Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 19, pp. 2263-2268, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00426-X

NEW CLASS OF POTENT LIGANDS FOR THE HUMAN PERIPHERAL CANNABINOID RECEPTOR

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ABSTRACT: A new class of potent ligand for the human peripheral cannabinoid (hCB_2) receptor is described. Two indole analogs **13** and **17** exhibited nanomolar potencies (K_i) with good selectivity for the hCB_2 receptor over the human central cannabinoid (hCB_1) receptor. Copyright © 1996 Elsevier Science Ltd

Introduction

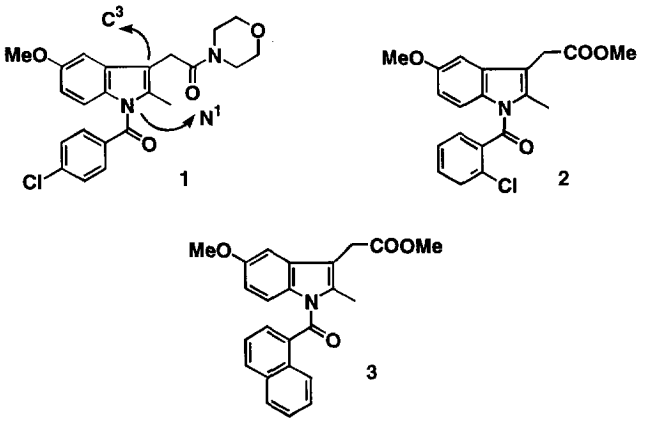
In 1993, a human peripheral cannabinoid (hCB_2) receptor, member of the growing class of G-protein-coupled receptors, was disclosed.[1] The discovery of a cannabinoid receptor distinct from the known human central cannabinoid (hCB_1) receptor,[2] led to renewed interest in the medicinal properties of cannabinoids. Pharmacological investigations on the use of cannabis, of which tetrahydrocannabinol (Δ^9-THC) is the main active constituent, have demonstrated anti-emetic,[3] analgesic,[4] and anti-inflammatory[5] effects. Other studies have also suggested that Δ^9-THC could be used in the treatment of glaucoma,[6] asthma,[7] motion disorder,[8] and muscle spasms.[9] In spite of this large potential for therapeutic benefits, the use of cannabinoids has been limited by their undesirable psychotropic properties.[10] The work described in this paper stems from the hypothesis that a selective and potent ligand for hCB_2 receptor would show therapeutically useful effects.[11]

The search for a hCB_2 receptor ligand was initiated by submitting a large number of compounds to *in vitro* binding assays[12,13] on the hCB receptors. These compounds included a series of analogs selected through a topological similarity search using WIN-55212-2,[14] a known potent ligand to the hCB receptor,[15] as the template. Three indole derivatives (e.g. **1**, **2**,[16] and **3**,[17] Fig. 1) were found to have moderate activity on the hCB_2 receptor (Tables 1, and 4). They were used as leads in two potential series of either N^1-benzoyl or N^1-naphthoyl indole analogs.

Discussion

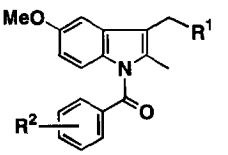
The work on the N^1-benzoyl indole series initiated by the lead compounds **1** and **2** was mainly focused on the significance of substitution at N^1 and C^3. For compounds bearing an identical benzoyl substituent at N^1, the presence of a morpholine unit attached at C^3 was beneficial for potency on the hCB_2 receptor, as indicated by the relative potencies of indomethacin **4**, indomethacin methyl ester **5**, the morpholinyl amide **1** and the corresponding amine **6** (Table 1). The morpholine moiety may be linked to the indole core through an ethylene tether (**6**) or a acetyl tether (**1**) with minimal change in potency on the hCB_2 receptor.

Figure 1.



The substitution pattern on the N^1-benzoyl group influences potency in this series. The *ortho*-chloro substituted N^1-benzoyl indole **2** showed increased hCB_2 potency (≈10-fold) compared to the *para*-chloro analog **5**. Similarly, for compounds bearing a morpholine unit at C^3, the *ortho*-chloro substituted indole **7** was more potent (≈6-fold) than the *para*-chloro analog **1**.

Table 1. Analogs of indoles 1 and 2

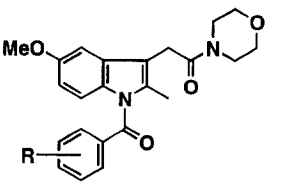


Compound	R^1	R^2	K_i [a](nM) hCB_2	K_i [a](nM) hCB_1
1	C(O)-N-morpholinyl	4-Cl	435 ± 43	>20000 (n = 2)
2	COOMe	2-Cl	397 ± 27	1720 ± 425
4	COOH	4-Cl	>20000 (n = 3)	>20000 (n = 2)
5	COOMe	4-Cl	4021 ± 1977	>20000 (n = 2)
6	CH_2-N-morpholinyl	4-Cl	213 ± 25	10253 ± 4848
7	C(O)-N-morpholinyl	2-Cl	69 ± 4	3600 ± 706

(a) CB receptor filtration binding assays were performed using recombinant CB_1[12] or CB_2[13] receptors. K_i values represent the mean ± S.E.M. from three independent determinations performed in duplicate unless otherwise indicated.

