British Journal of Pharmacology (2008) 153, 335–346
 0007–1188/08 $30.00
www.brjpharmacol.org

REVIEW

Targeting the cannabinoid CB2 receptor: modelling and structural determinants of CB2 selective ligands

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Recent developments indicate that CB2 receptor ligands have the potential to become therapeutically important. To explore this potential, it is necessary to develop compounds with high affinity for the CB2 receptor and little affinity for the CB1 receptor. This review will discuss structure-activity relations at both receptors for classical cannabinoids and cannabimimetic indoles. Examples of CB2 selective ligands from both classes of compounds are presented and the structural features leading to selectivity are described. Two approaches, receptor mutations and molecular modelling, have been employed to investigate the interaction of ligands with both cannabinoid receptors. These results obtained from these techniques are discussed.
British Journal of Pharmacology (2008) **153,** 335–346; doi:10.1038/sj.bjp.0707567; published online 5 November 2007

Keywords: cannabinoid receptor 1; cannabinoid receptor 2; molecular modelling; mutations; selectivity; endocannabinoids; classical cannabinoids; aminoalkylindole

Abbreviations: CB1, cannabinoid receptor subtype 1; CB2, cannabinoid receptor subtype 2; GPCR, G protein-coupled receptor; HHC, (−)-9-nor-9β-hydroxyhexahydrocannabinol; SAR, structure–activity relationships; TM, transmembrane

Introduction

Following the discovery of the endocannabinoid system it has become apparent that research in the cannabinoid field shows promise of becoming important therapeutically. To date, two distinct cannabinoid receptors designated CB1 and CB2 have been identified in mammalian tissues and have been cloned (Matsuda *et al*., 1990; Munro *et al*., 1993). In addition, a new cannabinoid receptor has been suggested (Baker *et al*., 2006) although the finding has not been verified (Petitet *et al*., 2006). While CB1 is located principally in the central nervous system, CB2 is found in tissues of the immune system, such as the spleen, tonsils and thymus (Galiegue *et al*., 1995). Several fatty acid derivatives, isolated from both nervous system and peripheral tissues, for example *N*-arachidonoylethanolamine (Devane *et al*., 1992) and sn-2-arachidonoylglycerol (Mechoulam *et al*., 1995), have been identified as endogenous ligands for cannabinoid receptors (Sugiura *et al*., 1995).

This review will concentrate on structure–activity relationships (SAR) of CB2 selective ligands with an emphasis on classical cannabinoids and cannabimimetic indoles. In addition, we will present an overview on molecular modelling and mutational studies carried to understand the requirements of selective binding to the CB2 receptor.

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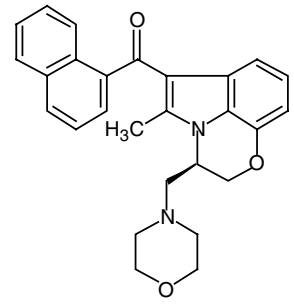
Received 25 June 2007; revised 12 September 2007; accepted 15 October 2007; published online 5 November 2007

Structural requirements for CB2 receptor selectivity

The first report of ligand selectivity for the CB2 receptor was by Felder *et al*. (1995), who found that WIN-55,212-2 has approximately 19-fold selectivity for the CB2 receptor, relative to the CB1 receptor. The following year four reports appeared that described additional selective ligands for the cannabinoid CB2 receptor (Gallant *et al*., 1996; Gareau *et al*., 1996; Huffman *et al*., 1996; Showalter *et al*., 1996). Two of these selective ligands are cannabimimetic indoles, JWH-015 (**2**) (Showalter *et al*., 1996) and L-768242 (**3**) (Gallant *et al*., 1996). The other CB2 selective ligands are classical cannabinoids, JWH-051 (**4**), JWH-057 (**5**) (**4**), L759633 (**6**) and L759656 (**7**) (Gareau *et al*., 1996). Gareau *et al*. also described 1-deoxy-Δ^8-THC-DMH (**5**). Although all seven of these compounds are selective for the CB2 receptor, only indoles **2** and **3** and classical cannabinoids **6** and **7** were reported to have the desirable combination of high affinity for the CB2 receptor and little affinity for the CB1 receptor. WIN-55,212-2 (**1**) and classical cannabinoids **4** and **5** have not only high affinity for the CB2 receptor, but they also have high affinity for the CB1 receptor.



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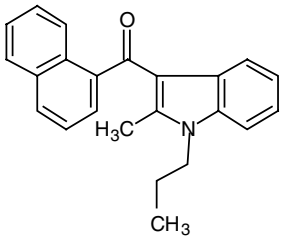

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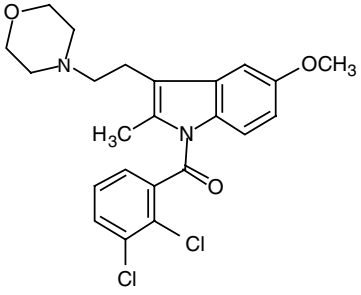

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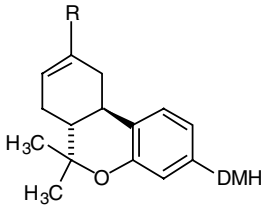

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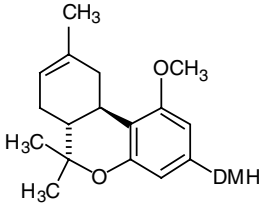
5 R = CH_3



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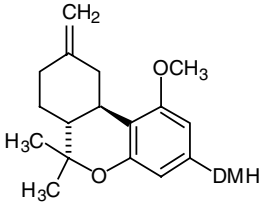

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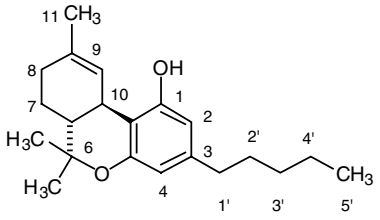
DMH = 1,1-dimethylheptyl

The discovery of these early CB2 selective ligands, combined with the recognition of the importance of the CB2 receptor, has led to intense efforts over the past decade to develop new, highly selective ligands for the CB2 receptor, which are devoid of the psychoactive effects that are mediated through the CB1 receptor (Huffman, 2005). Although these selective ligands belong to a number of structural classes, classical cannabinoids and cannabimimetic indoles have been the most systematically studied and there are sufficient data available to permit some insight into the structural features of these compounds that lead to high affinity for the CB2 receptor with little affinity for the CB1 receptor. Because the criteria for this selectivity are based upon the SAR for the respective classes of cannabinoids at each receptor, this review will discuss the SAR for classical cannabinoids at each receptor, and then the requirements for CB2 receptor selectivity will be developed. The requirements for CB2 selectivity for the cannabimimetis indoles will be developed in a similar manner, although the SAR for this class of cannabinoids is not as well developed as those for classical cannabinoids.

Classical cannabinoids

Classical cannabinoids are those compounds that are structurally based upon or developed from that of Δ^9-THC (**8**), and SAR for this class of cannabinoids at the CB1 receptor has been developed, which will be briefly summarized (Razdan, 1986; Seltzman, 1999). The absolute stereochemistry depicted in structure **8** is necessary for significant CB1 receptor affinity as is an alkyl side chain at C-3 of at least three, but optimally five to eight, carbon atoms. The presence of a *gem*dimethyl group at C-1′ or 1′,2′-dimethyl substituents leads to enhanced CB1 receptor affinity, which is maximized with a dimethylheptyl side chain. A suitable substituent and geometry at C-9 are also necessary for significant CB1 receptor affinity and in general an 11- or 11-nor-9-hydroxyl group enhances CB1 receptor affinity. A phenolic hydroxyl group at C-1 is usually essential for binding to the CB1 receptor, but alternatively a C-11 hydroxyl group can serve as a surrogate for a hydrogen bonding interaction with the receptor (Gareau *et al*., 1996; Huffman *et al*., 2002).

