Letter

Development of highly affine and selective fluorinated cannabinoid type 2 receptor ligands

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Development of highly affine and selective fluorinated cannabinoid type 2 receptor ligands

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KEYWORDS: Cannabinoid receptor type 2, Imidazole, Binding affinity, Fluorine, Positron emission tomography.

ABSTRACT: Cannabinoid type 2 receptors (CB_2 receptors) are involved in various pathological processes, and the visualization of their *in vivo* availability with positron emission tomography (PET) is of high interest. The study focusses on the introduction of fluorine into the structure of the highly affine and selective CB_2 receptor ligand *N*-(adamantan-1-yl)-5-ethyl-2-methyl-1-phenyl-1*H*-imidazole-4-carboxamide (**5**). A novel series of compounds was developed by modifying (i) the adamantane-3-position, (ii) the imidazole-*N*-phenyl ring, and (iii) the imidazole-2-position, and the impact on the CB_2 binding affinity and selectivity towards cannabinoid type 1 receptors (CB_1) was evaluated. This study identified compound **15** as one of the most potent ($K_i(CB_2)$ = 0.29 nM) and selective (CB_1/CB_2 >10000) CB_2 receptor ligands discovered so far, eligible for the development of an ^{18}F-labeled PET radiotracer.

Cannabis sativa and its extracts have been used for centuries as therapeutic agent and recreational use.[1] The isolation and structure elucidation of the main psychoactive constituent, (–)-*trans*-Δ^9-tetrahydrocannabinol (THC, **1**) by Mechoulam and co-workers[2] led to the identification of the endocannabinoid system consisting of neuromodulatory lipids and their (G-protein coupled) receptors.[3] Two types of cannabinoid receptors have been well characterized so far, namely the cannabinoid receptor type 1 (CB_1 receptor)[4] and the cannabinoid receptor type 2 (CB_2 receptor).[5] Further receptors like GPR55 and GPR18 are proposed to belong to the cannabinoid family. However, the research on this subtypes is at early stage.[6] CB_1 receptors are located at neurons and their activation is responsible for the psychotropic effect of **1**. Recently, the crystal structure of the CB_1 receptors has been reported facilitating future molecular modeling based drug design and ligand optimization studies for this receptor subtype.[7] CB_2 receptors are non-psychotropic, mainly located peripherally and involved in several immune diseases, neuropathic pain, and cancer.[8] CB_2 receptors are also expressed at low levels in the healthy brain. The physiological role of CB_2 receptors in the brain has long been controversially discussed.[9]

Recent evidence suggests that neuronal CB_2 receptors are involved in the regulation of hippocampal neurotransmission and in signaling pathways in the mouse brain cortex *via* different G_α protein subunits.[10] CB_2 receptors are able to form heteromers with other receptors including CB_1 (mainly post-synaptic) leading to new functionalities and therefore new signaling pathways.[11] In pathological conditions, up-regulation of CB_2 receptors has been reported in association with traumatic brain injury (TBI), neurodegeneration, and apoptosis in several cancer cell lines.[12] Moreover, the neuroprotective role of a CB_2 receptor inverse agonist was demonstrated in a mouse model of TBI.[13]

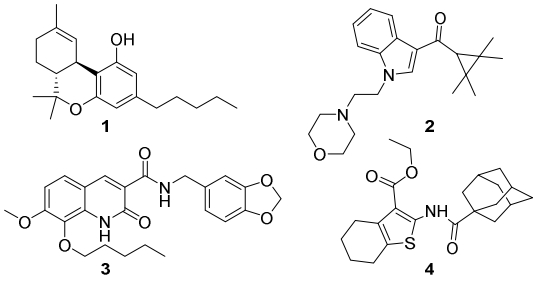


Figure 1. The structures of representative CB_2 ligands.

