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

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**Title**: Small changes for long term impact : optimization of structure kinetic properties : a case of CCR2 antagonists
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# CHAPTER 4

## WHEN STRUCTURE–AFFINITY RELATIONSHIPS MEET STRUCTURE–KINETICS RELATIONSHIPS: 3-((INDEN-1-YL)AMINO)-1-ISOPROPYL-CYCLOPENTANE-1-CARBOXAMIDES AS CCR2 ANTAGONISTS

*This chapter was based upon:*

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(manuscript in preparation)

## ABSTRACT

Chemokine ligand 2 (CCL2) mediates chemotaxis of monocytes to inflammatory sites *via* interaction with its G protein–coupled receptor CCR2. Preclinical animal models suggest that the CCL2-CCR2 axis has a critical role in the development and maintenance of inflammatory disease states (e.g., multiple sclerosis, atherosclerosis, insulin resistance, restenosis, and neuropathic pain), which can be treated through inhibition of the CCR2 receptor. However, in clinical trials high-affinity inhibitors of CCR2 have often demonstrated a lack of efficacy. We have previously described a new approach for the design of high–affinity CCR2 antagonists, by taking their residence time (RT) on the receptor into account. Here, we report our findings on both structure–affinity relationship (SAR) and structure–kinetic relationship (SKR) studies for a series of 3-((inden-1-yl)amino)-1-isopropyl-cyclopentane-1-carboxamides as CCR2 antagonists. SAR studies showed that this class of compounds tolerates a vast diversity of substituents on the indenyl ring with only small changes in affinity. However, the SKR is affected greatly by minor modifications of the structure. The combination of SAR and SKR in the hit-to-lead process resulted in the discovery of a new high–affinity and long–residence–time CCR2 antagonist (compound **15a**, $K_i$ = 2.4 nM; RT = 714 min).

## INTRODUCTION

Chemokines are a class of endogenous pro-inflammatory proteins that act through activation and recruitment of leukocytes and other cell types in a range of inflammatory and non-inflammatory conditions. However, inappropriate overexpression of such proteins is implicated in a variety of disease conditions.[1] Both C-C Chemokine Ligand 2 (CCL2) and its cognate receptor C-C Chemokine Receptor 2 (CCR2) are involved in various autoimmune or inflammation-associated diseases. Blockade of the CCL2-CCR2 axis via either genetic or pharmacologic intervention has proven efficacious in animal models of multiple sclerosis, atherosclerosis, insulin resistance, restenosis, and neuropathic pain.[2-4]

Fuelled by such promising preclinical data, there has been an increasing interest in advancing antagonists of CCR2 into clinical trials. However, till now all small molecules tested have failed in clinical trials because of lack of efficacy, e.g., MK-0812 (orthosteric CCR2 antagonist for the treatment of rheumatoid arthritis and multiple sclerosis)[5] and AZD-2423 (potent, orally bioavailable, non-competitive, negative allosteric modulator of the CCR2 chemokine receptor for treatment of neuropathic pain).[6] A humanized anti-CCR2 antibody (MLN-1202) did not show efficacy either in patients with rheumatoid arthritis and multiple sclerosis.[7] However, administration of the antibody reduced the numbers of circulating monocytes in peripheral blood.[8] Moreover, a study of MNL-1202 in patients with risk factors for atherosclerosis demonstrated that treatment was able to reduce C–reactive protein levels.[9] This shows that CCR2 antagonism can have important biological effects in humans.

Apparently, to be efficacious in treatment of CCR2–related diseases, high–affinity antagonism is not enough. Moreover, blockade of CCR2 can cause an increase in endogenous CCL2 levels[8] which will compete again with the administered drug. To be able to withstand this increasing concentration of endogenous ligand, the drug should be slowly dissociating.

Previously we reported that by using an additional parameter in drug design, the so-called residence time (RT) on the receptor, we generated high–affinity and longer–residence–time CCR2 antagonists based on a (1*S*,3*R*)-3-amino-N-(3,5-bis(trifluoromethyl)benzyl)-1-

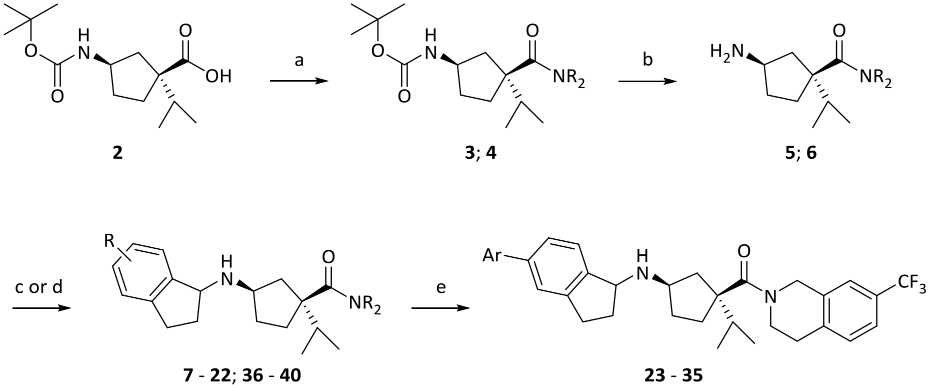
isopropylcyclopentane-1-carboxamide scaffold.[10] We explored different substituents and ring systems on the 3-amino group of the scaffold and observed that the longest residence time was found with an indane ring system. Although potent and long–residence–time compounds were identified in that study, we now sought to prolong the RT and define more detailed structure–kinetics relationships for the CCR2 receptor. In the present study we evaluated different amide groups for the 3-((inden-1-yl)amino)-1-isopropyl-cyclopentane-1-carboxamide scaffold based on their RT and explored a broad chemical space around the indane ring system to define the SAR and SKR for 3-((inden-1-yl)amino)-1-isopropyl-cyclopentane-1-carboxamides as CCR2 antagonists.

## RESULTS AND DISCUSSION

### Chemistry

The synthesis of *N*-(3,5-bis(trifluoromethyl)benzyl)-3-((2,3-dihydro-1*H*-inden-1-yl)amino)-1-isopropylcyclopentane-1-carboxamide **1** and (1*S*,3*R*)-3-((tert-butoxycarbonyl)amino)-1-isopropylcyclopentane-1-carboxylic acid **2** was achieved following the approach reported earlier by our group.[10] From acid **2** and 1-(4-(trifluoromethyl)pyridin-2-yl)piperazine or 7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride via a peptide coupling reaction were generated amides **3** and **4** under bromo-tris-pyrrolidino phosphoniumhexafluorophosphate (PyBroP) conditions.[11]

Scheme 1.[a]

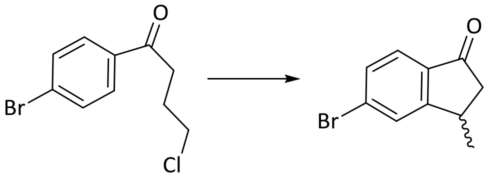


[a]Reagents and conditions: a) 1-(4-(trifluoromethyl)pyridin-2-yl)piperazine or 7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride, PyBrOP, DIPEA, DMAP, DCM, room temperature, 24 h, 82 - 90%; b) TFA, DCM, room temperature, 1 h, 90 - 98%; c) for array synthesis – corresponding indanone (1.05 equiv), 5-ethyl-2-methylpyridine borane (PEMB) (2.1 equiv), AcOH (1.05 equiv), NMP, 65 °C, 24 h, (compounds **7**; **9 – 13** and **17 – 22**); d) i) corresponding indanone (1,1 eq.), Ti(O-*i*-Pr)$_4$ (3 eq.), THF, 48 h, ii) $NaBH_4$, EtOH 16 h, room temperature, 8 – 41% (compounds **8**; **14 – 16** and **36 – 40**); e) array synthesis - compound **15**, corresponding aryl boronic acid, $Na_2CO_3$, Pd($PPh_3$)$_4$, toluene/NMP/$H_2O$, 80°C, 24 h, (compounds **23–35**).

Subsequently, a solution of TFA in DCM (1:1) was used to remove the *N*-Boc protecting group which yielded amines **5** and **6**. Compounds **7**, **9 – 13** and **17a** – **22** were generated from amines **5** and **6** using an array of different indanones with the 5-ethyl-2-methylpyridine borane complex (PEMB) under conditions reported by Burkhardt and Coleridge (Scheme 1).[12] In the case of the 5-$CF_3$ derivative (compound **17**) diastereomers were separated during the purification, however, only the first diastereomer to elute (**17a**) had a sufficient purity to be tested in bioassays. Compounds **8**, **14** – **16** and **36** – **40** were synthesized from **6** in a Ti(O*i*-Pr)$_4$ promoted reductive amination reaction with different indanones. Compounds **23 – 35** were synthesized via Suzuki–coupling of compound **15** with the corresponding arylboronic acids.

The synthesis of 3-Me; 5-Br indanone **42** was achieved by intramolecular cyclization of commercially available 1-(4-bromophenyl)-4-chlorobutan-1-one **41** following a procedure reported in patent literature (Scheme 2).[13]

Scheme 2.[a]

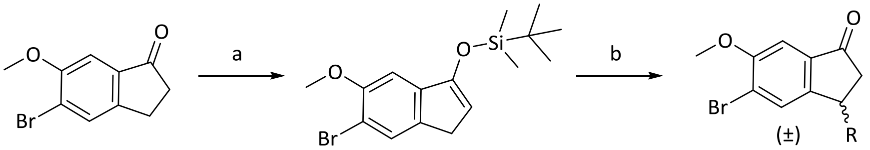


**41** **42** (±)

[a]Reagents and conditions: a) i) NaCl, $AlCl_3$, melt at 130 °C, ii) 4`-bromo-4-chloro-butyrophenone, 180 °C, 30 min, 91%.

The 3-alkyl; 5-Br; 6-OMe indanones were synthesized as shown in Scheme 3. The reaction of 5-Br; 6-OMe indanone **43** with *tert*-butyldimethylchlorosilane and DBU in benzene gave ((6-bromo-5-methoxy-1*H*-inden-3-yl)oxy)(*tert*-butyl)dimethylsilane (**44**). Deprotonation of **44** with *n*-BuLi and reaction of the lithium salt with methyl iodide or ethyl iodide in THF and subsequent quenching of the reaction mixture with 12 M HCl resulted in 5-bromo-6-methoxy-3-alkyl-indanones (**45**, **46**).

Scheme 3.[a]

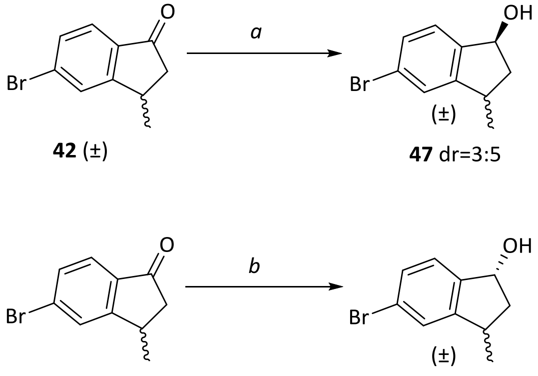


**43** **44** **45** R = Me
**46** R = Et

[a]Reagents and conditions: a) TBDMS-Cl, DBU, 0 °C → room temperature, 99%; b) i)LDA, 1 h, –78 °C → –35 °C, → –78 °C, 1 h, corresponding alkyliodide; ii) 12 M HCl, 82%.

The racemic (1*S*)-5-bromo-3-methyl-2,3-dihydro-1*H*-indanol **(47)** and racemic (1*R*)-5-bromo-3-methyl-2,3-dihydro-1*H*-indanol **(48)** were prepared via catalytic enantioselective reduction of racemic 5-bromo-3-methyl-2,3-dihydro-1*H*-indanone **(42)** using the (*R*)-methyl-CBS-oxazaborolidine and (*S*)-methyl-CBS-oxazaborolidine catalysts, respectively, with *N*,*N*-diethylaniline borane as reducing agent providing excellent enantioselectivity (Scheme 4).[14]

Scheme 4. [a]



**42** (±)

**47** dr=3:5

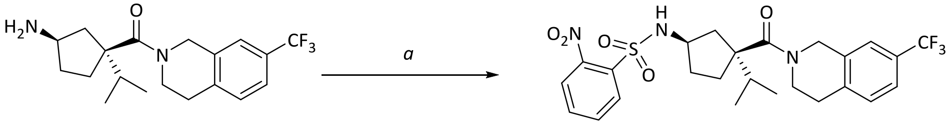
**42** (±)

**48** dr=3:5

[a] Reagents and conditions: a) (*R*)-(+)-2-methyl-CBS-oxazaborolidine catalyst, *N*,*N*,diethylaniline borane, toluene, 3 h, room temperature, 97 %. b) (*S*)-(+)-2-methyl-CBS-oxazaborolidine catalyst, *N*,*N*, diethylaniline borane, toluene, 3 h, room temperature, 99 %.

