Identification and Biological Evaluation of 4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (5-Trifluoromethylpyridin-2-yl)amide, a High Affinity TRPV1 (VR1) Vanilloid Receptor Antagonist

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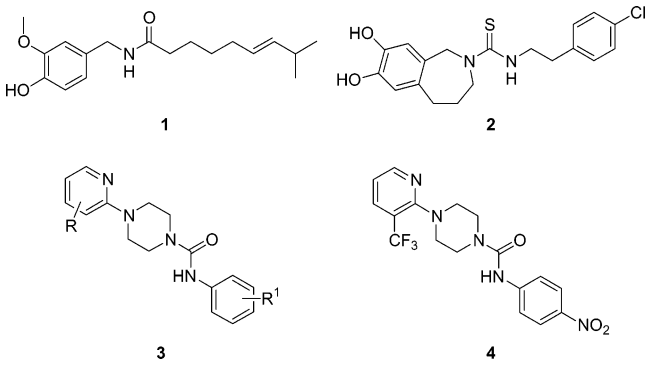
High throughput screening using the recombinant human TRPV1 receptor was used to identify a series of pyridinylpiperazine ureas (**3**) as TRPV1 vanilloid receptor ligands. Exploration of the structure−activity relationships by parallel synthesis identified the essential pharmacophoric elements for antagonism that permitted further optimization via targeted synthesis to provide a potent orally bioavailable and selective TRPV1 modulator **41** active in several in vivo models.

Introduction

The transient receptor potential (TRP) family represents a large class of ion channels characterized by their permeability to Ca^{2+} together with a common proposed primary structure consisting of six membrane spanning domains. The family may be divided into several classes based on sequence homology and functional behavior.[1,2] Of the families, TRPV1 (VR1) is the best characterized. The TRPV1 receptor has received considerable attention following the cloning of the rat[3] and human[4] isoforms together with the more recent characterization of the TRPV1 knockout mice by two groups.[5,6] Thus the knockout studies demonstrated that TRPV1 is the cellular target of capsaicin (**1**), the active component of chili peppers, and plays a role in the signaling of noxious stimuli, including heat and pH. Additionally the receptor is activated by a range of inflammatory mediators including bradykinin, lipoxygenase products, and the endocannabinoid anandamide.[7,8,9] Although capsaicin activates TRPV1 and is an irritant upon topical application, it ultimately leads to decreased sensitivity to painful stimuli and has consequently found therapeutic application for the treatment of pain associated with arthritis and diabetic neuropathy. The analgesic effects of TRPV1 agonists, including capsaicin, range from receptor desensitization to sensory neurite retraction depending on the degree of exposure. A close analogue of capsaicin, capsazepine (CPZ) (**2**), behaves as a competitive antagonist at TRPV1 and has been shown to afford anti-hyperalgesic effects not only against capsaicin challenge but also against other inflammatory stimuli.[10,11] Given the analgesic effects observed for both TRPV1 agonists and antagonists, TRPV1 receptor ligands represent an important opportunity for the discovery of novel analgesic agents which has attracted the attention of several pharmaceutical companies and academic laboratories.[7,12] Thus, we initiated a high throughput screening (HTS) of our corporate compound collection using a fluorescence cell-based assay utilizing the Ca^{2+} permeability of TRPV1.[13] This led to the identification of several series of agonists and antagonists, including a series of pyridinylpiperazine ureas (**3**),[14] a structural motif that was subsequently described by several other groups.[15−18] Activity validation for a representative hit (**4**) was accomplished via resynthesis and retesting which then prompted a detailed evaluation of SAR.



Chemistry

The low purity of the purchased libraries from which most of the HTS hits were obtained confounded

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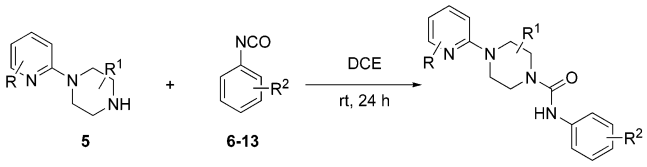
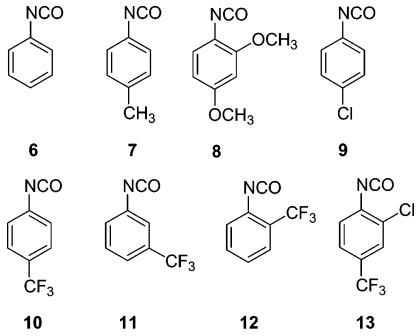
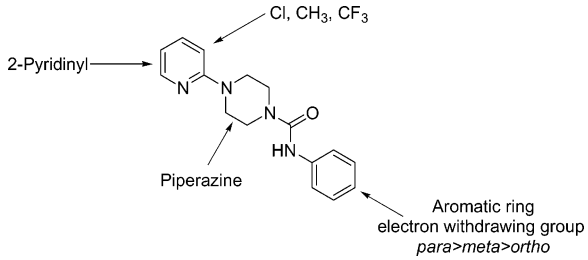
Scheme 1. Library Synthesis

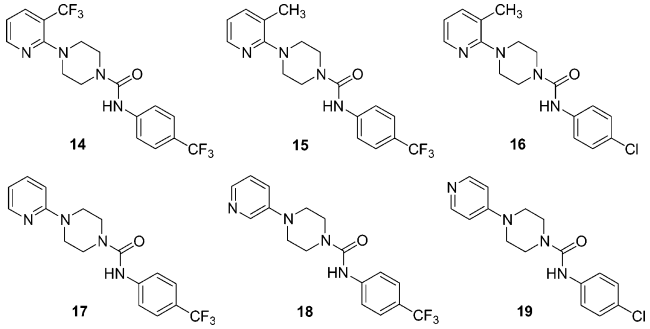


meaningful interpretation of structure−activity relationships (SARs). A broad range of highly pure analogues of **4** were therefore obtained using a straightforward matrix synthesis approach, which expedited SAR investigations. The synthetic approach focused upon the formation of the urea linkage (Scheme 1), which was readily constructed via the condensation of commercially available aryl isocyanates with substituted piperazines. The piperazine component, when not available commercially, was obtained via the condensation of a halopyridine with piperazine.[19] Thus heating the appropriate halopyridine with excess piperazine, to minimize the formation of disubstituted piperazines, in either *n*-butanol or acetonitrile, gave the corresponding pyridinylpiperazine in good yield. With less reactive halopyridines heating in neat piperazine was necessary. Eight commercially available aryl isocyanates **6**−**13**

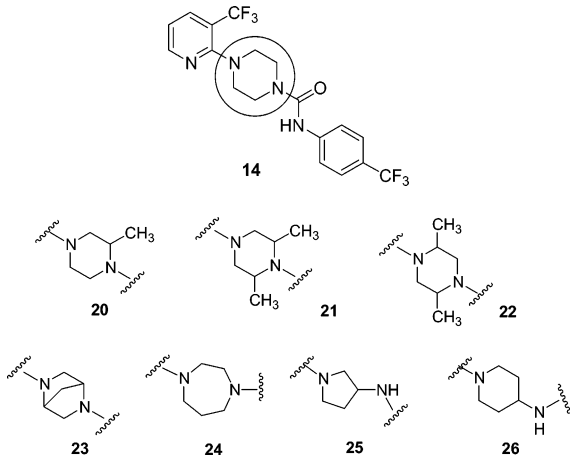


were selected and used to generate three 48-member libraries which identified both TRPV1 agonists and antagonists. The first library (Figure 1) demonstrated the desirability of an electron-withdrawing group in the para position of the aniline fragment for antagonist activity and suggested that 3-substituted pyridin-2-ylpiperazines were favored. In the second library (Figure 2) which contained no 3-substituted pyridines only low affinity agonists and antagonists were obtained. The third library (Figure 3) was most informative and clearly demonstrated the importance of a 3-substituted pyridin-2-ylpiperazine (3-Cl, 3-CH_3, and 3-CF_3) and a *p*-trifluoromethyl group in the aniline fragment. A fourth library, not shown, prepared from aliphatic isocyanates and 3-substituted pyridin-2-ylpiperazines afforded only inactive compounds, suggesting the need for an aromatic urea.





With the intrinsic activity of the pyridinylpiperazine template confirmed, we turned our attention to a more thorough investigation of SAR at the human receptor via targeted synthesis. To this end, specific changes to the pyridine, piperazine, and aniline fragments were made. When the pyridine point of attachment was examined, **17** and **18**, it was immediately apparent that the pyridin-2-ylpiperazine was optimal. A range of modifications to or replacements for the piperazine (**20**−**26**) showed that the piperazine ring was tolerant of



small substituents (e.g. **20**) but further substitution (**21**−**23**), ring expansion (**24**) or replacement with 3-aminopyrrolidine (**25**) or 4-aminopiperidine (**26**) afforded considerably less active compounds. Removal of a single piperazine nitrogen (**27** and **28**), using the chemistry of Scheme 2, afforded a less active compound as did removal of both a piperazine and the pyridine nitrogens, using the chemistry of Scheme 3 (**29** and **30**).

Prompted by the promising in vitro activity of **14**, particularly the behavior of the compound as an antagonist across species toward a range of stimuli, we sought to determine the in vivo efficacy of **14**. This was hindered by the poor physical properties of **14**, low solubility resulted in difficulty of formulation and prevented pharmacokinetic/pharmacodynamic evaluation. Therefore heterocyclic replacements for the aniline fragment of **14** were prepared. This was readily achieved by condensing pyridinylpiperazine **5m** with a range of aminoquinoline and aminopyridinephenyl carbamates.[20] However, both unsubstituted aminoquinolines and aminopyridines (**31**−**39**) were either less potent antagonists than **14** or agonists (**31**, **37**, **38**). Nonetheless, when a trifluoromethyl substituent was introduced into the pyridine ring, corresponding to the substitution in **14**,

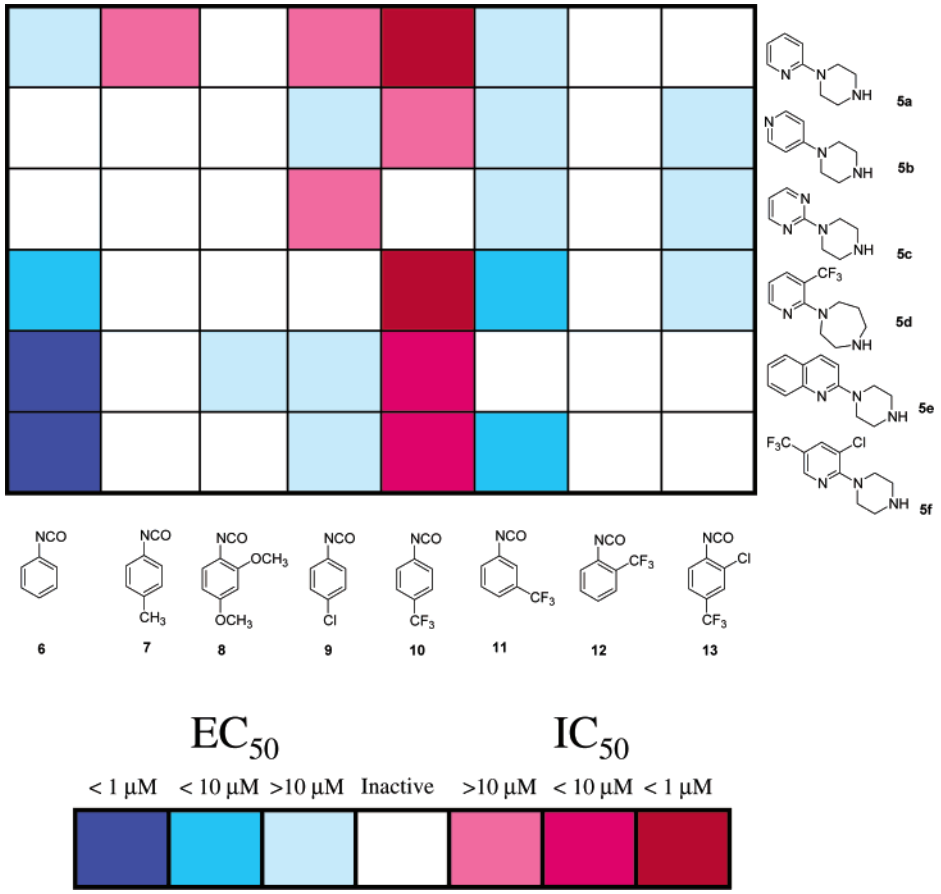


Figure 1. Library 1.

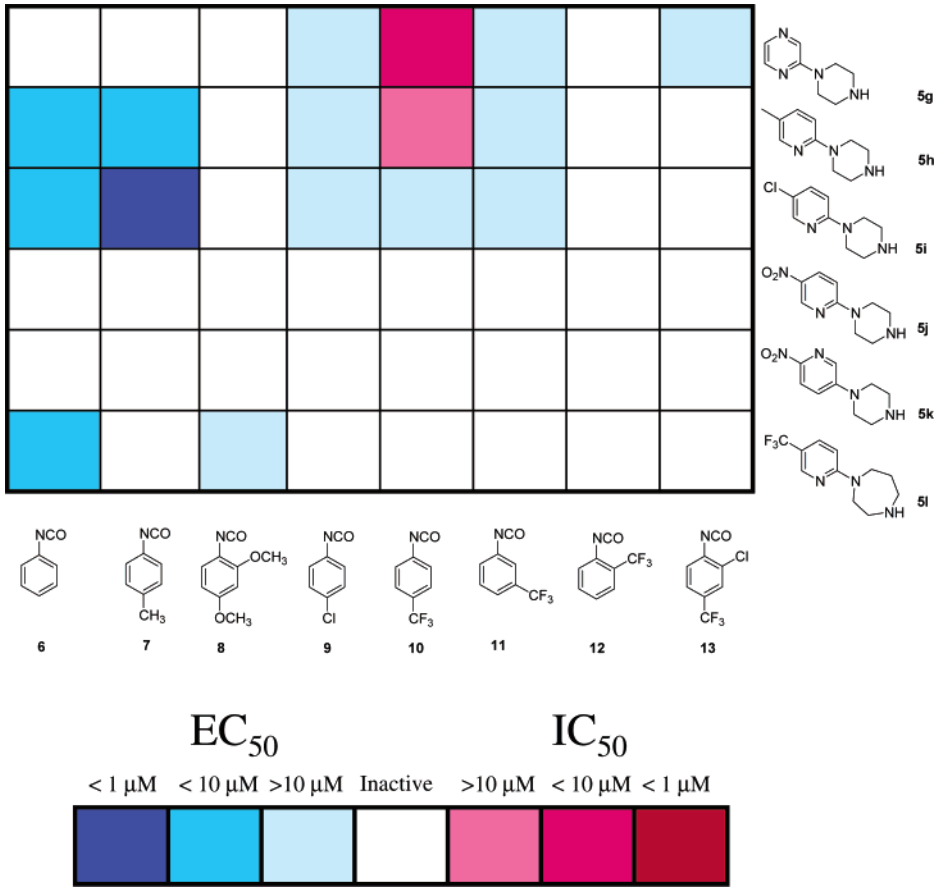


Figure 2. Library 2.

