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Article

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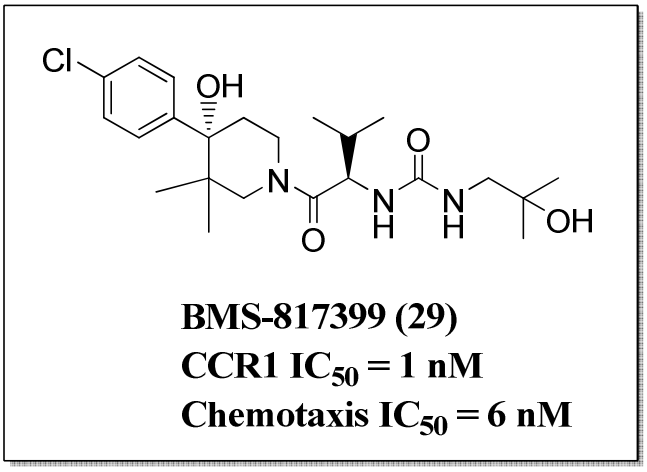
Graphical Abstract

ABSTRACT: High affinity, functionally potent, urea-based antagonists of CCR1 have been discovered. Modulation of PXR transactivation has revealed the selective and orally bioavailable CCR1 antagonist BMS-817399 (**29**), which entered clinical trials for the treatment of rheumatoid arthritis.



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INTRODUCTION.

CCR1 (CC Chemokine Receptor-1) is a G-protein coupled receptor (GPCR) that is expressed on the surface of monocytes/macrophages, osteoclasts, T-cells, and neutrophils.[1] Eleven different chemokines, including MIP-1α (CCL3), RANTES (CCL5), and Leukotactin-1 (CCL15), interact with this receptor to induce leukocyte activation and migration, processes that are critical for the progression of inflammatory diseases.[2]

Pre-clinical studies in rodents suggested that CCR1 blockade represents a viable strategy for the development of therapies for inflammatory disorders.[3] Several small molecule antagonists of CCR1 have

appeared in the literature[4,5] and a number have advanced to human clinical trials (Figure 1).[6] These include BX-471 (**1**)[7] for multiple sclerosis[8], CP-481715 (**2**)[9] for rheumatoid arthritis (RA)[6,10,11], AZD-4818 (**3**)[12] for chronic obstructive pulmonary disease[13], and MLN-3897 (**4**)[14] for RA.[15] Unfortunately, these molecules failed to achieve their targeted clinical endpoints in Phase 2 studies.

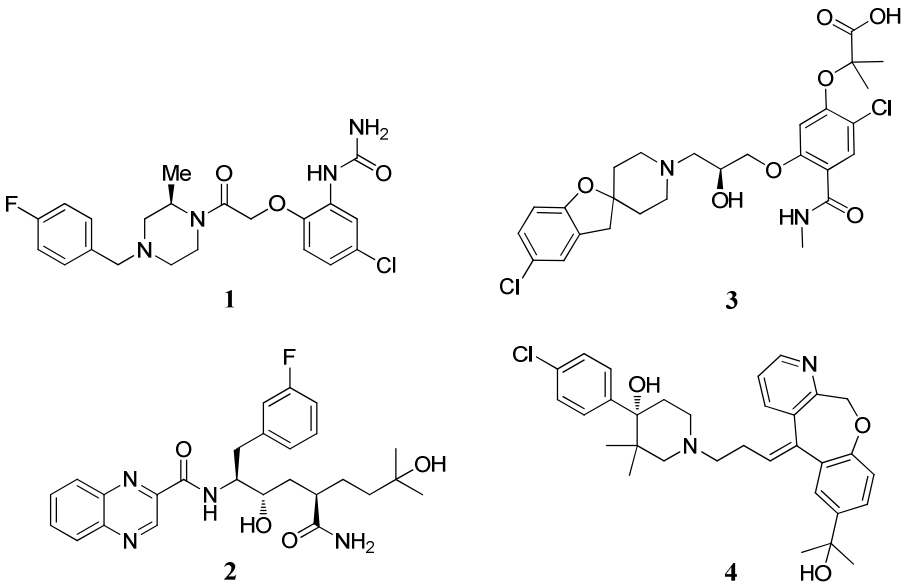


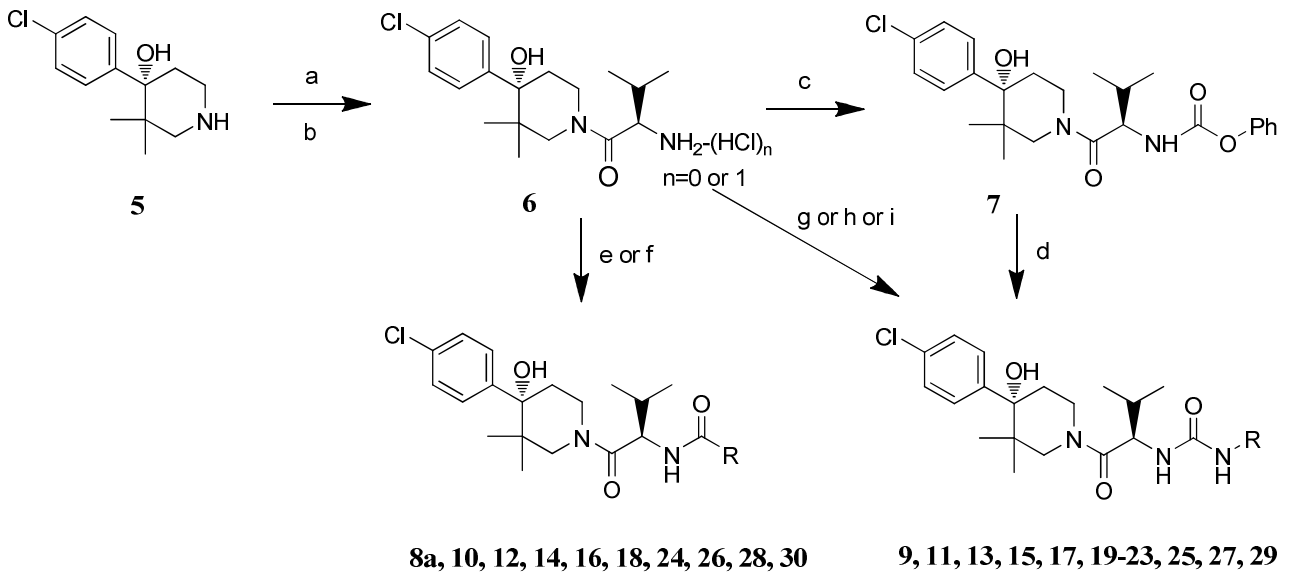
Figure 1. Structures of CCR1 antagonists with reported data from Phase 2 clinical trials.

In spite of earlier Phase 2 clinical failures, CCR1 has continued to be implicated in maintaining the population of inflammatory cells within the synovial tissues and fluid of joints in human RA patients.[10,16] A number of authors have suggested that the Phase 2 clinical failures were due to inadequate blockade of the CCR1 receptor over the course of the trial.[2b,16] One molecule countering that trend is the CCR1 antagonist CCX354[17] (structure unknown), which recently showed clinical efficacy in a three month Phase 2 RA study.[18,19,20] We have also been exploring CCR1 antagonists and have previously reported on our efforts leading to BMS-457 (**8a**).[21,22] We now report the discovery of BMS-817399 (**29**), which entered Phase 2 human clinal trials for the treatment of rheumatoid arthritis.

CHEMISTRY

The syntheses of the CCR1 antagonists in this report are shown in Scheme 1. (*S*)-4-(4-Chlorophenyl)-3,3-dimethylpiperidin-4-ol[23] (**5**) was coupled with BOC-D-valine followed by acid

Scheme 1. Synthesis of amide and urea CCR1 antagonists.[a]



[a](a) BOC-D-Val, HOBT, EDC, TEA, DCM, rt, 24h. (b) 4N HCl in dioxane, dioxane, 2h, rt, 44%. (c) PhO(CO)Cl, TEA, DCM, 1.5h, 0 ^{o}C, 53%. (d) RNH_2, TEA, MeCN, 20-30 min. microwave, 100 ^{o}C. (e) RCOOH, EDC, HOBT, DIEA, DCM and/or DMF, rt, 1-24h. (f) free base of **6** (obtained via chiral normal-phase HPLC), α,α-dimethyl-γ-butyrolactone, neat, 120 ^{o}C, 6h, 30%. (g) RNH-(CO)-OPh, TEA, MeCN, microwave, 100 ^{o}C, 1h then 150 ^{o}C, 1h. (h) 1-pyrrolidinecarbonyl chloride, TEA, DCM, rt, 20h, 86%. (i) RNCO, TEA, THF, rt.

deprotection to yield valine amide **6**. This could be either coupled with carboxylic acids to yield amides **8a**, **10**, **12**, **14**, **16**, **18**, **24**, **26**, **28**, and **30** or converted to carbamate **7** then reacted with amines to yield ureas **9**, **11**, **13**, **15**, **17**, **19, 22**, **25**, **27**, and **29**. The coupling partners can be reversed so that phenyl carbamates of amines, such as *R*- or *S*-*sec*-butylamine, were reacted with valine amide **6** to yield ureas **20** and **21**. Reaction of **6** with an aminecarbonylchloride such as pyrrolidinylcarbonylchloride yielded urea **23**. Amide **30** was obtained by reacting the free base of **6** (obtained via chiral normal-phase HPLC) with α,α-dimethyl-γ-butyrolactone neat at 120 ^{o}C.

RESULTS AND DISCUSSION

We have previously disclosed the discovery of **8a** (Figure 2), a potent CCR1 antagonist nominated for advanced pre-clinical development.[22] While this compound fulfilled the advancement criteria (excellent target affinity, functional potency, selectivity, *in vitro* safety, and pharmacokinetics), mild QT prolongation[24] was observed in a rabbit cardiovascular safety model. This was later confirmed in a conscious telemetrized dog study, thereby halting the development of this compound. Additionally, *in vivo* conversion to ketone (**8b**, Figure 2) was observed in rats. This ketone displays increased pregnane X receptor (PXR) transactivation. Increased PXR transactivation leads to upregulation of CYP3A4 in an attempt by the body to clear foreign substances, including drugs, via their metabolism. Thus increased PXR transactivation can potentially lead to undesirable drug-drug interactions.[25] Although ketone **8b** was not initially observed in significant concentrations in human, rat, and mouse liver microsomes, incubation of **8a** at higher concentrations revealed more significant conversion to **8b** (data not shown).

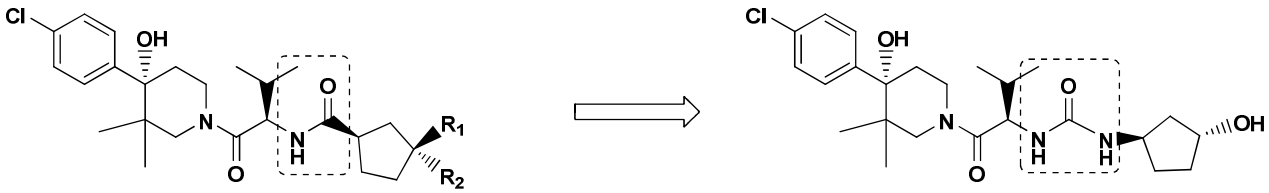


Figure 2. Replacement of the rightmost amide of preclinical candidate **8a** with a urea.

To seek out new SAR in an effort to circumvent these cardiovascular and metabolism issues, we replaced the rightmost amide of **8a** with a variety of differently substituted ureas yielding compounds such as **9**. We hypothesized that the more polar urea could modulate the liabilities associated with **8a** while also providing an additional conformational restriction of the terminal substituent. Fortunately, the ureas were often equipotent to their amide counterparts in both the binding and chemotaxis assays. For

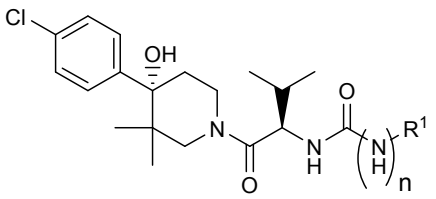
example, phenylurea **11** was equipotent with phenylamide **10** with respect to CCR1 binding affinity (Table 1, IC_{50} column).[22] Likewise, cyclopentylurea **13** was equipotent with cyclopentylamide **12** with respect to both binding affinity and chemotaxis inhibition. The metabolic stability of compounds in mouse, rat, and human liver microsomes was determined in order to see whether there is the potential to a) form undesired potentially toxic or pharmacologically inactive metabolites due to phase I metabolism or b) to accumulate in the body due to lack of metabolism. Liver microsome stability is therefore a tool which can to some extent predict the metabolic fate of compounds and is expressed as % remaining intact after incubation with the microsomes. Both cyclopentylamide **12** and cyclopentylurea **13** exhibited poor liver microsome stability (see Table 1, column entitled "% remaining in liver microsomes"). From experience, we knew that a single hydroxyl group could have a powerful effect on metabolic stability.[26,27] The addition of a 3-hydroxyl group to amide **12** yielded four diastereomeric cyclopentanol amides (**8a**, **14**, **16**, and **18**) of which two showed a dramatic improvement in liver microsome stability (**8a** and **14**) while the other two only moderate (**16** and **18**).[22] Unfortunately, **8a** and **14** were converted to the ketone *in vitro* when assayed at higher concentrations in liver microsomes (data not shown). We synthesized the four diastereomers of 3-hydroxycyclopentylurea, namely **9**, **15**, **17**, and **19.** The ureas were once again essentially equipotent with their corresponding amides with regards to binding affinity as well as in the inhibition of THP-1 cell chemotaxis, with the only marginal outlier being urea **9**. Three hydroxycyclopentylureas showed a dramatic improvement in liver microsome stability (**9**, **15**, and **19**) over cyclopentylurea **13,** while **17** showed only a moderate improvement. Although metabolically more stable in liver microsomes than **13**, cyclopentylurea **9** was less potent in inhibiting chemotaxis while **15** and **19** displayed PXR transactivation activity (**15**: EC_{50} = 5.7 μM; **19**: EC_{50} = 19 μM; these micromolar EC_{50} values, even though weak, are considered to show some transactivation activity). Thus, none of the 3-hydroxycyclopentylureas were progressed further.

We synthesized a wide variety of urea analogs at R_1, only some of which are listed in Table 1 (compounds **20**-**23**). Many of these urea analogs at R_1 yielded good CCR1 binding affinity.

Unfortunately, many analogs, including **20** – **23**, displayed PXR transactivation activity and/or human liver microsomal instability.

