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

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CHAPTER 5

EVALUATION OF (4-ARYLPIPERIDIN-1-YL)CYCLOPENTANECARBOXAMIDES AS HIGH AFFINITY AND LONG RESIDENCE TIME ANTAGONISTS FOR THE CCR2 RECEPTOR

This chapter was based upon:

M. Vilums, A.J.M. Zweemer, A. Dilanchian, J.P.D. van Veldhoven, H. de Vries, J. Brussee, D. Stamos, J. Saunders, L.H. Heitman, A.P. IJzerman.

(manuscript in preparation)

ABSTRACT

Preclinical animal models suggest that the CCL2/CCR2 axis plays an important role in the development and maintenance of inflammatory disease states (e.g., multiple sclerosis, atherosclerosis, asthma, diabetes, and neuropathic pain), which could be treated through inhibition of the CCR2 receptor. However, until now all high–affinity CCR2 antagonists that were advanced into clinical trials have failed due to the lack of efficacy. We have previously described a new approach for the design of CCR2 antagonists by the use of structure–kinetics relationships (SKR). Here we report new findings on the SAR and SKR of the reference compound MK-0483, its diastereomers, and structural analogues of it as CCR2 antagonists. On the "right–hand" side of the molecules the 7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline group generally yields better affinity and longer drug–target residence time (RT). On the "left–hand" side SAR of the phenyl ring suggests that lipophilic hydrogen bond accepting substituents on the 3-position are favourable. However, SKR suggests that a lipophilic group with a certain size is desired (e.g. 3-Br, 3-*i*-Pr), as present in compounds **21** and **22** (K_i = 2.8 and 3.6 nM, RT = 243 and 266 min, respectively). Alternatively a shielded hydrogen bond can also prolong the residence time; this was most prominently observed in MK-0483 (K_i = 1.2 nM, RT = 724 min) and its close analogue **26** (K_i = 7.8 nM) with a short residence time.

INTRODUCTION

Chemotactic cytokines (chemokines) play a vital role in the activation and regulation of leukocyte trafficking[1] and are involved in immunomodulation and host–defence mechanisms.[2, 3] Their chemoattractant activity is mediated through activation of cell–surface seven–transmembrane spanning G protein–coupled receptors (GPCRs). It seems that most of the chemokines are promiscuous in their actions since they can bind to numerous receptors.[4] However, monocyte chemoattractant protein-1 (MCP-1/CCL2) only binds and activates the CC-chemokine receptor 2 (CCR2) and the axis of CCL2/CCR2 has been suggested to be involved in various autoimmune and inflammation-associated diseases (e.g. multiple sclerosis, atherosclerosis, asthma, diabetes, and neuropathic pain).[5-9]

As a consequence there has been an increasing interest in advancing CCR2 receptor antagonists into clinical studies. However, thus far, high–affinity CCR2 antagonists have failed to show efficacy in phase 2 clinical trials. Recently several reviews[10-12] have suggested to incorporate an additional parameter coined "drug–target residence time (RT)" in the early drug discovery process. RT is thought to be correlated to drug efficacy, and could serve as an early criterion to diminish the attrition rate in later stages of drug development. We have previously described how the implementation of this additional parameter in the hit–to–lead optimization process helped us to distinguish between different structures for optimization.[13] Instead of proceeding with the highest affinity hit (**1**) (Figure 1) having a very short RT (a close analogue of MK-0812, which failed to show efficacy in clinical trials) we continued with a structure having moderate affinity only but with longer RT on the CCR2 receptor.

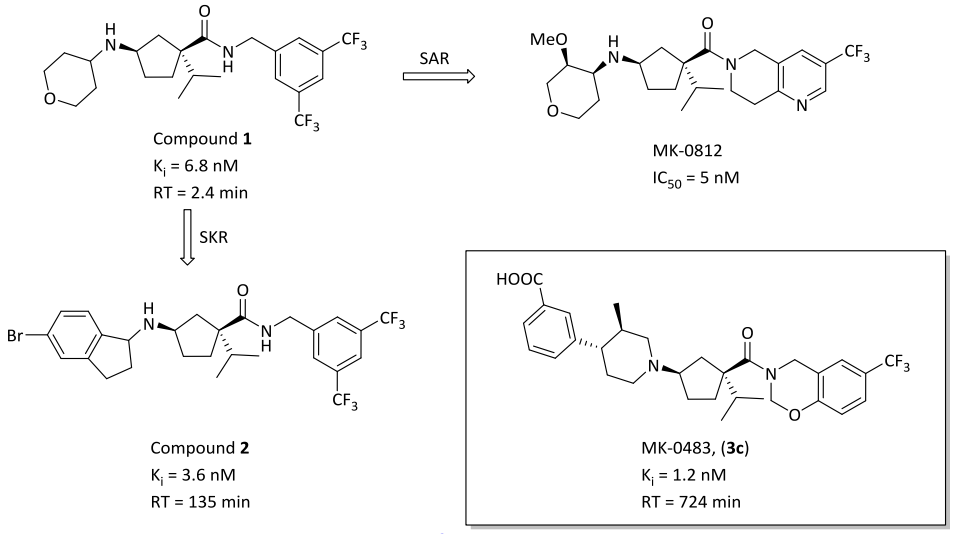


Figure 1. Structures of CCR2 antagonists **1**, **2**,[13] MK-0812 (Merck`s clinical candidate) [14] and MK0483 (Merck`s back-up clinical candidate).[15]

In the optimization process we improved both parameters simultaneously yielding a high–affinity and long RT CCR2 antagonist (**2**). However, the Merck research group has also reported on the discovery of MK-0483 (**3c**) a potent and orally bioavailable CCR2 and CCR5 dual antagonist, having a very slow dissociation halflife from the CCR2 receptor ($T_{1/2}$ > 9 h).[15] Struthers and Pasternak[14] described MK-0483 (**3c**) as the backup compound of the clinical candidate MK-0812, however, after the failure of MK-0812 in the clinical trials there is no information on further advancement of MK-0483 (**3c**) despite its slow dissociation kinetics.

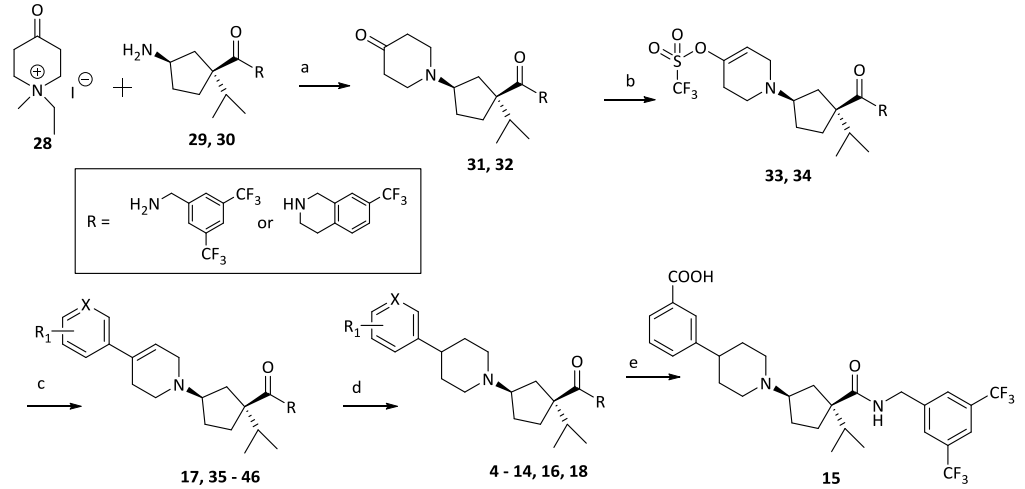
In this study, we used the knowledge from our previous findings[13] to evaluate the binding kinetics of MK-0483 (**3c**), its diastereomers (**3a**, **b**, **d**) and structural analogues that we synthesized (**4 - 27**) and to determine the structure–kinetics relationships (SKR) on the CCR2 receptor for this class of compounds. In a step–by–step manner we classified substituents on the 4 position of the piperidine ring to assess their importance in binding kinetics. In addition, we evaluated different amide substituents on the right–hand side of the molecule for the same purpose.

RESULTS AND DISCUSSION

Chemistry

Synthesis of MK-0483 (**3c**) and its diastereomers (**3a**, **b**, **d**) was achieved following the synthetic approach reported by Pasternak et.al.[15] For the synthesis of MK-0483 analogues (**4** - **18**) we designed a new synthetic route, which allowed us to incorporate different substituents directly on the *cis*-cyclopentane isomer in the one before last step of the synthesis (Scheme 1).

Scheme 1.[a]

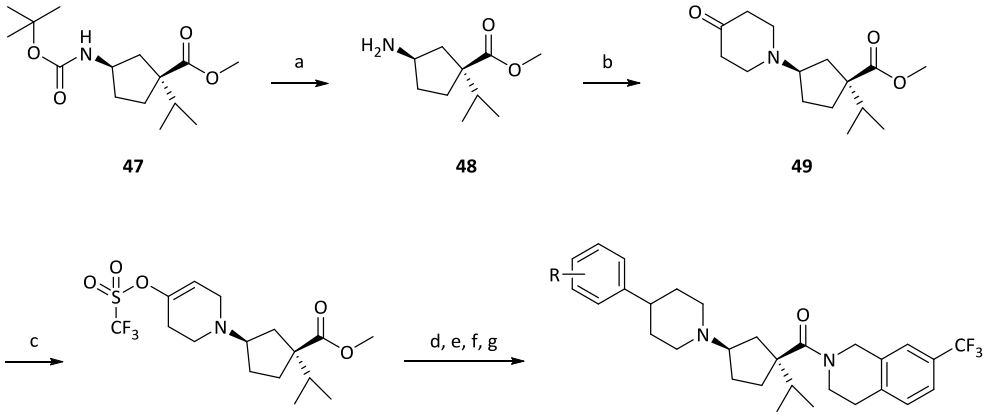


[a]Reagents and conditions: a) K_2CO_3, EtOH/H_2O (3:1), reflux, 5 h, (25-72%; b) i) LDA, dry THF, –78 °C → –20 °C; ii) *N*-phenyl-bis(trifluoromethanesulfonimide), –78 °C → room temperature, (21-42%); c) corresponding arylboronic acid, LiCl, 2 M $Na_2CO_{3(aq)}$, $Pd(PPh_3)_4$, DME, 90 °C, 3.5 h, (20-99%); d)Pd/C, $Pd(OAc)_2$, H_2 1 atm, MeOH or THF, (9-70%); e) ester **16**, 4 M $LiOH_{(aq)}$, H_2O/EtOH, 50 °C, 1.5 h, (53%). For designation of R_1, see Table 2 and 3.

