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Highly potent and selective cannabinoid receptor 2 agonists: Initial hit optimization of an adamantyl hit series identified from high-through-put screening

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ABSTRACT

A series of highly potent & selective adamantane derived CB2 agonists was identified in a high-throughput screen. A SAR was established and physicochemical properties were significantly improved. This was accompanied by potency of the compounds on the Q63R variant and varying β-arrestin data which will support the insight into their relevance for the in vivo situation.

Throughout evolution the mammalian body has developed numerous protective mechanisms to prevent and limit tissue injury caused by various types of neuronal as well as non-neuronal insults. Lipid signaling through activation of cannabinoid 2 (CB2) receptors is thought to be an important part of this protective machinery.[1] Inflammation/tissue injury causes a rapid increase in local endocannabinoid levels which leads to a fast modulation of signaling pathways in immune and other cells. It has been reported that endocannabinoids, endocannbinoid-like and/or synthetic CB2 receptor agonists positively affect a large number of pathological conditions, spanning from cardiovascular,[2] over gastrointestinal,[3] liver,[4] kidney,[5] lung,[6] neurodegenerative[7] and psychiatric[8] disorders to pain,[9] cancer,[10] bone,[11] reproductive system[12] and skin pathologies.[13] Prototypical CB2 receptor agonists range from endogeneous ligands such as anandamide (AEA)[14] and 2-arachidonoyl glycerol (2-AG)[15] over the plant-derived Δ9-tetrahydrocannabinol ((−)-Δ^9-THC)[14] to exogenous cannabinoid type agonists such as HU-308,[14] JWH-133[16] and HU-910[4] as well as even newer non-cannabinoid type agonists which are described in several recent overviews.[17] These agonists have various degrees of activities and possess different selectivity against the cannabinoid receptor 1 (CB1).[18]

With the goal to identify new CB2 receptor agonist hit structures, a high-throughput screen was performed. Various hit clusters were found. One cluster entailed a considerable number of adamantyl-derivatives with more than 30 members suggesting a specific interaction of this moiety with the CB2 receptor. A very preliminary SAR could be deduced from that data set. In general, all compounds showed high selectivity towards the CB1 receptor with considerably potency range at the CB2 receptor. Representative examples are shown in Table 1.

The majority of compounds in this cluster were amide based derivatives, however also carbamoylthioyl-amides were found, like example **1** with an EC_{50} of 1.4 μM at the CB2 receptor and being inactive at the CB1 receptor. Heteroaryl derivatives were active as well, exemplified with thiazolyl-derivative **2** which was already active in the high nano-molar range. Substituted aryl-amide derivatives yielded a very preliminary SAR. The substitution on the aryl-moiety seemed to be determining the activity at the CB2 receptor. Substituents in the 4-position (like in example **3**) yielded compounds active in the μM range. The nature of the substituent in the 2-position influenced the activity considerably. Derivative **4** was active with 100 nM at the CB2 receptor, compound **5** was active in the low nano-molar range (EC_{50} = 15 nM) and both

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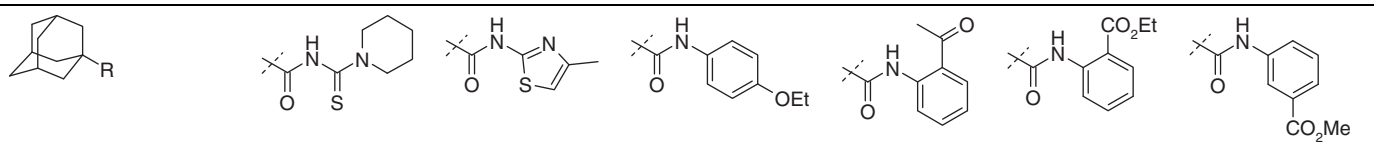
Table 1
Initial SAR from representative members of the adamantyl hit cluster[19]



No.	1	2	3	4	5	6
hCB2: cAMP EC_{50} (μM)	1.4	0.6	1.2	0.1	0.015	>10
hCB2: β-arrestin EC_{50} (μM)	0.5	0.9	—	—	0.076	—
hCB1: cAMP EC_{50} (μM)	>10	>10	>10	>10	>10	>10
*c*log*P*, MW	4.0, 306	3.5/327	4.4/299	4.1/297	4.7/313	4.2/313

