Bioisosteric replacement of central 1,2,4-oxadiazole ring of high affinity CB_2 ligands by regioisomeric 1,3,4-oxadiazole ring

Dominik Heimann,[a,§] Corinna Lueg,[a,§] Henk de Vries,[b] Bastian Frehland,[a] Dirk Schepmann,[a] Laura H. Heitman[b] and Bernhard Wünsch[a,c]

[§] Both authors contributed equally to this work.

[a] Institut für Pharmazeutische und Medizinische Chemie der Universität Münster, Corrensstraße 48, D-48149 Münster, Germany.

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

[b] Division of Medicinal Chemistry, Leiden Academic Centre for Drug Research, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands.

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

Abstract

It has been reported that bioisosteric replacement of an 1,2,4-oxadiazole ring by an 1,3,4-oxadiazole ring leads to higher polarity, reduced metabolic degradation by human liver microsomes and reduced interaction with hERG channels. In a seven to eight step synthesis 1,3,4-oxadiazles **9a-c** were synthesized as bioisosteric analogs of high-affinity but rather lipophilic CB_2 ligands **1a-c** containing an 1,2,4-oxadiazole ring. The 1,3,4-oxadiazole derivatives **9a** and **9b** show 10- and 50-fold reduced CB_2 affinity compared to the 1,2,4-oxadiazole derivatives **1a** and **1b**, respectively. However, the 1,3,4-oxadiazole **9a** has high CB_2 affinity (K_i = 25 nM) and high selectivity over the CB_1

receptor.

Key words

CB_2 ligands; bioisosterism; 1,2,4-oxadiazoles; 1,3,4-oxadiazoles; carbazolamides; fluorinated PET tracer;

1. Introduction

The $G_{i/o}$ protein-coupled CB_2 receptor belongs to the endogenous cannabinoid (endocannabinoid) system. After its discovery, it was referred to as the peripheral cannabinoid receptor since it could initially only be detected in peripheral organs (e.g. reproductive, cardiovascular, gastrointestinal and respiratory system).[1,2,3,4,5] Especially on immune cells (e.g. macrophages, T lymphocytes, B lymphocytes and natural killer cells) the CB_2 receptor is highly expressed.[6] In 2002, the presence of the CB_2 receptor was shown on microglia, i.e. immune cells in the central nervous system (CNS).[7] Under normal conditions, the CB_2 receptor expression in the CNS is rather low, whereas inflammatory processes let the concentration rise.[8] Anti-inflammatory effects were observed in numerous *in vitro* and *in vivo* models after activation of central CB_2 receptors.[9] Therefore, CB_2 agonists are promising compounds for the treatment of many neurodegenerative, neuroinflammatory and neuroimmunological diseases.[9]

Positron emission tomography (PET) is an imaging method that allows the visualization and time-dependent quantification of tracers, which possess a good affinity/selectivity profile towards a specific target (e.g. receptor) and contains a positron-emitting isotope like ^{18}F or ^{11}C. PET tracers can contribute to better understand biochemical processes like the development and severity of neuroinflammatory processes.

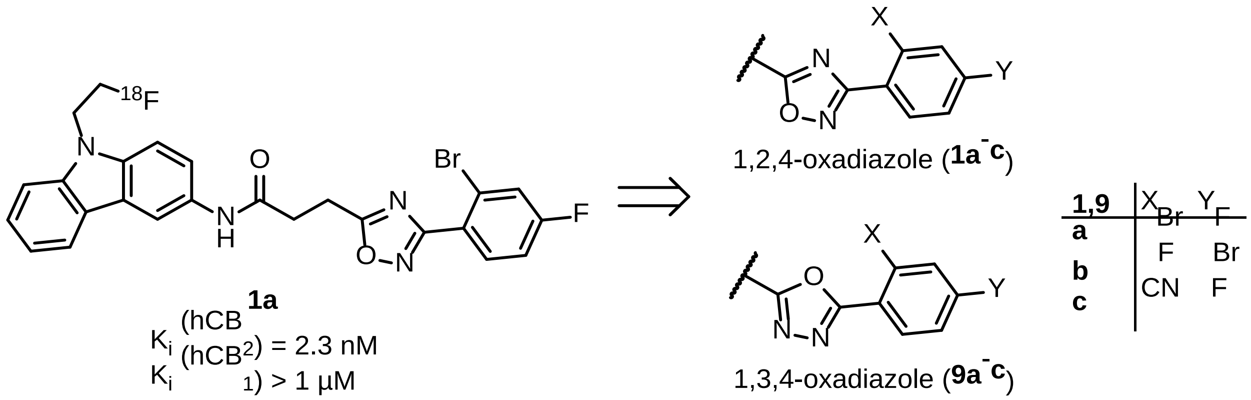


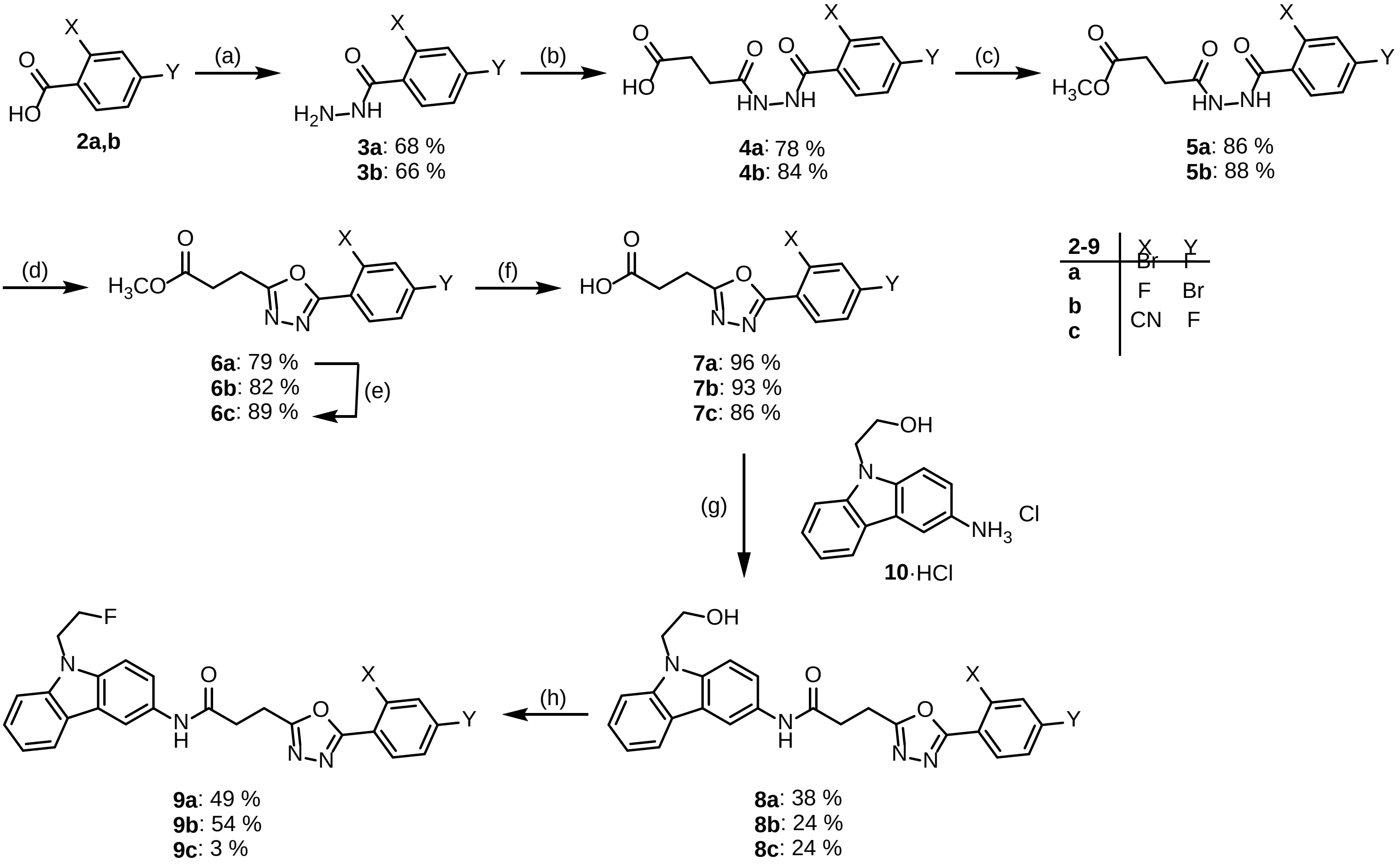
Figure 1. Lead compound **1a** and comparison of the 1,2,4-oxadiazole moiety of **1a** with the 1,3,4-oxadiazole moiety.

In 2013, the fluorine-18 labeled PET tracer $[^{18}F]$**1a** for imaging of CB_2 receptors has been reported (Figure 1). Although the 1,2,4-oxadiazole derivative **1a** displayed high CB_2 affinity ($K_i = 2.3$ nM) and high selectivity over the CB_1 subtype ($K_i > 1$ µM),[10,11] the high lipophilicity ($\log D_{7.4} = 3.8 – 4.2$) inhibited its broad application as PET tracer. In particular, the poor solubility in polar, parenterally administrable solvents (e.g. physiological saline solution) was recognized as problem.

Very recently, Boström *et al.* reported the concept of bioisosteric replacement of the 1,2,4-oxadiazole ring by an 1,3,4-oxadiazole ring resulting in reduced lipophilicity, higher metabolic stability during incubation with human liver microsomes and lower interactions with the hERG potassium channel.[12] Particularly, in case of rather lipophilic compounds ($\log D_{7.4} > 2.0$), replacement of the 1,2,4-oxadiazole ring by the more polar 1,3,4-oxadiazole ring leads to higher solubility in aqueous systems. However, the relative orientation of the substituents in 2- and 5-position is very similar in both ring systems.

