SYNTHESIS AND EVALUATION OF PHENOTHIAZINE DERIVATIVE FOR ANTIDEPRESSANTS ACTIVITY

1MR. VIJAY VINAYAK PAWAR MASTER OF PHARMACY PHARMACEUTICAL CHEMISTRY
SWAMI VIVEKANAND SANSATHA’S INSTITUTE OF PHARMACY, MUNGASE, MALEGAON (NASHIK), INDIA

ABSTRACT:

This study was aimed at the synthesis of fused Phenothiazine derivatives containing heterocyclic moiety. The synthesized compounds were tested for their preliminary tests, physical constants, TLC, IR, 1H-NMR Spectra and CHN analysis confirmed the structures of the final compounds. Antidepressant activity of all the synthesized compounds was evaluated by despair swim test by using Sprague Dawley Rats. Standard drug Imipramine was used as the control. In the despair swim test, all the synthesized derivatives showed antidepressant activity. Among them three Compounds (A7, A8, and A10) showed significant antidepressant activity comparing with control drug imipramine. These results are useful for the further investigation in the future.

Keywords: Antidepressant activities, Despair swim test, Phenothiazine and Sprague Dawley Rats.

1.0. INTRODUCTION.

Depression is estimated to affect 350 million people. The World Mental Health Survey conducted in 17 countries found that on average about 1 in 20 people reported having an episode of depression in the previous year. Depressive disorders often start at a young age; they reduce people’s functioning and often are recurring. For these reasons, depression is the leading cause of disability worldwide in terms of total years lost due to disability. The demand for curbing depression and other mental health conditions is on the rise globally. A recent World Health Assembly called on the World Health Organization and its member states to take action in this direction (WHO, 2012). Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration. Moreover, depression often comes with symptoms of anxiety. These problems can become chronic
or recurrent and lead to substantial impairments in an individual’s ability to take care of his or her everyday responsibilities. At its worst, depression can lead to suicide. Almost 1 million lives are lost yearly due to suicide, which translates to 3000 suicide deaths every day. For every person who completes a suicide, 20 or more may attempt to end his or her life.

A **depressant**, or central depressant, is a drug or endogenous compound that lowers neurotransmission levels, which is to depress or reduce arousal or stimulation, in various areas of the brain. Depressants are also occasionally referred to as "downers" as they lower the level of arousal when taken. Stimulants or "uppers" increase mental and/or physical function, they are the functional opposites of depressants.

Depressants are widely used throughout the world as prescription medicines and as illicit substances. When these are used, effects often include ataxia, anxiolysis, pain relief, sedation or somnolence, and cognitive/memory impairment, as well as in some instances euphoria, dissociation, muscle relaxation, lowered blood pressure or heart rate, respiratory depression, and anticonvulsant effects, and even complete anesthesia or death at high doses.

Depressants exert their effects through a number of different pharmacological mechanisms, the most prominent of which include facilitation of GABA, and inhibition of glutamatergic or monoaminergic activity. Other examples are chemicals that modify the electrical signaling inside the body. The most prominent of these being bromides and

### Types

**Alcohol**

Distilled (concentrated) alcoholic beverages, often called "hard liquor", roughly eight times more alcoholic than beer.

An alcoholic beverage (often referred to simply as spirits) is a drink that contains alcohol, an anesthetic that has been used as a psychoactive drug for several millennia. Ethanol is the oldest recreational drug still used by humans. Ethanol can cause alcohol intoxication when consumed. Alcoholic beverages are divided into three general classes for taxation and regulation of production: beers, wines, and spirits (distilled beverages). They are legally consumed in most countries around the world. More than 100 countries have laws regulating their
production, sale, and consumption.\(^2\)

The most common way to measure intoxication for legal or medical purposes is through blood alcohol content (also called blood alcohol concentration or blood alcohol level). It is usually expressed as a percentage of alcohol in the blood in units of mass of alcohol per volume of blood, or mass of alcohol per mass of blood, depending on the country. For instance, in North America a blood alcohol content of 0.10 (0.10% or one tenth of one percent) means that there are 0.10 g of alcohol for every dL of blood.\(^3\)

**Barbiturates**

Barbiturates are effective in relieving the conditions that they are designed to address. They are also commonly used for unapproved purposes, physically addictive, and have serious potential for overdose. When, in the late 1950s, many thought that the social cost of barbiturates was beginning to outweigh the medical benefits, a serious search began for a replacement drug. Most people still using barbiturates today do so in the prevention of seizures or in mild form for relief from the symptoms of migraines.

**Benzodiazepines**

A benzodiazepine (sometimes colloquially "benzo", often abbreviated "BZD") is a psychoactive drug whose core chemical structure is the fusion of a benzene ring and a diazepine ring. The first such drug, chlordiazepoxide (Librium), was discovered accidentally by Leo Sternbach in 1955, and made available in 1960 by Hoffmann–La Roche, which has also marketed the benzodiazepine diazepam (Valium) since 1963.

Benzodiazepines enhance the effect of the neurotransmitter gamma-aminobutyric acid (GABA) at the GABA\(_\text{A}\) receptor, resulting in sedative, hypnotic (sleep-inducing), anxiolytic (anti-anxiety), anticonvulsant, and muscle relaxant properties; also seen in the applied pharmacology of high doses of many shorter-acting benzodiazepines are amnesic-dissociative actions. These properties make benzodiazepines useful in treating anxiety, insomnia, agitation, seizures, muscle spasms, alcohol withdrawal and as a premedication for medical or dental procedures. Benzodiazepines are categorized as either short-, intermediate-, or long- acting. Short- and intermediate-acting benzodiazepines are preferred for the treatment of insomnia; longer-acting benzodiazepines are recommended for the treatment of anxiety.

In general, benzodiazepines are safe and effective in the short term, although cognitive impairments and
paradoxical effects such as aggression or behavioral disinhibition occasionally occur. A minority react reverse and contrary to what would normally be expected. For example, a state of panic may worsen considerably following intake of a benzodiazepine. Long-term use is controversial due to concerns about adverse psychological and physical effects, increased questioning of effectiveness, and, because benzodiazepines are prone to cause tolerance, physical dependence, and, upon cessation of use after long-term use, a withdrawal syndrome. Due to adverse effects associated with the long-term use of benzodiazepines, withdrawal from benzodiazepines, in general, leads to improved physical and mental health. The elderly are at an increased risk of suffering from both short- and long-term adverse effects.

There is controversy concerning the safety of benzodiazepines in pregnancy. While they are not major teratogens, uncertainty remains as to whether they cause cleft palate in a small number of babies and whether neurobehavioural effects occur as a result of prenatal exposure; they are known to cause withdrawal symptoms in the newborn. Benzodiazepines can be taken in overdoses and can cause dangerous deep unconsciousness. However, they are much less toxic than their predecessors, the barbiturates, and death rarely results when a benzodiazepine is the only drug taken; however, when combined with other central nervous system depressants such as alcohol and opiates, the potential for toxicity and fatal overdose increases. Benzodiazepines are commonly misused and taken in combination with other drugs of abuse. In addition, all benzodiazepines are listed in Beers List, which is significant in clinical practice. [4]

**Opioids**

Contrary to popular misconception, opioids are not depressants in the classical sense. They do produce central nervous system depression, however, they also excite certain areas of the central nervous system. To remain true to the term 'depressant' - opioids cannot be classified as such. For opioid agonists and opium derivatives, these are classified differently. Analgesic or narcotic correctly identifies these drugs. However, they do have depressant actions nonetheless.

