

## Optimization of pharmacokinetic properties by modification of a carbazole-based cannabinoid receptor subtype 2 (CB<sub>2</sub>) ligand

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### Abstract

Recently, the development of the fluorinated PET tracer [<sup>18</sup>F]**1a** for imaging of CB<sub>2</sub> receptors in the central nervous system was reported. [<sup>18</sup>F]**1a** showed high CB<sub>2</sub> affinity and selectivity over the CB<sub>1</sub> subtype, but rapid biotransformation in mice. In addition to the amide hydrolysis, oxidative *N*-dealkylation and carbazole oxidation were postulated as main metabolic pathways. Based on these results, novel carbazole derivatives with additional 6-substituents (**23a**, **24a**), modified hydrogenation state (**26a**) and enlarged fluoroalkyl substituent (**13a**, **13b**) were synthesized and pharmacologically evaluated. The key step in the synthesis of substituted carbazoles **23a**, **24a** and **26a** was a Fischer indole synthesis. Nucleophilic substitution of tosylated lactate **5** by carbazole anion

provided the fluoroisopropyl derivatives **13a** and **13b**. Partial hydrogenation of the aromatic carbazole system (**26a**) was not tolerated by the CB<sub>2</sub> receptor. A methylsulfonyl moiety in 6-position (**24a**) led to considerably reduced CB<sub>2</sub> affinity, whereas a 6-methoxy moiety (**23a**) was well tolerated. An additional methyl moiety in the fluoroethyl side chain of **1a** resulted in fluoroisopropyl derivatives **13** with unchanged high CB<sub>2</sub> affinity and CB<sub>2</sub> : CB<sub>1</sub> selectivity. Compared with the fluoroethyl derivative **1a**, the carbazole *N*-atom of the fluoroisopropyl derivative **13a** ( $K_i(\text{CB}_2) = 2.9 \text{ nM}$ ) is better shielded against the attack of CYP enzymes as formation of *N*-oxides was not observed and *N*-dealkylation took place to a less amount.

### Key words

Cannabinoid CB<sub>2</sub> receptor ligands; carbazole; fluoroisopropyl side chain; Fischer indole synthesis; structure affinity relationships, selectivity; metabolic stability; identification of metabolites, PET

## 1. Introduction

The first medical use of *Cannabis sativa* L. for the treatment of rheumatic pain, constipation and malaria, as well as the toxic effects, were reported in one of the oldest pharmacopeia, the *pen-ts'ao ching* [1]. It is assumed that this book was written in the first century A.D. based on oral traditions from the years around 2700 B.C. [2]. However, cannabis did not find a broad medical application in the western world until the midst 19<sup>th</sup> century, as reflected by the first medical conference on Cannabis in 1860, organized by the Ohio State Medical Society. The preliminary peak of the medical use was reached at the end of the 19<sup>th</sup> century, however, in the first decades

of the 20th century, the use decreased again due to political reasons and the development of new synthetic drugs such as acetylsalicylic acid and barbiturates [2]. In 1964, the isolation and characterization of  $\Delta^9$ -tetrahydrocannabinol (THC) and cannabidiol (CBD) stimulated the discovery of the endogenous cannabinoid (endocannabinoid) system in the following decades. After cloning of the cannabinoid receptor subtypes CB<sub>1</sub> [3] and CB<sub>2</sub> [4] in the early 1990s, it was possible to develop selective compounds for CB<sub>1</sub> and CB<sub>2</sub> receptors, respectively. Both CB receptor subtypes belong to the class of G<sub>i/o</sub> protein-coupled receptors (GPCR) and show an amino acid sequence homology of 44 % [5], but differ primarily in their expression pattern. The CB<sub>1</sub> receptor is one of the most common GPCR of the central nervous system (CNS) and is present in all four brain regions (cerebrum, diencephalon, cerebellum, brainstem) [6]. In these regions the CB<sub>1</sub> receptor plays an important modulating role for the release of other excitatory and inhibitory neurotransmitters (e.g. GABA) [7], especially in the synapses of neurons. The psychoactive side effects of THC, such as dysphoria and concentration disorders, are also attributed to activation of the CB<sub>1</sub> receptor.

In contrast, the expression of the CB<sub>2</sub> receptor in the CNS under normal conditions is rather low. However, the expression of the CB<sub>2</sub> receptor can be increased up to 100-fold under inflammatory conditions (neuroinflammation) [8]. In many test systems, it could be shown that activation of the CB<sub>2</sub> receptor reduces the release of numerous inflammatory mediators, such as IL-1, IL-6 and tumor necrosis factor  $\alpha$  (TNF  $\alpha$ ), as well as increases the release of anti-inflammatory factors (e.g. IL-10), thus resulting in an overall anti-inflammatory effect [9]. Therefore, the CB<sub>2</sub> receptor is regarded as a potential target for drugs directed for the treatment of many neuroimmunological and neurodegenerative diseases including depression, schizophrenia, Alzheimer's

disease, multiple sclerosis, amyotrophic lateral sclerosis, down syndrome, and Huntington's disease [9],[10],[11],[12],[13].

The positron emission tomography (PET) is an imaging modality that enables visualization and quantification of receptors under healthy and pathological conditions. The site of bound radiochemically labeled compound is visualized *in vivo* by the simultaneous detection of two gamma photons. The preferably used radioisotope is  $^{18}\text{F}$ , which has a half-life of 110 min compared to  $^{11}\text{C}$  with a half-life of 20 min [14].

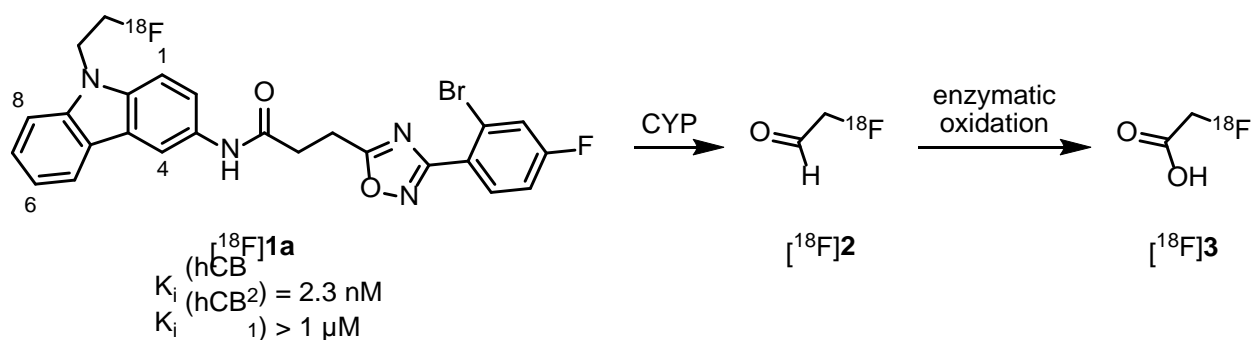
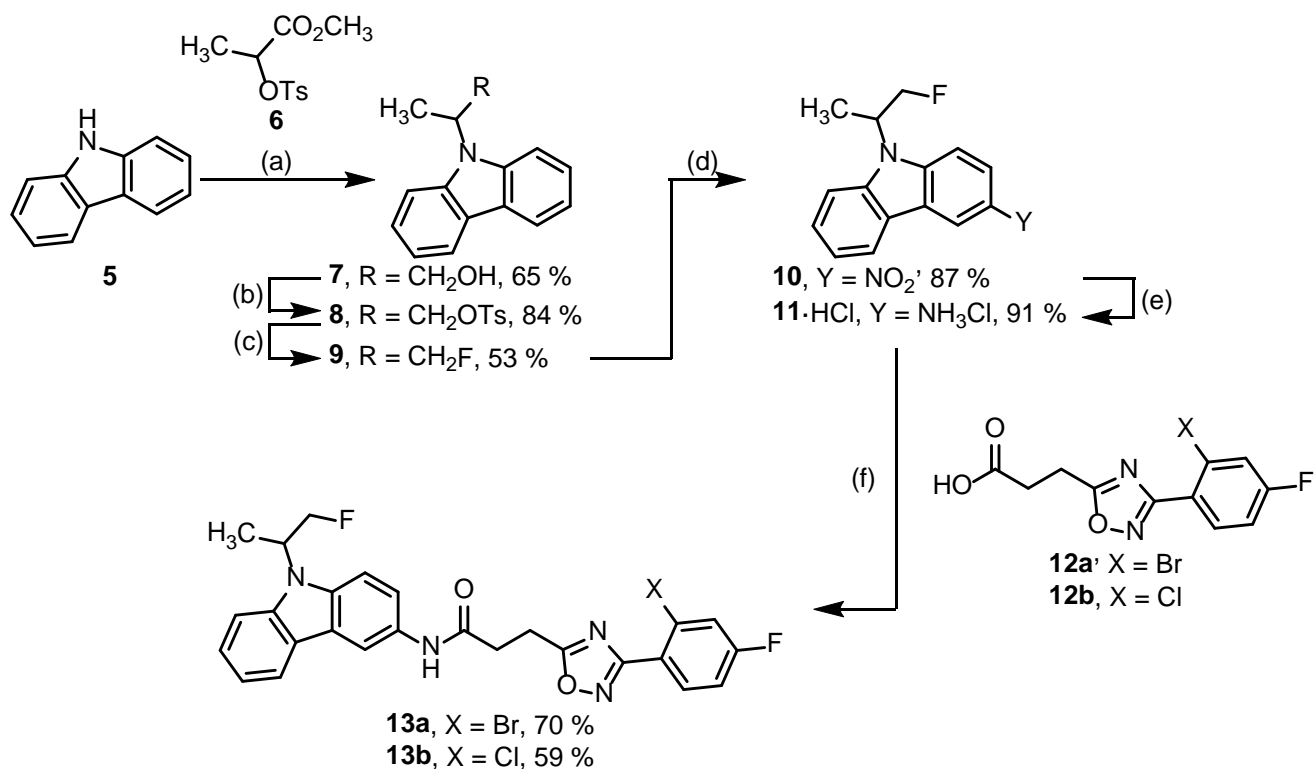


Figure 1. Postulated biotransformation of  $[^{18}\text{F}]\mathbf{1a}$ .

In 2013, we described the synthesis, radiosynthesis and biological evaluation of the  $\text{CB}_2$  receptor PET tracer  $[^{18}\text{F}]\mathbf{1a}$  containing a (phenyl-oxadiazolyl)propionamide scaffold [15],[16]. The ligand showed a high  $\text{CB}_2$  affinity ( $K_i(\text{hCB}_2) = 2.3 \text{ nM}$ ), excellent selectivity over the  $\text{CB}_1$  receptor (ca. 500-fold), penetration into the brain and low tendency to lose  $[^{18}\text{F}]\text{fluoride}$  *in vivo*. On the other hand,  $\mathbf{1a}$  showed some disadvantages like a rapid metabolism and relatively high lipophilicity ( $\log D_{7.4} = 3.82 - 4.21$ , recorded by HPLC). During *in vivo* experiments, a very polar radiometabolite was detected by radio-HPLC. It was postulated that  $[^{18}\text{F}]\text{fluoroacetic acid}$   $[^{18}\text{F}]\mathbf{3}$  was formed by oxidative cleavage of the  $[^{18}\text{F}]\text{fluoroethyl}$  moiety at the carbazole system (Figure 1). In this work, we aim to synthesize fluorinated  $\text{CB}_2$  receptor ligands with a sterically more demanding fluoroisopropyl residue in 9-position of the carbazole system to inhibit oxidative degradation by CYP enzymes. Moreover, different substituents in 6-position

of the carbazole scaffold of **1a** should be introduced to inhibit metabolic oxidation at 6-position, reduce the lipophilicity and modulate the electron density in the aromatic system.

## 2. Synthesis

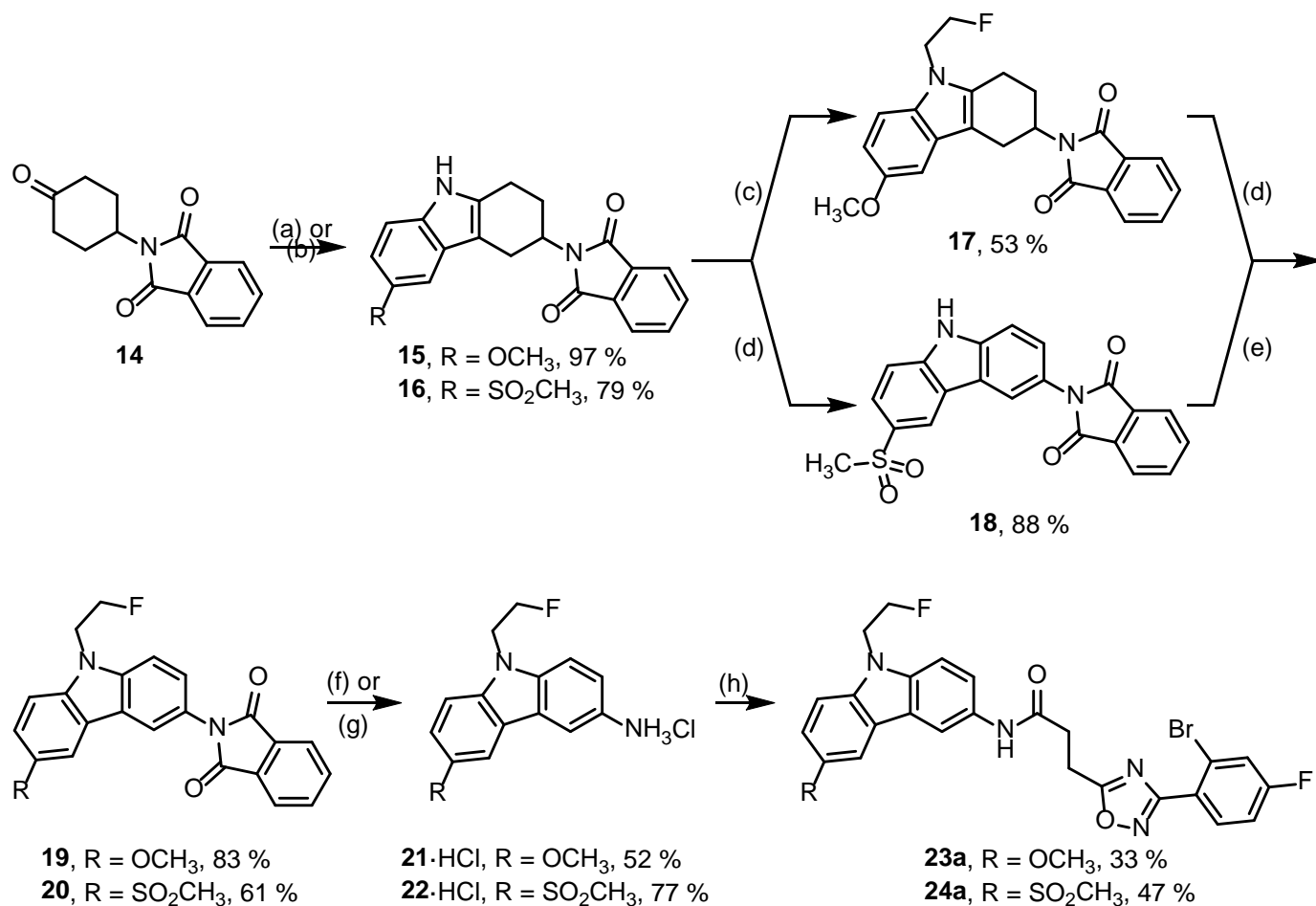


Scheme 1. Reagents and reaction conditions: (a) 1. NaH, DMF, rt → 110 °C; 2. LiAlH<sub>4</sub>, THF, rt → reflux. (b) tosyl chloride, NEt<sub>3</sub>, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, rt. (c) TBAF·3H<sub>2</sub>O, 60 °C. (d) HNO<sub>3</sub> 65 %, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C. (e) 1. H<sub>2</sub>, Pd/C 10 %, THF, 1 bar, rt; 2. HCl in Et<sub>2</sub>O. (f) COMU<sup>®</sup>, EtN/Pr<sub>2</sub>, THF, rt.

