

Interactions of *Magnolia* and *Ziziphus* extracts with selected central nervous system receptors

Uwe Koetter¹, Marilyn Barrett², Svenja Lacher³, Aliaa Abdelrahman³, Deanne Dolnick^{4†}

¹Dr. Koetter Consulting, Uttwil, Switzerland

²Pharmacognosy Consulting, Mill Valley CA, USA

³Pharmaceutical Institute, University of Bonn, Bonn, Germany

⁴Next Pharmaceuticals, Salinas CA, USA

†Corresponding author

E-mail addresses:

UK: koettu@mac.com

MB: marilyn@pharmacognosy.com

SL: svenja.lacher@uni-bonn.de

AA: aabdelra@uni-bonn.de

DD: ddolnick@nextpharmaceuticals.com

Abstract

Background

Magnolia officinalis bark and *Ziziphus spinosa* seed have a history of use in traditional Asian medicine for mild anxiety, nervousness and sleep-related problems. A proprietary combination of extracts from these plant materials has demonstrated effectiveness in alleviating sleep difficulties in an open-label clinical assessment.

Methods

In vitro radioligand binding assays were conducted with the fixed combination of extracts and the component extracts to investigate possible mechanisms for the clinical observations. The receptors selected for screening were chosen because they are involved in central nervous system functions related to relaxation and/or sleep. The selected receptor /receptor subtypes were: adenosine A₁, dopamine (transporter, D₁, D_{2S}, D₃, D_{4.4} and D₅), serotonin (transporter, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{4e}, 5-HT₆ and 5-HT₇) and the GABA benzodiazepine receptor. Extracts of *Magnolia* and *Ziziphus* and their combination (Seditol[®]) were initially screened at a concentration of 100µg/ml.

Results

Interactions were demonstrated between Seditol, and/or one of its constituent extracts of *Magnolia* and *Ziziphus*, with the adenosine A₁ receptor, dopamine transport and dopamine D₅ receptor (antagonist activity), serotonin receptors (5HT_{1B} and 5-HT₆ antagonist activity), and the GABA benzodiazepine receptor.

Conclusions

The interactions in the receptor binding models are consistent with the reported anxiolytic and sleep-inducing activities of extracts of Magnolia, Ziziphus and their combination.

The results provide further indications of the mode of action of these plant extracts.

Background

Magnolia officinalis bark and *Ziziphus spinosa* seed have a history of use in traditional Asian medicine for mild anxiety, nervousness and sleep-related problems. Both are listed in the Pharmacopeia of the Peoples Republic of China (English Edition, 2005).

Additionally, *Magnolia* bark is listed in the Japanese Pharmacopeia XIV (English Edition, 2001).

Extracts of both *M. officinalis* bark and *Z. spinosa* seed have demonstrated activity in rodent studies. *Magnolia* extracts, in traditional combinations with other herbs, have demonstrated anti-depressant effects in the tail suspension test, the forced swimming test and anxiolytic activity in the elevated plus-maze assay in mice.[1,2] The activity in the plus-maze assay was traced to a constituent in *Magnolia* bark; namely honokiol.[2]

An extract of *Z. spinosa* demonstrated anxiolytic activity in the elevated plus maze assay and the black and white test in mice. Furthermore, the extract demonstrated a sedative effect in prolonging hexobarbital-induced sleeping time in mice.[3] Activity guided fractionation identified spinosin as an active constituent of *Z. spinosa*. [4] Spinosin augmented pentobarbital-induced sleep, increasing sleep time and reducing sleep latency in mice.[5]

Extracts of *M. officinalis* bark and *Z. spinosa* seed are combined in a proprietary product called Seditol that is produced by Next Pharmaceuticals. Seditol is characterized as containing a minimum of 2.7% honokiol and 0.1% spinosin. Seditol is a dietary

supplement, marketed for the improvement of sleep difficulties associated with restlessness, stress or anxiety.

Seditol has been tested for tolerability and benefit in 295 volunteers with mild to moderate sleep difficulties in an open label study. The participants took the product for a minimum of 2 weeks and over 80% reported that Seditol helped them to relax, assisted in a restful sleep and was effective in reducing fatigue due to lack of sleep. [6]

In order to better understand the pharmacological targets for Seditol, the product and its component extracts (Magnolia and Ziziphus) were tested for binding affinity with a number of central nervous system receptors associated with relaxation and sleep. The selected receptor /receptor subtypes included adenosine A₁, dopamine (transporter, D₁, D_{2S}, D₃, D_{4.4} and D₅), serotonin (transporter, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{4e}, 5-HT₆ and 5-HT₇) and the GABA benzodiazepine receptor.

