The Cannabinoid Receptors

The Receptors

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The Cannabinoid Receptors

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Preface

The identification of the cannabinoid CB1 receptor as the mediator of short-term and a mediator of long-term retrograde inhibition of synaptic transmission has changed the cannabinoid field profoundly. For with this discovery, the CB1 receptor moved from a G-protein-coupled receptor (GPCR) associated predominantly with the drug abuse field, into the neuroscience mainstream. Compared with other neurotransmitter systems, the endocannabinoid system is quite unique. The endogenous cannabinoid ligands, N-arachidonoylethanolamine (anandamide) and sn-2-arachidonoylglycerol (2-AG) are not small cationic ligands stored in vesicles, but rather are lipophilic ligands synthesized on demand from the lipid bilayer itself. Previously discovered ligands of the CB1/CB2 receptors, including those derived from cannabis, share the characteristic of high lipophilicity with these endogenous cannabinoids.

The CB1 receptor has been shown to have a high level of ligand-independent activation (i.e., constitutive activity) in transfected cell lines, as well as in cells that naturally express the CB1 receptor. This property likely is essential for the receptor to maintain a *cannabinoid tone* in the central nervous system (CNS). This property also permits the receptor to be modulated not only by agonists, but also by inverse agonists. The CB2 receptor, found predominantly in the immune system, also exhibits high levels of constitutive activity and inverse agonists for CB2 have been identified.

The cannabinoid receptors CB1 and CB2 each couple to intracellular G proteins (predominantly via Gi/Go proteins) in order to transduce agonist binding into a cellular response. Signaling by the two receptors can differ markedly, as indeed can signal transduction through each individual receptor in response to various ligands. The divergence of signaling is regulated at various stages – from G-protein coupling to activation of effectors, and in many cases appears to be cell-type specific. The intracellular domains important for G-protein coupling differ between each receptor subtype, and differential G-protein activation by agonists has been characterized. Mutation studies of CB1/CB2 have identified regions important for ligand binding, activation, and desensitization. Receptor modeling studies combined with mutation studies have been able to identify the molecular toggle switch for

CB1 activation and have led to molecular design criteria, for example, for the production of neutral antagonists.

Over the centuries, the plant for which the cannabinoid receptor was named, *Cannabis sativa L.*, has been used for a myriad of medicinal purposes, as well as for its psychotomimetic effects. Studies with CB1 knockout mice have shown that the CB1 receptor is primarily responsible for mediating the effects of the psychoactive principal in cannabis, Δ^9-THC. Physiological and behavioral analysis of CB1-knockout mice has provided important new insights into CB1 receptor function in mammals, which include roles in learning and memory, analgesia, appetite regulation, neuroprotection, as well as endocannabinoid-mediated retrograde signaling at synapses. Today, cannabinoid agonists have been suggested to have potential therapeutic uses such as appetite stimulants, analgesics, antiemetics, antidiarrheals, antispasmodics, tumor antiproliferative agents, antiglaucoma agents, and as agents for the treatment of diseases associated with inappropriate retention of aversive memories such as post-traumatic stress disorders and phobias. Cannabinoid CB1 antagonists/inverse agonists have been suggested to have potential therapeutic uses as appetite suppressants and as agents that improve memory.

This book is designed to introduce newcomers to the cannabinoid field. It begins at the molecular level with cannabinoid ligand synthesis and structure–activity relationships; then moves to the molecular pharmacology of the cannabinoid receptors and the endocannabinoid system; and, culminates in the whole animal pharmacology and therapeutic applications for cannabinoid drugs. New putative cannabinoid receptors are also discussed here, as are challenges for future research. It is hoped that this book will serve as a useful guidebook to what continues to be a fascinating field.

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Part I
Cannabinoid Receptor Ligands and Structure–Activity Relationships

Structure–Activity Relationships of Classical Cannabinoids

Raj K. Razdan

Abstract In this chapter an overview of the more recent developments in the structure–activity relationships (SARs) of classical cannabinoids is discussed, especially the profound pharmacological effects produced by various chemical entities in the side chain at C-3, the hydroxyl at C-1, C-11, and hydroxyalkyl chains at C-6. Also cardiovascular studies point to the presence of a novel cannabinoid subtype receptor and the antagonist activity of cannabidiol has opened up new areas for research. Ligands, which had either a unique pharmacological profile, were potent agonists, partial agonists/antagonists, or were CB2 selective, were identified, generating leads with the potential to be drugs in the treatment of various diseases.