For the synthesis of CB<sub>2</sub> ligands **13** with fluoroisopropyl side chain racemic, methyl lactate **4** was tosylated [17] and the product **6** was reacted with deprotonated carbazole **5**. Subsequent reduction of the ester with LiAlH<sub>4</sub> afforded alcohol **7** in 65 % yield. Treatment of **7** with tosyl chloride under basic conditions yielded tosylate **8**, which was reacted with TBAF·3H<sub>2</sub>O in a nucleophilic substitution under solvent-free conditions. Fluoroisopropylcarbazole **9** was nitrated with nitric acid 65 % at 0 °C. Hydrogenation

of **10** catalyzed by Pd/C provided the primary aromatic amine **11**, which was precipitated as HCl salt **11**·HCl. In the last step, carbazolumine·HCl **11**·HCl was acylated with carboxylic acids **12a** and **12b** in the presence of COMU<sup>®</sup> to yield amides **13a** and **13b** (Scheme 1). The 1,2,4-oxadiazol building blocks **11** and **12** were obtained by NH<sub>2</sub>OH addition to 2-bromo- and 2-chloro-4-fluorobenzonitrile followed by acylation with succinic anhydride [15], [18], [19].

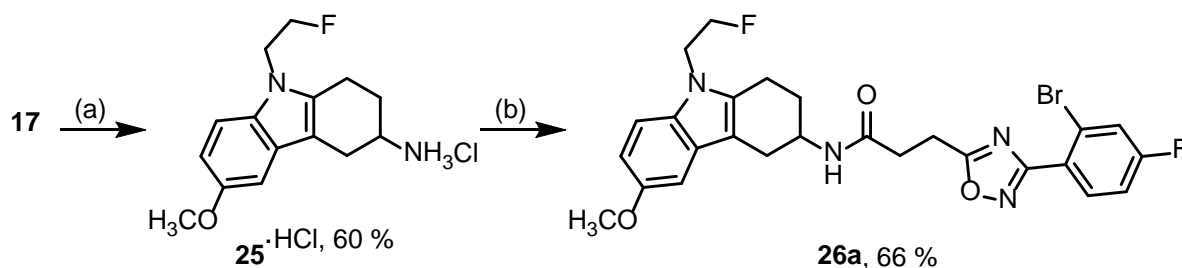
The Craig plot [20] was used for the selection of suitable substituents in 6-position. A methoxy group was selected as an electron-donating substituent and a methylsulfonyl moiety as a polar electron-withdrawing substituent. Moreover, the two substituents are sterically more demanding and possess additional H-bond acceptors.



Scheme 2. Reagents and reaction conditions: (a) 4-methoxyphenylhydrazine hydrochloride, EtOH, reflux. (b) 4-(methylsulfonyl)phenylhydrazine, AcOH, reflux. (c) NaH, DMF, TsOCH<sub>2</sub>CH<sub>2</sub>F, 0 °C → 95 °C. (d) DDQ, THF, reflux. (e) Cs<sub>2</sub>CO<sub>3</sub>, DMF, TsOCH<sub>2</sub>CH<sub>2</sub>F, 0 °C → rt. (f) 1. **19**, H<sub>2</sub>N-NH<sub>2</sub>·H<sub>2</sub>O, EtOH, reflux; 2. HCl in Et<sub>2</sub>O. (g) 1. **20**, H<sub>2</sub>N-NH<sub>2</sub>·H<sub>2</sub>O, EtOH, CH<sub>2</sub>Cl<sub>2</sub>, 40 °C; 2. HCl in Et<sub>2</sub>O. (h) **12a**, COMU<sup>®</sup>, EtN*i*Pr<sub>2</sub>, THF, rt.

To obtain carbazoles with a substituent in 6-position, 4-methoxy- and 4-(methylsulfonyl)phenylhydrazine were reacted with cyclohexanone derivative **14** in a Fischer indole synthesis [21]. The required 4-methylsulfonylphenylhydrazine was synthesized by nucleophilic aromatic substitution of 4-chlorophenyl methyl sulfone with hydrazine according to the literature [22]. Tetrahydrocarbazole **15** was alkylated with fluoroethyl tosylate and DDQ was used for the oxidation to afford the carbazole **19**. For the synthesis of carbazole **20** the aromatization was carried out first and afterwards

the fluoroethyl moiety was introduced using  $\text{Cs}_2\text{CO}_3$  instead of  $\text{NaH}$ . Hydrazinolysis of the phthalimides **19** and **20** led to the primary amines **21** and **22**, which were acylated with propionic acid **12a** to obtain amides **23a** and **24a** (Scheme 2).



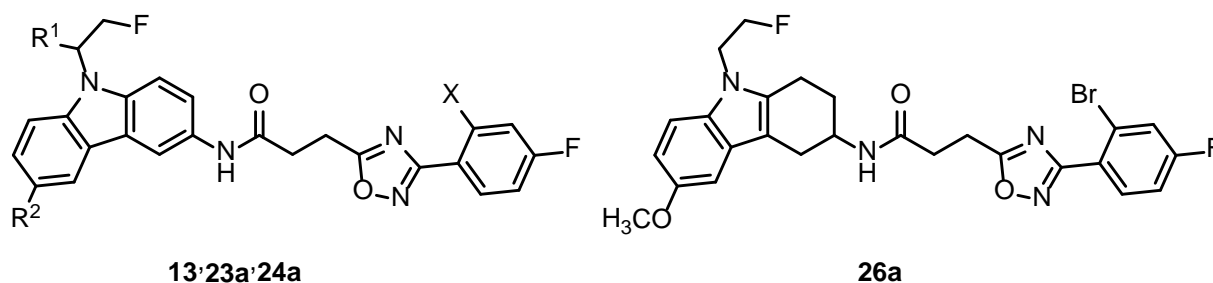
Scheme 3. Reagents and reaction conditions: (a) 1.  $\text{H}_2\text{N-NH}_2\cdot\text{H}_2\text{O}$ , EtOH, reflux; 2. HCl in  $\text{Et}_2\text{O}$ . (b) **12a**, COMU<sup>®</sup>,  $\text{EtN/Pr}_2$ , THF, rt.

Furthermore, the tetrahydrocarbazole **26a** was synthesized to determine the effects of the less planar scaffold on  $\text{CB}_2$  affinity. Therefore, tetrahydrocarbazole **17** was subjected to hydrazinolysis and **25·HCl** was coupled with propionic acid **12a** to yield amide **26a** (Scheme 3).



### 3. Receptor affinity

Table 1. CB<sub>1</sub> and CB<sub>2</sub> receptor affinity of fluorosopropyl derivatives **13**, compounds **23** and **24** with a substituent in 6-position and tetrahydrocarbazole derivative **26a**.



compd	R <sup>1</sup>	R <sup>2</sup>	X	K <sub>i</sub> (hCB <sub>2</sub> ) ± SEM [nM] <sup>a)</sup>	displacement (hCB <sub>1</sub> ) <sup>b)</sup>
<b>1a</b>	H	H	Br	2.9 ± 0.4	22 % <sup>c)</sup>
<b>13a</b>	CH <sub>3</sub>	H	Br	2.9 ± 0.4	12 %
<b>13b</b>	CH <sub>3</sub>	H	Cl	1.5 ± 0.1	10 %
<b>23a</b>	H	OCH <sub>3</sub>	Br	56 ± 7	3 %
<b>24a</b>	H	SO <sub>2</sub> CH <sub>3</sub>	Br	1137 ± 124	8 %
<b>26a</b>				1 % <sup>b)</sup>	10 %
CP 55,940				8.4 ± 0.2	9.3 ± 0.1
WIN 55,212-2				8.6 ± 0.2	8.7 ± 0.2
HU 210				9.8 ± 0.04	9.6 ± 0.1

a) The reported K<sub>i</sub>-values are mean values of three independent experiments (n = 3).

b) Due to the low hCB<sub>1</sub> affinity, only the radioligand displacement at a test compound concentration of 1 μM is given. Mean value of two independent experiments (n = 2).

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

The fluoroisopropyl derivatives **13a** and **13b** show similar high CB<sub>2</sub> affinity as the fluoroethyl derivative **1a**. Also, the affinity of both compounds to the CB<sub>1</sub> receptor is very low indicating high selectivity over the CB<sub>1</sub> receptor.

Compared to a proton, the 6-methoxy group of **23a** has a similar polarity, but is sterically more demanding, possesses an additional H-bond acceptor and increases the electron density of the carbazole system. With a  $K_i$  value of 56 nM, the CB<sub>2</sub> affinity of **23a** is by 19-fold decreased compared to the lead compound **1a**. In contrast, the methylsulfonyl group is a very polar substituent, possesses two additional H-bond acceptors, decreases the electron density in the carbazole system and is sterically much more demanding than a proton. Compared to the lead compound **1a**, the CB<sub>2</sub> affinity of sulfone **24a** is 400-fold decreased.

Due to the sp<sup>3</sup>-hybridized C-atoms in 1- to 4-position, tetrahydrocarbazole **26a** is no longer planar, which is not tolerated by the CB<sub>2</sub> receptor. At a test compound concentration of 1 μM, the tetrahydrocarbazole **26a** could only displace 1 % of the radioligand. Obviously, the  $K_i$  value is greater than 1 μM.

#### 4. Metabolism studies of **13a**

*In vivo* studies of [<sup>18</sup>F]**1a** in mice revealed low metabolic stability as only 35 % of intact radiotracer [<sup>18</sup>F]**1a** were detected 60 min after injection [15]. Furthermore, the *in vitro* stability over time was determined by incubation with mouse liver microsomes and metabolite structures were identified in our lab using LC-MS-MS [23]. After an incubation time of 90 min, 69.8 ± 0.5 % (SEM) of **1a** remained intact. The hydrolysis of the amide bond was identified as major clearance pathway, which was also reported and described in *in vivo* experiments with rats and mice [15], [19]. In addition, the oxidative *N*-dealkylation of the carbazole-*N*-atom **1a** was postulated, resulting in a loss of the F-atom of the potential positron emitter. Therefore, the effect of an additional methyl moiety in α-position to the tertiary amine (**13a**) on the metabolism was investigated.

Fluoroisopropylcarbazole **13a** was incubated with mouse liver microsomes or mouse plasma. The resulting samples were analyzed using LC-qToF-MS, which allowed identification of metabolites through exact masses and fragmentation experiments.

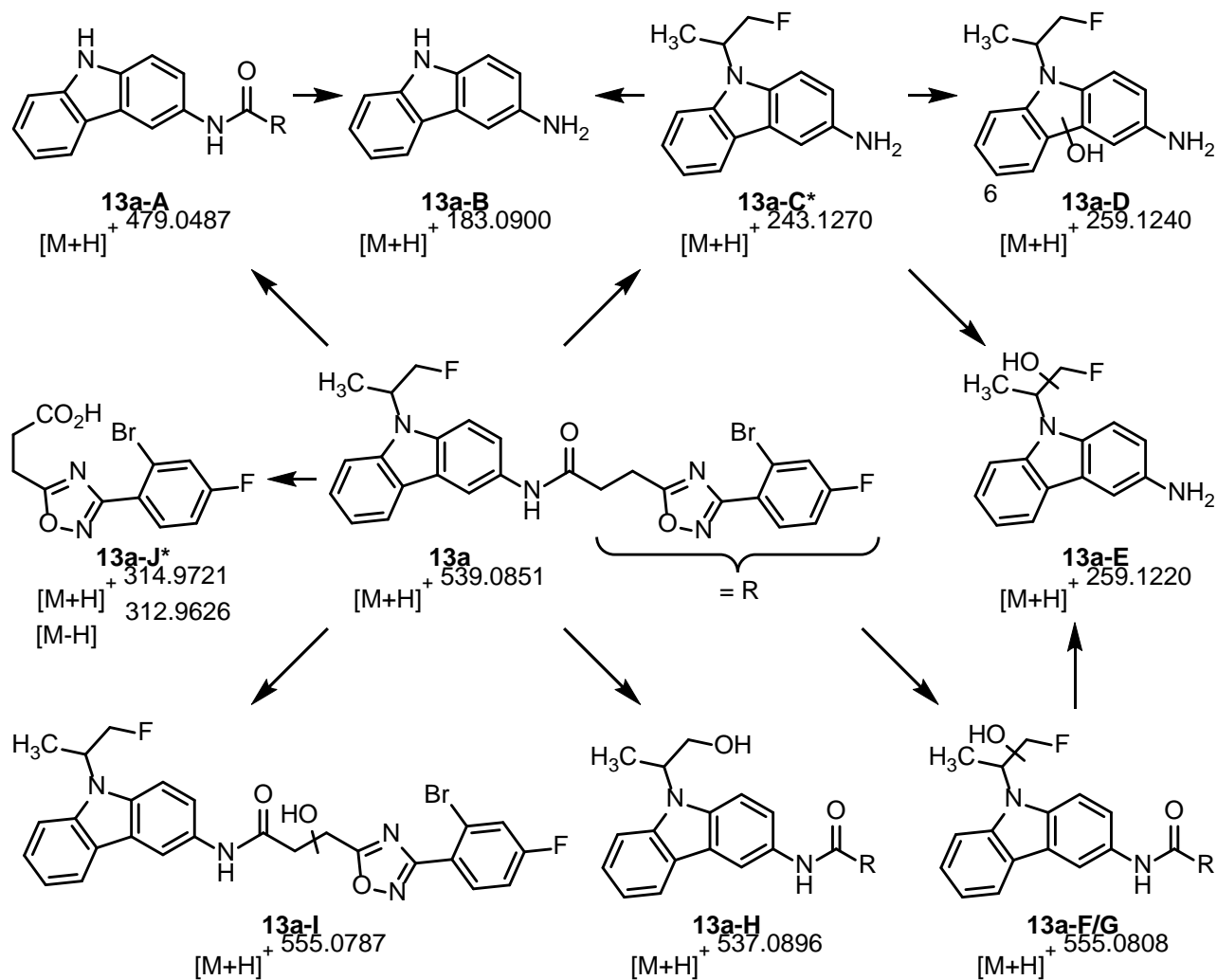


Figure 2. Proposed structures of metabolites identified 90 min after incubation of **13a** with mouse liver microsomes and NADPH. \* The marked metabolites were also formed without NADPH and in murine blood serum.