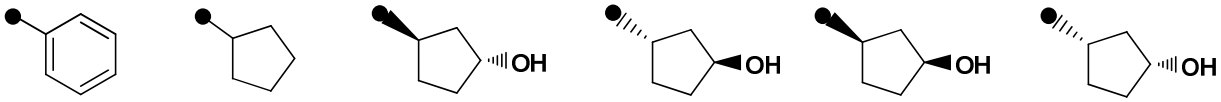
The published X-ray crystal structure of PXR revealed that it has a large binding pocket that allows it to interact with a wide range of hydrophobic substrates.[28,29] The addition of a polar hydroxyl group to cyclopentane yielded mixed results, inhibiting PXR transactivation activity in only four (**8a**, **9**, **14**, and **17**) out of eight compounds (**8a**, **9**, **14-19**) (Table 1). We continued to apply this strategy to acyclic alkyl amides and ureas and the results are shown in Table 2. Surprisingly, *S*-isopropanol urea **25** had a more potent binding IC_{50} than its corresponding amide **24**. In addition, urea **25** did not induce PXR transactivation and displayed respectable liver microsome stability. Compound **25**, however, could potentially have its secondary hydroxyl group metabolized to the ketone. For the *R*-isopropanol series, both amide **26** and urea **27** displayed equipotent CCR1 binding affinities. However, urea **27**, did not show any PXR transactivation activity, reminiscent of the lack of observed PXR transactivation activity with urea **25**. The strategy apparently worked with the additional polar hydroxyl group, eliminating the PXR transactivation activity for the ureas. To alleviate the risk for secondary alcohol metabolism, we converted urea-isopropanols **25** and **27** to the achiral tertiary alcohol **29** via the substitution of a single methyl group. Compound **29** exhibited CCR1 binding affinity and chemotaxis inhibition potencies of 1 and 6 nM, respectively, while displaying no PXR transactivation up to 25 μM. Furthermore, tertiary alcohol **29** did not exhibit the metabolic instability issues in liver microsomes seen with secondary alcohols **8a** and **14** (Table 1). Compound **29** also demonstrated generally favorable pharmacokinetic (PK) profiles in all species tested. The calculated oral bioavailabilities (F%) of **29** when dosed as a solution in the mouse, rat, dog and cynomolgus monkey were 79%, 46%, 100% and 40%, respectively (Figure 3).

Table 1. Binding and Chemotaxis Data for Ureas versus Amides.



amide: n=0
urea: n=1

R_1 =



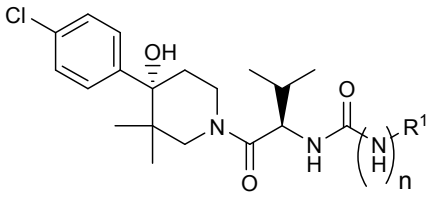
10, 11 **12, 13** **8a, 9** **14, 15** **16, 17** **18, 19**

20 **21** **22** **23**

Compound	n	CCR1 binding affinity[a] IC_{50} (nM)	Inhibition of MIP-1α-induced chemotaxis[a] IC_{50} (nM)	Inhibition of PXR trans-activation[a,d] A: EC_{20}(μM) B: EC_{50}(μM)	% remaining in liver micro-somes (h,r,m)[e]	Issues
10[22]	0	18	10±5 (2)	A, 1.1	N.A.[f]	Potency
11	1	20±18 (2)	N.D.[b]	N.D.	N.D.	Potency
12[22,g]	0	1±0.5 (177)	3±2 (72)	A, 0.5	47,50,26	PXR, metstab[c]
13	1	2±0.5 (2)	3±0 (2)	A, 7.1	77,57,68	PXR, metstab
8a[22]	0	2±0.6 (4)	2±0.1 (2)	A, 41	98,87,88	ketone formation
9	1	4	19±8 (4)	B, >50	100,84,100	chemotaxis
14[22]	0	6	12±3 (2)	A, >50	95,73,90	ketone formation
15	1	3	10±3 (2)	B, 5.7	99,96,100	PXR
16[22]	0	1±0.2 (2)	3±2 (2)	A, 5.3	43,75,60	PXR, metstab
17	1	2	4±1 (2)	B, >50	76,75,86	metstab
18[22]	0	2	1±0.1 (2)	A, 1.8	67,67,65	PXR, metstab
19	1	3	6±3 (2)	B, 19	92,83,100	PXR
20	1	3	6±0.3 (2)	A, 5.2	59,85,72	PXR, metstab
21	1	2	2±0.3 (2)	A, 8.7	65,89,87	PXR, metstab
22	1	1	N.D.	A, 2.2	3,1,2	PXR, metstab
23	1	9	N.D.	A, 0.8	2,11,19	PXR, metstab

[a]Values without standard deviations represent a single determination. Values in parentheses represent number of determinations. Assay variability was measured using compound **12** as an internal standard with <50% variability for the CCR1 binding assay (n = 177) and <66% for the chemotaxis inhibition assay (n = 72). [b]Not done. [c]metstab = metabolic instability in human, rat, and/or mouse liver microsomes. [d]Listing of both EC_{20} and EC_{50} values denotes a change in the assay reporting format during the course of the work. [e]Compounds were incubated at 37 °C for 10 minutes in the presence of human (h), rat (r), and mouse (m) liver microsomes. Substrate concentration = 3 μM. [f]Not available.

Table 2. Hydroxyl-substituted aliphatic amides and ureas.



amide: n=0
urea: n=1

Compound	n	R^1	CCR1 binding affinity[a] IC_{50} (nM) (n)	Inhibition of MIP-1α-induced chemotaxis[a] IC_{50} (nM) (n)	Inhibition of PXR trans-activation[a], EC_{50} (μM)	% remaining in liver micro-somes (h,r,m)[d]	Issues
24	0	S-$CH_2CH(OH)CH_3$	11	N.D.[b]	N.D.	N.D.	potency
25	1	S-$CH_2CH(OH)CH_3$	2	7±2 (2)	>25	88,79,95 (0.5 μM)	metstab[c]
26	0	R-$CH_2CH(OH)CH_3$	2	N.D.	7.8	87,79,85	PXR, metstab
27	1	R-$CH_2CH(OH)CH_3$	2±0.4 (6)	6±2 (3)	>25	88,78,86 (0.5 μM)	metstab
28	0	$CH_2C(OH)(CH_3)_2$	3	N.D.	2.3	100,84,100	PXR
29	1	$CH_2C(OH)(CH_3)_2$	1±0.4 (6)	6±2 (8)	>25	100,74,86 (0.5 μM)	**NONE**
30	0	$CH_2CH_2C(OH)(CH_3)_2$	2±0.3 (3)	22±12 (2)	25	91,35,74 (0.5 μM)	chemotaxis, PXR

[a]Values without standard deviations represent a single determination. Values in parentheses represent number of determinations. Assay variability was measured using compound **12** as an internal standard with <50% variability for the CCR1 binding assay (n = 177) and <66% for the chemotaxis inhibition assay (n = 72). [b]Not done. [c]metstab = metabolic instability in human, rat, and/or mouse liver microsomes. [d]Compounds were incubated at 37 °C for 10 minutes in the presence of human (h), rat (r), and mouse (m) liver microsomes. Substrate concentration = 3 μM unless specified as 0.5 μM.

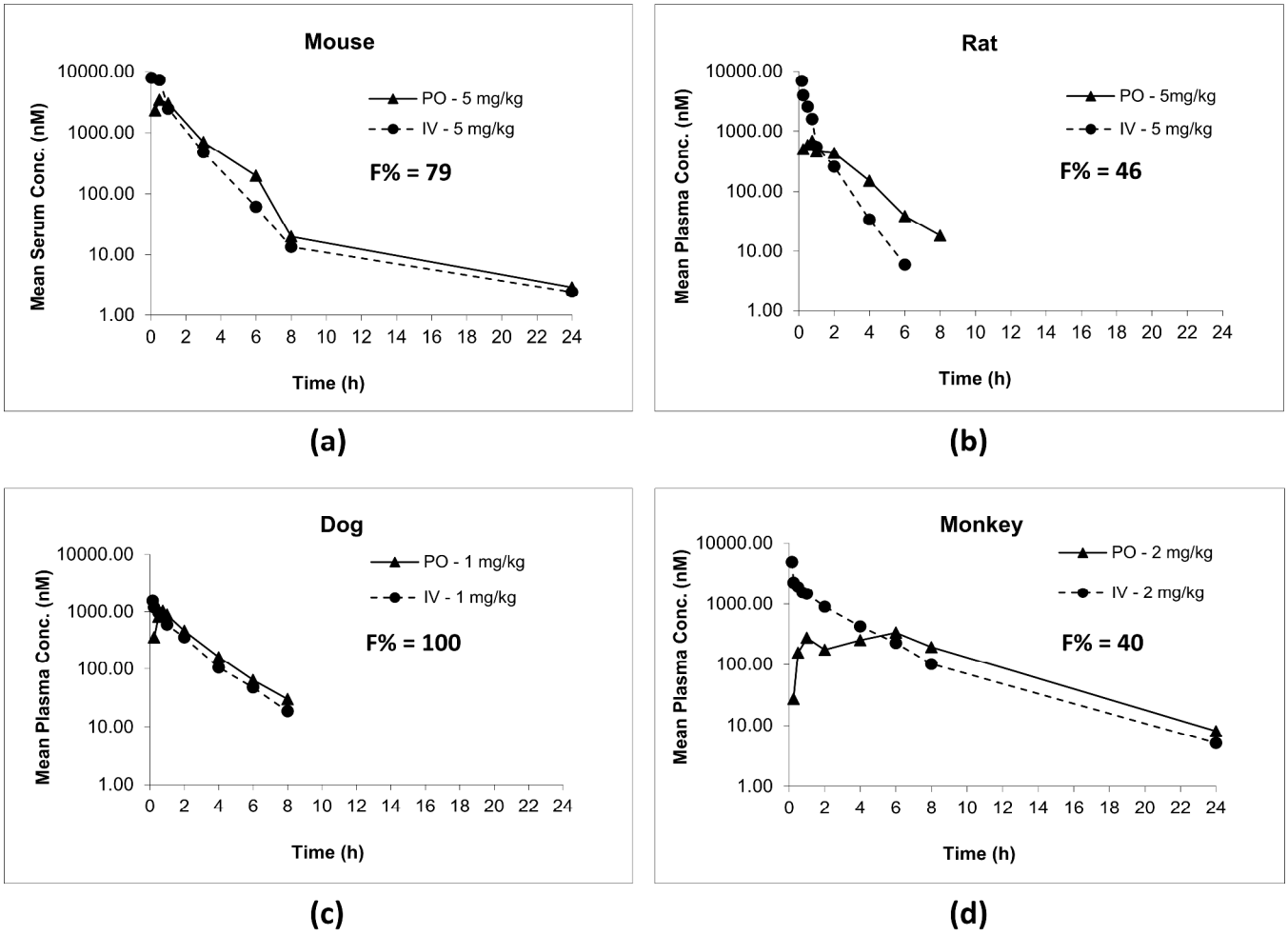


Figure 3. Intravenous (i.v.) and oral (p.o.) PK curves together with bioavailabilities (%F) for compound **29** in the (a) mouse, (b) rat, (c) dog, and (d) cyno.

Compound **29** binds to human peripheral blood mononuclear cells (PBMCs) with an IC_{50} = 4.2 ± 0.9 nM using ^{125}I-hMIP-1α as the ligand. Again, using ^{125}I-hMIP-1α as the ligand, compound **29** did not bind to mouse, rat (both non-PBMC), and monkey (PBMC) CCR1 (IC_{50}s > 50,000 nM each) and only bound marginally to dog CCR1 (PBMC) with an IC_{50} = 635 ± 785 nM (n=4). Most compounds in the program displayed selectivity only for human CCR1 (data not shown) due most likely to reduced CCR1 homology amongst different species.[30]

In addition to MIP-1α (CCL3), compound **29** potently inhibited chemotaxis induced by other CCR1 ligands. These data are summarized in Table 3.

Table 3. Inhibition (IC_{50}, nM) of different chemokine-induced chemotaxis by compound 29.

MIP-1α CCL3	RANTES CCL5	MCP-3 CCL7	HCC-1 CCL14a	LKN-1[c] CCL15	HCC-4 CCL16	MPIF-1[b] CCL23
5.5± 2.4 (8)[a]	3.3± 1.5 (2)	9.3± 2.8 (2)[b]	7.5± 0.1 (2)	16.2± 6.0 (6)	3.2± 2.3 (2)	14.3± 4.6 (2)

[a]Number in parentheses = n. [b]Based on 50% of a Ymax of 60%. [c]Δ-24 N-truncated forms.

CCR1 activation stimulates a variety of responses that include chemotaxis and cellular adhesion mediated partly via the up-regulation of the β_2 integrin receptor, CD11b.[31] Compound **29** blocked MIP-1α- and LKN-1-mediated up-regulation of CD11b in human whole blood with IC_{50} values of 25 ± 10 nM and 42 ± 8 nM, respectively. This measurement in whole blood defines an early critical event in cellular arrest at the endothelial surface. We infer from these data that compound **29** is not highly protein bound in human blood since the MIP-1α CD11b IC_{50} value is only a few fold different or the same as the IC_{50} values for binding and chemotaxis in the presence of MIP-1α (1 and 6 nM, respectively, Table 2). Actual protein binding measurements showed that it is 19 % free in human serum.

As mentioned earlier, several authors believe failure of CCR1 antagonists in the clinic was possibly due to inadequate blockade of the CCR1 receptor over time. The oral bioavailabilities of compound **29** in the species tested should enable coverage of multiples of the human whole blood functional CD11b IC_{90} (200 nM) over 24 hours in the clinic and results will be reported in the future.

To test the effect of CCR1 antagonism on chemotaxis driven by synovial fluid (SF) from patients with RA, we obtained human SF samples and confirmed that they were chemotactic for THP-1 cells, giving a window similar to that elicited by commercial CCR1 ligands. Compound **29** completely inhibited

chemotaxis in 8/14 samples. The mean IC_{50} for the eight samples was 12.9 ± 7.1 nM. In the remaining 6 samples, inhibition was either absent (1/14) or incomplete (5/14) at a maximum compound concentration of 100 nM. This may be due to the presence of chemotactic agents that act independently of CCR1.

Compound **29** did not bind to other CC family receptors, including CCR2, CCR3, CCR4, CCR5, CXCR2, CXCR3, and CXCR5. Nor did compound **29** bind to a broad panel of other G-protein coupled receptors and biogenic amine transporters. Advanced counter screen profiling did not reveal any significant safety (hERG, Na^+, Ca^{2+} channel), or drug-drug interaction (cytochrome P450 enzyme) issues. Because of its potency in the various assays described heretofore and its clean counterscreen profile, compound **29** was advanced into extensive pre-clinical safety evaluation prior to human clinical trials. This compound entered Phase II testing in rheumatoid arthritis patients.

