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7-Hydroxynaphthalen-1-yl-urea and -amide antagonists of human vanilloid receptor 1

Mark E. McDonnell,[a] Sui-Po Zhang,[a] Nadia Nasser,[b] Adrienne E. Dubin[b] and Scott L. Dax[a,*]

[a]*Johnson & Johnson Pharmaceutical Research and Development, Welsh and McKean Roads, Spring House, PA 19477, USA*
[b]*Johnson & Johnson Pharmaceutical Research and Development, 3210 Merryfield Row, San Diego, CA 92121, USA*

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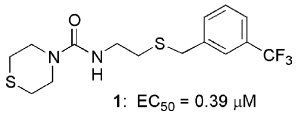
Abstract—A series of structurally simple 7-hydroxynaphthalenyl ureas and amides were discovered to be potent ligands of human vanilloid receptor 1 (VR1). 1-(7-Hydroxynaphthalen-1-yl)-3-(4-trifluoromethylbenzyl)urea **5f** exhibited nanomolar binding affinity ($K_i = 1.0$ nM) and upon capsaicin challenge, behaved as a potent functional antagonist ($IC_{50} = 4$ nM). The synthesis and structure–activity relationships (SARs) for the series are described.

The pungency and antinociceptive properties of *Capsicum* species have been recognized and used therapeutically for centuries. The active ingredient, capsaicin,[1] is small molecule agonist of vanilloid receptor type 1, a non-selective ligand- and temperature-gated cation channel. Opening of the vanilloid receptor 1 (VR1) channel leads to influx of cations (particularly calcium), and subsequent depolarization of subpopulations of mammalian sensory C- and Aδ-fibers causing initially excitation of primary afferents followed by neuronal desensitization. These pharmacological actions make capsaicin creams effective in relieving minor muscle aches, but limited in clinical utility, due to pungency.

The cloning of human and rat VR1[2–4] has reinvigorated research in vanilloids with the promise of developing novel therapeutic agents.[5,6] In this regard, we chose to pursue antagonists of VR1, hypothesizing that direct blockade of the channel could provide a therapeutic means to treat pain without pungency that is observed with some agonists such as capsaicin. From a high throughput functional assay, employing a Fluorometric Imaging Plate Reader (FLIPR™) technology, we identified urea **1** as a sub-micromolar agonist.



1: EC_{50} = 0.39 μM

Optimization via chemical synthesis, described herein, rapidly afforded a series of hydroxynaphthenyl ureas and amides that bind to human VR1 with nanomolar affinity and behave as functional antagonists of VR1, in the presence of capsaicin.

N-Hydroxynaphthenyl ureas were synthesized in straightforward fashion (Scheme 1). Aminonaphthol **2** was reacted with phenylchloroformate in the presence of an aqueous base to form the corresponding phenylcarbamate **3**. Subsequent reaction of hydroxynaphthalen-1-yl-carbamic acid phenyl ester **3** with amines such as substituted anilines, benzylamines, and phenethylamines (**4**), in dimethylsulfoxide (DMSO), afforded the urea final products **5** in good yields. A trialkylamine base was added to the reaction when the amine was only available as an acid addition salt. The choice of dimethylsulfoxide as solvent was important with respect to obtaining clean and efficient conversion of carbamate **3** to urea **5** under mild conditions.[7] Separately, aminonaphthol **2** could be directly reacted with isocyanates to give **5**. Furthermore, amide congeners **6** were prepared simply by coupling aminonaphthol with benzoic, phenylacetic, or dihydrocinnamic acids, or by reacting aminonaphthol with the corresponding acid chloride. *O*-Protecting groups could be employed to

* Corresponding author. Tel.: +1-215-628-5211; fax: +1-215-628-3297; e-mail: sdax@prdus.jnj.com

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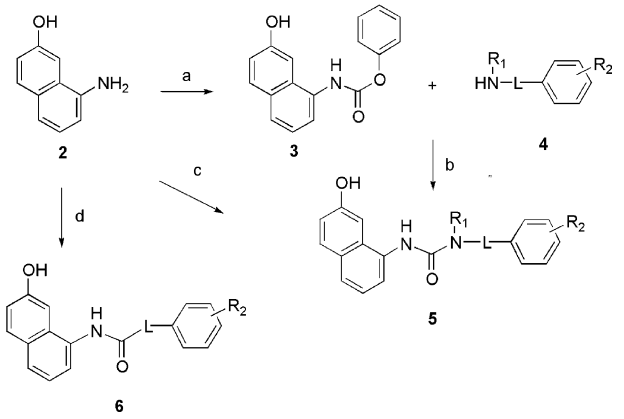
mask the naphthol (–OH) moiety in cases in which functionality, other than a phenolic center, was problematic. Along these lines, preliminary studies indicate that the free phenol is necessary for activity. *O*-Methylation abolished VR1 affinity and functional activity. Similarly, simple naphthalenyl congeners, lacking the 8-OH moiety, were poorly active (data not shown).

