Discovery of CC Chemokine Receptor-3 (CCR3) Antagonists with Picomolar Potency

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Starting with our previously described[20] class of CC chemokine receptor-3 (CCR3) antagonist, we improved the potency by replacing the phenyl linker of **1** with a cyclohexyl linker and by replacing the 4-benzylpiperidine with a 3-benzylpiperidine. The resulting compound, **32**, is a potent and selective antagonist of CCR3. SAR studies showed that the 3-acetylphenyl urea of **32** could be replaced with heterocyclic ureas or heterocyclic-substituted phenyl ureas and still maintain the potency (inhibition of eotaxin-induced chemotaxis) of this class of compounds in the low-picomolar range (IC_{50} = 10−60 pM), representing some of the most potent CCR3 antagonists reported to date. The potency of **32** for mouse CCR3 (chemotaxis IC_{50} = 41 nM) and its oral bioavailability in mice (20% F) were adequate to assess the efficacy in animal models of allergic airway inflammation. Oral administration of **32** reduced eosinophil recruitment into the lungs in a dose-dependent manner in these animal models. On the basis of its overall potency, selectivity, efficacy, and safety profile, the benzenesulfonate salt of **32**, designated DPC168, entered phase I clinical trials.

Introduction

Since 1980, the frequency of asthma has almost doubled. The disease now affects 8−10% of the adult population of the U.S. and is the leading cause of hospitalization among children less than 15 years of age. While the principal clinical manifestation of asthma is reversible airway obstruction, it is also recognized as an inflammatory disease.[1]

In allergic asthma, the pulmonary airways are characterized by an inflammatory infiltrate in which eosinophils often comprise the major component. Within the bronchial mucosa, eosinophils degranulate, releasing toxic proteins that promote tissue damage and bronchoreactivity.[2] They also release fibrogenic factors such as transforming growth factor-β (TGFβ) that are implicated in chronic airway remodeling.[3] Although correlative evidence links eosinophils with asthma symptomatology, a direct role for these cells in the disease is controversial, largely as a result of the failed clinical trials of two humanized anti-IL-5 antibodies. Although the antibody regimens dramatically reduced the numbers of circulating eosinophils, airway function was not significantly improved. Subsequent studies, however, have shown that, despite near elimination from the blood, eosinophils were reduced in the airway tissue by only 55%. Airway remodeling, however, was significantly reduced.[4] On the basis of these results, the original negative interpretation of the studies is undergoing reassessment.[5]

IL-5 mobilizes eosinophils from the bone marrow into the blood. Their recruitment into the pulmonary airways, however, occurs through the action of chemokines. Chemokines are small secreted proteins that chemoattract and activate leukocytes of a variety of types, sometimes under normal, homeostatic conditions, at other times during inflammation. They are classified into two main groups, CXC and CC, based on the positions of the first two of their conserved N-terminal cysteine residues. Both groups mediate their effects through seven transmembrane (7TM) G-protein-coupled receptors (GPCRs).

CC chemokine receptor-3 (CCR3) was originally cloned from human eosinophils[6] and is regarded as the principal mediator of eosinophil chemotaxis. CCR3 is also known to be expressed on a variety of inflammatory cells associated with allergic responses and prominent in allergic tissues such as the nose, airways, and skin. These cells include basophils,[7] mast cells,[8] and Th2 lymphocytes.[9] CCR3 is thought to mediate the migration of cells from the blood into the tissue and their subsequent activation. In addition to its role in migratory cells, CCR3 is also expressed on resident tissue cells such as airway epithelium.[10]

CCR3 has 11 known ligands, but several, including eotaxin (CC ligand 11, CCL11),[11b] eotaxin-2 (CCL24),[11c] and eotaxin-3 (CCL26),[11d] are exclusive high-affinity agonists of CCR3. Eotaxin has also been described as an antagonist of CCR2,[12] a partial agonist of CCR2b,[13] and a full agonist of CCR5.[12] Eotaxin-3 is an antagonist of CCR2 and stimulates the negative chemotaxis (repulsion) of monocytes in vitro.[14] The role of CCR3 in the eosinophilia seen in asthmatic airways is suggested by the fact that its ligands are expressed in asthma at high levels that correlate with disease severity and poor airway function.

CCR3 knock-out mice are fully functional and reproduce normally. In ovalbumin aerosol models of allergic

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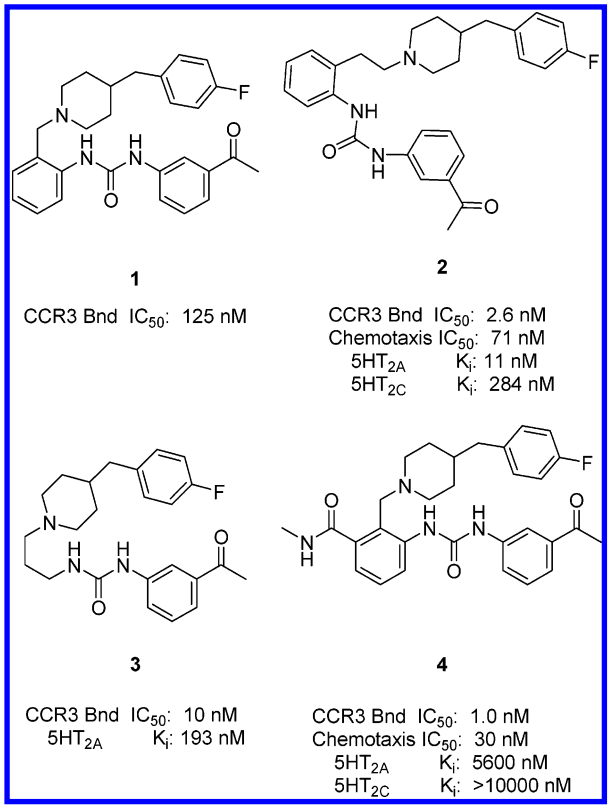


Figure 1. Structure of compounds discussed in text.

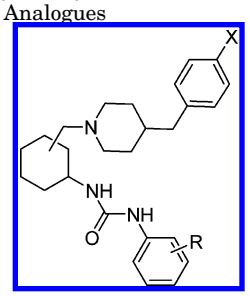
airway inflammation, they exhibit reduced lung eosinophilia (>50%) relative to wild-type mice.[15] A similar reduction in eosinophilia is seen in eotaxin postallergen challenge knock-out mice.[16]

The studies cited above suggest that CCR3 is a prominent mediator of allergic responses in the airways and that antagonizing the receptor will lead to a reduction in airway inflammation and remodeling. The potential importance of CCR3 in allergic inflammation has made this receptor a target of drug development,[17] and we[18] and others[19] have previously reported on small molecule CCR3 antagonists. In this paper, we provide further details of our efforts in this area.

Results and Discussion

Discovery of Cyclohexyl Linker. We have previously reported on the discovery of a potent class of CCR3 receptor antagonists.[20] The important pharmacophore of the class consists of a benzylpiperidine linked to a phenyl urea through a benzyl group[20] as in **1**, a phenethyl[20] group as in **2**, or a propyl[21] chain as in **3** (Figure 1). These compounds are very selective for CCR3 over other chemokine receptors that were tested (CCR1, CCR2, CCR5); however, they showed significant affinity for other 7TM GPCRs that were examined. For example, the phenethyl linker analogue **2** displayed potent affinity for the serotonin receptors and in particular the $5HT_{2A}$ ($K_i = 11$ nM) and $5HT_{2C}$ ($K_i = 284$ nM) subtypes, while the propyl linker analogue **3** had affinity for $5HT_{2A}$ ($K_i = 193$ nM) as shown in Figure 1. Although the benzyl-linked analogues (e.g., **4**) were more selective ($5HT_{2A}$ $K_i = 5600$ nM), this class of compounds had less desirable physicochemical properties. They were much less water soluble, much more lipophilic, and highly protein bound (>99%) and were inherently less potent

Table 1. SAR of Cyclohexyl Linker Stereochemistry in the 4-Benzylpiperidine Analogues



compd	stereochemistry[a]	X	R	CCR3[b] IC_{50} (nM)	$5HT_{2A}/5HT_{2C}$ K_i (nM)[c]	D2 K_i (nM)[c]
5	1,2 cis	H	3-OMe	423	NT[d]	NT[d]
6	1,2 trans	H	3-OMe	67	NT[d]	NT[d]
7	1,2 trans	F	3-OMe	12	869/2315	271
8	1,2 trans	F	3-Ac	4	601/>10 000	103
9	1,3 cis/trans mix	F	3-OMe	78	NT[d]	NT[d]
10	1,3 cis/trans mix	F	3-Ac	41	NT[d]	NT[d]

[a] All compounds in the table are racemic. [b] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown. [c] The value represents an average of usually two determinations with a deviation of ±30% of the mean value shown. [d] NT, not tested.

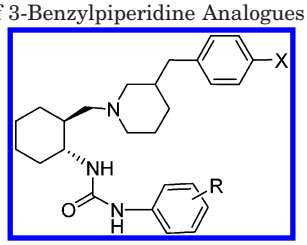
(compare **1** vs **2**). We hypothesized that most of these deficiencies could be attributed to the lower basicity of the piperidine nitrogen in the benzyl linker series compared to the phenethyl or propyl linker series.

To test this hypothesis, we synthesized a series of compounds where the central phenyl ring of **1** was replaced with a cyclohexyl ring. For this initial survey, we started our synthesis with the commercially available racemic cis or trans 2-hydroxymethyl-1-cyclohexylamine and 3-aminocyclohexane carboxylic acid (cis/trans mixture) and the results are summarized in Table 1.

The 1,2-cis isomer **5** has the same potency as the corresponding benzyl linker[20] (data not shown) and is clearly less potent than the 1,2-trans isomer **6** (Table 1). The 1,3 isomers (**9**, **10**, Table 1) are also less potent than the 1,2-trans isomers. Introduction of the 4-fluoro substituent on the benzylpiperidine group improves the potency of **6** ($IC_{50} = 67$ nM) about 5-fold to give **7** ($IC_{50} = 12$ nM). This is less than the 10-fold improvement observed for the phenethyl[20] or propyl[21] series but better than the 3-fold improvement seen with the benzyl[20] series. With the use of a more optimal substituent on the phenyl urea, the CCR3 binding potency is further improved (to $IC_{50} = 4$ nM) for the acetyl analogue **8**, which is a significant increase over the corresponding benzyl analogue **1** ($IC_{50} = 125$ nM, Figure 1). The structure−activity relationship (SAR) for serotonin selectivity of the cyclohexyl series mirrors the SAR noted for the 4-fluorobenzylpiperidine effect, namely, that its behavior is between that of the benzyl and phenethyl/propyl linker series. Thus, the serotonin selectivity of the trans 1,2-cyclohexyl linker analogue **8** ($5HT_{2A}$ $K_i = 600$ nM) is not as good as the benzyl analogue **4** ($5HT_{2A}$ $K_i = 5600$ nM, Figure 1), but it is better than the phenethyl **2** or the propyl **3** analogues (Figure 1). While the serotonin selectivity could be modulated, **8** showed potent affinity for the dopamine D2 receptor ($K_i = 103$ nM) as shown in Table 1.

To further improve selectivity, we examined replacing the 4-benzylpiperidine of these compounds with 3-ben-

Table 2. SAR of 3-Benzylpiperidine Analogues



compd[a]	X	R	CCR3[b] IC_{50} (nM)	$5HT_{2A}/5HT_{2C}$ K_i (nM)[c]	D2 K_i (nM)[c]
11	H	3-OMe	28	10 000/10 000	911
12	H	3-CN	9	9000/10 000	1010
13	H	3-Ac	3	6000/10 000	550
14	F	3-OMe	14	NT[d]	NT[d]
15	F	3-CN	12	NT[d]	NT[d]
16	F	3-Ac	3	1333/10 000	1000

[a] All compounds in the table are mixtures of diastereomers. [b] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown. [c] The value represents an average of usually two determinations with a deviation of ±30% of the mean value shown. [d] NT, not tested.

zylpiperidine.[18] Again, for our initial survey we used racemic 3-benzylpiperidine and racemic 1,2-trans cyclohexyl components for the analogues synthesized, and the data are summarized in Table 2. The potency of the 3-benzylpiperidine analogues for the CCR3 receptor is slightly better than the 4-benzylpiperidines (compare **6** and **11**). The 4-fluoro substituent on 3-benzylpiperidine shows little (compare **11** and **14**) to no improvement (compare **13** and **16**) in potency. However, there is a more pronounced improvement in selectivity against other GPCRs that were tested. The serotonin selectivity generally improves for all the compounds tested. For example, the 3-acetylphenyl urea analogue improves 2-fold in going from the 4-benzylpiperidine analogue **8** ($5HT_{2A}$ K_i = 601 nM) to the 3-benzylpiperidine analogue **16** ($5HT_{2A}$ K_i = 1333 nM). More significantly, there is a substantial improvement in selectivity against dopamine D2 of **16** (K_i = 1000 nM) over analogue **8** (K_i = 103 nM).

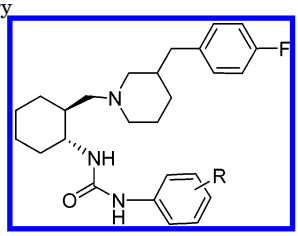
Chemistry

Having established that the cyclohexyl linker analogues could yield potent CCR3 antagonists, we needed to establish the preferred absolute configuration for this series. The absolute configuration about the cyclohexyl ring was initially established by resolving a synthetic intermediate by chiral HPLC or by using the previously described enzymatic procedure[23] using Lipase PS to give the (*R*,*R*) *N*-CBZ amino alcohol **18**. In either case, the resolved intermediate was converted to the 4-benzylpiperidine analogue **8**. The (+)-isomer **8** (CCR3 IC_{50} = 1.8 nM) was more potent than the (−)-isomer **8** (CCR3 IC_{50} = 43 nM). This established that the desired amino alcohol **17** required the *R*,*R* configuration.

To determine the preferred configuration about the 3-benzylpiperidine portion, the BOC-protected 3-(4-fluorobenzyl)piperidine **25** was resolved by chiral HPLC and used to synthesize the analogues summarized in Table 3. The data in Table 3 show that there is very little difference in binding affinity between the (*R*)- and (*S*)-3-(4-fluorobenzyl)piperidine analogues. This result is in contrast to the 16-fold difference in potency that is observed in the propyl linker series[22] in which the (*S*)-enantiomer is preferred. The selectivity against other GPCRs is also not very different between the (*R*)- and (*S*)-3-(4-fluorobenzyl)piperidine in the trans cyclohexyl linker series as shown in Table 3. However, when a functional assay of CCR3 receptor antagonism was used, the (*S*)-3-(4-fluorobenzyl)piperidine analogue **32** (chemotaxis IC_{50} = 0.034 nM) was clearly more potent than the (*R*)-3-(4-fluorobenzyl)piperidine analogue **29** (chemotaxis IC_{50} = 0.364 nM). This established the (1*R*,2*S*)-2-(((*S*)-3-(4-fluorobenzyl)piperidin-1-yl)methyl)-cyclohexanamine core as the preferred isomer.

The required starting material, the chiral (*R*,*R*) amino alcohol **17**, was obtained following the literature[24] synthetic procedures. All subsequently described analogues in this report were synthesized from **17** using the synthetic sequence outlined in Scheme 1. Thus, the amino alcohol **17** was protected as the benzylcarbamate by treatment with benzyl chloroformate to give the protected amine **18**. The *N*-CBZ amino alcohol **18** was subjected to Swern oxidation conditions to give the aldehyde **19**. Reductive amination of **19** with benzylpiperidine gave **20**. The hydrogenation of **20** gave the free amine **21**, which was treated with isocyanates to give the final urea analogues. Alternatively, the amine **21** was treated with phenyl carbamates to give the ureas.

