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## ANTHRACENE DERIVATIVES AS AN ANTI BREAST CANCER

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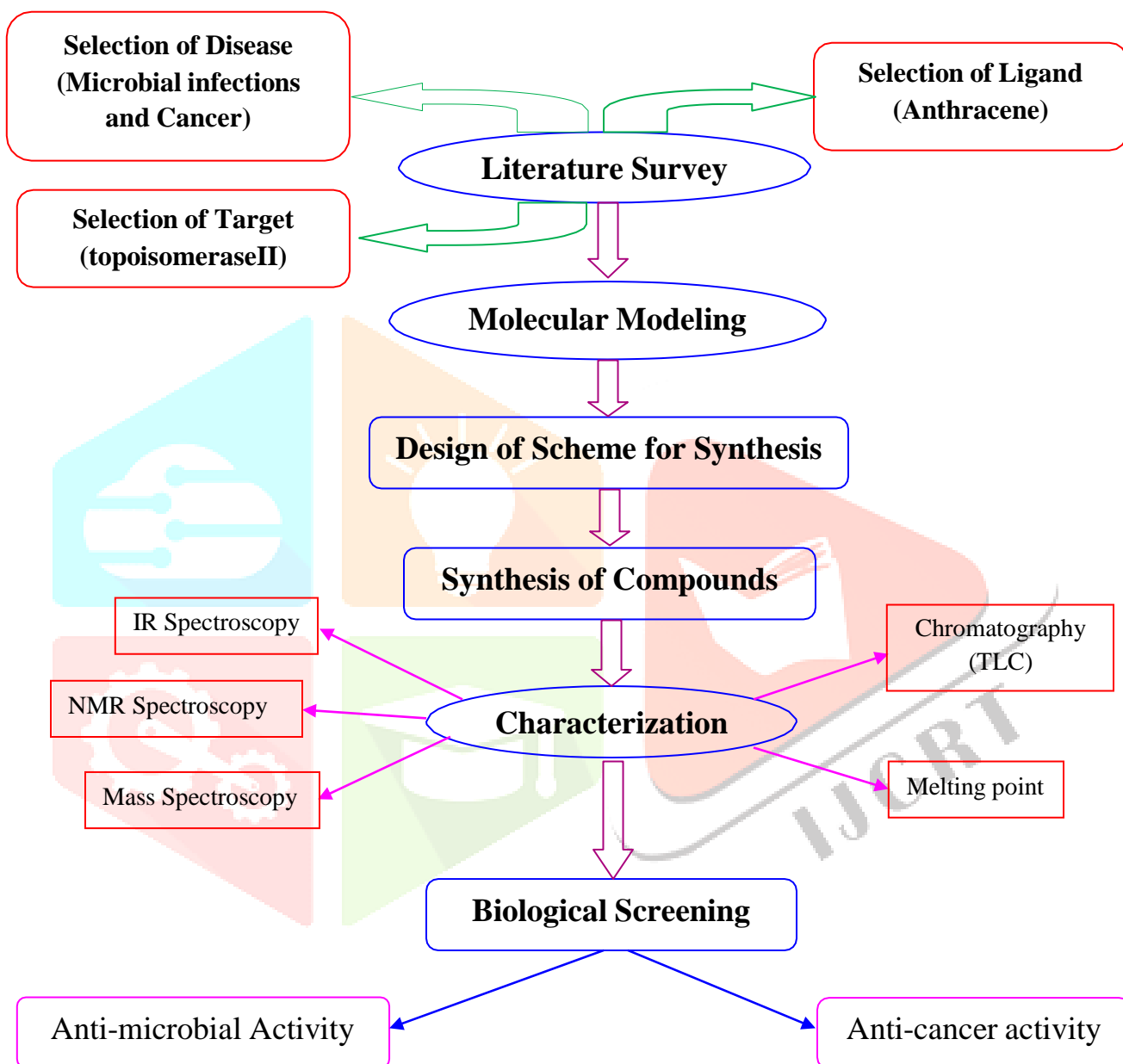
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### Abstract:

New potential chemotherapeutic strategies are required to overcome multidrug resistance (MDR) in cancer. In this study, Antibacterial activities of the synthesized compounds were evaluated against gram (+) *Staphylococcus aureus* and gram (-) *Klebsilla aerogenes* by measuring zone of inhibition. The antifungal activities of the synthesized compounds were evaluated against *Candida albicans* by disc diffusion method. Compounds screened for antibacterial activity against *Bacillus subtilis*, *Escherichia coli*. and antifungal activity against *Candida albicans*, *Aspergillus Niger* by cup plate method.

All the compounds of the series were screened for their antibacterial and antifungal activity studies. Substitution of hydroxyl and halo groups emerged as active in both antibacterial and antifungal screening. The reported antibacterial activity of 3-benzoyl-2-oxo/thioxo-1, 2, 3, 4- tetrahydropyrimidine analogues. 5-Acyl-6-methyl-4-substituted-2-oxo/thioxo-1,2,3,4- tetrahydropyrimidines were synthesized by cyclocondensation reaction between appropriate aldehyde, acetoacetate and urea/thiourea in presence of aluminium chloride and hydrochloric acid which upon treatment with benzoyl chloride in presence of aluminium chloride in presence of pyridine in benzene furnish the title compounds. The compounds have been tested for antibacterial activity against *staphylococcus aureus*.

**PLAN OF WORK:**

## 02. INTRODUCTION

### 2.1 Selection of disease:

#### 2.1.1 Infectious diseases:

Infection is invasion or colonization of the body by pathogenic microorganisms. Human beings have defense mechanism to keep healthy. Unbroken skin and mucous membranes are effective barriers against microbial invasion. Within the body, certain cells and certain specialized proteins called antibodies can work together to destroy microbes. In spite of defense humans are still susceptible to pathogens. Persons who are weak, sick and have cancer or are diabetic have increased susceptibility to infections.

Infectious disease is primary cause of death worldwide. Infectious disease mortality decreased markedly during most of the 20<sup>th</sup> century. However between 1980 and 1992 the death rate from infectious disease increased 58%. The sharp increase in infectious disease deaths in 1918 and 1919 was caused by influenza pandemic, which killed more than 20 million people worldwide. This episode illustrates the volatility of infectious disease death rates.

Infections are classified according to the extent to which host body is affected. Local infection is one in which invading microorganisms are limited to relatively small area of the body. Systemic infection is one in which microorganisms are spread throughout the body by the blood or lymph. Agents of the local infection enter a blood or lymphatic vessel and spread to other specified areas of the body called focal infection. The presence of bacteria in blood is known as bacteremia and if the bacteria actually multiply in blood the condition is called sepsis. Toxemia is the presence of toxins in blood. Viremia refers as presence of viruses in blood. Primary infection is acute infection that causes initial illness. Secondary infection caused by opportunistic pathogen after the primary infection has weakened the body's defense.

##### 2.1.1.1 Diseases:

Disease occurs when an infection results in any change from a state of health. Disease is an abnormal state in which part or all of the body is not properly adjusted or incapable of performing its normal functions. Majority of bacteria are harmless or beneficial, quite a

few bacteria are pathogenic. Pathogenic bacteria are bacteria that cause bacterial infection and are major cause of human death. One of the bacterial diseases with highest disease burden is tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, which kills about 2 million people a year, mostly in sub-Saharan Africa. Pathogenic bacteria contribute to diseases, such as pneumonia, tetanus, typhoid fever, diphtheria, syphilis, leprosy, tuberculosis, cholera and food borne illness. Microorganisms can cause tissue damage by releasing a variety of toxins or destructive enzymes. *Clostridium tetanus* releases toxins which produce shock and sepsis. Each species of pathogen has a characteristic spectrum of interactions with its human hosts. Some organisms, such as *Staphylococcus* or *Streptococcus*, can cause skin infections, pneumonia, meningitis and even overwhelming sepsis, a systemic inflammatory response producing shock, massive vasodilation and death. Yet these organisms are also part of the normal human flora and usually exist on the skin or in the nose without causing any disease at all. Other organisms invariably cause disease in humans, such as the rickettsia, which are obligate intracellular parasites able to grow and reproduce only within the cells of other organisms. One species of rickettsia causes typhus, while another causes rocky mountain spotted fever. *Chlamydia*, another phylum of obligate intracellular parasites, contains species that can cause pneumonia, or urinary tract infection and may be involved in coronary heart disease. *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, and *Mycobacterium avium* are opportunistic pathogens and cause disease mainly in people suffering from immunosuppression or cystic fibrosis. Microbial diseases can be prevented by immunizations and controlled by drugs<sup>1,2</sup>.

### 2.1.1. 2 Development of Disease:

Development of disease follows certain sequence.

**Incubation period:** It is time interval between initial infection and first appearance of any signs or symptoms. Time of incubation period depends on specific microorganism involved, its virulence and number of microorganisms and resistance of the host.

**Prodromal period:** This period is relatively short period that follows the period of incubation in some diseases and characterized by early, mild symptoms of diseases.

**Period of illness:** During period of illness disease is most acute. Person exhibits over signs and symptoms of disease, such as fever, chills, muscle pain, sore throat and GIT disturbances. During the period of illness, number of white blood cells may increase or decrease. Generally patient immune response and other defense mechanisms overcome the pathogen and period of illness ends.

**Period of convalescence:** Person regains strength and the body returns to its prediseased state. Recovery has occurred.

### 2.1.1. 3 Anti-microbial Agents:

An antimicrobial drug is a chemical substance that destroys pathogenic microorganisms with minimal damage to host tissues. Antibiotics have revolutionized the medical care in the 20<sup>th</sup> century. With the discovery of antibiotics people were convinced that infectious diseases might someday be wiped out. Diseases that were once life threatening, such as pneumonia, had become curable. The success of antibiotics in therapy related fields has made them one of the most important products of the drug industry today. However, the emergence of superbugs *i.e.* bacteria that resist the effects of the most powerful antibiotics are posing a great challenge to the field of medicines. Thus scientists are working to find new ways to defeat bacteria that are increasingly resistant to the antibiotics already available.

### 2.1.1. 4 Mechanisms of anti-microbial action:

#### A. Inhibition of cell metabolism

Antibacterial agents which inhibit cell metabolism are called antimetabolites. These compounds inhibit the metabolism of a microorganism, but not the metabolism of the host. They do this by inhibiting an enzyme-catalysed reaction which is present in the bacterial cell, but not in animal cells *e.g.* sulfonamides.

#### B. Inhibition of bacterial cell wall synthesis

Inhibition of cell wall synthesis leads to bacterial cell lysis (bursting) and death *e.g.* penicillin.

#### C. Interactions with the plasma membrane

Some antibacterial agents interact with the plasma membrane of bacterial cells to affect membrane permeability. This has fatal results for the cell *e.g.* tyrothricin.

#### D. Disruption of protein synthesis

Disruption of protein synthesis means that essential enzymes required for the cells survival can no longer be made *e.g.* chloramphenicol.

#### E. Inhibition of nucleic acid transcription and replication

Inhibition of nucleic acid function prevents cell division and the synthesis of essential enzymes *e.g.* nalidixic acid.

##### 2.1.1. 5 Drug resistance:

Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drug, chemicals, or other agents designed to cure or prevent the infection. The bacterial cell may change the structure of its cell membrane and prevent the drug from entering the cell. Alternatively, an enzyme may be produced which destroys the drug. If the drug is targeting a specific enzyme, then the bacterium may synthesize an excess of the enzyme. Thus the bacteria survive and continue to multiply causing more harm. Widespread use of antibiotics promotes the spread of antibiotic resistance. Definition of resistance is based on bacterial susceptibility to antibacterial agent. This is typically achieved by determining the minimal inhibitory concentration that inhibits the growth of bacteria. Thus resistance is defined as “bacteria that are not inhibited by the systemic concentration of the agent with normal dosage schedule and fall in the minimal inhibitory concentration ranges”. Likewise the multiple drug resistance is defined as the resistance to two or more drugs or drug classes.

##### 2.1.1. 6 Mechanism of antibiotic resistance:

Bacteria may display antibiotic resistance by one or more of the following mechanisms:

- A. They may lack a target for the antibiotic. For example Chlamydia does not have peptidoglycan and are not affected by the action of penicillin.
- B. The antibiotic target may be inaccessible. For example peptidoglycan in Gram- negative bacteria is not easily entered to penicillins that cannot penetrate the Gram-negative outer membrane of the cell and efflux pumps can actively pump out antibiotics from cells. For example resistance to tetracyclines by Gram negative bacterial strain occurs in this way.
- C. The antibiotic target may be modified to prevent the action of the drug. For example trimethoprim resistance is affected by alterations in to the DHFR target

enzyme. Quinolone resistance is manifest by mutations in the DNA gyrase, which prevent the formation of drug-ligand complex.

- D. The antibiotic may be chemically modified. For example chloramphenicol resistance is mostly affected by acetylation by using chloramphenicol acetyltransferase enzyme<sup>3</sup>.

### 2.1.2 Cancer:

Approximately 29% of world's unhealthy population is suffering from cancer. It shoes its major role in deaths worldwide. An estimated 12.66 million people were diagnosed with cancer across the world in 2008 .This equates to around 188 cases for every 100,000 people (using the crude rate). The number of new cases ranged from 67,000 in middle Africa to 3.72 million in eastern Asia. As expected from the size of Asia's population, the majority of cases (48%) occurred there. Just four cancer sites – lung, female breast, colorectal and stomach accounted for two-fifths (41%) of the world's total<sup>4</sup>.

Cancer is a disease in which there is uncontrolled multiplication and spread within the body of the body's own cells. It is a potentially fatal disease caused mainly by environmental factors that mutate genes encoding critical cell-regulatory proteins. There are over 200 different known cancers that afflict humans. The cancer-causing agents (carcinogens) can be present in food and water, in the air, and in chemicals and sunlight that people are exposed to. Since epithelial cells cover the skin, line the respiratory and alimentary tracts, and metabolize ingested carcinogens, it is not surprising that over 90% of cancers occurs in epithelia<sup>5</sup>.

In terms of behavior, tumours are either 'benign' or 'malignant'. Benign tumours are generally slow-growing expansive masses that compress rather than invade surrounding tissue. As such they generally pose little threat, except when growing in a confined space like the skull, and can usually be readily excised. However, many so- called benign tumours have malignant potential, notably those occurring in the large intestine, and these should be removed before malignancy develops. Malignant tumours are usually rapidly growing, invading surrounding tissue and, most significantly,

colonizing distant organs. The ability of tumour cells to detach from the original mass (the primary tumour) and set up a metastasis (secondary tumour) discontinuous with the primary is unequivocal proof of malignancy. Tumours are also classified according to their tissue of origin; recognition of the parent tissue in a lymph node metastasis could establish the location of a hitherto undiagnosed primary tumour. Much of cell behaviour (division and differentiation) is governed by the effects of polypeptide growth factors which, because of their water-soluble nature, cannot diffuse through the plasma membrane of the cell, instead interacting with membrane-bound glycoprotein receptors that transduce the first message (the growth factor or ligand) into a series of intracellular signals that promote or inhibit the transcription of specific genes<sup>6</sup>.

### 2.1.2.1 Cancer pathophysiology:

Cancers are caused by a series of mutations. Each mutation alters the behavior of the cell somewhat. Cancer is fundamentally a disease of failure of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, the genes which regulate cell growth and differentiation must be altered. The affected genes are divided into two broad categories. Oncogenes are genes which promote cell growth and reproduction. Tumor suppressor genes are genes which inhibit cell division and survival. Malignant transformation can occur through the formation of novel oncogenes, the inappropriate over-expression of normal oncogenes, or by the under-expression or disabling of tumor suppressor genes. Typically, changes in many genes are required to transform a normal cell into a cancer cell<sup>7</sup>.

Genetic changes can occur at different levels and by different mechanisms. The gain or loss of an entire chromosome can occur through errors in mitosis. More common are mutations, which are changes in the nucleotide sequence of genomic DNA. Large-scale mutations involve the deletion or gain of a portion of a chromosome. Genomic amplification occurs when a cell gains many copies (often 20 or more) of a small chromosomal locus, usually containing one or more oncogenes and adjacent genetic material. Translocation occurs when two separate chromosomal regions become abnormally fused, often at a characteristic location. A well-known example of this is the Philadelphia chromosome, or translocation of chromosomes 9 and 22, which occurs in

chronic myelogenous leukemia, and results in production of the BCR-abl fusion protein, an oncogenic tyrosine kinase. Small-scale mutations include point mutations, deletions, and insertions, which may occur in the promoter region of a gene and affect its expression, or may occur in the gene's coding sequence and alter the function or stability of its protein product. Disruption of a single gene may also result from integration of genomic material from a DNA virus or retrovirus, and resulting in the expression of *viral* oncogenes in the affected cell and its descendants. Replication of the enormous amount of data contained within the DNA of living cells will probabilistically result in some errors (mutations). Complex error correction and prevention is built into the process, and safeguards the cell against cancer. If significant error occurs, the damaged cell can "self-destruct" through programmed cell death, termed apoptosis. If the error control processes fail, then the mutations will survive and be passed along to daughter cells. Some environments make errors more likely to arise and propagate. Such environments can include the presence of disruptive substances called carcinogens, repeated physical injury, heat, ionising radiation, or hypoxia<sup>8</sup>.

#### 2.1.2.2 Selection of Specific Cancer Type: Breast Cancer:

Female breast cancer incidence rates vary nearly five-fold across the regions of the world. In 2008, rates ranged from around 20 per 100,000 in Eastern and Middle Africa to 90 per 100,000 in Western Europe. The countries with the highest incidence rates in 2008 were Belgium and Denmark (109 and 101 per 100,000, respectively). The incidence rate for UK women was high at 11th highest out of 184 countries worldwide.

Breast cancer is the most common cause of death from cancer in women worldwide, estimated to be responsible for almost 460,000 deaths in 2008. There is less variation in female breast cancer mortality across the regions of the world, largely due to better survival in the (high incidence) developed countries, with rates ranging from 6 per 100,000 in Eastern Asia to 19 per 100,000 in Southern and Western Africa in 2008<sup>9</sup>.

By studying types and statistics of various cancers, it is concluded that Breast Cancer is era where lot more work is needed as it ranks second in all cancer types and

first in cancers associated with women worldwide. That's why we have particularly selected breast cancer as our target of research.

## Breast Cancer

Breast cancer is a malignant tumor that starts in the cells of the breast. A malignant tumor is a group of cancer cells that can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. The disease occurs almost entirely in women, but men can get it too.

The first noticeable symptom of breast cancer is typically a lump that feels different from the rest of the breast tissue. More than 80% of breast cancer cases are discovered when the woman feels a lump. The earliest breast cancers are detected by a mammogram. Lumps found in lymph nodes located in the armpits can also indicate breast cancer. Indications of breast cancer other than a lump may include thickening different from the other breast tissue, one breast becoming larger or lower, a nipple changing position or shape or becoming inverted, skin puckering or dimpling, a rash on or around a nipple, discharge from nipple/s, constant pain in part of the breast or armpit, and swelling beneath the armpit or around the collarbone. Pain ("mastodynia") is an unreliable tool in determining the presence or absence of breast cancer, but may be indicative of other breast health issues. Inflammatory breast cancer is a particular type of breast cancer which can pose a substantial diagnostic challenge. Symptoms may resemble a breast inflammation and may include itching, pain, swelling, nipple inversion, warmth and redness throughout the breast, as well as an orange-peel texture to the skin referred to as *peau d'orange*; the absence of a discernible lump delays detection dangerously. Another reported symptom complex of breast cancer is Paget's disease of the breast. This syndrome presents as eczematoid skin changes such as redness and mild flaking of the nipple skin. As Paget's advances, symptoms may include tingling, itching, increased sensitivity, burning, and pain. There may also be discharge from the nipple. Approximately half of women diagnosed with Paget's also have a lump in the breast. In rare cases, what initially appears as a fibroadenoma (hard movable lump) could in fact be a phyllodes tumor. Phyllodes tumors are formed within the stroma (connective tissue) of

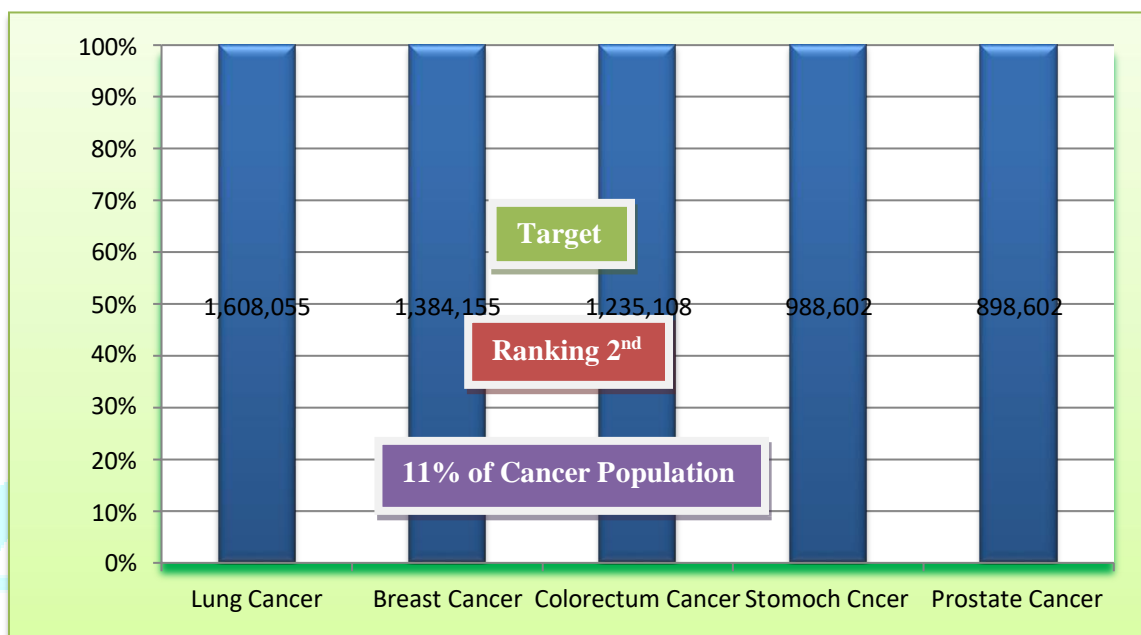
the breast and contain glandular as well as stromal tissue. Phyllodes tumors are not staged in the usual sense; they are classified on the basis of their appearance under the microscope as benign, borderline, or malignant.

Occasionally, breast cancer presents as metastatic disease, that is, cancer that has spread beyond the original organ. Metastatic breast cancer will cause symptoms that depend on the location of metastasis. Common sites of metastasis include bone, liver, lung and brain. Unexplained weight loss can occasionally herald an occult breast cancer, as can symptoms of fevers or chills. Bone or joint pains can sometimes be manifestations of metastatic breast cancer, as can jaundice or neurological symptoms. These symptoms are called *non-specific*, meaning they could be manifestations of many other illnesses. Most symptoms of breast disorders, including most lumps, do not turn out to represent underlying breast cancer. Less than 20% of lumps for example are cancer and benign breast diseases such as mastitis and fibroadenoma of the breast are more common causes of breast disorder symptoms. Nevertheless, the appearance of a new symptom should be taken seriously by both patients and their doctors, because of the possibility of an underlying breast cancer at almost any age<sup>10</sup>.

### 2.1.2.3 Breast Cancer Statistics:

Breast cancer is by far the most common cancer diagnosed in women worldwide (ranking second in both sexes combined). An estimated 1.38 million women across the world were diagnosed with breast cancer in 2008 (Fig. 2.2) accounting for nearly a quarter (23%) of all cancers diagnosed in women (11% of the total in men and women). Incidence is generally high in the developed countries and markedly lower in developing countries, though differences in population sizes mean that approximately equal numbers of cases were diagnosed in the developed and developing regions in 2008 (around 690,000 cases each). Breast cancer incidence has increased in most countries worldwide in the last decades, with the most rapid increases occurring in many of the developing countries. Reproductive behavior and the use of exogenous hormones, as well as differences in weight, exercise, diet and alcohol consumption, are thought to underlie the differences.

It has been estimated that breast cancer rates in developed countries could be half that of current rates if women had more children and breastfed for longer<sup>4</sup>.



**Fig 2.1 Cancer Type Incidence Statistics**

#### 2.1.2.4 Breast cancer pathophysiology:

Normal cells divide as many times as needed and stop. They attach to other cells and stay in place in tissues. Cells become cancerous when they lose their ability to stop dividing, to attach to other cells, to stay where they belong, and to die at the proper time. Normal cells will commit cell suicide (apoptosis) when they are no longer needed. Until then, they are protected from cell suicide by several protein clusters and pathways. One of the protective pathways is the PI3K/AKT pathway; another is the RAS/MEK/ERK pathway. Sometimes the genes along these protective pathways are mutated in a way that turns them permanently "on", rendering the cell incapable of committing suicide when it is no longer needed. This is one of the steps that causes cancer in combination with other mutations. Normally, the PTEN protein turns off the PI3K/AKT pathway when the cell is ready for cell suicide. In some breast cancers, the gene for the PTEN protein is mutated, so the PI3K/AKT pathway is stuck in the "on" position, and the cancer cell does not commit suicide. Mutations that can lead to breast cancer have been experimentally linked to estrogen exposure. Failure of immune surveillance, the removal of malignant cells

throughout one's life by the immune system. Abnormal growth factor signaling in the interaction between stromal cells and epithelial cells can facilitate malignant cell growth. In breast adipose tissue, over expression of leptin leads to increased cell proliferation and cancer.

In the United States, 10 to 20 percent of patients with breast cancer and patients with ovarian cancer have a first- or second-degree relative with one of these diseases. The familial tendency to develop these cancers is called hereditary breast—ovarian cancer syndrome. The best known of these, the BRCA mutations, confer a lifetime risk of breast cancer of between 60 and 85 percent and a lifetime risk of ovarian cancer of between 15 and 40 percent. Some mutations associated with cancer, such as *p53*, *BRCA1* and *BRCA2*, occur in mechanisms to correct errors in DNA. These mutations are either inherited or acquired after birth. Presumably, they allow further mutations, which allow uncontrolled division, lack of attachment, and metastasis to distant organs. However there is strong evidence of residual risk variation that goes well beyond hereditary BRCA gene mutations between carrier families. This is caused by unobserved risk factors. This implicates environmental and other causes as triggers for breast cancers. The inherited mutation in *BRCA1* or *BRCA2* genes can interfere with repair of DNA cross links and DNA double strand breaks (known functions of the encoded protein). These carcinogens cause DNA damage such as DNA cross links and double strand breaks that often require repairs by pathways containing *BRCA1* and *BRCA2*. However, mutations in *BRCA* genes account for only 2 to 3 percent of all breast cancers. About half of hereditary breast—ovarian cancer syndromes involve unknown genes<sup>10</sup>.

### 2.1.3 Selection of target: Dihydrofolate reductase enzyme:

Dihydrofolate reductase, or DHFR, is an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as electron donor, which can be converted to the kinds of tetrahydrofolate cofactors used in 1-carbon transfer chemistry. In humans, the DHFR enzyme is encoded by the *DHFR* gene.<sup>11, 12</sup> It is found in the q11→q22 region of chromosome 5.<sup>13</sup> Bacterial species possesses distinct DHFR enzymes (based on their

pattern of binding diaminoheterocyclic molecules), but mammalian DHFRs are highly similar.<sup>14</sup>

DHFR has a critical role in regulating the amount of tetrahydrofolate in the cell. Tetrahydrofolate and its derivatives are essential for purine and thymidylate synthesis, which are important for cell proliferation and cell growth.<sup>15</sup> DHFR plays a central role in the synthesis of nucleic acid precursors, and it has been shown that mutant cells that completely lack DHFR require glycine, an amino acid, and thymidine to grow.<sup>[16]</sup> DHFR has also been demonstrated as an enzyme involved in the salvage of tetrahydrobiopterin from dihydrobiopterin.<sup>17</sup>

DHFR can be targeted in the treatment of cancer. DHFR is responsible for the levels of tetrahydrofolate in a cell, and the inhibition of DHFR can limit the growth and proliferation of cells that are characteristic of cancer. Methotrexate, a competitive inhibitor of DHFR, is one such anticancer drug that inhibits DHFR.<sup>18</sup> Other drugs include trimethoprim and pyrimethamine. These three are widely used as antitumor and antimicrobial agents.<sup>19</sup> Whether or not these are potent anticancer agents is unclear.

Trimethoprim has shown to have activity against a variety of Gram-positive bacterial pathogens. However, resistance to trimethoprim and other drugs aimed at DHFR can arise due to a variety of mechanisms, limiting the success of their therapeutical uses. Resistance can arise from DHFR gene amplification, mutations in DHFR, decrease in the uptake of the drugs, among others. Regardless, trimethoprim and sulfamethoxazole in combination has been used as an antibacterial agent for decades.<sup>20</sup>

Folic acid is necessary for growth,<sup>21</sup> and the pathway of the metabolism of folic acid is a target in developing treatments for cancer and microbial infections. DHFR is one such target. A regimen of fluorouracil, doxorubicin, and methotrexate was shown to prolong survival in patients with advanced gastric cancer. Further studies into inhibitors of DHFR can lead to more ways to treat cancer and microbial infections.

### 03. Research envisaged

#### 03.1 Need of work:

With a wide range of antibacterial agents available in medicine, it may seem surprising that medicinal chemists are still actively seeking new and improved antibacterial agents. The reason is mainly due to emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens which make the treatment of infectious diseases an important and pressing global problem. Drugs, which are active today, may become inactive after several years. Though some of the drugs are highly effective, they are associated with toxic side effects. That's why bacterial infection is one of challenge due to drug resistance and toxicity<sup>1</sup>.

Approximately 29% of world's unhealthy population is suffering from cancer. It shows its major role in deaths worldwide. An estimated 12.66 million people were diagnosed with cancer across the world in 2008. This equates to around 188 cases for every 100,000 people (using the crude rate). The number of new cases ranged from 67,000 in middle Africa to 3.72 million in eastern Asia. As expected from the size of Asia's population, the majority of cases (48%) occurred there. Just four cancer sites – lung, female breast, colorectal and stomach accounted for two-fifths (41%) of the world's total<sup>2</sup>.

Therefore, a substantial research for the discovery and synthesis of new more and more potent, safer drugs than the existing ones classes of antimicrobial and anti-cancer agents is needed.

#### 03.2 Aim and Objective:

The literature reveals that derivatives of anthracene possess versatile biological activities such as antibacterial, antifungal, anti-inflammatory, anticancer, antiviral, antioxidant, calcium channel blocker and antihypertensive. Anthracene derivatives are also reported to have powerful activities such as antituberculosis, anticancer, antiarrhythmic, antibacterial, antifungal and anticonvulsant activities. Fluoroquinolone derivatives are also reported to have powerful antimicrobial activity. It has been observed that the incorporation of more than one bioactive heterocyclic moiety into a single framework

may result into the production of novel heterocycles with enhanced bioactivity and biological properties such as potency, selectivity, toxicity and metabolic stability. It is well known that anthracene and fused heterocyclic anthracene derivatives are of great biological interest, especially as antiviral, antitumor and antimicrobial agents. This prompts us to synthesize a series of novel fused anthracene derivatives and evaluate their antimicrobial activity<sup>3-7</sup>.

The chemistry and the synthesis of anthracene 9,11 dione have been attracting widespread attention in recent years. The present popularity of these anthracene 9,11 dione is mainly due to their close structural relationship to the clinically important 8,10 diaza benzo anthracene 9,11 dione anti-microbial and anti-cancer agents. Anthracene 9,11 dione is known as a versatile heterocyclic compound which has been subjected to a large variety of structural modifications in order to synthesize derivatives with different biological properties. Their various condensed derivatives are reported to possess calcium antagonist, anti-inflammatory, analgesic, antitumor, antidepressant, antibacterial, and antifungal effects. The anthracene 9,11 dione can be synthesized by biginelli reaction by condensation of aromatic aldehyde, beta naphthol and dimedone. Multi-component reactions (MCRs) are useful organic reactions involving three or more starting materials which react to give a product. They constitute a major part in the present day organic synthesis with advantages ranging from lower reaction times, increased reaction rates to higher yields and reproducibility<sup>8-9</sup>.

The versatile utility of anthracene has pharmacological interest, prompts us to prepare series of anthracene derivative.

In the present work we aim:

1. To perform molecular modeling by using Vlife MDS 4.3 software
2. To establish the scheme for synthesis of selected compounds from molecular modeling.
3. To achieve the synthesis of series of anthracene 9,11 dione.
4. To characterize the synthesized compound by physical data melting point, thin layer chromatography and spectral analysis like infrared spectroscopy, nuclear magnetic resonance spectroscopy and mass spectroscopy.

5. To perform biological screening of the synthesized compounds for % topoisomerase II enzyme inhibition assay, antimicrobial activity and in-vitro anti- cancer activity.
6. To carry out 2D QSAR and 3D QSAR for study of the compounds for % topoisomerase II enzyme inhibition assay and anti-microbial activity.

### 03.1 Topoisomerase enzyme receptor structure

**Topoisomerases** are enzymes that regulate the overwinding or underwinding of [DNA](#). The winding problem of DNA arises due to the intertwined nature of its double-helical structure. During [DNA replication](#) and [transcription](#), DNA becomes overwound ahead of a replication fork. If left unabated, this torsion would eventually stop the ability of RNA & DNA polymerase involved in these processes to continue down the DNA strand.

In order to prevent and correct these types of topological problems caused by the double helix, topoisomerases bind to either single-stranded or double-stranded DNA and cut the phosphate backbone of the DNA. This intermediate break allows the DNA to be untangled or unwound, and, at the end of these processes, the DNA backbone is resealed again. Since the overall chemical composition and connectivity of the DNA do not change, the tangled and untangled DNAs are chemical isomers, differing only in their global topology, thus their name. Topoisomerases are [isomerase enzymes](#) that act on the [topology of DNA](#).<sup>[1]</sup>

Bacterial topoisomerase and human topoisomerase proceed via the same mechanism for replication and transcription.

#### Discovery

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**James C. Wang** was the first to discover a topoisomerase when he identified *E. coli* topoisomerase I. Topo EC-codes are as follows: type I, [EC 5.99.1.2](#); type II: [EC 5.99.1.3](#). His discovery was made in the 1970s.

#### Function

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The [double-helical](#) configuration that DNA strands naturally reside, makes them difficult to separate and yet they must be separated by [helicase](#) enzymes, if other enzymes are

to transcribe the sequences that encode proteins, or if chromosomes are to be replicated. In so-called circular DNA, in which double-helical DNA is bent around and joined in a circle, the two strands are topologically linked, or knotted. Otherwise identical loops of DNA, having different numbers of twists, are topoisomers, and cannot be interconverted by any process that does not involve the breaking of DNA strands. Topoisomerases catalyze and guide the unknotting or unkinking of DNA<sup>[2]</sup> by creating transient breaks in the DNA using a conserved Tyrosine as the catalytic residue.<sup>[1]</sup>

The insertion of (viral) DNA into chromosomes and other forms of recombination can also require the action of topoisomerases.

### Clinical significance

Many drugs operate through interference with the topoisomerases [1]. The broad-spectrum fluoroquinolone antibiotics act by disrupting the function of bacterial type II topoisomerases. These small molecule inhibitors act as efficient anti-bacterial agents by hijacking the natural ability of topoisomerase to create breaks in chromosomal DNA.

Some chemotherapy drugs called topoisomerase inhibitors work by interfering with mammalian-type eukaryotic topoisomerases in cancer cells. This induces breaks in the DNA that ultimately lead to programmed cell death (apoptosis). This DNA-damaging effect, outside of its potential curative properties, may lead to secondary neoplasms in the patient.

Topoisomerase I is the antigen recognized by Anti Scl-70 antibodies in scleroderma.

### Topological problems

There are three main types of topology: supercoiling, knotting, and catenation. Outside of the essential processes of replication or transcription, DNA must be kept as compact as possible, and these three states help this cause. However, when transcription or replication occurs, DNA must be free, and these states seriously hinder the processes. In addition, during replication, the newly replicated duplex of DNA and the original duplex of DNA become intertwined and must be completely separated in order to ensure genomic integrity as a cell divides. As a transcription bubble proceeds, DNA ahead of the transcription fork becomes overwound, or positively supercoiled, while DNA behind the transcription bubble becomes underwound, or negatively supercoiled. As replication occurs, DNA ahead of the

replication bubble becomes positively supercoiled, while DNA behind the replication fork becomes entangled forming precatenanes. One of the most essential topological problems occurs at the very end of replication, when daughter chromosomes must be fully disentangled before mitosis occurs. Topoisomerase IIA plays an essential role in resolving these topological problems.

### Classes

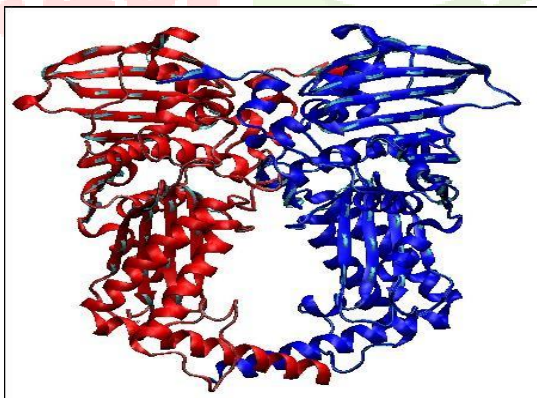
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Topoisomerases can fix these topological problems and are separated into two types depending on the number of strands cut in one round of action:<sup>[3]</sup> Both these classes of enzyme utilize a conserved tyrosine. However these enzymes are structurally and mechanistically different.

- A **type I topoisomerase** cuts one strand of a DNA double helix, relaxation occurs, and then the cut strand is reannealed. Cutting one strand allows the part of the molecule on one side of the cut to rotate around the uncut strand, thereby reducing stress from too much or too little twist in the helix. Such stress is introduced when the DNA strand is "supercoiled" or uncoiled to or from higher orders of coiling. Type I topoisomerases are subdivided into two subclasses: type IA topoisomerases, which share many structural and mechanistic features with the type II topoisomerases, and type IB topoisomerases, which utilize a controlled rotary mechanism. Examples of type IA topoisomerases include topo I and topo III. In the past, type IB topoisomerases were referred to as eukaryotic topo I, but IB topoisomerases are present in all three domains of life. Like type II topoisomerases, type IA topoisomerases form a covalent intermediate with the 5' end of DNA, whereas the IB topoisomerases form a covalent intermediate with the 3' end of DNA. Recently, a type IC topoisomerase has been identified, called topo V. While it is structurally unique from type IA and IB topoisomerases, it shares a similar mechanism with type IB topoisomerase.
- A **type II topoisomerase** cuts both strands of one DNA double helix, passes another unbroken DNA helix through it, and then **reanneals** the cut strands. This class is also split into two subclasses: type IIA and type IIB topoisomerases, which possess similar structure and mechanisms. Examples of type IIA topoisomerases include eukaryotic topo II, E. coli gyrase, and E. coli topo IV. Examples of type IIB topoisomerase include topo VI. Type II topoisomerases utilize ATP hydrolysis.

Topoisomerase	IA	IB	IIA	IIB
Metal Dependence	Yes	No	Yes	Yes
ATP Dependence	No	No	Yes	Yes
Single- or Double-Stranded cleavage?	SS	SS	DS	DS
Cleavage Polarity	5'	3'	5'	5'
Change in L	$\pm 1$	$\pm N$	$\pm 2$	$\pm 2$

Both type I and type II topoisomerases change the **linking number** (L) of DNA. Type IA topoisomerases change the linking number by one, type IB and type IC topoisomerases change the linking number by any integer, whereas type IIA and type IIB topoisomerases change the linking number by two.



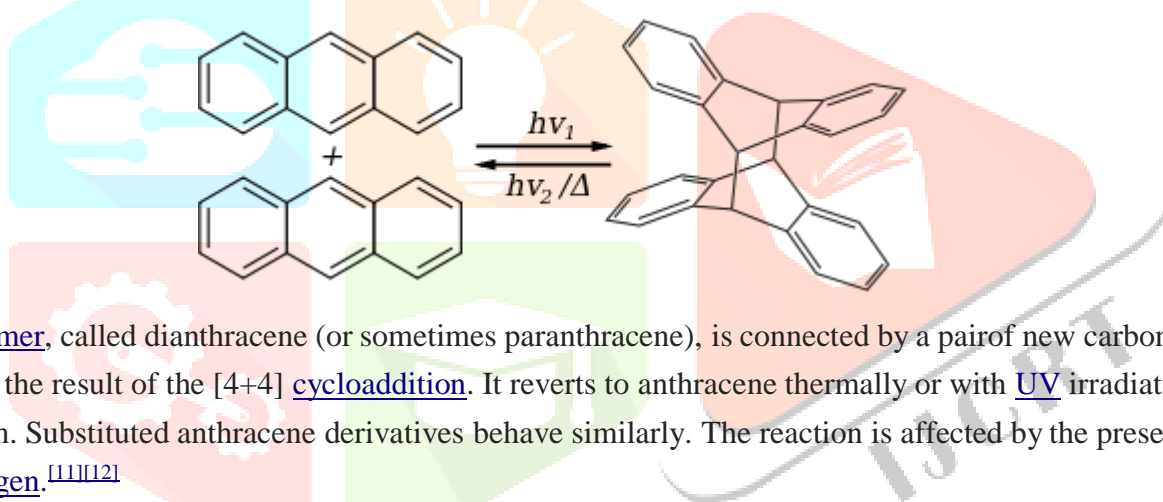
## 04. SELECTION OF LIGAND SKELETON

### 4.1 Chemistry of Anthracene

**Anthracene** is a solid **polycyclic aromatic hydrocarbon** (PAH) of formula  $C_{14}H_{10}$ , consisting of three fused **benzene** rings. It is a component of **coal tar**. Anthracene is used in the **production** of the **red dye alizarin** and other dyes. Anthracene is colorless but exhibits a blue (400-500 nm peak) **fluorescence** under **ultraviolet** light. Coal tar, which contains around 1.5% anthracene, remains a major source of this material. Common impurities are **phenanthrene** and **carbazole**. A classic laboratory method for the preparation of anthracene is by cyclodehydration of o-methyl- or o-methylene-substituted diarylketones in the so-called **Elbs reaction**.

