Published in final edited form as:

Phytother Res. 2013 June ; 27(6): 818–828. doi:10.1002/ptr.4795.

High throughput Screening to Identify Natural Human Monoamine Oxidase B Inhibitors

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Abstract

Age-related increase in monoamine oxidase B (MAO-B) may contribute to CNS neurodegenerative diseases. Moreover, MAO-B inhibitors are used in the treatment of idiopathic Parkinson disease as preliminary monotherapy or adjunct therapy with L-dopa. To date, meager natural sources of MAO-B inhibitors have been identified, and the relative strength, potency and rank of many plants relative to standard drugs such as Selegiline (L-deprenyl, Eldepryl) are not known. In this work, we developed and utilized a high throughput enzyme microarray format to screen and evaluate 905 natural product extracts (0.025–.7 mg/ml) to inhibit *human* MAO-B derived from BTI-TN-5B1-4 cells infected with recombinant baculovirus. The protein sequence of purified enzyme was confirmed using 1D gel electrophoresis-matrix assisted laser desorption ionization-time-of-flight-tandem mass spectroscopy, and enzyme activity was confirmed by [1] substrate conversion (3-mM benzylamine) to H₂O₂ and [2] benzaldehyde. Of the 905 natural extracts tested, the lowest IC₅₀s [<0.07 mg/ml] were obtained with extracts of Amur Corktree (*Phellodendron amurense*), Bakuchi Seed (*Cyamopsis psoraloides*), Licorice Root (*Glycyrrhiza glabra/uralensis*), Babchi (*Psoralea corylifolia seed*). The data also show, albeit to a lesser extent, inhibitory properties of herbs originating from the mint family (*Lamiaceae*) and Turmeric, Comfrey, Bringraj, Skullcap, Kava-kava, Wild Indigo, Gentian and Green Tea. In conclusion, the data reflect relative potency information by rank of commonly used herbs and plants that contain human MAO-B inhibitory properties in their natural form.

Keywords

monoamine oxidase; Parkinson's; herbs; natural medicine; MPTP; dopamine

INTRODUCTION

Monoamine oxidase (MAO, EC 1.4.3.4) is a mitochondrial membrane bound flavin-containing enzyme that catalyzes the oxidative deamination of monoamine neurotransmitters such as dopamine (DA). Due to the function role of MAO in regulation of DAergic neurotransmission, enzyme inhibitors have become of relevant pharmacological interest for

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Conflict of Interest

The authors have declared that there is no conflict of interest.

treating diseases associated with DAergic malfunction such as Parkinson's disease (PD) and depression (Drozak and Kozlowski, 2006; Horn *et al.*, 2010). In the case of PD, type B MAO (MAO-B) inhibitors are used in early to advanced disease states, alone or adjunct to DA agonists such as levodopa, pramipexole, ropinirole, rotigotine or entacapone (Rascol *et al.*, 2011). MAO-B inhibitors are primarily used to modulate symptomatic defect in motor control, by augmenting efficacy of Sinemet creating rise in synaptic DA concentrations (Talati *et al.*, 2009) and in some cases providing a time delay for PD patient requirement to commence traditional levodopa treatment (Lohle and Reichmann, 2011).

Much of the literature suggests that MAO-B inhibitors not only modulate DAergic function, but also impart neurological protection (Polanski *et al.*, 2011) and thereby slow the progressive nature of PD (Lohle and Reichmann, 2011; Fedorova *et al.*, 2011; Pagonabarraga and Kulisevsky, 2010). However, the protective effects of MAO-B inhibitors are still in question, with uncertainty as to distinction between symptomatic or etiological relief associated with the inhibition of MAO or other properties of this class of drugs (Kassubek *et al.*, 2010; van Laar *et al.*, 2010). For example, some studies show that the N-propargyl moiety in/or metabolites formed from rasagiline such as 1-(R)-aminoindan antagonize apoptosis, thereby introducing powerful neuroprotective properties. (Weinreb *et al.*, 2010) Independent of MAO inhibition, rasagiline also prevents mitochondrial permeability, cytochrome c release, casapase activation, DNA damage and affords rise in neurotrophic factors (Naoi and Maruyama, 2009) and neurorescue against post- N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-mediated damage in mice. (Mandel *et al.*, 2007)

A rise in capacity or expression of MAO-B is believed to contribute to age-related neurological injury due to aggravated accumulation of 3,4-dihydroxyphenylacetaldehyde, which is readily metabolized to 3,4-dihydroxyphenylacetic acid, free radicals and orthoquinones which provoke oxidative neurological damage. (Anderson *et al.*, 2011; Jinsmaa *et al.*, 2011) Transgenic mice over expressing MAO-B show substantial oxidative stress and inflammation within the SN, with elevated levels of H₂O₂ which can oxidize DA to dopaminochrome (a mitochondrial toxin) (Mallajosyula *et al.*, 2008; Siddiqui *et al.*, 2010)

While inhibitors of type B monoamine oxidase (MAO-B) such as rasagiline and L-deprenyl are candidate drugs for treatment of PD, it has also been suggested that natural products as in the case of *Banisteriopsis caapi* contain MAO-B inhibitory properties. (Wang *et al.*, 2010). With public interest in this enzyme for treatment of PD and neurological diseases associated with aging of the brain, and very little information on standardized screenings for natural MAO-B inhibitors, we screen and rank 905 products sold worldwide. Extracts included diverse range of natural products from sectioned fresh fruits, fresh vegetables, seeds, rinds, herbs, roots cut and dried to cultural spices often used as Chinese and Indian traditional medicines.

METHODS AND MATERIALS

Hanks Balanced Salt Solution, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), iodoacetamide, DL-Dithiothreitol (DTT), ethanol, 96 well plates, vanillic acid, 4-aminoantipyrine, general reagents and supplies, MAOB and supplies were all purchased from Sigma Scientific (Sigma, St Louis MO). Natural products were provided by Frontier Natural Products Co-op (Norway, IA), Monterey Bay Spice Company (Watsonville, CA), Mountain Rose Herbs (Eugene, OR), Mayway Traditional Chinese Herbs (Oakland, California), Kalyx Natural Marketplace (Camden, NY), Futureceuticals (Mokena, IL), organic fruit vegetable markets and Florida Food Products Inc. (Eustis, FL).

