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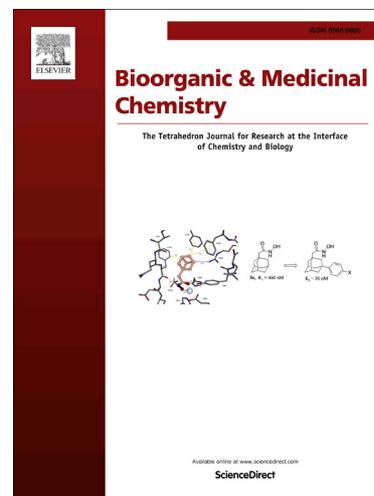
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**Synthesis and biological evaluation of spirocyclic antagonists of CCR2
(chemokine CC receptor subtype 2)**

Ann Kathrin Strunz,^[a] Annelien J.M. Zweemer,^[b] Christina Weiss,^[c] Dirk Schepmann,^[a] Anna Junker,^[a] Laura Heitman,^[b] Michael Koch,^[c] Bernhard Wünsch*^[a,d]

^[a] Institut für Pharmazeutische und Medizinische Chemie, Westfälische Wilhelms-Universität Münster, Corrensstraße 48, 48149 Münster, Germany.

Tel.: +49-251-8333311; Fax: +49-251-8332144; E-mail: wuensch@uni-muenster.de

^[b] Leiden Academic Centre for Drug Research (LACDR), Leiden University, Division of Medicinal Chemistry, Einsteinweg 55, 2333 CC Leiden, The Netherlands.

^[c] Bayer Pharma AG, Global Drug Discovery - Lead Discovery Wuppertal, Aprather Weg 18a, Gebäude 456, D-42096 Wuppertal, Germany.

^[d] Cells-in-Motion Cluster of Excellence ((EXC 1003 – CiM), Westfälische Wilhelms-Universität Münster, Germany.

Abstract

Activation of chemokine CC receptors subtype 2 (CCR2) plays an important role in chronic inflammatory processes such as atherosclerosis, multiple sclerosis and rheumatoid arthritis. A diverse set of spirocyclic butanamides **4** (N-benzyl-4-(3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamides) was prepared by different combination of spirocyclic piperidines **8** (3,4-dihydrospiro[[2]benzopyran-1,4'-piperidines]) and γ -halobutanamides **11**. A key step in the synthesis of spirocyclic piperidines **8** was an Oxa-Pictet-Spengler reaction of β -phenylethanols **5** with piperidone acetal **6**. The substituted γ -hydroxybutanamides **11c-e** were prepared by

hydroxyethylation of methyl acetates **13** with ethylene sulfate giving the γ -lactones **14c** and **14e**. Aminolysis of the γ -lactones **14c** and **14e** with benzylamines provided the γ -hydroxybutanamides **15c-e**, which were converted into the bromides **11c-e** by an *Appel* reaction using polymer-bound PPh_3 . In radioligand binding assays the spirocyclic butanamides **4** did not displace the iodinated radioligand ^{125}I -CCL2 from the human CCR2. However, in the Ca^{2+} -flux assay using human CCR2 strong antagonistic activity of butanamides **4** was detected. Analysis of the IC_{50} -values led to clear relationships between the structure and the inhibition of the Ca^{2+} -flux. **4g** (4-(3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)-N-[3,5-bis(trifluoromethylbenzyl)]-2-(4-fluorophenyl)butanamide) and **4o** (N-[3,5-bis(trifluoromethyl)benzyl]-2-cyclopropyl-4-(3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide) represent the most potent CCR2 antagonists with IC_{50} -values of 89 nM and 17 nM, respectively. Micromolar activities were found in the β -arrestin recruitment assay with murine CCR2, but the structure-activity-relationships detected in the Ca^{2+} -flux assay were confirmed.

Keywords

Chemokine CC receptor subtype 2 (CCR2) antagonists; Oxa-Pictet-Spengler reaction; ethylene sulfate; Appel reaction; polymer-bound triphenylphosphine; spirocyclic piperidines; receptor binding studies; Ca^{2+} flux; β -arrestin recruitment; structure-activity-relationships

1. Introduction

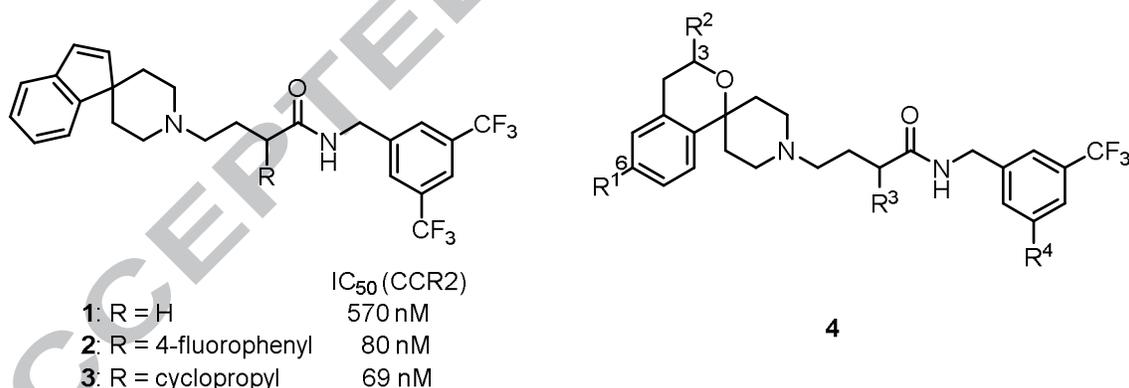
The class of chemokines (= chemotactic cytokines) consists of several chemoattractant proteins with 70 – 130 amino acids (8 - 14 kDa). Depending on the position of four conserved cysteine residues forming disulfide bonds, chemokines are divided into two major (CC and CXC chemokines) and two minor (C and CX₃C chemokines) groups. These chemokines interact with the corresponding CC, CXC, C, and CX₃C receptors belonging to class A (rhodopsin class) of G protein-coupled receptors. Chemokines and their receptors form a network, regulating the activation and migration of immune cells in the organism.¹ During the last years 26 chemokine receptors and more than 45 endogenous ligands have been identified.² Among those, the chemokine CC receptor subtype 2 (CCR2), mainly expressed on monocytes, T lymphocytes, dendritic cells and endothelial cells, is of high interest as a key target in the therapy of chronic inflammatory diseases including atherosclerosis, asthma, Morbus Crohn, rheumatoid arthritis, and multiple sclerosis.³

In the therapy of atherosclerosis CCR2 has become a promising target because of the interaction with its selective endogenous ligand monocyte chemoattractant protein 1 (MCP1). In addition to MCP1 CCR2 binds CCL7, CCL8, and CCL13 as agonists, whereas CCL11 and CCL26 are antagonists at CCR2.⁴ MCP1, systematically termed CCL2, plays an important role in the recruitment of monocytes from the blood into the subendothelial tissue, which is known to be an early key step in the formation of atherosclerotic plaques. Mechanic injury and toxins cause lesions of the arterial wall and lead to migration of monocytes, mediated by several adhesion molecules and chemokine receptors. In the arterial wall monocytes develop to macrophages, which turn into foam cells by the uptake of blood lipids.⁵⁻⁷ Advanced plaques become unstable and can suddenly rupture. Plaques release their content to the blood,

resulting in platelet aggregation and occlusion of the affected artery. On a long-term basis stroke, myocardial infarction and thrombosis can occur as serious complications.

Since the late 1990s, CCR2 antagonists with diverse structural elements have been reported.⁸⁻¹⁰ In 2006 Butora et al. published a new series of promising spiro[indene-1,4'-piperidines] (Figure 1). Whereas the butanamide **1** without a substituent at the linear alkyl chain shows an IC₅₀ value of 570 nM, the introduction of a *p*-fluorophenyl- (**2**) or cyclopropyl moiety (**3**) in α -position of the central butanamide increased the CCR2 affinity considerably. In case of α -substituted butanamides **2** and **3** (*S*)-configured enantiomers seem to show higher CCR2 affinity than their (*R*)-enantiomers.¹¹

Figure 1. Spirocyclic CCR2 antagonists **1-3** serve as lead compounds.



Starting from the lead compounds **1-3** we developed a new series of CCR2 antagonists **4** based on the spiro[[2]benzopyran-1,4'-piperidine] system. The O-atom within the ring system of **4** together with the substituent in 3-position should increase the polarity of the rather lipophilic spiro[indene-piperidines] **1-3**. Moreover the

synthetic strategy allows the introduction of various substituents in the 2-benzopyran system. In particular the introduction of a phenolic hydroxy moiety in position 6 is envisaged resulting in a precursor for the development of [^{11}C]- and [^{18}F]-labeled tracers for positron emission tomography (PET). In addition to variations in the 2-benzopyran system, *p*-fluorophenyl and cyclopropyl substituents should be introduced into the α -position of the butanamide (R^3) and fluoro and trifluoromethyl groups (R^4) were planned as substituents of the benzyl ring.¹¹⁻¹³

2. Synthesis

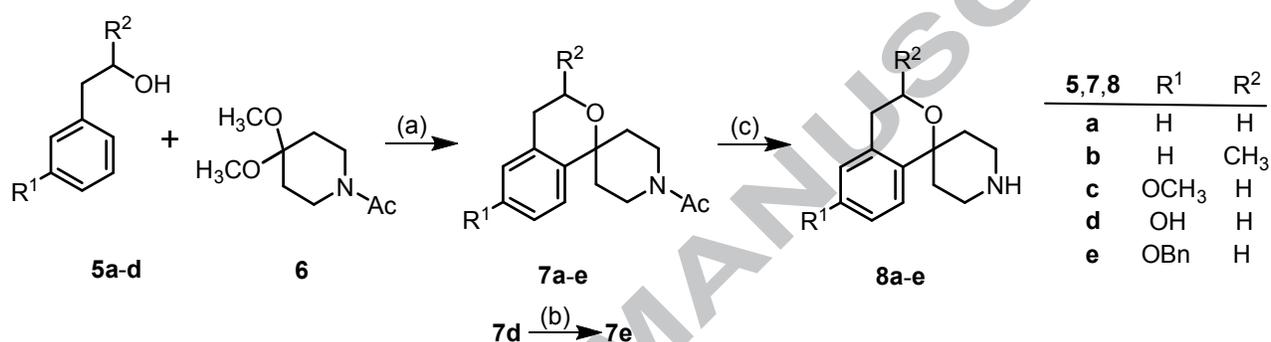
For the synthesis of the designed spirocyclic butanamides **4** a building block system was envisaged. At first the spirocyclic piperidines **8** and the γ -halobutanamides **11** were prepared and subsequently these building blocks were combined to generate differently substituted CCR2 ligands **4**.

The key step in the synthesis of the spirocyclic piperidines **8** was an Oxa-Pictet-Spengler reaction.¹⁴ Since 1-acetylpiperidin-4-one did not react with β -phenylethanol **5a**, the dimethyl acetal **6** was employed. *p*-Toluenesulfonic acid in boiling acetonitrile did not catalyze the reaction of phenylethanol **5a** with dimethyl acetal **6**, so that the Lewis acid $\text{BF}_3 \cdot \text{Et}_2\text{O}$ was used. At room temperature the transformation stopped at the intermediate mixed acetal. However microwave irradiation of the reaction mixture and addition of $\text{Bi}(\text{OTf})_3$ provided the spirocyclic piperidines **7a-d** in 24-64 % yields.¹⁵

(Scheme 1)

For the connection of the spirocyclic building blocks with the γ -halobutanamide building blocks **11** the secondary amines **8** were prepared by hydrolysis of the

acetamides **7** with NaOH. Whereas the secondary amines **8a-c** were isolated in 82-99 % yields, the hydroxy substituted derivative **8d** was isolated in only 12 % yield. The low yield of **8d** was due to incomplete extraction of the zwitterionic aminophenol from the water layer during work-up. Therefore the phenol **7d** was protected with a benzyl protective group (**7e**) before hydrolysis, which should be removed at the end of the synthesis. Hydrolysis of the acetamide **7e** with NaOH in dioxane led to a clean conversion and the benzyl ether **8e** was isolated in 34 % yield.



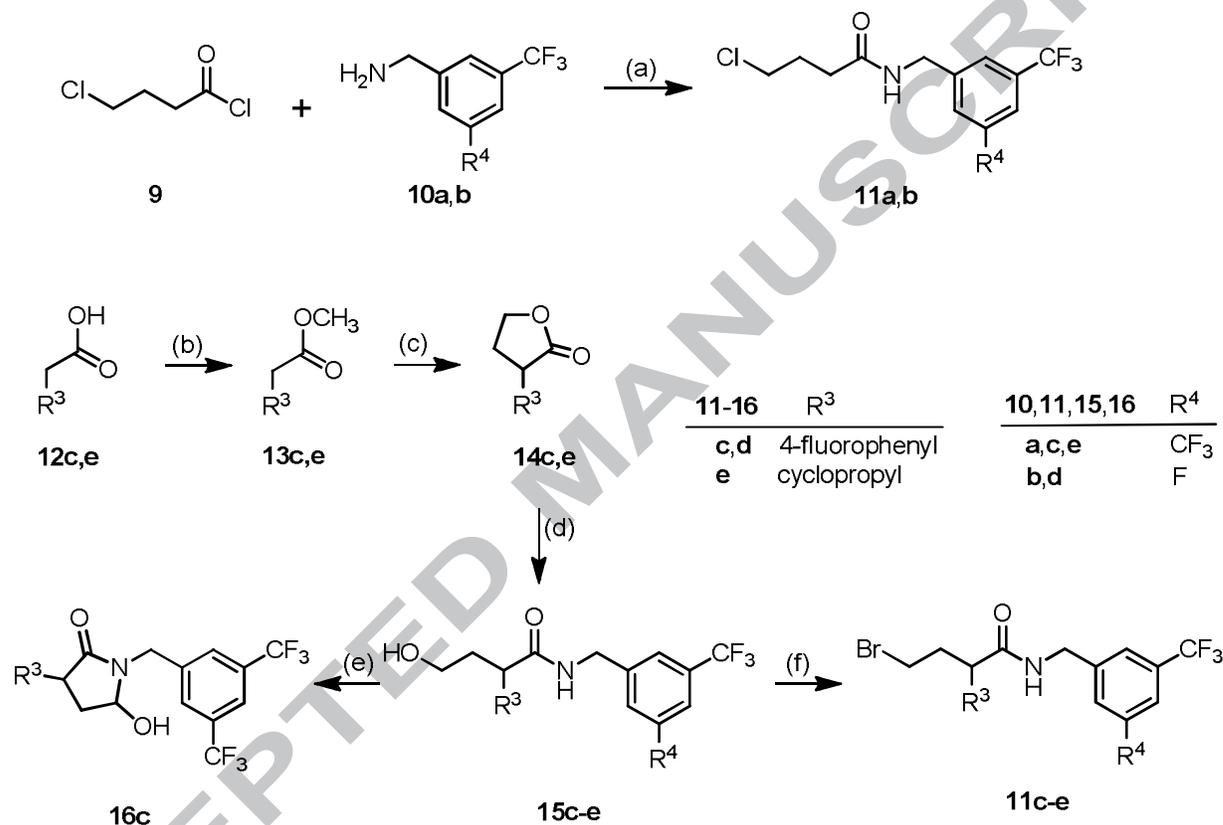
Scheme 1: Synthesis of the spirocyclic piperidines **8a-e**.

Reagents and reaction conditions: (a) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, $\text{Bi}(\text{OTf})_3$, CH_3CN , microwave irradiation 2 h, 150 °C, 400 W, 24-64 %. (b) Benzyl bromide, K_2CO_3 , CH_3CN , reflux, 16 h, 60 %. (c) NaOH 2 M, reflux, 16 h, **8a-c** 82-99 %; **8d** 12 %; NaOH 2 M, dioxane, reflux, 16 h, **8e** 34 %.

The γ -chlorobutanamides **11a** and **11b** without a further substituent in α -position of the butanoyl chain were obtained by acylation of benzylamines **10a** and **10b** with γ -chlorobutanoyl chloride (**9**).¹⁶ (Scheme 2)

The synthesis of the butanamides **11c** and **11d** with an α -(4-fluorophenyl) substituent started with hydroxyethylation of ester **13c**, which was obtained by esterification of the acid **12c** with $\text{CH}_3\text{OH}/\text{H}_2\text{SO}_4$. For this purpose ester **13c** was deprotonated with LiHMDS and the enolate was then trapped with ethylene sulfate.

After hydrolysis of the resulting monoester of sulfuric acid with NaOH, γ -lactone **14c** was isolated in 52 % yield. The cyclopropyl substituted γ -lactone **14e** was prepared in the same manner starting with cyclopropylacetic acid (**12e**). In order to achieve high yields the volatility of the γ -lactone **14e** during evaporation of solvents has to be taken into account.



Scheme 2: Synthesis of γ -halobutanamides **11a-e**.

Reagents and reaction conditions: (a) Na₂CO₃, CH₃CN, rt, 16 h, 89-96 %. (b) conc. H₂SO₄, CH₃OH, reflux, 16 h, 45-75 %. (c) 1. LiHMDS, ethylene sulfate, THF, -15 °C, 2 h; 2. ethanolic NaOH, reflux, 16 h, 52-59 %. (d) **10a,b**, AlCl₃, CH₂Cl₂, 0 °C → rt, 16 h, **15c,d** 23 %; **10a**, 1,2,4-triazole, diazabicycloundecene, CDCl₃, rt, 48 h, **15e** 7.1 %. (e) Dess-Martin-Periodinane, CH₂Cl₂, rt, 1 h, 73 %. (f) CBr₄, polymer-bound triphenylphosphine, CH₃CN, 0 °C → rt, 48 h, 73-99 %.

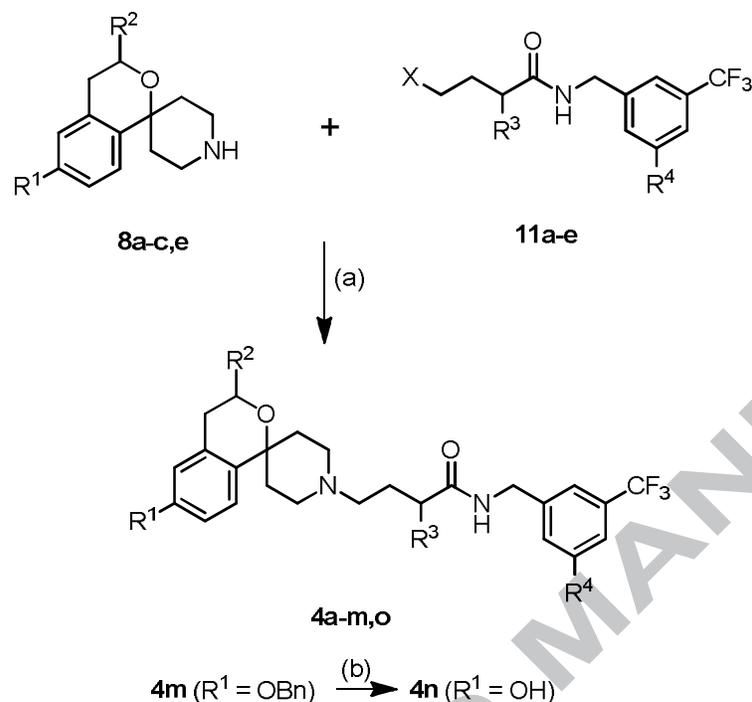
The aminolysis of the γ -lactone **14c** with benzylamines **10a,b** turned out to be very problematic, since the transformations were not complete. However after addition of

the Lewis acid AlCl_3 the butanamides **15c** and **15d** were isolated in pure form. For the aminolysis of the cyclopropyl substituted γ -lactone **14e** with benzylamine **10a** the acyl transfer catalyst 1,2,4-triazole had to be added to obtain the butanamide **15e**.¹⁷

Unexpectedly all attempts to activate the alcohols **15c-e** with methanesulfonyl chloride or *p*-toluenesulfonyl chloride failed to give the corresponding sulfonates. Therefore the alcohol **15c** was oxidized with Dess-Martin-Periodinane. Instead of the expected aldehyde the cyclic hemiaminal **16c** was obtained in 73 % yield. The same product has already been obtained by ozonolysis of the corresponding allyl derivative.¹⁸ However, the hemiaminal did not react with the spirocyclic piperidines **8** under reductive amination conditions ($\text{NaBH}(\text{OAc})_3$) via opening of the hemiaminal. Therefore activation of the alcohols **15c-e** by conversion into the bromides **11c-e** was considered next. The *Appel* reaction of the alcohol **15c** with CBr_4 and PPh_3 led to a clean conversion. The ^1H NMR spectrum of the non-purified bromide **11c** indicated complete and clean conversion. However all attempts to remove PPh_3 and/or PPh_3O from the bromide **11c** or from the alkylation product **4g** failed. Therefore polymer-bound PPh_3 was used instead of soluble PPh_3 in the *Appel* reaction. Simple filtration provided the pure bromides **11c-e** in 73-99 % yields.

In the final step the piperidines **8** were alkylated with the γ -halobutanamides **11** to give a diverse set of spirocyclic butanamides **4**. In order to optimize the conversion various reaction conditions were applied for different combinations (Scheme 3). The benzyl protective group of the benzyl ether **4m** was removed hydrogenolytically to afford the phenol **4n**. According to our standard HPLC method the purity of the final test compounds had to be higher than 95 %. To achieve this quality criterion different

purification methods including preparative HPLC had to be used. The extensive purification procedures reduced the yields.



Scheme 3: Synthesis of final test compounds **4a-o**.

