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Substituted aryl pyrimidines as potent and soluble TRPV1 antagonists

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ABSTRACT

Clinical candidate AMG 517 (**1**) is a potent antagonist toward multiple modes of activation of TRPV1; however, it suffers from poor solubility. Analogs with various substituents at the R region of **3** were prepared to improve the solubility while maintaining the potent TRPV1 activity of **1**. Compounds were identified that maintained potency, had good pharmacokinetic properties, and improved solubility relative to **1**.

The TRPV1 receptor (also known as Vanilloid Receptor 1 (VR1)) is a non-selective cation channel in the Transient Receptor Potential superfamily of ion channels. TRPV1 is activated by physical stimuli such as heat (>43 °C) and protons (low pH), endogenous stimuli such as anandamide as well as exogenous ligands such as capsaicin (CAP) and resiniferatoxin.[1–4] TRPV1 expression is increased after inflammatory injury in rodents,[5] and TRPV1 knockout mice show reduced thermal hypersensitivity after inflammatory tissue injury.[6,7] TRPV1 antagonists show a decrease in thermal and mechanical hyperalgesia in rats in inflammatory and neuropathic pain models.[8–11] This has resulted in the TRPV1 receptor being an attractive target in the treatment of pain.[12]

We have recently reported the identification of our clinical candidate **1** (AMG 517) which is a potent TRPV1 antagonist that blocks multiple modes of activation including capsaicin and acid (Fig. 1).[13] While AMG 517 demonstrated good potency and in vivo efficacy in rat in the capsaicin-induced flinch model and in the complete Freund's adjuvant (CFA)-induced inflammatory pain model, it suffered from poor solubility, which led to solubility limiting absorption at higher doses. We have reported on strategies to address this issue including replacing the phenyl ring in AMG 517 with a saturated heterocycle[14] and incorporating solubilizing groups at the 2-position of the pyrimidine ring[15] of AMG 517. In this letter, we describe an alternative approach to address the low solubility of AMG 517.

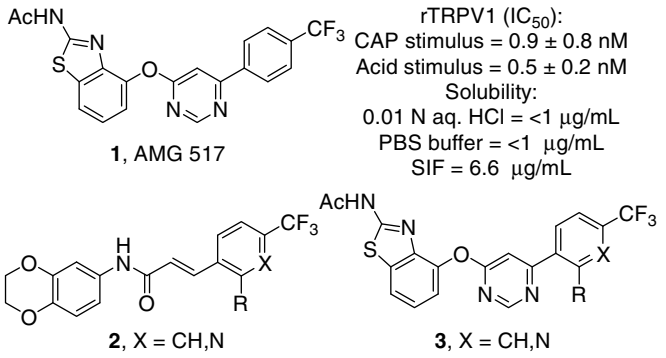

Figure 1. Clinical candidate AMG 517 (**1**) and related TRPV1 antagonist series (**2** and **3**).

In addition to pyrimidine derivatives such as AMG 517, we have also reported on a series of cinnamide TRPV1 antagonists (**2**) in which substitution at the region denoted as R was tolerated with respect to potency.[16] Based on evidence which demonstrated that our pyrimidine core functioned as a cyclized cinnamide analog,[17] we believed that incorporation of substituents in the R region of **3** would similarly be tolerated. With this in mind, a variety of derivatives with modifications in the R region of **3** were considered with the goal of improving solubility while maintaining reasonable pharmacokinetic properties[18] and potency relative to AMG 517.