Herbal extraction

Plant and herbal extracts were macerated, diced, chopped and homogenized in 100% ethanol at 50 mg/ml. Samples were placed on a rocker shaker for 24 h and stored in air tight containers at -20°C in the dark. All serial dilutions were made using a diluent consisting of HBSS with 10-mM HEPES adjusted to a pH 7.4.

MALDI MS MS protein identification

Human MAO-B was derived from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA for human MAO-B (Sigma, St Louis MO). The protein was validated by proteomic analysis using matrix assisted laser desorption ionisation (MALDI) Mass Spec (MS/MS) and analyzed by Mascot ID. Briefly, 10 μg of pure enzyme was solubilized, denatured and subjected to 1 D SDS page gel electrophoresis using a 5–20% Tris-HCL gradient gel with a running buffer 25 mM Tris, 192 mM glycine, 0.1% SDS at 200 V for 35 min. High intensity bands for MAO-B at 60 Kd were excised, followed by in gel digestion of peptides with trypsin following reduction/alkylation with DTT and iodoacetamide respectively. Samples were analyzed using MALDI MS/MS (Applied Biosystems) and protein sequence identified by Mascot analysis.

Monoamine oxidase activity

A continuous MAO-B assay was used to conduct high throughput screenings with slight modifications (Holt and Palcic, 2006). Briefly, MAO-B was prepared in HBSS containing 10-mM HEPES, pH 7.4 at a concentration of 3.5 U/ml HBSS. The enzyme and treatments were distributed in 96-well microplates and incubated at RT for 10 min. The substrate [benzylamine] (final working concentration 3 mM) and chromogen from a 5 $\times$ stock [5-mM vanillic acid, 2.5-mM 4-aminoantipyrine and 4 U/ml of horseradish peroxidase type II] were added to each well. Addition of the substrate (1:5 v/v) and the chromogenic solution (1:5 v/v) initiated the reaction, which required 18–24 h at RT for completion. Product formation was quantified every 4 h. At time zero, plates were quantified for O.D. at 490 nm to obtain a pre plate reading accounting for background. For data analysis, the post plates readings at time 4, 8, 12, 16, 20 and 24 h were obtained by subtracting the time zero pre-plate reading to monitor only the continuous product accumulation (increase in O.D at 490 nm).

High throughput design

We designed a model for rapid screening based on the concept used in PCR microarrays for gene amplification. An enzyme microarray format was adopted to where a 96-well plate contained known concentration of enzyme, and 905 treatments of equal concentration dissolved in buffered HBSS were incubated with the enzyme, prior to start of the reaction with the substrate and chromogen. After addition of the substrate, a curve for time-dependent product formation was monitored continuously over 24 h. A first tier investigation was established at a final working concentration of 0.7 mg/ml for each herbal extract. Any compounds that inhibited MAO-B with in the first tier screen below 50% of control were then placed in a second (final concentration = .4 mg/ml), third tier (final concentration = .2 mg/ml) and fourth tier (final concentration = .07 mg/ml). Extracts were ranked for potency, and the most potent were further evaluated for IC_{50} s along with deprenyl as a positive control. The enzyme microarray format is very rapid, reproducible and validated by triplicates and corroborated by a four tier evaluation process.

Benzaldehyde production

Quantification of benzaldehyde was continuously monitored using a Shimadzu high-performance liquid chromatography (HPLC) system equipped with an SPD-20A UV detector (set at 254 nm), a workstation containing EZSTART version 7.4 software and an

SS420X instrument interface docked to a Waters Autosampler Model 717 Plus (Shimadzu Scientific Instruments, Inc. US; Waters Corp., Milford, MA). Benzaldehyde standard curves were established, and samples taken from the enzyme reaction chamber were mixed with 2x distilled water and then 50:50 with methanol prior to injection. The flow rate was isocratic at 1.2 ml/min. The mobile phase consisted of 10-mM sodium phosphate (pH 2.7) mixed at 45:55 with methanol. The column was a TSK-gel ODS-100 V 5 μ m 4.6 mm (id) $\times$ 15.0 cm (L); (Tosoh Corporation), the run time was 6 mins and injection volume 25 μ l.

Determination of H⁺ concentration

Although we used 10-mM HEPES buffered HBSS (pH 7.4) as a diluent, due to the high number of compounds and potential for pH to contribute to inhibitory effects in the enzyme reaction, in particular for acidic fruits, a high throughput evaluation for pH was conducted in 96-well plates using standard phenol red indicator dye. Briefly, extract matrix blanks for extracts displaying inhibitory properties were evaluated for influence on pH by establishing a pre-plate reading at 550 nm using a Spectra 190-MAX UV spectrophotometric detector (Molecular devices, Sunnydale, CA, USA) and post plate reading after addition of phenol red stock solution (0.2 mg/ml water) in HBSS (15% v/v) to each well. The change in pH was immediately assessed relative to control blanks at 550 nm relative to control blanks. Any extracts that altered the pH of the reaction vessel were buffered to pH 7.2 and re-evaluated at neutral pH for MAO-B inhibition.

Determination of H₂O₂ Non enzymatic stoichiometric radical scavenging

Due to the high number of compounds and potential for antioxidant effects to yield a false positive for MAO-B inhibitor identification (an enzyme reaction that monitors continuous production of H₂O₂), all extracts at concentrations demonstrating MAO-B inhibitor activity were evaluated for ability to scavenge peroxide 30 μ M. Briefly, H₂O₂ was incubated in the presence of experimental compounds at RT for 20 min. A pre-plate reading was obtained where the data was subtracted from the final value to eliminate background interference. The chromogenic reagent (5x stock [5-mM vanillic acid, 2.5-mM 4-aminoantipyrine and 4 U/ml of horseradish per-oxidase type II]) was added to each sample and incubated for 10 min at 37°C. Samples were analyzed at 490 nm using a Spectra 190-MAX UV spectrophotometric detector (Molecular devices, Sunnydale, CA, USA). Extracts showing ability to scavenge peroxide were validated by HPLC via quantification of benzaldehyde.

