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Kavalactones and the endocannabinoid system: The plant-derived yangonin is a novel CB_1 receptor ligand

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ABSTRACT

To investigate the possible interactions between kavalactone-based molecules and proteins of the endocannabinoid system and provide novel and synthetically accessible structural scaffolds for the design of cannabinoid receptor ligands sharing pharmacological properties with kavapyrones, a preliminary SAR analysis was performed on five commercially available natural kavalactones and nine kavalactone-analogues properly synthesized. These compounds were investigated for assessing their cannabinoid receptor binding affinity and capability of inhibiting the activity of the two major metabolic enzymes of the endocannabinoid system, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Among the molecules tested, only yangonin exhibited affinity for the human recombinant CB_1 receptor with a $K_i = 0.72\ \mu M$ and selectivity vs. the CB_2 receptor ($K_i > 10\ \mu M$). None of the compounds exhibited strong inhibitory effects on the two enzymes analyzed. The CB_1 receptor affinity of yangonin suggests that the endocannabinoid system might contribute to the complex human psychopharmacology of the traditional kava drink and the anxiolytic preparations obtained from the kava plant.

1. Introduction

Kava ("intoxicating pepper"; *Piper methysticum* Forster) is a perennial tropical shrub widely cultivated in the South Pacific Island Countries. Kava is also the name of the aqueous beverage prepared from the roots of the plant; it has been used by the islanders for social ceremonies for centuries, though recreational kava-drinking has recently become popular [1–4]. Preparations made from various parts of the plant have also been used in local medicine for a range of illnesses, including fever, pain, headache and migraines, respiratory problems, sleeping difficulties, diarrhea or constipation, skin diseases, urogenital and menstrual problems, convulsion, and weight reduction [4]. By the early twentieth century, kava products became available also in Europe for the treatment of hypertension, gonorrhea and certain nervous conditions [5]. In the 1990s kava organic extracts, usually sold in encapsulated and tincture forms as over-the-counter preparations, attracted considerable interest worldwide as a clinically proven treatment for anxiety, depression, insomnia and stress [6]. Among the additional subtle pharmacological and behavioral effects of kava drinking are anesthesia and astringency on the tongue and the inner lining of the mouth, as well as sociability, and mild euphoria lasting for several hours. Small doses were reported to produce mild stimulant effects while larger dosages cause muscle relaxation, ataxia, paraesthesia, and somnolence. Low, non-sedating acute doses of kava do not appear to impair cognitive performance [7–9], though long-term studies are lacking. It must also be noted that early reports do not contain data on the composition and dose of the drink.

Nevertheless, regular and heavy use of kava is not without risks: dermopathy, liver and kidney problems, gastrointestinal distress, as well as impaired vision have been reported [10]. Of these, of particular concern is hepatotoxicity, which has been linked to the poor metabolizing capacity of susceptible individuals, drug interactions, extreme dosages, and mould or other impurities of kava [11,12]. Since 2002 several countries have banned the sales of kava extracts, although there is now a rekindled interest in standardized kava preparations as herbal medicines [13].

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, arachidonoyl ethanolamine (anandamide); CB, cannabinoid; COX, cyclooxygenase; GABA, γ-aminobutyric acid; MAGL, monoacylglycerol lipase; SAR, structure–activity relationship; THC, Δ^9-tetrahydrocannabinol.