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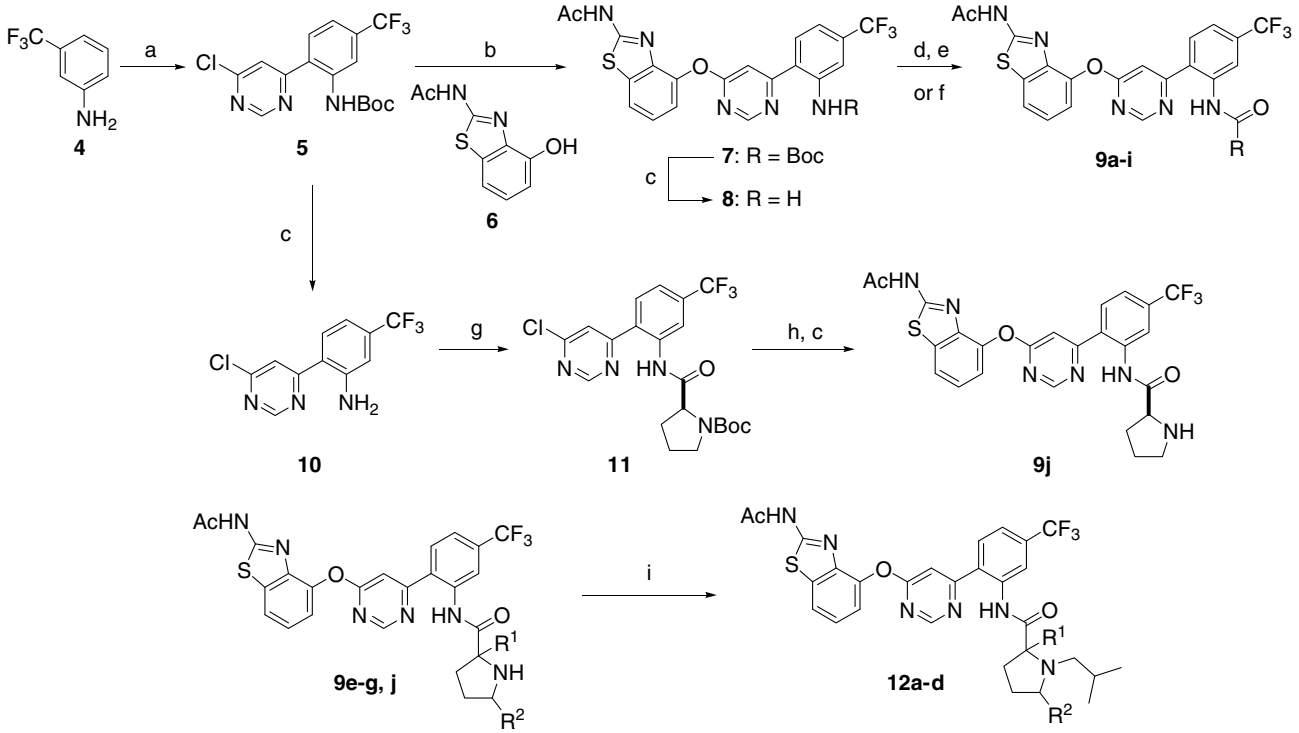
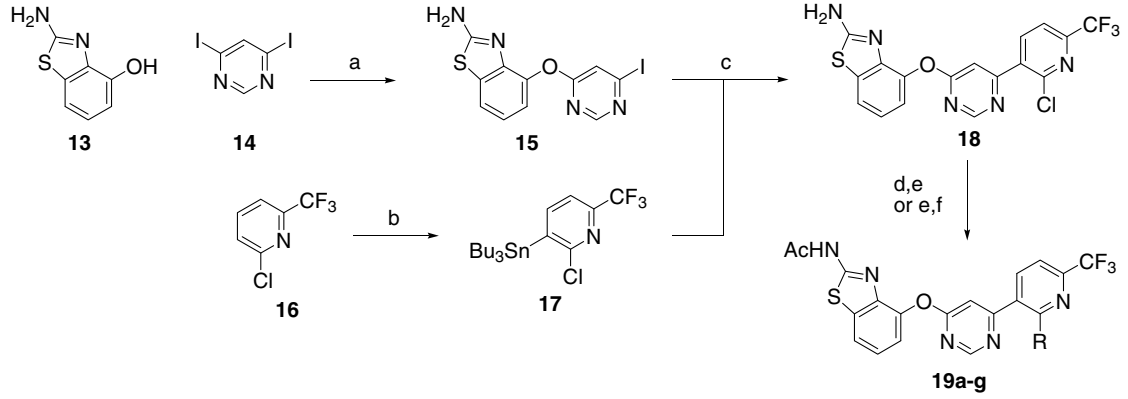
In this study, two sets of analogs (**3**) were investigated: an amide-linked series wherein R = NHC(=O)R′; X = CH and an amine-linked series wherein R = NR′$_2$; X = N. The amide linker was chosen based on the limited chemical reactivity of the aniline nitrogen when R = NH$_2$, X = CH and amidation reactions were one of the few reactions that were generally successful with this substrate. This poor reactivity is likely due to the steric crowding of the *ortho*-*pyrimidine* group and the electron-withdrawing nature of the CF$_3$ group. Hence, we had limited success in performing reductive aminations on this position. However, when X = N and R = Cl, amine-linked derivatives were readily accessible through chloride displacement with amines. To improve the solubility of the analogs, we attached a variety of ionizable groups and/or bulky alkyl groups. We postulated that the ionizable groups would improve solubility in acidic media, while the bulky alkyl groups would help reduce the crystal packing stability resulting in improved compound dissolution.

