In Vitro Structure−Activity Relationship and In Vivo Characterization of 1-(Aryl)-3-(4-(amino)benzyl)urea Transient Receptor Potential Vanilloid 1 Antagonists

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The synthesis and structure−activity relationship of 1-(aryl)-3-(4-(amino)benzyl)urea transient receptor potential vanilloid 1 (TRPV1) antagonists are described. A variety of cyclic amine substituents are well tolerated at the 4-position of the benzyl group on compounds containing either an isoquinoline or indazole heterocyclic core. These compounds are potent antagonists of capsaicin activation of the TRPV1 receptor in vitro. Analogues, such as compound **45**, have been identified that have good in vivo activity in animal models of pain. Further optimization of **45** resulted in compound **58** with substantially improved microsome stability and oral bioavailability, as well as in vivo activity.

Introduction

The TRPV1[a] receptor, also known as VR1, is a member of the transient receptor potential (TRP) superfamily.[1−3] TRP receptors are ion channels that are characterized by six transmembrane spanning regions with a pore-forming region between the fifth and sixth membrane spanning units. The TRPV1 receptor is a nonselective cation channel. Upon TRPV1 activation, both sodium and calcium ions enter the cell through the channel pore, resulting in cell membrane depolarization. This depolarization increases neuronal excitability, leading to action potential firing and transmission of a noxious nerve impulse to the spinal cord. TRPV1 activation also leads to release of substance P and CGRP, which enhance peripheral sensitization of tissue.[4] The TRPV1 receptor is expressed on sensory neurons in C and Aδ fibers, the somata of which are located in sensory ganglia with peripheral projections innervating the skin, muscles, joints, gut, and central terminals projecting to the spinal dorsal horn. TRPV1 receptors are expressed on both the peripheral and central terminals of these neurons, suggesting that these receptors can be activated both in the periphery and in the spinal cord. In addition, the TRPV1 receptor has also been found in several brain regions,[5,6] including regions involved in pain transmission. The TRPV1 receptor is often termed a polymodal receptor, since it can be activated not only by endogenous lipid agonists such anandamide[7,8] but also by heat and acidic pH (<6),[9,10] as well as the vanilloid capsaicin.[11]

Molecular mechanisms involved in the enhanced painful sensations under pathological conditions have become better understood. Among the molecular mechanisms recently described to play a critical role in pain transmission is the TRPV1 receptor.[12] Data obtained using TRPV1 knockout mice suggest that the TRPV1 receptor plays a role in the development of sensory sensitization to noxious heat and/or inflammation.[13,14]

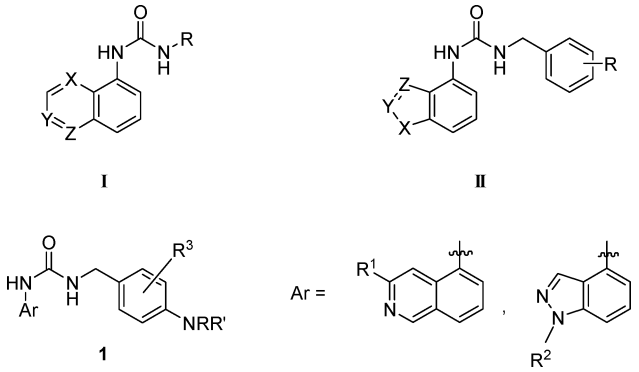

Figure 1. Structures of 6,6-fused ring (**I**) and 5,6-fused ring (**II**) heterocyclic ureas and general structure of 1-(aryl)-3-(4-(amino)benzyl)-urea (**1**) TRPV1 antagonists.

TRPV1 receptor antagonists such as capsazepine, which blocks activation of the channel in response to capsaicin, acid, and heat, reduce inflammation-induced hyperalgesia in animal models.[15] Thus, the TRPV1 receptor presents an opportunity for development of selective antagonists as agents to treat pathological pain.

Recently, we reported the identification of TRPV1 antagonists within a series of 6,6-fused ring heterocyclic ureas (**I**),[16] as well as the structure−activity studies of a series of 5,6-fused ring heterocyclic ureas (**II**)[17] (Figure 1). These investigations focused primarily on the optimization of the heteroaromatic moiety on the left-hand side of the molecule, with some preliminary SAR around the urea linker and the lipophilic group on the right side. In an effort to better understand this class of compounds as TRPV1 antagonists, we have extensively investigated the effect of modifying the lipophilic portion of the molecule on in vitro potency, in vivo efficacy, pharmacokinetic parameters, and physical properties. Herein, we report the in vitro SAR and in vivo characterization of a series of 1-(aryl)-3-(4-(amino)benzyl)-urea analogues (**1**) as potent and selective TRPV1 antagonists.

Chemistry

The synthesis of 1-(aryl)-3-(4-(amino)benzyl)urea analogues is shown in a general sense in Scheme 1. In the case of the

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[a] Abbreviations: TRPV1, transient receptor potential vanilloid 1; TRP, transient receptor potential; VR1, vanilloid receptor 1; CFA, complete Freund's adjuvant.

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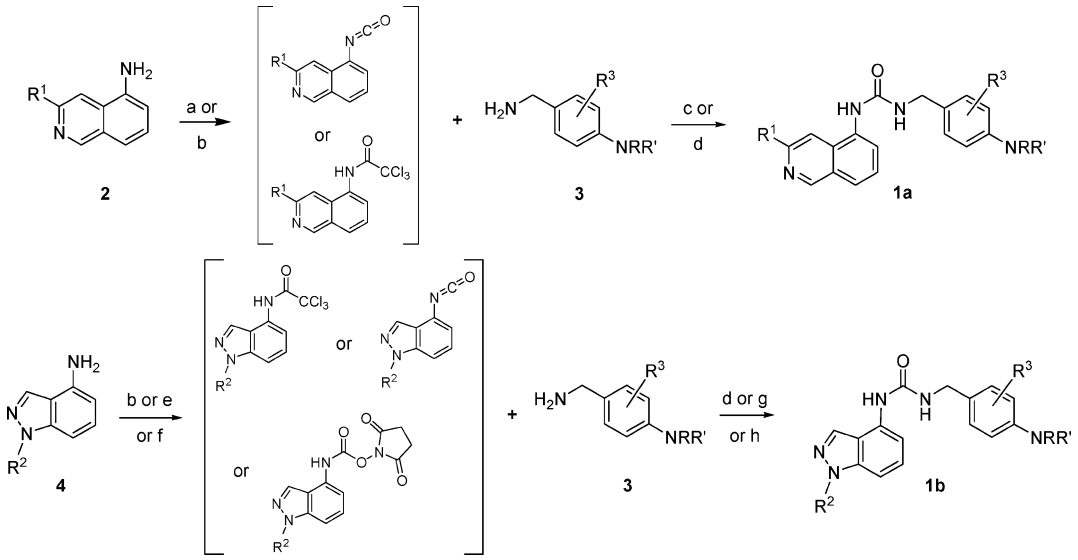
Scheme 1[a]



[a] Reagents and conditions: (a) $COCl_2$, DMAP, CH_2Cl_2, 0 °C to room temp; (b) trichloroacetyl chloride, Et_3N, CH_2Cl_2, 0 °C to room temp; (c) Et_2O; (d) DBU, MeCN, reflux; (e) $COCl_2$, toluene, reflux; (f) *N*,*N*′-disuccinimidyl carbonate, MeCN; (g) Et_3N, Et_2O; (h) diisopropylethylamine, DMF.

isoquinoline derivatives (**1**, Ar = isoquinolinyl), two methods were used to form the desired urea from isoquinoline amines **2** and benzylamines **3**. In the first method, an appropriately substituted **3** was coupled to the desired isoquinoline moiety (R^1 = H, CH_3) via the isoquinoline isocyanate in diethyl ether at room temperature to yield the urea **1a** in good yield. The isoquinoline isocyanates, in turn, were prepared by reacting **2** with phosgene in the presence of dimethylaminopyridine in methylene chloride at 0 °C. After warming to room temperature overnight followed by concentration, the mixture was then diluted by diethyl ether and filtered to yield a stock solution of isocyanate that was stored cold and used as desired. Alternatively, the second method involved conversion of the isoquinoline moiety (R^1 = Cl, NHAc) to the *N*-5-trichloroacetamide by reaction of **2** with trichloroacetyl chloride and triethylamine in methylene chloride. The crude trichloroacetamide was then further reacted with **3** and DBU in acetonitrile at reflux to afford **1a**.