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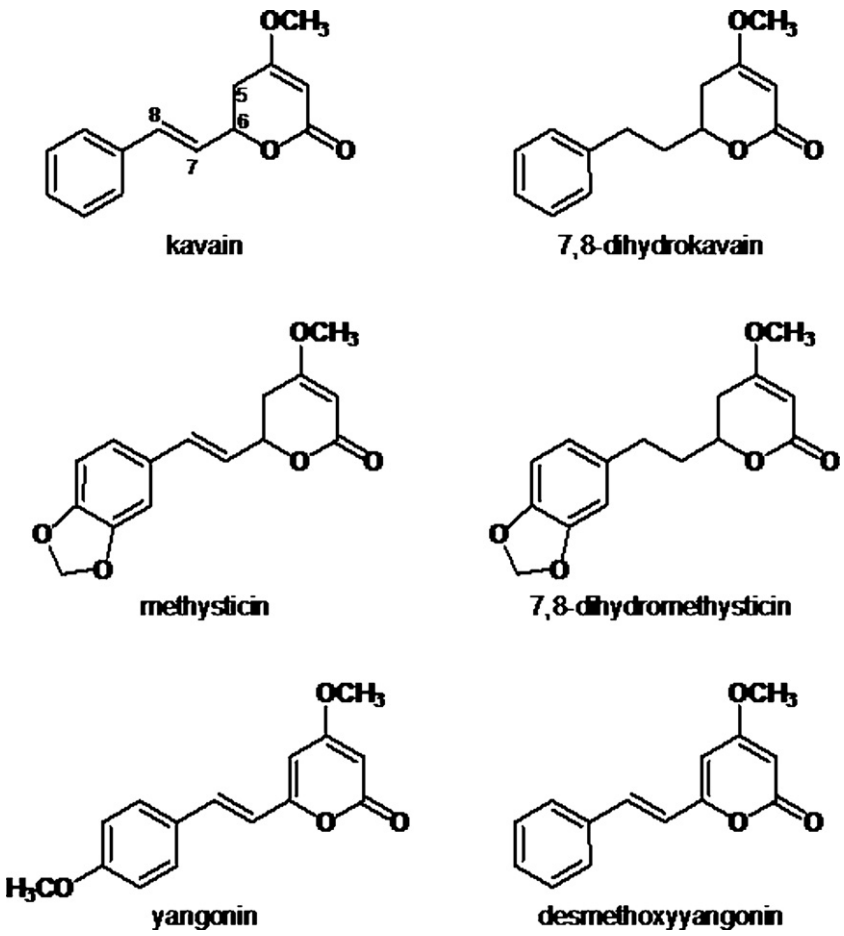

Fig. 1. Chemical structures of the six major kavalactones.

The investigation of the chemical constituents of kava dates back to the 1860s. The pharmacologically most important compounds isolated from the root are lipid-soluble α-pyrone derivatives, the kavalactones (or kavapyrones), of which 18 have been characterized [4]. The most abundant kavalactones are kavain, 7,8-dihydrokavain, methysticin, 7,8-dihydromethysticin, yangonin and desmethoxyyangonin (Fig. 1). The total kavalactone-content of the dried roots ranges from 3 to 20% and depends on the cultivar (chemotype), geographical location, environmental conditions, age of the plant, and time of harvest [14,15]. The pharmacology and underlying mechanisms of action of kava extract and kavalactones are only partially understood. Four major molecular target types have been identified and characterized [4,13]: (a) γ-aminobutyric acid (GABA) and benzodiazepine receptor sites; (b) voltage-gated Na^+ and Ca^{2+} ion channels; (c) monoamine uptake and catabolism; (d) arachidonate cascade. Each kavalactone has a unique pharmacological profile and the overall activity of the root extract is more pronounced than that of the individual constituents, which is suggestive of synergism [4]. Despite the fact that some of the pharmacological effects (e.g. sedation, ataxia, hyperthermia and bloodshot eyes) as well as analgesic and mood enhancer actions of kava resemble those of *Cannabis*[1] [1–3], nothing is known so far of the possible interaction of kava and kavalactones with the endocannabinoid system, which comprises two G-protein-coupled receptors, the cannabinoid type-1 (CB_1) and type-2 (CB_2) receptors, the two major endogenous ligands of such receptors, known as endocannabinoids, such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and the enzymes for endocannabinoid metabolism (see [16] for a recent review). The present study was initiated to examine the cannabinoid receptor binding affinity of the five commercially available kavalactones (desmethoxyyangonin only became available during the revision process of this article), namely (±)-kavain, 7,8-dihydrokavain, methysticin, 7,8-dihydromethysticin, and yangonin (Fig. 1), as well as of a novel series of synthetic compounds, chemically related to kavain (with various substituents at the sn-4 and sn-6 positions of the dihydropyran ring and two open-ring molecules) (Fig. 2), in order to perform a preliminary SAR analysis. We also evaluated the potential indirect agonist action of these 14 compounds, i.e. their capability to inhibit the enzymatic hydrolysis of AEA and 2-AG by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively, and hence to prolong endocannabinoid tonic action at cannabinoid receptors.