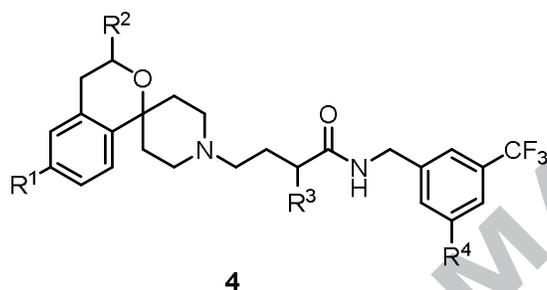
Reagents and reaction conditions: (a) K_2CO_3 , THF, reflux, 16 h, **4a-c,e,f** 3.5-43 %; THF, 0 °C \rightarrow 40 °C, 48 h, **4d,g-i** 3-44 %; diisopropylethylamine, BuNI, DMF, microwave irradiation, 1 h, 203 °C, 150 W, **4m,o** 27-34 %. (b) H_2 , Pd/C, 1 bar, CH_3OH , 1 h, 48%. Definition of the residues R^1 - R^4 is given in Table 1.

3. CCR2 affinity and antagonistic activity

The CCR2 affinity of the spirocyclic butanamides **4** was determined in radioligand displacement assays with membranes of U2OS cells stably expressing the human CCR2 (U2OS-CCR2) and the iodinated endogenous agonist ^{125}I -CCL2 as radioligand.¹⁹ Moreover the antagonistic activity of the compounds **4** at the CCR2 was analyzed in two complementary functional assays. An intracellular Ca^{2+} -flux assay employing the Chem-1 cell line stably transfected with human CCR2B and

recombinant human CCL2 was performed. Inhibition of β -arrestin recruitment was determined using the U2OS β -arrestin cell line transfected with murine CCR2. It should be noted that human and murine CCR2 share 80 % sequence identity.²⁰ This species difference accounts for different effects in the Ca^{2+} -flux and β -arrestin recruitment assay. The CCR2 affinities and the CCR2 antagonistic activities of the spirocyclic compounds **4** are summarized in Table 1.

Table 1: CCR2 affinity and antagonistic activity of spirocyclic butanamides **4**.



compd.	R ¹	R ²	R ³	R ⁴	¹²⁵ I-CCL2 displacement [%] ^a	IC ₅₀ human CCR2 [μM] ^b (Ca ²⁺ flux)	IC ₅₀ murine CCR2 [μM] ^b (β -arrestin)
4a	H	H	H	CF ₃	10	0.684	24
4b	H	CH ₃	H	CF ₃	-12	7.54	7.9
4c	OCH ₃	H	H	CF ₃	1	1.24	18
4d	H	H	H	F	4	0.18	>30
4e	H	CH ₃	H	F	-10	12.0	>30
4f	OCH ₃	H	H	F	-1	0.318	>30
4g (WMS-46-12)	H	H	4-F-C ₆ H ₄	CF ₃	25	0.089	11.2
4h	H	CH ₃	4-F-C ₆ H ₄	CF ₃	0	0.462	3.67
4i (WMS-46-09)	OCH ₃	H	4-F-C ₆ H ₄	CF ₃	nd	0.163	4.16
4j	H	H	4-F-C ₆ H ₄	F	7	0.173	3.63
4k	H	CH ₃	4-F-C ₆ H ₄	F	9	1.60	8.01
4l	OCH ₃	H	4-F-C ₆ H ₄	F	-7	0.353	4.63

4n	OH	H	4-F-C ₆ H ₄	F	nd	6.10	>30
4o (WMS-46-14)	H	H	c-C ₃ H ₅	CF ₃	nd	0.017	3.3
TAK779					K _i = 2.0 ± 0.7 nM ^c	0.95 nM	23

^a displacement of the radioligand ¹²⁵I-CCL2 from human CCR2 [%], measured at a concentration of 1 μM of the test compound.

^b The IC₅₀-values in the functional Ca²⁺-flux assay (human CCR2) and the β-arrestin recruitment assay (murine CCR2) are given in μM, respectively. In the Ca²⁺-flux assay CCL2 was added in 5 nM concentration, in the β-arrestin recruitment assay the CCL2 concentration was 3 nM.

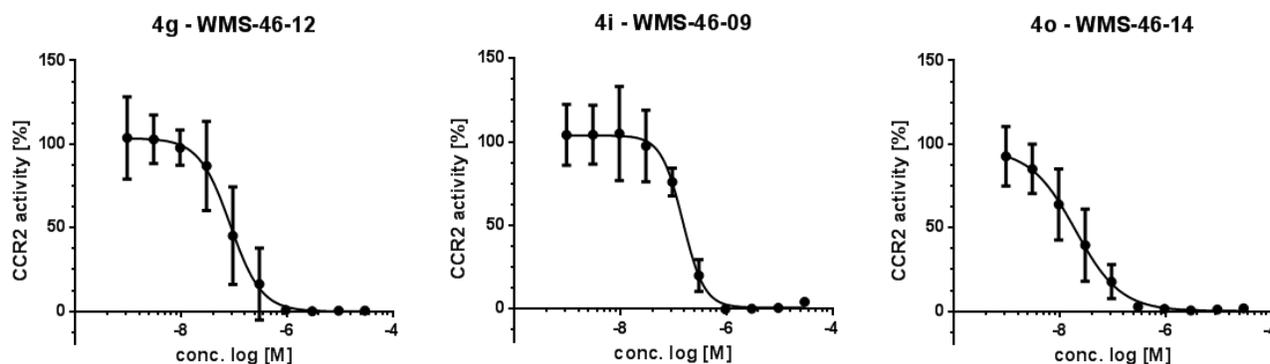
^c For TAK779 the exact K_i-value was determined. In literature an IC₅₀-value of 27 nM is given for TAK779.^{21,22}

nd = not determined.

At a concentration of 1 μM the test compounds **4** could not significantly displace the radioligand ¹²⁵I-CCL2 (conc. 0.1 nM) from human CCR2.

However, in the Ca²⁺-flux assay considerable effects of the spirocyclic butanamides **4** were detected, which allows the discussion of structure-activity relationships. Concerning the substitution pattern of the 2-benzopyran system, the compounds **4a**, **4d**, **4g**, and **4j** without a further substituent show the highest activity, respectively. A methoxy moiety in position 6 leads to slight reduction of the activity (compare **4d/4f**, **4g/4i**), whereas a methyl moiety in 3-position reduces the activity considerably (compare **4d/4e**, **4g/4h**).

Figure 2: Dose response curves of the most potent CCR2 antagonists in the Ca^{2+} -flux assay.



Introduction of the 4-fluorophenyl moiety into the butanamide chain increased the antagonistic activity remarkably (compare **4a-f** with **4g-l**). A cyclopropyl ring in α -position to the amide (compound **4o**) led to a further increase in the CCR2 antagonistic activity. (Table 1, Figure 2)

Compounds with two CF_3 -groups at the benzyl moiety show generally higher inhibition of Ca^{2+} -flux than their analogs with one CF_3 moiety and one fluorine atom ((compare **4g-4i** with **4j-4l**).

The phenol **4n** does not inhibit the Ca^{2+} -flux. However the corresponding methyl ether **4l** showed a promising IC_{50} -value of 353 nM indicating that modification of the phenolic OH group could result in potent CCR2 antagonists. In particular the introduction of fluoroalkyl substituents with the aim to develop a fluorinated PET tracer is considered.

Finally the 4-fluorophenyl and the cyclopropyl derivatives **4g** and **4o** without further substituents in the 2-benzopyran system and with two CF_3 -moieties at the

benzylamine part represent the most potent ligands of this series of compounds with IC₅₀-values of 89 and 17 nM, respectively. Figure 2 shows full inhibitory activity of **4g**, **4i** and **4o** in the Ca²⁺-flux assay.

In the β -arrestin recruitment assay using murine CCR2 IC₅₀-values in the micromolar range were found. The most potent antagonists in this assay are the 4-fluorophenyl substituted derivatives **4h**, **4i**, **4j**, and **4l** and the cyclopropyl derivative **4o** with IC₅₀-values between 3 and 5 μ M. Although the IC₅₀-values in this assay are considerably higher than in the Ca²⁺-flux assay, the tendency of increased antagonistic activity of 4-fluorophenyl and, moreover, cyclopropyl derivatives is confirmed herein. With exception of **4g**, the IC₅₀-values of bis(trifluoromethyl)benzylamides are lower than the IC₅₀-values of the corresponding 3-fluoro-5-(trifluoromethyl)benzyl analogs.

4. Conclusion

A diverse set of spirocyclic butanamides **4** has been synthesized by combination of various building blocks **8** and **11**. Whereas the spirocyclic butanamides **4** cannot compete with the radioligand ¹²⁵I-CCL2, the Ca²⁺-flux was inhibited depending on the structure and concentration of the ligands. It is assumed that the spirocyclic butanamides **4** are able to interact with the human CCR2 protein and inhibit the intracellular mobilization of Ca²⁺-ions. Interestingly, this class of compounds does not disturb the interaction of the human CCR2 with its large endogenous ligand CCL2, but inhibits the Ca²⁺ flux. The inhibition of the murine CCR2 coupled with β -arrestin recruitment is much lower compared with inhibition of the human CCR2. It is assumed that the different sequences of the receptor proteins are responsible for the different effects of the ligands. In previous studies¹⁹ it has been shown that different small molecule CCR2 inhibitors interact with different binding sites (orthosteric and

allosteric binding sites) resulting in different modes of inhibition. Therefore it is postulated that the CCR2 antagonists of type **4** block the Ca^{2+} -flux via interaction with an allosteric binding site, whilst not disturbing the interaction of the large endogenous ligand CCL2. Altogether, the described compounds represent a novel class of negative allosteric modulators of CCR2.

5. Experimental

5.1. Chemistry

5.1.1. General

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. Acetonitrile and dimethylformamide were dried over molecular sieves. CH_2Cl_2 was distilled from CaH_2 , methanol was distilled from magnesium methanolate and tetrahydrofuran was distilled from sodium/benzophenone. Thin layer chromatography (tlc): Silica gel 60 F254 plates (Merck). Flash chromatography (fc): Silica gel 60, 40–64 μm (Merck); parentheses include: diameter of the column, length, fraction size, R_f value, eluent. Melting point: Melting point apparatus SMP3 (Stuart Scientific), uncorrected. Microwave assisted reactions were carried out in a single mode cavity CEM Discover LabMate Synthesiser with Discover-PC-Software (CEM Corporation) or a multi mode system MARS (CEM Corporation). MS: microTOF-Q II (Bruker Daltonics); APCI, atmospheric pressure chemical ionization. IR: FT-IR-480 plus (Jasco) or FT-IR Prestige 21 (Shimadzu) equipped with ATR technique. Nuclear magnetic resonance (NMR) spectra were recorded on Agilent 600-MR (600 MHz for ^1H , 151 MHz for ^{13}C), Agilent 400-MR spectrometer (400 MHz for ^1H , 101 MHz for ^{13}C) or Varian AS 400 Mercury Plus NMR spectrometer; δ in ppm related to tetramethylsilane and measured referring to CDCl_3 ($\delta = 7.26$ ppm (^1H NMR) and $\delta = 77.2$ ppm (^{13}C NMR)) and CD_3OD ($\delta = 3.31$ ppm (^1H NMR) and $\delta = 49.0$ ppm (^{13}C

NMR)); coupling constants are given with 0.5 Hz resolution. Analytical HPLC: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; interface: D-7000; column: LiChrospher[®] 60 RP-select B (5 μ m); LiChroCART[®] 250-4 mm cartridge; flow rate: 1.0 mL/min; injection volume: 5.0 μ L; detection at λ = 210 nm; solvents: A: water with 0.05 % (v/v) trifluoroacetic acid; B: acetonitrile with 0.05 % (v/v) trifluoroacetic acid: gradient elution: (A %): 0-4 min: 90%, 4-29 min: 90 \rightarrow 0%, 29-31 min: 0%, 31-31.5 min: 0 \rightarrow 90 %, 31.5-40 min: 90%. The purity of all compounds was determined by this method. The purity of all test compounds is higher than 95 %. Preparative HPLC: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7150; interface: D-7000; column: Phenomenex Gemini C18 110 A 250-21.2 mm (5 μ m; flow rate: 20.00 mL/min; injection volume: 200.0 μ L; detection at λ = 254 nm; solvent: acetonitrile:water 70:30 with 0.05 % (v/v) NH₃.

5.1.2. 1-(4,4-Dimethoxypiperidin-1-yl)ethan-1-one (6)²³

1-Acetylpiperidin-4-one (8.52 g, 60 mmol) was dissolved in CH₃OH (12 mL). Trimethyl orthoformate (32.34 g, 0.3 mol) and *p*-toluenesulfonic acid (521 mg, 3.0 mmol) were added quickly and the solution was stirred overnight at rt. The transformation was terminated by addition of 10 % aqueous NaHCO₃ and the mixture was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and concentrated under reduced pressure. The crude product was used in the next reaction step without further purification. Pale yellow oil, yield 11.0 g (98 %). C₉H₁₇NO₃ (187.2 g/mol). MS (APCI): *m/z* = 188.1291 (calcd. 188.1281 for C₉H₁₈NO₃ [MH⁺]). ¹H NMR (DMSO-d₆): δ [ppm] = 1.57 (t, *J* = 5.8 Hz, 2H, N(CH₂CH₂)₂), 1.67 (t, *J* = 5.8 Hz, 2H, N(CH₂CH₂)₂), 1.99 (s, 3H, NCOCH₃), 3.10 (s, 6H, OCH₃), 3.34 – 3.42 (m, 4H, N(CH₂CH₂)₂). ¹³C NMR (DMSO-d₆): δ [ppm] = 21.7 (1C, CH₃), 32.3 (1C, N(CH₂CH₂)₂), 33.1 (1C, N(CH₂CH₂)₂), 38.4 (1C, N(CH₂CH₂)₂), 43.3 (1C,

$N(CH_2CH_2)_2$, 47.6 (2C, OCH₃), 98.5 (1C, C(OCH₃)₂), 168.5 (1C, C=O). IR: $\tilde{\nu}$ [cm⁻¹] = 2943 (C-H), 1639 (C=O), 1107, 1045 (C-O).

5.1.3. 1-(3,4-Dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)ethan-1-one (7a)

2-Phenylethanol **5a** (2.48 g, 20.3 mmol) was dissolved in CH₃CN (40 mL) under nitrogen flow and the solution was given into 8 microwave vials, 5 mL each. A solution of piperidone acetal **6** (4.49 g, 24.0 mmol) in CH₃CN (3.2 mL) was prepared. 0.4 mL of this solution were added to each mixture and the mixtures were stirred at rt for 30 min. BF₃·Et₂O (1.8 mL) was added to each vial dropwise under ice cooling and the mixtures were stirred at rt overnight. After addition of Bi(OTf)₃ (16 mg, 0.02 mmol) to each vial, the reaction mixtures were heated under microwave irradiation for 120 min at 150 °C and 400 W, respectively. The transformation was terminated by addition of 10 % aqueous NaHCO₃ to the combined mixtures. Layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3x). Combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The crude product was coated on silica gel and then purified by flash column chromatography (Ø 8 cm, length 12 cm, cyclohexane:EtOAc 1:4 → EtOAc, fraction size 65 mL, R_f = 0.19 (CH₂Cl₂:MetOH 96:4)). The isolated product was recrystallized from diisopropyl ether. Colorless solid, mp 152 °C, yield 1.53 g (31 %). Purity (HPLC): 96.4 %, t_R = 16.95 min. C₁₅H₁₉NO₂ (245.3 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.88 (dd, J = 11.0 / 4.6 Hz, 2H, N(CH₂CH₂)₂), 1.99 (td, J = 13.3 / 4.7 Hz, 2H, N(CH₂CH₂)₂), 2.15 (s, 3H, NCOCH₃), 2.82 (t, J = 5.5 Hz, 2H, PhCH₂CH₂), 3.01 (td, J = 12.0 / 4.9 Hz, 1H, N(CH₂CH₂)₂), 3.51 (td, J = 13.1 / 3.2 Hz, 1H, N(CH₂CH₂)₂), 3.76 – 3.82 (m, 1H, N(CH₂CH₂)₂), 3.93 (t, J = 5.5 Hz, 2H, PhCH₂CH₂), 4.41 – 4.48 (m, 1H, N(CH₂CH₂)₂), 7.08 – 7.19 (m, 4H, H_{arom}). ¹³C NMR (CD₃OD): δ [ppm] = 19.8 (1C, COCH₃), 29.1 (1C, PhCH₂CH₂), 35.9 (1C, N(CH₂CH₂)₂), 36.6 (1C, N(CH₂CH₂)₂), 37.5

(1C, N(CH₂CH₂)₂), 42.4 (1C, N(CH₂CH₂)₂), 58.8 (1C, PhCH₂CH₂), 72.9 (1C, ArCO), 124.9 (1C, C-8_{arom}), 125.96 and 126.04 (2C, C-5_{arom}, C-6_{arom}), 128.5 (1C, C-7_{arom}), 133.5 (1C, C-4_{arom}), 140.8 (1C, C-8_{arom}), 170.1 (1C, C=O). MS (APCI): m/z = 246.1487 (calcd. 246.1489 for C₁₅H₂₀NO₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 1643 (C=O), 1088 (C-O), 760, 694 (C-H_{arom}).

5.1.4. 1-(3-Methyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)ethanone (7b)

1-Phenylpropan-2-ol **5b** (2.80 g, 20.4 mmol) was dissolved in CH₃CN (40 mL) under nitrogen flow and the solution was given into 8 microwave vials, 5 mL each. Acetal **6** (4.62 g, 24.7 mmol) was dissolved in CH₃CN (3.2 mL). 0.4 mL of this solution were added to each mixture and the mixtures were stirred at rt for 30 min. BF₃·Et₂O (1.8 mL) was added dropwise to each vial under ice cooling and the mixture was stirred at rt overnight. The reaction mixtures were heated under microwave irradiation for 120 min at 150 °C and 400 W, respectively. The transformation was terminated by addition of 10 % aqueous NaHCO₃ to the combined mixtures. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The crude product was coated on silica gel and then purified by flash column chromatography (Ø 8 cm, length 17 cm, cyclohexane: EtOAc 20:80 → EtOAc, fraction size 65 mL, R_f = 0.18 (CH₂Cl₂: CH₃OH 96:4)). Colorless solid, mp 128 °C, yield 4.42 g (64 %). Purity (HPLC): 98.6 %, t_R = 18.43 min. C₁₆H₂₁NO₂ (259.3 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.35 (d, *J* = 6.1 Hz, 3x 0.5 H, CH₃), 1.35 (d, *J* = 6.1 Hz, 3x 0.5 H, CH₃), 1.61 – 1.76 (m, 1H, N(CH₂CH₂)₂), 1.83 (td, *J* = 13.8 / 4.6 Hz, 1H, N(CH₂CH₂)₂), 2.03 (td, *J* = 13.3 / 4.9 Hz, 1H, N(CH₂CH₂)₂), 2.08 – 2.15 (m, 1H, N(CH₂CH₂)₂), 2.16 (s, 3H, NCOCH₃), 2.67 – 2.70 (m, 2H, PhCH₂CH), 3.01 (td, *J* =

13.1 / 2.9 Hz, 0.5 H, N(CH₂CH₂)₂), 3.10 (td, *J* = 13.1 / 2.9 Hz, 0.5 H, N(CH₂CH₂)₂), 3.52 (td, *J* = 13.2 / 2.8 Hz, 0.5 H, N(CH₂CH₂)₂), 3.62 (td, *J* = 13.1 / 2.8 Hz, 0.5 H, N(CH₂CH₂)₂), 3.75 – 3.84 (m, 1H, N(CH₂CH₂)₂), 3.89 – 3.99 (m, 1H, PhCH₂CH), 4.40 – 4.49 (m, 1H, N(CH₂CH₂)₂), 7.05 – 7.21 (m, 4H, CH_{arom}). ¹³C NMR (CD₃OD): δ [ppm] = 19.83 (0.5 C, COCH₃), 19.85 (0.5 C, COCH₃), 20.62 (0.5 C, CH₃), 20.63 (0.5 C, CH₃), 29.3 (1C, CH₂CHO), 34.0 and 34.6 (1C, N(CH₂CH₂)₂), 37.4 and 37.6 (1C, N(CH₂CH₂)₂), 38.4 and 39.1 (1C, N(CH₂CH₂)₂), 42.3 and 42.4 (1C, N(CH₂CH₂)₂), 64.3 and 64.4 (1C, CH₂CHO), 73.60 and 73.62 (1C, ArCO), 124.65 and 124.66 (1C, C-8_{arom}), 125.92 and 125.93 (2C, C-5_{arom}, C-6_{arom}), 128.36 and 128.37 (1C, C-7_{arom}), 133.7 (1C, C-4_{arom}), 140.56 and 140.57 (1C, C-8_{arom}), 170.09 and 170.13 (1C, C=O). MS (APCI): *m/z* = 260.1634 (calcd. 260.1645 for C₁₆H₂₂NO₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 1628 (C=O), 1064 (C-O), 756, 702 (C-H_{arom}).

