Kavalactone Pharmacophores for Major Cellular Drug Targets

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Abstract: A number of studies have identified differential kavalactone activity against a variety of molecular targets, including P-glycoprotein (Pgp), platelet monoamine oxidase (MAO-B), transcription factor binding domains, pregnane X (PXR) and GABA receptors, and cytochrome P450 and cyclo-oxygenase (COX) enzymes. The molecular structure of the kavalactones possesses a pharmacophore for several of these targets. In most cases, conformational stability is more significant than the substituents present. The analysis of these pharmacophores provides important insights for future medicinal chemistry-based approaches to kavalactone-type drugs.

Keywords: Kavalactone, pharmacophore, molecular targets, conformational isomerism.

INTRODUCTION

Kava (*Piper methysticum*) is a native plant traditionally used as an intoxicating beverage by the indigenous people of the South Pacific [1]. Kava products have been used medicinally for a range of conditions including anxiety, stress and insomnia [2]. The beverage or extract from the rhizome of the plant has pharmacokinetic and pharmacodynamic interactions with drugs as diverse as alcohol, caffeine, benzodiazepines and catecholamines [3] and kavalactones are believed to be the main active components responsible for these effects [4]. Further studies with purified kavalactones have reported differential effects on various molecular targets, enabling identification of the responsible pharmacophores. We review here these studies and the relationship between kavalactone structure and activity.

P-Glycoprotein (Pgp)

P-glycoprotein (Pgp) is a 170 kDa ATP-activated, phosphorylated glycoprotein responsible for transporting substrates across cell membranes [5]. The ability of kavalactones to inhibit Pgp activity was measured using a fluorescent calcein uptake assay [6]. In this assay, inhibition of Pgp leads to an increased intracellular accumulation of calcein. All kavalactones showed a moderate to potent effect on the intracellular accumulation of calcein and can be divided into three distinct groups (see Fig. (**1**) for structures) based on their f_2 values (the concentration needed to double baseline fluorescence); kavain/dihydrokavain (88.1 ± 25.2 and 88.6 ± 13.9 μM), methysticin/dihydromethysticin (47.5 ± 17.1 and 54.6 ± 10.1 μM), desmethoxyyangonin (17.1 ± 0.6 μM). The f_2 value of yangonin could not be determined due to its lipophilicity, but compared with desmethoxyyangonin, the inhibition curve was shifted to the left. Thus the inhibitory potency of yangonin is comparable to or even greater than that of desmethoxyyangonin.

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Monoamine Oxidase Type B (MAO-B)

Monoamine oxidase type A and type B (MAO-A and MAO-B) catalyse the oxidative deamination of monoamines in neurotransmitters (endogenous) and in the diet (exogenous). MAO-A preferentially degrades serotonin and norepinephrine, while MAO-B preferentially degrades phenylethylamines and benzilamines. Dopamine is a substrate for both forms [7]. One study examining the effects of kavalactones on platelet monoamine oxidase (MAO-B) found that the order of potency for inhibition was; desmethoxyyangonin > (+/-)-methysticin > yangonin > (+/-)-dihydromethysticin > (+/-)- dihydrokavain > (+/-)-kavain [8]. The IC_{50} (μM) in intact and disrupted platelets for desmethoxyyangonin was 28.1 ± 12.9 and 0.12 ± 0.02, respectively. For kavain, these values were >400 and 40.5 ± 10.6 μM respectively.

Cyclo-Oxygenase Enzymes (COX)

Cyclo-oxygenase enzymes convert arachidonic acid into prostaglandins [9]. The effect of kavalactones on COX-1 and COX-2 isoenzymes was determined by measuring the rate of oxygen uptake in a cyclo-oxygenase inhibitory assay [10]. Dihydrokavain showed the highest COX-1 inhibitory activity (approx. 58%) and yangonin showed the highest COX-2 inhibitory activity (approx. 34%). The minimum kavalactone inhibition for each COX enzyme was approx. 25%. Three non-steroidal anti-inflammatory drugs (NSAIDs) were included as positive controls. Naproxen was the most effective in each case, resulting in approx. 32% inhibition of COX-1 and approx. 28% inhibition of COX-2. Kavalactones are thus reasonable inhibitors of cyclo-oxygenase enzymes.

