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(54) Title: SIPHONOCILONE AND RELATED COMPOUNDS AND USES THEREOF

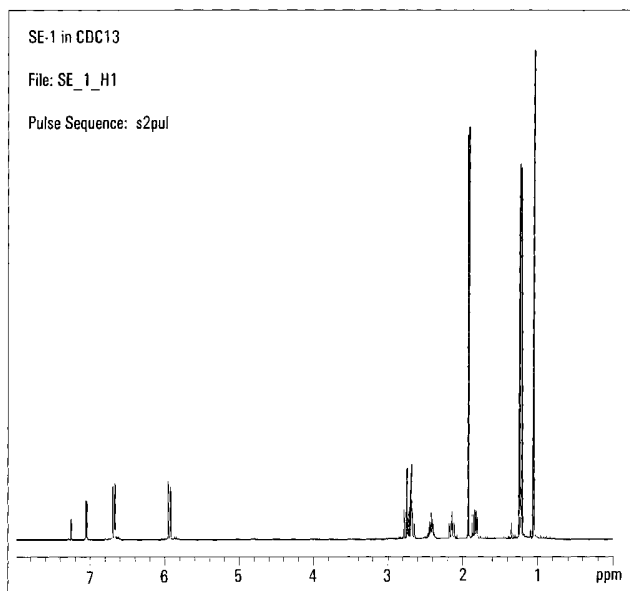


Figure 1  $^1\text{H}$  spectrum of the compound in  $\text{CDCl}_3$   
(referenced to residual solvent peak at 7.26ppm).

(57) Abstract: This invention relates to the compound (4 $\alpha$ H-3,5 $\alpha$ ,8 $\alpha$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one) known as siphonochilone, and related compounds, useful in mammals in supporting, promoting and maintaining health, and in the amelioration, prevention and treatment of central nervous system (CNS) disorders or conditions, unit dosages forms containing the compounds, delivery systems for the compounds, and methods for preparing the compounds. More specifically, it relates to plant extracts containing the compounds and uses thereof for the support, promotion and maintenance of health and the amelioration, prevention and treatment of such disorders or conditions. The invention also relates to methods of preparing a plant extract containing the compounds.



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**Declarations under Rule 4.17:**

- *as to the identity of the inventor (Rule 4.17(i))*
- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *of inventorship (Rule 4.17(iv))*

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- *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

## **SIPHONOCHILONE AND RELATED COMPOUNDS AND USES THEREOF**

### **FIELD OF INVENTION**

5 This invention relates to compounds useful in mammals in supporting, promoting and maintaining health, and in the amelioration, prevention and treatment of central nervous system (CNS) disorders or conditions, unit dosages forms containing the compounds, delivery systems for the compounds, and methods for preparing the compounds. More specifically, it relates to plant extracts containing the compounds and uses thereof for  
10 the support, promotion and maintenance of health and the amelioration, prevention and treatment of such disorders or conditions. The invention also relates to methods of preparing a plant extract containing the compounds.

### **BACKGROUND TO THE INVENTION**

15 Holzapfel et al. (Furanoterpenoids from *Siphonochilus aethiopicus*. Phytochemistry 59, 405-407, 2002) disclosed the first isolation and structural elucidation using NMR of two new furanoterpenoid derivatives from *Siphonochilus aethiopicus* of the family Zingiberaceae, namely, 4 $\alpha$ H-3,5 $\alpha$ ,8 $\alpha$  $\beta$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-  
20 8-one (known as siphonochilone), and 2-hydroxy-4 $\alpha$ H-3,5 $\alpha$ ,8 $\alpha$  $\beta$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one.

*Siphonochilus aethiopicus*, also known as African Ginger, is a member of the family Zingiberaceae, and has a mainly tropical, eastern and southern African distribution. The  
25 roots and rhizomes are known to be used as a spice to flavour food and in traditional herbal medicine for treating fevers, colds, 'flu, sinusitis, coughs, headache, asthma, malaria, hysteria, candida, epilepsy, menstrual cramps. The rhizomes of *Siphonochilus aethiopicus* have been reported in the scientific literature to have anti-inflammatory, anti-candidal, antibacterial and antifungal activities.

30 The use of compounds and extracts of *Siphonochilus aethiopicus* in the preparation of a medicament for use in the treatment or prophylaxis of allergic diseases, including

asthma and atopy, have been disclosed in patent applications (*Horak, 2009, US Patent Application 20090082433; and Horak, 2010, US Patent Application 20100168227*).

## DISCLOSURE OF THE INVENTION

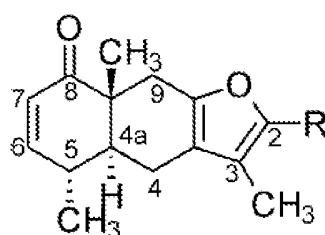
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The inventors have most surprisingly found that the use by ingestion, inhalation, or topical application of the furanoterpenoid (4 $\alpha$ H-3,5 $\alpha$ ,8 $\beta$ -trimethyl-4a,9-tetrahydronaphtho[2,3-b]-furan-8-one) known as siphonochilone, and related compounds, particularly the administration of specified low doses of the isolated pure compound

10 siphonochilone, has an effect on CNS related conditions or disorders. By way of example, the aforesaid use surprisingly results in rapid onset of calming, stress-relieving, sedative, hypnotic, and anxiolytic activities.

According to a first aspect of the invention, there is provided a compound of formula I:

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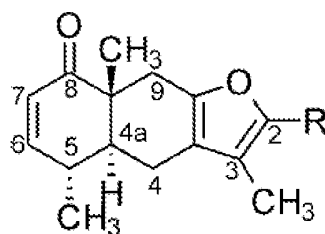


where R is selected from H, OH, OCH<sub>3</sub>, or mixtures thereof, for use as a medicament for ameliorating, treating or preventing a Central Nervous System (CNS) condition or disorder.

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According to the invention, there is also provided the use of a compound of formula I:



where R is selected from H,

10 OH, OCH<sub>3</sub>, or mixtures thereof, in the manufacture of a medicament for ameliorating, treating or preventing a Central Nervous System (CNS) condition or disorder.

In this specification the term “medicament” and “medicinal compositions” are intended to include functional foodstuffs and beverages, dietary supplements, botanical medicines,  
15 as well as pharmaceutical and cosmeceutical compositions.

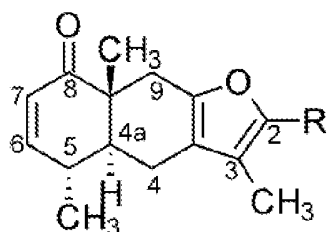
Preferably, the condition or disorder is selected from the group consisting of anxiety states, social phobia, panic attacks, obsessive compulsive disorder, insomnia, hyperactivity, impulsivity, agitation, seizures including febrile convulsions, psychosis,  
20 mania, aggression, nausea, vomiting, travel sickness, agitation, irritability during weight reduction programs, pre-menstrual syndrome (PMS), headache including tension headache, nervousness, agitation, irritability and insomnia in weight-reduction, nervousness, agitation, irritability and insomnia in alcohol and drug withdrawal, nervousness, agitation, irritability and insomnia in tobacco and nicotine withdrawal,  
25 nervousness, agitation, irritability and insomnia in tobacco and nicotine use, nervousness, agitation irritability and insomnia in caffeine use, agitation, nervousness, agitation irritability and insomnia from excess caffeine consumption, stress, post-traumatic stress, agitation anxiety and disruptive behaviour in dementia and Alzheimers disease, adjunctive treatment of pain that is exacerbated by anxiety and/or pain  
30 exacerbated by poor sleep, neuropathic pain, soothing, ameliorating and reducing the subjective experience of stress, supporting and maintaining a calm, composed, serene outlook, reducing nervous tension, nervous irritability, social discomfort; supporting and enhancing the onset and quality of sleep.

Thus, it will be appreciated that the invention, by ameliorating or preventing CNS conditions or disorders, in addition to being useful in treating specific disorders, finds substantial utility in the maintenance and promotion of health which results in the general enhancement, support and maintenance of well-being in individuals, and prevention of development of disease.

Thus, the medicament may be used as a replacement for *Piper methysticum* (Kava) extracts and fractions where the role of the *Piper methysticum* in the medicament is intended to promote and maintain health or treat or prevent a Central Nervous System (CNS) condition. Similarly, the medicament may be used as a replacement for extracts, fractions and isolated compounds from other CNS active natural products and botanicals including but not limited to melatonin, tryptophan, L-theanine, polyunsaturated fatty acids, and botanicals from the genera Avena, Ballota, Boswellia, Camellia, Cannabis, Catha, Centella, Chamaemelum, Chrysanthemum, Citrus, Coffea, Cola, Commiphora, Crocus, Curcuma, Cyclopia, Cymbopogon, Cytisus, Elettaria, Eleutherococcus, Ginkgo, Humulus, Hypericum, Ilex, Lavandula, Leonotis, Lobelia, Matricaria, Melissa, Mentha, Mesembryanthemum, Myristica, Nardostachys, Nepeta, Nicotiana, Ocimum, Origanum, Panax, Papaver, Passiflora, Piper, Rauvolfia, Rhodiola, Rosmarinus, Salvia, Sceletium, Scutellaria, Sutherlandia, Valeriana, Withania, Zizyphus, where the role of the botanical in the medicament is intended to promote and maintain health, and ameliorate, treat or prevent a Central Nervous System (CNS) condition.

The inventors have also surprisingly found, after extensive study of the binding of the compound of Formula 1 to 68 pharmacological targets in the presence of control radiolabelled receptor-specific ligands, that the compound of Formula 1 has marked selectivity and significant activity on the melatonin MT1 receptor in the presence of radiolabelled melatonin as reference compound.

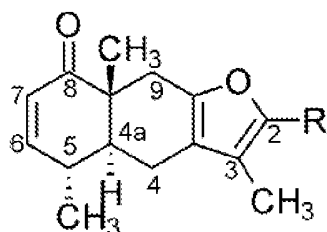
Thus, according to a further aspect of the invention, there is provided a compound of formula I:



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where R is selected from H, OH, OCH<sub>3</sub>, or mixtures thereof, for use as a medicament for ameliorating, treating or preventing a disease or condition that responds to treatment with a melatonin agonist.

15 There is also provided the use of a compound of formula I:



25 where R is selected from H, OH, OCH<sub>3</sub>, or mixtures thereof, in the manufacture of a medicament for use in ameliorating, treating or preventing a disease or condition that responds to treatment with a melatonin agonist.

The disease or condition may include disturbances in the onset, duration and quality of sleep, intrinsic sleep disorders including insomnia, extrinsic sleep disorders, circadian rhythm disorders (including time-zone change syndrome (jet lag), shift work sleep disorder, irregular sleep-wake pattern, delayed sleep phase syndrome, advanced sleep phase syndrome, non-24 hour sleep-wake syndrome), parasomnias, sleep disorder associated with internal medical or psychiatric disorders (including chronic obstructive pulmonary disease, Alzheimer's disease, Parkinson's disease, cerebrovascular

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dementia, schizophrenia, depression, anxiety), neurodegenerative diseases (including senile dementia, Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, spinocerebellar degeneration, multiple sclerosis (MS), psychoneurotic diseases (e.g., depression, anxiety, bipolar disorder, posttraumatic stress disorder (PTSD), seasonal affective disorder, schizophrenia), memory disorders (cognitive decline in the elderly, senile dementia), ischemic central nervous system disorders (including cerebral infarction, cerebral haemorrhage, brain oedema), central nervous system injury (including head trauma, spinal cord injury, whiplash injury), vascular dementia (including multi-infarct dementia), cancer (including brain tumour, pituitary adenoma, glioma, acoustic schwannoma, retinoblastoma, thyroid cancer, pharyngeal cancer, laryngeal cancer, cancer of the tongue, thymoma, mesothelial tumor, breast cancer, lung cancer, non-small cell lung cancer, small cell lung cancer, gastric cancer, esophageal cancer, duodenal cancer, colorectal cancer, colon cancer, rectal cancer, liver cancer, hepatocellular carcinoma, pancreatic cancer, pancreatic endocrine tumour, biliary tract cancer, gall bladder cancer, penile cancer, kidney cancer, renal pelvic cancer, ureteral cancer, renal cell cancer, testis tumour, prostate cancer, urinary bladder cancer, vulvar cancer, uterine cancer, cancer of uterine cervix, cancer of uterine body, uterine sarcoma, chorionic disease, vaginal cancer, ovary cancer, ovarian germ cell tumour, skin cancer, malignant melanoma, mycosis fungoides, basal cell tumour, soft tissue sarcoma, malignant lymphoma, Hodgkin's disease, osteomyelodysplasia syndrome, multiple myeloma, leukemia, acute myelocytic leukemia, chronic myelocytic leukemia, acute lymphatic leukemia, chronic lymphatic leukemia, adult T cell leukemia, chronic myeloproliferative disease, pancreatic endocrine tumor, fibrous histiocytoma, leiomyosarcoma, rhabdomyosarcoma, unknown primary cancer and the like), hyperinsulinemia, metabolic syndrome, obesity, diabetes, diabetic complications (e.g., diabetic retinopathy, diabetic neuropathy, diabetic nephropathy and the like), hypertriglyceridemia (hyperlipidemia), hypertension, circulatory disease (including ischemic cardiac diseases, arteriosclerosis), lower urinary tract disease or disorder (including dysuria, urinary incontinence), osteoporosis, reproductive and neuroendocrine diseases, convulsion, glaucoma, headache, and irritable bowel syndrome. In addition, melatonin agonists are effective for immunoregulation,



amelioration, prevention, and treatment of stress, for cognitive enhancement, and for tranquilization.

Therefore, the medicament as a CNS-active compound with CNS activity modulated by activity on the melatonin MT1 receptor may be used in compositions as a replacement for melatonin, and melatonin agonists, including agomelatin and ramelteon, in medicaments intended to maintain and promote health and ameliorate, treat or prevent a Central Nervous System (CNS) condition.