In total, ten metabolites of compound **13a** were detected (Figure 2). Oxidative N-dealkylation led to metabolite **13a-A**, and following amide-hydrolysis to primary aromatic amine **13a-B**. This metabolite could also be formed by hydrolysis of the parent compound **13a** and subsequent N-dealkylation of **13a-C**. Two additional primary aromatic amines **13a-D** and **13a-E** resulted from hydroxylation of **13a-C**. The exact

position of the OH moiety at the carbazole ring of metabolite **13a-D** is unknown. However, the 6-position is most likely bearing the hydroxy moiety. Moreover, the two metabolites **13a-F** and **13a-G**, hydroxylated in the fluoroisopropyl side chain, could be detected ( $t_R = 11.82$  min and 12.14 min), which possess almost the same fragmentation pattern (see supporting information, chapter 2). Therefore, it is assumed, that the hydroxy groups are attached to the terminal alkyl moieties of the isopropyl side chain, since a hydroxy moiety at the C-atom in the middle would lead to an unstable hemiaminal. Metabolite **13a-H** was obtained by defluorination. Although this metabolite was formed in minor amounts, the F-atom of the potential positron emitter is lost. In contrast to the identified metabolites of **1a** [23], formation of a carbazole *N*-oxide was not observed. It is assumed that the sterically demanding fluoroisopropyl moiety is shielding the carbazole N-atom from oxidative attack by CYP enzymes. The fragmentation pattern of metabolite **13a-I** bearing the OH moiety at the trimethylene spacer connecting the carbazole and 1,2,4-oxadiazole rings is given in Figure 3.

Since fragmentation of metabolite **13a-I** led to a carbazole fragment ( $m/z$  243.1305) without OH-moiety, which is formed by cleavage of the amide bond, hydroxylation of the carbazole and fluoroisopropyl substructures was excluded. (Figure 3) Fragment  $m/z$  285.9750 proved hydroxylation of the phenyloxadiazolylpropyl part of the molecule. Furthermore, fragment  $m/z$  214.9611 with a diazirine ring excluded hydroxylation of the phenyl ring, and thus confirmed the position of the OH group in the trimethylene linker. Diazirine derivatives, resulting from 1,2,4-oxadiazole fragmentation under electron impact ionization conditions, have already been described by Pihlaja *et al.* [24].

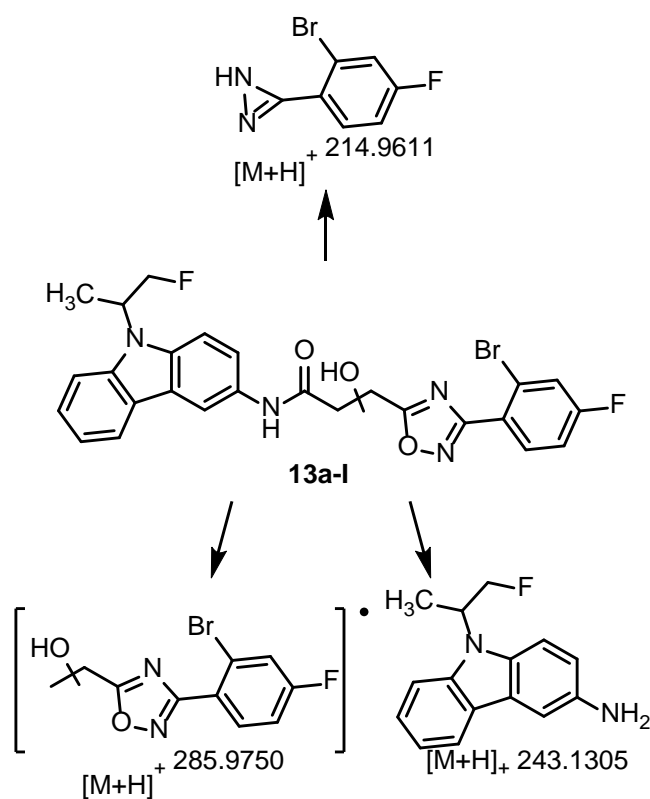


Figure 3. Fragmentation of metabolite **13a-I**.

In order to show the effect of the additional methyl moiety of the fluoroisopropyl derivative **13a** on oxidative *N*-dealkylation the fluoroethyl and fluoroisopropyl derivatives **1a** and **13a** were incubated under the same conditions with mouse liver microsome preparations and NADPH. The extracted ion chromatograms (EICs) of the resulting *N*-unsubstituted carbazole **13a-A** (= **1a-A**) are compared in Figure 4. According to the EICs, the additional methyl moiety reduced oxidative *N*-dealkylation of **13a** approximately by two-thirds after 90 min in comparison with the oxidative *N*-dealkylation of the fluoroethyl moiety.

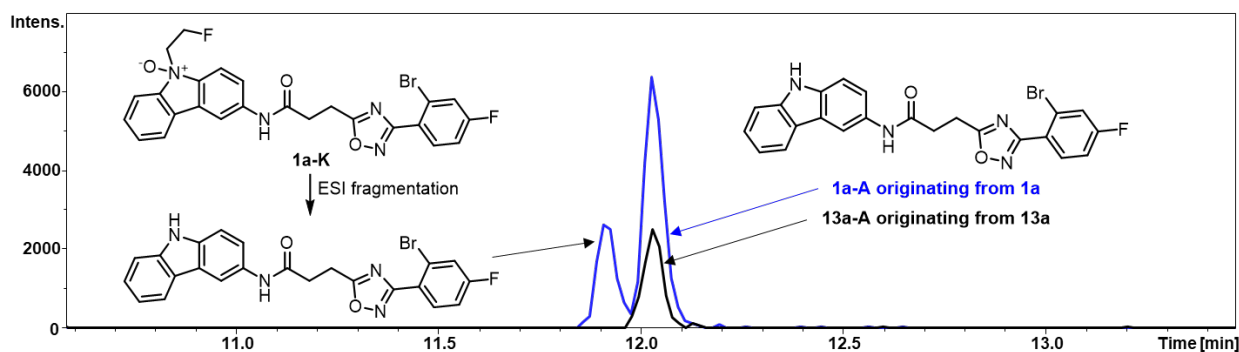


Figure 4. EICs  $m/z$  479.0513 for the incubations of **1a** and **13a** with mouse liver microsomes and NADPH. The left blue peak resulted from the in-source fragmentation of metabolite **1a-K** [23].

## 5. Conclusion

In order to inhibit *N*-dealkylation and oxidation of the *N*-substituted carbazole moiety of the potent CB<sub>2</sub> agonist **1a** ( $K_i$  (hCB<sub>2</sub>) = 2.9 nM) novel ligands with a modified carbazole substitution pattern were synthesized. The key step of the synthesis of CB<sub>2</sub> ligands with modified substituents in 6-position or with a partly hydrogenated carbazole scaffold was a Fischer indole synthesis with *N*-protected 4-aminocyclohexanone **14** and substituted phenylhydrazines. Whereas a methylsulfonyl moiety in 6-position of the carbazole system (**24a**) was not tolerated by the CB<sub>2</sub> receptor, the methoxy derivative **23a** showed considerable CB<sub>2</sub> affinity ( $K_i$  = 56 nM). Partial hydrogenation of the carbazole to form a tetrahydrocarbazole system (**26a**) led to complete loss of CB<sub>2</sub> affinity indicating that a planar aromatic ring system is essential to achieve strong interactions with the CB<sub>2</sub> receptor. Introduction of an additional methyl moiety into the fluoroethyl side chain of **1a** resulted in the fluoroisopropyl derivatives **13a** ( $K_i$  (hCB<sub>2</sub>) = 2.9 nM) and **13b** ( $K_i$  (hCB<sub>2</sub>) = 1.5 nM) with almost the same CB<sub>2</sub> affinity and selectivity over the CB<sub>1</sub> subtype. Investigation of the *in vitro* metabolism of the fluoroisopropyl derivative **13a** with murine microsomes and subsequent LC-MS-MS analysis revealed ten metabolites in small amounts. In comparison to **1a** [23], formation

of an *N*-oxide was not observed and the extent of oxidative *N*-dealkylation was reduced to one third. It can be concluded that the additional methyl moiety of the fluoroisopropyl derivatives **13** does not reduce CB<sub>2</sub> affinity, but is able to shield the carbazole *N*-atom from oxidative attack by microsomal CYP enzymes. Thus, the fluoroisopropyl moiety represents a promising substituent for the development of carbazole-based PET tracers for the selective imaging of CB<sub>2</sub> receptors in the CNS.

## 6. Experimental

### 6.1 Chemistry, General Methods

Oxygen and moisture sensitive reactions were carried out under nitrogen, dried with silica gel with moisture indicator (orange gel, Merck) and in dry glassware (Schlenk flask or Schlenk tube). Temperatures were controlled with dry ice/acetone (-78 °C), ice/water (0 °C), Cryostat (Julabo FT 901 or Huber TC100E-F), magnetic stirrer MR 3001 K (Heidolph) or RCT CL (IKA®), together with temperature controller EKT HeiCon (Heidolph) or VT-5 (VWR) and PEG or silicone bath. All solvents were of analytical grade quality. Demineralized water was used. THF was distilled from sodium/benzophenone. Methanol was distilled from magnesium methanolate. CH<sub>3</sub>CN and ethanol abs. were dried with molecular sieves (3 Å); DMF, ethyl acetate and toluene were dried with molecular sieves (4 Å). Thin layer chromatography (tlc): tlc silica gel 60 F<sub>254</sub> on aluminum sheets (Merck). Flash chromatography (fc): Silica gel 60, 40–63 μm (Merck); parentheses include: diameter of the column (∅), length of the stationary phase (l), fraction size (v) and eluent. Melting point: Melting point system MP50 (Mettler Toledo), open capillary, uncorrected. MS: MicroTOFQII mass spectrometer (Bruker Daltonics); deviations of the found exact masses from the calculated exact masses were 5 ppm or less; the data were analyzed with DataAnalysis (Bruker). NMR: NMR spectra were recorded on Agilent DD2 400 MHz

and 600 MHz spectrometers; chemical shifts ( $\delta$ ) are reported in parts per million (ppm) against the reference substance tetramethylsilane and calculated using the solvent residual peak of the undeuterated solvent. IR: FT/IR IRAffinity-1 IR spectrometer (Shimadzu) using ATR technique.

## 6.2 HPLC method for the determination of the purity

Equipment 1: Pump: L-7100, degasser: L-7614, autosampler: L-7200, UV detector: L-7400, interface: D-7000, data transfer: D-line, data acquisition: HSM-Software (all from LaChrom, Merck Hitachi); Equipment 2: Pump: LPG-3400SD, degasser: DG-1210, autosampler: ACC-3000T, UV-detector: VWD-3400RS, interface: DIONEX UltiMate 3000, data acquisition: Chromeleon 7 (Thermo Fisher Scientific); column: LiChropher<sup>®</sup> 60 RP-select B (5  $\mu$ m), LiChroCART<sup>®</sup> 250-4 mm cartridge; flow rate: 1.0 mL/min; injection volume: 5.0  $\mu$ L; detection at  $\lambda = 210$  nm; solvents: A: demineralized 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 - 31.5min: gradient from 0 % to 90 %; 31.5 - 40 min: 90 %.

## 6.3 Synthetic procedures

### 6.3.1 2-(Carbazol-9-yl)propan-1-ol (**7**) [25]

Preparation of this compound is described in the literature [25] following a different synthesis route.

Under N<sub>2</sub> atmosphere, carbazole (**5**, 1.79 g, 11 mmol, 1 eq.) was dissolved in dry DMF (54 mL). NaH (60 % dispersion in Paraffin Oil, 0.99 g, 25 mmol, 2.3 eq.) was added and the mixture was stirred for 30 min. After the dropwise addition of tosylate **6** (6.38 g, 25 mmol, 2.3 eq.), the reaction mixture was stirred at 110 °C for 43 h. Water (10 mL) and a saturated Na<sub>2</sub>CO<sub>3</sub> solution (40 mL) were added and the mixture was extracted



with ethyl acetate (200 mL). The organic layer was washed with water (2 x 50 mL) and brine (50 mL), dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure.  $\text{LiAlH}_4$  (1.63 g, 42 mmol, 4 eq.) was suspended in dry THF (130 mL) and the ester dissolved in THF (20 mL) was added over 15 min. The mixture was heated at reflux for 16 h before it was quenched with a NaOH solution (1M, 10 mL) and water (40 mL). Insoluble impurities were removed by filtration and washed with ethyl acetate (200 mL). The organic layer was separated from the aqueous layer and washed with brine (50 mL). Afterwards, the ethyl acetate layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure. The residue was purified by fc with a gradient ( $\varnothing = 5$  cm,  $l = 18$  cm,  $v = 60$  mL, cyclohexane/ethyl acetate 85:15, 70:30,  $R_f = 0.35$  (cyclohexane/ethyl acetate 7:3)). Beige solid, mp 117 °C, yield 1.56 g (65 %). Purity (HPLC): 98.6 % ( $t_R = 20.4$  min).  $\text{C}_{15}\text{H}_{15}\text{NO}$  (225.3 g/mol). Exact mass (APCI):  $m/z = 226.1224$  (calcd. 226.1226 for  $\text{C}_{15}\text{H}_{16}\text{NO} [\text{M}+\text{H}^+]$ ).  $^1\text{H}$  NMR (600 MHz,  $\text{DMSO-D}_6$ ):  $\delta$  (ppm) = 1.59 (d,  $J = 7.2$  Hz, 3H,  $\text{CH}_3$ ), 3.85 - 3.90 (m, 1H,  $\text{CH}_2\text{OH}$ ), 4.00 - 4.06 (m, 1H,  $\text{CH}_2\text{OH}$ ), 4.88 - 4.95 (m, 2H, NCH,  $\text{CH}_2\text{OH}$ ), 7.17 (t,  $J = 7.4$  Hz, 2H, 3-H, 6-H), 7.40 (t,  $J = 7.7$  Hz, 2H, 2-H, 7-H), 7.65 (d,  $J = 8.3$  Hz, 2H, 1-H, 8-H), 8.14 (d,  $J = 7.7$  Hz, 2H, 4-H, 5-H).  $^{13}\text{C}$  NMR (151 MHz,  $\text{DMSO-D}_6$ ):  $\delta$  (ppm) = 15.8 (1C,  $\text{CH}_3$ ), 53.3 (1C, NCH), 63.3 (1C,  $\text{CH}_2\text{OH}$ ), 111.1 (2C, C-1, C-8), 118.8 (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), 140.1 (2C, C-8a, C-9a). FTIR (neat):  $\tilde{\nu}$  ( $\text{cm}^{-1}$ ) = 3325 (m, O-H), 3062 (w, C-H, arom), 2877 (w, C-H, aliph), 1593 (s, C-C, arom).

