ฤทธิ์ต้านซึมเศร้าของสารสกัดจากกะทกรกในหนูถีบจักรและความเกี่ยวข้องของรีเซพเตอร์ D_1 และ 5-HT_{1A}

นายภานุ วิจักขณาลัญจ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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ANTIDEPRESSANT ACTIVITY OF EXTRACTS OF *PASSIFLORA FOETIDA* IN MICE AND THE INVOLVEMENT OF D_1 AND 5-HT_{1A} RECEPTORS

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ภานุ วิจักขณาลัญฉ์ : ฤทธิ์ต้านซึมเศร้าของสารสกัดจากกะทกรกในหนูถีบจักรและความเกี่ยวข้องของรีเซพเตอร์ D_1 และ 5-HT_{1A} (ANTIDEPRESSANT ACTIVITY OF EXTRACTS OF *PASSIFLORA FOETIDA* IN MICE AND THE INVOLVEMENT OF D_1 AND 5-HT_{1A} RECEPTORS) อ. ที่ปรึกษา: ผศ. ดร. สุรชัย อัญเชิญ, 63 หน้า. ISBN 974-17-5780-8.

การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบฤทธิ์ต้านซึมเศร้าของสารสกัดจากต้นกะทกรกด้วยแบบจำลองภาวะซึมเศร้าในหนูถีบจักรและทดสอบว่าการออกฤทธิ์ดังกล่าวเกี่ยวข้องกับรีเซพเตอร์โดปามีน (D1) และซีโรโตนิน (5-HT1A) หรือไม่ การทดสอบผลของการฉีดสารสกัดจากต้นกะทกรกเข้าช่องท้องในขนาด 50 มิลลิกรัมต่อกิโลกรัมแบบ subacute ต่อ locomotor activity พบว่าสารสกัดจากต้นกะทกรกทุก subfractions ไม่มีฤทธิ์กระตุ้นระบบประสาทส่วนกลางเมื่อเทียบกับกลุ่มควบคุม การทดสอบฤทธิ์ต้านซึมเศร้าทำในสัตว์ทดลองโดยใช้แบบจำลองการว่ายน้ำในที่กว้างของหนูถีบจักร (Open space swimming test) และการฉีดสารสกัดจากกะทกรกเข้าช่องท้องในขนาด 50 มิลลิกรัมต่อกิโลกรัม พบว่าสารสกัดจากกะทกรกใน subfractions PF003-1 และ PF003-2 สามารถลดเวลาไม่ว่ายน้ำของหนูถีบจักรได้อย่างมีนัยสำคัญ ซึ่งฤทธิ์ต้านซึมเศร้าดังกล่าวมีรูปแบบคล้ายกับ Imipramine ซึ่งใช้เป็นกลุ่มควบคุมผลบวก ส่วน subfractions อื่นๆ นั้นไม่มีฤทธิ์ต้านซึมเศร้า การทดสอบความเกี่ยวข้องระหว่างฤทธิ์ต้านซึมเศร้ากับรีเซพเตอร์โดปามีน (D_1) และซีโรโตนิน (5-HT_{1A}) ทำโดยการใช้สาร SCH 23390 ซึ่งเป็น dopamine D_1 antagonist ขนาด 0.05 มิลลิกรัมต่อกิโลกรัมและ WAY 100635 ซึ่งเป็น serotonin 5-HT_{1A} antagonist ขนาด 0.3 มิลลิกรัมต่อกิโลกรัม โดยฉีดสารเหล่านี้เข้าช่องท้องก่อนให้สารสกัดจากต้นกะทกรก 15 นาที ผลการทดลองแสดงว่า antagonists ทั้งสองสามารถยับยั้งฤทธิ์ต้านซึมเศร้าของสารสกัดจากต้นกะทกรก PF003-1 และ PF003-2 ได้อย่างชัดเจน ซึ่งชี้แนะว่าฤทธิ์ต้านซึมเศร้าของสารสกัดจากกะทกรกอาจเกิดจากการทำงานผ่านระบบประสาท dopaminergic และ serotoninergic ในสมอง ดังนั้นการศึกษาครั้งนี้ชี้แนะว่าสารสกัดจากต้นกะทกรก (PF003-1 และ PF003-2) มีศักยภาพต้านซึมเศร้า โดยอาจนำไปวิจัยและพัฒนาต่อเพื่อใช้ประโยชน์ในการรักษาผู้ป่วยโรคซึมเศร้าได้

ภาควิชา เภสัชวิทยา.....ลายมือชื่อนิสิต.....

สาขาวิชา เภสัชวิทยา.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

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The present study was aimed to investigate the potential antidepressant activity of *Passiflora foetida* (PF) extracts on the animal model of depression and the possible involvement of dopaminergic (DA) and serotoninergic (5-HT) mechanisms in their antidepressant activity. The acute intraperitoneal (IP) administration of 50 mg/kg PF extracts in mice revealed no CNS stimulant effects as measured by locomotor activity. Antidepressant activity was tested by using a mouse model of depression, the open space swimming test. Subacute IP administration of 50 mg/kg of PF extracts, subfraction PF003-1 and PF003-2, resulted in a significant reduction of the decrease in mobility time of mice. This antidepressant-like effect was comparable to that observed in a classical antidepressant drug, imipramine. Other subfractions of PF did not show apparent antidepressant activity. The involvement of serotoninergic and dopaminergic mechanisms in the antidepressant activity of PF extracts was investigated by using a dopamine D_1 antagonist, SCH 23390 (0.05 mg/kg, IP) and a serotonin 5-HT_{1A} antagonist, WAY 100635 (0.3 mg/kg, IP). The antidepressant effect of PF extracts, PF003-1 and PF003-2, was markedly abolished by prior IP administration of these antagonists, individually or in combination. Therefore, the results suggested that the antidepressant activity of PF extracts may mediate through dopaminergic and serotoninergic mechanisms in the brain. In conclusion, the present study suggested that PF extracts (subfractions PF003-1 and PF003-2) possessed potential antidepressant effects which could be of therapeutic interest for using in the treatment of patients with depressive disorders.

Department of Pharmacology.....Student's signature.....
Field of study Pharmacology.....Advisor's signature.....
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LIST OF ABBREVIATIONS

PF	=	*Passiflora foetida* L.
DA	=	Dopamine
5-HT	=	5-hydroxytryptamine; serotonin
DALYs	=	Disability Adjusted Life Years
YLLs	=	Years of life lost
YLDs	=	Years lived with disability
HIV	=	Human immune-deficiency virus
AIDS	=	Acquired immune-deficiency syndrome
COPD	=	Chronic obstructive pulmonary disease
ECT	=	Electroconvulsive therapy
NAc	=	Nucleus accumbens
HP	=	Hippocampus
PFC	=	Prefrontal cortex
VTA	=	Ventral tegmental area
LC	=	Locus coeruleus
DR	=	Dorsal raphe
DLPFC	=	Dorsolateral prefrontal cortex
NA	=	Noradrenaline
cDNA	=	Complementary deoxyribonucleic acid

mRNA	=	Messenger ribonucleic acid
TRH	=	Tryptophan hydroxylase
TH	=	Tyroxine hydroxylase
COMT	=	Catechol-O-methyltransferase
MAO	=	Monoamine oxidase
CNS	=	Central nervous system
PD	=	Parkinson’s disease
GABA	=	Gamma-aminobutyric acid
SSRIs	=	Selective serotonin reuptake inhibitors
OCD	=	Obsessive-compulsive disorder
SERT	=	Serotonin transporter
FST	=	Forced swimming test
DMSO	=	Dimethylsulfoxide
NSS	=	0.9% Normal saline solution
IP	=	Intraperitoneal
h	=	Hour

CHAPTER I

INTRODUCTION

Major depressive disorder is a common disorder, widely distributed in the population, and usually associated with substantial symptom severity and role impairment (Kessler et al., 2003). Between 1987 and 1997, there was a marked increase in the proportion of the population who received outpatient treatment for depression, but fewer outpatient visits and less use of psychotherapy (Olfson et al., 2002), leading to loss of productivity, functional decline, and increased mortality. Appropriate therapy improves the daily functioning and overall health of patients with depression (Stewart et al., 2003).

Major depression is defined by depressed mood or loss of interest in nearly all activities (or both) for at least two weeks, accompanied by a minimum of three or four of the following symptoms (for a total of at least five symptoms altogether): insomnia or hypersomnia, feelings of worthlessness or excessive guilt, fatigue or loss of energy, diminished ability to think or concentrate, substantial change in appetite or weight, psychomotor agitation or retardation, and recurrent thoughts of death or suicide. Among patients with major depression, the level of illness ranges from mild to severe (Table 1) (Williams et al., 2002).

Other psychiatric disorders frequently coexist with depression. In patients with concurrent anxiety, the provider should treat the depression first, since doing so may improve the symptoms of both disorders. Patients with a history of mania (elevated mood, increased energy, and impulsivity), psychosis (hearing voices or seeing things that are not there), or another major psychiatric illness should be referred for psychiatric evaluation. Substance abuse, which is common among depressed patients, must not be considered a contraindication to therapy. Aggressive treatment of depression can decrease the use of tobacco, alcohol, and possibly other drugs of abuse (Whooley and Simon, 2000).

Table 1 Diagnostic category for depression.

Diagnostic Category	Criteria	Duration
Minor depression	2 to 4 depressive symptoms, including depressed mood or anhedonia*	≥2 wk
Dysthymia	3 or 4 dysthymic symptoms, including depressed mood**	≥2 yr
Major depression	≥5 depressive symptoms, including depressed mood or anhedonia	≥2 wk
Mild	Few (if any) symptoms in excess of those require for the diagnosis; minimal impairment in functioning	
Moderate	Greater number and intensity of depressive symptoms; moderate impairment in functioning	
Severe	Marked intensity and pervasiveness of depressive symptoms; substantial impairment in functioning	

* Depressive symptoms include depressed mood, anhedonia, weight change, sleep disturbance, psychomotor problems, lack of energy, excessive guilt, poor concentration, and suicidal ideation.

** Dysthymic symptoms include depressed mood, poor appetite or over eating, sleep disturbance, lack of energy, low self-esteem, poor concentration, and hopelessness.

The costs of untreated depression in terms of health care expense, absenteeism, and diminished work productivity are enormous (Buckley et al., 2004). An estimated 121 million people currently suffer from depression. An estimated 5.8% of men and 9.5% of women will experience a depressive episode in any given year. These figures can, however, vary across different populations (World Health Organization [WHO], **online**, October 2, 2001).

The price of health problems the population are forced to pay normally includes the loss of life and disability which affect the quality of life of those who have to endure the pains associated with the diseases. In assessing the impact on society of health problems, the World Health Organization and World Bank have

developed a new indicator index called Disability Adjusted Life Years, or DALYs. The purpose is to make the comparative measurement of the damage caused by diseases, be it untimely death and trauma or disability, under a single index. Previously, measurement tended to be confined to one particular aspect such as mortality rate or morbidity rate, causing mental health problems to be relegated to the bottom of the list of public health problems because the mortality rate of mental health problems is relatively low.

The analysis of DALYs index takes into consideration two major components:

1. Years of life lost, or YLLs, which is derived from mortality data including accurate identification of the cause of death, classification of the cause of death by gender and age group;

2. Years lived with disability, or YLDs, which takes into account disability weight that includes incidence rate, remission rate, and risk ratio of risk factors that may affect the disease.

The formula may be given thus: DALYs = YLLs + YLDs. In this formula, 1(one) DALY is equivalent to the loss of 1 year of life span due to illness.

According to a study of the burdens or costs of diseases and injuries in Thailand in 1999, mental health problems are ranked in the top 20 diseases that represent the major cause of the DALYs.

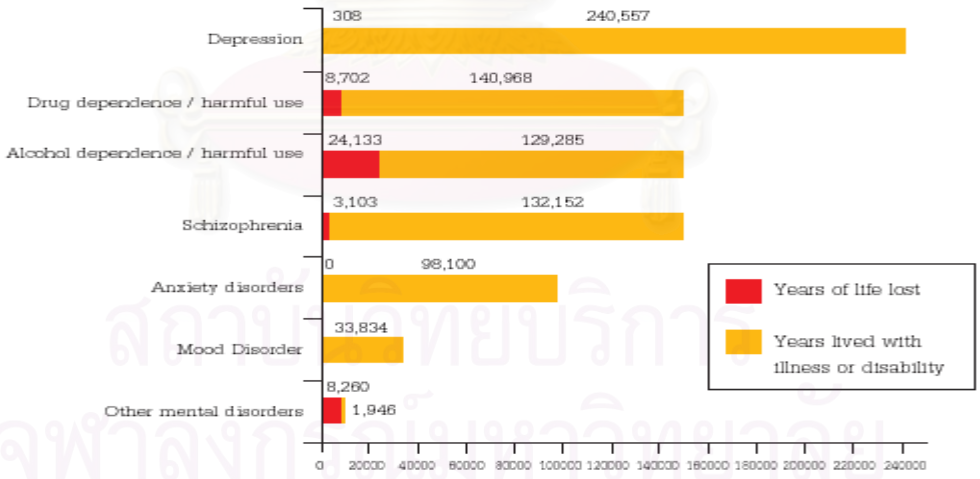
In the male group, depressive disorder has the DALYs value in the 15th position. In the female group, depressive disorder is ranked in the 4th place (Table 2).

According to the study on the burdens of diseases and injuries in Thailand in 1999, particularly with respect to mental health and psychiatric problems, the highest DALYs value occurs in depressive disorder sufferers, but it represents the Years Lived with Disability (YLDs) value alone. It shows that those afflicted with depressive disorder, even though they manage to avoid an untimely death, still have to endure the pain associated with the illness longer than any other mental illness. They are followed by sufferers from drug abuse and alcoholism that have similar DALYs values. But when looked at from the perspective of years of life lost (YLLs), alcoholism sufferers are higher in DALYs than other groups, prompting an epidemiological observation on the pattern of the disease and other disorders that could follow as a consequence, e.g. suicide resulting from depressive disorder, health problem resulting from drug addiction, and brain damage suffered by drinkers as a result of accidents (Figure 1).

Table 2 Diseases that were major cause of the DALYs in Thailand in 1999 (by gender)

Rank	Males	DALYs	%	Females	DALYs	%
1	HIV/AIDS	960,087	17	HIV/AIDS	372,947	9
2	Traffic accidents	510,907	9	Stroke	280,673	7
3	Stroke	267,567	5	Diabetes	267,158	7
4	Liver cancer	248,083	4	Depression	145,336	4
5	Diabetes	168,372	3	Liver cancer	118,384	3
6	Ischemic heart disease	164,094	3	Osteoarthritis	117,994	3
7	COPD (emphysema)	156,861	3	Traffic accidents	114,963	3
8	Homicide and violence	156,371	3	Anemia	112,990	3
9	Suicides	147,988	3	Ischemic heart disease	109,592	3
10	Drug dependence/ harmful use	137,703	2	Cataracts	96,091	2
11	Alcohol dependence/ harmful use	130,654	2	COPD (emphysema)	93,387	2
12	Cirrhosis	117,527	2	Deafness	87,612	2
13	Lung cancer	106,120	2	Lower respiratory tract infections	84,819	2
14	Drowning	98,464	2	Low birth weight	83,879	2
15	Depression	95,530	2	Dementia	70,191	2
16	Osteoarthritis	93,749	2	Anxiety disorders	66,992	2
17	Tuberculosis	93,695	2	Schizophrenia	60,800	2
18	Deafness	93,497	2	Tuberculosis	60,643	2
19	Low birth weight	91,934	2	Birth trauma & asphyxia	57,489	1
20	Anemia	87,610	2	Nephritis & nephrosis	55,258	1

Source: Bureau of Health Policy and Strategy, Ministry of Public Health

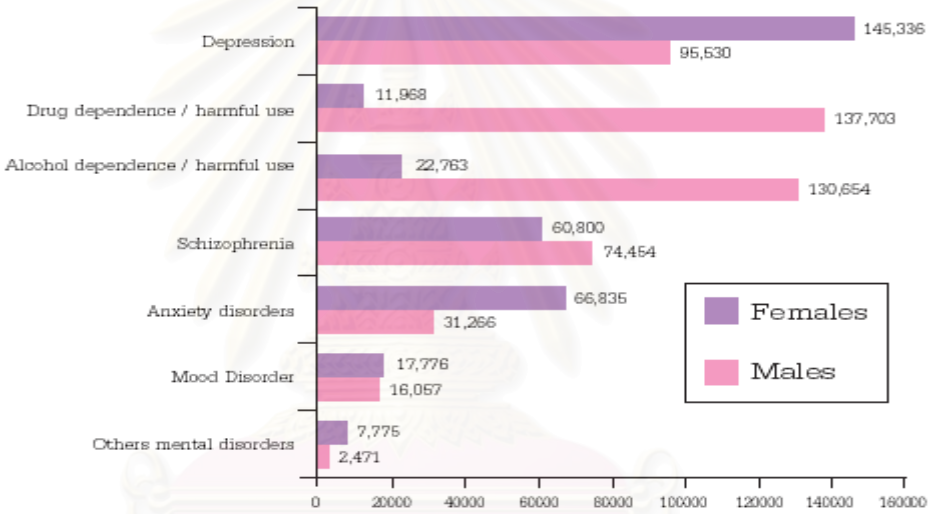


Source: Bureau of Health Policy and Strategy, Ministry of Public Health, 1999

Figure 1 Comparison of mental health burdens, 1999

When considered by gender, the largest groups of females were found to suffer from depressive disorder, followed by anxiety neurosis and schizophrenia. It showed that females ran the highest risk of developing depressive disorder while

males ran the highest risk of facing drug abuse and alcoholism. The fact that women were very vulnerable to depressive disorder and anxiety state certainly affected how they lived their day-to-day life, work and family relations and adversely rubbed off on their children as well. For men who were subjected to drug or alcohol abuse, they were liable to run into accidents, had disabilities, or were unemployed, causing the State to lose its labor force at an untimely date and depriving families of their heads or breadwinners. In some cases, they even became the burden of their families and society. All these cases must be put under epidemiological investigation to pinpoint causes and looked for appropriate counter-measures to alleviate the severity of the disorders (Figure 2).



