Minireview

Natural cannabinoids: Templates for drug discovery

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Abstract

Recent studies have elucidated the biosynthetic pathway of cannabinoids and have highlighted the preference for a C-3 \(n\)-pentyl side chain in the most prominently represented cannabinoids from *Cannabis sativa* and their medicinally important decarboxylation products. The corresponding C-3 \(n\)-propyl side chain containing cannabinoids are also found, although in lesser quantities. Structure–activity relationship (SAR) studies performed on \(\Delta^9\)-tetrahydrocannabinol (\(\Delta^9\)-THC), the key psychoactive ingredient of *Cannabis*, and its synthetic analogues have identified the C-3 side chain as the key pharmacophore for ligand affinity and selectivity for the known cannabinoid receptors and for pharmacological potency. Interestingly, the terminal \(n\)-pentyl saturated hydrocarbon side chain of endocannabinoids also plays a corresponding crucial role in conferring similar properties. This review briefly summarizes the biosynthesis of cannabinoids and endocannabinoids and focuses on their side chain SAR.

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Keywords: \(\Delta^9\)-Tetrahydrocannabinol; Biosynthesis; Anandamide; Endocannabinoids; Side chain SAR

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Introduction

Natural products have long been the source of a great majority of drugs and drug candidates. *Cannabis sativa* L. is one of the oldest known medicinal plants and has been extensively studied with respect to its phytochemistry. The plant biosynthesizes a total of 483 identified chemical entities belonging to different chemical classes (ElSohly, 2002), of which the cannabinoids are the most distinctive class of compounds, known to exist only in this plant. There are 66 known plant-derived cannabinoids, the most prevalent of which are the tetrahydrocannabinins (THCs), the cannabidiols (CBDs), and the cannabinoids (CBNs). The next most abundant cannabinoids are the cannabigerols (CBGs), the cannabinolchromenes (CBCs), and cannabinoinds (CBNDS) (Fig. 1). Most cannabinoids contain 21 carbon atoms, but there are some variations in the length of the C-3 side chain attached to the aromatic ring. In the most common homologues, the \(n\)-pentyl...
side chain is replaced with an n-propyl (De Zeeuw et al., 1972; Vree et al., 1972). These analogues are named using the suffix "varin" and are designated as THCV, CBDV, or CBNV, as examples. Cannabinoids with one (Vree et al., 1972) and four (Smith, 1997) carbons also exist but are minor components.

Classical cannabinoids (CCs) are ABC tricyclic terpenoid compounds bearing a benzopyran moiety (Fig. 2) and are insoluble in water but soluble in lipids, alcohols, and other non-polar organic solvents. These phenolic derivatives are more water soluble as their phenolate salts formed under strong alkaline conditions. \((-\Delta^9\text{-THC})\) is formed by the decarboxylation of its non-psychoactive precursor \(\Delta^9\text{-THCA}\) (1) by the action of light or heat during storage or smoking (Claussen and Korte, 1968; Yamauchi et al., 1967), or under alkaline conditions. \(\Delta^9\text{-THCA}\) (1) is biosynthesized by a well-established pathway involving the action of several specific enzymes which will be presented in the next section.

The identification of cannabinoid receptors in the brain suggested the presence of an endogenous ligand. The search for such a compound led to the discovery of the first endocannabinoid \(N\)-arachidonoyl ethanolamine (AEA, anandamide, 3) (Devane et al., 1992), a highly lipophilic compound susceptible to both oxidation (Burstein et al., 2000; Kozak et al., 2004) and hydrolysis (Cravatt et al., 1996; Giang and Cravatt, 1997; Willoughby et al., 1997). Anandamide (3) was shown to bind to the CB1 receptor with modest affinity \((K_i = 61\,\text{nM})\), to have low affinity for the CB2 receptor \((K_i = 1930\,\text{nM})\) (Lin et al., 1998), and behaves as a partial agonist in the biochemical and pharmacological tests used to characterize cannabinoid activity. Its role as a neurotransmitter or neuromodulator is supported by its pharmacological profile as well as its mechanisms of biosynthesis and bioinactivation.

