



Synthesis, Analysis and Docking Study of Substituted Pyrazole Derivatives Containing Amide Linkage For Anti Cancer Activity

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INTRODUCTION:

Cancer is abnormal cell division without control which also invade other tissue. Cancer cells can spread to other part of the body through the blood and lymph systems. Cancers are caused by combined genetic and non-genetic changes induced by environmental factors that trigger in appropriate activation or inactivation of specific genes leading to neoplastic transformations, or abnormal cell growth. There is a lack of information about key cellular events that occur in early stages of cancer development as well as environmental factors and internal causes that trigger these changes. Many cancers form solid tumours, which are masses of tissue. Cancers of the blood, such as leukemia, generally do not form solid tumors. Cancerous tumors are malignant, which means they can spread into, or invade nearby tissues. In addition, as these tumors grow some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumors far from the original tumor. Unlike malignant tumors, benign tumors do not spread into, or invade, nearby tissues. Benign tumors can sometimes be quite large however. When removed, they usually don't grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life threatening.⁽¹⁾

CANCER GROWTH:

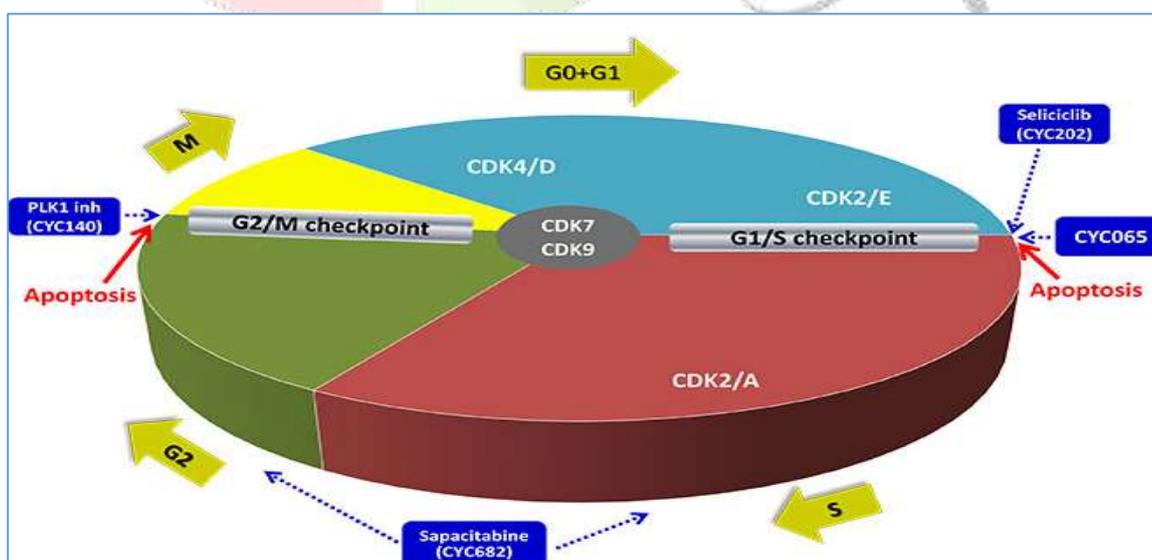
Cancer is caused by changes to genes that control the way our cells function, especially how they grow and divide (cancer cells). Genetic changes in cancer can be inherited from our parents. They can also arise during a person's lifetime as a result of errors that occur as cells divide or because of damage to DNA caused by certain environmental exposures. Cancer-causing environmental exposures include substances, such as the chemicals in tobacco smoke, and radiation, such as ultraviolet rays from the sun. Each person's cancer has a unique combination of genetic changes. As the cancer continues to grow, additional changes will occur. Even within the same tumor, different cells may have different genetic changes. In general, cancer cells have more

genetic changes, such as mutations in DNA, than normal cells. Some of these changes may have nothing to do with the cancer; they may be the result of the cancer, rather than its cause.

The cancer spread due to metastasis, cancer cells break away from where they first formed (primary cancer), travel through the blood or lymph system, and form new tumors (metastatic tumors) in other parts of the body. The metastatic tumor is the same type of cancer as the primary tumor. A cancer that has spread from the place where it first started to another place in the body is called metastatic cancer. The process by which cancer cells spread to other parts of the body is called metastasis. Metastatic cancer has the same name and the same type of cancer cells as the original, or primary, cancer. For example, breast cancer that spreads to and forms a metastatic tumor in the lung is metastatic breast cancer, not lung cancer. Under a microscope, metastatic cancer cells generally look the same as cells of the original cancer. Moreover, metastatic cancer cells and cells of the original cancer usually have some molecular features in common, such as the presence of specific chromosome changes. Treatment may help prolong the lives of some people with metastatic cancer. In general, though, the primary goal of treatments for metastatic cancer is to control the growth of the cancer or to relieve symptoms caused by it. Metastatic tumors can cause severe damage to how the body functions, and most people who die of cancer die of metastatic disease.

Cell cycle

The cell cycle, the process by which cells progress and divide, lies at the heart of cancer. In normal cells, the cell cycle is controlled by a complex series of signalling pathways by which a cell grows, replicates its DNA and divides. This process also includes mechanisms to ensure errors are corrected, and if not, the cells commit suicide (apoptosis). In cancer, as a result of genetic mutations, this regulatory process malfunctions, resulting in uncontrolled cell proliferation.

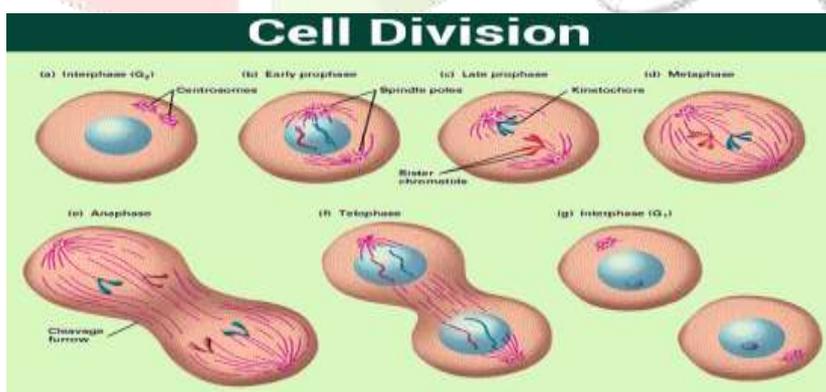


The cell division involves a complex series of molecular and biochemical signaling pathways that cue a cell to divide. The process of cell division, also called mitosis, is accomplished through four phases:

1. the G₁, or gap, phase, in which the cell grows and prepares to synthesize DNA;
2. the S, or synthesis, phase, in which the cell synthesizes DNA;
3. the G₂, or second gap, phase, in which the cell prepares to divide; and
4. The M, or mitosis, phase, in which cell division occurs.

As a cell approaches the end of the G₁ phase it is controlled at a vital checkpoint, called G₁/S, where the cell determines whether or not to replicate its DNA. At this checkpoint the cell is checked for DNA damage to ensure that it has all the necessary cellular machinery to allow for successful cell division. As a result of this check, which involves the interactions of various proteins, a "molecular switch" is toggled on or off. Cells with intact DNA continue to S phase; cells with damaged DNA that cannot be repaired are arrested and "commit suicide" through apoptosis, or programmed cell death.

A second such checkpoint occurs at the G₂ phase following the synthesis of DNA in S phase but before cell division in M phase. Cells use a complex set of enzymes called kinases to control various steps in the cell cycle. Cyclin Dependent Kinases, or CDKs, are a specific enzyme family that use signals to switch on cell cycle mechanisms. CDKs themselves are activated by forming complexes with cyclins, another group of regulatory proteins only present for short periods in the cell cycle. When functioning properly, cell cycle regulatory proteins act as the body's own tumor suppressors by controlling cell growth and inducing the death of damaged cells. Genetic mutations causing the malfunction or absence of one or more of the regulatory proteins at cell cycle checkpoints can result in the "molecular switch" being turned permanently on, permitting uncontrolled multiplication of the cell, leading to carcinogenesis, or tumor development.



TYPES OF CANCER:

Cancer can result from abnormal proliferation of any of the different kinds of cells in the body, so there are more than a hundred distinct types of cancer, which can vary substantially in their behaviour and response to treatment.

The most important tissue in cancer pathology is the distinction between two tumors.

- 1) Benign tumors
- 2) malignant tumors

BENIGN TUMOR-

Benign tumors such as a common skin wart, remains confined to its original location, neither invading surrounding normal tissue nor spreading to distant body sites. Whereas benign tumors can usually be removed surgically, the spread of malignant tumors to distant body sites frequently makes them resistant to such localized treatment.

MALIGNANT TUMORS-

A malignant tumor, however, is capable of both invading surrounding normal tissue and spreading throughout the body via the circulatory or lymphatic systems (metastasis). Only malignant tumors are properly referred to as cancers, and it is their ability to invade and metastasize that makes cancer so dangerous.

CARCINOMA-

Carcinomas are the most common type of cancer. They are formed by epithelial cells, which are the cells that cover the inside and outside surfaces of the body. There are many types of epithelial cells, which often have a column-like shape when viewed under a microscope. Carcinomas that begin in different epithelial cell types have specific names.

SARCOMA-

Soft tissue sarcoma forms in soft tissues of the body, including muscle, tendons, fat, blood vessels, lymph vessels, nerves, and tissue around joints. Sarcomas are cancers that form in bone and soft tissues, including muscle, fat, blood vessels, lymph vessels, and fibrous tissue (such as tendons and ligaments).

LEUKEMIA-

Cancers that begin in the blood-forming tissue of the bone marrow are called leukaemia's. These cancers do not form solid tumors. Instead, large numbers of abnormal white blood cells (leukemia cells and leukemic blast cells) build up in the blood and bone marrow, crowding out normal blood cells. There are four common types of leukemia, which are grouped based on how quickly the disease gets worse (acute or chronic) and on the type of blood cell the cancer starts in (lymphoblastic or myeloid). The low level of normal blood cells can make it harder for the body to get oxygen to its tissues, control bleeding, or fight infections.

LYMPHOMA-

Lymphoma is cancer that begins in lymphocytes (T cells or B cells). These are disease-fighting white blood cells that are part of the immune system. In lymphoma, abnormal lymphocytes build up in lymph nodes and lymph vessels, as well as in other organs of the body.

MULTIPLE MYELOMA-

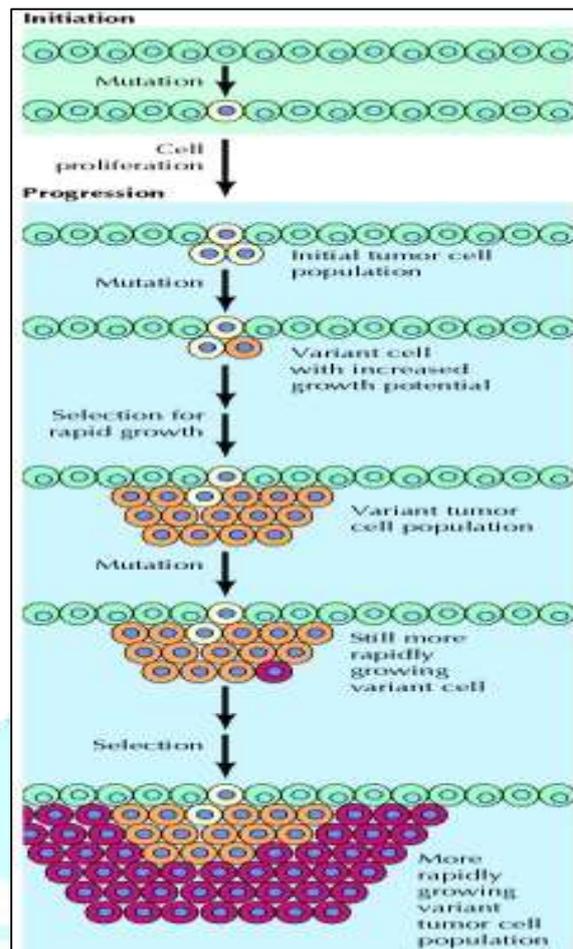
Multiple myeloma is cancer that begins in plasma cells, another type of immune cell. The abnormal plasma cells, called myeloma cells, build up in the bone marrow and form tumors in bones all through the body. Multiple myeloma is also called plasma cell myeloma and Kahler disease.

MELANOMA-

Melanoma is cancer that begins in cells that become melanocytes, which are specialized cells that make melanin (the pigment that gives skin its colour).⁽²⁾

DEVELOPMENT OF CANCER:

The cellular level, the development of cancer is viewed as a multistep process involving mutation and selection for cells with progressively increasing capacity for proliferation, survival, invasion, and metastasis (Fig1). The first step in the process, tumor initiation, is thought to be the result of a genetic alteration leading to abnormal proliferation of a single cell. Cell proliferation then leads to the outgrowth of a population of clonally derived tumor cells. Tumor progression continues as additional mutations occur within cells of the tumor population. Some of these mutations confer a selective advantage to the cell, such as more rapid growth, and the descendants of a cell bearing such a mutation will consequently become dominant within the tumor population. The process is called clonal selection, since a new clone of tumor cells has evolved on the basis of its increased growth rate or other properties (such as survival, invasion, or metastasis) that confer a selective advantage. Clonal selection continues throughout tumor development, so tumors continuously become more rapid-growing and increasing.



CAUSES OF CANCER:

Substances that cause cancer, called carcinogens, have been identified both by studies in experimental animals and by epidemiological analysis of cancer frequencies in human populations (e.g., the high incidence of lung cancer among cigarette smokers). Since the development of malignancy is a complex multistep process, many factors may affect the likelihood that cancer will develop, and it is overly simplistic to speak of single causes of most cancers. None the less, many agents, including radiation, chemicals, and viruses, have been found to induce cancer in both experimental animals and humans.

ANTICANCER DRUG:

Anticancer drug, also called antineoplastic drug, any drug that is effective in the treatment of malignant, or cancerous, disease. There are several major classes of anticancer drugs; these include alkylating agents, antimetabolites, natural products, and hormones. ⁽³⁾

Discovery of anticancer drug started after 1940's (when nitrogen mustard was used).