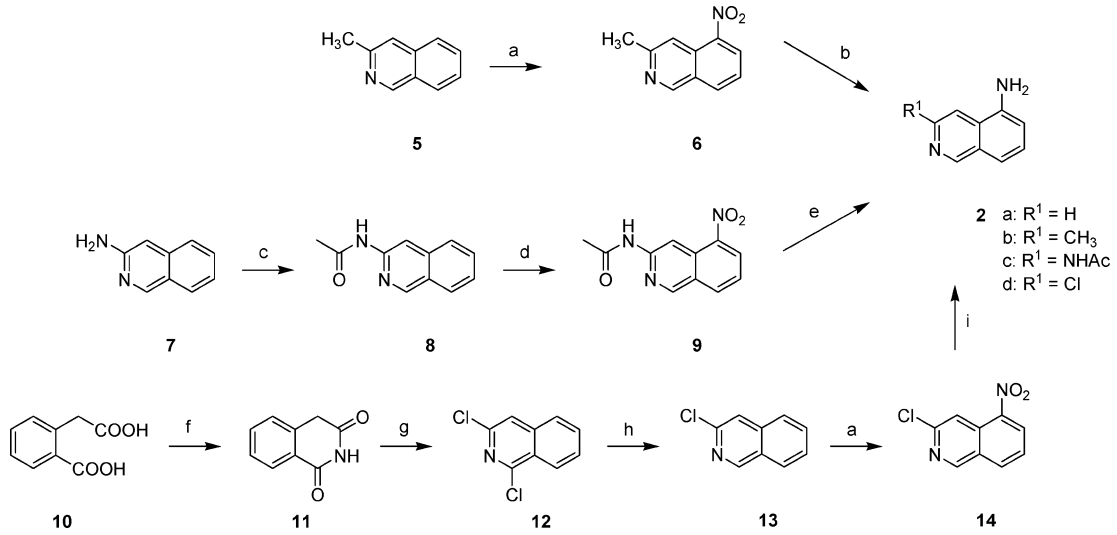
The choice of which of the methods to use for the formation of the critical urea linker was dictated by the ability to form the intermediate isoquinoline isocyanate. The isocyanate method was preferred because the urea formation tended to be a very clean reaction and resulted in relatively easy isolation of pure desired product. However, formation of the requisite isoquinoline isocyanate was an inconstant and unpredictable process, which forced development of the alternative procedure of utilizing the isoquinoline trichloroacetamide intermediate. This method was more robust and could be utilized for any of the desired isoquinoline substrates, regardless of the substitution, but usually required more effort in the purification and isolation of the final product. In addition, shelf life of the isoquinoline trichloroacetamide was longer than that of the isocyanate.

In the case of the indazole compounds (**1**, Ar = indazolyl), three different methods were employed to form the urea through the coupling of **3** to the indazole amine **4**. The first method utilized formation of an indazole isocyanate by reaction of **4** (R^2 = CO_2CH_3, CH_3) with phosgene, this time in toluene at reflux, followed by concentration and then dilution by diethyl ether and triethylamine to furnish a stock solution of indazole isocyanate. An aliquot of this solution was then reacted with **3** in diethyl ether to afford **1b** in high yield. The second method involved conversion of **4** (R^2 = CO_2CH_3) to its succinimidyl carbamate using *N*,*N*′-disuccinimidyl carbonate in acetonitrile. The advantage to this method was that the intermediate succinimidyl carbamate could be synthesized on a large scale and stored indefinitely. When needed, it was easily weighed out and reacted with **3** in dimethylformamide in the presence of Hünig's base to give **1b** in very high yield. The third method was utilized for some of the analogues possessing a methyl group on the N-1 indazole nitrogen. In this case **4** (R^2 = CH_3) was reacted with trichloroacetyl chloride and triethylamine in methylene chloride to give the crude trichloroacetamide that was then further reacted with **3** and DBU in acetonitrile at reflux to furnish **1b** in modest yield after column chromatography.

Here, the choice of method for formation of the critical urea linker was largely a function of convenience. Early in the investigation the preferred method for synthesis of analogues where R^2 = H was through the isocyanate. While still requiring skillful handling, formation of the indazole isocyanate intermediate was not nearly as capricious as in the case of isoquinoline. Later, a more convenient method was developed that utilized the intermediate indazole succinimidyl carbamate. This intermediate carbamate could easily be prepared on a large scale and stored indefinitely. The reaction to form the urea was very clean and high-yielding, with easy isolation of the product. In addition, this avoided using large amounts of phosgene when selected analogues were scaled up for advanced preclinical characterization. Finally, for many of the analogues where R^2 = CH_3, the indazole trichloroacetamide was utilized, owing to the predictable nature of its preparation and reaction with the desired benzylamines **3**.

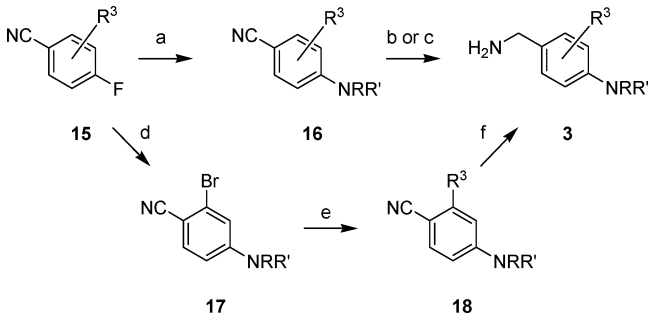
The synthesis of custom isoquinoline amines **2** (R^1 = CH_3, NHAc, Cl) is shown in Scheme 2. The key step was nitration of the substituted isoquinolines **5**, **8**, and **13** using potassium nitrate or sodium nitrite in sulfuric acid at 0 °C, followed by reduction of the resulting nitro intermediates **6** and **9** by catalytic hydrogenation and of **14** by iron in acetic acid. While the nitration substrates **5** and **8** were obtained easily, **13** required more synthetic effort. Homophthalic acid **10** was cyclized to imide **11** with ammonium hydroxide at high temperature followed by conversion to dichloride **12** with dichlorophenylphosphine oxide neat at 160 °C. **13** was then furnished by selective dechlorination of the C-1 chlorine by tin and hydrochloric acid in acetic acid.

Scheme 2[a]



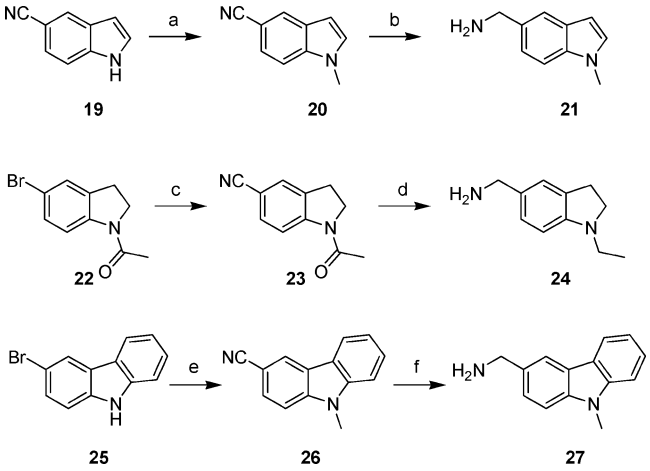
[a] Reagents and conditions: (a) KNO_3, H_2SO_4, 0 °C; (b) H_2 (1 atm), 10% Pd/C, 1:1 EtOH/THF; (c) Ac_2O, Et_3N, CH_2Cl_2; (d) $NaNO_2$, H_2SO_4, 0 °C; (e) H_2 (60 psi), 10% Pd/C, MeOH; (f) NH_4OH, *o*-dichlorobenzene, 200 °C; (g) $PhP(O)Cl_2$, 160 °C; (h) Sn^0HOAc/HCl, 60 °C; (i) Fe^0HOAc, 60 °C.

Scheme 3[a]



[a] Reagents and conditions: (a) HNRR′, DMSO, 120 °C; (b) $LiAlH_4$, THF, reflux; (c) BH_3·THF, THF, reflux; (d) HNRR′, diisopropylethylamine, DMSO, 130 °C, microwave; (e) R^3MgBr, $ZnCl_2$, $Pd(dppf)_2Cl_2$, dioxane, reflux; (f) 60 psi of H_2, Raney Ni, MeOH/NH_3.

Scheme 4[a]



[a] Reagents and conditions: (a) (i) KOH, acetone, 0 °C; (ii) MeI, 0 °C to room temp; (b) BH_3·THF, THF, reflux; (c) CuCN, NMP, reflux; (d) $LiAlH_4$, THF, reflux; (e) (i) NaH, MeI, DMF, 60 °C; (ii) CuCN, NMP, reflux; (f) $LiAlH_4$, THF, 0 °C to room temp.

