Universiteit Leiden
The Netherlands

Small changes for long term impact : optimization of structure kinetic properties : a case of CCR2 antagonists

Vilums, M.

Citation
Vilums, M. (2014, November 20). *Small changes for long term impact : optimization of structure kinetic properties : a case of CCR2 antagonists*. Retrieved from https://hdl.handle.net/1887/29811

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/29811

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

Leiden University Repository

The handle http://hdl.handle.net/1887/29811 holds various files of this Leiden University dissertation.

Author: Vilums, Maris
Title: Small changes for long term impact : optimization of structure kinetic properties : a case of CCR2 antagonists
Issue Date: 2014-11-20

CHAPTER 6

DESIGN AND SYNTHESIS OF NOVEL SMALL MOLECULE CCR2 ANTAGONISTS: EVALUATION OF 4-AMINOPIPERIDINE DERIVATIVES

This chapter was based upon:

M. Vilums, A.J.M. Zweemer, S. Dekkers, Y. Askar, H. de Vries, J. Saunders, D. Stamos, J. Brussee, L.H. Heitman, A.P. IJzerman.

(manuscript in preparation)

ABSTRACT

A novel *N*-(2-oxo-2-(piperidin-4-ylamino)ethyl)-3-(trifluoromethyl)benzamide series of human CCR2 chemokine receptor antagonists was identified. With a pharmacophore model based on known CCR2 antagonists a new core scaffold was designed, analogues of it synthesized and structure–affinity relationship studies derived yielding a new high affinity CCR2 antagonist *N*-(2-((1-(4-(3-methoxyphenyl)cyclohexyl)piperidin-4-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide.

The CC chemokine CCL2, through its interaction with the CCR2 G protein–coupled receptor, plays an important role in the recruitment of monocytes, natural killer cells, dendritic cells and T-lymphocytes.[1] Research on CCL2 knockout (KO) and CCR2 KO mice suggests that inhibition of the CCL2/CCR2 axis may be beneficial in the treatment of inflammatory diseases.[2] The pair is thought to be involved in atherosclerosis,[3] insulin resistance,[4] multiple sclerosis,[5] neuropathic pain[6] and asthma.[7] Different *in vitro* and *in vivo* models have shown the usefulness of small molecule CCR2 antagonists to inhibit the chemotactic response of CCL2.[8-10]Consequently, the pharmaceutical industry has devoted considerable efforts to the development of CCR2 antagonists to combat these diseases. A vast number of different scaffolds used in the design of CCR2 antagonists has been described.[11, 12]

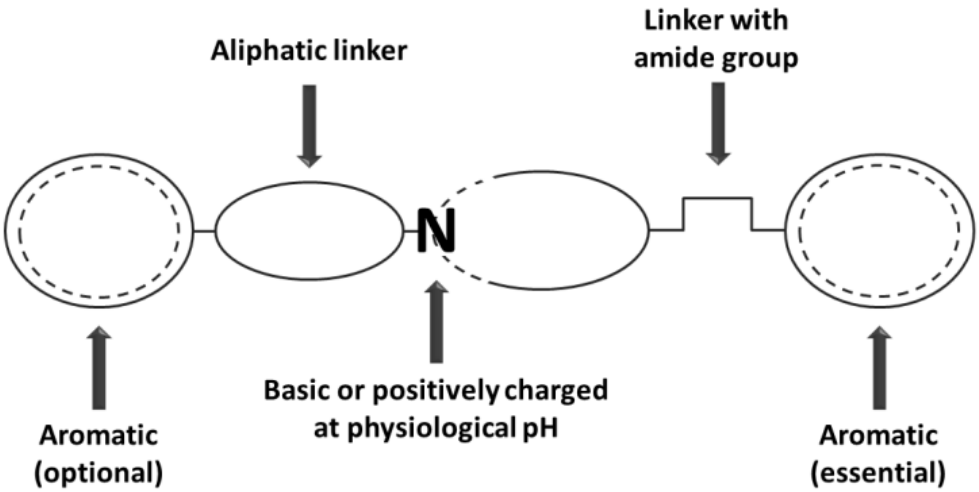


Figure 1. Pharmacophore of CCR2 antagonists.

However, the bulk of these antagonists share the same structural motifs: a basic nitrogen atom in the center, flanked by two aromatic rings of which one is connected to the nitrogen atom with an amide containing linker and the other with an aliphatic linker (Figure 1). In some cases the latter aromatic ring is missing and only the aliphatic group is left on one side.[12] Usually, the central nitrogen is part of an aliphatic heterocycle (e.g. piperidine, **1**,[13] pyrrolidine, **2**[14] and INCB3344[14, 15] or azetidine, JNJ lead[16]) (Figure 2).

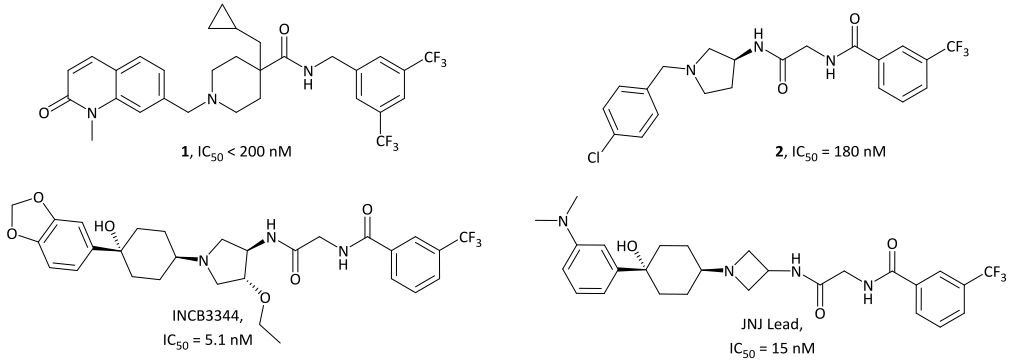
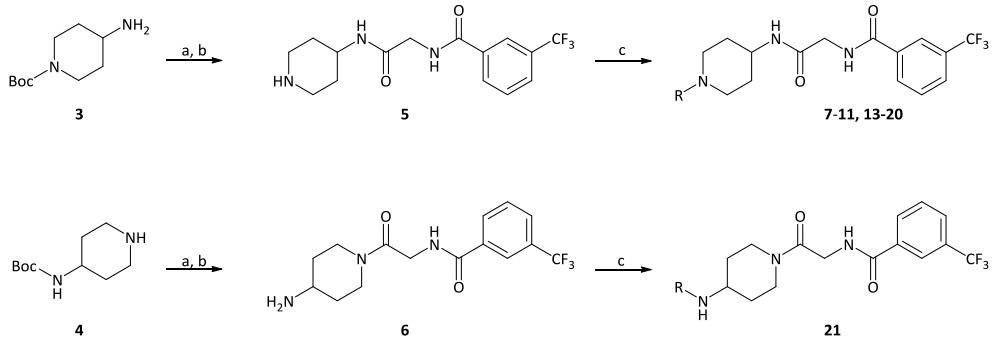


Figure 2. Known CCR2 antagonists.

In this paper, we describe our efforts towards the identification of a new class of CCR2 receptor antagonists. Using the structural knowledge of CCR2 antagonists as in figure 2 we generated hybrid scaffolds based on a piperidine ring. We explored different linkers between the basic nitrogen and aromatic groups as well as different substituents on the aromatic group. All compounds were evaluated in a ^{125}I-CCL2 displacement assay on a human bone osteosarcoma (U2OS) cell membrane preparation expressing CCR2 as described previously by our group.[17]

Scheme 1.[a]



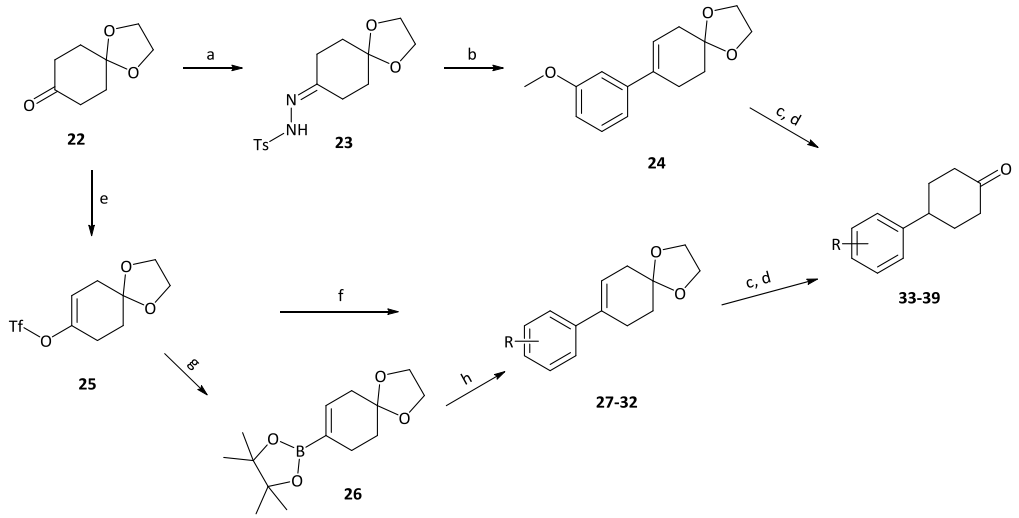
[a]Reagents and conditions: a) PyBrOP, DIPEA, DMAP, N-(3-(trifluoromethyl)benzoyl)glycine, DCM, MS 4 Å, room temperature; b) dry 3 M HCl in MeOH, room temperature, yield in two steps: 57-89%; c) corresponding aldehyde or ketone, $NaBH(OAc)_3$, AcOH, DCE, room temperature, (2-64%).

