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N-Pyridin-3-yl- and *N*-quinolin-3-yl-benzamides: Modulators of Human Vanilloid Receptor 1 (TRPV1)

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Abstract—High throughput screening of our compound library revealed a series of *N*-pyridyl-3-benzamides as low micromolar agonists of the human TRPV1 receptor. Synthesis of analogs in this series led to the discovery of a series of *N*-quinolin-3-yl-benzamides as low nanomolar antagonists of human TRPV1.

Research toward the discovery of new analgesics agents has focused on identifying ion channels specific to nociceptors and developing agents that modulate the flow of ions through these channels. Several years ago, the human vanilloid receptor type 1 (TRPV1) channel was identified isolated and cloned from afferent nociceptors.[1] TRPV1 is activated by capsaicin, the hot component of chili peppers, as well as other naturally occurring agonists, such as resiniferatoxin (RTX). The channel is also activated by painful stimuli, such as noxious heat, protons and certain endogenous lipophilic agonists, such as anadamide.[2] Since the discovery of this channel, much effort has been put forth in the identification of TRPV1 modulators as potential therapeutics.[3] Our group has reported several distinct series of novel TRPV1 antagonists[4] (see Fig. 1).

One of the more active leads identified by screening our internal compound library was 4-pentyl-*N*-pyridin-3-yl-benzamide, **1e**, a submicromolar agonist of TRPV1.

We observed several structural similarities between capsaicin and the initial hit including an alkyl chain, a central amide bond, and an aromatic group. Thus we undertook a study to investigate the structure–activity relationships (SAR) in this series.

3-Aminopyridine was coupled to 4-alkylsubstituted benzoic acids, using standard coupling conditions (HBTU and diisopropylethylamine in DMF) yielding a series of pyridylbenzamides that were evaluated in a cell-based fluorescent TRPV1 functional assay.[5] Binding affinity (K_i) was determined using an assay that measures the displacement of radiolabeled resiniferatoxin ([^{3}H]-RTX) from the human TRPV1 receptor[6] (see Table 1).

Varying the length of the alkyl chain had a modest effect on the potency of these compounds in the functional assay, the most potent agonist being the *n*-butyl derivative, **1d**. The compound that showed the best binding affinity in this series was the *n*-pentyl derivative, **1e**. The *N*-methylamide **1i** was inactive in both receptor binding and functional assays suggesting that the NH proton is involved in the interaction with the TRPV1 receptor.

In most cases, varying the group that linked the pyridine to the alkylbenzene resulted in a loss of both functional activity as well as binding affinity (Table 2). Changing the amide to an aminomethylene (**1k**) or to the reverse amide (**1l**) preserved the 2-atom linkage but resulted in a loss of binding and functional activity. Insertion of a 1-carbon linking group on either side of amide (**1m**, **1n**) also resulted in analogs with poor activity.

Keywords: TRPV1; Antagonists; Quinolinyl benzamide.
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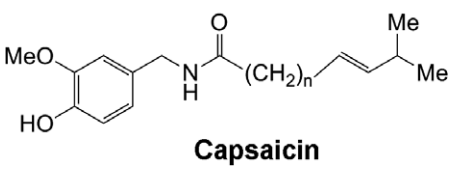
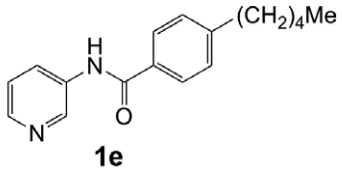


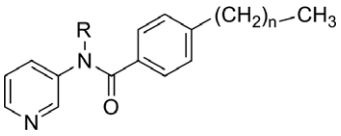
Capsaicin



1e

Figure 1. Effect of compound **6i** on CFA-induced hyperalgesia in the rat.

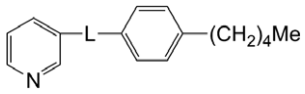
Table 1. Human TRPV1 binding affinities and functional activity of 4-alkyl-*N*-pyridin-3-yl-benzamides



Compound	*n*	R	Binding affinity K_i (nM)	Functional activity EC_{50} (μM)
1a	0	H	NT	>10.0
1b	1	H	NT	2.7
1c	2	H	NT	0.7
1d	3	H	626	0.3
1e	5	H	421	1.7
1f	6	H	1740	3.7
1g	7	H	546	2.9
1h	9	H	607	>30
1i	4	CH_3	15,000	>30

NT, not tested.

