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

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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 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.

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 CX_3C chemokines) groups. These chemokines interact with the corresponding CC, CXC, C, and CX_3C 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 chemotactic 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

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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] (Fig. 1). Whereas the butanamide **1** without a substituent at the linear alkyl chain shows an IC_{50} 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]

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 $BF_3{\cdot}Et_2O$ was used. At room temperature the transformation stopped at the intermediate mixed acetal. However microwave irradiation of the reaction mixture and addition of $Bi(OTf)_3$ 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.

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 CH_3OH/H_2SO_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.

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 ($NaBH(OAc)_3$) 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 ^{1}H 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

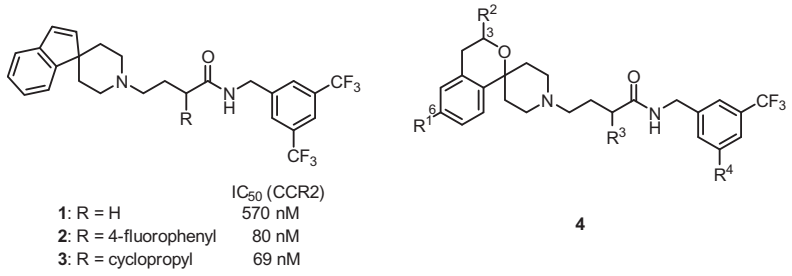
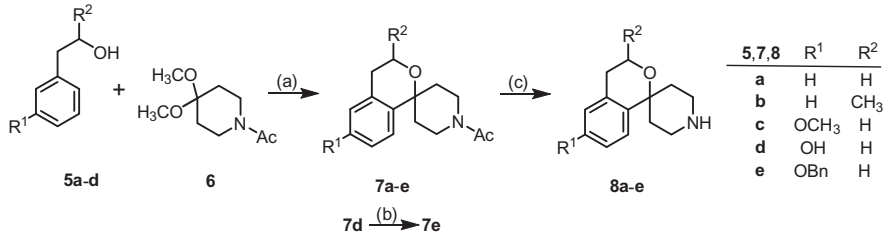
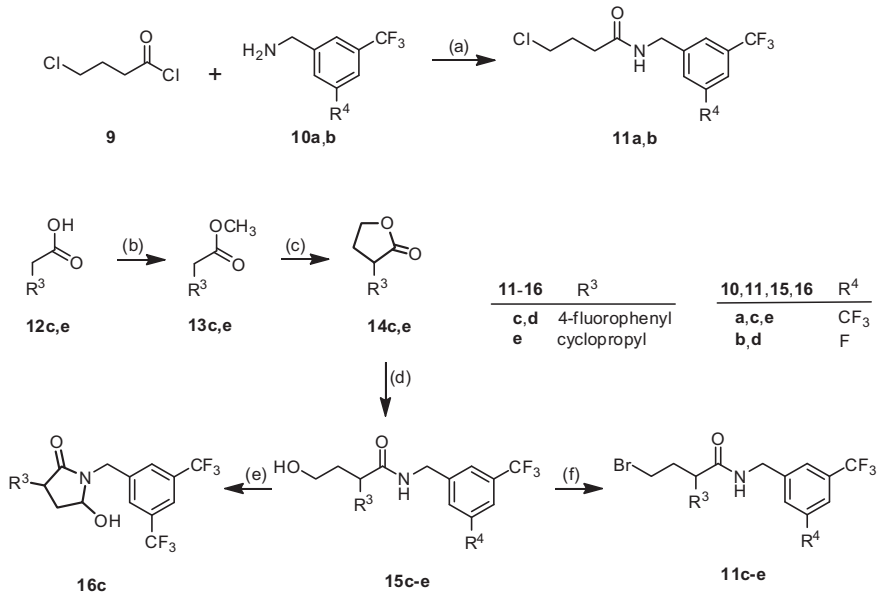


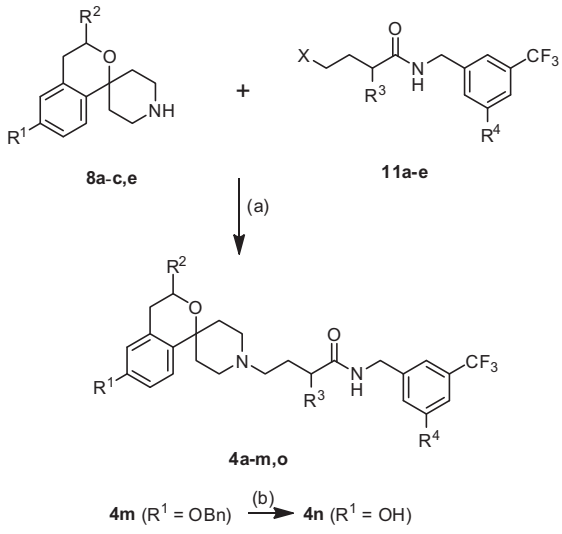
Figure 1. Spirocyclic CCR2 antagonists **1**–**3** serve as lead compounds.



Scheme 1. Synthesis of the spirocyclic piperidines **8a**–**e**. Reagents and reaction conditions: (a) $BF_3{\cdot}Et_2O$, $Bi(OTf)_3$, 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%.



Scheme 2. Synthesis of γ-halobutanamides **11a**–**e**. Reagents and reaction conditions: (a) Na_2CO_3, CH_3CN, rt, 16 h, 89–96%; (b) concd H_2SO_4, CH_3OH, 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**, $AlCl_3$, CH_2Cl_2, 0 °C → rt, 16 h, **15c**,**d** 23%; **10a**, 1,2,4-triazole, diazabicycloundecene, $CDCl_3$, rt, 48 h, **15e** 7.1%; (e) Dess–Martin-Periodinane, CH_2Cl_2, rt, 1 h, 73%; (f) CBr_4, polymer-bound triphenylphosphine, CH_3CN, 0 °C → rt, 48 h, 73–99%.



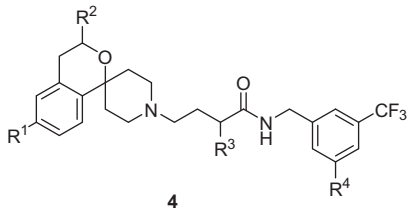
Scheme 3. Synthesis of final test compounds **4a**–**o**. Reagents and reaction conditions: (a) K_2CO_3, THF, reflux, 16 h, **4a**–**c**,**e**,**f** 3.5–43%; THF, 0 °C → 40 °C, 48 h, **4d**,**g**–**l** 3–44%; diisopropylethylamine, BuNI, DMF, microwave irradiation, 1 h, 203 °C, 150 W, **4m**,**o** 27–34%; (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.

(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.

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**



4

Compd	R^1	R^2	R^3	R^4	^{125}I-CCL2 displacement[a] (%)	IC_{50} human CCR2[b] (μM) (Ca^{2+} flux)	IC_{50} murine CCR2[b] (μM) (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_6H_4	CF_3	25	0.089	11.2
4h	H	CH_3	4-F-C_6H_4	CF_3	0	0.462	3.67
4i (WMS-46-09)	OCH_3	H	4-F-C_6H_4	CF_3	nd	0.163	4.16
4j	H	H	4-F-C_6H_4	F	7	0.173	3.63
4k	H	CH_3	4-F-C_6H_4	F	9	1.60	8.01
4l	OCH_3	H	4-F-C_6H_4	F	−7	0.353	4.63
4n	OH	H	4-F-C_6H_4	F	nd	6.10	>30
4o (WMS-46-14)	H	H	c-C_3H_5	CF_3	nd	0.017	3.3
TAK779					K_i = 2.0 ± 0.7 nM[c]	0.95 nM	23

nd = not determined.
[a] Displacement of the radioligand ^{125}I-CCL2 from human CCR2 (%), measured at a concentration of 1 μM of the test compound.
[b] The IC_{50}-values in the functional Ca^{2+}-flux assay (human CCR2) and the β-arrestin recruitment assay (murine CCR2) are given in μM, respectively. In the Ca^{2+}-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 IC_{50}-value of 27 nM is given for TAK779.[21,22]

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

However, in the Ca^{2+}-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**).

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, Fig. 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 IC_{50}-values of 89 and 17 nM, respectively. Figure 2 shows full inhibitory activity of **4g**, **4i** and **4o** in the Ca^{2+}-flux assay.

In the β-arrestin recruitment assay using murine CCR2 IC_{50}-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 IC_{50}-values between 3 and 5 μM. Although the IC_{50}-values in this assay are considerably higher than in the Ca^{2+}-flux assay, the tendency of increased antagonistic activity of 4-fluorophenyl and, moreover, cyclopropyl derivatives is confirmed herein. With exception of **4g**, the IC_{50}-values of bis(trifluoromethyl)benzylamides are lower than the IC_{50}-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 ^{125}I-CCL2, the Ca^{2+}-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 Ca^{2+}-ions. Interestingly, this class of compounds does not disturb the interaction of the human CCR2 with its large endogenous ligand CCL2, but inhibits the Ca^{2+} 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 studies[19] it has been shown that different

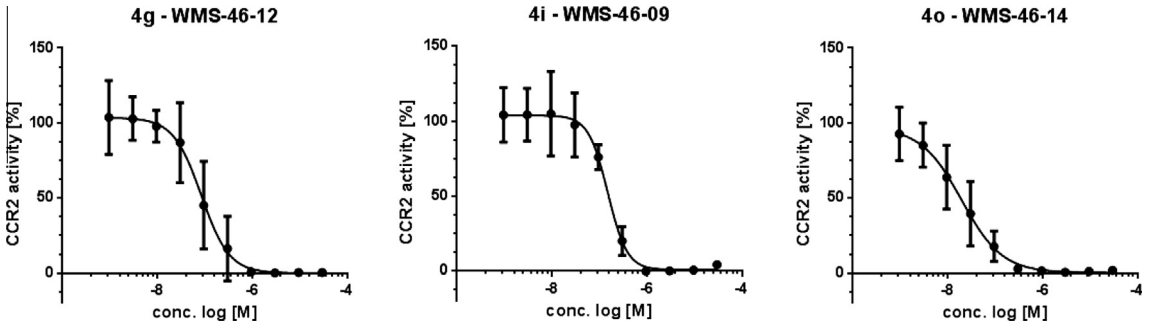


Figure 2. Dose response curves of the most potent CCR2 antagonists in the Ca^{2+}-flux assay.

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 Daltronics); 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 ^{1}H, 151 MHz for ^{13}C), Agilent 400-MR spectrometer (400 MHz for ^{1}H, 101 MHz for ^{13}C) or Varian AS 400 Mercury Plus NMR spectrometer; δ in ppm related to tetramethylsilane and measured referring to $CDCl_3$ (δ = 7.26 ppm (^{1}H NMR) and δ = 77.2 ppm (^{13}C NMR)) and CD_3OD (δ = 3.31 ppm (^{1}H NMR) and δ = 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 μm); LiChroCART® 250-4 mm cartridge; flow rate: 1.0 mL/min; injection volume: 5.0 μL; 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 → 0%, 29–31 min: 0%, 31–31.5 min: 0 → 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 μm; flow rate: 20.00 mL/min; injection volume: 200.0 μL; detection at λ = 254 nm; solvent: acetonitrile/water 70:30 with 0.05% (v/v) NH_3.