The synthetic methods to arrive at these compounds are depicted in schemes 1, 2 and 3. The commercially available *N*-Boc-protected piperidineamines **3** and **4** were used in a peptide–coupling reaction with *N*-(3-(trifluoromethyl)benzoyl)glycine under bromo-tris-pyrrolidino

phosphoniumhexafluorophosphate (PyBroP) conditions.[18] Subsequent removal of the Boc-protecting group with dry HCl in methanol produced the free amines **5** and **6**. These amines were used in reductive amination reactions with different aldehydes and ketones to yield the desired products **7**-**11**, **13**-**21** (Scheme 1).

For the synthesis of the desired ketones we first used a synthetic route via hydrazone intermediates (Scheme 2) under conditions described by Barluenga et al.[19] Commercially available ketone **22** was reacted with tosylhydrazide to generate hydrazone **23**, which was used subsequently in a Pd–catalyzed cross–coupling reaction to generate **24** with moderate yield.

Scheme 2.[a]

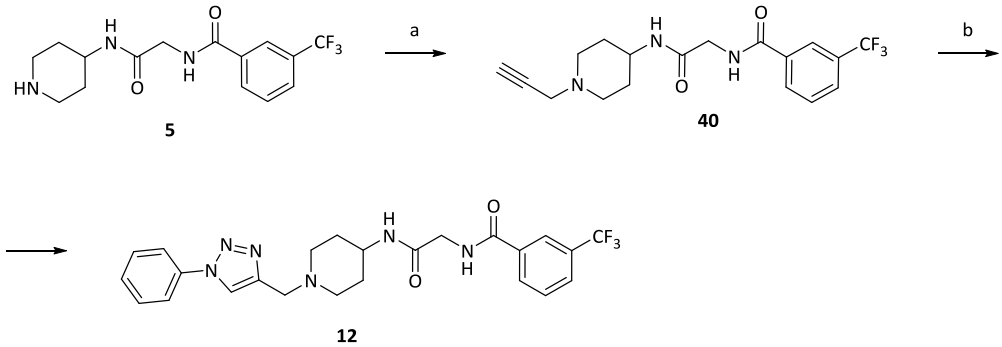


[a]Reagents and conditions: a) *p*-toluenesulfonhydrazide, dioxane, 130 °C, 45 minutes, MW, (65%); b) $Pd_2(dba)_3$, XPhos, Li*t*-BuO 1.0 M in hexanes, dioxane, 110 °C, 2 h, MW, (24%); c) Pd/C 10% wt, $Pd(OAc)_2$, MeOH, H_2, room temperature, 4-12 h, (85-99%) ; d) $FeCl_3 \bullet 6H_2O$, acetone, DCM, 5-12 h, room temperature, (42-99%); e) i)1.3 eq. LDA, THF, under N_2, –78 °C → –25 °C, 2 h, cool down to –78 °C; ii) *N*-phenyl-bis(trifluoromethanesulfonimide), –78 °C → room temperature, 24 h, (80%); f) 3,4-(methylenedioxy)phenylboronic acid, KF, $Pd(dppf)Cl_2$, room temperature overnight, (58%); g) $PdCl_2$, PPh_3, bis(pinacolato)diboron, KOPh, toluene, under N_2, 4 h at 50 °C, 24 h at room temperature, (59%); h) $Pd(PPh_3)_4$, corresponding arylhalogen, Na_2CO_3 2 M in H_2O, dioxane, under N_2, 80 °C, 5 h, MW, (80-99%).

However, attempts to use this method with other substituents on the phenyl ring resulted in very poor yields or no product at all. Another synthetic route was therefore chosen to yield the desired ketones. The acetal protected cyclohexanone **22** was deprotonated with

lithiumdiisopropylamide (LDA) and reacted with *N*-phenyl-bis(trifluoromethanesulfonimide) to generate triflate **25**. This compound was used directly in a Suzuki–coupling with 3,4-methylenedioxyphenylboronic acid, however, to introduce other substituents we decided to transform the triflate in boronic ester **26** with bis(pinacolato)diboron. This allowed us to use a wider range of arylhalogens as coupling partners in the Suzuki–coupling to eventually generate the desired intermediates with good overall yields. Subsequently, reduction of the double bond and removal of the acetal protecting group yielded the desired ketones **33**-**39**, which were used in reductive amination reactions to yield the final compounds.

Scheme 3.[a]



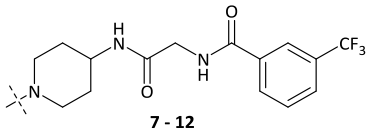
[a]Reagents and conditions: a) propargyl bromide, K_2CO_3, acetone, reflux overnight, (99%); b) iodobenzene, proline, Na_2CO_3, NaN_3, ascorbic acid, $CuSO_4 \bullet 5H_2O$, DMSO/H_2O 3:1, 80 °C, 48 h, (4%).

To explore the influence of a methylenetriazole group as a linker between the piperidine and phenyl moieties we used click chemistry (Scheme 3). First, we alkylated the piperidine of compound **5** with propargyl bromide to generate compound **40**, which was used in a further reaction with sodium azide and iodobenzene in the presence of proline, ascorbic acid and $CuSO_4$ as described by Feldman et al.[20]

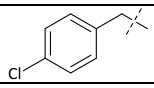
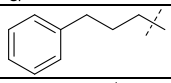
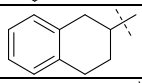
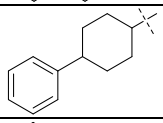
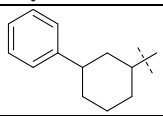
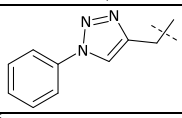
As mentioned before we combined the different scaffolds from two known CCR2 antagonists (compound **1** of Epix Delaware;[13] compound **2** from Tejin[14]) to generate a hybrid scaffold by transfecting the *N*-(3-(trifluoromethyl)benzoyl)glycine part onto the piperidine ring. We argued that the expansion of the central ring to piperidine (compared to INCB3344 and JNJ Lead) might have a minor effect only on the configuration of the molecule. However, the 4-

chlorobenzyl group (compound **7**) which had yielded good affinity in combination with the pyrrolidine scaffold[14] (compound **2**), provided no affinity in the case of piperidine (Table 1).

Table 1. CCR2 affinities of compounds **7**-**12**.



7 - 12

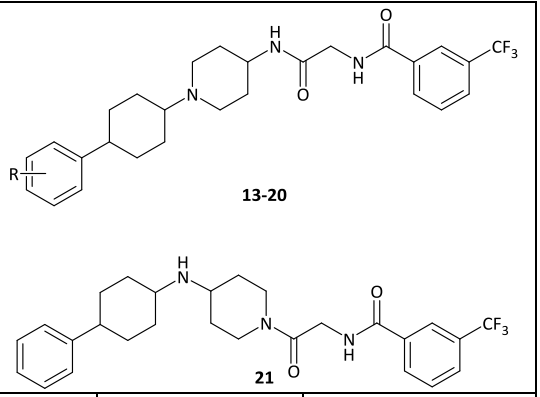
R	Nr.	K_i, (nM) ± SEM (n = 3)[a]
	**7**	0%
	**8**	6%
	**9**	24%
	**10**	74 ± 9
	**11**	10%
	**12**	12%

[a]Human CCR2 binding affinity in [^{125}I]CCL2 assay or % displ. at 1µM of [^{125}I]CCL2 binding

Extending the linker to propyl (compound **8**) had a minor effect on the affinity and the rigidification of the linker into tetrahydronaphthalene (compound **9**) yielded negligible improvement (displacement at 1 µM concentration of 6% and 24%, respectively). However, separation of the rings into a 4-phenylcyclohexyl group (compound **10**) resulted in a boost of affinity (K_i = 74 nM). To explore the correct location of the phenyl ring we moved it to the 3 position on the cyclohexane ring (compound **11**), which resulted in a complete loss of affinity. In addition, the cyclohexane`s exchange to methylenetriazole as a linker (compound **12**) did not yield any affinity either. Apparently, the distance, 3D orientation and lipophilicity provided by the cyclohexane moiety is just right for the binding of these molecules to the

CCR2 receptor and any deviation from it results in complete loss of affinity. This could also be the reason why the 4-aryl-cyclohexane motif is used in so many pyrrolidine[15, 21] and azetidine[16, 22] derivatives (e.g. INCB3344, JNJ Lead, see figure 2). We continued the SAR studies with different substituents on the phenyl ring of the 4-phenyl-cyclohexyl group. Introduction of a methyl group on different positions indicated that substitution on the 2 and 4 positions (compounds **13** and **15**) decreased the affinity (Table 2).