- Morphine
- Codeine
MISCELLANEOUS

- Alpha and beta blockers (Carvedilol, Propranolol, atenolol, etc.)
- Anticholinergics (Atropine, hyoscyamine, scopolamine, etc.)
- Anticonvulsants (Valproic acid, carbamazepine, lamotrigine, etc.)
- Antihistamines (Diphenhydramine, doxylamine, promethazine, etc.)
- Antipsychotics (Haloperidol, chlorpromazine, clozapine, etc.)
- Dissociatives (Dextromethorphan, ketamine, phencyclidine, nitrous oxide, etc.)
- Hypnotics (Zolpidem, zopiclone, chloral hydrate, chloroform, etc.)
- Muscle relaxants (Baclofen, carisoprodol, cyclobenzaprine, etc.)
- Sedatives (Gamma-hydroxybutyrate, etc.)

COMBINATIONS

Combining multiple depressants can be very dangerous because the central nervous system's depressive properties has been proposed to increase exponentially instead of linearly.\cite{citation needed} This characteristic makes depressants a common choice for deliberate overdoses in the case of suicide. The use of alcohol or benzodiazepines along with the usual dose of heroin is often the cause of overdose deaths in opiate addicts.

1.1 DIAGNOSIS OF DEPRESSION

The diagnosis of mental disorders is often believed to be more difficult than diagnosis of somatic or general medical, disorders, since there is no definitive lesion, laboratory test, or abnormality in brain tissue that can identify the illness.\cite{5}

A diagnosis of depression is confirmed when the patient meets established criteria which target the symptoms typically associated with depression. To meet the established criteria, a patient must exhibit either depressed mood or diminished interest or pleasure in usual activities and must have at least five symptoms from the following:

1. Significant weight loss or weight gain, or decrease or increase in appetite.
2. Insomnia or hypersomnia.
3. Psychomotor agitation or retardation as observed by others.
4. Fatigue or loss of energy.
5. Feelings of worthlessness, or inappropriate guilt.
6. Diminished ability to think or to concentrate.
7. Recurrent thoughts of death, suicidal ideation, suicide attempt or a specific plan for suicide.\cite{6}

Various rating scales have been developed that may help to demonstrate the severity of depressive disorder distinguish a predominantly anxious patient from a depressed patient. Biochemical tests are generally not
particularly helpful in determining the treatment plan or management of affective disorders. The dexamethasone suppression test is still used by some clinicians as an aid to diagnosis, but it must be considered as having limited value in practice.

Within the UK, mental and behavioural disorders are commonly classified using the international classification of diseases, ICD 10 (WHO 1992). The American Psychiatric Association (1994) has developed a precise system of diagnosis, based on the description of symptoms in the diagnostic and statistical manual of mental disorders (DSM).

Bipolar disorder is frequently misdiagnosed, and consequently patients are often inappropriately treated. Several problems contribute to the misdiagnosis of bipolar disorder. Patients often consider their manic symptoms to be normal and fail to realize that they might require treatment. Secondly, symptoms are highly variable, ranging from impulsive behaviour or substance abuse, to fluctuations in energy levels, and are often attributed to disorders other than bipolar disorder. [7]

The identification of target symptoms may be useful in evaluating the response to treatment. In routine clinical practice, antidepressant medication should not generally be used to treat patients with mild depression. Non-pharmacological strategies are preferable in this group.

Rating Scales

Various rating scales can be used to assist with the assessment of the severity of the disorder. Two of the more commonly used rating scales are the Back Depression Inventory and the Hamilton Depression Rating Scale.

- Back Depression Inventory
  - This is a self-reporting scale looking at 21 depressive symptoms. The subject is asked to read a series of statements and mark on a scale of 1-4 how severe symptoms are.
  - The higher the score, the more severely depressed a person may be.
- Hamilton Depression Rating Scale. [8]
  - This rating scale is used by a healthcare professional at the end of an interview to rate the severity of the depression.

Dexamethasone Suppression Test. [9]

This test involves the administration of 1 mg of dexamethasone at 11 p.m., which is said to coincide with the low point of cortisol secretion. It would be expected that normally dexamethasone would suppress the secretion of cortisol for about 24 hours.

Blood samples are taken the following day, at 8 a.m., 4 p.m. and 11 p.m. If it is found that serum cortisol levels are elevated between 9 and 24 hours after the administration of dexamethasone and then this is taken as a positive result, i.e. dexamethasone has failed to suppress normal cortisol secretion.

It is important to note that this test is not specific to depression and other disorders may account for an
apparent positive result. Similarly, there may be a high proportion of depressed people who show a negative result with this test. [10]

1.2 TREATMENT OF DEPRESSION

The aim of the treatment is to prevent harm and to relieve distress or to be prophylactic. It is important to differentiate symptoms of the disorder from the premorbid personality. This means the diagnosis will primarily influence the way in which these drugs are used rather than the choice of drug per se. There are several drugs available in market, which are used as antidepressants.

Standard drugs used in treatment of Depression. [11]

1. Drugs which block both NE and 5-HT reuptake. Imipramine, Clomipramine, Amitriptyline, Doxepin.
2. Drugs which mainly block NE reuptake.
   Desipramine, Lofepramine, Protryptiline, Maprotiline, Amoxapine, Lofepramine.
3. Selective Serotonin reuptake inhibitors (SSRIs).
   Sertraline, Fluoxetine, Fluoxamine, Paroxetine, Citalopram.
4. Atypical Antidepressants.
   Trazadone, Nefazodone, Bupropion, Mirtazapine, Mianserin, Venlafaxine.
5. MAO Inhibitors.
   b) Selective MAO-A inhibitors. Moclobemide.

Fig 1.6.1: -Schematic diagram showing some of the potential sites of action of antidepressant drugs. [12]

Despite the availability of many new antidepressants, the therapeutic effectiveness of these agents has changed little since the discovery of the antidepressant properties of Imipramine in the late 1950s. There is limited
Evidence to support the notion that MAOIs are less effective than tricyclics in hospitalized patients, but more effective in so called atypical depression. Overall, the SSRI antidepressants appear to be better tolerated than tricyclics. However, although less well tolerated, limited evidence suggests that vanalafaxine may be more effective than SSRIs in hospitalized patients. Overall, the current evidence indicates that any claim of overall superiority of one antidepressant over another would be premature.

Withdrawal of antidepressants should normally be undertaken gradually. Following abrupt discontinuation patients may experience symptoms of withdrawal that include gastrointestinal symptoms, together with headache, giddiness, sweating, shaking and insomnia. In addition, extra pyramidal reaction may be associated with abrupt withdrawal from some of the SSRI antidepressants. Following successful treatment, antidepressants should be gradually reduced over a period of 4 weeks. This period should be increased if patients experience problems, or where medication has been given for extended periods.

Generally, the long half life of fluoxetine enables the drug to be stopped without the need for tapering from the standard anti-depressant dose of 20 mg. Patients taking MAOIs may experience psychomotor agitation following discontinuation. Patients should be warned that there is a risk of problems with abrupt discontinuation. Other considerations which have to be taken into account are side effects, contraindications, toxicity in overdose, patient preference and clinician familiarity.

Generally speaking, the older drugs have a poorer side effect and toxicity profile than the more recently introduced agents.