Source: Bureau of Health Policy and Strategy, Ministry of Public Health, 1999.

Figure 2 Comparison of mental health burdens by gender, 1999

According to a report on the rate of mental illnesses per a population of 100,000 in Thailand from fiscal 1997 to 2001, the trend of depressive disorder patients could not be pinpointed as to what direction it was headed, with both an upswing and a downswing occurring (Figures 3).

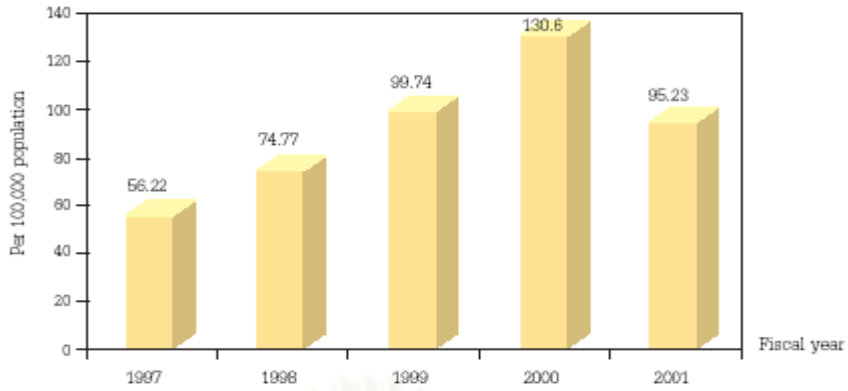
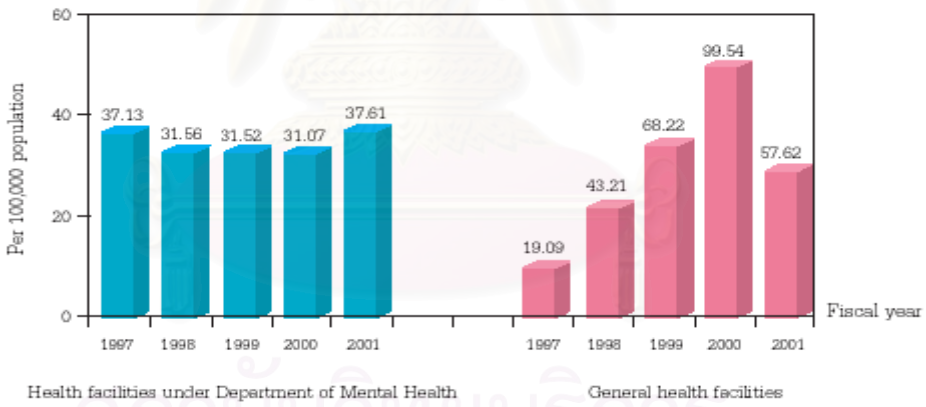


Figure 3 Rates of depressive disorder patients per a population of 100,000 in Thailand, 1997-2001

By health facilities used, the trends of depressive disorder patients at both health facilities under the Department of Mental Health and general health facilities cannot be pinpointed, with both an upswing and a downswing occurring at both types of health facilities, owing perhaps to the diagnostic results of depressive disorder being hidden among other illnesses (Figure 4).



Note: The data do not include Bangkok.

Source: Department of Mental Health, Ministry of Public Health.

Figure 4 Rates of depressive disorder patients per a population of 100,000 in Thailand at general health facilities, 1997-2001

In contrast to our limited understanding of depression, there are many effective treatments. The large majority (80%) of people with depression shows some improvement with any of several antidepressant medications (Department of Mental Health, **online**, October 9, 2004) or electroconvulsive therapy (ECT) (Sackeim et al.,

2001). In addition, several forms of psychotherapy (in particular, cognitive and behavioral therapies) can be effective for patients with mild to moderate cases, and the combination of medication and psychotherapy can exert a synergistic effect (Simon et al., 2004; Treatment for Adolescents with Depression Study [TADS] team, 2004; Reynolds III et al., 1999).

How antidepressant medications achieve their therapeutic benefit is not completely understood. No single drug has proved more effective than any other for the relief of depressive symptoms, but specific drugs may be more useful for associated conditions such as panic disorder (Pollack et al., 2003), neuropathic pain (Saarto and Wiffen, 2005), or obsessive–compulsive disorder (Greist et al., 2003) (Table 3).

Several studies suggest that depression is under treated in primary care. It appears that only 25% of depressed patients are prescribed antidepressant medications (Cassano and Fava, 2002).

Factors that may be considered in selecting a particular antidepressant include a previous response to that medication, a family history of a response to the same medication, and anticipated side effects (Table 4). Although selective serotonin-reuptake inhibitors cost more than tricyclic antidepressants, the total costs of treatment with these two drug classes are usually similar because of the increased number of visits associated with switching to the more expensive medications in some patients who begin with tricyclic agents (Whooley and Simon, 2000). Although newer antidepressants are generally less toxic than tricyclics in cases of overdose, antidepressants have not been convincingly shown to affect the long term outcome of depression or suicide rates (Moncrieff and Kirsch, 2005).

Table 3 Selected antidepressants for used in medical outpatients*

Category and Generic Name	Trade Name	Initial Dose†	Target Dose‡	Step-up Dose‡	Other Indications	Cost per Month§ (US dollar)
Serotonin- and Norepinephrine-reuptake inhibitors						
Tricyclics (tertiary amines)						
Amitriptyline	Elavil, Endep	25 mg at bedtime	100 mg at bedtime	150 mg at bedtime	Chronic pain, delusions, ¶ insomnia, migraine, post therpetic neuralgia	8.43
Doxepin	Sinequan	25 mg at bedtime	100 mg at bedtime	150-200 mg at bedtime	Alcoholism, ¶ insomnia, post-traumatic stress disorder	11.85
Imipramine	Tofranil	25 mg at bedtime	100 mg at bedtime	150-200 mg at bedtime	Enuresis, ¶ insomnia, panic disorder, post-traumatic stress disorder, obsessive-compulsive disorder	42.24
Tricyclics (secondary amines)						
Desipramine	Norpramin	25 mg at bedtime	100 mg at bedtime	150-200 mg at bedtime	Attention-deficit disorder, ¶ bulimia, diabetic neuropathy, posttherpetic neuralgia	32.98
Nortriptyline	Aventyl, Pamelor	25 mg at bedtime	50-75 mg at bedtime	100-150 mg at bedtime	Attention-deficit disorder, chronic low back pain, irritable bowel syndrome, diabetic neuropathy	45.88-70.65
Bicyclic						
Venlafaxine	Effexor	37.5 mg twice daily	75 mg twice daily	100-150 mg twice daily	Anxiety disorder, ¶ neuropathic pain, obsessive-compulsive disorder	78.71
	Effexor XR	37.5 mg daily	75-150 mg daily	225 mg daily		69.65-75.86
Selective serotonin-reuptake inhibitors						
Citalopram	Celexa	20 mg daily	20 mg daily	40 mg daily	Obsessive-compulsive disorder, diabetic neuropathy, post-stroke depression, ¶ panic disorder	60.51
Fluoxetine	Prozac	20 mg every morning	20 mg every morning	40-60 mg daily	Bulimia, ¶ obsessive-compulsive disorder	67.36
Paroxetine	Paxil	20 mg daily	20 mg daily	50 mg daily	Obsessive-compulsive disorder, ¶ panic disorder, migraine, ¶ social phobia	69.86

Sertraline	Zoloft	50 mg every morning	100 mg every morning	150-200 mg every morning	Obsessive-compulsive disorder, ¶ panic disorder, post-traumatic stress disorder	72.29
Serotonin antagonist						
Mirtazapine	Remeron	15 mg at bedtime	30 mg at bedtime	45 mg at bedtime	Anxiety, insomnia	71.83
Norepinephrine- and dopamine-reuptake inhibitors						
Bupropion	Wellbutrin	75 mg twice daily	150 mg twice daily	150 mg three times daily	Attention-deficit disorder, smoking cessation, ¶ post-traumatic stress disorder	96.11
	Wellbutrin SR	150 mg every morning	150 mg twice daily	200 mg twice daily		91.64
Serotonin antagonist and reuptake inhibitors						
Nefazodone	Serzone	100 mg twice daily	150 mg twice daily	300 mg twice daily	Panic disorder, post-traumatic stress disorder	74.11
Trazodone‖	Desyrel	50 mg at bedtime	200 mg at bedtime	200 mg twice daily	Insomnia	21.98

*The information provided in this table is intended only as a guide. Providers should refer to the package inserts or consult with a pharmacist for individual dosage recommendations, precautions, and drug interactions.

†The initial dose should be reduced in frail or elderly patients and in patients with hepatic or renal dysfunction.

‡The target dose is the dose likely to be effective for a typical patient. The step-up dose is the dose above which most patients would not derive additional benefit (Whooley and Simon, 2000, cited in McEvoy, 2000).

ξThe average wholesale price in U.S. dollars for a 30-day supply of the target dose is given. Generic prices are used, when available (Whooley and Simon, 2000, cited in 2000 Drug topics red book, 2000).

¶The drug is approved by the Food and Drug Administration for this indication.

‖Trazodone is too sedating at therapeutic doses for depression; it is best used in lower doses (50–100 mg at bedtime) as adjunctive therapy for patients with insomnia.

Table 4 Frequency of side effects of antidepressant medications*

Medication	Sedation	Agitation	Anti-cholinergic effects†	Postural hypotension	Gastrointestinal upset	Sexual dysfunction	Weight gain	Weight loss
Serotonin- and norepinephrine-reuptake inhibitors								
Tricyclics (tertiary amines)								
Amitriptyline	++++	0	++++	+++	+	+	++	0
Doxepin	++++	0	++++	+++	+	+	+	0
Imipramine	++++	0	++++	+++	+	+	+	0
Tricyclics (secondary amines)								
Desipramine	+++	0	+++	++	+	+	+	0
Nortriptyline	+++	0	+++	++	+	+	+	0
Bicyclic								
Venlafaxine‡	++	+	++	0	+++	++	0	+
Selective serotonin-reuptake inhibitors								
Citalopram	0	0	+	0	++	+	+	+
Fluoxetine	+	++	+	0	++	++	+	+
Paroxetine	++	0	+	0	++	++	+	+
Sertraline	+	+	+	0	++	++	+	+
Serotonin antagonist								
Mirtazapine	+++	0	++	+	0	0	++	0
Norepinephrine- and dopamine-reuptake inhibitor								
Bupropion	+	++	++	0	++	0	+	++
Serotonin antagonists and reuptake inhibitors								
Nefazodone	++	0	++	+	+	0	0	0
Trazodone	++++	0	++	+	+	0	+	+

*0 denotes none; +, minimal (<5 percent of patients); ++, low frequency (5–20 percent); +++, moderate frequency (21–40 percent); ++++, high frequency (>40 percent).

†Side effects may include dry mouth, dry eyes, blurred vision, constipation, urinary retention, tachycardia, or confusion.

‡Venlafaxine may cause a dose-related elevation in diastolic blood pressure; monitoring of blood pressure is recommended.

CHAPTER II

LITERATURE REVIEW

Pathophysiology of Depression

Knowledge of the function of different brain regions under normal conditions suggests the aspects of depression to which they may contribute. Neocortex (Bremner et al., 2003) and hippocampus (Tanaka et al., 2004) may mediate cognitive aspects of depression, such as memory impairments and feelings of worthlessness, hopelessness, guilt, doom, and suicidal. The striatum (particularly the ventral striatum or nucleus accumbens [NAc]) and amygdala, and related brain areas, are important in emotional memory, and could as a result mediate the anhedonia (decreased drive and reward for pleasurable activities), anxiety, and reduced motivation that predominate in many patients (Schwartz et al., 2003). Given the prominence of so-called neurovegetative symptoms of depression, including too much or too little sleep, appetite, and energy, as well as a loss of interest in sex and other pleasurable activities, a role for the hypothalamus has also been speculated (Wheatland, 2005). Of course, these various brain regions operate in a series of highly interacting parallel circuits, which perhaps begins to formulate a neural circuitry involved in depression (Figure 5).

Neurobiological basic researches as well as clinical studies have revealed that monoaminergic pathways are highly responsive to aversive stimuli and play a crucial role in the control of affect, cognition, emotion, and behaviors, all of which are profoundly disrupted in depressive states. Accordingly, a perturbation of monoaminergic transmission is implicated in the etiology of depressive disorders (Figure 6) (Kalia, 2005).

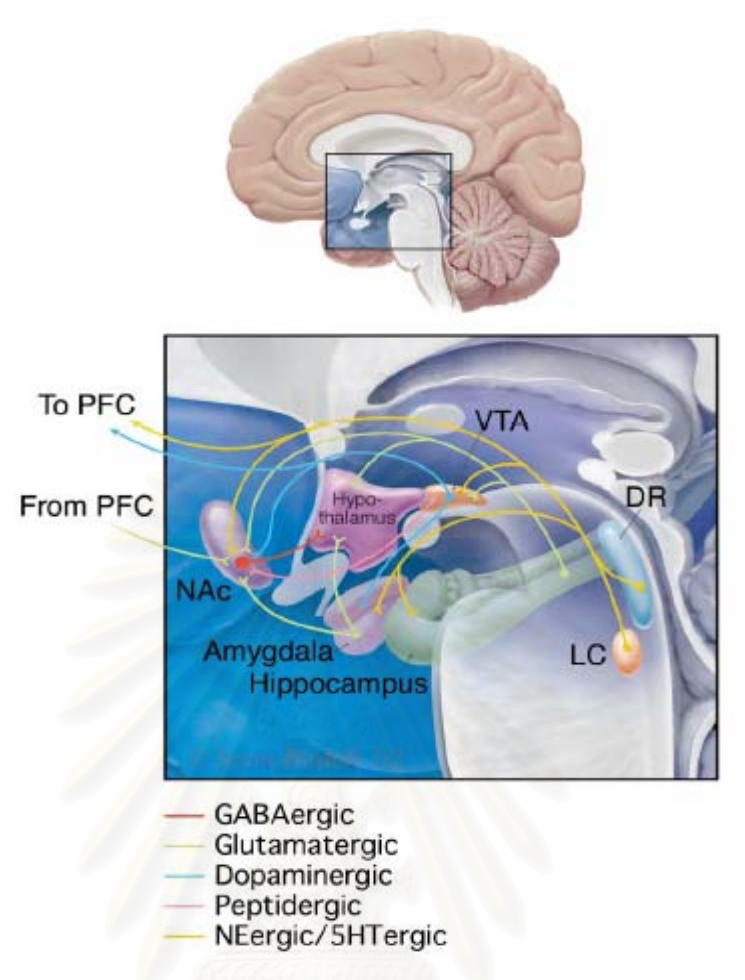


Figure 5 Neural Circuitry of Depression.

