Novel Transient Receptor Potential Vanilloid 1 Receptor Antagonists for the Treatment of Pain: Structure−Activity Relationships for Ureas with Quinoline, Isoquinoline, Quinazoline, Phthalazine, Quinoxaline, and Cinnoline Moieties

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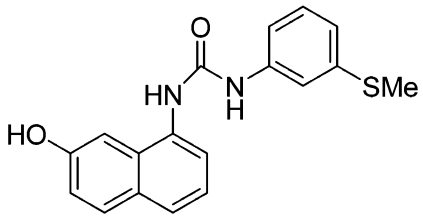
Novel transient receptor potential vanilloid 1 (TRPV1) receptor antagonists with various bicyclic heteroaromatic pharmacophores were synthesized, and their in vitro activity in blocking capsaicin activation of TRPV1 was assessed. On the basis of the contribution of these pharmacophores to the in vitro potency, they were ranked in the order of 5-isoquinoline > 8-quinoline = 8-quinazoline > 8-isoquinoline ≥ cinnoline ≈ phthalazine ≈ quinoxaline ≈ 5-quinoline. The 5-isoquinoline-containing compound **14a** (hTRPV1 IC_{50} = 4 nM) exhibited 46% oral bioavailability and in vivo activity in animal models of visceral and inflammatory pain. Pharmacokinetic and pharmacological properties of **14a** are substantial improvements over the profile of the high-throughput screening hit **1** (hTRPV1 IC_{50} = 22 nM), which was not efficacious in animal pain models and was not orally bioavailable.

Introduction

Vanilloid receptor subtype VR1 is a ligand-gated, Ca^{2+}-permeable cation channel present in peripheral sensory neurons, spinal cord, various brain regions, and nonneuronal tissues such as skin and bladder.[1] This receptor is currently referred to as TRPV1 because of its structural homology with the superfamily of transient receptor potential (TRP) channels.[2−4] Several "vanilloid" molecules such as capsaicin, the pungent ingredient of hot chili peppers, and resiniferatoxin (RTX) can activate TRPV1, causing burning pain by excitation of primary sensory neurons.[5] Other known TRPV1 activators include noxious heat and low extracellular pH,[6,7] endogenous lipid anandamide,[8,9] and other endogenous capsaicin-like substances.[10]

Interest in the TRPV1 receptor as a therapeutic target for pain management has increased since two research groups reported that mice lacking TRPV1 do not develop thermal hyperalgesia after acute inflammation.[11,12] Until recently, agonists were the major focus of the TRPV1 ligand development due to the analgesic effect resulting from the receptor desensitization.[13] However, all natural vanilloid receptor agonists, including capsaicin and RTX, cause an initial burning effect that complicates effective therapy. Attempts to make synthetic agonists with good separation between analgesic effects and the excitatory effects were not successful.[14] To avoid this persisting side effect found in TRPV1 agonists, a focused consideration has been given to competitive antagonists as novel analgesic drugs. Several of these compounds were synthesized and used as pharmacological tools for detailed investigation of TRPV1 function.[15−18] Szallasi and Appendino in their recent review article summarized the developments in this area and gave a broad assessment of the potential of TRPV1 as a new target for pain management.[19]



1 (hVR1 IC_{50} = 22 nM)

Figure 1. HTS hit **1**.

The search for our own TRPV1 antagonist started with compound **1** (Figure 1) identified by high-throughput screening (HTS) of the Abbott compound library. This 7-hydroxynaphthalene urea exhibited an IC_{50} value of 22 nM in the capsaicin-induced calcium influx assay using cells transfected with hTRPV1. Despite potent cellular activity, **1** did not exhibit in vivo activity in animal models of inflammatory pain and was not orally bioavailable. Structure−activity relationship studies from our lab (not shown) and others[20,21] on 7-hydroxynaphthalene ureas such as **1** revealed the importance of the hydroxyl group for the activity. Presumably, the hydroxyl group is engaged in a hydrogen-bonding interaction with TRPV1 to ensure effective binding. However, this hydroxyl group appears to present a serious liability for the pharmacokinetic properties of this class of compounds by providing a potential site for fast metabolism. To address this issue, we replaced the hydroxy-naphthyl group with a variety of 6,6-fused heteroaromatic moieties with one and two nitrogen

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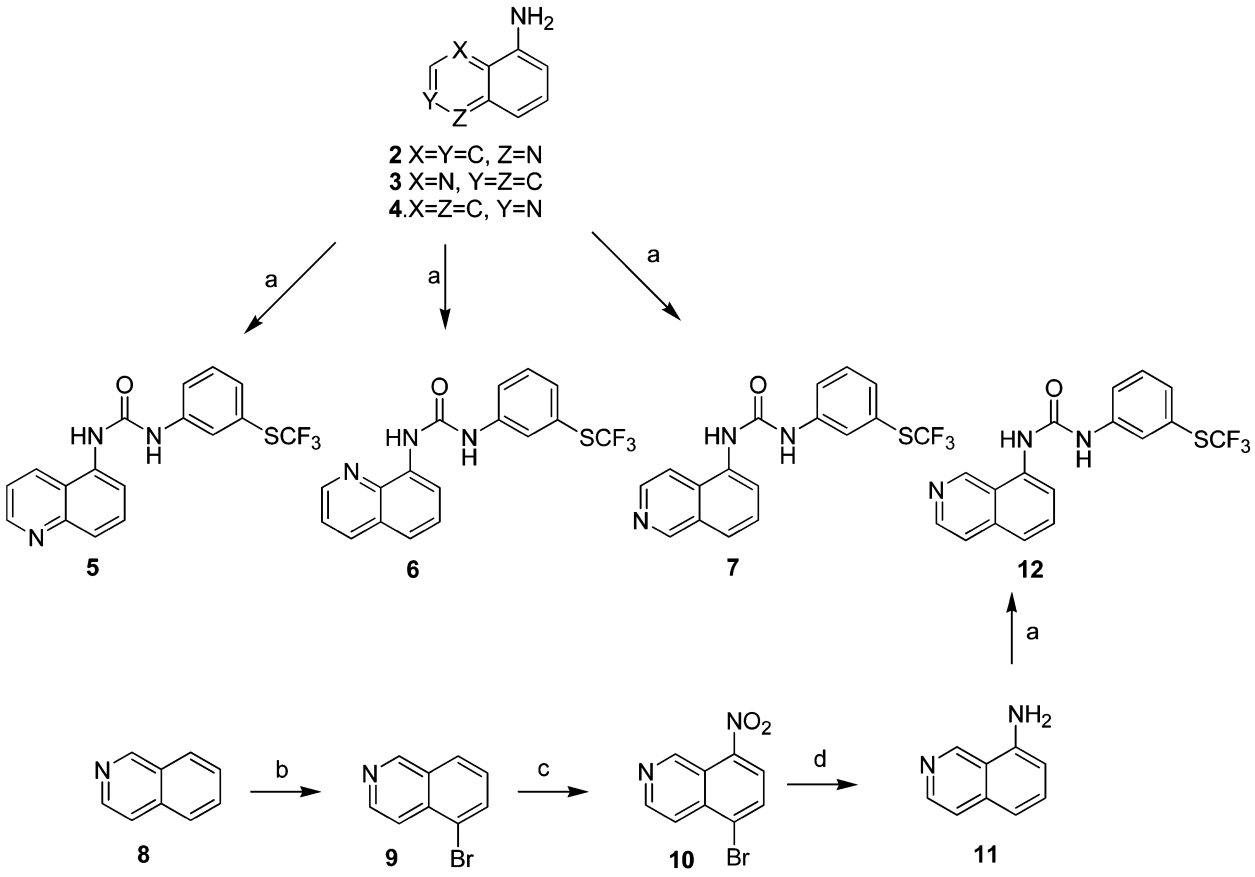
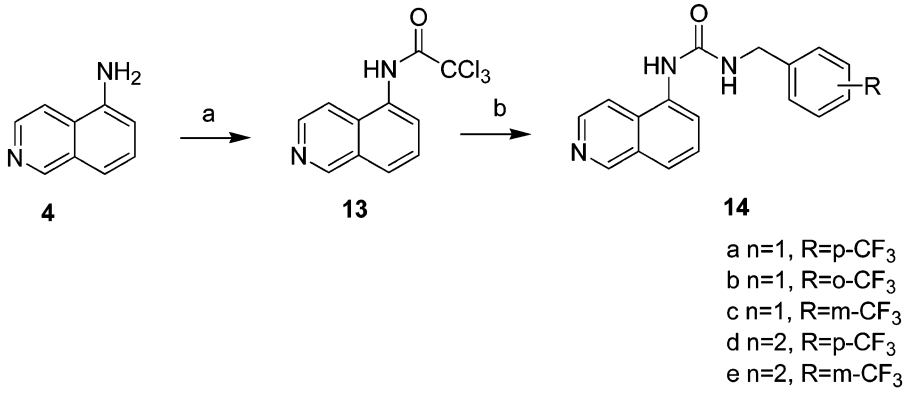
Scheme 1[a]



