

Supporting Information

Identification and characterization of Kava-derived compounds mediating TNF- α suppression

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General compound synthesis and purification details. All reagents for chemical synthesis were purchased from Aldrich Chemical Company (St. Louis, MO) and were used as received. Chemical reactions were carried out under a nitrogen atmosphere and monitored by thin layer chromatography using Merck 60 F254 precoated silica gel plates (0.25 mm thickness). Analytical LC was performed on a Waters Acquity UPLC with PDA, ELS and SQ detectors. An Acquity UPLC BEH 2.1 x 50 mm 1.7 μ M C18 column was used for analytical LC. ^1H NMR spectra were recorded at 400 MHz at ambient temperature with CDCl_3 as solvent unless otherwise stated. ^{13}C NMR spectra were recorded at 100.0 MHz at ambient temperature with CDCl_3 as solvent unless otherwise stated. Chemical shifts are reported in parts per million relative to CDCl_3 (^1H , δ 7.24; ^{13}C , δ 77.0). Data for ^1H NMR are reported as follows: chemical shift, integration, multiplicity (app = apparent, par obsc = partially obscure, ovrlp = overlapping, s = singlet, d = doublet, t = triplet, q = quartet, qt = quintuplet, m = multiplet) and coupling constants are reported as values in hertz. All ^{13}C NMR spectra were recorded with complete proton decoupling. High-resolution mass spectra were obtained in the Boston University Chemical Instrumentation Center using a Waters Q-TOF mass spectrometer. Flash chromatography was performed on using silica gel 60N of Kanto Chemical C. Int., Tokyo, Japan. Analytical LC was performed on a Waters Acquity UPLC with PDA, ELS and SQ detectors. An Acquity UPLC BEH 2.1 x 50 mm 1.7 μ M C18 column was used for analytical LC.

ADME/PK experiments. In vitro ADME and pharmackinetic analyses were performed at Apredica, Inc (Watertown, MA). Samples were analyzed by LC/MS/MS using either an Agilent 6410 mass spectrometer coupled with an Agilent 1200 HPLC and a CTC PAL chilled autosampler, all controlled by MassHunter software (Agilent), or an ABI2000 mass spectrometer coupled with an Agilent 1100 HPLC and a CTC PAL chilled autosampler, all controlled by Analyst software (ABI). After separation on a C18 reverse phase HPLC column (Agilent, Waters, or equivalent) using an acetonitrile-water gradient system, peaks were analyzed by mass spectrometry (MS) using ESI ionization in MRM mode.

Permeability assay: CaCo-2 cells grown in tissue culture flasks were trypsinized, suspended in medium, and the suspensions were applied to wells of a collagen-coated BioCoat Cell Environment in 24-well format (BD Biosciences) at 24,500 cells per well. The cells were allowed to grow and differentiate for three weeks, feeding at 2-day intervals. For Apical to Basolateral (A->B) permeability, the test agent was added to the apical (A) side and amount of permeation is determined on the basolateral (B) side; for Basolateral to Apical (B>A) permeability, the test agent was added to the B side and the amount of permeation is determined on the A side. The A-side buffer contained 100 PM Lucifer yellow dye, in Transport Buffer (1.98 g/L glucose in 10 mM HEPES, 1x Hank's Balanced Salt Solution) pH 6.5, and the B-side buffer was Transport Buffer, pH 7.4. CaCo-2 cells were incubated with these buffers for 2 h., and the receiver side buffer was removed for analysis by LC/MS/MS. To verify the CaCo-2 cell monolayers are properly formed, aliquots of the cell buffers were analyzed by fluorescence to determine the transport of the impermeable dye Lucifer Yellow.

Plasma stability screen, the test agent was incubated in duplicate with plasma at 37 °C. The reaction contains plasma and 2% DMSO. At the indicated times, an aliquot was removed from each experimental and control reaction and mixed with an three volumes of ice-cold Stop Solution (methanol containing haloperidol, diclofenac, or other internal standard). Stopped reactions were incubated at least ten minutes at -20 °C. The samples were centrifuged to remove precipitated protein, and the supernatants were analyzed by LC/MS/MS to quantitate the remaining parent. Data were converted to % remaining by dividing by the time zero concentration value.

PBS express solubility. Serial dilutions of test agent were prepared in test agent at 100x the final concentration. Test agent solutions were diluted 100-fold into PBS in a 96-well plate and mixed. The absorbance of the PBS-containing plate was measured prior to adding the test agents to determine the background absorbance. After 45 min and 16 hr, the presence of precipitate were then detected by

turbidity absorbance at 540 nm). An absorbance value of greater than (mean + 3x standard deviation of the blank), after subtracting the pre-experiment background, was indicative of turbidity. The solubility limit was reported as the highest experimental concentration with no evidence of turbidity.