Keywords Classical cannabinoids · Tetrahydrocannabinols · Structure activity relationships · Endocannabinoid system · Cannabinoid receptors · Vanilloid receptors · Pharmacological activity · Cannabinol · Cannabidiol · Cardiovascular activity

1 Introduction

It is well known [1–5] that cannabinoid research developed from the study of the pharmacological effects of the plant material from marijuana (*Cannabis sativa*). Earlier work by Adams and Todd had shown on the basis of degradation studies and ultraviolet (UV) that the natural material had a basic tricyclic benzopyran structure with a double bond either at the Δ^9- or Δ^8-position in the alicyclic ring (Fig. 1). They also showed that analogs with a double bond in the 6a,10a-position are not found in the plant, are UVactive, and had a pharmacological profile similar to the natural (–)-Δ^9-Tetrahydrocannabinol (THC) isolated from the plant. Adams and Loewe, using the dog-ataxia test, carried out extensive SAR studies in the series and established that the biological activity varied with the

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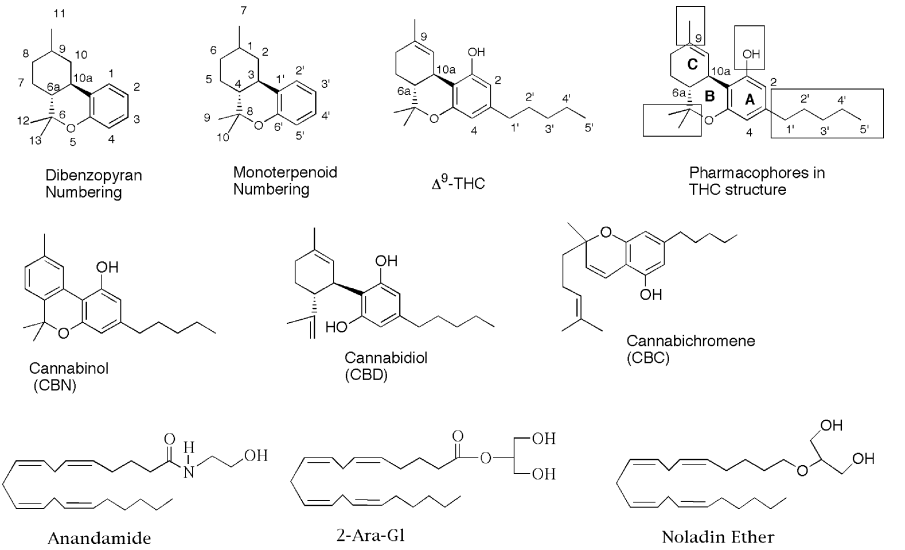


Fig. 1 Tetrahydrocannabinol numbering system, structures of selected natural products, and endocannabinoid system

position of the alkyl substituent in the side chain at C-3. The most potent compound in the series was found to be the 1′,2′-dimethylheptyl-pyran (DMHP) derivative which was about 500 times more potent than synhexyl, an analog with a n-hexyl side chain. They also showed that the natural THC (Δ^9-THC) was several-fold more active than the synthetic analog synhexyl. This work led to the development of various heterocyclic analogs of DMHP and resulted in potent agonists like SP-1, BRL 4664, etc. (Fig. 2). In 1964, the

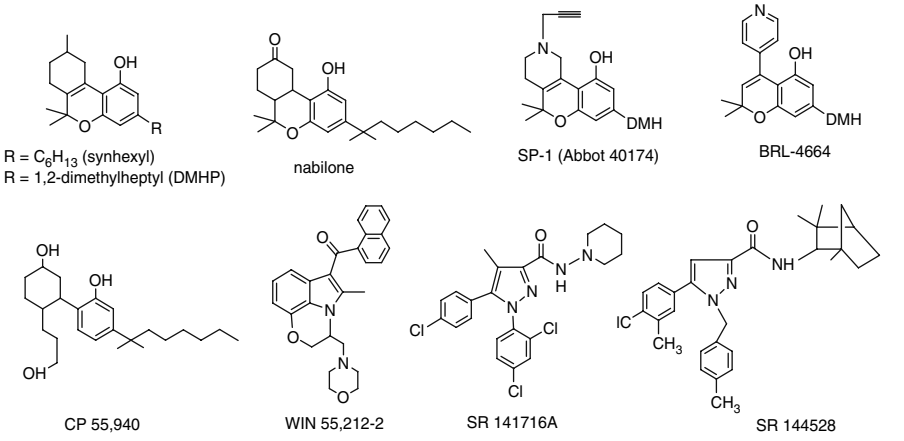


Fig. 2 Structures of selected cannabinoids

elegant work of Gaoni and Mechoulam [6] established, on the basis of nuclear magnetic resonance (NMR), that the position of the double bond in the alicyclic ring was in the Δ^9-position. It was thus determined that the active constituent of the plant, Δ^9-THC, is an ABC-tricyclic ring system having a benzopyran moiety (Fig. 1). At this time several analogs were synthesized and some were isolated from the plant, for example, Cannabinol (CBN) which had the same template as Δ^9-THC. Extensive SARs were developed in the series and these compounds were designated as *Classical Cannabinoids*. Hence, this class includes the natural product (–)-Δ^9-THC, the more stable and nearly equiactive isomer (–)-Δ^8-THC, other active constituents of the plant such as the CBN analogs mentioned above, cannabidiol (CBD) etc., as well as their synthetic analogs especially the $\Delta^{6a,10a}$ analogs developed by Adams and Todd. Although several synthetic strategies have been developed for the synthesis of analogs and metabolites in the Δ^9-THC series [7], most of the SAR studies in the cannabinoid field in the past several years have been carried out in the Δ^8-THC series, dictated mainly by ease of synthesis and the fact that the pharmacological profile of Δ^8-THC is very similar to Δ^9-THC, both in potency and activity.

