Indoles and Related Compounds as Cannabinoid Ligands

Clementina Manera, Tiziano Tuccinardi, Adriano Martinelli*

Dipartimento di Scienze Farmaceutiche, Università di Pisa, via Bonanno 6, 56126 Pisa, Italy

Abstract: The discovery of the endocannabinoid system has lead to great strides in research development. At present, two cannabinoid receptors, CB1R and CB2R, are known. They belong to Class A rhodopsin-like GPCRs, and possess a different tissue distribution. Many synthetic compounds have been synthesized and tested for their cannabinoid activity. A particular class among them, the aminoalkylindole derivatives (typified by WIN55212-2) are hypothesized to interact in a binding site different from the main cannabinoid agonists.

In this review we report the main aminoalkylindole derivatives, and other compounds which are hypothesized to interact in the same binding site. Furthermore we analyze the pharmacological profiles, the mutagenesis data and the computational models that describe their interaction in the cannabinoid receptors, evaluating the most important aspects for their activity and selectivity.

Key Words: Cannabinoid receptors, CB1R, CB2R, aminoalkylindoles, WIN55212-2.

INTRODUCTION

The term cannabinoid was first used to describe the tricyclic natural compounds from *Cannabis sativa* L, of which the (-)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC, (**1**)), was shown to be the principal psychoactive component of hashish and marijuana [1].

The pharmacological effects of cannabinoids are considered to be mediated through at least two G-protein-coupled seven-transmembrane receptors (GPCRs), namely CB1R and CB2R. However, recent evidence has been presented for the existence of a third cannabinoid receptor, which has been detected in the mouse brain [2].

The CB1R subtype is mainly located in the central nervous system, with the highest density in the cerebellum, the basal ganglia, the substantia nigra pars compacta, and some regions of the globus pallidus. CB1R is also present in peripheral organs such as the adrenal glands, bone marrow, lung, testis, and uterus [3]. The CB1R is nowadays extensively studied due to its implication both in the therapeutic and psychoactive effects of cannabinoids in the central nervous system. Transduction mechanisms of CB1R involve inhibition of cAMP production through inhibition of adenylate cyclase [4], inhibition of calcium influx [5,6], activation of potassium channels [7], and activation of the MAP kinase pathway [8].

The CB2R was originally identified from macrophages present in the spleen, and it is expressed primarily in cells associated with the immune system, like spleen, thymus, and tonsils [9]. Two mechanisms have been identified for the transduction mechanisms of CB2R: inhibition of adenylate cyclase [10] and stimulation of mitogen activated protein kinase [11]. Furthermore unlike CB1R, the CB2R does not have effect on ion channels [12].

*Address correspondence to this author at the Dipartimento di Scienze Farmaceutiche, Università di Pisa, *via* Bonanno 6, 56126 Pisa, Italy; Fax: ++39 050 2219605; E-mail: marti@farm.unipi.it

The physiological and putative therapeutic potential of the CB2R largely remains unexplored; however, recent data indicate that CB2R participates in the control of peripheral pain [13], inflammation [14], osteoporosis [15], growth of malignant gliomas [16], tumors of immune origin [17], and immunological disorders such as multiple sclerosis [18]. Furthermore, CB2R agents could be exploited for prevention of Alzheimer's disease pathology, given the presence of the CB2R in the brain microglial cells [19,20], and it has recently been shown that CB2R agonists might provide neuroprotection by blockade of microglial activation [21]. Finally, selective CB2R agonists may be the basis for developing new drugs for the treatment of amyotrophic lateral sclerosis [22].

Cannabinoid receptor agonists can be divided into four structurally distinct classes of compounds. These include classic cannabinoids like Δ^9-THC (**1**) (see Fig. (**1**)), nonclassical cannabinoids, represented by CP55940 (**2**), aminoalkylindoles, such as WIN55212-2 (**3**), and endogenous cannabinoids such as arachidonylethanolamide, also called anandamide (**5**) (AEA) [23].

Aminoalkylindole derivatives are structurally dissimilar from the other classes, and are hypothesized to interact in a binding site different from that of the other cannabinoid receptors (CBRs) agonists. This class of ligands appeared to be quite interesting due not only to their particular molecular structure but also to the selectivity properties that some of them have shown.

As for other class of GPCR ligands, also in the case of this kind of compounds, the building through homology procedure of models of CBRs proved to be fundamental for the design of new improved ligands.

The aim of this paper is to give an updated review about the main published results in the field of the aminoalkylindole derivatives tested as CBRs ligands and all the ligands that are supposed to interact in the aminoalkylindole binding site.

1389-5575/08 $55.00+.00

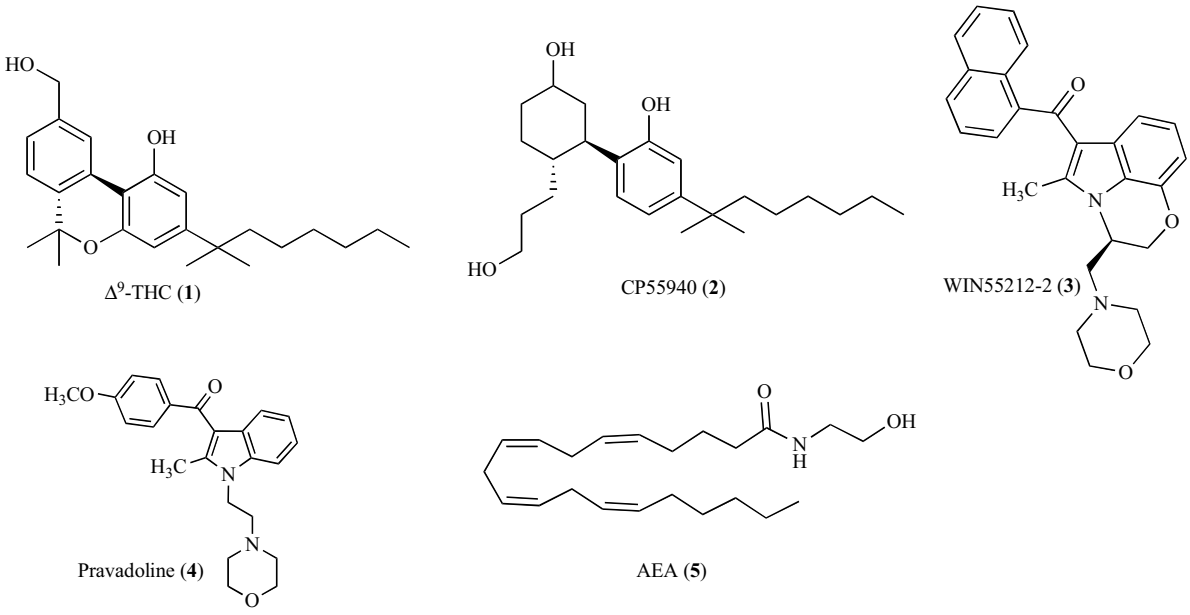


Fig. (1). Chemical structures of the most well-known cannabinoid ligands.

Many reviews have been written in recent years regarding the cannabinoid receptors [23-29] in particular very recently Huffman reported a review concerning the ligand of the CB2R [24] and Raitio and co-workers reported an analysis of the data concerning the CB2R [27]. Differently from all these reviews here we reported from a pharmaceutical and modelling point of view the analysis of the aminoalkylindole derivatives and all the compounds that are supposed to interact in the aminoalkylindole binding site. In this review, all these compounds are identified as aminoalkylindole binding site (AAIBS) derivatives.

AAIBS DERIVATIVES

Aminoalkylindoles and Analogs

Some years ago in the course of a program directed toward the development of non-steroidal anti-inflammatory drugs, a group at Sterling–Winthrop reported that Pravadoline (**4**) (see Fig. (**1**)), an indole derivative, and related compounds unexpectedly inhibited contractions of the electrically stimulated mouse vas deferens [30]. These aminoalkylindoles (AAIs) were found to inhibit adenylate cyclase, to be antinociceptive and to interact with a G-coupled protein in the brain that was subsequently identified as CB1R subtype. Moreover these aminoalkylindoles exhibit typical cannabinoid pharmacology *in vivo* [31,32].

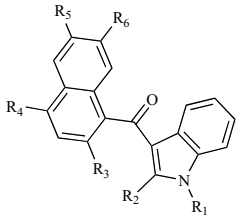
One rigid aminoalkylindole, WIN55212-2 (**3**) (see Fig. (**1**)), has shown particularly high affinity for both the CB1R and CB2R with a modest selectivity on CB2R and has been employed extensively in a number of investigations into the pharmacology of this group of compounds [33]. In particular some studies indicate that the isomer *s-trans* of WIN55212-2 (**3**), as well as of the all 2-methyl-aminoalkylindoles, is the isomer with the higher CB1R and CB2R affinities and the higher pharmacological potency [23]. Recently was reported that WIN55212-3 which is known as the inactive isomer of WIN55212-2 (**3**) is a competitive neutral antagonist of the human CB2R. In contrast WIN55212-3 acts as partial inverse agonist at the human CB1R [34].

From these early studies some preliminary SAR were developed [30,31,35,36]. In particular it was suggested that a group larger than methyl at C-2 of the indole nucleus greatly reduces potency and a hydrogen at the same position was slightly superior to a methyl group. Furthermore a bicyclic aroyl group, usually 1-naphthoyl or a substituted 1-naphthoyl group, at C-3 is essential for cannabinoid activity as well as an aminoalkyl group, usually substituted aminoethyl.

Subsequently some studies established that the aminoalkyl group is not necessary for cannabimimetic activity and could be replaced by an alkyl group [37,38]. In particular 1-penthyl derivative **JWH-007** has an high affinity for the CB1R (see Table **1**). On the contrary the 1-propyl analogs, **JWH-015** and **JWH-046** (see Table **1**) have relatively high affinity for the CB2R, and weak affinity for the CB1R [33]. For this reason several *N*-propyl indoles were prepared to obtain CB2R selective compounds (see Table **1**).

