

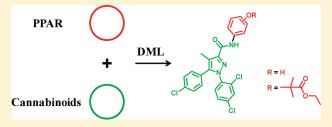
# Discovery of Potent Dual PPAR $\alpha$ Agonists/CB1 Ligands

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**ABSTRACT:** This letter describes the synthesis and in vitro and in vivo evaluation of dual ligands targeting the cannabinoid and peroxisome proliferator-activated receptors (PPAR). These compounds were obtained from fusing the pharmacophores of fibrates and the diarylpyrazole rimonabant, a cannabinoid receptor antagonist. They are the first examples of dual compounds with nanomolar affinity for both PPAR $\alpha$  and cannabinoid receptors. Besides, lead compound 2 proved to be CB1 selective. Unexpectedly, the phenol intermediates tested were equipotent (compound 1



as compared to 2) or even more potent (compound 3 as compared with 4). This discovery opens the way to design new dual ligands.

**KEYWORDS:** Dual ligands, PPARα, cannabinoids, rimonabant, fibrates, neuroprotective

Peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of nuclear receptors involved in the regulation of glucose and lipid metabolism, as well as in adipogenesis, feeding, and other processes such as inflammation and neuroprotection. Therefore, they are interesting targets in medicinal chemistry. Among the three isoforms, PPAR $\alpha$  is a relevant target for pharmaceutical development, oleoylethanolamide (OEA) being its endogenous ligand.<sup>2</sup> PPARa agonists (e.g., fenofibrate, clofibrate) are capable of lowering triglycerides and raising high-density lipoprotein levels. The limitations of currently approved fibrates are their poor selectivity, weak affinity to rodent and human cloned receptors, and the fact that they require high micromolar concentrations to be effective. Recent advances in this field have led to the discovery of the so-called second generation of PPAR $\alpha$  agonists such as BMS-687453, which showed affinity in the nanomolar range.<sup>3</sup> On the other hand, the therapeutic properties of cannabinoids including plantderived compounds as well as synthetic derivatives are due to the interactions with the cannabinoid receptors CB1 and CB2. The identification of these G-protein-coupled receptors<sup>4</sup> responsible for many biochemical processes prompted the discovery of anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) as endogenous ligands.

The endocannabinoid system comprising the endocannabinoids, cannabinoid receptors, and the proteins involved in regulation mechanisms, including the anandamide transporter (ANT), fatty acid amide hydrolase (FAAH), and monoacylglycerol lipase (MAGL), is a relevant therapeutic target. Among the synthetic ligands of these receptors, the cannabinoid receptor antagonist rimonabant proved effective in reducing weight gain and plasma lipids, although side effects banned it as a marketed drug.

PPARs are sensors of fatty acid levels. There is increasing evidence suggesting that endocannabinoids or related compounds may activate PPARs. One of the endogenous ligands that has nanomolar affinity for the PPAR $\alpha$  receptor is OEA. The first study that established the interactions between cannabinoids and PPARs was published in 2002, in which the endocannabinoid 2-AG proved to increase the transcriptional activity of PPAR $\alpha$ . Additional significance comes from the fact that both cannabinoid antagonists and PPAR $\alpha$  agonists may be useful as therapeutic agents for neuroprotective treatment. In Interaction

Received: April 6, 2011
Accepted: September 16, 2011
Published: September 16, 2011

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Figure 1. Structures of dual PPAR $\alpha$ -cannabinoid compounds 1-4.

Cannabinoids (endogenous, phytoderived, and synthetic) do not bind to or activate PPARs to the same extent as available synthetic PPAR ligands; therefore, they may work successfully as partial agonists of PPARs, reducing the potential carcinogenic effects of PPAR agonists. For this reason, cannabinoid actions at PPARs would provide an alternative mechanism for cannabinoids as therapeutic agents.

It has been reported that combining cannabinoids with other compounds (other cannabinoids or PPAR agonists) increases their therapeutic potential. For example, the combination of OEA (PPAR $\alpha$  agonist) with rimonabant (CB1 antagonist) resulted in more effective suppression of feeding and increased weight loss. Another example can be found in the combination of AEA (CB1) and GW7647 (PPAR $\alpha$  agonist) leading to synergistic effects on reducing pain behavior. <sup>13</sup>

We have previously reported oleylethanolamide analogues as PPAR $\alpha$  activators <sup>14</sup> and cannabinoid antagonist derivatives containing a 1,2,4-triazole motif. <sup>15</sup> We now report molecules incorporating cannabinoid and PPAR activities into a single structure using the strategy known as "designed multiple ligands" (DML). <sup>16</sup> Hence, we decided to integrate in one molecule the pharmacophore of the fibrates, fenofibrate (PPAR $\alpha$  agonist), and a structural motif of rimonabant, a proven CB1 antagonist/inverse agonist (Figure 1).