In order to prove the feasibility of bioisosteric replacement of the 1,2,4-oxadiazole ring of potent CB_2 ligands such as **1**, the regioisomeric 1,3,4-oxadiazole derivatives **9** should be synthesized and pharmacologically evaluated (Figure 1).

2. Synthesis



Scheme 1. Reagents and reaction conditions: (a) 1. $SOCl_2$, DMF, toluene, 95 °C; 2. $N_2H_4{\cdot}H_2O$, CH_2Cl_2, 40 °C. (b) succinic anhydride, EtOAc, 40 °C, rt. (c) $SOCl_2$, DMF, MeOH, 0 °C → rt. (d) $SOCl_2$, DMF, toluene, Na_2SO_4, 95 °C. (e) CuCN, $H_3CC(O)N(CH_3)_2$, 155 °C. (f) LiOH, THF/H_2O, rt. (g) COMU®, NEt_3, DMF, 45 °C. (h) XtalFluor-E®, $NEt_3{\cdot}3HF$, CH_2Cl_2, -78 °C → rt.

The 1,3,4-oxadiazole derivatives **9a**-**c** were synthesized starting from regioisomeric 2,4-disubstituted benzoic acids **2a** and **2b** (Scheme 1). After activation with $SOCl_2$, the benzoyl halides were reacted with $N_2H_4 \cdot H_2O$ to afford the benzohydrazides **3a** and **3b**. The second acylation of the hydryzine moiety was carried out with succinic anhydride to give diacylhydrazines **4a** and **4b**. After esterification of the acids **4a** and **4b** with methanol, condensation of the diacylhydrazines **5a** and **5b** was performed with $SOCl_2$ in the presence of catalytic amounts of DMF providing the 1,3,4-oxadiazoles **6a** and **6b**. The addition of anhydrous Na_2SO_4 increased the yield from 10 % to 79 % for **6a** and 82 % for **6b**, respectively. Treatment of the bromo derivative **6a** with CuCN under *Rosenmund-von-Braun* conditions led to the nitrile **6c** in 89 % yield. Hydrolysis of the esters **6a**-**c** with LiOH provided the carboxylic acids **7a**-**c**, which were coupled with the carbazolamine **10** and COMU® to yield the amides **8a-c**. Carbazolamine **10** was obtained by hydroxyethylation of carbazole followed by nitration and catalytic hydrogenation according to literature.[13] Deoxofluorination of alcohols **8a-c** with XtalFluoro-E® (diethylaminodifluorosulfonium tetrafluoroborate) gave the aliphatic fluorides **9a-c**. The yields of the final reaction step were not optimized, since we were predominatly interested in very pure samples for biological testing.

3. Receptor affinity

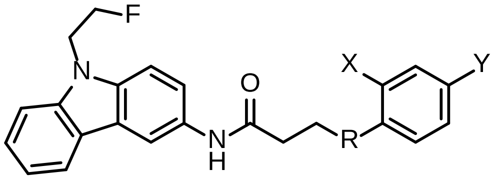
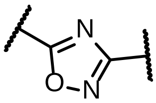
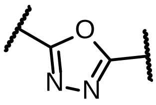


Table 1. CB_1 and CB_2 receptor affinity of 1,3,4- and 1,2,4-oxadiazole regioisomers **1a-c** and **9a-c**.

Compd	R	X	Y	K_i (hCB_2) ± SEM [nM][a]	displacement (hCB_1)[b]
1a		Br	F	2.9 ± 0.41[d]	- 13 %[c]
1b		F	Br	6.7 ± 1.0[d]	- 5 %
1c		CN	F	270 ± 32[d]	- 8 %
9a		Br	F	25 ± 4.1	22 %[c]
9b		F	Br	318 ± 55	- 8 %
9c		CN	F	219 ± 16	- 1 %
CP 55,940				8.44 ± 0.18	9.26 ± 0.12
WIN 55,212-2				8.57 ± 0.16	8.72 ± 0.24
HU 210				9.78 ± 0.04	9.55 ± 0.06

[a] The reported K_i-values are mean values of three independent experiments (n = 3).

[b] Due to the low hCB_1 affinity, only the radioligand displacement at a test compound concentration of 1 µM is given as mean value of two independent experiments (n = 2).

[c] Mean value of four experiments (n = 4).

[d] The CB_2 affinity of lead compounds **1a-c** has been recorded previously in another laboratory.[11]

The CB_2 and CB_1 receptor affinities were assessed in competition binding experiments with fragments of CHO-K1 cells expressing the human CB_1 or CB_2 receptor, respectively. [^{3}H]CP-55,940 served as radioligand in both assays. The non-specific binding of the radioligand [^{3}H]CP-55,940 was determined with rimonabant (SR141716A) and AM630, respectively.

The regioisomeric bromofluorophenyl derivative **1a** and **1b** containing the 1,2,4-oxadiazole ring show high CB_2 affinity with K_i values of 2.9 nM and 6.7 nM, respectively. Introduction of a cyano group as pseudohalogen in 2-position of the phenyl ring led to 100-fold decreased CB_2 affinity of **1c** compared to the bromo compound **1a**.

Replacement of the central 1,2,4-oxadiazle ring of **1** by the regioisomeric 1,3,4-oxadiazole ring led to 10- and 50-fold reduced CB_2 affinity of **9a** and **9b**, respectively. The nitrile **9c** displays almost the same CB_2 affinity as the low affinity regioisomer **1c**. Due to the negligible CB_1 affinity all compounds show high CB_2 : CB_1 selectivity, independent on the structure of the oxadiazole ring and the substitution pattern of the phenyl ring.

The CB_2 affinity of alcohols **8a-c** was also determined in the described assay. However, **8a-c** did not compete with the radioligand even at the high concentration of 1 µM. This result is in good agreement with with results obtained for the regioisomeric 1,2,4-oxadiazoles with hydroxyethyl moiety at the carbazole-N-atom.[11]

4. Conclusion

The aim of this study was to investigate, whether the rather lipophilic 1,2,4-oxadiazole ring of potent CB_2 ligands **1** can be replaced bioisosterically by the more polar 1,3,4-oxadiazole ring. For this purpose, three pairs of regioisomeric 1,2,4- and 1,3,4-oxadiazoles **1a-c** and **9a-c** were prepared and pharmacologically evaluated. *In vitro* radioligand binding studies revealed that displacement of the 1,2,4-oxadiazole ring of the high affinity ligands **1a** and **1b** by the regioisomeric 1,3,4-oxadiazole ring in **9a** and **9b** led to 10- and 50-fold reduced CB_2 affinity, respectively. Nevertheless, the bromofluoro derivative **9a** displays CB_2 affinity in the low nanomolar range (K_i = 25 nM) and high CB_2 : CB_1 selectivity.

5. Experimental

5.1 Chemistry, General Methods

Unless otherwise noted, moisture sensitive reactions were conducted under dry nitrogen. THF was dried with sodium/benzophenone and was freshly distilled before use. 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, eluent, fraction size, R_f value. Melting point: Melting point apparatus SMP 3 (Stuart Scientific), uncorrected. MS: MAT GCQ (Thermo-Finnigan); IR: IR spectrophotometer 480Plus FT-ATR-IR (Jasco). ^1H NMR (400 MHz), ^{13}C NMR (100 MHz): Unity Mercury Plus 400 spectrometer (Varian); δ in ppm related to tetramethylsilane; coupling constants are given with 0.5 Hz resolution. HPLC method for determination of the product purity: Merck Hitachi Equipment; UV detector: L-7400; autosampler: L-7200; pump: L-7100; degasser: L-7614; Method: column: LiChrospher® 60 RP-select B (5 µm), 250-4 mm cartridge; flow rate: 1.00 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: gradient from 90 % to 0 %, 29-31 min: 0 %, 31-31.5 min: gradient from 0 % to 90 %, 31.5-40 min: 90 %.

5.2 Synthetic procedures

5.2.1 2-Bromo-4-fluorobenzohydrazide (3a)

Preparation of this compound is described in literature[14] following a different synthesis route.

Under N_2, $SOCl_2$ (1.5 mL, 20.7 mmol) was added to a suspension of 2-bromo-4-fluorobenzoic acid (**2a**, 3.0 g, 13.7 mmol) and DMF (0.05 mL) in toluene (25 mL). The mixture was stirred at 95 °C for 1.5 h. After cooling down to rt, the mixture was concentrated *in vacuo* to give 4-bromo-2-fluorobenzoyl chloride. Without further purification 2-bromo-4-fluorobenzoyl chloride was dissolved in CH_2Cl_2 (200 mL) and hydrazine monohydrate (64 % in H_2O, 2.8 mL, 35.8 mmol) was added. The reaction mixture was stirred for 4.5 h at 45 °C. The organic solvent was removed under reduced pressure and the residue was purified by fc (d = 5.5 cm, l = 10 cm, cyclohexane/ethyl acetate 10:90, R_f 0.38 (ethyl acetate)). Colorless solid, mp 97 - 99 °C, yield 2.2 g (68 %). $C_7H_6BrFN_2O$ (233.0 g/mol). Exact mass (APCI): m/z = calcd. for $C_7H_6{}^{79}BrFN_2OH$ 232.9720 found 232.9699. Purity (HPLC): 83.4 % (t_R = 6.29 min). ^1H NMR (DMSO-D_6): δ (ppm) = 4.49 (s, 2H, NH-N*H_2*), 7.31 (td, *J* = 8.5/2.5 Hz, 1H, 5-H), 7.42 (dd, *J* = 8.8/6.1 Hz, 1H, 6-H), 7.64 (dd, *J* = 8.5/2.5 Hz, 1H, 3-H), 9.56 (s, 1H, N*H*-NH_2). ^{13}C NMR (DMSO-D_6): δ (ppm) = 115.3 (d, *J* = 21.3 Hz, 1C, C-5), 121.1 (m, 2C, C-2, C-3), 131.9 (d, *J* = 9.1 Hz, 1C, C-6), 135.1 (d, *J* = 3.5 Hz, 1C, C-1), 163.0 (d, *J* = 251.2 Hz, 1C, C-4), 166.5 (1C, C=O). IR (neat): ʋ (cm^{-1}) = 3321 (w, NH_2), 3159 (m, -NH-), 1654 (s, C=O).