The synthesis of the amide-linked series is outlined in Scheme 1. Boc-protection of (3-trifluoromethyl)aniline (**4**) followed by a regioselective borylation[19,20] and subsequent Suzuki coupling with 4,6-dichloropyrimidine gave chloropyrimidine **5**. Deprotonation of hydroxybenzothiazole **6**[14] followed by the addition of intermediate **5** afforded disubstituted pyrimidine **7**. Removal of the Boc group afforded aniline **8** which was coupled with various acid chlorides or carboxylic acids to provide amides **9a–i**. The two carboxylic acid coupling reagents that were used were EDC and PyClU.[21] PyClU was used for sterically hindered carboxylic acids such as (*S*)-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine-2-carboxylic acid that would not undergo amide formation with EDC. Conveniently, the Boc group was cleaved to give **9e** directly during the amide cou-

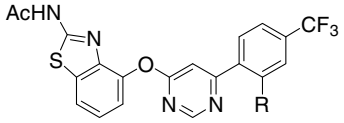


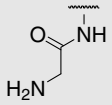
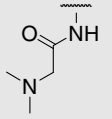
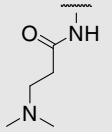
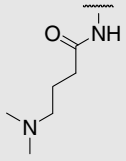
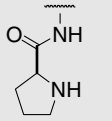
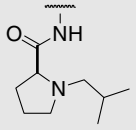
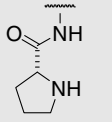
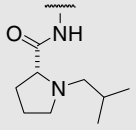
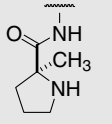
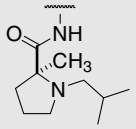
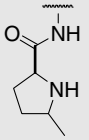
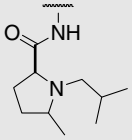
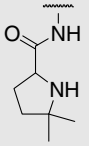
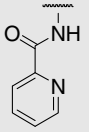
Scheme 1. Reagents and conditions: (a) i—(Boc)$_2$O, tol., reflux, 94%; ii—*sec*-BuLi, THF, B(OMe)$_3$, −70 °C then 1 M HCl; iii—4,6-dichloropyrimidine, Pd(OAc)$_2$, PPh$_3$, Na$_2$CO$_3$, CH$_3$CN, 70 °C, 36% over two steps; (b) NaH, DMF, rt, 83%; (c) TFA, CH$_2$Cl$_2$, rt, 87–91%; (d) i—carboxylic acid, EDC, CH$_2$Cl$_2$, 61–91%; ii—TFA, CH$_2$Cl$_2$, rt, 78–82%; (e) acid chloride, pyr., CH$_2$Cl$_2$, 32–79%; (f) (*S*)-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine-2-carboxylic acid, PyClU, *i*-Pr$_2$NEt, CH$_2$Cl$_2$, 45%; (g) (2)-*S*-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester, EDC, CH$_2$Cl$_2$, 84%; (h) **6**, K$_2$CO$_3$, DMF, 100 °C, 79%; (i) isobutyraldehyde, NaBH(OAc)$_3$, CH$_3$CN, 38–59%.



Scheme 2. Reagents and conditions: (a) K$_2$CO$_3$, DMSO, 80 °C, 78%; (b) LDA, Bu$_3$SnCl, THF, 79%; (c) Pd(PPh$_3$)$_4$, CuI, DMF, 51%; (d) Ac$_2$O, pyr. or Ac$_2$O, DMAP, CH$_2$Cl$_2$, 38-76%; (e) HCOOH, Pd(OAc)$_2$, PPh$_3$, DMF, 68%; (f) alkylamine, DMSO, heat, 17–58%.

Table 1
In vitro $^{45}Ca^{2+}$ influx activity and solubility of *ortho*-amides in rat TRPV1-expressing CHO cells



	R	CAP stimulus IC_{50}[a] (nM)	Aqueous solubility (μg/mL)		
			0.01 N HCl	PBS buffer	SIF
1		0.9 ± 0.8	<1	<1	6.6
9a		>4000	>200	25	83
9b		4.2 ± 0.2	100	2.4	61
9c		130[b]	81	3.9	30
9d		520 ± 40	>200	33	55
9j		13 ± 2	NA	NA	NA
12a		0.36 ± 0.5	>200	6.0	39
9e		8.2[b]	140	15	86
12b		0.53 ± 0.07	>200	1	39
9f		2.8 ± 0.3	>200	54	180
12c		0.68 ± 0.04	>200	1	37
9g		2.4 ± 0.2	120	7.4	59
12d		0.069 ± 0.021	180	1	29
9h		1.2 ± 0.4	>200	1.8	79
9i		1.0 ± 0.4	18	1	75

