ANTICANCER POTENTIAL OF NEW N-ACETYL PYRAZOLINE DERIVATIVES OF 1, 3-DIARYL/HETEROARYL PROPENONES: SYNTHESIS AND EVALUATION

Thesis submitted to the Central University of Punjab

For the award of

Master of Pharmacy

In

Medicinal Chemistry

BY

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October, 2013

CERTIFICATE

I declare that the thesis entitled "Anticancer Potential of New N-Acetyl Pyrazoline Derivatives of 1, 3-Diaryl/HeteroarylPropenones: Synthesis and Evaluation" has been prepared by me under the guidance of Dr. Raj Kumar, Assistant Professor, Centre for Chemical and Pharmaceutical Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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Acknowledgement

First and foremost I thank my God Almighty for His grace and mercy towards me and helping me at every stage of my work, giving me the strength, the wisdom and the will to complete my work diligently .

It gives me immense pleasure to express my sincere and whole hearted gratitude to my supervisor **Dr. Raj Kumar,** Assistant Professor,Centre for Chemical and Pharmaceutical Sciences, School of Basic and Applied Sciences,Central University of Punjab,Bathinda for his motivation, illuminative guidance and sincere advices throughout the course of this project. His guidance helped me in all the time.

I am grateful beyond words to **Dr. Sandeep Singh**, Assistant Professor, Central University of Punjab,Bathinda for lending his expertise and finesse for the biological work performed in the Tissue Culture Laboratory. I also appreciate his fervor with which he had helped me carry out all the experiments.

I know it sounds like a cliché' when I say that without **Dr. Raj Kumar** and **Dr.Sandeep Singh** my research work would have been incomplete but that's the truth. During the period of last one year I have asked numerous irritating, silly and may be foolish questions but they always had the patience to give the answers to them.

I feel indebted and convey my regards to Dean Academic Affairs/ COC, **Professor P. Rama Rao**, Central University of Punjab,Bathinda for the laboratory facilities, consumables and congenial working environment required for conducting the study.

I also extend my thanks to all the faculty members,**Dr. VikasJaitak, Dr. Vinod Kumar, Dr. Harish Holla** for their kind cooperation and support whenever required.

At this juncture, I would also like to thank **Mr. Pushpendera Singh**, Ph.D Scholar in Biocsiences for helping me with the biological work. He took out time in between his classes to wind my work up and make me familiar with the basics of biology.I would also like to thank **Ms**. **NandiniGautam** and **Ms. Gurpreet Kaur**, Ph.D Scholars, Environmental Science & Technology for helping me in my work.

I want to especially thank **Mr.Darpan,** Research Associate, Centre for Chemical and Pharmaceutical Sciences, School of Basic and Applied Sciences,Central University of Punjab,Bathindafor helping me with the interpretation of the NMR spectra.

CERTIFICATE

I certify that JIMI MARIN ALEX has preparedher thesisentitled “Anticancer Potential of New N-acetyl Pyrazoline Derivatives of 1, 3-Diaryl/HeteroarylPropenones: Synthesis and Evaluation”, for the award of M.Pharm. (Medicinal Chemistry) degree of the CentralUniversity of Punjab, under my guidance. She has carried out this work at the Centre for Chemical and Pharmaceutical Sciences, School of Basic and Applied Sciences, Central University of Punjab.

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ABSTRACT

Anticancer Potential of New N-Acetyl Pyrazoline Derivatives of 1, 3-Diaryl/HeteroarylPropenones: Synthesis and Evaluation

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School of Studies	:	Basic and Applied Sciences
Key words	:	1, 3-Diaryl propenones, pyrazolines, MTT assay, anticancer potential, antioxidant activity.

Pyrazoles, categorized as nitrogen-containing heterocycles, are well known for their interminable participation in the field of perpetual research and development of therapeutical active agents. As a consequence pyrazoles became an inevitable core of numerous drugs having diverse activities. The broad spectrum of activities portrayed by the pyrazoles instigated the researchers to modify the pyrazole ring as 4,5-dihydro-1H-pyrazoles commonly known as 2-pyrazolines. This modification played a determining role in defining the biological activities of several compounds. The presence of aromatic/heterocyclic substituents on the pyrazoline ring only served to accentuate these activities. Literature survey also revealed that substitution such as amide group, acetyl groupetc.at N1 of the pyrazoline also played a decisive role in deciding the biological activity. The vast information obtained from literature survey stimulated us to synthesize compounds having 2-pyrazoline as the core moiety of which either the C3 or C5 was substituted with heterocyclic ring in addition to acetyl moiety at the N1 of the pyrazoline. The compounds were assessed for their anticancer potential against four cancer cell- MCF-7, H-460, T-47 D and A-549. MTT assay was carried out for testing the cell viability. The assay results revealed that certain compounds showed anticancer potential because these agents inhibited the proliferation of breast cancer cell lines but not against lung cancer cell line. Compounds showing good activity against the cancer cell lines were also evaluated for their antioxidant property especially against reactive oxygen species.

