

## In Vitro Receptor Binding and Enzyme Inhibition by *Hypericum perforatum* Extract

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*Hypericum perforatum* L. Hypericaceae (St. John's wort), has been used since the time of ancient Greece for its many medicinal properties. Modern usage is still quite diverse and includes wound healing, kidney and lung ailments, insomnia and depression. This plant has been known to contain a red pigment, hypericin, and similar compounds, which have been assumed to be the primary active constituent(s) in this plant genus. A crude *Hypericum* extract was tested in a battery of 39 *in vitro* receptor assays, and two enzyme assays. A sample of pure hypericin was also tested. Hypericin had affinity only for NMDA receptors while the crude extract had significant receptor affinity for adenosine (nonspecific), GABA<sub>A</sub>, GABA<sub>B</sub>, benzodiazepine, inositol triphosphate, and monoamine oxidase (MAO) A and B. With the exception of GABA<sub>A</sub> and GABA<sub>B</sub>, the concentrations of *Hypericum* extract required for these *in vitro* activities are unlikely to be attained after oral administration in whole animals or humans. These data are consistent with recent pharmacologic evidence suggesting that other constituents of this plant may be of greater importance for the reported psychotherapeutic activity. Alternative pharmacologic mechanisms for *Hypericum*'s antidepressant activity are critically reviewed and the possible importance of GABA receptor binding in the pharmacology of *Hypericum* is highlighted. Some of these results have been previously reported (Cott, 1995; Cott, 1996; Cott and Misra, 1997).

### Methods

Through an NIMH screening contact (NovaScreen®, Baltimore, Maryland) a commercially available crude extract from the fresh flowers and buds of *Hypericum perforatum* (Herb Pharm, Williams, Oregon 97544) containing ~0.1% hypericin was dried under vacuum, dissolved in 4% DMSO, and diluted to an initial concentration of 5 µg/ml for *in vitro* assay in a battery of 39 receptor types, and two enzyme systems (Table 1). In addition, a sample of hypericin ~95% pure, Sigma Chem. Co.) was tested. Receptor assays showing at least 50% displacement of radioligand (or 50% inhibition of MAO) were considered "hits". Approximate K<sub>i</sub> determinations were then calculated from an IC<sub>50</sub> curve (where  $K_i = IC_{50}/(1 + K_d/[L])$ ) using 5 concentrations (50, 5, 0.5, 0.05, and 0.005 µg) of extract per ml of total assay volume.

**Table 1** Receptor binding profile of *Hypericum perforatum* crude extract (5 µg/ml)

Receptor	% inhibition	Reference/K <sub>i</sub> (nM)	
Adrenergic (α <sub>1</sub> )	-105	Prazosin	0.58
Adrenergic (α <sub>2</sub> )	-193	Phentolamine	1.53
Adrenergic (β)	-280	Alprenolol	1.07
Dopamine (DA <sub>1</sub> )	-61	SCH 23390	6.37
Dopamine (DA <sub>2</sub> )	-75	Sulpiride	3.16
<b>Serotonin (5HT<sub>1</sub>)</b>	<b>100</b>	Serotonin	5.15
Serotonin (5HT <sub>2</sub> )	-1499	Methysergide	0.77
Quisqualate	-69	Quisqualic Acid	8.89
Kainate	-1049	Kainic Acid	2523.36
Glycine (strychnine)	-13028	Strychnine Nitrate	1061.64
<b>GABA<sub>A</sub></b>	<b>100</b>	Muscimol	4.81
<b>GABA<sub>B</sub></b>	<b>96</b>	Baclofen	125.24
NMDA	-530	NMDA	14244.23
<b>Adenosine</b>	<b>104</b>	NECA	944.91
Angiotensin II	-522	Angiotensin II	1.36
Bombesin	-1668	GRP	2.12
Substance P	-2789	Substance P	9.36
Substance K	-3578	Neurokinin A	0.97
Neurotensin	-115	Neurotensin	0.99
Neuropeptide Y	-196	Neuropeptide Y	1.85
Somatostatin	-1147	Somatostatin	0.06
Arg-Vasopressin 1	39	Arg-Vasopressin 1	26.19
CCK <sub>A</sub>	-554	CCK	0.15
CCK <sub>B</sub>	-311	CCK	0.56
VIP	-6574	VIP	2.21
ANF <sub>1</sub>	-664	ANF	0.09
NGF	-2767	NGF	7.37
EGF	-373	EGF	0.74
<b>Forskolin</b>	<b>64</b>	Forskolin	39.53
Phorbol Ester	36	PDBU	7.77
<b>Inositol Tri-phosphate</b>	<b>90</b>	IP3	23.90
Calcium (N)	-285	W-Conotoxin	0.05
Calcium (T & L)	17	Nifedipine	1.17
Chloride	12	TBPS	0.47
Potassium	-716	Apamin	0.51
Glycine (non-strychnine)	-66	Glycine	323.31
MK-801	-26	MK-801	1.22
PCP	-2749	PCP	41.74
<b>Benzodiazepine</b>	<b>65</b>	RO-151788	0.07
<b>MAO<sub>A</sub></b>	<b>93</b>	Clorgyline	1.00
<b>MAO<sub>B</sub></b>	<b>49</b>	Deprenyl	10.00

