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Development, synthesis and structure-activity-relationships of inhibitors of the macrophage infectivity potentiator (Mip) proteins of *Legionella pneumophila* and *Burkholderia pseudomallei*

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Abstract

The bacteria *Burkholderia pseudomallei* and *Legionella pneumophila* cause severe diseases like melioidosis and Legionnaire's disease with high mortality rates despite antibiotic treatment. Due to increasing antibiotic resistances against these and other Gram-negative bacteria, alternative therapeutical strategies are in urgent demand. As a virulence factor, the macrophage infectivity potentiator (Mip) protein constitutes an attractive target. The Mip proteins of *B. pseudomallei* and *L. pneumophila* exhibit peptidyl-prolyl *cis*/*trans* isomerase (PPIase) activity and belong to the PPIase superfamily. In previous studies, the pipecolic acid moiety proved to be a valuable scaffold for inhibiting this PPIase activity. Thus, a library of pipecolic acid derivatives was established guided by structural information and computational analyses of the binding site and possible binding modes. Stability and toxicity considerations were taken into account in iterative extensions of the library. Synthesis and evaluation of the compounds in PPIase assays resulted in highly active inhibitors. The activities can be interpreted in terms of a common binding mode obtained by docking calculations.

Keywords:

Burkholderia pseudomallei
Legionella pneumophila
macrophage infectivity potentiator protein
synthesis
docking analysis
structure-activity-relationships

Abbrevations: Mip, macrophage infectivity potentiator; PPIase, peptidyl-prolyl *cis*/*trans* isomerase; LpMip, *L. pneumophila* Mip; BpMip, *B. pseudomallei* Mip; SAR, structure-activity relationship; HSQC, Heteronuclear Single Quantum Coherence; NMM, *N*-methylmorpholine; DIPEA, *N*, *N*-diisopropylethylamine; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; DMAP dimethylaminopyridine; HOBt, 1-hydroxybenzotriazol; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

1. Introduction

The diseases melioidosis and Legionnaire's disease are triggered by the pathogens *Burkholderia pseudomallei* and *Legionella pneumophila*, respectively. *B. pseudomallei* is endemic throughout the tropics, and is predicted to cause 89,000 deaths globally per annum.[1] Infection can occur by inoculation of skin lesions or less frequently by inhalation.[2] In addition, *B. pseudomallei* is listed as a Tier 1 biological agent by the U.S. Centers for Disease Control and Prevention.[3]

L. pneumophila infections also generally occur following inhalation of contaminated aerosols.[4] The main sources of infection are sanitary facilities such as showers and whirlpools, but also air conditioning systems and cooling towers.[5] While Legionnaire's disease manifests in an acute pneumonia, melioidosis symptoms are hard to distinguish from those of tuberculosis. Symptoms range from localized cutaneous abscesses to multi-organ involvement and septic shocks.[6] Despite protracted treatment with antibiotics, a high mortality rate is reported.[7-9]

Due to an increasing resistance to antibiotics, the treatment of infections by Gram-negative bacteria is becoming a difficult issue.[10-12] Therefore, it is important to search for new antibacterial strategies. One particular opportunity is to target virulence factors: these are not the targets for current antibiotics, commensal organisms should be spared, and the selective pressure for resistance should be lower.[13, 14] An important virulence factor for both *B. pseudomallei* and *L. pneumophila* is the macrophage infectivity potentiator (Mip) protein. The Mip protein from both species shows peptidyl-prolyl *cis*/*trans* isomerase (PPIase) activity.[15, 16] Inhibition of the PPIase may prevent the infection. The already intensively studied *L. pneumophila* Mip (LpMip) has been shown to play an important role in invasion and dissemination of the bacteria.[17, 18] LpMip is responsible for the penetration of the lung tissue. A guinea pig infection model with wild-type and an LpMip mutant revealed a contribution of Mip to bacterial prevalence within the lung. Furthermore, it was shown that LpMip interacts with collagen IV, the predominant collagen in the human lung.[19]

Norville *et al*. discovered two Mip paralogues (BpMip and BPSL0918) in the genome of *B. pseudomallei*. Since BPSL0918 did not show any PPIase activity, but knock-out had serious effects

on intracellular survival, it is speculated that BPSL0918 acts as a chaperone that is important for intracellular survival of the pathogen.[20] However, BpMip exhibits PPIase activity and is important in the infection process. Norville *et al*.[16] demonstrated that without BpMip there is reduced intracellular survival of *B. pseudomallei* in macrophage cells. In addition, the BpMip mutant strain was significantly attenuated in a BALB/c mouse model. Structural comparison between BpMip and LpMip showed a high homology in the PPIase domain.[21, 22] Mip proteins have been associated with virulence in a range of microbial pathogens, including *Trypanosoma cruzi,*[23] *Chlamydia trachomatis,*[24] and *Neisseria gonorrhoeae.*[25]

L. pneumophila and *B. pseudomallei* Mips belong to the FK506 binding proteins (FKBPs) family. FKBPs form stable complexes with the immunosuppressive natural products FK506 and rapamycin. The human FKBP12 complex with these drugs then inhibits the calcineurin and mTOR complex, respectively.[26-29] Both drugs show low nanomolar inhibition of Mips from a range of species. Due to the immunosuppressive effects, these compounds are not suitable for treatment of Legionnaire's disease and melioidosis. However, they demonstrate that Mips are druggable, and so these proteins are attractive targets for novel inhibitors.

To this end, we previously designed pipecolic acid derivatives to inhibit LpMip using structure-based design, synthesis, and biological screening. NMR-HSQC experiments confirmed that the developed compounds bind to the same hydrophobic binding pocket as rapamycin.[30] Some of these pipecolic acid esters were similarly efficient towards the BpMip. In addition, it was demonstrated that the compounds were not immunosuppressive and that they were able to reduce the cytotoxic effects of *B. pseudomallei* in macrophage cells.[31]

The previously described compounds proved that novel, non-immunosuppressive compounds targeting Mips can be designed. However, the identified compounds did not have suitable properties for therapeutic use. In this work, a library of pipecolic acid derivatives was established. Using hot-spot analyses and exploratory docking experiments against both LpMip and BpMip, possible substituents were suggested. Stability and toxicity considerations were taken into account in iterative

extensions of the library. Synthesis and evaluation of the compounds in LpMip and BpMip inhibition assays of the best candidates resulted in highly active inhibitors.

2. Results and Discussions

2.1. Library development

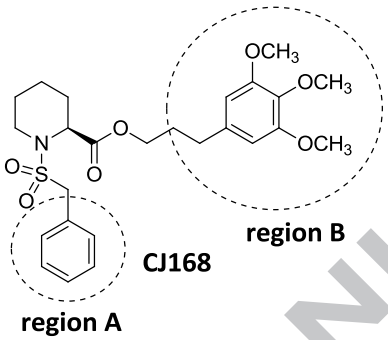


Figure 1: Lead structure from the study of Juli *et al.*[30] highlighting the pipecolic acid scaffold and the two regions addressed for modification and optimization.

Inhibitor **CJ168** (see Figure 1) of LpMip[30] and BpMip[31] was used as starting point to develop a library of pipecolic acid derivatives for exploring structure-activity relationships (SARs) and, possibly, obtaining compounds with improved activity, higher solubility, and lower toxicity. While the central pipecolic acid scaffold should be maintained as essential recognition unit for the MIP binding pocket, two regions were available for modification (see Figure 1): the phenyl ring of the benzylsulfonamide group (region A) and the ester part with the alkyl chain and the terminal trimethoxyphenyl group (region B).

Hot-spot analyses were carried out using GRID[32] (version 22c) to determine preferential interaction sites for various probe atoms in the binding pockets of LpMip and BpMip and identify yet unaddressed areas. As the binding mode of **CJ168** is available from a crystal structure with BpMip,[31] which compares very well with the postulated binding mode obtained by docking for LpMip,[30] modifications in regions A and B can be suggested on the basis of the determined hot spots. The binding pockets of BpMip and LpMip are highly homologous (see Figure 2). Marked differences occur

only in the subpocket formed by the Ala94-Gly95-Gly96-Val97-Ile98 loop in BpMip and the Val114-Gly115-Gly116-Pro117-Ile118 loop in LpMip, respectively (see Figure 2).

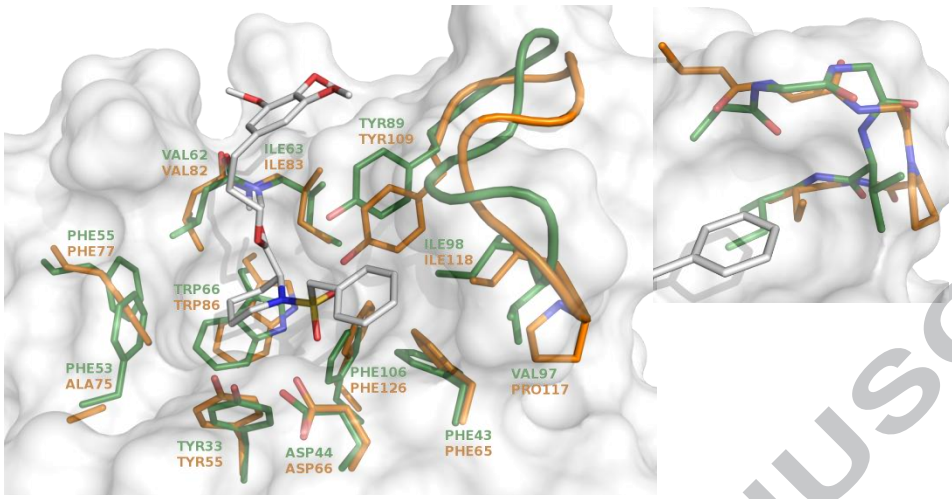


Figure 2: Comparison of the binding pockets of BpMip (green, chain C of PDB 4GGQ[31]) and LpMip (orange, model 4 of PDB 2VCD[22]). The surface and the ligand (**CJ168**, grey sticks) are shown for BpMip. The insert at the right highlights the differences in the loop sequence and conformation.

In BpMip, clear hot spots for hydrophobic substituents in *para*-position of the phenyl ring of **CJ168** were observed (see Figure 3). Furthermore, a suitably placed hydrogen-bond donor function could be able to address the carbonyl groups of Phe43 and Ala94. In LpMip, the corresponding polar hot spots were even more pronounced, suggesting donor functions to address the carbonyl groups of Phe65 and Val114 and an acceptor function for an interaction with the Gly116 NH. In contrast, the hydrophobic hot spots in this region were less well pronounced in LpMip, suggesting at best a small hydrophobic substituent in *meta*-position of the phenyl ring.

Modifications of the trimethoxyphenyl ring of region B were planned with the goal of improving solubility and, possibly, enhancing surface complementarity. In fact, from the BpMip crystal structures and the LpMip docking modes, the trimethoxyphenyl group appears to bind rather non-specifically. Although hot spots for chloro probes could be found around this ring, the preference for (phenolic) OH-probes was much more pronounced. Given the surface exposure of this interaction area, it is, however, unclear how much an additional hydrogen bond could really contribute to the

affinity. It would already be sufficient if a gain in solubility could be obtained by modifications in this region without losing much potency.

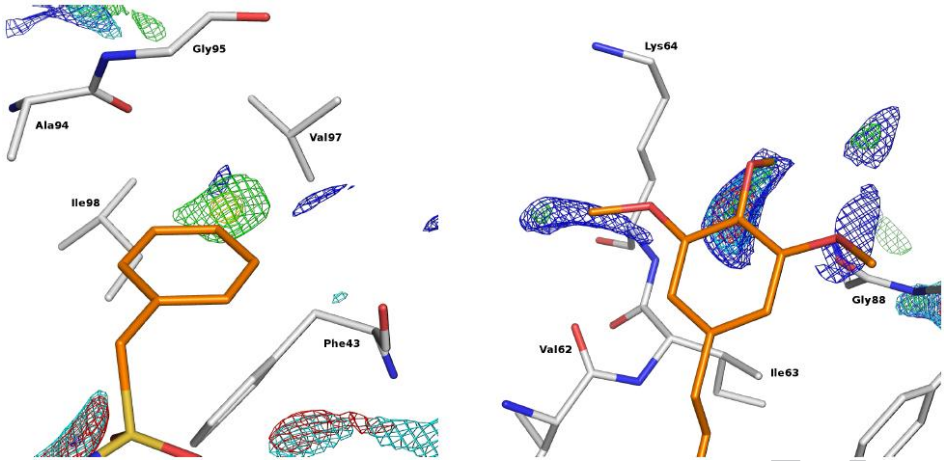


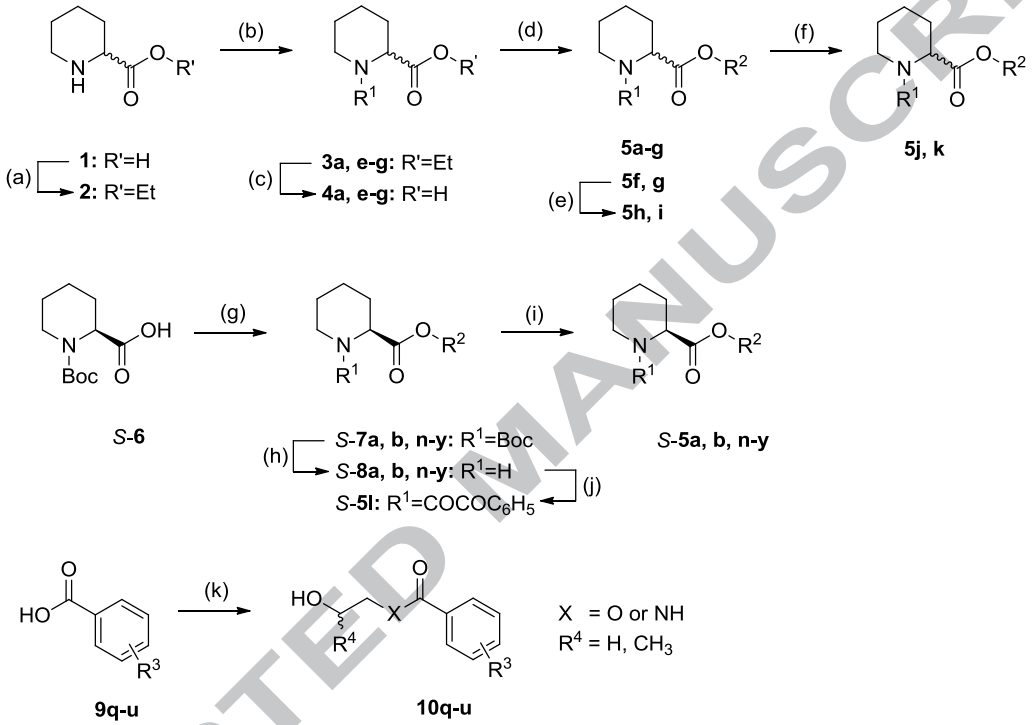
Figure 3: Characteristic hot spots of BpMip in the binding areas of region A (left) and region B (right) of lead compound **CJ168** (shown in orange in PDB structure 4G50[3]). The following probes were used (with color and contouring level in kcal/mol specified in parenthesis): methyl (yellow, -4.0), fluoro (cyan, -3.0), chloro (green, -5.0), phenolic OH (blue, -7.0), carbonyl-O (red, 5.0).