To prepare for the subsequent coupling, amine **6** was protected as the 2-nitrobenzenesulfonamide **(49)** (Scheme 5).[15]

Scheme 5. [a]



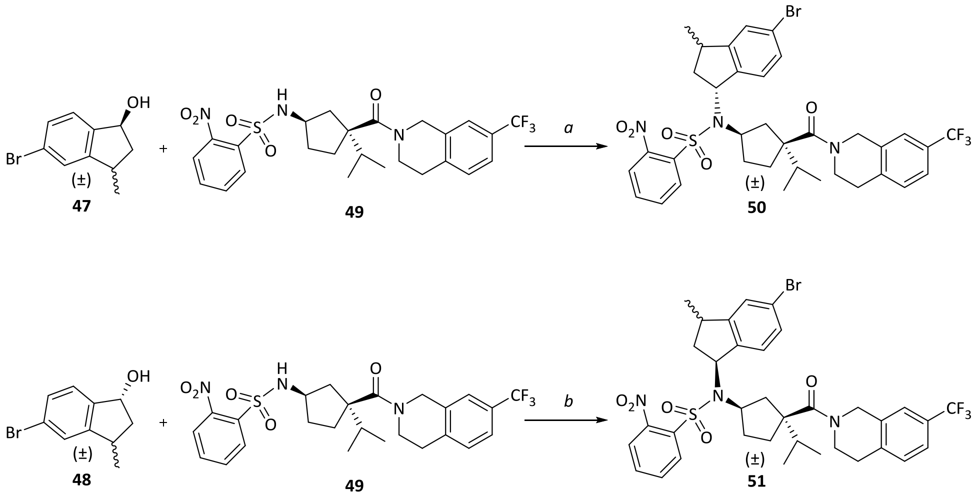
**6**

**49**

[a] Reagents and conditions: a) 2-nitrobenzenesulfonyl chloride, DIPEA, $CH_2Cl_2$, 1 h, room temperature, 98 %.

The respective racemic alcohols **(47)** and **(48)** when treated with **(49)** under Fukuyama–Mitsunobu conditions, resulted in *N*-alkylation to afford the 2-nitrobenzenesulfonamides **(50)** and **(51)** (scheme 6).

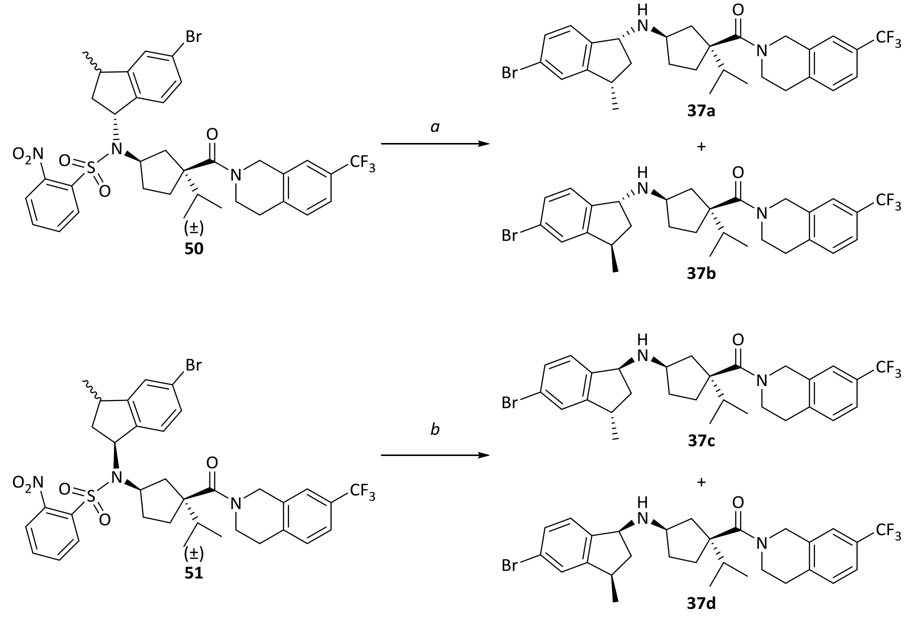
Scheme 6. [a]



[a]Reagents and conditions: a and b) DEAD, $PPh_3$, THF, –78 °C to room temperature.

Selective deprotection of the 2-nitrobenzenesulfonamides **(50)** and **(51)** with thiophenol and $K_2CO_3$ gave the desired diastereomers **37a, 37b, 37c** and **37d** (Scheme 7).

Scheme 7.[a]



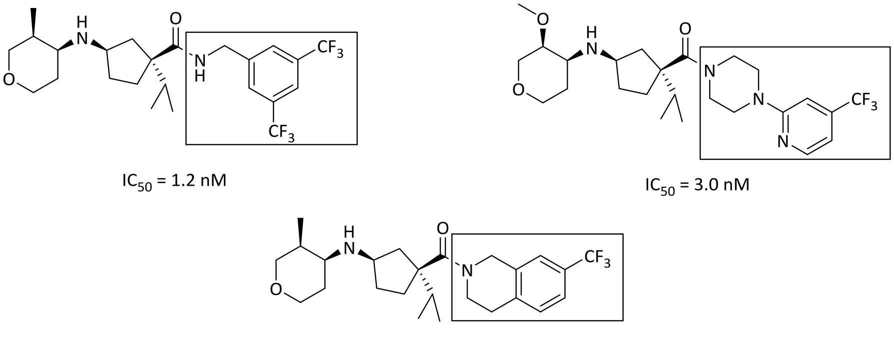
[a]Reagents and conditions: a and b) PhSH, $K_2CO_3$, DMF, room temperature.

## 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.[16] 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.[10]

## Structure–Affinity Relationships and Structure–Kinetics Relationships

In the past few years several distinctly different amide groups have been disclosed for the general CCR2 scaffold of 3-amino-1-isopropylcyclopentanecarboxamides, with many final compounds displaying high and often very similar affinities (Figure 1).[17-19]



$IC_{50}$ = 1.2 nM

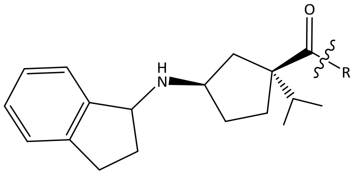
$IC_{50}$ = 3.0 nM

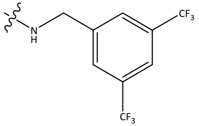
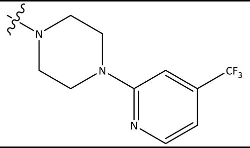
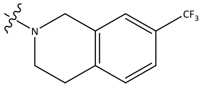
$IC_{50}$ = 1 nM

Figure 1. CCR2 antagonists from Merck and Pfizer based on a 3-amino-1-isopropylcyclopentanecarboxamide scaffold with different amide groups.[17-19]

In the current study we decided to keep the 3-((inden-1-yl)amino)-1-isopropyl-cyclopentane-1-carboxamide scaffold that was central in our previous report,[10] and investigated the effect on affinity and RT of three different amide groups (Table 1). When we changed the 3,5-bis(trifluoromethyl)benzyl group (compound **1**) to a 1-(4-(trifluoromethyl)pyridin-2-yl)piperazine group (compound **7**) the affinity was improved 3-fold, while a rigidification of the benzyl group into the 7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline group (compound **8**) yielded an even higher, 20-fold increase in affinity (compounds **1**, **7** and **8**; $K_i$ = 50 nM, 15 nM and 2.2 nM, respectively).

Table 1. Binding Affinities, KRI and Residence Time of compounds **1**; **7** and **8**

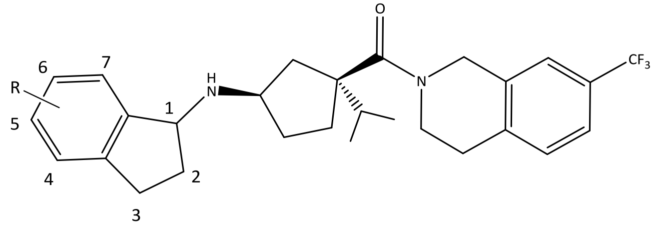


| Nr. | R | $K_i$ (nM) ± SEM (*n*=3) | KRI (*n*=2) | RT (min) |
|---|---|---|---|---|
| **1** |  | 50 ± 8 | 0.8 (0.7/0.8) | 9.1 ± 1.7 |
| **7** |  | 15 ± 1 | 0.5 (0.5/0.5) | 8.3 ± 2.8 |
| **8** |  | 2.2 ± 0.6 | 1.0 (0.9/1.0) | 21 ± 3 |

In kinetic tests of these compounds (kinetic rate index (KRI)[20] and RT) we learned that for longer receptor occupancy smaller and less flexible groups are preferred (KRI = 0.8, 0.5 and 1.0; RT = 9.1, 8.3 and 21 min, for **1**, **7** and **8**, respectively).

Encouraged by these results we decided to continue with compound **8** and investigate different substituents on the indenyl group (Table 2).The rigidification of the right-hand side of the structure improved the affinity in general, while the order of substituents on the indenyl group was hardly affected when compared to our previous findings on the 3,5-bis(trifluoromethyl)benzylamide derivatives (compound **1**).[10] Substitution on the 4-position decreased the affinity. Introduction of 4-Me (compound **9**) led to a 15-fold decrease. However, more polar groups were better tolerated. The 4-$NH_2$ substituent (compound **10**) led to a 2-fold decrease only, while 4-CN and 4-OH groups caused a 7- and 10-fold decrease (compounds **11** and **12**; $K_i$ = 15 and 21 nM, respectively).

Table 2. Binding Affinities, KRI and Residence Time of compounds **8**–**22**

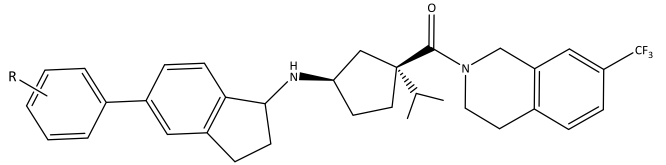


| Nr. | R | $K_i$ (nM) ± SEM ($n$=3) | KRI ($n$=2) | RT (min) |
|---|---|---|---|---|
| **8** | H | 2.2 ± 0.6 | 1.0 (0.9/1.0) | 21 ± 3 |
| **9** | 4-Me | 31 ± 2 | 0.7 (0.7/0.7) | - |
| **10** | 4-NH2 | 4.6 ± 1.0 | 0.7 (0.7/0.8) | - |
| **11** | 4-CN | 15 ± 5 | 0.6 (0.6/0.6) | - |
| **12** | 4-OH | 21 ± 3 | 0.7 (0.7/0.7) | - |
| **13** | 5-F | 4.9 ± 1.6 | 0.9 (0.9/0.9) | 55 ± 6 |
| **14** | 5-Cl | 1.6 ± 0.7 | 1.2 (1.0/1.3) | 100 ± 20 |
| **15** | 5-Br | 2.3 ± 0.6 | 1.3 (1.3/1.3) | 213 ± 32 |
| **16** | 5-I | 4.4 ± 0.9 | 1.3 (1.3/1.2) | 103 ± 9 |
| **17a** | 5-$CF_3$ | 13 ± 5 | 1.4 (1.5/1.3) | 667 ± 222 |
| **18** | 6-Me | 23 ± 6 | 0.6 (0.6/0.5) | - |
| **19** | 6-CN | 13 ± 8 | 0.6 (0.6/0.7) | - |
| **20** | 6-Cl | 7.9 ± 2.0 | 0.6 (0.6/0.6) | - |
| **21** | 5;6-di-OMe | 1.2 ± 0.3 | 1.0 (1.1/0.9) | 63 ± 5 |
| **22** | 4,7-di OMe | 49 ± 7 | 0.8 (0.8/0.8) | - |

Substituents on the 5 position had little effect on affinity. 5-F (compound **13**) led to a 2-fold decrease, but with an increase in the size of the halogen, 5-Cl and 5-Br, the affinity was regained (compounds **14** and **15**). However, 5-I (compound **16**) apparently was too big and led to a 2-fold decrease. An even bigger decrease was observed with the introduction of a $CF_3$ group (compound **17a**) often considered as a bioisostere of chlorine. However, this substitution pattern resulted in an 8-fold decrease compared to the chloro compound. On the 6 position neither an electron donating group (compound **18**), nor a strongly electron withdrawing group (compound **19**) was tolerated, while the 6-Cl substituent (compound **20**) led to a small decrease in affinity. However, the highest affinity compound **21** was obtained by double substitution on the 5 and 6 positions with methoxy groups. The corresponding regioisomer with 4,7-di-OMe (compound **22**) displayed a 40-fold decrease in affinity compared to compound **21**.