2. Materials and methods

2.1. Drugs and chemicals

7,8-Dihydrokavain, methysticin, 7,8-dihydromethysticin and yangonin, all isolated from *Piper methysticum*, were obtained from PhytoLab GmbH & Co. KG (Vestenbergsreuth, Germany). D,L-kavain was synthetic and also obtained from PhytoLab GmbH & Co. KG.

[1] "Auch mit der Wirkung des Lattisch und der des Haschisch ist die Kawawirkung verglichen worden." In Ref. [1, p. 44].

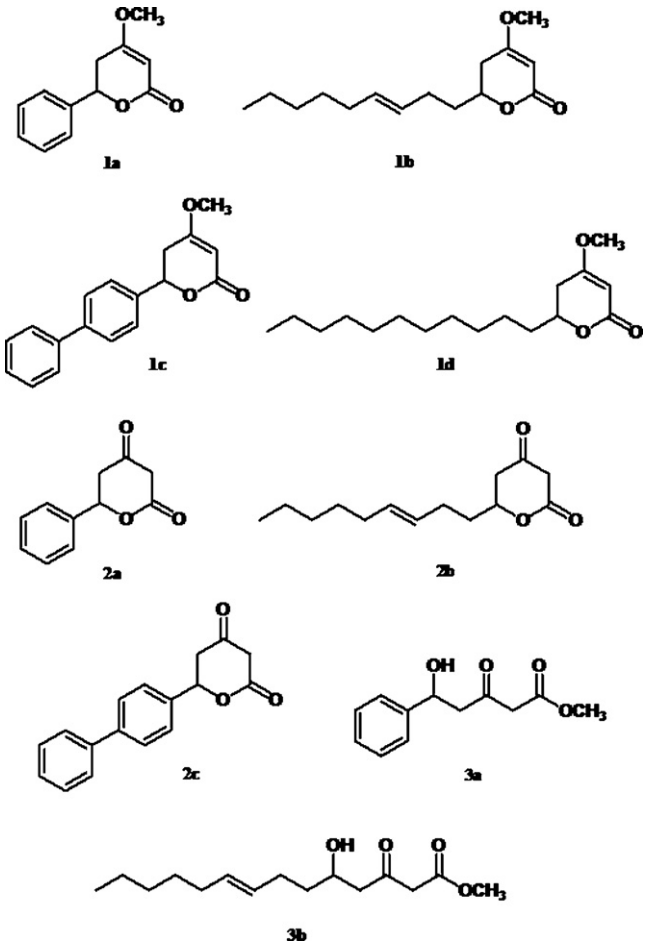

Fig. 2. Chemical structures of the novel synthetic analogues.

The purity of all kavalactones was >97%, as stated by the supplier. 2-AG (purity >95%) was purchased from Cayman Chemicals (Ann Arbor, MI, USA), while 2-arachidonoyl-[^{3}H]glycerol (specific activity 40 Ci/mmol) was from ARC, Inc. (St. Louis, MO, USA). Test compounds were dissolved in DMSO to make serial dilutions that were used the same day. WIN 55212-2 was obtained from Tocris Cookson Ltd. (Avonmouth, Bristol, UK). [^{3}H]CP-55,940 (specific activity 144 Ci/mmol) was purchased by Perkin Elmer (Milan, Italy).

2.2. Chemical synthesis of racemic kavain analogues

Synthesis of the new compounds was accomplished as depicted in Fig. 1 of the Supplementary Materials. All kavain analogues **1a-d** were prepared from the appropriate aldehyde **5** in only three steps. The iodine-promoted vinylogous aldol addition of Chan's diene **4** [17] to different aldehydes (**5a–d**), promoted by molecular iodine and followed by desilylation with trifluoroacetic acid, gave the desired δ-hydroxy-β-ketoesters **3a–d** in high yields [18]. Treatment of **3a–d** with K_2CO_3 in MeOH gave directly β-keto-δ-lactones **2a–d**, which could be either isolated by acidic work-up or treated immediately with dimethyl sulfate for the synthesis of the corresponding kavalactone analogues **1a–d**. Due to its simplicity, inexpensiveness, efficiency and versatility this synthetic strategy is very attractive and, by using different classes of aldehydes, potentially useful in the generation of a library of structurally diverse compounds. The spectroscopic and spectrometric characterization of the purified synthetic compounds is reported in the Supplementary Materials.