Transcription Factors

Kavalactones have very specific effects on transcription factors. Nuclear factor κB (NF-κB) is an inducible transcription factor which regulates expression of over 200 genes involved in immunity, inflammation and cell growth [11]. In an *in vitro* luciferase-based assay for NF-κB activity, the IC_{50} for methysticin (0.19 ± 0.01 μg/ml = 0.69 μM) was over 100-fold less than that for dihydromethysticin (20 ± 3 μg/ml = 72.4 μM) [12]. The least active kavalactone was dihydrokavain, with an IC_{50} of 60 ± 8 μg/ml (258 μM).

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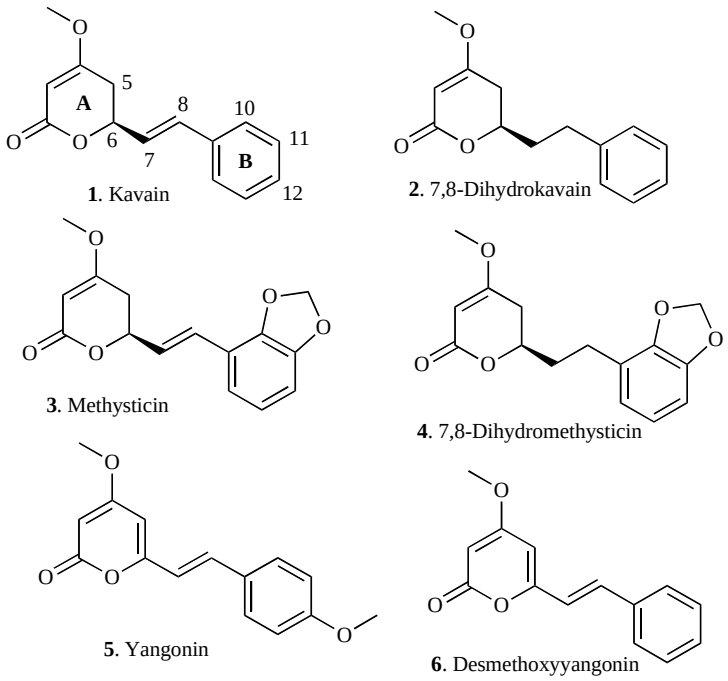

Fig. (1). Structures of the six main kavalactones. The carbon positions and the rings are labeled for kavain. The chemical formulae are kavain ($C_{14}H_{14}O_3$), 7,8-dihydrokavain ($C_{14}H_{16}O_3$), methysticin ($C_{15}H_{14}O_5$), 7,8-dihydromethysticin ($C_{15}H_{16}O_5$), yangonin ($C_{15}H_{14}O_4$) and desmethoxyyangonin (= 5,6-dehydrokavain = $C_{14}H_{12}O_3$). Compounds 1 to 4 have enantiomers. Compounds 5 and 6 are achiral.

Tumour necrosis factor α (TNF-α) is an inflammatory cytokine. Both kavain and dihydrokavain reduced TNFα secretion in a lipopolysaccharide-stimulated human acute monocytic leukaemia derived cell line (THP-1) by at least ten-fold compared to methysticin and dihydromethysticin, demonstrating a differential effect on the transcription factor lipopolysaccharide-induced TNFα activating factor (LITAF) [13].

Cytochrome P450 Superfamily and Pregnane X Receptor (PXR)