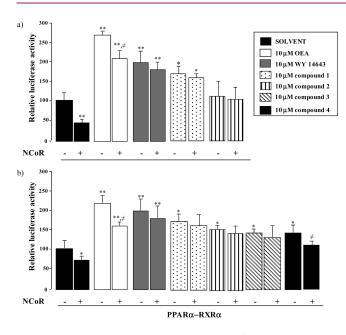
The procedure employed in our laboratory for the synthesis of compounds 1-4 is depicted in Scheme 1. The synthesis began with the carboxylate derivative 5, accessible through a two-step synthesis reported in the literature. The trimethyl aluminummediated amide formation between compound 5 and 3-aminophenol was completed in 3 min and 30 s at 125 °C under microwave irradiation yielding compound 1. This methodology avoided the traditional ester hydrolysis, acid activation to obtain in three steps the amide derivative, apart from reducing the reaction time from 18 h to 3 min using a microwave reactor. Deprotonation of compound 1, followed by treatment with ethyl 2-bromo-2-methylpropionate gave the target compound 2. A similar synthetic route using 4-aminophenol instead of 3-aminophenol was followed for the synthesis of phenol derivative 3 and the fibratelike 4. Deprotonation of compound 3 and nucleophilic substitution of the corresponding bromo derivative yielded compound 4.

### Scheme 1. Synthesis of Compounds 1-4

 $^a$  (i) 3-Aminophenol, Al(CH $_3$ ) $_3$  in 2.0 M heptane, THF, 125 °C, 3 min 30 s. (ii) Ethyl 2-bromo-2-methylpropionate, K $_2$ CO $_3$ , CH $_3$ CN, 90 °C, 18 h. (iii) 4-Aminophenol, Al(CH $_3$ ) $_3$  in 2.0 M heptane, THF, 125 °C, 3 min 30 s. (iv) Ethyl 2-bromo-2-methylpropionate, K $_2$ CO $_3$ , CH $_3$ CN, 90 °C, 18 h.

The compounds reported in this study were first evaluated for basal and ligand-induced activity of PPAR $\alpha$  (Figure 2).

Luciferase reporter gene assays were performed with extracts from MCF-7 cells that were transiently transfected with a reporter construct containing four copies of the human CPTI DR1-type RE (for PPARs) and the indicated expression vectors for PPAR $\alpha$ , RXR $\alpha$ , and NCoR (CoR). Cells were treated for 16 h with a 10 µM concentration of different compounds: DMSO (solvent), the reference PPAR $\alpha$  agonist WY14643, the endogenous PPAR $\alpha$ , agonist OEA, and compounds 1 and 2 (Figure 2a). MD simulation of luciferase activity was normalized to the basal activity of PPAR $\alpha$ -RXR $\alpha$ -SRC1 in the presence of the solvent (DMSO). Details on the methodology have been previously published.<sup>14</sup> Compounds 1 and 2 displayed agonistic activity at the PPAR $\alpha$  receptor at high nanomolar concentrations (Table 1). They were similar in potency to fibrate WY14643, although compound 2 was slightly more potent than compound 1 but 4— 5-fold less potent than the natural ligand oleylethanolamide.



**Figure 2.** Basal and ligand-induced activity of PPARα. Columns represent the mean of at least three experiments, and bars indicate standard deviations. Two-tailed, paired Student's test was performed, and P values were calculated in reference to the respective solvent control (\*P < 0.01, \*\*P < 0.001, or \*P < 0.01) to compare every ligand in the absence or presence of NCoR. (a) After 16 h of incubation. (b) After 6 h of incubation.

Table 1. Pharmacological Properties of Compounds  $1-4^a$ 

compd	PPAR $lpha$ activation EC <sub>50</sub> (nM) $^b$	PPAR $lpha$ activation EC $_{50}$ (nM) $^{c}$
OEA	$152\pm31$	$185 \pm 64$
WY14643	$650 \pm 134$	$542\pm98$
1	$989 \pm 175$	$887 \pm 185$
2	$743 \pm 109$	$856 \pm 166$
3	ND	$971\pm180$
4	ND	$1480\pm253$

 $^a$  EC<sub>50</sub> values calculated in the presence of the different compounds by GraphPad Prism 4. Results are the means  $\pm$  SEMs of three experiments. ND, no data.  $^b$  Sixteen hours of incubation.  $^c$  Six hours of incubation.