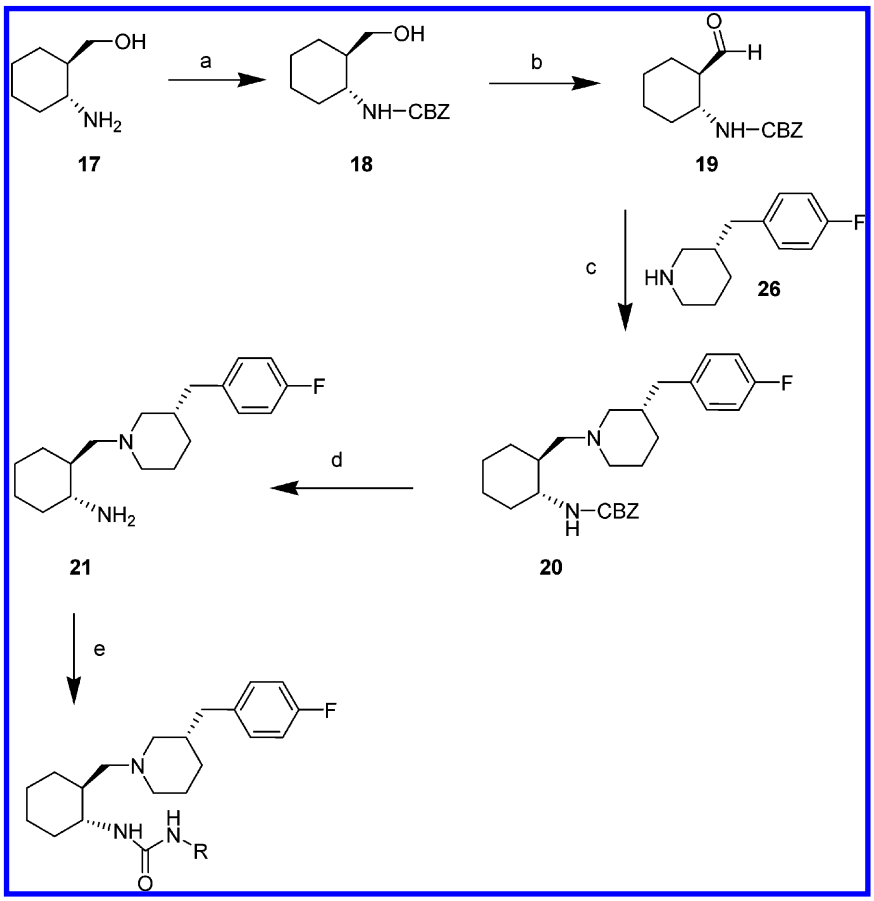
Table 3. SAR of 3-Benzylpiperidine Stereochemistry



compd[a]	piperidine stereochemistry	R	CCR3[b] IC_{50} nM	chemotaxis IC_{50} (nM)	$5HT_{2A}/5HT_{2C}$ K_i (nM)[c]	D2 K_i (nM)[c]
27	*R*	3-OMe	29	NT[d]	NT[d]	NT[d]
28	*R*	3-CN	9	NT[d]	4300/3000	1000
29	*R*	3-Ac	2.5	0.364[e]	5000/2400	702
30	*S*	3-OMe	14	NT[d]	NT[d]	NT[d]
31	*S*	3-CN	9	NT[d]	1800/2600	550
32	*S*	3-Ac	2.0	0.034 ± 0.019	1000/6000	1020

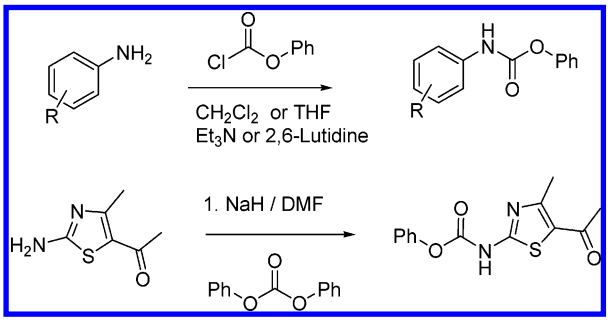
[a] All compounds in the table are homochiral with the absolute configuration of the cyclohexyl as shown. [b] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown. [c] The value represents an average of usually two determinations with a deviation of ±30% of the mean value shown. [d] NT, not tested. [e] Value was measured once.

Scheme 1. Synthesis of Urea Analogues[a]



[a] Key: (a) benzyl chloroformate/Na_2CO_3(aq)/CH_2Cl_2; (b) Swern oxidation; (c) $NaBH(OAc)_3$; (d) H_2/10% Pd/C; (e) R−N=C=O/THF or RHNCOOPh/THF.

Scheme 2. Synthesis of Phenyl Carbamates



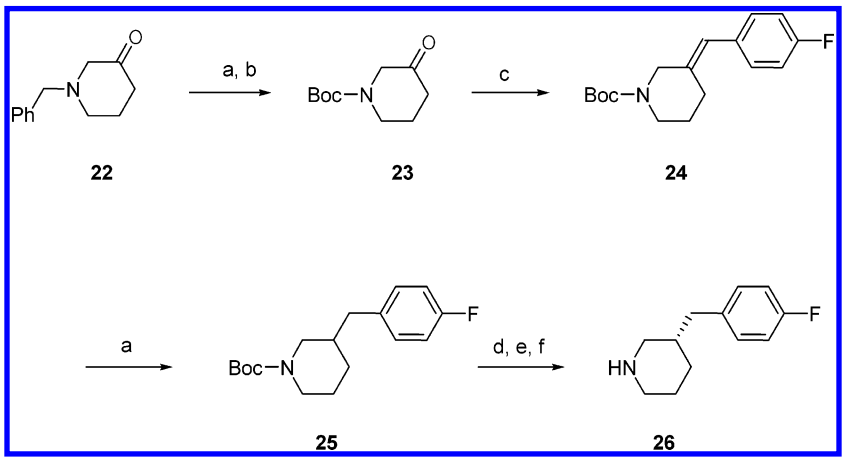
The phenyl carbamates were readily available from amines by treatment with phenyl chloroformate (Scheme 2). However, this procedure did not always work well with some amines, especially the heterocyclic amines. In these cases, the amine was first converted to the sodium salt (NaH in DMF) and then treated with diphenyl carbonate to give good yields of the desired phenyl carbamate (Scheme 2). The phenyl carbamates reacted readily with the amine **21** to give the urea analogues.

The 3-benzylpiperidine fragment was prepared[25] from commercially available *N*-benzyl-3-piperidone (**22**) as shown in Scheme 3. First, the benzyl group was converted to *N*-BOC-3-piperidone (**23**) by a catalytic hydrogenolysis, followed by acylation with di-*tert*-butyl dicarbonate. A Wittig reaction with 4-fluoro-benzylphosphonium chloride and *n*-butyllithium to give the olefin **24** was followed by a catalytic hydrogenation to afford BOC-protected 3-(4-fluorobenzyl)piperidine **25**. This compound was initially resolved by a preparative chiral HPLC to give the required (*S*)-(+)-isomer. However, a practical large-scale resolution method was developed, which uses (*R*)-(−)-mandelic acid. Thus, **25** was deprotected to give the free amine 3-(4-fluorobenzyl)piperidine, which was resolved by the formation of a diastereomeric salt to selectively crystallize the (*S*)-(+)-3-(4-fluorobenzyl)piperidine mandelic acid salt (98% ee after two recrystallizations).[26]

In Vitro Structure−Activity Relationship (SAR)

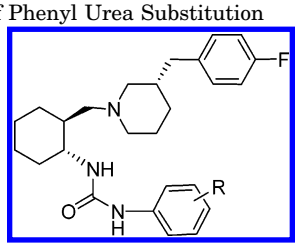
Binding SAR. The evaluation of the in vitro pharmacological properties of the compounds was performed using the binding assay previously described.[20] We examined a series of substituents on the phenyl urea, and the results are summarized in Table 4. The unsubstituted phenyl urea **33** itself is a potent CCR3 antagonist with a binding IC_{50} = 2.5 nM. Substitution at the 2, 3, or 4 position of the phenyl ring with electron-donating groups such as a methoxy (compounds **35**, **30**, **36**, Table 4) or electron-withdrawing groups such as a fluoride (**37**−**39**) was examined. The 2- or 4-isomers all give similarly potent compounds. However, the 3-isomers are less potent with the 3-methoxy analogue **30** decreasing to an IC_{50} = 18 nM and the 3-fluoro analogue **38** decreasing to an IC_{50} = 10 nM. The trend continued with the larger halogens, with the 3-chloro (**43**) or 3-bromo (**47**) analogues (IC_{50} = 31, 33 nM, respectively) losing some potency. The disubstituted analogues are

Scheme 3. Synthesis of (*S*)-3-(4-Fluorobenzyl)piperidine[a]



[a] Key: (a) H_2/10% Pd/C/MeOH; (b) di-*tert*-butyl-dicarbonate/$NaHCO_3$(aq)/THF; (c) (4-F-benzyl)$P(Ph)_3Cl$/BuLi/THF; (d) 1. 4N HCl/dioxane 2, Na_2CO_3; (e) (*R*)-(−)-mandelic acid/AcCN, recrystallize; (f) NaOH (aq)/ether.

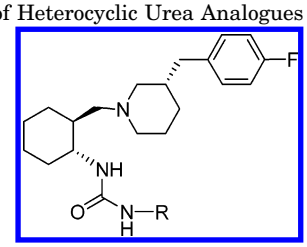
Table 4. SAR of Phenyl Urea Substitution



compd	R	CCR3 IC_{50} (nM)[a]	compd	R	CCR3 IC_{50} (nM)[a]
33	H	2.5	**44**	4-Cl	8
34	2-Me	1.6	**45**	3,5-Cl	26
35	2-OMe	1.4	**46**	3,4-Cl	53
30	3-OMe	18	**47**	3-Br	33
36	4-OMe	2.0	**48**	4-Br	6
37	2-F	1.8	**31**	3-CN	8.8
38	3-F	10	**49**	3-CN-4-F	30
39	4-F	5	**32**	3-Ac	2.0
40	3,4-F	12	**50**	4-Ac	3.0
41	3-F-4-Me	4	**51**	3,5-Ac	1.1
42	5-F-2-Me	4.6	**52**	3-Ac-4-F	1.5
43	3-Cl	31			

[a] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown.

Table 5. SAR of Heterocyclic Urea Analogues



compd	R	CCR3 IC_{50} (nM)[a]
53	indol-6-yl	1.6
54	indol-5-yl	0.4
55	2,3-dimethyl-indol-6-yl	1.8
56	indazol-5-yl	0.3
57	indazol-6-yl	0.7
58	3-Cl-indazol-5-yl	1.3
59	indolin-5-yl	1.7
60	benzo[*d*]thiazol-6-yl	1.2
61	benzimidazol-5-yl	1.0
62	1,3,4-thiadiazol-2-yl	2.3
63	thiazol-2-yl	1.9
64	4-methylthiazol-2-yl	1.4
65	5-acetyl-4-methylthiazol-2-yl	0.9
66	4-chlorobenzo[*d*]-thiazol-2-yl	4.0
67	1-methyl-pyrazol-3-yl	1.9
68	5-methylisoxazol-3-yl	2.7
69	pyridin-4-yl	1.1
70	3-cyano-4-(1H-pyrazol-1-yl)phenyl	2.0
71	4-(1H-1,2,4-triazol-1-yl)phenyl	2.2
72	3-(5-methyl-1H-tetrazol-1-yl)phenyl	1.4
73	2-(1-methyl-1H-tetrazol-5-yl)phenyl	0.9
74	3-(1-methyl-1H-tetrazol-5-yl)phenyl	0.7
75	4-(1-methyl-1H-tetrazol-5-yl)phenyl	0.7
76	3,5-bis-(1-methyl-1H-tetrazol-5-yl)phenyl	1.0

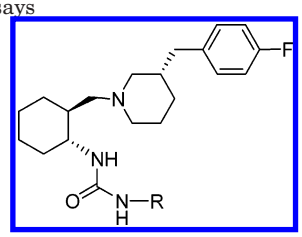
[a] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown.

also less potent; for example, the 3,5-dichloro **45** (IC_{50} = 26 nM) and 3,4-dichloro analogues **46** (IC_{50} = 53 nM) both give weaker compounds. However, with electron-withdrawing, hydrogen-bond-accepting substituents, all isomers give potent compounds. For example, the 3-acetyl isomer **32** (IC_{50} = 2.0 nM) and the 4-acetyl isomer **50** (IC_{50} = 3.0 nM) as well as the 3,5-diacetyl analogue **51** (IC_{50} = 1.1 nM) and the 3-acetyl-4-fluoro analogue **52** (IC_{50} = 1.5 nM) all provide potent compounds.

The apparent benefit of having hydrogen-bond-accepting groups prompted us to explore the use of heterocycle-containing urea groups. Several benzo-fused heterocycles, five- and six-membered ring heterocycles, and heterocyclic-substituted phenyl ureas were prepared, and the data for representative examples are summarized in Table 5. As the data in Table 5 highlights, they were all uniformly potent. Thus, the 5- or 6-isomeric indoles (**53**−**55**) and indazoles (**56**−**58**), benzthiazole (**60**), and benzimidazole (**61**) all have binding potencies of about 1 nM or less. Five-membered ring heterocycles such as 2-thiadiazole (**62**), 2-thiazoles (**63**−**66**), pyrazole (**67**), and isoxazole (**68**) as well as the six-membered ring pyridine urea (**69**) are all equally potent. Phenyl ureas substituted with pyrazoles (**70**), triazoles (**71**), and tetrazoles (**72**−**76**) are all potent without regard to the position of the heterocycle on the phenyl ring.

Functional Assays. Selected compounds were further characterized in functional assays using freshly isolated human eosinophils, as previously[20] described. The first assay used fluorescent imaging plate reader (FLIPR) technology to monitor intracellular calcium

Table 6. SAR of Urea Analogues in Functional Assays



compd	R	CCR3 IC_{50} (nM)[a]	Ca^{2+} IC_{50} (nM)[b]	chemotaxis IC_{50} (nM)
33	Ph	2.5	17	
34	2-Me-Ph	1.6		>100[c]
35	2-OMe-Ph	1.4	10	115 ± 60
36	4-OMe-Ph	2.0		1.0[c]
39	4-F-Ph	5	50	
48	4-Br-Ph	6	50	
41	3-F-4-Me-Ph	4	35	
32	3-Ac-Ph	2.0	8	0.034 ± 0.019
52	3-Ac-4-F-Ph	1.5	4	0.015 ± 0.003
51	3,5-Ac-Ph	1.1	2	0.007 ± 0.007
54	indol-5-yl	0.4	2	0.016 ± 0.001
56	indazol-5-yl	0.3	2	0.045 ± 0.015
57	indazol-6-yl	0.7	4	0.060 ± 0.008
65	5-acetyl-4-methylthiazol-2-yl	0.9	4	0.030 ± 0.020
66	4-chlorobenzo[*d*]-thiazol-2-yl	4.0		0.090[c]
70	3-cyano-4-(1H-pyrazol-1-yl)phenyl	2.0	4	
72	3-(5-methyl-1H-tetrazol-1-yl)phenyl	1.4	2	0.100[c]
74	3-(1-methyl-1H-tetrazol-5-yl)phenyl	0.7	1	0.010 ± 0.010
75	4-(1-methyl-1H-tetrazol-5-yl)phenyl	0.7		0.250[c]
76	3,5-bis-(1-methyl-1H-tetrazol-5-yl)phenyl	1.0	2	0.042[c]

[a] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown. [b] The value represents an average of usually two determinations with a deviation of ±50% of the mean value shown. [c] Value was measured once.

mobilization stimulated by eotaxin (CCL11) at 10 nM. The second assay determined eosinophil chemotaxis across a membrane in response to eotaxin (CCL11) at 10 nM. The results are summarized in Table 6. These compounds are functional antagonists since the compounds themselves did not stimulate calcium mobilization, chemotaxis, or random chemokinesis. While it is hard to discriminate between different analogues based on CCR3 receptor binding affinities (Tables 4 and 5), the differences between them become apparent in the functional assays.

Calcium Mobilization SAR. In the calcium mobilization assay the IC_{50} potency range is from about 1 to 50 nM and clusters around two features. Analogues with simple phenyl ureas have IC_{50}'s in the double-digit range, and analogues with heterocycle-containing ureas have IC_{50}'s in the low single-digit range. Thus, the parent phenyl urea **33** (IC_{50} = 17 nM), the 2-methoxyphenyl **35** (IC_{50} = 10 nM), and the 4-fluorophenyl **39** (IC_{50} = 50 nM) are some of the weaker compounds listed in Table 6 and are about 10-fold from their binding IC_{50} potency. The heterocyclic-substituted phenyl ureas show a better correlation between binding and the calcium mobilization assays. For example, the pyrazole-phenyl **70** (Ca^{2+} IC_{50} = 4 nM) or tetrazole-phenyl **72** (Ca^{2+} IC_{50} = 2 nM) are within 2-fold of their binding potency (Table 6). The correlation between binding and calcium mobilization assays for the heterocyclic ureas is again not as good. Thus, while the binding of the indole **54**, indazoles **56** and **57**, and thiazole **65** are all below 1 nM, the calcium mobilization IC_{50}'s range from 2 to 4 nM, about 5-fold from their binding numbers.

Chemotaxis SAR. A more pronounced distinction among the various analogues is observed in the chemotaxis assay. The 2-substituted phenyl urea analogues **34** and **35** are weak antagonists with IC_{50}'s more than 100 nM, while the 4-substituted phenyl ureas, such as the 4-methoxyphenyl **36** (IC_{50} = 1 nM), are more potent. However, substitution at the 3-position of the phenyl urea with a hydrogen-bond-accepting group, such as an acetyl (**32**, **52**) or a heterocycle (**72**, **74**, **75**), gives extremely potent compounds with chemotaxis IC_{50}'s in the 10−50 pM range. The nitrogen-linked tetrazole analogue **72** (IC_{50} = 0.100 nM), although very potent, is 10-fold weaker than the corresponding carbon-linked analogue **74** (IC_{50} = 0.010 nM). The 3-position on the phenyl urea seems to be the best for the heterocyclic substituent since the 4-tetrazole analogue **75** (IC_{50} = 0.250 nM) is more than 10-fold weaker than the 3-isomer **74**. The 3,5-disubstitued phenyl analogues, substituted with acetyl (**51**; IC_{50} = 0.007 nM) or tetrazole (**76**; IC_{50} = 0.042 nM), are also very potent. The heterocyclic ureas, such as indole **54**, indazoles **56** and **57**, and thiazoles **65**, also all give extremely potent compounds as summarized in Table 6.

There seem to be several specific structural requirements in order to obtain compounds with extremely potent inhibition of chemotaxis. One is the requirement for a hydrogen-bond-accepting group on the urea, such as an acetyl or a heterocycle, as discussed above. However, other parts of the molecule, such as the benzyl piperidine portion, are also important. For example, as noted earlier, the (*R*)-3-(4-fluorobenzyl)piperidine analogue **29** (chemotaxis IC_{50} = 0.364 nM) is clearly less potent than (*S*)-isomer **32** (chemotaxis IC_{50} = 0.034 nM).