5.1.5. 1-(6-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin-1'-yl])ethan-1-one (7c)

2-(3-Methoxyphenyl)ethanol **5a** (3.16 g, 20.8 mmol) was dissolved in CH₃CN (40 mL) under nitrogen flow and the solution was given into 8 microwave vials, 5 mL each. A solution of piperidone acetal **6** (4.57 g, 24.4 mmol) in CH₃CN (3.2 mL) was prepared. 0.4 mL of this solution were added to each mixture and the mixtures were stirred at rt for 30 min. BF₃·Et₂O (1.8 mL) was added to each vial dropwise under ice cooling and the mixtures were stirred at rt overnight. After addition of Bi(OTf)₃ (16 mg, 0.02 mmol) to each vial, reaction mixtures were heated under microwave irradiation for 120 min at 150 °C and 400 W, respectively. The transformation was terminated by addition of 10 % aqueous NaHCO₃ to the combined mixtures. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed under

reduced pressure. The crude product was coated on silica gel and then purified by flash column chromatography (\varnothing 8 cm, length 15 cm, cyclohexane:EtOAc 20:80, fraction size 65 mL, R_f = 0.20 (CH₂Cl₂:CH₃OH 96:4)). The isolated product was recrystallized from iPr₂O. Colorless solid, mp 110 °C, yield 1.56 g (27 %). Purity (HPLC): 93.7 %, t_R = 17.74 min. C₁₆H₂₁NO₃ (275.3 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.82 – 1.99 (m, 4H, N(CH₂CH₂)₂), 2.15 (s, 3H, NCOCH₃), 2.79 (t, J = 5.5 Hz, 2H, PhCH₂CH₂), 2.99 (td, J = 12.4 / 4.6 Hz, 1H, N(CH₂CH₂)₂), 3.51 (td, J = 12.7 / 3.4 Hz, 1H, N(CH₂CH₂)₂), 3.76 (s, 3H, OCH₃), 3.92 (t, J = 5.5 Hz, 2H, PhCH₂CH₂), 4.14 – 4.19 (m, 1H, N(CH₂CH₂)₂), 4.41 - 4.47 (m, 1H, N(CH₂CH₂)₂), 6.66 (d, J = 2.7 Hz, 1H, 5-*H*_{arom}), 6.75 (dd, J = 8.6 / 2.8 Hz, 1H, 7-*H*_{arom}), 7.05 (d, J = 8.7 Hz, 1H, 8-*H*_{arom}). ¹³C NMR (CD₃OD): δ [ppm] = 19.8 (1C, COCH₃), 29.4 (1C, CH₂CH₂O), 36.0 (1C, N(CH₂CH₂)₂), 36.8 (1C, N(CH₂CH₂)₂), 37.5 (1C, N(CH₂CH₂)₂), 42.4 (1C, N(CH₂CH₂)₂), 54.2 (1C, OCH₃), 58.7 (1C, CH₂CH₂O), 72.7 (1C, ArCO), 112.3 (1C, C-7_{arom}), 112.8 (1C, C-5_{arom}), 126.1 (1C, C-8_{arom}), 133.0 (1C, C-4_{arom}), 134.9 (1C, C-8_{arom}), 158.0 (1C, C-6_{arom}), 170.1 (1C, C=O). MS (APCI): m/z = 276.1610 (calcd. 276.1594 for C₁₆H₂₂NO₃ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 1640 (C=O), 1501 (OCH₃), 1085 (C-O), 822, 683 (C-H_{arom}).

5.1.6. 1-(6-Hydroxy-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]]-1'-yl)ethanone (7d)

2-(3-Hydroxyphenyl)ethanol **5d** (2.23 g, 16.2 mmol) was dissolved in CH₃CN (40 mL) under nitrogen flow and the solution was given into 8 microwave vials, 5 mL each. A solution of piperidone acetal **6** (3.70 g, 19.8 mmol) in CH₃CN (3.2 mL) was prepared. 0.4 mL of this solution were added to each mixture and the mixtures were stirred at rt for 30 min. BF₃·Et₂O (1.8 mL) was added to each vial dropwise under ice cooling and the mixture was stirred at rt overnight. After addition of Bi(OTf)₃ (16 mg, 0.02 mmol) to

each vial, the reaction mixtures were heated under microwave irradiation for 120 min at 150 °C and 400 W, respectively. Then 10 % aqueous NaHCO₃ was added to the combined mixtures. The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The crude product was coated on silica gel and then purified by flash column chromatography (Ø 8 cm, length 10 cm, cyclohexane:EtOAc 20:80 → EtOAc:CH₃OH 80:20, fraction size 65 ml, R_f = 0.1 (CH₂Cl₂:CH₃OH 96:4)). The isolated product was recrystallized from diisopropyl ether. Colorless solid, mp 239 °C, yield 1.01 g (24 %). Purity (HPLC): 92.6 %, t_R = 14.57 min. C₁₅H₁₉NO₃ (261.3 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.76 – 2.02 (m, 4H, N(CH₂CH₂)₂), 2.14 (s, 3H, NCOCH₃), 2.73 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 2.98 (td, *J* = 12.4 / 4.1 Hz, 1H, N(CH₂CH₂)₂), 3.50 (td, *J* = 12.9 / 3.7 Hz, 1H, N(CH₂CH₂)₂), 3.73 – 3.80 (m, 1H, N(CH₂CH₂)₂), 3.89 (t, *J* = 5.3 Hz, 2H, PhCH₂CH₂), 4.38 – 4.46 (m, 1H, N(CH₂CH₂)₂), 6.49 – 6.53 (m, 1H, 5-*H*_{arom}), 6.62 (dd, *J* = 8.5 / 2.5 Hz, 1H, 7-*H*_{arom}), 6.94 (d, *J* = 8.5 Hz, 1H, 8-*H*_{arom}), a signal for the OH proton is not seen in the spectrum. ¹³C NMR (CD₃OD): δ [ppm] = 19.8 (1C, NCOCH₃), 29.3 (1C, PhCH₂CH₂), 36.1 (1C, N(CH₂CH₂)₂), 36.8 (1C, N(CH₂CH₂)₂), 37.6 (1C, N(CH₂CH₂)₂), 42.4 (1C, N(CH₂CH₂)₂), 58.7 (1C, PhCH₂CH₂), 72.7 (1C, ArCO), 113.4 (1C, C-7_{arom}), 114.3 (1C, C-5_{arom}), 126.0 (1C, C-8_{arom}), 131.8 (1C, C-4_{arom}), 134.8 (1C, C-8_{aarom}), 155.3 (1C, C-6_{arom}), 170.1 (1C, C=O). MS (APCI): *m/z* = 262.1433 (calcd. 262.1438 for C₁₅H₂₀NO₃ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2978 (C-H), 1585 (C=O), 1450 (O-H), 1088 (C-O).

5.1.7. 1-[6-(Benzyloxy)-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl]ethan-1-one (7e)

7d (499 mg, 1.9 mmol) and benzyl bromide (479 mg, 2.8 mmol) were dissolved in CH₃CN (50 mL). After addition of K₂CO₃ (1.08 g, 7.8 mmol) the solution was heated to reflux overnight. Cooled to rt, K₂CO₃ was filtered off and CH₃CN was removed under reduced pressure. The residue was recrystallized from diisopropyl ether, R_f = 0.92, (CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5). Colorless solid, mp 183 °C, yield 443 mg (60 %). Purity (HPLC): 96.3 %, t_R = 22.50 min. C₂₂H₂₅NO₃ (351.4 g/mol). ¹H NMR (CDCl₃): δ [ppm] = 1.77 – 1.94 (m, 4H, N(CH₂CH₂)₂), 2.14 (s, 3H, NCOCH₃), 2.81 (q, J = 5.1 Hz, 2H, PhCH₂CH₂), 2.96 (td, J = 12.9 / 3.1 Hz, 1H, N(CH₂CH₂)₂), 3.51 (td, J = 12.8 / 3.3 Hz, 1H, N(CH₂CH₂)₂), 3.62 – 3.70 (m, 1H, N(CH₂CH₂)₂), 3.91 (t, J = 5.4 Hz, 2H, PhCH₂CH₂), 4.50 – 4.60 (m, 1H, N(CH₂CH₂)₂), 5.03 (s, 1H, PhCH₂O), 5.05 (s, 1H, PhCH₂O), 6.71 (d, J = 1.8 Hz, 1H, 5-H_{arom}), 6.84 (td, J = 8.6 / 2.7 Hz, 1H, 7-H_{arom}), 6.97 (d, J = 8.7 Hz, 1H, 8-H_{arom}), 7.32 – 7.44 (m, 5H, H'_{arom}). ¹³C NMR (CDCl₃): δ [ppm] = 21.9 (1C, NCOCH₃), 29.8 (1C, PhCH₂CH₂), 36.3 (1C, N(CH₂CH₂)₂), 37.5 (1C, N(CH₂CH₂)₂), 37.8 (1C, N(CH₂CH₂)₂), 42.6 (1C, N(CH₂CH₂)₂), 59.0 (1C, PhCH₂CH₂), 70.0 (1C, PhCH₂O), 72.9 (1C, ArCO), 113.4 (1C, C-7_{arom}), 114.4 (1C, C-5_{arom}), 126.4 (1C, C-8_{arom}), 127.4 and 128.6 (4C, C-2'_{arom}, C-3'_{arom}, C-5'_{arom}, C-6'_{arom}), 133.5 (1C, C-4_{aarom}), 135.1 (1C, C-8_{aarom}), 128.0 and 136.9 (2C, C-1'_{arom}, C-4'_{arom}), 157.1 (1C, C-6_{arom}), 168.9 (1C, C=O). MS (APCI): m/z = 352.1918 (calcd. 352.1907 for C₂₂H₂₆NO₃ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2928 (C-H), 1632 (C=O), 1119 (C-O), 764, 694 (C-H_{arom}).

5.1.8. 3,4-Dihydrospiro[[2]benzopyran-1,4'-piperidine] (8a)

A solution of **7a** (619 mg, 2.5 mmol) in 2 M NaOH (40 mL) was heated to reflux overnight. The reaction mixture was cooled to rt and extracted with CH₂Cl₂ (3x). The

combined organic layers were dried (Na_2SO_4), filtered and the filtrate was concentrated under reduced pressure. The product was used in the next reaction step without further purification, $R_f = 0.22$ (CH_2Cl_2 :MeOH 70:30 + 1% ethyldimethylamine). Colorless solid, mp 95 °C, yield 418 mg (82 %). Purity (HPLC): 97.0 %, $t_R = 12.03$ min. $\text{C}_{13}\text{H}_{17}\text{NO}$ (203.3 g/mol). ^1H NMR (CD_3OD): δ [ppm] = 1.84 (dd, $J = 15.7 / 1.8$ Hz, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 1.93 (td, $J = 12.6 / 4.6$ Hz, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.80 (t, $J = 5.6$ Hz, 2H, PhCH_2CH_2), 2.86 (dd, $J = 11.9 / 4.1$ Hz, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 3.04 (td, $J = 12.5 / 3.1$ Hz, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 3.91 (t, $J = 5.5$ Hz, 2H, PhCH_2CH_2), 7.07 – 7.20 (m, 4H, H_{arom}), a signal for the NH proton is not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 29.1 (1C, PhCH_2CH_2), 35.4 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 40.8 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 58.7 (1C, PhCH_2CH_2), 72.5 (1C, ArCO), 124.8 (1C, $C_{8\text{arom}}$), 126.0 and 126.1 (2C, $C_{5\text{arom}}$, $C_{6\text{arom}}$), 128.5 (1C, $C_{7\text{arom}}$), 133.4 (1C, $C_{4\text{arom}}$), 141.1 (1C, $C_{8\text{arom}}$). MS (APCI): $m/z = 204.1422$ (calcd. 204.1383 for $\text{C}_{13}\text{H}_{18}\text{NO}$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 1085 (C-O), 760, 690 (C- H_{arom}).

5.1.9. 3-Methyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidine] (8b)

A solution of **7b** (503 mg, 1.9 mmol) in 2 M NaOH (33 mL) was heated to reflux overnight. The reaction mixture was cooled to rt and extracted with CH_2Cl_2 (3x). The combined organic layers were dried (Na_2SO_4), filtered and the filtrate was concentrated under reduced pressure. The crude product was used in the next reaction step without further purification, $R_f = 0.11$ (CH_2Cl_2 : CH_3OH 70:30 + 1% ethyldimethylamine). Colorless solid, mp 99 °C, yield 421 mg (99 %). Purity (HPLC): 97.6 %, $t_R = 14.49$ min. $\text{C}_{14}\text{H}_{19}\text{NO}$ (217.3 g/mol). ^1H NMR (CD_3OD): δ (ppm) = 1.32 (d, $J = 6.1$ Hz, 3H, CH_3), 1.58 (dd, $J = 13.8 / 2.5$ Hz, 1H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 1.74 (td, $J = 14.1 / 4.1$ Hz, 1H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.02 (dd, $J = 14.7 / 2.0$ Hz, 1H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.09 (td, $J = 13.3 / 4.7$ Hz, 1H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.58 – 2.68 (m, 2H, PhCH_2CH), 2.80 – 2.88

(m, 2H, N(CH₂CH₂)₂), 3.02 (td, *J* = 12.6 / 2.8 Hz, 1H, N(CH₂CH₂)₂), 3.12 (td, *J* = 12.6 / 2.9 Hz, 1H, N(CH₂CH₂)₂), 3.84 – 3.92 (m, 1H, PhCH₂CH), 7.02 – 7.22 (m, 4H, CH_{arom}), a signal for the NH proton is not seen in the spectrum. ¹³C NMR (CD₃OD): δ [ppm] = 20.7 (1C, CH₃), 30.2 (1C, CH₂CHO), 34.3 (1C, N(CH₂CH₂)₂), 35.5 (0.5C, N(CH₂CH₂)₂), 36.7 (0.5C, N(CH₂CH₂)₂), 38.8 (1C, N(CH₂CH₂)₂), 41.0 (0.5C, N(CH₂CH₂)₂), 41.2 (0.5C, N(CH₂CH₂)₂), 64.0 (1C, CH₂CHO), 73.7 (1C, ArCO), 124.8 (1C, C-8_{arom}), 125.85 and 125.88 (2C, C-5_{arom}, C-6_{arom}), 128.3 (1C, C-7_{arom}), 133.6 (1C, C-4_{arom}), 141.6 (1C, C-8_{arom}). MS (APCI): 218.1535 (calcd. 218.1539 for C₁₄H₂₀NO [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2924 (C-H), 1643 (C=O), 1064 (C-O), 760 (C-H_{arom}).

5.1.10. 6-Methoxy-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidine] (8c)

A solution of **7c** (233 mg, 0.85 mmol) in 2 M NaOH (10 mL) was heated to reflux overnight. The reaction mixture was cooled to rt and extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the filtrate was concentrated under reduced pressure. The crude product was used in the next reaction step without further purification, R_f = 0.11 (CH₂Cl₂:CH₃OH 70:30 + 1% ethyldimethylamine). Colorless solid, mp 136 °C, yield 193 mg (98 %). C₁₄H₁₉NO₂ (233.3 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.86 (dd, *J* = 15.5 / 2.0 Hz, 2H, N(CH₂CH₂)₂), 1.95 (td, *J* = 13.9 / 4.4 Hz, 2H, N(CH₂CH₂)₂), 2.78 (t, *J* = 5.5 Hz, 2H, PhCH₂), 2.94 (dd, *J* = 12.2 / 3.4 Hz, 2H, N(CH₂CH₂)₂), 3.09 (td, *J* = 12.4 / 3.2 Hz, 2H, N(CH₂CH₂)₂), 3.76 (s, 3H, OCH₃), 3.89 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 6.66 (d, *J* = 2.7 Hz, 1H, 5-*H*_{arom}), 6.77 (dd, *J* = 8.7 / 2.7 Hz, 1H, 7-*H*_{arom}), 7.09 (d, *J* = 8.7 Hz, 1H, 8-*H*_{arom}), a signal for the NH proton is not seen in the spectrum. ¹³C NMR (CD₃OD): δ [ppm] = 29.4 (1C, PhCH₂CH₂O), 35.2 (1C, N(CH₂CH₂)₂), 38.2 (1C, N(CH₂CH₂)₂), 40.7 (2C, N(CH₂CH₂)₂), 54.2 (1C, OCH₃), 58.7 (1C, CH₂CH₂O), 72.0 (1C, ArCO), 112.4 (1C, C-7_{arom}), 112.8 (1C, C-5_{arom}), 125.9 (1C, C-8_{arom}), 134.8 (1C, C-8_{arom}), 158.0

(1C, C-6_{arom}), a signal for the C-atom of C-4 is not seen in the spectrum. MS (APCI): $m/z = 234.1529$ (calcd. 234.1489 for C₁₄H₂₀NO₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 1501 (OCH₃), 1085 (C-O), 810, 606 (C-H_{arom}).

5.1.11. 3,4-Dihydrospiro[[2]benzopyran-1,4'-piperidin]-6-ol (8d)

A solution of **7d** (391 mg, 1.5 mmol) in 2 M NaOH (25 mL) was heated to reflux overnight. The reaction mixture was cooled to rt. The pH value was adjusted to pH 8-10 and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were dried (Na₂SO₄), filtered and the filtrate was concentrated under reduced pressure. Colorless oil, yield 40 mg (12 %). C₁₃H₁₇NO₂ (219.3 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.77 - 1.96 (m, 4H, N(CH₂CH₂)₂), 2.71 (t, $J = 5.5$ Hz, 2H, PhCH₂CH₂), 2.88 (dd, $J = 11.2 / 3.4$ Hz, 2H, N(CH₂CH₂)₂), 3.05 (td, $J = 11.9 / 2.7$ Hz, 2H, N(CH₂CH₂)₂), 3.86 (t, $J = 5.5$ Hz, 2H, PhCH₂CH₂), 6.50 (d, $J = 1.9$ Hz, 1H, 5-*H*_{arom}), 6.58 - 6.68 (m, 1H, 7-*H*_{arom}), 6.98 (d, $J = 8.5$ Hz, 1H, 8-*H*_{arom}), a signal for the NH proton is not seen in the spectrum.

5.1.12. 6-(Benzyloxy)-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin] (8e)

7e (495 mg, 1.4 mmol) was suspended in dioxane (24 mL). 2 M NaOH (52 mL) was added and the mixture was heated to reflux overnight. The reaction mixture was then cooled to rt and extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the filtrate was concentrated under reduced pressure. The crude product was dissolved in CH₂Cl₂ and a saturated solution of HCl in Et₂O was added. Resulting crystals were filtered, washed and dissolved in water. The pH value was adjusted to pH 10 with 2 M NaOH and the aqueous layer was extracted with CH₂Cl₂ (3x). Combined organic layers were dried (Na₂SO₄), filtered and the filtrate was concentrated in vacuo, $R_f = 0.20$ (CH₂Cl₂: CH₃OH:NH₃ 95:4.5:0.5). Colorless

solid, mp 136 °C, yield 150.1 mg (34 %). Purity (HPLC): 95.6 %, t_R = 17.86 min. $C_{20}H_{23}NO_2$ (309.4 g/mol). 1H NMR ($CDCl_3$): δ [ppm] = 1.89 (d, broad, J = 12.5 Hz, 2H, $N(CH_2CH_2)_2$), 1.98 (td, J = 13.6 / 4.6 Hz, 2H, $N(CH_2CH_2)_2$), 2.80 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 2.89 (d, broad, J = 11.1 Hz, 2H, $N(CH_2CH_2)_2$), 3.13 (td, J = 12.1 / 2.8 Hz, 2H, $N(CH_2CH_2)_2$), 3.89 (t, J = 5.2 Hz, 2H, $PhCH_2CH_2$), 5.03 (s, 2H, $PhCH_2O$), 6.71 (d, J = 2.1 Hz, 1H, 5- H_{arom}), 6.85 (dd, J = 8.3 / 3.0 Hz, 1H, 7- H_{arom}), 7.11 (d, J = 8.7 Hz, 1H, 8- H_{arom}), 7.30 – 7.46 (m, 5H, H'_{arom}), a signal for the NH proton is not seen in the spectrum. ^{13}C NMR ($CDCl_3$): δ [ppm] = 30.1 (1C, $PhCH_2CH_2$), 37.8 (2C, $N(CH_2CH_2)_2$), 42.3 (2C, $N(CH_2CH_2)_2$), 58.8 (1C, $PhCH_2CH_2$), 70.1 (1C, $PhCH_2O$), 73.5 (1C, $ArCO$), 113.4 (1C, $C-7_{arom}$), 114.4 (1C, $C-5_{arom}$), 126.8 (1C, $C-8_{arom}$), 127.6 and 128.1 (4C, $C-2'_{arom}$, $C-3'_{arom}$, $C-5'_{arom}$, $C-6'_{arom}$), 135.07 and 135.12 (2C, $C-4a_{arom}$, $C-8a_{arom}$), 128.7 and 137.2 (2C, $C-1'_{arom}$, $C-4'_{arom}$), 157.0 (1C, $C-6_{arom}$). MS (APCI): m/z = 310.1801 (calcd. 310.1802 for $C_{20}H_{24}NO_2$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 2936 (C-H), 1088 (C-O), 737, 698 (C- H_{arom}).

5.1.13. N-[3,5-Bis(trifluoromethyl)benzyl]-4-chlorobutanamide (11a)

3,5-Bis(trifluoromethyl)benzylamine **10a** (172 mg, 0.7 mmol) and Na_2CO_3 (78 mg, 0.7 mmol) were dissolved in CH_3CN (5 ml) and 4-chlorobutanoyl chloride **9** (107 mg, 0.8 mmol) was added dropwise. The solution was stirred overnight at rt. The pH value was adjusted to pH 7 with saturated aqueous $NaHCO_3$ to stop the transformation and the mixture was extracted with EtOAc (3x). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The product was used in the next reaction step without further purification, R_f = 0.58 (cyclohexane:EtOAc 40:60). Colorless solid, mp 79 °C, yield 236 mg (96 %). Purity (HPLC): 95.6 %, t_R = 20.59 min. $C_{13}H_{12}ClF_6NO$ (347.7 g/mol). 1H NMR ($CDCl_3$): δ [ppm] = 2.16 (quint, J = 6.5 Hz, 2H, $ClCH_2CH_2CH_2$), 2.46 (t, J = 7.1 Hz, 2H, $ClCH_2CH_2CH_2$), 3.61 (t, J = 6.1

Hz, 2H, ClCH₂CH₂CH₂), 4.56 (d, $J = 6.0$ Hz, 2H, PhCH₂NH), 6.00 (s, broad, 1H, NH), 7.72 (s, 2H, 2-*H*_{arom}, 6-*H*_{arom}), 7.78 (s, 1H, 4-*H*_{arom}). ¹³C NMR (CDCl₃): δ [ppm] = 27.8 (1C, ClCH₂CH₂CH₂), 32.9 (1C, ClCH₂CH₂CH₂), 42.7 (1C, PhCH₂NH), 44.3 (1C, ClCH₂CH₂CH₂), 121.5 (hept, $J = 3.9$ Hz, 1C, C-4_{arom}), 123.1 (q, $J = 271.2$ Hz, 2C, CF₃), 127.6 (2C, C-2_{arom}, C-6_{arom}), 132.0 (q, $J = 33.5$ Hz, 2C, C-3_{arom}, C-5_{arom}), 141.0 (1C, C-1_{arom}), 171.9 (1C, C=O). MS (APCI): $m/z = 348.0594$ (calcd. 348.0584 for C₁₃H₁₃ClF₆NO [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 1644 (C=O), 1276 (C-F), 705, 681 (C-H_{arom}).