CLASSIFICATION OF ANTICANCER AGENTS:**Cytotoxic Agents:**

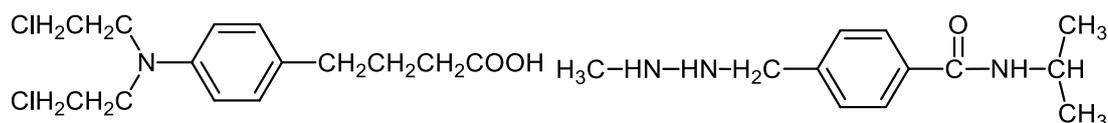
1. Alkylating agents – a) Nitrogen mustard – mechlorethamine, Cyclophosphamide, Melphan, ifosphamide
b) Ethylenimine – thiotepa
 - c) Alkylsulfonate – busulfan
 - d) Nitrosoureas – carmustine, lomustine
 - e) Triazine - decarbazine
 - f) Methyl hydrazine - procarbazine
2. Platinum co-ordination – cisplatin, carboplatin, oxaliplatin
3. Antimetabolite- a) Folate antagonists – methotrexate, pemetrexed
b) purine antagonists – 6- mercaptopurine, 6- thioguanine, azathioprine,
 - c) pyrimidine antagonists – 5-fluorouracil, capecitabine, cytarabine
4. Microtubule damaging agent- 1) Vinca alkaloids - vincristine, vinblastine
 - 2) Taxanes- paclitaxel, docetaxel, estramustine
5. Topoisomerase- 1 inhibitor – topotecan, irinotecan
6. Topoisomerase-2 inhibitor- etoposide
7. Antibiotic- actinomycin, doxorubicin, epirubicin
8. Miscellaneous- hydroxyurea, L- asparaginase

TARGETED DRUGS:

1. Tyrosine protein kinase inhibitor- imatinib, nilotinib
2. EGF receptor inhibitor – gefitinib, erotinib
3. Angiogenesis inhibitor- bevacizumab
4. Proteasome inhibitor – bortezomib

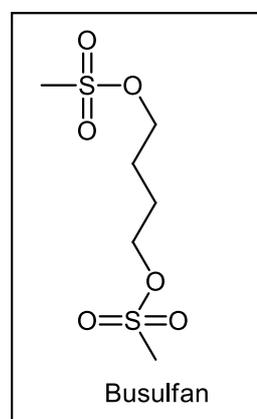
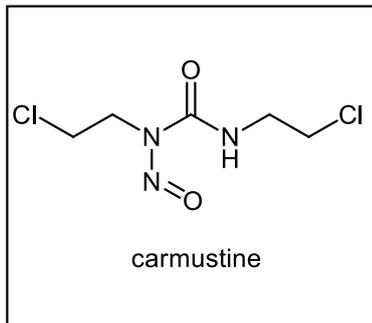
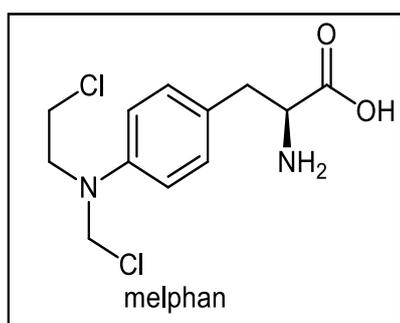
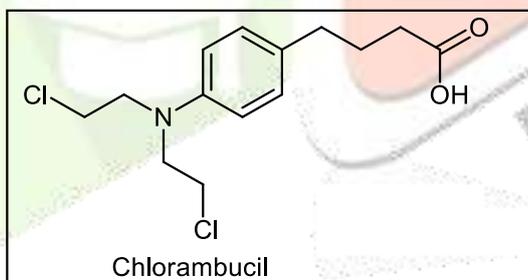
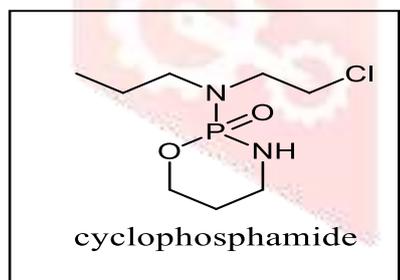
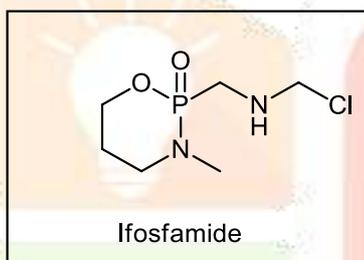
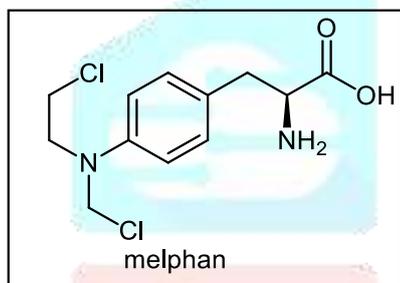
HORMONAL DRUGS:

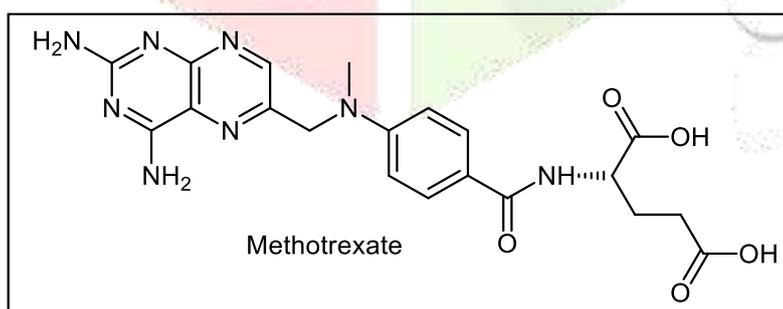
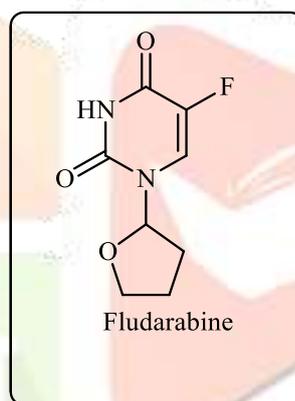
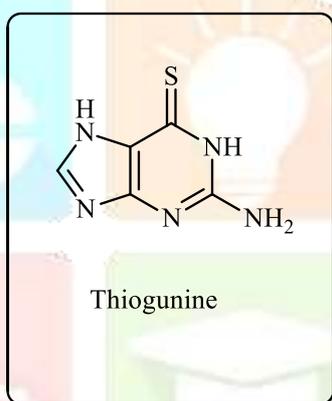
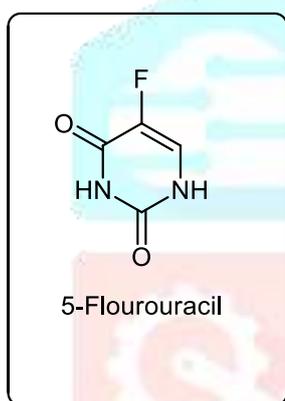
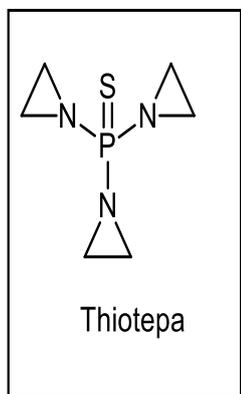
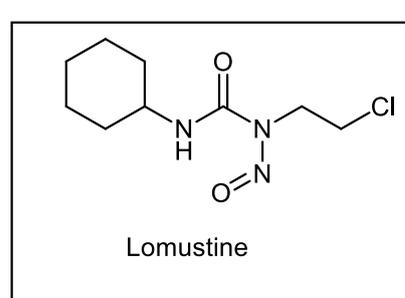
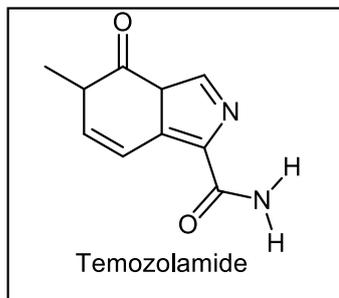
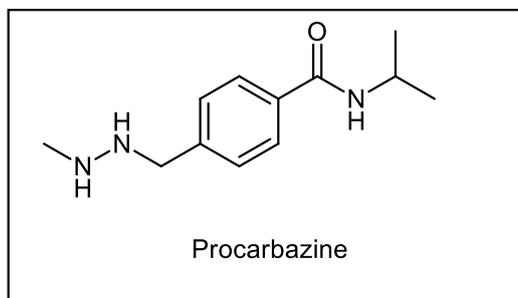
1. Glucocorticoid – prednisolone
2. Estrogen – fosfesstrol
3. Selective estrogen receptor modulation – tamoxifen
4. Antiandrogen- flutamide
5. GNRH analogue- nafarelin, triptorelin
6. Progestin – hydroxyprogesterone acetate

STRUCTURES OF SOME ANTICANCER DRUGS:

Procarbazine

Chlorambucil





IMPORTANCE OF PYRAZOLE:

Pyrazole is known to be one of the most potential families of nitrogen-containing compounds. Pyrazole derivatives exhibit a broad spectrum of biological profiles, for instance, anti-tubercular, anti-AIDS, anti-malarial, anti-microbial, antitumor, anticancer, and antifungal.

Pyrazole derivatives were discovered in 1883 by Ludwig Knorr. Pyrazole, a five-membered heterocyclic containing two nitrogen atoms, pyrazole compounds and their pharmacological effect on humans, they are classified as alkaloid although they are rare in nature.

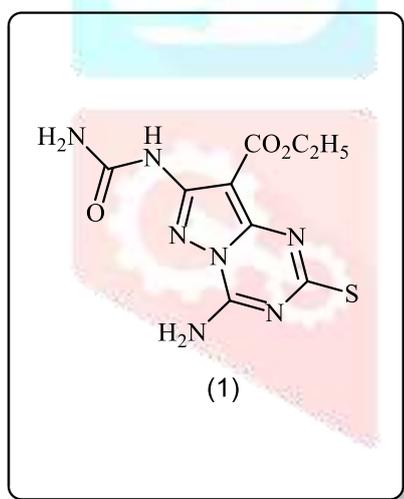
The molecular formula of pyrazole is $C_3H_4N_2$ which has 6 π electrons delocalized over the ring forming an aromatic system. ^{(4), (42)}

Importance's of amide:

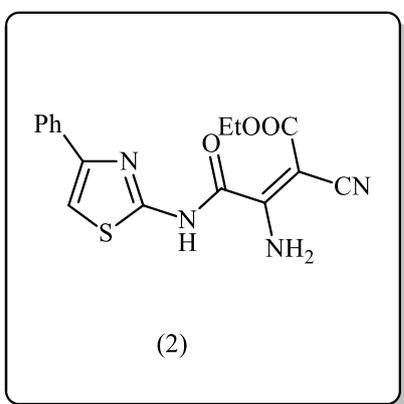
An amide linkage, $-CO-NH-$, is a core structural unit in the skeleton of proteins and pervasive in nature. Amide-based molecules are considered to be suitable for drug research due to their biological compatibility. Development of new amide derivatives has been actively pursued due to their potent application in vast biological activities, such as fungicidal, herbicidal, insecticidal, anti-cancer, and antibacterial, etc. ⁽⁵⁾

REVIEW OF LITERATURES OF PYRAZOLE AND AMIDE

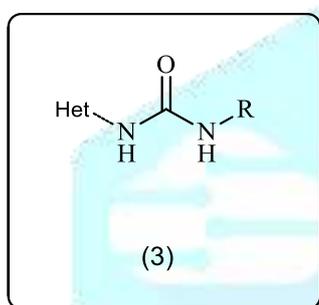
Bakr. et al. Reported the synthesis of pyrazolo [1,5a] [1, 3, 5] triazine-8-carboxylic acid ethyl ester 244 from the reaction of amino pyrazolyl urea derivative. ⁽⁶⁾



Ranjana et al. reported synthesis of ethyl 1, 3, 4-triphenyl-1H-pyrazolo [3, 4-b] pyridine-6-carboxylate (23) from the reaction of 5-aminopyrazole ($R = Ph$, 16) and ethyl 2, 4-dioxo-4-phenylbutanoate. Electron-donating groups on the contrary decreased the electrophilicity of the carbonyl carbon and hence resulted in lower yields. ⁽⁷⁾ Synthetic utility of 2-amino-4-substituted-thiazoles, their reactions and biological activities have been surveyed and are presented in this review. 2-Amino-4-phenylthiazole 3 was reacted with diethyl 3-amino-2-cyano-2-pentenoate to yield the corresponding amide derivatives (2).



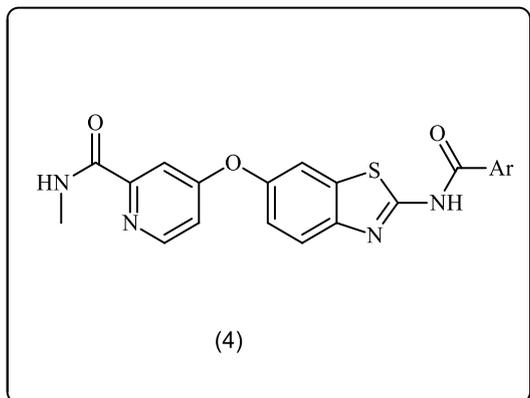
Jose *et al.* reported Pharmacophores Identification and Scaffold Exploration to Discover Novel, Potent, and Chemically Stable Inhibitors of Acid Ceramidase in Melanoma Cell. In this regard, Benzimidazole derivatives showed the best balance between potency and stability.⁽⁸⁾



Compound	Heteryl moiety	R
1		
2		
3		
4		
5		
6		
7		
8		

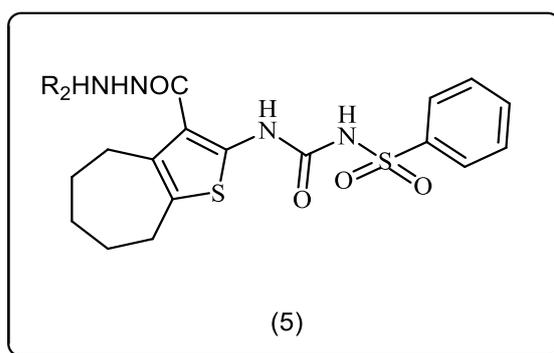
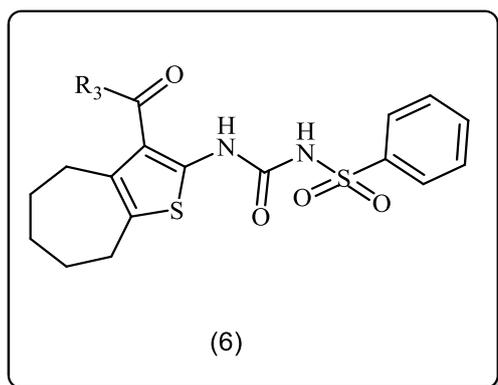
Ju-Hyeon Lee *et al.* A new series of benzothiazole amide and urea derivatives tethered with the privileged pyridyl amide moiety by ether linkage at the 6-position of benzothiazole (22 final compounds) has been

designed and synthesized as potent anticancer sorafenib analogues. urea member is the best derivative with superior potency and efficacy compared to sorafenib as well as notable extended spectrum activity covering 57 human cancer cell lines.⁽⁹⁾

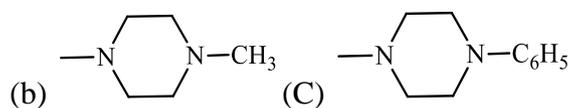


Compound	Ar
4b	3,5-(CF ₃) ₂ -C ₆ H ₃ -
5a	4-Cl-3-CF ₃ -C ₆ H ₃ -NH
5b	3, 5-(CF ₃) ₂ -C ₆ H ₃ -NH
5c	2,4-Cl ₂ -C ₆ H ₃ -NH

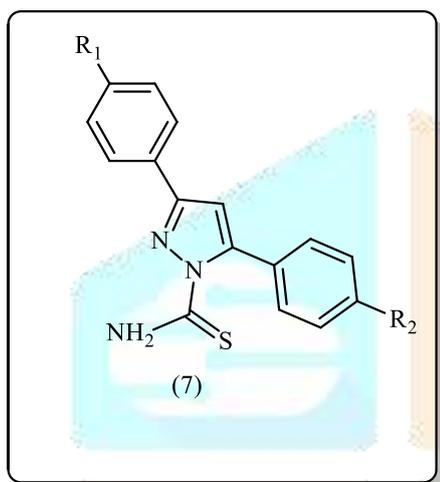
Magda *et al.* reported the substituted piperazinylcarbonyl)-5, 6, 7, 8-tetrahydro-4H-cyclohepta[b]thiophen-2-yl] ureas have been synthesized and tested for their antitumor activity. Molecular modelling and pharmacophores prediction methods have been used to study the antitumor activity of the most active compounds compared with the least active species by means of the molecular mechanic method.⁽¹⁰⁾



R³ = HNCH₃H₇ (a), HNC₄H₉,

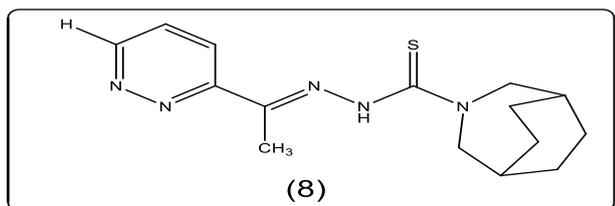


Muhammad *et al.* Reported Based on the docking results; novel pyrazole derivatives were synthesized and characterized by IR, ^1H NMR, ^{13}C NMR and Mass spectroscopy. These compounds were screened for antioxidant activity by DPPH radical scavenging activity and anticancer activity against breast cancer cell line (MCF-7) and lung cancer cell line (A549) with MTT assay. Compounds, (7) showed significant anticancer activity against MCF-7 and A549 cell lines which was comparable to the positive control doxorubicin. ⁽¹¹⁾