In the past decades the development of selective CB_2 receptor ligands was in the focus of medicinal chemistry research.[14, 15] However, despite the high number of ligands specifically developed for this target, only a few compounds were considered for clinical trials and none of them has currently been approved for human use.[11] When targeting the cannabinoid receptors in the brain, the predominantly disfavored pharmacological properties of the CB_2 ligands are caused by the hydrophobic nature of the binding site of cannabinoid receptors suitable for binding of the highly lipophilic natural ligands (e.g. **1**, $LogD_{7.4}$ = 6.9). Unlike **1** which passes the blood-brain barrier (BBB), most of the peripherally administered lipophilic substances do not and show high nonspecific binding to fat tissue.[16] Profiling studies were performed to identify ligands with the best molecular pharmacology for targeting the CB_2 receptors, which despite high lipophilic-

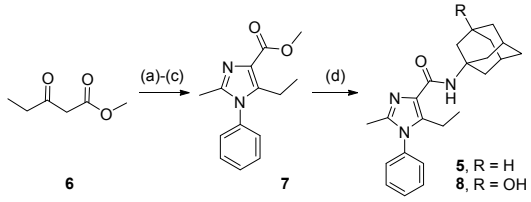
ity are likely to reach the brain, mainly due to the low polar surface area.[17] However, despite the promising therapeutic and diagnostic potential the development of CB_2 receptor ligands with improved neuropharmacological properties remains a challenging task.

The medicinal chemistry of the CB_2 receptor ligands covers various structural motifs.[15] Beside the classical tetrahydrocannabinol-derived cannabinoid ligands,[18] *N*-alkylindole-3-carboxamide is one of the most popular building block of cannabinoid receptor ligands, and efforts to develop CB_2 receptor selective ligands proved to be fruitful as shown by compound **2** (A-796260) in Figure 1.[19] Quinoline is also a widely used scaffold for the development of CB_2 receptor ligands and representatively exemplified in Figure 1 by compound **3** (JTE-907).[20] Unlike indoles, the quinoline type ligands generally possess high selectivity towards the CB_1 receptor. In the past years, several ^{11}C- and ^{18}F-labeled ligands have been developed for this scaffold[21, 22] including [^{11}C]NE40, the first and only CB_2 receptor radioligand which has been tested in humans to date.[23] More recently, a novel series of pyrrole and thiophene derived compounds has been reported, some of them with high affinity for the CB_2 receptor and selectivity towards the CB_1 receptors as exemplified by compound **4** ($K_i(CB_2)$ = 2.15 nM, $K_i(CB_1)$ = 1008 nM).[24] Consequently, the imaging properties of an ^{11}C-labeled analog of **4** have been investigated by *in vitro* and *in vivo* autoradiography and PET studies by Haider and co-workers.[25]

In our previous efforts to develop fluorinated CB_2 receptor (radio)ligands we used oxazole, thiazole[26] and indole[27] as scaffolds. Recently, we reported the development of a highly affine and selective ^{18}F-labeled CB_2 radiotracer ($K_i(CB_2)$ = 0.4 nM, $K_i(CB_1)$ = 380 nM) and proved its suitability in a mouse model of neuroinflammation,[26] however, this radioligand suffers from low metabolic stability *in vivo*. Here, we redirected our focus on the structure of the highly affine and selective *N*-(adamantan-1-yl)-5-ethyl-2-methyl-1-phenyl-1*H*-imidazole-4-carboxamide (**5**, Scheme 1).[28] Compound **5** was reported by Lange and co-workers[28] as a result of a thorough SAR study and its suitable pharmacological properties have been highlighted (e.g.: CB_1/CB_2 >10000, MW = 349, $LogP_{HPLC}$ = 3.5, PSA = 47).