A second important endocannabinoid, 2-arachidonoylglycerol (2-AG, 4), binds weakly to both CB1 \((K_i = 472\,\text{nM})\) and CB2 \((K_i = 1400\,\text{nM})\) receptors (Mechoulam et al., 1995). 2-AG (4) was isolated from intestinal (Mechoulam et al., 1995) and brain tissues (Stella et al., 1997) and is present in the brain at concentrations approximately 170-fold higher than AEA (3) (Stella et al., 1997).
The common structural features between the plant-derived cannabinoid receptor agonist Δ⁹-THC (2) and the endocannabinoid agonists (AEA (3) and 2-AG (4)) are that both classes of compounds have a polar head group and a hydrophobic chain with a terminal n-pentyl group. Recent work has provided evidence that ligands from both classes have common binding sites (Li et al., 2005; Tian et al., 2005; Picone et al., in press). This is substantiated by structure–activity relationship (SAR) work which revealed a number of similarities between the two classes of cannabinoids. The SAR studies performed on classical cannabinoids represented by Δ⁹-THC (2) and its next generation analogues, the non-classical (Johnson and Melvin, 1986; Little et al., 1988) and hybrid cannabinoids (Chu et al., 2003; Drake et al., 1998; Harrington et al., 2000; Makriyannis and Rapaka, 1990; Thakur et al., 2002; Tius et al., 1997, 1994), have recognized four pharmacophores within the cannabinoid prototype: a phenolic hydroxyl (PH), a lipophilic side chain (SC), a northern aliphatic hydroxyl (NAH), and a southern aliphatic hydroxyl (SAH) (for reviews see: Howlett et al., 2002; Khanolkar et al., 2000; Makriyannis and Rapaka, 1990; Palmer et al., 2000, 2002; Thakur et al., 2005a,b). This review focuses on the SAR of the “lipophilic side chain,” the key pharmacophore which plays a crucial role in determining ligand affinity and selectivity towards cannabinoid receptors as well as pharmacological potency.

In order to provide a clear picture regarding the biochemical origin of the n-pentyl side chain and how it is incorporated into the cannabinoid template, the biosynthesis of Δ⁹-THCA (1) and other major cannabinoid components of Cannabis will be summarized below. The key steps in the biosynthesis of the endocannabinoids will also be highlighted.

### Biosynthesis of cannabinoids and endocannabinoids

Prior to 1990, the precursors of all terpenoids, isopentenyl diphosphate (IPP, 5) and dimethylallyl diphosphate (DMAAP, 6) were believed to be biosynthesized via the mevalonate pathway (Shoyama et al., 1975). Later, it was shown that many plant terpenoids including cannabinoids are biosynthesized via the deoxyxylulose phosphate pathway (Eisenreich et al., 1998; Fellermeier et al., 2001; Rohmer, 1999). The established biosynthetic pathway of Δ⁹-THC acid (1) and other major cannabinoids has been summarized in Scheme 1.

Geranyl pyrophosphate (GPP, 7), obtained from IPP (5) and DMAPP (6), was also shown to partially isomerize to neryl pyrophosphate (NPP, 8). Although there is no direct evidence about the biosynthesis of olivetolic acid (12), its chemical structure suggests a biosynthetic route involving cyclization of a polyketide compound 11 (Raharjo et al., 2004a,b,c). This polyketide is formed by condensation of one molecule of n-hexanoyl-CoA (9) with three molecules of malonyl-CoA (10) catalyzed by polyketide synthase (PKS). Condensation of olivetolic acid (12) and GPP (7) gives cannabigerol acid (CBGA, 13) and is catalyzed by the enzyme geranylpyrophosphate:olivetolate geranyltransferase (GOT) (Fellermeier and Zenk, 1998). This enzyme also accepts NPP (8) as a minor substrate to give cannabigerolic acid (CBNA, 14), which is the precursor for cannabidiolic acid (CBDA, 16) (Taura et al., 1996).

