Polycyclic Maleimide-based Scaffold as New Privileged Structure for Navigating the Cannabinoid System Opportunities

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S Supporting Information

ABSTRACT: The discovery of the relevant role played by a dysregulation of the endogenous cannabinoid system in several pathological conditions has prompted an extensive research in this field. In this Letter, a series of cannabinoid receptor ligands bearing a previously unexplored polycyclic scaffold was designed and synthesized, in order to evaluate the potential of a new easily affordable privileged structure. The new compounds showed an appreciable affinity and a significant selectivity for the CB2 receptor and are endowed with an intriguing noncompetitive antagonist behavior. Due to the ability of the polycyclic structure to be easily modified in different ways, these compounds could represent convenient chemical tools to be exploited in order to better understand the endocannabinoid system impact on physiopathological conditions.

KEYWORDS: Polycyclic scaffold, Diels–Alder reaction, endocannabinoid system, cannabinoid receptors, CB2 antagonist

The presence of an endogenous cannabinoid system (ECS) was discovered while attempting to understand the effects induced in humans by the use of *Cannabis Sativa*.¹ It has now become clear that ECS dysregulation is connected to pathological conditions, and thus, its modulation has gained enormous potential for intervention in multiple areas of human health. ECS is a neuromodulatory system found both in the brain and in the periphery. It consists of two G protein-coupled receptors, known as the cannabinoid type 1 (CB1) and type 2 (CB2) receptors, endogenous ligands, of which anandamide (*N*-arachidonoyl-ethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) are the best characterized, and the enzymes that regulate their production and degradation.¹ The CB1 receptors (CB1Rs) are primarily located in the central nervous system (CNS) and represent a therapeutic target that may impact pathways that mediate pain, hunger, neurodegenerative disorders, and drug-seeking behavior, even if detrimental side effects, including psychoactivity, depression, and suicidal thoughts, could be observed. On the contrary, the CB2 receptors (CB2Rs) are mainly distributed in peripheral tissues and immune cells, and therefore, they play significant roles in pathologies involving an inflammatory component (such as pain, inflammatory bowel disease, atherosclerosis, osteoporosis, and cancer).² In particular, with respect to cancer, pharmacological activation of the CB2R has been shown to produce antitumor effects in different cancer types. Changes in the expression of this receptor were reported in human cancers and a correlation between its expression, histologic grade, and prognosis has been demonstrated in

breast cancer,³ glioma, hepatocellular carcinoma, pancreatic cancer, endometrial carcinoma, and leukemia.^{4–6}

However, the presence of CB2-positive cells in the brain during injury and in inflammatory neurodegenerative disorders might provide a novel strategy for cannabinoid-mediated intervention against stroke-induced neurodegeneration, without the unwanted psychoactive effects related to CB1R stimulation.⁷ CB2Rs are also detected in glial cells, and in particular, they are overexpressed in A β plaque-associated microglia, suggesting a crucial role in Alzheimer's disease (AD).⁸ Indeed, several studies have shown that A β -mediated activation of microglia induces the production of various proinflammatory mediators that cause neuronal dysfunction and cell death, suggesting its involvement in AD.⁹ The identification of cannabinoid receptors and their endogenous lipid ligands has triggered an exponential growth of studies aiming at exploring the ECS and its regulatory functions in health and disease. Notably, in spite of the relevant number of CB1 and CB2 ligands currently in clinical trials, only few synthetic compounds have reached the market. The development of new structures able to bind these receptors can thus be considered a valuable goal in a medicinal chemistry perspective. In this context, the use of "privileged structures",

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term first proposed by Evans¹⁰ in the late 80s and widened in its meaning during the years, could represent a feasible strategy in the drug discovery process. A privileged structure can be described as a simple structural subunit able, when properly substituted, to bind different targets. Therefore, the availability of versatile and easily affordable or adaptable structures represents a valuable advantage in exploring new validated privileged scaffolds.¹¹ In recent years, our research group has revalued the simple and convenient Diels–Alder adduct between anthracene and maleimide (**1**, Figure 1) from a

Figure 1. Design strategy for the studied compounds 2–13.

medicinal chemistry point of view. Indeed, this polycyclic structure could easily be modified in different ways, i.e., by selecting the properly substituted anthracene derivative and/or by introducing appropriate side chains on the imide nitrogen. Following this strategy, compounds showing different activities were discovered, such as calcium antagonists,^{12,13} glycogen synthase kinase (GSK-3- β) inhibitors,¹³ chemosensitizers, and proapoptotic agents.¹⁴ In this Letter, taking into account that

literature reports 1,5-diphenylimidazolidine-2,4-dione derivatives as cannabinoid receptor ligands (**A**, Figure 1),¹⁵ the potential of the polycyclic core **1** as privileged structure in designing new compounds able to bind these receptors was explored. To this aim, polymethylene chains (6–8 methylene units) were first introduced on the imide nitrogen, to mimic the side chain of classical natural and synthetic ligands. Then, commercially available lipophilic-substituted anthracenes were coupled with maleimide in order to obtain a small series of variously decorated compounds (**2–13**, Figure 1).