Taking these considerations and synthetic accessibility issues into account, a large variety of derivatives was considered for preparation, which ultimately led to the compounds summarized in Table 1 and discussed in more detail below. All suggested molecules were subjected to exploratory docking calculations (results not shown) to avoid the preparation of unsuitable, non-fitting compounds. A more detailed docking analysis was carried out for the ultimately prepared compounds, as further described below.

2.2. Chemistry

Synthesis of racemic compounds. The synthesis of the pipecolic acid derivatives was carried out following the methods of Juli *et al.*[30] Briefly, synthesis was started with conversion of piperidine-2-carboxylic acid with 3 equiv. of thionyl chloride in ethanol to prepare ethyl piperidine-2-carboxylate (see Figure 4). Subsequently, the sulfonamides were obtained by reaction of the ethyl piperidine-2-carboxylate and 3 equiv. *N*-methylmorpholine (NMM) and *N*, *N*-diisopropylethylamine (DIPEA), respectively, and equimolar amounts of the corresponding carbonyl or sulfonyl chloride. The ester

was hydrolyzed using lithium hydroxide to give the free pipecolic acids **4a, e**-**g**. The esters **5a**-**g** were synthesized using 1 equiv. of the free acids, 1 equiv. of the corresponding alcohol, 1.5 equiv. of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC·HCl) and catalytic amounts of dimethylaminopyridine (DMAP).



Reagents and conditions: (a) $SOCl_2$, EtOH, reflux, 5 h; (b) corresponding carbonyl or sulfonyl chloride, NMM or DIPEA, CH_2Cl_2, 0 °C, 6 - 24 h; (c) LiOH, MeOH, 0 C, 1 h (d) corresponding alcohol, EDC·HCl, DMAP, CH_2Cl_2, 0 °C, 24 h, (e) H_2, Pd/C, RT, 24 h; (f) Me_2CO, Na_2SO_4, H_2, cat. Pd/C, RT, 24 h. (g) corresponding alcohol, EDC·HCl, DMAP, CH_2Cl_2, 0 °C, 24 h; (h) TFA, CH_2Cl_2; (i) corresponding carbonyl or sulfonyl chloride, NMM or DIPEA, CH_2Cl_2, 0 °C, 6 - 24 h; (j) phenylglyoxalic acid, EDC·HCl, HOBt, CH_2Cl_2, 0 °C, 24 h, (k) corresponding alcohol or amine, EDC·HCl, DMAP or HOBt, CH_2Cl_2, 0 °C, 24 h.

Figure 4: Synthesis scheme of inhibitors.

Synthesis of compounds 5j and 5k. To reduce the nitro-group to an amino-group, **5f** or **5g** was dissolved in EtOAc and hydrogenated at RT and 10 bar hydrogen with catalytic amounts of Pd/C to obtain **5h**, **5i**. Using a modified procedure from Fache *et al*.[33] 1 equiv. of **5h** or **5i**, 1.1 equiv. of Me_2CO, 4 equiv. of Na_2SO_4 and catalytic amounts of Pd/C were hydrogenated at RT and 10 bar hydrogen to obtain compounds **5j**, **5k**.

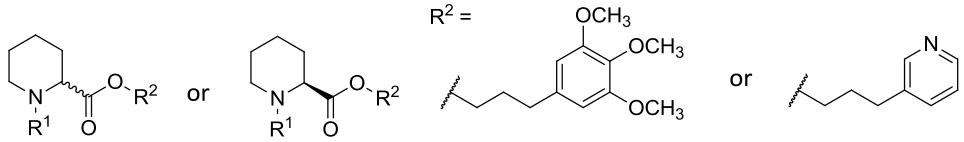
Synthesis of pure *S*-enantiomers. To synthesize the *S*-enantiomeric piperidine-2-carboxylate derivatives, an esterification of (*S*)-1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid (1 equiv.) was

performed, using the corresponding alcohol (1.0 equiv.), EDC·HCl (1.5 equiv.) and DMAP (0.2 equiv.). Afterwards, the protection group was cleaved with trifluoroacetic acid in CH_2Cl_2. Subsequently, the sulfonamides were obtained by reaction of the ethyl piperidine-2-carboxylate and 3 equiv. NMM or DIPEA and equimolar amounts of the corresponding sulfonyl chloride.

For the synthesis of *S*-**5l** an amidation of *S*-**8a** (1 equiv.) with phenylglyoxalic acid (1.0 equiv.) was performed, using EDC·HCl (1.3 equiv.) and 1-hydroxybenzotriazol (HOBt) (0.5 equiv.). *S*-**5v** and *S*-**5w** were obtained in a one pot synthesis: the corresponding alcohol was generated *in situ* and the ester established according to the procedures above. For compound *S*-**5y**, a benzylic protection group was used to prevent side reactions with the phenolic moiety. To synthesize compound *S*-**5z** in the first step of the synthesis an amidation was carried out according to the given procedure of *S*-**5l**.

Synthesis of corresponding alcohols. To obtain the alcohol derivatives (**10q**-**u**), an esterification or amidation with the corresponding benzoic acid (1 equiv.) was carried out, using the corresponding alcohol or amine (1.0 equiv.), EDC·HCl (1.5 equiv.) and DMAP or HOBt (0.2 equiv.). The alcohols (**10q**-**u**) were used in the *S*-enantiomeric synthesis to yield compounds **5q**-**u**. For the derivatives **5v**-**y** a one pot synthesis combining alcohol synthesis and the first step of the *S*-enantiomeric synthesis was established.

Table 1: Substitution pattern of piperidine-2-carboxylic acid derivatives.



3-(3,4,5-trimethoxyphenyl)propyl = Trim

3-(pyridin-3-yl)propyl = Pyr

racemates	R^1	R^2
5a	$-SO_2-CH_2-C_6H_5$	Trim
5b	$-SO_2-CH_2-C_6H_5$	Pyr
5c	$-SO_2-CH_2-C_6H_5$	$-C_2H_4-OH$
5d	$-SO_2-CH_2-C_6H_5$	$-CH(CH_3)_2$
5e	$-CO-CH_2-O-C_6H_4$-*p*-Cl	Pyr
5f	$-SO_2-CH_2-C_6H_4$-*p*-NO_2	Pyr
5g	$-SO_2-CH_2-C_6H_4$-*m*-NO_2	Pyr
5h	$-SO_2-CH_2-C_6H_4$-*p*-NH_2	Pyr
5i	$-SO_2-CH_2-C_6H_4$-*m*-NH_2	Pyr
5j	$-SO_2-CH_2-C_6H_4$-*m*-NH-$CH(CH_3)_2$	Pyr
5k	$-SO_2-CH_2-C_6H_4$-*p*-NH-$CH(CH_3)_2$	Pyr
R- or *S*-enantiomers		
S-**5a**	$-SO_2-CH_2-C_6H_5$	Trim
R-**5a**	$-SO_2-CH_2-C_6H_5$	Trim
S-**5b**	$-SO_2-CH_2-C_6H_5$	Pyr
S-**5l**	$-CO-CO-C_6H_4$	Pyr
S-**5m***	$-SO_2-CH_2-C_6H_4$-*p*-Cl	Pyr
S-**5n**	$-SO_2-CH_2-C_6H_4$-*m*-Cl	Pyr
S-**5o**	$-SO_2-CH_2-C_6H_4$-*p*-F	Pyr
S-**5p**	$-SO_2-CH_2-C_6H_4$-*m*-NO_2	Pyr
S-**5q**	$-SO_2-CH_2-C_6H_5$	$-C_2H_4-OCO-3-C_5H_4N$
S-**5r**	$-SO_2-CH_2-C_6H_5$	$-C_2H_4-NHCO-3-C_5H_4N$
S-**5s**	$-SO_2-CH_2-C_6H_5$	$-C_2H_4-O-3-C_5H_4N$
S-**5t**	$-SO_2-CH_2-C_6H_5$	$-C_2H_4-OCO-C_6H_3$-3, 5-$(O(CH_2)_2OCH_3)_2$
S-**5u**	$-SO_2-CH_2-C_6H_5$	$-C_2H_4-NHCO-C_6H_3$-3, 5-$(O(CH_2)_2OCH_3)_2$
S-**5v**	$-SO_2-CH_2-C_6H_5$	$-CH(S-CH_3)CH_2-NHCO-3-C_5H_4N$
S-**5w**	$-SO_2-CH_2-C_6H_5$	$-CH(R-CH_3)CH_2-NHCO-3-C_5H_4N$
S-**5x**	$-SO_2-CH_2-C_6H_5$	$-CH(S-CH_3)CH_2-NHCO-3-C_6H_2$-3,4,5-
S-**5y**	$-SO_2-CH_2-C_6H_5$	$-C_2H_4-NHCO-C_6H_3$-3-OCH_3, 4-OH
S-**5z**	$-SO_2-CH_2-C_6H_5$	NH-$CH(CH_3)_2$[#]

*: Synthesized according to the procedure for racemic compounds. [#]: Amide instead of ester.

Structure-activity-relationships. Table 2 shows the Mip inhibitory and cytotoxicity data. In previous studies compound **CJ168** (resynthesized here as **5a**/*S*-**5a**) showed an IC_{50} value (LpMip) of 9 µM[30] for the racemic compound. Evaluation of the enantiomers of **5a** against LpMip indicate an enantioselective effect (IC_{50} of *S*-**5a** = 6 µM[30] versus IC_{50} of *R*-**5a** = 12.8 µM) of the *S*-enantiomer which Holt *et al*.[34] had observed for piperidine-2-carboxylates as FKBP inhibitors. Previous docking studies had already revealed that the R-enantiomer can be placed in the LpMIP pocket only in a somewhat distorted orientation, leading to a less favorable hydrophobic score.[30] With its higher potency the *S*-enantiomer served as a starting structure to develop further inhibitors.

By replacing the trimethoxyphenyl group of **5a** with a pyridinyl group (**5b**) the activity was maintained but the toxicity in macrophages (J774.1) was reduced significantly (IC_{50} value => 100 µM). Furthermore, this substitution reduced the molecular weight and the logP, and dramatically increased the solubility of the compound. Accordingly, the pyridinyl group was preferred over the trimethoxyphenyl group for all subsequent derivatives exploring region A.

Table 2: PPIase activity, cytotoxicity and selectivity index of piperidine-2-carboxylic acid derivatives.