Table 2. CCR2 affinities of compounds **13**-**21**.



Nr.	R	K_i, (nM) ± SEM (n = 3)[a]
13	2-Me	26%
14	3-Me	270 ± 20
15	4-Me	38%
16	3-OMe	66 ± 12
17	3,5-di-OMe	41%
18	2,6-di-OMe	42%
19	4-OH	139 ± 35
20	3,4-OCH_2O-	90 ± 18
21	-	31%

[a]Human CCR2 binding affinity in [^{125}I]CCL2 assay or % displ. at 1μM of [^{125}I]CCL2 binding

The 3 position can tolerate substitution, albeit with a slight decrease in affinity (**14**, K_i = 270 nM). Changing the methyl to methoxy resulted in a regain of the affinity (compound **16**, K_i = 66 nM) pointing to a possible H–bond formation in the receptor binding pocket. However, insertion of two methoxy groups on either the 3,5 or 2,6 positions (compounds **17, 18**) yielded a decrease in affinity (displacement of 41% and 42%, respectively). Inserting a hydroxyl group on the 4 position (compound **19**) was tolerated with a twofold affinity

decrease compared to **10**. Combining substituents of **16** and **19** into a 3,4-methylendioxy group retained the affinity (compound **20**, K_i = 90 nM) which is in accordance with observations from the pyrrolidine[15] and azetidine[16] series. Finally, we wanted to explore the possibility of reversing the piperidine ring (compound **21**) in the same fashion as it was described for pyrrolidines[23] where it had only minimal effect on the binding affinity. However, in our case of the piperidine moiety such reversal substantially decreased the affinity for the CCR2 receptor.

In conclusion, we have synthesized a novel series of *N*-(2-oxo-2-(piperidin-4-ylamino)ethyl)-3-(trifluoromethyl)benzamide derivatives and compounds substituted with 4-arylcyclohexanes were identified as good hits for CCR2 antagonism and might be considered for further optimization.

EXPERIMENTAL SECTION

Chemistry

All solvents and reagents were purchased from commercial sources and were of analytical grade. Demineralized water is simply referred to as H_2O, because it was used in all cases, unless stated otherwise (i.e., brine). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 400 liquid spectrometer (^{1}H NMR, 400 MHz; ^{13}C NMR, 101 MHz). Chemical shifts are reported in parts per million (ppm), are designated by δ, and are downfield to the internal standard tetramethylsilane (TMS). Coupling constants are reported in hertz and are designated as *J*. *a* and *b* indicating different diastereomers. Analytical purity of the final compounds was determined by high-performance liquid chromatography (HPLC) with a Phenomenex Gemini 3 µm C18 110A column (50 × 4.6 mm, 3 µm), measuring UV absorbance at 254 nm. The sample preparation and HPLC method for compounds were as follows: 0.3–0.8 mg of compound was dissolved in 1 mL of a 1:1:1 mixture of $CH_3CN/H_2O/t$-BuOH and eluted from the column within 15 min at a flow rate of 1.3 mL/min. The elution method was set up as follows: 1–4 min isocratic system of H_2O/CH_3CN/1% TFA in H_2O, 80:10:10, from the 4th min, a gradient was applied from 80:10:10 to 0:90:10 within 9 min, followed by 1 min of equilibration at 0:90:10 and 1 min at 80:10:10. All compounds showed a single peak at the designated retention time and are at least 95% pure. Microwave reactions were done using Biotage Initiator microwave synthesizer. Thin-layer chromatography (TLC) was routinely consulted to monitor the progress of reactions, using aluminum-coated Merck silica gel F^{254} plates. Purification by column chromatography was achieved by use of Grace Davison Davisil silica column material (LC60A, 30–200 µm). The procedure for a series of similar compounds is given as a general procedure for all within that series, annotated by the numbers of the compounds.

*N-(2-oxo-2-(piperidin-4-ylamino)ethyl)-3-(trifluoromethyl)benzamide (**5**).* To 2 g, 10 mmol (1 equiv.)of 4-amino-1-boc-piperidine (**3**) dissolved in 30 mL DCM was added 2.47 g, 10 mmol (1 equiv.) of *N*-(3-(trifluoromethyl)benzoyl)glycine, 4.66 g, 10 mmol (1 equiv.) of PyBroP, 1 g, 8 mmol (0.8 equiv.) of 4-dimethylaminopyridine, 5.10 mL, 30 mmol (3 equiv.) of *N*,*N*-diisopropylethylamine and molecular sieves

4 Å. The reaction mixture was stirred for 24 h at room temperature. The product was partitioned between DCM/1M NaOH. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The intermediate was purified by column chromatography (50/50 EtOAc in DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (s, 1H), 8.00 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 7.6 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 4.17 (d, J = 4.8 Hz, 2H), 4.00–3.92 (m, 3H), 2.89 (t, J = 11.6 Hz, 2H), 1.91 (d, J = 2.8 Hz, 2H), 1.45–1.37 (m, 11H). Subsequently, *tert*-butyl 4-(2-(3-(trifluoromethyl)benzamido)acetamido)piperidine-1-carboxylate was added to a dry solution of 3M HCl in MeOH. The reaction was stirred at room temperature for 2.5 hours. Upon completion, the reaction was neutralized with 1M NaOH (aq.) to pH = 10 and the methanol was evaporated. Extraction with DCM and subsequent drying over $MgSO_4$ and evaporation yielded the product. Overall yield = 57%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.80 (s, 1H), 8.61 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 7.6 Hz, 1H), 6.54 (s, 1H), 4.86 (d, J = 7.6 Hz, 2H), 3.92–3.87 (m, 3H), 2.63 (t, J = 10.2 Hz, 2H), 1.89–1.86 (m, 2H), 1.32–1.30 (m, 2H).

*N-(2-oxo-2-(piperidin-4-ylamino)ethyl)-3-(trifluoromethyl)benzamide (**6**).* To 1 equivalent of *tert*-butyl piperidin-4-ylcarbamate (**4**) dissolved in DCM was added 1 equivalent of *N*-(3-(trifluoromethyl)benzoyl)glycine, 1 equivalent of PyBroP, 0.8 equivalents of 4-dimethylaminopyridine, 3 equivalents of *N*,*N*-diisopropylethylamine and molecular sieves 4 Å. The reaction mixture was stirred for 24 h at room temperature. The product was extracted with DCM/ 1 M NaOH. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The intermediate was purified by column chromatography (50/50 EtOAc in DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.13 (s, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.59 (t, J = 8.0 Hz, 1H), 7.47 (br s, 1H), 4.65–4.50 (m, 2H), 4.70 (dd, J = 22.4, 3.6, Hz, 2H), 3.82–3.72 (m, 2H), 3.23–3.15 (m, 1H), 2.88 (t, J = 12.0 Hz, 1H), 2.15–2.00 (m, 2H), 1.47 (s, 9H), 1.41–1.30 (m, 2H). *tert*-butyl (1-((3-(trifluoromethyl)benzoyl)glycyl)piperidin-4-yl)carbamate was added to a dry solution of 3M HCl in MeOH. The reaction was stirred at room temperature for 2.5 hours. Upon completion, the reaction was neutralized with 1M NaOH (aq.) to pH = 10 and the methanol was evaporated. Extraction with DCM and subsequent drying on $MgSO_4$ and evaporation yielded the product. Overall yield = 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.00 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.59 (d, J = 7.6 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 4.31 (d, J = 12.0 Hz, 2H), 4.40–4.10 (m, 2H), 3.68 (d, J = 12.0 Hz, 1H), 3.10–2.65 (m, 4H), 1.83–1.70 (m, 2H), 1.28–1.14 (m, 2H).

*General procedure for the preparation of compounds **7**, **10**, **16** and **19**.*

A round-bottom flask was purged with N_2 gas before adding DCM. *N*-(2-oxo-2-(piperidin-4-lamino)ethyl)-3-(trifluoromethyl)benzamide (**5**) was added together with the corresponding aldehyde or ketone in a 1:1 ratio. In the case of ketones, 1 equivalent of acetic acid was added. 2 equivalents of $NaBH(OAc)_3$ were added and the reaction mixture was stirred with 4 Å MS for 48 hours at room temperature. The reaction was quenched with 1M NaOH (aq.) and extracted with DCM. The organics were washed with brine and dried over $MgSO_4$. Purification by column chromatography was performed using an eluent system of 90:9:1 DCM:MeOH:NH_4OH.

