SYNTHESIS, STRUCTURAL CHARACTERIZATION AND MOLECULAR DOCKING OF NOVEL PHENYTOIN

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MASTER OF SCIENCE IN CHEMISTRY

By

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PG AND RESEARCH

DEPARTMENT OF CHEMISTRY

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CERTIFICATE

This is to certify that the dissertation entitled **"SYNTHESIS, STRUCTURAL CHARACTERIZATION AND MOLECULAR DOCKING OF NOVEL PHENYTOIN DERIVATIVE"** submitted by Mr. **PUGAZHENDHI S (CHPS22006)**in partial fulfilment of the requirements for the degree of Master of Science in Chemistry of National College (Autonomous), is based on his studies under my guidance. It is further certified that this dissertation or any part thereof has not been submitted elsewhere for any other degree.

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CONTENT

ABBREVIATIONS

FDA	-	Food and drug Administration
GABA	-	Gamma-aminobutyric acid
DPH	-	Diphenhydramine
HONO	-	Nitrous acid
CYO	-	Cytochrome p450
OTC	-	Over the counter
ATC	-	Anatomical therapeutic chemical
CSF	-	Cerebrospinal fluid
PHT	-	Phenytoin
AEDs	-	Antiepileptic drugs
CNS	-	Central Nervous System
FDA	-	Food and Drug Administration
DPH	-	Diphenylhexatriene
HPLC	-	High Performance Liquid Chromatography
TLC	-	Thin Layer Chromatography
ATR-FTIR	-	Attenuated Total Reflection –Fourier Transform Infra-red
NMR	-	Nuclear Magnetic Resonance
DMSO	-	Dimethyl Sulfoxide

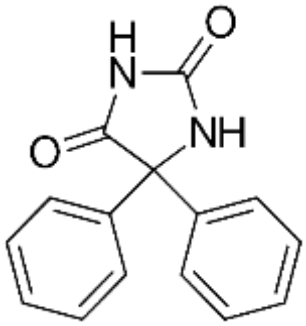
CHAPTER-I

INTRODUCTION

CHAPTER 1
INTRODUCTION

Introduction

It is used as a photosensitive agent in photo curable coatings, as a precursors for some pharmaceutically important compound such as the antiepileptic phenytoin and anticonvulsant dilantin, as an aluminium electrolytic capacitors. As a potent activator of microsomal epoxide hydrolase in vitro, as a photosensitive agent. It is unique because it produces a large number of reduction products. Benzils are used as inhibitors of carboxyl esterase enzymes, proteins involved in the metabolism of esterified drugs and xenobiotic. Phenytoin is the oldest non sedative antimnseizure drug. It is also known as muscle relaxant and anti-arrhythmic. It is an antiepileptic drug which is also called anticonvulsant. The concentration of phenytoin must be maintained in the plasma. Phenytoin is composed of five membered hydantoin with two phenyl groups at the five positions. Phenytoin has three member molecule associated with urea and although has pharmacological tool. It is an anticonvulsant and used to treat various types of disorders such as epilepsy[1].



PHENYTOIN

Multistep synthesis of phenytoin

Benzil is a-diketone and an organic compound. Diketone have the two ketonic groups and have two alkyl groups which may be same or different such as benzil which have the same phenyl groups. Benzil is a yellow crystalline solid. Its m.p is 95°C. It has the long C-C bond which is 1.5A°. The PhCO centres are planar. The benzoyl group pairs are twisted with respect to each other and having the 11 dihedral angle. The molecular formula of benzil is

$(C_6H_5C0)^2$. Benzil is used to form the phenytoin which is medicine in the treatment of epilepsy. Phenytoin, also known as Phenytoinis sold under the name Dilantin, is an anticonvulsant medication commonly prescribed for seizure disorders and less commonly prescribed for bipolar disorder or anxiety. Common anticonvulsant medications like Phenytoin include phenobarbital, carbamazepine, oxcarbazepine and gabapentin[2].

Phenytoin also known as dilantin or eptoin, is a commonly used antiepileptic drug (FDA approved). It suppresses the abnormal brain activity during seizure by reducing electrical conductance among the brain cells. Epilepsy caused by the rapid flow of sodium ions from neurons. It decreases the flow of sodium ions by the release of GABA. Phenytoin reduces the flow of calcium and sodium ions and at high frequency the flow of neurons is low which causes to prevent from the electrical activity of seizure. Phenytoin reduces the release of neurotransmitter and glutamate through them the body's central nervous system is stimulated and increases the release of GABA through it the body's central nervous system is suppressant. The first action of Phenytoin is decrease the effect of seizure on motor cortex. In the past few years this drug has also been implicated in inhibiting breast tumour growth and metastasis[3]. Phenytoin has also been shown to accelerate wound healing in both animals and humans[4]and has been proven to have antimicrobial activity as well[5]. Research teams have been working on manipulating the Dilantin molecule to enhance its positiveactivities[6]. New bivalent ligands derived from phenytoinhave been shown to provide even better anticonvulsant activity[7]. Similarly hybrids between phenytoin and thiosemicarbazide, 1, 3, 4-oxadiazole, 1, 3, 4- thiadiazole or 1, 2, 4-triazole also show promising results for anticonvulsant activity. Other antiepileptic Dilantin derivative drugs can be synthesized by reacting 2, 5-Dioxo-4, and 4- diphenylimidazolidine-1- carboxylic acid with methyl ester of different amino acids and substituted benzhydrols in pyridine and in the presence of N, N dicyclohexyl carbodimide[8].

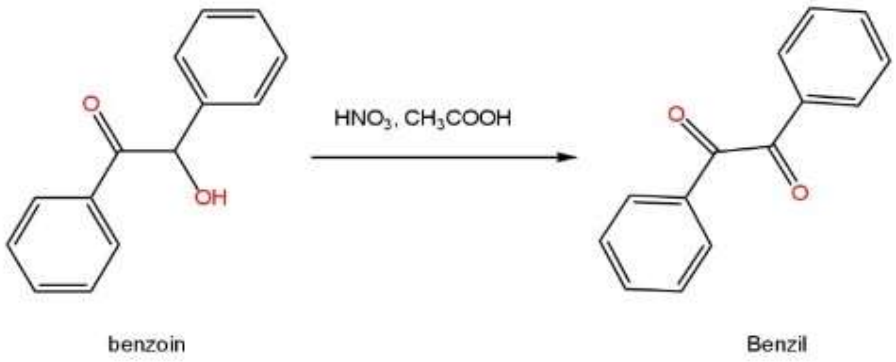


Figure 1.Synthesis of Benzil

In a multistep process to synthesize Dilantin, starting with Benzaldehyde to make Benzoin and using this product to create Benzil. Then this Benzil is finally used to make Dilantin (Figure 1). Benzaldehyde dimerizes to benzoin. Benzoin on oxidation gives benzil and it is further transformed by benzilic acid rearrangement to 5, 5 diphenylhydantoin. The hydantoin, 5, 5- diphenylhydantoin (5, 5-diphenyl-2, 4- imidazolidinedione; DPH) has diverse effects on the biochemistry of the central nervous system. However in the form of its sodium salt (Phenytoin sodium; e.g., "Dilantin") DPH is of value as an anticonvulsant for the control of grand mal and psychomotor epilepsy. There is a rate determining attack by the urea anion on one carbonyl group of benzil followed by rapid cyclization and finally, slow rearrangement to the product anion. It is likely that the formation of this latter anion (or the imido-anion) is the driving force behind the rearrangement[9].