Testing these compounds in the high–throughput dual–point competition association assay showed that the abovementioned rigidification on the right–hand side of the molecule affects RT only for 5-substituted indenyl derivatives, with most of them having KRI values higher than unity. These compounds were tested in a full competition association assay and the highest affinity compound **21** had a RT of 63 min. Halogen substituents had size–dependent effects on KRI values. When tested for RT, indeed, increasing size correlated with longer residence times except for 5-I **16** where we observed a decrease in RT, as was the case for its affinity. However, compound **17a** (single diastereomer) displayed a more than 6-fold increase in RT compared to the 5-Cl compound (**14**). This indicates that affinity does not correlate with the residence time in this series of compounds. All other compounds showed KRI values below unity and thus showed a behavior comparable to the benzyl derivatives reported earlier.[10]
Next, we explored the 5 position by incorporating an additional aromatic system. Previously Xue et al[21] had shown this approach to be successful in a pyrolidine series of CCR2 antagonists. However, for our structures, an added unsubstituted phenyl ring (compound **23**) resulted in a dramatic decrease of affinity (Table 3). Adding a 2-Me group (compound **24**) yielded a small increase while 3-Me (compound **25**) did not improve the affinity compared to unsubstituted **23**. Incorporation of a cyano group on the 3 or 4 position (compounds **26** and **27**) resulted in a regain of affinity into the nanomolar range. The same effect was observed with a methoxy group (compounds **29** and **30**). However, 2-OMe (compound **28**) was detrimental for affinity.

Table 3. Binding Affinities, KRI and Residence Time of compounds **23**–**30**

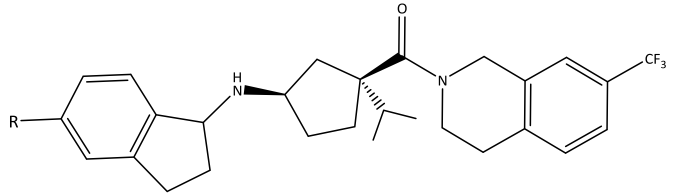


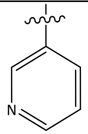
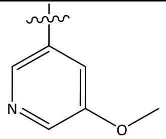
| Nr. | R | $K_i$ (nM) ± SEM ($n$=3) | KRI ($n$=2) |
|---|---|---|---|
| **23** | H | 28%$^a$ | - |
| **24** | 2-Me | 46%$^a$ | - |
| **25** | 3-Me | 24%$^a$ | - |
| **26** | 3-CN | 55 ± 14 | 0.7 (0.7/0.7) |
| **27** | 4-CN | 11 ± 4.7 | 0.9 (0.8/1.0) |
| **28** | 2-OMe | 2%$^a$ | - |
| **29** | 3-OMe | 39 ± 6 | 0.8 (0.8/0.8) |
| **30** | 4-OMe | 75 ± 23 | 0.9 (0.9/0.9) |

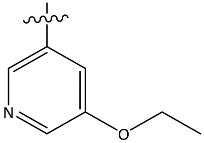
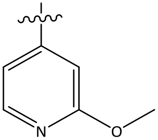
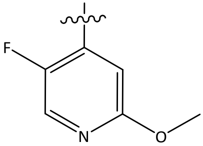
$^a$Percent displacement at 1 $\mu$M $^{125}$I-CCL2

These findings suggest that the space filling and hydrogen–accepting properties are more important for binding than the electronic properties of the substituents. Possible hydrogen bonding may also play a role when the phenyl ring was exchanged for 3-pyridine (compound **31**; $K_i$ = 10 nM) (Table 4).

Table 4. Binding Affinities, KRI and Residence Time of compounds **31**–**35**



| Nr. | R | $K_i$ (nM) ± SEM ($n$=3) | KRI ($n$=2) |
|---|---|---|---|
| **31** |  | 10 ± 4.5 | 0.7 (0.6/0.7) |
| **32** |  | 41 ± 8 | 0.8 (0.7/0.8) |

| | | | |
|---|---|---|---|
| **33** |  | 10 ± 4 | 0.5 (0.4/0.6) |
| **34** |  | 23 ± 7 | 0.6 (0.6/0.6) |
| **35** |  | 12 ± 1 | 0.8 (0.8/0.8) |

Incorporation of a 2-OMe group (compound **32**) resulted in a decrease of affinity, with an affinity comparable to its phenyl analogue **29**. Extending the substituent to an ethoxy group (compound **33**) is in accordance with the idea of space filling properties, as it yielded a gain in affinity compared to **32**. Changing the location of the nitrogen atom in the pyridine ring to the 4-position (compound **34**) improved the affinity by 2-fold vs **32**. An additional gain in affinity was reached by incorporating a fluorine atom on the 5 position (compound **35**). In general, this series of compounds suggests there is enough space in the binding pocket to accommodate another aromatic ring with preferably hydrogen bond accepting properties. However, when these compounds were tested in the dual-point competition association assay none of them showed KRI values above 1.

Another approach to investigate SAR and SKR in more detail was based on the superimposition of structure **8** with the structure of MK-0483, which has been reported as a CCR2 antagonist with a receptor dissociation time ($T_{1/2}$) of over 9 h (Figure 2).[22]

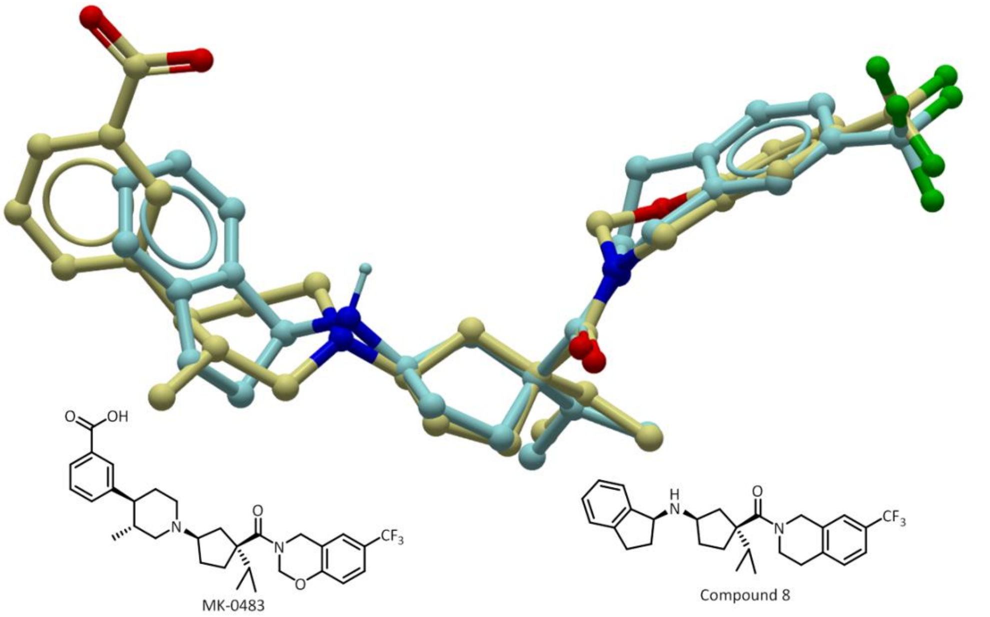
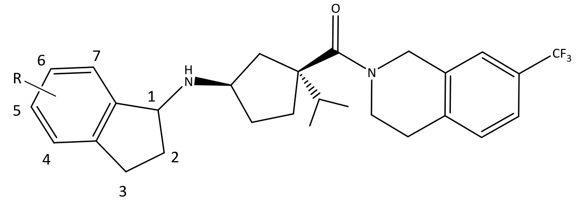


Figure 2. Superimposition of MK-0483 (yellow) with compound **8** (cyan) using "ICM-Pro 3.7b, Molsoft LLC".

In the superimposition the 3-position of the indane ring of compound **8** overlaps with the 3 position of the piperidine ring of MK-0483. So we decided to incorporate a methyl group on the 3 position of the indenyl system to yield compound **36** (Table 5).

Table 5. Binding Affinities, KRI and Residence Time of compounds **36**–**40**.

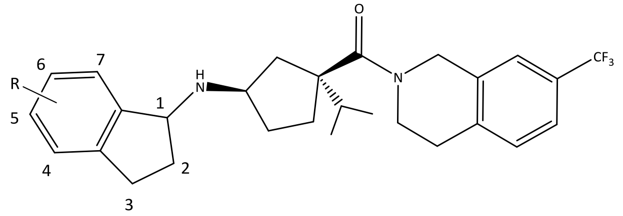


| Nr. | R | $K_i$ (nM) ± SEM ($n$=3) | KRI ($n$=2) | RT (min) |
|---|---|---|---|---|
| **36** | 3-Me | 3.4 ± 0.5 | 0.7 (0.6/0.7) | - |
| **37** | 5-Br, 3-Me | 2.0 ± 0.2 | 1.3 (1.3/1.2) | 345 ± 48 |
| **38** | 5-Br, 6-OMe | 4.5 ± 1.0 | 1.3 (1.3/1.2) | 323 ± 10 |
| **39** | 5-Br, 3-Me, 6-OMe | 13 ± 4 | 1.5 (1.6/1.3) | 238 ± 11 |
| **40** | 5-Br, 3-Et, 6-OMe | 83 ± 5 | 1.3 (1.3/1.3) | 179 ± 10 |

This had a minor effect on the affinity, but caused a significant decrease in KRI value. However, the combination of a 3-Me and 5-Br substituent (compound **37**) yielded an increase in RT while affinity remained unchanged. This is an indication that the 3-Me group *per se* is not in direct contact with the receptor binding site. However, it could play an important role in shielding of a sub-pocket in combination with 5-Br; a similar idea was put forward by Schmidtke et al.[23] in calculations on hydrogen bond shielding. Intrigued by our findings, we decided to combine the substituents of the long RT compound **15** (5-Br) and highest affinity compound **21** (6-OMe) in one structure **38**. This combination prolonged the RT, but the affinity was decreased by 2-fold. Our next step was to make a hybrid of compounds **37** and **38** to incorporate 3-Me; 5-Br; 6-OMe substituents on the indenyl group in one compound **39**. Next to this, we also extended the methyl group into an ethyl group (compound **40**). Unfortunately, these changes yielded 5- and 40-fold decreases in affinity, respectively, however, their RT was not affected when compared to 5-Br (compound **15**).

We then decided to separate compound **15** into diastereomers (Table 6) by preparative supercritical fluid chromatography (SFC). Similar to our previous findings[10] the first compound to elute also showed a higher affinity. However, the difference in affinity between the diastereomers was only 10-fold in the case of compounds **15a** and **15b**. In addition, compound **37** was resynthesized using a different method to yield all four diastereomers, which were separated (**37a**-**d**). *R*-diastereomers **37a** and **37b** retained high affinity, while *S*-diastereomers **37c** and **37d** had only sub-micromolar affinity values ($K_i$ = 1.7, 4.6, 199 and 137 nM, respectively).