2.3. Receptor binding assays

The pharmacological activity of kavalactones was evaluated in terms of percent inhibition of radioligand binding and K_i. In both cases, competitive binding assays were performed using membranes from HEK-293 cells transfected with the human recombinant CB_1 receptor (B_{max} = 3.6 pmol/mg protein) and human recombinant CB_2 receptor (B_{max} = 5.2 pmol/mg protein). Receptors were incubated with [^{3}H]CP-55,940 (0.4 nM/Kd = 0.12 nM and 0.53 nM/Kd = 0.18 nM, respectively, for CB_1 and CB_2 receptors) as the high-affinity-ligand and displaced with 10 μM WIN 55212-2 as the heterologous competitor for non-specific binding (K_i values of 9.2 nM and 2.1 nM for the CB_1 and CB_2 receptor, respectively). Varying concentrations of compounds (0.1, 0.5, 1.0, 5.0, and 10.0 μM) or vehicle (DMSO) were tested following the procedure described by the manufacturer (Perkin Elmer, Milan, Italy). Displacement curves were generated by incubating drugs with [^{3}H]CP-55,940 for 90 min at 30 °C. K_i values were calculated by applying the Cheng-Prusoff equation to the IC_{50} values (obtained using the GraphPad® program) for the displacement of the bound radioligand by increasing concentrations of the test compound. Data are reported as means of n = 3 experiments.

2.4. FAAH inhibition studies

The effect of varying concentrations of compounds (0.5, 1.0, 5.0, and 10.0 μM) and vehicle (DMSO) on AEA hydrolysis was measured by incubating the membrane fractions from rat brain (70 μg/sample) in Tris–HCl 50 mM, at pH 9 for 30 min at 37 °C in presence of [^{14}C]-AEA properly diluted with unlabelled AEA. After incubation, the amount of [^{14}C]ethanolamine produced enzymatically was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volume-equivalents of $CHCl_3$/MeOH 1:1 (by volume). Data are expressed as the concentration exerting 50% inhibition of AEA hydrolysis (IC_{50}), calculated by GraphPad®. Data are reported as means of n = 3 experiments.

2.5. MAGL inhibition studies

The effect of varying concentrations of compounds (0.5, 1.0, 5.0, and 10.0 μM) and vehicle (DMSO) on 2-AG hydrolysis was measured by incubating the 10,000xg cytosolic fraction of COS cells (100 μg/sample) in Tris–HCl 50 mM, at pH 7.0 at 37 °C for 20 min, with synthetic 2-arachidonoyl-[^{3}H]-glycerol properly diluted with unlabelled 2-AG. After incubation, the amount of [^{3}H]glycerol produced was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volume-equivalents of $CHCl_3$/MeOH 1:1 (by volume). Data are expressed as the concentration exerting 50% inhibition of 2-AG hydrolysis (IC_{50}), calculated by GraphPad®. Data are reported as means of n = 3 experiments.

3. Results

3.1. Affinity of the compounds for CB_1 and CB_2 receptors

This study examined the affinity of five kavalactones and nine synthetic derivatives for the human recombinant CB_1 and CB_2 receptors. The calculated IC_{50} and K_i values are reported in Table 1. For comparison, the corresponding values of Δ^9-tetrahydrocannabinol (THC), the well-characterized *Cannabis* component and cannabinoid CB_1 and CB_2 receptor agonist, are also shown in Table 1. Among all the compounds tested, only yangonin exhibited the ability to displace bound radioligand, indicating a measurable affinity for CB_1 receptors. In particular, yangonin

Table 1
Effect of five natural kavalactones and nine synthetic derivatives on [^{3}H]-CP55940 binding to the human recombinant cannabinoid receptors. The activity, expressed as IC_{50}, was determined by non-linear regression of the inhibition of radioligand binding exerted by increasing concentrations of test compounds. When the IC_{50} was lower than 10 μM, the K_i values were calculated by applying the Cheng–Prusoff equation to the IC_{50} values (obtained by GraphPad®). Percent of binding displacement at the maximum concentration tested is also reported. Δ^9-THC and WIN 55212-2, tested under the same experimental conditions, are shown as reference compound. When applicable, data are reported as means ± *SD* of three experiments.

