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N-Isoquinolin-5-yl-*N*′-aralkyl-urea and -amide antagonists of human vanilloid receptor 1

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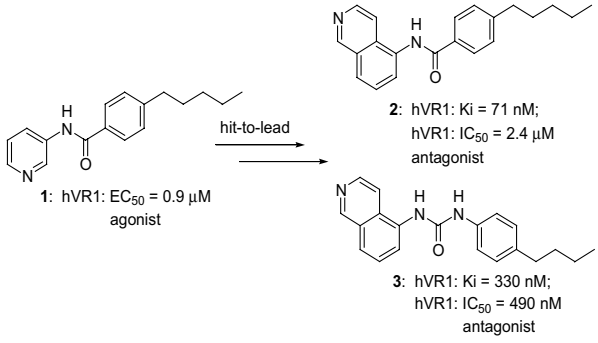
Abstract—Starting from a low micromolar agonist lead identified by high-throughput screening, series of *N*-isoquinolin-5-yl-*N*′-aralkyl ureas and analogous amides were developed as potent antagonists of human vanilloid receptor 1 (VR1). The synthesis and structure–activity relationships (SAR) of the series are described.

The discovery and cloning of vanilloid receptors has ushered in a new era of pharmaceutically based research aimed at delivering novel analgesics. Our labs[1–4] and others[5,6] have reported on various series of small molecules that modulate vanilloid receptor type 1 (VR1). Moreover, some congeners are reported to be effective in rodent models of pain. For example, a series of *N*-phenyl-4-pyridin-2-yl-piperazine carboxamides, exemplified by the potent VR1 antagonist BCTC (*N*-(4-*tert*-butyl-phenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1-(2*H*)-carboxamide), have been shown to reverse behavioral effects of acute, inflammatory, and neuropathic pain in rats.[7]

From a high throughput functional assay, employing FLIPR™ (Fluorometric Imaging Plate Reader) technology and HEK cells transfected with hVR1,[2] we identified 4-pentyl-*N*-pyridin-3-yl-benzamide (**1**) as a sub-micromolar agonist. Our decision to pursue this 'hit' arose from recognition that this molecule possessed structural features similar to the naturally occurring VR1 agonist capsaicin, from which the first VR1 antagonist, capsazepine, was elaborated. Thus, in keeping with the approach Sandoz researchers undertook in developing capsaicin SAR,[8–10] our efforts focused upon exploration of three distinct regions of our lead molecule, specifically the polar heterocyclic 'head' portion, apparently responsible for conferring functional activity toward the channel, a urea or amide linking group, and lastly, a lipophilic 'tail'. In the first regard, we observed that replacement of the 3-pyridyl group with an isoquinoline moiety resulted in a dramatic switch in functional activity, namely conversion of VR1 agonists to antagonists. Agonists stimulate calcium flux (effective concentrations (EC_{50}) values reported) whereas antagonists block capsaicin-induced flux (reported as inhibitory concentrations (IC_{50}) values). With *N*-isoquinolin-5-yl-4-pentyl-benzamide (**2**) and *N*-isoquinolin-5-yl-*N*′-4-butylphenyl-urea (**3**) serving as prototypes, we synthesized and evaluated urea and amide analogs in attempt to enhance in vitro potency.



As reported herein, these efforts led to optimized isoquinolin-5-yl-ureas and amides that possess

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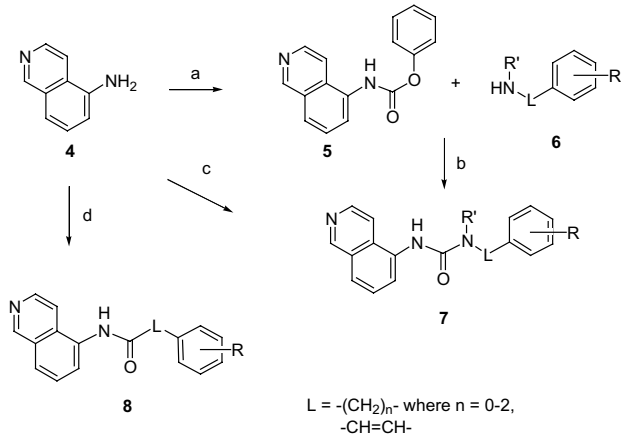
sub-nanomolar binding affinity (K_i values given) for human VR1 in a transfected cell line and behave as functional antagonists of VR1 at nanomolar concentrations, upon capsaicin challenge.