		BpMip			LpMip	
comp.	cytotoxicity, J774.1 IC_{50}, [µM]	IC_{50} [µM]	K_i [µM]	Cytotoxicity-affinity (CA)	IC_{50} [µM]	Cytotoxicity-affinity (CA)
5a[30]	44.5	0.8 ± 0.1	1.25 ± 0.3	35.6	9.0 ± 0.7	4.9
S-**5a**[30]	48.4	0.12 ± 0.09	0.16 ± 0.02	303	6.0 ± 0.7	8.1
R-**5a**	66.9	1.9 ± 0.3	1.2 ± 0.3	55.8	12.8 ± 6.2	5.2
5b	> 100	0.69 ± 0.3	0.57 ± 0.16	> 175	10.7 ± 1.8	> 9.4
S-**5b**	> 100	0.26 ±0.08	0.15 ± 0.03	> 667	5.8 ± 0.5	> 17.2
5c	> 100	1.6 ± 0.3	2.8 ± 0.4	> 35.7	94 ± 26	> 1.1
5d	> 100	4.7 ± 1.2	1.8 ± 0.6	> 55.6	79.2 ± 3.6	> 1.3
5e	50.9	6.3 ± 4	17 ± 4	3.0	89.3 ± 5.7	0.6
5f	56.7	0.3 ± 0.1	0.17 ± 0.03	334	3.9 ± 0.3	14.5
5g	46.7	n. d.	n. d.	-	8.7 ± 0.7	5.4
5h	> 100	3.5 ± 1.7	4.2 ± 0.7	> 23.8	21.4 ± 3.9	> 4.7
5i	62.3	n. d.	n. d.	-	> 100	< 0.6
5j	57.4	3.8 ± 1.5	4.3 ± 0.5	13.3	41.5 ± 9.7	1.4
5k	44.3	-	4.5 ± 1.7	9.8	44.5 ± 16	1.0
S-**5l**	88.1	3.2 ± 1.2	5.5 ± 1.1	16.0	142 ± 17.5	0.6
S-**5m**	33.6	0.21 ± 0.08	0.13 ± 0.02	259	2.4 ± 0.1	14.0
S-**5n**	43.3	0.23 ± 0.08	0.18 ± 0.01	241	7.5 ± 1.0	5.8
S-**5o**	> 100	0.072 ± 0.03	0.097 ± 0.02	> 1031	5.7 ± 0.8	> 17.5
S-**5p**	48.2	0.48 ± 0.17	0.46 ± 0.06	105	7.9 ± 1.2	6.1
S-**5q**	> 100	0.22 ± 0.08	0.091 ± 0.03	> 1099	2.6 ± 0.6	> 38.5
S-**5r**	> 100	0.42 ± 0.18	0.29 ± 0.06	> 345	11.4 ± 1.7	> 8.8
S-**5s**	> 100	2.2 ± 0.4	0.25 ± 0.06	> 400	7.7 ± 2.5	> 13
S-**5t**	> 80*	0.42 ± 0.05	0.47 ± 0.07	> 170	8.6 ± 1.3	> 9.3
S-**5u**	> 100	0.58 ± 0.08	0.98 ± 0.29	> 102	5.6 ± 0.7	> 18.0
S-**5v**	> 100	0.32 ± 0.12	0.26 ± 0.04	> 385	3.5 ± 0.5	> 28.6
S-**5w**	> 100	0.99 ± 0.4	0.81 ± 0.13	> 124	11.5 ± 2.2	> 8.7
S-**5x**	> 100	0.58 ± 0.16	0.74 ± 0.25	> 102-	2.2 ± 0.1	> 46
S-**5y**	> 100	0.16 ± 0.08	0.093 ± 0.01	> 1075	3.2 ± 0.4	> 31.3
S-**5z**	> 100	6.2 ± 0.7	9.2 ± 1.2	> 10.9	> 100	1

* Precipitation at given concentration. n.d.: not determined

From comparison of PPIase assay data of LpMip and BpMip it could be concluded that *para*-substituted benzyl sulfonamides showed slightly better inhibitory effects than *meta*-substituted counterparts (cf. *S*-**5m** and *S*-**5n**, **5f** and **5g**, **5h** and **5i**). Additionally, several compounds with *para*-substituents exhibited better IC_{50} values than their unsubstituted counterparts. In fact, for LpMip the compound *S*-**5m** showed the best inhibition compared to the unsubstituted substance *S*-**5b**. For BpMip the compound with the smallest halogen *S*-**5o** (fluorine) has the best IC_{50} value (0.072 µM).

Moreover, larger substituents like isopropyl in *para*- or *meta*-position significantly reduced activity (**5j**, **5k**). To evaluate the necessity of the benzyl sulfonamide for activity, the amide (**5e**) and the diketone (*S*-**5l**) were synthesized. Both of them, as well as other amide and diketone derivatives not reported here, showed only weak inhibition of LpMip and BpMip. This indicated that the benzyl sulfonamide is the moiety of choice for inhibiting these two Mips.

The influence of the moiety at the ester of the piperidine-2-carboxylate (region B) was also investigated. Compounds with a second ester or an amide group connected to the aryl ring, but also with ethers in the alkyl chain were synthesized and the aromatic substituent at the phenyl group was exchanged. Compounds carrying small isopropyl and 2-hydroxyethyl moieties showed significantly lower anti-PPIase-activity. This indicated that, in spite of the rather exposed position of the piperidine-2-carboxylate function[30] (region B in Figure 1; cf. also Figure 2), it makes a significant contribution to the overall binding affinity.

Nearly all diester compounds showed a higher activity against LpMip than the references **5b**/*S*-**5b** or **5a**/*S*-**5a**. For BpMip, *S*-**5q** is the compound exhibiting the best anti-PPIase-activity. However, these results have to be discussed critically because the diester compounds showed limited stability in buffer media (see below). Since a substantial inhibitory activity is observed, it is likely that the hydrolysis product is still active. By exchanging the benzoic ester with an amide (*S*-**5q** ↔ *S*-**5r**, *S*-**5t** ↔ *S*-**5u**) the chemical stability could be enhanced significantly. Furthermore, compound *S*-**5y**, being most similar to rapamycin, showed the best K_i value for BpMip which was 0.093 µM. To further improve the chemical stability, a methyl group was introduced next to the ester moiety of the piperidine-2-carboxylate. An *S*-configuration of the stereo center showed a better anti-PPIase-activity than the *R*-configured one (*S*-**5v** ↔ *S*-**5w**). *S*-**5s**, a representative of the ether derivatives, revealed a good activity for BpMip ranging between the diester and the amide derivative. With other variations (e.g., substituting the piperidine-2-carboxylate for an amide) no increase in activity was detected (*S*-**5z** ↔ **5d**). To conclude, substitution of region B is worthwhile to improve *in vitro* activity.

NMR-HSQC analysis. To vertify the binding of the newly synthesized compounds to LpMip NMR-HSQC experiments were carried out according to.[30] Therefore, the chemical shift changes induced by the Mip inhibitors were determined. Figure 5 displays the patterns of chemical shift changes of compounds *S*-**5b** and *S*-**5q**, representatively. In analogy to Juli *et al*.[30] the titration of ^{15}N labeled MIP$^{77\text{-}213}$ with the substances *S*-**5b** and *S*-**5q** resulted in significant chemical shift changes of the amide resonances which mirror the affinity to the binding pocket. Since not only the protons being shifted are the same but also the extent of shift is similar, a strong binding to the Mip domain and the Mip cavity is very likely. Moreover, the affected residues are located in the binding pocket for rapamycin. Akin results were obtained for all new inhibitors which were subjected to the NMR measurement. Due to solubility problems a determination of valid dissociation constant was not possible.

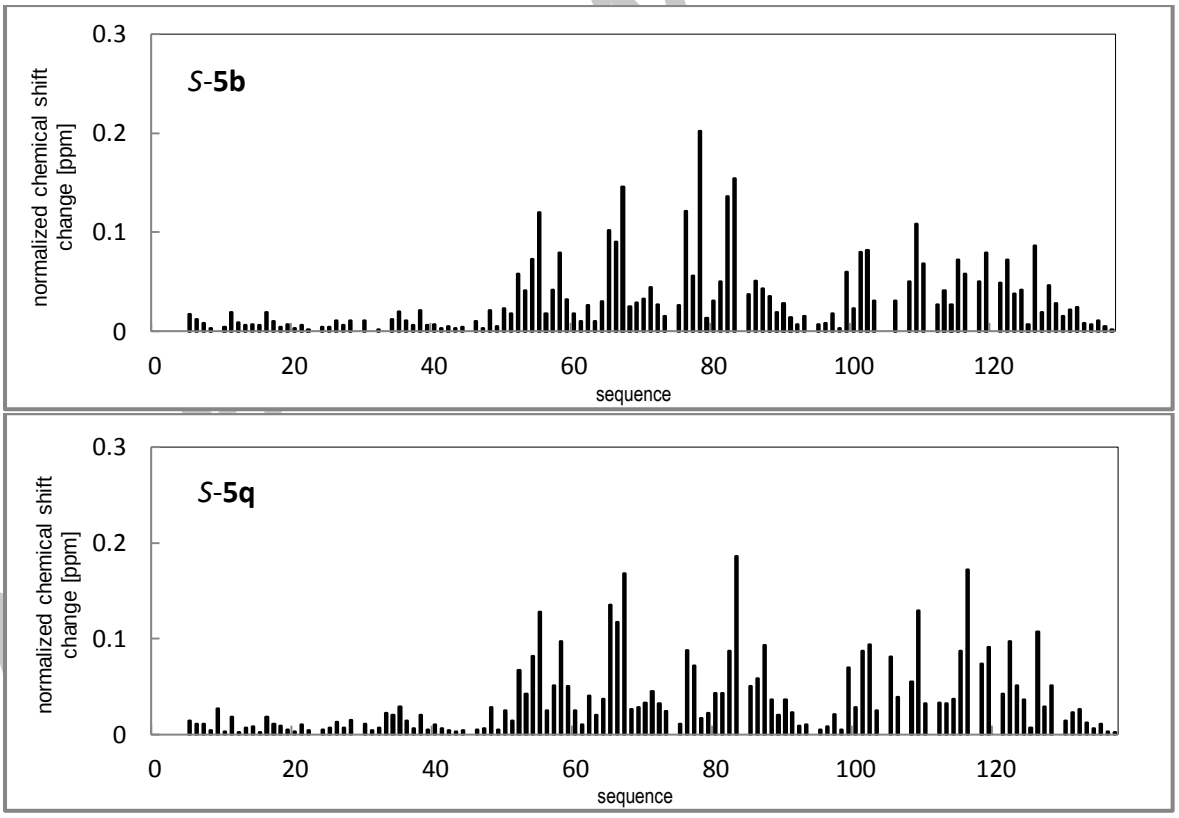


Figure 5: NMR-HSQC experiments with *S*-**5b** and *S*-**5q**.

Docking analysis. To investigate the binding modes of the inhibitors and to analyze the binding model as well as the scoring approach in light of the experimental activity data, docking calculations

were carried out for all compounds reported in Table 1 as described in the experimental section (4.4). The crystal structure of the lead compound **CJ168** (= *S*-**5a**, corresponding to ligand **CJ168**) complexed with BpMip (PDB structure 4G50[31]) served as reference for the entire analysis and provided the possibility to evaluate the docking protocol by redocking ligand **CJ168** back to the uncomplexed 4G50 structure. As shown in Figure 6, the experimental binding mode is reproduced very well, indicating a reliable docking protocol. A more detailed discussion of this finding and further redocking results are provided in the Supplementary Material (Table S1).

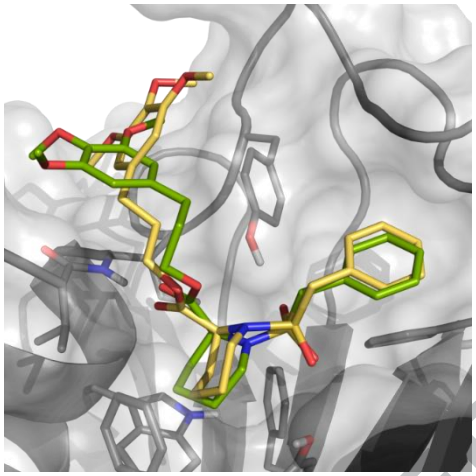


Figure 6: Redocking of CJ168 to BpMip (crystal pose of PDB structure 4G50 chain A shown in lime, docked pose in yellow).

Docking to BpMip: For all compounds, docking poses showing the same binding mode as the lead structure **CJ168** were obtained with good scores (the scores for the top pose of each compound are reported in Table S2 of the Supplementary Material). A quantitative correlation between the scores and the experimental affinities cannot be expected, given the narrow affinity range of less than 2.3 pK_i units for BpMIP (1.8 pIC_{50} units for LpMIP). Nevertheless, correlations within particular subsets can be observed and pairwise comparisons reflect structure-activity relationship (SAR) trends. These are discussed in detail in the Supplementary Material.

Docking to LpMip: For virtually all compounds, the scores obtained for the LpMip poses are less favorable than the scores for the corresponding BpMip poses. This may be related to the fact that only a complex structure with rapamycin is available for docking to LpMip, whereas for BpMip the

complex with lead compound **CJ168** provides a protein conformation which is already better adapted to the class of ligands investigated here. Besides that, it is also more difficult to correctly capture the relative trends in LpMip activity since the range of the IC_{50} values spans less than two orders of magnitude (2.2 – 142 µM). Nevertheless, the relatively low (i.e., less favorable) scores obtained for the smaller ligands (**5c**, **5d**, *S*-**5z**) and the "non-sulfonamide" compounds (**5e**, *S*-**5l**) correctly point to the weaker activity of these substances. Further details are available as Supplementary Material.

Stability of compounds. For the different assays, analytical methods have been developed to assess the stability of the synthesized substances. To study the stability of the compounds in the PPIase assay, different dilutions were prepared, and the substances were incubated at 25 °C and 40 °C for 24 h in methanol with 10 mM HEPES buffer (pH = 7.8). Samples were analysed by HPLC after 0 and 24 h. Whilst the parent compounds *S*-**5a** and *S*-**5b** (99 %) were highly stable in buffer for 24 h, the more active derivatives *S*-**5q** (40 %) and *S*-**5t** (85 %) showed a considerable decomposition in buffer. All other examined samples **5c**, **5d**, *S*-**5o**, *S*-**5s**, *S*-**5u**, *S*-**5v** and *S*-**5z** showed stabilities higher than 95 %. Of note, all unstable compounds carry two ester functions in the molecule (see Figure 7).

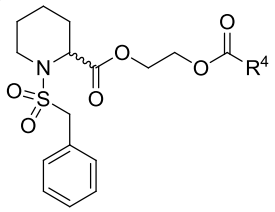


Figure 7: basic structure of the unstable compounds.

In addition, the stability of the inhibitors in the infection assay was evaluated. Compounds were incubated with murine macrophages (J774.1) at 37 °C in RPMI medium for 0, 1, and 24 h. HPLC analysis mirrored the results of the buffer stability study. As expected, the diesters showed the lowest stability (*S*-**5q** and *S*-**5t**). The other tested inhibitors (**5c**, *S*-**5r**, *S*-**5s**, and *S*-**5v**) were stable in the applied assay systems (see Figure 8). It is remarkable that compound **5c** displayed a good stability, indicating that the ester at the piperidine ring is stable under these experimental conditions. Similar incubation experiments were performed for *S*-**5r** in human plasma. A decrease of the *S*-**5r**

concentration was observed after 1, 4 and 24 h, being 5, 20 and 50 percent respectively. These data demonstrate that most of the inhibitors, except the diester compounds, show a sufficient stability in buffer, cell media, and plasma.