Numbers in bold are "hits"

**Results**

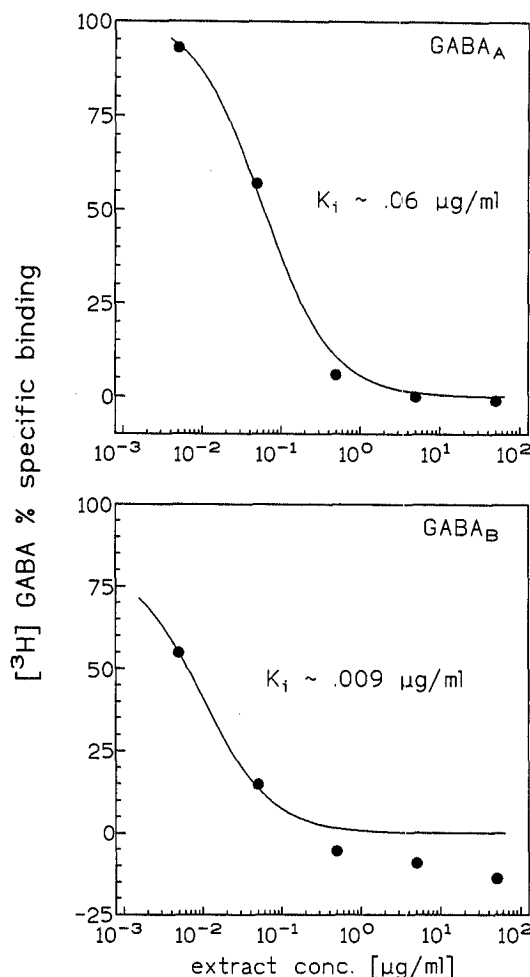
*Hypericum* extract showed considerable binding affinity in several receptor assays (Table 1). Receptors in bold represent hits and include adenosine, GABA<sub>A</sub>, GABA<sub>B</sub>, 5HT<sub>1</sub>, benzodiazepine (central), forskolin, inositol triphosphate (IP3), and the enzymes, monoamine oxidase type A and B (MAO<sub>A</sub>, MAO<sub>B</sub>). The assays that showed initial activity were tested with a quick IC<sub>50</sub> curve and are summarized in Table 2.

With the exception of forskolin, all receptors showing initial activity were confirmed in the quick IC<sub>50</sub> studies. Of the receptors bound by *hypericum* extract, GABA (both types A and B) are of the greatest magnitude by far (K<sub>i</sub> ~ 75 ng/ml and 6 ng/ml, respectively). The binding curves for these GABA receptor subtypes are shown in Fig. 1.