[a] Unless otherwise indicated, each IC_{50} value represents the average of at least two independent experiments with three replicates at each concentration (±SEM).
[b] The result of one experiment with three replicates at each concentration.

pling of (*S*)-1-(*tert*-butoxycarbonyl)-2-methylpyrrolidine-2-carboxylic acid with **8** using PyClU. Compound **9j** was made by an alternative sequence in which the benzothiazole moiety was incorporated after amide formation. In this case, chloropyrimidine **5** was first deprotected to give aniline **10**. Amide formation on **10** with EDC and (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid afforded Boc-protected pyrrolidine **11** which underwent chloride displacement with benzothiazole **6** followed by Boc-deprotection to afford **9j**. Finally, compounds **9e–g** and **9j** underwent reductive aminations with isobutyraldehyde to afford alkylated pyrrolidine analogs, **12a–d**.

The synthesis of the amine-linked series is shown in Scheme 2. Displacement of one of the iodine atoms on 4,6-diiodopyrimidine **14**[14] with hydroxybenzothiazole **13** under basic conditions afforded iodopyrimidine **15**. The aryl tin Stille coupling partner **17** was formed by regioselective stannylation of chloropyridine **16**.[22] Stille coupling of **15** and **17** afforded chloropyridine **18**. In the case of **19a**, where R = H, **18** underwent acetylation of the 2-aminobenzothiazole group first followed by reduction of the chloride. For **19b–g**, various amines were added to intermediate **18** followed by acetylation of the 2-aminobenzothiazole group to give analogs **19b–g**.

The IC_{50} data described herein are obtained from a $^{45}Ca^{2+}$ uptake assay on rat TRPV1 expressing CHO cells preincubated with compound that was activated with either capsaicin or acid.[17] No significant difference in inhibiting either stimulant was observed for these series.[13] None of the compounds showed agonist activity in a separate assay. Solubility data were obtained in three different aqueous media, 0.01 N HCl, PBS buffer (pH 7), and simulated intestinal fluid (SIF) by a Symyx automated method.[23]

Initially, we examined a set of amide-linked derivatives (Table 1). In this *ortho*-amide series, we found that while primary amine **9a** was soluble in 0.01 N HCl, it showed >4 μM potency toward capsaicin activation. In contrast, the corresponding dimethylamino analog, **9b**, maintained reasonable solubility in 0.01 N HCl, and was

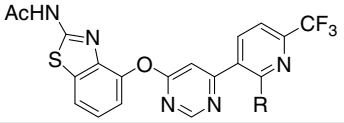
much more potent suggesting that lipophilic groups were favored in this region of the receptor. Compounds **9b–d** examined various distances between the phenyl ring and the basic nitrogen. With increasing length, potency dropped two orders of magnitude (**9b** to **9d**). With this information, we maintained the three-atom separation between the phenyl ring and the basic nitrogen, and investigated a series of pyrrolidine derivatives (**9e–h, 9j**, and **12a–d**). The unsubstituted pyrrolidines, **9e** and **9j** showed a small loss in potency relative to the open-chain compound **9b**, while the tertiary amine analog, **12a**, and its enantiomer, **12b**, were subnanomolar TRPV1 antagonists, again suggesting that the receptor prefers lipophilic groups in this region. Unfortunately, these two potent and soluble compounds suffered from high clearance in vivo (>4 L/h/kg).[24] Incubation of **12a** in rat hepatocytes[25] identified both the amide hydrolysis product and the N-dealkylation product as metabolites.

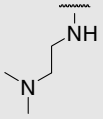
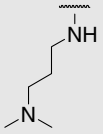
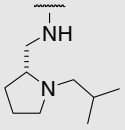
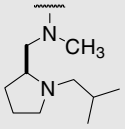
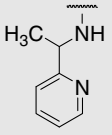
One strategy we investigated to suppress the metabolism of **12a** was to incorporate flanking methyl groups on either side of the pyrrolidine nitrogen. For example, **9f** and **12c** which contained a methyl group between the pyrrolidine nitrogen and the amide carbonyl maintained good potency and solubility. However, the in vivo clearance for these compounds was also high (>3.4 L/h/kg). Compounds **9g** and **12d** contained a single methyl group at the 5-position of the pyrrolidine ring. The N-alkylated analog, **12d**, showed good potency and solubility in 0.01 N HCl but high in vivo clearance (4.7 L/h/kg). The unalkylated secondary amine, **9g**, lost a small amount of potency and maintained good solubility in 0.01 N HCl; however, the in vivo clearance was reduced significantly (0.62 L/h/kg). Unfortunately, **9g** and its precursors were an inseparable 1:1 mixture of diastereomers epimeric at the methyl group. Placing two geminal methyl groups α to the pyrrolidine nitrogen such as in **9h** also afforded a potent and soluble compound, but again the in vivo clearance was high (4.9 L/h/kg). Interestingly while the flanking methyl groups did not lower the in vivo clearance in general, they did demonstrate that secondary amines could achieve <3 nM potency by increasing the lipophilic character around the pyrrolidine nitrogen.