The medicament, as a CNS-active compound, including but not limited to CNS activity mediated by the melatonin MT1 receptor, may be used as a replacement for CNS active compounds in medicaments intended to ameliorate, treat or prevent Central Nervous System (CNS) conditions, including as a replacement for, but not limited to, GABA agonists, benzodiazepines, nicotine, cannabinoid CB<sub>2</sub> receptor agonists, antihistamines, dopamine antagonists, serotonin receptor agonists and antagonists, 5-HT uptake inhibitors, nor-adrenalin-uptake inhibitors, phosphodiesterase inhibitors, sigma receptor antagonists, glycine uptake inhibitors, glutamate inhibitors.

The compound may be derived from a plant extract or material of a plant of the family *Zingiberaceae*. Preferably, the plant extract or material is selected from the genera *Siphonochilus*, *Kaempferia*, *Cienkowskia* or *Cienkowskiella*, or mixtures thereof. Preferably, the plant extract or material is selected from the species *Siphonochilus aethiopicus*, *Siphonochilus kirkii*, *Siphonochilus natalensis*, *Siphonochilus nigericus*, *Kaempferia aethiopica*, *Kaempferia galangal*, *Kaempferia natalensis*, *Kaempferia ethelae*, *Kaempferia nigerica*, *Kaempferia rosea*, *Cienkowskia aethiopica* or *Cienkowskiella aethiopica*, or mixtures thereof. In a most preferred embodiment of the invention, the species is *Siphonochilus aethiopicus*.

Preferably, the compound used is the furanoterpenoid compound, siphonochilone, 4 $\alpha$ H-3,5 $\alpha$ ,8 $\alpha$  $\beta$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one (i.e. where R is H). The compound may be in the form of a racemic mixture or in the form of one of its stereoisomers, or analogues or metabolites thereof.

The medicament may be in a unit dosage form containing from about 1 microgram to about 50 milligrams, preferably from about 10 micrograms and to about 10 milligrams of the compound. The dose is desirably prepared for administration about 1 to 3 times a day according to the symptoms.

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The applicant has most surprisingly found that low doses of the isolated pure compound, furanoterpenoid 4a $\alpha$ H-3,5 $\alpha$ ,8a $\beta$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one, results in rapid onset of calming, stress-relieving, sedative, hypnotic, and anxiolytic activities, with a dose-response whereby low servings or doses have subtle activity that can be usefully applied in functional food, functional beverage, and cosmeceutical formulations intended to support and maintain health and well-being. The applicant has also surprisingly found that there is a dose-response whereby higher doses have more marked sedative, hypnotic and anxiolytic activities that can be applied in formulations of botanical medicines and pharmaceuticals intended to prevent, treat or

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15 mitigate disease.

The applicant has further found that compositions of extracts of *Siphonochilus aethiopicus* containing a known and defined unit serving or dose of the compound of Formula 1 have calming, stress-relieving, sedative, hypnotic, and anxiolytic activities.

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Compositions of the invention can be formulated in unit servings and unit doses in functional foods, functional beverages, botanical medicines, pharmaceuticals, veterinary foods and medicines, aromatherapy products, and cosmeceuticals intended to promote and maintain the health, well-being, and quality of life of mammals through calming, stress-relieving, sleep-improving activities, and for ameliorating, preventing or treating

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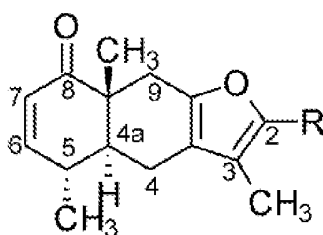
anxiety states, social phobia, panic attacks, obsessive compulsive disorder, insomnia, hyperactivity, impulsivity, agitation, seizures including febrile convulsions, psychosis, mania, aggression, nausea, vomiting, travel sickness, agitation, irritability during weight reduction programs, pre-menstrual syndrome (PMS), headache including tension headache, nervousness, agitation, irritability and insomnia in weight-reduction,

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nervousness, agitation, irritability and insomnia in alcohol and drug withdrawal, nervousness, agitation, irritability and insomnia in tobacco and nicotine withdrawal, nervousness, agitation, irritability and insomnia in tobacco and nicotine use, nervousness, agitation irritability and insomnia in caffeine use, agitation, nervousness,

agitation irritability and insomnia from excess caffeine consumption, stress, post-traumatic stress, agitation anxiety and disruptive behaviour in dementia and Alzheimers disease, pain that is exacerbated by anxiety and/or pain exacerbated by poor sleep, neuropathic pain, and for soothing the subjective experience of stress, for supporting  
5 and maintaining a calm, composed, serene outlook; reducing nervous tension, nervous irritability, social discomfort; supporting and enhancing the onset and quality of sleep.

According to a further aspect of the invention there is provided a medicinal composition useful for ameliorating, treating or preventing a Central Nervous System (CNS)  
10 condition or disorder, which composition includes as an active ingredient a compound of formula I:



20 where R is selected from H, OH, OCH<sub>3</sub>, or mixtures thereof, wherein the composition is in a unit dosage form and contains from 1 microgram to 50 milligrams of the active ingredient.

Preferably, the composition is in a unit dosage form that contains from 10 micrograms to  
25 10 milligrams of the active ingredient.

The active ingredient may be in the form the form of a plant extract or material of a plant of the family *Zingiberaceae*. Preferably, the plant extract or material is selected from the genera *Siphonochilus*, *Kaempferia*, *Cienkowskia* or *Cienkowskiella*, or mixtures thereof.

30 Preferably, the plant extract or material is selected from the species *Siphonochilus aethiopicus*, *Siphonochilus kirkii*, *Siphonochilus natalensis*, *Siphonochilus nigericus*, *Kaempferia aethiopica*, *Kaempferia galangal*, *Kaempferia natalensis*, *Kaempferia ethelae*, *Kaempferia nigerica*, *Kaempferia rosea*, *Cienkowskia aethiopica* or

*Cienkowskiella aethiopica*, or mixtures thereof. In a most preferred embodiment of the invention, the species is *Siphonochilus aethiopicus*.

5 The extract may be an essential oil or supercritical CO<sub>2</sub> extract containing at least 0.1%, preferably at least 1%, and most preferably at least 10% by weight of the compound of formula I.

10 The extract may be an aqueous extract, an ethanolic extract, an aqueous-ethanolic extract, a glycerine extract, a propylene glycol extract, extracts using naturally occurring oils, and more preferably edible oils, including but not limited to olive oil, cottonseed oil, peanut oil, grape-seed oil, almond oil, hemp seed oil, sesame seed oil, coconut oil, cocoa butter, linseed oil, fish oil, krill oil, tigernut oil, and vitamin E containing oils (such as, but not limited to, corn oil, soybean oil, wheat germ oil, sunflower, and safflower oils).

15 The aqueous extract, ethanolic extract, or aqueous-ethanolic extract may be spray-dried, or preferably spray freeze-dried into powder or granule form.

20 The composition may be provided in the form of a tea, a powder (including a dissolvable or effervescent powder), granules, a tablet (including a sustained release tablet, or a dissolvable or effervescent tablet), a chewable gum, a lozenge, a capsule, a softgel capsule, a syrup, an elixir, a tincture or other liquid with a squeeze or dropper top, an oral spray, a metred dose oral spray, a suppository, a dissolvable strip or disc, an inhaler (including a metred dose inhaler), an intranasal spray, including a metred dose  
25 intranasal spray, a transdermal patch, (including a sustained release transdermal patch), an ampoule of injectable liquid, a cartridge for use in an electronic vaporiser for inhalation, or in a cartridge for use in an electronic cigarette, a topical gel, a cream, an ointment or a lotion.

30 The composition or medicament may be in the form of a beverage, including, but not limited to, a fruit juice, a dairy beverage including a fermented dairy beverage, a malted drink, a chocolate drink, a drink base in powdered, granulated or concentrated liquid or syrup form, carbonated and still waters, plain or flavoured water, plain or coloured

water, (sweetened or unsweetened or with taste-modifiers), a still or carbonated soft drink, an alcohol-containing wine, a beer, a spirit, a cocktail, or a mixer.

The composition or medicament may be in the form of a foodstuff, including, but not limited to a fruit bar or snack bar, a chocolate-containing product, a meal replacement formulation, a slimming formulation, yoghurt, a cold or frozen dessert, a plain or flavoured powder, a chewing gum, an ice-lollies, an ice cream, jelly babies and other candies and confections.

- 10 The composition or medicament may also be in the form of a pet food, for example, pellets, granules, powders, sauces, canned or liquid foods.

The composition or medicament may be provided in the form of aromatherapy candles, vaporisers, incense sticks, pillows, cushions, soft toys, pets' cushions and blankets.

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Preferably, the condition is selected from the group consisting of anxiety states, social phobia, panic attacks, obsessive compulsive disorder, insomnia, hyperactivity, impulsivity, agitation, seizures including febrile convulsions, psychosis, mania, aggression, nausea, vomiting, travel sickness, agitation, irritability during weight reduction programs, pre-menstrual syndrome (PMS), headache including tension headache, nervousness, agitation, irritability and insomnia in weight-reduction, nervousness, agitation, irritability and insomnia in alcohol and drug withdrawal, nervousness, agitation, irritability and insomnia in tobacco and nicotine withdrawal, nervousness, agitation, irritability and insomnia in tobacco and nicotine use, nervousness, agitation irritability and insomnia in caffeine use, agitation, nervousness, agitation irritability and insomnia from excess caffeine consumption, stress, post-traumatic stress, agitation anxiety and disruptive behaviour in dementia and Alzheimers disease; adjunctive treatment of pain that is exacerbated by anxiety and/or pain exacerbated by poor sleep, neuropathic pain, soothing, ameliorating and reducing the subjective experience of stress, supporting and maintaining a calm, composed, serene outlook; reducing nervous tension, nervous irritability, social discomfort; supporting and enhancing the onset and quality of sleep.

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Thus, it will be appreciated that the invention, by providing CNS activity, in addition to being useful in treating specific disorders, finds substantial utility in the maintenance, and promotion of health which results in the general enhancement, support and maintenance of well-being in individuals, and prevention of development of disease.

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Preferably, the compound is the furanoterpenoid compound, siphonochilone, 4 $\alpha$ H-3,5 $\alpha$ ,8 $\alpha$  $\beta$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one (i.e. where R is H). The compound may be in the form of a racemic mixture or in the form of one of its stereoisomers or analogues or metabolites thereof.

10

The composition or medicament may include at least one other CNS active extract, fraction or isolated compound as an ingredient or ingredients to provide a combination treatment with modifying, additive or synergistic CNS activity. These ingredients include, but are not limited to caffeine, nicotine, theobromine, melatonin, taurine, tryptophan, L-theanine, polyunsaturated fatty acids, fermented and unfermented dairy products, and botanicals from the genera Avena, Ballota, Boswellia, Camellia, Cannabis, Catha, Centella, Chamaemelum, Chrysanthemum, Citrus, Coffea, Cola, Commiphora, Crocus, Curcuma, Cyclopia, Cymbopogon, Cytisus, Elettaria, Eleutherococcus, Ginkgo, Hibiscus, Humulus, Hypericum, Ilex, Lavandula, Leonotis, Lobelia, Matricaria, Melissa, Mentha, Mesembryanthemum, Myristica, Nardostachys, Nepeta, Nicotiana, Ocimum, Origanum, Panax, Papaver, Passiflora, Piper, Rauvolfia, Rhodiola, Rosmarinus, Salvia, Sceletium, Scutellaria, Sutherlandia, Theobroma, Valeriana, Withania, Zizyphus, where the role of the botanical in the medicament is intended to promote and maintain health, and ameliorate, treat or prevent a Central Nervous System (CNS) condition.

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*Siphonochilus aethiopicus*, in addition to containing the compound of formula 1, has multiple active components including flavonoids and diarylheptanoids similar to curcumin known or anticipated to have antiinflammatory activity, and is, thus, also, suited for development into a polymolecular phytomedicine in addition to the isolated pure compound drugs.

30

The invention also extends to a method of modulating, ameliorating, treating or preventing a Central Nervous System (CNS) condition or disorder as described above which method comprises administering to an individual a compound of formula I.

5 The invention also extends to a method of modulating, ameliorating, treating or preventing a disease or condition that responds to treatment with a melatonin agonist as described above which method comprises administering to an individual a compound of formula I.

10 According to another aspect of the invention there is provided a method of preparing an extract from a plant material selected from the family *Zingiberaceae* which method includes:

- (a) subjecting a first amount of plant material selected from the family *Zingiberaceae* to a first extraction step to form a first essential oil or carbon dioxide extract and a residual plant material; and
- 15 (b) subjecting said residual plant material to a further solvent extraction step to form a second extract; and
- (c) combining said first essential oil or carbon dioxide extract and said second extract to form a final extract.

20 In a preferred embodiment of the invention, the first extraction step may be an extraction step in which the volatile oil and other components are extracted using supercritical or subcritical carbon dioxide.

25 In another embodiment of the invention, the first extraction step may be steam distillation a step, including low pressure steam distillation.

The further solvent extraction step may be carried out using a solvent selected from water, ethanol or an ethanol-water mix.

30 Preferably, the plant material is selected from the genera *Siphonochilus*, *Kaempferia*, *Cienkowskia* or *Cienkowskiella*, or mixtures thereof. More preferably, the plant extract or material is selected from the species *Siphonochilus aethiopicus*, *Siphonochilus kirkii*, *Siphonochilus natalensis*, *Siphonochilus nigericus*, *Kaempferia aethiopica*, *Kaempferia*

*galangal*, *Kaempferia natalensis*, *Kaempferia ethelae*, *Kaempferia nigerica*, *Kaempferia rosea*, *Cienkowskia aethiopica* or *Cienkowskiella aethiopica*, or mixtures thereof. In a most preferred embodiment of the invention, the species is *Siphonochilus aethiopicus*.

- 5 The plant material may be in the form of a rhizome and/or root of the plant.

Preferably, the supercritical / subcritical carbon dioxide extraction step is conducted at a pressure of 95 to 275 bars (9500 to 27500 kPa), most preferably, at 110bars (11000kpa).

