6-disubstituted pyrimidines as potent and selective TRPV1 antagonists.[11,12] These studies culminated in the identification of our first TRPV1 clinical candidate, **1** (AMG 517, Fig. 1), a potent and orally bioavailable TRPV1 antagonist (in vitro IC_{50} values <1 nM in both capsaicin- (CAP) and acid (pH 5)-mediated assays[13]; rat F_{oral} = 32%). In addition, compound **1** was shown to be effective in a rodent 'on-target' biochemical challenge model (capsaicin-induced flinch) and was anti-hyperalgesic in a model of inflammatory pain (CFA-induced thermal hyperalgesia).[12] However, compound **1** suffered from low aqueous solubility which presented challenges in its formulation development. Therefore, our goal was to identify analogues of **1** that had increased aqueous solubility while maintaining TRPV1 potency. Previously we have reported one successful approach toward this end that involved the replacement of the 4-(trifluoromethyl)phenyl group of compound **1** with various saturated aza-heterocycles.[14] As an alternative approach to increasing aqueous solubility, we studied the effect of adding additional ionizable or bulky substituents to the 2- or 5-positions of the pyrimidine core (X or Y; compound **2**, Fig. 1). In this paper, we report the design, synthesis, and evaluation of this series of trisubstituted pyrimidines. The structure–activity relationship (SAR) studies reported herein led to the identification of potent TRPV1 antagonists with significantly improved aqueous solubilities.

For the evaluation of TRPV1 antagonist activity, we measured the inhibition of capsaicin (CAP)- and acid (pH 5)-

Keywords: Trisubstituted pyrimidines; Vanilloid receptor-1; Antagonists; Aqueous solubility.
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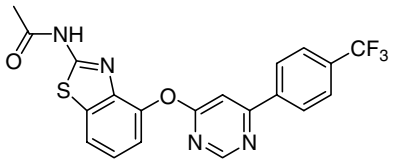
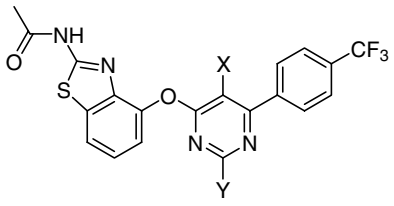


1
(AMG 517)

rTRPV1 (CAP): IC_{50} = 0.9 ± 0.8 nM
rTRPV1 (pH 5): IC_{50} = 0.5 ± 0.4 nM

Solubility
0.01 N HCl < 1.0 μg/mL
PBS < 1.0 μg/mL
SIF 6.6 μg/mL



2

Figure 1. Lead compound **1** and generic substituted pyrimidine targets **2**.

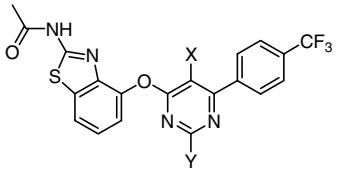
induced influx of $^{45}Ca^{2+}$ into CHO cells expressing the rat TRPV1. Functional activities [IC_{50} (nM)] are reported in Tables 1–3. All compounds reported herein behaved as antagonists.[15] Results are reported as the average of at least two independent experiments with three replicates at each concentration (±SEM). To determine the aqueous equilibrium solubility of these compounds, an automated screening solubility assay in three media [0.01 N HCl, phosphate-buffered saline (PBS, pH 7.4), and fasted-state simulated intestinal fluid (SIF, pH 6.8)] was used.[16] The solubility range tested was 1–200 μg/mL.

Our strategy was to introduce ionizable groups to the pyrimidine core of **1** to reduce the lipophilicity, or bulky substituents to disrupt packing forces in the solid state.[17] To determine the optimum placement for the additional substituent, we began our SAR studies by first examining the effect of introducing small substituents at the 2- or 5-positions of the pyrimidine ring (e.g., Me, NH_2, and NMe_2; compounds **7a**–**f**, Table 1). The synthetic methods used to prepare these trisubstituted pyrimidines are outlined in Scheme 1. Compounds **7a**–**f** were synthesized by the reaction of *N*-(4-hydroxybenzo[*d*]thiazol-2-yl)acetamide **3**[14] with 4-chloride of pyrimidines **4a**–**f** followed by Suzuki couplings with commercially available 4-(trifluoromethyl)phenylboronic acid **8**. The TRPV1 assay results and aqueous solubility data are reported in Table 1.