The figure shows a highly simplified summary of a series of neural circuits in the brain that may contribute to depressive symptoms. While most research in the depression field has focused on hippocampus (HP) and frontal cortex (e.g., prefrontal cortex [PFC]), there is the increasing realization that several subcortical structures implicated in reward, fear, and motivation are also critically involved. These include the nucleus accumbens (NAc), amygdala, and hypothalamus. The figure shows only a subset of the many known interconnections among these various brain regions. The figure also shows the innervations of several of these brain regions by monoaminergic neurons. The ventral tegmental area (VTA) provides dopaminergic input to the NAc, amygdala, PFC, and other limbic structures.

Norepinephrine (from the locus coeruleus or LC) and serotonin (from the dorsal raphe [DR] and other raphe nuclei) innervate all of the regions shown in the figure. In addition, there are strong connections between the hypothalamus and the VTA-NAc pathway (Nestler, Barrot, et al., 2002; Harvey et al., 2005).

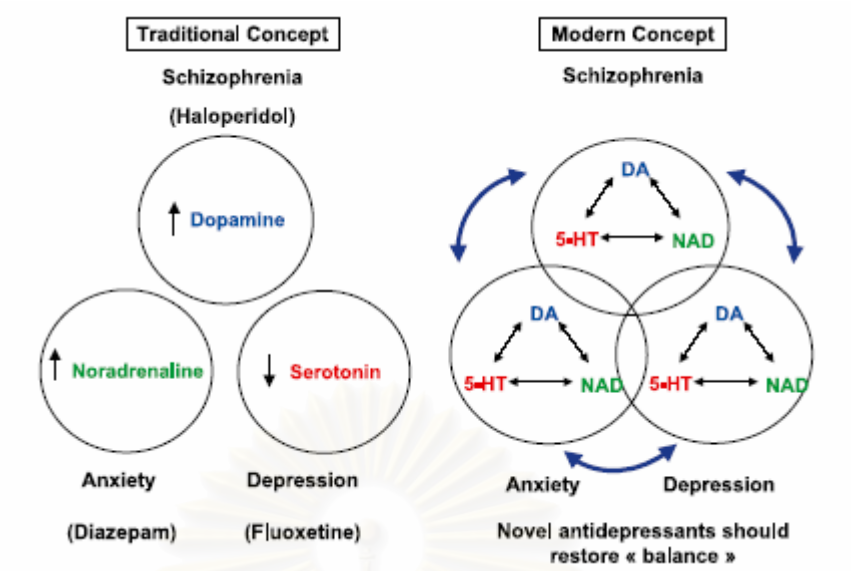


Figure 6 Depressive states are often co morbid with other psychiatric disorders, all of which involve complex alterations in monoaminergic transmission. Attribution of disorders to unitary changes in the activity of one monoamine is a simplification in view of complex patterns of changes in monoaminergic transmission and equilibrium in depressive and other psychiatric states.

All clinically available antidepressants directly harness monoaminegic mechanisms in that they interact with enzymes for catabolism, transporters for reuptake, or receptors expressing their actions. And all operatively behave as multitarget agents. These broad-based actions are commensurate with the widespread perturbation of monoaminergic networks (and other modulators) in depressive states. Indeed, the involvement of monoamines in depressive states cannot be subsumed by a simplistic formula of “insufficient release.” Rather, they are implicated in a complex region and receptor-dependent manner. Accordingly, there is a need for antidepressant strategies which reestablish the perturbed equilibrium amongst corticolimbic monoaminergic pathways (Figure 7) (Millan, 2004).

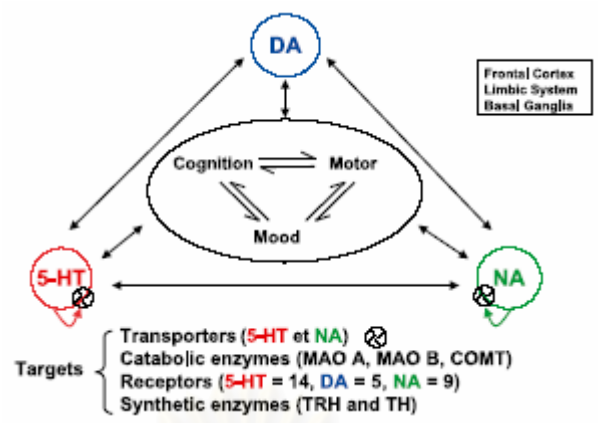


Figure 7 Corticolimbic monoaminergic mechanisms control mood, cognition, and motor behavior – functions profoundly disrupted in depressive states. For restoration of perturbed monoaminergic transmission and homeostasis, a multiplicity of targets is available. TRH = tryptophan hydroxylase; TH = tyrosine hydroxylase; COMT = catechol-O-methyltransferase; MAO = monoamine oxidase.

However, it is not the enhancement of monoaminergic signaling per se, but rather long-term, adaptive changes that may underlie the therapeutic effect. These include functional and structural changes (Schloss and Henn, 2004).

Dopamine

To understand the function of dopamine (DA) and dopaminergic compounds, it is important to consider sites of action in both the CNS and in some peripheral tissues, including the cardiovascular system and the kidney. DA participates in the control of many physiological functions. In the CNS, DA receptors are widely expressed and thus, are involved in a range of functions, including the control of locomotion, cognition, and emotion, in addition to neuroendocrine secretion. All effects occur through interactions with membrane receptors that belong to the family of seven transmembrane domain G-protein-coupled receptors.

DA receptors are the primary targets of drug action in the pharmacological treatment of various diseases, such as schizophrenia, Parkinson's disease (PD), and migraine. D_2-receptors have been implicated in the pathophysiology of schizophrenia and PD.

Dopamine receptors

At least six different forms of the DA receptors cloned from the brain have been reported. The D_1 class of DA receptor has been divided into D_1 and D_5 receptor subtypes (D_{1A} and D_{1B} in rats; D_{1B} is also known as D_5 in humans), and the D_2 class comprises D_{2S}-, D_{2L}-, D_3-, and D_4-receptor subtypes (see Table 5). When activated, the D_1-like receptor subtypes stimulate adenylate cyclase activity, whereas the D_2-like receptor subtypes generally inhibit adenylate cyclase activity (Table 5).

Table 5 Classification of the dopamine receptors

	D_1 family		D_2 family		
	D_1	D_5	D_2	D_3	D_4
Agonists	SKF-38393 *R*(+)SKF-81297 Dihydrexedine	Dopamine SKF-38393	Quinpirole Bromocriptine (+)PHNO	Quinpirole Pergolide 7-OH-DPAT	Dopamine
Antagonists	SCH-23390 α-Flupenthixol SKF-83566 SCH-39166	SCH-23390	Spiperone Raclopride Sulpiride Haloperidol U-101958	UH-232 Nafadotride (+)S-14297	Spiperone Clozapine U-101387
Function					
Adenylate cyclase	Stimulates	Stimulates	Inhibits	?	?
Phosphoinositol turnover	?	?	Inhibits	?	?
Molecular structure					
Size (amino acid residues)	446	447	414 (short) 443 (long)	446	387
mRNA size	3.8 kb	3 kb	2.5 kb	8.3 kb	5.3 kb
Distribution					
Brain	Striatum	Hypothalamus	Striatum	Olfactory tubercle	Frontal cortex
	Nucleus accumbens	Hippocampus	Substantia nigra	Hypothalamus	Midbrain
Periphery	-	-	Heart	-	Heart
Archetypic tissue	Parathyroid gland	-	Pituitary gland	-	-

R(+)SKF-81297, *R*(+)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-benzazepine; (+)*S*-14297, (+)-7-(*N*,*N*-dipropylamino)-5,6,7,8-tetrahydronaphtho[2,3-*b*]dihydro-2,3-furane; 7-OH-DPAT, 7-hydroxy-diphenylaminotetralin; (+)PHNO, 9-hydroxy-4-propyl-naphthoxazine; PD-128907,*R*(+)-*trans*-3,4,4*a*,10*b*-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano[4,3-*b*]-1,4-oxazine-9-ol; U-101387, (*S*)-(2)-(4-[4-{isochroman-1-yl}ethyl]piperazin-1-yl)benzenesulphonamide; U-101958, 3-isopropoxy-*N*-methyl-*N*-(1-[phenylmethyl]-4-piperidinyl)-2-pyridinylamine.

Central dopaminergic systems

The central dopaminergic neuron system is comprised of three main pathways.

1. The nigrostriatal pathway originates in the substantia nigra (A9 region), projects to the striatum, and is involved in extrapyramidal motor function.
2. The mesolimbic/mesocortical pathways originate in the ventral tegmental area (A10) and project to cortical structures considered to be crucial for cognitive function and motivation.
3. The tubuloinfundibular pathway originates in the hypothalamus (A12), projects to the hypophysis, and is involved in neuroendocrine regulation (Cooper et al., 2003).

Mesolimbocortical DA is implicated in reward and reinforcement mechanisms, as shown by the observation that administration of psychostimulants and drugs of abuse elicits an increase in DA release in the mesolimbic areas, whereas withdrawal of these drugs results in a reduction of dopaminergic transmission. There is also a general agreement that mesolimbocortical DA plays a role in learning and memory (Cami and Farre, 2003). The prefrontal cortex, defined as the essential cortical projection area of the thalamic mediodorsal nucleus, is implicated in the control of locomotion activity and plays a major role in cognitive processes, as well as in affective and emotional behaviors (Kodama et al., 2004; Jiao et al., 2005).

The D_1-receptor is the most widespread DA receptor and expressed at higher levels than any other DA receptor. D_1 mRNA and protein have been found in the striatum, the nucleus accumbens, olfactory tubercle, caudate putamen, septum, amygdala, hippocampus, and cerebellum (Cooper et al., 2003). In addition, D_1-receptors have been detected in the limbic system, hypothalamus, and thalamus. D_1-receptors in the entopeduncular nucleus and in the substantia nigra pars reticulata are preferentially localized on striatal gamma-aminobutyric acid (GABA)ergic neurons co-expressing substance P. Lesion studies in the brain with 6-OH-DA failed to reduce the density of D_1-binding sites in the substantia nigra and striatum pathways, suggesting that in contrast to the localization of D_2-receptors, D_1-receptors in nigra are primarily presynaptic (on afferent projections) and in the caudate-putamen are primarily postsynaptic (Emilien et at., 1999).

A dysfunction of mesolimbic and mesocortical dopaminergic pathways is primarily implicated in the melancholic and cognitive features of depression,

respectively. Interestingly, a common trait of antidepressants is an enhancement in extracellular levels of dopamine in the frontal cortex, exerted by two basic mechanisms: (1) blockade of cortical noradrenaline transporters, which – since they greatly outnumber dopamine transporters in this region – are responsible for taking up dopamine and (2) blockade of α_2-adrenoceptors and 5-HT_{2C} receptors, which tonically inhibit mesocortical vs. subcortical dopaminergic projections (Millan et al., 2000a, 2000b). Although antidepressants do not, in general, enhance dopamine release in nucleus accumbens, they "strengthen" dopaminergic signaling and elicit adaptive changes in mesolimbic D_2/D_3 receptors: clarification of underlying mechanisms could lead to more effective treatment of anhedonia. D_2/D_3 receptor agonists, such as pramipexole, reveal marked antidepressant in humans (Reichmann et al., 2003). Dopamine D_2/D_3 receptor agonists act by stimulation of D_2 sites. An alternative approach may be D_3 receptor antagonists since, in models of mood, motor function, and cognition. They exert actions opposite to dopamine D_2 receptor antagonists that aggravate depressive states. Finally, blockade of inhibitory D_2 autoreceptors, which enhances dopamine levels in the nucleus accumbens and frontal cortex, may be involved in antidepressant properties of the antipsychotic, amisulpride (Millan, 2004; Cassano and Jori, 2002). Most of the available evidence show that the increased sensitivity to dopamine receptor stimulation induced by chronic antidepressant treatments is related to an increased dopamine D_2-like (i.e. D_2 and D_3) receptor function (Klimek et al., 2002), and a decreased dopamine D_1 receptor number and sensitivity. Moreover, these changes are most prominent in the limbic areas, i.e. those areas innervated by dopamine neurons in the ventral tegmental area, thus supporting the view that they might indeed be important in the therapeutic effect of these drugs. The down-regulation of dopamine D_1 receptors might play a role in the changes of dopamine D_2-like receptor function. Indeed, it is well established that dopamine D_1 and D_2 receptors are functionally linked and that the dopamine D_1 receptors play a permissive role for the expression of postsynaptic stimulant responses mediated by dopamine D_2 receptor activation (D'Aquila et al., 2000). An enhanced neurotransmission at the dopamine D_1 receptor level might result in an antidepressant effect; results from several laboratories have shown that acute or sub-acute administration of selective dopamine D_1 receptor agonists has an antidepressant effect similar to that of chronic imipramine in two animal models of depression, the forced

swimming test and the learned helplessness (Gambarana et al., 1995). And the chronic-stress-induced depressive state is caused by a D_1 receptor-mediated hypodopaminergic mechanism in the PFC (Mizoguchi et al., 2002).

Moreover, acute treatment with the dopamine D_1 receptor antagonist SCH 23390, but not with the dopamine D_2-like receptor antagonist L-sulpiride, suppresses the effect of chronic imipramine in the learned helplessness model of depression (Gambarana et al., 1995). These observations provide support to the hypothesis that an enhanced dopamine transmission at the dopamine D_1 receptor level induced by chronic antidepressants might play an important role in their mechanism of action, and conversely, that changes in the sensitivity of dopamine D_1 receptors might be involved in the pathogenesis of mood disorders. In summary, release studies show that all antidepressants, either acutely or chronically administered, interfere with dopamine release. However, the only effect shared by all antidepressant drugs, that might be relevant to their therapeutic effect, appears to be the increase of dopamine release in the prefrontal cortex after acute administration (D'Aquila et al., 2000).

Serotonin and Depression

5-hydroxytryptamine (5-HT, serotonin) has been implicated in the etiology of many disease states and in particular in mental illnesses such as depression, anxiety, schizophrenia, obsessive compulsive disorder (OCD), panic disorders (Michels and Marzuk, 1993a, 1993b), and eating disorders (Becker et al., 1999). Many currently used treatments of these disorders are thought to act through the modulation of serotoninergic tone.

During the past 10 years, many 5-HT receptor subtypes have been characterized, and to date at least 15 different receptors for this neurotransmitter have been identified. Initially, receptors were characterized using pharmacological tools, but more recently, molecular biology techniques have allowed the cloning and sequencing of these receptors encoded by different genes. Knowledge of 5-HT receptor cDNA sequences has also allowed antibody and antisense techniques to be employed.

All 5-HT receptor subtypes, except one (the 5-HT_3 type), are members of the G-protein-coupled superfamily. Their stimulation affects various enzymes (adenylate cyclase, phospholipases A and C) and cation channels (especially K^+ and Ca^{++}

channels) through the activation of intracellular specific G proteins (Figure 8) (Siegel et al., 1999).