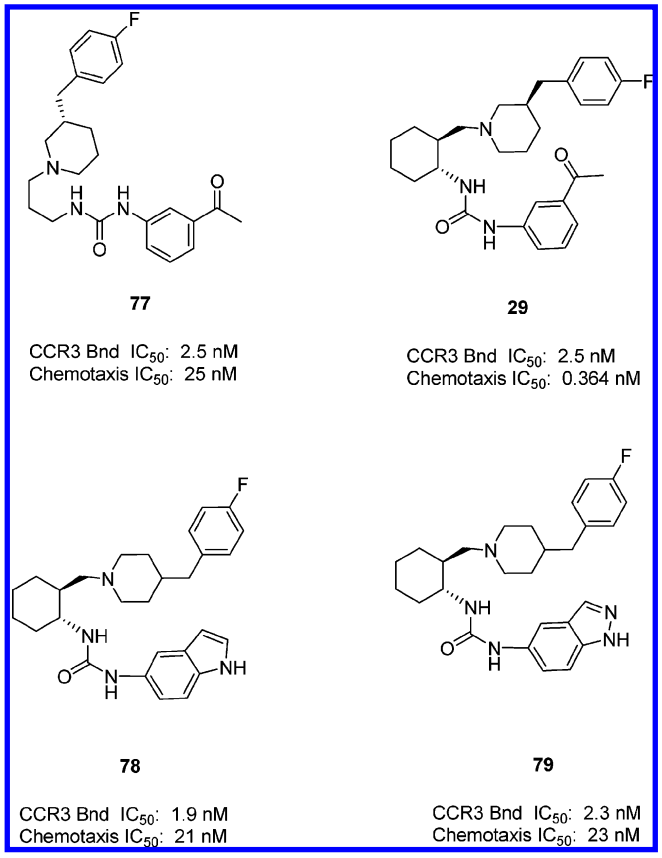


Figure 2. Chemotaxis SAR of compounds discussed in text.

An even more dramatic difference in potency is seen between the 4-(4-fluorobenzyl)piperidine and the 3-(4-fluorobenzyl)piperidine isomers. As shown in Figure 2, the indole **78** (IC_{50} = 21 nM) and the indazole **79** (IC_{50} = 23 nM) urea analogues in the 4-(4-fluorobenzyl)-piperidine series are both about 1000-fold less potent than the corresponding (*S*)-3-(4-fluorobenzyl)piperidine series analogues **54** (IC_{50} = 0.016 nM) and **56** (IC_{50} = 0.045 nM) in Table 6.

In addition, the conformational rigidity provided by the trans cyclohexyl linker is also critical for the potency. Again, there is large (about a 500-fold) difference in potency between the propyl linker series analogue **77** (IC_{50} = 25 nM in Figure 2) and the corresponding cyclohexyl-linked analogue **32** (IC_{50} = 0.034 nM in Table 6).

Although the disconnection between the measured binding affinity and the functional (calcium mobilization and chemotaxis) assays is not understood, it is clear that they are measuring different aspects of receptor pharmacology. The fact that a Chinese hamster ovary (CHO) cell line is used for the binding assay does not seem to be an explanation. We have also measured the binding affinities of several analogues using freshly isolated human eosinophils and found only a small difference (2- to 3-fold) in binding that does not account for the large difference in functional activity using the same cells (see Table 8).

Current models[27] of GPCR activation, such as the extended ternary complex model,[28] propose different conformational states of the receptor. Ligands having different binding affinities for these various receptor conformational states are able to produce a continuum of receptor function.[29] Viewed in this light, all our antagonists seem to be able to displace the natural ligand, eotaxin, equally well from the receptor, but they vary widely in their ability to interfere with the different conformations of the receptor responsible for the various functional activities observed. It would appear that the conformation of the receptor required for signal transduction leading to calcium mobilization is different from the conformation required for chemotaxis.[30]

The picomolar potency of compounds in the chemotaxis assay relative to their nanomolar potency in the binding assay suggests that the compounds inhibit chemotaxis at fractional receptor occupancy. We postulate that such compounds stabilize a ternary complex in which the receptor is coupled to inactive G (GDP) proteins.[31,32] The sequestration of G proteins in the complex makes them unavailable for shuttling to receptors that are free to bind natural ligand. If the chemotaxis-relevant G protein is present in limiting amounts relative to receptor, functional inhibition would be achieved even at low receptor occupancy.[33] Intuitively, this seems reasonable for a functionality such as chemotaxis which depends on the ability to sense a concentration gradient of the chemokine. Since compounds in the calcium assay manifest nanomolar rather than picomolar potency, however, we assume that calcium mobilization signals through a different G protein or through one that is more poorly coupled and thus requires a higher receptor occupancy. Again, this seems reasonable if calcium mobilization is associated with eosinophil activation at the inflammation site where the maximum concentration of chemokine would be present.

Recent reports[34−36] suggest that the extracellular signal-regulated kinase (ERK) signaling pathway is important for eotaxin-induced chemotaxis of eosinophils and is blocked by MEK inhibitors. In agreement with this, we find that a selected group of compounds, with a wide range of chemotaxis potencies, inhibited eotaxin-dependent ERK phosphorylation with potencies similar to those in the chemotaxis assay (data not shown).[37]

Since our objective is to develop a therapeutic agent that inhibits the migration of eosinophils into tissues, we concentrated our efforts on analogues that showed the most potency in our chemotaxis assay, since that measured the functional activity we wanted to optimize.

Selectivity. Many of the antagonists in this study were examined against a panel of other 7TM GPCR to determine their selectivity against other receptors. The panel included other chemokine receptors (CCR1, CCR2, CCR5, CXCR1, and CXCR2) as well as biogenic amine and transporters, and the data for a few selected analogues are summarized in Table 7. The selectivity of the compounds highlighted in Table 7 is representative of the vast majority of this class of CCR3 antagonist. They are more than 100-fold selective for CCR3 over the other GPCRs using the CCR3 binding IC_{50} and greater than 1000-fold selective using the chemotaxis IC_{50} values. In general, they show some affinity for the serotonin $5HT_{2A}$ receptor subtype, dopamine D2, and the dopamine and norepinephrine transporters as noted in Table 7.

We also examined the affinity of our compounds for the CCR3 receptors of other species of interest. We studied rat, mouse, and Cynomolgus monkey eosinophils in our CCR3 binding assay or in the chemotaxis

Table 7. GPCR Activities of Selected Analogues (nM)

assay	**32**	**65**	**74**
CCR3 IC_{50}[a]	2.0	0.9	0.7
CCR1 IC_{50}[b]	>10 000	>10 000	>10 000
CCR2 IC_{50}[b]	>10 000	>10 000	>10 000
CCR5 IC_{50}[b]	>1000	>1000	>1000
CXCR1 IC_{50}[b]	>10 000	>10 000	>10 000
CXCR2 IC_{50}[b]	>10 000	>10 000	>10 000
$5HT_{2A}$ IC_{50}[b]	920	1415	3120
$5HT_{2C}$ IC_{50}[b]	>1000	>1000	2600
D2 IC_{50}[b]	1019	321	487
serotonin transporter, K_i	2938	2350	3900
dopamine transporter, K_i	492	718	2260
norepinephrine transporter, K_i	946	611	1260

[a] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown. [b] The value represents an average of usually two determinations with a deviation of ±30% of the mean value shown.

Table 8. Cross Reactivity of Selected Analogues (nM)

assay	**32**	**65**	**74**
CCR3/CHO, IC_{50}[a]	2.0	0.9	0.7
	Human eos[b]		
CCR3, IC_{50}[a]	0.8	0.5	0.5
chemotaxis, IC_{50}	0.034 ± 0.019	0.030 ± 0.020	0.010 ± 0.010
calcium, IC_{50}[a]	8	4	1
	Mice eos		
CCR3, IC_{50}	54 ± 21	9 ± 4	20 ± 8
chemotaxis, IC_{50}	41 ± 8	10 ± 3	20 ± 10
	Rat eos		
CCR3, IC_{50}	>1000	>1000	>1000
	Cyno eos		
chemotaxis, IC_{50}	0.039 ± 0.018	0.007 ± 0.001	0.010 ± 0.010

[a] The value represents an average of usually three determinations with a standard deviation of ±20−50% of the mean value shown. [b] eos, eosinophils.

Table 9. PK Parameters of Selected Analogues

	compounds		
PK parameter	**32**	**65**	**74**
mouse F (%)	20	17	15
mouse $t_{1/2}$ (h)	2.0	0.9	1.2
mouse CL ((L/h)/kg)	1.8	6.8	1.7
cyno F (%)	8	3	6
cyno $t_{1/2}$ (h)	4.0	5.0	4.3
cyno CL ((L/h)/kg)	2.1	2.1	1.3
chimp F (%)	22	10	15
chimp $t_{1/2}$ (h)	5.0	5.0	2.9
chimp CL ((L/h)/kg)	1.2	1.6	0.9
protein binding (hu) (%)	96.3	96.9	92.0
Caco-2, Papp (cm/s)	11×10^{-6}	6×10^{-6}	2×10^{-6}

functional assay, and the data are summarized in Table 8. No compounds that were tested bind to rat CCR3. Most did show affinity for mouse CCR3, with the acetylphenyl urea analogue **32** (IC_{50} = 54 nM) being less potent than the thiazole urea **65** (IC_{50} = 9 nM) or tetrazole-containing phenyl urea analogue **74** (IC_{50} = 20 nM). The chemotaxis potency in the mouse correlates well with the binding potency observed for these compounds. Determining the binding potency was not feasible for Cynomolgus monkey eosinophils, but in the chemotaxis assay these compounds exhibit low picomolar potency.

In Vivo Pharmacology

Pharmacokinetics. Several analogues were examined for their oral bioavailability in species of interest, and the data are summarized in Table 9. The permeability for most analogues, as measured using the Caco-2 assay, was reasonable. They were generally highly protein bound (92−97%) and showed low to moderate oral bioavailability, driven mainly by moderate to high clearance in all species. The oral bioavailability was best in the mouse and the chimp (15−22% F) and less in the cyno (3−8% F), as summarized in Table 9.

Animal Models of Eosinophil Migration. Having established that these compounds were potent CCR3 antagonists in vitro, we next assessed their efficacy in vivo. Since many of these compounds were active against mouse CCR3 and had reasonable oral bioavailability in mice, we examined these compounds in two murine models of allergic airway inflammation.[38] In the first, mouse eotaxin, delivered intranasally to mice previously sensitized to intraperitoneal (ip) injections of ovalbumin (OVA), elicited a selective pulmonary eosinophilia evident at 6 h in the bronchoalveolar lavage (BAL) fluid. In the second model, an aerosolized OVA challenge of OVA-sensitized mice stimulated pulmonary inflammation in which eosinophils were a major component in the BAL fluid at 24 h. The results are summarized in Table 10.

In the eotaxin challenge model, a dose dependent inhibition of eosinophil recruitment into the lungs was observed for all the compounds tested. The efficacy of the compounds in this model was not uniform. While both **32** and **65** showed efficacy consistent with their mouse chemotaxis potency and pharmacokinetics (see Tables 8 and 9), **74** was less efficacious than expected.

To test the efficacy in the OVA challenge model, the compounds were administered orally at two doses, corresponding to the EC_{50} (low dose) and 2-fold the EC_{90} (high dose) in the eotaxin challenge model. The effectiveness of **32** in the OVA challenge model was in line with expectations of 86% reduction of eosinophil infiltration observed at the high dose. In contrast, the thiazole urea analogue **65** gave a similar response at both doses with 60−69% reduction of eosinophils.

Clinical Candidate DPC168. Compounds were next subjected to an extensive array of 117 selectivity assays that included neurotransmitter, steroid, eicosanoid, hormone, brain and gut peptide receptors, binding sites on ion channels and transporters, and second messenger and transport assays (performed by NovaScreen at 100 nM and 10 μM). This included all the 7TM GPCR assays

Table 10. In Vivo Efficacy of Selected Analogues (po)

	eotaxin challenge model (mice)		OVA challenge model (mice) percent inhibition of eosinophils	
compd	EC_{50} (mg/kg)	EC_{90} (mg/kg)	low dose	high dose
32	20	45	50% @ 30 mg/kg	86% @ 100 mg/kg
65	2.7	12.4	60% @ 3 mg/kg	69% @ 30 mg/kg
74	40	70		

available. In these assays, **32** exhibited greater than 100-fold selectivity in all assays (compared to the CCR3 binding IC_{50}). Additionally, in vivo 5-day safety-assessment studies were conducted in rats and dogs. In these studies, **32** was well tolerated in both rats and dogs without significant GI, CV, or CNS side effects up to the maximum dose used (60 and 30 mg/kg, respectively).

Thus, while **32** was not ideal, with some affinity for the dopamine transporter (K_i = 492, Table 7), it is such a potent inhibitor of eosinophil chemotaxis (IC_{50} = 0.034 nM) that the potential therapeutic window was satisfactory. This evaluation was also corroborated by the safety assessment of **32** in rats and dogs. On the basis of its overall potency, efficacy, and safety profile, **32**, as the benzenesulfonate salt, was advanced into phase I clinical trials and designated as DPC168.

Summary and Conclusions

Starting with our previously described[20] class of CCR3 receptor antagonist, we improved the potency by replacing the phenyl linker of **1** with a cyclohexyl linker and by replacing the 4-benzylpiperidine with a 3-benzylpiperidine. The resulting compound, **32**, is a potent and selective antagonist of CCR3. SAR studies showed that the 3-acetylphenyl urea of **32** could be replaced with heterocyclic ureas or heterocyclic-substituted phenyl ureas and still maintain the potency (inhibition of eotaxin-induced chemotaxis) of this class of compounds in the low picomolar range, representing some of the most potent CCR3 antagonists reported to date. The potency of **32** for mouse CCR3 (chemotaxis IC_{50} = 41 nM) and its oral bioavailability in mice (20% F) were adequate to assess the efficacy in animal models of allergic airway inflammation. Oral administration of **32** reduced eosinophil recruitment into the lungs in a dose-dependent manner in these animal models. The benzenesulfonate salt of **32**, designated DPC168, entered phase I clinical trials, and results from these studies will be reported in due course. Our continuing efforts to further improve this class of compounds will also be reported in the near future.

Experimental Section

General. All reactions were carried out under an atmosphere of dry nitrogen. Commercial reagents were used without further purification. ^{1}H NMR spectra were recorded using tetramethylsilane (TMS) as an internal standard. TLC was performed on E. Merck 15710 silica gel plates. Medium-pressure liquid chromatography (MPLC) was carried out using EM Science silica gel 60 (230−400 mesh). HPLC analysis was carried out using Jasco PV-987 pumps, Jasco UV-975 detectors, and DuPont Zorbax Sil or Zorbax NH_2 1-in. preparative columns. All final targets were obtained as noncrystalline amorphous solids unless specified otherwise. Mass spectra were measured with an HP5988A mass spectrometer with particle beam interface using NH_3 for chemical ionization or a Finnigan MAT 8230 mass spectrometer with NH_3-DCI or VG TRIO 2000 for electrospray ionization (ESI). High-resolution mass spectra were measured on a VG 70-VSE instrument with NH_3 chemical ionization. Elemental analysis was performed by Quantitative Technologies, Inc., Bound Brook, NJ. For compounds where analysis was not obtained, HPLC analysis was used, and purity was determined to be >95%.

Racemic analogues (**5**−**16**) in Tables 1 and 2 were synthesized from the commercial (±)-*cis*-2-hydroxymethyl-1-cyclohexylamine hydrochloride (catalog no. 26586) and (±)-*trans*-2-hydroxymethyl-1-cyclohexylamine hydrochloride (catalog no. 26587) available from Acros Organics. The 3-aminocyclohexanecarboxylic acid (catalog no. 26587) was obtained from TCI America. The synthetic sequence used for the racemic compounds is analogous to that described in detail below for the chiral compounds using the same reaction conditions summarized in Scheme 1.

1-((±)-2-*cis*)-2-((4-Benzylpiperidin-1-yl)methyl)cyclohexyl)-3-(3-methoxyphenyl)urea (5). This was synthesized using the procedure outlined in Scheme 1, except using commercially available 4-benzylpiperidine. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.30−7.09 (m, 10 H), 6.93 (dd, J = 1.5 Hz, J = 8 Hz, 1 H), 6.61 (dd, J = 2 Hz, J = 8 Hz, 1 H), 3.87 (bs, 1 H), 3.81 (s, 3 H), 3.20 (bs, 1 H), 2.80 (bs, 1 H), 2.51 (m, 2 H), 2.08−1.20 (m, 18 H). MS ESI $(M + H)^+$ = 436.3. Anal. Calcd for $(C_{27}H_{37}N_3O_2 \cdot (CHCl_3)_{0.6})$: C, 65.36; H, 7.47; N, 8.28. Found: C, 65.60; H, 7.48; N, 8.12.

1-((±)-2-*trans*-((4-Benzylpiperidin-1-yl)methyl)cyclohexyl)-3-(3-methoxyphenyl)urea (6). This was synthesized using the procedure outlined in Scheme 1, except using commercially available 4-benzylpiperidine. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.30−7.09 (m, 8 H), 6.78 (dd, J = 1.5 Hz, J = 8 Hz, 1 H), 6.60 (dd, J = 2 Hz, J = 8 Hz, 1 H), 6.35 (bs, 1 H), 3.81 (s, 3 H), 3.28 (dt, J = 3.3 Hz, J = 10 Hz,1 H), 3.05 (bd, J = 11 Hz, 1 H), 2.76 (bd, J = 11 Hz, 1 H), 2.57−2.33 (m, 4 H), 2.08−1.89 (m, 2 H), 1.83−0.90 (m, 14 H). MS ESI $(M + H)^+$ = 436.3. Anal. Calcd for $(C_{27}H_{37}N_3O_2 \cdot (H_2O)_{0.66})$: C, 72.47; H, 8.63; N, 9.39. Found: C, 72.46; H, 8.59; N, 9.18.