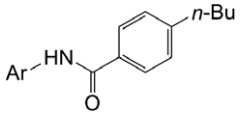
Table 2. Human TRPV1 binding affinities and functional activity of derivatives with various linking groups



Compound	L	Binding affinity K_i (nM)	Functional activity EC_{50} (μM)
1e	–NHCO–	421	1.7
1k	$–NHCH_2–$	>3000	>5.0
1l	–CONH–	>3000	>5.0
1m	$–CH_2NHCO–$	>3000	4.2
1n	$–NHCOCH_2–$	7260	>5.0

Replacement of the pyridine of compound **1d** with other aromatic groups had a profound effect on both binding and function (Table 3). A simple benzene replacement resulted in a compound, **2**, with little binding or functional activity. The naphthol, **3**, isoquinoline, **4** and quinoline, **5**, amide analogs showed improved binding potency 8- to 20-fold over **1d**. Significantly, these analogs were the first to exhibit antagonist activity. This attenuation of functional activity prompted our group to further investigate these series. Appendino et al. reported that insertion of an iodine atom in the 6′ position in the vanillyl moiety of TRPV1 agonists caused a similar shift in functional activity from agonist to antagonist.[7] The authors suggest the change in functional activity may be due to a difference in the way the 6′-iodo derivatives bind to the TRPV1 receptor. Within our own series, the explanation for the shift in functional activity is not clear.

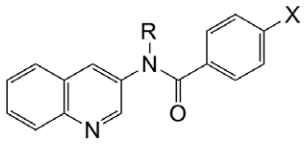
Table 3. Human TRPV1 binding affinities and functional activity of benzamide compounds



Compound	Ar	Binding affinity K_i (nM)	Functional activity IC_{50} (μM)
1d	3-Pyr	626	EC_{50} = 0.3
2	Ph	6272	>30
3	7-OH-1 Naphthyl	29	0.042
4	5-Isoquinoline	72	2.4
5	3-Quinoline	53	0.23

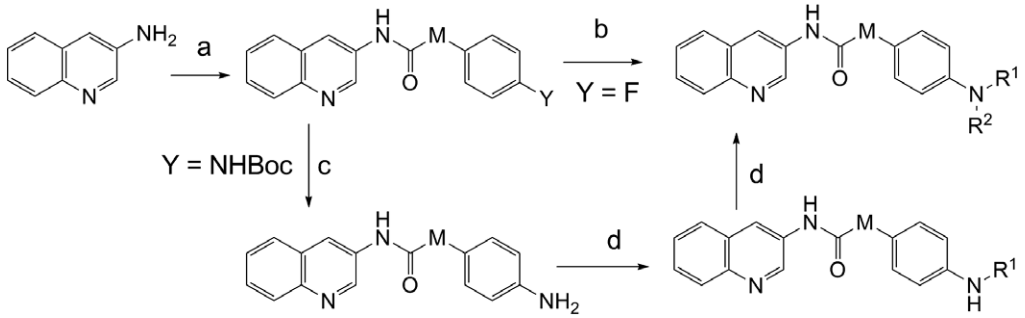
While the naphthol compound **3** showed excellent antagonist activity, the series suffered from metabolic liability due to the phenolic group and was not further developed. Modification of the terminal alkyl group in the amidoquinoline series, **5**, had a profound effect on the binding affinity and the potency in the calcium flux assay (Table 4). Increasing the chain length of the alkyl group from propyl, **5a**, to heptyl, **5d**, resulted in decreased functional potency and binding affinity. Further increases in chain length resulted in significant reduction in binding and functional activity. The bulky *tert*-butyl group imparted good binding affinity as well as good

Table 4. Human TRPV1 binding affinities and functional activity of 3-quinolinyl benzamides



Compound	R	X	Binding affinity K_i (nM)	Functional activity IC_{50} (μM)
5a	H	*n*-Propyl	60	0.13
5b	H	*n*-Hexyl	190	0.15
5c	H	*n*-Heptyl	3520	>30
5d	H	*n*-Octyl	2080	>30
5e	H	*n*-Nonyl	980	>30
5f	H	*tert*-Butyl	24	0.041
5g	Me	*n*-Propyl	NT	>30

NT, not tested.



