Bioorganic & Medicinal Chemistry Letters 21 (2011) 1442–1446

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of INCB10820/PF-4178903, a potent, selective, and orally bioavailable dual CCR2 and CCR5 antagonist

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ARTICLE INFO

Article history:
Received 20 September 2010
Revised 5 January 2011
Accepted 6 January 2011
Available online 11 January 2011

Key words:
CCR2
CCR5
Chemokine
Antagonist

ABSTRACT

We report the discovery of a potent, selective, and orally bioavailable dual CCR2 and CCR5 antagonist (3*S*,4*S*)-*N*-[(1R,3S)-3-isopropyl-3-({4-[4-(trifluoromethyl)pyridin-2-yl]piperazin-1-yl}carbonyl)cyclopentyl]-3-methoxytetrahydro-2*H*-pyran-4-amine (**19**). After evaluation in 28-day toxicology studies, compound **19** (INCB10820/PF-4178903) was selected as a clinical candidate.

Monocytes, a subset of leukocytes, and macrophages, which are derived from monocytes, are well-characterized mediators of tissue destruction in chronic inflammatory and autoimmune diseases.[1,2] Excessive recruitment of these cells to sites of inflammation is presumed to be harmful to these diseases. The trafficking of monocytes/macrophages to sites of inflammation is driven by monocyte chemoattractant protein-1 (MCP-1, CCL2) through interaction with its specific receptor, CCR2, which is a member of the super family of seven-transmembrane G-protein-coupled receptors (GPCRs) and is predominantly expressed on monocytes.[3] Binding of MCP-1 to CCR2 induces chemotaxis, resulting in directed migration of monocytes/macrophages to disease sites where MCP-1 expression is elevated.[4] Studies in rodent models by genetic deletion of either MCP-1[5] or CCR2[6–8] and use of peptidyl CCR2 antagonists[9] or anti-MCP-1 antibodies[10] have pointed to a critical role of MCP-1/CCR2 in rheumatoid arthritis,[9,10] multiple sclerosis,[11–13] atherosclerosis,[8,14–16] and neuropathic pain,[17] and strongly suggest that CCR2 is an attractive target for potential therapeutic intervention of these diseases. As a result, inhibition of CCR2 has emerged as a novel therapeutic approach for pharmaceutical research.

A variety of structural types of small molecule CCR2 antagonists have been disclosed in the past few years.[18,19] Among them, our series of CCR2 antagonists (structure I, Fig. 1)[20] share a common pharmacophore with the Merck series of CCR2 antagonists (structure II, Fig. 1),[21–24] with a basic amine on the left-hand side, a carbonyl in the middle of the molecule and a hydrophobic aromatic ring on the right-hand side. The central carbonyl and the right-hand side aryl are separated by a three-atom linker in I while the linker in II is two-atom long. We wondered whether the one-atom shorter linker in II is long enough to place the aryl group at a position for optimal interaction with the CCR2 receptor and decided to explore a slightly longer linker utilizing the 3-aminocyclopentanecarboxamide core. As piperidine, 1,2,5,6-tetrahydropyridine and piperazine possess a length of about three atoms counting from the 1-position to the 4-position, these heterocycles were chosen as potential linkers for our initial exploration (structure III, Fig. 1). Thus, a series of piperidine, 1,2,5,6-tetrahydropyridine and piperazine (1*S*,3*R*)-3-amino-1-isopropylcarboxamide were synthesized with tetrahydropyran-2*H*-4-yl as a substituent on the left-hand side amine.

The synthesis started with the commercially available (1*R*,4*S*)-4-[(*tert*-butoxycarbonyl)amino]cyclopent-2-ene-1-carboxylic acid **1**. The carboxylic acid was converted to methyl ester by treatment with iodomethane/potassium carbonate in DMF at room

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doi:10.1016/j.bmcl.2011.01.015

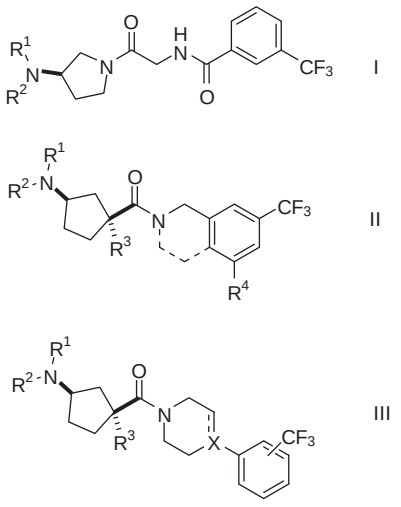


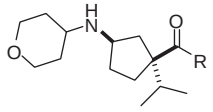
Figure 1.