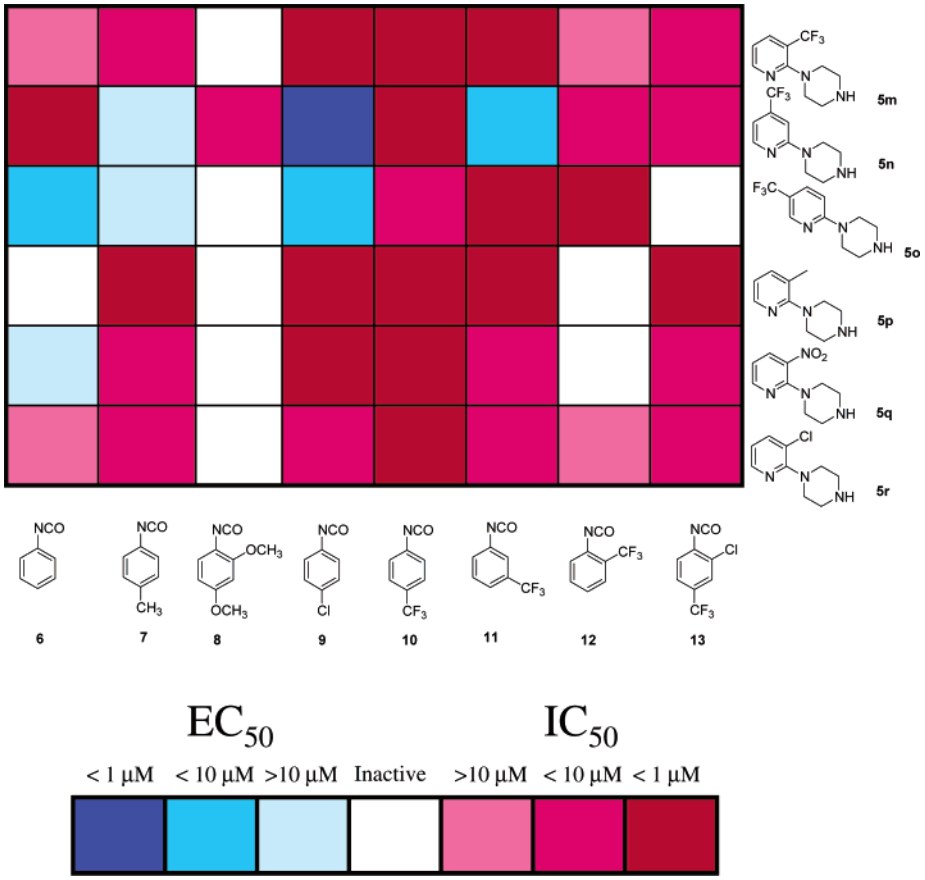
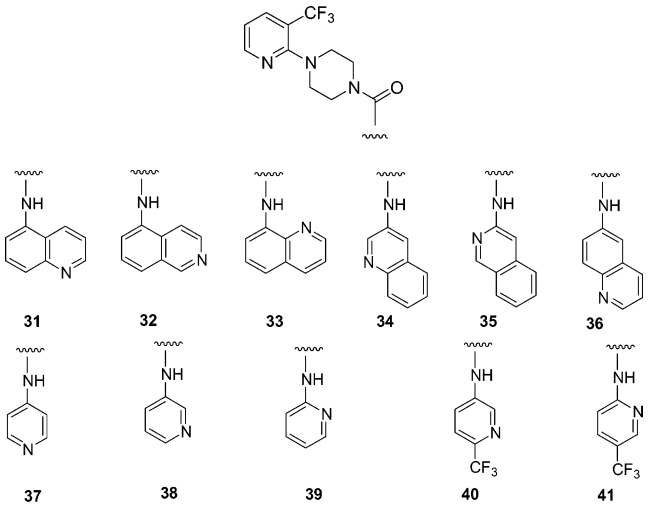


Figure 3. Library 3.

potency was improved (**40** and **41**). Notably **41** exhibited acceptable pharmacokinetic properties.

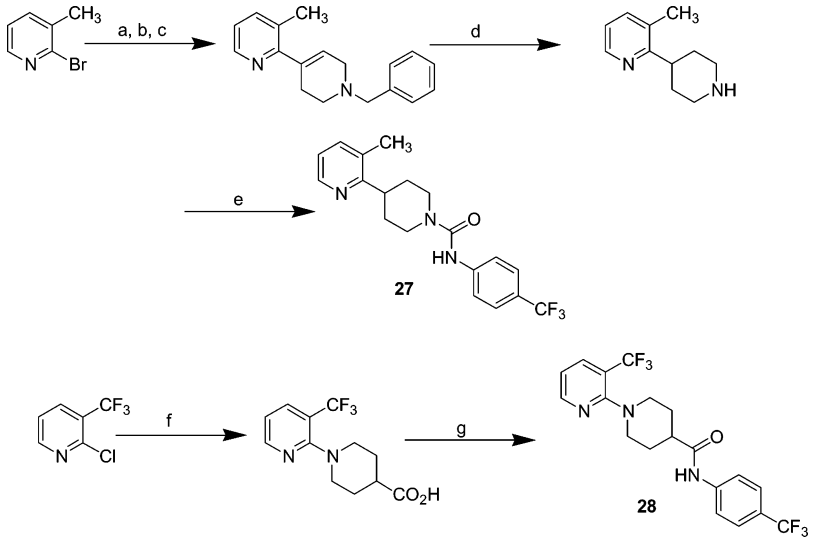


Biological Results

In Vitro Evaluation. Modulation of TRPV1 activity was assessed in vitro by measuring compound- or capsaicin-induced Ca^{2+} flux using FLIPR and HEK293 cells stably expressing recombinant human and rat TRPV1 (hTRPV1-HEK293 and rTRPV1-HEK293, respectively) as previously described.[21,13,22] Intracellular Ca^{2+} levels were measured in TRPV1-expressing cells during exposure to compounds. A concentration dependent increase in Ca^{2+} influx was observed using both human and rat cell lines for **19**, **30**, **31**, **37**, and **38** (Table 1). The responses elicited by these compounds were mediated by TRPV1: they required extracellular Ca^{2+} and TRPV1 expression and were blocked by the TRPV1 antagonists ruthenium red and capsazepine. The efficacy of agonists was normalized to the maximum response induced by capsaicin. Data are expressed as the $EC_{50} \pm$ SEM (Table 1). With the exception of **19**, these agonists elicited slowly desensitizing or sustained responses with steep concentration dependence (n_H values > 1.5). The unsubstituted pyridines **37** and **38** were full agonists, and the quinoline **31** was a partial agonist with low intrinsic activity at both human and rat TRPV1. Compound **19** revealed fast desensitizing kinetics and the EC_{50} value and Hill coefficient could not be accurately determined. Interestingly, the intrinsic activity of **30** differed significantly at the rat and human receptors. While **30** was a full agonist at rTRPV1, it was a partial agonist at the human receptor (Table 1). Nearly the opposite result was observed with **31**. Compounds exhibiting partial agonism concomitantly decreased the subsequent response to capsaicin applied near its EC_{80}. The block was surmountable by supramaximal concentrations of capsaicin compatible with the dependence of partial agonism on relative potencies and concentrations of the competing compounds. These results suggest that species differences in efficacy are independent of cell line.[23,24] The direct measure of channel activity using whole cell patch clamp methods will be required to determine the relative intrinsic efficacies of the agonists described here.

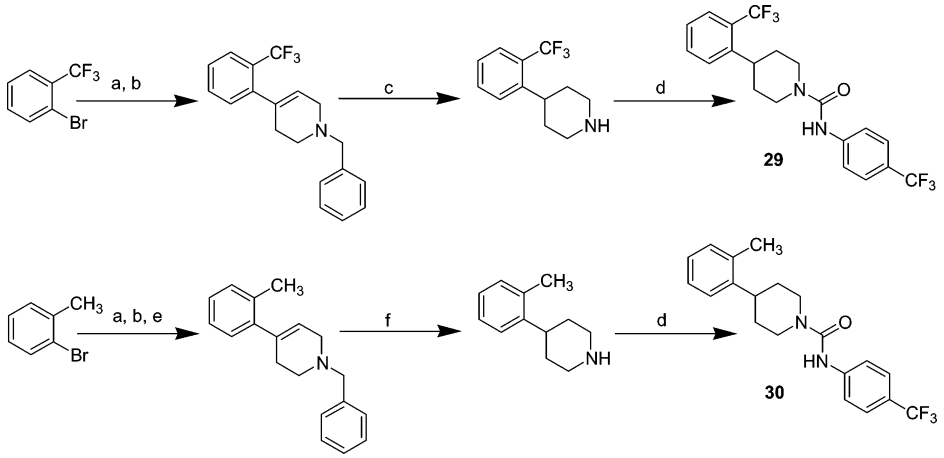
Most compounds had no detectable agonist activity but inhibited the influx of Ca^{2+} induced by the subse-

Scheme 2. Removal of Single Piperazine Nitrogen[a]



[a] Reagents and conditions: (a) (i) *n*-BuLi, −78 °C, THF; (b) *N*-benzyl-4-piperidone; THF; (c) $SOCl_2$, 18 h; (d) 10% Pd−C, EtOH/H_2, 60 psi, 18 h; (e) 4-trifluoromethylphenyl isocyanate, 1,2-dichloroethane, 18 h; (f) piperidine-4-carboxylic acid, CH_3CN, 80 °C, 18 h; (g) 4-trifluoromethylaniline, HATU, EtN(*i*-Pr)$_2$, CH_2Cl_2 16 h.

Scheme 3. Removal of Pyridine Nitrogen and a Single Piperazine Nitrogen[a]



[a] Reagents and conditions: (a) *n*-BuLi, −78 °C, THF; (b) *N*-benzyl-4-piperidone; (c) 10% Pd−C, EtOH/H_2, 60 psi; (d) 4-trifluoromethylphenylisocyanate, 1,2-dichloroethane, 18 h; (e) concentrated HCl−MeOH (1:1), reflux, 18 h; (f) 20% Pd(OH)$_2$, EtOH/H_2, 60 psi, 18 h.

quent addition of capsaicin applied at its EC_{80} in a dose dependent manner. Data are expressed as the $IC_{50} \pm$ SEM (Table 1). The rank order of potency to inhibit capsaicin-induced Ca^{2+} influx at the rat receptor was similar to that at hTRPV1.

Compounds **18** and **27** were partial agonists with low efficacy at the rat receptor (Table 1) but showed no detectable agonist activity up to 30 μM at the human receptor in Ca^{2+} flux and fluorescence membrane potential (not shown) assays. Instead, **18** and **27** blocked the response to low (15 nM) but not high (3 μM) capsaicin concentrations at human TRPV1.

We evaluated the mechanism of inhibition experiments by performing capsaicin concentration dependence in the presence and absence of compound **14**. Blockade of capsaicin-induced Ca^{2+} flux by compound **14** was surmountable and appeared to be competitive at both rat (Figure 4a) and human TRPV1. Furthermore, compound **14** displaced [^{3}H]-resiniferatoxin (RTX) binding with a K_i of 38 ± 15 nM ($n = 2$). Compound **41** has been previously shown to be a competitive antagonist at native capsaicin receptors expressed in rat dorsal root ganglion nociceptive neurons using the whole cell voltage clamp method.[22]

Since TRPV1 receptors are stimulated by endogenous proinflammatory substances, we tested whether TRPV1 antagonists could inhibit the activity elicited by low pH, PKC-dependent phosphorylation, and anandamide (see Biology Methods). The potency of selected compounds (**4**, **14**, and **41**) to inhibit acid-, phorbol 12-myristate-13-acetate (PMA)-, and anandamide-induced Ca^{2+} influx mediated by TRPV1 was determined using FLIPR. All three compounds effectively inhibited the responses induced by each of these stimuli at both human (Table 2) and rat (Table 3) receptors. Furthermore, activation of human TRPV1 by heat (45 °C) was completely blocked by 300 nM **14** and 10 μM ruthenium red (data not shown).

We evaluated the mechanism of inhibition by measuring the PMA concentration dependence in the presence and absence of compound **14**. Blockade of PMA-induced Ca^{2+} flux by compound **14** was concentration dependent and insurmountable and appeared to be noncompetitive at both rat (Figure 4b) and human

Table 1. Potency at Recombinant TRPV1[a]

compd	human EC_{50} (nM)	efficacy (%)	human IC_{50} (nM)	SEM (n)	rat EC_{50} (nM)	efficacy (%)	rat IC_{50} (nM)	SEM (n)
4			74	15 (6)			100	12 (3)
14			57	9 (13)			90	12 (4)
15			290	172 (3)			194	53 (3)
16			113	6 (4)			409	221 (3)
17			1220	353 (4)			863	262 (3)
18			5030	2540 (3)	2670	18 ± 3		735 (3)
19	7240	100		2250 (4)	3600	>40		800 (2)
20			64	9 (4)			104	58 (4)
21			2450	808 (4)			3720	908 (3)
22			2290	47 (3)			2280	125 (2)
23			>10000	(3)			>10000	(2)
24			538	116 (3)			1180	198 (2)
25	>10000, IA[b]		>10000	(3)			13100	6710 (3)
26			5030	2570 (3)			4360	860 (2)
27			2170	837 (4)	1960	24 ± 2		390 (4)
28			965	65 (2)			>10000	(2)
29			1310	107 (3)			1480	125 (2)
30	1930	8 ± 1		175 (2)	1720	86 ± 3		302 (3)
31	5260	36 ± 10		2750 (3)	8300	2		1700 (3)
32			2280	522 (4)	7290	11	>10000, >10000	(3)
33			8130	2890 (3)			14700	4650 (2)
34			526	70 (5)			2300	700 (3)
35			367	69 (3)			1010	590 (2)
36			908	99 (4)			3975	25 (2)
37	1160	120 ± 12		318 (3)	1280	100 ± 0		300 (3)
38	618	121 ± 11		178 (3)	275	105 ± 5		90 (4)
39			12100	4890 (2)			>10000	(3)
40			334	13 (3)			605	95 (2)
41			65	16 (8)			102	12 (6)
capsazepine			74	14 (5)			365	135 (2)
ruthenium red			260	36 (5)			220	55 (3)

[a] Values are mean ± SEM for number of determinations in parentheses. [b] IA: inactive (no detectable effect up to 30 μM).