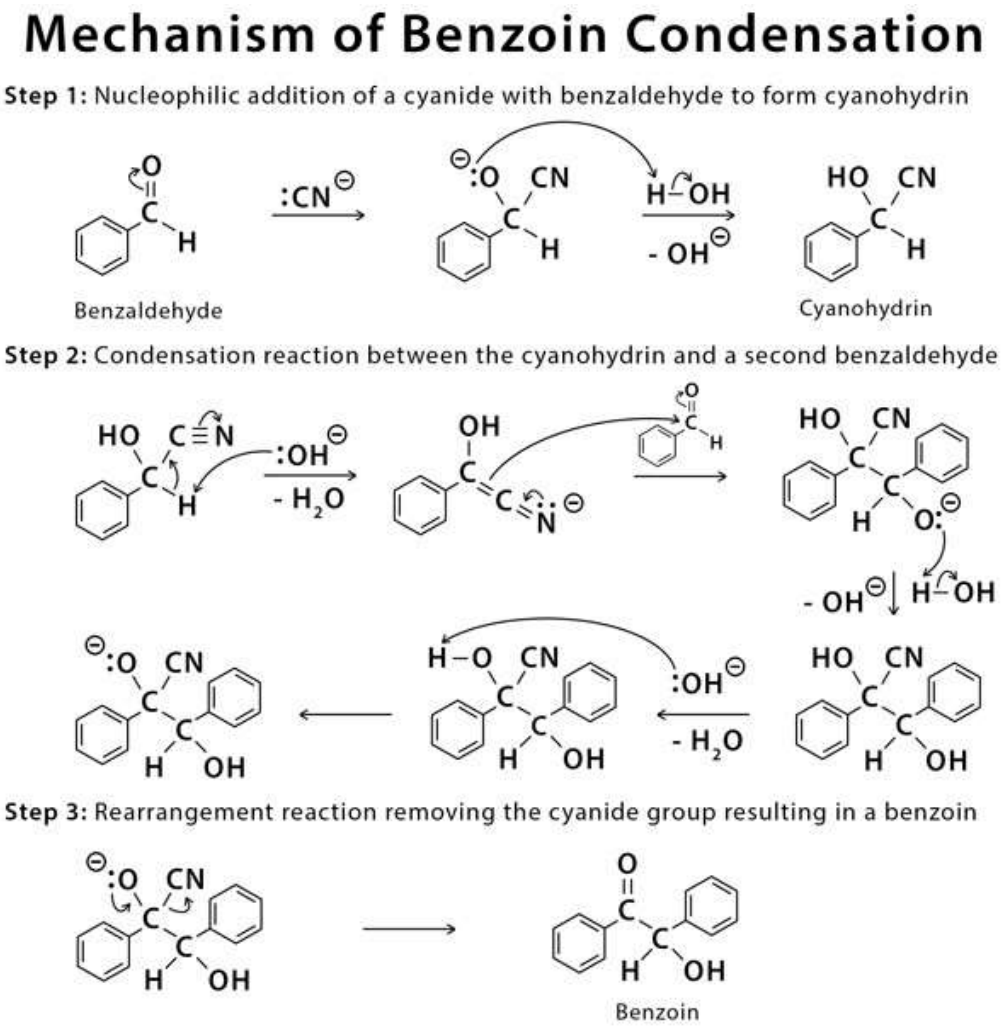


Figure 2. Benzoin condensation mechanism

Benzoin is a hydroxy ketone attached to two phenyl groups. It appears as off white crystals with a light camphor-like odor. It is synthesized from benzaldehyde in benzoin condensation (Figure 2). Benzoin is used as precursor to benzyl by organic oxidation. This reaction is typically catalysed by cyanide ion but here greener alternative of using thiamine hydrochloride (vitamin B1) catalyst was used. For formation of benzoin, other experimental studies have shown that irradiation of solid 5-cyclodextrin complexes of benzaldehyde produces optically active benzoin as a major photo-product with an enantiomeric excess of up to 15% but extendedphotolysis will result in formation of other unnecessary products during this method.

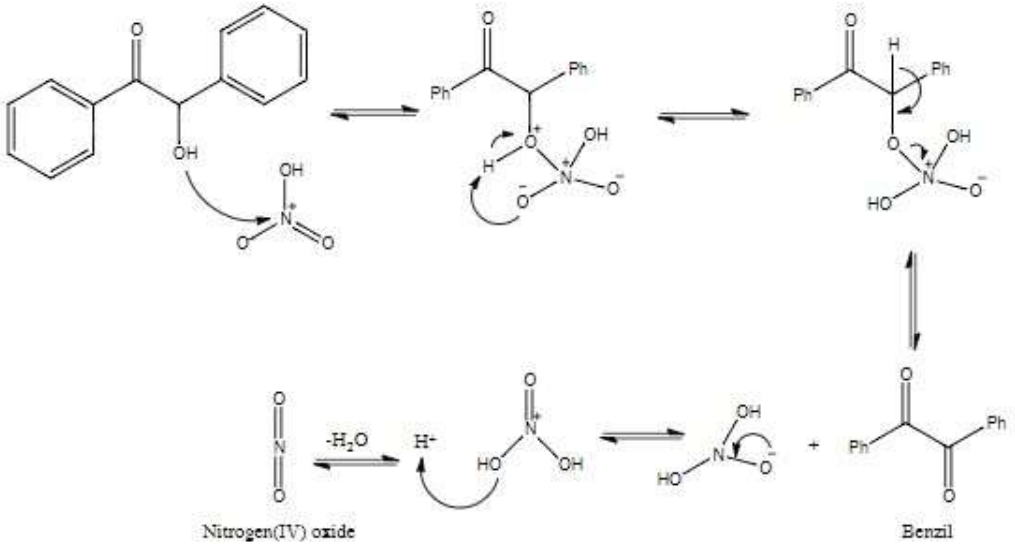


Figure 3. Benzil formation mechanism

Benzil (systematically known as 1, 2-diphenyl-1, 2-ethanedione) is an organic compound with the formula $(C_6H_5CO)^2$, generally abbreviated as $(PhCO)^2$. This yellow solid is one of the most common diketones. Its main use is as a photo initiator in polymer chemistry. Most benzil is consumed for use in the free-radical curing of polymer networks. Ultraviolet radiation decomposes benzil, generating free-radical species within the material, promoting the formation of cross-links. Benzil is a standard building block in organic synthesis. It condenses with amines to give diketimine ligands. A classic organic reaction of benzil is the benzilic acid rearrangement, in which base catalyses the conversion of benzil to benzilic acid. This reactivity is exploited in the preparation of the drug phenytoin. In this experiment Benzil was synthesized by the oxidation of benzoin using nitric acid in the presence of acetic acid (Figure.3)

Under these conditions, nitric acid produces nitronium ion. Nitronium ion is as an intermediate in the nitration of benzene. Nitronium ion can also oxidize alcohols. In this reaction, it is reduced to nitrous acid (HONO) and the alcohol in benzoin is oxidized to a carbonyl[10].

Synthesis of phenytoin

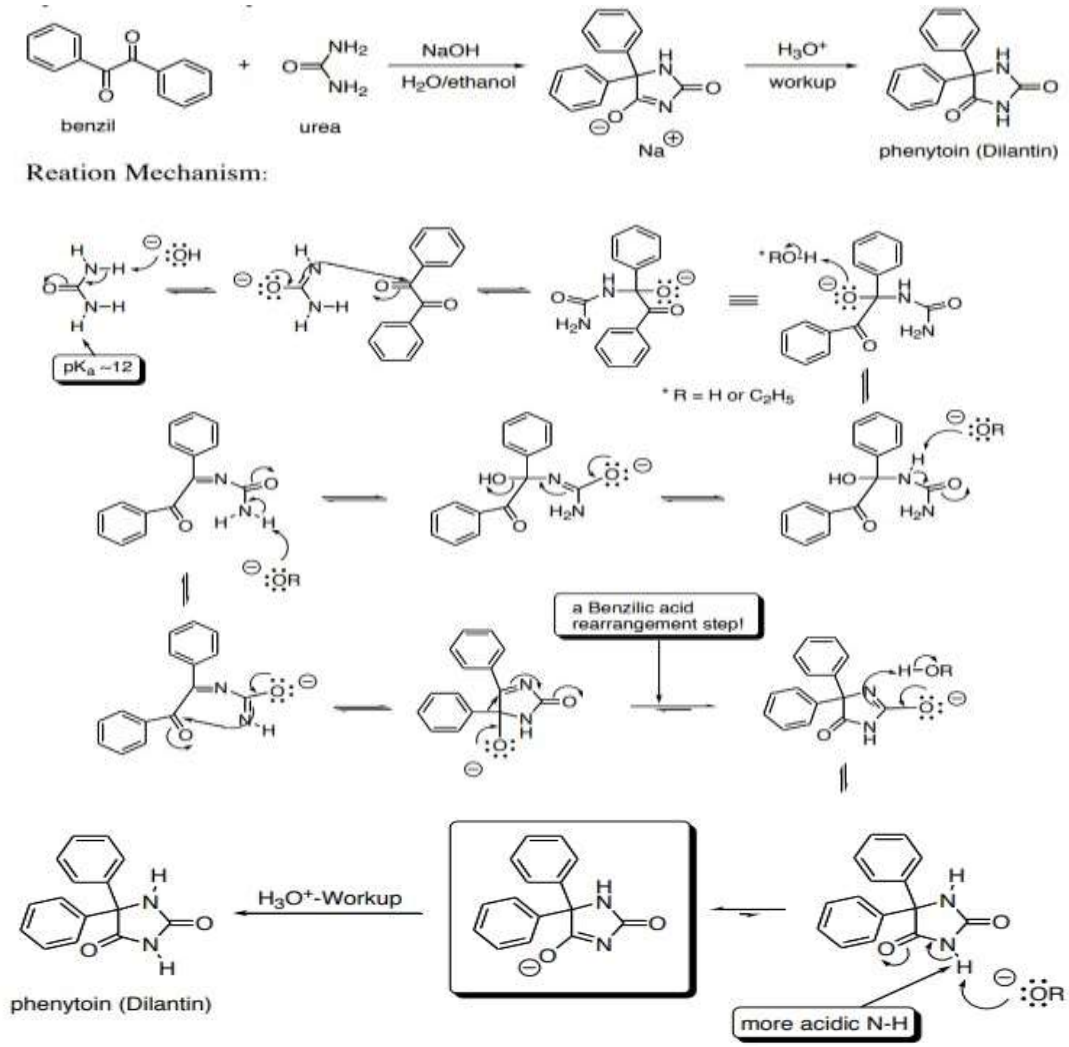


Figure 4. Phenytoin formation mechanism

Role of nitro phenytoin derivative

It's primarily used to prevent seizures, particularly tonic-clonic seizures that impact the entire brain or partial seizures that impact sections of the brain. Phenytoin uses also include treating neuropathic pain and irregular heartbeat. In some cases, phenytoin may also be used for the treatment of mood disorders[11].

Phenytoin and its Use in Medicine

Epilepsy is one of the most important neurological disorders. About 50 million people (1% of the world population) worldwide are affected. The annual incidence ranges

from 20 to 70 cases per 100’000 and the prevalence is 0.4 to 0.8%. Generalized epilepsies occur in approximately one-third of patients. The treatment of epilepsy is based on stopping seizures or minimizing their frequency as well as mitigating undesirable side-effects. Phenytoin (PHT) is a well-established and one of the most widely prescribed anticonvulsants for the treatment and the prevention of seizures and status epilepticus. The use of PHT in adults with severe traumatic brain injuries before and after neurosurgical intervention has been shown to be effective as prophylaxis. The risk for an early posttraumatic seizure after acute, traumatic brain injuries can be diminished significantly. The use of antiepileptic drugs to treat patients who have developed post-traumatic epilepsy is common practice. PHT is the only antiepileptic drug for which an optimal therapeutic range (serum concentrations) is clearly defined and which is effective in preventing early seizures after acute brain injury[11].