Oxidocyclization of CBGA (13) to cannabichromenic acid (CBCA, 15) is catalyzed by the oxidoreductase enzyme cannabichromenic acid synthase (Morimoto et al., 1998). CBNA (14) is also converted to CBCA (15), although to a lesser extent by the action of this same enzyme. Cannabidiolic acid synthase (CBDA synthase) catalyzes the oxidocyclization of CBGA (13) to CBDA (16) (Taura et al., 1996). The above studies showed that cannabidiolic acid (CBDA, 16) is predominately biosynthesized from cannabigerolic acid (CBGA, 13) and to a lesser extent from cannabigerolic acid (CBNA, 14).

The key cannabinoid precursor Δ⁸-tetrahydrocannabinolic acid (THCA, 1) is formed from CBGA (13) through stereo-selective oxidocyclization involving tetrahydrocannabinolic acid synthase (THCA synthase) (Sirikantaramas et al., 2004; Taura et al., 1995). THCA synthase is a monomeric flavin-labeled enzyme that oxidizes CBGA (13) via a mechanism similar to that of berberine bridge enzyme (BBE) and requires molecular oxygen (Sirikantaramas et al., 2004), unlike CBDA synthase and CBCA synthase, which do not require other co-enzymes, co-factors, or molecular oxygen for enzymatic action (Morimoto et al., 1998; Taura et al., 1995, 1996). CBNA (14) is also converted to THCA (1) by the action of this enzyme, and it was proposed that THCA (1) is formed via a common intermediate in the reactions of CBGA (13) and CBNA (14) (Taura et al., 1995). It is worth noting here that THCA synthase does not convert the corresponding neutral cannabinoids, cannabigerol and cannabigerol to Δ⁸-THC (2), indicating that the presence of the carboxyl group in these substrates is essential for the enzymatic cyclization of terpene moieties.

The biosynthesis of anandamide (AEA, 3) and 2-arachidonoylglycerol (2-AG, 4) in neural cells has been well-reviewed (De Petrocellis et al., 2004; Piomelli, 2004; Ueda et al., 2005).
The biosynthesis of AEA (3) involves the enzymatic transfer of an arachidonoyl group from the sn-1 position of phosphatidylcholine (PC) to the head group of a phosphatidylethanolamine (PE) by an N-acyltransferase (NAT) (Di Marzo et al., 1994). Anandamide (3) is then released by an N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) (Okamoto et al., 2004; Ueda et al., 2005), and possibly to some extent via the corresponding N-arachidonoyllysophosphatidylethanolamine (NA-lyso-PE) (Sun et al., 2004). The biosynthesis of 2-AG (4) from 1,2-diacylglycerols (1,2-DAG) is enzymatically catalyzed by an sn-1-selective diacylglycerol lipase (sn-1-DAGL) (Bisogno et al., 2003; Sugiura et al., 2002). The two known endocannabinoids AEA (3) and 2-AG (4) differ only in their head groups, are both derived from arachidonic acid, and each has the biologically important terminal n-pentyl tail.
Side chain SAR of cannabinoids and endocannabinoids

Variation of the n-pentyl group of natural cannabinoids and endocannabinoids can lead to wide variations in affinity and selectivity for the cannabinoid receptors as well as their pharmacological potencies. In a very early SAR finding, the importance of the side chain was first demonstrated by Adams (Adams, 1942; Adams et al., 1949) where the importance of the side chain was first demonstrated by Adams pharmacological potencies. In a very early SAR finding, the selectivity for the cannabinoid receptors as well as their heptyl analogue was shown to be 100-fold more potent than the n-hexyl analogue in the Δ9,10a-THC series. Subsequent SAR studies on classical cannabinoids (CCs) have recognized the C-3 alkyl side chain as the most critical pharmacophoric group (Howlett et al., 2002; Khanolkar et al., 2000; Makriyannis and Rapaka, 1990; Palmer et al., 2000, 2002; Thakur et al., 2005a). Therefore, the focus of this review of the SAR studies of analogues derived from cannabinoids and endocannabinoids is on the terminal n-pentyl tail.