(Jimi Marin Alex) (Dr. Raj Kumar)

I would also like to thank the members of the non-teaching staff, especially **Mr. Rajesh Tiwari, Mr. Gopal,** and **Mr. Ashwini** for the invaluable help that they gave me in various experiments.

I thank my **Dad, Amma and younger brother** for their support through all this and for bearing up with me especially on the frustrated days when I used to vent my anger at them and also for understanding me. Above all, I want to thank them for upholding me in their everyday prayers as well as many other people who regularly keep me in their prayers.

Last but not at all the least I want thank my friends (who used stay up late writing their thesis) for the enjoyable time we had through those long nights. It was a great time that we spend in A.C. reading room. Without some of these people thesis writing would have been a trying process. Thanks guys for the wonderful moments filled with laughter and jokes!!!!

Jimi Marin Alex

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LIST OF ABBREVIATIONS

Sr. No.	Full Form	Abbreviations
1	Deoxyribonucleic acid	DNA
2	Epidermal growth factor receptor	EGFR
3	Vascular endothelial growth factor	VEGF
4	Nuclear Factor-κB	NF- κB
5	Mammalian target of rapamycin	mTOR
6	Phosphatidylinositol 3-kinases	PI3-K
7	Heat shock Protien-90	HSP-90
8	v-raf murine sarcoma viral oncogene homolog B	BRAF
9	Adenosine triphosphate	ATP
10	Xanthine Oxidase	XO
11	Minimum Inhibitory Concentration	MIC
12	Nuclear Magnetic Resonance	NMR
13	Tetramethylsilane	TMS

14	Ultraviolet	UV
15	Fourier Transform Infrared	FT-IR
16	Melting Point	M.P.
17	Doublet	d
18	Singlet	s
19	Multiplet	m
20	Triplet	t
21	Doublet of doublet	dd
22	Coupling constant	J
23	Parts per million	Ppm
24	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide	MTT
25	Dimethysulfoxide	DMSO
26	Sodium dodecyl sulphate	SDS
27	Roswell Park Memorial Institute	RPMI
28	Dulbecco's Modified Eagle's medium	DMEM

29	Fetal Bovine Serum	FBS
30	Phosphate Buffer Saline	PBS
31	Micromolar	µM
32	Namometer	nm
33	Standard Deviation	S.D.
34	Dihydrodichlorofluroscien diacetate	H_2DCFDA
35	Reactive Oxygen Species	ROS
36	Red Blood Cells	RBC
37	Sodium Hydroxide	NaOH
38	Thin Layer Chromatography	TLC
39	Hertz	Hz
40	Infiltrating Ductal Carcinoma	IDC