It may also occur in the **interstellar medium**.<sup>[9]</sup> More than 20% of the **carbon** in the universe may be associated with PAHs, including anthracene.<sup>[1]</sup>

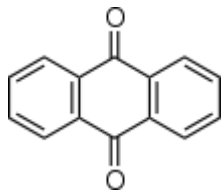
Anthracene **photodimerizes** by the action of **UV** light:



The **dimer**, called dianthracene (or sometimes paranthracene), is connected by a pair of new carbon-carbon bonds, the result of the [4+4] **cycloaddition**. It reverts to anthracene thermally or with **UV** irradiation below 300 nm. Substituted anthracene derivatives behave similarly. The reaction is affected by the presence of **oxygen**.<sup>[11][12]</sup>

Reduction of anthracene with alkali metals yields the deeply colored radical anion salts  $M^+[anthracene]^-$  ( $M = Li, Na, K$ ). Hydrogenation gives 9,10-**dihydroanthracene**, preserving the aromaticity of the two flanking rings.

Chemical **oxidation** occurs readily, giving **anthraquinone**,  $C_{14}H_8O_2$  (below), for example using **hydrogen peroxide** and **vanadyl acetylacetonate**.<sup>[13]</sup>



Anthracene, a wide band-gap [organic semiconductor](#) is used as a [scintillator](#) for detectors of high energy [photons](#), [electrons](#) and [alpha particles](#). Plastics, such as [polyvinyltoluene](#), can be doped with anthracene to produce a plastic scintillator that is approximately water-equivalent for use in [radiation therapy dosimetry](#). Anthracene's [emission spectrum](#) peaks at between 400 nm and 440 nm.

It is also used in [wood preservatives](#), [insecticides](#), and coating [materials](#).

Anthracene is one of the three components (the other two being potassium perchlorate and sulfur) which are used to produce the black smoke released during a Papal Conclave.<sup>1</sup>**Derivatives**

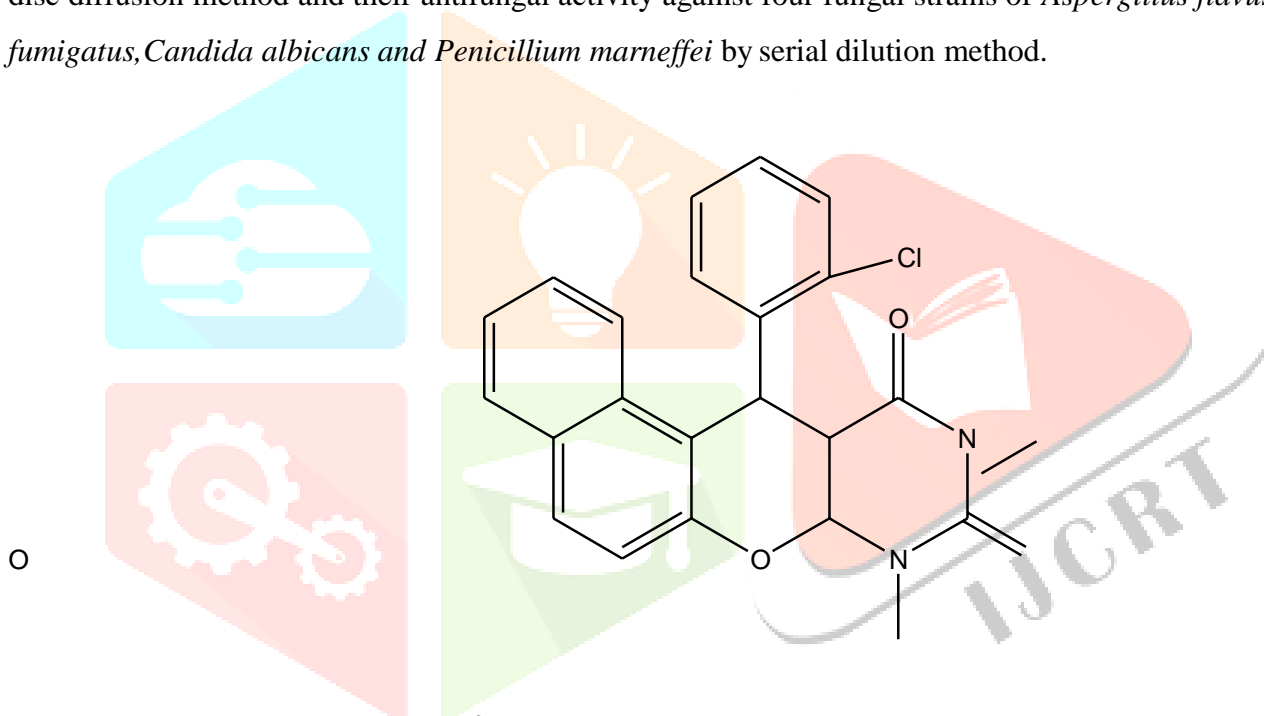
A variety of anthracene derivatives find specialized uses. Derivatives having a [hydroxyl group](#) are 1-hydroxyanthracene and 2-hydroxyanthracene, homologous to [phenol](#) and [naphthols](#), and hydroxyanthracene (also called anthrol, and anthracenol)<sup>[16][17]</sup> are [pharmacologically](#) active. Anthracene may also be found with multiple hydroxyl groups, as in [9,10-dihydroxyanthracene](#).

## 05. LITERATURE REVIEW

### 5.1 Antimicrobial Activity:

5.1.1 M. Ashok *et al*<sup>1</sup> (2007) were reported convenient one pot synthesis of some novel derivatives of anthracene 9,11 dione possessing 2-chlorobenzaldehyde moiety and evaluation of their antibacterial and antifungal activities.

All the synthesized compounds were screened for their *in vitro* antibacterial activity against standard strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, *Klebsiella pneumonia* by disc diffusion method and their antifungal activity against four fungal strains of *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Penicillium marneffe* by serial dilution method.

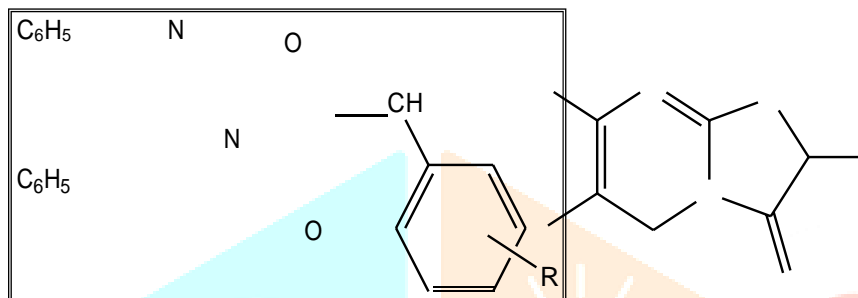


5.1.2 J. D. Akbari *et al*<sup>2</sup> (2008) were reported synthesis and antimicrobial activity of some new anthracene 9,11 dione derivatives and compounds exhibited significant inhibition on bacterial and fungal growth.

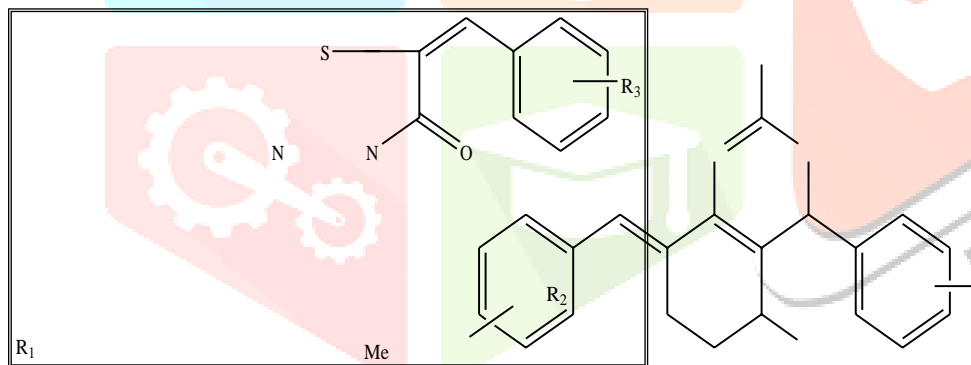
The *in vitro* antimicrobial activity of the synthesized compounds was tested against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* using the disc diffusion method.

4.1.3 P. Muthumani *et al*<sup>3</sup> (2011) were reported synthesis and antimicrobial activity of some novel pyrimidine derivatives.

Antibacterial activities of the synthesized compounds were evaluated against gram (+) *Staphylococcus aureus* and gram (-) *Klebsilla aerogenes* by measuring zone of inhibition. The antifungal activities of the synthesized compounds were evaluated against *Candida albicans* by disc diffusion method.

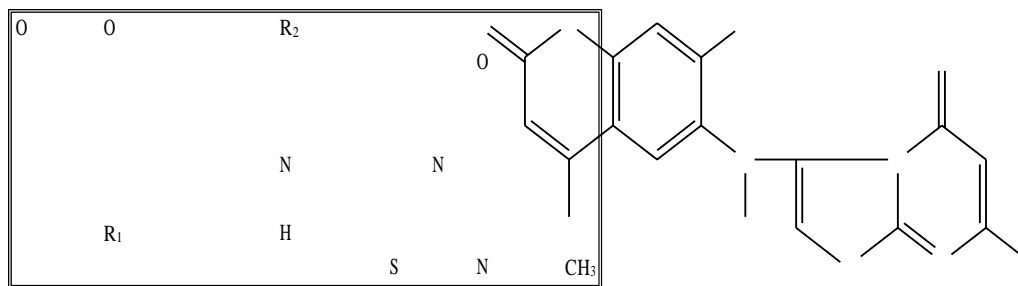


4.1.4 A. E. Galil *et al*<sup>4</sup> (2010) reported antimicrobial activity of 2,6-dibenzylidene-3-methylcyclohexanone.



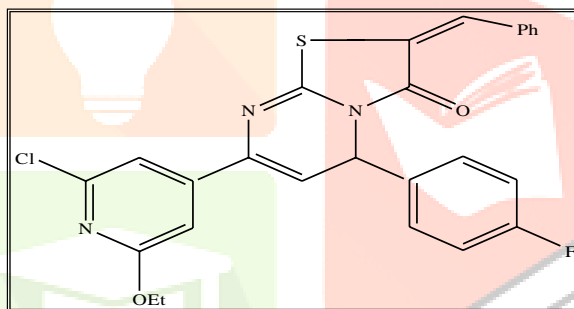
Author concludes that thiazolo pyrimidine and substituted thiazolo pyrimidine moieties fused to 3-methylcyclohexane ring are essential for antimicrobial activities.

4.1.5 V.V. Mulwad *et al*<sup>5</sup> (2010) were shown synthesis and antimicrobial activity of 7-methyl-3-(2-oxo-2H-benzopyran-6-yl)-5H-1, 4-thiazolo-[3, 2-a] pyrimidin-5-one and its derivatives.

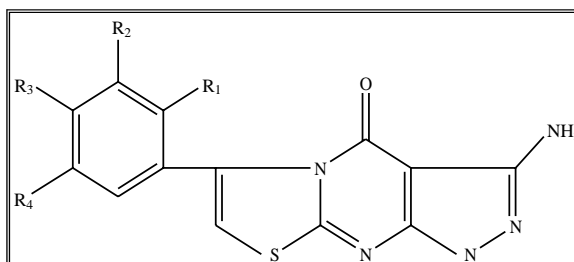


Compounds screened for antibacterial activity against *Bacillus subtilis*, *Escherichia coli*. and antifungal activity against *Candida albicans*, *Aspergillus Niger* by cup plate method.

4.1.6 A.E. Galil, E. Amr *et al*<sup>6</sup> (2009) was reported synthesis and antimicrobial activity of thiopyrimidine and thiazolopyrimidine derivatives.

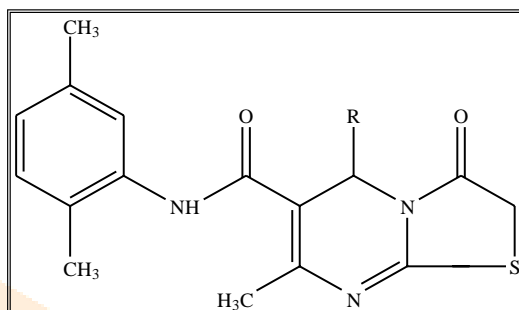


4.1.7 C. N. Khobragade *et al*<sup>7</sup> (2009) were reported synthesis and antimicrobial activity of novel pyrazolo [3,4-d] pyrimidine derivatives.

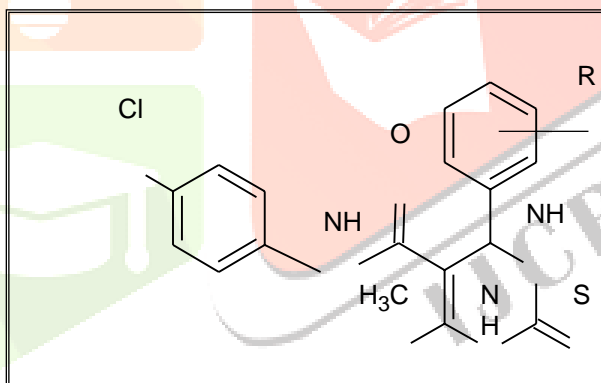


All the compounds of the series were screened for their antibacterial and antifungal activity studies. Substitution of hydroxyl and halo groups emerged as active in both antibacterial and antifungal screening.

4.1.8 M. J. Solanki *et al*<sup>8</sup> (2011) were reported synthesis and antimicrobial activity of novel pyrazolo [3,4-d] pyrimidine derivatives.

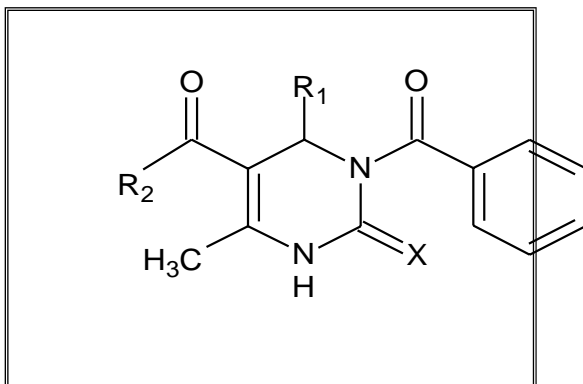


4.1.9 Joshi K. K. *et al*<sup>9</sup> (2009) were reported the synthesis N-(4-chlorophenyl)-6-methyl- 4-aryl-2-thioxo- 1, 2, 3, 4- tetrahydropyrimidine-5-carboxamide derivatives as antimicrobial activity.



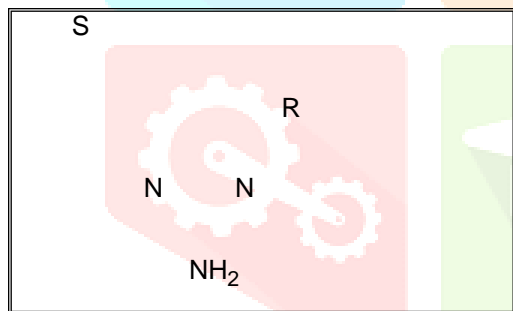
4.1.10 Ramesh L. Sawant *et al*<sup>10</sup>(2008) were reported antibacterial activity of 3-benzoyl-2-oxo/thioxo-1, 2, 3, 4-tetrahydropyrimidine analogues. 5-Acyl-6-methyl-4-substituted-2- oxo/thioxo-1,2,3,4-tetrahydropyrimidines were synthesized by cyclocondensation reaction between appropriate aldehyde, acetoacetate and urea/thiourea in presence of aluminium chloride and hydrochloric acid which upon treatment with benzoyl chloride in presence of aluminium chloride in presence of pyridine in benzene furnish the title

compounds. The compounds have been tested for antibacterial activity against *staphylococcus aureus*.



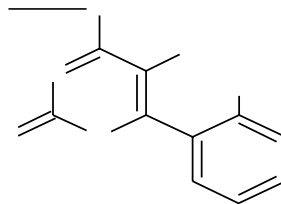
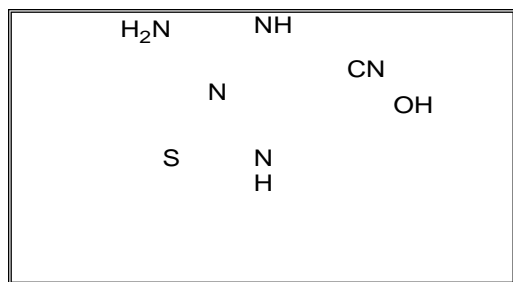
## 4.2 Anticancer Activity

**4.2.1** Kulakarni S. V *et al*<sup>11</sup> (2009) were reported Design, synthesis and anticanceractivity of some new pyrimidine derivatives.

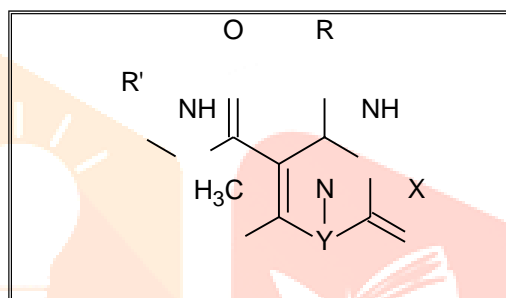


**4.2.2** O. M. fathalla *et al*<sup>12</sup> (2010) were reported Synthesis, antibacterial and anticancer evaluation of some pyrimidine derivatives. Activity performed by measurement of Potential **4.2.3** Cytotoxicity by Sulfo-Rhodamine-B stain (SRB) Assay. The various novel pyrimidine derivatives showed significant growth inhibition towards liver cancer cell in comparison to 5-Flurouracil.

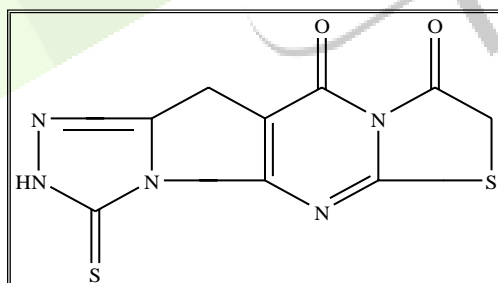




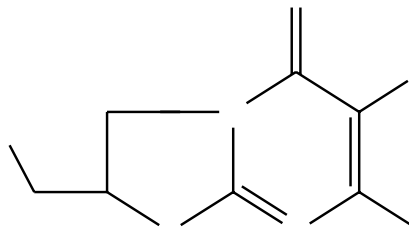
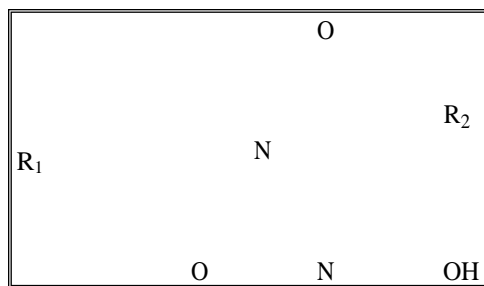
**4.2.4** B. R. Prashantha kumar *et al*<sup>13</sup> (2009) were reported novel biginelli dihydropyrimidines with potential anticancer activity. The synthesized Biginelli compounds were subjected for their *in vitro* anticancer activity against MCF-7 human breast cancer cells.



**4.2.5** A.A. Abu-Hashem *et al*<sup>14</sup> (2011) were reported synthesis and antitumor activities of some new thiazolopyrimidines, pyrrolothiazolopyrimidines and triazolopyrrolothiazolopyrimidines derivatives in vitro ehrlich ascites assay.



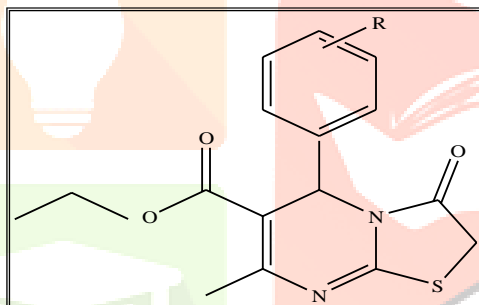
**4.2.6** C. Jarry *et al*<sup>15</sup> (2008) were reported antileukemia activity of 7-hydroxy-2-substituted-methyl- 5H-oxazolo[3,2-a ]pyrimidin-5-one derivatives.



They were tested for their ability to inhibit proliferation in human Bcr-Abl<sup>+</sup> leukemic cells.

### 4.3 Antioxidant Activity:

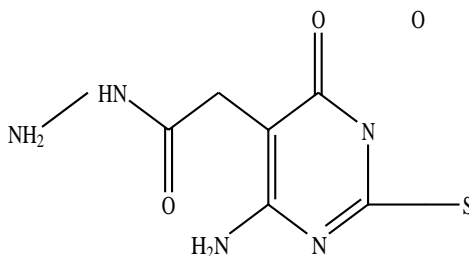
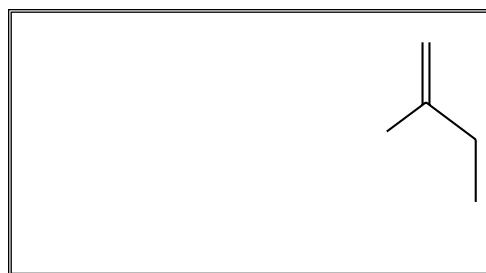
**4.3.1** A. Pathak *et al*<sup>16</sup> (2010) were reported synthesis and antioxidant activity of ethyl-5- (substituted phenyl)-7-methyl-3-oxo-3, 5-dihydro-2H-thiazolo (3, 2-a)-pyrimidine-6- carboxylates by invitro free radical scavenging activity using DPPH (2, 2-diphenyl-1- picryl hydrazyl) reduction method.



R = 2, 3, 4 -OCH<sub>3</sub>

Among the synthesized compounds above structures showed good activity compared to the standard.

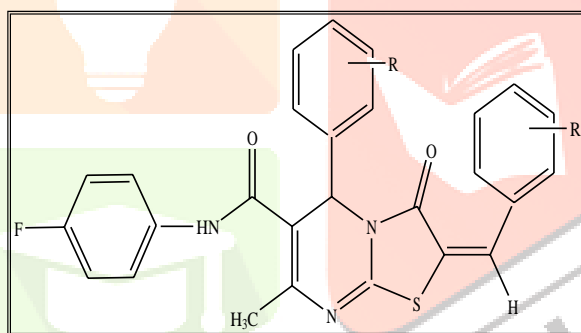
**4.3.2** A.A. Abu-Hashem *et al*<sup>14</sup> (2011) were reported synthesis and antioxidant activity of thiazolo pyrimidine.



All compounds were tested for antioxidant activity as reflected in the ability to inhibit lipid peroxidation in rat brain and kidney homogenates and rate erythrocyte hemolysis.

## 4.4 Analgesic and Antiinflammatory Activity

**4.4.1** S. A. Khan *et al*<sup>17</sup> (2010) reported synthesis and pharmacological evaluation of newer thiazolo (3, 2-a) pyrimidine for antiinflammatory and antinociceptive activity. Antiinflammatory activity was assessed by the rat paw edema method and antinociceptive activity was evaluated by thermal stimulus technique.



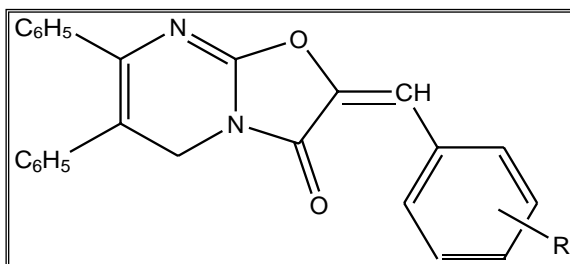
Compounds with an aryl ring substituted with a smaller electron withdrawing group at the fourth position displayed better activity than the other derivatives. Substitutions with a halogen atom at both the aryl rings resulted in increased antiinflammatory activity.

**4.4.2** P. Muthumani *et al*<sup>18</sup> (2010) were reported synthesis and analgesic, antiinflammatory activity of some novel pyrimidine derivatives.

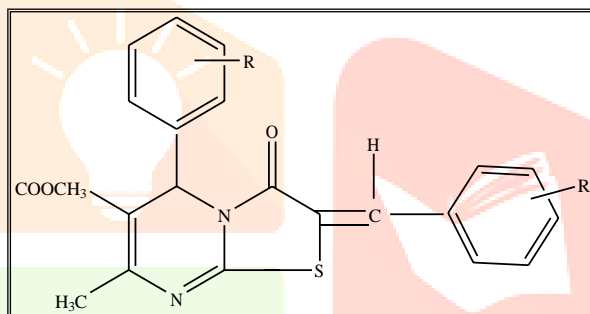
Antiinflammatory activity was measured using the carrageenan induced paw edema in the rats. Paw volume was measured using the mercury displacement techniques with the help of a plethysmometer.

Analgesic activity of the compounds by Eddy's hot plate method was evaluated.

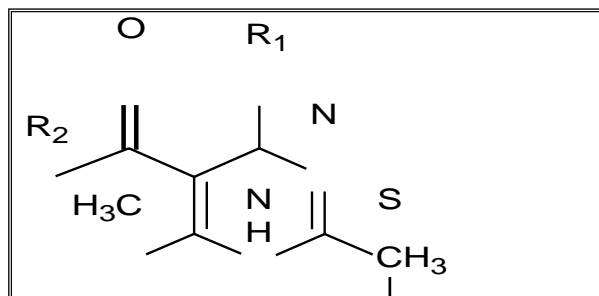
Compounds showed analgesic activity less than the reference standard.



**4.4.3** B. Tozkoparan *et al*<sup>19</sup> (2011) were reported synthesis and antiinflammatoryactivities of some thiazolo [3, 2-a] pyrimidine derivatives.

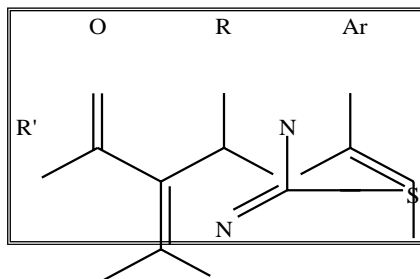


**4.4.4** Ramesh L. Sawant *et al*<sup>20</sup> (2011) were reported analgesic activity of 2-methylthio- 1,4-dihydropyrimidines. Analgesic activity performed by acetic acid induced Writhing method. Compound 4-chlorophenyl group at fourth position of 1, 4-dihydropyrimidine and ethyl ester at fifth position showed good analgesic activity. Compound with 4- chlorophenyl group at fourth position of 1, 4-dihydropyrimidine and methyl ester at fifth position showed lowest analgesic activity.



## 4.5 Acetylcholinesterase inhibitors:

**4.5.1** Hui Zhi *et al*<sup>21</sup> (2008) were reported design, synthesis and biological evaluation of 3-substituted 5H-thiazolo [3, 2-a] pyrimidine derivatives as acetylcholinesterase inhibitors.

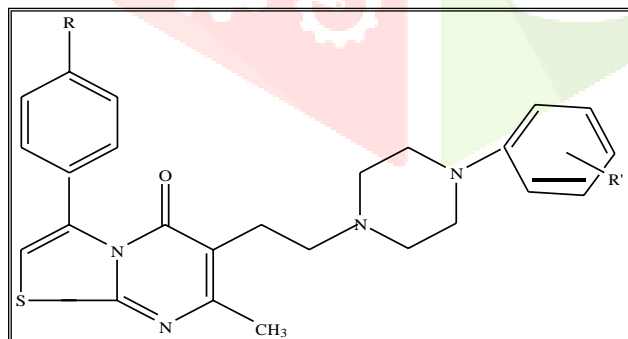


R= 4-hydroxyphenyl; R'=methyl; Ar=4-chlorophenyl

Among the synthesized compounds above compound showed good acetylcholinesterase inhibitor activity. The results demonstrate the effectiveness and validity of the virtual screening approach especially of the docking screening approach, and provide a starting point for the development of novel drugs to treat Alzheimer's disease.

## 4.6 Antihistaminic Activity:

**4.6.1** F. M. Awadallah *et al*<sup>22</sup> (2008) was shown novel 5H- thiazolo [3,2-a] pyrimidin-5-one derivatives as 5-HT<sub>2A</sub> receptor antagonists.

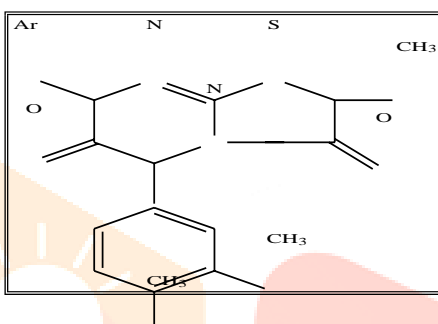


Novel 5H-thiazolo [3, 2-a] pyrimidin-5-one derivatives linked through ethylene bridge to various phenylpiperazine groups were prepared for evaluation as 5-HT<sub>2A</sub> receptor antagonists. Compounds were tested for their antagonist activity on 5-HT<sub>2A</sub> receptors using inhibition of 5-Hydroxytryptophan (5-HTP) induced head twitches in mice.

Pharmacophore modelling study, based on a hypothetical pharmacophore template generated from a set of diverse known active ligands, revealed good fitting of the designed compounds to the generated hypothetical pharmacophore.

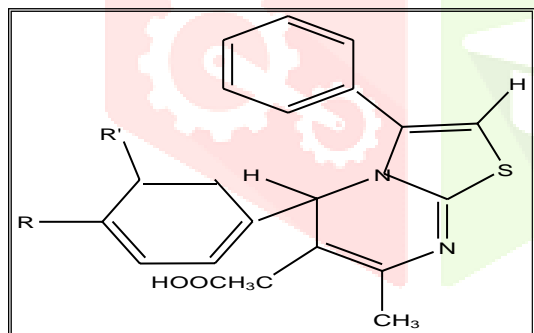
## 4.7 Antiviral activity:

4.7.1 S. F. Mohamed *et al*<sup>23</sup> (2010) were reported antiviral activity of thiazolopyrimidine derivatives.



## 4.8 Calcium Antagonists:

4.8.1 W. Wiegrebe *et al*<sup>24</sup> (1992) were reported thiazolo [3, 2-a] pyrimidine derivatives as calcium antagonists.



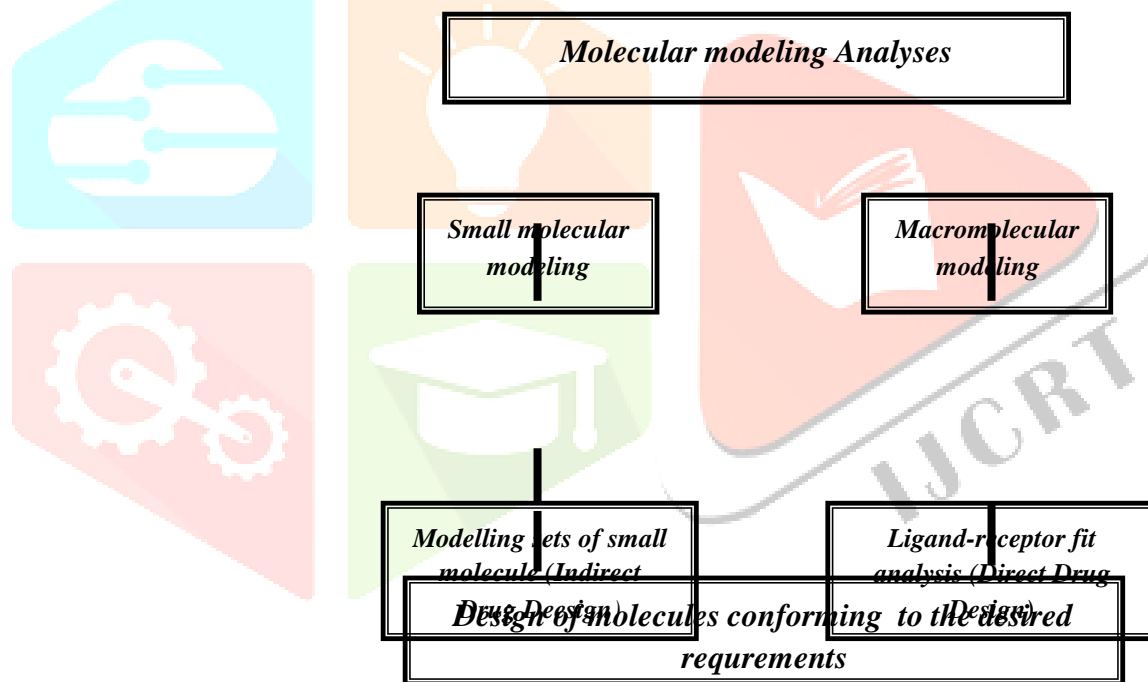
Calcium antagonistic activities of these compounds were evaluated in K<sup>+</sup>-depolarized rataorta.

## 06. MOLECULAR MODELING

### 06.1 Molecular Modeling and Computational Chemistry:

Medicinal chemists today are facing many complicated challenges. The most demanding and perhaps the most rewarding one is the rational design of new therapeutic agents for treating human diseases.

The definition currently accepted of what molecular modeling can be stated as “molecular modeling is anything that requires the use of a computer to paint, describe or evaluate any aspect of the properties of the structure of a molecule”. Methods used in the molecular modeling are regarding automatic structure generation, analysis of three- dimensional (3D) databases and construction of protein models by techniques based on sequence homology, diversity analysis, docking of ligand. Molecular modeling has widened the horizons of pharmaceutical research by providing tools for finding new leads.



**Fig. 6.1 Conceptual Frame in Computer-Aided Drug Design**

Thus, today molecular modeling is regarded as a field concerned with the use of all sort of different strategies to model and to deduce information of a system at the atomic level.

On the other hand, this discipline includes all methodologies used in computational chemistry, like computation of the energy of a molecular system, energy minimization *Monte Carlo* methods or molecular dynamics. In other words, it is possible to conclude that computational chemistry is the nucleus of molecular modeling. Identification of biomolecular moieties involved in the interaction with a specific receptor permits to understand the molecular mechanism responsible of its specific biological activity. In turn, this knowledge is aimed at designing new active molecules that can be successfully used as drugs. Due to the fact that simulation accuracy is limited to the precision of the constructed models, when it is possible, computational simulations have to be compared with experimental results to confirm model accuracy and to modify them if necessary, in order to obtain better representations of the system.

## 06.2 Preparation of Library and Molecular Docking

Library of novel 900 quinazolinone based analogues was prepared on the random basis. During the preparation of library care was taken to avoid the long chain substitution at sixth and seventh position while second, third, fourth and fifth position was mainly focused for substitution. All these analogues were subjected to molecular docking and docking score was observed.

Molecular docking is an efficient tool for investigating receptor-ligand interactions and for virtual screening, which plays a key role in rational drug design, especially when the crystal structure of a receptor or enzyme is available.

It is widely accepted that drug activity is obtained through the molecular binding of one molecule (the ligand) to the pocket of another, usually larger, molecule (the receptor), which is commonly a protein. In their binding conformations, the molecules exhibit geometric and chemical complementarity, both of which are essential for successful drug activity. Molecular docking helps in studying drug/ ligand or receptor/ protein interactions by identifying the suitable active sites in protein, obtaining the best geometry of ligand- receptor complex and calculating the energy of interaction for different ligands to design more effective ligands. Furthermore, the use of a colour code to indicate the nature of the atoms and functional groups present in the three dimensional

structures also enables the medicinal chemist to investigate the binding of the ligand to the target site.

Molecular mechanics also enables the medicinal chemist to calculate the binding energy of a ligand. This is the energy lost when the ligand binds to its target site, that is

$$E_{\text{Binding}} = E_{\text{Target}} + E_{\text{Ligand}} - E_{\text{Target + Bound ligand}}$$

All the quantities on the right hand side of the equation may be calculated using molecular mechanics force fields. However, it should be remembered that in many cases the binding of a drug to its target should be weak, because in most cases it has to be able to leave the target after it has activated that site. A major problem with docking procedures is that the conformation adopted by a ligand when it binds to its target site will depend on the energy of the molecular environment at that site. This means that, although a ligand may have the right pharmacophore, its global minimum energy conformer is not necessarily the conformation that binds to the target site, that is:

Global minimum energy conformer  $\rightarrow$  bioactive conformer

However, it is normally assumed that the conformers that bind to target sites will be those with a minimum potential energy. Since molecules may have large numbers of such meta stable conformers a number of techniques, such as the Metropolis Monte Carlo method and comparative molecular field analysis (CoMFA) have been developed to determine the effect of conformational changes on the effectiveness of docking procedures.

Docking procedures have also been adapted to design possible leads. The computer is used to fit suitable structural fragments into the docking area. These fragments are joined to make molecules that fit the docking site. This procedure is referred to as *De novo* design.

The target or receptor is either experimentally known or theoretically generated through knowledge based protein modeling or homology modeling. The molecular docking tool has been developed to obtain a preferred geometry of interaction of ligand - receptor complexes having minimum interaction energy based on different scoring functions viz. only electrostatics, sum of steric and electrostatic (parameters from MMFF force field) and Dock Score. V-Life MDS uses genetic algorithm (GA), Piecewise Linear Pair wise Potential (PLP) and Grid algorithms to minimize the interaction energy between ligand - receptor.

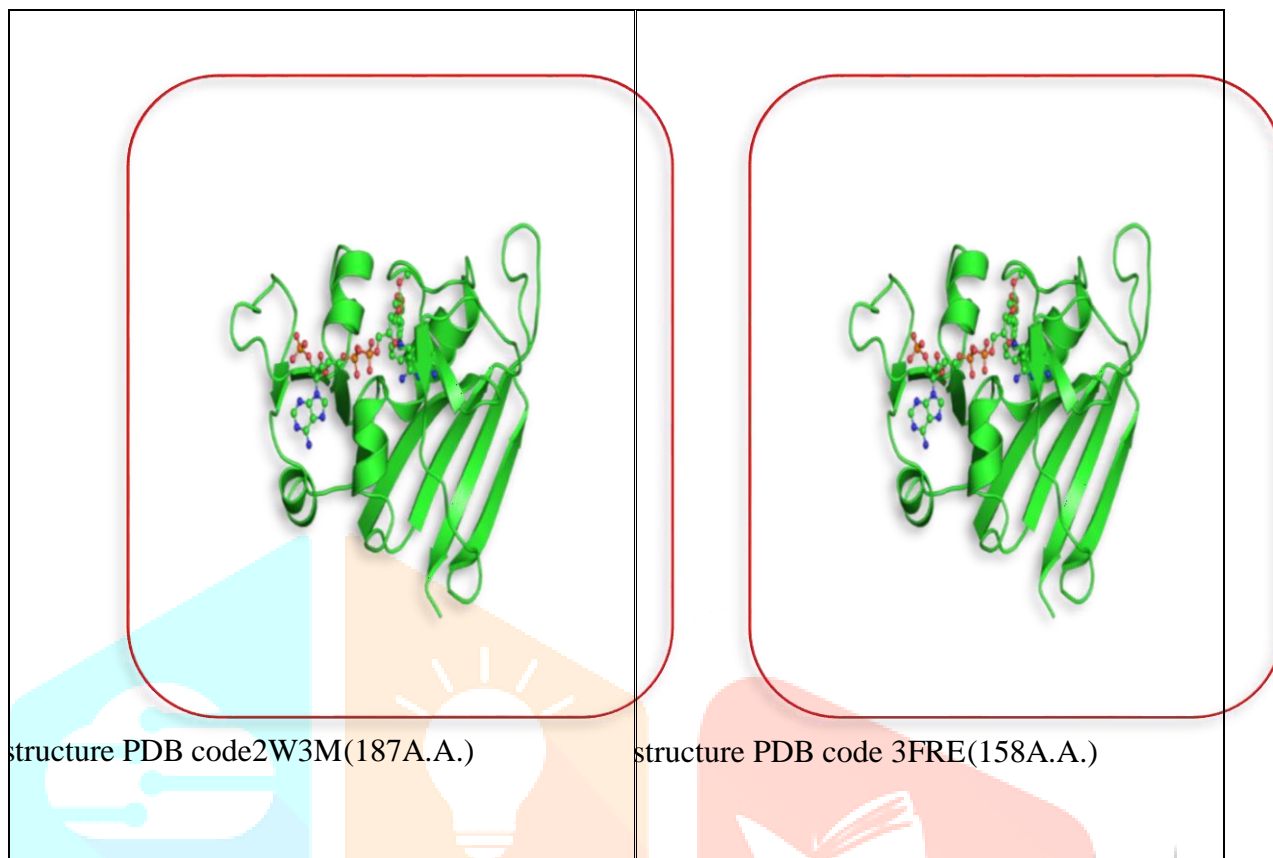
One key aspect of molecular modeling is calculating the energy of conformations and interactions using methods ranging from quantum mechanics to purely empirical energy functions. Molecular docking energy evaluations are usually carried out with the help of a scoring function. Developing these scoring functions is a major challenge in structure based drug design. Efficiency and accuracy of geometric modeling of the binding process to obtain correct docking solutions depends on scoring function. Usually scoring functions are based on force fields that were initially designed to simulate the function of proteins (based on enthalpy).