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The invention also extends to a method of preparing an extract containing the compound of Formula 1 from a plant material selected from the family *Zingiberaceae* which method includes subjecting an amount of plant material selected from the family *Zingiberaceae* to an extraction step using subcritical or supercritical carbon dioxide as the solvent system to form a carbon dioxide extract.

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## SPECIFIC DESCRIPTION OF THE INVENTION

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The invention is now described according to the following non-limiting examples and with reference to the accompanying diagrammatic drawings in which the figures represents the following:

*Figure 1:* shows the  $^1\text{H}$  spectrum of the compound, 4a $\alpha$ H-3,5 $\alpha$ ,8a $\beta$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one, dissolved in  $\text{CDCl}_3$  as described in example 1 (referenced to residual solvent peak at 7.26ppm).

*Figure 2:* shows the  $^{13}\text{C}$  spectrum of the compound described in figured 1 (referenced to residual solvent peak at 77.0 ppm).

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*Figure 3:* shows the DEPT NMR spectrum of the compound described in figure 1.



- Figure 4:* shows the  $^1\text{H}$ - $^1\text{H}$  cosy spectrum of the compound described in figure 1.
- Figure 5:* shows the  $^1\text{H}$ - $^{13}\text{C}$  hsqc NMR spectrum of the compound described in figure 1
- Figure 6:* shows the  $^1\text{H}$ - $^{13}\text{C}$  hmhc NMR spectrum of the compound described in figure 1.
- Figure 7:* shows the  $^1\text{H}$ - $^1\text{H}$  noesy spectrum of the compound described in figure 1 (800ms mixing time).
- Figure 8:* shows thin Layer Chromatogram (TLC) of sample of freeze spray-dried pressed sap of fresh *Siphonochilus aethiopicus* rhizome compared with a sub-sample of the same batch of fresh sap by conventional spray-drying, showing a markedly reduced amount of the compound of Formula 1 of the invention after conventional spray drying.
- Figure 9:* shows results of siphonochilone at a concentration of 10 $\mu\text{M}$  on radioligand binding assay targets 1-34 expressed as % inhibition of control specific binding.
- Figure 10:* shows results of siphonochilone at a concentration of 10 $\mu\text{M}$  on radioligand binding assay targets 35-68 expressed as % inhibition of control specific binding.
- Figure 11:* shows the  $\text{IC}_{50}$  (Inhibitory Constant) of the isolated compound siphonochilone on the melatonin MT1 receptor for 8 test concentrations.

## EXAMPLE 1

### Isolation and characterization of pure compound.

5 3 kg of fresh fleshy roots and conical rhizomes of cultivated *Siphonochilus aethiopicus* were cleaned of soil, thoroughly rinsed in water, coarsely chopped, and then briefly pulsed to rough consistency with a blender to form a pulp of plant material. The pulp of moist material was added to 6 litres of distilled water, and hydro-distilled in a standard stainless steel reflux still for three and a half hours. The condensed essential oil and  
10 water were collected in the separating funnel of the still and were left to stand at room temperature overnight. Crystals formed on the interface between the oil and water, comprising the compound of Formula 1. The crystals were collected by filtering using a sintered glass funnel. The crystals were rinsed with distilled water, and re-filtered with a sintered glass funnel. A total of 2.25g of the compound of Formula 1 was obtained.

15 For structural elucidation 20mg of the crystalline isolated compound was dissolved in 700  $\mu$ l CDCl<sup>3</sup>.

*Instrumentation:* Varian Unity Inova 400 NMR spectrometer with a <sup>1</sup>H frequency of 400  
20 MHz and a <sup>13</sup>C frequency of 100 MHz . 5mm dual broad band PFG probe with a probe temperature of 25 °C.

### Experimental:

<sup>1</sup>H, <sup>13</sup>C, DEPT, <sup>1</sup>H-<sup>1</sup>H cosy, <sup>1</sup>H-<sup>13</sup>C hsqc, <sup>1</sup>H-<sup>13</sup>C hmbc and <sup>1</sup>H-<sup>1</sup>H and noesy NMR  
25 experiments were carried out (see Figures 1-7). The <sup>1</sup>H spectrum was referenced to the residual solvent peak at 7.26 ppm and the <sup>13</sup>C spectrum to the centre peak of the CDCl<sub>3</sub> triplet at 77.0 ppm.

### Results:

30 The <sup>1</sup>H, <sup>13</sup>C, DEPT, <sup>1</sup>H-<sup>1</sup>H cosy, <sup>1</sup>H-<sup>13</sup>C hsqc, <sup>1</sup>H-<sup>13</sup>C hmbc and <sup>1</sup>H-<sup>1</sup>H noesy NMR spectra are shown in Figures 1 to 7 respectively. From the <sup>1</sup>H, <sup>13</sup>C, DEPT and EI-MS spectra it was determined that the molecular formula of the compound is C<sub>15</sub>H<sub>18</sub>O<sub>2</sub>. Further analysis of these spectra were able to provide other structural information about the molecule such as the presence of a carbonyl carbon, a cis double bond and in total

3 CH<sub>3</sub>, 2 CH<sub>2</sub>, 5 CH and 5 quaternary carbon groups. Using this information and the 2D spectra the basic framework of the molecule could be established. With this information a comparison with reported studies confirmed the structure to be that of the molecule shown in Formula 1. Study of the 2D spectra confirmed the structure to be the

5 furanoterpenoid known as siphonochilone that has been previously reported in literature. A comparison of the chemical shifts of the <sup>1</sup>H and <sup>13</sup>C spectra were found to correspond closely with those found in the literature. The 2D noesy spectrum collected showed correlations from Me-8a to H-5 and from Me-5 to H-4a which indicates that the molecule has the same stereochemistry as that reported in the literature for Formula 1.

Position	δ <sub>C</sub>	δ <sub>H</sub> (J Hz)
2	137.45	7.06, 1H, br m
3	119.01	-----
3a	114.60	-----
4	22.63	2.71, 1H, overlapping ddd, <i>J</i> = 15.7, 5.4, 1.8 2.15, 1H, dddd, <i>J</i> = 15.7, 10.8, 3.2, 1.5
4a	45.02	1.85, 1H, ddd, <i>J</i> = 10.8, 10.0 5.4,
5	34.30	2.43, 1H, dqdd, <i>J</i> = 10.0, 7.2, 2.7, 2.1
6	154.27	6.69, 1H, dd, <i>J</i> = 10.1, 2.1
7	126.54	5.95, 1H, dd, <i>J</i> = 10.1, 2.7
8	204.06	-----
8a	44.99	-----
9	32.01	2.77, 1H, br dd, <i>J</i> = 16.7, 1.5 2.68, 1H, br dd, <i>J</i> = 16.7, 1.8
9a	149.19	-----
3-Me	8.20	1.93, 3, d, <i>J</i> = 1.3
5-Me	18.86	1.24, 3H, d, <i>J</i> = 7.2
8a-Me	16.67	1.06, 3H, s

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR assignments for the isolated pure compound of Formula 1.

## EXAMPLE 2.

### Edible oil extraction, softgel formulation.

200g of fresh roots and rhizomes of *Siphonochilus aethiopicus* were cleaned of soil and washed thoroughly in water, sliced into 2 to 3 mm thick pieces, and freeze-dried in a

commercial food-grade freeze-drier. The freeze-dried roots and rhizomes were finely

ground using a blender, and 500 ml of wheat germ oil was added. The mixture was stirred and allowed to macerate on a heating plate for five hours at 70°C, with intermittent stirring. Using a mechanical scissor-jack press, the mixture was pressed through a perforated stainless steel container lined with a double layer of cellulose perlite filter paper to recover the wheat-germ oil extract fraction. The concentration of the compound of Formula 1 in the oil extract was determined using GC-MS (combined gas chromatography-mass spectroscopy), according to the method of Viljoen, A.M. et al. 2001. (*The essential oil composition of the roots and rhizomes of Siphonochilus aethiopicus*. *South African Journal of Botany* 67: 115–116).

The 200g of dry rhizomes yielded a concentration of 246µg of the compound of Formula 1 per 1000mg of extract, as determined using using GC-MS (combined gas chromatography-mass spectroscopy), according to the method of Viljoen, A.M. et al. 2001. (*The essential oil composition of the roots and rhizomes of Siphonochilus aethiopicus*. *South African Journal of Botany* 67: 115–116).

100µg of the compound of Formula 1 in 406.5g wheat-germ oil extract was then diluted with 93.5g additional wheat germ oil to make up a dosage unit total weight of 500mg, encapsulated in a gelatin-based softgel.

### EXAMPLE 3.

#### Cosmeceutical

20mg of the isolated pure compound of Formula 1 was added to 100g of safflower oil, warmed to 90°C and sonicated to dissolve and uniformly disperse the compound. The safflower oil containing the dissolved / dispersed compound of Formula 1 was formulated into an aqueous cream base with preservatives, and sealed in individual sachets such that a single sachet of 2.5ml of cream contains 200µg of compound of Formula 1. The sachet of cream was applied evenly to the face in the mornings as a stress-reducing and moisturising day cream.

EXAMPLE 4.Tension Headache

5 2mg of volatile oil of *Siphonochilus aethiopicus* was produced by vacuum steam distillation. This volatile oil, containing 347 µg of the compound of Formula 1, was dissolved in 1ml of warm grape-seed oil. 0.5ml of this oil was gentle rubbed until absorbed into both temples of a volunteer suffering from recurrent tension headache due to concentration on intense computer work. The volunteer continued working on the  
10 computer task, and reported feeling markedly less stress and tension twenty minutes after application, and noting that the tension headache present before the topical application had surprisingly resolved while working in spite of intense concentration required to complete the work tasks at hand.

15 EXAMPLE 5.Thin film delivery

200 µg of the compound of Formula 1 was formulated in a thin film of ingestible  
20 dissolvable polymer strip, sealed in a foil sachet. In other embodiments of the invention, in addition to the active compound and edible polymers, the strip may include a sweetening agent, saliva stimulating agent, flavouring agent, colouring agent, and stabilizing and thickening agents. The strip is taken 10 minutes before retiring to bed, or on retiring to bed, and allowed to dissolve in the mouth, the resulting saliva and  
25 dissolved strip is swallowed.

EXAMPLE 6.Inhalation of metred dose

30

A crystal of the compound of Formula 1 weighing 5.3mg was placed in a 5ml glass vial sealed with a flexible plastic lid on a laboratory heating plate. The heat was gradually increased to melting point and the heating increased until the compound was

completely vaporised at about 115 °C. An empty 2ml volume glass syringe with depressed plunger, with an attached stainless steel 10 gauge needle was pre-heated in boiling water, the needle inserted through the lid, a second 18 gauge syringe needle was inserted through the lid to act as an air-inlet, and 2ml of the vapour in the vial was  
5 drawn into the syringe. The barrel of the syringe was separated from the needle, the nozzle of the syringe placed within the nares, and the plunger depressed slowly to eject a volume of 0.5ml of vapour while inhaling deeply and steadily through the nose, and then the breath held for 10 seconds. Inhalation of a single volume of a 0.5ml of this vapour contains a metred dose of approximately 500µg of the compound of Formula 1.  
10 A single volunteer after administering this dose, within two minutes, most surprisingly experienced a tangible and appreciable feeling of deep mental relaxation, and a sense of equanimity lasting some 3 to 4 hours.

#### EXAMPLE 7.

15

##### Metred dose inhaler

The compound of Formula 1 is dissolved or suspended in a suitable propellant or powder and included in a pressurized canister. The metered dose inhaler canister is  
20 attached to a plastic, hand-operated actuator. On activation, the metered-dose inhaler releases a fixed dose of the compound of Formula 1 in in aerosol form. The person in need must first fully exhale, place the mouth-piece of the device into the mouth, and having just started to inhale at a moderate rate, depress the canister to release the metred dose of compound. The aerosolized medication is drawn into the lungs by  
25 continuing to inhale deeply before holding the breath for about 10 seconds to allow the aerosol to settle onto the walls of the respiratory system. The metered dose inhaler can be used, in single or repeated doses, for self-administering the required dose of the compound of Formula 1 to treat, without limiting the uses, acute anxiety states including phobias and panic attack, and to prevent or mitigate such states in the event that the  
30 precipitating situation is known to the user.

EXAMPLE 8.

Tablets weighing 300mg and containing 250 µg of the compound of Formula 1 were pressed after granulating the compound of Formula 1 with the excipients starch, calcium phosphate, and magnesium stearate. Two adult volunteers with social discomfort each took two tablets an hour before each of 3 separate stress-inducing social occasions, and without imbibing alcoholic beverages at the occasions as they normally would to help cope with social situations. Both reported they surprisingly found they could enjoy the occasions, and had not felt uncomfortable, anxious or stressed.

EXAMPLE 9Sustained release formulation

400 µg of the compound of Formula 1 is formulated as a once-a-day sustained release anxiolytic tablet by pressing into tablets after granulating with 200mg pectin, 2.5mg magnesium stearate and 50mg lactose. A once-a-day sustained release dosage form reduces the possibility of sedation as a side-effect, and increases medication compliance.

EXAMPLE 10.Oral spray for inducing and promoting restful night's sleep

A tincture was prepared by extracting 300g of milled freshly freeze-dried sliced rhizomes of *Siphonochilus aethiopicus* in 1000ml of 70% ethanol-water. The mixture was stored in a closed stainless steel container, and allowed to macerate for five days with daily intermittent stirring at room temperature. The mixture was then filtered through filter paper in a glass funnel to give 677ml of filtrate, which was then diluted to make 1000ml of solution by adding distilled water. The solution was analysed using the method of Viljoen et al., 2001 (*The essential oil composition of the roots and rhizomes of Siphonochilus aethiopicus. South African Journal of Botany* 67: 115–116), and compared to reference compound of Formula 1 by capillary GC-MS. The concentration

of the compound of Formula 1 in the ethanol water solution was found to be 274µg/ml. 20ml of the tincture solution was measured into an amber glass bottle fitted with a plastic spray cap where 10 depression of the spray cap releases 1.0ml.