Choice of Antidepressants:

As no antidepressant has been found to be more effective than any other, the choice will be determined by other factors. In some areas, cost has become a major factor in choice. In general the SSRI drugs appear to have a better side effect profile and are less toxic following overdose. For most people with moderate-to-severe depression, the use of a SSRI as the first-line choice is appropriate. If an SSRI is not appropriate then alternative agents include lofepramine. As long as there are no contraindications, then previous response and tolerance to a particular drug or patient preference should also be considered. The quantities of medication supplied to these patients should be carefully monitored. [13]

1.3 OTHER TREATMENTS

1.3.1 Electroconvulsive therapy

Electroconvulsive therapy (ECT) is a procedure whereby pulses of electricity are sent through the brain via two electrodes, usually one on each temple, to induce a seizure while the patient is under a short general Anesthetic. Hospital psychiatrists may recommend ECT for cases of severe major depression which have not responded to antidepressant medication or, less often, psychotherapy or supportive interventions [14].

Electroconvulsive therapy (ECT) would only be considered after referral to a psychiatrist. Although it is said to have a faster onset of action, its effects are fairly short-lived and antidepressants are normally required to prevent relapse. Although the treatment itself is considered safe, there are risks from the anaesthetic agent, and some patients suffer short-term memory loss following treatment.
1.3.2 Non Drug Treatment

In addition to drug treatment, it is important to consider the patient’s wider social, cultural and environmental circumstances. Although for some people the main element of treatment will be pharmacological, most patients will need help and support to cope with depression. Specific non-pharmacological interventions such as cognitive behavior therapy (CBT) can be as effective as drug treatments. For some people a combination of antidepressants and CBT is required. Other forms of psychotherapy are also available from specialist services. For mild forms of depression non-drug strategies are generally considered as the first line treatment.

1.3.3 St John’s wort (Hypericum perforatum)

Extracts of hypericum are effective in the short-term management of mild or moderately severe depression. It contains many active substances, one of which, hyperforin, is a monoamine reuptake inhibitor, mild MAO inhibitor and a stimulant at GABA receptors. Hypericum may induce the metabolism of other drugs, which may lead to toxicity on discontinuation. There is currently insufficient evidence to support the use of this product in severe depression [15].

1.3.4 Psychological treatments

There are a wide range of psychological treatments for depression. The main ones include: Cognitive Behavior Therapy (CBT)

1.3.5 Mindfulness Meditation

Mindfulness is about being aware of what is happening in the present on a moment by moment basis, while not making judgments about whether we like or don’t like what we find. It is used to help people deal with some of the symptoms of depression including worry.

1.3.6 Interpersonal Therapy (IPT)

The causes of depression or our vulnerabilities to developing depression can often be traced to aspects of social functioning (work, relationships, and social roles) and personality. The goal of IPT is to help people understand how their vulnerabilities can lead to current depression or the risk of developing depression in the future.

1.3.7 Psychotherapy

Psychotherapy usually extends over several months or years during which a relationship is built up between a therapist and their patient. This relationship is then used to explore how events in a person’s past have led to their current depression.

1.3.8 Counseling

Counseling encompasses a broad set of approaches and goals that are essentially aimed at helping an individual to solve long standing problems within their family situation, at work or to resolve sudden major problems (crisis counseling).

1.3.9 Self-help and alternative therapies

There are also a wide range of self-help measures and alternate therapies which can be useful for some types of depression, either alone or in conjunction with physical treatments (such as antidepressants) or psychological treatments. The more biological types of depression (melancholic and psychotic depression) are very unlikely to respond to self-help and alternative therapies alone. However, these therapies can be valuable
adjuncts to physical treatments.

Self-help and alternative therapies that may be useful for depression are:

1. Meditation
2. Relaxation and meditation techniques
3. Good nutrition
4. Alcohol and drug avoidance
5. Exercise
6. Light therapy
7. Yoga
8. Acupuncture

1.4 Tricyclic Antidepressants:

Chemical structure of the prototypical and first marketed tricyclic antidepressant imipramine.

Tricyclic antidepressants (TCAs) are chemical compounds used primarily as antidepressants. TCAs were first discovered in the early 1950s and marketed later in the decade. They are named after their chemical structure, which contains three rings of atoms. Tetracyclic antidepressants (TeCAs), which contain four rings of atoms, are a closely related group of antidepressant compounds.

Although TCAs are sometimes prescribed, they have been largely replaced in clinical use in most parts of the world by newer antidepressants such as selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs) and norepinephrine reuptake inhibitors (NRIs). Adverse effects have been found to be of a similar level between TCAs and SSRIs.

The first TCA reported for the treatment of depression was imipramine, a dibenzazepine analogue of chlorpromazine code-named G22355. It was not originally targeted for the treatment of depression. The drug's tendency to induce manic effects was "later described as 'in some patients, quite disastrous'". The paradoxical observation of a sedative inducing mania led to testing with depressed patients. The first trial of imipramine took place in 1955 and the first report of antidepressant effects was published by Swiss psychiatrist Roland Kuhn in 1957. Some testing of Geigy’s imipramine, then known as Tofranil, took place at the Münsterlingen Hospital near Konstanz. Geigy later became Ciba-Geigy and eventually Novartis.
The majority of the TCAs act primarily as serotonin-norepinephrine reuptake inhibitors (SNRIs) by blocking the serotonin transporter (SERT) and the norepinephrine transporter (NET), respectively, which results in an elevation of the synaptic concentrations of these neurotransmitters, and therefore an enhancement of neurotransmission.[20][21] Notably, with the sole exception of amineptine, the TCAs have negligible affinity for the dopamine transporter (DAT), and therefore have no efficacy as dopamine reuptake inhibitors (DRIs).[20] Both serotonin and norepinephrine have been highly implicated in depression and anxiety, and it has been shown that facilitation of their activity has beneficial effects on these mental disorders.[21]

**Side effects**

Many side effects may be related to the antimuscarinic properties of the TCAs. Such side effects are relatively common and may include dry mouth, dry nose, blurry vision, lowered gastrointestinal motility or constipation, urinary retention, cognitive and/or memory impairment, and increased body temperature. Other side effects may include drowsiness, anxiety, emotional blunting (apathy/anhedonia), confusion, restlessness, dizziness, akathisia, hypersensitivity.

**Interactions**

The TCAs are highly metabolised by the cytochrome P450 hepatic enzymes. Drugs that inhibit cytochrome P450 (for example, cimetidine, methylphenidate, fluoxetine, antipsychotics, and calcium channel blockers) may produce decreases in the TCAs’ metabolism, leading to increases in their blood concentrations and accompanying toxicity.[22] Drugs that prolong the QT interval including antiarrhythmics such as quinidine, the antihistamines astemizole and terfenadine, and some antipsychotics may increase the chance of ventricular dysrhythmias. TCAs may enhance the response to alcohol and the effects of barbiturates and other CNS depressants. Side effects may also be enhanced by other drugs that have antimuscarinic properties.

**Overdose**

TCA overdose is a significant cause of fatal drug poisoning. The severe morbidity and mortality associated with these drugs is well documented due to their cardiovascular and neurological toxicity. Additionally, it is a serious problem in the pediatric population due to their inherent toxicity[23] and the availability of these in the home when prescribed for bed wetting and depression. In the event of a known or suspected overdose medical assistance should be sought immediately.

**List of TCAs**

Those that preferentially inhibit the reuptake of serotonin (by at least 10 fold over norepinephrine) include:

- **Clomipramine** (Anafranil) (~200× selective for serotonin over norepinephrine reuptake)
- **Imipramine** (Tofranil, Janimine, Praminil)
Those that preferentially inhibit the reuptake of norepinephrine (by at least 10 fold over serotonin) include:

- Desipramine (Norpramin, Pertofrane)
- Dibenzepin‡ (Noveril, Victoril)
- Lofepramine§ (Lomont, Gamanil)
- Nortriptyline (Pamelor, Aventyl, Norpress)

2.0. AIM AND OBJECTIVES

Various substituted phenothiazine heterocyclic ring containing derivatives are known but its practical applications have been known very little. The Literature review suggests the synthesis of substituted phenothiazine derivatives and their remarkable antidepressant, anticonvulsant, antipsychotics and antitumor activities.