Seizures

Tonic-clonic seizers: Mainly used in the prophylactic management of tonic-clonic seizers with complex symptomatology (psychomotor seizures). A period of 5-10 days of dosing may be required to achieve anticonvulsant effects.

Focal seizers: Mainly used to project against the development of focal seizers with complex symptomatology (psychomotor and temporal lobe seizures).Also effective in controlling focal seizures with autonomic symptoms.

Absence seizures: Not used in treatment of pure absence seizures due to risk for increasing frequency of seizures due to risk for increasing frequency of seizures. However, can be used in combination with other anticonvulsants during combined absence and tonic-clonic seizures.

Seizures during surgery: A 2018 meta-analysis found that early antiepileptic treatment with either phenytoin or phenobarbital reduced the risk of seizure in the first week after neurosurgery for brain tumours.

Status epilepticus: Considered after failed treatment using a benzodiazepine due to slow onset of action[12].

Other

Abnormal heart rhythms: may be used in the treatment of ventricular tachycardia and sudden episodes of atrial tachycardia after other antiarrhythmic.

Medications or cardioversion has failed. It is a class Ib antiarrhythmic.

Digoxin toxicity: Intravenous phenytoin formulation is a medication of choice for arrhythmias caused by cardiac glycoside toxicity.

Trigeminal neuralgia: Second choice drug to carbamazepine[12].

Special considerations

Phenytoin has a narrow therapeutic index. Its therapeutic range for an anticonvulsant effect is 10-20 ml and for an antiarrhythmic effect 10-20 ml.

The most common cause of phenytoin intoxication self-medication, which accounts for than thirty percent of the cases.

Avoid giving intramuscular formulation unless necessary due to skin cell death and local tissue destruction.

Elderly patients may show earlier signs of toxicity.

In the obese, ideal body weight should be used for dosing calculations.

Pregnancy: Pregnancy category D due to risk of fetal hydantoin syndrome and fetal bleeding. However, optimal seizure control.

Breastfeeding: The manufacturer does not recommend breastfeeding since low concentrations of phenytoin are excreted in breast milk.

Liver disease: Do not use oral loading dose. Consider using decreased maintenance dose.

Kidney disease: Do not use oral loading dose. Can begin with standard maintenance dose and adjust as needed.

Intravenous use in contraindicated in patients with sinus bradycardia, sinoatrial block, second-or-third-degree atrioventricular block, Stokes-Adams syndrome, or hypersensitivity to phenytoin, other hydantoins or any ingredient in the respective formulation[13].

Phenytoin interaction

A drug interaction is a change in one drug's effect when administered with another drug, food, or other substance. For example, two or more drugs taken together can change the way a drug works in your body. This possibly could make one or more of the drugs less safe or could cause them not to work as they should. It is important to know a little about the difference between a drug interaction and a side effect. A side effect, also known as an adverse effect, is caused by a single drug. Side effects can occur with the normal use of a drug and sometimes can be predicted and treated. A side effect, if severe enough, may require your doctor to stop the drug or lower the dosage. An example of a side effect is the drowsiness caused by certain antihistamines.

A drug interaction is caused by two or more drugs, foods, or other substances taken by the same person. A drug interaction is a situation in which a substance (usually another drug) affects the activity of a drug when both are administered together. This action can be synergistic (when the drug's effect is increased) or antagonistic (when the drug's effect is decreased) or a new effect can be produced that neither produces on its own. These interactions may occur out of accidental misuse or due to lack of knowledge about the active ingredients involved in the relevant substances. The incidence and severity of drug interactions are on the rise as more medications are brought to market. Following the absorption, distribution, metabolism and excretion model of pharmacokinetics, will provide varied mechanism of drugdrug, food - drug and drug-herb interactions most likely to cause harm. Understanding the mechanism of drug interactions will assist all clinicians in avoiding these serious, often preventable events. Drug-drug interactions occur when one therapeutic agent either alters the concentration (pharmacokinetic interactions) or the biological effect of another agent (Pharmacodynamics interactions).

Pharmacokinetic drug-drug interactions can occur at the level of absorption, distribution, or clearance of the affected agent. Many drugs are eliminated by metabolism. The microsomal reactions that have been studied the most involve cytochrome P (CYP)-450 family of enzymes, of which a few are responsible for the majority of metabolic reactions involving drugs. These include the forms CYP1A2,CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Enzyme inhibition refers to the decrease in metabolic enzyme activity due to the presence of an inhibitor. Enzyme induction is associated with an increase in enzyme activity. For drugs that are substrates of the isoenzyme induced, the effect is to lower the concentration of these substrates. The clinical consequence of the presence of an inducing agent and the resultant decrease in concentration of the substrate may mean a loss of efficacy. Conditions when more than one drug is administered[14].

A) Use of drugs acting by different mechanisms of actions for the effective therapy of disease e.g., multiple drug therapy for the tuberculosis, leprosy, cancer etc.
B) Treatment of multiple disorders simultaneously with different drugs e.g., hypertension and diabetics, hypertension and other cardiac disorders, hypertension and rheumatoid arthritis, hypertension and asthma etc.
C) Use of OTC drugs e.g., Aspirin, Paracetamol, antacids etc., along with drugs for the treatment of other disorders. Antacids, H2 receptor antagonists, NSAIDS, cough and

cold preparations and the anti-asthmatic drugs constitute the major part of the OTC drugs. Americans filled a record 2.4 billion prescriptions in 1996. Although this may be seen as a good thing, it also can present problems. There has never before been so much opportunity for confusion, drug interactions, side effects, and the improper use of medications. In addition: Americans over age 65 comprise 12 percent of the population, but they consume about 30 percent of all prescription drugs and 40 percent of overthecounter (OTC or non-prescription) drugs. When two to four different drugs are taken, the potential for interaction is 6 percent, but the risk increases to 50percent with five drugs and to almost 100 percent with eight drugs. The average older person takes four or five drugs daily. Drug interactions are responsible for 3 percent to 10 percent of admissions of older patients to the hospital, which costs an estimated $20 million annually in the United States. These facts illustrate the importance of everyone (and especially older people) being knowledgeable about drug interaction[14].

Pharmacology

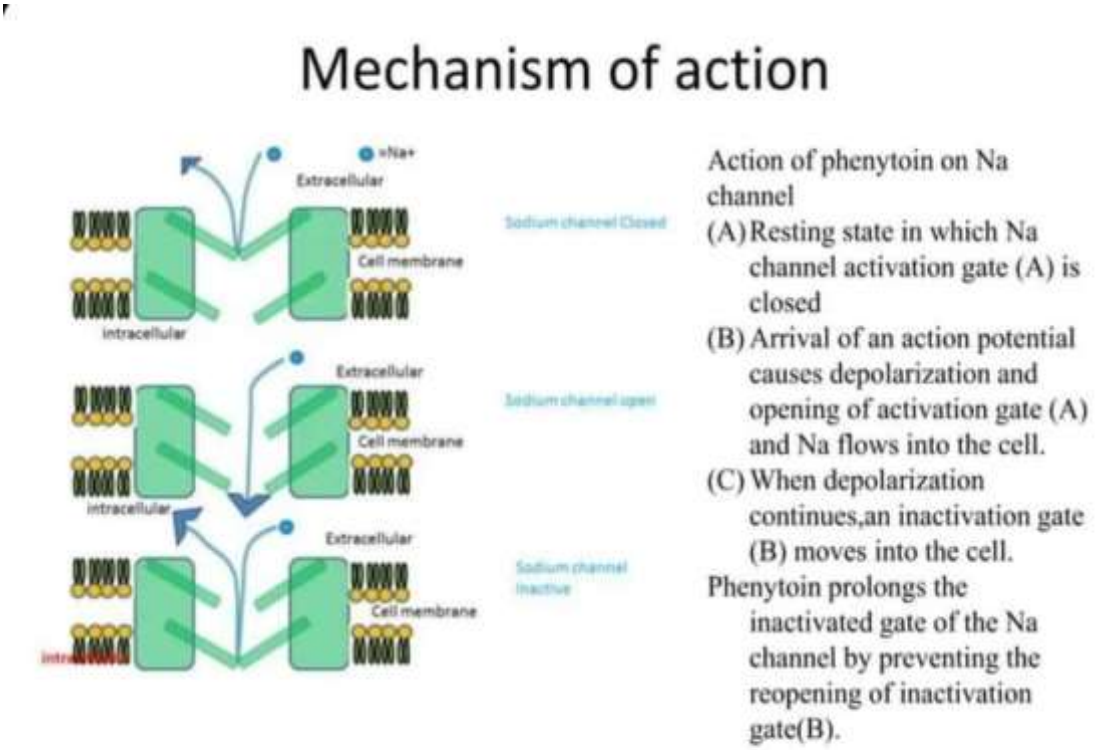
Mechanism of action

Phenytoin is believed to protect against seizures by causing voltage-dependent block ofvoltage gated sodium channels. This blocks sustained high frequency repetitive firing ofaction potentials. This is accomplished by reducing the amplitude of sodium-dependent action potentials through enhancing steady-state inactivation. Sodium channels exist in three main conformations: the resting state, the open state, and the inactive state[15].

Phenytoin binds preferentially to the inactive form of the sodium channel. Because it takes time for the bound drug to disassociate from the inactive channel, there is a time-dependent block of the channel. Since the fraction of inactive channels is increased by membranedepolarizationas well as by repetitive firing, the binding to the inactive state by phenytoin sodium can produce voltage-dependent, use-dependent and time-dependent block of sodium-dependent action potentials[16].

The primary site of action appears to be themotor cortexwhere spread of seizure activity is inhibited[12]. Possibly by promoting sodium efflux from neurons, phenytoin tends to stabilize the threshold against hyper excitability caused by excessive stimulation or environmental changes capable of reducing membrane sodium gradient. This includes the reduction ofpost-tetanic potentiationat synapses which prevents cortical seizure foci from

detonating adjacent cortical areas. Phenytoin reduces the maximal activity of brain stem centers responsible for the tonic phase of generalized tonic-clonic seizures.



