INTRODUCTION

Mental depression has been recognized as one of the major health problems affecting the mankind. The disease not only affects the individual but also causes a great social, health and economic burden to the society (Baldwin and Hirschfeld, 2005). It is estimated that approximately 300 million people worldwide suffer from major depressive disorder (Kessler et al., 1994) and nearly 35-40% of suicides are considered to be related to mental depression as the underlying cause (Vijayakumar, 2005). At one time it was considered as a progressive neurodegenerative disorder (commonly observed in elderly people), but in recent years it is increasingly seen in young adults, adolescents and even in children (Millan, 2004) (Fig.1). Depression, particularly in lower age groups, untreated can affect the performance and learning, social interactions, development of normal peer relationships, self-esteem and life acquisitions. This can further indulge the individual to various antisocial activities like substance abuse, disruptive behaviors, violence, aggression, legal troubles, and even suicidal ideations (Sadock and Sadock, 2000). It is observed that women (in all the age groups) are prone to develop depressive disorders more than men, with the life time prevalence of 20% in women as compared to 10% in men, respectively (15-24 years age group) (Sadock and Sadock, 2000) (Fig.1).

![Epidemiology of depressive illness](image)

Fig. 1. Epidemiology of depressive illness (Data from National Comorbidity Survey, Adapted from Sadock and Sadock, 2000).
Depression is usually defined by the presence of certain behaviors and thought patterns. Various international agencies have described the diagnostic criteria of depressive disorders. These characteristics are commonly employed to diagnose the disease. Amongst the most prominent clinical manuals are the Diagnostic and Statistical Manual of Mental Disorders (3rd edition) (DSM-III) given by the American Psychiatric Association (APA) in 1980 and its revised version DSM-III-R (APA, 1987), DSM-IV (APA, 1994), DSM-IV-TR (text revision) (APA, 2000) and ICD-10 [the International Classification of Diseases (10th revision), World Health Organization 1992] (Berton and Nestler, 2006).

Some of the core symptoms of depression include low mood, markedly reduced interest or pleasure in all the activities, appetite changes, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feeling of worthlessness or excessive guilt, reduced ability to think or concentrate and morbid thought of death or recurrent suicidal ideation. There may be diurnal fluctuations of these symptoms (with symptoms more severe during early morning) (Nestler et al., 2002). To characterize the individual as “depressed”, these symptoms must be felt at least for 2 weeks continually. Unfortunately, depression is usually a recurring, and sometimes a chronic condition (Baldwin and Hirschfeld, 2005).

As per DSM-IV-TR, major depressive disorder can be divided into different subtypes as defined below (Table 1). The clinical importance of differentiating these subtypes is that treatment approach may vary according to the subtypes of depression. Melancholic depression also referred to as typical depression has the primary symptoms of non-reactive mood, in which the mood does not fit, even temporarily, when something good happens to the person, or loss of pleasure in all or almost all enjoyable activities (Lam et al., 2005). In contrast, patients with atypical features present with a reactive mood state (where mood can improve transiently in response to something good that happens). Other subtypes include psychotic depression with features such as hallucinations and delusions.
### Table 1: Subtypes of depression with clinical implications