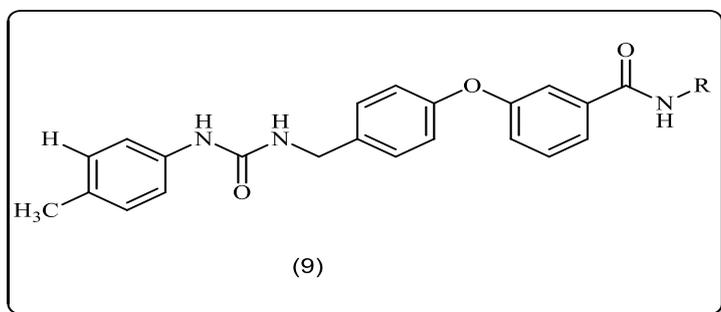


$\text{R}_1 = \text{CH}_3, \text{H}, \text{R}_2 = \text{Ph}$,

Johnny *et al.* reported Synthesis, Cytotoxicity, and Antitumor Activity of Copper (II) and Iron (II) Complexes of 4N-Azabicyclo [3.2.2] nonane Thiosemicarbazones Derived from Acyl Diazines. A series of thiosemicarbazones (TSCs) (bearing a 4N-azabicyclo [3.2.2] nonane moiety) derived from 3-acylpyridazines, 4-acetylpyrimidines, and 2-acetylpyrazines (1-8) were synthesized as potential antitumor agents. ⁽¹²⁾

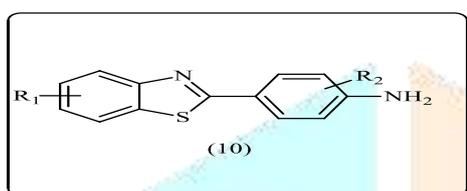


Sikka *et al.* reported synthesized a series of some new benzyl urea derivatives which were screened for their inhibitory activities on MX-1, HepG2, Ketr3 and HT-29 cell lines. Compounds 30 showed greater inhibitory activity against HT-29 and MX-1, respectively. ⁽¹³⁾



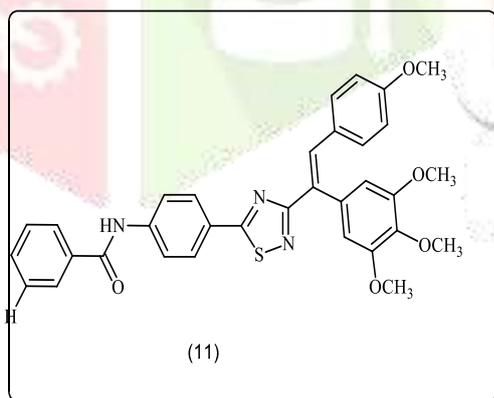
R= 3 pyridyl

Tracey *et al.* reported Antitumor Benzothiazoles. 3.1 Synthesis of 2-(4-Aminophenyl) benzothiazoles and Evaluation of Their Activities against Breast Cancer Cell Lines in Vitro and in Vivo, Compound (10) showed the most potent growth inhibition against the ER+ (MCF-7 and BO) and ER- (MT-1 and MT-3) tumors.⁽¹⁴⁾

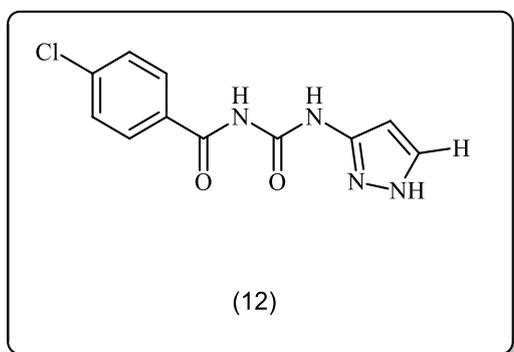


R₁=H, R₂=3Me

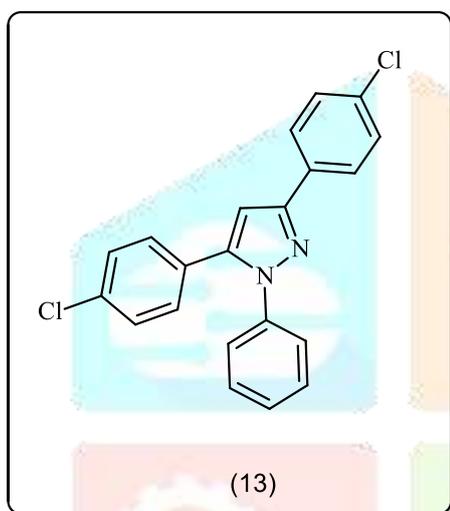
Ragha *et al.* reported Design, Synthesis, and Anticancer Activity of Amide Derivatives of Structurally Modified Combretastatin-A4. the compound is tested for their anticancer activity towards human cancer cell lines, MCF-7 (breast), A-549 (lung), Colo-205 (colon), and A-2780 (ovarian).⁽¹⁵⁾



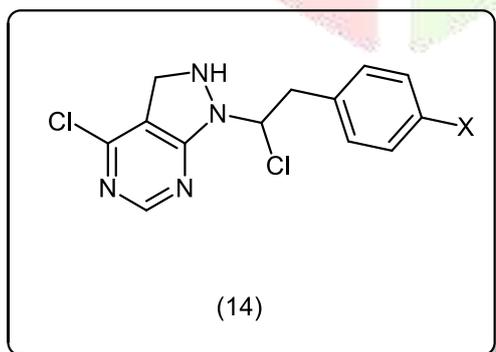
George *et al.* Reported New Potential Antitumor Pyrazole Derivatives: Synthesis and Cytotoxic Evaluation, New pyrazole derivatives were designed and synthesized as potential protein kinase inhibitors in the view to develop specific antitumortherapies.⁽¹⁶⁾



Khalid *et al.* reported the Synthesis and Pharmacological Activities of Pyrazole Derivatives: This review highlights the different synthesis methods and the pharmacological properties of pyrazole derivatives. Studies on the synthesis and biological activity of pyrazole.⁽¹⁷⁾

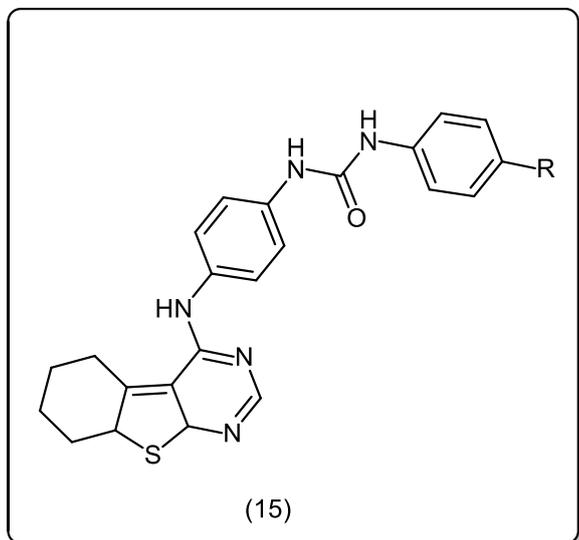


Fabrizio *et al.* reported the optimization and synthesis of pyrazolo [3, 4-d] pyrimidine as Abl inhibitor and antiproliferative agents toward human leukaemia cell lines.⁽¹⁸⁾



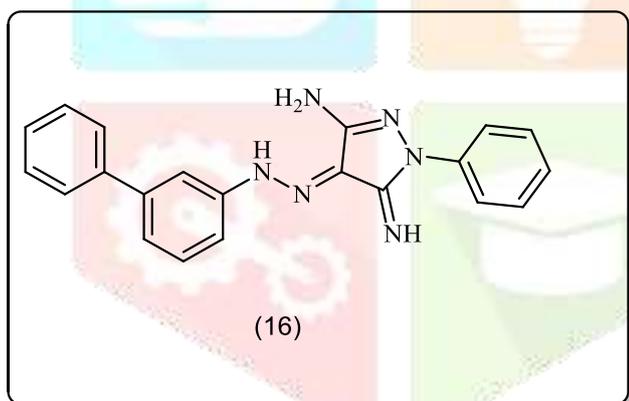
X =H, Cl, F

Eman *et al.* reported the Design and synthesis of thienopyrimidine urea derivatives with potential cytotoxic and pro-apoptotic activity against breast cancer cell line MCF-.7 The compound shows potent anticancer activity.⁽¹⁹⁾

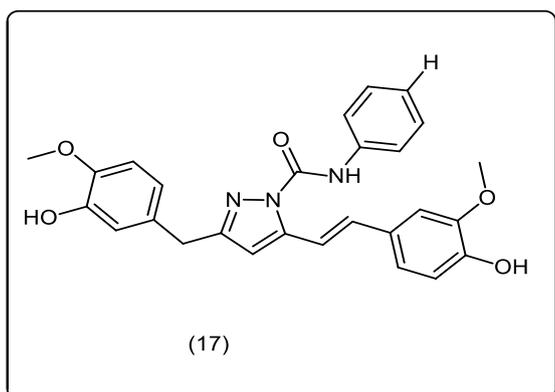


R=2, 6-(Cl)

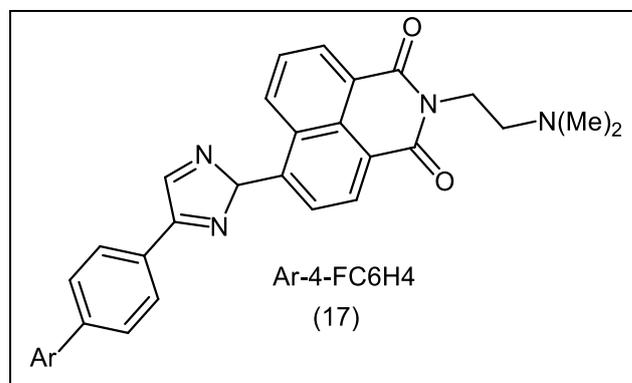
Ahmed *et al.* reported Design, Synthesis and Anticancer Screening of Novel Pyrazole Derivatives Linking to Benzimidazole, Benzoxazole and Benzothiazole. compounds 3a-c and 5a-i were screened for their anticancer activities against breast carcinoma (T47D) and human hepatocarcinoma cell lines (Huh7) compared with Doxorubicin. ⁽²⁰⁾



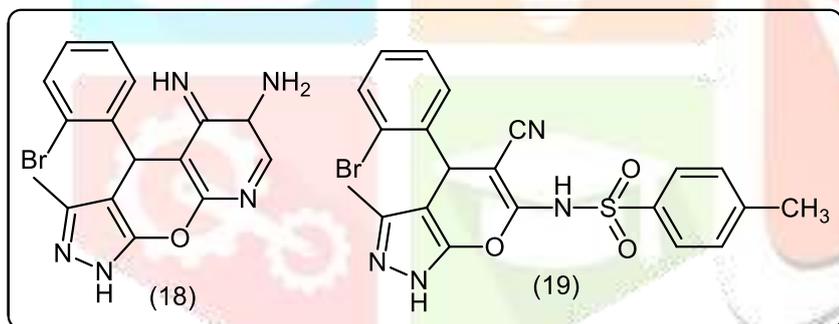
Mohamed *et al.* reported the Synthesis, Characterisation, and In Vitro Anticancer Activity of Curcumin Analogues Bearing Pyrazole/Pyrimidine Ring Targeting EGFR Tyrosine Kinase. The anticancer effects were evaluated on a panel of 60 cell lines, according to the National Cancer Institute (NCI) screening protocol. ⁽²¹⁾



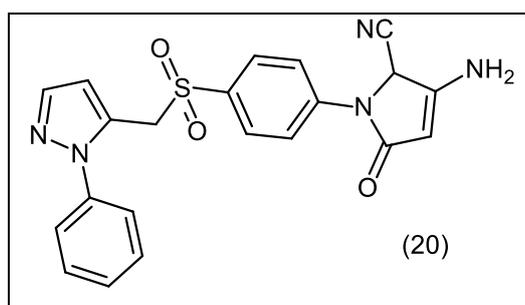
Bayu *et al.* Reported the synthesis of 4-pyrazoly1,8-naphthalimide derivatives (22a-1) [28]. All compounds were toxic against MCF-7 and HeLa that have IC₅₀ values of 0.51-17.01 μM (onMCF-7) and 3.09-16.60 μM (on HeLa). Toward these cells, compound 22b, 22h, have higher cytotoxicity than ammonified (control). On A549 cell, most of the synthesized compounds have good anticancer activity (IC₅₀ values between 5.09) (22)



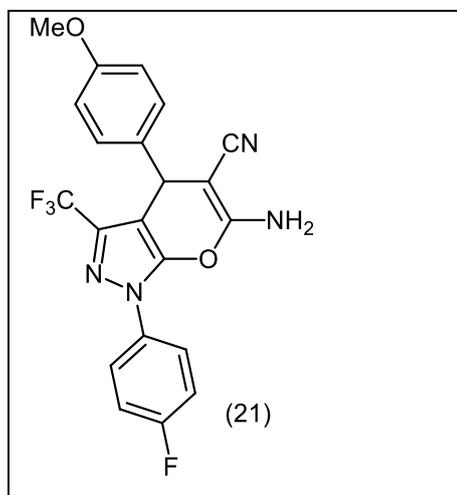
Nashwa *et al.* reported the novel anticancer fused pyrazole derivatives as EGFR and VEGFR-2 dual TK inhibitors. In vitro EGFR and VEGFR-2 inhibitory activity were performed for the synthesized compounds, and the results identified compound 3 as the most potent EGFR inhibitor (IC₅₀ = 0.06 μM) and compound (17) as the most potent VEGFR-2 inhibitor (IC₅₀ = 0.22 μM). (23)



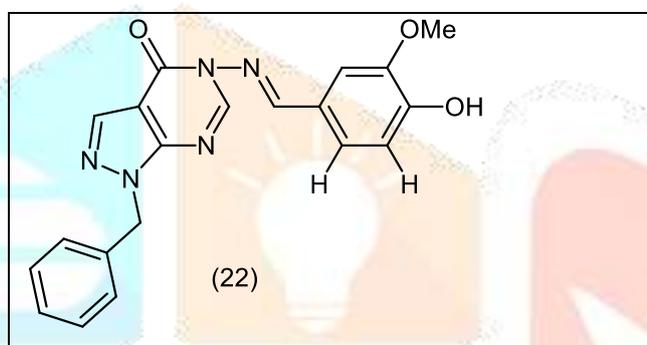
Saleh *et al.* reported the Novel Antitumor Acetamide, Pyrrole, Pyrrolo pyrimidine, Thiocyanate, Hydra zone, Pyrazole, Isothiocyanate and Thiophene Derivatives Containing a Biologically Active Pyrazole Moiety. Compound 20 was more effective than the reference drug, doxorubicin. (24)



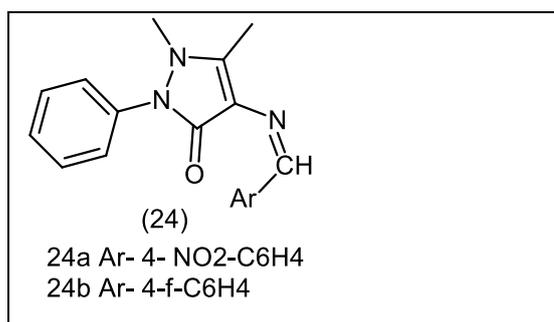
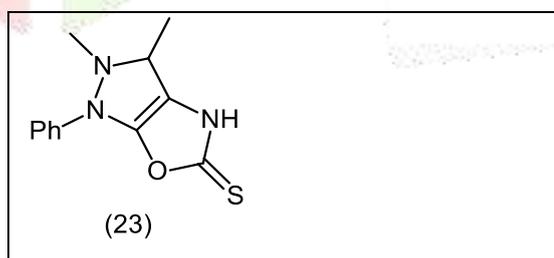
Bhartendu *et al.* reported A green and clean pathway: One pot, multicomponent, visible light assisted synthesis of pyrano [2, 3-c] Pyrazole under catalyst-free and solvent-free conditions, (25)