(–)-Δ^9-THC is a partial agonist and binds equally well to the two G-protein-coupled receptors, CB1 and CB2, discovered in mammalian tissue. CB1 occurs both inside and outside the central nervous system (CNS) and CB2 is found mainly in the periphery [8–10]. The initial SAR studies of classical cannabinoids had pointed to three pharmacophores in the template, the most important being a lipophilic side chain at C-3, the hydroxyl at C-1, and a hydroxyl group at C-11. As a result, several potent agonists were developed. At about the same time the work at Pfizer resulted in the development of nonclassical cannabinoids, such as (–)-CP 55,940 and (–)-CP 55,244, which pointed toward the additional fourth pharmacophore, the southern aliphatic hydroxyl (SAH), and led to the discovery of the CB1 receptor. With this background most of the structural modifications for SAR studies [4, 5, 10–12] were carried out in the pharmacophores mentioned above. The importance of these sites has been borne out by molecular modeling studies [13–16] and is well accepted in the field.

In this article, an overview of the more recent developments in the SAR of classical cannabinoids is presented. These developments have resulted in several ligands which are either potent CB1 agonists, partial agonists/antagonists, or CB2 selective agonists.

2 SAR Studies

2.1 Aliphatic Side Chain at C-3

In recent years extensive SAR studies were carried out on modification of the aliphatic side chain including the effect of chain length and its substitution by methyl groups, substitution by various groups such as halogen, cyano, amido,

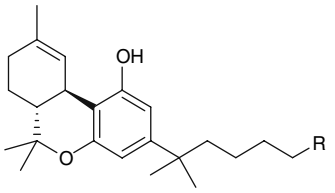
etc., at different carbons particularly the terminal carbon of the chain, the introduction of the rigid acetylene group/double bond at various positions in the side chain and other changes in the side chain.

2.1.1 Chain Length and Its Substitution by Methyl Groups

Cannabinoid activity is retained if the alkyl chain has minimum three to eight carbon atoms, the optimum being five to seven carbons, and is enhanced by the presence of a gem-dimethyl group at C-1′. Activity is also enhanced by the substitution of a 1′,2′-dimethylheptyl group. The former branching pattern is generally used and preferred, as the latter pattern introduces two chiral centers and leads to threo and erythro diastereomeric mixtures. A systematic study of the effect of a methyl substituent on each carbon of the n-pentyl side chain of Δ^9-THC indicates [17–19] that 1′- or 2′-methyl analogs are the most potent and there is relatively little difference between the R and S isomers in either set of compounds. A series of 1′,1′-dimethylalkyl-Δ^8-THC analogs with side chains of 2–12 carbon atoms was studied and showed that even the undecyl analog had significant affinity and was inactive in vivo. A quantitative SAR study of these analogs showed that, for optimum affinity and potency, the side chain must be of a length which will permit its terminus to loop back in proximity to the phenolic ring of the cannabinoid.

2.1.2 Substitution by Various Groups

The nature of the substituent has a profound effect on activity; the substitution of the terminal carbon of the n-pentyl chain in (–)-Δ^8-THCs by a halogen [20] such as a bromo, iodo, or a trifluoromethyl group increased the binding affinity and potency in the tetrad tests 2–40 times while the 5′-fluoro derivative was less active compared to (–)-Δ^8-THC. Similar modest effects in pharmacological profile were noted with the azido and amino substitutions [21]. However, a study of several cyano analogs of 1′,1′-gem-dimethyl-Δ^8-THC showed [22] that they had very high CB1-binding affinity (0.36–13 nM) and high in vivo potency as agonists. Two analogs, **1** and **2** (Fig. 3) had extremely high potency (ED_{50}, 0.0047 and 0.006 mg/kg for the tail-flick and spontaneous activity respectively) in the tetrad tests. The dimethylcarboxamido analog **3** also showed a similar profile of enhanced binding affinity and in vivo activity. In contrast the sulfonamido group can lead to compounds, as in **4**, with a unique profile, which have high binding affinity but are practically devoid of agonist effects. This provides a lead for the development of antagonists with a template different from Sanofi's pyrazole-based CB1 antagonist, SR 141716A. Furthermore, these side-chain derivatives have provided further insights in this cannabinoid pharmacophore. Traditionally, it has been assumed that a hydrophobic pocket accommodates the side chain but this study suggests that, in this region, the presence of a nitrile or a carboxamide group, which is polar but not negatively charged, enhances the interaction between the ligand and the receptor.