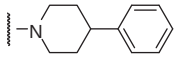
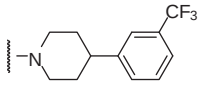
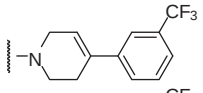
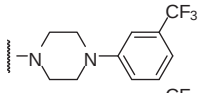
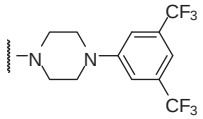
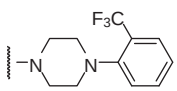
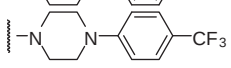
temperature. The resulting ester was subjected to an alkylation with isopropyl iodide using lithium hexamethyldisilazide (LHMDS) to provide the alkylated product **2** as a mixture of cis and trans diastereomers (4:1 ratio). The minor trans diastereomer was removed by crystallization following hydrolysis of the ester to an acid. The resulting enantiopure acid was subjected to a hydrogenation using Pd/C as the catalyst to afford the saturated carboxylic acid **3**. Coupling of acid **3** with 4-arylpiperidine, 4-aryl-1,2,5,6-tetrahydropyridine, or 4-arylpiperazine using BOP as the coupling agent provided the amide **4**. Following removal of the Boc group using TFA/CH_2Cl_2, the resulting amine was subjected to a reductive amination with tetrahydropyran-4-one to afford the final compounds **6**–**12**.

With a phenyl at the 4-position of the piperidine, compound **6** showed no activity, with 0% inhibition at 1000 nM in the MCP-1 binding assay[25] (Table 1). When the phenyl group was substituted with a CF_3 at the 3-position, compound **7** had an IC50 of 904 nM, demonstrating a tremendous contribution of the added CF_3 group to the binding affinity. Replacement of piperidine in **7**, in which the phenyl ring is orthogonal to the piperidine ring, with 1,2,5,6-tetrahydropyridine and piperazine provided compounds **8** and **9**, in which the two ring systems of phenyl and 1,2,5,6-tetrahydropyridine in **8** or phenyl and piperazine in **9** are coplanar or near coplanar. This replacement resulted in an improvement of almost 20-fold for **8** and 52-fold for **9** in CCR2 binding affinity, demonstrating a favored interaction of the 3-trifluoromethylphenyl with the receptor once the orientation of the two ring systems is coplanar or near coplanar. The 2.6-fold tighter binding affinity in **9** than in **8** suggested that piperazine is a better linker than 1,2,5,6-tetrahydropyridine despite the similarity in orientation of the two ring systems. Adding another CF_3 at the 5-position on phenyl (**10**) in **9** or moving the CF_3 in **9** from 3-position to 2-position (**11**) or 4-position (**12**) resulted in a loss in binding activity.

Having identified piperazine as a suitable linker, we carried the *N*-(3-trifluoromethylphenyl)piperazinyl moiety on the right-hand side in **9** (CCR2 binding IC_{50} = 18 nM) forward for SAR studies at the 3-position of the tetrahydropyran on the left-hand side, with methyl, ethyl, methoxy, ethoxy, or methoxymethyl as substituents (Table 2). These analogs (**13**–**17**) were synthesized following the procedures described in Scheme 1. The reductive amination in the last step of synthesis with a racemic 3-alkyltetrahydropyran-4-one afforded the final compounds as a mixture of 4 diastereomers which were separated by preparative chiral HPLC to give two major diastereomers (cis isomers) and two minor diastereomers (trans isomers). The 4 diastereomers of the methyl and ethyl

Table 1
Identification of a linker



Compd	R	CCR2 binding[a] IC_{50} (nM)
6		0% @ 1000 nM
7		904
8		46
9		18
10		213
11		19% @ 1000 nM
12		40% @ 1000 nM

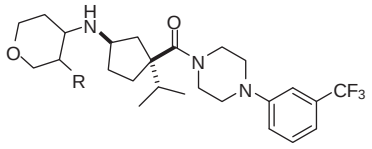
[a] Human CCR2 binding affinity from MCP-1 assay.