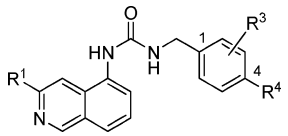
The requisite benzylamines **3** were prepared in a straightforward manner as shown in Scheme 3. Commercially available 4-fluorobenzonitriles **15** were reacted with the desired secondary amine fragments by heating in DMSO, typically in a sealed tube at 120 °C, to yield 4-aminobenzonitriles **16** in good yield. Alternatively, when custom substitution on the phenyl ring was desired, the amine was reacted with **15** (R^3 = 2-Br) in the presence of Hünig's base in DMSO at 130 °C in a microwave reactor to yield 4-aminobenzonitriles **17**. Installation of alkyl groups on **17** to give nitriles **18** was then accomplished via Sonogashira or Negishi type couplings. Subsequent reduction of nitriles **16** and **18** using a variety of methods, such as catalytic hydrogenation with Raney nickel or chemical reduction with lithium aluminum hydride or borane tetrahydrofuran complex, furnished amines **3**. Coupling of **3** to the heteroaromatic moiety via the aryl isocyanate or other activated species as described above resulted in the target urea compounds **1** in good overall yield.

The bicyclic amines **21**, **24**, and **27** were synthesized as shown in Scheme 4. Commercially available 5-cyanoindole (**19**) was first N-methylated using potassium hydroxide and methyliodide in acetone, and the resultant nitrile **20** was reduced with borane tetrahydrofuran complex to afford **21** in 28% yield. The synthesis of amine **24** started with 5-bromo-1-acetylindoline (**22**) that was cyanated with copper(I) cyanide in *N*-methylpyrrolidinone at reflux to yield nitrile **23** in quantitative yield after overnight Soxhlet extraction. Reduction of **23** with lithium aluminum hydride in THF at reflux furnished **24** in 65% yield. Finally, amine **27** was synthesized starting from 3-bromocarbazole (**25**) through a series of standard reactions including N-methylation with sodium hydride and methyl iodide and cyanation using copper(I) cyanide in *N*-methylpyrrolidinone at reflux to yield nitrile **26** in nearly quantitative yield. Nitrile **26** was then reduced with lithium aluminum hydride in THF at 0 °C to give **27** in 45% yield. Amines **21**, **24**, and **27** were coupled with the heteroaromatic groups using the methods described above to furnish the target urea compounds **1**.

Results and Discussion

The impetus for the present investigation was improvement of the physical properties of this general class of compounds. One strategy involved introducing hydrogen-bonding atoms such as oxygen and nitrogen in the benzyl or similar hydrophobic substituent. An amino group, if appropriately basic, could potentially provide an ionizable group to improve solubility. Even though aromatic amines are only one-millionth as basic as alkylamines, they are still protonated even in dilute acidic

Table 1. In Vitro Biological Activity of Isoquinoline Analogues in the Human TRPV1 Ca^{2+} Influx Assay[a]



Compd	R^1	R^3	R^4	IC_{50} (nM)	Compd	R^1	R^3	R^4	IC_{50} (nM)
28[16]	H	H	CF_3	2.1 ± 0.2	**39**	CH_3	H		3.8 ± 0.5
29	H	H	NMe_2	157 ± 57	**40**	NH_2	H		9.8 ± 0.8
30	H	H		5.3 ± 1.1	**41**	Cl	H		156 ± 30
31	H	H		7.3 ± 1.0	**42**	H	2-Cl		5.2 ± 0.6
32	H	H		3.3 ± 0.3	**43**	H	2-CF_3		5.7 ± 1.3
33	H	H		4.7 ± 0.7	**44**	H	3-CF_3		4.6 ± 1.7
34	H	H		9.3 ± 1.9	**45**	H	3-F		2.8 ± 0.3
35	H	H		91 ± 30	**46**	H	3,5-diF		9.3 ± 2.7
36	H	H		258 ± 70	**47**	CH_3	3-F		15.5 ± 6.7
37	H	H		23.8 ± 7.4	**48**	CH_3	3-F		7360 ± 144
38	H	H		3.3 ± 0.3	**49**	NH_2	3-CF_3		14.0 ± 3.0

[a] All values are the mean ± SEM of at least three separate experiments run in triplicate.

solutions. From earlier SAR studies in a 7-hydroxynaphthalen-1-ylurea series, it was found that although the 4-amino group was not tolerated as a benzyl substituent, the 4-dimethylamino group was well tolerated (1450 vs 14 nM). Thus, we began our investigation for the present study with the dimethylamino group and expanded it to include other dialkylamines. The compounds were evaluated for their ability to inhibit activation of the recombinant human TRPV1 receptor by capsaicin in a calcium influx functional assay. The activities of lead analogues were also measured using a recombinant rat TRPV1 receptor.

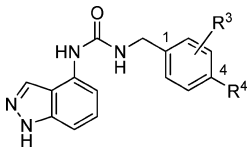
As shown in Table 1, in the case where the left-side heteroaromatic group was isoquinoline, substitution at the 4-position on the benzyl moiety with a variety of dialkylamino groups led to analogues with very good antagonist potency at the TRPV1 receptor. Although the potency of the dimethylamino group (compound **29**) was over 70-fold lower than the prototypical lipophilic trifluoromethyl substituent (compound **28**), increasing the lipophilicity of the amino substituent increased the potency to the level of **28**. For example, incorporation of pyrrolidine, piperidine, azepane, and azocane rings (compounds **30**−**33**) all resulted in analogues with single-digit nanomolar potency. Substitution at the 4-position on the piperidine ring, such as methyl, was well tolerated (compound **34**), as was the inclusion of the heteroatoms oxygen and sulfur in the ring, as in the morpholine and thiomorpholine analogues (compounds **46** and **37**). However, the sterically demanding 2,6-dimethylmorpholinyl group had a deleterious effect on the TRPV1 antagonist potency (compound **36**) as did the isoindoline analogue **48**. On the other hand, analogues **38** and **49** with the bulky bicyclic tropane group had very good potency with IC_{50} values of 3.3 and 14 nM, respectively.

The effect of substitution on the isoquinoline ring on in vitro potency and physicochemical properties was also examined. Analogues in this series with methyl, amino, and chloro substituents at the 3-position of the isoquinoline ring were synthesized and evaluated for TRPV1 antagonist activity. Representative examples are shown in Table 1. It was found that electron-donating groups such as amino and methyl had little or no effect on the in vitro potency compared with hydrogen (compare **39** and **40** to **32**). On the other hand, electron-withdrawing groups such as chloro appeared to cause a decrease in in vitro potency. For example, the potency of the morpholine analogue **41** was 1.7-fold less than the hydro analogue **35** (156 vs 91 nM).

The effects of substitution on the phenyl ring were also investigated. As shown in Table 1, substitution on the phenyl ring was well tolerated. Groups such as trifluromethyl and the halogens chlorine and fluorine, whether ortho or meta to the azepane ring, all yielded analogues with very good in vitro potency. For example, fluoro analogue **45** was found to have an IC_{50} at the human TRPV1 receptor of 2.8 nM.

Compounds containing the indazole rather than the isoquinoline heterocycle were also synthesized and evaluated. As shown in Table 2, some differences in the in vitro potency were observed when the isoquinoline heterocycle was replaced with indazole. For example, in the indazole series, there was a significant decrease in activity for compounds with substitution meta to the azepane compared to the ortho substituted analogues (e.g., compare **50**−**53** of Table 2 to **42**−**45** of Table 1,

Table 2. In Vitro Biological Activity of Indazole Analogues in the Human TRPV1 Ca^{2+} Influx Assay[a]



Compd	R^3	R^4	IC_{50} (nM)	Compd	R^3	R^4	IC_{50} (nM)
50	2-Cl		190 ± 52	**64**	2,5-diF		21.3 ± 1.6
51	2-CF_3		133 ± 12	**65**	2,5-diF		82.0 ± 15.4
52	3-CF_3		29.5 ± 5.0	**66**	2,5-diF		157 ± 37.2
53	3-F		14.2 ± 1.6	**67**[b]			1850 ± 477
54	3,5-diF		39.3 ± 4.7	**68**			285 ± 68
55	H		18.0 ± 5.2	**69**[b]			305 ± 18
56	2-Cl		58.8 ± 6.6	**70**			902 ± 93
57	2-CF_3		54.5 ± 2.1				
58	3-CF_3		26.9 ± 5.8	**71**	3-CH_3		19.8 ± 4.8
59	3-Cl		20.4 ± 5.2	**72**	2-CH_3		29.0 ± 4.9
60	3-Br		43.2 ± 4.8	**73**			22.4 ± 10.1
61	3-F		14.8 ± 3.0	**74**[b]			19.0 ± 3.1
62	3,5-diF		12.6 ± 2.7	**75**			35.3 ± 6.5
63	2,3-diF		25.5 ± 8.1	**76**			10.3 ± 2.6