A group at Pfizer developed a series of bicyclic nonclassical cannabinoids, of which CP-47,497 (**9**) and CP-55,940 (**10**) are typical examples (Melvin *et al*., 1984; Johnson and Melvin, 1986). Both of these compounds have high affinity for the CB1 receptor, however the hydroxypropyl group present in CP-55,940 (**10**) enhances affinity relative to CP-47,497 (**9**) (Devane *et al*., 1988). This series of compounds was designed based upon the potent synthetic cannabinoid, (−)-9-nor-9β-hydroxyhexahydrocannabinol (HHC, **11**), as a template and have similar SAR to the classical cannabinoids.



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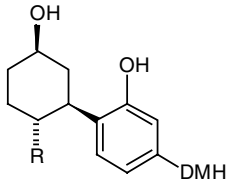
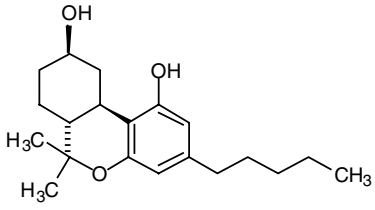


9 R = H
10 R = $CH_2CH_2CH_2OH$



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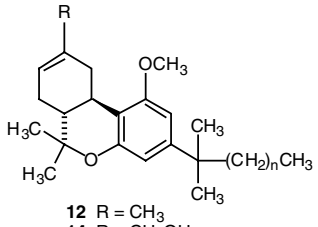
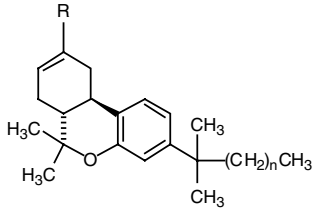
The SAR at the CB2 receptor for classical cannabinoids have not been developed to the extent of the SAR at the CB1 receptor. In 1996 it was reported that two 1-deoxy-Δ^8-THC analogues, JWH-051 (**4**) and JWH-057 (**5**), have very high affinity for the CB2 receptor, but both also have high affinity for the CB1 receptor (Huffman *et al*., 1996). Using these two compounds, plus L759633 (**6**), as leads, 1-methoxy (**12**),

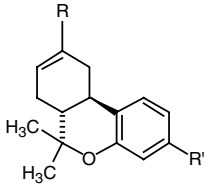
1-deoxy (**13**) and 11-hydroxy Δ^8-THC analogues (**14** and **15**) were prepared (Huffman *et al*., 1999, 2002). Initially, three 1-deoxy-Δ^8-THC analogues with unbranched alkyl chains (**16**, R = CH_3, R′ = C_4H_9 to C_6H_{13}) were synthesized and their affinities for the CB1 and CB2 receptors were determined. The 3-butyl (**16**, R = CH_3, R′ = C_4H_9) and 3-pentyl (**16**, R = CH_3, R′ = C_5H_{11}) analogues have CB2 receptor affinities in the 30–50 nM range and very little ($K_i \geqslant 2500$ nM) affinity for the CB1 receptor; however, the 3-hexyl compound (**16**, R = CH_3, R′ = C_6H_{13}) has very modest affinity for the CB2 receptor ($K_i = 273 \pm 63$ nM) and very little affinity for the CB1 receptor. 1-Deoxy-11-hydroxy-Δ^8-THC (**16**, R = CH_2OH, R′ = C_5H_{11}) was also prepared and while it has 10-fold selectivity for the CB2 receptor, it has considerably lower affinity for the CB2 receptor than its dimethylheptyl analogue (**4**).



12 R = CH_3
14 R = CH_2OH



13 R = CH_3
15 R = CH_2OH



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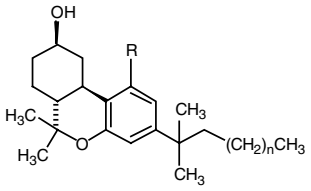
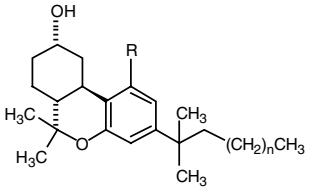
In the 1-deoxy-3-(1′,1′-dimethylalkyl)-Δ^8-THC series (**13**, $n = 0$–7), the 3-(1′,1′-dimethylpropyl) (**13**, $n = 1$) to dimethylheptyl (**5**, also **13**, $n = 5$) compounds have very high CB2 receptor affinities, from 3 to 9 nM, and the dimethylethyl (**13**, $n = 0$) and dimethyloctyl (**13**, $n = 6$) analogues have CB2 receptor affinities in the 60–80 nM range (Huffman *et al*., 1999). All of these compounds are selective for the CB2 receptor, and in particular JWH-133 (**13**, $n = 2$) with $K_i = 3.4 \pm 1.0$ nM for the CB2 receptor exhibits nearly 200-fold selectivity. The dimethylheptyl and dimethyloctyl compounds have significant affinity for the CB1 receptor.

Using L759633 (**6**, also **12**, $n = 5$) as a lead compound, a similar series of 1-methoxy-3-(1′,1′-dimethylalkyl)-Δ^8-THC analogues (**12**, $n = 0$–4) was prepared (Huffman *et al*., 2002). The lower members of this homologous series (**12**, $n = 0$–2) have little affinity for either the CB1 or CB2 receptor, while the dimethylpentyl (JWH-226) and dimethylhexyl (JWH-229) analogues (**12**, $n = 3$ and 4) are 93- and 174-fold selective for the CB2 receptor and both have $K_i = < 45$ nM for the CB2 receptor. The dimethylheptyl compound (**6**, also **12**, $n = 5$) is less selective for the CB2 receptor than either the dimethylpentyl or dimethylhexyl analogue and has somewhat lower affinity for the CB2 receptor than the dimethylpentyl compound.

1-Deoxy-11-hydroxy-Δ^8-THC-DMH (**4**, also **15**, $n = 5$) has exceptionally high CB2 receptor affinity, which is nearly 100-fold greater than that of 1-deoxy-Δ^8-THC-DMH (**5**) (Huffman *et al*., 1996). It was thought that this observation could be exploited to obtain additional selective ligands for the CB2 receptor (Huffman *et al*., 2002). A series of 1-deoxy-11-hydroxy-3-(1′,1′-dimethylalkyl)-Δ^8-THC analogues (**15**, $n = 0$–4) was prepared and their affinities for the CB1 and CB2 receptors were determined. This entire series of compounds has excellent affinity for the CB2 receptor, with $K_i = 0.5$–18 nM; however, the dimethylpentyl (**15**, $n = 3$) and dimethylhexyl (**15**, $n = 4$) analogues also have high affinity for the CB1 receptor ($K_i = 8.8 \pm 1.4$ and 1.8 ± 0.3 nM, respectively). The dimethylpropyl analogue (**15**, $n = 1$) is the most selective of this series of compounds with 33-fold selectivity for the CB2 receptor. The trends in the 1-methoxy-11-hydroxy-3-(1′,1′-dimethylalkyl)-Δ^8-THC series (**14**, $n = 0$–5) are similar to those in the 1-deoxy-11-hydroxy series. However, neither the dimethylethyl (**14**, $n = 0$) nor dimethylpropyl (**14**, $n = 1$) analogues have high affinity for either receptor ($K_i \geqslant 85$ nM) and only one member of this series exhibits significant selectivity for the CB2 receptor. The dimethylbutyl analogue (**14**, $n = 2$) is 12-fold selective with $K_i = 28 \pm 1$ nM at the CB2 receptor. Although the higher members of this series have affinity for the CB2 receptor of less than 5 nM, they also have CB1 receptor affinities of 14–43 nM.



