Sembragiline: a novel, selective monoamine oxidase type B inhibitor for the treatment of Alzheimer's disease

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Supplementary Materials

Supplemental Methods

IC_{50} (nM)	Human (Gottowik et al., 1993)		Rat (Da Prada et al., 1990; Da Prada et al., 1989)	
	MAO-B	MAO-A	MAO-B	MAO-A
Lazabemide	6.9	>10	37	>10,000
Harmaline	>10,000	3.7	>10,000	7
RO0411049	>10,000	27.0	>10,000	20
Clorgyline	-	-	670	1

Supplementary table 1: Summary of IC_{50} values for various inhibitors mentioned in the text for human and rat MAO-B and MAO-A

Selectivity of sembragiline

Assay	Origin	Ligand	% Inhibition of control
Receptors / Binding sites			
Adrenergic α_1 (non-selective)	Rat cerebral cortex	[^{3}H]prazosin	-6
Adrenergic α_2 (non-selective)	Rat cerebral cortex	[^{3}H]RX 821002	10
Adrenergic β_1 (*h*)	Human recombinant (Sf9 cells)	[^{3}H](-)CGP 12177	12
Adrenergic β_2 (*h*)	Human recombinant (Sf9 cells)	[^{3}H](-)CGP 12177	8
Adrenergic β_3 (*h*)	SK-N-MC cells	[^{125}I]CYP	14
Angiotensin AT_1 (*h*)	Human recombinant (CHO cells)	[^{125}I][Sar^1,Ile^8]-ATII	15
Angiotensin AT_2 (*h*)	Human recombinant (Hela cells)	[^{125}I]CGP42112A	1
Benzodiazepine (BZD, central)	rat cerebral cortex	[^{3}H]flunitrazepam	19
Cannabinoid CB_1 (*h*)	Human recombinant (HEK 293 cells)	[^{3}H]WIN55212-2	5
Cannabinoid CB_2 (*h*)	Human recombinant (HEK 293 cells)	[^{3}H]WIN55212-2	5
Cholecystokinin CCK_A (*h*) (CCK_1)	Human recombinant (NM-3T3 cells)	[^{3}H]devazepide	17
Cholecystokinin CCK_B (*h*) (CCK_2)	Human recombinant (HEK-293 cells)	[^{125}I]CCK-8	-12
Corticotropin Releasing Factor (CRF_1)	Rat pituitary gland	[^{125}I]Tyr^0-CRF	0
Dopamine D_1 (*h*)	Human recombinant (L cells)	[^{3}H]SCH23390	-10
Dopamine D_{2S} (*h*)	Human recombinant (CHO cells)	[^{3}H]spiperone	-6
Dopamine D_3 (*h*)	Human recombinant (CHO cells)	[^{3}H]spiperone	33
$GABA_A$	Rat cerebral cortex	[^{3}H]muscimol	-6
$GABA_{B(1B)}$ (*h*)	Human recombinant (HEK 293 cells)	[^{3}H]CGP 54626	-12
α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)	Rat cerebral cortex	[^{3}H]AMPA	6
Kainate	Rat cerebral cortex	[^{3}H]kainic acid	3
N-methyl-D-aspartate (NMDA)	Rat cerebral cortex	[^{3}H]CGP 39653	5
Glycine (strychnine-sensitive)	Rat spinal cord	[^{3}H]strychnine	23
Glycine (strychnine-insensitive)	Rat cerebral cortex	[^{3}H]MDL 105,519	-5