Medicinal chemist utilities of substituted phenothiazine derivatives to promote the synthesis of new potential phenothiazine derivatives and evaluate its possible pharmacological activities like antidepressant activity,

1. To carry out the literature survey and review on substituted phenothiazine to establish the method of synthesis for the proposed compounds.
2. To synthesize the title compounds by appropriate methods.
3. To carry out the preliminary tests such as physical constant determination, solubility, TLC and chemical tests.
4. To confirm the structures of the synthesized compounds by IR, ¹HNMR.
5. To evaluate the synthesized compounds for their antidepressant activities.
6. To evaluate the synthesized compounds for their antidepressant activities.

3.0 LITERATURE REVIEW OF PHENOTHIAZINE

The extensive survey of literature has revealed that the Phenothiazine derivatives posses wide range of biological activities including antibacterial, antimicrobial, anti-inflammatory, analgesic, antitubercular, anticonvulsant, antidepressant activities.

1) Upendra Kumar Kushwaha et al., synthesized a series of 2-chloro-10-[2-{3'-chloro-2'-oxo-4'-(substitutedphenyl)-1’-azetidinyl]thiazol—4-yl]-phenothiazine 2-chloro-10-[2-{3’-chloro-2’-oxo-4’-(substitutedphenyl)-1azetidinyl]oxazol—4-yl] phenothiazine and evaluated for Anti-inflammatory and Analgesic activity.\textsuperscript{[51]}

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2) A Rajasekaran et al., synthesized Schiff bases and Azetidiones derivatives which are screened for Anti-inflammatory activity, Anti-tubercular, and Antimicrobial activity.\textsuperscript{[52]}

3) S. K. Srivastava et al., synthesized a new series of 2-arylidenylamino 5-{[- N10-2-chlorophenothiazin} 1, 3, 4-thiadiazoles and 1-(5’-{-N10-2 chlorophenothiazin} 1’, 3’, 4’-thiadiazol-2-yl)-4-substitute-3-chloro-2-azetidiones and screened for Antimicrobial and Anti-inflammatory activity.\textsuperscript{[53]}

4) Aaron B. Bate et al., synthesized quaternized chlorpromazine, triflupromazine and Promethazine derivatives and examined for Anti-tubercular agents against actively growing and non-replicating mycobacterium tuberculosis H37RV.\textsuperscript{[54]}

5) Peter B. Madrid et al., synthesized a series of substituted Phenothiazine and screened for Anti-tubercular activity against mycobacterium tuberculosis H37RV.\textsuperscript{[55]}
6) Amit R. Trivedi et al., synthesized some novel 3-methyl-1-(10H-phenothiazine-2-yl)-4-phenyl-6-hydroxy-4, 5-dihydro-1H-pyrozolo[3,4- d]pyrimidines and screened for mycobacterium tuberculosis H37RV.\(^{[56]}\)

7) Mia L. Laws et al., synthesized novel series of 3-carboalkoxy-2-methyl-2,3- dihydro-1H-phenothiazine-4[10H]-one derivative and studied for Anticonvulsant activity.\(^{[57]}\)

8) R.K. Upadhyay et al., synthesized chalcones derivatives of 1-[2(10-p-chlorobenzyl)phenothiazine]-3-(substituted aryl)-2-propen-1-ones and studied for Antimicrobial activity by filter paper disc and diffusion method against, Enterococcus faecium, Enterococcus faecalis, Escherichia Coli, and Bacillus subtilis fungicidal study by using Candida albicans and Aspergillus niger.\(^{[58]}\)

9) Turan Zitouni G et al., synthesize some 10-[2-(4-Amino-5-mercapto-1,2,4- triazol-3-yl)ethyl]phenothiazine and screened for Antidepressent and Anxiolytic activity.\(^{[59]}\)

10) Jignesh P. Raval et al., synthesized a novel series of substituted cynopyridinyl phenothiazine ,
acetamido-cynopyridinyl phenothiazine, and 8-cyno-triazo-pyridinyl phenothiazine and evaluated for Antimicrobial activity by using Bacterial strains such as staphylococcus aureas, pseudomonas auregenosa, shigella flexneri and for fungal study cerevesae vitae, Candida albicans and Aspergillus niger.\cite{60}
11) A R Bhat *et al.*, synthesized some new 10-[α-4-substituted benzamide- aminobenzyl] phenothiazine and screened for Antimicrobial activity by using *Staphylococcus aureus*, *Escherichia Coli* similarly with *Candida albicans* and *Aspergillus niger*.\[^{61}\]

![Structure 1](image1)

12) Ashok K. Yadav *et al.*, synthesized some new series of N-(2',3',5'-tri-o-benzoyl-β-D-ribofuranosyl)-6-(4-chloro-2-methoxy-5-methylanilino/2-methyl aniline)-9-chloro-5-acetoxy/5-methoxy-12H-benzo[β]phenothiazines and evaluated for gram negative bacteria like *Escherichia Coli* gram positive bacteria like *Staphylococcus aureus* and *Aspergillus niger*, *Aspergillus flavas* and fusarium oxysporium.\[^{62}\]

![Structure 2](image2)

13) S D Srivastava *et al.*, synthesized a series of 2-benzylidenylamino-5- (10N-phenothiazinomethyl)-1,3,4-thiadiazole and 1-[5-(10N-phenothiazimiethyl)- 1',3', 4'–thiadazole-2'-yl]-4-phenyl-2-azetidinone and screened for Antifungal activity against *C.pannical*, *C.albicans* and *r.oryzace*.\[^{63}\]

![Structure 3](image3)
14) Noboru Motohashi et al., synthesized a series of N-Acyl phenothiazine and screened for Anti-HIV.\[64\]

\[
\begin{array}{c}
\text{(H}_3\text{C)}_3 \\
\begin{array}{c}
\text{O} \\
\text{N}
\end{array}
\end{array}
\]

15) Kiran Bajaj et al., synthesized a new series of N-[2-aryl-3-arylaminoethylene-2,3- dihydro-1,5-benzothiazepin-4yl]phenothiazine and N-[2-aryl-3-arylaminoethylene-2,3-dihydro-1,5-benzoazepin-4yl]phenothiazine which is evaluated for psychotropic activity.\[65\]

\[
\begin{array}{c}
R= \text{H, 4} \cdot \text{OCH}_3 \\
R_1 = \text{H, 2} \cdot \text{Cl, 3} \cdot \text{Cl}
\end{array}
\]

16) Neelam Jaiswal et al., synthesized some new 10-[(3,5-diaryl-2-pyrazolin-1-yl)acetyl]phenothiazine and screened for in vitro activity of rat brain pyruvate oxidase and Anticonvulsant Activity.\[66\]
17) A Kumar et al., synthesized a novel series of Schiff’s bases and 3-chloro-2-oxo-1-[(2-oxo-2(10H-phenothiazine-10-yl)ethyl]amino]-4substituted phenyl-2,3-dihydroazetium and studied for Cardiovascular Activity.\[67\]

18) V K. Pandey et al., synthesized some new 3-arylamino/imido alkyl-10-[(4-methoxyphenyl/phenyl) phenyl amino methyl]-10H-phenothiazine-2ols and screened for their Antiviral Activity against two animal viruses viz Japanese encephalitis virus (JEV) P20778 and Herpes simplex virus type-I (HSV-I) 753166.\[68\]

19) Abha Bishnoi et al., synthesized a series of some new 10-(α-p-benzimidazolyl-aminobenzyl)phenothiazine and all the compound are evaluated for Antiviral
activity against JEV and HSV – I \textit{in-vitro} and Antifungal activity against fusarium solani.\cite{69}

20) Tanaji N. Bansode \textit{et al.}, synthesized a new N-acyl derivatives of phenothiazine by two different method and screened for Antibacterial activity by using Bacillus subtilis, Escherichia Coli, Staphylococcus aureas and Pseudomonas aeruginosa fungal studies by Aspergillus niger and Aspergillus fumigates.\cite{70}

21) Sharad Kumar \textit{et al.}, synthesized a substituted (4-((10H-phenothiazine-10yl)-(phenyl))-methylamino-(phenyl)-1H-benzo[d]imidazol-1-yl)-methylamino and screened for Antimicrobial activity against Escherichia Coli, Bacillus subtilis, and Staphylococcus aureas.\cite{71}
22) G.S. KALWANIA. Et al. synthesized The 5, 5-Dioxide Derivatives of Phenothiazines. They synthesized title compounds have screened for their antimicrobial activity.