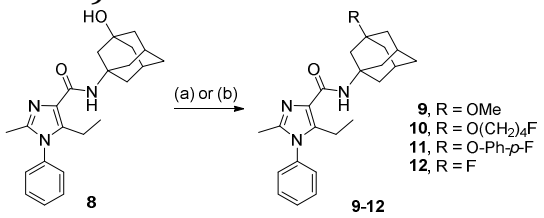
The aim of the presented work was the synthesis of a fluorinated CB_2 ligand based on the structure of compound **5** (Scheme 1) as prerequisite for the development of a novel ^{18}F-labeled radiotracer for imaging of the cerebral CB_2 receptors with PET. The newly synthesized derivative should retain the high CB_2 affinity and selectivity of the lead compound **5** and should contain a fluorine atom at a position which allows a facile incorporation of ^{18}F. Previous SAR studies performed on various CB_2 ligands, including the work which led to the identification of **5**[28] highlighted the need of a lipophilic (e.g. tetramethylcyclopropyl, myrtanyl or adamantyl) subunit as pharmacophore. However, recent studies showed the possibility to hydroxylate the adamantane 3-position without loss of affinity towards the CB_2 receptors[22, 25] opening the possibility to fluoroalkoxylate this position of the molecule. On the other side, the phenyl subunit has been less explored and its ability to tolerate fluoroderivatization needs to be investigated especially due to the robustness and metabolic stability of aryl fluorides.[29] In parallel we decided to introduce fluorine also at the imidazole-2-position and check the influence on the CB_2 receptor binding affinity.

Scheme 1. Synthesis of the lead 5 and derivative 8[a]



[a]Reagents and conditions: (a) $NaNO_2$, $AcOH/H_2O$, 0 °C to rt., 2 h (77%); (b) H_2, Pd/C, Ac_2O, AcOH 1 atm., rt., 20 h (quantitative); (c) aniline, TFA, butyronitrile, 117 °C, 1.5 h (24%); (d) i. LiOH, H_2O/MeOH, 70 °C, 5 h; ii. 1M HCl, rt.; iii. 1-adamantylamine for **5** and 3-amino-1-hydroxyadamantane for **8**, BOP, Et_3N, DCM, rt., 20 h (30% over two steps).[28]

Scheme 2. Synthesis of adamantane functionalized derivatives 9-11[a]



[a]Reagents and conditions: (a) RX, NaH, DMF (**9**, 14%; **10**, 19%; **11**, 42%); (b) DAST, DCM, −78 °C to rt. (**12**, >90%).

The synthesis of the lead compound **5** and its hydroxyadamantane derivative **8** was performed as described and depicted in Scheme 1 starting from the commercially available methyl 3-oxopentanoate (**6**).[28] Nitration of **6** gave the respective oxime which was further acetylated with acetic anhydride under catalytic (H_2, Pd/C) reductive reactions conditions, followed by condensation with aniline in presence of TFA at elevated temperature and concluded with cycloaromatization to imidazole **7**. Ester hydrolysis followed by Castro's reagent (BOP) mediated amide bond formation with amantadine or 3-aminoadamantan-1-ol as coupling partner delivered compounds **5** and **8** respectively (Scheme 1).

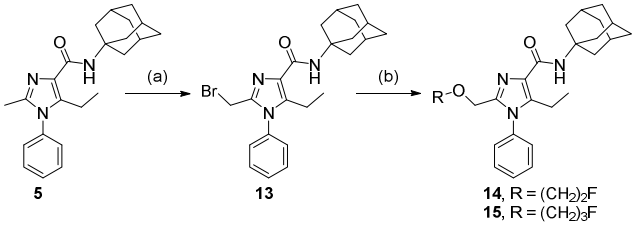
With large amounts of **8** in our hands various reactions conditions were investigated to etherify the alcohol of the adamantine subunit. First, compound **8** was reacted with excess of MeI under deprotonative reaction conditions (NaH) in DMF to provide **9** in 14% yield after 22 hours at room temperature. Large amounts of **8** remained unreacted. The reaction yield could not be enhanced by increasing the reaction time or the temperature. Attempts to synthesize a fluoroethoxy or a fluoropropoxy analogue by reacting the alcohol **8** with various electrophiles (e.g. 1-fluoro-2-iodoethane, 1-fluoro-3-iodopropane, and the corresponding mesylates and triflates) in presence of a base (NaH up to 5 equivalents) failed in delivering the desired ether presumably due to the low nucleophilicity of the bulky alcohol combined with the volatility and instability of the nucleophiles at elevated temperature. However, the 1-fluorobuthoxy derivative **10** was synthe-

sized in 19% yield by using 10 equivalents of 1-fluoro-4-bromobutane at 80 °C for 22 hours. Benzylether **11** was also synthesized according to this procedure (Scheme 2).