1-((±)-2-*trans*-((4-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-methoxyphenyl)urea (7). This was synthesized using the procedure outlined in Scheme 1, except using commercially available 4-benzylpiperidine. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.66 (bs, 1 H), 7.20 (t, J = 8 Hz, 1 H), 7.06−7.02 (m, 3 H), 6.98−6.92 (m, 2 H), 6.75 (bd, J = 7 Hz, 1 H), 6.61 (dd, J = 2 Hz, J = 8 Hz, 1 H), 6.11 (bs, 1 H), 3.81 (s, 3 H), 3.26 (m, 1 H), 2.98 (bd, J = 11 Hz, 1 H), 2.68 (bd, J = 11 Hz, 1 H), 2.41−2.32 (m, 4 H), 2.02 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.89 (dt, J = 2 Hz, J = 11 Hz,1 H), 1.71−0.78 (m, 14 H). MS ESI $(M + H)^+$ = 454.3. Anal. Calcd for $(C_{27}H_{36}FN_3O_2 \cdot (H_2O)_{0.33})$: C, 70.57; H, 8.04; N, 9.14. Found: C, 70.44; H, 7.92; N, 9.04.

1-((±)-2-*trans*-((4-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-acetylphenyl)urea (8). This was synthesized using the procedure outlined in Scheme 1, except using 4-(4-fluorobenzyl)piperidine. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.95 (bs, 1 H), 7.62 (d, J = 2 Hz, 1 H), 7.59 (d, J = 2 Hz, 1 H), 7.56 (bs, 1 H), 7.36 (t, J = 8 Hz, 1 H), 7.08−7.02 (m, 2 H), 6.99−6.93 (m, 2 H), 6.57 (bs, 1 H), 3.28 (m, 1 H), 3.07 (bd, J = 10 Hz, 1 H), 2.79 (bd, J = 10 Hz, 1 H), 2.61 (s, 3 H), 2.44 (d, J = 7 Hz, 2 H), 2.50−2.33 (m, 2 H), 2.11−1.95 (m, 3 H), 1.84−0.98 (m, 13 H). MS ESI $(M + H)^+$ = 466.4. Anal. Calcd for $(C_{28}H_{36}FN_3O_2 \cdot (H_2O)_{0.66})$: C, 70.43; H, 7.88; N, 8.80. Found: C, 70.46; H, 7.65; N, 8.72.

1-((1*R*,2*S*)-2-((4-(4-Fluorobenzyl)piperidin-1-yl)methyl-)cyclohexyl)-3-(3-acetylphenyl)urea (+)-8. This was synthesized using the procedure outlined for (±)-**8**, except that the *tert*-butyl 2-*trans*-((4-(4-fluorobenzyl)piperidin-1-yl)methyl)cyclohexylcarbamate intermediate was resolved by chiral HPLC (Chiralpak AD) and the first isomer obtained from the column was used for the remainder of the synthesis to give (+)-**8**. $[\alpha]^{25}_D$ = +18.2° (MeOH, c = 0.446 g/dL). ^{1}H NMR (300 MHz, $CDCl_3$) same as racemic above. MS ESI $(M + H)^+$ = 466.3. Anal. Calcd for $(C_{28}H_{36}FN_3O_2 \cdot (H_2O)_{0.50})$: C, 70.86; H, 7.86; N, 8.85. Found: C, 70.98; H, 7.72; N, 8.78.

1-((1*S*,2*R*)-2-((4-(4-Fluorobenzyl)piperidin-1-yl)methyl-)cyclohexyl)-3-(3-acetylphenyl)urea (−)-8. This was synthesized using the procedure outlined for (±)-**8**, except that the *tert*-butyl 2-*trans*-((4-(4-fluorobenzyl)piperidin-1-yl)methyl)cyclohexylcarbamate intermediate was resolved by chiral HPLC (Chiralpak AD) and the second isomer obtained from the column was used to give (−)-**8**. $[\alpha]^{25}_D$ = −20.5° (MeOH, c = 0.434 g/dL). ^{1}H NMR (300 MHz, $CDCl_3$) same as racemic above. MS ESI $(M + H)^+$ = 466.3. Anal. Calcd for $(C_{28}H_{36}FN_3O_2 \cdot (H_2O)_{0.50})$: C, 70.86; H, 7.86; N, 8.85. Found: C, 71.00; H, 7.66; N, 8.89.

1-((±)-3-((4-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-methoxyphenyl)urea (9). This was synthesized using the procedure outlined in Scheme 1, except using racemic 3-hydroxymethyl-1-cyclohexylamine (cis/trans) as the starting amine and 4-(4-fluorobenzyl)piperidine. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.20 (t, J = 8 Hz, 1 H), 7.06−7.02 (m, 2 H), 6.98−6.92 (m, 3 H), 6.75 (bd, J = 7 Hz, 1 H), 6.62 (dd, J = 2 Hz, J = 8 Hz, 1 H), 6.30 (bs, 1 H), 4.67 (d, J = 8 Hz, 1 H), 3.79 (s, 3 H), 3.66 (m, 1 H), 2.79 (bt, J = 11 Hz, 2 H), 2.08 (d, J = 7 Hz, 2 H), 2.08−1.92 (m, 1 H), 1.85−0.68 (m, 15 H). MS ESI (M + H)$^+$ = 454.3. Anal. Calcd for ($C_{27}H_{36}FN_3O_2$·$(H_2O)_{0.10}$): C, 71.21; H, 8.01; N, 9.23. Found: C, 71.06; H, 8.06; N, 9.05.

1-((±)-3-((4-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-acetylphenyl)urea (10). This was synthesized using the procedure outlined in Scheme 1, except using racemic 3-hydroxymethyl-1-cyclohexylamine (cis/trans) as the starting amine and 4-(4-fluorobenzyl)piperidine. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.82 (bs, 1 H), 7.78 (d, J = 8 Hz, 1 H), 7.60 (d, J = 8 Hz, 1 H), 7.38 (t, J = 8 Hz, 1 H), 7.09−7.04 (m, 2 H), 6.98−6.92 (m, 3 H), 4.96 (d, J = 8 Hz, 1 H), 3.64 (m, 1 H), 2.79 (bt, J = 10 Hz, 2 H), 2.60 (s, 3 H), 2.47 (d, J = 7 Hz, 2 H), 2.08−1.98 (m, 3 H), 1.85−0.68 (m, 15 H). MS ESI (M + H)$^+$ = 466.3. Anal. Calcd for ($C_{28}H_{36}FN_3O_2$·$(H_2O)_{0.50}$): C, 70.86; H, 7.86; N, 8.85. Found: C, 70.91; H, 7.89; N, 8.70.

1-(((*R*/*S*-2-*trans*)-2-(((*R*/*S*)-3-Benzylpiperidin-1-yl)methyl)cyclohexyl)-3-(3-methoxyphenyl)urea (11). This was synthesized using the procedure outlined in Scheme 1, except using racemic 3-benzylpiperidine. The NMR shows a mixture of two diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.60 (bs, 0.5 H), 7.40 (bs, 0.5 H), 7.27 (m, 1 H), 7.20−7.08 (m, 5 H), 7.03 (m, 1 H), 6.74 (m, 1 H), 6.59−6.51 (m, 1 H), 6.09 and 6.05 (bs, 1 H), 3.79 and 3.75 (s, 3 H), 3.22 (m, 1 H), 2.96 (bd, J = 10 Hz, 1 H), 2.61 (m, 1 H), 2.42−2.30 (m, 4 H), 2.05−1.85 (m, 1 H), 1.80−0.85 (m, 15 H). MS ESI (M + H)$^+$ = 436.3. Anal. Calcd for ($C_{27}H_{37}N_3O_2$): C, 74.45; H, 8.56; N, 9.65. Found: C, 74.23; H, 8.68; N, 9.45.

1-(((*R*/*S*-2-*trans*)-2-(((*R*/*S*)-3-Benzylpiperidin-1-yl)methyl)cyclohexyl)-3-(3-cyanophenyl)urea (12). This was synthesized using the procedure outlined in Scheme 1, except using racemic 3-benzylpiperidine. The NMR shows a mixture of two diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.90 (bs, 0.5 H), 7.70 (bs, 0.5 H), 7.71 (m, 1 H), 7.55 (m, 1 H), 7.38−7.20 (m, 3 H), 7.19−7.13 (m, 3 H), 7.00 (m, 1 H), 6.26 and 6.16 (bs, 1 H), 3.21 (m, 1 H), 3.00 (bd, J = 9 Hz, 1 H), 2.69 (m, 1 H), 2.42−2.30 (m, 4 H), 2.10−1.95 (m, 1 H), 1.80−0.85 (m, 15 H). MS ESI (M + H)$^+$ = 431.3. Anal. Calcd for ($C_{27}H_{34}N_4O$·$(H_2O)_{0.25}$): C, 74.53; H, 7.99; N, 12.88. Found: C, 74.52; H, 8.07; N, 12.85.

1-(((*R*/*S*)-2-*trans*)-2-(((*R*/*S*)-3-Benzylpiperidin-1-yl)methyl)cyclohexyl)-3-(3-acetylphenyl)urea (13). This was synthesized using the procedure outlined in Scheme 1, except using racemic 3-benzylpiperidine. The NMR shows a mixture of two diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.92 and 7.87 (t, J = 2 Hz, 1 H), 7.80 (bs, 0.5 H), 7.65 (bs, 0.5 H), 7.65−7.50 (m, 2 H), 7.41−7.20 (m, 2 H), 7.19−7.03 (m, 3 H), 7.00 − 6.97 (m, 1 H), 6.31 and 6.22 (bs, 1 H), 3.21 (m, 1 H), 2.97 (bd, J = 9 Hz, 1 H), 2.69 (m, 1 H), 2.60 and 2.56 (s, 3 H), 2.42−2.30 (m, 4 H), 2.10−1.95 (m, 1 H), 1.85−0.85 (m, 15 H). MS ESI (M + H)$^+$ = 448.3. Anal. Calcd for ($C_{28}H_{37}N_3O_2$·$(H_2O)_{0.25}$): C, 74.38; H, 8.36; N, 9.29. Found: C, 74.46; H, 8.28; N, 9.29.

1-(((*R*/*S*)-2-*trans*)-2-(((*R*/*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-methoxyphenyl)urea (14). This was synthesized using the procedure outlined in Scheme 1, except using racemic 3-(4-fluorobenzyl)piperidine. The NMR shows a mixture of two diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.60 (bs, 0.5 H), 7.40 (bs, 0.5 H), 7.20−7.08 (m, 3 H), 7.03−6.90 (m, 2 H), 6.82 (m, 1 H), 6.76 (m, 1 H), 6.59−6.51 (m, 1 H), 6.10 and 6.06 (bs, 1 H), 3.79 and 3.75 (s, 3 H), 3.22 (m, 1 H), 2.96 (bd, J = 10 Hz, 1 H), 2.61 (m, 1 H), 2.42−2.30 (m, 4 H), 2.05−1.85 (m, 1 H), 1.80−0.85 (m, 15 H). MS ESI (M + H)$^+$ = 454.3. Anal. Calcd for ($C_{27}H_{36}FN_3O_2$): C, 71.49; H, 8.00; N, 9.26. Found: C, 71.19; H, 8.01; N, 9.20.

1-(((*R*/*S*)-2-*trans*)-2-(((*R*/*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-cyanophenyl)urea (15). This was synthesized using the procedure outlined in Scheme 1, except using racemic 3-(4-fluorobenzyl)piperidine. The NMR shows a mixture of two diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.69 (m, 1 H), 7.55 (m, 1 H), 7.39−7.20 (m, 3 H), 7.19−7.13 (m, 1 H), 7.01−6.85 (m, 2 H), 6.82 (m, 1 H), 6.30 and 6.22 (bs, 1 H), 3.21 (m, 1 H), 3.00 (m, 1 H), 2.69 (m, 1 H), 2.49−2.30 (m, 4 H), 2.10−1.95 (m, 1 H), 1.80−0.85 (m, 15 H). MS ESI (M + H)$^+$ = 449.3. Anal. Calcd for ($C_{27}H_{33}FN_4O$·$(H_2O)_{0.25}$): C, 71.58; H, 7.45; N, 12.37. Found: C, 71.47; H, 7.48; N, 12.27.

1-(((*R*/*S*)-2-*trans*)-2-(((*R*/*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-acetylphenyl)urea (16). This was synthesized using the procedure outlined in Scheme 1, except using racemic 3-(4-fluorobenzyl)piperidine. The NMR shows a mixture of two diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.85 (m, 1 H), 7.75 (bs, 0.5 H), 7.65−7.50 (m, 2 H), 7.41−7.20 (m, 1.5 H), 7.19−7.03 (m, 1 H), 7.00−6.87 (m, 2 H), 6.76 (m, 1 H), 6.31 and 6.22 (bs, 1 H), 3.21 (m, 1 H), 2.97 (m, 1 H), 2.69 (m, 1 H), 2.61 and 2.69 (s, 3 H), 2.42−2.28 (m, 4 H), 2.10−1.95 (m, 1 H), 1.85−0.80 (m, 15 H). MS ESI (M + H)$^+$ = 466.3. Anal. Calcd for ($C_{28}H_{36}FN_3O_2$·$(H_2O)_{0.25}$): C, 71.54; H, 7.83; N, 8.94. Found: C, 71.49; H, 7.85; N, 8.99.

As examples of the synthetic procedures used in this work, the synthesis of **32**, DPC168, and **65**, DPC-A37818, are described in detail below. All other compounds were made using essentially the same chemistry.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-acetylphenyl)urea (32). Synthesis of (*S*)-3-(4-Fluorobenzyl)piperidine (26). To a stirring solution of *N*-benzyl-3-piperidone hydrochloride hydrate (4.2 g, 18.6 mmol) and 10% palladium on carbon (0.8 g) in degassed methanol (200 mL) was added hydrogen gas to 55 psi. The reaction mixture was stirred for 16 h and then filtered through a pad of Celite. The Celite was washed with methanol (200 mL). The filtrates were combined and concentrated in vacuo to a colorless oil. The oil was dissolved in tetrahydrofuran (THF, 200 mL) and then treated with di-*tert*-butyl-dicarbonate (5.27 g, 24.1 mmol) and saturated aqueous sodium bicarbonate (50 mL). The reaction mixture was stirred for 4 h and then concentrated in vacuo to a white solid. The solid was partioned between EtOAc and 1 N HCl. The organic layer was separated, washed with 1 N NaOH and brine, dried over Na_2SO_4, and evaporated in vacuo to a colorless oil. The oil was purified by flash chromatography (silica gel, hexane/EtOAc 3:1) to yield 2.93 g of *tert*-butyl 3-oxopiperidine-1-carboxylate **23** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 3.99 (s, 2 H), 3.58 (t, J = 6.3 Hz, 2 H), 2.46 (t, J = 6.3 Hz, 2 H), 1.97 (p, J = 6.3 Hz, 2 H), 1.45 (s, 9 H).

To a stirring solution of (4-fluorophenylmethyl)triphenylphosphonium chloride (17.68 g, 43.5 mmol) in dry THF (60 mL) at −78 °C was added 2.5 M *n*-butyllithium in hexane (14.6 mL, 36.5 mmol). The reaction mixture was warmed to 0 °C for 1 h, and the piperidone **23** (3.46 g, 17.4 mmol) in THF (60 mL) was added. The mixture was stirred at room temperature for 1 h and then heated to reflux for 16 h. The reaction mixture was cooled to room temperature, and the reaction was quenched by the addition of saturated aqueous NH_4Cl. The reaction mixture was extracted with EtOAc (3 × 100 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, and evaporated in vacuo to a pale yellow oil. The oil was purified by flash chromatography (silica gel, hexane/EtOAc 9:1) to yield 3.82 g of a mixture of *E* and *Z* isomers of olefin **24** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.22−7.14 (m, 2 H), 7.04−6.98 (m, 2 H), 6.36 (s, 0.33 H), 6.28 (s, 0.67 H), 4.14 (s, 1.34 H), 4.00 (s, 0.66 H) 3.50 (app t, J = 5.5 Hz, 2 H), 2.47 (t, J = 5.1 Hz, 0.66 H), 2.39 (t, J = 5.1 Hz, 1.34 H), 1.75−1.68 (m, 1.34 H), 1.65−1.57 (m, 0.66 H), 1.48 (s, 9 H).

To a stirring solution of the olefin **24** (3.82 g, 13.1 mmol) and 10% palladium on carbon (0.76 g) in degassed methanol (200 mL) was added hydrogen gas to 55 psi. The reaction mixture was stirred for 16 h and then filtered through a pad of Celite. The Celite was washed with methanol (200 mL). The

filtrates were combined and concentrated in vacuo to yield 2.76 g of *N*-BOC-3-(4-fluorobenzyl)piperidine **25** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.12−7.07 (m, 2 H), 6.98−6.93 (m, 2 H), 3.89 (dt, J = 13.2 Hz, 4.0 Hz, 1 H), 3.84−3.74 (m, 1 H), 2.57−2.43 (m, 4 H), 1.75−1.60 (m, 4 H), 1.42 (s, 9 H), 1.15−1.09 (m, 1 H).

N-BOC-3-(4-fluorobenzyl)piperidine **25** (5 g) was dissolved in 30 mL of 4 N HCl in dioxane. Some initial gassing occurred which eventually subsided. After 1 h, the mixture was neutralized with aqueous Na_2CO_3, and the dioxane was evaporated off. The residue was extracted with ether, and the combined ether extracts were dried over $MgSO_4$ and evaporated off to give 2.6 g of the free amine (±)-**26** as an oil.