We started with a sequenced Hoffman elimination and conjugated addition[16] of *N*,*N*-ethyl-methyl-4-oxo-piperidinium iodide[17] (**28**) to amines **29** and **30**, which were obtained following the synthetic approaches described earlier by our group.[18] Piperidones **31** and **32** were deprotonated with lithium diisopropylamide (LDA) and subsequently treated with *N*-phenyl-bis(trifluoromethanesulfonimide) to generate triflates **33** and **34**. These triflates were used in a Suzuki–coupling with different arylboronic acids, and subsequent hydrogenation of the

formed olefins (**17**, **35** - **46**) resulted in the final compounds (**4 - 14**, **16**, **18**) with the desired groups on the 4 position of the piperidine ring. Additional saponification of compound **16** yielded benzoic acid **15**. Any attempts to introduce halogen substituents on the aryl group using this synthetic route resulted in dehalogenation products during the hydrogenation step. We argued that the hydrogenation could be more selective if less bulky structures were used. Therefore we decided to reverse the synthetic route and modify the left–hand side of the molecules prior to peptide coupling on the right–hand side (Scheme 2). The synthesis of (1*S*,3*R*)-methyl-3-((*tert*-butoxycarbonyl)amino)-1-isopropylcyclopentanecarboxylate **47** was achieved following the synthetic approach reported by Kothandaraman et al.[19] The TFA mediated *N*-Boc deprotection yielded amine **48**, which was used in a coupling reaction with the piperidone salt **28**. The coupling was performed in several smaller batches in parallel as big scale reactions resulted in poor yields. Subsequently the triflate **50** was generated form the piperidone **49** under the same conditions as for compounds **33** and **34**. The triflate **50** was coupled with corresponding boronic acids and the obtained olefins were hydrogenated using PtO_2. In this case only traces of the dehalogenated products were observed.

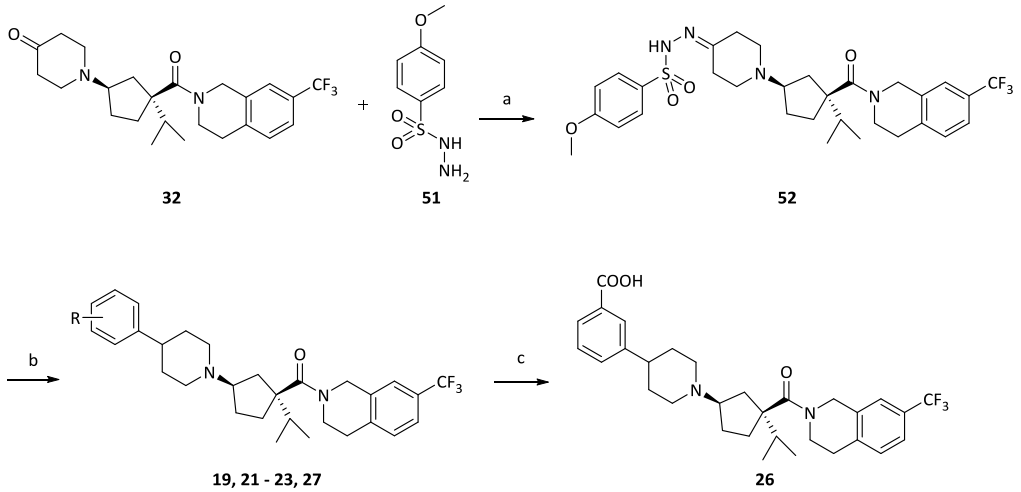
Scheme 2.[a]



[a]Reagents and conditions: a) TFA, DCM, 2 h, room temperature (98%); b) K_2CO_3, EtOH/H_2O (3:1), reflux, 3 h (56%); c) i) LDA, dry THF, –100 °C → –40 °C; ii) *N*-phenyl-bis(trifluoromethanesulfonimide), –80 °C → room temperature (67%); d) corresponding arylboronic acid, LiCl, 2 M $Na_2CO_{3(aq)}$, $Pd(PPh_3)_4$, DME, 90 °C, 3.5 h; e)PtO_2, H_2 2 atm, THF, room temperature, 10-15 min; f) 4 M $LiOH_{(aq)}$, H_2O/EtOH, reflux, 3 h; g) 7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride, PyBrOP, DIPEA, DMAP, DCM, room temperature, 48 h, (yield in four steps = 0.8-9%). For designation of R, see Table 3.

Next, a saponification of the esters and peptide coupling under bromo-tris-pyrrolidino phosphoniumhexafluorophosphate (PyBroP) conditions yielded final compounds **20**, **24**, and **25**. However, this approach gave poor overall yields and was abandoned for further synthesis. Recently, Allwood et al.[20] described a new, metal–free reductive coupling of saturated heterocyclic sulfonylhydrazones with boronic acids as an alternative to the tedious step–by–step route depicted in scheme 1. Analogous to the method described by Allwood et al., the tosylhydrazone **52** was obtained in a quantitative yield from piperidone intermediate **32** and sulfonylhydrazide **51** (Scheme 3).[20] Subsequently, the reductive coupling of the corresponding arylboronic acids and the tosylhydrazone **52** resulted in the desired products, which were purified by preparative HPLC yielding the final compounds **19**, **21 – 23**, **27** as TFA salts. The methylester **27** was saponified to give the carboxylic acid derivative **26** as a white HCl salt.

Scheme 3.[a]



[a]Reagents and conditions: a) MeOH, room temperature, 4 h (100%); b) corresponding boronic acid, Cs_2CO_3, dry 1,4-dioxane, 100 °C, 18 h, (8-23%); c) ester **27**, 4 M $LiOH_{(aq)}$, H_2O/EtOH, 50 °C, 2 h, (65%). For designation of R, see Table 3.

Biology

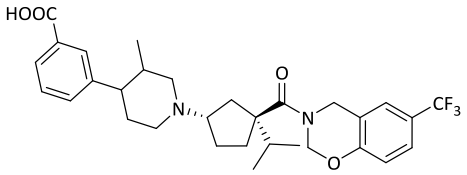
To determine their binding affinity all compounds were tested in a ^{125}I-CCL2 radioligand displacement assay on U2OS–CCR2 membrane preparations as described previously by our group.[21] Next to all four MK-0483 diastereomers compounds with affinities lower than or

equal to 100 nM were subsequently screened in a [^{3}H]INCB3344 dual point competition association assay on U2OS–CCR2 membrane preparations to determine their kinetic–rate–index (KRI), which served as an indicator for the magnitude of the RT. Compounds with a KRI >1 were finally tested in the full competition association assay to determine the RT, as described previously by our group.[18]

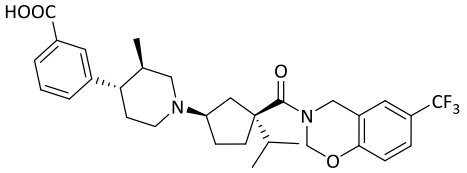
Structure–Affinity Relationships and Structure–Kinetics Relationships

The 3-piperidinylcyclopentanecarboxamide scaffold has been extensively evaluated by the Merck research group for CCR2 receptor binding.[22-24] All these efforts resulted in the discovery of MK-0483, which, upon tritiation, served as a radioligand. This [^{3}H]–MK-0483 bound to monocytes and appeared to have very slow receptor dissociation kinetics.[15] We decided to resynthesize MK-0483 and its diastereomers and to evaluate these four compounds in our various binding assays on the CCR2 receptor to determine the structural components responsible for both the high affinity and the long RT of MK-0483. Following the synthetic approach reported by Pasternak et.al.[15] we were able to generate the same four diastereomers and assigned them **3a-d** according to the sequence of elution form the preparative chiral HPLC, respectively. First, we determined their affinity in a ^{125}I-CCL2 competition displacement assay in which the *trans*-cyclopentane isomers (**3a** and **3b**) showed only moderate affinity (K_i = 59 and 383 nM, respectively) (Table 1). However, the *cis*-cyclopentane isomers (**3c** and **3d**) had high affinity for the CCR2 receptor (K_i = 1.2 and 11 nM, respectively) which is in accordance with the reported values.[15] Next, we evaluated all four diastereomers in the competition–association assay as this could help us to understand the importance of 3D conformation for SKR.

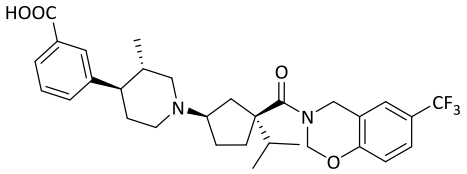
Table 1. Binding affinities, association and dissociation rate constants, and residence times of MK-0483 and its diastereomers



3a, b



3c, (MK-0483)



3d

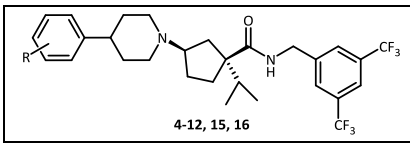
Nr.	K_i (nM) ± SEM (n = 3)	k_{on} (nM^{-1} min^{-1})	k_{off} (min^{-1})	RT (min)
3a	59 ± 2	0.00044 ± 0.0004	0.0028 ± 0.0004	357 ± 51
3b	383 ± 19	0.000058 ± 0.000007	0.0037 ± 0.0007	270 ± 51
3c, (MK-0483)	1.2 ± 0.1	0.020 ± 0.002	0.0014 ± 0.00004	724 ± 20
3d	11 ± 2.6	0.013 ± 0.001	0.018 ± 0.002	56 ± 6

Surprisingly, both *trans*-isomers (**3a** and **3b**), despite their moderate affinity, had similar and very slow dissociation characteristics translating in residence times longer than 4 hours (RT = 357 and 270 min, respectively) (Table 1), whereas the association rate constants displayed a 7-fold difference (k_{on} = 0.00044 and 0.000058 nM^{-1} min^{-1}, respectively) causing also the difference in affinity. The opposite was observed in the case of *cis*-isomers **3c** and **3d**. For these compounds, the association rate constants were similar and the dissociation rate constants showed a more than 12-fold difference, yielding residence times of 724 (**3c**) and 56 min (**3d**), respectively, which is also in line with the reported halflife value for MK-0483 (**3c**) by the Merck researchers.[15] Apparently, the *cis*-isomers have the best conformation for the eventual binding state on the receptor (affinity), reach that state quickly as can be gauged from their relatively fast k_{on} values, and stay there for prolonged times (Table 1). The only difference for the *trans*-isomers is their relatively slow association rate, but still they are capable of binding to the CCR2 receptor, and display long residence times as well.