analogs exhibited a similar pattern in potency. The two major diastereomers (**13a–b** and **14a–b**) were more potent than the parent compound **9** in binding affinity while the two minor isomers (**13c–d** and **14c–d**) were less active than **9**. One of the major diastereomers (**13a** and **14a**) is about threefold more active than the other major diastereomer (**13b** and **14b**) while one of the minor diastereomers (**13d** and **14d**) is about 6–10-fold less active than the other minor isomer (**13c** and **14c**). The one-carbon longer ethyl group did not offer a better binding affinity than the methyl group although they were equally effective in the functional chemotaxis[25] (**13a** and **14a**). When the ketone is a racemic 3-alkoxytetrahydropyran-4-one or 3-methoxymethyltetrahydropyran-4-one, the reductive amination was more selective and only two major diastereomers (cis isomers) were isolated by preparative chiral HPLC. As seen with the methyl and ethyl analogs, the methoxy analogs (**15a–b**) were more potent than the parent compound **9** in binding affinity and one of the diastereomers (**15a**) is about threefold more active than the other diastereomer (**15b**). By comparison, **15a** is similar to **13a** and **14a** in binding affinity but about threefold less active than **13a** and **14a** in chemotaxis. With one-atom longer, the ethoxy (**16a–b**) and methoxymethyl (**17a–b**) were less active than the methoxy analogs (**15a–b**) and even the parent compound **9**, indicating that the 3-position of the tetrahydropyran moiety can only tolerate a group with a length of up to two atoms.

CCR1 and CCR5 are two closely related receptors of CCR2. A counterscreen against these two receptors revealed that this series of compounds were inactive against CCR1 (IC_{50} >10 μM) but some of them displayed potent CCR5 activity using a MIP-1β binding assay.[26] Without a substituent at the 3-position on the tetrahydropyran, compound **9** displayed an IC_{50} of 283 nM in antagonizing the binding of MIP-1β to CCR5, which was about 16-fold less active than the CCR2 binding affinity. Substitution at the 3-position on the tetrahydropyran with methyl, ethyl, methoxy, ethoxy and methoxymethyl all provided the first diastereomers with most potent CCR5 activity and with an improved CCR5 activity over **9**, the same trend seen for CCR2 activity. In contrast to the CCR2 activity, the two- to three-atom long substituents (**14a**, **15a**, and **16a**) appeared to provide better CCR5 activity than the one-atom long substituent (**13a**) and the three-atom long substituent (**16a**) was as effective as the two-atom long substituents (**14a** and **15a**). The only exception is the three-atom long methoxymethyl substituent (**17a**) which is even worse than the one-atom long methyl substituent (**13a**) for CCR5 activity. Since CCR5 is implicated in a variety of autoimmune diseases,[27] we decided to identify a dual CCR2 and CCR5 antagonist by taking advantage of the CCR5 activity from this series of compounds. Among the analogs generated from modifications on the left-hand side, compounds **14a** and **15a** emerged as two attractive dual antagonists as they displayed IC_{50} values of single-digit nanomolar in CCR2 activity with only fourfold less CCR5 activity. However, it would be ideal if a dual CCR2 and CCR5 antagonist exhibits similar activity for both receptors. To achieve this, we turned our SAR studies back to the right-hand side by replacing the phenyl with different heteroaryls in an attempt to improve the CCR5 activity.