Table 6. Binding Affinities, KRI and Residence Time of Separated Diastereomers. (**15**, **15a**, **15b**, **37**, **37a-d**)



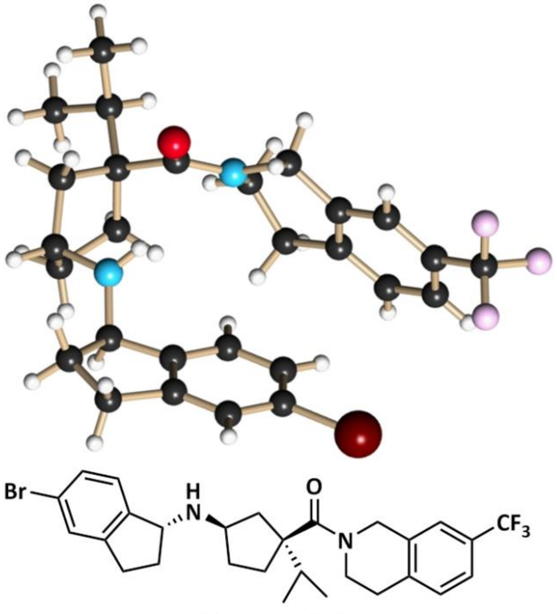
| Nr. | R | $K_i$ (nM) ± SEM ($n$=3) | KRI ($n$=2) | RT (min) |
|---|---|---|---|---|
| **15** | 5-Br | 2.3 ± 0.6 | 1.3 (1.3/1.3) | 213 ± 32 |
| **15a** | 5-Br | 2.4 ± 1.2 | 1.7 (1.7/1.7) | 714 ± 153 |
| **15b** | 5-Br | 24 ± 9 | 0.8 (0.7/0.9) | 15 ± 4 |
| **37** | 5-Br, 3-Me | 2.0 ± 0.2 | 1.3 (1.3/1.2) | 345 ± 48 |
| **37a** | 5-Br, 3-Me | 1.7 ± 0.1 | 1.4 (1.4/1.4) | 588 ± 208 |
| **37b** | 5-Br, 3-Me | 4.6 ± 0.1 | 1.2 (1.2/1.1) | 208 ± 35 |
| **37c** | 5-Br, 3-Me | 199 ± 47 | - | - |
| **37d** | 5-Br, 3-Me | 137 ± 15 | - | - |

In the RT measurements, a distinct difference was observed for the different diastereomers in the case of 7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline compounds on the amide part, quite the opposite of our previous findings for the flexible benzylamide derivatives.[10] The diastereomers of compound **15** had very different dissociation rate constants while the association rate constants were similar (Table 7).

Table 7. Kinetic data of **15a**, **b**, **17a** and **37a**, **b** compounds

| Nr. | $K_i$ (nM) ± SEM (n=3) | $k_{on}$ (nM$^{-1}$ min$^{-1}$) | $k_{off}$ (min$^{-1}$) | RT (min) |
|---|---|---|---|---|
| **15a** | 2.4 ± 1.2 | 0.0080 ± 0.0011 | 0.0014 ± 0.0003 | 714 ± 153 |
| **15b** | 24 ± 9 | 0.0059 ± 0.001 | 0.066 ± 0.017 | 15± 4 |
| **17a** | 13 ± 5 | 0.0032 ± 0.0004 | 0.0015 ± 0.0005 | 667 ± 222 |
| **37a** | 1.7 ± 0.1 | 0.0044 ± 0.0003 | 0.0017 ± 0.0006 | 588 ± 208 |
| **37b** | 4.6 ± 0.1 | 0.0026 ± 0.0006 | 0.0048 ± 0.0008 | 208 ± 35 |

We succeeded in crystalizing compound **15a** for single–crystal X-ray diffraction analysis. Based on the crystallographic analysis, the absolute configuration of compound **15a** was the *R*-isomer (Figure 3). Apparently, the rigidification stabilizes specific interactions of the *R*-isomer in the binding site of the CCR2, which results in smaller dissociation rate constants. This is also in accordance with **37a** and **37b**, however, the additional methyl group on the indane ring should be positioned in the 3*R*-conformation (**37a**) resulting in an almost 3-fold longer RT than the 3*S*-diastereomer (**37b**).



Compound **15a**

Figure 3. X-ray structure of ((1*S*,3*R*)-3-(((*R*)-5-bromo-2,3-dihydro-1*H*-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)methanone (**15a**).

## CONCLUSION

We have evaluated the SAR and SKR of 3-((inden-1-yl)amino)-1-isopropyl-cyclopentane-1-carboxamide derivatives as CCR2 antagonists. On the right-hand side of the molecule the 7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline group is optimal to increase residence time. On the left-hand side, the indane ring can accommodate very different substituents, as many compounds maintain nanomolar affinity. However, lipophilic and electron-withdrawing substituents (e.g. Cl, Br, I, CF$_3$) on the 5-position of the indane ring are crucial for long residence time. Moreover, compound **17a** (5-CF$_3$) having a modest (compared to other compounds in the series) affinity of 13 nM and a long residence time (RT = 667 min) is a good example that affinity and residence time do not necessarily. It also stresses the importance of additional screening for residence time in the early stages of drug discovery. In addition, small changes of the structure can have a big impact on residence time, e.g., the incorporation of a 5-Br (**15a**) substituent yielded a more than 30-fold increase in residence time (714 min). From this perspective compound **15a** should be evaluated in CCR2–related disease animal models to assess the usefulness of prolonged inhibition of CCR2. In general, this work provides methodology to retrieve the kinetic parameters from the vast number of high affinity compounds in the early stages of the drug discovery, which could help to provide better drug candidates for the later stages of drug development.

## 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). $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker AV 400 liquid spectrometer ($^1H$ NMR, 400 MHz; $^{13}C$ NMR, 100 MHz) or on a Bruker 500 MHz Avance III NMR spectrometer (compounds **15a**, **b** and **37a – d**) at ambient temperature. Chemical shifts are reported in parts per million (ppm), are designated by $\delta$, 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 for compounds **8**; **14 – 16** and **36** – **40** were as follows: 0.3–0.8 mg of compound was dissolved in 1 mL of a 1:1:1 mixture of $CH_3CN/H_2O/t\text{-}BuOH$ 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. Enantiomeric excess was accomplished using chiral SFC. For **47** and **48** the column was Chiralpak AD-H (250 x 4.6mm), 5µm. The mobile phase condition of 10% MeOH with 20 mM $NH_3$ and 90% $CO_2$ was applied at a flow rate of 3.0 mL/min at 254 nm. For **37a** and **37b** the column was Phenomenex Lux-4 (250 x 4.6 mm), 5 µm. The mobile phase condition of 20% *i*-propanol (IPA) with 1.0% DEA and 80% $CO_2$ was applied at a flow rate of 3.0 mL/min at 254 nm. For **37c** and **37d** the column was Regis RR-Whelko (250 x 4.6 mm), 5 µm. The mobile phase condition of 25% IPA with 1.0% diethylamine (DEA) and 75% $CO_2$ was applied at a flow rate of 3.0 mL/min at 254 nm. High–resolution mass spectral analyses (HRMS) were performed on LTQ-Orbitrap FTMS operated in a positive ionization mode with an electrospray ionization (ESI) source, with the following conditions: mobile phase A, 0.1% formic acid in water; mobile phase B, 0.08% formic acid in $CH_3CN$; gradient, 10–80% B in 26 min; and flow rate, 0.4 mL/min. Preparative HPLC (for compounds **7**; **9 – 13** and **18** – **35**) was performed on a Waters Auto Purification HPLC–ultraviolet (UV) system with a diode array detector using a Luna C18 Phenomenex column (75 × 30 mm, 5 µm), and a linear gradient from 1 to 99% of mobile phase B was applied. Mobile phase A consisted of 5 mM HCl solution, and mobile phase B consisted of acetonitrile. The flow rate was 50 mL/min. Liquid chromatography–mass spectrometry (LC–MS) analyses were performed using an Onyx C18 monolithic column (50 × 4.6 mm, 5 µm), and a linear gradient from 1 to 99% mobile phase B was applied. Mobile phase A consisted of 0.05% TFA in water, and mobile phase B consisted of 0.035% TFA in acetonitrile. The flow rate was 1.2 mL/min. Separations of enantiomers were accomplished using chiral SFC. The column was Phenomenex Lux-4 (250 × 10 mm, 5 µm). The mobile phase condition of 10% MeOH with 20 mM $NH_3$ and 90% $CO_2$ was applied at a flow rate of 10 mL/min. Optical rotations were measured in ethanol at 20 °C on a Perkin-Elmer polarimeter (Wavelength = 589 nm). The single crystal X-ray diffraction studies were carried out on a Bruker Kappa APEX-II CCD diffractometer equipped with Mo $K_{\alpha}$ radiation (λ = 0.71073 Å). 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.

Synthesis of (1*S*,3*R*)-3-(tert-butoxycarbonylamino)-1-isopropylcyclopentanecarboxylic acid (**2**) was achieved following the synthetic approach reported earlier by our group.[10]

*General Procedure for the Synthesis of Compounds **3** and **4**.*

Compound **2** (1 equiv) was dissolved in 25 ml DCM. To this mixture the corresponding amine (1 equiv) was added and subsequentaly DiPEA (3 equiv), PyBrOP (1 equiv) and DMAP (0.8 equiv). The reaction mixture was stirred for 24 hours at room temperature. The product was partitioned between DCM and 1 M citric acid solution in water and then with DCM/1M NaOH. The organic layer was dried with $MgSO_4$ and evaporated. The product was purified by column chromatography (0-100% ethyl acetate in DCM).

*tert-Butyl ((1R,3S)-3-isopropyl-3-(4-(4-(trifluoromethyl)pyridin-2-yl)piperazine-1-carbonyl)cyclopentyl)carbamate (**3**).*[18] Yield = 90%. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.22 (d, *J* = 4.8 Hz, 1H), 6.77–6.72 (m, 2H), 4.94 (br s, 1H), 3.82 (br s, 1H), 3.70–3.61 (m, 4H), 3.59–3.45 (m, 4H), 2.11–1.90 (m, 3H), 1.80–1.61 (m, 4H), 1.31 (s, 9H), 0.81–0.72 (m, 6H).

*tert-Butyl ((1R,3S)-3-isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)carbamate (**4**).*[24] Yield = 82%. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.45 (d, *J* = 7.6 Hz, 1H), 7.40 (s, 1H), 7.28 (d, *J* = 7.6, 1H), 4.97–4.61 (m, 3H), 4.00–3.73 (m, 3H), 2.94 (br s, 2H), 2.30–2.05 (m, 3H), 1.88–1.67 (m, 3H), 1.59 (br s, 1H), 1.42 (s, 9H), 0.91–0.84 (m, 6H).

*((1S,3R)-3-Amino-1-isopropylcyclopentyl)(4-(4-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)methanone (**5**).*[18] Trifluoroacetic acid (4 mL) was added to a solution of compound **3** (1.20 g, 2.48 mmol) in 10 mL of DCM. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was neutralized with 1 M NaOH and extracted with DCM. The organic layer was dried with $MgSO_4$, filtered, and evaporated to give the product as yellow crystals (0.86 g, 90%). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 8.20 (d, *J* = 4.8 Hz, 1H), 6.77–6.72 (m, 2H), 3.72–3.38 (m, 8H), 3.20–3.10 (m, 1H), 2.45–2.30 (m, 1H), 2.07–1.90 (m, 2H), 1.80–1.35 (m, 4H), 0.83–0.70 (m, 6H).

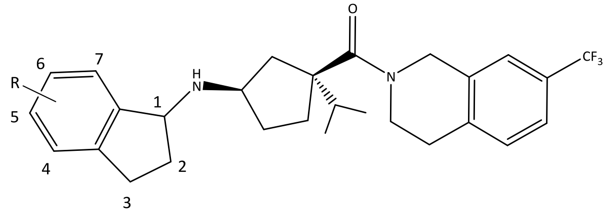
*((1S,3R)-3-Amino-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**6**).*[24] Trifluoroacetic acid (10 mL) was added to a solution of compound **4** (3.27 g, 7.2 mmol) in 10 mL of DCM. The reaction mixture was stirred for 1 h at room temperature. The reaction mixture was neutralized with 1 M NaOH and extracted with DCM. The organic layer was dried with $MgSO_4$, filtered, and evaporated to give the product as yellow crystals (2.50 g, 98%). $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 7.40 (d, *J* = 7.6 Hz, 1H), 7.36 (s, 1H), 7.24 (d, *J* = 7.6, 1H), 4.76 (s, 2H), 3.81 (br s, 2H), 3.32–3.22 (m, 1H), 2.91 (br s, 2H), 2.53–2.45 (m, 1H), 2.18–1.73 (m, 4H), 1.69–1.60 (m, 1H), 1.40–1.35 (m, 1H), 0.91–0.84 (m, 6H).