4.0. THEORETICAL DISCUSSION

Phenothiazine:

10H-phenothiazine Heterocyclic compounds plays important role in medicinal chemistry, nitrogen containing heterocyclic with a sulfur atom are considered as an important class of compounds in medicinal chemistry because of their interesting diversified biological application.\textsuperscript{1-5} 10H-Phenothiazine (PTZ) is a tricyclic aromatic compound linked via bridges of sulfur and nitrogen. In this ring system it consists of two benzene rings Ortho fused to 1, 4 – thiazine ring. It is also called as Dibenzothiazine, Thiodiphenylamine.\textsuperscript{6-8} Phenothiazine derivatives are very useful precursors for the development of molecules of biological interest.\textsuperscript{9-11}

Phenothiazine derivatives are considered to be important chemical synths of various physiological significances and pharmaceutical utilities. They possess a variety of biological activity including anti-implamatory, antitubercular, anticonvulscent, antimicrobial and antidepressant activities.

Methods of Synthesis:

1. Sinha Shweta et al in the year 2011 had been reported the synthesis of N-
Chapter 4

Theoretical Discussion

Coroacetyl)Phenothiazine by solution of 10H- Phenothiazine in dry benzene, chloracetyl chloride reflux for 50-60°C.\[^72\]

\[
\begin{align*}
\text{H} & \quad \text{ClCH}_2\text{COCl} \\
\text{S} & \quad \text{C}_6\text{H}_6 \text{reflux}
\end{align*}
\]

Fig: 1 (synthesis of N-2 - coroacetyl)phenothiazine by solution of 10H-phenothiazine in dry benzene, chloracetyl chloride reflux for 50 - 60°C)

2. Nagaraj Naik et.al. Novel series of phenothiazine derivatives obtained by the phenothiazine-aryl amines conjugates via acetyl group.\[^73\]

\[
\begin{align*}
\text{NH}_2 & \quad \text{HS} \quad \text{CuI, proline K}_2\text{Co}_3 \\
\text{Br} & \quad \text{2 methoxy ethanol}
\end{align*}
\]

\[
\begin{align*}
\text{S} & \quad \text{N} \\
\text{R}^5 & \quad \text{R}^4
\end{align*}
\]

\[
\begin{align*}
\text{THF RT 6hr} & \quad \text{Cl} \quad \text{NH}_2 \\
\text{THF reflux 6hr} & \quad \text{Cl}
\end{align*}
\]
3. Majumder Alok et. Phenothiazine and its 3-methyl, 3-chloro, and 5-nitro derivatives have been prepared by a new method which consists of aniline, P-chloro aniline and P-nitro aniline respectively with cyclohexanone.(from schiff’s base)[74]

\[ X = H, \text{CH}_3, \text{Cl, NO}_2 \]

Fig.3: synthesis of phenothiazine dvt aniline, p-chloroaniline, p-nitro aniline, respectively with cyclohexanol

4. Luiza Gaina et.al. microwave-assisted synthesis of Phenothiazine derivatives.[75]

Fig.4: synthesis of phenothiazine dvt by microwave assisted method

\[ R' \]
5. Souad J. Lafta. Synthesis of new heterocyclic derivatives of phenothiazine.\cite{76}

![Chemical structure](image1)

Fig. 5: synthesis of new heterocyclic dvt phenothiazine

6. Radu Gropeanu. Synthesis of Iodine phenothiazines.\cite{77}

![Chemical structure](image2)

Fig. 6: synthesis of Iodine phenothiazine
7. Pawan Kumar Swarnkar et al. a series of new phenothiazine dvt were synthesized with the objective for evaluation as antimicrobials.\[78\]

Fig. 7: synthesis of phenothiazine dvt by 2-amino, 6-substituted benzothiazole
8. Ritu Sharma et al. synthesis of $\alpha$-azetidone dvt of phenothiazine.\cite{79}

\[ \text{BrCH}_2\text{CH}_2\text{SCH}_2 \rightarrow \text{N} \]

\[ \text{H} \]

\[ \text{ArCHO} \]

\[ \text{CH}_2\text{ArNHCON} \]

\[ \text{Ar} = \text{substituted phenyl ring} \]

Fig. 8: synthesis of $\alpha$-azetidone dvt of phenothiazine

9. Seyed Ahmad Ali Golriz, synthesis of phenothiazine based monomers & polymers.\cite{80}

\[ \text{O} \]

\[ \text{O} \]

\[ \text{HN} \]

\[ \text{S} \]

\[ \text{Cl} \]

base, Cu catalyst

Fig. 9: synthesis of phenothiazine based monomers and polymers (pahenothiazine molecule attached to the N-position)
10. Prof Univ, Dr. Lonel Mongalagiu, Synthesis & optical properties of some new azaheterocyclic dyes.\textsuperscript{[81]}

\[
\begin{align*}
\text{CH}_3\text{N} & \quad \text{S} \quad \text{CHO} \\
& \quad \text{N} \quad \text{S} \quad \text{Z}_3 \quad \text{CH}_3\text{N} \\
& \quad \text{Z}_1 \quad \text{Z}_2 \quad \text{Z}_3 \quad \text{Z}_4
\end{align*}
\]

Fig. 10: synthesis of some new azaheterocyclic dyes

\[\hat{R} = \text{me, Et} \]
\[\text{Z}_1 = \text{me, HZ}_2 = \text{me, HZ}_3 = \text{me, H} \]

11. Alan R. Katritzky et.al specific synthesis of 1- substituted phenothiazine using carbon dioxide protection of the NH grp during lithiation.\textsuperscript{[82]}

\[
\begin{align*}
\text{S} & \quad \text{n-C}_4\text{H}_9\text{Li, THF, CO}_2 \quad \text{NH} \\
& \quad \text{S} \quad \text{t-C}_4\text{H}_9\text{Li} \quad \text{NH} \\
& \quad \text{S} \quad \text{CO}_2\text{Li} \quad \text{NH} \\
& \quad \text{S} \quad \text{CO}_2\text{Li} \quad \text{Li}
\end{align*}
\]

Fig. 11: synthesis of 1 -substituted phenothiazine using CO\textsubscript{2} protection of the NH grp during lithiation
12. I. C. Shukla, et.al synthesis of phenothiazine from 2-amino-2-nitrodiphenyl sulphides.\textsuperscript{[83]}

\[ \begin{align*}
R_1 & \quad \text{CHONH} \\
R_2 & \\
R_3 & \\
R_4 &
\end{align*} \]

formylation 90% HCOOH

\[ \begin{align*}
R_1 &= \text{Cl} \\
R &= \text{CF}_3 \\
R &= \text{H,F} \\
R &= \text{H,Cl}
\end{align*} \]

Fig.12: synthesis of 2-amino-2-nitrodiphenyl sulphides
5.0. EXPERIMENTAL

Materials and Methods:

The identification and characterization of the prepared compounds were carried out by the following procedure to ascertain that all prepared compounds were of different chemical nature than the respective parent compound.