### 6.3.2 2-(Carbazol-9-yl)propyl 4-methylbenzenesulfonate (**8**)

Under  $\text{N}_2$  atmosphere, tosyl chloride (2.55 g, 13 mmol, 2 eq.) was dissolved in dry  $\text{CH}_2\text{Cl}_2$  (30 mL), dry pyridine (10 mL) and triethylamine (1.4 mL, 10 mmol, 1.5 eq.). Carbazole derivative **7** (1.50 g, 6.7 mmol, 1 eq.) dissolved in dry  $\text{CH}_2\text{Cl}_2$  (10 mL) was

added dropwise over 10 min. The reaction mixture was stirred at room temperature for 17 h followed by evaporation to dryness in vacuo. The residue was dissolved in HCl (1 M, 20 mL) and the solvent was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 20 mL). The combined organic layers were washed with a saturated Na<sub>2</sub>CO<sub>3</sub> solution (20 mL), which was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 20 mL). After drying (Na<sub>2</sub>SO<sub>4</sub>), the combined CH<sub>2</sub>Cl<sub>2</sub> layers were concentrated under reduced pressure. The residue was purified by fc ( $\phi$  = 4 cm, l = 18 cm, v = 30 mL, cyclohexane/ethyl acetate 90:10, R<sub>f</sub> = 0.39 (cyclohexane/ethyl acetate 8:2)). Colorless solid, mp 93 °C, yield 2.14 g (84 %). Purity (HPLC): 99.7 % (t<sub>R</sub> = 24.5 min). C<sub>22</sub>H<sub>21</sub>NO<sub>3</sub>S (379.5 g/mol). Exact mass (APCI): m/z = 380.1310 (calcd. 380.1315 for C<sub>22</sub>H<sub>22</sub>NO<sub>3</sub>S [M+H<sup>+</sup>]). <sup>1</sup>H NMR (400 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 1.57 (d, J = 7.2 Hz, 3H, CHCH<sub>3</sub>), 2.29 (s, 3H, Ar-CH<sub>3</sub>), 4.45 (dd, J = 10.7/4.1 Hz, 1H, CH<sub>2</sub>), 4.70 (dd, J = 10.7/9.6 Hz, 1H, CH<sub>2</sub>), 5.18 - 5.25 (m, 1H, NCH), 7.03 (d, J = 8.4 Hz, 2H, 3-H<sub>phenyl</sub>, 5-H<sub>phenyl</sub>), 7.18 (t, J = 7.5 Hz, 2H, 3-H<sub>carb</sub>, 6-H<sub>carb</sub>), 7.21 (d, J = 8.3 Hz, 2H, 2-H<sub>phenyl</sub>, 6-H<sub>phenyl</sub>), 7.34 (t, J = 7.6 Hz, 2H, 2-H<sub>carb</sub>, 7-H<sub>carb</sub>), 7.51 (d, J = 8.3 Hz, 2H, 1-H<sub>carb</sub>, 8-H<sub>carb</sub>), 8.11 (d, J = 7.7 Hz, 2H, 4-H<sub>carb</sub>, 5-H<sub>carb</sub>). <sup>13</sup>C NMR (101 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 14.7 (1C, CHCH<sub>3</sub>), 21.1 (1C, Ar-CH<sub>3</sub>), 49.2 (1C, NCH), 70.4 (1C, CH<sub>2</sub>), 110.2 (2C, C-1<sub>carb</sub>, C-8<sub>carb</sub>), 118.8 (2C, C-3<sub>carb</sub>, C-6<sub>carb</sub>), 120.1 (2C, C-4<sub>carb</sub>, C-5<sub>carb</sub>), 122.6 (2C, C-4<sub>a</sub>carb, C-4<sub>b</sub>carb), 125.5 (2C, C-2<sub>carb</sub>, C-7<sub>carb</sub>), 126.8 (2C, C-2<sub>phenyl</sub>, C-6<sub>phenyl</sub>), 129.6 (2C, C-3<sub>phenyl</sub>, C-5<sub>phenyl</sub>), 131.3 (1C, C-1<sub>phenyl</sub>), 139.0 (2C, C-8<sub>a</sub>carb, C-9<sub>a</sub>carb), 144.5 (1C, C-4<sub>phenyl</sub>). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2943 (w, C-H, aliph), 1597 (w, C-C, arom), 1354 (s, SO<sub>3</sub>), 1165 (s, SO<sub>3</sub>).

### 6.3.3 9-(1-Fluoropropan-2-yl)carbazole (**9**)

TBAF·3H<sub>2</sub>O (2.71 g, 8.6 mmol, 2.2 eq.) was added to tosylate **8** (1.48 g, 3.9 mmol, 1 eq.) and the mixture was stirred at 60 °C for 23 h. After addition of ethyl acetate (80 mL), the organic layer was washed with water (2 x 40 mL) and brine (40 mL), dried

(Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. The residue was purified by fc ( $\emptyset = 4$  cm,  $l = 16$  cm,  $v = 30$  mL, cyclohexane/ethyl acetate 99:1,  $R_f = 0.65$  (cyclohexane/ethyl acetate 8:2)). Colorless solid, mp 82 - 83 °C, yield 0.472 g (53 %). Purity (HPLC): 96.4 % ( $t_R = 23.0$  min). C<sub>15</sub>H<sub>14</sub>FN (227.3 g/mol). Exact mass (APCI):  $m/z = 228.1185$  (calcd. 228.1183 for C<sub>15</sub>H<sub>15</sub>FN [M+H<sup>+</sup>]). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 1.78 (dd,  $J = 7.2/1.5$  Hz, 3H, CH<sub>3</sub>), 4.84 (ddd,  $J = 33.2/9.0/5.8$  Hz, 1H, CH<sub>2</sub>F), 4.96 (ddd,  $J = 33.7/9.0/5.8$  Hz, 1H, CH<sub>2</sub>F), 5.05 - 5.19 (m, 1H, NCH), 7.25 (ddd,  $J = 7.9/6.9/1.2$  Hz, 2H, 3-H, 6-H), 7.45 (ddd,  $J = 8.3/6.9/1.2$  Hz, 2H, 2-H, 7-H), 7.49 (dt,  $J = 8.2/1.0$  Hz, 2H, 1-H, 8-H), 8.11 (dt,  $J = 7.8/0.9$  Hz, 2H, 4-H, 5-H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 14.9 (d,  $J = 5.0$  Hz, CH<sub>3</sub>), 50.9 (d,  $J = 21.8$  Hz, 1C, NCH), 84.3 (d,  $J = 175.0$  Hz, 1C, CH<sub>2</sub>F), 109.9 (2C, C-1, C-8), 119.3 (2C, C-3, C-6), 120.5 (2C, C-4, C-5), 123.7 (2C, C-4a, C-4b), 125.8 (2C, C-2, C-7), 139.8 (2C, C-8a, C-9a). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3059 (w, C-H, arom), 2951 (w, C-H, aliph), 1593 (m, C-C, arom).

#### 6.3.4 9-(1-Fluoropropan-2-yl)-3-nitrocarbazole (**10**)

Fluoroisopropylcarbazole **9** (0.537 g, 2.4 mmol, 1 eq.) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (12 mL) and cooled down to 0 °C. HNO<sub>3</sub> 65 % (250  $\mu$ L, 3.6 mmol, 1.5 eq.) was added and the solution was stirred at 0 °C for 4 h. Afterwards, the reaction mixture was diluted with water (5 mL), neutralized with a saturated NaHCO<sub>3</sub> solution and the aqueous layer was diluted with water to 10 mL. After evaporation of CH<sub>2</sub>Cl<sub>2</sub> in vacuo, the aqueous layer was extracted with ethyl acetate (1 x 20 mL, 2 x 10 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated in vacuo. The residue was purified by fc ( $\emptyset = 3$  cm,  $l = 15$  cm,  $v = 20$  mL, cyclohexane/ethyl acetate 30:70,  $R_f = 0.60$  (cyclohexane/ethyl acetate 6:4)). Yellow solid, mp 192 - 193 °C, yield 0.561 g (87 %). Purity (HPLC): 98.6 % ( $t_R = 22.6$  min). C<sub>15</sub>H<sub>13</sub>FN<sub>2</sub>O<sub>2</sub> (272.3 g/mol). Exact mass (APCI):  $m/z = 273.1047$  (calcd. 273.1034 for C<sub>15</sub>H<sub>14</sub>FN<sub>2</sub>O<sub>2</sub> [M+H<sup>+</sup>]). <sup>1</sup>H NMR (400 MHz,

DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 1.67 (dd,  $J$  = 7.2/0.9 Hz, 3H, CH<sub>3</sub>), 4.86 (ddd,  $J$  = 45.7/10.0/4.2 Hz, 1H, CH<sub>2</sub>F), 5.08 (ddd,  $J$  = 48.3/9.8/8.7 Hz, 1H, CH<sub>2</sub>F), 5.34 - 5.52 (m, 1H, NCH), 7.32 - 7.37 (m, 1H, 6-H), 7.56 (ddd,  $J$  = 8.4/7.2/1.3 Hz, 1H, 7-H), 7.85 (d,  $J$  = 8.4 Hz, 1H, 8-H), 7.91 (d,  $J$  = 9.2 Hz, 1H, 1-H), 8.31 (dd,  $J$  = 9.2/2.4 Hz, 1H, 2-H), 8.43 (d,  $J$  = 7.8 Hz, 1H, 5-H), 9.18 (d,  $J$  = 2.2 Hz, 1H, 4-H). <sup>13</sup>C NMR (151 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 13.9 (d,  $J$  = 6.9 Hz, 1C, CH<sub>3</sub>), 51.1 (d,  $J$  = 19.3 Hz, 1C, NCH), 83.6 (d,  $J$  = 170.5 Hz, 1C, CH<sub>2</sub>F), 110.7 (1C, C-1), 111.6 (1C, C-8), 117.1 (1C, C-4), 120.7 (1C, C-6), 121.1 (1C, C-2), 121.5 (1C, C-5), 122.5 (1C, C-4a), 122.7 (1C, C-4b), 127.4 (1C, C-7), 140.1 (1C, C-3), 140.7 (1C, C-8a), 143.0 (1C, C-9a). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2966 (w, C-H, aliph), 1593 (w, C-C, arom), 1508 (m, C-C, arom), 1315 (s, NO<sub>2</sub>).

### 6.3.5 9-(1-Fluoropropan-2-yl)carbazol-3-ammonium chloride (**11**·HCl)

Under N<sub>2</sub> atmosphere, nitrocarbazole **10** (0.528 g, 1.9 mmol, 1 eq.) was dissolved in dry THF (52 mL). Pd/C 10 % (80 mg) was added and the mixture was stirred for 24 h under H<sub>2</sub> atmosphere (balloon). After filtration through Celite<sup>®</sup>, the mixture was concentrated under reduced pressure and the residue was dissolved in Et<sub>2</sub>O. A solution of HCl in Et<sub>2</sub>O (2 M, 1.0 mL, 2.0 mmol, 1.03 eq.) was added dropwise until the salt **11**·HCl precipitated completely. The precipitate was filtered off, washed with cold Et<sub>2</sub>O and dried under reduced pressure.  $R_f$  = 0.52 (cyclohexane/ethyl acetate 3:7). Grey solid, mp 220 - 240 °C (decomposition), yield 0.492 g (91 %). Purity (HPLC): 97.9 % ( $t_R$  = 15.4 min). C<sub>15</sub>H<sub>16</sub>ClFN<sub>2</sub> (278.8 g/mol). Exact mass (APCI):  $m/z$  = 243.1283 (calcd. 243.1292 for C<sub>15</sub>H<sub>16</sub>FN<sub>2</sub> [M+H<sup>+</sup>]). <sup>1</sup>H NMR (400 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 1.64 (d,  $J$  = 6.9 Hz, 3H, CH<sub>3</sub>), 4.84 (ddd,  $J$  = 45.8/9.9/4.4 Hz, 1H, CH<sub>2</sub>F), 5.05 (dt,  $J$  = 48.3/9.1 Hz, 1H, CH<sub>2</sub>F), 5.27 - 5.43 (m, 1H, NCH), 7.25 (t,  $J$  = 7.4 Hz, 1H, 6-H), 7.41 - 7.56 (m, 2H, 2-H, 7-H), 7.77 (d,  $J$  = 8.3 Hz, 1H, 8-H), 7.84 (d,  $J$  = 8.8 Hz, 1H, 1-H), 8.15 (d,  $J$  = 2.2 Hz, 1H, 4-H), 8.20 (d,  $J$  = 7.7 Hz, 1H, 5-H), 10.45 (s, 3H, -NH<sub>3</sub><sup>+</sup>).

$^{13}\text{C}$  NMR (101 MHz, DMSO- $\text{D}_6$ ):  $\delta$  (ppm) = 13.9 (d,  $J$  = 6.9 Hz, 1C,  $\text{CH}_3$ ), 50.5 (d,  $J$  = 19.4 Hz, 1C, NCH), 83.6 (d,  $J$  = 170.4 Hz, 1C,  $\text{CH}_2\text{F}$ ), 110.9 (1C, C-8), 111.4 (1C, C-1), 114.7 (1C, C-4), 119.4 (1C, C-6), 120.5 (1C, C-5), 120.6 (1C, C-2), 121.9 (1C, C-4b), 122.8 (1C, C-4a), 123.1 (1C, C-3), 126.5 (1C, C-7), 138.5 (1C, C-9a), 140.1 (1C, C-8a). FTIR (neat):  $\tilde{\nu}$  ( $\text{cm}^{-1}$ ) = 3452 (w, N-H), 2854 (m, C-H, aliph).