Side chain length and branching

Decreasing the length of the n-pentyl side chain of Δ9-THC by two carbons (i.e. C-3 n-propyl) reduces potency by 75% (Razdan, 1986). Shortening the length of the side chain in Δ8-THC by one carbon atom (i.e. C-3 n-butyl) results in reduction of affinity for CB1 (Ki = 65 nM) as well as potency, while increasing the side chain length to heptyl, heptyl and octyl provides a systematic increase in affinity (Kd values ranging from 41 to 8.5 nM) and potency (Martin et al., 1999).

The effect of side chain branching with the presence of 1’,1’-dimethyl groups was also further explored for other side chains in tetrahydrocannabinols (Fig. 4, 17–23). Much of the tetrahydrocannabinol SAR involves Δ9-THC (17), the equipotent and thermodynamically more stable isomer of Δ8-THC (2). Thus, 1’,1’-dimethylthyl-Δ9-THC (CB1, Ki = 14.0 nM), 1’,1’-dimethylbutyl-Δ9-THC (CB1, Ki = 10.9 nM), 1’,1’-dimethylpentyl-Δ9-THC (18) (CB1, Ki = 3.9 nM), 1’,1’-dimethylhexyl-Δ9-THC (CB1, Ki = 2.7 nM), and 1’,1’-dimethylpentyl-Δ9-THC (19) (CB1, Ki = 0.77 nM) show significant increases in their binding affinities for the CB1 receptor (Huffman et al., 2003) compared to their non-branched congeners. Affinities increase with increasing chain length up until 1’,1’-dimethyloctyl-Δ9-THC, where it then decreases with further increasing side chain length. The dimethylheptyl side chain was found to be optimal based on pharmacological testing. Replacing the C-3 n-pentyl side chain of THC with a 1’,1’-dimethylheptyl side chain increased the binding affinity 53-fold (Martin et al., 1993). This enhancement was also reflected in the tetrad of the behavioral tests for these compounds namely, spontaneous activity (SA) (14-fold), tail flick (TF) (35-fold), hypothermia (RT) (35-fold) and ring immobility (30-fold) (Martin et al., 1993). Thus, the 1’,1’-dimethyl branching in the side chain brings about significant improvements in affinity and potency of cannabinoids. For this reason the dimethylheptyl side chain has found extensive use during the exploration of other pharmacophores in classical, non-classical, and hybrid cannabinoids (Palmer et al., 2002; Thakur et al., 2005a).

Early on, Adams et al. (1948) found that 1’,2’-dimethyl substitution in the hexyl side chain of the Δ6a,10a-THC analogue (synhexyl) greatly increased potency. This study was performed with a mixture of the eight steroisomers from the three chiral centers. Aaron and Ferguson (1968) first described the synthesis of these eight individual isomers (Δ6a,10a-THCs) and their pharmacological studies which found two of them to be very potent. Subsequently, the synthesis of stereoisomeric mixtures of 3-(1’,2’-dimethylheptyl)-Δ9- and Δ9-THC analogues was reported (Edery et al., 1972; Petrzilka et al., 1969). More recently, the synthesis of all four isomers of 1’,2’-dimethylheptylresorcinol and their corresponding Δ9-THC analogues was carried out by Huffman et al. (1997a), Liddle and Huffman (2001) and Liddle et al. (1998), who showed that the 1’S,2’R isomer 20 has the greatest affinity for CB1 (Ki = 0.46 nM) and is the most potent diastereomer in vivo.

The stereochemical features of an alkyl substituent on the side chain were further explored in a series of Δ9-THC analogues bearing a methyl group at either the C-1’ (21, 22), C-2’, C-3’ or C-4’ (23) positions of the side chain (Huffman et al.