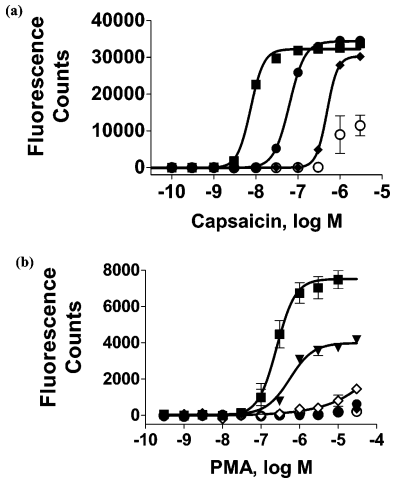


Figure 4. Antagonist **14** competitively inhibited the capsaicin-induced increase in intracellular Ca^{2+} and noncompetitively inhibited the PMA-induced response. (a) rTRPV1-HEK293 cells were incubated for 3 min in vehicle (square), or compound **14** at 100 nM (solid circle), 1000 nM (diamond), or 3000 nM (clear circle), and the Ca^{2+} response (in fluorescence counts) elicited by exposure to the indicated concentrations of capsaicin is shown. Each point is the main ± SEM of duplicate values. Block by **14** was surmountable. The EC_{50} for capsaicin was 8 nM [6 to 9 nM, 95% confidence intervals], 63 nM [60 to 65 nM], and 490 nM [464 to 517] in the absence, and presence of 100 and 1000 nM **14**, respectively. The Hill coefficients were similar (2.8 [1.8−3.7, 95% confidence interval], 2.3 [2.2−2.5], and 3.4 [3.1−3.8], respectively), consistent with competitive inhibition. (b) rTRPV1-HEK293 cells were incubated for 3 min in vehicle (square), or compound **14** at 10 nM (triangle), 30 nM (clear diamond), 100 nM (solid circle), 1000 nM (diamond), or 3000 nM (clear circle), and the Ca^{2+} response (in fluorescence counts) elicited by exposure to the indicated concentrations of PMA is shown. Block was insurmountable, consistent with noncompetitive mechanism of inhibition. Sigmoidal fits were not constrained (GraphPad Prism). Error bars represent the SEM, and in cases where they are not visible, they are smaller than the size of the symbol.

Table 2. Antagonism of Recombinant Human TRPV1 Activated by a Panel of Stimuli in a Ca^{2+} Influx in Vitro Assay (IC_{50} in nM)

compound	low pH (nM)	PMA (nM)	anandamide (nM)
4	54 ± 14 (6)	38 ± 11 (3)	104 ± 23 (4)
14	20 ± 5 (3)	39 ± 17 (3)	45 ± 14 (5)
41	16 ± 4 (3)	13 ± 3 (3)	75 ± 29 (5)
capsazepine (CPZ)	64 ± 27 (4)	195 ± 39 (5)	85 ± 51 (3)
ruthenium red (RR)	283 ± 89 (2)	510 ± 159 (3)	391 ± 31 (3)

TRPV1. The protein kinase C (PKC) inhibitors bisindolylmaleimide I and staurosporine completely blocked the effect of the phorbol esters PMA and phorbol 12,-13-dibutyrate (PDBu) (data not shown) but did not occlude subsequent capsaicin-induced TRPV1 activity.

The ability of **41** (as well as **4** and **14**) to inhibit responses evoked by protons and phosphorylation may account for the efficacy of this class of molecules in rodent models of inflammatory pain.[17] Compound **41**, but not capsazepine, potently inhibited PMA-induced responses at rat TRPV1 (Table 3).

In Vivo Evaluation. Pharmacokinetics Pharmacokinetic assessment of **41** was performed in the rat at doses of 2.4 μmol/kg iv bolus, and 24 μmol/kg by the oral (po) and intraperitoneal (ip) routes (n = 2−4). The elimination half-life after iv administration was 7.4 ± 0.8 h (mean ± SE), with a clearance of 0.29 ± 0.04 L/h and a calculated volume of distribution of 3.1 ± 0.6 L. Oral bioavailability was 100%, and ip bioavailability was 66%. Peak plasma concentrations of 0.9 ± 0.2 μmol/L (po) and 0.05 ± 0.02 μmol/L (ip) occurred between 2 and 3 h after administration.

Pharmacological Models. Several in vivo effects of vanilloid agonists are readily quantifiable and thus are useful for pharmacological evaluation of potential antagonists. The noxious effects of topical capsaicin are familiar to most. We studied the ability of **41** to prevent sensory effects produced by local injection of capsaicin

Table 3. Antagonism of Recombinant Rat TRPV1 Activated by a Panel of Stimuli in a Ca^{2+} Influx in Vitro Assay (IC_{50} in nM)

compound	low pH (nM)	PMA (nM)	anandamide (nM)
4	117 ± 27 (3)	21	104 ± 44 (3)
14	13 ± 4 (4)	14 ± 2 (3)	17 ± 5 (4)
41	16 ± 5 (3)	17 ± 5 (3)	74 ± 18 (5)
capsazepine (CPZ)	6550 ± 2000 (4)	5550, >3000, >3000 (3)	270 ± 104 (3)
ruthenium red (RR)	246 ± 85 (2)	397 ± 67 (3)	359 ± 107 (3)

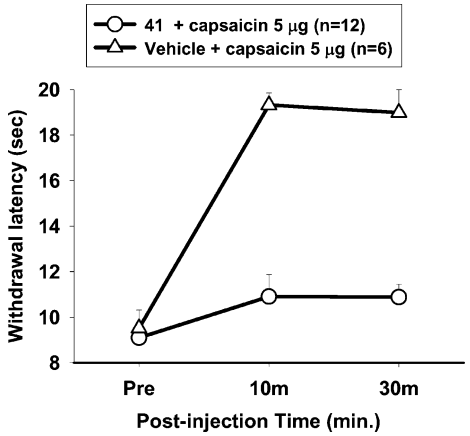


Figure 5. Compound **41** prevents capsaicin-evoked thermal desensitization. Rats dosed with 24 μmol/kg **41**, po, 1 h before intraplantar capsaicin, 5 μg, to one hindpaw, maintained normal thermal hindpaw paw withdrawal latencies (*X*-axis, latency to withdrawal, seconds) in a modified Hargreaves device. Control group rats exhibited marked prolongation of thermal thresholds indicating desensitization due to activation of TRPV1 receptors. $N = 6-12$ per group

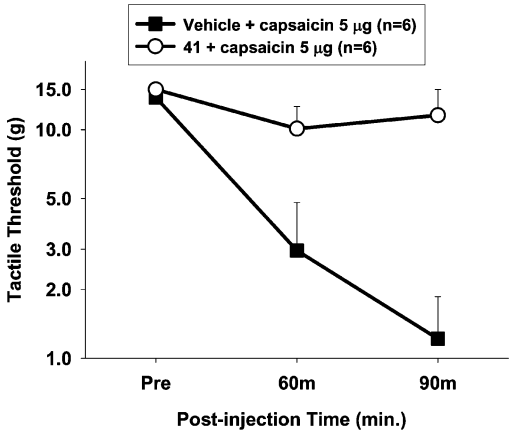


Figure 6. Compound **41** prevents capsaicin-evoked tactile allodynia in rats orally dosed with 24 μmol/kg compound **41** 90 min prior to intraplantar administration of capsaicin, 5 mg, to one hindpaw, maintained normal paw withdrawal thresholds in the range of 12−15 g, to probing with calibrated von Frey filaments. Control group rats developed profound sensitivity to this ordinarily inoffensive light touch. *X*-axis, semilog representation of 50% paw withdrawal threshold, grams. $N = 6$ per group.

or *N*-arachidonoyl-dopamine (NADA), recently characterized as a full agonist at TRPV1.[25] Mammals treated with systemic capsaicin show a dramatic hypothermic response that is absent in mice lacking the TRPV1 gene.[5] We recorded the effects of **41** on body temperature and examined its ability to prevent hypothermia produced by systemic administration of capsaicin. Of note, no adverse effects of **41** on any aspect of rat behavior were observed at the highest doses evaluated (24 μmol/kg ip, and 72 μmol/kg, po)

Sensory Effects. In preliminary experiments (data not shown), intradermal injection of capsaicin into the plantar surface of one hind paw produced very brief local thermal hyperalgesia (less than 10 min), followed by a more prolonged period of decreased responses to thermal stimulation (thermal desensitization) in the immediate area of the injection, lasting approximately 20 min. Thermal desensitization appeared maximal at 5 μg of capsaicin, with an ED_{50} of 3 μg. Localized thermal desensitization was accompanied by more slowly developing and persistent tactile allodynia, or sensitivity to ordinarily innocuous touch, in the area surrounding the injection, beginning approximately 30 min after injection and lasting at least 1 h. NADA produced sustained thermal hyperalgesia alone, without thermal desensitization or tactile allodynia.

Thermal Desensitization. Pretreatment with **41** (24 μmol/kg po) 1 h before intraplantar capsaicin (5 μg) completely prevented thermal desensitization; withdrawal latencies were unchanged from baseline throughout the observation period (Figure 5). In contrast, vehicle-pretreated animals showed a large thermal desensitization: latencies increased from a baseline of 9.5±0.8 s, to 19.3 ± 0.5 s at 10 min, sustained through 30 min (Figure 5; $p < 0.05$, two-way ANOVA).

Tactile Allodynia. A dose of **41** (24 μmol/kg po) 90 min prior to intraplantar capsaicin (10 μg) prevented tactile allodynia. Rats pretreated with **41** maintained normal tactile withdrawal thresholds of 11.6 ± 1.8 g, whereas among controls, withdrawal threshold decreased from 13.8 ± 0.6 g to 1.2 ± 0.3 g ($p < 0.05$, two-way ANOVA) (Figure 6)

Thermal Hyperalgesia. Pretreatment with **41** (24 μmol/kg ip) 1 h before paw injection completely prevented thermal hyperalgesia produced by intraplantar NADA, 5 μg. Animals pretreated with vehicle alone demonstrated robust thermal hyperalgesia, with an approximately 50% decrease in thermal withdrawal latencies at 30 and 60 min of observation that was still detectable at 90 min, $p < 0.05$, two-way ANOVA (Figure 7).

Thermoregulatory Effects. Hyperthermia. The effects of **41** alone on core body temperature were tested in awake rats. The highest dose of **41** tested (72 μmol/kg) resulted in a slight but significant increase in maximum body temperature compared to vehicle treated rats, 38.2 ± 0.2 °C vs 37.4 ± 0.1 °C;(Figure 8, $p < 0.001$, one-way ANOVA, $n = 4-7$ per group). Lower doses of **41** had no significant effect.

Hypothermia. Preliminary experiments defined the hypothermic dose−response to subcutaneously (sc) administered capsaicin. A maximum response of a decrease of 3 °C was produced by 1 mg/kg (ED_{50} was 0.4 mg/kg, data not shown). After administration of 1 mg/kg capsaicin, core temperature nadir was 34.6 ± 0.2 °C in the vehicle-pretreated group (Figure 9). Pretreatment

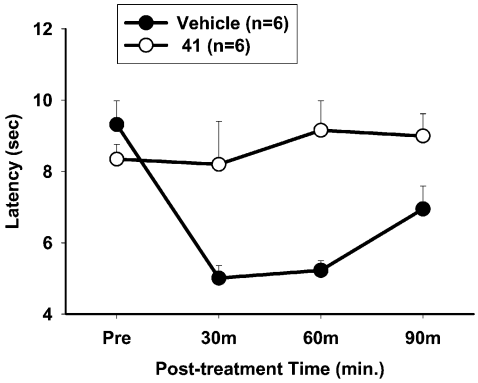


Figure 7. Compound **41** prevents NADA-evoked thermal hyperalgesia. Pretreatment with 24 μmol/kg of compound **41**, ip, completely prevented the reduction of paw withdrawal latencies (modified Hargreaves device) evoked in control rats by the intraplantar administration of 5 μg of *N*-arachidonoyldopamine, a full TRPV1 agonist. *X*-axis: withdrawal latency, seconds. $N = 6$ per group.

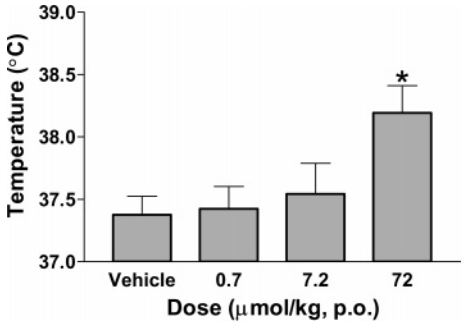


Figure 8. Compound **41** causes mild hyperthermia. Core body temperature was monitored telemetrically using intraperitoneally implanted sensors. Peak temperature during an *X* minute observation period after oral administration of **41** was significantly higher in the 72 μmol/kg dose group only ($p < 0.0001$, one-way ANOVA). *X*-axis: Peak core temperature, degrees Celsius. $N = 4-8$ per group.

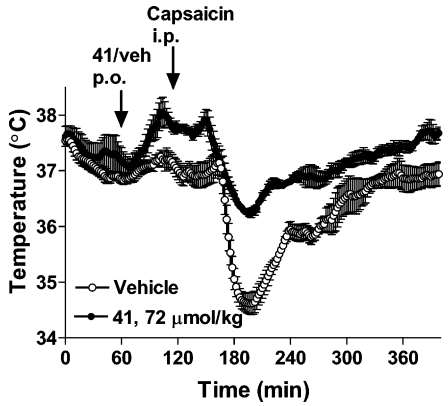


Figure 9. Oral pretreatment with **41** partially prevents core hypothermia evoked by sc capsaicin. Complete recording traces are shown for the highest dose treatment group (72 μmol/kg) and the vehicle group. Mild hyperthermia is evident in the compound treated group ($N = 7$), with a slight dip in core temperature below pretreatment baseline after capsaicin administration. Pronounced hypothermia is evident in the vehicle group ($N = 4$) after capsaicin.

with **41** (range 0.7 to 72 μmol/kg) prevented hypothermia in a dose dependent manner, with an ED_{50} of 38 μmol/kg (Figure 10). Only the highest dose was significant compared to vehicle, with an efficacy of 64 ± 5% ($p < 0.0001$, one-way ANOVA).

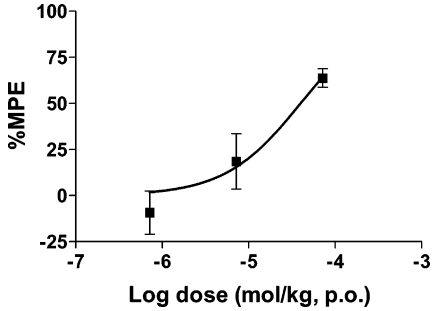


Figure 10. Oral pretreatment with **41** partially prevents capsaicin-evoked hypothermia in a dose−response manner. Percent of maximum possible efficacy (%MPE) to prevent capsaicin-evoked hypothermia was evaluated by ranging treatment outcomes between mean minimum pretreatment temperature baseline (no change from this baseline after intervention = 100% MPE), and mean core temperature nadir produced by capsaicin (maximum physiological perturbation = 0% MPE). *X*-axis: log dose in mol/kg; *Y*-axis, % MPE. The calculated ED_{50} to prevent hypothermia was 16 μmol/kg, po. $N = 4-7$ per group.