Methods

Test Material

Seditol is a proprietary blend of a patented extract of the bark of *Magnolia officinalis* Rehder & Wilson [Magnoliaceae] and an extract of the seeds of *Ziziphus spinosa* (Buhge) Hu ex. Chen.(syn. *Ziziphus jujube* var. *spinosa* (Bunge) Hu ex HF Chow) [Fam. Rhamnaceae]. The magnolia extract is the subject of two US patents (Nos. 6,582,735 and 6,814,987) describing composition and methods of preventing, treating in managing sleeplessness, restlessness and weight gain due to stress or lack of sleep. Seditol is

characterized as containing a minimum of 2.7% honokiol, an active constituent of *Magnolia officinalis* bark and 0.1% spinosin, a chemical marker of quality for *Ziziphus spinosa* seeds.

Radioligand binding assays

Adenosine binding assays

The binding assays were conducted as described elsewhere.[7] In short, the extracts were investigated in radioligand binding assays at A₁ adenosine receptors of rat brain cortical membranes using the A₁-selective radioligand [³H]2-chloro-N⁶-cyclopentyladenosine ([³H]CCPA). The extracts were dissolved in dimethyl sulfoxide (DMSO) and a final concentration of 2.5% DMSO was used in the assays. Membranes were preincubated with 0.2 I.U./mg protein of adenosine deaminase in order to remove endogenous adenosine. Radioligand binding to rat brain cortical membranes was carried out in TRIS-HCl buffer 50mM, pH 7.4. Assays were performed by incubating the mixtures at 23°C for 90 min. Nonspecific binding was defined using 10 μM of 2-chloroadenosine (CADO). [³H]CCPA was used in a final concentration of 1 nM. Protein (ca. 50 μg per well containing a final volume of 0.2 ml) was added to start the reaction. Incubations were terminated by rapid filtration using a Brandel 96-channel cell harvester (Brandel, Gaithersburg, Maryland, USA) through Packard 96-well GF/B-glass fibre filter plates. Filters were rinsed three times with 0.2 ml of ice-cold TRIS-HCl buffer 50 mM, pH 7.4, each. Radioactivity of the wet 96-well filter plates was counted after 9 h of preincubation with 40μl of Microscint-20 scintillation cocktail (Packard Bioscience). All experiments were performed in triplicate.

Data were analyzed using GraphPad PRISM[®] Version 4.0 (San Diego, CA, USA). For nonlinear regression analysis, the Cheng-Prusoff equation and K_D -values of 0.2nM for [³H]CCPA, 8nM for [³H]MSX-2, 0.41nM for [³H]PSB-603 and 4.9nM for [³H]PSB-11 were used to calculate K_i -values from IC_{50} -values.

Dopamine, Serotonin and GABA central binding

In vitro binding assays were performed using the general procedures shown in Table 1. The in vitro cellular functional assays were performed using the general procedures shown in Table 2. The experimental conditions are summarized in Table 3 and 4, respectively. All receptors were human cloned, except for BZD and 5-HT_{1B}, which were endogenous to the rat cerebral cortex and the Chinese hamster ovary (CHO) cells, respectively.

The extracts were dissolved in DMSO at a concentration of 30 mg/ml and then diluted in water or saline for initial screening at a concentration of 100µg/ml. Selected assays were repeated at a concentration of 10 µg/ml. Each determination was performed in duplicate. In each experiment, the respective reference compound was tested at a minimum of eight concentrations in duplicate to obtain a competition curve in order to validate the experiment.

For the receptor binding studies, the specific radioligand binding to the receptors is defined as the difference between total binding and nonspecific binding determined in the presence of an excess of unlabeled ligand. Results are expressed as a percent of control

specific binding and as a percent inhibition of control specific binding obtained in the presence of the tested extracts.

For the cellular functional assays, the specific radioligand binding to the receptors is defined as the difference between total response and measured specific agonist response determined in the presence of an excess of ligand. Results are expressed as a percent of control specific response or as a percent inhibition of control specific agonist response obtained in the presence of the tested extracts.

GABA_A binding assays

The binding assays were conducted as described elsewhere.[8] Briefly, the experiments were conducted with GABA_A receptors expressed from *Xenopus laevis*. Stage V-VI oocytes were prepared and cRNA injected as previously described. Female *Xenopus laevis* (NASCO, USA) were anesthetized by exposing them for 15 minutes to a 0.2 % MS-222 (methanesulfonate salt of 3-aminobenzoic acid ethyl ester; Sandoz, Germany) solution before surgically removing parts of the ovaries. Follicle membranes from isolated oocytes were enzymatically digested with 2mg/ml collagenase (Type 1A, Sigma, Germany). Synthesis of capped run-off poly(A⁺) cRNA transcripts was obtained from linearized cDNA templates (pCMV vector). One day after enzymatic isolation, the oocytes were injected with 50 nl of DEPC-treated water (diethylpyrocarbonate, Sigma, Germany) containing the different rat cRNAs at a concentration of approximately 300-3000 pg/nl/subunit. The amount of cRNA was determined by means of a NanoDrop ND-1000 (Kisker-biotech, Steinfurt, Germany). To ensure expression of the gamma2S