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 CH_3OH (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 $NaHCO_3$ and the mixture was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), 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%). $C_9H_{17}NO_3$ (187.2 g/mol). MS (APCI): m/z = 188.1291 (calcd 188.1281 for $C_9H_{18}NO_3$ [MH^+]). ^{1}H NMR (DMSO-d_6): δ [ppm] = 1.57 (t, J = 5.8 Hz, 2H, N(CH_2CH_2)$_2$), 1.67 (t, J = 5.8 Hz, 2H, N(CH_2CH_2)$_2$), 1.99 (s, 3H, NCOCH_3), 3.10 (s, 6H, OCH_3), 3.34–3.42 (m, 4H, N(CH_2CH_2)$_2$). ^{13}C NMR (DMSO-d_6): δ [ppm] = 21.7 (1C, CH_3), 32.3 (1C, N(CH_2CH_2)$_2$), 33.1 (1C, N(CH_2CH_2)$_2$), 38.4 (1C, N(CH_2CH_2)$_2$), 43.3 (1C, N(CH_2CH_2)$_2$), 47.6 (2C, OCH_3), 98.5 (1C, C(OCH_3)$_2$), 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 CH_3CN (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 CH_3CN (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. $BF_3{\cdot}Et_2O$ (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 $NaHCO_3$ to the combined mixtures. Layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3×). Combined organic layers were dried (Na_2SO_4), 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 (CH_2Cl_2/MeOH 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. $C_{15}H_{19}NO_2$ (245.3 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.88 (dd, J = 11.0/4.6 Hz, 2H, N(CH_2CH_2)$_2$), 1.99 (td, J = 13.3/4.7 Hz, 2H, N(CH_2CH_2)$_2$), 2.15 (s, 3H, NCOCH_3), 2.82 (t, J = 5.5 Hz, 2H, PhCH_2CH_2), 3.01 (td, J = 12.0/4.9 Hz, 1H, N(CH_2CH_2)$_2$), 3.51 (td, J = 13.1/3.2 Hz, 1H, N(CH_2CH_2)$_2$), 3.76–3.82 (m, 1H, N(CH_2CH_2)$_2$), 3.93 (t, J = 5.5 Hz, 2H, PhCH$_2$CH_2), 4.41–4.48 (m, 1H, N(CH_2CH_2)$_2$),

7.08–7.19 (m, 4H, H_{arom}). ^{13}C NMR (CD_3OD): δ [ppm] = 19.8 (1C, $COCH_3$), 29.1 (1C, $PhCH_2CH_2$), 35.9 (1C, $N(CH_2CH_2)_2$), 36.6 (1C, $N(CH_2CH_2)_2$), 37.5 (1C, $N(CH_2CH_2)_2$), 42.4 (1C, $N(CH_2CH_2)_2$), 58.8 (1C, $PhCH_2CH_2$), 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-4a$_{arom}$), 140.8 (1C, C-8a$_{arom}$), 170.1 (1C, C=O). MS (APCI): m/z = 246.1487 (calcd 246.1489 for $C_{15}H_{20}NO_2$ [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 CH_3CN (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 CH_3CN (3.2 mL). 0.4 mL of this solution were added to each mixture and the mixtures were stirred at rt for 30 min. $BF_3{\cdot}Et_2O$ (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 $NaHCO_3$ to the combined mixtures. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), 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 (CH_2Cl_2/CH_3OH 96:4)). Colorless solid, mp 128 °C, yield 4.42 g (64%). Purity (HPLC): 98.6%, t_R = 18.43 min. $C_{16}H_{21}NO_2$ (259.3 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.35 (d, J = 6.1 Hz, 3× 0.5 H, CH_3), 1.35 (d, J = 6.1 Hz, 3× 0.5 H, CH_3), 1.61–1.76 (m, 1H, $N(CH_2CH_2)_2$), 1.83 (td, J = 13.8/4.6 Hz, 1H, $N(CH_2CH_2)_2$), 2.03 (td, J = 13.3/4.9 Hz, 1H, $N(CH_2CH_2)_2$), 2.08–2.15 (m, 1H, $N(CH_2CH_2)_2$), 2.16 (s, 3H, $NCOCH_3$), 2.67–2.70 (m, 2H, $PhCH_2CH$), 3.01 (td, J = 13.1/2.9 Hz, 0.5 H, $N(CH_2CH_2)_2$), 3.10 (td, J = 13.1/2.9 Hz, 0.5 H, $N(CH_2CH_2)_2$), 3.52 (td, J = 13.2/2.8 Hz, 0.5 H, $N(CH_2CH_2)_2$), 3.62 (td, J = 13.1/2.8 Hz, 0.5 H, $N(CH_2CH_2)_2$), 3.75–3.84 (m, 1H, $N(CH_2CH_2)_2$), 3.89–3.99 (m, 1H, $PhCH_2CH$), 4.40–4.49 (m, 1H, $N(CH_2CH_2)_2$), 7.05–7.21 (m, 4H, CH_{arom}). ^{13}C NMR (CD_3OD): δ [ppm] = 19.83 (0.5C, $COCH_3$), 19.85 (0.5C, $COCH_3$), 20.62 (0.5C, CH_3), 20.63 (0.5C, CH_3), 29.3 (1C, CH_2CHO), 34.0 and 34.6 (1C, $N(CH_2CH_2)_2$), 37.4 and 37.6 (1C, $N(CH_2CH_2)_2$), 38.4 and 39.1 (1C, $N(CH_2CH_2)_2$), 42.3 and 42.4 (1C, $N(CH_2CH_2)_2$), 64.3 and 64.4 (1C, CH_2CHO), 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-4a$_{arom}$), 140.56 and 140.57 (1C, C-8a$_{arom}$), 170.09 and 170.13 (1C, C=O). MS (APCI): m/z = 260.1634 (calcd 260.1645 for $C_{16}H_{22}NO_2$ [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 CH_3CN (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 CH_3CN (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. $BF_3{\cdot}Et_2O$ (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 $NaHCO_3$ to the combined mixtures. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), 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 15 cm, cyclohexane/EtOAc 20:80, fraction size 65 mL, R_f = 0.20 (CH_2Cl_2/CH_3OH 96:4)). The isolated product was recrystallized from iPr_2O. Colorless solid, mp 110 °C, yield 1.56 g (27%). Purity (HPLC): 93.7%, t_R = 17.74 min. $C_{16}H_{21}NO_3$ (275.3 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.82–1.99 (m, 4H, $N(CH_2CH_2)_2$), 2.15 (s, 3H, $NCOCH_3$), 2.79 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 2.99 (td, J = 12.4/4.6 Hz, 1H, $N(CH_2CH_2)_2$), 3.51 (td, J = 12.7/3.4 Hz, 1H, $N(CH_2CH_2)_2$), 3.76 (s, 3H, OCH_3), 3.92 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 4.14–4.19 (m, 1H, $N(CH_2CH_2)_2$), 4.41–4.47 (m, 1H, $N(CH_2CH_2)_2$), 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}). ^{13}C NMR (CD_3OD): δ [ppm] = 19.8 (1C, $COCH_3$), 29.4 (1C, CH_2CH_2O), 36.0 (1C, $N(CH_2CH_2)_2$), 36.8 (1C, $N(CH_2CH_2)_2$), 37.5 (1C, $N(CH_2CH_2)_2$), 42.4 (1C, $N(CH_2CH_2)_2$), 54.2 (1C, OCH_3), 58.7 (1C, CH_2CH_2O), 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-4a$_{arom}$), 134.9 (1C, C-8a$_{arom}$), 158.0 (1C, C-6$_{arom}$), 170.1 (1C, C=O). MS (APCI): m/z = 276.1610 (calcd 276.1594 for $C_{16}H_{22}NO_3$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 1640 (C=O), 1501 (OCH_3), 1085 (C—O), 822, 683 (C—H$_{arom}$).

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

2-(3-Hydroxyphenyl)ethanol **5d** (2.23 g, 16.2 mmol) was dissolved in CH_3CN (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 CH_3CN (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. $BF_3{\cdot}Et_2O$ (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 $NaHCO_3$ was added to the combined mixtures. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), 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/CH_3OH 80:20, fraction size 65 mL, R_f = 0.1 (CH_2Cl_2/CH_3OH 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. $C_{15}H_{19}NO_3$ (261.3 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.76–2.02 (m, 4H, $N(CH_2CH_2)_2$), 2.14 (s, 3H, $NCOCH_3$), 2.73 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 2.98 (td, J = 12.4/4.1 Hz, 1H, $N(CH_2CH_2)_2$), 3.50 (td, J = 12.9/3.7 Hz, 1H, $N(CH_2CH_2)_2$), 3.73–3.80 (m, 1H, $N(CH_2CH_2)_2$), 3.89 (t, J = 5.3 Hz, 2H, $PhCH_2CH_2$), 4.38–4.46 (m, 1H, $N(CH_2CH_2)_2$), 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. ^{13}C NMR (CD_3OD): δ [ppm] = 19.8 (1C, $NCOCH_3$), 29.3 (1C, $PhCH_2CH_2$), 36.1 (1C, $N(CH_2CH_2)_2$), 36.8 (1C, $N(CH_2CH_2)_2$), 37.6 (1C, $N(CH_2CH_2)_2$), 42.4 (1C, $N(CH_2CH_2)_2$), 58.7 (1C, $PhCH_2CH_2$), 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-4a$_{arom}$), 134.8 (1C, C-8a$_{arom}$), 155.3 (1C, C-6$_{arom}$), 170.1 (1C, C=O). MS (APCI): m/z = 262.1433 (calcd 262.1438 for $C_{15}H_{20}NO_3$ [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 CH_3CN (50 mL). After addition of K_2CO_3 (1.08 g,