Rationale for PXR Selectivity

A 2.8Å resolution X-ray crystal structure of amide **28** in PXR[32] (Figure 4A) showed that the piperidine amide carbonyl and hydroxyl are forming hydrogen bonds with Q285 and H407, respectively. In addition to these direct interactions, the piperidine amide carbonyl is H-bonded to S247 through a bridging water. An intramolecular hydrogen bond is also observed between the isopropanol OH and the isopropyl amide NH. This stabilizes a compact ligand conformation that is compatible with the PXR ligand binding site. A similar scenario can be invoked for amide **30** when overlapped onto amide **28** in the observed binding site of PXR. A compact conformation can be achieved when the isopropanol OH is hydrogen bonded to the bridging water with only the terminal isopropanol groups being slightly shifted from one another to enable accomodation (Figure 4B). However, the rigidity of the urea linker in **25**, **27** and **29** forces these compounds into a more extended conformation that is predicted to cause steric clashes within the PXR binding site. An alignment of the most similar conformation of urea **29** onto amide **28** yields a steric interaction between one of the isopropanol *gem*-dimethyls and PXR's binding pocket, in

particular with F288 (Figure 4C). Furthermore, all of the lowest energy rotational conformers for both the *cis*- and *trans*-urea isomers of compound **29** displayed steric interactions with PXR's binding pocket (Figure 5). Thus there is no low energy urea conformation available to **29** that avoids a steric clash with the PXR binding site while maintaining the polar interactions with H407 and Q285. The urea moieties of compounds **25** and **27** would likely mimic the steric clashes within the PXR binding site of compound **29**.

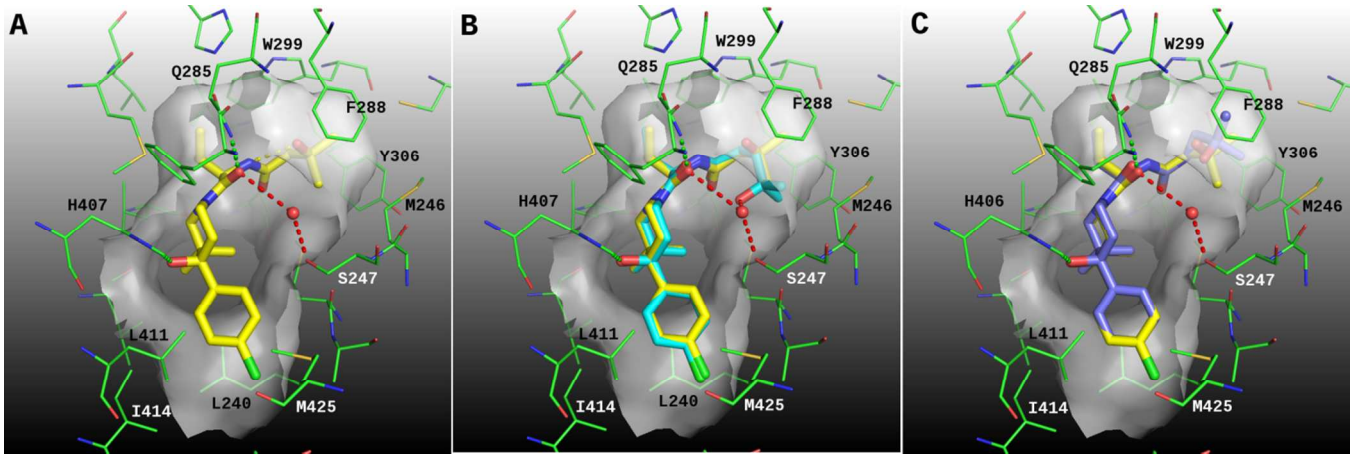


Figure 4. (A) Crystal structure of compound **28** in PXR. Compound **28** is represented in yellow tubes, and PXR residues within 4Å are green lines. The binding site is shown as a surface view with the surface of the front most residues turned off for clarity. Hydrogen bonds to H407 and Q285 are shown as green dashed lines, hydrogen bonds to the bridging water (red sphere) are shown as red dashed lines and the intramolecular hydrogen bond as a yellow dashed line. (B) Predicted binding mode of compound **30** (cyan) in PXR. Hydrogen bonds are colored as above. (C) Superposition of the best fitting conformation of compound **29** onto compound **28** from the PXR crystal structure. Steric clashes with F288 that block binding of compound **29** can be seen by the penetration of the gem-dimethyl moiety through the surface.

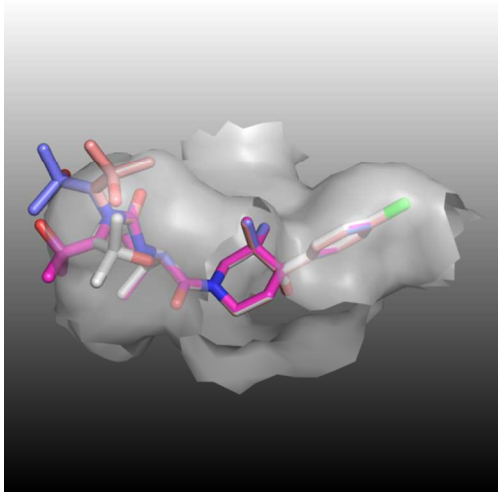


Figure 5. Surface view of the PXR ligand binding site with low energy conformations of compound **29** superimposed. The low energy *cis*-urea conformations are shown in white and salmon sticks. The low energy *trans*-ureas are shown as magenta and purple. No conformation is completely enclosed within the surface, indicating the presumed steric clashes between the urea and the PXR binding site.

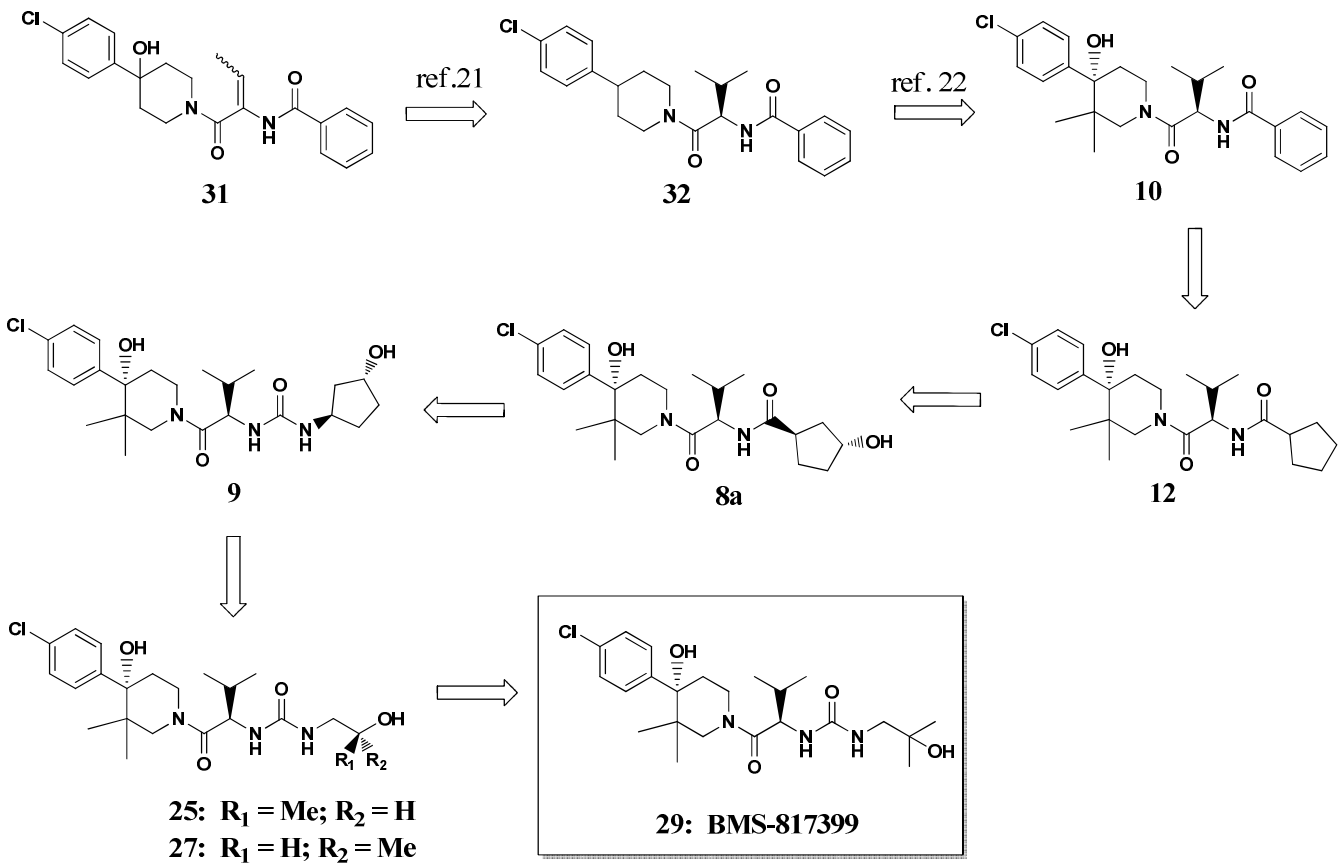
Conclusion.

The entire sequence of events leading to the discovery of compound **29** is shown in Scheme 2. Beginning with lead **31**, which was obtained from screening our corporate compound collection, it was discovered that the D-valine linker is optimal as shown in benzamide **32**.[21] Replacement of the piperidine with a *gem*-dimethylpiperidinol yielded benzamide **10** with improved chemotaxis inhibition potency.[22] Replacement of the benzamide with an alkyl/cycloalkyl amide (cyclopentane amide **12**) led to yet another increase in binding and chemotaxis inhibition potencies.[22] However, these alkyl/cycloalkyl ureas suffered from metabolic instability in human liver microsomes and exhibited increased PXR transactivation activity. We hypothesized that the addition of a hydroxyl group should increase metabolic stability and decrease PXR transactivation activity. Indeed this proved to be the case with hyroxycyclopentane **8a** compared to its des-hydroxy parent **12**. We were fortunate that replacement of the amide linker with a urea maintained binding potency (**9**) as well as metabolic stability and low PXR transactivation activity. Unfortunately, the hydroxycyclopentylureas could not be progressed. We then turned out attention to the

acyclic alkyl ureas such as **25** which were more potent than their amide counterparts (**24**) with regards to binding affinity. Both acyclic alkyl ureas **25** and **27** displayed a complete lack of PXR transactivation activity unlike their amide counterpart (**26**). Conversion of the secondary alcohols of **25** and **27** to a metabolically more stable and achiral tertiary alcohol via the substitution of a single methyl group resulted in the discovery of clinical candidate BMS-817399 (**29**). The polar hydroxyl group in **29** initially was thought to eliminate PXR activation activity by providing a repulsive interaction with PXR's hydrophobic pocket. We now believe it is the rigidity of the urea linker that induces a more extended conformation in which there is no conformation devoid of steric clashes within the PXR binding site, resulting in selectivity.

In conclusion, SAR development of lead **8a** led us to compound **29**, a potent, selective, and orally bioavailable CCR1 antagonist suitable for clinical development. Additional studies on the structure-activity relationships of compound **8a** led to a different structural solution to the selectivity problems, and these will be the subject of a future report.

Scheme 2.



Experimental Section.

Proton magnetic resonance (^{1}H NMR) spectra were recorded on either a Bruker Avance 400 or a JEOL Eclipse 500 spectrometer and are reported in ppm relative to the reference solvent of the sample in which they were run. HPLC and LCMS analyses were conducted using a Shimadzu SCL-10A liquid chromatograph and a SPD UV-vis detector at 220 nm with the MS detection performed with either a Micromass Platform LC spectrometer or a Waters Micromass ZQ spectrometer. All flash column chromatography was performed on EM Science silica gel 60 (particle size of 40-60 μm). All reagents were purchased from commercial sources and used without further purification unless otherwise noted. All reactions were performed under an inert atmosphere. HPLC analyses were performed using the following conditions. All final compounds had an HPLC purity of ≥ 95% unless specifically mentioned.

HPLC Methods. HPLC analyses and preparatory reverse-phase scale purifications were performed using the following conditions.

Method A. A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (solvent B); t = 0 min, 10% B; t= 15 min, 100% B was employed on a SunFire C18 3.5 μ 3.5 mm x 150 mm column. Flow rate was 0.5 mL/min and UV detection was set to 220 nm. The LC column was maintained at ambient temperature.

Method B. A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (solvent B); t = 0 min, 10%B; t = 15 min, 100%B (20 min.) was employed on a XBridge Ph 3.5 μ 3.0 mm x 150 mm column. Flow rate was 0.5 mL/min and UV detection was set to 220 nm. The LC column was maintained at ambient temperature.

Method C. A linear gradient using 10% methanol, 90% water, 0.1% TFA (solvent A) and 90% methanol, 10% water, and 0.1% TFA (solvent B); t = 0 min, 20%B; t = 14 min, 100%B was employed on a Waters Sunfire C-18 19 mm x 50 mm column. Flow rate was 20 mL/min and UV detection was set to 220 nm. The LC column was maintained at ambient temperature. Fractions containing the product were concentrated *in vacuo* to yield product.

Method D. An isocratic run using a polar organic phase consisting of a 4:1 mixture of methanol/isopropanol with 0.1% diethylamine was employed on a Chiralpak AD-H 5 x 25 cm, 5 μM column. Flow rate was 60 mL/min and UV detection was set to 220 nm. The HPLC column was maintained at 35 ^{o}C. Fractions containing the product were concentrated *in vacuo*.

Method E. A linear gradient using 100% water, 0.05% TFA (solvent A) and 100% acetonitrile and 0.05% TFA (solvent B); t = 0 min, 0%B; t = 30 min, 100%B was employed on a Phenomenex Luna 5μ C-18(2) 21.2 mm x 250 mm column. Flow rate was 15 mL/min and UV detection was set to 220 nm. The LC column was maintained at ambient temperature. Fractions containing the product were lyophilized to yield product.

(R)-2-Amino-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methylbutan-1-one, HCl (6). **Part A.** Synthesis of tert-butyl (R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate. (R)-2-(tert-Butoxycarbonylamino)-3-methylbutanoic acid (BOC-D-Val) (13.16 g, 60.6 mmol, 1.1 eq), EDC (11.61 g, 60.6 mmol, 1.1 eq), and HOBT (9.27 g, 60.6 mmol, 1.1 eq) were mixed in methylene chloride (200 mL) at room temperature with stirring until a clear solution was obtained. This was followed by the addition of (S)-4-(4-chlorophenyl)-3,3-dimethylpiperidin-4-ol[24] (13.2 g, 55.1 mmol, 1.0 eq), followed by triethylamine (9.21 mL, 66.1 mmol, 1.2 eq) via an addition funnel over 5 minutes at room temperature. A slight exotherm was observed. The reaction was stirred overnight. Workup entailed rinsing the organic layer with water (1 X 50 mL), 1N HCl (1 X 50 mL), saturated sodium bicarbonate (1 X 50 mL), and brine (1 X 50 mL). The organic layer was dried over anhydrous sodium sulfate. Concentration under reduced pressure provided 28 grams of a light tan glass. The crude product was purified over silica gel in 9:1 to 3:1 to 1:1 hexanes/EtOAc to 100% EtOAc. tert-Butyl (R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (24 g, 54.7 mmol) was obtained as a white glass. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 - 7.29 (m, 4H), 5.39 (dd, *J*=18.0, 9.2 Hz, 1H), 4.71 - 4.63 (m, 0.5H), 4.57 (td, *J*=9.8, 5.7 Hz, 1H), 4.17 - 4.10 (m, 0.5H), 3.89 (d, *J*=13.1 Hz, 0.5H), 3.68 - 3.55 (m, 1H), 3.39 (d, *J*=13.1 Hz, 0.5H), 3.15 (td, *J*=13.0, 3.0 Hz, 0.5H), 3.05 (d, *J*=13.1 Hz, 0.5H), 2.73 - 2.65 (m, 0.5H), 2.65 - 2.57 (m, 0.5H), 2.03 - 1.87 (m, 1H), 1.69 - 1.60 (m, 2H), 1.58 - 1.51 (m, 0.5H), 1.47 - 1.40 (m, 9H), 1.06 (d, *J*=6.8 Hz, 1H), 0.94 (d, *J*=6.8 Hz, 3H), 0.90 (d, *J*=6.8 Hz, 2H), 0.85 (d, *J*=2.5 Hz, 3H), 0.78 (d, *J*=11.3 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{18}H_{28}ClN_2O_2$ $[M + H]^+$ 439.24. Found, 439.28.