Furthermore, the DAST promoted fluorodeoxygenation of **8** was performed to deliver **12** in nearly quantitative yield. However, a "classical" (S_N2) radiofluorination procedure would not be applicable for the radiosynthesis of [^{18}F]**12** and other methods would need to be developed.

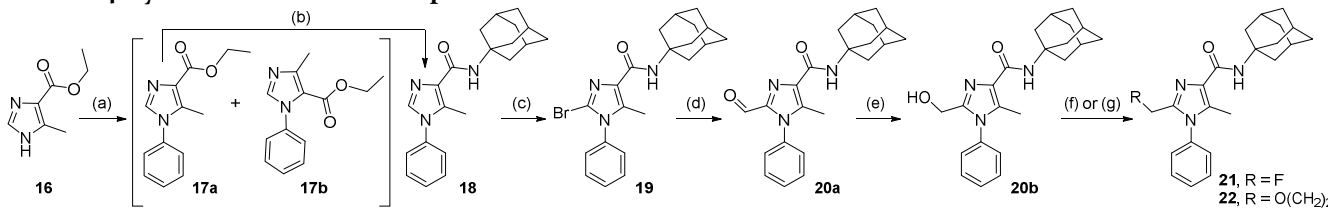
It has previously been shown that both methyl and ethyl substituents are well tolerated at the imidazole-2-position without altering the binding affinity towards CB_2 receptors and therefore, we redirected our attention at this position in our efforts to introduce the fluorine atom. Treatment of **5** with NBS under radical reactions conditions employing AIBN delivered a complex reaction mixture from which **13** was isolated in 30% yield. The low yield and the difficult purification can be explained by the competitive high reactivity of the imidazole-5-ethyl group. Treatment of **13** with 2-fluoroethanol and 3-fluoropropanol in presence of Cs_2CO_3, smoothly provided ethers **14** and **15**, respectively. Encouraged by these preliminary results, we decided to develop an alternative, more efficient method for the functionalization of imidazole at the 2-position. For this, we designed the 2-bromoimidazole key intermediate **19** starting from the commercially available **16**. In the first step, Chan-Lam coupling resulted in formation of **17a** (24% yield, together with its regioisomer, **17b**, 34% yield, Scheme 4),[28] which was then coupled with 1-adamantylamine to the amide **18**, and further selectively brominated with NBS at the second position to give **19**. Bouveault reaction, using DMF as reactant formed the respective aldehyde (**20a**) which was smoothly reduced by $NaBH_4$ in presence of MeOH to give alcohol **20b**. Compound **20b** was converted by the DAST mediated fluorodeoxygenation into the fluoromethyl derivative **21** in good yield and *via* Williamson ether synthesis into the fluoroethoxy derivative **22**.

Scheme 3. Synthesis of imidazole-2-position functionalized derivatives 14 and 15[a]



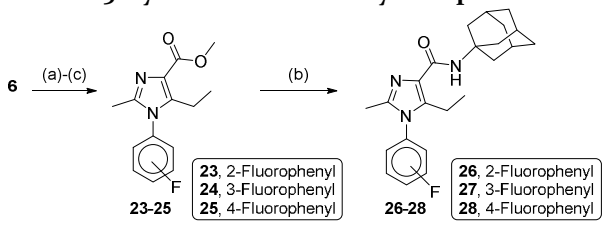
[a]Reagents and conditions: (a) NBS, AIBN, CCl_4, 77 °C, 6 h (30%); (b) R-OH, Cs_2CO_3, MeCN, 40 °C, 60 min (>90%).

Scheme 4. Synthesis of imidazole-2-position functionalized derivatives 21 and 22[a]



[a]Reagents and conditions: (a) $C_6H_7BO_2$, CuI (cat.) EtOH/H_2O, 85 °C, 60 h (**17a**, 24%, **17b**, 34%); (b) 1-adamantylamine, $AlMe_3$, DCM, 35 °C (40% from **17a**) (c) NBS, MeCN, rt., 4 h (85%); (d) i. LDA, THF, –78 °C, 30 min, ii. DMF, –78 °C to rt.; (e) $NaBH_4$, MeOH, 0 °C, 30 min (30% over two steps); (f) DAST, DCM, –78 °C, 30 min, **21**, 91%; (g) 1-bromo-2-fluoroethane, DMF, NaH, **22**, 83%.