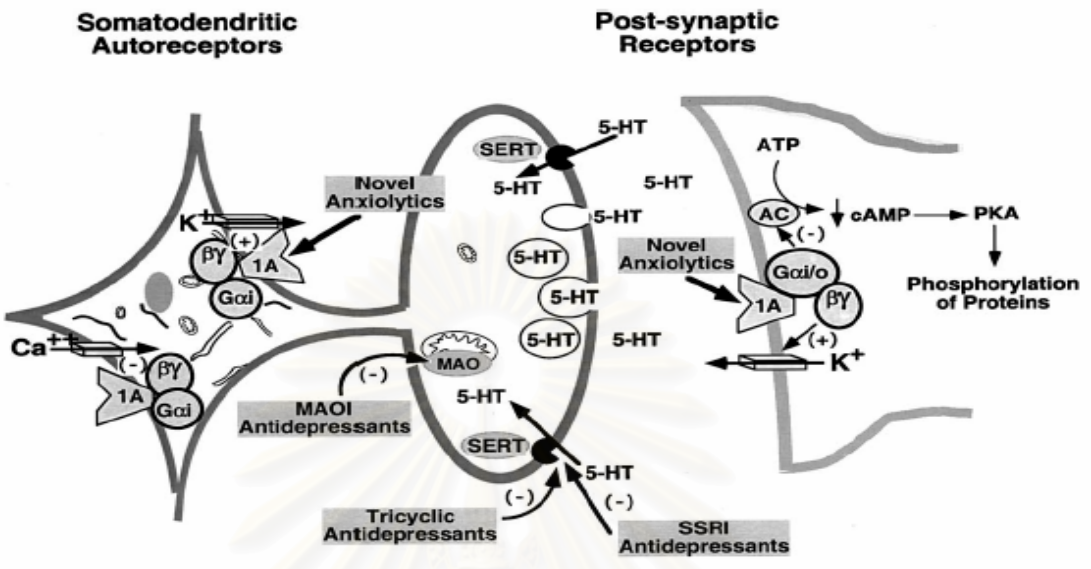


Figure 8 Anxiolytic and antidepressant drug effects on serotonergic neurotransmission. The 5-HT_{1A} receptor is located on serotonergic cell bodies and dendrites and functions as the somatodendritic autoreceptor. In terminal field areas of serotonergic innervation, the 5-HT_{1A} receptor is located postsynaptically. The azapirone compounds are agonists at the 5-HT_{1A} receptor and comprise a new class of psychoactive agents with anxiolytic and antidepressant activity. By blocking the serotonin transporter (SERT) or inhibiting monoamine oxidase (MAO), antidepressant drugs increase the synaptic concentration of the neurotransmitter 5-HT.

The 5-HT_{1A} Receptors

This receptor, together with the 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} receptors, forms a first group sharing the capacity to inhibit adenylyl cyclase when they are stimulated. Selective agonists and a selective radioligand of the 5-HT_{1A} receptor were developed early, which explains why this receptor is the best known among the 15 different 5-HT receptors that have been cloned, sequenced, and pharmacologically characterized to date. This receptor is widely distributed in the central nervous system. The studies showed that 5-HT_{1A} receptor binding sites in rat brain are especially abundant in the gyrus dentatus and CA1 area of Ammon's horn in the hippocampus, the lateral septum, the entorhinal and frontal cortex, and the dorsal raphe nucleus. Significant but lower expression of 5-HT_{1A} receptors was also reported

in some thalamic and hypothalamic nuclei. In contrast, these receptors are hardly detected in the striatum, substantia nigra, and cerebellum (Lanfumey and Hamon, 2000).

In the DLPFC (dorsolateral prefrontal cortex) and HC (hippocampus), there is a significant decrease in 5-HT_{1A} mRNA of subjects with major depression disorder. Alterations in 5-HT_{1A} mRNA levels in the brains of subjects with mood disorders add further support for hypothesis of dysregulation of the serotonergic system in these psychiatric disorders (Lopez-Figueroa et al., 2004).

The role of serotonin in the treatment of depressive and anxiety disorders is underscored by the therapeutic action of selective 5-HT reuptake inhibitors acting to enhance the degree of activation of various 5-HT receptor subtypes. The 5-HT_{1A} receptors are particularly relevant to the antidepressant and anxiolytic responses in human beings (Fricchione, 2004). They are located presynaptically in the raphe nuclei, where they act as cell body autoreceptors to inhibit the firing rate of 5-HT neurons, and are located postsynaptically in limbic and cortical regions, where they also attenuate firing activity.

Functions of Serotonin$_{1A}$ Receptors

5-HT_{1A} receptors are present on the soma and dendrites of 5-HT neurons and on postsynaptic neurons in the brain and spinal cord. On 5-HT neurons, 5-HT_{1A} receptors exert a negative feedback influence on firing activity: when activated by an excess amount of 5-HT, or by an exogenous agonist, they hyperpolarize 5-HT neurons, thereby slowing down their pacemaker firing activity. Because 5-HT release is proportional to the firing rate of 5-HT neurons, the excessive activation of 5-HT_{1A} autoreceptors results in a decrease of 5-HT release in projecting structures (Rueter et al., 1997). The postsynaptic 5-HT_{1A} receptors, which are particularly abundant in limbic structures, commonly exert an inhibitory function on neuronal activity as well. Therefore the net effect of the systemic administration of a 5-HT_{1A} receptor agonist results in a net decrease of 5-HT transmission at all postsynaptic 5-HT receptors, except those of the 5-HT_{1A} subtype. With respect to 5-HT_{1A} signal transfer in postsynaptic areas, the response to the systemic administration of a 5-HT_{1A} agonist represents a composite of decreased 5-HT release (resulting from 5-HT_{1A} autoreceptor activation) and direct occupation of the postsynaptic 5-HT_{1A} receptors (Blier and Ward, 2003; Cowen, 2000).

Relevance of Serotonin$_{1A}$ Receptors in the Antidepressant Response

Several lines of preclinical data suggest that postsynaptic 5-HT$_{1A}$ receptors are particularly important to the antidepressant response, and this body of evidence is growing. For instance, behavioral models of stress and antidepressant drug effects in animals, such as the forced swimming test and chronic mild stress, have consistently shown that activation of postsynaptic 5-HT$_{1A}$ receptors produce changes similar to those of conventional antidepressants. Interestingly, the phenomenon of neurogenesis, recently shown to occur with various types of antidepressant treatments in the hippocampus, appears to be mediated by the activation of 5-HT$_{1A}$ receptor (Dremencov et al., 2003; Fujita et al., 2000). These results thus provide further tentative support for the hypothesis that an enhancement of 5-HT$_{1A}$ transmission in the forebrain underlies the antidepressant response (Blier and Ward, 2003).

The increase in serotonin neurotransmission, due to somatodendritic autoreceptor desensitization following agonist or antidepressant treatment, to normo-sensitive 5-HT$_{1A}$ receptors in certain brain regions (e.g. hippocampus or cortex) and to sub-sensitive 5-HT$_{1A}$ receptors in other brain regions (e.g. amygdala or hypothalamus) also underlies the therapeutic efficacy of these drugs (Figure 9) (Hensler, 2003).

Animal models of depression

As defined by the American Psychiatric Association, depression is a heterogeneous disorder often manifested with symptoms at the psychological, behavioral and physiological levels. As with all diseases, approximations of both the disorder and the actions of corrective medications in laboratory animals are essential for the development of effective therapies. The wide spectrums of disruptions that characterize depression highlight the difficulty posing researchers to mimic the disorder in the laboratory. Indeed, two human symptoms, recurring thoughts of death or suicide, or excessive thoughts of guilt, are impossible to model in laboratory animals. The question remains impenetrable as to whether we can ever know whether a laboratory animal is ‘depressed’. Nonetheless, numerous attempts have been made to create animal models of depression, or at least of the symptoms of depression, and criteria for their evaluation have been established (Anisman and Matheson, 2005).

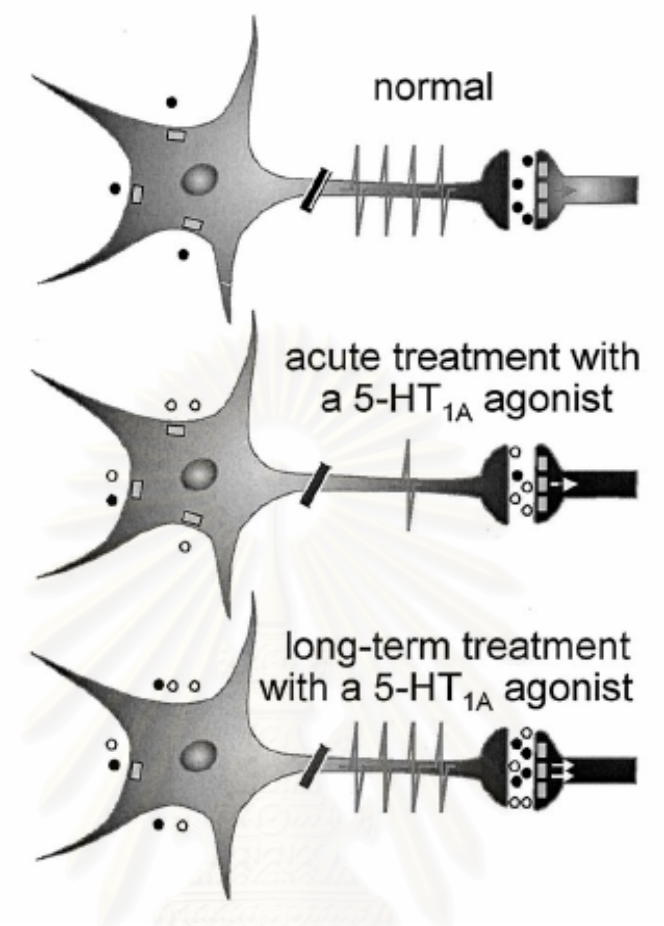


Figure 9 Diagrams summarizing the effect of acute and long-term treatments with 5-HT_{1A} agonists on the function of the 5-HT system. The rectangles represent the 5-HT_{1A} autoreceptors on the cell body of 5-HT neurons on the left and on postsynaptic neuronal elements on the right. The filled circles represent the 5-HT molecules and the open circles the 5-HT_{1A} agonist. The numbers of action potentials on the axons represent the firing rates of 5-HT neurons. The small arrows within the postsynaptic element represent and are proportioned to 5-HT_{1A} neurotransmission. Note the disappearance of the rectangles in the cell body of 5-HT neurons but not on the postsynaptic neurons. This corresponds to the desensitization of the presynaptic but not postsynaptic 5-HT_{1A} receptors (in the hippocampus) after long-term treatment with 5-HT_{1A} agonists.

Some of the most widely cited criteria were developed by McKinney and Bunney more than 30 years ago (Cryan et al., 2002). They proposed that the minimum requirements for an animal model of depression are: (1) it is 'reasonably analogous' to the human disorder in its manifestations or symptomatology; (2) there is a behavioral change that can be monitored objectively; (3) the behavioral changes observed should be reversed by the same treatment modalities that are effective in humans; and (4) it should be reproducible between investigators.

Various paradigms have been developed and are instrumental in detecting the antidepressant-like potential of novel compounds in preclinical settings. The models commonly used are diverse and were developed originally based on the behavioral consequences of stress, drug, lesion or genetic manipulations (Table 6) (Cryan et al., 2002).

Several animal models of depression have been developed, largely based on the effectiveness of known antidepressants or responses to stress, due to the current lack of known depression-vulnerability genes for development of animal models. These models have been used as research tool to screen effective antidepressant agents. The forced swim test (FST) was developed by Porsolt and colleagues (Porsolt, Le Pichon, and Jalfre, 1977) in the rat and, subsequently, in the mouse (Porsolt, Bertin, and Jalfre, 1977). This test is the most widely used tool for assessing antidepressant activity preclinically. The widespread use of this model is largely a result of its ease of use, reliability across laboratories and ability to detect a broad spectrum of antidepressant agents. The forced swim test exhibits a predictive value in detecting antidepressant action but does not reliably detect selective serotonin reuptake inhibitors, including failed responses to chronic antidepressant treatment. It needs a modified scoring for achieving improved detection. About half of the rats in the forced swim test were reported to dive under the water surface, a behavior that was rarely observed and not reliably altered by any of the antidepressants tested by other investigators. The diving behavior resembles responses to trauma, as in underwater trauma studies.

Table 6 Widely used rodent models sensitive to the effects of antidepressant agents

Animal model	Ease of use	Reliability	Specificity	Applicable to mice	Comments
Forced swim test	High	High	High	Yes	Sensitive to acute antidepressant treatments; does not reliably detect SSRIs
Modified forced swim test	High	High	High	?	Sensitive to acute antidepressant treatments; differentiates antidepressants from different classes including SSRIs
Tail suspension test	High	High	High	Yes	Sensitive to acute antidepressant treatments; certain strains climb their tail
Olfactory bulbectomy	Medium	High	High	Yes	Behavioral effects evidence only following chronic treatment; mechanism of action poorly understood
Learned helplessness	Medium	Medium	High	Yes	Sensitive to short-term antidepressant treatments; ethical restrictions in some countries
DRL-72	Medium	Medium	Medium	?	Sensitive to short-term antidepressant treatments
Neonatal clomipramine	Medium	Medium	?	Yes	Only limited testing of antidepressant have been conducted
Prenatal stress	Medium	?	?	Yes	Only limited testing of antidepressant have been conducted
Chronic mild stress	Low	Low	High	Yes	Reliability has been questioned repeatedly; behavioral effects evident only following chronic treatment
Resident intruder	Low	?	Medium	?	Distinguishable behavioral effects only following chronic treatment; requires further validation in other laboratories
Drug-withdrawal-induced changes in ICSS	low	high	Medium	Yes	Requires further validation; cannot assess baseline strain differences easily

DRL-72, differential reinforcement of low-rate 72 second schedule; ICSS, intracranial self-stimulation; SSRIs, selective serotonin reuptake inhibitors

Depression is characterized by a lack of ‘‘motivation’’ rather than a lack of ‘‘physical space’’ to move around. In an open space swimming test, the model uses a large pool, with the induced depressive behavior resulting from a lack of motivation (hopelessness), rather than a lack of “physical space”. The results suggest that the open space swimming test is highly predictive of antidepressant action and is more sensitive to the drug treatments. The measurement is more objective than that of the forced swimming test and does not involve judging and scoring the animals’ movement or lack of movement by investigators. The demonstrated effectiveness of four major types of antidepressants: imipramine, a prototypical tricyclic antidepressant, iproniazid, a monoamine oxidase inhibitor, mianserin, an atypical antidepressant, and alaproclate, an SSRI, all significantly reduced the immobility. The results suggest that the effects on the test are not restricted to a particular underlying molecular mechanism of action. Thus, this swimming test shows promising potential as a screen for novel antidepressants and, perhaps, for revealing some of the underlying pathophysiology of depression.

The open space swimming test satisfies (or better satisfies than the leading forced swimming model) three of the four minimum requirements for an animal model of depression, with one (reliability across laboratories) remaining to be tested. These are (1) reasonably analogous to the human disorder in its manifestations or symptomatology; (2) the existence of a behavioral change that can be monitored objectively; (3) reversibility of the behavioral change by the same treatments that are effective in human; and (4) reproducible between investigators.

The open space swimming model has several advantages. First, one obvious advantage of this test over the leading forced swimming test is that, unlike those time-sampling judgment and scoring in the forced swimming test, no human judging and scoring are involved in this test so that the monitoring in this test is more objective, offering a possible improvement of reproducibility across laboratories as well (second advantage). Third, in the forced swimming test, the extent of the behaviors (such as vigorous, moderate, or mild swimming mobility) is not reflected in the score counts, but is directly measured in this test. In addition, no climbing behavior was observed in the open space model, probably due to the large space available. This removes another artificial judgment as whether climbing should account for more than or equal to active swimming in the forced swimming test. Fourth, this test does not limit the

animal's movement due to space restriction and probably mimics the disorder in human more closely (Sun and Alkon, 2003, 2004).

Herbal medicine and the treatment of depression

Herbal medicine is an increasingly common form of alternative therapy in the United States. A 1997 survey estimated that 12.1 percent of adults in the United States had used an herbal medicine in the previous 12 months (as compared with 2.5 percent in 1990), resulting in out-of-pocket payments of $5.1 billion. Among those who had used herbal medicine, 15.1 percent had seen an alternative medicine practitioner, with a total of 10.5 million office visits, 19.8 percent of which had been completely or partially covered by insurance. Several countries (e.g., Germany, France, Sweden, and Australia) have implemented strategies for licensing herbal remedies. Since ancient times some plants have been used to relieve depressive symptoms, the use of their extracts is gaining increased acceptance by both the medical profession and patients. Among such plants, *Passiflora incarnat*a Linn. (Passionflower, Maypop, Apricot vine), St. John's Wort (*Hypericum perforatum*) (De Smet, 2000), Product Catuama [the association of four hydroalcoholic extracts obtained from the following plants: Trichilia catigua (Meliaceae), Paullinia cupana (Sapindaceae), Ptychopetalum olcaloides (Olacaceae) and Zinziber officinalis (Zinziberaceae)] (Campos et al., 2004), and *Aniba riparia* (Sousa et al., 2004) are used as antidepressant in some countries.