Data analysis

Statistical analysis was performed using Graph Pad Prism (version 3.0; Graph Pad Software Inc. San Diego, CA, USA) with significance of difference between the groups assessed using a one-way ANOVA, followed by Tukey post hoc means comparison test, a two-way ANOVA or Student's *t* test. IC50s were determined by regression analysis using Origin Software (OriginLab, Northampton, MA).

RESULTS

Method validation was established by monitoring the continuous time-dependent product formation in the presence of a substrate (benzylamine 2 mM) $\pm$ MAO-B $\pm$ Selegiline (L-deprenyl) (100 μ M) (Fig. 1a [H₂O₂] and Fig. 1b [benzaldehyde]). The data show a slow but steady rate of reaction, resulting in time-dependent product formation with high signal/noise ratio. Protein sequencing using MALDI MS/MS and analysis by Mascot ID showed a positive hit for human MAO-B with a 95% confidence interval for peptide/sequence mass (Fig. 2). MAO-B positive controls were established using a known inhibitor [L-deprenyl] which showed significant potency and a complete loss of product formation [H₂O₂] and [benzylamine] at 1 μ M (Fig. 3a and 3b). The data reflect a greater sensitivity observed by

HPLC quantification of benzaldehyde than colorimetric assessment of H₂O₂ despite similar trends in enzyme inhibition.

A high throughput enzyme microarray model was developed and used in this work as described in Fig. 4. We designed a model for rapid screening based on a similar concept often applied in microarray analysis for quantitative real-time RT-PCR. However, instead of gene amplification, an enzyme microarray format was adopted to where a 96-well plate contained known concentration of enzyme and 905 treatments of equal concentration dissolved in buffered HBSS were incubated with the enzyme, prior to start of the reaction. After addition of the substrate, a curve for time-dependent product formation was monitored continuously over 24 h. A first tier investigation was established at a final working concentration of 0.7 mg/ml for each herbal extract. Any extract that inhibited MAO-B with in the first tier screen below 50% of control was then subject to a second (final concentration = .4 mg/ml), third tier (final concentration = .2 mg/ml) and fourth tier (final concentration = .07 mg/ml). A total of 905 extracts were evaluated at a final working concentration of .7 mg/ml. Of these, 133 demonstrated an IC₅₀ < 0.7 mg/ml. Of the 133 retested at .4 mg/ml, 66 extracts showed an IC₅₀ < 0.4 mg/ml. Of the 66 retested at .2 mg/ml, 26 extracts showed an IC₅₀ < 0.2 mg/ml. Of the 26 retested at .2 mg/ml, five extracts showed IC₅₀ < 0.07 mg/ml. All extracts showing inhibitory properties were evaluated for potential interfering variable of pH shifts or radical scavenging abilities (which would render false positive based on MAO-B activity based on formation of H₂O₂). False positives were eliminated by adjusted pH and confirmed by benzaldehyde product accumulation by HPLC. All inhibitors are listed in Table 1 where extracts demonstrating the greatest potency are listed as Level 1 (strongest) IC₅₀ < .07 mg/ml, followed by Level 2 (strong) IC₅₀ < .2 mg/ml, Level 3 (moderately strong) IC₅₀ > .2 < .4 mg/ml, Level 4 (moderate) (IC₅₀ > .4 < .7 mg/ml) and Level 5 (weak) IC₅₀ = .7 mg/kg. The most potent natural MAO-B inhibitors were identified, confirmed and an IC₅₀ established (Fig. 5). The findings in this study yield evidence to suggest there are a number of natural products commonly used worldwide which have capacity to inhibit human MAO-B enzyme. However, in the therapeutic range, the most likely candidates were Amur Cork tree, Licorice, Psoralea Fruit and Bakuchi.

DISCUSSION

In this study, we screened 905 natural product extracts to elucidate potential sources of human MAO-B inhibitors. The data elucidate a number of new potential sources and specify the relatively few that inhibited MAO-B within therapeutic range. The herbal extracts showing greatest potency included Amur Corktree (*Phellodendron amurense*), Licorice Root (*Glycyrrhiza glabra* and *Glycyrrhiza uralensis*), Psoralea Fruit (*Psoralea corylifolia* (PC)) and Bakuchi Seed (*Cyamopsis psoralioides*). While it is outside the scope of this paper to review all MAO-B inhibitors identified, we will focus the discussion on the most potent elucidated.

New findings presented from this work show potent MAO-B inhibitory capacity inherent within Bakuchi seed, an otherwise understudied ayurvedic historical medicine most known for treatment of vitiligo, where it is associated with regeneration of melanocytes (Dhanik *et al.*, 2011). It is of interest to note that this plant also contains constituents such as psoralens or hydroxybakuchiol which promote skin pigmentation (Khushboo *et al.*, 2010) augment melanin dispersal (Sultan and Ali, 2011) and augment central DAergic function due to direct inhibition of the DA transporter, making it a likely candidate for treatment of PD (Zhao *et al.*, 2007).

In this study, one of the most interesting findings (also corroborated by others) was the MAO-B inhibitory effects of psoralens or furocoumarins (derived from PC seeds), which

impart anti-depressant effects via reduction of stress evoked plasma cortisol (Chen *et al.*, 2005; Chen *et al.*, 2007; Kong *et al.*, 2001) and protect against MPTP-induced DAergic loss within substantia nigra in mice. (Zhao *et al.*, 2009) The anti-depressant properties of PC also correspond to the elevation of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid, as well as rise in striatal DA, effects which are concomitant to reduction of stress-evoked serum corticotropin-releasing factor and adrenal corticotropin-releasing hormone, suggesting a number of neurotransmitter systems are affected. (Yi *et al.*, 2008; Xu *et al.*, 2008).