Scheme 5. Synthesis of fluoroaryl compounds 26-28[a]



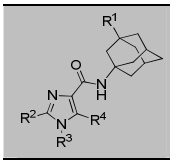
[a]Reagents and conditions: for (a)-(b) see Scheme 1; (c) fluoroaniline, TFA, butyronitrile, 117 °C, 1.5 h (**23**, 12% yield; **24**, 15% yield; **25**, 18% yield); (d) 1-adamantylamine, $AlMe_3$, DCM, 35 °C, 22 h (**26**, 31 % yield; **27**, 47% yield; **28**, 33% yield).

To further explore the structural options of compound **5**, the impact of the introduction of a fluorine atom at the phenyl ring on the CB_1/CB_2 binding affinities was investigated. Therefore, the three regioisomers **26**, **27** and **28** were synthesized by using the corresponding fluoroanilines (*o*-, *m*-, and *p*-, respectively) as source of fluorine by using the same synthesis sequence as shown in Scheme 1. Weinreb amidation employing $AlMe_3$ delivered the fluoroaryl derivatives **26**-**28** in moderate yield (Scheme 5, 3-5% yield over four steps).

All the herein reported compounds were investigated *in vitro* for their binding affinities towards the CB_2 and CB_1 receptors according to a protocol well established in our lab.[26] For the CB_2 assay, [^{3}H](*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN55.212-2, **29**) was used as competitive radioligand (K_D = 2.1 nM) together with increasing concentrations of the synthesized (100 pM to 10 µM) on CHO cells stably transfected with the human CB_2 receptor (by courtesy of Prof. Prather, University Arkansas for Medical Sciences, Little Rock, USA). The non-specific binding was determined by using 10 µM [^{3}H]**29**. The CB_1 binding affinity was determined with hCB_1-CHO obtained from Euroscreen, Gosselies, Belgium and [^{3}H](–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol (CP55.940, **30**) as competitive radioligand.[26]

As reflected by the K_i values in Table 1 the hydroxylation at the adamantane subunit in compound **5** led to a drastic drop of affinity towards CB_2 receptors proving the need of a lipophilic partial structure at this site of the molecule (compound **8**).

Table 1. In vitro binding affinity (K_i) at the human CB_2 and CB_1 receptors



	R^1	R^2	R^3	R^4	$K_i(CB_2)$ [nM][a]	$K_i(CB_1)$ [nM][a]
5	H	Me	Phenyl	Et	2.98 ± 0.33 (1.03 ± 0.2)[b]	>10000
8	OH	Me	Phenyl	Et	101 ± 18.5	>1000
9	OMe	Me	Phenyl	Et	69.3 ± 15.7	>10000
10	$O(CH_2)_4F$	Me	Phenyl	Et	13.9 ± 1.8	>10000
11	4-Fluorobenzyl ether	Me	Phenyl	Et	7.44 ± 1.0	NA
12	F	Me	Phenyl	Et	1.0 ± 0.2	32620
14	H	$CH_2O(CH_2)_2F$	Phenyl	Et	1.1 ± 0.2	7600
15	H	$CH_2O(CH_2)_3F$	Phenyl	Et	0.29 ± 0.02	>10000
18	H	H	Phenyl	Me	201 ± 16.5	>10000
21	H	CH_2F	Phenyl	Me	4.16 ± 3.5	5000
22	H	$CH_2O(CH_2)_2F$	Phenyl	Me	3.45 ± 0.6	9200
26	H	Me	2-Fluorophenyl	Et	5.56 ± 0.28	>10000
27	H	Me	3-Fluorophenyl	Et	3.41 ± 0.18	>10000
28	H	Me	4-Fluorophenyl	Et	10.2 ± 0.2	>10000

[a]Means (±) of two to three experiments run in triplicate; [b]K_i of compound **5** as reported in [28]; NA = not available.