According to Schemes 1 and 2, the reported compounds were prepared via Diels–Alder cycloaddition, refluxing in toluene or xylene the properly selected anthracenes with maleimide (final compounds **1**, **11**, and **12** and intermediates **16–19**) or maleic anhydride (to obtain **20**). When the selected anthracenes were not commercially available, they were prepared as reported in Scheme 1, i.e., by reductive amination, reacting anthracen-9-carboxaldehyde with heptylamine followed by treatment with NaBH₄ (**15**) or by alkylation of 9-hydroxymethylantracene with heptyl bromide (**14**). Finally, the N-substituted compounds **2**, **3**, **5–10**, and **13** were obtained by alkylation of the corresponding Diels–Alder adduct with the selected bromoalkane. Compound **4** was synthesized by treating intermediate **20** with heptylamine in refluxing toluene in a Dean–Stark apparatus (Scheme 2).

The effect of the new compounds on [³H]-CP55940 binding to human recombinant cannabinoid CB1 and CB2 receptors

Scheme 1. Synthesis of the Compounds 1–3 and 5–13^a

^aReagents and conditions: (i) heptylamine, NaBH₄, EtOH, rt; (ii) 1-bromoheptane, NaH, THF, rt/100 °C; (iii) maleimide, toluene/xylene, reflux; (iv) 1-bromoheptane, *t*BuOK, DMSO, rt; (v) 1-bromohexane, *t*-BuOK, DMSO, rt.

Scheme 2. Synthesis of Compound 4^a

^aReagents and conditions: (i) maleic anhydride, toluene, reflux; (ii) N-octylamine, toluene, reflux.

Table 1. Effect of Compounds 1–13 on [³H]-CP55940 Binding to the Human Recombinant CB1 and CB2 Receptors

compd	R	R ₁	R ₂	IC ₅₀ on CB ₁ (μM)	K _i on CB ₁ (μM)	IC ₅₀ on CB ₂ (μM)	K _i on CB ₂ (μM)
1	H	H	H	>10	>10	>10	>10
2	C ₆ H ₁₃	H	H	>10	>10	8.14 ± 2.3	2.06 ± 0.6
3	C ₇ H ₁₅	H	H	9.35 ± 1.2	3.77 ± 0.5	7.94 ± 1.6	2.01 ± 0.4
4	C ₈ H ₁₇	H	H	>10	>10	8.74 ± 2.1	2.22 ± 0.5
5	C ₇ H ₁₅	Br	H	>10	>10	>10	>10
6	C ₇ H ₁₅	Cl	H	>10	>10	9.88 ± 1.1	2.51 ± 0.2
7	C ₇ H ₁₅	CH ₃	H	>10	>10	3.86 ± 0.3	0.98 ± 0.1
8	C ₆ H ₁₃	CH ₃	H	>10	>10	3.93 ± 0.8	1.01 ± 0.2
9	C ₇ H ₁₅	CH ₂ NHC ₇ H ₁₅	H	>10	>10	>10	>10
10	C ₇ H ₁₅	CH ₂ OC ₇ H ₁₅	H	>10	>10	>10	>10
11	H	CH ₂ NHC ₇ H ₁₅	H	>10	>10	3.93 ± 0.6	1.01 ± 0.1
12	H	CH ₂ OC ₇ H ₁₅	H	>10	>10	0.9 ± 0.1	0.23 ± 0.01
13	C ₇ H ₁₅	H	Cl	>10	>10	6.85 ± 0.8	1.74 ± 0.02

was analyzed. The activities, expressed as IC₅₀, were determined by nonlinear regression of the inhibition of radioligand binding exerted by increasing concentrations of test compounds. When the IC₅₀ value was lower than 10 μM, the K_i value was calculated by applying the Cheng–Prusoff equation to the IC₅₀ value. The binding data of the new compounds for CB1R and CB2R are reported in Table 1.

From these data, it can be noticed that the unsubstituted polycyclic scaffold 1 was devoid of activity on both receptors. Conversely, compounds carrying side chains of six (2) and eight (4) methylene units showed an appreciable selectivity for CB2R, with a K_i in the low micromolar range. Unexpectedly, compound 3, bearing an *N*-heptyl chain, showed a comparable affinity for both receptors, with only a slight preference for CB2. For a better understanding of this behavior, and to evaluate the impact of the substitution pattern on selectivity, the *N*-heptyl chain was maintained, and different substituents were inserted in positions 9 and 2 of the structural core (R₁ and R₂, respectively, Figure 1). The introduction of halogens in position 9 (R₁) led to intriguing results: compound 5, bearing a Br atom, proved to be inactive on both receptors, while the corresponding Cl derivative 6 showed a selectivity profile comparable to 2. Interestingly, a similar trend was also noticed when the Cl was introduced as R₂ (13). However, in this *N*-heptyl R₁-substituted subset, the most interesting result was obtained introducing a methyl to give compound 7, endowed with high selectivity for CB2R and a K_i value in the submicromolar range. Considering that compound 2, with R₁ = H and bearing a six methylene units side chain showed considerable selectivity for the CB2R,