CHAPTER I

INTRODUCTION

The past decades have witnessed the dawn of new diseases, understanding of the pathophysiology of these diseases, finding druggable targets and charting out effective therapeutic regimens for them. Although perpetual research has yielded answers to most of these problems, many of them still remain unresolved and difficult to answer. One such aggressive disease condition that warrants a desperate need to be cured is cancer. Cancer is the leading cause of mortality second only to the cardiovascular diseases (Simone, 1992) and according to WHO 2008 cancer is one of the four leading threats to human health and development. Cancer is not just a single disease but a group of disease (Cancer facts and figures, 2013; Patrick, 2009) characterized by inappropriately controlled cell replication gradually leading to disruption of normal physiology, metabolism or structure. These cancer cells lose the specialized characteristics that distinguish one cell from another (Gilman and Goodman, 2011). Numerous factors are held responsible such as diet and nutrition, chemicals, environment, radiations, occupation, age and hormones (Munoz-Pinedo et al., 2012; Simone, 1992). Epidemiological factors alone cannot be categorized as the cause of cancer development but cancer may also result as a consequence of genetic alterations. Cancer Facts & Figures 2013 estimated that 1,660,290 new cancer cases are expected to be diagnosed in 2013 (Cancer facts and figures, 2013). Of the 10 million new cancer cases seen each year worldwide, nearly 5.5 million cases are reported from the less developed countries. Cancer prevalence in India is estimated to be around 2.5 million with over 8, 00,000 new cases and 5,500,000 deaths occurring each year (I.A.R.C., 2012). Significant reduction in these statistics is yet to be seen despite the various treatment strategies inclusive of surgery, radiation, chemotherapy, hormonal therapy, biological therapy and targeted therapy that have been implemented (Hughes et al., 2004; Suzuki et al., 1995). Besides being costly, the anticancer drugs are known to be highly toxic and developing resistance rapidly (Piekarski and Jelinska, 2013). These limitations have maneuvered research towards the development of novel diagnostic, treatment and preventive approaches to combat cancer. This has encouraged us to synthesize compounds that may offer to be a panacea to the most life threatening disease of the recent times.

CHAPTER II

REVIEW OF LITERATURE

2.1 Anticancer Drugs

In response to the desperate concerns raised by the global cancer statistics efforts were made to synthesize compounds that would help combat cancer. An ideal anticancer agent has been aptly defined (Leighton, 1969) as " one which has selectivity for the cancer cells; simultaneously causing no harm to the normal cells ". Compounds designed to target cancer differ not only in their structures but also show myriad biological activities (Gilman and Goodman, 2011) (**Table 2.1; Figure 2.1**). These agents justified their designation of 'chemotherapeutic agents' by orchestrating events that would inhibit the development of invasive cancer by either blocking the DNA damage that triggers carcinogenesis or arresting/ reversing the progression of premalignant cells in the already affected cells (Hong and Sporn, 1997). Alkylating agents (**1-7**) led the way for strategizing the cancer chemotherapy initiated by the accidental spill of sulphur mustard which brought about tumor regression during World War I (DeVita and Chu, 2008). The property of the DNA alkylating agents to act as cytotoxic and antitumor compounds was attributed to their ability to bind to DNA covalently. Their place in drug market was short lived owing to a number of side effects arising primarily due to non-selectivity which resulted in bone marrow toxicity, mucosal toxicity and neurotoxicity. Moreover, it was observed that resistance developed rapidly with these drugs (Gilman and Goodman, 2011; Gottesman, 2002). These disadvantages provided the necessary impetus to the scientists to explore alternatives which resulted in the introduction of antimetabolites. The strategy employed with antimetabolites were to inhibit the synthesis of DNA precursor thereby inhibiting DNA replication (Folic Acid analogs, **8**) or by getting incorporated into DNA thus blocking its elongation and function (pyrimidine, **9** and purine, **10** analogs). Accompanied with disadvantages, this class of compounds also failed to maintain its presence for a long time instigating the researchers to turn their focus towards mitosis and the process it entails, especially microtubules. Microtubules are the structures credited for the formation of mitotic spindle which helps in transporting the daughter chromosomes to the separate poles of the dividing cell. This significant role played by the microtubules in cell division has made them a desirable target in cancer chemotherapy to develop potent anticancer compounds (**11-13**). Rapidly developing

resistance was the main drawback with this class of drugs as well (Perez, 2009). Apart from these, hormones were also seen to play an important part in the development of cancer. The introduction of hormonal therapy helped in preventing disease recurrence following surgical resection; especially of the localized breast, colorectal and lung cancer led to the introduction of the field of hormonal therapy (**14-18**) (Bonneterre et al., 2000; Gilman and Goodman, 2011; Troisi et al., 1995).

Table 2.1: Various Chemotherapeutic Agents

ALKYLATING AGENTS	1. Nitrogen Mustard	Mechlorethamine **(1)**, Cyclophosphamide, Melphalan
	2. Ethylamine and methylamines	Altretamine **(2)**
	3. Methylhydrazine Derivative	Procarbazine **(3)**
	4. Alkyl Sulphonate	Busulphan **(4)**
	5. Nitrosourea	Carmustine **(5)**, Lomustine
	6. Triazines	Dacarbazine **(6)**
	7. Platinum coordinat-ion complex	Cisplatin **(7),** Carboplatin
ANTIMETABOLITES	1. Folic acid analogue	Methotrexate **(8)**
	2. Pyrimidine analogue	5-Thiouracil, Cytarabine **(9)**
	3. Purine analogue	Cladribine **(10)**, Fludarbine, Pentostatin
ANTIMITOTIC AGENTS	1. Antagonize polymerisation	Vinca alkaloids: Vinchristine, Vinblastine **(11)**, Vinorelbine
	2. Antagonize Depolymerisation	Taxanes: Paclitaxel, Epipodophyllotoxin, Etoposide **(12)**
	3. Microtubule nucleation at multiple sites	Epothilones: Ixabepilone **(13)**