### 6.3.6 3-[3-(2-Bromo-4-fluorophenyl)-1,2,4-oxadiazol-5-yl]-N-[9-(1-fluoropropan-2-yl)carbazol-3-yl]propanamide (**13a**)

Under  $\text{N}_2$  atmosphere, *N*-ethyl-*N,N*-diisopropylamine (0.14 mL, 0.83 mmol, 3 eq.) and COMU<sup>®</sup> (155 mg, 0.36 mmol, 1.3 eq.) were added to a solution of carboxylic acid **12a** (96 mg, 0.31 mmol, 1.1 eq.) in dry THF (3 mL). After the reaction mixture had been stirred at room temperature for 50 min, carbazolumine hydrochloride **11**·HCl (78 mg, 0.28 mmol, 1 eq.) was added and stirring was continued for 24 h. Afterwards, all volatiles were removed under reduced pressure and the residue was dissolved in ethyl acetate (30 mL). The organic solvent was washed with water (2 x 10 mL) and brine (50 mL). After drying ( $\text{Na}_2\text{SO}_4$ ), the organic layer was concentrated in vacuo. The residue was purified by fc with a gradient ( $\phi$  = 2 cm,  $l$  = 15 cm,  $v$  = 10 mL, cyclohexane/ethyl acetate/triethylamine 70:30:1, 65:35:1,  $R_f$  = 0.64 (cyclohexane/ethyl acetate 4:6)). Yellowish solid, mp 147 - 148 °C, yield 105 mg (70 %). Purity (HPLC): 96.5 % ( $t_R$  = 23.8 min).  $\text{C}_{26}\text{H}_{21}\text{BrF}_2\text{N}_4\text{O}_2$  (539.4 g/mol). Exact mass (APCI):  $m/z$  = 539.0895 (calcd. 539.0889 for  $\text{C}_{26}\text{H}_{22}^{79}\text{BrF}_2\text{N}_4\text{O}_2$  [ $\text{M}+\text{H}^+$ ]).  $^1\text{H}$  NMR (400 MHz, DMSO- $\text{D}_6$ ):  $\delta$  (ppm) = 1.61 (d,  $J$  = 6.4 Hz, 3H,  $\text{CH}_3$ ), 2.99 (t,  $J$  = 6.9 Hz, 2H,  $\text{CH}_2\text{CH}_2\text{CONH}$ ), 3.36 (t,  $J$  = 7.0 Hz, 2H,  $\text{CH}_2\text{CH}_2\text{CONH}$ ), 4.82 (ddd,  $J$  = 46.0/9.7/4.4 Hz, 1H,  $\text{CH}_2\text{F}$ ), 5.02 (dt,  $J$  = 48.2/9.0 Hz, 1H,  $\text{CH}_2\text{F}$ ), 5.16 - 5.34 (m, 1H, NCH), 7.17 (t,  $J$  = 7.4 Hz, 1H, 6- $\text{H}_{\text{carb}}$ ), 7.36 - 7.48 (m, 2H, 7- $\text{H}_{\text{carb}}$ , 5- $\text{H}_{\text{phenyl}}$ ), 7.51 (dd,  $J$  = 8.9/1.9 Hz, 1H, 2- $\text{H}_{\text{carb}}$ ), 7.65 (d,  $J$  = 8.7 Hz, 1H, 1- $\text{H}_{\text{carb}}$ ), 7.68 (d,  $J$  = 8.5 Hz, 1H, 8- $\text{H}_{\text{carb}}$ ), 7.82 (dd,  $J$  = 8.6/2.5

Hz, 1H, 3-H<sub>phenyl</sub>), 7.88 (dd,  $J = 8.7/6.1$  Hz, 1H, 6-H<sub>phenyl</sub>), 8.04 (d,  $J = 7.6$  Hz, 1H, 5-H<sub>carb</sub>), 8.41 (d,  $J = 1.7$  Hz, 1H, 4-H<sub>carb</sub>), 10.15 (s, 1H, CONH). <sup>13</sup>C NMR (151 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 14.0 (d,  $J = 6.8$  Hz, 1C, CH<sub>3</sub>), 21.8 (1C, CH<sub>2</sub>CH<sub>2</sub>CONH), 32.0 (1C, CH<sub>2</sub>CH<sub>2</sub>CONH), 50.3 (d,  $J = 19.6$  Hz, 1C, NCH), 83.7 (d,  $J = 170.4$  Hz, 1C, CH<sub>2</sub>F), 110.5 (2C, C-1<sub>carb</sub>, C-8<sub>carb</sub>), 110.8 (1C, C-4<sub>carb</sub>), 115.5 (d,  $J = 21.6$  Hz, 1C, C-5<sub>phenyl</sub>), 118.7 (1C, C-2<sub>carb</sub>), 118.7 (1C, C-6<sub>carb</sub>), 120.0 (1C, C-5<sub>carb</sub>), 121.4 (d,  $J = 25.1$  Hz, 1C, C-3<sub>phenyl</sub>), 122.1 (d,  $J = 10.2$  Hz, 1C, C-2<sub>phenyl</sub>), 122.4 (2C, C-4<sub>a</sub>carb, C-4<sub>b</sub>carb), 124.4 (d,  $J = 3.5$  Hz, 1C, C-1<sub>phenyl</sub>), 125.7 (1C, C-7<sub>carb</sub>), 131.2 (1C, C-3<sub>carb</sub>), 133.6 (d,  $J = 9.4$  Hz, 1C, C-6<sub>phenyl</sub>), 135.9 (1C, C-9<sub>a</sub>carb), 140.0 (1C, C-8<sub>a</sub>carb), 162.8 (d,  $J = 253.2$  Hz, 1C, C-4<sub>phenyl</sub>), 166.6 (1C, C-3<sub>oxadiazole</sub>), 168.5 (1C, C=O), 179.6 (1C, C-5<sub>oxadiazole</sub>). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3279 (w, NH), 2924 (w, C-H, aliph), 1643 (m, C=O), 1593 (m, C-C, arom), 1550 (m, C-C, arom).

### 6.3.7 3-[3-(2-Chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl]-N-[9-(1-fluoropropan-2-yl)carbazol-3-yl]propanamide (**13b**)

Under N<sub>2</sub> atmosphere, *N*-ethyl-*N,N*-diisopropylamine (0.16 mL, 0.91 mmol, 3 eq.) and COMU<sup>®</sup> (169 mg, 0.39 mmol, 1.3 eq.) were added to a solution of carboxylic acid **12b** (90 mg, 0.33 mmol, 1.1 eq.) in dry THF (3 mL). After the reaction mixture had been stirred at room temperature for 30 min, carbazolumine hydrochloride **11**·HCl (85 mg, 0.30 mmol, 1 eq.) was added and stirring was continued for 24 h. Afterwards, all volatiles were removed under reduced pressure and the residue was dissolved in ethyl acetate (30 mL). The organic layer was washed with NaOH solution (1 M, 10 mL), water (10 mL) and brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. The residue was purified by fc ( $\phi = 2$  cm,  $l = 15$  cm,  $v = 10$  mL, cyclohexane/ethyl acetate 70:30,  $R_f = 0.64$  (cyclohexane/ethyl acetate 4:6)). Beige solid, mp 157 - 159 °C, yield 88 mg (59 %). Purity (HPLC): 98.2 % ( $t_R = 23.3$  min). C<sub>26</sub>H<sub>21</sub>ClF<sub>2</sub>N<sub>4</sub>O<sub>2</sub> (494.9 g/mol).

Exact mass (APCI):  $m/z = 495.1393$  (calcd. 495.1394 for  $C_{26}H_{22}^{35}ClF_2N_4O_2 [M+H^+]$ ).

$^1H$  NMR (600 MHz, DMSO- $D_6$ ):  $\delta$  (ppm) = 1.61 (d,  $J = 6.9$  Hz, 3H,  $CH_3$ ), 3.00 (t,  $J = 6.9$  Hz, 2H,  $CH_2CH_2CONH$ ), 3.36 (t,  $J = 6.9$  Hz, 2H,  $CH_2CH_2CONH$ ), 4.81 (ddd,  $J = 45.9/9.8/4.4$  Hz, 1H,  $CH_2F$ ), 5.02 (dt,  $J = 48.1/9.0$  Hz, 1H,  $CH_2F$ ), 5.19 - 5.30 (m, 1H, NCH), 7.17 (t,  $J = 7.4$  Hz, 1H, 6- $H_{carb}$ ), 7.38 - 7.44 (m, 2H, 3- $H_{phenyl}$ , 7- $H_{carb}$ ), 7.51 (dd,  $J = 8.8/1.6$  Hz, 1H, 2- $H_{carb}$ ), 7.63 - 7.70 (m, 3H, 1- $H_{carb}$ , 8- $H_{carb}$ , 5- $H_{phenyl}$ ), 7.97 (dd,  $J = 8.6/6.2$  Hz, 1H, 6- $H_{phenyl}$ ), 8.04 (d,  $J = 7.7$  Hz, 1H, 5- $H_{carb}$ ), 8.41 (d,  $J = 1.3$  Hz, 1H, 4- $H_{carb}$ ), 10.15 (s, 1H, CONH).  $^{13}C$  NMR (151 MHz, DMSO- $D_6$ ):  $\delta$  (ppm) = 14.0 (d,  $J = 6.7$  Hz, 1C,  $CH_3$ ), 21.8 (1C,  $CH_2CH_2CONH$ ), 32.0 (1C,  $CH_2CH_2CONH$ ), 50.3 (d,  $J = 19.6$  Hz, 1C, NCH), 83.7 (d,  $J = 170.4$  Hz, 1C,  $CH_2F$ ), 110.5 (2C, C-1 $_{carb}$ , C-8 $_{carb}$ ), 110.8 (1C, C-4 $_{carb}$ ), 115.2 (d,  $J = 21.7$  Hz, 1C, C-3 $_{phenyl}$ ), 118.4 (d,  $J = 25.5$  Hz, 1C, C-5 $_{phenyl}$ ), 118.7 (1C, C-2 $_{carb}$ ), 118.7 (1C, C-6 $_{carb}$ ), 120.0 (1C, C-5 $_{carb}$ ), 122.3 (d,  $J = 3.4$  Hz, 1C, C-1 $_{phenyl}$ ), 122.4 (2C, C-4a $_{carb}$ , C-4b $_{carb}$ ), 125.7 (1C, C-7 $_{carb}$ ), 131.2 (1C, C-3 $_{carb}$ ), 133.4 (d,  $J = 9.7$  Hz, 1C, C-6 $_{phenyl}$ ), 133.5 (d,  $J = 11.1$  Hz, 1C, C-2 $_{phenyl}$ ), 135.9 (1C, C-9a $_{carb}$ ), 139.9 (1C, C-8a $_{carb}$ ), 163.0 (d,  $J = 252.5$  Hz, 1C, C-4 $_{phenyl}$ ), 165.7 (1C, C-3 $_{oxadiazole}$ ), 168.6 (1C, C=O), 179.6 (1C, C-5 $_{oxadiazole}$ ). FTIR (neat):  $\tilde{\nu}$  ( $cm^{-1}$ ) = 3282 (w, NH), 2927 (w, C-H, aliph), 1639 (m, C=O), 1593 (m, C-C, arom), 1550 (m, C-C, arom).

### 6.3.8 *N*-(6-Methoxy-1,2,3,4-tetrahydrocarbazol-3-yl)phthalimide (**15**) [21]

A solution of *N*-(4-oxocyclohexyl)phthalimide (**14**, 5.00 g, 21 mmol, 1 eq.) and 4-methoxyphenylhydrazine hydrochloride (3.59 g, 21 mmol, 1 eq.) in dry ethanol (100 mL) was heated at reflux for 2.5 h. After cooling down to room temperature, the precipitate was filtrated off and washed with ethanol 96 % (3 x 10 mL). The solid was dried under reduced pressure and freeze-dried overnight.  $R_f = 0.66$  (cyclohexane/ethyl acetate/dimethylethylamine 5:5:0.2). Colorless solid, mp 222 - 223 °C, yield 6.88 g (97 %). Purity (HPLC): 84.1 % ( $t_R = 22.2$  min).  $C_{21}H_{18}N_2O_3$  (346.4 g/mol). Exact mass

(APCI):  $m/z = 347.1389$  (calcd. 347.1390 for  $C_{21}H_{19}N_2O_3 [M+H^+]$ ).  $^1H$  NMR (400 MHz, DMSO- $D_6$ ):  $\delta$  (ppm) = 2.03 - 2.11 (m, 1H, 2-H), 2.67 (tt,  $J = 12.3/6.4$  Hz, 1H, 2-H), 2.80 - 2.98 (m, 3H, 1- $CH_2$ , 4-H), 3.20 - 3.28 (m, 1H, 4-H), 3.71 (s, 3H,  $OCH_3$ ), 4.43 - 4.52 (m, 1H, 3-H), 6.65 (dd,  $J = 8.7/2.4$  Hz, 1H, 7-H), 6.82 (d,  $J = 2.4$  Hz, 1H, 5-H), 7.15 (d,  $J = 8.7$  Hz, 1H, 8-H), 7.84 - 7.91 (m, 4H, 4- $H_{phth}$ , 5- $H_{phth}$ , 6- $H_{phth}$ , 7- $H_{phth}$ ), 10.61 (s, 1H, NH).  $^{13}C$  NMR (101 MHz, DMSO- $D_6$ ):  $\delta$  (ppm) = 22.6 (1C, C-1), 24.7 (1C, C-4), 26.5 (1C, C-2), 47.8 (1C, C-3), 55.3 (1C,  $OCH_3$ ), 99.7 (1C, C-5), 106.3 (1C, C-4a), 109.9 (1C, C-7), 111.2 (1C, C-8), 123.0 (2C, C-4 $_{phth}$ , C-7 $_{phth}$ ), 127.2 (1C, C-4b), 131.3 (1C, C-8a), 131.5 (2C, C-3a $_{phth}$ , C-7a $_{phth}$ ), 134.2 (1C, C-9a), 134.4 (2C, C-5 $_{phth}$ , C-6 $_{phth}$ ), 153.0 (1C, C-6), 167.9 (2C, C=O). FTIR (neat):  $\tilde{\nu}$  ( $cm^{-1}$ ) = 3425 (w, N-H), 3379 (w, C-H, arom), 2924 (w, C-H, aliph), 1697 (s, C=O), 1597 (w, C-C, arom).