The Grid based docking is a rigid and exhaustive docking method. In this method, after unique conformers of the ligand are generated, the receptor cavity of interest is chosen by the user and a grid is generated around the cavity (default grid interval size 1 Å). Cavity points are found and the centre of mass of the ligand is moved to each cavity point. All rotations of ligand are scanned at each cavity point where ligand is placed (step size of rotation could be typically 100-150 as an example). For each rotational pose of the ligand is generated and the corresponding bumps are checked for each pose of ligand. The score is calculated for each valid pose (determined by the cut off criteria fed by user in terms of max no of allowed bumps) and the pose of the ligand with the best score is given as output to user. VLife MDS provides a facility to dock different ligands in protein binding sites chosen by the user.

#### 06.2.1 Target structure:

The structure of target was obtained from the protein data bank (PDB code 2W3M and 3FRE). The receptor was in complex with folic acid and trimethoprim.

PDB 2W3M was used for anticancer drug design having total 187 amino acids. VAL 1A and ASP 186B are terminal amino acids in 2W3M. PDB 3FRE was used for anti- microbial drug design having total 158 amino acids. THR 1X and ASP LYS 157X are terminal amino acids in 3FRE.



#### 06.2.2 Methodology and cavity identification:

Docking studies of the prepared library of pyrimidine analogues was done on Vlife MDS 4.3 using Gasteiger and grid based docking method. The crystal structure of DHFR enzyme receptor was obtained from PDB (PDB entry 2W3M and 3FRE). By using V-life MDS, 2W3M and 3FRE was open in MDS sheet and saved as sybale format by removing water molecule and then this receptor structure was used further for docking purpose.

The 2D structure of the compounds were drawn by using 2D draw molecule option of Vlife and then converted into the 3D by exporting to window of MDS sheet. The 3D structures were then energetically minimized up to the rms gradient of 0.01 using Merck Molecular Force Field (MMFF). Docking methodology is shown in fig. no 6.3

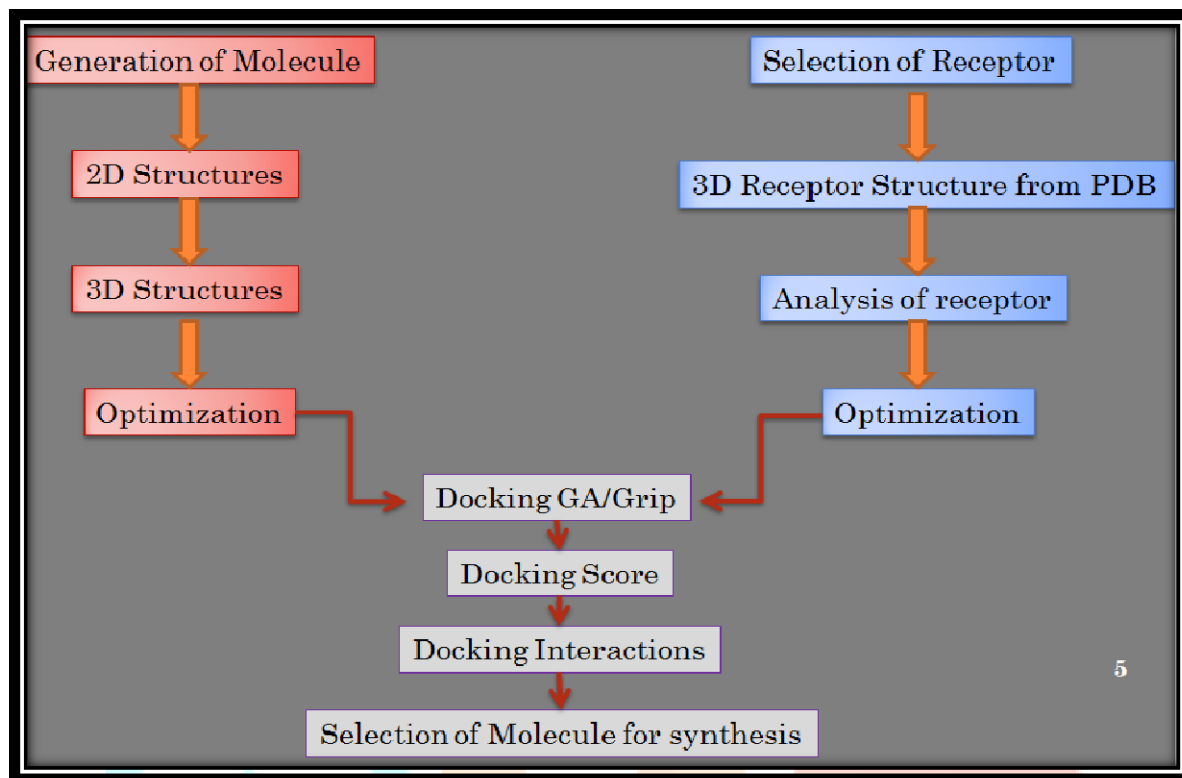


Fig. 6.4 Docking methodology

By click on biopredicta and then open biopredicta tool; goes to cavity determination option from software cavities of receptor were determined. The cavities in the receptor were mapped to assign an appropriate active site, the basic feature used to map the cavities were the surface mapping of the receptor and identifying the geometric voids as well as scaling the void for its hydrophobic characteristics. Hence all the cavities that are present in receptor are identified and ranked based on their size and hydrophobic surface area. Cavity no.1 was selected for docking of PDB 2W3M and 3FRE receptor. Cavity no.1 of 2W3M have volume  $17942\text{\AA}^3$  and surface area =  $17035.01\text{ \AA}^2$  which is highlighted in red color in **Fig.6.4**. Cavity no.1 of 3FRE have volume  $3205\text{\AA}^3$  and surface area =  $4709.06\text{\AA}^2$  which is highlighted in greenish color in **Fig.6.5**.

The active site for docking was defined as all atoms within  $5\text{\AA}$  radius. From biopredicta tool open docking and then batch grid docking. Batch docking shows browsing of receptor, ligand (Compound) and result generated saved in output file. Compounds saved in output file as a docked ligand format with proper conformation

which will further use in checking binding interaction. Results generated were saved as log file in output folder.

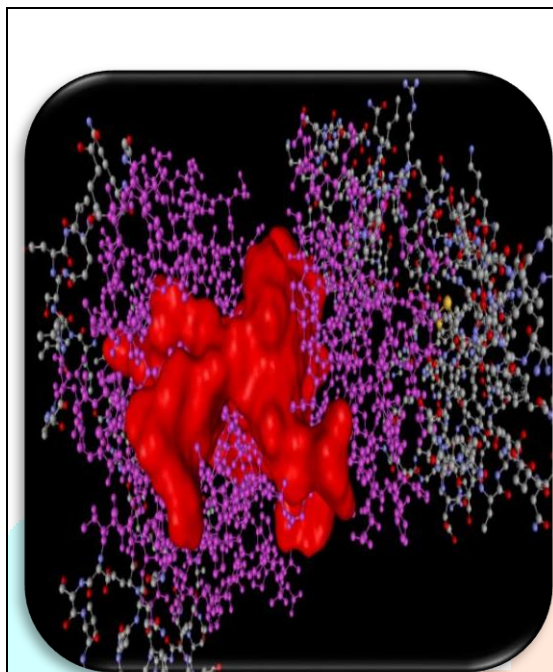


Fig. 6.5 2W3M cavity no. 1

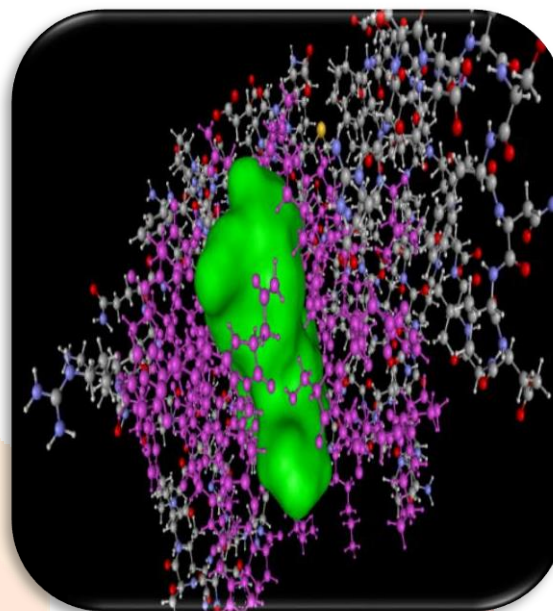
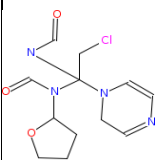
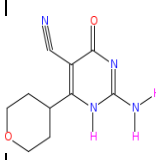
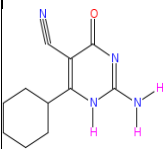
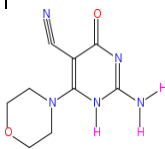
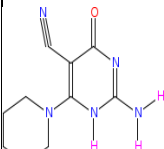
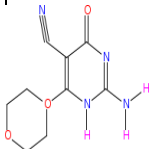
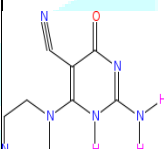
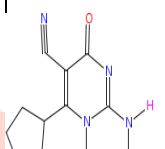
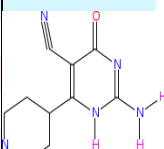
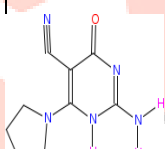
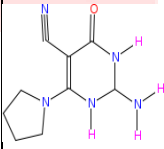
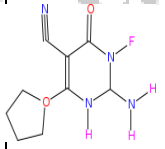
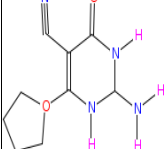
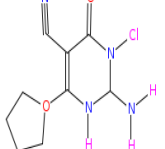


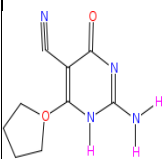
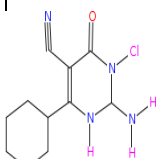
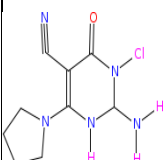
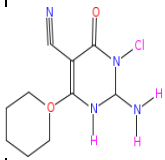
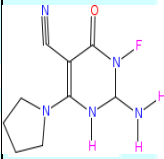
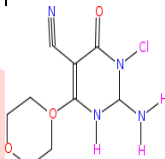
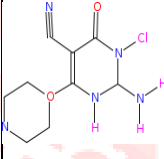
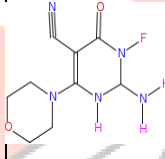
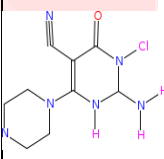
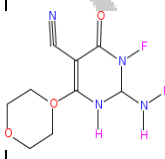
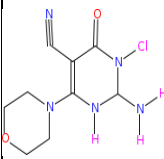
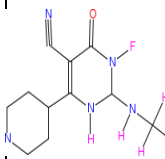
Fig. 6.6 3FRE cavity no. 1

### 06.2.3 Docking results:

Table no. 06.1 Docking score result

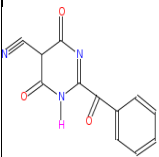
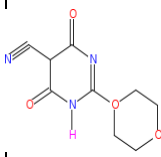
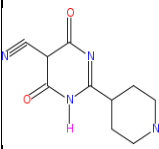
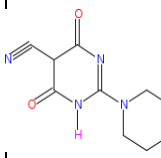
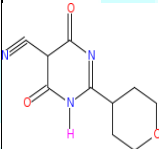
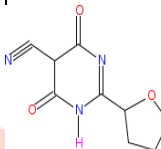
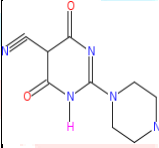
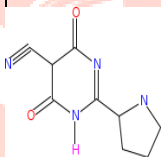
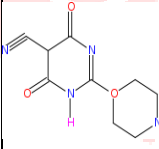
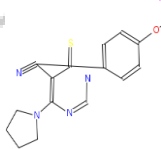
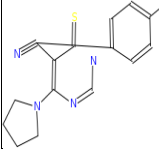
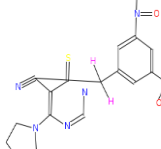
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4	1u		-4.543399	9	1z		-4.429177
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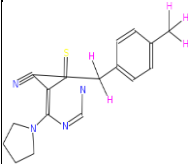
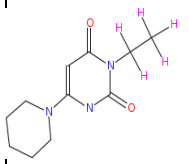
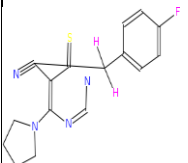
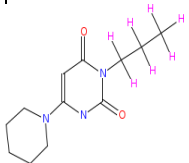
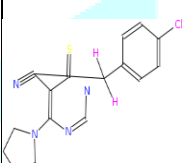
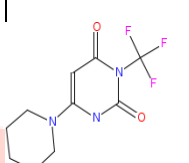
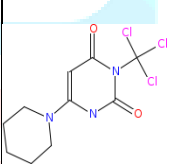
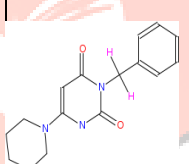
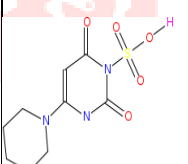
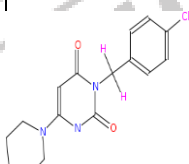
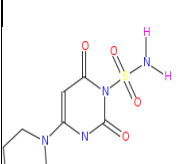
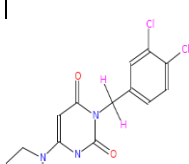
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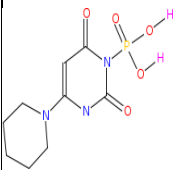
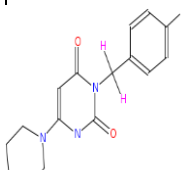
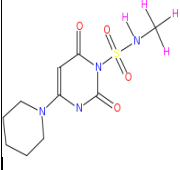
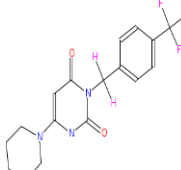
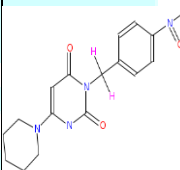
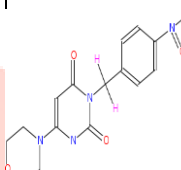
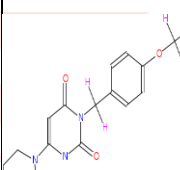
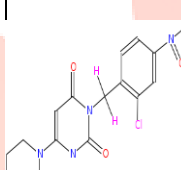
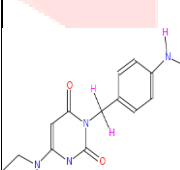
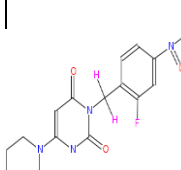
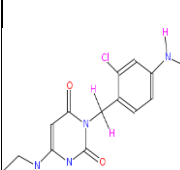
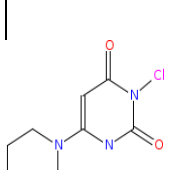
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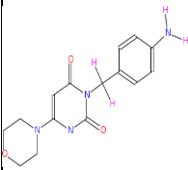
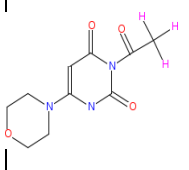
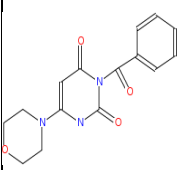
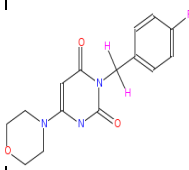
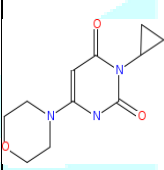
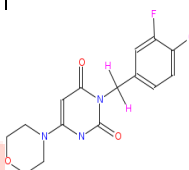
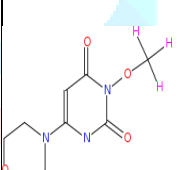
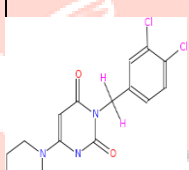
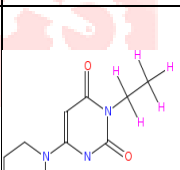
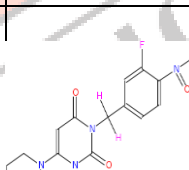
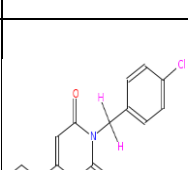
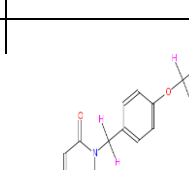
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42	3i		-4.816826	47	3n		-4.084500
43	3j		-3.713055	48	3o		-3.825821
44	3k		-4.937042	49	3p		-2.954136
45	3l		-4.799076	50	3q		-4.157927

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53	3t		-4.454208	58	3y		-4.515141
54	3u		-4.887077	59	3z		-4.398973
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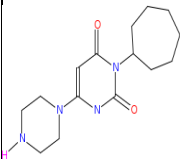
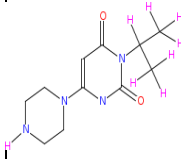
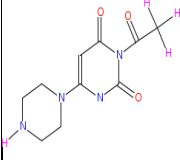
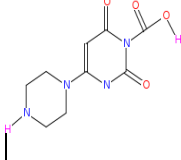
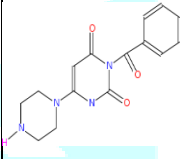
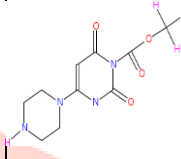
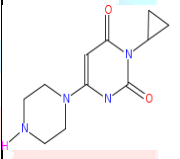
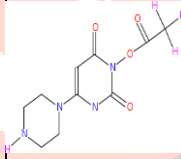
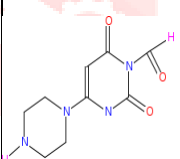
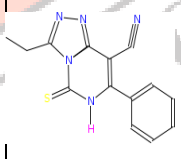
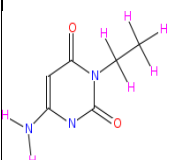
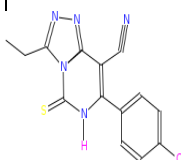
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65	4f		-3.018975	70	4k		-3.552788
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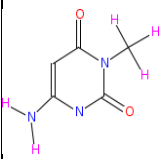
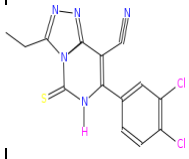
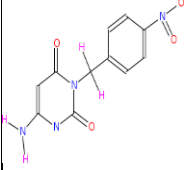
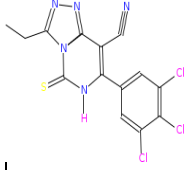
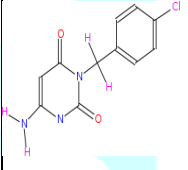
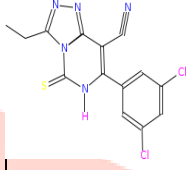
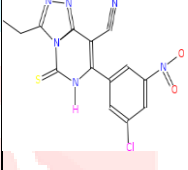
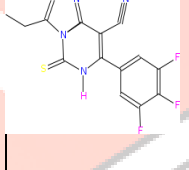
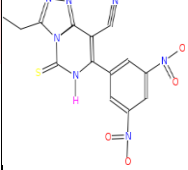
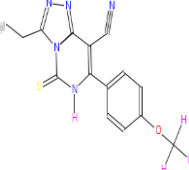
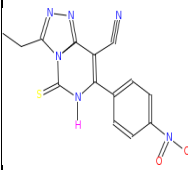
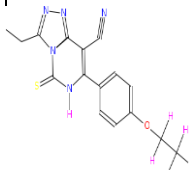
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74	4p		-3.713055	79	4u		-2.644648
75	4q		-4.937042	80	4v		-3.018975
81	4w		-4.799076	86	5b		-3.169153
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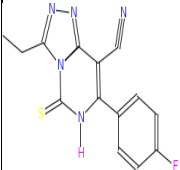
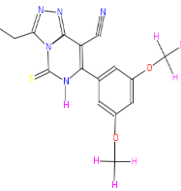
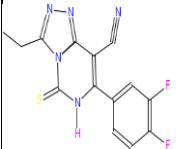
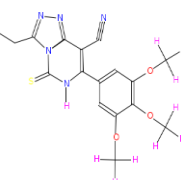
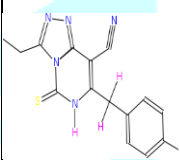
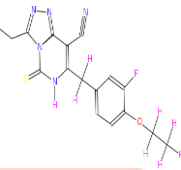
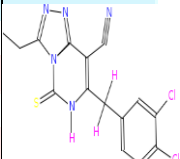
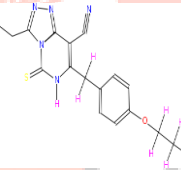
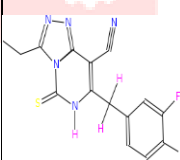
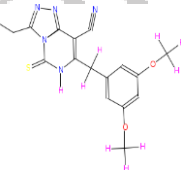
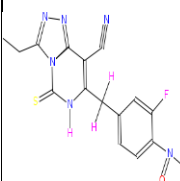
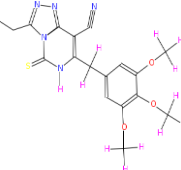
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92	5i		-2.872770	97	5n		-1.859843
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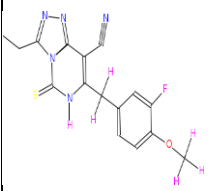
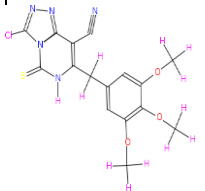
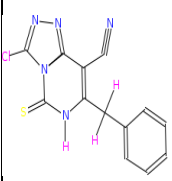
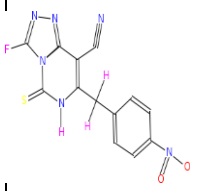
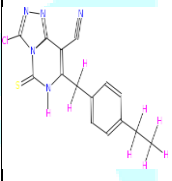
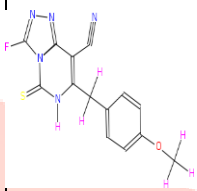
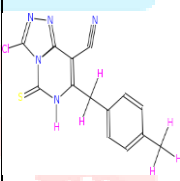
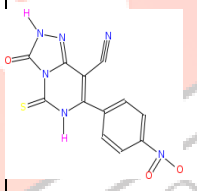
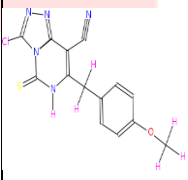
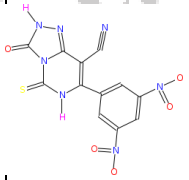
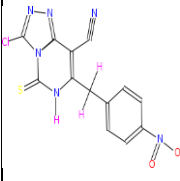
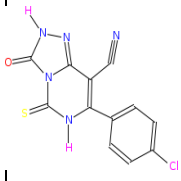
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103	5t		-3.412892	108	5y		-3.897591
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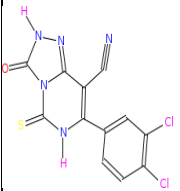
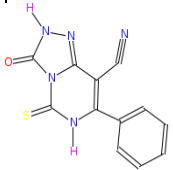
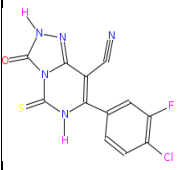
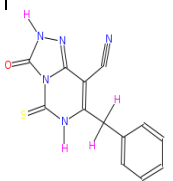
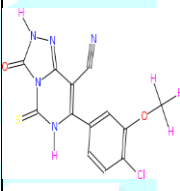
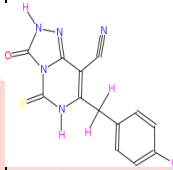
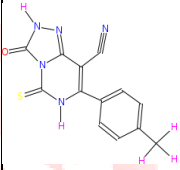
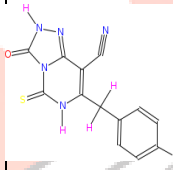
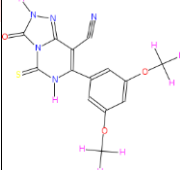
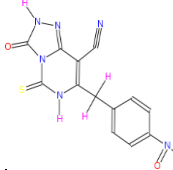
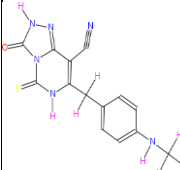
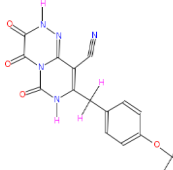
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114	6e		-3.663190	119	6j		-3.011446
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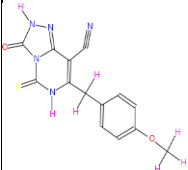
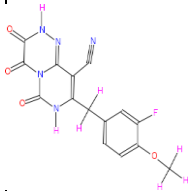
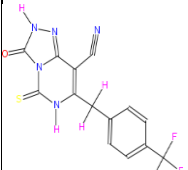
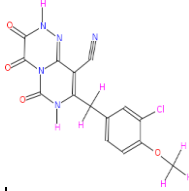
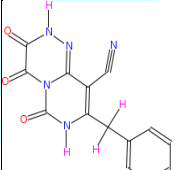
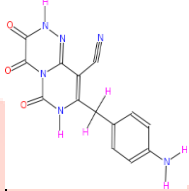
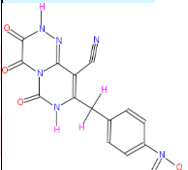
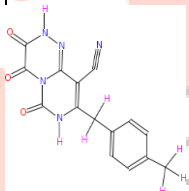
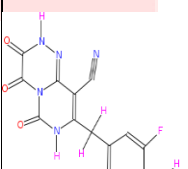
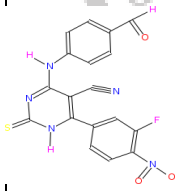
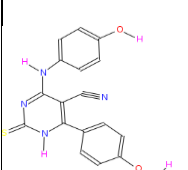
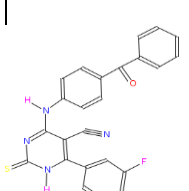
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125	6p		3.905382	130	6u		-2.853757
131	6v		-3.960534	136	7a		-3.858939
132	6w		-4.507489	137	7b		-4.677759

133	6x		-4.116265	138	7c		-4.988944
134	6y		-3.567000	139	7d		-4.917652
135	6z		-3.161491	140	7e		-5.110830
141	7f		-4.421368	146	7k		-4.620093
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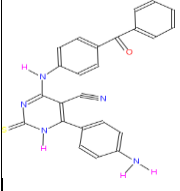
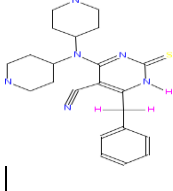
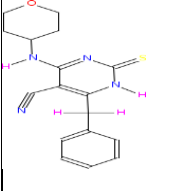
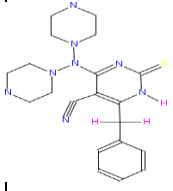
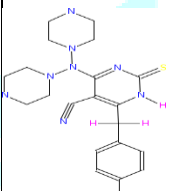
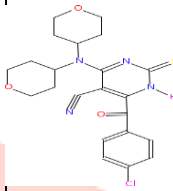
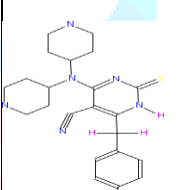
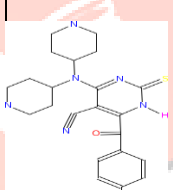
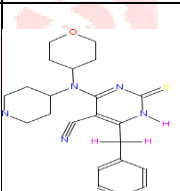
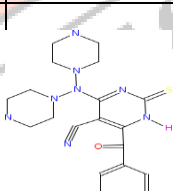
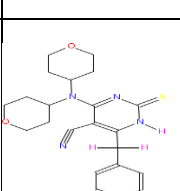
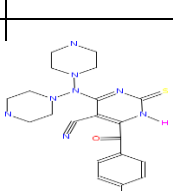
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152	7q		-4.269085	157	7v		-4.043108
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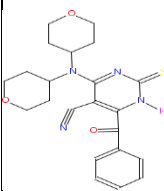
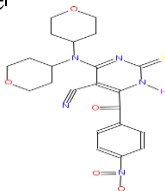
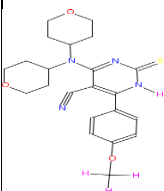
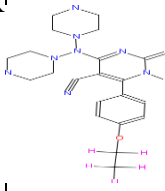
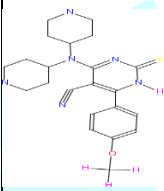
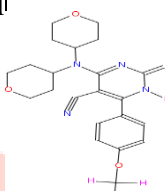
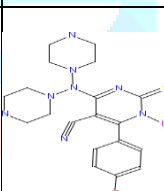
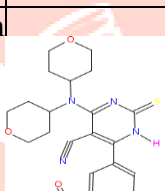
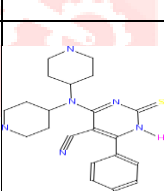
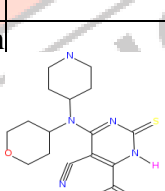
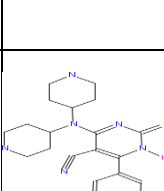
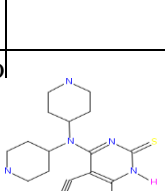
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162	8a		-4.232711	167	8f		-4.442810
163	8b		-4.067793	168	8g		-3.826260
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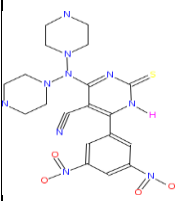
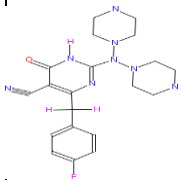
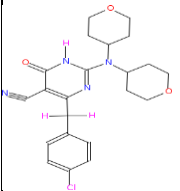
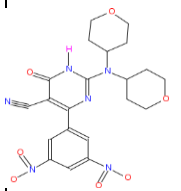
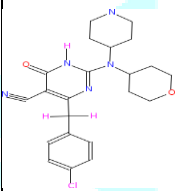
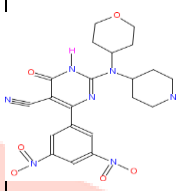
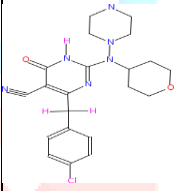
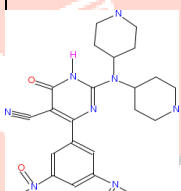
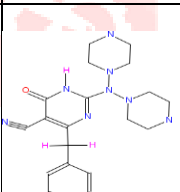
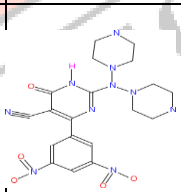
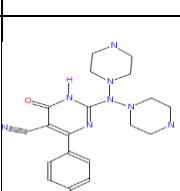
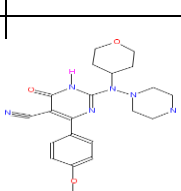
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174	8m		-4.280534	179	8r		-4.469692
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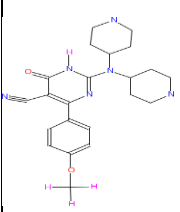
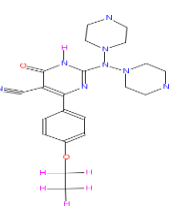
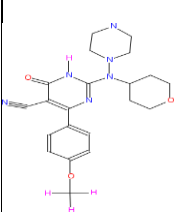
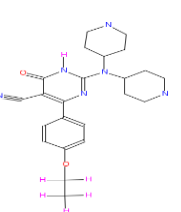
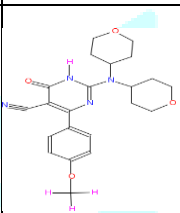
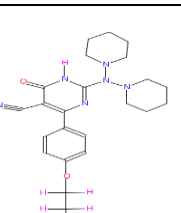
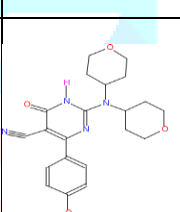
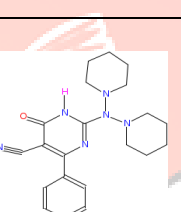
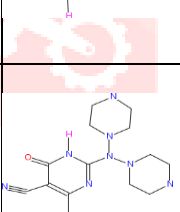
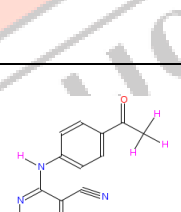
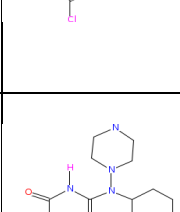
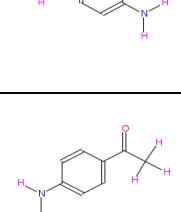
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185	8x		-3.511585	190	9c		-2.750001
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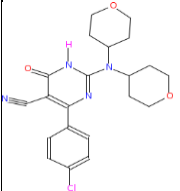
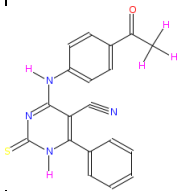
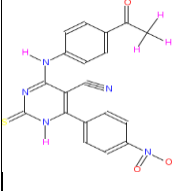
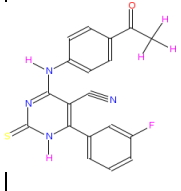
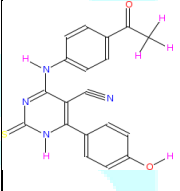
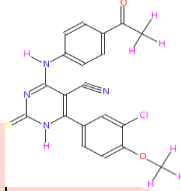
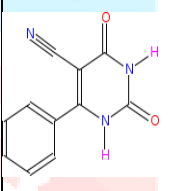
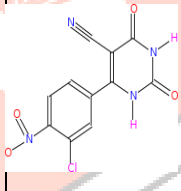
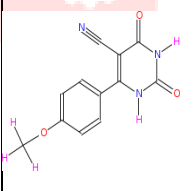
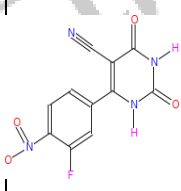
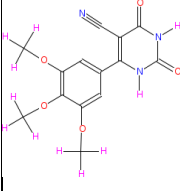
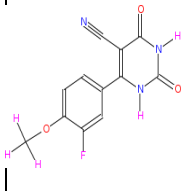
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201	9w		-3.148514	206	10q		-2.145345
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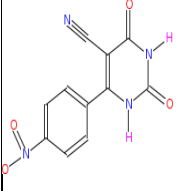
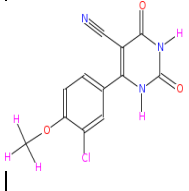
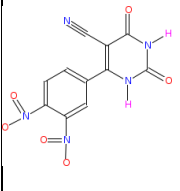
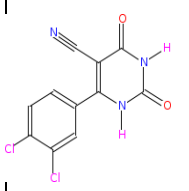
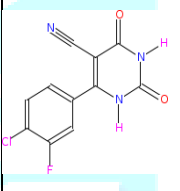
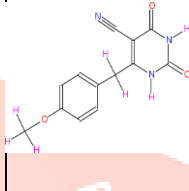
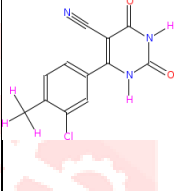
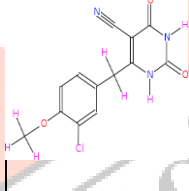
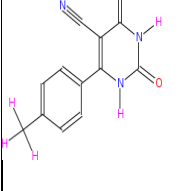
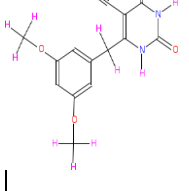
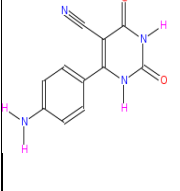
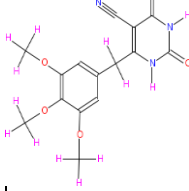
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212	10w		-3.900422	217	11b		-3.468556
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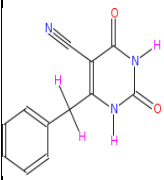
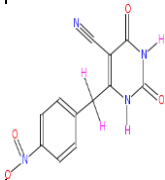
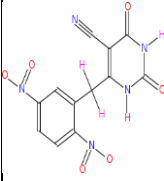
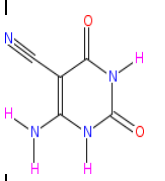
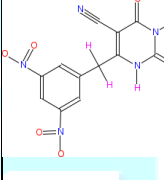
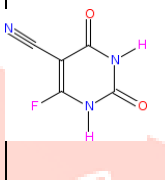
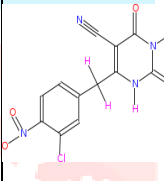
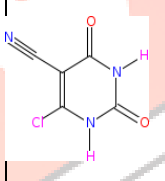
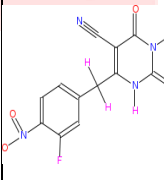
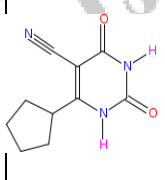
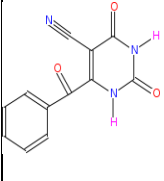
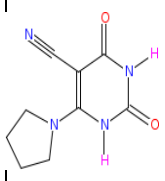
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222	11g		-3.398930	227	11l		-3.639210
223	11h		-4.303102	228	11m		-1.873283
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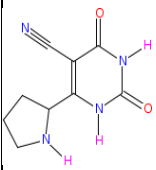
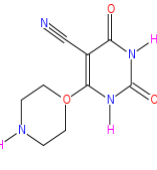
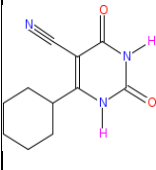
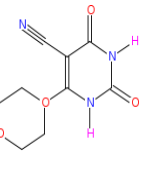
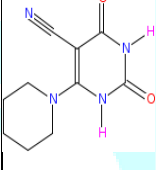
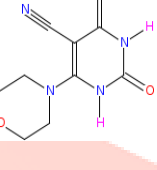
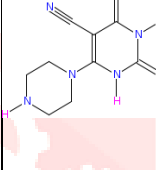
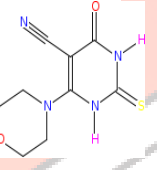
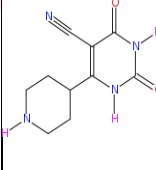
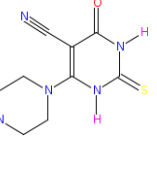
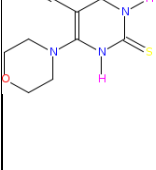
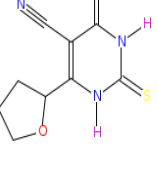
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232	11q		-3.095412	237	11v		-2.923298
233	11r		-3.441934	238	11w		-2.191706
234	11s		-2.716310	239	11x		-2.072358
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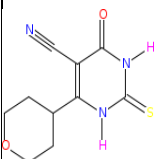
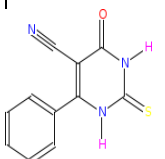
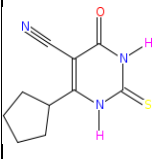
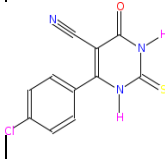
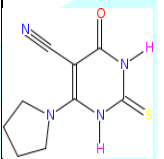
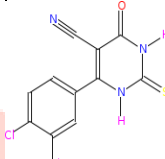
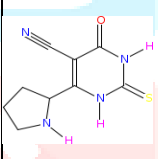
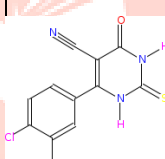
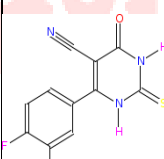
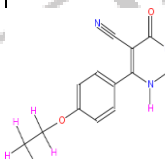
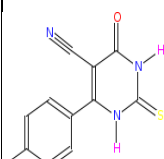
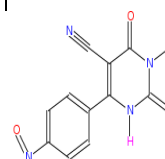
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243	12b		-1.833354	248	12g		-2.934447
244	12c		-2.064308	249	12h		-2.637720
245	12d		-2.105344	250	12i		-3.800956
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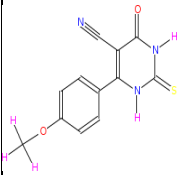
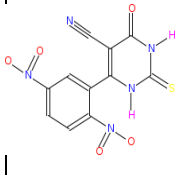
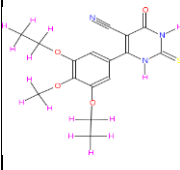
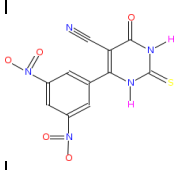
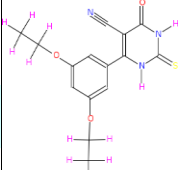
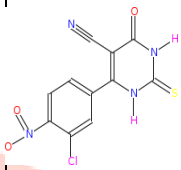
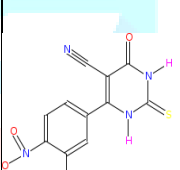
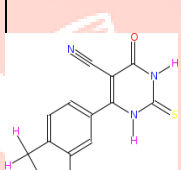
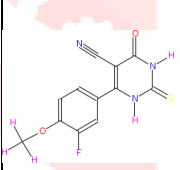
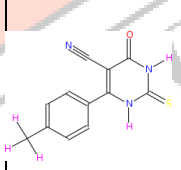
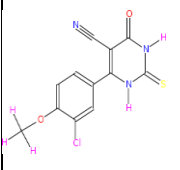
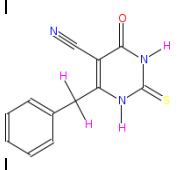
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255	12n		-3.951177	260	12s		-4.754674
261	13a		-5.010238	266	13f		-5.605707
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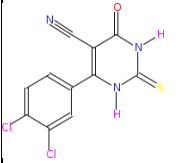
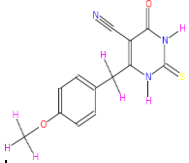
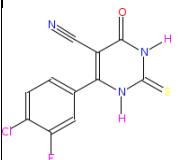
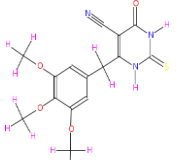
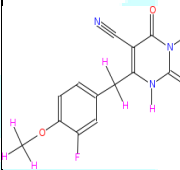
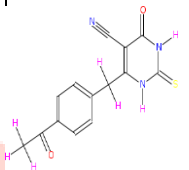
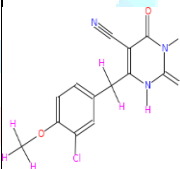
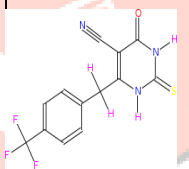
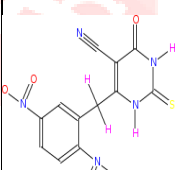
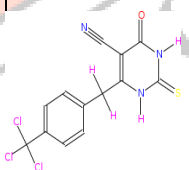
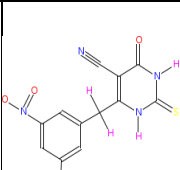
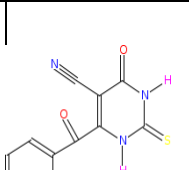
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265	13e		-2.988729	270	13j		-5.303599
271	13k		-5.199943	276	13p		-3.244580
272	13l		-3.770917	277	13q		-4.789086
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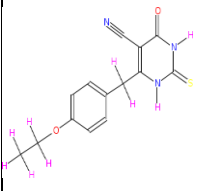
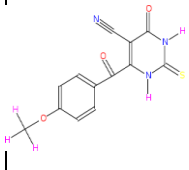
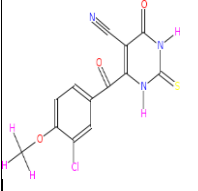
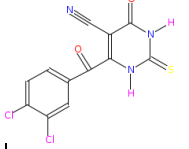
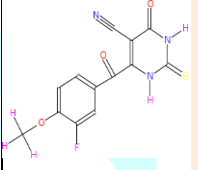
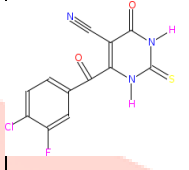
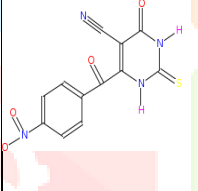
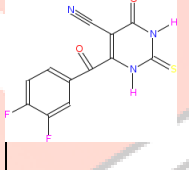
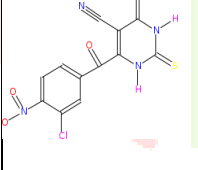
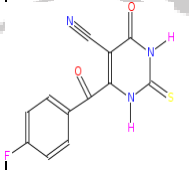
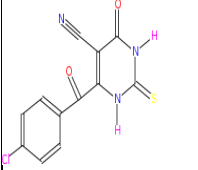
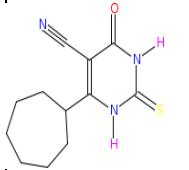
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281	13u		-4.487984	286	13z		-5.115248
282	13v		-3.675171	287	14a		-4.804716
283	13w		-3.925524	288	14b		-5.138929
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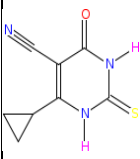
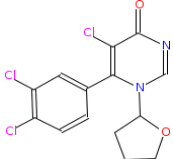
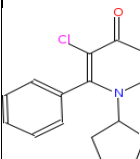
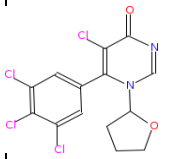
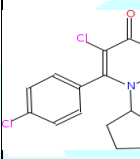
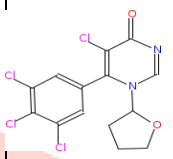
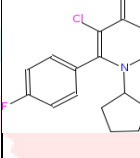
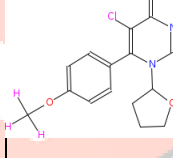
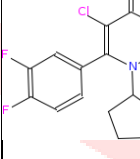
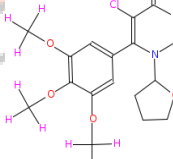
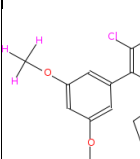
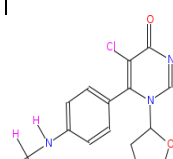
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292	14f		-4.365852	297	14k		-4.949817
293	14g		-4.950690	298	14l		-4.336707
294	14h		-4.298543	299	14m		-4.396033
295	14i		-4.174539	300	14n		-4.017758
301	14o		-4.501035	306	14t		-3.709904