Unless the responsible components are metabolized before they enter the general circulation, plasma levels sufficient to bind GABA receptors would be predicted; however, studies of intrinsic activity have not yet been done. The significance of this GABA binding is unknown at the present, but there is a considerable literature on the role of GABA in affective disorders. GABA<sub>B</sub> stimulation has been found to enhance β-receptor down-regulation during imipramine treatment (Enna et al., 1986). Nielsen et al. (1990) have reported antidepressant effects with the GABAergic agent, fengabine, in depressed outpatients. Petty et al. (1992; 1993) have reported that GABA plasma levels are low in both bipolar and unipolar depression, and have argued that benzodiazepines (which enhance GABA<sub>A</sub> activity) may be effective antidepressants as well as anxiolytics (Petty et al., 1995). GABA neuronal systems also modulate dopamine and dopamine-induced behaviors (Cott et al., 1976; Cott and Engel, 1977).

The inhibition of MAO is consistent with previous reports (Thiede and Walper, 1994; Bladt and Wagner, 1994; Suzuki et al., 1994) (Fig. 2). Unlike the crude extract, however, hypericin (95% pure) lacked significant MAO<sub>A</sub> or MAO<sub>B</sub> inhibition at concentrations up to 10 μM (Table 3).

Hypericin did show affinity for the NMDA receptor (K<sub>i</sub> ~ 1 μM; Fig. 3). This action may play a role in its reported antiviral activity, since NMDA antagonists prevent gp120-induced neurotoxicity (Diop et al., 1994). Hypericin is currently in early clinical trials in the U.S. as an antiviral (Meruelo et al., 1988; Bombardelli and Morazzoni, 1995). Studies have shown that both hypericin and pseudohypericin inhibit a variety of encapsulated viruses, including herpes simplex (Weber et al.,

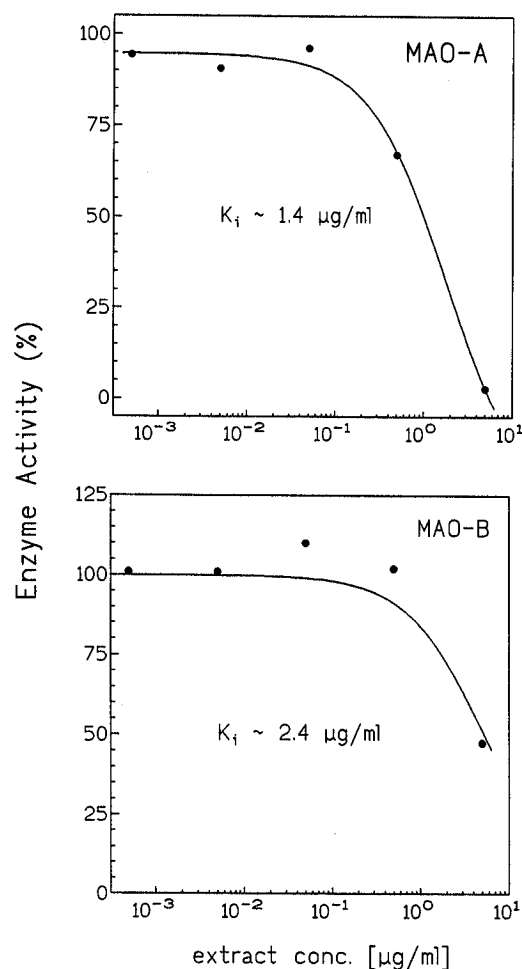


**Fig. 1** Effects of *Hypericum perforatum* on GABA receptor binding. GABA<sub>A</sub> binding was measured in bovine cerebellar membranes using [<sup>3</sup>H] GABA at 5.0 nM as a ligand. GABA<sub>B</sub> binding was measured in rat cortex membranes using [<sup>3</sup>H] GABA at 5.0 nM as a ligand in the presence of 50 μM isoguvacine to block GABA<sub>A</sub> sites.