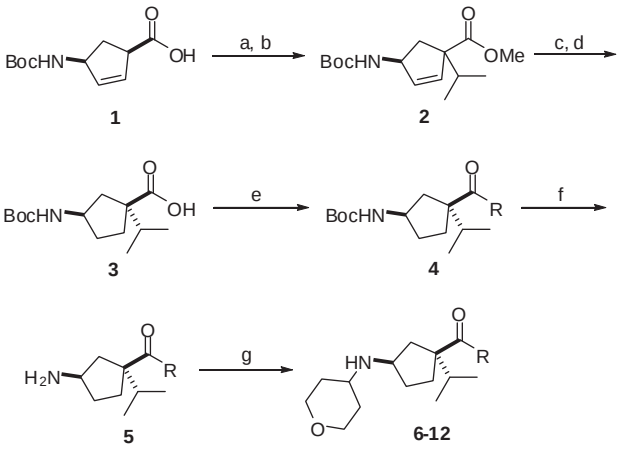
Since the ethyl analog **14a** did not offer any advantage in CCR2 and CCR5 activity over the methoxy analog **15a** and the more lipophilic ethyl group could be a potential issue in metabolic stability and hERG potassium channel activity, only the 3-methoxytetrahydropyan-4-yl group on the left-hand side was carried over to the next stage of SAR studies which involved incorporating different heteroaryls on the right-hand side. The biological data of the most active diastereomers for this set of heteroaryl analogs, which were synthesized following the procedures described in Scheme 1, is presented in Table 3. As drawn in the structure above Table 3, the absolute stereochemistry of the two substituents on the tetrahydropyran ring in these most active diastereomers were characterized as 3*S*,4*S* by X-ray crystallography and/or NMR. Replacement of the 3-trifluoromethylphenyl in **15a** with 6-trifluoromethylpyridin-2-yl provided compound **18** with CCR2 and CCR5 binding affinity similar to **15a** but an 11-fold loss in a human whole blood assay (Alexa assay). The Alexa assay was developed at Incyte to assess the binding affinity of a CCR2 antagonist while taking protein binding into account as it is conducted in an environment of whole blood.[28] Moving the nitrogen away from the position between the piperazine and the CF_3 in **18** to the position where the piperazine is at 2-position and the CF_3 is at 4-position provided analog **19** which was about twofold more potent in CCR2 activity than **18**, with an IC_{50} of 3.0 nM in binding affinity, 3.2 nM in chemotaxis and 3.9 nM in whole blood activity. More importantly, the CCR5 binding affinity was improved from an IC_{50} of 23 nM in **15a** to an IC_{50} of 5.3 nM in **19**, a fourfold enhancement.

Table 2
Biological data for 3-substituted tetrahydropyran analogs



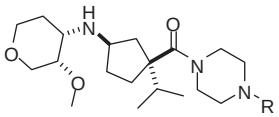
Compd	R	CCR2 IC_{50} (nM)		CCR5[c] binding IC_{50} (nM)
		Binding[a]	CTX[b]	
9	H	18		283
13a	Methyl (major)	3.1	2.5	77
13b	Methyl (major)	10.5	5.7	108
13c	Methyl (minor)	28		370
13d	Methyl (minor)	175		>1000
14a	Ethyl (major)	4.6	2.3	18
14b	Ethyl (major)	11.5		312
14c	Ethyl (minor)	46		546
14d	Ethyl (minor)	462		1000
15a	Methoxy	5.7	6.9	23
15b	Methoxy	15		204
16a	Ethoxy	45.5		22.5
16b	Ethoxy	66		206
17a	Methoxymethyl	36		201
17b	Methoxymethyl	46.5		572

[a] Human CCR2 binding affinity from MCP-1 assay.
[b] Human CCR2 functional chemotaxis.
[c] CCR5 binding affinity from MIP-1β assay.



Scheme 1. Reagents and conditions: (a) MeI, K_2CO_3, DMF, rt, 99%; (b) LHMDS, isopropyl iodide, THF, −24 °C, overnight, 78%; (c) LiOH, H_2O, THF, MeOH, reflux, overnight, crystallization, 56%; (d) H_2, Pd/C, EtOH, 96%; (e) 4-arylpiperidine, 4-aryl-1,2,5,6-tetrahydropyridine or 4-arylpiperazine, BOP, TEA, CH_2Cl_2 or DMF; (f) TFA, CH_2Cl_2; (g) tetrahydropyran-4-one, $Na(OAc)_3BH$, TEA, CH_2Cl_2.

Table 3
Biological data for pyridine and pyrimidine analogs



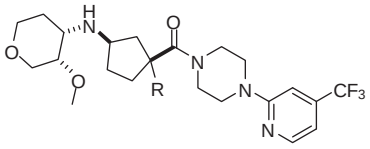
Compd	R	CCR2 IC_{50} (nM)		Alexa[c] IC_{50} (nM)	CCR5 IC_{50} (nM)	
		Binding[a]	CTX[b]		Binding[d]	CTX[e]
15a		5.7	6.9	7	23	
18		6.2	6.2	79	26.5	
19		3.0	3.2	3.9	5.3	4.3
20		11	8.9	17	34.8	

[a] Human CCR2 binding affinity from MCP-1 assay.
[b] Human CCR2 functional chemotaxis.
[c] Human whole blood binding assay.
[d] Human CCR5 binding affinity from MIP-1β assay.
[e] Human CCR5 functional chemotaxis

In a CCR5 chemotaxis functional assay,[26] **19** displayed an IC_{50} of 4.3 nM, which is almost as potent as its CCR2 chemotaxis activity. Replacement of 3-trifluoromethylphenyl in **15a** with 4-trifluoromethylpyrimidin-2-yl provided analog **20** which is slightly less active than **15a** in both CCR2 and CCR5 activity.