5.2.2 4-Bromo-2-fluorobenzohydrazide (3b)

Preparation of this compound is described in literature[14,15] following a different synthesis route.

Under N_2, $SOCl_2$ (1.5 mL, 20.7 mmol) was added to a suspension of 4-bromo-2-fluorobenzoic acid (**2b**, 3.0 g, 13.7 mmol) and DMF (0.05 mL) in toluene (25 mL). The mixture was stirred at 95 °C for 1.5 h. After cooling down to rt, the mixture was concentrated *in vacuo* to give 4-bromo-2-fluorobenzoyl chloride. Without further purification 2-bromo-4-fluorobenzoyl chloride was solved in CH_2Cl_2 (200 mL) and hydrazine monohydrate (64.0 % in H_2O, 2.8 mL, 35.8 mmol) was added. The reaction mixture was stirred for 4.5 h at 45 °C. The organic solvent was removed under reduced pressure and the residue was purified by fc (d = 4.0 cm, l = 10 cm, cyclohexane/ethyl acetate 10:90, R_f 0.38 (ethyl acetate)). Colorless solid, mp 98 - 101 °C, yield 2.1 g (66 %). $C_7H_6BrFN_2O$ (233.0 g/mol). Exact mass (APCI): m/z = calcd. for $C_7H_6{}^{79}BrFN_2OH$ 232.9720 found 232.9729. Purity (HPLC): 98.7 % (t_R = 8.11 min). ^1H NMR (DMSO-D_6): δ (ppm) = 4.56 (s, 2H, NH-N*H_2*) 7.46 - 7.53 (m, 2H, 3-H, 5-H), 7.47 (d, *J* = 10.4 Hz, 1H, 6-H), 9.60 (s, 1H, N*H*-NH_2). ^{13}C NMR (DMSO-D_6): δ (ppm) = 120.2 (d, *J* = 25.8 Hz, 1C, C-1), 123.2 (d, *J* = 15.3 Hz, 1C, C-3), 124.4 (d, *J* = 9.1 Hz, 1C, C-6), 128.3 (d, *J* = 3.6 Hz, 1C, C-5), 132.1 (d, *J* = 3.9 Hz, 1C, C-4), 159.6 (d, *J* = 254.0 Hz, 1C, C-2), 163.1 (1C, C=O). IR (neat): υ (cm^{-1}) = 3321 (w, NH_2), 3130 (m, -NH-), 1658 (s, C=O).

5.2.3 4-[2-(2-Bromo-4-fluorobenzoyl)hydrazine-1-yl]-4-oxobutanoic acid (4a)

3a (1.0 g, 4.26 mmol) and succinic anhydride (1.1 g, 10.7 mmol) were suspended in ethyl acetate (350 mL) and the mixture was stirred for 3.5 h at rt. The reation mixture was diluted with diethyl ether (350 mL) and stirred overnight. The resulting precipitate was filtered off and washed with petroleum ether. The crude product was used without further purification (R_f 0.37 (ethyl acetate/formic acid 1:0.01)). Colorless solid, mp 185 - 186 °C, yield 1.1 g (78 %). $C_{11}H_{10}BrFN_2O_4$ (333.1 g/mol). Exact mass (APCI): m/z = calcd. for $C_{11}H_{10}{}^{79}BrFN_2O_4H$ 332.9881 found 332.9896. ^1H NMR (DMSO-D_6): δ (ppm) = 2.44 (t, *J* = 5.3 Hz, 2H, $CH_2\mathit{CH_2}CO_2H$), 2.47 (t, *J* = 5.2 Hz, 2H, $C\mathit{H_2}CH_2$ CO_2H), 7.36 (td, *J* = 8.5/2.4 Hz, 1H, 5-H), 7.50 (dd, *J* = 8.5/6.1 Hz, 1H, 6-H), 7.67 (dd, *J* = 8.7/2.4 Hz, 1H, 3-H), 10.08 (s, 1H, Ar-C(=O)-N*H*), 10.27 (s, 1H, -CH_2-C(=O)-N*H*).

5.2.4 4-[2-(4-Bromo-2-fluorobenzoyl)hydrazine-1-yl]-4-oxobutanoic acid (4b)

3b (1.0 g, 4.26 mmol) and succinic anhydride (1.1 g, 10.7 mmol) were suspended in ethyl acetate (350 mL) and the mixture was stirred for 3 h at rt. The reation mixture was diluted with diethyl ether (350 mL) and stirred overnight. The resulting precipitate was filtered and washed with petroleum ether. The crude product was used without further purification (R_f 0.40 (ethyl acetate/formic acid 1:0.01)). Colorless solid, mp 190 - 194 °C, yield 1.2 g (84 %). $C_{11}H_{10}BrFN_2O_4$ (333.1 g/mol). Exact mass (APCI): m/z = calcd. for $C_{11}H_{10}{}^{79}BrFN_2O_4H$ 332.9881 found 332.9878. ^1H NMR (DMSO-D_6): δ (ppm) = 2.44 (t, *J* = 6.0 Hz, 2H, $CH_2\mathit{CH_2}CO_2H$), 2.47 (t, *J* = 5.5 Hz, 2H, $C\mathit{H_2}CH_2CO_2H$), 7.52 - 7.55 (m, 2H, 3-H, 5-H), 8.07 (d, *J* = 10.4 Hz, 1H, 6-H), 10.06 (s, 1H, Ar-C(=O)-N*H*), 10.27 (s, 1H, -CH_2-C(=O)-N*H*).

5.2.5 Methyl 4-[2-(2-bromo-4-fluorobenzoyl)hydrazine-1-yl]-4-oxobutanoate (5a)

Under N_2, $SOCl_2$ (0.33 mL, 4.5 mmol) was added to a suspension of **4a** (1.0 g, 3.0 mmol) and DMF (0.05 mL) in CH_3OH (50 mL) at 0 °C. The reaction mixture was stirred at rt for 1 h. The mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate and washed with brine. The organic solvent was removed under reduced pressure and the residue was purified by fc (d = 4 cm, l = 10 cm, cyclohexane/ethyl acetate 10:90, R_f 0.50 (ethyl acetate)). Colorless solid, mp

146 - 48 °C, yield 900 mg (86 %). $C_{12}H_{12}BrFN_2O_4$ (347.1 g/mol). Exact mass (APCI): m/z = calcd. for $C_{12}H_{12}{}^{79}BrFN_2O_4H$ 347.0037 found 347.0033. ^1H NMR (DMSO-D$_6$): δ (ppm) = 2.46 (t, *J* = 7.0 Hz, 2H, $CH_2\mathit{CH_2}CO_2CH_3$), 2.56 (t, *J* = 7.0 Hz, 2H, $C\mathit{H_2}CH_2CO_2CH_3$), 3.59 (s, 3H, $CH_2CH_2CO_2C\mathit{H_3}$), 7.36 (td, *J* = 8.5, 2.5 Hz, 1H, 5-H), 7.49 (dd, *J* = 8.6/6.1 Hz, 1H, 6-H), 7.68 (dd, *J* = 8.8/2.5 Hz, 1H, 3-H), 10.11 (s, 1H, Ar-C(=O)-N*H*), 10.28 (s, 1H, -CH$_2$-C(=O)-N*H*). IR (neat): ʋ (cm^{-1}) = 3321 (w, NH$_2$), 3130 (m, -NH-),1720 (m, (C=O)-NH), 1654 (m, C=O$_{ester}$).

5.2.6 Methyl 4-[2-(4-bromo-2-fluorobenzoyl)hydrazine-1-yl]-4-oxobutanoate (5b)

Under N$_2$, SOCl$_2$ (0.2 mL, 3.1 mmol) was added to a suspension of **4b** (687 mg, 2.1 mmol) and DMF (0.05 mL) in CH_3OH (25 mL) at 0 °C. The reaction mixture was stirred at rt for 40 min. The resulting reaction mixture was stirred at rt for 1 h. The mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate and washed with brine. The organic solvent was removed under reduced pressure and the residue was purified by fc (d = 3.5 cm, l = 10 cm, cyclohexane/ethyl acetate 10:90, R$_f$ 0.50 (ethyl acetate)). Colorless solid, mp 148 -149 °C, yield 613 mg (88 %). $C_{12}H_{12}BrFN_2O_4$ (347.1 g/mol). Exact mass (APCI): m/z = calcd. for $C_{12}H_{12}{}^{79}BrFN_2O_4H$ 347.0037 found 347.0039. ^1H NMR (CDCl$_3$): δ (ppm) = 2.89 (t, *J* = 7.0 Hz, 2H, $CH_2\mathit{CH_2}CO_2CH_3$), 3.12 (t, *J* = 7.0 Hz, 2H, $C\mathit{H_2}CH_2CO_2CH_3$), 3.72 (s, 3H, $CH_2CH_2CO_2C\mathit{H_3}$), 7.37 (dd, *J* = 8.5/2.5 Hz, 1H, -CH$_2$-C(=O)-N*H*), 7.45 (dd, *J* = 8.4/1.8 Hz, 1H, Ar-C(=O)-N*H*), 7.98 (t, *J* = 8.3 Hz, 1H, 6-H), 8.80 (d, *J* = 5.9 Hz, 1H, 3-H), 9.15 - 9.25 (m, 1H, 5-H). IR (neat): ʋ (cm^{-1}) = 3321 (w, -NH$_2$), 3130 (m, -NH-),1720 (m, (C=O)-NH), 1654 (m, C=O$_{ester}$).