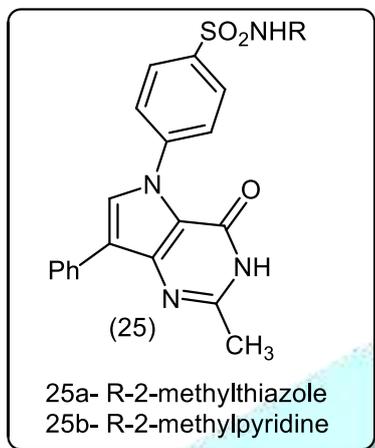
Chauhan *et al.* reported Medicinal attributes of pyrazolo [3, 4-d] pyrimidines a review for good anticancer activity ⁽²⁶⁾



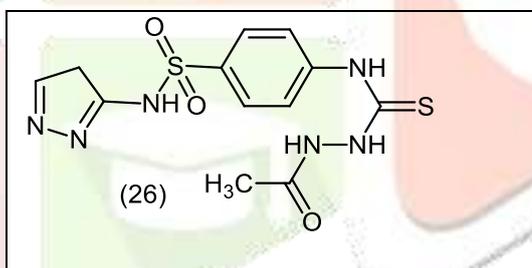
El-Gazzar *et al.* reported Novel Pyrazole Derivatives as Anticancer and Radio sensitizing Agents The results of in-vitro anticancer evaluation showed that compounds 23 and 24a,24b were the most potent compounds on HEPG2 (IC 50 = 2.6 and 4.2 µg/ml).⁽²⁷⁾



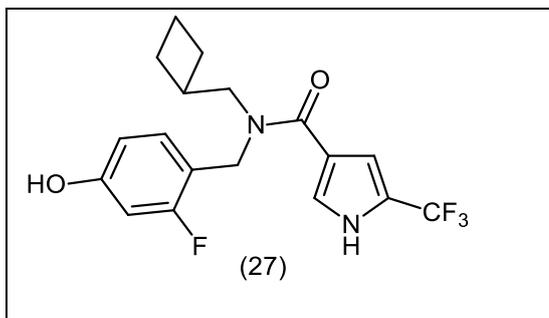
Mostafa et al. reported Synthesis of novel pyrrole and pyrrolo [2,3-d] pyrimidine derivatives bearing sulfonamide moiety for evaluation as anticancer and radio sensitizing agents, the structures of these compounds were confirmed by elemental analysis, IR, ^1H NMR and mass spectral data. All the newly synthesized compounds were evaluated for their in vitro cytotoxicity against liver and breast cancer cell line (HEPG2 and MCF7). Most of the screened compounds showed interesting cytotoxic activities compared with the used reference drug (doxorubicin).⁽²⁸⁾



Mohamed et al. reported Synthesis, structural characterization and anticancer evaluation of pyrazole derivatives, the active compound 26 could be considered as useful templates for further development to obtain more potent anti-cancer agent.⁽²⁹⁾

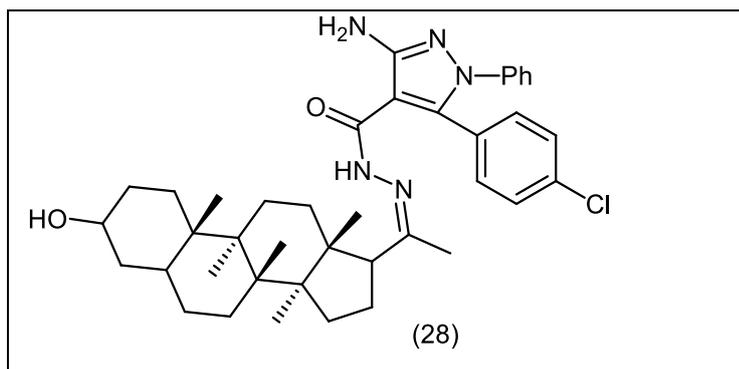


Khalid et al. reported Synthesis and Pharmacological Activities of Pyrazole Derivatives: A Review, the compound 215 as hPKM2 activator with ($\text{AC}_{50} = 0.011 \mu\text{M}$) as the most active anticancer agent with IC_{50} of 5.94 and 6.40 μM against A549 and NCI-H1299 cell lines respectively.⁽³⁰⁾

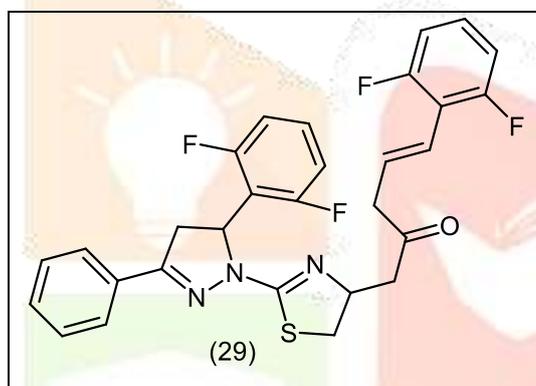


Smaail et al. reported Synthesis and Pharmacological Activities of Pyrazole Derivatives: A Review, the compound 28 was found to exhibit much higher inhibitory effects towards adenocarcinoma (MCF-7), non-

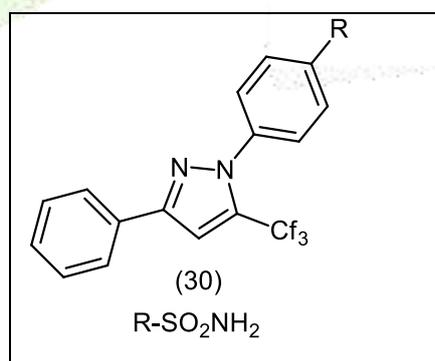
small cell lung cancer (NCI-H460) and CNS cancer (SF-268), with IC₅₀ values 0.01, 0.02 and 0.04 μ M respectively. ⁽³¹⁾



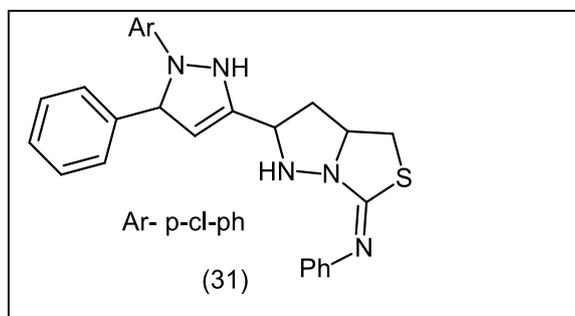
Ansari *et al.* reported Review: biologically active pyrazole derivatives novel 4,5-dihydropyrazole derivatives (29) exhibiting excellent anticancer activity compared to the reference drug cisplatin. ⁽³²⁾



Gosselin *et al.* reported Highly Regioselective Synthesis of 1-Aryl-3, 4, 5-Substituted Pyrazole. ⁽³³⁾



Flefel, *et al.* reported Synthesis and Anticancer Activity of New Substituted Pyrazoles and Their Derived 1, 2, 4-Triazoles and Sugar Derivatives. ⁽³⁴⁾



NEED OF STUDY:

The growth and proliferation of cells take place in an uncontrolled manner in cancer. Cancer leads to a tremendous mortality regardless of the recent advances in the development of clinically authorized anticancer agents. Increasing recurrence of mammalian tumors and severe side-effects of chemotherapeutic agents reduce the clinical efficacy of a large variety of anticancer agents that are currently being used. Thus, there is always a constant need to develop alternative or synergistic anticancer drugs with minimal side-effects. One in 4 deaths in the United States is due to cancer. A total of 1,638,910 new cancer cases and 577,190 deaths from cancer are reported in the United States in 2012. ⁽³⁵⁾

- Although there are many kinds of cancer, only a few occur frequently (Table 1). More than a million cases of cancer are diagnosed annually in the United States, and more than half million of Americans die of cancer each year. Cancer is among the leading causes of death worldwide. In 2012, there were 14.1 million new cases and 8.2 million cancer-related deaths worldwide.
- 57% of new cancer cases in 2012 occurred in less developed regions of the world that include Central America and parts of Africa and Asia; 65% of cancer deaths also occurred in these regions.
- The number of new cancer cases per year is expected to rise to 23.6 million by 2030. The International Agency for Research on Cancer Exit Disclaimer has more information about cancer statistics across the world.

Cancers of 10 different body sites account for more than 75% of this total cancer incidence. The four most common cancers, accounting for more than by far the most lethal and is responsible for nearly 30% of all cancer death. Refer table 1 for different types of cancer with their cases in USA. Hence there is need for the development of newer anticancer agents with better properties.

Table 1. Ten Most Frequent Cancers in the USA		
Cancer site	Cases per year	Deaths per year
Breast	184,200 (15.1%)	41,200 (7.5%)
Prostate	180,400 (14.8%)	31,900 (5.8%)
Lung	164,100 (13.4%)	156,900 (28.4%)
Colon/rectum	130,200 (10.7%)	56,300 (10.2%)
Lymphomas	62,300 (5.1%)	27,500 (5.0%)
Bladder	53,200 (4.4%)	12,200 (2.2%)
Uterus	48,900 (4.0%)	11,100 (2.0%)
Skin (melanoma)	47,700 (3.9%)	7,700 (1.4%)
Kidney	31,200 (2.6%)	11,900 (2.2%)
Leukaemia's	30,800 (2.5%)	12,100 (2.2%)
Subtotal	933,000 (76.5%)	368,800 (66.8%)
All sites	1,220,100 (100%)	552,200 (100%)

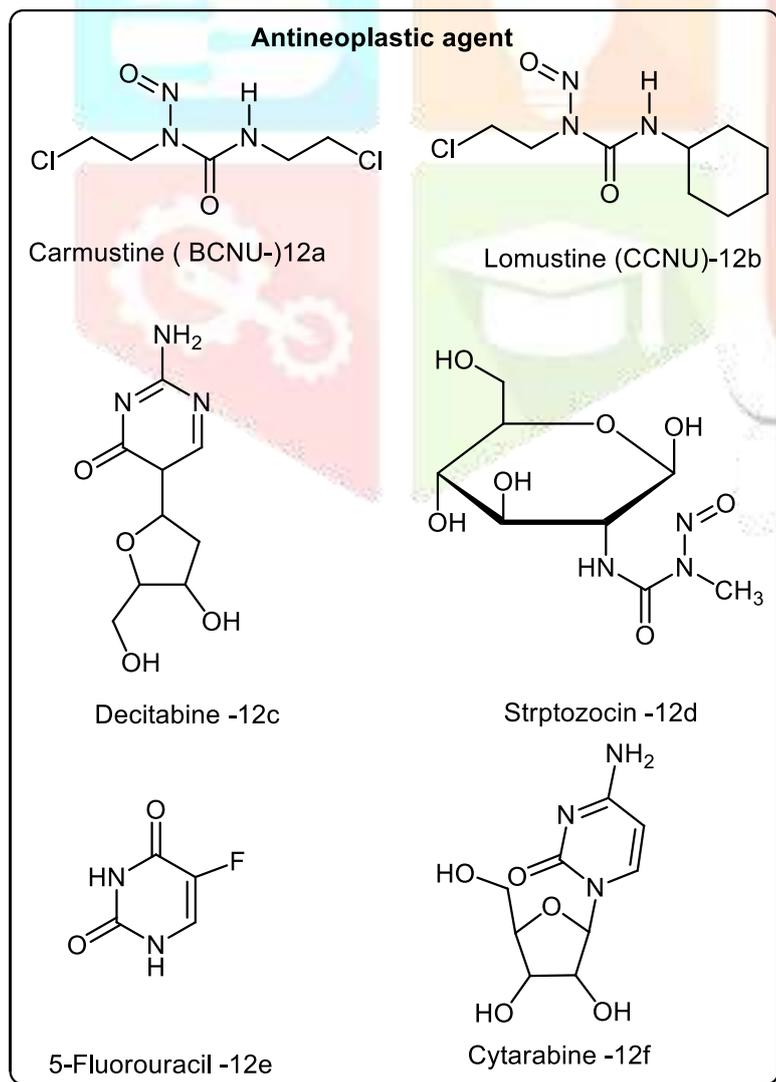
OBJECTIVE:

Paul Ehrlich in 1900 given the meaning of pharmacophore as molecular framework that carries (phoros) the essential features responsible for a drug's (pharmacon) biological activity. A pharmacophore represents the spatial arrangement of steric and electronic features that is essential for the molecule to interact with a specific biological target and to trigger (or block) its biological response⁽³⁶⁾

The identification of pharmacophore is important step in understanding the interactions between a receptor and a ligand, which depends on size of active, various steric- electrostatic and hydrophobic contacts. Some contacts or sites are more important than others. The specific interactions that are crucial for ligand recognition and binding by the receptor are thermal pharmacophore. These interactions are directly involved in the structural integrity of receptor or in the mechanism of its action. Pharmacophore model is derived from a set of known ligands for a target. Pharmacophore hypothesis are generated by the multiple conformations of the set of molecules. When protein is unknown, it is more efficient Different methods The conformation search can be quite large and can be approaches using following methods – Systematic search method –

Distance geometry method – Clique detection algorithm. Nowadays pharmacophore approaches became one of the foremost tools in drug discovery after the past century's development. Numerous ligand-based and structure-based strategies are developed for improved pharmacophore modelling with success and extensively applied in virtual screening, de novo design and lead improvement⁽³⁷⁾ Pharmacophores are used as queries for recovering likely leads from structural databases for designing molecules with specific desired attributes and for evaluating similarity and variety of molecules manipulation pharmacophore fingerprints. It may be used to align molecules based on the 3D arrangement of chemical structures or to improve prognostic 3D quantitative structural activity relationship (QSAR) models.⁽³⁸⁾ Similarly, Virtual screening is a computational process used in the areas of drug discovery and development to explore libraries of small ligands which can be suitably bound to their target proteins or enzymes while docking is a phenomenon of predicting the orientations of molecules in the bounded stable complex.

All the drug substances contain one or more pharmacophore viz. -COOH, -OH, -NH₂, COOR, -NHCONH-, -CONH₂, SO₂NH₂, aromatic ring, etc. Amongst all the functional group, the amide and urea derivatives were found to present in many drug substances of different category. Hence it was envisage synthesizing heterocyclic ring with urea linkage a pharmacophor. Many anticancer drugs also contain the urea linkage.