Metabolism of phenytoin

PHT is metabolised nearly completely in the liver. Only about 5% of a dosage is excreted unchanged and the metabolites have no important anticonvulsive effect. PHT is a substrate of the cytochrome P450 (CYP) superfamily, especially of the CYP 2C19 and CYP 2C9 subfamilies, and is therefore subject to various genetic polymorphisms. In the world's population exist more than 10 different mutations of CYP 2C19, resulting in poor metabolisers and extensive metabolisers. There can be up to a twofold difference in the hepatic elimination rate, which leads to different PHT concentrations. Furthermore, the frequencies of different mutations vary among ethnic populations, so that experiences in the dosage of PHT cannot directly be transferred from one population to another[17].

Pharmacodynamics

Phenytoin is an anticonvulsant with a narrow therapeutic index. Although the recommended therapeutic range is cited to be between 10-20 mg/L, differences in albumin

levels, genetics, comorbidities, and body composition can make achieving an ideal phenytoin dose challenging. For example, studies have confirmed that phenytoin metabolism is impacted by CYP2C9 genotype polymorphisms and possibly by CYP2C19 genotype polymorphisms (the latter has not been as extensively studied).It is worth nothing that although phenytoin is highly protein bound, only the fraction unbound is able to exert a pharmacological effect. Therefore, factors that reduce or increase the percentage of protein bound phenytoin (for example: concomitant administration of drugs that can cause displacement from protein binding sites) can have a marked impact on phenytoin therapy[18].

Pharmacokinetic properties

Phenytoin elimination kinetics show mixed-order, non-linear elimination behaviour at therapeutic concentrations. Where phenytoin is at low concentration it is cleared byfirst order kinetics, and at high concentrations byzero order kinetics. A small increase in dose may lead to a large increase in drug concentration as elimination becomes saturated. The time to reach steady state is often longer than 2 weeks.

Absorption

Phenytoin is absorbed from the small intestine after oral administration. Various formulation factors may affect the bioavailability of phenytoin, however, non-linear techniques have estimated absorption to be essentially complete. After absorption it is distributed into body fluid including CSF. Its volume of distribution has been estimated to be between 0.52 and 1.19 litres/kg, and it is highly protein bound (usually 90% in adults).

Distribution

The plasma half-life of phenytoin in man averages 22 hours with a range of 7 to 42 hours. Steady state therapeutic drug levels are achieved at least 7 to 10 days after initiation of therapy[19].

Biotransformation

Phenytoin is hydroxylated in the liver by an enzyme system which is saturable. Small incremental doses may produce very substantial increases in serum levels when these are in the upper range of therapeutic concentrations.

Elimination

The parameters controlling elimination are also subject to wide interpatient variation. The serum level achieved by a given dose is therefore also subject to wide variation.

Special Populations

Patients with Renal or Hepatic Disease: Increased fraction of unbound phenytoin in patients with renal or hepatic disease, or in those with hypoalbuminemia or hyperbilirubinemia has been reported.

Chemical and Physical Characteristics of phenytoin

PHT is a Phenytoin nitro derivative (3-[(E)-[(3-nitrocyclohexyl)methylidene]amino]-5,5-diphenylimidazolidine-2,4-dione) with the structure formula $C_{22}H_{16}N_4O_4$ and the molecular weight of 400.39 g/mol. PHT is nearly insoluble in water and ethanol 96%, but is soluble in alkaline solutions because of the weak acidic character (pKa = 8.33). PHT is a white crystalline, odourless, and flavourless powder.

Indications and usage

Phenytoin is indicated for the control of generalized tonic-clonic (grand mal) and complex partial (psychomotor, temporal lobe) seizures and prevention and treatment of seizures occurring during or following neurosurgery. Phenytoin serum level determinations may be necessary for optimal dosage adjustments

Contraindications

Phenytoin is contraindicated in those patients who are hypersensitive to phenytoin or other hydantoins[21].

Warnings

Abrupt withdrawal of phenytoin in epileptic patients may precipitate status epilepticus. When, in the judgment of the clinician, the need for dosage reduction, discontinuation, or substitution of alternative antiepileptic medication arises, this should be done gradually. However, in the event 2 of an allergic or hypersensitivity reaction, rapid substitution of alternative therapy may be necessary. In this case, alternative therapy should be an antiepileptic drug not belonging to the hydantoin chemical class[22].

Precautions

The liver is the chief site of biotransformation of phenytoin; patients with impaired liver function, elderly patients, or those who are gravely ill may show early signs of toxicity. A small percentage of individuals who have been treated with phenytoin have been shown to metabolize the drug slowly. Slow metabolism may be due to limited enzyme availability and lack of induction; it appears to be genetically determined. Phenytoin should be discontinued if a skin rash appears. If the rash is exfoliative, purpuric, or bullous or if lupus erythematosus, Stevens-Johnson syndrome, or toxic epidermal necrolysis is suspected, use of this drug should not be resumed and alternative therapy should be considered. If the rash is of a milder type (measles-like or scarlatiniform), therapy may be resumed after the rash has completely disappeared. If the rash recurs upon reinstitution of therapy, further phenytoin medication is contraindicated[24].

Phenytoin and other hydantoins are contraindicated in patients who have experienced phenytoin hypersensitivity. Additionally, caution should be exercised if using structurally similar compounds (e.g., barbiturates, succinimides, oxazolidinediones, and other related compounds) in these same patients. Hyperglycaemia, resulting from the drug's inhibitory effects on insulin release, has been reported. Phenytoin may also raise the serum glucose level in diabetic patients. Osteomalacia has been associated with phenytoin therapy and is considered to be due to phenytoin's interference with vitamin D metabolism. Phenytoin is not indicated for seizures due to hypoglycaemic or other metabolic causes. Appropriate diagnostic procedures should be performed as indicated. Phenytoin is not effective for absence (petit mal) seizures. If tonic-clonic (grand mal) and absence (petit mal) seizures are

present, combined drug therapy is needed. Serum levels of phenytoin sustained above the optimal range may produce confusional states referred to as “delirium,” “psychosis,” or “encephalopathy,” or rarely irreversible cerebellar dysfunction. Accordingly, at the first sign of acute toxicity, plasma levels are recommended. Dose reduction of phenytoin therapy is indicated if plasma levels are excessive; if symptoms persist, termination is recommended[25].

Adverse reactions

Central Nervous System

The most common manifestations encountered with phenytoin therapy are preferable to this system and are usually dose-related. These include nystagmus, ataxia, slurred speech, decreased coordination, and mental confusion. Dizziness, insomnia, transient nervousness, motor twitching, and headaches have also been observed. There have also been rare reports of phenytoin-induced dyskinesia’s, including chorea, dystonia, tremor, and asterixis, similar to those induced by phenothiazine and other neuroleptic drugs. A predominantly sensory peripheral polyneuropathy has been observed in patients receiving long-term phenytoin therapy. Gastrointestinal System: Nausea, vomiting, constipation, toxic hepatitis, and liver damage. Integumentary System: Dermatological manifestations sometimes accompanied by fever have included scarlatiniform or morbilliform rashes. A morbilliform rash (measles-like) is the most common; other types of dermatitis are seen more rarely. Other more serious forms which may be fatal have included bullous, exfoliative or purpuric dermatitis, lupus erythematosus, Stevens-Johnson syndrome, and toxic epidermal necrolysis. Haemopoietin System: Haemopoietin complications, some fatal, have occasionally been reported in association with administration of phenytoin. These have included thrombocytopenia, leukopenia, granulocytopenia, agranulocytosis, and pancytopenia with or without bone marrow suppression. While macrocytosis and megaloblastic anaemia have occurred, these conditions usually respond to folic acid therapy. Lymphadenopathy including benign lymph node hyperplasia, pseudo lymphoma, lymphoma, and Hodgkin’s disease have been reported[26].

Over dosage

The lethal dose in pediatric patients is not known. The lethal dose in adults is estimated to be 2 to 5 grams. The initial symptoms are nystagmus, ataxia, and dysarthria. Other signs are tremor, hyperreflexia, lethargy, slurred speech, nausea, vomiting. The patient may become comatose and hypotensive. Death is due to respiratory and circulatory

depression. There are marked variations among individuals with respect to phenytoin plasma levels where toxicity may occur. Nystagmus, on lateral gaze, usually appears at 20 mcg/mL, ataxia at 30 mcg/mL; dysarthria and lethargy appear when the plasma concentration is over 40 mcg/mL, but as high a concentration as 50 mcg/mL has been reported without evidence of toxicity. As much as 25 times the therapeutic dose has been taken to result in a serum concentration over 100 mcg/mL with complete recovery. Treatment: Treatment is nonspecific since there is no known antidote. The adequacy of the respiratory and circulatory systems should be carefully observed and appropriate supportive measures employed. Haemodialysis can be considered since phenytoin is not completely bound to plasma proteins. Total exchange transfusion has been used in the treatment of severe intoxication in pediatric patients. In acute over dosage, the possibility of other CNS depressants, including alcohol, should be borne in mind[27].

Clinical uses

Phenytoin is used as an antiarrhythmic drug due to its effects on ion channel and receptors on myocardial cell membranes. Primarily,phenytoin shortens the action and inhibits the rapid inwared sodium current. It may be useful in the treatemenrt of ventricular tachycardia.

It is used to treat patients with epilepsy with hypersensitivity to antiepileptic drugs.Phenytoin is a drug that is sometimes used in patients with intractable trigeminal neuragia.