Using the morpholinylacetyl unit at C^3, the effect of halogen substitution on the N^1-benzoyl group was investigated (Table 2). The *meta*-chloro substituted indole **8** showed a decrease in potency on the hCB_2 receptor relative to **7**. Of the three dihalogenated-benzoyl analogs (**9**, **10**, and **11**) only the N^1-(2,3-dichlorobenzoyl) indole **9** showed a significant increase in potency (K_i = 14 nM for hCB_2) and selectivity (K_i hCB_1 / K_i hCB_2 = 146) over **7**.

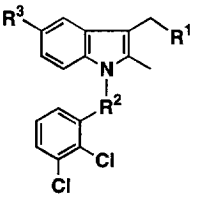
Table 2. Analogs of indole 7



Compound	R	K_i (nM) hCB_2	K_i (nM) hCB_1
7	2-Cl	69 ± 4	3600 ± 706
8	3-Cl	354 ± 45	16800, 13000
9	2-Cl, 3-Cl	14 ± 6	2043 ± 183
10	2-Cl, 4-F	134 ± 11	5570 ± 1441
11	2-Cl, 6-Cl	59 ± 7	2553 ± 611

The SAR studies around the 2,3-dichlorobenzoyl indole **9** were pursued by investigating the effect of the N^1-carbonyl, the C^3 residue and the substitution on the indole core (Table 3). Replacing the carbonyl function of the N^1-benzoyl indole **9** by a methylene unit led to the N^1-benzyl indole **12**, and resulted in a dramatic loss in potency for the hCB_2 receptor. Conversely, the key morpholine moiety may be linked to the indole core, through either an ethylene tether (**13**) or an acetyl tether (**9**) with comparable profile. Truncation of the ethylene tether of indole **13** by one methylene unit led to no loss of potency on the hCB_2 receptor, as exemplified by **14**. Finally, the presence of a methoxy unit at C^5 on the indole core had no effect on potency as shown by the K_i of **13** vs **15** on both receptors .

Table 3. Analogs of indole 9

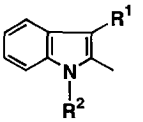


Compound	R^1	R^2	R^3	K_i (nM) hCB_2	K_i (nM) hCB_1
9	(CO)-N-morpholinyl	C(O)	OCH_3	14 ± 6	2043 ± 183
12	(CO)-N-morpholinyl	CH_2	OCH_3	1046 ± 367	>20000 (n = 2)
13	CH_2-N-morpholinyl	C(O)	OCH_3	12.0 ± 0.2	1917 ± 381
14	N-morpholinyl	C(O)	OCH_3	22 ± 5	3363 ± 856
15	CH_2-N-morpholinyl	C(O)	H	27 ± 2	3193 ± 881

The relative potencies of the *ortho*-chloro substituted N^1-benzoyl indole **2** (Table 1) and the N^1-naphthoyl analog **3** (Table 4) suggests that these substituents may be topologically similar. This hypothesis was substantiated by the increased potency of the 2,3-disubstituted N^1-benzoyl indole **9** (K_i = 14 nM for hCB_2) over the monosubstituted analogs **7** (K_i = 69 nM for hCB_2) and **8** (K_i = 354 nM for hCB_2). Consequently, it appears likely that the 2,3-dichlorobenzoyl and the 1-naphthoyl pharmacophores have similar binding modes on the cannabinoid receptors.

The results of the SAR studies performed in the N^1-benzoyl indole series were ported to the N^1-naphthoyl indole series. A morpholine unit was introduced at C^3 with an ethylene tether to give the indole **16** which was equipotent to the lead compound **3**. Potency was increased dramatically by removing one methylene in the C^3 tether of **16** resulting in compound **17** which has a K_i of 8.5 nM on the hCB_2 receptor. A similar transformation in the N^1-benzoyl series (**14** versus **13**) had limited effect on potency. The presence of a carbonyl unit as a tether between the indole and the naphthyl unit was critical, the N^1-naphthylindole **18** was relatively inactive on the hCB_2 receptor. This is in line with the observation made in the N^1-benzoyl series (see **12**, Table 3).