	R	CB1 (K_i, nM)	
1. (O-581)	-CN	0.36 ± 0.14	Potent agonist
2. (O-774)	$-CH_2CN$	0.6 ± 0.05	Potent agonist
3. (O-1125)	$-CON(CH_3)_2$	0.86 ± 0.06	Potent agonist
4. (O-606)	-CO-NH–⟨⟩–SO_2NH_2	29 ± 6	No agonist effects

Fig. 3 Effect of various substituents on the terminal carbon of the C-3 chain

2.1.3 Introduction of Regions of Planarity (Acetylene Groups) or Rigid Angles (Cis-Double Bonds) at Various Positions in the Side Chain [23–25]

Although it is well known that the flexible nature of the side chain plays a crucial role in the activation of the cannabinoid receptor, the precise nature of this interaction is not clear. A series of analogs with structurally restrained side chains of varying lengths were therefore studied in mice for their effect on binding affinity and potency. It was found that receptor affinity was the same for the acetylene and saturated side-chain analogs, whereas double bond substitution increased affinity 10-fold. Moreover, the relationship between affinity and potency in some of the acetylene derivatives was found to be 10-fold less than that of Δ^8-THC; however, this potency/affinity ratio was restored when the triple bond was changed to a cis-double bond. Additionally, an acetylene at C2′–C3′ in the octyl and nonyl side chains (**9** and **10**) showed antinociception selectivity by as much as 70-fold (Fig. 4). In contrast, several high-affinity acetylene derivatives, especially those with cyano substitutions at the terminus of the side chain (e.g., O-823, **5**) were partial agonists or were inactive. Some of these low-efficacy, high-affinity ligands, such as O-823, antagonized [25] the effects of cannabinoids in the guinea pig ileum. In a follow-up study they were examined in the GTPγS-binding assay and found to be devoid of agonist effects [26, 27]. These compounds were effective antagonists in that they blocked the agonist effects of several potent cannabinoids in this assay, but were not very effective in blocking the pharmacological effects of Δ^9-THC in vivo. Pretreatment with low doses of these compounds was without efficacy on the in vivo effects of THC in mice whereas high doses tended to increase rather than diminish the effects of Δ^9-THC. It appears that they have very weak agonist effects that mask their antagonist effects.

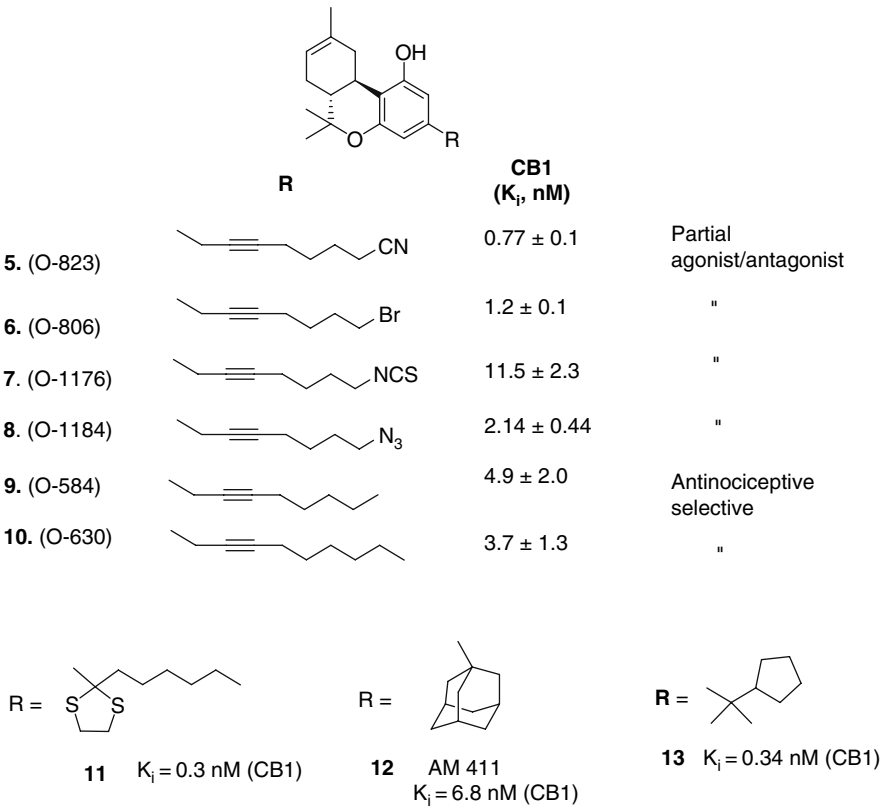


Fig. 4 Selected tetrahydrocannabinols with planar regions and larger groups in the side chain

These side-chain derivatives are the first compounds structurally related to THC that possess partial agonist/antagonist properties. At present an explanation for these unique effects is lacking. The pharmacological selectivity exhibited by some analogs may be explained by multiple transduction pathways for the CB1 receptors. This is supported by recent [28, 29] suggestions of the coupling of CB1receptors to both Gs and Gi/o proteins. However, it is possible that these agonists are interacting with as-yet-unidentified receptors. They seem unlikely to be CB2 receptors as their presence in the brain is questionable and CB2-selective analogs are not active in the tetrad tests.