*General Procedure for the Synthesis of Compounds **7**, **9 – 13** and **18 – 23**.*

To a series of 1.5 mL glass tubes was added amine **5** or **6** in NMP (0.95 M, 0.095 mmol), followed by solutions of different indanones (0.5 M, 0.1 mmol) in NMP, and these mixtures were subsequently treated with acetic acid (0.1 mmol), followed by 5-ethyl-2-methyl-pyridine borane (PEMB) (0.2 mmol). The reaction mixture was heated at 65°C on a reaction block for 24 h. The reaction mixtures were purified directly using an automated mass-guided reverse-phase HPLC, and product containing fractions were concentrated to give final products of >90% purity as judged by LC–MS (average of 220 and 254 nm traces).

*((1S,3R)-3-((2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(4-(4-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)methanone (**7**)*. Purity (Average of 220 and 254 nm) = 95.4, LC-MS = $501^+$, $t_R$ = 1.75 min.

Table 8. Purity, $M^+$ and Retention Times of Compounds **9-13, 18-22**.



| Nr. | R | % Purity (Average of 220 and 254 nm) | Mol wt | $M^+$ | Retention time (min) |
|---|---|---|---|---|---|
| **9** | 4-Me | 97.3 | 484.3 | 485.4 | 1.70 |
| **10** | 4-NH2 | 99.3 | 485.3 | 486.4 | 1.39 |
| **11** | 4-CN | 97.4 | 495.3 | 496.3 | 1.57 |
| **12** | 4-OH | 92.5 | 486.3 | 487.3 | 1.56 |
| **13** | 5-F | 92.5 | 488.3 | 489.4 | 1.67 |
| **18** | 6-Me | 97.9 | 484.3 | 485.2 | 1.72 |
| **19** | 6-CN | 95.1 | 495.3 | 496.3 | 1.62 |
| **20** | 6-Cl | 98.9 | 504.2 | 505.3 | 1.71 |
| **21** | 5;6-di OMe | 96.1 | 530.3 | 531.3 | 1.58 |
| **22** | 4;7-di OMe | 92.3 | 530.3 | 531.3 | 1.74 |

*General Procedure for the Synthesis of Compounds **8**, **14 – 16** and **36 – 40**.*

In a 5 mL glass tube, amine **6** (1 equiv) dissolved in 1 mL of dry THF, and the corresponding indanone (1.2 equiv) dissolved in 1 mL of dry THF were loaded. Mixture was flushed with nitrogen gas and the tube was capped. Through the septa Ti(O-*i*Pr)$_4$ (3 equiv) was added and the reaction mixture was stirred for 48 h at room temperature. Then the tube was decapped and $NaBH_4$ (5 equiv) and 0.5 mL of absolute EtOH were added, and stirred for 16 h. The reaction mixture was quenched with $H_2O$ and resulting inorganic precipitate was filtered off and washed with DCM. The filtrate was extracted with DCM/$H_2O$. The organic layer was dried with $MgSO_4$ and evaporated. The product was purified by column chromatography (40% ethyl acetate in DCM).

*((1S,3R)-3-((2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**8**)*. Yield = 34%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.57–7.37 (m, 2H), 7.37–7.10 (m, 5H), 4.91–4.75 (m, 2H), 4.37–4.20 (m, 1H), 3.85 (s, 2H), 3.36–3.20 (m, 1H), 3.09–2.88 (m, 3H), 2.88–2.74 (m, 1H), 2.58 (br s, 1H), 2.49–2.33 (m, 1H), 2.25–1.87 (m, 4H), 1.87–1.72 (m, 2H), 1.72–1.56 (m, 1H), 1.48–1.35 (m, 1H), 1.01–0.79 (m, 6H). LC–MS: $471^+$; $t_R$: 9.79 min.

*((1S,3R)-3-((5-chloro-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**14**)*. Yield = 32%. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.53–7.34 (m, 2H), 7.30–7.18 (m, 4H), 4.90–4.70 (m, 2H), 4.30–4.13 (m, 1H), 3.90–3.75 (m, 2H), 3.30–3.14 (m, 1H), 3.02–2.84 (m, 3H), 2.82–2.69 (m, 1H), 2.62–2.34 (m, 2H), 2.20–1.54 (m, 7H), 1.38 (br.s, 1H), 0.98–0.76 (m, 6H). LC–MS: $505^+$; $t_R$: 9.82 min.

*((1S,3R)-3-((5-bromo-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**15**).* Yield = 14%. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.81–7.04 (m, 6H), 4.79 (br s, 2H), 4.21 (dd, $J$ = 15.3, 8.7 Hz, 1H), 3.85 (br s, 2H), 3.23 (d, $J$ = 6.5 Hz, 1H), 2.94 (d, $J$ = 4.3 Hz, 2H), 2.89–2.71 (m, 2H), 2.69–2.40 (m, 2H), 2.34 – 1.20 (m, 8H), 1.19–0.73 (m, 6H). LC–MS: 549$^+$; $t_R$: 10.19 min.

*((1S,3R)-3-(((R)-5-bromo-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**15a**).* $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 9.05 (br s, 1H), 7.70 (br s, 1H), 7.61 (br s, 2H), 7.54 (d, $J$ = 8.1 Hz, 1H), 7.52 (d, $J$ = 8.1 Hz, 1H), 7.41 (d, $J$ = 8.1 Hz, 1H), 4.85–4.68 (m, 3H), 3.85–3.70 (m, 2H), 3.20–3.10 (m, 1H), 3.00–2.82 (m, 3H), 2.60–2.32 (m, 4 H), 2.30–2.03 (m, 4H), 1.70–1.50 (m, 2 H), 0.90 (d, $J$ = 6.1 Hz, 3H) 0.65 (d, $J$ = 6.1 Hz, 3H),

$^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 174.6, 148.3, 148.3, 137.7, 135.3, 129.9, 129.5, 128.5 (d, $J_{C\text{-}F}$ = 11.65 Hz), 128.4, 128.1, 126.7 (q, $J_{C\text{-}F}$ = 222.65 Hz), 123.9, 123.7, 123.2, 60.9, 60.9, 57.3, 57.2, 56.2, 35.3, 32.9, 30.1, 29.9, 28.8, 28.8, 28.2, 18.3, 18.3.

**HRMS** calcd for ($C_{28}H_{33}BrF_3N_2O$) [M + H]$^+$ 549.1723, found 549.1708.

**SFC** chiral purity: 100 % ee.

**15a** = $[\alpha]_D^{20}$ = - 5.1 ($c$ = 0.65, EtOH).

*((1S,3R)-3-(((S)-5-bromo-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**15b**).* $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 9.61 (br s, 1H), 7.70 (br s, 1H), 7.66 (br s, 2H), 7.54 (d, $J$ = 8.1 Hz, 1H), 7.52 (d, $J$ = 8.1 Hz, 1H), 7.41 (d, $J$ = 8.1 Hz, 1H), 4.85–4.68 (m, 3H), 3.85–3.70 (m, 2H), 3.20–2.86 (m, 1H), 3.00–2.82 (m, 3H), 2.46–2.32 (m, 4 H), 2.30–2.03 (m, 4H), 1.70–1.50 (m, 2 H), 0.90 (d, $J$ = 6.1 Hz, 3H) 0.65 (d, $J$ = 6.1 Hz, 3H),

$^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: ppm 174.5, 148.3, 148.3, 137.7, 137.7, 130.3, 129.9, 128.5 (d, $J_{C\text{-}F}$ = 11.65 Hz), 128.4, 128.4, 126.7 (q, $J_{C\text{-}F}$ = 222.65 Hz), 123.9, 123.6, 123.3, 60.9, 60.9, 57.3, 57.2, 56.2, 35.4, 32.9, 30.1, 29.9, 28.8, 28.8, 28.2, 18.3, 18.3.

HRMS calcd for ($C_{28}H_{33}BrF_3N_2O$) [M + H]$^+$ 549.1723, found 549.1718.

SFC chiral purity: 97.4 % ee.

**15b** = $[\alpha]_D^{20}$ = -0.9 ($c$ = 0.68, EtOH)

*((1S,3R)-3-((5-iodo-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**16**).* Yield = 28%. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.55 (s, 1H), 7.48–7.40 (m, 3H), 7.28 (s, 1H), 7.05 (d, $J$ = 7.9 Hz, 0.5H$^a$), 6.99 (d, $J$ = 7.9 Hz, 0.5H$^b$), 4.79 (s, 2H), 4.24–4.16 (m, 1H), 3.84 (br s, 2H), 3.22 (q, $J$ = 6.0 Hz, 1H), 2.93 (s, 3H), 2.82–2.72 (m, 1H), 2.53 (br s, 1H), 2.43–2.34 (m, 1H), 2.16–1.82 (m, 4H), 1.80–1.60 (m, 3H), 1.38 (br s, 1H), 0.93 (d, $J$ = 5.7 Hz, 3H$^a$); 0.87 (d, $J$ = 5.7 Hz, 3H$^b$). *a* and *b* are indicated for different diastereomers. LC–MS: 597$^+$; $t_R$: 10.06 min.

*((1S,3R)-1-isopropyl-3-((3-methyl-2,3-dihydro-1H-inden-1-yl)amino)cyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**36**).* Yield = 41%. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.58–7.05 (m, 7H), 4.79 (d, $J$ = 16.5 Hz, 2H), 4.35–4.11 (m, 1H), 3.87 (s, 2H), 3.27 (s, 1H), 3.03 (dd, $J$ = 17.9, 15.0 Hz, 1H), 2.96 (s, 2H), 2.73–2.61 (m, 2H), 2.32–1.84 (m, 4H), 1.67 (dd, $J$ = 17.8, 11.2 Hz, 2H), 1.55–1.20 (m, 5H), 1.11–0.75 (m, 6H). LC–MS: 485$^+$; $t_R$: 10.08 min.

*((1S,3R)-3-((5-bromo-3-methyl-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**37**).* Yield = 8%. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 7.52–7.34 (m, 2H), 7.34–7.17 (m, 3H), 7.14 (d, $J$ = 7.8 Hz, 1Ha), 7.08 (d, $J$ = 8.0 Hz, 1Hb), 4.90–4.70 (m, 2H), 4.20–4.00 (m, 1H), 3.86 (s, 2H), 3.32–3.25 (m, 1H), 3.13–2.84 (m, 3H), 2.81–2.61 (m, 1H), 2.54 (br s, 1H), 2.28–2.05 (m, 2H), 1.94–1.58 (m, 4H), 1.50–1.16 (m, 5H), 1.03–0.79 (m, 6H). LC–MS: 563$^+$, 565$^+$; $t_R$: 10.00 min.

*((1S,3R)-3-((5-bromo-6-methoxy-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**38**).* Yield = 27%. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.54 – 7.18 (m, 4H), 6.92 (s, $1H^a$); 6.84 (s, $1H^b$), 4.90–4.70 (m, 2H), 4.30–4.20 (m, 1H), 3.98–3.66 (m, 5H), 3.36–3.13 (m, 1H), 3.01–2.81 (m, 3H), 2.80–2.66 (m, 1H), 2.44 (br s, 2H), 2.32–1.98 (m, 4H), 1.98–1.56 (m, 3H), 1.40 (m, 1H), 0.93 (d, *J* = 5.7Hz, $3H^a$); 0.87 (d, *J* = 5.7Hz, $3H^b$). *a* and *b* are indicated for different diastereomers. LC–MS: $579^+$, $581^+$; $t_R$: 10.06 min.

*((1S,3R)-3-((5-bromo-6-methoxy-3-methyl-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**39**).* Yield = 20%. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.54–7.35 (m, 2H), 7.35–7.20 (m, 2H), 6.91 (s, $0.25H^a$), 6.84 (s, $0.75H^b$), 4.95–4.68 (m, 2H), 4.24–4.03 (m, 1H), 3.88–3.79 (m, 5H), 3.35–3.16 (m, 1H), 3.07–2.85 (m, 3H), 2.81–2.64 (m, 1H), 2.56 (s, 1H), 2.20–2.05 (m, 3H), 1.92–1.85 (m, 2H), 1.69–1.64 (m, 1H), 1.55–1.37 (m, 1H), 1.36–1.18 (m, 4H), 1.02–0.78 (m, 6H). *a* and *b* are indicated for different diastereomers. LC–MS: $593^+$, $595^+$; $t_R$: 9.35 min.