The genus *Passiflora*, comprising about 500 species, is the largest in family Passifloraceae (the Passion flower family). The species of this genus are distributed in the warm temperate and tropical regions of the New World; they are much rarer in Asia, Australia, and tropical Africa. The use of *Passiflora* as a medicine was lauded for the first time by a Spanish researcher Monardus in Peru in 1569 as the beautiful flowers of *Passiflora* appeared to him to be symbolic of the passion of Christ. Various species of *Passiflora* have been used extensively in the traditional system of therapeutics in many countries.

Passiflora foetida (PF) leaf infusion has been used to treat hysteria and insomnia in Nigeria. The plant is widely cultivated in India. The leaves are applied on the head to treat giddiness and headache; a decoction is given in bilious-ness and asthma. The fruit is used as an emetic. In La Reunion, the leaves are considered

emmenagogue and are also prescribed in hysteria. In Brazil, the herb is used in the form of lotions or poultices for erysipelas, and skin diseases with inflammation. Moreover, some reports indicated the use of *Passiflora foetida* in depression and anxiety (Duke, 2002; Li, 2000).

Alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds are known in *Passiflora foetida* Linn. Apart from previous constituents, various miscellaneous phyto-constituents reported from *Passiflora foetida* include: flavonoids pachypodol, 7,4′-dimethoxyapigenin, 4′,7-*O*-dimethyl-naringenin, 3,5-dihydroxyflavanone, C-glycosyl flavonoids chrysoeriol, apigenin, isovitexin, vitexin, 2″-xylosylvitexin, luteolin-7-β-D-glucoside, kaempferol; Cyanohydrin glycosides tetraphyllin A, tetraphyllin B, tetraphyllin B sulphate, deidaclin, volkenin; Fatty acids linoleic acid, linolenic acid; and alpha-pyrones, pasifloricins (Dhawan et al., 2004).

Recently, crude extracts of *Passiflora foetida* had been tested for the antidepressant activity (Unchern et al, unpublished observations). It was found that they increased the active swimming time (motivational behavior) of mice in the open-space swimming test. At present, the mechanism of antidepressant activity of *Passiflora foetida* is still unknown. However, preliminary study suggested that the extracts of *Passiflora foetida* possessed marked binding affinity to D_1 and 5-HT_{1A} receptors from rat brains and significant antidepressant activity in mice (Meksuriyen et al, unpublished observations). Therefore, the extracts of *Passiflora foetida* or its subfractions might be potential candidates for using in the treatment of depression.

In the light of above considerations, the present study was designed to investigate the antidepressant activity of *Passiflora foetida* subfractions. Firstly, the locomotor activity test has been used to evaluate CNS stimulating or depressing effects of *Passiflora foetida* extracts on motor behavior that may interfere with motivational behavior in the open-space swimming test. The major difference between antidepressants and CNS stimulants in clinical practice is that, in the usual therapeutic doses stimulants have marked effect on normal individuals, whereas the mood-elevating effects of antidepressants are visible only in patients who are clinically depressed (Glick and Goldfarb, 1976). Secondly, open-space swimming test was used to evaluate antidepressant effect of the *Passiflora foetida* subfractions. Thirdly, the involvement of serotoninergic and dopaminergic mechanisms in the antidepressant effect of *Passiflora foetida* extracts was clarified using specific

receptor antagonists; WAY-100635 (serotonergic 5-HT_{1A} receptor antagonist) and SCH 23390 (dopaminergic D_1 receptor antagonist).

CHAPTER III

MATERIALS AND METHODS

1. Experimental animals

Male Swiss albino mice, weighing 20-25 g, were purchased from National Laboratory Animal Center (Salaya campus, Mahidol University, Nakornpathom, Thailand). Mice were housed in groups of 10-12 per cage in a temperature-controlled environment (25 ± 2 °C) under a 12-h light–dark cycle (light on at 08:30 AM) with free access to food and water for at least 1 week prior to testing. All behavioral tests were done between 09:00 AM and 06:00 PM. Mice were randomly assigned to different groups and moved to the test room in their home cages at least 1 h before trials. All procedures with animals were conducted according to guidelines of the Animal Care and Use Committee, National Institute of Health, and were approved by the Research Ethical Committee of the Faculty of Pharmaceutical Sciences.

2. Drugs and Chemicals

Passiflora foetida L. (PF) extracts (sub-fractions PF002-1, PF002-2, PF002-3, PF002-4, PF002-5, PF002-6, PF002-7, PF003-1, PF003-2, PF003-3, PF003-4 and PF003-5) were kindly provided by Associated Professor Dr. Rutt Suttisri, Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Imipramine hydrochloride (Sigma)

SCH 23390 (Sigma)

WAY 100635 (Sigma)

Dimethylsulfoxide [DMSO; $(CH_3)_2SO$] (BDH)

0.9% Normal saline solution

Passiflora foetida L. subfractions were dissolved with 25% DMSO in 0.9% normal saline solution. Imipramine, SCH 23390 and WAY 100635 were dissolved in 0.9% normal saline solution. All drugs were injected intraperitoneally (IP) in a constant volume of 0.5 ml/100 g body weight. Control groups received 25% DMSO

in 0.9% normal saline solution and/or 0.9% normal saline solution (according to the experimental conditions) in the same volume as that of the treated groups.

3. Apparatus

Locomotor activity test: Activity cage model 7430 (7431+ 7432), UGO Basile, Comerico-verese, Italy.

Open space swimming test: A plastic circular pool.

4. Locomotor activity test

Mice, in groups of 8, were treated with 25% DMSO in 0.9% normal saline solution and/or 0.9% normal saline solution (controls), Imipramine (15 mg/kg; positive control) and PF sub-fractions (50 mg/kg) at 23, 3, and 1 h before the trial. Mice were placed individually in a transparent Activity cage (23 cm length × 35 cm width × 20 cm height) for 30 min of acclimatization. Thirty steel bars at the floor have 3 mm diameter and are spaced 11 mm apart, connected to the circuit which are insulated from each other. The odd bars are grounded. The even bars are active. The bridges that the animal breaks with its paw disconnect one or more active bars with ground, thereby producing random configurations which changes as the animal moves. The activity was recorded by the electronic counting circuit. After vehicle or drug administration, the locomotor activity of animals was continuously recorded for 60 min (modified from Perez-Garcia et al., 1999).

5. Open-space swimming test

Mice were randomly assigned to different groups (fourteen each) and were moved to the test room in their home cages at least 1 h before trials. Mice were placed individually in a circular pool, which has a diameter of 75 cm and a height of 30 cm and was filled with H_2O (24 ± 1 °C) to 20 cm height. The tests for different drug treatment and control groups were done in a counterbalanced order. No escape was provided in these trials. Mice were free to swim (or not to swim) for 15 min and then removed and returned to their home cage after drying. The observer was obscured from sight of the mice during the trials. The same procedure (15 min sessions per day) was followed 24 h later for 3 more days. 25% DMSO in 0.9% normal saline solution and/or 0.9% normal saline solution (0.5 ml/100 g body weight) was used in control groups, Imipramine (15 mg/kg × 3 per day, IP) dissolved in saline was used in positive control groups, and PF002 and PF003 sub-fractions (50 mg/kg × 3 per day,

IP) dissolved in 25% DMSO in normal saline solution were used in treatment groups. All injections were administered during the swimming trial sessions at 23, 3, and 1 h before the 2nd, 3rd, and 4th trial sessions, respectively. The rationale for the three doses before test trials is the more consistent predictive effects than those of a single dose. The mobility time included all times during the entire 15 min that a mouse showed active swimming/searching as well as slow drifts. Active swimming was defined as those swimming motions a mouse making to move around in the pool. (Modified from Sun and Alkon, 2003, 2004)

6. Test for the effects of D_1- and 5-HT_{1A} receptors on open space swimming

The selective D_1 receptor antagonist, SCH 23390, and the selective 5-HT_{1A} receptor antagonist, WAY 100635, were dissolved in normal saline solution and injected intraperitoneally 15 min prior to administrating PF sub-fractions. The test was done only with sub-fractions that had shown antidepressant effect in previous experiments. Mice were divided in groups of 10 and treatments were given as shown below:

Group	Drugs	Administration
1	0.9% NSS + DMSO	Drugs and vehicle were administered during swimming trial sessions at 23 h, 3 h, and 1 h before the second, third, and fourth trial session in the open space swimming test, respectively.
2	0.9% NSS + PF subfraction	
3	SCH 23390 + DMSO	
4	SCH 23390 + PF subfraction	
5	WAY 100635 + DMSO	
6	WAY 100635 + PF subfraction	
7	SCH 23390 + WAY 100635 + DMSO	
8	SCH 23390 + WAY 100635 + PF subfraction	

7. Statistical analysis

Results were expressed as mean ± SEM of experimental data obtained from 8-12 mice. Differences among means were tested by one-way analysis of variance

(ANOVA) followed by LSD post hoc test for multiple-group comparison. $P < 0.05$ was considered to be statistically different.

CHAPTER IV

RESULTS

1. Locomotor activity test

Spontaneous locomotor activity of mice receiving vehicles (DMSO and NSS), Imipramine, 5-HT_{1A}-receptor antagonist (WAY 100635), D_1-receptor antagonist (SCH 23390), and *Passiflora foetida* subfractions (PF002 and PF003 series) is shown in Table 7.

Table 7 Spontaneous locomotor activity of mice receiving vehicles (DMSO and NSS), Imipramine, 5-HT_{1A}-receptor antagonist (WAY 100635), D_1-receptor antagonist (SCH 23390), and *Passiflora foetida* subfractions

Experimental treatment	N	Locomotor activity (Counts in 60 min; Mean ± SEM)
DMSO	8	1002 ± 241
NSS	8	1006 ± 241
IMIPRAMINE	8	840 ± 240
WAY 100635	8	353 ± 68[#]
SCH 23390	8	308 ± 85[#]
WAY + SCH	8	79 ± 27[#]
PF002-1	8	1096 ± 347
PF002-2	8	741 ± 153
PF002-3	8	1452 ± 199
PF002-4	8	799 ± 231
PF002-5	8	1212 ± 254
PF002-6	8	803 ± 247
PF002-7	8	601 ± 155
PF003-1	8	838 ± 138
PF003-2	8	589 ± 113
PF003-3	8	457 ± 192
PF003-4	8	522 ± 207
PF003-5	8	225 ± 51[*]
PF003-(3-5)	7	322 ± 81[*]

[#] $P < 0.05$ versus NSS; [*] $P < 0.05$ versus DMSO

Locomotor activity test was used to evaluate the CNS stimulant effect of *Passiflora foetida* sub-fractions. As shown in Table 7, SCH 23390, SCH 23390 plus WAY 100635, PF003-(3-5), and PF003-5 significantly decreased locomotor activity as compared to their respective controls. Imipramine (a classical antidepressant drug), PF002-series, and PF003-1 to PF003-4 produced no significant effects on locomotor activity as compared with their vehicle. It should be noted that DMSO had no effect on locomotor activity as compared to NSS.

Judging from the above mentioned results, no stimulant effect of *Passiflora foetida* on motor activity was found in any subfractions. Therefore, all subfractions were further tested in the animal model of depression.

2. Effects of *Passiflora foetida* subfractions on the open space swimming test

Mice injected with vehicle (NSS and DMSO) showed a gradual reduction in the mobility time over successive trials (Figure 10). The mobility time included all the time that mice moved during the entire 15 min, as caused by active swimming/searching as well as slow drifts, which were caused by apparently non-searching movement of the legs, in the overall movement. Active swimming is defined as when a mouse is making active swimming motions as those to move around in the pool. Unlike the behavior patterns reported in the forced swimming test, no climbing on the wall was observed, probably due to the large space available to the mice. As the trials progressed, the control mice showed progressively less and briefer intermittent periods of active swimming. Typically, a control mouse did not make any movements other than those just sufficient to keep its head above the water surface (immobility), a characteristic behavior that is taken as an indicator of depression in the forced swimming test.

Imipramine was used in this study as the positive control to determine whether depressive behavior that induced by the open space swimming test was sensitive to antidepressant treatment. Imipramine administration clearly attenuated the reduction in mobility time over trials, compared with that of the control group. The imipramine-treated mice showed more and long-lasting periods of active swimming/searching, measured as the mobility time. Statistical analysis revealed significant differences

starting from the 2nd trial to the 4th trial ($P < 0.05$), which indicated persistent increase in motivational behavior of mice that received the antidepressant treatment.

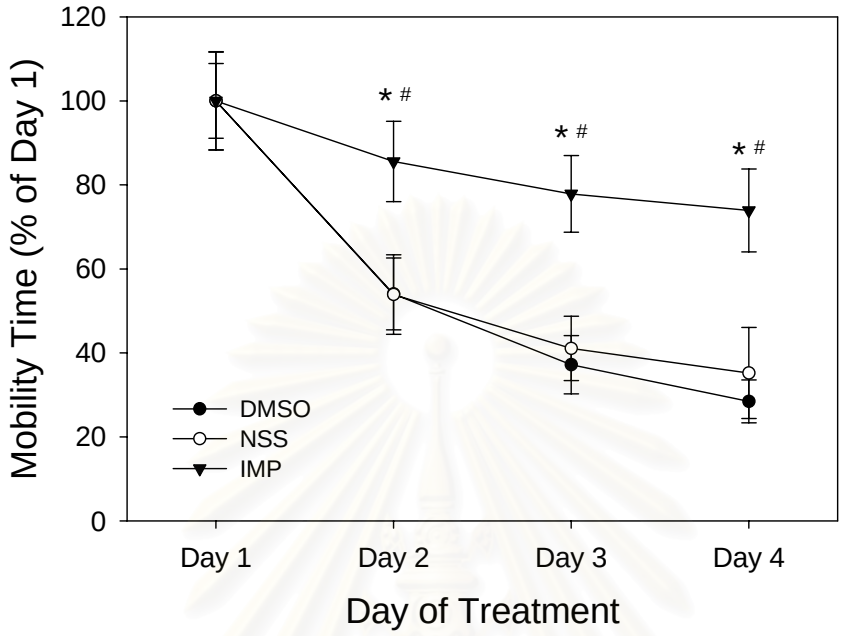


Figure 10 The effects of imipramine (IMP; 15 mg/kg × 3/day, IP; administered between the open space swimming trials) on the mobility time in the open space swimming test in mice. Each point represents the mean ± SEM value from 14 mice per group. Mice were placed individually in a circular pool for 15 min/session/day and the same procedure was followed 24 h later for 3 days.

* $P < 0.05$ versus DMSO, # $P < 0.05$ versus NSS

PF003-1 was effective in reducing the decrease in mobility time in the open space swimming test (Figure 11), as compared with that of a control group. Statistical analysis yielded a significant difference between the groups ($P < 0.05$) indicating that the active swimming of the mice that were injected with PF003-1 was significantly higher than that of the mice that were injected with vehicle only (from the 2nd trial to the 4th trial).

