Full Paper

Hydroxyalkylation with Cyclic Sulfates: Synthesis of Carbazole Derived CB_2 Ligands with Increased Polarity

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In order to increase the polarity of the potent CB_2 ligand **1a**, the homologous hydroxyalkyl carbazoles **2a–c** were prepared and pharmacologically evaluated. An important step in the synthesis is the hydroxyalkylation of carbazole with cyclic sulfates providing the 2-hydroxyethyl and 3-hydroxypropyl derivatives **5a** and **5b** in a one-step reaction. The final propionamides **2a–c** were prepared using the recently reported coupling reagent COMU®. The X-ray crystal structure of **2c** displays an almost coplanar arrangement of the 3-phenyl-1,2,4-oxadiazole biaryl system. The increased polarity of **2a** is associated with an almost 100-fold reduced CB_2 affinity. The 3-hydroxypropyl derivative **2b** represents the best compromise between lipophilicity and CB_2 affinity ($K_i = 33$ nM).

Keywords: Carbazole derivatives / CB_2 receptor ligands / Cyclic sulfates / Structure affinity relationships / X-ray crystal structure analysis

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Introduction

The endocannabinoid system exerts an important role in the modulation of many physiological responses, in *e.g.*, the central nervous system, the immune system, and cardiovascular system [1]. It consists of the endogenous ligands anandamide (*N*-arachidonoyl ethanolamine, AEA) and 2-*O*-arachidonoylglycerol (2-AG), which mediate their effects through the G-protein coupled cannabinoid CB_1 and CB_2 receptors. Furthermore, the enzymes catalyzing the key steps of the biosynthesis (*e.g.*, *N*-acyltransferase, phosphodiesterase D, diacylglycerollipase) and degradation of endocannabinoids (*e.g.*, fatty acid amide hydrolase, monoacylglycerollipase) belong to the endocannabinoid system [2]. In the last decade, several clinical studies were performed with cannabinoid receptor ligands [3–8]. Dronabinol (Δ^9-THC, Marinol®) has been approved as antiemetic and appetite stimulating drug for AIDS and tumor patients. The semisynthetic Δ^9-THC analog nabilon (Cesamet®) is clinically used for the treatment of chemotherapy induced emesis [3–5].

The clinical use of CB_1 receptor antagonists and inverse CB_1 agonists like rimonabant is limited due to the development of tolerance and severe side effects including depression and elevated suicide potential [9]. However, there is an increasing interest for the development of selective agonists for CB_2 receptors, which are found in the immune system [10, 11], but also in the central nervous system [12–15]. CB_2 receptor agonists show antihyperalgesic effects in different models of pain, including acute, inflammatory, and neuropathic pain [16–18]. In different models of neuroinflammation, CB_2 receptor agonists led to anti-inflammatory effects by *e.g.*, reduced activation of microglia and reduced expression of pro-inflammatory cytokines [16, 19–23]. Neuroinflammation is associated with the progression of numerous chronic neurodegenerative diseases including Alzheimer's disease [20], multiple sclerosis [23], and Huntington's disease [24]. Considering the role of CB_2 receptors in the regulation of neuronal proliferation and survival [25–27] and the

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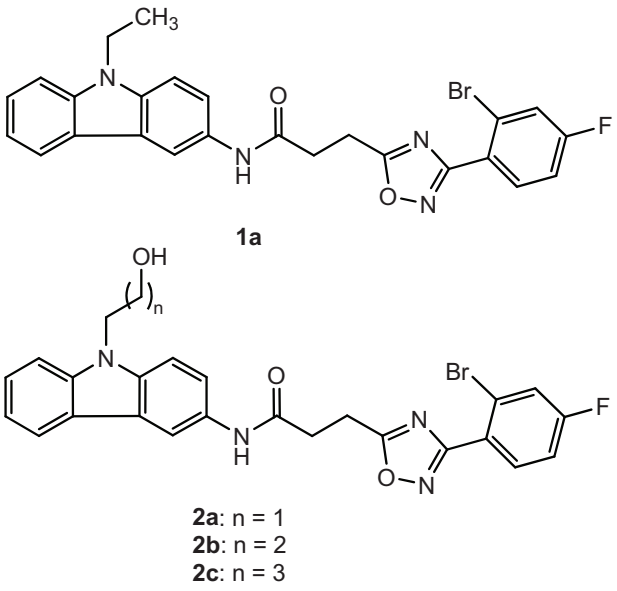


Figure 1. Designed CB_2 ligands **2** with increased polarity due to an additional hydroxy moiety in the carbazole *N*-alkyl side chain.

upregulation of CB_2 receptors in neuroinflammatory and neurodegenerative diseases [28–30], selective CB_2 receptor agonists represent an innovative therapeutic approach for the treatment of neurodegenerative disorders avoiding psychotropic side effects [31–33].

Recently Cheng et al. [34] reported carbazole based CB_2 agonists of type **1** bearing different substituents at the phenyl ring in 3-position of the 1,2,4-oxadiazole ring. Compounds of type **1** reveal high CB_2 agonistic activity and selectivity against the CB_1 subtype (Fig. 1). According to the method of Cheng et al., the 2-bromo-4-fluorophenyl derivative **1a** was prepared and a K_i-value (CB_2 affinity) of 4.3 nM was determined [35]. In order to improve the bioavailability of the potent but very lipophilic CB_2 agonist **1a**, we planned to enhance the polarity of **1a**. For this purpose the ethyl side chain in 9-position of the carbazole moiety should be replaced by a hydroxyalkyl substituent (**2**). A particular aim of this project was to study whether a rather polar hydroxy group within the side chain of the lipophilic carbazole region is tolerated by the CB_2 receptor.

At first the log*D*-values (pH 7.4) of the CB_2 agonist **1a** and the envisaged hydroxyalkyl analogs **2a–c** were calculated with ChemDraw Ultra 12. Table 1 shows that introduction of a hydroxy group into the ethyl side chain (**2a**) decreased the log*D* value by almost one order of magnitude from 5.99 (**1a**) to 5.13 (**2a**). Elongation of the side chain to a 3-hydroxpropyl (**2b**) and furthermore a 4-hydroxybutyl (**2c**) group led to increased lipophilicity, but even the log*D* value of the most lipophilic hydroxybutyl compound **2c** was still below the log*D* value of the parent ethyl derivative **1a**.

Results and discussion

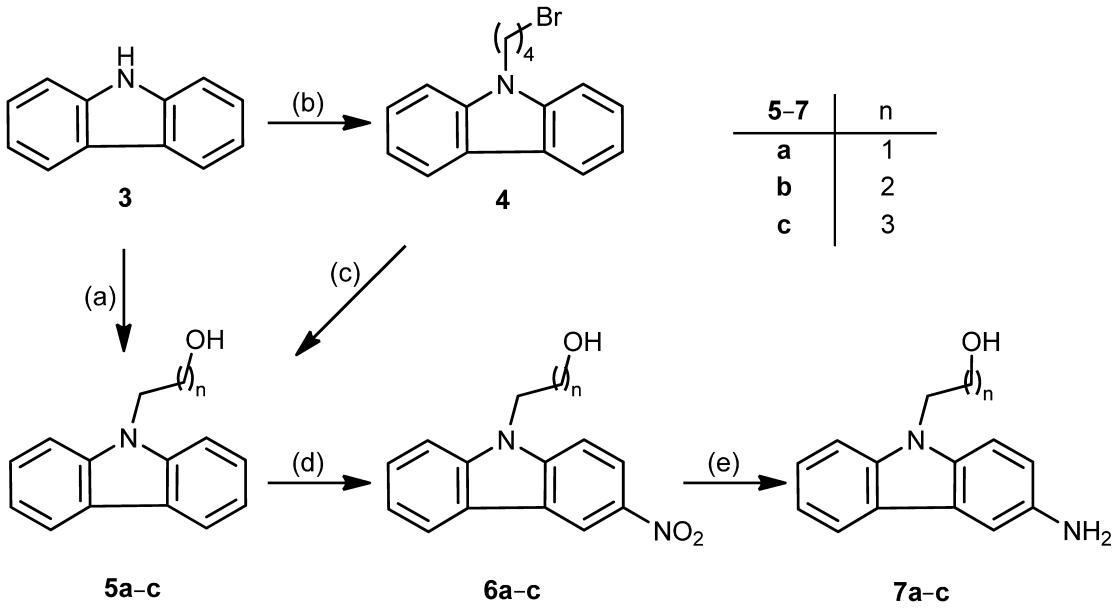
Synthesis

The synthesis of the hydroxyalkyl substituted carbazole derivatives **2a** and **2b** started with deprotonation of carbazole (**3**) with *n*-BuLi, reaction of the carbazolyl anion with ethylene sulfate or trimethylene sulfate and subsequent hydrolysis with diluted H_2SO_4 to afford the hydroxyethyl and hydroxypropyl derivatives **5a** and **5b**, in 68 and 70% yield, respectively (Scheme 1). In the literature the synthesis of **5a** and **5b** has been reported by alkylation of carbazole (**3**) with halogenated ethanol or propanol deriviatives [36–38]. The crystalline cyclic ethylene sulfate represents a non-toxic but reactive alternative for haloethanols and gaseous and very toxic oxirane. Like ethylene sulfate the homologous trimethylene sulfate also represents a non-toxic, non-volatile but reactive solid. However, there are only few examples using these cyclic sulfates for the introduction of a 2-hydroxyethyl [39–41] or 3-hydroxypropyl moiety [42–44]. Moreover, hydroxyalkylation of *N*-nucleophiles with cyclic sulfates has not been reported so far. The hydroxybutyl derivative **5c** was obtained by reaction of the carbazolyl anion with 1,4-dibromobutane and subsequent treatment with NaOH.