[a] All values are the mean ± SEM of at least three separate experiments run in triplicate. [b] Compound contains a methyl group at the N-1 indazole nitrogen.

respectively). As with the isoquinoline analogues, replacement of the azepane with tropane was well tolerated in the indazole series (compound **55**, 18 nM). For these derivatives, the same potency trend was observed for the ortho and meta substitution in the case of chloro and trifluoromethyl analogues (e.g., compounds **56**−**59**). However, when larger substituents such as benzyl (compounds **73** and **74**) and 3,3-dimethyl-1-butyl (compound **76**) were incorporated meta to the tropane group, very good potencies were observed. Further derivatization of the tropane itself resulted in a decrease in potency. For example, the ketal **65** and ketone **66** were approximately 4-fold and 7-fold less potent than the simple tropane **64**, respectively. When the amino group was fused to the phenyl ring to form an indole, indoline, or carbazole ring system, the compounds were less potent, with IC_{50} values at the human TRPV1 receptor greater than 280 nM. Finally, as seen previously,[17] methylation of the N-1 indazole nitrogen was also well tolerated and yielded analogues with potencies similar to those of the N−H analogues (e.g., compounds **68**, **69**, **73**, and **74**).

Selected analogues from both the isoquinoline and indazole series were evaluated for microsome stability (Table 3). Compound **45** had low microsomal stability, particularly with human liver microsomes. We devised a strategy to modify **45** to improve the oral bioavailability and metabolic stability while at the same time maintaining the favorable characteristics. We focused on two areas that were potentially responsible for the rapid metabolism: the cyclic amine moiety (N-dealkylation) and the phenyl ring (aromatic hydroxylation). The stability of compound **45** was improved by modification of one or both regions of the molecule. For example, while not as stable to rat microsomes, compound **44** was approximately 2-fold more stable to human liver microsomes, and the tropane analogue **38** was more stable to microsomes from both species. Combination of these two effects led to compound **58**, which had stability similar to that of **45** with rat liver microsomes but was almost 3-fold more stable with human microsomes. Pharmacokinetic

Table 3. In Vitro Metabolic Stability of Selected TRPV1 Antagonists[a]

	rate disappearance (pmol min^{-1} mg^{-1}) liver microsomes	
compd	rat	human
28	383	135
45	366	1223
44	689	643
38	247	818
58	315	445
61	394	516

[a] Data represent rate of disappearance of compound after 30 min of incubation with liver microsomes using an initial concentration of 0.50 μM compound as described in ref 18. Data were validated using terfenadine as a positive control and were within the expected range.

Table 4. In Vivo Activity[a] and Pharmacokinetic Profile[b] of Compounds **45** and **58**

characteristic	**45**	**58**
hVR1 IC_{50} (nM)	2.8 ± 0.3	26.9 ± 5.8
CFA[c] (thermal hyperalgesia) ED_{50}, po (μmol kg^{-1})	30	20
iv V_β (L kg^{-1})	1.8	2.1
CL_p (L h^{-1} kg^{-1})	1.8	1.0
$t_{1/2}$ (h)	0.69	1.4
C_{max}, po (μg mL^{-1})	0.15	0.24
F of rat, dog; po (%)	10, 18	21, 46
plasma protein binding of rat, human (%)	nd[d]	98.6, 97.0

[a] ED_{50} values were determined from three-part dose response experiment, with 6−12 animals per dose group. [b] Pharmacokinetic results were determined in rat (three animals per group, each iv and oral) following administration of 10 μmol kg^{-1}. [c] 95% confidence intervals: **45**, 22−36 μmol kg^{-1}; **58**, 12−27 μmol/kg. [d] nd = not determined.

analysis of **45** and **58** revealed substantially greater oral bioavailability of **58** with 21% and 46% in rat and dog, respectively (Table 4). Both compounds were orally active in the CFA model of inflammatory pain and exhibited similar activity. The weaker in vitro potency of **58** was balanced by greater oral bioavailability. In assays performed by CEREP for selectivity, compound **58** was found to be more than 370-fold selective for TRPV1 compared to a diverse array of receptors, ion channels, reuptake sites, and enzymes, although weak binding (IC_{50} > 5 μM) to the adenosine A_3 and peripheral BZD receptors as well as the Na^+ channel (site 2) was observed. The compound was further profiled and found to be negative in the Ames and micronucleus assays. In addition, **58** had no effect in the rat and dog cardiovascular safety evaluation up to 72× the therapeutic plasma levels. On the other hand, the measured aqueous solubility of **58** was determined to be 14 μg/mL at pH 2, while at pH 6.8 the solubility was <1 ng/mL. Thus, only very modest improvements in aqueous solubility were able to be achieved through incorporation of various 4-dialkylamino substituents on the benzyl moiety of this class of compounds.

Conclusion

The synthesis, in vitro SAR, and in vivo characterization of a series of 1-(aryl)-3-(4-(amino)benzyl)urea TRPV1 antagonists were presented. Many of the analogues had excellent potency (single-digit nanomolar) at the human TRPV1 receptor. Two compounds (**45** and **58**) had sufficient in vivo potency in an animal pain model to warrant further profiling for drug development. Compound **58** had many desirable characteristics of a drug candidate, such as potency, efficacy, selectivity, cardiovascular safety, and oral bioavailability. Even though the original goal of significantly improving the aqueous solubility of this class of compounds was not realized, the poor metabolic stability of **45** in the presence of human liver microsomes was improved by the replacement of the azepane ring with the tropane of **58**. In addition, compound **58** exhibited a better pharmacokinetic profile than **45** in both rats and dogs.

Experimental Section

^{1}H NMR spectra were obtained at 300 MHz using tetramethylsilane as internal standard. The mass spectra (electron spray ionization (ESI) and dissolvable chemical ionization (DCI)) and high-resolution mass spectra were recorded on Finnigin-4000 instruments. Elemental combustion analysis results were obtained from Robertson Microlit Laboratories or Quantitative Technologies, Inc., and were within ±0.4% of theoretical values. Flash column chromatography was carried out on EM Science silica gel 60 (230−400 mesh) or Analogix Super Flash columns. Reactions were routinely conducted under an inert atmosphere (N_2) using commercial high-purity solvents as received. Methyl 4-amino-1*H*-indazole-1-carboxylate (**4**, $R^2 = CO_2CH_3$) and 1-methyl-1*H*-indazole-4-ylamine (**4**, $R^2 = CH_3$) was synthesized as described in ref 17. 5-Aminoisoquinoline (**2a**) was obtained from commercial sources. Compounds isolated as the hydrochloride salt as indicated were prepared by treatment of the free amine with ethereal HCl or ethanolic HCl in either ethanol or methanol followed by precipitation of the salt by ethyl ether, if needed, and collection by filtration.

1-(4-(Dimethylamino)benzyl)-3-(isoquinolin-5-yl)urea (29). A solution of 2,2,2-trichloro-*N*-(isoquinolin-5-yl)acetamide[16] (580 mg, 2.0 mmol), 4-dimethylaminobenzylamine dihydrochloride (450 mg, 2.0 mmol), and DBU (1.1 mL, 7.4 mmol) in MeCN (50 mL) was heated to reflux for 2 h. Additional DBU (1.1 mL, 7.4 mmol) was added, and heating was continued for 3 h. The mixture was cooled and concentrated in vacuo. Ethyl acetate and water were added to the residue, and the resulting precipitate was collected by filtration, washed with EtOAc, and dried under vacuum. The result was 460 mg (72%) of the title compound as a tan solid. ^{1}H NMR (DMSO-d_6): δ 9.26 (s, 1H), 8.72 (s, 1H), 8.52 (d, J = 5.8 Hz, 1H), 8.32 (d, J = 6.8 Hz, 1H), 7.93 (d, J = 6.1 Hz, 1H), 7.67−7.78 (m, 1H), 7.59 (t, J = 8.0 Hz, 1H), 7.18 (d, J = 8.5 Hz, 2H), 6.97 (m, 1H), 6.72 (d, J = 8.8 Hz, 2H), 4.23 (d, J = 5.4 Hz, 2H), 2.86 (s, 6H). MS (ESI$^+$): *m*/*z* 321 $(M + H)^+$. Anal. ($C_{19}H_{20}N_4O \cdot 0.7H_2O$) C, H, N.