From this initial set of compounds we found that the introduction of substituents at the 5-position of the pyrimidine ring resulted in a significant decrease in TRPV1 potency (**7a**–**c**; Table 1). In contrast, adding substituents at the 2-position of pyrimidine was tolerated (**7d**–**f**; Table 1). Compound **7b** showed a 270-fold decrease in potency in the capsaicin-mediated assay and 190-fold decrease in the pH-mediated assay. However, the 2-NH_2substituted analogue **7e** was only 12-fold less potent than **1** in the capsaicin-mediated assay and 10-fold less potent in the pH-mediated assay. Furthermore, introduction of the bulkier NMe_2 group at position 5 of the pyrimidine (**7c**) resulted in an additional loss of potency, relative to **7b**, while the NMe_2 group at position 2 of the pyrimidine (**7f**) resulted in comparable potency to **7e**. As shown in Table 1, the small loss in activity of **7d**–**f** relative to**1** was offset by some modest improvements in aqueous solubility. These results suggested that substituents may be tolerated at position 2 of the pyrimidine, but not at position 5.

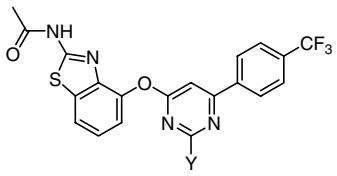
With the 2-position of the pyrimidine ring identified as the suitable position for substitution, we expanded our

Table 1. SAR and aqueous solubility: small substituents on the 2- and 5-positions of the pyrimidine core



Compound	X	Y	rTRPV1 IC_{50} (nM)		Solubility (μg/mL)		
			Capsaicin	pH	0.01 HCl	PBS	SIF
1	H	H	0.9 ± 0.8	0.5 ± 0.4	<1.0	<1.0	6.6
7a	Me	H	130 ± 30	30 ± 4	⩽1.0	1.7	⩽1.0
7b	NH_2	H	240 ± 70	93 ± 10	8.6	6.1	5.2
7c	NMe_2	H	>4000	660 ± 80	1.2	1.3	⩽1.0
7d	H	Me	9.3 ± 2	1.4 ± 0.5	14	2.0	⩽1.0
7e	H	NH_2	12 ± 1	4.8 ± 0.9	4.7	8.8	3.5
7f	H	NMe_2	11 ± 9	4.5 ± 0.6	21	⩽1.0	1.9

Table 2. SAR and aqueous solubility: 2-substituted pyrimidines (directly-attached heterocycles)



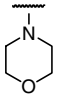
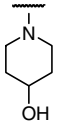
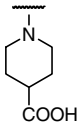
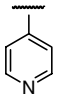
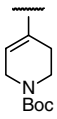
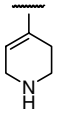
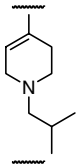
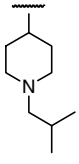
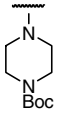
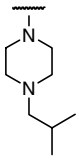
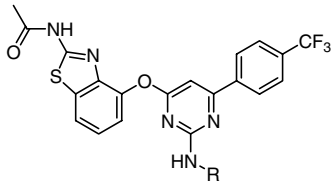
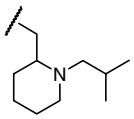
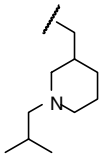
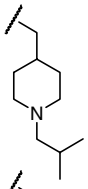
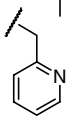
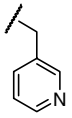
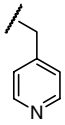
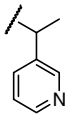
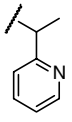
Compound	Y	rTRPV1 IC_{50} (nM)		Solubility (μg/mL)		
		Capsaicin	pH	0.01 HCl	PBS	SIF
11a		28 ± 1	1.1 ± 0.3	⩽1.0	⩽1.0	1.4
11b		5.1 ± 1.4	1.5 ± 0.1	⩽1.0	⩽1.0	2.8
11c		39 ± 3	31 ± 3	⩽1.0	4.6	4.7
11d		35 ± 6	90 ± 1	21	2.0	12
11e		410 ± 90	27 ± 1	⩽1.0	6.0	3.4
11f		6.2 ± 0.2	1.1 ± 0.3	15	⩽1.0	31
11g		1.8 ± 0.2	0.6 ± 0.1	⩽1.0	5.2	19
12		910 ± 600	190 ± 20	8.4	1.7	120
13		3.2 ± 0.8	0.4 ± 0.1	52	5.5	72
14		100 ± 50	100 ± 4	75	8.9	180
11h		43 ± 0.2	3.4 ± 0.6	⩽1.0	⩽1.0	67
18		12 ± 3	4.0 ± 0.4	16	⩽1.0	39