This was, however, rather unexpected when compared with the related quinoline-2-one-3-carboxamide[22] thiophene-2-carboxamide derived CB_2[25] receptor ligands, for which the hydroxylation of the adamantan subunit was tolerated without loss of binding affinity. Methoxylation at this position led to a slight increase in affinity whereas butoxylation nearly restored the low-nanomolar binding affinity of the starting compound **5** profiling the lipophilicity dependent binding mode towards the CB_2 receptor. Additionally, the implementation of the 4-fluorobenzyl as substituent at this position further improved the binding affinity towards the CB_2 receptors. Replacement of the hydroxyl group by fluorine yielded derivative **12** which slightly surpassed the low-nanomolar affinity of the lead molecule with a K_i value of 1 nM.

Derivatization performed at the imidazole-2-position revealed the distinct need of a substituent, as proven by the low CB_2 binding affinity of the non-substituted compound **18** (Table 1).

Accordingly, the binding data of imidazole-2-fluoromethyl derivative **21** correlated with the low-nanomolar CB_2 affinity of the lead compound **5**. The introduction of an ether function and simultaneous chain elongation at this position did not considerably alter the binding affinity as demonstrated by the imidazole-2-fluoroethoxylated compounds **14** and **22**. Notably, the use of a fluoropropoxy ether at this part of the molecule enhanced the binding affinity towards the CB_2 receptors to sub-nanomolar level (0.29 nM, Table 1). Furthermore, the three fluorophenyl regioisomers (**26**, **27** and **28** respectively) possess high CB_2 affinity ($K_i(CB_2)$ ≤10 nM) and CB_1/CB_2 selectivity (>1000) revealing a moderate influence of the phenyl ring on the ligand binding mode to the CB_2 receptor. Considering this, the implementation of a 2-fluoropyridin subunit at this position (not described) might be tolerated too affording ligands with enhanced eligibility for aromatic radiofluorination. Notably, for all the herein reported derivatives the CB_1 receptor affinity remained constantly low (>1 µM).

In summary, a novel series of CB_2 fluorinated 1-aryl-imidazole-4-yl-carboxamide CB_2 receptor ligands has been synthesized by varying the 1-aryl subunit, the imidazole 2-position, and the adamantane. First *in vitro* investigations revealed a moderate tolerability of an ether function at the adamantane subunit with a binding affinity towards the CB_2 receptor in good correlation with the lipophilicity of the alkoxy group. On the other hand the introduction of the fluorine at the phenyl ring has only a minor impact on the CB_2 receptor binding affinity. Despite the unfavorable electron density for an S_N2 substitution, the radiofluorination at this position might be possible due to the recent developments in the methodology of the late-stage ^{18}F-labeling.[30] However, the implementation of more suitable surrogates at this subunit (e.g. 2-fluoropyridine) needs to be considered. The most suitable position for fluoroderivatization was discovered to be the imidazole position-2. As a result, the 2-(3-fluoropropoxy)methyl-1*H*-imidazole derivative **15** was identified with sub-nanomolar binding affinity (K_i = 0.29 nM) combined with an excellent selectivity against the CB_1 receptor subtype (CB_1/CB_2 >10000).

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org and includes detailed experimental pro-

cedures, compounds characterization and ^{1}H NMR of final compounds.

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Author Contributions

R.-P. M. designed the study. R.-P. M. and K.H. conceived and performed the chemical syntheses. W. D.-C. and P. B. planned and performed the radioligand binding studies. All authors contributed to and approved the final version of the manuscript.

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ABBREVIATIONS

AA, ammonium acetate; CB_2, cannabinoid receptors type 2; CB_1, cannabinoid receptors type 1; PET, positron emission tomography; DMF, *N*,*N*-dimethylformamide; BOP, (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; Et_3N, triethylamine; CHO, chinese hamster ovary; EA, ethyl acetate; PE, Petroleum ether (35-60 °C).

REFERENCES

1. Hanus, L. O.; Meyer, S. M.; Munoz, E.; Taglialatela-Scafati, O.; Appendino, G. Phytocannabinoids: a unified critical inventory. *Nat. Prod. Rep*. **2016**, *12*, 1357-1392.

2. Gaoni, Y.; Mechoulam, R. Isolation, structure, and partial synthesis of an active constituent of Hashish. *J. Am. Chem. Soc*. **1964**, *86*, 1646-1647.