5.1.14. 4-Chloro-N-[3-fluoro-5-(trifluoromethyl)benzyl]butanamide (11b)

3-Fluoro-5-(trifluoromethyl)benzylamine **10b** (968 mg, 5.0 mmol) and Na₂CO₃ (551 mg, 5.2 mmol) were dissolved in CH₃CN (33 mL) and 4-chlorobutanoyl chloride **9** (707 mg, 5.0 mmol) was added dropwise. The solution was stirred overnight at rt. The pH value was adjusted to pH 7 with saturated aqueous NaHCO₃ to stop the transformation and the mixture was extracted with EtOAc (3x). The organic layers were combined, dried (Na₂SO₄), filtered and the solvent was evaporated in vacuum. A further purification of the crude product was not necessary, R_f = 0.58 (cyclohexane:EtOAc 40:60). Colorless solid, mp 60 °C, yield 1334 mg (89 %). Purity (HPLC): 90.8 %, t_R = 19.40 min. C₁₂H₁₂ClF₄NO (297.7 g/mol). ¹H NMR (CDCl₃): δ [ppm] = 2.15 (quint, $J = 6.6$ Hz, 2H, ClCH₂CH₂CH₂), 2.45 (t, 7.1 Hz, 2H, ClCH₂CH₂CH₂), 3.61 (t, $J = 6.1$ Hz, 2H, ClCH₂CH₂CH₂), 4.48 (d, $J = 6.2$ Hz, 2H, PhCH₂NH), 6.04 (s, broad, 1H, NH), 7.16 - 7.25 (m, 2H, 2-*H*_{arom}, 4-*H*_{arom}), 7.31 (s, 1H, 6-*H*_{arom}). ¹³C NMR (CDCl₃): δ [ppm] = 27.9 (1C, ClCH₂CH₂CH₂), 33.0 (1C, ClCH₂CH₂CH₂), 42.7 (d, $J = 1.5$, 1C, PhCH₂NH), 44.4 (1C, ClCH₂CH₂CH₂), 111.9 (dq, $J = 24.5 / 3.9$ Hz, 1C, C-4_{arom}), 118.0 (d, $J = 22.0$ Hz, 1C, C-2_{arom}), 119.9 (quint, $J = 3.6$ Hz, 1C, C-6_{arom}), 132.8 (qd, $J = 33.3 / 8.1$ Hz, 1C, C-5_{arom}), 142.3 (d, $J = 7.7$ Hz, 1C, C-1_{arom}), 162.6 (d, $J = 249.2$ Hz, 1C, C-3_{arom}), 171.8 (1C, C=O), the signal for

the C-atom of the CF_3 group is not seen in the spectrum. MS (APCI): $m/z = 298.0642$ (calcd. 298.0616 for $\text{C}_{12}\text{H}_{13}\text{ClF}_4\text{NO}$ $[\text{MH}^+]$). IR: $\tilde{\nu}$ [cm^{-1}] = 1639 (C=O), 1231 (C-F), 1169 (C-F_{arom}), 876, 698 (C-H_{arom}).

5.1.15.N-[3,5-Bis(trifluoromethyl)benzyl]-4-bromo-2-(4-fluorophenyl)butanamide (11c)

15c (503 mg, 1.19 mmol) and CBr_4 (593 mg, 1.78 mmol) were dissolved in CH_3CN (25 mL) and cooled to 0 °C. Then triphenylphosphine (1.85 g, polymer-bound, 1.6 mmol/g) was added, during 30 min at 0 °C. The mixture was stirred at rt for 2 d. The mixture was filtered over Celite® and water was added to the eluent. The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were washed with water, dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure. The crude product was used in the next reaction step without further purification, $R_f = 0.90$ ($\text{CH}_2\text{Cl}_2:\text{EtOAc}$ 40:60). Brown oil, yield 419.7 mg (73 %). Purity (HPLC): 62.5 %, $t_R = 24.44$ min. $\text{C}_{19}\text{H}_{15}\text{BrF}_7\text{NO}$ (486.2 g/mol). ^1H NMR (CDCl_3): δ [ppm] = 2.26 (dtd, $J = 14.8 / 7.5 / 4.7$ Hz, 1H, $\text{BrCH}_2\text{CH}_2\text{CH}$), 2.66 (dtd, $J = 14.9 / 7.5 / 4.7$ Hz, 1H, $\text{BrCH}_2\text{CH}_2\text{CH}$), 3.26 (ddd, $J = 10.2 / 7.9 / 4.6$ Hz, 1H, $\text{BrCH}_2\text{CH}_2\text{CH}$), 3.46 (ddd, $J = 10.2 / 7.2 / 4.7$ Hz, 1H, $\text{BrCH}_2\text{CH}_2\text{CH}$), 3.79 (t, $J = 7.4$ Hz, 1H, $\text{BrCH}_2\text{CH}_2\text{CH}$), 4.44 (dd, $J = 15.8 / 5.8$ Hz, 1H, PhCH_2NH), 4.62 (dd, $J = 15.8 / 6.6$ Hz, 1H, PhCH_2NH), 5.95 (s, broad, 1H, NH), 7.06 (t, $J = 8.6$ Hz, 2H, 3- H_{arom} , 5- H_{arom}), 7.31 (dd, $J = 8.7 / 5.2$ Hz, 2H, 2- H_{arom} , 6- H_{arom}), 7.56 (s, 2H, 2'- H_{arom} , 6'- H_{arom}), 7.75 (s, 1H, 4'- H_{arom}). ^{13}C NMR (CDCl_3): δ [ppm] = 31.8 (1C, $\text{BrCH}_2\text{CH}_2\text{CH}$), 35.6 (1C, $\text{BrCH}_2\text{CH}_2\text{CH}$), 42.6 (1C, PhCH_2NH), 49.8 (1C, $\text{BrCH}_2\text{CH}_2\text{CH}$), 116.2 (d, $J = 21.7$ Hz, 2C, C-3_{arom}, C-5_{arom}), 121.4 (hept, $J = 3.7$ Hz, 1C, C-4'_{arom}), 123.1 (q, $J = 274.2$ Hz, 2C, CF_3), 127.2 (q, $J = 3.8$ Hz, 2C, C-2'_{arom}, C-6'_{arom}), 129.5 (d, $J = 8.1$ Hz, 2C, C-2_{arom}, C-6_{arom}), 131.9 (q, $J = 33.3$ Hz, 2C, C-3'_{arom}, C-5'_{arom}), 133.9 (d, $J = 3.4$ Hz, 1C,

$C-1_{\text{arom}}$), 140.9 (1C, $C-1'_{\text{arom}}$), 162.4 (d, $J = 247.6$ Hz, 1C, $C-4_{\text{arom}}$), 172.5 (1C, $C=O$). MS (APCI): $m/z = 486.0295$ (calcd. 486.0298 for $C_{19}H_{16}BrF_7NO$ [MH^+]). IR: ν [cm^{-1}] = 1670 ($C=O$), 1277 ($C-F$), 1169 ($C-F_{\text{arom}}$), 721, 660 ($C-H_{\text{arom}}$).

5.1.16.4-Bromo-2-(4-fluorophenyl)-N-[3-fluoro-5-(trifluoromethyl)benzyl]butanamide (11d)

15d (796 mg, 2.13 mmol) and $CBBr_4$ (761 mg, 2.29 mmol) were dissolved in CH_3CN (40 mL) and cooled to 0 °C. Then triphenylphosphine (3.33 g, polymer-bound, 1.6 mmol/g) was added during 30 min at 0 °C. The mixture was stirred at rt overnight. The mixture was filtered over Celite® and water was added. The aqueous layer was extracted with Et_2O (3x), the combined organic layers were washed with water, dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure. The crude product was used in the next reaction step without further purification, $R_f = 0.60$ ($CH_2Cl_2:EtOAc$ 40:60). Brown oil, yield 920 mg (99 %). Purity (HPLC): 47.25 %, $t_R = 23.51$ min. $C_{18}H_{15}BrF_5NO$ (436.2 g/mol). 1H NMR ($CDCl_3$): δ [ppm] = 2.26 (dtd, $J = 12.2 / 7.4 / 4.8$ Hz, 1H, $BrCH_2CH_2CH$), 2.66 (dtd, $J = 12.3 / 7.4 / 4.7$ Hz, 1H, $BrCH_2CH_2CH$), 3.25 (ddd, $J = 10.3 / 8.0 / 4.7$ Hz, 1H, $BrCH_2CH_2CH$), 3.46 (ddd, $J = 10.3 / 7.1 / 4.8$ Hz, 1H, $BrCH_2CH_2CH$), 3.76 (t, $J = 7.4$ Hz, 1H, $BrCH_2CH_2CH$), 4.41 (dd, $J = 15.7 / 5.9$ Hz, 1H, $PhCH_2NH$), 4.51 (dd, $J = 15.6 / 6.3$ Hz, 1H, $PhCH_2NH$), 5.86 (s, broad, 1H, NH), 7.03 (d, $J = 6.4$ Hz, 1H, $2'-H_{\text{arom}}$), 7.06 (t, $J = 8.5$ Hz, 2H, $3-H_{\text{arom}}$, $5-H_{\text{arom}}$), 7.16 (s, 1H, $6'-H_{\text{arom}}$), 7.20 (d, $J = 8.3$ Hz, 1H, $4'-H_{\text{arom}}$), 7.32 (dd, $J = 8.7 / 5.3$ Hz, 2H, $2-H_{\text{arom}}$, $6-H_{\text{arom}}$). ^{13}C NMR ($CDCl_3$): δ [ppm] = 31.8 (1C, $BrCH_2CH_2CH$), 35.6 (1C, $BrCH_2CH_2CH$), 42.7 (1C, $PhCH_2NH$), 49.9 (1C, $BrCH_2CH_2CH$), 111.9 (dq, $J = 24.4 / 3.8$ Hz, 1C, $C-4'_{\text{arom}}$), 116.2 (d, $J = 21.7$ Hz, 2C, $C-3_{\text{arom}}$, $C-5_{\text{arom}}$), 117.7 (d, $J = 20.6$ Hz, 1C, $C-2'_{\text{arom}}$), 119.5 (quint, $J = 3.7$ Hz, 1C, $C-6'_{\text{arom}}$), 129.5 (d, $J = 8.0$ Hz, 2C, $C-2_{\text{arom}}$, $C-6_{\text{arom}}$), 133.9 (d, $J = 3.4$ Hz, 1C, $C-1_{\text{arom}}$),

142.1 (d, $J = 7.6$ Hz, 1C, C-1'_{arom}), 162.4 (d, $J = 247.6$ Hz, 1C, C-3'_{arom}), 162.6 (d, $J = 249.9$ Hz, 1C, C-4_{arom}), 172.4 (1C, C=O), signals for the C-atom of the CF₃ group and for C-5' are not seen in the spectrum. MS (APCI): $m/z = 436.0301$ (calcd. 436.0330 for C₁₈H₁₆BrF₅NO [MH⁺]). IR: ν [cm⁻¹] = 1651 (C=O), 1227 (C-F), 1169 (C-F_{arom}), 664 (C-Br).

5.1.17.N-[3,5-Bis(trifluoromethyl)benzyl]-4-bromo-2-cyclopropylbutanamide

(11e)

15e (60 mg, 0.16 mmol) and CBr₄ (100 mg, 0.30 mmol) were dissolved in CH₃CN (4 mL) and cooled to 0 °C. Then triphenylphosphine (266 mg, polymer-bound, 1.6 mmol/g) was added during 30 min at 0 °C. The mixture was stirred at rt overnight. The mixture was filtered over Celite[®] and water was added to the eluent. The aqueous layer was extracted with CH₂Cl₂ (3x), the combined organic layers were washed with water, dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The crude product was used in the next reaction step without further purification, R_f = 0.40 (CH₂Cl₂: CH₃OH:NH₃ 95:4.5:0.5). Brown oil, yield 33 mg (47 %). Purity (HPLC): 54 %, $t_R = 23.32$ min. C₁₆H₁₆BrF₆NO (432.2 g/mol). ¹H NMR (CDCl₃): δ [ppm] = 0.25 – 0.33 (m, 2H, CH_{2cycloprop}), 0.59 – 0.71 (m, 2H, CH_{2cycloprop}), 0.95 – 1.05 (m, 1H, CH_{cycloprop}), 1.74 (td, $J = 9.2 / 5.4$ Hz, 1H, BrCH₂CH₂CH), 2.08 – 2.16 (m, 1H, BrCH₂CH₂CH), 2.42 (ddt, $J = 14.3 / 8.8 / 5.3$ Hz, 1H, BrCH₂CH₂CH), 3.43 (ddd, $J = 10.2 / 9.2 / 4.9$ Hz, 1H, BrCH₂CH₂CH), 3.56 – 3.66 (m, 1H, BrCH₂CH₂CH), 4.53 (dd, $J = 15.8 / 6.1$ Hz, 1H, PhCH₂NH), 4.70 (dd, $J = 15.8 / 6.2$ Hz, 1H, PhCH₂NH), 6.19 (s, broad, 1H, NH), 7.75 (s, 2H, 2-H_{arom}, 6-H_{arom}), 7.79 (s, 1H, 4-H_{arom}). ¹³C NMR (CDCl₃): δ [ppm] = 3.9 (1C, CH_{2cycloprop}), 4.5 (1C, CH_{2cycloprop}), 13.7 (1C, CH_{cycloprop}), 32.2 (1C, BrCH₂CH₂CH), 35.0 (1C, BrCH₂CH₂CH), 42.5 (1C, PhCH₂NH), 50.0 (1C, BrCH₂CH₂CH), 121.4 (hept, $J = 3.9$ Hz, 1C, C-4_{arom}), 123.2 (q, $J = 272.2$ Hz, 2C,

CF₃), 127.3 (q, $J = 3.1$ Hz, 2C, C-2_{arom}, C-6_{arom}), 132.0 (q, $J = 33.2$ Hz, 2C, C-3_{arom}, C-5_{arom}) 141.2 (1C, C-1_{arom}), 174.3 (1C, C=O). MS (APCI): $m/z = 432.0436$ (calcd. 432.0392 for C₁₆H₁₇BrF₆NO [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2924 (C-H), 1647 (C=O), 1277 (C-F).

5.1.18. Methyl 2-(4-fluorophenyl)acetate (**13c**)²⁴

2-(4-Fluorophenyl)acetic acid **12c** (5.00 g, 32.4 mmol) was dissolved in CH₃OH (6.5 mL). Conc. H₂SO₄ (0.3 mL) was added and the mixture was heated to reflux overnight. Water was added, the organic layer was separated and the aqueous layer was extracted with Et₂O (3x). The combined organic layers were washed with saturated aqueous NaHCO₃ and water to remove the acid. Then the organic layers were dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure, $R_f = 0.23$ (CH₂Cl₂: EtOAc 40:60). Colorless oil, yield 4.07 g (75 %). C₉H₉FO₂ (168.2 g/mol). ¹H NMR (CDCl₃): δ [ppm] = 3.60 (s, 2H, PhCH₂), 3.69 (s, 3H, CO₂CH₃), 6.96 – 7.05 (m, 2H, 3-*H*_{arom}, 5-*H*_{arom}), 7.19 – 7.28 (m, 2H, 2-*H*_{arom}, 4-*H*_{arom}). ¹³C NMR (CDCl₃): δ [ppm] = 40.3 (1C, CH₂), 52.1 (1C, CO₂CH₃), 115.4 (d, $J = 21.3$ Hz, 2C, C-3_{arom}, C-5_{arom}), 129.6 (d, $J = 3.4$ Hz, 1C, C-1_{arom}), 130.8 (d, $J = 8.0$ Hz, 2C, C-2_{arom}, C-6_{arom}), 162.0 (d, $J = 241.5$ Hz, 1C, C-4_{arom}), 174.9 (1C, C=O). MS (APCI): $m/z = 169.0654$ (calcd. 169.0659 for C₉H₁₀FO₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2955 (C-H), 1736 (C=O), 1153 (C-F_{arom}), 822 (C-H_{arom}).

5.1.19. Methyl 2-cyclopropylacetate (**13e**)²⁵

2-(Cyclopropyl)acetic acid **12e** (2.43 g, 24.2 mmol) was dissolved in CH₃OH (4 mL). Conc. H₂SO₄ (0.15 mL) was added and the mixture was heated to reflux overnight. Water was added, the organic layer was separated and the aqueous layer was extracted with Et₂O (3x). The combined organic layers were washed with saturated

aqueous NaHCO₃ and water. Then the organic layers were dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The crude product was used in the next reaction step without further purification. Pale yellow oil, yield 1.23 g (45 %). C₆H₁₀O₂ (114.1 g/mol). ¹H NMR (CDCl₃): δ [ppm] = 0.14 – 0.19 (m, 2H, CH_{2cycloprop}), 0.52 – 0.57 (m, 2H, CH_{2cycloprop}), 1.02 – 1.10 (m, 1H, CH_{cycloprop}), 2.22 (d, *J* = 7.1 Hz, 2H, CH₂), 3.69 (s, 3H, CO₂CH₃). ¹³C NMR (CDCl₃): δ [ppm] = 4.4 (2C, CH_{2cycloprop}), 6.9 (1C, CH_{cycloprop}), 39.2 (1C, CH₂), 51.5 (1C, CO₂CH₃), 173.7 (1C, C=O). MS (APCI): *m/z* = 115.0772 (calcd. 115.0754 for C₆H₁₁O₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2951 (C-H), 1736 (C=O), 1169 (C-O).

5.1.20. 3-(4-Fluorophenyl)-4,5-dihydrofuran-2(3H)-one (14c)

Ester **13c** (2.72 g, 16.2 mmol) was dissolved in THF (146 mL) and the solution was cooled down to -15 °C (acetone / dry ice). 0.1 M LiHMDS (in THF, 18.4 mL) was added and the mixture was stirred at -15 °C for 30 min. Cyclic sulfate (5.06 g, 40.7 mmol) was dissolved in THF (57 mL) and cooled to -15 °C simultaneously. The solution of the enolate of **13c** was transferred to the THF solution of cyclic sulfate via canula and the mixture was stirred for 2 h at -15 °C. The mixture was warmed to rt, the solvent was removed in vacuo and ethanolic NaOH (146 mL ethanol:water 2:1, 3.27 g NaOH) was added. The resulting solution was heated to reflux overnight. The pH value of the reaction mixture was then adjusted to pH 3 with ¼ conc. H₂SO₄ and the aqueous layer was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (Ø 4.5 cm, length 14 cm, CH₂Cl₂:EtOAc 90:10, fraction size 30 mL, R_f = 0.26 (CH₂Cl₂:EtOAc 40:60)). Brown oil, yield 1.51 g (52 %). C₁₀H₉FO₂ (180.2 g/mol). ¹H NMR (CDCl₃): δ [ppm] = 2.41 (dddd, *J* = 12.8 / 10.7 / 9.6 / 8.3 Hz, 1H, 4-*H*), 2.72 (dddd, *J* = 12.8 / 9.0 / 6.6 / 3.0 Hz, 1H, 4-

H), 3.79 (dd, $J = 10.6 / 8.8$ Hz, 1H, 3-*H*), 4.34 (td, $J = 9.4 / 6.5$ Hz, 1H, 5-*H*), 4.48 (td, $J = 8.7 / 3.0$ Hz, 1H, 5-*H*), 7.02 – 7.07 (m, 2H, 3- H_{arom} , 5- H_{arom}), 7.24 – 7.28 (m, 2H, 2- H_{arom} , 6- H_{arom}). ^{13}C NMR (CDCl_3): δ [ppm] = 31.6 (1C, C-4), 44.7 (1C, C-3), 66.4 (1C, C-5), 115.5 and 115.8 (d, each $J = 21.5$ Hz, 2C, C-3 $_{\text{arom}}$, C-5 $_{\text{arom}}$), 129.5 and 130.9 (d, each $J = 8.1$ Hz, 2C, C-2 $_{\text{arom}}$, C-6 $_{\text{arom}}$), 132.2 (d, $J = 3.4$ Hz, 1C, C-1 $_{\text{arom}}$), 162.2 (d, $J = 246.7$ Hz, 1C, C-4 $_{\text{arom}}$), 177.1 (1C, C=O). MS (APCI): $m/z = 181.0653$ (calcd. 181.0659 for $\text{C}_{10}\text{H}_{10}\text{FO}_2$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 1762 (C=O), 1219 (C-F $_{\text{arom}}$), 1150 (C-O), 814 (C-H $_{\text{arom}}$).