17 R = OCH_3
19 R = H



18 R = OCH_3
20 R = H

Based upon the high affinity of HHC (**11**) for the CB1 receptor and the CB2 receptor selectivity shown by various 1-deoxy- and 1-methoxy-Δ^8-THC analogues, a series of 11-nor-1-methoxy-9-hydroxyhexahydrocannabinols (**17** and **18**, $n = 1$–4) and their 1-deoxy analogues (**19** and **20**, $n = 1$–3) were synthesized and their affinities for the CB1 and CB2 receptors were determined (Marriott *et al*., 2006). The 1-deoxy-3-dimethylheptyl compounds have been described previously (Huffman *et al*., 1999). In the 1-methoxy series with both a 9β- (**17**) and 9α-hydroxyl group (**18**), the CB1 receptor affinities increases as the C-3 alkyl chain increases from four to seven carbon atoms. Both dimethylheptyl compounds (**17**, $n = 5$ and **18**, $n = 5$) have moderate affinity for the CB1 receptor in the 100–135 nM range. The other compounds in both the 9β- and 9α-hydroxy series have weak CB1 receptor affinities with $K_i = 376$–3905 nM. The lowest members of these homologous series (**17** and **18** $n = 1$) have little affinity for the CB2 receptor ($K_i = 198 \pm 23$ and 589 ± 65 nM, respectively). The other members of these two series have K_i from 10 to 38 nM, with the exception of **18**, $n = 2$, with $K_i = 69 \pm 6$ nM. These compounds exhibit 4- to 33-fold selectivity for the CB2 receptor and the 9β-ol has higher

affinity than the corresponding 9α-ol at both the CB1 and CB2 receptors. JWH-350 with 33-fold selectivity for the CB2 receptor and high CB2 receptor affinity ($K_i = 12 \pm 1$ nM) has the desirable combination of excellent CB2 affinity combined with little affinity for the CB1 receptor.

In the 1-deoxy-11-nor-9-hydroxy-HHC series (**19** and **20**, $n = 1$–4), the trends at both the CB1 and CB2 receptors are similar with little affinity for the CB1 receptor for the lowest members of the series (**19** and **20**, $n = 1$). The CB1 receptor affinity increases with increasing alkyl chain length until the dimethylheptyl compounds (**19** and **20**, $n = 4$), which have very high CB1 receptor affinity ($K_i = 7.9 \pm 0.9$ and 28 ± 3 nM, respectively) (Marriott *et al*., 2006). The CB2 receptor affinities of the entire series of 9β-hydroxy-HHCs (**19**, $n = 1$–4) are uniformly high, with $K_i = 36 \pm 3$ nM for **19**, $n = 1$ to 2.7 ± 0.1 nM for **19**, $n = 3$. With the exception of the highest member of this homologous series these compounds exhibit 22- to 29-fold selectivity for the CB2 receptor. Both the 9β- and 9α-hydroxy-3-dimethylheptyl compounds (**19** and **20**, $n = 4$) exhibit little selectivity for either receptor and have high affinity for both receptors. The lower members of the 9α-hydroxy series (**20**, $n = 1$–3) have CB1 receptor affinities that increase from $K_i = 4589$ nM for **20**, $n = 1$ to $K_i = 127$ nM for **20**, $n = 3$. The CB2 receptors of HHCs **20**, $n = 1$–3, are considerably greater than their CB1 affinities and also increase with the length of the alkyl chain. For the lowest member of the series (**20**, $n = 1$) $K_i = 153 \pm 15$ nM increasing to 34 ± 5 nM for **20**, $n = 3$. As is the case with the 1-methoxy-HHCs (**17** and **18**), the 9β-hydroxy compounds (**19**) have greater affinity for both the CB1 and CB2 receptors than the 9α-epimers. The three lowest members of the 9β-hydroxy series (**19**, $n = 1$–3) are 22- to 30-fold selective for the CB2 receptor. Two of them, JWH-336 (**19**, $n = 1$) with 30-fold selectivity and good affinity for the CB2 receptor ($K_i = 36 \pm 3$ nM) and JWH-300 (**19**, $n = 2$) with $K_i = 5.3 \pm 0.1$ nM, 22-fold selective for the CB2 receptor, combine good affinity for the CB2 receptor with weak affinity for the CB1 receptor. The 9β-hydroxy-3-dimethylhexyl compound is 23-fold selective for the CB2 receptor, but has moderate affinity ($K_i = 63 \pm 3$ nM) for the CB1 receptor.

It is apparent that for useful CB2 selectivity a combination of little affinity and low efficacy at the CB1 receptor combined with moderate to high affinity for the CB2 receptor is essential. Classical cannabinoids with a phenolic hydroxyl group related to Δ^9-THC (**8**) and nonclassical cannabinoid CP-55,940 have very little selectivity for either the CB1 or CB2 receptor (Pertwee, 1999). This extends to the 1′,1′-dimethyl alkyl Δ^8-THC series in which even the lowest member of the series with a two carbon alkyl chain has very high affinity for the CB1 receptor (Huffman *et al*., 2003) and the dimethylethyl to dimethylpentyl analogues have CB2 receptor affinities that are virtually identical to their CB1 receptor affinities (Huffman JW, unpublished work). The principal structural features necessary for classical cannabinoids to interact with the CB1 receptor are the C-1 phenolic hydroxyl group and an unbranced C-3 alkyl side chain containing at least three carbon atoms (Razdan, 1986; Seltzman, 1999). Most approaches to CB2 selective ligands have been based upon the observation that JWH-051 (**4**) and JWH-057 (**5**), 1-deoxy-Δ^8-THC analogues (Huffman *et al*., 1996) and L59633, a 1-methoxy derivative of Δ^8-THC-DMH (**6**) (Gareau *et al*., 1996), are selective ligands for the CB2 receptor. These compounds have replaced the phenolic hydroxyl group of THC with either a methoxy group or a hydrogen.