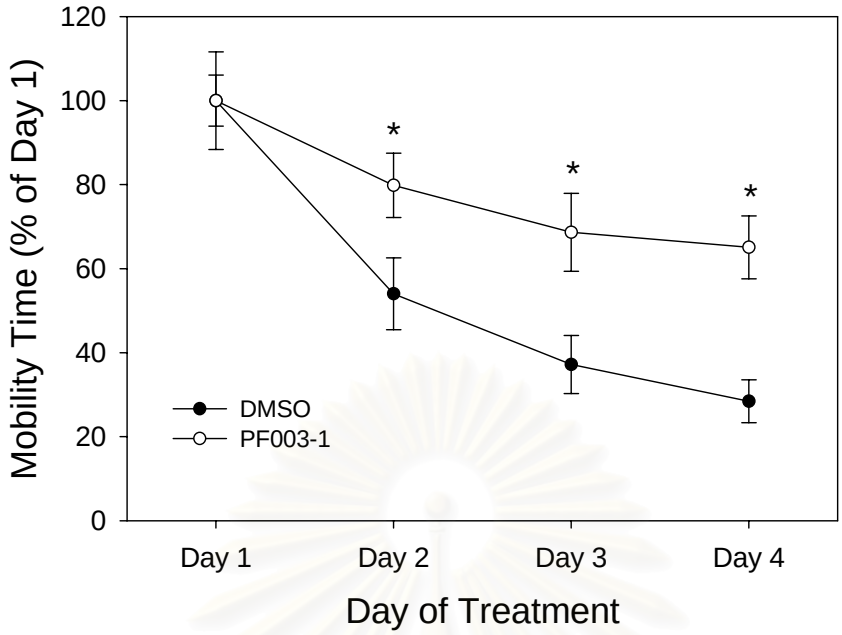


Figure 11 The effects of PF003-1 (50 mg/kg × 3/day, IP; administered between the open space swimming trials) on the mobility time in the open space swimming test in mice. Each point represents the mean ± SEM value from 14 mice per group.

* $P < 0.05$ versus DMSO

Comparable to PF003-1, PF003-2 was effective in reducing the decrease in mobility time in the open space swimming test (Figure 12), as compared with that of a control group. Statistical analysis yielded a significant difference between the groups ($P < 0.05$) indicating that the active swimming of the mice that were injected with PF003-2 was significantly higher than that of the mice that were injected with vehicle only. However, PF003-2 was effective from the 3rd trial to the 4th trial.

On the contrary, sub-fractions of PF002-series and PF003-(3-5) were not effective in attenuating the reduction in mobility time of mice in open-swimming test as compared to control groups. And the summary effects of all substances were shown in table 8.

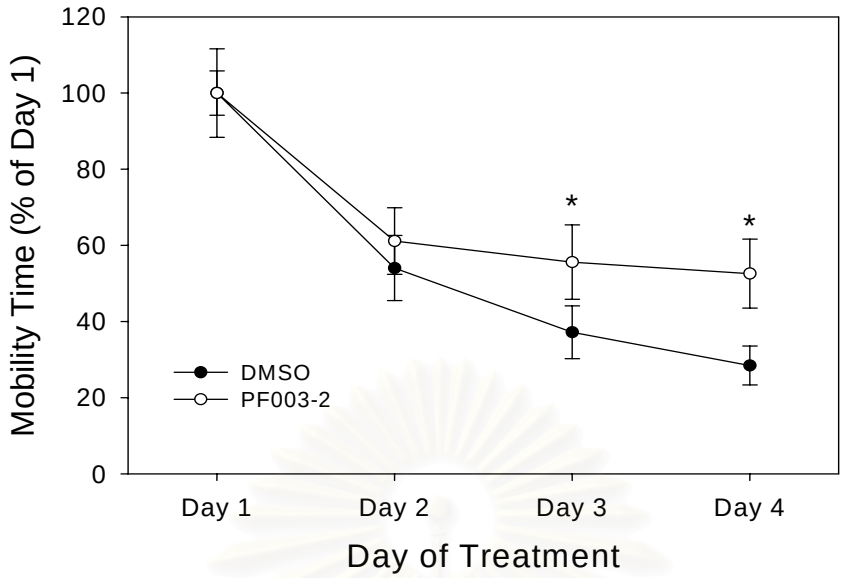


Figure 12 The effects of PF003-2 (50 mg/kg × 3/day, IP; administered between the open space swimming trials) on the mobility time in the open space swimming test in mice. Each point represents the mean ± SEM value from 14 mice per group.

* $P < 0.05$ versus DMSO

Table 8 Summary effects of Imipramine (positive control group), PF002-series, and PF003-series on the mobility time in mice.

Treatment	N	Mobility Time in Minutes (Mean ± SEM)			
		Day 1	Day 2	Day 3	Day 4
DMSO	14	10.10 ± 1.09	5.46 ± 0.80	3.76 ± 0.65	2.87 ± 0.48
NSS	14	9.48 ± 1.03	5.11 ± 0.83	3.89 ± 0.67	3.34 ± 0.95
Imipramine	14	11.16 ± 0.92	9.55 ± 0.99#	8.69 ± 0.94#	8.24 ± 1.02#
PF002-(1-4)	14	9.83 ± 0.90	4.74 ± 0.99	3.32 ± 0.84	3.23 ± 0.76
PF002-(5-6)	14	9.73 ± 0.60	5.75 ± 0.83	3.38 ± 0.66	3.10 ± 0.33
PF002-7	14	11.38 ± 0.97	5.76 ± 0.78	4.43 ± 0.60	4.20 ± 0.37
PF003-1	14	12.60 ± 0.71	10.06 ± 0.89*	8.65 ± 1.08*	8.20 ± 0.87*
PF003-2	14	12.61 ± 0.68	7.71 ± 1.02	7.01 ± 1.14*	6.63 ± 1.06*
PF003-(3-5)	14	9.11 ± 1.04	5.56 ± 1.34	4.21 ± 1.36	3.08 ± 1.03

* $P < 0.05$ versus DMSO, # $P < 0.05$ versus NSS

3. Effects of 5-HT_{1A}- and D_1-receptor antagonist on antidepressant effect of PF003-1 and PF003-2 in open space swimming test

The mice injected with vehicle (NSS and DMSO) and antagonists showed a gradual reduction (Figure 13) in the mobility time. The mobility time includes all the time that mice moved during the entire 15 min, as mentioned in previous experiments. No significant differences were found in the antagonist-treated groups as compared to a control group.

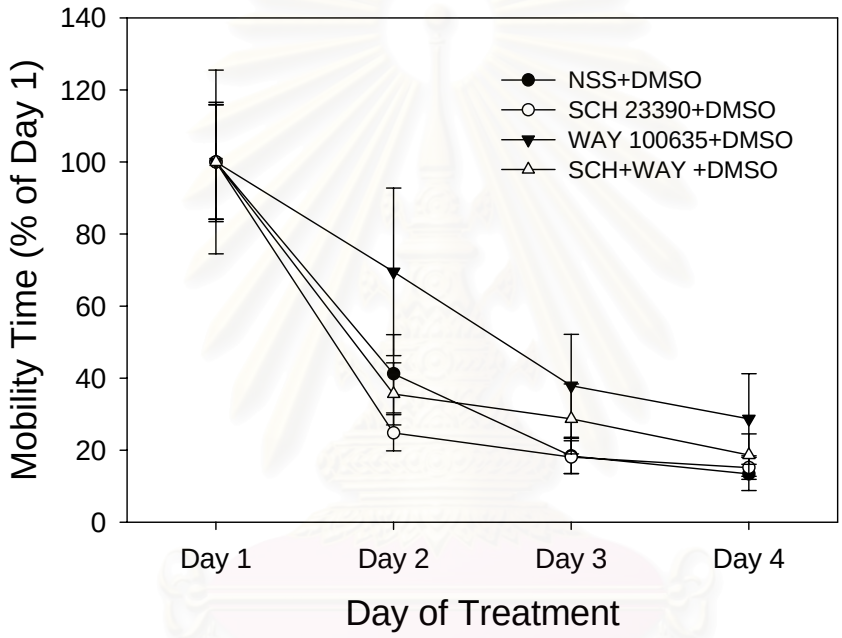


Figure 13 The effects of NSS+DMSO, SCH 23390+DMSO, WAY 100635+DMSO, and SCH+WAY+DMSO (0.5 ml/100 gm body weight × 3/day, IP; administered between the open space swimming trials) on mobility time in the open space swimming test in mice. Each point represents the mean ± SEM value from 12 mice per group.

Similar to previous experiments, PF003-1 was effective in reducing the decrease in mobility time in the open space swimming test (Figure 14), as compared with that of a NSS+DMSO group. Statistical analysis yielded a significant difference between the groups ($P < 0.05$) indicating that the active swimming of the mice that were injected with PF003-1 was significantly higher than that of the mice that were injected with vehicles only (from the 2nd trial to the 4th trial).

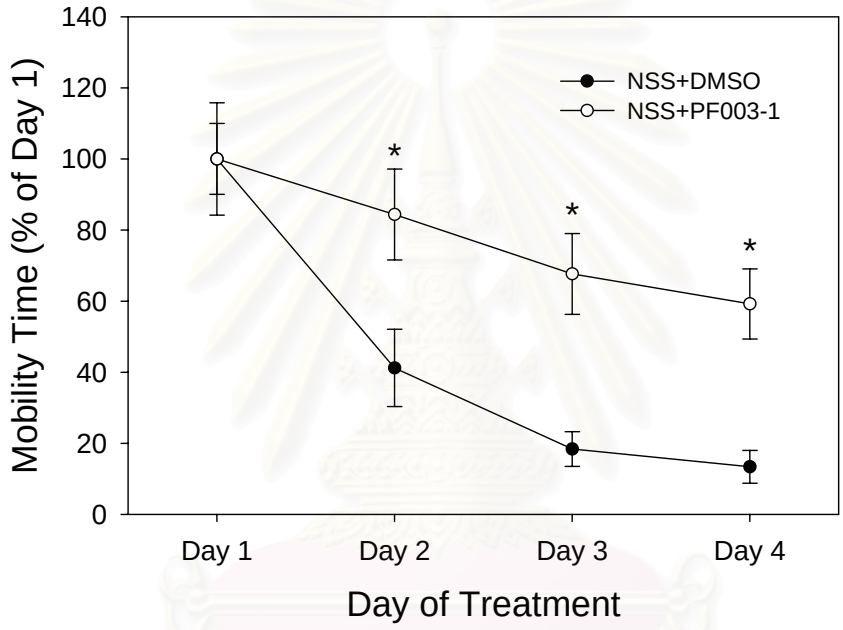


Figure 14 The effects of PF003-1 (50 mg/kg × 3/day, IP; administered between the open space swimming trials) on the mobility time in the open space swimming test in mice. Each point represents the mean ± SEM value from 12 mice per group. NSS was injected 15 minutes prior to PF003-1.

* $P < 0.05$ versus NSS+DMSO

The anti-immobility effect of PF003-1 is decreased when pretreated with SCH 23390 (a D_1-receptor antagonist; 0.05 mg/kg), WAY 100635 (a $5HT_{1A}$-receptor antagonist; 0.3 mg/kg), and a combination of SCH 23390 and WAY 100635 (Figure 15). The anti-immobility effect of PF003-1 was significantly attenuated by the D_1 antagonist SCH 23390 at the 2nd and the 4th trial, while $5HT_{1A}$ antagonist WAY 100635 was significantly reduced the effect of PF003-1 at the 3rd and the 4th trial. However, the combination of SCH23390 and WAY 100635 was showed the significant reduction of the anti-immobility effect of PF003-1 from the 2nd to the 4th trial.

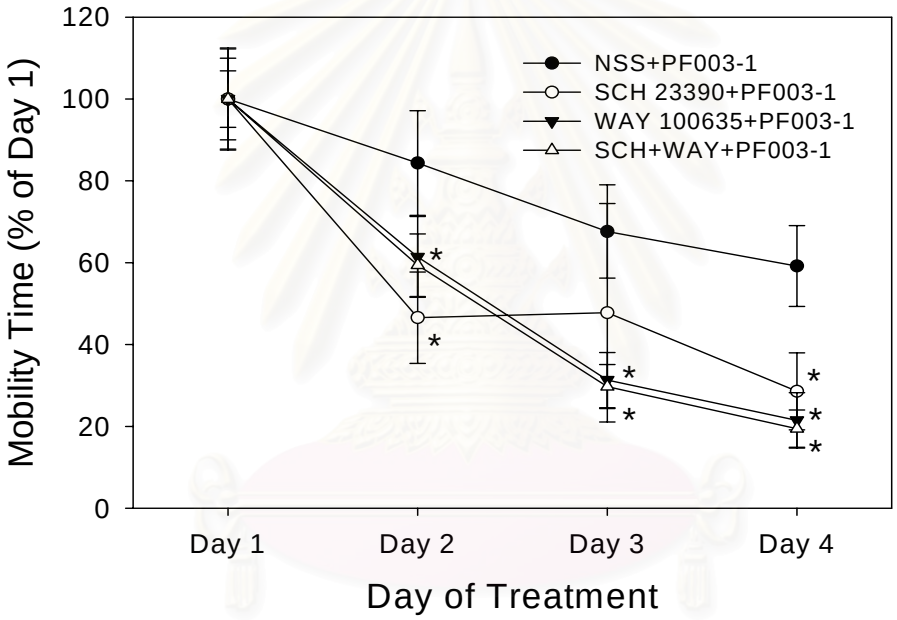


Figure 15 The effects of pretreatment with 5-HT_{1A}- and D_1-receptor antagonists (WAY 100635 and SCH 23390, respectively); [IP × 3/day, administered 15 min prior to PF003-1; between the open space swimming trials] on the mobility time in the open space swimming test in mice. Each point represents the mean ± SEM value from 12 mice per group.

* $P < 0.05$ versus NSS+PF003-1

In comparison to PF003-1, PF003-2 was effective in reducing the decrease in mobility time in the open space swimming test (Fig. 16), as compared with that of the NSS+DMSO group. Statistical analysis showed a significant difference between the groups ($P < 0.05$) indicating that the active swimming of the mice that were injected with PF003-2 was significantly higher than that of the mice that were injected with vehicles only (from the 2nd trial to the 4th trial).

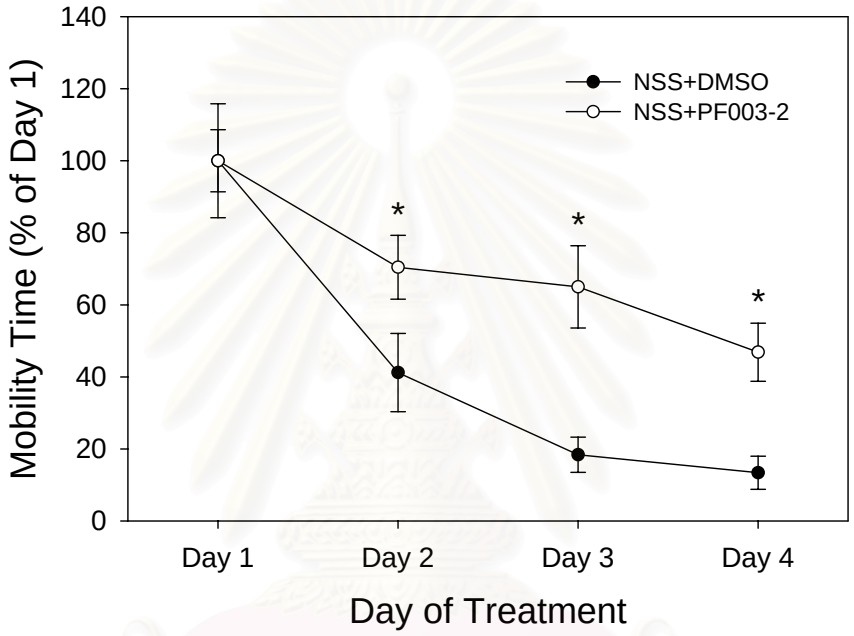


Figure 16 The effect of PF003-2 (50 mg/kg × 3/day, IP; administered between the open space swimming trials) on the mobility time in the open space swimming test in mice. Each point represents the mean ± SEM value from 12 mice per group. NSS was injected 15 minutes prior to PF003-2.

* $P < 0.05$ versus NSS+DMSO

The anti-immobility effect of PF003-2 is decreased when pretreated with SCH 23390 (a D_1-receptor antagonist; 0.05 mg/kg), WAY 100635 (a $5HT_{1A}$-receptor antagonist; 0.3 mg/kg), and a combination of SCH 23390 and WAY 100635 (Figure 17). The anti-immobility effect of PF003-2 was significantly attenuated by SCH 23390, WAY 100635 and the combination of SCH23390 and WAY 100635 and the effects were seen from the 2nd to the 4th trial.