In order to develop SAR for both the CB1R and CB2R, a number of additional indole derivatives were prepared and their affinity was evaluated [39,40]. It was found that a *n*-pentyl nitrogen substituent is optimum for CB1R affinity and that CB1R affinity decreases dramatically when *N*-alkyl substituent of three or less carbon atoms or longer than six carbon atoms is attached to the indole nitrogen (see Table **1**). In agreement with the Wintrop data, a 2-methyl group slightly decreases affinity at the CB1R, relative to an unsubstituted 2-position. Moreover with regards to the 3-(1-naphthoyl) substituents, it was found that a 7-methyl substituent as in **JWH-046** and **JWH-048** has little effect on either CB1R or CB2R affinity while a 4-methoxy-1-naphthoyl group at C-3 of the indole, as in **JWH-098** slightly enhances CB1R affinity (see Table **1**). As regards larger 4-alkoxyl groups, they make the compound inactive.

Table 1. *N*-alkyl-3-(1-naphthoyl)indole Derivatives with their CB1R and CB2R Receptor Affinities[a]



Compd.	R_1	R_2	R_3	R_4	R_5	R_6	CB1R Ki (nM)	CB2R Ki (nM)
WIN55212-2							1.89±0.09	0.28±0.16
JWH-004	C_6H_{13}	CH_3	H	H	H	H	48±13	4.02±1.46
JWH-007	C_5H_{11}	CH_3	H	H	H	H	9.5±4.5	2.9±2.6
JWH-009	C_7H_{15}	CH_3	H	H	H	H	311±106	141±14.5
JWH-015	C_3H_7	CH_3	H	H	H	H	164±22	13.8±4.6
JWH-016	C_4H_9	CH_3	H	H	H	H	22±1.5	4.29±1.63
JWH-018	C_5H_{11}	H	H	H	H	H	9±5	2.9±2.6
JWH-019	C_6H_{13}	H	H	H	H	H	9.8±2	5.5±2
JWH-020	C_7H_{15}	H	H	H	H	H	128±17	205±20
JWH-042	CH_3	CH_3	H	H	H	H	>10000	5050±192
JWH-043	C_2H_5	CH_3	H	H	H	H	1180±44	964±242
JWH-046	C_3H_7	CH_3	H	H	H	CH_3	343±38	16±5
JWH-047	C_4H_9	CH_3	H	H	H	CH_3	58.7±3	3.47±1.8
JWH-048	C_5H_{11}	CH_3	H	H	H	CH_3	10.7±1.0	0.49±0.1
JWH-049	C_6H_{13}	CH_3	H	H	H	CH_3	55.1±17	32.3±2.4
JWH-050	C_7H_{15}	CH_3	H	H	H	CH_3	342±6	526±133
JWH-070	CH_3	H	H	H	H	H	>10000	>10000
JWH-071	C_2H_5	H	H	H	H	H	1340±123	2940±852
JWH-072	C_3H_7	H	H	H	H	H	1050±55	170±54
JWH-073	C_4H_9	H	H	H	H	H	8.9±1.8	38±24
JWH-076	C_3H_7	H	H	H	H	CH_3	214±11	106±46
JWH-077	CH_3	H	H	OCH_3	H	H	>10000	>10000
JWH-078	C_2H_5	H	H	OCH_3	H	H	817±60	633±116
JWH-079	C_3H_7	H	H	OCH_3	H	H	63±3	32±6
JWH-080	C_4H_9	H	H	OCH_3	H	H	5.6±1	2.21±1.3
JWH-081	C_5H_{11}	H	H	OCH_3	H	H	1.2±0.03	12.4±2.23
JWH-082	C_6H_{13}	H	H	OCH_3	H	H	5.3±0.8	6.4±0.94
JWH-083	C_7H_{15}	H	H	OCH_3	H	H	106±12	102±50
JWH-093	C_3H_7	C_4H_9	H	OCH_3	H	H	40.7±2.8	59.1±10.5
JWH-094	C_3H_7	CH_3	H	OCH_3	H	H	476±67	97.3±2.7

(Table 1. Contd….)

Compd.	R_1	R_2	R_3	R_4	R_5	R_6	CB1R Ki (nM)	CB2R Ki (nM)
JWH-095	C_4H_9	C_5H_{11}	H	OCH_3	H	H	140±4.3	312±83
JWH-096	C_4H_9	CH_3	H	OCH_3	H	H	33.7±2.9	13.3±5.6
JWH-097	C_5H_{11}	C_6H_{13}	H	OCH_3	H	H	455±28	121±15
JWH-098	C_5H_{11}	CH_3	H	OCH_3	H	H	4.5±0.1	1.88±0.3
JWH-099	C_6H_{13}	CH_3	H	OCH_3	H	H	35.3±9	17.8±2.87
JWH-100	C_7H_{15}	CH_3	H	OCH_3	H	H	381±102	155±74.3
JWH-120	C_3H_7	H	H	CH_3	H	H	1054±31	6.1±0.7
JWH-122	C_5H_{11}	H	H	CH_3	H	H	0.69±0.5	1.2±1.2
JWH-148	C_3H_7	CH_3	H	CH_3	H	H	123±8	14±1.0
JWH-149	C_5H_{11}	CH_3	H	CH_3	H	H	5.0±2.1	0.73±0.03
JWH-151	C_3H_7	CH_3	H	H	OCH_3	H	>10000	30±1.1
JWH-153	C_5H_{11}	CH_3	H	H	OCH_3	H	250±24	11±0.5
JWH-159	C_5H_{11}	CH_3	H	H	H	OCH_3	45±1	10.4±1.4
JWH-160	C_3H_7	CH_3	H	H	H	OCH_3	1568±201	441±110
JWH-163	C_3H_7	H	H	H	OCH_3	H	2358±215	138±12
JWH-164	C_5H_{11}	H	H	H	H	OCH_3	6.6±0.7	6.9±0.2
JWH-165	C_3H_7	H	H	H	H	OCH_3	204±26	71±8
JWH-166	C_5H_{11}	H	H	H	OCH_3	H	44±10	1.9±0.08
JWH-180	C_3H_7	H	H	C_3H_7	H	H	26±2	9.6±2.0
JWH-181	C_5H_{11}	CH_3	H	C_3H_7	H	H	1.3±0.1	0.62±0.04
JWH-182	C_5H_{11}	H	H	C_3H_7	H	H	0.65±0.03	1.1±0.1
JWH-189	C_3H_7	CH_3	H	C_3H_7	H	H	52±2	12±0.8
JWH-210	C_5H_{11}	H	H	C_2H_5	H	H	0.46±0.03	0.69±0.01
JWH-211	C_3H_7	CH_3	H	C_2H_5	H	H	70±0.8	12±0.8
JWH-212	C_3H_7	H	H	C_2H_5	H	H	33±0.9	10±1.2
JWH-213	C_5H_{11}	CH_3	H	C_2H_5	H	H	1.5±0.2	0.42±0.05
JWH-234	C_5H_{11}	H	H	H	H	C_2H_5	8.4±1.8	3.8±0.6
JWH-235	C_3H_7	H	H	H	H	C_2H_5	338±34	123±34
JWH-236	C_3H_7	CH_3	H	H	H	C_2H_5	1351±204	240±63
JWH-239	C_3H_7	H	H	C_4H_9	H	H	342±20	52±6
JWH-240	C_5H_{11}	H	H	C_4H_9	H	H	14±1	7.2±1.3
JWH-241	C_3H_7	CH_3	H	C_4H_9	H	H	147±20	49±7
JWH-242	C_5H_{11}	CH_3	H	C_4H_9	H	H	42±9	6.5±0.3
JWH-258	C_5H_{11}	H	H	OC_2H_5	H	H	4.6±0.6	10.5±1.3
JWH-259	C_3H_7	H	H	OC_2H_5	H	H	220±29	74±7
JWH-260	C_5H_{11}	CH_3	H	OC_2H_5	H	H	29±0.4	25±1.9

(Table 1. Contd….)

Compd.	R_1	R_2	R_3	R_4	R_5	R_6	CB1R Ki (nM)	CB2R Ki (nM)
JWH-261	C_3H_7	CH_3	H	OC_2H_5	H	H	767±105	221±14
JWH-262	C_5H_{11}	CH_3	H	H	H	C_2H_5	28±3	5.6±0.7
JWH-265	C_3H_7	H	OCH_3	H	H	H	3788±323	80±13
JWH-266	C_3H_7	CH_3	OCH_3	H	H	H	>10000	455±55
JWH-267	C_5H_{11}	H	OCH_3	H	H	H	381±16	7.2±0.14
JWH-268	C_5H_{11}	CH_3	OCH_3	H	H	H	1379±193	40±0.6

[a]Data taken from [33,39,48].

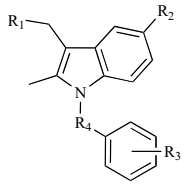
The SAR at the CB2R are very similar to those at the CB1R; however, differently from CB1R subtype, a methoxy-group at position 4 of the 1-naphthoyl system determined an important general increase of CB2R affinity [24,39].

Further *N*-benzoyl-, *N*-naphtoyl-, and *N*-2-ethyl(morpholinyl)-indoles have been reported as CB2R selective ligands [41]. In particular two potent compounds (**17** and **22** of Table **2**) with Ki of 12 nM and 8.5 nM, respectively, for the human CB2R. They exhibit good selectivity over the human CB1R (CB1R/CB2R = 160 for **17** and 103 for **22**).

Additionally substituted C-3 carboxamide indoles have been reported as CB2R ligands characterized by a high CB2R/CB1R selectivity (see Table **3**) [42,43].

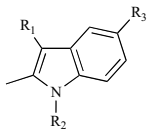
In several patents some 3-arylketoneindoles with *N*-1-alkyl chains, the ends of the which there are heterocycles or other functional groups are described [44,45], including AM1221 (**34**) and AM1241 (**35**) (see Table **4**). This last compound has been studied in animal models of neurophatic pain [46]. More recently novel CB2R selective indoles such as **36** characterized by sulphide, sulfoxide, sulphonamide and ether group on the N1 alkyl chain were reported [47].