In the 1-deoxy-3-(1′,1′-dimethylalkyl)-Δ^8-THC series (**13**) there is from fair to high affinity for the CB2 receptor with an alkyl side chain of two to eight carbons (Huffman *et al*., 1999). CB1 receptor affinity does not become significant ($K_i = < 200$ nM) until a seven carbon chain is present. A limited number of examples of straight chain analogues of **13** have been evaluated and the lack of branching in 1-deoxy-Δ^8-THC and its 3-butyl and 3-hexyl analogues leads to a significant decrease in CB2 receptor affinity; however, both deoxy-Δ^8-THC and its 3-butyl homologue have the combination of good affinity for the CB2 receptor and little affinity for the CB1 receptor (Huffman *et al*., 1999). The 11-hydroxy derivatives of the 1-deoxy-3-(1′,1′-dimethylalkyl)-Δ^8-THCs (**15**) have considerably enhanced CB2 receptor affinities, but with the exception of the lowest members of this series (**15**, $n = 0$ and 1) this series of compounds has significant CB1 receptor affinity (Huffman *et al*., 2002). As suggested for JWH-051 (**4**), the 11-hydroxyl may serve as a surrogate for the phenolic hydroxyl in a hydrogen bonding interaction with the CB1 receptor (Huffman *et al*., 1996). The trends for CB1 and CB2 receptor affinities for the 9-hydroxy-HHCs (**19** and **20**) are similar to those of the 11-hydroxy-Δ^8-THC compounds (Marriott *et al*., 2006). That is, the lowest members of the series (**19** and **20**, $n = 1$) have from good to modest affinity for the CB2 receptor with little affinity for the CB1 receptor. The higher homologues, particularly in the 9β-hydroxy series (**19**), have relatively high affinity for the CB1 receptor, possibly due to interaction of the 9-hydroxyl group with the receptor.

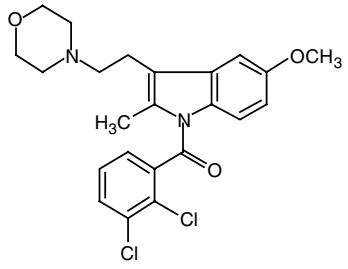
The CB1 and CB2 receptor affinities for the 1-methoxy-Δ^8-THC series (**12**) are significantly less than those of the corresponding 1-deoxy cannabinoids (Huffman *et al*., 2002). Only the three highest homologues of this series (**12**, $n =$ 3–5) have significant affinity for the CB2 receptor. None of these 1-methoxy compounds have appreciable affinity for the CB1 receptor. For the 11-hydroxy-1-methoxy compounds (**14**) the two lowest members of the series (**14**, $n = 0$ and 1) have relatively little affinity for the CB2 receptor and although higher members of the series have good to very high affinity for the CB2 receptor, they also have high CB1 affinity. For the 11-nor-9-hydroxy-1-methoxy-HHC series, the lowest members (**17** and **18**, $n = 1$) have little affinity for either receptor. The CB2 affinities for the next higher homologues (**17** and **18**, $n = 2$ and 3) increase by about an order of magnitude, while the CB1 affinities increase only somewhat (Marriott *et al*., 2006).

In general, for optimal CB2 receptor selectivity in the classical cannabinoid series, the C-1 phenolic hydroxy is replaced by hydrogen, a 1-methoxy substituent is not as effective. Hydroxyl substituents at C-9 or C-11 increase CB2 receptor affinity, but also increase CB1 affinity. In the 1-deoxy series, the length of the C-3 alkyl substituent does not materially affect CB2 receptor affinity, although it very much affects CB1 affinity. At this point relatively little systematic data have been published concerning the effect

upon receptor selectivity of other substituents at C-1, C-9 or various positions in the side chain.

Cannabimimetic indoles

The first cannabimimetic indole that was reported to show selectivity for the CB2 receptor was WIN-55,212-2 (**1**); however, although this compound is CB2 selective, it has very high affinity for the CB1 receptor (Felder *et al*., 1995). The first reported indole derivative that exhibited the desirable combination of good affinity for the CB2 receptor and weak affinity at the CB1 receptor was JWH-015 (**2**) with $K_i = 13.8 \pm 4.6$ nM at the CB2 receptor and $K_i = 164 \pm 22$ nM at CB1 receptor (Showalter *et al*., 1996; Aung *et al*., 2000). Almost simultaneously the Merck Frosst group reported that a structurally different indole derivative, L768242 (**21**) and several related compounds have good affinity for the CB2 receptor and little affinity at the CB1 receptor (Gallant *et al*., 1996). L768242, with $K_i = 12.0 \pm 0.2$ nM at the CB2 receptor is greater than 150-fold selective at the CB2 receptor.



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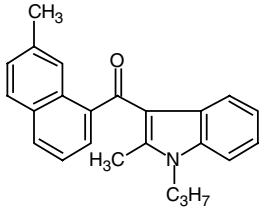

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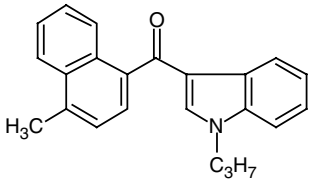

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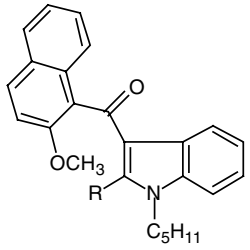
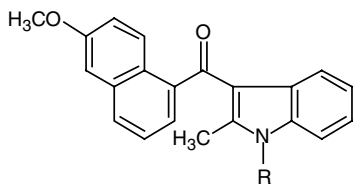
In an effort to expand upon the selectivity for the CB2 receptor exhibited by JWH-015 (**2**), the Clemson group synthesized a number of 1-alkyl-3-(1-naphthoyl)indoles and their 2-methyl analogues with various length *N*-alkyl substituents and a variety of groups appended to the naphthalene ring (Wiley *et al*., 1998; Aung *et al*., 2000; Huffman *et al*., 2005b). These studies led to the development of preliminary SAR, particularly at the CB1 receptor, for *N*-alkyl-3-(1-naphthoyl)indoles and the discovery of seven additional indole derivatives with good to high affinity for the CB2 receptor (Huffman *et al*., 2005b). One of these compounds, JWH-046 (**22**), is very similar in structure to JWH-015 (**2**) and is also very similar in its affinities for the CB1 and CB2 receptors (Aung *et al*., 2000). A third highly selective cannabimimetic indole, JWH-120 (**23**), exhibits 173-fold selectivity for the CB2 receptor, with CB2 receptor affinity of 6.1 ± 0.7 nM (Huffman *et al*., 2005b). This compound contains a methyl substituent on the naphthalene ring, but lacks the methyl group at C-2 of the indole, which is present in JWH-015 (**2**) and JWH-046 (**22**). Compounds **2**, **22** and **23** are all *N*-propyl indole derivatives, compounds that usually have significantly lower CB1 receptor affinity than their higher homologues, while the length of the *N*-alkyl group has considerably less effect upon CB2 receptor affinity.

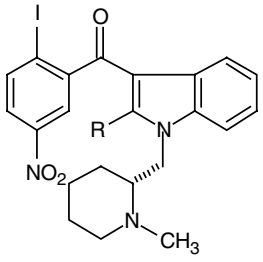


24 R = H
25 R = CH_3

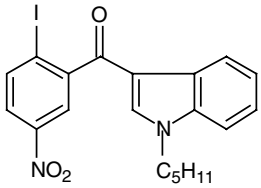


26 R = C_3H_7
27 R = C_5H_{11}

Four 3-(methoxy-1-naphthoyl)indoles were identified that have the combination of weak CB1 receptor affinity combined with good to excellent affinity for the CB2 receptor (Huffman *et al*., 2005b). Two of these compounds, JWH-267 and JWH-268, are 1-pentyl-3-(2-methoxy-1-naphthoyl)indoles (**24** and **25**, respectively), which differ only in the presence of an indole 2-methyl substituent in **25**. JWH-267 (**24**), which lacks the C-2 methyl group, has very high affinity for the CB2 receptor ($K_i = 7.2 \pm 0.14$ nM) and exhibits 53-fold selectivity for the CB2 receptor. The 2-methyl analogue, JWH-268 (**25**) has somewhat less affinity for the CB2 receptor with $K_i = 40 \pm 0.6$ nM and is 34-fold selective for the CB2 receptor. The other two CB2 selective indoles are both 3-(6-methoxy-1-naphthoyl)indole derivatives, which differ only in the substituent appended to the indole nitrogen. The *N*-propyl compound, JWH-151 (**26**), shows greater than 333-fold selectivity for the CB2 receptor with essentially no affinity for the CB1 receptor and good ($K_i = 30 \pm 1$ nM) affinity at the CB2 receptor. JWH-153 (**27**), the *N*-pentyl analogue of JWH-151, has very high affinity for the CB2 receptor ($K_i = 11 \pm 0.5$ nM) and has 23-fold selectivity for the CB2 receptor.