<table>
<thead>
<tr>
<th>Depression: subtypes with features</th>
<th>Key symptoms</th>
<th>Clinical consideration</th>
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<tbody>
<tr>
<td>Melancholic features</td>
<td>Distinct quality of depressed mood, worsening of mood in morning, early morning waking, marked psychomotor changes, significant anorexia or weight loss, excessive or inappropriate guilt</td>
<td>Often more severe and may be more likely to respond to biological interventions</td>
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<tr>
<td>Atypical features</td>
<td>Reactive mood state, oversleeping, increased appetite and weight gain, interpersonal rejection sensitivity</td>
<td>Associated with early age of onset, chronic course and history of trauma/abuse</td>
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<tr>
<td>Psychotic features</td>
<td>Presence of hallucinations or delusions (especially delusions of guilt)</td>
<td>Antidepressant + antipsychotic drug + electroconvulsive therapy</td>
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<tr>
<td>Catatonic features</td>
<td>Presence of catatonic signs and symptoms (rigidity and psychomotor excitation)</td>
<td>Acute catatonia responds to injectible lorazepam</td>
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<tr>
<td>Seasonal (winter) pattern</td>
<td>Regular onset of depressive episodes during the winter with summer remission, associated with atypical features such as oversleeping, overeating with carbohydrate craving and weight gain</td>
<td>Bright light therapy or antidepressants</td>
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<tr>
<td>Postpartum onset</td>
<td>Onset of depressive episode within 4 weeks postpartum, may be associated with psychotic features</td>
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<tr>
<td>Rapid cycling</td>
<td>4 or more episodes of mania/hypomania and depression (or switches between states in a year)</td>
<td>Lithium is less effective than anticonvulsants</td>
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Adapted from Lam et al., 2005
Catatonic subtype is not commonly encountered in clinical practice, but this includes features of catatonia such as rigidity or psychomotor agitation/excitement (Lam et al., 2005). The proper identification of depression-relevant symptoms is important for the accurate diagnosis of depression, development of treatment strategies and measurement of outcome (Vaccarino et al., 2008).

The overall management of people with depression is often far from ideal. Stigma and discrimination make people who might be suffering from depression reluctant to present for treatment (Baldwin and Hirschfeld, 2005). Even after the patient presents with psychological symptoms, the recognition for depression by doctors and other health professionals is variable and often poor. A large number of antidepressant drugs are available for the treatment of the disease but unfortunately, none of them meet the profile of an ideal agent, with side effects limiting their use in some patients (Baldwin and Hirschfeld, 2005). Therefore, it is not only important to understand the exact pathophysiology of depression but also the limitations of the current drug therapy.

Many brain regions namely neocortex and hippocampus (mediates cognitive aspects such as memory impairment and feeling of worthlessness, guilt and suicidability associated in depression), striatum particularly nucleus accumbens and amygdala (mediates emotional memory, anhedonia, anxiety and reduced motivation), and hypothalamus (neurovegetative symptoms of depression such as too much or too little sleep, appetite and energy, as well as loss of sex drive) have been implicated in the regulation of behavior, emotions and thought processes which are intimately involved in mental depression (Nestler et al., 2002). These brain regions are known to operate in a series of highly interacting parallel neuronal circuits and are highly innervated by monoaminergic pathways (Nestler et al., 2002).

Ever since the discovery of neurotransmitters, various theories have been put forward in understanding the exact pathophysiology of mental
depression. **The monoaminergic theory of mental depression** (also known as classical theory of depression) proposes that depression is due to the deficiency of monoamines viz. norepinephrine (NE), serotonin (5-HT), and/or dopamine (DA) in the brain (Millan, 2004). These biogenic amines are known to be important for regulation of cognition, mood, thermoregulation, sexual functions, and various endocrine functions involved in homeostasis (Millan, 2004). Deficiency or degeneration of one or any of the three monoamines can lead to the clinical pathology of mental depression (Deecher et al., 2006). Although opportunities to study monoamine metabolism and monoaminergic functions in the human brain are very limited but, taking the results of all the research strategies together, there are strong indications that central monoamine metabolism may be disturbed in depression.

**Norepinephrine (NE):** Norepinephrine is the neurotransmitter often associated with the “fight or flight” response to stress. Noradrenergic neurons arise from the locus ceruleus of the brain stem and innervates the frontal cortex (where it regulates cognition and attention), and cerebellum (involved in certain motor movements) regions of the brain (Stahl and Briley, 2004). Low levels of norepinephrine are associated with a lack of alertness, poor memory, and depressive symptoms. The concentration of 3-methoxy-4-hydroxyphenyl-glycol (MHPG), a major metabolite of norepinephrine is also known to be decreased in the patients suffering from mental depression (Muscettola et al., 1984).