Nitration of the hydroxyalkylcarbazoles **5a–c** was performed with concentrated nitric acid at 5–10°C to give the mononitro derivatives **6a–c** in 54–67% yields. Reduction of the nitrocarbazoles **6a–c** with H_2 in the presence of Pd/C provided the primary aromatic amines **7a–c**, which were isolated as HCl salts.

The propionic acid **9**, which was required for the preparation of the envisaged CB_2 ligands **2**, was prepared according to literature [34]. Addition of hydroxylamine to the benzonitrile **8** provided an intermediate amide oxime, which was reacted with succinic anhydride to afford the oxadiazolylpropionic acid **9** (Scheme 2). The final coupling of the carbazol-3-amines **7** with the propionic acid **9** was induced by the recently developed coupling reagent COMU® [45–47], which led to the amides **2a–c**.

Recrystallization of the 4-hydroxybutyl derivative **2c** with cyclohexane/ethyl acetate led to crystals suitable for X-ray crystal structure analysis. In Fig. 2 the structure of **2c** is shown. In the solid state the 4-hydroxybutyl and the propionamide substructures are almost linearly arranged. The dihedral angle defined by the 2-bromo-4-fluorophenyl and the 1,2,4-oxadiazolyl planes is 9.7° and can be compared with the corresponding angle of the cyanophenyl derivative (11.2°) obtained from X-ray crystal structure experiments [35]. The almost coplanar orientation of the biaryl substructure is possible because of the missing substituents in the *o*-position

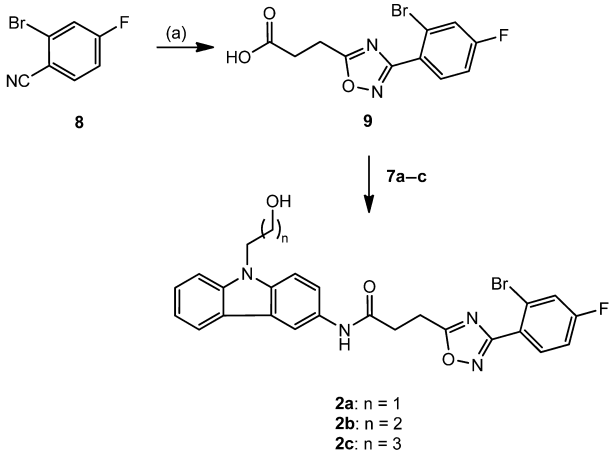


5–7	n
a	1
b	2
c	3

Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78°C, 30 min, then ethylene sulfate or trimethylene sulfate, −78°C, 1 h, then rt, 24 h, then H_2SO_4, reflux, 24 h, 68% (**5a**), 70% (**5b**). (b) *n*-BuLi, THF, −78°C, 30 min, then $Br(CH_2)_4Br$, 0°C, 1 h, then rt, 24 h, 62%. (c) NaOH, Bu_4NI, diglyme, reflux, 45 min, 90% (**5c**). (d) HNO_3 conc., CH_2Cl_2, 5–10°C, 54–67%. (e) H_2 (balloon), Pd/C, THF, rt, 24 h, 90–96% of HCl salt.

of the 1,2,4-oxadiazole ring, although a large bromo atom is present in *o*-position of the phenyl moiety. The distance between the oxadiazole N-atom and the bromo atom (N3-Br1) is 3.005 Å, indicating a weak interaction between these atoms. This interaction could contribute to the planar orientation of the two aromatic rings.

The amide group of **2c** displays a classical intermolecular hydrogen bond between the N–H moiety and the O=C moiety of a second molecule. The distance between the proton and the O-atom is 2.840 Å and the corresponding angle N–H-O(=C) is 164°. Furthermore the amide is not coplanar with the tricyclic system. The torsion angle is around 50°, which might be due to the crystal packing effect.



Scheme 2. Reagents and conditions: (a) 1. $NH_2OH{\cdot}HCl$, Na_2CO_3, H_2O, CH_3OH, 86°C, workup; 2. succinic anhydride, DMF, 148°C, 30 min, 45%. (b) COMU®, NEt_3, DMF, <10°C, 24 h, 30–80%.

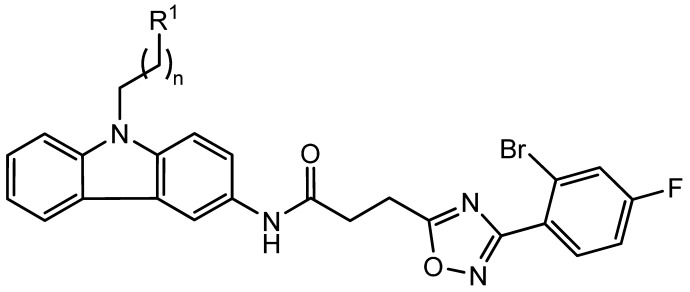
Receptor affinity

Competition experiments with the radioligand [^{3}H]CP-55,940 were performed to determine the CB_2 receptor affinities of the carbazole derivatives **2a–c**. In the CB_2 assay the test compounds competed with the radioligand [^{3}H]CP-55,940 to interact with a rather low amount of CB_2 receptors of a membrane preparation generated from CHO cells stably transfected with the gene for the human CB_2 receptor [48, 49]. The CB_2 affinities of the carbazole derivatives are summarized in Table 1.

The data in Table 1 show that introduction of a polar hydroxy moiety into the ethyl side chain of **1a** ($K_i = 4.3$ nM) leads to a considerable reduction of the CB_2 affinity. The 2-hydroxyethyl derivative **2a** is almost 100-fold less active than the ethyl derivative **1a**. The lower CB_2 affinity of **2a** might be due to the higher polarity: the additional hydroxy moiety in the ethyl side chain may inhibit lipophilic interactions of the carbazole part of the molecule with lipophilic parts of the binding pocket of the CB_2 receptor.

Elongation of the N-substituent to a 3-hydroxypropyl side chain (**2b**) leads to increased lipophilicity and CB_2 affinity

Table 1. CB_2 receptor affinities of carbazole derivatives **1a** and **2a–c** and reference compounds.



Compd.	R^1	n	CB_2 affinity $K_i \pm$ SEM (nM) ($n = 3$)	log$D$[a)] (pH 7.4)
1a	H	1	4.3 ± 3.0[35]	5.99
2a	OH	1	367[b)]	5.13
2b	OH	2	33 ± 11	5.24
2c	OH	3	96 ± 29	5.69
CP 55,940			27 ± 6	
WIN 55,212			70 ± 27	
HU 210			18 ± 5	

[a)] Calculated (ChemDraw) log$D$ values at a pH of 7.4.
[b)] Due to low affinity, the CB_2 affinity was only recorded once.