In view of above fact and in continuation of in continuation of work on Pyrazole derivatives ⁽³⁹⁻⁴⁰⁾, it was planned to synthesize Pyrazole and amide derivatives as anticancer derivatives. It was also reported in the literature ^(16,17) that the compound with pyrazole and amide linkage exhibit anticancer activity. Hence the following compound were planned for synthesis. These compounds will be evaluated for *in-vitro* for anticancer activity against four human cancer cell lines viz. against MCF-7 (human breast cancer cell line), HeLa (human cervical cancer cell line), SKMEL-2 (human melanoma cancer cell line) and HL-60 (human leukemia cancer cell line). Their GI_{50} were determined in micromolar concentration. ⁽⁴¹⁾

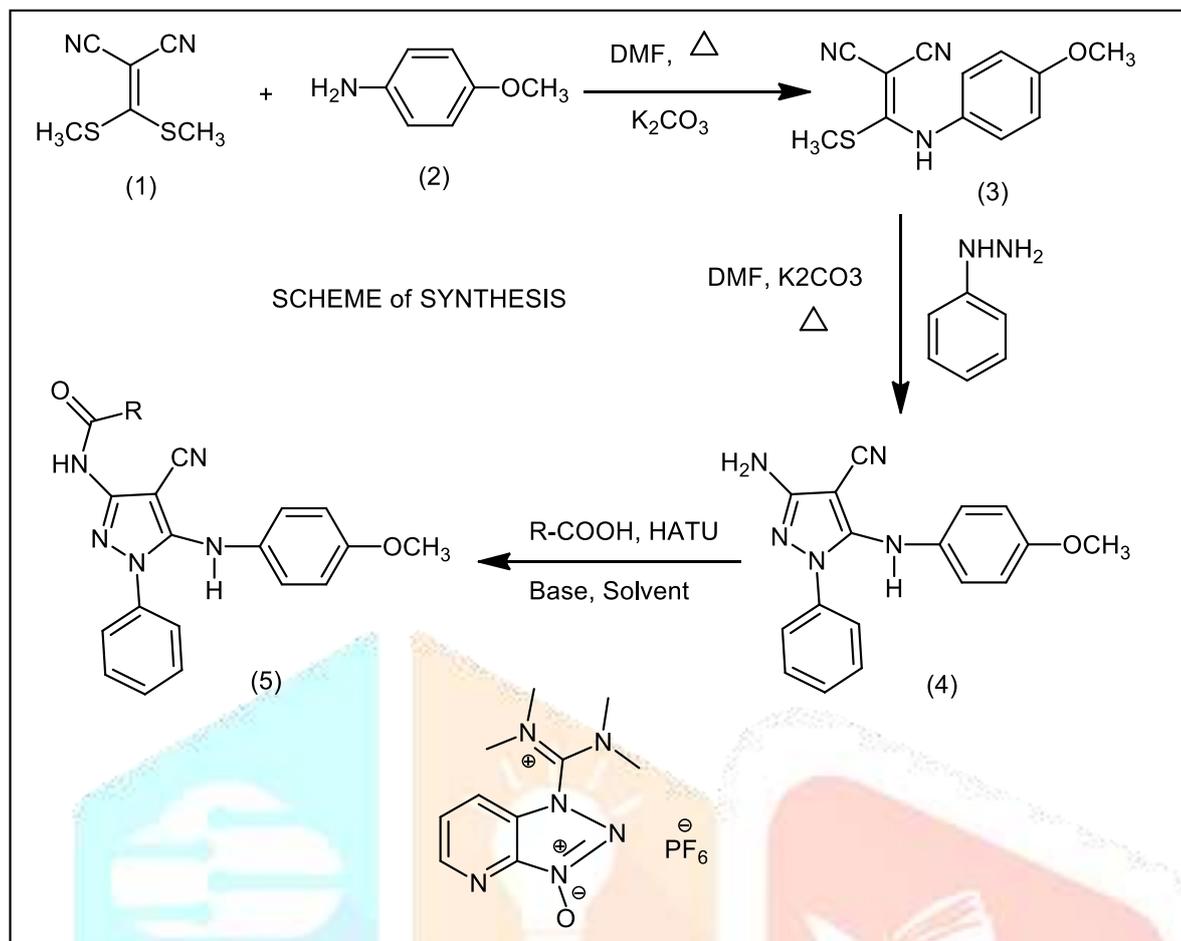
The objective of the present work includes-

1. Design a molecule by molecular docking for anticancer activity,
2. Synthesize a new compounds
3. Characterization of new compounds,

PLAN OF WORK:

- Selection of topic, literature survey and feasibility study.
- Procurement of chemical and special apparatus.
- Synthesis of starting compounds, intermediates and final compound.
- Characterisation and conformation of synthesized compound by chemical spectra and analytical method.
- Docking study and Physico chemical properties
- Dissertation writing.

The target of compound will be synthesized by following route /scheme



HATU - Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium
(1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate)

RCOOH - Carboxylic acid viz Benzoic acid, Acetic acid, 5-Chloro-2-methyl benzoic acid, picolinic acid, Nicotinic acid, Salicylic acid, p-Nitrobenzoic acid

MATERIAL AND METHODS:

Chemicals used were obtained from Research Lab and Merck. The reaction was carried out by conventional method. Melting points were determined in open capillaries using melting point apparatus and were uncorrected. The purity of synthesized compounds ascertained by TLC using silica gel-G plate as a stationary phase and iodine vapours as a visualising agent. The structure of synthesized compound was confirmed by IR, NMR and Mass spectral analysis. The IR spectra were recorded on JASCO FTIR-4100, NMR spectra recorded on BRUKER AVANCE II 400 spectrometer using $CDCl_3$ and DMSO as solvents with TMS as internal standard and Mass spectra were recorded on 1200 L Varian LC/MS instruments.

Table 2: List of equipment

Sr.no	Instrument	Specification	Application
1	Digital weighing balance	BL-220H Shimadzu corporation, Japan	Weighing
2	Hot plate	Round, 10inch dia	Heating
3	Hot air oven	DOLPHIN TH(lab oven)	Drying
4	Magnetic stirrer	REMI (1MHL)	Stirring
5	Vacuum pump	(induction motor) 200/220v,2AMP	Filtration
6	FTIR spectrophotometer	JASCO FTIR-4100	Spectral analysis
7	¹ HNMR Spectrophotometer	BRUKER AVANCE-400	Spectral analysis
8	Mass spectrometer	MACRO MASS	Spectral analysis

Table 3- Raw material characterisation

Sr.no	Compound	Mol. formula./mol. weight (gm/mol)	m.p/b.p (°C)	State/ Appearance	Solubility
1	Nicotinic acid	C ₅ H ₄ NO ₂ 123.11	237	White	Water.
2	Tri ethyl amine	C ₆ H ₁₅ N 101.1	89	Colourless liquid	Water miscible in Ethanol, acetone
3	Potassium carbonate	K ₂ CO ₃ 138	891	Granular powder	Miscible with water
4	Acetic acid	C ₂ H ₄ O ₂ 60.052	118	Colourless liquid	miscible in water
5	Benzoic acid	C ₆ H ₆ COOH 122.12	122	Colourless solid	Slightly soluble in water, acetone alcohol
6	Malono nitrile	C ₃ H ₂ N ₂ 66.06	20	White crystalline solid	Water

7	Dimethyl formamide	C ₃ H ₇ NO 73.09	153	Colourless liquid	Miscible with Water
8	Phenyl hydrazine	C ₆ H ₈ N ₂ 108.14	19.8	Pale yellow oily liquid	Ether, chloroform, benzene
9	Picolinic acid	C ₆ H ₅ NO ₂ 123.11	139	White solid	Glacial acetic acid, water, ethanol
10	HATU	C ₁₀ H ₁₅ F ₆ N ₆ OP 380.235	183	White crystalline solid	DMSO
11	p-anisidine	C ₇ H ₉ NO 123	56-59	White crystals	Sparingly soluble in water, soluble in ethanol, acetone, benzene.

❖ EXPERIMENTAL WORK:

❖ Synthesis of following compounds

1. *N*-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) benzamide (**5a**)
2. 2-(4-((4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) carbamoyl) benzamido) acetic acid (**5b**)
3. 5-chloro-*N*-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl)2-methylbenzamide (**5c**)
4. *N*-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) isonicotinamide (**5d**)
5. *N*-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl)picolinamide (**5e**)
6. *N*-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) acetamide (**5f**)

SYNTHESIS**4-amino-5-((4-methoxyphenyl) amino)-1-phenyl-2,3-dihydro-1H-pyrazole-3-carbonitrile**

The desired target compounds were synthesized by reacting phenyl hydrazine (1) with 2-Bis(methylthio)methylene malononitrile (2) independently. The reaction was carried out in dimethylformamide in presence of anhydrous K_2CO_3 for 6.30 hours. The reaction mixture was poured in ice cold water, and product obtained was filtered and was with water recrystallise from rectified spirit.

Molecular Formula- $C_{17}H_{17}N_5S O$ **Molecular Weight-** 293**Melting point-** 86-90°C**Yield-** 0.039g (60.73%)**Mobile phase-** Benzene: Methanol (1.5:0.5)**Rf value-** 0.7**Synthesis of N-(4-cyno-5-((4-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) benzamide (5a)**

A mixture of benzoic acid (0.0005mole) and HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) (0.0005mole) in Dimethyl Formamide (DMF) 3 ml was stirred for 15 minutes at room temperature in conical flask. To this mixture 4-amino-5-((4-methoxyphenyl) amino)-1-phenyl-2,3-dihydro-1H-pyrazole-3-carbonitrile (0.0005mole), DMF (1ml) and triethyl amine (TEA) (0.001mole) was poured slowly and again stirred at room temperature for 30 minutes. After stirring is completed, the mixture was poured in ice cold water to obtained precipitate. The reaction mixture was filtered and the solvent was removed under vacuum the product was collected and dried in air.

Molecular Formula- $C_{24}H_{19}N_5O_2$ **Molecular Weight-** 409**Melting point-** 123-127°C**Yield-** 0.098g (percent)**Mobile phase-** Toluene: Methanol (1.5:0.5)**Rf value-** 0.4**Synthesis 2-(4-((4-cyno-5-((4-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) carbamoyl) benzamido) acetic acid (5b)**

A mixture of 2- benzamidoacetic acid (0.0005mole) and HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) (0.0005mole) in Dimethyl Formamide (DMF) 3 ml was stirred for 15 minutes at room temperature in conical flask. To this, mixture 4-amino-5-((4-methoxyphenyl) amino)-1-phenyl-2,3-dihydro-1H-pyrazole-3-carbonitrile(0.0005mole), DMF (1ml) and ethyl Di- isopropyl amine (0.001mole) was poured slowly and again stirred at room temperature for 30 minutes. After stirring is completed, the mixture was

poured in ice cold water to obtained precipitate. The reaction mixture was filtered and the solvent was removed under vacuum. The product was collected and dried in air.

Molecular Formula- $C_{27}H_{22}N_6 O_5$

Molecular Weight- 510s.

Melting point- 143-145°C

Yield- 0.046g (92%)

Mobile phase- Toluene: Methanol (1.4:0.3)

Rf value- 0.6

Synthesis 5-chloro-N-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl)2-methylbenzamide (5c)

A mixture of 5-chloro-2-methyl benzoic acid (0.0005mole) and HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) (0.0005mole) in Dimethyl Formamide (DMF) 3 ml was stirred for 15 minutes at room temperature in conical flask. To this, a mixture 4-amino-5-((4-methoxyphenyl) amino)-1-phenyl-2,3-dihydro-1H-pyrazole-3-carbonitrile (0.0005mole), DMF (1ml) and ethyl Di- isopropyl amine (0.001mole) was poured slowly and again stirred at room temperature for 30 minutes. After stirring is completed, the mixture was poured in ice cold water to obtained precipitate. The reaction mixture was filtered and the solvent was removed under vacuum. The product was collected and dried in air.

Molecular Formula- $C_{25}H_{20}ClN_5O_2$

Molecular Weight- 457.

Melting point- 120-122°C

Yield- 0.108g

Mobile phase- Toluene: Methanol (1.4:0.3)

Rf value- 0.4

Synthesis N-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) isonicotinamide(5d)

A mixture of nicotinic acid (0.0005mole) and HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) (0.0005mole) in Dimethyl Formamide (DMF) 3 ml was stirred for 15 minutes at room temperature in conical flask. To this, a mixture 4-amino-5-((4-methoxyphenyl) amino)-1-phenyl-2,3-dihydro-1H-pyrazole-3-carbonitrile (0.0005mole), DMF (1ml) and ethyl Di- isopropyl amine (0.001mole) was poured slowly and again stirred at room temperature for 30 minutes. After stirring is completed, the mixture was poured in ice cold water to obtained precipitate. The reaction mixture was filtered and the solvent was removed using vacuum. The product was collected and dried in air.

Molecular Formula- $C_{23}H_{18}N_6 O_2$

Molecular Weight- 410.43

Melting point- 139-142°C

Yield- 0.022g

Mobile phase- Toluene: Methanol (1.4:0.3)

Rf value- 0.4

Synthesis N-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) picolinamide(5e)

A mixture of picolinic acid (0.0005mole) and HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) (0.0005mole) in Dimethyl Formamide (DMF) 3 ml was stirred for 15 minutes at room temperature in conical flask. To this, a mixture 4-amino-5-((4-methoxyphenyl) amino)-1-phenyl-2,3-dihydro-1H-pyrazole-3-carbonitrile(0.0005mole), DMF (1ml) and ethyl Di- isopropyl amine (0.001mole) was poured slowly and again stirred at room temperature for 30 minutes. After stirring is completed, the mixture was poured in ice cold water to obtained precipitate. The reaction mixture was filtered and the solvent was removed under vacuum. The product was collected and dried in air.

Molecular Formula- $C_{23}H_{18}N_6O_2$

Molecular Weight- 457.

Melting point- 144-146°C

Yield- 0.043g

Mobile phase-Toluene: Methanol (1.4:0.3)

Rf value- 0.4

Synthesis *N*-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1Hpyrazol-3-yl) acetamide (5f)

A mixture of acetic acid (0.0005mole) and HATU (Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium) (0.0005mole) in Dimethyl Formamide (DMF) 3 ml was stirred for 15 minutes at room temperature in conical flask. To this, a mixture 4-amino-5-((4-methoxyphenyl)samino)-1-phenyl-2,3-dihydro-1H-pyrazole-3-carbonitrile(0.0005mole), DMF (1ml) and ethyl Di- isopropyl amine (0.001mole) was poured slowly and again stirred at room temperature for 30 minutes. After stirring is completed, the mixture was poured in ice cold water to obtained participate. The reaction mixture was filtered and the solvent was removed under vacuum. The product was collected and dried in air.

Molecular Formula- $C_{19}H_{17}N_5O_2$

Molecular Weight- 417.

Melting point- 150-15°C

Yield- 0.031g

Mobile phase-Toluene: Methanol (1.4:0.3)

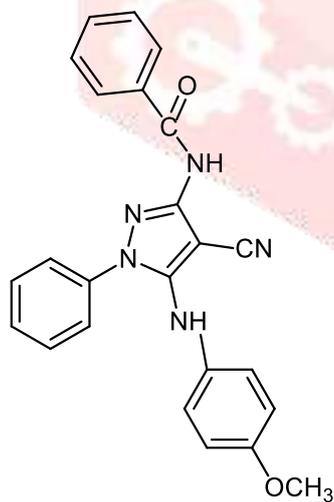
Rf value- 0.6

RESULT AND DISCUSSION:

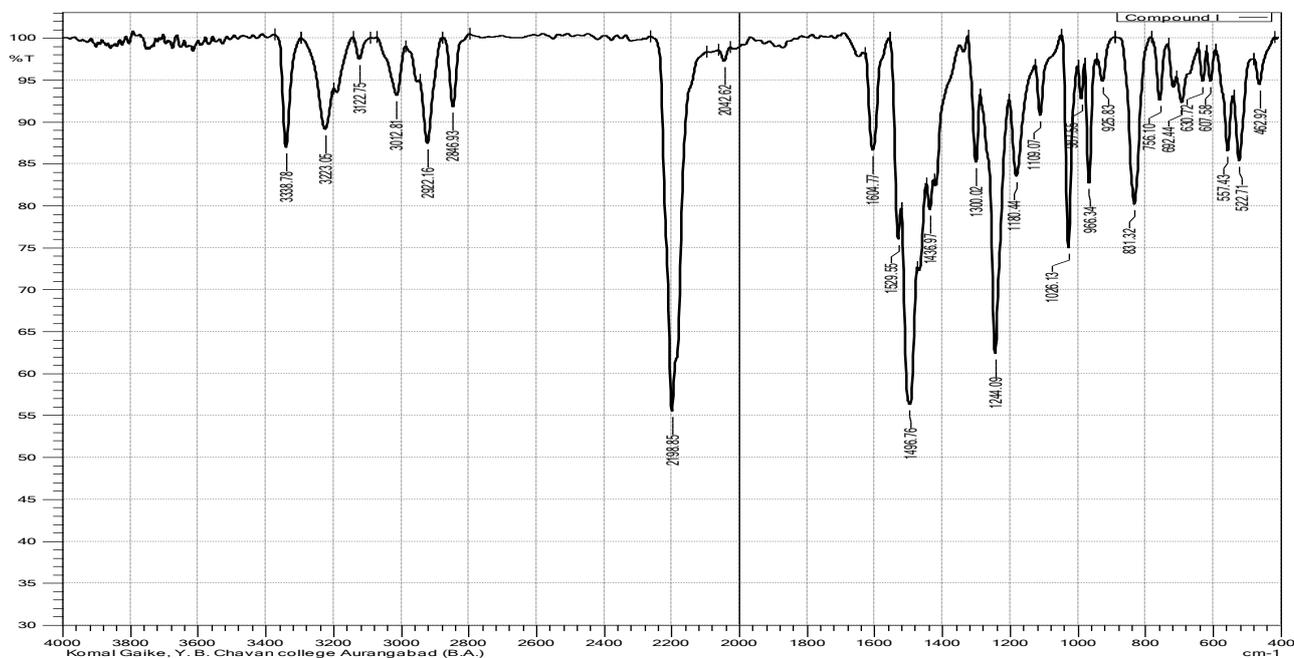
- ❖ The reaction will be carried out in the laboratory. Required chemicals are available in laboratory and the chemicals such as carbon disulphide, DMF, DMSO, ethyl cyano acetate, phenyl hydrazine, TEA, Chloro methyl benzoic acid, aromatic amines, sodium nitrite, ethanol, phenyl isocyanate, acetone.
- ❖ Glassware's such as RBF, condenser, Petri plate, measuring cylinder, beaker, and other common glassware's.
- ❖ Instruments / machines such as Autoclave, Deep Freezer, Hot air oven, Heating mantle, stirrer, UV cabinet, IR spectrophotometer, NMR spectrophotometer, Mass spectrophotometer, incubator.
- ❖ The above resources are available at college except NMR and Mass spectrophotometer.