[a] Reagents: (a) 3-trifluoromethylthiophenyl isocyanate, toluene, rt; (b) (i) NBS, concentrated H_2SO_4, −10 °C to rt, 24 h; (ii) NH_4OH; (c) KNO_3, concentrated H_2SO_4, rt, 1 h, 46% for two steps; (d) H_2, 10% Pd/C, DMF−Et_3N, 72%.

Scheme 2[a]



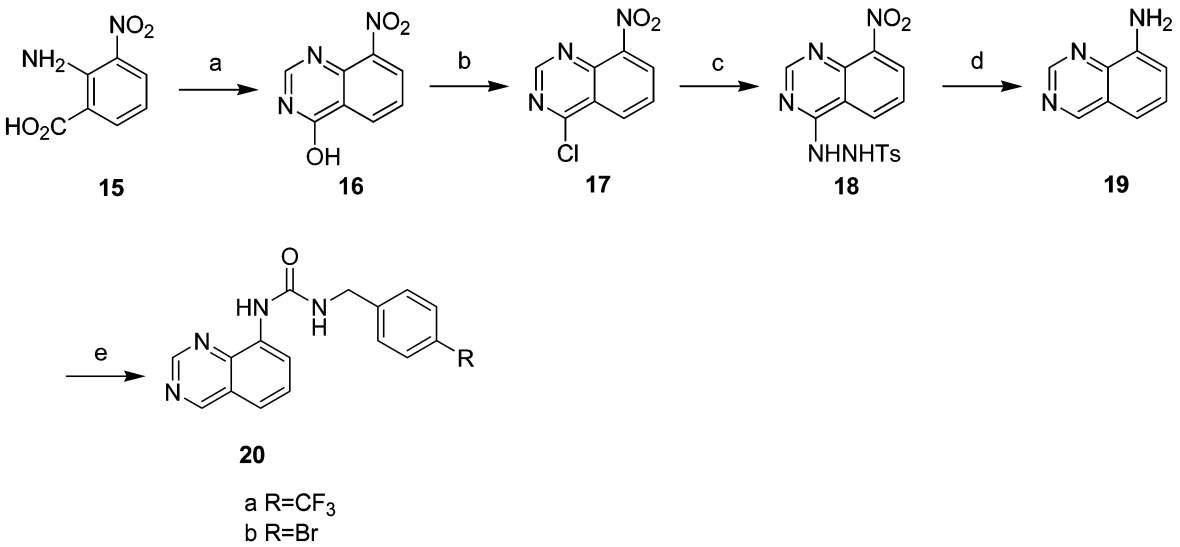
[a] Reagents: (a) Cl_3CCOCl, CH_2Cl_2, Et_3N, rt, 14 h, 65%; (b) trifluoromethylbenzylamine, MeCN, DBU, reflux, 10 h, 41%

atoms, which in several cases resulted in compounds similar to or more potent than the 7-hydroxynaphthalene lead.

Chemistry

The compounds **5**, **6**, and **7** (Scheme 1) with 5- and 8-quinoline as well as 5-isoquinoline moieties were prepared by acylation of corresponding commercially available amines **2**, **3**, and **4** with 4-(trifluoromethylthio)phenyl isocyanate. 8-Aminoisoquinoline (**11**), a starting material for the synthesis of **12**, was prepared from isoquinoline in a three-step procedure including bromination, nitration, and hydrogenation. For preparative purposes, the first two steps can be combined, which obviates the necessity for the isolation of the bromo intermediate **9**. We believe our method has practical advantages over the recently reported synthesis[22] of 8-aminoisoquinoline (**11**) that consisted of diazotization/iodination of 5-aminoisoquinoline followed by nitration and hydrogenation. When the isocyanate component for the right side fragment introduction was not readily available, the synthetic scheme was revised (Scheme 2) to make the acylating agent (isocyanate or its surrogate) from the heteroaromatic left side fragment. Although it was possible to make isocyanate from the amine **4** by reacting it with phosgene in CH_2Cl_2 in the presence of DMAP, the product decomposed after removal of the solvents, and therefore it needed to be

Scheme 3[a]



[a] Reagents: (a) formamidine acetate, $CH_3OCH_2CH_2OH$, reflux, 24 h, 91%; (b) PCl_5, $POCl_3$, $HCONH_2$, reflux, 3 h; (c) p-Me-$C_6H_4SO_2NHNH_2$, CH_2Cl_2, rt, 16 h; (d) (i) H_2, 10% Pd/C, EtOH; (ii) aq. NaOH, reflux, 3 h; (e) (i) Cl_3CCOCl, CH_2Cl_2, Et_3N, rt, 16 h; (ii) benzyl amine, DBU, MeCN, reflux, 10 h.

kept as an ether solution at low temperatures or used as soon as it was prepared. A more practical approach consisted of preparing trichloroacetamide **13** as a stable solid that reacted with a variety of amines in the acetonitrile in the presence of DBU[23] to provide target ureas such as **14a**−**e** with 40−70% yield.