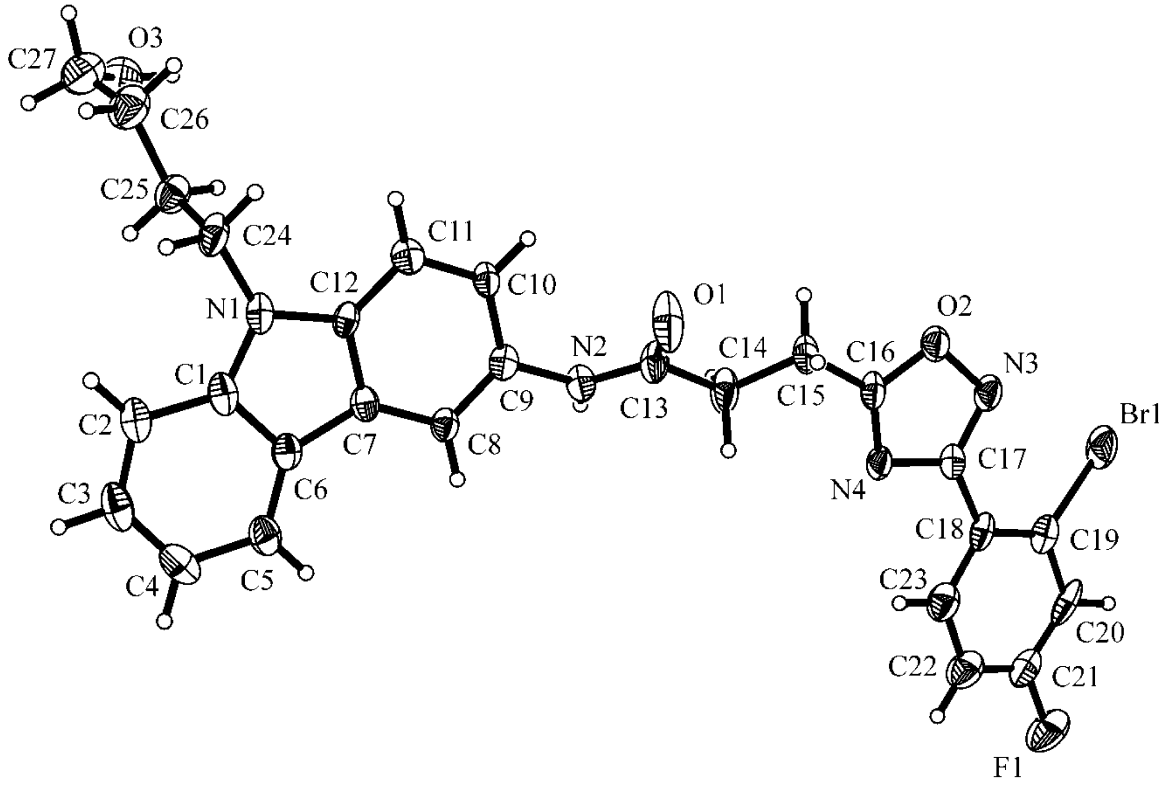


Figure 2. A view of the molecular structure of the 4-hydroxybutyl derivative **2c**: selected bond length (Å) and angles (°): N1-C1 1.368(8); N1-C12 1.387(6); N1-C24 1.431(8); N2-C9 1.427(8); N2-C13 1.323(8); O1-C13 1.223(7); N3-O2 1.424(6); N3-C17 1.307(8); N4-C17 1.386 (7); C19-Br1 1.891(8); C21-F1 1.332(9); C1-N1-C24-C25 -84.7(8); N4-C17-C18-C19 169.9(6): N3-C17-C18-C19 -10.3(10); C13-N2-C9-C10 49.5(5); C13-N2-C9-C8 −133.0(8)°. N3-Br1 3.005(5); C17-N3-Br1 91.3; C19-Br1-N3 73.1. Intermolecular H-bond: N2-H02…O1 2.840(7) Å, 164(5).

compared with **2a**. Although the lipophilicity is further increased by the 4-hydroxybutyl side chain of **2c**, the CB_2 affinity is reduced ($K_i = 96$ nM). The 3-hydroxypropyl derivative **2b** represents the best compromise between lipophilicity and CB_2 affinity.

Conclusions

Introduction of a hydroxy moiety into the alkyl side chain attached to the carbazole N-atom leads to CB_2 ligands with moderate affinity compared with the CB_2 agonist **1a** bearing an ethyl substituent at the carbazole ring system. However the hydroxy group is tolerated by the CB_2 receptor, since the CB_2 affinity of the hydroxypropyl derivative **2b** is still very high. The novel developed strategy of the one-pot introduction of hydroxyalkyl substituents via cyclic sulfates allows the fast access of these type of ligands and, additionally, the further modification of the properties of the carbazole N-substituent.

Experimental

Chemistry, general

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). ^{1}H 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^{-1}; injection volume: 5.0 μL; detection at $\lambda = 210$ 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%.

General procedures

*General Procedure A for the hydroxyalkylation of carbazole **3***

Under N_2 9*H*-carbazole (**3**, 1.0 equiv.) was dissolved in THF (1 mL per 100 mg) and cooled down to −78°C. *n*-BuLi (ca. 1.6 M in *n*-hexane, 1.2 equiv.) was added dropwise and the mixture was stirred for 30 min at −78°C. Then a solution of the respective cyclic sulfate (1.1 equiv.) in THF (15 mL) was added and the mixture was stirred for 1 h at −78°C and 24 h at rt. H_2O (5 mL) was added, the mixture was acidified with H_2SO_4 conc. and heated to reflux for 24 h. Afterwards, the solution was neutralized with 5 M NaOH, brine was added and the mixture was extracted with CH_2Cl_2. The organic layers were dried (Na_2SO_4), concentrated under reduced pressure and the residue was purified by fc.

*General Procedure B for the nitration of 9-(hydroxyalkyl)-carbazoles **5***

The respective 9-(hydroxyalkyl)carbazole **5** (1.0 equiv.) was dissolved in CH_2Cl_2 (2 mL per 100 mg) and cooled down to 5–10°C. Concentrated nitric acid (1.5 equiv.) was added dropwise under vigorous stirring. Stirring was continued at <10°C until the starting material was transformed completely. H_2O (0.25 mL per 1 mL CH_2Cl_2) was added, the reaction mixture was neutralized with $NaHCO_3$, brine was added and the mixture was extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4), concentrated under reduced pressure, the residue was purified by fc.

*General Procedure C for the reduction of 3-nitro-9-(hydroxyalkyl)carbazoles **6***

The respective 3-nitro-9-(hydroxyalkyl)carbazole **6** (1.0 equiv.) was dissolved in THF (2 mL per 100 mg) and Pd/C (10 wt.% loading) was added. The reaction mixture was stirred for 24 h at rt under H_2 (balloon). The catalyst was removed by filtration over Celite® and the filtrate was concentrated under reduced pressure. The residue was dissolved in Et_2O. Under N_2, $HCl \cdot Et_2O$ (2 M, 2.0 equiv.) was added dropwise under vigorous stirring to produce the respective HCl salt. The precipitate was filtered, washed with cold MeOH, dried (Na_2SO_4) and used without further purification.

*General Procedure D for the synthesis of 3-(3-phenyl)-1,2,4-oxadiazol-5-yl)propanoic acid **9** [34]*

$NH_2OH \cdot HCl$ (1.0 equiv.) was dissolved in H_2O (0.4 mL per mmol), Na_2CO_3 (0.5 equiv.) was added and the mixture was stirred for 25 min at rt. A solution of benzonitrile **8** (1.0 equiv.) in CH_3OH (1.8 mL per 1 mmol) was added under vigorous stirring and the mixture was stirred at 86°C until the nitrile **8** was converted. Then, CH_3OH was removed under reduced pressure, the residue was diluted with brine and the mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. Succinic anhydride (0.9 equiv.) and DMF (0.5 mL) were added to the residue and the reaction mixture was stirred for 30 min at 148°C. The solvent was removed *in vacuo* and the residue was purified by fc (cyclohexane/ethyl acetate/formic acid 50:50:0.01). The final product was purified by recrystallization (CH_2Cl_2/H_2O).

*General Procedure E for the COMU® coupling of 1,2,4-oxadiazole carboxylic acid 9 with 3-aminocarbazoles **7***

COMU® (1.2–1.5 equiv.) was added to a mixture of 3-(1,2,4-oxadiazolyl)propanooic acid **9** (1.0 equiv.) and triethylamine (2.0–3.0 equiv.) in DMF and the mixture was stirred for 30 min at rt. The reaction mixture was cooled down to 0°C and a solution of the respective 3-aminocarbazole hydrochloride **7** (1.0 equiv.) in DMF was added dropwise. This mixture was stirred for 24 h at <10°C. Then H_2O and brine were added, the mixture was extracted with $CHCl_3$, the combined organic layers were dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by fc and finally by recrystallization with CH_2Cl_2.