While future research will have to be initiated on PC and Bakuchi seed, a plethora of existing work demonstrates a range of therapeutic values that exist outside the scope of PD, including a potential to treat cancer and osteoarthritis, (Ming *et al.*, 2011), reduce bone loss (Lim *et al.*, 2009) treat bacterial/viral infections or inflammatory related disease (Szliszka *et al.*, 2011; Yang *et al.*, 2011; Li *et al.*, 2011; Khushboo *et al.*, 2010). In addition, constituents within PC have been well characterized and known to include several classes of compounds; e.g. psoralenoside (benzofuran glycosides), psoralen, isopsoralen, psoralidin (coumarins), bakuchiol (meroterpenes) (Qiu *et al.*, 2011; Song *et al.*, 2011) corylifolean, corylifolin, bakuchicin, isobavachin, bavachinin, bavachalcone, isobavachalcone, corylin, corylidin, bavachromene, astragalin, p-hydroxybenzoic acid stigmasterol, triaconate and β -sitosterol-D-glucoside (Khushboo, Jadhav, 2010).

Glycyrrhiza glabra/uralensis

In a strikingly similar pattern to that of PC, existing research also shows capacity of Glycyrrhiza glabra to reduce stress response in mice through elevating brain concentrations of norepinephrine and DA comparable to that of tricyclic anti-depressant drugs; imipramine and fluoxetine (Dhingra and Sharma, 2006). The data from the current work also corroborate the effects of glycyrrhiza uralensis and glabra roots to have MAO inhibitor capacity with reported IC₅₀ at .03 mg/ml (Tanaka *et al.*, 1987; Hatano *et al.*, 1991a) where the same Hatano *et al.* also define a number of licorice constituents such as licopyranocoumarin licocoumarone and glycyrrhisoflavone being responsible for MAO inhibition. (Hatano *et al.*, 1991b).

Licorice is a highly studied natural medicine, with therapeutic values ranging from anti-inflammatory properties in microglial BV2 cells, neuroprotective properties against 6-hydroxydopamine cytotoxicity/MPTP nigrostriatal DAergic degeneration in mice (Kim *et al.*, in press) and reduction of glutamate-mediated excitotoxicity associated with hippocampal neuronal cell death. (Yang *et al.*, 2012) Many of these therapeutic properties appear to enhance the case for licorice in therapeutic potential application for PD.

Unlike, other herbs referenced in this study, there is meager research on therapeutic effects or characterization of Amur Corktree *Phellodendron amurense* in relation to any aspect of DAergic neurotransmission. However, recent interest in this plant surrounds its anti-inflammatory and anti-cancer properties, in addition to preventing osteoarticular cartilage and chondrocyte destruction. (Xian *et al.*, 2011; Kim *et al.*, 2011; Ghosh *et al.*, 2010) Future research will be required to further investigate therapeutic aspects of Amur Corktree in processes inherent to degenerative disease specific to PD.

As a note, in the past, we had investigated a number of compounds for MAO inhibitory activity in foods on a very small scale and at that time had eluded to the fact that green tea catechins and curcumin were likely candidate MAO-B inhibitors. (Mazzio *et al.*, 1998) Since then, a number of studies have examined this and shown green tea components such as epigallocatechin gallate and curcumin supplementation exert reduction of brain MAO-B enzyme activity in rats (Lin *et al.*, 2010), effects which are synergist with rasagiline in PD mice models (Ashizawa and Sano, 1990) and administration which has reversed MPTP

induced loss of DA. (Rajeswari and Sabesan, 2008) The findings from this current study broaden our view of potential MAO-B candidates yielding a number of herbs potentially more or equally as promising to green tea or turmeric.

The identification of new natural or synthetic MAO-B inhibitors which provide therapeutic advantage in treatment of PD must be viewed in light of differential selectivity from MAO-A, due to risk of hypertensive crisis in the presence of tyramine containing foods. Although, it is estimated that the adverse effects are less than previously believed (Gillman, 2011), in particular for rasagiline selectivity when used at the recommended dose (Chen and Wilkinson, 2012; Isaacson, 2010) (Goren *et al.*, 2010), future potential drug development should include a tyramine challenge control for *in vivo* animal studies.

In conclusion, the findings from this paper open up new areas for future research specifying specific natural plants with capacity to inhibit MAO-B, that could lead to medicines with greater capacity to treat diseases associated with maladaptive DAergic function with in the CNS.

Acknowledgments

This research was supported by a grant from NIH NCRR RCMI program (G12RR 03020) and the National Institute of Minority Health and Health Disparities, NIH (8G12MD007582-28 and 1P20 MD006738-01).

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Colorimetric : Time Dependent *Human* MAO-B Product formation
[Hydrogen Peroxide] $\pm$ Enzyme $\pm$ Selegiline (L-deprenyl) (100 μ M)

HPLC: Time Dependent *Human* MAO-B Product formation
[Benzylaldehyde] $\pm$ Enzyme $\pm$ Selegiline (L-deprenyl) (100 μ M)

Figure 1.

A. *Human* MAO-B activity - Time-dependent H₂O₂ product formation from 3-mM benzylamine in the presence or absence of *human* MAO-B, and in the presence of 3-mM benzylamine + 100 μ M of deprenyl. The data represent μ M H₂O₂ produced from 0–18 h (incubation at RT) and are presented as the Mean $\pm$ S.E.M, $n=4$. Significance of difference for product formation between the Time 0 control *versus* 2–18 h was determined using a one-way ANOVA followed by a Tukey post hoc test * $p < .05$. **B.** *Human* MAO-B activity - Time-dependent benzaldehyde product formation from 3-mM benzylamine in the presence or absence of *human* MAO-B, and in the presence of 3-mM benzylamine + 100 μ M of deprenyl. The data represent μ M benzaldehyde produced from 1–12 h (incubation at RT) and are presented as the Mean $\pm$ S.E.M, $n=4$. Significance of difference for product formation between the time 1 h *versus* 6 and 12 h was determined using a one-way ANOVA followed by a Tukey post hoc test. * $p < .05$. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

1D Gel Electrophoresis : MALDI -TOF MS MS Analysis of Enzyme

Figure 2.