28 R = H
30 R = CH_3

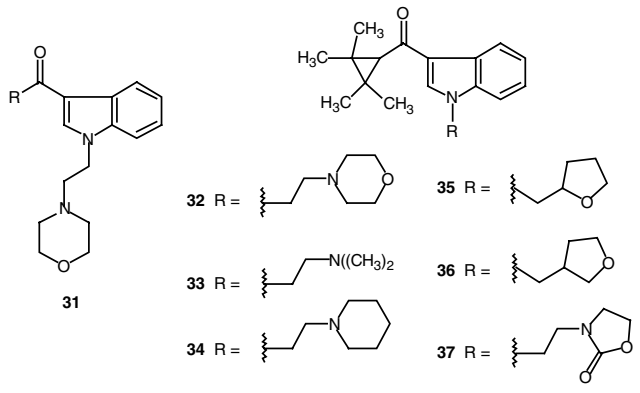


29

AM1241 (**28**), with high affinity ($K_i = 1.6$ nM) and nearly 350-fold selectivity for the CB2 receptor as the racemate was developed by the Makriyannis group (Deng, 2000; Ibrahim *et al*., 2003). The *R*-(+) enantiomer (depicted) has somewhat higher affinity for both the CB1 and CB2 receptors than the

S-(–) enatiomer. AM1241 was designed based upon the observation that AM1202 (**29**), with a pentyl group, rather than an *N*-methyl-2-piperidinylmethyl substituent on nitrogen, has modest, slightly greater than 4-fold, selectivity for the CB2 receptor. Both an *N*-methyl-2-piperidinyl and pentyl substituent on the indole nitrogen usually provide maximum affinity for both the CB1 and CB2 receptors. The *N*-methyl-2-piperidinylmethyl nitrogen substituent on AM1241 was chosen based in part upon this observation and in part upon the presence of a chiral centre in the piperidine moiety. The rationale for this selection was that with a chiral centre it was possible that one enantiomer would provide greater selectivity than the other. A 2-methyl analogue of AM1241 (**30**) was also prepared, however it was less selective (51-fold) with significantly lower affinity for both receptors (Deng, 2000).

A group at Abbott Laboratories has reported the preparation and *in vitro* pharmacology for three series of cannabimimetic indoles, several of which exhibit excellent selectivity for the CB2 receptor (Dart *et al*., 2006). Initially, a series of nine compounds was prepared in which the group R is one of a variety of cycloalkanes and the indole nitrogen substituent is a morpholinoethyl group (**31**). One of these compounds, A-796260 (**32**), with a tetramethylcyclopropyl group is a highly selective, potent CB2 selective agonist with $K_i = 0.77$ nM at the CB2 receptor and very slight affinity for the CB1 receptor ($K_i = 2100$ nM). Using A-796260 as a lead compound, nine additional aminoalkylindoles were synthesized in which the morpholinoethyl group was replaced with other substituted amino groups. Although none of these compounds are as selective as A-796260, two of them, **33** and **34**, have good affinity for the CB2 receptor and exhibited 555- and 469-fold selectivity for the CB2 receptor.



The Abbot group prepared a third series of 16 analogues of A-796260 (**32**) with a variety of neutral side chains (Dart *et al*., 2006). Again, none of these compounds are as selective as A-796260, however several of them (**35**, **36** and **37**) combine high affinity for the CB2 receptor ($K_i = 0.18$–2.7 nM) with weak affinity for the CB1 receptor. These compounds show 230- to 950-fold selectivity for the CB2 receptor. Several additional side chains containing hydroxyl, haloalkyl and alkoxyalkyl groups were also described and several of these compounds are also selective ligands for the CB2 receptor. In particular, the hydroxyalkyl compounds have excellent CB2 receptor affinity and are 168- to greater than 490-fold selective for the CB2 receptor.

In their early work on aminoalkylindoles, the Sterling group found that a 3-(1-naphthoyl) substituent provides considerably greater affinity for the CB1 receptor than a benzoyl or substituted benzoyl group (Bell *et al*., 1991). Subsequent work by the Clemson group indicated that a methoxyl or small alkyl group (methyl, ethyl, propyl) at the 4-position of the naphthoyl group enhances CB1 receptor affinity (Huffman *et al*., 2005a). A methyl or methoxy at the 7-position of the naphthoyl group has little effect on affinity, however a methoxy at C-6 attenuates CB1 receptor affinity, while a 2-methoxy has a strongly adverse effect upon CB1 affinity. An indole 2-methyl group reduces CB1 receptor affinity somewhat relative to the unsubstituted compound, while larger C-2 substituents reduce CB1 receptor affinity considerably (Bell *et al*., 1991; Huffman *et al*., 2005a).

The other principal variation in structure that has been examined is the nature of the group appended to the indole nitrogen. CB1 receptor affinity is maximized by a pentyl or an *N*-methyl-2-piperidinylmethyl nitrogen substituent (Bell *et al*., 1991; Huffman *et al*., 2005a). Morpholinoethyl, piperidinoethyl, butyl or hexyl substituents attenuate CB1 receptor affinity somewhat. Most other heterocyclic, amino and alkyl substituents larger than hexyl or smaller than butyl lead to a considerable decrease in CB1 receptor affinity. Small alkyl groups, such as methyl, ethyl or propyl, at C-4 of the naphthoyl group usually enhance affinity for both receptors, but have a more profound effect upon CB1 receptor affinity.

Many cannabimimetic indoles, with both *N*-aminoalkyl and *N*-alkyl substituents, have been described and their CB2 receptor affinities have been determined (Bell *et al*., 1991; Aung *et al*., 2000; Deng, 2000; Ibrahim *et al*., 2003; Huffman *et al*., 2005a). However it is difficult at this point to present comprehensive SAR for these compounds at the CB2 receptor. The trends in terms of SAR at the CB2 receptor are similar to those at the CB1 receptor; however in general, the effects of structural variations are considerably less than those for the corresponding changes upon CB1 receptor affinity. In particular, an *N*-pentyl substituent usually provides high CB1 and CB2 receptor affinities, while an *N*-propyl group greatly attenuates CB1 affinity, but has much less effect upon CB2 binding.