5.2.7 Methyl 3-[5-(2-bromo-4-fluorophenyl)-1,3,4-oxadiazol-2-yl]propanoate (6a)

Under N_2, a mixture of **5a** (200 mg, 0.6 mmol), DMF (0.05 mL) and toluene (15 mL) was heated to 75 °C. Na_2SO_4 (ca. 0.5 – 1.0 g) and $SOCl_2$ (0.04 mL, 0.5 mmol) were added and the mixture was heated to 95 °C for 2.5 h. The mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and the solution was washed with brine. The organic solvent was removed under reduced pressure and the residue was purified by fc (d = 4 cm, l = 10 cm, cyclohexane/ethyl acetate 10:90, R_f 0.75 (ethyl acetate)). Colorless solid, mp 75 - 77 °C, yield 150 mg (79 %). $C_{12}H_{10}BrFN_2O_3$ (329.0 g/mol). Exact mass (APCI): m/z = calcd. for $C_{12}H_{10}{}^{79}BrFN_2O_3H$ 328.9932 found 328.9922. Purity (HPLC): 96.9 % (t_R = 17.57 min). ^1H NMR (DMSO-D_6): δ (ppm) = 2.89 (t, *J* = 7.0 Hz, 2H, $CH_2\mathit{CH_2}CO_2CH_3$), 3.12 (t, *J* = 7.0 Hz, 2H, $C\mathit{H_2}CH_2CO_2CH_3$), 3.63 (s, 3H, $CH_2CH_2CO_2C\mathit{H_3}$), 7.51 (td, *J* = 8.5/2.5 Hz, 1H, 5-H), 7.89 (dd, *J* = 8.6/6.1 Hz, 1H, 6-H), 7.96 (dd, *J* = 8.8/2.5 Hz, 1H, 3-H). ^{13}C NMR (DMSO-D_6): δ (ppm) = 20.4 (1C, $\mathit{C}H_2CH_2CO_2CH_3$), 29.5 (1C, $CH_2\mathit{C}H_2CO_2CH_3$), 51.7 (1C, $CH_2CH_2CO_2\mathit{C}H_3$), 115.8 (d, *J* = 21.9 Hz, 1C, C-5), 121.7 (d, *J* = 3.6 Hz, 1C, C-1), 121.7 (d, *J* = 25.3 Hz, 1C, C-3), 121.9 (d, *J* = 10.3 Hz, 1C, C-2), 133.5 (d, *J* = 9.6 Hz, 1C, C-6), 162.1 (1C, $C\text{-}5_{\text{oxadiazole}}$), 163.2 (d, *J* = 249.3 Hz, 1C, C-4), 166.3 (1C, $C\text{-}2_{\text{oxadiazole}}$), 171.8 (1C, $\mathit{C}O_2CH_3$). IR (neat): υ (cm^{-1}) = 3074 (m, C-H, arom), 2958 (m, C-H, aliph), 1735 (s, C=O).

5.2.8 Methyl 3-[5-(4-bromo-2-fluorophenyl)-1,3,4-oxadiazol-2-yl]propanoate (6b)

Under N_2, a mixture of **5b** (200 mg, 0.6 mmol), DMF (0.05 mL) and toluene (15 mL) was heated to 75 °C. Na_2SO_4 (ca. 0.5 – 1.0 g) and $SOCl_2$ (0.04 mL, 0.5 mmol) were added and the mixture was heated to 95 °C for 2.5 h. The mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and the solution was washed with brine. The organic solvent was removed under reduced pressure and the residue was purified by fc (d = 5 cm, l = 10 cm, cyclohexane/ethyl acetate 10:90, R_f 0.75 (ethyl acetate)).

Colorless solid, mp 76 - 77 °C, yield 156 mg (82 %). $C_{12}H_{10}BrFN_2O_3$ (329.0 g/mol). Exact mass (APCI): m/z = calcd. for $C_{12}H_{10}{}^{79}BrFN_2O_3H$ 328.9932 found 328.9945. Purity (HPLC): 98.9 % (t_R = 15.17 min). ^{1}H NMR (DMSO-D$_6$): δ (ppm) 2.89 (t, *J* = 7.0 Hz, 2H, $CH_2\mathit{CH_2}CO_2CH_3$), 3.12 (t, *J* = 7.0 Hz, 2H, $C\mathit{H_2}CH_2CO_2CH_3$), 3.63 (s, 3H, $CH_2CH_2CO_2C\mathit{H_3}$), 7.51 (td, *J* = 8.5/2.5 Hz, 1H, 6-H), 7.89 (dd, *J* = 8.6/6.1 Hz, 1H, 3-H), 7.96 (dd, *J* = 8.8/6.0 Hz, 1H, 5-H). ^{13}C NMR (DMSO-D$_6$): δ (ppm) = 20.4 (1C, $\mathit{C}H_2CH_2CO_2CH_3$), 29.5 (1C, $CH_2\mathit{C}H_2CO_2CH_3$), 51.7 (1C, $CH_2CH_2CO_2\mathit{C}H_3$), 115.82 (d, *J* = 21.9 Hz, 1C, C-3), 121.4 – 122.1 (m, 3C, C-1, C-4, C-5), 133.5 (d, *J* = 9.7 Hz, 1C, C-6), 162.5 (d, *J* = 12.3 Hz, 1C, C-5$_{\text{oxadiazole}}$), 163.2 (d, *J* = 249.3 Hz, 1C, C-2), 166.3 (1C, C-2$_{\text{oxadiazole}}$), 171.7 (1C, $\mathit{C}O_2CH_3$). IR (neat): υ (cm^{-1}) = 3074 (m, C-H, arom), 2958 (m, C-H, aliph), 1735 (s, C=O).

5.2.9 Methyl 3-[5-(2-cyano-4-fluorophenyl)-1,3,4-oxadiazol-2-yl]propanoate (6c)

6a (200 mg, 0.6 mmol) and CuCN (271 mg, 3.1 mmol) were suspended in *N,N*-dimethylacetamide (5 mL) under N_2. The mixture was stirred at 155 °C for 12.5 h before cooling to room temperature. Saturated NH_4Cl (2 mL) and then ethyl acetate (8 mL) were added. The precipitated CuCN was filtered off and the organic layer was collected and washed once with brine. The organic layer was dried (Na_2SO_4), evaporated under reduced pressure and the residue was purified by fc (d = 2.5 cm, l = 10 cm, cyclohexane/ethyl acetate 75:25, R_f 0.68 (ethyl acetate)). Pale yellow oil, yield 147 mg (89 %). $C_{13}H_{10}FN_3O_3$ (275.2 g/mol). Exact mass (APCI): m/z = calcd. for $C_{13}H_{10}FN_3O_3H$ 276.0779 found 276.0808. Purity (HPLC): 96.7 % (t_R = 15.15 min). ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.96 (t, J = 7.3 Hz, 2H, $C\mathit{H_2}CH_2CO_2CH_3$), 3.31 (t, *J* = 7.3 Hz, 2H, $CH_2C\mathit{H}_2CO_2CH_3$), 3.63 (s, 3H, $CH_2CH_2CO_2C\mathit{H_3}$), 7.47 (td, *J* = 8.9/7.6/2.6 Hz, 1H, 5-H), 7.55 (dd, *J* = 7.8/2.6 Hz, 1H, 3-H), 8.26 (dd, *J* = 8.9/5.2 Hz, 1H, 6-H). ^{13}C NMR (DMSO-D$_6$): δ (ppm) = 21.6 (1C, $\mathit{C}H_2CH_2CO_2CH_3$), 30.7 (1C, $CH_2\mathit{C}H_2CO_2CH_3$), 51.8

(1C, $CH_2CH_2CO_2CH_3$), 111.9 (d, *J* = 10.3 Hz, 1C, C-2), 116.1 (1C, *C*N), 121.6 (d, *J* = 21.9 Hz, 1C, C-5), 122.5 (d, *J* = 26.1 Hz, 1C, C-3), 125.0 (d, *J* = 3.6 Hz, 1C, C-1), 132.6 (d, *J* = 9.4 Hz, 1C, C-6), 162.9 (d, *J* = 252.1 Hz, 1C, C-4), 165.0 (1C, C-$5_{\text{oxadiazole}}$), 171.8 (1C, C-$2_{\text{oxadiazole}}$), 179.9 (1C, CO_2CH_3). IR (neat): υ (cm^{-1}) = 2233 (w, CN), 1735 (s, C=O).