The spectra of compound are given below with interpretation.

The compound were synthesized and found to be pure. Their melting points were taken and will be sent for analysis followed by activity. The following work will be under taken: -Characterization of this compound by NMR, Mass, IR and biological activity –invitro cell line screening on MCF-7 (human breast cancer cell line), HeLa (human cervical cancer cell line), SKMEL-2 (human melanoma cancer cell line) and HL-60 (human leukemia cancer cell line). will be carried out after synthesized and Characterization of compound.⁽⁴²⁻⁴³⁾

IR spectrum 1: N-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) benzamide (5a)

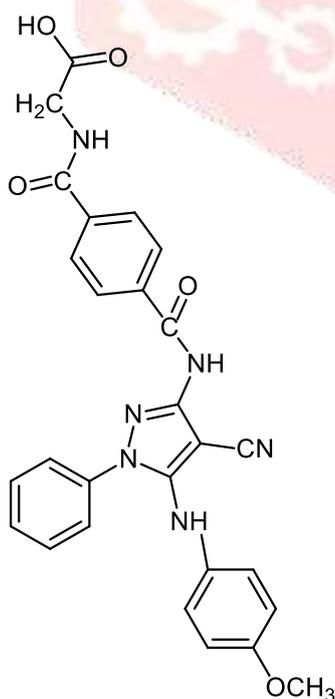
(5a)



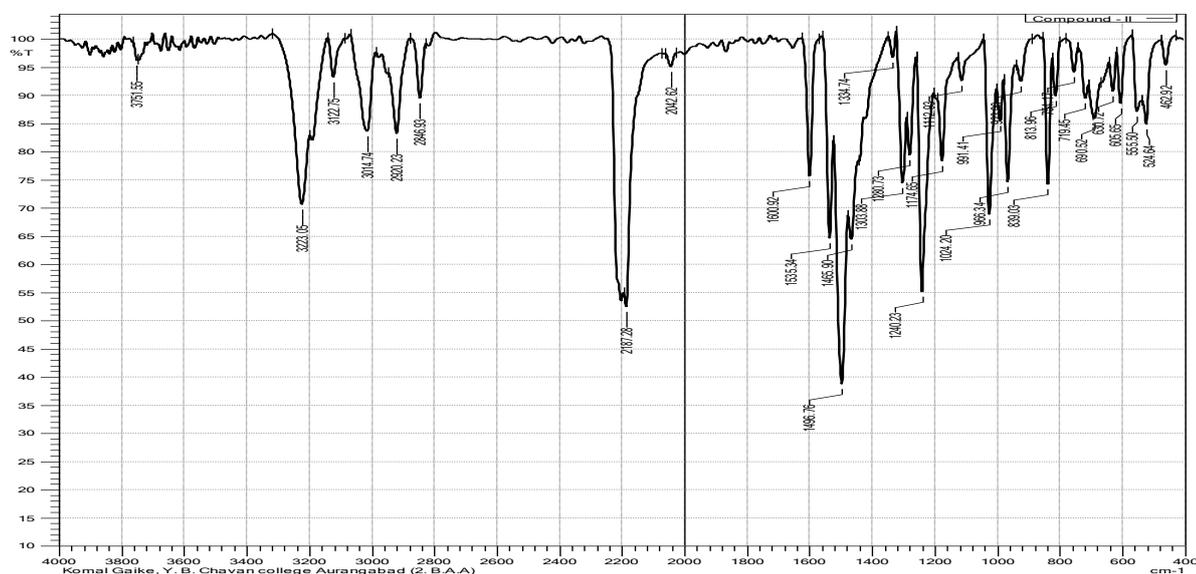
INTERPRETATION OF SPECTRUM OF COMPOUND (5a)

Sr. no.	Functional group	Wave no. (cm ⁻¹)
1	N-H stretching primary	3336.85
2	Aromatic C-H stretching	2922.16
3	C=O stretching	1651.07
4	CONH stretching amide	1692.92
5	Sec amine NH	3223.05
6	C-N stretching	2198.85

IR spectrum 2: 2-(4-((4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) carbamoyl) benzamido) acetic acid (5b)



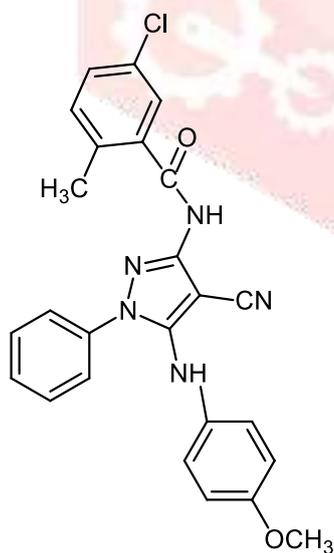
(5b)



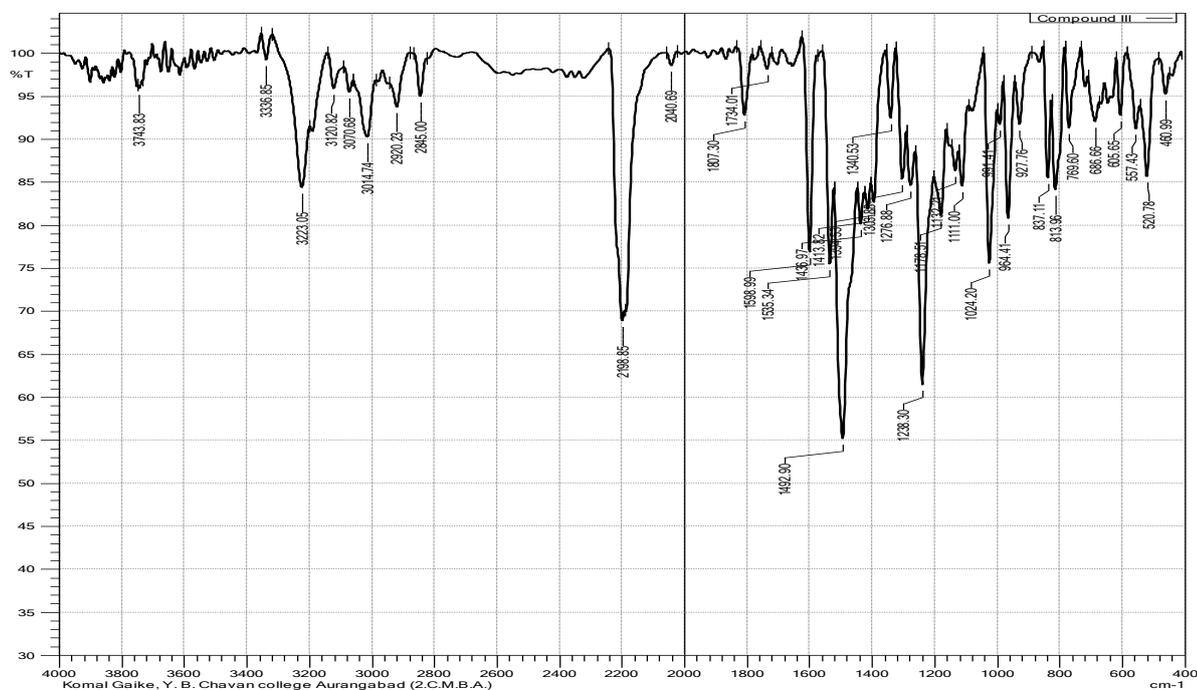
INTERPRETATION OF SPECTRUM OF COMPOUND (5b)

Sr. no.	Functional group	Wave no. (cm ⁻¹)
1	N-H stretching primary	3751.55
2	Aromatic C-H stretching	2920.23
3	COO stretching	1496.76
4	CONH stretching amide	1651.07
5	Sec amine NH	3223.05
6	C-N stretching	2187.28
7	C=O amide	1600.92

IR spectrum 3: 5-chloro-N-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl)2-methylbenzamide (5c)



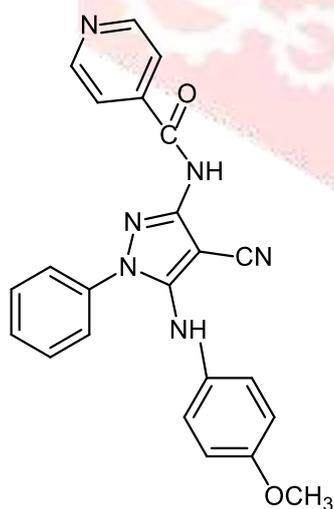
(5c)



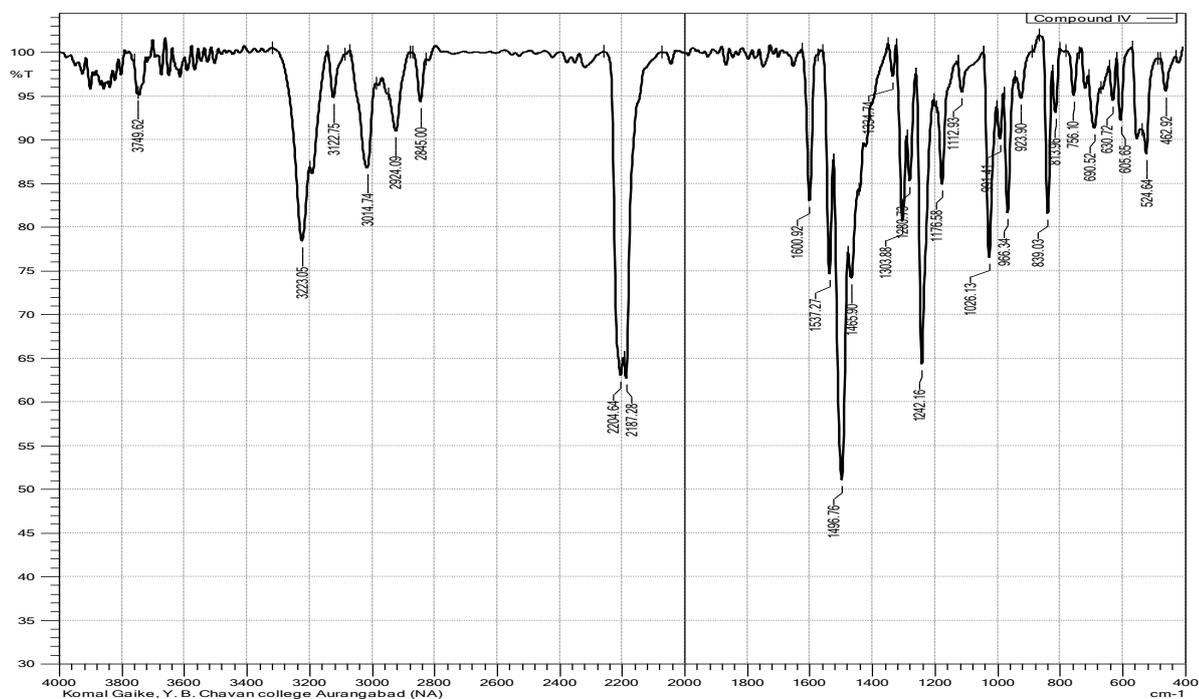
INTERPRETATION OF SPECTRUM OF COMPOUND (5c)

Sr. no.	Functional group	Wave no. (cm ⁻¹)
1	N-H stretching primary	3223.05
2	Aromatic C-H stretching	2920.23
3	C=O stretching	1734.07
4	CONH stretching amide	1598.99
5	Sec amine NH	3336.85
6	C-N stretching	2198.85

IR spectrum 4: N-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) isonicotinamide(5d)



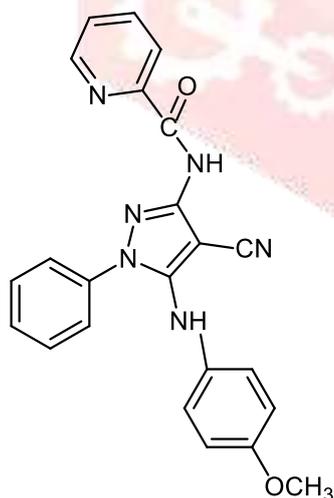
(5d)



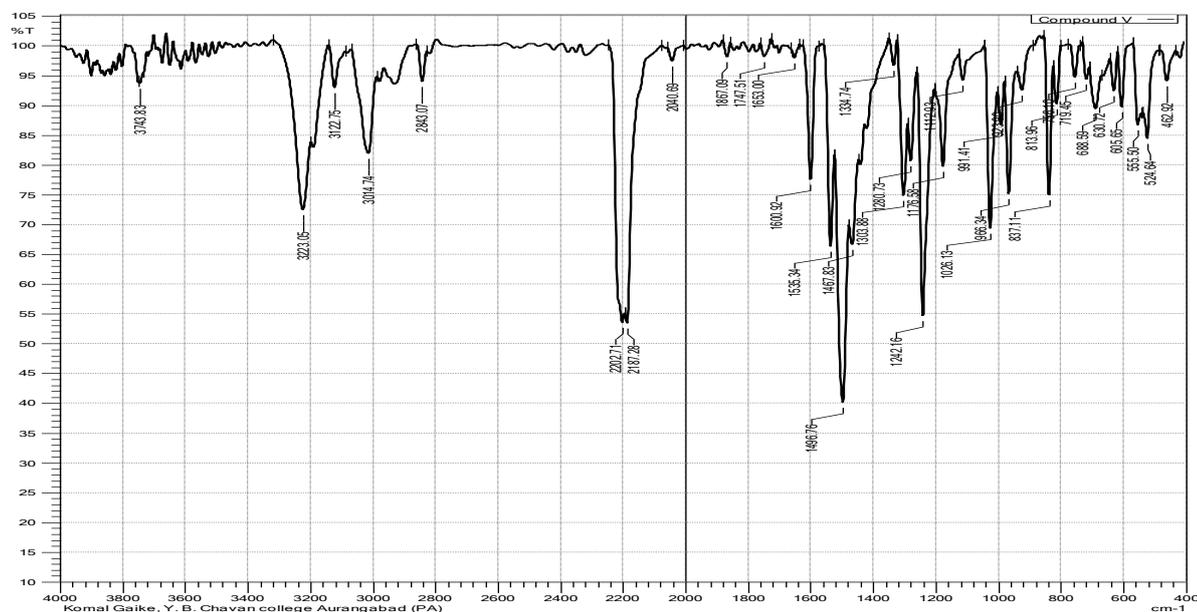
INTERPRETATION OF SPECTRUM OF COMPOUND (5d)