General Procedures for the Preparation of Compounds 30−38: (4-(Pyrrolidin-1-yl)phenyl)methanamine (3, R^3 = H, R^4 = Pyrrolidinyl). A solution of 4-fluorobenzonitrile (1.10 g, 9.08 mmol) and pyrrolidine (2.58 g, 36.3 mmol) in DMSO (15 mL) was heated to 120 °C in a sealed tube for 2 h. The mixture was cooled to room temperature and partitioned between Et_2O and H_2O. The separated aqueous phase was extracted with Et_2O, and the combined organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was placed under high vacuum overnight, resulting in 1.55 g (99%) of 4-(pyrrolidin-1-yl)benzonitrile as a light-orange solid. ^{1}H NMR (DMSO-d_6): δ 7.52 (m, 2H), 6.59 (m, 2H), 3.28 (m, 4H), 1.96 (m, 4H). MS (ESI$^+$): *m*/*z* 173 $(M + H)^+$. This intermediate nitrile (1.55 g, 9.00 mmol) was taken up in 40 mL of THF followed by addition of solid $LiAlH_4$ (1.37 g, 36.0 mmol) in several small portions. Upon complete addition the mixture was heated to reflux for 1 h. The mixture was then cooled to 0 °C, and the reaction was quenched by careful addition of solid $Na_2SO_4 \cdot 10H_2O$. After being stirred for 1 h, the mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The result was 1.48 g (93%) of the title compound as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 7.10 (m, 2H), 6.46 (m, 2H), 3.57 (s, 2H), 3.29 (br s, 2H + H_2O), 3.18 (m, 4H), 1.93 (m, 4H). MS (ESI$^+$): *m*/*z* 160 $(M - NH_2)^+$.

1-(Isoquinolin-5-yl)-3-(4-(pyrrolidin-1-yl)benzyl)urea Hydrochloride (30). To a flask containing 20 mL of CH_2Cl_2 at 0 °C was added a solution of phosgene in toluene (20% w/w, 1.50 mL, 2.84 mmol). A solution of DMAP in 5 mL of CH_2Cl_2 was then added dropwise followed by addition of solid 5-aminoisoquinoline (340.0 mg, 2.36 mmol). The mixture was allowed to warm to ambient temperature and was stirred for 18 h. The mixture was concentrated to approximately 5 mL, diluted by Et_2O (40 mL), and then filtered. The filtrate was then diluted with Et_2O to a final volume of 100 mL to give a stock solution of 5-isocyanatoisoquinoline of approximately 0.024 M.

An aliquot of 5-isocyanatoisoquinoline solution (72 mL, 1.7 mmol) was added dropwise to (4-(pyrrolidin-1-yl)phenyl)methanamine (304 mg, 1.7 mmol) in 5 mL of THF at ambient temperature. The mixture was stirred overnight and concentrated to a volume of approximately 20 mL. The resulting precipitate was collected by vacuum filtration, washed with Et_2O, and dried under high vacuum. The hydrochloride salt was formed by treatment of an ethanolic solution of the product with ethereal HCl. The result was 212 mg (29%) of the title compound as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 9.81 (s, 1H), 9.57 (br s, 1H), 8.79 (m, 1H), 8.65−8.73 (m, 2H), 8.10 (d, J = 8.1 Hz, 1H), 7.92 (t, J = 8.0 Hz, 1H), 7.49 (m, 1H), 7.21 (d, J = 9.0 Hz, 2H), 6.67 (m, 2H), 4.26

(m, 2H), 3.24 (m, 4H), 1.96 (m, 4H). MS (ESI^+): *m*/*z* 347 ($M + H)^+$. Anal. ($C_{21}H_{22}N_4O \cdot 2.25HCl$) C, H, N.

1-(3-Aminoisoquinolin-5-yl)-3-(4-(azepan-1-yl)benzyl)urea (40): *N*-(5-Aminoisoquinolin-3-yl)acetamide (2c). To a solution of isoquinolin-3-amine (2.00 g, 13.9 mmol) in CH_2Cl_2 (80 mL) were added triethylamine (2.02 mL, 14.5 mmol) and acetic anhydride (4.10 mL, 43.4 mmol). The mixture was stirred for 18 h at ambient temperature. The volatiles were evaporated, and the residue was chased with toluene and concentrated in vacuo to give crude *N*-(isoquinolin-3-yl)acetamide, which was then dissolved in 30 mL of concentrated H_2SO_4 and cooled to 0 °C. Sodium nitrite (1.45 g, 17.1 mmol) was added in small portions while maintaining reaction temperature at 0 °C. After 30 min the reaction was quenched with ice, the mixture was basified with concentrated NH_4-OH, and the yellow solid was filtered, washed with water, and dried under vacuum to afford 2.55 g (79%) of *N*-(5-nitroisoquinolin-3-yl)acetamide (**9**). ^1H NMR (DMSO-d_6): δ 10.99 (bs, 1H), 9.37 (s, 1H), 9.16 (s, 1H), 8.61 (d, $J = 7.8$ Hz, 1H), 8.50 (d, $J = 8.1$ Hz, 1H), 7.70 (t, $J = 8.0$ Hz, 1H), 2.19 (s, 3H). MS (ESI^+) *m*/*z* 232 ($M + H)^+$.

Compound **9** (2.40 g, 10.4 mmol) was hydrogenated at 60 psi of hydrogen with 10% Pd/C (240 mg) in methanol (200 mL) for 4 h at ambient temperature. The catalyst was filtered and the solvent evaporated to afford 1.85 g (88%) of **2c** as a brown solid. ^1H NMR (DMSO-d_6): δ 10.41 (s, 1H), 8.92 (s, 1H), 8.41 (s, 1H), 7.18−7.26 (m, 2H), 6.81−6.88 (m, 1H), 5.60 (s, 2H), 2.13 (s, 3H). MS (ESI^+) *m*/*z* 202 ($M + H)^+$.

***N*-(3-Acetamidoisoquinolin-5-yl)-2,2,2-trichloroacetamide.** Triethylamine (215 μL, 1.54 mmol) was added dropwise to a solution of **2c** (282 mg, 1.40 mmol) and trichloroacetyl chloride (172 μL, 1.54 mmol) in CH_2Cl_2 at 0 °C. The mixture was then allowed to warm to ambient temperature. After 4 h the reaction mixture was diluted by CH_2Cl_2 and washed with saturated $NaHCO_3$ solution and brine, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (4% CH_3OH/CH_2Cl_2) yielded 384 mg (79%) of the title compound as a brown solid. ^1H NMR (DMSO-d_6): δ 11.07 (s, 1H), 10.64 (s, 1H), 9.20 (s, 1H), 8.57 (s, 1H), 8.07 (d, $J = 8.1$ Hz, 1H), 7.64 (d, $J = 6.0$ Hz, 1H), 7.56 (t, $J = 7.5$ Hz, 1H), 2.13 (s, 3H). MS (ESI^+) *m*/*z* 347 ($M + H)^+$.

***N*-(5-(3-(4-(Azepan-1-yl)benzyl)ureido)isoquinolin-3-yl)acetamide.** A solution of *N*-(3-acetamidoisoquinolin-5-yl)-2,2,2-trichloroacetamide (239 mg, 0.69 mmol), (4-(azepan-1-yl)phenyl)-methanamine (141 mg, 0.69 mmol, synthesized as described for compound **39**), and DBU (258 μL, 1.7 mmol) in MeCN (20 mL) was heated to reflux. After 10 h the mixture was cooled to ambient temperature and concentrated in vacuo, and the residue was partitioned between EtOAc and saturated $NaHCO_3$ solution. The separated aqueous phase was extracted with EtOAc, and the combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (1−3% CH_3OH/CH_2Cl_2) yielded 136 mg (46%) of the title compound as a light-brown solid. ^1H NMR (DMSO-d_6): δ 10.57 (s, 1H), 9.07 (s, 1H), 8.52 (s, 1H), 8.45 (s, 1H), 8.12 (d, $J = 6.8$ Hz, 1H), 7.68 (d, $J = 8.1$ Hz, 1H), 7.43 (t, $J = 8.0$ Hz, 1H), 7.14 (d, $J = 8.5$ Hz, 2H), 7.04 (s, 1H), 6.66 (d, $J = 8.8$ Hz, 2H), 4.19 (d, $J = 5.4$ Hz, 2H), 3.44 (m, 4H), 2.14 (s, 3H), 1.66−1.76 (m, 4H), 1.41−1.48 (m, 4H). MS (ESI^+) *m*/*z* 432 ($M + H)^+$.