5.2.10 3-[5-(2-Bromo-4-fluorophenyl)-1,3,4-oxadiazol-2-yl]propanoic acid (7a)

6a (550 mg, 1.7 mmol) and LiOH (350 mg, 8.4 mmol) were dissolved in THF (60 mL) and H_2O (20 mL) and the mixture was stirred for 20 min at rt. Afterwards, the mixture was neutralized with 1 M H_2SO_4, diluted with ethyl acetate and washed twice with brine. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was washed with CH_2Cl_2 and purified by fc (ethyl acetate/formic acid 1:0.01, R_f 0.78). Colorless solid, mp 152 - 154 °C, yield 500 mg (96 %). $C_{11}H_8BrFN_2O_3$ (315.0 g/mol). Exact mass (APCI): m/z = calcd. for $C_{11}H_8{}^{79}BrFN_2O_3H$ 314.9775 found 314.9773. Purity (HPLC): 90.5 % (t_R = 18.41 min). ^{1}H NMR (DMSO-D_6): δ (ppm) = 3.00 (t, *J* = 7.2 Hz, 2H, $CH_2CH_2CO_2H$), 3.28 (t, *J* = 7.1 Hz, 2H, $CH_2CH_2CO_2H$), 7.18 (td, *J* = 8.8/2.5 Hz, 1H, 5-H), 7.49 (dd, *J* = 8.2/2.5 Hz, 1H, 3-H), 7.85 (dd, J = 8.8/5.9 Hz, 1H, 6-H). ^{13}C NMR (DMSO-D_6): δ (ppm) = 21.4 (1C, $CH_2CH_2CO_2H$), 29.8 (1C, $CH_2CH_2CO_2CH_3$), 115.8 (d, *J* = 21.9 Hz, 1C, C-5), 121.6 (d, *J* = 24.7 Hz, 1C, C-3), 121.7 (d, *J* = 3.6 Hz, 1C, C-1), 122.0 (d, *J* = 10.4 Hz, 1C, C-2), 133.5 (d, *J* = 9.6 Hz, 1C, C-6), 162.1 (1C, C-$5_{\text{oxadiazole}}$), 163.2 (d, *J* = 254.7 Hz, 1C, C-4), 166.6 (1C, C-$2_{\text{oxadiazole}}$), 171.8 (1C, CO_2H). IR (neat): υ (cm^{-1}) = 3170-2350 (m, COOH), 1705 (s, C=O).

5.2.11 3-[5-(4-Bromo-2-fluorophenyl)-1,3,4-oxadiazol-2-yl]propanoic acid (7b)

6b (450 mg, 1.4 mmol) and LiOH (290 mg, 6.9 mmol) were dissolved in THF (60 mL) and H_2O (20 mL) and the mixture was stirred for 20 min at rt. Afterwards, the mixture was neutralized with 1 M H_2SO_4, diluted with ethyl acetate and washed twice with brine. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was washed with CH_2Cl_2 and purified by fc (ethyl acetate/formic acid 1:0.01, R_f 0.80). Colorless solid, mp 152 - 155 °C, yield 409 mg (93 %). $C_{11}H_8BrFN_2O_3$ (315.0 g/mol). Exact mass (APCI): m/z = calcd. for $C_{11}H_8{}^{79}BrFN_2O_3H$ 314.9775 found 314.9760. Purity (HPLC): 98.6 % (t_R = 15.19 min). ^{1}H NMR (DMSO-D_6): δ (ppm) = 3.02 (t, *J* = 7.2 Hz, 2H, $CH_2\mathit{CH_2}CO_2H$), 3.29 (t, *J* = 7.1 Hz, 2H, $C\mathit{H_2C}H_2CO_2H$), 7.32 – 7.47 (m, 2H, 3-H, 5-H), 7.93 (t, *J* = 7.9 Hz, 1H, 6-H). ^{13}C NMR (DMSO-D_6): δ (ppm) = 21.9 (1C, $\mathit{C}H_2CH_2CO_2H$), 30.9 (1C, $CH_2\mathit{C}H_2CO_2CH_3$), 120.6 (d, *J* = 24.3 Hz, 2C, C-1, C-3), 128.5 (d, *J* = 3.8 Hz, 1C, C-5), 131.8 (d, *J* = 11.4 Hz, 2C, C-4, C-6), 156.0 (d, *J* = 10.6 Hz, 1C, C-$5_{oxadiazole}$), 160.5 (d, *J* = 260.6 Hz, 1C, C-2), 175.4 (1C, C-$2_{oxadiazole}$), 178.1 (1C, $\mathit{C}O_2H$). IR (neat): υ (cm^{-1}) = 3170-2310 (m, COOH), 1705 (s, C=O).

5.2.12 3-[5-(2-Cyano-4-fluorophenyl)-1,3,4-oxadiazol-2-yl]propanoic acid (7c)

6c (550 mg, 2.0 mmol) and LiOH (420 mg, 10.0 mmol) were dissolved in THF (60 mL) and H_2O (20 mL) and the mixture was stirred for 15 min at rt. Afterwards, the reaction was neutralized with 1 M H_2SO_4, diluted with CH_2Cl_2 and washed twice with brine. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was washed with CH_2Cl_2 and purified by fc (ethyl acetate/formic acid 1:0.01, R_f 0.66). Colorless solid, mp 139 - 140 °C, yield 450 mg (86 %). $C_{12}H_8FN_3O_3$ (261.2 g/mol). Exact mass (APCI): m/z = calcd. for $C_{12}H_8FN_3O_3H$ 262.0622 found 262.0635. Purity (HPLC): 86.8 % (t_R = 17.41 min). ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.01 (t, *J* = 7.1 Hz, 2H, $CH_2C\mathit{H_2}CO_2H$), 3.30 (t, *J* = 7.1 Hz, 2H, $C\mathit{H_2}CH_2CO_2H$), 7.43 (ddd, *J* = 8.7/7.7/2.7 Hz, 1H, 5-H), 7.54 (dd, *J* = 8.2/2.7 Hz, 1H, 3-H), 8.16 (dd, *J* = 8.7/6.0 Hz, 1H, 6-H). ^{13}C NMR (DMSO-D_6): δ (ppm) = 20.5 (1C, $\mathit{C}H_2CH_2$ CO_2H), 29.7 (1C, $CH_2\mathit{C}H_2$

CO_2H),, 111.35 (d, *J* = 10.4 Hz, 1C, C-2), 115.9 (1C, *C*N), 121.7 (d, *J* = 22.1 Hz, 1C, C-5), 122.3 (d, *J* = 3.5 Hz, 1C, C-1), 122.6 (d, *J* = 26.2 Hz, 1C, C-3), 131.7 (d, *J* = 9.4 Hz, 1C, C-6), 161.0 (1C, C-$5_{oxadiazole}$), 162.9 (d, *J* = 252.8 Hz, 1C, C-4), 165.0 16.9 (1C, C-$2_{oxadiazole}$), 172.8 (1C, $\mathit{C}O_2H$). IR (neat): ʋ (cm^{-1}) = 2229 (w, CN), 1728 (s, C=O).

5.2.13 3-[5-(2-Bromo-4-fluorophenyl)-1,3,4-oxadiazol-2-yl]-N-[9-(2-hydroxyethyl)-9H-carbazol-3-yl]propanamide (8a)

COMU® (489 mg, 1.1 mmol) was added to a mixture of carboxylic acid **7a** (400 mg, 1.0 mmol) and triethylamine (0.35 mL, 2.5 mmol) in DMF (15 mL), and the mixture was stirred for 30 min at rt. The reaction mixture was cooled down to 0 °C and a solution of carbazole hydrochloride **10**·HCl (215 mg, 0.9 mmol) in DMF was added dropwise. This mixture was stirred for 24 h at 45 °C. Then H_2O and brine were added and the resulting precipitate was filtered off and dissolved in ethyl acetate. Brine was added and the aqueous layer was extracted with ethyl acetate until the product was extracted completely. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by fc (d = 8 cm, l = 7 cm, cyclohexane/ethyl acetate 75:25, R_f 0.62 (ethyl acetate)). The product was recrystallized from CH_2Cl_2. Colorless solid, mp 213-215 °C, yield 200 mg (38 %). $C_{25}H_{20}BrFN_4O_3$ (523.4 g/mol). Exact mass (ESI): m/z = calcd. for $C_{25}H_{20}{}^{79}BrFN_4O_3H$ 523.0776 found 523.0768. Purity (HPLC): 98.1 % (t_R = 19.09 min). ^1H NMR (DMSO-D_6): δ (ppm) = 2.95 (t, *J* = 7.0 Hz, 2H, $CH_2C\mathit{H_2}CONH$), 3.29 (t, *J* = 7.1 Hz, 2H, $C\mathit{H_2}CH_2CONH$), 3.70 – 7.34 (m, 2H, $NCH_2C\mathit{H_2}OH$), 4.40 (t, *J* = 5.0 Hz, 2H, $NC\mathit{H_2}CH_2OH$), 4.87 (t, *J* = 5.2 Hz, 1H, $NCH_2CH_2O\mathit{H}$), 7.16 (t, *J* = 7.3 Hz, 1H, 6-H_{carb}), 7.42 (t, *J* = 7.5 Hz, 1H, 7-H_{carb}), 7.48 (td, *J* = 8.9/1.8 Hz, 1H, 5-H_{phenyl}), 7.49 – 7.55 (m, 2H, 1-H_{carb}, 2-H_{carb}), 7.57 (d, *J* = 8.2 Hz, 1H, 8-H_{carb}), 7.87 (dd, *J* = 8.6/2.1 Hz, 1H, 3-H_{phenyl}), 7.97 (dd, *J* = 8.5/6.1 Hz, 1H, 6-H_{phenyl}), 8.03 (d, *J* = 7.8 Hz, 1H, 5-H_{carb}), 8.41

(s, 1H, 4-H$_{carb}$), 10.13 (s, 1H, CON*H*). ^{13}C NMR (DMSO-D$_6$): δ (ppm) = 21.4 (1C, *C*H$_2$CH$_2$CONH), 32.7 (1C, CH$_2$*C*H$_2$CONH), 46.0 (1C, N*C*H$_2$CH$_2$OH), 60.2 (1C, NCH$_2$*C*H$_2$OH), 110.2 (1C, C-8$_{carb}$), 110.3 (1C, C-1$_{carb}$), 111.5 (1C, C-4$_{carb}$), 116.5 (d, *J* = 21.8 Hz, 1C, C-5$_{phenyl}$), 119.2 (1C, C-2$_{carb}$), 119.3 (1C, C-6$_{carb}$), 120.6 (1C, C-5$_{carb}$), 122.2 (1C, C-4a$_{carb}$), 122.4 (3C, C-1$_{phenyl}$, C-2$_{phenyl}$, C-3$_{phenyl}$), 122.6 (1C, C-4b$_{carb}$), 126.3 (1C, C-3$_{carb}$), 131.7 (1C, C-7$_{carb}$), 134.2 (d, *J* = 9.6 Hz, 1C, C-6$_{phenyl}$), 137.7 (1C, C-9a$_{carb}$), 141.5 (1C, C-8a$_{carb}$), 162.76 (1C, C-2$_{oxadiazole}$), 163.8 (d, *J* = 253.4 Hz, 1C, C-4$_{phenyl}$), 167.6 (1C, C-5$_{oxadiazole}$), 169.2 (1C, *C*ONH). IR (neat): υ (cm^{-1}) = 3305 (m, N-H), 3051 (m, C-H, arom), 2928 (m, C-H, aliph), 1685 (s, C=O).