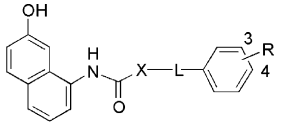
Compounds **5–6** were assayed for binding affinity at hVR1 using a modified version of the method previously reported by Szallasi and Blumberg (Table 1).[8] Membranes harvested from a HEK293 cell line stably expressing human VR1 cDNA (hVR1/HEK293)[9] were incubated with [^{3}H]-RTX and the test compounds. After incubation and centrifugation, bound [^{3}H]-RTX was quantified and from this, K_i values were determined. Ureas **5** and amides **6** were also assayed for ability to antagonize capsaicin-induced calcium flux using a Ca^{2+}-sensitive fluorescent dye and FLIPR™ technology (Molecular Devices, Inc.).[10] hVR1/HEK Cells[4] were first challenged by exposure to compound (**5** and **6**) to determine if they possess inherent agonist activity. Antagonist potency was subsequently determined using a similar protocol except that after incubation with compound, cells were challenged with capsaicin at a concentration eliciting ~80% maximal response.



Scheme 1. (a) PhOC(O)Cl/aq $NaHCO_3/CH_2Cl_2$ (92%); (b) DMSO (45–97%); (c) Ar-L-N=C=O/MeCN, microwave, 100 °C 5 min (45%); (d) Ar-L-CO_2H, PyBROP, DIPEA, DMF; or Ar-L-C(O)Cl/aq $NaHCO_3/CH_2Cl_2$ (13–39%).

Our starting point was 1-benzyl-3-(7-hydroxynaphthalen-1-yl)-urea **5a** which had good binding affinity (K_i = 94 nM) and exhibited significant functional antagonism of VR1 (IC_{50} = 45 nM). *N*-Alkylated congeners were poorly active in terms of binding affinity (N-Me **5l** > N-Me **5b** ~N-Et **5c** > *N*-benzyl **5d**) suggestive of a requisite free NH group. The carbamate intermediate **4** was also inactive (IC_{50} = 6300 nM). However, in the case of the ureas, addition of lipophilic inert electron-withdrawing groups onto the benzylic portion of the urea enhanced both binding affinity and functional antagonist potency. The preferred site of substitution was the distal end of the molecule; specifically *para*-substitution was slightly favored over *meta*-analogues and *ortho*-analogues (not shown). Thus, 3-trifluoromethylated urea **5e** represented a significant

Table 1. Human VR1 binding affinities and functional activity of ureas **5** and amides **6**[a]



5; X = NH
6: X = -CH$_2$-

	X	L	R	Binding affinity K_i (nM)	Functional activity IC_{50} (nM)
4	O	Nil	(H)	Ia	6300
5a	NH	–CH_2–	(H)	94	45
5b	NMe	–CH_2–	(H)	Ia[a]	Ia
5c	NEt	–CH_2–	(H)	1110	Ia
5d	NCH_2Ph	–CH_2–	(H)	3280	Ia
5e	NH	–CH_2–	3-CF_3	2.1	26
5f	NH	–CH_2–	4-CF_3	1.0	4
5g	NH	–CH(CH_3)–	4-CF_3	4.0	nd[b]
5h	NH	–CH_2–	4-OCF_3	1.0	27
5i	NH	–CH_2–	3,4-diF	7.0	nd
5j	NH	–CH_2–	2,4-diCl	2.8	nd
5k	NH	–CH_2–	3,4-diCl	2.0	15
5l	NMe	–CH_2–	3,4-diCl	581	nd
5m	NH	–$(CH_2)_2$–	(H)	53	nd
5n	NH	–$(CH_2)_2$–	3,4-diCl	1.0	90
6k	–CH_2–	–CH_2–	3,4-diCl	4.6	17
6o	–CH_2–	–CH_2–	4-Cl	9.8	21
6p	–CH_2–	Nil	3,4-diCl	1830	770
6q[c]	Nil	Nil	4-Cl	28% inh @10 μM	320
CPZ	—	—	—	120	100

[a] Ia, inactive (> 10 uM).
[b] nd, not done.
[c] Percent inhibition @ 10 uM; CPZ, capsazepine.

breakthrough in being nearly two orders of magnitude more potent in terms of binding affinity ($K_i = 2.1$ nM) and 2-fold more potent as a functional antagonist ($IC_{50} = 26$ nM) compared to the unsubstituted analogue **5a**. 1-(7-Hydroxynaphthalen-1-yl)-3-(4-trifluoromethylbenzyl)-urea **5f** was even more potent with a K_i value of 1 nM and an IC_{50} value of 4 nM. Methylation at the benzylic carbon center retained strong VR1 affinity (**5g**: $K_i = 4$ nM). The trifluoromethylether congener **5h** also exhibited exquisite binding affinity ($K_i = 1$ nM) but was less active in the functional assay ($IC_{50} = 27$ nM) relative to the directly trifluoromethylated **5f**. The incorporation of additional halogen substituents had relatively minor impact on binding and functional activity, evident by highly active **5i** (3,4-difluoro), **5j** (2,4-dichloro) and **5k**[11] (3,4-dichloro) which all displayed low nanomolar affinity for VR1.