Table 2. ***N*-benzoyl-*N*-naphtoyl- and *N*-2-ethyl(morpholinyl)-indoles with their CB1R and CB2R Receptor Affinities[a]**



Compd.	R_1	R_2	R_3	R_4	CB1R Ki (nM)	CB2R Ki (nM)
6	C(O)-N-morph	OCH_3	4-Cl	C(O)	>20000	435±43
7	COOMe	OCH_3	2-Cl	C(O)	1720±425	397±27
8	COOH	OCH_3	4-Cl	C(O)	>20000	>20000
9	COOMe	OCH_3	4-Cl	C(O)	>20000	4021±1977
10	CH_2-N-morph	OCH_3	4-Cl	C(O)	>10000	213±25
11	C(O)-N-morph	OCH_3	2-Cl	C(O)	3600±706	69±4
12	C(O)-N-morph	OCH_3	3-Cl	C(O)	>10000	354±45
13	C(O)-N-morph	OCH_3	2-Cl,3-Cl	C(O)	2043±183	14±6
14	C(O)-N-morph	OCH_3	2-Cl,4-F	C(O)	5570±1441	134±11
15	C(O)-N-morph	OCH_3	2-Cl,6-Cl	C(O)	2553±611	59±7
16	C(O)-N-morph	OCH_3	2-Cl,3-Cl	CH_2	>20000	1046±367
17	CH_2-N-morph	OCH_3	2-Cl,3-Cl	C(O)	1917±381	12±0.2
18	N-morph	OCH_3	2-Cl,3-Cl	C(O)	3363±856	22±5
19	CH_2-N-morph	H	2-Cl,3-Cl	C(O)	3193±881	27±2

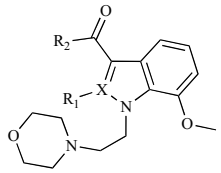
(Table 2. Contd….)



Compd	R_1	R_2	R_3	CB1R	CB2R
20	CH_2COOMe	1-naphthoyl	OCH_3	>1890	142±21
21	CH_2CH_2-N-morph	1-naphthoyl	H	2183±825	216±33
22	CH_2-N-morph	1-naphthoyl	H	877±222	8.5±1.6
23	CH_2-N-morph	1-naphthyl	H	6680±2359	>2700
24	1-naphthoyl	CH_2CH_2-N-morph	H	638±172	14±0.4

[a]Data taken from [41].

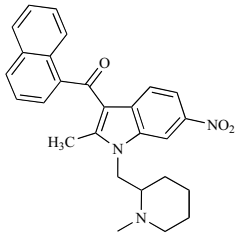
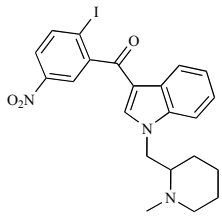
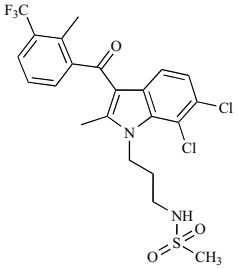
Table 3. *N*-2-ethyl(morpholinyl)-3-carboxamide Indoles with their CB1R and CB2R Receptor Affinities[a]



Compd	R_1	R_2	X	CB1R Ki (nM)	CB2R Ki (nM)
25	CH_3	HN[(*S*) 3-phenylpropan-2-yl methyl estere]	CH	4000	8
26	H	HN[(1*S*)-fenchyl]	CH	245±52	11±3
27	CH_3	HN[(1*S*)-fenchyl]	CH	8±2	29±6
28	C_2H_5	HN[(1*S*)-fenchyl]	CH	ND	110±23
29	C_3H_7	HN[(1*S*)-fenchyl]	CH	ND	6%
30	-	HN[(1*S*)-fenchyl]	N	24±10	2.0±0.5
31	-	HN[α-(2-pyridyl)benzyl]	N	162±60	146±79
32	-	HN[(2)-methoxybenzyl]	N	ND	77±20
33	-	N[(N-2-propyl)(2-chloro-6-fluorobenzyl)]	N	ND	69±26

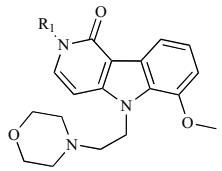
[a]Data taken from [42,43]. ND means Not Determined.

Table 4. Chemical Structures of AM1221 (34), AM1241 (35) and Compound 36 with their CB1R and CB2R Receptor Affinities[a]

AM1221 (34)		AM1241 (35)		Compd 36	
					
CB1R Ki (nM)	**CB2R Ki (nM)**	**CB1R Ki (nM)**	**CB2R Ki (nM)**	**CB1R Ki (nM)**	**CB2R Ki (nM)**
52 nM	**0.28 nM**	**285 nM**	**0.53 nM**	**ND**	**ND**

[a]Data taken from [44]. ND means Not Determined.

Table 5. Some Indolopyridone Derivatives with their CB1R and CB2R Receptor Affinities[a]



Compd	R_1	CB1R Ki (nM)	CB2R Ki (nM)
37	(1S)-fenchyl	16±4	1.0±0.2
38	(2)-methoxybenzyl	3700±1000	67±23
39	α-(2-pyridyl)benzyl	ND	13%[b]

[a]Data taken from [43]. [b] % inhibition at 500 μM. ND means Not Determined.

Subsequently were reported some tricyclic compounds structurally analogs of indole derivatives which shows considerable selectivity for the CB2R including compounds **37** and **38** [43] of Table **5**. In particular compound **37** showed anti-inflammatory properties *in vivo* [43].

Recently with the aim to prepare CB2R selective ligands, a number of additional cannabimimetic indoles have been prepared and their affinities for the CB1R and CB2R were reported [48]. These compounds are characterized by propyl or pentyl group as substituent on the indole nitrogen and the naphthoyl group at C-3 contains various alkyl and alkoxy substituents. Furthermore the indole is either unsubstituted at C-2 or contains a 2-methyl group. (see Table **1**).

The results for these compounds indicate that the CB1R affinity is enhanced considerably by the presence of small alkyl groups (methyl, ethyl, and propyl) or methoxy substituent at C-4 of the naphthoyl group. On the contrary the same alkyl substituents at C-7 and the methoxy group at C-6 or C7 determined a little effect on affinity. In this work **JWH-120**, **JWH-151** and **JWH-267** were reported as new highly selective CB2R agonists [48].

In the same year a new class of cannabimimetic indoles, with unsubstituted or substituted 3-phenylacetyl substituents, has been prepared. Two of these compounds, **JWH-251** and **JWH-302**, have 5-fold selectivity for the CB1R with modest affinity for the CB2R (see Table **6**) [49].

More recently in order to confirm the hypothesis that the indole derivatives interact with the CB1R primarily by aromatic stacking, two series of 1-alkyl-2-aryl-4-(1-naphthoyl) pyrroles were synthesized (see Table **7**) [50].

Several compounds show CB1R affinities in the 6–30 nM range. The high affinities of these pyrroles support the hypothesis that, like the indole derivatives, they interact with the CB1R by aromatic stacking [49].

Oxoquinolines and Oxonaphthyridines

In a patent of Japan Tobacco Inc. [51] 2-oxoquinoline structures that act selectively at cannabinoid receptor, especially at the CB2R, were reported. The most selective and active compounds is **43** (see Table **8**), which behaves as an inverse agonist *in vitro* and possesses anti-inflammatory properties *in vivo*. However several compounds described in the patent possess high selectivity for the CB2R with Ki(CB1R)/Ki(CB2R) ratio from 20000 to 70000.

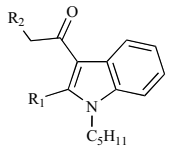
Subsequently in order to obtain more potent and effective CB2R inverse agonists several analogues of **43** were prepared and the analysis of the structure-activity relationships was reported [52]. As a result, all the compounds were defined as full CB2R inverse agonists, and additionally, except for two 3,4-dihydroxyphenylalkylamides, were found to be equally potent as SR144528 [52].

More recently our research group reported a new series of 1,8-naphthyridin-4(1H)-on-3-carboxamide and quinolin-4(1H)-on-3-carboxamide derivatives which exhibit remarkable affinity and selectivity at CB2R (see Table **9**) [53,54]. In particular compound **85**, which presented *p*-fluorobenzyl and carboxycycloheptylamide substituents bound in the 1 and 3 positions of the 1,8-naphthyiridine-4-one nucleus, showed a high CB2R affinity with a Ki of 1.0 nM. Moreover the *N*-cyclohexyl-7-chloro-1-(2-morpholin-4-ylethyl)quinolin-4(1*H*)-on-3-carboxamide (**101**) possessed a remarkable affinity, with Ki of 3.3 nM, which was also accompanied by a high selectivity for the CB2R (Ki(CB1R)/ Ki(CB2R) ratio greater than 303). Furthermore the [^{35}S]GTPγ binding assay and functional studies on human basophils indicated that these compounds behaved as CB1R and CB2R agonists. It is also possible to hypothesise that in order to obtain a good CB2R/CB1R selectivity and CB2R affinity seemed to be required the presence of a nonaromatic carboxamide substituent in position 3 and a lipophilic substituent with an H bond acceptor in position 1.

Furthermore a set of 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives were reported as CB2R agonists (see Table **10**) [55].

The results indicate that these derivatives exhibited a CB2R selectivity, in particular, derivative **112** showed a high selectivity for the CB2R (Ki(CB1R)/ Ki(CB2R)= 143). Moreover, in the [^{35}S]-GTPγ binding assay, all the compounds behaved as CB2R agonists.