### 6.3.9 *N*-[6-(Methylsulfonyl)-1,2,3,4-tetrahydrocarbazol-3-yl]phthalimide (**16**)

A solution of *N*-(4-oxocyclohexyl)phthalimide (**14**, 3.53 g, 15 mmol, 1 eq.) and 4-(methylsulfonyl)phenylhydrazine (2.70 g, 15 mmol, 1 eq.) in glacial acetic acid (110 mL) was heated at reflux for 47 h. The mixture was concentrated in vacuo and the residue was dissolved in  $CH_2Cl_2$  (200 mL). Afterwards, the organic layer was washed with water (2 x 70 mL) and brine (70 mL), dried ( $Na_2SO_4$ ) and concentrated under reduced pressure. The residue was purified by fc with a gradient ( $\phi = 6$  cm,  $l = 13$  cm,  $v = 60$  mL, cyclohexane/ $CH_2Cl_2$  60:40, 50:50, 30:70, 0:100,  $CH_2Cl_2$ /methanol 100:10,  $R_f = 0.51$  (cyclohexane/ethyl acetate/formic acid 3:7:0.2)). Pale yellow solid, mp 282 - 284 °C, yield 4.50 g (79 %). Purity (HPLC): 86.6 % ( $t_R = 19.6$  min).  $C_{21}H_{18}N_2O_4S$  (394.5 g/mol). Exact mass (APCI):  $m/z = 395.1072$  (calcd. 395.1060 for  $C_{21}H_{19}N_2O_4S [M+H^+]$ ).  $^1H$  NMR (600 MHz, DMSO- $D_6$ ):  $\delta$  (ppm) = 2.11 (d,  $J = 12.3$  Hz, 1H, 2-H), 2.64 - 2.74 (m, 1H, 2-H), 2.92 - 3.01 (m, 3H, 1- $CH_2$ , 4-H), 3.12 (s, 3H,  $CH_3$ ), 3.29 - 3.33 (m, 1H, 4-H), 4.47 - 4.54 (m, 1H, 3-H), 7.49 (d,  $J =$



8.6 Hz, 1H, 8-H), 7.56 (dd,  $J = 8.6/1.4$  Hz, 1H, 7-H), 7.84 - 7.93 (m, 5H, 5-H, 4-H<sub>phth</sub>, 5-H<sub>phth</sub>, 6-H<sub>phth</sub>, 7-H<sub>phth</sub>), 11.45 (s, 1H, NH). <sup>13</sup>C NMR (151 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 22.5 (1C, C-1), 24.2 (1C, C-4), 26.2 (1C, C-2), 44.6 (1C, CH<sub>3</sub>), 47.4 (1C, C-3), 108.3 (1C, C-4a), 111.2 (1C, C-8), 117.3 (1C, C-5), 118.9 (1C, C-7), 123.0 (2C, C-4<sub>phth</sub>, C-7<sub>phth</sub>), 126.3 (1C, C-4b), 130.7 (1C, C-6), 131.5 (2C, C-3a<sub>phth</sub>, C-7a<sub>phth</sub>), 134.5 (2C, C-5<sub>phth</sub>, C-6<sub>phth</sub>), 136.8 (1C, C-9a), 138.5 (1C, C-8a), 167.9 (2C, C=O). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3348 (m, N-H), 2939 (w, C-H, aliph), 1697 (s, C=O), 1620 (w, C-C, arom), 1130 (s, SO<sub>2</sub>).

6.3.10 *N*-[9-(2-Fluoroethyl)-6-methoxy-1,2,3,4-tetrahydrocarbazol-3-yl]phthalimide  
(17)

Under N<sub>2</sub> atmosphere, tetrahydrocarbazole **15** (3.00 g, 8.7 mmol, 1 eq.) was dissolved in dry DMF (43 mL) and NaH (60 % dispersion in Paraffin Oil, 0.866 g, 18 mmol, 2.5 eq.) was added at 0 °C. After stirring at 0 °C for 30 min, fluoroethyl tosylate (2.46 g, 11 mmol, 1.3 eq.) was added slowly to the reaction mixture. The mixture was heated at 95 °C for 2.5 h. Water (5 mL) was added, the reaction mixture was concentrated in vacuo and the residue was dissolved in ethyl acetate (300 mL). The organic layer was washed with saturated Na<sub>2</sub>CO<sub>3</sub> solution (2 x 100 mL) and water (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure.  $R_f = 0.67$  (cyclohexane/ethyl acetate/dimethylethylamine 6:4:0.2). Pale yellow solid, mp 150 -190 °C (decomposition), yield 1.81 g (53 %). Purity (HPLC): 99.2 % ( $t_R = 23.3$  min). C<sub>23</sub>H<sub>21</sub>FN<sub>2</sub>O<sub>3</sub> (392.4 g/mol). Exact mass (APCI):  $m/z = 393.1606$  (calcd. 393.1609 for C<sub>23</sub>H<sub>22</sub>FN<sub>2</sub>O<sub>3</sub> [M+H<sup>+</sup>]). <sup>1</sup>H NMR (400 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 2.08 - 2.18 (m, 1H, 2-H), 2.66 (tt,  $J = 12.2/7.2$  Hz, 1H, 2-H), 2.81 - 3.01 (m, 3H, 1-CH<sub>2</sub>, 4-H), 3.25 (t,  $J = 13.0$  Hz, 1H, 4-H), 3.72 (s, 3H, OCH<sub>3</sub>), 4.31 - 4.51 (m, 3H, CH<sub>2</sub>CH<sub>2</sub>F, 3-H), 4.65 (dt,  $J = 47.5/4.3$  Hz, 2H, CH<sub>2</sub>F), 6.72 (dd,  $J = 8.8/2.2$  Hz, 1H, 7-H), 6.86 (d,  $J = 2.1$  Hz, 1H,

5-H), 7.31 (d,  $J = 8.8$  Hz, 1H, 8-H), 7.83 - 7.93 (m, 4H, 4-H<sub>phth</sub>, 5-H<sub>phth</sub>, 6-H<sub>phth</sub>, 7-H<sub>phth</sub>).  
<sup>13</sup>C NMR (101 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 21.5 (1C, C-1), 24.6 (1C, C-4), 26.3 (1C, C-2), 43.0 (d,  $J = 20.3$  Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 47.6 (1C, C-3), 55.3 (1C, OCH<sub>3</sub>), 82.9 (d,  $J = 167.5$  Hz, 1C, CH<sub>2</sub>F), 99.9 (1C, C-5), 106.6 (1C, C-4a), 110.1 (2C, C-7, C-8), 123.0 (2C, C-4<sub>phth</sub>, C-7<sub>phth</sub>), 126.8 (1C, C-4b), 131.5 (2C, C-3<sub>aphth</sub>, C-7<sub>aphth</sub>), 131.7 (1C, C-8a), 134.5 (2C, C-5<sub>phth</sub>, C-6<sub>phth</sub>), 135.2 (1C, C-9a), 153.4 (1C, C-6), 167.9 (2C, C=O).  
 FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2931 (w, C-H, aliph), 1701 (s, C=O), 1589 (w, C-C, arom).

### 6.3.11 *N*-[6-(Methylsulfonyl)carbazol-3-yl]phthalimide (**18**)

Tetrahydrocarbazole **16** (2.00 g, 5.1 mmol, 1 eq.) was dissolved in THF (40 mL) and DDQ (2.88 g, 13 mmol, 2.5 eq.) was added to the solution. The reaction mixture was heated at reflux for 3 h. After removing the solvent under reduced pressure, the residue was filtered, washed with water (350 mL), dried under reduced pressure and freeze-dried overnight.  $R_f = 0.45$  (cyclohexane/ethyl acetate/formic acid 4:6:0.2). Pale yellow solid, mp > 300 °C, yield 1.75 g (88 %). Purity (HPLC): 75.9 % ( $t_R = 18.7$  min). C<sub>21</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>S (390.4 g/mol). Exact mass (APCI):  $m/z = 391.0735$  (calcd. 391.0747 for C<sub>21</sub>H<sub>15</sub>N<sub>2</sub>O<sub>4</sub>S [M+H<sup>+</sup>]). <sup>1</sup>H NMR (600 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 3.24 (s, 3H, CH<sub>3</sub>), 7.55 (dd,  $J = 8.5/1.9$  Hz, 1H, 2-H), 7.72 (d,  $J = 8.5$  Hz, 1H, 1-H), 7.75 (d,  $J = 8.6$  Hz, 1H, 8-H), 7.93 - 7.95 (m, 3H, 7-H, 5-H<sub>phth</sub>, 6-H<sub>phth</sub>), 8.00 - 8.02 (m, 2H, 4-H<sub>phth</sub>, 7-H<sub>phth</sub>), 8.37 (d,  $J = 1.7$  Hz, 1H, 4-H), 8.74 (d,  $J = 1.8$  Hz, 1H, 5-H), 12.09 (s, 1H, NH). <sup>13</sup>C NMR (151 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 44.5 (1C, CH<sub>3</sub>), 111.8 (1C, C-1), 111.8 (1C, C-8), 120.5 (1C, C-4), 120.6 (1C, C-5), 121.9 (1C, C-4b), 122.1 (1C, C-3), 123.4 (2C, C-4<sub>phth</sub>, C-7<sub>phth</sub>), 124.0 (1C, C-4a), 124.5 (1C, C-7), 126.6 (1C, C-2), 131.2 (1C, C-6), 131.6 (2C, C-3<sub>aphth</sub>, C-7<sub>aphth</sub>), 134.8 (2C, C-5<sub>phth</sub>, C-6<sub>phth</sub>), 140.0 (1C, C-9a), 142.7 (1C, C-8a), 167.6 (2C, C=O). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3352 (m, N-H), 2920 (w, C-H, aliph), 1705 (s, C=O), 1604 (w, C-C, arom), 1134 (s, SO<sub>2</sub>).

### 6.3.12 *N*-[9-(2-Fluoroethyl)-6-methoxycarbazol-3-yl]phthalimide (**19**)

Tetrahydrocarbazole **17** (1.40 g, 3.6 mmol, 1 eq.) was dissolved in THF (28 mL) and DDQ (2.02 g, 8.9 mmol, 2.5 eq.) was added to the solution. The reaction mixture was heated at reflux for 2 h. After evaporation of the solvent under reduced pressure, the residue was filtered and washed with ethyl acetate (250 mL) and CH<sub>2</sub>Cl<sub>2</sub> (70 mL). The solvents were evaporated under reduced pressure and the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (200 mL). Afterwards, the organic layer was washed with a saturated Na<sub>2</sub>CO<sub>3</sub> solution (70 mL), a saturated NH<sub>4</sub>Cl solution (70 mL), water (2 x 70 mL) and brine (70 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The resulting product was used without further purification. *R*<sub>f</sub> = 0.44 (cyclohexane/ethyl acetate/dimethylethylamine 5:5:0.2). Dark yellow solid, mp 222 °C, yield 1.15 g (83 %). C<sub>23</sub>H<sub>17</sub>FN<sub>2</sub>O<sub>3</sub> (388.4 g/mol). Compound was purified by fc with a gradient (∅ = 2 cm, l = 14 cm, v = 10 mL, cyclohexane/ethyl acetate/dimethylethylamine 80:20:1, 75:25:1, 60:40:1) leading to a purity (HPLC) of 96.9 % (t<sub>R</sub> = 21.4 min). Exact mass (APCI): *m/z* = 389.1284 (calcd. 389.1296 for C<sub>23</sub>H<sub>18</sub>FN<sub>2</sub>O<sub>3</sub> [M+H<sup>+</sup>]). <sup>1</sup>H NMR (600 MHz, DMSO-D<sub>6</sub>): δ (ppm) = 3.85 (s, 3H, OCH<sub>3</sub>), 4.72 - 4.86 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>F), 7.14 (dd, *J* = 8.9/2.5 Hz, 1H, 7-H), 7.46 (dd, *J* = 8.7/2.0 Hz, 1H, 2-H), 7.60 (d, *J* = 8.9 Hz, 1H, 8-H), 7.71 (d, *J* = 8.7 Hz, 1H, 1-H), 7.73 (d, *J* = 2.4 Hz, 1H, 5-H), 7.91 - 7.95 (m, 2H, 5-H<sub>phth</sub>, 6-H<sub>phth</sub>), 7.98 - 8.02 (m, 2H, 4-H<sub>phth</sub>, 7-H<sub>phth</sub>), 8.20 (d, *J* = 1.9 Hz, 1H, 4-H). <sup>13</sup>C NMR (151 MHz, DMSO-D<sub>6</sub>): δ (ppm) = 43.1 (d, *J* = 20.1 Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 55.6 (1C, OCH<sub>3</sub>), 82.7 (d, *J* = 167.7 Hz, 1C, CH<sub>2</sub>F), 103.2 (1C, C-5), 109.7 (1C, C-1), 110.7 (1C, C-8), 115.4 (1C, C-7), 119.9 (1C, C-4), 122.1 (1C, C-4a), 122.3 (1C, C-4b), 122.7 (1C, C-3), 123.4 (2C, C-4<sub>phth</sub>, C-7<sub>phth</sub>), 125.2 (1C, C-2), 131.6 (2C, C-3a<sub>phth</sub>, C-7a<sub>phth</sub>), 134.7 (2C, C-5<sub>phth</sub>, C-6<sub>phth</sub>), 135.6 (1C, C-8a),

140.1 (1C, C-9a), 153.6 (1C, C-6), 167.7 (2C, C=O). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2924 (w, C-H, aliph), 1716 (s, C=O).