To assess the stability of the compounds in the assay, two different experiments changing the incubation time were performed. Cells were treated for 6 h with a  $10\,\mu\mathrm{M}$  concentration of different compounds: DMSO (solvent), WY14643, OEA, and compounds 1-4 (Figure 2b). These results were consistent with the previous ones after 16 h of incubation, and the phenol derivatives 1 and 3 showed agonistic activity at the PPAR $\alpha$  receptor at high nanomolar concentrations. Compound 2 was the more potent of the series, whereas its homologue 4 with a para substitution at the benzene ring was less potent with an EC<sub>50</sub> of 1.5  $\mu$ M.

The compounds were screened for cannabinoid activity in radioligand binding assays (Table 2). All compounds were found to displace [ ${}^{3}$ H]-CP55940 binding from cannabinoid CB1 receptors at nanomolar concentrations.  $K_{i}$  values of compounds 1 and 2 were very similar to the reference agonist WIN 55,212-2, while compound 3 with a  $K_{i}$  of 4.6 nM was more potent than the

Table 2. CB1 Receptor Affinity<sup>a</sup>

compd	CB1 $K_{i}$ (nM)
rimonabant	$7.3 \pm 0.97$
WIN 55,212-2	$45.6 \pm 8.6$
1	$52 \pm 22$
2	$65 \pm 17$
3	$4.6 \pm 0.96$
4	$109 \pm 50$

<sup>a</sup> Data are mean values of n = 3 separate experiments and are expressed as  $K_i$  (nM) for CB1 and binding.

reference compound rimonabant. Compound 4 was the least potent of the series. The diarylpyrazole scaffold used for designing the compounds and the lack of potency at the cannabinoid CB2 receptor (data not shown) are usually associated with selective cannabinoid CB1 receptors antagonist activity.

The biological activity of compounds 2 (fibratelike) and 3 (*p*-phenol) was evaluated carrying out pharmacological experiments in the isolated mouse vas deferens (MVD).<sup>18</sup> It is a commonly used tissue to study and characterize cannabinoid effects as agonists or antagonists.<sup>19</sup> Compound 2 was chosen as the best dual compound of the series and compound 3 because of having a binding constant (4.6 nM) similar to rimonabant (7.3 nM). The effect of the compounds was always compared with that of reference drugs: arachidonyl-2-chloroethylamide (ACEA) and AM251 as cannabinoid CB<sub>1</sub> selective agonist and antagonist, respectively.

The MVD is a nerve-smooth muscle preparation that serves as a highly sensitive and quantitative functional in vitro bioassay for cannabinoid receptor agonists. Additionally, it is commonly used as a bioassay for competitive surmountable cannabinoid receptor antagonists and also provides a means for distinguishing neutral cannabinoid antagonists from inverse agonists. The bioassay of cannabinoid receptor agonists relies on the ability of these ligands to produce concentration-related decreases in the amplitude of electrically evoked contractions of the vas deferens by acting on prejunctional neuronal receptors and inhibiting the release of the contractile neurotransmitters such as noradrenaline and ATP. The bioassay of competitive surmountable cannabinoid receptor antagonists examines the ability of these compounds to produce dextral shifts in cannabinoid receptor agonist log concentration-response curves in electrically stimulated tissues. 20-22

Data obtained from compounds 2 and 3 at the tested concentrations  $(10^{-7}-1.8\times10^{-5} \,\mathrm{M})$  did not reveal any effect either on the nonstimulated basal recording or on the electrically induced contractile response on MVD (the % of inhibition of the electrically evoked contractions was <10% and not different from that induced by the vehicle), so it could be established that they both lack a direct (activation or blockade) or indirect (induction or inhibition of neurotransmitter release) intrinsic activity on a large number of well-known receptors that play a role in the basal tone or on the electrically induced contraction in this tissue (adrenergic, purinergic, cannabinoid,  $\delta$ ,  $\mu$ , and  $\kappa$  opioid receptors).