5.1.21. 3-Cyclopropyl-4,5-dihydrofuran-2(3*H*)-one (14e)

13e (1.02 g, 8.9 mmol) was dissolved in THF (70 mL) and cooled down to -15 °C (acetone / dry ice). 1 M LiHMDS solution (in THF, 10.5 mL) was added and the mixture was stirred at -15 °C for 30 min. Cyclic sulfate (2.88 g, 23.2 mmol) was dissolved in THF (36 mL) and cooled to -15 °C simultaneously. Deprotonated 13e was then transferred to the solution of cyclic sulfate in THF via canula and the mixture was stirred for 2 h at -15 °C. After warming to rt, THF was removed in vacuum and ethanolic NaOH (76 mL ethanol:water 2:1, 1.75 g NaOH) was added. The mixture was heated to reflux overnight. The pH value of the reaction mixture was adjusted to pH 3 by addition of $\frac{1}{4}$ conc. H_2SO_4 and the aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was immediately (!) used in the next reaction step without further purification. Brown oil, yield 665.5 mg (59 %). $\text{C}_7\text{H}_{10}\text{O}_2$ (126.2 g/mol). ^1H NMR (CDCl_3): δ [ppm] = 0.21 - 0.34 (m, 1H, $\text{CH}_{2\text{cycloprop}}$), 0.37 - 0.47 (m, 1H, $\text{CH}_{2\text{cycloprop}}$), 0.50 - 0.56 (m, 1H, $\text{CH}_{2\text{cycloprop}}$), 0.64 - 0.72 (m, 1H, $\text{CH}_{2\text{cycloprop}}$), 1.17 - 1.28 (m, 1H, $\text{CH}_{\text{cycloprop}}$), 2.01 - 2.08 (m, 1H, 4-*H*), 2.10 - 2.21 (m, 1H, 4-*H*), 2.24 - 2.33 (m, 1H, 3-*H*), 4.14 - 4.23 (m, 1H, 5-*H*), 4.29 - 4.40 (m, 1H, 5-

H). ^{13}C NMR (CDCl_3): δ [ppm] = 2.3 (1C, $\text{CH}_{2\text{cycloprop}}$), 3.6 (1C, $\text{CH}_{2\text{cycloprop}}$), 11.3 (1C, $\text{CH}_{\text{cycloprop}}$), 28.4 (1C, C-4), 61.9 (1C, C-5), 66.4 (1C, C-3), 178.6 (1C, C=O). MS (APCI): m/z = 127.0766 (calcd. 127.0754 for $\text{C}_7\text{H}_{11}\text{O}_2$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 2970 (C-H), 1709 (C=O), 1184 (C-O).

5.1.22.N-[3,5-Bis(trifluoromethyl)benzyl]-2-(4-fluorophenyl)-4-hydroxybutanamide (15c)

14c (1.05 g, 0.6 mmol) was dissolved in CH_2Cl_2 (20 mL) and AlCl_3 (1.51 g, 1.1 mmol) was added. The mixture was cooled to 0 °C and 3,5-bis(trifluoromethyl)benzylamine **10a** (4.10 g, 16.8 mmol) dissolved in CH_2Cl_2 (10 mL) was added dropwise. The reaction mixture was then stirred at rt overnight. The transformation was stopped by addition of saturated aqueous NH_4Cl solution. Then water and CH_2Cl_2 were added and the aqueous layer was extracted with CH_2Cl_2 (3x). Combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (\varnothing 2 cm, length 14 cm, CH_2Cl_2 : EtOAc 40:60, fraction size 10 mL, R_f = 0.27 (CH_2Cl_2 :EtOAc 40:60)). Colorless solid, mp 91 °C, yield 565 mg (23 %). Purity (HPLC): 91.0 %, t_R = 19.53 min. $\text{C}_{19}\text{H}_{16}\text{F}_7\text{NO}_2$ (423.3 g/mol). ^1H NMR (CDCl_3): δ [ppm] = 1.99 (dtd, J = 14.3 / 6.5 / 4.5 Hz, 1H, $\text{HOCH}_2\text{CH}_2\text{CH}$), 2.34 – 2.47 (m, 1H, $\text{HOCH}_2\text{CH}_2\text{CH}$), 3.57 – 3.64 (m, 1H, $\text{HOCH}_2\text{CH}_2\text{CH}$), 3.66 – 3.76 (m, 2H, $\text{HOCH}_2\text{CH}_2\text{CH}$), 4.46 (dd J = 15.8 / 6.0 Hz, 1H, PhCH_2NH), 4.59 (dd J = 15.8 / 6.4 Hz, 1H, PhCH_2NH), 6.06 (s, broad, 1H, NH), 7.04 (t, J = 8.6 Hz, 2H, 3- H_{arom} , 5- H_{arom}), 7.30 (dd, J = 8.6 / 5.3 Hz, 2H, 2- H_{arom} , 6- H_{arom}), 7.57 (s, 2H, 2'- H_{arom} , 6'- H_{arom}), 7.74 (s, 1H, 4'- H_{arom}), a signal for the OH proton is not seen in the spectrum. ^{13}C NMR (CDCl_3): δ [ppm] = 35.8 (1C, $\text{HOCH}_2\text{CH}_2\text{CH}$), 42.6 (1C, PhCH_2NH), 49.0 (1C, $\text{HOCH}_2\text{CH}_2\text{CH}$), 60.4 (1C, $\text{HOCH}_2\text{CH}_2\text{CH}$), 116.0 (d, J = 21.6 Hz, 2C, C-3 $_{\text{arom}}$, C-5 $_{\text{arom}}$), 121.3 (hept, J = 3.9 Hz,

1C, C-4'_{arom}), 123.1 (q, $J = 272.8$ Hz, 2C, CF₃), 127.2 (2C, C-2'_{arom}, C-6'_{arom}), 129.4 (d, $J = 8.0$ Hz, 2C, C-2_{arom}, C-6_{arom}), 131.9 (q, $J = 33.8$ Hz, 2C, C-3'_{arom}, C-5'_{arom}), 135.1 (d, $J = 3.6$ Hz, 1C, C-1_{arom}), 141.0 (1C, C-1'_{arom}), 161.2 (d, $J = 241.5$ Hz, 1C, C-4_{arom}), 173.9 (1C, C=O). MS (APCI): $m/z = 424.1169$ (calcd. 424.1142 for C₁₉H₁₇F₇NO₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 3294 (O-H), 1651 (C=O), 1277 (C-F_{arom}), 1169 (C-O).

5.1.23.2-(4-Fluorophenyl)-N-[3-fluoro-5-(trifluoromethyl)benzyl]-4-hydroxybutanamide (15d)

14c (313 mg, 1.7 mmol) and 3-fluoro-5-(trifluoromethyl)benzylamine **10b** (340 mg, 1.8 mmol) were dissolved in CH₃CN (15 mL). Na₂CO₃ (184 mg, 1.7 mmol) was added and the mixture was heated to reflux overnight. After cooling to rt, water was added and the pH value was adjusted to pH 3 with 1 M aqueous HCl. The separated aqueous layer was extracted with EtOAc (3x). Combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (\emptyset 4 cm, length 15 cm, CH₂Cl₂:EtOAc 40:60, fraction size 30 mL, R_f = 0.23 (CH₂Cl₂:EtOAc 40:60)). Colorless solid, mp 98 °C, yield 147 mg (23 %). Purity (HPLC): 93.6 %, t_R = 20.2 min. C₁₈H₁₆F₅NO₂ (373.3 g/mol). ¹H NMR (CDCl₃): δ [ppm] = 1.92 – 2.01 (m, 1H, HOCH₂CH₂CH), 2.33 – 2.44 (m, 1H, HOCH₂CH₂CH), 3.54 – 3.61 (m, 1H, HOCH₂CH₂CH), 3.65 – 3.72 (m, 2H, HOCH₂CH₂CH), 4.36 – 4.49 (m, 2H, PhCH₂NH), 5.93 (s, broad, 1H, NH), 7.03 (t, $J = 8.6$ Hz, 2H, 3-*H*_{arom}, 5-*H*_{arom}), 7.13 – 7.19 (m, 3H, 2'-*H*_{arom}, 4'-*H*_{arom}, 6'-*H*_{arom}), 7.28 (dd, $J = 8.7 / 5.3$ Hz, 2H, 2-*H*_{arom}, 6-*H*_{arom}), a signal for the OH proton is not seen in the spectrum. ¹³C NMR (CDCl₃): δ [ppm] = 35.9 (1C, HOCH₂CH₂CH), 42.7 (1C, PhCH₂NH), 49.11 (1C, HOCH₂CH₂CH), 60.5 (1C, HOCH₂CH₂CH), 111.8 (1C, C-4'_{arom}) 116.0 (d, $J = 21.4$ Hz, 2C, C-3_{arom}, C-5_{arom}),

117.7 (d, $J = 22.0$ Hz, 1C, C-2'arom), 119.5 (quint, $J = 4.5$ Hz, 1C, C-6'arom), 129.5 (d, $J = 8.1$ Hz, 2C, C-2arom, C-6arom), 135.1 (d, $J = 3.4$ Hz, 1C, C-1arom), 142.2 (d, $J = 7.4$ Hz, 1C, C-1'arom), 162.2 (d, $J = 246.8$ Hz, 1C, C-3'arom), 173.8 (1C, C=O), signals for C-atom of the CF₃ group, for C-4 and C-5' are not seen in the spectrum. MS (APCI): $m/z = 374.1177$ (calcd. 374.1174 for C₁₈H₁₇F₅NO₂ [MH⁺]). IR: ν [cm⁻¹] = 3298 (O-H), 1643 (C=O), 1223 (C-F), 1169 (C-F_{arom}).

5.1.24. N-[3,5-Bis(trifluoromethyl)benzyl]-2-cyclopropyl-4-hydroxybutanamide (15e)

14e (303 mg, 2.4 mmol) and (3,5-bis(trifluoromethyl)benzylamine **10a** (500 mg, 2.1 mmol) were dissolved in deuteriochloroform (3 mL); 1,2,4-triazole (34 mg, 0.5 mmol) and diazabicycloundecene (62 mg, 0.4 mmol) were added and the mixture was stirred at rt for 2 d. Afterwards the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (\varnothing 4 cm, length 17 cm, CH₂Cl₂: EtOAc 40:60 \rightarrow CH₂Cl₂:EtOAc 25:75, fraction size 30 mL, R_f = 0.25 (CH₂Cl₂:EtOAc 40:60)). Pale yellow solid, mp 92 °C, yield 54 mg (7.1 %). Purity (HPLC): 90.1 %, t_R = 19.95 min. C₁₆H₁₇F₆NO₂ (369.3 g/mol). ¹H NMR (CDCl₃): δ [ppm] = 0.24 - 0.30 (m, 2H, CH_{2cycloprop}), 0.63 - 0.69 (m, 2H, CH_{2cycloprop}), 0.97 - 1.07 (m, 1H, CH_{cycloprop}), 1.62 - 1.73 (m, 1H, HOCH₂CH₂CH), 1.95 - 2.06 (m, 2H, HOCH₂CH₂CH), 3.76 (t, $J = 6.0$ Hz, 2H, HOCH₂CH₂CH), 4.56 (dd, $J = 15.7 / 6.0$ Hz, 1H, PhCH₂NH), 4.66 (dd, $J = 15.8 / 6.0$ Hz, 1H, PhCH₂NH), 6.43 (s, broad, 1H, NH), 7.75 (s, 2H, 2-H_{arom}, 6-H_{arom}), 7.79 (1H, 4-H_{arom}). ¹³C NMR (CDCl₃): δ [ppm] = 4.4 (1C, CH_{2cycloprop}), 4.6 (1C, CH_{2cycloprop}), 13.7 (1C, CH_{cycloprop}), 34.7 (1C, HOCH₂CH₂CH), 42.5 (1C, PhCH₂NH), 49.4 (1C, HOCH₂CH₂CH), 60.7 (1C, HOCH₂CH₂CH), 121.3 (hept, $J = 3.9$ Hz, 1C, C-4arom), 127.2 - 127.5 (m, 2C, C-2arom, C-6arom), 131.9 (q, $J = 33.4$ Hz, 2C, C-3arom, C-5arom), 141.3 (1C, C-1arom), 175.9 (1C,

C=O), signals for the two C-atoms of the CF₃ groups are not seen in the spectrum.

MS (APCI): $m/z = 370.1252$ (calcd. 370.1236 for C₁₆H₁₈F₆NO₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 3306 (O-H), 2931 (C-H), 1647 (C=O), 1281 (C-F_{arom}), 1122 (C-O).

5.1.25. 1-[3,5-Bis(trifluoromethyl)benzyl]-3-(4-fluorophenyl)-5-hydroxy-pyrrolidin-2-one (16c)¹⁸

15c (104 mg, 0.3 mmol) was dissolved in CH₂Cl₂ (1 mL) and Dess-Martin-periodinane (118 mg, 0.3 mmol) was added. The reaction mixture was stirred at rt for 60 min. The transformation was stopped by addition of diethyl ether (5 mL) and 1.3 M NaOH (2 mL) and the mixture was stirred for 10 min. The organic layer was separated, washed with 1.3 M NaOH and water, dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (Ø 2 cm, length 12 cm, CH₂Cl₂: EtOAc 40:60, fraction size 10 mL, R_f = 0.81 (CH₂Cl₂:EtOAc 40:60)). Colorless solid, yield 75 mg (73 %). C₁₉H₁₄F₇NO₂ (421.3 g/mol). ¹H NMR (CDCl₃): δ [ppm] = 2.35 – 2.50 (m, 1H, NCH(OH)CH₂), 2.87 – 2.97 (m, 1H, NCH(OH)CH₂), 3.72 (dd, $J = 9.8 / 6.4$ Hz, 0.5 H, NCOCHPh), 3.98 (dd, $J = 9.2 / 8.1$ Hz, 0.5 H, NCOCHPh), 4.42 (d, $J = 15.3$ Hz, 0.5H, PhCH₂N), 4.51 (d, $J = 15.1$ Hz, 0.5H, PhCH₂N), 4.84 (d, $J = 15.2$ Hz, 0.5H, PhCH₂N), 4.93 (d, $J = 15.2$ Hz, 0.5H, PhCH₂N), 5.17 - 5.21 (m, 0.5H, NCH(OH)CH₂), 5.21 - 5.24 (m, 0.5H, NCH(OH)CH₂), 7.03 (t, $J = 8.5$ Hz, 2H, 3-*H*_{arom}, 5-*H*_{arom}), 7.16 (dd, $J = 8.7 / 5.3$ Hz, 1H, 2-*H*_{arom}, 6-*H*_{arom}), 7.32 (dd, $J = 8.5 / 5.3$ Hz, 1H, 2-*H*_{arom}, 6-*H*_{arom}), 7.75 – 7.83 (m, 3H, 2'-*H*_{arom}, 4'-*H*_{arom}, 6'-*H*_{arom}), a signal for the OH proton is not seen in the spectrum. ¹³C NMR (CDCl₃): δ [ppm] = 37.5 (0.5 C, NCH(OH)CH₂), 38.3 (0.5 C, NCH(OH)CH₂), 43.5 (1C, PhCH₂N), 44.9 (0.5 C, NCOCHPh), 46.1 (0.5 C, NCOCHPh), 81.0 (0.5 C, NCH(OH)CH₂), 81.2 (0.5 C, NCH(OH)CH₂), 115.7 and 115.9 (d, each $J = 8.8$ Hz, 2C, C-3_{arom}, C-5_{arom}), 121.6 – 121.9 (m, 1C, C-4'_{arom}),

128.3 and 128.4 (q, each $J = 3.7$ Hz, 2C, C-2_{arom}, C-6_{arom}), 129.3 and 129.5 (d, each $J = 8.4$ Hz, 2C, C-2'_{arom}, C-6'_{arom}), 132.1 (q, $J = 33.4$ Hz, 2C, C-3'_{arom}, C-5'_{arom}), 134.2 (d, $J = 3.1$ Hz, 0.5 C, C-1_{arom}), 134.5 (d, $J = 3.1$ Hz, 0.5 C, C-1_{arom}), 139.4 (d, $J = 1.5$ Hz, 1C, C-1'_{arom}), 162.1 (d, $J = 245.3$ Hz, 1C, C-4_{arom}), 174.6 (d, $J = 91.2$ Hz, 1C, NCOCHPh), signals for the C-atoms of the CF₃ groups are not seen in the spectrum.

5.1.26. N-[3,5-Bis(trifluoromethyl)benzyl]-4-(3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4a)

8a (108 mg, 0.5 mmol) was dissolved in THF (20 mL) and **11a** (312 mg, 0.9 mmol) and K₂CO₃ (874 mg, 6.3 mmol) were added. The mixture was heated to reflux overnight. K₂CO₃ was filtered off and the solvent was removed under reduced pressure. The crude product was coated on silica gel and then purified by flash column chromatography twice (Ø 4 cm, length 14 cm, CH₂Cl₂:EtOAc 50:50 + 1 % ethyldimethylamine → EtOAc 100 + 1% ethyldimethylamine → acetone 100 + 1% ethyldimethylamine, fraction size 30 mL; Ø 4 cm, length 15 cm, CH₂Cl₂:MetOH:NH₃ 94.2:5:0.8, fraction size 30 mL; R_f = 0.17 (CH₂Cl₂:MetOH:NH₃ 95:4.5:0.5)). Yellow oil, yield 118 mg (43 %). Purity (HPLC): 97.6 %, t_R = 20.00 min. C₂₆H₂₈F₆N₂O₂ (514.5 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.85 – 1.89 (m, 2H, NCH₂CH₂CH₂), 1.91 – 1.96 (m, 2H, N(CH₂CH₂)₂), 2.06 (td, $J = 13.8 / 4.1$ Hz, 2H, N(CH₂CH₂)₂), 2.35 (t, $J = 7.3$ Hz, 2H, NCH₂CH₂CH₂), 2.47 – 2.53 (m, 2H, NCH₂), 2.54 – 2.58 (m, 2H, NCH₂), 2.80 (t, $J = 5.5$ Hz, 2H, PhCH₂CH₂), 2.82 – 2.88 (m, 2H, NCH₂), 3.89 (t, $J = 5.5$ Hz, 2H, PhCH₂CH₂), 4.53 (s, 2H, PhCH₂NH), 7.07 – 7.19 (m, 4H, H_{arom}), 7.87 (s, 1H, 4'-H_{arom}), 7.90 (s, 2H, 2'-H_{arom}, 6'-H_{arom}), a signal for the NH proton is not seen in the spectrum. ¹³C NMR (CD₃OD): δ [ppm] = 22.2 (1C, NCH₂CH₂CH₂), 29.1 (1C, PhCH₂CH₂), 33.3 (1C, NCH₂CH₂CH₂), 35.7 (2C, N(CH₂CH₂)₂), 41.8 (1C, PhCH₂NH), 48.8 (2C, N(CH₂CH₂)₂), 57.6 (1C, NCH₂CH₂CH₂), 58.5 (1C, PhCH₂CH₂), 72.4 (1C,

ArCO), 120.5 (m, 1C, C-4'_{arom}), 124.8 (1C, C-8_{arom}), 125.88 and 125.94 (2C, C-5_{arom}, C-6_{arom}), 127.7 (m, 2C, C-2'_{arom}, C-6'_{arom}), 128.5 (1C, C-7_{arom}), 131.4 (q, $J = 33.2$ Hz, 2C, C-3'_{arom}, C-5'_{arom}), 133.6 (1C, C-4_{aarom}), 141.2 (1C, C-8_{aarom}), 142.4 (1C, C-1'_{arom}), 174.3 (1C, C=O), signals for the C-atoms of the CF₃ groups are not seen in the spectrum. MS (APCI): $m/z = 515.2161$ (calcd. 515.2155 for C₂₆H₂₉F₆N₂O₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 1651 (C=O), 1277 (C-F), 756, 683 (C-H_{arom}).

5.1.27.N-[3,5-Bis(trifluoromethyl)benzyl]-4-(3-methyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4b)

Spiropiperidine **8b** (410 mg, 1.9 mmol) was dissolved in THF (56 mL) and **11a** (787 mg, 2.3 mmol) and K₂CO₃ (2.09 g, 14.9 mmol) were added. The mixture was heated to reflux overnight. K₂CO₃ was filtered off and the solvent was removed under reduced pressure. The crude product was coated on silica gel and then purified by flash column chromatography twice (\varnothing 4 cm, length 14 cm, CH₂Cl₂:EtOAc 50:50 + 1 % dimethylethylamine → EtOAc 100 + 1 % ethyldimethylamine → acetone 100 + 1 % ethyldimethylamine, fraction size 30 mL; R_f = 0.17 (CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5)). The isolated product was recrystallized from methyl *tert*-butyl ether:diisopropyl ether 50:50. Pale yellow solid, mp 125 °C, yield 86 mg (9 %). Purity (HPLC): 98 %, t_R = 21.35 min. C₂₇H₃₀F₆N₂O₂ (528.5 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.31 (d, $J = 6.1$ Hz, 3H, CH₃), 1.62 (dd, $J = 13.6 / 4.6$ Hz, 1H, N(CH₂CH₂)₂), 1.84 (td, $J = 13.5 / 4.4$ Hz, 1H, N(CH₂CH₂)₂), 1.87 – 1.93 (m, 2H, NCH₂CH₂CH₂), 2.07 (dd, $J = 14.3 / 2.6$ Hz, 1H, N(CH₂CH₂)₂), 2.20 (td, $J = 13.2 / 4.6$ Hz, 1H, N(CH₂CH₂)₂), 2.33 (t, $J = 7.3$ Hz, 2H, NCH₂CH₂CH₂), 2.40 – 2.45 (m, 3H, NCH₂), 2.53 (td, $J = 12.3 / 2.4$ Hz, 1H, NCH₂), 2.60 – 2.69 (m, 2H, PhCH₂CH₂), 2.72 – 2.81 (m, 2H, NCH₂), 3.84 - 3.93 (m, 1H, PhCH₂CH), 4.53 (s, 2H, PhCH₂NH), 7.03 – 7.17 (m, 4H, H_{arom}), 7.87 (s, broad, 1H, 4'-H_{arom}), 7.88 (s, 2H, 2'-H_{arom}, 6'-H_{arom}), a signal for the NH proton is not seen in

the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 20.6 (1C, CH_3), 22.5 (1C, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 33.5 (1C, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 34.0 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 36.6 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 38.3 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 41.8 (1C, PhCH_2CH), 48.8 (0.5C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 48.9 (0.5C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 57.7 (1C, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 64.0 (1C, PhCH_2CH), 73.3 (1C, ArCO), 120.5 (hept, $J = 3.9$, 1C, $\text{C-4}'_{\text{arom}}$), 123.4 (q, $J = 270.6$ Hz, 2C, CF_3), 124.6 (1C, C-8_{arom}), 125.8 and 125.9 (2C, C-5_{arom} , C-6_{arom}), 127.5 – 127.8 (m, 2C, $\text{C-2}'_{\text{arom}}$, $\text{C-6}'_{\text{arom}}$), 128.3 (1C, C-7_{arom}), 131.4 (q, $J = 33.2$ Hz, 2C, $\text{C-3}'_{\text{arom}}$, $\text{C-5}'_{\text{arom}}$), 133.9 (1C, $\text{C-4a}_{\text{arom}}$), 141.2 (1C, $\text{C-1}'_{\text{arom}}$), 142.5 (1C, $\text{C-8a}_{\text{arom}}$), 174.4 (1C, C=O). MS (APCI): $m/z = 529.2268$ (calcd. 529.2284 for $\text{C}_{27}\text{H}_{31}\text{F}_6\text{N}_2\text{O}_2$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 1643 (C=O), 1281 (C-F), 1120 (C-O), 756, 679 (C-H_{arom}).