subunit cRNAs encoding for $\alpha 1$, $\beta 2$ and $\gamma 2S$ subunits were mixed in a ratio of 1:1:10. Oocytes were stored at 18°C in ND96 solution. Voltage clamp measurements were performed on the 1st and 2nd days after cRNA injection. Electrophysiological experiments were performed by the two-microelectrode voltage clamp method making use of a TURBO TEC 01C amplifier (npi electronic GmbH, Tamm, Germany) at a holding potential of -70mV. The bath solution contained 90mM NaCl, 1mM KCl, 1mM MgCl₂, 1mM CaCl₂ and 5mM HEPES (pH 7.4).

GABA and the magnolia extract were applied by means of an automated fast perfusion system.[9] To elicit I_{GABA} , the chamber was perfused with 120 μ l of GABA-containing solution at volume rates of 300 μ l/s. The magnolia extract was solved in DMSO at a concentration of 10 mg/ml and then diluted in bath solution to 10 μ g/ml, 50 μ g/ml and 100 μ g/ml. Indicated concentrations of the magnolia extract were co-applied with GABA EC₃₋₁₀ (effective concentration of GABA that induces 3-10 % of maximal GABA-evoked current). Percent potentiation of I_{GABA} by the magnolia extract was calculated using formula: $(R/C-1)*100\%$, where R is the amplitude of the chloride current evoked by co-application of control GABA EC₃₋₁₀ and the indicated concentration of the magnolia extract, and C is the amplitude of the chloride current evoked by application of control GABA EC₃₋₁₀ alone. Bar graphs were built using Origin software (OriginLab Corporation, USA). Statistical significance was calculated using unpaired Student t-test with a confidence interval of $P<0.05$.

Results

The receptor binding results for selected receptors associated with the neurotransmitters dopamine, serotonin and GABA are summarized in Table 5. Results of interactions with the adenosine A₁ receptor are depicted in Figure 1 and the results of interactions with the GABA benzodiazepine receptor are depicted in Figure 2.

Adenosine:

Seditol had a small interaction with the adenosine A₁ receptor (14±1% inhibition of radioligand binding at 100µg/ml). This effect was due to the Magnolia extract as the Ziziphus extract was not active when tested individually at this concentration. The Magnolia extract exhibited 48±15 % inhibition of radioligand binding at 100µg/ml and a K_i of 9.2±1.1µg/ml (Figure 1). Further testing investigated the nature of this interaction. The Magnolia extract at a concentration of 10µg/ml completely inhibited forskolin-stimulated cAMP accumulation in CHO cells expressing the adenosine A₁ receptor. As this result was considered likely to be an artifact, the functional properties of the magnolia extract were investigated in GTP-shift experiments using rat cortex membrane preparation containing adenosine A₁ receptors. The magnolia extract did not cause a GTP-shift in this experiment.

Dopamine:

The selected receptor/receptor subtypes associated with dopamine included the transporter, D₁, D_{2S}, D₃, D_{4.4} and D₅. Seditol and the Magnolia extract bound with the dopamine transporter and the D₅ receptor (Table 5). Seditol inhibited binding to the DA

transporter by 64% at 100µg/ml and 11% at 10µg/ml. Magnolia inhibited binding to the DA transporter by 39% at 10µg/ml with no results being determined at 100µg/ml due to interference in the test system. The Ziziphus extract was not active at 100µg/ml in this system. Seditol interacted modestly with the D₅ receptor with 21% inhibition of control at 100µg/ml. This activity was due to the Magnolia extract as it caused a 66% inhibition at 100µg/ml and 21% inhibition at 10µg/ml. The Ziziphus extract was not active at 100µg/ml in this system.

Serotonin:

The selected receptor/receptor subtypes associated with serotonin included the transporter, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{4e}, 5-HT₆ and 5-HT₇. Seditol bound modestly as an antagonist to the 5-HT_{1B} and 5-HT₆ receptors (Table 5). Seditol displayed an antagonist effect with the 5-HT_{1B} receptor with 29% inhibition of control at a concentration of 100µg/ml. This activity is due to the presence of the Ziziphus extract, which caused 23% inhibition of control binding at 100µg/ml. The Magnolia extract, when tested separately had no antagonist effect at 100µg/ml and instead had an agonist effect, with 102 % inhibition of control at 100µg/ml. However the Magnolia extract appears not to interfere with the activity of the Ziziphus extract when the two are combined in Seditol. Seditol also displayed an antagonist effect with the 5-HT₆ receptor with 20% inhibition of control at 100µg/ml. This activity is due to the presence of the Magnolia extract which caused a 15% inhibition of control at 10µg/ml and 48% inhibition of control at 100µg/ml.