**Serotonin (5-HT):** From a phylogenetic standpoint, serotonin (5-HT) is one of the oldest neurotransmitter present in the brain (Sjoerdsma and Palfreyman, 1990). In the human brain, 5-HT containing neurons are highly localized in specific clusters in the brain stem and spinal cord (Tork, 1990). From these sites, the cells send out axons that innervates the diverse areas throughout the brain. For example, serotonergic neurons (like noradrenergic neurons) are known to project to frontal cortex
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(regulate mood), limbic areas (regulate emotions and anxiety), and to the hypothalamus (which regulates eating behavior, appetite, weight, sex drive and pleasure) (Stahl and Briley, 2004). Given the nature of extensive distribution, any dysfunction in the serotoninergic pathway would be implicated as a pathology in major depression (Graeff, 1997).

**Dopamine (DA):** In recent years, besides the above said two major neurotransmitters, many investigators have explored the role of dopaminergic systems in mental depression (Kapur and Mann, 1992; Brown and Gershon, 1993). Anhedonia (i.e. inability to gain pleasure from enjoyable experiences) is a frequent symptom of depression and is associated with a dysfunction of the dopaminergic reward system. Also, depressed patients are known to display an up-regulation of D2 dopamine receptor density in basal ganglia/cerebellum in comparison with healthy subjects (D’haenen and Bossuyt, 1994). This is proposed to be compensatory mechanism of the brain in response to decreased dopamine levels in depressed patients. The involvement of dopaminergic system in depression is further strengthened by the fact that bupropion, a selective inhibitor of dopamine reuptake is known to possess antidepressant activity (Prica et al., 2008). Similarly, various D2/D3 dopamine receptor agonists such as ropinirole and pramipexole are known to demonstrate antidepressant activity when used as therapeutic agents in depression associated with Parkinson’s disease (Rogoz and Skuza, 2006). Interestingly, most of the antidepressants belonging to different class, namely, tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) or dual reuptake inhibitors of serotonin and norepinephrine (SNRIs), are known to enhance the dopaminergic neurotransmission when administered at higher doses. For example, desipramine (a potent inhibitor of noradrenaline reuptake) or fluoxetine (a SSRI) is known to increase extracellular concentrations of dopamine in the prefrontal cortex, the area mainly involved in solving complex problems,
cognition and in the expression of personality and appropriate social behavior (Ainsworth et al., 1998).

The monoaminergic hypothesis is further strengthened by the fact that reserpine when administered to animals or humans depletes brain catecholamine and produces symptoms similar to mental depression. The antidepressants are known to effectively reverse the reserpine-induced behavioral syndrome (O’Leary et al., 2007a). Further, the correlations between monoamines and depression was encouraged by the findings that first generation antidepressant drugs enhanced the availability of norepinephrine and serotonin at their respective receptor systems. Tricyclic antidepressants (TCAs) do this via reuptake inhibition, and monoamine oxidase (MAO) inhibitors act via inhibition of degradation (Delgado, 2004). Newer antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and dopamine reuptake inhibitors (DRIs) also fit into this hypothesis and aim to correct the deficiency of monoamines in conditions of mental depression.