- 5 Two adult volunteers sensitive to caffeine – unable to fall asleep if they drink caffeine-containing drinks after about 16:00 - were given 300ml of caffeinated filter coffee at 19:00. Twenty minutes before retiring to bed, both self-administered by mouth 0.5ml of spray containing 137µg of the compound of Formula 1. The following morning both volunteers reported that they had been most surprised to find that they had felt distinctly  
10 drowsy about ten minutes or so after taking the oral spray, soon fell asleep, and awoke to feel refreshed after a night of undisturbed sleep.

#### EXAMPLE 11

##### 15 Extraction by freeze spray drying

227 ml freshly expressed liquid was prepared by blending 3kg of washed and cleaned freshly harvested cultivated roots and rhizomes of *Siphonochilus aethiopicus*, pressing the slurry through a bench-top filter-press lined with filter fabric, and then filtering the  
20 resulting liquid under negative pressure through a glass funnel lined with filter paper. 100g of the filtrate was spray dried on a mini spray drier without addition of excipients to yield 4.7g of dry extract, and 100 ml of the filtrate freeze spray dried on a laboratory spray freeze dryer without the addition of excipients to yield 5.4g of dry extract. Thin layer Chromatograms were prepared by extracting a 0.47g sample of the spray dried  
25 extract (equivalent to 10g of filtrate) and 0.47g of the spray-dried extract (equivalent to 8.7g of filtrate) with 5ml of ethanol. After filtration and evaporation the residue was taken up in 0.5 ml of ethanol. A chloroform: ethanol: acetic acid (94:5:1) solvent system was used, and vanillin: sulphuric acid was used as the spray reagent. The R<sub>f</sub> value for the compound of Formula 1 is 71.

30

Figure 8 shows a Thin Layer Chromatogram (TLC) of a sample of freeze spray-dried pressed sap of fresh *Siphonochilus aethiopicus* rhizome made in accordance with this example (left of the figure) compared with a sub-sample of the same batch of fresh sap



by conventional spray-drying (right), showing a markedly reduced amount of the compound of Formula 1 of the invention (circled uppermost compound marked 1) after conventional spray drying. Thus, there is a surprisingly greater relative concentration of the compound of Formula 1 in the extract with freeze spray-drying compared with conventional spray-drying.

## EXAMPLE 12

### Liquid CO<sub>2</sub> extraction

Coarsely ground freeze-dried sliced rhizome of *Siphonochilus aethiopicus* was packed into a suitable pressured vessel. A volume of liquid carbon dioxide at the ratio of approximately 10ml of liquid carbon dioxide per 1g of plant material was allowed to pass through the raw material at a flow rate of approximately 2ml/min. The liquid carbon dioxide was then collected, the pressure released and carbon dioxide allowed to vent into the atmosphere. Removal of any added co-solvent or residual moisture was completed by drying in a vacuum desiccator. The residual extract in the collection vessel was an oil or semi-solid depending on the exact extraction conditions.

The following range of extraction conditions were employed:

SAMPLE	PRESSURE	TEMP	CO-SOLVENT	% YIELD	% COMPOUND OF FORMULA 1 (w/w)
Siph 1	100 bars	25°C	None	1.1	10.5
Siph 2	95 bars	36°C	None	1.0	10.8
Siph 3	100 bars	25°C	10% ethanol	4.5	5.3
Siph 4	275 bars	36°C	None	1.0	10.8

The content of the compound of Formula 1 was determined by the GC-MS method described by Viljoen et al., 2001. Sub-critical liquid CO<sub>2</sub> (25°C / 100 bars) liquid CO<sub>2</sub> close to the critical temp (36°C / 95 bars ) and supercritical CO<sub>2</sub> significantly above the critical temperature (36°C / 275 bars ) all gave products containing useful proportions of the compound of Formula 1. The extraction method for Siph 2 and Siph 4 are provided for the purposes of comparison only with an embodiment of the extraction

method of the invention carried out on Siph 1 and Siph 3. The addition of 10% ethanol co-solvent has been found to increase the overall yield of extract but thus significantly decreased the concentration of the compound of Formula 1 as other more polar compounds were also extracted.

5

Due to the health, safety and environmental concerns associated with some organic solvents and possible solvent contamination of the final plant extract, subcritical and supercritical fluid extraction using CO<sub>2</sub> as the extraction medium, offers an excellent alternative to the use of chemical and other solvents. Carbon dioxide leaves no residue post-extraction, and causes no damage or alteration to the phytochemical profile of the extract. Liquid carbon dioxide has a high selectivity, being able to solubilise low molar mass compounds of moderate polarity whilst leaving behind in the matrix higher molecular weight lipids, waxes and pigments which would otherwise increase the bulk of an extract and dilute the content of the active compounds. Liquid carbon dioxide is also superior to non-polar organic solvents in that it is non-flammable, so that the solvent can be safely vented to the atmosphere avoiding waste disposal and recycling costs. The intrinsically non-toxic and highly volatile nature of carbon dioxide solves the problems of elimination of residual levels of harmful solvents from the product.

## 20 EXAMPLE 13

### Workplace stress-reducing aromatherapy candle

1kg of unscented paraffin wax is melted together in a pot on low heat, and allowed to melt, candle colouring wax is added as desired. 4 ml of fragrant essential oil of *Siphonochilus aethiopicus* obtained by low-pressure steam distillation, and containing 623 mg of the compound of Formula 1, determined by GC-MS analysis following the method of Viljoen, 2001, is combined with ten drops each of vanilla oil and sandalwood oil, and the oils added to the molten wax. The wax is stirred to evenly disperse the fragrant oils through throughout the wax. New wicks are suspended from the middle of wooden rods over the mouths of 100ml volume glass candle jars. Molten wax is poured into each jar, and when set the excess wick is trimmed. The synergistic soothing fragrances are released into the air when the candle is lit.

EXAMPLE 14Extraction and re-extraction

5

A liquid extract of *Siphonochilus aethiopicus* rhizomes was prepared and formulated by combining 610mg of a supercritical CO<sub>2</sub> extract from 100g fresh rhizome of *Siphonochilus aethiopicus* together with 2.83g of extract prepared by re-extracting the original residual post-CO<sub>2</sub> extracted plant material using 70% ethanol water, followed by  
10 filtration and drying under vacuum. The two extracts (610mg CO<sub>2</sub> extract and 2.83g ethanolic extract) were dissolved into 50ml of 45% ethanol water.

0.25ml of this tincture solution, containing 18500µg of the compound of Formula 1 (determined by GC-MS analysis following the method of Viljoen, 2001), is diluted in  
15 warm water sweetened with honey and taken to treat insomnia due to menstrual cramps, exploiting both the sleep-enhancing activity of the compound of Formula 1, with the cyclooxygenase inhibitory activity of the ethanolic extract.

EXAMPLE 15.

20

Melatonin MT1 receptor radioligand binding assay

Siphonochilone dissolved in DMSO was tested at a concentration of 10µM on the following panel of radioligand binding assays using control radioligands:

25 A1 (h) (antagonist radioligand), reference compound DPCPX; A2A (h) (agonist radioligand) reference compound NECA; A3 (h) (agonist radioligand), reference compound IB-MECA; alpha 1 (non-selective) (antagonist radioligand), reference compound prazosin; alpha 2 (non-selective) (antagonist radioligand), reference compound yohimbine; beta 1 (h) (agonist radioligand), reference compound atenolol;  
30 beta 2 (h) (agonist radioligand), reference compound ICI 118551; AT1 (h) (antagonist radioligand), reference compound saralacin; AT2 (h) (agonist radioligand), reference compound angiotensin II; BZD (central) (agonist radioligand), reference compound diazepam; BZD (peripheral) (antagonist radioligand), reference compound PK11195;

BB (non-selective) (agonist radioligand), reference compound bombesin; B2 (h) (agonist radioligand) reference compound NPC567; CB1 (h) (agonist radioligand), reference compound CP 55940; CCK1 (CCKA) (h) (agonist radioligand), reference compound CCK8s; CCK2 (CCKB) (h) (agonist radioligand), reference compound CCK8s; D1 (h) (antagonist radioligand), reference compound SCH 23390; D2 (h) (antagonist radioligand) reference compound (+)butaclamol; D3 (h) (antagonist radioligand) reference compound (+)butaclamol; D4.4 (h) (antagonist radioligand) reference compound clozapine; D5 (h) (antagonist radioligand) reference compound SCH 23390; ETA (h) (agonist radioligand) reference compound endothelin-1; ETB (h) (agonist radioligand) reference compound endothelin-3; GABA (non-selective) (agonist radioligand) reference compound GABA; GAL1 (h) (agonist radioligand) reference compound galanin; GAL2 (h) (agonist radioligand) reference compound galanin; CXCR2 (IL-8B) (h) (agonist radioligand) reference compound IL-8; CCR1 (h) (agonist radioligand) reference compound MIP-1 alpha; H1 (h) (antagonist radioligand) reference compound pyrilamine; H2 (h) (antagonist radioligand) reference compound cimetidine; MT1 (ML1A) (h) (agonist radioligand) reference compound melatonin; M1 (h) (antagonist radioligand) reference compound pirenzepine; M2 (h) (antagonist radioligand) reference compound methoctramine ; M3 (h) (antagonist radioligand) reference compound 4-DAMP; M4 (h) (antagonist radioligand) reference compound 4-DAMP; M5 (h) (antagonist radioligand) reference compound 4-DAMP; NK1 (h) (agonist radioligand) reference compound [Sar9, Met(O2)11]-SP; NK2 (h) (agonist radioligand) reference compound [Nleu10]-NKA (4-10); NK3 (h) (antagonist radioligand) reference compound SB 222200; Y1 (h) (agonist radioligand) reference compound NPY; Y2 (h) (agonist radioligand) reference compound NPY; NTS1 (NT1) (h) (agonist radioligand) reference compound neurotensin; delta 2 (DOP) (h) (agonist radioligand) reference compound DPDPE; kappa (KOP) (agonist radioligand) U 50488; mu (MOP) (h) (agonist radioligand) reference compound DAMGO ; NOP (ORL1) (h) (agonist radioligand) reference compound nociception ; PCP (antagonist radioligand) reference compound MK801; EP2 (h) (agonist radioligand) reference compound PGE2; EP4 (h) (agonist radioligand) reference compound PGE2; IP (PGI2) (h) (agonist radioligand) reference compound iloprost; P2X (agonist radioligand) reference compound alpha ,beta -MeATP; P2Y (agonist radioligand) reference compound dATPalpha S; 5-HT1A (h) (agonist radioligand) reference compound 8-OH-DPAT; 5-HT1B (antagonist

radioligand) reference compound serotonin; 5-HT2A (h) (antagonist radioligand) reference compound ketanserin; 5-HT2B (h) (agonist radioligand) reference compound ( $\pm$ )DOI; 5-HT2C (h) (antagonist radioligand) reference compound RS 102221; 5-HT3 (h) (antagonist radioligand) reference compound MDL 72222; 5-HT5a (h) (agonist radioligand) reference compound serotonin; 5-HT6 (h) (agonist radioligand) reference compound serotonin; 5-HT7 (h) (agonist radioligand) reference compound serotonin; sst (non-selective) (agonist radioligand) reference compound somatostatin-14; GR (h) (agonist radioligand) dexamethasone ; VPAC1 (VIP1) (h) (agonist radioligand) reference compound VIP; Ca<sup>2+</sup> channel (L, verapamil site) (antagonist radioligand) reference compound D600; KV channel (antagonist radioligand) reference compound alpha dendrotoxin; SKCa channel (antagonist radioligand) reference compound apamin.

Experiments were performed in duplicate. The specific ligand binding to the receptors was defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand. The results are expressed as a percent of control specific binding ((measured specific binding/control specific binding)  $\times 100$ ) obtained in the presence of radiolabelled control ligands. The results are presented in bar graph form in Figures 9 and 10. The results demonstrate that the isolated compound siphonochilone has selective activity on the melatonin MT1 receptor.

In order to determine the IC<sub>50</sub> (Inhibitory Constant) of the isolated compound siphonochilone on the melatonin MT1 receptor, 8 test concentrations of the compound were tested on the MT1 (ML1A) (h) receptor, with melatonin as the agonist radioligand. The experiment was run in duplicate. The results are presented in Table 2 below, and as a graph as Figure 11. The IC<sub>50</sub> was determined to be 1.3E-5 M and the Hills' coefficient nH: 1.1

Test concentration of siphonochilone (M)	% of Control Specific Binding		
	1 <sup>st</sup> sample set	2 <sup>nd</sup> sample set	Mean
3.0E-08	116.7	102.6	109.6
1.0E-07	112.4	96.0	104.2
3.0E-07	120.7	101.3	111.0
1.0E-06	119.0	97.8	108.9
3.0E-06	90.3	81.3	85.8
1.0E-05	78.7	55.6	67.2
3.0E-05	30.0	24.5	27.3
1.0E-04	14.1	10.4	12.2

Table 2. Results for 8 concentrations of siphonochilone in duplicate on % control specific binding on MT1 receptor on the MT1 (ML1A) (h) receptor, with melatonin as the agonist radioligand.

5 The inventors have most surprisingly found that the known compound furanoterpenoid (4 $\alpha$ H-3,5 $\alpha$ ,8 $\beta$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one) and related compounds, display a properties that make the compound(s) useful in treating or preventing CNS reacted conditions. In particular, the administration of specified low doses of the isolated pure compound, results in rapid onset of calming, stress-relieving, sedative, hypnotic, and anxiolytic activities. The compound has also found to be useful in cosmeceutical formulations intended to support health and well-being. The applicant has also surprisingly found that higher doses have more marked sedative, hypnotic and anxiolytic activities that can be applied in formulations of botanical medicines and pharmaceuticals intended to prevent, treat or mitigate disease.

