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3-ALKYL-(5,5'-DIPHENYL)IMIDAZOLIDINEDIONES AS NEW CANNABINOID RECEPTOR LIGANDS

Martial Kanyonyo, Sophie J. Govaerts, Emmanuel Hermans[#], Jacques H. Poupaert and Didier M. Lambert[*]

Unité de Chimie Pharmaceutique et de Radiopharmacie, Avenue E. Mounier 73.40 & [#]Unité de Pharmacologie Expérimentale, Avenue Hippocrate 54.10 Université Catholique de Louvain, 1200 Bruxelles, Belgium

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Abstract : Twenty-four 3-alkyl-(5,5'-diphenyl)imidazolidinediones were synthesized and evaluated as new cannabinoid receptor ligands. Three compounds exhibited a Ki value around 100 nM against [^{3}H]-SR 141716A binding obtained from human CB_1 transfected CHO cells membranes. The lack of change of affinity in the presence of a non hydrolyzable GTP analogue seems to indicate they are cannabinoid antagonists.

Two sub-classes of cannabinoid receptors (CB_1 and CB_2) have been characterized and both belong to G protein coupled receptor superfamily[1]. The CB_1 which was first evidenced by autoradiography and radioligand binding studies using [^{3}H]-CP55940 was cloned from rat, human and mouse. It is expressed in the brain and some peripheral tissues including testis, small intestine, urinary bladder and *vas deferens*. An alternative spliced form of CB_1, christened CB_{1A}, has also been described[2] but so far, no peculiar property in terms of ligand recognition and receptor activation has been shown for this variant. The CB_2 was discovered by sequence homology and is predominantly found in the immune system (spleen, tonsils, immune cells). The discovery of cannabinoid receptors led to the identification of endogenous lipid compounds such as anandamide and 2-arachidonylglycerol which bind to cannabinoid receptors. Until now, agonists for these receptors belong to three distinct chemical classes[3] : molecules derived from tetrahydrocannabinol (THC), the aminoalkylindoles derived from pravadoline and the fatty acid amides and esters derived from anandamide, the first described endogenous ligand. This diversity of structures is paralleled with a variety of origins : THC was isolated from a plant, pravadoline is a synthetic molecule and anandamide was isolated from mammalian brain. Three classes of antagonists have been described so far : SR 141716A[4] (scheme 1) and SR 144528[5] are diarylpyrazoles, LY-320135 is an arylbenzofuran[6] and AM630 is an aminoalkylindole[7].

Huffman et al.[8-9] found that simplified derivatives of aminoalkylindoles such as 1-alkyl-3-(1-naphthoyl)-pyrroles also exhibit a significant affinity for cannabinoid receptors[8-10]. The more potent ligand was the *n*-pentyl derivative (JWH 030, scheme 1) with a Ki of 87 nM on brain cannabinoid receptors. On analyzing the structures of 1-alkyl-3-(1-naphthoyl)pyrroles and of diarylpyrazoles antagonists, we decided to investigate whether a 5,5'-diphenylhydantoin nucleus may constitute a new template for CB recognition

* Phone : +32 2 7647347 ; Fax : +32 2 7647363 ; E-mail : lambert@cmfa.ucl.ac.be

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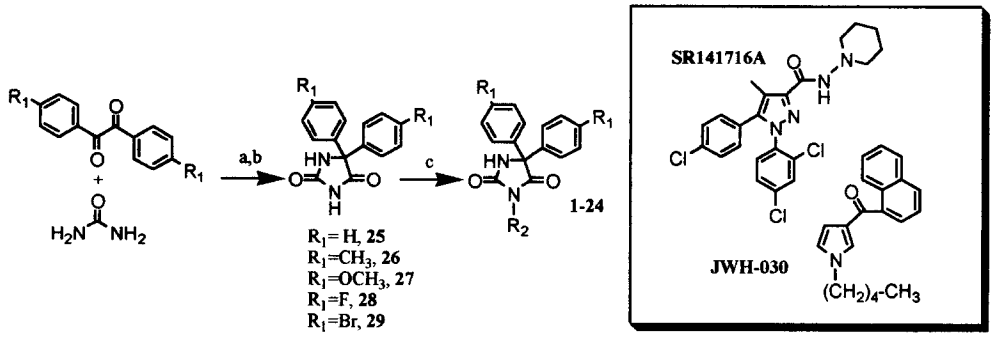
considering that the diphenyl rings may mimic the phenyl rings in the reference molecules and the N_3 nitrogen of the hydantoin would be useful for further *N*-alkylations. A series of 3-alkylated 5,5'-diphenylimidazolidinediones (**1-24**) was prepared and tested by radioligand binding assay on transfected cells stably expressing the human CB_1. 3-Alkyl 5,5'-diphenylimidazolinediones were readily obtained in two steps, summarized in scheme 1. Benzil or the corresponding substituted benzils, urea and KOH were stirred in refluxing ethanol during two hours. After cooling and washing with 0.5 N NaOH, the glycolureide product was discarded by filtration. After the addition of acetic acid to the filtrate, a precipitate was obtained providing **25-29** in 65-88 % yields. In a second step, the alkylation of **25-29** (1mmol) by an excess (1.2 mmol) of bromo or chloro alkyl chains, was carried out in anhydrous dimethylformamide in the presence of K_2CO_3 at room temperature overnight.