Toxicology

- Neurotoxicity
- Other toxicities

Neurotoxicity

The neurotoxic effects depend on the plasma concentration of phenytoin.Neurotoxic effects can range from ataxia, tremors,nystagmus,hyperactivy,

Lethargy and eventually coma and death.At high concentrations,phenytoin may cause seizures.How ever, this is rare, therefore it is necessary to search for other causes in patients with seizures with phenytoin over dose.

The general correlation of side effects with total plasma phenytoin concentration (values obtained through most labaratories)

Other toxicity

The unusual adverse effect known as “purple Glove Syndrome” can happen when phenytoin is administered intravenously(IV). It is characterized by increased limb edema, discolouration, and ischemia, which can result in limb amputation and severe skin necrosis. The blood-brone crystallization of phenytoin is the cause of this condition.Phenytoin hypersensitivity whichoften appears 1 week to 1 month after theraphy starts, is another hazardous side effect. Fever, rash, and involvement of several internal organs (hepatitis, myocarditis, pneumonitis) are its defining features.Chronic phenytoin use may result in megaloblastic anemia from peripheral neuropathy,lupus like disease, or folate insufficiency.This is an acute overdose occurrence that is not frequently documented.

Molecular docking

Docking attempts to find the best matching between two molecules. Docking is the computational determination of binding affinity between molecules (protein structure and ligand).

Docking is the most popular and integrity part of computational data based screening method of compounds in Pharmaceutical Research for drug Discovery efforts. The molecular docking is an important part of virtual screening, means “Ligand-based Screening” to find out the active compound as a template and also focus on comparative molecular similarity analysis of compounds with known and unknown activity by algorithm method. Also helps to predict the toxicity study of designing the formulation or synthesis of New Chemical Entity (NCE) in now a day of Pharmaceutical Research Developments. Docking is an important part of drug designing field of molecular modelling system in which the orientation by means of interaction through an H - bond or Vander Waals force of one molecule (ligand) to a second molecule (macro molecule or target protein) were bound with each other to form a stable complex. The orientation directly refers to the strength of bond association or bond affinity between these two molecules and also predicted the scoring functions. The scoring function directly influences the biological activity of that relevant molecule Docking

1.1 REVIEW OF LITERATURE

Sachin sheshra fawade et al.have examined phenytoin is the anticonvulsants class of drugs. The FDA approved phenytoin in 1939 for the treatement of epilepsy.Due to its narrow therapeutic index,the drug has use in the treatement of generalized tonic-clonic seizers,complex partial seizers, status epilpticus, trigeminal neuralgia and behaviour disorders. The base catalyzed reaction between benzil, urea and acid base is used for synthesis of phenytoin. This protocol gave rapid access to the phenytoin compound in very good yield.

Abhijith Kadam et al.have Synthesized Phenytoin by condensation of benzil and urea in presence of base using ethanol as solvent which itself acts as CNS stimulant. Removal of solvent after synthesis is most difficult and non-assured process.In case of phenytoin transformation in polymorphism plays an important role when solvent other than water is used.Therefore by application of green chemistry principle phenytoin was synthesized by condensation of benzil and urea in presence of base and water as green solvent.

Toufan Parman et al.have said that Phenytoin and related xenobiotics can be bioactivated by embryonic prostaglandin H synthase(PHS) to a teratogenic free radical intermediate. The mechanism of free radical formation was evaluated using photolytic oxidation with sodium persulfate and by EPR spectrometry. Characterization of the products by mass spectrometry suggested that phenytoin photolyzes to a nitrogen-centred radical that rapidly undergoes ring opening to form a carbon-centered radical.

Saadi M.Det al. have performed new reaction routs for the some of the derivatives of phenytoin.These reactions include acylation, halaoenation, reduction, and nucleophilic substitutions. Most of the derivatives showed variable chemical reactivities and thermal stability, and the N_1 and N_3 disubstituted analogue were found much less stable, and hydrolyzes easily in the reaction medium.

Tadatoshi Tanino et al. have aimed to further clarify the pharmacokinetic characteristics of phenytoin (DPH) and its derivatives , DPH-1- methylnicotinate (MNDPH), valeroyl DPH (VADPH) and valproyl DPH (VPDPH), in plasma and brain, we have investigated their physicochemical properties and probuilding characteristics.

Agrawal et al .have said that Phenytoin is a classical anticonvulsant drug used in the treatment of epilepsy. It is a Biopharmaceutics classificatioc system class II drug that has poor aqueous solubility, which affects dissolution rate. The main objective of this study was to enhance the dissolution rate of phenytoin and formulate the optimized chewable tablet.

Shweta Verma et al. have researched about the new Phenytoin derivaives have been synthesized, characterized and compared for CNS activity.The synthesis was carried out in three steps.

Snehal Gaikwad et al.have said that phenytoin sodium is a high –yeilding chemically synthesized anticonvulsant drug, which is prescribed to be taken orally or by slow intravenous injection .It stabilizes the excitable membranes of cardiac muscle and neuronal cells by decreasing the restng fluxes of sodium and inducing chemical depolarisations .It is widely used to control tonic-clonic (grand mal) seizure,partial(focal) seizures and prophylactic seizers during neurosurgery or post-traumatic injury to the head.

Masahiro Iwaki et al. have said that to gain to the site of action of anticonvulsants in the brain, drugs must cross one more barriers.In contrast to the capillary endothelial junction in other tissues, the endothelial cells in brain capillaries are joined by continuous tight intercellular junctions,indicating that drugs must pass through the cells, rather than between them in order to be distributed in to the brain.

Sonam Rani et al. have studied that the moiety of Phenytoin is also found it used in treatement of several other disorders such as heart disease, arrhythmias, microbial infections, and CNS disorder.

1.2 AIM AND SCOPE

Phenytoin is commonly used to treat epilepsy. It is an enzyme inducing drug that helps metabolize other anticonvulsant drugs in the body which slows impulses in the brain that causes seizures. Phenytoin reacts with many other drugs because it has some very unique properties. It is loosely bounded to plasma proteins, and largely metabolized by cytochrome enzymes. Since it is loosely bound to these enzymes and proteins, the occurrence of inhibition and metabolism with other drugs is frequent. The main mechanism of phenytoin is inhibiting collagenase. This is very useful when treating skin related diseases and injuries. A

specific example of the effect of dilantin on a cellular level is examining patients with epidermolysis bullosa. People who suffer from this disease have an increased level of collagenase which removes polysaccharides from the connective tissue and increases blistering. By inhibiting collagenase with phenytoin, blistering of the skin decreases. Additionally, phenytoin is known to increase the expression of growth factor B in macrophages which promotes the healing of ulcers and traumatic wounds. Phenytoin has many different uses and can treat a variety of diseases which makes it a very essential drug in the medical field. There were many disadvantages in this improved such as shorter reaction time, higher product purity, lower environmental pollution and higher product yield. ATR-IR, ^{1}H NMR and ^{13}C NMR has been used for the determination of functional groups present in the molecule of synthesized derivative. The molecular docking was performed for the synthesized compounds to P4HB receptor in order to rationalize their anticonvulsant, epilepsy and seizures activities in a qualitative way.

CHAPTER II

METHODS AND MATERIALS

2. METHODS AND MATERIAL

Apparatus and chemicals used Apparatus used

1. Condenser
2. Beaker (400ml)
3. Beaker (100ml)
4. Mantel
5. Glass rod
6. Watch glass
7. Funnel
8. Pipette
9. R B flask
10. Spatula
11. Magnetic stirrer
12. Magnetic pellet

Chemicals used

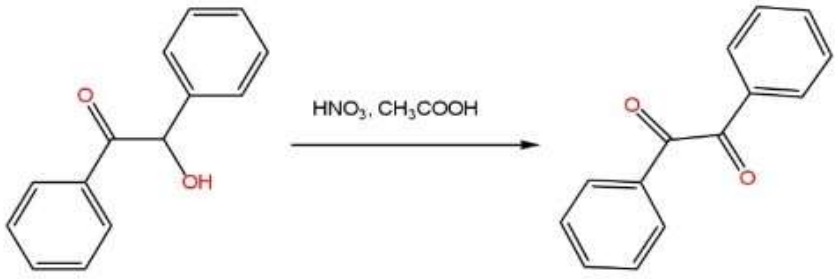
1. Nitric acid
2. Urea
3. Sodium hydroxide
4. Ethanol
5. Hydrazinehydrochloride
6. Hydroxy Benzaldehyde
7. Acetic acid
8. Methanol

SYNTHETIC METHOD

Scheme 1

Preparation of Benzyl (compound 2)

Place 2.0 g (0.094 mol) of the crude benzoin and 10ml of concentrated nitric acid in a 250-ml round-bottomed flask. Heat on a boiling water bath (in the fume cupboard) with occasional shaking until the evolution of oxides of nitrogen has ceased (about 1.5 hours). Pour the reaction mixture into 300-400 ml of cold water contained in a beaker, stir well until the oil crystallizes completely as a yellow solid. Filter the crude benzil at the pump, and wash it thoroughly with water to remove the nitric acid. Recrystallized from ethanol or rectified spirit (about 2.5 ml per gram) [28].