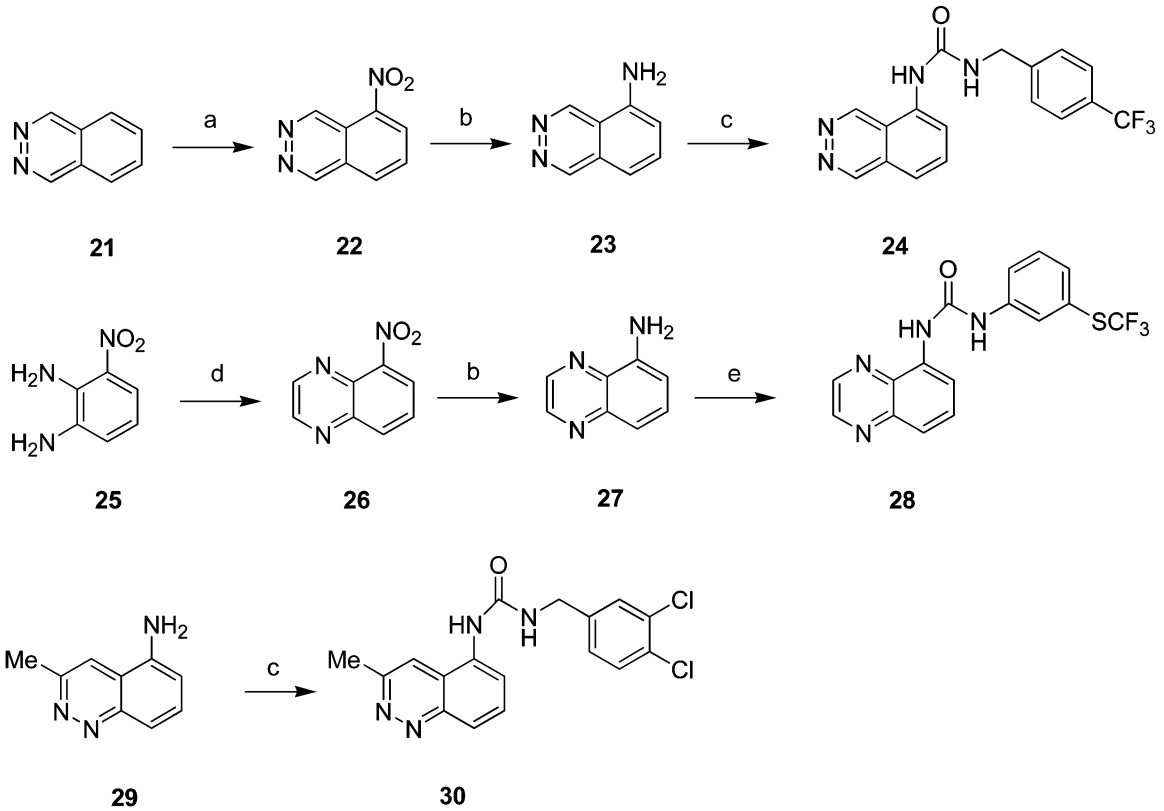
Molecules with two nitrogen-containing bicyclic fragments such as quinazoline, phthalazine, quinoxaline, and cinnoline were the next synthetic targets. The synthesis of quinazoline urea **20** is outlined in Scheme 3. Reaction between 2-amino-3-nitro-benzoic acid (**15**) and formamidine acetate furnished the quinazoline core **16** with 91% crude yield. The latter was subjected to a four-step synthetic sequence of chlorination, substitution of the chlorine with tosylhydrazine, catalytic hydrogenation, and tosylhydrazine removal to obtain 8-aminoquinazoline (**19**) in 25% yield for four steps. This synthetic sequence represents a substantially modified and improved version of the previously reported procedure.[24] Phthalazine urea **24** (Scheme 4) was synthesized in four steps from the phthalazine (**21**). Regioselective nitration afforded 5-nitro-phthalazine (**22**),[25] which was converted to amino derivative **23** by catalytic hydrogenation and further elaborated to **24** through the corresponding trichloroacetamide in the same manner as shown in Schemes 2 and 3. The key step in the synthesis of quinoxaline urea **28** was the cyclization of the 3-nitrobenzene-1,2-diamine (**25**) to the 5-nitro-quinoxaline (**26**) upon addition of glyoxal. Cinnoline urea **30** was prepared from commercially available amine **29** by the trichloroacetamide method. The trichloroacetamide method worked well also for the synthesis of carbamates **31** (Scheme 5), although yields were generally lower than those obtained for the urea formation.

Results and Discussion

The compounds were evaluated for their ability to block activation of the recombinant human TRPV1 receptor by capsaicin. The activities of the lead compounds were also measured by a Ca^{2+} influx assay using rat recombinant TRPV1 receptor. Because of the polar nature of the hydroxynaphthalene moiety in the HTS hit **1**, we considered that the atomic charge distribution on the far left side of the molecule would be important for binding. Shown in Figure 2 are the electrostatic maps for the quantum mechanically derived atomic charges for the naphthalene and isoquinoline rings of compounds **1**, **7**, and **12**, respectively. Compounds **1** and **7** are significantly more potent than compound **12** in the in vitro assay. The variation in the charge pattern of the rings in structures **1** and **7** varied from that observed for the less potent compound **12**, although an unambiguous pattern did not emerge from this limited study. To further understand the role of charge distribution, we then synthesized a variety of additional analogues with further variation of this charge pattern. Bicyclic heteroaromatic cores were prepared that contained one and two atoms on the ring originally substituted in **1** by a hydroxy group.

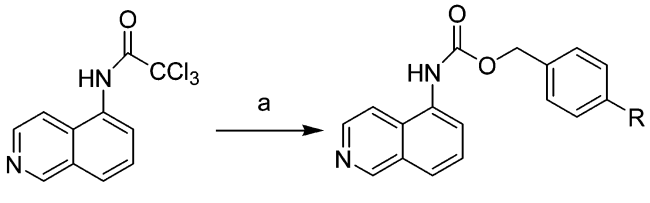
The screening of compounds with single nitrogen-containing bicyclic cores for their antagonistic activity at human TRPV1 (Table 1) revealed that the isoquinoline derivative **7** (IC_{50} = 44 nM) exhibited superior potency to the regioisomeric isoquinoline **12** (IC_{50} = 1100 nM) as well as the two compounds **5** (IC_{50} = 1500 nM) and **6** (IC_{50} = 420 nM) possessing a quinoline core. Extensive structure−activity relationship studies on the right side of compound **7** (not discussed here)[26,27] demonstrated that 3-mono-, 4-mono-, and 3,4-di-substituted benzyl groups yielded high in vitro potency. Thus, the combination of the 4-trifluoromethylbenzyl group on one side and the 5-isoquinoline moiety on the other side of the urea linker afforded the compound **14a** with inhibitory potency of 4 nM at human TRPV1 and 24 nM at rat TRPV1. Evaluation of bicyclic cores containing two nitrogen atoms was performed both to define the structural requirements for the pharmacophore and to identify possible benefits and limitations regarding their effects on physicochemical properties. The quinazoline core containing TRPV1 antagonist **20a** (IC_{50} = 42 nM) was more potent than the regioisomeric phthalazine compound **24** (IC_{50} = 175 nM) and the cinnoline

Scheme 4[a]



[a] Reagents: (a) KNO_3, concentrated H_2SO_4, 70−80 °C, 16 h; (b) H_2, 10% Pd/C, EtOH, 23 h; (c) (i) Cl_3CCOCl, CH_2Cl_2, Et_3N, rt, 16 h; (ii) benzyl amine, DBU, MeCN, reflux, 10 h; (d) glyoxal (aq. 40%), EtOH, reflux, 2 h, then rt, 16 h; (e) 3-trifluoromethylthiophenyl isocyanate, toluene, rt, 48 h, 16% for three steps.

Scheme 5[a]



a R=CF_3
b R=Me
c R=Cl
d R=Br
e R=t-Bu

[a] Reagents: (a) benzyl alcohol, DBU, MeCN, reflux, 10 h.

compound **30** (IC_{50} =189 nM). All three compounds contained benzyl groups on the right side of the molecules. Although compounds **20a** and **30** had different patterns of substitution on the benzyl group (CF_3 vs di-Cl), these substituents had similar binding affinities to the receptor. The weakest TRPV1 antagonist was the quinoxaline derivative **28** with IC_{50} = 1700 nM. However, this compound contained a phenyl group on the right side of the molecule versus benzyl groups in the other regioisomers **20a**, **24**, and **30**. Because benzyl groups provided about a 10-fold increase in in vitro activity compared with phenyl groups (e.g., compare compound **7** and **14a**), we considered the quinoxaline core to be quite similar to the phthalazine and cinnoline cores with regard to their impact on TRPV1 potency. Although the in vitro potency of these three pharmacophores was inferior to the quinazoline pharmacophore, the extremely low aqueous solubility of quinazoline-containing compound **20a** prevented a more elaborate investigation of this class of compounds.

Thus, on the basis of in vitro potency, the 5-isoquinolinyl group was chosen as the best core among the eight bicyclic heteroaromatics evaluated. As an example, compound **14a** with an isoquinoline fragment exhibited 10-fold greater potency than its closest competitor, the quinazoline derivative **20a** (4 nM vs 42 nM). Schild analysis using the calcium influx assay demonstrated that compound **14a** was a competitive antagonist of capsaicin at the human TRPV1 receptor because it shifted the concentration−effect curve of capsaicin to the right in a parallel fashion without reducing the maximum response. Some of the TRPV1 antagonists described in this paper were tested for selectivity. In studies performed by CEREP, compound **14a** was found to be greater than 2500-fold selective for TRPV1 compared with a diverse array of neurotransmitter receptors, peptide receptors, ion channels, reuptake sites, and enzymes, although did show weak micromolar binding to the BZD (peripheral) and 5-HT_{2C} receptors.