compound 7 was modified by substituting the heptyl chain with an hexyl one, attempting to further increase activity and selectivity. The obtained derivative 8 showed the same selectivity for CB2 as 7 and a comparable affinity. Taken together, these first data clearly indicate that the presence of a side chain of appropriate length is a crucial prerequisite for receptor affinity and that small structural differences markedly affect potency and selectivity. Accordingly, the CH₃ proved to be the best R₁ substituent. Starting from this result and to better investigate the role of lipophilicity, a further heptyl chain was bound to the methyl group of 7 through an amine (9) or ether (10) linker, unfortunately leading to a loss of activity. In contrast, a remarkable increase in CB2R binding affinity was observed when the *N*-heptyl chain was removed from 9 and 10, leading to derivatives 11 and 12, respectively. In particular, the ether compound 12 proved to be the most active in the whole series, with a K_i value = 0.23 μM. These last results suggest for compounds 11 and 12 an optimal global length of the side chain (globally nine atoms) and the best positioning (R₁) among the compounds reported in this series. The ether linker was also slightly preferred to the amine one. However, it seems clear that the simultaneous presence of long chains in both *N*- and R₁ led to a loss of activity, maybe due to unfavorable steric contribution.

Compound 12 was then selected to explore the functional activity at CB2R by a cAMP Hunter assay, to measure how it modulates intracellular cAMP levels in CHO cells over-expressing CB2R. When tested under the stimulus of NKH-477 (a water-soluble analog of forskolin), compound 12 did not lead cAMP content back to the basal condition up to the

highest concentration tested (25 μM , data not shown). Moreover, the selected compound did not show capability of displacing an EC_{80} CB2R-ligand challenge (3 μM of JWH-133), suggesting that it could act as a noncompetitive antagonist (data not shown). Schild plot analysis showed antagonist-induced parallel shifts of agonist concentration–response curves (Figure 2A) and confirmed a noncompetitive behavior, as the slope of linear regression was less than unity (Figure 2B).

Figure 2. Concentration–response curves of compound 12 in cAMP-based functional assay. (A) JWH-133 dose–response curves alone and in the presence of various concentrations of compound 12. (B) Schild plot analysis and slope value of linear regression derived by plotting the $\log(\text{CR} - 1)$ against the $\log$ of all the concentrations tested in the presence of compound 12. CR (concentration ratio) was calculated by dividing the EC_{50} of JWH133 in the presence of compound 12 by the concentration producing the same response in its absence.

From these data, the presence of an allosteric site on the CB2R could be speculated, leading to potential therapeutic advantages: an allosteric modulator may induce a selective “tuning” of drug effects, differently from an orthosteric ligand, which may also continuously affect receptor function. In this respect, allosteric modulation at CB2R could be regarded as an unexplored stimulating approach for treating a number of pathological conditions, as it has been shown for CB1R.

Compound 12, the most promising cannabinoid ligand in this series, contains a polycyclic core previously not investigated in this field that could indeed be recognized as a new privileged structure in medicinal chemistry. In this respect, the choice of a suitable set of substituents allowing discrimination between different targets represents an undeniable prerequisite for ensuring the lack of unwanted effects. Considering that compounds bearing this structural motif had been already reported as calcium channel ligands and GSK-3 β inhibitors,¹³ the binding of 12 to these targets was also evaluated. In particular, both the percentage blockade of Ca^{2+} entry elicited by K^+ 70 mM-evoked depolarization, evaluated in

SH-SY5Y cells,¹³ and the percentage inhibition of GSK-3 β , assessed by means of the LANCE Ultra TR-FRET assay,¹³ proved to be very low (data not shown), corroborating the ability of this derivative to differentiate between diverse unrelated targets.

In summary, in this study an easily affordable privileged structure has been exploited in order to target cannabinoid receptors, and a series of selective CB2R ligands was identified. The ability of the new compounds to act as noncompetitive antagonists can be deemed as a relevant added value, considering that, despite several studies involving CB1 ligands, less is known about CB2R potential. Moreover, even though the relevant number of new structurally unrelated scaffolds was patented in the last years as CB2R ligands, only few of them were reported as antagonists.¹⁶ Therefore, these compounds can be considered as useful tools for further assessing the impact of CB2Rs in physiopathological conditions and for paving the way to a further optimization process leading to a better understanding of the opportunities offered by the cannabinoid system in health and disease.

■ ASSOCIATED CONTENT

Supporting Information

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Synthetic procedures, analytical data, and biological assay details (PDF)

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■ DEDICATION

Dedicated to Professor Piero Valenti on the occasion of his 80th birthday.

■ ABBREVIATIONS

ECS, endogenous cannabinoid system; AEA, N-arachidonoyl-ethanolamine; 2-AG, 2-arachidonylglycerol; CB1R, CB1

receptors; CB2R, CB2 receptors; AD, Alzheimer's disease; GSK-3 β , glycogen synthase kinase.

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