7.8 mmol) the solution was heated to reflux overnight. Cooled to rt, K_2CO_3 was filtered off and CH_3CN was removed under reduced pressure. The residue was recrystallized from diisopropyl ether, R_f = 0.92, (CH_2Cl_2/CH_3OH/NH_3 95:4.5:0.5). Colorless solid, mp 183 °C, yield 443 mg (60%). Purity (HPLC): 96.3%, t_R = 22.50 min. $C_{22}H_{25}NO_3$ (351.4 g/mol). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.77–1.94 (m, 4H, N(CH_2CH_2)$_2$), 2.14 (s, 3H, NCOCH_3), 2.81 (q, J = 5.1 Hz, 2H, PhCH_2CH_2), 2.96 (td, J = 12.9/3.1 Hz, 1H, N(CH_2CH_2)$_2$), 3.51 (td, J = 12.8/3.3 Hz, 1H, N(CH_2CH_2)$_2$), 3.62–3.70 (m, 1H, N(CH_2CH_2)$_2$), 3.91 (t, J = 5.4 Hz, 2H, PhCH$_2$CH_2), 4.50–4.60 (m, 1H, N(CH_2CH_2)$_2$), 5.03 (s, 1H, PhCH_2O), 5.05 (s, 1H, PhCH_2O), 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}). ^{13}C NMR ($CDCl_3$): δ [ppm] = 21.9 (1C, NCOCH_3), 29.8 (1C, PhCH_2CH_2), 36.3 (1C, N(CH_2CH_2)$_2$), 37.5 (1C, N(CH_2CH_2)$_2$), 37.8 (1C, N(CH_2CH_2)$_2$), 42.6 (1C, N(CH_2CH_2)$_2$), 59.0 (1C, PhCH$_2$CH_2), 70.0 (1C, PhCH_2O), 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-4a$_{arom}$), 135.1 (1C, C-8a$_{arom}$), 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 $C_{22}H_{26}NO_3$ [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 CH_2Cl_2 (3×). 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. $C_{13}H_{17}NO$ (203.3 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.84 (dd, J = 15.7/1.8 Hz, 2H, N(CH_2CH_2)$_2$), 1.93 (td, J = 12.6/4.6 Hz, 2H, N(CH_2CH_2)$_2$), 2.80 (t, J = 5.6 Hz, 2H, PhCH_2CH$_2$), 2.86 (dd, J = 11.9/4.1 Hz, 2H, N(CH_2CH_2)$_2$), 3.04 (td, J = 12.5/3.1 Hz, 2H, N(CH_2CH_2)$_2$), 3.91 (t, J = 5.5 Hz, 2H, PhCH$_2$CH_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, N(CH_2CH_2)$_2$), 40.8 (2C, N(CH_2CH_2)$_2$), 58.7 (1C, PhCH$_2$CH_2), 72.5 (1C, ArCO), 124.8 (1C, C-8$_{arom}$), 126.0 and 126.1 (2C, C-5$_{arom}$, C-6$_{arom}$), 128.5 (1C, C-7$_{arom}$), 133.4 (1C, C-4a$_{arom}$), 141.1 (1C, C-8a$_{arom}$). MS (APCI): m/z = 204.1422 (calcd 204.1383 for $C_{13}H_{18}NO$ [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 (3×). 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. $C_{14}H_{19}NO$ (217.3 g/mol). ^{1}H 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, N(CH_2CH_2)$_2$), 1.74 (td, J = 14.1/4.1 Hz, 1H, N(CH_2CH_2)$_2$), 2.02 (dd, J = 14.7/2.0 Hz, 1H, N(CH_2CH_2)$_2$), 2.09 (td, J = 13.3/4.7 Hz, 1H, N(CH_2CH_2)$_2$), 2.58–2.68 (m, 2H, PhCH_2CH), 2.80–2.88 (m, 2H, N(CH_2CH_2)$_2$), 3.02 (td, J = 12.6/2.8 Hz, 1H, N(CH_2CH_2)$_2$), 3.12 (td, J = 12.6/2.9 Hz, 1H, N(CH_2CH_2)$_2$), 3.84–3.92 (m, 1H, PhCH$_2$CH), 7.02–7.22 (m, 4H, CH_{arom}), a signal for the NH proton is not seen in the spectrum. ^{13}C NMR (CD_3OD): δ [ppm] = 20.7 (1C, CH_3), 30.2 (1C, CH_2CHO), 34.3 (1C, N(CH_2CH_2)$_2$), 35.5 (0.5C, N(CH_2CH_2)$_2$), 36.7 (0.5C, N(CH_2CH_2)$_2$), 38.8 (1C, N(CH_2CH_2)$_2$), 41.0 (0.5C, N(CH_2CH_2)$_2$), 41.2 (0.5C, N(CH_2CH_2)$_2$), 64.0 (1C, CH_2CHO), 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-4a$_{arom}$), 141.6 (1C, C-8a$_{arom}$). MS (APCI): 218.1535 (calcd 218.1539 for $C_{14}H_{20}NO$ [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 CH_2Cl_2 (3×). 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 136 °C, yield 193 mg (98%). $C_{14}H_{19}NO_2$ (233.3 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.86 (dd, J = 15.5/2.0 Hz, 2H, N(CH_2CH_2)$_2$), 1.95 (td, J = 13.9/4.4 Hz, 2H, N(CH_2CH_2)$_2$, 2.78 (t, J = 5.5 Hz, 2H, PhCH_2), 2.94 (dd, J = 12.2/3.4 Hz, 2H, N(CH_2CH_2)$_2$), 3.09 (td, J = 12.4/3.2 Hz, 2H, N(CH_2CH_2)$_2$), 3.76 (s, 3H, OCH_3), 3.89 (t, J = 5.5 Hz, 2H, PhCH$_2$CH_2), 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. ^{13}C NMR (CD_3OD): δ [ppm] = 29.4 (1C, PhCH_2CH_2O), 35.2 (1C, N(CH_2CH_2)$_2$), 38.2 (1C, N(CH_2CH_2)$_2$), 40.7 (2C, N(CH_2CH_2)$_2$), 54.2 (1C, OCH_3), 58.7 (1C, CH_2CH_2O), 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-8a$_{arom}$), 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 $C_{14}H_{20}NO_2$ [MH$^+$]). IR: $\tilde{\nu}$ [cm^{-1}] = 1501 (OCH_3), 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 (3×). The combined organic layers were dried (Na_2SO_4), filtered and the filtrate was concentrated under reduced pressure. Colorless oil, yield 40 mg (12%). $C_{13}H_{17}NO_2$ (219.3 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.77–1.96 (m, 4H, N(CH_2CH_2)$_2$), 2.71 (t, J = 5.5 Hz, 2H, PhCH_2CH$_2$), 2.88 (dd, J = 11.2/3.4 Hz, 2H, N(CH_2CH_2)$_2$), 3.05 (td, J = 11.9/2.7 Hz, 2H, N(CH_2CH_2)$_2$), 3.86 (t, J = 5.5 Hz, 2H, PhCH$_2$CH_2), 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 CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered and the filtrate was concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 and a saturated solution of HCl in Et_2O 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 CH_2Cl_2 (3×). Combined organic layers were dried (Na_2SO_4), filtered and the filtrate was concentrated in vacuo, R_f = 0.20 (CH_2Cl_2/CH_3OH/NH_3 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). ^{1}H 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$_2$CH_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$_2$CH$_2$), 37.8 (2C, N(CH$_2$CH$_2$)$_2$), 42.3 (2C, N(CH$_2$CH$_2$)$_2$), 58.8 (1C, PhCH$_2$CH$_2$), 70.1 (1C, PhCH$_2$O), 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 (3×). 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). ^{1}H NMR (CDCl$_3$): δ [ppm] = 2.16 (quint, J = 6.5 Hz, 2H, ClCH$_2$CH_2CH$_2$), 2.46 (t, J = 7.1 Hz, 2H, ClCH$_2$CH$_2$CH_2), 3.61 (t, J = 6.1 Hz, 2H, ClCH_2CH$_2$CH$_2$), 4.56 (d, J = 6.0 Hz, 2H, PhCH_2NH), 6.00 (s, broad, 1H, NH), 7.72 (s, 2H, 2-H_{arom}, 6-H_{arom}), 7.78 (s, 1H, 4-H_{arom}). ^{13}C NMR (CDCl$_3$): δ [ppm] = 27.8 (1C, ClCH$_2$CH$_2$CH$_2$), 32.9 (1C, ClCH$_2$CH$_2$CH$_2$), 42.7 (1C, PhCH$_2$NH), 44.3 (1C, ClCH$_2$CH$_2$CH$_2$), 121.5 (hept, J = 3.9 Hz, 1C, C-4$_{arom}$), 123.1 (q, J = 271.2 Hz, 2C, CF_3), 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 $C_{13}H^{13}ClF_6NO$ [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 Na_2CO_3 (551 mg, 5.2 mmol) were dissolved in CH_3CN (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 $NaHCO_3$ to stop the transformation and the mixture was extracted with EtOAc (3×). The organic layers were combined, dried (Na_2SO_4), 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 $C_{12}H_{12}ClF_4NO$ (297.7 g/mol). ^{1}H NMR (CDCl$_3$): δ [ppm] = 2.15 (quint, J = 6.6 Hz, 2H, ClCH$_2$CH_2CH$_2$), 2.45 (t, 7.1 Hz, 2H, ClCH$_2$CH$_2$CH_2), 3.61 (t, J = 6.1 Hz, 2H, ClCH_2CH$_2$CH$_2$), 4.48 (d, J = 6.2 Hz, 2H, PhCH_2NH), 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}). ^{13}C NMR (CDCl$_3$): δ [ppm] = 27.9 (1C, ClCH$_2$CH$_2$CH$_2$), 33.0 (1C, ClCH$_2$CH$_2$CH$_2$), 42.7 (d, J = 1.5, 1C, PhCH$_2$NH), 44.4 (1C, ClCH$_2$CH$_2$CH$_2$), 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 $C_{12}H_{13}ClF_4NO$ [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 (3×) 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 (CH_2Cl_2/EtOAc 40:60). Brown oil, yield 419.7 mg (73%). Purity (HPLC): 62.5%, t_R = 24.44 min $C_{19}H_{15}BrF_7NO$ (486.2 g/mol). ^{1}H NMR (CDCl$_3$): δ [ppm] = 2.26 (dtd, J = 14.8/7.5/4.7 Hz, 1H, BrCH$_2$CH_2CH), 2.66 (dtd, J = 14.9/7.5/4.7 Hz, 1H, BrCH$_2$CH_2CH), 3.26 (ddd, J = 10.2/7.9/4.6 Hz, 1H, BrCH_2CH$_2$CH), 3.46 (ddd, J = 10.2/7.2/4.7 Hz, 1H, BrCH_2CH$_2$CH), 3.79 (t, J = 7.4 Hz, 1H, BrCH$_2$CH$_2$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, BrCH$_2$CH$_2$CH), 35.6 (1C, BrCH$_2$CH$_2$CH), 42.6 (1C, PhCH$_2$NH), 49.8 (1C, BrCH$_2$CH$_2$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$_{arom}$), 140.9 (1C, C-1′$_{arom}$), 162.4 (d, J = 247.6 Hz, 1C, C-4$_{arom}$), 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$_{arom}$), 721, 660 (C—H$_{arom}$).