Cytochrome P450 enzymes are membrane-bound proteins found in the endoplasmic reticulum of hepatocytes and gastrointestinal mucosal cells. There is disagreement between different models, species and preparations as to which cytochrome P450 enzymes are inhibited and which are induced by kava [14]. Five individual enzymes within the cytochrome P450 superfamily of enzymes (containing over 50 families) are responsible for the metabolism of more than 90% of drugs - CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 [15]. CYP3A4 is the most abundant enzyme, and it is involved in the metabolism of approximately 50% to 60% of drugs. One study found that kava induces CYP3A4 [16]. The most common means of cytochrome P450 induction appears to involve the orphan nuclear receptors such as pregnane X receptors (PXR) and constitutive androstane receptors [17]. Activation of PXR and induction of CYP3A23 was measured in rat hepatocytes treated with desmethoxyyangonin, dihydrokavain, dihydromethysticin, kavain, methysticin, or yangonin [18]. Desmethoxyyangonin, dihydromethysticin and pregnenolone 16α-carbonitrile (PCN) induced the expression of CYP3A23 approximately seven-fold. Inhibition of mRNA synthesis abolished this induction suggesting that these agents act by increasing the levels of CYP3A23 mRNA. PXR activity induced by dihydromethysticin and desmethoxyyangonin was only around 10% of that induced by PCN when used at concentrations which elicited similar induction of CYPA23, suggesting that PXR plays a limited role in CYP3A23 induction in response to dihydromethysticin and desmethoxyyangonin.

γ-Aminobutyric Acid (GABA) Receptors

γ-Aminobutyric acid is the chief inhibitory neurotransmitter in the mammalian central nervous system [19]. *In vitro* and *in vivo* studies indicate that the pharmacological activities of kavalactones are not due to a direct effect on GABA receptors. When yangonin, desmethoxyyangonin, kavain, dihydrokavain, methysticin and dihydromethysticin were tested for competitive activity against GABA on binding sites in rat and mouse brain membranes, there was a 22-26% decrease in binding of GABA at forebrain $GABA_a$ sites for dihydromethysticin, yangonin and tetrahydroyangonin at 1 mM concentrations [20]. However, these effects disappeared following lipid extraction of the membranes, despite an increase in specific GABA binding. Any pharmacological effects of kavalactones on GABA receptors may thus be due to remodeling of endogenous lipids surrounding the receptor, rather than direct interactions with the receptor. This explanation is supported by the lipophilicity of the kavalactones. These observations are consistent with a study in mice which showed that flumazenil, a competitive benzodiazepine receptor antagonist, blocked both the anxiolytic and sedative effects of diazepam but did not block behavioural effects caused by kava.

DISCUSSION

Published experimental data from these studies suggests links between kavalactone structure, conformation and bio-

Table 1. Differential Activity of Kavalactones for Molecular Drug Targets

Target	Assay	Activity	Ref
P-glycoprotein (Pgp)	Fluorescent calcein uptake assay (f_2 values)	Desmethoxyyangonin (17.1 ± 0.6 μM) /yangonin > methysticin /dihydromethysticin (47.5 ± 17.1 and 54.6 ± 10.1 μM) > kavain/dihydrokavain (88.1 ± 25.2 and 88.6 ± 13.9 μM)	[6]
Monoamine oxidase type B (MAO-B)	Inhibition of 2-phenylethylamine-[ethyl-1-^{14}C] hydrochloride (PEA) solution in intact/disrupted platelets (IC_{50} μM)	Desmethoxyyangonin (28.1 ± 12.9 / 0.12 ± 0.02 μM) > methysticin (39.5 ± 17.9 / 0.67 ± 0.12 μM) > yangonin > dihydromethysticin > dihydrokavain > kavain (>400 / 40.5 ± 10.6 μM)	[8]
Cyclo-oxygenase 1 (COX-1)	Cyclo-oxygenase inhibition assay (% inhibition)	Dihydrokavain (58%) > methysticin (42%) > desmethoxyyangonin (39%) > yangonin (36%) > kavain (34%) > dihydromethysticin (25%)	[10]
Cyclo-oxygenase 2 (COX-2)	Cyclo-oxygenase inhibition assay (% inhibition)	Yangonin (34%) > dihydromethysticin (32%) > dihydrokavain (28%) > methysticin (26%) > kavain (25%) > desmethoxyyangonin (23%)	[10]
Nuclear factor κB (NF- κB)	Luciferase reporter gene assay (IC_{50} μM)	Methysticin (0.69 ± 0.04 μM) > dihydromethysticin (72.4 ± 10.7 μM) > kavain (139 ± 18.9 μM) > desmethoxyyangonin (144.6 ± 30.7 μM) > dihydrokavain (258.3 ± 30 μM)	[12]
Lipopolysaccharide-induced TNFα activating factor (LITAF)	Lipopolysaccharide-induced TNFα activating factor (LITAF) inhibition (% inhibition)	Kavain (97.3%) > dihydrokavain (94.6%) > methysticin (35.7%) > dihydromethysticin (34.8%)	[13]

activity. Table **1** and Fig. (**2**) summarise the activity of the kavalactones against the molecular targets discussed.