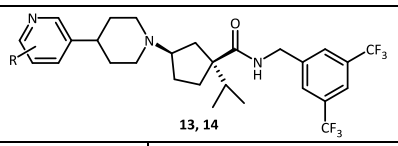
Bis(trifluoromethyl)benzyl Derivatives 4-16

From the above we chose to continue with *cis*-analogues of MK-0483 and decided to synthesize several new analogues of MK-0483 and a small number of known[15, 24] compounds to describe the SAR and SKR for the CCR2 receptor in this series of structures. In the first array we generated the more flexible (on the right–hand side) bis(trifluoromethyl)benzyl derivatives with different substituents on the aromatic ring connected to the piperidine (**4-16**) (Table 2). The unsubstituted phenyl compound (**4**) had an affinity of 1.5 nM for CCR2, which was best in class. However, in the dual–point competition–association assay it yielded a KRI below unity (KRI = 0.7), indicative of a short RT. Incorporation of the 2-methoxy group (compound **5**) resulted in a decrease of both the affinity and the KRI value (K_i = 28 nM, KRI = 0.6). The 3-methoxy group (compound **6**) yielded a small regain in the affinity (correlating with the reported values)[24] and the KRI value (K_i = 5.9 nM, KRI = 0.7). However, the 4-methoxy derivative (**7**), despite its moderate affinity (K_i = 29 nM), had a KRI value of 0.8. The double substitution of 3,4-di-methoxy (compound **8**) resulted in a big decrease in both the affinity and the KRI value. Closing the methoxy groups to yield a benzo[1,3]dioxole ring (compound **9**) yielded a minor improvement in affinity, however, it did not enhance the KRI value (KRI = 0.6). Lipophilic groups on the 4 position (e.g. 4-OCF_3, 4-*t*-Bu) resulted in an even bigger decrease of the affinity (compound **10**, 4-OCF_3) or a complete loss of the affinity (compound **11**, 4-*t*-Bu). The hydrophilic 4-hydroxy group (compound **12**) boosted affinity somewhat but still had a lower KRI value compared to the 4-methoxy derivative (**7**).

Table 2. Binding affinities and KRI values of compounds **4**-**16**.



4-12, 15, 16



13, 14

Nr.	R	K_i (nM) ± SEM (n = 3)	KRI (n=2)
4	H	1.5 ±0.1	0.7 (0.6/0.7)
5	2-OMe	28 ± 2	0.6 (0.5/0.7)
6	3-OMe	5.9 ± 1.7	0.7 (0.6/0.8)
7	4-OMe	29 ± 2	0.8 (0.7/0.9)
8	3,4-di-OMe	48 ± 1	0.6 (0.5/0.6)

9	3,4-OCH_2O-	17 ± 3	0.6 (0.6/0.7)
10	4-OCF_3	122 ± 20	-
11	4-*t*Bu	7%[a]	-
12	4-OH	17 ± 2	0.6 (0.6/0.6)
13	H	5.4 ± 0.9	0.6 (0.6/0.6)
14	4-OMe	76 ± 14	0.5 0.5/0.6)
15	3-COOH	22 ± 6	0.8 (0.8/0.8)
16	3-COOMe	7.9 ± 1.6	0.6 (0.5/0.7)

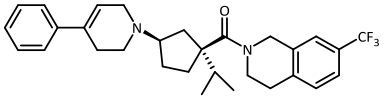
[a]Percent displacement at 1 μM ^{125}I-CCL2.

Comparing the 3-pyridine (**13**) with phenyl ring (**4**) also resulted in a minor decrease in affinity (correlating with the reported values)[24] and the KRI value (K_i = 5.4 nM, KRI = 0.6). The combination of 4-methoxy and 3-pyridine (compound **14**) yielded a 50-fold decrease in affinity compared to **4** and the smallest KRI value observed in this study (KRI = 0.5). Apparently, any substitution on the phenyl ring is not favorable and results in a decrease in affinity compared to the unsubstituted phenyl ring (**4**), although the 3-substituents are tolerated more than others. The evaluation of direct analogues of MK-0483, such as the 3-carboxylic acid (compound **15**), resulted in a 14-fold decrease in affinity (K_i = 22 nM, in agreement with the reported values)[24] compared to **4,** however, the KRI value increased to 0.8, whereas the methyl ester (**16**) had better affinity than the acid (K_i = 7.9 nM), but a smaller KRI value.

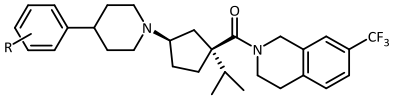
7-(Trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline Derivatives 17-27

The rigidification on the right–hand side into the 7-(trifluoromethyl)-1,2,3,4-tetrahyroisoquinoline group provided compounds **17-27**. The unsubstituted phenyl intermediate (olefin **17**) had a 14-fold lower affinity than its saturated analogue **18** and two-fold shorter RT (Table 3) indicating that the phenyl ring should be positioned in an angle to the piperidine ring for optimal binding.

Table 3. Binding affinities, KRI values and residence times of compounds **17**-**27**.



17



18-27

Nr.	R	K_i (nM) ± SEM (n = 3)	KRI (n=2)	RT (min)
17	-	70 ± 25	1.1 (1.1/1.0)	38 ± 4
18	H	4.4 ± 0.7	1.1 (1.2/1.0)	91 ± 25
19	3-OMe	0.95 ± 0.22	0.9 (0.8/0.9)	-
20	3-Cl	2.6 ± 0.3	1.0 (0.9/1.2)	200 ± 29
21	3-Br	2.8 ± 0.2	1.0 (1.0/1.1)	243 ± 45
22	3-*i*Pr	3.6 ± 0.4	1.0 (1.0/0.9)	266 ± 48
23	3-CF_3	5.3 ± 0.3	1.0 (0.9/1.1)	158 ± 35
24	4-Cl	2.0 ± 0.3	0.8 (0.8/0.8)	-
25	4-CF_3	7.2 ± 0.2	0.8 (0.8/0.9)	-
26	3-COOH	7.8 ± 1.4	0.7 (0.7/0.7)	-
27	3-COOMe	0.91 ± 0.25	0.8 (0.8/0.8)	-

From the bis(trifluoromethyl)benzyl series (Table 2) we learned that substituents on the 3 position were better tolerated so therefore we focused on this position in this array of compounds. The 3-methoxy derivative (**19**) had excellent affinity (K_i = 0.95 nM), but the KRI was below unity. Changing from 3-methoxy to 3-chloro (**20**) resulted in a small decrease in affinity, but an increase in RT. The 3-bromo derivative (**21**) had similar affinity as 3-chloro, but a longer RT. We have previously reported a similar correlation in another series of CCR2 antagonists where the methoxy substituents yielded the best affinity compounds, while the halogen derivatives had a longer RT.[13] The 3-isopropyl substituent (**22**) being similar in size to the bromine but having electron donating properties yielded only a minor decrease in the affinity and a minor increase in the RT, thus suggesting the importance of the space–filling properties of the substituents. The 3-trifluoromethyl derivative (**23**) (bioisoster of chlorine) showed similar results as **20** (3-Cl). In our effort to increase the RT we also explored the 4 position by introducing the lipophilic 4-chloro (**24**) and 4-trifluoromethyl (**25**) groups. However, despite the good affinity, both compounds had KRI values below unity (KRI = 0.8 for both).

A direct analogue of MK-0483 with an acid group on the 3 position (**26**) resulted in a minor decrease in affinity (comparable to the reported values),[15] but we noticed a substantial

decrease in RT (KRI = 0.7). Apparently, either the additional methyl group on the 3 position of the piperidine ring, the oxygen atom in the 3,4-dihydro-2*H*-benzo[e][1,3]oxazine ring or the combination of both is responsible for the long residence time of MK-0483. The compound with a corresponding methyl ester (**27**) yielded the best affinity in this study, although it had a short RT (K_i = 0.91 nM, KRI = 0.8).

CONCLUSIONS

We have evaluated the SAR and SKR of MK-0483 (**3c**), its diastereomers (**3a**, **b**, **d**) and structural analogues (**4** - **27**) as CCR2 antagonists. On the right–hand side of the molecules a rigid 7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline moiety yields better affinity than the more flexible bis(trifluoromethyl)benzyl substituent and generally prolongs the drug–target residence time. On the left–hand side SAR of the phenyl ring suggests that lipophilic hydrogen bond accepting substituents on the 3 position (e.g. 3-OMe (**19**), 3-COOMe (**27**)) are vital for improved affinity. However, the SKR suggests that to have long RT a lipophilic group with a certain size is desired (e.g. 3-Br (**21**), 3-*i*-Pr (**22**)) or alternatively (in the case of MK-0483 (**3c**)) a carboxylic acid group can be used. However, the acid group *per se* does not provide the long RT for these structures. Possibly the additional methyl group on the piperidine ring (as seen in MK-0483 (**3c**)) provides shielding of a hydrogen bond formed between the acid group and the receptor. We have reported similar observations on shielding in another series of CCR2 antagonists, moreover, a similar idea was put forward by Schmidtke et al.[25] in calculations on hydrogen bond shielding. Likewise, the additional oxygen atom in the 3,4-dihydro-2*H*-benzo[e][1,3]oxazine ring of MK-0483 (**3c**) could make an additional hydrogen bond and cause its long RT as the hydrogen bond would be shielded by the ring system itself. In conclusion, this study contributes to one of the first detailed structure–kinetics relationships for CCR2 antagonists which can help to develop better drug candidates. Moreover, the value of the long RT drugs has already been proven on other targets, although only in retrospect.[10] From this perspective, MK-0483 (**3c**) would be a suitable candidate to be deliberately advanced into clinical studies due to its long RT. If it failed due to lack of efficacy one could

argue that blockade of CCR2 (alone) is not sufficient to treat the many immunological disorders that were mentioned in the Introduction.

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, 100 MHz) at ambient temperature. 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*. 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 was 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. Preparative HPLC was performed on a Schimatzu HPLC–ultraviolet (UV) system using a Gemini C18 Phenomenex column (100 × 10 mm, 5 µm), and a linear gradient from 10 to 90% of mobile phase B was applied, keeping mobile phase C constant at 10%. Mobile phase A consisted of H_2O, mobile phase B consisted of acetonitrile, and mobile phase C consisted of 1% TFA solution in H_2O. The flow rate was 5 mL/min. Liquid chromatography–mass spectrometry (LC–MS) analyses were performed using Thermo Finnigan Surveyor - LCQ Advantage Max LC-MS system and a Gemini C18 Phenomenex column (50 × 4.6 mm, 3 µm). 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. 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.

*General procedure for the synthesis of compounds **4 – 14, 16**.*

In a round–bottom flask a mixture of the corresponding olefin (**35** – **46**), Pd/C 5% wt (5 mol%) and palladium acetate (1.5 mol %) were dissolved in MeOH and stirred overnight with a hydrogen balloon. The reaction mixture was filtered through Celite and purified by preparative HPLC purification system.

*(1S,3R)-N-(3,5-bis(trifluoromethyl)benzyl)-1-isopropyl-3-(4-phenylpiperidin-1-yl)cyclopentane-1-carboxamide (TFA salt) (**4**)*. Yield = 69%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.62 (s,1H), 7.76–7.74 (m, 3H), 7.60 (s, 1H), 7.37–7.17 (m, 5H), 4.60–4.43 (m, 2H), 3.76–3.65 (m, 2H), 3.43 (s, 1H), 2.92–2.71 (m, 3H), 2.61–2.56 (m, 1H), 2.29–2.01 (m, 8H), 2.92–2.85 (m, 1H), 1.81–7.23 (m, 1H), 0.88–0.87 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 176.8, 161.5, 161.2, 142.6, 141.7, 131.9, 131.6, 129.2, 129.0, 127.9, 127.4, 126.6, 124.7, 122.0, 117.5, 67.5, 57.3, 52.9, 51.8, 43.3, 40.3, 34.8, 32.5, 31.9, 30.5, 27.9, 18.6, 17.8; MS peak: $541^+[H^+]$; HPLC: t_r = 8.9 min.