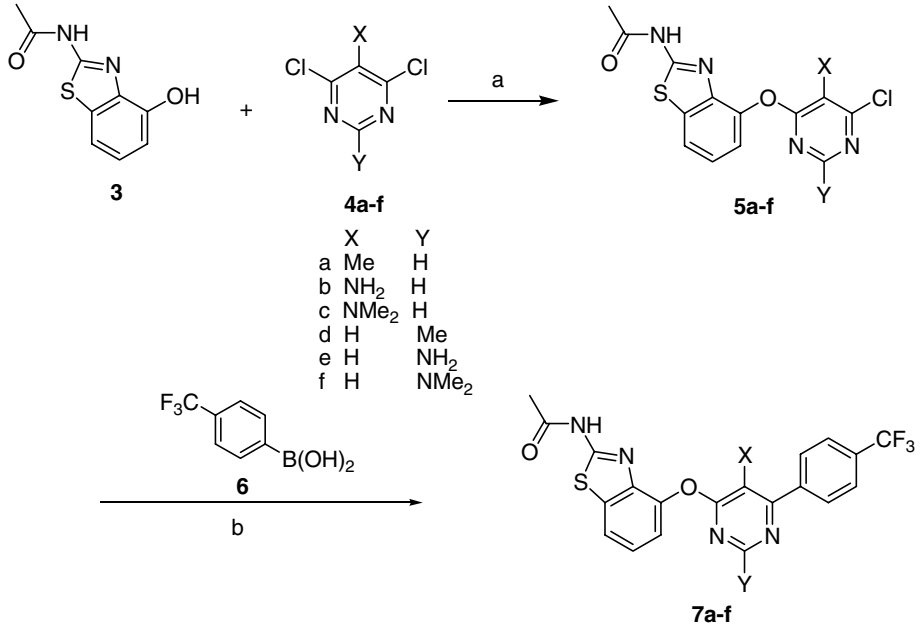
Table 3. SAR and aqueous solubility: 2-substituted pyrimidines (aminomethyl-linked heterocycles)



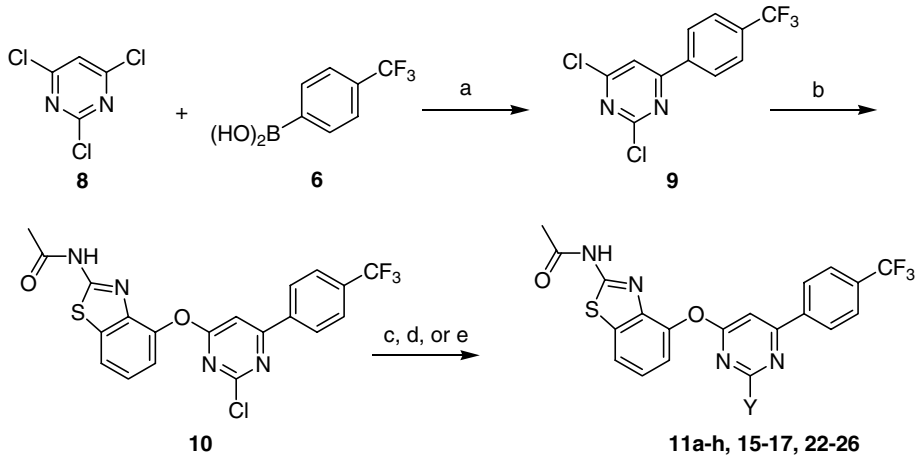
Compound	R	rTRPV1 IC_{50} (nM)		Solubility (μg/mL)		
		Capsaicin	pH	0.01 HCl	PBS	SIF
19		6.1 ± 0.7	11 ± 2	⩾200	9.1	190
20		20 ± 6	15 ± 1	⩾200	15	⩾200
21		51 ± 10	64 ± 10	⩾200	9.4	⩾200
22		5.9 ± 0.3	2.8 ± 0.3	160	2.3	49
23		7.8 ± 1	4.2 ± 2	21	2.9	20
24		6.1 ± 1	3.9 ± 0.8	⩾200	⩽1.0	5.0
25		8.7 ± 2	9.3 ± 1	⩾200	7.2	62
26[20]		1.5 ± 0.2	1.6 ± 0.2	⩾200	6.7	150