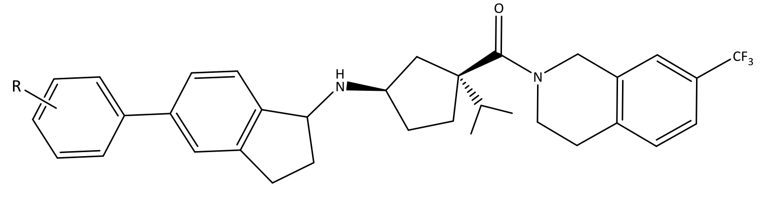
*((1S,3R)-3-((5-bromo-3-ethyl-6-methoxy-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**40**).* Yield = 17%. $^1$H NMR (400 MHz, $CDCl_3$) *δ*: 7.67–7.44 (m, 2H), 7.44–7.34 (m, 1H), 7.30 (s, 1H), 7.14 (s, 1H), 6.46 (s, 2H), 5.01–4.76 (m, 2H), 4.70 (d, *J* = 7.3 Hz, 1H), 4.09–3.71 (m, 6H), 3.19–2.64 (m, 4H), 2.51–2.14 (m, 4H), 2.13–1.70 (m, 5H), 1.56–1.39 (m, 1H), 1.16–0.77 (m, 9H). LC–MS: $607^+$, $609^+$; $t_R$: 9.60 min.

*((1S,3R)-1-isopropyl-3-((5-(trifluoromethyl)-2,3-dihydro-1H-inden-1-yl)amino)cyclopentyl)(7-(trifluoromethyl)-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**17a**)*. In a 50 mL round–bottom flask, to a solution of 1 equivalent of amine **6** in anhydrous methanol was added 1 equivalent of 5-(trifluoromethyl)-2,3-dihydro-1*H*-inden-1-one which was subsequently treated with 2 equivalents of acetic acid, followed by 4 equivalents of 5-ethyl-2-methyl-pyridine borane (PEMB). The reaction mixture was heated at 65°C for 24 h. Reaction mixture was monitored by reverse phase UPLC ($t_R$ : 0.53) and was carefully quenched with concentrated HCl, then water was added to the reaction mixture and extracted with dichloromethane. The combined organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. Reraction mixture was purified by flash chromatography (0-20 % $CH_2Cl_2$/MeOH) to afford product **17** (mixture of diastereomers). Further purification by reverse-phase HPLC using a gradient from 1 to 99% mobile phase B (mobile phase A = 0.1% HCl in water, mobile phase B = 0.1% HCl in $CH_3CN$) resulted in the separation of the two diastereomers **17 a** and **17 b** as HCl salt. Yield: **17a** = 19.5 %, 96% purity,UPLC-MS: $539^+$; $t_R$: 1.45 min and **17b** = 8.0%, 83% purity, UPLC-MS: $539^+$; $t_R$: 1.48 min. **17a** : $^1$H NMR (400 MHz, $CDCl_3$ ) δ 10.09 (s, 1H), 9.24 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.57 (s, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.46–7.38 (m, 1H), 7.35 (s, 1H), 7.29–7.20 (m, 1H), 4.90–4.62 (m, 3H), 3.84 (s, 1H), 3.68 (t, *J* = 8.7 Hz, 1H), 3.48 (q, *J* = 7.8, 7.0 Hz, 1H), 2.93 (ddt, *J* = 19.3, 11.4, 5.0 Hz, 3H), 2.68 (d, *J* = 14.1 Hz, 1H), 2.64–2.37 (m, 3H), 2.18–1.97 (m, 3H), 1.92 (dd, *J* = 14.2, 7.4 Hz, 1H), 1.80 (s, 1H), 1.68 (d, *J* = 12.1 Hz, 1H), 0.84 (dd, *J* = 20.0, 6.6 Hz, 6H).

*General Procedure for the Synthesis of Compounds **23 – 35**.*

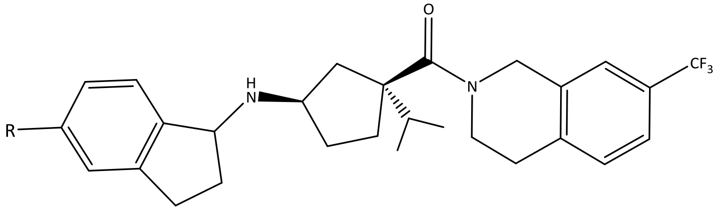
To a series of 1.5 mL glass tubes was added **15** in toluene ( 0.1 mmol) followed by solutions of different aryl boronic acids (0.5 M, 0.2 mmol) in NMP and these mixtures were subsequently treated with $Na_2CO_3$ solution ( 1 M, 0.2 mmol) followed by $Pd(PPh_3)_4$ in toluene (0.05 eq, 0.005 mmol). The reaction mixtures (0.15 M) were capped and heated at 80°C on a reaction block overnight. The reaction mixtures were purified directly using an automated mass–guided reverse phase–HPLC, and product containing fractions were concentrated to give final products >90% purity as judged by LC-MS (average of 220 nm and 254 nm traces).

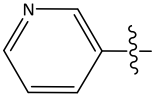
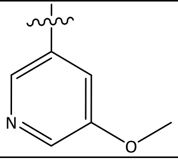
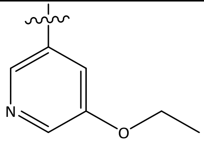
Table 9. Purity, $M^+$ and retention times of compounds **23-30**.

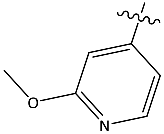
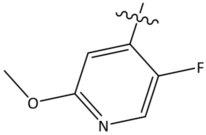


| Nr. | R | % Purity (Average of 220 and 254 nm) | Mol wt | M$^+$ | Retention time (min) |
|---|---|---|---|---|---|
| **23** | H | 96.8 | 546.29 | 547.2 | 1.92 |
| **24** | 2-Me | 91.5 | 560.3 | 561.3 | 1.92 |
| **25** | 3-Me | 99.5 | 560.3 | 561.3 | 1.96 |
| **26** | 3-CN | 99.38 | 571.3 | 572.3 | 1.77 |
| **27** | 4-CN | 99.5 | 571.3 | 572.2 | 1.76 |
| **28** | 2-OMe | 92.9 | 576.3 | 577.3 | 1.89 |
| **29** | 3-OMe | 97.7 | 576.3 | 577.3 | 1.66 |
| **30** | 4-OMe | 99.0 | 576.3 | 577.3 | 1.83 |

Table 10. Purity, M$^+$ and retention times of compounds **31-35**.



| Nr. | R | % Purity (Average of 220 and 254 nm) | Mol wt | M$^+$ | Retention time (min) |
|---|---|---|---|---|---|
| **31** |  | 99.9 | 547.3 | 548.2 | 1.40 |
| **32** |  | 99.9 | 577.3 | 578.3 | 1.45 |
| **33** |  | 99.9 | 591.3 | 592.3 | 1.43 |

| | | | | | |
|---|---|---|---|---|---|
| **34** |  | 93.7 | 577.3 | 578.3 | 1.66 |
| **35** |  | 96.8 | 595.3 | 596.3 | 1.79 |

*(1S,3R)-N-(3,5-bis(trifluoromethyl)benzyl)-3-(((1S,3R)-5-bromo-3-methyl-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentanecarboxamide (**37a**) and (1S,3R)-N-(3,5-bis(trifluoromethyl)benzyl)-3-(((1S,3S)-5-bromo-3-methyl-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentanecarboxamide (**37b**).* In a 20 mL scintillation vial, to a solution of **50** (1.00 g, 1.34 mmol) in DMF (10 mL) was added $K_2CO_3$ ( 0.55 g, 4.01 mmol) and benzenethiol (274.4 µL, 2.67 mmol), and the reaction mixture was stirred at room temperature for 30 min. The reaction was monitored by reverse phase UPLC ($t_R$: 0.73 and 0.74). To the reaction mixture was added water (20 mL) followed by extraction with $CH_2Cl_2$ (4 × 100 mL). The combined organic layer was washed with water, brine, dried over $Na_2SO_4$, and concentrated *in vacuo*. The brown residue was purified by flash chromatography (0-10% $CH_2Cl_2$/MeOH) to afford **37a** (0.10 g) and **37b** (0.10 g) with 80% diastereomeric excess purity by SFC analysis. Further purification was carried out by reverse–phase preparatory HPLC-UV with a gradient from 1 to 99% mobile phase B (mobile phase A = 0.1% HCl in water, mobile phase B= 0.1% HCl in $CH_3CN$) resulting in pure **37a** HCl salt and **37b** HCl salt as a white solid. Yield: **37a** = 14.7 mg, 1.8%, UPLC-MS: $563^+$, $565^+$; $t_R$: 2.45 min. $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$: 9.06 (br s, 1 H), 7.66 (bs, 1 H), 7.60 (m, 1 H), 7.52 (d, $J$ = 8.1 Hz, 1 H), 7.50 (m, 1 H), 7.47 (m, 1 H), 7.41 (d, $J$ = 8.1 Hz, 1 H), 4.70 (m, 2 H), 4.69 (dd, $J$ = 15.7, 7.3 Hz, 1 H), 3.77 (m, 2 H), 3.51 (s, 1 H), 3.16 (dt, $J$ = 14.3, 7.0 Hz, 1 H), 2.91 (m, 2 H), 2.76–1.68 (m, 2 H), 2.39–2.24 (m, 2 H), 2.17–1.59 (m, 2 H), 2.12 (m, 1 H), 1.64 (m, 2 H), 1.33 (d, $J$ = 6.8 Hz, 3 H), 0.89-0.75 (d, $J$ = 6.1 Hz, 6 H).

$^{13}C$ NMR (125 MHz, DMSO-$d_6$) $\delta$: 175.3, 150.9, 144.6, 139.3, 135.0, 130.3, 129.9, 128.6 (d, $J_{C\text{-}F}$ = 11.65 Hz), 126.9, 126.4 (q, $J_{C\text{-}F}$ = 222.65 Hz), 126.9, 123.6, 123.3, 120.1, 60.0, 55.7, 57.7, 47.8, 42.8, 38.5, 37.2, 36.6, 32.9, 29.9, 28.8, 28.8, 19.9, 18.3, 18.3

ROESY (NOE) (500 MHz, DMSO-$d_6$) δ ppm 7.50 [1.33 (H-30)], 4.69 [2.76 (H-6), 1.68 (H-6 weak), 2.17 (H-12), 3.16 (H-7)].

HRMS calcd for ($C_{29}H_{34}BrF_3N_2O$) $[M + H]^+$ 563.1879, found 563.1883.

SFC chiral purity: 99.1% ee.

**37a** = $[\alpha]_D^{20}$ = + 12.2 ($c$ = 0.43, EtOH)

**37b** = 17.3 mg, 2.1%, UPLC-MS: $563^+$, $565^+$; $t_R$: 2.49 min. $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$: 9.06 (bs, 1 H), 7.66 (bs, 1 H), 7.60 (m, 1 H), 7.52 (d, $J$ = 8.1 Hz, 1 H), 7.50 (m, 1 H), 7.47 (m, 1 H), 7.41 (d, $J$ = 8.1 Hz, 1 H), 4.70 (m, 2 H), 4.69 (dd, $J$ = 15.7, 7.3 Hz, 1 H), 3.77 (m, 2 H), 3.52 (m, 1 H), 3.41 (s, 1 H), 2.91 (m, 2 H), 2.91 (m, 2 H), 2.50–1.98 (m, 2 H), 2.39–2.24 (m, 2 H), 2.17–1.59 (m, 2 H), 2.12 (m, 1 H), 1.64 (m, 2 H), 1.25 (d, $J$ = 6.8 Hz, 3 H), 0.89–0.75 (d, $J$ = 6.1 Hz, 6 H).