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(2-methoxyphenyl)piperidin-1-yl)cyclopentane-1-carboxamide (TFA salt) (**5**)*. Yield = 64%, ^{1}H NMR (400 MHz, CD_3CN) δ: 8.77 (br s, 1H), 7.92 (s, 3H), 7.46 (t, J = 4.8 Hz 1H), 7.27 (t, J = 20.0Hz, 1H), 7.16 (dd, J^1=J^2= 1.2 Hz, 1H), 7.01–6.97 (m, 2H), 4.58–4.53 (m, 2H), 3.84 (s, 3H), 3.74 (d, J =12.0 Hz, 1H), 3.60–3.51 (m, 2H), 3.24–3.15 (m, 1H), 3.04–2.95 (m, 2H), 2.45 (dd, J^1 =7.6 Hz, J^2 =6.8 Hz, 1H), 2.19–1.97 (m, 7H), 1.84–1.67 (m, 2H), 0.90–0.86 (m, 6H). ^{13}C NMR (101 MHz, CD_3CN) δ: 157.1, 142.9, 131.3, 128.1, 126.9, 126.4, 121.0, 117.4, 114.7, 111.0, 66.9, 57.3, 55.2, 55.0, 51.6, 42.5, 33.7, 33.4, 32.3, 31.5, 29.0, 27.8, 18.0, 16.9; MS peak : 571$^+$ [H$^+$]; HPLC t_r = 8.9 min.

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(3-methoxyphenyl)piperidin-1-yl)cyclopentane-1-carboxamide (TFA salt) (**6**)*. Yield = 16%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.74 (s, 1H), 7.79–7.77 (m, 3H), 7.60 (s, 1H), 7.25–7.11 (m, 2H), 6.83–6.18 (m, 2H), 4.65–4.46 (m, 2H), 3.85–3.83 (m, 4H), 3.45–3.21 (m, 4H), 2.89–2.72 (m, 4H), 2.33–1.97 (m, 8H), 1.72 (s, 1H), 0.91–0.89 (m, 6H)); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 176.9, 160.0, 144.2, 141.8, 131.8, 129.9, 127.9, 124.6, 118.3, 112.7, 112.2, 99.9, 67.6, 56.9, 55.6, 55.2, 52.9, 52.1, 43.1, 40.6, 35.0, 30.2, 30.1, 28.1, 18.7, 17.8; MS peak: 571$^+$ [H$^+$]; HPLC: t_r = 8.9 min.

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(4-methoxyphenyl)piperidin-1-yl)cyclopentane-1-carboxamide (TFA salt) (**7**)*. Yield = 9%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.72 (s, 1H), 7.77–7.75 (m, 3H), 7.0 (s, 1H), 7.12 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 4.65–4.41 (m, 2H), 3.81 (s, 3H), 3.79–3.68 (m, 2H), 3.33 (s, 1H), 2.82–2.63 (m, 4H), 2.32–2.25 (m, 9H), 1.96–1.93 (m, 1H), 0.88–0.87 (m, 6H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ: 142.0, 135.0, 128.1, 127.8, 121.3, 114.5, 67.8, 57.2, 55.5, 53.2, 52.4, 43.4, 39.9, 35.2, 32.3, 30.7, 30.6, 28.3, 18.9, 18.0; MS peak: 571$^+$ [H$^+$]; HPLC: t_r = 9.1 min.

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-3-(4-(3,4-dimethoxyphenyl)piperidin-1-yl)-1-isopropylcyclopentane-1-carboxamide (TFA salt) (**8**)*. Yield = 37%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.48 (s, 1H), 7.77–7.75 (m, 3H), 7.59 (s, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.74 (d, J = 7.2 Hz, 2H), 4.64–4.43 (m, 2H), 3.89–3.86 (m, 6H), 3.76–3.68 (m, 2H), 3.36 (s, 1H), 2.83–2.67 (m, 4H), 2.32–1.94 (m, 9H), 1.72–1.66 (m, 1H), 0.89–0.87 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 149.4, 148.3 141.9, 135.5, 131.9, 131.6, 128.0, 121.2, 112.0, 111.5, 109.7, 67.7, 57, 2, 43.3, 40.3, 35.1, 32.6, 32.2, 30.6, 30.5, 28.2, 18.8, 18.0; MS peak: 601$^+$ [H$^+$]; HPLC: t_r = 8.7min.

*(1S,3R)-3-(4-(Benzo[d][1,3]dioxol-5-yl)piperidin-1-yl)-N-(3,5-bis(trifluoromethyl)benzyl)-1-isopropylcyclopentane-1-carboxamide (TFA salt) (**9**)*. Yield = 38%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.72 (s, 1H), 7.76–7.75 (m, 3H), 7.57 (s, 1H), 6.78 (d, J = 10.0 Hz, 1H), 6.67 (d, J = 10.4 Hz, 1H), 6.63 (s, 1H), 5.93 (s, 2H), 4.63–5.51 (m, 2H), 3.76–3.67 (m, 2H), 3.35 (s, 1H) 2.83–2.70 (m, 2H), 2.69–2.61 (m, 2H), 2.31–1.86 (m, 8H), 1.72–1.64 (m, 1H), 0.88–0.86 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 176.2, 148.1, 146.8, 141.9, 136.7, 131.9, 131.6, 128.0, 121.2, 119.7, 108.7, 107.2, 101.2, 67.7, 57.1, 52.9, 52.1, 43.3, 40.4, 35.1, 32.4, 32.2, 30.6, 28.1, 18.8, 17.9; MS peak: 585$^+$ [H$^+$]; HPLC: t_r = 8.9 min.

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(4-(trifluoromethoxy)phenyl)piperidin-1-yl)cyclopentane-1-carboxamide (TFA salt) (**10**)*. Yield = 32%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.06, (s, 1H), 7.76, (s, 3H), 7.35 (s, 1H), 2.23–2.17 (m, 4H), 4.64–4.43 (m, 2H), 3.79–3.70 (m, 2H), 3.42 (s, 2H), 2.88–2.72 (m, 3H), 2.66–2.60 (m, 1H), 2.34–1.86 (m, 9H), 1.75–1.61 (m, 1H), 0.91–0.89 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 176.3, 148.5, 141.6, 141.2, 132.1, 131.7, 128.1, 124.8, 12.0, 121.6, 121.4, 67.7, 57.2, 52.9, 51.8, 43.3, 39.9, 35.2, 32.7, 31.9, 30.4, 30.3, 27.9, 18.7, 18.0; MS peak: 625$^+$ [H$^+$]; HPLC: t_r = 9.4 min.

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-3-(4-(4-(tert-butyl)phenyl)piperidin-1-yl)-1-isopropylcyclopentane-1-carboxamide (TFA salt) (**11**)*. Yield = 17%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.76 (s, 1H), 9.12 (s, 1H), 7.83–7.78 (m, 4H), 7.63 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 0.5 H^a), 7.17 (d, J = 8.4 Hz, 0.5H^b), 4.63–4.47 (m, 2H), 3.83–3.37 (m, 6H), 2.87–1.73 (m, 11H), 1.41 (s, 4.5H^a), 1.35 (s, 4.5H^b), 0.95–0.93 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 177.2, 156.9, 156.8, 143.5, 141.8, 141.6, 139.6, 130.4, 127.6, 126.3, 124.4, 121.2, 70.1, 67.6, 59.2, 56.9, 52.9, 43.3, 43.1, 39.9, 34.5, 32.3, 31.1, 30.3, 18.9, 18.7, 17.8, 17.7; MS peak: 597$^+$ [H$^+$]; HPLC: t_r = 9.8 min. *a* and *b* for different rotamers.

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-3-(4-(4-hydroxyphenyl)piperidin-1-yl)-1-isopropylcyclopentane-1-carboxamide (TFA salt) (**12**)*. Yield = 31%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.63 (s, 1H), 7.92 (s, 3H), 7.52 (s, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.79 (d, *J* = 8.0 Hz, 2H), 4.51–4.50 (m, 2H), 3.62–3.46 (m, 3H), 2.95–2.75 (m, 3H), 2.45–1.97 (m, 10H), 1.80–1.70 (m, 2H), 0.89–0.86 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 155.9, 143.2, 135.3, 128.3, 127.7, 120.8, 117.4, 115.4, 66.6, 56.8, 42.2, 51.5, 42.5, 38.6, 34.4, 33.0, 30.5, 27.6, 17.7, 17.2; MS peak: 557^{+} [H^{+}]; HPLC: t_r = 8.5 min.

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(pyridin-3-yl)piperidin-1-yl)cyclopentane-1-carboxamide (TFA salt) (**13**)*. Yield = 55%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.11 (s, 1H), 8.94 (s, 1H), 8.65 (d, *J* = 5.2 Hz, 1H), 8.43 (d, *J* = 8.0 Hz, 1H), 7.86 (t, *J* = 5.6 Hz, 1H), 7.76–7.74 (m, 3H), 7.52 (s, 1H), 4.64–4.47 (m, 2H), 3.82–3.74 (m, 2H), 3.43–3.39 (m,1H), 2.68–2.47 (m, 3H), 2.17–1.69 (m, 8H), 1.23 (s, 2H), 0.91–0.87 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 178.45, 148.73, 147.8, 142.5, 140.7, 133.9, 132.0, 131.9, 131.7, 131.6, 131.3, 127.7, 124.7, 123.6, 121.9, 121.1, 65.9, 57.1, 53.4, 52.9, 51.3, 50.0, 47.4, 44.2, 42.8, 39.7, 38.5, 38.2, 35.5, 35.1, 33.9, 33.2, 32.9, 29.7, 29.1, 28.3, 26.2, 23.8, 19.3, 19.2, 17.5; MS peak: 542^{+} [H^{+}]; HPLC: t_r = 7.9 min.

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(6-methoxypyridin-3-yl)piperidin-1-yl)cyclopentane-1-carboxamide (TFA salt) (**14**)*. Yield = 70%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.18 (s, 1H), 8.25 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 1H), 7.35 (s, 1H), 7.30–7.25 (m, 3H), 6.95 (d, *J* = 8.8 Hz, 1H), 4.63–4.46 (m, 2H), 4.03 (s, 3H), 3.78–3.69 (m, 2H), 3.41–3.39 (m, 1H), 2.95–2.89 (m, 3H), 2.62–2.56 (m, 1H), 2.30–1.67 (m, 10H), 0.80–0.86 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 176.4, 162.5, 161.9, 161.5, 161.2, 160.8, 142.5, 141.7, 140.6, 132.3, 132.0, 131.7, 131.3, 128.0, 124.7, 122.0, 121.4, 121.3, 117.3, 114.5, 111.3, 67.6, 57.3, 44.6, 52.6, 51.6, 43.3, 36.8, 34.9, 32.6, 31.9, 30.0, 29.9, 28.1, 18.7, 17.9 ; MS peak: 572^{+} [H^{+}]; HPLC: t_r = 8.0 min.

