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4-Aminopyrimidine tetrahydronaphthols: A series of novel vanilloid receptor-1 antagonists with improved solubility properties

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Abstract—8-(6-(4-(Trifluoromethyl)phenyl)pyrimidin-4-ylamino)-1,2,3,4-tetrahydronaphthalen-2-ol (**4**) and analogs (**5**–**10**) were shown to be potent inhibitors of human and rat TRPV1 in vitro with increased solubility over our previous series. Synthesis, SAR, and improvements in metabolic stability and absorption of these compounds are described herein.

Agonism of the ion channel vanilloid receptor-1[1] (TRPV1 or VR1) by capsaicin, the pungent component naturally occurring in chili peppers, causes a rapid influx of cations into the nerve cell setting off a cascade of events that result in a burning sensation. This sensation should be familiar to anyone who has enjoyed a spicy curry. Stimulation of the neuron by this route leads ultimately to desensitization to subsequent capsaicin application as well as a decreased sensitivity to noxious thermal stimuli. Conversely, mice lacking the TRPV1 gene, while grossly normal, exhibit a reduced response to painful thermal stimuli in inflammatory pain models.[2] These observations in knock-out mice have provided a rationale for the pursuit of small molecule TRPV1 antagonists as a treatment for inflammatory pain.[3]

We have reported on a series of pyrimidine-based TRPV1 antagonists with excellent in vitro and in vivo properties.[4] From this series, AMG 517 (**1**, Fig. 1) was selected for evaluation in human clinical trials. We then initiated a program aiming to further improve the profile of the series. Specifically, due to the poor solubility of compound **1** we encountered difficulties in formulation and observed solubility-limited absorption at higher doses in animal studies. Previously, we have shown that incorporation of groups containing a basic amine at the 2- or 6-position of the pyrimidine core of **1** resulted in improved solubility while potency was maintained.[5] We also pursued an alternative twofold approach to improving solubility: (1) increase the basicity of the core and (2) reduce crystallinity via partial saturation. Herein we describe a set of tetrahydronaphthol analogs of **1** (compounds **4**–**10**, Figs. 1 and 2) that achieved our goal of improved solubility following this alternative strategy.

Previous SAR studies provided us with an understanding of the three key features required for optimal interaction of antagonists like **1** with the TRPV1 receptor: (1) a 2-oxo or 2-amino- pyrimidine or pyridine core, (2) a lipophilic region on one end of the molecule, and (3) an aromatic group with hydrogen-bonding elements at the other end. Early on in our high-throughput screening (HTS) efforts we had uncovered another series of analogs with low nM potency, the naphthol ureas[6] exemplified by compound **2** (Fig. 1) with an IC_{50} = 13 nM [as measured by inhibition of capsaicin-induced $^{45}Ca^{2+}$ influx into human TRPV1-expressing Chinese hamster ovary (CHO) cells].[7] This urea series provided the same requisite key interactions with the receptor, that is, a urea mimicking the oxopyrimidine core flanked by a lipophilic aromatic group and a hydrogen bond-donating naphthol. However, the urea series suffered from low solubility and poor pharmacokinetic properties. Nevertheless, we felt that the naphthol

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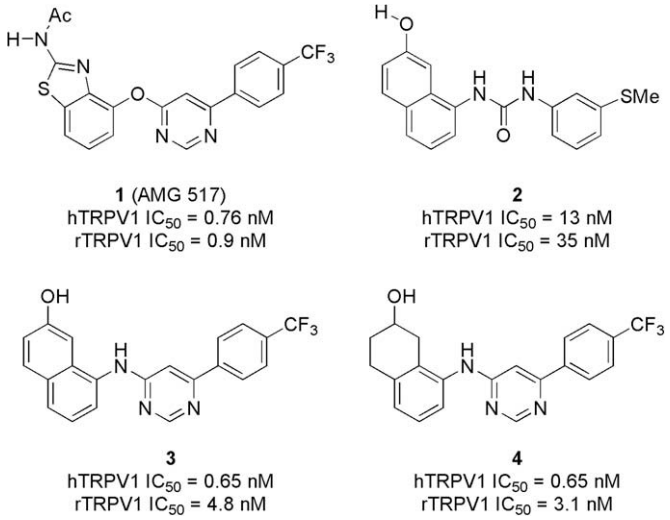


Figure 1. Clinical candidate **1** (AMG 517), naphthol urea HTS hit **2**, hybrid analog **3**, tetrahydronaphthol analog **4**.