We had carried out SAR studies on the left-hand side and right-hand side while retaining the isopropyl at the central region of the molecule, leading to the most attractive dual CCR2 and CCR5 antagonist **19**. To get an insight into whether the isopropyl is the most preferred group at the central region of the molecule, the isopropyl was replaced with different substituents while maintaining the 3-methoxytetrahydropyran-4-yl on the left-hand side and 4-trifluoromethylpyridin-2-yl on the right-hand side (Table 4). The methyl analog **21** exhibited very weak CCR2 binding affinity (IC_{50} = 221 nM) while the ethyl and *n*-propyl analogs **22** and **23** were more active than the methyl analog but still about threefold less active than the isopropyl compound **19** in CCR2 binding, indicating that longer (two- to three-atom length) and sterically bulky (branched) group at this position is preferred. However, branching at the β-position of the substituent is inferior as the isobutyl and cyclopropylmethyl analogs **24** and **25** were not as potent as the *n*-propyl analog **23** in CCR2 binding affinity. Methoxymethyl has a similar chain length as *n*-propyl but is more polar than *n*-propyl. The slightly less CCR2 binding activity for the methoxymethyl analog **26** compared to the *n*-propyl analog **23** indicated that polar group at this position is less tolerated. This can also be seen in the ethoxymethyl analog **27**, the methoxyethyl analog **28**, the tetrahydrofuran-3-yl analog **29**, the pyridine-3-ylmethyl analog **30**, the 2-hydroxy-2-propyl analog **31**, the 3-fluoro-1-propyl analog **32** and the 2,2,2-trifluoro-1-hydroxy-1-ethyl analog **33**. The less tolerability of a polar group at this position was further confirmed by removing the hydroxyl group in **33**. Removal of the hydroxyl group in **33** provided the trifluoroethyl analog **34** which is fourfold in CCR2 binding affinity and fivefold in CCR2 whole blood activity more active than the hydroxyl analog **33** and is as potent as **19** in CCR2 and CCR5 binding affinity and CCR2 whole blood activity, although the trifluoroethyl analog **34** is still about 2–3-fold less active than **19** in CCR2 and CCR5 chemotaxis activity. In addition to polarity, chain length may partially attribute to the loss in CCR2 binding affinity for compounds **27**–**30** as the four-atom alkoxyalkyl in **27** and **28** is more detrimental to binding than the three-atom methoxymethyl in **26**. The complete loss in CCR2 binding activity for compound **31** could stem from the 2-hydroxy-2-propyl group being too polar and too bulky.

Table 4
Modification on **19** at 1-position of cyclopentanecarboxamide



Compd	R	CCR2 IC_{50} (nM)		Alexa[c] IC_{50} (nM)	CCR5 IC_{50} (nM)	
		Binding[a]	CTX[b]		Binding[d]	CTX[e]
19	Isopropyl	3.0	3.2	3.9	5.3	4.3
21	Methyl	221				
22	Ethyl	9.3		31.7		
23	*n*-Propyl	10.6		41		
24	Isobutyl	22.7		53.0		
25	Cyclopropylmethyl	27.6		67.9		
26	Methoxymethyl	19.5				
27	Ethoxymethyl	34				
28	Methoxyethyl	116				
29	Tetrahydrofuran-3-yl	232				
30	Pyridin-3-ylmethyl	294				
31	2-Hydroxy-2-propyl	>1000				
32	3-Fluoro-1-propyl	18.5		39.5		
33	2,2,2-Trifluoro-1-hydroxy-1-ethyl	13.5		17		
34	2,2,2-Trifluoro-1-ethyl	3.4	9.0	3.2	8.4	11.1

[a] Human CCR2 binding affinity from MCP-1 assay.
[b] Human CCR2 functional chemotaxis.
[c] Human whole blood binding assay.
[d] Human CCR5 binding affinity from MIP-1β assay.
[e] Human CCR5 functional chemotaxis.