1994) and the HI-virus associated with AIDS (Meruelo et al., 1988; Lavie et al., 1989; Lopez-Bazzocchi et al., 1991). These researchers have concluded that hypericin and pseudohypericin display a unique and uncommonly effective antiviral therapy.

Assay Concentration (μg/ml)	% inhibition in various assays							
	Adeno-sine	GABA <sub>A</sub>	GABA <sub>B</sub>	5HT <sub>1</sub>	BDZ	IP3	MAO <sub>A</sub>	MAO <sub>B</sub>
0.005	-2	7	45	12	-4	-3	9	-1
0.05	13	43	85	12	0	16	4	-10
0.5	17	94	105	9	0	13	33	-2
5.0	20	100	109	12	19	40	97	53
50	71	101	114	54	65	107	-	-
- K <sub>i</sub> (μg/ml)	1	0.06	0.009	25	24	10	1.4	2.4

**Table 2** Receptors and enzymes affected by *Hypericum perforatum* crude extract



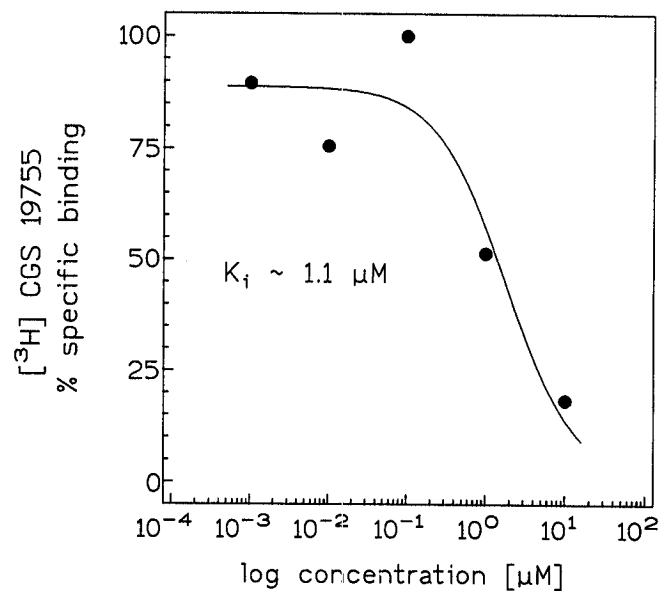
**Fig. 2** Effects of *Hypericum perforatum* on MAO<sub>A</sub> (above) and MAO<sub>B</sub> (below). MAO<sub>A</sub> inhibition was measured in rat liver mitochondrial membranes using [<sup>14</sup>C] Serotonin as substrate. MAO<sub>B</sub> inhibition was measured in rat brain membranes using [<sup>14</sup>C] Phenylethylamine as substrate.

**Table 3** Receptor binding/enzyme inhibition by hypericin (95%)

conc. (µM)	% inhibition		
	MAO-A	MAO-B	NMDA
.001	-	-	10.5
.01	-	-	24.5
.1	-	-	-3
1	27.0	-2.0	48.5
10	20.8	-20.7	81.7
-K <sub>i</sub>	-	-	1.1

## Discussion

St. John's wort has become increasingly popular in Germany for the treatment of mild-to-moderate depression. It has now been tested in more than 3000 patients against placebo and various active medications (Harrer et al., 1994; Hübner et al., 1994; Martinez et al., 1994; Sommer and Harrer, 1994; Vorbach



**Fig. 3** Effects of Hypericin on NMDA receptors. N-methyl-D-aspartate (NMDA) binding was measured in rat forebrain membranes using [<sup>3</sup>H] CGS 19755 at 14 nM as a ligand.

et al., 1994; Woelk et al., 1994) and has been approved by the regulatory authorities in Germany for the treatment of depression. A recent review of the clinical efficacy is presented by Linde et al. (1996), yet the mechanism for this effect remains unclear.