5.1.16. 4-Bromo-2-(4-fluorophenyl)-*N*-[3-fluoro-5-(trifluoromethyl)benzyl]butanamide (11d)

15d (796 mg, 2.13 mmol) and CBr_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 (3×), 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). ^{1}H NMR (CDCl$_3$): δ [ppm] = 2.26 (dtd, J = 12.2/7.4/4.8 Hz, 1H, BrCH$_2$CH_2CH), 2.66 (dtd, J = 12.3/7.4/4.7 Hz, 1H, BrCH$_2$CH_2CH), 3.25 (ddd, J = 10.3/8.0/4.7 Hz, 1H, BrCH_2CH$_2$CH), 3.46 (ddd, J = 10.3/7.1/4.8 Hz, 1H, BrCH_2CH$_2$CH), 3.76 (t, J = 7.4 Hz, 1H, BrCH$_2$CH$_2$CH), 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_{arom}), 7.06 (t, J = 8.5 Hz, 2H, 3-H_{arom}, 5-H_{arom}), 7.16 (s, 1H, 6′-H_{arom}), 7.20 (d, J = 8.3 Hz, 1H, 4′-H_{arom}), 7.32 (dd, J = 8.7/5.3 Hz, 2H, 2-H_{arom}, 6-H_{arom}). ^{13}C NMR (CDCl$_3$): δ [ppm] = 31.8 (1C, BrCH$_2$CH$_2$CH), 35.6 (1C, BrCH$_2$CH$_2$CH), 42.7 (1C, PhCH$_2$NH), 49.9 (1C, BrCH$_2$CH$_2$CH), 111.9 (dq, J = 24.4/3.8 Hz, 1C, C-4′$_{arom}$), 116.2 (d, J = 21.7 Hz, 2C, C-3$_{arom}$, C-5$_{arom}$), 117.7 (d, J = 20.6 Hz, 1C, C-2′$_{arom}$), 119.5 (quint, J = 3.7 Hz, 1C, C-6′$_{arom}$), 129.5 (d, J = 8.0 Hz, 2C, C-2$_{arom}$, C-6$_{arom}$), 133.9 (d, J = 3.4 Hz, 1C, C-1$_{arom}$), 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 CF_3 group and for C-5′ are not seen in the spectrum. MS (APCI): m/z = 436.0301 (calcd 436.0330 for $C_{18}H_{16}BrF_5NO$ [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 CBr_4 (100 mg, 0.30 mmol) were dissolved in CH_3CN (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 war stirred at rt overnight. The mixture was filtered over Celite® and water was added to the eluent. The aqueous layer was extracted with CH_2Cl_2 (3×), 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 = 0.40 (CH_2Cl_2/CH_3OH/NH_3 95:4.5:0.5). Brown oil, yield 33 mg (47%). Purity (HPLC): 54%, t_R = 23.32 min. $C_{16}H_{16}BrF_6NO$ (432.2 g/mol). ^{1}H NMR ($CDCl_3$): δ [ppm] = 0.25–0.33 (m, 2H, C$H_{2cycloprop}$), 0.59–0.71 (m, 2H, C$H_{2cycloprop}$), 0.95–1.05 (m, 1H, C$H_{cycloprop}$), 1.74 (td, J = 9.2/5.4 Hz, 1H, $BrCH_2CH_2CH$), 2.08–2.16 (m, 1H, $BrCH_2CH_2CH$), 2.42 (ddt, J = 14.3/8.8/5.3 Hz, 1H, $BrCH_2CH_2CH$), 3.43 (ddd, J = 10.2/9.2/4.9 Hz, 1H, $BrCH_2CH_2CH$), 3.56–3.66 m, 1H, $BrCH_2CH_2CH$), 4.53 (dd, J = 15.8/6.1 Hz, 1H, $PhCH_2NH$), 4.70 (dd, J = 15.8/6.2 Hz, 1H, $PhCH_2NH$), 6.19 (s, broad, 1H, NH), 7.75 (s, 2H, 2-H_{arom}, 6-H_{arom}), 7.79 (s, 1H, 4-H_{arom}). ^{13}C NMR ($CDCl_3$): δ [ppm] = 3.9 (1C, C$H_{2cycloprop}$), 4.5 (1C, C$H_{2cycloprop}$), 13.7 (1C, C$H_{cycloprop}$), 32.2 (1C, $BrCH_2CH_2CH$), 35.0 (1C, $BrCH_2CH_2CH$), 42.5 (1C, $PhCH_2NH$), 50.0 (1C, $BrCH_2CH_2CH$), 121.4 (hept, J = 3.9 Hz, 1C, C-4_{arom}), 123.2 (q, J = 272.2 Hz, 2C, CF_3), 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 $C_{16}H_{17}BrF_6NO$ [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 CH_3OH (6.5 mL). Conc. H_2SO_4 (0.3 mL) was added and the mixture was headed to reflux overnight. Water was added, the organic layer was separated and the aqueous layer was extracted with Et_2O (3×). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water to remove the acid. Then the organic layers were dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure, R_f = 0.23 (CH_2Cl_2/EtOAc 40:60). Colorless oil, yield 4.07 g (75%). $C_9H_9FO_2$ (168.2 g/mol). ^{1}H NMR ($CDCl_3$): δ [ppm] = 3.60 (s, 2H, $PhCH_2$), 3.69 (s, 3H, CO_2CH_3), 6.96–7.05 (m, 2H, 3-H_{arom}, 5-H_{arom}), 7.19–7.28 (m, 2H, 2-H_{arom}, 4-H_{arom}). ^{13}C NMR ($CDCl_3$): δ [ppm] = 40.3 (1C, CH_2), 52.1 (1C, CO_2CH_3), 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 $C_9H_{10}FO_2$ [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 CH_3OH (4 mL). Conc. H_2SO_4 (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 Et_2O (3×). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and water. Then the organic layers were 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. Pale yellow oil, yield 1.23 g (45%). $C_6H_{10}O_2$ (114.1 g/mol). ^{1}H NMR ($CDCl_3$): δ [ppm] = 0.14–0.19 (m, 2H, C$H_{2cycloprop}$), 0.52–0.57 (m, 2H, C$H_{2cycloprop}$), 1.02–1.10 (m, 1H, C$H_{cycloprop}$), 2.22 (d, J = 7.1 Hz, 2H, CH_2), 3.69 (s, 3H, CO_2CH_3). ^{13}C NMR ($CDCl_3$): δ [ppm] = 4.4 (2C, C$H_{2cycloprop}$), 6.9 (1C, C$H_{cycloprop}$), 39.2 (1C, CH_2), 51.5 (1C, CO_2CH_3), 173.7 (1C, C═O). MS (APCI): m/z = 115.0772 (calcd 115.0754 for $C_6H_{11}O_2$ [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(3*H*)-one (14c)