5.1.28.N-[3,5-Bis(trifluoromethyl)benzyl]-4-(6-methoxy-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4c)

Spiropiperidine **8c** (596 mg, 2.6 mmol) was dissolved in THF (88 mL) and chlorobutanamide **11a** (1.05 g, 3.0 mmol) and K_2CO_3 (2.92 g, 21.0 mmol) were added. The mixture was heated to reflux overnight. K_2CO_3 was filtered off and the solvent was removed under reduced pressure. The crude product was coated on silica gel and then purified by flash column chromatography three times (\varnothing 3 cm, length 10 cm, cyclohexane:EtOAc 50:50 + 1% ethyldimethylamine, fraction size 65 mL; \varnothing 4 cm, length 15 cm, CH_2Cl_2 : CH_3OH : NH_3 94.2:5:0.8, fraction size 30 mL; \varnothing 2 cm, length 15 cm, CH_2Cl_2 : CH_3OH : NH_3 94.2:5:0.8, fraction size 10 mL; $R_f = 0.20$, (CH_2Cl_2 : CH_3OH : NH_3 95:4.5:0.5)). Pale yellow oil, yield 383 mg (28 %). Purity (HPLC): 96.3 %, $t_R = 21.70$ min. $\text{C}_{27}\text{H}_{30}\text{F}_6\text{N}_2\text{O}_3$ (544.5 g/mol). ^1H NMR (CD_3OD): δ [ppm] = 1.84 – 1.90 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 1.90 – 1.95 (m, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.03 (td, $J = 13.6 / 4.4$ Hz, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.36 (t, $J = 7.2$ Hz, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 2.49 – 2.56 (m, 2H, NCH_2), 2.56 – 2.61 (m, 2H, NCH_2), 2.77 (t, $J = 5.5$ Hz, 2H, PhCH_2CH_2),

2.82 – 2.90 (m, 2H, NCH₂), 3.75 (s, 3H, OCH₃), 3.87 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 4.53 (s, 2H, PhCH₂NH), 6.65 (d, *J* = 2.6 Hz, 1H, 5-*H*_{arom}), 6.76 (dd, *J* = 8.7 / 2.7 Hz, 1H, 7-*H*_{arom}), 7.06 (d, *J* = 8.7 Hz, 1H, 8-*H*_{arom}), 7.87 (s, 1H, 4'-*H*_{arom}), 7.90 (s, 2H, 2'-*H*_{arom}, 6'-*H*_{arom}), a signal for the NH proton is not seen in the spectrum. ¹³C NMR (CD₃OD): δ [ppm] = 22.0 (1C, NCH₂CH₂CH₂), 29.4 (1C, PhCH₂CH₂), 33.3 (1C, NCH₂CH₂CH₂), 35.7 (2C, N(CH₂CH₂)₂), 41.9 (1C, PhCH₂NH), 48.9 (2C, N(CH₂CH₂)), 54.2 (1C, OCH₃), 57.5 (1C, NCH₂CH₂CH₂), 58.5 (1C, PhCH₂CH₂), 72.1 (1C, ArCO), 112.3 (1C, C-7_{arom}), 112.8 (1C, C-5_{arom}), 120.5 (hept, *J* = 3.7 Hz, 1C, C-4'_{arom}), 123.4 (q, *J* = 271.0 Hz, 2C, CF₃), 125.9 (1C, C-8_{arom}), 127.6 – 127.9 (m, 2C, C-2'_{arom}, C-6'_{arom}), 131.4 (q, *J* = 33.6 Hz, 2C, C-3'_{arom}, C-5'_{arom}), 133.2 (1C, C-4_{aarom}), 135.0 (1C, C-8_{aarom}), 142.4 (1C, C-1'_{arom}), 158.0 (1C, C-6_{arom}), 174.3 (1C, C=O). MS (APCI): *m/z* = 545.2258 (calcd. 545.2233 for C₂₇H₃₁F₆N₂O₃ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 1651 (C=O), 1277 (C-F), 737, 683 (C-H_{arom}).

5.1.29.4-(3,4-Dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)-N-[3-fluoro-5-(trifluoromethyl)benzyl]butanamide (4d)

Spiropiperidine **8a** (264 mg, 1.3 mmol) was dissolved in THF (3 mL) and the solution was cooled to 0 °C. Then **11b** (134 mg, 0.5 mmol) was added dropwise over 30 min and the mixture was warmed to rt and stirred for 2 d at 40 °C. Water was added and the mixture was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed in vacuo. The crude product was coated on silica gel and then purified by flash column chromatography twice (Ø 2 cm, length 22 cm, CH₂Cl₂:CH₃OH:NH₃ 84.2:15:0.8, fraction size 10 mL; Ø 2 cm, length 14 cm, CH₂Cl₂:CH₃OH:NH₃ 84.2:15:0.8, fraction size 10 mL; R_f = 0.17 (CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5)). Pale yellow oil, yield 19.3 mg (9.3 %). Purity (HPLC): 97.1 %, t_R = 18.48 min. C₂₅H₂₈F₄N₂O₂ (464.5 g/mol). ¹H NMR (CD₃OD): δ

[ppm] = 1.83 – 1.90 (m, 2H, NCH₂CH₂CH₂), 1.91 - 1.95 (m, 2H, N(CH₂CH₂)₂), 2.05 (td, *J* = 13.5 / 4.4 Hz, 2H, N(CH₂CH₂)₂), 2.34 (t, *J* = 7.3 Hz, 2H, NCH₂CH₂CH₂), 2.48 – 2.58 (m, 4H, NCH₂), 2.79 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 2.84 (d, broad, *J* = 11.0 Hz, 2H, N(CH₂CH₂)₂), 3.88 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 4.45 (s, 2H, PhCH₂NH), 7.06 – 7.20 (m, 4H, *H*_{arom}), 7.34 (d, *J* = 9.5 Hz, 2H, 2'-*H*_{arom}, 4'-*H*_{arom}), 7.45 (s, 1H, 6'-*H*_{arom}), a signal for the NH proton is not seen in the spectrum. ¹³C NMR (CD₃OD): δ [ppm] = 22.2 (1C, NCH₂CH₂CH₂), 29.1 (1C, PhCH₂CH₂), 33.4 (1C, NCH₂CH₂CH₂), 35.7 (2C, N(CH₂CH₂)₂), 41.8 (d, *J* = 1.5 Hz, 1C, PhCH₂NH), 48.8 (2C, N(CH₂CH₂)₂), 57.6 (1C, NCH₂CH₂CH₂), 58.5 (1C, PhCH₂CH₂), 72.4 (1C, ArCO), 111.0 (dq, *J* = 25.2 / 3.9 Hz, 1C, C-4'_{arom}), 111.0 (1C, C-6_{arom}), 117.8 (d, *J* = 22.3 Hz, 1C, C-2'_{arom}), 119.7 (quint, *J* = 3.9 Hz, 1C, C-6'_{arom}), 124.8 (1C, C-8_{arom}), 125.9 (d, *J* = 7.5 Hz, 1C, C-5_{arom}), 128.5 (1C, C-7_{arom}), 133.6 (1C, C-4_{aarom}), 141.2 (1C, C-8_{aarom}), 143.7 (d, *J* = 7.4 Hz, 1C, C-1'_{arom}), 162.6 (d, *J* = 247.1 Hz, 1C, C-3'_{arom}), 174.3 (1C, C=O), signals for the C-atom of the CF₃ group and for C-5' are not seen in the spectrum. MS (APCI): *m/z* = 465.2193 (calcd. 465.2160 for C₂₅H₂₉F₄N₂O₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2924 (C-H), 1651 (C=O), 1230 (C-F), 1126 (C-F_{arom}), 1085 (C-O), 760, 698 (C-H_{arom}).

5.1.30.N-[3-Fluoro-5-(trifluoromethyl)benzyl]-4-(3-methyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4e)

Spiropiperidine **8b** (379 mg, 1.7 mmol) was dissolved in THF (55 mL) and **11b** (631 mg, 2.1 mmol) and K₂CO₃ (1.99 g, 14.5 mmol) were added. The mixture was heated to reflux overnight. K₂CO₃ was filtered off and the solvent was removed under reduced pressure. The crude product was coated on silica gel and then purified by flash column chromatography (Ø 4 cm, length 15 cm, CH₂Cl₂:EtOAc 50:50 + 1 % ethyldimethylamine, fraction size 30 mL; R_f = 0.21(CH₂Cl₂: CH₃OH:NH₃ 95:4.5:0.5)). Colorless oil, yield 30 mg (3.5 %). Purity (HPLC): 97.7 %, t_R = 19.84 min.

$C_{26}H_{30}F_4N_2O_2$ (478.5 g/mol). 1H NMR (CD_3OD): δ [ppm] = 1.33 (d, J = 6.1 Hz, 3H, CH_3), 1.67 (dd, J = 13.8 / 2.6 Hz, 1H, $N(CH_2CH_2)_2$), 1.88 (td, J = 11.5 / 2.8 Hz, 1H, $N(CH_2CH_2)_2$), 1.92 – 1.99 (m, 2H, $NCH_2CH_2CH_2$), 2.13 (dd, J = 14.5 / 2.6 Hz, 1H, $N(CH_2CH_2)_2$), 2.24 (td, J = 13.3 / 4.5 Hz, 1H, $N(CH_2CH_2)_2$), 2.37 (t, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2$), 2.52 – 2.63 (m, 4H, $N(CH_2CH_2)_2$), 2.64 – 2.73 (m, 2H, $PhCH_2CH$), 2.83 – 2.96 (m, 2H, $NCH_2CH_2CH_2$), 3.86 – 3.93 (m, 1H, $PhCH_2CH$), 4.47 (s, 2H, $PhCH_2NH$), 7.04 – 7.19 (m, 4H, H_{arom}), 7.35 (d, J = 8.9 Hz, 2H, $2'-H_{arom}$, $4'-H_{arom}$), 7.47 (s, 1H, $6'-H_{arom}$), a signal for the NH proton is not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 20.8 (1C, CH_3), 22.1 (1C, $NCH_2CH_2CH_2$), 29.2 (1C, $PhCH_2CH$), 33.3 (1C, $NCH_2CH_2CH_2$), 33.8 (1C, $N(CH_2CH_2)_2$), 36.6 (1C, $N(CH_2CH_2)_2$), 38.1 (1C, $N(CH_2CH_2)_2$), 41.8 (d, J = 1.7 Hz, 1C, $PhCH_2NH$), 48.8 (1C, $N(CH_2CH_2)_2$), 57.6 (1C, $NCH_2CH_2CH_2$), 64.1 (1C, $PhCH_2CH$), 73.1 (1C, $ArCO$), 110.9 (dq, J = 25.3 / 4.0 Hz, 1C, $C-4'_{arom}$), 117.8 (d, J = 22.5 Hz, 1C, $C-2'_{arom}$), 119.7 (quint, J = 3.7, 1C, $C-6'_{arom}$), 124.5 (1C, $C-8_{arom}$), 125.9 and 126.0 (2C, $C-5_{arom}$, $C-6_{arom}$), 128.4 (1C, $C-7_{arom}$), 132.2 (qd, J = 33.0 / 8.2 Hz, $C-5'_{arom}$), 133.9 (1C, $C-4a_{arom}$), 140.9 (1C, $C-8a_{arom}$), 143.7 (d, J = 7.7 Hz, 1C, $C-1'_{arom}$), 162.6 (d, J = 245.6 Hz, 1C, $C-3'$), 174.2 (1C, $C=O$), the signal for the C-atom of the CF_3 group is not seen in the spectrum. MS (APCI): m/z = 479.2299 (calcd. 479.2316 for $C_{26}H_{31}F_4N_2O_2$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 3245 (N-H), 2928 (C-H), 1651 (C=O), 1230 (C-F), 1169 (C-F_{arom}), 1085 (C-O), 756, 698 (C-H_{arom}).

5.1.31. **N-[3-Fluoro-5-(trifluoromethyl)benzyl]-4-(6-methoxy-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4f)**

Spiropiperidine **8c** (625 mg, 2.7 mmol) was dissolved in THF (92 mL) and **11b** (959 mg, 3.2 mmol) and K_2CO_3 (2.99 g, 21.7 mmol) were added. The mixture was heated to reflux overnight. K_2CO_3 was filtered off and the solvent was removed under

reduced pressure. The crude product was coated on silica gel and then purified by flash column chromatography (\varnothing 8 cm, length 10 cm, CH_2Cl_2 :EtOAc 50:50 + 1 % ethyldimethylamine fraction size 65 mL; $R_f = 0.17$ (CH_2Cl_2 : CH_3OH : NH_3 95:4.5:0.5)). Colorless oil, yield 356 mg (27 %). Purity (HPLC): 96.5 %, $t_R = 18.99$ min. $\text{C}_{26}\text{H}_{30}\text{F}_4\text{N}_2\text{O}_3$ (494.5 g/mol). ^1H NMR (CD_3OD): δ [ppm] = 1.68 – 1.77 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 1.77 – 1.84 (m, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 1.90 (td, $J = 13.8 / 4.5$ Hz, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.23 (t, $J = 7.3$ Hz, 2H, $\text{NHCH}_2\text{CH}_2\text{CH}_2$), 2.31 – 2.44 (m, 4H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.65 (t, $J = 5.6$ Hz, 2H, PhCH_2CH_2), 2.67 – 2.75 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 3.65 (s, 3H, OCH_3), 3.76 (t, $J = 5.5$ Hz, 2H, PhCH_2CH_2), 4.35 (s, 2H, PhCH_2NH), 6.54 (d, $J = 2.7$ Hz, 1H, 5- H_{arom}), 6.65 (dd, $J = 8.7 / 2.7$ Hz, 1H, 7- H_{arom}), 6.96 (d, $J = 8.7$ Hz, 1H, 8- H_{arom}), 7.24 (d, $J = 9.1$ Hz, 2H, 2'- H_{arom} , 4'- H_{arom}), 7.36 (s, 1H, 6'- H_{arom}), a signal for the NH proton is not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 22.2 (1C, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 29.3 (d, $J = 20.3$ Hz, 1C, $\text{CH}_2\text{CH}_2\text{O}$), 33.4 (1C, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 35.9 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 41.8 (d, $J = 1.8$ Hz, 1C, PhCH_2NH), 48.9 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 54.2 (1C, OCH_3), 57.8 (1C, $\text{NCH}_2\text{CH}_2\text{CH}_2$), 58.4 (1C, $\text{CH}_2\text{CH}_2\text{O}$), 72.3 (1C, ArCO), 110.7 (dq, $J = 24.8 / 3.7$ Hz, 1C, C-4' $_{\text{arom}}$), 112.2 (1C, C-7 $_{\text{arom}}$), 112.8 (1C, C-5 $_{\text{arom}}$), 117.8 (d, $J = 22.4$ Hz, 1C, C-2' $_{\text{arom}}$), 119.7 (quint, $J = 3.7$ Hz, 1C, C-6' $_{\text{arom}}$), 125.9 (1C, C-8 $_{\text{arom}}$), 133.4 (1C, C-4a $_{\text{arom}}$), 134.9 (1C, C-8a $_{\text{arom}}$), 143.7 (d, $J = 7.8$ Hz, 1C, C-1' $_{\text{arom}}$), 158.0 (1C, C-6 $_{\text{arom}}$), 162.6 (d, $J = 245.0$ Hz, 1C, C-3' $_{\text{arom}}$), 174.3 (1C, C=O), signals for the C-atom of the CF_3 group and for C-5' are not seen in the spectrum. MS (APCI): $m/z = 495.2254$ (calcd. 495.2265 for $\text{C}_{26}\text{H}_{31}\text{F}_4\text{N}_2\text{O}_3$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 3245 (N-H), 2924 (C-H), 1651 (C=O), 1501 (OCH_3), 1230 (C-F), 1169 (C-F $_{\text{arom}}$), 1085 (C-O), 810, 698 (C-H $_{\text{arom}}$).

5.1.32. 4-(3,4-Dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)-N-[3,5-bis(trifluoromethylbenzyl)]-2-(4-fluorophenyl)butanamide (4g, WMS-46-12)

8a (224 mg, 1.1 mmol) was dissolved in THF (6 mL) and the solution was cooled to 0 °C. **11c** (195 mg, 0.40 mmol) solved in THF (4 mL) was added dropwise over 30 min. Then the mixture was warmed to rt and stirred for 2 d at 40 °C. Then water was added and the mixture was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed in vacuo. The crude product was coated on silica gel and then purified by flash column chromatography (Ø 2 cm, length 15 cm, CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5, fraction size 10 mL, R_f = 0.11 (CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5)). Pale yellow solid, mp 85 °C, yield 104.5 mg (43 %). Purity (HPLC): 96.8 %, t_R = 21.24 min. C₃₂H₃₁F₇N₂O₂ (608.6 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.94 – 1.99 (m, 2H, N(CH₂CH₂)₂), 2.00 – 2.06 (m, 1H, NCH₂CH₂CH), 2.11(td, *J* = 13.13 / 4.07, 2H, N(CH₂CH₂)₂), 2.36 – 2.46 (m, 1H, NCH₂CH₂CH), 2.60 – 2.77 (m, 4H, NCH₂), 2.80 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 3.04 (t, broad, *J* = 14.0 Hz, 2H, N(CH₂CH₂)₂), 3.65 (dd, *J* = 8.5 / 6.4 Hz, 1H, NCH₂CH₂CH), 3.89 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 4.41 (d, *J* = 15.8 Hz, 1H, PhCH₂NH), 4.59 (d, *J* = 15.8 Hz, 1H, PhCH₂NH), 7.06 (t, *J* = 8.8 Hz, 2H, 3'-H_{arom}, 5'-H_{arom}), 7.09 – 7.20 (m, 4H, 5-H_{arom}, 6-H_{arom}, 7-H_{arom}, 8-H_{arom}), 7.39 (dd, *J* = 8.7 / 5.3 Hz, 2H, 2'-H_{arom}, 6'-H_{arom}), 7.70 (s, 2H, 2''-H_{arom}, 6''-H_{arom}), 7.80 (s, 1H, 4''-H_{arom}), a signal for the NH proton is not seen in the spectrum. ¹³C NMR (CD₃OD): δ [ppm] = 28.8 (1C, NCH₂CH₂CH), 29.0 (1C, PhCH₂CH₂), 35.0 (2C, N(CH₂CH₂)₂), 41.7 (1C, PhCH₂NH), 48.7 (1C, N(CH₂CH₂)₂), 48.9 (1C, N(CH₂CH₂)₂), 49.2 (1C, NCH₂CH₂CH), 55.6 (1C, NCH₂CH₂CH), 58.7 (1C, PhCH₂CH₂), 71.6 (1C, ArCO), 115.2 (d, *J* = 10.7 Hz, 2C, C-3'_{arom}, C-5'_{arom}), 120.4 (hept, *J* = 4.0 Hz, 1C, C-4''_{arom}), 123.3 (q, *J* = 273.3 Hz, 2C, CF₃), 124.6 (1C, C-8_{arom}), 126.0 and 126.2 (2C, C-5_{arom}, C-6_{arom}), 127.1 – 127.3 (m, 2C, C-2''_{arom}, C-6''_{arom}), 128.6 (1C, C-7_{arom}), 129.1 (d, *J* = 8.0 Hz, 2C, C-2'_{arom}, C-

6'arom), 131.3 (q, $J = 33.3$ Hz, 2C, C-3''arom, C-5''arom), 133.6 (1C, C-4aarom), 135.3 (d, $J = 3.0$ Hz, 1C, C-1'arom), 140.3 (1C, C-1''arom), 142.3 (1C, C-8aarom), 162.3 (d, $J = 244.3$ Hz, 1C, C-4'arom), 174.0 (1C, C=O). MS (APCI): $m/z = 609.2361$ (calcd. 609.2347 for $C_{32}H_{32}F_7N_2O_2$ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 1655 (C=O), 1277 (C-F), 1130 (C-F_{arom}), 756, 683 (C-H_{arom}).