Table 6. 1-Pentyl-3-phenylacetylindoles with their CB1R and CB2R Receptor Affinities[a]



Compd.	R_1	R_2	CB1R Ki (nM)	CB2R Ki (nM)
JWH-167	H	PhAc	90±17	159±14
JWH-205	CH_3	PhAc	124±23	180±9
JWH-251	H	2-CH_3-PhAc	29±3	146±36
JWH-252	CH_3	2-CH_3-PhAc	23±3	19±1
JWH-208	H	4-CH_3-PhAc	179±7	570±127
JWH-209	CH_3	4-CH_3-PhAc	746±49	1353±270
JWH-250	H	2-OCH_3-PhAc	11±2	33±2
JWH-306	CH_3	2-OCH_3-PhAc	25±1	82±11
JWH-302	H	3-OCH_3-PhAc	17±2	89±15
JWH-253	CH_3	3-OCH_3-PhAc	62±10	84±12
JWH-201	H	4-OCH_3-PhAc	1064±21	444±14
JWH-202	CH_3	4-OCH_3-PhAc	1678±63	645±6
JWH-311	H	2-F-PhAc	23±2	39±3
JWH-314	CH_3	2-F-PhAc	39±2	76±4
JWH-312	H	3-F-PhAc	72±7	91±20
JWH-315	CH_3	3-F-PhAc	430±24	182±23
JWH-313	H	4-F-PhAc	422±19	365±92
JWH-316	CH_3	4-F-PhAc	2862±670	781105
JWH-203	H	2-Cl-PhAc	8.0±0.9	7.0±1.3
JWH-204	CH_3	2-Cl-PhAc	13±1	25±1
JWH-237	H	3-Cl-PhAc	38±10	106±2
JWH-303	CH_3	3-Cl-PhAc	117±10	138±12
JWH-206	H	4-Cl-PhAc	389±25	498±37
JWH-207	CH_3	4-Cl-PhAc	1598±134	3723±10
JWH-249	H	2-Br-PhAc	8.4±1.8	20±2
JWH-305	CH_3	2-Br-PhAc	15±1.8	29±5
JWH-248	H	4-Br-PhAc	1028±39	657±19
JWH-304	CH_3	4-Br-PhAc	3363±332	2679±688

[a]Data taken from [49]. PhAc indicates Phenylacetyl.

CBRs MODELLING

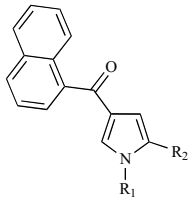
Both CB1R and CB2R are seven-transmembrane (TM) receptors that belong to the rhodopsin-like family Class A of GPCRs.

The modelling of a GPCR is generally made through a homology procedure which starts from the alignment with a template and, successively, a refinement which takes into account all the available experimental data concerning the receptors and the interaction receptor-ligands.

Alignment

Fig. (**2**) shows the CLUSTALW [56] alignment for human CB1R and CB2R.

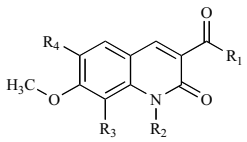
Table 7. 1-Alkyl-2-aryl-4-(1-naphthoyl)pyrroles with their CB1R and CB2R Receptor Affinities[a]



Compd.	R_1	R_2	CB1R Ki (nM)	CB2R Ki (nM)
JWH-156	C_3H_7	C_6H_5	404±18	104±18
JWH-150	C_4H_9	C_6H_5	60±1	15±2
JWH-145	C_5H_{11}	C_6H_5	14±2	6.4±0.4
JWH-147	C_6H_{13}	C_6H_5	11±1	7.1±0.2
JWH-146	C_7H_{15}	C_6H_5	21±2	62±5
JWH-370	C_5H_{11}	*o*-CH_3-Ph	5.6±0.4	4.0±0.5
JWH-365	C_5H_{11}	*o*-C_2H_7-Ph	17±1	3.4±0.2
JWH-373	C_5H_{11}	*o*-C_4H_9-Ph	60±3	69±2
JWH-292	C_5H_{11}	*o*-OCH_3-Ph	29±1	20±1
JWH-307	C_5H_{11}	*o*-F-Ph	7.7±1.8	3.3±0.2
JWH-369	C_5H_{11}	*o*-Cl-Ph	7.9±0.4	5.2±0.3
JWH-372	C_5H_{11}	*o*-CF_3-Ph	77±2	8.2±0.2
JWH-346	C_5H_{11}	*m*-CH_3-Ph	67±6	39±2
JWH-367	C_5H_{11}	*m*-OCH_3-Ph	53±2	23±1
JWH-368	C_5H_{11}	*m*-F-Ph	16±1	9.1±0.7
JWH-246	C_5H_{11}	*m*-Cl-Ph	70±4	16±1
JWH-363	C_5H_{11}	*m*-CF_3-Ph	245±5	71±1
JWH-293	C_5H_{11}	*m*-NO_2-Ph	100±5	41±4
JWH-244	C_5H_{11}	*p*-CH_3-Ph	130±6	18±1
JWH-364	C_5H_{11}	*p*-C_2H_7-Ph	34±3	29±1
JWH-371	C_5H_{11}	*p*-C_4H_9-Ph	42±1	64±2
JWH-243	C_5H_{11}	*p*-OCH_3-Ph	285±40	41±3
JWH-308	C_5H_{11}	*p*-F-Ph	41±1	33±2
JWH-245	C_5H_{11}	*p*-Cl-Ph	276±4	25±2
JWH-348	C_5H_{11}	*p*-CF_3-Ph	218±19	53±1
JWH-309	C_5H_{11}	1-Naphthyl	41±3	49±7
JWH-347	C_5H_{11}	2-Naphthyl	333±17	169±17
JWH-366	C_5H_{11}	3-Pyridyl	191±12	24±1

[a]Data taken from [50].

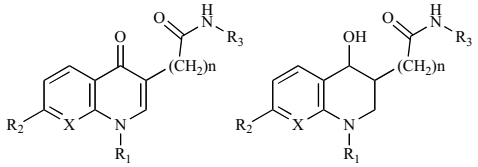
Table 8. Some 2-oxoquinoline Derivatives with their CB1R and CB2R Receptor Affinities[a,b]



Compd.	R_1	R_2	R_3	R_4	CB1R Ki (nM)	CB2R Ki (nM)
40	OCH_3	H	H	OC_5H_{11}	3671	0.014
41	OH	H	OC_4H_9	H	3247	0.77
42	OH	H	OC_3H_7	H	905	0.032
43	HN[benzo[d][1,3]dioxol-4-yl]	H	OC_5H_{11}	H	3436	0.087
44	HN[2-(pyridin-4-yl)ethyl]	H	OC_2H_5	H	609	0.02
45	HN[4-fluorophenethyl]	H	OC_4H_9	H	249	0.016
46	HN[2-(pyridin-4-yl)ethyl]	H	OC_4H_9	H	208	0.01
47	HN[4-fluorophenethyl]	H	OC_3H_7	H	336	0.021
48	HN[4-fluorophenethyl]	H	H	OC_5H_{11}	2398	0.036
49	HN[4-fluorophenethyl]	CH_3	H	OC_5H_{11}	864	0.043

[a]Data taken from [51]. [b]Table reports only the most CB2R/CB1R selective compounds described in the Patent.

Table 9. 1,8-naphthyridin-4(1H)-on-3-carboxamide and quinolin-4(1H)-on-3-carboxamide Derivatives with their CB1R and CB2R Receptor Affinities[a]



50-95, 99-101 **96-98**

Compd.	R1	R2	R3	X	n	CB1R Ki (nM)	CB2R Ki (nM)
50	H	CH_3	cyclohexyl	N	0	>1000	>1000
51	H	CH_3	benzyl	N	0	>1000	>1000
52	ethylmorph	CH_3	cyclohexyl	N	0	>1000	100±8
53	ethylmorph	CH_3	morph	N	0	>1000	>1000
54	ethylmorph	CH_3	CH_2cyclohexyl	N	0	>1000	117±15
55	ethylmorph	CH_3	N-CH_3pipz	N	0	>1000	>1000
56	ethylmorph	CH_3	benzyl	N	0	>1000	475±25
57	ethylmorph	CH_3	4-CH_3cyclohexyl	N	0	537±24	30±2
58	ethylmorph	CH_3	cyclopentyl	N	0	>1000	50±4
59	ethylmorph	CH_3	cycloheptyl	N	0	560±33	22±2
60	ethylmorph	CH_3	isopentyl	N	0	>1000	50±3
61	ethylmorph	CH_3	*p*-Cl-benzyl	N	0	>1000	>1000

(Table 9. Contd….)

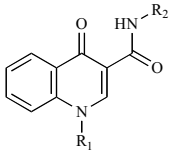
Compd.	R1	R2	R3	X	n	CB1R Ki (nM)	CB2R Ki (nM)
62	benzyl	CH_3	cyclohexyl	N	0	127±13	10±0.5
63	benzyl	CH_3	benzyl	N	0	>1000	>1000
64	benzyl	CH_3	*p*-Cl-benzyl	N	0	>1000	>1000
65	*o*-F-benzyl	CH_3	cyclohexyl	N	0	208±17	44±2
66	*o*-F-benzyl	CH_3	benzyl	N	0	>1000	600±60
67	*p*-F-benzyl	CH_3	cyclohexyl	N	0	15±1.8	5.5±0.4
68	*p*-F-benzyl	CH_3	benzyl	N	0	457±40	65.3±6
69	*n*-hexyl	CH_3	cyclohexyl	N	0	95±3	8.0±0.2
70	*n*-hexyl	CH_3	benzyl	N	0	>1000	325±25
71	*n*-butyl	CH_3	cyclohexyl	N	0	262±10.4	17.5±1
72	*n*-butyl	CH_3	benzyl	N	0	>1000	>1000
73	ethylmorph	NH_2	benzyl	N	0	>1000	>1000
74	ethylmorph	NH_2	cyclohexyl	N	0	>1000	>1000
75	ethylmorph	Cl	cyclohexyl	N	0	>1000	25±1.8
76	ethylmorph	CH_3	benzyl	N	1	>1000	>1000
77	benzyl	CH_3	benzyl	N	1	>1000	729±82
78	ethylmorph	CH_3	cyclohexyl	N	1	>1000	>1000
79	benzyl	CH_3	cyclohexyl	N	1	>1000	530±50
80	*n*-hexyl	CH_3	cyclohexyl	N	1	>1000	>1000
81	*n*-butyl	CH_3	cyclohexyl	N	1	>1000	>1000
82	*p*-F-benzyl	CH_3	4-CH_3cyclohexyl	N	0	8.7±1.6	1.4±0.1
83	*o*-F-benzyl	CH_3	4-CH_3cyclohexyl	N	0	37.5±5.4	8.4±0.3
84	benzyl	CH_3	cycloheptyl	N	0	143.2±9.1	5.1±1.3
85	*p*-F-benzyl	CH_3	cycloheptyl	N	0	4.3±0.6	1.0±0.1
86	*o*-F-benzyl	CH_3	cycloheptyl	N	0	149.4±1.8	13.4±4.7
87	benzyl	Cl	cyclohexyl	N	0	463.6±1.1	24.6±4.7
88	*p*-F-benzyl	Cl	cyclohexyl	N	0	495.0±39.4	21.4±1.0
89	*o*-F-benzyl	Cl	cyclohexyl	N	0	171.2±12.3	18.1±2.7
90	1-ethyl-4-phenylpip	CH_3	cyclohexyl	N	0	>1000	>1000
91	phenethyl	CH_3	cyclohexyl	N	0	>1000	16.3±1.2
92	*p*-OCH_3benzyl	CH_3	cyclohexyl	N	0	>1000	35.8±2.1
93	*p*-F-benzyl	H	cyclohexyl	N	0	384.1±25.3	13.0±1.4
94	benzyl	H	cyclohexyl	N	0	>1000	48.6±12.0
95	ethylmorph	H	cyclohexyl	N	0	>1000	67.2±11.6
96	*o*-F-benzyl	CH_3	cyclohexyl	N	0	>1000	>1000
97	ethylmorph	CH_3	4-CH_3cyclohexyl	N	0	>1000	>1000