Ester ^{13}C (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 ^{13}C 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 1/4 concd H_2SO_4 and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography (Ø 4.5 cm, length 14 cm, CH_2Cl_2/EtOAc 90:10, fraction size 30 mL, R_f = 0.26 (CH_2Cl_2/EtOAc 40:60)). Brown oil, yield 1.51 g (52%). $C_{10}H_9FO_2$ (180.2 g/mol). ^{1}H NMR ($CDCl_3$): 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_{arom}, C-5_{arom}), 129.5 and 130.9 (d, each J = 8.1 Hz, 2C, C-2_{arom}, C-6_{arom}), 132.2 (d, J = 3.4 Hz, 1C, C-1_{arom}), 162.2 (d, J = 246.7 Hz, 1C, C-4_{arom}), 177.1 (1C, C═O). MS (APCI): m/z = 181.0653 (calcd 181.0659 for $C_{10}H_{10}FO_2$ [MH^+]). IR: $\tilde{\nu}$ [cm^{-1}] = 1762 (C═O), 1219 (C—F_{arom}), 1150 (C—O), 814 (C—H_{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 1/4 concd H_2SO_4 and the aqueous layer was extracted with CH_2Cl_2 (3×). 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%). $C_7H_{10}O_2$ (126.2 g/mol). ^{1}H NMR ($CDCl_3$): δ [ppm] = 0.21–0.34 (m, 1H, C$H_{2cycloprop}$), 0.37–0.47 (m, 1H, C$H_{2cycloprop}$), 0.50–0.56 (m, 1H, C$H_{2cycloprop}$), 0.64–0.72 (m, 1H, C$H_{2cycloprop}$), 1.17–1.28 (m, 1H, C$H_{cycloprop}$), 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, C$H_{2cycloprop}$), 3.6 (1C, C$H_{2cycloprop}$), 11.3 (1C, C$H_{cycloprop}$), 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 $C_7H_{11}O_2$ [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 (3×). 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 (Ø 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. $C_{19}H_{16}F_7NO_2$ (423.3 g/mol). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.99 (dtd, J = 14.3/6.5/4.5 Hz, 1H, HOCH$_2$C*H*$_2$CH), 2.34–2.47 (m, 1H, HOCH$_2$C*H*$_2$CH), 3.57–3.64 (m, 1H, HOCH$_2$CH$_2$C*H*), 3.66–3.76 (m, 2H, HOC*H*$_2$CH$_2$CH), 4.46 (dd J = 15.8/6.0 Hz, 1H, PhC*H*$_2$NH), 4.59 (dd J = 15.8/6.4 Hz, 1H, PhC*H*$_2$NH), 6.06 (s, broad, 1H, N*H*), 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, HOCH$_2$*C*H$_2$CH), 42.6 (1C, PhCH$_2$NH), 49.0 (1C, HOCH$_2$CH$_2$*C*H), 60.4 (1C, HO*C*H$_2$CH$_2$CH), 116.0 (d, J = 21.6 Hz, 2C, *C*-3$_{arom}$, *C*-5$_{arom}$), 121.3 (hept, J = 3.9 Hz, 1C, *C*-4′$_{arom}$), 123.1 (q, J = 272.8 Hz, 2C, *C*F$_3$), 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 $C_{19}H_{17}F_7NO_2$ [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 CH_3CN (15 mL). Na_2CO_3 (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 (3×). 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 (Ø 4 cm, length 15 cm, CH_2Cl_2/EtOAc 40:60, fraction size 30 mL, R_f = 0.23 (CH_2Cl_2/EtOAc 40:60)). Colorless solid, mp 98 °C, yield 147 mg (23%). Purity (HPLC): 93.6%, t_R = 20.2 min. $C_{18}H_{16}F_5NO_2$ (373.3 g/mol). ^{1}H NMR ($CDCl_3$): δ [ppm] = 1.92–2.01 (m, 1H, HOCH$_2$C*H*$_2$CH), 2.33–2.44 (m, 1H, HOCH$_2$C*H*$_2$CH), 3.54–3.61 (m, 1H, HOCH$_2$CH$_2$C*H*), 3.65–3.72 (m, 2H, HOC*H*$_2$CH$_2$CH), 4.36–4.49 (m, 2H, PhC*H*$_2$NH), 5.93 (s, broad, 1H, N*H*), 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. ^{13}C NMR ($CDCl_3$): δ [ppm] = 35.9 (1C, HOCH$_2$*C*H$_2$CH), 42.7 (1C, PhCH$_2$NH), 49.11 (1C, HOCH$_2$CH$_2$*C*H), 60.5 (1C, HO*C*H$_2$CH$_2$CH), 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*-2$_{arom}$, *C*-6$_{arom}$), 135.1 (d, J = 3.4 Hz, 1C, *C*-1$_{arom}$), 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 *C*F$_3$ group, for *C*-4 and *C*-5′ are not seen in the spectrum. MS (APCI): m/z = 374.1177 (calcd 374.1174 for $C_{18}H_{17}F_5NO_2$ [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 deuterochloroform (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 (Ø 4 cm, length 17 cm, CH_2Cl_2/EtOAc 40:60 → CH_2Cl_2/EtOAc 25:75, fraction size 30 mL, R_f = 0.25 (CH_2Cl_2/EtOAc 40:60)). Pale yellow solid, mp 92 °C, yield 54 mg (7.1%). Purity (HPLC): 90.1%, t_R = 19.95 min. $C_{16}H_{17}F_6NO_2$ (369.3 g/mol). ^{1}H NMR ($CDCl_3$): δ [ppm] = 0.24–0.30 (m, 2H, C*H*$_{2cycloprop}$), 0.63–0.69 (m, 2H, C*H*$_{2cycloprop}$), 0.97–1.07 (m, 1H, C*H*$_{cycloprop}$), 1.62–1.73 (m, 1H, HOCH$_2$CH$_2$C*H*), 1.95–2.06 (m, 2H, HOCH$_2$C*H*$_2$CH), 3.76 (t, J = 6.0 Hz, 2H, HOC*H*$_2$CH$_2$CH), 4.56 (dd, J = 15.7/6.0 Hz, 1H, PhC*H*$_2$NH), 4.66 (dd, J = 15.8/6.0 Hz, 1H, PhC*H*$_2$NH), 6.43 (s, broad, 1H, N*H*), 7.75 (s, 2H, 2-*H*$_{arom}$, 6-*H*$_{arom}$), 7.79 (1H, 4-*H*$_{arom}$). ^{13}C NMR ($CDCl_3$): δ [ppm] = 4.4 (1C, *C*H$_{2cycloprop}$), 4.6 (1C, *C*H$_{2cycloprop}$), 13.7 (1C, *C*H$_{cycloprop}$), 34.7 (1C, HOCH$_2$CH$_2$CH), 42.5 (1C, PhCH$_2$NH), 49.4 (1C, HOCH$_2$CH$_2$*C*H), 60.7 (1C, HO*C*H$_2$CH$_2$CH), 121.3 (hept, J = 3.9 Hz, 1C, *C*-4$_{arom}$), 127.2–127.5 (m, 2C, *C*-2$_{arom}$, *C*-6$_{arom}$), 131.9 (q, J = 33.4 Hz, 2C, *C*-3$_{arom}$, *C*-5$_{arom}$),141.3 (1C, *C*-1$_{arom}$), 175.9 (1C, *C*=O), signals for the two C-atoms of the *C*F$_3$ groups are not seen in the spectrum. MS (APCI): m/z = 370.1252 (calcd 370.1236 for $C_{16}H_{18}F_6NO_2$ [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 CH_2Cl_2 (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 (Na_2SO_4), filtered and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (Ø 2 cm, length 12 cm, CH_2Cl_2/EtOAc 40:60, fraction size 10 mL, R_f = 0.81 (CH_2Cl_2/EtOAc 40:60)). Colorless solid, yield 75 mg (73%). $C_{19}H_{14}F_7NO_2$ (421.3 g/mol). ^{1}H NMR ($CDCl_3$): δ [ppm] = 2.35–2.50 (m, 1H, NCH(OH)C*H*$_2$), 2.87–2.97 (m, 1H, NCH(OH)C*H*$_2$),3.72 (dd, J = 9.8/6.4 Hz, 0.5 H, NCOC*H*Ph), 3.98 (dd, J = 9.2/8.1 Hz, 0.5 H, NCOC*H*Ph), 4.42 (d, J = 15.3 Hz, 0.5H, PhC*H*$_2$N), 4.51 (d, J = 15.1 Hz, 0.5H, PhC*H*$_2$N), 4.84 (d, J = 15.2 Hz, 0.5H, PhC*H*$_2$N), 4.93 (d, J = 15.2 Hz, 0.5H, PhC*H*$_2$N), 5.17–5.21 (m, 0.5H, NC*H*(OH)CH$_2$)), 5.21–5.24 (m, 0.5H, NC*H*(OH)CH$_2$), 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. ^{13}C NMR ($CDCl_3$): δ [ppm] = 37.5 (0.5C, NCH(OH)*C*H$_2$), 38.3 (0.5C, NCH(OH)CH$_2$), 43.5 (1C, PhCH$_2$N), 44.9 (0.5C, NCOCHPh), 46.1 (0.5C, NCOCHPh), 81.0 (0.5C, NCH(OH)CH$_2$), 81.2 (0.5C, NCH(OH)CH$_2$), 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, 0.5C, *C*-1$_{arom}$), 134.5 (d, J = 3.1 Hz, 0.5C, *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 CF_3 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 K_2CO_3 (874 mg, 6.3 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 twice (Ø 4 cm, length 14 cm, CH_2Cl_2/EtOAc 50:50 + 1% ethyldimethylamine → EtOAc 100 + 1% ethyldimethylamine → acetone 100 + 1% ethyldimethylamine, fraction size 30 mL; Ø 4 cm, length 15 cm, CH_2Cl_2/MeOH/NH_3 94.2:5:0.8, fraction size 30 mL; R_f = 0.17 (CH_2Cl_2/MeOH/NH_3 95:4.5:0.5)). Yellow oil, yield 118 mg (43%). Purity (HPLC): 97.6%, t_R = 20.00 min. $C_{26}H_{28}F_6N_2O_2$ (514.5 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.85–1.89 (m, 2H, $NCH_2CH_2CH_2$), 1.91–1.96 (m, 2H, $N(CH_2CH_2)_2$), 2.06 (td, J = 13.8/4.1 Hz, 2H, $N(CH_2CH_2)_2$), 2.35 (t, J = 7.3 Hz, 2H, $NCH_2CH_2CH_2$), 2.47–2.53 (m, 2H, NCH_2), 2.54–2.58 (m, 2H, NCH_2), 2.80 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 2.82–2.88 (m, 2H, NCH_2), 3.89 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 4.53 (s, 2H, $PhCH_2NH$), 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. ^{13}C NMR (CD_3OD): δ [ppm] = 22.2 (1C, $NCH_2CH_2CH_2$), 29.1 (1C, $PhCH_2CH_2$), 33.3 (1C, $NCH_2CH_2CH_2$), 35.7 (2C, $N(CH_2CH_2)_2$), 41.8 (1C, $PhCH_2NH$), 48.8 (2C, $N(CH_2CH_2)_2$), 57.6 (1C, $NCH_2CH_2CH_2$), 58.5 (1C, $PhCH_2CH_2$), 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-4a$_{arom}$), 141.2 (1C, C-8a$_{arom}$), 142.4 (1C, C-1′$_{arom}$), 174.3 (1C, C=O), signals for the C-atoms of the CF_3 groups are not seen in the spectrum. MS (APCI): m/z = 515.2161 (calcd 515.2155 for $C_{26}H_{29}F_6N_2O_2$ [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 K_2CO_3 (2.09 g, 14.9 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 twice (Ø 4 cm, length 14 cm, CH_2Cl_2/EtOAc 50:50 + 1% dimethylethylamine → EtOAc 100 + 1% ethyldimethylamine → acetone 100 + 1% ethyldimethylamine, fraction size 30 mL; R_f = 0.17 (CH_2Cl_2/CH_3OH/NH_3 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. $C_{27}H_{30}F_6N_2O_2$ (528.5 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.31 (d, J = 6.1 Hz, 3H, CH_3), 1.62 (dd, J = 13.6/4.6 Hz, 1H, $N(CH_2CH_2)_2$), 1.84 (td, J = 13.5/4.4 Hz, 1H, $N(CH_2CH_2)_2$), 1.87–1.93 (m, 2H, NCH_2CH_2 CH_2), 2.07 (dd, J = 14.3/2.6 Hz, 1H, $N(CH_2CH_2)_2$), 2.20 (td, J = 13.2/4.6 Hz, 1H, $N(CH_2CH_2)_2$), 2.33 (t, J = 7.3 Hz, 2H, $NCH_2CH_2CH_2$), 2.40–2.45 (m, 3H, NCH_2), 2.53 (td, J = 12.3/2.4 Hz, 1H, NCH_2), 2.60–2.69 (m, 2H, $PhCH_2CH_2$), 2.72–2.81 (m, 2H, NCH_2), 3.84–3.93 (m, 1H, $PhCH_2CH$), 4.53 (s, 2H, $PhCH_2NH$), 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, $NCH_2CH_2CH_2$), 33.5 (1C, $NCH_2CH_2CH_2$), 34.0 (1C, $N(CH_2CH_2)_2$), 36.6 (1C, $N(CH_2CH_2)_2$), 38.3 (2C, $N(CH_2CH_2)_2$, 41.8 (1C, $PhCH_2CH$), 48.8 (0.5C, $N(CH_2CH_2)_2$), 48.9 (0.5C, $N(CH_2CH_2)_2$), 57.7 (1C, $NCH_2CH_2CH_2$), 64.0 (1C, $PhCH_2CH$), 73.3 (1C, ArCO), 120.5 (hept, J = 3.9, 1C, C-4′$_{arom}$), 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, C-2′$_{arom}$, C-6′$_{arom}$), 128.3 (1C, C-7$_{arom}$), 131.4 (q, J = 33.2 Hz, 2C, C-3′$_{arom}$, C-5′$_{arom}$), 133.9 (1C, C-4a$_{arom}$), 141.2 (1C, C-1′$_{arom}$), 142.5 (1C, C-8a$_{arom}$), 174.4 (1C, C=O). MS (APCI): m/z = 529.2268 (calcd 529.2284 for $C_{27}H_{31}F_6N_2O_2$ [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 (Ø 3 cm, length 10 cm, cyclohexane/EtOAc 50:50 + 1% ethyldimethylamine, fraction size 65 mL; Ø 4 cm, length 15 cm, CH_2Cl_2/CH_3OH/NH_3 94.2:5:0.8, fraction size 30 mL; Ø 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. $C_{27}H_{30}F_6N_2O_3$ (544.5 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.84–1.90 (m, 2H, $NCH_2CH_2CH_2$), 1.90–1.95 (m, 2H, $N(CH_2CH_2)_2$), 2.03 (td, J = 13.6/4.4 Hz, 2H, $N(CH_2CH_2)_2$), 2.36 (t, J = 7.2 Hz, 2H, $NCH_2CH_2CH_2$), 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, NCH_2), 3.75 (s, 3H, OCH_3), 3.87 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 4.53 (s, 2H, $PhCH_2NH$), 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. ^{13}C NMR (CD_3OD): δ [ppm] = 22.0 (1C, $NCH_2CH_2CH_2$), 29.4 (1C, $PhCH_2CH_2$), 33.3 (1C, $NCH_2CH_2CH_2$), 35.7 (2C, $N(CH_2CH_2)_2$), 41.9 (1C, $PhCH_2NH$), 48.9 (2C, $N(CH_2CH_2)$,54.2 (1C, OCH_3), 57.5 (1C, $NCH_2CH_2CH_2$), 58.5 (1C, $PhCH_2CH_2$), 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, CF_3), 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-4a$_{arom}$), 135.0 (1C, C-8a$_{arom}$), 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 $C_{27}H_{31}F_6N_2O_3$ [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 CH_2Cl_2 (3×). 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 twice (Ø 2 cm, length 22 cm, CH_2Cl_2/CH_3OH/NH_3 84.2:15:0.8, fraction size 10 mL; Ø 2 cm, length 14 cm, CH_2Cl_2/CH_3OH/NH_3 84.2:15:0.8, fraction size 10 mL; R_f = 0.17 (CH_2Cl_2/CH_3OH/NH_3 95:4.5:0.5)). Pale yellow oil, yield 19.3 mg (9.3%). Purity (HPLC): 97.1%, t_R = 18.48 min. $C_{25}H_{28}F_4N_2O_2$ (464.5 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.83–1.90 (m, 2H, $NCH_2CH_2CH_2$), 1.91–1.95 (m, 2H, $N(CH_2CH_2)_2$), 2.05 (td, J = 13.5/4.4 Hz, 2H, $N(CH_2CH_2)_2$), 2.34 (t, J = 7.3 Hz, 2H, $NCH_2CH_2CH_2$), 2.48–2.58 (m, 4H, NCH_2), 2.79 (t, J = 5.5 Hz, 2H,