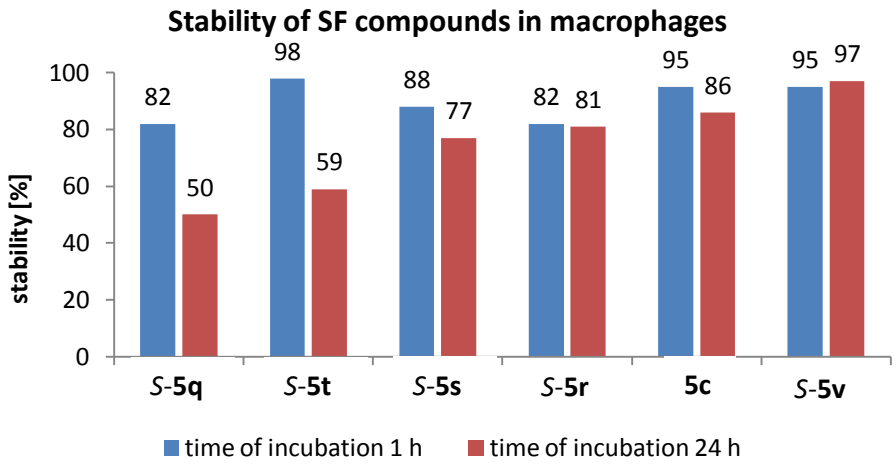


Figure 8: Stability of inhibitors in cell media.

3. Conclusions

Mips are challenging targets which is mirrored in the flat SARs obtained and which can be explained by binding pockets which do not provide a lot of interaction partners. Nevertheless, the established compound library revealed Mip inhibitors with promising anti-PPIase activity in the nanomolar range of concentration and low cytotoxicity. A structure-activity relationship analysis showed that a *para*-substitution of the benzyl moiety of the sulfonamide is slightly preferred to a *meta*-substitution. Large substituents like isopropyl at the benzyl moiety of the sulfonamide in *meta*- or *para*-position resulted in a poor inhibition of the PPIase activity. Diester-compounds showed good activities; however, stability tests demonstrated that they were unstable in buffer media and therefore are not considered suitable for further investigations. Applying a replacement of the ester with an amide, the chemical stability could be clearly improved while the inhibitory activity remains almost the same. Moreover, the compound *S*-**5x** showed the best cytotoxicity-affinity (CA) value for LpMip (IC_{50} = 2.2 µM, CA ≥ 46), whilst S-5o K_i = 0.0937 µM, CA ≥> 1099) and *S*-**5y** (K_i = 0.093 µM, CA ≥> 1075) had the best CA value for BpMip.

4. Experimental Section.

4.1. General information

All chemicals were purchased from Sigma-Aldrich Chemicals (Schnelldorf, Germany), Acros Organics (Geel, Belgium), Bachem (Bubendorf, Switzerland), and VWR International (Darmstadt, Germany), and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on an AV 400 nuclear magnetic resonance spectrometer (^{1}H 400.132 MHz, ^{13}C 100.613 MHz) (Bruker BioSpin MRI GmbH, Ettlingen, Germany). As internal standard, the signals of the deuterated solvent were used (chloroform-*d*: ^{1}H 7.26 ppm, ^{13}C 77.16 ppm; DMSO-d_6: ^{1}H 2.50 ppm, ^{13}C 39.52 ppm). The abbreviations (s) singlet, (d) doublet, (t) triplet, (q) quartet, (dd) doublet of doublet, (ddd) doublet of doublet of doublet, (br) broad signal, (m) multiplet were applied during spectra interpretation. Coupling constants are given in Hertz. Thin layer chromatography (TLC) was carried out on SIL G-25 silica gel plates (Macherey-Nagel, Düren, Germany) using petrol ether and ethyl acetate or ethyl acetate and methanol as eluent. For purification, the Puriflash-430-system and Puriflash-Silica-STD columns (10 - 50 µm, 12 and 25 g) were used (Interchim, Montluçon, France). IR spectra were recorded on a Jasco FT-IR-6100 FT-IR spectrometer (Jasco, Gross-Umstadt, Germany). Melting points were determined with the MPD350:BM 3.5 melting point apparatus (Sanyo, Gallenkamp BV, Netherland) and are reported uncorrected. ESI-MS was conducted in positive mode; for LC/MS, the following conditions were used: column: Agilent Zorbax SB-CN (50 x 4.6 mm, 3.5 µm particle size (Agilent Technologies, Böblingen, Germany); mobile phase: A) Acetonitrile + 0.1 % acetic acid, B) H_2O + 0.1 % acetic acid; gradient: 95 % A (0 - 5 min), 95 % A → 10 % A (5 - 10 min), 10 % A (10 - 15 min), flow: 0.4 ml/min. MS detection mode: ESI, nebulizer pressure: 40 psi, drying gas flow: 5 L/min, drying gas temperature: 350 °C. Purity of compounds was determined by HPLC using an Agilent HPLC system (1100 series, Agilent Technologies, Böblingen, Germany) with UV detection at λ = 254 nm. An Inertsil ODS-2 C_{18} column (150 × 4.6 mm, 5 µm particle size; MZ-Analysetechnik, Mainz, Germany) was used as the stationary phase. The mobile phase was a mixrure of A) a 10 mM KH_2PO_4 buffer solution (pH = 7.4) and B) methanol. A gradient elution (method I; 0-15 min: 30 → 90 % (B), 15-18

min: 90 % (B), 18-20 min: 90 → 30 % (B), 20-25 min: 30 % (B), as well as an isocratic elution (method II) with 30 % of A) and 70 % of (B) was applied. All final products had a purifty ≥ 95 %.

4.2. Synthesis procedures

4.2.1. Synthesis of Ethyl piperidine-2-carboxylate (2). Piperidine-2-carboxylic acid (1 equiv.) was dissolved in 40 mL of anhydrous ethanol. Thionyl chloride (3 equiv.) was added dropwise and refluxed. After the reaction was completed (TLC control), the solvent was removed *in vacuo*. The residue was suspended in 20 mL of aqueous $NaHCO_3$ solution and washed with 4 x 30 mL $CHCl_3$. The organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo* to yield compound **2**.

Spectroscopic data was acquired following the methods of Juli *et al*.[30]

4.2.2. General Procedure for the synthesis of Compounds 3a, e-g. Compound **2** (1 equiv.) was dissolved in 40 mL of anhydrous CH_2Cl_2, and NMM or DIPEA (3 equiv.) was added at 0 °C followed by the corresponding carbonyl or sulfonyl chloride (1 equiv.). The mixture was stirred until completion of the reaction (TLC control) and subsequently washed with 4 x 30 mL 2 M HCl and H_2O. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo*. If necessary, a subsequent purification by flash-chromatography was performed to obtain compounds **3a, e**-**g**.

The preparation of **1-(Benzylsulfonyl)piperidine-2-carboxylate** (**3a**) and **(*S*)-1-(Benzylsulfonyl)piperidine-2-carboxylate** (*S*-**3a**) has already been described.[30]

4.2.2.1. Ethyl (*R*)-1-(benzylsulfonyl)piperidine-2-carboxylate (*R*-**3a**) was obtained as a yellow oil (0.34 g, 57 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.43-7.50 (m, 2H), 7.31-7.42 (m, 3H), 4.57 (d, 1H, *J* = 3.9 Hz), 4.26-4.19 (m, 4H), 3.42-3.49 (m, 1H), 3.16 (ddd, 1H, *J* = 12.7, 12.7, 3.1 Hz), 2.12-2.22 (m, 1H), 1.53-1.74 (m, 3H), 1.37-1.52 (m, 1H), 1.20-1.27 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 131.0

(2C), 129.4, 128.5 (2C), 128.4, 61.4, 58.8, 56.0, 43.4, 27.8, 25.1, 20.3, 14.2; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3026, 2949, 2864, 1708, 1377, 1325, 1180, 1125, 797, 696.

4.2.2.2. 1-(2-(4-Chlorophenoxy)acetyl)piperidine-2-carboxylat (**3e**) was obtained as a yellow oil (0.54 g, 87 %). The ^{1}H-NMR showed the presence of a 77 to 23 mixture of rotamers. Rotamer a: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.20-7.25 (m, 2H), 6.85-6.91 (m, 2H), 5.28 (d, 1H, *J* = 5.4 Hz), 4.65-4.80 (m, 3H), 4.10-4.25 (m, 2H),3.77-3.85 (m, 1H), 3.31 (ddd, 1H, *J* = 13.0, 13.0, 2.9 Hz), 2.23-2.34 (m, 1H), 1.56-1.77 (m, 3H), 1.27-1.55 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 167.6, 156.7, 129.4 (2C), 126.5, 116.0 (2C), 67.4, 61.3, 52.3, 43.0, 26.5, 25.2, 20.9, 14.2; rotamer b (only different signals to a are shown): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.45-4.52 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.6, 167.5, 156.5, 129.5 (2C), 126.6, 115.9 (2C), 68.2, 61.7, 55.8, 40.0, 27.3, 24.5, 20.8; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3055, 2941, 2861, 1732, 1660, 1491, 1367, 1227, 1161, 1018, 823.

4.2.2.3. Ethyl 1-((4-nitrobenzyl)sulfonyl)piperidine-2-carboxylate (**3f**) was obtained as a yellow oil (0.2 g, 88 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20-8.26 (m, 2H), 7.66-7.72 (m, 2H), 4.70 (d, 1H, *J* = 4.4 Hz), 4.41 (AB-spin system, 1H, J_{AB} = 13.6 Hz), 4.39 (AB-spin system, 1H, J_{AB} = 13.6 Hz), 4.20-4.33 (m, 2H), 3.45-3.55 (m, 1H), 3.10 (ddd, 1H, *J* = 12.6, 12.6, 2.9 Hz), 2.21-2.29 (m, 1H), 1.69-1.81 (m, 2H), 1.43-1.65 (m, 2H), 1.33 (t, 3H, *J* = 7.1 Hz), 1.21-1.28 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 148.0, 136.7, 132.1 (2C), 123.7 (2C), 61.7, 58.0, 56.2, 43.5, 28.0, 25.1, 20.4, 14.2; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3080, 2938, 2857, 1732 (m) 1520, 1344, 1320, 1180, 1128, 857.

4.2.2.4. Ethyl 1-((3-nitrobenzyl)sulfonyl)piperidine-2-carboxylate (**3g**) was obtained as a yellow oil (0.7 g, 93 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.37 (t, 1H, *J* = 2.0 Hz), 8.22 (ddd, 1H, *J* = 8.0, 2.0, 1.0 Hz), 7.81-7.86 (m, 1H), 7.56 (t, 1H, *J* = 8.0 Hz), 4.68 (d, 1H, *J* = 4.5 Hz), 4.41 (AB-spin system, 1H, J_{AB} = 13.6 Hz), 4.38 (AB-spin system, 1H, J_{AB} = 13.6 Hz), 4.20-4.35 (m, 2H), 3.46-3.57 (m, 1H), 3.13 (ddd, 1H, *J* = 12.6, 12.6, 3.1 Hz), 2.19-2.31 (m, 1H), 1.69-1.80 (m, 2H), 1.42-1.66 (m, 2H), 1.33 (t, 3H, *J* = 7.1 Hz),

1.23-1.30 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 148.3, 137.1, 131.6, 129.5, 126.0, 123.4, 61.7, 57.7, 56.2, 43.5, 27.9, 25.2, 20.4, 14.2; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3089, 2942, 2862, 1732, 1527, 1351, 1336, 1208, 1181, 1148, 1129, 811.

4.2.3. General Procedure for the Synthesis of Compounds 4a, e-g. Compounds **4a, e-g** were synthesized using a procedure described by Holt *et al*.[34] Therefore, compound **3a, e-g** (1 equiv.) was dissolved in 30 mL of methanol and cooled to 0 °C. To this mixture a solution of lithium hydroxide (10 mL, 1 M) was added dropwise and stirred for 1 h. After the reaction was completed (TLC control), 10% HCl was added to adopt a pH value of 1. The free carboxylic acid was extracted with 4 x 30 mL CH_2Cl_2. The combined organic layers were washed with 4 x 30 mL H_2O and brine and then dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* to give compounds **4a, e-g**.

The preperation of **1-(Benzylsulfonyl)piperidine-2-carboxylic acid** (**4a**) and **(*S*)-1-(Benzylsulfonyl)piperidine-2-carboxylic acid** (*S*-**4a**) has already been described.[30]

4.2.3.1. (*R*)-1-(Benzylsulfonyl)piperidine-2-carboxylic acid (*R*-**4a**) was obtained as a yellow oil (0.31 g, 99 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41-7.47 (m, 2H), 7.33-7.41 (m, 3H), 4.58 (d, 1H, *J* = 5.0 Hz), 4.27 (s, 2H), 3.42-3.52 (m, 1H), 3.15 (ddd, 1H, *J* = 12.7, 12.7, 3.1 Hz), 2.12-2.23 (m, 1H), 1.55-1.78 (m, 3H), 1.37-1.50 (m, 1H), 1.26-1.37 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 176.1, 130.9 (2C), 129.1, 128.6 (3C), 58.9, 55.6, 43.5, 27.5, 24.9, 20.3; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3027, 2950, 2864, 2560, 1708, 1444, 1326, 1228, 1180, 1125, 696.