Interleukin-2	CTLL-2 cells	[^{125}I]IL-2	-3
Interleukin-6 (*h*)	U-266 cells	[^{125}I]IL-6	5
Histamine H_1 (*h*)	Human recombinant (HEK-293 cells)	[^{3}H]pyrilamine	11
Histamine H_2	Guinea pig striatum	[^{125}I]APT	19
Histamine H_3	Rat cerebral cortex	[^{3}H](R)α-Me-histamine	-4
Insulin	Rat liver	[^{125}I]insulin	18
Muscarinic M_1 (*h*)	Human recombinant (CHO cells)	[^{3}H]pirenzepine	6
Muscarinic M_2 (*h*)	Human recombinant (CHO cells)	[^{3}H]AF-DX 384	6
Muscarinic M_3 (*h*)	Human recombinant (CHO cells)	[^{3}H]4-DAMP	12
Neurokinin NK_1 (*h*)	U-373MG cells	[^{3}H] [Sar_9,$Met(0_2)^{II}$]-SP	-2
Neurokinin NK_2 (*h*)	Human recombinant (CHO cells)	[^{125}I]NKA	6
Neuropeptide Y Y_1 (*h*)	Human recombinant (Sf9 cells)	[^{125}I]peptideYY	14
Neuropeptide Y Y_2 (*h*)	KAN-TS cells	[^{125}I]peptideYY	-3
Nicotinic (neuronal) (alpha-BGTX-insensitive)	Rat cerebral cortex	[^{3}H]cystine	-15
Nicotinic (neuronal) (alpha-BGTX-sensitive)	Rat cerebral cortex	[^{125}I]α-bungarotoxin	-10
Nicotinic (muscle type)	BC3H-1 cells	[^{125}I]α-bungarotoxin	0
Opioid δ (DOP)	Guinea pig cerebral cortex	[^{3}H]DADLE	-1
Opioid κ (KOP)	Guinea pig cerebellum	[^{3}H]U 69593	-1
Opioid μ (h) (MOP)	Human recombinant (CHO cells)	[^{3}H]DAMGO	-7
Nociceptin ORLI (*h*) (NOP)	Human recombinant (HEK-293 cells)	[^{3}H]Nociceptin	2
Ouabain	MDCK cells	[^{3}H]Ouabain	12
Phenylcyclidine (NMDA)	Rat cerebral cortex	[^{3}H]TCP	9
Purinergic P_2X	Rat urinary bladder	[^{3}H]a,ß-MeATP	-8
Purinergic P_2Y	Rat cerebral cortex	[^{35}D]dATPαS	-1
Serotonin 5-HT (non-selective)	Rat cerebral cortex	[^{3}H]a,ß-MeATP	-9
Serotonin $5HT_{1A}$ (*h*)	Human recombinant (CHO cells)	[^{3}H]8-OH-DPAT	21
Serotonin $5HT_{2A}$ (*h*)	Human recombinant (HEK-293 cells)	[^{3}H]ketanserin	14
Serotonin $5HT_{2B}$ (*h*)	Human recombinant (CHO cells)	[^{3}H]LSD	17
Serotonin $5HT_{2C}$ (*h*)	Human recombinant (CHO cells)	[^{3}H]mesulergine	3
Serotonin $5HT_3$ (*h*)	Human recombinant (HEK-293 cells)	[^{3}H]BRL 43694	7
Serotonin $5HT_6$	Rat recombinant (HEK-293 cells)	[^{3}H]LSD	-5
Serotonin $5HT_7$	Rat recombinant (HEK-293 cells)	[^{3}H]LSD	1
Glucocorticoid (*h*) (GR)	IM-9 cells (cytosol)	[^{3}H]triamcinolone	-28
Oestrogen (*h*) (ER)	MCF-7 cells (cytosol)	[^{3}H]estradiol	-1
Androgen (*h*) (AR)	LNCaP cells (cytosol)	[^{3}H]methyltrienolone	-12