SAR by exploring larger substituents in this position. The synthesis of compounds **11a**–**h** and **22**–**26** is outlined in Scheme 2. For these derivatives, the 2-position of the pyrimidine was modified via late stage diversification of intermediate **10** by either adding a primary or secondary amine or by a Suzuki reaction with an appropriate boronic acid. The required intermediate (**10**) was prepared by a Suzuki coupling of 2,4,6-trichloropyrimidine (**8**) and 4-(trifluoromethyl)phenylboronic acid (**6**) followed by an aryl ether formation with *N*-(4-hydroxybenzo[*d*]thiazol-2-yl)acetamide (**3**).[18]

In some cases additional modifications to the heterocyclic substituents were required to prepare the final target compounds Schemes 3 and 4. For example, the Boc group of tetrahydropyridine derivative **11g** was removed under acidic conditions to give the corresponding free amine **12** (Scheme 3). The isobutyl derivative **13** was obtained by reductive amination of compound **12** with isobutyraldehyde. Additionally, the double bond of **13** was reduced by $Pd(OH)_2$-catalyzed hydrogenation to give the corresponding saturated piperidine analogue **14**. Similarly, the final isobutyl piperazine and piperidine



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 50 °C, 90%; (b) $Pd(PPh_3)_2Cl_2$, Na_2CO_3, DME, EtOH, H_2O, microwave, 120 °C, 10 min, 75–90%.

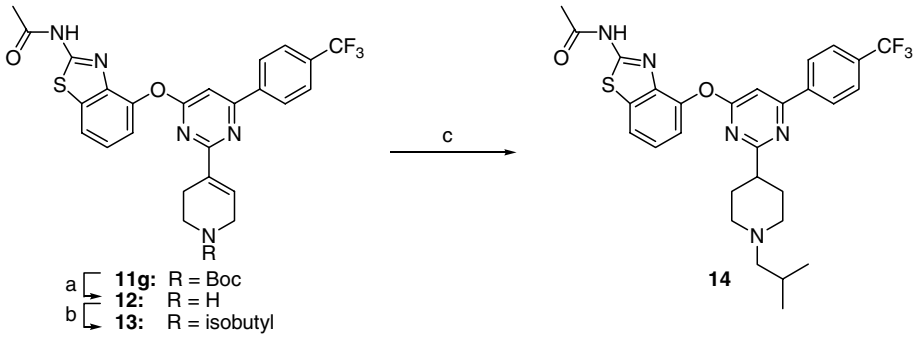


Scheme 2. Reagents and conditions: (a) $Pd(OAc)_2$, PPh_3, K_2CO_3, DME, 95 °C, 50%[19]; (b) **3**, K_2CO_3, DMF, 50 °C, 90%; (c) 2 equiv amine, EtOH, microwave, 140 °C, 30 min, 45–90%; (d) pyridin-4-ylboronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, DME, EtOH, H_2O, microwave, 120 °C, 10 min, 85%; (e) *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydro-1(2H)-pyridinecarboxylate, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 95 °C, 52%.