2.1.4 Other Changes in the Side Chain

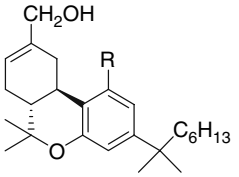
The substitution of the 2′-carbon by a cyclopentyl group with 1′,1′-gem-dimethyl group in the side chain (**13**, Fig. 4) retained [30] very high affinity for both CB1 ($K_i = 0.34$ nM) and CB2 ($K_i = 0.39$ nM) receptors. Even the cycloheptyl analog

retained high affinity to both CB1 ($K_i = 0.94$ nM) and CB2 ($K_i = 0.22$ nM) receptors. It was also found that the 1′,1′-dimethyl substitution in THCs can be replaced by cyclic moieties such as dithiolane, dioxolane, cyclopenty,l etc., with retention of potent affinity to both CB1 and CB2 receptors. In vitro pharmacological testing found the dithiolane analog **11** to be a potent CB1 agonist ($K_i = 0.32$ nM) [31]. Similarly, substitution of an 1-adamantyl group in place of the n-pentyl side chain of Δ^8-THC provided [32] a potent and efficacious CB1 agonist AM411 (**12**). The activity of these THC analogs suggests the presence of a quite large subsite within the binding pocket of CB1 and CB2 receptors.

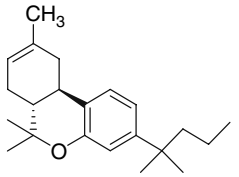
2.2 The Hydroxyl at C-1

From traditional cannabinoid SAR it was known that the presence of a phenolic hydroxyl is very important for eliciting CB1 affinity, and its substitution by a methoxy group, hydrogen or fluorine atom, decreased both CB1- and CB2-binding affinities with marked effects on CB1. Recent work [33–35] has shown (Fig. 5) that even 1-deoxy or 1-methoxy-THCs, appropriately substituted at C-3 and C-11 can retain potent activity at both CB1 and CB2 receptors, and the 1-methoxy-THCs show more CB2 selectivity. SAR studies have indicated that either eliminating the hydroxyl group at C-1 (1-deoxy analogs) or changing it to a methoxy and at the same time decreasing the length of the DMH side chain at C-3 (1′,1′-dimethylbutyl, DMB, being optimal, e.g., **16**) enhances CB2 selectivity, which can be affected by the presence of a hydroxymethyl or an exo-cyclic group at C-9. In a very recent report [36] on the activity of 2′R- and 2′S- 1-methoxy and 1-deoxy-3-(2′-methylalkyl)- Δ^8-THCs with alkyl side chains of three to seven carbon atoms, it was found that all these compounds had greater affinity for the CB2 than the CB1 receptor. Some of them had good affinity for CB2 ($K_i = 13$–47 nM) and little for CB1 ($K_i = 1493$ to $>10{,}000$ nM) receptors. Also in the 1-deoxy series, the 2′S-methyl compounds generally showed greater affinity for the CB2 receptor than the corresponding 2′R isomer (see discussion of CB2 selectivity below).

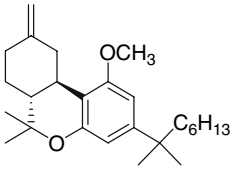
From SAR studies has emerged the development of water-soluble cannabinoids, an area of growing importance and interest. Since cannabinoids are generally very lipid-soluble, solubilizing agents for pharmacological studies are used, but these agents, which have pharmacological effects of their own, can be avoided by making available cannabinoids which are water-soluble. By the formation of various esters of phenols, which hydrolyze at different rates, a series of water-soluble cannabinoids [37, 38] was developed in the early 1970s. A similar approach, and applying it to the recently developed potent THC agonists, led to the development of O-1057 (**20**, Fig. 6). It is a potent agonist [39, 40] and has affinity for both CB1 ($K_i = 8.36$ nM) and CB2 ($K_i = 7.95$ nM) receptors. It inhibits forskolin-stimulated cyclic AMP



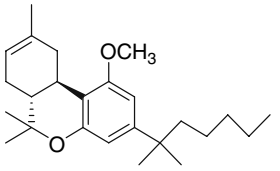
	CB1 (k_i, nM)	CB2 (k_i, nM)	CB1/CB2
14. R = OH (HU-210)	0.73 ± 0.11	0.52 ± 0.05	1.4
15. R = H JWH-051	1.2 ± 0.1	0.032 ± 0.019	37.5



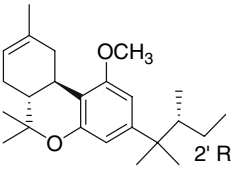
16. 677 ± 132 | 3.4 ± 1.0 | 199



17. > 20,000 | 19 ± 4 | 1052



18. JWH- 229 | 3134 ± 110 | 18 ± 2 | 174



19. JWH- 359 | 2918 ± 450 | 13 ± 0.2 | 224

Fig. 5 Selected tetrahydrocannabinols with CB2 selectivity

production by both CB1- and CB2-transfected CHO cells and has a potency similar to that of CP 55,940 and exceeding that of Δ^9-THC, especially as an analgesic ($ED_{50} = 0.02$ mg/kg, i.v.) and was antagonized by SR 141716A. At present, the potential of O-1057 for clinical application as an analgesic is under investigation.