Table 4. Analogs of indole 3



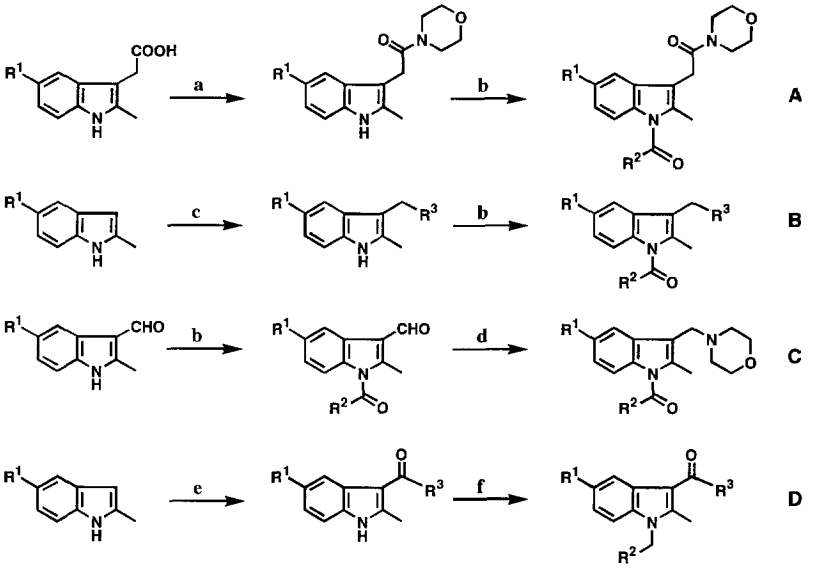
Compound	R^1	R^2	K_i (nM) hCB_2	K_i (nM) hCB_1
3			142 ± 21	4610, 1890
16	CH_2CH_2-N-morpholinyl	1-naphthoyl	216 ± 33	2183 ± 825
17	CH_2-N-morpholinyl	1-naphthoyl	8.5 ± 1.6	877 ± 222
18	CH_2-N-morpholinyl	1-naphthyl	7840, 2700	6680 ± 2359
19	1-naphthoyl	CH_2CH_2-N-morpholinyl	14.0 ± 0.4	638 ± 172

Publications[18-22] and patents have appeared in recent years on novel series of cannabinoid receptor ligands. It was shown that a number of acyclic analogs of WIN-55212-2,[14] such as WIN-56098[23] were potent ligands on the hCB_1 receptor. These aminoalkyl indoles are structurally related to the N^1-naphthoyl indole series described in this paper. These two series differ by the position of the naphthoyl and alkylmorpholinyl substituents on the indole core. In the WIN series the naphthoyl is located at C^3 and the alkylmorpholinyl at N^1 as illustrated by indole **19**,[21,22] which was prepared and tested. Comparing these series suggests that substituents at N^1 and C^3 may be somewhat interchangeable (**16** versus **19**) and that the loss of affinity for the hCB_2 receptor in the N^1-naphthoyl indole series, can be alleviated by shortening the tether at C^3 as exemplified by indole **17**. Similar observation of N^1 and C^3 interchangeability in indoles have been reported[19] earlier. The indole moiety can thus be thought of as a scaffold on which to position the key aryl and alkylmorpholinyl groups.

Chemistry

Indoles of type **A** (Tables 1, 2, and 3) having a morpholine subunit attached through an amide bond were prepared by a DCC type coupling[24] on the commercially available 5-methoxyindole-3-acetic acid followed by a N^1-acylation of the indole potassium salt[25] using the appropriate acid chloride. Indoles of type **B** (Tables 1, and 3) were prepared by a C^3-alkylation of the indole zinc salt[26] followed by the previously described N^1-acylation. The synthesis of indoles of type **C** (Table 3) started with a N^1-acylation followed by reductive amination.[27] Indoles of type **D** (Tables 3 and 4) were prepared by C^3-acylation of an indole zinc salt followed by a N^1-alkylation of the indole sodium salt.

Scheme 1. General preparation of C^3 and N^1 substituted indoles



[a] Reagents : (a) DCC, morpholine, THF (b) i. KHMDS, HMPA, THF ii. RCOCl
(c) i. MeMgCl, $ZnCl_2$, Et_2O ii. R^3CH_2Cl (d) $NaCNBH_3$, morpholine, MeOH, HCl
(e) i. MeMgCl, $ZnCl_2$, Et_2O ii. R^3COCl (f) i. NaH, DMF ii. R^2CH_2Cl

Conclusion

We have described SAR studies in two indole series that allowed the identification of compounds with nanomolar potency on the hCB_2 receptor and selectivity against the hCB_1 receptor. Some key features are the presence of either a 2,3-dihalogenatedbenzoyl or a 1-naphthoyl residue at the N^1 position and a alkylmorpholinyl residue at C^3. Substitution at the indole C^5 position had no influence on the affinity for the hCB_2 receptor. We have identified two potent compounds **13** (L-768,242) and **17** (L-759,787) with K_i of 12 nM and 8.5 nM, respectively, for the hCB_2 receptor. They exhibit good selectivity over the hCB_1 receptor (hCB_1/hCB_2 = 160 for **13** and 103 for **17**). Studies on the pharmacology of these compounds are being pursued and will be reported in the near future.

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(*Received in USA* 29 *May* 1996; *accepted* 23 *August* 1996)