302	14p		-3.965385	307	14u		-4.421953
303	14q		-3.098552	308	14v		-4.757928
304	14r		-4.116758	309	14w		-4.855990
305	14s		-3.784990	310	14x		-3.779552
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312	14z		-4.452018	317	15e		-4.815258

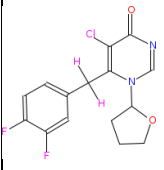
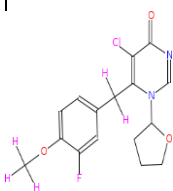
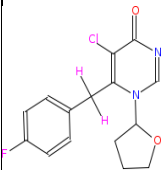
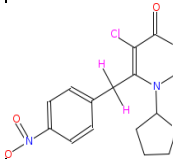
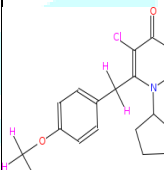
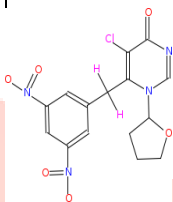
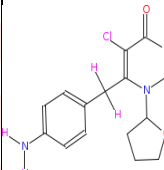
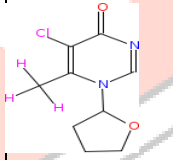
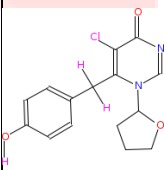
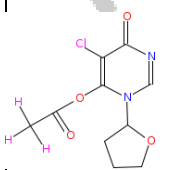
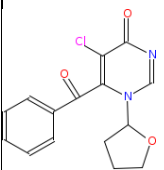
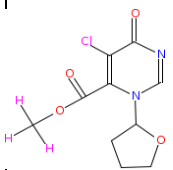
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321	15i		-4.253608	326	15n		-4.492146
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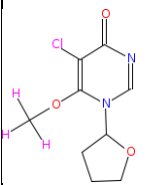
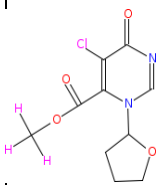
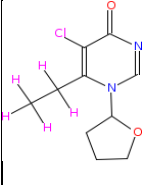
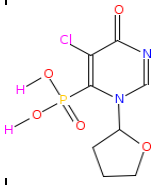
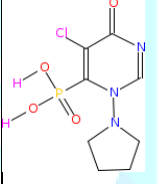
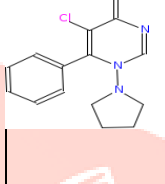
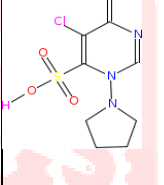
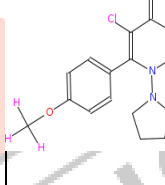
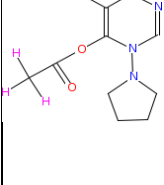
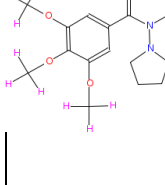
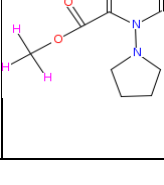
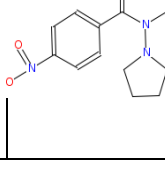
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325	15m		-4.498693	330	15r		-2.524178
331	15s		-4.121758	336	15x		-4.163085
332	15t		-3.797939	337	15y		-4.393760
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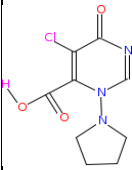
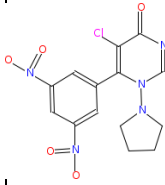
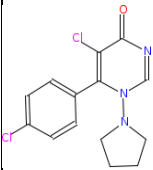
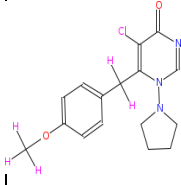
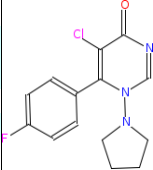
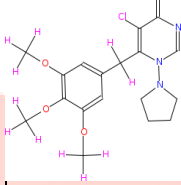
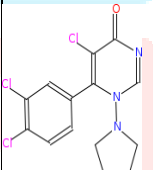
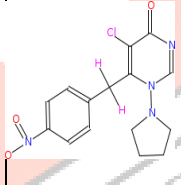
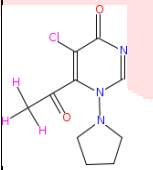
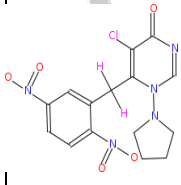
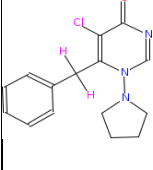
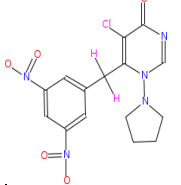
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342	16d		-4.277469	347	16i		-4.372262
343	16e		-4.310887	348	16j		-4.364820
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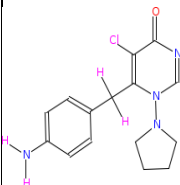
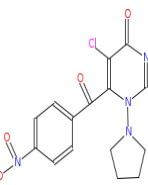
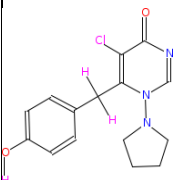
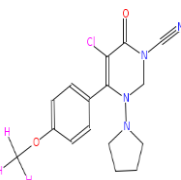
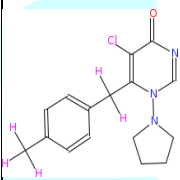
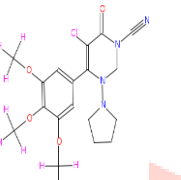
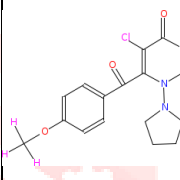
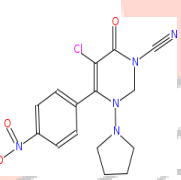
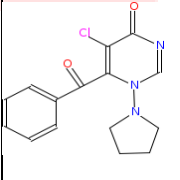
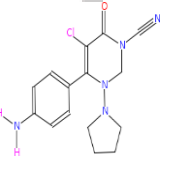
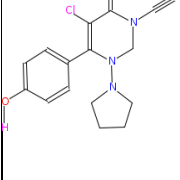
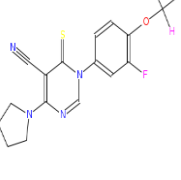
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353	16o		-4.825975	358	16t		-4.896991
354	16p		-4.325259	359	16u		-3.797137
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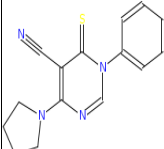
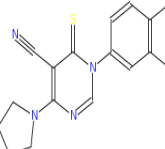
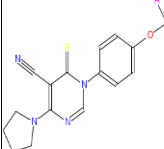
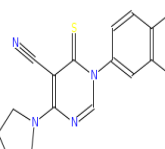
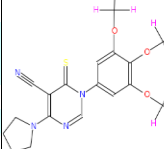
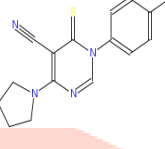
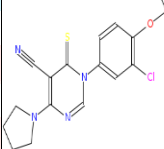
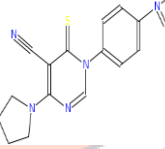
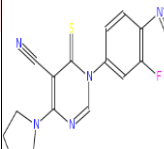
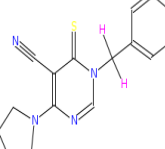
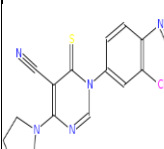
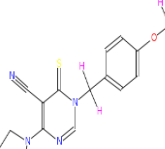
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364	16z		-2.509477	369	17e		-4.104365
365	17a		-4.244486	370	17f		-3.573649
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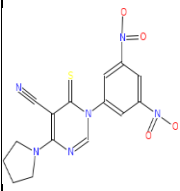
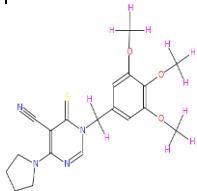
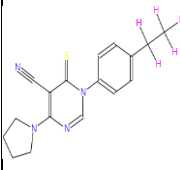
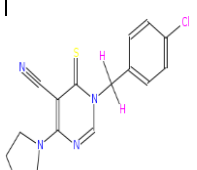
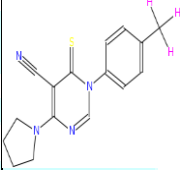
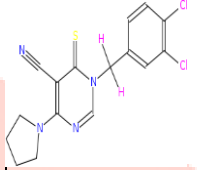
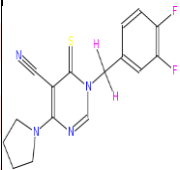
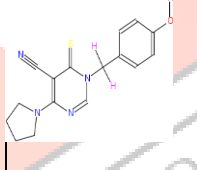
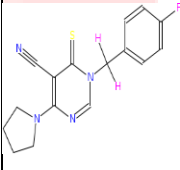
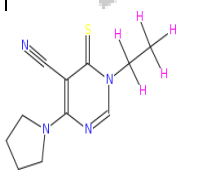
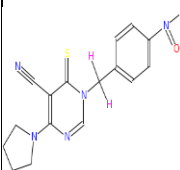
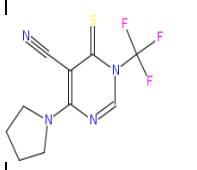
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375	17k		-3.892049	380	17p		-3.745782
381	17q		-4.423487	386	17v		-4.168869
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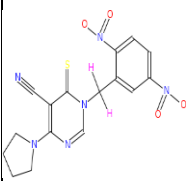
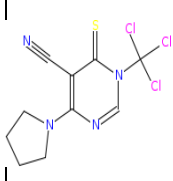
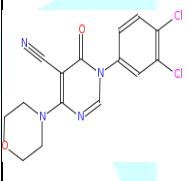
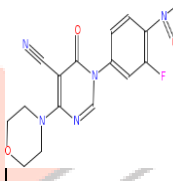
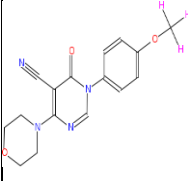
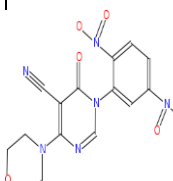
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391	18a		-3.695127	396	18f		-4.247932
392	18b		-4.181167	397	18g		-4.523481
393	18c		-3.352344	398	18h		-2.345411
394	18d		-3.498106	399	18i		-4.189879

395	18e		-4.529960	400	18j		-4.445924
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402	18l		-4.444359	407	18q		-2.264583
403	18m		-5.020778	408	18r		-3.489998
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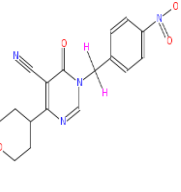
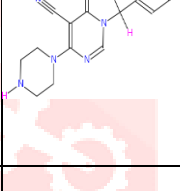
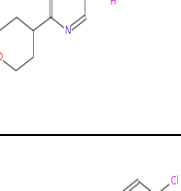
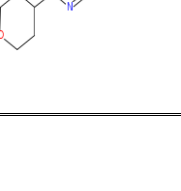
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414	18x		-3.487054	419	19c		-3.806633
415	18y		-3.898279	420	19d		-3.352320
421	19e		-3.030760	426	19j		-4.306638

422	19f		-4.832666	427	19k		-4.869977
423	19g		-4.379154	428	19l		-5.069025
424	19h		-4.466372	429	19m		-4.799637
425	19i		-4.756878	430	19n		-4.368801
431	19o		-4.063650	436	19t		-4.521274
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435	19s		-4.865306	440	19x		-4.480608
441	19y		-4.043732	446	20d		-4.621195
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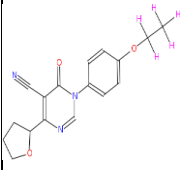
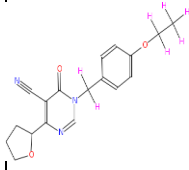
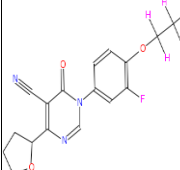
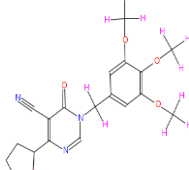
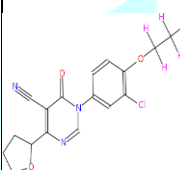
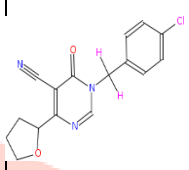
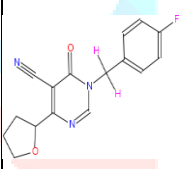
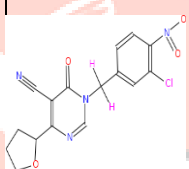
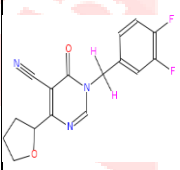
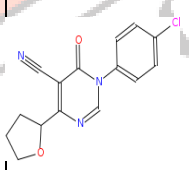
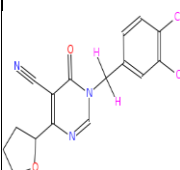
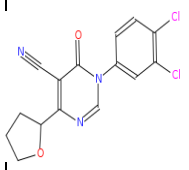
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452	20j		-2.985790	457	20p		-4.446892
453	20k		-5.041253	458	20q		-3.661193
454	20l		-3.985512	459	20r		-4.167859

455	20n		-4.366531	460	20s		-4.169955
461	20t		-4.532674	466	20y		-4.434250
462	20u		-3.927186	467	20z		-4.953069
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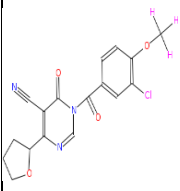
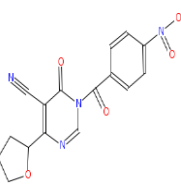
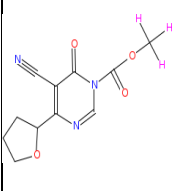
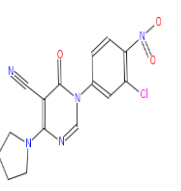
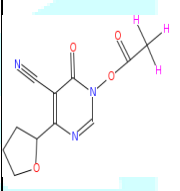
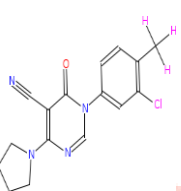
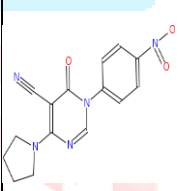
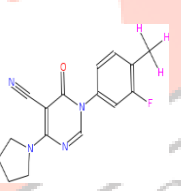
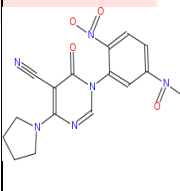
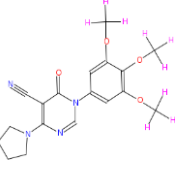
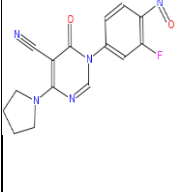
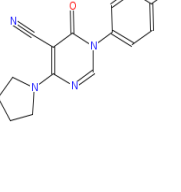
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473	21g		-4.699584	478	21l		-3.390888
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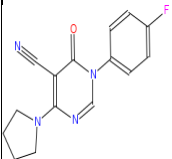
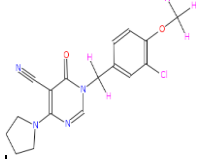
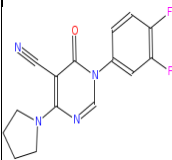
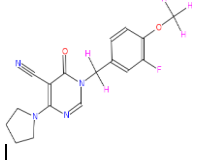
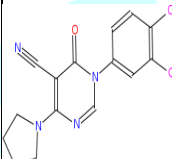
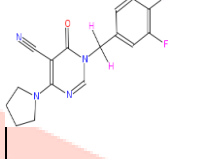
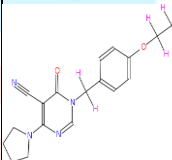
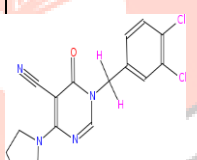
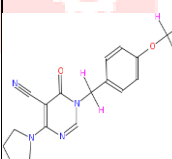
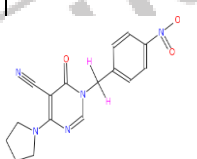
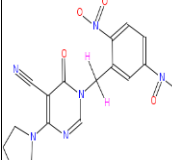
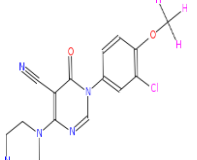
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482	21q		-4.411104	487	21w		-4.545979
483	21s		-4.543544	488	21x		-4.428000
484	21t		-3.473671	489	21y		-4.848165
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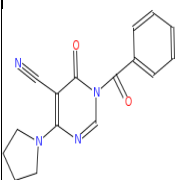
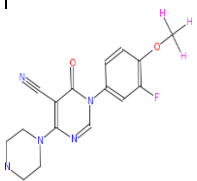
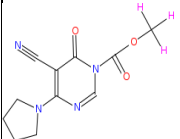
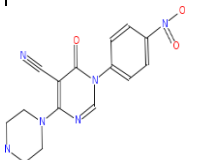
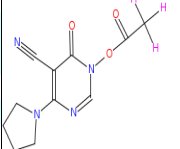
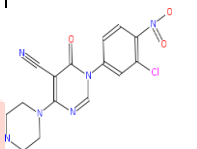
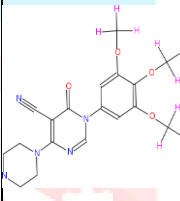
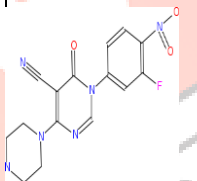
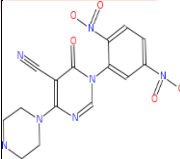
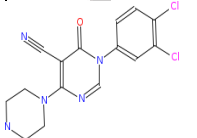
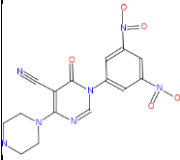
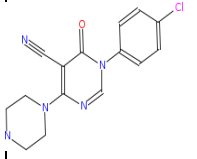
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494	22d		-3.813569	499	22i		-3.566322
495	22e		-3.974473	500	22j		-4.297402
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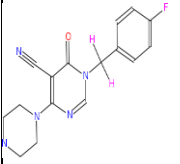
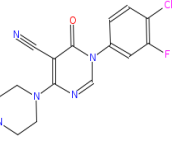
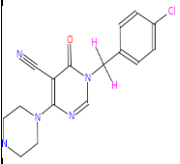
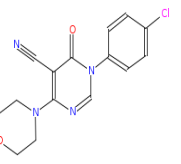
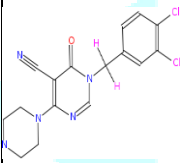
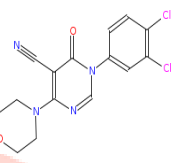
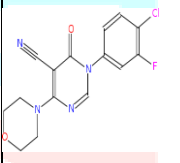
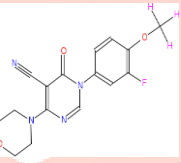
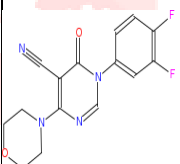
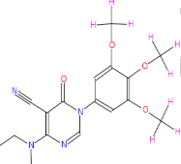
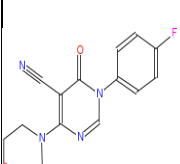
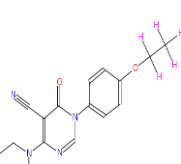
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511	22u		-4.363696	516	22z		-3.744416
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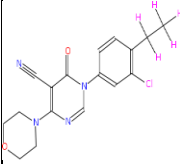
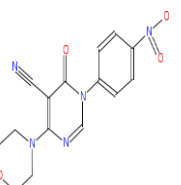
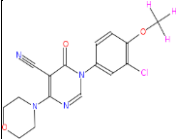
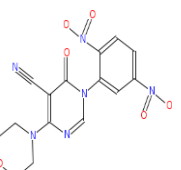
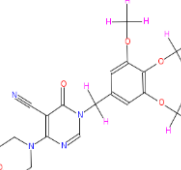
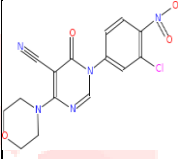
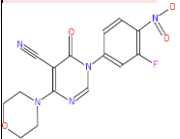
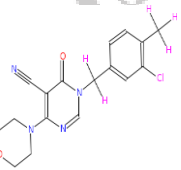
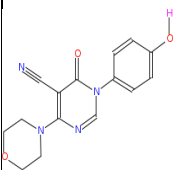
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522	23f		-3.908731	527	23k		-4.685674
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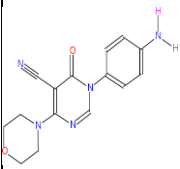
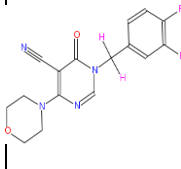
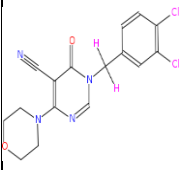
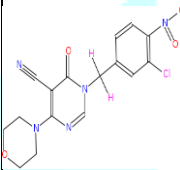
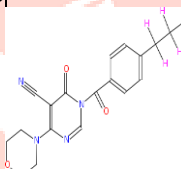
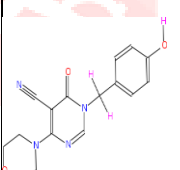
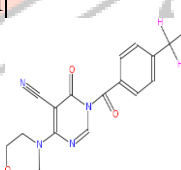
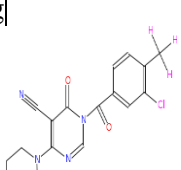
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541	23y		-4.671801	546	24d		-3.948968
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543	24a		-5.298300	548	24f		-3.768958
544	24b		-3.540991	549	24g		-4.771296
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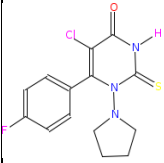
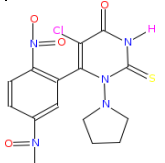
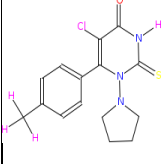
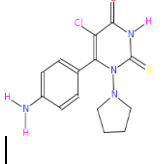
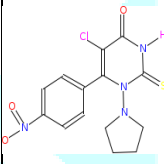
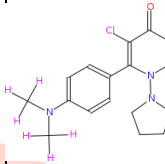
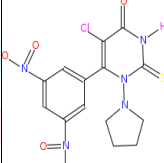
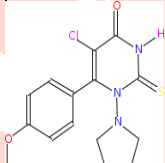
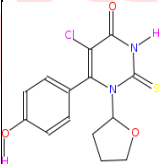
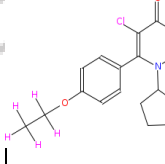
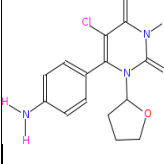
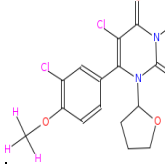
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554	24l		-3.800076	559	24q		-4.256575
555	24m		-3.707378	560	24r		-4.460067
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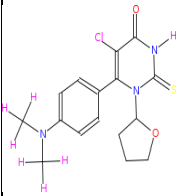
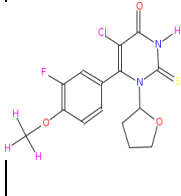
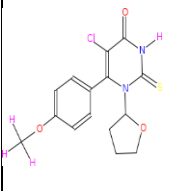
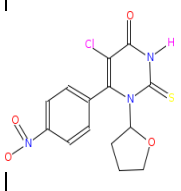
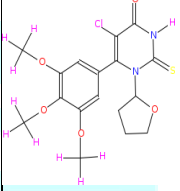
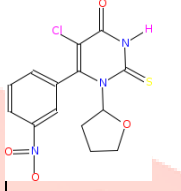
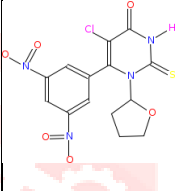
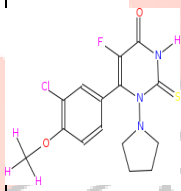
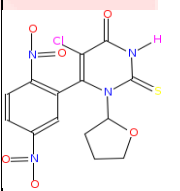
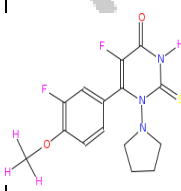
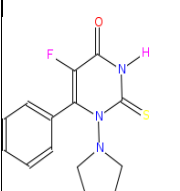
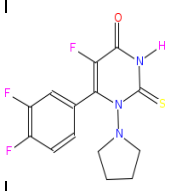
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571	25c		-4.376011	576	25h		-4.005055
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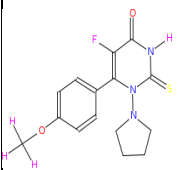
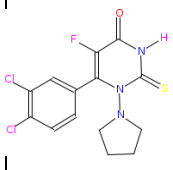
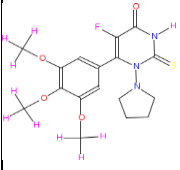
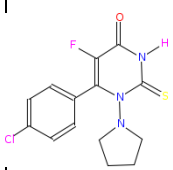
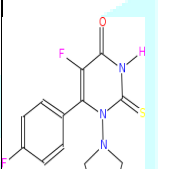
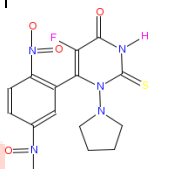
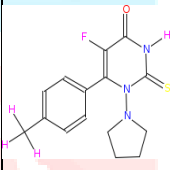
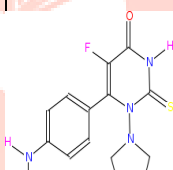
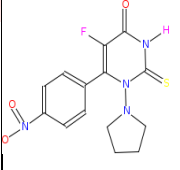
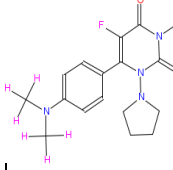
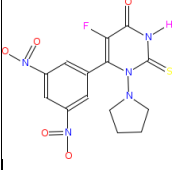
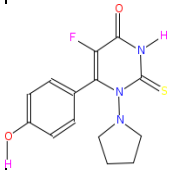
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581	25m		-3.607952	586	25s		-4.526787
582	25n		-5.239381	587	25t		-4.646255
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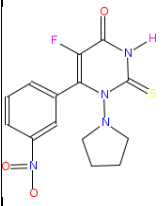
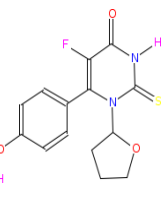
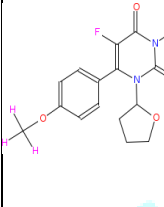
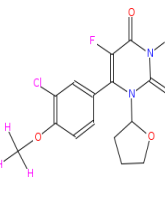
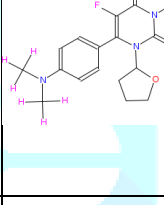
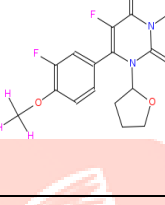
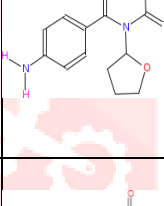
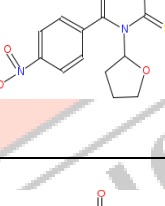
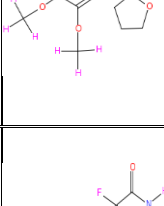
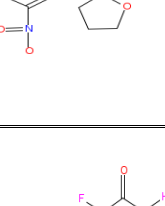
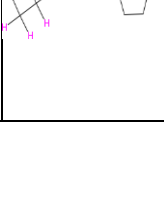
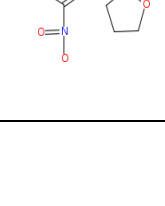
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592	25y		-4.812243	597	26d		-4.827365
593	25z		-3.577975	598	26e		-3.599957
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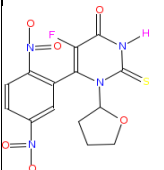
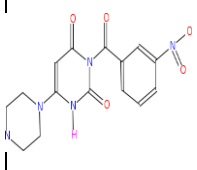
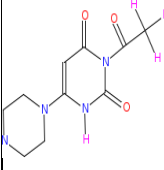
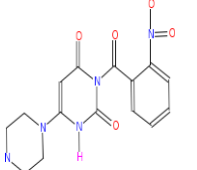
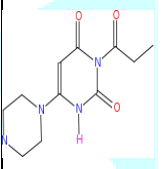
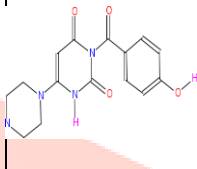
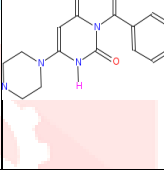
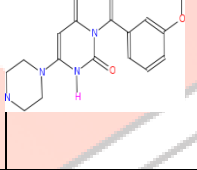
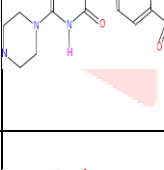
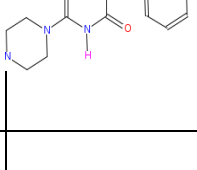
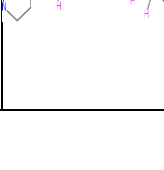
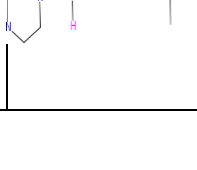
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603	26j		-3.889632	608	26o		-4.250826
604	26k		-3.903588	609	26p		-3.788779
605	26l		-4.189697	610	26q		-4.657095
611	26r		-4.532080	616	26w		-3.534443

612	26s		-4.621198	617	26x		-4.284106
613	26t		-4.416054	618	26y		-4.260864
614	26u		-4.435655	619	26z		-3.347567
615	26v		-3.756515	620	27a		-4.614666
621	27b		-4.399317	626	27g		-3.819634
622	27c		-4.398298	627	27h		-4.383648

623	27d		-3.932794	628	27i		-3.642334
624	27e		-3.744478	629	27j		-3.320933
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631	27l		-1.997263	636	27q		-4.548842
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634	27o		-3.549612	639	27t		-4.320038
635	27p		-1.450219	640	27u		-3.575643
641	27v		-3.999520	646	28a		-3.528582
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644	27y		-3.554367	649	28d		-4.340799

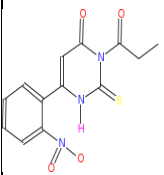
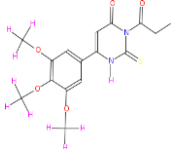
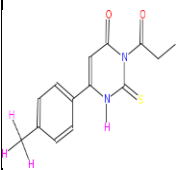
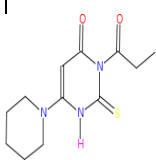
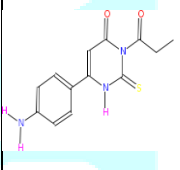
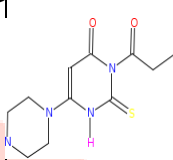
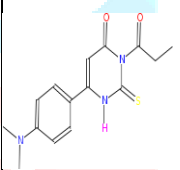
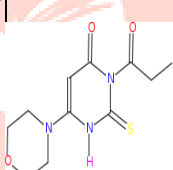
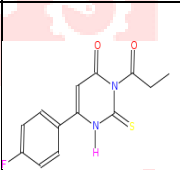
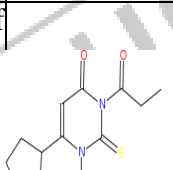
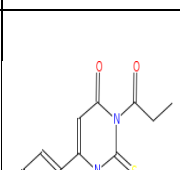
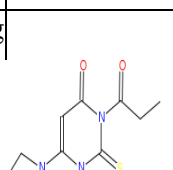
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653	28h		-4.464760	658	28m		-3.750700
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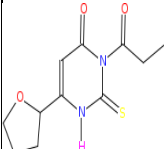
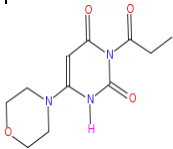
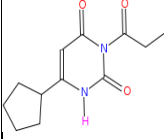
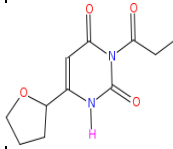
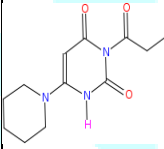
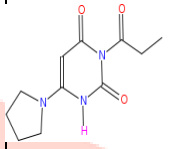
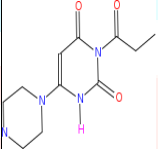
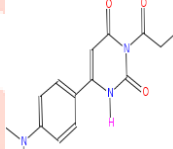
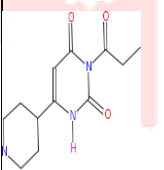
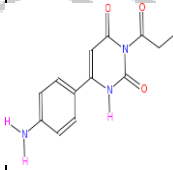
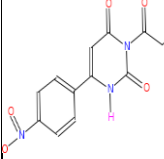
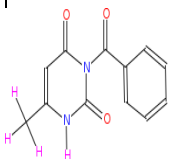
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662	29a		-1.573313	667	29f		-4.429177
663	29b		-1.833354	668	29g		-4.323399
664	29c		-2.064308	669	29h		-3.240237
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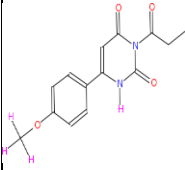
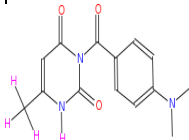
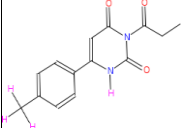
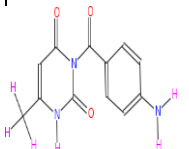
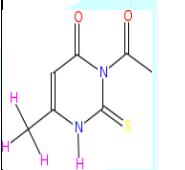
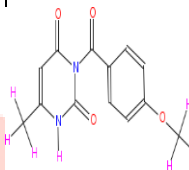
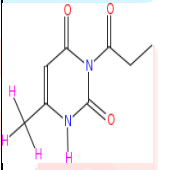
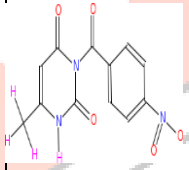
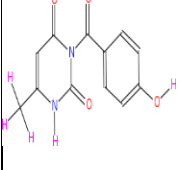
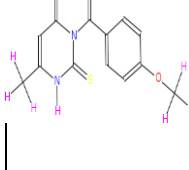
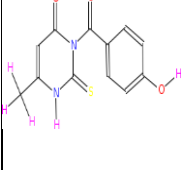
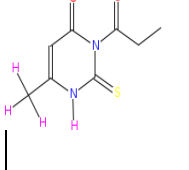
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674	29m		-2.061378	679	29r		-3.982544
675	29n		-3.951177	680	29s		-3.936351
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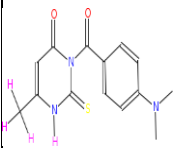
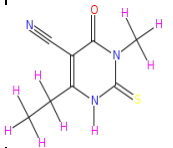
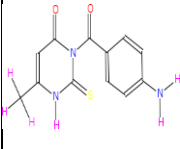
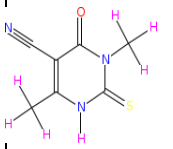
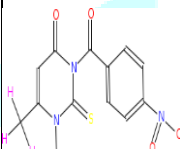
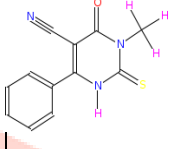
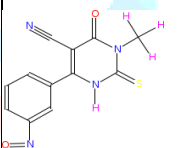
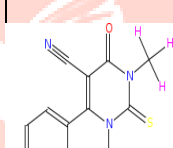
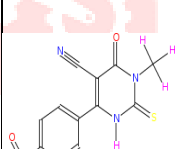
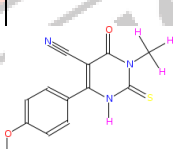
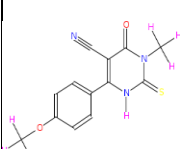
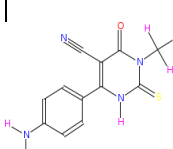
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691	30d		-5.199943	696	30i		-4.330076
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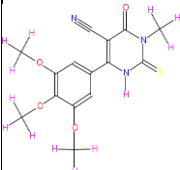
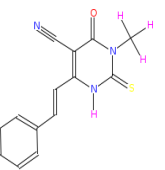
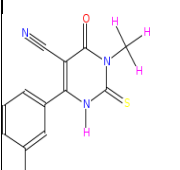
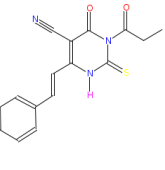
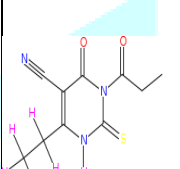
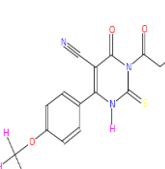
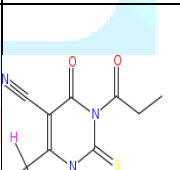
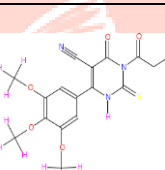
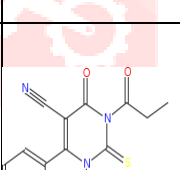
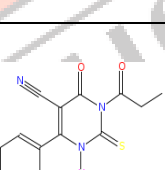
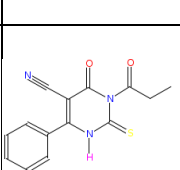
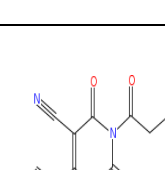
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701	30n		-4.487984	706	30s		-3.825821
702	30o		-3.675171	707	30t		-2.954136
703	30p		-2.910738	708	30u		-4.157927
704	30q		-4.440204	709	30v		-4.320418