Experimental procedures

*9-(4-Bromobutyl)-9H-carbazole (**4**)*

Under N_2 9*H*-carbazole (6.0 g, 35.9 mmol) was dissolved in THF (60 mL) and cooled down to −78°C. *n*-BuLi (1.6 M in *n*-hexane, 29.2 mL, 46.7 mmol) was added dropwise and the mixture was stirred for 30 min at −78°C. This mixture was added via a cannula to a solution of 1,4-dibromobutane (16.5 mL, 140 mmol) in THF (15 mL) at 0°C and the mixture was stirred for 1 h at 0°C and for 24 h at rt. Then H_2O (5 mL) was added, the solution was neutralized with 5 M NaOH, brine was added and the mixture was extracted with CH_2Cl_2. The organic layers were dried (Na_2SO_4), concentrated *in vacuo* and the residue was purified by fc [$d = 8$ cm, $l = 14$ cm, cyclohexane, R_f 0.43 (cyclohexane/ethyl acetate 90:10)]. Colorless needles, mp 62–64°C, yield 7.3 g (62%). $C_{16}H_{16}BrN$ (302.2 g mol^{-1}). Exact mass (APCI): m/z = calcd. for $C_{16}H_{16}{}^{79}BrNH$ 302.0539; Found: 302.0521. Purity (HPLC): 95.61% ($t_R = 19.58$ min). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.88–1.97 (m, 2H, $NCH_2CH_2CH_2CH_2Br$), 2.03–2.12 (m, 2H, $NCH_2CH_2CH_2CH_2Br$), 3.39 (t, $J = 6.5$ Hz, 2H, $NCH_2CH_2CH_2CH_2Br$), 4.37 (t, $J = 6.9$ Hz, 2H, $NCH_2CH_2CH_2CH_2Br$), 7.24 (t, $J = 6.3$ Hz, 2H, 3-H, 6-H), 7.41 (t, $J = 8.2$ Hz, 2H, 2-H, 7-H), 7.46–7.49 (m, $J = 7.6$ Hz, 2H, 1-H, 8-H), 8.11 (d, $J = 7.8$ Hz, 2H, 4-H, 5-H). ^{13}C NMR ($CDCl_3$): δ (ppm) = 30.4 (1C, $NCH_2CH_2CH_2CH_2Br$), 31.1 (1C, $NCH_2CH_2CH_2CH_2Br$), 33.3 (1C, $NCH_2CH_2CH_2CH_2Br$), 42.3 (1C, $NCH_2CH_2CH_2CH_2Br$), 108.8 (2C, C-1,C-8), 120.5 (2C, C-4,C-5), 118.7 (2C, C-3, C-6), 123.0 (2C, C-4a, C-4b), 125.9 (2C, C-2, C-7), 140.4 (2C, C-8a, C-9a). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3051 (m, C–H, arom), 2958, 2927 (m, C–H, aliph), 651, 617 (s, CH_2–Br).

*2-(9H-Carbazol-9-yl)ethan-1-ol (**5a**)*

Following the General Procedure A, *n*-BuLi (1.2 M in *n*-hexane, 32.4 mL, 38.9 mmol, 1.2 equiv.) was added dropwise to 9*H*-carbazole (5.43 g, 32.5 mmol, 1.0 equiv.) in THF (55 mL) and, after 30 min, a solution of ethylene sulfate (4.51 g, 36.3 mmol, 1.1 equiv.) in THF (15 mL) was added. The crude product was purified by fc ($d = 8$ cm, $l = 12$ cm, cyclohexane/ethyl acetate 65:35, R_f 0.42). Pale yellow solid, mp 79–81°C, yield 4.66 g (68%). $C_{14}H_{13}NO$ (211.3 g mol^{-1}). Exact mass (APCI): m/z = calcd. for $C_{14}H_{13}NOH$ 212.1069; Found: 212.1060. Purity (HPLC): 98.1% ($t_R = 19.28$ min). ^{1}H NMR (DMSO-D_6): δ (ppm) = 3.86 (quart, $J = 5.9$ Hz, 2H, NCH_2CH_2OH), 4.47 (t, $J = 5.5$ Hz, 2H, NCH_2CH_2OH), 4.99 (t, $J = 5.0$ Hz, 1H, NCH_2CH_2OH), 7.24 (t, $J = 7.4$ Hz, 2H, 3-H, 6-H), 7.48 (t, $J = 7.6$ Hz, 2H, 2-H, 7-H), 8.18 (d, $J = 7.8$ Hz, 2H, 4-H, 5-H). ^{13}C NMR ($CDCl_3$): δ (ppm) = 45.3 (1C, NCH_2CH_2OH), 59.6 (1C, NCH_2CH_2OH), 109.6 (2C, C-1, C-8), 118.6 (2C, C-3, C-6), 120.2 (2C, C-4, C-5), 122.2 (2C, C-4a, C-4b), 125.0 (2C, C-2, C-7), 140.5 (2C,C-8a, C-9a). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3356 (m, O–H), 3045, 3019 (w, C–H, arom), 2943 (w, C–H, aliph).

*3-(9H-Carbazol-9-yl)propan-1-ol (**5b**)*

Following the General Procedure A, *n*-BuLi (1.3 M in *n*-hexane, 29.9 mL, 38.9 mmol, 1.2 equiv.) was added dropwise to 9*H*-carbazole (5.43 g, 32.5 mmol, 1.0 equiv.) in THF (55 mL) and, after 30 min a solution of trimethylene sulfate (5.01 g, 36.3 mmol, 1.1 equiv.) in THF (15 mL) was added. The crude product was purified by fc ($d = 8$ cm, $l = 12$ cm, cyclohexane/ethyl acetate 65:35, R_f 0.45). Colorless solid, mp 102–105°C, yield 5.1 g (70%). $C_{15}H_{15}NO$ (225.3 g mol^{-1}). Exact mass (APCI): m/z = calcd. for $C_{15}H_{15}NOH$ 226.1226; Found: 226.1129. Purity (HPLC): 97.8% ($t_R = 19.35$ min). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.90 (dt, $J = 12.4/6.4/6.0$ Hz, 2H, $NCH_2CH_2CH_2OH$), 3.39 (t, $J = 5.9$ Hz, 2H, $NCH_2CH_2CH_2OH$), 4.25 (t, $J = 6.6$ Hz, 2H, $NCH_2CH_2CH_2OH$), 7.04 (m, 4H, 2-H, 3-H, 6-H, 7-H), 7.27 (d, 2H, 1-H, 8-H), 7.91 (d, $J = 7.7$ Hz, 2H, 4-H, 5-H). ^{13}C NMR ($CDCl_3$): δ (ppm) = 31.5 (1C, $NCH_2CH_2CH_2OH$), 39.3 (1C, $NCH_2CH_2CH_2OH$), 59.7 (1C, $NCH_2CH_2CH_2OH$), 108.8 (2C, C-1, C-8), 119.0 (2C, C-3, C-6), 120.5 (2C, C-4, C-5), 123.0 (2C, C-4a, C-4b), 125.8 (2C, C-2, C-7), 140.6 (2C, C-8a, C-9a). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3344 (m, O–H), 3047, 3020 (w, C–H, arom), 2943 (m, C–H, aliph).

*4-(9H-Carbazol-9-yl)butan-1-ol (**5c**)*

Solid NaOH (79.4 mg, 1.99 mmol) was added to a solution of bromide **4** (120 mg, 0.397 mmol) and tetrabutylammonium iodide (44 mg, 0.12 mmol) in diglyme (5 mL) and the mixture was heated to reflux for 45 min. After neutralization with 5 M HCl and saturated solution of $NaHCO_3$, brine was added to the reaction mixture. The mixture was extracted with $CHCl_3$, the organic layers were dried (Na_2SO_4), concentrated under reduced pressure and the residue was purified by fc [$d = 2$ cm, $l = 14$ cm, cyclohexane/ethyl acetate 75:25, R_f 0.25 (cyclohexane/ethyl acetate 60:40)]. Colorless solid, mp 68–69°C, yield 85.4 mg (90%). $C_{16}H_{17}NO$ (239.3 g/mol). Exact mass (APCI): m/z = calcd. for $C_{16}H_{17}NOH$ 240.1388; Found: 240.1383. Purity (HPLC): 99.2% ($t_R = 19.33$ min). ^{1}H NMR (DMSO-D_6): δ (ppm) = 1.39–1.47 (m, 2H, $NCH_2CH_2CH_2CH_2OH$), 1.75–1.84 (m, 2H, $NCH_2CH_2CH_2CH_2OH$), 3.38 (m, 2H, $NCH_2CH_2CH_2CH_2OH$), 4.41 (t, $J = 5.2$ Hz, 2H, $NCH_2CH_2CH_2CH_2OH$), 7.19 (t, $J = 6.3$ Hz, 2H, 3-H, 6-H), 7.45 (t, $J = 7.5$ Hz, 2H, 2-H, 7-H), 7.60 (d, $J = 8.2$ Hz, 2H, 1-H, 8-H), 8.14 (d, $J = 7.8$ Hz, 2H, 4-H, 5-H). ^{13}C NMR (DMSO-D_6): δ (ppm) = 25.4 (s, 1C, $NCH_2CH_2CH_2CH_2OH$), 30.1 (s, 1C, $NCH_2CH_2CH_2CH_2OH$), 42.3 (s, 1C, $NCH_2CH_2CH_2CH_2OH$), 60.6 (s, 1C, $NCH_2CH_2CH_2CH_2OH$), 109.4 (s, 2C, C-1, C-8), 118.8 (s, 2C, C-3, C-6), 120.4 (s, 2C, C-4, C-5), 122.1 (s, 2C, C-4a, C-5a), 125.8 (s, 2C, C-2, C-7), 140.1 (s, 2C, C-8a, C-9a). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3244 (m, O–H), 3051 (m, C–H, arom), 2935 (m, C–H, aliph).