GABA:

Seditol and the Ziziphus extract did not interact with the GABA central benzodiazepine receptor at a concentration of 100µg/ml. However, individually the magnolia extract had a strong interaction, causing a 61% inhibition at that concentration. The activity of the magnolia extract was eliminated when with the concentration was reduced 10 fold to 10µg/ml (Table 1). This explains why there was no activity demonstrated with Seditol. The magnolia extract strongly potentiated the GABA activated chloride current at the benzodiazepine subunits of the GABA receptor in a bell-shaped dose-response curve with maximum effect at 50µg/ml (Figure 2).

Discussion

In the present series of assays, we demonstrated interactions with the adenosine A₁ receptor, dopamine transporter and dopamine D₅ receptor (antagonist activity), serotonin receptors (5HT_{1B} and 5-HT₆ antagonist activity) and the GABA benzodiazepine receptor. These interactions give us clues as to the potential mode of action of the anxiolytic and sedative properties attributed to Seditol.

Seditol demonstrated an interaction with the adenosine A₁ receptor that appears to be due to the constituent Magnolia extract. Adenosine A₁ is known to play an important role in the initiation of sleep. During the day, adenosine accumulates in neurons and in the evening it is released into the nerve synapses where it prepares the body to enter into a relaxed state.[10] The Magnolia extract was tested for adenosine A₁ agonist activity. The rationale for this is that it was hypothesized that the Magnolia extract might act in a similar means to an extract of Valerian root (*Valeriana officinalis* L). Previously it has been reported that a valerian extract counteracted functional central arousal caused by the

oral administration of caffeine, a well known adenosine antagonist.[11] Results with the Valerian extract indicate that it is a partial agonist of adenosine A₁. [7] The GTP-shift experiments with the Magnolia extract indicate that it may be either a partial agonist in analogy with the Valerian extract, or an antagonist.

Dopamine is a neurotransmitter that plays an important role in mood. Dopamine is increased as an internal reward system leading to feelings associated with pleasure and contentment. Dopamine transporter inhibitors (in this case Seditol) increase the level of dopamine in neural synapses increasing and prolonging the action of dopamine. The dopamine transporter is a target for the development of pharmacotherapies for a number of central disorders including attention deficit hyperactivity disorder (ADHD), obesity, depression, and stimulant abuse.[12] D₁ and D₅ receptor antagonism appear to play a role in the development of fear or anxiety.[13] Seditol and the Magnolia extract displayed D₅ antagonistic activity. In addition, there was a suggestion that Ziziphus may be a D₁ antagonist.

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in a number of physiological functions including sleep, appetite, pain perception, and sexual activity. Several pathological states such as migraine, depression, and anxiety have been linked to the serotonergic system. To date, there are 14 known serotonin receptor subtypes through which serotonin exerts its actions. One receptor in particular, known as 5-HT_{1B}, plays a crucial role in regulating serotonin transmission in the brain. Recent studies have suggested a role for the 5-HT_{1B} receptor in depression, as well as in obsessive-

compulsive disorder, drug addiction, anxiety, aggression and sleep. 5-HT_{1B} antagonists reduce the latency to onset of anxiolytic behavior and play a role in stress regulation with activity comparable to diazepam.[14] Seditol exhibited a mild 5-HT_{1B} antagonist activity that appears to be due to the presence of the Ziziphus extract.

Seditol also displayed a modest antagonist effect with the 5-HT₆ receptor. This activity appears to be due to the presence of the Magnolia extract. Selective 5-HT₆ antagonists may have potential as anxiolytics and antidepressants. [15]

A previous study demonstrated an interaction between a Ziziphus seed extract (drug extract ratio of 7.3:1) and the serotonin receptor subtypes 5-HT_{1A} and 5-HT₂ with a concentration of 10 mg/ml.[16] This study used a different Ziziphus extract in a concentration 100 times lower than that used in the study above and did not get similar results.

Gamma-aminobutyric acid (GABA) is a neurotransmitter that modulates arousal and attention, anxiety, sleep and muscle tone. Sedation and relief from anxiety are the effects of agents acting on GABAergic neurotransmission. Benzodiazepines are a class of drugs that act in this manner, reducing anxiety and inducing sleep through interactions with the GABA_A receptor.

