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Metabolically labile cannabinoid esters: A 'soft drug' approach for the development of cannabinoid-based therapeutic drugs

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Abstract—Biphenylic ester derivatives, designed by using a 'soft-drug' approach, proved to possess good binding properties toward cannabinoid CB_1 and CB_2 receptors and, at the same time, their metabolically labile ester portion would promote a rapid systemic inactivation. This may constitute a possible solution to the psychotropic side effects encountered when cannabinoids are therapeutically employed as local analgesic or antiglaucoma agents.

The medical applications of *Cannabis sativa* derivatives have been known for many centuries, but their approved use was commonly discontinued in the early 1900s due to the well-known psychotropic side effects. Nowadays, at least two receptor subtypes, CB_1 and CB_2, have been recognized within the mammalian cannabinergic system. CB_1 is mainly found in central and peripheral neurons, whereas CB_2 exists primarily in immune cells.[1] It has been shown that stimulation not only of CB_1, but also of CB_2 receptors causes analgesic effects.[2] Moreover, stimulation of the ocular CB_1 receptor produces a reduction of intraocular pressure (IOP), indicating a route by which new anti-glaucoma drugs may be developed.[3] Therefore, molecules able to stimulate CB-receptors have the potential to be used locally as analgesic or anti-glaucoma agents. The main problem in the therapeutic use of CB-agonists is the difficulty in limiting the occurrence of central psychotropic effects. A possible solution is given by the 'soft drug approach'.[4]

Soft drugs are bioactive molecules deliberately designed to go through a rapid, efficient, and predictable metabolic inactivation after having exerted their effects at the site of action.[4] In many cases the use of soft drugs has led to the development of safer marketed drugs since they are endowed with the appropriate local activity, whereas they are devoid of systemic side effects.[5] The soft drug approach would, therefore, be particularly suitable for the development of cannabinoids to be used as locally active analgesic or anti-glaucoma agents, without causing the unwanted central effects. These considerations have already inspired some excellent examples of 'soft-cannabinoids' possessing CB_1-mediated IOP lowering properties, to be developed as safe anti-glaucoma agents.[6]

We focused our attention on structurally simple biphenylic derivatives that had already been reported to be good CB-ligands[7a,b] and potent CB-agonists.[7c] In particular, compound **1** (Fig. 1) showed K_is of 79 and 2 nM, on CB_1 and CB_2, respectively.[7b]

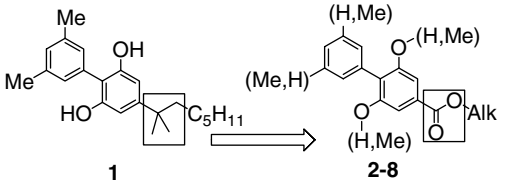

Figure 1. Structural derivation of metabolically labile esters **2**–**8** (see Table 1) from biphenylic CB-ligand **1**.

Keywords: Cannabinoids; CB_1; CB_2; Soft-drugs; Retrometabolic.
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We were inspired by this class of biphenyl cannabimimetic derivatives because their simple molecular structures guarantee a greater synthetic accessibility compared to classical cannabinoids, and, most importantly, they generally display high potencies of action, which have been considered as a useful feature for their application in the treatment of pain and glaucoma.[7c]

Therefore, we envisaged the possibility of inserting a metabolically labile ester group within the linear aliphatic side chain of biphenyl derivative **1**, in an attempt to develop soft-cannabinoid analogues (**2**–**8**, Table 1). Compound **2** is the direct ester analogue of parent aliphatic CB-ligand **1**, obtained by simply replacing the *gem*-dimethyl-substituted ethylene portion of **1** with an ester –C(O)O– group. The linear five-carbon-aliphatic side chain (*n*-C_5H_{11}) of the ester group in **2** was chosen in order to maintain an overall seven-carbon-length as in **1**. Moreover, we also wanted to verify the effect on CB_1/CB_2 affinity due to small structural modifications on several molecular portions of the biphenylic core originally present in **1**. Changes in the methyl and/or free OH aryl substituents, as well as in the length of the linear aliphatic ester chain, were obtained in the series of new compounds **3**–**8**.

These newly designed ester-type soft cannabinoids should act only locally, as long as they preserve their structural integrity. However, as soon as they are systematically absorbed, they are predicted to undergo a rapid hydrolytic inactivation that leads to inactive carboxylic acids and aliphatic alcohols, as the metabolic products (Fig. 2).