15

Dietary supplements and herbal medicines containing milled raw materials and extracts of *Piper methysticum* (Kava) enjoyed popular use in many countries in herbal products for improving sleep, and for stress and anxiety. Fear that some kava-containing products were responsible for hepatotoxicity, including some fatalities from liver failure,

led some regulatory authorities to remove Kava-containing products from the market. Thus, in view of the potential risk of kava containing products, and more due to consumers concerns about the safety of kava, there is a need for new experiential botanical ingredients with calming, sleep-supporting and anxiolytic activities. It is an advantage of the invention the medicaments or compositions according to the invention will provide an alternative to Kava for improving sleep and for relieving stress and anxiety while at the same time alleviating possible risks associated therewith.

The extraction of a relatively narrow fraction of plant compounds for the production of a volatile oil fraction during steam distillation, or for the production of an extract using liquid CO<sub>2</sub> leaves behind plant material with residual potentially useful bioactive compounds. The method of the invention also provides for this already-extracted plant material to be re-extracted using a different extraction technique and solvent system, and the resulting second extract to be combined with the first extract. This technique gives a fuller spectrum of phytochemicals, with additive or synergistic biological activities.

A further advantage is that the closer the phytochemical spectrum of a plant extract is to the phytochemical spectrum of the safely traditionally consumed plant part, and the closer the serving or dose of the plant extract consumed correlates with a safe traditional serving size or dose, the greater the provisional assumption of safety.

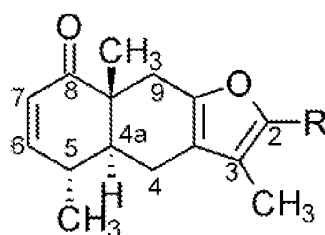
Investment in pre-clinical and clinical safety studies of the broad phytochemistry combined extract carries less inherent risk than for completely novel isolated compounds or extracts with narrow fractions of the original plant phytochemistry.

Due to the surprising and significant activity on the melatonin MT<sub>1</sub> receptor the compound of formula I has substantial utility in treating a number of diseases that respond to treatment with a melatonin agonist.

**CLAIMS:**

1. A compound of formula I:

5



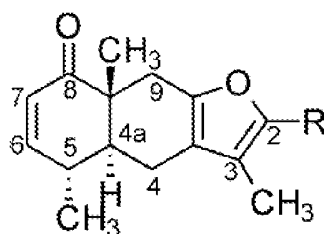
where R is selected from H, OH, OCH<sub>3</sub>, or mixtures thereof, for use as a medicament for ameliorating, treating or preventing a Central Nervous System (CNS) condition or disorder.

15

2. A compound as claimed in claim 1, in which the condition or disorder is selected from the group consisting of anxiety states, social phobia, panic attacks, obsessive compulsive disorder, insomnia, hyperactivity, impulsivity, agitation, seizures including febrile convulsions, psychosis, mania, aggression, nausea, vomiting, travel sickness, agitation, irritability during weight reduction programs, pre-menstrual syndrome (PMS), headache including tension headache, nervousness, agitation, irritability and insomnia in weight-reduction, nervousness, agitation, irritability and insomnia in alcohol and drug withdrawal, nervousness, agitation, irritability and insomnia in tobacco and nicotine withdrawal, nervousness, agitation, irritability and insomnia in tobacco and nicotine use, nervousness, agitation irritability and insomnia in caffeine use, agitation, nervousness, agitation irritability and insomnia from excess caffeine consumption, stress, post-traumatic stress, agitation anxiety and disruptive behaviour in dementia and Alzheimers disease, adjunctive treatment of pain that is exacerbated by anxiety and/or pain exacerbated by poor sleep, neuropathic pain, soothing, ameliorating and reducing the subjective experience of stress, supporting and maintaining a calm, composed, serene outlook, reducing nervous tension, nervous irritability, social discomfort; supporting and enhancing the onset and quality of sleep.



3. A compound of formula I:



- 10 where R is selected from H, OH, OCH<sub>3</sub>, or mixtures thereof, for use as a medicament for ameliorating, treating or preventing a disease or condition that responds to treatment with a melatonin agonist.

4. A compound as claimed in claim 3, in which the disease or condition is selected
- 15 from the group consisting of disturbances in the onset, duration and quality of sleep, intrinsic sleep disorders including insomnia, extrinsic sleep disorders, circadian rhythm disorders (including time-zone change syndrome (jet lag), shift work sleep disorder, irregular sleep-wake pattern, delayed sleep phase syndrome, advanced sleep phase syndrome, non-24 hour sleep-wake syndrome), parasomnias, sleep disorder associated
- 20 with internal medical or psychiatric disorders (including chronic obstructive pulmonary disease, Alzheimer's disease, Parkinson's disease, cerebrovascular dementia, schizophrenia, depression, anxiety), neurodegenerative diseases (including senile dementia, Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, spinocerebellar
- 25 degeneration, multiple sclerosis (MS), psychoneurotic diseases (e.g., depression, anxiety, bipolar disorder, posttraumatic stress disorder (PTSD), seasonal affective disorder, schizophrenia), memory disorders (cognitive decline in the elderly, senile dementia), ischemic central nervous system disorders (including cerebral infarction, cerebral haemorrhage, brain oedema), central nervous system injury (including head
- 30 trauma, spinal cord injury, whiplash injury), vascular dementia (including multi-infarct dementia), cancer (including brain tumour, pituitary adenoma, glioma, acoustic schwannoma, retinoblastoma, thyroid cancer, pharyngeal cancer, laryngeal cancer, cancer of the tongue, thymoma, mesothelial tumor, breast cancer, lung cancer, non-

small cell lung cancer, small cell lung cancer, gastric cancer, esophageal cancer, duodenal cancer, colorectal cancer, colon cancer, rectal cancer, liver cancer, hepatocellular carcinoma, pancreatic cancer, pancreatic endocrine tumour, biliary tract cancer, gall bladder cancer, penile cancer, kidney cancer, renal pelvic cancer, ureteral cancer, renal cell cancer, testis tumour, prostate cancer, urinary bladder cancer, vulvar cancer, uterine cancer, cancer of uterine cervix, cancer of uterine body, uterine sarcoma, chorionic disease, vaginal cancer, ovary cancer, ovarian germ cell tumour, skin cancer, malignant melanoma, mycosis fungoides, basal cell tumour, soft tissue sarcoma, malignant lymphoma, Hodgkin's disease, osteomyelodysplasia syndrome, multiple myeloma, leukemia, acute myelocytic leukemia, chronic myelocytic leukemia, acute lymphatic leukemia, chronic lymphatic leukemia, adult T cell leukemia, chronic myeloproliferative disease, pancreatic endocrine tumor, fibrous histiocytoma, leiomyosarcoma, rhabdomyosarcoma, unknown primary cancer and the like), hyperinsulinemia, metabolic syndrome, obesity, diabetes, diabetic complications (e.g., diabetic retinopathy, diabetic neuropathy, diabetic nephropathy and the like), hypertriglyceridemia (hyperlipidemia), hypertension, circulatory disease (including ischemic cardiac diseases, arteriosclerosis), lower urinary tract disease or disorder (including dysuria, urinary incontinence), osteoporosis, reproductive and neuroendocrine diseases, convulsion, glaucoma, headache, and irritable bowel syndrome, immunoregulation, amelioration, prevention and treatment of stress, cognitive enhancement, and tranquilization.

5. A compound as claimed in anyone of claims 1 to 4 inclusive, in which the compound is derived from a plant extract or material of a plant of the family *Zingiberaceae*.

6. A compound as claimed in claim 5, in which the plant extract or material is selected from the genera *Siphonochilus*, *Kaempferia*, *Cienkowskia* or *Cienkowskiella*, or mixtures thereof.

7. A compound as claimed in claim 6, in which the plant extract or material is selected from the species *Siphonochilus aethiopicus*, *Siphonochilus kirkii*, *Siphonochilus natalensis*, *Siphonochilus nigericus*, *Kaempferia aethiopica*, *Kaempferia*

*galangal*, *Kaempferia natalensis*, *Kaempferia ethelae*, *Kaempferia nigerica*, *Kaempferia rosea*, *Cienkowskia aethiopica* or *Cienkowskiella aethiopica*, or mixtures thereof.

8. A compound as claimed in claim 7, in which the species is *Siphonochilus aethiopicus*.

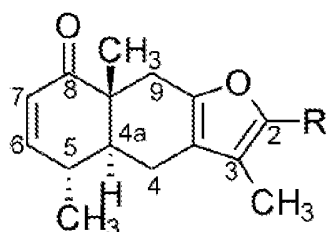
9. A compound as claimed in any one of claims 1 to 8 inclusive, in which the compound is siphonochilone (4 $\alpha$ H-3,5 $\alpha$ ,8 $\beta$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one).

10. A compound as claimed in any one of claims 1 to 9 inclusive, in which the medicament is in a unit dosage form containing from 1 microgram to 50 milligrams of the compound.

11. A compound as claimed in claim 10, in which the medicament is in a unit dosage form containing from 10 micrograms to 10 milligrams of the compound.

12. Use as claimed in claim 10 or claim 11, in which the dosage form is for administration from 1 to 3 times daily.

13. A medicinal composition useful for ameliorating, treating or preventing a Central Nervous System (CNS) condition or disorder, which composition includes as an active ingredient a compound of formula I:



where R is selected from H, OH, OCH, or mixtures thereof, and wherein the composition is in a unit dosage form containing from 1 microgram to 50 milligrams of the active ingredient.

5 14. The composition as claimed in claim 13, in which the unit dosage form contains from 10 micrograms to 10 milligrams of the active ingredient.

15. The composition as claimed in claims 13 or 14, in which the active ingredient is derived from a plant extract or material of a plant of the family *Zingiberaceae*.

10

16. The composition as claimed in claim 15, in which the plant extract or material is selected from the genera *Siphonochilus*, *Kaempferia*, *Cienkowskia* or *Cienkowskiella*, or mixtures thereof.

15 17. The composition of claim 16, in which the plant extract or material is selected from the species *Siphonochilus aethiopicus*, *Siphonochilus kirkii*, *Siphonochilus natalensis*, *Siphonochilus nigericus*, *Kaempferia aethiopica*, *Kaempferia galangal*, *Kaempferia natalensis*, *Kaempferia ethelae*, *Kaempferia nigerica*, *Kaempferia rosea*, *Cienkowskia aethiopica* or *Cienkowskiella aethiopica*, or mixtures thereof.

20

18. The composition as claimed in claim 17, in which the species is *Siphonochilus aethiopicus*.

25 19. The composition as claimed in claims 15 to 18, in which the extract is an essential oil or supercritical CO<sub>2</sub> extract containing at least 0.1% by weight of the compound of formula I.

20. The composition as claimed in claim 19, in which the extract contains at least 1% by weight of the compound of formula I.

30

21. The composition as claimed in claim 20, in which the extract contains at least 10% by weight of the compound of formula I.

22. The composition as claimed in claims 13 to 21 inclusive, in which the condition or disorder is selected from the group consisting of anxiety states, social phobia, panic attacks, obsessive compulsive disorder, insomnia, hyperactivity, impulsivity, agitation, seizures including febrile convulsions, psychosis, mania, aggression, nausea, vomiting, travel sickness, agitation, irritability during weight reduction programs, pre-menstrual syndrome (PMS), headache including tension headache, nervousness, agitation, irritability and insomnia in weight-reduction, nervousness, agitation, irritability and insomnia in alcohol and drug withdrawal, nervousness, agitation, irritability and insomnia in tobacco and nicotine withdrawal, nervousness, agitation, irritability and insomnia in tobacco and nicotine use, nervousness, agitation irritability and insomnia in caffeine use, agitation, nervousness, agitation irritability and insomnia from excess caffeine consumption, stress, post-traumatic stress, agitation anxiety and disruptive behaviour in dementia and Alzheimers disease, adjunctive treatment of pain that is exacerbated by anxiety and/or pain exacerbated by poor sleep, neuropathic pain, soothing, ameliorating and reducing the subjective experience of stress, supporting and maintaining a calm, composed, serene outlook, reducing nervous tension, nervous irritability, social discomfort, supporting and enhancing the onset and quality of sleep.

23. The composition as claimed in claims 13 to 22 in which the active ingredient is the compound siphonochilone (4 $\alpha$ H-3,5 $\alpha$ ,8 $\alpha$  $\beta$ -trimethyl-4a,9-tetrahydro-naphtho[2,3-b]-furan-8-one).

24. The composition as claimed in claims 13 to 23, which includes at least one other CNS active extract, fraction or isolated compound as an active ingredient or ingredients.

25. The composition as claimed in claim 24, in which the other ingredient or ingredients include caffeine, nicotine, theobromine, melatonin, taurine, tryptophan, L-theanine, polyunsaturated fatty acids, fermented and unfermented dairy products, botanicals from the genera Avena, Ballota, Boswellia, Camellia, Cannabis, Catha, Centella, Chamaemelum, Chrysanthemum, Citrus, Coffea, Cola, Commiphora, Crocus, Curcuma, Cyclopia, Cymbopogon, Cytisus, Elettaria, Eleutherococcus, Ginkgo, Hibiscus, Humulus, Hypericum, Ilex, Lavandula, Leonotis, Lobelia, Matricaria, Melissa,

Mentha, Mesembryanthemum, Myristica, Nardostachys, Nepeta, Nicotiana, Ocimum, Origanum, Panax, Papaver, Passiflora, Piper, Rauvolfia, Rhodiola, Rosmarinus, Salvia, Sceletium, Scutellaria, Sutherlandia, Theobroma, Valeriana, Withania and Zizyphus.

5     26.     A method of preparing an extract from a plant material selected from the family *Zingiberaceae* which method includes:

10            (a)     subjecting a first amount of plant material selected from the family *Zingiberaceae* to a first extraction step to form a first essential oil or carbon dioxide extract and a residual plant material; and

              (b)     subjecting said residual plant material to a further solvent extraction step to form a second extract; and

15            (c)     combining said first essential oil or carbon dioxide extract and said second extract to form a final extract.

20     27.     The method as claimed in claim 26, in which the first extraction step produces a volatile oil fraction using supercritical or subcritical carbon dioxide.