Mascot results for protein identification by peptide mass fingerprinting of *Human* MAO-B tryptic digest analyzed by MALDI-TOF/TOF-MS. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

Figure 3.

A. Deprenyl inhibitory effects on *Human* MAO-B. The data represent product formation (μM benzylamine) produced at 24 h (incubation at RT) in the presence or absence of deprenyl (82 – 1000 nM) and are presented as the Mean $\pm$ S.E.M, $n=4$. Significance of difference for product formation between the controls *versus* deprenyl was determined using a one-way ANOVA followed by a Tukey post hoc test. * $p<.05$. **B.** Deprenyl inhibitory effects on *Human* MAO-B. The data represent product formation (μM H₂O₂) produced at 24 h (incubation at RT) in the presence or absence of deprenyl (82 – 375 nM) and are presented as the Mean $\pm$ S.E.M, $n=4$. Significance of difference between the controls *versus* activity in the presence of deprenyl was determined using a one-way ANOVA followed by a Tukey post hoc test. * $p<.05$. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

Figure 4.

A high throughput enzyme experimental microarray design. 905 extracts were evaluated for capacity to inhibit *Human* MAO-B. A first tier screening was conducted at a final working concentration of 0.7 mg/ml for each herbal extract. Enzyme activity was continuously monitored over a 24-h period. Extracts demonstrating an $IC_{50} < 0.7$ mg/ml were screened through a tier 2 screening at .4 mg/ml. Extracts demonstrating an IC_{50} at < 0.4 mg/ml were screened through a tier 3 screening at .2 mg/ml. Extracts demonstrating an IC_{50} at < 0.2 mg/ml were screened through a tier 4 screening at .07 mg/ml.. All extracts showing inhibitory properties were evaluated for potential interfering variable of pH shifts or radical scavenging abilities (which would render false positive based on MAO-B activity based on formation of H_2O_2). This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

Figure 5.

Most potent herbal extract inhibitors of *human* MAO-B activity. The data represent product formation (H_2O_2) as % control produced at 24 h (incubation at RT) in the presence or absence of extracts (.025–.8 mg/ml) and are presented as the Mean, $n=4$. IC_{50} concentrations were established from a sigmoidal fit dose–response equation and significance of difference between the controls *versus* treatment was determined using a one-way ANOVA followed by a Tukey post hoc test * $p<.05$. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

Table 1

Human MAO-B inhibitors by potency. Extracts demonstrating the greatest potency are listed as Level 1 (strongest) IC₅₀ <.07 mg/ml, followed by Level 2 (strong) IC₅₀ <.2 mg/ml, Level 3 (moderately strong) IC₅₀ >.2<.4 mg/ml, Level 4 (moderate) (IC₅₀>.4 < .7 mg/ml) and Level 5 (weak) IC₅₀= .7 mg/kg
Natural plant sources of *human* MAO-B inhibitors

LEVEL	ID	Common name	Scientific name	Inhibitory conc.
LEVEL 1	A9	Amur Cork Tree	<i>Phellodendron amurense</i>	IC ₅₀ <.07 mg/ml
	B54	Bakuchi Seed ^a	<i>Cyamopsis psoralioides</i>	IC ₅₀ <.07 mg/ml
	L2	Licorice Root	<i>Glycyrrhiza glabra</i>	IC ₅₀ <.07 mg/ml
	G22	Gan Cao ^a	<i>Glycyrrhiza uralensis root</i>	IC ₅₀ <.07 mg/ml
	P58	Psoralea Fruit Babchi ^a	<i>Psoralea corylifolia</i>	IC ₅₀ <.07 mg/ml
LEVEL 2	P15	Beefsteakplant ^a	<i>Perilla frutescens leaf</i>	IC ₅₀ <.2 mg/ml
	W4	Black Pepper	<i>Piper nigrum</i>	IC ₅₀ <.2 mg/ml
	B15	Bringraj 'False Daisy'	<i>Eclipta erecta</i>	IC ₅₀ <.2 mg/ml
	C34	Chinese arborvitae ^a	<i>Truja, Platycladus orientalis twig/leaf</i>	IC ₅₀ <.2 mg/ml
	C2	Comfrey	<i>Symphytum officinale leaf</i>	IC ₅₀ <.2 mg/ml
	C84	Curry powder	<i>Coriander, turmeric, cumin, fenugreek, red pepper</i>	IC ₅₀ <.2 mg/ml
	F4	Figwort	<i>Scrophularia nodosa</i>	IC ₅₀ <.2 mg/ml
	S54	Gloryvine Stem	<i>Sargentodoxa cuneata</i>	IC ₅₀ <.2 mg/ml
	XT3	Green Tea	<i>Camellia sinensis</i>	IC ₅₀ <.2 mg/ml
	H20	Herb de province	<i>Oregano, thyme, savory, lavender, basil, sage, rosemary</i>	IC ₅₀ <.2 mg/ml
	K8	Kava Kava	<i>Piper methysticum</i>	IC ₅₀ <.2 mg/ml
	L45	Ladys' Mantle	<i>Alchemilla vulgaris</i>	IC ₅₀ <.2 mg/ml
	A37	Lesser Galangal	<i>Alpinia officinarum Root</i>	IC ₅₀ <.2 mg/ml
	L35	Red Henna	<i>Lawsonia inermis</i>	IC ₅₀ <.2 mg/ml
	C53	Sappanwood	<i>Caesalpinia sappan wood</i>	IC ₅₀ <.2 mg/ml
	D10	Thick-stemmed Wood ^a	<i>Dryopteris crassirhizoma</i>	IC ₅₀ <.2 mg/ml
	T3	Turmeric ^a	<i>Curcuma Longa</i>	IC ₅₀ <.2 mg/ml
	W13	Watercress	<i>Nasturtium officinale</i>	IC ₅₀ <.2 mg/ml
	W3	Wild Indigo Root	<i>Baptisia tinctoria</i>	IC ₅₀ <.2 mg/ml
	W10	Wild lettuce	<i>Lactuca virosa</i>	IC ₅₀ <.2 mg/ml
	G6	Yellow Gentian Root	<i>Gentiana lutea</i>	IC ₅₀ <.2 mg/ml
LEVEL 3	A4	Alfalfa	<i>Medicago sativa</i>	IC ₅₀ >.2 <.4 mg/ml
	A48	Alpinia	<i>Alpinia katsumadai seed</i>	IC ₅₀ >.2 <.4 mg/ml
	B48	Bay Leaf	<i>Laurus nobilis</i>	IC ₅₀ >.2 <.4 mg/ml
	B13	Bayberry Root bark ^a	<i>Morella cerifera</i>	IC ₅₀ >.2 <.4 mg/ml
	C103	Cedar Berries	<i>Juniperus monosperma</i>	IC ₅₀ >.2 <.4 mg/ml
	C101	Celandine	<i>Chelidonium majus</i>	IC ₅₀ >.2 <.4 mg/ml
	S56	Common hedgenettle	<i>Stachys officinales</i>	IC ₅₀ >.2 <.4 mg/ml