Resolution of 3-(4-Fluorobenzyl)piperidine (26). Two grams of the crude racemic 3-(4-fluorobenzyl)piperidine was dissolved in 25 mL of acetonitrile and heated to reflux. The solution was hazy. To this was added 1.56 g (1 equiv) of (*R*)-(−)-mandelic acid dissolved in 15 mL of acetonitrile. Some initial precipitation occurred when the cooler solution was added, but it did redissolve when refluxing resumed. The heat was turned off, and a small amount of enantiomerically pure salt was added as the temperature dropped. At first, the seed crystals dissolved, but when the temperature dropped to 75 °C, they remained suspended in the stirred solution. After a few more degrees of cooling, crystal growth was obvious. Cooling was continued at the rate of 1 deg/min. At 50 °C, the solution was filtered to recover 0.9 g of salt, which melted at 164 °C. It was recrystallized from acetonitrile twice to give (*S*)-(+)-3-(4-fluorobenzyl)piperidine mandelic acid salt in 98% ee and melting at 168−171 °C. The salt was extracted into a mixture of ether and 1 N NaOH. The ether extract was washed with water and brine, dried over sodium sulfate, and evaporated to give (*S*)-3-(4-fluorobenzyl)piperidine **26** as the free base, which was used in subsequent reductive amination.

(1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexanamine (21). The preparation of ((1*R*,2*R*)-2-aminocyclohexyl)methanol **17** was obtained following the literature procedure[24b] except that we used LAH to reduce the intermediate ester. To a solution of ((1*R*,2*R*)-2-aminocyclohexyl)methanol **17** (9.5 g, 73.8 mmol) in CH_2Cl_2 (200 mL) is added 200 mL of an aqueous solution of Na_2CO_3 (15 g, 141 mmol). While the mixture is stirred, benzyl chloroformate (12.6 g, 73.8 mmol) is added slowly, and the mixture is stirred at room temperature for 1 h. The organic layer is separated and washed with water and brine. The organic solvent is removed on a rotary evaporator to give a white solid. The solid is recrystallized from hexane to give 16.3 g (62 mmol) of the alcohol **18** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.40−7.29 (m, 5 H), 5.11 (s, 2 H), 4.71 (bd, 1 H), 3.76−3.71 (m, 1 H), 3.53−3.28 (m, 3 H), 2.00−1.95 (m, 1 H), 1.90−1.09 (m, 8 H). MS AP$^+$ $(M + H)^+$ = 264.3 (100%).

A solution of DMSO (36 g, 430 mmol) in CH_2Cl_2 (200 mL) is cooled to −78°C. To this solution is added dropwise oxalyl chloride (27.41 g, 216 mmol), and the resulting solution is stirred for an additional 10 min. A solution of alcohol **18** (38 g, 144 mmol) in CH_2Cl_2 (150 mL) is added via an addition funnel and stirred for 10 min. Then, Et_3N (58 g, 570 mmol) is added and the solution is stirred for 20 min; the ice bath is removed, and the solution is stirred for an additional 30 min. The solution is diluted with water, and the organic layer is separated and washed with water, 1 N HCl, and brine. The organic layer is dried over Na_2SO_4, filtered, and concentrated to give 38 g of aldehyde **19** as a white solid. The solid is recrystallized from hexane to give 19.7 g of a first crop of aldehyde **19** as white needles. A second crop gave an additional 11 g. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 9.59 (d, 3.6 Hz, 1 H), 7.38−7.28 (m, 5 H), 5.07 (m, 2 H), 4.69 (m, 1 H), 3.84 (m, 21 H), 2.19−2.11 (m,1 H), 2.09−2.01 (m, 1 H), 1.86−1.75 (m, 3 H), 1.54−1.17 (m, 4 H).

A solution of aldehyde **19** (19.6 g, 75 mmol) and (3*S*)-3-(4-fluorophenylmethyl)piperidine **26** (14.5 g, 75 mmol) in dichloroethane (400 mL) was treated with $Na(OAc)_3BH$ (32 g, 152 mmol) and stirred overnight at room temperature. The resulting solution was poured slowly into a stirred mixture of ice/water/1 N NaOH and stirred for 20 min. The organic layer was separated and washed with water and brine. The solution was dried over $MgSO_4$; the organic solvent was removed under vacuum, and the residue was chromatographed on basic alumina (50% EtOAc/hexane) to give 32.1 g (73 mmol) of amine **20**. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.79 (bs, 1 H), 7.38−7.29 (m, 5 H), 6.95−6.84 (m, 4 H), 5.08 (m, 2 H), 3.06 (m, 1 H), 2.80 (m, 1 H), 2.55−2.36 (m, 2 H), 2.30 (dd, J = 9 Hz, J = 13 Hz, 1 H), 2.05 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.81−0.90 (m, 16 H).

A solution of **20** (32 g, 73 mmol) in MeOH was treated with 8 g of 10% Pd/C and hydrogenated at 50 psi overnight in a Parr apparatus. The mixture was filtered and the filtrate concentrated on a rotary evaporator to give 20 g (65 mmol) of the amine **21**, which was used without further purification.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-acetylphenyl)urea (32). A solution of amine **21** (10 g, 32.8 mmol) in THF is treated with 3-acetylyphenyl isocyanate (5.3 g, 32.8 mmol), and the mixture is stirred for 30 min. The solvent is removed on a rotary evaporator, and the residue is chromatographed on silica gel (0.5:4.5:95 $NH_4OH/MeOH/CH_2Cl_2$) to give 11 g of urea **32** as a solid. The urea **32** was further purified by a second chromatography on silica gel (40:60:1 EtAc/Hex/TEA) and final recrystallization from ether to give a crystalline solid. Mp 115−117 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.86 (m, 1 H), 7.78 (bs, 1 H), 7.68−7.64 (m, 1 H), 7.62−7.59(m, 1 H), 7.38 (t, J = 8 Hz, 1 H), 6.95−6.90 (m, 2 H), 6.79−6.72 (m, 2 H), 6.25 (s, 1 H), 3.21 (dt, J = 3 Hz, 11 Hz, 1 H), 3.00−2.97 (m, 1 H), 2.66−2.56 (m, 1 H), 2.61 (s, 3 H), 2.44−2.32 (m, 4 H), 2.06 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.80−0.86 (m, 15 H). MS ESI: $(M + H)^+$ = 466.3 (100%). Anal. Calcd for $C_{28}H_{36}N_3O_2F$: C, 72.23; H, 7.70; N, 9.02. Found: C, 72.33; H, 7.91; N, 9.00.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-acetylphenyl)urea·Hydrochloride (32·HCl). A solution of **32** (15 g, 32 mmol) in 300 mL of THF was cooled in an ice bath and treated dropwise with 36 mL of a 1 M HCl/ether solution. The resulting solution was stirred for 30 min and concentrated in vacuo. The resulting solid was triturated with ether and the resulting white solid dried under high vacuum overnight to give 16 g of the hydrochloride salt designated as IQ657. Mp 58−60 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ TMS: 9.61 (s, 1 H), 9.15 (s, 1 H), 8.00 (m, 1 H), 7.63−7.61 (m, 1 H), 7.51−7.49 (m, 1 H), 7.39−7.34 (m, 1 H), 7.22−7.17 (m, 2 H), 7.09−7.04 (m, 2 H), 6.86 (d, J = 8 Hz, 1 H), 3.47−3.31 (m, 4 H), 3.11 (m, 1 H), 2.98−2.82 (m, 2 H), 2.67−2.62 (dd, J = 5 Hz, J = 13 Hz, 1 H), 2.58−2.50 (m, 2 H), 2.52 (s, 3 H), 2.39 (dd, J = 8 Hz, J = 13 Hz, 1 H), 2.16−2.06 (m, 2 H), 1.84−1.556 (m, 7 H), 1.30−1.00 (m, 4 H). Anal. Calcd for $C_{28}H_{37}N_3O_2FCl\cdot H_2O\cdot THF_{0.25}$: C, 64.73; H, 7.68; N, 7.81. Found: C, 64.89; H, 7.41; N, 7.81.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(3-acetylphenyl)urea-benzenesulfonate (32·Besylate): DPC168. Benzenesulfonic acid monohydrate (1.06 g, 6 mmol) was dried by azeotroping off the water of a benzene solution (twice) and adding the dried acid solution to a solution of **32** (2.81 g, 6 mmol) in toluene (40 mL). The solvents were removed in vacuo (twice) and the resulting residue was recrystallized twice from toluene and dried under high vacuum overnight to give 2.77 g of benzenesulfonic salt, designated DPC168, as a white solid. Mp 157−159 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ TMS: 9.46 (s, 1 H), 8.77 (s, 1 H), 8.29 (s, 1 H), 7.93 (m, 2 H), 7.55 (m, 2 H), 7.43 (m, 3 H), 7.31 (t, J = 8 Hz, 1 H), 6.95 (m, 2 H), 6.83 (m, 2 H), 6.64 (d, J = 9 Hz, 1 H), 3.66 (m, 1 H), 3.56 (m, 1 H), 3.40 (m, 2 H), 2.57 (s, 3 H), 2.56−2.41 (m, 4 H), 2.37−2.29 (m, 2 H), 2.11−1.90 (m, 2 H), 1.86−1.70 (m, 6 H), 1.33−1.23 (m, 4 H), 1.00 (m, 1 H). Anal. Calcd for $C_{34}H_{42}N_3O_5FS$: C, 65.47; H, 6.80; N, 6.75; S, 5.14. Found: C, 65.48; H, 6.80; N, 6.70; S, 5.35.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(5-acetyl-4-methylthiazol-2-yl)urea (65): DPC-A37818. Synthesis of Phenyl 5-Acetyl-4-methylthiazol-2-ylcarbamate. In a round-bottom flask, NaH 60% dispersion in mineral oil (3.07 g, 77 mmol) was washed

2× with hexane and suspended in DMF. Then 2-amino-5-acetyl-4-methyl-thiazole (10.0 g, 64 mmol) was added and stirred while cooling in an ice bath. Stirring continued until the NaH was consumed. Diphenyl carbonate (34 g, 160 mmol) was added while cooling, and after the addition was complete, the reaction mixture was stirred for an additional 30 min at room temperature. The DMF was removed on a rotary evaporator (high vacuum, 40 °C) to yield a brown residue. This residue was dissolved in 1 L of $CHCl_3$ and washed successively with 2 L of 0.5 N HCl, 2 × 1 L of water, and finally with 1 L of brine. The aqueous portions were back extracted twice with 300 mL of $CHCl_3$. The combined organic fractions were dried over anhydrous sodium sulfate, filtered, and concentrated on a rotary evaporator to give a white solid. This was chromatographed on silica (15−70% EtOAc/hexane) to give 15 g of the desired carbamate as a white solid. Mp 180−182 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 11.42 (bs, 1 H), 7.47−7.40 (m, 2 H), 7.33−7.27 (m, 1 H), 7.22−7.18 (m, 2 H), 2.72 (s, 3 H), 2.50 (s, 3 H). ESI MS: $(M + H)^+ = 277.1$. Anal. Calcd for $C_{13}H_{12}N_2O_3S$: C, 56.51; H, 4.39; N, 10.14; S, 11.60. Found: C, 56.42; H, 4.31; N, 10.11; S, 11.74.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(5-acetyl-4-methylthiazol-2-yl)urea (65): DPC-A37818. A solution of (1*R*,2*S*)-2-[(3*S*)-3-(4-fluorobenzyl)piperidin-1-ylmethyl]cyclohexylamine (62.8 g, 206.3 mmol) and phenyl 5-acetyl-4-methylthiazol-2-ylcarbamate (57 g, 206.3 mmol) in a mixture of AcCN (6 L), $CHCl_3$ (2 L), and DMF (1 L) was stirred at room temperature overnight. The solvents were removed under vacuum on a rotary evaporator, and the residue was dissolved in methylene chloride, washed with water and brine, and then dried over Na_2SO_4. The drying agent was filtered off, and the filtrate was concentrated on a rotary evaporator to give a solid. The solid was recrystallized from acetonitrile (3 L) and EtOH (1 L) to give a first crop of 75 g of the desired urea. The mother liquor was concentrated, and the solid was recrystallized from acetonitrile to give an additional 17 g of urea **65** designated as DPC-A37818. Mp 174−175°C. $[\alpha]^{25}_D = -40.4°$ ($CHCl_3$, $c = 0.304$ g/dL). ^{1}H NMR (500 MHz, DMSO-d_6) δ TMS: 10.74 (bs, 1 H), 7.09−7.05 (m, 2 H), 6.98−6.930 (m, 2 H), 6.72 (bd, $J = 8$ Hz, 1 H), 3.29−3.23 (m, 1 H), 2.69−2.67 (m, 1 H), 2.57 (bd, $J = 10$ Hz, 1 H), 2.52 (s, 3 H), 2.43 (m, 1 H), 2.41 (s, 3 H), 2.33−2.26 (m, 2 H), 1.97−1.93 (m, 1 H), 1.84−1.37 (m, 11 H), 1.24−1.10 (m, 3 H), 0.96−0.79 (m, 2 H). ESI MS: $(M + H)^+ = 487.3$; HRMS: $(M + H)^+ = 487.256$ 1. Anal. Calcd for $C_{26}H_{35}FN_4O_2S$: C, 64.17; H, 7.26; N, 11.51; S, 6.60. Found: C, 64.22; H, 7.34; N, 11.45; S, 6.67.

1-((1*R*,2*S*)-2-(((*R*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-methoxyphenyl)urea (27). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.27 (bs, 1 H), 7.16−7.01 (m, 4 H), 7.00−6.95 (m, 2 H), 6.74 (dd, $J = 1$ Hz, $J = 8$ Hz, 1 H), 6.54 (m, 1 H), 6.13 (bs, 1 H), 3.76 (s, 3 H), 3.26 (dt, $J = 10$ Hz, $J = 3$ Hz, 1 H), 2.89 (bd, $J = 10$ Hz, 1 H), 2.67 (bd, $J = 10$ Hz, 1 H), 2.53 (m, 1 H), 2.41−2.37 (m, 4 H), 2.02 (dd, $J = 2$ Hz, $J = 13$ Hz, 1 H), 1.93 (bt, $J = 11$ Hz, 1 H), 1.80−0.80 (m, 14 H). MS ESI $(M + H)^+ = 454.3$. Anal. Calcd for ($C_{27}H_{36}FN_3O_2$): C, 71.49; H, 8.00; N, 9.26. Found: C, 71.23; H, 8.01; N, 9.15.

1-((1*R*,2*S*)-2-(((*R*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-cyanophenyl)urea (28). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.71 (m, 1 H), 7.56 (bs, 1 H), 7.56−7.52 (m, 1 H), 7.37 (m, 1 H), 7.35−7.29 (m, 1 H), 7.25 (m, 1 H), 7.13−7.08 (m, 2 H), 7.02−6.96 (m, 2 H), 6.31 (bs, 1 H), 3.23 (dt, $J = 10$ Hz, $J = 4$ Hz, 1 H), 2.96 (bd, $J = 10$ Hz, 1 H), 2.72 (bd, $J = 10$ Hz, 1 H), 2.58 (dd, $J = 5$ Hz, $J = 13$ Hz, 1 H), 2.45−2.32 (m, 4 H), 2.10−1.9 (m, 2 H), 1.83−0.85 (m, 14 H). MS ESI $(M + H)^+ = 449.3$. Anal. Calcd for ($C_{27}H_{33}FN_4O\cdot(H_2O)_{0.33}$): C, 71.35; H, 7.46; N, 12.33. Found: C, 71.24; H, 7.29; N, 12.29.