*2-(3-Nitro-9H-carbazol-9-yl)ethan-1-ol (**6a**)*

According to the General Procedure B a solution of **5a** (5.11 g, 24.2 mmol) in CH_2Cl_2 (100 mL) was treated with HNO_3 conc. (1.7 mL, 36.3 mmol). The product was purified by fc ($d = 8$ cm, $l = 12$ cm, cyclohexane/ethyl acetate 50:50, R_f 0.27). Pale yellow solid, mp 230°C, yield 3.34 g (54%). $C_{14}H_{12}N_2O_3$ (256.3 g mol^{-1}). Exact mass (APCI): m/z = calcd. for $C_{14}H_{12}N_2O_3Na$ 279.0740; Found: 279.0740. Purity (HPLC): 97.8% ($t_R = 18.87$ min). ^{1}H NMR ($CDCl_3$): δ (ppm) = 4.13 (t, $J = 5.3$ Hz, 2H, NCH_2CH_2OH), 4.55 (t, $J = 5,3$ Hz, 2H, NCH_2CH_2OH), 7.38 (t, $J = 8.0$ Hz, 1H, 6-H), 7.51–7.60 (m, 3H, 1-H, 7-H, 8-H), 8.13 (d, $J = 7.5$ Hz, 1H, 5-H), 8.39 (dd, $J = 8.9/2.0$ Hz, 1H, 2-H), 9.03 (d, $J = 2.2$ Hz, 1H, 4-H). ^{13}C NMR (DMSO-D_6): δ (ppm) = 39.4 (1C, NCH_2CH_2OH), 59.6 (1C, NCH_2CH_2OH), 110.2 (1C, C-8), 110.9 (1C, C-1), 117.3 (2C, C-4, C-6), 120.7 (1C, C-5), 121.3 (1C, C-2), 122.0 (1C, C-4b), 122.3 (1C, C-7), 127.4 (1C, C-4a), 139.9 (1C, C-3), 141.8 (1C, C-8a), 144.1 (1C, C-9a). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3414 (s, O–H), 3051 (m, C–H, arom), 1492, 1323 (s, NO_2).

*3-(3-Nitro-9H-carbazol-9-yl)propan-1-ol (**6b**)*

According to the General Procedure B a solution of **5b** (5.0 g, 25.0 mmol) in CH_2Cl_2 (100 mL) was treated with HNO_3 conc. (1.75 mL, 37.5 mmol). The product was purified by fc ($d = 5$ cm, $l = 11$ cm, cyclohexane/ethyl acetate 50:50, R_f 0.31). Yellow solid, mp 188–190°C, yield 4.25 g (62%). $C_{15}H_{14}N_2O_3$ (270.3 g mol^{-1}).

Exact mass (APCI): m/z = calcd. for $C_{15}H_{14}N_2O_3H$ 271.1077; Found: 279.1066. Purity (HPLC): 95.6% (t_R = 19.58 min). ^{1}H NMR ($CDCl_3$): δ (ppm) = 1.60 (s, 1H, $NCH_2CH_2CH_2OH$), 2.14 (quint, J = 6.3 Hz, 2H, $NCH_2CH_2CH_2OH$), 3.64 (m, 2H, $NCH_2CH_2CH_2OH$), 4.54 (t, J = 6.7 Hz, 2H, $NCH_2CH_2CH_2OH$), 7.35 (t, J = 7.8 Hz, 1H, 6-H), 7.49–7.60 (m, 3H, 1-H, 7-H, 8-H), 8.15 (d, J = 8.1 Hz, 1H, 5-H), 8.37 (dd, J = 9.1/2.3 Hz, 1H, 2-H), 9.00 (d, J = 2.2 Hz, 1H, 4-H). ^{13}C NMR (DMSO-D_6): δ (ppm) = 31.6 (1C, $NCH_2CH_2CH_2OH$), 39.3 (1C, $NCH_2CH_2CH_2OH$), 57.7 (1C, $NCH_2CH_2CH_2OH$), 109.5 (1C, C-8), 110.3 (1C, C-1), 117.3 (1C, C-6), 120.6 (1C, C-4), 121.3 (1C, C-5), 121.4 (1C, C-2)121.8 (1C, C-4b), 122.2 (1C, C-7), 127.4 (1C, C-4a), 139.9 (1C, C-3), 141.6 (1C, C-8a), 143.3 (1C, C-9a). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3344 (s, O–H), 3041 (m, C–H, arom), 2935 (m, C–H, aliph), 1495, 1323 (s, NO_2).

*4-(3-Nitro-9H-carbazol-9-yl)butan-1-ol (**6c**)*

According to the General Procedure B a solution of **5c** (2.5 g, 10.45 mmol) in CH_2Cl_2 (50 mL) was treated with HNO_3 conc. (0.73 mL, 15.68 mmol). The product was purified by fc (d = 6 cm, l = 12 cm, cyclohexane/ethyl acetate 50:50, R_f 0.28). Yellow solid, mp 87–89°C, yield 1.99 g (67%). $C_{16}H_{16}N_2O_3$ (270.3 g mol^{-1}). Exact mass (APCI): m/z = calcd. for $C_{16}H_{16}N_2O_3H$ 285.1234; Found: 279.1238. Purity (HPLC): 99.4% (t_R = 19.50 min). ^{1}H NMR (DMSO-D_6): δ (ppm) = 1.43 (m, 2H, $NCH_2CH_2CH_2CH_2OH$), 1.83 (m, 2H, $NCH_2CH_2CH_2CH_2OH$), 3.38 (m, 2H, $NCH_2CH_2CH_2CH_2OH$), 4.43 (t, J = 5.1 Hz, 1H, $NCH_2CH_2CH_2CH_2OH$), 4.52 (t, J = 7.1 Hz, 2H, $NCH_2CH_2CH_2CH_2OH$), 7.34 (t, J = 7.5 Hz, 1H, 6-H), 7.59 (t, J = 7.7 Hz, 1H, 7-H), 7.75 (d, J = 8.3 Hz, 1H, 8-H), 7.82 (d, J = 9.1 Hz, 1H, 5-H), 8.35 (dd, J = 9.1/2.3 Hz 1H, 2-H), 8.42 (d, J = 7.9 Hz, 1H, 1-H), 9.19 (d, J = 2.2 Hz, 1H, 4-H). ^{13}C NMR (DMSO-D_6): δ (ppm) = 25.4 (1C, $NCH_2CH_2CH_2CH_2OH$), 29.9 (1C, $NCH_2CH_2CH_2CH_2OH$), 42.9 (1C, $NCH_2CH_2CH_2CH_2OH$), 60.5 (1C, $NCH_2CH_2CH_2CH_2OH$), 109.7 (1C, C-8), 110.6 (1C, C-1), 117.5 (1C, C-6), 120.7 (1C, C-4), 121.4 (1C, C-5), 121.6 (1C, C-2), 122.0 (1C, C-4b), 122.3 (1C, C-7), 127.6 (1C, C-4a), 140,0 (1C, C-3), 141.5 (1C, C-8a), 143.4 (1C, C-9a). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3244 (s, O–H), 3043 (m, C–H, arom), 2935 (m, C–H, aliph), 1495, 1323 (s, NO_2).

*2-(3-Amino-9H-carbazol-9-yl)ethan-1-ol hydrochloride (**7a** HCl)*

Following the General Procedure C, **6a** (500 mg, 1.95 mmol, 1.0 equiv.) was dissolved in THF (10 mL), Pd/C (100 mg) was added and the mixture was stirred under H_2 atmosphere (balloon) for 18 h. The amine was precipitated with $HCl \cdot Et_2O$ [2 mol L^{-1}, 2 mL, 4 mmol, 2.0 equiv., R_f 0.35 (cyclohexane/ethyl acetate 30:70)]. Colorless solid, mp 230°C, yield 492 mg (96%). $C_{14}H_{15}ClN_2O$ (262.7 g mol^{-1}). Exact mass (APCI): m/z = calcd. for $C_{14}H_{15}N_2O$ 217.1179; Found 217.1179. Purity (HPLC): 99.0% (t_R = 11.64 min). ^{1}H NMR (**7a**·HCl, DMSO-D_6): δ (ppm) = 3.78 (t, J = 5.5 Hz, 2H, NCH_2CH_2OH), 4.47 (t, J = 5.5 Hz, 2H, NCH_2CH_2OH), 7.24 (t, J = 7.4 Hz, 1H, 6-H), 7.45 (dd, J = 8.7/2.1 Hz, 1H, 2-H), 7.50 (t, J = 7.7 Hz, 1H, 7-H), 7.66 (d, J = 8.3 Hz, 1H, 1-H), 7.74 (d, J = 8.7 Hz, 1H, 8-H), 8.12 (d, J = 2.1 Hz, 1H, 4-H), 8.18 (d, J = 7.7 Hz, 1H, 5-H), 10.4 (s, 2H, $-NH_2$). ^{13}C NMR (**7a**·HCl, DMSO-D_6): δ (ppm) = 45.5 (1C, NCH_2CH_2OH), 59.7 (1C, NCH_2CH_2OH), 110.2 (1C, C-1), 110.8 (1C, C-8), 114.8 (1C, C-4), 119.3 (1C, C-2), 120.6 (1C, C-6), 121.5 (1C, C-5), 122.3 (1C, C-4b), 122.8 (1C, C-7), 126.7 (2C, C-4a, C-9a), 139.7 (1C, C-8a), 141.2 (1C, C-3). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3344 (s, O–H), 3041 (m, C–H, arom), 2935 (m, C–H, aliph).