$^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 175.3, 150.9, 144.6, 139.3, 135.0, 130.3, 129.9, 128.6 (d, $J_{C\text{-}F}$ = 11.65 Hz), 126.9, 126.9, 126.4 (q, $J_{C\text{-}F}$ = 222.65 Hz), 123.6, 123.3, 120.1, 59.89, 57.7, 55.7, 47.8, 42.8, 38.0, 37.4, 36.6, 32.9, 29.9, 28.8, 28.8, 19.9, 18.3, 18.3

ROESY (NOE) (500 MHz, DMSO-$d_6$) $\delta$: ppm 7.50 [3.52 (H-7)], 1.25 (H-30 weak)], 4.69 [2.50 (H-6), 1.98 (H-6 weak)].

HRMS calcd for ($C_{29}H_{34}BrF_3N_2O$) $[M + H]^+$ 563.1879, found 563.1883.

SFC chiral purity: 95.2% ee.

**37b** = $[\alpha]_D^{20}$ = + 13.9 ($c$ = 0.50, EtOH)

*(1S,3R)-N-(3,5-bis(trifluoromethyl)benzyl)-3-(((1R,3R)-5-bromo-3-methyl-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentanecarboxamide (**37c**) and (1S,3R)-N-(3,5-bis(trifluoromethyl)benzyl)-3-(((1R,3S)-5-bromo-3-methyl-2,3-dihydro-1H-inden-1-yl)amino)-1-isopropylcyclopentanecarboxamide (**37d**).* In a 20 mL scintillation vial, to a solution of **51** (1.00 g, 1.34 mmol) in DMF (10 mL) was added $K_2CO_3$ ( 0.55 g, 4.01 mmol) and benzenethiol (274.4 µL, 2.67 mmol), and the reaction mixture was stirred at room temperature for 30 min. The reaction was monitored by reverse phase UPLC ($t_R$: 0.76). To the reaction mixture was added water (20 mL) followed by extraction with $CH_2Cl_2$ (4 × 100 mL). The combined organic layer was washed with water, brine, dried over $Na_2SO_4$, and concentrated *in vacuo*. The brown residue was purified by flash chromatography (0-10% $CH_2Cl_2$/MeOH) to afford **37c** (50 mg) and **37d** (90 mg) both with 50% diastereomeric excess purity. Further purification was carried out by reverse–phase preparatory HPLC-UV with a gradient from 1 to 99% mobile phase B (mobile phase A = 0.1% HCl in water, mobile phase B= 0.1% HCl in $CH_3CN$) resulting in **37c** HCl salt and **37d** HCl salt as a white solid, both again with 70% diastereomeric excess purity. Therefore further purification was carried out on a Gilson purification instrument with a normal phase silica column and a diode array detector using a Luna Phenomenex column (50mm × 21mm, 5µm), and a linear gradient from 1 to 10% ($CH_2Cl_2$/MeOH) of mobile phase was applied. Mobile phase A consisted of $CH_2Cl_2$ and mobile phase B consisted of 10% MeOH/$CH_2Cl_2$. Yield: **37c** = 13.1 mg, 1.7%, UPLC-MS: $563^+$, $565^+$; $t_R$: 2.52 min. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 7.66 (bs, 1 H), 7.60 (m, 1 H), 7.52 (d, $J$ = 8.1 Hz, 1 H), 7.41 (d, $J$ = 8.1 Hz, 1 H), 7.38 (m, 1 H), 7.30 (m, 1 H), 4.70 (m, 2 H), 4.01 (m, 1 H), 3.77 (m, 2 H), 3.51 (s, 1 H), 3.14 (m, 1 H), 2.91 (m, 2 H), 2.57–1.20 (m, 2 H), 2.39–2.24 (m, 2 H), 2.17–1.59 (m, 2 H), 2.12 (m, 1 H), 1.64 (m, 2 H), 1.24 (d, $J$ = 6.8 Hz, 3 H), 0.89–0.75 (d, $J$ = 6.1 Hz, 6 H).

$^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 175.3, 150.9, 144.6, 139.3, 135.0, 130.3, 129.9, 128.6(d, $J_{C\text{-}F}$ = 11.65 Hz), 126.9, 126.4 (q, $J_{C\text{-}F}$ = 222.65 Hz), 123.6, 123.4, 123.3 120.1, 60.0, 58.4, 57.7, 55.7, 47.8, 44.8, 42.8, 36.6, 32.9, 29.9, 28.8, 28.8, 19.9, 18.3, 18.3

HRMS calcd for ($C_{29}H_{34}BrF_3N_2O$) $[M + H]^+$ 563.1879, found 563.1883.

SFC chiral purity: 95.0% ee.

**37c** = $[\alpha]_D^{20}$ = + 1.9 ($c$ = 1.9, EtOH).

**37d** = 12.7 mg, 1.7%, UPLC-MS: $563^+$, $565^+$; $t_R$: 2.52 min. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$: 9.06 (bs, 1 H), 7.66 (bs, 1 H), 7.60 (m, 1 H), 7.52 (d, $J$ = 8.1 Hz, 1 H), 7.50 (m, 1 H), 7.47 (m, 1 H), 7.41 (d, $J$ = 8.1 Hz, 1 H), 4.70 (m, 2 H), 4.12 (dd, $J$ = 15.7, 7.3 Hz, 1 H), 3.77 (m, 2 H), 3.51 (s, 1 H), 3.23 (dt, $J$ = 14.3, 7.0 Hz,1 H), 2.91 (m, 2 H), 2.76–1.68 (m, 2 H), 2.39–2.24 (m, 2 H), 2.17–1.59 (m, 2 H), 2.12 (m, 1 H), 1.64 (m, 2 H), 1.16 (d, $J$ = 6.8 Hz, 3 H), 0.89–0.75 (d, $J$ = 6.1 Hz, 6 H).

$^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$: 175.3, 150.9, 144.6, 139.3, 135.0, 130.3, 129.9, 128.6(d, $J_{C\text{-}F}$ = 11.65 Hz), 126.9, 126.9, 126.4 (q, $J_{C\text{-}F}$ = 222.65 Hz), 123.6, 123.3 120.1, 60.0, 57.7, 55.7, 47.8, 42.8, 38.5, 37.2, 36.6, 32.9, 29.9, 28.8, 28.8, 20.9, 18.3, 18.3

HRMS calcd for ($C_{29}H_{34}BrF_3N_2O$) $[M + H]^+$ 563.1879, found 563.1857.

SFC chiral purity: 95.3% ee.

**37d** = $[\alpha]_D^{20}$ = - 0.9 (*c* = 0.9, EtOH).

*5-bromo-3-methyl-2,3-dihydro-1H-inden-1-one (**42**).*[13] In a 50 mL round-bottom flask NaCl (3.7 g, 63.0 mmol) and $AlCl_3$ (15.0 g, 115 mmol) were loaded and heated at 130 °C until completely melted. Next, 4`-bromo-4-chloro-butyrophenone (**41**) (3.0 g, 11.5 mmol) was added and the reaction mixture was heated at 180 °C for 30 min. After cooling to room temperature, the reaction mixture was slowly poured on an ice/1N HCl mixture – an exothermic reaction was observed. The reaction mixture was extracted with DCM, dried with $MgSO_4$ and the organic solvent was evaporated to yield 2.35 g (yield 91%, with 90% purity) of light brown crystals which were used in the next step without purification. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.70 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 3.50–3.40 (m, 1H), 2.96 (ABX, *J* = 19.2 Hz$^a$, *J* = 7.6 Hz$^b$, 1H), 2.30 (ABX, *J* = 18.8 Hz$^a$, *J* = 3.6 Hz$^b$, 1H), 1.43 (d, *J* = 7.2 Hz, 3H).

*((6-bromo-5-methoxy-1H-inden-3-yl)oxy)(tert-butyl)dimethylsilane (**44**).* In a 25 mL round–bottom flask 5-bromo-6-methoxy-2,3-dihydro-1*H*-inden-1-one (**43**) (0.96 g, 4.0 mmol) was dissolved in 8 mL of toluene and cooled to 0 °C. TBDMS-Cl solution in toluene (1.95 mL, 6.5 mmol) was added, followed by dropwise addition of DBU (1.12 mL, 7.5 mmol). The reaction mixture was stirred at 0 °C for 10 min and continued to be stirred at room temperature for 7 days. The reaction mixture was extracted with $Et_2O/H_2O$, dried with $MgSO_4$, and evaporated. Yield = 1.40 g (98%), which was used in the next step without purification. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.55 (s, 1H), 6.94 (s, 1H), 5.47 (t, *J* = 2.4 Hz, 1H), 3.96 (s, 3H), 3.22 (d, *J* = 2.4 Hz, 2H), 1.05 (s, 9H), 0.29 (s, 6H).

*5-bromo-6-methoxy-3-methyl-2,3-dihydro-1H-inden-1-one (**45**).* In a 20 mL reaction tube a 2 M solution of LDA (0.6 mL, 1.2 mmol) in THF/heptane/ethylbenzene was mixed with 5 mL of dry THF. The reaction mixture was cooled down to –78 °C and compound **44** (0.35 g, 1 mmol) (dissolved in 1 mL of dry THF) was added. The reaction mixture was warmed to –35 °C during 1 h and then cooled back to –78 °C. Methyl iodide (0.08 mL, 1.3 mmol) was added and the reaction mixture was warmed to room temperature over 2 h and left stirring overnight. 37% HCl in water (0.27 mL, 3.2 mmol) was added and this mixture was stirred for 3 h. The reaction mixture was extracted with DCM/$H_2O$, dried with $MgSO_4$, and evaporated. The product was purified by column chromatography with DCM as eluent. Yield = 0.21 g (82%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.61 (s,1H), 7.03 (s, 1H), 3.83 (s, 3H), 3.35 – 3.25 (m, 1H), 2.85 (ABX, *J* = 18.8 Hz$^a$, *J* = 7.6 Hz$^b$, 1H), 2.18 (ABX, *J* = 19.2 Hz$^a$, *J* = 3.2 Hz$^b$, 1H), 1.29 (d, *J* = 7.2 Hz, 3H).

*5-bromo-6-methoxy-3-ethyl-2,3-dihydro-1H-inden-1-one (**46**).* In a 20 mL reaction tube a 2 M solution of LDA (0.6 mL, 1.2 mmol) was dissolved in 5 mL of dry THF. The reaction mixture was cooled down to –78 °C and compound **44** (0.35 g, 1 mmol) (dissolved in 1 mL of dry THF) was added. The reaction mixture was warmed to –35 °C during 1 h and then cooled back to –78 °C. Ethyl iodide (0.10 mL, 1.3 mmol) was added and the reaction mixture was warmed to room temperature during 2 h and left stirring overnight. 37% HCl in water (0.27 mL, 3.2 mmol) was added and stirred for 3 h. The reaction mixture was extracted with DCM/$H_2O$, dried with $MgSO_4$, and evaporated. The product was purified by column chromatography with DCM as eluent. Yield = 0.24 g (89%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.67 (s,1H), 7.11 (s, 1H), 3.88 (s, 3H), 3.35–3.25 (m, 1H), 2.81 (ABX, *J* = 19.2 Hz$^a$, *J* = 7.2 Hz$^b$, 1H), 2.32 (ABX, *J* = 19.2 Hz$^a$, *J* = 3.2 Hz$^b$, 1H), 1.95–1.85 (m, 1H), 1.55–1.45 (m, 1H), 0.93 (t, *J* = 7.2 Hz, 3H).