(Table 9. Contd….)

Compd.	R1	R2	R3	X	n	CB1R Ki (nM)	CB2R Ki (nM)
98	benzyl	CH_3	cyclohexyl	N	0	>1000	>1000
99	ethylmorph	Cl	4-CH_3cyclohexyl	N	0	>1000	40.5±7.7
100	benzyl	H	cyclohexyl	H	0	>1000	4.8±0.4
101	ethylmorph	Cl	cyclohexyl	H	0	>1000	3.3±0.4

[a]Data taken from [53,54].

Table 10. 4-oxo-1,4-dihydroquinoline-3-carboxamide Derivatives with their CB1R and CB2R Receptor Affinities[a]



Compd.	R_1	R_2	CB1R Ki (nM)	CB2R Ki (nM)
102	C_4H_9	1-naphthyl		455±63
103	C_5H_{11}	1-naphthyl	4083±375	371±34
104	C_6H_{13}	1-naphthyl		844±78
105	C_5H_{11}	benzyl		>1000
106	C_5H_{11}	2-phenylethyl		201±28
107	C_5H_{11}	3-phenylpropyl		>1000
108	C_5H_{11}	3,4-dichlorophenyl		>1000
109	C_5H_{11}	4-cyanophenyl		772±72
110	C_5H_{11}	2-(benzo[1,3]dioxol-5-yl)ethyl		426±39
111	C_5H_{11}	1-adamantyl		16.4±1.5
112	C_5H_{11}	2-adamantyl	1925±179	13.4±1.2
113	C_5H_{11}	1-(3,5-dimethyl)adamantyl		15.8±1.4
114	benzyl	1-(3,5-dimethyl)adamantyl		664±62
115	C_5H_{11}	(*RS*)-1-phenylethyl		70.8±9.1
115*R*	C_5H_{11}	(*R*)-1-phenylethyl	1154±108	37.1±3.4
115*S*	C_5H_{11}	(*S*)-1-phenylethyl		784±71
116	C_5H_{11}	(*RS*)-1-(2-naphthyl)ethyl		>1000
116*R*	C_5H_{11}	(*R*)-1-(2-naphthyl)ethyl		584±54
116*S*	C_5H_{11}	(*S*)-1-(2-naphthyl)ethyl		>5000
117	C_5H_{11}	(*RS*)-1-(1-naphthyl)ethyl		174±16
117*R*	C_5H_{11}	(*R*)-1-(1-naphthyl)ethyl		125±12
117*S*	C_5H_{11}	(*S*)-1-(1-naphthyl)ethyl		>1000
118	C_5H_{11}	(*RS*)-1-(1,2,3,4-tetrahydro-naphthyl)	1045±96	60.2±5.5

[a]Data taken from [55].

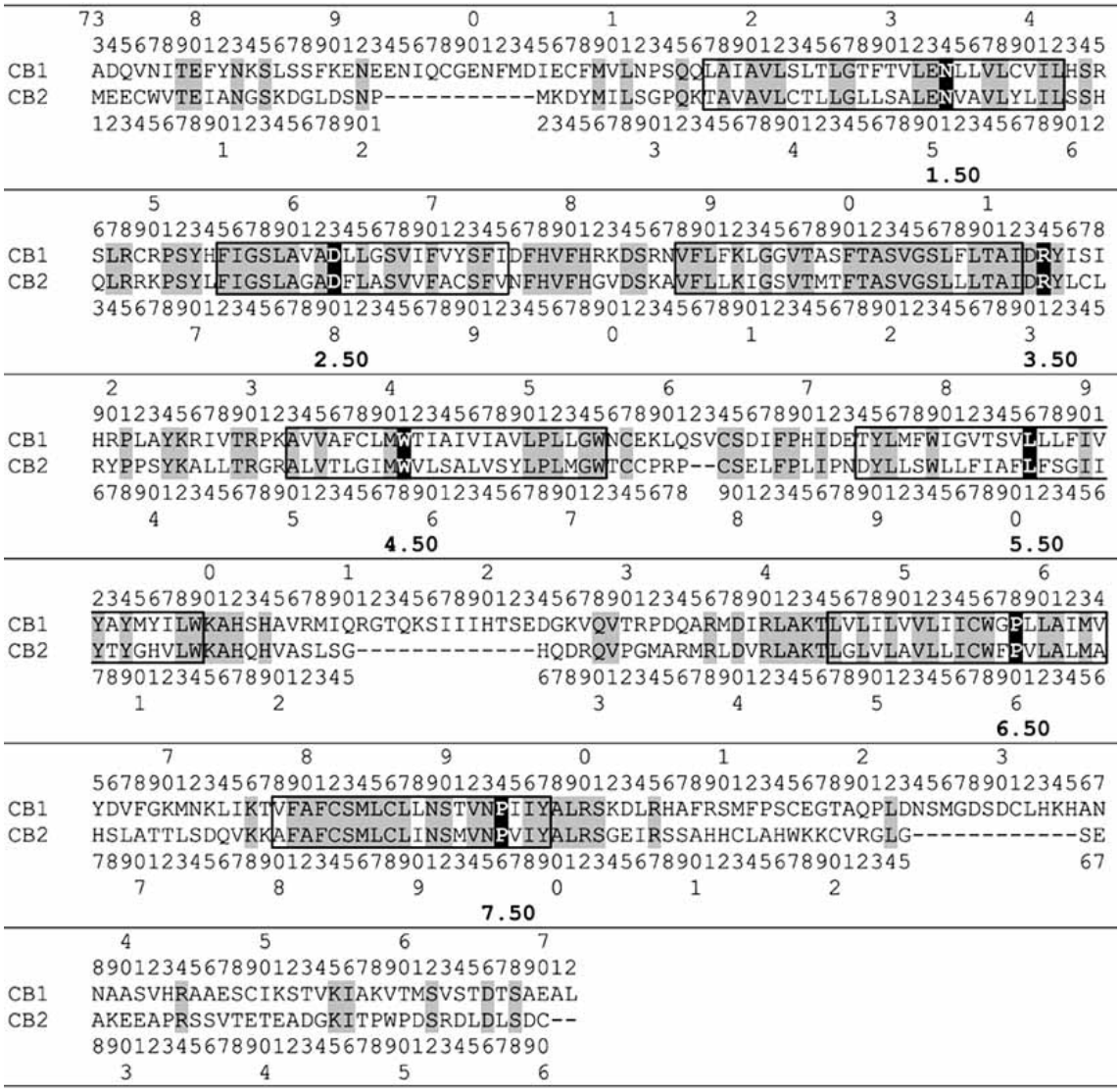


Fig. (2). Alignment of human CBRs amino acid sequences. Conserved residues are indicated in grey. Residues shown in black, indicated as x.50 (where x is the helix number) are the most conserved residues according to the Ballesteros and Weinstein [58]. The length of the TM domains was obtained from the GPCR Data Bank [57].

Mutagenesis Studies

Extensive mutagenesis studies have been carried out for CBRs. Many of these studies reported also the affinity variation of WIN55212-2 (**3**). Tables **11** and **12** report the CB1R and CB2R residues investigated by means of site directed mutagenesis in which the WIN55212-2 (**3**) affinity variation was evaluated.

The available data for the CB1R suggest that it is probable an interaction of WIN55212-2 (**3**) with residues F3.36 (200), W5.43(279) and W6.48(356).

Fig. (**3A**) summarized in a three-dimensional visualization the mutations that influences the affinity of WIN55212-2 (**3**). From this analysis it seems that the hypothetical WIN55212-2 (**3**) binding site should be delimited by TM3, TM5 and TM6, even if there are not information about the influence of TM4 and EL2.

The mutagenesis data available for the CB2R suggest that binding site of WIN55212-2 (**3**) should be localized in the same region described for the CB1R subtype (see Fig. (**3B**)). In more detail the ligand could interact with S3.31 (112), S4.53(161), and F5.46(197). Residues W4.64(172), C174(EL2), C179, and Y299(7.53) should possess a structural role for the maintenance of a particular conformation of the receptor, since their mutation determines the loss of binding for many ligands.

Otherwise residue Y5.39(190) could possess a structural role since its mutation determines a decrease of affinity for WIN55212-2 (**3**) and also AEA (**5**) but it is in proximity of the hypothetical WIN55212-2 (**3**) binding site thus it could also interact with the ligand.