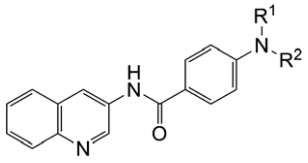
Scheme 1. Reagents and conditions: (a) HBTU, DIEA, BOC-NHPhCO$_2$H, CH$_3$CN, heat; (b) HNR^{1}R^2, DMSO, 100 °C; (c) TFA, DCM; (d) aldehyde, ketone, or paraformaldehyde, Me$_4$NBH(OAc)$_3$, DCE, heat.

antagonist potency (**5f**). As was seen previously in the pyridyl series, N-methylation of the amide, **5g**, resulted in the loss of both functional and binding activity.

In the course of this study, we determined that tertiary amines were suitable replacements for the terminal alkyl group. The quinoline *p*-benzamides were readily prepared[8] by coupling 3-aminoquinoline with Boc-protected *p*-amino benzoic acid, using HBTU coupling conditions, followed by deprotection to yield the free amine. The *N*-alkyl groups were then installed sequentially by reductive amination with a suitable carbonyl component. Alternatively, 3-aminoquinoline was coupled to *p*-fluorobenzoic acid followed by the reaction with an appropriate amine at high temperature in DMSO (Scheme 1).

As shown in Table 5, there was a significant effect of the chain length of R^1 on the binding affinity. The *n*-propyl compound **6a**, exhibited only modest binding affinity for the human TRPV1 receptor, while the longer *n*-octyl analog **6f** exhibited a 10-fold increase in binding affinity. Interestingly, this trend seems to be opposite to that seen in the simpler alkyl series (see Table 4). However, the 14-carbon chain derivative, **6h**, showed a lack of binding or functional activity. The *N*-methyl-*N*-cyclohexyl analog **6i** exhibited excellent binding and antagonist potency at the human TRPV1 receptor. Replacement of the *N*-methyl with NH (**6j**) reduced both binding and functional activity, as did the replacement of the *N*-methyl substituent with the larger *N*-propyl group (**6k**). Insertion of a methylene between the N and the cyclohexyl group gave a compound (**6l**) with a similar binding affinity but with somewhat reduced antagonist potency. During the course of this SAR study, discrepancies were sometimes seen between the functional and binding activities of the compounds. These slight differences could be attributed to the nature of the cellular-based functional assay as compared to the radioligand binding assay.[5,6]

Table 5. Human TRPV1 binding affinities and functional activity of 3-quinolinyl *p*-amino benzamides



Compound	R^1	R^2	Binding affinity K_i (nM)	Functional activity IC_{50} (μM)
6a	*n*-Propyl	Me	267	0.39
6b	*n*-Butyl	Me	83	0.066
6c	*n*-Pentyl	Me	38	0.054
6d	*n*-Hexyl	Me	70	0.086
6e	*n*-Heptyl	Me	24	0.12
6f	*n*-Octyl	Me	15	0.19
6g	*n*-Nonyl	Me	23	0.15
6h	$(CH_2)_{13}Me$	Me	1471	>30
6i	***c*-Hexyl**	**Me**	**8**	**0.022**
6j	*c*-Hexyl	H	156	10
6k	*c*-Hexyl	*p*-*n*-Propyl	85	10
6l	CH_2-*c*-hexyl	Me	16	0.17

Compound **6i** was further examined for its in vitro properties and pharmacokinetic profile. Against other activators of the human TRPV1 receptor such as low pH, anandamide, and the phorbol ester PMA, **6i** was a functional antagonist with IC_{50} values of 0.16 μM, 0.033 μM and 0.035 μM, respectively. Selectivity of compound **6i** was demonstrated when assayed at 10 μM against a panel of >50 different receptors, ion channels, and transporters (Cerep; Paris, France), exhibiting <50% inhibition at all targets tested. In the presence of rat or human liver microsomes, **6i** showed modest in vitro rat and human metabolic stability (45% and 25% remaining after 15 min, respectively). The pharmacokinetic profile of compound **6i** was studied following intravenous (iv) and oral (po) dosing in male Sprague–Dawley rats. The in vivo clearance of **6i** was moderate (20 mL/min/kg), the elimination half-life ($t_{1/2}$) was equal to 100 min and the compound had good oral bioavailability (F = 70%). Subsequently, compound **6i** was evaluated for in vivo efficacy in an established rodent model of hyperalgesia.[9] In the rat, at an oral dose of 30 mg/kg, compound **6i** produced a significant (>80%) reversal of thermal hyperalgesia induced by complete Freund's adjuvant (CFA) (Fig. 2).