705	30r		-4.660487	710	30w		-4.520437
711	30x		-4.543399	716	31c		-4.515141
712	30y		-4.583955	717	31d		-4.398973
713	30z		-3.162625	718	31e		-3.552788
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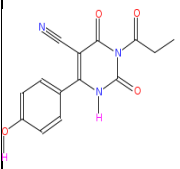
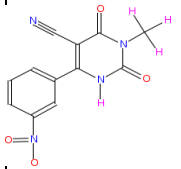
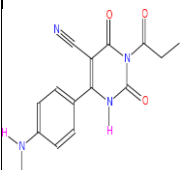
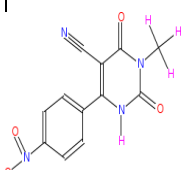
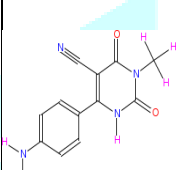
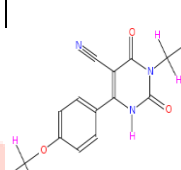
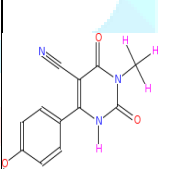
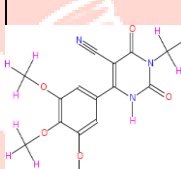
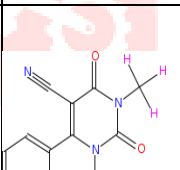
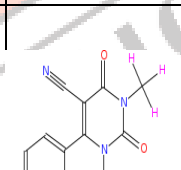
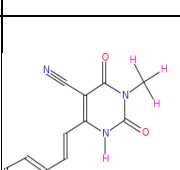
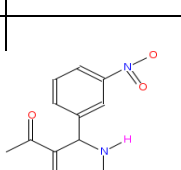
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723	31j		-3.401577	728	31p		-3.552788
724	31l		-2.539450	729	31q		-4.128493
725	31m		-3.164620	730	31r		-3.982544
731	31s		-3.350069	736	31w		-3.936351

732	31t		-3.579170	737	31x		-2.644648
733	31u		-0.347136	738	31y		-3.018975
734	31v		-2.850885	739	31z		-3.169153
735	31v		-4.216313	740	32a		-2.477860
741	32b		-4.179420	746	32g		-2.768214
742	32c		-4.415052	747	32h		-2.244581

743	32d		-4.972067	748	32i		-1.591610
744	32e		-4.816826	749	32j		-0.519627
745	32f		-3.713055	750	32k		-1.859843
751	32l		-4.937042	756	32q		0.335012
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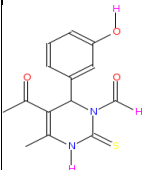
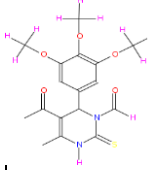
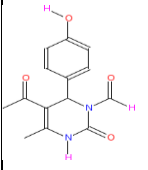
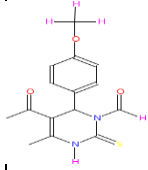
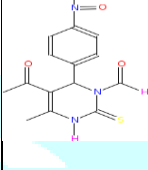
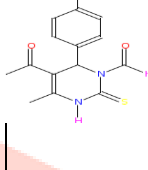
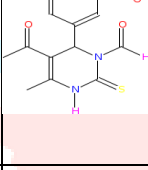
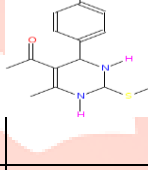
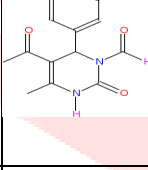
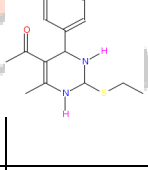
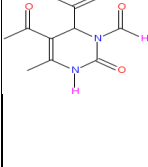
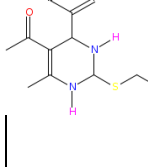
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761	32v		-4.887077	766	33a		-3.897591
762	32w		-4.777392	767	33b		-1.996366
763	32x		-4.128493	768	33c		-1.750336
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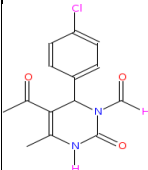
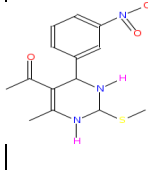
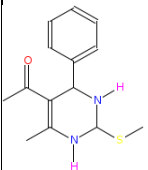
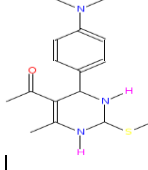
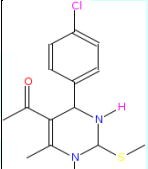
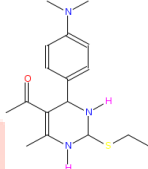
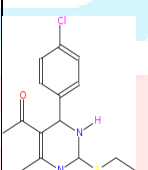
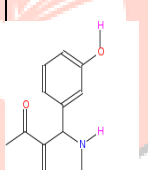
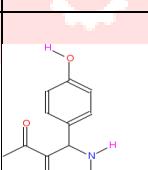
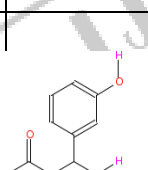
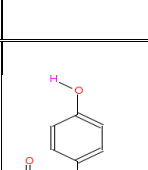
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772	33g		-3.018975	777	33l		-3.011446
773	33h		-4.415052	778	33m		-3.127130
774	33i		-4.972067	779	33n		-3.436488
775	33j		-4.816826	780	33o		-3.994591

781	33p		-3.713055	786	33u		-3.401185
782	33q		-4.937042	787	33v		-1.248823
783	33r		-4.799076	788	33w		-2.424660
784	33s		-4.325091	789	33x		-3.419863
785	33t		-4.415052	790	33y		-3.412892
791	33z		-4.972067	796	34e		-3.704842

792	34a		-3.301119	797	34f		-2.882619
793	34b		-0.625637	798	34g		-4.279670
794	34c		-2.872770	799	34h		-1.333442
795	34d		-3.110456	800	34i		-2.966944
801	34j		-1.827941	806	34o		-3.663190
802	34k		-1.248823	807	34p		-3.088192

803	34l		-2.424660	808	34q		-3.362113
804	34m		-3.419863	809	34r		-2.066932
805	34n		-3.412892	810	34s		-3.784437
811	34t		-3.704842	816	34y		-2.535225
812	34u		-2.882619	817	34z		3.905382
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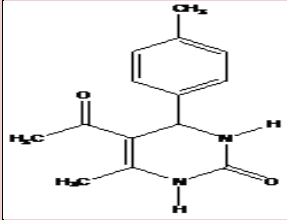
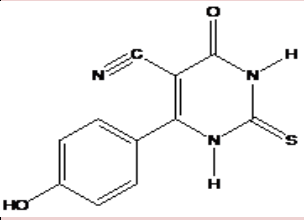
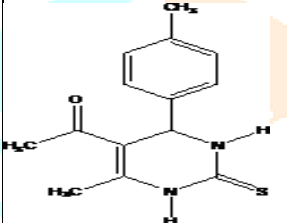
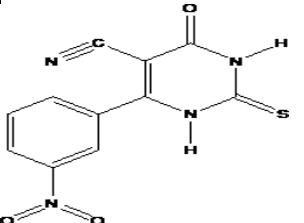
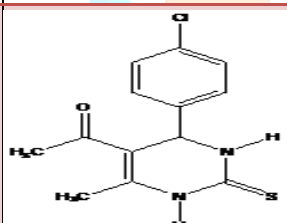
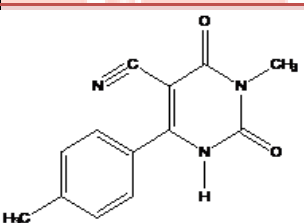
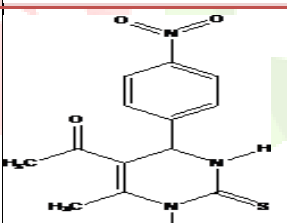
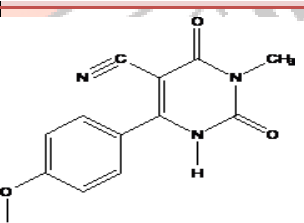
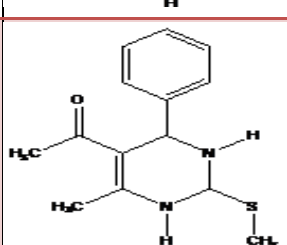
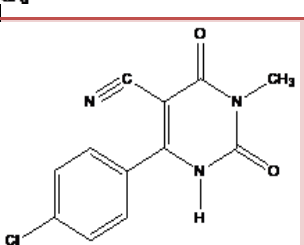
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815	34x		-2.966944	820	35c		-4.116265
821	35d		-3.663190	826	35i		-3.567000
822	35e		-3.088192	827	35j		-3.161491
823	35f		-3.362113	828	35k		-4.421368
824	35g		-2.066932	829	35l		-2.968208

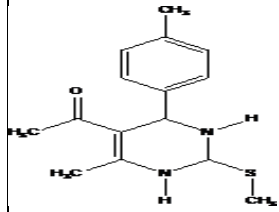
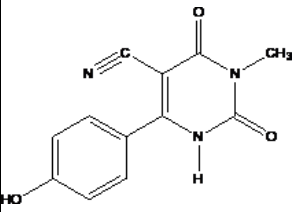
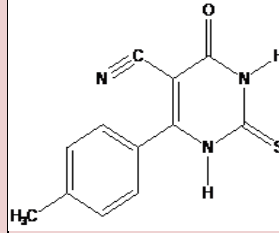
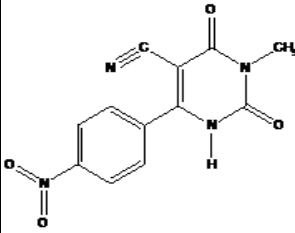
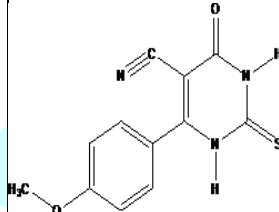
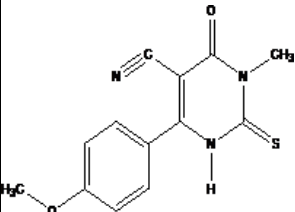
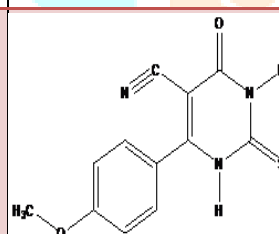
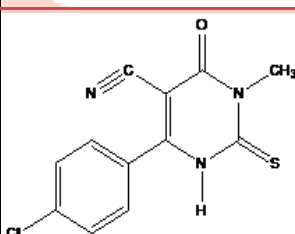
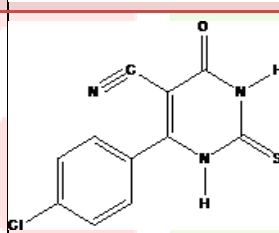
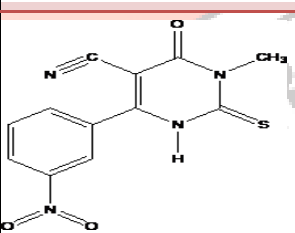
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831	35r		-2.535225	836	35w		-4.963976
832	35s		3.905382	837	35x		-3.308057
833	35t		-3.960534	838	35y		-4.896991
834	35u		-4.507489	839	35z		-3.797137
835	35v		-4.116265				

## 06.3 Selection of best docked analogues for synthesis:

## 06.3.1 Selected 20 best docked analogues for synthesis:

Table no. 6.2 20 best docked analogues for synthesis

Comp.code	Structure	Dock score	Comp. code	Structure	Dock score
HDB-1		-3.847	RLS-4		-2.537
HDB-2		-3.761	RLS-5		-1.820
HDB-3		-3.331	RLS-6		-3.008
HDB-4		-4.067	RLS-7		-2.287
HDB-5		-3.466	RLS-8		-3.055

HDB-6		-4.135	RLS-9		-3.340
HDB-7		-2.274	RLS-10		-2.649
RLS-1		-3.704	RLS-11		-3.102
RLS-2		-2.958	RLS-12		-3.156
RLS-3		-2.541	RLS-13		-3.324
			MTX STD	MTHOTREXATE	-1.576

#### 06.4 Docking interactions of active best docked analogues:

For checking binding interaction first open receptor in MDS followed by compound which saved as ligand dock file. From tool option click on merge molecule so that compound and receptor is merged together. From biopredicta tool edit this complex and select ligand and receptor structure afterward check its interaction.

#### 06.4.1 Docking interaction of compounds with microbial DHFR on PDB code3FRE:

##### 06.4.1.1 Docking interactions of HDB-2:

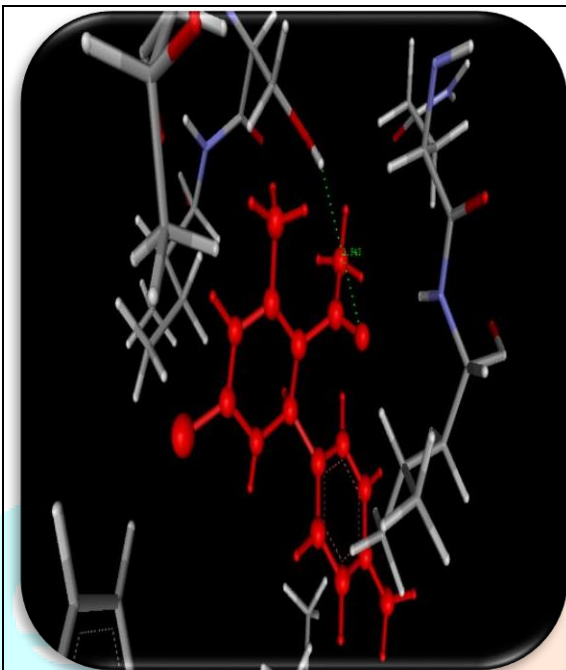


Fig. 6.7 HDB-2 Hydrogen bonding

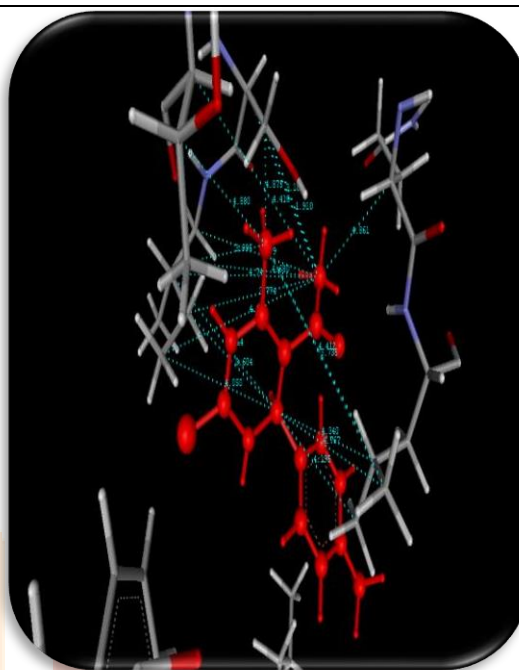


Fig. 6.8 HDB-2 Hydrophobic bonding

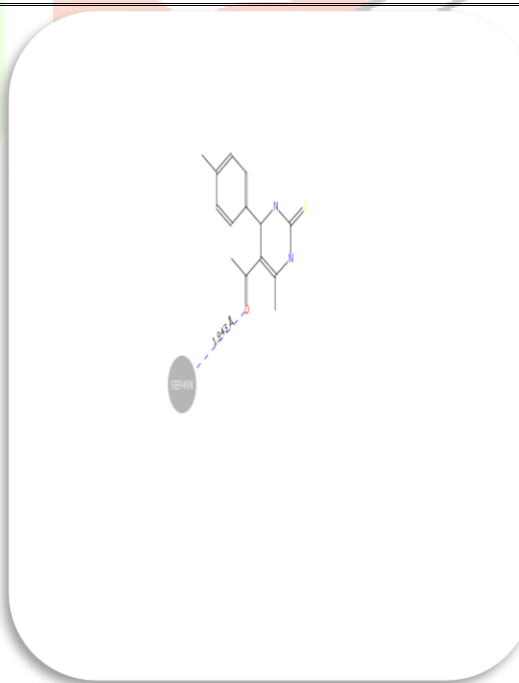
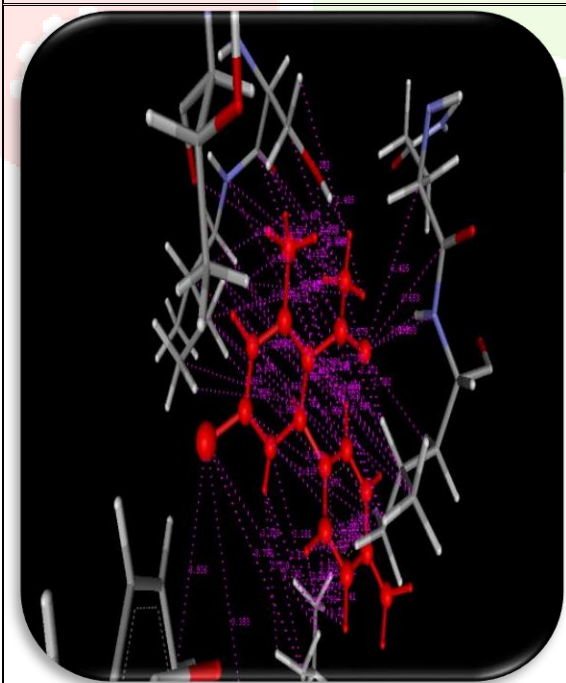


Fig. 6.9 HDB-2 VdW bonding

Fig. 6.10 2D HDB-2 Hydrogen bonding

#### 06.4.1.2 Docking interactions of HDB-6:

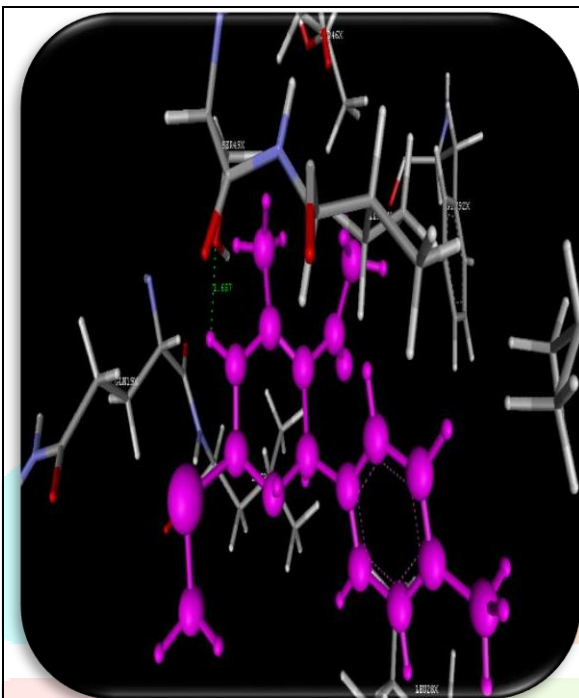


Fig. 6.11 HDB-6 Hydrogen bonding

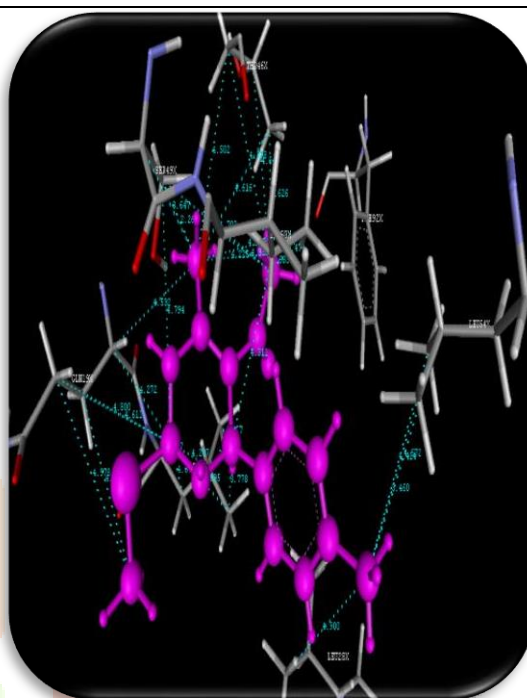
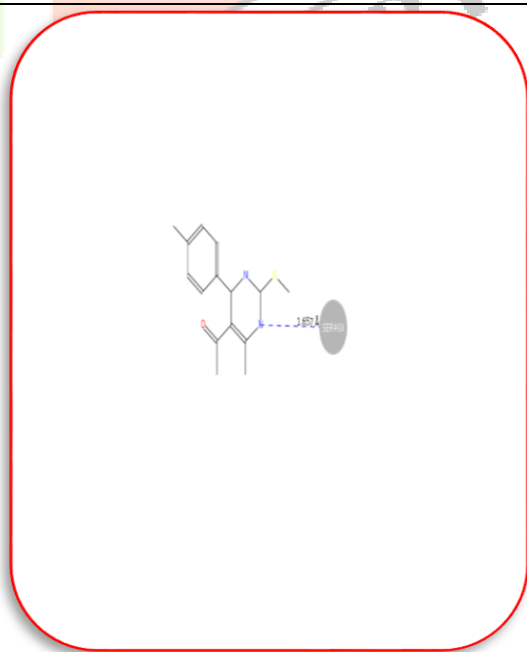
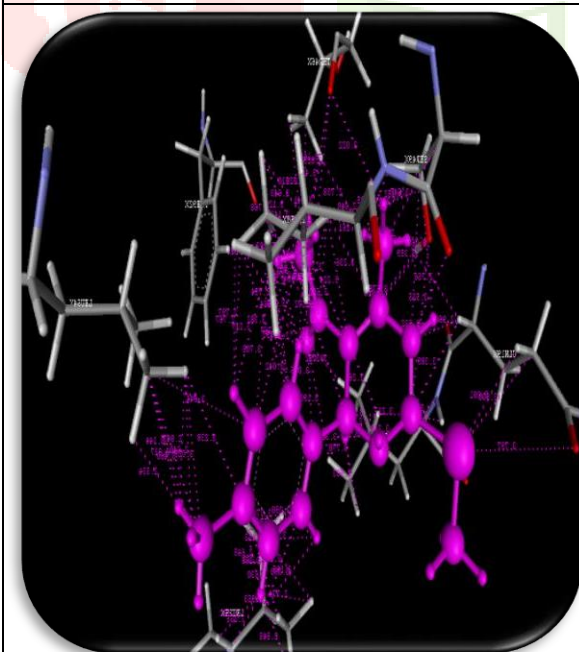
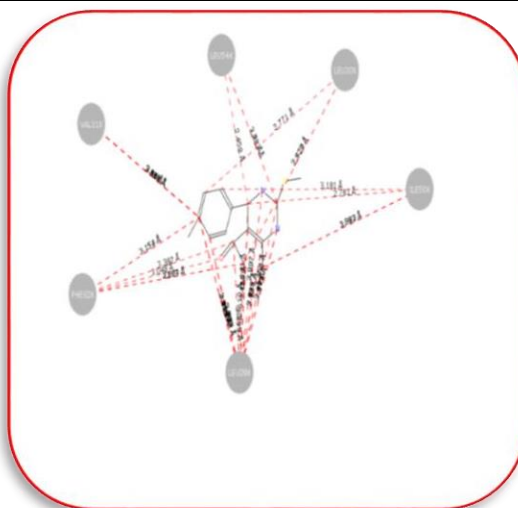
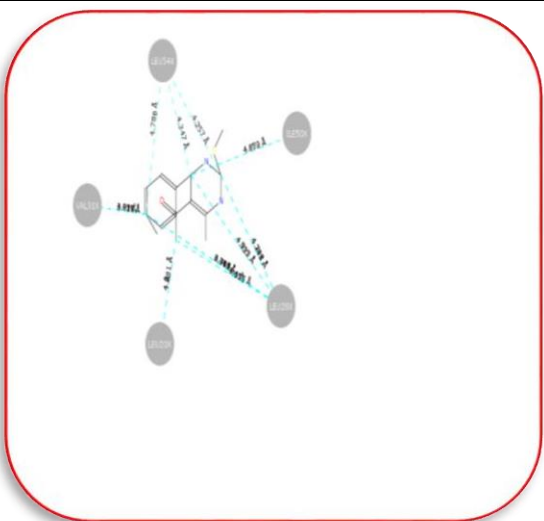


Fig. 6.12 HDB-6 Hydrophobic bonding

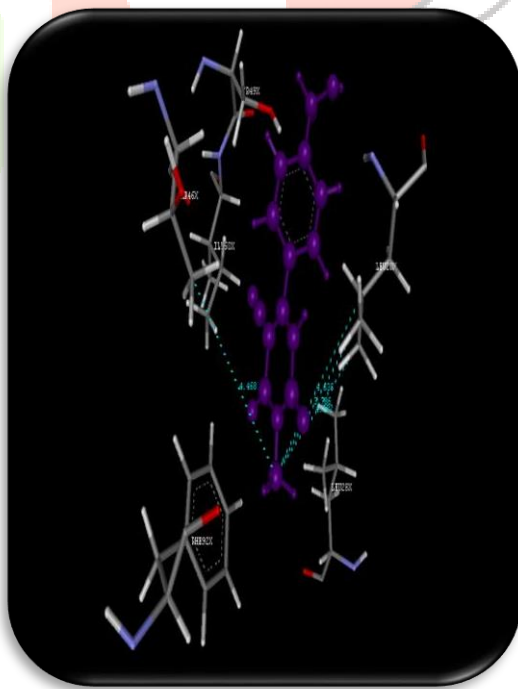
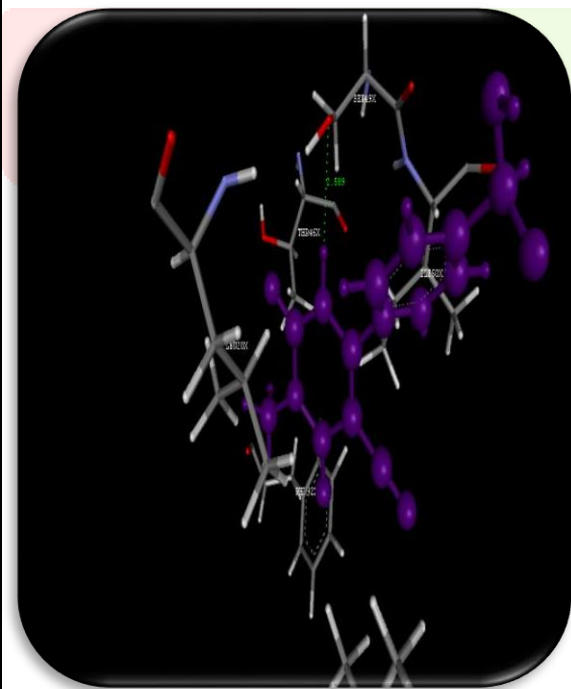


**Fig. 6.14 2D HDB-6 Hydrogen bonding**

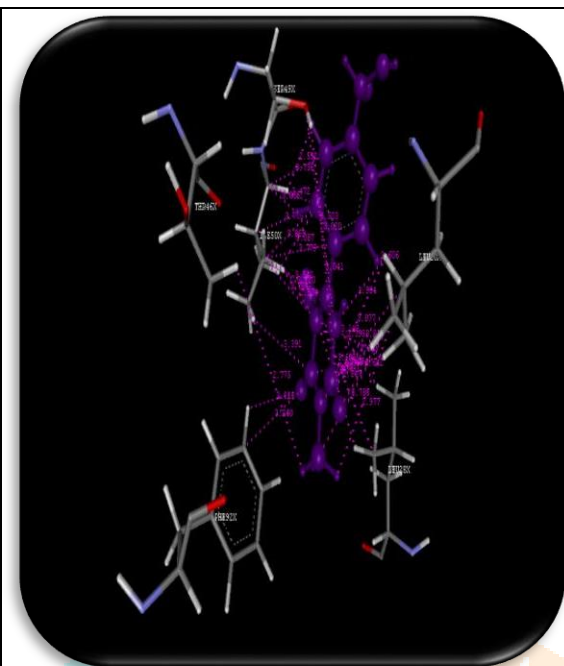
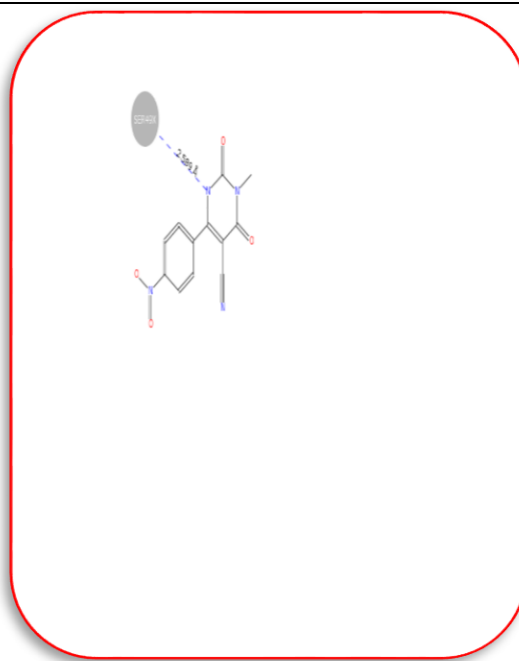


**Fig. 6.16 HDB-6 VdW bonding**

#### 06.4.1.3 Docking interactions images of RLS-10:



**Fig. 6.18 RLS-10 Hydrophobic bonding**

**Fig. 6.19 RLS-10 VdW bonding****Fig. 6.20 2D RLS-10 Hydrogen bonding**

#### 06.4.1.4 Docking interactions images of Trimethoprim:

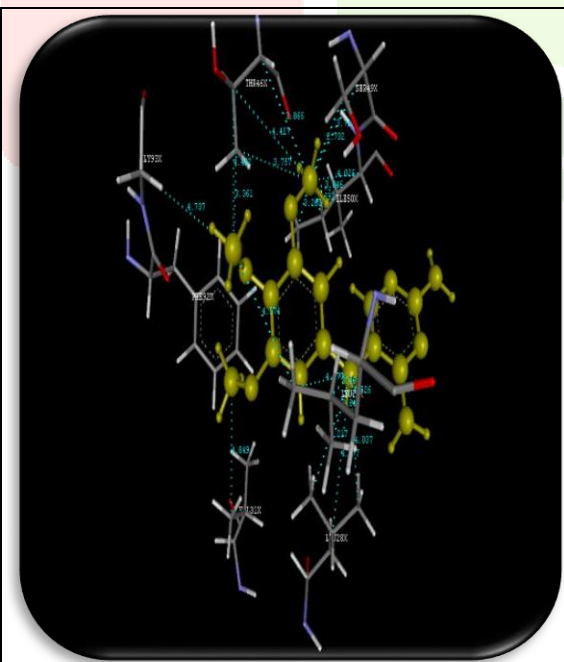
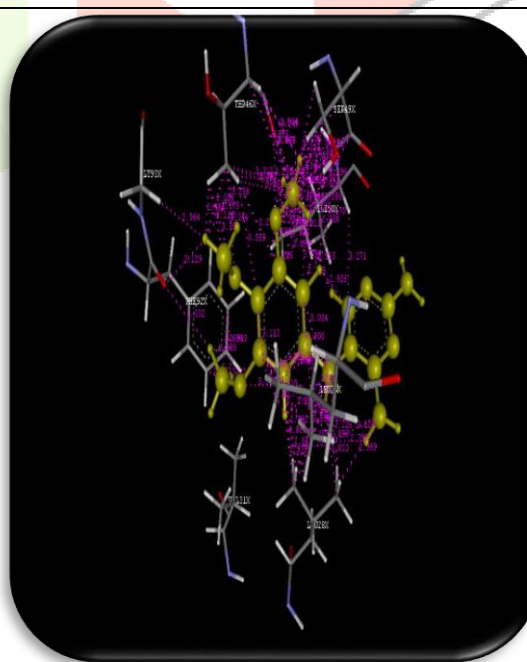
**Fig. 6.21 TMP Hydrophobic bonding****Fig. 6.22 TMP VdW bonding**

Table no. 6.3 Docking interaction of compounds with microbial DHFR on PDB code3FRE

Comp.	Hydrogen bonding	Hydrophobic bonding	VdW bonding
<b>HDB-2</b>	SER49X 1.943	GLN19X 4.861 LEU20X 4.340 THR46X 4.418 SER49X 3.182 ILE50X 4.469	GLN19X 3.426 LEU20X 3.393 LEU28X 3.819 THR46X 3.515 SER49X 3.283 ILE50X 3.636 PHE92X 3.936
<b>HDB-6</b>	SER49X 1.657	GLN19X 4.272 LEU20X 4.937 LEU28X 4.900 THR46X 4.898 SER49X 3.647 ILE50X 4.933 LEU54X 4.572	GLN19X 2.968 LEU20X 3.020 LEU28X 3.255 THR46X 3.456 SER49X 3.152 ILE50X 3.255 LEU54X 3.644 PHE92X 3.056
<b>RLS-10</b>	SER49X 2.589	LEU20X 4.635 THR46X 4.468	LEU20X 3.812 LEU28X 3.793 THR46X 2.775 SER49X 3.503 ILE50X 3.472 PHE92X 3.240
<b>STD TMP</b>	---	LEU20X 4.526 LEU28X 4.777 VAL31X 4.849 THR46X 3.865 SER49X 3.718 ILE50X 4.025 GLY93X 4.737	LEU20X 3.461 LEU28X 3.033 THR46X 3.865 SER49X 3.718 ILE50X 3.383 PHE92X 3.032 GLY93X 2.564

#### 06.4.2 Docking interaction images of compounds with cancer cell DHFR on PDBcode 2W3M:

##### 06.4.2.1 Docking interaction images of HDB-2:

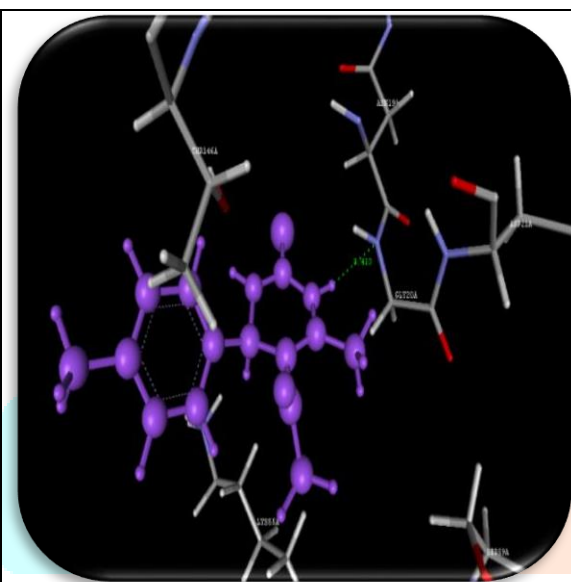


Fig. 6.23 HDB-2 Hydrogen bonding

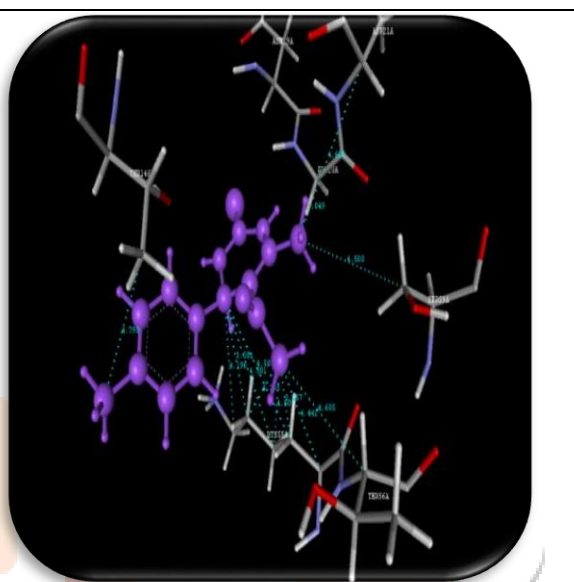


Fig. 6.24 HDB-2 Hydrophobic bonding

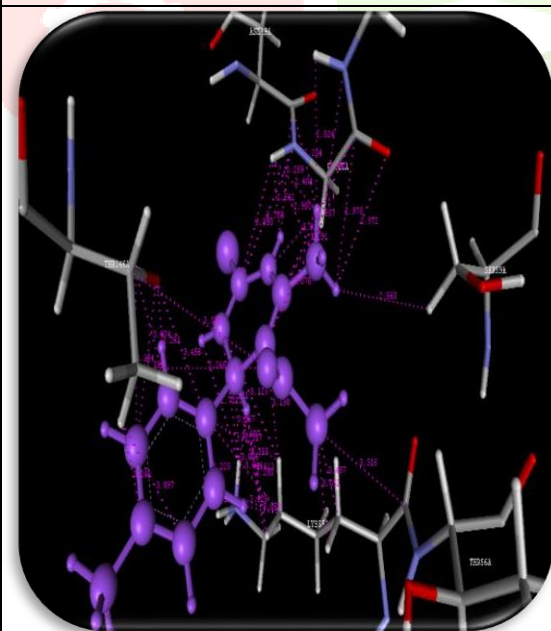


Fig. 6.25 HDB-2 VdW bonding

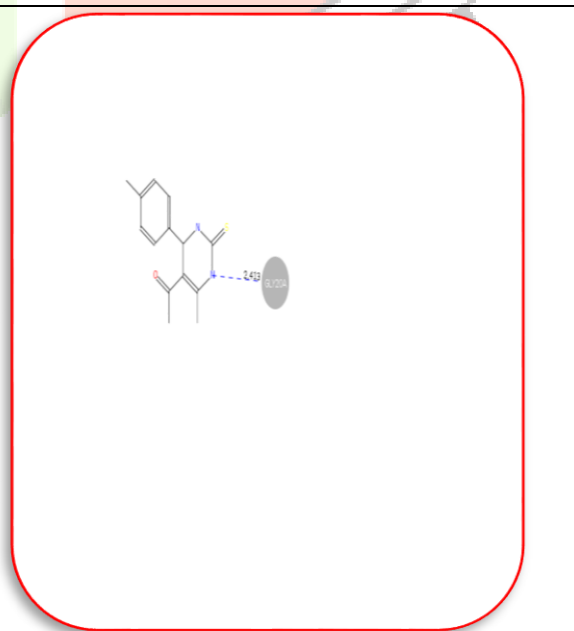
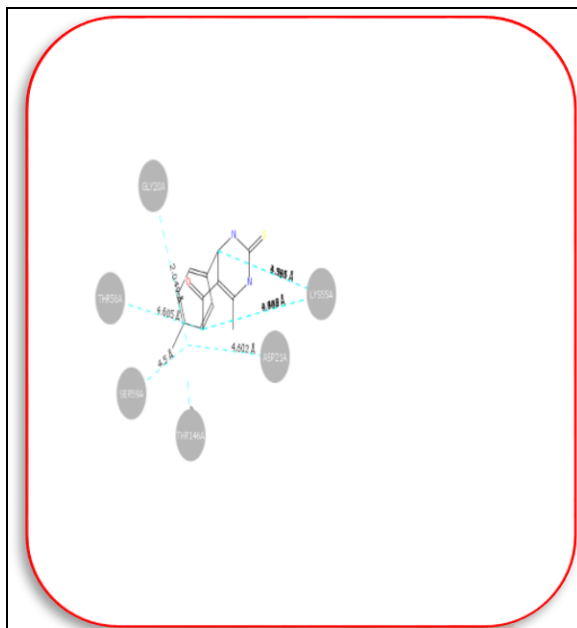
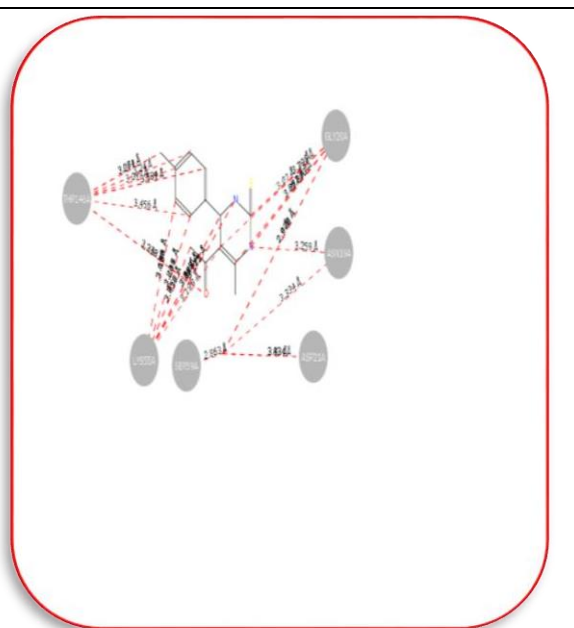