Part B. tert-Butyl (R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (from part A) (24 g, 54.7 mmol, 1 eq) was dissolved in dioxane (50 mL) at room

temperature with stirring. To this solution at rt was added 4N HCl in dioxane (27.3 mL, 109 mmol, 2 eq) dropwise via an addition funnel over 5 minutes. After 4 hours, LCMS detects about 80% completion. Another one equivalent of 4N HCl in dioxane was added thereto. After 2 additional hours, the reaction was evaporated 5 times from methylene chloride to yield crude product as a white glass. The glass was stirred in 200 mL of acetone overnight. The precipitate was filtered and redissoved in methanol, followed by the addition of ethyl ether and the mixture was evaporated to yield a white amorphous glass which was subsequently dried under high vacuum for 8 hours. Product (R)-2-amino-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methylbutan-1-one, HCl (10.0 g, 26.6 mmol, 44% yield over two steps) was obtained as an amorphous white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (d, *J*=8.8 Hz, 1H), 7.49 (d, *J*=8.8 Hz, 1H), 7.33 (d, *J*=8.8 Hz, 2H), 4.62 - 4.53 (m, 0.5H), 4.39 (dd, *J*=12.3, 4.4 Hz, 1H), 4.04 (dd, *J*=13.0, 0.5 Hz, 1H), 3.88 (d, *J*=13.2 Hz, 0.5H), 3.78 - 3.72 (m, 0.5H), 3.71 - 3.62 (m, 2H), 3.61 - 3.55 (m, 0.5H), 3.28 – 3.21 (m, 0.5H), 3.21 – 3.15 (m, 0.5H), 2.82 - 2.60 (m, 1H), 2.35 (dq, *J*=11.4, 6.9 Hz, 0.5H), 2.27 - 2.13 (m, 0.5H), 1.63 (d, *J*=14.1 Hz, 0.5H), 1.56 (d, *J*=13.6 Hz, 0.5H), 1.22 (d, *J*=7.0 Hz, 1.5H), 1.10 (t, *J*=7.7 Hz, 3H), 1.01 (d, *J*=7.0 Hz, 1.5H), 0.89 - 0.80 (m, 4.5H), 0.77 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_1H_2ClN_2O_2$ $[M + H]^+$ 339.18. Found, 339.24.

Phenyl (R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (7). (R)-2-Amino-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methylbutan-1-one, HCl (**6**) (1.0 g, 2.66 mmol, 1 eq) was added to methylene chloride (15 mL) with stirring at rt under nitrogen. Triethylamine (0.743 mL, 5.33 mmol, 2 eq) was added and the colorless solution was cooled to 0 °C. To this was added a methylene chloride solution of phenyl chloroformate (0.417 g, 2.66 mmol, 1 eq) dropwise via an addition funnel over 5 minutes. After 1 hour, LCMS showed the reaction to be about 90% complete. An additional 0.2 equivilents of TEA were added followed by 0.2 equivalents of phenyl chloroformate at 0 °C. The organic mixture was rinsed with 1N HCl (1 x 15 mL),

followed by saturated aqueous sodium carbonate (1 x 15 mL) and brine (1 x 15 mL). The organic layer was dried over anhydrous sodium sulfate. The mixture was filtered and the solvent removed *in vacuo* to provide a colorless oil. The oil was stirred at rt with 20 mL of 1:1 acetonitrile/heptane mixture and the precipitate then filtered and dried yielding phenyl (R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (650 mg, 1.42 mmol, 53% yield) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 - 7.45 (m, 2H), 7.40 - 7.28 (m, 4H), 7.25 - 7.17 (m, 1H), 7.12 (d, *J*=8.6, Hz, 1H), 7.08 (d, *J*=8.6, Hz, 1H), 4.60 (m, 1H), 4.07 (d, *J*=13.0 Hz, 1H), 3.70 - 3.59 (m, 1H), 3.48 (d, *J*=13.1, Hz, 1H), 3.25 - 3.18 (m, 0.5H), 3.15 (d, *J*=12.3 Hz, 0.5H), 2.78 - 2.59 (m, 1H), 2.21 (dq, *J*=13.5, 6.8, Hz, 0.5H), 2.09 (dq, *J*=13.6, 6.7 Hz, 0.5H), 1.61 (d, *J*=14.1 Hz, 0.5H), 1.53 (d, *J*=13.6 Hz, 1H), 1.11 (d, *J*=6.8 Hz, 1.5H), 1.07 (d, *J*=6.8 Hz, 1.5H), 1.01 (dd, *J*=6.8, 4.2 Hz, 3H), 0.84 - 0.79 (m, 4.5H), 0.77 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_{25}H_{32}ClN_2O_4$ $[M + H]^+$ 459.21. Found, 459.13.

1-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-(2-hydroxy-2-methylpropyl)urea (29). Phenyl (R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (**7**) (920 mg, 2.004 mmol, 1 eq) was added to acetonitrile (10 mL). To this mixture was then added triethylamine (0.279 mL, 2.004 mmol, 1 eq) and 1-amino-2-methylpropan-2-ol (179 mg, 2.004 mmol, 1 eq). The reaction was heated at 100 °C for 30 minutes in the microwave. Upon cooling, a precipitate formed. Ethyl ether (10 mL) was added and the mixture was stirred for 10 minutes. The precipitate was filtered and the product obtained was dried under high vacuum to yield (638 mg, 1.54 mmol, 70% yield) of a white solid.

^{1}H NMR (500 MHz, CD_3OD) (approx. 1:1 mixture of rotamers) δ 7.47 (dd, *J*=15.4, 8.8 Hz, 2H), 7.31 (dd, *J*=8.5, 5.2 Hz, 2H), 4.71 (dd, *J*=12.1, 6.1 Hz, 1H), 4.54 (ddd, *J*=12.9, 2.5, 2.2 Hz, 0.5H), 4.08 – 3.98 (m, 1H), 3.68 – 3.58 (m,1H), 3.48 (dd, *J*=12.9, 1.4 Hz, 0.5H), 3.21 – 3.13 (m, 1H), 3.14 – 3.06 (m, 2H), 2.70 (td, *J*=13.6, 4.97 Hz, 0.5H), 2.61 (td, *J*=13.5, 5.0 Hz, 0.5H), 2.09 (dq, *J*=13.2, 6.6 Hz, 0.5H), 1.95

(dq, *J*=13.3, 6.7 Hz, 0.5H), 1.60 (ddd, *J*=13.9, 2.5, 2.3 Hz, 0.5H), 1.51 (ddd, *J*=14.2, 2.6, 2.5 Hz, 0.5H), 1.16 (s, 3H), 1.14 (d, J=1.7 Hz, 3H), 1.05 (d, *J*=7.2 Hz, 1.5H), 0.98, (d, *J*=7.2 Hz, 1.5H), 0.94 (d, *J*=6.6 Hz, 1.5H), 0.91 (d, *J*=6.6 Hz, 1.5H), 0.82 (s, 1.5H), 0.81 (s, 1.5H), 0.79 (s, 1.5H), 0.75 (s, 1.5H). ^{13}C NMR (126 MHz, CD_3OD) (mixture of rotamers) δ 173.62, 173.30, 161.14, 160.78, 144.78, 144.62, 133.82 (2 C, s), 130.20 (4 C, s), 128.28 (4 C, s), 76.01, 75.95, 71.70, 71.66, 55.87, 55.22, 55.08, 51.77 (2 C, s), 51.08, 43.01, 40.38, 39.86, 39.29, 34.78, 33.67, 33.12, 32.43, 27.16 (2 C, s), 27.06 (2 C, s), 23.13, 22.79, 21.35, 21.11, 20.32, 19.82, 17.94, 17.71. (LCMS (ESI) m/z Calcd for $C_{23}H_{36}ClN_3O_4$ $[M + H]^+$ 454.25. Found, 454.32. HPLC Purity: 96.8%, t_r = 7.66 min (method A); 99.1%, t_r = 8.23 min (method B).

N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (10). (R)-2-Amino-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methylbutan-1-one, HCl salt (**6**) (30 mg, 0.089 mmol, 1.0 eq), benzoic acid (10.81 mg, 0.089 mmol, 1.0 eq), EDC (20.37 mg, 0.106 mmol, 1.2 eq), HOBT (16.27 mg, 0.106 mmol, 1.2 eq) and N,N-diisopropylethylamine (0.031 mL, 0.177 mmol, 2.0 eq) were mixed in methylene chloride (3 mL) and DMF (0.5 mL) at rt with stirring for 1 h. The product was purified by reverse-phase preparative HPLC (Method C) (t_r = 12.54 min). Product fractions were collected and the solvent removed *in vacuo* to yield N-((R)-1-((S)-4-(4- chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (30 mg, 0.064 mmol, 73% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.87 - 7.80 (m, 2H), 7.59 - 7.44 (m, 5H), 7.32 (d, *J*=8.6 Hz, 1H), 7.29 (d, *J*=8.6 Hz, 1H), 5.11 (d, *J*=7.7 Hz, 0.5H), 5.04 (d, *J*=7.5 Hz, 0.5H), 4.64 - 4.55 (m, 0.5H), 4.21 (d, *J*=11.2 Hz, 0.5H), 4.30 – 4.10 (m, 0.5H), 3.71 - 3.59 (m, 2H), 3.22 (td, *J*=12.9, 3.0 Hz, 0.5H), 3.15 (d, *J*=12.8 Hz,

0.5H), 2.75 (td, *J*=13.5, 4.6 Hz, 0.5H), 2.64 (td, *J*=13.6, 4.8 Hz, 0.5H), 2.35 - 2.25 (m, 0.5H), 2.18 (dq, *J*=14.0, 6.9 Hz, 0.5H), 1.64 (d, *J*=14.3 Hz, 0.5H), 1.53 (d, *J*=13.6 Hz, 0.5H), 1.12 (d, *J*=6.6 Hz, 1.5H), 1.07 (d, *J*=6.6 Hz, 1.5H), 1.00 (d, *J*=6.8 Hz, 3H), 0.84 (d, *J*=10.1 Hz, 3H), 0.79 (d, *J*=5.9 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{25}H_{31}ClN_2O_3$ $[M + H]^+$ 443.21. Found, 443.50. HPLC Purity: 98.0%, t_r = 9.81 min (method A); 99.1%, t_r = 11.24 min (method B).

(R)-1-sec-Butyl-3-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)urea (20).

Part A. Phenyl (R)-sec-butylcarbamate. (R)-Butan-2-amine (1.00 g, 13.7 mmol, 1 eq) was dissolved in acetonitrile (10 mL) at rt under nitrogen with stirring. To this solution was added triethylamine (3.81 mL, 27.3 mmol, 2 eq) and the reaction cooled to 0 ^oC with an ice bath. An acetonitrile solution of phenyl chloroformate (1.72 mL, 13.7 mmol, 1 eq) was then added dropwise via an addition funnel over 5 minutes. A precipitate formed. After 3 hours, the solvent was evaporated followed by the addition of ethyl acetate (10 mL) and water (10 mL). The layers were separated and the ethyl acetate layer was rinsed with 1N HCl (2 x 15 mL), and brine (1 x 15 mL). The organic layer was dried over sodium sulfate and the solvent removed *in vacuo* to yield a colorless oil. The oil was purified over silica gel in 9:1 n-heptane/ethyl acetate. The product (R)-phenyl sec-butylcarbamate (1.20 g, 6.21 mmol) was obtained as a colorless oil which solidified upon standing. ^1H NMR (400 MHz, CD_3OD) δ 7.38 - 7.33 (m, 2H), 7.21 - 7.16 (m, 1H), 7.08 (d, *J*=7.9 Hz, 2H), 3.57 (sxt, *J*=6.7 Hz, 1H), 1.53 (quin, *J*=7.1 Hz, 2H), 1.18 (d, *J*=7.0 Hz, 3H), 0.97 (t, *J*=7.5 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{11}H_{16}NO_2$ $[M + H]^+$ 194.12. Found, 194.22.

Part B. (R)-2-Amino-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methylbutan-1-one, HCl (**6**) (40 mg, 0.107 mmol, 1 eq), (R)-phenyl sec-butylcarbamate (from part A) (20.59 mg, 0.107

mmol, 1 eq), and triethylamine (0.030 ml, 0.215 mmol, 2 eq) were mixed in acetonitrile (3 mL) at rt. The mixture was heated in the microwave at 100 ^{o}C for 1 hour. LCMS showed product and both starting materials to be present. The reaction was transferred to a new microwave tube. Additional triethylamine (0.030 ml, 0.215 mmol, 2 eq) was added and the contents heated once more in the microwave at 150 ^{o}C for 1 hour. The solvent was removed and the residue was purified over silica gel in 3:1 hexanes/EtOAc to 100% EtOAc. The product 1-sec-butyl-3-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)urea (37 mg, 0.084 mmol, 79% yield) was obtained as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.48 (d, *J*=8.6 Hz, 2H), 7.31 (d, *J*=8.6 Hz, 2H), 6.10 (dd, *J*=15.6, 8.6 Hz, 0.5H), 5.96 (dd, *J*=16.5, 9.4 Hz, 0.5H), 4.77 - 4.66 (m, 1H), 4.59 - 4.49 (m, 0.5H), 4.02 (d, *J*=11.4 Hz, 1H), 3.70 - 3.53 (m, 2H), 3.53 - 3.45 (m, 0.5H), 3.22 - 3.15 (m, 0.5H), 3.12 (d, *J*=13.2 Hz, 0.5H), 2.76 - 2.56 (m, 1H), 2.15 - 2.02 (m, 0.5H), 1.94 (dq, *J*=13.2, 6.7 Hz, 0.5H), 1.61 (d, *J*=14.1 Hz, 0.5H), 1.51 (d, *J*=14.1 Hz, 0.5H), 1.48 - 1.36 (m, 2H), 1.29 (br. s., 0.5H), 1.12 - 1.03 (m, 4.5H), 1.00 - 0.84 (m, 8.5H), 0.84 - 0.78 (m, 4.5H), 0.75 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_{23}H_{37}ClN_3O_3$ $[M + H]^+$ 438.25. Found, 438.33. HPLC Purity: 93.4%, t_r = 9.06 min (method A); 95.0%, t_r = 10.19 min (method B).