1-((1*R*,2*S*)-2-(((*R*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-acetylphenyl)urea (29). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.86 (m, 1 H), 7.78 (bs, 1 H), 7.66−7.62 (m, 1 H), 7.57−7.54 (m, 1 H), 7.35 (t, $J = 8$ Hz, 1 H), 7.12−7.07 (m, 2 H), 7.02−6.96 (m, 2 H), 6.26 (s, 1 H), 3.28 (m, 1 H), 2.94 (bd, $J = 10$ Hz, 1 H), 2.71 (bd, $J = 12$ Hz, 1 H), 2.58 (dd, $J = 5$ Hz, $J = 13$ Hz, 1 H), 2.57 (s, 3 H), 2.44−2.33 (m, 3 H), 2.06 (dd, $J = 2$ Hz, $J = 12$ Hz, 1 H), 1.98 (m, 1 H), 1.78−0.85 (m, 14 H). MS ESI: $(M + H)^+ = 466.3$.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-methoxyphenyl)urea (30). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.60 (bs, 1 H), 7.17 (t, $J = 8$ Hz, 1 H), 7.09 (t, $J = 2$ Hz, 1 H), 6.85−6.76 (m, 2 H), 6.73 (dd, $J = 1.5$ Hz, $J = 8$ Hz, 1 H), 6.59 (dd, $J = 2$ Hz, $J = 8$ Hz, 1 H), 6.04 (bs, 1 H), 3.80 (s, 3 H), 3.22 (m, 1 H), 2.96 (bd, $J = 10$ Hz, 1 H), 2.61 (bd, $J = 10$ Hz, 1 H), 2.39−2.30 (m, 4 H), 2.04 (dd, $J = 2$ Hz, $J = 13$ Hz, 1 H), 1.83−0.85 (m, 15 H). MS ESI $(M + H)^+ = 454.2$. HRMS: calcd for $C_{27}H_{37}FN_3O_2$ $(M + H)^+$, 454.287 0; found, 454.2883. Anal. Calcd for ($C_{27}H_{36}FN_3O_2$): C, 71.49; H, 8.00; N, 9.26. Found: C, 71.13; H, 8.00; N, 9.08.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-cyanophenyl)urea (31). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.86 (bs, 1 H), 7.68 (m, 1 H), 7.56−7.52 (m, 1 H), 7.37 (m, 1 H), 7.30−7.27 (m, 1 H), 6.96−6.92 (m, 2 H), 6.85−6.78 (m, 2 H), 6.15 (bs, 1 H), 3.19 (m, 1 H), 2.99 (bd, $J = 10$ Hz, 1 H), 2.65 (bd, $J = 10$ Hz, 1 H), 2.44−2.32 (m, 4 H), 2.08 (dd, $J = 2$ Hz, $J = 12$ Hz, 1 H), 1.83−0.85 (m, 15 H). MS ESI $(M + H)^+ = 449.2$. HRMS: calcd for $C_{27}H_{34}FN_4O$ $(M + H)^+$, 449.271 7; found, 449.2712. Anal. Calcd for ($C_{27}H_{33}FN_4O\cdot(H_2O)_{0.25}$): C, 71.58; H, 7.45; N, 12.37. Found: C, 71.72; H, 7.52; N, 12.27.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-phenylurea (33). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.29−7.26 (m, 4 H), 7.05−6.99 (m, 1 H), 6.96−6.91 (m, 2 H), 6.86−6.80 (m, 2 H), 6.04 (bs, 1 H), 3.22 (m, 1 H), 2.94 (bd, $J = 10$ Hz, 1 H), 2.61 (bd, $J = 10$ Hz, 1 H), 2.39−2.30 (m, 4 H), 2.02 (dd, $J = 2$ Hz, $J = 13$ Hz, 1 H), 1.80−0.81 (m, 15 H). MS ESI $(M + H)^+ = 424.2$. HRMS: calcd for $C_{26}H_{35}FN_3O$ $(M + H)^+$, 424.276 4; found, 424.275 5. Anal. Calcd for ($C_{26}H_{34}FN_3O\cdot(H_2O)_{0.25}$): C, 72.95; H, 8.12; N, 9.82. Found: C, 72.91; H, 8.10; N, 9.73.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-*o*-tolylurea (34). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.57 (d, $J = 8$ Hz, 1 H), 7.19−7.14 (m, 2 H), 7.08−6.85 (m, 6 H), 5.79 (bs, 1 H), 3.24 (m, 1 H), 2.85 (m, 1 H), 2.56 (bm, 1 H), 2.46−2.25 (m, 4 H), 2.22 (s, 3 H), 2.00 (dd, $J = 12$ Hz, $J = 2.5$ Hz, 1 H), 1.70−0.83 (m, 15 H). MS ESI $(M + H)^+ = 438.3$. HRMS: calcd for $C_{27}H_{37}FN_3O$ $(M + H)^+$, 439.292 1; found, 438.292 3. Anal. Calcd for ($C_{27}H_{36}FN_3O\cdot(H_2O)_{0.2}$): C, 73.50; H, 8.32; N, 9.52. Found: C, 73.59; H, 8.42; N, 9.54.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(2-methoxyphenyl)urea (35). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.18 (m, 1 H), 6.99−6.80 (m, 5 H), 6.94−6.88 (m, 2 H), 3.76 (s, 3 H), 3.20 (m, 1 H), 2.95 (m, 1 H), 2.58 (bm, 1 H), 2.46−2.31 (m, 4 H), 2.08 (dd, $J = 12$ Hz, $J = 2.5$ Hz, 1 H), 1.80−0.88 (m, 15 H). MS ESI $(M + H)^+ = 454.3$. HRMS: calcd for $C_{27}H_{37}FN_3O_2$ $(M + H)^+$, 454.287 0; found, 454.287 6. Anal. Calcd for ($C_{27}H_{36}FN_3O_2$): C, 71.49; H, 8.00; N, 9.26. Found: C, 71.18; H, 7.91; N, 9.13.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(4-methoxyphenyl)urea (36). ^{1}H NMR (400 MHz, $CDCl_3$) δ TMS: 7.18 (m, 2 H), 7.09 (bs, 1 H), 7.00−6.96 (m, 2 H), 6.90−6.85 (m, 2 H), 6.81 (m, 2 H), 5.95 (bs, 1 H), 3.74 (s, 3 H), 3.27 (m, 1 H), 2.91 (m, 1 H), 2.64 (m, 1 H), 2.43−2.30 (m, 4 H), 2.04 (dd, $J = 13$ Hz, $J = 3$ Hz, 1 H), 1.80−0.88 (m, 15 H). MS ESI $(M + H)^+ = 454.46$.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(2-fluorophenyl)urea (37). ^{1}H NMR (400 MHz, $CDCl_3$) δ TMS: 8.13 (m, 1 H), 8.00 (bs, 1 H), 7.12−7.03 (m, 2 H), 6.97−6.91 (m, 3 H), 6.79−6.75 (m, 2 H), 6.21 (bs, 1 H), 3.19 (dt, $J = 3$ Hz, $J = 10$ Hz, 1 H), 2.99 (bd, $J = 10$ Hz, 1 H), 2.61 (bd, $J = 10$ Hz, 1 H), 2.40 (d, $J = 7$ Hz, 2 H), 2.35−2.31 (m, 2 H), 2.07 (dd, $J = 2$ Hz, $J = 12$ Hz, 1 H), 1.83−0.85 (m, 15 H). MS ESI $(M + H)^+ = 442.47$.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-fluorophenyl)urea (38). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.89 (bs, 1 H), 7.23−7.17 (m, 2 H), 6.97−6.91 (m, 3 H), 6.85−6.78 (m, 2 H), 6.71 (dt, $J = 2.2$ Hz, $J = 8$ Hz, 1 H), 6.11 (bs, 1 H), 3.21 (dt, $J = 3$ Hz, $J = 10$ Hz, 1 H), 2.99 (bd, $J = 10$ Hz, 1 H), 2.63 (bd, $J = 10$ Hz, 1 H), 2.40

(d, J = 7.3 Hz, 2 H), 2.35−2.31 (m, 2 H), 2.08 (dd, J = 2 Hz, J = 12 Hz, 1 H), 1.83−0.85 (m, 15 H). MS ESI $(M + H)^+$ = 442.2. HRMS: calcd for $C_{26}H_{34}F_2N_3O$ $(M + H)^+$, 442.267 0; found, 442.2693. Anal. Calcd for ($C_{26}H_{33}F_2N_3O$): C, 70.72; H, 7.53; N, 9.52. Found: C, 70.50; H, 7.53; N, 9.33.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(4-fluorophenyl)urea (39). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.26−7.20 (m, 2 H), 7.01−6.93 (m, 4 H), 6.88−6.80 (m, 2 H), 5.99 (bs, 1 H), 3.21 (m, 1 H), 2.94 (bd, J = 10 Hz, 1 H), 2.61 (bd, J = 10 Hz, 1 H), 2.41−2.30 (m, 4 H), 2.03 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.80−0.81 (m, 15 H). MS ESI $(M + H)^+$ = 442.2. HRMS: calcd for $C_{26}H_{34}F_2N_3O$ $(M + H)^+$, 442.267 0; found, 442.269 6. Anal. Calcd for ($C_{26}H_{33}F_2N_3O$): C, 70.72; H, 7.53; N, 9.52. Found: C, 70.54; H, 7.58; N, 9.35.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3,4-difluorophenyl)urea (40). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS): 7.65 (bs, 1 H), 7.38−7.30 (m, 1 H), 7.09−6.81 (m, 6 H), 6.14 (bs, 1 H), 3.20 (dt, J = 3 Hz, J = 10 Hz, 1 H), 2.96 (bd, J = 10 Hz, 1 H), 2.63 (bd, J = 10 Hz, 1 H), 2.41 (d, J = 7 Hz, 2 H), 2.38−2.31 (m, 2 H), 2.08 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.80−0.85 (m, 15 H). MS ESI $(M + H)^+$ = 460.3. HRMS: calcd for $C_{26}H_{33}F_3N_3O$ $(M + H)^+$, 460.257 6; found, 460.258 7. Anal. Calcd for ($C_{26}H_{32}F_3N_3O \cdot (H_2O)_{0.25}$): C, 67.29; H, 7.06; N, 9.05. Found: C, 67.19; H, 6.97; N, 8.98.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-fluoro-4-methylphenyl)urea (41). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.60 (bs, 1 H), 7.12 (dd, J = 1.8 Hz, J = 12 Hz, 1 H), 7.08−6.81 (m, 6 H), 6.07 (bs, 1 H), 3.21 (dt, J = 3 Hz, J = 10 Hz, 1 H), 2.96 (bd, J = 10 Hz, 1 H), 2.61 (bd, J = 10 Hz, 1 H), 2.39−2.30 (m, 4 H), 2.19 (d, J = 1.5 Hz, 3 H), 2.01 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.80−0.83 (m, 15 H). MS ESI $(M + H)^+$ = 456.3. HRMS: calcd for $C_{27}H_{36}F_2N_3O$ $(M + H)^+$, 456.282 6; found, 456.282 4. Anal. Calcd for ($C_{27}H_{35}F_2N_3O$): C, 71.18; H, 7.74; N, 9.22. Found: C, 70.93; H, 7.65; N, 9.13.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(5-fluoro-2-methylphenyl)urea (42). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.62 (dd, J = 11 Hz, J = 2.2 Hz, 1 H), 7.06 (m, 1 H), 6.98−6.82 (m, 5 H), 6.66 (dt, J = 2.5 Hz, J = 8 Hz, 1 H), 5.83 (bs, 1 H), 3.22 (m, 1 H), 2.94 (m, 1 H), 2.60 (bm, 1 H), 2.45−2.30 (m, 4 H), 2.14 (s, 3 H), 2.05 (dd, J = 12 Hz, J = 2 Hz, 1 H), 1.80−0.83 (m, 15 H). MS ESI $(M + H)^+$ = 456.3. HRMS: calcd for $C_{27}H_{36}F_2N_3O$ $(M + H)^+$, 456.282 6; found, 456.285 6. Anal. Calcd for ($C_{27}H_{35}F_2N_3O \cdot (H_2O)_{0.2}$): C, 70.62; H, 7.77; N, 9.15. Found: C, 70.62; H, 7.63; N, 8.96.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-chlorophenyl)urea (43). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.85 (bs, 1 H), 7.37 (s, 1 H), 7.21−7.18 (m, 2 H), 7.01−6.81 (m, 5 H), 6.07 (bs, 1 H), 3.20 (dt, J = 3 Hz, J = 10 Hz, 1 H), 3.00 (bd, J = 10 Hz, 1 H), 2.64 (bd, J = 10 Hz, 1 H), 2.40 (d, J = 7 Hz, 2 H), 2.39−2.31 (m, 2 H), 2.08 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.80−0.86 (m, 15 H). MS ESI $(M + H)^+$ = 458.3. HRMS: calcd for $C_{26}H_{34}ClFN_3O$ $(M + H)^+$, 458.237 4; found, 458.236 5. Anal. Calcd for ($C_{26}H_{33}ClFN_3O$): C, 68.18; H, 7.26; N, 9.17. Found: C, 68.09; H, 7.27; N, 8.99.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(4-chlorophenyl)urea (44). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.65 (bs, 1 H), 7.26 (bs, 2 H), 7.24 (bs, 2 H), 6.96−6.91 (m, 4 H), 6.87−6.80 (m, 2 H), 6.03 (bs, 1 H), 3.19 (dt, J = 3 Hz, J = 10 Hz, 1 H), 2.97 (bd, J = 10 Hz, 1 H), 2.62 (bd, J = 10 Hz, 1 H), 2.40 (d, J = 7.3 Hz, 2 H), 2.38−2.31 (m, 2 H), 2.05 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.82−0.82 (m, 15 H). MS ESI $(M + H)^+$ = 458.3. HRMS: calcd for $C_{26}H_{34}ClFN_3O$ $(M + H)^+$, 458.237 4; found, 458.237 7. Anal. Calcd for ($C_{26}H_{33}ClFN_3O$): C, 68.18; H, 7.26; N, 9.17. Found: C, 68.56; H, 7.41; N, 9.04.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3,5-dichlorophenyl)urea (45). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.85 (bs, 1 H), 7.30 (d, J = 1.9 Hz, 2 H), 7.01−6.84 (m, 5 H), 6.13 (bs, 1 H), 3.19 (dt, J = 3 Hz, J = 10 Hz, 1 H), 3.01 (bd, J = 10 Hz, 1 H), 2.65 (bd, J = 10 Hz, 1 H), 2.42 (d, J = 7 Hz, 2 H), 2.39−2.31 (m, 2 H), 2.08 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.84−0.85 (m, 15 H). MS ESI $(M + H)^+$ = 492.3. HRMS: calcd for $C_{26}H_{33}Cl_2FN_3O$ $(M + H)^+$, 492.198 5; found, 492.198 4. Anal. Calcd for ($C_{26}H_{32}Cl_2FN_3O$): C, 63.41; H, 6.55; N, 8.53. Found: C, 63.13; H, 6.55; N, 8.37.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3,4-dichlorophenyl)urea (46). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.50 (d, J = 2.5 Hz, 1 H), 7.32 (d, J = 9 Hz, 1 H), 7.17 (dd, J = 2.5 Hz, J = 9 Hz, 1 H), 6.97−6.91 (m, 2 H), 6.88−6.80 (m, 2 H), 6.05 (bs, 1 H), 3.19 (m, 1 H), 2.99 (bd, J = 10 Hz, 1 H), 2.64 (bd, J = 10 Hz, 1 H), 2.43−2.31 (m, 4 H), 2.08 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.83−0.81 (m, 15 H). MS ESI $(M + H)^+$ = 492.1. HRMS: calcd for $C_{26}H_{33}Cl_2FN_3O$ $(M + H)^+$, 492.198 5; found, 492.197 5. Anal. Calcd for ($C_{26}H_{32}Cl_2FN_3O$): C, 63.41; H, 6.55; N, 8.53. Found: C, 63.44; H, 6.62; N, 8.37.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-bromophenyl)urea (47). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.75 (bs, 1 H), 7.52 (m, 1 H), 7.28−7.22 (m, 1 H), 7.16−7.13 (m, 2 H), 6.97−6.92 (m, 4 H), 6.87−6.81 (m, 2 H), 6.06 (bs, 1 H), 3.20 (dt, J = 3 Hz, J = 10 Hz, 1 H), 2.99 (bd, J = 10 Hz, 1 H), 2.63 (bd, J = 10 Hz, 1 H), 2.40 (d, J = 7.3 Hz, 2 H), 2.35−2.31 (m, 2 H), 2.07 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.82−0.82 (m, 15 H). MS ESI $(M + H)^+$ = 502.3. HRMS: calcd for $C_{26}H_{34}BrFN_3O$ $(M + H)^+$, 502.186 9; found, 502.187 3. Anal. Calcd for ($C_{26}H_{33}BrFN_3O$): C, 62.15; H, 6.62; N, 8.36. Found: C, 62.17; H, 6.65; N, 8.24.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(4-bromophenyl)urea (48). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.65 (bs, 1 H), 7.48−7.36 (m, 2 H), 7.22−7.19 (m, 2 H), 6.96−6.91 (m, 4 H), 6.87−6.80 (m, 2 H), 6.03 (bs, 1 H), 3.19 (dt, J = 3 Hz, J = 10 Hz, 1 H), 2.97 (bd, J = 10 Hz, 1 H), 2.61 (bd, J = 10 Hz, 1 H), 2.40 (d, J = 7.3 Hz, 2 H), 2.35−2.31 (m, 2 H), 2.05 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.82−0.82 (m, 15 H). MS ESI $(M + H)^+$ = 502.0. HRMS: calcd for $C_{26}H_{34}BrFN_3O$ $(M + H)^+$, 502.186 9; found, 502.186 8. Anal. Calcd for ($C_{26}H_{33}BrFN_3O$): C, 62.15; H, 6.62; N, 8.36. Found: C, 62.11; H, 6.66; N, 8.21.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-cyano-4-fluorophenyl)urea (49). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.77 (bs, 1 H), 7.62 (dd, J = 3 Hz, J = 5 Hz, 1 H), 7.54−7.48 (m, 1 H), 7.12 (t, J = 9 Hz, 1 H), 6.96−6.88 (m, 2 H), 6.87−6.80 (m, 2 H), 6.08 (bs, 1 H), 3.18 (dt, J = 3 Hz, J = 10 Hz, 1 H), 2.99 (bd, J = 10 Hz, 1 H), 2.66 (bd, J = 10 Hz, 1 H), 2.44 (d, J = 7.3 Hz, 2 H), 2.39−2.31 (m, 2 H), 2.09 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.82−0.82 (m, 15 H). MS ESI $(M + H)^+$ = 467.3.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(4-acetylphenyl)urea (50). ^{1}H NMR (400 MHz, $CDCl_3$) δ TMS: 7.86 (m, 3 H), 7.42 (m, 2 H), 6.93−6.90 (m, 2 H), 6.80−6.76 (m, 2 H), 6.30 (bs, 1 H), 3.31 (dt, J = 3 Hz, 11 Hz, 1 H), 3.06 (m, 1 H), 2.66−2.56 (m, 1 H), 2.54 (s, 3 H), 2.46−2.32 (m, 4 H), 2.10 (m, 1 H), 1.90−0.86 (m, 15 H). MS ESI: $(M + H)^+$ = 466.46.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3,5-diacetylphenyl)urea (51). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.22 (d, J = 1.5 Hz, 2 H), 8.19 (d, J = 1.5 Hz, 1 H), 6.95−6.91 (m, 2 H), 6.73−6.67 (m, 2 H), 6.42 (bs, 1 H), 3.22 (m, 1 H), 3.05 (bd, J = 12 Hz, 1 H), 2.68 (s, 6 H), 2.66 (bd, J = 12 Hz, 1 H), 2.47−2.35 (m, 4 H), 2.08 (d, J = 13 Hz, 1 H), 1.80−0.95 (m, 16 H). MS ESI $(M + H)^+$ = 508.4. HRMS: calcd for $C_{30}H_{39}FN_3O_3$ $(M + H)^+$, 508.297 5; found, 508.297 5. Anal. Calcd for ($C_{30}H_{38}FN_3O_3 \cdot (H_2O)_{0.5}$): C, 69.74; H, 7.61; N, 8.13. Found: C, 69.63; H, 7.51; N, 8.04.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-acetyl-4-fluorophenyl)urea (52). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.87−7.83 (m, 1 H), 7.65 (bs, 1 H), 7.47 (dd, J = 3 Hz, J = 6 Hz, 1 H), 7.09 (dd, J = 11 Hz, J = 1 Hz, 1 H), 6.98−6.94 (m, 2 H), 6.84−6.78 (m, 2 H), 6.16 (bs, 1 H), 3.19 (dt, J = 3 Hz, J = 10 Hz, 1 H), 2.99 (bd, J = 10 Hz, 1 H), 2.66 (d, J = 5 Hz, 1 H), 2.66 (m, 1 H), 2.44−