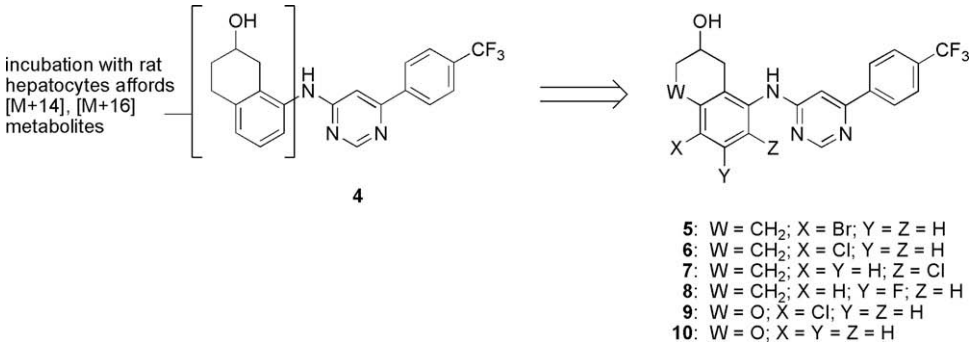


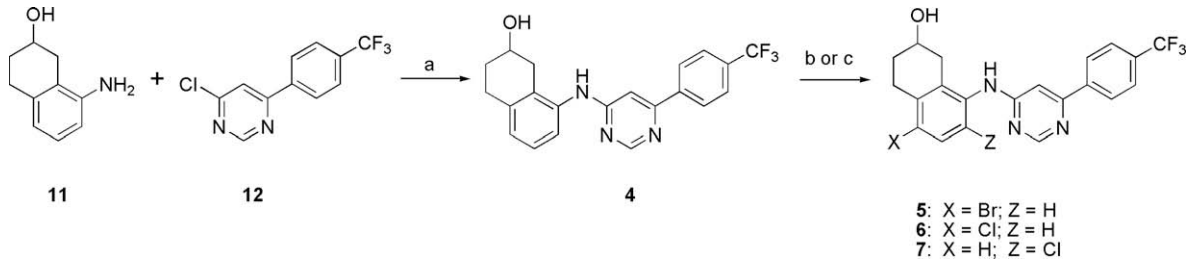
Figure 2. Putative sites of metabolism on compound **4** and targets **5**–**10** designed to block the metabolic soft spot.

moiety provided a new direction in SAR development for the pyrimidine series. Combining the trifluoromethylphenylpyrimidine of compound **1** with the 1-aminonaphthol of urea **2** resulted in **3**[8] (Fig. 1), with an IC_{50} = 0.65 nM. This combination of features resulted in an increase in potency over the urea analog **2**; however, we observed only a modest improvement in solubility over compound **1** (Table 1). The slight but measurable improvement in solubility, particularly in aqueous 0.01 N HCl (0.003 mg/mL), we attributed to the increased basicity of the aminopyrimidine versus the oxopyrimidine core.[9] By partially saturating the naphthol ring, affording tetrahydronaphthol analog **4** (Fig. 1), a much more significant improvement in solubility was gained and excellent potency was maintained. Thus, compound **4** blocked capsaicin-induced calcium influx into hTRPV1-expressing CHO cells with an IC_{50} = 0.65 nM, in rTRPV1 with an IC_{50} = 3.1 nM, and had solubilities of 0.19 mg/mL in 0.01 N HCl, 0.023 mg/mL in pH 7.4 phosphate buffered saline, and 0.060 mg/mL in simulated intestinal fluid (Table 1).[10]