## Search for an antidepressant mechanism

While previous studies report that hypericin inhibits MAO at concentrations of 50 µg/ml (e.g., Suzuki et al., 1984), others have failed to confirm this effect (Demisch et al., 1989; Thiede and Walper, 1994; Bladt and Wagner, 1994). One explanation is that the hypericin used by Suzuki was apparently an extract of only 80% purity. It is possible that one or more constituents of the remaining 20% of this preparation could account for this weak enzyme inhibition. This possibility is supported by Bladt and Wagner (1994) which shows that the *Hypericum* fractions with the greatest MAO inhibition contain the highest concentration of flavonoids. Computer modeling of *Hypericum* constituents also suggests flavonoids as the most likely MAO inhibitor fraction (Höltje and Walper, 1993).

The MAO inhibition shown for *Hypericum* may not be pharmacologically relevant, since it could not be confirmed *in vivo*. Bladt and Wagner (1994) reported that no MAO inhibition was seen *ex vivo* after administration of 300 mg/kg *Hypericum* extract to rats. Whether this is due to rapid metabolism of the active compounds or other reasons cannot be said with certainty at this point. However, pharmacokinetic studies with the *Hypericum* extract, LI 160, showed plasma levels of only 1.5 ng/ml after a single 300 mg dose and 8.5 ng/ml at steady state in human volunteers (Staffeldt et al., 1994). These blood levels are several orders of magnitude below the concentrations needed to inhibit MAO.

Other proposed mechanisms involve effects on serotonin. Müller and Rossel (1994) report that *Hypericum* extract inhibits serotonin receptor expression and Perovic and Müller (1995) reported inhibition of serotonin uptake. Both these effects require high concentrations of extract, however, and are not likely to be achieved in the whole animal.

Another novel proposal is that *Hypericum* extract (concentrations not provided) reduces cytokine expression (interleukin-6) (Thiele et al., 1994). The hypothesis is that interleukins are capable of inducing depression in susceptible individuals (Smith, 1991). The field of psychoneuroimmunology is perhaps too new to give a definitive answer regarding this mechanism in the near future, but the link between depression and the immune system is still drawing attention (Kook et al., 1995).

It is clear that additional studies of pharmacologic mechanism must be performed. It is conceivable that the very high affinity of *Hypericum* extract for GABA receptors presented here may be important. Future studies should characterize *Hypericum's* effect with greater sophistication to determine if, and how, GABA receptors are affected in whole animals.

## Conclusion

The climate for botanical medicines in the United States is rapidly evolving. The World Health Organization (WHO) released its "Guidelines for The Assessment of Herbal Medicines" on March 13, 1992 (Akerle, 1992). These guidelines represent a major step in standardization of the way developed countries can incorporate traditional medicines within modern health care systems (Cott, 1995; Cott and Misra, 1997). The essence of these guidelines is that the historical use of a substance is a valid form of safety and efficacy information, in the absence of scientific documentation to the contrary. It is noteworthy that the U.S. Congress has demonstrated its interest in unconventional medical practices by establishing the NIH Office of Alternative Medicine. The prevalence of unconventional medical treatments sought by the American public can no longer be ignored (Eisenberg et al., 1993; Marwick, 1995). Together with the Dietary Supplement Health and Education Act of 1994, these events will surely promote a healthier climate for natural products research.

*Hypericum* is clearly one of the leading psychotherapeutic phytomedicines. Mainstream American researchers will remain unconvinced of the therapeutic efficacy until studies are conducted under an FDA (Food and Drug Administration) approved IND (Investigational New Drug) application in major depressive disorder using DSM-IV diagnostic criteria. The shortcomings of the current controlled clinical trials of *Hypericum* have been pointed out in a recent review (Linde et al., 1996). They include lack of well-characterized severely depressed patient populations, heterogeneity of diagnoses, lack of intent-to-treat analyses, lack of long-term studies, lack of control over compliance, low dosages of comparison medications, and shortage of publications in English-language journals. In addition, the lack of a viable pharmacologic mechanism or the assurance of which components within the plant are critical for therapeutic effect (necessary in order to standardize formulations) will continue to create skepticism among psychopharmacologists and regulatory authorities.

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