The mutagenesis data suggest also a different localization of the binding site of WIN55212-2 (**3**) with respect to the others classes of CBRs agonists.

Table 11. Mutational Analysis for CB1R Agonists Interaction

		WIN55212-2	CP55940	HU210	AEA
S114A[59]	1.30	/	Decrease	/	/
Q115A[59]	1.31	/	Decrease	/	/
D163N/E[60]	2.50	Decrease	little changes	/	little changes
D163N[61]	2.50	No changes	/	/	/
H181PG[59]	EC1	No changes	ND	/	/
V188PG[59]	EC1	No changes	ND	/	/
F189A[62]	3.25	No changes	/	/	decrease
K192A[63]	3.28	No changes	decrease	decrease	decrease
G195S[64]	3.31	Increase	/	/	/
A198M[64]	3.34	No changes	/	/	/
F200A[62,65]	3.36	Decrease	/	/	No changes
L207A[66]	3.43	Increase	Increase	/	/
T210I[67]	3.46	Increase	Increase	Increase	/
T210A[67]	3.46	decrease	decrease	decrease	/
Y275F[68]	5.39	decrease	/	/	decrease
W279A[62]	5.43	decrease	/	/	No changes
V282F[69]	5.46	Increase	No changes	No changes	No changes
W356A[62]	6.48	decrease	/	/	No changes
M371PG[59]	EC3	No changes	No changes	/	/

Table 12. Mutational Analysis for CB2R Agonists Interaction

		WIN55212-2	CP55940	HU210	AEA
D80N/E[60]	2.50	No changes	No changes	/	/
K109A[70]	3.28	No changes	No changes	/	little decrease
K109A/S112G[70]	3.28/3.31	decrease	ND	/	ND
R131A[71]	3.50	No changes	/	No changes	No changes
S161A[72]	4.53	Increase	Increase	/	/
V164I[72]	4.56	No changes	No changes	/	/
S165A[72]	4.57	No changes	No changes	/	/
W172A[73]	4.64	ND	ND	ND	/
W172F/Y[73]	4.64	decrease	No changes	No changes	/
C174S[72]	EL2	ND	ND	/	/
C175S[72]	EL2	Increase	Decrease	/	/
R177S[72]	EL2	No changes	No changes	/	/
C179S[72]	EL2	ND	ND	/	/

(Table 12. Contd….)

		WIN55212-2	CP55940	HU210	AEA
Y190F[68]	5.39	Decrease	/	/	Decrease
S193G[72]	5.42	No changes	No changes	/	/
F197V[69]	5.46	Decrease	No changes	No changes	No changes
L201P[74]	5.50	ND	/	ND	ND
Y209A[74]	5.58	Little changes	/	Little changes	Little changes
Y299A[75]	7.53	ND	/	ND	ND
C313A[75]		No changes	/	No changes	No changes
C320A[75]		No changes	/	No changes	No changes

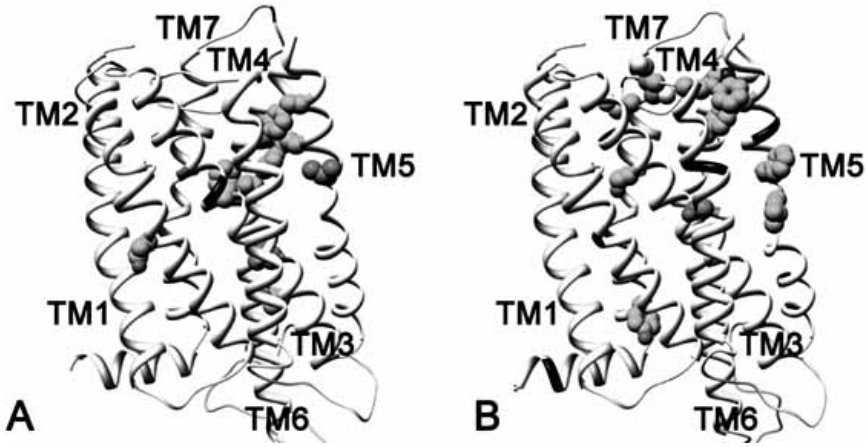


Fig. (3). Ribbon representation of CB1R (A) and CB2R (B) receptors. Residues whose mutation does not affect WIN55212-2 affinity are shown in black ribbons and residues whose mutation determines a change in WIN55212-2 affinity are highlighted in CPK.

In the CB1R subtype, mutation in the first extracellular loop (EC1) determined affinity variations only for CP55940 (**2**).

The K3.28(192)A mutation determined affinity variation for CP55940 (**2**), HU210 and AEA (**5**) whereas the WIN 55212-2 (**3**) affinity resulted in no change. The F3.36(200)A mutation determined a decrease of affinity only for WIN 55212-2 (**3**) whereas for the other agonists, the affinity resulted in no change. Finally the V5.46(282)F mutation caused an increased of affinity only for WIN55212-2 (**3**).

In the CB2R the mutation of F5.46(197) with valine is the only one that causes an affinity variation only for WIN 55212-2 (**3**).

As above mentioned WIN552212-2 (**3**) displays a certain degree of CB2R/CB1R selectivity and analysing the mutagenesis study it is possible hypothesizes that the higher CB2R affinity could be correlated with the direct or indirect interaction of the ligand with the non-conserved S3.31(112) and F5.46(197) that in the CB1R are substituted with glycine and valine respectively. The mutation of these two residues in the CB2R determined a decrease of WIN55212-2 (**3**) affinity, furthermore in the CB1R the mutation of G195(3.31) and V5.46(282) with serine and phenylalanine respectively caused an increase of the WIN55212-2 (**3**) affinity.

NMR Studies

The NMR method is frequently used for the analysis of the 3D structure of GPCRs loops. In 2002 Ulfers and co-workers [76,77] reported the determination of the third intracellular loop of the human CB1R and successively Choi and co-workers reported the conformational study of the cytoplasmic helix 8 of the CB1R [78] and CB2R [79].

At present preliminary studies revealed the possibility of investigating through NMR methodologies also the conformations of the CBRs TM domains and their orientation in the lipid bilayer [80,81].

WIN55212-2 Preferred Conformation

By combining the use of high-resolution 2D NMR and modelling studies it was suggested that the naphthyl ring is oriented off the plane of the benzoxazine ring by approximately 59° with the carbonyl group pointing towards the methyl group. Furthermore the axial morpholinomethyl conformation is preferred in order to relieve steric interactions [82].

Modelling Studies

In 1999 Song and co-workers reported the first docking of WIN55212-2 (**3**) inside CBRs [69]. Using the 7.5 Å resolution projection map of frog rhodopsin [83] as a reference structure they constructed both human CB1R and CB2R subtypes. WIN55212-2 (**3**) was manually docked into the TM3-4-5 bundle and then minimized.

The *s-trans*-conformer of the ligand interacts into both CB1R and CB2R models with F3.25 and W5.43 through the naphthyl ring and the indole group interacted with F3.36. Furthermore in the CB2R the indole ring of the ligand interacted also with the non-conserved F5.46(197).

In 2003 McAllister and co-workers reported a docking study of various ligands inside a model of the activated state of CB1R [62]. This model was constructed using the crystal structure of bovine rhodopsin [84] as a template and taking into account the experimental results for rhodopsin and the β-2-adrenergic receptor concerning the receptor activation [85-89].

In this CB1R model WIN55212-2 (**3**) was inserted among TM3, 4, 5 and 6. There are no hydrogen-bonding interactions between the ligand and the receptor and the indole ring interacted with F3.36(200) and the naphthyl ring with W5.43 (279) and W6.48(356).

In 2004 Salo and co-workers [90] reported the construction and analysis of the human CB1R. In their model, the indole core of WIN55212-2 (**3**) directly interacts with F3.36 (200) and the naphthyl group stacks directly with W5.43 (279) and Y5.39(275).

Finally in 2006 our research group reported a model of CB1R and CB2R activated conformation [91].

As for the CB1R model proposed by McAllister and co-workers [62], the two CBRs were built in their activated conformation taking into account the experimental results for rhodopsin and the β-2-adrenergic receptor concerning the receptor activation.

In both models WIN55212-2 (**3**) was placed among TM3, 4, 5 and 6; in the CB1R, the binding site was characterised by a lipophilic pocket delimited by F3.36(200), W5.43(279) and W6.48(356), which principally interact through aromatic stacking with the naphthyl ring the first two and with the indole ring system the last one. With regards with the morpholinic group, it was positioned in a secondary lipophilic pocket formed by L3.26(190), P4.60(251) and L4.61(252).

The CB2R binding site was similar to the CB1R one, with a primary lipophilic pocket delimited by F3.36(117), W5.43(194), W6.48(258), but the WIN55212-2 (**3**) orientation was slightly different. In the CB2R site, the ligand veers away from F3.36(117), since it feels the effect of a strong interaction with F5.46(197) in agreement with mutagenesis data. As regards the secondary lipophilic pocket in which the morpholinic group was positioned, the substituent interacted with L3.27(108), P4.60(168) and L4.61(169), and the non-conserved S3.31(112) formed a hydrogen bond with the oxygen atom of the morpholinic group. This last interaction could be in an agreement with the mutagenesis data reported by Chin and co-workers that suggests for this residue an important role in the CB2R subtype. The CB2R model reported in this article has been also used for the design of new CB2R agonists and for a preliminary virtual screening study [92].

CONCLUSIONS

During the past 10 years the endocannabinoid system (ECS) has shown to have a significant role in certain physiological events in particular for the distribution of the cannabinoid receptors (CB1R and CB2R) in the central system as well as in the peripheral tissue. For these reasons the interest in the cannabinoid pharmacology has rapidly increased and the ECS is considered as a target for the drug discovery. It is well known that the CB1R participates in the appetite stimulation, nausea suppression, cognition and memory, pain perception, and the regulation of intra ocular pressure. In particular, selective CB1R antagonists are currently under investigation in clinical human studies for treating obesity and may be a helpful tool to stop smoking. In contrast, Δ^9-THC (**1**) is currently marketed to reduce emesis and/or prevent cachexia in AIDS or cancer patients. Unlike the CB1R, the physiological and putative therapeutic potential of the CB2R largely remains unexplored. However, selective ligands could be useful for the treatment of pain, inflammation, osteoporosis, growth of malignant gliomas, tumors of the immune origin, for prevention of Alzheimer's disease and for the treatment of amyotrophic lateral sclerosis.