*N-(2-((1-(4-chlorobenzyl)piperidin-4-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide (**7**).* The product was obtained in a yield of 62%. ^{1}H NMR (400 MHz, Acetone-d6) δ: 8.35–8.20 (m, 3H), 7.90 (d, J = 7.6 Hz, 1H), 7.75 (t, J = 7.6 Hz, 1H), 7.25–7.42 (m, 5H), 4.06 (d, J = 5.6 Hz, 2H), 3.70–3.80 (m, 1H), 3.49 (s, 2H), 2.80 (d, J = 11.6 Hz, 2H), 2.16–2.05 (m, 2H), 1.88–1.80 (m, 2H), 1.58–1.47 (m, 2H). ^{13}C NMR (101Hz, Acetone-d6) δ: 168.0, 165.3, 138.0, 135.4, 132.0, 131.1, 130.4, 130.0, 129.5, 128.1, 127.8, 127.8, 125.5, 124.1, 124.1, 61.6, 52.1, 46.6, 43.1, 31.8. LC/MS mass found: 454^+, 456^+ [H^+]. Purity: 96.9 %.

*N-(2-oxo-2-((1-(4-phenylcyclohexyl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**10**).* The product was obtained with a yield of 43%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.17 (s, 1H), 8.10–8.00 (m, 2H), 7.76 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.35–7.18 (m, 5H), 7.08 (d, J = 8.0 Hz, 0.3H),^{a} 7.00 (d, J = 8.0 Hz, 0.7H),^{b} 4.20 (d, J = 5.2 Hz, 2H), 3.88–3.76 (m, 1H), 3.10–2.90 (m, 2H), 2.77–2.69 (m, 1H), 2.50–2.38 (m, 1H), 2.30 (br s, 1H), 2.11 (t, J = 10.6 Hz, 1H), 2.05–1.88 (m, 6H), 1.68–1.40 (m, 6H). ^{13}C NMR (101Hz, $CDCl_3$) δ: 168.5,^{a} 168.5,^{b} 166.4, 146.8,^{a} 146.8,^{b} 134.2, 131.3, 130.9, 130.4, 129.2, 128.4, 128.3,

127.1, 126.8, 126.0, 125.7, 125.0, 124.5, 122.3, 63.4, 59.2, 48.6, 47.8, 47.3, 44.2, 44.1, 42.2, 33.6, 32.3, 28.7, 28.5, 27.9. LC/MS mass found: 488^+ [H^+]. Purity: 99.0 %. *a* and *b* are indicated for different diastereomers.

*N-(2-((1-(4-(3-methoxyphenyl)cyclohexyl)piperidin-4-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide (**16**).* This final compound was obtained with a yield of 6%. ^{1}H NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 8.13 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 7.64–7.55 (m, 2H), 7.24–7.19 (m, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.74–6.72 (m, 1H), 6.63 (s, NH), 4.15 (d, *J* = 4.8 Hz, 2H), 3.80 (s, 3H), 3.02 (t, *J* = 13.2 Hz, 2H), 2.71 (t, *J* = 4.4 Hz, 1H), 2.62–2.45 (m, 2H), 2.18 (s, 1H), 2.11–1.86 (m, 6H), 1.74–1.43 (m, 6H). ^{13}C NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 168.2, 166.4, 134.5, 130.5, 129.5, 129.4, 124.5, 119.7, 119.3, 113.6, 112.9, 111.2, 110.5, 77.5, 77.2, 76.8, 55.3, 48.6, 44.1, 43.9, 33.4, 28.6. LC/MS mass found: 518^+ [H^+]. Purity: 97.1 %.

*N-(2-((1-(4-(4-hydroxyphenyl)cyclohexyl)piperidin-4-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide (**19**).* The product was obtained with a yield of 40%. ^{1}H NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 8.13 (s, 1H), 8.03 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.76 (dd, J^1 = 4.8 Hz, J^2 =4.4 Hz, 2H), 4.07 (s, 2H), 3.77 (q, *J* = 4.0 Hz, 1H), 2.94 (d, *J* = 9.2 Hz, 2H), 2.71 (s, 1H), 2.40–2.52 (m, 3H), 2.12 (t, *J* = 10.8 Hz, 1H), 1.98–1.88 (m, 5H), 1.75 (d, *J* = 4.8 Hz, 1H), 1.65–1.49 (m, 4H), 1.41 (t, *J* = 10.4 Hz, 2H). ^{13}C NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 168.3, 154.7, 154.3, 138.2, 134.4, 131.0, 130.6, 129.3, 128.5, 128.2, 127.7, 124.4, 115.3, 115.2, 63.6, 48.6, 48.0, 47.0, 43.5, 43.2, 33.8, 31.8, 31.7, 29.0, 28.5, 26.8. LC/MS mass found: 504^+ [H^+]. Purity: 96.3 %.

*General procedure for the preparation of the final compounds **8, 9, 11, 13, 14, 18, 20** and **21.***

A 5 mL vial was loaded with 0.5 mmol (1 equiv.) of the corresponding aldehyde or ketone together with 0.5 mmol (1 equiv.) of piperidine **5** or **6**. In the case of ketones 1.25 mmol (2.5 equiv.) of acetic acid were added. 1 mL of MeOH was added and left to stir at room temperature for 30 minutes. 1 mmol (2 equiv.) of PEMB were added, dropwise. The reaction mixture heated at 60 °C overnight. The crude reaction mixture was directly poured onto a column and purified using the eluent system of 97:2:1 DCM:MeOH:NH_4OH.

*N-(2-oxo-2-((1-(3-phenylpropyl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**8**).* Yield = 10%, ^{1}H NMR (400 MHz, MeOD-d4) δ: 8.18 (s, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.67 (t, *J* = 8.0 Hz, 1H), 7.26 (t, *J* = 8.0 Hz, 2H), 7.19–7.14 (m, 3H), 4.0 (s, 2H), 3.82–3.77 (m, 1H), 3.15 (d, *J* = 12.4 Hz, 2H), 2.66 (q, *J* = 6.8 Hz, 4H), 2.47 (t, *J* = 11.2 Hz, 2H), 1.97–1.88 (m, 4H), 1.66 (q, *J* = 11.2 Hz, 2H). LC/MS: 448^+ [H^+]. Purity: 96.7%.

*N-(2-oxo-2-((1-(1,2,3,4-tetrahydronaphthalen-2-yl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**9**).* Yield = 77%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.01 (t, *J* = 5.6 Hz, 1H), 8.22 (s, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 7.94–7.90 (m, 2H), 7.76 (t, *J* = 7.6 Hz, 1H), 7.05 (s, 4H), 3.88 (d, *J* = 5.6 Hz, 2H), 3.58–3.50 (m, 1H), 3.34–2.67 (m, 7H), 2.89–2.25 (m, 2H), 1.97 (d, *J* = 12.0 Hz, 1H), 1.75 (d, *J* = 11.6 Hz, 2H), 1.57–1.53 (m, 1H), 1.45 (q, *J* = 10.8 Hz, 2H). LC/MS: 460^+ [H^+]. Purity: 99.0%.

*N-(2-oxo-2-((1-(3-phenylcyclohexyl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**11**).* Yield = 3%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.23 (s, 1H), 8.10 (s, 1H), 8.02 (d, *J* = 7.2 Hz, 1H), 7.75 (dd, J^1 = 8.0 Hz, J^2 = 7.6 Hz, 2H), 7.54 (t, *J* = 15.6 Hz, 1H), 7.34–7.12 (m, 4H), 7.20 (d, *J* = 4.8 Hz, 2H), 3.98 (s, 1H), 3.47 (d, *J* = 7.2 Hz, 1H), 3.35 (s, 1H), 3.15 (m, 1H), 2.92–2.86 (m, 4H) 2.56 (d, *J* = 11.6 Hz, 1H), 2.30–2.05 (m, 4H), 2.05–1.98 (m, 2H), 1.96–1.93 (m, 2H), 1.74 (t, *J* = 10.8 Hz, 1H), 1.65–1.60 (m, 1H). LC/MS: 488^+ [H^+]. Purity: 99.0%.

*N-(2-oxo-2-((1-(4-(2-methy-phenyl)cyclohexyl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**13**).* The product was obtained with a yield of 13%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.15 (s, 1H), 8.05–8.00 (m, 2H), 7.74 (d, *J* = 7.6 Hz, 1H), 7.54 (t, *J* = 7.6 Hz, 1H), 7.17–7.07 (m, 3H), 6.91 (d, *J* = 7.6 Hz, 1H), 4.19 (d, *J* = 4.8 Hz, 2H), 3.82–3.80 (m, 1H), 3.02–2.79 (m, 3H), 2.47–2.36 (m, 1H), 2.33 (s, 3H), 2.25 (s, 1H), 2.07–1.95 (m, 5H), 1.89–1.79 (m, 2H), 1.57–1.47 (m, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 168.4, 166.5,

145.7, 144.8, 135.3, 134.4, 131.2, 130.4, 129.3, 128.5, 126.2, 125.7, 125.2, 124.5, 122.4, 63.6, 58.0, 48.9, 47.9, 47.4, 44.2, 39.8, 32.7, 29.2, 27.5, 19.5. LC/MS: 502^+ [H^+]. Purity: 95.3%.