HORMONAL ANTAGONISTS	1. Antiestrogen	Tamoxifen **(14)**, Toremifen
	2. Estrogen	Diethylstilbestrol **(15)**
	3. Aromatase inhibitor	Anastrozole **(16)**
	4. Antiandrogen	Flutamide **(17)**
	5. Progestins	Medroxyprogestrone acetate **(18)**

Despite the cornucopia of chemotherapeutic agents, no momentous therapeutic success has been achieved since a large number of these drugs have narrow therapeutic index and/or develop resistance rapidly. This motivated the researchers to search for alternatives which would assist in the successful eradication of cancer. Thus, field of targeted therapies was introduced.

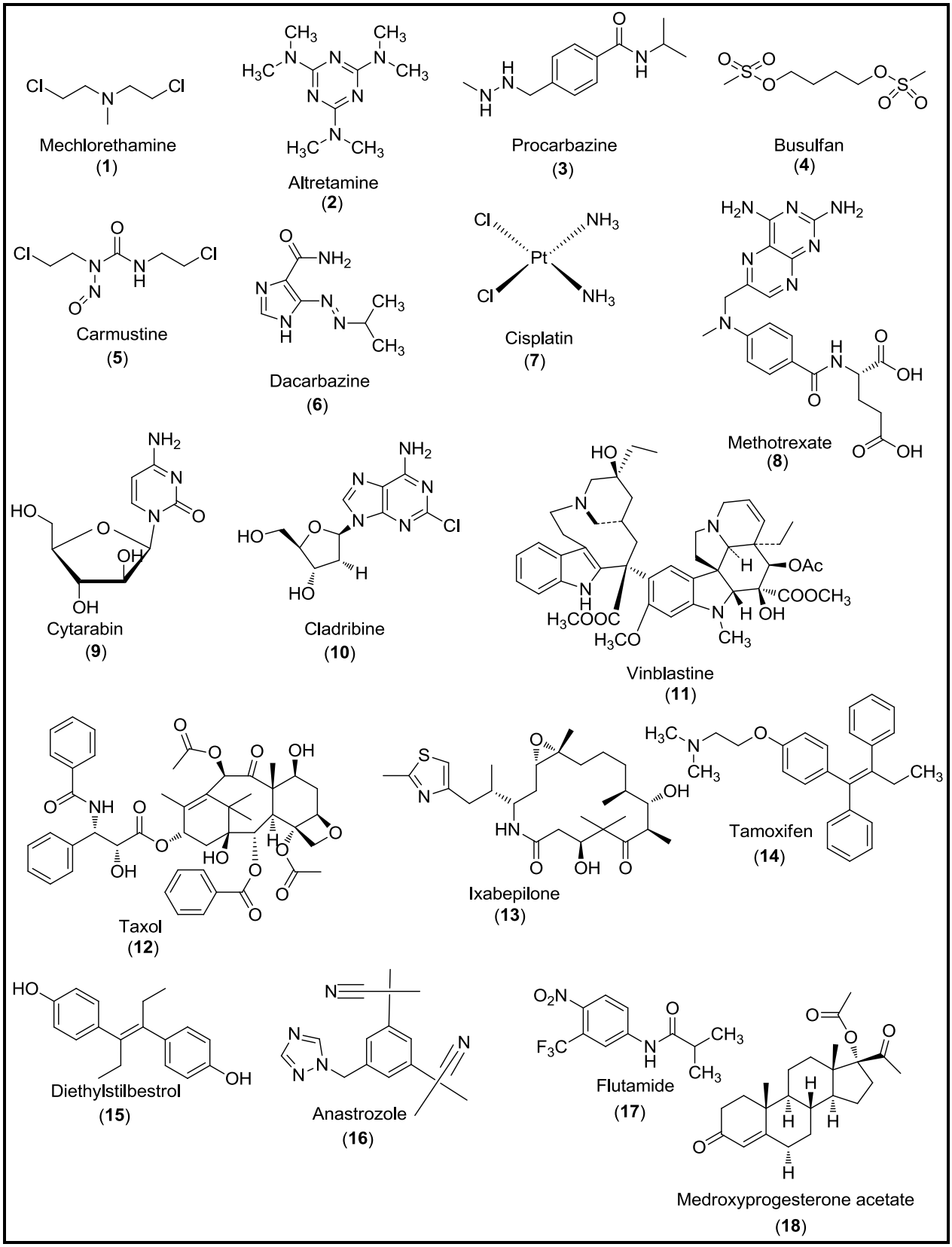


Figure 2.1 Various Anticancer Agents Used

2.2 Cancer Targets

As the problem of selectivity still remained unresolved with use of anticancer drugs (Kerbel, 1998) and the phenomenon of multidrug resistance became lucid and more vivid with research (Duan et al., 2012; Liscovitch and Lavie, 2002), the participation of the various signaling mechanism and pathways (Dubois, 2000; Kyriakis, 2009; Lemmon and Schlessinger, 2010; Luttun et al., 2004) become clearer. This necessitated the need to discover, trace the mechanisms and outline the pathways. As a consequence, a number of targets (**Table 2.2; Figure 2.2**) were discovered and methods to inhibit them or activate them were defined which proved to be a better alternative than the treatments applied earlier.

Table 2.2: Some of the Cancer Targets

	Cancer Targets	**Drugs**
1.	Epidermal Growth Factor Receptor	
	• bind to kinase domain	Erlotininb **(19)**, Gefitinib
	• bind to extracellular domain of EGFR	Cetuximab, Panitumumab
2.	Vascular Endothelial Growth Factor-A	Bevacizumab
3.	Tyrosine Kinase (BCR-ABL)	Imatinib **(20)**, Dasatinib, Nilotinib
4.	Proteasomes	Bortezomib **(21)**, Carfilzomib, ONX0912
5.	Nuclear Factor-κB	Disulfiram **(22)**, dithiocarbamates
6.	Survivin	