PhCH_2CH$_2$), 2.84 (d, broad, J = 11.0 Hz, 2H, N(CH_2CH$_2$)$_2$), 3.88 (t, J = 5.5 Hz, 2H, PhCH$_2$CH_2), 4.45 (s, 2H, PhCH_2NH), 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. ^{13}C NMR (CD$_3$OD): δ [ppm] = 22.2 (1C, NCH$_2$CH$_2$CH$_2$), 29.1 (1C, PhCH$_2$CH$_2$), 33.4 (1C, NCH$_2$CH$_2$CH$_2$), 35.7 (2C, N(CH$_2$CH$_2$)$_2$), 41.8 (d, J = 1.5 Hz, 1C, PhCH$_2$NH), 48.8 (2C, N(CH$_2$CH$_2$)$_2$), 57.6 (1C, NCH$_2$CH$_2$CH$_2$), 58.5 (1C, PhCH$_2$CH$_2$), 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-4a$_{arom}$), 141.2 (1C, C-8a$_{arom}$), 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 CF$_3$ group and for C-5′ are not seen in the spectrum. MS (APCI): m/z = 465.2193 (calcd 465.2160 for C$_{25}$H$_{29}$F$_4$N$_2$O$_2$ [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 K$_2$CO$_3$ (1.99 g, 14.5 mmol) were added. The mixture was heated to reflux overnight. K$_2$CO$_3$ 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 (Ø 4 cm, length 15 cm, CH$_2$Cl$_2$/EtOAc 50:50 + 1% ethyldimethylamine, fraction size 30 mL; R_f = 0.21 (CH$_2$Cl$_2$/CH$_3$OH/NH$_3$ 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$_4$N$_2$O$_2$ (478.5 g/mol). ^{1}H NMR (CD$_3$OD): δ [ppm] = 1.33 (d, J = 6.1 Hz, 3H, CH_3), 1.67 (dd, J = 13.8/2.6 Hz, 1H, N(CH$_2$CH_2)$_2$), 1.88 (td, J = 11.5/2.8 Hz, 1H, N(CH$_2$CH_2)$_2$), 1.92–1.99 (m, 2H, NCH$_2$CH_2CH$_2$), 2.13 (dd, J = 14.5/2.6 Hz, 1H, N(CH$_2$CH_2)$_2$), 2.24 (td, J = 13.3/4.5 Hz, 1H, N(CH$_2$CH_2)$_2$), 2.37 (t, J = 7.2 Hz, 2H, NCH$_2$CH$_2$CH_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$_2$CH$_2$), 3.86–3.93 (m, 1H, PhCH$_2$CH), 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$_3$OD): δ [ppm] = 20.8 (1C, CH_3), 22.1 (1C, NCH$_2$CH_2CH$_2$), 29.2 (1C, PhCH$_2$CH), 33.3 (1C, NCH$_2$CH$_2$CH_2), 33.8 (1C, N(CH$_2$CH$_2$)$_2$), 36.6 (1C, N(CH$_2$CH$_2$)$_2$), 38.1 (1C, N(CH$_2$CH$_2$)$_2$), 41.8 (d, J = 1.7 Hz, 1C, PhCH$_2$NH), 48.8 (1C, N(CH$_2$CH$_2$)$_2$), 57.6 (1C, NCH$_2$CH$_2$CH$_2$), 64.1 (1C, PhCH$_2$CH), 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$_4$N$_2$O$_2$ [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$_2$CO$_3$ (2.99 g, 21.7 mmol) were added. The mixture was heated to reflux overnight. K$_2$CO$_3$ 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 (Ø 8 cm, length 10 cm, CH$_2$Cl$_2$/EtOAc 50:50 + 1% ethyldimethylamine fraction size 65 mL; R_f = 0.17 (CH$_2$Cl$_2$/CH$_3$OH/NH$_3$ 95:4.5:0.5)). Colorless oil, yield 356 mg (27%). Purity (HPLC): 96.5%, t_R = 18.99 min C$_{26}$H$_{30}$F$_4$N$_2$O$_3$ (494.5 g/mol). ^{1}H NMR (CD$_3$OD): δ [ppm] = 1.68–1.77 (m, 2H, NCH$_2$CH_2CH$_2$), 1.77–1.84 (m, 2H, N(CH$_2$CH_2)$_2$), 1.90 (td, J = 13.8/4.5 Hz, 2H, N(CH$_2$CH_2)$_2$), 2.23 (t, J = 7.3 Hz, 2H, NHCH$_2$CH$_2$CH_2), 2.31–2.44 (m, 4H, N(CH_2CH$_2$)$_2$), 2.65 (t, J = 5.6 Hz, 2H, PhCH_2CH$_2$), 2.67–2.75 (m, 2H, NCH_2CH$_2$CH$_2$), 3.65 (s, 3H, OCH_3), 3.76 (t, J = 5.5 Hz, 2H, PhCH$_2$CH_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$_3$OD): δ [ppm] = 22.2 (1C, NCH$_2$CH$_2$CH$_2$), 29.3 (d, J = 20.3 Hz, 1C, CH$_2$CH$_2$O), 33.4 (1C, NCH$_2$CH$_2$CH$_2$), 35.9 (2C, N(CH$_2$CH$_2$)$_2$), 41.8 (d, J = 1.8 Hz, 1C, PhCH$_2$NH), 48.9 (2C, N(CH$_2$CH$_2$)$_2$), 54.2 (1C, OCH$_3$), 57.8 (1C, NCH$_2$CH$_2$CH$_2$), 58.4 (1C, CH$_2$CH$_2$O), 72.3 (1C, ArCO), 110.7 (dq, J = 24.8/3.7 Hz, 1C, C-4′$_{arom}$), 112.2 (1C, C-7$_{arom}$), 112.8 (1C, C-5$_{arom}$), 117.8 (d, J = 22.4 Hz, 1C, C-2′$_{arom}$), 119.7 (quint, J = 3.7 Hz, 1C, C-6′$_{arom}$), 125.9 (1C, C-8$_{arom}$), 133.4 (1C, C-4a$_{arom}$), 134.9 (1C, C-8a$_{arom}$), 143.7 (d, J = 7.8 Hz, 1C, C-1′$_{arom}$), 158.0 (1C, C-6$_{arom}$), 162.6 (d, J = 245.0 Hz, 1C, C-3′$_{arom}$), 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 C$_{26}$H$_{31}$F$_4$N$_2$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$_{arom}$), 1085 (C—O), 810, 698 (C—H$_{arom}$).