Conclusion

Following the identification of a lead series of TRPV1 ligands from HTS, we undertook a detailed investigation of SAR. Using both matrix and targeted synthesis approaches, the key elements for potent TRPV1 antagonism were determined. The essential template for antagonism is a 4-pyridiny-2-ylpiperazine-1-carboxylic acid phenylamide with the most potent compounds bearing a 3-subsitiuent on the pyridine and an electron-withdrawing group in the para-position of the phenylamide fragment. Two of the examples **4** and **14** were extensively profiled in vitro in comparison with capsazepine (CPZ) and ruthenium red (RR). The behavior of **4** and **14** was comparable across species following receptor activation by a range of stimuli including capsaicin, protons (acid), PKC phosphorylation (PMA), and the reactive oxygen species H_2O_2 (data not shown) but in vivo evaluation was problematic. Replacing the phenylamide fragment of **4** and **14** with a *p*-trifluoromethyl-2-pyridinamide afforded compound **41** with a similar in vitro profile to compounds **4** and **14**. In contrast to **4** and **14**, compound **41** was orally bioavailable with a favorable pharmacokinetic profile. In vivo studies demonstrate that **41** is an efficacious TRPV1 antagonist in a variety of models. The compound showed the ability to fully compete with effective doses of two locally administered potent TRPV1 agonists in vivo. These models are useful preliminary systems for the assessment of compounds since their pharmacology is well defined, and they offer substantial experimental windows for dose−effect assessment.

While prophylactic activity versus systemically administered capsaicin was observed, **41** provided less than full (68%) reversal of hypothermia at a dose that was 3-fold higher than that required for full prophylaxis of locally administered TRPV1 agonist effects. The reasons for this are not known. The pharmacology of thermoregulation is complex and includes both central and peripheral mechanisms. However, since **41** caused mild hyperthermia, possibly by decreasing a constitutively active endogenous vanilloid pathway, it is possible

that additional compensatory nonvanilloid thermoregulatory mechanisms were activated by predosing with **41** that contributed to the hypothermia.

In the course of exploring the SAR for this series, several weak TRPV1 agonists were identified (**19**, **30**, **31**, **37**, and **38**), although these observations were not explored further other than to recognize the restricted SAR for antagonist activity. Additionally two compounds (**18** and **27**) showed species differences, behaving as weak antagonists and weak agonists at the human and rat TRPV1 receptors, respectively. One final, noteworthy observation is the difference observed for the potency of CPZ at the recombinant human versus rat receptors. While the behavior of CPZ to block proton-induced responses was described previously,[26,27,28] this is the first report of the species-dependent potency of CPZ to block PKC-mediated activation under physiological conditions.

In conclusion the present studies have led to the discovery of TRPV1 ligands that exhibit potent antagonist activities. Thus the initial lead series of compounds, identified via parallel synthesis, were modified via targeted synthesis to obtain competitive antagonists. Although these analogues showed good in vitro functional activity across species, further modifications were made to obtain a compound (**41**) with acceptable physical properties permitting more detailed in vivo evaluation. Thus examination of **41**, particularly in several pharmacological models, demonstrates that **41** provides a tool to determine the therapeutic potential of selective TRPV1 antagonists.

Experimental Section

Anhydrous solvents were obtained from a GlassContour solvent dispensing system. Chromatography was performed using prepacked ISCO Redisep and Biotage silica cartridges. ^{1}H (400, 500 MHz) and ^{13}C (101 MHz) NMR spectra were recorded on a Bruker 400 and 500. Chemical shifts are reported in parts per million downfield from an internal Me_4Si standard. Mass spectra were recorded on a Hewlett-Packard 1100MSD, using electrospray ionization (ESI). Melting points are uncorrected and obtained on a MelTemp apparatus. Combustion analyses were performed by Desert Analytics (Tuscon, AZ). Reagents were purchased from commercial suppliers and were used without purification unless otherwise noted. The following intermediates were obtained from the commercial suppliers indicated and used without further purification: 1-(4-pyridyl)piperazine (Lancaster) (**5b**), 1-(2-pyrimidyl)piperazine (Aldrich) (**5c**), 1-[3-(trifluoromethyl)-2-pyridyl]-1,4-diazepane (Apollo) (**5d**), quipazine [2-(1-piperazinyl)quinoline] (Sigma) (**5e**), 1-[3-chloro-5-(trifluoromethyl)-2-pyridyl]piperazine (Maybridge) (**5f**), 1-(2-pyrazinyl)piperazine (Apollo) (**5g**), 1-[5-(trifluoromethyl)-2-pyridyl]-1,4-diazepane (Oakwood) (**5l**), 1-[5-trifluoromethyl)-2-pyridyl]piperazine (Apollo) (**5o**), 1-(2-pyridyl)piperazine (Aldrich), 4-nitrophenyl isocyanate (Aldrich), phenyl isocyanate (Aldrich) (**6**), *p*-tolyl isocyanate (Aldrich) (**7**), 2,4-dimethoxyphenyl isocyanate (Aldrich) (**8**), 4-chlorophenyl isocyanate (Aldrich) (**9**), 4-(trifluoromethyl)phenyl isocyanate (Aldrich) (**10**), 3-(trifluoromethyl)-phenyl isocyanate (Aldrich) (**11**), 2-(trifluoromethyl)phenyl isocyanate (Aldrich) (**12**), 2-chloro-4-(trifluoromethyl)phenyl isocyanate (Aldrich) (**13**).

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (4-Nitrophenyl)amide (4). A solution of 1-(3-trifluoromethyl-pyridin-2-yl)piperazine (**5m**) (231 mg, 1 mmol) and 4-nitrophenyl isocyanate (104 mg, 1 mmol) in 1,2 dichloroethane (15 mL) was stirred at room temperature for 18 h. The solvent was evaporated, and chromatography of the resulting residue (10−40% ethyl acetate:hexanes) gave the title compound as a yellow solid (297 mg, 75%): ^{1}H NMR ($(CD_3)_2$-SO) δ 9.30 (s, 1H), 8.55 (dd, J = 4.7, 1.2 Hz, 1H), 8.18−8.15 (m, 2H), 8.11 (dd, J = 7.8, 1.7 Hz, 1H), 7.76−7.72 (m, 2H), 7.24 (dd, J = 7.8, 4.8 Hz, 1H), 3.65−3.62 (m, 4H), 3.24−3.21 (m, 4H), MS *m/z* 396.2 [MH$^+$]. Anal. ($C_{17}H_{16}F_3N_5O_3$) C, H, N.

Preparation of Pyridinylpiperazines. 1-Pyridin-3-ylpiperazine (5a). To a suspension of 3-bromopyridine (475 mg, 3.0 mmol), piperazine (1.55 g, 18 mmol), and Na*t*OBu (400 mg, 4.2 mmol) in *o*-xylenes (5 mL) were added $Pd(OAc)_2$ (14 mg, 0.3 mmol) and $P(tBu)_3$ (49 mg, 0.3 mmol). The mixture was heated to 120 °C. After 18 h the reaction was diluted with dichloromethane and filtered through a pad of Celite. The organic solvent was evaporated, and chromatography of the residue (5−9% 2 M ammonia in methanol:dichloromethane) gave the 1-pyridin-3-ylpiperazine intermediate (55 mg, 11%). ^{1}H NMR (CD_3OD) δ 8.22 (d, J = 2.8 Hz, 1H), 7.98 (J = 4.7, 1.2 Hz, 1H), 7.43−7.40 (m, 1H), 7.30−7.27 (m, 1H), 3.22−3.20 (m, 4H), 3.02−2.99 (m, 4H), MS *m/z* 164.2 [MH$^+$].

Method A, Representative Procedure. 1-(3-Trifluoromethylpyridin-2-yl)piperazine (5m). A solution of 2-chloro-3-trifluoromethylpyridine (10 g, 0.055 mol) and piperazine (47 g, 0.55 mol) in 1-butanol (450 mL) was heated at reflux temperature. After 18 h, the reaction mixture was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was diluted with 1 N NaOH (200 mL) and extracted with ethyl acetate (3 × 250 mL). The organic layers were combined and dried (Na_2SO_4), and the solvent was evaporated. Chromatography of the resulting residue (5−10% 2 M ammonia in methanol:dichloromethane) gave the title compound as a solid (10 g, 79%): ^{1}H NMR (CD_3-OD) δ 8.46 (dd, J = 7.2, 4.8 Hz, 1H), 7.99 (dd, J = 7.8, 1.7 Hz, 1H), 7.14 (dd, J = 7.8, 4.8 Hz, 1H), 3.21−3.19 (m, 4H), 2.96−2.93 (m, 4H), MS *m/z* 232.1 [MH$^+$].

Method B, Representative Procedure. 1-(3-Methylpyridin-2-yl)piperazine (5p). A neat mixture of 2-chloro-3-methylpyridine (5 mL, 0.05 mol) and piperazine (20 g, 0.23 mol) was heated at 135 °C for 24 h. The reaction mixture was cooled to room temperature, diluted with 1 N $NaHCO_3$ (500 mL), and extracted with dichloromethane (3 × 500 mL). The organic layers were combined and dried (Na_2SO_4), and the solvent was evaporated. Chromatography of the resulting residue (4−10% 2 M ammonia in methanol:dichloromethane) gave the title compound as an oil (5.7 g, 70%): ^{1}H NMR (CD_3-OD) δ 8.06 (dd, J = 5.0, 1.4 Hz, 1H), 7.52 (dd, J = 7.4, 1.7 Hz, 1H), 6.94 (dd, J = 7.4, 5.0 Hz, 1H), 3.10−3.08 (m, 4H) 2.99−2.97 (m, 4H), MS *m/z* 178.4 [MH$^+$].

Method C. Representative Procedure. 1-(3-Nitropyridin-2-yl)piperazine (5q). A solution of 2-chloro-3-nitropyridine (10 g, 0.06 mol) and piperazine (13.6 g, 0.16 mol) in acetonitrile was heated at reflux temperature. After 18 h, the reaction mixture was cooled and the solvent was removed under reduced pressure. The residue was diluted with 1 N NaOH (200 mL) and extracted with ethyl acetate (3 × 200 mL). The organic layers were combined and dried (Na_2SO_4), and the solvent was evaporated. Chromatography of the resulting residue (5% 2 M ammonia in methanol:dichloromethane) gave the title compound as a yellow solid (3.1 g, 24%): ^{1}H NMR (CD_3OD) δ 8.35 (dd, J = 4.6, 1.7 Hz, 1H), 8.18 (dd, J = 8.1, 1.7 Hz, 1H), 6.85 (dd, J = 8.1, 4.6 Hz, 1H), 3.41−3.38 (m, 4H), 2.91−2.89 (m, 4H), MS *m/z* 209.1 [MH$^+$].

1-(5-Methylpyridin-2-yl)piperazine (5h). The title compound was prepared from 2-chloro-5-methylpyridine and piperazine according to method B (46%). ^{1}H NMR (CD_3OD) δ 7.92 (d, J = 1.7 Hz, 1H), 7.42 (dd, J = 8.6, 2.3 Hz, 1H), 6.75 (d, J = 8.6 Hz, 1H), 3.41−3.39 (m, 4H), 2.93−2.91 (m, 4H), 2.19 (s, 3H), MS *m/z* 178.4 [MH$^+$].

1-(5-Chloropyridin-2-yl)piperazine (5i). The title compound was prepared from 2,5-dichloropyridine and piperazine according to method A (78%). ^{1}H NMR (CD_3OD) δ d 8.04 (d, J = 1.7 Hz, 1H), 7.51 (dd, J = 9.1, 2.7 Hz, 1H), 6.78 (d, J = 9.1 Hz, 1H), 3.49−3.46 (m, 4H), 2.91−2.89 (m, 4H), MS *m/z* 198.1 [MH$^+$].

1-(5-Nitropyridin-2-yl)piperazine (5j). The title compound was prepared from 2-bromo-5-nitropyridine and pip-

erazine according to method C (38%). ^{1}H NMR (CD_3OD) δ 8.96 (d, J = 2.7 Hz, 1H), 8.22 (dd, J = 9.6, 2.8 Hz, 1H), 6.82 (d, J = 9.6 Hz, 1H), 3.77 (t, J = 5.2 Hz, 4H), 2.90 (t, J = 5.2 Hz, 4H), MS *m/z* 209.4 [MH^+].

1-(6-Nitropyridin-3-yl)piperazine (5k). The title compound was prepared from 5-bromo-2-nitropyridine and piperazine according to method C (47%). ^{1}H NMR (CD_3OD) δ 8.18 (d, J = 9.3 Hz, 1H), 8.14 (d, J = 3.1 Hz, 1H), 7.46 (dd, J = 9.3, 3.1 Hz, 1H), 3.49−3.46 (4H, m), 2.99−2.97 (4H, m), MS *m/z* 209.1 [MH^+].

1-(4-Trifluoromethylpyridin-2-yl)piperazine (5n). The title compound was prepared from 2-chloro-4-trifluoromethylpyridine and piperazine according to method A (70%). ^{1}H NMR (CD_3OD) δ 8.28 (d, J = 5.2 Hz, 1H), 6.98 (s, 1H), 6.83 (d, J = 5.2 Hz, 1H), 3.59−3.57 (m, 4H), 2.93−2.90 (m, 4H), MS *m/z* 232.1 [MH^+].

1-(3-Chloropyridin-2-yl)piperazine (5r). The title compound was prepared from 2,3-dichloropyridine and piperazine according to method A (90%). ^{1}H NMR (CD_3OD) δ 8.16 (dd, J = 4.8, 1.6 Hz, 1H), 7.71 (dd, J = 7.8, 1.6 Hz, 1H), 6.95 (dd, J = 7.8, 4.8 Hz, 1H), 3.31−3.28 (m, 4H), 2.99−2.97 (m, 4H), MS *m/z* 198.4 [MH^+].

3-Methyl-1-(3-trifluoromethylpyridin-2-yl)piperazine (5s). The title compound was prepared from 2-chloro-3-trifluoromethylpyridine and 2-methylpiperazine according to method A (76%). ^{1}H NMR (CD_3OD) δ 8.46−8.45 (m, 1H), 7.99 (dd, J = 7.8, 1.7 Hz, 1H), 7.14 (dd, J = 7.7, 4.8 Hz, 1H), 3.42−3.40 (m, 2H), 3.01−2.94 (m, 4H), 2.67−2.61 (m, 1H), 1.10 (d, J = 6.5 Hz, 3H), MS *m/z* 246.1 [MH^+].

3,5-Dimethyl-1-(3-trifluoromethylpyridin-2-yl)piperazine (5t). The title compound was prepared from 2-chloro-3-trifluoromethylpyridine and 2,6-dimethylpiperazine according to method A (17%). ^{1}H NMR (CD_3OD) δ 8.44 (dd, J = 4.8, 1.2 Hz, 1H), 7.98 (dd, J = 7.8, 1.7 Hz, 1H), 7.13−7.10 (m, 1H), 3.44−3.41 (m, 2H), 3.03−2.98 (m, 2H), 2.57 (t, J = 12.1 Hz, 2H), 1.10 (d, J = 6.5 Hz, 6H), MS *m/z* 260.2 [MH^+].