1-(3-Aminoisoquinolin-5-yl)-3-(4-(azepan-1-yl)benzyl)urea. A solution of *N*-(5-(3-(4-(azepan-1-yl)benzyl)ureido)isoquinolin-3-yl)-acetamide (130 mg, 0.30 mmol) in 48% HBr (8 mL) was heated to 60 °C for 1 h. The solution was cooled to ambient temperature and basified with concentrated ammonium hydroxide solution, and the product was extracted with EtOAc. The organic phase was concentrated in vacuo and the residue was purified by flash chromatography (1−5% CH_3OH/CH_2Cl_2), which gave 93 mg (79%) of compound **40** as a yellow solid. ^1H NMR (DMSO-d_6): δ 8.76 (s, 1H), 8.19 (s, 1H), 7.88 (d, $J = 6.4$ Hz, 1H), 7.45 (d, $J = 8.1$ Hz, 1H), 7.03−7.15 (m, 3H), 6.76 (t, $J = 5.4$ Hz, 1H), 6.61−6.71 (m, 3H), 5.90 (s, 2H), 4.17 (d, $J = 5.4$ Hz, 2H), 3.44 (m, 4H), 1.71 (m, 4H), 1.45 (m, 4H). MS (ESI^+) *m*/*z* 390 ($M + H)^+$. Anal. ($C_{23}H_{27}N_5O \cdot 0.4H_2O$) C, H, N.

1-(4-Azepan-1-yl-2-chlorobenzyl)-3-(1*H*-indazol-4-yl)urea (50): Methyl 4-(3-(4-(Azepan-1-yl)-2-chlorobenzyl)ureido)-1*H*-indazole-1-carboxylate. A solution of phosgene in toluene (20% w/w, 9.52 mL, 18.0 mmol) was added to a suspension of methyl 4-amino-1*H*-indazole-1-carboxylate[17] (1.72 g, 9.00 mmol) in toluene (300 mL), and the mixture was heated to reflux. After 3.5 h the solution was cooled and concentrated in vacuo. The residue was taken up in diethyl ether (150 mL) and triethylamine (10 mL) and filtered. The filtrate was diluted with Et_2O to a final volume of 325 mL to give a stock solution of methyl 4-isocyanato-1*H*-indazole-1-carboxylate of approximately 0.028 M.

A solution of (4-(azepan-1-yl)-2-chlorophenyl)methanamine (1.16 g, 4.86 mmol, synthesized from 2-chloro-4-fluorobenzonitrile and hexamethyleneimine as described for compound **30**) in THF (10 mL) was added to an aliquot of 4-isocyanato-1*H*-indazole-1-carboxylate solution (175 mL, 4.86 mmol), and the mixture was stirred for 18 h at ambient temperature. The precipitate was collected by filtration, washed with Et_2O, and dried under vacuum, resulting in 1.68 g (76%) of the title compound as a white solid. ^1H NMR (DMSO-d_6): δ 9.01 (s, 1H), 8.42 (s, 1H), 7.85 (d, $J = 7.5$ Hz, 1H), 7.67 (d, $J = 8.5$ Hz, 1H), 7.47 (t, $J = 8.1$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 1H), 6.60−6.72 (m, 3H), 4.28 (d, $J = 5.4$ Hz, 2H), 4.03 (s, 3H), 3.43 (m, 4H), 1.70 (m, 4H), 1.44 (m, 4H). MS (ESI^+) *m*/*z* 456 ($M + H)^+$.

1-(4-Azepan-1-yl-2-chlorobenzyl)-3-(1*H*-indazol-4-yl)urea. To a suspension of methyl 4-(3-(4-(azepan-1-yl)-2-chlorobenzyl)-ureido)-1*H*-indazole-1-carboxylate (1.60 g, 3.51 mmol) in methanol (40 mL) was added 20 mL of NaOH/MeOH solution (1 g/ 20 mL). After 30 min the reaction mixture was diluted with water and extracted with ethyl acetate. The combined organics were washed with water and brine and dried (Mg_2SO_4), and the solvent was removed in vacuo. The residue was triturated with Et_2O and the solid collected by vacuum filtration to afford 1.14 g (82%) of the title compound as a white solid. ^1H NMR (DMSO-d_6): δ 12.96 (br s, 1H), 9.00 (s, 1H), 8.14 (s, 1H), 7.65 (d, $J = 7.5$ Hz, 1H), 7.14−7.25 (m, 2H), 6.97−7.07 (m, 2H), 6.60−6.72 (m, 2H), 4.26 (d, $J = 5.4$ Hz, 2H), 3.43 (m, 4H), 1.70 (m, 4H), 1.44 (m, 4H). MS (ESI^+) *m*/*z* 398 ($M + H)^+$. Anal. ($C_{21}H_{24}ClN_5O$) C, H, N.

1-(2,5-Difluoro-4-(8-azaspiro[bicyclo[3.2.1]octane-3,2′-[1,3]dioxolane]-8-yl)benzyl)-3-(1*H*-indazol-4-yl)urea (65): 8-Azaspiro[bicyclo[3.2.1]octane-3,2′-[1,3]dioxolane]. A solution of *N*-carbethoxy-4-tropinone (10.7 g, 54.2 mmol), ethylene glycol (3.70 g, 59.5 mmol), and *p*-toluenesulfonic acid (1.03 g, 5.41 mmol) in toluene (160 mL) was heated to reflux for 16 h. The solution was cooled, diluted by EtOAc, and washed with saturated $NaHCO_3$ solution and brine, dried (Na_2SO_4), and concentrated in vacuo to give 13.1 g (100%) of the desired ketal as a pale-brown oil. A sample of the ketal (4.48 g, 18.6 mmol), hydrazine hydrate (5.00 mL, 103 mmol), and potassium hydroxide (30.0 g, 535 mmol) in 150 mL of ethylene glycol was heated to reflux for 2 h. The solution was cooled and poured into water, and the product was extracted with Et_2O, then CH_2Cl_2. The combined organic extract was washed with brine, dried (Na_2SO_4), and concentrated in vacuo, resulting in 2.49 g (79%) of the title compound. ^1H NMR (DMSO-d_6): δ 3.87 (t, $J = 6.4$ Hz, 2H), 3.69 (t, $J = 6.3$ Hz, 2H), 3.38 (m, 2H), 2.02 (br s, 1H), 1.87 (m, 2H), 1.68 (m, 4H), 1.54 (m, 2H). MS (ESI^+) *m*/*z* 170 ($M + H)^+$.

(2,5-Difluoro-4-(8-azaspiro[bicyclo[3.2.1]octane-3,2′-[1,3]dioxolane]-8-yl)phenyl)methanamine (3, R^3 = 2,5-Difluoro, R^4 = 4-Tropinone Ethylenedioxy Ketal). A solution of 2,4,5-trifluorobenzonitrile (0.97 g, 6.21 mmol) and 8-azaspiro[bicyclo[3.2.1]-octane-3,2′-[1,3]dioxolane] (1.05 g, 6.21 mmol) in DMSO (10 mL) was heated to 120 °C. After 4 h the mixture was cooled and partitioned between EtOAc and H_2O. The aqueous phase was extracted with EtOAc, and the combined organic extract was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was crystallized from 1:1 diethyl ether/hexane, resulting in 1.23 g, (65%) of the intermediate nitrile as a tan powder. This nitrile (1.23 g, 3.99 mmol) was taken up in 10 mL of THF followed by addition of 1M $BH_3 \cdot$THF (12.0 mL, 12.0 mmol), and the mixture was heated to reflux. After 2 h the solution

was cooled to ambient temperature, and the reaction was quenched by careful addition of 3 N NaOH solution and stirred for 30 min. The mixture was partitioned between EtOAc and H_2O, and the separated organic phase was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (5−10% CH_3OH/CH_2Cl_2) yielded 0.91 g (73%) of the title compound as a colorless oil. ^{1}H NMR (DMSO-d_6): δ 7.18 (dd, J = 14.6, 7.1 Hz, 1H), 6.77 (dd, J = 12.6, 7.5 Hz, 1H), 4.28 (m, 2H), 3.94 (t, J = 6.4 Hz, 2H), 3.70 (t, J = 6.4 Hz, 2H), 3.61 (s, 2H), 2.03 (m, 2H), 1.64−1.94 (m, 8H). MS (ESI$^+$) m/z 294 $(M - NH_2)^+$.