Conversion of the urea moiety into an amide linkage retained VR1 activity. For example, 3-(3,4-dichlorophenyl)-*N*-(7-hydroxynaphthalen-1-yl)-propionamide **6k**[12] possessed similar affinity and potency ($K_i = 4.6$ nM; $IC_{50} = 17$ nM) as the urea analogue **5k**. The monochloro analogue **6o** was also potent ($K_i = 9.8$ nM; $IC_{50} = 21$ nM). However, moving the aryl moiety closer to the amide center significantly reduced activity, evident by both 2-(3,4-dichlorophenyl)-*N*-(7-hydroxynaphthalen-1-yl)-acetamide **6p**[13] and 3,4-dichloro-*N*-(7-hydroxynaphthalen-1-yl)-benzamide **6q**.[14]

In conclusion, ureas and amides derived from 8-aminonaphthalen-2-ol and containing an appropriately positioned halosubstituted aryl moiety, display low nanomolar affinity for human VR1 and behave as functional antagonists of the channel upon capsaicin challenge. These structurally simple molecules may provide a starting point for the development of VR1 antagonists as a potentially new family of analgesic agents. Indeed, *N*-[4-(methylsulfonylamino)benzyl]-thioureas[15] recently have been disclosed as VR1 antagonists, thus demonstrating that simple benzylic ureas, if appropriately functionalized, can effectively modulate VR1.

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9. **[^{3}H]-RTX binding assay using hVR1/HEK293 cell membranes**. Cloning and generation of stable cell lines expressing human VR1. Human VR1 was cloned and stably expressed in HEK293 cells (hVR1/HEK293) as described by Grant et al. in *J. Pharm. Exp. Ther.* **2002**, *300*, 9. **Preparation of membranes**. hVR1/HEK293 were homogenized with a Polytron twice and centrifuged at 3000 rpm for 10 min in HEPES buffer containing 20 mM HEPES, pH 7.4, NaCl 5.8 mM, sucrose 320 mM, $MgCl_2$ 2 mM, $CaCl_2$ 0.75 mM and KCl 5 mM. The supernatant was centrifuged at 18,000 rpm for 20 min. The pellet was saved in a tube and 10 mL assay buffer was added into the tube. The pellet and buffer were mixed with a Polytron. **Incubation procedure**. Incubations for 60 min at 37 °C were performed in a total volume of 0.5 mL that contained 120 μg/mL membrane protein and 0.3–0.6 nM [^{3}H]-RTX (NEN, Boston, MA, USA) in the HEPES buffer. After incubation, the samples were cooled on ice and 100 μg of α-acid glycoprotein were added followed by centrifugation at 13,000 rpm for 15 min. The supernatant was aspirated and the tips of tubes were cut off into 6-mL vials. Nonspecific binding was measured in the presence of 200 nM unlabeled RTX in 4 mL scintillation liquid using a Packard scintillation counter. **Data analysis**. Percent (%)) inhibition = (total) binding − total binding in prescence of compound */100 (total) binding − non specific binding. K_i values were obtained from Prism (GraphPad, San Diego, CA, USA) calculated using equation of Cheng–Prusoff ($K_i = IC_{50}/(1 + [LIGAND])/K_d$).
10. hVR1/HEK cells[4] were seeded on poly-D-lysine coated 96-well, black-walled plates (BD 354640) and 2 days later loaded with Fluo-3/AM for 1 h and subsequently tested for agonist-induced increases in intracellular Ca^{2+} levels using FLIPR™ technology. Cells were challenged with single concentrations of compound (**3**, **4**, and **5**) and intracellular Ca^{++} was measured for 3 min prior to the addition of CAP to all wells to achieve a final CAP concentration of 15 nM to fully activate VR1. Antagonist potency was determined using the protocol described by McDonnell et al. (*Bioorg. Med. Chem.* **2002**, *12*, 1189). Data were analyzed using Prism software to calculate IC_{50} values.
11. **1-(3,4-Dichlorobenzyl)-3-(7-hydroxynaphthalen-1-yl)-urea (5k)**. 3,4-Dichlorobenzylamine (53 uL, 0.39 mmol) and (7-hydroxynaphthalen-1-yl)-carbamic acid phenyl ester (125 mg, 0.35 mmol) were combined and stirred at ambient temperature in DMSO (3 mL) overnight. The product was purified by directly injecting the crude reaction onto a reverse phase prep-HPLC (gradient 10–90% MeCN, 0.1% TFA). The appropriate fractions were lyophilized to yield 1-(7-hydroxynaphthalen-1-yl)-3-(3,4-dichloro-phenylmethyl)-urea (yield = 63 mg, 49%). MS (MH^+) 362.4; analytical reverse-phase HPLC (gradient 10–90% MeCN, 0.1% TFA) $t_R = 4.23$ min, 99%. ^{1}H NMR (CD_3OD) δ 4.35 (s, 2H), 7.09 (dd, 1H, $J = 8.8$, 2.4 Hz), 7.21–7.26 (m, 3H), 7.44–7.49 (m, 3H), 7.62 (d, 1H, $J = 8.2$ Hz), 7.73 (d, 1H, $J = 8.9$ Hz); HRMS: calcd for $C_{18}H_{14}N_2Cl_2O_2$: 360.0433; found 360.0432.