*3-(1-((1R,3S)-3-((3,5-bis(trifluoromethyl)benzyl)carbamoyl)-3-isopropylcyclopentyl)piperidin-4-yl)benzoic acid (**15**)*. In a 50 mL round–bottom flask compound **16** (0.058 g, 0.128 mmol) was dissolved in EtOH (10 mL). Subsequently, 4 M LiOH (0.3 mL, 1.2 mmol) in H_2O was added and the reaction mixture was stirred for 1.5 hours at 50 °C. EtOH was evaporated in vacuum. The reaction mixture was partitioned between brine and chloroform and the pH was adjusted to 7 with 1 M HCl (aq.). The organic layer was collected, dried over $MgSO_4$ and concentrated in vacuum, yielding compound **15** as a HCl salt (0.042 g, 53 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.34 (s, 1H), 8.04 (s, 1H), 7.76–7.75 (m, 3H), 7.64 (s, 1H), 7.22–7.20 (m, 2H), 4.64–4.58 (m, 1H), 4.27–4.22 (m, 1H), 3.88 (d, *J* = 11.2 Hz, 1H), 3.78 (d, *J* = 11.2 Hz, 1H), 3.49–3.42 (m, 1H), 3.14–3.12 (m, 1h), 2.90–2.65 (m, 4H), 2.36–2.32 (m, 1H), 2.19–1.96 (m, 5H), 1.93–1.88 (m, 2H), 1.69–1.68 (m, 1H), 0.93–0.89 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 176.3, 173.1, 142.9, 142.5, 136.6, 131.4, 131.2, 131.0, 128.2, 128.0, 127.9, 125.2, 124.7, 122.0, 120.6, 68.1, 57.6, 53.5, 52.7, 43.2, 40.2, 35.2, 34.3, 32.8, 30.6, 29.4, 26.9, 19.3, 17.8. ; MS peak: 585^{+} [H^{+}]; HPLC: t_r = 9.2 min.

*Methyl 3-(1-((1R,3S)-3-((3,5-bis(trifluoromethyl)benzyl)carbamoyl)-3-isopropylcyclopentyl)piperidin-4-yl)benzoate (TFA salt) (**16**)*. Yield = 69%, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.66 (s, 1H), 7.85 (s, 1H), 7.78–7.76 (m, 3H), 7.36 (t, *J* = 7.7 Hz, 1H), 7.26 (d, *J* = 7.7 Hz, 1H), 4.57 (d, *J* = 5.6 Hz, 2H), 3.91 (s, 3H), 3.26 (d, *J* = 11.0 Hz, 1H), 3.12 (d, *J* = 11.0 Hz, 1H), 2.75 (s, 1H), 2.65–2.49 (m, 1H), 2.32–2.27 (m, 1H), 2.18–1.93 (m, 4H), 1.93–1.76 (m, 4H), 1.76–1.63 (m, 2H), 1.60–1.30 (m, 2H), 0.94–0.89 (m, 6H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ: 178.7, 167.1, 145.8, 142.5, 131.8, 131.5, 131.0, 128.5, 127.9, 127.5, 124.6, 121.9, 121.0, 65.7, 56.9, 53.5, 52.1, 51.1, 42.6, 41.9, 35.4, 33.6, 33.3, 29.9, 19.3, 17.3; MS peak: 599^{+} [H^{+}]; HPLC: t_r = 9.7 min.

*((1S,3R)-1-isopropyl-3-(4-phenyl-3,6-dihydropyridin-1(2H)-yl)cyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**17**)*. In a 5 mL microwave tube a mixture of triflate **34** (0.06 g, 0.1 mmol), phenylboronic acid (0.018 g, 1.5 mmol), LiCl (0.012 g, 0.3 mmol), 2 M Na_2CO_3 in H_2O (0.15 mL 0.3 mmol) and tetrakis(triphenylphosphine)palladium(0) (5 mol%) was dissolved in DME (1 mL) under a nitrogen atmosphere. The reaction mixture was heated under microwave irradiation at 90 °C for 5.5 hours. The reaction mixture was partitioned between DCM and H_2O. The organic layer was dried with

$MgSO_4$ and concentrated in vacuum. The product was purified by preparative HPLC system, yielding compound **17** as TFA salt (0.014 g, 23%). ^{1}H NMR (400 MHz, CD_3CN) δ: 7.50–7.28 (m, 8H), 6.00 (s, 1H), 4.90–4.60 (m, 3H), 4.30 (d, J = 16.0 Hz, 0.5H^a), 4.20 (d, J = 16.0 Hz, 0.5H^b), 3.90–3.55 (m, 4H), 3.34 (br s, 0.5H^a), 3.20–2.90 (m, 3.5H), 2.80–2.65 (m, 3H), 2.40–1.95 (m, 4H), 1.72 (br s, 1H), 0.93 (d, J = 6.4 Hz, 3H^a), 0.86 (d, J = 6.4 Hz, 3H^b); MS peak: 497$^+$ [H$^+$]; HPLC: t_r = 8.7 min. *a* and *b* indicating different rotamers.

*((1S,3R)-1-isopropyl-3-(4-phenylpiperidin-1-yl)cyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**18**)*. In a 10 mL round–bottom flask compound **17** (6.6 mg, 0.011 mmol) was dissolved in 5 mL of THF. To the reaction mixture Pd/C 5% wt (5 mol%) and palladium acetate (1.5 mol %) were added. The reaction mixture was flushed with hydrogen and kept under hydrogen atmosphere with a hydrogen balloon. The reaction mixture was heated at 50 °C for 30 seconds and stirring continued at room temperature for 2 hours. After the reaction was finished the mixture was filtered over celite and the product was purified by preparative HPLC system, yielding compound **18** as TFA salt (4.5 mg, 67%). ^{1}H NMR (400 MHz, CD_3CN) δ: 7.45–7.18 (m, 8H), 4.85–4.68 (m, 2H), 3.90–3.60 (m, 3H), 3.40 (br s, 1H), 3.00–1.80 (m, 17H), 1.64 (br s, 1H), 0.91 (d, J = 6.4 Hz, 3H), 0.82 (d, J = 6.4 Hz, 3H); MS peak: 499$^+$ [H$^+$]; HPLC: t_r = 8.7 min.

*General procedure for the synthesis of compounds **19**, **21 – 23** and **27**.*

Into an oven dried glass tube were added the tosylhydrazone **52** (140 mg, 0.23 mmol, 1 equiv.), the corresponding boronic acid (0.34 mmol, 1.5 equiv.), Cs_2CO_3 (110 mg, 0.34 mmol, 1.5 equiv. dried at 100 °C overnight) and then sealed with a crimp top with septum. High vacuum was applied for 30 minutes after which it was backfilled with nitrogen and 1 mL of dry 1,4-dioxane was added. Subsequently, the mixture was degassed 4 times by applying a vacuum and a nitrogen atmosphere sequentially. The reaction mixture was heated at 100 °C for 18 hours. TLC showed full conversion of the tosylhydrazone (EtOAc, $KMnO_4$ spray to visualize). The cooled reaction mixture was quenched with 2 mL of an aqueous saturated $NaHCO_3$ solution, extracted 3 times with DCM, dried over $MgSO_4$ and concentrated in vacuum. The crude yellow oil was pre–purified by column chromatography followed by preparative HPLC purification to give the final compounds **19**, **21 – 23** and **27** as TFA salts as colorless solidified oils.

*1-((1R,3S)-3-isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)-4-(3-methoxyphenyl)piperidin-1-ium 2,2,2-trifluoroacetate (**19**)*. Yield = 10%. ^{1}H NMR (400 MHz, CD_3CN) δ: 10.66 (br s, 1H, NH), 7.53 (s, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.23 (td, J = 7.2, 2.0 Hz, 1H), 6.82–6.70 (m, 3H), 4.78–4.68 (m, 2H), 3.78–3.65 (m, 6H), 3.60 (d, J = 12.0 Hz, 1H), 3.40–3.28 (m, 1H), 2.95–2.86 (m, 4H), 2.81–2.74 (m, 1H), 2.57–2.52 (m, 1H), 2.48–2.35 (m, 2H), 2.11–1.98 (m, 6H), 1.72–1.56 (m, 2H), 0.89 (d, J = 6.4 Hz, 3H), 0.75 (d, J = 6.4 Hz, 3H). LC–MS: 529$^+$ [H$^+$]; HPLC t_r: 8.09 min.

*4-(3-Bromophenyl)-1-((1R,3S)-3-isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)piperidin-1-ium 2,2,2-trifluoroacetate (**21**)*. Yield = 8%. ^{1}H NMR (400 MHz, CD_3CN) δ: 10.84 (br s, 1H, NH), 7.52 (s, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.42–7.37 (m, 2H), 7.33 (d, J = 8.0 Hz, 1H), 7.27–7.21 (m, 2H), 4.78–4.68 (m, 2H), 3.85–3.65 (m, 3H), 3.60 (d, J = 12.6 Hz, 1H), 3.40–3.28 (m, 1H), 2.96–2.77 (m, 5H), 2.57–2.52 (m, 1H), 2.48–2.35 (m, 2H), 2.16–1.98 (m, 6H), 1.68–1.56 (m, 2H), 0.89 (d, J = 6.4 Hz, 3H), 0.75 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ: 147.0, 134.8, 130.6, 129.9, 129.8, 129.6, 125.7, 123.3, 123.0, 122.2, 56.4, 56.0, 52.3, 51.6, 39.1, 34.7, 32.6, 29.9, 28.1, 27.7, 17.4, 17.1. LC–MS: 578$^+$ [H$^+$]; t_R: 8.87 min.

*1-((1R,3S)-3-Isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)-4-(3-isopropylphenyl)piperidin-1-ium 2,2,2-trifluoroacetate (**22**)*. Yield = 23%. ^{1}H NMR (400 MHz, CD_3CN) δ: 9.25 (br s, 1H, NH), 7.54 (s, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.24 (t, J = 7.6 Hz, 1H), 7.14–7.10 (m, 2H), 7.03 (d, J = 7.6 Hz, 1H), 4.82–4.71 (m, 2H), 3.90–3.69 (m, 3H), 3.60 (d, J = 12.2 Hz, 1H), 3.42–3.31 (m, 1H), 3.00–2.78 (m, 5H), 2.54–2.32 (m, 3H), 2.22–2.08 (m, 2H), 2.07–1.96 (m, 4H), 1.67–1.59 (m, 2H), 1.20 (d, J = 7.0 Hz, 6H), 0.88 (d, J = 6.4 Hz, 3H), 0.79 (d, J = 6.4 Hz, 3H). ^{13}C NMR (101 MHz,

CD_3CN) δ: 149.4, 144.1, 134.7, 129.7, 128.7, 127.7, 124.9, 124.0, 123.3, 67.3, 56.5, 52.8, 51.9, 39.3, 34.0, 32.3, 30.4, 30.3, 28.3, 17.4, 17.2. LC–MS: 541^+ [H^+]; t_R: 9.22 min.