*(1S)-5-bromo-3-methyl-2,3-dihydro-1H-inden-1-ol (**47**).* In a 50 mL round bottom flask under $N_2$ atmosphere, (*R*)-(+)-2-methyl-CBS-oxazaborolidine catalyst (73.9 mg, 0.27 mmol, 1 M in toluene) in anhydrous toluene (5 mL) was added to *N*,*N*-diethylaniline borane (1.58 mL, 8.88 mmol) at room temperature (25°C). To this solution **42** ( 0.50 g, 2.22 mmol) in toluene (20 mL) was added dropwise over

5 h, and the resulting mixture was stirred overnight at room temperature (25°C). Reaction was monitored by reverse phase UPLC ($t_R$ : 0.59). The reaction mixture was carefully quenched with methanol (5 mL) and 1 M HCl (1 mL) and extracted with ethyl acetate (4 × 50 mL). The combined organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated *in vacuo*. The white residue was purified by flash chromatography (0-30 % ethyl acetate/hexanes) to afford the white crystalline solid **47** as a racemic mixture of diastereomers in a ratio 3:5. Yield = 0.49 g, 2.16 mmol (97%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.41–7.32 (m, 3H), 7.29–7.22 (m, 3H), 5.20 (dd, *J* = 6.4, 3.1 Hz, 1H), 5.16–5.08 (m, 1H), 3.43 (h, *J* = 7.0 Hz, 1H), 3.05 (h, *J* = 7.1 Hz, 1H), 2.76 (dt, *J* = 12.7, 7.1 Hz, 1H), 2.25 (ddd, *J* = 13.6, 7.4, 3.1 Hz, 1H), 1.98 (dt, *J* = 13.4, 6.6 Hz, 1H), 1.49 (ddd, *J* = 12.7, 8.9, 7.6 Hz, 1H), 1.34 (d, *J* = 6.8 Hz, 3H), 1.26 (dd, *J* = 7.1, 2.4 Hz, 2H). HPLC (Chiralpak AD-H column, 10% MeOH with 20 nM $NH_3$, 90% $CO_2$, 3.0 mL/min, 254 nm, ee = 95.8%. UPLC-MS: $209^+$, $211^+$; $t_R$: 1.91 min.

*(1R)-5-bromo-3-methyl-2,3-dihydro-1H-inden-1-ol (**48**)*. In a 50 mL round bottom flask under $N_2$, (*S*)-(+)-2-methyl-CBS-oxazaborolidine catalyst (73.9 mg, 0.27 mmol, 1 M in toluene) in anhydrous toluene (5 mL) was added to *N*,*N*-diethylaniline borane (1.58 mL, 8.88 mmol) at room temperature (25°C). To this solution **42** ( 0.50 g, 2.22 mmol) in toluene (20 mL) was added dropwise over 5 h, and the resulting mixture was stirred overnight at room temperature (25°C). The reaction was monitored by reverse phase UPLC ($t_R$ : 0.58). The reaction mixture was carefully quenched with methanol (5 mL) and 1 N HCl (1 mL) and then extracted with ethyl acetate (4 × 50 mL). The combined organic layer washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The white residue was purified by flash chromatography (0-30 % ethyl acetate/hexanes) to afford the white crystalline solid **48** as a racemic mixture in a ratio (3:5). Yield = 0.50 g, 2.20 mmol (99%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.37 (dddt, *J* = 8.4, 5.6, 2.8, 1.2 Hz, 4H), 7.28–7.22 (m, 2H), 5.20 (dd, *J* = 6.4, 3.1 Hz, 1H), 5.12 (t, *J* = 7.3 Hz, 1H), 3.43 (h, *J* = 7.0 Hz, 1H), 3.12–2.97 (m, 1H), 2.76 (dt, *J* = 12.7, 7.1 Hz, 1H), 2.25 (ddd, *J* = 13.6, 7.4, 3.1 Hz, 1H), 1.98 (dt, *J* = 13.4, 6.6 Hz, 1H), 1.49 (ddd, *J* = 12.7, 8.9, 7.7 Hz, 1H), 1.34 (d, *J* = 6.8 Hz, 3H), 1.26 (d, *J* = 7.0 Hz, 2H). HPLC (Chiralpak AD-H column, 10% MeOH with 20 nM $NH_3$, 90% $CO_2$, 3.0 mL/min, 254 nm, ee = 96.2%. UPLC-MS: $209^+$, $211^+$; $t_R$: 1.92, 1.95 min.

*N-((1R,3S)-3-isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)-2-nitrobenzenesulfonamide (**49**)*. In a 50 mL round bottom flask, to a solution of amine **6** (1.00 g, 2.82 mmol) in anhydrous $CH_2Cl_2$ (10 mL) was added 2-nitrobenzenesulfonyl chloride (0.75 mg, 3.38 mmol) followed by DIPEA (1.70 mL, 9.73 mmol) and stirred at room temperature (25°C) for 1 h. The reaction was monitored by reverse phase UPLC ($t_R$ : 0.77). The reaction mixture was concentrated in vacuo to obtained the crude product (yellow oil). The product was purified by flash chromatography (40-80 % ethyl acetate/hexanes) to afford the light yellow foamy solid **49**. Yield = 1.50 g (98%).$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.16–8.09 (m, 1H), 7.82–7.75 (m, 1H), 7.75–7.64 (m, 2H), 7.48–7.40 (m, 1H), 7.36 (s, 1H), 7.27 (d, *J* = 4.9 Hz, 1H), 6.05 (s, 1H), 4.81 (s, 1H), 4.71 (d, *J* = 16.4 Hz, 1H), 3.94 (d, *J* = 16.7 Hz, 1H), 3.81 (dq, *J* = 8.7, 4.3 Hz, 1H), 3.76–3.64 (m, 1H), 2.90 (tt, *J* = 16.6, 6.4 Hz, 2H), 2.40 (d, *J* = 12.9 Hz, 1H), 2.14 (pent., *J* = 6.7 Hz, 1H), 1.82 (d, *J* = 16.1 Hz, 3H), 1.67 (dd, *J* = 8.6, 4.5 Hz, 1H), 1.58–1.40 (m, 1H), 0.87 (d, *J* = 6.7 Hz, 3H), 0.76 (d, *J* = 6.7 Hz, 3H). UPLC-MS: $540^+$; $t_R$: 2.85 min.

*N-((1R)-5-bromo-3-methyl-2,3-dihydro-1H-inden-1-yl)-N-((1R,3S)-3-isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)-2-nitrobenzenesulfonamide (**50**)*. In a 100 mL round-bottom flask, to a cooled solution (–78 °C, acetone/dry ice bath) of $PPh_3$ (1.23 g, 4.69 mmol) in anhydrous THF (20 mL) was added DEAD (0.82 g, 4.69 mmol) and stirred for 30 min, followed by addition of **47** (0.40 g, 1.76 mmol). The pink colored reaction mixture was allowed to stir for 30 min maintaining the temperature at –78 °C. To this solution, **49** (0.63 g, 1.17 mmol) was added. The reaction mixture was stirred for 6 h at –78 °C and allowed to warm up to room temperature overnight. The reaction was monitored by reverse phase UPLC ($t_R$ : 0.93). The reaction mixture was concentrated in vacuo to obtained a pink-colored residue. The crude residue was purified by flash chromatography (0-10 % $CH_2Cl_2$/MeOH) to afford the light pink foamy solid **50** ( purity 80%). Yield = 1.00 g (<100%). UPLC-MS: $540^+$; $t_R$: 3.53 min. Without further purification, **50** was taken forward in the following deprotection step.

*N-((1S)-5-bromo-3-methyl-2,3-dihydro-1H-inden-1-yl)-N-((1R,3S)-3-isopropyl-3-(7-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)cyclopentyl)-2-nitrobenzenesulfonamide (**51**)*. In a 100 mL round-bottom flask, to a cooled solution (–78 °C, acetone/ dry ice bath) of $PPh_3$ (1.23 g, 4.69 mmol) in anhydrous THF (20 mL) was added DEAD (0.82 g, 4.69 mmol) and stirred for 30 min, followed by addition of **48** (0.40 g, 1.76 mmol). The pink colored reaction mixture was allowed to stir for 30 min maintaining the temperature at –78 °C. To this solution, **49** (0.63 g, 1.17 mmol) was added. The reaction mixture was stirred for 6 h at –78 °C and allowed to warm up to room temperature overnight. The reaction was monitored by reverse phase UPLC ($t_R$ : 0.93). The reaction mixture was concentrated *in vacuo* to obtained a pink color residue. The crude residue was purified by flash chromatography (0-10 % $CH_2Cl_2$/MeOH) to afford the light pink foamy solid **55** ( purity 80%). Yield = 1.00 g (<100%). UPLC-MS: $540^+$; $t_R$: 3.53 min. Without further purification, **51** was taken forward in the following deprotection step.

## X_Ray Data

### Experimental Summary for (15a)

The single crystal X-ray diffraction studies were carried out on a Bruker Kappa APEX-II CCD diffractometer equipped with Mo $K_\alpha$ radiation (λ = 0.71073 Å). A 0.30 x 0.25 x 0.20 mm colorless block was mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100(2) K using ϕ and ω scans. Crystal-to-detector distance was 40 mm and exposure time was 5 seconds per frame using a scan width of 0.5°. Data collection was 98.6% complete to 25.00° in $\vartheta$. A total of 6816 reflections were collected covering the indices, -7<=h<=4, -12<=k<=19, -30<=l<=25. 4615 reflections were found to be symmetry independent, with a $R_{int}$ of 0.0235. Indexing and unit cell refinement indicated a primitive, orthorhombic lattice. The space group was found to be $P2_12_12_1$. The data were integrated using the Bruker SAINT software program and scaled using the SADABS software program. Solution by direct methods (SHELXS) produced a complete phasing model consistent with the proposed structure. All nonhydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-97). All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-97.

## Biology

*Chemicals and reagents.* $^{125}$I-CCL2 (2200 Ci/mmol) was purchased from Perkin-Elmer (Waltham, MA). INCB3344 was synthesized as described previously.[25, 26] [$^3$H]INCB3344 (specific activity 32 Ci mmol$^{-1}$) was custom-labeled by Vitrax (Placentia, CA) for which a dehydrogenated precursor of INCB3344 was provided. Tango™ CCR2-*bla* U2OS cells stably expressing human CCR2 were obtained from Invitrogen (Carlsbad, CA).

*Cell culture and membrane preparation.* U2OS cells stably expressing the human CCR2 receptor (Invitrogen, Carlsbad, CA) were cultured in McCoys5a medium supplemented with 10% fetal calf serum, 2 mM glutamine, 0.1 mM non-essential amino acids (NEAA), 25 mM HEPES, 1 mM sodium pyruvate, 100

IU/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml G418, 50 µg/ml hygromycin and 125 µg/ml zeocin in a humidified atmosphere at 37°C and 5% $CO_2$. Cell culture and membrane preparation were performed as described previously.[16]

*$^{125}$I-CCL2 displacement assay.* Binding assays were performed as described previously.[16]

*[$^3$H]INCB3344 dual point competition association assay.* Kinetic rate index (KRI) values of unlabeled ligands were determined using the dual-point competition association assay as described previously.[10]

*[$^3$H]INCB3344 competition association assay.* The kinetic parameters of unlabeled ligands were determined using the competition association assay described earlier by our group.[10]

*Data Analysis.* All experiments were analyzed using the nonlinear regression curve fitting program Prism 5 (GraphPad, San Diego, CA). For radioligand displacement data $K_i$ values were calculated from $IC_{50}$ values using the Cheng and Prusoff equation.[27]

**Abbreviations**

Boc, tert-butyloxycarbonyl; CCL2, chemokine ligand 2; CCR2, chemokine receptor 2; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; DBU, 1,8-diazabicycloundec-7-ene; DEA, diethylamine; DEAD, Diethyl azodicarboxylate; DCM, dichloromethane; DiPEA, N,N-diisopropylethylamine; DMAP, N,N-dimethylaminopyridine; DMF, dimethylformamide; DPM, disintegrations per minute; ESI, electrospray ionisation; FTMS, fourier transform mass spectrometer; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; hERG, human Ether-à-go-go-Related Gene; HPLC, high-performance liquid chromatography; HRMS, high resolution mass spectral analyses; IPA, *iso*-propanol; KRI, kinetic rate index; LDA, lithium diisopropylamide; LC-MS, liquid chromatography – mass spectrometer; NEAA, non-essential amino acids; NMP, N-methylpyrrolidone; NMR, nuclear magnetic resonance; PEI, polyethylenimine; PEMB, 5-ethyl-2-methylpyridine borane; PyBrOP, bromo-tris-pyrrolidino phosphoniumhexafluorophosphate; RT, residence time; SAR, structure-affinity relationships; SFC, supercritical fluid chromatography; SKR, structure-kinetic relationships; TBDMS-Cl, tert-butyldimethylsilyl chloride; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; TMS, tetramethylsilane; Tris, tris(hydroxymethyl)aminomethane; U2OS, human bone osteosarcoma cells; UPLC, ultra performance liquid chromatography; UV, ultraviolet.

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