4.2.3.2. 1-(2-(4-Chlorophenoxy)acetyl)piperidine-2-carboxylic acid (**4e**) was obtained as a yellow oil (0.21 g, 92 %). The ^{1}H-NMR showed the presence of a 77 to 23 mixture of rotamers. Rotamer a: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.19-7.25 (m, 2H), 6.84-6.89 (m, 2H), 5.33 (d, 1H, *J* = 5.0 Hz), 4.62-4.86 (m, 2H), 3.78-3.86 (m, 1H), 3.30 (ddd, 1H, *J* = 12.8, 12.8, 2.7 Hz), 2.26-2.35 (m, 1H), 1.60-1.81 (m, 3H), 1.34-1.55 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.9, 168.3, 156.5, 129.4 (2C), 126.6, 116.1 (2C),

67.2, 52.3, 43.1, 26.3, 25.0, 20.8; Rotamer b (only different signals to a are shown): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.82-4.86 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.4, 167.9, 156.4, 129.5 (2C), 126.7, 115.9 (2C), 68.1, 55.6, 40.1, 27.1, 24.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3027, 2933, 2863, 1707, 1490, 1422, 1227, 1160, 1016, 823.

4.2.3.3. 1-((4-Nitrobenzyl)sulfonyl)piperidine-2-carboxylic acid (**4f**) was obtained as a yellow oil (0.07 g, 95 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20-8.26 (m, 2H), 7.61-7.67 (m, 2H), 4.71 (d, 1H, *J* = 4.6 Hz), 4.38 (s, 2H), 3.47-3.58 (m, 1H), 3.13 (ddd, 1H, *J* = 12.7, 12.7, 3.0 Hz), 2.21-2.32 (m, 1H), 1.61-1.82 (m, 1H), 1.42-1.56 (m, 1H), 1.27-1.40 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.7, 148.0, 136.7, 131.9 (2C), 123.7 (2C), 58.1, 55.8, 43.6, 27.8, 25.0, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3076, 2945, 2880, 1716, 1598, 1519, 1343, 1318, 1125, 1109, 841.

4.2.3.4. 1-((3-Nitrobenzyl)sulfonyl)piperidine-2-carboxylic acid (**4g**) was obtained as a yellow oil (0.22 g, 96 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.32 (t, 1H, *J* = 2.0 Hz), 8.20 (ddd, 1H, *J* = 8.1, 2.0, 1.0 Hz), 7.78-7.82 (m, 1H), 7.54 (t, 1H, *J* = 8.1 Hz), 4.77 (d, 1H, *J* = 4.9 Hz), 4.36 (s, 2H), 3.44-3.52 (m, 1H), 3.09 (ddd, 1H, *J* = 12.7, 12.7, 3.0 Hz), 2.21-2.29 (m, 1H), 1.57-1.81 (m, 3H), 1.17-1.57 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.9, 148.2, 137.1, 131.3, 129.6, 126.0, 123.5, 57.9, 55.8, 43.7, 27.8, 25.1, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3088, 2943, 2862, 1715, 1504, 1334, 1258, 1182, 1129, 960, 840.

4.2.4. General Procedure for the Synthesis of Compounds 5a-g. For synthesis of compounds **5a**-**g** a procedure from Dong *et al*.[35] was used. Compound **4a, e**-**g** (1 equiv.), the corresponding alcohol (1.0 equiv.), EDC·HCl (1.5 equiv.), and DMAP (0.2 equiv.), were dissolved in 20 mL of anhydrous CH_2Cl_2. This mixture was stirred overnight. After the reaction was completed (TLC control) a subsequent purification by flash-chromatography was performed to obtain compound **5a**-**g**.

3-(3,4,5-Trimethoxyphenyl)propyl 1-(benzylsulfonyl)piperidine-2-carboxylate (**5a**) and **(*S*)-3-(3,4,5-Trimethoxyphenyl)propyl 1-(benzylsulfonyl)piperidine-2-carboxylate** (*S*-**5a**) have already been described.[30]

4.2.4.1. (*R*)-3-(3,4,5-Trimethoxyphenyl)propyl 1-(benzylsulfonyl)piperidine-2-carboxylate (*R*-**5a**) was obtained as a yellow oil (0.45 g, 81 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41-7.48 (m, 2H), 7.32-7.40 (m, 3H), 6.41 (s, 2H), 4.53 (d, 1H, *J* = 4.7 Hz), 4.27 (s, 2H), 4.16-4.24 (m, 2H), 3.84 (s, 6H), 3.82 (s, 3H), 3.40-3.49 (m, 1H), 3.17 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.65 (t, 2H, *J* = 7.6 Hz), 2.11-2.18 (m, 1H), 1.94-2.03 (m, 2H), 1.54-1.73 (m, 3H), 1.37-1.49 (m, 1H), 1.17-1.30 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.5, 153.2 (2C), 136.7, 131.0 (2C), 129.3, 128.5 (3C), 105.4 (2C), 64.6, 60.8, 58.9, 56.1 (3C), 43.5, 32.4, 30.3, 27.8, 25.0, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3064, 2940, 2859, 1731, 1589, 1455, 1335, 1237, 1177, 1122, 698 (m); HPLC purity: 98 % (method I); ESI-MS: m/z 492.4 $[M+H]^+$.

4.2.4.2. 3-(Pyridin-3-yl)propyl 1-(benzylsulfonyl)piperidine-2-carboxylate (**5b**) was obtained as a colourless oil (0.66 g, 93 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.45-8.48 (m, 2H), 7.51-7.56 (m, 1H), 7.43-7.48 (m, 2H), 7.33-7.40 (m, 3H), 7.24 (ddd, 1H, *J* = 7.8, 4.9, 0.7 Hz), 4.50 (d, 1H, *J* = 4.4 Hz), 4.13-4.31 (m, 4H), 3.39-3.50 (m, 1H), 3.16 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.73 (t, 2H, *J* = 7.8 Hz), 2.09-2.16 (m, 1H), 1.96-2.03 (m, 2H), 1.55-1.72 (m, 3H), 1.35-1.49 (m, 1H), 1.15-1.23 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 149.7, 147.5, 136.3, 136.1, 130.9 (2C), 129.3, 128.5 (3C), 123.5, 64.3, 58.9, 56.1, 43.5, 29.9, 29.3, 27.7, 24.9, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3032, 2945, 2860, 1732, 1575, 1335, 1147, 1127, 1109, 738, 698 (m); HPLC purity: > 99 % (method I); ESI-MS: m/z 403.4 $[M+H]^+$.

4.2.4.3. 2-Hydroxyethyl 1-(benzylsulfonyl)piperidine-2-carboxylate (**5c**) was obtained as a colourless oil (0.41 g, 71 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.34-7.45 (m, 5H), 4.34-4.43 (m, 1H), 4.26 (s, 2H), 4.18-4.28 (m, 2H), 3.83 (q, 2H, *J* = 4.8 Hz), 3.34-3.43 (m, 1H), 3.13 (ddd, 1H, *J* = 12.8, 12.7, 2.9 Hz), 2.46 (t, 1H, *J* = 5.6 Hz), 2.11-2.20 (m, 1H), 1.34-1.72 (m, 4H), 1.18-1.29 (m, 1H); ^{13}C-NMR (100 MHz,

$CDCl_3$) δ 171.4, 130.9 (2C), 129.1, 128.7, 128.6 (2C), 66.9, 60.8, 58.8, 56.3, 43.8, 27.4, 24.8, 20.2; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3491 (br), 3065, 3034, 2944, 2861, 1733, 1455, 1334, 1322, 1198, 1177, 1147, 1125, 1059, 739, 697 (s); HPLC purity: > 99 % (method I); ESI-MS: m/z 328.3 $[M+H]^+$.

4.2.4.4. Isopropyl 1-(benzylsulfonyl)piperidine-2-carboxylate (**5d**) was obtained as a white powder (0.09 g, 39 %); mp 64-68 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.44-7.49 (m, 2H), 7.32-7.39 (m, 3H), 5.10 (sept, 1H, *J* = 6.3 Hz), 4.54 (d, 1H, *J* = 4.5 Hz), 4.28 (s, 2H), 3.42-3.49 (m, 1H), 3.16 (ddd, 1H, *J* = 12.7, 12.7, 3.1 Hz), 2.12-2.21 (m, 1H), 1.54-1.72 (m, 1H), 1.38-1.51 (m, 1H), 1.16-1.31 (m, 7H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.9, 131.0 (2C), 129.4, 128.5 (2C), 128.4, 69.1, 58.7, 56.1, 43.4, 27.8, 25.1, 21.8 (2C), 20.3; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3041, 2934, 2853, 1721, 1498, 1336, 1210, 1183, 1142, 1126, 735, 697 (m); HPLC purity: 96 % (method II); ESI-MS: m/z 326.2 $[M+H]^+$.

4.2.4.5. 3-(Pyridin-3-yl)propyl 1-(2-(4-chlorophenoxy)acetyl)piperidine-2-carboxylate (**5e**) was obtained as a yellow oil (0.26 g, 88 %). The ^{1}H-NMR showed the presence of a 77 to 23 mixture of rotamers. Rotamer a: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.42-8.48 (m, 2H), 7.44-7.51 (m, 1H), 7.19-7.24 (m, 3H), 6.84-6.90 (m, 2H), 5.30 (d, 1H, *J* = 5.1 Hz), 4.65-4.82 (m, 2H), 4.07-4.19 (m, 2H), 3.79-3.87 (m, 1H), 3.31 (ddd, 1H, *J* = 13.0, 13.1, 3.0 Hz), 2.61-2.76 (m, 2H), 2.21-2.32 (m, 1H), 1.87-1.98 (m, 2H), 1.60-1.79 (m, 3H), 1.27-1.53 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 167.7, 156.6, 149.7, 147.6, 136.4, 135.9, 129.4, 126.5, 123.4, 116.0, 67.4, 64.2, 52.4, 43.1, 29.8, 29.2, 26.5, 25.2, 20.9. Rotamer b (only different signals to a are shown): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.65-4.82 (m, 3H), 4.47-4.53 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.6, 167.5, 156.5, 129.5 (2C), 115.9 (2C), 68.4, 64.6, 55.9, 40.1, 29.3, 27.3, 24.5, 20.8; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3031, 2942, 2860, 1732, 1661, 1594, 1491, 1422, 1227, 1161, 1016, 824 (m); HPLC purity: 96 % (method II); ESI-MS: m/z 417.4 $[M+H]^+$.

4.2.4.6. 3-(Pyridin-3-yl)propyl 1-((4-nitrobenzyl)sulfonyl)piperidine-2-carboxylate (**5f**) was obtained as a yellow oil (0.22 g, 52 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.45-8.49 (m, 2H), 8.20-8.25 (m, 2H),

7.65-7.70 (m, 2H), 7.49-7.54 (m, 1H), 7.24 (ddd, 1H, *J* = 7.8, 4.8, 0.7 Hz), 4.66 (d, 1H, *J* = 4.7 Hz), 4.39 (AB-spin system, 1H, J_{AB} = 13.6 Hz), 4.37 (AB-spin system, 1H, J_{AB} = 13.6 Hz), 4.15-4.28 (m, 2H), 3.45-3.52 (m, 1H), 3.11 (ddd, 1H *J* = 12.7, 12.7, 3.0 Hz), 2.73 (t, 2H, *J* = 7.7 Hz), 2.16-2.27 (m, 1H), 1.98-2.08 (m, 2H), 1.58-1.79 (m, 3H), 1.41-1.55 (m, 1H), 1.15-1.29 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 149.9, 148.0, 147.8, 136.6, 136.0, 135.8, 132.0 (2C), 123.6 (2C), 123.5, 64.6, 58.0, 56.2, 43.6, 29.8, 29.3, 28.0, 25.0, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3031, 2943, 2858, 1732, 1606, 1519, 1446, 1344, 1197, 1177, 1128, 812 (m); HPLC purity: > 99 % (method II); ESI-MS: m/z 448.4 $[M+H]^+$.

4.2.4.7. 3-(Pyridin-3-yl)propyl 1-((3-nitrobenzyl)sulfonyl)piperidine-2-carboxylate (**5g**) was obtained as a yellow oil (0.82 g, 65 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.45-8.51 (m, 2H), 8.36 (t, 1H, *J* = 1.8 Hz), 8.22 (ddd, 1H, *J* = 8.1, 1.8, 0.9 Hz), 7.80-7.87 (m, 1H), 7.53-7.60 (m, 1H), 7.23-7.30 (m, 1H), 4.63 (d, 1H, *J* = 3.4 Hz), 4.39 (AB-spin system, 1H, J_{AB} = 14.0 Hz), 4.37 (AB-spin system, 1H, J_{AB} = 14.0 Hz), 4.17-4.30 (m, 2H), 3.46-3.54 (m, 1H), 3.13 (ddd, 1H, *J* = 12.7, 12.7, 3.0 Hz), 2.74 (t, 2H, *J* = 7.7 Hz), 2.17-2.28 (m, 1H), 1.99-2.07 (m, 2H), 1.59-1.79 (m, 3H), 1.42-1.57 (m, 1H), 1.16-1.30 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.3, 149.5, 148.3, 147.4, 137.1, 136.3, 136.2, 131.5, 129.6, 126.0, 123.6, 123.5, 64.6, 57.8, 56.2, 43.6, 29.9, 29.3, 27.9, 25.1, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3087, 3030, 2944, 2860, 1732, 1526, 1351, 1336, 1301, 1148, 1128, 811 (m); HPLC purity: 99 % (method I); ESI-MS: m/z 448.4 $[M+H]^+$.