2,5-Dimethyl-1-(3-trifluoromethylpyridin-2-yl)piperazine (5u). The title compound was prepared from 2-chloro-3-trifluoromethylpyridine and 2,5-dimethylpiperazine according to method B (16%). ^{1}H NMR (CD_3OD) δ 8.65 (dd, J = 4.8, 1.2 Hz, 1H), 8.10 (dd, J = 7.8, 1.7 Hz, 1H), 7.40−7.36 (m, 1H), 3.35−3.32 (m, 1H), 3.04−3.01 (m, 2H), 2.93−2.90 (m, 1H), 2.66−2.60 (m, 1H), 2.50−2.44 (m, 1H), 1.05 (d, J = 6.6 Hz, 3H), 0.74 (d, J = 6.5 Hz, 3H),), MS *m/z* 260.2 [MH^+].

2-(3-Trifluoromethylpyridin-2-yl)-2,5-diazabicyclo[2.2.1]heptane (5v). The title compound was prepared from 2-chloro-3-trifluoromethylpyridine and 2,5-diazobicycloheptane according to method A (92%). ^{1}H NMR (CD_3OD) δ 8.77 (dd, J = 4.8, 1.2 Hz, 1H), 7.86 (dd, J = 7.8, 1.7 Hz, 1H), 6.78−6.75 (m, 1H), 4.83 (s, 1H), 3.78−3.75 (m, 2H), 3.18 (dd, J = 10.5, 1.5 Hz, 1H), 3.00 (dd, J = 10.3, 2.2 Hz, 1H), 1.90 (d, J = 9.9 Hz, 1H), 1.76 (d, J = 9.9 Hz, 1H), MS *m/z* 244.1 [MH^+].

1-(3-Trifluoromethylpyridin-2-yl)pyrrolidin-3-ylamine (5w). The title compound was prepared from 2-chloro-3-trifluoromethylpyridine and 3-aminopyrrolidine according to method A (29%). ^{1}H NMR (CD_3OD) δ 8.25 (dd, J = 4.8, 1.2 Hz, 1H), 7.88 (dd, J = 7.8, 1.7 Hz, 1H), 6.75 (dd, J = 7.7, 4.8 Hz, 1H), 3.80−3.50 (m, 4H), 3.36−3.30 (m, 1H), 2.20−2.10 (m, 1H), 1.82−1.75 (m, 1H), MS *m/z* 232.1 [MH^+].

3′-Trifluoromethyl-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-4-ylamine (5x). To a solution of 2-chloro-3-trifluoromethylpyridine (1.81 g, 10.0 mmol) in acetonitrile (100 mL) were added piperidin-4-ylcarbamic acid *tert*-butyl ester (2.20 g, 11.0 mmol) and triethylamine (11 g, 11.0 mmol). The reaction mixture was heated at reflux temperature for 16 h. The solvent was evaporated and the residue dissolved in dichloromethane (50 mL), washed with brine (2 × 50 mL), dried (Na_2SO_4), and evaporated to afford (3′-trifluoromethyl-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-4-yl)carbamic acid *tert*-butyl ester (2.9 g, 8.0 mmol) as an oil, which was used without further purification. The intermediate oil was dissolved in a mixture of dichloromethane:trifluoroacetic acid (1:1; 50 mL) and stirred at room temperature for 2 h. The solvent was evaporated and the residue was purified by silica gel chromatography (eluent: CH_2Cl_2:2% MeOH) to yield the title compound (1.8 g, 92%). ^{1}H NMR (CD_3OD) δ 8.2 (d, J = 4 Hz, 1H), 7.5 (d, J = 9 Hz, 1H), 6.5 (d, J = 11 Hz, 1H), 3.2−3.4 (m, 2H), 3.1 (m, 1H), 2.9−3.1 (m, 2H), 1.5−1.7 (m, 4H), MS *m/z* 246.4 [MH^+].

Preparation of Pyridinylpiperazinyl Ureas: Library Synthesis Procedure. To each well of a 6 × 8, 48 well polyfiltronic plate was added a 0.2 M solution (1.5 mL) of the appropriate amine (**5**) in 1,2 dichloroethane followed by a 0.2 M solution (1.5 mL) of the corresponding phenyl isocyanate (**6**−**13**) in 1,2-dichloroethane. The plate was covered and shaken overnight at room temperature. The solvent was removed via a speedvac system (Savant) to afford the urea products in near quantitative yield (0.3 mmol of compound, exact yields were not determined). Characterization of the library compounds was accomplished using MS and ^{1}H NMR.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (14). The title compound was prepared according to the library synthesis procedure using **5m** and 4-(trifluoromethyl)phenyl isocyanate (**10**). ^{1}H NMR (CD_3OD) δ 7.21 (dd, J = 7.7, 4.8 Hz, 1H), 3.71−3.69 (m, 4H), 3.29−3.27 (m, 4H), MS *m/z* 419.2 [MH^+], Anal. ($C_{18}H_{16}F_6N_4O$) C, H, N.

4-(3-Methylpyridin-2-yl)piperazine-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (15). The title compound was prepared according to the library synthesis procedure using **5p** and 4-(trifluoromethyl)phenyl isocyanate (**10**). ^{1}H NMR (($CD_3)_2SO$)) δ 9.00 (s, 1H), 8.12 (dd, J = 4.8, 1.8 Hz, 1H), 7.71 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.8 Hz, 2H), 7.53 (d, J = 7.1 Hz, 1H), 3.64−3.60 (m, 4H), 3.10−3.07 (m, 4H), 2.28 (s, 3H), MS *m/z* 365.2 [MH^+], Anal. ($C_{18}H_{19}F_3N_4O$) C, H, N.

4-(3-Methylpyridin-2-yl)piperazine-1-carboxylic Acid (4-chlorophenyl)amide (16). The title compound was prepared according to the library synthesis procedure using **5p** and 4-chlorophenyl isocyanate (**9**). ^{1}H NMR (($CD_3)_2SO$) δ 8.72 (s, 1H), 8.12 (d, J = 4.8 Hz, 1H), 7.55−7.50 (m, 2H), 7.30−7.27 (m, 2H), 6.97−6.94 (m, 1H), 3.60−3.58 (m, 4H), 3.08−3.06 (m, 4H), 2.27 (s, 3H), MS *m/z* 331.2 [MH^+], Anal. ($C_{17}H_{19}ClN_4O$) C, H, N.

4-Pyridin-2-ylpiperazine-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (17). The title compound was prepared according to the library synthesis procedure using 1-(2-pyridyl)piperazine (Aldrich) and 4-(trifluoromethyl)phenyl isocyanate (**10**). ^{1}H NMR (($CD_3)_2SO$)) δ 8.98 (s, 1H), 8.57−8.55 (m, 1H), 8.10 (dd, J = 7.8, 1.7 Hz, 1H), 7.70 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.7 Hz, 2H), 7.26−7.22 (m, 1H), 3.64−3.60 (m, 4H), 3.24−3.20 (m, 4H), MS *m/z* 351.0 [MH^+], Anal. ($C_{17}H_{17}F_3N_4O$) C, H, N: calcd. C: 58.28, H: 4.89, N: 15.99; found C: 58.44, H: 4.84, N: 15.95.

4-Pyridin-3-ylpiperazine-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (18). The title compound was prepared in a similar manner as **4** using **5a** and 4-(trifluoromethyl)-phenyl isocyanate (**10**) (93%). ^{1}H NMR (CD_3OD) δ 8.28 (d, J = 2.82, 1H), 8.01 (J = 4.6, 1.2 Hz, 1H), 7.61−7.54 (m, 4H), 7.49−7.46 (m, 1H), 7.34−7.31 (m, 1H), 3.75−3.73 (m, 4H), 3.32−3.30 (m, 4H), MS *m/z* 351.2 [MH^+], Anal. ($C_{17}H_{17}F_3N_4O$) C, H, N.

4-Pyridin-4-ylpiperazine-1-carboxylic Acid (4-Chlorophenyl)amide (19). The title compound was prepared according to the library synthesis procedure using (**5b**) and 4-chlorophenyl isocyanate (**9**). ^{1}H NMR (($CD_3)_2SO$)) δ 9.01 (s, 1H), 8.19 (d, J = 5.3 Hz, 2H), 7.65 (d, J = 8.6 Hz, 2H), 7.55(d, J = 8.8 Hz, 2H), 6.81 (d, J = 6.5 Hz, 2H), 3.58−3.54 (m, 4H), 3.36−3.33 (m, 4H), MS *m/z* 351.0 [MH^+], Anal. ($C_{17}H_{17}F_3N_4O$) C, H, N.

2-Methyl-4-(3-trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (20). The title compound was prepared in a similar manner as **4** using **5s** and 4-(trifluoromethyl)phenyl isocyanate (**10**) (70%). ^{1}H NMR (($CD_3)_2SO$)) δ 8.90 (s, 1H), 8.58 (d, J = 3.6 Hz, 1H), 8.12 (dd, J = 7.8, 1.6 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.59 (d, J = 8.7 Hz, 1H), 7.27 (dd, J = 7.6, 4.9 Hz, 1H), 4.90−4.48 (m, 1H), 3.99 (d, J = 13.0 Hz, 1H), 3.42 (d, J = 12.2 Hz, 1H), 3.26−3.25 (m, 2H), 3.13 (dd, J = 12.5, 3.5 Hz, 1H), 2.94 (t, J = 12.0 Hz, 1H), 1.24 (d, J = 6.6 Hz, 3H) MS *m/z* 433.4 [MH^+],

Anal. ($C_{18}H_{19}F_3N_4O$) C, H, N: calcd. C: 52.78, H: 4.20, N: 12.96; found C: 53.01, H: 4.25, N: 12.94

2,6-Dimethyl-4-(3-trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (21). The title compound was prepared in a similar manner as **4** using **5t** and 4-(trifluoromethyl)phenyl isocyanate (**10**) (67%). ^{1}H NMR ((CD_3)$_2$SO)) δ 8.74 (s, 1H), 8.65 (dd, J = 4.8, 1.2 Hz, 1H), 8.18 (dd, J = 7.8, 1.8 Hz, 1H), 7.75 (d, J = 8.4 Hz, 2H), 7.59 (d, J 8.6 Hz, 2H), 7.38 (dd, J = 7.1, 4.8 Hz, 1H), 4.41−4.38 (m, 2H), 3.20 (d, J = 11.9 Hz, 2H), 3.05 (dd, J = 12.4, 4.3 Hz, 2H), 1.36 (d, J = 6.8 Hz, 6H), MS *m/z* 447.3 [MH$^+$], Anal. ($C_{20}H_{20}F_6N_4O$) C, H, N.

2,5-Dimethyl-4-(3-trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (22). The title compound was prepared in a similar manner as **4** using **5u** and 4-(trifluoromethyl)phenyl isocyanate (**10**) (30%). ^{1}H NMR ((CD_3)$_2$SO)) δ 8.84 (s, 1H), 8.52 (dd, J = 4.8, 1.2 Hz, 1H), 8.08 (dd, J = 7.8, 1.6 Hz, 1H), 7.71 (d, J = 8.6 Hz, 2H), 7.59 (d, J = 8.6 Hz, 2H), 7.17 (dd, J = 7.1, 4.8 Hz, 1H), 4.46 (bs, 1H), 4.05−4.02 (m, 1H), 3.82 (dd, J = 13.2, 2.6 Hz, 1H), 3.65 (dd, J = 13.3, 3.9 Hz, 1H), 3.53 (dd, J = 13.2, 3.6 Hz, 1H), 3.08 (d, J = 12.2 Hz, 1H), 1.70 (d, J = 6.6 Hz, 3H), 1.02 (d, J = 6.5 Hz, 3H), MS *m/z* 447.3 [MH$^+$], Anal. ($C_{20}H_{20}F_6N_4O$) C, H, N.

5-(3-Trifluoromethylpyridin-2-yl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylic Acid (4-Trifluoromethylphenyl)-amide (23). The title compound was prepared in a similar manner as **4** using **5v** and 4-(trifluoromethyl)phenyl isocyanate (**10**) (46%). ^{1}H NMR ((CD_3)$_2$SO) δ 8.66 (s, 1H), 8.37 (dd, J = 4.7, 1.5 Hz, 1H), 7.95 (dd, J = 7.8, 1.7 Hz, 1H), 7.70 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 6.87 (dd, J = 7.8, 4.7 Hz, 1H), 4.91 (s, 1H), 4.78 (s, 1H), 3.76 (d, J = 9.3 Hz, 1H), 3.60−3.57 (m, 2H), 3.16 (d, J = 5.2 Hz, 1H), 1.95 (bs, 2H), MS *m/z* 431.3 [MH$^+$], Anal. ($C_{19}H_{16}F_6N_4O$) C, H, N.

4-(3-Trifluoromethylpyridin-2-yl)-[1,4]diazepane-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (24). The title compound was prepared according to the library synthesis procedure using **5d** and 4-(trifluoromethyl)phenyl isocyanate (**10**). ^{1}H NMR ((CD_3)$_2$SO)) δ 8.67 (s, 1H), 8.38 (dd, J = 4.7, 1.8 Hz, 1H), 7.97 (dd, J = 7.8, 1.9 Hz, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 6.98 (dd, J = 7.6, 4.3 Hz, 1H), 3.74−3.70 (m, 2H), 3.60−3.50 (m, 6H), 1.97−1.90 (m, 2H), MS *m/z* 433.1 [MH$^+$], Anal. ($C_{19}H_{18}F_6N_4O$) C, H, N.

1-(4-Trifluoromethylphenyl)-3-[1-(3-trifluoromethylpyridin-2-yl)pyrrolidin-3-yl]-urea (25). The title compound was prepared in a similar manner as **4** using **5w** and 4-(trifluoromethyl)phenyl isocyanate (**10**) (88%). ^{1}H NMR ((CD_3)$_2$SO)) δ 8.29 (d, J = 4.6 Hz, 1H), 7.90 (dd, J = 7.6, 1.5 Hz, 1H), 7.56−7.51 (m, 4H), 6.78 (dd, J = 7.8, 4.8 Hz, 1H), 4.41−4.38 (m, 1H), 3.90−3.86 (m, 1H), 3.80−3.75 (m, 1H), 3.70−3.67 (m, 1H), 3.50 (dd, J = 10.4, 3.8 Hz, 1H), 2.28−2.24 (m, 1H), 2.02−1.99 (m, 1H), MS *m/z* 419.2 [MH$^+$], Anal. ($C_{18}H_{16}F_6N_4O$) C, H, N.