3. Reggio, P. H. Endocannabinoid binding to the cannabinoid receptors: What is known and what remains unknown. *Curr. Med. Chem*. **2010**, *17*, 1468-1486.

4. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **1990**, *346*, 561-564.

5. Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **1993**, *365*, 61-65.

6. Brown, A. J. Novel cannabinoid receptors. *Br. J. Pharmacol*. **2007**, *152*, 567-575.

7. Shao, Z.; Yin, J.; Chapman, K.; Grzemska, M.; Clark, L.; Wang, J.; Rosenbaum, D. M. High-resolution crystal structure of the human CB1 cannabinoid receptor. *Nature* **2016**, doi: 10.1038/nature20613.

8. Chakravarti, B.; Ravi, J.; Ganju, R. K. Cannabinoids as therapeutic agents in cancer: current status and future implications. *Oncotarget* **2014**, *5*, 5852-5872.

9. Rogers, N. Cannabinoid receptor with an identity crisis' gets a second look. *Nat. Med*. **2015**, *21*, 966-967.

10. Kendall, D. A.; Yudowski, G. A. Cannabinoid receptors in the central nervous system: their signaling and roles in disease. *Front. Cell. Neurosci*. **2017**, 10: 294.

11. Navarro, G.; Morales, P.; Rodríguez-Cueto, C.; Fernández-Ruiz, J.; Jagerovic, N.; Franco, R. Targeting cannabinoid CB2 receptors in the central nervous system. Medicinal chemistry approaches with focus on neurodegenerative disorders. *Front. Neurosci*. **2016**, 10:406.

12. Giacoppo, S.; Mandolino, G.; Galuppo, M.; Bramanti, P.; Mazzon, E. Cannabinoids: new promising agents in the treatment of neurological diseases. *Molecules* **2014**, *19*, 18781-18816.

13. Bu, W.; Ren, H.; Deng, Y.; Del Mar, N.; Guley, N. M.; Moore, B. M.; Honig, M. G.; Reiner, A. Mild traumatic brain injury produces neuron loss that can be rescued by modulating microglial activation using a CB2 receptor inverse agonist. Front. Neurosc. **2016**, 10:449.

14. Morales, P.; Hernandez-Folgado, L.; Goya, P.; Jagerovic, N. Cannabinoid receptor 2 (CB2) agonists and antagonists: a patent update. *Expert Opin. Ther. Patents* **2016**, *26*, 843-856.

15. Aghazadeh Tabrizi, M.; Baraldi, P. G.; Borea, P. A.; Varani, K. Medicinal chemistry, pharmacology, and potential therapeutic benefits of cannabinoid CB2 receptor agonists. *Chem. Rev*. **2016**, *116*, 519-560.

16. Pajouhesh, H.; Lenz, G. R. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx*. **2005**, *2*, 541-553.

17. Soethoudt, M.; Grether, U.; Fingerle, J.; Grim, T. W.; Fezza, F.; de Petrocellis, L.; Ullmer, C.; Rothenhausler, B.; Perret, C.; van Gils, N.; Finlay, D.; MacDonald, C.; Chicca, A.; Gens, M. D.; Stuart, J.; de Vries, H.; Mastrangelo, N.; Xia, L.; Alachouzos, G.; Baggelaar, M. P.; Martella, A.; Mock, E. D.; Deng, H.; Heitman, L. H.; Connor, M. Cannabinoid CB2 receptor ligand profiling reveals biased signalling and off-target activity. *Nat. Commun*. **2017**, *8*, 13958.

18. Bow, E. W.; Rimoldi, J. M. The structure–function relationships of classical cannabinoids: CB1/CB2 modulation. *Perspect. Medicin. Chem*. **2016**, *8*, 17-39.

19. Eissenstat, M. A.; Bell, M. R.; D'Ambra, T. E.; Alexander, E. J.; Daum, S. J.; Ackerman, J. H.; Gruett, M. D.; Kumar, V.; Estep, K. G. Aminoalkylindoles: structure-activity relationships of novel cannabinoid mimetics. *J. Med. Chem*. **1995**, *38*, 3094-3105.