A structural feature of most of the CB2 selective 3-aroyl cannabimimetic indoles reported to date is the combination of either a nitrogen or 3-aroyl substituent that attenuates CB1 receptor affinity with a 3-aroyl or nitrogen substituent that imparts from moderate to high CB1 affinity. JWH-015 (**2**) is typical of this structural type, with a 3-naphthoyl group in combination with an *N*-pentyl substituent, which provides a compound with high CB2 receptor affinity ($K_i = 9.5 \pm 4$ nM), however with an *N*-propyl group CB1 affinity decreases to 164 nM (Showalter *et al*., 1996; Huffman *et al*., 2005b). The *N*-pentyl compound has very high CB2 receptor affinity ($K_i = 2.9 \pm 3$ nM), while the *N*-propyl compound JWH-015 has slightly lower affinity for the CB2 receptor ($K_i = 13.8 \pm 4.6$ nM), but combines good affinity for the CB2 receptor with low affinity for the CB1 receptor. Other CB2-selective indoles reported by the Clemson group have similar combinations of either a nitrogen substituent

that leads to reduced CB1 receptor affinity or a group a the 3-position of the indole that attenuates CB1 affinity. JWH-267 (**24**) and JWH-268 (**25**) have a 2-methoxy-1-naphthoyl group at C-3 of the indole, which leads to very diminished CB1 receptor affinity, although both compounds have *N*-pentyl groups (Huffman *et al*., 2005b). JWH-151 (**26**) contains a 3-(6-methoxy-1-naphthoyl)indole, which leads to diminished CB1 receptor affinity, while JWH-153 (**27**) also contains a 6-methoxy-1-naphthoyl unit with an *N*-pentyl group, but in this case it is the indole 2-methyl group that contributes to diminished CB1 affinity.

AM1241 (**29**) and its 2-methyl analogue (**30**) both contain an *N*-methyl-2-piperidinylmethyl group, which normally imparts enhanced CB1 receptor affinity, however the presence of a monocyclic substituted benzoyl group contributes to diminished CB1 receptor affinity for these compounds (22, 23). Similarly, the Abbott compounds (**31**–**37**) contain an aliphatic group at the 3-position of the indole, which greatly diminishes CB1 receptor affinity (Dart *et al*., 2006). However, when the substituent on the indole nitrogen is *N*-methyl-2-piperidinylmethyl, the compound has very high CB1 receptor affinity. Changing the nitrogen substituent to morpholinoethyl reduces CB1 affinity from 5.5 to 2100 nM.

Although one can make generalizations regarding SAR at the CB2 receptor and concerning the structural features that lead to selectivity for the CB2 receptor, these generalizations are not even semi-quantitative. For instance, it is known that an *N*-methyl-2-piperidinylmethyl substituent on the indole nitrogen enhances CB1 receptor affinity relative to a morpholinoethyl substituent (D'Ambra *et al*., 1996), however the greater than 350-fold increase in CB1 receptor affinity substituting an *N*-methyl-2-piperidinylmethyl group for the morpholinoethyl group present in AM1241 (**29**) would not have been predicted. Similarly, JWH-015 (**2**) has $K_i = 164 \pm 22$ nM at CB1 receptor and $K_i = 13.8 \pm 4.6$ nM at CB2 receptor (Huffman *et al*., 2005a). However, in contrast to the usual SAR, JWH-072, the analogue of **2**, which lacks the 2-methyl substituent, has $K_i = 1055 \pm 55$ nM at the CB1 receptor and $K_i = 170 \pm 54$ nM at CB2 receptor. Another highly selective ligand for the CB2 receptor that does not follow the normal SAR for cannabimimetic indoles is JWH-120 (**23**) (Huffman *et al*., 2005a). This compound has $K_i = 1054 \pm 31$ nM at the CB1 receptor and $K_i = 6.1 \pm 0.7$ nM at the CB2 receptor. The analogue with a 2-methyl group (JWH-148) has nearly 10-fold greater affinity for the CB1 receptor with $K_i = 123 \pm 8$ nM. The CB2 affinity for JWH-148 is also slightly lower than for JWH-120 ($K_i = 14 \pm 1$ nM).

Mutational studies of CB2 receptor

A recent review (Raitio *et al*., 2005) systematically discussed CB2 receptor mutations reported prior to 2005. In the present review only the most important findings prior to 2005 (see Table 1) will be discussed and work reported after 2005 will be emphasized.

In every transmembrane (TM) domain, there are residues that have been probed for their importance in ligand binding or signal transduction. In accordance with the general belief that G protein-coupled receptor (GPCR) ligands interact primarily with TM helices 3–5, mutational studies have concentrated on these helices. The effects of the D60.3N charge-neutralizing mutation of the CB1 and CB2 receptors have been analysed by Nebane *et al*. (2006). The binding of CP55940 (**10**) (K_d and B_{max}) to CB1/2 receptors was not affected by D60.3N mutation (as compared to the wild-type receptors). Also the mutant receptors showed constitutive activity similar to the wild-type receptors. However, the CB agonist CP55940 (**10**) was less effective at inhibiting forskolin-induced cAMP accumulations in the CB1/2 D6.30N-transfected cells than in the CB1/2 wild-type-transfected cells. The decreased maximum response of the mutant receptors indicates altered G protein–receptor interactions, since the binding of CP55940 (**10**) was not altered. This study strongly indicates that the D6.30 is not crucial for ligand binding but is important for full activation of the receptor. In addition, a salt bridge between R3.50 and D6.30 common to other GPCRs is either not present in CB1/2 receptors or is not important for maintaining the CB1 and CB2 receptors in an inactive state.

Another revealing study concentrated on cysteine effects on ligand binding (Zhang *et al*., 2005). Based on 10 CB2 cysteine mutants, Zhang *et al*. found that C2.59 is responsible for MTS ethylammonium effects upon the CB2 receptor. This in turn indicates that C2.59 is located within the ligand-binding cavity of CB2 (although perhaps close to the border of the cavity), which contrasts with the lipid facing location of position 2.59 in rhodopsin (Palczewski *et al*., 2000).

Molecular modelling of CB2 receptor

While SAR at the CB2 receptor (for both classical cannabinoids and indoles) is not completely developed, as noted above, some trends have become apparent. Since our aim is to design structurally novel CB2 selective ligands, any method that could predict target structures *a priori* would be more than welcome. Single-point mutations and molecular modelling, when combined with extensive SAR data, may be the best tools to reach this target. While SAR can provide useful information based on indirect observations, the use of mutations and molecular modelling can increase the available information and allow an understanding of how CB2 selectivity can be achieved.

Almost all earlier modelling and mutation studies were concentrated on CB1 and only recently have such studies been carried out for the CB2 receptor. A majority of the published studies of the CB2 receptor deal with indole-derivatives or CP55940 (**10**). One of the first such studies was from Reggio *et al*. (1998), in which the s-trans-configuration of WIN-55,212-2 (**1**) was suggested as the bioactive conformation for binding to the CB2 receptor. In addition it was suggested that aromatic stacking is an important factor for CB binding of indole compounds. Later it was suggested that upon binding there exist some aromatic interactions between the naphthyl ring of WIN-55,212-2 (**1**) and the residues F3.25 and W5.43. In addition, the indole ring of WIN-55,212-2 (1) and residues F3.36, W.43 and F5.46 also

Table 1 Mutations and receptor chimera studies at CB2: importance of the specific amino-acid residues or receptor domains for ligand recognition and/or receptor activation