The pharmacokinetic profile of **14a** in rats was characterized by low plasma clearance (CLp = 0.6 L/(h kg)), reasonable oral bioavailability (F = 46%), but a low volume of distribution (V_β = 0.6 L/kg) and a short plasma elimination half-life ($T_{1/2}$ = 0.6 h, iv). As shown in Table 2, compound **14a** was active in the acetic acid induced visceral pain model showing 30% effect at the dose of 100 μmol/kg (po). Moreover, compound **14a**

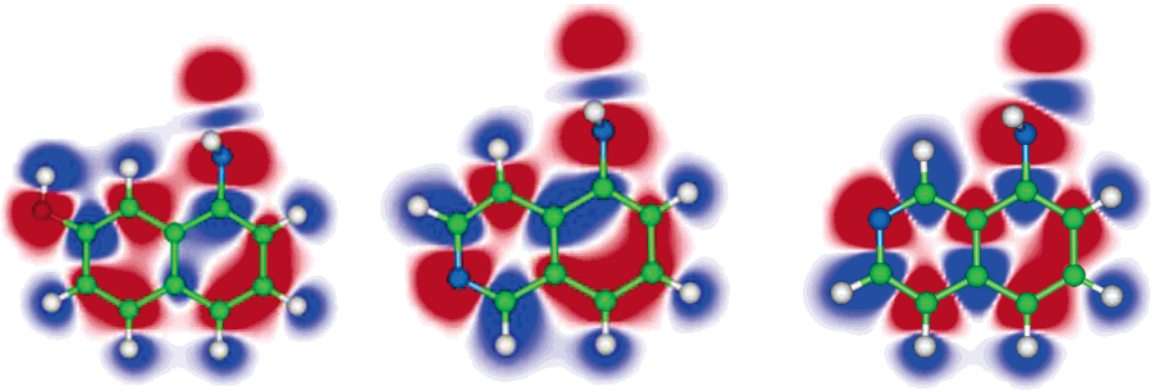
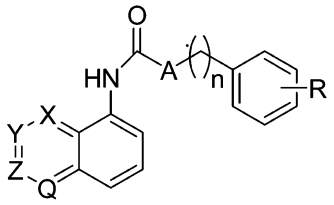


Figure 2. Electrostatic map showing the atomic charges in the naphthalene or isoquinoline rings of VR1 antagonists **1**, **7**, and **12**, respectively. The attached urea appendages of these compounds were included in the calculations but are not shown in these pictures. Atom charges were calculated by fitting to the electrostatic potential of the HF/6-31G* wave function. The electrostatic field using these point charges was calculated using the DelPhi protocol. The map corresponds to the plane of the aromatic system. Blue = positive charge, Red = negative charge.

Table 1. In Vitro Biological Activity of VR1 Antagonists in Human VR1 Ca^{2+} Influx Assay[a]



compd	X	Y	Z	Q	A	*n*	R	IC_{50} (nM)
5	CH	CH	CH	N	NH	0	3-SCF3	1500 ± 400
6	N	CH	CH	CH	NH	0	3-SCF3	420 ± 40
7	CH	CH	N	CH	NH	0	3-SCF3	44 ± 18
12	CH	N	CH	CH	NH	0	3-SCF3	1100 ± 150
14a	CH	CH	N	CH	NH	1	4-CF3	4 ± 3
14b	CH	CH	N	CH	NH	1	2-CF3	87 ± 25
14c	CH	CH	N	CH	NH	1	3-CF3	51 ± 2
14d	CH	CH	N	CH	NH	2	4-CF3	19 ± 6
14e	CH	CH	N	CH	NH	2	3-CF3	20 ± 5
20a	N	CH	N	CH	NH	1	4-CF3	42 ± 6
20b	N	CH	N	CH	NH	1	4-Br	170 ± 60
24	CH	N	N	CH	NH	1	4-CF3	175 ± 40
28	N	CH	CH	N	NH	0	3-SCF3	1700 ± 350
30[b]	CH	CH	N	N	NH	1	3,4-di-Cl	189 ± 25
31a	CH	CH	N	CH	O	1	4-CF3	37 ± 12
31b	CH	CH	N	CH	O	1	4-Me	313 ± 70
31c	CH	CH	N	CH	O	1	4-Cl	110 ± 5
31d	CH	CH	N	CH	O	1	4-Br	318 ± 110
31e	CH	CH	N	CH	O	1	4-t-Bu	223 ± 70

[a] All values are the mean ± SEM of at least three separate observations run in triplicate. [b] Compound contains methyl group at 3-position.

reduced thermal hyperalgesia in the complete Freund's adjuvant (CFA) model of chronic inflammatory pain with an ED_{50} value of 30 μmol/kg (po).

Attempts to further modulate properties of isoquinoline analogues by replacing the urea linker with carbamate led to the generally less potent and less stable compounds **31a**−**e**.

Conclusion

To identify TRPV1 antagonists with in vivo activity in animal pain models, the hydroxynaphthyl fragment of the high-throughput screening hit **1** (hTRPV1 IC_{50} = 22 nM) was replaced with a variety of nitrogen-containing bicyclic heteroaromatics with the goal of discovering compounds with at least similar in vitro potency and improved pharmacokinetic and physicochemical properties. On the basis of the experimental hTRPV1 IC_{50} values and some theoretical correlations, the strength of the pharmacophores was ranked in the order of 5-isoquinoline > 8-quinoline = 8-quinazoline > 8-isoquinoline ≥ cinnoline ≈ phthalazine ≈ quinoxaline ≈ 5-quinoline. In particular, compound **14a** with a 5-isoquinoline core displayed IC_{50} values of 4 and 24 nM in blocking capsaicin activation of human and rat TRPV1, respectively. Due to the better pharmacokinetic properties and higher aqueous solubility, compound **14a** was active in animal models of visceral pain and chronic inflammatory pain. These data represent an encouraging foundation for the discovery of TRPV1 antagonists as novel analgesics.

Table 2. In Vitro Potency, in Vivo Activity,[a] and Pharmacokinetic Profile[b] of TRPV1 Antagonist **14**a

human TRPV1 IC_{50} (nM)	4 ± 3
rat TRPV1 IC_{50} (nM)	24 ± 9
chronic inflammatory pain (CFA; thermal hyperalgesia) ED_{50} μmol/kg	30 (po)
visceral pain (ACA)	30% @ 100 μmol/kg (po)
iv V_β (L/kg)	0.6
Clp (L/(h kg))	0.61
$T_{1/2}$ (h)	0.6
ip C_{max} (μg/mL)	3.0
F (%)	83
po C_{max} (μg/mL)	1.6
F (%)	46
plasma protein binding in rat (%)	97.1
plasma protein binding in human (%)	98.0

[a] The data represent mean ± SEM; n = 6 per dose group. [b] Pharmacokinetic parameters determined in rats following administration of 10 μmol/kg.

Experimental Section

^{1}H NMR spectra were obtained at 300 and 400 MHz using tetramethylsilane as an internal standard. The mass spectra (electron spray ionization (ESI) and dissolvable chemical ionization (DCI)) and high-resolution mass spectra were recorded on a Finnigin-400 instrument. Elemental combustion analyses were within ±0.4% of theoretical values and obtained from Robertson Microlit Laboratories. Chromatographic separations were performed on silica gel 60 (230−400 mesh).