*1-((1R,3S)-3-Isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)-4-(3-(trifluoromethyl)phenyl)piperidin-1-ium 2,2,2-trifluoroacetate (**23**)*. Yield = 9%. ^{1}H NMR (400 MHz, CD_3CN) δ: 10.80 (br s, 1H, NH), 7.60–7.49 (m, 5H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 4.81–4.66 (m, 2H), 3.94–3.69 (m, 3H), 3.62 (d, *J* = 12.0 Hz, 1H), 3.42–3.30 (m, 1H), 3.02–2.83 (m, 5H), 2.61–2.40 (m, 3H), 2.14–1.98 (m, 6H), 1.72–1.55 (m, 2H), 0.89 (d, *J* = 6.4 Hz, 3H), 0.75 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ: 146.5, 135.7, 131.7, 130.5, 124.5, 124.3, 68.3, 57.0, 53.2, 52.6, 40.1, 35.7, 33.6, 30.9, 29.1, 18.4, 18.1. LC–MS: 567^+ [H^+]; t_R: 8.88 min.

*1-((1R,3S)-3-Isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)-4-(3-(methoxycarbonyl)phenyl)piperidin-1-ium 2,2,2-trifluoroacetate (**27**)*. Yield = 14%. ^{1}H NMR (400 MHz, CD_3CN) δ: 10.94 (br s, 1H, NH), 7.86–7.84 (m, 2H), 7.52 (s, 1H), 7.50–7.41 (m, 3H), 7.33 (d, *J* = 8.0 Hz, 1H), 4.81–4.66 (m, 2H), 3.92–3.65 (m, 5H), 3.62 (d, *J* = 12.1 Hz, 1H), 3.42–3.30 (m, 1H), 3.02–2.82 (m, 5H), 2.60–2.40 (m, 3H), 2.17–1.96 (m, 6H), 1.72–1.55 (m, 2H), 0.89 (d, *J* = 6.4Hz, 3H), 0.74 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ: 166.7, 144.9, 134.8, 131.5, 130.6, 129.6, 129.0, 127.8, 127.6, 123.3, 67.4, 56.0, 52.3, 51.8, 51.7, 39.2, 34.8, 32.6, 30.0, 28.1, 17.4, 17.1. LC–MS: 557^+ [H^+]; t_R: 8.49 min.

*General procedure for synthesis of compounds **20**, **24**, **25**.*

In a 5 mL microwave tube a solution of compound **50** (0.5 mmol, 1 equiv.) in 3 mL of DME was mixed with corresponding arylboronic acid (0.65 mmol, 1.3 equiv), LiCl (1.5 mmol, 3 equiv), 2 M Na_2CO_3 (1.5 mmol, 3 equiv) solution in H_2O and $Pd(PPh_3)_4$ (5 mol%). The reaction mixture was heated under microwave irradiation at 90 °C for 30 min – 2.5 hours. The reaction mixture was partitioned between DCM/H_2O. The organic layer was dried with $MgSO_4$ and concentrated in vacuum. The product was purified by column chromatography on silica gel with an eluent system consisting of DCM and increasing amounts of EtOAc (0-50%). The corresponding olefin was dissolved in THF and transferred to a 20 mL microwave tube. To the reaction mixture PtO_2 (20 wt%) and acetic acid (3 equiv) were added. The tube was capped, flushed with H_2 gas, and additionally pressurized with 20 mL of H_2 gas using a 20 mL syringe. The reaction was monitored using TLC/MS. The reaction was complete after 10 to 15 min. In the case of halogen substituents, if longer reaction times were used a de-halogenation was observed. Next, the corresponding esters were dissolved in EtOH and transferred to a 50 mL round–bottom flask. To the reaction mixture 4 M LiOH (10 equiv) in H_2O was added and the reaction mixture was refluxed for 3 hours. EtOH was evaporated in vacuum. The reaction mixture was acidified with 1 M HCl and the product was extracted with DCM. The organic layer was dried with $MgSO_4$ and concentrated in vacuum. The corresponding acid was dissolved in DCM and transferred to a 10 mL round–bottom flask. To the reaction mixture 7-trifluoromethyl-1,2,3,4-tetrahydroisoquinoline HCl salt (2 equiv) was added followed by the addition of DIPEA (12 equiv), PyBrOP (3 equiv), DMAP (1.5 equiv), and several beads of 4 Å molecular sieves. The reaction mixture was stirred for 48 hours at room temperature. The reaction mixture was extracted with DCM/H_2O, dried with $MgSO_4$ and concentrated in vacuum. The product was purified by preparative HPLC. All intermediates were checked by MS and were advanced to the next step without further analysis.

*((1S,3R)-3-(4-(3-chlorophenyl)piperidin-1-yl)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone TFA salt (**20**)*. Overall yield = 9%, ^{1}H NMR (400 MHz, CD_3CN) δ: 8.51 (br s, 1H), 7.50–7.40 (m, 2H), 7.30–7.16 (m, 4H), 7.09 (d, *J* = 6.8 Hz, 1H), 4.81 (br s, 2H), 4.00–3.65 (m, 4H), 3.52 (br s, 1H), 3.15–2.75 (m, 5H), 2.60–2.40 (m, 2H), 2.35–2.00 (m, 7H), 1.79 (br s, 1H), 0.89 (br s, 6H). MS peak: 533^+ [H^+]; HPLC: t_r = 8.82 min.

*((1S,3R)-3-(4-(4-Chlorophenyl)piperidin-1-yl)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone TFA salt (**24**)*. Overall yield = 0.8%, ^{1}H NMR (400 MHz, CD_3CN) δ: 10.81 (br s, 1H), 7.50–7.24 (m, 5H), 7.15 (d, *J* = 8.4 Hz, 2H), 4.40–4.20 (m, 2H), 3.86 (d, *J* = 11.6 Hz, 2H),

3.72 (d, *J* = 11.2 Hz, 2H), 3.52 (br s, 1H), 3.10–2.60 (m, 7H), 2.35–2.05 (m, 7H), 1.95–1.80 (m, 1H), 1.75–1.62 (m, 1H), 0.95–0.83 (m, 6H). MS peak: 533^+ [H^+]; HPLC: t_r = 8.96 min.

*((1S,3R)-1-Isopropyl-3-(4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)cyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone TFA salt (**25**)*. Overall yield = 2%, ^{1}H NMR (400 MHz, CD_3CN) *δ*: 9.43 (br s, 1H), 7.70–7.29 (m, 7H), 4.81 (s, 2H), 3.95–3.74 (m, 4H), 3.53 (br s, 1H), 3.10–2.85 (m, 5H), 2.65–2.45 (m, 2H), 2.30–2.10 (m, 7H), 1.95–1.70 (m, 2H), 0.95–0.83 (m, 6H). MS peak: 567^+ [H^+]; HPLC: t_r = 9.07 min.

*4-(3-carboxyphenyl)-1-((1R,3S)-3-isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)piperidin-1-ium chloride (**26**)*. Ester **27** (25 mg, 0.045 mmol, 1.0 equiv.) was dissolved in a mixture of EtOH (5 mL), water (2 mL) and an 4M LiOH (aq.) (113µL, 0.45 mmol, 10.0 equiv.) was added. After 2 hours at 50 °C all of **27** was consumed as shown by TLC (EtOAc, $KMnO_4$ spray to visualize). The ethanol was evaporated and the pH was adjusted to pH = 1 with a 3M HCl (aq.) solution. The white precipitate was filtered off, rinsed with water and dried in vacuum to yield compound **26** as HCl salt. Yield = 17 mg, 65%. ^{1}H NMR (400 MHz, CD_3CN) *δ*: 7.88–7.84 (m, 2H), 7.52 (s, 1H), 7.50–7.45 (m, 2H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 4.80–4.70 (m, 2H), 3.90–3.63 (m, 3H), 3.58 (d, *J* = 12.8 Hz, 1H), 3.40–3.29 (m, 1H), 3.01–2.85 (m, 5H), 2.70–2.40 (m, 3H and water), 2.24–2.05 (m, 6H), 1.82–1.75 (m, 1H), 1.67–1.59 (m ,1H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.76 (d, *J* = 6.4 Hz, 3H). LC–MS: 543^+ [H^+]; HPLC t_r: 8.10 min.

*1-Ethyl-1-methyl-4-oxo-piperidin-1-ium iodide (**28**)*. In a 20 mL microwave tube a solution of 1-methyl-4-piperidone (2.2 mL, 17.9 mmol) in acetone (15 mL) was mixed with ethyl iodide (1.43 mL, 17.9 mmol) under nitrogen atmosphere. The reaction mixture was stirred in the microwave at 60 °C for 5 hours forming yellow solids. The solids were filtered, washed with acetone and dried under vacuum to yield yellow salt **28** (4.35 g, 90%). The compound was used in next step as is.

*Synthesis of (1S,3R)-3-amino-N-(3,5-bis(trifluoromethyl)benzyl)-1-isopropylcyclopentane-1-carboxamide (**29**)* was achieved following the synthetic approach reported by our group earlier.[18]

*Synthesis of ((1S,3R)-3-amino-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**30**)* was achieved following the synthetic approach reported earlier by our group.