The irreversible inhibitors of monoamine oxidase (MAO) are known to block the mitochondrial degradation of enzyme and increase the brain levels of norepinephrine, serotonin and/or dopamine (Bortolato et al., 2008). They are considered to be having good efficacy, however, these drugs are poorly tolerated, because of their propensity to irreversible inhibiting the monoamine oxidase enzyme. Moreover, MAOIs are associated with side effects and drug-drug or food-drug interactions (Rapaport, 2007). Unlike the earlier inhibitors, moclobemide, a reversible inhibitor of monoamine oxidase-A (RIMAs) has better safety profile as compared to conventional or first generation MAO-inhibitors (Schatzberg, 2002). Similarly, the tricyclic antidepressants such as imipramine possessed plethora of side effects due to their multitarget profile of actions which included the antagonism of muscarinic, \( \alpha \)-adrenergic, and histaminergic receptors (Schatzberg, 2002).
Selective serotonin reuptake inhibitors (SSRIs), categorized as the second-generation antidepressants are known to specifically inhibit the reuptake of serotonin specifically and are considered relatively safe as compared to TCAs or MAOIs. Accordingly, they are universally prescribed for the long term control of depressive state (Millan, 2003; Shorter and Tyrer, 2003). However, SSRIs do not possess better efficacy when compared to tricyclic antidepressants (Anderson, 2000), which lead to the introduction of other newer class of antidepressants viz. dual reuptake inhibitors of serotonin and noradrenaline (SNRIs), exemplified by venlafaxine (Demitrack, 2002). Venlafaxine is considered to be more efficacious and it lacked the unwanted receptor interactions that were observed with tricyclics (Blier, 2003; Davidson et al., 2003). Venlafaxine has provided one of the first opportunities to examine the hypothesis that a specific dual-action antidepressant would provide a superior clinical response as compared to any single-action agent (Demitrack, 2002). At lower concentration, it has relatively higher affinity for serotonin transport site, virtually identical to that of SSRI. It is only at higher concentration that the drug inhibits the reuptake of both serotonin and norepinephrine resulting in a more complete dual inhibition (Owens et al., 1997; Redrobe et al., 1998; Frazer, 2001). Venlafaxine has not been shown to inhibit monoamine oxidase enzyme (Muth et al., 1986). Venlafaxine is found to be active after both intraperitoneal and per oral administration (Burnett and Dinan, 1998). The drug is advantageous over conventional antidepressants, as it has low potential for any stimulatory, sedative and proconvulsant effects; together with a low risk of drug interactions (Burnett and Dinan, 1998). Duloxetine is another new dual-action antidepressant that also inhibits the reuptake of both serotonin and norepinephrine (Bymaster et al., 2001). It has been reported that duloxetine has a better profile as it inhibits the reuptake of serotonin and norepinephrine more potently as compared to venlafaxine (Bymaster et al., 2001).
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Triple reuptake inhibitor concept: Recently, the concept of triple reuptake inhibitors has been widely debated (Skolnick et al., 2006). It is argued that the drugs which will inhibit the reuptake of all the three neurotransmitters viz. norepinephrine, serotonin and dopamine could be advantageous over the first two generation of antidepressants (Skolnick et al., 2006). However, no currently marketed antidepressant inhibits the reuptake of all the three neurotransmitters linked to depression. There are presently two molecules in clinical trials that belong to the category of triple reuptake inhibitors. These molecules include GlaxoSmithKline's NS2359 and Merck's DOV216303. Both candidates are in phase 2 clinical trial. A drug inhibiting reuptake of all the three of these neurotransmitters is believed to produce more rapid onset of action and greater efficacy with lesser side-effects than traditional antidepressants (Skolnick et al., 2006; Chen and Skolnick, 2007).

However, there are several limitations of monoaminergic hypothesis of mental depression (Hindmarch, 2002).

1. Although it is clearly established that monoamine-based therapies such as TCAs and SSRIs are clinically effective antidepressants, however, the clinical effect is often noticeable after at least 2 to 4 weeks (Nierenberg, 2001). The lag-time in the onset of action of antidepressants can be explained by the activation of inhibitory autoreceptors (5-HT1A serotonin and α2-adrenergic) on serotonergic and noradrenergic neurons which initially attenuate the effects of antidepressants on synaptic transmitter levels (Nutt, 2002). Subsequently, these autoreceptors are known to become desensitized, allowing the emergence of an overt antidepressant response. This theory has led to the proposition that antagonists at these autoreceptors such as pindolol, a 5-HT1A serotonin autoreceptor antagonist (Geretsegger et al., 2008) may be useful adjuncts to antidepressant treatment (Nutt, 2002). Another selective 5-HT1A serotonin receptor antagonist such as WAY-100635 is known to
augment SSRI- and SNRI-induced changes in cortical serotonin levels (Beyer et al., 2002). Similarly, antidepressants, when given in combination with α2 adrenergic autoreceptor antagonists such as yohimbine or idazoxan can potentially elevate levels of all the three monoamines (Schechter et al., 2005).