N-Isoquinolin-5-yl-*N'*-aralkyl ureas **7** were prepared in a straightforward manner, from the reaction of phenyl carbamates of 5-aminoisoquinolines with anilines, benzyl amines, or phenethylamines (**6**), using dimethylsulfoxide as solvent. The intermediate isoquinolin-5-yl-carbamic acid phenyl ester **5** was obtained via reaction of 5-aminoisoquinoline with phenylchloroformate. Separately, 5-aminoisoquinoline **4** could be directly reacted with phenethyl-, benzyl- or, aryl-isocyanates to yield ureas **7**. *N*-Isoquinolin-5-yl-aralkylamides **8** were generated from the coupling of aminoisoquinolines to benzoic, phenylacetic, or phenylpropionic acids using standard protocols. Urea products **7** could be directly isolated from the reaction by precipitation;[13–15] amide adducts **8** were obtained via extraction and purification by chromatography[16] (Scheme 1).

Isoquinolin-5-yl-derived ureas and amides **7**–**8** were assayed for binding affinity at hVR1 by measuring displacement of radiolabeled resiniferatoxin (RTX), employing a modified version of the method previously reported by Szallasi and Blumberg.[11] The compounds were also assayed for their ability to antagonize capsaicin-induced calcium flux using a Ca^{2+}-sensitive fluorescent dye and FLIPR™ technology (Molecular Devices, Inc.)[12] under conditions previously reported by our group.[1–3]

Both isoquinolin-5-yl-ureas and -amides **7**–**8** exhibited excellent binding affinity to VR1 and behaved as functional antagonists of the channel (Table 1). Overall, binding affinity (K_i values) correlated with functional inhibitory concentrations in terms of potency, within an order of magnitude, despite the use of different agonists in each assay, namely resiniferatoxin (RTX) in the case of binding and capsaicin (CAP) as challenge for functional antagonism. Furthermore, antagonists from this series shifted the capsaicin concentration dependence to the right in a parallel fashion in whole cell patch clamp experiments on endogenously expressed rat VR1 (Dubin et al., unpublished observations). Collectively, these data allow us to speculate that isoquinolin-5-yl-ureas and -amides act upon a common binding site also shared by capsaicin and resiniferatoxin. Furthermore, these small molecule hVR1 antagonists did not exhibit any overt cytotoxic effects during the course of conducting in vitro evaluations.



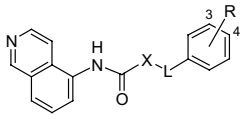
Scheme 1. (a) PhOC(O)Cl/aq $NaHCO_3/CH_2Cl_2$ (92%); (b) DMSO (45–97%); (c) Ar–L–N=C=O/CH_2Cl_2 (50–80%); (d) Ar–L–CO_2H, HBTU, or HATU, DIEA/DMF; or Ar–L–C(O)Cl, DIEA/CH_3CN (20–65%).

Optimized compounds were found to be one to two orders of magnitude more potent than capsazepine. Carbamate precursor **5** was inactive and N-methylation of the urea and amide functionality abolished activity at hVR1 (data not shown). In addition to an unmasked (free N–H) urea or amide linking group, a requirement for good in vitro potency is the proper positioning of an isoquinoline group and an aryl moiety that contains a highly lipophilic substituent at the distal end. The preferred spacing groups (L) are short alkyl moieties with benzylic versions (L = –CH_2–) being optimal as illustrated by benzylic ureas **7j** and **7i** compared to their phenyl/phenethyl homologs **7c**/**7r**, and **7b**/**7q**, respectively. This general trend carries over to highly potent propionamide congeners. Interestingly, this feature was noted in a series of 7-hydroxynaphthalen-1-yl-urea and -amide VR1 antagonists previously disclosed.[2]

With respect to aryl substituents, lipophilicity appears to be an overriding factor rather than electronics since both alkylated and halogenated congeners are exceptionally potent. Thus although the *t*-butyl benzyl and phenethyl ureas **7l** and **7s** are exquisitely potent, the halogenated analog **7k** is comparable. However, compounds with less lipophilic aryl tails, such as monosubstituted variations, are significantly less active (e.g., compare **7b** vs **7d** and **7g** vs **7k**). Moreover, the placement of nonlipophilic electron-donating aryl substituents is detrimental as evidenced by the methoxylated compounds **7e** and **7n**. However, in contrast, the trifluoromethoxylated congeners (**7f**,**o**) exhibit low nanomolar potency. A compound lacking any aryl substitution **7m** was poorly active further supporting the necessity of added lipophilicity to the distal end of the aralkyl urea in order to maximize in vitro potency. Propionamides **8a**–**d** and cinnamides **8e**–**f** followed similar trends in that the more lipophilic aryl substituents were preferred (compare **8c** vs **8a**). Collectively, the results of our studies suggest that a preliminary working pharmacophore for VR1 binding and antagonism consists simply of an isoquinolinyl-derived urea or amide coupled to a lipophilic tail. However, it is clear from our previous research,[2,4] as well as work from other laboratories,[5,6] that heterocycles and functional groups other than an isoquinoline moiety can confer antagonist properties to similarly designed small molecules.