Magnolia extract was found to have a strong effect on the benzodiazepine binding site of the GABA receptor, working in the same direction as benzodiazepines. Previous studies

have reported that a constituent of Magnolia, honokiol, interacts with the GABA receptor.[4,17,18] Magnolol, another constituent of Magnolia, has been shown to be active in the oocyte assay used in this study.[19] Honokiol and magnolol demonstrated activity in the plus maze test with a dose of 0.2 mg/kg for 7 days. Honokiol exhibited activity in the mice at a dose similar to that of diazepam (a benzodiazepine), but did not exhibit the side effects known to be produced by this class of drugs.[20,21]

A Ziziphus extract (drug extract ratio of 7.3:1) has been reported to bind to the GABA_A site.[16] However, these studies did not duplicate those results. The reason may be a difference in the concentration tested, as the positive results were obtained with a concentration of 10mg/ml, 100 times higher than used in these experiments.

Conclusions

The receptors selected for screening were chosen because they are involved in various mental functions related to relaxation, stress, cognition, and sleep. We have demonstrated an interaction between Seditol and/or one of its constituent extracts of Magnolia and Ziziphus with the adenosine A₁ receptor, the dopamine transporter and dopamine D₅ receptor (antagonist activity), serotonin receptors (5HT_{1B} and 5-HT₆ antagonist activity) and the GABA benzodiazepine receptor. The adenosine A₁ and GABA benzodiazepine receptors play a role in the regulation of sleep. The dopamine, serotonin and GABA benzodiazepine receptors are implicated in the management of anxiety and stress.

The affinity of the Magnolia extract with the A₁ and GABA receptors is in line with earlier reports. The modest interaction of Magnolia as an antagonist with 5-HT₆ has to the best of our knowledge not been previously reported. In addition, this may be the first report of interaction between the Ziziphus extract and the 5-HT_{1B} receptor.

The activities in the receptor binding models contribute to an explanation of the mode of action of the extracts of Magnolia, Ziziphus and their combination (Seditol). These results support the subjective experience with Seditol of promoting relaxation allowing consumers to fall asleep faster and more easily. Further investigations using an in-vivo model, involving bioavailability and biotransformation, are important to verify these findings.

Competing interests

This research was supported by Next Pharmaceuticals, Salinas, CA. UK and MB are consultants to Next Pharmaceuticals.

Authors' contributions

UK, MB and DD designed and coordinated the study. SL and AA conducted the adenosine receptor binding studies. UK and MB assisted with the interpretation of the data. MB, UK and DD drafted the manuscript.

Acknowledgements

We acknowledge the support of Next Pharmaceuticals for this work. We thank the laboratory of Christa E. Müller at the University of Bonn, Pharmaceutical Institute for the

adenosine work. We thank Dr. I. Baburin in the Department of Pharmacology and Toxicology, University of Vienna, for the GABA oocyte work.

References

1. Luo L, Nong Wang J, Kong LD, Jiang QG, Tan RX: **Antidepressant effects of Banxia Houpu decoction, a traditional Chinese medicinal empirical formula.** *J Ethnopharmacol.* 2000, 73: 277-281
2. Kuribara H, Kishi E, Hattori N, Okada M, Maruyama Y: **The anxiolytic effect of two oriental herbal drugs in Japan attributed to honokiol from magnolia bark.** *J Pharm Pharmacol.* 2000, 52:1425-1429
3. Peng WH, Hsieh MT, Lee YS, Lin YC, Liao J: **Anxiolytic effect of seed of Ziziphus jujuba in mouse models of anxiety.** *J Ethnopharmacol.* 2000, 72: 435-441
4. Li YJ, Bi KS: **Study on the therapeutic material basis of traditional Chinese medicinal preparation suanzaoren decoction.** *Chem Pharm Bull (Tokyo)* 2006, 54: 847-851
5. Wang LE, Bai YJ, Shi XR, Cui XY, Cui SY, Zhang F, Zhang QY, Zhao YY, Zhang YH: **Spinosin, a C-glycoside flavonoid from semen Ziziphi Spinozae, potentiated pentobarbital-induced sleep via the serotonergic system.** *Pharmacol Biochem Behav.* 2008, 90(3):399-403.
6. LaValle J, Pelletier M, LaValle L, Barrett M, Uwe K, Dolnick D: **A proprietary blend of *Magnolia* and *Ziziphus* extracts assists with sleep: an open-label assessment.** (manuscript submitted for publication in BMC Complementary and Alternative Medicine).