5.2.14 3-[5-(4-Bromo-2-fluorophenyl)-1,3,4-oxadiazol-2-yl]-N-[9-(2-hydroxyethyl)-9H-carbazol-3-yl]propanamide (8b)

COMU® (290 mg, 1.2 mmol) was added to a mixture of carboxylic acid **7b** (300 mg, 0.9 mmol) and triethylamine (0.4 mL, 2.8 mmol) in DMF (15 mL), and the mixture was stirred for 30 min at rt. The reaction mixture was cooled down to 0 °C and a solution of carbazole hydrochloride **10**·HCl (200 mg, 0.9 mmol) in DMF was added dropwise. This mixture was stirred for 24 h at 45 °C. Then H_2O and brine were added and the resulting precipitate was filtered off and dissolved in ethyl acetate. Brine was added and the aqueous layer was extracted with ethyl acetate until the product was extracted completely. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by fc (d = 5 cm, l = 6 cm, cyclohexane/ethyl acetate 50:50, R$_f$ 0.18 (ethyl acetate)). The product was recrystallized from CH_2Cl_2. Colorless solid, mp 202 - 203 °C, yield 220 mg (24 %). $C_{25}H_{20}BrFN_4O_3$ (523.4 g/mol). Exact mass (ESI): m/z = calcd. for $C_{25}H_{20}{}^{79}BrFN_4O_3H$ 523.0776 found 523.0763. Purity (HPLC): 84.1 % (t$_R$ = 19.07 min). ^{1}H NMR (DMSO-D$_6$): δ (ppm) = 2.95 (t, *J* = 6.9 Hz, 2H, CH$_2$*CH$_2$*CONH), 3.28 (t, *J* = 7.0 Hz, 2H,

CH_2CH_2CONH), 3.71 – 3.79 (m, 2H, NCH_2CH_2OH), 4.40 (t, *J* = 5.6 Hz, 2H, NCH_2CH_2OH), 4.86 (t, *J* = 5.6 Hz, 1H, NCH_2CH_2OH), 7.15 (t, *J* = 7.4 Hz, 1H, 6-H_{carb}), 7.42 (t, *J* = 7.2 Hz, 1H, 7-H_{carb}), 7.48 – 7.55 (m, 2H, 1-H_{carb}, 2-H_{carb}), 7.57 (d, *J* = 8.3 Hz, 1H, 8-H_{carb}), 7.65 (dd, *J* = 8.4/1.4 Hz, 1H, 5-H_{phenyl}), 7.89 (dd, *J* = 10.3/1.7 Hz, 1H, 3-H_{phenyl}), 7.95 (t, *J* = 8.1 Hz, 1H, 6-H_{phenyl}), 8.02 (d, *J* = 7.8 Hz, 1H, 5-H_{carb}), 8.40 (s, 1H, 4-H_{carb}), 10.12 (s, 1H, CON*H*). ^{13}C NMR (DMSO-D_6): δ (ppm) = 21.7 (1C, CH_2CH_2CONH), 32.0 (1C, CH_2CH_2CONH), 45.3 (1C, NCH_2CH_2OH), 59.6 (1C, NCH_2CH_2OH), 109.5 (1C, C-8_{carb}), 109.7 (1C, C-1_{carb}), 110.9 (1C, C-4_{carb}), 111.3 (d, *J* = 21.7 Hz, 1C, C-3_{phenyl}), 118.5 (1C, C-2_{carb}), 118.7 (1C, C-6_{carb}), 120.0 (1C, C-5_{carb}), 120.7 (d, *J* = 24.9 Hz, 1C, C-1_{phenyl}), 121.8 (d, *J* = 10.1 Hz, 1C, C-4_{phenyl}), 125.6 (2C, C-$4a_{carb}$, C-$4b_{carb}$), 125.9 (d, *J* = 10.7 Hz, 1C, C-6_{phenyl}), 128.7 (d, *J* = 3.6 Hz, 1C, C-5_{phenyl}), 130.1 (1C, C-3_{carb}), 130.7 (1C, C-7_{carb}), 137.0 (1C, C-$9a_{carb}$), 140.9 (1C, C-$8a_{carb}$), 158.9 (d, *J* = 260.8 Hz, 1C, C-2_{phenyl}), 160.0 (d, *J* = 5.5 Hz, 1C, C-$2_{oxadiazole}$), 166.9 (1C, C-$5_{oxadiazole}$), 168.6 (1C, *C*ONH). IR (neat): υ (cm^{-1}) = 3305 (m, N-H), 2924 (m, C-H, aliph), 1685 (s, C=O).

5.2.15 3-[5-(2-Cyano-4-fluorophenyl)-1,3,4-oxadiazol-2-yl]-N-[9-(2-hydroxyethyl)-9H-carbazol-3-yl]propanamide (8c)

COMU® (550 mg, 1.3 mmol) was added to a mixture of carboxylic acid **7c** (280 mg, 1.1 mmol) and triethylamine (0.4 mL, 2.8 mmol) in DMF (15 mL), and the mixture was stirred for 30 min at rt. The reaction mixture was cooled down to 0 °C and a solution of carbazole hydrochloride **10**·HCl (200 mg, 0.9 mmol) in DMF was added dropwise. This mixture was stirred for 24 h at 45 °C. Then H_2O and brine were added and the resulting precipitate was filtered off and dissolved in ethyl acetate. Brine was added and the aqueous layer was extracted with ethyl acetate until the product was extracted completely. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by fc (d = 5 cm, l = 7 cm, cyclohexane/ethyl acetate 50:50, R_f 0.28 (ethyl acetate)). The product was recrystallized from CH_2Cl_2. Colorless solid, mp 215 °C, yield 121 mg (24 %). $C_{26}H_{20}FN_5O_3$ (469.5 g/mol). Exact mass (APCI): m/z = calcd. for $C_{26}H_{20}FN_5O_3H$ 470.1652 found 470.1623. Purity (HPLC): 93.2 % (t_R = 19.76 min). ^{1}H NMR (DMSO-D_6): δ (ppm) = 2.97 (t, *J* = 7.0 Hz, 2H, CH_2CH_2CONH), 3.29 (t, *J* = 7.1 Hz, 2H, CH_2CH_2CONH), 3.76 (q, *J* = 5.5 Hz, 2H, NCH_2CH_2OH), 4.40 (t, *J* = 5.7 Hz, 2H, NCH_2CH_2OH), 4.85 (t, *J* = 5.4 Hz, 1H, NCH_2CH_2OH), 7.15 (t, *J* = 7.5 Hz, 1H, 6-H_{carb}), 7.42 (t, *J* = 7.6 Hz, 1H, 7-H_{carb}), 7.51 – 7.55 (m, 2H, 1-H_{carb}, 2-H_{carb}), 7.57 (d, *J* = 8.2 Hz, 1H, 8-H_{carb}), 7.81 (td, *J* = 8.5/2.7 Hz, 1H, 5-H_{phenyl}), 8.02 (d, *J* = 7.7 Hz, 1H, 5-H_{carb}), 8.16 (dd, *J* = 8.7/2.7 Hz, 1H, 3-H_{phenyl}), 8.20 (dd, *J* = 8.9/5.3 Hz, 1H, 6-H_{phenyl}), 8.40 (s, 1H, 4-H_{carb}), 10.12 (s, 1H, CONH). ^{13}C NMR (DMSO-D_6): δ (ppm) = 20.8 (1C, CH_2CH_2CONH), 31.8 (1C, CH_2CH_2CONH), 45.3 (1C, NCH_2CH_2OH), 59.5 (1C, NCH_2CH_2OH), 109.4 (1C, C-8_{carb}), 109.6 (1C, C-1_{carb}), 110.9 (1C, C-4_{carb}), 115.8 (d, *J* = 3.1 Hz, 1C, CN), 116.2 (d, *J* = 10.2 Hz, 1C, C-2_{phenyl}), 118.4 (1C, C-2_{carb}), 118.7 (1C, C-6_{carb}), 120.0 (1C, C-5_{carb}), 121.6 (d, *J* = 20.4 Hz, 1C, C-3_{phenyl}), 121.7 (1C, C-$4a_{carb}$), 121.9 (1C, C-$4b_{carb}$), 122.3 (d, *J* = 3.3 Hz, 1C, C-1_{phenyl}), 122.5 (d, *J* = 26.7 Hz, 1C, C-5_{phenyl}), 125.6 (1C, C-7_{carb}), 131.0 (1C, C-3_{carb}), 131.7 (d, *J* = 9.3 Hz, 1C, C-6_{phenyl}), 137.0 (1C, C-$9a_{carb}$), 140.8 (1C, C-$8a_{carb}$), 160.9 (1C, C-$2_{oxadiazole}$), 162.9 (d, *J* = 252.6 Hz, 1C, C-4_{phenyl}), 167.3 (1C, C-$5_{oxadiazole}$), 168.5 (1C, CONH). IR (neat): υ (cm^{-1}) = 3464 (w, O-H), 3324 (m, N-H), 2935 (m, C-H, aliph), 2233 (w, CN), 1689 (s, C=O).