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 CH$_2$Cl$_2$ (3×). The combined organic layers were dried (Na$_2$SO$_4$), 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, CH$_2$Cl$_2$/CH$_3$OH/NH$_3$ 95:4.5:0.5, fraction size 10 mL, R_f = 0.11 (CH$_2$Cl$_2$/CH$_3$OH/NH$_3$ 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. C$_{32}$H$_{31}$F$_7$N$_2$O$_2$ (608.6 g/mol). ^{1}H NMR (CD$_3$OD): δ [ppm] = 1.94–1.99 (m, 2H, N(CH$_2$CH_2)$_2$), 2.00–2.06 (m, 1H, NCH$_2$CH_2CH), 2.11 (td, J = 13.13/4.07, 2H, N(CH$_2$CH_2)$_2$), 2.36–2.46 (m, 1H, NCH$_2$CH_2CH), 2.60–2.77 (m, 4H, NCH_2), 2.80 (t, J = 5.5 Hz, 2H, PhCH_2CH$_2$), 3.04 (t, broad, J = 14.0 Hz, 2H, N(CH_2CH$_2$)$_2$), 3.65 (dd, J = 8.5/6.4 Hz, 1H, NCH$_2$CH$_2$CH), 3.89 (t, J = 5.5 Hz, 2H, PhCH$_2$CH_2), 4.41 (d, J = 15.8 Hz, 1H, PhCH_2NH), 4.59 (d, J = 15.8 Hz, 1H, PhCH_2NH), 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. ^{13}C NMR (CD$_3$OD): δ [ppm] = 28.8 (1C, NCH$_2$CH$_2$CH), 29.0 (1C, PhCH$_2$CH$_2$), 35.0 (2C, N(CH$_2$CH$_2$)$_2$), 41.7 (1C, PhCH$_2$NH), 48.7 (1C, N(CH$_2$CH$_2$)$_2$), 48.9 (1C, N(CH$_2$CH$_2$)$_2$), 49.2 (1C, NCH$_2$CH$_2$CH), 55.6 (1C, NCH$_2$CH$_2$CH), 58.7 (1C, PhCH$_2$CH$_2$), 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, CF$_3$), 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-4a$_{arom}$), 135.3 (d, J = 3.0 Hz, 1C, C-1′$_{arom}$), 140.3 (1C, C-1″$_{arom}$),142.3 (1C, C-8a$_{arom}$), 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$_7$N$_2$O$_2$ [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 CH_2Cl_2 (3×). 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 (Ø 2 cm, length 15 cm, $CH_2Cl_2/CH_3OH/NH_3$ 95:4.5:0.5, fraction size 10 mL; R_f = 0.09 ($CH_2Cl_2/CH_3OH/NH_3$ 95:4.5:0.5). Pale yellow solid, mp 85 °C, yield 102 mg (44%). Purity (HPLC): 97.3%, t_R = 22.21 min. $C_{33}H_{33}F_7N_2O_2$ (622.6 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.31 (d, J = 6.1 Hz, 3H, CH_3), 1.64–1.74 (m, 1H, N(CH_2CH_2)$_2$), 1.92 (td, J = 13.9/4.3 Hz, 1H, N(CH_2CH_2)$_2$), 1.99–2.10 (m, 1H, NCH_2CH_2CH), 2.09–2.19 (m, 1H, N(CH_2CH_2)$_2$), 2.25 (td, J = 13.5/4.5 Hz, 1H, N(CH_2CH_2)$_2$), 2.35–2.47 (m, 1H, NCH_2CH_2CH), 2.55–2.63 (m, 2H, NCH_2), 2.61–2.71 (m, 2H, PhCH_2CH), 2.72–2.86 (m, 2H, NCH_2), 2.90–3.06 (m, 2H, NCH_2), 3.64 (dd, J = 8.5/6.4 Hz, 1H, NCH_2CH_2CH), 3.84–3.93 (m, 1H, $PhCH_2CH$), 4.43 (d, J = 15.8 Hz, 1H, PhCH_2NH), 4.58 (d, J = 15.7 Hz, 1H, PhCH_2NH), 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, NCH_2CH_2CH), 33.3 (1C, N(CH_2CH_2)$_2$), 36.5 (1C, PhCH_2CH), 37.6 (1C, N(CH_2CH_2)$_2$), 41.7 (1C, $PhCH_2NH$), 48.7 (0.5C, N(CH_2CH_2)$_2$), 48.8 (0.5C, N(CH_2CH_2)$_2$), 49.0 (0.5C, N(CH_2CH_2)$_2$), 49.1 (0.5C, N(CH_2CH_2)$_2$), 49.3 (1C, NCH_2CH_2CH), 55.8 (1C, NCH_2CH_2CH), 64.3 (1C, $PhCH_2CH$), 72.6 (1C, ArCO), 115.1 (d, J = 20.2 Hz, 2C, C-3′$_{arom}$, C-5′$_{arom}$), 120.4 (hept, J = 3.9 Hz, 1C, C-4″$_{arom}$), 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, C-2″$_{arom}$, C-6″$_{arom}$), 128.4 (1C, C-7$_{arom}$), 129.0 (d, J = 8.0 Hz, 2C, C-2′$_{arom}$, C-6′$_{arom}$), 131.3 (q, J = 33.0 Hz, 2C, C-3″$_{arom}$, C-5″$_{arom}$), 133.9 (1C, C-4a$_{arom}$), 136.4 (d, J = 2.8 Hz, 1C, C-1′$_{arom}$), 140.4 (1C, C-8a$_{arom}$), 142.3 (1C, C-1″$_{arom}$), 162.2 (d, J = 250.8 Hz, 1C, C-4′$_{arom}$), 174.0 (1C, C=O). MS (APCI): m/z = 623.2498 (calcd 623.2503 for $C_{33}H_{34}F_7N_2O_2$ [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 (3×). 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 (Ø 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. $C_{33}H_{33}F_7N_2O_3$ (638.6 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.92–2.00 (m, 2H, N(CH_2CH_2)$_2$), 2.04–2.12 (m, 2H, N(CH_2CH_2)$_2$), 2.38–2.49 (m, 1H, NCH_2CH_2CH), 2.66–2.75 (m, 1H, NCH_2CH_2CH), 2.78 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 2.80–2.95 (m, 3H, NCH_2), 3.03–3.17 (m, 3H, NCH_2), 3.66 (t, J = 7.4 Hz, 1H, NCH_2CH_2CH), 3.75 (s, 3H, OCH_3), 3.87 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 4.40 (d, J = 15.8 Hz, 1H, PhCH_2NH), 4.60 (d, J = 15.8 Hz, 1H, PhCH_2NH), 6.66 (d, J = 2.6 Hz, 1H, 5-H_{arom}), 6.77 (dd, J = 8.7/2.7 Hz, 1 H, 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. ^{13}C NMR (CD_3OD): δ [ppm] = 28.6 (1C, NCH_2CH_2CH), 29.2 (1C, $PhCH_2CH_2$), 34.9 (2C, N(CH_2CH_2)$_2$), 41.7 (1C, PhCH_2NH), 48.8 (1C, N(CH_2CH_2)$_2$), 49.0 (1C, N(CH_2CH_2)$_2$), 49.1 (1C, NCH_2CH_2CH), 54.2 (1C, OCH_3), 55.5 (1C, NCH_2CH_2CH), 58.7 (1C, $PhCH_2CH_2$), 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, CF_3), 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 $C_{33}H_{34}F_7N_2O_3$ [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 (3×). 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 (Ø 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)). 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. $C_{31}H_{31}F_5N_2O_2$ (558.6 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.91–2.01 (m, 2H, N(CH_2CH_2)$_2$), 2.00–2.08 (m, 1H, NCH_2CH_2CH), 2.11 (td, J = 13.8/4.6 Hz, 2H, N(CH_2CH_2)$_2$), 2.35–2.46 (m, 1H, NCH_2CH_2CH), 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, N(CH_2CH_2)$_2$), 3.64 (dd, J = 8.4/6.5 Hz, 1H, NCH_2CH_2CH), 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, NCH_2CH_2CH), 29.0 (1C, $PhCH_2CH_2$), 35.0 (2C, N(CH_2CH_2)$_2$), 41.7 (1C, PhCH_2NH), 48.8 (1C, N(CH_2CH_2)$_2$), 48.9 (1C, N(CH_2CH_2)$_2$), 49.2 (1C, NCH_2CH_2CH), 55.6 (1C, NCH_2CH_2CH), 58.7 (1C, $PhCH_2CH_2$), 71.8 (1C, ArCO), 110.8 (dd, J = 25.2/3.8 Hz, 1C, C-4″$_{arom}$), 115.1 (d, J = 21.6 Hz, 2C, C-3′$_{arom}$, C-5′$_{arom}$), 117.5 (d, J = 22.9 Hz, 1C, C-2″$_{arom}$), 119.3 (quint, J = 4.1 Hz, 1C, C-6″$_{arom}$), 124.6 (1C, C-8$_{arom}$), 126.0 (1C, C-6$_{arom}$), 126.3 (1C, C-5$_{arom}$), 128.6 (1C, C-7$_{arom}$), 129.1 (d, J = 8.3 Hz, 2C, C-2′$_{arom}$, C-6′$_{arom}$), 132.2 (qd, J = 26.1/8.4 Hz, 1C, C-5″$_{arom}$), 133.7 (1C, C-4a$_{arom}$), 136.3 (1C, C-1′$_{arom}$), 141.0 (1C, C-8a$_{arom}$), 143.5 (d, J = 7.5 Hz,1C, C-1″$_{arom}$), 162.3 (d, J = 244.9 Hz, 1C, C-3″$_{arom}$), 162.5 (d, J = 247.6 Hz, 1C, C-4′$_{arom}$), 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 (3×). 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 (Ø 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). ^{1}H NMR (CD_3OD): δ [ppm] = 1.33 (d, *J* = 6.1 Hz, 3H, C*H*$_3$), 1.71–1.79 (m, 1H, N(CH$_2$C*H*$_2$)$_2$), 1.98 (td, *J* = 13.9/4.2 Hz, 1H, N(CH$_2$C*H*$_2$)$_2$), 2.05–2.15 (m, 1H, NCH$_2$C*H*$_2$CH), 2.18–2.25 (m, 1H, N(CH$_2$C*H*$_2$)$_2$), 2.30 (td, *J* = 13.7/4.5 Hz, 1H, N(CH$_2$C*H*$_2$)$_2$), 2.39–2.50 (m, 1H, NCH$_2$C*H*$_2$CH), 2.60–2.73 (m, 2H, PhC*H*$_2$CH), 2.74–2.85 (m, 2H, NC*H*$_2$), 2.89–3.05 (m, 2H, NC*H*$_2$), 3.10–3.21 (m, 2H, NC*H*$_2$), 3.66 (dd, *J* = 8.5/6.4 Hz, 1H, NCH$_2$CH$_2$C*H*), 3.85–3.93 (m, 1H, PhCH$_2$C*H*), 4.36 (d, *J* = 15.7 Hz, 1H, PhC*H*$_2$NH), 4.50 (d, *J* = 15.7 Hz, 1H, PhC*H*$_2$NH), 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, *C*H$_3$), 29.2 (1C, NCH$_2$*C*H$_2$CH), 33.8 (1C, N(CH$_2$*C*H$_2$)$_2$), 36.6 (1C, PhCH$_2$*C*H), 38.1 (1C, N(CH$_2$*C*H$_2$)$_2$), 41.7 (1C, PhCH$_2$NH), 48.7 (0.5C, N(*C*H$_2$CH$_2$)$_2$), 48.8 (0.5C, N(*C*H$_2$CH$_2$)$_2$), 49.0 (0.5C, N(*C*H$_2$CH$_2$)$_2$), 49.2 (0.5C, N(*C*H$_2$CH$_2$)$_2$), 49.6 (1C, NCH$_2$CH$_2$CH), 56.2 (1C, NCH$_2$CH$_2$*C*H), 64.1 (1C, PhCH$_2$*C*H), 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*-4a$_{arom}$), 135.8 (1C, *C*-1′$_{arom}$), 141.0 (1C, *C*-8a$_{arom}$), 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 *C*F$_3$ group and for *C*-5″ are not seen in the spectrum. MS (APCI): *m*/*z* = 573.2558 (calcd 573.2535 for $C_{32}H_{34}F_5N_2O_2$ [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 (4l)