5.1.33.N-[3,5-Bis(trifluoromethylbenzyl)-2-(4-fluorophenyl)-4-(3-methyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4h)

8b (246 mg, 1.3 mmol) was dissolved in THF (6 mL) and the solution was cooled to 0 °C. **11c** (180 mg, 0.37 mmol) dissolved in THF (4 mL) was added dropwise over 30 min. Then the mixture was warmed to rt and stirred for 2 d at 40 °C. Then water was added and the mixture was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed in vacuo. The crude product was coated on silica gel and then purified by flash column chromatography (Ø 2 cm, length 15 cm, CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5, fraction size 10 mL; R_f = 0.09 (CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5). Pale yellow solid, mp 85 °C, yield 102 mg (44 %). Purity (HPLC): 97.3 %, t_R = 22.21 min. C₃₃H₃₃F₇N₂O₂ (622.6 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.31 (d, $J = 6.1$ Hz, 3H, CH₃), 1.64 – 1.74 (m, 1H, N(CH₂CH₂)₂), 1.92 (td, $J = 13.9 / 4.3$ Hz, 1H, N(CH₂CH₂)₂), 1.99 – 2.10 (m, 1H, NCH₂CH₂CH), 2.09 – 2.19 (m, 1H, N(CH₂CH₂)₂), 2.25 (td, $J = 13.5 / 4.5$ Hz, 1H, N(CH₂CH₂)₂), 2.35 – 2.47 (m, 1H, NCH₂CH₂CH), 2.55 – 2.63 (m, 2H, NCH₂), 2.61 – 2.71 (m, 2H, PhCH₂CH), 2.72 – 2.86 (m, 2H, NCH₂), 2.90 – 3.06 (m, 2H, NCH₂), 3.64 (dd, $J = 8.5 / 6.4$ Hz, 1H, NCH₂CH₂CH), 3.84 – 3.93 (m, 1H, PhCH₂CH), 4.43 (d, $J = 15.8$ Hz, 1H, PhCH₂NH), 4.58 (d, $J = 15.7$ Hz, 1H, PhCH₂NH), 7.03 – 7.09 (m, 2H, 3'-H_{arom}, 5'-H_{arom}), 7.09 – 7.19 (m, 4H, 5-H_{arom}, 6-H_{arom}, 7-H_{arom}, 8-H_{arom}), 7.40 (dd, $J = 8.8 / 5.3$ Hz, 2H, 2'-H_{arom}, 6'-H_{arom}), 7.70 (s, 2H, 2''-H_{arom}, 6''-H_{arom}), 7.80 (s, 1H, 4''-H_{arom}), a signal for the NH

proton is not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 20.6 (1C, CH_3), 29.1 (1C, $\text{NCH}_2\text{CH}_2\text{CH}$), 33.3 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 36.5 (1C, PhCH_2CH), 37.6 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 41.7 (1C, PhCH_2NH), 48.7 (0.5 C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 48.8 (0.5 C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 49.0 (0.5 C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 49.1 (0.5 C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 49.3 (1C, $\text{NCH}_2\text{CH}_2\text{CH}$), 55.8 (1C, $\text{NCH}_2\text{CH}_2\text{CH}$), 64.3 (1C, PhCH_2CH), 72.6 (1C, ArCO), 115.1 (d, $J = 20.2$ Hz, 2C, $\text{C-3}'_{\text{arom}}$, $\text{C-5}'_{\text{arom}}$), 120.4 (hept, $J = 3.9$ Hz, 1C, $\text{C-4}''_{\text{arom}}$), 123.3 (q, $J = 273.2$ Hz, 2C, CF_3), 124.4 (1C, C-8_{arom}), 125.9 and 126.1 (2C, C-5_{arom} , C-6_{arom}), 127.2 (2C, $\text{C-2}''_{\text{arom}}$, $\text{C-6}''_{\text{arom}}$), 128.4 (1C, C-7_{arom}), 129.0 (d, $J = 8.0$ Hz, 2C, $\text{C-2}'_{\text{arom}}$, $\text{C-6}'_{\text{arom}}$), 131.3 (q, $J = 33.0$ Hz, 2C, $\text{C-3}''_{\text{arom}}$, $\text{C-5}''_{\text{arom}}$), 133.9 (1C, $\text{C-4a}_{\text{arom}}$), 136.4 (d, $J = 2.8$ Hz, 1C, $\text{C-1}'_{\text{arom}}$), 140.4 (1C, $\text{C-8a}_{\text{arom}}$), 142.3 (1C, $\text{C-1}''_{\text{arom}}$), 162.2 (d, $J = 250.8$ Hz, 1C, $\text{C-4}'_{\text{arom}}$), 174.0 (1C, C=O). MS (APCI): $m/z = 623.2498$ (calcd. 623.2503 for $\text{C}_{33}\text{H}_{34}\text{F}_7\text{N}_2\text{O}_2$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 2978 (C-H), 1651 (C=O), 1277 (C-F), 1172 (C-F_{arom}), 1130 (C-O).

5.1.34.N-[3,5-Bis(trifluoromethyl)benzyl]-2-(4-fluorophenyl)-4-(6-methoxy-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4i, WMS-46-09)

8c (200 mg, 0.86 mmol) was dissolved in THF (6 mL) and the solution was cooled to 0 °C. **11c** (147 mg, 0.30 mmol) dissolved in THF (4 mL) was added dropwise over 30 min. Then the mixture was warmed to rt and stirred for 2 d at 40 °C. Then water was added and the mixture was extracted with CH_2Cl_2 (3x). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was coated on silica gel and then purified by flash column chromatography (\varnothing 2 cm, length 17 cm, CH_2Cl_2 : CH_3OH : NH_3 95:4.5:0.5, fraction size 10 mL; $R_f = 0.14$ (CH_2Cl_2 : CH_3OH : NH_3 95:4.5:0.5)). The product was recrystallized from CH_2Cl_2 and then purified by preparative HPLC (Method 2). Yellow solid, mp 88 °C, yield 6.2 mg (3.2 %). Purity (HPLC): 95.4 %, $t_R = 21.18$ min. $\text{C}_{33}\text{H}_{33}\text{F}_7\text{N}_2\text{O}_3$ (638.6 g/mol). ^1H NMR

(CD₃OD): δ [ppm] = 1.92 -2.00 (m, 2H, N(CH₂CH₂)₂), 2.04 – 2.12 (m, 2H, N(CH₂CH₂)₂), 2.38 – 2.49 (m, 1H, NCH₂CH₂CH), 2.66 – 2.75 (m, 1H, NCH₂CH₂CH), 2.78 (t, J = 5.5 Hz, 2H, PhCH₂CH₂), 2.80 – 2.95 (m, 3H, NCH₂), 3.03 – 3.17 (m, 3H, NCH₂), 3.66 (t, J = 7.4 Hz, 1H, NCH₂CH₂CH), 3.75 (s, 3H, OCH₃), 3.87 (t, J = 5.5 Hz, 2H, PhCH₂CH₂), 4.40 (d, J = 15.8 Hz, 1H, PhCH₂NH), 4.60 (d, J = 15.8 Hz, 1H, PhCH₂NH), 6.66 (d, J = 2.6 Hz, 1H, 5- H_{arom}), 6.77 (dd, J = 8.7 / 2.7 Hz, 1H, 7- H_{arom}), 7.01 - 7.12 (m, 3H, 8- H_{arom} , 3'- H_{arom} , 5'- H_{arom}), 7.39 (dd, J = 8.6 / 5.4 Hz, 2H, 2'- H_{arom} , 6'- H_{arom}), 7.69 (s, 2H, 2''- H_{arom} , 6''- H_{arom}), 7.80 (s, 1H, 4''- H_{arom}), a signal for the NH proton is not seen in the spectrum. ¹³C NMR (CD₃OD): δ [ppm] = 28.6 (1C, NCH₂CH₂CH), 29.2 (1C, PhCH₂CH₂), 34.9 (2C, N(CH₂CH₂)₂), 41.7 (1C, PhCH₂NH), 48.8 (1C, N(CH₂CH₂)₂), 49.0 (1C, N(CH₂CH₂)₂), 49.1 (1C, NCH₂CH₂CH), 54.2 (1C, OCH₃), 55.5 (1C, NCH₂CH₂CH), 58.7 (1C, PhCH₂CH₂), 71.3 (1C, ArCO), 112.4 (1C, C-7_{arom}), 113.0 (1C, C-5_{arom}), 115.2 (d, J = 21.9 Hz, 2C, C-3'_{arom}, C-5'_{arom}), 120.2 – 120.6 (m, 1C, C-4''_{arom}), 123.3 (q, J = 269.4 Hz, 2C, CF₃), 125.7 (1C, C-8_{arom}), 127.2 (q, J = 3.4 Hz, 2C, C-2''_{arom}, C-6''_{arom}), 129.1 (d, J = 8.1 Hz, 2C, C-2'_{arom}, C-6'_{arom}), 131.3 (q, J = 33.2 Hz, 2C, C-3''_{arom}, C-5''_{arom}), 135.1 (1C, C-1'_{arom}), 142.3 (1C, C-1''_{arom}), 158.2 (1C, C-6_{arom}), 162.3 (d, J = 243.5 Hz, 1C, C-4'_{arom}), 173.8 (1C, C=O), signals for the C-atoms C-4a and C-8a are not seen in the spectrum. MS (APCI): m/z = 639.2463 (calcd. 639.2452 for C₃₃H₃₄F₇N₂O₃ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2932 (C-H), 1659 (C=O), 1277 (C-F), 1169 (C-F_{arom}), 1126 (C-O).

5.1.35.4-(3,4-Dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)-N-2-(4-fluorophenyl)-[3-fluoro-5-(trifluoromethylbenzyl)]butanamide (4j)

8a (300 mg, 1.48 mmol) was dissolved in THF (6 mL) and the solution was cooled to 0 °C. **11d** (247 mg, 0.57 mmol) dissolved in THF (4 mL) was added dropwise over 30 min. Then the mixture was warmed to rt and stirred for 2 d at 40 °C. Then water was

added and the mixture was extracted with CH_2Cl_2 (3x). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was coated on silica gel and then purified by flash column chromatography (\varnothing 2 cm, length 15 cm, $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}:\text{NH}_3$ 95:4.5:0.5, fraction size 10 mL, $R_f = 0.16$ ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}:\text{NH}_3$ 95:4.5:0.5)). The product was recrystallized from CH_2Cl_2 and finally purified by preparative HPLC (Method 2). Pale yellow oil, yield 9.7 mg (3.0 %). Purity (HPLC): 97.9 %, $t_R = 20.53$ min. $\text{C}_{31}\text{H}_{31}\text{F}_5\text{N}_2\text{O}_2$ (558.6 g/mol). ^1H NMR (CD_3OD): δ [ppm] = 1.91 – 2.01 (m, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.00 – 2.08 (m, 1H, $\text{NCH}_2\text{CH}_2\text{CH}$), 2.11 (td, $J = 13.8 / 4.6$ Hz, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.35 – 2.46 (m, 1H, $\text{NCH}_2\text{CH}_2\text{CH}$), 2.62 – 2.76 (m, 2H, NCH_2), 2.80 (t, $J = 5.5$ Hz, 2H, PhCH_2CH_2), 2.81 – 2.89 (m, 2H, NCH_2), 3.05 (t, broad, $J = 13.8$ Hz, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 3.64 (dd, $J = 8.4 / 6.5$ Hz, 1H, $\text{NCH}_2\text{CH}_2\text{CH}$), 3.89 (t, $J = 5.5$ Hz, 2H, PhCH_2CH_2), 4.36 (d, $J = 15.7$ Hz, 1H, PhCH_2NH), 4.49 (d, $J = 15.7$ Hz, 1H, PhCH_2NH), 7.07 (t, $J = 8.8$ Hz, 2H, 3'- H_{arom} , 5'- H_{arom}), 7.08 – 7.20 (m, 5H, 5- H_{arom} , 6- H_{arom} , 7- H_{arom} , 8- H_{arom} , 2''- H_{arom}), 7.25 (s, 1H, 6''- H_{arom}), 7.29 (d, $J = 8.5$ Hz, 1H, 4''- H_{arom}), 7.40 (dd, $J = 8.8 / 5.3$ Hz, 2H, 2'- H_{arom} , 6'- H_{arom}), a signal for the NH proton is not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 28.8 (1C, $\text{NCH}_2\text{CH}_2\text{CH}$), 29.0 (1C, PhCH_2CH_2), 35.0 (2C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 41.7 (1C, PhCH_2NH), 48.8 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 48.9 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 49.2 (1C, $\text{NCH}_2\text{CH}_2\text{CH}$), 55.6 (1C, $\text{NCH}_2\text{CH}_2\text{CH}$), 58.7 (1C, PhCH_2CH_2), 71.8 (1C, ArCO), 110.8 (dd, $J = 25.2 / 3.8$ Hz, 1C, C-4'' $_{\text{arom}}$), 115.1 (d, $J = 21.6$ Hz, 2C, C-3' $_{\text{arom}}$, C-5' $_{\text{arom}}$), 117.5 (d, $J = 22.9$ Hz, 1C, C-2'' $_{\text{arom}}$), 119.3 (quint, $J = 4.1$ Hz, 1C, C-6'' $_{\text{arom}}$), 124.6 (1C, C-8 $_{\text{arom}}$), 126.0 (1C, C-6 $_{\text{arom}}$), 126.3 (1C, C-5 $_{\text{arom}}$), 128.6 (1C, C-7 $_{\text{arom}}$), 129.1 (d, $J = 8.3$ Hz, 2C, C-2' $_{\text{arom}}$, C-6' $_{\text{arom}}$), 132.2 (qd, $J = 26.1 / 8.4$ Hz, 1C, C-5'' $_{\text{arom}}$), 133.7 (1C, C-4a $_{\text{arom}}$), 136.3 (1C, C-1' $_{\text{arom}}$), 141.0 (1C, C-8a $_{\text{arom}}$), 143.5 (d, $J = 7.5$ Hz, 1C, C-1'' $_{\text{arom}}$), 162.3 (d, $J = 244.9$ Hz, 1C, C-3'' $_{\text{arom}}$), 162.5 (d, $J = 247.6$ Hz, 1C, C-4' $_{\text{arom}}$), 173.9 (1C, C=O), the signal for the C-atom of the CF_3 group is not seen

in the spectrum. MS (APCI): $m/z = 559.2392$ (calcd. 559.2378 for $C_{31}H_{32}F_5N_2O_2$ $[MH^+]$). IR: $\tilde{\nu}$ [cm^{-1}] = 2970 (C-H), 1651 (C=O), 1227 (C-F), 1169 (C-F_{arom}), 1126 (C-O).

5.1.36.2-(4-Fluorophenyl)-N-[3-fluoro-5-trifluoromethylbenzyl]-4-(3-methyl-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4k)

8b (260 mg, 1.19 mmol) was dissolved in THF (6 mL) and the solution was cooled to 0 °C. **11d** (176 mg, 0.40 mmol) dissolved in THF (4 mL) was added dropwise over 30 min. Then the mixture was warmed to rt and stirred for 2 d at 40 °C. Then water was added and the mixture was extracted with CH_2Cl_2 (3x). The combined organic layers were dried (Na_2SO_4), filtered and the solvent was removed in vacuo. The crude product was coated on silica gel and then purified by flash column chromatography (\varnothing 2 cm, length 15 cm, $CH_2Cl_2:CH_3OH:NH_3$ 95:4.5:0.5, fraction size 10 mL; $R_f = 0.16$ ($CH_2Cl_2:CH_3OH:NH_3$ 95:4.5:0.5)). Pale yellow solid, mp 92 °C, yield 43 mg (19 %). Purity (HPLC): 91.1 %, $t_R = 21.19$ min. $C_{32}H_{33}F_5N_2O_2$ (572.6 g/mol). 1H NMR (CD_3OD): δ [ppm] = 1.33 (d, $J = 6.1$ Hz, 3H, CH_3), 1.71 – 1.79 (m, 1H, $N(CH_2CH_2)_2$), 1.98 (td, $J = 13.9 / 4.2$ Hz, 1H, $N(CH_2CH_2)_2$), 2.05 – 2.15 (m, 1H, NCH_2CH_2CH), 2.18 – 2.25 (m, 1H, $N(CH_2CH_2)_2$), 2.30 (td, $J = 13.7 / 4.5$ Hz, 1H, $N(CH_2CH_2)_2$), 2.39 – 2.50 (m, 1H, NCH_2CH_2CH), 2.60 – 2.73 (m, 2H, $PhCH_2CH$), 2.74 – 2.85 (m, 2H, NCH_2), 2.89 – 3.05 (m, 2H, NCH_2), 3.10 – 3.21 (m, 2H, NCH_2), 3.66 (dd, $J = 8.5 / 6.4$ Hz, 1H, NCH_2CH_2CH), 3.85 – 3.93 (m, 1H, $PhCH_2CH$), 4.36 (d, $J = 15.7$ Hz, 1H, $PhCH_2NH$), 4.50 (d, $J = 15.7$ Hz, 1H, $PhCH_2NH$), 7.02 – 7.06 (m, 1H, 7- H_{arom}), 7.05 (t, $J = 8.7$ Hz, 2H, 3'- H_{arom} , 5'- H_{arom}), 7.09 – 7.14 (m, 3H, 5- H_{arom} , 6- H_{arom} , 8- H_{arom}), 7.16 (d, $J = 9.1$ Hz, 1H, 2''- H_{arom}), 7.26 (s, 1H, 6''- H_{arom}), 7.28 (d, $J = 8.5$ Hz, 1 Hz, 4''- H_{arom}), 7.39 (dd, $J = 8.7 / 5.4$ Hz, 2H, 2'- H_{arom} , 6'- H_{arom}), a signal for the NH proton is not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 20.6 (1C,

CH₃), 29.2 (1C, NCH₂CH₂CH), 33.8 (1C, N(CH₂CH₂)₂), 36.6 (1C, PhCH₂CH), 38.1 (1C, N(CH₂CH₂)₂), 41.7 (1C, PhCH₂NH), 48.7 (0.5 C, N(CH₂CH₂)₂), 48.8 (0.5 C, N(CH₂CH₂)₂), 49.0 (0.5 C, N(CH₂CH₂)₂), 49.2 (0.5 C, N(CH₂CH₂)₂), 49.6 (1C, NCH₂CH₂CH), 56.2 (1C, NCH₂CH₂CH), 64.1 (1C, PhCH₂CH), 73.1 (1C, ArCO), 110.7, (dq, $J = 25.1 / 3.9$ Hz, 1C, C-4''_{arom}), 115.0 (d, $J = 22.2$ Hz, 2C, C-3'_{arom}, C-5'_{arom}), 117.4 (d, $J = 21.3$ Hz, 1C, C-2''_{arom}), 119.3 (quint, $J = 3.9$ Hz, 1C, C-6''_{arom}), 124.5 (1C, C-8_{arom}), 125.8 and 126.9 (2C, C-5_{arom}, C-6_{arom}), 128.4 (1C, C-7_{arom}), 129.1 (d, $J = 8.0$ Hz, 2C, C-2'_{arom}, C-6'_{arom}), 133.9 (1C, C-4_{aarom}), 135.8 (1C, C-1'_{arom}), 141.0 (1C, C-8_{aarom}), 143.6 (d, $J = 7.5$ Hz, 1C, C-1''_{arom}), 162.2 (d, $J = 244.4$ Hz, 1C, C-3''_{arom}), 162.5 (d, $J = 247.5$ Hz, 1C, C-4'_{arom}), 174.3 (1C, C=O), signals for the C-atom of the CF₃ group and for C-5'' are not seen in the spectrum. MS (APCI): $m/z = 573.2558$ (calcd. 573.2535 for C₃₂H₃₄F₅N₂O₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2928 (C-H), 1651 (C=O), 1227 (C-F), 1126 (C-O).

5.1.37.2-(4-Fluorophenyl)-N-[3-fluoro-5-(trifluoromethyl)benzyl]-4-(6-methoxy-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4I)

8c (304 mg, 1.3 mmol) was dissolved in THF (6 mL) and the solution was cooled to 0 °C. **11d** (214 mg, 0.49 mmol) dissolved in THF (4 mL) was added dropwise over 30 min. Then the mixture was warmed to rt and stirred for 2 d at 40 °C. Then water was added and the mixture was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed in vacuo. The crude product was coated on silica gel and then purified by flash column chromatography (Ø 2 cm, length 15 cm, CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5, fraction size 10 mL; R_f = 0.23 (CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5)) and recrystallized from CH₂Cl₂. Yellow oil, yield 44 mg (5.7 %). Purity (HPLC): 82.1 %, t_R = 20.52 min. C₃₂H₃₃F₅N₂O₃ (588.6 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.91 - 2.00 (m, 2H, N(CH₂CH₂)₂), 2.09 (td, $J = 13.2 / 4.3$

Hz, 2H, N(CH₂CH₂)₂), 2.36 – 2.48 (m, 1H, NCH₂CH₂CH), 2.65 – 2.73 (m, 1H, NCH₂CH₂CH), 2.77 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 2.78 – 2.96 (m, 3H, NCH₂), 3.02 – 3.14 (m, 3H, NCH₂), 3.64 (t, *J* = 7.41 Hz, 1H, NCH₂CH₂CH), 3.75 (s, 3H, OCH₃), 3.87 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 4.35 (d, *J* = 15.7 Hz, 1H, PhCH₂NH), 4.49 (d, *J* = 15.7 Hz, 1H, PhCH₂NH), 6.66 (d, *J* = 2.7 Hz, 1H, 5-*H*_{arom}), 6.77 (dd, *J* = 8.7 / 2.7 Hz, 1H, 7-*H*_{arom}), 7.04 (d, *J* = 8.7 Hz, 1H, 8-*H*_{arom}), 7.08 (t, *J* = 8.9 Hz, 2H, 3'-*H*_{arom}, 5'-*H*_{arom}), 7.16 (d, *J* = 8.5 Hz, 1H, 2''-*H*_{arom}), 7.25 (s, 1H, 6''-*H*_{arom}), 7.29 (d, *J* = 8.7 Hz, 1H, 4''-*H*_{arom}), 7.40 (dd, *J* = 8.6 / 5.3 Hz, 2H, 2'-*H*_{arom}, 6'-*H*_{arom}), a signal for the NH proton is not seen in the spectrum. ¹³C NMR (CD₃OD): δ [ppm] = 29.2 (1C, NCH₂CH₂CH), 29.4 (1C, PhCH₂CH₂), 35.9 (2C, N(CH₂CH₂)₂), 41.7 (1C, PhCH₂NH), 48.8 (1C, N(CH₂CH₂)₂), 49.1 (1C, N(CH₂CH₂)₂), 49.6 (1C, NCH₂CH₂CH), 54.2 (1C, OCH₃), 56.2 (1C, NCH₂CH₂CH), 58.4 (1C, PhCH₂CH₂), 72.2 (1C, ArCO), 110.7 (dq, *J* = 25.0 / 3.8 Hz, 1C, C-4''_{arom}), 112.2 (1C, C-7_{arom}), 112.8 (1C, C-5_{arom}), 115.0 (d, *J* = 22.5 Hz, 2C, C-3'_{arom}, C-5'_{arom}), 117.4 (d, *J* = 22.6 Hz, 1C, C-2''_{arom}), 119.3 (quint, *J* = 3.4 Hz, 1C, C-6''_{arom}), 125.9 (1C, C-8_{arom}), 129.1 (d, *J* = 8.9 Hz, 2C, C-2'_{arom}, C-6'_{arom}), 132.1 (qd, *J* = 33.2 / 8.4 Hz, 1C, C-5''_{arom}), 133.4 (1C, C-4a_{arom}), 134.9 (1C, C-8a_{arom}), 135.8 (d, *J* = 3.0 Hz, 1C, C-1'_{arom}), 143.6 (d, *J* = 7.3 Hz, 1C, C-1''_{arom}), 158.0 (1C, C-6_{arom}), 162.1 (d, *J* = 244.5 Hz, 1C, C-3''_{arom}), 162.5 (d, *J* = 247.6 Hz, 1C, C-4'_{arom}), 174.3 (1C, C=O), the signal for the C-atom of the CF₃ group is not seen in the spectrum. MS (APCI): *m/z* = 589.2497 (calcd. 589.2484 for C₃₂H₃₄F₅N₂O₃ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2924 (C-H), 1647 (C=O), 1226 (C-F), 1169 (C-F_{arom}), 1126 (C-O).