*N-(2-oxo-2-((1-(4-(3-methyl-phenyl)cyclohexyl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**14**).* The product was obtained with a yield of 12%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.13 (s, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.96 (d, J = 4.8 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.18 (t, J = 8.0 Hz, 1H), 7.07 (s, 1H), 6.99 (d, J = 9.6 Hz, 2H), 6.92 (d, J = 8.0 Hz, ½H^a), 6.85 (d, J = 7.6 Hz, ½H^b), 4.16 (d, J = 4.8 Hz, 2H), 3.90–3.70 (m, 1H), 2.96–2.88 (m, 2H), 2.66–2.62 (m, 1H), 2.44–2.26 (m, 5H), 2.11–2.05 (m, 1H), 2.00–1.85 (m, 6H), 1.62–1.35 (m, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 168.4, 166.5, 147.0, 137.9, 134.4, 131.1, 130.5, 129.3, 128.5, 128.3, 127.8, 125.1, 124.5, 124.1, 123.8, 63.5, 59.2, 48.7, 47.9, 47.4, 44.3, 44.1, 42.4, 33.7, 32.5, 28.7, 28.1, 21.6. LC/MS: 502^+ [H^+]. Purity: 98.6%.

*N-(2-((1-(4-(2,6-dimethoxyphenyl)cyclohexyl)piperidin-4-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide (**18**).* The product was obtained with a yield of 30%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.15 (s, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.99 (s, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.55 (t, J = 7.6 Hz, 1H), 7.12–7.07 (m, 1H), 6.85 (s, 1H), 6.53 (d, J = 7.6 Hz, 2H), 4.18–4.13 (m, 2H), 3.83–3.78 (m, 1H), 3.77 (s, 6H), 3.32–3.26 (m, 1H), 3.02–2.96 (m, 2H), 2.46–2.36 (m, 2H), 2.26 (s, 1H), 2.15–1.93 (m, 6H), 1.66–1.53 (m, 2H), 1.49–1.39 (m, 2H), 1.25 (d, J = 6.8 Hz, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 168.4, 166.5, 158.8, 134.4, 131.1, 129.3, 128.5, 126.9, 125.1, 124.5, 123.8, 122.4, 104.8, 57.8, 55.9, 51.5, 47.5, 44.1, 40.5, 34.6, 33.9, 32.7, 32.0, 29.6, 29.0, 24.2. LC/MS: 548^+ [H^+]. Purity: 96.7 %.

*N-(2-((1-(4-(benzo[d][1,3]dioxol-5-yl)cyclohexyl)piperidin-4-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide (**20**).* The product was obtained with a yield of 24%. ^{1}H NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 8.13 (s, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.77–7.73 (m, 2H), 7.56 (t, J = 8.0 Hz, 1H), 6.76–6.63 (m, 4H), 5.91 (s, 2H), 4.16 (d, J = 4.8 Hz, 2H), 3.84–3.76 (m, 1H), 2.93 (t, J = 12.4 Hz, 2H), 2.60 (s, 1H), 2.49–2.33 (m, 2H), 2.27 (s, 1H), 2.09 (t, J = 11.2 H, 1H), 1.98–1.84 (m, 6H), 1.60–1.44 (m, 4H), 1.42–1.38 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 168.2, 166.5, 147.6, 145.6, 141.1, 134.4, 131.3, 130.5, 129.3, 128.5, 124.5, 119.7, 108.2, 107.7, 107.3, 100.9, 63.5, 59.2, 48.7, 47.9, 47.3, 44.1, 42.3, 34.0, 32.4, 28.8, 28.0. LC/MS: 532^+ [H^+]. Purity: 95.5%.

*N-(2-oxo-2-4-[(4-phenylcyclohexyl)amino]piperidin-1-ylethyl)-3-(trifluoromethyl)benzamide (**21**).* Yield = 2%, ^{1}H NMR (400 MHz, MeOH-d4) δ: 8.21 (s, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.88 (d, J = 7.6 Hz, 1H), 7.70 (t, J = 7.6 Hz, 1H), 7.30–7.16 (m, 5H), 4.69 (d, J = 13.6 Hz, 1H), 4.45 (d, J = 16.4 Hz, 1H), 4.21–4.15 (m ,2H), 3.65–3.58 (m, 1H), 3.52 (s, 2H), 3.39–3.36 (m, 1H), 3.26–3.23 (m, 1H), 2.79 (t, J = 12.4 Hz, 1H), 2.59 (t, J = 11.6 Hz, 1H), 2.28–2.15 (m, 4H), 2.04–2.01 (m, 2H), 1.73–1.49 (m, 6H). LC/MS: 488^+ [H^+]. Purity: 99.6%.

*N-(2-oxo-2-((1-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**12**).* To a 50 mL round-bottom flask equipped with magnetic stirrer 0.72 mmol (1 equiv.) of iodobenzene, 0.8 mmol (1.1 equiv.) of *N*-(2-oxo-2-((1-(prop-2-yn-1-yl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**40**), 0.16 mmol (0.25 equiv.) of proline, 0.10 mmol (0.14 equiv.) of Na_2CO_3, 0.96 mmol (1.3 equiv.) of NaN_3 and 0.08 mmol (0.12 equiv.) of ascorbic acid were added and dissolved in a mixture of DMSO and H_2O (3:1). Then, 0.08 mmol (0.12 equiv.) of $CuSO_4$•$5H_2O$ were added and the reaction was heated at 80 °C for 48h. The mixture was quenched with 3% NH_4OH, extracted 5 times with 10 mL EtOAc, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Solids were recrystallized from a mixture of H_2O / Acetone = 1:1 and washed with petroleum ether. Yield = 4%. ^{1}H NMR (400 MHz, Acetone –d6) δ: 8.43 (s, 1H), 8.23–8.20 (m, 2H), 7.92–7.90 (m, 2H), 7.76 (t, J = 7.6 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 7.48 (m, 1H), 7.47 (t, J = 7.2 Hz, 1H), 7.32 (d, J = 6.4 Hz, 1H), 4.04 (d, J = 5.2 Hz, 2H), 3.69 (s, 2H), 2.22 (t, J = 11.2 Hz, 2H) 2.16 (s, 1H), 2.14 (s, 1H), 1.95 (s, 1H), 1.85 (m, 2H), 1.54 (q, J = 8.4 Hz, 1H), 1.21 (d, J = 4.8 Hz, 2H). LC/MS: 487^+ [H^+]. Purity: 95.7%.

*General procedure for the preparation compounds **15** and **17**.*

A round-bottom flask was loaded with 0.25 mmol (1 equiv.) of *N*-(2-oxo-2-(piperidin-4-ylamino)ethyl)-3-(trifluoromethyl)benzamide (**5**) and 0.25 mmol (1 equiv.) of the corresponding ketone. 40 mL of benzene together with 10 mol% p-toluenesulfonic acid and 4 Å MS were added. The reaction mixture was left to

reflux with a Dean-Stark setup for 48 hours. Then, to the cooled mixture 2.5 mmol (10 equiv.) sodium borohydride were added portionwise and the reaction was left to stir at room temperature for 24 h. The reaction mixture was quenched with 1M NaOH and extracted with DCM. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The product was purified by column chromatography (90:9:1 DCM:MeOH:NH_4OH).

*N-(2-oxo-2-((1-(4-(p-tolyl)cyclohexyl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**15**).* The product was obtained with a yield of 10%. ^{1}H NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 8.10 (s, 1H), 8.02 (s, 1H), 7.90 (s, 1H), 7.70 (d, J = 7.2 Hz, 1H), 7.53–7.52 (m, 1H), 7.17–7.02 (m, 4H), 4.09 (s, 2H), 3.94 (s, 1H), 3.47–3.21 (m, 3H), 2.98 (s, 1H), 2.84 (s, 2H), 2.46–2.40 (m, 1H), 2.31 (m, 4H), 2.17 (s, 1H), 2.09–2.01 (m, 4H), 1.85–1.78 (m, 2H), 1.70–1.51 (m, 3H). ^{13}C NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 141.9, 139.3, 136.3, 135.8, 134.6. 130.9, 130.7, 129.4, 128.4, 127.0, 126.6, 125.2, 124.5, 122.5, 65.5, 48.5, 44.8, 43.5, 42.7, 36.0. 32.5, 28.6, 26.9, 22.9, 21.1. LC/MS: 502^+ [H^+]. Purity: 98.9%.