- Physical constants.
- Thin Layer Chromatography (TLC)
- FT-Infrared Spectroscopy (FT-IR)
- Nuclear Magnetic Resonance Spectroscopy (\( ^1H \)-NMR)
- Elemental Analysis (C,H,N)

Melting Point Determination:

The melting points of the organic compounds were determined by open capillary in a heavy liquid paraffin bath. Melting point is a valuable criterion of purity for an organic compound, as a pure crystal is having definite and sharp melting point. The purity should not be assumed but must be established by observation of any changes in the melting point when the compound is subjected to purification by recrystallization.

Thin Layer Chromatography:

Chromatography is an important technique to identify the formulation of new compounds and also to determine the purity of the compounds. The Rf value is the characteristic for each compound.

Fourier Transformer-Infrared Spectroscopy (FT-IR):

FTIR can be routinely used to identify the functional groups and for quality control of raw materials/finished products. Beckmann MicrolabIR 600 Spectrophotometer offers fast throughput and rapid access to reliable and dependable IR results. High signal to noise ratio makes FT-IR more useful for difficult samples. It has resolution of 1 cm\(^{-1}\) and scans range of 4000cm\(^{-1}\) to 250 cm\(^{-1}\).IR spectra were recorded on spectrophotometer using KBr disc method.

Nuclear Magnetic Resonance Spectroscopy (\( ^1H \)-NMR):

\(^1H\)NMR spectra were recorded on sophisticated multinuclear NMR Spectrometer model Avance- II (Bruker), DMSO-d6 as internal standards. The instrument is equipped with a Gyro magnet of field strength 9.4 T. Its \(^1H\) frequency is 400 MHz.
SCHEME.

\[ \text{Cl} + \text{H}_2\text{N} - \text{NH} - \text{R} \rightarrow \text{DMF} \xrightarrow{\text{KOH}} \text{O} - \text{COOH} \]

\[ \text{S/I}_2 \rightarrow \text{HN} - \text{O} - \text{COOH} \]

\[ \text{S} \rightarrow \text{H}_2\text{SO}_4 \xrightarrow{\text{C}_2\text{H}_5\text{OH}} \]

\[ \text{O} \xrightarrow{\text{NH}_2\text{NH}_2} \text{S} \]

\[ \text{Ar CHO} \rightarrow \text{N} - \text{O} - \text{NH} - \text{CH} - \text{Ar} \]

\[ \text{S} \]
5.1 Procedure for Scheme

**Step 1**] General procedure for the preparation of 7, 8 or 9 substituted aniline Benzoic acid derivatives:
Equimolar amount of substituted aniline was added to a chloro benzoic acid in 20 mL of DMF and 0.1 percent of potassium hydroxide solution and the reaction mixture was heated under refluxed at about 80°C temperature, for 2 h. TLC indicated the end of reaction. The mixture was cooled by addition of a water/ice mixture. The solid was filtered in excellent yield.

**Step 2**] General procedure for the preparation of 7, 8 or 9 substituted 10H- phenothiazine 1 carboxylic acid derivatives:
Equimolar amount of 7, 8 or 9 substituted Anilino Benzoic acid was added to a solution of sulfur powder and iodine in 5 mL of ethanol. Reaction mixture was heated under reflux with stirring for about 2 h and poured into ice/water mixture. The precipitate was filtered and washed with cold water.

**Step 3**] Synthesis of derivatives of ethyl 10H-phenothiazine-1-carboxylate: 0.01 mole of 10H-phenothiazine-1-carboxylic acid was reflux with conc. H$_2$SO$_4$ using ethanol as solvent for 1 hour in 250ml RBF. After which the resulting reaction mixture was kept in ice cold water.

**Step 4**] Synthesis of derivatives of 10H-phenothiazine-1-carbohydrazide:
0.01 mole of compound A was reflux with 3-4 ml of hydrazine hydrate for 1 hour. After which the resulting reaction mixture was allowed to cool in ice bath. The resulting precipitate was collected and dried.
Step 5] Synthesis of derivatives of N’-benzylidene-10H-phenothiazine-1- carbohydrazide:
0.01 mole of compound B was reflux with 0.01 mole of substituted aromatic aldehyde for 1 hour. After which the resulting reaction mixture was allowed to cool in ice bath. The resulting precipitate was collected and dried and recrystallized from ethanol.

**FOR COMPOUNDS A₁-A₁₂**

<table>
<thead>
<tr>
<th>Comp. Code</th>
<th>Ar</th>
<th>R</th>
<th>Comp. Code</th>
<th>Ar</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td></td>
<td>NO₂</td>
<td>A₇</td>
<td></td>
<td>Cl</td>
</tr>
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<tr>
<td>A₃</td>
<td></td>
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<td>A₉</td>
<td></td>
<td>Cl</td>
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<td>Cl</td>
<td>Cl</td>
<td>A₁₀</td>
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<td>NO₂</td>
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<td>NO₂</td>
<td>A₁₁</td>
<td></td>
<td>NO₂</td>
</tr>
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</table>
Table no. 01: Analytical & Physicochemical data of the synthesized compounds (A₁-A₁₂)

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Mol. Formula</th>
<th>Mol. Wt.</th>
<th>M. P. °C</th>
<th>Yield %</th>
<th>Elemental analyses</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Calculated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>A₁</td>
<td>C₂₀H₁₄N₄O₃S</td>
<td>390.14</td>
<td>210-215</td>
<td>70</td>
<td>61.53</td>
</tr>
<tr>
<td>A₂</td>
<td>C₂₁H₁₆CIN₃O₂S</td>
<td>409.88</td>
<td>188-195</td>
<td>68</td>
<td>61.53</td>
</tr>
<tr>
<td>A₃</td>
<td>C₂₀H₁₄ClN₃O₂S</td>
<td>395.86</td>
<td>235-240</td>
<td>72</td>
<td>60.68</td>
</tr>
<tr>
<td>A₄</td>
<td>C₂₂H₁₆ClN₃OS</td>
<td>405.89</td>
<td>218-225</td>
<td>62</td>
<td>65.10</td>
</tr>
<tr>
<td>A₅</td>
<td>C₂₀H₁₃ClN₄O₃S</td>
<td>425.96</td>
<td>220-230</td>
<td>70</td>
<td>56.54</td>
</tr>
<tr>
<td>A₆</td>
<td>C₂₀H₁₃ClN₄O₃S</td>
<td>425.86</td>
<td>236-240</td>
<td>62</td>
<td>54.03</td>
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<tr>
<td>A₇</td>
<td>C₂₀H₁₂Cl₂N₄O₄S</td>
<td>475.30</td>
<td>212-215</td>
<td>71</td>
<td>50.54</td>
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<tr>
<td>A₈</td>
<td>C₂₀H₁₃BrN₄O₃S</td>
<td>470.31</td>
<td>216-223</td>
<td>70</td>
<td>51.18</td>
</tr>
<tr>
<td>A₉</td>
<td>C₂₁H₁₅Cl₂N₃OS</td>
<td>428.3</td>
<td>222-227</td>
<td>73</td>
<td>58.89</td>
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<tr>
<td>A₁₀</td>
<td>C₂₀H₁₂N₆O₇S</td>
<td>480.41</td>
<td>240-245</td>
<td>75</td>
<td>50.00</td>
</tr>
<tr>
<td>A₁₁</td>
<td>C₂₀H₁₃N₅O₆S</td>
<td>451.41</td>
<td>238-245</td>
<td>77</td>
<td>53.21</td>
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<tr>
<td>A₁₂</td>
<td>C₂₂H₁₉N₅O₃S</td>
<td>433.48</td>
<td>219-235</td>
<td>72</td>
<td>60.96</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Comp. Name</td>
<td>Structure</td>
<td>IUPAC names</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
<td>-----------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td><img src="structure1.png" alt="Structure Image" /></td>
<td><em>(E)</em>-N'-benzylidene-7-nitro-10H-phenothiazine-1-carbohydrazide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td><img src="structure2.png" alt="Structure Image" /></td>
<td><em>(E)</em>-7-chloro-N'-((4-methoxybenzylidene)-10H-phenothiazine-1-carbohydrazide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>A&lt;sub&gt;3&lt;/sub&gt;</td>
<td><img src="structure3.png" alt="Structure Image" /></td>
<td><em>(E)</em>-8-chloro-N'-((2-hydroxybenzylidene)-10H-phenothiazine-1-carbohydrazide</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5. **A₅**