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 CH_2Cl_2 (3×). 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 (Ø 2 cm, length 15 cm, $CH_2Cl_2/CH_3OH/NH_3$ 95:4.5:0.5, fraction size 10 mL; R_f = 0.23 ($CH_2Cl_2/CH_3OH/NH_3$ 95:4.5:0.5)) and recrystallized from CH_2Cl_2. Yellow oil, yield 44 mg (5.7%). Purity (HPLC): 82.1%, t_R = 20.52 min. $C_{32}H_{33}F_5N_2O_3$ (588.6 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.91–2.00 (m, 2H, N(CH$_2$C*H*$_2$)$_2$), 2.09 (td, *J* = 13.2/4.3 Hz, 2H, N(CH$_2$C*H*$_2$)$_2$), 2.36–2.48 (m, 1H, NCH$_2$C*H*$_2$CH), 2.65–2.73 (m, 1H, NCH$_2$C*H*$_2$CH), 2.77 (t, *J* = 5.5 Hz, 2H, PhC*H*$_2$CH$_2$), 2.78–2.96 (m, 3H, NC*H*$_2$), 3.02–3.14 (m, 3H, NC*H*$_2$), 3.64 (t, *J* = 7.41 Hz, 1H, NCH$_2$CH$_2$C*H*), 3.75 (s, 3H, OC*H*$_3$), 3.87 (t, *J* = 5.5 Hz, 2H, PhCH$_2$C*H*$_2$), 4.35 (d, *J* = 15.7 Hz, 1H, PhC*H*$_2$NH), 4.49 (d, *J* = 15.7 Hz, 1H, PhC*H*$_2$NH), 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. ^{13}C NMR (CD_3OD): δ [ppm] = 29.2 (1C, NCH$_2$*C*H$_2$CH), 29.4 (1C, PhCH$_2$*C*H$_2$), 35.9 (2C, N(CH$_2$*C*H$_2$)$_2$), 41.7 (1C, PhCH$_2$NH), 48.8 (1C, N(*C*H$_2$CH$_2$)$_2$), 49.1 (1C, N(*C*H$_2$CH$_2$)$_2$), 49.6 (1C, NCH$_2$CH$_2$*C*H), 54.2 (1C, O*C*H$_3$), 56.2 (1C, NC*H*$_2$CH$_2$CH), 58.4 (1C, PhCH$_2$*C*H$_2$), 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 *C*F$_3$ group is not seen in the spectrum. MS (APCI): *m*/*z* = 589.2497 (calcd 589.2484 for $C_{32}H_{34}F_5N_2O_3$ [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 $NaHCO_3$ was added and the aqueous layer was extracted with CH_2Cl_2 (3×). The 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 (Ø 2 cm, length 17 cm, $CH_2Cl_2/CH_3OH/NH_3$ 95:4.5:0.5, fraction size 10 mL, R_f = 0.18 ($CH_2Cl_2/CH_3OH/NH_3$ 95:4.5:0.5)). Yellow oil, yield 17 mg (27%). Purity (HPLC): 49.9%, t_R = 23.67 min $C_{38}H_{37}F_5N_2O_3$ (664.7 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.90–1.97 (m, 2H, N(CH$_2$C*H*$_2$)$_2$), 2.03–2.11 (m, 2H, N(CH$_2$C*H*$_2$)$_2$), 2.36–2.44 (m, 1H, NCH$_2$C*H*$_2$CH), 2.61–2.67 (m, 1H, NCH$_2$C*H*$_2$CH), 2.68–2.73 (m, 1H, NC*H*$_2$), 2.75 (t, *J* = 5.4 Hz, 2H, PhC*H*$_2$CH$_2$), 2.78–2.85 (m, 3H, NC*H*$_2$), 2.99–3.09 (m, 2H, NC*H*), 3.63–3.69 (m, 1H, NCH$_2$CH$_2$C*H*), 3.85 (t, *J* = 5.5 Hz, 2H, PhCH$_2$C*H*$_2$), 4.34 (d, *J* = 15.7 Hz, 1H, PhC*H*$_2$NH), 4.48 (d, *J* = 15.7 Hz, 1H, PhC*H*$_2$NH), 5.04 (s, 2H, PhCH$_2$O), 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 $C_{38}H_{38}F_5N_2O_3$ [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 CH_3OH (0.8 mL) and Pd/C (10% w/w, 22 mg, 0.2 mmol) was added. The mixture was stirred under H_2-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 (Ø 1 cm, length 18 cm, $CH_2Cl_2/CH_3OH/NH_3$ 95:4.5:0.5, fraction size 5 mL, R_f = 0.05 ($CH_2Cl_2/CH_3OH/NH_3$ 95:4.5:0.5)). Brown oil, yield 5.2 mg (48%). Purity

(HPLC): 80.3%, t_R = 20.39 min $C_{31}H_{31}F_5N_2O_3$ (574.6 g/mol). ^{1}H NMR (CD_3OD): δ [ppm] = 1.79–1.84 (m, 2H, $N(CH_2CH_2)_2$), 1.96 (td, J = 13.4/4.6 Hz, 2H, $N(CH_2CH_2)_2$), 2.30–2.35 (m, 2H, NCH_2CH_2CH), 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, NCH_2CH_2CH), 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, NCH_2CH_2CH), 29.9 (1C, $PhCH_2CH_2$), 36.13 (1C, $N(CH_2CH_2)_2$), 36.14 (1C, $N(CH_2CH_2)_2$), 41.7 (1C, $PhCH_2NH$), 48.8 (1C, $N(CH_2CH_2)_2$), 49.1 (1C, $N(CH_2CH_2)_2$), 49.7 (1C, NCH_2CH_2CH), 56.4 (1C, NCH_2CH_2CH), 58.4 (1C, $PhCH_2CH_2$), 72.5 (1C, ArCO), 110.7 (dq, J = 24.9/4.0 Hz, 1C, C-4″$_{arom}$), 113.3 (1C, C-7$_{arom}$), 114.3 (1C, C-5$_{arom}$), 114.9 (d, J = 22.0 Hz, 2C, C-3′$_{arom}$, C-5′$_{arom}$), 117.4 (d, J = 22.5 Hz, 1C, C-2″$_{arom}$), 119.3 (quint, J = 3.9 Hz,1C, C-6″$_{arom}$), 125.9 (1C, C-8$_{arom}$), 129.1 (d, J = 8.1 Hz, 2C, C-2′$_{arom}$, C-6′$_{arom}$), 132.3 (1C, C-4a$_{arom}$), 134.8 (1C, C-8a$_{arom}$), 135.9 (d, J = 3.5 Hz, 1C, C-1′$_{arom}$), 143.6 (d, J = 7.5 Hz, 1C, C-1″$_{arom}$), 155.3 (1C, C-6$_{arom}$), 162.1 (d, J = 244.5 Hz, 1C, C-3″$_{arom}$), 162.5 (d, J = 248.0 Hz, 1C, C-4′$_{arom}$), 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 $NaHCO_3$ was added and the aqueous layer was extracted with CH_2Cl_2 (3×). The 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 twice (Ø 1 cm, length 28 cm, $CH_2Cl_2/CH_3OH/NH_3$ 96.5:3:0.5, fraction size 5 mL; Ø 1 cm, length 22 cm, $CH_2Cl_2/CH_3OH/NH_3$ 96.5:3:0.5, fraction size 5 mL; R_f = 0.08 ($CH_2Cl_2/CH_3OH/NH_3$ 96.5:3:0.5)). Yellow oil, yield 26 mg (34%). Purity (HPLC): 93.9%, t_R = 21.86 min. $C_{29}H_{32}F_6N_2O_2$ (554.6 g/mol). ^{1}H NMR (CD_3OD): 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, NCH_2CH_2CH), 0.92–1.01 (m, 1H, NCH_2CH_2CH), 0.97–1.04 (m, 1H, $CH_{cycloprop}$), 1.60 (td, J = 9.3/4.9 Hz, 1 H, NCH_2CH_2CH), 1.93–2.03 (m, 2H, $N(CH_2CH_2)_2$), 2.06–2.13 (m, 2H, $N(CH_2CH_2)_2$), 2.60–2.70 (m, 2H, NCH_2), 2.81 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 2.82–2.90 (m, 2H, NCH_2), 3.02–3.08 (m, 2H, NCH_2), 3.90 (t, J = 5.5 Hz, 2H, $PhCH_2CH_2$), 4.49 (d, J = 15.7 Hz, 1H, $PhCH_2NH$), 4.63 (d, J = 15.5 Hz, 1H, $PhCH_2NH$), 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. ^{13}C NMR (CD_3OD): δ [ppm] = 2.7 (1C, $CH_{2cycloprop}$), 3.7 (1C, $CH_{2cycloprop}$), 13.7 (1C, $CH_{cycloprop}$), 29.0 (1C, NCH_2CH_2CH), 29.2 (1C, $PhCH_2CH_2$), 34.9 (2C, $N(CH_2CH_2)_2$), 41.7 (1C, $PhCH_2NH$), 48.6 (1C, $N(CH_2CH_2)_2$), 49.0 (1C, $N(CH_2CH_2)_2$), 55.6 (1C, NCH_2CH_2CH), 58.8 (1C, $PhCH_2CH_2$), 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 CF_3 groups, for C-3′ and C-5′ are not seen in the spectrum. MS (APCI): m/z = 555.2455 (calcd 555.2441 for $C_{29}H_{33}F_6N_2O_2$ [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

5.2.1.1. Materials. [^{125}I]-CCL2 (81.4 GBq/μmol (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).

5.2.1.2. Cell culture and membrane preparation. U2OS cells stably expressing the human CCR2 (Invitrogen, Carlsbad, CA) were cultured in McCoys5a 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 μg/mL streptomycin, 100 μg/mL G418, 50 μg/mL hygromycin, and 125 μg/mL zeocin in a humidified atmosphere at 37 °C and 5% CO_2. Cell culture and membrane preparation were performed as described previously.[19]

5.2.1.3. ^{125}I-CCL2 binding assays. Binding assays were performed in a 100-μl reaction volume containing 50 mM Tris–HCl buffer (pH 7.4), 5 mM $MgCl_2$, 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 ^{125}I-CCL2 using at least 6 concentrations of competing ligand for 150 min 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).

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

5.2.2. Functional CCR2 assays

5.2.2.1. 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.

5.2.2.2. Measurement of cellular 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 μg/mL geniticine and non-essential amino acids (NEAA)). The cells were transferred into Optimem (supplemented by 5% FCS, 50 U/mL penicillin and 50 μg/mL streptomycin and NEAA) and seeded into 384-well plates (μCLEAR/black Greiner Bio One) at a density of 5000 cells/25 μL. Cells were incubated for approximately 24 h at 37 °C, 5% CO_2. Before the assay medium was removed and the cells were incubated with Fluo-4 solution (25 μL Tyrode's solution containing 3 μM Fluo-4 AM (1 mM DMSO stock solution), 0.4 mg/mL brilliant black, 2.5 mM probenicid, 0.03% pluronic F-127) for 60 min at 37 °C, 5% CO_2. 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 $CaCl_2$ and 0.05% BSA. Compounds (10 μL per well) were added and cells were incubated for 10 min at 37 °C, 5% CO_2. Then 20 μL of agonist solution (recombinant human CCL2 (PeproTech, 300-04) in Tyrode's solution with 0.05% BSA) were added. CCL2 was applied at EC_{50}, 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. IC_{50} values were fitted using a 4 parameter logistic function (Hill function).

5.2.2.3. 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% CO_2. 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 $CaCl_2$ and 0,05% BSA. Compounds (10 μL per well) were added and cells were incubated for 10 min at 37 °C, 5% CO_2. Then 20 μL of agonist solution (recombinant murine CCL2 (PeproTech, 250-10) in Tyrodés solution with 0.05% BSA) were added. CCL2 was applied at EC_{50}, 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. IC_{50} values were fitted using a 4 parameter logistic function (Hill function).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.02.019.

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