Sample	IC_{50}, CB_1	K_i, CB_1	Max effect at CB_1 (% displacement)	IC_{50}, CB_2	K_i, CB_2	Max effect at CB_2 (% displacement)
7,8-Dihydrokavain	>10 μM	>10 μM	10 μM (23.63 ± 3.21%)	>10 μM	>10 μM	10 μM (8.16 ± 3.21%)
7,8-Dihydromethysticin	>10 μM	>10 μM	10 μM (20.13 ± 2.15%)	>10 μM	>10 μM	10 μM (12.58 ±6.11%)
D,L-Kavain	>10 μM	>10 μM	10 μM (14.10 ± 4.13%)	>10 μM	>10 μM	10 μM (16.14 ± 1.34%)
Methysticin	>10 μM	>10 μM	10 μM (29.09 ± 8.22%)	>10 μM	>10 μM	10 μM (9.55 ± 2.21%)
Yangonin	1.79 ± 0.53 μM	0.72 ± 0.21 μM	25 μM (98.40 ± 5.63%)	>10 μM	>10 μM	10 μM (38.24 ± 12.34%)
1a	>10 μM	>10 μM	10 μM (8.20 ± 4.17%)	>10 μM	>10 μM	10 μM (4.04 ± 2.21%)
1b	>10 μM	>10 μM	10 μM (16.40 ± 3.33%)	>10 μM	>10 μM	10 μM (45.40 ± 5.46%)
1c	>10 μM	>10 μM	10 μM (0 ± 1.22%)	>10 μM	>10 μM	10 μM (19.50 ± 4.17%)
1d	>10 μM	>10 μM	10 μM (10.36 ± 3.84%)	>10 μM	>10 μM	10 μM (25.67 ± 3.87%)
2a	>10 μM	>10 μM	10 μM (20.47 ± 5.65%)	>10 μM	>10 μM	10 μM (3.49 ± 0.84%)
2b	>10 μM	>10 μM	10 μM (2.90 ± 0.82%)	>10 μM	>10 μM	10 μM (6.60 ± 2.26%)
2c	>10 μM	>10 μM	10 μM (8.20 ± 2.41%)	>10 μM	>10 μM	10 μM (0 ± 0.52%)
3a	>10 μM	>10 μM	10 μM (16.47 ± 2.41%)	>10 μM	>10 μM	10 μM (8.82 ± 4.11%)
3b	>10 μM	>10 μM	10 μM (2.30 ± 1.11%)	>10 μM	>10 μM	10 μM (13.15 ± 3.52%)
Δ^9-THC	–	0.0041 μM		–	0.0089 μM	

showed selectivity for the CB_1 receptor with a K_i = 0.72 μM; its affinity for the CB_2 receptor was significantly lower (K_i > 10 μM), although a 38.2% inhibition of specific binding was noted at 10 μM, the highest concentration tested (Fig. 3). The other compounds were much weaker ligands at both receptors, their displacement of the radioligand being always lower than 50% at 10 μM, the highest concentration tested (Table 1). However, some compounds, **1b** and **1d** in particular, did produce significant displacement of radioligand from CB_2 but not CB_1, receptors (Table 1), thus raising the possibility that also selective CB_2 ligands might be developed in the future from the further chemical modification of these molecules. THC and WIN 55212-2 produced the expected displacement in the binding experiments (Fig. 3).

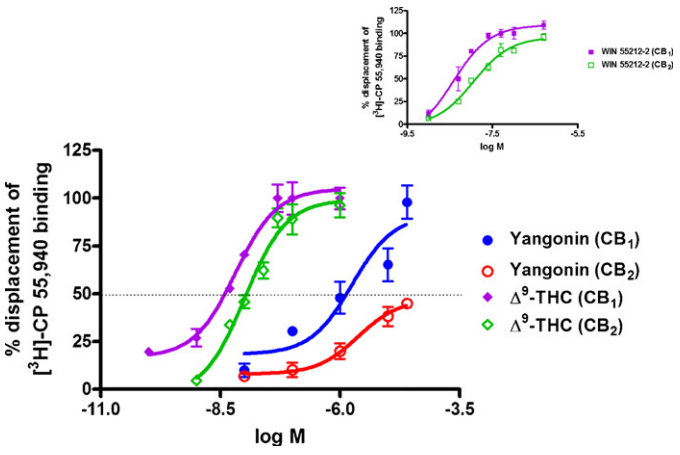

Fig. 3. Displacement curves of yangonin and the reference compound, THC. Also the displacement curves of WIN 55212-2, used for the determination of non-specific binding, are shown. Data are reported as mean ± SE of three experiments.