*(1S,3R)-N-(3,5-bis(trifluoromethyl)benzyl)-1-isopropyl-3-(4-oxopiperidin-1-yl)cyclopentane-1-carboxamide (**31**)*. In 250 mL round–bottom flask a solution of amine **29** (2.0 g, 5.0 mmol) dissolved in ethanol (75 mL) and H_2O (25 mL) was stirred at 40 °C. Compound **28** (2.02 g, 7.5 mmol) was dissolved in H_2O (30 mL) and added dropwise within 5 minutes to the reaction mixture. Then K_2CO_3 (1.4 g, 10 mmol) was added and the reaction mixture was refluxed for 5 hours, followed by an additional 12 hour stirring at room temperature. Ethanol was removed in vacuum and the reaction mixture was partitioned between DCM/ H_2O. The organic layer was dried over $MgSO_4$ and concentrated in vacuum. Compound **31** was purified by column chromatography on silica gel with an eluent system consisting of DCM and increasing amounts of EtOAc (50-100%). Yield =1.7 g, (72%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.77–7.70 (m, 3H), 7.49 (s, 1H), 4.56 (d, *J* = 5.6 Hz, 2H), 2.83–2.76 (m, 5H), 2.38–2.27 (m, 4H), 2.19–1.90 (m, 5H), 1.70–1.53 (m, 2H), 1.15 (s, 1H), 0.92–0.89 (m, 6H)

*1-((1R,3S)-3-isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)piperidin-4-one (**32**)*. In a 20 mL microwave tube a solution of amine **30** (0.71 g, 2.0 mmol) in ethanol (10 mL) and H_2O (3 mL) was stirred at 40 °C. Compound **28** (0.81 g, 3 mmol) was dissolved in H_2O (3 mL) and added dropwise during 5 minutes to the reaction mixture. Then K_2CO_3 (0.56 g, 4 mmol) was added and the reaction mixture was heated under microwave irradiation at 100 °C for 3 hours. Ethanol was removed in vacuum and the reaction mixture was extracted with DCM/ 1M NaOH solution in H_2O. The organic layer was dried over $MgSO_4$ and concentrated in vacuum. Compound **32** was purified by column chromatography on silica gel with an eluent system consisting of DCM and increasing amounts of EtOAc (50-100%). Yield =0.22 g, (25%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.40–7.30 (m, 2H), 7.21 (d, *J* = 8 Hz, 1H), 4.80–4.60 (m, 2H), 3.77 (br s, 2H), 2.88 (br s, 2H), 2.72 (br s, 3H), 2.63–2.53

(m, 2H), 2.41–2.38 (m, 3H), 2.24–2.18 (m, 1H), 2.05–1.80 (m, 4H), 1.60–1.30 (m, 3H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.73 (d, *J* = 6,8 Hz, 3H).

*1-((1R,3S)-3-((3,5-bis(trifluoromethyl)benzyl)carbamoyl)-3-isopropylcyclopentyl)-1,2,3,6-tetrahydropyridin-4-yl trifluoromethanesulfonate (**33**)*. In a 100 mL round–bottom flask a solution of LDA (2M in heptanes, 2.2 mL, 2.88 mmol) in dry THF (20 mL) was cooled down to –78 °C under a nitrogen atmosphere. The ketone compound **31** (0.55 g, 1.15 mmol) was dissolved in dry THF (15 mL) and added dropwise to the reaction mixture. Vigorous stirring was necessary. The reaction mixture was stirred for 1 hour at –78 °C. After 1 hour, the reaction mixture was slowly warmed to –20 °C and kept at this temperature for 1 hour. Subsequently the reaction mixture was cooled down to –78 °C and *N*-phenyl-bis(trifluoromethanesulfonimide) (0.65 g, 2.3 mmol) dissolved in dry THF (8 mL) was added dropwise to the reaction mixture. After the addition was complete, the reaction mixture was slowly brought to room temperature and stirred overnight. The reaction mixture was quenched with EtOH, concentrated in vacuum and partitioned between DCM/H_2O. The organic layer was dried with $MgSO_4$ and concentrated in vacuum. The product was purified by column chromatography on silica gel with an eluent system consisting of DCM and increasing amounts of EtOAc (0-30%), yielding the triflate compound **33** (0.3 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (t, *J* = 5.6 Hz, 1H), 7.76–7.72 (m, 3H), 5.68 (s, 1H), 4.49 (d, *J* = 5.6 Hz, 2H), 3.20–3.06 (m, 2H), 2.89–2.82 (m, 2H), 2.67–2.60 (m, 1H), 2.40–2.20 (m, 3H), 2.04–1.79 (m, 4H), 1.73–1.59 (m, 2H), 0.91(d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H).

*1-((1R,3S)-3-((3,5-bis(trifluoromethyl)benzyl)carbamoyl)-3-isopropylcyclopentyl)-1,2,3,6-tetrahydropyridin-4-yl trifluoromethanesulfonate (**34**)*. In a 20 mL microwave tube a solution of LDA (2M in heptanes, 0.33 mL, 0.65 mmol) in THF (5 mL) was cooled down to –78 °C under nitrogen atmosphere. The ketone compound **32** (0.22 g, 0.5 mmol) was dissolved in dry THF (5 mL) and added dropwise to the reaction mixture. Vigorous stirring was necessary. The reaction mixture was stirred for 1 hour at –78 °C. After 1 hour, the reaction mixture was slowly warmed to –20 °C and kept at this temperature for 1.5 hours. Subsequently the reaction mixture was cooled down to –78 °C and *N*-phenyl-bis(trifluoromethanesulfonimide) (0.23 g, 0.65 mmol) dissolved in THF (5 mL) was added dropwise to the reaction mixture. After the addition was complete, the reaction mixture was slowly brought to room temperature and stirred overnight. The reaction mixture was quenched with EtOH, concentrated in vacuum and partitioned between DCM/H_2O. The organic layer was dried with $MgSO_4$ and concentrated in vacuum. The product was purified by column chromatography on silica gel with an eluent system consisting of DCM and increasing amounts of EtOAc (0-20%), yielding the triflate compound **34** (0.06 g, 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44 (d, *J* = 8 Hz, 1H), 7.38 (s, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 5.71 (s, 1H), 4.90–4.57 (m, 2H), 3.82 (br s, 2H), 3.17 (s, 2H), 2.93 (d, *J* = 5.2 Hz, 2H), 2.80–2.60 (m, 4H), 2.43 (br s, 2H), 2.25–2.15 (m, 1H), 2.08–1.82 (m, 3H), 1.60–1.38 (m, 2H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.80 (d, *J* = 6.4 Hz, 3H).

*General synthesis of compounds **35** – **46**.*

In a 5 mL microwave tube a mixture of triflate **33** (0.25 mmol, 1 equiv.), corresponding arylboronic acid (0.35 mmol, 1.4 equiv.), LiCl (0.75 mmol, 3 equiv.), 2 M Na_2CO_3 in H_2O (0.75 mmol, 3 equiv.) and tetrakis(triphenylphosphine)palladium(0) (5 mol%) was dissolved in DME (4 mL) under a nitrogen atmosphere. The reaction was heated under microwave irraditaion at 90 °C for 3.5 hours. DME was evaporated in vacuum and the reaction mixture was partitioned between 2 M Na_2CO_3/DCM. The organic layer was dried with $MgSO_4$ and concentrated in vacuum. The products were purified by column chromatography on silica gel with an eluent system consisting of DCM and increasing amounts of EtOAc (0-50%). LC–MS was used for confirmation of the products before using them in the next step.

(1*S*,3*R*)-*N*-(3,5-bis(trifluoromethyl)benzyl)-1-isopropyl-3-(4-phenyl-3,6-dihydropyridin-1(2*H*)-yl)cyclopentane-1-carboxamide (**35**). Yield = 40 %, MS peak: 539^+ [H^+].

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(2-methoxyphenyl)-3,6-dihydropyridin-1(2H)-yl)cyclopentane-1-carboxamide (**36**)*. Yield = 21%, MS peak: 569^+ [H^+].

*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(3-methoxyphenyl)-3,6-dihydropyridin-1(2H)-yl)cyclopentane-1-carboxamide (**37**).* Yield = 20%, MS peak: 569^+ [H^+].
*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(4-methoxyphenyl)-3,6-dihydropyridin-1(2H)-yl)cyclopentane-1-carboxamide (**38**).* Yield = 99% (without purification), MS peak: 569^+ [H^+].
*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-3-(4-(3,4-dimethoxyphenyl)-3,6-dihydropyridin-1(2H)-yl)-1-isopropylcyclopentane-1-carboxamide (**39**)*. Yield = 30%, MS peak: 599^+ [H^+].
*(1S,3R)-3-(4-(Benzo[d][1,3]dioxol-5-yl)-3,6-dihydropyridin-1(2H)-yl)-N-(3,5-bis(trifluoromethyl)benzyl)-1-isopropylcyclopentane-1-carboxamide (**40**)*. Yield = 41%, MS peak: 583^+ [H^+].
*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(4-(4-(trifluoromethoxy)phenyl)-3,6-dihydropyridin-1(2H)-yl)cyclopentane-1-carboxamide (**41**)*. Yield = 51%, MS peak: 623^+ [H^+].
*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-3-(4-(4-(tert-butyl)phenyl)-3,6-dihydropyridin-1(2H)-yl)-1-isopropylcyclopentane-1-carboxamide (**42**)*. Yield = 32%, MS peak: 595^+ [H^+].
*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-3-(4-(4-hydroxyphenyl)-3,6-dihydropyridin-1(2H)-yl)-1-isopropylcyclopentane-1-carboxamide (**43**)*. Yield = 25%, MS peak: 555^+ [H^+].
*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-3-(3',6'-dihydro-[3,4'-bipyridin]-1'(2'H)-yl)-1-isopropylcyclopentane-1-carboxamide (**44**)*. Yield = 55%, MS peak: 540^+ [H^+].
*(1S,3R)-N-(3,5-bis(Trifluoromethyl)benzyl)-1-isopropyl-3-(6-methoxy-3',6'-dihydro-[3,4'-bipyridin]-1'(2'H)-yl)cyclopentane-1-carboxamide (**45**)*. Yield = 59%, MS peak: 570^+ [H^+].
*Methyl 3-(1-((1R,3S)-3-((3,5-bis(trifluoromethyl)benzyl)carbamoyl)-3-isopropylcyclopentyl)-1,2,3,6-tetrahydropyridin-4-yl)benzoate (**46**)*. Yield = 53%, MS peak: 597^+ [H^+].
*Synthesis of (1S,3R)-methyl-3-((tert-butoxycarbonyl)amino)-1-isopropylcyclopentanecarboxylate (**47**)* was achieved following the synthetic approach reported by Kothandaraman S. et al.[19]
*Methyl (1S,3R)-3-amino-1-isopropylcyclopentane-1-carboxylate (**48**)*. In a 100 mL round–bottom flask compound **47** (11.4 g, 40 mmol) was dissolved in a mixture of DCM (30 mL) and TFA (20 mL). The reaction mixture was stirred at room temperature for 2 hours. After completion, the reaction mixture was basified to pH 14 with 2 M NaOH solution in H_2O and extracted with DCM. The organic layer was dried with $MgSO_4$ and concentrated in vacuum. Yield = 7.3 g (98%). The crude product was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.57 (s, 3H), 3.25–3.18 (m, 1H), 2.20–2.12 (m, 1H), 1.90–1.70 (m, 4H), 1.48–1.35 (m, 2H), 1.28–1.15 (m, 2H), 0.80–0.71 (m, 6H).
*Methyl (1S,3R)-1-isopropyl-3-(4-oxopiperidin-1-yl)cyclopentane-1-carboxylate (**49**)*. In 7 separate batches of 100 mL round–bottom flasks a solution of amine **48** (0.74 g, 4.0 mmol) dissolved in ethanol (20 mL) and H_2O (8 mL) was stirred at 40 °C. Compound **28** (1.61 g, 6 mmol) was dissolved in H_2O (8 mL) and added dropwise during 5 minutes to the reaction mixture. Then K_2CO_3 (1.12 g, 8 mmol) was added and the reaction mixture was refluxed for 3 hours. All batches were combined and ethanol was removed in vacuum and the reaction mixture was partitioned between DCM/H_2O. The organic layer was dried over $MgSO_4$ and concentrated in vacuum. The product was purified by column chromatography on silica gel with an eluent system consisting of DCM and increasing amounts of EtOAc (0-100%). Combined yield = 4.19 g, (56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.55 (s, 3H), 2.63 (t, J = 6.4 Hz, 4H), 2.59–2.50 (m, 1H), 2.29 (t, J = 6.4 Hz, 4H), 2.16–2.12 (m, 1H), 2.01–1.95 (m, 1H), 1.87–1.74 (m, 3H), 1.37 (t, J = 4.8 Hz, 2H), 0.74 (d, J = 6.8 Hz, 3H), 0.71 (d, J = 6.8 Hz, 3H).
*Methyl (1S,3R)-1-isopropyl-3-(4-(((trifluoromethyl)sulfonyl)oxy)-3,6-dihydropyridin-1(2H)-yl)cyclopentane-1-carboxylate (**50**)*. In a 250 mL round–bottom flask a solution of LDA (2M in heptanes, 11 mL, 22 mmol) in dry THF (100 mL) was cooled down to –100 °C under a nitrogen atmosphere. The ketone compound **49** (4.19 g, 15.6 mmol) was dissolved in dry THF (20 mL) and added dropwise to the reaction mixture keeping the temperature below –78 °C. Vigorous stirring was necessary. The reaction mixture was stirred for 3 hours, slowly rising the temperature to –40 °C. Subsequently, the reaction mixture was cooled down to –80 °C and *N*-phenyl-bis(trifluoromethanesulfonimide) (7.86 g, 22 mmol) dissolved in dry THF (10 mL) was added dropwise to the reaction mixture keeping the temperature of