5.2.16 General procedure for the fluorination of the alcohols 8a-c with XtalFluor-E®

Under N_2, diethylaminodifluorosulfonium tetrafluoroborate (XtalFluor-E®, 1.5 - 3.0 eq.) was suspended in CH_2Cl_2. Triethylamine trihydrofluoride (1.5 - 3.0 eq.) and a solution of the respective alcohol **8** (1.0 eq.) in CH_2Cl_2 were added to the suspension via cannula at - 78 °C. The resulting mixture was warmed up to rt during 1 or 3 h. An aqueous solution of Na_2CO_3 (5 % m/m) was added and the reaction mixture was stirred for 15 min at rt. After addition of brine the mixture was extracted with CH_2Cl_2 until the product was extracted completely. The organic layer was dried (Na_2SO_4), the organic concentrated *in vacuo*, the product was recrystallized from ethyl acetate.

5.2.17 3-[5-(2-Bromo-4-fluorophenyl)-1,3,4-oxadiazol-2-yl]-N-[9-(2-fluoroethyl)-9H-carbazol-3-yl]propanamide (9a)

According to the General Procedure, **8a** (120 mg, 0.2 mmol) was treated with XtalFluor-E® (78 mg, 0.3 mmol) and triethylamine trihydrofluoride (0.1 mL, 0.6 mmol) in CH_2Cl_2 (20 mL) at - 78 °C. The product was purified by fc (d = 3 cm, l = 10 cm, cyclohexane/ethyl acetate 25:75, R_f 0.60 (ethyl acetate)). Colorless solid, mp 203 - 205 °C, yield 60 mg (49 %). $C_{25}H_{19}BrF_2N_4O_2$ (525.3 g/mol). Exact mass (APCI): m/z = calcd. for $C_{25}H_{19}{}^{79}BrF_2N_4O_2H$ 525.0732 found 525.0738. Purity (HPLC): 93.0 % (t_R = 21.86 min). ^1H NMR (DMSO-D$_6$): δ (ppm) = 2.95 (t, *J* = 7.0 Hz, 2H, CH_2CH_2CONH), 3.29 (t, *J* = 7.1 Hz, 2H, CH_2CH_2CONH), 4.70 (dt, *J* = 14.7/4.3 Hz, 2H, NCH_2CH_2F), 4.79 (dt, *J* = 38.5/4.4 Hz, 2H, NCH_2CH_2F), 7.18 (t, *J* = 7.1 Hz, 1H, 6-H_{carb}), 7.44 (t, *J* = 7.2 Hz, 1H, 7-H_{carb}), 7.48 (ddd, *J* = 8.7/8.3/2.7 Hz Hz, 1H, 5-H_{phenyl}), 7.54 (dd, *J* = 8.0, 1H, 1-H_{carb}), 7.57 (d, *J* = 8.8/1.8, 1H, 2-H_{carb}), 7.60 (d, *J* = 8.2 Hz, 1H, 8-H_{carb}), 7.88 (dd, *J* = 8.6/2.6 Hz, 1H, 3-H_{phenyl}), 7.97 (dd, *J* = 8.8/6.0 Hz, 1H, 6-H_{phenyl}), 8.05 (d, *J* = 7.6 Hz, 1H, 5-H_{carb}), 8.43 (s, 1H, 4-H_{carb}), 10.15 (s, 1H, CON*H*). ^{13}C NMR (DMSO-D$_6$): δ (ppm) = 20.7 (1C, CH_2CH_2CONH), 32.0 (1C, CH_2CH_2CONH), 42.9 (d, *J* = 20.3 Hz, 1C, NCH_2CH_2F), 82.6 (d, *J* = 167.4 Hz, 1C, NCH_2CH_2F), 109.5 (1C, C-8$_{carb}$), 109.6 (1C, C-1$_{carb}$), 110.8 (1 C, C-4$_{carb}$), 115.8 (d, *J* = 21.8 Hz,1C, C-5$_{phenyl}$), 119.2 (1C, C-2$_{carb}$), 119.3 (1C, C-6$_{carb}$), 120.0 (1C, C-5$_{carb}$), 121.2 – 121.4 (5C, C-4a$_{carb}$, C-4b$_{carb}$, C-1$_{phenyl}$, C-2$_{phenyl}$, C-3$_{phenyl}$), 125.8 (1C, C-7$_{carb}$), 131.4 (1C, C-3$_{carb}$), 133.5 (d, *J* = 9.6 Hz, 1C, C-6$_{phenyl}$), 136.7 (1C, C-9a$_{carb}$), 140.6 (1C, C-8a$_{carb}$), 162.1 (1C, C-2$_{oxadiazole}$), 163.2 (d, *J* = 254.6 Hz, 1C, C-4$_{phenyl}$), 166.9 (1C, C-5$_{oxadiazole}$), 168.6 (1C, *C*ONH). IR (neat): ʋ (cm^{-1}) = 3267 (m, N-H), 2958 (m, C-H, aliph), 1689 (s, C=O).

5.2.18 3-[5-(4-Bromo-2-fluorophenyl)-1,3,4-oxadiazol-2-yl]-N-[9-(2-fluoroethyl)-9H-carbazol-3-yl]propanamide (9b)

According to the General Procedure, **8b** (200 mg, 0.4 mmol) was treated with XtalFluor-E® (130 mg, 0.6 mmol) and triethylamine trihydrofluoride (0.2 mL, 1.2 mmol) in CH_2Cl_2 (30 mL) at - 78 °C. The product was purified by fc (d = 3 cm, l = 12 cm, cyclohexane/ethyl acetate 50:50, R$_f$ 0.60 (ethyl acetate)). Colorless solid, mp 207 - 208 °C, yield 110 mg (54 %). $C_{25}H_{19}BrF_2N_4O_2$ (525.3 g/mol). Exact mass (APCI): m/z = calcd. for $C_{25}H_{19}{}^{79}BrF_2N_4O_2H$ 525.0732 found 525.0732. Purity (HPLC): 95.2 % (t$_R$ = 21.83 min). ^{1}H NMR (DMSO-D$_6$): δ (ppm) = 2.95 (t, *J* = 7.1 Hz, 2H, CH_2CH_2CONH), 3.29 (t, *J* = 6.9 Hz, 2H, CH_2CH_2CONH), 4.69 (dt, *J* = 14.4/4.5 Hz, 2H, NCH_2CH_2F), 4.79 (dt, *J* = 34.8/4.5 Hz, 2H, NCH_2CH_2F), 7.18 (t, *J* = 7.4 Hz, 1H, 6-H$_{carb}$), 7.43 (t, *J* = 7.7 Hz, 1H, 7-H$_{carb}$), 7.49 - 7.58 (m, 3H, 1-H$_{carb}$, 2-H$_{carb}$, 5-H$_{phenyl}$), 7.60 (d, *J* = 8.3 Hz, 1H, 8-H$_{carb}$), 7.64 (dd, *J* = 8.7/1.7 Hz, 1H, 3-H$_{phenyl}$), 7.95 (t, *J* = 8.1 Hz, 1H, 6-H$_{phenyl}$), 8.04 (d, *J* = 7.8 Hz, 1H, 5-H$_{carb}$), 8.41 (s, 1H, 4-H$_{carb}$), 10.15 (s, 1H, CON*H*). ^{13}C NMR (DMSO-D$_6$): δ (ppm) = 21.9 (1C, *C*H_2CH_2CONH), 31.9 (1C, CH_2*C*H_2CONH), 42.9 (d, *J* = 19.7 Hz, 1C, N*C*H_2CH_2F), 82.6 (d, *J* = 167.9 Hz, 1C, NCH_2*C*H_2F), 109.5 (1C, C-8$_{carb}$), 109.6 (1C, C-1$_{carb}$), 111.0 (1 C, C-4$_{carb}$), 111.8 (d, *J* = 10.3 Hz,1C, C-4$_{phenyl}$), 116.2 (1C, C-2$_{carb}$), 118.9 (1C, C-6$_{carb}$), 120.1 (1C, C-5$_{carb}$), 121.5 (d, *J* = 21.7 Hz, 1C, C-1$_{phenyl}$), 121.9 (1C, C-4a$_{carb}$), 122.1 (1C, C-4b$_{carb}$), 122.5 (d, *J* = 26.1 Hz, 1C, C-3$_{phenyl}$), 125.1 (d, *J* = 3.5 Hz,1C, C-5$_{phenyl}$), 125.8 (1C, C-7$_{carb}$), 131.3 (1C, C-3$_{carb}$), 132.5 (d, *J* = 9.4 Hz, 1C, C-6$_{phenyl}$), 136.8 (1C, C-9a$_{carb}$), 140.6 (1C, C-8a$_{carb}$), 162.8 (d, *J* = 252.1 Hz, 1C, C-2$_{phenyl}$), 165.0 (1C, C-2$_{oxadiazole}$), 168.5 (1C, C-5$_{oxadiazole}$), 180.6 (1C, *C*ONH). IR (neat): υ (cm^{-1}) = 3267 (m, N-H), 2958 (m, C-H, aliph), 1689 (s, C=O).