compounds had EC_{50}s well above 10 μM at the CB1 receptor. Shifting the ester moiety to position **3** yielded inactive derivative **6**. The ability of CB2 agonists to recruit β-arrestin, which ultimately leads to the steric inhibition of G protein coupling and termination of signaling independent of G protein classes, was determined. The fact that various partial agonists might constitutively stimulate receptor activation without causing β-arrestin recruitment might be considered as a crucial parameter for the translation of in vitro potency into in vivo efficacy.[20] Compounds **1** and **2** showed an EC_{50} in the β-arrestin assay in the high nano-molar range and derivative **5** was active with an EC_{50} of 76 nM. Calculated molecular properties of these derivatives revealed in general a low molecular weight of ~300 Da and *c*log*P* values ~4. This adamantyl-cluster offered a preliminary SAR on the human CB2 and CB1 cAMP read-out and on the activation potential of the human CB2 β-arrestin. Several derivatives in this adamantyl-cluster displayed high ligand efficiencies[21] with *c*log*P* values generally ~4 or above. JWH-133 and HU-910 are both highly lipophilic compounds (*c*log*P*: 7.99 and 8.79, respectively) and active in the cAMP assay in the low nano-molar range (4.3 nM and 5.3 nM, respectively) which corroborated our confidence into the HTS derived adamantyl-cluster being a valuable starting point for optimization. Adamantane groups are well known moieties in approved drugs (i.e., Amantadine or Vildagliptin) useful in the treatment of diseases related to the CNS as well as diseases to be treated peripherally.[22] The adamantane moiety, optimally substituted, can entail favorable properties like high bioavailability, good metabolic stability and half-life. Literature analysis[23] yielded a competitor compound **7**, an analog of a clinically evaluated derivative, which was reported with sub-nano molar activity at the CB2 receptor to have some selectivity for the CB1 receptor. Superimposition and fitting into the homology model of the hit structure **5** and adamantane derivative **7** yielded similar specific interactions thus even further supporting our confidence in this hit-cluster in combination with CB2 agonism (Fig. 1).

There is a nice match of pharmacophoric features of the hydrophobic adamantane, the aromatic moiety as well as donor/acceptor features of the amide and inverse amide, respectively. The vector at the aromatic moiety has the same directionality. Thus, the conformation of the HTS hit is determined through intra-molecular H-bond interaction and therefore suggested the replacement of the anthranilic acid moiety through bicyclic systems offering opportunities for optimization of these CB2 receptor agonists. To prove this initial hypothesis, adamantane concept compounds with varying bi-cyclic amide substitutions were designed. The synthesis entails a one-step transformation of an activated adamantane acid derivative with the respective amine moiety to access novel derivatives (**8–11**) which were profiled under various aspects; that is, CB2 function and binding, additionally CB2 β-arrestin function and preliminary molecular properties (i.e., lipohilicity (log*D*), solubility (LYSA), permeability (Pe) and stability in human microsomes (MAB-maximal achievable bioavailability)[24] (Table 2).[25] Furthermore, functional activity on the Q63R variation of the CB2 receptor was assessed. This relevant polymorphism is suggested to be associated, for example, with liver damage in obese children,[26] increases of risk of celiac disease,[27] and with the risk for schizophrenia[28] and therefore might have significant impact on the efficacy of CB2 receptor agonist derived drugs for the human situation.

As expected from our 'modeling hypothesis' the tetrahydroquinoline is a good replacement for the anthranilic acid moiety. Already the un-substituted compound **8** was active in the nano-molar range on functional CB2 with high selectivity for CB1.[29] Micromolar activity in the binding assay was observed, the compound was though inactive on human CB2 β-arrestin. In the CB2 human Q63R variant the compound was as well active in the nano-molar range. Preliminary molecular properties assessment confirmed the compound to be insoluble with a MAB value of 23% in human microsomes. Further substitution of the tetrahydroquinoline with a methoxy functionality yielded derivative **9** which was even more

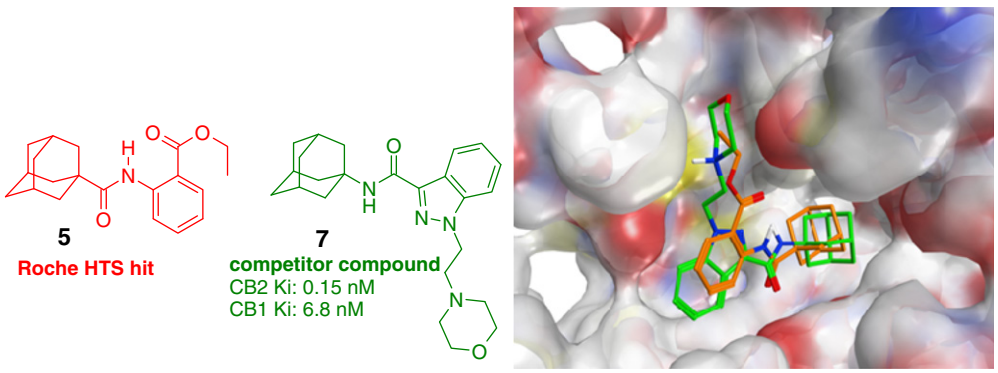


Figure 1. Superimposition of compound **5** (orange) and **7** (green) in the binding site of a CB2 homology model.