5.1.38. 4-[6-(Benzyloxy)-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl]-2-(4-fluorophenyl)-N-[3-fluoro-5-(trifluoromethyl)benzyl]]butanamide (4m)

8e (59 mg, 0.19 mmol) and bromide **11d** (40 mg, 0.09 mmol) were dissolved in DMF (4 mL). Diisopropylethylamine (21 mg, 0.16 mmol) and tetrabutylammonium iodide

(20 mg, 0.05 mmol) were added and the reaction mixture was heated under microwave irradiation for 60 min at 203 °C, 38 psi and 150 W. Then 10 % aqueous NaHCO₃ was added and the aqueous layer was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (Ø 2 cm, length 17 cm, CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5, fraction size 10 ml, R_f = 0.18 (CH₂Cl₂:CH₃OH:NH₃ 95:4.5:0.5)). Yellow oil, yield 17 mg (27 %). Purity (HPLC): 49.9 %, t_R = 23.67 min. C₃₈H₃₇F₅N₂O₃ (664.7 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 1.90 – 1.97 (m, 2H, N(CH₂CH₂)₂), 2.03 – 2.11 (m, 2H, N(CH₂CH₂)₂), 2.36 – 2.44 (m, 1H, NCH₂CH₂CH), 2.61 – 2.67 (m, 1H, NCH₂CH₂CH), 2.68 – 2.73 (m, 1H, NCH₂), 2.75 (t, *J* = 5.4 Hz, 2H, PhCH₂CH₂), 2.78 – 2.85 (m, 3H, NCH₂), 2.99 – 3.09 (m, 2H, NCH), 3.63 – 3.69 (m, 1H, NCH₂CH₂CH), 3.85 (t, *J* = 5.5 Hz, 2H, PhCH₂CH₂), 4.34 (d, *J* = 15.7 Hz, 1H, PhCH₂NH), 4.48 (d, *J* = 15.7 Hz, 1H, PhCH₂NH), 5.04 (s, 2H, PhCH₂O), 6.73 (d, *J* = 2.6 Hz, 1H, 5-*H*_{arom}), 6.83 (dd, *J* = 8.6 / 2.7 Hz, 1H, 7-*H*_{arom}), 7.03 – 7.04 (m, 1H, 8-*H*_{arom}), 7.03 – 7.12 (m, 2H, 3'-*H*_{arom}, 5'-*H*_{arom}), 7.14 (m, 1H, 2''-*H*_{arom}), 7.24 (s, 1H, 6''-*H*_{arom}), 7.27 – 7.31 (m, 1H, 4''-*H*_{arom}), 7.32 – 7.36 (m, 2H, 2'-*H*_{arom}, 6'-*H*_{arom}), 7.35 – 7.42 (m, 5H, *H*^{'''}_{arom}), a signal for the NH proton is not seen in the spectrum. MS (APCI): *m/z* = 665.2766 (calcd. 665.2797 for C₃₈H₃₈F₅N₂O₃ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2924 (C-H), 1655 (C=O), 1227 (C-F), 1126 (C-F_{arom}).

5.1.39. 2-(4-Fluorophenyl)-N-[3-fluoro-5-(trifluoromethyl)benzyl]-4-(6-hydroxy-3,4-dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4n)

4m was dissolved in CH₃OH (0.8 mL) and Pd/C (10% w/w, 22 mg, 0.2 mmol) was added. The mixture was stirred under H₂-atmosphere in a hydrogenation apparatus

(1 bar) for 1 h. The reaction mixture was then filtered over Celite® and the eluent was concentrated in vacuo. The resulting crude product was purified by flash column chromatography (\varnothing 1 cm, length 18 cm, $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}:\text{NH}_3$ 95:4.5:0.5, fraction size 5 ml, $R_f = 0.05$ ($\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}:\text{NH}_3$ 95:4.5:0.5)). Brown oil, yield 5.2 mg (48 %). Purity (HPLC): 80.3 %, $t_R = 20.39$ min. $\text{C}_{31}\text{H}_{31}\text{F}_5\text{N}_2\text{O}_3$ (574.6 g/mol). ^1H NMR (CD_3OD): δ [ppm] = 1.79 – 1.84 (m, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 1.96 (td, $J = 13.4 / 4.6$ Hz, 2H, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 2.30 – 2.35 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}$), 2.35 – 2.42 (m, 3H, NCH_2), 2.69 (t, $J = 5.5$ Hz, 2H, PhCH_2CH_2), 2.71 – 2.82 (m, 3H, NCH_2), 3.58 (t, $J = 7.4$ Hz, 1H, $\text{NCH}_2\text{CH}_2\text{CH}$), 3.83 (t, $J = 5.5$ Hz, 2H, PhCH_2CH_2), 4.38 (d, $J = 15.7$ Hz, 1H, PhCH_2NH), 4.44 (d, $J = 15.7$ Hz, 1H, PhCH_2NH), 6.49 (d, $J = 2.6$ Hz, 1H, 5- H_{arom}), 6.60 (dd, $J = 8.5 / 2.7$ Hz, 1H, 7- H_{arom}), 6.95 (d, $J = 8.5$ Hz, 1H, 8- H_{arom}), 7.05 (d, $J = 8.7$ Hz, 2H, 3'- H_{arom} , 5'- H_{arom}), 7.16 (d, $J = 9.6$ Hz, 1H, 4''- H_{arom}), 7.25 – 7.30 (m, 2H, 2''- H_{arom} , 6''- H_{arom}), 7.39 (dd, $J = 8.7 / 5.4$ Hz, 2H, 2'- H_{arom} , 6'- H_{arom}), signals for the NH and OH protons are not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 29.4 (1C, $\text{NCH}_2\text{CH}_2\text{CH}$), 29.9 (1C, PhCH_2CH_2), 36.13 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 36.14 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 41.7 (1C, PhCH_2NH), 48.8 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 49.1 (1C, $\text{N}(\text{CH}_2\text{CH}_2)_2$), 49.7 (1C, $\text{NCH}_2\text{CH}_2\text{CH}$), 56.4 (1C, $\text{NCH}_2\text{CH}_2\text{CH}$), 58.4 (1C, PhCH_2CH_2), 72.5 (1C, ArCO), 110.7 (dq, $J = 24.9 / 4.0$ Hz, 1C, C-4'' $_{\text{arom}}$), 113.3 (1C, C-7 $_{\text{arom}}$), 114.3 (1C, C-5 $_{\text{arom}}$), 114.9 (d, $J = 22.0$ Hz, 2C, C-3' $_{\text{arom}}$, C-5' $_{\text{arom}}$), 117.4 (d, $J = 22.5$ Hz, 1C, C-2'' $_{\text{arom}}$), 119.3 (quint, $J = 3.9$ Hz, 1C, C-6'' $_{\text{arom}}$), 125.9 (1C, C-8 $_{\text{arom}}$), 129.1 (d, $J = 8.1$ Hz, 2C, C-2' $_{\text{arom}}$, C-6' $_{\text{arom}}$), 132.3 (1C, C-4a $_{\text{arom}}$), 134.8 (1C, C-8a $_{\text{arom}}$), 135.9 (d, $J = 3.5$ Hz, 1C, C-1' $_{\text{arom}}$), 143.6 (d, $J = 7.5$ Hz, 1C, C-1'' $_{\text{arom}}$), 155.3 (1C, C-6 $_{\text{arom}}$), 162.1 (d, $J = 244.5$ Hz, 1C, C-3'' $_{\text{arom}}$), 162.5 (d, $J = 248.0$ Hz, 1C, C-4' $_{\text{arom}}$), 174.4 (1C, C=O), signals for the C-atom of the CF_3 group and for C-5'' are not seen in the

spectrum. MS (APCI): $m/z = 575.2336$ (calcd. 575.2328 for $C_{31}H_{32}F_5N_2O_3 [MH^+]$). IR:

$\tilde{\nu} [cm^{-1}] = 2928$ (C-H), 1643 (C=O), 1227 (C-F), 1126 (C-F_{arom}).

5.1.40.N-[3,5-Bis(trifluoromethyl)benzyl]-2-cyclopropyl-4-(3,4-

dihydrospiro[[2]benzopyran-1,4'-piperidin]-1'-yl)butanamide (4o, WMS-46-14)

8a (70 mg, 0.34 mmol) and bromide **15e** (61 mg, 0.14 mmol) were solved in DMF (6 mL). Diisopropylethylamine (39 mg, 0.30 mmol) and tetrabutylammonium iodide (27 mg, 0.07 mmol) were added and the reaction mixture was heated under microwave irradiation for 60 min at 203 °C, 38 psi and 150 W. Then 10 % aqueous NaHCO₃ was added and the aqueous layer was extracted with CH₂Cl₂ (3x). The combined organic layers were dried (Na₂SO₄), filtered and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography twice (Ø 1 cm, length 28 cm, CH₂Cl₂:CH₃OH:NH₃ 96.5:3:0.5, fraction size 5 ml; Ø 1 cm, length 22 cm, CH₂Cl₂:CH₃OH:NH₃ 96.5:3:0.5, fraction size 5 ml; R_f = 0.08 (CH₂Cl₂:CH₃OH:NH₃ 96.5:3:0.5)). Yellow oil, yield 26 mg (34 %). Purity (HPLC): 93.9 %, t_R = 21.86 min. C₂₉H₃₂F₆N₂O₂ (554.6 g/mol). ¹H NMR (CD₃OD): δ [ppm] = 0.25 (dq, $J = 9.4 / 5.0$ Hz, 1H, CH_{2cycloprop}), 0.35 (dq, $J = 9.9 / 5.0$ Hz, 1H, CH_{2cycloprop}), 0.54 (tt, $J = 9.2 / 4.9$ Hz, 1H, CH_{2cycloprop}), 0.65 (tt, $J = 9.4 / 4.3$ Hz, 1H, CH_{2cycloprop}), 0.85 – 0.91 (m, 1H, NCH₂CH₂CH), 0.92 – 1.01 (m, 1H, NCH₂CH₂CH), 0.97 – 1.04 (m, 1H, CH_{cycloprop}), 1.60 (td, $J = 9.3 / 4.9$ Hz, 1 H, NCH₂CH₂CH), 1.93 – 2.03 (m, 2H, N(CH₂CH₂)₂), 2.06 – 2.13 (m, 2H, N(CH₂CH₂)₂), 2.60 – 2.70 (m, 2H, NCH₂), 2.81 (t, $J = 5.5$ Hz, 2H, PhCH₂CH₂), 2.82 – 2.90 (m, 2H, NCH₂), 3.02 – 3.08 (m, 2H, NCH₂), 3.90 (t, $J = 5.5$ Hz, 2H, PhCH₂CH₂), 4.49 (d, $J = 15.7$ Hz, 1H, PhCH₂NH), 4.63 (d, $J = 15.5$ Hz, 1H, PhCH₂NH), 7.10 – 7.21 (m, 4H, H_{arom}), 7.86 (s, 1H, 4'-H_{arom}), 7.91 (s, 2H, 2'-H_{arom}, 6'-H_{arom}), a signal for the NH proton is not seen in the spectrum. ¹³C

NMR (CD₃OD): δ [ppm] = 2.7 (1C, CH₂_{cycloprop}), 3.7 (1C, CH₂_{cycloprop}), 13.7 (1C, CH_{cycloprop}), 29.0 (1C, NCH₂CH₂CH), 29.2 (1C, PhCH₂CH₂), 34.9 (2C, N(CH₂CH₂)₂), 41.7 (1C, PhCH₂NH), 48.6 (1C, N(CH₂CH₂)₂), 49.0 (1C, N(CH₂CH₂)₂), 55.6 (1C, NCH₂CH₂CH), 58.8 (1C, PhCH₂CH₂), 120.5 (hept, $J = 3.9$ Hz, 1C, C-4'_{arom}), 124.6 (1C, C-8_{arom}), 126.0 and 126.3 (2C, C-5_{arom}, C-6_{arom}), 127.3 – 127.6 (m, 2C, C-2'_{arom}, C-6'_{arom}), 128.6 (1C, C-7_{arom}), 133.7 (1C, C-4a_{arom}), 135.5 (1C, C-1'_{arom}), 142.5 (1C, C-8a_{arom}), 208.6 (1C, C=O), signals for the C-atoms of the ArCO group, the CF₃ groups, for C-3' and C-5' are not seen in the spectrum. MS (APCI): $m/z = 555.2455$ (calcd. 555.2441 for C₂₉H₃₃F₆N₂O₂ [MH⁺]). IR: $\tilde{\nu}$ [cm⁻¹] = 2920 (C-H), 1647 (C=O), 1276 (C-F), 1169 (C-F_{arom}), 1126 (C-O).

5.2. Pharmacology

5.2.1. Radioligand CCR2 binding assay

Materials

[¹²⁵I]-CCL2 (81.4 GBq/ μ mol (2200 Ci/mmol)) was purchased from Perkin-Elmer (Waltham, MA). INCB3344 was synthesized as described previously.²⁶ Tango CCR2-*bla* U2OS cells stably expressing human CCR2 were obtained from Invitrogen (Carlsbad, CA).

Cell culture and membrane preparation

U2OS cells stably expressing the human CCR2 (Invitrogen, Carlsbad, CA) were cultured in McCoy5a medium supplemented with 10% fetal calf serum, 2 mM glutamine, 0.1 mM non-essential amino acids (NEAAs), 25 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 μ g/mL streptomycin, 100 μ g/mL G418, 50 μ g/mL hygromycin,

and 125 µg/mL zeocin in a humidified atmosphere at 37 °C and 5% CO₂. Cell culture and membrane preparation were performed as described previously.¹⁹

¹²⁵I-CCL2 binding assays

Binding assays were performed in a 100-µl reaction volume containing 50 mM Tris-HCl buffer (pH 7.4), 5 mM MgCl₂, 0.1% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonic acid (CHAPS) and 15 µg of membrane protein at 37 °C. Nonspecific binding was determined with 10 µM INCB3344. Displacement assays were performed with 0.1 nM ¹²⁵I-CCL2 using at least 6 concentrations of competing ligand for 150 minutes of incubation. The HP D300 digital dispenser from Tecan (Männedorf, Switzerland) was used to dispense the compounds in DMSO directly into the assay plate. Incubations were terminated by dilution with ice-cold 50 mM Tris-HCl buffer supplemented with 0.05% CHAPS and 0.5 M NaCl. Separation of bound from free radioligand was performed by rapid filtration through a 96-well GF/B filter plate precoated with 0.25% polyethylenimine using a PerkinElmer Filtermate-harvester (PerkinElmer, Groningen, The Netherlands). Filters were washed 10 times with ice-cold wash buffer, and 25 µl of Microscint scintillation cocktail (PerkinElmer) was added to each well; the filter-bound radioactivity was determined by scintillation spectrometry using the P-E 1450 Microbeta Wallac Trilux scintillation counter (PerkinElmer).

Data analysis

All experiments were analyzed using the nonlinear regression curve fitting program Prism 5 (GraphPad, San Diego, CA). For radioligand displacement data, K_i values were calculated from IC₅₀ values using the Cheng and Prusoff equation.²⁷

5.2.2. Functional CCR2 assays

Materials

Chem-1 cell line transfected with human CCR2 (ChemiSCREEN™ CCR2B Calcium-Optimized FLIPR Cell Line, Merck Millipore) was used for the intracellular calcium flux assay. U2OS β -arrestin cell line transfected with murine CCR2 (93-0543C3, DiscoverX Corporation, Ltd.) was used for the β -arrestin recruitment assay. Chemicals and reagents were purchased from different commercial sources and of analytical grade.

Measurement of intracellular calcium flux

Chem-1 cells transfected with human CCR2 were cultured in DMEM high glucose medium (supplemented by 10 % FCS, 1 mM pyruvate, 15 mM HEPES, 500 μ g/ml geniticine and non-essential amino acids (NEAA)). The cells were transferred into Optimem (supplemented by 5% FCS, 50 U/ml penicilline and 50 μ g/ml streptomycine and NEAA) and seeded into 384-well plates (μ CLEAR/black Greiner Bio One) at a density of 5000 cells/25 μ l. Cells were incubated for approximately 24 h at 37 °C, 5 % CO₂. Before the assay medium was removed and the cells were incubated with Fluo-4 solution (25 μ l Tyrode's solution containing 3 μ M Fluo-4 AM (1 mM DMSO stock solution), 0.4 mg/ml brilliant black, 2.5 mM probenicide, 0.03 % pluronic F-127) for 60 min at 37 °C, 5% CO₂. The compounds were dissolved in DMSO with 10 mM stock concentration followed by further dilution with DMSO in 1/3.16 steps. Required test solutions for the assay were obtained by dilution with Tyrode's solution containing 2 mM CaCl₂ and 0,05 % BSA. Compounds (10 μ L per well) were added and cells were incubated for 10 min at 37 °C, 5 % CO₂. Then 20 μ l of agonist solution (recombinant human CCL2 (PeproTech, 300-04) in Tyrode's solution with 0.05 % BSA) were added. CCL2 was applied at EC₅₀, which was determined in an experiment prior to

compound testing (approximately 5 nM). Fluorescence intensity (excitation: 485 nm, emission: 520 nm) was measured for 120 s in 1.0 s intervals by a proprietary fluorescence measuring device. The effect of each concentration was recorded four times. The mean values of the four experiments were used to generate one sigmoidal curve. IC₅₀ values were fitted using a 4 parameter logistic function (Hill function).

β-Arrestin recruitment assay

U2OS β-arrestin cell line transfected with murine CCR2 were cultured in MEM Eagle medium (supplemented by 10 % FCS, 50 U/ml penicilline, 50 µg/ml streptomycine, 250 µg/ml hygromycine and 500 µg/ml geniticine). The cells were transferred into Optimem (supplemented by 1 % FCS, 50 U/ml penicilline and 50 µg/ml streptomycine) and seeded into 384-well plates (µCLEAR/black Greiner Bio One) at a density of 2000 cells/25 µl. Cells were incubated for approximately 24 h at 37 °C, 5 % CO₂. The compounds were dissolved in DMSO with 10 mM stock concentration followed by further dilution with DMSO in 1/3.16 steps. Required test solutions for the assay were obtained by dilution with Tyrode's solution containing 2 mM CaCl₂ and 0,05 % BSA. Compounds (10 µL per well) were added and cells were incubated for 10 min at 37 °C, 5 % CO₂. Then 20 µl of agonist solution (recombinant murine CCL2 (PeproTech, 250-10) in Tyrode's solution with 0.05 % BSA) were added. CCL2 was applied at EC₅₀, which was determined in an experiment prior to compound testing (approximately 3 nM).

After 90 min of incubation at room temperature, 50 µL of detection reagent (93-001, DiscoverX Corporation, Ltd.) per well were added. After additional 60 min of incubation at room temperature luminescent signal was detected by a proprietary luminescence-measuring device. The effect of each concentration was recorded four

times. The mean values of the four experiments were used to generate one sigmoidal curve. IC₅₀ values were fitted using a 4 parameter logistic function (Hill function).

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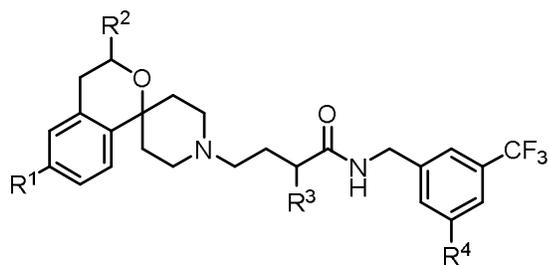
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Graphical Abstract

CCR2 antagonistic activity (Ca^{2+} flux)
 $R^1 = R^2 = \text{H} > R^1 = \text{OCH}_3, R^2 = \text{H} > R^1 = \text{H}, R^2 = \text{CH}_3$
 $R^3 = \text{cyclopropyl} > R^3 = \text{4-fluorophenyl} > R^3 = \text{H}$
 $R^4 = \text{CF}_3 > R^4 = \text{F}$

ACCEPTED MANUSCRIPT