1-Quinolin-5-yl-3-(3-trifluoromethylsulfanyl-phenyl)-urea (5). A solution of 5-aminoquinoline (**2**) (0.25 g, 1.67

mmol) and 3-trifluoromethylthiophenyl isocyanate (0.37 g, 1.67 mmol) in toluene (30 mL) was stirred for 3 h at ambient temperature. A precipitate collected by filtration was washed with toluene and dried under vacuum to give 0.39 g (64%) of the desired product **5**. ^{1}H NMR (DMSO-d_6): δ 9.35 (s, 1 H), 8.97 (m, 2H), 8.50 (d, J = 7.5 Hz, 1H), 8.00 (m, 2H), 7.80−7.60 (m, 4H), 7.50 (t, J = 7.5 Hz, 1H), 7.34 (d, J = 7.5 Hz, 1H). MS (ESI+) *m/e*: 364 (M + H)$^+$. Anal. Calcd ($C_{17}H_{12}F_3N_3$-OS): C, H, N.

1-Quinoline-8-yl-3-(3-trifluoromethylsulfanyl-phenyl)-urea (6). This compound was prepared from 8-aminoquinoline (**3**) using the procedure described for the synthesis of **5** (yield 66%). ^{1}NMR (DMSO-d_6): δ 10.18 (s, 1 H), 9.77 (s, 1 H), 8.96 (m, 1 H), 8.59 (m, 1H), 8.40 (dd, J = 1.0 and 7.5 Hz, 1H), 8.08 (s, 1H), 7.61 (m, 4H), 7.48 (t, J = 7.5 Hz, 1 H), 7.32 (d, J = 7.5 Hz, 1H). MS (ESI+) *m/e*: 364 (M + H)$^+$. Anal. Calcd ($C_{17}H_{12}F_3N_3OS$): C, H, N.

1-Isoquinoline-5-yl-3-(3-trifluoromethylsulfanyl-phenyl)-urea (7). This compound was prepared from 5-aminoisoquinoline (**4**) using the procedure described for the synthesis of **5** (yield 53%). ^{1}H NMR (DMSO-d_6): δ 9.35 (s, 1H), 9.32 (s, 1H), 8.96 (s, 1H), 8.59 (d, J = 6.0 Hz, 1H), 8.25 (d, J = 7.0 Hz, 1H), 8.00−8.08 (m, 1 H), 7.95 (d, J = 6.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.67 (t, J = 8.0 Hz, 1H), 7.56−7.63 (m, 1 H), 7.49 (t, J = 8.0 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H). MS (ESI+) *m/e*: 364 (M + H)$^+$. Anal. Calcd ($C_{17}H_{12}F_3N_3OS$): C, H, N.

5-Bromo-8-nitroisoquinoline (10). To a −10 °C solution of isoquinoline (**8**) (11 g, 85 mmol) in concentrated H_2SO_4 (100 mL) was added *N*-bromosuccinimide (17.8 g, 100 mmol) by portions. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. A 10 mL aliquot of the reaction mixture was removed, poured onto ice, neutralized with concentrated ammonium hydroxide (∼30 mL), and extracted with ether. The combined ether layers were dried over $MgSO_4$, and the solvent was removed under vacuum. This yielded 1.51 g of 5-bromoisoquinoline (**9**), contaminated with <10% of 5,8-dibromoisoquinoline. Potassium nitrate (10.1 g, 100 mmol) was added to the remaining reaction mixture. After 1 h, the reaction mixture was poured onto ice and neutralized with concentrated NH_4OH (∼300 mL). The crude product was collected by filtration and allowed to dry. Recrystalization from methanol gave 5-bromo-8-nitroisoquinoline (**10**) (8.83 g, 46%). ^{1}H NMR (DMSO-d_6): δ 9.81 (broad s, 1H), 8.87 (d, J = 6.0 Hz, 1H), 8.38 (m, 2H), 8.17 (dd, J = 1.0 and 6.0 Hz, 1H). MS (DCI/NH_3) *m/e*: 253 (M + H)$^+$, Br pattern observed.

8-Aminoisoquinoline (11). A mixture of 5-bromo-8-nitroisoquinoline (**10**) (8.83 g, 34.9 mmol) and 10% Pd/C (600 mg) in DMF (200 mL) and Et_3N (5.3 mL) was hydrogenated at 60 psi for 1 h. The catalyst was removed by filtration, and the solvent was evaporated under vacuum. The residue was taken into water, the aqueous solution was extracted with ether, and the combined organic extracts were dried over $MgSO_4$. The ether was removed under vacuum to give 8-aminoisoquinone (**11**) (3.6 g, 72%). ^{1}H NMR (DMSO-d_6): δ 9.45 (s, 1H), 8.34 (d, J = 6.0 Hz, 1H), 7.56 (d, J = 6.0 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 7.01 (d, J = 7.5 Hz, 1H), 6.75 (d, J = 7.5 Hz, 1H), 6.20 (broad s, 2H). MS (DCI/NH_3) *m/e*: 145 (M + H)$^+$.

1-Isoquinolin-8-yl-3-(3-trifluoromethylsulfanyl-phenyl)-urea (12). This compound was prepared from 8-aminoisoquinoline (**11**) using the procedure described for the synthesis of **5** (yield 48%). ^{1}H NMR (DMSO-d_6): δ 9.53 (s, 1H), 9.35 (s, 1H), 9.20 (s, 1H), 8.52 (d, J = 6.0 Hz, 1H), 8.12 (m, 1H), 8.05 (m, 1 H), 7.83 (d, J = 6.0 Hz, 1H), 7.78 (t, J = 7.5 Hz, 1H), 7.70−7.58 (m, 2H), 7.50 (t, J = 7.5 Hz, 1H), 7.35 (d, J = 7.5 Hz, 1H). MS (ESI+) *m/e*: 364 (M + H)$^+$. Anal. Calcd ($C_{17}H_{12}F_3N_3OS$): C, H, N.

2,2,2-Trichloro-*N*-isoquinolin-5-yl-acetamide (13) (Scheme 1). To a +5 °C solution of 5-aminoisoquinoline (**4**) (1.0 g, 6.9 mmol) in dichloromethane (40 mL) and Et_3N (1 mL) was added dropwise trichloroacetyl chloride (1.38 g, 7.6 mmol). The reaction mixture was stirred at ambient temperature for 14 h, concentrated, and diluted with ethyl acetate. The solution was washed with 1 N HCl, the aqueous layer was separated, treated with aqueous $NaHCO_3$, and extracted with ethyl acetate. Combined organic phases were washed with water, separated, and concentrated. The solid residue was suspended in ethyl acetate (5 mL), and the mixture was filtered to obtain pure **13** (1.3 g, 65%) as a tan solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.20 (broad s, 1H), 9.41, (s, 1H), 8.60 (d, J = 6.0 Hz, 1H), 8.18 (m, 1H), 7.77 (m, 2H), 7.66 (d, J = 6.0 Hz, 1H). MS (DCI/NH_3) *m/e*: 289 (M + H)$^+$.

General Procedure for the Preparation of Ureas from Corresponding Trichloroacetamides: *N*-Isoquinolin-5-yl-*N*′-(4-trifluoromethylbenzyl)-urea (14a). A solution of 2,2,2-trichloro-*N*-isoquinolin-5-yl-acetamide (**13**) (10.0 g, 34.7 mmol), 4-trifluoromethylbenzylamine (6.39 g, 36.6 mmol), and DBU (13.0 mL, 86.75 mmol) in MeCN (250 mL) was refluxed for 10 h. The mixture was cooled, concentrated, diluted with ethyl acetate, and washed twice with aqueous ammonium chloride. The organic layer was separated and concentrated under vacuum The solid obtained was suspended in ethyl acetate and filtered to obtain 4.9 g (41%) of **14a** as a tan solid.^{1}H NMR (300 MHz, DMSO-d_6): δ 9.26 (s, 1H), 8.82 (s, 1H), 8.52 (d, J = 6.0 Hz, 1H), 8.26 (d, J = 7.5 Hz, 1H), 7.94 (d, J = 6.0 Hz, 1H), 7.71 (m, 3H), 7.58 (m, 3H), 7.20 (t, J = 6.0 Hz, 1H), 4.48 (d, J = 6.0 Hz, 2H). MS (DCI/NH_3) *m/e*: 346 (M + H)$^+$. Anal. Calcd ($C_{18}H_{14}F_3N_3O$·0.05H_2O): C, H, N.