In summary, we report upon a series of isoquinolin-5-yl-ureas and -amides that bind to human VR1 with nanomolar affinity and behave as functional antagonists of VR1, upon capsaicin challenge, with similar potency. These structurally simple molecules, recently disclosed

Table 1. Human VR1 binding affinities and functional activity of *N*-aralkyl-*N*′-isoquinolin-5-yl-derived ureas **7** and amides **8**[a]



	X	L	R	Binding affinity K_i (nM)	Functional activity IC_{50} (nM)
7a	NH	Nil	3-CF_3	19	32
7b	NH	Nil	4-CF_3	160	225
7c	NH	Nil	3,4-Di-Cl	17	170
7d	NH	Nil	3,5-Di-CF_3	1.4	32
7e	NH	–CH_2–	4-CH_3O–	193	45
7f	NH	–CH_2–	4-CF_3O–	1.3	3
7g	NH	–CH_2–	4-Cl	31	6
7h	NH	–CH_2–	3-CF_3	23	11
7i	NH	–CH_2–	4-CF_3	5	3
7j	NH	–CH_2–	3,4-Di-Cl	9	4
7k	NH	–CH_2–	4-Cl, 3-CF_3	0.8	6
7l	NH	–CH_2–	4-*t*-Bu	0.3	3
7m	NH	–$(CH_2)_2$–	(H)	1440	1000
7n	NH	–$(CH_2)_2$–	4-CH_3O–	1290	680
7o	NH	–$(CH_2)_2$–	4-CF_3O–	6	11
7p	NH	–$(CH_2)_2$–	3-CF_3	41	19
7q	NH	–$(CH_2)_2$–	4-CF_3	22	19
7r	NH	–$(CH_2)_2$–	3,4-Di-Cl	17	10
7s	NH	–$(CH_2)_2$–	4-*t*-Bu	1.3	3
8a	–$(CH_2)_2$–		4-Cl	98	74
8b	–$(CH_2)_2$–		4-CF_3	60	38
8c	–$(CH_2)_2$–		4-*t*-Amyl	4	13
8d	–$(CH_2)_2$–		4-*t*-Bu	11	410
8e	–CH=CH–		(H)	400	1500
8f	–CH=CH–		4-*t*-Bu	1.2	5
Capsazepine				120	100

in patent applications,[17,18] might serve as a template for the design of future VR1 antagonists with the goal of providing novel therapeutics for the treatment of pain in humans.

Acknowledgements

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11. [^{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. *J. Pharm. Exp. Ther*. **2002**, *300*. *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) 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 presence of compound)*100/(total binding−nonspecific binding). K_i values were obtained from Prism (GraphPad, San Diego, CA) calculated using equation of Cheng–Prusoff ($K_i = IC_{50}/(1 + [LIGAND]/K_d)$).