2.33 (m, 4 H), 2.07 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.81−0.82 (m, 15 H). MS ESI (M + H)$^+$ = 484.3.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(1H-indol-6-yl)urea (53). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.51 (bs, 1 H), 7.58 (s, 1 H), 7.48 (d, J = 8 Hz, 1 H), 7.09 (m, 1 H), 6.99−6.79 (m, 5 H), 6.45 (s, 1 H), 6.33 (bs, 1 H), 3.28 (dt, J = 10 Hz, J = 4 Hz, 1 H), 2.83 (m, 1 H), 2.57 (m, 1 H), 2.40−2.20 (m, 4 H), 2.01 (dd, J = 13 Hz, J = 3 Hz, 1 H), 1.80−0.70 (m, 15 H). MS ESI (M + H)$^+$ = 463.3. Anal. Calcd for ($C_{28}H_{35}FN_4O\cdot(H_2O)_{0.10}$): C, 71.64; H, 7.53; N, 11.89. Found: C, 71.82; H, 7.69; N, 11.51.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(1H-indol-5-yl)urea (54). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.25 (bs, 1 H), 7.50 (m, 1 H), 7.29 (m, 1 H), 7.20 (m, 1 H), 7.07 (dd, J = 2 Hz, J = 8 Hz, 1 H), 6.96−6.86 (m, 4 H), 6.46 (m, 1 H), 6.09 (bs, 1 H), 3.26 (m, 1 H), 2.79 (bd, J = 10 Hz, 1 H), 2.56 (bd, J = 10 Hz, 1 H), 2.38−2.00 (m, 4 H), 1.99 (dd, J = 4 Hz, J = 12 Hz, 1 H), 1.83−0.85 (m, 16 H). MS ESI (M + H)$^+$ = 463.3.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(2,3-dimethyl-1H-indol-6-yl)urea (55). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.81 (bs, 1 H), 7.38 (s, 1 H), 7.14 (d, J = 7 Hz, 1 H), 6.97−6.84 (m, 5 H), 6.10 (bs, 1 H), 3.11 (dt, J = 11 Hz, J = 4 Hz, 1 H), 2.75 (m, 1 H), 2.55 (m, 1 H), 2.40−2.20 (m, 4 H), 2.34 (s, 3 H), 2.17 (s, 3 H), 2.01 (dd, J = 12 Hz, J = 4 Hz, 1 H), 1.80−0.70 (m, 15 H). MS ESI (M + H)$^+$ = 491.4. Anal. Calcd for ($C_{30}H_{39}FN_4O\cdot(H_2O)_{0.50}$): C, 72.11; H, 8.07; N, 11.21. Found: C, 72.01; H, 7.99; N, 11.00.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(1H-indazol-5-yl)urea (56). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 10.45 (bs, 1 H), 7.94 (bs, 1 H), 7.69 (bs, 1 H), 7.60 (bs, 1 H), 7.38 (m, 1 H), 7.25 9m, 1 H), 6.97−6.81 (m, 4 H), 6.26 (bs, 1 H), 3.28 (dt, J = 3 Hz, J = 10 Hz, 1 H), 2.92 (bd, J = 10 Hz, 1 H), 2.65 (bd, J = 10 Hz, 1 H), 2.42−2.28 (m, 4 H), 2.06 (dd, J = 2 Hz, J = 13 Hz, 1 H), 1.80−0.83 (m, 15 H). MS ESI (M + H)$^+$ = 464.4. HRMS: calcd for $C_{27}H_{35}FN_5O$ (M + H)$^+$, 464.282 6; found, 464.282 8.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(1H-indazol-6-yl)urea (57). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.97 (s, 1 H), 7.95 (d, J = 9 Hz, 1 H), 7.59 (d, J = 9 Hz, 1 H), 7.52 (bs, 1 H), 6.93−6.89 (m, 2 H), 6.82−6.73 (m, 3 H), 3.30 (m, 1 H), 2.95 (bd, J = 10 Hz, 1 H), 2.66 (bd, J = 10 Hz, 1 H), 2.45−2.29 (m, 4 H), 2.06 (dd, J = 3 Hz, J = 13 Hz, 1 H), 1.81−1.42 (m, 7 H), 1.39−0.84 (m, 8 H). MS ESI (M + H)$^+$ = 464.4. HRMS: calcd for $C_{27}H_{35}FN_5O$ (M + H)$^+$, 464.282 6; found, 464.283 7. Anal. Calcd for ($C_{27}H_{34}FN_5O\cdot(H_2O)_{0.75}$): C, 67.97; H, 7.50; N, 14.68. Found: C, 68.17; H, 7.35; N, 14.64.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-chloro-1H-indazol-5-yl)urea (58). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.92 (bs, 1 H), 7.79 (m, 1 H), 7.30−7.23 (m, 3 H), 7.00 (bs, 1 H), 6.97−6.89 (m, 2 H), 6.85−6.81 (m, 2 H), 3.40 (m, 1 H), 3.30 (m, 1 H), 3.02 (bd, J = 8 Hz, 1 H), 2.85 (m, 1 H), 2.45−2.28 (m, 3 H), 2.19−1.95 (m, 4 H), 1.80−1.42 (m, 5 H), 1.39−0.84 (m, 7 H). MS ESI (M + H)$^+$ = 498.3.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(indolin-5-yl)urea (59). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.06−6.99 (m, 3 H), 6.94−6.88 (m, 2 H), 6.81 (d, J = 8 Hz, 1 H), 6.53 (d, J = 8 Hz, 1 H), 5.81 (bs, 1 H), 3.53 (m, 2 H), 3.25 (m, 1 H), 2.96 (m, 2 H), 2.80 (bd, J = 10 Hz, 1 H), 2.58 (bd, J = 9 Hz, 1 H), 2.43−2.25 (m, 4 H), 2.00 (dd, J = 12 Hz, J = 2.5 Hz, 1 H), 1.80−0.86 (m, 15 H). MS ESI (M + H)$^+$ = 465.4. HRMS: calcd for $C_{28}H_{38}FN_4O$ (M + H)$^+$, 465.303 0; found, 465.304 6. Anal. Calcd for ($C_{28}H_{37}FN_4O\cdot(H_2O)_{0.75}$): C, 70.34; H, 8.12; N, 11.72. Found: C, 70.36; H, 7.85; N, 11.49

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(benzo[*d*]thiazol-6-yl)urea (60). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.87 (s, 1 H), 8.22 (d, J = 2.2 Hz, 1 H), 8.01 (d, J = 8.8 Hz, 1 H), 7.78 (bs, 1 H), 7.20 (dd, J = 8.8 Hz, J = 2.2 Hz, 1 H), 6.94−6.89 (m, 2 H), 6.82−6.76 (m, 2 H), 6.23 (bs, 1 H), 3.24 (m, 1 H), 2.96 (bd, J = 10 Hz, 1 H), 2.63 (bd, J = 10 Hz, 1 H), 2.41−2.32 (m, 4 H), 2.08 (dd, J = 12.2 Hz, J = 2.2 Hz, 1 H), 1.80−0.86 (m, 15 H). MS ESI (M + H)$^+$ = 481.4. HRMS: calcd for $C_{27}H_{34}FN_4OS$ (M + H)$^+$, 481.243 7; found, 481.244 3. Anal. Calcd for ($C_{27}H_{33}FN_4OS\cdot(H_2O)_{0.25}$): C, 66.84; H, 6.96; N, 11.55. Found: C, 66.71; H, 6.88; N, 11.53.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3H-benzo[*d*]imidazol-5-yl)urea (61). ^{1}H NMR (400 MHz, $CDCl_3$) δ TMS: 7.86 (s, 1 H), 7.80 (m, 2 H), 7.51 (bs, 1 H), 7.01 (m, 1 H), 6.99−6.75 (m, 5 H), 3.30 (dt, J = 10 Hz, J = 3 Hz, 1 H), 2.86 (m, 1 H), 2.63 (m, 1 H), 2.43−2.25 (m, 4 H), 2.10 (dd, J = 13 Hz, J = 3 Hz, 1 H), 1.80−0.70 (m, 15 H). MS ESI (M + H)$^+$ = 464.4. Anal. Calcd for ($C_{27}H_{34}FN_5O\cdot H_2O$): C, 67.34; H, 7.53; N, 14.54. Found: C, 67.01; H, 7.52; N, 14.32.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(1,3,4-thiadiazol-2-yl)urea (62). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.67 (s, 1 H), 7.06−6.91 (m, 2 H), 6.79−6.73 (m, 2 H), 3.36 (m, 1 H), 2.88 (bd, J = 10 Hz, 1 H), 2.59 (bd, J = 10 Hz, 1 H), 2.49−2.15 (m, 4 H), 2.09 (dd, J = 13 Hz, J = 4.4 Hz, 1 H), 1.85−0.83 (m, 15 H). MS ESI (M + H)$^+$ = 432.3. HRMS: calcd for $C_{22}H_{31}FN_5OS$ (M + H)$^+$, 432.223 3; found, 432.224 9. Anal. Calcd for ($C_{22}H_{30}FN_5OS\cdot(H_2O)_{0.2}$): C, 60.72; H, 7.04; N, 16.09. Found: C, 60.72; H, 7.03; N, 15.82.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(thiazol-2-yl)urea (63). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.37 (d, J = 3.6 Hz, 1 H), 7.01−6.97 (m, 2 H), 6.87 (d, J = 3.6 Hz, 1 H), 6.75−6.69 (m, 2 H), 3.28 (m, 1 H), 2.93 (bd, J = 10 Hz, 1 H), 2.53 (bd, J = 10 Hz, 1 H), 2.49−2.22 (m, 4 H), 2.32 (s, 3 H), 2.06 (dd, J = 13 Hz, J = 2.5 Hz, 1 H), 1.80−0.86 (m, 15 H). MS ESI (M + H)$^+$ = 431.4. HRMS: calcd for $C_{23}H_{32}FN_4OS$ (M + H)$^+$, 431.228 1; found, 431.228 4. Anal. Calcd for ($C_{23}H_{31}FN_4OS$): C, 64.16; H, 7.26; N, 13.01. Found: C, 63.79; H, 7.24; N, 12.86.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(4-methylthiazol-2-yl)urea (64). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.16 (bs, 1 H), 7.07−6.97 (m, 2 H), 6.74−6.69 (m, 2 H), 6.41 (d, J = 0.8 Hz, 1 H), 3.24 (m, 1 H), 2.93 (bd, J = 10 Hz, 1 H), 2.53 (bd, J = 10 Hz, 1 H), 2.49−2.22 (m, 4 H), 2.32 (s, 3 H), 2.06 (dd, J = 13 Hz, J = 2.5 Hz, 1 H), 1.80−0.86 (m, 15 H). MS ESI (M + H)$^+$ = 445.4. HRMS: calcd for $C_{24}H_{34}FN_4OS$ (M + H)$^+$, 445.243 7; found, 445.243 5. Anal. Calcd for ($C_{24}H_{33}FN_4OS\cdot(H_2O)_{0.1}$): C, 64.57; H, 7.50; N, 12.55. Found: C, 64.42; H, 7.54; N, 12.44.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(4-chlorobenzo[*d*]thiazol-2-yl)-urea (66). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.68 (dd, J = 1.1 Hz, J = 8.0 Hz, 1 H), 7.44 ((dd, J = 1.1 Hz, J = 7.6 Hz, 1 H), 7.20 (t, J = 8.0 Hz, 1 H), 7.04−6.99 (m, 2 H), 6.70−6.64 (m, 2 H), 3.27 (m, 1 H), 3.00 (bd, J = 11 Hz, 1 H), 2.63 (bd, J = 11 Hz, 1 H), 2.45−2.31 (m, 4 H), 2.12 (dd, J = 11 Hz, J = 2.5 Hz, 1 H), 1.88−0.86 (m, 15 H). MS ESI (M + H)$^+$ = 515.4. HRMS: calcd for $C_{27}H_{33}ClFN_4OS$ (M + H)$^+$, 515.204 8; found, 515.206 1. Anal. Calcd for ($C_{27}H_{32}ClFN_4OS$): C, 62.96; H, 6.26; N, 10.88. Found: C, 62.84; H, 6.37; N, 10.56.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(1-methyl-1H-pyrazol-3-yl)urea (67). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.19 (d, J = 2.2 Hz, 1 H), 7.17−7.02 (m, 2 H), 6.89−6.83 (m, 2 H), 6.56 (bs, 1 H), 6.01 (d, J = 1.5 Hz, 1 H), 3.76 (s, 3 H), 3.36 (m, 1 H), 2.79 (bd, J = 10 Hz, 1 H), 2.68 (bd, J = 8 Hz, 1 H), 2.51−2.28 (m, 3 H), 2.18 (bd, J = 13 hz, 1 H), 2.08 (dd, J = 13 Hz, J = 2.5 Hz, 1 H), 1.98−0.82 (m, 15 H). MS ESI (M + H)$^+$ = 428.4. HRMS: calcd for $C_{24}H_{35}FN_5O$ (M + H)$^+$, 428.282 6; found, 428.283 1. Anal. Calcd for ($C_{24}H_{34}FN_5O\cdot(H_2O)_{0.33}$): C, 66.50; H, 8.06; N, 16.15. Found: C, 66.41; H, 8.01; N, 16.26.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(5-methylisoxazol-3-yl)urea (68). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 7.33 (bs, 1 H), 7.11−6.98 (m, 2 H), 6.89−6.83 (m, 2 H), 6.18 (s, 1 H), 3.31 (m, 1 H), 2.85 (bd, J = 10 Hz, 1 H), 2.65 (bd, J = 8 Hz, 1 H), 2.49−2.28 (m, 3 H), 2.37 (s, 3 H), 2.18 (bd, J = 13 Hz, 1 H), 2.08 (dd, J = 13 Hz, J = 2.5 Hz, 1 H), 1.90−0.82 (m, 15 H). MS ESI (M + H)$^+$ = 429.4. HRMS: calcd for $C_{24}H_{34}FN_4O_2$ (M + H)$^+$, 429.266 6;

found, 429.264 8. Anal. Calcd for ($C_{24}H_{33}FN_4O_2$·$(H_2O)_{0.1}$): C, 66.98; H, 7.78; N, 13.02. Found: C, 66.72; H, 7.80; N, 12.82.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(pyridin-4-yl)urea (69). ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 8.38 (d, J = 7 Hz, 2 H), 7.57 (s, J = 7 Hz, 1 H), 7.31 (d, J = 7 Hz, 2 H), 7.00−6.84 (m, 2 H), 6.84−6.70 (m, 2 H), 3.15 (m, 1 H), 3.07 (m, 1 H), 2.76 (m, 1 H), 2.60−2.46 (m, 1 H), 2.42 (d, J = 7 Hz, 2 H), 2.20 (m, 2 H), 2.00−1.80 (m, 3 H), 1.80−1.40 (m, 7 H), 1.20−0.80 (m, 4 H). MS ESI $(M + H)^+$ = 425.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-cyano-4-(1H-pyrazol-1-yl)phenyl)urea (70). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.04 (m, 1 H), 7.79 (m, 2 H), 7.79 (bs, 1 H), 7.64 (m, 2 H), 6.99−6.86 (m, 2 H), 6.81−6.82 (m, 2 H), 6.53 (m, 1 H), 6.28 (bs, 1 H), 3.20 (m, 1 H), 3.02 (bd, J = 11 Hz, 1 H), 2.68 (bd, J = 11 Hz, 1 H), 2.45 (d, J = 7 Hz, 2 H), 2.39−2.35 (m, 2 H), 2.08 (dd, J = 13 Hz, J = 2.5 Hz, 1 H), 1.90−0.82 (m, 15 H). MS ESI $(M + H)^+$ = 515.3. Anal. Calcd for ($C_{30}H_{35}FN_6O$·$(H_2O)_{0.5}$): C, 68.81; H, 6.93; N, 16.05. Found: C, 68.80; H, 6.74; N, 15.98.

1-(4-(1H-1,2,4-Triazol-1-yl)phenyl)-3-((1*R*,2*S*)-2-(((*S*)-3-(4-fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)urea (71). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.47 (s, 1 H), 8.09 (s, 1 H), 7.72 (bs, 1 H), 7.57 (m, 2 H), 7.46 (m, 2 H), 6.99−6.93 (m, 2 H), 6.82 (m, 2 H), 6.30 (bs, 1 H), 3.22 (dt, J = 10 Hz, J = 3 Hz, 1 H), 3.00 (m, 1 H), 2.42−2.35 (m, 4 H), 2.10 (m, 1 H), 1.85−0.75 (m, 15 H). MS ESI $(M + H)^+$ = 491.3. Anal. Calcd for ($C_{28}H_{35}FN_6O$·$(H_2O)_{0.25}$): C, 67.92; H, 7.23; N, 16.97. Found: C, 67.86; H, 7.30; N, 16.81.