Pharmacokinetics. Compounds were assessed for their pharmacokinetic properties in female CD-1 mice. The animals were housed five per cage in a single room, and supplied with water and a commercial rodent diet, ad libitum, for the duration of the study. Compounds were formulated in 5% ethanol/95% PBS buffer and dosed via tail vein (iv) or oral gavage (po). Blood samples were collected by cardiac puncture and placed into tubes containing EDTA as anticoagulant. The samples were centrifuged at 13000 rpm for 5 minutes at 4°C, and plasma was collected and stored at -80°C.

List of representative extracts and natural products from the GNC collection.

Ursodiol
Nisoldipine
Gotu Kola
Flax Seed Oil
Boswellia
Haloperidol
Salsalate
Zalcitabine
Acetyl-L-carnitine
Rutin
Levaquin
Cranberry
Damiana
Ginger
Lomotil
Sporanox
St. John's Wort
Alpha Lipoic Acid
Vitamin B-2
Kayexalate
Lindane
Vitamin B-6

Panthenic Acid
Natural E 100
Tranlycypromine sulfate
Potent Acidophilus
Cat's Claw
L. Carnitine
Nitrofurantoin
Prilosec (omeprazole)
Terbinafine
Chloroxine
(+/-)-alpha-tocopherol
Folic Acid
Calcium Complete
Glucosamine sulfate
Sodium cocoyl isethionate
Boron
Royal Jelly
Melatonin 3
Medrol
Phytonadione
Eupatorium perfoliatum
DHEA
Dandelion Root
Alfalfa
Mitotane
Crotamiton
Bentoquatam
Valerian Root
Taurine
Primrose oil
L. Glutamine
Black cohosh
Inosine
Fo-Ti
MSM
L-Tyrosine
Kava kava root
Feverfew
Chromium
Juniper
Creatine Monohydrate
Niacinamide
Shiitake Mushroom

Goldenseal Root
Siberian ginseng
Ashwagandha
L. Arginine
Vitamin D
Blue-Green Algae
Astragalus
Eyebright
Tomato lycopene
Kelp
Red clover
Cayenne
Cascara sagrada
Horse Chestnut
DHA
Milk Thistle
Butcher's broom
Mct
Kudzu root
Vitamin b-12
Borage oil
Phosphatidyl
Bromelain
Devil's Claw Root
Shark cartilage
Echinacea root
Psyllium seed husk
Lutein
Vitamin A
Iron
L-Phenylalanine
Vitamin B-1
Pyruvate fuel
Yucca
Horsetail
Biotin
Vitamin C
Vitex
Pau d'arco
Bilberry
L-ornithine
Guarana
Green tea

Diet fuel
Ephedra
Reishi Mushroom
Copper
Ginkgo Biloba
Aloe vera gel
Saw palmetto
Enada NADH
Odorless garlic
DMAE
Red Raspberry
Beta-Carotene
Chickweed
Elderberry extract
PABA
Chlorophyll
Brewer's yeast
Yohimbized
5-HTP
White Ginseng Root
Chondroitin Sulfate
Maitake mushroom
White willow
Nettle
Hops
Cell forte
Manganese
Grape seed
Octacosanol 2000 mcg
Marshmallow
Elecampane root
Yarrow flowers
Histidine complex
Mullein
Colostrum plus
L-glutamic acid 500mg
Lemon balm
Glycine
Quercetin +c
Acidophilus
Turmeric
Vitamin e-400
Yellow dock

Lutein
Pc-55
L-cysteine
Butcher's broom
L-carnitine
Vitamin b-12
Niacinamide 100
Vitamin b-1 300
L-arginine 500
Shark cartilage
White oak bark
Beta-carotene 15
Calcium complete
Ginkgo biloba
Feverfew
Pantothenic acid
Chromium 200
Chamomile
Gymnema sylvestre
Tonalin
Blue green algae
Nutraflora fos
Triple alfalfa
Gamma-oryzanol 200
Catnip
Lobelia
Aloe vera gel
Horehound
Bitter melon powder
Boron 3
MSM
White stevia
Biotin 300
Black cohosh
Schizandra
Vitamin a 100000
Vitamin c-500
Androstenedione
Artichoke extract
Boswellia
Avena sativa
PS 100
Folic acid

Copper
Natural e-100
Vitamin k
L- methionine 500 mg
Arnica montana 30x
Blue cohosh
Coltsfoot
Blueberry leaf
Barberry
Angelica
Tea tree oil
Chitosan
Brewer's yeast
Fenugreek
Isoflavone caps
Ashwagandha
Maitake mushroom
Devil's claw