*3-(3-Amino-9H-carbazol-9-yl)propan-1-ol hydrochloride (**7b**·HCl)*

Following the General Procedure C, **6b** (1.3 g, 4.8 mmol, 1.0 equiv.) was dissolved in THF (26 mL), Pd/C (260 mg) was added and the mixture was stirred under H_2 atmosphere (balloon) for 24 h. The amine was precipitated with $HCl \cdot Et_2O$ [2 mol L^{-1}, 4.8 mL, 9.6 mmol, 2.0 equiv., R_f 0.37 (cyclohexane/ethyl acetate 15:85)]. Colorless solid, mp 228–230°C, yield 1.21 g (91%). $C_{14}H_{15}ClN_2O$ (276.8 g mol^{-1}). Exact mass (APCI): m/z = calcd. for $C_{15}H_{17}N_2O$ 241.1335; Found 241.1324. Purity (HPLC): 97.8% (t_R = 12.64 min). ^{1}H NMR (**7b**·HCl, DMSO-D_6): δ (ppm) = 1.91 (quint, J = 6.5 Hz, 2H, $NCH_2CH_2CH_2OH$), 3.78 (t, J = 5.5 Hz, 2H, $NCH_2CH_2CH_2OH$), 4.47 (t, J = 5.5 Hz, 2H, $NCH_2CH_2CH_2OH$), 7.24 (t, J = 7.4 Hz, 1H, 6-H), 7.45 (dd, J = 8.7, 2.1 Hz, 1H, 2-H), 7.50 (t, J = 7,7 Hz, 1H, 7-H), 7.66 (d, J = 8.3 Hz, 1H, 1-H), 7.73 (d, J = 8.7 Hz, 1H, 8-H), 8.13 (d, J = 2.1 Hz, 1H, 4-H), 8.19 (d, J = 7.7 Hz, 1H, 5-H), 10.4 (s, 2H, $-NH_2$). ^{13}C NMR (**7b**·HCl, DMSO-D_6): δ (ppm) = 31.7 (s, 1C, $NCH_2CH_2CH_2OH$), 45.5 (s, 1C, $NCH_2CH_2CH_2OH$), 57.9 (s, 1C, $NCH_2CH_2CH_2OH$), 109.7 (s, 1C, C-4), 110.2 (s, 1C, C-8), 114.8 (s, 1C, C-1), 119.2 (s, 1C, C-2), 120.6 (s, 1C, C-6), 121.3 (s, 1C, C-5), 122.2 (s, 1C, C-4b), 122.8 (s, 1C, C-7), 126.6 (s, 2C, C-4a, C-9a), 139.1 (s, 1C, C-8a), 140.7 (s, 1C, C-3). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3263 (s, $-NH_2$), 2889 (m, C–H, aliph).

*4-(3-Amino-9H-carbazol-9-yl)butan-1-ol hydrochloride (**7c**·HCl)*

Following the General Procedure C, **6c** (500 mg, 1.71 mmol, 1.0 equiv.) was dissolved in THF (10 mL), Pd/C (150 mg) was added and the mixture was stirred under H_2 atmosphere (balloon) for 24 h. The amine was precipitated with $HCl \cdot Et_2O$ [2 mol L^{-1}, 1.7 mL, 3.4 mmol, 2.0 equiv., R_f 0.37 (cyclohexane/ethyl acetate 15:85)]. Colorless solid, mp 182°C, yield 448.3 mg (90%). $C_{16}H_{19}ClN_2O$ (290.8 g mol^{-1}). Exact mass (APCI): m/z = calcd. for $C_{16}H_{19}N_2O$ 255.1492; Found: 241.1475. Purity (HPLC): 97.6% (t_R = 13.16 min). ^{1}H NMR (**7c**·HCl, DMSO-D_6): δ (ppm) = 1.41 (quint, J = 6.5 Hz, 2H, $NCH_2CH_2CH_2CH_2OH$), 1.80 (quint, J = 6.5 Hz, 2H, $NCH_2CH_2CH_2CH_2OH$), 3.39 (t, J = 5.5 Hz, 2H, $NCH_2CH_2CH_2CH_2OH$), 4.44 (t, J = 5.5 Hz, 2H, $NCH_2CH_2CH_2CH_2OH$), 7.24 (t, J = 7.4 Hz, 1H, 6-H), 7.46 (dd, J = 8.7/2.1 Hz, 1H, 2-H), 7.52 (t, J = 7,7 Hz, 1H, 7-H), 7.66 (d, J = 8.3 Hz, 1H, 1-H), 7.74 (d, J = 8.7 Hz, 1H, 8-H), 8.12 (d, J = 2.1 Hz, 1H, 4-H), 8.20 (d, J = 7.7 Hz, 1H, 5-H), 10.4 (s, 2H, $-NH_2$). ^{13}C NMR (**7c**·HCl, DMSO-D_6): δ (ppm) = 25.4 (1C, $NCH_2CH_2CH_2CH_2OH$), 30.0 (1C, $NCH_2CH_2CH_2CH_2OH$), 42.5 (1C, $NCH_2CH_2CH_2CH_2OH$), 60.5.9 (1C, $NCH_2CH_2CH_2CH_2OH$), 109.9 (1C, C-4), 110.4 (1C, C-8), 114.9 (1C, C-1), 119.4 (1C, C-2), 120.7 (1C, C-6), 120.8 (1C, C-5), 121.4 (1C, C-4b), 122.3 (1C, C-7), 122.8 (1C, C-4a), 126.8 (1C, C-9a), 139.2 (1C, C-8a), 140.8 (1C, C-3). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3282 (s, $-NH_2$), 2885 (m, C–H, aliph).

*3-[3-(2-Bromo-4-fluorophenyl)-1,2,4-oxadiazol-5-yl]-propanoic acid (**9**) [34]*

According to General Procedure D, 2-bromo-4-fluorobenzonitrile (**8**, 5.0 g, 25.0 mmol) was reacted with $NH_2OH \cdot HCl$ (5.21 g, 75.0 mmol) and Na_2CO_3 (3.98 g, 37.5 mmol) in H_2O (15 mL) and CH_3OH (45 mL). The intermediate was purified by fc (d = 8 cm, l = 12 cm, cyclohexane/ethyl acetate 50:50, R_f 0.27, yield 492 mg, 39%) and subsequently reacted with succinic anhydride (2.5 g, 25.0 mmol) in DMF (0.5 mL). The product was purified by recrystallization [CH_2Cl_2/H_2O, R_f 0.46 (cyclohexane/ethyl acetate/formic acid 50:50:0.01)]. Colorless solid, mp 114–116°C, yield 2.9 g (45%). $C_{11}H_8BrFN_2O_3$ (315.1 g/mol). Exact mass (APCI): m/z = calcd.

for $C_{11}H_8{}^{79}BrFN_2O_3H$ 314.9775; Found: 314.9760. Purity (HPLC): 99.9% ($t_R = 17.15$ min). ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.02 (t, $J = 7.1$ Hz, 2H, $CH_2CH_2CO_2H$), 3.29 (t, $J = 7.1$ Hz, 2H, $CH_2CH_2CO_2H$), 7.15 (ddd, $J = 8.7/7.7/2.6$ Hz, 1H, 5-H), 7.47 (dd, $J = 8.2/2.6$ Hz, 1H, 3-H), 7.85 (dd, $J = 8.7/6.0$ Hz, 1H, 6-H). ^{13}C NMR (DMSO-D_6): δ (ppm) = 21.6 (1C, $CH_2CH_2CO_2H$), 29.8 (1C, $CH_2CH_2CO_2H$), 114.7 (d, $J = 21.5$ Hz, 1C, C-5), 121.5 (d, $J = 24.7$ Hz, 1C, C-3), 127.2 (1C, C-2), 129.4 (1C, C-1), 133.2 (d, $J = 9.1$ Hz, 1C, C-6), 163.2 (d, $J = 255.7$ Hz, 1C, C-4), 167.0 (1C, C-$3_{oxadiazole}$), 175.6 (1C, CO_2H), 177.6 (1C, C-$5_{oxadiazole}$). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3097–2403 (m, COOH), 1708 (s, C=O).