A locked molecular configuration, through extended conjugation, appears to be essential for the kavalactone inhibition of P-glycoprotein. The dienolide desmethoxyyangonin was the most active kavalactone. The kavalactones with the least activity were the enolide kavain and its dihydro derivative, 7,8-dihydrokavain. The only chemical difference between desmethoxyyangonin and kavain is the lack of the double bond between carbon 5 and 6 in ring A of kavain - Fig. (**1**). The presence of a stereogenic centre (chiral carbon) at position 6 of kavain, due to the reduced carbon-carbon double bond, disrupts the bioactivity of the pharmacophore. Given the almost identical activities of kavain and dihydrokavain, the reduction of the cinnamyl chain appears to have negligible effect on activity.

The only chemical difference between kavain and methysticin is the presence of the methylenedioxy (ketal) group on ring B. Since methysticin, and its dihydro derivative, 7,8-dihydromethysticin, have activity intermediate between that of desmethoxyyangonin and kavain/dihydrokavain, this ketal group may partially compensate for the lack of the double bond between carbon 5 and carbon 6 in ring A.

For monoamine oxidase B effects, a locked molecular conformation may be even more important for bioactivity. The increase in potency of desmethoxyyangonin compared to kavain against Pgp was around five times, whereas for MAO-B it was one to two orders of magnitude. This difference may be partially due to the use of a racemic mixture in the MAO-B study, whereas it was not specified whether the pure enantiomer was used in the Pgp study. This is a limitation which makes it more difficult to make conclusive comparisons between these studies.

Dihydrokavain was clearly the most inhibitory kavalactone against COX-1, causing approximately 50% more inhibition than the next most potent kavalactone (methysticin). It was almost 100% more potent than kavain. This comparison with kavain suggests that conformational flexibility at the carbon 7 to 8 bond is important for bioactivity. These experiments used only the (+)-enantiomers so a specific enantiomeric conformation may also be important. Conformational flexibility may be less important for COX-2 since the fixed configuration of yangonin and the flexible structure of dihydromethysticin each led to similar levels of inhibition.

The IC_{50} of methysticin for NF-κB binding was over 100-fold less than that for dihydromethysticin. As the only structural difference between these molecules is the reduction of the cinnamyl side-chain on the enolide methysticin to give dihydromethysticin, the rigid configuration provided by the double bond between carbon 7 and 8 appears to be very important for bioactivity in this interaction. Once again, only the (+)-enantiomers were used in this study, so stereospecific effects may also be important.

The pharmacophore responsible for kavalactone inhibition of TNF-α expression/LITAF binding appears to include the functional groups on the A ring, while the addition of the ketal group to ring B of methysticin and dihydromethysticin probably interferes with the docking of these agents. The activity of the enolides, kavain and methysticin, is not substantially affected by the reduction of the cinnamyl chain to dihydrokavain and dihydromethysticin, implying that kavalactone interaction with LITAF is not dependent upon conformational isomerism.