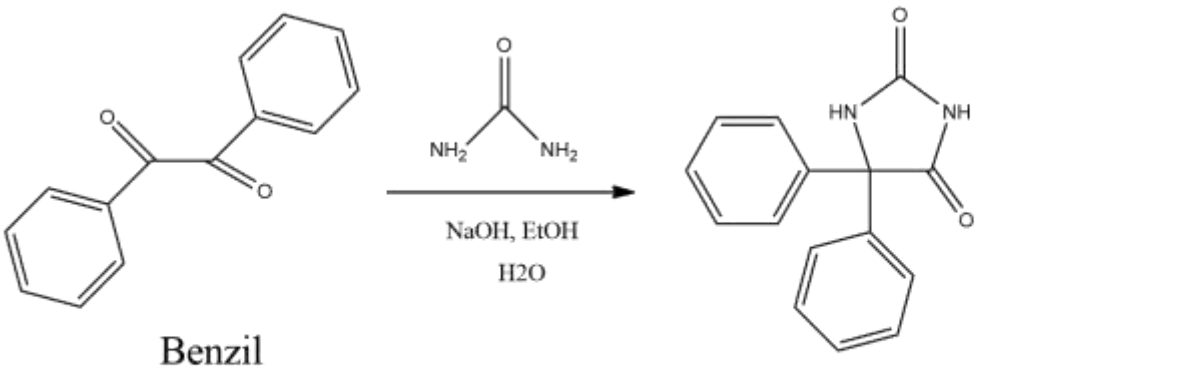
benzoin Benzil

Compound 1 **Compound2**

Scheme 2

Preparation of phenytoin (compound 3)

Place 5.3 g (0.025 mol) of benzil, 3.0 g (0.05 mol) of urea, 15 ml of aqueous sodium hydroxide solution (30%) and 75 ml of ethanol in a round bottomed flask of 100 ml capacity. Set up a reflux condenser with the flask and boil using an electric heating mantle for at least 2 h. Cool to room temperature, pour the reaction mixture into 125 ml of water and mix carefully. Allow the reaction mixture to stand for 15 min and then filter the product under suction to remove an insoluble by-product. Render the filtrate strongly acidic with concentrated hydrochloric acid, cool in ice water and immediately filter off the precipitated product under suction. Recrystallise at least once from industrial spirit to obtain about 2.8 g (44%) of pure 5, 5- diphenylhydantoin, and m.p. 297-298 ° C[29].



Benzil

Phenytoin

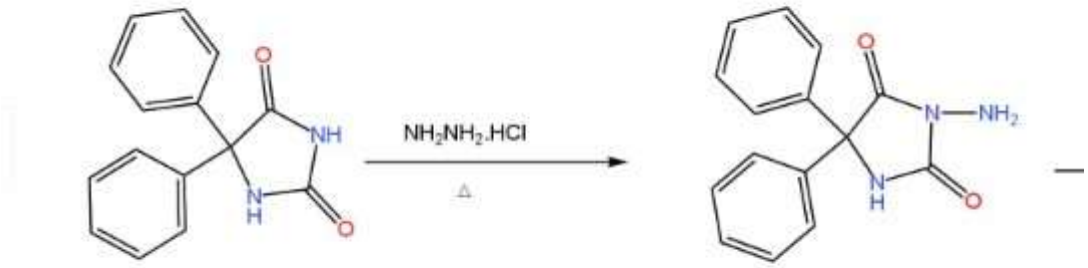
5,5-diphenylimidazolidine-2,4-dione

Compound 2 **Compound 3**

Scheme-3

Preparation of 3-amino-5, 5-diphenylimidazolidine-2,4dione (compound 4)

Begin by weighing out 1.5g of dilantin and 1.2g hydrazine hydrochloride. Dissolve the dilatin in 15ml of water in a 50ml round bottom flask .add the hydrazine hydrochloride to the dilatin solution and stir until it is completely dissolved. Heat the reaction mixture to 80-90^0C and maintain this temperature for 2 hours with stirring. After 2 hours allow the reaction mixture to cool to room temperature .filter the resulting solid product and wash it with cold water .dry the product under vacuum or in an oven at 60^0C until a constant weight is achieved. The resulting product is 3-amino-5, 5 diphenylimidazolidine-2, 4-dione.the purity of the product can be confirmed by performing a melting point determination and or using analytical techniques such as TLC.[30].



Phenytoin

(5,5-diphenylimidazolidine-2,4-dione)

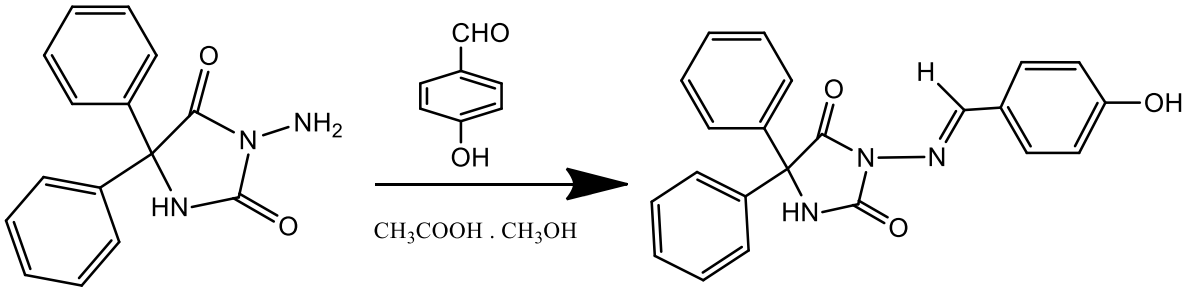
3-amino-5,5-diphenylimidazolidine-2,4-dione

Compound 3 **Compound 4**

Scheme 4 preparation of phenytoin hydroxyderivative (compound 5)

(E)-3-((4-hydroxy benzlidene)amino)-5-5-diphenylimidazolidine2, 4-dione

In a round-bottom flask equipped with a magnetic stir bar, add 3-Amino-5, 5 diphenylimidazolidine-2, 4-dione and 4- hydroxybenzaldehyde. Add acetic acid and stir the mixture until the solids dissolve completely. Add methanol to the flask and stir the mixture for 30 minutes. After stirring, the reaction mixture will have turned yellow. The reaction mixture is now ready to be filtered. The reaction mixture is now ready to be filtered. Filter the solid through a Buchner funnel and wash with cold methanol. Collect the solid and dry it under vacuum to obtain the product.



3-amino-5,5-diphenylimidazolidine-2,4-dione **(E)-3-((4-hydroxybenzylidene)amino)-5,5-diphenylimidazolidine-2,4dione**

,

Compound 4 **Compound 5**

Solubility

The amount of solvent required for complete saturation of 1g of phenytoin was found out.Solubility in water,ethanol,methanol and DMSO was observed.

Meltng point

The MP was determined using digital melting point apparatus (model S-972). One end of the capillary tube was scaled and dried dilantin was filled by jabbing the open end of the tube.The tube was inverted and gently tapped on the benchtop to cause the solid to fall to the closed end and was filled up to 2-3 mm of height .The tube was placed into the melting ponit apparatus.Once the temperature of 20^0C below the expected melting point was reached,the temperature was further increased at the rate of not more than 1^0every 30s .The melting point range was recorded from the first visible drop of liquid to a completely melted solid.

ATR-IR Spectroscopy

Attenuated Total Reflection (ATR) is a measurement technique used in IR spectroscopy, the most common measurement technique used in fact. ATR is one of the more popular sampling techniques used by FT-IR spectroscopists because it is quick, non-destructive and requires no sample preparation.

The ATR-FTIR technique makes it possible to study materials which are non-transparent to infrared radiation in a pristine condition.It utilizes internal reflection to generate an evanescent wave that penetrates the sample, providing valuable molecular information.

NMR Spectroscopy

Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy or magnetic resonance spectroscopy (MRS), is a spectroscopic technique to observe local magnetic fields around atomic nuclei.

^{1}H NMR spectra:

^{1}H NMR spectra were recorded on a BRUKER AVANCE spectrometer operating at 500 MHz. Samples were prepared by dissolving 10 mg of compound in 0.5 ml of $CDCl_3$. Tetramethylsilane (TMS) was used as an internal standard.

^{13}C NMR SPECTRA:

^{13}C NMR Spectra were recorded on a BRUKER AVANCE spectrometer operating at 100 MHz. Samples were prepared by dissolving 10 mg of compound in 0.5 ml of $CDCl_3$. Tetramethylsilane (TMS) was used as an internal standard.

CHAPTER - III

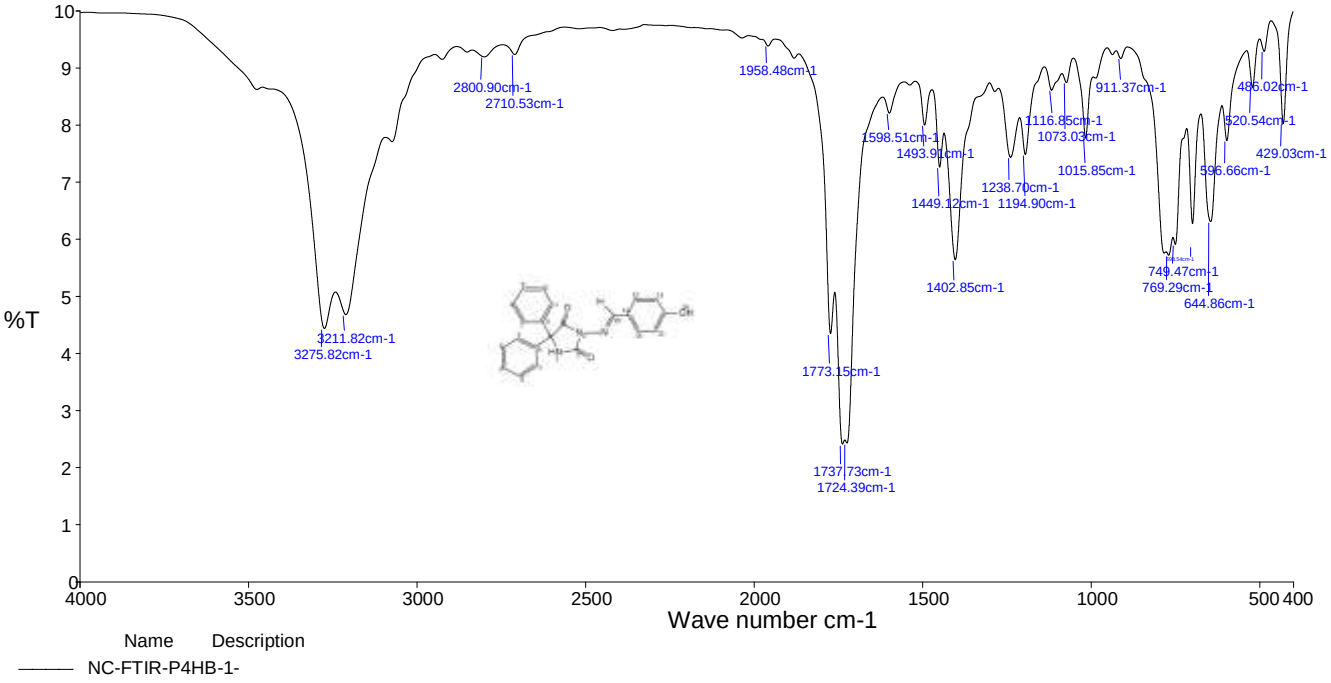
RESULT AND DISCUSSION

CHAPTER – III
RESULT AND DISCUSSION

IR Spectral data of(E)-3-((4-hydroxy benzlidene)amino)-5-diphenylilidazolidine 2,4-dione (Compound 5)