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

According to the General Procedure E, a solution of propanoic acid **9** (400 mg, 1.3 mmol), **7a**·HCl (222 mg, 0.9 mmol), COMU® (652 mg, 1.5 mmol) and triethylamine (0.54 mL, 3.9 mmol) in DMF (15 mL) was stirred for 24 h at <10°C. The residue was purified by fc [$d = 5$ cm, $l = 15$ cm, cyclohexane/ethyl acetate 15:85, R_f 0.51 (ethyl acetate)]. Colorless solid, mp 163–165°C, yield 368 mg (80%). $C_{25}H_{20}BrFN_4O_3$ (523.4 g mol^{-1}). Exact mass (ESI): m/z = calcd. for $C_{25}H_{20}{}^{79}BrFN_4O_3H$ 523.0776; Found: 523.0783. Purity (HPLC): 97.7% ($t_R = 20.24$ min). ^{1}H NMR (DMSO-D_6): δ (ppm) = 2.99 (t, $J = 7.0$ Hz, 2H, CH_2CH_2CONH), 3.35 (t, $J = 7.1$ Hz, 2H, CH_2CH_2CONH), 3.76 (q, $J = 5.6$ Hz, 2H, NCH_2CH_2OH), 4.40 (t, $J = 5.5$ Hz, 2H, NCH_2CH_2OH), 4.86 (t, $J = 5.4$ Hz, 1H, NCH_2CH_2OH), 7.15 (t, $J = 7.4$ Hz, 1H, 6-H_{carb}), 7.38–7.48 (m, 2H, 7-H_{carb}, 5-H_{phenyl}), 7.49–7.55 (m, 2H, 1-H_{carb}, 2-H_{carb}), 7.57 (d, $J = 8.3$ Hz, 1H, 8-H_{carb}), 7.83 (dd, $J = 8.6/2.6$ Hz, 1H, 3-H_{phenyl}), 7.89 (dd, $J = 8.7/6.1$ Hz, 1H, 6-H_{phenyl}), 8.02 (d, $J = 7.8$ Hz, 1H, 5-H_{carb}), 8.39 (s, 1H, 4-H_{carb}), 10.13 (s, 1H, CONH). ^{13}C NMR (DMSO-D_6): δ (ppm) = 21.8 (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}), 115.5 (d, $J = 21.6$ Hz,1C, C-5_{phenyl}), 118.5 (1C, C-2_{carb}), 118.7 (1C, C-6_{carb}), 120.0 (1C, C-5_{carb}), 121.4 (d, $J = 24.9$ Hz, 1C, C-3_{phenyl}), 121.7 (1C, C-$4a_{carb}$), 122.0 (1C, C-$4b_{carb}$), 122.2 (d, $J = 10.1$ Hz, 1C, C-2_{phenyl}), 124.5 (d, $J = 3.4$ Hz,1C, C-1_{phenyl}), 125.6 (1C, C-7_{carb}), 131.0 (1C, C-3_{carb}), 133.6 (d, $J = 9.3$ Hz, 1C, C-6_{phenyl}), 137.0 (1C, C-$9a_{carb}$), 140.9 (1C, C-$8a_{carb}$), 162.9 (d, $J = 253.4$ Hz, 1C, C-4_{phenyl}), 166.4 (1C, C-$3_{oxadiazole}$), 168.5 (1C, CONH), 179.6 (1C, C-$5_{oxadiazole}$). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3421 (m, O–H), 3329 (m, N–H), 3078 (m, C-H, arom), 2935 (m, C–H, aliph), 1678 (s, NH–C=O).

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

According to the General Procedure E, a solution of propanoic acid **9** (453 mg, 1.4 mmol), **7b**·HCl (400 mg, 1.4 mmol), COMU® (740 mg, 1.7 mmol) and triethylamine (0.4 mL, 2.8 mmol) in DMF (15 mL) was stirred for 24 h at <10°C. The residue was purified by fc [$d = 6$ cm, $l = 10$ cm, cyclohexane/ethyl acetate 5:95, R_f 0.20 (ethyl acetate)]. Colorless solid, mp 155–157°C, yield 229 mg (30%). $C_{26}H_{22}BrFN_4O_3$ (537.4 g mol^{-1}). Exact mass (ESI): m/z = calcd. for $C_{26}H_{22}{}^{79}BrFN_4O_3H$ 537.0935; Found: 537.0932. Purity (HPLC): 96.6% ($t_R = 20.53$ min). ^{1}H NMR (DMSO-D_6): δ (ppm) = 1.82–1.91 (m, 2H, $NCH_2CH_2CH_2OH$), 2.97 (t, $J = 7.1$ Hz, 2H, CH_2CH_2CONH), 3.12 (t, $J = 7.2$ Hz, 2H, CH_2CH_2CONH), 3.33–3.36 (m, 2H, $NCH_2CH_2CH_2OH$), 4.40 (t, $J = 6.7$ Hz, 2H, $NCH_2CH_2CH_2OH$), 4.86 (t, $J = 4.9$ Hz, 1H, NCH_2CH_2OH), 7.13 (t, $J = 7.5$ Hz, 1H, 6-H_{carb}), 7.38–7.47 (m, 2H, 7-H_{carb}, 5-H_{phenyl}), 7.52–7.54 (m, 2H, 1-H_{carb}, 2-H_{carb}), 7.57 (d, $J = 8.3$ Hz, 1H, 8-H_{carb}), 7.78–7.90 (m, $J = 8.6/2.5$ Hz, 1H, 3-H_{phenyl}, 6-H_{phenyl}), 8.01 (d, $J = 7.8$ Hz, 1H, 5-H_{carb}), 8.38 (s, 1H, 4-H_{carb}), 10.12 (s, 1H, CONH). ^{13}C NMR (DMSO-D_6): δ (ppm) = 21.1 (1C, CH_2CH_2CONH), 31.8 (1C, $NCH_2CH_2CH_2OH$), 32.0 (1C, CH_2CH_2CONH), 58.0 (1C, $NCH_2CH_2CH_2OH$), 109.1 (1C, C-8_{carb}), 109.3 (1C, C-1_{carb}), 111.1 (1C, C-4_{carb}), 115.5 (d, $J = 21.8$ Hz, 1C, C-5_{phenyl}), 118.5 (1C, C-2_{carb}), 118.9 (1C, C-6_{carb}), 120.1 (1C, C-5_{carb}), 121.4 (d, $J = 24.6$ Hz, 1C, C-3_{phenyl}), 121.7 (1C, C-$4a_{carb}$), 121.9 (1C, C-$4b_{carb}$), 122.2 (d, $J = 10.1$ Hz, 1C, C-2_{phenyl}), 122.3 (d, $J = 8.7$ Hz, 1C, C-2_{phenyl}), 125.8 (d, $J = 10.6$ Hz, 1C, C-6_{phenyl}), 126.9 (1C, C-7_{carb}), 129.8 (1C, C-8_{carb}), 131.1 (d, $J = 3.8$ Hz, 1C, C-1_{phenyl}), 136.7 (1C, C-$9a_{carb}$), 141.4 (1C, C-$8a_{carb}$), 163.7 (d, $J = 258.9$ Hz, 1C, C-4_{phenyl}), 167.4 (1C, C-$3_{oxadiazole}$), 168.6 (1C, CONH), 179.9 (1C, C-$5_{oxadiazole}$). IR (neat): $\tilde{\nu}$ (cm^{-1}) = 3667 (m, O–H), 3290 (m, N–H), 1654 (s, NH–C=O).

*3-[3-(2-Bromo-4-fluorophenyl)-1,2,4-oxadiazol-5-yl]-N-[9-(4-hydroxybutyl)-9H-carbazol-3-yl]propanamide (**2c**)*