![Fig. 4. Δ9-THC analogues with variations in side chain length and branching.](image-url)
onset and shorter duration of action than $\Delta^8$-THC (17), whereas two other analogues, 3'-methyl-$\Delta^8$-THC (CB1, $K_i = 54$ nM for racemic, $K_i = 38$ nM for $3'R$, $K_i = 53$ nM for $3'S$) and 4'-methyl-$\Delta^8$-THC (23, $K_i = 141$ nM), showed decreased affinities. Of the two C-1’-methyl analogues, the 1’R isomer 21 exhibited higher CB1 affinity ($K_i = 7.6$ nM) and potency (TF and RT tests) compared with the 1’S analogue 22 ($K_i = 20$ nM). The in vivo pharmacological data (SA, TF and RT test) for 1’-methyl, 2’-methyl, and 4’-methyl isomers were found to be consistent with their receptor affinities. In another study it was observed that the 3’S epimer of 3’-hydroxy-$\Delta^8$-THC was significantly more potent than the 3’R isomer (Martin et al., 1984). Thus, it can be concluded that as the branching moves away from the aromatic A ring, the binding affinities and potencies of the ligands consistently decrease, and that both factors, branching site and stereochemistry within the chain, influence the affinity and potency of cannabinoids.

In the early 1990s soon after the discovery of anandamide (3), it was realized that the terminal n-pentyl tail of endocannabinoids may play an analogous role to that of the n-pentyl side chain of THC (2). It was observed by Felder et al. (1993) that when the arachidonic acid moiety of AEA (3) was replaced with docosahexaenoic acid, the resulting structurally related amide analogue with a terminal ethyl group compared with the n-pentyl tail of AEA (3) exhibited a great reduction in affinity for CB1 and potency.

Replacement of the n-pentyl side chain in the AEA (3) template (Fig. 5) with n-hexyl ($K_i = 24$ nM), n-heptyl ($K_i = 55$ nM) and n-octyl chains (24, $K_i = 18$ nM) resulted in a similar trend in CB1 binding affinities as in the classical cannabinoids (Ryan et al., 1997). However, these three analogues failed to produce significant pharmacological effects in vivo. The replacement of 5-carbon chain with the dimethylalkyl chain improved affinity and increased potency in both in vitro and in vivo. Like the THCs, the dimethylheptyl analogue 25 exhibited the highest potency (Ryan et al., 1997; Seltzman et al., 1997). When the geminal dimethyl branching was moved one carbon towards the terminal methyl, the resulting ligand 26 exhibited a decrease in CB1 binding affinity compared to 25. This observation is also analogous to that observed for classical cannabinoids. The corresponding monomethyl substituted analog 27 showed a smaller decrease in CB1 binding affinity. Thus, a comparison of affinities and potencies of AEA analogues with THC analogues revealed that similar trends were found when the chain length and branching were varied. However, the magnitude of enhanced potency when the side chain was varied from straight to branched was greater for THC analogues compared to the AEA analogues (Ryan et al., 1997).

**Effects of side chain substituents**

The side chain seems to be a place of choice for halogen substitution and a considerable enhancement in affinity for CB1 was observed by substitution at the terminal carbon of the side chain with bulkier halogens (Br, I) producing the largest effects (Charalambous et al., 1991a; Martin et al., 1993; Nikas et al., 2004). Thus, the 5’-Br-$\Delta^8$-THC and 5’-Br-1’,1’-dimethylpentyl-$\Delta^8$-THC (AM087, 28, Fig. 6) exhibited 7.6 and 0.43 nM affinities, respectively, for the CB1 receptor, whereas 5’-Br-1’,1’-dimethylheptyl-$\Delta^8$-THC showed a slight reduction in affinity (1.27 nM) compared to 28. 5’-I-$\Delta^8$-THC has high affinity (CB1, $K_i = 7.8$ nM) as compared to (−)-1’-iodohexyl-$\Delta^8$-THC (CB1, $K_i = 328$ nM) which exhibits an 8-fold reduction in affinity compared to $\Delta^8$-THC (17) (Nikas et al. 2004). Although (−)-5’-18F-$\Delta^8$-THC had a slightly reduced affinity (CB1, $K_i = 57$ nM) compared to $\Delta^8$-THC (17), this fluorinated compound has served as a positron imaging (PET) probe for...
experiments aimed at studying localization of cannabinoid receptors in the brains of primates (Charalambous et al., 1991b).