Methyl 4-((2,5-Dioxopyrrolidin-1-yloxy)carbonylamino)-1*H*-indazole-1-carboxylate. Methyl 4-amino-1*H*-indazole-1-carboxylate[17] (1.9 g, 10 mmol) and disuccinimidyl carbonate (2.8 g, 11 mmol) were mixed in acetonitrile (100 mL) for 48 h under a nitrogen atmosphere. The solid was filtered off, washed with acetonitrile (10 mL), and dried under vacuum at ambient temperature to give 2.56 g (77%) of the title compound as an off-white solid.

Methyl 4-(3-(2,5-Difluoro-4-(8-azaspiro[bicyclo[3.2.1]octane-3,2′-[1,3]dioxolane]-8-yl)benzyl)ureido)-1*H*-indazole-1-carboxylate. Solid methyl 4-((2,5-dioxopyrrolidin-1-yloxy)carbonylamino)-1*H*-indazole-1-carboxylate (0.89 g, 2.69 mmol) was added to a solution of (2,5-difluoro-4-(8-azaspiro[bicyclo[3.2.1]octane-3,2′-[1,3]dioxolane]-8-yl)phenyl)methanamine (0.84 g, 2.69 mmol) and diisopropylethylamine (0.47 mL, 2.69 mmol) in DMF (15 mL) at ambient temperature. After 30 min water was added, and the resulting precipitate was collected by filtration, washed with water, and dried under high vacuum. The result was 1.27 g (90%) of the title compound as a tan solid. ^{1}H NMR (DMSO-d_6): δ 9.00 (s, 1H), 8.42 (s, 1H), 7.82 (d, J = 7.5 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 7.48 (t, J = 8.1 Hz, 1H), 7.13 (dd, J = 14.2, 7.1 Hz, 1H), 6.87 (dd, J = 12.7, 7.6 Hz, 1H), 6.76 (t, J = 5.6 Hz, 1H), 4.24−4.35 (m, 4H), 4.03 (s, 3H), 3.94 (t, J = 6.4 Hz, 2H), 3.70 (t, J = 6.4 Hz, 2H), 2.03 (m, 2H), 1.85 (m, 4H), 1.70 (m, 2H). MS (ESI$^+$) m/z 528 $(M + H)^+$.

1-(2,5-Difluoro-4-(8-azaspiro[bicyclo[3.2.1]octane-3,2′-[1,3]dioxolane]-8-yl)benzyl)-3-(1*H*-indazol-4-yl)urea. To a suspension of methyl 4-(3-(2,5-difluoro-4-(8-azaspiro[bicyclo[3.2.1]octane-3,2′-[1,3]dioxolane]-8-yl)benzyl)ureido)-1*H*-indazole-1-carboxylate (1.20 g, 2.27 mmol) in methanol (15 mL) was added 6 mL of NaOH/MeOH solution (1 g/20 mL). After 30 min the reaction mixture was diluted with water and extracted with ethyl acetate. The combined organics were washed with water and brine and dried (Mg_2SO_4), and the solvent was removed in vacuo. The residue was triturated with Et_2O and the solid collected by vacuum filtration to afford 1.06 g (99%) of compound **65** as a tan solid. ^{1}H NMR (DMSO-d_6): δ 12.99 (s, 1H), 8.71 (s, 1H), 8.07 (s, 1H), 7.62 (d, J = 7.5 Hz, 1H), 7.02−7.24 (m, 3H), 6.87 (dd, J = 12.6, 7.8 Hz, 1H), 6.74 (t, J = 5.6 Hz, 1H), 4.21−4.36 (m, 4H), 3.94 (t, J = 6.4 Hz, 2H), 3.70 (t, J = 6.4 Hz, 2H), 2.04 (m, 2H), 1.87 (m, 4H), 1.70 (m, 2H). MS (ESI$^+$) m/z 470 $(M + H)^+$. Anal. ($C_{24}H_{25}F_2N_5O_3$) C, H, N.

1-(1-Methyl-1*H*-indazol-4-yl)-3-((1-methyl-1*H*-indol-5-yl)-methyl)urea (67): 2,2,2-Trichloro-*N*-(1-methyl-1*H*-indazol-4-yl)-acetamide. A mixture of 1-methyl-1*H*-indazole-4-ylamine[17] (1.32 g, 8.98 mmol) and triethylamine (1.30 mL, 9.34 mmol) in CH_2Cl_2 (50 mL) at 0 °C was treated with trichloroacetyl chloride (1.30 mL, 11.6 mmol) dropwise. The mixture was slowly warmed to room temperature overnight. After removal of volatiles in vacuo, the residue was chromatographed on silica gel (98:2 CH_2Cl_2/CH_3OH, eluant) to afford 465 mg (18%) of the title compound as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 11.11 (s, 1H), 7.92 (s, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.43 (m, 1H), 7.19 (d, J = 7.1 Hz, 1H), 4.06 (s, 3H).

(1-Methyl-1*H*-indol-5-yl)methanamine (21). To a solution of 5-cyanoindole (1.0 g, 7.0 mmol) in acetone (25 mL) at 0 °C was added powdered KOH (2.0 g, 35 mmol), followed by methyl iodide (0.90 mL, 14 mmol). The mixture was stirred at room temperature for 1 h and then treated with dry toluene (10 mL). The mixture was filtered, and the filtrate was dried over Na_2SO_4 and evaporated in vacuo to afford 0.90 g (82%) of the *N*-methylindole **20** as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 8.09 (s, 1H), 7.64 (d, J = 8.5 Hz, 1H), 7.55 (d, J = 3.4 Hz, 1H), 7.50 (dd, J = 8.4, 1.7 Hz, 1H), 6.59 (d, J = 3.0 Hz, 1H), 3.85 (s, 3H). MS (APCI$^+$) m/z 157.0 $(M + H)^+$.

To a solution of **20** (0.90 g, 5.8 mmol) in THF (40 mL) was added 1 M BH_3·THF (21 mL, 21 mmol) dropwise via addition funnel. The mixture was refluxed overnight, cooled to 0 °C, and quenched carefully with 2 N HCl, and the volatiles were removed in vacuo. The remaining aqueous layer was washed with ether, then basified with 5 N NaOH and extracted with EtOAc. The extracts were finally washed with H_2O and brine, dried (Na_2SO_4), and concentrated in vacuo to afford 310 mg (28%) of compound **21** as a yellow oil, which was used without further purification.

1-(1-Methyl-1*H*-indazol-4-yl)-3-((1-methyl-1*H*-indol-5-yl)-methyl)urea. A solution of **21** (305 mg, 1.91 mmol), 2,2,2-trichloro-*N*-(1-methyl-1*H*-indazol-4-yl)acetamide (462 mg, 1.59 mmol), and DBU (0.52 mL, 3.48 mmol) in acetonitrile (40 mL) was refluxed overnight. After the mixture was cooled to room temperature, the volatiles were removed in vacuo and the residue was taken up in EtOAc and washed with saturated NH_4Cl solution. After the mixture was dried (Na_2SO_4) and concentrated in vacuo, the residue was chromatographed on silica gel (99:1 to 98:2 CH_2Cl_2/CH_3OH, eluant gradient) to afford 320 mg (60%) of compound **67** as a tan solid. ^{1}H NMR (DMSO-d_6): δ 8.70 (s, 1H), 8.02 (s, 1H), 7.69 (d, J = 7.5 Hz, 1H), 7.51 (s, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.30 (d, J = 3.1 Hz, 1H), 7.25 (m, 2H), 7.14 (m, 1H), 6.72 (m, 1H), 6.40 (d, J = 3.1 Hz, 1H), 4.41 (d, J = 5.8 Hz, 2H), 3.99 (s, 3H), 3.78 (s, 3H). MS (ESI$^+$) m/z 334 $(M + H)^+$, 356 $(M + Na)^+$. Anal. ($C_{19}H_{19}N_5O$) C, H, N.