12. hVR1/HEK cells[3] were seeded on poly-D-lysine coated 96-well, black-walled plates (BD 354640) and 1–2 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 on line with compounds (**7** and **8**) at final concentrations ranging from 0.3 nM to 30 μM in half-log increments. Intracellular Ca^{2+} was measured for 3 min prior to the addition of CAP to all wells to achieve a final CAP concentration of 15 nM (EC_{80}). 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.
13. 1-Isoquinolin-5-yl-3-(4-trifluoromethyl-phenyl)-urea (**7b**): The 5-aminoisoquinoline (0.002 mol, 0.29 g) was dissolved in 10 mL of methylene chloride. The 4-trifluoromethyl-phenyl isocyanate (0.0022 mol, 0.41 g) was slowly added via syringe to the stirred solution. The reaction mixture was stirred at room temperature for 4 h. The resultant precipitate was collected by vacuum filtration. The collected solid was triturated with hexane (2 × 5 mL). This material was recrystallized from ethyl acetate to yield product (0.48 g, 72%; mp 188–190 °C). ^{1}H NMR (MeOH-d_4): δ 7.2 (d, H), 7.4 (t, 1H), 7.5 (d, 1H), 7.6 (t, 1H), 7.7 (d, 1H), 7.8 (s, 1H), 7.9 (d, 1H), 8.35 (d, 1H), 8.6 (d, 1H), 9.2 (s, 1H); ^{13}C NMR ($CDCl_3$, CD_3OD): δ 119, 123, 124 (2C), 125, 126, 127 (2C), 129, 130, 134, 143 (2C), 153, 154 (C═O); IR (KBr, thin film, cm^{-1}): 3253, 1605 (C═O), 1557, 1317, 1113, 1066, 840; MS (MH^+): 332.4; HRMS (m/z): MH^+ calcd for $C_{17}H_{13}F_3N_3O$, 332.101; found, 332.101.
14. 1-Isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea (**7i**): Synthesized in the same manner as (**7b**) substituting 4-trifluoromethyl-benzyl isocyanate for 4-trifluoromethyl-phenyl isocyanate (2.1 g, 60%; mp 216–218 °C). ^{1}H NMR (MeOH-d_4): δ 4.4 (s, 2H), 7.4–7.6 (m, 5H), 7.7 (d, 1H), 7.8 (d, 1H), 8.1 (d, 1H), 8.3 (d, 1H), 9.1 (s, 1H); ^{13}C NMR ($CDCl_3$, CD_3OD): δ 44 (CH_2), 115 (2C), 123 (2C), 126 (2C), 128 (2C), 130 (3C), 134, 142 (2C), 144, 153, 157 (C═O); IR (KBr, thin film, cm^{-1}): 3276, 1636 (C═O), 1568, 1327, 1111, 1068, 828; MS (MH^+): 346.1; HRMS (m/z): MH^+ calcd for $C_{18}H_{15}F_3N_3O$, 346.117; found, 346.117.
15. 1-Isoquinolin-5-yl-3-[2-(4-trifluoromethyl-phenyl)-ethyl]-urea (**7q**): Synthesized in the same manner as (**7b**) substituting 4-trifluoromethyl phenethyl isocyanate for 4-trifluoromethyl-phenyl isocyanate (0.56 g, 78%; mp 214–216 °C). ^{1}H NMR (MeOH-d_4): δ 3.0 (t, 2H), 3.6 (t, 2H), 7.5–7.6 (m, 4H), 7.65 (t, 1H), 7.75 (d, 1H), 7.85 (d, 1H), 8.2 (d, 1H), 8.5 (d, 1H), 9.2 (s, 1H); ^{13}C NMR ($CDCl_3$, CD_3OD): δ 36 (CH_2), 41 (CH_2), 115, 123 (2C), 124, 126 (3C), 128, 129 (2C), 130, 134, 142, 144, 153, 157 (C═O); IR (KBr, thin film, cm^{-1}): 3303, 1633 (C═O), 1578, 1332, 1127, 1071, 827; MS (MH^+): 360.5; HRMS (m/z): MH^+ calcd for $C_{19}H_{16}F_3N_3O$, 360.132; found, 360.120.
16. *N*-Isoquinolin-5-yl-3-(4-trifluoromethyl-phenyl)-propionamide (**8b**): 4-(Trifluoro-methyl)hydrocinnamic acid (0.0018 mol, 0.39 g) was dissolved in 25 mL CH_2Cl_2, followed by the addition of diisopropylethyl amine (0.0018 mol, 0.23 g). *O*-(7-Aza-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexa-fluorophosphate (HATU) (0.0021 mol, 0.80 g), was added and the reaction mixture was stirred at room temperature for 15 min. 5-Aminoisoquinoline (0.0015 mol, 0.22 g) was added and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was washed with 25 mL of saturated sodium bicarbonate, with 25 mL of brine, dried over magnesium sulfate, and evaporated in vacuo. The residue was chromatographed on silica gel eluting with 3% MeOH/CH_2Cl_2. The product was obtained as an orange-brown solid (0.25 g, 48%; mp 155–157 °C). ^{1}H NMR (MeOH-d_4): δ 2.9 (t, 2H), 3.2 (t, 3H), 7.5–7.65 (m, 3H), 7.7–7.8 (m, 3H), 7.9–8.0 (m, 2H), 8.5 (d, 1H), 9.2 (s, 1H); ^{13}C NMR ($CDCl_3$, CD_3OD): δ 38 (CH_2), 43 (CH_2), 116, 126 (3C), 127, 128, 129 (4C), 131, 132, 142, 145, 152, 173 (C═O); IR (KBr, thin film, cm^{-1}): 3303, 1633 (C═O), 1578, 1332, 1127, 1071, 827; MS (MH^+): 345.121; HRMS (m/z): MH^+ calcd for $C_{19}H_{16}F_3N_2O$, 344.3; found, 345.122.
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