***N*-Isoquinolin-5-yl-*N*′-[2-(trifluoromethyl)benzyl]-urea (14b).** To CH_2Cl_2 (300 mL) at 0 °C were added phosgene (20 mL, 20% solution in toluene) and solution of DMAP (10.0 g, 82 mmol) in CH_2Cl_2 (50 mL) dropwise. To the resulting milky suspension was added dropwise a solution of 5-aminoisoquinoline (**4**) (5.0 g, 34.9 mmol) in CH_2Cl_2 (100 mL). The mixture was allowed to warm to room temperature and then stirred for 16 h. The solvent was removed under reduced pressure. The solid residue was extracted with diethyl ether (400 mL). The diethyl ether solution of the resulting isocyanate was filtered, and a 20 mL aliquot (∼1.75 mmol) was used directly for the next step. This solution was added to a solution of 2-(2-trifluoromethyl-phenyl)-ethylamine (0.26 g, 1.5 mmol) in diethyl ether (10 mL), the mixture was stirred for 2 h and filtered, and the filter cake was washed with diethyl ether to provide the title compound as an off-white solid (0.22 g, 42%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.29 (s, 1H), 8.88 (s, 1H), 8.58 (d, J = 6.0 Hz, 1H), 8.30 (d, J = 7.5 Hz, 1H), 7.99 (d, J = 6.0 Hz, 1H), 7.80−7.53 (m, 5H), 7.50 (t, J = 7.5 Hz, 1H), 7.18 (t, J = 4.5 Hz, 1H), 4.58 (d, J = 6.0 Hz, 2H). MS(ESI): *m/e*: 346 (M + H)$^+$.

***N*-Isoquinolin-5-yl-*N*′-[3-(trifluoromethyl)benzyl]-urea (14c).** This compound was synthesized from **13** and 3-(trifluoromethyl)benzylamine according to the procedure described for the synthesis of **14a** (32%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.27 (s, 1H), 8.82 (broad s, 1H), 8.53 (d, J = 6.0 Hz, 1H), 8.25 (m, 1H), 7.94 (d, J = 6.0 Hz, 1H), 7.55−7.79 (m, 6H), 7.18 (t, J = 6.0 Hz, 1H), 4.47 (d, J = 6.0 Hz, 2H); MS (ESI−) *m/e*: 344 (M − H)$^-$. Anal. Calcd ($C_{18}H_{14}F_3N_3O$): C, H, N.

***N*-Isoquinolin-5-yl-*N*′-[2-(4-trifluoromethyl-phenyl)-ethyl]-urea (14d).** This compound was synthesized from **13** and 2-[4-(trifluoromethyl)phenyl]ethylamine according to the procedure described for the synthesis of **14a** (yield 48%). ^{1}H NMR (DMSO-d_6): δ 9.26 (s, 1H), 8.64 (s, 1H), 8.51 (d, J = 6.0 Hz, 1H), 8.23 (d, J = 7.5 Hz, 1H), 7.87 (d, J = 6.0 Hz, 1H), 7.73 (d, J = 7.5 Hz, 1H), 7.64 (s, 1H), 7.59 (m, 4H), 6.62 (t, J = 4.5 Hz, 1H), 3.46 (m, 2H), 2.91 (t, J = 6.0 Hz, 2H). MS (ESI+) *m/e*: 360 (M + H)$^+$. Anal. Calcd ($C_{19}H_{16}F_3N_3O$): C, H, N.

***N*-Isoquinolin-5-yl-*N*′-[2-(3-trifluoromethyl-phenyl)-ethyl]-urea (14e).** This compound was synthesized from **13** and 2-[3-(trifluoromethyl)phenyl]ethylamine according to the procedure described for the synthesis of **14a** (yield 62%). ^{1}H NMR (DMSO-d_6): δ 9.26 (s, 1H), 8.62 (s, 1H), 8.52 (d, J = 6.0 Hz, 1H), 8.22 (d, J = 7.5 Hz, 1H), 7.87 (d, J = 6.0 Hz, 1H), 7.73 (d, J = 7.5 Hz, 1H), 7.64 (s, 1H), 7.59 (m, 4H), 6.60 (t, J = 4.5 Hz, 1H), 3.45 (m, 2H), 2.91 (t, J = 6.0 Hz, 2H). MS (ESI+) *m/e*: 360 (M + H)$^+$.

8-Nitro-quinazolin-4-ol (16) (Scheme 2). A solution of 2-amino-3-nitro-benzoic acid (**15**) (5.12 g, 28 mmol) in meth-

oxyethanol (100 mL) was treated with formamidine acetate (13 g, 125 mmol) under reflux for 17 h. A second portion of formamidine acetate (13 g, 125 mmol) was then added, and the reflux was continued for 7 more hours. The mixture was cooled, and the solvent was removed under vacuum. The residue was taken in saturated $NaHCO_3$ and extracted with ethyl acetate. The organic layers were combined, washed with saturated $NaHCO_3$, and dried with magnesium sulfate, and the solvent was removed under vacuum to give desired **16** (4.84 g, 91%), which was used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.70 (broad s, 1H), 8.35 (dd, J = 1.5 and 8 Hz, 1H), 8.28 (dd, J = 1.5 and 8 Hz, 1H), 8.25 (s, 1H), 7.67 (m, 1H). MS (ESI) *m/e*: 192 $(M + H)^+$

4-Chloro-8-nitro-quinazoline (17). To a solution of 8-nitro-quinazolin-4-ol (**16**) (3.56 g, 18.6 mmol) in phosphorus oxychloride (17 mL) and formamide (40 μL) was added phosphorus pentachloride (4.37 g, 21 mmol), and the reaction was heated to reflux for 3 h, cooled, diluted with ethyl ether, and filtered to give 2.50 g of 4-chloro-8-nitro-quinazoline, which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.21 (s, 1H), 8.55 (dd, J = 1.5 and 8.5 Hz, 1H), 8.35 (dd, J = 1.5 and 7.5 Hz, 1H), 7.86 (m, 1H). MS (DCI/NH_3) *m/e*: 210 $(M + H)^+$. Cl pattern observed.

***N*-(8-Nitro-quinazolin-5-yl)-*N*′-*p*-tolyl-hydrazine (18).** 4-Chloro-8-nitro-quinazoline (**17**) (2.60 g, 12.4 mmol) and *p*-toluenesulfonhydrazide (2.31 g, 12.4 mmol) were suspended in 60 mL of dichloromethane, and the reaction mixture was stirred at ambient temperature for 16 h. The solvent was removed under vacuum to give 5.00 g of the desired product, which was used without further purification.

Quinazolin-8-ylamine (19). A solution of crude quinazoline derivative **18** (5.00 g, 13.9 mmol) in ethanol (60 mL) was subjected to hydrogenation at atmospheric pressure over 10% palladium on carbon (100 mg) at ambient temperature for 24 h, at which time additional solvent and catalyst were added, and the reaction continued another 17 h. The reaction was then filtered to remove the catalyst. Sodium hydroxide (150 mL of 1 N solution) was then added to the filtrate, which was heated to reflux for 3 h. The reaction mixture was cooled, and the solvent was removed under vacuum. The residue was diluted with water and extracted with ethyl ether. The organic layers were combined, dried over $MgSO_4$, and evaporated under vacuum to give desired **19** (0.69 g, 25% for four steps from **16**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.40 (s, 1H), 9.14 (s, 1H), 7.45 (m, 1H), 7.20 (dd, J = 1.5 and 8 Hz, 1H), 7.07 (dd, J = 1.5 and 7.5 Hz, 1H,), 6.07 (broad s, 2H). MS (DCI/NH_3) *m/e*: 146 $(M + H)^+$.