Residue(s)/ domain(s)	*Effect of the mutation at CB2*	*Reference*
D2.50(80)	D2.50N: no change in CP55940, HU-210, Δ^9-THC or WIN55,212-2 binding; signalling abolished D2.50E: no change in CP55940, HU-210, Δ^9-THC or WIN55,212-2 binding; signalling by CP55940 and WIN55,212-2 significantly reduced	(Tao and Abood, 1998)
C2.59(89)	C2.59S binding of HU-243 is not affected by MTSEA treatment, while it is abolished in case of mutants C6.47S, C7.38S, C.42S and a double mutant C7.38S/C7.42S C2.5	(Zhang *et al*., 2005)
TM3	When TM3 of CB1 was exchanged for TM3 of CB2, binding of WIN55,212-2, JWH-015 and JWH-018 was enhanced	(Chin *et al*., 1999)
K3.28(109)	K3.28A: no significant effect on binding of CP55940, Δ^9-THC, WIN55,212-2 or AEA; 10-fold reduction in JWH-015 binding; no effect on signalling by CP55940 or WIN55,212-2 K3.28R: no effect on binding or signalling	(Tao *et al*., 1999b)
K3.28(109) S3.31(112)	K3.28AS3.31G: loss of affinity for CP55940, Δ^9-THC and AEA; ability to bind WIN55,212-2 and JWH-015 retained; signalling by CP55940 abolished, and by WIN55,212-2 drastically reduced	(Tao *et al*., 1999b)
S3.31(112)	CB1/G3.31(195)S: enhancement of WIN55,212-2 binding	(Chin *et al*., 1999)
M3.34(115)	CB1/A3.34(198)M: no effect on WIN55,212-2 binding	(Chin *et al*., 1999)
D3.49(130)	D3.49A: binding affinity of [^{3}H]HU-243 significantly decreased; downstream signalling defective for receptor activation by HU-210 and WIN55,212-2	(Rhee *et al*., 2000a)
D3.49(130)	D3.49A: HU-243, CP55940 and WIN55,212-2 binding abolished; signalling by HU-210, WIN55,212-2 and AEA abolished; constitutive activity lost	(Feng and Song, 2003)
R3.50(131)	R3.50A: no significant change in binding affinities of HU-210 and WIN55,212-2; only a slight effect on signalling (signalling by HU-210 affected more than by WIN55,212-2)	(Rhee *et al*., 2000a)
R3.50(131)	R3.50A: no change in binding of HU-210, WIN55,212-2, AEA or SR144528; signalling by HU-210, WIN55,212-2 and AEA abolished; constitutive activity lost	(Feng and Song, 2003)
Y3.51(132)	Y3.51A: no effect on binding affinities of HU-210 and WIN55,212-2; signalling markedly reduced	(Rhee *et al*., 2000a)
D3.49(130) R3.50(131) Y3.51(132)	DRY3.49-3.51AAA: binding affinity of [^{3}H]HU-243 significantly decreased; downstream signalling defective for receptor activation by HU-210 and WIN55,212-2	(Rhee *et al*., 2000a)
TM4-E2-TM5	When exchanged for the same region of CB1, binding of CP55940 completely eliminated; the region important for the high-affinity binding of SR144528 and WIN55,212-2	(Shire *et al*., 1999, 1996)
W4.50(158)	W4.50F: HU-243 binding and downstream signalling by HU-210, 2-AG and WIN55,212-2 retained W4.50A/Y: agonist binding and signalling eliminated	(Rhee *et al*., 2000b)
S4.53(161)	S4.53A: no change in CP55940 or WIN55,212-2 binding and activity, loss of SR144528 binding and activity	(Gouldson *et al*., 2000)
V4.56(164)	V4.56I: no significant effect on the binding affinities of CP55940, SR144528 or WIN55,212-2	(Gouldson *et al*., 2000)
S4.57(165)	S4.57A: no change in CP55940 or WIN55,212-2 binding and activity, loss of SR144528 binding and activity	(Gouldson *et al*., 2000)
W4.64(172)	W4.64F/Y: no effect on HU-243, HU-210 and CP55940 binding; a slightly reduced WIN55,212-2 binding; downstream signalling by HU-210, WIN55,212-2 and 2-AG retained W4.64A/L: agonist binding and signalling eliminated	(Rhee *et al*., 2000b)
C4.66(174)	C4.66S: binding of CP55940, WIN55,212-2, Δ^9-THC, AEA and SR144528 eliminated	(Shire *et al*., 1996; Gouldson *et al*., 2000)
C4.67(175)	C4.67S: no change in CP55940 binding or activity, loss of SR144528 binding and activity, eightfold reduced binding and activity of WIN55,212-2	(Gouldson *et al*., 2000)
R(177)	R177S: no significant effect on the binding affinities of CP55940, SR144528 or WIN55,212-2	(Gouldson *et al*., 2000)
C(179)	C179S: binding of CP55940, WIN55,212-2, Δ^9-THC, AEA and SR144528 eliminated	(Shire *et al*., 1996; Gouldson *et al*., 2000)
E2 loop	When exchanged for the E2 loop of CB1, CP55940 binding completely eliminated	(Shire *et al*., 1996)
Y5.39(190)	Y5.39F: no significant change in binding of CP55940, Δ^9-THC, WIN55,212-2, JWH-051 or JWH-015; 24-fold drop in AEA binding; no effect on signal transduction Y5.39I: ligand binding capacity lost; signal transduction abolished	(McAllister *et al*., 2002)
S5.42(193)	S5.42G: no significant effect on the binding affinities of CP55940, SR144528 or WIN55,212-2	(Gouldson *et al*., 2000)
F5.46(197)	F5.46V: 14-fold decrease in WIN55,212-2 affinity; no change in the affinities of HU-210, CP55940 and AEA; important for the selectivity of WIN55,212-2 for CB2	(Song *et al*., 1999)
L5.50(201)	L5.50P: WIN55,212-2 binding abolished; complete loss of signalling by HU-210, AEA and WIN55,212-2	(Song and Feng, 2002)
Y5.58(209)	Y5.58A: binding affinities of HU-210, AEA and WIN55,212-2 slightly reduced; complete loss of signalling by HU-210, AEA and WIN55,212-2	(Song and Feng, 2002)
A6.34(244)	A6.34E: HU-243, CP55940 and WIN55,212-2 binding abolished; signalling by HU-210 and WIN55,212-2 severely reduced, and by AEA completely abolished; constitutive activity lost	(Feng and Song, 2003)
D6.30(240)	D6.30N: no effect on binding of CP55940; no effect on constitutive activity, no effect on signalling by CP55940	
S7.39(285)	S7.39A: no effect on signal transduction of HU-210, CP55940 and WIN55,212-2; binding affinity of HU-243 somewhat decreased	(Rhee, 2002)
S7.46(292)	S7.46A: important for signal transduction of HU-210 and CP55940, but not WIN55,212-2; binding affinity of HU-243 somewhat decreased	(Rhee, 2002)
Y7.53(299)	Y7.53A: complete loss of HU-210, WIN55,212-2, AEA and HU-243 binding; downstream signalling by HU-210 and WIN55,212-2 severely impaired; signalling by AEA abolished	(Feng and Song, 2001)
C(313)	C313A: no effect on ligand binding; downstream signalling by HU-210 and WIN55,212-2 severely impaired; signalling by AEA abolished	(Feng and Song, 2001)
C(320)	C320A: no effect on ligand binding; downstream signalling by HU-210 and WIN55,212-2 severely impaired; signalling by AEA abolished	(Feng and Song, 2001)

interact with the receptor via aromatic interactions (Song *et al.*, 1999). These data are in accord with findings of Tuccinardi *et al.* (2006), which report a strong favourable interaction between the naphthyl ring of WIN-55,212-2 (**1**) and CB2 residues F5.46/W5.43 in a docking/molecular dynamics experiment. Similar interactions have been reported (Yates *et al.*, 2005) between CB2 receptor residues and JWH-015 (**2**). The other principal interaction between a CB2-receptor model and WIN-55,212-2 (**1**) is a hydrogen bond between the morpholine ring and S3.31.