Fig. 6.26 2D HDB-2 Hydrogen bonding

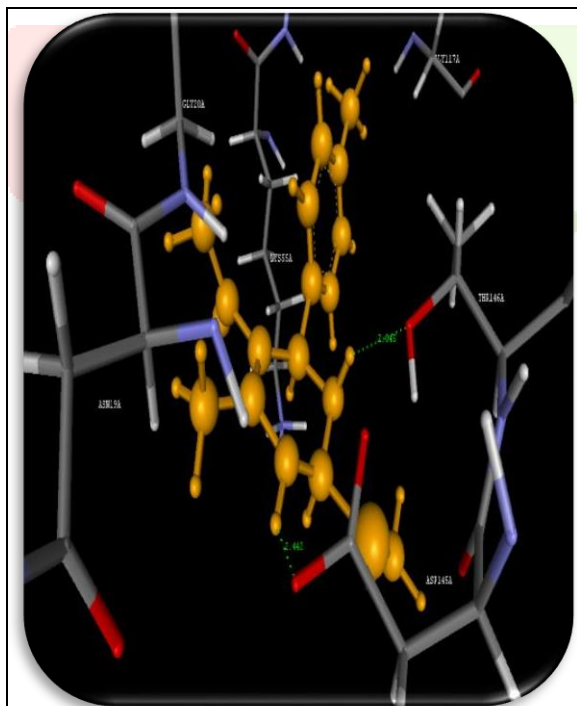


**Fig. 6.27 2D HDB-2 Hydrophobic bonding**

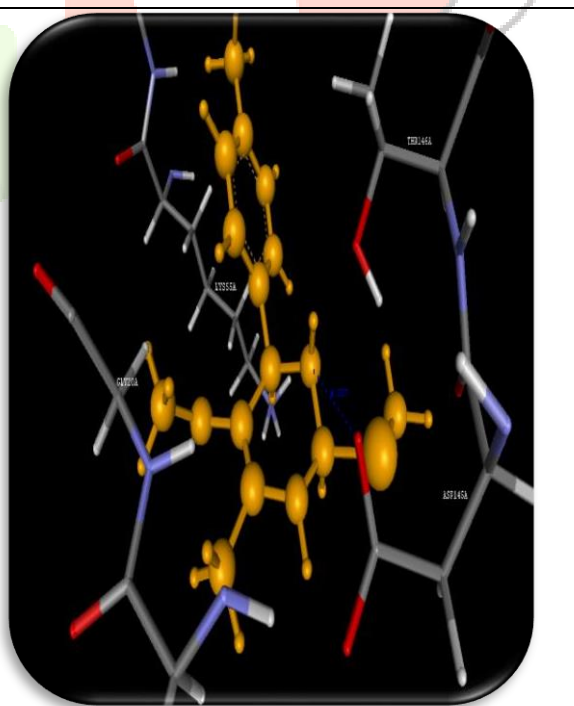


**Fig. 6.28 2D HDB-2 VdW bonding**

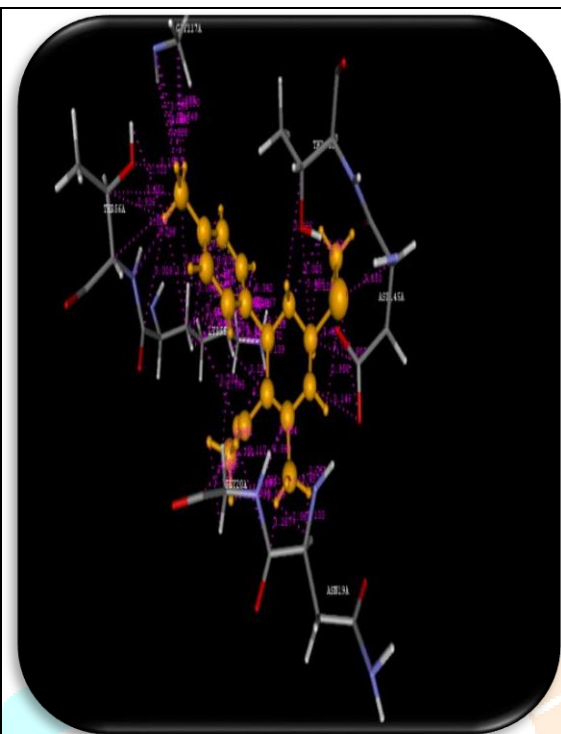
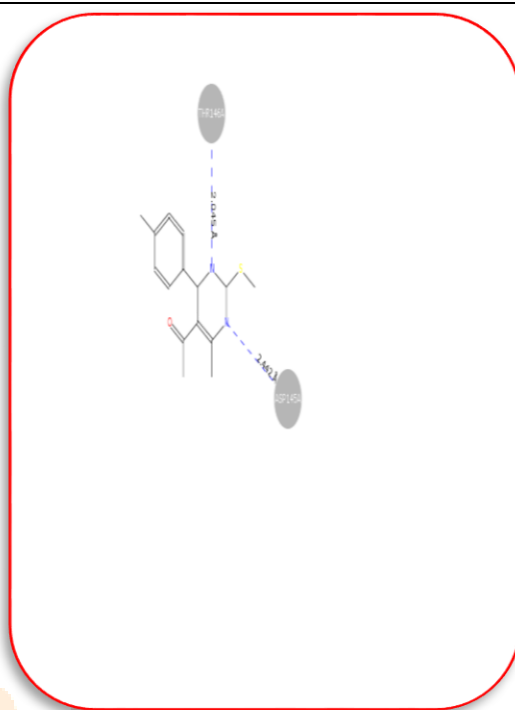
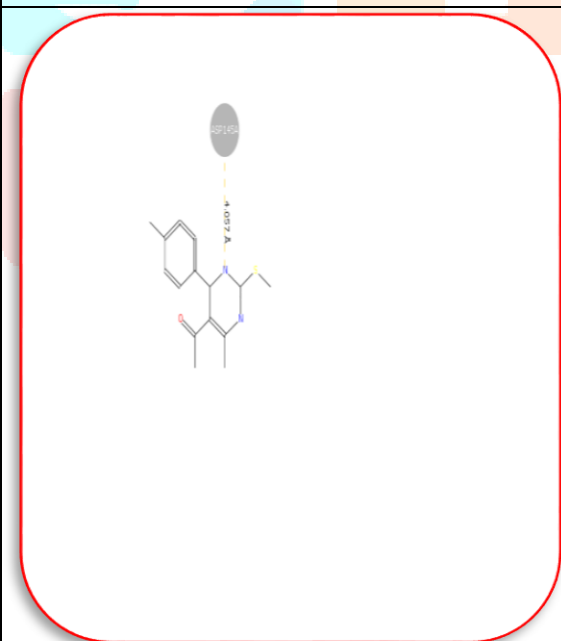
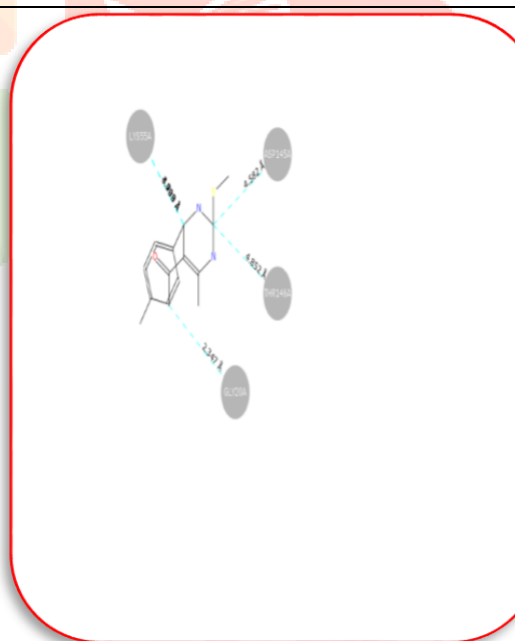
#### 06.4.2.2 Docking interaction images of HDB-6:



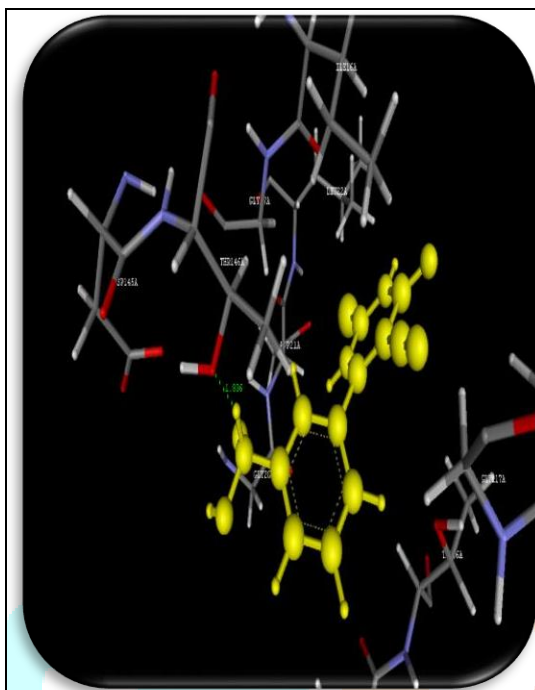
**Fig. 6.29 HDB-6 Hydrogen bonding**



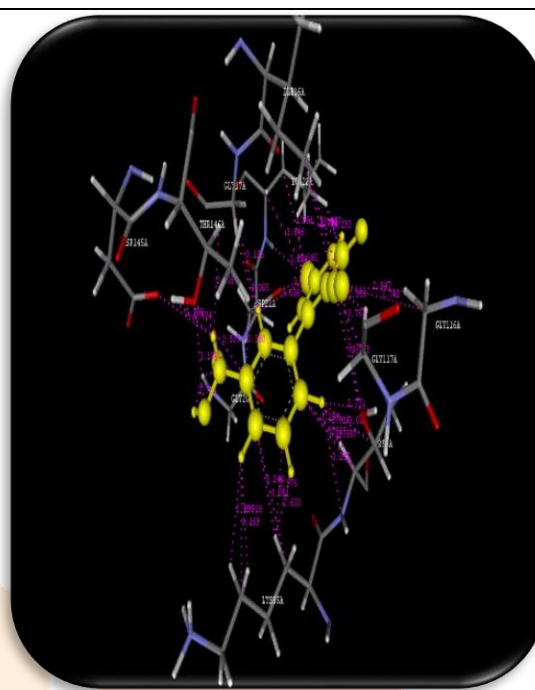
**Fig. 6.30 HDB-6 Charge interactions**

**Fig. 6.31 HDB-6 VdW bonding****Fig. 6.32 2D HDB-6 Hydrogen bonding****Fig. 6.33 2D HDB-6 Charge interaction****Fig. 6.34 2D HDB-6 VdW bonding**

#### 06.4.2.3 Docking interaction images of RLS-5:

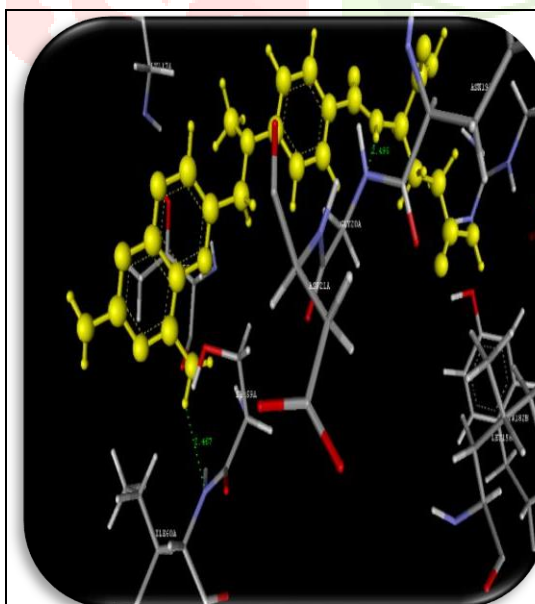


**Fig. 6.35 RIS-5 Hydrogen bonding**

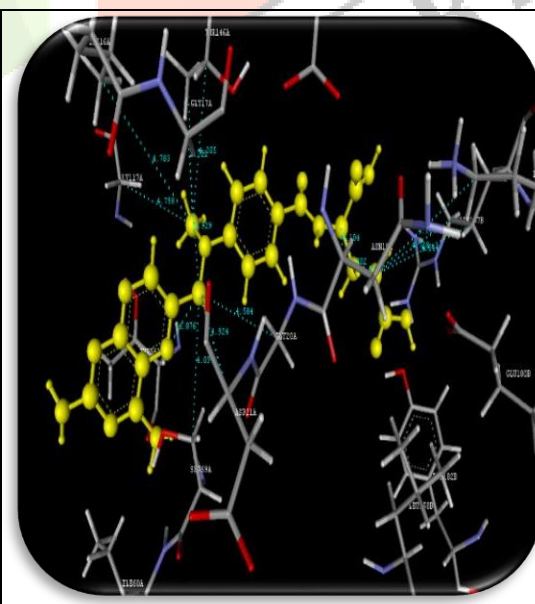


**Fig. 6.36 RLS-5 Hydrogen bonding**

#### 06.4.2.4 Docking interaction images of Methotrexate:



**Fig. 6.37 MTX Hydrogen bonding**



**Fig. 6.38 MTX Hydrophobic bonding**

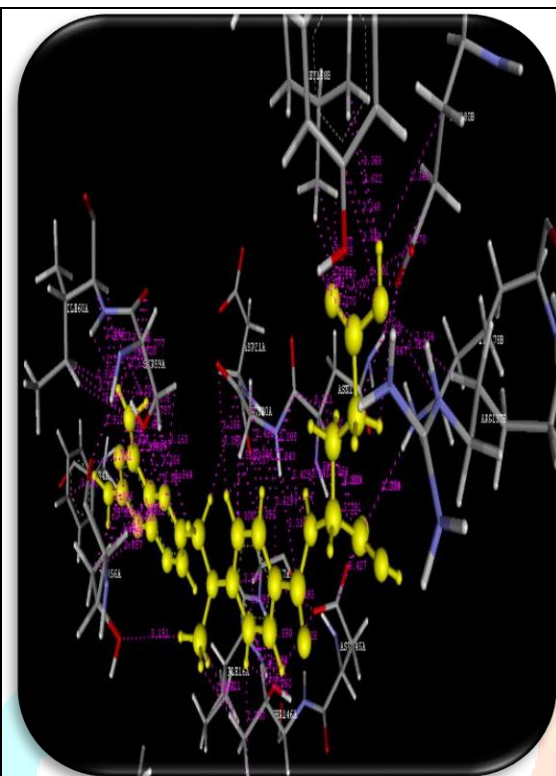
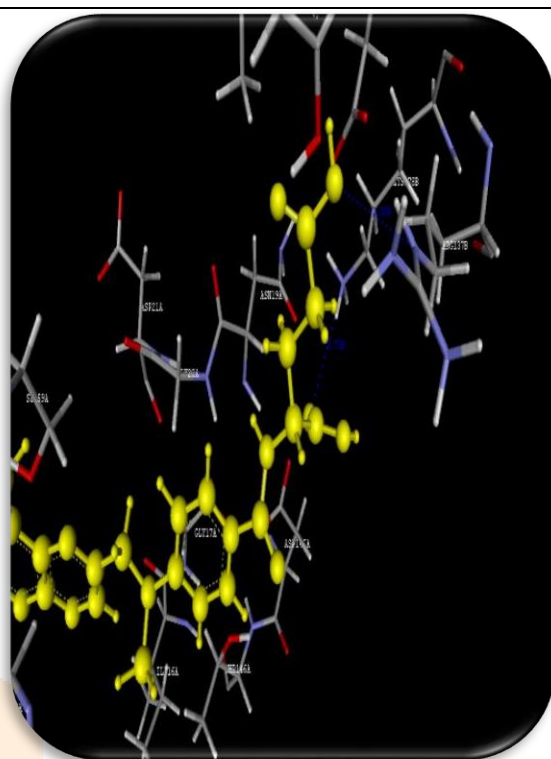
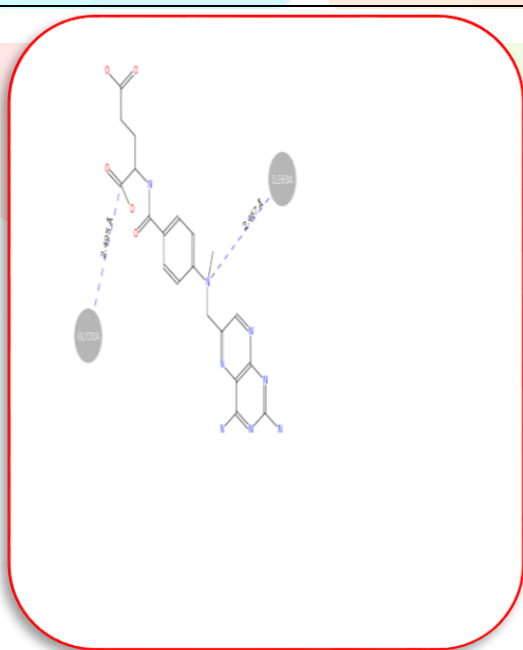
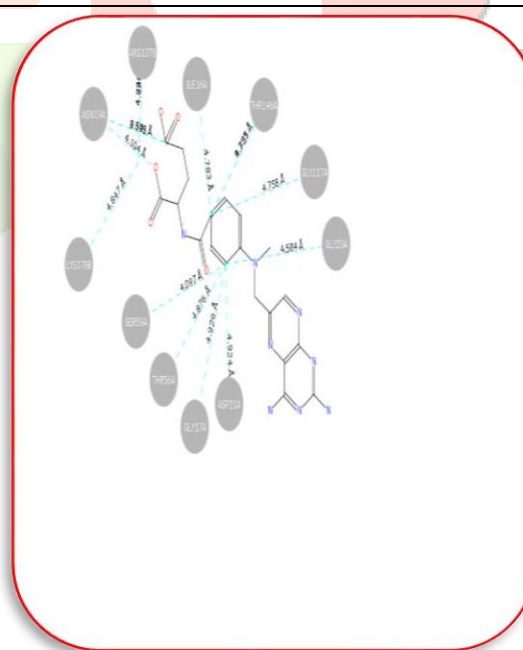
**Fig. 6.39 MTX VdW bonding****Fig. 6.40 MTX Charge interactions****Fig. 6.41 2D MTX Hydrogen bonding****Fig. 6.42 2D MTX Hydrophobic bonding**

Table no. 6.4 Docking interaction of compounds with cancer cell DHFR on PDBcode 2W3M

Comp.	Hydrogenbonding	hydrophobicbonding	VdW bonding	Charge interactions
<b>HDB-2</b>	SER49X 1.943	GLN19X 4.861 LEU20X 4.340 THR46X 4.418 SER49X 3.182 ILE50X 4.469	GLN19X 3.426 LEU20X 3.393 LEU28X 3.819 VAL31X 3.353 THR46X 3.515 SER49X 3.407 ILE50X 3.636 PHE92X 3.936	---
<b>HDB-6</b>	THR146A 2.045 ASP145A 2.442	ASN19A 3.109 GLY20A 2.347 LYS55A 4.232 THR56A 3.804 THR146A 4.651 ASP145A 4.582 GLY117A 3.548	ASN19A 3.497 GLY20A 3.569 LYS55A 3.018 THR56A 3.298 GLY117A 3.668 ASP145A 3.853 THR146A 3.366	ASP145A 4.057
<b>RLS-5</b>	THR146A 1.836	---	1 ILE16A 3.794 GLY17A 3.126 ASP21A 3.656 LEU22A 3.846 LYS55A 3.851 THR56A 3.205 GLY116A 3.742 THR146A 3.503 ASP145A 3.106	---
<b>STD MTX</b>	GLY20A- 2.495 ILE60A - 2.467	GLY117A- 4.758 THR146A- 4.355 ARG137B- 4.728 LYS178B- 4.847	GLY20A - 3.390 GLY17A - 3.293 ASN19A - 2.941 PHE34A- 3.398	LYS178B- 3.738

### 06.5 Discussion:

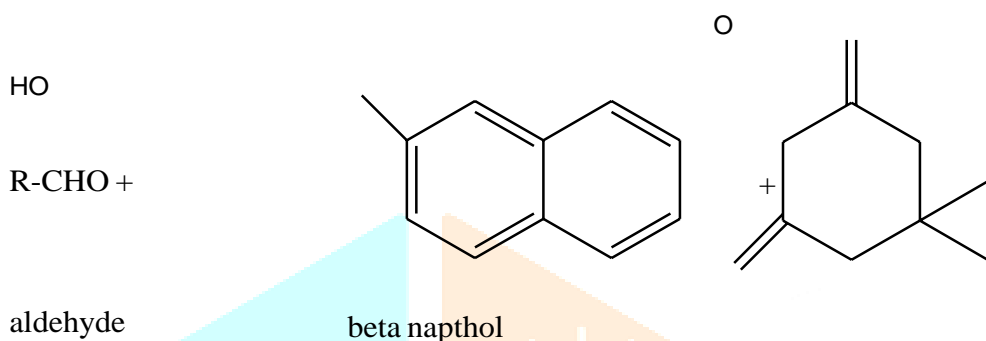
From the above molecular modeling and docking study compounds were selected on the basis of docking score, docking interactions and interaction distance in comparison with trimethoprim and methotrexate. Selected compounds have docking score less than standard compounds with same docking interactions and matching interaction distance with standard.



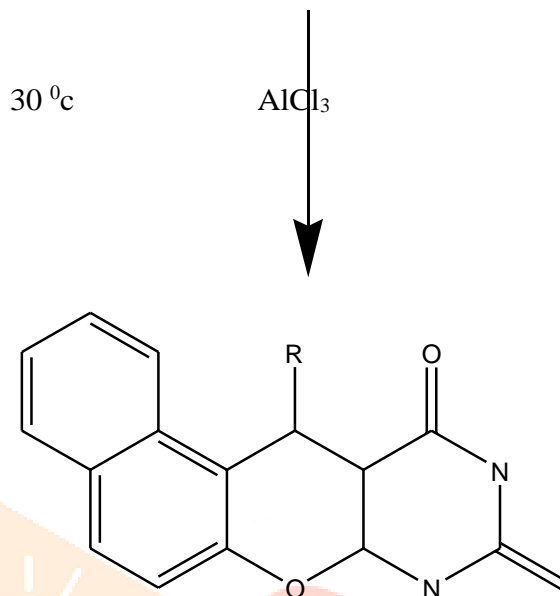
## 07. DESIGN OF SCHEME:

For the synthesis of 8,10 di methyl-12aryl -8,12 dihydro-7oxa-8,10diazabenzanthracene 9,11 dione schemes were designed.

### 07.1 Reaction scheme-I:<sup>1,2</sup>



O  
1,3 dicarbonyl compound



8,10 dimethyl-12 aryl -8,12 dihydro-7oxa 8,10 diazabenzanthracene 9,11dione

## 07.2 Mechanism of Reaction scheme

The step involves aluminium chloride catalysed one pot synthesis of 8,10 dimethyl- 12aryl-8,12 dihydro-7 oxa-8,10 di benzo anthracene 9,11 dione.

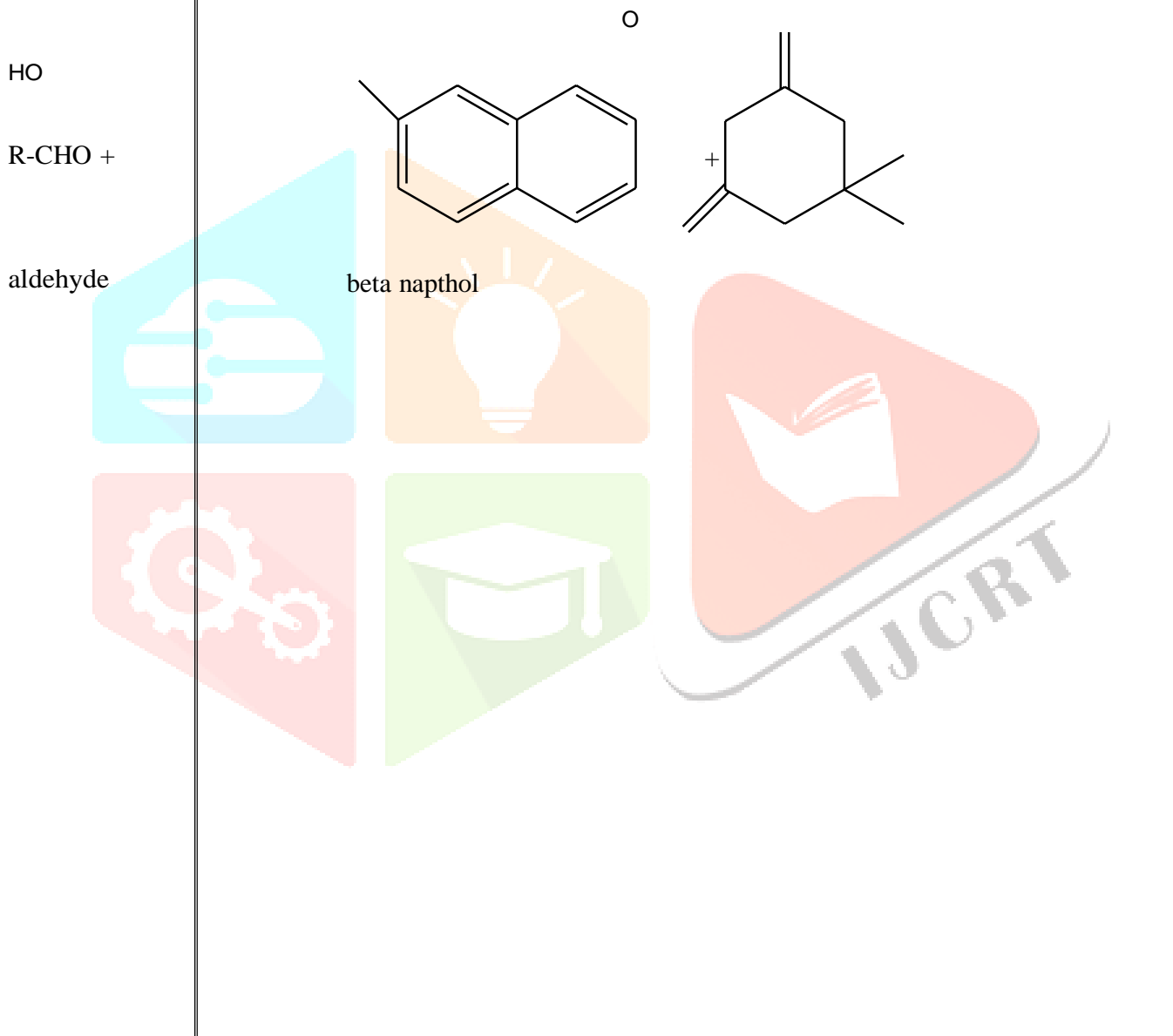
<sup>4</sup>.various aromatic aldehyde containing electron withdrawing group& electron donating group substitutes at ortho, para,meta shows equal ease of towards reaction.

Efficient one pot condensation of beta naphthol,aldehyde,cyclic 1-3 dicarbonyl compound had been achieved with molecular iodine as a catalyst under microwave irradiation thus variety of tetra hydro benzo (a)xanthene11 one di benzo(a)anthracene 9 ,11 dione.



## 08. SYNTHESIS OF COMPOUNDS:

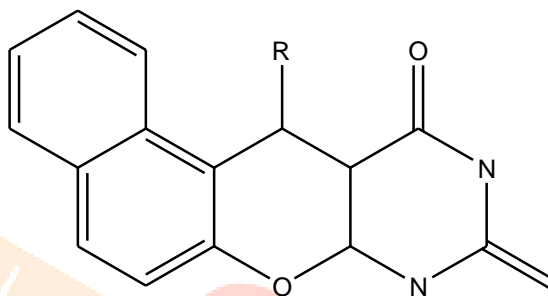
### 08.1 Scheme



O  
1,3 dicarbonyl compound

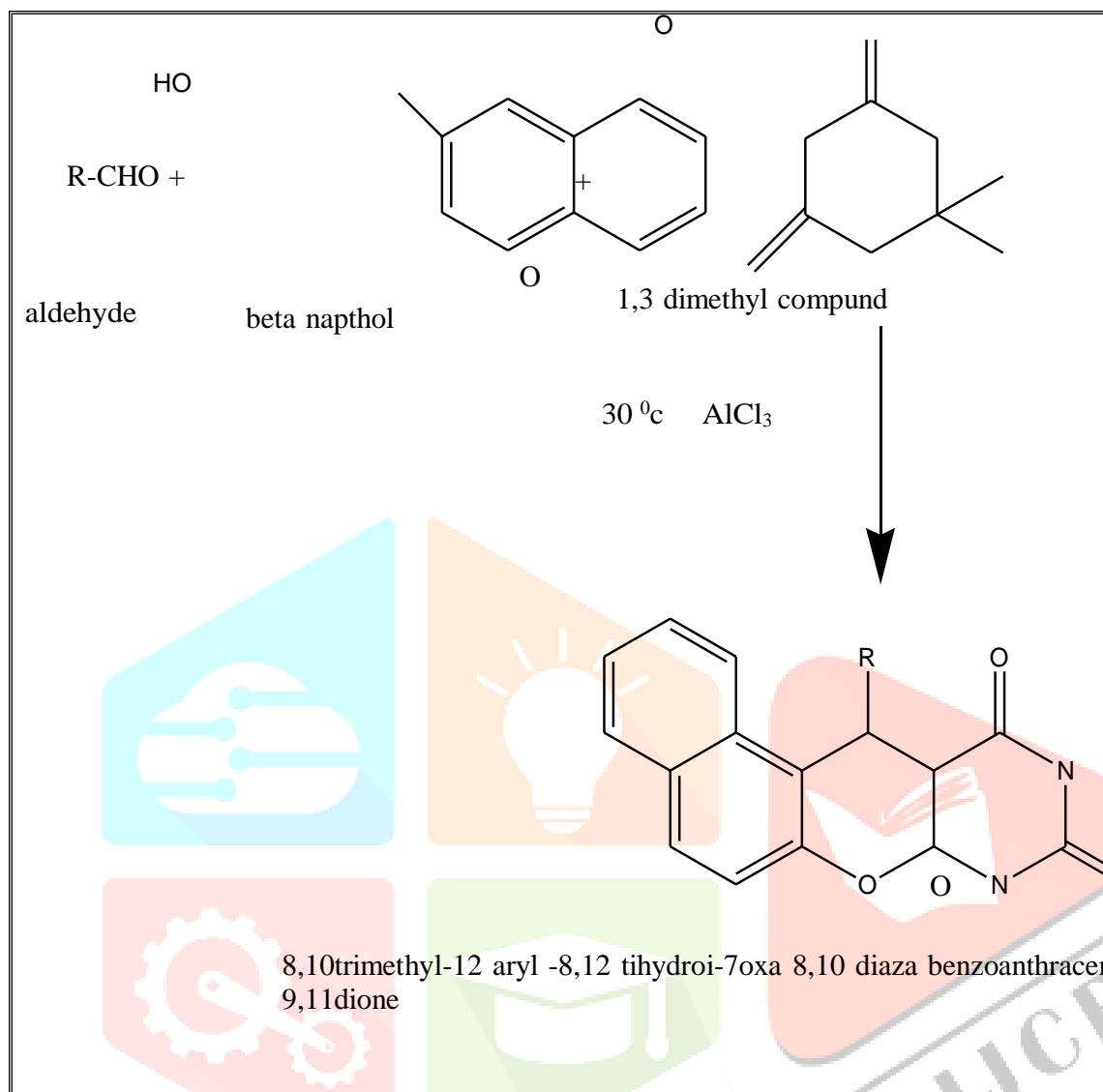
30 °c

AlCl<sub>3</sub>



8,10 dimethyl-12 aryl -8,12 dihydro-7oxa 8,10 diazabenzanthracene 9,11dione

O



#### 08.1.1 General procedure for scheme-::

##### Step: General procedure for synthesis of 8,10 diaza benzoanthracene 9,11 dione analogues:

These compounds were synthesized by the reported cyclocondensation reaction between aldehyde, beta naphthol and dimedone. The mixture of appropriate aldehyde (0.02 mol), beta naphthol (0.02 mol), dimedone (0.03 mol), aluminium chloride (0.01 mol), were refluxed for 4-6 hours. The solid thus separated on cooling was filtered, washed with coldmethanol, dried and recrystallized from methanol.

**1) 8,10 dimethyl-12chloro -8,12 dihydro-7 oxa-8,10 diaza benzo anthracene9,11dione (pvv-1):**

A mixture of 2-chlorobenzaldehyde, beta naphthol, dimedone,& alluminium chloride was taken in round bottom flask and refluxed for 4-6 hrs.

Melting point: 175°C, yield: 84.85%.

**2) 8,10 dimethyl-12 benzo chloro -8,12 dihydro-7 oxa-8,10 diaza benzo anthracene9,11 dione (pvv-2):**

A mixture 3 chloro benzaldehyde, beta naphthol, dimedone, alluminium chloride was taken in round bottom flask and refluxed for 4-6 hrs.

Melting point: 187°C, yield: 82.87%.

**3) 8,10 dimethyl-12 benzo chloro -8,12 dihydro-7 oxa-8,10 diaza benzo anthracene 9,11 dione (pvv-3):**

A mixture of 4-chlorobenzaldehyde, beta naphthol, dimedone, alluminium chloride was taken in round bottom flask and refluxed for 4-6 hrs.

Melting point: 149°C, yield: 83.77%.

**4) 8,10 dimethyl-12 benzo nitro -8,12 dihydro-7 oxa-8,10 diaza benzo anthracene 9,11 dione (pvv-4):**

A mixture of 4-nitrobenzaldehyde, betanaphthol, dimedone, alluminium chloride was taken in round bottom flask and refluxed for 4-6 hrs.

Melting point: 131°C, yield:

Melting point: 149°C, yield: 83.77%.

**5) 8,10 dimethyl-12 benzo 3-nitro -8,12 dihydro-7 oxa-8,10 diaza benzo anthracene 9,11 dione (pvv-5):**

A mixture of 3-nitrobenzaldehyde, betanaphthol, dimedone,aluminium chloride was taken in volumetric flask undergoes microbial irradiation

Reaction time: 1 h, Melting point: 208°C, yield: 85.23 %

**6) 8,10 dimethyl-12 benzo para hydro -8,12 dihydro-7 oxa-8,10 diaza benzo anthracene 9,11 dione (pvv-4):**

A mixture of p-hydro benzaldehyde, betanaphthol, dimedone,aluminium chloride was taken in volumetric flask undergoes microbial irradiation reaction time :1 h,

- 7) **8,10 dimethyl-12 salicylaldehyde -8,12 dihydro-7 oxa-8,10 diaza benzo anthracene 9,11 dione (pvv-4):**A mixture of salicylaldehyde , betanaphthol, dimedone,aluminium chloride was taken in volumetric flask undergoes microbial irradiation reaction time :**1 h**,

Reaction time: 2 h, Melting point: 202°C, yield: 73.20 %

- 8) **8,10 dimethyl-12 anise aldehyde -8,12 dihydro-7 oxa-8,10 diaza benzo anthracene 9,11 dione (pvv-4):**A mixture of anise aldehyde , betanaphthol, dimedone,aluminium chloride was taken in volumetric flask undergoes microbial irradiation reaction time :**1 h**,

Reaction time: 2 h, Melting point: 210°C, yield: 78.40 %

### 08.3 Merck index table:

Table no. 08.1 Merck index

Merck Index No.	Chemical Name (Mol. Form.)	Mol. Wt.	Density g/ml	Physical Constant		Solubility	Caution
				MP °C	BP °C		
1481	Acetyl acetone (C <sub>5</sub> H <sub>8</sub> O <sub>2</sub> )	100.12	0.98	-23	140	Soluble in water, alcohol, ether, chloroform, hot benzene	-

335	Aluminium chloride (AlCl <sub>3</sub> )	133.34	-	-	-	Soluble in organic solvent	Anhydrous form is strong irritant
1057	Benzaldehyde (C <sub>7</sub> H <sub>6</sub> O)	106.12	1.04	-56.5	179	Miscible with alcohol, ether, oils	Narcotic in high conc. May cause contact dermatitis
2214	4-Chloro Benzaldehyde (C <sub>7</sub> H <sub>5</sub> ClO)	140.56	1.119	46	214	Alcohol, Ether	-
3285	Dimethylsulfoxide	78.13	1.1	18.55	189		-Rapidly absorb through skin
3792	Ethyl acetate (C <sub>4</sub> H <sub>8</sub> O <sub>2</sub> )	88.10	0.902	-83	77	Miscible with alcohol, acetone, chloroform	Irritating to eyes, nose and throat
4801	Hydrochloric acid (HCl)	36.5	1.05	-	-		-Corrosive burns due to inhalation of acid fumes.
4856	4-Hydroxy benzaldehyde (C <sub>7</sub> H <sub>6</sub> O <sub>2</sub> )	122.12	-	-	-	Alcohol, Ether, acetone	
5984	Methanol (CH <sub>4</sub> O)	32.04	0.79	-97.8	64	Miscible with water, ethanol, ether, benzene	Irritation of eyes, skin and mucous membrane.
6110	Methyl iodide (CH <sub>3</sub> I)	141.94	2.28	-66.5	42.5	Miscible with alcohol, ether	Overexposure cause nausea, vomiting, vertigo, ataxia
6684	<i>p</i> -Nitrobenzaldehyde (C <sub>7</sub> H <sub>5</sub> NO <sub>2</sub> )	151.12	-	106-107	-	Soluble in alcohol, benzene	-
	<i>m</i> -Nitrobenzaldehyde	151.12	-	58	16	Alcohol, Ether,	-

	pyde (C <sub>7</sub> H <sub>5</sub> NO <sub>2</sub> )				4	Chloroform	
<b>8060</b>	Pyridine (C <sub>5</sub> H <sub>5</sub> N)	79.10	0.982	-41.6	115.2	Sol in water, alcohol, ether, petether, oils	Headache, nervousness, dizziness and insomnia
<b>9443</b>	Thiourea (CH <sub>4</sub> N <sub>2</sub> S)	76.12	-	176-178	-	Soluble in water, alcohol and ether	Chronic administration in rats has resulted in hepatic tumors
<b>8613</b>	Sodium ethoxide (C <sub>2</sub> H <sub>5</sub> NaO)	68.05	-	260	91	Soluble in water and alcohol	-



## 09. IDENTIFICATION AND CHARACTERIZATION:

### 09. Identification and Characterization<sup>1-4</sup>

The Identification and Characterization of the compounds was carried out by the following methods.

- 1) Melting point
- 2) Thin layer chromatography
- 3) Infrared spectroscopy
- 4) Nuclear magnetic resonance spectroscopy
- 5) Mass spectroscopy

#### 09.1 Melting Point<sup>1</sup>

The melting points of the organic compounds were determined by open capillary tube method. Melting point is the valuable criteria for an organic compound as pure crystal is having definite and sharp melting point. Determining the melting point is a simple and fast method used in many diverse areas of chemistry to obtain a first impression of the purity of a substance. This is because even small quantities of impurities change the melting point, or at least clearly enlarge its melting range. Melting points were determined by using Veego electronic (VMP-D) apparatus.

#### 09.2 Thin Layer Chromatography<sup>2</sup>

Chromatography is an important technique to identify the formation of the new compounds and also to determine the purity of the compounds. The R<sub>f</sub> value is the characteristics for each compounds. Thin layer chromatography is performed to monitor the completion of reaction. TLC is a method in which a thin layer of some inert material is used as a substrate. A layer of oxides is made from slurry of powder in a suitable inert solvent. In the present work, silica gel G (Loba) is used as coating material. The solvent used for development of TLC was benzene: ethyl acetate in various concentrations.

#### 09.3 Infrared Spectroscopy<sup>3,4</sup>

The infrared radiation spectroscopy is concerned about the absorption of infrared radiation by a molecule and exhibits characteristics absorption spectra. The most useful

range for analytical purpose lies between 4000 and 400  $\text{cm}^{-1}$ . After absorption of IR radiations by a chemical substance, molecules of substance vibrate at many rates of vibrations giving rise to closely packed absorption bands, commonly known as IR absorption spectrum. Although the IR spectrum is characteristic of the entire molecule, it is true that certain groups of atoms give rise to bands at or near the same frequency regardless of structure of the rest of the molecule. It is the persistence of these characteristic bands that permits the chemist to obtain useful structural information by simple inspection and reference to generalized charts of characteristic group frequencies. Different bonds (C-C, C-O, C=O, O-H, N-H etc) have different vibrational frequencies, and we can detect the presence of these bands in an organic molecule by identifying this characteristic frequency as an absorption band in the infrared spectrum.

The IR spectra of compounds were recorded on a JASCO FT-IR 4100 spectrometer.

#### 09.4 Nuclear magnetic Resonance Spectroscopy<sup>3,4</sup>

Nuclear magnetic resonance (NMR) is a form of absorption spectroscopy. It is concerned with the absorption of certain energy by spinning nuclei in a magnetic field when irradiated by certain energy radiation perpendicular to it. NMR is concerned with the magnetic properties of certain atomic nuclei, notably the nucleus of hydrogen atom the proton. Studying a molecule by NMR spectroscopy enables us to record differences in the magnetic properties of the various magnetic nuclei present, and to deduce in large measure what the positions of these nuclei are within the molecule.

The  $^1\text{H}$  NMR spectra were obtained on a BRUKER AVANCEV II 400 NMR spectrometer in DMSO as solvent and TMS as internal standard, chemical shifts are given in ppm.

#### 09.5 Mass spectroscopy<sup>3,4</sup>

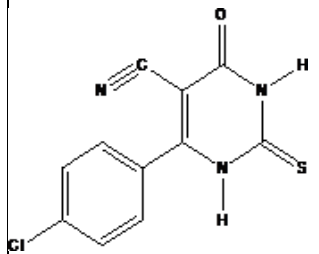
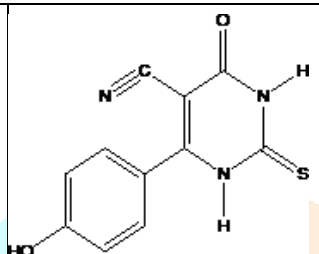
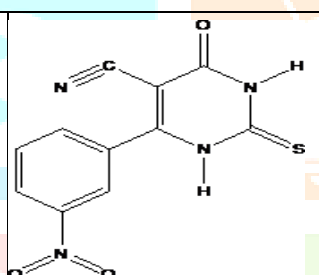
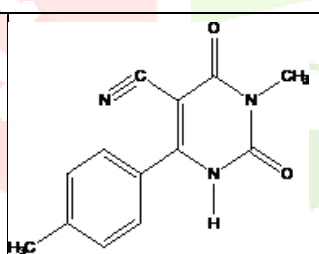
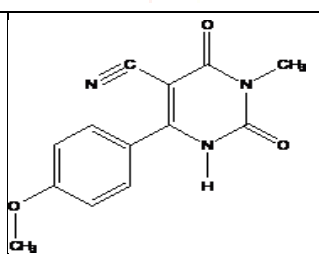
Mass spectrometry is an analytical tool used for measuring the molecular mass of a sample. Mass spectra are obtained after ionisation of samples by different ionization techniques. Molecular mass of the compound is determined by mass to charge ( $m/z$ ) ratio.