1-(4-Trifluoromethylphenyl)-3-(3′-trifluoromethyl-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-4-yl)urea (26). A solution of (**5x**) (0.245 g, 1.0 mmol) in dichloromethane (20 mL) was treated with 4-trifluoromethyl phenyl isocyanate (**10**) (0.16 mL, 1.1 mmol) and stirred at room temperature for 5 h. The reaction mixture was diluted with saturated $NaHCO_3$ solution (20 mL) and the organic layer separated, washed with brine (20 mL), dried over Na_2SO_4 and evaporated to afford an oil. Trituration with 60:40 hexane:ethyl acetate precipitated the title compound as a cream-colored powder (0.349 g, 81%): ^{1}H NMR ((CD_3)$_2$SO)) 8.8 (s, 1H), 8.5 (d, J = 3.6 Hz, 1H), 8.0 (d, J = 7.7 Hz, 1H), 7.7 (m, 2H), 7.2 (d, J = 4.9 Hz, 1H), 6.4 (d, J = 7.7 Hz, 1H), 3.7 (m, 1H), 3.4−3.5 (bd, J = 12.7 Hz, 2H), 2.9 (t, J = 10.7 Hz, 2H), 2.0 (bd, J = 9.7 Hz, 2H), 1.5 (bq, J = 9.9 Hz, 2H). MS(CI) *m/z* 433 (MH+). Anal. ($C_{19}H_{18}F_6N_4O$): C, H, N.

3-Methyl-3′,4′,5′,6′-tetrahydro-2′*H*-[2,4′]bipyridinyl-1′-carboxylic Acid (4-Trifluoromethylphenyl)amide (27). Step A: 1′-Benzyl-3-methyl-2′,3′,5′,6′-tetrahydro-1′*H*-[2,4′]-bipyridinyl-4′-ol. 2.5 M *n*-BuLi in hexanes (25 mL, 0.06 mol) was placed in a dry flask and cooled to −78 °C. 2-Bromo-3-methyl pyridine (0.06 mol, 6.5 mL) dissolved in tetrahydrofuran (150 mL) was added dropwise to the flask and stirred for 1 h at −78 °C. A solution of 1-benzyl-4-piperidone (10.8 mL, 0.06 mol) dissolved in tetrahydrofuran was then added dropwise to the cold solution. The reaction mixture was stirred for 18 h, warmed to room temperature, and treated with saturated NH_4Cl solution (20 mL). The solvent was removed under reduced pressure and the residue diluted with 1 N $NaHCO_3$ and extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, dried (Na_2SO_4), and evaporated. Chromatography of the residue (2−6% 2 M ammonia in methanol: dichloromethane) gave the title compound (10 g, 61%). ^{1}H NMR (CD_3OD) δ 8.31 (d, J = 4.7 Hz, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.39−7.27 (m, 5H), 7.17 (dd, J = 7.6, 4.7, 1H), 3.61 (s, 2H), 2.82−2.79 (m, 2H), 2.65−2.59 (m, 5H), 2.45−2.37 (m, 2H), 1.76−1.72 (m, 2H), MS *m/z* 283.3 [MH$^+$].

Step B: 1′-Benzyl-3-methyl-1′,2′,3′,6′-tetrahydro-[2,4′]-bipyridinyl. A solution of thionyl chloride (20 mL) was cooled to 0 °C and stirred for 30 min. 1′-Benzyl-3-methyl-2′,3′,5′,6′-tetrahydro-1′*H*-[2,4′]bipyridinyl-4′-ol (1.5 g, 5.3 mmol) was added dropwise and the reaction warmed to room temperature. After 18 h excess thionyl chloride was evaporated and the residue chromatographed (2−6% 2 M ammonia in methanol: dichloromethane) to afford the title compound (0.503 g, 35%). ^{1}H NMR (CD_3OD) δ 8.29−8.28 (m, 1H), 7.65 (d, J = 6.9 Hz, 1H), 7.42−7.26 (m, 5H), 7.19 (dd, J = 7.7, 4.9 Hz, 1H), 5.75−5.74 (m, 1H), 3.69 (s, 2H), 3.20−3.18 (m, 2H), 2.80−2.77 (m, 2H), 2.51−2.48 (m, 2H), 2.37 (s, 3H), MS *m/z* 265.2 [MH$^+$].

Step C: 3-Methyl-1′,2′,3′,4′,5′,6′-hexahydro-[2,4′]bipyridinyl. 10% Pd/C (280 mg) in ethanol was added to a solution of 1′-benzyl-3-methyl-1′,2′,3′,6′-tetrahydro-[2,4′]bipyridinyl (480 mg, 1.80 mmol) in ethanol. Nitrogen was bubbled through the solution, and the reaction was hydrogenated (H_2) at 60 psi. After 18 h the reaction mixture was filtered through Celite and washed with methanol, and the solvent was evaporated. Chromatography of the residue (4−9% 2 M ammonia in methanol) gave the title compound (60 mg, 20%). ^{1}H NMR (CD_3OD) δ 8.30 (d, J = 4.8 Hz, 1H), 7.56 (d, J = 7.6 Hz, 1H), 7.13 (dd, J = 7.6, 4.8 Hz, 1H), 3.25−3.22 (m, 2H), 3.17−3.13 (m, 1H), 2.38 (s, 3H), 1.97−1.85 (m, 2H), 1.79−1.75 (m, 2H), MS *m/z* 177.3 [MH$^+$].

Step D: The title compound (**27**) was prepared in a similar fashion as **4** using 3-Methyl-1′,2′,3′,4′,5′,6′-hexahydro-[2,4′]-bipyridinyl and 4-trifluoromethyl phenyl isocyanate (**10**) ^{1}H NMR (CD_3OD) δ 8.30 (dd, J = 4.8, 1.1 Hz, 1H), 7.60−7.53 (m, 5H), 7.14 (dd, J = 7.6, 4.8 Hz, 1H), 4.37−4.33 (m, 2H), 3.27−3.23 (m, 1H), 2.42 (s, 3H), 1.95−1.88 (m, 2H), 1.82−1.79 (m, 2H), MS *m/z* 364.2 [MH$^+$], Anal. ($C_{19}H_{20}F_3N_3O$) C, H, N.

3′-Trifluoromethyl-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-4-carboxylic Acid (4-Trifluoromethylphenyl)amide (28). Step A: 3′-Trifluoromethyl-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-4-carboxylic Acid. To a solution of 2-chloro-3-trifluoromethylpyridine (1.81 g, 10.0 mmol) in 100 mL of acetonitrile was added piperidin-4-carboxylic acid (1.29 g, 11.0 mmol), and the reaction mixture was heated at reflux temperature for 16 h. The solvent was evaporated, and the residue was stirred with saturated $NaHCO_3$ solution (50 mL). The precipitated solid (2.05 g, 75%) was collected by vacuum filtration and used, Step B, without further purification. ^{1}H NMR ((CD_3)$_2$SO)) δ 11.0 (bs, 1H), 8.1 (d, J = 4 Hz, 1H), 7.6 (d, J = 9 Hz, 1H), 6.5(dd, J = 4, 9 Hz, 1H), 3.3−3.5 (m, 2H), (2.8−3.1,m, 3H), 1.4−1.6 (m, 4H), MS *m/z* 275.0 [MH$^+$].

Step B: To a solution of 3′-trifluoromethyl-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-4-carboxylic acid (0.413 g, 1.5 mmol) in dichloromethane (20 mL) were added diisopropylethylamine and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (0.456 g, 1.0 mmol). The reaction mixture was then stirred at room temperature for 10 min. 4-Trifluoromethylaniline (0.177 g, 1.1 mmol) was added and the reaction mixture stirred for an additional 16 h. The reaction mixture was diluted with saturated $NaHCO_3$ (20 mL), and the organic layer was separated, washed with brine (20 mL), dried over Na_2SO_4, and evaporated. The product was chromatographed on silica gel eluting with CH_2Cl_2:2% MeOH to afford the title compound **28**, as a white solid (0.283 g, 68%): ^{1}H NMR

($(CD_3)_2SO$)) δ 8.5 (d, J = 3.6 Hz, 1H), 8.1 (d, J = 9.5 Hz, 1H), 7.8 (d, J = 8.6 Hz, 2H), 7.7 (d, J = 8.6 Hz, 2H), 7.1 (dd, J = 4.8, 5.1 Hz, 1H), 3.6 (d, J = 12.7 Hz, 2H), 2.9 (t, J = 11.2 Hz, 2H), 2.6 (m, 1H), 1.7−1.9 (m, 4H). MS(CI) *m/z* 418 (MH^+). Anal. ($C_{19}H_{17}F_6N_3O$) C, H, N.

4-(2-Trifluoromethylphenyl)piperidine-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (29). Step A: 1-Benzyl-4-(2-trifluoromethylphenyl)-1,2,3,6-tetrahydropyridine. *n*-BuLi, 2.5 M in hexane (34 mL, 85 mmol), was added dropwise to a solution of 2-trifluoromethylbromobenzene (16.88 g, 75 mmol) in anhydrous THF (300 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 1 h and at 0 °C for 10 min. The reaction mixture was recooled at −78 °C, and a solution of *N*-benzylpiperidone (14.2 g, 75 mmol) in THF (50 mL) was added slowly at −78 °C. The reaction mixture was stirred for a further 2 h at −78 °C whereupon saturated ammonium chloride solution (100 mL) was added. The cooling bath was removed and the reaction mixture warmed to room temperature. The reaction mixture was extracted with dichloromethane (3 × 250 mL), and the combined organic extracts were washed with water (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and evaporated under reduced pressure to yield 25.56 g of the crude product. The crude material was purified on a silica gel column using an ethyl acetate/hexane gradient system (0:100 to 30:70). Evaporation of the solvent under reduced pressure afforded the title compound (16 g, 63.6%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.75 (d, J = 8.08 Hz, 1H), 7.57 (d, J = 7.83 Hz, 1H), 7.48 (t, J = 7.83 Hz, 1H), 7.37−7.22 (m, 6H), 3.58 (s, 2H), 2.83−2.75 (m, 2H), 2.59−2.51 (m, 2H), 2.30−2.20 (m, 5H). MS-ESI *m/z* 336.2 $[MH]^+$.

Step B: 4-(2-Trifluoromethylphenyl)piperidine. 1-Benzyl-4-(2-trifluoromethylphenyl)-1,2,3,6-tetrahydropyridine (1 g) was dissolved into ethanol treated with Pd/C 10% (50 mg) and hydrogenated in the Parr bottle at 60 psi for 96 h. The reaction mixture was filtered and recharged with fresh catalyst after 24 and 48 h. Finally the reaction mixture was filtered, and solvent was evaporated to dryness to yield the title compound (0.5 g 70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.69−7.60 (m, 1H), 7.57−7.22 (m, 3H), 3.51 (bs, 1H), 3.30−3.97 (m, 3H), 2.80 (bs, 1H), 2.38−2.02 (m, 2H), 1.92−1.58 (m, 3H). MS-ESI *m/z* 230.2 $[MH]^+$.

Step C: 4-(2-Trifluoromethylphenyl)piperidine was treated with 4-trifluoromethylphenyl isocyanate (**10**) as described for the preparation of **4**, above, to yield the title compound (**29**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.65 (d, J = 7.83 Hz, 1H), 7.59−7.48 (m, 5H), 7.42 (d, J = 7.83 Hz, 1H), 7.33 (t, J = 7.58 Hz, 1H), 6.77 (bs, 1H), 4.28−4.18 (m, 2H), 3.25−3.00 (m, 3H), 1.97−1.71 (m, 4H). MS-ESI *m/z* 417.4 $[MH]^+$. Anal. ($C_{20}H_{18}F_6N_2O$) C, H, N.

4-*o*-Tolylpiperidine-1-carboxylic Acid (4-Trifluoromethylphenyl)amide (30). Step A: 1-Benzyl-4-*o*-tolylpiperidin-4-ol. A solution of 2-bromotoluene (12.83 g, 75 mmol) in THF (300 mL) was added dropwise over 0.5 h to a solution of *n*-BuLi (2.5 M) in hexane cooled to −78 °C. The mixture was stirred for 1 h at −78 °C, and a solution of 1-benzylpiperidone (14.2 g, 75 mmol) in THF (100 mL) was added dropwise over a period of 0.5 h, maintaining the reaction temperature at −78 °C. The resulting mixture was stirred at −78 °C for 0.5 h and at −30 °C for 1.5 h whereupon a saturated solution of ammonium chloride (20 mL) was added. The reaction mixture was stirred and warmed to room temperature. Water (200 mL) was added to the reaction mixture and extracted with dichloromethane (3 × 120 mL). The combined organic extracts were washed with water, dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure to yield crude product (22.5 g) as an oil. The crude product was purified by flash chromatography (2 M NH_3 in MeOH/dichloromethane, 0:100 to 5:95) to afford the title compound (13.12 g, 62.2%) as an oil. ^{1}H NMR ($CDCl_3$ 400 MHz): δ 7.44−7.12 (m, 9H), 3.63 (s, 2H), 2.89−2.80 (m, 2H), 2.65−2.54 (m, 6H), 2.34−2.21 (m, 2H), 2.01−1.93 (m, 2H). MS-ESI *m/z* 282.3 $[MH]^+$.

Step B: 1-Benzyl-4-*o*-tolyl-1,2,3,6-tetrahydropyridine. A solution of 4-o-tolylpiperidin-4-ol (3.5 g, 12.44 mmol) in a mixture of methanol (25 mL) and concentrated HCl (25 mL) was heated at reflux temperature for 3 h. The reaction mixture was cooled and the solvent removed under reduced pressure to give crude product as a hydrochloride salt (3.42 g). The salt was converted to the free base (aq NaOH/dichloromethane) and purified by column chromatography eluting with ethyl acetate/hexane (0:100 to 10:90) to afford the title compound (2.1 g 64%). ^{1}H NMR (DMSO, 400 MHz): δ 7.66−7.37 (m,-2H), 7.51−7.43 (m, 4H), 7.22−7.13 (m, 3H), 5.54 (s, 1H), 3.78−3.39 (m, 4H), 3.30−3.14 (m, 1H), 2.94−2.75 (m, 1H), 2.47−2.36 (m, 2H), 2.27 (s, 3H). MS-ESI *m/z* 264.4 $[MH]^+$.

Step C: 4-*o*-Tolylpiperidine. To a solution of 1-Benzyl-4-*o*-tolyl-1,2,3,6-tetrahydropyridine (2 g, 7.59 mmol) in ethanol (80 mL) was added palladium hydroxide 20 wt % (Degussa type E 101) (1.5 g), and the mixture was hydrogenated at 60 psi for 40 h. The reaction mixture was filtered through a pad of Celite and the filtrate evaporated to give the title compound (1.3 g, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.28−7.06 (m, 4H), 3.29−3.18 (m, 2H), 2.90−2.74 (m, 3H), 2.70 (s, 1H), 2.35 (s, 3H), 1.82−1.61 (m, 4H). MS-ESI *m/z* 176.2 $[MH]^+$.