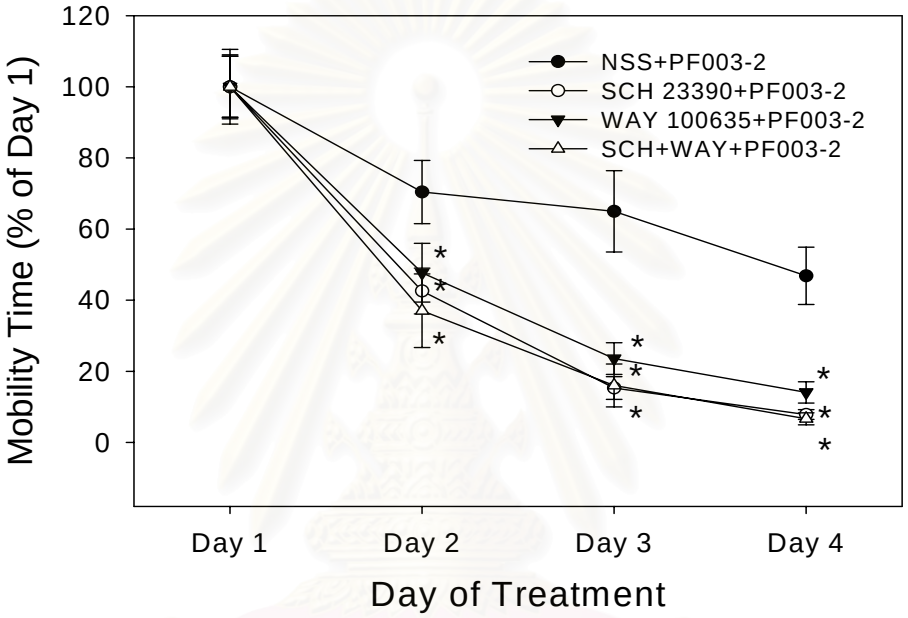


Figure 17 The effects of pretreatment with 5-HT_{1A}- and D_1-receptor antagonists (WAY 100635 and SCH 23390, respectively); IP × 3/day, administered 15 min prior to PF003-2; between the open space swimming trials] on the mobility time in the open space swimming test in mice. Each point represents the mean ± SEM value from 12 mice per group.

* $P < 0.05$ versus NSS+PF003-2

CHAPTER V

DISCUSSION

In the present study, effects of *Passiflora foetida* extracts in sub-fractions were studied with animal behavioral models (locomotor activity and open space swimming tests) to evaluate their possible antidepressant activity. Regarding to animal models of depression, it has been believed by many researchers that the immobility observed in the water (forced swimming test) is a relatively specific indicator of depressive phenomenon. However, the current paradigm precludes the disadvantage of forced swimming test as a model of depression. The open swimming test employed the mobility (active swimming/searching) as a central component of depressive behavior and this endpoint is not subjected to false positive responses to CNS stimulants.

Locomotors activity test was used for screening CNS actions, thereby providing information about motor behavioral stimulation/depression by *Passiflora foetida* subfractions. The results demonstrate that the *Passiflora foetida* sub-fractions (PF002- and PF003-series) did not possess CNS stimulant effect. Administration of tested agents at multiple doses which relatively simulated the experimental condition of antidepressant testing (50 mg/kg × 3/day) did not significantly change motor activity of naive mice. Therefore, this observation supports the hypothesis that, to be considered as a potential antidepressant, a drug must reduce immobility in forced swimming test at doses that do not stimulate locomotion (Porsolt, Bertin and Jalfre, 1977).

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors. The most widely used animal models for antidepressant screening is the forced swimming test. Although the relationship between immobility (a posture thought to reflect a state of ‘‘behavior despair’’ in which animals have given up the hope to escape) and depression remains controversial, it is well demonstrated that drugs with antidepressant activity reduce the time during which the animals remain immobile.

The open space swimming test was used in this experiment, because, this test does not limit the animals movement due to space restriction and probably mimics the disorder in human more closely. It is the lack of motivation (opportunities or hope) rather than the restricted ‘‘physical space’’ that largely (though not exclusively) defines the human disease. This is largely based on reasonable assumptions at this stage rather than on solid experimental data so that it remains to be evaluated whether such a comparison with human depression is valid.

In the second part, we firstly demonstrated that imipramine, the classical antidepressant, can represent its effect in this modified open space swimming test in mice. As the original model, four major classes of effective antidepressants (imipramine, a tricyclic antidepressant; iproniazid, a monoamine oxidase inhibitor; mianserin, atypical antidepressant, and alaproclate, a selective serotonin-reuptake inhibitor) showed significantly antidepressant effect. Imipramine was appropriate to be the positive control in this study because it represents pharmacological actions through the serotoninergic, noradrenergic, and dopaminergic systems (Gambarana et al., 1995).

In the experiment, the sub-acute administration of PF003-1 and PF003-2 at doses of 50 mg/kg (3 doses/day, for 3 consecutive days) significantly attenuated the reduction in mobility time, which suggested that these sub-fractions of *Passiflora foetida* possess an antidepressant-like action. The results demonstrated that PF003-1 has antidepressant effect in the same magnitude as imipramine (mobility time, 8.20±0.87 VS 8.24±1.02 of PF003-1 and imipramine, respectively; in the fourth session) and slightly higher than PF003-2 (but not significant).

Surprisingly, PF002-7 that had a similar receptor binding profile to PF003-1 and PF003-2 did not show antidepressant effect.

The classical theory of the biochemical foundation of depression hypothesizes a decrease in central synaptic neurotransmission secondary to the deficiency in monoaminergic neurotransmitters, serotonin and/or noradrenaline. The role for a serotonin deficit is supported by several lines of evidence and all clinically effective antidepressants increase the amount of monoamines available in the synaptic cleft, by inhibiting reuptake mechanism (tricyclics) or by inhibiting enzymatic catabolism (MAOIs). The major role suggested for serotonin deficiency in this theory led to the

development of a large number of compounds intended to increase serotonin neurotransmission, particularly by blocking serotonin reuptake using: the selective serotonin reuptake inhibitors (SSRIs), and, more recently by blocking both serotonin and noradrenaline reuptake with the serotonin noradrenaline reuptake inhibitors (SNRIs).

In addition to antidepressant medications, herbal remedies such as *Hypericum perforatum*, an herbaceous perennial plant that also known as “St. John’s wort”, is used popularly as a natural antidepressant. Although some clinical and experimental studies suggest it has some properties similar to conventional antidepressants, the proposed mechanism of action seems to be multiple: a non-selective blockade of the reuptake of serotonin, noradrenaline and dopamine; an increase in density of serotoninergic and dopaminergic receptors and an increased affinity for GABAergic receptors. Moreover, the inhibition of monoamine oxidase enzyme activity has been involved (Rodríguez-Landa and Contreras, 2003). The hydroalcoholic extract obtained from aerial parts of *Siphocampylus verticillatu*, a Brazilian medicinal plant. Its action seems to involve an interaction with adrenergic, dopaminergic, glutamatergic and serotoninergic systems (Rodrigues et al., 2002), and *Curcuma longa* is a major constituent of Xiaoyao-san, the traditional Chinese medicinal formula, which has been used effectively to treat depression-related diseases in China. In addition, the neurochemical assays showed that curcumin produced a marked increase of serotonin and noradrenaline levels at 10 mg/kg in both the frontal cortex and hippocampus. Dopamine levels were also increased in the frontal cortex and the striatum. Moreover, curcumin was found to inhibit monoamine oxidase activity in the mouse brain. These findings suggest that the antidepressant-like effects of curcumin may involve the central monoaminergic neurotransmitter systems (Xu et al., 2005).

Although the classical antidepressant drugs are known to interfere mainly with the availability of serotonin and noradrenaline, several lines of evidence have recently suggested that some of their actions are associated with dopamine pathway modulation (Campos et al.,). Some important points should be considered: (1) the role of noradrenaline and mainly serotonin for the pathogenesis of depression has been largely explored, but newly reported evidence indicates a potential role for dopamine in this pathology (2) most currently available therapies for the treatment of depression are based on the modulation of the serotoninergic system, but the search

for new therapeutic options is still needed because of the lack of effect on some patients or the great number of collateral effects exhibited by these drugs; (3) some commercially available drugs, such as bupropion and nomifensine, which preferentially interfere with dopamine mechanisms, have been employed with success for the treatment of refractory bipolar depression or addiction (Yamada et al., 2004; Campos et al., 2005).

Some compound from plants showed its antidepressant effect that involved dopaminergic transmission, for examples, the flavonoid apigenin can decrease immobility in the forced swimming test that may be mediated by the dopaminergic (D_2 receptor) mechanisms in the mouse brain (Nakazawa et al., 2003); hyperforin, the hydroalcoholic extracts of *Hypericum perforatum*, (at concentrations of 0.1–1 μM) showed non-specific presynaptic effects, resulting in the nonselective inhibition of the uptake of many neurotransmitters, and the interaction with dopamine D_1 and opioid receptors (Mennini and Gobbi, 2004), and the Brazilian medicinal plant, the extracts of *Trichilia catigua*, also showed the evidence for a dopamine-mediated antidepressant-like effect (Campos et al., 2005).

In this study, the involvement of dopamine D_1 receptor and serotonin 5-HT_1 receptor in the antidepressant activity of *Passiflora foetida* subfractions was assessed by using specific D_1 and 5-HT_1 receptor antagonists (SCH 23390 and WAY 100635, respectively). SCH 23390 markedly reversed the effects induced by *Passiflora foetida* extracts in the open space swimming test, thereby supporting the involvement of dopaminergic mechanism in their antidepressant action.

The anti-immobility effects of *Passiflora foetida* extracts were mostly prevented by the D_1 receptor antagonist, SCH 23390, at doses devoid of locomotor impairment, suggesting that their anti-immobility effect may be mediated through D_1 dopamine receptor activation. D_1 dopamine agonists have been reported to be effective in animal models of depression by some authors (D'Aquila et al., 1994; Gambarana et al., 1995) but not by others (Campos et al., 2004). In contrast, functional interaction between D_1 and D_2 receptors has been well documented in various behaviors; they are known to interact synergistically in some behaviors (Campos et al., 2005).

A large number of experimental and clinical studies indicate that the serotonin (5-HT) system is strongly implicated in the neural regulation of mood and several

pieces of evidence have implicated abnormalities in 5-HT neurotransmission in the pathophysiology of depression. Several studies indicate that an enhancement of 5-HT neurotransmission underlies the therapeutic response to various types of antidepressant treatments. Drugs affecting 5-HT neurotransmission, such as those inhibiting 5-HT reuptakes at nerve terminals, or inhibiting its metabolism (monoamine oxidase inhibitors), are effective in depression.

Several reports have suggested that 5-HT_{1A} receptors are involved in the mechanism of action of antidepressant drugs. Such *Limacia scanden* could be attributed either to release of endogenous serotonin or inhibition of 5-HT reuptake in the CNS (Hwi and Lay, 1998), the methanolic extract of *Clitoria ternatea* (CT) mediated via serotonin and acetylcholine (Jain et al., 2003). Considering that 5-HT_{1A} receptors have an important role in mood disorders, the involvement of the 5-HT system in the antidepressant-like effect of *Passiflora foetida* extracts was determined in the present study by using 5-HT_{1A} antagonist to examine the behavioral responses to *Passiflora foetida* extracts in the open space swimming test.

The involvement of 5-HT_{1A} receptors in the antidepressant-like effect of *Passiflora foetida* extracts is strongly suggested by the results showing that pretreatment of mice with 5-HT_{1A} antagonist WAY 100635 nearly completely abolished the anti-immobility effect of *Passiflora foetida* subfractions PF003-1 and PF003-2 in the open space swimming test.

The co-administration of SCH 23390 and WAY 100635 totally reversed antidepressant effects of *Passiflora foetida* subfractions PF003-1 and PF003-2. This finding suggested that antidepressant activity of *Passiflora foetida* may be mediated mostly via dopaminergic and serotoninergic mechanisms.

Taken together, the experimental results suggest the involvement of both DA_1 and 5-HT_{1A} receptors in the antidepressant-like effect of *Passiflora foetida* extracts in the open space swimming test. How the activation of DA_1 and 5-HT_{1A} receptors that caused by *Passiflora foetida* extracts could lead to apparent antidepressant activity in animal models is still unknown at present.

Recently, a preliminary study suggested that the extracts of *Passiflora foetida*, especially PF003-1 and PF003-2, possessed marked binding affinity to D_1 and 5-HT_{1A} receptors from rat brains (Meksuriyen et al, unpublished observations). Therefore,

Passiflora foetida extracts may mediate the antidepressant activity through direct or indirect activation of these two monoaminergic receptors.

The inhibition of the monoamine reuptake, mainly of serotonin and dopamine, of *Passiflora foetida* was found in other plant such as Product Catuama (Campos et al., 2004). These data suggest that the antidepressant-like effect of *Passiflora foetida* on the open space swimming test may be, alternatively, due to an increase in monoaminergic transmission, resulting from monoamine uptake inhibition, more potently of dopamine and serotonin.

In conclusion, the present study employed the open space swimming test to investigate potential antidepressant activity of *Passiflora foetida* extracts. This new animal model for depression has clear advantages due to improved objectivity in monitoring, closer resemblance to human conditions, and high predictability of antidepressant activity (Sun and Alkon., 2003). The experimental results suggest that *Passiflora foetida* extracts, namely, subfractions PF003-1 and PF003-2, apparently show marked antidepressant effects. However, their mechanism of action is still unclear at present. The possible antidepressant mechanisms that were suggested by this study involve direct or indirect dopaminergic and serotoninergic activation in the CNS. They might be due to the activation of DA_1 and 5-HT_{1A} receptors in the CNS. Alternatively, they could possibly be due to the inhibition of neuronal monoamine uptake, most potently of dopamine and serotonin. This effect could be caused by an action of *Passiflora foetida* on monoamine transporters, most likely lead to the elevated neurotransmitter levels in the synaptic clefts. Last but not least, they might be due to the inhibition of MAO activity in monoaminergic nerve endings. Further investigations focusing on investigating the effects of *Passiflora foetida* extracts on cerebral extracellular monoamines levels, presynaptic neuronal reuptake of monoamines, and MAO enzymatic activity, should be beneficial to characterize the specific monoaminergic systems and mechanisms responsible for the antidepressant-like effect of *Passiflora foetida*.