Scheme 1 : synthesis and structures of target compounds (**1-24**) compared to SR141716A and JWH030



Conditions and reagents : a) KOH, C_2H_5OH, reflux b) CH_3COOH and c) R_2-Cl or R_2-Br, K_2CO_3, DMF

The affinity of **1-24** for the CB_1 was determined by measuring their ability to displace the high affinity radioligand [^{3}H]-SR 141716A from a membrane preparation of CHO cells expressing human CB_1[11]. Final molecules as well as the hydantoin intermediates **25-29** were screened at a first dose of 10 μM and when >60% displacement of the specific radioactivity bound was obtained, they were further tested at 1 μM (Table 1). None of the intermediates **25-29** showed a significant displacement of the radioligand at 10 μM (data not shown). Whatever the nature of the R_1 substituent, the affinity for CB_1 increased with an increase in the length of alkyl chains as for the case of 1-alkyl-3-(1-naphthoyl)pyrroles. The dibromo derivatives showed the highest affinity.

Three compounds, i.e. the 3-ethylmorpholino-5,5'-di-*p*-bromophenylimidazolidinedione **20**, 3-(1-hydroxypropyl)-5,5'-di-*p*-bromophenylimidazolidinedione **21**, 3-heptyl-5,5'-di-*p*-bromophenyl imidazolidinedione **23** were selected for further pharmacological evaluations and their Ki are shown in Table 2. Compared to reference cannabinoids, the 3-alkyl-5,5-di-*p*-bromophenylimidazolidinediones exhibited an affinity inferior to those of classical cannabinoids (HU210, CP 55940) but superior to that of the reference aminoalkylindole

tested (Win55212-2). The Ki obtained for **20**, **21** and **23** were close to the value described in the literature for 1-pentyl-3-naphthoylpyrrole[10].

Table 1 Displacement of [^{3}H]-SR 141716A binding to CB_1 CHO cells membranes by compounds **1-24**

Compounds	R_1	n	R_2	% of displacement at 10 μM	% of displacement at 1 μM
1	H	2	$-N(CH_2CH_2)_2O$	< 5	-
2	H	2	$-N(CH_2)_5$	<15	-
3	H	2	$-N(CH_3)_2$	<5	-
4	H	2	$-CH_3$	<20	-
5	H	3	$-CH_3$	25.1 ± 2.2	-
6	H	4	$-CH_3$	35.4 ± 2.9	-
7	H	5	$-CH_3$	35.6 ± 1.5	-
8	H	7	$-CH_3$	61.2 ± 4.7	-
9	H	1	$-C_6H_5$	40.6 ± 3.9	-
10	H	0	$-CH(CH_3)_2$	<5	-
11	CH_3	2	$-N(CH_2CH_2)_2O$	23.9 ± 1.9	-
12	CH_3	5	$-CH_3$	46.8 ± 3.9	-
13	CH_3	6	$-CH_3$	51.3 ± 3.8	-
14	OCH_3	2	$-N(CH_2CH_2)_2O$	21.7 ± 1.7	-
15	OCH_3	5	$-CH_3$	66.6 ± 5.3	23.0 ± 1.9
16	F	2	$-N(CH_2CH_2)_2O$	30.3 ± 2.1	-
17	F	5	$-CH_3$	40.6 ± 3.1	-
18	F	6	$-CH_3$	51.4 ± 2.9	-
19	F	7	$-CH_3$	62.5 ± 5.7	15.3 ± 1.1
20	Br	2	$-N(CH_2CH_2)_2O$	91.2 ± 7.3	54.1 ± 4.5
21	Br	3	-OH	88.4 ± 6.7	50.2 ± 5.3
22	Br	5	$-CH_3$	72.1 ± 5.3	30.2 ± 1.9
23	Br	6	$-CH_3$	89.2 ± 7.6	51.5 ± 3.4
24	Br	7	$-CH_3$	80.0 ± 6.0	48.2 ± 3.8

Results are expressed as the percentages of the displaced specific radioactivity (mean ±sem, n=3-5)

Table 2 Ki determinations of **20**, **21** and **23** and reference cannabinoids

Compounds	Ki (nM) against [^{3}H]SR 141716A
20	70.3 ± 4.3
21	103.2 ± 6.8
23	97.9 ± 5.5
HU210	0.82 ± 0.04
CP 55940	5.2 ± 0.3[a]
SR 141716A	8.9 ± 0.4[a]
Win55212-2	152.2 ± 9.3[a]

Ki are expressed as means ± sem (n=3-5)

Reference values[13] for CP55940, SR141716A and Win55212-2 are 20.7 ± 4.8, 1.18 ± 0.1 and 21.8 ± 6.1 nM respectively.

Guanylyl nucleotides are known to disrupt the functional coupling of G-protein coupled receptors, resulting in decreased affinity of agonists. This constitutes an useful assay to distinguish between agonists and antagonists, the later being unaffected by such uncoupling[14]. In our hands, addition of 5'-guanylyimidodiphosphate (Gpp(NH)p) (50μM) to the binding assay significantly reduced the affinity of CP 55940 (IC_{50} values : agonist –Gpp(NH)p, 14.4 nM; +Gpp(NH)p, 27.5 nM) but was without effect on the binding of **20**, **21** and **23**, supporting evidence for their antagonist properties at the CB_1 receptor.

In conclusion, these molecules represent a new chemical class of cannabinoid ligands and may constitute a new template for further syntheses and pharmacological evaluation of drugs interacting with CB_1 in order to depict receptor topology.

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