As an example of the tetrazole containing phenyl urea analogues, a detailed procedure for **74** is given below. The other analogues where synthesized using similar procedures.

Preparation of Phenyl 3-(1-Methyl-1H-tetrazol-5-yl)-phenylcarbamate. 3-Nitrobenzoyl chloride (7.00 g, 37.7 mmol) was dissolved in tetrahydrofuran (300 mL), and methylamine (41.5 mL of a 2.0 M solution in tetrahydrofuran, 82.9 mmol) was added. The reaction mixture was stirred for 2 h. The reaction mixture was diluted with ethyl acetate and washed three times with water. The organic layer was dried over sodium sulfate and concentrated to provide *N*-methyl-3-nitro-benzamide as a solid (6.38 g, 94%) which was used without further purification. ^{1}H NMR (300 MHz, CDCl3), δ TMS: 8.84 (bs, 1 H), 8.67 (m, 1 H), 8.37 (dd, J = 8, 2 Hz, 1 H), 8.28 (d, J = 7 Hz, 1 H), 7.78 (dd, J = 8, 7 Hz, 1 H), 2.83 (m, 3 H). MS ESI $(M + H)^+$ = 181.

N-Methyl-3-nitro-benzamide (30.0 g, 167 mmol) was dissolved in acetonitrile (835 mL); sodium azide (10.9 g, 167 mmol) was added, and the mixture was cooled in an ice bath. Trifluoromethanesulfonic anhydride (29 mL, 172 mmol) was added dropwise at a rate which maintained the internal temperature below 3 °C. The reaction mixture was stirred for 3.5 h at 0 °C. The solution was diluted with dichloromethane and then was poured into 1 N aqueous sodium hydroxide (100 mL). The organic layer was separated, dried over sodium sulfate, and concentrated under vacuum to 50 mL, and water was added to precipitate a yellow solid (18.46 g, 54%). A second crop of 1-methyl-5-(3-nitrophenyl)tetrazole crystals was obtained by concentrating the filtrate in vacuo and adding it to boiling ethyl acetate. When the mixture was cooled to 0 °C, additional material (6.07 g, 18%) was isolated by filtration. ^{1}H NMR (300 MHz, $CDCl_3$), δ TMS: 8.67 (m, 1 H), 8.49 (dd, J = 8, 2 Hz, 1 H), 8.31 (d, J = 8 Hz, 1 H), 7.94 (dd, J = 8, 8 Hz, 1 H), 4.22 (s, 3 H).

1-Methyl-5-(3-nitrophenyl)tetrazole (28.8 g, 140 mmol) was dissolved in ethyl acetate (430 mL) and methanol (1270 mL). Palladium on carbon (2.7 g, 10 wt %) was added, and the mixture was shaken under a hydrogen atmosphere (60 psi) for 1.5 h. The mixture was filtered, and the filtrate was concentrated under vacuum to give 1-methyl-5-(3-aminophenyl)tetrazole as a white solid (24.0 g, 98%) which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$), δ TMS: 7.21 (dd, J = 8, 7 Hz, 1 H), 6.99 (s, 1 H), 6.90 (d, J = 7 Hz, 1 H), 6.76 (d, J = 8 Hz, 1 H), 5.44 (bs, 2 H), 4.10 (s, 3 H).

1-Methyl-5-(3-aminophenyl)tetrazole (24.0 g, 137 mmol) was dissolved in dichloromethane (1.4 L), and 2,6-lutidine (44.1 g, 411 mmol) was added. Phenyl chloroformate (21.2 g, 136 mmol) was added in four portions over 15 min, and the mixture was stirred for 1.5 h. The mixture was poured into 1 N aqueous hydrochloric acid (200 mL), and the mixture was extracted three times with dichloromethane (200 mL). The combined organic layers were washed with saturated aqueous sodium chloride, dried over sodium sulfate, and concentrated under vacuum. The crude brown material was dissolved in hot toluene, filtered, and allowed to precipitate at 0 °C to give a white solid (34.1 g). The filtrate was concentrated and recrystallized from toluene to give an additional crop of phenyl 3-(1-methyl-1H-tetrazol-5-yl)phenylcarbamate as off-white crystals (3.44 g, 93% total). ^{1}H NMR (300 MHz, $CDCl_3$), δ TMS: 10.51 (bs, 1 H), 8.01 (s, 1 H), 7.71 (dt, J = 7, 2 Hz, 1 H), 7.55 (m, 2 H), 7.41 (m, 2 H), 7.24 (m, 2 H), 4.14 (s, 3 H).

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-(1-methyl-1H-tetrazol-5-yl)phenyl)-urea (74). A solution of (1*R*,2*S*)-2-(((*S*)-3-(4-fluorobenzyl)-piperidin-1-yl)methyl)cyclohexanamine (30.7 g, 101 mmol) and phenyl 3-(1-methyl-1H-tetrazol-5-yl)phenylcarbamate (29.8 g, 101 mmol) in acetonitrile (800 mL) was stirred overnight at room temperature. The mixture was concentrated in vacuo, and the residue was purified over silica gel, eluting with 60:40 CH_2Cl_2/EtOAc−95:5 CH_2Cl_2/MeOH−90:10 CH_2Cl_2/MeOH, to yield 48.5 g of a white powder. This powder was dried overnight under vacuum at 53 °C to remove residual solvents, to yield 43.7 g of white powder as product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.05−7.92 (m, 1 H), 7.80 (bs, 1 H), 7.56−7.36 (m, 3 H), 6.96 (dd, J = 9 Hz, J = 5 Hz, 2 H), 6.76 (t, J = 9 Hz, 2 H), 6.39 (bs, 1 H), 4.22 (s, 3 H), 3.22 (dt, J = 3 Hz, J = 10 Hz, 1 H), 3.02 (d, J = 11 Hz, 1 H), 2.69 (d, J = 10 Hz, 1 H), 2.49−2.26 (m, 4 H), 2.12 (dd, J = 13 Hz, J = 2 Hz, 1 H), 1.98−0.75 (m, 15 H). HRMS $(M + H)^+$ = 506.303 7. Anal. Calcd for ($C_{28}H_{36}FN_7O$): C, 66.51; H, 7.18; N, 19.39; F, 3.76. Found: C, 66.18; H, 7.14; N, 19.18; F, 4.06.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(3-(5-methyl-1H-tetrazol-1-yl)phenyl)-urea (72). ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.75 (s, 1 H), 7.44 (t, J = 7 Hz, 1 H), 7.30−7.20 (m, 2 H), 7.10 (d, J = 7 Hz, 1 H), 7.00−6.85 (m, 2 H), 6.77 (t, J = 7 Hz, 2 H), 6.36 (s, 1 H), 3.28−3.10 (m, 1 H), 3.05−2.90 (m, 1 H), 2.75−2.60 (m, 1 H), 2.60 (s, 3 H), 2.50−2.25 (m, 4 H), 2.15−2.00 (m, 1 H), 1.90−1.55 (m, 9 H), 1.55−1.10 (m, 4 H), 1.10−0.85 (m, 2 H). MS ESI $(M + H)^+$ = 506.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(2-(1-methyl-1H-tetrazol-5-yl)phenyl)-urea (73). ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 8.85 (s, 1 H), 8.40 (d, J = 7 Hz, 1 H), 8.20 (bs, 1 H), 7.57 (t, J = 7 Hz, 1 H), 7.38 (d, J = 7 Hz, 1 H), 7.11 (t, J = 7 Hz, 1 H), 6.95−6.60 (m, 2 H), 6.51 (t, J = 7 Hz, 2 H), 4.13 (s, 3 H), 3.20−2.90 (m, 2 H), 2.65−2.20 (m, 5 H), 2.20−1.95 (m, 2 H), 1.95−1.10 (m, 13 H), 1.10−0.85 (m, 3 H). MS ESI $(M + H)^+$ = 506.

1-((1*R*,2*S*)-2-(((*S*)-3-(4-Fluorobenzyl)piperidin-1-yl)-methyl)cyclohexyl)-3-(4-(1-methyl-1H-tetrazol-5-yl)phenyl)-urea (75). ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 7.65 (d, J = 7 Hz, 1 H), 7.54 (d, J = 7 Hz, 1 H), 7.00−6.85 (m, 2 H), 6.80−6.60 (m, 3 H), 4.12 (s, 3 H), 3.36−3.15 (m, 1 H), 3.10−2.90 (m, 1 H), 2.70 (d, J = 7 Hz, 1 H), 2.50−2.20 (m, 4 H), 2.10 (d, J = 7 Hz, 1 H), 1.95−1.59 (m, 11 H), 1.59−0.85 (m, 4H). MS ESI $(M + H)^+$ = 506.

1-(3,5-Bis(1-methyl-1H-tetrazol-5-yl)phenyl)-3-((1*R*,2*S*)-2-(((*S*)-3-(4-fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)urea (76). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.23 (d, J = 1.83 Hz, 2 H), 7.85 (t, J = 1.65 Hz, 1 H), 7.19 (m, 1 H), 6.98 (m, 2 H), 6.70 (t, J = 8.79 Hz, 2 H), 4.31 (s, 6 H), 3.25 (m, 1 H), 3.00 (d, J = 11.35 Hz, 1 H), 2.70 (d, J = 7.32 Hz, 1 H), 2.36 (m, 4 H), 2.11 (dd, J = 12.82, 2.56 Hz, 1 H), 1.85−1.70 (m, 9 H), 1.65−0.80 (m, 6 H). MS ESI $(M + H)^+$ = 588.5

1-((1*R*,2*S*)-2-((4-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(1H-indol-5-yl)urea (78). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 8.27 (bs, 1 H), 7.52 (m, 1 H), 7.35 (d, J = 11 Hz, 1 H), 7.23 (m, 1 H), 7.07 (dd, J = 2 Hz, J = 9 Hz, 1 h), 6.92 (d, J = 7 Hz, 4 H), 6.54 (m, 1 H), 6.12 (bs, 1 H), 3.31

(m, 1 H), 2.86 (bd, J = 11 Hz, 1 H), 2.61 (bd, J = 11 Hz, 1 H), 2.41−2.30 (m, 2 H), 1.99−1.90 (m, 3 H), 1.80−0.90 (m, 15 H). MS ESI $(M + H)^+ = 463.3$.

1-((1*R*,2*S*)-2-((4-(4-Fluorobenzyl)piperidin-1-yl)methyl)cyclohexyl)-3-(1H-indazol-5-yl)urea (79). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 10.42 (bs, 1 H), 8.04 (m, 1 H), 7.70 (m, 1 H), 7.45 (d, J = 9 Hz, 1 H), 7.28 (m, 1 H), 6.98−6.89 (m, 4 H), 6.27 (bs, 1 H), 3.28 (m, 1 H), 2.93 (bd, J = 12 Hz, 1 H), 2.66 (bd, J = 12 Hz, 1 H), 2.44−2.33 (m, 2 H), 2.09 (m, 2 H), 1.99 (dd, J = 13 Hz, J = 2.5 Hz, 1 H), 1.82 (m, 1 H), 1.78−0.80 (m, 14 H). MS ESI $(M + H)^+ = 464.2$.

Biological Assays. CCR3−Receptor Binding. Millipore filter plates (no. MABVN1250) are treated with 5 μg/mL protamine in phosphate buffered saline, pH 7.2, for 10 min at room temperature. Plates are washed three times with phosphate buffered saline and incubated with phosphate buffered saline for 30 min at room temperature. For binding, 50 μL of binding buffer (0.5% bovine serum albumen, 20 mM HEPES buffer, and 5 mM magnesium chloride in RPMI 1640 media) with or without a test concentration of a compound present at a known concentration is combined with 50 μL of ^{125}I-labeled human eotaxin (to give a final concentration of 150 pM radioligand) and 50 μL of cell suspension in binding buffer containing 5×10^5 total cells. Cells used for the binding assay are CHO cell lines transfected with a gene expressing human CCR3 as described by Daugherty.[17i] The mixture of compound, cells, and radioligand is incubated at room temperature for 30 min. Plates are placed onto a vacuum manifold, vacuum is applied, and the plates are washed three times with binding buffer with 0.5 M NaCl added. The plastic skirt is removed from the plate, and the plate is allowed to air-dry; the wells are punched out, and the cpm are counted. The percent inhibition of binding is calculated using the total count obtained in the absence of any competing compound or chemokine ligand and the background binding determined by addition of 100 nM eotaxin in place of the test compound.

Human Eosinophil Chemotaxis Assay. Neuroprobe MBA96 96-well chemotaxis chambers with Neuroprobe poly(vinylpyrrolidone)-free polycarbonate PFD5 5-μm filters in place are warmed in a 37 °C incubator prior to the assay. Freshly isolated human eosinophils[39] are suspended in RPMI 1640 with 0.1% bovine serum albumin at 1×10^6 cells/mL and warmed in a 37 °C incubator prior to the assay. A 20 nM solution of human eotaxin in RPMI 1640 with 0.1% bovine serum albumin is warmed in a 37 °C incubator prior to the assay. The eosinophil suspension and the 20 nM eotaxin solution are each mixed 1:1 with prewarmed RPMI 1640 with 0.1% bovine serum albumin with or without a dilution of a test compound that is at 2-fold the desired final concentration. The filter is separated, and the eotaxin/compound mixture is placed into the bottom part of the chemotaxis chamber. The filter and upper chamber are assembled, and 200 μL volume of the cell suspension/compound mixture is added to the appropriate wells of the upper chamber. The upper chamber is covered with a plate sealer, and the assembled unit is placed in a 37 °C incubator for 45 min. After incubation, the plate sealer is removed and all remaining cell suspension is aspirated off. The chamber is disassembled, and unmigrated cells are washed away with phosphate buffered saline and then the filter is wiped with a rubber tipped squeegee. The filter is allowed to completely dry and stained with Wright Giemsa. Migrated cells are enumerated by microscopy.

Calcium Mobilization Assay. Intracellular calcium flux was measured as the increase in fluorescence emitted by the calcium-binding fluorophore, fluo-3, when preloaded cells were stimulated with CCR3 ligand. Freshly isolated eosinophils were loaded with fluorophore by resuspending them in a HEPES-buffered PBS solution containing 5 μM fluo-3 and incubating for 60 min at 37 °C. After being washed twice to remove excess fluorophore, cells were resuspended in binding buffer (without phenol red) and plated into 96-well plates at 2×10^5/well. Plates were placed individually in a FLIPR-1 (Molecular Devices, CA) that uses an argon-ion laser to excite the cells and robotically adds reagents while monitoring changes in fluorescence in all wells simultaneously. To determine the IC_{50}, compound or buffer alone was added and cells were incubated for 5 min; eotaxin was then added to a final concentration of 10 nM. The fluorescence shift was monitored, and the base-to-peak excursion was computed automatically. All conditions were tested in duplicate, and the mean shift per condition was determined. The inhibition achieved by graded concentrations of compound was calculated as a percentage of the compound-free eotaxin control.

Animal Models of Eosinophil Migration. 1. Animals. Female Balb/c (18−20 g) and male C57/B6 (18−20 g) mice were obtained from Charles River Laboratories (Raleigh, NC). Animals were housed at the Bristol Myers Squibb animal care facility under a 12 h light−dark cycle and were allowed food and water ad libitum. All animal protocols were reviewed and approved by the Bristol Myers Squibb Wilmington Site Animal Care and Use Committee.

2. Sensitization of Mice to Ovalbumin. Mice received an intraperitoneal (ip) injection of 10 μg of ovalbumin (OVA; Sigma, St. Louis, MO) and 1 mg of aluminum hydroxide (in a volume of 0.1 mL) on days 1 and 8. On days 15−17, mice were subjected to either a single intranasal instillation of murine eotaxin or an aerosolized OVA challenge.

3. Intranasal (IN) Administration of Eotaxin. OVA-sensitized Balb/c mice were administered either vehicle (PBS + 0.1% low endotoxin BSA) or 10 μg of murine recombinant eotaxin (carrier-free mr eotaxin; R & D Systems, Minneapolis, MN) in 50 μL volume, under Metofane (Mallinckrodt Veterinary Inc., Mundelein, IL) anesthesia. In some experiments, oral gavage was used to administer vehicle (0.25% methylcellulose) or test compound 30 min before the eotaxin challenge. Six hours following eotaxin administration, bronchoalveolar lavage (BAL) was performed with 1 mL of PBS (Ca^{2+}/Mg^{2+}-free) containing 10 mM EDTA (lavage buffer). BAL samples were centrifuged (320*g*, 10 min) and resuspended in 200 μL of lavage buffer containing 0.1% BSA (resuspension buffer). An aliquot of the cell suspension was used for total counts, performed using a Neubauer hemacytometer, and for cytospin preparations used to count differentials after Wright's Giemsa staining (Sigma, St. Louis, MO).

4. Aerosol Antigen Challenge. OVA-sensitized C57/Bl 6 mice (11−12 per group) were placed in a plexiglass pie chamber connected to a nebulizer (Pari Respiratory Equipment, Midlothianm, VA) driven by compressed air. Mice were exposed to an aerosol of either vehicle (saline containing 0.01% Tween 20) or 1% OVA for 30 min. Mice were treated with either vehicle (0.25% methycellulose) or test compound every 8 h, beginning at 24 h prior to aerosol challenge. The fourth dose was administered 30 min prior to OVA challenge, and the mice received two more doses at 8 h intervals following OVA challenge during the course of the experiment. BAL was performed 24 h following OVA challenge as described above. The BAL samples were centrifuged (320*g*, 10 min) and resuspended in 500 μL of resuspension buffer. Aliquots of the cell suspension were used for total counts, performed using a Technicon H1E automated hematology analyzer (Bayer Diagnostics, Tarryton, NY), and for differential counts on Wright's Giemsa-stained cytospin preparations.

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