4.2.4.8. 3-(Pyridin-3-yl)propyl 1-((4-aminobenzyl)sulfonyl)piperidine-2-carboxylate (**5h**) was obtained as a orange oil (0.05 g, 38 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.42-8.48 (m, 2H), 7.49-7.53 (m, 1H), 7.18-7.24 (m, 3H), 6.62-6.67 (m, 2H), 4.47 (d, 1H, *J* = 4.9 Hz), 4.08-4.25 (m, 4H), 3.67 (s, 2H), 3.39-3.46 (m, 1H), 3.16 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.72 (t, 2H, *J* = 7.8 Hz), 2.06-2.16 (m, 1H), 1.93-2.16 (m, 2H), 1.54-1.72 (m, 3H), 1.13-1.49 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.5, 149.8, 147.6, 146.8, 136.2, 135.9, 131.9 (2C), 123.4, 118.6, 114.9 (2C), 64.2, 58.0, 56.0, 43.5, 29.9, 29.2,

27.7, 25.0, 20.3; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3452, 3372, 3031, 2927, 2856, 1731, 1613, 1517, 1445, 1323, 1298, 1176, 1126, 823 (m); HPLC purity: 97 % (method II); ESI-MS: m/z 418.4 $[M+H]^+$.

4.2.4.9. 3-(Pyridin-3-yl)propyl 1-((3-aminobenzyl)sulfonyl)piperidine-2-carboxylate (**5i**) was obtained as a orange oil (0.16 g, 69 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.44-8.49 (m, 2H), 7.52-7.56 (m, 1H), 7.24 (ddd, 1H, *J* = 7.8, 4.9, 0.8 Hz), 7.10-7.15 (m, 1H), 6.77-6.81 (m, 2H), 6.63-6.67 (m, 1H), 4.51 (d, 1H, *J* = 4.7 Hz), 4.08-4.25 (m, 4H), 3.44-3.52 (m, 1H), 3.32 (s, 2H), 3.19 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.73 (t, 2H, *J* = 7.8 Hz), 2.08-2.15 (m, 1H), 1.95-2.03 (m, 2H), 1.56-1.72 (m, 3H), 1.37-1.50 (m, 1H), 1.16-1.29 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.5, 149.6, 147.4, 146.6, 136.4, 136.2, 130.2, 129.4, 123.5, 121.1, 117.3, 115.2, 64.2, 58.9, 56.1, 43.5, 29.9, 29.2, 27.7, 24.9, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3447, 3370, 3031, 2944, 2859, 1731, 1625, 1606, 1590, 1462, 1322, 1147, 1126, 965, 767 (m); HPLC purity: >99 % (method I); ESI-MS: m/z 418.4 $[M+H]^+$.

4.2.4.10. (*S*)-3-(Pyridin-3-yl)propyl 1-((4-chlorobenzyl)sulfonyl)piperidine-2-carboxylate (*S*-**5m**) was obtained as a yellow oil (0.20 g, 86 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.46-8.51 (m, 2H), 7.57-7.62 (m, 1H), 7.38-7.43 (m, 2H), 7.32-7.36 (m, 2H), 7.28-7.32 (m, 1H), 4.55 (d, 1H, *J* = 4.9 Hz), 4.14-4.28 (m, 4H), 3.40-3.50 (m, 1H), 3.14 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.75 (t, 2H, *J* = 7.8 Hz), 2.12-2.20 (m, 1H), 1.97-2.06 (m, 2H), 1.57-1.76 (m, 3H), 1.38-1.50 (m, 1H), 1.15-1.25 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 149.1, 147.0, 136.7, 136.6, 134.7, 132.3 (2C), 128.8 (2C), 127.8, 123.7, 64.3, 58.1, 56.1, 43.6, 29.9, 29.3, 27.9, 25.0, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3031, 2944, 2859, 1732, 1492, 1335, 1301, 1176, 1128, 826 (m); HPLC purity: 97 % (method I); ESI-MS: m/z 437.4 $[M+H]^+$.

4.2.5. General Procedure for the Synthesis of Compounds 5j and 5k. To reduce the nitro to an amino group the starting material **5f** or **5g** was dissolved in 20 mL EtOAc and hydrogenated at RT and 10 bar hydrogen with catalytic amounts of Pd/C. After the reaction was completed the Pd/C was filtrated and the solvent was evaporated to yield compound **5h** or **5i**. Without further purification a modified

procedure from Fache *et al*.[33] was used to synthesize compounds **5j and k**. Therefore, 1 equiv. of **5h** or **5i**, respectively, 1.1 equiv. of Me_2CO, 4 equiv. of Na_2SO_4 and catalytic amounts of Pd/C were dissolved in 20 mL CH_2Cl_2 and hydrogenated at RT and 10 bar hydrogen. After the reaction was finished (TLC control) Pd/C was filtered off and the crude product was purified by flash-chromatography to obtain compounds **5j**, 5**k**.

4.2.5.1. 3-(Pyridin-3-yl)propyl 1-((3-(isopropylamino)benzyl)sulfonyl)piperidine-2-carboxylate (**5j**) was obtained as a colouless oil (0.01 g, 9 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.43-8.49 (m, 2H), 7.48-7.55 (m, 1H), 7.22 (ddd, 1H, *J* = 7.8, 4.8, 0.7 Hz), 7.13 (t, 1H, *J* = 7.7), 6.67-6.71 (m, 1H), 6.65-6.67 (m, 1H), 6.55 (ddd, 1H, *J* = 7.7, 2.2, 0.7 Hz), 4.51 (d, 1H, *J* = 4.7 Hz), 4.11-4.25 (m, 4H), 3.63 (sept, 1H, *J* = 6.3 Hz), 3.45-3.53 (m, 1H), 3.19 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.72 (t, 2H, *J* = 7.8 Hz), 2.07-2.15 (m, 1H), 1.94-2.05 (m, 2H), 1.55-1.72 (m, 3H), 1.33-1.50 (m, 1H), 1.13-1.29 (m, 7H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.5, 149.9, 147.6, 147.6, 136.3, 135.9, 130.1, 129.3, 123.4, 119.4, 115.5, 113.3, 64.2, 59.2, 56.0, 44.1, 43.4, 30.0, 29.2, 27.7, 24.9, 22.9 (2C), 20.3; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3389, 3031, 2961, 2861, 1732, 1605, 1491, 1323, 1175, 1127, 792 (m); HPLC purity: 95 % (method II); ESI-MS: m/z 460.3 $[M+H]^+$.

4.2.5.2. 3-(Pyridin-3-yl)propyl 1-((4-(isopropylamino)benzyl)sulfonyl)piperidine-2-carboxylate (**5k**) was obtained as a colourless oil (0.04 g, 21 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.43-8.47 (m, 2H), 7.49-7.53 (m, 1H), 7.18-7.23 (m, 3H), 6.51-6.56 (m, 2H), 4.47 (d, 1H, *J* = 4.8 Hz), 4.08-4.24 (m, 4H), 3.61 (sept, 1H, *J* = 6.4 Hz), 3.39-3.47 (m, 1H), 3.16 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.71 (t, 2H, *J* = 7.8 Hz), 2.05-2.14 (m, 1H), 1.93-2.03 (m, 2H), 1.52-1.71 (m, 3H), 1.35-1.49 (m, 1H), 1.13-1.28 (m, 7H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.5, 149.8, 147.7, 147.6, 136.2, 135.9, 131.8 (2C), 123.4, 116.7, 112.9 (2C), 64.1, 58.5, 56.0, 44.1, 43.4, 29.9, 29.2, 27.7, 24.9, 22.8 (2C), 20.3; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3389, 3029, 2927, 2858, 1732, 1613, 1520, 1323, 1174, 1127, 826 (m); HPLC purity: 96 % (method II); ESI-MS: m/z 460.4 $[M+H]^+$.

4.2.5.3. (*S*)-3-(Pyridin-3-yl)propyl 1-(2-oxo-2-phenylacetyl)piperidine-2-carboxylate (*S*-**5l**). For the synthesis of *S*-**5l** an amidation with **8a** (1 equiv.) and phenylglyoxalic acid (1.0 equiv.) in 20 mL anhydrous CH_2Cl_2 was performed at 0 °C using EDC·HCl (1.3 equiv.) and HOBt (0.5 equiv.). The mixture was stirred until completion (TLC control) and after purification by means of flash-chromatography the compound was obtained as a yellow oil (0.20 g, 62 %). The ^{1}H-NMR showed the presence of a 77 to 23 mixture of rotamers. Rotamer a: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.40-8.50 (m, 2H), 8.01-8.05 (m, 2H), 7.58-7.67 (m, 1H), 7.43-7.55 (m, 3H), 7.19-7.25 (m, 1H), 5.42 (d, 1H, *J* = 5.2 Hz), 4.26 (t, 2H, *J* = 6.4 Hz), 3.48-3.55 (m, 1H), 3.26 (ddd, 1H, *J* = 13.0, 13.1, 3.0 Hz), 2.75 (t, 2H, *J* = 7.8 Hz), 2.01-2.10 (m, 2H), 1.31-1.94 (m, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.6, 170.4, 167.4, 149.9, 147.8, 136.1, 135.9, 134.8, 133.2, 129.7 (2C), 129.1 (2C), 123.4, 64.6, 51.6, 44.3, 30.0, 29.3, 26.4, 24.9, 21.2. Rotamer b (only different signals to a are shown): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.96-7.99 (m, 2H), 4.61-4.68 (m, 1H), 4.39 (d, 1H, *J* = 4.9), 3.03 (ddd, 1H, *J* = 13.3, 13.3, 3.0 Hz), 2.61 (t, 2H, *J* = 7.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.2, 170.3, 147.7, 135.8, 133.1, 129.9 (2C), 128.9 (2C), 123.3, 64.5, 56.6, 39.3, 29.8, 29.1, 27.3, 24.4, 21.0; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3058, 3031, 2943, 2860, 1734, 1678, 1639, 1444, 1228, 1161, 794, 714 (m); HPLC purity: 98 % (method I); ESI-MS: m/z 381.3 $[M+H]^+$.

4.2.6. General Procedure for the Synthesis of Compounds *S*-5a, b, n-y. According to a procedure of Choi *et al.*[36] (*S*)-1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid (1 equiv.) was dissolved in 20 mL anhydrous CH_2Cl_2 and at 0 °C the corresponding alcohol (1.0 equiv.), EDC·HCl (1.5 equiv.) and DMAP (0.2 equiv.) were added. After the reaction was completed (TLC control) the organic layer was washed twice with water and the solvent was evaporated *in vacuo*. The boc-protection group was cleaved with 2 mL trifluoroacetic acid in 20 mL CH_2Cl_2. After 24 h the reaction was neutralized with saturated $NaHCO_3$ extracted with 3 x 30 mL CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated to yield compound **8a, b, n-y** which was used in the next step without further purification. Compound **8a, b, n-y** (1 equiv.) was dissolved in 40 mL of anhydrous CH_2Cl_2, and NMM or DIPEA (3 equiv.) was added at 0 °C followed by corresponding sulfonyl chloride

(1 equiv.), respectively. The mixture was stirred until completion (TLC) and subsequently the solvent removed *in vacuo*. After purification by means of flash-chromatography compounds *S*-**5a, b, n**-**y** were obtained.

4.2.6.1. (*S*)-3-(Pyridin-3-yl)propyl 1-(benzylsulfonyl)piperidine-2-carboxylate (*S*-**5b**) was obtained as a colourless oil (0.22 g, 80 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.45-8.48 (m, 2H), 77.52 (ddd, 1H, *J* = 7.8, 2.2, 1.7 Hz), 7.43-7.48 (m, 2H), 7.33-7.40 (m, 3H), 7.23 (ddd, 1H, *J* = 7.8, 4.9, 0.7 Hz), 4.51 (d, 1H, *J* = 4.3 Hz), 4.13-4.31 (m, 4H), 3.39-3.50 (m, 1H), 3.16 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.73 (t, 2H, *J* = 7.8 Hz), 2.09-2.16 (m, 1H), 1.96-2.03 (m, 2H), 1.55-1.72 (m, 3H), 1.35-1.49 (m, 1H), 1.14 (ddq, 1H, *J* = 12.4, 12.4, 3.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.5, 149.7, 147.7, 136.2, 135.9, 131.0 (2C), 129.3, 128.6 (3C), 123.4, 64.3, 58.9, 56.1, 43.5, 30.0, 29.3, 27.8, 25.0, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3032, 2945, 2859,1732, 1575, 1335, 1147, 1127, 1109, 738, 697 (m); HPLC purity: 97 % (method I); ESI-MS: m/z 403.4 $[M+H]^+$.

4.2.6.2. (*S*)-3-(pyridin-3-yl)propyl 1-((3-chlorobenzyl)sulfonyl)piperidine-2-carboxylate (*S*-**5n**) was obtained as a yellow oil (0.85 g, 83 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.46-8.49 (m, 2H), 7.54 (ddd, 1H, *J* = 7.8, 2.2, 1.7 Hz), 7.46 (t, 1H, *J* = 1.6 Hz), 7.28-7.37 (m, 3H), 7.23-7.27 (m, 1H), 4.55 (d, 1H, *J* = 4.8 Hz), 4.08-4.29 (m, 4H), 3.43-3.50 (m, 1H), 3.16 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.73 (t, 2H, *J* = 7.8 Hz), 2.10-2.24 (m, 1H), 1.95-2.07 (m, 2H), 1.57-1.77 (m, 3H), 1.38-1.52 (m, 1H), 1.15-1.30 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 149.6, 147.4, 136.3, 136.2, 134.3, 131.2, 131.0, 129.8, 129.2, 128.7, 123.5, 64.4, 58.2, 56.1, 43.6, 29.9, 29.3, 27.8, 25.0, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3031, 2944, 2860, 1732, 1597, 1423, 1335, 1177, 1148, 1128, 795 (m); HPLC purity: 97 % (method II); ESI-MS: m/z 437.5 $[M+H]^+$.