\[(E)-N'-(2\text{-chlorobenzylidene})-8\text{-nitro}-10H\text{-phenothiazine}-1\text{-carbohydrazide}\]

6. **A₆**

\[(E)-N'-(3\text{-chlorobenzylidene})-8\text{-nitro}-10H\text{-phenothiazine}-1\text{-carbohydrazide}\]

7. **A₇**

\[(Z)-7,9\text{-dichloro-}N'-(2\text{-hydroxy}-5\text{-nitrobenzylidene})-10H\text{-phenothiazine}-1\text{-carbohydrazide}\]

8. **A₈**

\[(Z)-7\text{-bromo-}N'-(2\text{-nitrobenzylidene})-10H\text{-phenothiazine}-1\text{-carbohydrazide}\]
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>9.</td>
<td>$A_9$</td>
<td>(Z)-6,9-dichloro-$N'$-(3-methylbenzylidene)-10$H$-phenothiazine-1-carbohydrazide</td>
</tr>
<tr>
<td>10.</td>
<td>$A_{10}$</td>
<td>(Z)-7,9-dinitro-$N'$-(3-nitrobenzylidene)-10$H$-phenothiazine-1-carbohydrazide</td>
</tr>
<tr>
<td>11.</td>
<td>$A_{11}$</td>
<td>(Z)-$N'$-(4-hydroxybenzylidene)-7,9-dinitro-10$H$-phenothiazine-1-carbohydrazide</td>
</tr>
<tr>
<td>12.</td>
<td>$A_{12}$</td>
<td>(Z)-$N'$-(4-(dimethylamino)benzylidene)-8-nitro-10$H$-phenothiazine-1-carbohydrazide</td>
</tr>
</tbody>
</table>
6.0. SPECTRAL DATA

❖ Infrared Spectra:

The peaks in IR spectrum gave an idea about the probable structure of the compound. IR region ranges between 4000-400 cm\(^{-1}\). The derivatives including intermediates were recorded on Beckmann (JASCO) FTIR-spectrophotometer spectrometer, which showed different vibration levels of molecules by using potassium bromide (KBr) pellet technique.

❖ \(^1\)H-NMR Spectra:

NMR Spectroscopy enables us to record differences in magnetic properties of the various magnetic nuclei present and to deduce in the large measure about the position of these nuclei within the molecule. We can deduce how many different kinds of environment are there in the molecules and also which atoms are present in neighboring groups. The proton NMR spectra, enables us to know different chemical and magnetic environments corresponding to protons in molecules.

\(^1\)H-NMR of the title compounds were recorded on sophisticated multinuclear BRUKER AVANCE II 400 NMR, DMSO-d6 as internal standards. The instrument is equipped with a Gyro magnet of field strength 9.4T. Its \(^1\)H frequency is 400 MHz the chemical shift data were expressed as δ-values related to TMS.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Comp. Name</th>
<th>Structure</th>
<th>FTIR (KBr, cm(^{-1}))</th>
<th>(^1)H NMR (CDCl(_3), ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>A(_2)</td>
<td></td>
<td>3050.23 (ArCHstr.), 1615.11 (C=Ostr.), 1520.32 (-C=N str.), 1240.36 (-C-N str.), 3240.23 (-N-H str.), 1330.32 (-C-O str.)</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>A(_3)</td>
<td></td>
<td>850.22 (-C-Cl str.), 3015.23 (Ar-CH str.), 1685.11 (-C=O str.), 1510.32 (-C=N str.), 1250.36 (-C-N str.)</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>A₄</td>
<td><img src="image" alt="Structure A₄" /></td>
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<tr>
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<td>-------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3100.25 (Ar-CH str.), 830.23 (-C-Cl str.), 1635.12 (-C=O str.), 1490.2 (-C=N str.), 1270.16 (-C-N str.), 3270.23 (-N-H str.), 1340.33 (-C-O str.).</td>
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<td></td>
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</table>

<table>
<thead>
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<th>5.</th>
<th>A₅</th>
<th><img src="image" alt="Structure A₅" /></th>
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<td></td>
<td>1255.36 (-N-O str.), 3110.23 (Ar-CH str.), 1615.11 (-C=O str.), 1510.32 (-C=N str.), 1260.36 (-C-N str.), 3250.23 (-N-H str.), 1370 (-N-O str.), 1320.32 (-C-O str.).</td>
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</table>

<table>
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<th>6.</th>
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<th><img src="image" alt="Structure A₆" /></th>
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</thead>
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<tr>
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<td></td>
<td>3050.23 (Ar-CH str.), 1605.11 (-C=O str.), 1520.32 (-C=N str.), 1240.36 (-C-N str.), 3250.23 (-N-H str.), 1320.32 (-C-O str.), 945.20 (-C-Cl str.).</td>
</tr>
</tbody>
</table>

<table>
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</thead>
<tbody>
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<td></td>
<td></td>
<td>3650.21 (-OH str.), 3010.23 (Ar-CH str.), 1615.11 (-C=O str.), 1500.32 (-C=N str.), 1250.36 (-C-N str.), 3250.23 (-N-H str.), 1320.32 (-C-O str.).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.26 aromatic C-OH, 9.88 sec amine, 7.41 phenothiazine, 7.77 benzylidenimin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>8.</th>
<th>A₈</th>
<th><img src="image" alt="Structure A₈" /></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3020.23 (Ar-CH str.), 3600.23 (-OH str.), 1715.11 (-C=O str.), 1510.32 (-C=N str.), 1250.36 (-C-N str.), 3270.23 (-N-H str.), 1320.32 (-C-O str.).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NH 9.88 sec amine, 7.08 phenothiazine, 7.98 benzylidenimin 7.16 1-benzene</td>
</tr>
</tbody>
</table>
9. $A_9$

3010.23 (Ar-CH str.), 1680.11 (-C=O str.), 1540.32 (-C=N str.), 1260.36 (-C-N str.), 1340 (-N-O str.), 3250.23 (-N-H str.), 1320.32 (-C-O str.).

10. $A_{10}$

3010.23 (Ar-CH str.), 1685.11 (-C=O str.), 1510.32 (-C=N str.), 1320 (-N-O str.), 1250.36 (-C-N str.), 3250.23 (-N-H str.), 1320.32 (-C-O str.).