Step D: 4-*o*-Tolylpiperidine was treated with 4-trifluoromethylphenyl isocyanate (**10**) as described for the preparation of **4**, above, to yield the title compound (**30**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.58−7.47 (m, 4H), 7.23−7.09 (m, 4H), 6.69 (bs, 1H), 4.30−4.20 (m, 2H), 3.11−2.89 (m, 3H), 2.38 (s, 3H), 1.93−1.83 (m, 2H), 1.82−1.66 (m, 2H). MS-ESI *m/z* 363.3 $[MH]^+$. Anal. ($C_{20}H_{21}F_3N_2O$) C, H, N.

Representative Procedure for the Preparation of Heterocyclic Carbamic Acid Phenyl Esters Used for the Synthesis of Compounds 31−41. To a solution of the appropriate heterocyclic amine (1 equiv), in anhydrous THF or dioxane cooled to 0 °C, was added phenyl chloroformate (1.05 equiv) dropwise, keeping the temperature below 5 °C. The reaction mixture was stirred at room temperature until complete disappearance of starting material was observed by TLC. The reaction mixture was diluted with either ethyl acetate or dichloromethane and the resulting solution washed successively with 0.5 M HCl solution, 1 N NaOH solution, brine, and water. The organic fraction was dried over Na_2SO_4, filtered, and evaporated to yield crude carbamate. The crude product was purified on silica gel column eluting with ethyl acetate/hexane or acetone/hexane or methanol/dichloromethane systems.

Quinolin-5-ylcarbamic Acid Phenyl Ester. The title compound was prepared from quinolin-5-ylamine as described in the general procedure above. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.98 (dd, J = 4.6, 1.5 Hz, 1H), 8.87 (d, J = 8.7 Hz, 1H), 7.97−7.89 (m, 3H), 7.75 (dd, J = 8.7, 4.5 Hz, 1H), 7.45−7.41 (m, 2H), 7.29−7.25 (m, 3H), MS-ESI *m/z* 265.4 $[MH]^+$.

Isoquinolin-5-ylcarbamic Acid Phenyl Ester. The title compound was prepared from isoquinolin-5-ylamine as described in the general procedure above. Purification of the crude product was accomplished via a silica gel column eluting with acetone/dichloromethane (0:100 to 6:94) (72%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.28 (d, J = 0.80 Hz, 1H), 8.58 (d, J = 5.81 Hz, 1H), 8.20 (br s, 1H), 7.86−7.68 (m, 3H), 7.62 (t, J = 8.08 Hz, 1H), 7.40 (t, J = 8.08 Hz, 2H), 7.30−7.19 (m, 3H). MS-ESI *m/z* 265.4 $[MH]^+$.

Quinolin-8-ylcarbamic Acid Phenyl Ester. The title compound was prepared from quinolin-8-ylamine as described in the general procedure above. ^{1}H NMR [($(CD_3)_2SO$, 400 MHz] δ 9.74 (s, 1H), 8.95 (dd, J = 4.2, 1.7 Hz, 1H), 8.45 (dd, J = 8.3, 1.6 Hz, 1H), 8.25 (dd, J = 7.6, 1.1 Hz, 1H), 7.72−7.63 (m, 3H), 7.48−7.44 (m, 2H), 7.30−7.29 (m, 3H),), MS-ESI *m/z* 265.4 $[MH]^+$.

Quinolin-3-ylcarbamic Acid Phenyl Ester. The title compound was prepared from quinolin-3-ylamine as described in the general procedure above. ^{1}H NMR (CD_3OD, 400 MHz) δ 9.14 (d, J = 2.4 Hz, 1H), 8.78 (d, J = 1.7 Hz, 1H), 8.07−8.02 (m, 2H), 7.85−7.83 (m, 1H), 7.75−7.73 (m, 1H), 7.46−7.42 (m, 2H), 7.28−7.25 (m, 3H), MS-ESI *m/z* 265.4 $[MH]^+$.

Isoquinolin-3-ylcarbamic Acid Phenyl Ester. The title compound was prepared from isoquinolin-3-ylamine as described in the general procedure above. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.73 (s, 1H), 9.15 (s, 1H), 8.38 (s, 1H), 7.88 (d, J =

8.34 Hz, 1H), 7.79 (d, J = 8.34, 1H), 7.67−7.62 (m, 1H), 7.49−7.43 (m, 3H), 7.33−7.25 (m, 3H). MS-ESI *m/z* 265.4 $[MH]^+$.

Quinolin-6-ylcarbamic Acid Phenyl Ester. The title compound was prepared from quinolin-6-ylamine as described in the general procedure above. Purification of the crude product was accomplished via silica gel column eluting with 1:1 ethyl acetate:hexane (82%). ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 10.62 (s, 1H), 8.78 (dd, J = 4.2, 1.6 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 2.0 Hz, 1H), 8.00 (d, J = 9.1 Hz, 1H), 7.82 (dd, J = 9.1, 2.4 Hz, 1H), 7.50−7.43 (m, 3H), 7.30−7.27 (m, 3H). MS-ESI *m/z* 265.1 $[MH]^+$.

Pyridin-4-ylcarbamic Acid Phenyl Ester. The title compound was prepared from pyridin-4-ylamine as described in the general procedure above. Purification of the crude product was accomplished via silica gel column eluting with acetone/dichloromethane (0:100 to 5:95) yielding (16%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.54−8.49 (m, 2H), 8.02 (br s, 1H), 7.46−7.37 (m, 4H), 7.30−7.15 (m, 3H). MS-ESI *m/z* 215.2 $[MH]^+$.

Pyridin-3-ylcarbamic Acid Phenyl Ester. The title compound was prepared from pyridin-3-ylamine as described in the general procedure above. Purification of the crude product was accomplished via silica gel column eluting with ethyl acetate/hexane (0:100 to 50:50) yielding 42.8%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.61 (d, J = 2.74 Hz, 1H), 8.48−8.34 (m, 2H), 8.15 (m, 1H), 7.45−7.36 (m, 2H), 7.33−7.16 (m, 4H). MS-ESI *m/z* 215.2 $[MH]^+$.

Pyridin-2-ylcarbamic Acid Phenyl Ester. The title compound was prepared from pyridin-2-ylamine as described in the general procedure above. Purification of the crude product was accomplished via silica gel column eluting with ethyl acetate/hexane (0:100 to 20:80) yielding 27.4%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.19 (br s, 1H), 8.46−8.40 (m, 1H), 8.08 (d, J = 8.59 Hz, 1H), 7.77−7.70 (m, 1H), 7.47−7.40 (m, 2H), 7.31−7.20 (m, 3H), 7.06−7.00 (m, 1H). MS-ESI *m/z* 215.2 $[MH]^+$.

(6-Trifluoromethylpyridin-3-yl)carbamic Acid Phenyl Ester. The title compound was prepared from 6-trifluoromethyl-pyridin-3-ylamine as described in the general procedure above. Purification of the crude product was accomplished via silica gel column eluting with ethyl acetate/hexane (1:1) yielding (35%). ^{1}H NMR (CD_3OD, 400 MHz) δ 9.01 (d, J = 2.5 Hz, 1H), 8.48 (dd, J = 8.6, 2.3 Hz, 1H), 8.01 (d, J = 8.6 Hz, 1H), 7.68−7.63 (m, 2H), 7.50−7.44 (m, 3H). MS-ESI 283.1 $[MH]^+$.

(5-Trifluoromethylpyridin-2-yl)carbamic Acid Phenyl Ester. The title compound was prepared from 5-trifluoromethyl-pyridin-3-ylamine as described in the general procedure above. Purification of the crude product was accomplished via silica gel column eluting with ethyl acetate/hexane (1:1) (85%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.60−8.56 (m, 1H), 8.12−8.04 (m, 2H), 7.45−7.41 (m, 2H), 7.29−7.21 (m, 3H), MS-ESI 283.3 $[MH]^+$.

General Procedure for the Preparation of Heterocyclic Ureas 31−41. A mixture of 1-(3-trifluoromethyl-pyridin-2-yl)piperazine (**5m**) (1.00 mmol) and the appropriate heterocyclic carbamic acid phenyl ester (0.95 mmol) were taken into a reaction vial equipped with a rubber septum in anhydrous DMSO (7 mL). The resulting mixture was shaken over a mechanical shaker for 18 h at room temperature. The contents of the vial were diluted with ethyl acetate (40 mL) and washed successively with water (2 × 40 mL), 1 N NaOH (1 × 25 mL), and water (2 × 25 mL), dried over Na_2SO_4, filtered, and evaporated to dryness to give crude product. The crude product was purified by silica gel column chromatography or HPLC.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid Quinolin-5-ylamide (31). 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with quinolin-5-ylcarbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.84 (dd, J = 4.30, 1.52 Hz, 1H), 8.54−8.50 (m, 1H), 8.46−8.41 (m, 1H), 8.06 (dd, J = 7.83, 1.77 Hz, 1H), 7.91 (d, J = 8.59 Hz, 1H), 7.79−7.72 (m, 1H), 7.58−7.49 (m, 2H), 7.25−7.19 (m, 1H), 3.81−3.74 (m, 4H), 3.37−3.31 (m, 4H). MS-ESI *m/z* 402.2 $[MH]^+$. Anal. ($C_{20}H_{18}F_3N_5O$) C, H, N.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid Isoquinolin-5-ylamide (32). 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with isoquinolin-5-ylcarbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD, 400 MHz) δ 9.22 (d, J = 1.01 Hz, 1H), 8.54−8.50 (m, 1H), 8.43 (d, J = 6.06 Hz, 1H), 8.08−8.04 (dd, J = 7.83, 1.77 Hz, 1H), 7.98−7.94 (m, 1H), 7.87−7.84 (m, 1H), 7.70−7.64 (m, 2H), 7.24−7.19 (m, 1H), 3.83−3.73 (m, 4H), 3.35−3.31 (m, 4H). MS-ESI *m/z* 402.2 $[MH]^+$. Anal. ($C_{18}H_{19}F_3N_4O$) C, H, N: calcd. C: 59.85, H: 4.52, N: 17.45; found C: 59.80, H: 4.48, N: 17.40.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid Quinolin-8-ylamide (33). 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with quinolin-8-ylcarbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.82−8.80 (dd, J = 4.30, 1.52 Hz, 1H), 8.51−8.47 (m, 1H), 8.37−8.34 (dd, J = 9.1, 4.04 Hz, 1H), 8.28−8.22 (dd, J = 8.34, 1.52 Hz, 1H), 8.05−8.00 (m, 1H), 7.54−7.48 (m, 3H), 7.21−7.16 (m, 1H), 3.80−3.73 (m, 4H), 3.36−3.31 (m, 4H). MS-ESI *m/z* 402.2 $[MH]^+$. Anal. ($C_{20}H_{18}F_3N_5O$) C, H, N.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid Quinolin-3-ylamide (34). 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with quinolin-3-ylcarbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.32 (d, J = 2.53, 1H), 6.94−6.90 (dd, J = 4.80, 1.26 Hz, 1H), 6.79 (d, J = 2.53, 1H), 6.48−6.44 (dd, J = 7.83, 1.77 Hz, 1H), 6.36 (d, J = 8.59 Hz, 1H), 6.24 (d, J = 8.08 Hz, 1H), 6.08−6.01 (m, 1H), 6.00−5.94 (m, 1H), 5.87−5.80 (m, 1H), 2.20−2.12 (m, 4H), 1.74−1.71 (m, 4H). MS-ESI *m/z* 402.2 $[MH]^+$. Anal. ($C_{20}H_{18}F_3N_5O$) C, H, N.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid Isoquinolin-3-ylamide 35. 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with isoquinolin-3-ylcarbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD, 400 MHz) δ 9.00 (br, s, 1H), 8.52−8.48 (m, 1H), 8.12 (br s, 1H), 8.06−8.02 (dd, J = 7.83−1.52 Hz, 1H), 7.96 (d, J = 8.34 Hz, 1H), 7.79 (d, J = 8.34 Hz, 1H), 7.69−7.61 (m, 1H), 7.50−7.43 (m, 1H), 7.24−7.17 (m, 1H), 3.77−3.71 (m, 4H), 3.33−3.29 (m, 4H). MS-ESI *m/z* 402.2 $[MH]^+$. Anal. ($C_{18}H_{19}F_3N_4O$) C, H, N: calcd. C: 59.85, H: 4.52, N: 17.45; found C: 59.90, H: 4.54, N: 17.48.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid quinolin-6-ylamide 36. 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with quinolin-6-ylcarbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.65−8.62 M, 1H), 8.46−8.42 (m, 1H), 8.20−8.14 (m, 1H), 8.00−7.94 (m, 2H), 7.87 (d, J = 9.10 Hz, 1H), 7.77−7.72 (m, 1H), 7.43−7.38 (dd, J = 8.34, 4.30 Hz, 1H), 7.17−7.12 (m, 1H), 3.70−3.64 (m, 4H), 3.26−3.23 (m, 4H). MS-ESI *m/z* 402.2 $[MH]^+$. Anal. ($C_{20}H_{18}F_3N_5O$) C, H, N.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid Pyridin-4-ylamide 37. 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with pyridin-4-ylcarbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.51−8.48 (dd, J = 4.80, 1.01 Hz, 1H), 8.31−8.26 (dd, J = 6.32, 1.52 Hz, 2H), 8.06−8.02 (dd, J = 7.83, 1.52 Hz, 1H), 7.53−7.49 (dd, J = 6.32, 1.52 Hz, 2H), 7.23−7.18 (m, 1H), 3.73−3.66 (m, 4H), 3.29−3.24 (m, 4H). MS-ESI *m/z* 402.2 $[MH]^+$. Anal. ($C_{16}H_{16}F_3N_5O$) C, H, N.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid Pyridin-3-ylamide 38. 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with pyridin-3-ylcarbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.61−8.58 (m, 1H), 8.51−8.48 (dd, J = 4.8, 1.26 Hz, 1H), 8.18−8.15 (dd, J = 4.8, 1.26 Hz, 1H), 8.06−8.01 (dd, J = 7.83, 1.52 Hz, 1H), 7.95−7.90 (m, 1H), 7.37−7.32 (m, 1H), 7.22−7.17 (m,

1H), 3.72−3.66 (m, 4H), 3.29−3.24 (m, 4H). MS-ESI *m/z* 402.2 $[MH]^+$. Anal. ($C_{16}H_{16}F_3N_5O$) C, H, N.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid Pyridin-2-ylamide 39. 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with pyridin-3-ylcarbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.44−8.34 (m, 1H), 8.27−8.17 (m, 1H), 7.97 (dd, J = 7.83, 1.77 Hz, 1H), 7.50 (d, J = 8.59, 1H), 7.38−7.32 (m, 1H), 7.17−7.11 (m, 1H), 3.73−3.67 (m, 4H), 3.23−3.21 (m, 4H). MS-ESI *m/z* 402.2 $[MH]^+$. Anal. ($C_{16}H_{16}F_3N_5O$) C, H, N.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (6-Trifluoromethylpyridin-3-yl)amide 40. 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with (6-trifluoromethylpyridin-3-yl)carbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD) δ 8.75 (d, J = 2.5 Hz, 1H), 8.52−8.50 (m, 1H), 8.16−8.12 (m, 1H), 8.07−8.04 (m, 1H), 7.72 (d, J = 8.6 Hz, 1H), 7.24−7.20 (m, 1H), 3.74−3.70 (m, 4H), 3.30−3.26 (m, 4H), MS-ESI *m/z* 420.2 $[MH]^+$. Anal. ($C_{17}H_{15}F_6N_5O$) C, H, N.