1-(4-(8-Azabicyclo[3.2.1]octan-8-yl)-3-methylbenzyl)-3-(1*H*-indazol-4-yl)urea Trifluoroacetate (71): (4-(8-Azabicyclo[3.2.1]-octan-8-yl)-3-methylphenyl)methanamine (3, R^3 = 3-CH_3, R^4 = Tropanyl). A mixture of 4-bromo-3-methylbenzonitrile (700 mg, 3.57 mmol), 8-azabicyclo[3.2.1]octane hydrochloride[19] (630 mg, 4.27 mmol), BINAP (200 mg, 0.32 mmol), $Pd_2(dba)_3$ (100 mg, 0.11 mmol), and sodium *tert*-butoxide (850 mg, 8.84 mmol) in toluene (100 mL) was heated to reflux. After 18 h the mixture was cooled to ambient temperature, filtered through Celite, and concentrated in vacuo. The residue was column-chromatographed on SiO_2, eluting with 25% EtOAc/hexane which yielded 4-(8-azabicyclo-[3.2.1]octan-8-yl)-3-methylbenzonitrile that was contaminated by an unidentifiable side product. A solution of this mixture in THF (30 mL) was added dropwise to a mixture of $LiAlH_4$ (531 mg, 13.4 mmol) in THF (75 mL), and the mixture was heated to reflux. After 1.5 h the mixture was cooled to 0 °C and the reaction was quenched by careful addition of Na_2SO_4·$10H_2O$. The mixture was stirred for 30 min, filtered through Celite, and concentrated in vacuo to give 710 mg (86%) of the title compound as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 7.05 (d, J = 2.0 Hz, 1H), 6.96 (dd, J = 8.1, 2.0 Hz, 1H), 6.76 (d, J = 8.1 Hz, 1H) 3.63 (m, 2H), 3.57 (s, 2H), 3.31 (s, 2H), 2.26 (br s, 3H), 1.44−1.91 (m, 10H).

Compound **71** was synthesized using (4-(8-azabicyclo[3.2.1]-octan-8-yl)-3-methylphenyl)methanamine and methyl 4-amino-1*H*-indazole-1-carboxylate[17] and the process described for compound **50**. The product was purified by HPLC (Waters reverse-phase column 40 mm × 100 mm, 0.1% TFA/H_2O/MeCN), which afforded **71** as a white solid (yield 41%). ^{1}H NMR (DMSO-d_6): δ 12.95 (s, 1H), 9.14 (s, 1H), 8.28 (s, 1H), 7.67 (d, J = 7.1 Hz, 1H), 7.18 (m, 2H), 7.09 (d, J = 1.7 Hz, 1H), 7.01 (m, 2H), 6.81 (d, J = 8.1 Hz, 1H), 4.21 (d, J = 5.8 Hz, 2H), 3.65 (m, 2H), 2.28 (s, 3H), 1.73−1.92 (m, 4H), 1.65 (m, 3H), 1.50 (m, 3H). MS (ESI$^+$) m/z 390 $(M + H)^+$. HRMS calcd ($C_{23}H_{27}N_5O$): 390.228 84. Obsvd: 390.228 83.

1-(2-Benzyl-4-(8-azabicyclo[3.2.1]octan-8-yl)benzyl)-3-(1*H*-indazol-4-yl)urea (73): (2-Benzyl-4-(8-azabicyclo[3.2.1]octan-8-yl)phenyl)methanamine (3, R^3 = 3-Benzyl, R^4 = Tropanyl). 2-Bromo-4-fluorobenzonitrile (1.25 g, 6.25 mmol), 8-azabicyclo-[3.2.1]octane hydrochloride[19] (1.02 g, 6.87 mmol), and diisopropylethylamine (2.18 mL, 12.5 mmol) were combined in DMSO (15 mL) and heated to 130 °C for 25 min in a microwave reactor. The mixture was partitioned between EtOAc and half-saturated $NaHCO_3$ solution. The separated organic phase was washed with

water and brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was triturated with Et_2O and the solid collected by vacuum filtration to give 0.85 g (47%) of 4-(8-azabicyclo[3.2.1]-octan-8-yl)-2-bromobenzonitrile (**17**, R^4 = tropanyl) as a light-yellow solid. ^{1}H NMR (DMSO-d_6): δ 7.57 (d, J = 8.8 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 6.84 (dd, J = 8.8, 2.37 Hz, 1H), 4.36 (m, 2H), 1.76−2.03 (m, 5H), 1.64 (m, 2H), 1.31−1.50 (m, 3H). MS (ESI$^+$) m/z 291/293 $(M + H)^+$.

A 2 M solution of benzylmagnesium bromide in THF (2.30 mL, 4.60 mmol) was added dropwise to a mixture of $ZnCl_2$ (2.30 mmol) in dioxane (15 mL) at ambient temperature. The mixture was stirred for 30 min followed by addition of solid bromonitrile **17** (335 mg, 1.15 mmol) and Pd(dppf)$_2$Cl$_2$. The mixture was heated to reflux for 3 h, then cooled to ambient temperature, quenched with methanol, and diluted by EtOAc and 3 M NaOH solution. The mixture was poured into water, and the separated organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (20% EtOAc/hexane) yielded 316 mg (91%) of 2-benzyl-4-(8-azabicyclo[3.2.1]octan-8-yl)benzonitrile (**18**, R^3 = benzyl, R^4 = tropanyl) as a colorless oil. ^{1}H NMR (DMSO-d_6): δ 7.47 (d, J = 8.82 Hz, 1H), 7.15−7.34 (m, 5H), 6.84 (d, J = 2.4 Hz, 1H), 6.71 (dd, J = 8.7, 2.5 Hz, 1H), 4.30 (m, 2H), 4.00 (s, 2H), 1.76−2.02 (m, 5H), 1.64 (m, 2H), 1.44 (m, 1H), 1.32 (m, 2H). MS (ESI$^+$) m/z 303 $(M + H)^+$.

The intermediate benzylnitrile **18** from above (310 mg, 1.03 mmol) was hydrogenated at 60 psi in the presence of Raney nickel (3 g) in 30 mL of MeOH/NH_3 at ambient temperature. After 2 h the catalyst was filtered and the filtrate concentrated in vacuo. The residue was purified by flash chromatography (5−10% CH_3OH/CH_2Cl_2) to give 243 mg (76%) of (2-benzyl-4-(8-azabicyclo[3.2.1]-octan-8-yl)phenyl)methanamine as a pale-brown oil. ^{1}H NMR (DMSO-d_6): δ 7.26 (m, 2H), 7.16 (m, 4H), 6.60 (m, 2H), 4.10 (m, 2H), 3.95 (s, 2H), 3.55 (s, 2H), 2.08 (br s, 2H), 1.94 (m, 2H), 1.68−1.87 (m, 5H), 1.36 (m, 1H), 1.20 (m, 2H). MS (ESI$^+$) m/z 307 $(M + H)^+$.

Compound **73** was synthesized using (2-benzyl-4-(8-azabicyclo-[3.2.1]octan-8-yl)phenyl)methanamine and methyl 4-amino-1*H*-indazole-1-carboxylate[17] and the process described for compound **65** (yield 87%). ^{1}H NMR (DMSO-d_6): δ 12.87 (s, 1H), 8.53 (s, 1H), 8.20 (s, 1H), 8.02 (s, 1H), 7.66 (d, J = 6.8 Hz, 1H), 7.10−7.29 (m, 6H), 7.03 (d, J = 9.0 Hz, 1H), 6.63 (m, 2H), 6.46 (t, J = 5.1 Hz, 1H), 4.21 (d, J = 5.1 Hz, 2H), 4.13 (m, 2H), 3.99 (s, 2H), 1.99 (m, 2H), 1.81 (m, 5H), 1.41 (m, 1H), 1.21 (m, 2H). MS (ESI$^+$) m/z 466 $(M + H)^+$. Anal. ($C_{29}H_{31}N_5O$) C, H, N.

1-(2-Benzyl-4-(8-azabicyclo[3.2.1]octan-8-yl)benzyl)-3-(1-methyl-1*H*-indazol-4-yl)urea (74). To a solution of compound **73** (292 mg, 0.627 mmol) in DMSO (5 mL) was added NaH (60% in oil, 28 mg, 0.70 mmol) at ambient temperature. After 1.5 h, dimethyl sulfate (66 μL, 0.69 mmol) was added. The reaction was allowed to proceed for 1 h, and then the mixture was partitioned between EtOAc and half-saturated NaCl solution. The separated organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (40−75% EtOAc/hexane) gave 122 mg (69%) of the title compound as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.61 (s, 1H), 8.00 (s, 1H), 7.68 (d, J = 7.5 Hz, 1H), 7.09−7.31 (m, 8H), 6.65 (m, 2H), 6.50 (t, J = 5.1 Hz, 1H), 4.18 (d, J = 5.1 Hz, 2H), 4.12 (m, 2H), 3.99 (m, 5H), 1.95 (m, 2H), 1.66−1.86 (m, 5H), 1.38 (m, 1H), 1.20 (m, 2H). MS (ESI$^+$) m/z 480 $(M + H)^+$. Anal. ($C_{30}H_{33}N_5O$) C, H, N.

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Supporting Information Available: Experimental procedures for synthesis of **31**−**39**, **41**−**49**, **51**−**64**, **66**, **68**−**70**, **72**, **75**, and **76**; details of the Ca^{2+} influx functional in vitro assay and complete Freund's adjuvant in vivo assay; and results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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