28.     The method as claimed in claim 27, in which the first extraction step is carried out using steam distillation.

25     29.     The method as claimed in claim 29, the further solvent extraction step is carried out using a solvent selected from water, ethanol or an ethanol-water mix.

30     30.     The method as claimed in anyone of claims 26 to 29, in which the plant extract or material is selected from the genera *Siphonochilus*, *Kaempferia*, *Cienkowskia* or *Cienkowskiella*, or mixtures thereof.

31.     The method as claimed in claim 30, in which the plant extract or material is selected from the species *Siphonochilus aethiopicus*, *Siphonochilus kirkii*, *Siphonochilus natalensis*, *Siphonochilus nigericus*, *Kaempferia aethiopica*, *Kaempferia*

*galangal*, *Kaempferia natalensis*, *Kaempferia ethelae*, *Kaempferia nigerica*, *Kaempferia rosea*, *Cienkowskia aethiopica* or *Cienkowskiella aethiopica*, or mixtures thereof.

32. The method as claimed in claim 31, in which the species is *Siphonochilus aethiopicus*.

33. The method as claimed in 27, in which the supercritical or subcritical carbon dioxide extraction step is conducted at a pressure of 95 to 275 bars (9500 to 275,000 kPa).

34. The method as claimed in 33 in which the extraction step is conducted at a pressure of 110bars (11,000 kPa).

35. A method of preparing an extract containing the compound of Formula 1 from a plant material selected from the family *Zingiberaceae* which method includes subjecting an amount of plant material selected from the family *Zingiberaceae* to an extraction step using subcritical or supercritical carbon dioxide as the solvent system to form a carbon dioxide extract.

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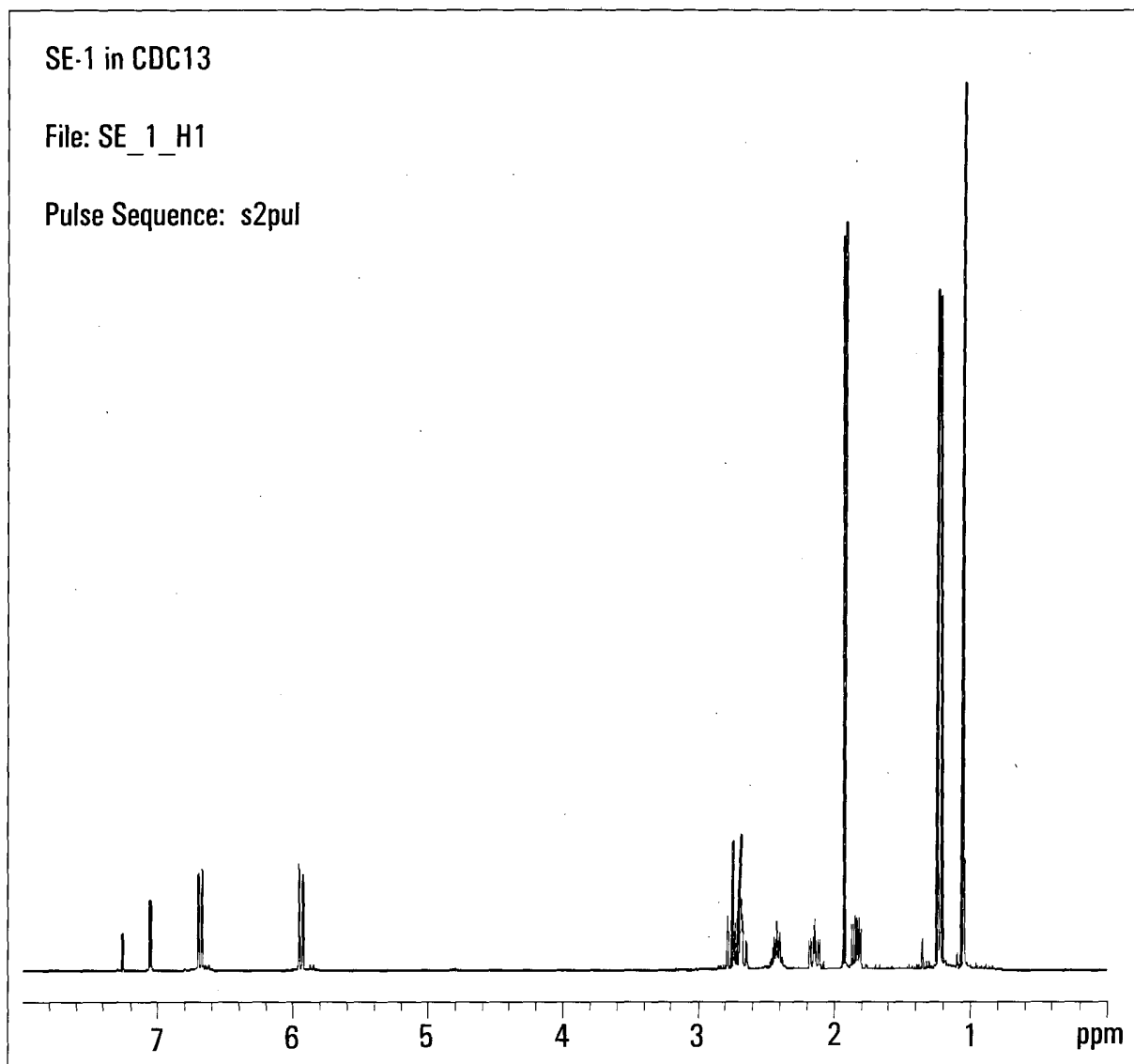


Figure 1 <sup>1</sup>H spectrum of the compound in CDCl<sub>3</sub>  
(referenced to residual solvent peak at 7.26ppm).



2/11

File: SE\_1\_C13

Pulse Sequence: s2pul

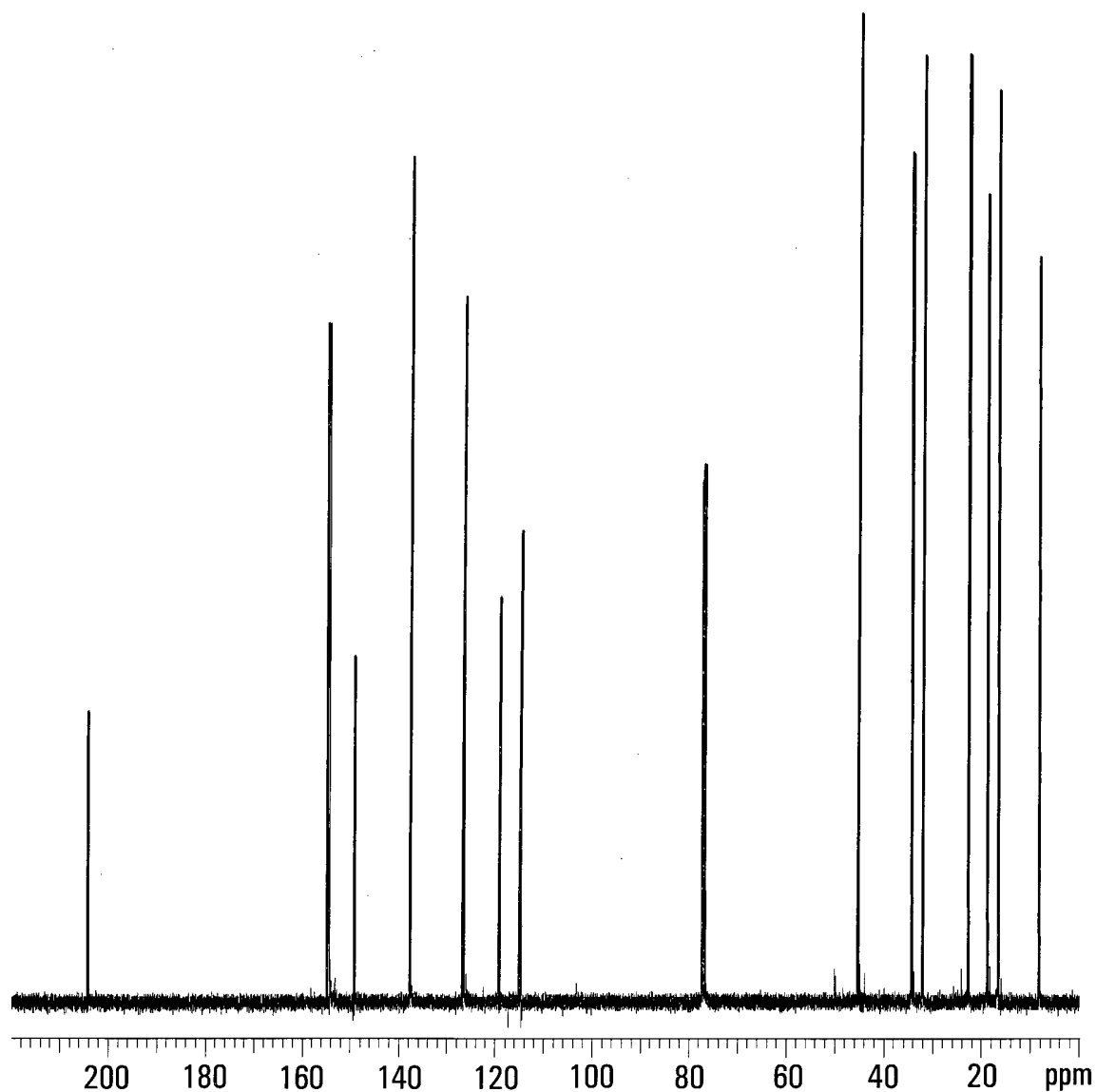
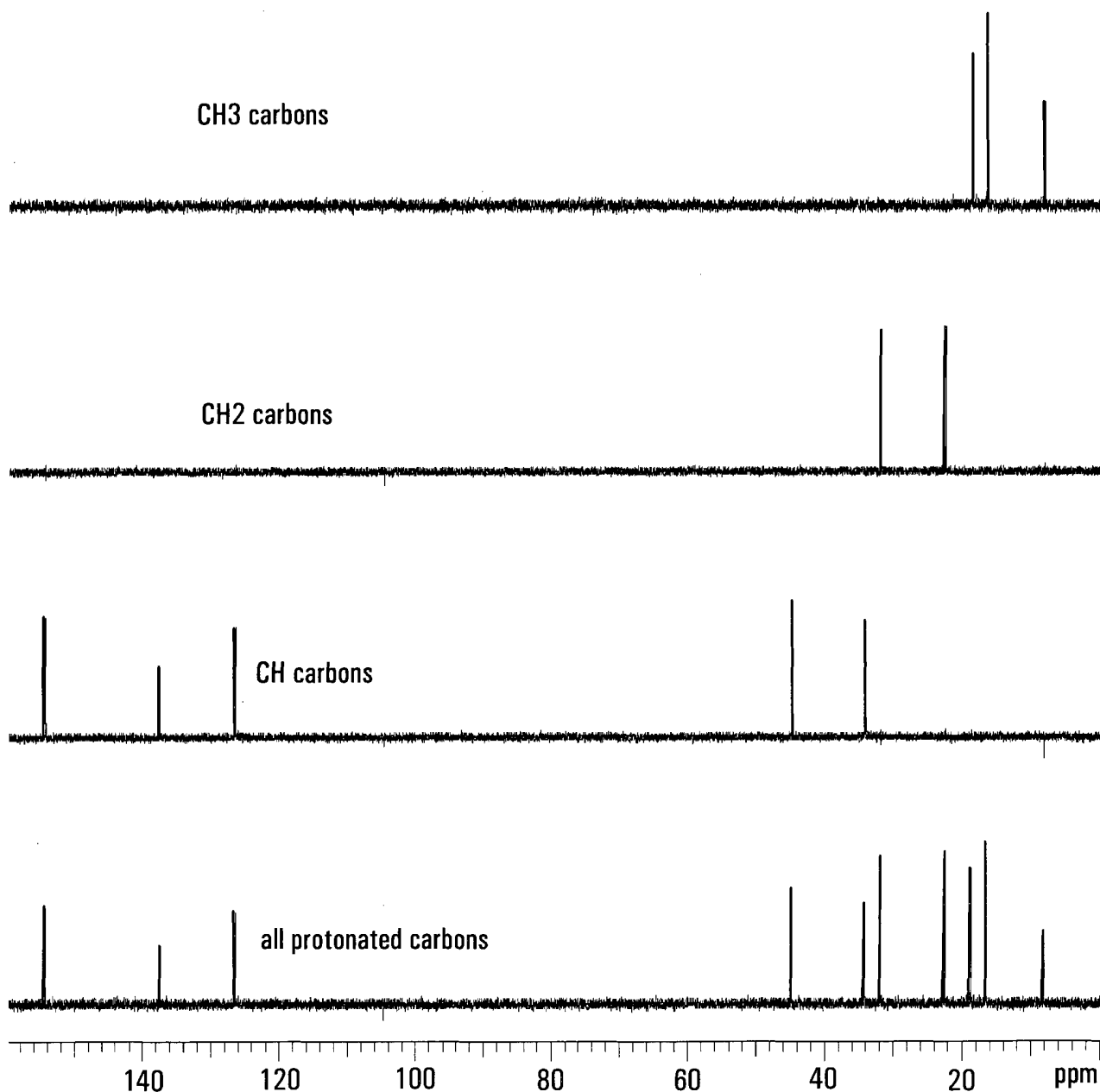


Figure 2  $^{13}\text{C}$  spectrum of the compound in  $\text{CDCl}_3$   
(referenced to residual solvent peak at 77.0 ppm).