4.2.6.3. (*S*)-3-(Pyridin-3-yl)propyl 1-((4-fluorobenzyl)sulfonyl)piperidine-2-carboxylate (*S*-**5o**) was obtained as a yellow oil (0.23 g, 91 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.44-8.49 (m, 2H), 7.53 (ddd, 1H,

J = 7.8, 2.0, 1.7 Hz), 7.41-7.46 (m, 2H), 7.22-7.26 (m, 1H), 7.02-7.09 (m, 2H), 4.55 (d, 1H, *J* = 4.8 Hz), 4.08-4.28 (m, 4H), 3.40-3.47 (m, 1H), 3.13 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz), 2.73 (t, 2H, *J* = 7.8 Hz), 2.12-2.20 (m, 1H), 1.95-2.06 (m, 2H), 1.55-1.75 (m, 3H), 1.36-1.52 (m, 1H), 1.14-1.28 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 162.9 (d, $^1J_{CF}$=246.4 Hz), 149.7, 147.5, 136.3, 136.1, 132.7 (2C, d, J_{CF} = 8.3 Hz), 125.2 (d, J_{CF} = 3.3 Hz), 123.5, 115.6 (2C, d, J_{CF} = 21.5 Hz), 64.3, 57.9, 56.1, 43.5, 29.9, 29.3, 27.9, 25.0, 20.4; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3031, 2946, 2861, 1732, 1508, 1335, 1176, 1127, 841 (m); HPLC purity: 98 % (method II); ESI-MS: m/z 421.4 $[M+H]^+$.

4.2.6.4. (*S*)-3-(Pyridin-3-yl)propyl 1-((3-nitrobenzyl)sulfonyl)piperidine-2-carboxylate (*S*-**5p**) was obtained as a yellow oil (0.27 g, 68 %). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.42 (d, 1H, *J* = 1.8 Hz), 8.40 (dd, 1H, *J* = 4.8, 1.8 Hz), 8.33-8.36 (m, 1H), 8.24 (ddd, 1H, *J* = 8.1, 2.3, 0.9 Hz), 7.86-7.91 (m, 1H), 7.71 (t, 1H, *J* = 8.1 Hz), 7.60-7.66 (m, 1H), 7.30 (ddd, 1H, *J* = 7.8, 4.8, 0.6 Hz), 4.71 (AB-spin system, 1H, J_{AB} = 13.6 Hz), 4.66 (AB-spin system, 1H, J_{AB} = 13.6 Hz), 4.38 (d, 1H, *J* = 5.0 Hz), 4.04-4.18 (m, 2H), 3.39-3.47 (m, 1H), 3.14 (ddd, 1H, *J* = 12.8, 12.7, 2.7 Hz), 2.67 (t, 2H, *J* = 7.7 Hz), 1.96-2.03 (m, 1H), 1.87-1.96 (m, 2H), 1.50-1.67 (m, 3H), 1.21-1.36 (m, 1H), 1.03-1.20 (m, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 170.9, 149.5, 147.4, 147.2, 137.5, 136.4, 135.7, 132.3, 129.7, 125.5, 123.3, 123.0, 63.9, 56.2, 55.1, 42.7, 29.4, 28.3, 27.1, 24.2, 19.6; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3087, 3031, 2943, 2860, 1732, 1576, 1526, 1351, 1336, 1301, 1148, 1128, 880 (w); HPLC purity: 97 % (method I); ESI-MS: m/z 448.4 $[M+H]^+$.

4.2.6.5. (*S*)-2-((1-(Benzylsulfonyl)piperidine-2-carbonyl)oxy)ethyl nicotinate (*S*-**5q**) was obtained as a colourless oil (0.17 g, 58 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.22 (dd, 1H, *J* = 2.2, 0.8 Hz), 8.78 (dd, 1H, *J* = 4.9, 1.7 Hz), 8.35 (td, 1H, *J* = 8.0, 1.9 Hz), 7.31-7.46 (m, 6H), 4.45-4.63 (m, 5H), 4.24 (s, 2H), 3.39-3.47 (m, 1H), 3.15 (ddd, *J* = 12.8, 12.8, 3.1 Hz), 2.08-2.18 (m, 1H), 1.51-1.69 (m, 3H), 1.34-1.47 (m, 1H), 1.13-1.25 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 164.6, 152.9, 150.3, 137.9, 130.9 (2C), 129.2, 128.5 (3C), 125.8, 123.7, 3.0, 62.7, 58.8, 56.0, 43.4, 27.8, 24.9, 20.3; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3062,

3034, 2946, 2861, 1723, 1590, 1455, 1336, 1277, 1175, 1126, 1107, 739, 697 (m); HPLC purity: 96 % (method I); ESI-MS: m/z 433.3 $[M+H]^+$.

4.2.6.6. (*S*)-2-(Nicotinamido)ethyl 1-(benzylsulfonyl)piperidine-2-carboxylate (*S*-**5r**) was obtained as a colourless oil (0.09 g, 72 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.12 (dd, 1H, *J* = 2.3, 0.8 Hz), 8.72 (dd, 1H, *J* = 4.8, 1.7 Hz), 8.19 (ddd, 1H, *J* = 8.0, 2.3, 1.7 Hz), 7.31-7.41 (m, 7H), 4.49 (ddd, 1H, *J* = 11.2, 6.3, 3.4 Hz), 4.26 (s, 2H), 4.16-4.22 (m, 2H), 3.72-3.87 (m, 2H), 3.27-3.35 (m, 1H), 3.08 (ddd, 1H *J* = 12.8, 12.9, 2.8 Hz), 2.08-2.16 (m, 1H), 1.57-1.66 (m, 2H), 1.30-1.44 (m, 2H), 1.09-1.22 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.1, 165.6, 152.1, 148.7, 135.3, 130.8 (2C), 129.6 (2C), 128.9 (2C), 128.7, 123.2, 64.7, 59.1, 56.7, 44.2, 38.7, 26.9, 24.5, 20.0; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3371, 3065, 3031, 2944, 2860, 1736, 1650, 1591, 1496, 1334, 1196, 1176, 1125, 1109, 781, 739, 697 (m); HPLC purity: 97 % (method I); ESI-MS: m/z 432.4 $[M+H]^+$.

4.2.6.7. (*S*)-2-(Pyridin-3-yloxy)ethyl 1-(benzylsulfonyl)piperidine-2-carboxylate (*S*-**5s**) was obtained as an orange oil (0.11 g, 68 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.31 (d, 1H, *J* = 2.3 Hz), 8.25 (dd, 1H, *J* = 4.3, 1.6 Hz), 7.33-7.47 (m, 5H), 7.19-7.25 (m, 2H), 4.48-4.62 (m, 3H), 4.24-4.28 (m, 4H), 3.40-3.48 (m, 1H), 3.16 (ddd, 1H, *J* = 12.8, 12.8, 3.1 Hz), 2.10-2.18 (m, 1H), 1.53-1.71 (m, 3H), 1.36-1.49 (m, 1H), 1.15-1.28 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 154.6, 142.6, 137.7, 130.9 (2C), 129.2, 128.6 (2C), 128.5, 124.0, 121.6, 66.1, 63.2, 58.8, 56.0, 43.5, 27.8, 24.9, 20.3; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3062, 3034, 2943, 2861, 1737, 1576, 1455, 1335, 1265, 1174, 1147, 1126, 739, 697 (m); HPLC purity: 96 % (method II); ESI-MS: m/z 405.4 $[M+H]^+$.

4.2.6.8. (*S*)-2-((3,5-Bis(2-methoxyethoxy)benzoyl)oxy)ethyl 1-(benzylsulfonyl)piperidine-2-carboxylate (*S*-**5t**) was obtained as a colourless oil (0.56 g, 93 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.32-7.45 (m, 5H), 7.19 (d, 2H, *J* = 2.4 Hz), 6.72 (t, 1H, *J* = 2.4 Hz), 4.42-4.61 (m, 5H), 4.24 (s, 2H), 4.08-4.14 (m, 4H), 3.71-3.75 (m, 4H), 3.40-3.48 (m, 7H), 3.13 (ddd, 1H, *J* = 12.8, 12.8, 3.0 Hz),

2.11-2.18 (m, 1H), 1.50-1.69 (m, 3H), 1.34-1.47 (m, 1H), 1.14-1.28 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.3, 166.0, 159.8 (2C), 131.3, 131.0 (2C), 129.3, 128.5 (3C), 108.2 (2C), 107.0, 70.8 (2C), 67.6 (2C), 62.9, 62.6, 59.2 (2C), 58.8, 55.9, 43.4, 27.8, 25.0, 20.3; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3065, 3034, 2936, 2885, 1719, 1593, 1444, 1336, 1299, 1171, 1122, 1063, 738, 698 (m); HPLC purity: 96 % (method II); ESI-MS: m/z 580.3 $[M+H]^+$.

4.2.6.9. (*S*)-2-(3,5-Bis(2-methoxyethoxy)benzamido)ethyl 1-(benzylsulfonyl)piperidine-2-carboxylate (*S*-**5u**) was obtained as a colourless oil (0.14 g, 86 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.35-7.42 (m, 5H), 7.01-7.06 (m, 3H), 6.66 (t, 1H, *J* = 2.3 Hz), 4.43 (ddd, 1H, *J* = 11.1, 6.0, 3.9), 4.14-4.28 (m, 8H), 3.72-3.79 (m, 6H), 3.44 (s, 6H), 3.28-3.35 (m, 1H), 3.07 (ddd, 1H, *J* = 12.8, 12.8, 2.9 Hz), 2.08-2.15 (m, 1H), 1.54-1.60 (m, 2H), 1.30-1.47 (m, 2H), 1.09-1.24 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.2, 167.1, 159.9 (2C), 136.0, 130.8 (2C), 129.1, 128.8, 128.6 (2C), 105.7, 105.7, 70.9 (2C), 67.5 (2C), 64.7, 59.2 (2C), 58.9, 56.5, 44.0, 38.8, 27.1, 24.6, 20.1; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3364, 3062, 3034, 2930, 2882, 1737, 1656, 1590, 1496, 1441, 1323, 1171, 1148, 1121, 1059, 739, 697 (m); HPLC purity: 97 % (method II); ESI-MS: m/z 579.6 $[M+H]^+$.

4.2.6.10. (*S*)-(*S*)-1-(Nicotinamido)propan-2-yl 1-(benzylsulfonyl)piperidine-2-carboxylate (*S*-**5v**) was obtained as a colourless oil (0.06 g, 65 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.12 (dd, 1H, *J* = 2.3, 0.8 Hz), 8.70 (dd, 1H, *J* = 4.8, 1.7 Hz), 8.18 (ddd, 1H, *J* = 8.0, 2.3, 1.7 Hz), 7.36-7.44 (m, 6H), 7.32 (ddd, 1H, *J* = 8.0, 4.8, 0.8 Hz), 5.14 (dq, 1H, *J* = 6.4, 2.7 Hz), 4.25 (s, 2H), 4.07 (d, 1H, *J* = 3.0 Hz), 3.80 (ddd, 1H, *J* = 14.3, 6.4, 2.7 Hz), 3.57 (ddd, 1H, *J* = 14.3, 6.9, 6.0), 3.27-3.35 (m, 1H), 3.09 (ddd, 1H, *J* = 12.9, 13.0, 2.7 Hz), 2.09-2.18 (m, 1H), 1.56-1.66 (m, 2H), 1.22-1.41 (m, 5H), 0.99-1.16 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.7, 166.0, 152.0, 148.8, 135.3, 130.7 (2C), 129.7, 128.9, 128.7 (2C), 123.1, 72.2, 59.3, 56.9, 44.2, 43.5, 27.6, 25.0, 20.1, 17.2; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3362, 3062, 3034, 2935, 2860, 1732, 1655, 1591, 1537, 1496, 1455, 1321, 1198, 1179, 1147, 1124, 738, 698 (s); HPLC purity: 98 % (method I); ESI-MS: m/z 446.4 $[M+H]^+$.

4.2.6.11. (*S*)-(*R*)-1-(Nicotinamido)propan-2-yl 1-(benzylsulfonyl)piperidine-2-carboxylate (*S*-**5w**) was obtained as a colourless oil (0.43 g, 70 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.11 (dd, 1H, *J* = 2.3, 0.8 Hz), 8.71 (dd, 1H, *J* = 4.8, 1.7 Hz), 8.17 (ddd, 1H, *J* = 8.0, 2.3, 1.7 Hz), 7.35-7.41 (m, 5H), 7.22 (t, 1H, *J* = 5.0 Hz), 7.33 (ddd, 1H, *J* = 8.0, 4.8, 0.8 Hz), 5.06-5.15 (m, 1H), 4.19-4.25 (m, 3H), 3.65-3.77 (m, 2H), 3.23-3.31 (m, 1H), 3.04 (ddd, 1H, *J* = 12.5, 12.7, 2.8 Hz), 2.03-2.10 (m, 1H), 1.54-1.64 (m, 2H), 1.22-1.46 (m, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.7, 166.0, 152.2, 148.7, 135.2, 130.8 (2C), 129.7, 129.0, 128.8 (2C), 128.6, 123.2, 71.7, 59.0, 56.5, 44.2, 44.1, 26.7, 24.6, 19.8, 17.0; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3349, 3062, 3032, 2939, 2860, 1733, 1651, 1592, 1538, 1455, 1321, 1198, 1179, 1147, 1125, 738, 698 (s); HPLC purity: > 99 % (method I); ESI-MS: m/z 446.4 $[M+H]^+$.