7. Müller CE, Schumacher B, Brattström A, Abourashed EA, Koetter U: **Interactions of valerian extracts and a fixed valerian-hop extract combination with adenosine receptors.** *Life Sci.* 2002, 71(16):1939-49.
8. Khom S, Baburin I, Timin EN, Hohaus A, Sieghart W, Hering S: **Pharmacological properties of GABA_A receptors containing gamma1 subunits.** *Mol Pharmacol* 2006, 69(2):640-9.
9. Baburin I, Beyl S, Hering S: **Automated fast perfusion of *Xenopus* oocytes for drug screening.** *Pflugers Arch* 2006, 453(1):117-23.
10. Basheer R, Strecker RE, Thakkar MM, McCarley RW: **Adenosine and sleep-wake regulation.** *Prog Neurobiol.* 2004, 73(6):379-96.
11. Schellenberg R, Sauer S, Abourashed EA, Koetter U, Brattström A: **The fixed combination of valerian and hops (Ze91019) acts via a central adenosine mechanism.** *Planta Med.* 2004, 70(7):594-7.
12. Runyon SP, Carroll FI: **Dopamine transporter ligands: recent developments and therapeutic potential.** *Curr Top Med Chem.* 2006, 6(17):1825-43.
13. Inoue T, Izumi T, Li XB, Kitaichi Y, Nakagawa S, Koyama T: **Effect of a dopamine D1/5 receptor antagonist on haloperidol-induced inhibition of the acquisition of conditioned fear.** *Eur J Pharmacol.* 2005, 519(3):253-8.
14. Tatarczyńska E, Kłodzińska A, Stachowicz K, Chojnacka-Wójcik E: **Effects of a selective 5-HT1B receptor agonist and antagonists in animal models of anxiety and depression.** *Behav Pharmacol.* 2004, 15(8):523-34.

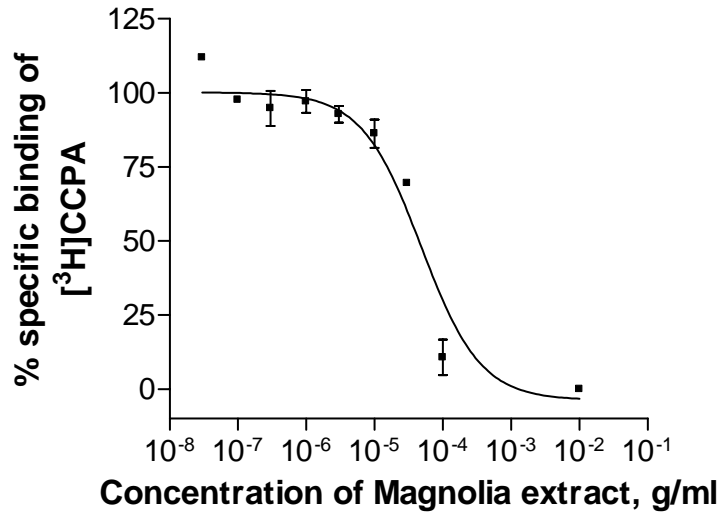
15. Wesolowska A, Nikiforuk A: **Effects of the brain-penetrant and selective 5-HT₆ receptor antagonist SB-399885 in animal models of anxiety and depression.**
Neuropharmacology. 2007, 52(5):1274-83.
16. Liao JF, Jan YM, Huang SY, Wang HH, Yu LL, Chen CF: **Evaluation with receptor binding assay on the water extracts of ten CNS-active Chinese herbal drugs.**
Proc Natl Sci Counc Repub China B 1995, 19:151-158
17. Ai J, Wang X, Nielsen M: **Honokiol and magnolol selectively interact with GABAA receptor subtypes in vitro.** *Pharmacology* 2001, 63:34-41
18. Squires RF, Ai J, Witt MR, Kahnberg P, Saederup E, Sterner O, Nielsen M: **Honokiol and magnolol increase the number of [3H] muscimol binding sites three-fold in rat forebrain membranes in vitro using a filtration assay, by allosterically increasing the affinities of low-affinity sites.** *Neurochem Res* 1999; 24:1593-1602
19. Kim HJ, Baburin I, Khom S, Hering S, Hamburger M: **HPLC-based activity profiling approach for the discovery of GABAA receptor ligands using an automated two microelectrode voltage clamp assay on *Xenopus* oocytes.**
Planta Med. 2008, 74(5):521-6.
20. Kuribara H, Kishi E, Hattori N, Yuzurihara M, Maruyama Y: **Application of the elevated plus-maze test in mice for evaluation of the content of honokiol in water extracts of magnolia.** *Phytother Res* 1999, 13:593-596
21. Kuribara H, Stavinoha WB, Maruyama Y: **Honokiol, a putative anxiolytic agent extracted from magnolia bark, has no diazepam-like side-effects in mice.** *J Pharm Pharmacol* 1999, 51:97-103