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File: SE\_1\_DEPT

Pulse Sequence: DEPT

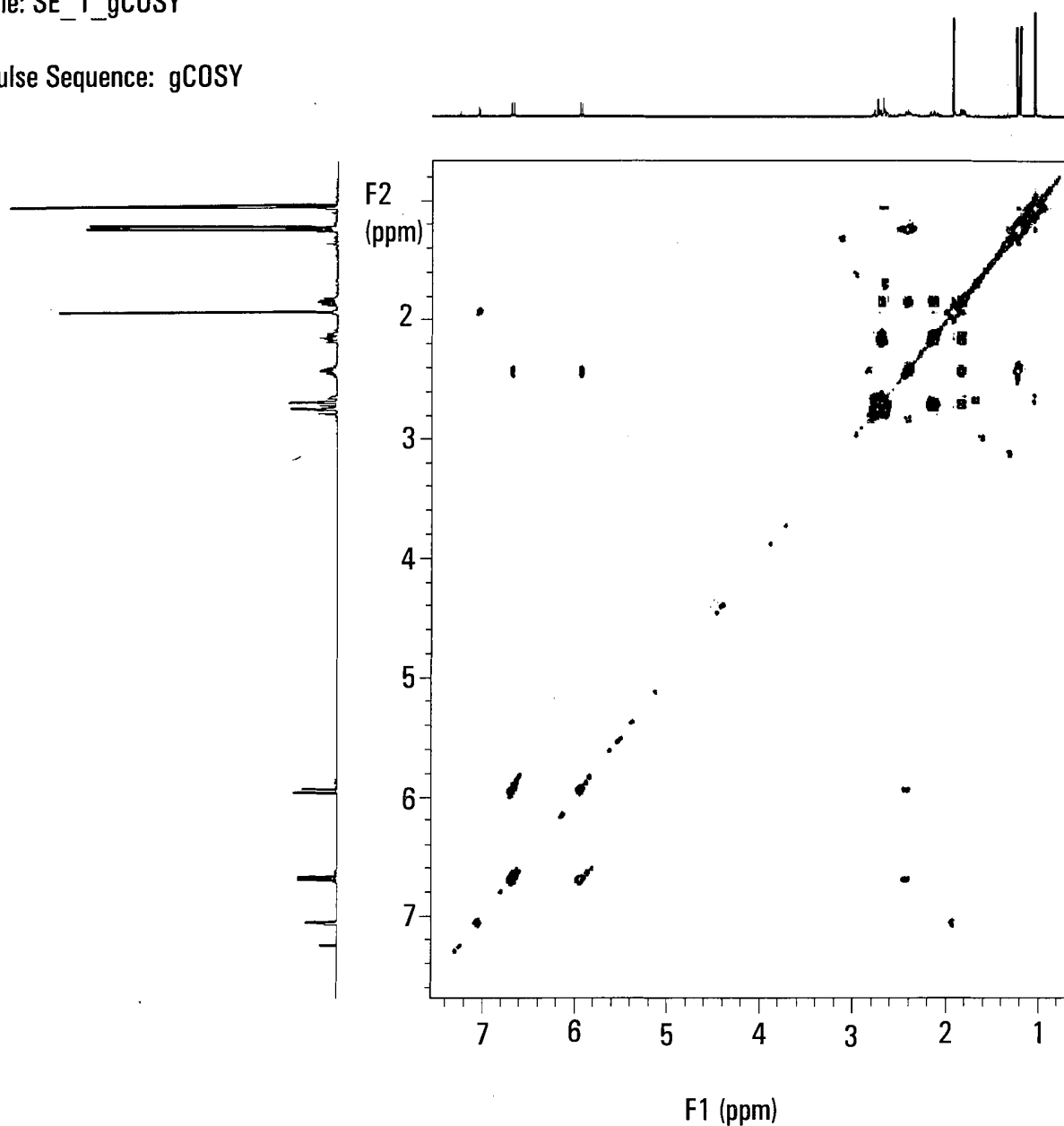
Figure 3 DEPT NMR spectrum of the compound in  $\text{CDCl}_3$

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SE-1 in CDC13

File: SE\_1\_gCOSY

**Pulse Sequence: gCOSY**



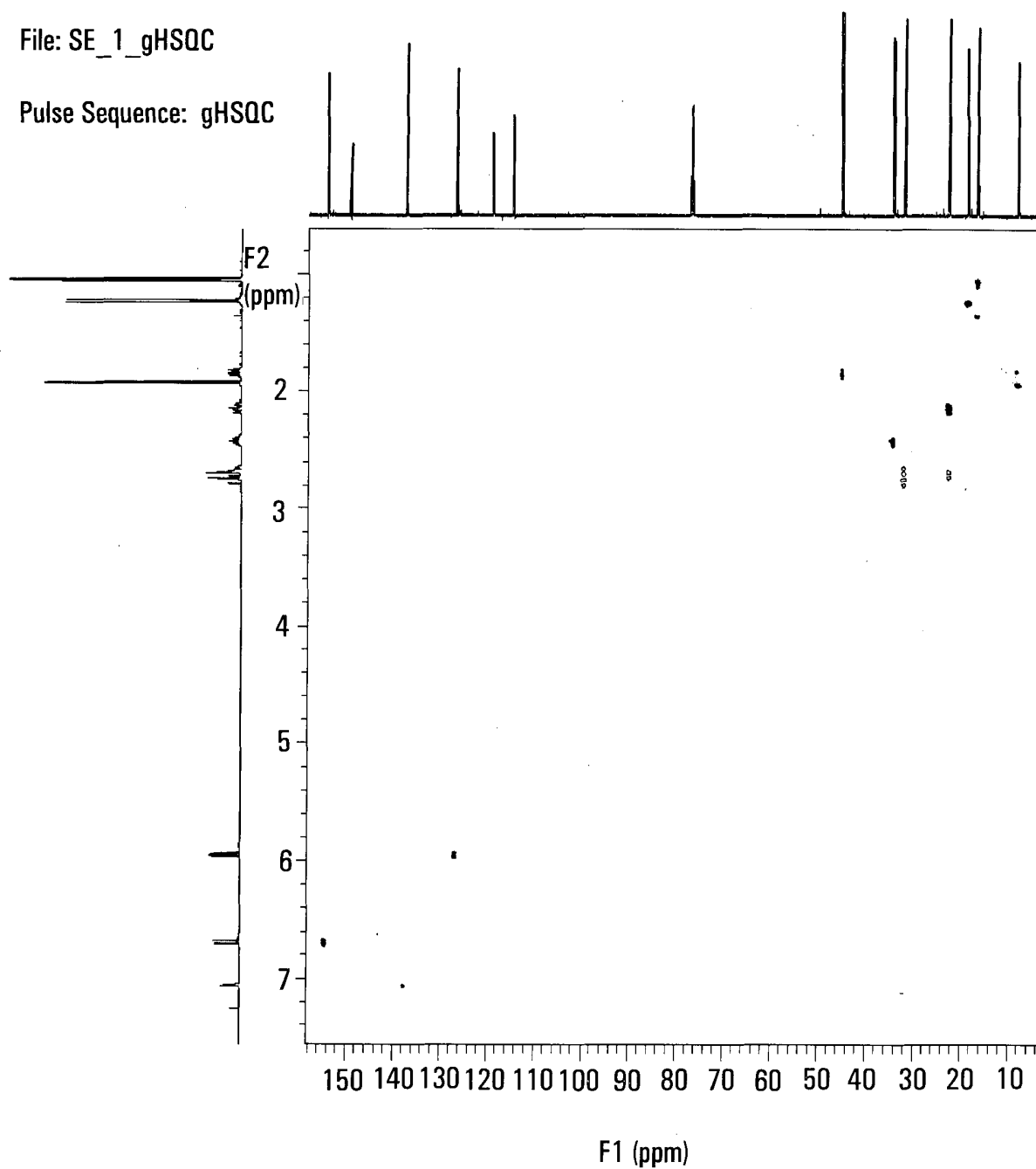
**Figure 4**  $^1\text{H}$ - $^1\text{H}$  cosy spectrum of the compound in  $\text{CDCl}_3$

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SE-1 in CDCl<sub>3</sub>

File: SE\_1\_gHSQC

Pulse Sequence: gHSQC

Figure 5 <sup>1</sup>H-<sup>13</sup>C hsqc NMR spectrum of the compound in CDCl<sub>3</sub>

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SE-1 in CDCl<sub>3</sub>

File: SE\_1\_gHMBC

Pulse Sequence: gHMBC

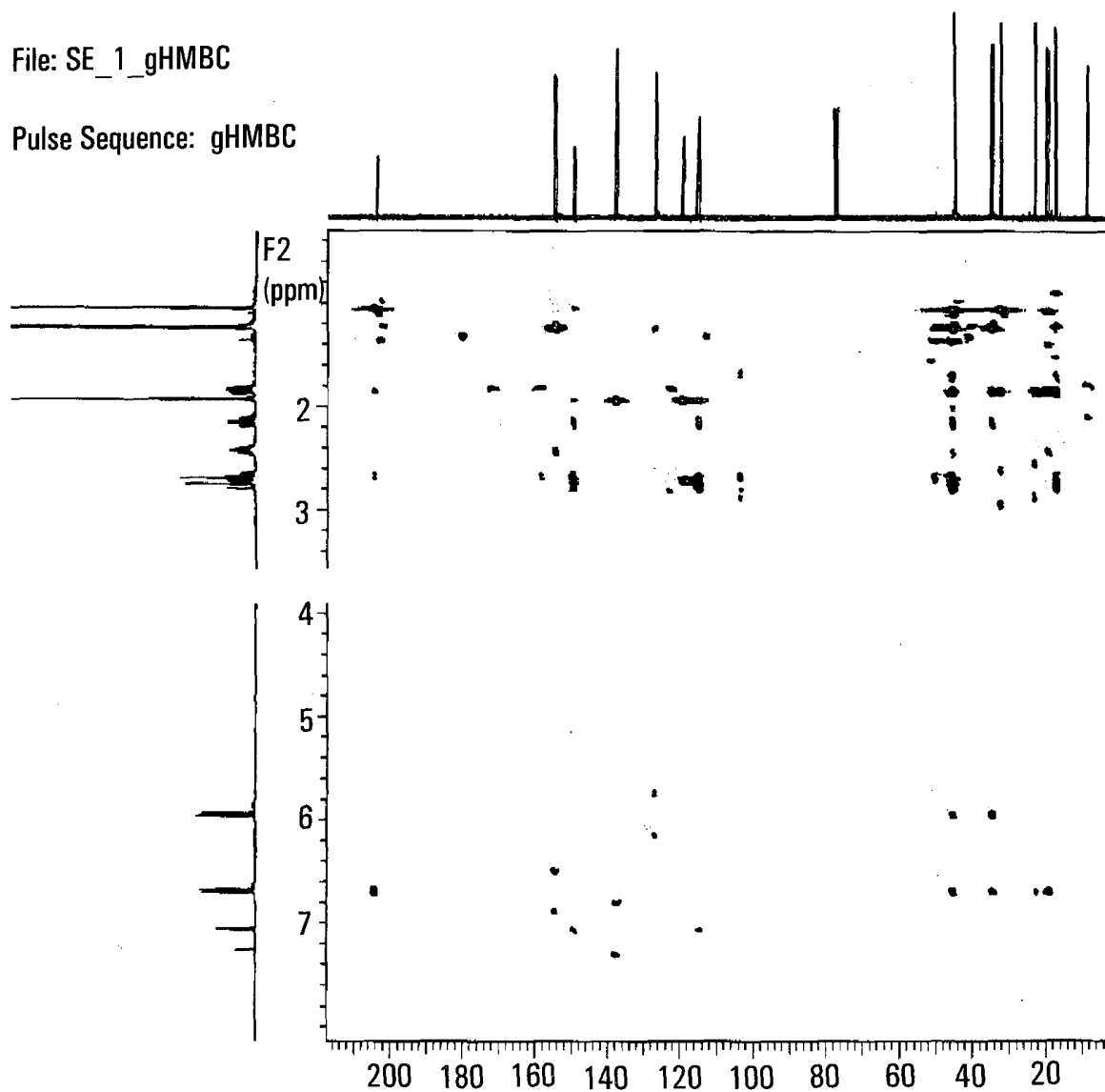


Figure 6  $^1\text{H}$ - $^{13}\text{C}$  hmbc NMR spectrum of the compound in  $\text{CDCl}_3$

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SE-1 in CDCl<sub>3</sub>

File: SE\_1\_NOESY

Pulse Sequence: NOESY

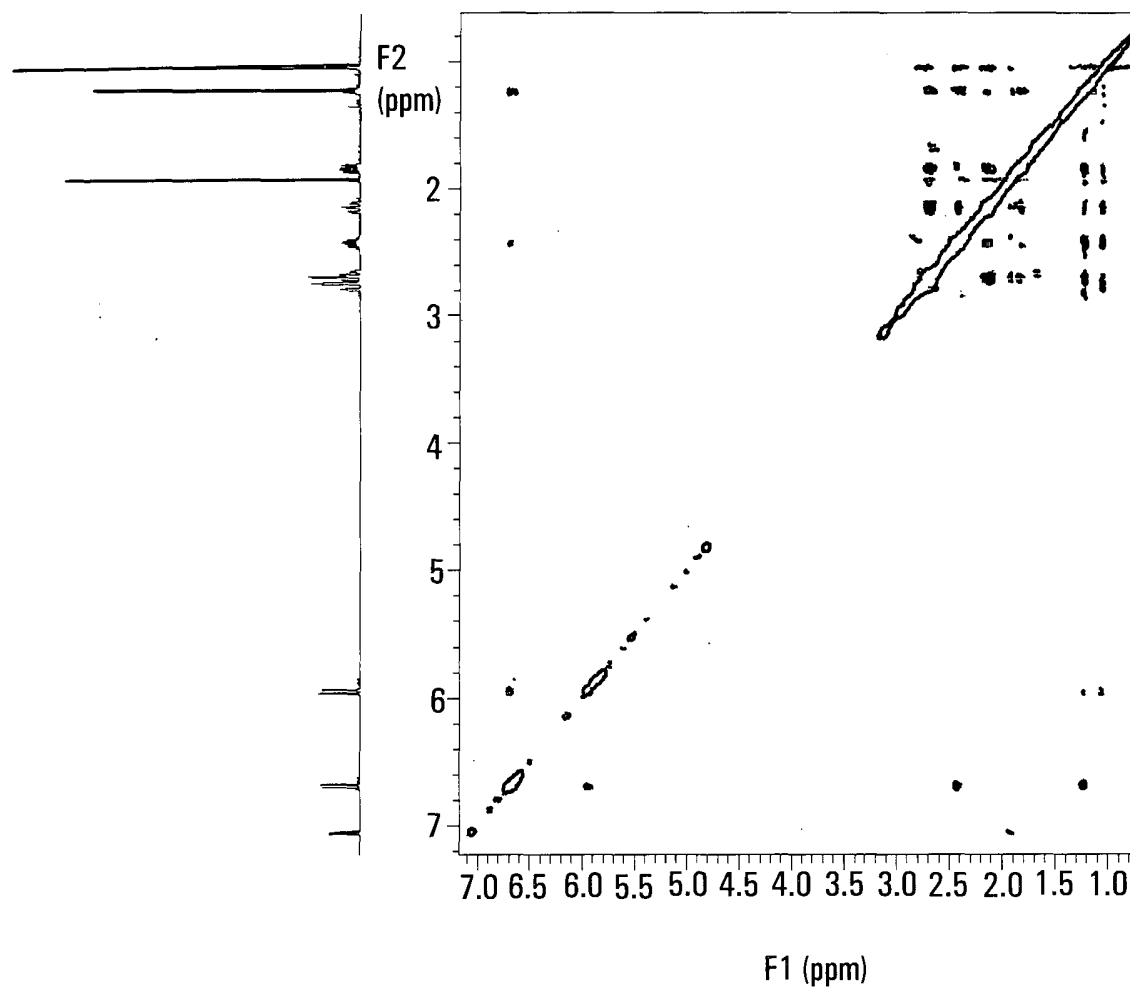


Figure 7 <sup>1</sup>H-<sup>1</sup>H noesy spectrum of the compound in CDCl<sub>3</sub>  
(800ms mixing time)

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Figure 8. Thin Layer Chromatogram (TLC) of sample of freeze spray-dried pressed sap of fresh *Siphonochilus aethiopicus* rhizome (left) compared with a sub-sample of the same batch of fresh sap by conventional spray-drying (right), showing markedly reduced amount of compound of Formula 1 of the invention (circled uppermost compound marked 1) after conventional spray drying.