4.2.6.12. (*S*)-(*S*)-1-(3,4,5-Trimethoxybenzamido)propan-2-yl 1-(benzylsulfonyl)piperidine-2-carboxylate (*S*-**5x**) was obtained as a white powder (0.40 g, 81 %); mp 67-70 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.33-7.42 (m, 5H), 7.13-7.18 (m, 3H), 5.14 (dq, 1H, *J* = 6.4, 2.7 Hz), 4.22 (AB-spin system, 1H, J_{AB} = 14.0 Hz), 4.19 (AB-spin system, 1H, J_{AB} = 14.0 Hz), 4.11 (d, 1H, *J* = 4.0 Hz), 3.90 (s, 6H), 3.89 (s, 3H), 3.80 (ddd, 1H, *J* = 14.3, 5.0, 2.6 Hz), 3.58 (ddd, 1H, *J* = 14.3, 6.9, 6.0 Hz), 3.27-3.34 (m, 1H), 3.12 (ddd, 1H *J* = 12.9, 13.0, 2.9 Hz), 2.14-2.21 (m, 1H), 1.60-1.69 (m, 1H), 1.29-1.44 (m, 6H), 1.03-1.15 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.6, 167.0, 153.0 (2C), 140.8, 130.7 (2C), 129.3, 129.0, 128.8, 128.7 (2C), 104.7 (2C), 72.6, 60.9, 59.3, 56.9, 56.2 (2C), 44.3, 43.7, 27.1, 24.6, 20.1, 17.3; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3392, 3062, 2943, 2861, 1732, 1650, 1583, 1497, 1333, 1180, 1122, 764, 698 (m); HPLC purity: > 99 % (method II); ESI-MS: m/z 535.4 $[M+H]^+$.

4.2.6.13. (*S*)-2-(4-Hydroxy-3-methoxybenzamido)ethyl 1-(benzylsulfonyl)piperidine-2-carboxylate (*S*-**5y**) was obtained as a white powder (0.05 g, 37 %); mp 74-79 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.47 (d, 1H, *J* = 2.0 Hz), 7.36-7.41 (m, 6H), 7.02 (t, 1H, *J* = 5.6 Hz), 6.90 (d, 1H, *J* = 8.3 Hz), 5.94 (s, 1H), 4.45 (ddd, 1H, *J* = 11.2, 6.4, 3.4), 4.17-4.29 (m, 4H), 3.92 (s, 3H), 3.68-3.85 (m, 2H), 3.27-3.36 (m, 1H), 3.08

(ddd, 1H, *J* = 12.7, 12.7, 3.1 Hz), 2.09-2.18 (m, 1H), 1.30-1.69 (m, 4H), 1.10-1.28 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 176.0, 171.2, 148.7, 146.3, 130.8 (2C), 129.0, 128.9, 128.7 (2C), 126.2, 120.7, 113.9, 110.4, 64.9, 59.1, 56.6, 56.1, 44.1, 38.8, 27.1, 24.6, 20.1; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3397, 2942, 2857, 1736, 1636, 1590, 1505, 1379, 1282, 1176, 1124, 737, 697 (m); HPLC purity: 97 % (method II); ESI-MS: m/z 447.4 $[M+H]^+$.

4.2.7. (*S*)-1-(Benzylsulfonyl)-*N*-isopropylpiperidine-2-carboxamide (*S*-**5z**) According to a procedure of Flynn *et al*.[37] (*S*)-1-(*tert*-butoxycarbonyl)piperidine-2-carboxylic acid (1 equiv.) was dissolved in 20 mL anhydrous CH_2Cl_2 and at 0 °C isopropylamine (1.0 equiv.), EDC·HCl (1.3 equiv.), and HOBt (0.2 equiv.) were added. After the reaction was completed (TLC control) the organic layer was washed twice with water and the solvent was evaporated *in vacuo*. The boc protection group was cleaved with 2 mL trifluoroacetic acid in 20 mL CH_2Cl_2. After 24 h the solution was neutralized with saturated $NaHCO_3$ and extracted with 3 x 30 mL of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated to yield compound **8z** which was used in the next step without further purification. Compound **8z** (1 equiv.) was dissolved in 40 mL of anhydrous CH_2Cl_2, and DIPEA (3 equiv.) was added at 0 °C followed by phenylmethanesulfonyl chloride (1 equiv.), respectively. The mixture was stirred until completion (TLC control) and subsequently the solvent was removed *in vacuo*. After purification by means of flash chromatography compound *S*-**5z** was obtained as a colourless oil (0.20 g, 53 %); mp 75-77 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.37-7.47 (m, 5H), 6.11 (d, *J* = 6.8 Hz), 4.29 (s, 2H), 4.22 (d, 1H, *J* = 5.1 Hz), 4.00-4.15 (m, 1H), 3.56-3.64 (m, 1H), 3.01 (ddd, 1H, *J* = 13.6, 13.3, 2.9 Hz), 2.18-2.26 (m, 1H), 1.20-1.65 (m, 5H), 1.14 (t, 6H, *J* = 6.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 168.7, 130.7 (2C), 128.9, 128.8 (3C), 58.7, 56.4, 43.7, 41.7, 25.8, 24.5, 22.7, 22.5, 19.8; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3424, 3065, 2942, 2868, 1665, 1516, 1455, 1369, 1325, 1173, 1131, 741, 701 (m); HPLC purity: 97 % (method II); ESI-MS: m/z 325.3 $[M+H]^+$.

4.2.8. General Procedure for the Synthesis of Compounds 10q-u. To obtain the alcohol derivatives, an esterification or amidation with the corresponding benzoic acid (1 equiv.) was carried out in 20 mL anhydrous CH_2Cl_2 at 0 °C using the corresponding alcohol or amine (1.0 equiv.), EDC·HCl (1.5 equiv.), and DMAP or HOBt (0.2 equiv.). After the reaction was completed (TLC control) a subsequent purification by flash-chromatography was performed to obtain compounds **10q**-**u**.

4.2.8.1. 2-Hydroxyethyl nicotinate (**10q**) was obtained as a colourless oil (0.62 g, 91 %). Spectroscopical data have already been described.[38]

4.2.8.2. *N*-(2-Hydroxyethyl)nicotinamide (**10r**) was obtained as a colourless oil (0.31 g, 77 %). Spectroscopical data have already been described.[39]

4.2.8.3. 2-(Pyridin-3-yloxy)ethanol (**10s**) was obtained as a yellow oil (0.15 g, 21 %). Spectroscopical have already been described.[40]

4.2.8.4. 2-Hydroxyethyl 3,5-bis(2-methoxyethoxy)benzoate (**10t**) was obtained as a colourless oil (0.43 g, 80 %). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.23 (d, 2H, *J* = 2.4 Hz), 6.74 (t, 1H, *J* = 2.4 Hz), 4.41-4.46 (m, 2H), 4.11-4.16 (m, 4H), 3.92-3.96 (m, 2H), 3.72-3.77 (m, 4H), 3.45 (s, 6H), 1.88 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 166.6, 159.8 (2C), 131.6, 108.3 (2C), 106.9, 70.8 (2C), 67.6 (2C), 66.8, 61.4, 59.2 (2C); IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3437 (br), 3097, 2929, 2822, 1715, 1593, 1299, 1169, 1062, 860.

4.2.8.5. *N*-(2-Hydroxyethyl)-3,5-bis(2-methoxyethoxy)benzamide (**10u**) was obtained as a colourless oil (0.33 g, 81 %). ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 8.36 (t, 1H, *J* = 5.4 Hz), 7.01 (d, 2H, *J* = 2.3 Hz), 6.65 (t, 1H, *J* = 2.3 Hz), 4.70, (t, 1H, *J* = 4.7 Hz), 4.09-4.14 (m, 4H), 3.63-3.68 (m, 4H), 3.47-3.52 (m, 2H), 3.27-3.33 (m, 8H); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 165.6, 159.3 (2C), 136.5, 105.7 (2C), 103.7, 70.2

(2C), 67.1 (2C), 59.6 (2C), 58.1, 42.1; IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3351, 3094, 2928, 2876, 1641, 1737, 1589, 1441, 1161, 1116, 1053, 844.

4.3. Biological assays

The activity of the compounds was determined by an enzyme coupled PPIase assay as described previously.[30, 41] In brief, 150 μM of the peptide substrate (i.e., succinamide-Ala-Phe-Pro-Phe-*p*-nitroalanine; Bachem, Bubendorf, Switzerland) dissolved in 35 mM HEPES buffer (pH = 8.0) was incubated for 6 min with 50 nM BpMip. Chymotrypsin was added to obtain a final concentration of 2.5 mg/mL in a final volume of 200 μL at 8 °C. The absorbance at λ = 390 nm was measured over 15 min; a blank determination at λ = 595 nm was subtracted, measured using an Optima plate reader (BMG Labtech GmbH, Ortenberg, Germany). Data were fitted to a first-order reaction, and inhibition constants were fit to the Morrison tight binding inhibition equation, using GraphPad v6.0.2. A detailed description is currently under review (Vivoli *et al*., in preparation).

Cytotoxicity. Cell viability was measured as described before.[42] Briefly, the compounds were dissolved in DMSO to a concentration of 20 mM and serially diluted in DMSO. 1 x 10^5 cells per mL of the J774.1 murine macrophage cell line (ATCC) were incubated in a volume of 200 µl in 96 well cell culture plates in the respective medium without phenol red with serial compound dilutions at 37 °C and 5 % CO_2. The final concentration of DMSO was 1 %. After 24 h of incubation, 10 % of an AlamarBlue solution was added. The IC_{50} value was calculated with respect to negative controls, i.e. without compounds, from the absorbance values measured at λ=550 nm using a microplate reader (Multiskan Ascent, Thermo Fisher Scientific, Braunschweig, Germany). The reference wavelength was set to λ = 630 nm.

4.4. Computational methods

Hot spot analyses for identification of yet unaddressed interactions were conducted using GRID[32] (version 22c). The 3D grids of the binding pockets were investigated via different hydrophobic (CH_3,

F, Cl) and hydrophilic (phenolic/carboxylic hydroxyl group, carbonylic oxygen) probes, using a mesh size of 0.2 Å and default settings for other parameters. Visualization and evaluation was conducted in PyMOL[43].

Docking calculations were carried out with GOLD[44] (docking suite version 5.2.2). *Protein and Ligand setup*: Structural data of protein-ligand complexes were retrieved from the Protein Data Bank[45] (PDB): LpMip bound to Rapamycin (PDB code: 2VCD[22]) and BpMip bound to CJ168 (equivalent to the lead structure **CJ168**, resynthesized here as *S*-**5a**, as shown in Figure 1 and Table 1) (PDB code: 4G50[31]). For 2VCD, conformer 4 out of the ensemble of 16 NMR structures was selected as described previously[30]; in case of 4G50, chain A was used for all docking calculations. The preparation of the proteins was carried out in MOE[46] (version 13.08): Alternate locations were selected after visual inspection, missing atoms were added and the system was protonated using Protonate3D[47] with default settings at pH = 7.4. Water and ligand molecules were deleted. Furthermore, after docking **CJ168** to LpMip, the thus received complex was minimized using the Amber12:EHT force field[48, 49] implemented in MOE with an RMS gradient of 0.1 kcal/mol/Å² to allow adaptations of the binding pocket to the sulfonamide scaffold. New ligand molecules derived from **CJ168** were set up manually in MOE (all as *S*-enantiomers) and energetically minimized using the implemented MMFF94s[50] force field applying an RMS gradient of 0.0001 kcal/mol/Å².

Docking Setup: For both proteins the binding region was defined as a sphere of 12.5 Å radius centered at the side chain of the central tryptophan residue in the MIP binding pocket (Trp86 in LpMip and Trp66 in BpMip, respectively). 50 poses were generated for each ligand with automatic "very flexible" settings, thus making the number of operations dependent on the properties of each ligand. Furthermore, a weak constraint was set to facilitate the saturation of the Ile63/83 backbone NH as hydrogen-bond donor in both proteins and reduce the number of docking results largely deviating from the prototypical binding mode and/or unspecifically sticking to the surface. ChemPLP[51] and Goldscore[52] implemented in GOLD were used as scoring functions, revealing

ChemPLP as the superior function in terms of the agreement with the subsequently determined binding affinities; results obtained with Goldscore are, hence, not further discussed here. All generated poses were rescored with DSX[53] using the potentials derived from the Cambridge Structural Database[54]. The best pose (as reported in Table S2 of the Supplementary Material) was then selected via a consensus scoring approach by rank considering both the ChemPLP and DSX ranks after visual inspection in PyMOL[43] to eliminate occasional misplaced but well-scored poses in which the central sulfonamide is flipped with respect to the crystal structure (4G50). This docking protocol was validated by redocking analyses using known crystal structures, as described in the Supplementary Material (Table S1 and associated discussion).

4.5. HSQC NMR experiments

The HSQC NMR experiments were performed as described in Juli et al. ref. 30.

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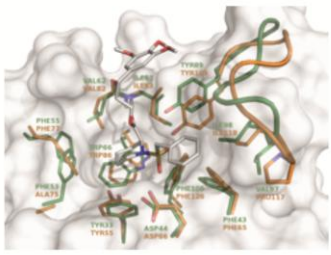
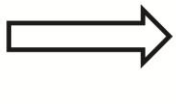
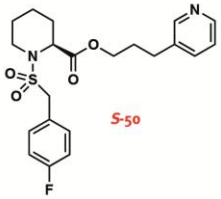
Graphical abstract



L. pneumophila/*B. pseudomallei*
Macrophage Infectivity Potentiator Protein





comp.	cytotoxicity (J774.1) IC_{50} [µM]	*B. pseudomallei* Mip IC_{50} [µM]	*L. pneumophila* Mip IC_{50} [µM]
S-50	› 100	0.072 ± 0.03	5.7 ± 0.8
S-5r	› 100	0.42 ± 0.18	11.4 ± 1.7