1-Quinazolin-8-yl-3-(4-trifluoromethyl-benzyl)-urea (20a). This compound was synthesized from quinazolin-8-ylamine (**19**) in two steps by using 4-trifluoromethylbenzylamine and the trichloroacetamide procedure described for the synthesis of **14a** from **4**. ^{1}H NMR (DMSO-d_6): δ 9.58 (s, 1H), 9.44 (s, 1H), 9.32 (s, 1H), 8.71 (dd, J = 4.5 and 6.0 Hz, 1H), 8.02 (t, J = 4.5 Hz, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.65 (m, 2H), 7.55 (d, J = 7.5 Hz, 2H), 4.46 (d, J = 6.0 Hz, 2H). MS *m/e*: 347 $(M + H)^+$. Anal. Calcd ($C_{17}H_{13}F_3N_4O \cdot C_2H_5OH$): C, H, N.

1-(4-Bromo-benzyl)-3-quinazolin-8-yl-urea (20b). This compound was synthesized from quinazolin-8-ylamine (**19**) in two steps by using 4-bromobenzylamine and the trichloroacetamide procedure described for the synthesis of **14a** from **4**. ^{1}H NMR (DMSO-d_6): δ 9.58 (s, 1H), 9.40 (s, 1H), 9.31 (s, 1H), 8.72 (m 1H), 7.95 (t, J = 6.0 Hz, 1H), 7.67 (m, 2H), 7.54 (d, J = 7.5 Hz, 2H), 7.30 (d, J = 7.5 Hz, 2H), 4.34 (d, J = 6.0 Hz, 2H). MS *m/e*: 357$(M + H)^+$. Br pattern observed. Anal. Calcd ($C_{16}H_{13}BrN_4O \cdot 0.8C_2H_5OH \cdot 0.8H_2O \cdot 2.4HCl$): C, H, N.

5-Nitro-phthalazine (22) (Scheme 3). To a solution of phthalazine (**21**) (5.00 g, 38.4 mmol) in concentrated H_2SO_4 (35 mL) was added potassium nitrate (18.60 g, 184 mmol). The reaction mixture was heated to 70−80 °C for 16 h, cooled, and poured onto ice. The mixture was then neutralized with concentrated ammonium hydroxide and extracted with ethyl acetate. The organic layers were combined and dried over $MgSO_4$, and the solvent was removed under vacuum. The residue was titurated with ethyl acetate and filtered to give 2.37 g of 5-nitro-phthalazine, which was used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.19 (s, 1H), 9.91 (s, 1H), 8.83 (d, J = 8.0 Hz, 1H,), 8.62 (d, J = 8.0 Hz, 1H), 8.25 (m, 1H). MS (DCI/NH_3) *m/e*: 176 $(M + H)^+$.

5-Amino-phthalazine (23). A mixture of 5-nitro-phthalazine (**22**) (1.05 g, 6 mmol) and 10% Pd/C (100 mg) in EtOH (50 mL) was hydrogenated at 1 atm for 23 h. The resulting mixture was filtered through Celite, and the solvent was removed under vacuum to give 0.97 g of crude 5-amino-phthalazine (**23**), which was used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.68 (s, 1H), 9.62 (s, 1H), 9.51 (s, 1H), 8.87 (s, 1H), 7.84 (m, 1H), 7.47 (d, J = 7.0 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H). MS (DCI/NH_3) *m/e*: 146 $(M + H)^+$.

1-Phthalazin-5-yl-3-(4-trifluoromethyl-benzyl)-urea Hydrochloride (24). This compound was synthesized from 5-amino-phthalazine (**23**) in two steps by using 4-trifluoromethylbenzylamine and the trichloroacetamide procedure described for the synthesis of **14a** from **4**. ^{1}H NMR (DMSO-d_6): δ 10.32 (s, 1H), 10.03 (s, 1H), 9.82 (s, 1H), 8.69 (d, J = 7.5 Hz, 1H), 8.13 (t, J = 7.5 Hz, 1H), 7.89 (d, J = 7.5 Hz, 1H), 7.72 (m, 3H), 7.58 (d, J = 7.5 Hz, 2H), 4.50 (d, J = 4.5 Hz, 2H). MS (ESI+) *m/e*: 347 $(M + H)^+$. Anal. Calcd ($C_{17}H_{13}F_3N_4O \cdot HCl \cdot H_2O$): C, H, N.

5-Nitro-quinoxaline (26). To a solution of 3-nitro-benzene-1,2-diamine (**25**) (1.53 g, 10 mmol) in EtOH (25 mL) was added glyoxal (3.4 mL, 40% in water, 30 mmol). The reaction mixture was heated to reflux for 2 h, cooled, stirred for 16 h, diluted with water, and extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, and evaporated under vacuum to give 2.23 g of desired product **26**, which was used without further purification. ^{1}H NMR (DMSO-d_6): δ 9.15 (d, J = 1.0 Hz, 1H), 9.13 (d, J = 1.0 Hz, 1H), 8.44 (m, 2H), 8.06 (d, J = 9.0 Hz, 1H), 8.02 (d, J = 9.0 Hz, 1H). MS (DCI/NH_3) *m/e*: 176 $(M + H)^+$.

5-Amino-quinoxaline (27). A mixture of 5-nitro-quinoxaline (**26**) prepared above and 10% Pd/C (223 mg) in EtOH (100 mL) was hydrogenated at 60 psi for 1 h. The catalyst was removed by filtration, the filtrate was evaporated under vacuum, and the residue was chromatographed on silica gel (EtOAc/hexane, 1:1) to give **27** (0.45 g, 31% for two steps). ^{1}H NMR (DMSO-d_6): δ 8.85 (d, J = 2.0 Hz, 1H,), 8.72 (d, J = 2.0 Hz 1H), 7.53 (t, J = 8.0 Hz, 1H), 7.18 (dd, J = 1.0 and 8.5 Hz, 1H), 6.61 (dd, J = 1.5 and 8.0 Hz, 1H), 6.14 (broad s, 2H). MS (DCI): 146 $(M + H)^+$.

1-Quinoxalin-5-yl-3-(3-trifluoromethylsulfanyl-phenyl)-urea (28). A solution of 5-amino-quinoxaline (**27**) (0.37 g, 2.5 mmol) and 3-trifluoromethylthiophenyl isocyanate (0.55 g, 2.5 mmol) in toluene (50 mL) was stirred for 48 h at ambient temperature. A precipitate collected by filtration was washed with toluene and dried under vacuum to give 0.48 g (53%) of the desired product **28**. ^{1}H NMR (DMSO-d_6): δ 10.14 (s, 1H), 9.71 (s, 1H), 9.05 (s, 1H), 8.96 (s, 1H), 8.61 (d, J = 7.5 Hz, 1H), 8.06 (s, 1H), 7.83 (t, J = 7.5 Hz, 1H), 7.70 (d, J = 7.5 Hz, 1H), 7.60 (d, J = 7.5 Hz, 1H), 7.49 (t, J = 7.5 Hz, 1H), 7.34 (d, J = 7.5 Hz, 1H). MS (ESI+) *m/e*: 365 $(M + H)^+$. Anal. Calcd ($C_{16}H_{11}F_3N_4OS$): C, H, N.

***N*-(3,4-Dichlorobenzyl)-*N*′-(3-methylcinnolin-5-yl)-urea (30).** This compound was synthesized from 5-amino-3-methylcinnoline (**29**) in two steps by using 3,4-dichlorobenzylamine and the trichloroacetamide procedure described for the synthesis of **14a** from **4**. ^{1}H NMR (DMSO-d_6): δ 8.93 (s, 1H), 8.19 (d, J = 4.5 Hz, 1H), 8.04 (m, 2H), 7.76 (t, J = 4.5 Hz, 1H), 7.59 (m, 2H), 7.34 (dd, J = 1.5 and 4.5 Hz, 1H), 7.10 (t, J = 4.5 Hz, 1H), 4.36 (d, J = 3.0 Hz, 2H), 2.88 (s, 3H). MS (ESI+) *m/e*: 362 $(M + H)^+$. Anal. Calcd ($C_{17}H_{14}Cl_2N_4O$): C, H, N.