N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)pyrrolidine-1-carboxamide (23). (R)-2-Amino-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methylbutan-1-one, HCl (**6**) (30 mg, 0.080 mmol, 1 eq), TEA (0.022 mL, 0.160 mmol, 2 eq) and pyrrolidine-1-carbonyl chloride (10.68 mg, 0.080 mmol, 1 eq) were mixed and stirred in methylene chloride (1 ml) at rt for 20 hours. The solvent was removed and the residue was purified over silica gel in 3:1 hexanes /EtOAc to 1:1 hexanes /EtOAc to 100% EtOAc. The product N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)pyrrolidine-1-carboxamide (30 mg, 0.069 mmol, 86 % yield) was obtained as a white solid. ^{1}H NMR (400 MHz,

CD_3OD) (mixture of rotamers) δ 7.45 (d, *J*=8.8 Hz, 1H), 7.43 (d, *J*=8.8 Hz, 1H), 7.28 (dd, *J*=8.3, 4.8 Hz, 2H), 5.62 (d, *J*=8.8 Hz, 1H), 5.54 (d, *J*=8.8 Hz, 1H), 4.78 - 4.72 (m, 0.5H), 4.72 - 4.66 (m, 0.5H), 4.52 (d, *J*=11.0 Hz, 0.5H), 4.10 - 3.97 (m, 1H), 3.65 - 3.55 (m, 1H), 3.49 - 3.43 (m, 0.5H), 3.20 - 3.13 (m, 0.5H), 3.13 - 3.05 (m, 0.5H), 2.73 - 2.54 (m, 1H), 2.14 - 2.02 (m, 0.5H), 2.01 - 1.85 (m, 4.5H), 1.84 - 1.78 (m, 0.5H), 1.58 (d, *J*=14.1 Hz, 0.5H), 1.48 (d, *J*=14.1 Hz, 0.5H), 1.28 – 1.12 (m, 1H), 1.03 (d, *J*=6.6 Hz, 1.5H), 0.96 (d, *J*=6.6 Hz, 1.5H), 0.91 (d, *J*=7.0 Hz, 1.5H), 0.88 (d, *J*=6.2 Hz, 1.5H), 0.78 (d, *J*=6.6 Hz, 3H), 0.74 (d, *J*=7.9 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{23}H_{35}ClN_3O_3$ $[M + H]^+$ 436.24. Found, 436.28. HPLC Purity: 96.9%, t_r = 8.74 min (method A); 96.3%, t_r = 9.77 min (method B).

N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-4-hydroxy-4-methylpentanamide (30). The free base of (R)-2-amino-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methylbutan-1-one, HCl salt, (**6**) was obtained from preparative chiral normal-phase HPLC (t_r = 10.27 min) (Method D). Thus (R)-2-amino-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methylbutan-1-one (40 mg, 0.118 mmol, 1 eq) and 5,5-dimethyldihydrofuran-2(3H)-one (40.4 mg, 0.354 mmol, 3 eq) were mixed together neat and heated in a closed vial behind a safety shield at 120 °C with stirring. The reaction became a dark amber solution during heating. After 6 hours, the heating was stopped. The reaction was evaporated and the residue purified by reverse-phase preparative HPLC (Method C) (t_r = 11.39 min). The product N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-4-hydroxy-4-methylpentanamide (16 mg, 0.035 mmol, 30%) was obtained as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.8 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.32 (d, *J*=8.8 Hz, 1H), 7.31 (d, *J*=8.8 Hz, 1H), 4.81 (d, *J*=7.3 Hz, 0.5H), 4.60 - 4.50 (m, 0.5H), 4.10 (dd, *J*=13.5, 2.1 Hz, 0.5H), 4.03 (dd, *J*=12.7, 1.8 Hz, 0.5H), 3.66 - 3.58 (m, 1H), 3.58 - 3.48 (m, 0.5H), 3.23 - 3.13 (m, 0.5H), 3.10 (d, *J*=12.7 Hz, 0.5H), 2.71 (td, *J*=13.5, 4.8 Hz, 0.5H), 2.61 (td, *J*=13.6, 4.7 Hz, 0.5H), 2.40 - 2.29 (m, 2H),

2.16 (dq, *J*=13.6, 6.8 Hz, 0.5H), 2.09 - 1.95 (dq, J = 13.6, 6.8 Hz, 0.5H), 1.80 – 1.70 (m, 2H), 1.60 (dt, *J*=13.8, 2.4 Hz, 0.5H), 1.52 (dt, *J*=13.8, 2.2 Hz, 0.5H), 1.20 (s, 3H), 1.19 (s, 3H), 1.05 (d, *J*=6.6 Hz, 1.5H), 1.00 (d, *J*=6.8 Hz, 1.5H), 0.93 (d, *J*=6.8 Hz, 3H), 0.83 (s, 1.5H), 0.81 (s, 1.5H), 0.76 (s, 3H). LCMS (ESI) m/z Calcd for $C_{24}H_{38}ClN_2O_4$ $[M + H]^+$ 453.25. Found, 453.28. HPLC Purity: 96.4%, t_r = 7.89 min (method A); 95.0%, t_r = 8.57 min (method B).

1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-phenylurea (11). (R)-2-Amino-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methylbutan-1-one, HCl (**6**) (25 mg, 0.067 mmol, 1 eq) was mixed with THF (2 mL) and methylene chloride (2 ml) at rt. To this mixture was added triethylamine (0.019 ml, 0.133 mmol, 2 eq) followed by phenyl isocyanate (0.015 ml, 0.133 mmol, 2 eq). After 1 hour, the solvent was removed *in vacuo* and the residue taken up in a minimum of THF to solubilize and the mixture purified over silica gel in 3:1 to 1:1 hexanes/EtOAc to 100% THF. The product 1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-phenylurea (9.0 mg, 0.020 mmol, 30 %) was obtained as a white solid. ^{1}H NMR (500 MHz, CD_3OD) (mixture of rotamers) δ 7.50 (d, *J*=8.8 Hz, 1H), 7.47 (d, *J*=8.5 Hz, 1H), 7.38 - 7.28 (m, 4H), 7.27 - 7.21 (m, 2H), 6.97 (q, *J*=7.5 Hz, 1H), 4.60 (s, 1H), 4.04 (d, *J*=11.0 Hz, 1H), 3.73 (t, *J*=6.3 Hz, 1H), 3.66 (d, *J*=13.2 Hz, 1H), 3.51 - 3.43 (m, 1H), 3.19 - 3.15 (m, 1H), 2.04 - 1.98 (m, 0.5H), 1.87 (dt, *J*=6.5, 3.2 Hz, 1H), 1.63 (d, *J*=14.0 Hz, 0.5H), 1.52 (d, *J*=14.8 Hz, 0.5H), 1.25 (t, *J*=7.1 Hz, 3H), 1.11 (d, *J*=6.6 Hz, 1.5H), 1.01 (d, *J*=6.9 Hz, 1.5H), 0.99 (d, *J*=6.6 Hz, 1.5H), 0.94 (d, *J*=6.9 Hz, 1.5H), 0.84 (d, *J*=4.9 Hz, 3H), 0.82 (s, 1.5H), 0.77 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_{25}H_{33}ClN_3O_3$ $[M + H]^+$ 458.22. Found, 458.28. HPLC Purity: 95.2%, t_r = 9.85 min (method A); 95.8%, t_r = 10.93 min (method B).

N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)cyclopentanecarboxamide (12). The title compound was synthesized by the procedure used to make N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (**10**). The crude product was purified via flash chromatography over silica gel in 9:1 to 1:1 Hexanes/EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ 7.42 - 7.38 (m, 1H), 7.37 - 7.29 (m, 3H), 6.33 (d, *J*=8.8 Hz, 0.5H), 6.27 (d, *J*=8.8 Hz, 0.5H), 4.97 (dt, *J*=8.9, 5.4 Hz, 1H), 4.71 - 4.63 (m, 0.5H), 4.13 (dd, *J*=13.2, 1.8 Hz, 0.5H), 3.95 (dt, *J*=13.3, 2.4 Hz, 0.5H), 3.60 (d, *J*=13.6 Hz, 0.5H), 3.42 (dd, *J*=13.4, 1.5 Hz, 0.5H), 3.17 (td, *J*=13.0, 3.1 Hz, 0.5H), 3.06 (d, *J*=13.2 Hz, 0.5H), 2.74 - 2.66 (m, 0.5H), 2.64 (d, *J*=5.7 Hz, 0.5H), 2.62 - 2.53 (m, 1H), 2.12 - 1.66 (m, 7H), 1.45 (d, *J*=13.6 Hz, 0.5H), 1.27 (br. s., 1H), 1.05 (d, *J*=6.6 Hz, 1.5H), 0.94 (dd, *J*=6.6, 3.1 Hz, 3H), 0.90 (d, *J*=7.0 Hz, 2.5H), 0.86 (d, *J*=3.5 Hz, 3H), 0.77 (d, *J*=1.3 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{24}H_{36}ClN_2O_3$ $[M + H]^+$ 435.24. Found, 435.20. HPLC Purity: 92.9%, t_r = 9.66 min (method A); 91.5%, t_r = 11.06 min (method B).

1-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-cyclopentylurea (13). The title compound was synthesized by the procedure used to make 1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-phenylurea (**11**). The crude product was purified via flash chromatography over silica gel in 3:1 to 1:1 hexanes/EtOAc to 100% EtOAc. ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.8 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.16 (d, *J*=8.8 Hz, 1H), 7.31 (d, *J*=8.8 Hz, 1H), 6.26 (dd, J = 15.2, 7.5 Hz, 0.5H), 5.91 (dd, J = 14.6, 9.4 Hz, 0.5H), 4.75 - 4.65 (m, 1H), 4.54 (d, *J*=8.4 Hz, 1H), 4.02 (d, *J*=10.8 Hz, 1H), 3.99 - 3.89 (m, 1H), 3.69 - 3.57 (m, 1H), 3.51 - 3.43 (m, 0.5H), 3.22 - 3.15 (m, 0.5H), 3.15 - 3.08 (m, 0.5H), 2.77 - 2.54 (m, 1H), 2.07 (dd, *J*=12.9, 6.7 Hz, 0.5H), 1.90 (ddd, *J*=18.5, 12.1, 6.6 Hz, 2.5H), 1.76 - 1.47 (m, 5H), 1.38 (dt, *J*=12.2, 6.0 Hz, 2H), 1.05 (d, *J*=6.8 Hz, 1.5H), 0.96 (d, *J*=6.8 Hz, 1.5H), 0.93 (d, *J*=6.8 Hz, 1.5H), 0.89 (d, *J*=6.8 Hz, 1.5H), 0.83 (s, 1.5H), 0.81 (d, *J*=2.6 Hz, 3H), 0.75 (s, 1.5H). LCMS

(ESI) m/z Calcd for $C_{24}H_{37}ClN_3O_3$ $[M + H]^+$ 450.25. Found, 450.36. HPLC Purity: 98.7%, t_r = 9.30 min (method A); 98.9%, t_r = 10.42 min (method B).

(1R,3R)-N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-hydroxycyclopentanecarboxamide (8a). The title compound was synthesized by the procedure used to make N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (**10**). The crude product was purified via flash chromatography over silica gel in 9:1 methylene chloride/ethyl acetate to 4:1 to 1:1 methylene chloride/ethyl acetate to 100% EtOAc to 9:1 ethyl acetate/methanol. ^{1}H NMR (500 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.8 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.31 (dd, *J*=8.8 Hz, 2H), 4.55 (d, *J*=12.6 Hz, 0.5H), 4.38 - 4.31 (m, 1H), 4.09 (d, *J*=13.2 Hz, 0.5H), 4.03 (d, *J*=12.6 Hz, 0.5H), 3.65 - 3.55 (m, 1H), 3.55 - 3.50 (m, 0.5H), 3.21 - 3.14 (m, 0.5H), 3.11 (d, *J*=12.6 Hz, 0.5H), 3.06 - 2.96 (m, 1H), 2.74 - 2.66 (m, 0.5H), 2.66 - 2.57 (m, 0.5H), 2.21 - 2.13 (m, 0.5H), 2.12 - 2.01 (m, 1.5H), 2.00 - 1.67 (m, 4H), 1.67 - 1.56 (m, 1.5H), 1.51 (d, *J*=13.7 Hz, 0.5H), 1.04 (d, *J*=6.6 Hz, 1.5H), 1.00 (d, *J*=6.6 Hz, 1.5H), 0.93 (m, 3H), 0.82 (d, *J*=4.9 Hz, 3H), 0.75 (d, *J*=7.7 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{24}H_{36}ClN_2O_4$ $[M + H]^+$ 451.24. Found, 451.33. HPLC Purity: 99.5%, t_r = 7.68 min (method A); 99.4%, t_r = 8.24 min (method B).

1-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-((1R,3R)-3-hydroxycyclopentyl)urea (9). The title compound was synthesized by the procedure used to make 1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-(2-hydroxy-2-methylpropyl)urea (**29**). The crude product was purified via flash chromatography over silica gel in 1:1 hexanes/EtOAc to 100% EtOAc to 9:1 methylene chloride/MeOH. ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.48 (d, *J*=8.8 Hz, 2H), 7.46 (d,

J=8.8 Hz, 2H), 7.32 (d, *J*=8.8 Hz, 2H), 7.31 (d, *J*=8.8 Hz, 2H), 4.70 (t, *J*=5.3 Hz, 1H), 4.53 (d, *J*=10.8 Hz, 0.5H), 4.30 (d, *J*=4.0 Hz, 1H), 4.23 - 4.11 (m, 1H), 4.02 (d, *J*=12.5 Hz, 1H), 3.62 (d, J=13.2 Hz, 1H), 3.50 - 3.43 (m, 0.5H), 3.22 - 3.15 (m, 0.5H), 3.15 - 3.08 (m, 0.5H), 2.76 - 2.56 (m, 1H), 2.18 - 1.88 (m, 4H), 1.69 - 1.46 (m, 3H), 1.36 (qd, *J*=13.9, 6.6 Hz, 1H), 1.05 (d, *J*=6.6 Hz, 1.5H), 0.95 (dd, *J*=8.7, 6.9 Hz, 3H), 0.89 (d, *J*=6.8 Hz, 1.5H), 0.83 (s, 1.5H), 0.80 (d, *J*=3.1 Hz, 3H), 0.75 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_{24}H_{37}ClN_3O_4$ $[M + H]^+$ 466.25. Found, 466.25. HPLC Purity: 95.3%, t_r = 7.51 min (method A); 95.3%, t_r = 7.91 min (method B).