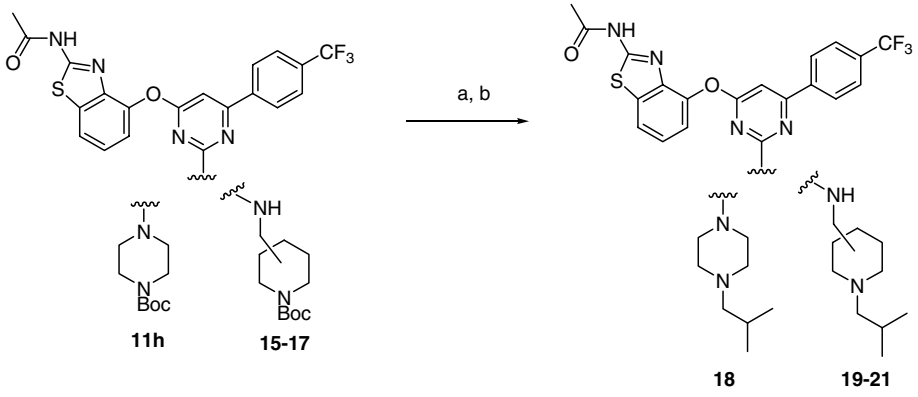
derivatives (**18** and **19**–**21**, respectively) were prepared by deprotection of the Boc group followed by reductive amination with isobutyraldehyde (Scheme 4).

In the second phase of this investigation, we examined the effect of the direct attachment of various heterocyclic rings (e.g., morpholino, pyridinyl, and piperidine analogues) to the 2-position of the pyrimidine core. As shown in Table 2, the morpholino and piperidine analogues **11a** and **b** maintained good potency, but with no improvement in aqueous solubility. Introduction of polar substituents on the piperidine ring (**11c**–**e**) resulted in much less potent TRPV1 antagonists, although some improvements in solubility were observed. For example, the 4-hydroxypiperidine analogue **11d** was 7-fold less potent than the piperidine derivative **11b** in the capsaicin-mediated assay and 60-fold less potent in the acid-mediated assay; however, the solubility of **11d** was greater than **11b** (21 μg/mL vs ⩽1.0 μg/mL in 0.01 HCl). Direct substitution of the pyrimidine core with a 4-pyridinyl group provided more promising results. As well as having improved solubility, compound **11f** was approximately equipotent to **11b** in the capsaicin-mediated assay and in acid-mediated assay. This result led us to investigate the introduction of 4-pyridyl, 4-piperidinyl, 4-piperazinyl derivatives at the 2-position of the pyrimidine in attempts to further enhance aqueous solubility.

Boc-protected tetrahydropyridine analogue **11g**, initially prepared as an intermediate, was quite potent with IC_{50} values of 1.8 and 0.6 nM in the CAP- and acid-mediated assays, respectively (Table 2). Deprotection of the Boc group was detrimental to activity (**12**); however, the potency was restored with the N-alkylation of **12** with the bulky isopropyl group. In this case, compound **13** re-



Scheme 3. Reagents and conditions: (a) 50% TFA/CH_2Cl_2, 98%; (b) $NaBH(OAc)_3$, isobutyraldehyde, 1,2-dichloroethane, DMF, 74%; (c) $Pd(OH)_2$, HCO_2NH_4, butanol, 120 °C, 49%.



Scheme 4. Reagents and conditions: (a) 50%TFA/CH_2Cl_2, 87%; (b) $NaBH(OAc)_3$, isobutyraldehyde, 1,2-dichloroethane, DMF, 62%.

tained good TRPV1 potency and the solubility was substantially improved over compound **1**. Unfortunately, a significant decrease in potency was observed in both the CAP- and acid-mediated assays with the reduction of the double bond of compound **13** indicating that an sp^3 center attached to pyrimidine C_2 was not well tolerated for this set of compounds (i.e., *N*-isobutyl piperidine derivative **14**).

Based on these results, we investigated piperazine derivatives that had a similar geometry at the pyrimidine C_2 as **11g** and **13**. The trends in potencies observed for piperazine derivatives **11h** and **18** were similar to their tetrahydropyridine counterparts, **11g** and **13**. For example, compound **18** maintained good TRPV1 potency (IC_{50} = 12.0 nM in CAP-mediated assay and IC_{50} = 4.0 nM in pH-mediated assay), but the solubility of this compound was <50 μg/mL (16 μg/mL, 0.01 N HCl; ⩽1.0 7 μg/mL, PBS; 39 μg/mL SIF, Table 2).