Sr. no.	Functional group	Wave no. (cm ⁻¹)
1	N-H stretching primary	3223.05
2	Aromatic C-H stretching	3014.74
3	C=O stretching	1537.27
4	CONH stretching amide	1600.92
5	Sec amine NH	3223.05
6	C-N stretching	2204.64

IR spectrum 5: N-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) picolinamide(5e)



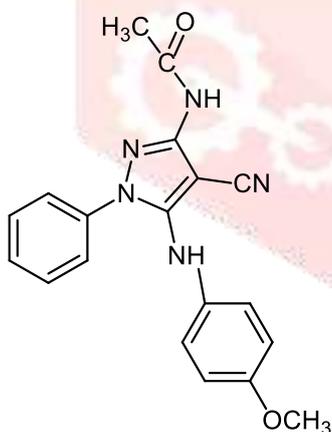
(5e)



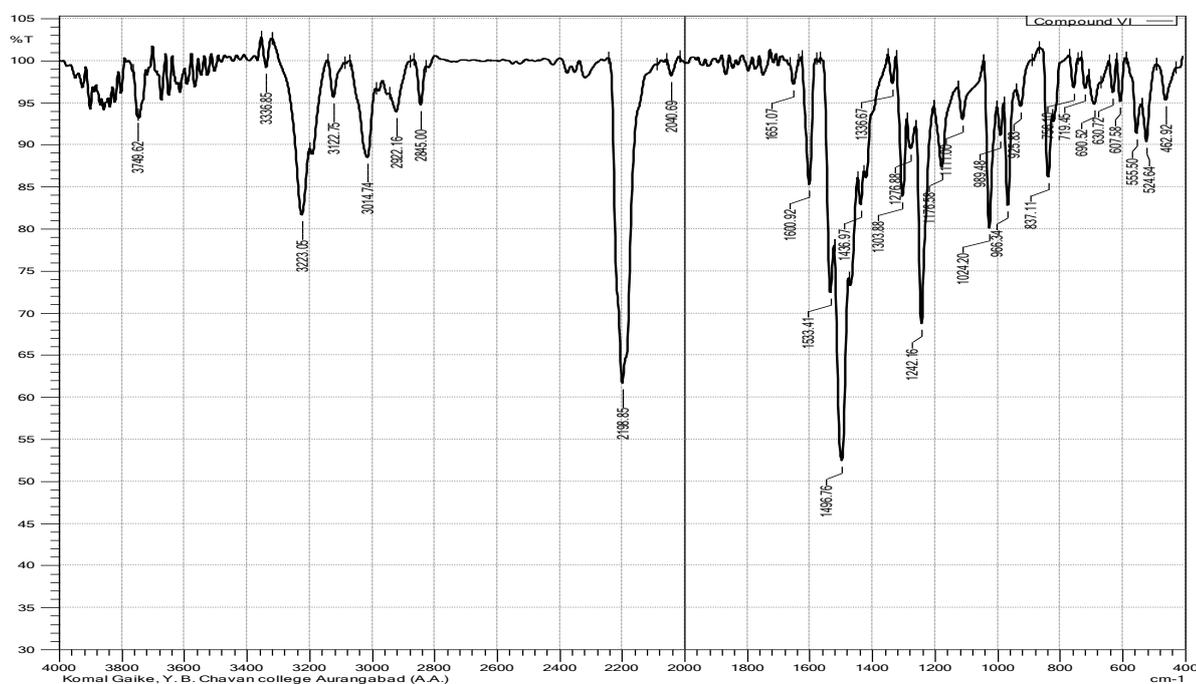
INTERPRETATION OF SPECTRUM OF COMPOUND (5e)

Sr. no.	Functional group	Wave no. (cm ⁻¹)
1	N-H stretching primary	3774.83
2	Aromatic C-H stretching	3022.14
3	C=O stretching	1631.00
4	CONH stretching amide	1600.92
5	Sec amine NH	3223.05
6	C-N stretching	2202.71

IR spectrum 6: *N*-(4-cyno-5-((-methoxyphenyl) amino)-1-phenyl-1Hpyrazol-3-yl) acetamide (5f)



(5f)



INTERPRETATION OF SPECTRUM OF COMPOUND (5f)

Sr. no.	Functional group	Wave no. (cm ⁻¹)
1	N-H stretching primary	3336.85
2	Aromatic C-H stretching	2922.16
3	C=O stretching	1651.07
4	CONH stretching amide	1692.92
5	Sec amine NH	3223.05
6	C-N stretching	2198.85

LIGAND RECEPTOR INTERACTION (DOCKING STUDY)

Molecular docking studies were performed using Glidev5.6 (Schrodinger, LLC). The coordinate for enzyme was taken from RCSB Protein Data Bank (3-poz) and prepared for docking using protein preparation wizard. Water molecules in the enzyme structure were removed. The bond orders and formal charges were added for hetero groups and the hydrogens were added to all atoms. The side chains that were not close to the binding cavity and not participate in salt bridges, were neutralized. After preparation, the structure was refined to optimize the hydrogen bond network using OPLS_2005 force field. This helps in reorientation of structure. The minimization was terminated when the energy converged or the RMSD reached a maximum cutoff of 0.30 Å. Grid was then defined around active site of enzyme by centering on ligand using default box size. The extra precision (XP) docking mode of Glide was used for docking of compounds, optimized by Lig prep, on generated grid of protein structure⁽⁴⁴⁾

In the present study six amide derivatives containing substituted pyrazole moiety (**5a-5f**) were taken for docking study. The interaction between the ligand and protein are shown in the molecular interactions images and docking score are represented in table below.

To rationalize most relevant SAR and to predict the binding affinity of synthesized compounds, the docking studies were carried out on X-ray coordinates of enzyme (PDB-3-poz) The accuracy of a docking procedure

was first evaluated by comparing binding conformation of co-crystallized inhibitor predicted by extraprecision (XP) Glide docking mode and experimental binding mode as determined by X-ray crystallography. The root means square deviations of 0.3 Å were found for rigid docking, which suggests the reliability of docking procedure. Docking score (G-score) of all compounds against Protein kinase inhibitor is shown in Table 4. The most of compounds showed good correlation of virtual docking score. All docked compounds adopted a similar conformation and position in the active binding site of enzyme. For most of compounds, the ligand-enzyme complex was primarily stabilized by hydrophobic interactions and specific polar hydrogen bonds. The side chain, amide group, nitrogen of Pyrazole were involved in the binding with amino acid Lys 745, Asn 842, Ala 722, Asp 855 of target enzyme. This indicates the importance of amide group in binding with enzyme. The docking score were found satisfactory ranging from -6.55 to -8.42 (Table 4)

Table 4: Docking score and Glide emodel of compound (5a-5f)

Compound no	Docking score	Glide emodel
5a	-7.899	-73.672
5b	-6.55	-82.371
5c	-7.977	-81.74
5d	-7.88	-82.017
5e	-7.658	-81.319
5f	-8.428	-73.672