Although modelling of classical cannabinoids at the CB2 receptor has not been reported often, there are some studies. It has been reported (Lu *et al.*, 2005) that adamantyl derivatives of classical tricyclic cannabinoids exhibit selectivity for either the CB1 or CB2 receptor. A pharmacophore model for these adamantyl derivatives, which differentiates CB1/CB2 selectivity based on conformational properties of the adamantyl substituent, was proposed. Recently (Durdagi *et al.*, 2007) a 3-Dimensional Quantitative Structure–Activity Relationship Analysis study with classical cannabinoid derivatives was carried out using Comparative Molecular Field Analysis and Comparative Molecular Similarity Analysis. The bioactive conformation of classical cannabinoids was proposed to be such that the C3-alkyl side chain is almost perpendicular to the plane of ring A. 3-Dimensional Quantitative Structure–Activity Relationship Analysis models for both the CB1 and CB2 receptor were based on these putative bioactive conformation, and statistically valid models were created.

Utilization of the rhodopsin template

Following the publication of the rhodopsin X-ray structure (Palczewski *et al.*, 2000) several new receptor models for CB2 have been constructed (Xie *et al.*, 2003; Montero *et al.*, 2005; Salo *et al.*, 2005; Yates *et al.*, 2005; Zhang *et al.*, 2005; Raduner *et al.*, 2006; Stern *et al.*, 2006; Tuccinardi *et al.*, 2006). These models have usually been used for retrospective analysis of binding properties and/or to explain the effects of individual experiments.

To understand the selectivity of CB2 receptor ligands, it would be necessary to use models of both CB1 and CB2 receptors and compare those models. Recently CB1 and CB2 homology models and automatic docking analysis were described (Tuccinardi *et al.*, 2006). These models were based upon the rhodopsin template (Palczewski *et al.*, 2000) and additional information was used to supplement the normal homology modelling procedures. TM helixes 3 and 6 are usually thought to rotate upon receptor activation, and this modification was adopted, as well as the straightening of TM6 and modification of rotameric switch residues W6.48 and F3.36 to trans-gauche (as proposed by Singh *et al.*, 2002). In a following step, molecular docking (Autodock, see Morris *et al.*, 1998) was used to study the binding of 96 ligands from the literature. Based upon docking analysis (evaluated using binding-free energy predictions) a CB2 selectivity mechanism was postulated. CB2/CB1 selectivity could be increased by the presence in the ligands of a lipophilic group able to interact with F5.46 in the CB2 receptor and a group able to form a H bond with S3.31 (Tuccinardi *et al.*, 2006). S3.31 had been predicted earlier to interact via an H-bond with the northern aliphatic hydroxyl of CP55940 (**10**) (Tao *et al.*, 1999a).

In another homology modelling study (Stern *et al.*, 2006) an activated CB2 model was constructed in a manner similar to that of Tuccinardi *et al.* (2006). Automatic molecular docking experiments were used with Gold-software (Jones *et al.*, 1997). The interaction between an adamantyl-derivative of 4-oxo-1,4,-dyhydroquinoline-3-carbozamide with residues F5.46, and W5.43 was detected. It was postulated that the binding cavity of the adamantyl-derivative may share to some extent the binding mode of WIN-55,212-2 (**1**).

A third comparison of CB1/CB2 models (Montero *et al.*, 2005) used a more conservative approach by keeping the trans-membrane helixes at the same positions as in the rhodopsin template. After completion of CB1 and CB2 models, molecular docking was again used to validate the protein models. In this review there appears to be some uncertainties in the material and methods. In the results, it is not completely clear exactly what molecular docking methods were used. There is little information regarding interacting residues and interaction types, although it has been reported that AM630 and SR144528 interact with W5.43 and F5.46.

Although new *N*-arachidonoylethanolamine derivatives (or endogenous cannabinoids in general) are infrequently analysed together with CB2 models, a recent study (Raduner *et al.*, 2006) has used this approach. The modelling of the CB2 receptor uses rhodopsin as a template (Palczewski *et al.*, 2000) and the molecular docking of some alkylamides into the putative CB2 binding pocket is an interesting point. The problem with alkylamides and other *N*-arachidonoylethanolamine-like compounds is the huge conformational space to be covered if normal docking methods are used. In this case, the Flexidock algorithm of Sybyl was used together with molecular dynamics/molecular mechanics minimization. In this approach the amide group of the alkylamide is headed into the hydrophilic pocket, surrounded by the residues D5.38 and Y5.39. The interaction between alkylamides and Y5.39 are mediated not only via a hydrogen bond (phenolic oxygen of Y5.39 and amide hydrogen of alkylamides) but also via interactions between Y5.39 and the C-2/C-3 double bond of an alkylamide. In one case (Salo *et al.*, 2005) the CB2 model constructed has been used to find new ligands for CB2 and not only to explain SAR data. In this case a new, although weak, CB2 selective ligand was found. The CB2 model was constructed without rotating TM helixes and thus the model is close to the inactive state of rhodopsin. The final model was utilized both in building the database queries (receptor-based pharmacophore) and in filtering the hit compounds by a docking and scoring method (consensus scoring).

CB2 models have also been used to design a fluorescent CB2 compound (Yates *et al.*, 2005). The authors used a homology model of the CB2 receptor and a *de novo* molecular design programme LigBuilder (Wang *et al.*, 2000) to predict the binding activity of a fluorescent derivative of the CB2 agonist JWH-015. While this prediction was not

perfect, this case represents an approach not often seen in literature.

The reason why comparative CB2 receptor models have not been described more frequently in virtual screening is not clear. Such models may have been used for predictions, but the results are either good enough (researchers are applying for patents, and are unable to publish the results), or the results are far from satisfactory. If the latter is true one should try to find an explanation. An obvious reason is the quality of the models used, and particularly the approach to modelling the active state of the CB2 receptor. It has been stated that activation of a GPCR requires large conformational changes in TM helixes (for an excellent review see Kobilka, 2007) and extensive modelling effort to reach and analyse an active state has been carried out (Niv *et al.*, 2006). The current hypothesis also includes large conformational movements upon GPCR activation (either constitutive or ligand induced) and these movements are general among different GPCR receptor types. However, the CB2 (and also CB1) receptors seem to differ in many senses from other class-A receptors (for example, Nebane *et al.*, 2006), and it may be quite challenging to predict how a CB2 receptor model should be manipulated to achieve an active state (although approach of Niv *et al.*, 2006 may offer a solution). Currently there is one structure of a photoactivated intermediate of GPCR, rhodopsin, at a resolution of 4.2 Å (Salom *et al.*, 2006). While this structure is not the activated form of the receptor, it still indicates that the scale of the movements of the TM helices is much smaller than earlier predicted using indirect biochemical methods.

Acknowledgements

JWH thanks the National Institute on Drug Abuse for support through grant DA03590. AP thanks Alexander von Humboldt Foundation.

Conflict of interest

The authors state no conflict of interest.

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