LEVEL	ID	Common name	Scientific name	Inhibitory conc.
	E3	Eucalyptus Leaf	<i>Eucalyptus globulus</i>	IC ₅₀ >.2 <.4 mg/ml
	E6	Eyebright	<i>Euphrasia officinalis</i>	IC ₅₀ >.2 <.4 mg/ml
	D18	Fringed Pink	<i>Dianthus superbus</i>	IC ₅₀ >.2 <.4 mg/ml
	G31	Garam Marsala	<i>Garam Marsala</i>	IC ₅₀ >.2 <.4 mg/ml
	G28	Gourmet pepper mill	<i>Gourmet pepper mill</i>	IC ₅₀ >.2 <.4 mg/ml
	G29	Gunpowder green tea	<i>Camellia sinensis</i>	IC ₅₀ >.2 <.4 mg/ml
	H22	Hyssop	<i>Hyssopus officinalis</i>	IC ₅₀ >.2 <.4 mg/ml
	P89	Indian long pepper	<i>Piper longum</i>	IC ₅₀ >.2 <.4 mg/ml
	I2	Isatis Leaf	<i>Isatis indigotica; Isatis tinctoria</i>	IC ₅₀ >.2 <.4 mg/ml
	I8	Italian spice herbal tea	<i>Italian spice herbal tea</i>	IC ₅₀ >.2 <.4 mg/ml
	K9	Kola Nut	<i>Cola acuminata</i>	IC ₅₀ >.2 <.4 mg/ml
	L43	Lemon Curry	<i>Lemon curry</i>	IC ₅₀ >.2 <.4 mg/ml
	L49	Lemon Verbana	<i>Aloysia triphylla</i>	IC ₅₀ >.2 <.4 mg/ml
	M20	Lilly Tree	<i>Magnolia denudata flower</i>	IC ₅₀ >.2 <.4 mg/ml
	L46	Linden Leaf	<i>Tilia europaea</i>	IC ₅₀ >.2 <.4 mg/ml
	M38	Maiden Hair Fern	<i>Adiantum capillus</i>	IC ₅₀ >.2 <.4 mg/ml
	M40	Motherwort	<i>Leonurus cardiaca</i>	IC ₅₀ >.2 <.4 mg/ml
	P4	Parsley	<i>Petroselinum crispum</i>	IC ₅₀ >.2 <.4 mg/ml
	R4	Red Clover	<i>Trifolium pratense</i>	IC ₅₀ >.2 <.4 mg/ml
	R9	Red Sandlewood	<i>Pterocarpus santalinus</i>	IC ₅₀ >.2 <.4 mg/ml
	L33	Rough bugleweed	<i>Lycopus lucidus</i>	IC ₅₀ >.2 <.4 mg/ml
	S61	Sage	<i>Salvia officinalis</i>	IC ₅₀ >.2 <.4 mg/ml
	S23	Sassafras	<i>Sassafras Albidum</i>	IC ₅₀ >.2 <.4 mg/ml
	E21	Scouringrush horsetail	<i>Equisetum Heimale</i>	IC ₅₀ >.2 <.4 mg/ml
	S32	Skull cap ^a	<i>Scutellaria barbata</i>	IC ₅₀ >.2 <.4 mg/ml
	S16	Skull cap ^a	<i>Scutellaria lateriflora</i>	IC ₅₀ >.2 <.4 mg/ml
	S64	Spearmint ^a	<i>Mentha spicata</i>	IC ₅₀ >.2 <.4 mg/ml
	S20	Stevia/Candyleaf	<i>Stevia rebaudiana</i>	IC ₅₀ >.2 <.4 mg/ml
	S15	Szechuan pepper	<i>Zanthoxylum bungeanum</i>	IC ₅₀ >.2 <.4 mg/ml
	W11	White Sage	<i>Salvia apiana</i>	IC ₅₀ >.2 <.4 mg/ml
	Y8	Yohimbe Bark	<i>Corynanthe yohimbe</i>	IC ₅₀ >.2 <.4 mg/ml
	Y7	Young Hyson Green Tea	<i>Camellia sinensis</i>	IC ₅₀ >.2 <.4 mg/ml
	V1	Zi Hua Di Ding	<i>Viola yedoensis herb</i>	IC ₅₀ >.2 <.4 mg/ml
LEVEL 4	ID	Common Name	Scientific Name	Inhibitory Conc.
	A67	Ancho chili pepers	<i>Capsicum annum</i>	IC ₅₀ >.4 <.7 mg/ml
	B19	Blackberry leaf	<i>Rubus fruticosus</i>	IC ₅₀ >.4 <.7 mg/ml
	B12	Blackberry Root	<i>Rubus fruticosus or Rubus Villosus</i>	IC ₅₀ >.4 <.7 mg/ml
	F17	Bladderwrack	<i>Fucus vesiculosus</i>	IC ₅₀ >.4 <.7 mg/ml
	B26	Blessed Thistle	<i>Cnicus benedictus</i>	IC ₅₀ >.4 <.7 mg/ml