Thyroid hormone (TH)	Rat liver	[^{125}I]T3	9
Thyrotropin-releasing hormone (TRH)	Rat cerebral cortex	[^{3}H]Me-TRH	0
Vasopressin V_1	A7r5 cells	[^{3}H]AVP	20
Vasopressin V_2	LLC-PK1 cells	[^{3}H]AVP	-7
Ca^{2+} channel (L, DHP site)	Rat cerebral cortex	[^{3}H](+)PN 200-110	-7
Ca^{2+} channel (L, diltiazem site)	Rat cerebral cortex	[^{3}H]diltiazen	-7
Ca^{2+} channel (L, verapamil site)	Rat cerebral cortex	[^{3}H](-)D 888	-3
Ca^{2+} channel (N)	Rat cerebral cortex	[125I] ω-conotoxin GVIA	-15
K^+ATP channel	Rat cerebral cortex	[^{3}H]glibencamide	3
K^+V channel	Rat cerebral cortex	[^{125}I]α-dendrotoxin	0
SK^+Ca channel	Rat cerebral cortex	[^{125}I]apamin	-2
Na^+ channel (site 1)	Rat cerebral cortex	[^{3}H]saxitoxin	-2
Na+ channel (site 2)	Rat cerebral cortex	[^{3}H]batrachotoxinin	0
NE transporter (*h*)	Human recombinant (MDCK cells)	[^{3}H]nisoxetine	13
DA transporter	Rat striatum	[^{3}H]GBR12935	2
GABA transporter	Rat cerebral cortex	[^{3}H]GABA	-11
5-HT transport (*h*)	Human recombinant (HEK-293 cells)	[^{3}H]paroxetine	8
Enzymes / Others		**Substrate/stimulus/tracer**	
Cyclooxygenase COX_1 (*h*)	Human platelets	arachidonic acid	-12
Cyclooxygenase COX_2 (*h*)	HUV-EC-C cells	arachidonic acid	6
Nitric oxide synthase inducible	RAW 264-7 cells	[^{3}H]arginine	24
Nitric oxide synthase Constitutive (cerebellar)	Rat cerebellum	[^{3}H]arginine	0
Nitric oxide synthase Constitutive *(h)* (endothelial)	HUV-EC-C cells	[^{3}H]arginine	9
Phophodiesterase I	Bovine brain	[^{3}H]cAMP	-1
Phophodiesterase II (*h*)	Differentiated U-937 cells	[^{3}H]cAMP	-9
Phophodiesterase III (*h*)	Human platelets	[^{3}H]cAMP	-9
Phophodiesterase IV (*h*)	U-937 cells	[^{3}H]cGMP	10
Phophodiesterase V (*h*)	Human platelets	[^{3}H]cGMP	1
Phophodiesterase VI	Bovine retina	[^{3}H]cGMP	3
β-secretase-1 (*h*) (BACE)	Human recombinant	Mca-S-E-V-N-L-D-A-E-F-R-K(Dnp)-R-R-NH_2	0
Adenylyl cyclase (stimulated)	Rat brain	ATP / forskolin	-11
ERK_2 kinase ($P42^{mapk}$)	Rat recombinant (*E.coli*)	[γ–^{33}P]ATP / MBP	1
MEK1 kinase	Rabbit recombinant (*E.coli*)	ATP / inactivated MAPK	-1
Protein kinase A (stimulated)	Bovine heart	[γ–^{33}P]ATP/histone H_1/ cAMP	-5
Protein kinase C	Rat brain		-14
Phospholipase C	Rat brain	[^{3}H]PIP_2	-5
Acetylcholineserase (*h*)	Human recombinant (HEK-293 cells)	AMTCh	15
Histamine N-methyl transferase	Rat kidney	[^{14}C]S-adenosyl-L-methionin/ histamine	-10

Tyrosine hydroxylase	Rat striatum	[^{3}H]tyrosine	-45
Superoxide O^{2-} scavenging / xanthine oxidase	Purified xanthine oxidase from bovine milk	hypoxanthine	-12
H_2O_2 scavanging	Cell-free system	scopoletin	-5
Lipid peroxidation	Rat liver microsomes	ascorbic acid	4
ATPase (H^+/K^+)	Rabbit stomach fundus	p-NPP	-4
ATPase (Na^+/K^+)	Dog kidney	ATP	-2

Supplemental Table 2. List of binding sites tested. Sembragiline was tested at concentration 10 μM. (*h*) indicates where the human receptor or enzyme was used. Selectivity screen was performed at Cerep, France according to their standard operating procedures (see www.cerep.com; for details).