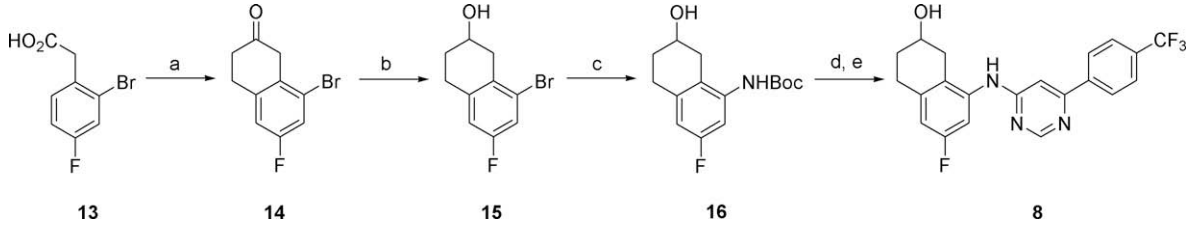
Encouraged by the improvement in solubility, we evaluated the pharmacokinetic profile of compound **4** in rats. Upon intravenous (i.v.) administration, tetrahydronaphthol **4** exhibited a very high rate of clearance (CL = 3900 mL/h/kg), close to the rate of rat hepatic blood flow. To determine whether the absolute configuration at the chiral center had any influence on potency or PK properties, compound **4** was resolved by chiral reverse-phase HPLC[11] to provide the antipodes (+)-**4** and (−)-**4**. We observed no significant difference in potency, solubility, or in vivo clearance for racemic **4** in comparison to the single enantiomers (Table 1). For this reason, we simplified our efforts by building the remaining SAR with racemates.

To account for the high clearance, we conducted metabolite identification studies on compound **4** in isolated rat hepatocytes.[12] These studies showed that compound **4** underwent Phase I metabolism restricted to the tetrahydronaphthol moiety with the formation of at least six metabolites [M+14 and M+16]. No significant direct Phase II conjugation was observed. We prepared a limited set of derivatives (compounds **5**–**10**, Fig. 2), selected based in part on synthetic accessibility, designed to block some of the possible sites of metabolism.

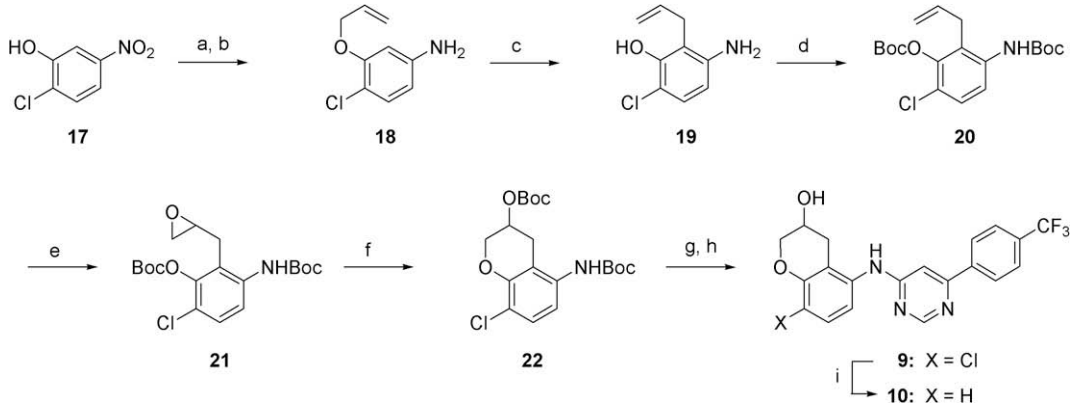
The syntheses of compounds **4**–**10** are illustrated in Schemes 1–3. As shown in Scheme 1, the tetrahydronaphthol analog **4** was prepared by heating 8-amino-1,2,3,4-tetrahydronaphthol[6b] (**11**) with 4-chloro-6-[4-(trifluoromethyl)phenyl]pyrimidine[4a] (**12**) in EtOH by microwave irradiation. Reaction of **4** with *N*-bromosuc-



Scheme 1. Reagents and conditions: (a) EtOH, 160 °C microwave (40%); (b) NBS, DMF (82%); (c) NCS, DMF: **6** (16%); **7** (4%).



Scheme 2. Reagents and conditions: (a) $SOCl_2$, DCM; ethylene, $AlCl_3$, DCM, −5 °C (54%); (b) $NaBH_4$, MeOH (77%); (c) $H_2NCO_2(t\text{-Bu})$, $Pd_2(dba)_3$, X-Phos, Cs_2CO_3, dioxane, 90 °C (18%); (d) 4 M HCl in dioxane (99%); (e) 4-chloro-6-[4-trifluoromethyl)phenyl]pyrimidine (**12**), EtOH, 160 °C microwave (13%).