LEVEL	ID	Common name	Scientific name	Inhibitory conc.
	C1	California Poppy	<i>Eschscholzia californica</i>	IC ₅₀ > .4 < .7 mg/ml
	C87	Catnip ^a	<i>Nepeta cataria</i>	IC ₅₀ > .4 < .7 mg/ml
	H16	Chameleon	<i>Houttuynia cordata herb</i>	IC ₅₀ > .4 < .7 mg/ml
	c80	Cilantro Leaf	<i>Coriandrum sativum</i>	IC ₅₀ > .4 < .7 mg/ml
	C39	Cinnamon Twig	<i>Cinnamomum cassia</i>	IC ₅₀ > .4 < .7 mg/ml
	C9	Cleavers	<i>Galium aparine</i>	IC ₅₀ > .4 < .7 mg/ml
	A49	Clematis trifoliata	<i>Akebia trifoliata fruit</i>	IC ₅₀ > .4 < .7 mg/ml
	D1	Damiana Leaf	<i>Turnera diffusa</i>	IC ₅₀ > .4 < .7 mg/ml
	E24	Earl Grey black tea	<i>Earl Grey black tea</i>	IC ₅₀ > .4 < .7 mg/ml
	E23	Elder berries	<i>Sambucus nigra</i>	IC ₅₀ > .4 < .7 mg/ml
	E2	Epipedium	<i>Epipedium grandiflorum</i>	IC ₅₀ > .4 < .7 mg/ml
	E15	Epipedium	<i>Epipedium koreanum</i>	IC ₅₀ > .4 < .7 mg/ml
	F2	Feverfew	<i>Tanacetum parthenium</i>	IC ₅₀ > .4 < .7 mg/ml
	C17	Garden Chervil	<i>Anthriscus cerefoilium</i>	IC ₅₀ > .4 < .7 mg/ml
	G30	German chamomile	<i>Matricaria recutita</i>	IC ₅₀ > .4 < .7 mg/ml
	G37	Goats Rue	<i>Galega officinalis</i>	IC ₅₀ > .4 < .7 mg/ml
	G8	Great Valley gumweed	<i>Grindelia camporum</i>	IC ₅₀ > .4 < .7 mg/ml
	L3	Gromwell root	<i>Lithospermum erythrorhizon root</i>	IC ₅₀ > .4 < .7 mg/ml
	G4	Gymnema	<i>Gymnema sylvestre</i>	IC ₅₀ > .4 < .7 mg/ml
	H3	Hops, <i>whole</i>	<i>Humulus lupulus</i>	IC ₅₀ > .4 < .7 mg/ml
	H8	Horsetail	<i>Equisetum arvense</i>	IC ₅₀ > .4 < .7 mg/ml
	P14	Japanese Indigo	<i>Polygonum tinctorium levis</i>	IC ₅₀ > .4 < .7 mg/ml
	K2	Kombu	<i>Laminaria setchellii</i>	IC ₅₀ > .4 < .7 mg/ml
	L6	Lily of the valley	<i>Convallaria majalis</i>	IC ₅₀ > .4 < .7 mg/ml
	L23	Lysimachia	<i>Lysimachia christinae</i>	IC ₅₀ > .4 < .7 mg/ml
	M32	Marjoram ^a	<i>Origanum majorana</i>	IC ₅₀ > .4 < .7 mg/ml
	M31	Melilot Herb	<i>Melilotus vulgaris</i>	IC ₅₀ > .4 < .7 mg/ml
	M34	Mint	<i>Mentha haplocalyx</i>	IC ₅₀ > .4 < .7 mg/ml
	M39	Mistle Toe	<i>Phoradendron flavescens</i>	IC ₅₀ > .4 < .7 mg/ml
	M8	Mugwort	<i>Artemisia vulgaris</i>	IC ₅₀ > .4 < .7 mg/ml
	M36	Mullein leaf	<i>Verbascum thapsus</i>	IC ₅₀ > .4 < .7 mg/ml
	P12	Papaya Leaf	<i>Carica papaya</i>	IC ₅₀ > .4 < .7 mg/ml
	P5	Peach leaf	<i>Prunus persica</i>	IC ₅₀ > .4 < .7 mg/ml
	P81	Pennyroyal	<i>Mentha pulegium</i>	IC ₅₀ > .4 < .7 mg/ml
	P56	Peony	<i>Paeonia suffruticosa root - bark</i>	IC ₅₀ > .4 < .7 mg/ml
	P79	Peppermint ^a	<i>Mentha piperita</i>	IC ₅₀ > .4 < .7 mg/ml
	P88	Periwinkle	<i>Vinca minor</i>	IC ₅₀ > .4 < .7 mg/ml
	P3	Plantain Herb	<i>Plantago asiatica</i>	IC ₅₀ > .4 < .7 mg/ml