Quantification of PEA, spermine and spermidine

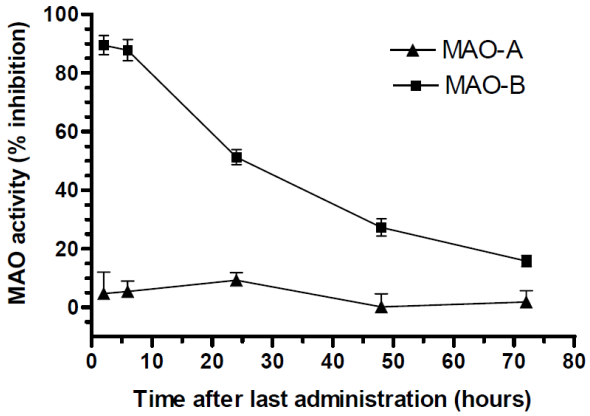
Analyte brain content was determined by HPLC with tandem mass spectrometry detection, using as internal standards D_4-phenylethylamine for PEA, D3-spermine (deuterated) spermine and none for spermidine. An aliquot of 25 μL of each experimental sample was mixed with 4 μL of internal standard solution. The resulting mixture was automatically derivatised with 20 μL SymDAQ™ (within an auto-sampler). After two minutes, 40 μL of the mixture was injected into the liquid chromatography (LC) system by an automated sample injector (SIL-20AD, Shimadzu, Japan). Chromatographic separation was performed on a Polaris C18 column (150 x 2 mm, particle size 3 μm) held at a temperature of 30°C in a gradient elution run, using eluent A (formic acid 0.1% v/v in ultrapurified H_2O) and eluent B (formic acid 0.1% v/v in acetonitrile). The flow (total flow: 0.25 mL/min) of the LC was diverted to waste for 4 minutes, after which it was switched to the mass spectrometer (MS) for detection of the analytes and internal standards. A post-column make-up flow of acetonitrile + 1.0% formic acid (0.10 mL/min) was added to the column effluent to enhance ionisation efficiency. MS analyses were performed using an API 5000 MS/MS system consisting of an API 5000 MS/MS analyser and a Turbo Ion Spray interface (both from Applied Biosystems, USA). The acquisitions were performed in positive ionisation mode, with ionisation spray voltage set at 5.5 kV and a probe temperature of 600 °C. The instrument was operated in multiple-reaction-monitoring (MRM) mode. Calibration curves ranging 0.05–100 nM for PEA, 1–2000 nM for spermine and 0.005–10.0 μM for spermidine were fitted using weighted (1/x) regression, and the sample concentrations were determined using these calibration curves. Accuracy was verified by quality control samples after each sample series. Concentrations were calculated with the Analyst™ data system (version 1.5.2, Applied Biosystems, the Netherlands).

Effect L-5-HTP-induced behavioural effects

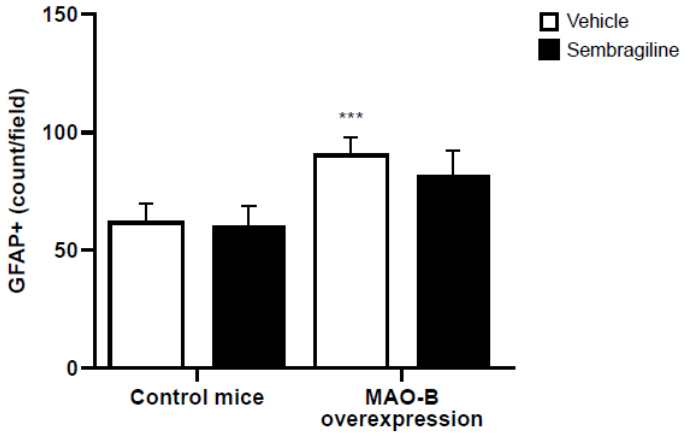
5-HT syndrome	Other behaviour
Forepaw trading	Scratching
Backward locomotion	Yawning
Hindlimb abduction	Grooming
Flat body posture	
Straub tail	
Wet dog shakes	
Tremor	

Supplemental Table 3: After L-5-HTP injection, rats were monitored for the occurrence of the specific behaviours of interest indicated above for 1 hour. Total frequency of all the individual behaviours was added into two compound scores represented by the "5-HT Syndrome" and the "Other behaviour" clustering.

Supplemental Results



Supplemental Figure 1. Time-course of MAO-A and MAO-B inhibition in the rat brain after oral administration of 0.3 mg/kg sembragiline once a day for 5 days**.** Mean ±SD, n=3.



Supplemental Figure 2. The effect of sembragiline on astrogliosis in the hippocampus. MAO-B mice with and without induction of MAO-B overexpression were treated with 3 mg/kg oral sembragiline or vehicle daily for 14 days. Quantification of GFAP+ hippocampal cell count. *** P<0.001 vs vehicle-treated control mice. Data represent mean ± SD, n=3.

Supplementary references

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