Image1: Molecular interactions of compound (5a) with active site of target

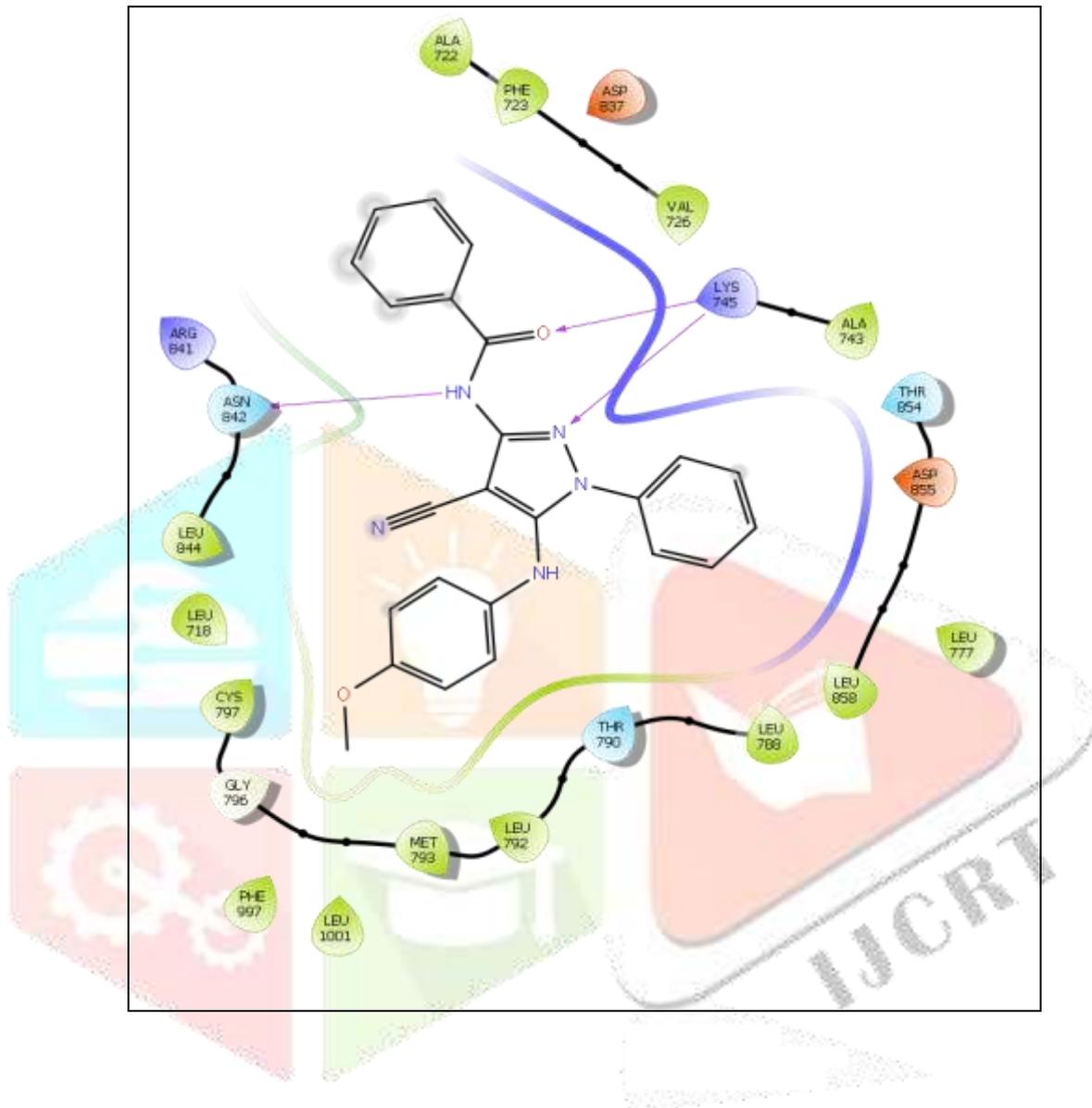


Image2: Molecular interactions of compound (5b) with active site of target

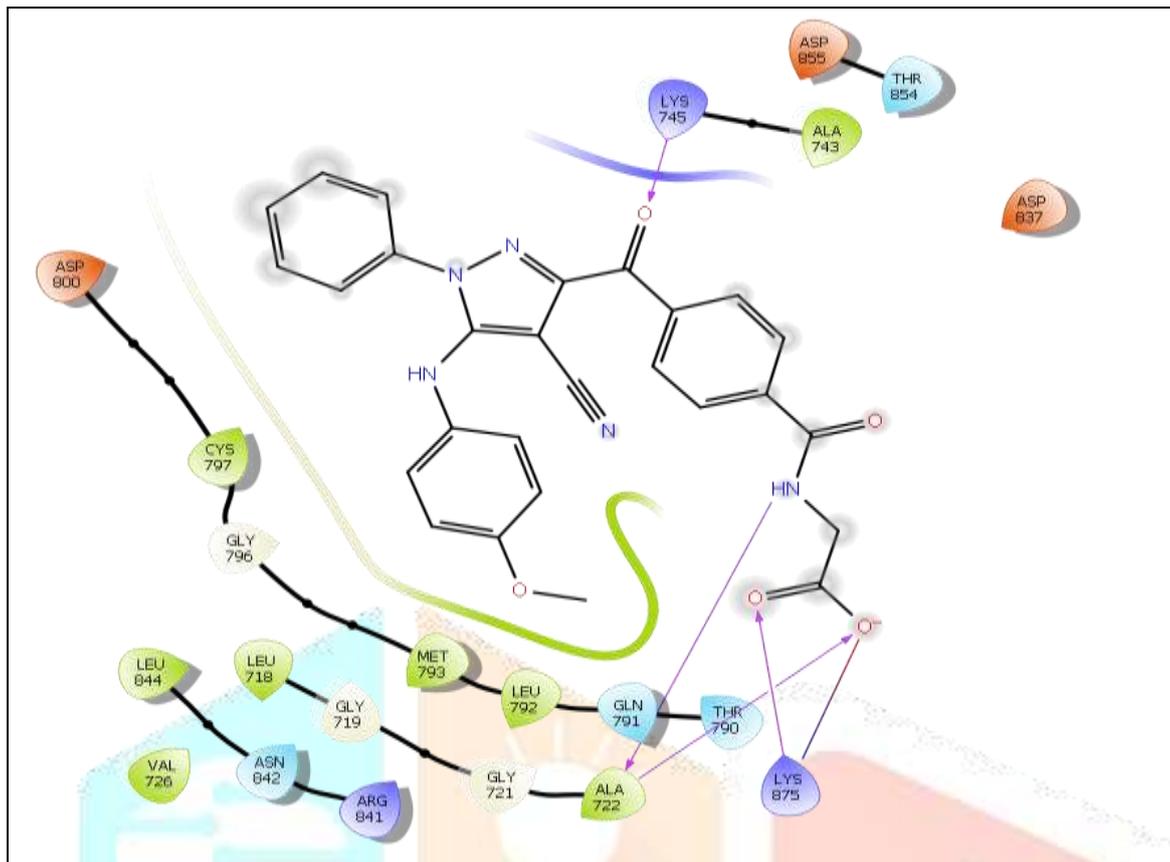


Image3: Molecular interactions of compound (5c) with active site of target

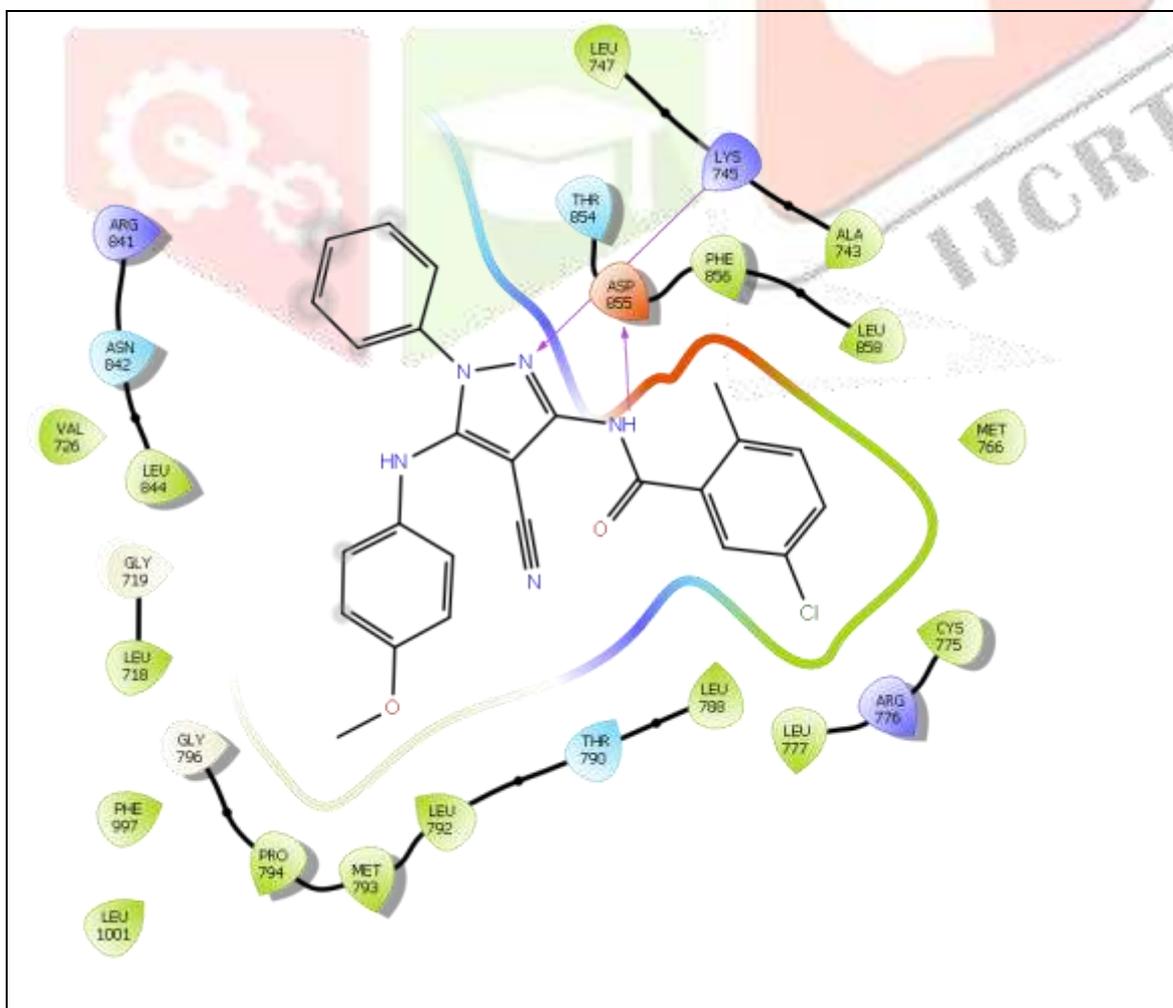
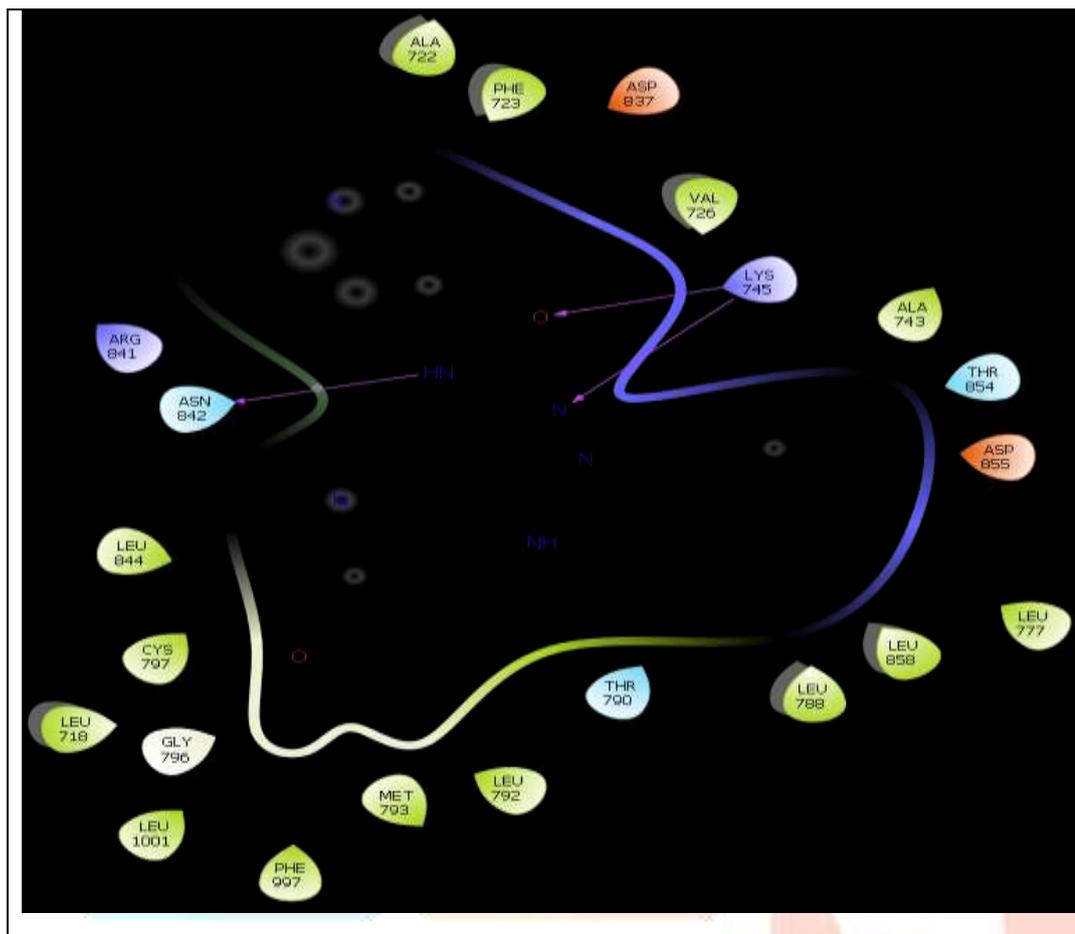
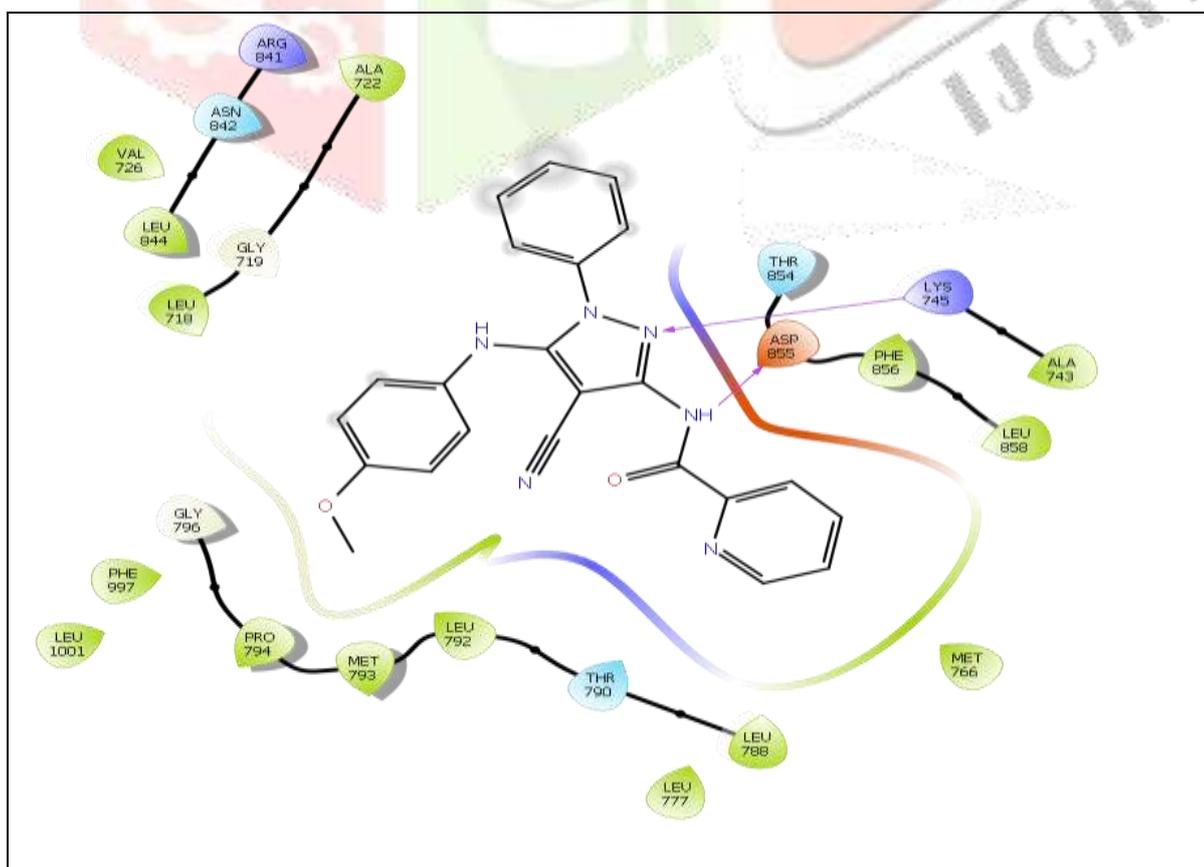
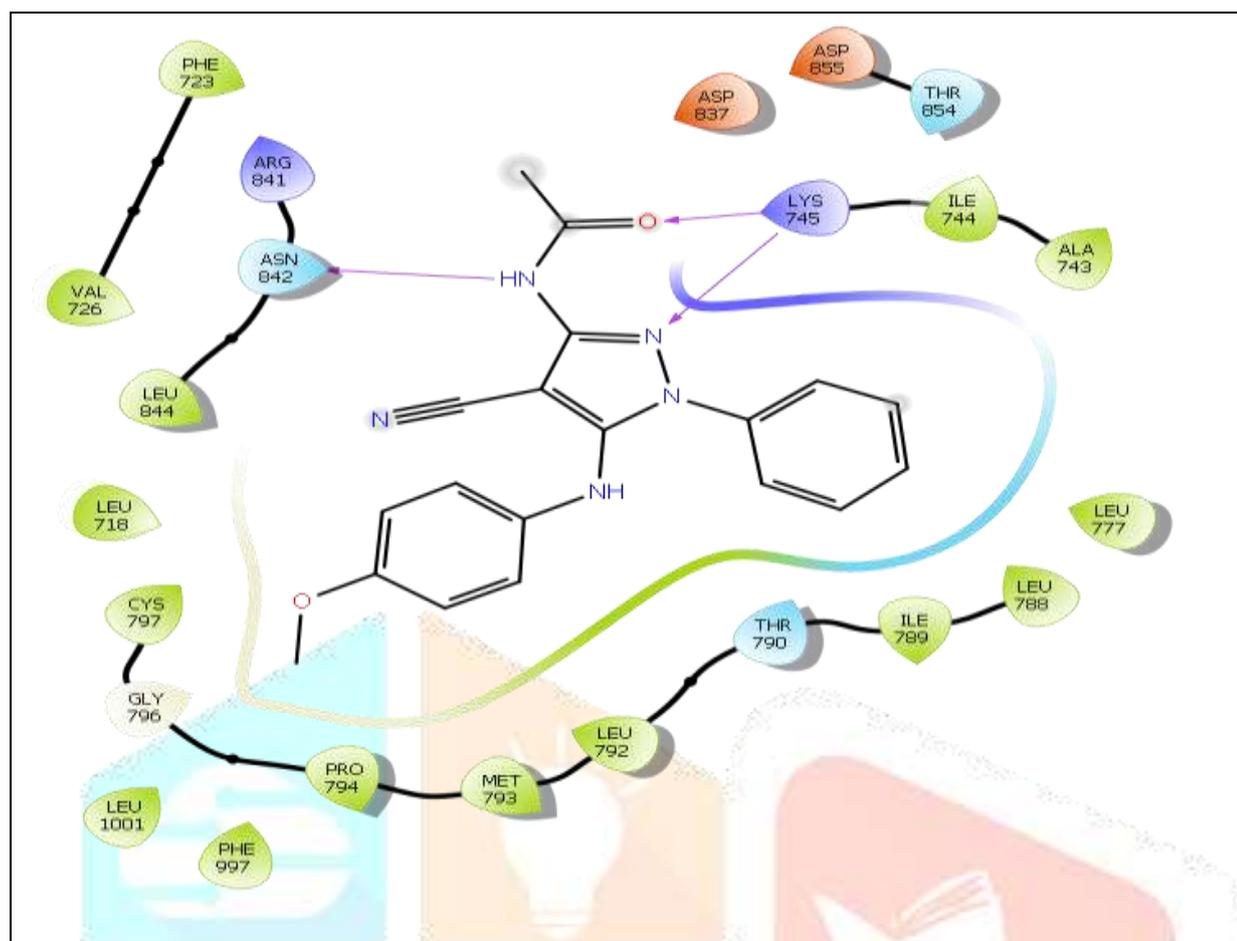


Image 4: Molecular interactions of compound (5d) with active site of target**Image5: Molecular interactions of compound (5e) with active site of target**

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Image6: Molecular interactions of compound (5f) with active site of target



ADME PROPERTIES DETERMINATION:

New chemical entities fail to reach the clinical stage because of their unfavorable absorption, distribution, metabolism, and elimination (ADME) parameters. Therefore, a computational study of synthesized compounds **5a** to **5f** was performed for assessment of ADME properties and value obtained is depicted in Table 4. Polar surface area (TPSA), and Lipinski's rule of five were recalculated using <http://www.SwissADME.ch/index.php> and Molinspiration online property calculation tool kit. From all these parameters, it can be observed that all the synthesized compounds exhibited excellent properties. Furthermore, these compounds had not violated Lipinski's rule of five and thus showing possible utility for developing the compound with good drug like properties. These molecules likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: logP (octanol–water partition coefficient) less than 5, molecular weight less than 500, number of hydrogen bond acceptors less than 10 and number of hydrogen bond donors less than 5. All the synthesized compounds followed the criteria for orally active drug and therefore, these compounds can be further developed as oral drug candidates. The results of this *in silico* ADME prediction analysis suggest that the synthesized compounds follow the computational assessment for good candidate.

Table no 5: In-silico drug like (physicochemical) properties of synthesized compound (5a-5f)

Comd. no.	MW	HBD	HBA	TPSA	Logp	WSOL	Lipinski violation
1	409.44	2	4	91.97	3.68	PS	0
2	510.50	4	7	158.37	2.72	MS	MW>500, NorO>10
3	457.91	2	4	91.97	4.53	PS	0
4	410.43	2	5	104.86	2.94	MS	0
5	410.43	2	5	104.86	2.98	MS	0
6	347.37		4	91.97	2.55	MS	0

Table no6: In-silico drug like (physicochemical) properties of synthesized compound (5a-5f)

Comd. no.	Pharmacokinetics (GI absorption and CYT inhibition)					
	GIA	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
1	High	Yes	Yes	Yes	Yes	Yes
2	Low	No	Yes	Yes	No	Yes
3	High	No	Yes	Yes	Yes	Yes
4	High	Yes	Yes	Yes	Yes	Yes
5	High	Yes	Yes	Yes	Yes	Yes
6	High	Yes	No	Yes	Yes	Yes

Abbreviation: MW molecular weight, HBA hydrogen bond acceptors, HBD hydrogen bond donor, TPSA Total polar surface area, Log (log of partition coefficient of compound between n-octanol and water), WSOL Water solubility, MS moderately soluble, GIA-GI absorption, CYT-Cytochrome p450 enzyme

SUMMARY

The dissertation entitled synthesis analysis and docking study of substituted pyrazole derivatives containing amide linkages for anticancer activity can be summarised as mentioned below.

1. We have designed and synthesized the new series of Six derivatives of N-(4-cyano-5-(4-methoxyphenyl) amino)-1-phenyl-1H-pyrazol-3-yl) benzamide by conventional methods and purity was checked by TLC.
2. All the synthesized compounds were characterised by spectral analysis like IR, NMR, spectroscopy and all the structural features are in agreement with spectral report.
3. Derivatives were synthesised for anti-cancer activity and their docking studies carried in Schrödinger software. The docking results are encouraging. This indicated that these compounds should be undertaken for further studies in future.
4. *In silico* ADME prediction of synthesized compound indicated that compounds had potential to develop a good oral drug candidate. These findings provide important information for the exploration of compounds **5ato 5f** as good candidate for further modification.

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