20. Mu, L.; Bieri, D.; Slavik, R.; Drandarov, K.; Müller, A.; Cermak, S.; Weber, M.; Schibli, R.; Kramer, S. D.; Ametamey, S. M. Radiolabeling and *in vitro*/*in vivo* evaluation of *N*-(1-adamantyl)-8-methoxy-4-oxo-1-phenyl-1,4-dihydroquinoline-3-carboxamide as a PET probe for imaging cannabinoid type 2 receptor. *J. Neurochem*. **2013**, *126*, 616-624.

21. Slavik, R.; Grether, U.; Müller Herde, A.; Gobbi, L.; Fingerle, J.; Ullmer, C.; Krämer, S. D.; Schibli, R.; Mu, L.; Ametamey, S. M. Discovery of a high affinity and selective pyridine analog as a potential positron emission tomography imaging agent for cannabinoid type 2 receptor. *J. Med. Chem*. **2015**, *58*, 4266-4277.

22. Slavik, R.; Herde, A. M.; Bieri, D.; Weber, M.; Schibli, R.; Kramer, S. D.; Ametamey, S. M.; Mu, L. Synthesis, radiolabeling and evaluation of novel 4-oxo-quinoline derivatives as PET tracers for imaging cannabinoid type 2 receptor. *Eur. J. Med. Chem*. **2015**, *92c*, 554-564.

23. Ahmad, R.; Postnov, A.; Bormans, G.; Versijpt, J.; Vandenbulcke, M.; Van Laere, K. Decreased in vivo availability of the cannabinoid type 2 receptor in Alzheimer's disease. *E. J. Nucl. Med. Mol. Imaging* **2016**, *43*, 2219-2227.

24. Osman, N. A.; Ligresti, A.; Klein, C. D.; Allarà, M.; Rabbito, A.; Di Marzo, V.; Abouzid, K. A.; Abadi, A. H. Discovery of novel tetrahydrobenzo[*b*]thiophene and pyrrole based scaffolds as potent and selective CB2 receptor ligands: The structural

elements controlling binding affinity, selectivity and functionality. *Eur. J. Med. Chem*. **2016**, *122*, 619-634.

25. Haider, A.; Müller Herde, A.; Slavik, R.; Weber, M.; Mugnaini, C.; Ligresti, A.; Schibli, R.; Mu, L.; Mensah Ametamey, S. Synthesis and biological evaluation of thiophene-based cannabinoid receptor type 2 radiotracers for PET imaging. *Front. Neurosci*. **2016**, 10:350.

26. Moldovan, R.-P.; Teodoro, R.; Gao, Y.; Deuther-Conrad, W.; Kranz, M.; Wang, Y.; Kuwabara, H.; Nakano, M.; Valentine, H.; Fischer, S.; Pomper, M. G.; Wong, D. F.; Dannals, R. F.; Brust, P.; Horti, A. G. Development of a high-affinity PET radioligand for imaging cannabinoid subtype 2 receptor. *J. Med. Chem.* **2016**, *59*, 7840-7855.

27. Moldovan, R.-P.; Deuther-Conrad, W.; Horti, A.; Brust, P. Synthesis and preliminary biological evaluation of indol-3-yl-oxoacetamides as potent cannabinoid receptor type 2 ligands. *Molecules* **2017**, *22*, 77.

28. Lange, J. H. M.; van der Neut, M. A. W.; Wals, H. C.; Kuil, G. D.; Borst, A. J. M.; Mulder, A.; den Hartog, A. P.; Zilaout, H.; Goutier, W.; van Stuivenberg, H. H.; van Vliet, B. J. Synthesis and SAR of novel imidazoles as potent and selective cannabinoid CB2 receptor antagonists with high binding efficiencies. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 1084-1089.

29. Kuchar, M.; Mamat, C. Methods to increase the metabolic stability of ^{18}F-radiotracers. Molecules **2015**, 20, 16186-16220.

30. Preshlock, S.; Tredwell, M.; Gouverneur, V. ^{18}F-Labeling of arenes and heteroarenes for applications in positron emission tomography. *Chem. Rev.* **2016**, *116*, 719-766.

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