Esters **2**–**8** were prepared as outlined in Scheme 1 from commercially available 4-bromo-3,5-dimethoxybenzoic acid **12**.

Treatment of **12** with refluxing thionyl chloride, followed by reaction of the resulting acid chloride with the appropriate alcohol in THF, afforded esters **13a**–**c**. Subsequent Pd-catalyzed cross-coupling reaction with the properly substituted arylboronic acid in the presence of $Pd(PPh_3)_4$ as the catalyst yielded biphenyl derivatives **4** and **14a**–**c**. In spite of the fact that the bromo-aryl group in intermediates **13a**–**c** is rather hindered because of the simultaneous presence of two *ortho*-methoxy substituents, the yields of this step were quite satisfactory, ranging between 64% and 75%. Final progressive demethylation steps of the aryl-methoxy groups were carried out by finely modulating the reaction conditions.

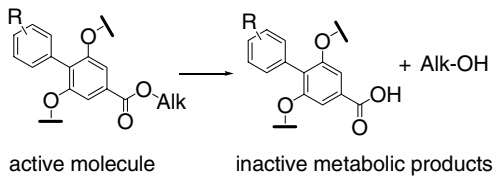
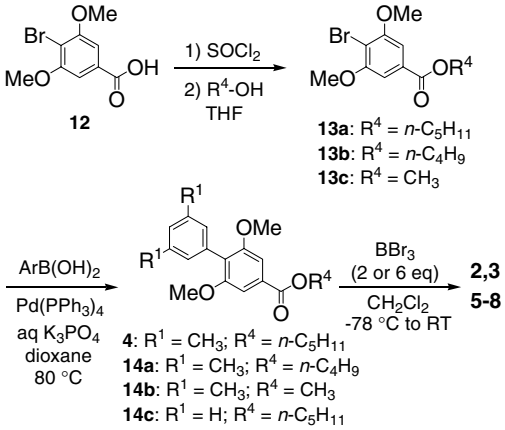
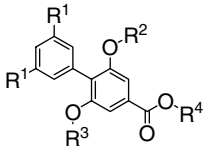


Figure 2. Predicted metabolic hydrolytic inactivation of ester-type soft cannabinoids.



Scheme 1. Synthesis of esters **2**–**8** from commercially available **12**.

Table 1. Binding affinity of esters **2**–**8** and carboxylic acids **9**–**11**



2-11

Compound	R^1	R^2	R^3	R^4	hCB_1 K_i[a] (μM)	hCB_2 K_{ia} (μM)
2	CH_3	H	H	*n*-C_5H_{11}	2.0	1.1
3	CH_3	CH_3	H	*n*-C_5H_{11}	1.4	0.27
4	CH_3	CH_3	CH_3	*n*-C_5H_{11}	>10	>10
5	H	H	H	*n*-C_5H_{11}	>10	4.0
6	CH_3	H	H	*n*-C_4H_9	2.6	3.2
7	CH_3	H	H	CH_3	>10	4.5
8	CH_3	CH_3	H	CH_3	>10	6.4
9	CH_3	H	H	H	>10	>10
10	CH_3	CH_3	H	H	>10	>10
11	CH_3	CH_3	CH_3	H	>10	>10

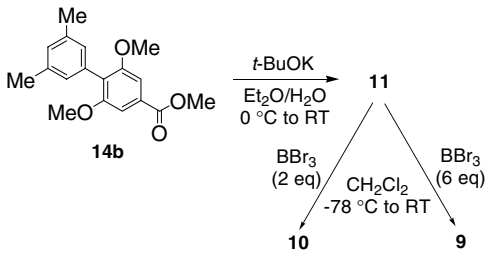
[a] Data represent mean values for at least three separate experiments performed in duplicate and are expressed as K_i (μM). hCB_1, human CB_1 receptor; hCB_2, human CB_2 receptor. Standard errors are not shown for the sake of clarity and were never higher than 10% of the means.