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## % Inhibition Control Specific Binding

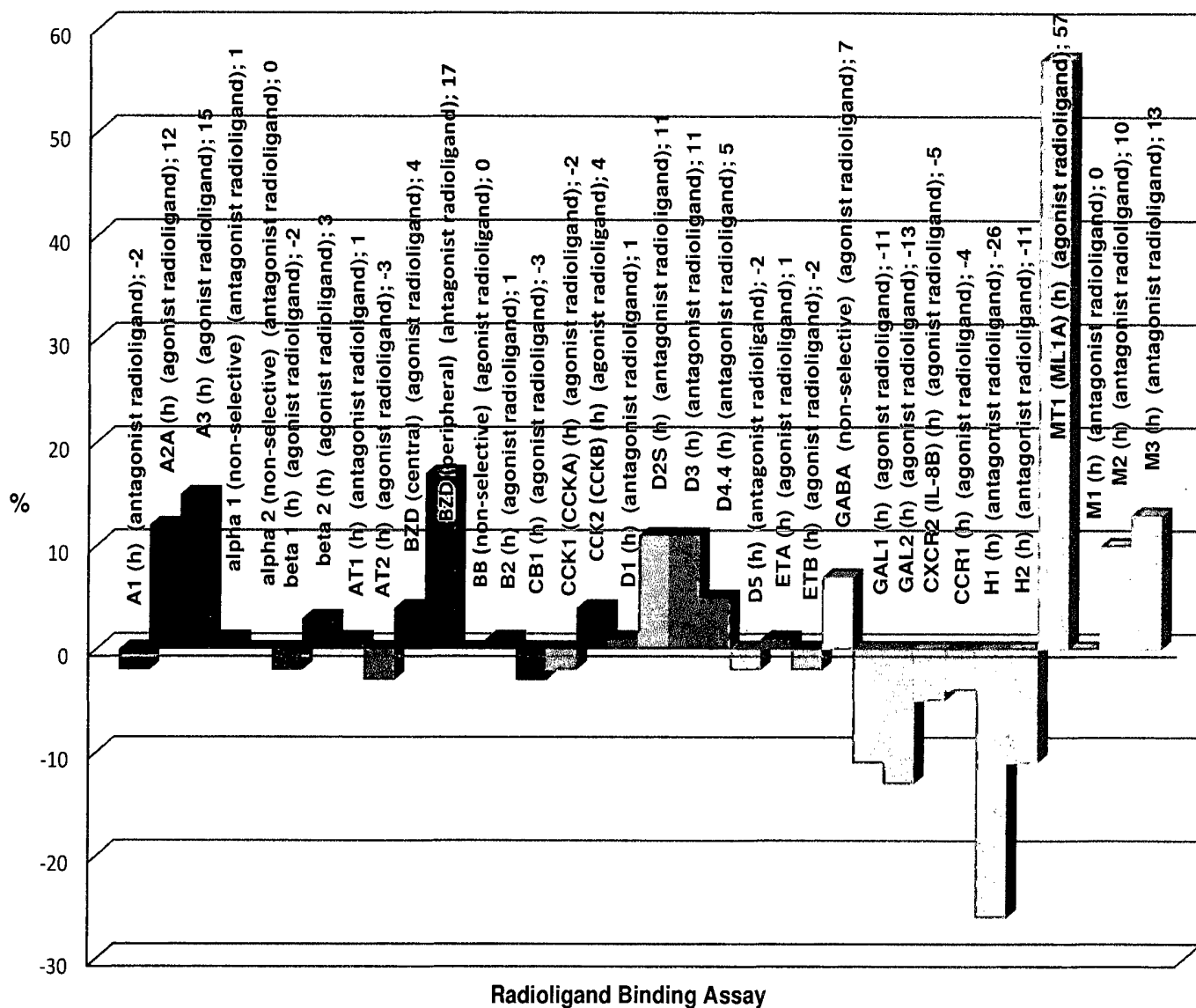


Figure 9: Bar graph of the results of isolated pure siphonochilone at a concentration of 10 $\mu$ M on radioligand binding assay targets 1-34 expressed as % inhibition of control specific binding.



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## % Inhibition Control Specific Binding

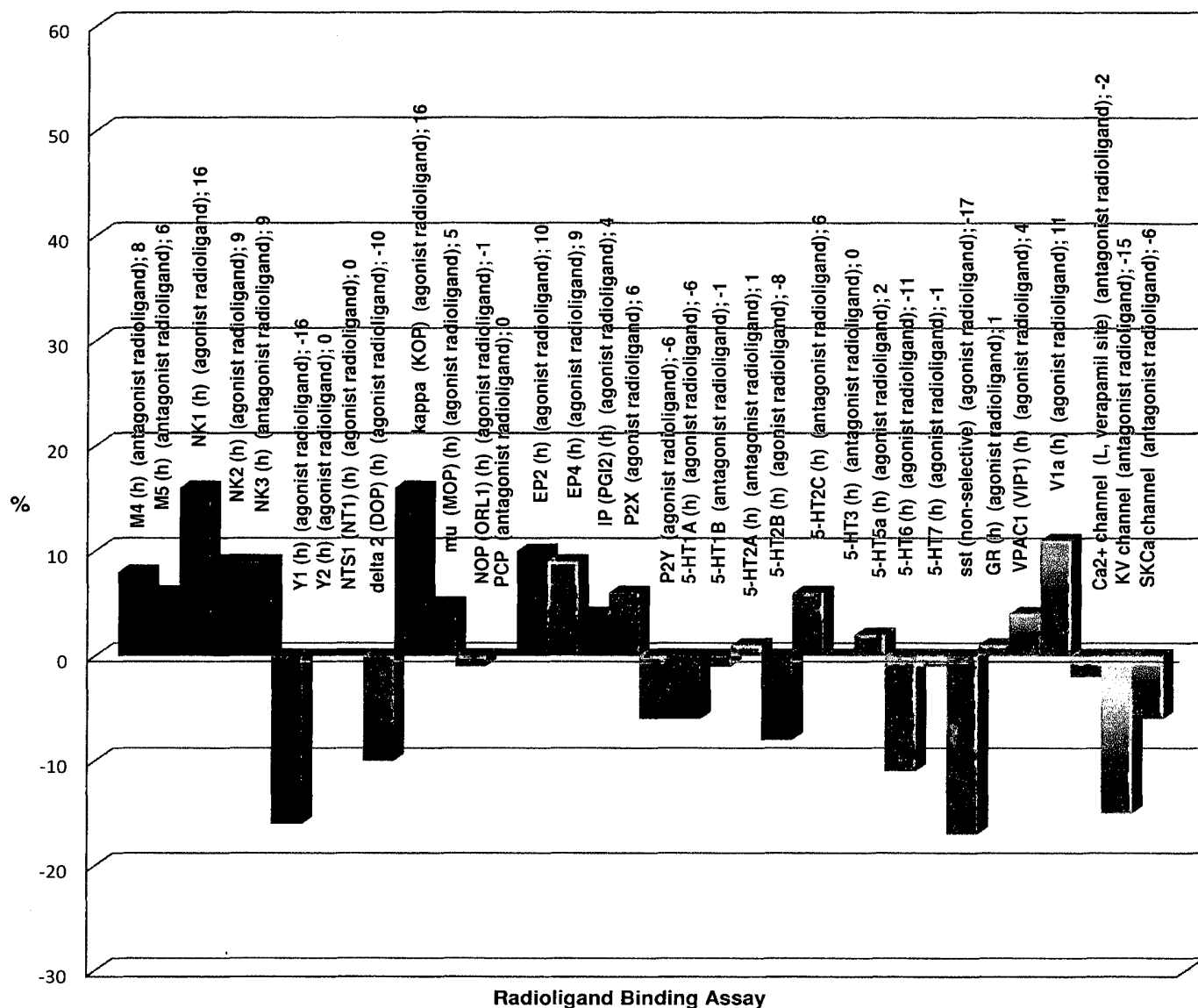


Figure 10: Bar graph of the results of isolated pure siphonochilone at a concentration of  $10\mu\text{M}$  on radioligand binding assay targets 35-68 expressed as % inhibition of control specific binding.

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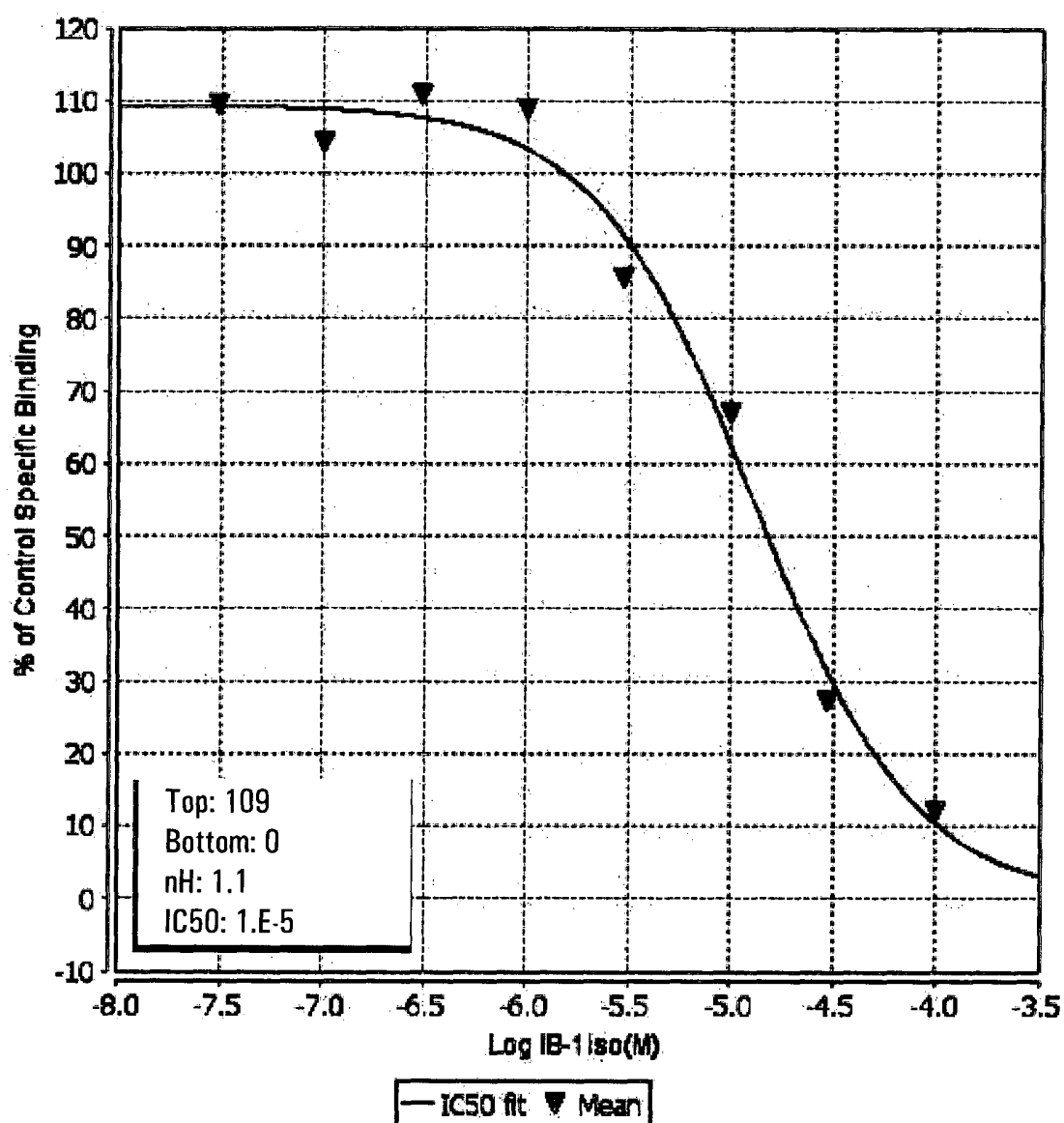


Figure 11: Competition curve for siphonochilone on MT1 (ML1A) (h) (agonist radioligand)