Incorporation of cyano and other groups at the terminus of the side chain (Fig. 6) was found to dramatically affect pharmacological potency in the $\Delta^8$-THC series with relatively little affect on binding affinity. Furthermore, it was observed that the presence of a cyano group doesn’t affect all pharmacological potency to the same degree. Thus, cyano substituted $\Delta^8$-THC analog (O-774, 29) exhibited similar affinity as 19, but substantial enhancement of in vivo potency {SA (12-fold); TF (4-fold); RT (8-fold)} (Crocker et al., 1999; Griffin et al., 2001; Singer et al., 1998). Shortening the side chain of O-774 (29) by one carbon led to 30, which retained both high affinity and high pharmacological potency. It was also observed that addition of a $p$-cyanophenoxy moiety (O-704, 31) was well tolerated with respect to receptor affinity, however it showed reduced potency compared to O-581 (29). Piperidinylamido substitution (O-856, 32) showed slightly reduced affinity and potency compared to $\Delta^8$-THC-DMH (19) (Singer et al., 1998).

A similar trend was observed with the endocannabinoids (Fig. 7). These studies were carried out with the FAAH-stable anandamide analogue $R$-$(+)$-methanandamide (AM356, 33), a metabolically stable template developed by Makriyannis (Abadji et al., 1994; Goutopoulos et al., 2001; Lin et al., 1998). The incorporation of a bromo (O-1860, 34) or a cyano (O-1812, 35) group at the C-20 along with geminal dimethyls at C-16 dramatically improved CB1 receptor affinities, whereas the corresponding hydroxyl analogue (O-1811, 36) exhibited diminished affinity (Di Marzo et al., 2001).

**Conformationally restricted side chain analogues**

The pharmacophoric conformation of the side chain of $\Delta^8$-THC (17) and its congeners represented in the cannabinoid

![Fig. 6. $\Delta^8$-THC analogues bearing different substituents at the terminus of the side chain.](image)

![Fig. 7. Tail modified analogues of $R$-$(+)$-methanandamide.](image)
receptor:ligand complex is not known. Based on a quantitative structure–activity relationship (QSAR) analysis of the side chain conformation in a variety of Δ8-THC analogues, Keimowitz et al. (2000) postulated that the length of the cannabinoid side chain as well as its ability to fold back placing the terminus close to ring structure are critical in determining the ligand’s affinity for CB1. This is consistent with the compact conformation of Δ8-THC (17) in a model membrane system determined using neutron diffraction (Martel et al., 1993) as well as the conformation of the 1′,1′-dimethylheptyl side chain of Pfizer’s bicyclic non-classical cannabinoid CP-47,497 which was determined using 2D NMR (Xie et al., 1994).

A significant degree of conformational restriction can be imposed upon the side chain (Fig. 8) either by the introduction of unsaturation (37–39) or with a new cyclic ring fused to the aromatic A ring (40, 41), thereby restricting the conformations the side chain could attain and leading to variations in biological responses. For all the analogues bearing unsaturation, it was observed that their CB1 affinities and efficacies were differentially altered. The analogue which

![Figure 8](image-url)  
**Fig. 8.** Side chain conformationally restricted analogues of Δ8-THC.
has the triple bond in the C-1' position (1'-heptyl-Δ⁸-THC, 37) and its cis double bond analogue 38 bound to both CB1 and CB2 receptors with high affinity (Martin et al., 2002; Papahatjis et al., 1998). It was observed that the efficacy of these compounds increased with heterosubstitution at the terminus of the unsaturated side chain. Analogues generated by the incorporation of a triple bond at the C-2' position of hexyl, octyl and nonyl (39) side chains of Δ⁸-THC exhibited 5- to 10-fold improved affinities compared to Δ⁸-THC (17). However, the in vivo potencies of these compounds varied greatly (Ryan et al., 1995). Analogues bearing unsaturation at the C-2' position and a polar moiety such as a CN or azido group at the terminal carbon of the side chain exhibited high affinities for cannabinoid receptors; however, they behaved either as partial agonists or antagonists compared to Δ⁸-THC (17) which is a full agonist (Martin et al., 1999).