the reaction below –80 °C. After the addition was complete, the reaction mixture was slowly brought to room temperature and stirred overnight. The reaction mixture was quenched with EtOH, concentrated in vacuum and partitioned between DCM/H_2O. The organic layer was dried with $MgSO_4$ and concentrated in vacuum. The product was purified by column chromatography on silica gel with an eluent system consisting of DCM and increasing amounts of EtOAc (0-10%), yielding the triflate compound **50** (4.2 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.73 (s, 1H), 3.70 (s, 3H), 3.25–3.12 (m, 2H), 2.80–2.67 (m, 3H), 2.46 (br s, 2H), 2.35–2.25 (m, 1H), 2.13–1.85 (m, 4H), 1.60–1.48 (m, 2H), 0.89 (d, *J* = 6.4 Hz, 3H), 0.86 (d, *J* = 6.4 Hz, 3H).

*N'-(1-((1R,3S)-3-isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)piperidin-4-ylidene)-4-methoxybenzenesulfonohydrazide (**52**)*. Sulfonylhydrazide **51** (365 mg, 1.80 mmol, 1.05 equiv.) was slurried in 3,5 mL of MeOH and piperidone **32** (750 mg, 1.72 mmol, 1.00 equiv.) was added at room temperature, resulting in a homogeneous reaction mixture. After 4 hours all of the piperidone was consumed shown by TLC (1/1 EtOAc/Pet. Ether). Methanol was removed in vacuum and the solidified oil (1.06 g, yield 100%) was used in the next reactions without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, *J* = 8.0 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.36 (s, 1H), 4.90–4.60 (m, 2H), 3.87 (s, 3H), 3.85–3.60 (m, 2H), 3.46 (s, 1H), 2.90 (s, 2H), 2.60–2.41 (m, 6H), 2.33 (br s, 3H), 2.19–2.14 (m, 1H), 2.00 (br s, 1H), 1.88–1.78 (m, 2H), 1.54–1.43 (m, 1H), 1.36–1.25 (m, 1H), 0.92 (d, J = 6.4 Hz, 3H), 0.75 (d, *J* = 6.4 Hz, 3H).

Abbreviations

Boc, *tert*-butyloxycarbonyl; CCL2, chemokine ligand 2; CCR2, chemokine receptor 2; CCR5, chemokine receptor 5; DCM, dichloromethane; DiPEA, *N*,*N*-diisopropylethylamine; DMAP, *N*,*N*-dimethylaminopyridine; DME, dimethoxyethane; EtOAc, ethylacetate; EtOH, ethanol; HPLC, high-performance liquid chromatography; ^{125}I-CCL2, ^{125}I–labelled chemokine ligand 2; KRI, kinetic rate index; LDA, lithium diisopropylamide; LC-MS, liquid chromatography – mass spectrometer; MCP-1, monocyte chemotactic protein-1; MeOH, methanol; NMR, nuclear magnetic resonance; PyBrOP, bromo-tris-pyrrolidino phosphoniumhexafluorophosphate; RT, residence time; SAR, structure–affinity relationships; SKR, structure–kinetic relationships; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; TMS, tetramethylsilane; U2OS, human bone osteosarcoma cells; UV, ultraviolet.

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REFERENCES.

1. Baggiolini, M. Chemokines and leukocyte traffic. *Nature* **1998,** 392, 565-568.
2. Bachmann, M. F.; Kopf, M.; Marsland, B. J. Chemokines: more than just road signs. *Nat Rev Immunol* **2006,** 6, 159-164.
3. Onuffer, J. J.; Horuk, R. Chemokines, chemokine receptors and small-molecule antagonists: recent developments. *Trends in Pharmacological Sciences* **2002,** 23, 459-467.
4. Tarrant, T. K.; Patel, D. D. Chemokines and leukocyte trafficking in rheumatoid arthritis. *Pathophysiology : the official journal of the International Society for Pathophysiology / ISP* **2006,** 13, 1-14.
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. 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.
7. Kanda, H.; Tateya, S.; Tamori, Y.; Kotani, K.; Hiasa, K.; Kitazawa, R.; Kitazawa, S.; Miyachi, H.; Maeda, S.; Egashira, K.; Kasuga, M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *The Journal of clinical investigation* **2006,** 116, 1494-505.
8. 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.
9. White, F. A.; Feldman, P.; Miller, R. J. Chemokine Signaling and the Management of Neuropathic Pain. *Molecular Interventions* **2009,** 9, 188-195.
10. Guo, D.; Hillger, J. M.; Ijzerman, A. P.; Heitman, L. H. Drug-Target Residence Time-A Case for G Protein-Coupled Receptors. *Medicinal Research Reviews* **2014**, Jul;34(4):856-92.
11. Swinney, D. C. Biochemical mechanisms of drug action: what does it take for success? *Nature Reviews Drug Discovery* **2004,** 3, 801-808.
12. Copeland, R. A.; Pompliano, D. L.; Meek, T. D. Drug-target residence time and its implications for lead optimization. *Nat Rev Drug Discov* **2006,** 5, 730-9.
13. 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.
14. Struthers, M.; Pasternak, A. CCR2 antagonists. *Current topics in medicinal chemistry* **2010,** 10, 1278-98.
15. Pasternak, A.; Goble, S. D.; Struthers, M.; Vicario, P. P.; Ayala, J. M.; Di Salvo, J.; Kilburn, R.; Wisniewski, T.; Demartino, J. A.; Mills, G. S.; Yang, L. Discovery of a potent orally bioavailable CCR2 and CCR5 dual antagonist. *Medicinal Chemistry Letters* **2010,** 1, 14-18.
16. Kuehne, M. E.; Muth, R. S. Total Syntheses of Yohimbe Alkaloids, with Stereoselection for the Normal, Allo, and 3-Epiallo Series, Based on Annelations of 4-Methoxy-1,2-Dihydropyridones. *Journal of Organic Chemistry* **1991,** 56, 2701-2712.
17. Crawford, J. B.; Chen, G.; Carpenter, B.; Wilson, T.; Ji, J.; Skerlj, R. T.; Bridger, G. J. Practical Convergent Laboratory-Scale Synthesis of a CCR5 Receptor Antagonist. *Org Process Res Dev* **2012,** 16, 109-116.
18. Vilums, M.; Zweemer, A. J.; Yu, Z.; de Vries, H.; Hillger, J. M.; Wapenaar, H.; Bollen, I. A.; 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.
19. Kothandaraman, S.; Donnely, K. L.; Butora, G.; Jiao, R.; Pasternak, A.; Morriello, G. J.; Goble, S. D.; Zhou, C.; Mills, S. G.; Maccoss, M.; Vicario, P. P.; Ayala, J. M.; Demartino, J. A.; Struthers, M.; Cascieri, M. A.; Yang, L. Design, synthesis, and structure-activity relationship of novel CCR2 antagonists. *Bioorganic & Medicinal Chemistry Letters* **2009,** 19, 1830-4.
20. Allwood, D. M.; Blakemore, D. C.; Brown, A. D.; Ley, S. V. Metal-Free Coupling of Saturated Heterocyclic Sulfonylhydrazones with Boronic Acids. *Journal of Organic Chemistry* **2014,** 79, 328-338.
21. Zweemer, A. J. M.;Nederpelt, I.; Vrieling, H.; Hafith, S.; Doornbos, M. L. J.; de Vries, H.; Abt, J.; Gross, R.; Stamos, D.; Saunders, J.; Smit, M. J.; Ijzreman, A. P.; Heitman, L. H. Multiple binding sites for small molecule antagonists at the chemokine receptor CCR2. *Mol. Pharmacol.* **2013** Oct;*84*(4):551-61.
22. Yang, L.; Butora, G.; Jiao, R. X.; Pasternak, A.; Zhou, C.; Parsons, W. H.; Mills, S. G.; Vicario, P. P.; Ayala, J. M.; Cascieri, M. A.; MacCoss, M. Discovery of 3-piperidinyl-1-cyclopentanecarboxamide as a novel scaffold for highly potent CC chemokine receptor 2 antagonists. *Journal of Medicinal Chemistry* **2007,** 50, 2609-11.
23. Butora, G.; Jiao, R.; Parsons, W. H.; Vicario, P. P.; Jin, H.; Ayala, J. M.; Cascieri, M. A.; Yang, L. 3-Amino-1-alkyl-cyclopentane carboxamides as small molecule antagonists of the human and murine CC chemokine receptor 2. *Bioorganic & Medicinal Chemistry Letters* **2007,** 17, 3636-41.
24. Pasternak, A.; Goble, S. D.; Vicario, P. P.; Di Salvo, J.; Ayala, J. M.; Struthers, M.; DeMartino, J. A.; Mills, S. G.; Yang, L. Potent heteroarylpiperidine and carboxyphenylpiperidine 1-alkyl-cyclopentane carboxamide CCR2 antagonists. *Bioorganic & Medicinal Chemistry Letters* **2008,** 18, 994-8.
25. Schmidtke, P.; Luque, F. J.; Murray, J. B.; Barril, X. Shielded Hydrogen Bonds as Structural Determinants of Binding Kinetics: Application in Drug Design. *Journal of the American Chemical Society* **2011,** 133, 18903-18910.