3.2. Inhibition of FAAH and MAGL activities

All compounds were examined for their inhibitory activity towards FAAH- and MAGL-like activities. As shown in Table 2, all compounds tested were very weak inhibitors of the two enzymes, with IC_{50} values >10 μM. However, for the natural-derived kavalactones we observed a distinct activity depending on the enzyme analyzed. In fact, except for dihydrokavain, all the molecules were found to weakly inhibit FAAH and, in contrast, to weakly stimulate MAGL activity at the highest concentration tested (10 μM) (Table 2).

4. Discussion

Based on some of the reported pharmacological actions of kava drink, we have studied here the effects of five kava-derived compounds and nine related synthetic compounds as direct or indirect ligands of cannabinoid receptors. We report that one of the kava constituents, yangonin, is a relatively good ligand of cannabinoid CB_1 receptors, although weaker than THC.

Previous studies have only partially clarified the potentially mode of the anxiolytic, sedative and other centrally mediated or peripheral actions of kava drink. Kavalactones, generally accepted as the main bioactive ingredients of this preparation, bind in vitro to several target sites with various affinities and elicit a wide array of physiological and behavioral effects in vivo that could account for the properties of various kava preparations with

Table 2
Effect of five natural kavalactones and nine synthetic derivatives on the two major catabolic enzymes involved in endocannabinoid degradation. The activity, expressed as IC_{50}, was determined by non-linear regression of the inhibition of total activity obtained incubating the enzyme in absence of compounds. Positive signs preceding the values indicate a stimulation of the total enzymatic activity. When applicable, data are reported as means ± *SD* of three experiments.

Sample	IC_{50}, FAAH	Max effect, FAAH (% inhibition)	IC_{50}, MAGL	Max effect, MAGL (% inhibition)
7,8-Dihydrokavain	>10 μM	10 μM (+1.80 ± 0.12%)	>10 μM	10 μM (+1.91 ± 0.25%)
7,8-Dihydromethysticin	>10 μM	10 μM (22.20 ± 6.33%)	>10 μM	10 μM (+3.25 ± 0.89%)
D,L-Kavain	>10 μM	10 μM (15.64 ± 4.25%)	>10 μM	10 μM (+12.12 ± 2.36%)
Methysticin	>10 μM	10 μM (16.37 ± 7.32%)	>10 μM	10 μM (+13.82 ± 4.56%)
Yangonin	>10 μM	10 μM (28.34 ± 4.41%)	>10 μM	10 μM (+17.39 ± 4.43%)
1a	>10 μM	10 μM (5.40 ± 1.34%)	>10 μM	10 μM (15.59 ± 4.81%)
1b	>10 μM	10 μM (6.63 ± 2.22%)	>10 μM	10 μM (20.28 ± 2.45%)
1c	>10 μM	10 μM (1.40 ± 0.23%)	>10 μM	10 μM (17.82 ± 3.15%)
1d	>10 μM	10 μM (1.91 ± 0.81%)	>10 μM	10 μM (16.88 ± 1.39%)
2a	>10 μM	10 μM (4.55 ± 0.33%)	>10 μM	10 μM (23.21 ± 3.56%)
2b	>10 μM	10 μM (5.28 ± 3.01%)	>10 μM	10 μM (17.47 ± 4.73%)
2c	>10 μM	10 μM (1.85 ± 0.81%)	>10 μM	10 μM (16.06 ± 0.97%)
3a	>10 μM	10 μM (15.29 ± 1.23%)	>10 μM	10 μM (25.20 ± 6.10%)
3b	>10 μM	10 μM (15.18 ± 4.05%)	>10 μM	10 μM (0 ± 0.96%)