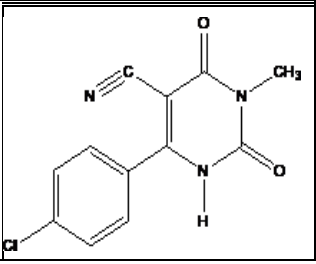
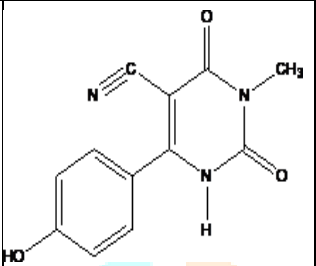
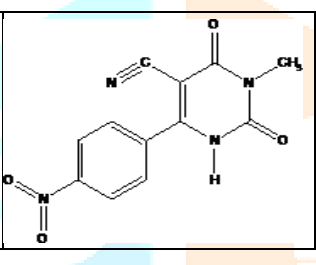
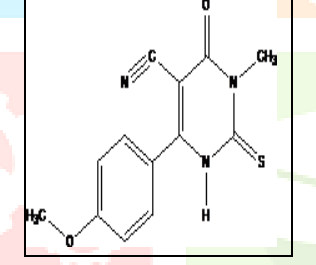
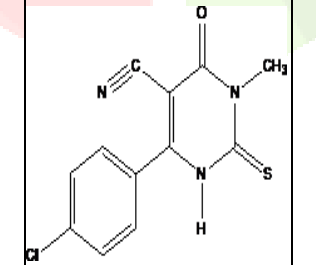
The mass spectra were obtained on WATERS Q-TOF MICROMASS (LC-MS).

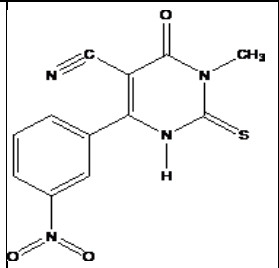
## 09.6 Physicochemical Characteristic of Compounds

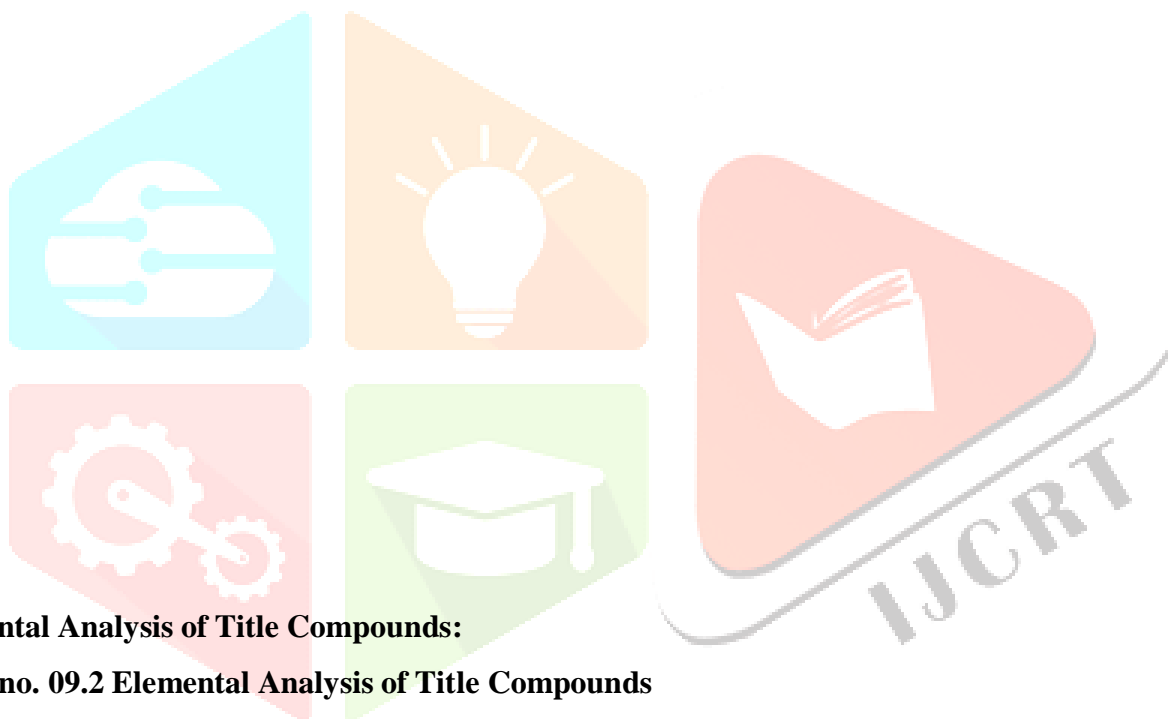
Table no. 09.1 Physicochemical Characteristic of Compounds

Molecular Code	Molecular Structure	Molecular Formula	Mol. Weight	Melting Point (°C)	Yield(%)	R <sub>f</sub> Value
HDB-1		C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	244	175	84.85	0.377
HDB-2		C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> OS	260	187	82.87	0.2
HDB-3		C <sub>13</sub> H <sub>13</sub> N <sub>2</sub> OSCl	280	149	83.77	0.370
HDB-4		C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S	291	131	69.67	0.3
HDB-5		C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> OS	262	109	77.33	0.339
HDB-6		C <sub>15</sub> H <sub>20</sub> N <sub>2</sub> OS	276	202	85.23	0.392
HDB-7		C <sub>14</sub> H <sub>17</sub> N <sub>3</sub> OS	294	161	73.20	0.26
RLS-1		C <sub>12</sub> H <sub>9</sub> N <sub>3</sub> OS	243	257	48.67	0.65
RLS-2						

		$C_{12}H_9N_3O_2S$	259	275	57.45	0.45
RLS-3		$C_{11}H_6N_3OSCl$	264	237	45.35	0.46
RLS-4		$C_{11}H_7N_3O_2S$	245	249	57.35	0.28
RLS-5		$C_{11}H_6N_4O_3S$	274	217	35.97	0.32
RLS-6		$C_{13}H_{11}N_3O_3$	241	162	75.59*	0.24
RLS-7		$C_{13}H_{11}N_3O_2$	257	215	69.45	0.78

RLS-8		$C_{12}H_8N_3O_2Cl$	261	169	69.45	0.1
RLS-9		$C_{12}H_9N_3O_3$	243	141-142	53.47	0.28
RLS-10		$C_{12}H_8N_4O_4$	272	256	45.45	0.40
RLS-11		$C_{13}H_{11}N_3O_2S$	273	245	58.45	0.36
RLS-12		$C_{12}H_8N_3OCl$	277	249	64.39	0.23

RLS-13		$C_{12}H_8N_4O_3S$	288	223	34.56	0.39
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### 09.7 Elemental Analysis of Title Compounds:

Table no. 09.2 Elemental Analysis of Title Compounds

Compound	Elements					
	C	H	O	N	S	Cl
HDB-1	69.83	6.60	13.10	11.47	---	---
HDB-2	64.58	6.19	6.17	10.86	12.32	---
HDB-3	55.61	4.67	5.70	9.98	11.42	12.63

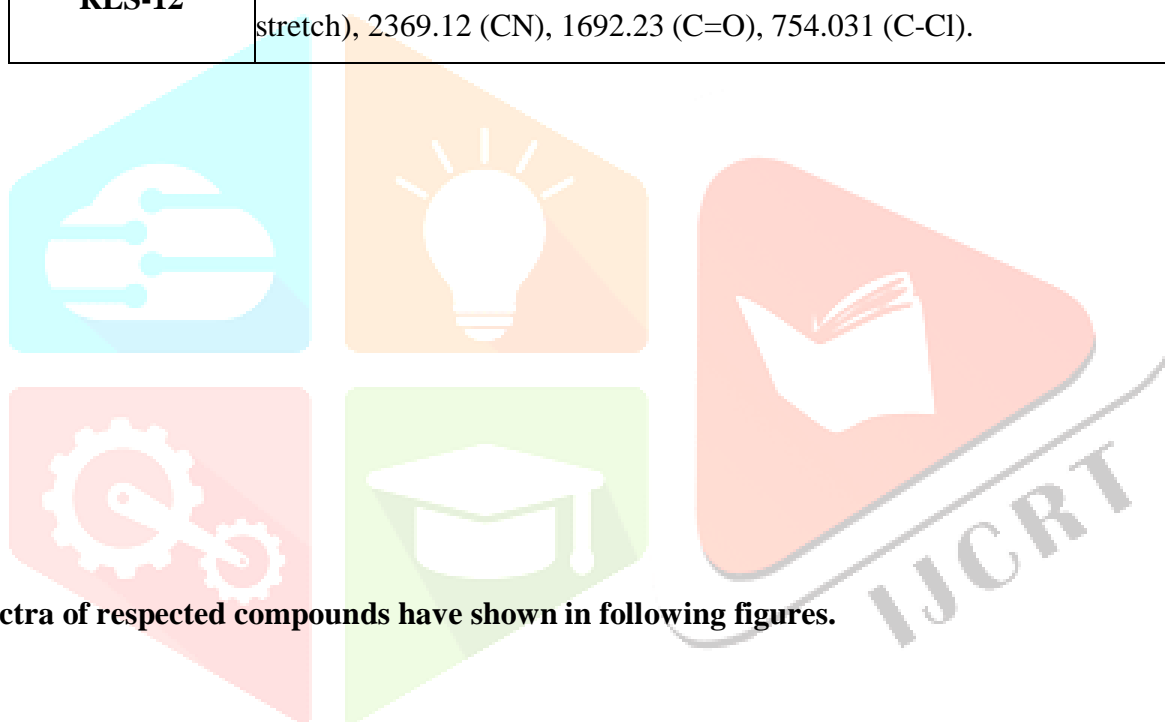
<b>HDB-4</b>	53.60	4.50	16.48	14.42	11.01	---
<b>HDB-5</b>	64.09	6.91	6.10	10.68	12.22	---
<b>HDB-6</b>	65.18	7.29	5.79	10.14	11.60	---
<b>HDB-7</b>	56.65	5.77	5.39	9.44	10.80	11.94
<b>RLS-1</b>	59.24	3.73	6.58	17.27	13.18	---
<b>RLS-2</b>	55.59	3.50	12.34	16.21	12.37	---
<b>RLS-3</b>	50.10	2.29	6.07	15.93	12.16	13.44
<b>RLS-4</b>	53.83	2.88	13.05	17.13	13.07	---
<b>RLS-5</b>	48.17	2.21	17.50	20.43	11.69	---
<b>RLS-6</b>	64.72	4.60	13.26	17.42	---	---
<b>RLS-7</b>	60.70	4.31	18.66	16.33	---	---
<b>RLS-8</b>	55.08	3.08	12.23	16.06	---	13.55
<b>RLS-9</b>	59.29	3.73	19.73	17.28	---	---
<b>RLS-10</b>	52.95	2.96	23.51	20.58	---	---
<b>RLS-11</b>	57.13	4.06	11.71	15.37	11.73	---
<b>RLS-12</b>	51.90	2.90	5.76	15.13	11.55	12.77
<b>RLS-13</b>	50	2.80	16.65	19.43	11.12	---

### 09.8 IR data of title compounds:

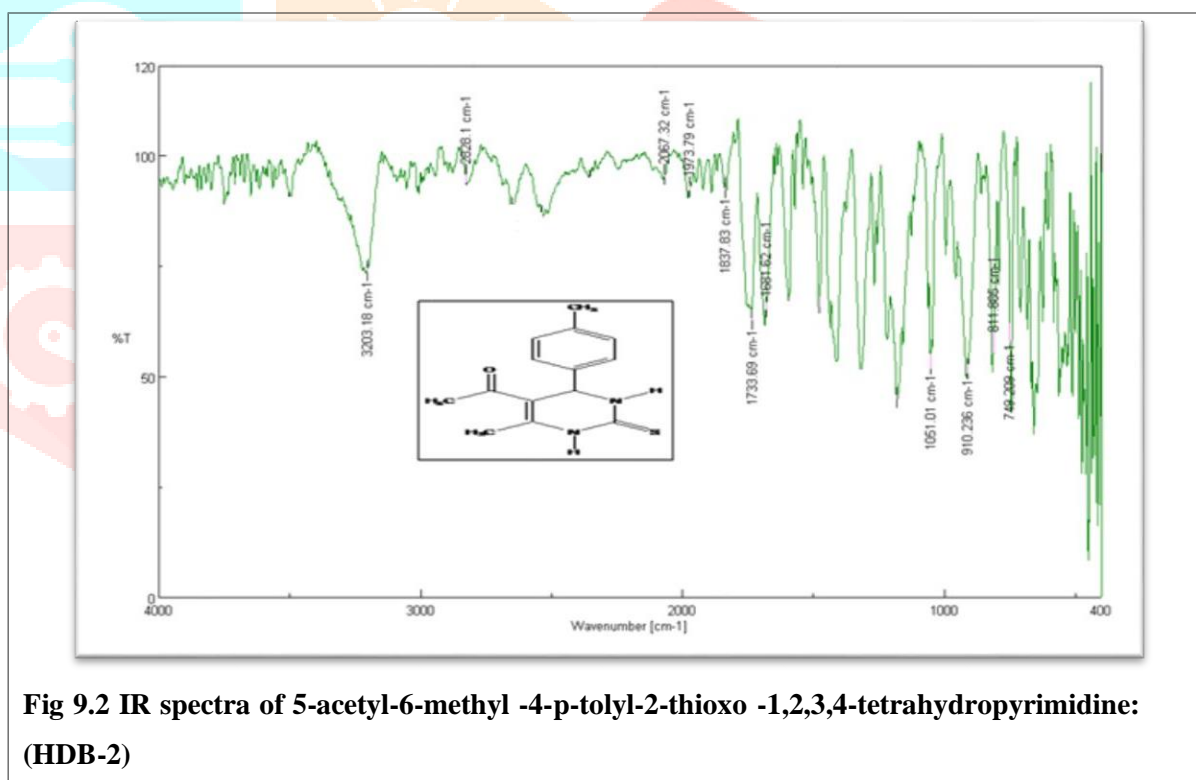
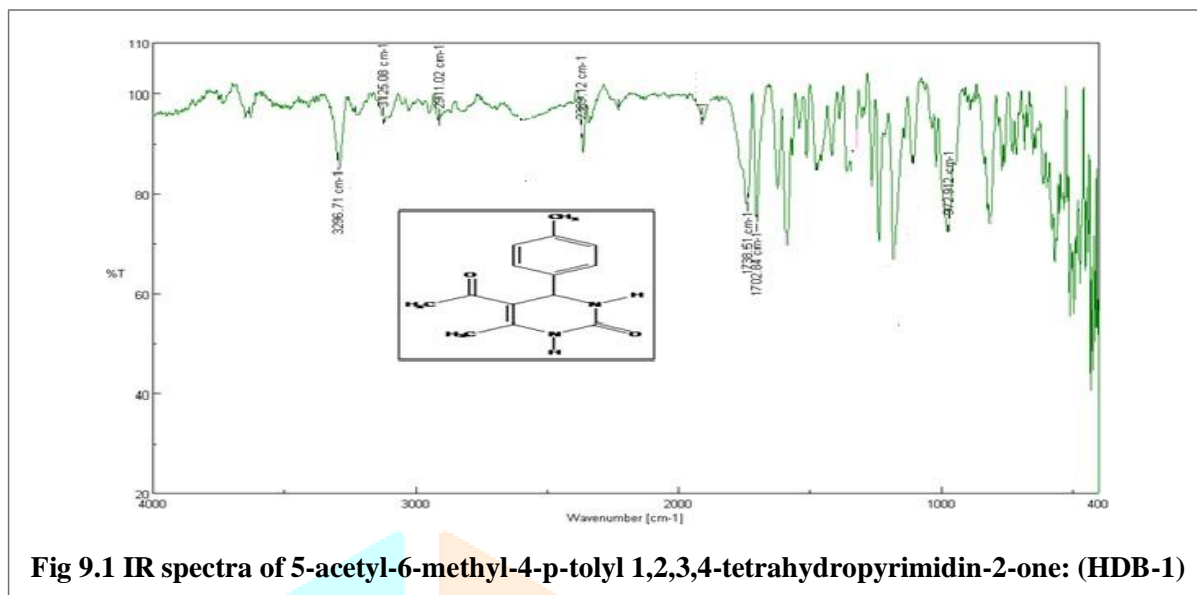
Table no. 09.3 IR data of title compounds:

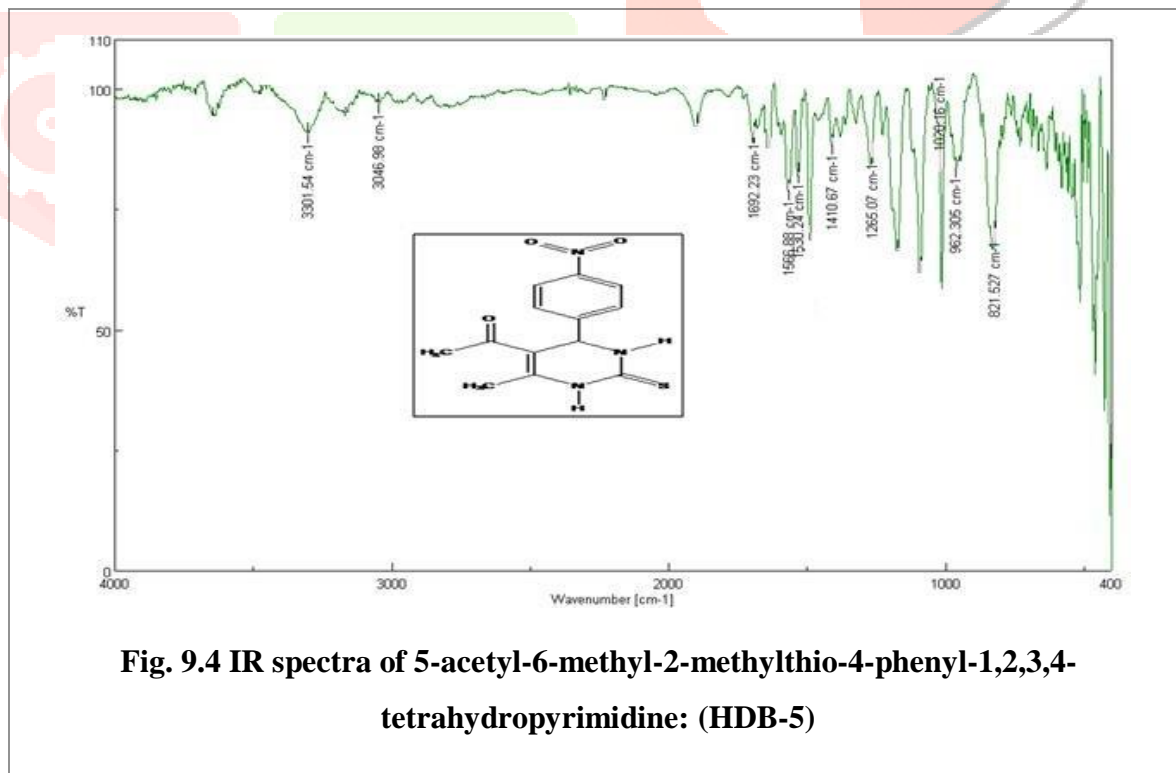
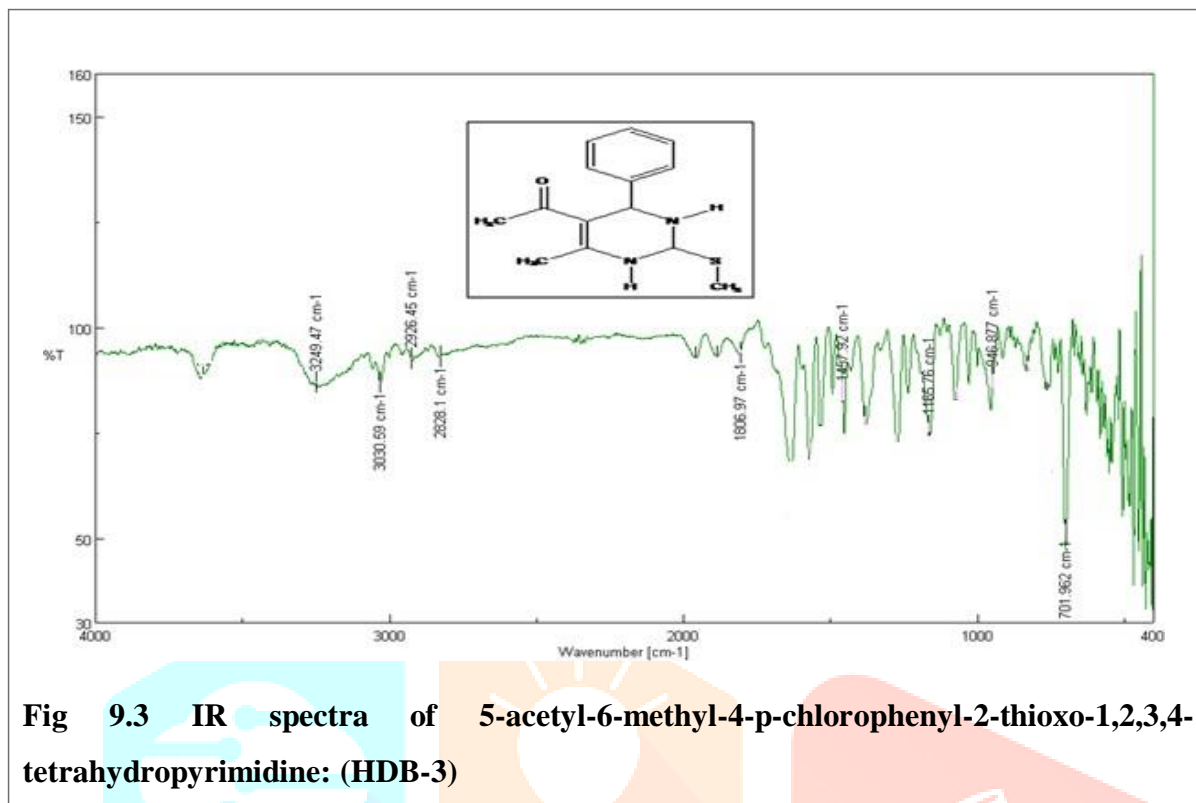
Compound	IR value (cm <sup>-1</sup> )
<b>HDB-1</b>	3296.71 (NH stretch), 2911.02 (CH stretch), 1738.51 (C=O), 1702.84 (C=O Amide), 912 (C-N).
<b>HDB-2</b>	3203.18 (NH stretch), 2828.1 (CH stretch), 1733.69 (C=O), 910 (C-N)
<b>HDB-3</b>	3301.54 (NH stretch), 3046.98 (CH stretch), 1692.23 (C=O), 821.527 (C-Cl), 916 (C-N).
<b>HDB-5</b>	3249.47 (NH stretch), 2926.45 (CH stretch), 3030.59 (CH stretch SP <sup>2</sup> ), 1806.51 (C=O), 962 (C-N).
<b>HDB-6</b>	3291.89 (NH stretch), 3020.94 (CH stretch SP <sup>2</sup> ), 2921.63 (CH stretch), 1697.05 (C=O), 957 (C-N).
<b>HDB-7</b>	3650 (NH stretch), 2832 (CH stretch), 2921.63 (CH stretch), 1697.05 (C=O), 957 (C-N).
<b>RLS-1</b>	3187.76 (NH stretch), 3099.05 (CH stretch SP <sup>2</sup> ), 1692 (C=O), 2228.13 (CN), 611 (C-O).
<b>RLS-2</b>	3187.76 (NH stretch), 3067.23 (CH stretch SP <sup>2</sup> ) 2942.84 (CH stretch), 1707.66 (C=O), 2234.13 (CN).
<b>RLS-3</b>	3208 (NH stretch), 3093.26 (CH stretch SP <sup>2</sup> ), 754.031 (C-Cl), 1707.66 (C=O), 2338.27 (CN), 903 (C-N).
<b>RLS-4</b>	3614.91 (OH stretch), 3307.32 (NH stretch), 2958.27 (CH stretch), 1785.76 (C=O), 2228.34 (CN), 837 (C-O).

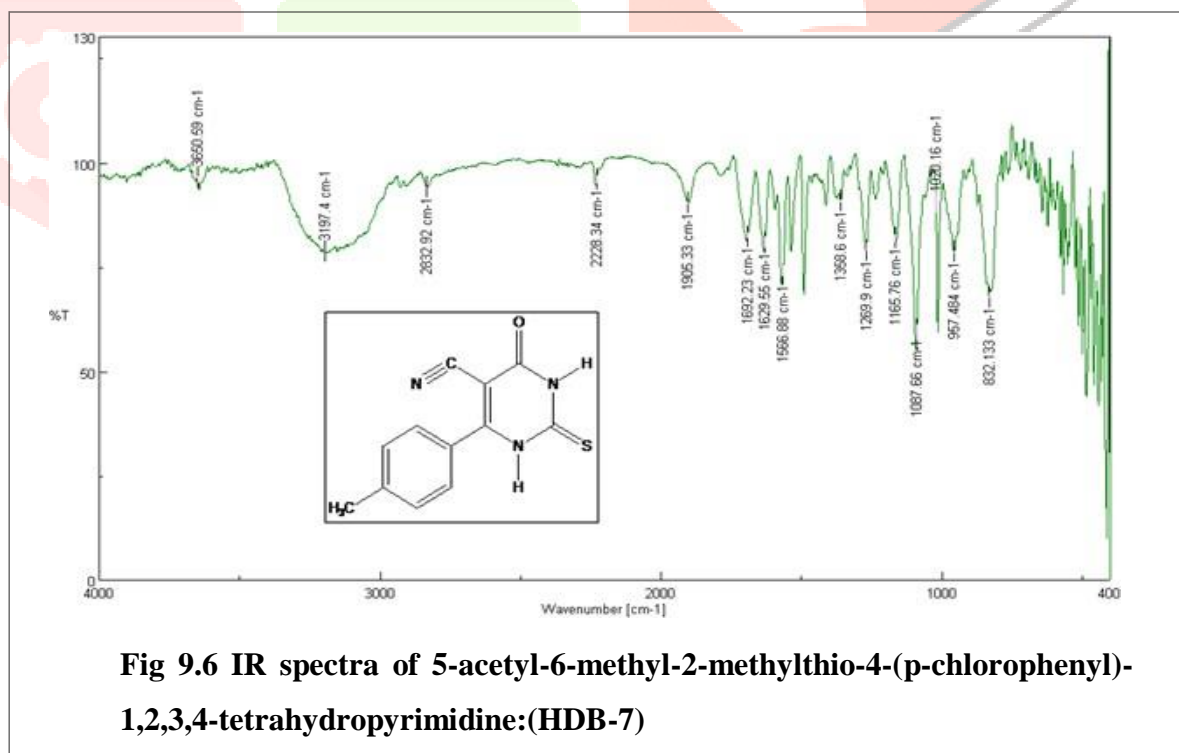
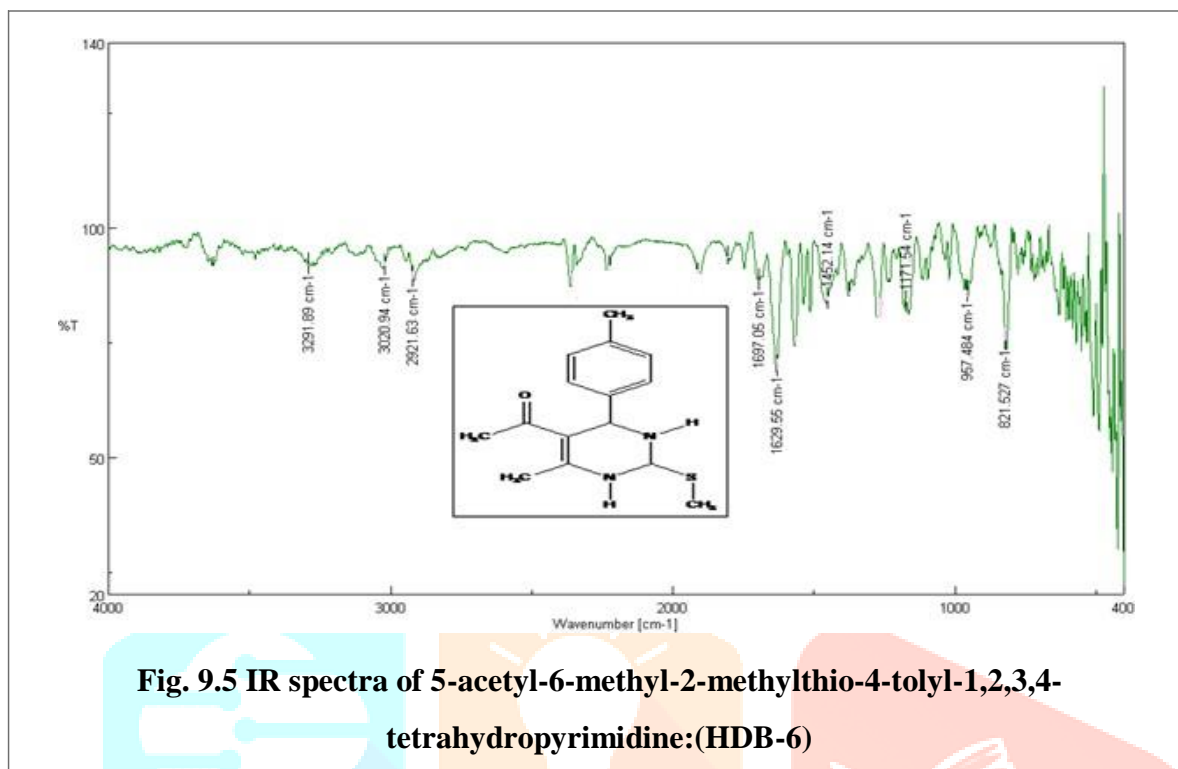
<b>RLS-7</b>	3197.4 (NH stretch), 2978.52 CH stretch), 1697.05(C=O), 2228.349 (CN), 931 (C-N),
<b>RLS-9</b>	3609.13 (OH stretch), 3312.14 (NH stretch), 2958.27 (CH stretch), 2228.34 (CN), 1785.76 (C=O).
<b>RLS-10</b>	3359.39 (NH stretch), 3088.44 (CH stretch SP2), 2989.12 (CH stretch), 2228.34 (CN), 1728.87 (C=O).
<b>RLS-12</b>	3181.97 (NH stretch), 3030.59 (CH stretch SP2), 2900.41 (CH stretch), 2369.12 (CN), 1692.23 (C=O), 754.031 (C-Cl).



IR spectra of respected compounds have shown in following figures.







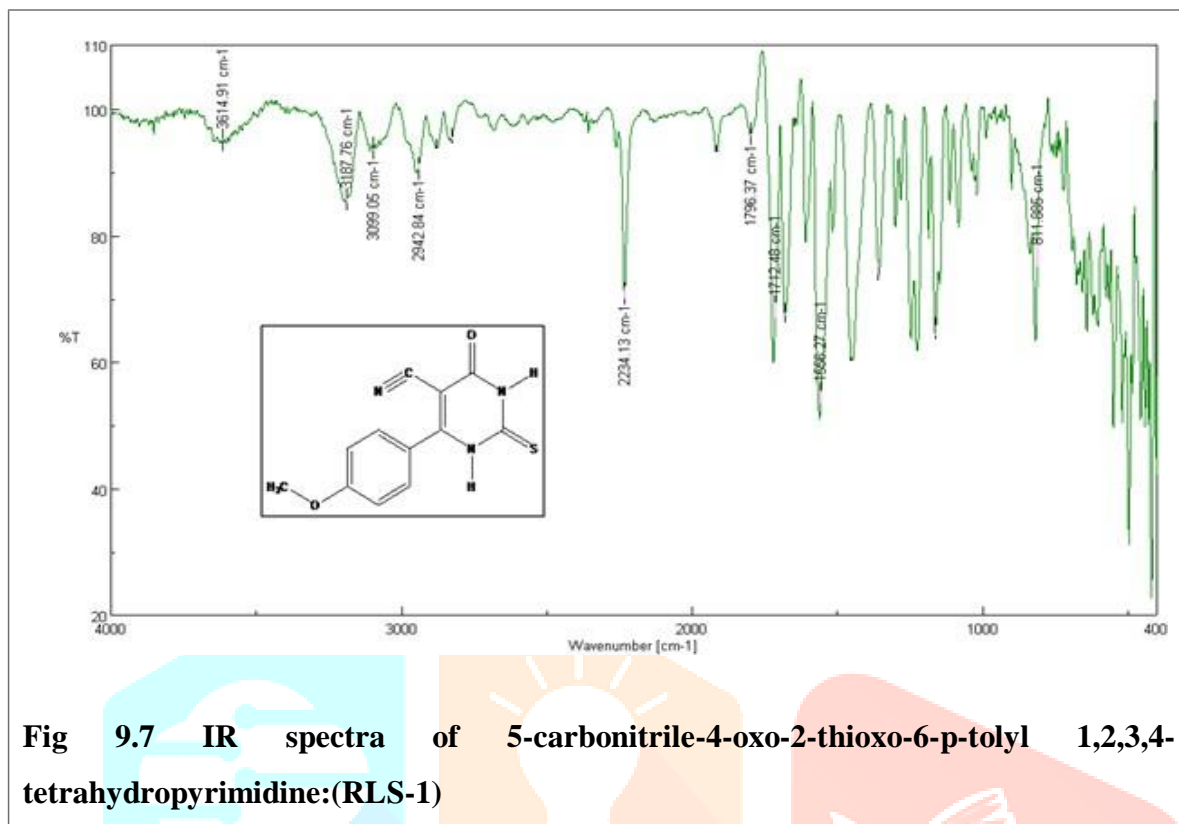


Fig 9.7 IR spectra of 5-carbonitrile-4-oxo-2-thioxo-6-p-tolyl 1,2,3,4-tetrahydropyrimidine:(RLS-1)