2. As discussed above, although many patients ultimately respond well to antidepressant treatment, others have an incomplete response or do not respond at all (Fava and Davidson, 1996).

3. Antidepressants also show effective anti-anxiety action (Sheehan et al., 1993).

4. Lastly drugs like tianeptine, which increase serotonin uptake, are also effective as antidepressants (Loo et al., 1999).

Besides the availability of large number of antidepressants, over 60% of patients with mental depression do not respond fully to the therapy. Half of them eventually will not respond at all and will be referred to as treatment resistant depression patients (Amital et al., 2008). Researchers are looking for some of the novel alternatives to explore the exact pathophysiology of depression. Besides the well-established monoaminergic theory of depression, the other proposed theories include the possible involvement of γ-amino butyric acid (GABAergic) system, N-methyl-d-aspartate (NMDA) receptor pathway, peptidergic, purinergic and more recently, neurotrophic factors and the involvement of hypothalamic-pituitary-adrenal (HPA) axis in depression. Besides this, there seems to be the involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate pathway and sigma receptor participation in the mechanisms of many antidepressants (Nestler et al., 2002).

L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) is an important signaling pathway that is reported to be involved in depression (Mantovani et al., 2003). Nitric oxide, a messenger molecule in the brain, synthesized from L-arginine by nitric oxide synthase (NOS) has
been implicated in neurotransmission, synaptic plasticity, learning, perception of pain, aggression and depression (Esplugues, 2002). Recent evidences have shown that the reduction of nitric oxide levels within the hippocampus can induce antidepressant-like effects, thus implicating endogenous hippocampal nitric oxide in the neurobiology of stress and depression (Joca and Guimares, 2006). Several of physiological actions of nitric oxide are mediated through its interaction with the heme iron of soluble guanylate cyclase (sGC), leading to enzyme activation and consequent increase in cyclic guanosine monophosphate (cGMP) (Kaster et al., 2005a; 2005b). Recent studies have shown the possibility that the inhibition of nitric oxide synthase (NOS) could be used as a strategy to enhance the clinical efficacy of serotonergic antidepressants (Harkin et al., 2004).

The role of GABAergic and glutamatergic neurotransmission in the pathophysiology of depression has been debated for sometime as one of the underlying pathologies in mental depression. Neurosteroids are the steroid molecules which are formed in the central and peripheral nervous systems independent of the supply by the steroidogenic endocrine glands (Reddy et al., 1998). These are known to modulate the GABAergic or glutamatergic neurotransmission. Dysregulation in the concentration of the neurosteroids (allo)pregnenolone and 3α,5α-tetrahydrodeoxycorticosterone (3α, 5α-THDOC) has been found in depressed patients. Available data indicates that neurosteroids are also involved in premenstrual dysphoric disorders, eating disorders, attention deficit and hyperactivity disorders, generalized anxiety disorders, panic disorders etc. Decreased levels of neurosteroids such as dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS) and pregnenolone sulfate (PS) has been associated with depression, cognitive dysfunction, ageing and other neurological disorders (Orentreich et al., 1984; Reddy et al., 1998). PS and DHEAS are negative allosteric modulators of the GABA_A receptors (Majewska, 1992) and positive modulators of N-methyl-d-aspartate
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(NMDA) receptor-mediated response (Murray and Gillies, 1997). However, the exact mechanism underlying in the beneficial effects of neurosteroids in depression is currently not understood.