Thus the research and the development of new potent and selective ligands for CB1R and CB2R is still of great importance in order to determine insight into the physiological role of each receptor.

The aminoalkylindole derivatives are structurally dissimilar from the other classes of traditional cannabinoids and endogenous cannabinoids. Some of these compounds have shown a slightly selectivity toward CB1R but most of them possess a high degree of selectivity for CB2R. Furthermore experimental data suggest that the aminoalkylindole derivatives could interact in a binding site different from that of the other CBRs agonists. Other compounds such as oxoquinolines and oxonaphthyridines are supposed to interact in the aminoalkylindole binding site and show remarkable affinity and selectivity at CB2R. In light of these considerations the class of AAIBS derivatives results very promising for the development of novel CB2R selective ligands which should afford further examination of the physiological role of CB2R and should be used for the treatment of several phatophysiological diseases such as immune disorder, Alzheimer's disease and amyotrophic lateral sclerosis.

REFERENCES

[1] Gaoni, Y.; Mechoulam R. *J. Am. Chem. Soc.*, **1964**, *86*, 1646.
[2] Breivogel, C.S.; Griffin, G.; Di Marzo, V.; Martin, B.R. *Mol. Pharmacol.*, **2001**, *60*, 155.
[3] Howlett, A.C.; Barth, F.; Bonner, T. I.; Cabral, G.; Casellas, P.; Devane, W.A.; Felder, C.C.; Herkenham, M.; Mackie, K.; Martin, B.R.; Mechoulam, R.; Pertwee, R.G. *Pharmacol. Rev.*, **2002**, *54*, 161.
[4] Howlett, A.C. *Neuropharmacology*, **1987**, *26*, 507.
[5] Mackie, K.; Hille, B. *Proc. Natl. Acad. Sci. U.S.A.*, **1992**, *89*, 3825.
[6] Gebremedhin, D.; Lange, A.R.; Campbell, W.B.; Hillard, C.J.; Harder, D.R. *Am. J. Physiol.*, **1999**, *276*, 2085.

[7] Deadwyler, S.A.; Hampson, R.E.; Mu, J.; Whyte, A.; Childers, S. *J. Pharmacol. Exp. Ther.*, **1995**, *273*, 734.

[8] Bouaboula, M.; Poinot-Chazel, C.; Bourrie, B.; Canat, X.; Calandra, B.; Rinaldi-Carmona, M.; Le Fur, G.; Casellas, P. *Biochem. J.*, **1995**, *312*, 637.

[9] Piomelli, D. *Nat. Rev. Neurosci.*, **2003**, *4*, 873.

[10] Pertwee R.G. *Pharmacol. Ther.*, **1997**, *74*, 129.

[11] Bouaboula, M.; Poinot-Chazel, C.; Marchand, J.; Canat, X.; Bourrie, B.; Rinaldi-Carmona, M.; Calandra, B.; Le Fur, G.; Casellas, P. *Eur. J. Biochem.*, **1996**, *237*, 704.

[12] Felder, C.C.; Joyce, K.E.; Briley, E.M.; Mansouri, J.; Mackie, K.; Blond, O.; Lai, Y.; Ma, A.L.; Mitchell, R.L. *Mol. Pharmacol.*, **1995**, *48*, 443.

[13] Ibrahim, M.M.; Porreca, F.; Lai, J.; Albrecht, P.J.; Rice, F.L.; Khodorova, A.; Davar, G.; Makriyannis, A.; Vanderah, T.W.; Mata, H.P.; Malan, T.P. Jr. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 3093.

[14] Iwamura, H.; Suzuki, H.; Ueda, Y.; Kaya, T.; Inaba, T. *J. Pharmacol. Exp. Ther.*, **2001**, *296*, 420.

[15] Ofek, O.; Karsak, M.; Leclerc, N.; Fogel, M.; Frenkel, B.; Wright, K.; Tam, J.; Attar-Namdar, M.; Kram, V.; Shohami, E.; Mechoulam, R.; Zimmer, A.; Bab, I. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 696.

[16] Sanchez, C.; de Ceballos, M.L.; del Pulgar, T.G.; Rueda, D.; Corbacho, C.; Velasco, G.; Galve-Roperh, I.; Huffman, J.W.; Ramon y Cajal, S.; Guzman, M. *Cancer Res.*, **2001**, *61*, 5784.

[17] McKallip, R.J.; Lombard, C.; Fisher, M.; Martin, B.R.; Ryu, S.; Grant, S.; Nagarkatti, P.S.; Nagarkatti, M. *Blood*, **2002**, *100*, 627.

[18] Pertwee, R.G. *Pharmacol. Ther.*, **2002**, *95*, 165.

[19] Franklin, A.; Stella, N. *Eur. J. Pharmacol.*, **2003**, *474*, 195.

[20] Stella, N. *Glia*, **2004**, *48*, 267.

[21] Ramirez, B.G.; Blazquez, C.; Gomez del Pulgar, T.; Guzman, M.; de Ceballos, M.L. *J. Neurosci.,* **2005**, *25*, 1904.

[22] Kim, K.; Moore, D.H.; Makriyannis, A.; Abood, M.E. *Eur. J. Pharmacol.*, **2006**, *542*, 100.

[23] Reggio, P.H. *Curr. Pharm. Des.*, **2003**, *9*, 1607.

[24] Huffman, J.W. *Curr. Pharm. Des.*, **2000**, *6*, 1323.

[25] Huffman, J.W. *Mini Rev. Med. Chem.*, **2005**, *5*, 641.

[26] Huffman, J.W.; Padgett, L.W. *Curr. Med. Chem.*, **2005**, *12*, 1395.

[27] Raitio, K.H.; Salo, O.M.H.; Nevalainen, T.; Poso, A.; Jaervinen, T. *Curr. Med. Chem.*, **2005**, *12*, 1217.

[28] Reggio, P.H. *Endocannabinoids*, **2006**, 11.

[29] Padgett, L.W. *Life Sci.*, **2005**, *77*, 1767.

[30] Bell, M.R.; D'Ambra, T.E.; Kumar, V.; Eissenstat, M.A.; Herrmann, J.L.; Wetzel, J.R.; Rosi, D.; Philion, R.E.; Daum, S.J.; Hlasta, D.J.; Kullnig, R.K.; Ackerman, J.H.; Haubrich, D.R.; Luttinger, D.A.; Baizman, E.R.; Miller, M.S.; Ward, S.J. *J. Med. Chem.*, **1991**, *34*, 1099.

[31] D'Ambra, T.E.; Estep, K.G.; Bell, M.R.; Eissenstat, M.A.; Josef, K.A.; Ward, J.; Haycock, D.A.; Baizman, E.R.; Casiano, F.M.; Beglin, N.C.; Chippari, S.M.; Grego, J.D.; Kullnig, R.K.; Daley, G.T. *J. Med. Chem.*, **1992**, *35*, 124.

[32] Compton, D.R.; Gold, L.H.; Ward, S.J.; Balster, R.L.; Martin, B.R. *J. Pharmacol. Exp. Ther.*, **1992**, *263*, 1118.

[33] Showalter, V.M.; Compton, D.R.; Martin, B.R.; Abood, M.E. *J. Pharmacol. Exp. Ther.*, **1996**, *278*, 989.

[34] Savinainen, J.R.; Kokkola, T.; Salo, O.M.; Poso, A.; Jarvinen, T.; Laitinen, J.T. *Br. J. Pharmacol.*, **2005**, *145*, 636.

[35] Eissenstat, M.A.; Bell, M.R.; D'Ambra, T.E.; Alexander, E.J.; Daum, S.J.; Ackerman, J.H.; Gruett, M.D.; Kumar, V.; Estep, K.G.; Olefirowicz, E.M., Wetzel, J.R.; Alexander, M.D.; Weaver, J.D.; Haycock, D.A.; Luttinger, D.A.; Casiano, F.M.; Chippari, S.M.; Kuster, J.E.; Stevenson, J.I.; Ward, S.J. *J. Med. Chem.*, **1995**, *38*, 3094.

[36] D'Ambra, T.E.; Eissenstat, M.A.; Abt, J.; Ackerman, J.H.; Bacon, E.R.; Bell, M.R.; Carabateas, P.M.; Josef, K.A.; Kumar, V.; Weaver, J.D.; Arnold, R.; Casiano, F.M.; Chippari, S.M.; Haycock, D.A.; Kuster, J.E.; Luttinger, D.A.; Stevenson, J.I.; Ward, S.J.; Hill, W.A.; Khanolkar A.; Makriyannis A. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 17.

[37] Huffman, J.W.; Dai, D.; Martin, B.R.; Compton, D.R. *Bioorg. Med. Chem. Lett.*, 1994, *4*, 563.

[38] Wiley, J.L.; Compton, D.R.; Dai, D.; Lainton, J.A.; Phillips, M.; Huffman, J.W.; Martin, B.R. *J. Pharmacol. Exp. Ther.*, **1998**, *285*, 995.

[39] Aung, M.M.; Griffin, G.; Huffman, J.W.; Wu, M.; Keel, C.; Yang, B.; Showalter, V.M.; Abood, M.E.; Martin, B.R. *Drug Alcohol Depend.*, **2000**, *60*, 133.

[40] Huffman, J.W. *Curr. Med. Chem.*, **1999**, *6*, 705.

[41] Gallant, M.; Dufresne, C.; Gareau, Y.; Guay, D.; Leblanc, Y.; Prasit, P.; Rochette, C.; Sawyer, N.; Slipetz, D.M., Tremblay, N.; Metters, K.M., Labelle, M. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 2263.