### 6.3.13 *N*-[9-(2-Fluoroethyl)-6-(methylsulfonyl)carbazol-3-yl]phthalimide (**20**)

Under N<sub>2</sub> atmosphere, carbazole derivative **18** (1.10 g, 2.8 mmol, 1 eq.) was dissolved in dry DMF (25 mL). Cs<sub>2</sub>CO<sub>3</sub> (1.84 g, 5.6 mmol, 2 eq.) was added at 0 °C. After stirring at 0 °C for 30 min, fluoroethyl tosylate (0.738 g, 3.4 mmol, 1.2 eq.) was added slowly to the reaction mixture. Stirring was continued for 18 h at room temperature. Afterwards, the solvent was removed in vacuo, the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (200 mL) and the solution was washed with water (3 x 60 mL). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. The residue was purified by fc with a gradient ( $\emptyset$  = 3 cm, l = 20 cm, v = 30 mL, cyclohexane/CH<sub>2</sub>Cl<sub>2</sub> 60:40, 50:50, 30:70, 15:85, 0:100, CH<sub>2</sub>Cl<sub>2</sub>/methanol 100:0.5, cyclohexane/CH<sub>2</sub>Cl<sub>2</sub>/dimethylethylamine 30:70:1, 15:85:0, R<sub>f</sub> = 0.60 (cyclohexane/ethyl acetate/dimethylethylamine 1:9:0.2)). Pale yellow solid, mp 289 - 292 °C, yield 0.756 g (61 %). Purity (HPLC): 96.0 % (t<sub>R</sub> = 20.0 min). C<sub>23</sub>H<sub>17</sub>FN<sub>2</sub>O<sub>4</sub>S (436.5 g/mol). Exact mass (APCI): m/z = 437.0965 (calcd. 437.0966 for C<sub>23</sub>H<sub>18</sub>FN<sub>2</sub>O<sub>4</sub>S [M+H<sup>+</sup>]). <sup>1</sup>H NMR (400 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 3.26 (s, 3H, CH<sub>3</sub>), 4.78 - 4.97 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>F), 7.63 (dd, J = 8.7/2.0 Hz, 1H, 2-H), 7.89 (d, J = 8.8 Hz, 1H, 1-H), 7.92 - 7.97 (m, 3H, 8-H, 5-H<sub>phth</sub>, 6-H<sub>phth</sub>), 7.99 - 8.05 (m, 3H, 7-H, 4-H<sub>phth</sub>, 7-H<sub>phth</sub>), 8.43 (d, J = 2.0 Hz, 1H, 4-H), 8.78 (d, J = 1.8 Hz, 1H, 5-H). <sup>13</sup>C NMR (151 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 43.4 (d, J = 19.9 Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 44.4 (1C, CH<sub>3</sub>), 83.1 (d, J = 163.3 Hz, 1C, CH<sub>2</sub>F), 110.6 (1C, C-8), 110.7 (1C, C-1), 120.5 (1C, C-4), 120.5 (1C, C-8), 121.6 (1C, C-4b), 121.9 (1C, C-4a), 123.4 (2C, C-4<sub>phth</sub>, C-7<sub>phth</sub>), 124.5 (1C, C-3), 124.7 (1C, C-7), 126.7 (1C, C-2), 131.6 (2C, C-3a<sub>phth</sub>, C-7a<sub>phth</sub>), 131.7 (1C, C-6), 134.8 (2C, C-5<sub>phth</sub>, C-6<sub>phth</sub>), 140.4 (1C, C-9a),

143.1 (1C, C-8a), 167.5 (2C, C=O). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2935 (w, C-H, aliph), 1712 (s, C=O), 1597 (w, C-C, arom), 1138 (s, SO<sub>2</sub>).

#### 6.3.14 9-(2-Fluoroethyl)-6-methoxycarabzol-3-ammonium chloride (**21**·HCl)

Hydrazine monohydrate (0.36 mL, 7.3 mmol, 3 eq.) was added to a solution of phthalimide **19** (0.947 g, 2.4 mmol, 1 eq.) in ethanol 96 % (25 mL). The reaction mixture was heated at reflux for 2.25 h. After cooling down to room temperature, the solution was filtered, the residue was washed with ethanol 96 % followed by evaporation of the solvent. The residue was dissolved in ethyl acetate (100 mL) and the organic layer was washed with NaOH solution (1 M, 30 mL), water (30 mL) and brine (30 mL). After drying (Na<sub>2</sub>SO<sub>4</sub>), the solvent was removed under reduced pressure. The residue was dissolved in Et<sub>2</sub>O, filtered and the product was precipitated by the addition of a solution of HCl in Et<sub>2</sub>O (2 M, 1.2 mL, 2.4 mmol, 1 eq.). The product was filtered off, washed with Et<sub>2</sub>O (10 mL) and dried under reduced pressure. R<sub>f</sub> = 0.50 (cyclohexane/ethyl acetate/dimethylethylamine 3:7:0.2). Grey solid, mp 180 - 210 °C (decomposition), yield 0.373 g (52 %). Purity (HPLC): 88.9 % (t<sub>R</sub> = 14.7 min). C<sub>15</sub>H<sub>16</sub>ClFN<sub>2</sub>O (294.8 g/mol). Exact mass (APCI): m/z = 259.1242 (calcd. 259.1241 for C<sub>15</sub>H<sub>16</sub>FN<sub>2</sub>O [M+H<sup>+</sup>]). <sup>1</sup>H NMR (600 MHz, DMSO-D<sub>6</sub>): δ (ppm) = 3.87 (s, 3H, OCH<sub>3</sub>), 4.59 - 4.91 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>F), 7.15 (dd, J = 8.9/2.5 Hz, 1H, 7-H), 7.45 (dd, J = 8.7/2.2 Hz, 1H, 2-H), 7.59 (d, J = 8.9 Hz, 1H, 8-H), 7.70 (d, J = 8.7 Hz, 1H, 1-H), 7.76 (d, J = 2.5 Hz, 1H, 5-H), 8.12 (d, J = 2.1 Hz, 1H, 4-H), 10.41 (s, 3H, -NH<sub>3</sub><sup>+</sup>). <sup>13</sup>C NMR (151 MHz, DMSO-D<sub>6</sub>): δ (ppm) = 43.1 (d, J = 20.0 Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 55.7 (1C, OCH<sub>3</sub>), 82.6 (d, J = 167.7 Hz, 1C, CH<sub>2</sub>F), 103.3 (1C, C-5), 110.5 (1C, C-1), 110.8 (1C, C-8), 114.9 (1C, C-4), 115.9 (1C, C-7), 120.5 (1C, C-2), 121.9 (1C, C-4b), 122.2 (1C, C-4a), 122.6 (1C, C-3), 135.8 (1C, C-8a), 139.8 (1C, C-9a), 153.7 (1C, C-6). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2858 (w, C-H, aliph), 1562 (w, C-C, arom).

### 6.3.15 9-(2-Fluoroethyl)-6-(methylsulfonyl)carbazol-3-ammonium chloride (**22**·HCl)

CH<sub>2</sub>Cl<sub>2</sub> (15 mL) and hydrazine monohydrate (32  $\mu$ L, 0.65 mmol, 2 eq.) were added to a stirred suspension of phthalimide **20** (0.143 g, 0.33 mmol, 1 eq.) in ethanol 96 % (3 mL). The mixture was heated at 40 °C for 20 h. After the mixture was cooled down to room temperature, phthalhydrazide was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (30 mL) and the solution was washed with NaOH solution (1 M, 3 x 10 mL), water (10 mL) and brine (10 mL). Drying (Na<sub>2</sub>SO<sub>4</sub>) and evaporation of the solvent under reduced pressure gave a residue, which was dissolved in THF (10 mL) and precipitated with a solution of HCl in Et<sub>2</sub>O (2 M, 0.16 mL, 0.33 mmol, 1 eq.). After filtration, the product was washed with Et<sub>2</sub>O (3 x 2 mL) and dried under reduced pressure. R<sub>f</sub> = 0.33 (ethyl acetate/dimethylethylamine 10:0.2). Colorless solid, mp 205 - 250 °C (decomposition), yield 86 mg (77 %). Purity (HPLC): 98.5 % (t<sub>R</sub> = 12.5 min). C<sub>15</sub>H<sub>16</sub>ClFN<sub>2</sub>O<sub>2</sub>S (342.8 g/mol). Exact mass (APCI): m/z = 307.0908 (calcd. 307.0911 for C<sub>15</sub>H<sub>16</sub>FN<sub>2</sub>O<sub>2</sub>S [M+H<sup>+</sup>]). <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  (ppm) = 3.27 (s, 3H, CH<sub>3</sub>), 4.76 - 4.91 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>F), 7.56 (dd, *J* = 8.7/2.1 Hz, 1H, 2-H), 7.85 (d, *J* = 8.7 Hz, 1H, 1-H), 7.92 (d, *J* = 8.7 Hz, 1H, 8-H), 8.02 (dd, *J* = 8.7/1.8 Hz, 1H, 7-H), 8.31 (d, *J* = 1.9 Hz, 1H, 4-H), 8.83 (d, *J* = 1.9 Hz, 1H, 5-H), 10.34 (s, 3H, -NH<sub>3</sub><sup>+</sup>). <sup>13</sup>C NMR (101 MHz, DMSO-*D*<sub>6</sub>):  $\delta$  (ppm) = 43.4 (d, *J* = 20.0 Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 44.4 (1C, CH<sub>3</sub>), 82.5 (d, *J* = 167.6 Hz, 1C, CH<sub>2</sub>F), 110.7 (1C, C-8), 111.4 (1C, C-1), 115.2 (1C, C-4), 120.8 (1C, C-4b), 121.3 (1C, C-5), 121.9 (1C, C-2), 122.2 (1C, C-4a), 124.9 (1C, C-7), 125.0 (1C, C-3), 131.7 (1C, C-6), 140.0 (1C, C-9a), 143.1 (1C, C-8a). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 2804 (w, C-H, aliph), 1597 (w, C-C, arom), 1103 (s, SO<sub>2</sub>).

6.3.16 3-[3-(2-Bromo-4-fluorophenyl)-1,2,4-oxadiazol-5-yl]-N-[9-(2-fluoroethyl)-6-methoxycarbazol-3-yl]propanamide (**23a**)

Under N<sub>2</sub> atmosphere, *N*-ethyl-*N,N*-diisopropylamine (0.11 mL, 0.65 mmol, 3 eq.) and COMU<sup>®</sup> (120 mg, 0.28 mmol, 1.3 eq.) were added to a solution of carboxylic acid **12a** (75 mg, 0.24 mmol, 1.1 eq.) in dry THF (3 mL). After the reaction mixture was stirred at room temperature for 30 min, carbazolamine hydrochloride **21**·HCl (64 mg, 0.22 mmol, 1 eq.) was added and stirring was continued for 23 h. All volatiles were removed under reduced pressure and the residue was suspended in ethyl acetate (5 mL) and water (5 mL). Afterwards, the suspension was filtered and the residue was washed with water (10 mL), ethyl acetate (4 mL), ethanol 96 % (4 mL) and CH<sub>2</sub>Cl<sub>2</sub> (4 mL). The product was dried under reduced pressure and freeze-dried overnight. R<sub>f</sub> = 0.44 (cyclohexane/ethyl acetate/formic acid 5:5:0.2). Colorless solid, mp 191 °C, yield 39 mg (33 %). Purity (HPLC): 96.9 % (t<sub>R</sub> = 23.0 min). C<sub>26</sub>H<sub>21</sub>BrF<sub>2</sub>N<sub>4</sub>O<sub>3</sub> (555.4 g/mol). Exact mass (APCI): m/z = 555.0842 (calcd. 555.0838 for C<sub>26</sub>H<sub>22</sub><sup>79</sup>BrF<sub>2</sub>N<sub>4</sub>O<sub>3</sub> [M+H<sup>+</sup>]). <sup>1</sup>H NMR (400 MHz, DMSO-D<sub>6</sub>): δ (ppm) = 2.99 (t, *J* = 6.9 Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>CONH), 3.36 - 3.38 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>CONH), 3.85 (s, 3H, OCH<sub>3</sub>), 4.60 - 4.82 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>F), 7.07 (dd, *J* = 8.8/2.5 Hz, 1H, 7-H<sub>carb</sub>), 7.42 - 7.48 (m, 2H, 2-H<sub>carb</sub>, 5-H<sub>phenyl</sub>), 7.49 - 7.52 (m, 2H, 1-H<sub>carb</sub>, 8-H<sub>carb</sub>), 7.58 (d, *J* = 2.4 Hz, 1H, 5-H<sub>carb</sub>), 7.83 (dd, *J* = 8.6/2.6 Hz, 1H, 3-H<sub>phenyl</sub>), 7.89 (dd, *J* = 8.8/6.1 Hz, 1H, 6-H<sub>phenyl</sub>), 8.43 (d, *J* = 1.7 Hz, 1H, 4-H<sub>carb</sub>), 10.12 (s, 1H, CONH). <sup>13</sup>C NMR (101 MHz, DMSO-D<sub>6</sub>): δ (ppm) = 21.8 (1C, CH<sub>2</sub>CH<sub>2</sub>CONH), 31.9 (1C, CH<sub>2</sub>CH<sub>2</sub>CONH), 43.0 (d, *J* = 20.2 Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 55.6 (1C, OCH<sub>3</sub>), 82.6 (d, *J* = 167.8 Hz, 1C, CH<sub>2</sub>F), 102.7 (1C, C-5<sub>carb</sub>), 109.4 (1C, C-1<sub>carb</sub>), 110.4 (1C, C-8<sub>carb</sub>), 111.1 (1C, C-4<sub>carb</sub>), 115.0 (1C, C-7<sub>carb</sub>), 115.5 (d, *J* = 21.6 Hz, 1C, C-5<sub>phenyl</sub>), 118.8 (1C, C-2<sub>carb</sub>), 121.4 (d, *J* = 25.0 Hz, 1C, C-3<sub>phenyl</sub>), 121.7 (1C, C-4<sub>a</sub>carb), 122.1 (d, *J* = 10.0 Hz, 1C, C-2<sub>phenyl</sub>), 122.4 (1C, C-4<sub>b</sub>carb), 124.4 (d, *J* = 3.4 Hz, 1C, C-1<sub>phenyl</sub>), 130.8 (1C, C-3<sub>carb</sub>), 133.6 (d, *J* = 9.4 Hz, 1C, C-

6<sub>phenyl</sub>), 135.6 (1C, C-8<sub>carb</sub>), 137.3 (1C, C-9<sub>carb</sub>), 153.2 (1C, C-6<sub>carb</sub>), 162.8 (d,  $J = 253.3$  Hz, 1C, C-4<sub>phenyl</sub>), 166.5 (1C, C-3<sub>oxadiazole</sub>), 168.5 (1C, C=O), 179.6 (1C, C-5<sub>oxadiazole</sub>). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3263 (w, N-H), 2931 (w, C-H, aliph), 1643 (s, C=O).