In addition to amides containing alkyl amines as solubilizing groups, we also synthesized heteroaromatic amides such as **9i** to try to avoid the high in vivo clearance that we observed in the pyrrolidine series. Pyridine amide **9i**, which maintained the three atom linker between the phenyl ring and the basic nitrogen, did show single digit potency and reasonable in vivo clearance (0.44 L/h/kg); however, no improvement in solubility was obtained. According to calculations, the conjugate acid of **9i** has a pK_a value of 1.6.[26] Therefore, this pyridine amide may not be sufficiently ionized in 0.01 N HCl (pH 2.0) to impart significant solubility at low pH.

In addition to the amide derivatives, we also explored a simple amine linkage between the phenyl ring and the solubilizing group. This modification would remove the possibility of amide hydrolysis that may contribute to the high in vivo clearance observed in the amide-linked derivatives. To facilitate the synthesis of the amine-linked derivatives, we employed a pyridine ring in which X = N in **3**. The data for the pyridine derivatives are shown in Table 2. Compound **19a**, the pyridine analog of AMG 517, showed about a 10-fold loss in potency, a slight improvement in solubility and low in vivo clearance (0.6 L/h/kg). The structure–activity relationships of this series of compounds generally paralleled the trends observed in the amide series but were typically 10-fold less potent. Dimethylamino derivatives **19b** and **19c** showed a threefold drop in potency, when the linker between the pyridyl ring and the basic nitrogen was increased from three to four atoms. This result is consistent with the amide series in which the optimal number of atoms between the aromatic ring and the basic nitrogen was also three. The pyrrolidine analog **19d** showed good potency and solubility, but as with the amide analogs, the in vivo clearance was high (2.5 L/h/kg). Hence, N-dealkylation may be the likely metabolic pathway for these compounds. Analog **19e** demonstrated that a methyl group on the aniline nitrogen significantly diminished potency. The aniline methyl may be removing the possible hydrogen bond between the aniline nitrogen and the pyrimidine ring or simply producing an unfavorable steric interaction that changes the conformation to a less optimal one for binding.

Table 2
In vitro $^{45}Ca^{2+}$ influx activity and solubility of 2-substituted pyridine analogs in rat TRPV1-expressing CHO cells



A	R	CAP stimulus IC_{50}[a] (nM)	Aqueous solubility (μg/mL)		
			0.01 N HCl	PBS buffer	SIF
19a		10 ± 1	4.9	5.9	1
19b		63 ± 6	NA	NA	NA
19c		210 ± 40	190	17	150
19d		17 ± 0.5	>200	6.3	170
19e		280 ± 30	>200	80	116
19f		0.48 ± 0.25	120	1.9	36

[a] Each IC_{50} value represents the average of at least two independent experiments with three replicates at each concentration (±SEM).

Based on the good potency and low clearance of the pyridine amide **9i**, we revisited pyridine substituents in the amine-linked series. In looking at analog **9i**, if the carbonyl of the amide was removed the pK_a of the molecule would be dramatically raised and the solubility at low pH may be improved. Compound **19f** exemplifies this with a calculated pK_a of 4.5. This compound did show subnanomolar potency and improved solubility in 0.01 N HCl relative to the pyridine substituted amide. In addition, **19f** demonstrated low in vivo clearance (0.11 L/h/kg), an elimination half life ($t_{½}$) of 4.7 h, a volume of distribution (V_{SS}) of 2.2 L/kg as well as moderate oral bioavailability (F_{oral} = 17%) and exposure ($AUC_{0-\infty}$ = 5530 ng h/mL).[18,27]

In conclusion, a series of TRPV1 antagonists were designed and synthesized with substitution at the R region of **3** with the goal of having improved solubility relative to clinical candidate AMG 517. One analog, **19f**, from the amine-linked series, showed improved

solubility relative to AMG 517, yet maintained good potency toward TRPV1 and good pharmacokinetic properties.

References and notes

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