(1S,3S)-N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-hydroxycyclopentanecarboxamide (14). The title compound was synthesized by the procedure used to make N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (**10**). The crude product was purified via preparative reverse-phase HPLC (Method E). ^{1}H NMR (500 MHz, CD_3OD) (mixture of rotamers) δ 7.51 - 7.44 (m, 2H), 7.34 - 7.28 (m, 2H), 4.55 (d, *J*=12.1 Hz, 0.5H), 4.36 (dt, *J*=5.5, 2.7 Hz, 1H), 4.13 - 4.01 (m, 1H), 3.66 - 3.55 (m, 1H), 3.53 – 3.50 (m, 1H), 3.28 - 3.07 (m, 2H), 3.02 (dq, *J*=16.9, 8.7 Hz, 1H), 2.75 - 2.66 (m, 0.5H), 2.62 (td, *J*=13.5, 4.9 Hz, 0.5H), 2.22 - 2.12 (m, 0.5H), 2.09 - 1.80 (m, 4.5H), 1.79 - 1.57 (m, 2.5H), 1.52 (d, *J*=13.7 Hz, 0.5H), 1.04 (d, *J*=6.6 Hz, 1.5H), 0.99 (d, *J*=7.1 Hz, 1.5H), 0.92 (d, *J*=6.6 Hz, 3H), 0.82 (d, *J*=5.5 Hz, 3H), 0.76 (d, *J*=5.5 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{24}H_{36}ClN_2O_4$ $[M + H]^+$ 451.24. Found, 451.50. HPLC Purity: 95.5%, t_r = 7.61 min (method A); 95.2%, t_r = 8.24 min (method B).

1-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-((1S,3S)-3-hydroxycyclopentyl)urea (15). The title compound was synthesized by the procedure used to make 1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-(2-hydroxy-2-methylpropyl)urea (**29**). The crude product was purified via

flash chromatography over silica gel in 1:1 hexanes/EtOAc to 100% EtOAc to 9:1 methylene chloride/MeOH. ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.52 - 7.44 (m, 2H), 7.31 (dd, *J*=8.3, 4.0 Hz, 2H), 4.84 - 4.66 (m, 1H), 4.65 - 4.50 (m, 1H), 4.30 (dt, *J*=6.4, 3.2 Hz, 0.5H), 4.23 - 3.98 (m, 2H), 3.70 - 3.58 (m, 1H), 3.48 (t, *J*=11.9 Hz, 0.5H), 3.22 - 3.08 (m, 1H), 2.76 - 2.56 (m, 1H), 2.19 - 1.85 (m, 4H), 1.68 - 1.48 (m, 2.5H), 1.38 (dt, *J*=14.9, 6.6 Hz, 1H), 1.24 (t, *J*=7.1 Hz, 0.5H), 1.15 (q, *J*=7.0 Hz, 1.5H), 1.08 - 1.03 (m, 1H), 1.01 - 0.86 (m, 5H), 0.85 - 0.79 (m, 4H), 0.79 - 0.73 (m, 2H). LCMS (ESI) m/z Calcd for $C_{24}H_{37}ClN_3O_4$ $[M + H]^+$ 466.25. Found, 466.37. HPLC Purity: 92.6%, t_r = 7.52 min (method A); 97.9%, t_r = 8.05 min (method B).

(1R,3S)-N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-hydroxycyclopentanecarboxamide (16). The title compound was synthesized by the procedure used to make N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (**10**). The crude product was purified via preparative reverse-phase HPLC (Method E). ^{1}H NMR (500 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.8 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.32 (d, *J*=8.8 Hz, 2H), 7.31 (d, J = 8.8 Hz, 2H), 4.55 (d, *J*=13.2 Hz, 0.5H), 4.25 (sxt, *J*=4.8 Hz, 1H), 4.08 (d, *J*=11.5 Hz, 0.5H), 4.03 (d, *J*=13.2 Hz, 0.5H), 3.66 - 3.59 (m, 1H), 3.55 - 3.49 (m, 0.5H), 3.22 - 3.17 (m, 0.5H), 3.11 (d, *J*=12.6 Hz, 0.5H), 2.85 (dq, *J*=15.3, 7.5 Hz, 1H), 2.71 (td, *J*=13.6, 4.7 Hz, 0.5H), 2.62 (td, *J*=13.3, 4.7 Hz, 0.5H), 2.21 - 2.07 (m, 1.5H), 2.07 - 1.99 (m, 0.5H), 1.98 - 1.85 (m, 2H), 1.83 - 1.69 (m, 3H), 1.61 (d, *J*=13.7 Hz, 0.5H), 1.52 (d, *J*=13.7 Hz, 0.5H), 1.05 (d, *J*=6.6 Hz, 1.5H), 1.00 (d, *J*=6.6 Hz, 1.5H), 0.93 (d, *J*=6.6 Hz, 3H), 0.82 (d, *J*=7.7 Hz, 3H), 0.76 (s, 3H). LCMS (ESI) m/z Calcd for $C_{24}H_{36}ClN_2O_4$ $[M + H]^+$ 451.24. Found, 451.50. HPLC Purity: 97.0%, t_r = 7.92 min (method A); 97.2%, t_r = 8.71 min (method B).

1-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-((1R,3S)-3-hydroxycyclopentyl)urea (17). The title compound was synthesized by the procedure used to make 1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-(2-hydroxy-2-methylpropyl)urea (**29**). The crude product was purified via flash chromatography over silica gel in 1:1 hexanes/EtOAc to 100% EtOAc to 9:1 methylene chloride/MeOH. ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.8 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.32 (d, *J*=8.8 Hz, 1H), 7.31 (d, *J*=8.8 Hz, 1H), 6.45 – 6.00 (m, 0.5H), 4.74 - 4.65 (m, 1H), 4.58 - 4.49 (m, 0.5H), 4.28 - 4.17 (m, 1H), 4.10 - 3.91 (m, 2H), 3.69 - 3.57 (m, 1H), 3.48 (d, *J*=13.0 Hz, 0.5H), 3.22 - 3.16 (m, 1H), 3.11 (d, *J*=13.0 Hz, 1H), 2.76 - 2.57 (m, 1H), 2.27 - 2.17 (m, 1H), 2.07 (dq, *J*=13.0, 6.5 Hz, 0.5H), 2.01 - 1.87 (m, 1.5H), 1.84 - 1.54 (m, 3H), 1.51 (d, *J*=14.1 Hz, 1H), 1.47 - 1.36 (m, 1H), 1.05 (d, *J*=6.6 Hz, 1.5H), 0.95 (dd, *J*=12.7, 6.8 Hz, 3H), 0.90 (d, *J*=6.6 Hz, 1.5H), 0.84 - 0.78 (m, 4.5H), 0.75 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_{24}H_{37}ClN_3O_4$ $[M + H]^+$ 466.25. Found, 466.41. HPLC Purity: 97.2%, t_r = 7.53 min (method A); 100%, t_r = 8.01 min (method B).

(1S,3R)-N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-hydroxycyclopentanecarboxamide (18). The title compound was synthesized by the procedure used to make N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (**10**). The crude product was purified via preparative reverse-phase HPLC (Method E). ^{1}H NMR (500 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.8 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.34 - 7.29 (m, 2H), 4.55 (d, *J*=13.2 Hz, 0.5H), 4.28 - 4.21 (m, 1H), 4.08 (d, *J*=13.2 Hz, 0.5H), 4.03 (d, *J*=12.6 Hz, 0.5H), 3.66 - 3.57 (m, 1H), 3.55 - 3.50 (m, 0.5H), 3.22 - 3.14 (m, 0.5H), 3.11 (d, *J*=12.6 Hz, 0.5H), 2.88 - 2.80 (m, 1H), 2.71 (td, *J*=13.5, 4.4 Hz, 0.5H), 2.62 (td, *J*=13.6, 4.7 Hz, 0.5H), 2.21 - 2.12 (m, 0.5H), 2.12 - 1.86 (m, 3.5H), 1.83 - 1.71 (m, 3H), 1.60 (d, *J*=14.3 Hz, 0.5H), 1.51 (d, *J*=14.3 Hz, 0.5H), 1.05 (d, *J*=7.1 Hz, 1.5H), 1.00 (d, *J*=6.6 Hz, 1.5H), 0.93 (d, *J*=6.6 Hz, 3H), 0.82 (d,

J=7.7 Hz, 3H), 0.76 (s, 3H). LCMS (ESI) m/z Calcd for $C_{24}H_{36}ClN_2O_4$ $[M + H]^+$ 451.24. Found, 451.50. HPLC Purity: 91.8%, t_r = 7.98 min (method A); 95.1%, t_r = 8.80 min (method B).

1-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-((1S,3R)-3-hydroxycyclopentyl)urea (19). The title compound was synthesized by the procedure used to make 1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-(2-hydroxy-2-methylpropyl)urea (**29**). The crude product was purified via flash chromatography over silica gel in 1:1 hexanes/EtOAc to 100% EtOAc to 9:1 methylene chloride/MeOH. ^{1}H NMR (500 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.5 Hz, 1H), 7.47 (d, *J*=8.5 Hz, 1H), 7.32 (d, *J*=8.5 Hz, 1H), 7.30 (d, *J*=8.5 Hz, 1H),6.13 - 6.02 (m, 0.5H), 4.82 (br. s., 0.5H), 4.73 - 4.66 (m, 1H), 4.54 (d, *J*=11.0 Hz, 0.5H), 4.26 - 4.18 (m, 1H), 4.08 - 3.93 (m, 2H), 3.73 (br. s., 0.5H), 3.62 (d, *J*=12.9 Hz, 1H), 3.48 (d, *J*=13.5 Hz, 0.5H), 3.21 - 3.15 (m, 0.5H), 3.11 (d, *J*=12.6 Hz, 0.5H), 2.70 (td, *J*=13.6, 4.7 Hz, 0.5H), 2.66 - 2.57 (m, 0.5H), 2.26 - 2.01 (m, 3H), 2.00 - 1.88 (m, 1.5H), 1.88 - 1.73 (m, 1.5H), 1.73 - 1.55 (m, 2.5H), 1.51 (d, *J*=13.7 Hz, 0.5H), 1.47 - 1.33 (m, 1H), 1.05 (d, *J*=6.6 Hz, 1.5H), 0.97 (d, *J*=6.6 Hz, 1.5H), 0.93 (d, *J*=6.6 Hz, 1.5H), 0.90 (d, *J*=6.6 Hz, 1.5H), 0.83 (s, 1.5H), 0.81 (d, *J*=2.7 Hz, 3H), 0.75 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_{24}H_{37}ClN_3O_4$ $[M + H]^+$ 466.25. Found, 466.26. HPLC Purity: 95.4%, t_r = 7.57 min (method A); 94.5%, t_r = 8.16 min (method B).

(S)-1-sec-Butyl-3-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)urea (21). The title compound was synthesized by the procedure used to make (R)-1-sec-butyl-3-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)urea (**20**). The crude product was purified via flash chromatography over silica gel in 3:1 Hexanes/EtOAc to 100% EtOAc. ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.48 (d, *J*=8.6 Hz, 1H), 7.46 (d, *J*=8.6 Hz, 1H), 7.32 (d, *J*=8.6 Hz, 1H), 7.31 (d, *J*=8.6 Hz, 1H), 6.07 (dd, *J*=15.3, 8.5 Hz,

0.5H), 5.93 (dd, *J*=15.5, 9.1 Hz, 0.5H), 4.75 - 4.67 (m, 1H), 4.58 - 4.50 (m, 0.5H), 4.09 - 3.98 (m, 1H), 3.68 - 3.54 (m, 2H), 3.52 - 3.45 (m, 0.5H), 3.22 - 3.16 (m, 0.5H), 3.15 - 3.07 (m, 0.5H), 2.77 - 2.55 (m, 1H), 2.07 (dq, *J*=13.0, 6.6 Hz, 0.5H), 1.94 (dq, *J*=13.3, 6.7 Hz, 0.5H), 1.61 (d, *J*=14.1 Hz, 0.5H), 1.51 (d, *J*=14.3 Hz, 0.5H), 1.47 - 1.36 (m, 2H), 1.13 - 1.02 (m, 4.5H), 1.00 - 0.84 (m, 8.5H), 0.84 - 0.78 (m, 4.5H), 0.76 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_{23}H_{37}ClN_3O_3$ $[M + H]^+$ 438.25. Found, 438.34. HPLC Purity: 97.5%, t_r = 9.06 min (method A); 98.1%, t_r = 10.18 min (method B).

1-Benzyl-3-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)urea (22). The title compound was synthesized by the procedure used to make 1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-phenylurea (**11**). The crude product was purified by reverse-phase preparative HPLC (Method C) (t_r = 12.63 min). ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.8 Hz, 1H), 7.46 (d, *J*=8.3 Hz, 1H), 7.40 – 7.15 (m, *J*=8.5, 6.8 Hz, 7H), 4.74 (t, *J*=5.7 Hz, 1H), 4.54 (d, *J*=13.6 Hz, 0.5H), 4.32 (s, 1H), 4.30 (s, 1H), 4.03 (d, *J*=11.0 Hz, 1H), 3.68 - 3.59 (m, 1H), 3.51 - 3.45 (m, 0.5H), 3.22 - 3.16 (m, 0.5H), 3.12 (d, *J*=12.7 Hz, 0.5H), 2.71 (td, *J*=13.5, 4.6 Hz, 0.5H), 2.62 (td, *J*=13.5, 5.1 Hz, 0.5H), 2.09 (dq, *J*=13.1, 6.5 Hz, 0.5H), 2.01 - 1.91 (m, 0.5H), 1.61 (d, *J*=14.1 Hz, 0.5H), 1.51 (d, *J*=13.6 Hz, 0.5H), 1.34 - 1.28 (m, 0.5H), 1.06 (d, *J*=6.6 Hz, 1.5H), 0.96 (dd, *J*=9.9, 6.8 Hz, 3H), 0.91 (d, *J*=6.6 Hz, 1.5H), 0.82 (d, *J*=4.0 Hz, 3H), 0.77 (d, *J*=9.2 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{26}H_{35}ClN_3O_3$ $[M + H]^+$ 472.24. Found, 472.00. HPLC Purity: 99.1%, t_r = 9.51 min (method A); 99.6%, t_r = 10.49 min (method B).