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APPENDICES

Table 1 Mobility Time of Mice in Vehicle (DMSO, NSS), Imipramine, PF002-Series, and PF003-Series in Open Space Swim Test

Group	Mobility Time				Group	Mobility Time			
	Day 1	Day 2	Day 3	Day 4		Day 1	Day 2	Day 3	Day 4
DMSO	5.03	2.23	1.38	0.30	NSS	14.78	5.83	3.38	1.43
DMSO	4.82	1.90	1.53	1.42	NSS	3.65	1.60	1.33	1.37
DMSO	8.60	10.93	8.27	5.55	NSS	12.33	6.40	5.77	2.22
DMSO	7.58	3.10	1.80	1.30	NSS	7.38	1.37	2.38	2.88
DMSO	5.03	3.18	1.97	1.43	NSS	11.38	8.70	7.72	12.42
DMSO	5.75	3.65	1.40	1.20	NSS	4.00	3.55	2.02	1.90
DMSO	12.68	5.12	3.68	2.90	NSS	12.87	4.98	3.87	2.85
DMSO	14.30	6.27	3.97	1.88	NSS	7.57	2.83	2.73	0.78
DMSO	13.17	5.55	3.60	5.43	NSS	14.88	13.62	10.52	10.75
DMSO	7.57	2.32	3.23	2.75	NSS	12.43	5.20	2.00	1.68
DMSO	14.83	6.23	4.25	3.50	NSS	5.63	3.32	2.67	1.28
DMSO	14.22	7.07	9.28	5.18	NSS	7.83	4.28	3.35	2.10
DMSO	14.05	7.97	3.20	2.35	NSS	6.38	5.10	2.85	2.18
DMSO	13.78	10.88	5.02	5.03	NSS	11.57	4.73	3.92	2.87
IMIPRAMINE	13.23	5.30	9.25	4.58	PF002-(1-4)	12.32	4.67	2.65	3.47
IMIPRAMINE	14.98	12.32	11.45	14.32	PF002-(1-4)	9.80	3.62	3.25	2.57
IMIPRAMINE	11.42	11.88	7.32	10.12	PF002-(1-4)	10.20	4.17	1.80	2.65
IMIPRAMINE	8.70	12.75	10.22	8.13	PF002-(1-4)	14.07	14.10	12.42	12.20
IMIPRAMINE	5.50	3.07	3.80	4.20	PF002-(1-4)	14.60	11.80	7.95	5.90
IMIPRAMINE	14.32	13.82	12.43	12.45	PF002-(1-4)	7.45	2.47	1.48	2.47
IMIPRAMINE	7.98	8.75	4.17	5.93	PF002-(1-4)	7.25	3.43	1.88	1.47
IMIPRAMINE	11.77	9.20	6.90	9.08	PF002-(1-4)	5.95	6.42	4.33	3.08
IMIPRAMINE	14.93	8.32	11.03	6.90	PF002-(1-4)	11.93	1.47	1.47	1.13
IMIPRAMINE	8.22	5.88	4.63	2.92	PF002-(1-4)	8.25	1.77	2.10	1.45
IMIPRAMINE	7.12	6.68	5.22	5.47	PF002-(1-4)	5.83	3.17	1.68	2.40
IMIPRAMINE	14.92	14.88	14.68	14.13	PF002-(1-4)	4.20	2.57	1.03	1.93
IMIPRAMINE	14.88	13.75	12.77	11.95	PF002-(1-4)	12.28	3.93	2.87	1.87
IMIPRAMINE	8.23	7.03	7.73	5.23	PF002-(1-4)	13.42	2.77	1.57	2.60
PF002-(5-6)	13.77	9.67	3.65	3.32	PF002-7 N	3.95	2.10	2.23	4.10
PF002-(5-6)	6.32	2.72	0.93	0.42	PF002-7 N	12.93	5.52	7.12	5.97
PF002-(5-6)	10.37	9.32	10.55	4.82	PF002-7 N	10.87	5.30	5.28	6.58
PF002-(5-6)	11.42	8.93	3.77	3.25	PF002-7 N	7.72	2.93	2.88	4.23
PF002-(5-6)	6.07	2.78	2.17	2.75	PF002-7 N	5.42	2.68	1.73	2.28
PF002-(5-6)	10.38	5.48	4.12	3.10	PF002-7 N	11.83	4.15	4.77	3.48
PF002-(5-6)	9.60	4.12	1.97	2.10	PF002-7 N	12.88	4.85	2.10	3.13
PF002-(5-6)	9.12	4.02	2.05	2.20	PF002-7 N	14.95	9.88	8.02	5.43
PF002-(5-6)	13.18	12.38	6.25	0.92	PF002-7 N	14.70	9.75	4.67	4.75
PF002-(5-6)	8.57	3.92	1.48	0.93	PF002-7 N	14.57	4.23	2.73	2.58
PF002-(5-6)	10.97	4.95	3.22	2.37	PF002-7 N	12.98	5.67	3.47	3.72
PF002-(5-6)	9.65	4.08	1.92	0.88	PF002-7 N	13.27	11.47	9.00	6.05
PF002-(5-6)	7.23	2.30	1.92	1.03	PF002-7 N	14.90	8.10	4.18	3.98
PF002-(5-6)	9.58	5.78	3.37	2.33	PF002-7 N	8.42	4.03	3.85	2.48

Table 1 Mobility Time of Mice in Vehicle (DMSO, NSS), Imipramine, PF002-Series, and PF003-Series in Open Space Swim Test (continued)

Group	Mobility Time			
	Day 1	Day 2	Day 3	Day 4
PF003-1	14.10	5.62	3.95	4.23
PF003-1	9.07	9.02	6.88	9.25
PF003-1	12.38	11.18	9.00	8.37
PF003-1	7.18	7.63	1.85	4.33
PF003-1	14.88	12.13	9.05	5.95
PF003-1	14.97	13.92	14.02	13.25
PF003-1	10.08	5.13	4.87	4.03
PF003-1	10.52	6.73	3.48	4.82
PF003-1	10.68	6.97	7.60	8.73
PF003-1	15.00	12.80	10.93	11.93
PF003-1	15.00	13.93	13.03	9.43
PF003-1	14.93	14.73	14.57	13.78
PF003-1	12.73	8.50	10.55	7.70
PF003-1	14.85	12.52	11.30	8.98

Group	Mobility Time			
	Day 1	Day 2	Day 3	Day 4
PF003-2	14.57	9.58	3.50	2.23
PF003-2	6.30	3.17	3.93	3.65
PF003-2	7.97	3.27	5.52	9.37
PF003-2	14.75	12.20	12.70	14.07
PF003-2	12.70	2.50	1.52	1.83
PF003-2	11.10	5.47	2.77	2.58
PF003-2	14.57	12.72	8.05	7.15
PF003-2	13.62	9.23	7.68	3.72
PF003-2	12.95	8.95	11.18	8.13
PF003-2	13.78	12.57	14.53	11.78
PF003-2	12.92	6.43	11.35	10.40
PF003-2	14.32	12.18	9.35	8.30
PF003-2	14.37	5.98	2.53	2.40
PF003-2	12.67	3.67	3.53	7.22

Table 2 Mobility Time of Mice in Vehicle (DMSO, NSS), Dopamine D_1 Receptor Antagonist (SCH 23390), and Serotonin 5-HT_{1A} Receptor Antagonist (WAY 100635) in Open Space Swim Test

Group	Mobility Time			
	Day 1	Day 2	Day 3	Day 4
NSS+DMSO	4.82	0.73	0.42	0.62
NSS+DMSO	4.93	1.37	0.85	0.70
NSS+DMSO	5.42	1.13	0.75	0.77
NSS+DMSO	5.45	0.90	1.08	1.25
NSS+DMSO	14.12	6.65	2.40	1.50
NSS+DMSO	6.18	3.07	3.00	3.47
NSS+DMSO	6.05	2.67	2.92	1.28
NSS+DMSO	2.02	0.60	0.28	0.20
NSS+DMSO	9.80	4.22	1.65	0.60
NSS+DMSO	9.58	6.65	0.92	0.55
NSS+DMSO	8.62	4.15	0.60	0.12
NSS+DMSO	7.18	2.53	0.60	0.22
WAY100635+DMSO	10.80	10.77	7.00	0.35
WAY100635+DMSO	3.80	1.10	0.48	0.65
WAY100635+DMSO	5.23	2.58	2.87	1.87
WAY100635+DMSO	1.43	0.23	0.93	1.22
WAY100635+DMSO	3.20	1.85	0.18	0.28
WAY100635+DMSO	0.80	0.20	0.32	0.22
WAY100635+DMSO	1.78	0.50	0.47	1.03
WAY100635+DMSO	12.63	8.60	6.20	7.60
WAY100635+DMSO	14.30	10.80	7.93	4.10
WAY100635+DMSO	12.75	5.15	1.35	0.28
WAY100635+DMSO	5.48	3.92	1.40	0.53
WAY100635+DMSO	11.13	12.22	2.42	5.75

Group	Mobility Time			
	Day 1	Day 2	Day 3	Day 4
SCH23390+DMSO	7.02	0.83	1.15	0.92
SCH23390+DMSO	4.15	1.08	0.78	0.48
SCH23390+DMSO	2.03	0.32	0.30	1.18
SCH23390+DMSO	10.25	2.30	2.73	2.27
SCH23390+DMSO	7.83	1.67	0.22	0.60
SCH23390+DMSO	4.10	2.13	1.10	0.63
SCH23390+DMSO	6.33	0.88	1.48	1.25
SCH23390+DMSO	3.48	0.25	0.17	0.35
SCH23390+DMSO	4.38	1.70	0.77	0.63
SCH23390+DMSO	3.87	1.38	0.63	0.25
SCH23390+DMSO	6.02	1.83	1.58	0.93
SCH23390+DMSO	10.97	3.08	1.80	1.17
SCH+WAY+DMSO	11.33	3.95	3.65	2.52
SCH+WAY+DMSO	1.55	0.15	1.95	0.20
SCH+WAY+DMSO	9.40	3.50	3.42	3.88
SCH+WAY+DMSO	1.97	0.82	7.83	1.60
SCH+WAY+DMSO	7.67	2.77	0.93	0.62
SCH+WAY+DMSO	10.17	2.70	0.22	0.35
SCH+WAY+DMSO	8.73	1.68	1.03	3.57
SCH+WAY+DMSO	7.83	0.50	0.57	0.55
SCH+WAY+DMSO	3.22	1.60	0.42	0.30
SCH+WAY+DMSO	9.62	4.17	2.08	1.50
SCH+WAY+DMSO	10.95	6.43	2.57	0.75
SCH+WAY+DMSO	9.30	4.38	1.62	1.25

Table 3 Mobility Time of PF003-1-Treated Mice When Treated with Dopamine D1 Receptor Antagonist (SCH 23390) and Serotonin 5-HT1A Receptor Antagonist (WAY 100635)

Group	Mobility Time			
	Day 1	Day 2	Day 3	Day4
NSS+PF003-1	9.37	4.88	2.55	2.22
NSS+PF003-1	7.37	4.43	3.07	2.78
NSS+PF003-1	8.87	6.23	6.28	6.13
NSS+PF003-1	7.05	12.58	6.95	5.98
NSS+PF003-1	9.27	10.70	8.70	5.25
NSS+PF003-1	7.65	6.90	3.67	3.02
NSS+PF003-1	7.03	6.88	4.93	4.97
NSS+PF003-1	13.85	13.58	11.53	12.35
NSS+PF003-1	8.65	4.22	5.27	5.02
NSS+PF003-1	9.37	7.30	5.35	6.15
NSS+PF003-1	15.00	13.72	12.28	7.93
NSS+PF003-1	13.05	6.87	8.23	7.18
WAY100635+PF003-1	13.47	12.27	3.98	1.50
WAY100635+PF003-1	14.13	9.90	5.17	7.70
WAY100635+PF003-1	11.60	5.32	9.40	2.82
WAY100635+PF003-1	5.73	0.90	0.40	0.27
WAY100635+PF003-1	12.97	10.57	5.33	4.33
WAY100635+PF003-1	12.70	6.45	6.72	5.50
WAY100635+PF003-1	14.22	11.78	3.12	0.30
WAY100635+PF003-1	14.78	6.33	3.75	0.40
WAY100635+PF003-1	10.95	3.67	1.85	2.50
WAY100635+PF003-1	14.23	10.75	2.38	0.65
WAY100635+PF003-1	14.33	9.43	2.30	1.65
WAY100635+PF003-1	12.82	5.95	3.20	5.00

Group	Mobility Time			
	Day 1	Day 2	Day 3	Day 4
SCH23390+PF003-1	8.48	2.35	2.98	1.30
SCH23390+PF003-1	4.02	1.32	0.73	0.67
SCH23390+PF003-1	12.07	4.42	0.62	1.92
SCH23390+PF003-1	9.90	4.58	1.52	1.05
SCH23390+PF003-1	12.10	8.77	6.48	5.43
SCH23390+PF003-1	6.25	1.70	0.95	0.98
SCH23390+PF003-1	10.02	1.90	2.08	1.38
SCH23390+PF003-1	9.33	6.40	23.00	3.45
SCH23390+PF003-1	8.22	4.92	3.65	4.08
SCH23390+PF003-1	10.28	7.43	4.57	7.28
SCH23390+PF003-1	3.85	1.82	0.73	0.60
SCH23390+PF003-1	5.23	0.85	0.33	0.35
SCH+WAY+PF003-1	7.40	3.18	4.28	2.55
SCH+WAY+PF003-1	9.55	4.57	1.87	2.60
SCH+WAY+PF003-1	3.85	1.62	0.32	0.18
SCH+WAY+PF003-1	13.08	6.38	2.63	0.17
SCH+WAY+PF003-1	7.83	7.10	3.58	2.25
SCH+WAY+PF003-1	11.57	6.98	3.22	0.37
SCH+WAY+PF003-1	7.92	3.37	2.07	2.03
SCH+WAY+PF003-1	6.45	5.80	3.72	1.93
SCH+WAY+PF003-1	3.15	3.23	0.73	0.85
SCH+WAY+PF003-1	8.80	3.77	2.02	0.98
SCH+WAY+PF003-1	8.42	5.37	2.43	1.47
SCH+WAY+PF003-1	7.12	5.15	1.42	3.13

Table 4 Mobility Time of PF003-2-Treated Mice When Treated with Dopamine D1 Receptor Antagonist (SCH 23390) and Serotonin 5-HT1A Receptor Antagonist (WAY 100635)

Group	Mobility Time			
	Day 1	Day 2	Day 3	Day4
NSS+PF003-2	14.83	8.83	4.37	3.37
NSS+PF003-2	11.27	9.82	7.72	6.10
NSS+PF003-2	10.10	8.58	6.57	4.95
NSS+PF003-2	9.75	5.05	11.08	5.57
NSS+PF003-2	8.38	5.17	3.77	4.18
NSS+PF003-2	10.30	5.02	1.97	1.32
NSS+PF003-2	9.37	4.65	4.87	4.08
NSS+PF003-2	12.27	9.43	5.80	3.95
NSS+PF003-2	9.05	6.02	4.88	3.48
NSS+PF003-2	14.30	12.52	11.58	9.97
NSS+PF003-2	6.07	7.82	5.47	2.97
NSS+PF003-2	8.30	4.40	12.48	8.15
WAY100635+PF003-2	4.97	2.60	1.52	0.75
WAY100635+PF003-2	10.45	9.38	4.15	2.25
WAY100635+PF003-2	11.65	6.03	3.58	2.15
WAY100635+PF003-2	8.28	2.63	1.48	0.35
WAY100635+PF003-2	8.85	4.18	1.85	1.42
WAY100635+PF003-2	6.45	4.47	2.53	1.67
WAY100635+PF003-2	9.62	3.87	1.05	0.87
WAY100635+PF003-2	9.07	3.65	2.37	1.72
WAY100635+PF003-2	11.52	3.42	1.00	0.35
WAY100635+PF003-2	11.95	6.67	3.77	2.47
WAY100635+PF003-2	9.92	3.05	1.15	0.77
WAY100635+PF003-2	5.85	1.85	1.15	0.53

Group	Mobility Time			
	Day 1	Day 2	Day 3	Day 4
SCH23390+PF003-2	3.72	1.18	1.07	0.60
SCH23390+PF003-2	11.57	2.87	1.08	0.80
SCH23390+PF003-2	5.98	3.55	1.42	0.73
SCH23390+PF003-2	11.87	3.52	1.42	0.43
SCH23390+PF003-2	12.50	7.15	2.80	1.33
SCH23390+PF003-2	12.53	3.12	0.93	0.75
SCH23390+PF003-2	8.37	3.20	0.75	0.30
SCH23390+PF003-2	8.57	5.68	1.55	1.47
SCH23390+PF003-2	7.47	2.80	0.57	0.37
SCH23390+PF003-2	8.27	4.75	0.97	0.42
SCH23390+PF003-2	11.18	6.33	3.40	0.78
SCH23390+PF003-2	9.73	3.43	1.15	0.78
SCH+WAY+PF003-2	5.77	1.42	0.47	0.25
SCH+WAY+PF003-2	11.08	10.75	2.52	1.00
SCH+WAY+PF003-2	11.67	6.22	1.97	0.38
SCH+WAY+PF003-2	11.70	5.72	1.60	0.32
SCH+WAY+PF003-2	11.47	5.68	6.53	1.52
SCH+WAY+PF003-2	8.30	2.33	0.85	0.23
SCH+WAY+PF003-2	13.38	2.33	1.13	0.72
SCH+WAY+PF003-2	8.97	1.43	0.50	0.22
SCH+WAY+PF003-2	8.20	0.93	0.43	0.37
SCH+WAY+PF003-2	9.45	3.25	1.43	1.28
SCH+WAY+PF003-2	6.48	1.57	1.12	1.18
SCH+WAY+PF003-2	12.32	2.37	0.47	0.43

CURRICULUM VITAE

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