NH 9.88 sec. Amine, 7.41 phenothiazine, 8.52 benzyldenimine, 8.85 – N=O from Benzene;

11. $A_{11}$

3100.28 (Ar-CH str.), 830.24 (-C-Cl str.), 1635.12 (-C=O str.), 1590.2 (-C=N str.), 1270.16 (-C-N str.), 3270.25 (-N-H str.), 1340.35 (-C-O str.).
7.0. ANTIDEPRESSANT ACTIVITY

ANTIDEPRESSANT ACTIVITY [84,85,86,87]

Behavioural despair was proposed as a model to test antidepressant activity by Porsolt et al. (1977, 1978). It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression.

PROCEDURE
Behavioral despair or forced swim test (FST) was proposed as a model to test antidepressant activity by Porsolt et al. (1977) It was suggested that mice or rats when forced to swim in restricted space from where they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. The behavioral despair test is employed to assess the antidepressant activity of synthesized derivatives. Sprague-Dawley rats of 200-270 gm in a group of two each were used and on the first day of the experiment (pretest session), rats were individually placed in a cylindrical recipient (Plexiglass cylinder) of dimensions (diameter, 10 cm; height, 25 cm) containing 10 cm of water 25°C. The animals were left to swim for 6 min before being removed, dried and returned to their cages. The procedure was repeated 24 h later, in 5 min swim session (test session). The synthesized compounds (25 mg kg-1), and imipramine, as a reference antidepressant drug (25 mg kg-1) were suspended in a 0.5 % aqueous solution of Na CMC (Corboxy Methyl Cellulose). The drugs were given by gavage in a standard volume of 10ml/kg body weight, 1 h prior to the test. Control animals received 0.5 % aqueous solution of Na CMC (Corboxy Methyl Cellulose). This test was performed after 1 hr, 5 hrs and 24 hrs of dose administration. For individual animal video recording was made. Then, the rats were dropped individually into the Plexiglass cylinder and left in the water for 6 min. After the first 2 min of the initial vigorous struggling, the animals were immobile. An immobility time is the time spent by rat floating in water without struggling, making only those moment necessary to keep the head above the water. The total duration of immobility was recorded during the last 5 min of the 6 min test session.

EVALUATION
Duration of immobility is measured in controls and animals treated with various doses of a test drug or standard. Antidepressant drugs, but also stimulants like amphetamine and caffeine, reduce duration of immobility. Dose-responses can be evaluated.

<table>
<thead>
<tr>
<th>Compound code.</th>
<th>Immobility time (sec.)</th>
<th>% Immobility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Hr</td>
<td>5 Hr</td>
</tr>
<tr>
<td>A₁</td>
<td>152.5</td>
<td>159</td>
</tr>
<tr>
<td>A₂</td>
<td>163.5</td>
<td>168</td>
</tr>
<tr>
<td>A₃</td>
<td>157.5</td>
<td>161</td>
</tr>
<tr>
<td>A₄</td>
<td>159</td>
<td>158.5</td>
</tr>
<tr>
<td>A₅</td>
<td>160.5</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>A₆</td>
<td>A₇</td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>154.5</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>158</td>
<td>153.5</td>
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<td>164</td>
<td>165.5</td>
</tr>
<tr>
<td></td>
<td>89.30</td>
<td>83.23</td>
</tr>
<tr>
<td></td>
<td>85.63</td>
<td>83.19</td>
</tr>
<tr>
<td></td>
<td>82.82</td>
<td>83.58</td>
</tr>
</tbody>
</table>

Fig. No 7.2: Antidepressant-like effects of N’-benzylidene-10H-phenothiazine-1- carbohydrazide derivatives test compounds in FST Data are presented as compared to control group. (Compounds code Vs Immobility time).
Fig. No 7.3: Antidepressant-like effects of $N^\prime$-benzylidene-10H-phenothiazine-1- carbohydrazide derivatives test compounds in FST Data are presented as compared to control group. (Compounds code Vs % Immobility)

![Graph showing percent immobility of compounds A1 to A12](image)

8.0. RESULT AND DISCUSSION

8.1 Antidepressant Activity

All the synthesized compounds were subjected to antidepressant activity study on Sprague-Dawley rats by despair swim test. Imipramine was used as standard control. The animals show more stable levels of immobility during the last four minutes of the session. The results showed that all the compounds showed antidepressant activity. Among them three Compounds (A7, A8 and A10) showed significant antidepressant activity comparing with standard control imipramine (Table 7.1 & Fig. 8.1)
Fig. 8.1: Antidepressant Activity (Forced Swim Test in Rat) of synthesized compounds

8.2: QSAR [94]

Intercorrelation between the descriptors in the final equations is less than 0.2. Best Equations correlating Log (% Immobility) with descriptors for series (A₁-A₁₂) generated are presented in Table no. 8.3.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Equation</th>
<th>N</th>
<th>S</th>
<th>r</th>
<th>r²</th>
<th>r²cv</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>series</td>
<td>Y = -0.199 *X₃ - 0.229 * X₁ - 1.553 * X₂ - 12.575</td>
<td>12</td>
<td>0.361</td>
<td>0.8066</td>
<td>0.6506</td>
<td>0.538</td>
<td>14.17</td>
</tr>
</tbody>
</table>

Where

Y = Log (% Immobility) X1: ClogP -

X2 = VAMP HOMO (Whole Molecule)

X3 = Dipole Moment Z Component (Whole Molecule) X4 = Inertia Moment 2 Length (Whole Molecule)

Significance of the terms –

N= No. of Molecules
s = standard error --- less is better

r = correlation coefficient – higher is better > 0.7, $r^2_{cv}$ = cross validated $r^2$ – higher is better > 0.5,

F Value = higher is better

Observed and predicted data and graphs are presented in Table no. 8.4 and Fig. 8.3. for Series

<table>
<thead>
<tr>
<th>Compound Code</th>
<th>Observed Value</th>
<th>Predicted Value</th>
<th>Residual value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1.9452</td>
<td>1.9354</td>
<td>0.0098</td>
</tr>
<tr>
<td>A2</td>
<td>1.9754</td>
<td>1.9625</td>
<td>0.0129</td>
</tr>
<tr>
<td>A3</td>
<td>1.9592</td>
<td>1.9624</td>
<td>-0.0032</td>
</tr>
<tr>
<td>A4</td>
<td>1.9633</td>
<td>1.9644</td>
<td>-0.0011</td>
</tr>
<tr>
<td>A5</td>
<td>1.9674</td>
<td>1.9587</td>
<td>0.0087</td>
</tr>
<tr>
<td>A6</td>
<td>1.9508</td>
<td>1.9432</td>
<td>0.0076</td>
</tr>
<tr>
<td>A7</td>
<td>1.9202</td>
<td>1.9189</td>
<td>0.0013</td>
</tr>
<tr>
<td>A8</td>
<td>1.9262</td>
<td>1.9543</td>
<td>-0.0281</td>
</tr>
<tr>
<td>A9</td>
<td>1.9646</td>
<td>1.9751</td>
<td>-0.0105</td>
</tr>
<tr>
<td>A10</td>
<td>1.9292</td>
<td>1.9312</td>
<td>-0.002</td>
</tr>
<tr>
<td>A11</td>
<td>1.9592</td>
<td>1.9615</td>
<td>-0.0023</td>
</tr>
<tr>
<td>A12</td>
<td>1.9365</td>
<td>1.9368</td>
<td>-0.0003</td>
</tr>
</tbody>
</table>

8.5: QSAR

Intercorrelation between the descriptors in the final equations is less than 0.2.

Fig. No. 8.6: a) Correlation graph and b) Histogram of observed and predicted log (% Immobility) data for 12 compounds

\[
y = 0.7518x + 0.4846 \\
R^2 = 0.6507
\]
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