different chemical compositions. The anxiolytic effect of kavalactones is thought to be due to modulation of GABA receptors. However, the results of receptor binding experiments with different kava-extracts provided mixed results: both enhancement and inhibition of [^{3}H]muscimol-binding were noted [19,20]. Nonetheless, pure kavalactones weakly but significantly enhanced in vitro the GABAergic response to [^{3}H]bicuculline methochloride, with kavain being the most active (28% enhancement at 0.1 μM) [21]. Animal studies confirmed the GABAergic contribution to the anxiolytic properties of kava. For example, in a recent mouse behavioral test, the anxiolytic and sedative profile of a kava extract (49.3% total kavalactone content) was similar to the $GABA_A$ receptor modulator diazepam, albeit the natural product was several hundred-fold less effective [22]. In a discriminative stimulus paradigm study in rats, a root extract rich in kavain (41%) partially substituted for chlordiazepoxide, the training anxiolytic drug [23]. Binding studies and electrophysiological experiments suggested the involvement of voltage-gated cation channels in kavalactone pharmacology [4]. For example, the binding of the Na^+ channel selective [^{3}H]batrachotoxinin-A 20-α-benzoate to rat brain synaptosomal preparation was inhibited by kavalactones with (+)-kavain being the most active (IC_{50} = 52.6 μM), [24]. The antiseizure effects of kava extracts and individual kavalactones observed against electroshock- or strychnine-induced convulsions in animals and in vitro models appear to be mediated by cation channel inhibition [4,25]. Blockade of Na^+ ion channels could account for the local anesthetic effect of kava. The psychoactivity of kavalactones has also been related to their effects on monoamine levels in the central nervous system, including the mesolimbic reward system. The extent and nature of these interactions, however, appear to be complex and depend on the dosage of the actual chemical as well as the brain region examined [26]. Kava preparations were also shown to interfere with monoamine metabolism by inhibiting human platelet monoamine oxidase-B with IC_{50} values of 24.0, 39.5 and 28.1 μM, for kava extract, desmethoxyyangonin and (±)-methysticin, respectively; (±)-kavain was inactive in this assay [27]. Finally, studies on the effects of kavalactones on arachidonic acid metabolism in vitro indicated that (+)-kavain inhibited arachidonic acid-induced platelet aggregation, ATP-release, and the synthesis of prostaglandin E_2 and thromboxane A_2 with IC_{50} values of 78, 115, 86 and 71 μM, respectively [28]. Another study on the effect of kavalactones on cyclooxygenase (COX) activity showed that the most active COX-1 inhibitor was dihydrokavain (58% inhibition at 430 μM), whereas the most active COX-2 inhibitor was yangonin (34% inhibition at 387 μM) [29].

In summary, the various psychoactive and physiological effects of kavalactones, alone or in combination, are complex and not fully understood. From in vitro studies, the $GABA_A$ receptor, affected by sub-micromolar to low micromolar concentrations of kavalactones, particularly kavain, methysticin and dihydromethysticin, appears to be the most sensitive pharmacological target, whereas effects on voltage-gated cation channels and monoamine and eicosanoid levels in vitro are observed at much higher concentrations, and in vivo could be due to interactions with other signaling systems. For these reasons, we investigated here the possibility that these compounds, and some of their synthetic derivatives, interact with cannabinoid receptors.

Of all the kavalactones and analogues examined in the current study, yangonin emerged as the most interesting compound. As reported in Table 1, the binding affinity of this kavalactone to the CB_1 receptor is measurable (K_i = 0.72 μM), being ~170-fold lower than that of THC, the main psychoactive constituent of Cannabis (K_i = 0.0041 μM). Nevertheless, its interaction with the endocannabinoid system could be pharmacologically relevant and contribute to the behavioral and physiological effects of kava. Indeed, the K_i of yangonin is commensurate with the reported value of 1.0 μM as the concentration of this kavalactone necessary to produce maximal enhancement of bicuculline methochloride binding to $GABA_A$ receptor complex [21]. However, further studies are