In the final phase of this investigation we examined the effect of projecting the pyrimidine 2 substituent out of the plane of the central core with the use of an aminomethyl linking group. The objective was to further destabilize the solid state packing forces and to explore deeper into the receptor pocket. We also incorporated basic amine functionality to the aminomethyl-piperidine derivatives (**19**–**21**) to improve solubility. All of these analogues had improved aqueous solubility, suggesting the benefit of the 2-aminomethyl linking group with respect to solubility. Additionally, with the appropriate position of isopropyl substituent, TRPV1 activity could be maintained. Although the piperidine analogues with the *N*-isopropyl at 3- or 4-positions showed reduced potency (**20** and **21**), the derivative having *N*-isopropyl at 2-position was more potent (**19**; IC_{50} = 6.1 nM in CAP-mediated assay and IC_{50} = 11 nM in pH-mediated assay).

Given the success of aminomethyl-linked piperidine **19**, we prepared a series of aminomethyl-linked pyridine analogues (i.e., **22**–**26**). These derivatives were synthesized according to the methods described in Scheme 2, starting with commercially available aminomethyl pyridines. All of these compounds had TRPV1 potencies of <10 nM. In addition, the introduction of an α-methyl substituent as additional bulk improved the aqueous solubility (Table 3). For example, compound **26** also showed better solubility than **22** in automated solubility assay.

Based on their excellent potencies and enhanced solubilities, compounds **13**, **19**, and **26** were selected for in vivo pharmacokinetic (PK) studies with intravenous (iv) dosing in Sprague–Dawley rats (Table 4). Unfortunately,

Table 4. Mean pharmacokinetic parameters following intravenous dose in Sprague–Dawley rats[a]

Compound	$AUC_{0-\infty}$ (ng h/mL)	CL (mL/h/kg)	*V*ss (mL/kg)	$t_{1/2}$ (h)
13	258	4040	6380	2.0
19	351	2860	8290	2.6
26	743	1350	6440	5.5

[a]Dosed at 1 mg/kg as a solution in DMSO. n = 2 animals per study. Interanimal variability was less than or equal to 30%.

following iv administration of 1 mg/kg, both **13** and **19** showed high rates of clearance (CL = 4040 mL/h/kg and CL = 2860 mL/h/kg, respectively). However, compound **26** demonstrated a modest rate of clearance (CL = 1350 mL/h/kg), a high volume of distribution ((*V*ss) 6440 mL/kg), and an elimination half-life ($t_{1/2}$) of 5.5 h.[21] In addition, compound **26** was relatively well absorbed following oral administration of 5 mg/kg (C_{max} = 280 ng/mL, $AUC_{0-\infty}$ = 899 ng h/mL) with a F_{oral} = 24%.

In summary, a series of trisubstituted pyrimidines were designed, synthesized, and evaluated for TRPV1 antagonist activities to block the capsaicin- and acid (pH 5)-induced uptake of $^{45}Ca^{2+}$ in CHO cell expressing rat TRPV1. An automated solubility screening assay was used as a valuable tool for the optimization of physical properties of this series. Structure–activity and structure–solubility studies led to the identification of compound **26**, which was among the most potent analogues (rTRPV1 CAP IC_{50} = 1.5 nM) in this series. This compound exhibited 24% oral bioavailability. Importantly, aqueous solubility of **26**($\geqslant$200 μg/mL, 0.01 HCl; 6.7 μg/mL, PBS; 150 μg/mL SIF) was substantially improved over compound **1**.

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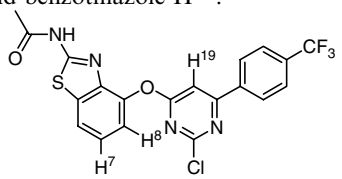

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20. Compound **26** was also active in the human TRPV1 assays with IC_{50} values of 2.1 and 1.7 nM in the CAP- and acid-mediated assays, respectively.
21. For comparison, the pharmacokinetic parameters for compound **1** are as follows: CL = 190 mL/h/kg, *V*ss = 1556 mL/kg, $AUC_{0-\infty}$ = 5400 ng h/mL, $t_{1/2}$ = 6.3 h, F_{oral} = 32%. For further details regarding the pharmacokinetic profile of compound **1**, see Ref. 12.