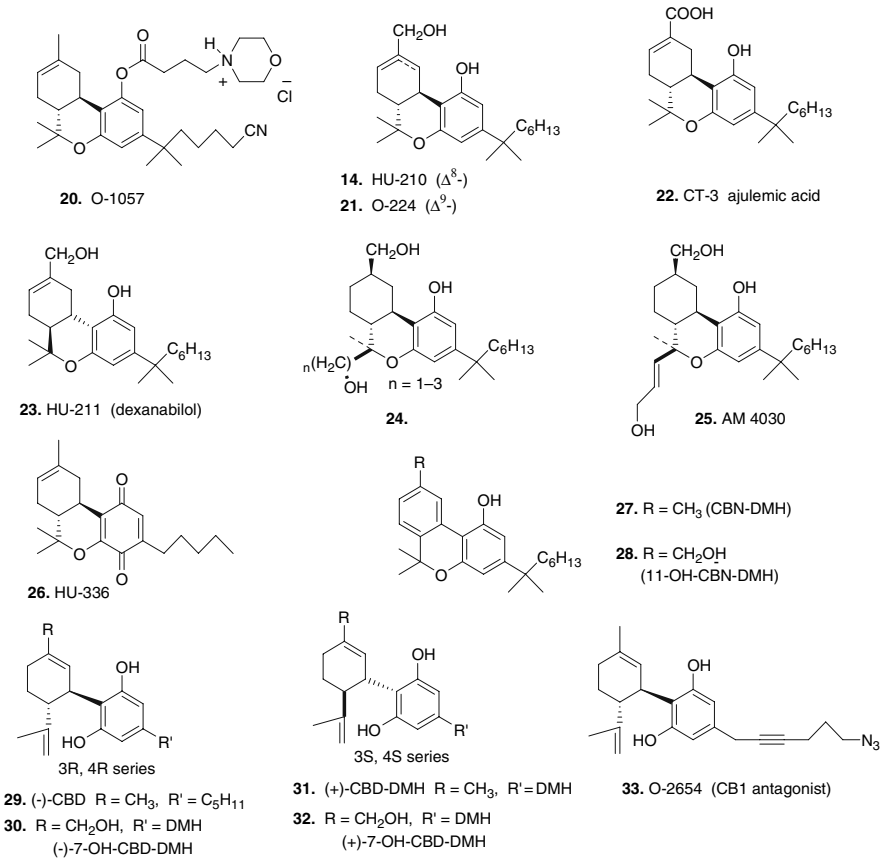


Fig. 6 Selected examples of CB1 ligands

2.3 The C-11 Position in the Alicyclic Ring

SAR studies [41–43] show that the presence of a hydroxyl group at C-11 is not a prerequisite for activity since the 9-nor compound retains activity. However, the metabolite of Δ^9-/Δ^8-THC, (i.e., 11-hydroxymethyl-THC) is approximately three times more active than the parent compound. With this background, the (–)-11-hydroxymethyl derivatives of both Δ^8- and Δ^9-THC-DMH (**14** and **21** respectively, Fig. 6) were synthesized and tested [44–49] for activity. They are (HU-210, **14** in the Δ^8- and O-224, **21** in the Δ^9-series) some of the most potent THCs known to be having very high CB1-binding affinities ($K_i = 0.7$ and 0.4 nM, respectively). It is interesting to note that the enantiomer of HU-210, (i.e., HU-211, dexanabilol **23**) is devoid of (–)-Δ^9-THC-like activity and is presently undergoing clinical development as a neuroprotective agent. This reinforces the importance of stereoselectivity in receptor–ligand interactions.

Even the C-9 aldehyde, also a metabolite, is quite potent ($K_i = 2$ nM) but the C-9 acid [50] has poor affinity ($K_i = 108$ nM) and is much less active. The lack of activity in the acid led to the development of CT-3 (ajulemic acid, **22**), which has shown analgesic/anti-inflammatory effects. It is presently under clinical development.

In general, the incorporation of a hydroxyl group at C-11 increases the binding affinity to CB1 receptors. The position of the double bond in the alicyclic ring has little effect on activity and is Δ^9- > Δ^8- > $\Delta^{6a,10a}$-. However, in the $\Delta^{7,8}$- isomers activity is retained when the 9-methyl group is *beta* but the *alpha*-isomer is much less active. When the double bond is eliminated by reduction, the hexahydro analogs are obtained, which have a 9α or 9β substitution. In this series [51, 52] the analogs retain high affinity and potency to both CB1 and CB2 receptors and it was found that the equatorial, 9β-methyl or hydroxymethyl, analogs are more potent than their axial (9α) counterparts. The activity is, however, adversely affected by substitution by a hydrogen or a fluorine atom at C-9. The presence of a ketone group, as in nabilone (Fig. 2) or a β-hydroxyl at C-9, retains activity. Nabilone is marketed as an agonist for the treatment of nausea in cancer therapy and as an appetite stimulant in AIDS patients.