An interesting finding is that compounds 2 and 3 (at the two tested concentrations  $10^{-6}$  and  $2\times10^{-6}$  M) significantly attenuate the inhibition induced by the selective CB<sub>1</sub> receptor agonist ACEA (Figure 3) as also occurred with the reference compound AM251. These data suggest that both behave as CB<sub>1</sub>

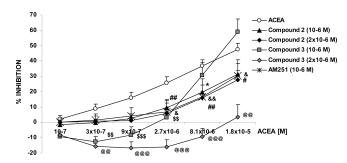


Figure 3. Functional activity of compounds 2 and 3 on MVD. Lines show the mean %  $\pm$  SEM (n=6-8) inhibition of the electrically induced contraction of the MVD induced by the addition of increasing concentrations of ACEA in control tissues or in tissues incubated with 2, 3, or AM251. Statistically significant difference:  $^*p < 0.05$ , 2 ( $10^{-6}$  M) + ACEA vs ACEA;  $^*p < 0.05$  and  $^{\#p} < 0.01$ , 2 ( $2 \times 10^{-6}$  M) + ACEA vs ACEA;  $^{\$\$}p < 0.01$  and  $^{\$\$\$\$}p < 0.001$ , 3 ( $10^{-6}$  M) + ACEA vs ACEA; and  $^{\$p} < 0.01$  and  $^{\$\$\$}p < 0.001$ , 3 ( $2 \times 10^{-6}$  M) + ACEA vs ACEA; and  $^{\$p} < 0.05$  and  $^{\$\$\$}p < 0.01$ , AM251 ( $10^{-6}$  M) + ACEA vs ACEA (two-way ANOVA test, Bonferroni's posthoc test).

receptor antagonists, compound **2** being as effective as AM251, whereas compound **3** is significantly more effective than AM251. From the shape of the curve, it could be suggested that it behaves like a competitive antagonist, although more work is required to confirm this possibility.

Regarding  $CB_2$  antagonism experiments, compound 2 failed in modifying the inhibitory effect of the selective  $CB_2$  receptor agonist JWH-015 (data not shown), so it could be suggested that it is a selective  $CB_1$  antagonist; on the other hand, phenol derivative 3 was able to inhibit the effect of JWH-015 (Supporting Information), although this effect was lower than that observed on  $CB_1$  receptor, suggesting a relatively selective  $CB_1$  effect.

Despite the affinity for both cannabinoid CB1 receptors and PPAR $\alpha$  receptors, the compounds did not display activity as feeding suppressants in vivo food deprived rats when administered intraperitoneally (Supporting Information). The lack of acute effects on feeding of these acyl derivatives may be attributable to several reasons. First, the in vitro profile of the compounds may not be manifested in vivo due to poor pharmacokinetics. Second, the compounds may affect non-CB1, non-PPAR $\alpha$ -mediated signaling mechanisms that control feeding. For instance, OEA is a feeding suppressant that acts not only at the PPAR $\alpha$  receptor but also on the orphan receptor GPR119 to inhibit feeding. Third, the lack of acute effects does not exclude the potential induction of changes in energy expenditure when these compounds are given chronically. All of these aspects will be addressed in future studies.

In summary, we have prepared and evaluated four compounds, three of which showed nanomolar affinity for both the PPAR $\alpha$  and the CB1 receptors. The middle-low potency at the PPAR $\alpha$  receptor similar to fibrates will probably lead to a hypolipemiant and neuroprotective profile, while the high affinity for the CB1 receptor may help to modulate metabolism. The intermediates phenol derivatives, 1 and 3, were found to be unexpectedly potent, offering new possibilities in the search for dual PPAR $\alpha$  agonists/CB1 ligands. Therefore, the four synthesized compounds can be considered interesting leads in the search for a new class of dual ligands capable of modulating metabolism with potential neuroprotective activities.

## ASSOCIATED CONTENT

Supporting Information. Synthetic experimental details, analytical data, and biological assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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#### **Funding Sources**

The present work has been supported by grants SAF2009-12422-C02-02, RTA (RED Trastornos Adictivos RD06/001/0014), the European Union's seventh Framework Programme (Health-F2-2008-223713, Reprobesity), and the following grants from the Spanish Ministry of Science and Innovation: SAF2010-20521, National Institute of Health 'Carlos III' Red de Trastornos Adictivos EU-ERDF (RD06/0001/0000), and CIBER-OBN EU-ERDF (CB06/03/1008). M.M.-G. is supported by the Research Stabilization Program of the Instituto de Salud Carlos III (CES 10/004).

#### ■ ACKNOWLEDGMENT

We thank Prof. J. Fernández Ruiz for generous use of the equipment to perform the binding studies and M. Gómez Cañas for her technical assistance.

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