5.2.19 3-[5-(2-Cyano-4-fluorophenyl)-1,3,4-oxadiazol-2-yl]-N-[9-(2-fluoroethyl)-9H-carbazol-3-yl]propanamide (9c)

According to the General Procedure, **8c** (100 mg, 0.2 mmol) was treated with XtalFluor-E® (73 mg, 0.3 mmol) and triethylamine trihydrofluoride (0.1 mL, 0.6 mmol) in CH_2Cl_2 (30 mL) at - 78 °C. The product was purified by fc (d = 3 cm, l = 12 cm, cyclohexane/ethyl acetate 50:50, R_f 0.60 (ethyl acetate)). Colorless solid, mp 209 - 211 °C, yield 25 mg (3 %). $C_{26}H_{19}F_2N_5O_2$ (471.2 g/mol). Exact mass (APCI): m/z = calcd. for $C_{26}H_{19}F_2N_5O_2H$ 472.1580 found 472.1596. Purity (HPLC): 95.5 % (t_R = 20.13 min). ^{1}H NMR (DMSO-D$_6$): δ (ppm) = 3.01 (t, *J* = 6.9 Hz, 2H, $CH_2C\mathit{H_2}$CONH), 3.34 (t, *J* = 6.9 Hz, 2H, $C\mathit{H_2}CH_2$CONH), 4.69 (dt, *J* = 14.6/4.2 Hz, 2H, $NC\mathit{H_2}CH_2F$), 4.78 (dt, *J* = 38.5/4.1 Hz, 2H, $NCH_2C\mathit{H_2}F$), 7.18 (t, *J* = 7.4 Hz, 1H, 6-H$_{carb}$), 7.44 (t, *J* = 7.6 Hz, 1H, 7-H$_{carb}$), 7.54 (dd, *J* = 8.9/ 2.0 Hz, 1H, 2-H$_{carb}$), 7.56 (d, *J* = 9.1 Hz, 1H, 1-H$_{carb}$), 7.60 (d, *J* = 8.4 Hz, 1H, 8-H$_{carb}$), 7.81 (ddd, *J* = 8.7/8.3/2.7 Hz, 1H, 5-H$_{phenyl}$), 8.04 (d, *J* = 7.8 Hz, 1H, 5-H$_{carb}$), 8.15 (dd, *J* = 8.6/2.7 Hz, 1H, 3-H$_{phenyl}$), 8.20 (dd, *J* = 8.9/5.3 Hz, 1H, 6-H$_{phenyl}$), 8.41 (s, 1H, 4-H$_{carb}$), 10.15 (s, 1H, CON*H*). ^{13}C NMR (DMSO-D$_6$): δ (ppm) = 20.8 (1C, $\mathit{C}H_2CH_2$CONH), 31.9 (1C, $CH_2\mathit{C}H_2$CONH), 45.3 (d, *J* = 19.7 Hz, 1C, $N\mathit{C}H_2CH_2F$), 82.6 (d, *J* = 167.8 Hz, 1C, $NCH_2\mathit{C}H_2F$), 109.4 (1C, C-8$_{carb}$), 109.6 (1C, C-1$_{carb}$), 109.6 (d, *J* = 7.2 Hz, 1C, C-2$_{phenyl}$), 111.0 (1C, C-4$_{carb}$), 118.8 (2C, C-2$_{carb}$, C-6$_{carb}$), 119.6 (d, *J* = 5.3 Hz, 1C, *C*N),120.0 (1C, C-5$_{carb}$), 121.6 (d, *J* = 22.0 Hz, 1C, C-5$_{phenyl}$), 121.9 (1C, C-4a$_{carb}$), 122.3 (1C, C-4b$_{carb}$), 122.5 (d, *J* = 3.1 Hz, 1C, C-1$_{phenyl}$), 122.5 (d, *J* = 26.6 Hz, 1C, C-3$_{phenyl}$), 125.8 (1C, C-7$_{carb}$), 131.7 (1C, C-3$_{carb}$), 131.8 (d, *J* = 10.8 Hz, 1C, C-6$_{phenyl}$), 136.8 (1C, C-9a$_{carb}$), 140.6 (1C, C-8a$_{carb}$), 160.1 (1C, C-2$_{oxadiazole}$), 161.7 (d, *J* = 252.6 Hz, 1C, C-4$_{phenyl}$), 167.3 (1C, C-5$_{oxadiazole}$), 168.6 (1C, *C*ONH). IR (neat): ʋ (cm^{-1}) = 3348 (m, N-H), 2924 (m, C-H, aliph), 2221 (w, CN), 1615 (s, C=O).

5.3 Receptor binding studies to determine CB_1 and CB_2 receptor affinity

[^3H]CP55940 displacement assays were used for the determination of affinity (K_i) values of ligands for the cannabinoid CB_1 and CB_2 receptors. Membrane aliquots containing 5 µg (CHOK1hCB_1_bgal) or 1 µg (CHOK1hCB_2_bgal) of membrane protein in 100 µL assay buffer (50 mM Tris–HCl, 5 mM $MgCl_2$, 0.1 % BSA, pH 7.4) were incubated at 30 °C for 1 h, in presence of 3.5 nM [^3H]CP55940 (CHOK1hCB_1_bgal) or 1.5 nM [^3H]CP55940 (CHOK1hCB_2_bgal). Initially, 1 µM of competing ligand was used, followed by six concentrations of competing ligand (between $10^{-5.5}$ M and $10^{-10.5}$ M) when more than 50 % displacement was found at 1 µM. Non-specific binding was determined in the presence of 10 µM AM630 (CHOK1hCB2_bgal) or 10 µM SR141716A (CHOK1hCB1_bgal). Incubation was terminated by rapid filtration through GF/C filters (Whatman International, Maidstone, UK), and followed by extensive washing using a Filtermate 96-well harvester (Perkin Elmer, Groningen, The Netherlands). Filter-bound radioactivity was determined by scintillation spectrometry using a 1450 Microbeta Wallac Trilux scintillation counter (Perkin Elmer).

Data analysis was performed by using the nonlinear regression curve fitting program GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). From displacement assays, IC_{50} values were obtained by non-linear regression analysis of the displacement curves. The obtained IC_{50} values were converted into K_i values using the Cheng Prusoff equation[16] to determine the affinity of the ligands using a K_D value of [^3H]CP55940 of 0.93 nM at CB_2R.

Acknowledgement

Financial support by the *Deutsche Forschungsgemeinschaft (DFG, collaborative research center 656 "Molecular Cardiovascular Imaging")* is gratefully acknowledged.

Conflict of interest

There is no conflict of interest.

References

1 S. Munro, K. L. Thomas and M. Abu-Shaar, *Nature*, 1993, **365**, 61–65.

2 M. Storr, E. Gaffal, D. Saur, V. Schusdziarra and H. D. Allescher, *Can. J. Physiol. Pharmacol.*, 2002, **80**, 67–76.

3 C. Zoratti, D. Kipmen-Korgun, K. Osibow, R. Malli and W. F. Graier, *Br. J. Pharmacol.*, 2003, **140**, 1351–1362.

4 Y. A. Shmist, I. Goncharov, M. Eichler, V. Shneyvays, A. Isaac, Z. Vogel and A. Shainberg, *Mol. Cell. Biochem.*, 2006, **283**, 75–83.

5 M. M. Ibrahim, F. Porreca, J. Lai, P. J. Albrecht, F. L. Rice, A. Khodorova, G. Davar, A. Makriyannis, T. W. Vanderah, H. P. Mata and T. P. Malan, JR, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 3093–3098.

6 B. K. Atwood and K. Mackie, *Br. J. Pharmacol.*, 2010, **160**, 467–479.

7 S. J. Carlisle, F. Marciano-Cabral, A. Staab, C. Ludwick and G. A. Cabral, *Int. Immunopharmacol.*, 2002, **2**, 69–82.

8 K. Maresz, E. J. Carrier, E. D. Ponomarev, C. J. Hillard and B. N. Dittel, *J. Neurochem.*, 2005, **95**, 437–445.

9 M. Roche and D. P. Finn, *Pharmaceuticals*, 2010, **3**, 2517–2553.

10 R. Teodoro, R.-P. Moldovan, C. Lueg, R. Günther, C. K. Donat, F.-A. Ludwig, S. Fischer, W. Deuther-Conrad, B. Wünsch and P. Brust, *Org. Med. Chem. Lett.*, 2013, **3**, 11.

11 C. Lueg, D. Schepmann, R. Günther, P. Brust and B. Wünsch, *Bioorg. Med. Chem.*, 2013, **21**, 7481–7498.

12 J. Boström, A. Hogner, A. Llinàs, E. Wellner and A. T. Plowright, *J. Med. Chem.*, 2012, **55**, 1817–1830.

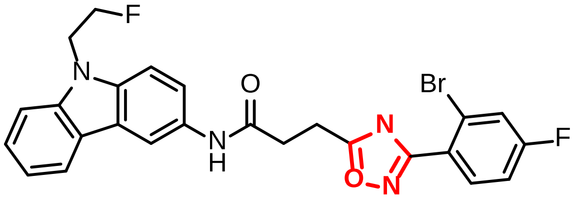
13 C. Lueg, F. Galla, B. Frehland, D. Schepmann, C. G. Daniliuc, W. Deuther-Conrad, P. Brust and B. Wünsch, *Arch. Pharm.*, 2014, **347**, 21–31.

14 M. Shailaja, M. Anitha, A. Manjula and B. V. Rao, *Indian J. Chem.*, 2010, **49B**, 1088–1097.

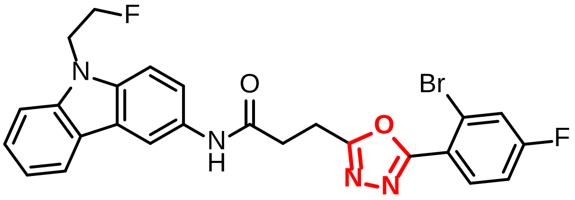
15 Y. Li, J. Liu, H. Zhang, X. Yang and Z. Liu, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2278–2282.

16 C. Yung-Chi and W. H. Prusoff, *Biochem. Pharmacol.*, 1973, **22**, 3099–3108.

Graphical Abstract



$K_i(CB_2)$ = 2.9 nM



$K_i(CB_2)$ = 25 nM

Highlights

Three pairs of regioisomeric 1,2,4- and 1,3,4-oxadiazoles were synthesized as selective CB$_2$ ligands. Although the 1,3,4-oxadiazoles should have better physicochemical and pharmacokinetic properties, the CB$_2$ affinity was reduced by the bioisosteric replacement.