In addition, **6i** was orally efficacious in a rodent model of experimental colitis.[10] These initial in vivo efficacy data gathered for compound **6i** support the continued study of the 3-quinolinyl *p*-aminobenzamide series of TRPV1 antagonists.

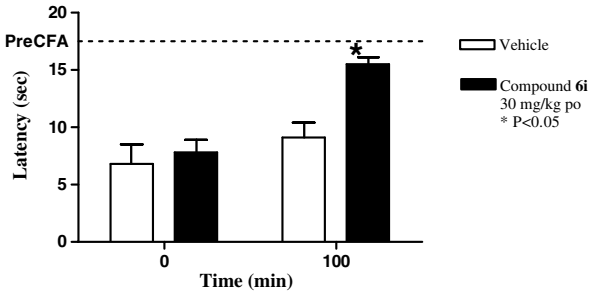


Figure 2.

In summary, a series of 3-pyridyl benzamide agonists of human TRPV1 receptor was identified through broad screening of our internal compound library. Modification of the initial HTS hit, compound **1e**, led to the discovery of novel 3-quinolinyl *p*-aminobenzamides that act as functional antagonists at the TRPV1 receptor. In particular, compound **6i** was extensively profiled in several in vitro and in vivo assays. Compound **6i** exhibited relatively high functional antagonist activity at the human TRPV1 receptor whether activated by capsaicin, low pH or phorbol ester PMA. Oral in vivo efficacy was demonstrated by **6i** in rodent models of inflammatory pain and colitis. These results suggest that further optimization of the 3-quinolinyl *p*-amino benzamide core to improve solubility, metabolic stability, and pharmacokinetic properties may lead to TRPV1 antagonists that have potential therapeutic utility for the treatment of inflammatory pain and gastrointestinal disorders.

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5. hTRPV1/HEK cells 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 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 TRPV1. 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).
6. [^{3}**H**]-*RTX binding assay using hVR1/HEK293 cell membranes.* Cloning and generation of stable cell lines expressing human TRPV1. Human TRPV1 was cloned and stably expressed in HEK293 cells (hVR1/HEK293) as described by Grant et al. in *J. Pharm. Exptl Ther.* **2002**, *300*, 9. *Preparation of membranes.* Human TRPV1/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 was 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. Non-specific 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 − non-specific binding). K_i values were obtained from Prism (GraphPad, San Diego, CA) calculated using equation of Cheng–Prusoff ($K_i = IC_{50}/(1 + [LIGAND]/K_d)$).
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8. *4-(Cyclohexyl-methyl-amino)-N-quinolin-3-yl-benzamide*. Tetramethylammonium triacetoxyborohydride (2.64 g, 0.010 mol) was added to a mixture of 4-cyclohexylamino-*N*-quinolin-3-yl-benzamide (1.58 g, 0.0046 mol) and paraformaldehyde (0.928 g, 0.032 mol) in 1,2-dichloroethane (50 mL). The resultant mixture was heated at reflux for 6 h. The solvent was evaporated in vacuo. The crude product was purified by flash chromatography eluting with a gradient of methanol (3–5%) in dichloromethane. The product was triturated with diethyl ether to give a colorless solid (1.21 g). MS (MH^+): 360; ^{1}H NMR (DMSO-d_6): δ 1.07–1.22 (m, 1H), 1.36–1.72 (m, 7H), 1.76–1.85 (m, 2H), 2.83 (s, 3H), 3.70–3.81 (m, 1H), 6.86 (d, J = 9 Hz, 2H), 7.55–7.69 (m, 2H), 7.91–7.98 (m, 4H), 8.81 (d, J = 2.2 Hz, 1H), 9.15 (d, J = 2.4 Hz, 1H) and 10.29 (s, 1H).
9. Complete Fruend's adjuvant (CFA; 100 μL emulsion of saline and heat-killed *Mycobacterium tuberculosis* in mineral oil) was injected into a single hind paw of male

Sprague–Dawley rats. Each rat was placed in a test chamber on a warm glass surface and allowed to acclimate for approximately 10 min. Response latencies to the radiant thermal stimulus were recorded for each animal prior to and 24 h following the injection of CFA. Immediately following the post-CFA latency assessment, test compound or vehicle was administered orally to the rats. Post-treatment withdrawal latencies were assessed at 100 min post-dose. Treatment effects on latencies were compared using a two-way ANOVA followed by Bonferroni post hoc test with significance set at $p < 0.05$.

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