Phenytoin was synthesized by condensation of benzil and urea in the presence of 30% NaOH solution and as a green solvent.It was obtined a soild melting in the range 296-297^{0}C. The product again treated with benzaldehyde in the acidic medium methanol as a solvent one more benzene ring is attached in the phenytoin.The soild state IR(ATR)Cm^{-1} .Spectrum of the compound reveals a characteristic aromatic strech at sharp N-H streching vibiration are seen at 3275.8cm^{-1}.The streching viberation of the C=O group are seen around at 1737.24cm^{-1}.

IR Spectral data of (E)-3-((4-hydroxy benzlidene)amino)-5-diphenylilidazolidine 2,4-dione (Compound 5)



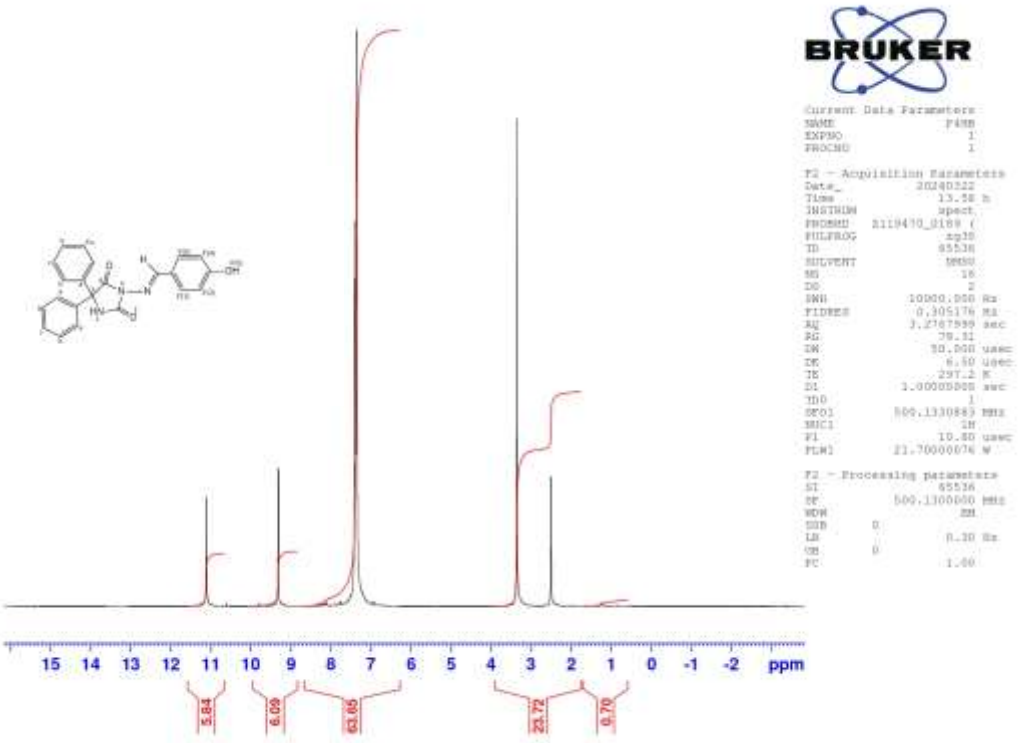
IR Spectral data of (compound 5)

S.NO	Functional group	IR(ATR)Cm^{-1}
1	C-H out of plane viberations of mono substituted phenyl ring	749.47,644.86
2	C-N stretching	1238.70
3	C=O stretching	1737.24
4	C-H stretching of aromatic ring	3211.82
5	N-H stretching	3275.8
6	O-H stretching	3275.82

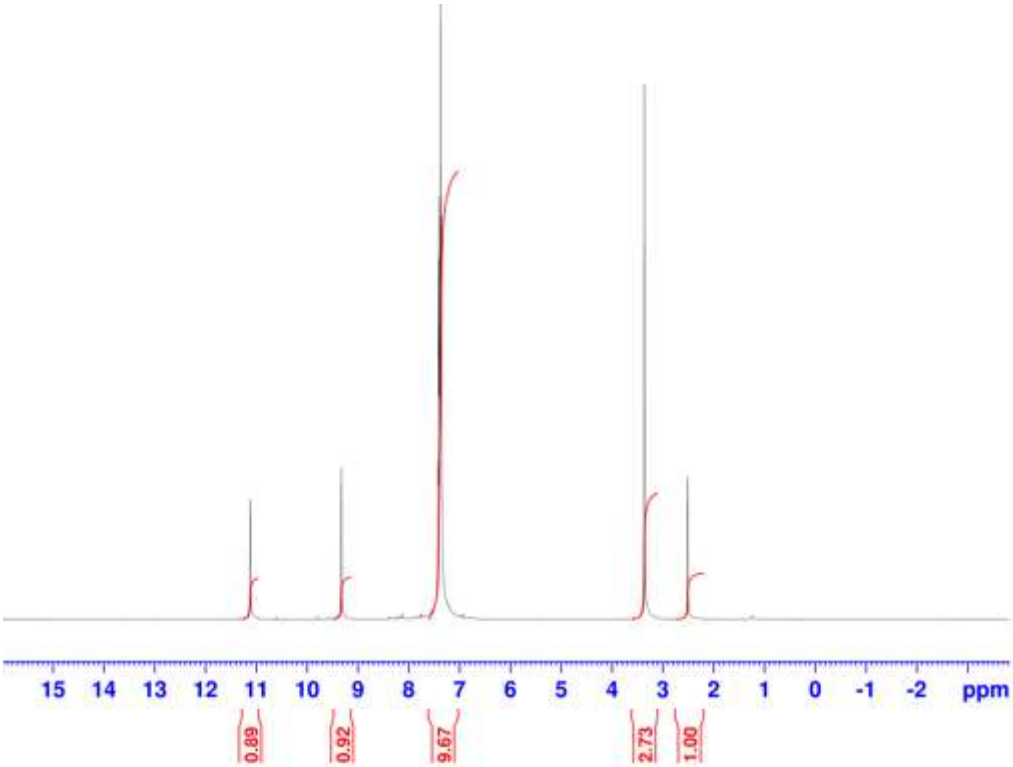
^{1}H NMR Spectral data of (E)-3-((4-hydroxy benzlidene)amino)-5-diphenylilidazolidine 2,4-dione

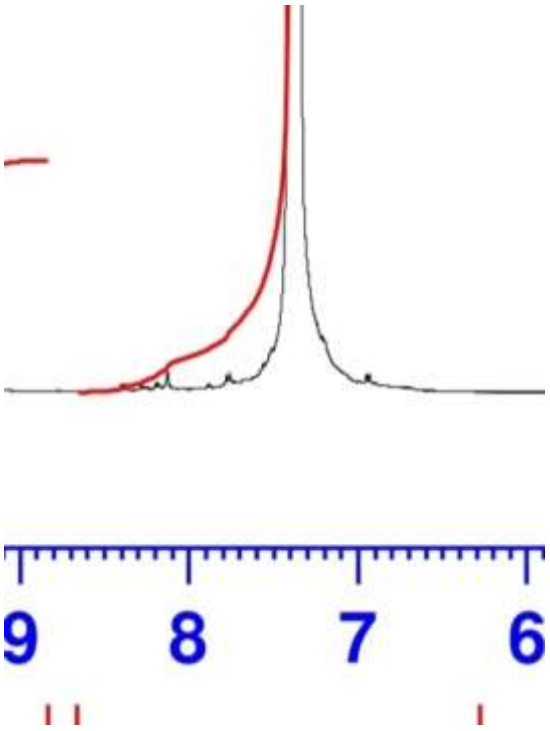
The ^{1}H NMR (DMSO,ppm) data for the compound **5** the signals observed between7.80-6.90ppm for the aromatic protons present in the phynatoin structure and the characteristics sinlet peaks at11.17ppm,9.32ppm and 5.35ppm assigned to proton attached to imide amide nitrogen respectively.