In details, when **4** was treated with only 2 equiv of boron tribromide at −78 °C, and then the reaction mixture was slowly allowed to reach rt, mono-demethylated product **3**[8] could be isolated by stopping the reaction immediately after TLC showed the consumption of starting material. On the other hand, exhaustive demethylation of **4** was achieved by using 6 equiv of BBr_3 and monitoring by TLC the consumption of both starting material and partially demethylated intermediate **3**, thus obtaining, di-hydroxy-substituted ester **2**.[9] The same procedures were applied to the mono- and di-demethylation of methyl ester **14b**, which, respectively, afforded compounds **8** and **7**. Only completely demethylated products **5** and **6** were instead obtained by BBr_3 treatment of precursors **14c** and **14a**, respectively.

We also synthesized carboxylic acids **9**–**11**, which are the predicted metabolic products of the newly designed soft-cannabinoids (Fig. 2), in order to test whether or not they preserved any (unwanted) affinity for the CB-receptors.

To this purpose, we started their synthesis (Scheme 2) from methyl ester **14b**, prepared as previously shown in Scheme 1, which was hydrolyzed by treatment with potassium *tert*-butoxide in a diethylether/water mixture, producing dimethylated carboxylic acid **11**.[10] Also in this case, it was possible to selectively obtain mono- and di-hydroxy-substituted products (**10** and **9**, respectively),[11] by tuning the BBr_3-promoted demethylation conditions as seen before.

Compounds **2**–**11** were tested for their affinity for human recombinant CB_1 and CB_2 receptors using previously described procedures and employing HEK cells transfected with either the hCB_1 or hCB_2 receptor and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl) phenyl]*trans*-4-(3-hydroxypropyl)cyclo-hexanol ([^{3}H]CP-55,940; K_d = 0.18 and 0.31 nM for CB_1 and CB_2 receptor, respectively) as the high affinity ligand, as described by the manufacturer (Perkin-Elmer, Italy).[12] Displacement curves were generated by incubating drugs with [^{3}H]-CP-55,940 (0.14 and 0.084 nM for CB_1 and CB_2 binding assay, respectively). In all cases, K_i values were calculated by applying the Cheng–Prusoff equation to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds. The resulting K_i values are reported in Table 1. In addition, in order to evaluate the metabolic transformations of the compound **3**, rat liver was homogenated in 50 mM Tris–HCl buffer, pH 7, and centrifuged at 800*g*, for 6 min at 4 °C. The supernatant (750 μg) and compound **3** (100 μg) were incubated in 50 mM Tris–HCl buffer, pH 7. The organic phase, after extraction of the incubation mixture with two volumes of $CHCl_3/CH_3OH$ (2:1 by vol), was lyophilized under vacuum and then fractionated by TLC (20 × 20 cm) on silica gel F_{254} (Merck) by using AcOEt/EtPt/AcOH (60:40:1) as the eluting system.



Scheme 2. Synthesis of carboxylic acids **9**–**11** from ester **14b**.

Compound **2**, the ester derivative possessing the closest structural resemblances to CB-ligand **1**, showed interesting affinity for both CB_1 and CB_2 receptors, with K_i values of 2.0 and 1.1 μM, respectively. The presence of a single methoxy-group in the place of one phenol OH, as in compound **3**, even enhanced the binding affinity of the resulting molecule, especially for the CB_2-subtype (K_i = 0.27 μM). On the contrary, the replacement of both phenol—with two MeO—groups as in **4** caused a complete loss of binding affinity for both receptor subtypes. The importance of the methyl-aryl substituents (R^1) was checked by assaying compound **5**, bearing an unsubstituted phenyl-ring: in this case the resulting molecule showed no affinity for CB_1, whereas it still maintained a certain level of affinity (K_i = 4.0 μM) for CB_2. These data show that the presence of two methyl-groups (R^1) in the 3′ and 5′ position of the phenyl-substituents is fundamental for CB_1-binding and is also beneficial for an efficient CB_2-affinity.

The effect of the length of the aliphatic ester chain was also investigated in *n*-butyl (**6**) and methyl (**7** and **8**) esters. When compared to *n*-pentyl ester **2**, the corresponding *n*-butyl ester **6** showed a nearly unmodified affinity for CB_1, whereas its binding for CB_2 was more negatively affected (K_i = 3.2 μM). Methyl ester **7** showed a reduction of affinity for both receptor subtypes, which led to a substantial loss of affinity for CB_1, still maintaining some binding on CB_2 (K_i = 4.5 μM). In this latter case, the transformation of a single phenol OH of **7** into a methoxy-group, as in **8**, did not cause the same binding improvement observed with their longer-chain analogues (cf. **2** and **3**); in fact, the affinity of **8** generally was even worse than that of **7**. Therefore, the most appropriate length of the linear aliphatic ester side chain turned out to be that of five carbon atoms (R^4 = *n*-C_5H_{11}), as in **2** and **3**. As a matter of fact, an alkyl chain of this extent, together with the ester linker, covers an approximately same spatial occupation as the seven-carbon fully-aliphatic side chain in parent CB-ligand **1**.