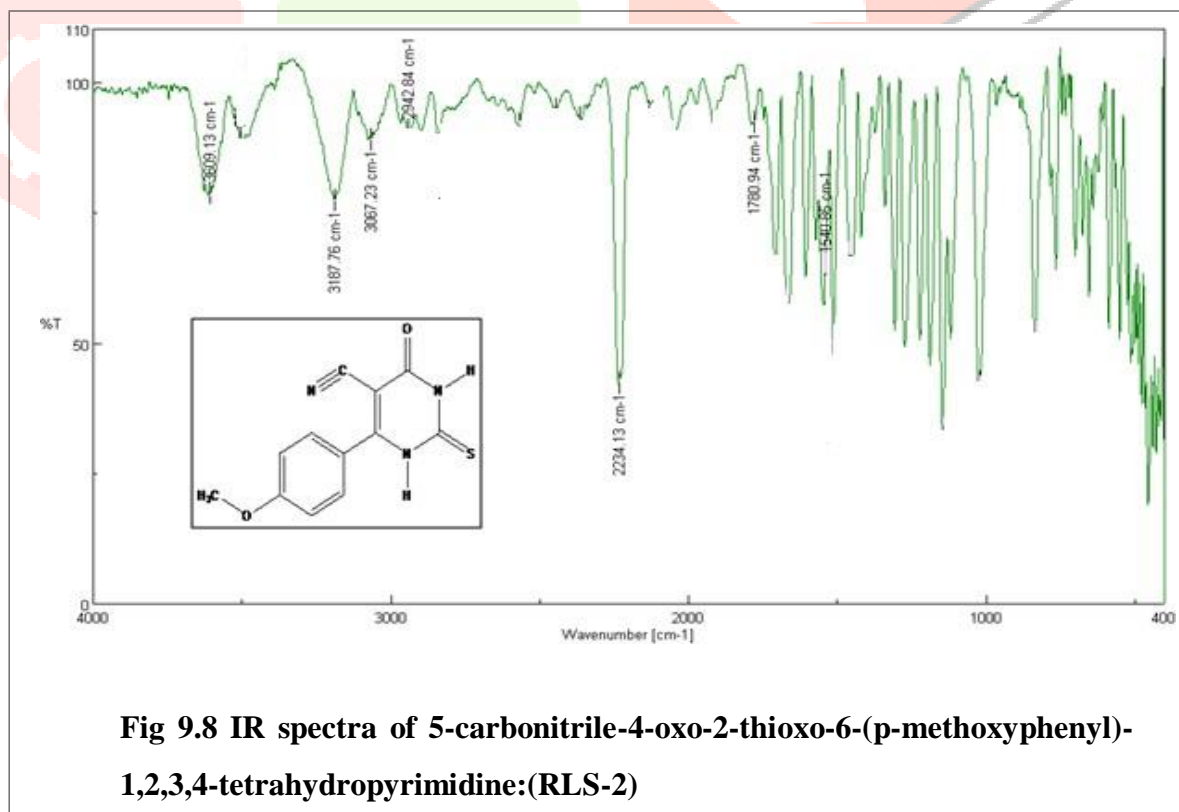
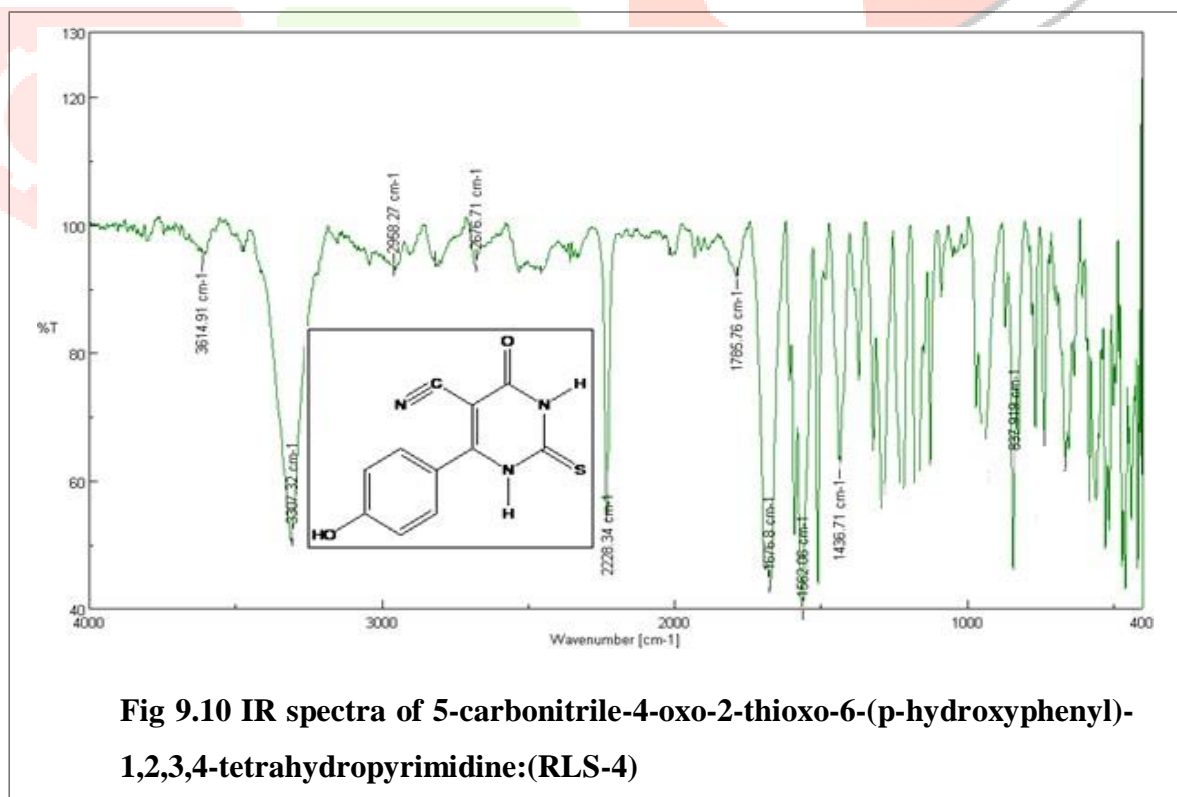
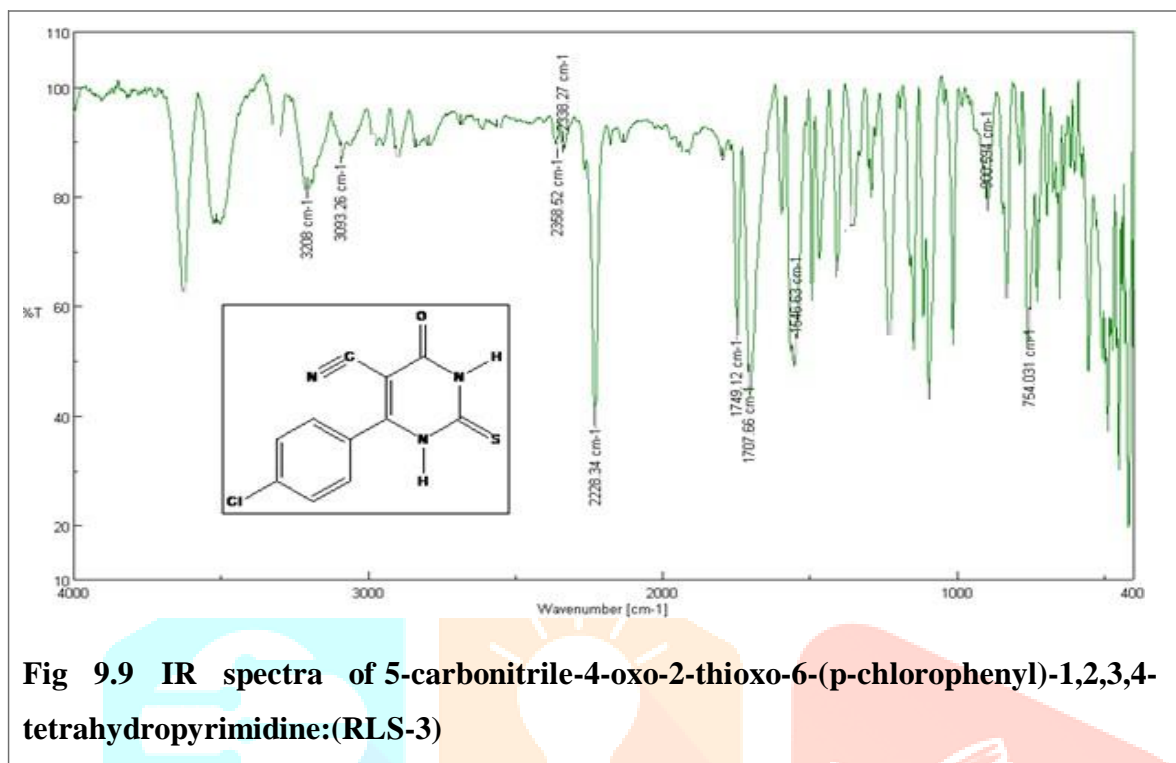
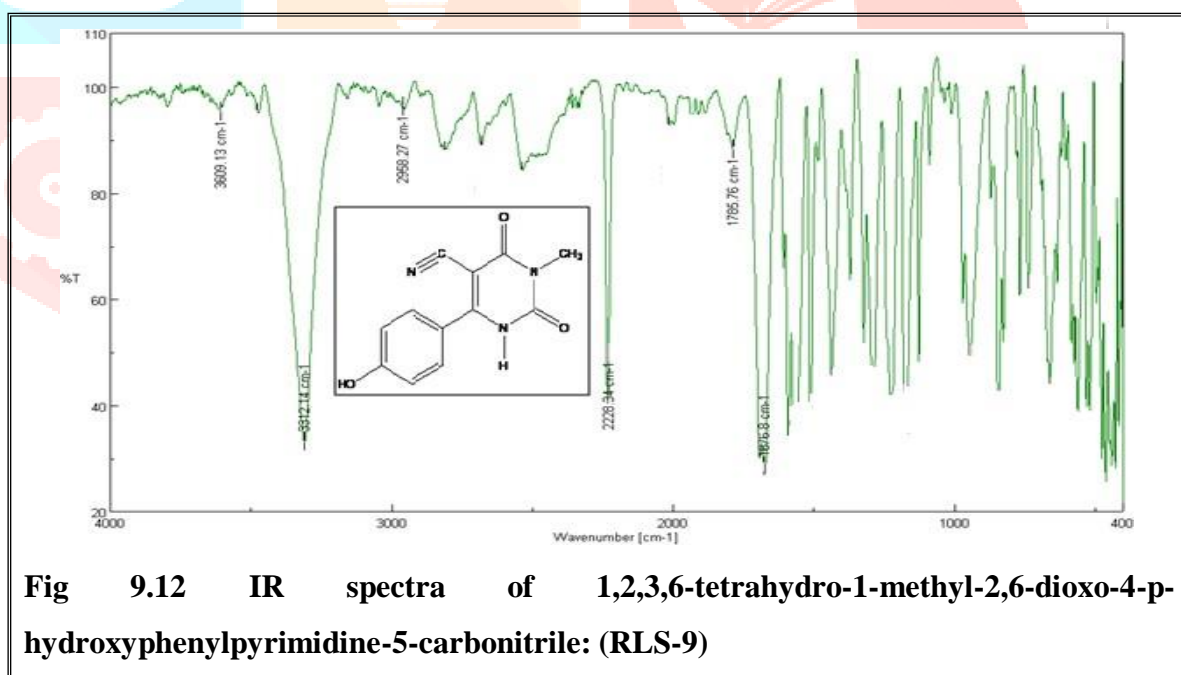
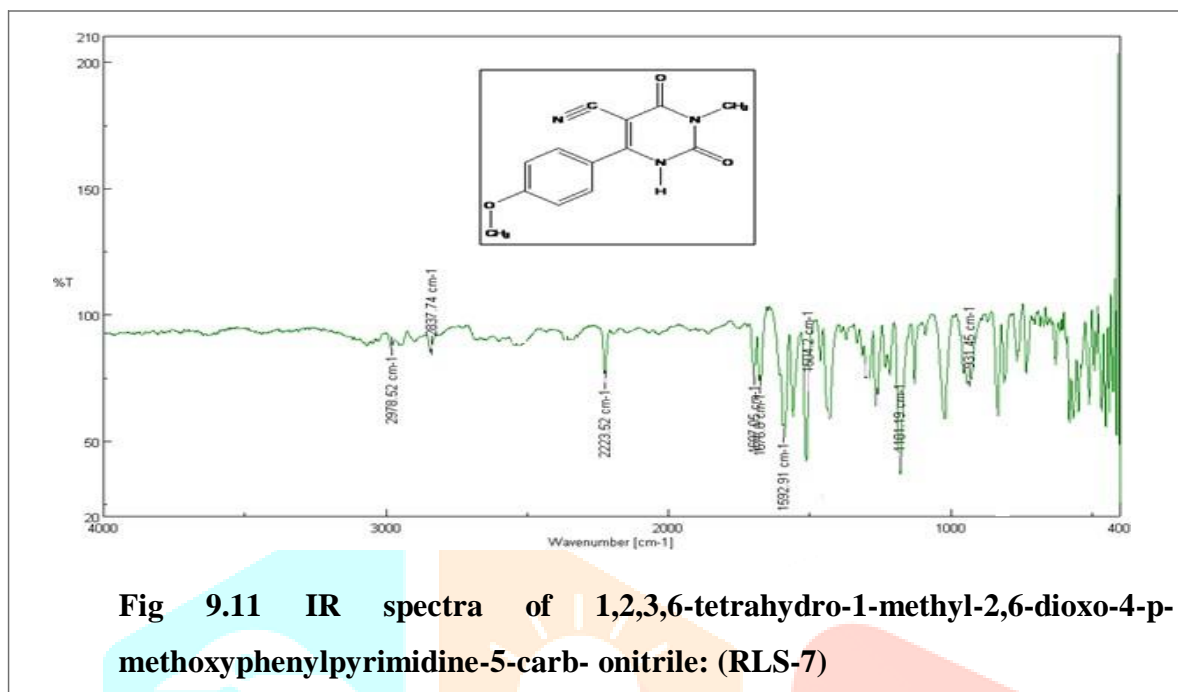
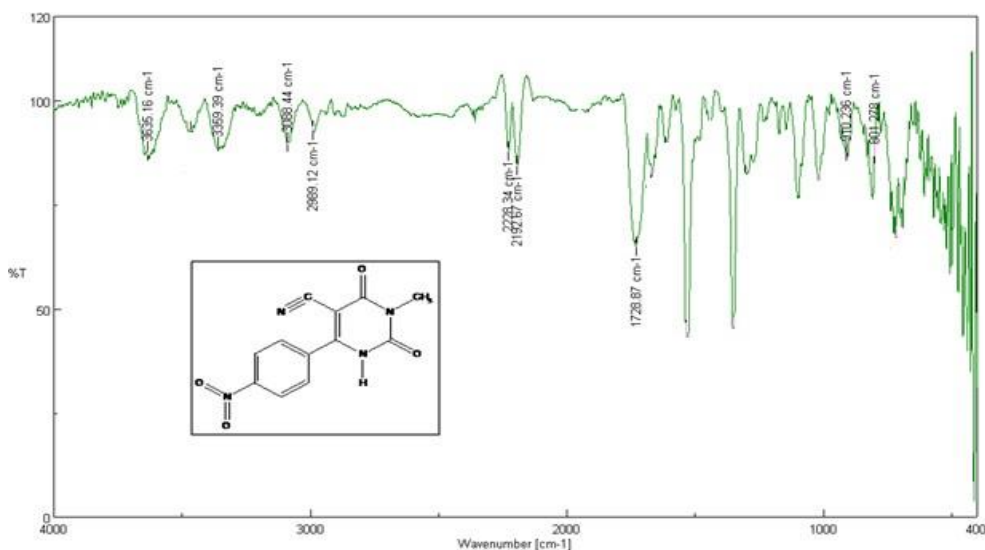


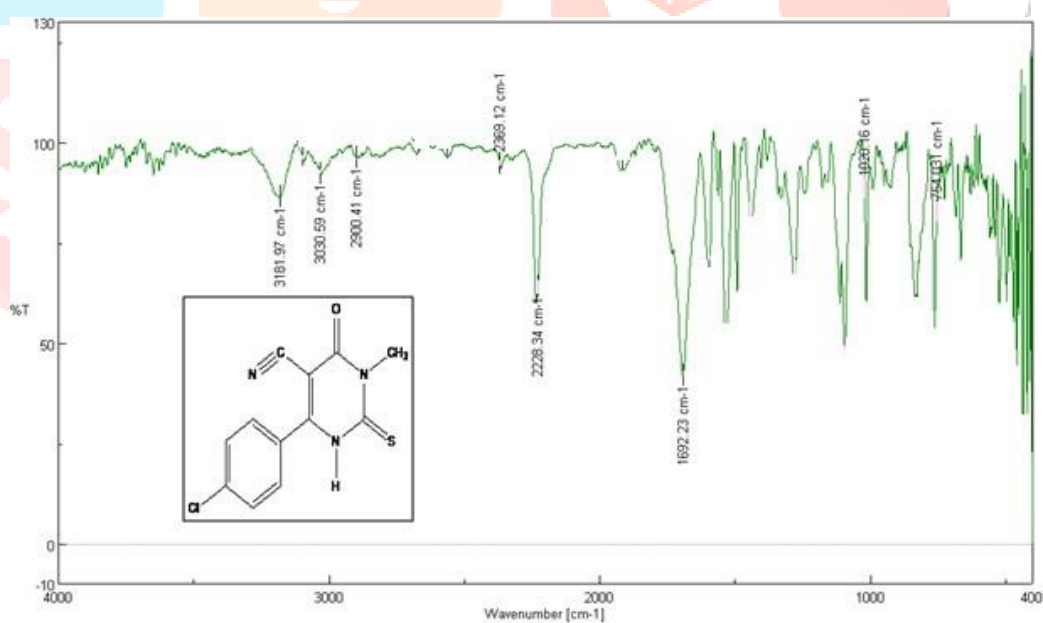
Fig 9.8 IR spectra of 5-carbonitrile-4-oxo-2-thioxo-6-(p-methoxyphenyl)-1,2,3,4-tetrahydropyrimidine:(RLS-2)







**Fig 9.13 IR spectra of 1,2,3,6-tetrahydro-1-methyl-2,6-dioxo-4-p-nitrophenylpyrimidine-5-carbonitrile: (RLS-10)**

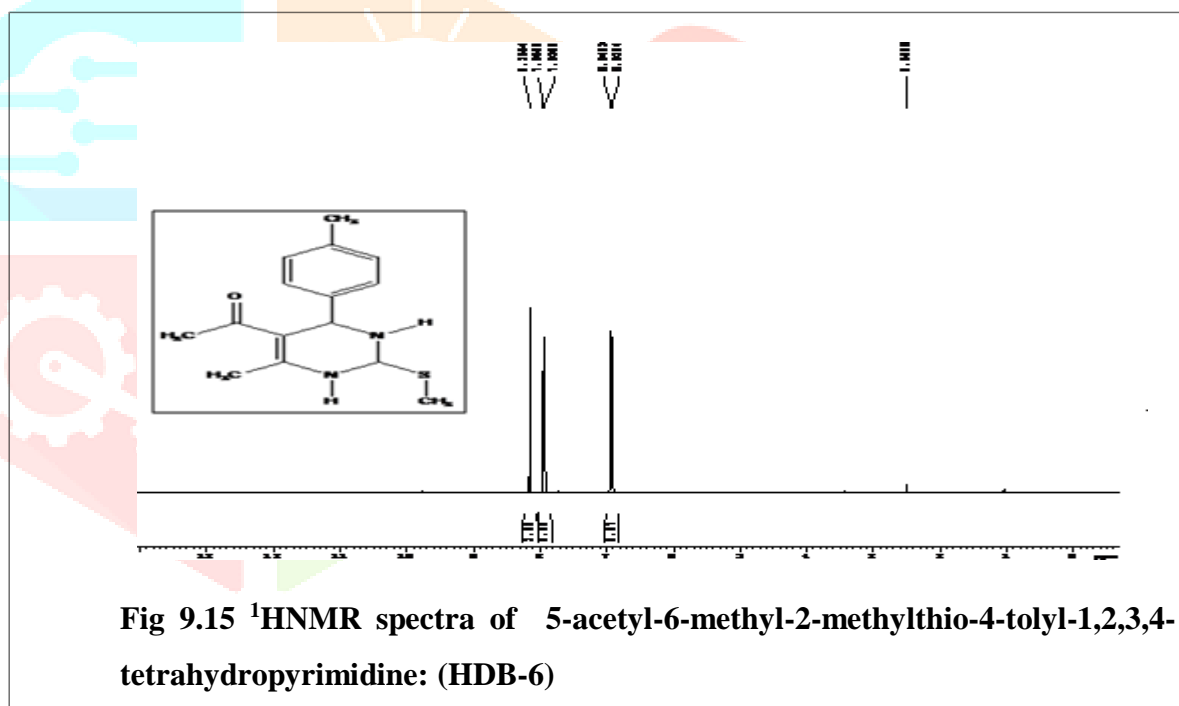


**Fig 9.14 IR spectra of 1,2,3,6-tetrahydro-4-(4-chlorophenyl)-1-methyl-6-oxo-2-dioxypyrimidine-5-carbonitrile: (RLS-12)**

09.9  $^1\text{H}$  NMR data of respected compounds: Table no. 09.4  $^1\text{H}$  NMR data of title compounds:

Compound	$^1\text{H}$ NMR
HDB-6	7.8-(d, 4H, CH benzene), 6.7-(s, 2H, methine), 1.1-(s, 6H, methyl), 2.5-(s, 2H, N-H), 1.2(s, 3H, methyl).
RLS-7	8.25-(d, 4H, benzene), 7.22-(s, 1H, NH), 3.86-(s, 3H, methyl), 2.50(s, 3H, methyl).
RLS-12	7.8-(d, 4H, Benzene), 3.36-(s, 3H, methyl), 3.19-(s, 1H, N-H).

$^1\text{H}$ NMR spectra of respected compounds are shown in following figure.



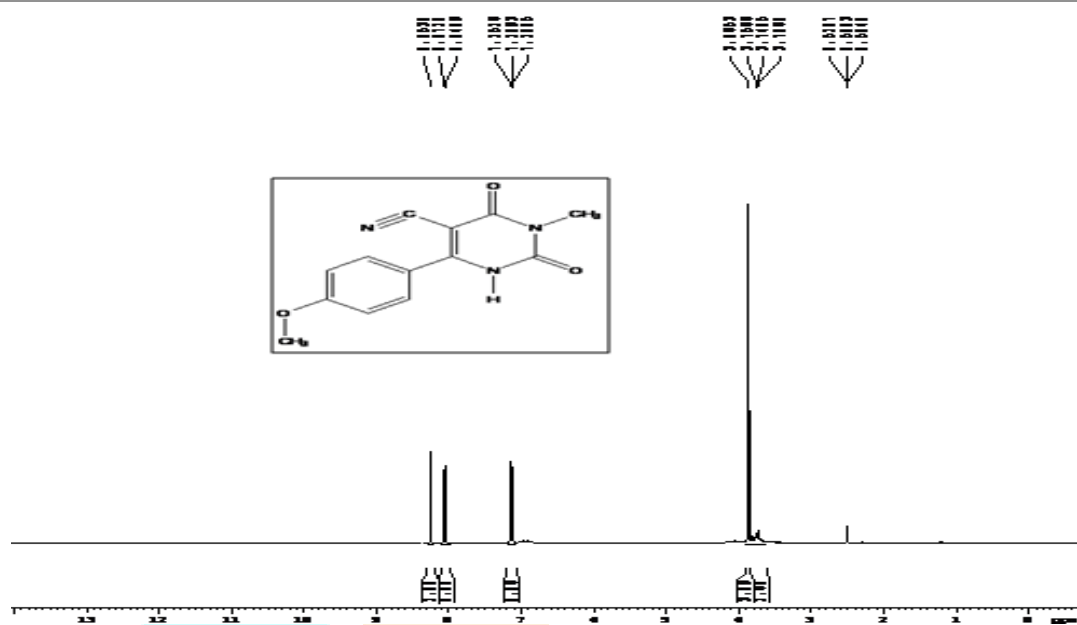


Fig 9.16 <sup>1</sup>H NMR spectra of 1,2,3,6-tetrahydro-1-methyl-2,6-dioxo-4-p-methoxyphenylpyrimidine-5-carbonitrile: (RLS-7)



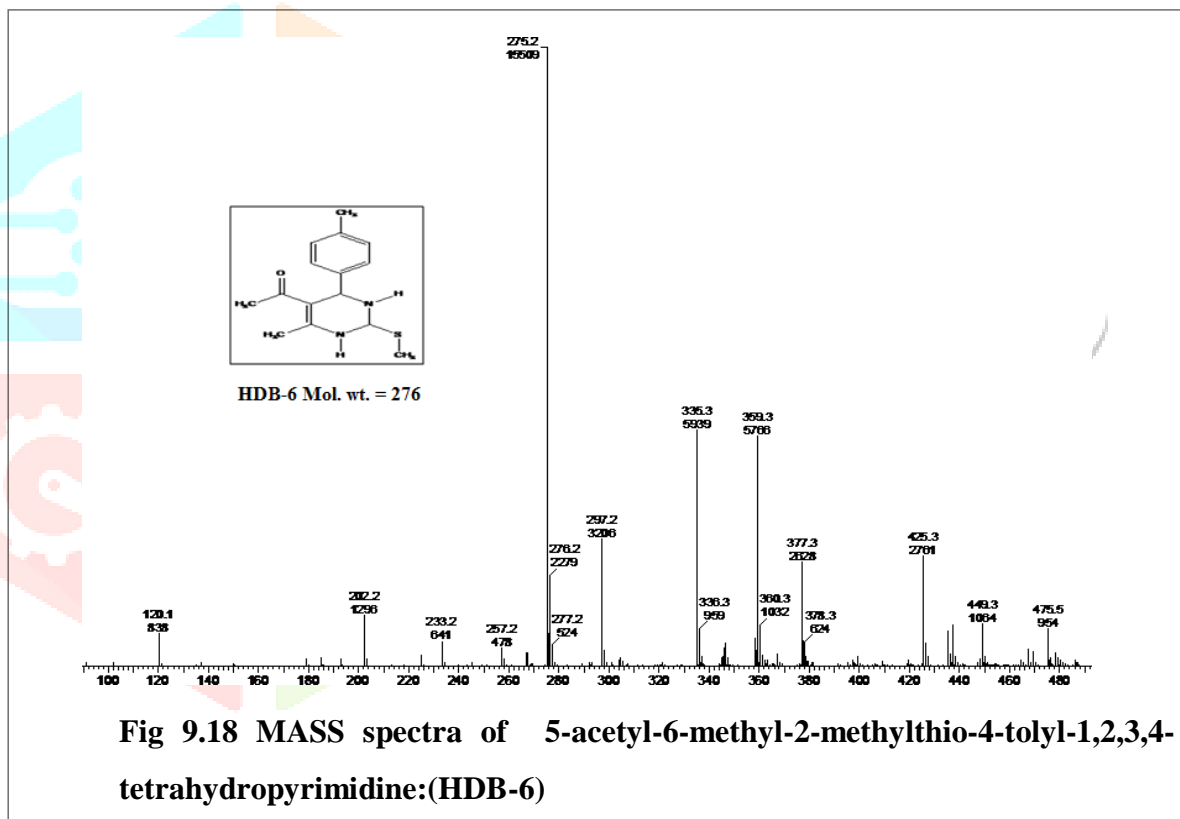
Fig 9.17 <sup>1</sup>H NMR spectra of 1,2,3,6-tetrahydro-4-(4-chlorophenyl)-1-methyl-6-oxo-2-dioxypyrimidine-5-carbonitrile: (RLS-12)

### 09.10 Mass Spectroscopy data of tital compounds:

Table no. 09.5 Mass Spectroscopy data of tital compounds:

Compound	m/z ratio value
HDB-6	276.2 (100%) , 277.2(18.0%) (M+1 peak)

Mass spectra of tital compound have shown in following figure.





## 10. BIOLOGICAL SCREENING

### 10.2 Anti-microbial Activity: <sup>5-11</sup>

An anti-microbial is substance that kills or inhibits their growth. Biological evaluation involves testing of the microbial susceptibility to chemotherapeutic agents. Determination of antimicrobial effectiveness against pathogens is essential for proper therapy. Antimicrobial susceptibility may be reported qualitatively, as sensitive, intermediate or resistant or quantitatively in terms of the concentration of agent which inhibits the growth of the organism. The organism being 'sensitive' means that the organism is inhibited by the anti-microbial agent at clinically attainable concentration; 'resistant' means that the growth of the organism is not inhibited. The basic principle of microbial assay lies in the comparison of the inhibition of the growth of bacteria by measured concentration of antibiotic to be investigated with that produced by known concentration of the standard preparation of the antibiotic having a known activity. The idea of the effectiveness of a chemotherapeutic on of agent against pathogen can obtained from the Minimum Inhibitory Concentration (MIC). Minimum inhibitory concentration is defined as the lowest concentration of antimicrobial agent that can inhibit the visible growth of microorganism after overnight incubation. MIC is important in diagnostic laboratories to confirm resistance of the microorganism to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.

#### 10.2.1 Methods:

The following two are the methods:

##### 10.2.1. 1. Diffusion method:

A Petri plate containing an agar medium is inoculated uniformly over its entire surface with standardized amount of the test organism. Filter paper disks impregnated with known concentration of chemotherapeutic agents are placed on the solidified agar

surface. During incubation, chemotherapeutic agents diffuse from the disks into the agar. The agent diffuses from the disk, lower its concentration. If the chemotherapeutic agent is effective, a zone of inhibition forms around the disk after a standardized incubation. A more advanced diffusion method, the E test, enables a lab technician to estimate the minimum inhibitory concentration. A plastic coated strips gradient of antibiotic concentration and MIC can be read from a scale printed on the strip.

### **10.2.1. 2. Broth Dilution method:**

Broth dilution is useful in determination of MIC and minimum bactericidal concentration (MBC) of an antimicrobial activity. MBC is lowest concentration of the antimicrobial agents that kills the test organism. The MIC is determined by making a sequence of decreasing concentration of the drug in a broth, which is then inoculated with the test bacteria. After incubation the turbidity may be read visually or by measuring the percentage transmittance or absorbance at 600 nm against a blank.

The tube dilution test is the standard method for determining levels of microbial resistance to an antimicrobial agent. Serial dilutions of the test agent are made in a liquid microbial growth medium which is inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration (highest dilution) of test agent preventing appearance of turbidity (growth) is considered to be the minimum inhibitory concentration.

## **10.2.2 Material and Methods:**

### **10.2.2.1 Requirements:**

#### **Apparatus**

Test tubes, Test tube stand, Bunsen burner, Culture media, Measuring cylinder, Inoculating loop, 1 mL and 5 mL Glass syringe.

#### **Equipments**

UV-Vis Spectrophotometer (Jasco)

Laminar airflow (The Popular Science Apparatus, India) Autoclave (Dolphin)

BOD Incubator ( Labline)

### Chemicals

Beef extract (Loba Chemie Pvt.Ltd.), Peptone (Loba Chemie Pvt.Ltd.), Sodium chloride(Qualigens), Distilled water, Dettol and Dimethylformamide ((Merck).

### Nutrient Broth

Nutrient broth is used to find growth of organism in a test tube and screw-caped culture tube. Test tube broth culture is advantageous in the sense that it can be easily handled. Microbes exist in the fluid independently or in a cluster form with minimum contamination.

Sr.No.	Ingredients	Quantity
1.	Beef extract	10.0 gm
2.	Peptone	10.0 gm
3.	Sodium chloride	5.0 gm
4.	Distilled water	q.s. up to 1000 mL

**Table no.10.4 Composition of nutrient broth**

#### 10.2.2.2 Preparation of Nutrient Broth:

Put the weighted amount of beef extract, sodium chloride and peptone in 500 mL of distilled water. Heat the mixture and agitate with glass rod to dissolve the ingredients. Add distilled water to make the final volume. Adjust the *pH* of the medium to 7.2-7.4 by adding acid or alkali.

#### 10.2.2.3 Sterilization of Equipments:

The operating procedures of an autoclave are as follows:

- 1) Autoclave was filled and heated till steam started building up.
- 2) Items to be sterilized were placed in the chamber.

- 3) The autoclave door was then closed and locked.
- 4) When temperature reached 100<sup>0</sup>C, operating valve was closed.
- 5) The jacket pressure was then checked.
  - 6) The sterilization time was measured from the moment temperature reached 121<sup>0</sup>C rather than 15 lb pressure, sterilized it for 15 min.
  - 7) After 15-20 min, the steam supply valve was closed and waits till the pressure falls to zero.
- 8) The autoclave door was unlocked and opened.
- 9) Autoclaved items were removed from the chamber.

#### 10.2.2.4 Preparation of Solutions:

##### Stock Solution of Test Compounds

The test compounds (5 mg) were dissolved in 1 mL of DMF and volume was adjusted upto 2 mL with DMF to produce a concentration of 2500 µg/mL.

##### Preparation of Standard Antibiotic Solution

ofloxacin was also prepared in DMF to obtain a concentration of 500 µg/ml to 3.9 µg/ml.

**Preparation of Saline Solution:** 0.9 gm sodium chloride dissolved in 100 mL distilled water.

##### Cultures

All pathogenic strains of bacteria were procured from National Collection of Industrial Microorganism (NCIM) Pune, India. The microorganisms, which were maintained by sub culturing, were used at regular intervals in nutrient agar medium.

Sr.No.	Standard Micro-organism	M CodeNo.
	<b>Gram + Ve</b>	
1	<i>Staphylococcus aureus</i>	2079

	<b>Gram – Ve</b>	
3	<i>Escherichia coli</i>	2089

Table no. 10.5 Microorganisms used for antibacterial activity

### 0.2.2.5 Preparation of Inoculums:

#### Bacterial Suspension:

Recently grown stock culture of each of the test organism was used to prepare subculture on the surface of a suitable volume of nutrient broth. The bacterial cultures were incubated at 30 to 35°C for 18 to 24 h.

#### Bacterial Saline Solution :

Bacterial suspension of 1 mL was added in 99 mL of sterile saline solution to prepare bacterial saline solution.

#### Control Parameters:

**Media Control:** Sterilized medium was kept for growth (48 h) so as to assure the sterility of the medium. If this control shows growth of any type, then the media were discarded.

**Culture Control:** The culture of the organism was inoculated in sterilized medium. If no growth was observed, then the culture was considered to be faulty. The fresh culture was prepared.

### 0.2.2.6 Precautions:

- 1) Add test solution and standard solution in the alternate manner in test tube.
- 2) Remaining microbial culture is to be discarded after adding disinfectant.
- 3) Dissolve an accurate quantity of the standard preparation of the antibiotic in suitable solvent. This stock solution should be stored in refrigerator and used within the period as specified in the pharmacopoeia.
- 4) Remember to mix the contents of each tube well between.

**0.2.2.7 Procedure:** <sup>12, 13</sup>

- 1) Into test tube rack, placed a one set of 9 sterile 13×100 mm test tubes labeled 1 through 9. Labeled one rack set I - control (blank), gram positive Set II- *Staphylococcus aureus*, gram negative Set III-*Escherichia coli*.
- 2) 0.6 ml of DMF in labeled 1 and 0.5 ml of DMF in labeled 2 through labeled 9 in Set I- III was added by using a sterile 1 ml glass syringe.
- 3) 0.4 ml test compound solution in labeled 1 test tube was added by using a sterile 1 ml glass syringe and then mixed it.
- 4) Using a sterile 1 ml glass syringe, test compound solution 0.5 ml from tube 1 to tube 2 was transferred. It was mixed well and then transferred 0.5 ml from tube 2 to tube 3. This procedure was continued through tube 9. Then 0.5 ml from test tube 9 was discarded.

**Table no. 10.6 Test compounds serial dilution-broth medium setup**

Additions (mL) to:	Test Tube Number										
	1	2	3	4	5	6	7	8	9	Media Contr-ol	Cultu -re Contr -ol
DMF	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Compound	0.4	Serial Dilutions									-
Medium	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.5	4.4
Test Culture	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-	0.1
Compound (µg/mL)	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	-	-
						5	2	1			

- 5) 4.4 ml of nutrient broth in labeled 1 to labeled 9 in set I-III was added by using sterile 5 ml glass syringe.
- 6) 0.1 ml of the 1:1000 dilution of the microorganism in set II and III was added by using a sterile 1 ml glass syringe.
- 7) At the same time two control tubes were prepared.
  - a) One containing the inoculated media control.
  - b) One containing the inoculated culture media control.
- 8) Incubated all sets of tube for 42-48 h at 37°C.
- 9) The growth of test organism was measured by determining the extinction at 600 nm of each of the solution in the tube against the blank (Set I).



### 10.2.3 Results of anti-microbial activity:

Sr.No.	Compoundcode	Anti-microbial activity (MIC) microgram	
		<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
1	HDB-1	62.5	62.5
2	HDB-2	7.81	7.81
3	HDB-3	62.5	125
4	HDB-4	31.25	62.5
5	HDB-5	62.5	62.5
6	HDB-6	7.81	15.31
7	HDB-7	62.5	62.5
8	RLS-1	125	250
9	RLS-2	125	250
10	RLS-3	62.5	125
11	RLS-4	125	125
12	RLS-5	62.5	62.52
13	RLS-6	15.62	62.5
14	RLS-7	31.25	62.5
15	RLS-8	31.25	62.5
16	RLS-9	62.5	125
17	RLS-10	15.62	15.62

18	RLS-11	125	250
19	RLS-12	31.25	62.5
20	RLS-13	31.25	62.5
STD	TMP	15.62	31.25

Table no. 10.7 Results of anti-microbial activity:

### 10.3 *In-vitro* anti-cancer activity:

#### 10.3.2 Results of in-vitro anti-cancer activity:

Sr.No.	Compound Code	GI <sub>50</sub> (μMol)
1	pvv-1	
2	pvv-2	
3	pvv-3	
4	pvv-4	
5	pvv-5	
6	pvv-6	
7	pvv-7	
8	pvv-8	
9	pvv-9	

10	pvv-10	
----	--------	--

Table no. 10.8 Results of *in-vitro* anti-cancer activity:

### Discussion:

Synthesized 10 analogues were subjected for , anti-microbial activity and in-vitro anti-cancer activity. .

In *in-vitro* anticancer activity any potent analogues was not found but analogues.



## 11. DISCUSSION

### 11.1 Molecular Docking:

Initially, molecular docking of 1,2,3,4-tetrahydro and 1,2,3,6-tetrahydro pyrimidine analogues shown good docking score when they were docked in DHFR Receptors using PDB 2W3M and 3FRE Docking score were ranging from -1.20 to -6.83. On the basis of docking study, 20 analogues out of 900 were selected for synthesis. Analogue **HDB-2**, **HDB-6**, **HDB-7**, **RLS-5**, **RLS-6**, **RLS-10** are best docked analogues. Interactions of these analogues with DHFR receptor shown in **fig. No. 6.7 to 6.42**

### 11.2 Synthetic Work:

Here in, we have reported a simple method for the synthesis of 1,2,3,4-tetrahydro pyrimidine and 1,2,3,6-tetrahydropyrimidine analogues. The reaction between substituted aldehyde, urea/thiourea and acetyl acetone in acidic condition yielded corresponding 1,2,3,4-tetrahydro pyrimidine which on reaction with methyl iodide and pyridine gives final substituted 1,2,3,4-tetrahydropyrimidine For the synthesis of 1,2,3,6-tetrahydropyrimidine analogues substituted aldehyde, substituted urea/thiourea and ethylcyanoacetate in sodium ethoxide is stirred for 48 hours. In this at first acyliminium ion formed which subsequently cyclize to the Biginelli dihydropyrimidines. structures of the synthesized compounds were characterized by melting point, TLC, IR spectroscopy, NMR spectroscopy and Mass spectroscopy.

### 11.3 Identification and Characterization:

**Table 9.3** showing IR data of the intermediate and final compounds. The IR spectrum of compound showed an absorption band at  $3240-3280\text{ cm}^{-1}$  due to N-H stretch,  $2996-3150\text{ cm}^{-1}$  due to C-H aromatic stretch. (C=O) was observed at  $1690-1850\text{ cm}^{-1}$ .

In **Table 9.4** the  $^1\text{H}$  NMR data of the synthesized compounds have been mentioned. The  $^1\text{H}$  NMR spectra of compound HDB-6 showed dublet at 7.8-(d, 4H, CH benzene), 6.7-(s, 2H, methine), 1.1-(s, 6H, methyl), 2.5-(s, 2H, N-H), 1.2(s, 3H, methyl); compound RLS-7 showed 8.25-(d, 4H, benzene), 7.22-(s, 1H, NH), 3.86-(s, 3H, methyl), 2.50(s, 3H, methyl) and compound RLS-12 shows 7.8-(d, 4H, Benzene), 3.36-(s, 3H, methyl), 3.19-(s, 1H, N-H).

**Table 9.5** showing Mass spectroscopy data of the compound HDB-6 having m/z ratio at 276.2 (100%), 277.2(18.0%). Chromatogram is performed by using benzene:ethyl acetate in various proportion.

## 11.4 Biological activity:

### 11.4.1 Dihydrofolatereductase enzyme assay:

The DHFR enzyme assay was performed on DHFR enzyme assay kit on microplate spectrophotometer and results are shown in table no. 10.3 results of % Inhibition of DHFR Enzyme, reveals that the compounds **HDB-2, HDB-3, RLS-5, RLS-6, RLS-7** shows better % DHFR enzyme inhibition than standard methotrexate.

### 11.4.2 Antimicrobial activity:

Antimicrobial activity of title compounds was performed by broth dilution method in the drug concentration range 3.90 to 1000 ppm against *E. coli* and *S. aureus*. The results of anti-microbial activity are shown as minimum inhibitory concentration (MIC). Compounds **HDB-2, HDB-6, RLS-6, RLS-10** shows better anti-microbial activity than standard trimethoprim which is shown in table no.10.7 result of antimicrobial activity.

### 11.4.3 Anticancer Activity:

The *in-vitro* anticancer activity was performed by **Sulforhodamine B (SRB)** assay and results shown in table no. 10.8 results of *in-vitro* anti-cancer activity. *In-vitro* anticancer screening reveals that the compounds **HDB-1, HDB-2, HDB-6, HDB-7** shows mild anticancer activity.

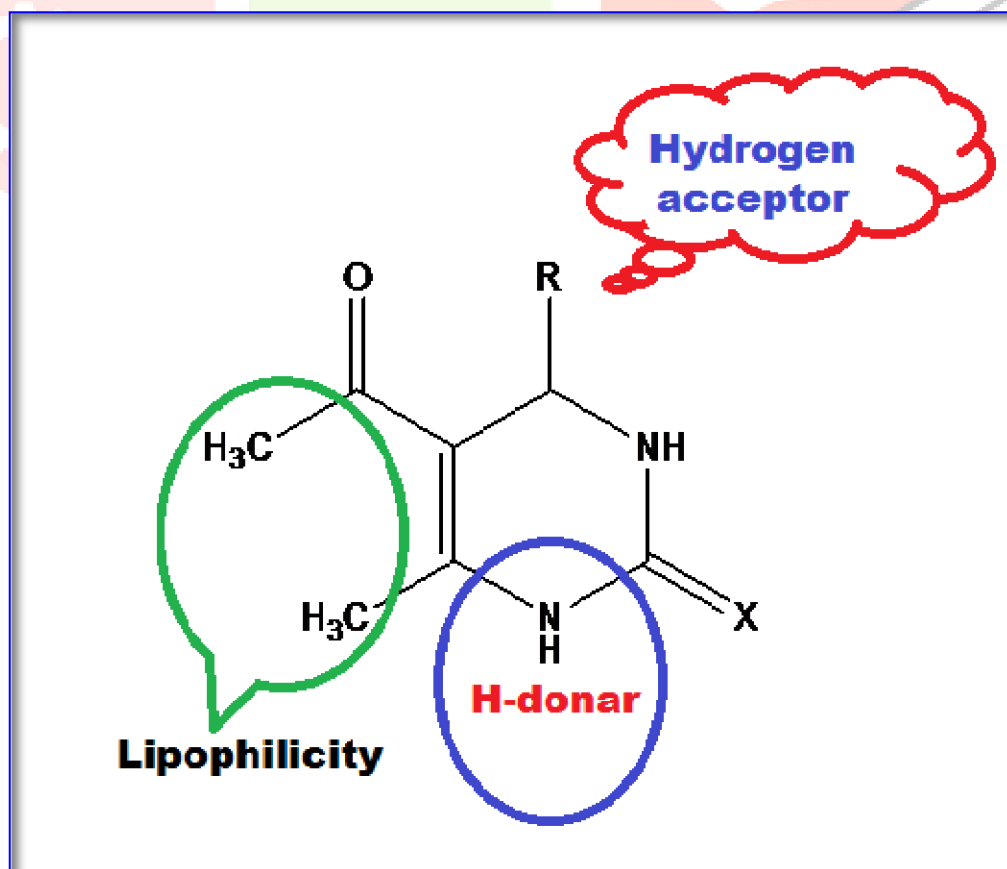
## 12. CONCLUSION:

1. The molecular docking was used to prioritized the Anthracene analogues astopoisomerase ii inhibitors for synthesis.
2. The 1,2,3,4-tetrahydropyrimidine and 1,2,3,6-tetrahydropyrimidine analogues were synthesized according to scheme.
3. Structures of title compounds were confirmed by IR, <sup>1</sup>HNMR & Mass spectra.
4. Compounds 5-Acetyl-6-methyl-4-p-tolyl 1,2,3,4-tetrahydropyrimidin-2-one (HDB-1); 5-Acetyl-6-methyl -4-p-tolyl-2-thioxo -1,2,3,4-tetrahydropyrimidine (HDB-2); 5-Carbonitrile-4-oxo-2-thioxo-6-(3-nitrophenyl)-1,2,3,4-tetrahydropyrimidine (RLS-5); 1,2,3,6-Tetrahydro-1-methyl-2,6-dioxo-4-p-tolypyrimidine-5-carbonitrile (RLS-6); 1,2,3,6-Tetrahydro-1-methyl-2,6-dioxo-4-p methoxyphenylpyrimidine-5-carbonitrile (RLS-7) shows potent DHFR Enzyme inhibition than standard Methotrexate.
5. Compound 5-Acetyl-6-methyl -4-p-tolyl-2-thioxo -1,2,3,4-tetrahydropyrimidine (HDB-2); 5-Acetyl-6-methyl-2-methylthio-4-tolyl-1,2,3,4-tetrahydropyrimidine (HDB-6); 1,2,3,6-Tetrahydro-1-methyl-2,6-dioxo-4-p-tolypyrimidine-5- carbonitrile (RLS-6); 1,2,3,6-Tetrahydro-1-methyl-2,6-dioxo-4-p-nitrophenylpyrimidine-5-carbonitrile (RLS-10) shows better anti-microbial activity on *S.aureus* and *E.coli* in comparison with standard Trimethoprim.

6. Compounds 5-Acetyl-6-methyl-4-p-tolyl 1,2,3,4-tetrahydropyrimidin-2-one (HDB-1); 5-Acetyl-6-methyl-4-p-tolyl-2-thioxo-1,2,3,4-tetrahydropyrimidine (HDB-2); 5-Acetyl-6-methyl-2-methylthio-4-tolyl-1,2,3,4-tetrahydropyrimidine (HDB-6); 5-Acetyl-6-methyl-2-methylthio-4-(p-chlorophenyl)-1,2,3,4-tetrahydropyrimidine (HDB-7) shows mild *in-vitro* anti-cancer activity on MCF7 cell line in comparison with standard MTX.
7. Thus from this study it is concluded that molecular modeling can become a basic tool for anti-microbial and anti-cancer drugs research so that active molecules can be designed.

### 13. FUTURE SCOPE:

1. After the completion of literature survey it was observed that Dihydro Folate Reductase Enzyme receptor literally needs a detail studies in the era of their role in anti-microbial and anti-cancer as there are many anti-microbial and anticancer agents who has been targeted to DHFR.
2. With the help of docking score, docking interactions and results of anti-microbial screening on *S.aureus*, *E.coli* and anticancer screening on breast cancer (MCF-7) cell line, it was observed and concluded that when we will place hydrogen bond donor at 1<sup>st</sup> position, hydrogen bond acceptor at 4<sup>th</sup> position and longer aliphatic chain at 5<sup>th</sup> and 6<sup>th</sup> position of pyrimidine skeleton; it will generate more potent analogues.



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