22. Speth RC, Wastek GJ, Yamamura HI: **Benzodiazepine receptors: temperature dependence of [³H]flunitrazepam binding.** *Life Sci.* 1979, 24:351-358.
23. Pristupa ZB, Wilson JM, Hoffman BJ, Kish SJ, Niznik HB: **Pharmacological heterogeneity of the cloned and native human dopamine transporter: disassociation of [³H]WIN 35,428 and [³H]GBR 12,935 binding.** *Mol. Pharmacol.* 1994, 45:125-135
24. Tatsumi M, Jansen K, Blakely RD, Richelson E: **Pharmacological profile of neuroleptics at human monoamine transporters.** *Eur. J. Pharmacol.* 1999, 368:277-283
25. Zhao QY, Grandy DK, Thambi L, Kushner JA, Van Tol HMM, Cone R, Pribnow D, Salon J, Bunzow JR, Civelli O: **Cloning and expression of human and rat D₁ dopamine receptors.** *Nature* 1990, 347:76-80
26. Missale C, Nash SR, Robison SW, Jaber M, Caron MG: **Dopamine Receptors: From Structure to Function.** *Physiol. Rev.* 1998, 78:189-225
27. Newman-Tancredi A, Verrielle L, Millan MJ: **Differential modulation by GTPgamma S of agonist and inverse agonist binding to h5-HT_{1a} receptors revealed by [³H]-WAY100,635.** *Brit. J. Pharmacol.* 2001, 132:518-524
27. Sunahara RK, Guan HC, O-Dowd BF, Seeman P, Laurier LG, NG G, George SR, Torchia J, Van Tol HMM, Niznik HB: **Cloning of the gene for a human dopamine D₅ receptor with higher affinity for dopamine than D₁.** *Nature* 1991, 350:614-619

28. Giles H, Landsdell SJ, Bolofo ML, Wilson HI, Martin GR: **Characterization of a 5-HT_{1B} receptor on CHO cells: functional responses in the absence of radioligand binding.** *Br. J. Pharmacol.* 1996, 117:1119-1126
29. Mialet J, Berque-Bestel I, Eftekhari P, Gastineau M, Giner M, Dahmoune Y, Donzeau-Gouge P, Hofbeke J, Langlois M, Sicsic S, Fishmeister R, Lezoualch F: **Isolation of the serotonergic 5-HT_{4(e)} receptor from human heart and comparative analysis of its pharmacological profile in C6-gial and CHO cell lines.** *Brit. J. Pharmacol* 2000, 129:771-781
30. Kohen R, Metcalf MA, Khan N, Druck T, Huebner K, Lachowicz JE, Meltzer HY, Sibley DR, Roth BL, Hamblin MW: **Cloning, characterisation and chromosomal localization of a human 5-HT₆ serotonin receptor.** *J. Neurochem.* 1996, 66:47-56
31. Adham N, Zgombick JM, Bard J, Branchek TA: **Functional characterization of the recombinant human 5-hydroxytryptamine_{7(a)} receptor isoform coupled to adenylate cyclase stimulation.** *J. Pharmacol. Exp. Ther.* 1998, 287:508-514