Most importantly, carboxylic acids **9**–**11**, which constitute the predicted metabolic products of their ester precursors, were completely devoid of any affinity for either receptor subtype. This inability to bind the CB-receptors by **9**–**11** is a fundamental requirement for the ester derivatives to be considered as possible soft-cannabinoid agents.

Finally, preliminary assays on metabolic transformations of the highest affinity compound **3** performed in rat liver homogenates confirmed that about 40% of this molecule is hydrolyzed after only 30 min of incubation at 37 °C. Although these first in vitro metabolic data are not sufficient to completely exclude the possible

occurrence of CNS side effects, they still constitute a starting basis for more in-depth evaluations. Further studies are currently under way to fully characterize the functional and metabolic behavior of compounds **2** and **3** and the kinetics of their hydrolytic inactivation.

The generally lower binding levels found with all the ester analogues of CB-ligand **1** could be largely expected, since the original bulky and lipophilic *gem*-dimethylethyl portion of **1** was replaced by the polar ester group, needed to provide a metabolically cleavable site. Nevertheless, the real challenge of this work was to keep some sufficient receptor affinity for compounds that were designed to: (i) be administered locally; (ii) lose their CB-affinity when they get into the systemic circulation. Therefore, K_i values reaching low- or sub-micromolar levels, such as those shown by **2** and **3**, may be good enough to achieve these goals. Moreover, this 'soft-cannabinoid' approach does not even require a particular receptor-subtype selectivity, since the selective peripheral action, devoid of central effects, would probably be supported by the programmed rapid metabolic inactivation of these derivatives.

Further studies will be devoted to a deeper pharmacological and metabolic characterization of the highest affinity esters **2** and **3**, as well as to the development of other biphenylic soft-CB-ligands, by moving the ester portion along the aliphatic side chain, in an attempt to verify the possibility of obtaining molecules possessing even better binding properties and/or more suitable metabolic behaviors.

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8. Compound **3** purified by column chromatography (Hex/EtOAc); white solid, mp 62 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.90–0.97 (m, 3H), 1.36–1.47 (m, 4H), 1.71–1.85 (m, 2H), 2.32 (s, 6H), 3.75 (s, 3H), 4.30 (t, 2H, J = 6.6 Hz), 6.92 (s, 2H), 6.95 (s, 1H), 7.20 (d, 1H, J = 1.5 Hz), 7.30 (d, 1H, J = 1.5 Hz), 8.11 (br s, 1H); MS-EI (70 eV) *m/e* 342 (M^+).
9. Compound **2** purified by column chromatography (Hex/EtOAc); white solid, mp 93 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.89–0.96 (m, 3H), 1.39–1.48 (m, 4H), 1.68–1.79 (m, 2H), 2.31 (s, 6H), 4.26 (t, 2H, J = 6.5 Hz), 6.95 (s, 1H), 6.99 (s, 2H), 7.18 (s, 2H), 8.08 (br s, 2H); MS-EI (70 eV) *m/e* 328 (M^+).
10. Compound **11**: white solid, mp 190 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.36 (s, 6H), 3.81 (s, 6H), 6.94 (s, 1H), 6.98 (s, 2H), 7.41 (s, 2H).
11. (a) Compound **9**: white solid, mp 206–208 °C; ^{1}H NMR (acetone-d_6, 200 MHz) δ 2.31 (s, 6H), 6.93 (s, 1H), 6.98 (s, 2H), 7.21 (s, 2H), 8.01 (br s, 2H). (b) Compound **10**: white solid, mp 198 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.30 (s, 6H), 3.76 (s, 3H), 6.93 (m, 3H), 7.22 (d, 1H, J = 1.3 Hz), 7.33 (d, 1H, J = 1.3 Hz), 8.09 (br s, 2H).
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