[42] Hynes, J. Jr.; Leftheris, K.; Wu, H.; Pandit, C.; Chen, P.; Norris, D.J.; Chen, B.C.; Zhao, R.; Kiener, P.A.; Chen, X.; Turk, L.A.; Patil-Koota, V.; Gillooly, K.M.; Shuster, D.J.; McIntyre, K.W. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 2399.

[43] Wrobleski, S.T.; Chen, P.; Hynes, J. Jr.; Lin, S.; Norris, D.J.; Pandit, C.R.; Spergel, S.; Wu, H.; Tokarski, J.S.; Chen, X.; Gillooly, K.M.; Kiener, P.A.; McIntyre, K.W.; Patil-Koota, V.; Shuster, D.J.; Turk, L.A.; Yang, G.; Leftheris, K. *J. Med. Chem.*, **2003**, *46*, 2110.

[44] Makriyannis, A.; Deng, H. In W.O. Patent 01/028557, **2001**.

[45] Makriyannis, A.; Deng, H.; Yale, G.D. In W.O. Patent 02/060447, **2002**.

[46] Malan, T.P. Jr.; Ibrahim, M.M.; Deng, H.; Liu, Q.; Mata, H.P.; Vanderah, T.; Porreca, F.; Makriyannis, A. *Pain*, **2001**, *93*, 239.

[47] Barth, F.; Congy, C.; Guillaumont, C.; Rinaldi, M.; Vasse, F.; Vernhet, C. In W.O. Patent 02/42269, **2002**.

[48] Huffman, J.W.; Zengin, G.; Wu, M.J.; Lu, J.; Hynd, G.; Bushell, K.; Thompson, A.L.; Bushell, S.; Tartal, C.; Hurst, D.P.; Reggio, P.H.; Selley, D.E.; Cassidy, M.P.; Wiley, J.L.; Martin, B.R. *Bioorg. Med. Chem.*, **2005**, *13*, 89.

[49] Huffman, J.W.; Szklennik, P.V.; Almond, A.; Bushell, K.; Selley, D.E.; He, H.; Cassidy, M.P.; Wiley, J.L.; Martin, B.R. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 4110.

[50] Huffman, J.W.; Padgett, L.W.; Isherwood, M.L.; Wiley, J.L.; Martin, B.R. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 5432.

[51] Inaba, T.; Kaya, T.; Iwamura, H.I. W.O. Patent 00/40562, **2000**.

[52] Raitio, K.H.; Savinainen, J.R.; Vepsalainen, J.; Laitinen, J.T.; Poso, A.; Jarvinen, T.; Nevalainen, T. *J. Med. Chem.*, **2006**, *49*, 2022.

[53] Ferrarini, P.L.; Calderone, V.; Cavallini, T.; Manera, C.; Saccomanni, G.; Pani, L.; Ruiu, S.; Gessa, G.L. *Bioorg. Med. Chem.,* **2004**, *12*, 1921.

[54] Manera, C.; Benetti, V.; Castelli, M.P.; Cavallini, T.; Lazzaretti, S.; Pibiri, F.; Saccomanni, G.; Tuccinardi, T.; Vannacci, A.; Martinelli, A.; Ferrarini, P.L. *J. Med. Chem.*, **2006**, *49*, 5947.

[55] Stern, E.; Muccioli, G.G.; Millet, R.; Goossens, J.F.; Farce, A.; Chavatte, P.; Poupaert, J.H.; Lambert, D.M.; Depreux, P.; Henichart, J.P. *J. Med. Chem.*, **2006**, *49*, 70.

[56] Thompson, J.D.; Higgins, D.G.; Gibson, T.J. *Nucleic Acids Res.*, **1994**, *22*, 4673.

[57] http://www.gpcr.org/7tm/

[58] Ballesteros, J.A.; Weinstein, H.W. *Methods Neurosci.*, **1995**, *25*, 366.

[59] Murphy, J.W.; Kendall, D.A. *Biochem. Pharmacol.*, **2003**, *65*, 1623.

[60] Tao, Q.; Abood, M.E. *J. Pharmacol. Exp. Ther.*, **1998**, 285, 651.

[61] Roche, J.P.; Bounds, S.; Brown, S.; Mackie, K. *Mol. Pharmacol.*, **1999**, *56*, 611.

[62] McAllister, S.D.; Rizvi, G.; Anavi-Goffer, S.; Hurst, D.P.; Barnett-Norris, J.; Lynch, D.L.; Reggio, P.H.; Abood, M.E. *J. Med. Chem.*, **2003**, *46*, 5139.

[63] Song, Z.H.; Bonner, T.I. *Mol. Pharmacol.*, **1996**, *49*, 891.

[64] Chin, C.N.; Murphy, J.W.; Huffman, J.W.; Kendall, D.A. *J. Pharmacol. Exp. Ther.*, **1999**, *291*, 837.

[65] Shen, C.P.; Xiao, J.C.; Armstrong, H.; Hagmann, W.; Fong, T.M. *Eur. J. Pharmacol.*, **2006**, *531*, 41.

[66] D'Antona, A.M.; Ahn, K.H.; Wang, L.; Mierke, D.F.; Lucas-Lenard, J.; Kendall, D.A. *Brain Res.*, **2006**, *1108*, 1.

[67] D'Antona, A.M.; Ahn, K.H.; Kendall, D.A. *Biochemistry*, **2006**, *45*, 5606.

[68] McAllister, S.D.; Tao, Q.; Barnett-Norris, J.; Buehner, K.; Hurst, D.P.; Guarnieri, F.; Reggio, P.H.; Nowell Harmon, K.W.; Cabral, G.A.; Abood, M.E. *Biochem. Pharmacol.*, **2002**, *63*, 2121.

[69] Song, Z.H.; Slowey, C.A.; Hurst, D.P.; Reggio, P.H. *Mol. Pharmacol.*, **1999**, *56*, 834.

[70] Tao, Q.; McAllister, S.D.; Andreassi, J.; Nowell Harmon, K.W.; Cabral, G.A.; Hurst, D.P.; Bachtel, K.; Ekman, M.C.; Reggio, P.H.; Abood, M.E. *Mol. Pharmacol.*, **1999**, *55*, 605.

[71] Feng, W.; Song, Z.H. *Biochem. Pharmacol.*, **2003**, *65*, 1077.

[72] Gouldson, P.; Calandra, B.; Legoux, P.; Kerneis, A.; Rinaldi-Carmona, M.; Barth, F.; Le Fur, G.; Ferrara, P.; Shire, D. *Eur. J. Pharmacol.*, **2000**, *401*, 17.
[73] Rhee, M.H.; Nevo, I.; Bayewitch, M.L.; Zagoory, O.; Vogel, Z. *J. Neurochem.*, **2000**, *75*, 2485.
[74] Song, Z.H.; Feng, W. *FEBS Lett.*, **2002**, *531*, 290.
[75] Feng, W.; Song, Z.H. *FEBS Lett.*, **2001**, *501*, 166.
[76] Ulfers, A.L.; McMurry, J.L.; Kendall, D.A.; Mierke, D.F. *Biochemistry*, **2002**, *41*, 11344.
[77] Ulfers, A.L.; McMurry, J.L.; Miller, A.; Wang, L.; Kendall, D.A.; Mierke, D.F. *Protein Sci.*, **2002**, *11*, 2526.
[78] Choi, G.; Guo, J.; Makriyannis, A. *Biochim. Biophys. Acta,* **2005**, *1668*, 1.
[79] Choi, G.; Landin, J.; Xie, X.Q. *J. Pept. Res.*, **2002**, *60*, 169.
[80] Tiburu, E.K.; Karp, E.S.; Birrane, G.; Struppe, J.O.; Chu, S.; Lorigan, G.A.; Avraham, S.; Avraham, H.K. *Biochemistry*, **2006**, *45*, 7356.
[81] Zheng, H.; Zhao, J.; Sheng, W.; Xie, X.Q. *Biopolymers*, **2006**, *83*, 46.
[82] Xie, X.Q.; Han, X.W.; Chen, J.Z.; Eissenstat, M.; Makriyannis, A. *J. Med. Chem.*, **1999**, *42*, 4021.
[83] Unger, V.M.; Hargrave, P.A.; Baldwin, J.M.; Schertler, G.F. *Nature*, **1997**, *389*, 203.
[84] Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C.A.; Motoshima, H.; Fox, B.A.; Le Trong, I.; Teller, D.C.; Okada, T.; Stenkamp, R.E.; Yamamoto, M.; Miyano, M. *Science*, **2000**, *289*, 739.
[85] Farrens, D.; Altenbach, C.; Ynag, K.; Hubbell, W.; Khorana, H. *Science*, **1996**, *274*, 768.
[86] Jensen, A. D.; Guarnieri, F.; Rasmussen, S.G.; Asmar, F.; Ballesteros, J.A.; Gether, U. *J Biol. Chem.*, **2001**, *276*, 9279.
[87] Javitch, J.A.; Fu, D.; Liapakis, G.; Chen, J. *J. Biol. Chem.*, **1997**, *272*, 18546.
[88] Lin, S.; Sakmar, T. *Biochemistry*, **1996**, *35*, 11149.
[89] Ballesteros, J.; Jensen, A.; Liapakis, G.; Rasmussen, S.; Shi, L.; Gether, U.; Javitch, J. *J. Biol. Chem.*, **2001**, *276*, 29171.
[90] Salo, O.M.; Lahtela-Kakkonen, M.; Gynther, J.; Jarvinen, T.; Poso, A. *J. Med. Chem.*, **2004**, *47*, 3048.
[91] Tuccinardi, T.; Ferrarini, P.L.; Manera, C.; Ortore, G.; Saccomanni, G.; Martinelli, A. *J. Med. Chem.*, **2006**, *49*, 984.
[92] Tuccinardi, T.; Cascio, M.G.; Di Marzo, V.; Manera, C.; Ortore, G.; Saccomanni, G.; Martinelli, A. *Lett. Drug Des. Disc.*, **2007**, *4*, 15.

Received: 15 March, 2007 Revised: 27 September, 2007 Accepted: 28 September, 2007