LEVEL	ID	Common name	Scientific name	Inhibitory conc.
	P85	Plantain Leaf	<i>Plantago major</i>	IC ₅₀ > .4 < .7 mg/ml
	R21	Red rose And Petals	<i>Rosa damascena</i>	IC ₅₀ > .4 < .7 mg/ml
	R2	Redroot	<i>Ceanothus americanus</i>	IC ₅₀ > .4 < .7 mg/ml
	C77	Senna Leaf	<i>Cassia angustifolia leaf</i>	IC ₅₀ > .4 < .7 mg/ml
	S19	Shepards Purse ^a	<i>Capsella bursa-pastoris</i>	IC ₅₀ > .4 < .7 mg/ml
	L7	Spice Bush	<i>Lindera aggregata root</i>	IC ₅₀ > .4 < .7 mg/ml
	S33	Spikemoss	<i>Selaginella doederleinii</i>	IC ₅₀ > .4 < .7 mg/ml
	S60	Spirulina powder	<i>Arthrospira platensis</i>	IC ₅₀ > .4 < .7 mg/ml
	S72	Spirulina	<i>Arthrospira platensis</i>	IC ₅₀ > .4 < .7 mg/ml
	C40	Spreading sneezeweed	<i>Centepida minima</i>	IC ₅₀ > .4 < .7 mg/ml
	S3	Strawberry Leaf	<i>Fragaria vesca</i>	IC ₅₀ > .4 < .7 mg/ml
	S28	Sweet Grass Braid	<i>Hierochlœ Odorata</i>	IC ₅₀ > .4 < .7 mg/ml
	T1	Tansy	<i>Tanacetum vulgare</i>	IC ₅₀ > .4 < .7 mg/ml
	T5	Tarragon	<i>Artemisia dracunculus</i>	IC ₅₀ > .4 < .7 mg/ml
	C95	Tea	<i>Celyon (black tea)</i>	IC ₅₀ > .4 < .7 mg/ml
	T2	Thyme Leaf ^a	<i>Thymus vulgaris</i>	IC ₅₀ > .4 < .7 mg/ml
	S68	Toothache Plant	<i>Spilanthes acmella</i>	IC ₅₀ > .4 < .7 mg/ml
	S22	Touch me not	<i>Speranskia tuberculata</i>	IC ₅₀ > .4 < .7 mg/ml
	T30	Turkey Rhubarb	<i>Rheum palmatum</i>	IC ₅₀ > .4 < .7 mg/ml
	V2	Vervain	<i>Verbena officinalis herb</i>	IC ₅₀ > .4 < .7 mg/ml
	W16	White Willow Bark	<i>Salix alba</i>	IC ₅₀ > .4 < .7 mg/ml
	P73	Wintergreen	<i>Pyrola calliantha</i>	IC ₅₀ > .4 < .7 mg/ml
	W8	Wormwood	<i>Artemisia absinthum</i>	IC ₅₀ > .4 < .7 mg/ml
	A44	Wormwood	<i>Artemisia capillaris</i>	IC ₅₀ > .4 < .7 mg/ml
LEVEL 5	A41	Agrimony	<i>Agrimonia pilosa</i>	IC ₅₀ ≈ .7 mg/kg
	S74	Alexandrian Senna	<i>Senna alexandrina</i>	IC ₅₀ ≈ .7 mg/kg
	A6	Asafoetida	<i>Ferula Assa-Foetida</i>	IC ₅₀ ≈ .7 mg/kg
	D9	BAI CAI GAN	<i>Dried Chinese Cabbage</i>	IC ₅₀ ≈ .7 mg/kg
	B27	Black Berry Powder	<i>Rubus armeniacus</i>	IC ₅₀ ≈ .7 mg/kg
	I7	Black Henna	<i>Indigofera tinctoria</i>	IC ₅₀ ≈ .7 mg/kg
	C91	Cayenne powder	<i>Capsicum annum</i>	IC ₅₀ ≈ .7 mg/kg
	V6	Chaste Tree Berry	<i>Vitex agnus-castus</i>	IC ₅₀ ≈ .7 mg/kg
	C12	Chickweed	<i>Stellaria media</i>	IC ₅₀ ≈ .7 mg/kg
	P29	Chinese Cinquefoil	<i>Potentilla chinensis</i>	IC ₅₀ ≈ .7 mg/kg
	T24	Dandelion Root	<i>Taraxacum mongolicum</i>	IC ₅₀ ≈ .7 mg/kg
	D5	Dill weed	<i>Anethum graveolens</i>	IC ₅₀ ≈ .7 mg/kg
	A26	Dogbane	<i>Apocynum venetum herb</i>	IC ₅₀ ≈ .7 mg/kg
	F20	Fennel seed ^a	<i>Foeniculum vulgare</i>	IC ₅₀ ≈ .7 mg/kg

LEVEL	ID	Common name	Scientific name	Inhibitory conc.
	F22	Fringe Bark Tree	<i>Chionanthus virginicus</i>	IC ₅₀ ≈.7 mg/kg
	F23	Fumitory	<i>Fumaria officinalis</i>	IC ₅₀ ≈.7 mg/kg
	G27	Garcinia cambogia	<i>Garcinia cambogia</i>	IC ₅₀ ≈.7 mg/kg
	G23	Gardenia	<i>Gardenia jasminoides fruit</i>	IC ₅₀ ≈.7 mg/kg
	S31	Japanese Catnip	<i>Schizonepeta tenuifolia</i>	IC ₅₀ ≈.7 mg/kg
	P22	Knotweed Grass	<i>Polygonum aviculare</i>	IC ₅₀ ≈.7 mg/kg
	M37	Malva Flower	<i>Malva sylvestris</i>	IC ₅₀ ≈.7 mg/kg
	N11	Nettle stinging	<i>Urtica dioica</i>	IC ₅₀ ≈.7 mg/kg
	O18	Oatstraw	<i>Avena sativa</i>	IC ₅₀ ≈.7 mg/kg
	O14	Orange peel	<i>Citrus sinensis</i>	IC ₅₀ ≈.7 mg/kg
	O12	Oregano leaf ^a	<i>Origanum vulgare</i>	IC ₅₀ ≈.7 mg/kg
	O2	Osha Root	<i>Ligusticum porteri</i>	IC ₅₀ ≈.7 mg/kg
	P27	Patrinia	<i>Patrinia villosa herb</i>	IC ₅₀ ≈.7 mg/kg
	P28	Purslane	<i>Portulaca oleracea</i>	IC ₅₀ ≈.7 mg/kg
	R8	Raspberry Leaf	<i>Rubus idaeus</i>	IC ₅₀ ≈.7 mg/kg
	H29	Strawflower	<i>Helichrysum foetidum</i>	IC ₅₀ ≈.7 mg/kg
	E25	Tea	<i>English breakfast black tea</i>	IC ₅₀ ≈.7 mg/kg
	I9	Tea	<i>Irish breakfast green tea</i>	IC ₅₀ ≈.7 mg/kg
	S41	The Holy Herb	<i>Siegesbeckia orientalis</i>	IC ₅₀ ≈.7 mg/kg
	P18	Tongue fern	<i>Pyrosia Lingua</i>	IC ₅₀ ≈.7 mg/kg
	G26	Wintergreen	<i>Gaultheria procumbens</i>	IC ₅₀ ≈.7 mg/kg

^aBotanical Replicates confirmed from various distributors.