Table 5
Pharmacokinetic property of compound **19**

Species	Dose (mg)	Cl (L/h/kg)	V_{dss} (L/kg)	$t_{1/2}$ (iv) (h)	AUC (po)(μM·h)	*F* (%)
Rat	5/10	3.8	17	5	3.0	84
Cyno	2.5/10	2.2	6.2	3	5.3	57

Owing to its superior in vitro profile, compound **19** was further evaluated. It was active against mouse CCR2, rat CCR2, and cynomolgus monkey CCR2 with a binding IC_{50} of 2.8, 2.9, and 4.6 nM, respectively, but inactive against canine CCR2, with an IC_{50} of >10 μM. It was also active against mouse CCR5 and cynomolgus monkey CCR5, with an IC_{50} of 71 and 4.0 nM, respectively. Counterscreen against a panel of >50 receptors, enzymes and ion channels proved that **19** is a selective dual CCR2 and CCR5 antagonist. Compound **19** did not inhibit the tested major isoforms of human CYP enzymes, with an IC_{50} of >25 μM against CYP3A4 and CYP2D6. In hERG potassium channel activity, **19** displayed an IC_{50} of 1.7 μM in a hERG patch clamp assay.

ADME profiling revealed that compound **19** exhibited a high permeability across Caco-2 monolayers with a value of 5.3 cm/s, and a high free fraction (fu = 56%) in human protein binding. When treated with human liver microsomes in vitro, compound **19** exhibited good metabolic stability, with a half-life ($t_{1/2}$) of 93 min. In vivo, moderate to high systemic clearance was observed in rats and cynomolgus monkeys, with Cl = 3.8 L/h/kg in rats and 2.2 L/h/kg in cynomolgus monkeys. However, the high volume of distribution in both species (Table 5) resulted in a moderate to long half-life for this compound, with $t_{1/2}$ = 5 h (iv) in rats and 3 h (iv) in cynomolgus monkeys. Compound **19** was orally well-absorbed, with an oral bioavailability (*F* %) of 84% in rats and 57% in cynomolgus monkeys.

In conclusion, we identified piperazine as a suitable linker between the carbonyl in the center and the aryl on the right-hand side using 3-aminocyclopentanecarboxamide as our core structure. Modifications on both left-hand and right-hand sides led to the discovery of a potent, selective and orally bioavailable dual CCR2 and CCR5 antagonist **19** ((3*S*,4*S*)-*N*-[(1*R*,3*S*)-3-isopropyl-3-({4-[4-(trifluoromethyl)pyridin-2-yl]piperazin-1-yl}carbonyl)cyclopentyl]-3-methoxytetrahydro-2*H*-pyran-4-amine). The potency and PK property of this compound predicted a low human dose of 20–50 mg twice-a-day dosing (BID).[29] Compound **19** (INCB10820/PF-4178903), after evaluation in 28-day toxicology studies, was selected as a clinical candidate. Further evaluation of this compound will be reported in due course.

Acknowledgments

We thank Lynn Leffet, Karen Gallagher, Patricia Feldman, Bitao Zhao, Yanlong Li, Robert Collins, and Gengjie Yang for technical assistance.

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28. A volume of 200 μl of whole blood collected in heparin coated vacutainer tubes (BD Biosciences) from normal volunteers is preincubated with nothing or with indicated amounts of a competitor (compound) for 5 min at rt. MCP-Alexa 488 at 30 nM final concentration together with an anti-CD14-allophycocyanin (APC)-conjugated antibody (Caltag, CA) at 1/200 is then added to the samples and further incubated for 30 min at rt, being protected from light. Washing of the samples is done using 3 mL of cold FACS buffer (FB, 3% FBS in PBS) followed by centrifugation (5 min/500 g/rt). The red blood cells are then lysed for 10 min at rt in 2 mL of FACS™ lysing solution (BD Biosciences) followed by a 5 min/500 g/rt centrifugation. The samples are then resuspended in 250 μl of FB containing 1% paraformaldehyde and analyzed by flow cytometry (Facs Calibur, BD Biosciences).
29. The human dose projection was based upon the human trough concentration required to cover the human whole blood (HWB) IC_{80}. The human trough concentration was obtained from the projected human PK by an allometric scaling of the rat PK.