*N-(2-((1-(4-(3,5-dimethoxyphenyl)cyclohexyl)piperidin-4-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide (**17**).* The product was obtained with a yield of 2%. ^{1}H NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 8.10 (s, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 8.0 Hz, 1H), 7.58 (t, J = 8.0 Hz, 1H), 7.30 (s, 1H), 7.17 (d, J = 7.6 Hz, 1H), 6.44 (d, J = 2.0 Hz, 1H), 6.32 (d, J = 2.4 Hz, 1H), 4.12 (t, J = 5.2 Hz, 2H), 4.05–3.97 (m, 1H), 3.78 (s, 6H), 3.55 (d, J = 10.4 Hz, 1H), 3.43 (d, J = 12.0 Hz, 1H), 3.25 (t, J = 10.4 Hz, 1H), 2.99 (s, 1H), 2.92 (s, 2H), 2.38 (d, J = 12.0 Hz, 2H), 2.25–2.02 (m, 5H), 1.94–1.85 (m, 2H), 1.69–1.56 (m, 1H). ^{13}C NMR (400 MHz, $CDCl_3$ + drop of MeOD) δ: 161.3, 144.8, 134.6, 130.6, 129.4, 124.5, 105.8, 105.0, 97.5, 65.5, 55.4, 48.6, 44.6, 43.5, 28.5, 22.9. LC/MS: 548^+ [H^+]. Purity: 97.7%.

*Synthesis of 4-methyl-N'-(1,4-dioxaspiro[4.5]decan-8-ylidene)benzenesulfonohydrazone (**23**).* In a microwave tube 3.2 mmol (1 equiv.) of 1,4-dioxaspiro[4,5]decan-8-one (**22**) was dissolved in 5 mL of dioxane and 3.5 mmol (1.1 equiv.) of tosylhydrazide was added. The reaction mixture was heated at 130 °C in the microwave for 1 h. Product crystallized upon cooling and was collected by filtration. Yield = 65%. ^{1}H NMR ($CDCl_3$) δ: 7.84 (d, J = 7.6 Hz, 2H), 7.61 (s, 1H), 7.31 (d, J = 7.6 Hz, 2H), 3.95 (s, 4H), 2.44–2.32 (m, 7H), 1.82–1.71 (m, 4H).

*Synthesis of 8-(3-methoxyphenyl)-1,4-dioxaspiro[4.5]dec-7-ene (**24**).* In a 20 mL microwave tube 2.0 mmol (1 equiv.) of 4-methyl-*N*'-(1,4-dioxaspiro[4.5]decan-8-ylidene)benzenesulfonohydrazone (**23**) is dissolved in 7 mL of dioxane and flushed with nitrogen gas. Next, 2 mol% of $Pd_2(dba)_3$, 4 mol% of 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (Xphos) and 2.8 equivalents of 1 M Li*t*-BuO in hexanes were added. The reaction mixture was stirred for 1 minute and 2.2 mmol (1.1 equiv.) of 3-bromoanisole was added. Reaction mixture was heated at 110 °C in the microwave for 24 h. Reaction mixture was quenched with saturated solution of $NaHCO_3$ in water and extracted with DCM. The organic layer was washed with brine and dried over $MgSO_4$. The product was purified by column chromatography (2:8 EtOAc/DCM). Yield = 24%. ^{1}H NMR ($CDCl_3$) δ: 7.21 (t, J = 8 Hz, 1H), 6.98 (d, J = 7.6 Hz, 1H), 6.93 (s, 1H), 6.77 (d, J = 8.4 Hz, 1H), 5.98 (s, 1H), 4.01 (s, 4H), 3.80 (s, 3H), 2.65 (br s, 2H), 2.46 (br s, 2H), 1.91 (t, J = 6.4 Hz, 2H).

*1,4-dioxaspiro[4.5]dec-7-en-8-yl trifluoromethanesulfonate (**25**).* An oven–dried round–bottom flask was flushed with N_2 gas and filled with 5 mL of dry THF. 3.9 mmol (1.3 equiv.) of 2 M solution of LDA in ethylbenzene was added. The reaction mixture was cooled to –78 °C. 3 mmol (1 equiv.) of ketone **22** was dissolved in 10 mL of dry THF and slowly added to the reaction mixture. The reaction mixture was stirred for 1 hour at –78 °C, half an hour at –25 °C and then cooled again to –78 °C. Next, 3.9 mmol (1.3 equiv.) of the *N*-phenyl-bis(trifluoromethanesulfonimide) was added to the reaction and the mixture was stirred for 4 hours at –78 °C after which it was stirred at room temperature overnight. The reaction mixture was quenched with H_2O and extracted with EtOAc. The organic layer was washed with brine (3x) and dried over $MgSO_4$. The product was purified by column chromatography (1:4 diethylether / petroleumether). Yield = 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.20 (t, J = 4.2 Hz, 1H), 4.04 (s, 4H), 2.81 – 2.75 (m, 1H), 2.48 (d, J = 7.6 Hz, 2H), 1.90 (t, J = 6.4 Hz, 2H).

*4,4,5,5-tetramethyl-2-(1,4-dioxaspiro[4.5]dec-7-en-8-yl)-1,3,2-dioxaborolane (**26**).* In a round–bottom flask 1.1 mmol (1.1 equiv.) of bis(pinacolato)diboron was dissolved in 50 mL of toluene and $PdCl_2(PPh_3)_2$ (0.03 equiv), triphenylphosphine (0.06 equiv) and potassium phenolate (1.5 equiv) were added. The reaction mixture was flushed with N_2 gas and 1 mmol (1 equiv.) of triflate **25** was added. The mixture was stirred at 50 °C under a nitrogen atmosphere. After 4 hours the heat source was removed and the reaction was stirred for 24 hours at room temperature. The reaction mixture was partitioned between H_2O/EtOAc and the organic layer was washed with brine and dried over $MgSO_4$. Column chromatography was performed using a gradient of 0-10% EtOAc in DCM as eluent. Yield = 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.47 (s, 1H), 3.98 (s, 4H), 2.39–2.36 (m, 4H), 1.73 (t, J = 6.4 Hz, 2H), 1.25 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 139.7, 108.0, 83.4, 64.5, 37.2, 31.2, 25.9, 25.0.

*Synthesis of 8-(2H-1,3-benzodioxol-5-yl)-1,4-dioxaspiro[4.5]dec-7-ene (**27**).* 0.7 mmol (1 equiv.) of triflate **25** was dissolved in 10 mL of dry THF. To the reaction mixture 0.77 mmol (1.1 equiv.) of 3,4-(methylenedioxy)phenylboronic acid, 2.31 mmol (3.3 equiv.) of potassium fluoride and 10 mol% of $Pd(dppf)Cl_2$ were added. The reaction mixture was stirred at room temperature overnight. Next, the reaction mixture was filtered over celite and purified with column chromatography (DCM). Yield = 58%. ^{1}H NMR δ: ($CDCl_3$): 6.89 (s, 1H), 6.85 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 8.0 Hz, 1H), 5.93 (s, 2H), 5.86 (t, J = 4.0 Hz, 1H), 4.01 (s, 4H), 2.60 (br s, 2H), 2.44 (s, 2H), 1.90 (t, J = 6.4 Hz, 2H).

*General procedure for the synthesis of compounds **28-32**.*

In a 20 mL microwave tube 0.75 mmol (1 equiv.) of the boron ester **26** was dissolved in 10 mL of dioxane and 5 mol% of $Pd(PPh_3)_4$ was added along with 0.75 mmol (1 equiv.) of the corresponding arylbromide. Next, 8 equivalents of 2 M Na_2CO_3 in H_2O solution was added and the reaction mixture was flushed with N_2 gas and capped. The reaction mixture was heated in the microwave for 10 hours at 80 °C. Upon completion, the reaction mixture was partitioned between DCM/H_2O and the organic layer was dried over $MgSO_4$. Column chromatography was performed with DCM as eluent.

*8-(2-methyl-phenyl)-1,4-dioxaspiro[4.5]dec-7-ene (**28**).* Yield = 90%, ^{1}H NMR ($CDCl_3$) δ: 7.16–7.06 (m, 4H), 5.44 (s, 1H), 4.01 (s, 4H), 2.45 (br s, 4H), 2.29 (s, 3H), 1.89 (t, J = 7.2 Hz, 2H).

*8-(3-methyl-phenyl)-1,4-dioxaspiro[4.5]dec-7-ene (**29**).* Yield = 99%, ^{1}H NMR ($CDCl_3$) δ: 7.23–7.16 (m, 3H), 7.03 (br s, 1H), 5.95 (s, 1H), 4.00 (s, 4H), 2.65 (br s, 2H), 2.46 (br s, 2H), 2.33 (s, 3H), 1.91 (t, J = 6.4 Hz, 2H).