4-(3-Trifluoromethylpyridin-2-yl)piperazine-1-carboxylic Acid (5-Trifluoromethylpyridin-2-yl)amide 41. 1-(3-Trifluoromethylpyridin-2-yl)piperazine (**5m**) was treated with (5-trifluoromethylpyridin-2-yl)carbamic acid phenyl ester as described in the general procedure, above, to give the title compound. ^{1}H NMR (CD_3OD) δ 8.53−8.50 (m, 2H), 8.06−8.03 (m, 1H), 8.00−7.94 (m, 2H), 7.23−7.19 m, 1H), 3.74−3.71 (m, 4H), 3.30−3.27 (m, 4H), MS-ESI *m/z* 420.1 $[MH]^+$. Anal. ($C_{17}H_{15}F_6N_5O$) C, H, N.

Biology Methods. Functional Characterization for Agonist/Antagonist Activity. The construction of the cell line stably expressing recombinant human TRPV1 was previously described.[13] The rat TRPV1 cDNA was PCR amplified from rat brain cDNA (Clontech) using two primers with forward primer (5′gactGAATTCGCCACCatggaacaacgggctagcttagac3′) and reverse primer (5′ actagcGCGGCCGCttatttctccctgggaccatggaat 3′) that were designed based on the Genbank sequence AF029310. The PCR conditions that used for amplification of rat TRPV1 is 94 °C 30 s, 65 °C 30 s, and 72 °C for 5 min for 40 cycles using Expand High Fidenity PCR System (Roche Biosciences). The PCR product was digested with EcoR1 and Not1 and cloned into pCINeo (Invitrogen). The insert region was sequenced to comfirm identity to AF029310. HEK293 cells were transfected with rat TRPV1 using methods previously described,[13] and cells surviving selection by Geneticin were generated by FACS (FACSVantage, Scripps Research Institute) and screened for activity on the FLIPR to yield a stably expressing rat TRPV1 line. Cells were grown in DMEM (high glucose) medium supplemented with 10% fetal bovine serum and antibiotics and maintained at 37 °C in a humidified atmosphere of 5% CO_2.

FLIPR protocols and analysis were as described previously.[21,13] Briefly, hTRPV1-HEK293 and rTRPV1-HEK293 were seeded at ∼50 K cells/well in 96-well poly-D-lysine-coated BD plates, incubated overnight, and then loaded with 4 μM calcium fluorescent dye Fluo-3/AM (acetoxymethylester) (Teflabs, Austin, TX) with Pluronic-F127 (Sigma, St. Louis, MO; 0.01%) at room temperature for 60 min. Prior to testing on the FLIPR (Molecular Devices, Sunnyvale, CA), extracellular dye was aspirated and cells were incubated in assay buffer (containing in mM: NaCl 128, KCl 2, $MgCl_2$ 1, dextrose 10, HEPES 20, $CaCl_2$ 2; pH 7.45). Cells were incubated in compounds at a final concentration ranging between 0.3 nM to 30 μM in half log increments for 3 min prior to application of capsaicin to achieve a final concentration of 15 nM. Raw fluorescence data were corrected by subtracting basal fluorescence counts at all time points. The relevant agonist signal was the smaller of the fluorescence values measured at the peak and at the end of the agonist incubation. The EC_{50} for capsaicin under these assay conditions was 6.1 ± 1.1 nM (n = 11) at the human recombinant TRPV1. The EC_{50} for capsaicin at rat TRPV1 was initially 8.0 ± 1.6 nM (n = 6), and IC_{50} values were determined using 15 nM capsaicin as the stimulus. However, later experiments using rTRPV1-HEK293 (but not hTRPV1-HEK293) cells revealed a higher EC_{50} for capsaicin, and IC_{50} determinations were made using 100 nM capsaicin as the stimulus. Interestingly, the EC_{50} for anandamide and PMA were unchanged at both cell lines.

The potency of selected compounds to inhibit acid-, phorbol ester-, and anandamide-induced Ca^{2+} influx mediated by TRPV1 was determined using FLIPR. Protons directly activate TRPV1.[29−31] Cells were challenged for about 5 min with low pH (pH 5.8−6.0) solution which produced a stable increase in basal intracellular Ca^{2+} mediated by TRPV1 activity compared to adjacent wells exposed to physiological pH. The high basal Ca^{2+} levels required sustained TRPV1 activity, and antagonist potency was determined by assessing the decrease in basal fluorescence over a 3 min period in antagonist.

The phorbol ester phorbol-12-myristate-13-acetate (PMA) was applied to activate TRPV1. In both hTRPV1-HEK293 and rTRPV1-HEK293 cell lines, PMA activated TRPV1 in a PKC dependent manner. The EC_{50} for PMA at either human and rat recombinant TRPV1 was 90 ± 20 nM (n = 9) and 130 ± 40 nM (n = 4), respectively. The active phorbol ester effect was blocked by ruthenium red and capsazepine and required extracellular Ca^{2+}. There was no detectable increase in intracellular Ca^{2+} levels by PMA on untransfected HEK293 cells. The rank order of potency for a panel of phorbol esters was similar to their rank order of potency to block classical PKC: phorbol 12,13-dibutyrate ∼ PMA > phorbol 12,13-diacetate ≫ phorbol 12,13-didecanoate. The inactive 4α-phorbol had no detectable effect. The PMA-induced increase in Ca^{2+} was occluded by preincubation in the PKC inhibitors bisindolylmaleimide (BIM) and staurosporine. Taken together, PMA activates both rat and human TRPV1 with similar potency. The efficacy of PMA compared to the full agonist capsaicin was about 50% but showed some variability between rat and human with PMA often being more efficacious at the human receptor. For IC_{50} determinations, cells were incubated in compound (**4**, **14**, **41**, capsazepine, or ruthenium red) for 3 min and subsequently exposed to PMA (300 nM). Fluorescence was monitored for 5−10 min.

Anandamide is a brain-derived cannabinoid ligand that is an agonist at TRPV1 at low μM concentrations.[32] Similar to its efficacy at other heterologous expression systems,[33,34] anandamide was a full agonist at recombinant human TRPV1 and rat TRPV1 in these studies. The EC_{50} of anandamide was 3.1 ± 0.04 μM (n = 5) and 4.7 ± 1.0 μM (n = 2) at human and rat receptors, respectively, and the IC_{50} to block the Ca^{2+} response after application of 10 μM anandamide was determined using FLIPR. Anandamide had no effect on untransfected cells. The pharmacology of the response elicited by anandamide was consistent with other reports.[33,34,35] The sigmoidal dose−response curves were analyzed by computerized curve-fitting, using a four-parameter logistic Hill equation in Prism (GraphPad, San Diego, CA). The EC_{50} (for agonists) or IC_{50} (for antagonists) value of a compound is the effective dose showing 50% of maximal effect. All data are expressed as means ± SEM for $n \geq 3$ determinations in duplicate.

In Vivo Characterization. Animals. The Institutional Animal Care and Use Committee of Johnson & Johnson Pharmaceutical Research and Development, L. L. C., approved all protocols. For behavioral experiments, rats (Harlan, Indianapolis, IN) were housed pairwise in cages with ad libitum access to standard rat chow and water. For behavioral experiments, male rats (200−250 g) maintained on a reverse 12 h day/night cycle were used; for temperature telemetry experiments, female rats (300 g) on a standard 12-h cycle were used.

Drugs and Reagents. Compound **41**, solubilized in poly(ethylene glycol) (PEG-400), was administered orally (po) in a volume of 3 mL/kg or intraperitoneally (ip). Capsaicin and *N*-arachidonoyldopamine (NADA) solubilized in 0.9% saline were administered via intraplantar injection in a volume of 30 μL. For hypothermia testing, capsaicin was formulated in 10% ethanol, 40% propanediol, and 50% PBS (phosphate-

buffered saline) and administered by subcutaneous injection (sc) in a volume of 1 mL/kg

Tactile Allodynia Testing. Tactile allodynia was measured as previously described.[36] Briefly, calibrated von Frey filaments of evenly incremental stiffness between 0.41 and 15.8 g (Stoelting, Wood Dale, IL) were applied perpendicularly to the plantar surface of the affected paw through wire-mesh observation cages, and the pressure at which the paw was withdrawn was noted. A 50% paw withdrawal threshold (PWT) was interpolated using an adaptation of the Dixon up−down method, noting positive and negative responses to sequentially applied stimuli.

Thermal Sensitivity Testing. Rats were placed in a modified Hargreaves[37] box (UARDG, La Jolla, CA)[38] with a glass floor maintained at 30 °C. Thermal sensitivity was assessed by focusing a light source on the undersurface of a hind paw and recording the latency to paw withdrawal. The intensity of the heat produced by the focused light beam was adjusted to induce paw withdrawal at 10 s in normal rats. Test animals were stimulated for a maximum of 20 s.

Intraplantar Capsaicin Model. Rats were dosed with vehicle or drug 1 h preinjection, placed under isoflurane gas anesthesia, and injected with capsaicin (5 or 10 μg in 30 μL of saline) intradermally into the plantar surface of one hind paw, and tested for thermal sensitivity 10, 30, and 60 min postinjection, and tactile allodynia 30, 60, and 90 min postinjection.

Intraplantar NADA Model. Rats were dosed with vehicle or drug 1 h preinjection, placed under isoflurane gas anesthesia, injected with intraplantar NADA (5 μg in 30 μL of saline) intradermally into the plantar surface of one hind paw, and tested for thermal sensitivity 30, 60, and 90 min postinjection; tactile allodynia 30 and 60 min postinjection.

Effects on Body Temperature. Female Sprague Dawley rats weighing approximately 300 g underwent intraperitoneal implantation of telemetric temperature transducers (DataSciences TA10TA-F40) under isoflurane/air anesthesia and were allowed to recover for at least 1 week. On the day of the experiment, telemetry receivers were placed under the home cages of surgically implanted animals. Body temperature was continuously monitored (every 2 min) remotely beginning 60 min prior to po administration of compound or vehicle (PEG-400) until 7 h after administration. Animals were dosed with **41** or vehicle by gentle oral gavage.

Capsaicin-Induced Hypothermia: Pharmacological Model. The dose-dependent prevention of capsaicin-evoked hypothermia was measured as follows. Rats were placed in telemetry cages and allowed to acclimate for 60 min. Minimum temperature recorded during this time was used as pretreatment baseline. Compound or vehicle was then gently administered by oral gavage, and telemetry recording was resumed. The maximum temperature recorded during this time was noted and compared to baseline. Sixty minutes later, capsaicin was injected sc. The capsaicin-induced hypothermic effect was calculated by comparing the baseline temperature prior to dosing with vehicle or compound to the minimum body temperature following capsaicin dose.

Data Handling. %MPE calculation: Data were normalized to 0−100% prophylactic effect as follows. Pretreatment baselines were used to represent normothermia or 100% prevention of hypothermia. The mean minimum body temperature attained after capsaicin injection in the group treated with vehicle alone was used to represent maximum hypothermia, or 0% hypothermia prevention. Data were normalized to this range of values using the formula,

$$\frac{\text{(observed value-minimum attainable temperature)}}{\text{(baseline-minimum temperature)}} \times 100$$

The ED_{50} was calculated using Prism (graph Pad, San Diego, CA).

Statistics. Statistics were calculated using Prism (Graph Pad, San Diego, CA) and SigmaStat (Systat Software Inc., Point Richmond, CA). One-way ANOVA with Dunnett's posthoc test was used to compare maximum body temperatures and minimum body temperatures. Two-way ANOVA with post-hoc testing using Bonferroni's method was used applied to behavioral data. Significance was chosen at $p < 0.05$.

Pharmacokinetic Methods. A rat in vivo system was used to determine the pharmacokinetic parameters of compound **41** after acute bolus oral and intravenous administration. One group of nine female Sprague Dawley rats (approximately 300 g body weight) was used. They were group-housed, provided food and water ad libitum, and maintained on a 12 h light and dark cycle. Animals were acclimated for at least 7 days after receipt from the vendor prior to investigations.

Three animals received a bolus dose of the TRPV1 receptor antagonist **41** at a dose of 1 mg/kg in a volume of 1 mL/kg in the lateral tail vein, three animals received a bolus dose of **41** in the peritoneal cavity at a dose of 1 mg/kg in a volume of 1 mL/kg or four animals by oral gavage at a dose of 10 mg/kg in a volume of 10 mL/kg. The dosing solution was prepared in 10% Solutol (PEG-660 hydroxystearate) in 5% dextrose in water at a concentration of 1 mg/mL.

Blood sampling followed dosing via venipuncture with a the tail vein needle over a time course. Blood samples consisted of 100 μL samples taken from the tail vein using a 23 gauge needle into heparinized Natelson blood collection tubes and expelled into 1.5 mL microcentrifuge tubes containing 300 μL of acetonitrile with a 1 μM internal standard. These blood samples were then centrifuged at 14000 rpm in a microcentrifuge for 3 min. The supernatant was retained and kept refrigerated until processed for analysis. The time course for sampling from the intravenous administered animals was as follows: 0.03, 0.08, 0.25, 0.5, 1, 3, 5, and 24 h. The time course for sampling from the intraperitoneal and oral administered animals was as follows: 0.5, 1, 2, 4, 6, and 24 h.

All blood samples were deproteinized by 1:4 dilution of the sample with acetonitrile with vigorous mixing. This sample was incubated for 5 min and then centrifuged at 14 000 rpm in a microcentrifuge for 4 min. The supernatant was recovered into autosampler vials, diluted 1:1 with sterile water, and capped. These samples were analyzed by LC-MS/MS to determine the plasma level of the compound by APCI positive mode by selected reaction monitoring. Data collection method: A Vydac SP C18 2.1 × 50 mm analytical column was used. The flow rate was maintained at 0.25 mL/min, and a sample volume of 40 μL was injected on the column.

Plasma level vs time plots were created, and the software package WinNonlin was used to analyze the data. Noncompartmental pharmacokinetic models were used to determine the AUC_{inf} (area under the curve extrapolated to infinity which measures animals' drug exposure over a time course and is used to calculate bioavailability), $AUMC_{inf}$, mean residence time (MRT), $T_{1/2}$, and clearance resulting from both routes of administration. The dose-corrected AUC_{inf} was compared between the two routes of administration to determine the oral bioavailability of the compound.

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Supporting Information Available: Microanalytical data for all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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