Isoquinolin-5-yl-carbamic Acid 4-Trifluoromethylbenzyl Ester (31a) (Scheme 4). This compound was synthesized from **13** and 4-trifluoromethylbenzyl alcohol according to the procedure described for the synthesis of **14a** (yield 31%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.90 (broad s, 1H), 9.30 (s, 1H), 8.52 (d, J = 6.0 Hz, 1H), 7.94 (m, 3H), 7.80 (d, J = 7.5

Hz, 2H), 7.70 (m, 3H), 5.30 (s, 2H). MS (DCI/NH_3) *m/e*: 347 $(M + H)^+$. Anal. Calcd ($C_{18}H_{13}F_3N_2O_2$): C, H, N.

Isoquinolin-5-yl-carbamic Acid 4-Methyl-benzyl Ester (31b). This compound was synthesized from **13** and 4-methylbenzyl alcohol according to the procedure described for the synthesis of **14a** (yield 33%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.80 (broad s, 1H), 9.30 (s, 1H), 8.52 (d, J = 6.0 Hz, 1H), 7.92 (m, 3H), 7.66 (t, J = 7.5 Hz, 1H), 7.38 (d, J = 7.5 Hz, 2H), 7.22 (d, J = 7.5 Hz, 2H), 5.18 (s, 2H), 2.32 (s, 3H). MS (DCI/NH_3) *m/e*: 293 $(M + H)^+$. Anal. Calcd ($C_{18}H_{16}N_2O_2$): C, H, N.

Isoquinolin-5-yl-carbamic Acid 4-Chloro-benzyl Ester (31c). This compound was synthesized from **13** and 4-chlorobenzyl alcohol according to the procedure described for the synthesis of **14a** (yield 30%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.82 (broad s, 1H), 9.30 (s, 1H), 8.50 (d, J = 6.0 Hz, 1H), 7.95 (m, 3H), 7.66 (t, J = 7.5 Hz, 1H), 7.50 (s, 4H), 5.20 (s, 2H). MS (DCI/NH_3) *m/e*: 313 $(M + H)^+$. Anal. Calcd ($C_{17}H_{13}ClN_2O_2$): C, H, N.

Isoquinolin-5-yl-carbamic Acid 4-Bromo-benzyl Ester (31d). This compound was synthesized from **13** and 4-bromobenzyl alcohol according to the procedure described for the synthesis of **14a** (yield 34%). The aliquot of the free base was converted to the hydrochloride salt by treating its CH_2Cl_2 solution with 1 M HCl in MeOH, evaporating, and drying under vacuum. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.23 (s, 1H), 9.86 (s, 1H), 8.69 (d, J = 6.0 Hz, 1H), 8.50 (d, J = 6.0 Hz, 1H), 8.30 (d, J = 7.5 Hz, 2H), 7.98 (t, J = 7.5 Hz, 1H), 7.60 (m, 2H), 7.44 (d, J = 7.5 Hz, 2H), 5.20 (s, 2H). MS (DCI/NH_3) *m/e*: 357 $(M + H)^+$. Anal. Calcd. ($C_{17}H_{13}BrN_2O_2 \cdot 1.0HCl$): C, H, N.

Isoquinolin-5-yl-carbamic Acid 4-*tert*-Butyl-benzyl Ester (31e). This compound was prepared by a two-step procedure described for the synthesis of **14b** and using 4-*tert*-butylbenzyl alcohol (yield 26%). The aliquot of the free base was converted to the hydrochloride salt by treating its CH_2Cl_2 solution with 1 M HCl in MeOH, evaporating, and drying under vacuum. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.10 (s, 1H), 9.71 (s, 1H), 8.62 (d, J = 6.0 Hz, 1H), 8.38 (d, J = 6.0 Hz, 1H), 8.20 (m, 2H), 7.90 (t, J = 7.5 Hz, 1H), 7.40 (m, 4H), 5.10 (s, 1H), 1.28 (s, 9H). MS (ESI) *m/e*: 335 $(M + H)^+$. Anal. Calcd ($C_{21}H_{22}N_2O_2 \cdot 1.0HCl$): C, H, N.

Computational Analysis. Molecular structures corresponding to the diaryl urea portions of compounds **1**, **7**, and **12** were built and energy optimized at the HF/6-31G* level using Gaussian software (Gaussian, Inc., Pittsburgh, PA). Atom charges were calculated by electrostatic field fitting of the HF/6-31G* wave function. The electrostatic field using these point charges was calculated using the DelPhi module of Insight II software (Accelrys, San Diego, CA). A contour of the electrostatic field was calculated on the plane of the aromatic system.

Ca^{2+} Influx Functional Assay for the Determination of in Vitro Activity. The functional antagonist activity of compounds at the TRPV1 receptor was determined with a Ca^{2+} influx assay by measuring the effect on capsaicin evoked increase in intracellular Ca^{2+} levels ($[Ca^{2+}]i$) using cells stably expressing recombinant human or rat TRPV1. All compounds were tested over an 11-point half-log concentration range. Compound solutions were prepared in D-PBS (4× final concentration) and diluted serially across 96-well v-bottom tissue culture plates using a Biomek 2000 robotic automation workstation (Beckman-Coulter, Inc., Fullerton, CA). A 0.2 μM solution of the TRPV1 agonist capsaicin was also prepared in D-PBS. The fluorescent Ca^{2+} chelating dye fluo-4 was used as an indicator of the relative levels of $[Ca^{2+}]i$ in a 96-well format using a fluorescence imaging plate reader (FLIPR; Molecular Devices, Sunnyvale, CA). Cells were grown to confluency in 96-well black-walled tissue culture plates. Then, prior to the assay, the cells were loaded with 100 μL per well of fluo-4 AM (2 μM, in D-PBS) for 1−2 h at 23 °C. Washing of the cells was performed to remove extracellular fluo-4 AM (2 × 1 mL D-PBS per well), and afterward, the cells in 100 μL of D-PBS were placed in the reading chamber of the FLIPR instrument. Fifty microliters of the compound solutions was added to the cells at the 10 s time mark of the experimental run. After 3 min, 50 μL of the capsaicin solution was added (0.05 μM final concentration; final volume = 200 μL) to activate the TRPV1 receptor. Fluorescence readings were made at 1−5 s intervals over the 240 s course of the experimental run. The peak increase in relative fluorescence units (minus baseline) was calculated and expressed as a percentage of the 0.05 μM capsaicin (control) response. Curve fits of the data were solved using a four-parameter logistic Hill equation in GraphPad Prism (GraphPad Software, Inc., San Diego, CA), and IC_{50} values were calculated.

The Model of Inflammatory Pain—Complete Freund's Adjuvant-Induced Thermal Hyperalgesia. Unilateral inflammation was induced by injecting 150 μL of a 50% solution of complete Freund's adjuvant (CFA) (Sigma Chemical Co., St. Louis, MO) in physiological saline into the plantar surface of the right hindpaw of the rat. The hyperalgesia to thermal stimulation was determined 48 h after CFA injection as previously described.[28,29] Compound **14a** was administered orally 1 h prior to testing.

Abdominal Constriction Assay in CD1 Mice. As previously described,[30] each animal received an intraperitoneal injection of 0.3 mL of 0.6% acetic acid in normal saline to evoke writhing. Abdominal constriction was defined as a mild constriction and elongation passing caudally along the abdominal wall, accompanied by a slight twisting of the trunk and followed by bilateral extension of the hind limbs. The total number of abdominal constrictions was recorded from 5 to 20 min after acetic acid injection. Compound **14a** was administered orally 1 h prior to acetic acid injection.

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Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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