needed to establish the functional, i.e. agonistic or antagonistic, nature of the interaction of yangonin with the CB_1 receptor. Surprisingly, all compounds were nearly inactive at inhibiting FAAH and MAGL activities, the two major catabolic enzymes involved in endocannabinoid degradation. FAAH and MAGL are typical serine-hydrolase presenting slight differences in their catalytic mechanism. In fact, while AEA hydrolysis is activated by a lysine residue, which acts as base [30], the hydrolysis of 2-AG is facilitated by proximal cysteine amino acids, as demonstrated by crystal structure studies [31]. Accordingly, lipophilic sulfhydryl-reagents such as maleimides [32,33] and isothiazolinones [34], as well as disulfiram [35], are effective inhibitors of this enzyme. Recently, an analogue of the known β-lactone serine hydrolase inhibitor tetrahydrolipstatin was also found to inhibit MAGL in vitro and to possess antinociceptive activity in vivo [36]. On the other hand, the detoxification of kavalactones in humans is postulated to involve an electrophilic opening of the lactone ring by the sulfhydryl group of glutathione (GSH) [37,38]. In fact, the observed hepatotoxicity of commercial kava extracts has been explained by the lack of plant-derived GSH in such preparations. Whether the reactivity of kavalactones towards critical sulfhydryl groups accounts for their slight enhancement of the MAGL-catalysed hydrolysis of 2-AG, observed here, requires further investigation. During the assay, such a reaction, if reversible, could protect the enzyme from partial oxidative inactivation by contaminants in the assay buffer and cell homogenate. Such a protective mechanism, involving redox-sensitive cysteine thiol groups, has recently been proposed for kavalactones [39].

One interesting issue is the question of whether or not the in vitro micromolar concentrations at which yangonin interacts with CB_1, determined here, might be relevant in vivo. Among kavalactones, only the human pharmacokinetics of kavain has been studied so far [40]. One and four hours after oral administration of a single dose of 800 mg (±)-kavain, the blood serum concentration of this compound was 0.043 μM and 0.17 μM, respectively. However, the effective total daily dose reported from clinical trials with commercial kava extract formulations is 60–240 mg of kavalactones [11]. On the other hand, traditional kava drinkers typically consume 0.1–0.5 l of the beverage, which is made by mixing ~150 g dried kava root with 1 l water, to afford approximately 1 l of kava drink. Assuming that the total kavalactone-content in the root is 10% and the six major kavalactones are present in equal amounts, 250–1250 mg yangonin might be ingested orally during a "kava session". This dose could provide serum concentrations of yangonin high enough to affect the CB_1 receptor in the central nervous system and, as a result, contribute to the overall psychopharmacological profile of the kava drink.

Apart from phytocannabinoids from *C. sativa*, there are only a few exogenous regulators of the endocannabinoid system of natural origin [41]. A skin irritant polyyne fatty alcohol, falcarinol, was recently found to be a covalent antagonist of the human CB_1 receptor (K_i = 0.594 μM) [42]. Lipophilic isobutylamides from *Echinacea purpurea* (L.) Moench, and *Echinacea angustifolia* DC, both widely used herbal medicines, were shown bind to the human CB_2 receptor (K_i ~ 0.060 μM) [43]. The sesquiterpene β-caryophyllene, present in many spices and food plants, and also in the essential oil of *C. sativa*, is an agonist of the human CB_2 receptor (K_i = 0.155 μM) [44]. Furthermore, a quinonemethide triterpene, pristimerin was shown to be an inhibitor of MAGL from rat brain (K_i = 0.093 μM) [45]. Recent studies have also revealed that several naturally occurring (iso)flavones, including kaempferol, biochanin A, daidzein and genistein, inhibit rodent and human FAAH preparations with IC_{50} values at micromolar concentrations [46,47]. Remarkably, yangonin, which possesses an extensive conjugated double bond system, bears little structural resemblance to these diverse "phytocannabinoids".

5. Conclusions

In conclusion, through the screening of five major natural kavalactones and nine synthetic derivatives on cannabinoid receptors and endocannabinoid metabolic enzymes, we identified yangonin as a unique and selective, though not very potent, CB_1 receptor ligand. The sub-micromolar affinity concentration determined here is within the range found for the $GABA_A$ affinity of some of the other kavalactones. The significance of our results is twofold. First, the CB_1 receptor affinity of yangonin might indicate the involvement of the endocannabinoid system in the complex human psychopharmacology of the traditional kava drink and also in the anxiolytic preparations obtained from the kava plant. Indeed, if at all due to CB_1-mediated effects, these anxiolytic effects would indicate for yangonin an agonist action, since CB_1 inverse agonists/antagonists such as rimonabant and taranabant have been shown to cause anxiogenic effects in humans [16]. Secondly, yangonin provides a novel, versatile and synthetically accessible structural scaffold for the design of new cannabinoid receptor ligands. Further pharmacological, including functional and behavioral, studies on the cannabimimetic activity of yangonin and other kavalactones as well as with a larger number of synthetic analogues, are thus warranted.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phrs.2012.04.003.

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