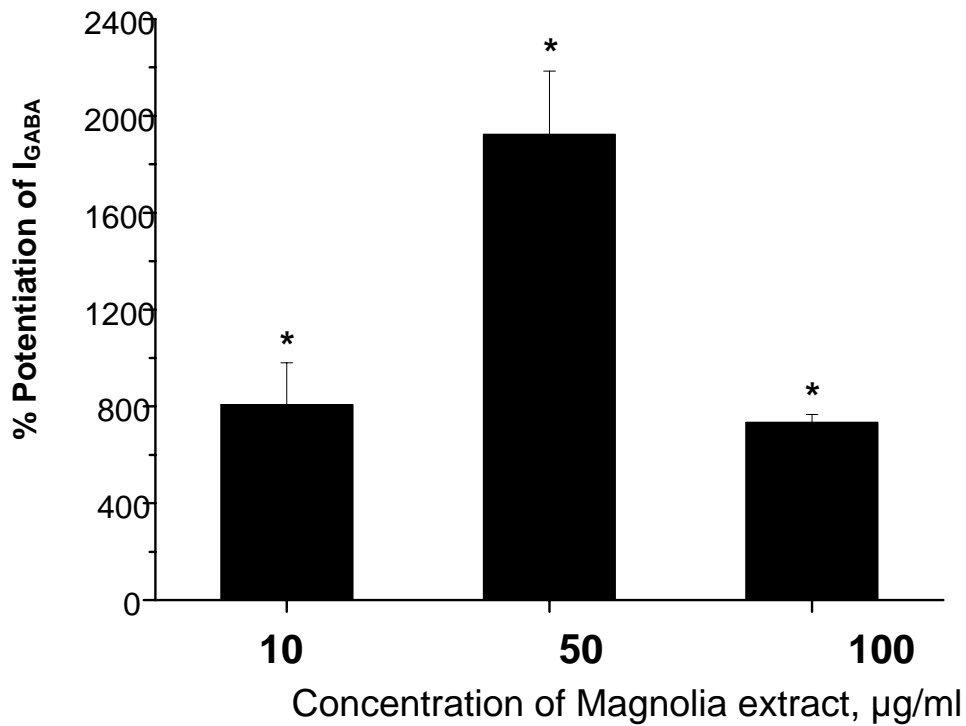
Figures

Figure 1 - Interaction of Magnolia extract with Adenosine A₁



Competition curve of Magnolia extract with rat adenosine A₁ receptor with K_i value of 9.2 μg/ml. Data points represent means of 2 independent experiments including standard errors of the mean.

Figure 2 - Interaction of Magnolia extract with GABA_A



Potentiation of the chloride current through GABA_A receptors composed of α 1, β 2 and γ 2S subunits by 10, 50 or 100 µg/ml of sample M. The Y axis indicates the increase compared to the control current in %. Data are given as mean \pm SEM from three different experiments. (*) Statistical significance from zero ($p < 0.05$).

Tables

Table 1 - Summary of BDZ and transporter receptor-binding assays

Receptor	Reference compound	Reference
BDZ	Diazepam	22
DA transporter	BTCP	23
5-HT transporter	Imipramine	24

Table 2 - Summary of dopamine and serotonin cellular functional assays

Receptor	Reference compound agonist effect	Reference compound antagonist effect	Reference
D ₁	Dopamine	SCH 23390	25
D _{2S}	Dopamine	(+)butaclamol	26
D ₃	Dopamine	(+)butaclamol	26
D _{4.4}	Dopamine	Clozapine	26
D ₅	Dopamine	SCH 23390	26
5-HT _{1A}	8-OH-DPAT	WAY 100635	27
5-HT _{1B}	Serotonin	Methiothepin	28
5-HT _{4E}	Serotonin	GR 113808	29
5-HT ₆	Serotonin	Methiothepin	30
5-HT ₇	Serotonin	Mesulergine	31

Table 3 - General experimental conditions for the BZD and transporter binding assays

Assay	Ligand	Conc. (nM)	Non-specific	Incubation
BZD	[3H]flunitrazepam	0.4	Diazepam (3 μ M)	60 min/4°C
DA transporter	[3H]BTCP	4	BTCP (10 μ M)	120 min/4°C
5-HT transporter	[3H]imipramine	2	Imipramine (10 μ M)	60 min/22°C

Table 4 - General experimental conditions for dopamine and serotonin cellular functional assays

Receptor	Stimulus agonistic effect (control)	Stimulus antagonistic effect (nM)	Incubation
D ₁	none (10 μ M dopamine)	dopamine (300)	30 min/22°C
D _{2S}	none (100 nM dopamine)	dopamine (30)	20 min/37°C
D ₃	none (30 nM dopamine)	dopamine (10)	10 min/37°C
D _{4.4}	none (300 nM dopamine)	dopamine (100)	10 min/37°C
D ₅	none (1 μ M dopamine)	dopamine (50)	30 min/22°C
5-HT _{1A}	none (1 nM 8-OH-DPAT)	8-OH-DPAT (10)	15 min/22°C
5-HT _{1B}	none (10 μ M serotonin)	serotonin (100)	30 min/37°C
5-HT _{4E}	none (1 μ M serotonin)	serotonin (30)	30 min/22°C
5-HT ₆	none (10 μ M serotonin)	serotonin (100)	45 min/37°C
5-HT ₇	none (10 μ M serotonin)	serotonin (100)	45 min/37°C

Table 5. Effects of extracts of Magnolia, Ziziphus and their combination (Seditol) on radioligand binding

Receptor	Magnolia	Ziziphus	Seditol
DA transporter	interference (39)	–	64 (11)
D ₁		antagonist: 14	–
D _{2S}		–	–
D ₃			–
D _{4.4}			–
D ₅	antagonist: 66 (21)	–	antagonist: 21
5-HT transporter	interference	13	–
5-HT _{1A}	–	–	–
5-HT _{1B}	agonist: 102	antagonist: 23	antagonist: 29
5-HT _{4E}			–
5-HT ₆	antagonist: 48 (15)	–	antagonist: 20
5-HT ₇			–
BZD	61	–	–

The results are expressed as a percent inhibition of control specific binding with a concentration of 100µg/ml (mean values; n=2). Occasional tests at a concentration of 10µg/ml are included in parenthesis. (-) Indicates an inhibition of less than 10%. Blank squares indicate that the test was not run.