Of late, the role of sigma ligands in the pathophysiology of depression has been considered for explaining the drug actions. Neurosteroids are reported to interact with sigma-1 site which regulated the intracellular levels of Ca²⁺. This action may have a role to play in their antidepressant action (Maurice et al., 2001; Urani et al., 2002). Nevertheless, it is still a matter of conjuncture whether sigma-1 modulation is more important or the direct actions at monoaminergic receptors in the antidepressant profiles of several sigma-1 receptor ligands (Tottori et al., 2001; Millan, 2003).

Animal models of depression: A major impediment in depression research is the lack of validated animal models. Many of the core symptoms of depression in man such as depressed mood, feeling of worthlessness or suicide tendency cannot be measured or expressed in laboratory animals. However, some of the tests like forced swim test (FST) described by Porsolt et al., 1977a or the tail suspension test (TST) described by Steru et al., 1985 which extensively employed as rodent models of depression show a great degree of predictability in testing antidepressant action of drugs. In FST, rats or mice are forced to swim inside a jar of specific dimensions containing water upto certain height and maintained at 23-25°C. The animals first struggle to escape for a brief period of time and then become immobile (surrender or show helplessness) or make only those movements necessary to keep their heads above water. Typically, a six- minute test is employed and immobility period is measured for the last four minute session or during the entire test period of six minutes. Similarly, in the TST, mice are suspended by the tail, using adhesive tape, to a horizontal bar. Typically, mice immediately show several escape-like behaviors, followed by surrender to the situation (helplessness behavior), which is again recorded as
immobility period. The “helplessness” episode as represented by immobility is considered to be a sign of dejection, one of the main symptoms of depression in humans and this behavior is amenable to treatment with antidepressant drugs. These two animal models have high degree of pharmacological predictability and validity since majority of antidepressants show positive response. Moreover, these models are simple, inexpensive and reliable, and have a high specificity in detecting antidepressant action of novel drugs. It is debated that, although these two behavioural paradigms are mainly employed to screen antidepressants, but, they involve different neuronal mechanisms (Renard et al., 2003). The last three decades of experimental evidences have shown that strain-difference and experimental situations are extremely important in minimizing inter-laboratory variations (Kulkarni and Parale, 1986; Parale and Kulkarni, 1986)

The present work reported in the thesis was undertaken to validate both the forced swim test (FST) and tail suspension test (TST), the two majorly employed animal models of despair using different category of antidepressant agents and also test the predictability parameter for testing New Molecular Entities (NMEs), respectively. ED50 values of various standard antidepressant drugs were calculated and compared using statistical analysis. The correlation of despair with target specific transmitter substance(s) namely, norepinephrine, serotonin and dopamine was studied. The detailed mechanism of antidepressant-like effect of various newer agents like venlafaxine, bupropion, neurosteroids (pregnenolone sulfate and dehydroepiandrosterone sulfate) and herbal preparation like berberine chloride were explored. The research work focuses on the involvement of monoaminergic and/or L-arginine-nitric oxide-cyclic guanosine monophosphate pathway in the antidepressant-like effect of these molecules. Further, attempts were also made to study the involvement of sigma receptors in their antidepressant activity. The new series antidepressant candidates (New Molecular Entities; NMEs) were
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synthesized (in collaboration with Professor S.V. Kessar, Department of Chemistry, Panjab University, Chandigarh) and tested for their biological profile using these animal models of depression. Various behavioral (locomotor activity, hyperalgesia, anxiety, memory), biochemical (measurement of lipid peroxidation, reduced glutathione, nitrite, myeloperoxidase, adrenal ascorbic acid) and neurochemical (measurement of norepinephrine, serotonin and dopamine) approaches have been made to understand the exact mechanism(s) involved in the antidepressant actions of these drugs. The work has been presented under different chapters (chapters 1 to 8) to highlight each aspect of the study.