6.3.17 *3-[3-(2-Bromo-4-fluorophenyl)-1,2,4-oxadiazol-5-yl]-N-[9-(2-fluoroethyl)-6-(methylsulfonyl)carbazol-3-yl]propanamide (24a)*

Under N<sub>2</sub> atmosphere, *N*-ethyl-*N,N*-diisopropylamine (91  $\mu$ L, 0.53 mmol, 3 eq.) and COMU<sup>®</sup> (98 mg, 0.23 mmol, 1.3 eq.) were added to a solution of carboxylic acid **12a** (61 mg, 0.19 mmol, 1.1 eq.) in dry THF (8 mL). After the reaction mixture was stirred at room temperature for 30 min, carbazolumine hydrochloride **22**·HCl (60 mg, 0.18 mmol, 1 eq.) was added and stirring was continued for 20 h. Afterwards, all volatiles were removed under reduced pressure and the residue was dissolved in ethyl acetate (30 mL). The organic layer was washed with NaOH solution (10 mL), water (2 x 10 mL) and brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. The residue was purified by fc with a gradient ( $\phi = 2$  cm,  $l = 15$  cm,  $v = 10$  mL, cyclohexane/CH<sub>2</sub>Cl<sub>2</sub> 40:60, 20:80, 0:100, CH<sub>2</sub>Cl<sub>2</sub>/methanol 100:0.5, 100:0.75, 100:1,  $R_f = 0.29$  (cyclohexane/ethyl acetate/formic acid 4:6:0.2)). Colorless solid, mp 212 - 213 °C, yield 49 mg (47 %). Purity (HPLC): 97.6 % ( $t_R = 21.1$  min). C<sub>26</sub>H<sub>21</sub>BrF<sub>2</sub>N<sub>4</sub>O<sub>4</sub>S (603.4 g/mol). Exact mass (APCI):  $m/z = 603.0508$  (calcd. 603.0508 for C<sub>26</sub>H<sub>22</sub><sup>79</sup>BrF<sub>2</sub>N<sub>4</sub>O<sub>4</sub>S [M+H<sup>+</sup>]). <sup>1</sup>H NMR (600 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 3.02 (t,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>CONH), 3.25 (s, 3H, CH<sub>3</sub>), 3.36 (t,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>CONH), 4.74 - 4.85 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>F), 7.45 (td,  $J = 8.5/2.6$  Hz, 1H, 5-H<sub>phenyl</sub>), 7.60 (dd,  $J = 8.8/1.8$  Hz, 1H, 2-H<sub>carb</sub>), 7.69 (d,  $J = 8.8$  Hz, 1H, 1-H<sub>carb</sub>), 7.80 - 7.86 (m, 2H, 8-H<sub>carb</sub>, 3-H<sub>phenyl</sub>), 7.89 (dd,  $J = 8.7/6.1$  Hz, 1H, 6-H<sub>phenyl</sub>), 7.95 (dd,  $J = 8.7/1.7$  Hz, 1H, 7-H<sub>carb</sub>), 8.61 (d,  $J = 1.8$  Hz, 1H, 4-H<sub>carb</sub>), 8.65 (d,  $J = 1.7$  Hz, 1H, 5-H<sub>carb</sub>), 10.25 (s, 1H, CONH). <sup>13</sup>C NMR (151 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 21.8 (1C, CH<sub>2</sub>CH<sub>2</sub>CONH), 32.0 (1C,



CH<sub>2</sub>CH<sub>2</sub>CONH), 43.3 (d,  $J = 19.9$  Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 44.4 (1C, CH<sub>3</sub>), 82.5 (d,  $J = 167.7$  Hz, 1C, CH<sub>2</sub>F), 110.2 (1C, C-8<sub>carb</sub>), 110.4 (1C, C-1<sub>carb</sub>), 111.2 (1C, C-4<sub>carb</sub>), 115.5 (d,  $J = 21.6$  Hz, 1C, C-5<sub>phenyl</sub>), 119.9 (1C, C-2<sub>carb</sub>), 120.1 (1C, C-5<sub>carb</sub>), 121.4 (d,  $J = 25.0$  Hz, 1C, C-3<sub>phenyl</sub>), 121.7 (1C, C-4<sub>a</sub>carb), 121.8 (1C, C-4<sub>b</sub>carb), 122.1 (d,  $J = 10.1$  Hz, 1C, C-2<sub>phenyl</sub>), 124.1 (1C, C-7<sub>carb</sub>), 124.4 (d,  $J = 3.4$  Hz, 1C, C-1<sub>phenyl</sub>), 131.0 (1C, C-6<sub>carb</sub>), 132.5 (1C, C-3<sub>carb</sub>), 133.6 (d,  $J = 9.4$  Hz, 1C, C-6<sub>phenyl</sub>), 137.5 (1C, C-9<sub>a</sub>carb), 142.9 (1C, C-8<sub>a</sub>carb), 162.8 (d,  $J = 253.2$  Hz, 1C, C-4<sub>phenyl</sub>), 166.5 (1C, C-3<sub>oxadiazole</sub>), 168.8 (1C, C=O), 179.6 (1C, C-5<sub>oxadiazole</sub>). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3325 (w, N-H), 2924 (w, C-H, aliph), 1678 (m, C=O), 1593 (w, C-C, arom), 1570 (m, C-C, arom), 1126 (s, SO<sub>2</sub>).

*6.3.18 9-(2-Fluoroethyl)-6-methoxy-1,2,3,4-tetrahydrocarbazol-3-ammonium chloride (25·HCl)*

Hydrazine monohydrate (66  $\mu$ L, 1.4 mmol, 2 eq.) was added to a solution of phthalimide **17** (268 mg, 0.68 mmol, 1 eq.) in ethanol 96 % (10 mL). The reaction mixture was heated at reflux for 2 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate (30 mL) and the organic layer was washed with NaOH solution (1 M, 20 mL). The NaOH solution was extracted with ethyl acetate (2 x 20 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The residue was dissolved in Et<sub>2</sub>O and filtered followed by the addition of a solution of HCl in Et<sub>2</sub>O (2 M, 0.34 mL, 0.68 mmol, 1.0 eq) until the salt **25**·HCl precipitated completely. The product was filtered off, washed with Et<sub>2</sub>O (5 mL) and dried under reduced pressure.  $R_f = 0.35$  (ethyl acetate/methanol/dimethylethylamine 7:3:0.2). Beige solid, mp 225 - 226 °C, yield 122 mg (60 %). Purity (HPLC): 96.6 % ( $t_R = 14.2$  min). C<sub>15</sub>H<sub>20</sub>ClFN<sub>2</sub>O (298.8 g/mol). Exact mass (APCI):  $m/z = 363.1555$  (calcd. 263.1554 for C<sub>15</sub>H<sub>20</sub>FN<sub>2</sub>O [M+H<sup>+</sup>]). <sup>1</sup>H NMR (400 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 1.86 - 1.99 (m, 1H, 2-H), 2.16 - 2.26 (m, 1H, 2-H),

2.66 (dd,  $J = 14.8/8.6$  Hz, 1H, 4-H), 2.73 - 2.93 (m, 2H, 1-CH<sub>2</sub>), 3.03 (dd,  $J = 16.6/3.3$  Hz, 1H, 4-H), 3.47 (s, broad, 1H, 3-H), 3.74 (s, 3H, OCH<sub>3</sub>), 4.33 (dt,  $J = 28.0/4.6$  Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>F), 4.61 (dt,  $J = 47.7/4.3$  Hz, 2H, CH<sub>2</sub>F), 6.71 (dd,  $J = 8.7/2.0$  Hz, 1H, 7-H), 6.87 (d,  $J = 2.0$  Hz, 1H, 5-H), 7.29 (d,  $J = 8.8$  Hz, 1H, 8-H), 8.30 (s, 3H, -NH<sub>3</sub><sup>+</sup>). <sup>13</sup>C NMR (101 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 19.6 (1C, C-1), 25.7 (1C, C-4), 26.6 (1C, C-2), 43.0 (d,  $J = 20.4$  Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 46.9 (1C, C-3), 55.4 (1C, OCH<sub>3</sub>), 82.9 (d,  $J = 167.5$  Hz, 1C, CH<sub>2</sub>F), 99.8 (1C, C-5), 104.7 (1C, C-4a), 110.2 (1C, C-8), 110.3 (1C, C-7), 126.7 (1C, C-4b), 131.7 (1C, C-8a), 134.9 (1C, C-9a), 153.5 (1C, C-6). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3452 (w, N-H), 2900 (m, C-H, aliph), 1589 (w, C-C, arom).

6.3.19 *3-[3-(2-Bromo-4-fluorophenyl)-1,2,4-oxadiazol-5-yl]-N-[9-(2-fluoroethyl)-6-methoxy-1,2,3,4-tetrahydrocarbazol-3-yl]propanamide (26a)*

Under N<sub>2</sub> atmosphere, *N*-ethyl-*N,N*-diisopropylamine (0.13 mL, 0.74 mmol, 3 eq.) and COMU® (137 mg, 0.32 mmol, 1.3 eq.) were added to a solution of carboxylic acid **12a** (85 mg, 0.27 mmol, 1.1 eq.) in dry THF (3 mL). After the reaction mixture was stirred at room temperature for 30 min, tetrahydrocarbazolamine hydrochloride **25**·HCl (74 mg, 0.25 mmol, 1 eq.) was added and stirring was continued for 23 h. Afterwards, all volatiles were removed under reduced pressure and the residue was dissolved in ethyl acetate (30 mL). The organic layer was washed with water (2 x 10 mL) and brine (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. The residue was purified by fc ( $\emptyset = 2$  cm,  $l = 15$  cm,  $v = 10$  mL, cyclohexane/ethyl acetate 70:30,  $R_f = 0.38$  (cyclohexane/ethyl acetate/formic acid 5:5:0.2)). Colorless solid, mp 159 °C, yield 91 mg (66 %). Purity (HPLC): 93.0 % ( $t_R = 22.7$  min). C<sub>26</sub>H<sub>25</sub>BrF<sub>2</sub>N<sub>4</sub>O<sub>3</sub> (559.4 g/mol). Exact mass (APCI):  $m/z = 559.1152$  (calcd. 559.1151 for C<sub>26</sub>H<sub>26</sub><sup>79</sup>BrF<sub>2</sub>N<sub>4</sub>O<sub>3</sub> [M+H<sup>+</sup>]). <sup>1</sup>H NMR (400 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 1.73 - 1.85 (m, 1H, 2-H<sub>carb</sub>), 1.94 - 2.03 (m, 1H, 2-H<sub>carb</sub>), 2.40 - 2.48 (m, 1H, 4-H<sub>carb</sub>), 2.72 (t,  $J = 7.4$  Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>CONH), 2.74

- 2.86 (m, 2H, 1-CH<sub>2carb</sub>), 2.90 (dd,  $J = 15.0/5.2$  Hz, 1H, 4-H<sub>carb</sub>), 3.24 (t,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>CONH), 3.74 (s, 3H, OCH<sub>3</sub>), 4.04 (dt,  $J = 12.0/7.2$  Hz, 1H, 3-H<sub>carb</sub>), 4.32 (dt,  $J = 27.8/4.1$  Hz, 2H, CH<sub>2</sub>CH<sub>2</sub>F), 4.62 (dt,  $J = 47.5/4.7$  Hz, 2H, CH<sub>2</sub>F), 6.70 (dd,  $J = 8.8/2.5$  Hz, 1H, 7-H<sub>carb</sub>), 6.83 (d,  $J = 2.4$  Hz, 1H, 5-H<sub>carb</sub>), 7.27 (d,  $J = 8.8$  Hz, 1H, 8-H<sub>carb</sub>), 7.46 (td,  $J = 8.4/2.6$  Hz, 1H, 5-H<sub>phenyl</sub>), 7.84 (dd,  $J = 8.6/2.6$  Hz, 1H, 3-H<sub>phenyl</sub>), 7.88 (dd,  $J = 8.7/6.1$  Hz, 1H, 6-H<sub>phenyl</sub>), 8.15 (d,  $J = 7.6$  Hz, 1H, CONH). <sup>13</sup>C NMR (101 MHz, DMSO-D<sub>6</sub>):  $\delta$  (ppm) = 20.0 (1C, C-1<sub>carb</sub>), 21.9 (1C, CH<sub>2</sub>CH<sub>2</sub>CONH), 27.3 (1C, C-4<sub>carb</sub>), 28.4 (1C, C-2<sub>carb</sub>), 31.2 (1C, CH<sub>2</sub>CH<sub>2</sub>CONH), 42.9 (d,  $J = 20.4$  Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 45.1 (1C, C-3<sub>carb</sub>), 55.4 (1C, OCH<sub>3</sub>), 82.9 (d,  $J = 167.6$  Hz, 1C, CH<sub>2</sub>CH<sub>2</sub>F), 99.8 (1C, C-5<sub>carb</sub>), 106.4 (1C, C-4a<sub>carb</sub>), 109.9 (2C, C-7<sub>carb</sub>, C-8<sub>carb</sub>), 115.5 (d,  $J = 21.6$  Hz, 1C, C-5<sub>phenyl</sub>), 121.4 (d,  $J = 25.1$  Hz, 1C, C-3<sub>phenyl</sub>), 122.1 (d,  $J = 10.1$  Hz, 1C, C-2<sub>phenyl</sub>), 124.5 (d,  $J = 3.5$  Hz, 1C, C-1<sub>phenyl</sub>), 127.1 (1C, C-4b<sub>carb</sub>), 131.6 (1C, C-8a<sub>carb</sub>), 133.6 (d,  $J = 9.4$  Hz, 1C, C-6<sub>phenyl</sub>), 135.3 (1C, C-9a<sub>carb</sub>), 153.3 (1C, C-6<sub>carb</sub>), 162.8 (d,  $J = 253.3$  Hz, 1C, C-4<sub>phenyl</sub>), 166.5 (1C, C-3<sub>oxadiazole</sub>), 169.4 (1C, C=O), 179.6 (1C, C-5<sub>oxadiazole</sub>). FTIR (neat):  $\tilde{\nu}$  (cm<sup>-1</sup>) = 3302 (w, N-H), 2931 (w, C-H, aliph), 1635 (s, C=O).

#### 6.4 Receptor binding studies

[<sup>3</sup>H]CP55940 displacement assays were used for the determination of affinity ( $K_i$ ) values of ligands for the cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptors. Membrane aliquots containing 5  $\mu$ g (CHOK1hCB<sub>1</sub>\_bgal) or 1  $\mu$ g (CHOK1hCB<sub>2</sub>\_bgal) of membrane protein in 100  $\mu$ L assay buffer (50 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, 0.1 % BSA, pH 7.4) were incubated at 30 °C for 1 h, in presence of 3.5 nM [<sup>3</sup>H]CP55940 (CHOK1hCB<sub>1</sub>\_bgal) or 1.5 nM [<sup>3</sup>H]CP55940 (CHOK1hCB<sub>2</sub>\_bgal). Initially, 1  $\mu$ M of competing ligand was used, followed by six concentrations of competing ligand (between 10<sup>-5.5</sup> M and 10<sup>-10.5</sup> M) when more than 50 % displacement was found at 1  $\mu$ M. Non-specific binding was determined in the presence of 10  $\mu$ M AM630 (CHOK1hCB<sub>2</sub>\_bgal) or 10  $\mu$ M

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 [26] to determine the affinity of the ligands using a  $K_D$  value of [ $^3H$ ]CP55940 of 0.93 nM at CB<sub>2</sub>R.

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## Graphical abstract