12. **3-(4-Chlorophenyl)-*N*-(7-hydroxynaphthalen-1-yl)-propionamide** (**6o**). Into a round-bottom flask equipped with a stir bar was added 5 mL DMF, 3-(4-chlorophenyl)propanoic acid (0.27 g, 1.2 mmol), *i*Pr_2NEt (0.22 mL, 1.26 mmol), 1-aminonapth-7-ol (0.2 g, 1.2 mmol) and lastly PyBroP (0.585 g, 1.25 mmol). The reaction was stirred overnight at room temperature. The product was purified by directly injecting the crude reaction onto a reverse phase prep-HPLC (gradient 10–90% MeCN, 0.1% TFA). The appropriate fractions were lyophilized to yield 3-(4-chlorophenyl) - *N* - (7 - hydroxynaphthalen - 1 - yl) - propionamide (yield = 150 mg, 13%). MS (MH^+) 325.8, Analytical reverse-phase HPLC (gradient 10–90% MeCN, 0.1% TFA) t_R = 3.5 min, ~96%. ^{1}H NMR (DMSO-d_6) δ 2.74 (m, 2H), 3.0 (m, 2H), 7.09 (dd, 1H, *J* = 8.8, 2.3 Hz), 7.19–7.39 (m, 7H), 7.45 (d, 1H, *J* = 7.2 Hz), 7.62 (d, 1H, *J* = 7.9 Hz), 7.76 (d, 1H, *J* = 8.9 Hz), 9.7 (s, 1H); HRMS: calcd for $C_{19}H_{16}NClO_2$: 325.0864; found 325.0869.
13. **2-(3,4-Dichlorophenyl)-*N*-(7-hydroxynaphthalen-1-yl)-acetamide** (**6p**). Synthesized in the same manner as **6o**, substituting 3,4-dichlorophenylacetic acid for 3-(4-chlorophenyl)propanoic acid. (yield = 84 mg, 39%). MS (MH^+) 347.8; analytical reverse-phase HPLC (gradient 10-90% MeCN, 0.1% TFA) t_R = 4.3 min, ~99%. ^{1}H NMR (CD_3OD) δ 3.83 (s, 2H), 7.09 (dd, 1H, *J* = 8.8, 2.4 Hz), 7.17 (d, 1H, *J* = 2.3 Hz), 7.23 (t, 1H, *J* = 8.0), 7.35–7.42 (m, 2H), 7.51 (d, 1H, *J* = 8.2 Hz), 7.61 (d, 1H, *J* = 2.0 Hz), 7.66 (d, 1H, *J* = 8.3 Hz), 7.74 (d, 1H, *J* = 8.9 Hz); HRMS: calcd for $C_{18}H_{13}NCl_2O_2$: 345.0318; found 345.0323.
14. **3,4-Dichloro-*N*-(7-hydroxynaphthalen-1-yl)-benzamide** (**6q**). Synthesized in the same manner as **6o**, substituting 3,4-dichlorobenzoic acid for-(4-chlorophenyl)propanoic acid. (yield = 6.2 mg, 3%). MS (MH^+) 333.3; analytical reverse-phase HPLC (gradient 10–90% MeCN, 0.1% TFA) t_R = 4.3 min, ~99%. ^{1}H NMR ($CD_3O\Delta$) δ 7.10 (dd, 1H, *J* = 9.0 Hz), 7.21 (s, 1H), 7.31 (t, 1H, *J* = 7.6), 7.35–7.42 (m, 2H), 7.46 (d, 1H, *J* = 7.3 Hz), 7.72–7.8 (m, 3H), 7.95 (d, 1H, *J* = 8.9 Hz), 8.24 (s, 1H); HRMS: calcd for $C_{17}H_{11}NCl_2O_2$: 331.0170; found 331.01668.

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