Scheme 3. Reagents and conditions: (a) allyl bromide, K_2CO_3, acetone, reflux (97%); (b) $SnCl_2$, EtOH, 70 °C (100%); (c) 1,2-dichlorobenzene, 190 °C (92%); (d) Boc_2O, THF; (e) *m*-CPBA (21% over steps d and e); (f) NaI, acetone, reflux (29%); (g) 4 M HCl in dioxane (100%); (h) 4-chloro-6-[4-trifluoromethyl)phenyl]pyrimidine (**12**), EtOH, 160 °C microwave (35%); (i) H_2, 10% Pd/C, MeOH (100%).

cinimide in DMF at room temperature afforded predominately the 5-bromo analog **5** in 82% yield. Alternatively, treatment of **4** with *N*-chlorosuccinimide provided a mixture of mono- and di-chlorinated products that were separated by silica gel chromatography followed by reversed-phase HPLC. In this way, modest yields of **6** (16%) and **7** (4%) were obtained.

According to Scheme 2, following a procedure described by Wikstrom et al.,[13] Friedel-Crafts acylation of ethylene with the acid chloride of **13** followed by ring closure provided 8-bromo-6-fluoro-2-tetralone (**14**). The tetralone was reduced to alcohol **15** with sodium borohydride. Palladium-mediated amidation[14] of bromide **15** with *tert*-butylcarbamate provided *N*-Boc-6-fluoro-8-aminotetrahydronaphthol (**16**). The aniline was revealed by deprotection with HCl in dioxane and reacted with 4-chloro-6-[4-trifluoro-methyl)phenyl]-pyrimidine (**12**) to afford **8**.

The chromanyl derivatives **9** and **10** were prepared according to Scheme 3. Formation of the allyl ether of 2-chloro-5-nitrophenol (**17**) followed by tin chloride reduction of the nitro group provided aniline **18**. The Claisen-rearranged product **19** was di-protected with Boc anhydride to afford **20**, and then oxidized with *m*-CPBA to epoxide **21**. NaI-catalyzed cyclization of epoxide **21**, as described by Otani,[15] afforded chroman **22**. After global deprotection with HCl in dioxane, the aniline was reacted with 4-chloro-6-[4-trifluoromethyl)-phenyl]pyrimidine (**12**) to afford **9**. Dehalogenation of **9** with hydrogen over Pd on carbon provided the final analog **10**.

The compounds were tested for their ability to block the capsaicin-induced uptake of $^{45}Ca^{2+}$ in human or rat TRPV1-expressing (hTRPV1 or rTRPV1) CHO cells.[7] Functional activity reported as mean $IC_{50} \pm SEM$ (nM) is summarized in Table 1. Results are the average

Table 1. In-vitro inhibition of capsaicin-induced $^{45}Ca^{2+}$ influx in hTRPV1 or rTRPV1-expressing CHO cells, solubility data, and in-vivo pharmacokinetic profile for compounds **1** and **3–10**

Compound	hTRPV1 IC_{50}[a] (nM)	rTRPV1 IC_{50}[a] (nM)	Solubility[b] (mg/mL)			CL, i.v. (mL/h/kg)	$t_{1/2}$, i.v. (h)	F, p.o. (%)	AUC, p.o. (ng h/mL)
			0.01 N HCl	PBS[c]	SIF[d]				
1	0.76 ± 0.04	0.9 ± 0.8	<0.0001	<0.0001	0.00066	190	6.3	32[e]	5300[e]
3	0.65 ± 0.08	4.8 ± 0.4	0.003	<0.0001	0.010	> 4000	0.7	—	—
4	0.65 ± 0.13	3.1 ± 0.2	0.19	0.023	0.060	3900	1.2	—	—
(+)-4	0.58 ± 0.04	5.7 ± 0.5	0.18	0.0056	0.060	4100	1.2		
(−)-4	0.62 ± 0.01	5.5 ± 1.4	0.16	0.0091	0.085	5800	0.9		
5	2.1 ± 0.5	9.6 ± 1.2	0.0072	0.0077	0.073	390	5.5	60[f]	7600[f]
6	1.4 ± 0.1	5.6 ± 0.5	0.022	0.027	0.032	500	6.6	34[f]	3400[f]
7	1.1 ± 0.3	11 ± 1.5	0.071	0.0125	0.030	—	—	—	—
8	1.1 ± 0.6	43 ± 8	—	—	—	—	—	—	—
9	2.9 ± 0.6	28 ± 11	0.136	0.0056	0.017	—	—	—	—
10	10 ± 1	190 ± 50	0.200	0.015	0.063	—	—	—	—