*8-(4-methyl-phenyl)-1,4-dioxaspiro[4.5]dec-7-ene (**30**).* Yield = 84%, ^{1}H NMR ($CDCl_3$) δ: 7.26 (d, J = 6.8 Hz, 2H), 7.10 (d, J = 6.8 Hz, 2H), 5.94 (s, 1H), 4.02 (s, 4H), 2.64 (br s, 2H), 2.46 (br s, 2H), 2.33 (s, 3H), 1.92 (t, J = 6.4 Hz, 2H).

*8-(3,5-dimethoxyphenyl)-1,4-dioxaspiro[4.5]dec-7-ene (**31**).* Yield = 80%, ^{1}H NMR ($CDCl_3$) δ: 6.54 (d, J = 2 Hz, 2H), 6.35 (t, J = 2.2 Hz, 1H), 5.97 (t, J = 4.0 Hz, 1H), 4.01 (s, 4H), 3.78 (s, 6H), 2.63 (br s, 2H), 2.46 (br s, 2H), 1.92 (t, J = 6.4 Hz, 2H).

*8-(2,6-dimethoxyphenyl)-1,4-dioxaspiro[4.5]dec-7-ene (**32**).* Yield = 90%, ^{1}H NMR ($CDCl_3$) δ: 7.15 (t, J = 8.4 Hz, 1H), 6.54 (d, J = 8.4 Hz, 2H), 5.47 (s, 1H), 4.01 (s, 4H), 3.78 (s, 6H), 2.47–2.40 (m, 4H), 1.90 (t, J = 6.4 Hz, 2H).

*General procedure for the hydrogenation of the double bond of compounds **24, 27-32**.*

A round–bottom flask was purged with hydrogen gas and the corresponding cyclohexene was added. 4 wt% of Pd/C (10% wt) was added together with 2 mol% $Pd(OAc)_2$ and MeOH was added and hydrogen balloon. The reaction mixture was flushed with hydrogen and stirred overnight under hydrogen atmosphere. When the reaction was finished, the reaction mixture was filtered through celite. Column chromatography was performed if necessary, using 100% DCM as eluent. The obtained yields were 85-99%. All products showed correct mass in TLC/MS.

*General procedure for the synthesis of compounds **33-39**.* To a round–bottom flask was added 1 equivalent of the corresponding acetal. Mixture of DCM and acetone (4:1) was added. Finally 3.5 equivalents of $FeCl_3 \cdot 6H_2O$ were added and the reaction mixture was stirred at room temperature for 3.5

hours. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with DCM, the organic layer was washed with brine and dried over $MgSO_4$. The product was purified by column chromatography (DCM).

*4-(2-methylphenyl)cyclohexan-1-one (**33**).* This compound was obtained in 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25–7.10 (m, 4H), 3.26–3.19 (m, 1H), 2.58–2.50 (m, 4H), 2.41 (s, 3H), 2.17–2.13 (m, 2H), 1.97–1.79 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 211.2, 142.9, 135.2, 130.6, 126.5, 125.0, 41.8, 38.5, 33.2, 19.5.

*4-(3-methylphenyl)cyclohexan-1-one (**34**).* This compound was obtained in 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25–7.19 (m, 1H), 7.04 (d, J = 8.0 Hz, 3H), 3.02–2.94 (m, 1H), 2.55–2.47 (m, 4H), 2.32 (s, 3H), 2.22–2.17 (m, 2H), 2.17–1.88 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 211.3, 144.9, 138.2, 128.6, 127.5, 123.8, 42.8, 41.5, 34.1, 21.5.

*4-(4-methylphenyl)cyclohexan-1-one (**35**).* This compound was obtained in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.18 (s, 4H), 3.08–3.00 (m, 1H), 2.60–2.50 (m, 4H), 2.36 (s, 3H), 2.26–2.21 (m, 2H), 2.02–1.96 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 211.3, 141.9, 136.2, 129.1, 126.6, 42.4, 41.5, 34.1, 21.1.

*4-(3-methoxyphenyl)cyclohexan-1-one (**36**).* This compound was obtained in 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27–7.23 (m, 1H), 6.84 (d, J = 7.6Hz, 1H), 6.77 (s, 1H), 6.78 (s, 1H), 3.80 (s, 3H), 3.04–2.97 (m, 1H), 2.53–2.49 (m, 4H), 2.25–2.20 (m, 2H), 1.11–1.88 (m, 2H).

*4-(3,5-dimethoxyphenyl)cyclohexan-1-one (**37**).* This compound was obtained in 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.40 (s, 2H), 6.34 (s, 1H), 3.78 (s, 6H), 2.99–2.93 (m, 1H), 2.51 (d, J = 4.4Hz, 2H), 2.47 (d, J = 4.4Hz, 2H), 2.23–2.20 (m, 2H), 1.98–1.93 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 211.2, 161.0, 147.4, 105.1, 98.2, 55.4, 43.2, 41.4, 33.9.

*4-(2,6-dimethoxyphenyl)cyclohexan-1-one (**38**).* This product was obtained in 42% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25–7.08 (m, 1H), 6.53 (q, J = 6.0 Hz, 2H), 3.80 (s, 6H), 2.57–2.47 (m, 5H), 1.91–1.87 (m, 2H), 1.80–1.65 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 213.2, 158.6, 127.4, 126.9, 120.5, 109.1, 104.4, 55.8, 42.1, 35.8, 33.9, 29.7, 27.3.

*4-(2H-1,3-benzodioxol-5-yl)cyclohexan-1-one (**39**).* This product was obtained in 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.77–6.69 (m, 3H), 5.91 (s, 2H), 2.99–2.92 (m, 1H), 2.54–2.47 (m, 4H), 2.20–2.16 (m, 2H), 1.93–1.82 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 211.2, 147.9, 146.2, 138.9, 119.6, 108.4, 107.2, 101.0, 42.7, 41.4, 34.3.

*N-(2-oxo-2-((1-(prop-2-yn-1-yl)piperidin-4-yl)amino)ethyl)-3-(trifluoromethyl)benzamide (**40**).* In a 50 mL round–bottom flask 1.1 mmol (1.1 equiv.) of *N*-(2-oxo-2-(piperidin-4-ylamino)ethyl)-3-(trifluoromethyl)benzamide (**5**) was dissolved in 15 mL of acetone. Subsequently, 1.1 mmol (1.1 equiv.) of K_2CO_3 and 1 mmol (1 equiv.) of propargyl bromide were added. The mixture was refluxed overnight. The product precipitated form the reaction mixture and was collected by filtration. Yield = 99%.^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.21–8.18 (m, 2H), 7.76 (d, J = 8.0 Hz, 1H), 7.61–7.57 (m, 1H), 4.71 (s, 2H), 4.54 (s, 2H), 4.19–4.15 (m, 3H), 3.98–3.95 (m, 2H), 3.83–3.81 (m, 2H), 3.07 (s, 2H), 1.26 (s, 1H).

Abbreviations

AcOH, acetic acid; Boc, *tert*-butyloxycarbonyl; CCL2, chemokine ligand 2; CCR2, chemokine receptor 2; DCE, dichloroethane; DCM, dichloromethane; DiPEA, *N*,*N*-diisopropylethylamine; DMAP, *N*,*N*-dimethylaminopyridine; DMSO, dimethylsulfoxide; INCB3344, *N*-(2-(((3*S*,4*S*)-1-(4-(benzo[d][1,3]dioxol-5-yl)-4-hydroxycyclohexyl)-4-ethoxypyrrolidin-3-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide; JNJ Lead, *N*-(2-((1-((1*R*,4*R*)-4-(3-(dimethylamino)phenyl)-4-hydroxycyclohexyl)azetidin-3-yl)amino)-2-oxoethyl)-3-(trifluoromethyl)benzamide; KO, knock-out; KOPh, potassium phenolate; LDA, lithium diisopropylamide; Li*t*-BuO, lithium *tert*-butoxide; MeOH, methanol; MW, microwave; MS, molecular sieves; $Pd_2(dba)_3$, tris(dibenzylideneacetone)dipalladium(0); $Pd(dppf)Cl_2$, [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane; $Pd(OAc)_2$, palladium acetate; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); PEMB, 5-ethyl-2-methyl-

pyridine borane; PPh_3, triphenylphosphine; PyBrOP, bromo-tris-pyrrolidino phosphoniumhexafluorophosphate; SAR, structure–affinity relationships; TFA, trifluoroacetic acid; THF, tetrahydrofuran; U2OS, Human Bone Osteosarcoma Cells; XPhos, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl.

ACKNOWLEDGEMENTS

This study was financially supported by the Dutch Top Institute Pharma, project number D1-301. We thank Dr. Julien Louvel and Jacobus van Veldhoven for their input in analytical data analysis. We acknowledge Prof. Dr. Martine Smit (Vrije Universiteit, Amsterdam, The Netherlands) for helpful comments and suggestions.