(S)-N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-hydroxybutanamide (24). The title compound was synthesized by the procedure used to make N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (**10**). The crude product was purified by reverse-phase preparative HPLC (Method C) (t_r =

10.57 min). ^{1}H NMR (500 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.5 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.31 (dd, *J*=8.5, 5.2 Hz, 2H), 4.91 (d, *J*=6.9 Hz, 0.5H), 4.55 (d, *J*=12.9 Hz, 0.5H), 4.16 - 4.06 (m, 1.5H), 4.03 (d, *J*=12.6 Hz, 0.5H), 3.66 - 3.60 (m, 1H), 3.51 (d, *J*=12.6 Hz, 0.5H), 3.22 - 3.13 (m, 0.5H), 3.11 (d, *J*=12.6 Hz, 1H), 2.71 (td, *J*=13.4, 4.8 Hz, 0.5H), 2.61 (td, *J*=13.5, 4.9 Hz, 0.5H), 2.44 - 2.39 (m, 0.5H), 2.39 (s, 0.5H), 2.37 (d, *J*=3.8 Hz, 0.5H), 2.34 (d, *J*=4.9 Hz, 0.5H), 2.16 (dq, *J*=13.5, 6.7 Hz, 0.5H), 2.06 - 1.97 (m, 1H), 1.61 (d, *J*=14.3 Hz, 0.5H), 1.52 (d, *J*=14.0 Hz, 0.5H), 1.22 - 1.18 (m, 3H), 1.06 (d, *J*=6.9 Hz, 1.5H), 1.01 (d, *J*=6.9 Hz, 1.5H), 0.94 (d, *J*=6.9 Hz, 3H), 0.82 (d, *J*=9.1 Hz, 3H), 0.77 (d, *J*=6.6 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{22}H_{33}ClN_2O_4Na$ $[M + Na]^+$ 447.20. Found, 447.04. HPLC Purity: 97.8%, t_r = 7.55 min (method A); 98.3%, t_r = 8.12 min (method B).

1-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-((S)-2-hydroxypropyl)urea (25). The title compound was synthesized by the procedure used to make 1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-(2-hydroxy-2-methylpropyl)urea (**29**). The crude product was purified via flash chromatography over silica gel in 1:1 hexanes/EtOAc to 100% EtOAc to 9:1 methylene chloride/methanol. ^{1}H NMR (400 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.8 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.32 (d, *J*=8.8 Hz, 1H), 7.31 (d, *J*=8.8 Hz, 1H), 4.70 (dd, *J*=8.5, 6.3 Hz, 1H), 4.54 (d, *J*=8.6 Hz, 0.5H), 4.14 - 4.00 (m, 1H), 3.76 (dquin, *J*=13.1, 6.6 Hz, 1H), 3.68 - 3.57 (m, 1H), 3.50 - 3.44 (m, 0.5H), 3.22 - 3.12 (m, 2H), 3.11 - 2.98 (m, 1H), 2.76 - 2.57 (m, 1H), 2.08 (dq, *J*=13.2, 6.6 Hz, 0.5H), 2.01 (s, 0.5H), 2.00 - 1.90 (m, 0.5H), 1.61 (d, *J*=14.3 Hz, 0.5H), 1.51 (d, *J*=13.8 Hz, 0.5H), 1.24 (t, *J*=7.1 Hz, 0.5H), 1.13 (dd, *J*=8.2, 6.5 Hz, 3H), 1.06 (d, *J*=6.8 Hz, 1.5H), 0.98 (d, *J*=6.8 Hz, 1.5H), 0.94 (d, *J*=6.8 Hz, 1.5H), 0.91 (d, *J*=6.8 Hz, 1.5H), 0.83 (s, 1.5H), 0.80 (d, *J*=4.2 Hz, 3H), 0.76 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_{22}H_{35}ClN_3O_4$ $[M + H]^+$ 440.23. Found, 440.23. HPLC Purity: 98.6%, t_r = 7.44 min (method A); 100%, t_r = 7.90 min (method B).

(R)-N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-hydroxybutanamide (26). The title compound was synthesized by the procedure used to make N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (**10**). The crude product was purified via flash chromatography by reverse-phase preparative HPLC (Method C) (t_r = 10.81 min). ^{1}H NMR (500 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.5 Hz, 1H), 7.46 (d, *J*=8.5 Hz, 1H), 7.32 (d, *J*=8.5 Hz, 1H), 7.30 (d, *J*=8.5 Hz, 1H), 4.91 (d, *J*=6.9 Hz, 0.5H), 4.59 - 4.52 (m, 0.5H), 4.17 - 4.05 (m, 1.5H), 4.03 (d, *J*=12.6 Hz, 0.5H), 3.66 - 3.61 (m, 1H), 3.50 (d, *J*=13.2 Hz, 0.5H), 3.25 - 3.14 (m, 0.5H), 3.11 (d, *J*=12.6 Hz, 0.5H), 2.71 (td, *J*=13.5, 4.8 Hz, 0.5H), 2.61 (td, *J*=13.5, 4.9 Hz, 0.5H), 2.42 - 2.35 (m, 1.5H), 2.16 (dq, *J*=13.4, 6.6 Hz, 0.5H), 2.08 - 1.97 (m, 0.5H), 1.61 (d, *J*=14.0 Hz, 0.5H), 1.52 (d, *J*=14.0 Hz, 0.5H), 1.21 (dd, *J*=6.2, 4.3 Hz, 3H), 1.06 (d, *J*=6.6 Hz, 1.5H), 1.01 (d, *J*=6.9 Hz, 1.5H), 0.95 (dd, *J*=6.7, 4.0 Hz, 3H), 0.82 (d, *J*=9.1 Hz, 3H), 0.77 (d, *J*=11.0 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{22}H_{34}ClN_2O_4$ $[M + H]^+$ 425.22. Found, 425.06. HPLC Purity: 96.6%, t_r = 7.66 min (method A); 95.1%, t_r = 8.23 min (method B).

1-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-((R)-2-hydroxypropyl)urea (27). The title compound was synthesized by the procedure used to make 1-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-(2-hydroxy-2-methylpropyl)urea (**29**). The crude product was purified via flash chromatography over silica gel in 1:1 hexanes/EtOAc to 100% EtOAc to 9:1 methylene chloride/methanol. ^{1}H NMR (400 MHz, CD_3OD) δ 7.49 (d, *J*=8.8 Hz, 1H), 7.46 (d, *J*=8.8 Hz, 1H), 7.32 (d, *J*=8.8 Hz, 1H), 7.31 (d, *J*=8.8 Hz, 1H), 4.71 (dd, *J*=8.5, 6.0 Hz, 1H), 4.58 - 4.51 (m, 0.5H), 4.03 (d, *J*=11.4 Hz, 1H), 3.76 (td, *J*=11.4, 6.4 Hz, 1H), 3.62 (d, *J*=13.0 Hz, 1H), 3.51 - 3.44 (m, 0.5H), 3.24 - 3.08 (m, 2H), 3.00 (ddd, *J*=13.6, 6.9, 1.6 Hz, 1H), 2.89 (s, 0.5H), 2.76 - 2.69 (m, 0.5H), 2.66 (dd, *J*=8.6, 4.6 Hz, 0.5H), 2.64 - 2.57 (m, 0.5H), 2.09 (dq, *J*=13.0, 6.6 Hz, 0.5H), 2.02 - 1.89 (m, 0.5H), 1.61 (d, *J*=13.8

Hz, 0.5H), 1.51 (d, *J*=13.8 Hz, 0.5H), 1.12 (t, *J*=6.2 Hz, 3H), 1.06 (d, *J*=6.6 Hz, 1.5H), 0.98 (d, *J*=6.8 Hz, 1.5H), 0.94 (d, *J*=6.8 Hz, 1.5H), 0.90 (d, *J*=6.8 Hz, 1.5H), 0.83 (s, 1.5H), 0.81 (d, *J*=1.8 Hz, 3H), 0.76 (s, 1.5H). LCMS (ESI) m/z Calcd for $C_{22}H_{35}ClN_3O_4$ $[M + H]^+$ 440.23. Found, 440.25. HPLC Purity: 98.9%, t_r = 7.45 min (method A); 99.3%, t_r = 7.92 min (method B).

N-((R)-1-((S)-4-(4-Chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-hydroxy-3-methylbutanamide (28). The title compound was synthesized by the procedure used to make N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)benzamide (**10**). The crude product was purified by reverse-phase preparative HPLC (Method C) (t_r = 11.29 min). ^{1}H NMR (500 MHz, CD_3OD) (mixture of rotamers) δ 7.49 (d, *J*=8.5 Hz, 1H), 7.46 (d, *J*=8.5 Hz, 1H), 7.31 (dd, *J*=8.5, 4.9 Hz, 2H), 4.95 (d, *J*=6.6 Hz, 0.5H), 4.88 (d, *J*=6.3 Hz, 0.5H), 4.54 (d, *J*=10.7 Hz, 0.5H), 4.03 (d, *J*=13.7 Hz, 0.5H), 3.64 (d, *J*=13.2 Hz, 1H), 3.50 (d, *J*=13.2 Hz, 0.5H), 3.18 (td, *J*=12.9, 2.7 Hz, 0.5H), 3.11 (d, *J*=12.9 Hz, 0.5H), 2.71 (td, *J*=13.5, 4.7 Hz, 0.5H), 2.61 (td, *J*=13.6, 4.9 Hz, 0.5H), 2.42 (s, 1H), 2.40 (d, *J*=2.2 Hz, 1H), 2.21 - 2.13 (m, 0.5H), 1.61 (d, *J*=14.3 Hz, 0.5H), 1.52 (d, *J*=14.3 Hz, 0.5H), 1.27 (d, *J*=4.1 Hz, 6H), 1.07 (d, *J*=6.6 Hz, 1.5H), 1.01 (d, *J*=6.9 Hz, 1.5H), 0.95 (t, *J*=6.5 Hz, 3H), 0.82 (d, *J*=11.5 Hz, 3H), 0.77 (d, *J*=12.6 Hz, 3H). LCMS (ESI) m/z Calcd for $C_{23}H_{36}ClN_2O_4$ $[M + H]^+$ 439.23. Found, 439.10. HPLC Purity: 97.5%, t_r = 8.08 min (method A); 98.6%, t_r = 8.91 min (method B).

Pharmacology.

CCR1 Binding assay. For radioligand competition studies, THP-1 human monocytic leukemia cells were used in combination with LS WGA PS beads (Amersham, Cat.#: RPNQ 0260 and [^{125}I]-MIP-1α (PerkinElmer, Cat. # NEX298) at a final concentration of 0.1 nM. Unlabeled MIP-1α was added in excess to determine non-specific binding. The binding assay yielded 7% radioligand bound. The [^{125}I]-MIP-1α K_d was 0.11 nM and the B_{max} was 222 (547 sites/cell). Plates were incubated at room temperature for 12 h

then analyzed by LEADseeker™. The competition data by compound over a range of concentrations was plotted as percentage inhibition of radioligand bound in the absence of test compound (percent of total signal). After correcting for non-specific binding, IC_{50} values were determined.

Human PBMC binding assay. Peripheral blood mononuclear cells (PBMC) were isolated from EDTA anti-coagulated donor blood using standard procedures and transferred to 96-well filter plates ^{125}I-MIP-1α was added at a final concentration of 0.03 nM. To determine non-specific binding, excess of an unlabeled small molecule was added, specifically a previous lead CCR1-selective compound from the same chemical series, N-((R)-1-((S)-4-(4-chlorophenyl)-4-hydroxy-3,3-dimethylpiperidin-1-yl)-3-methyl-1-oxobutan-2-yl)-3-sulfamoylbenzamide,[22] at a final concentration of 10 μM. All conditions were run in duplicate at a minimum. Assay plates were incubated for 60 min at rt. After air drying, filter were removed and gamma counted.

Cross-species binding assays on mouse, rat, cynomolgus monkey and dog.

The cells used were as follows: mouse: WEHI 274.1 cell line (ATCC Cat. #CRL-1679); rat: peritoneal elicited cells. Lewis rats were injected i.p. with 5 mL of 3% thioglycollate fluid 48 hours before the day of the assay. After being euthanized, the rats were injected i.p. with 20 mL of PBS or 10% FBS in PBS with 10 mM EDTA and lavaged i.p. to retrieve cells; cynomolgus monkey: PBMCs; dog: PBMCs. Cells were washed once in assay buffer (RPMI, 20 mM HEPES, 0.1% BSA) and then diluted to a final concentration of 5 × 106/mL in the same buffer. 125I-MIP-1α (NEN/Perkin Elmer, Cat. # NEX298) was diluted to a final concentration of 0.08 nM in assay buffer. Cells and radiolabeled ligand were added to the wells of a 96-well filter plate (Millipore MABVN 1250) to give a final cell density of 250 × 103/well. To determine non-specific binding, unlabeled hMIP-1α (R&D Systems, Cat. #270-LD) was added at a final concentration of 1 μM. Total binding was determined in the absence of unlabeled ligand or test compound. To determine the effects of compound **29**, the compound was diluted in DMSO and then 3-fold serial dilutions in assay buffer to give final concentrations ranging from 50,000 nM to 4.6 nM. All

conditions were run in duplicate. Assay plates were incubated for 60 min at RT then washed 3 times with wash buffer (assay buffer + 0.4M NaCl). After air-drying the plates, the filters were removed from individual wells and gamma counted for 1 min.

Chemotaxis assay. The chemotaxis assay was conducted with THP-1 human monocytic cells labeled with the fluorescent dye Calcein-AM. Ligand-induced chemotaxis was measured in a chemotaxis plate (Neuroprobe #101-8 ChemoTx® Disposable Chemotaxis Plates) as cellular fluorescence in the lower well following a 60 min incubation at 37ºC. The concentration of ligand used was the one that had previously been established as giving a maximum signal in a response that was typically bell-shaped (MIP-1α (CCL3) 1nM; RANTES (CCL5) 10nM; MCP-3 (CCL7) 2nM; HCC-1 (CCL14a) 1nM; LKN (CCL15) (Δ-24 N-truncated) 0.3nM; MPIF-1 (CCL23) (Δ-24 N-truncated) 1nM). The inhibition of chemotaxis achieved by graded concentrations of compound was calculated as a percentage of the compound-free ligand control. The small molecule compounds were in both compartments (upper and lower chambers) at equal concentrations.

Inhibition of CD11b upregulation in whole blood. To elicit CD11b upregulation, CCR1 ligand – either MIP-1α (R&D Systems, Cat. # 270-LD-10) or LKN-1 (R&D, Cat. # 628-LK, Δ-24 N-truncated) was added to EDTA-anticoagulated human venous blood at a final concentration of 50 nM and incubated for 30 min at 37°C. Cells were stained with anti-hCD11b-phycoerythrin (BD Pharmingen Cat. # 555388) and anti-hCD14-FITC (BD Pharmingen Cat. # 555397 and read on a FACS Caliber, gating on monocytes by forward scatter/side scatter and CD14+ staining. For inhibition studies, whole blood was preincubated with titered concentrations of compound for 1 hour.

Inhibition of THP-1 cell chemotaxis by synovial fluid. Samples of synovial fluid (SF) from human RA patients were obtained from Bioreclamation, Inc. (Hicksville, NY). Details of the severity or duration of RA were not available. SF was serially diluted and tested in the chemotaxis assay as described above. For compound inhibition, a dilution of SF was selected that gave a workable range (signal/noise $\geq$ 2.5).