The conformationally constrained tetracyclic analogues 40 and 41 were synthesized with a fourth ring in the tricyclic cannabinoid skeleton fused to the phenolic A ring (Khanolkar et al., 1999). Analogue 40, in which the side chain is fixed at a distance of 1–1.5 Å from the phenolic ring, has its side chain pointing downwards and has an 18-fold higher CB1 affinity compared to analogue 41 in which the side chain is fixed at a distance of at least 3–3.5 Å and has a lateral orientation. Analogue 40 also exhibits 3-fold higher affinity than analogue 41 for the CB2 receptor. These results suggest that the cannabinoid receptor affinities decreased significantly when the side chain is forced into a lateral orientation and further away from the phenolic ring.

Cyclic substituents at C-1' for probing a novel pharmacophoric subsite

As discussed earlier, a comparison of the binding data of n-heptyl-Δ⁸-THC and its 1',1'-dimethylheptyl analogue 19 suggested that the presence of the two C-1'-methyl groups enhanced the ligand’s affinity for CB receptors. To optimize the steric contribution due to C-1’ substitution, Papahatjis et al. synthesized novel analogues (Fig. 9, 42–47) in which the 1',1'-dimethyl group was replaced with 3- to 6-membered heterocyclic and carbocyclic rings (Papahatjis et al., 1998, 2001, 2002, 2003). The most interesting compounds which resulted from this work were the C-1'-dithiolane analogue 43 and C-1'-dioxolane analogue 44. These two compounds exhibited high affinities but no significant selectivity for either of the CB receptors. The corresponding C-1’-carbocyclic congener 46 also maintained a high affinity for CB1 and CB2 with a 3-fold preference for CB1. The C-1’-cyclopropyl analogues 45 and 47 also show high affinity for both receptors. This increase in the affinity of these analogues 42–47 was attributed to the presence of a hydrophobic subsite in both CB1 and CB2 in the vicinity of the benzylic position of the side chain. Thus, within the CB1 receptor the putative subsite appears to be indifferent to the presence of oxygen, sulfur or methylene groups attached to the C-1' position. Conversely, the CB2 receptor appears to show a preference for the smaller dioxolane five-membered ring compared to the slightly larger dithiolane or more hydrophobic cyclopentyl analogues.
Effects of other cyclic and bulky side chains

To add to our present understanding of the possible conformations of the side chain adopted during a ligand’s interaction with its active site, novel analogues carrying bulky and/or rigid aliphatic substituents at C-1’ were developed (Figs. 10, 11; 48–51) (Lu et al., 2005). Based on the binding affinities, it was observed that the bulky adamantyl group can easily be accommodated within CB1/CB2 binding site. The results also revealed that variation in adamantyl substituents can lead to higher affinities and selectivities for each of the two receptors depending on the relative orientation of the adamantyl group with respect to the tricyclic cannabinoid structure. Computational modeling suggested that the differences in affinities and selectivities can be explained on the basis of allowable conformational space for the adamantyl substituents. Thus, the 3-(1-adamantyl) group of the CB1 selective analogue AM411 (48), the first pharmacologically active (Jarbe et al., 2004; Luk et al., 2004; McLaughlin et al., 2005a,b) classical cannabinoid to be crystallized (Lu et al., 2005), orients within a spherical space in the direct proximity of the phenolic A ring. Conversely, in the analogues AM744 (49) and AM755 (50) which show CB2 preference, the allowable adamantyl conformations exist within a donut-like space that extends beyond that of the spherical conformational space of AM411 (48). Finally, a ligand AM757 (51) capable of occupying both spaces exhibits no CB1/CB2 selectivity (Fig. 12).