REFERENCES

1. Deshmane, S. L.; Kremlev, S.; Amini, S.; Sawaya, B. E. Monocyte Chemoattractant Protein-1 (MCP-1): An Overview. *Journal of Interferon and Cytokine Research* **2009,** 29, 313-326.
2. Daly, C.; Rollins, B. J. Monocyte chemoattractant protein-1 (CCL2) in inflammatory disease and adaptive immunity: therapeutic opportunities and controversies. *Microcirculation* **2003,** 10, 247-57.
3. Dawson, T. C.; Kuziel, W. A.; Osahar, T. A.; Maeda, N. Absence of CC chemokine receptor-2 reduces atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis* **1999,** 143, 205-11.
4. Kamei, N.; Tobe, K.; Suzuki, R.; Ohsugi, M.; Watanabe, T.; Kubota, N.; Ohtsuka-Kowatari, N.; Kumagai, K.; Sakamoto, K.; Kobayashi, M.; Yamauchi, T.; Ueki, K.; Oishi, Y.; Nishimura, S.; Manabe, I.; Hashimoto, H.; Ohnishi, Y.; Ogata, H.; Tokuyama, K.; Tsunoda, M.; Ide, T.; Murakami, K.; Nagai, R.; Kadowaki, T. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *Journal of Biological Chemistry* **2006,** 281, 26602-26614.
5. Fife, B. T.; Huffnagle, G. B.; Kuziel, W. A.; Karpus, W. J. CC chemokine receptor 2 is critical for induction of experimental autoimmune encephalomyelitis. *The Journal of Experimental Medicine* **2000,** 192, 899-905.
6. White, F. A.; Feldman, P.; Miller, R. J. Chemokine Signaling and the Management of Neuropathic Pain. *Molecular Interventions* **2009,** 9, 188-195.
7. Kim, Y. K.; Oh, H. B.; Lee, E. Y.; Gho, Y. S.; Lee, J. E.; Kim, Y. Y. Association between a genetic variation of CC chemokine receptor-2 and atopic asthma. *Allergy* **2007,** 62, 208-9.
8. Xia, M.; Sui, Z. Recent developments in CCR2 antagonists. *Expert Opinion on Therapeutic Patents* **2009,** 19, 295-303.
9. Xia, M.; Hou, C.; DeMong, D. E.; Pollack, S. R.; Pan, M.; Brackley, J. A.; Jain, N.; Gerchak, C.; Singer, M.; Malaviya, R.; Matheis, M.; Olini, G.; Cavender, D.; Wachter, M. Synthesis, structure-activity relationship and in vivo antiinflammatory efficacy of substituted dipiperidines as CCR2 antagonists. *Journal of Medicinal Chemistry* **2007,** 50, 5561-3.
10. Pease, J. E.; Horuk, R. Chemokine receptor antagonists: Part 1. *Expert Opinion on Therapeutic Patents* **2009,** 19, 39-58.
11. Struthers, M.; Pasternak, A. CCR2 antagonists. *Current Topics in Medicinal Chemistry* **2010,** 10, 1278-98.
12. Carter, P. H. Progress in the discovery of CC chemokine receptor 2 antagonists, 2009 - 2012. *Expert Opinion on Therapeutic Patents* **2013,** 23, 549-68.
13. Ben-Zeev, E.; Chen, D.; Fichman, M.; Ghosh, S.; Koerner, S. i.; Marantz, Y.; Melendez, R.; Mohanty, P.; Shacham, S.; Zhang, Z. Carboxamide compounds and their use as antagonists of the chemokine ccr2 receptor. *Patent WO2009076404,* **2009**.
14. Moree, W. J.; Kataoka, K.; Ramirez-Weinhouse, M. M.; Shiota, T.; Imai, M.; Sudo, M.; Tsutsumi, T.; Endo, N.; Muroga, Y.; Hada, T.; Tanaka, H.; Morita, T.; Greene, J.; Barnum, D.; Saunders, J.; Kato, Y.; Myers, P. L.; Tarby, C. M. Small molecule antagonists of the CCR2b receptor. Part 2: Discovery process and initial structure-activity relationships of diamine derivatives. *Bioorganic & Medicinal Chemistry Letters* **2004,** 14, 5413-5416.
15. Xue, C. B.; Wang, A.; Meloni, D.; Zhang, K.; Kong, L.; Feng, H.; Glenn, J.; Huang, T.; Zhang, Y.; Cao, G.; Anand, R.; Zheng, C.; Xia, M.; Han, Q.; Robinson, D. J.; Storace, L.; Shao, L.; Li, M.; Brodmerkel, C. M.; Covington, M.;

Scherle, P.; Diamond, S.; Yeleswaram, S.; Vaddi, K.; Newton, R.; Hollis, G.; Friedman, S.; Metcalf, B. Discovery of INCB3344, a potent, selective and orally bioavailable antagonist of human and murine CCR2. *Bioorganic & Medicinal Chemistry Letters* **2010,** 20, 7473-8.

16. Zhang, X.; Hufnagel, H.; Markotan, T.; Lanter, J.; Cai, C.; Hou, C.; Singer, M.; Opas, E.; McKenney, S.; Crysler, C.; Johnson, D.; Sui, Z. Overcoming hERG activity in the discovery of a series of 4-azetidinyl-1-aryl-cyclohexanes as CCR2 antagonists. *Bioorganic & Medicinal Chemistry Letters* **2011,** 21, 5577-82.

17. Vilums, M.; Zweemer, A. J. M.; Yu, Z. Y.; de Vries, H.; Hillger, J. M.; Wapenaar, H.; Bollen, I. A. E.; Barmare, F.; Gross, R.; Clemens, J.; Krenitsky, P.; Brussee, J.; Stamos, D.; Saunders, J.; Heitman, L. H.; IJzerman, A. P. Structure-Kinetic Relationships-An Overlooked Parameter in Hit-to-Lead Optimization: A Case of Cyclopentylamines as Chemokine Receptor 2 Antagonists. *Journal of Medicinal Chemistry* **2013,** 56, 7706-7714.

18. Frerot, E.; Coste, J.; Pantaloni, A.; Dufour, M. N.; Jouin, P. Pybop and Pybrop - 2 Reagents for the Difficult Coupling of the Alpha,Alpha-Dialkyl Amino-Acid, Aib. *Tetrahedron* **1991,** 47, 259-270.

19. Barluenga, J.; Quinones, N.; Cabal, M. P.; Aznar, F.; Valdes, C. Tosylhydrazide-Promoted Palladium-Catalyzed Reaction of beta-Aminoketones with o-Dihaloarenes: Combining Organocatalysis and Transition-Metal Catalysis. *Angewandte Chemie-International Edition* **2011,** 50, 2350-2353.

20. Feldman, A. K.; Colasson, B.; Fokin, V. V. One-pot synthesis of 1,4-disubstituted 1,2,3-triazoles from in situ generated azides. *Organic Letters* **2004,** 6, 3897-3899.

21. Xue, C. B.; Feng, H.; Cao, G. F.; Huang, T. S.; Glenn, J.; Anand, R.; Meloni, D.; Zhang, K.; Kong, L. Q.; Wang, A. L.; Zhang, Y. X.; Zheng, C. S.; Xia, M.; Chen, L. H.; Tanaka, H.; Han, Q.; Robinson, D. J.; Modi, D.; Storace, L.; Shao, L. X.; Sharief, V.; Li, M.; Galya, L. G.; Covington, M.; Scherle, P.; Diamond, S.; Emm, T.; Yeleswaram, S.; Contel, N.; Vaddi, K.; Newton, R.; Hollis, G.; Friedman, S.; Metcalf, B. Discovery of INCB3284, a Potent, Selective, and Orally Bioavailablle hCCR2 Antagonist. *Acs Medicinal Chemistry Letters* **2011,** 2, 450-454.

22. Zhang, X. Q.; Hou, C. F.; Hufnagel, H.; Singer, M.; Opas, E.; McKenney, S.; Johnson, D.; Sui, Z. H. Discovery of a 4-Azetidinyl-1-thiazoyl-cyclohexane CCR2 Antagonist as a Development Candidate. *Acs Medicinal Chemistry Letters* **2012,** 3, 1039-1044.

23. Xue, C. B.; Wang, A. L.; Han, Q.; Zhang, Y. X.; Cao, G. F.; Feng, H.; Huang, T. S.; Zheng, C. S.; Xia, M.; Zhang, K.; Kong, L. Q.; Glenn, J.; Anand, R.; Meloni, D.; Robinson, D. J.; Shao, L. X.; Storace, L.; Li, M.; Hughes, R. O.; Devraj, R.; Morton, P. A.; Rogier, D. J.; Covington, M.; Scherle, P.; Diamond, S.; Emm, T.; Yeleswaram, S.; Contel, N.; Vaddi, K.; Newton, R.; Hollis, G.; Metcalf, B. Discovery of INCB8761/PF-4136309, a Potent, Selective, and Orally Bioavailable CCR2 Antagonist. *Acs Medicinal Chemistry Letters* **2011,** 2, 913-918.