[a] Values are means of at least three measurements, ± standard error of the mean (SEM).
[b] Thermodynamic solubility measured in a high-throughput automated format (Ref. 10).
[c] Phosphate buffered saline, pH 7.4.
[d] Simulated intestinal fluid, pH 6.8.
[e] 3 mg/kg dose in 5% Tween 80/OraPlus.
[f] mg/kg dose in 5% Tween 80/Oraplus.

of at least three measurements. All compounds were tested in a separate assay for agonist activity; the compounds showed no agonist activity. Solubility was measured using a high-throughput SYMYX platform[10] in three aqueous media: 0.01 N HCl, pH 7.4 phosphate buffered saline (PBS), and pH 6.8 simulated intestinal fluid (SIF).

Each of the modifications made to compound **4** (e.g. compounds **5–10**) was tolerated to some extent, antagonist activity was maintained in the series and, for those compounds analyzed, solubility in all three media was significantly increased in comparison to compounds **1** and **3**. The 5-bromotetrahydronaphthol analog **5** was only threefold less potent than **4** in the human and rat TRPV1 assays (IC_{50} = 2.1 and 9.6 nM, respectively). Introduction of the bromine atom resulted in the desired reduction in clearance [$t_{1/2}$ = 5.5 h; CL = 390 mL/h/kg]. Compound **5**, dosed orally (p.o.) in rats as a 5 mg/kg suspension in 5% Tween 80/Oraplus, demonstrated good absorption [F_{oral} = 60%; AUC = 7600 ng h/mL]. In comparison to compound **4**, however, the solubility of **5** in 0.01 N HCl and PBS decreased (0.0072 and 0.0077 mg/mL, respectively). This result can be explained by increased lipophilicity imparted by the bromine atom. The 5-chloro analog **6** showed similar potencies to **4** and **5**, with IC_{50} = 1.4 nM in hTRPV1 and IC_{50} = 5.6 nM in rTRPV1. Like compound **5**, the pharmacokinetic profile of **6** [$t_{1/2}$= 6.6 h; CL = 500 mL/h/kg; F_{oral} = 34%] was an improvement over **4**. In addition, the solubility of **6** in 0.01 N HCl and PBS (0.022 and 0.027 mg/mL, respectively) was improved over compound **5**. The isomeric 7-chlorotetrahydronaphthol analog **7** demonstrated no significant advantage over **6** with regard to potency or solubility. The 6-fluoro analog **8** was more than 10-fold less potent in the rat TRPV1 assay relative to **4**. The chromanyl analogs **9** and **10** were also over one to two orders of magnitude less potent in the rTRPV1 assay (IC_{50} = 28 and 190 nM, respectively) although these analogs demonstrated very good solubility in aqueous 0.01 N HCl (0.136 and 0.200 mg/mL, respectively). Generally, the structural modifications made to **4** were better tolerated by the human receptor than the rat receptor. The combined properties of compounds **5** and **6** (potency, solubility, clearance and oral absorption) made these two compounds the best in the series.

In conclusion, by increasing the pK_a of **1** via the basic 2-aminopyrimidine core, incorporating tetrahydronaphthol as a replacement for *N*-acylamino-benzothiazole, and adding halogen atoms to block metabolism, we have designed 4-aminotetrahydronaphthol pyrimidines (e.g., **5** and **6**) with excellent in-vitro potencies, good pharmacokinetic properties, and improved solubilities over clinical candidate AMG 517 (**1**).

Acknowledgments

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11. The enantiomers were separated by preparative chiral phase HPLC on a Chiralcel^{TM} AD column; mobile phase 25% EtOH in hexane; flow rate 30 mL/min; (+)-**4** was the first to elute. The isolated antipodes were >99% enantiomeric purity as determined by chiral analytical LC.
12. In vitro metabolism was examined by incubating compound **4** at a concentration of 10 μM with rat hepatocytes (1 million cells/mL) for 4 h at 37 °C. Control incubations in the absence of cells were also carried out. Reactions were quenched with acetonitrile containing 0.05% formic acid. The supernatant was analyzed by reverse phase (YMC ODS-AQ, 4.6×250 mm, 5 μm) HPLC-MS/MS with radiometric detection and ion trap mass spectrometry using electrospray ionization. Hydrolysis experiments were conducted with glucoronidase and/or sulfatase to confirm the presence of glucoronide and/or sulfate metabolites.
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