^{1}H NMR Spectral data of (compound 5)



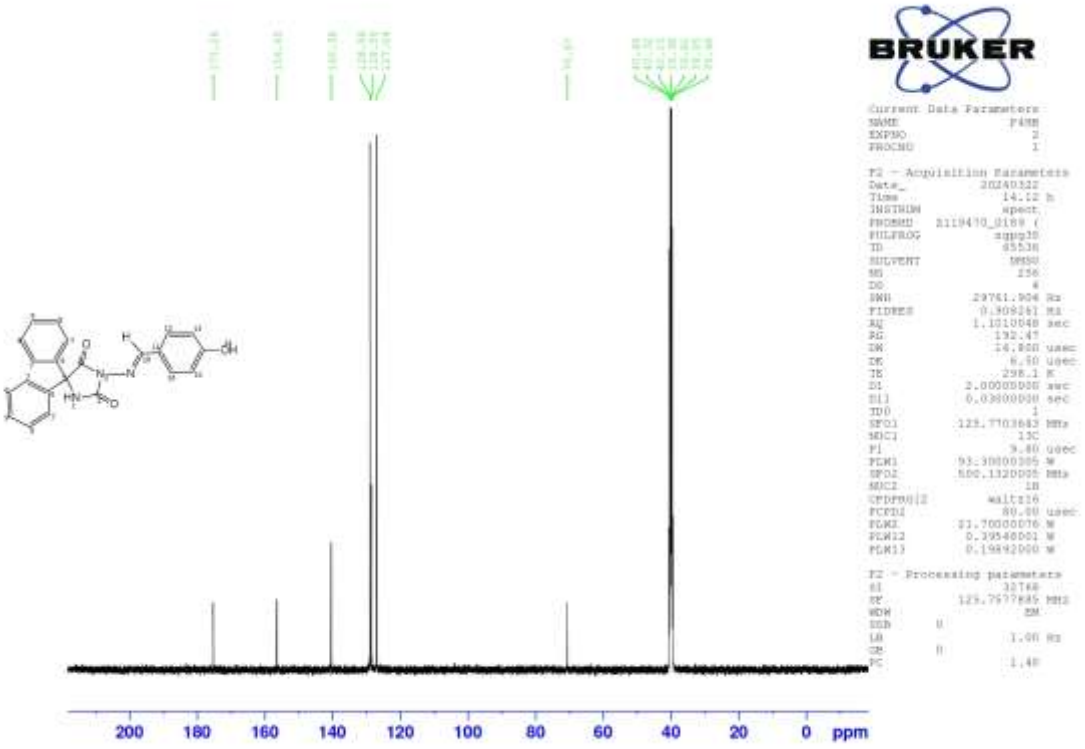
S.NO	Position of proton	Chemical shift(ppm)
1	NH	11.17
2	N=C-H	9.326
3	3`(a)	7.78
4	5’b	7.37
5	5’C	7.35
6	5’a	7.34
7	3’e	7.0
8	3’b,3`d	6.85
9	3'c(OH)	5.35


15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
0.89
0.92
9.67
2.73
1.00


9 8 7 6

^{13}C NMR Spectral data of (E)-3-((4-hydroxy benzlidene)amino)-5-diphenylimidazolidine2,4-dione (compound5)

The ^{13}C NMR (DMSO,ppm) data of the compound carbon signal appears at 140.38ppm confirms the hydroxy benzene attached in the phenytoin group.The aromatic carbon signals for compound **5** are observed from 140.38- 112.18ppm.



^{13}C NMR Spectral data of (Compound 5)

S.No	POSITION OF CARBON	CHEMICAL SHIFT(ppm)
1	4C	175.28
2	2,14C	156.45
3	10,6	140.38
4	7,8,9,	128.98-127.04
5	5	70.67

MOLECULAR DOCKING

Interactions between receptors and ligands at their best poses presented by maps depicting polar and lipophilic amino acids.

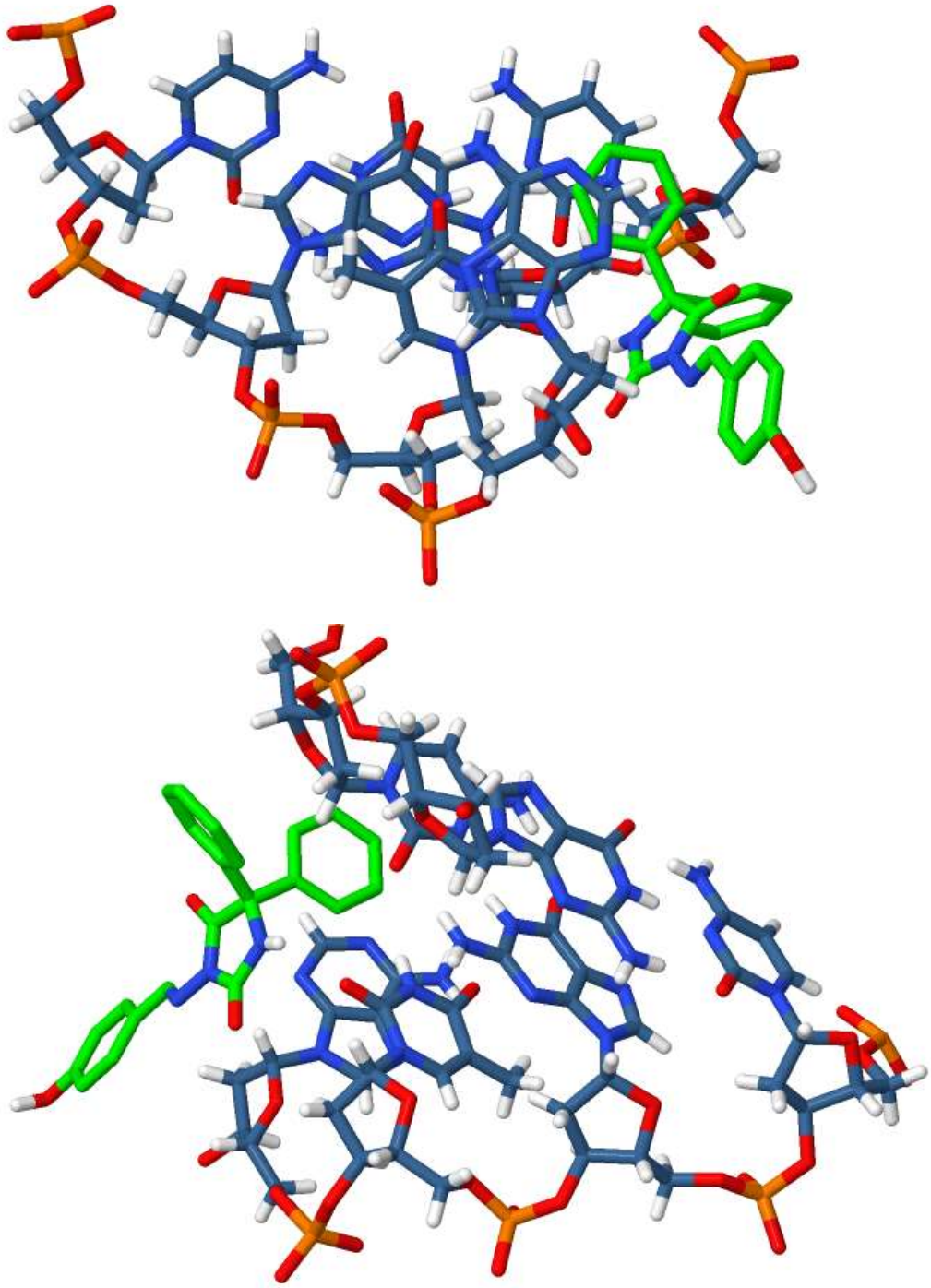


Figure 1. Interaction of standard drug (a) P4HB (Ligand) (b) with PDB-1pwf (Protein) in different angles

Molecular Docking

Docking calculations were exerted using AutoDock 4.2 software and a model of the open pore of the Na channel was utilized as a receptor. The implementing Lamarckian Genetic Algorithm (LGA) was adopted to perform the molecular docking studies. Final docked conformations were clustered using a tolerance of 1 A˚ root mean square deviation (RMSD).The docking score were observed at -5.26kcal/mol. So this compound would be a good one for further investigations and optimization to develop new antiepileptic drug. Docking results were in good harmony with experimental data and indicated that lowest binding energy belongs to compound **5**, which has strongest interactions with the active site of GABAA receptor. Interact surface value is observed 446.69. Compound **5** could be used for further investigation

Rank	**Est. Free Energy of Binding**	**Est. Inhibition Constant, Ki**	**vdW + Hbond + desolv Energy**	**Electrostatic Energy**	**Total Intermolec. Energy**	**Frequency**	**Interact Surface**
1	-3.86	1.48 kcal/mol	-5.26 kcal/mol	-0.02 kcal/mol	-5.28 kcal/mol	50%	446.692

CHAPTER – IV

CONCLUSION

CHAPTER – IV
CONCLUSION

While the synthesis an old reaction that has been many times revisted,the present work shows that is often worthwhile reinvesting so called old reactions that can be revistalized toproved interesting pharmacologicsl probes for the medical chemists. Phenytoin derivative has been prefaced in the laboratory they are playing important role in industry,biomedical fields and analytical chemistry.Phenytoin is the famous derivative of benzil which is widely used for the treatment of different dieases. Structure determination of benzyl deritavite compound were obtained by the use of FTIR and spectroscopy.FTIR and ^{1}H NMR spectra of phenytoin show the peak for C-N at 1238.70 cm^{-1} and CO at 1737.24 cm^{-1}

The ^{1}H NMR (DMSO,ppm) data for the compound **5** the signals observed between7.80-6.90ppm for the aromatic protons present in the phynatoin structure and the characteristics sinlet peaks at11.17ppm,9.32ppm and 5.35ppm assigned to proton attached to imide amide nitrogen respectively. The ^{13}C NMR (DMSO,ppm) data of the compound carbon signal appears at 140.38ppm confirms the hydroxy benzene attached in the phenytoin group.The aromatic carbon signals for compound **5** are observed from 140.38- 112.18ppm.

This docking analysis reveals that the phenyl ring of **5** which has the main role in drug-receptor interaction should be kept and in order to achieve better potency, an electronegative group can be provided at the meta position of N- aromatic part. So this compound would be a good candidate for further investigations and optimization to develop new antiepileptic, seizures and Anticonvulsant drug

CHAPTER - V

REFERENCE

CHAPTER-V
REFERENCE

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