According to the General Procedure E, a solution of propanoic acid **9** (275 mg, 0.87 mmol), **7c**·HCl (270 mg, 1.2 mmol), COMU® (560 mg, 1.3 mmol) and triethylamine (0.36 mL, 3.6 mmol) in DMF (15 mL) was stirred for 22.5 h at <10°C. The residue was purified by fc [$d = 5$ cm, $l = 15$ cm, cyclohexane/ethyl acetate 15:85, R_f 0.43 (ethyl acetate)]. Colorless solid, mp 158–161°C, yield 158 mg (33%). $C_{28}H_{26}BrFN_4O_3$ (551.4 g mol^{-1}). Exact mass (ESI): m/z = calcd. for $C_{28}H_{26}{}^{79}BrFN_4O_3H$ 551.1089; Found: 551.1052. Purity (HPLC): >99%. ^{1}H NMR (DMSO-D_6): δ (ppm) = 1.37–1.45 (m, 2H, $NCH_2CH_2CH_2CH_2OH$), 1.73–1.82 (m. 2H, $NCH_2CH_2CH_2CH_2OH$), 2.98 (t, $J = 7.0$ Hz, 2H, CH_2CH_2CONH), 3.36 (t, $J = 7.0$ Hz, 2H, CH_2CH_2CONH), 4.36 (q, $J = 6.7$ Hz, 2H, $NCH_2CH_2CH_2CH_2OH$), 4.46 (t, $J = 6.7$ Hz, 1H, $NCH_2CH_2CH_2CH_2O\underline{H}$), 7.16 (t, $J = 7.7$ Hz, 1H, 6-H_{carb}), 7.40–7.47 (m, 2H, 7-H_{carb}, 5-H_{phenyl}), 7.52–7.54 (m, 2H, 1-H_{carb}, 2-H_{carb}), 7.56 (d, $J = 8.2$ Hz, 1H, 8-H_{carb}), 7.81 (dd, $J = 8.4/2.4$ Hz, 1H, 3-H_{phenyl}), 7.87 (dd, $J = 8.7/6.0$ Hz, 1H, 6-H_{phenyl}), 8.02 (d, $J = 7.6$ Hz, 1H, 5-H_{carb}), 8.38 (s, 1H, 4-H_{carb}), 10.16 (s, 1H, CONH). ^{13}C NMR (DMSO-D_6): δ (ppm) = 21.81 (1C, CH_2CH_2CONH), 25.36 (1C, $NCH_2CH_2CH_2CH_2OH$), 29.98 (1C, $NCH_2CH_2CH_2CH_2OH$), 31.97 (1C, CH_2CH_2CONH), 42.19 (1C, $NCH_2CH_2CH_2CH_2OH$), 60.43 (1C, $NCH_2CH_2CH_2CH_2OH$), 109.2 (1C, C-8_{carb}), 109.4 (1C, C-1_{carb}), 111.0 (1C, C-4_{carb}), 115.5 (d, $J = 21.6$ Hz, 1C, C-5_{phenyl}), 118.5 (1C, C-2_{carb}), 118.8 (1C, C-6_{carb}), 120.1 (1C, C-5_{carb}), 121.4 (d, $J = 25.1$ Hz, 1C, C-3_{phenyl}), 121.7 (1C, C-$4a_{carb}$), 121.9 (1C, C-$4b_{carb}$), 122.2 (d, $J = 10.1$ Hz, 1C, C-2_{phenyl}), 124.5 (d, $J = 3.5$ Hz, 1C, C-1_{phenyl}), 125.7 (1C, C-7_{carb}), 131.0 (1C, C-3_{carb}), 133.6 (d, $J = 9.5$ Hz, 1C, C-6_{phenyl}), 136.1 (1C, C-$9a_{carb}$), 140.4 (1C, C-$8a_{carb}$), 162.9 (d, $J = 253.3$ Hz, 1C, C-4_{phenyl}), 166.6 (1C, C-$3_{oxadiazole}$), 168.5 (1C, CONH), 179.6 (1C, C-$5_{oxadiazole}$).

X-ray diffraction

X-ray diffraction, general

Datasets were collected with a Nonius KappaCCD diffractometer. Programs used: data collection, COLLECT [50], data reduction Denzo-SMN [51]; absorption correction, Denzo [52]; structure solution SHELXS-97 [53]; structure refinement SHELXL-97 [54]; and graphics, XP (BrukerAXS, 2000). Thermals ellipsoids are shown with 30% probability, R values are given for observed reflections, and wR^2 values are given for all reflections.

Exceptions and special features

An unidentified disordered solvent molecule was found in the asymmetrical unit and could not be satisfactorily refined. The program SQUEEZE [55] was therefore used to remove mathematically the effect of the solvent. The Flack parameter was refined to 0.01(2). The CH_2-OH group at C26 atom is disordered over two positions. Several restraints (SADI, SAME, and SIMU) were used in order to improve refinement stability.

X-ray crystal structure analysis of 2c

Formula $C_{27}H_{24}BrFN_4O_3$, $M = 551.41$, colorless crystal, $0.44 \times 0.08 \times 0.06$ mm, $a = 4.7209(1)$, $b = 14.4710(4)$, $c = 39.7695(13)$ Å, $V = 2716.90(13)$ Å^3, $\rho_{calc} = 1.348$ g cm^{-3}, $\mu = 1.554$ mm^{-1}, empirical absorption correction ($0.548 \leq T \leq 0.912$), $Z = 4$, orthorhombic, space group $P2_12_12_1$ (No. 19), $\lambda = 0.71073$ Å, $T = 223(2)$ K, ω and φ scans, 13,647 reflections collected ($\pm h$, $\pm k$, $\pm l$), $[(\sin\theta)/\lambda] = 0.60$ Å^{-1}, 3835 independent ($R_{int} = 0.053$) and 3414 observed reflections [$I > 2\sigma(I)$], 350 refined parameters, $R = 0.067$, $wR^2 = 0.169$, max. (min.) residual electron density 0.31 (-0.42) e Å^{-3}, the hydrogen at N_2 atom was refined freely; others were calculated and refined as riding atoms. CCDC 947453.

Experimental receptor binding studies

Materials

The recombinant CHO cells expressing the CB2 receptor were a generous donation of Prof. Paul Prather (Little Rock, Arkansas, USA). Cell incubator: Heracell 120 (Thermo Fisher Scientific, Langenselbold, Germany). Homogenizers: Elvehjem Potter (Braun Biotech International, Melsungen, Germany) and Soniprep 150 (MSE, London, UK). Centrifuges: cooling centrifuge model Rotina 35R (Hettich, Tuttlingen, Germany) and high-speed cooling centrifuge model Sorvall RC-5C plus (Thermo Fisher Scientific, Langenselbold, Germany). Multiplates: standard 96-well multiplates (Diagonal, Muenster, Germany). Shaker: self-made device with adjustable temperature and tumbling speed (scientific workshop of the institute). Vortexer: Vortex Genie 2 (Thermo Fisher Scientific, Langenselbold, Germany). Harvester: MicroBeta FilterMate-96 Harvester. Filter: Printed Filtermat Typ A and B. Scintillator: Meltilex (Typ A or B) solid state scintillator. Scintillation analyzer: MicroBeta Trilux (all Perkin Elmer LAS, Rodgau-Jügesheim, Germany). Chemicals and reagents were purchased from different commercial sources and were of analytical grade.

Cell culture and preparation of membrane homogenates from CB_2 cells

The cell culture was modified according to ref. [49].

CHO cells stably transfected with the gene for the human CB_2 receptor were grown in Dulbecco Modified Earl's Medium (DMEM) containing 10% of standardized FCS (Biochrom AG, Berlin, Germany) and 250 μg mL^{-1} Geneticin. The cells were harvested by scraping off the surface, resuspended in PBS buffer and pelleted (10 min, 5000 × *g*) and the number of cells was determined using an improved Neubauer's counting chamber (VWR, Darmstadt, Germany). Subsequently, the cells were lysed by sonication (4°C, 6 × 10 s cycles with breaks of 10 s). The resulting cell fragments were centrifuged with a high performance cool centrifuge (20,000 × *g*, 4°C). The supernatant was discarded and the pellet resuspended in a defined volume of TRIS buffer (50 mM TRIS, 12 mM $MgCl_2$, 4 mM EDTA, pH 7.4) yielding cell fragments of approximately 2,000,000 cells mL^{-1}. The suspension of membrane homogenates was sonicated again (4°C, 2 × 10 s cycles with breaks of 10 s) and stored at −80°C.

General protocol for the binding assays

The test compound solutions were prepared by dissolving approximately 10 μmol (usually 2–4 mg) of test compound in DMSO so that a 10 mM stock solution was obtained. To obtain the required test solutions for the assay, the DMSO stock solution was diluted with the respective assay buffer. The filtermats were presoaked in 0.5% aqueous polyethylenimine solution for 2 h at room temperature before use. All binding experiments were carried out in duplicates in 96-well multiplates. The concentrations given are the final concentrations in the assay. Generally, the assays were performed by addition of 50 μL of the respective assay buffer, 50 μL test compound solution in various concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} mol L^{-1}), 50 μL of corresponding radioligand solution and 50 μL of the respective receptor preparation into each well of the multiplate (total volume 200 μL). The receptor preparation was always added last. During the incubation, the multiplates were shaken at a speed of 500–600 rpm at the specified temperature. Unless otherwise noted, the assays were terminated after 120 min by rapid filtration using the harvester. During the filtration each well was washed five times with 300 μL of water. Subsequently, the filtermats were dried at 95°C. The solid scintillator was melted on the dried filtermats at a temperature of 95°C for 5 min. After solidifying of the scintillator at room temperature, the trapped radioactivity in the filtermats was measured with the scintillation analyzer. Each position on the filtermat corresponding to one well of the multiplate was measured for 5 min with the [^{3}H]-counting protocol. The overall counting efficiency was 20%. The IC_{50} values were calculated with the program GraphPad Prism® 3.0 (GraphPad Software, San Diego, CA, USA) by non-linear regression analysis. Subsequently, the IC_{50} values were transformed into K_i values using the equation of Cheng and Prusoff [56]. The K_i values of most active compounds are given as mean value ± SEM from three independent experiments.

Protocol of the CB_2 receptor binding assay

The assay was modified according to ref. [48]:

The assay was performed with the radioligand [^{3}H]CP-55,940 (Perkin Elmer). The CB_2 receptor containing cell membrane fragments (fragments of approximately 100000 cells well^{-1}) were incubated with various concentrations of the test compound, 1 nM [^{3}H]CP-55,940 and binding buffer (50 TRIS pH 7.4, 12 mM $MgCl_2$, 4 EDTA and 2% BSA) at 37°C. The non-specific binding was determined with 10 μM non-labeled CP-55,940. The K_d value of [^{3}H]CP-55,940 is 11.8 nM (determined by a saturation experiment).

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