2.4 Modifications at C-6 Position and the 1:4 Quinones in Ring A of Classical Cannabinoids

2.4.1 Modifications at C-6 Position

The SAR studies in the $\Delta^{6a,10a}$-series by Adams' group had shown that optimum activity was obtained by the presence of a gem-dimethyl group at C-6. Further SAR studies in our laboratory showed [53] that high activity and potency was retained in the (equatorial) 12β-hydroxy-Δ^8-THC. With this background Tius and Makriyannis' groups [12, 54, 55] developed hybrid cannabinoids, which incorporate the structural features of Δ^9-THC and the nonclassical cannabinoid CP 55,940. The binding affinity showed that the equatorial β-hydroxypropyl analog (**24**) had higher affinity than the α-axial epimer at C-6. Further analogs in the series were examined, which had restricted rotation at this site and resulted in very potent analogs such as AM 4030 (**25**, Fig. 6) with high binding affinity to CB1 ($K_i = 0.7$ nM) and CB2 ($K_i = 8.6$ nM).

2.4.2 1:4 Quinones in Ring A

In 1968, Mechoulam's group had reported [56] the formation of a hydroxyquinone from the oxidation of CBD, which cyclized to the corresponding Δ^8-THC under acid conditions. The structure of the CBN quinone derivative has now been confirmed by X-ray crystallography [57]. These 1:4 quinones in the Δ^8-THC (i.e., **26**), CBD and CBN series displayed antiproliferative activity in several human

cancer cell lines in vitro and the CBD analog significantly reduced cancer growth of HT-29 cancer in nude mice. It is interesting to note that these 1:4 quinones do not bind to CB1 receptors and the mechanism of their anticancer activity is unclear.

In summary, it is important to emphasize that classical cannabinoids, in general, bind to both CB1 and CB2 receptors and, as discussed above, it is clear that modifications at the hydroxyl at C-1 and at C-9 result in ligands with CB2 selectivity. An excellent discussion of CB2 selective ligands is reported in a recent review by John Huffman [58]. From a study of 1-methoxy- and 1-deoxy-Δ^8-THCs the following SAR conclusions were drawn: (1) the presence of a 1′,1′-dimethyl-alkyl side chain enhances both CB1- and CB2-binding affinities. However, the length of the chain has more effect on CB1 than on CB2. This is particularly more pronounced in the 1-deoxy-Δ^8-THC series where ligands with very short side chains retain good CB2 selectivity. (2) Introduction of an 11-hydroxy group enhances affinity for both receptors but the enhancement is more for CB1 affinity compared to CB2, and as a result CB2 selectivity is lowered. (3) In general the 1-methoxy analogs show lower binding affinities to both CB1 and CB2 receptors compared to their 1-deoxy counterparts.

The most CB2-selective compound found in the series [36] was JWH-359 (**19**, Fig. 5; $K_i = 2918$ nM for CB1 and 13 nM for CB2; CB1/CB2 = 224) and the compound with the highest affinity to CB2 was JWH-051 (**15**, $K_i = 0.032$ nM for CB2) but it has low selectivity (CB1/CB2 = 37.5) [33].

3 Other Cannabinoids Found in the Plant

It is well documented that numerous other cannabinoids are present in the plant but only a few such as CBN, CBD, and cannabichromene (CBC) have been studied for their biological activity. Limited SAR studies have been carried out in the CBN and CBD series and the conclusions are discussed below.

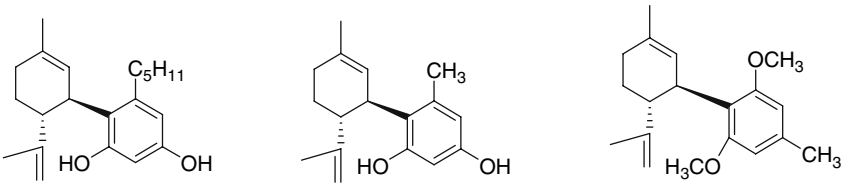
3.1 Cannabinol (CBN)

It is one of the first cannabinoids to be synthesized, which demonstrated the basic skeleton of the THC structure. Interest in CBN analogs increased after it was reported [59] that the affinity of CBN to CB2 receptors was greater than that of Δ^9-THC. The SAR studies were carried out in hopes of getting novel CB2-selective agonists with a CBN template, but none of the analogs showed high CB2 selectivity although some analogs showed high binding affinities to both CB1 and CB2 receptors [60, 61]. The SAR indicate the following: (1) There are differences in the binding profiles of THCs and CBN analogs, and the removal of the phenolic hydroxyl decreases CB1-binding affinity much more in the CBN series than in the THC series. Thus in the 3-(1′,1′-dimethylheptyl) analogs (e.g., **27**) there is a 400-fold decrease in the CBN series versus a 30-fold

decrease in the THC series. (2) In the 1-deoxy-CBN series, when a hydroxyl group is present at C-11, the side chain length has relatively little influence on the selectivity (CB1/CB2 ratio) in contrast to the finding in the THC series. (3) CB1/CB2 selectivity is reduced if the planarity of ring C is increased as in CBN analogs. (4) High CB2-binding affinity was found only when the phenolic hydroxyl was present (e.g., **28**). The only exception was in the 1-deoxy-CBN series when the hydroxyl was at C-11. Thus the presence of a hydroxyl group either at ring C or ring A enhances binding affinity to the CB2 receptor.