Not all experiments showing differential kavalactone activity towards a specific molecular target aid identification of a pharmacophore. In some cases, the actions of kavalactones

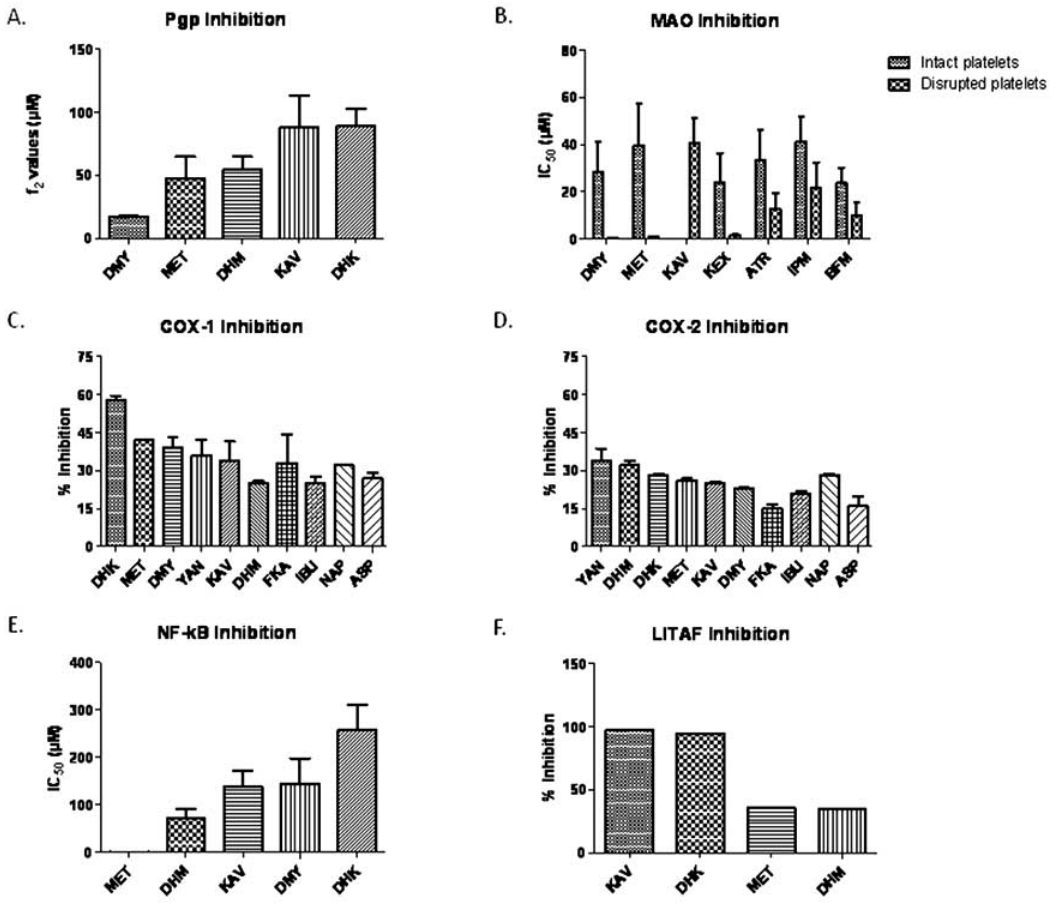

Fig. (2). Differential activity of kavalactones for molecular drug targets.

A. Pgp Inhibition - f_2 values are the concentration needed to double baseline fluorescence in a fluorescent calcein uptake assay. B. MAO Inhibition - KAV IC_{50} for intact platelets was beyond the scale of the graph (>400 μM). C. COX-1 Inhibition. D. COX-2 Inhibition. E. NF-κB Inhibition. F. LITAF Inhibition - No error bars were available.

Error bars are standard deviation. Reference molecules used in the original studies are also graphed.

ATR = Amitryptiline, ASP = Aspirin, BFM = Brofaromine, DMY = Desmethoxyyangonin, DHK = Dihydrokavain, DHM = Dihydromethysticin, FKA = Flavokavain A, IBU = Ibuprofen, IPM = Imipramine, KEX = Kava extract, KAV = Kavain, MET = Methysticin, NAP = Naproxen, YAN = Yangonin

were either minimal (pregnane X receptor), equivocal (cytochrome P450 enzymes) or non-specific (GABA receptors).

In conclusion, the conformational isomerism or configurational rigidity of the kavalactones is the most important feature in most of these molecular interactions, with chemical structure more important in LITAF binding and possibly COX-2. Using this information, simple chemical manipulations of the kavalactone structure could enable improvements in the selectivity of kavalactone-type drugs for particular cellular targets.

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