Liver Microsome Metabolism Assays. Test compound is received as a 3.5 mM stock solution in 100 percent DMSO. Compound is diluted to create a 50 μM acetonitrile (MeCN) solution containing 1.4% DMSO, which is then used as a 100x stock for incubation with microsomes. Each compound is tested in duplicate separately in each of three species in the Bristol-Myers Squibb (BMS) Metabolic Stability-Human, Rat, and Mouse assay panel. Compound **29**, NADPH and liver microsome solutions are combined for incubation in three steps: 152 μL of liver microsome suspension, protein concentration of 1. 1 mg/mL in 100 mM NaP_i, pH 7.4, 6.6 mM $MgC1_2$ buffer, is pre-warmed at 37° C.

1) 1.7 μL of 50 μM compound (98. 6% MeCN, 1.4% DMSO) is added to the same tube and pre-incubated at 37°C for 5 minutes.

2) The reaction is initiated by the addition of 17 μL of pre-warmed 10 mM NADPH solution in 100 mM NaP_i, pH 7.4.

3) Reaction components are mixed well, and 75 μL are immediately transferred into 150 μL quench/stop solution (zero-time point, T_0). Reactions are incubated at 37° C for 10 minutes and then an additional 75 μL aliquot is transferred into 150 μL quench solution. Acetonitrile containing 100 μM DMN (a UV standard for injection quality control), is used as the quench solution to terminate metabolic reactions.

4) Quenched mixtures are centrifuged at 1500 rpm (500x g) in an Allegra X-12 centrifuge, SX4750 rotor (Beckman Coulter Inc., Fullerton, Calif.) for fifteen minutes to pellet denatured microsomes. A volume of 90 μL of supernatant extract, containing the mixture of parent compound and its metabolites, is then transferred to a separate 96-well plate for UV-LC/MS-MS analysis to determine the percent of parent compound that is remaining in the mixture.

5)

Metabolic Stability Assay — Reaction Components

Reaction Components	Final Concentration in the Metabolic Stability Assay
Compound (Substrate)	0.5 μM (or 3 μM for earlier compounds)
NaPi Buffer, pH 7.4	100 mM
DMSO	0.014%
Acetonitrile	0.986%

Microsomes (human, rat, mouse) (BD/Gentest)	1 mg/mL protein
NADPH	1.0 mM
$MgCl_2$	6. 66 mM
37^o C Incubation time	0 minutes and 10 minutes
Quench/Stop Solution (MeCN + 100 μM DMN)	150 μL
Sample of Reaction	75 μL
Sedimentation of Denatured Microsome	15 minutes
UV-LC/MS analysis of supernatant	0. 17 μM

PXR Transactivation Assay. The cell culture medium used is DMEM. Lipofectamine 2000, PBS, heat-inactivated fetal bovine serum (FBS),trypsin-EDTA (0.25%), and penicillin-streptomycin werepurchased from GIBCO/Invitrogen (Carlsbad, Calif). Charcoal/dextran treated fetal bovine serum (FBS) was purchased from Hyclone (Logan, Utah). HepG2 cells were obtained from ATCC (Manassas, Va.). Human PXR-pcDNA3 and luciferase reporter containing CYP3A4 promoter, CYP3ALuc, were generated at Bristol-Myers Squibb. White tissueculture (TC)-surface 384-well plates were purchased from Perkin Elmer (Boston, Mass.). Luciferase substrate (Steady-Glo) was purchased from Promega (Madison, Wis.). Controlcompounds rifampicin, mifepristone, and sulfinpyrazone were purchased from Sigma (St. Louis, Mo.).

Culture of HepG2 cells is performed in T175 flasks using DMEM containing 10%FBS.The transfection mixture contains 1 μg/mL of PXR-pcDNA3 plasmid DNA, 20 μg/ml of Cyp3A-Luc plasmid DNA, 90 μL/mL of Lipofectamine 2000, and serum-free medium. After incubating at room temperature for 20 minutes, the transfection mixture (1 ml per flask) is applied to the cells in fresh medium (20mL per flask),and flasks incubated at 37 ^{o}C. (5% CO_2) overnight.

Cells in each flask are washed with PBS and 2 mL of Trypsin-EDTA (0.25%) is added and incubated for five minutes at 37 ^{o}C, 5% CO_2. The flasks are then tapped vigorously to break up cell aggregates. After the addition of 8 mL of DMEM containing 5% charcoal/dextran-treated FBS, the entire mixture is transferred to conical tubes. Cells are then centrifuged at 1000 rpm for 5 minutes. Cell pellets

are resuspended to a final count of ~7x10 cells/mL in freezing media (DMEM containing 20% serum and 10% DMSO). The cell suspension is aliquoted into 15 mL polypropylene tubes, 5 mL per tube. Cells are slowly frozen by placing in a styrofoam-insulated container at -80 ^{o}C overnight. Vials are transferred to an Ultracold (-140 ^{o}C) freezer after 24 hours for long-term storage. Vials of cryopreserved cells are thawed rapidly in a warm water bath for five minutes. Cells are pooled and diluted to 50 mL in a 50 mL conical vial. The thawed cells are centrifuged at 1500 rpm for 5 minutes to collect the cells and the supernatant discarded. Cells are then resuspended in fresh Media II (DMEM containing 5% charcoal/dextran-treated FBS, 1% Penicillin/Streptomycin, 100 μM non-essential amino acids, 1 mM sodium pyruvate, and 2 mM L-glutamine), counted using the Guava Cell Counter, and diluted to 1.6x10^{5} cells/ml in the same media.

Fifty microliters of cell mixture is added to wells in columns 1-23 of white tissue-culture treated 384-well plates containing 0.25 μL of test compound dissolved in 100% DMSO. Fifty microliters of Media II is added to wells in column 24. The plates are incubated at 37 ^{o}C (5% CO_2) for 24 hours, then 5 μL of Alamar Blue reagent (Trek Diagnostics, Cat #00-100) is added to each well. Plates are then incubated an additional two hours at 37 ^{o}C, (5% CO_2) and then one hour at room temperature. Fluorescence is read at Ex525/Em598. After the fluorescence is measured, 25 μL of luciferase substrate (Steady-Glo, Promega) is added to each well. The plates are incubated for fifteen minutes at room temperature, after which the luminescence is read on a PheraStar (BMG Labtech) plate reader. Rifampicin (10 μM), a well-known agonist of PXR, is included in each plate as an internal standard and positive control. The data is then expressed as percent control (% CTRL), where the control signal is the signal from the 10 μM rifampicin and the blank signal is that from the DMSO vehicle.

% CTRL = ((Compound signal —Blank signal)/(Control signal —Blank signal))*100

Compounds are tested at ten concentrations (2.5 nM-50 μM, 1:3 serial dilution). Assay results are reported as EC_{50}, the concentration of compound at which 50% of the maximal response is observed, and as YMAXOBS, the maximal response (highest percent CTRL) observed for that compound. The EC_{50} is defined as the concentration corresponding to half of the maximal response derived from the fitted 20-point curve as determined using a four-parameter logistic regression model. Additionally, compounds may also be reported as EC_{20} or EC_{60}.

Pharmacokinetics. All animal studies were performed under the approval of the Bristol-Myers Squibb (BMS) Animal Care and Use Committee and in accordance with the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Single-dose mouse pharmacokinetics. Male Balb-C mice (N = 9, 25–30 mg) received compound **29** once as an intravenous (IV) bolus dose via the tail vein (5 mg/kg; 5 mL/kg in 10:30:60 dimethyl acetamide (DMAC), PEG400, water) or by oral gavage (5 mg/kg, 10 mL/kg in 10:30:60 DMAC, PEG400, water). Mice receiving compound **29** orally were fasted over-night. Blood samples (~0.2 mL) were obtained by retro-orbital bleeding or cardiac puncture at 0.05 (IV dose only), 0.25 (oral (PO) dose only), 0.5, 1, 3, 6, 8, and 24 hours (h) post dose (N=3 at each time point) to obtain a composite pharmacokinetic profile.

Single-dose rat pharmacokinetics. Male Sprague-Dawley rats (N = 3 per dose route, 275–325 g) received compound **29** once as an IV bolus via the jugular vein (5 mL/kg) or by oral gavage (10 mL/kg). Rats receiving compound **29** orally were fasted over-night. Blood samples (~0.3 mL) were collected from the jugular vein into K_3EDTA-treated syringes. Serial blood samples were obtained predose and at 0.17 (IV dose only), 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, and 24 h post dose.

Single-dose dog pharmacokinetics. The pharmacokinetic profile of **29** was evaluated in male beagle dogs (9–11 kg). The IV and PO studies were conducted in a crossover design (N = 3) with a 1–2-week washout period in between doses. Prior to dosing, the cephalic vein was catheterized for IV dosing and

blood collection (IV and PO). Compound **29** was infused IV at 1 mg/kg (2 mL/kg in DMAC, PEG400, water (10:30:60)) over 5 minutes at a constant rate of 0.2 mL/kg/min. Dogs in the PO study were fasted overnight prior to dosing and were fed 4 hours after dosing. In the PO studies, **29** was administered by oral gavage at 1 mg/kg (2 mL/kg in DMAC, PEG400, water (10:30:60)). Serial blood samples were collected at 0.17 (IV dose only), 0.25, 0.5, 0.75, 1, 2, 4, 6, 8 and 24 h post dose.

Single-dose monkey pharmacokinetics. The pharmacokinetics of **29** were evaluated in male cynomolgus monkeys in a crossover-design. Following an overnight fast, 3 animals (3.5 to 4.2 kg) received **29** by IV infusion (2 mg/kg; 5 mL/kg in ethanol, PEG400, water (10:30:60)) at a constant rate of 0.2 mL/min/kg via the femoral vein. Blood samples (~0.3 mL) were collected from a femoral artery pre-dose and at 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, and 24h post dose. Following a >1–week washout, the same animals received **29** by oral gavage (2 mg/kg; 5 mL/kg in ethanol, PEG400, water (10:30:60)) and serial blood samples (~0.3 mL) were collected from a femoral artery predose and at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post dose.

In each of the above pharmacokinetic studies, all blood samples were centrifuged at 4 °C (1500–2000 × g) to obtain plasma. Plasma samples were stored at -20 °C until analysis for **29**.

Docking and minimization calculations.

Low energy conformations for compounds **29** and **30** were generated using the Conformational Search module of Macromodel, (Version 9.9, from Schrodinger, Inc.), MCMM extended sampling with the OPLS_2005 forcefield in vacuum. Conformations with energies greater 10 kcal/mol from the global minimum were rejected. The remaining conformations were placed into PXR ligand binding site by rigid superposition of the atoms within the chlorophenyl-dimethylpiperidine substructure. The resulting poses were then manually filtered to remove those which resulted in steric clashes with the receptor and lacked hydrogen bonds to the crystallographic water. Because no poses of **29** lacking steric clashes could be found, representative low energy conformations are shown in Figures 3 and 4.

Crystallization and structure determination of PXR with compound 28. Apo crystals of PXR/SRC-1 were grown overnight in hanging drops at 23 °C in 0.1M imidazole pH 7.8, 8-10% isopropanol. These apo crystals were transferred to 1 μL drops containing 5 mM compound **28** in the same well solution and soaked overnight. Crystals were harvested next day using 32% ethylene glycol as cryoprotectant and flashfrozen in Liquid Nitrogen. Diffraction data was collected at beamline 22-ID (SER-CAT) at the Advanced Photon Source at Argonne National Laboratory. Data was indexed, integrated, and scaled with HKL2000.[33] The crystals had symmetry consistent with space group $P4_32_12$ with cell dimensions a = 91.7Å, b = 91.7Å, c = 85.6Å and 1 molecule of PXR in the crystallographic asymmetric unit. Structure was determined by molecular replacement using the program PHASER[34] with a homology model of PXR based on the structure of PXR (2O9I)[35] as a search model. The structure was examined and manually fitted using program COOT.[36] The location of ligands in Ligand binding site was easily identified by inspection of electron density maps. The ligand (compound **28**) was automatically fit into the ligand density using the program AFITT (Open Eye Scientific software, Inc., Santa Fe, NM, USA) and refined using program autoBUSTER (Global Phasing LTD, Cambridge, UK).

Supporting Information

Single crystal X-ray diffraction results for compound **28** in PXR. This material is available free of charge via the Internet at http://pubs.acs.org.

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Abbreviations Used

TM, trans-membrane; RA, rheumatoid arthritis; CCL, CC chemokine ligand; PXR, pregnane X receptor; HOBt, 1-hydroxybenzotriazole; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; TEA, triethylamine; DCM, dichloromethane; rt, room temperature; DIEA, N,N-diisopropylethylamine; DMF, dimethylformamide; AcCN, acetonitrile; CYP P450, cytochrome P450, THF, tetrahydrofuran; eq, equivalent(s); ESI, electrospray ionization; LCMS, liquid chromatography-mass spectrometry.

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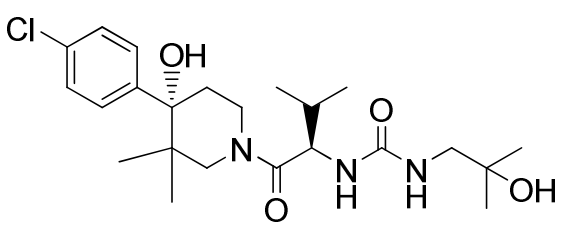
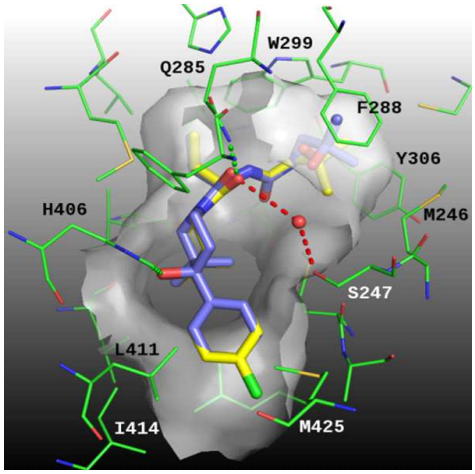
Table of Contents Graphic

The Discovery of CCR1 Antagonist, BMS-817399, for the Treatment of Rheumatoid Arthritis

Joseph B. Santella, III*, Daniel S. Gardner, John V. Duncia*, Hong Wu, Murali Dhar, Cullen Cavallaro, Andrew J. Tebben, Percy H. Carter, Joel C. Barrish, Melissa Yarde, Stephanie W. Briceno, Mary Ellen Cvijic, R. Robert Grafstrom, Richard Liu, Sima R. Patel, Andrew J.Watson, Guchen Yang, Anne V. Rose, Rodney D. Vickery, Janet Caceres-Cortes, Christian Caporuscio, Daniel M. Camac, Javed A. Khan, Yongmi An, William R. Foster, Paul Davies, and John Hynes, Jr.



BMS-817399 (29)
CCR1 IC_{50} = 1 nM
Chemotaxis IC_{50} = 6 nM



Superposition of compound **29** (purple) onto compound **28** (yellow) from the PXR crystal structure.