3.2 (–)-Cannabidiol (CBD, 29)

It is one of the major constituents of the plant and does not possess any of the psychotropic effects of Δ^9-THC but has several pharmacological effects which have been confirmed in vitro assays and in animal and human tests. CBD does not bind to CB1 or CB2 receptors and even its DMH analogs [62] bind very weakly to both receptors. Thus (–)-CBD-DMH binds to the CB1 receptors with a K_i above 10 μM and to the CB2 receptors with a K_i of 1800 nM. However, much higher affinities to both CB1 and CB2 were found in the enantiomer (+)-CBD-DMH (**31**) ($K_i = 17.4$ and 211 nM, respectively). A similar pattern was observed in the 7-hydroxy series, (+)-7-OH-CBD-DMH (**32**) bound with a K_i of 2.5 nM to CB1 and 44 nM to CB2, while the values were 4400 and 671 nM, respectively, in the (–)-enantiomer (3R, 4R series, **30**). Similarly the metabolites with an acid group at C-7 showed differences in the (+) – and (–) – series. It can be concluded that in CBDs, higher binding affinity to CB1 and CB2 is found in the 3S, 4S series compared to the natural 3R, 4R series. However, it should be noted that not all cannabinoid activities are CB1/CB2-mediated. The pharmacological interaction of CBD and its analogs with vanilloid receptors, their activity for cellular uptake, and their enzymatic hydrolysis of anandamide were reported [63] recently. There is now a great deal of interest in CBD for its therapeutic potential, for example, in the management of epilepsy, as an anti-inflammatory agent, as a neuroprotective antioxidant, etc. Pertwee's group has recently shown [64] that CBD can antagonize the cannabinoid agonists R-(+)-WIN 55,212 and CP 55,940 in the mouse isolated vas deferens, and CBD shares this ability with the CB1 antagonist SR 141716A. It was also found that CBD produces this antagonism at concentrations well below those at which it binds to CB1 receptors and antagonizes α_1-adrenoceptor agonists insurmountably. An SAR study of various CBD analogs showed [65] that O-2654 (**33**, Fig. 6), an analog in which the 4′-pentyl group of CBD was replaced by a 6″-azido-2″-hexyne side chain, was as potent as CBD in producing surmountable antagonism of R-(+)-WIN 55,212 in vasa deferentia. However, this antagonism was produced with a potency ($K_B = 85.7$ nM) which was similar to its CB1-binding affinity (114 nM) suggesting that it is a competitive CB1 receptor antagonist.



34. abnormal-CBD

35. O-1602 agonist

36. O-1918 antagonist

Fig. 7 Selected ligands with cardiovascular activity

This is in contrast to that of CBD. Furthermore it appears that O-2654 may be a neutral CB1 receptor antagonist. This is an interesting finding as it provides a potential template for the development of silent antagonists of CB1 receptors.

Cardiovascular activity is another area where CBD is involved. Cardiovascular studies of cannabinoids by Kunos and co-workers [66–69] has led to the postulation of an endothelial site, distinct from CB1 or CB2 receptors, that contributes to anandamide-induced vasodilation in the mesenteric circulation and possibly elsewhere. The non-CB1 endothelial receptor is coupled to Gi/Go, and abnormal-CBD (**34**, Fig. 7), which does not bind to CB1 receptors, is an agonist and CBD is an antagonist. SAR studies in the two series resulted in the development of O-1602 (**35**) as a more potent agonist and O-1918 (**36**) as a more potent antagonist. Both of them have a methyl group in the benzene ring in place of the n-pentyl side chain, as longer chains decreased activity. The role of cannabinoids in the regulation of blood pressure is most interesting and there is a distinct possibility that these studies will result in ligands, useful in the treatment of cardiovascular disease.

3.3 Cannabichromene (CBC)

CBC is also found in significant quantities in the plant. Except that it is an antimicrobial agent [70] very little is known about its pharmacological effects and no significant SAR studies have been reported in recent years.

4 Concluding Remarks

Much progress has been reported in recent years on SAR studies and on our understanding of the interaction of classical cannabinoids with the cannabinoid CB1 and CB2 receptors. Several important pharmacophore areas have been defined and this should help in the future design of selective ligands and the possible discovery of novel cannabinoid subtype receptors. With the identification

of the *endocannabinoid system*, the acceptance of cannabinoids in medical treatment is assured. From this field one is hopeful that several new drugs will soon become available.

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