To better understand the conformational and steric requirements of the hydrophobic pocket of the receptor where the C-3 side chain resides, Nadipuram et al. (2003) synthesized several Δ⁸-THC analogues (Fig. 13) in which the cycloalkyl groups of 5, 6 and 7 carbons were attached at the C-1’ position. The C-1’ position was further substituted with either 1’,1’-dimethyl (52a–52c) or a 1’,1’-dithiolane functionality. The geminal dimethyl analogues 52a–52c exhibited subnanomolar affinities. Within the geminal dimethyl analogues, 1’,1’-dimethyl-1’-cyclopentyl 52a had the highest affinity for the CB1 receptor ($K_i = 0.34 \text{nM}$) whereas the 1’,1’-dimethyl-1’-cycloheptyl analogue 52c exhibited greater affinity for the CB2 receptor. Ligand 52b exhibited similar affinities for cannabinoid receptors as 1’,1’-dimethylheptyl-Δ⁸-THC (19). However, despite their relatively high affinities, none of these ligands exhibited much CB subtype receptor selectivity. The corresponding dithiolane analogues, where the 1’,1’-dimethyl unit was replaced by a 1’,1’-dithiolane ring, exhibited relatively diminished activities (Nadipuram et al., 2003). Later, Krishnamurthy et al. (2003) reported the synthesis of Δ⁸-THC analogues in which the cycloalkyl side chain was replaced with a phenyl ring thus retaining steric bulk and conformational constraint. These analogues 53a–53d incorporated 1’,1’-

Fig. 12. The aromatic rings are perpendicular to the plane of the page with the adamantyl substituent closest to the viewer and the carbocyclic ring furthest from the viewer. Global energy minimum conformers are shown in green tube display. (a) This figure illustrates the union of the van der Waals’ volume (purple grid) of all accessible conformers of the CB1 selective compound AM411 (48). (b) This figure illustrates the Unique Volume Map (yellow grid) calculated using all conformers of the CB1 selective compound AM411 (48) and of the CB2 selective compound AM755 (50). The yellow grid area shows the region of space into which conformers of AM755 (50) protrude that is not shared with the accessible conformers of AM411 (48). (c) This figure illustrates the Unique Volume Map (red grid) calculated using all conformers of the CB2 selective compound AM755 (50) and of the non-selective compound AM757 (51). The red grid area shows the region of space into which conformers of AM755 (50) protrude that is not shared with the accessible conformers of AM757 (51) (Reproduced with permission from J. Med. Chem. 2005, 48, 4576–4585. Copyright 2005 Am. Chem. Soc.).

Fig. 13. Δ⁸-THC analogues bearing different cyclic side chains.
dimethyl, 1',1'-dithiolane, methylene, or carbonyl groups at the C-1' position. These compounds exhibited significantly different binding profiles compared to their cycloalkyl congeners. The dimethyl analogue 53a exhibited high CB2 affinity (Kᵢ = 0.91 nM) and 13-fold selectivity over CB1. Analogues 53b and 53c both exhibited diminished affinity and selectivity. Analogue 53d, bearing a polar C-1' keto group, showed reduced affinity for both CB1 and CB2 receptors as was previously shown by Papahatjis et al. (1998) in other THC analogues, but exhibited nearly 13-fold selectivity for CB2.

Conclusion

Over the past 50 years, much research has been carried out directed towards the development of SAR of the classical cannabinoids, primarily using Δ⁸-THC (17) as a template. It has been shown that the key pharmacophore, the n-pentyl chain present in tetrahydrocannabinols and other cannabis constituents, is incorporated during the biosynthesis of olivetolic acid (12). Variation of the n-pentyl side chain of the classical cannabinoids or variation of the n-pentyl tail of the endocannabinoids leads to striking variations in affinities and selectivities of these ligands towards the cannabinoid receptors as well as their pharmacological potencies. The SAR of these analogs have added to our understanding about the steric requirements of CB1 and CB2 receptors and suggested the presence of pharmacophoric subsites within the CB1/CB2 binding domains. Moreover, the design and synthesis of later generation ligands has helped in gaining an insight into ligand binding site(s) and structural features required for receptor activation. Such information has been used in the design of high affinity and receptor subtype selective ligands that may serve as useful pharmacological probes as well as drug prototypes of medications for CB receptor mediated pathologies.

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References