	• Suppressing survivin expression	Antisense, Ribozyme, siRNA, shRNA
	• Antagonizing survivin function	Dominant-negative survivin or by Cdk inhibitors
7.	PI-3 Kinases	Sirolimus **(23)**, Temsirolimus, Everosilomus
8.	HSP-90	

	• Additively or synergistically	1.17-allylamino,17-dimthoxygeldana-mycin (17AAG) + trastuzumab (breast cancer) 2.17-allylamino,17-dimthoxygeldana-mycin (17AAG) + cisplatin (colon cancer cell lines)
	• Individually	VER 49009 **(24)**, PU24FCI
9.	Cancer Stem Cells	Salinomycin **(25)**
10.	Tumor Suppressor Gene, p-53	Engineered oncolytic virus, ONYX-015
11.	Topoisomerase Enzyme	
	• Topoisomerase I	Irinotecan, Topotecan **(26)**, Camptothecin
	• Topoisomerase II	Etoposide **(27)**, Doxorubicin, Daunorubicin
12.	Epigenetic Therapy	
	• DNA methylation	5-azacytidine **(28)**,5-aza-2´-deoxycytidine
	• Histone Deactetylation	Suberoylanilidehydroxamic acid (SAHA) **(29)**, Hydroxymic acid

Epidermal growth factor receptor (EGFR), also known as ErbB1 or HER 1 (Liu et al., 2009) was the first receptor tyrosine kinase that helped the researchers to understand the role of EGFR in cancer (Paul and Mukhopadhyay, 2004) especially in human epithelial cancers wherein the activation or expression of the EGFR are altered (Hynes and Lane, 2005). These inhibitors may either block the enzymatic function of the EGFR (**19**) by competitively inhibiting ATP binding at the active site of the kinase or bind specifically to the extracellular domain of EGFR (Gilman and Goodman, 2011). Bevacizumab (Avastin) is a humanized antibody directed against VEGF-A (vascular endothelial growth factor) (Ferrara et al., 2005). The role of angiogenesis is central to the development of tumors which to a large extent is mediated by VEGF and consequently their level are increased in cancer especially in breast cancer, thus making them an attractive target (Adams et al., 2000; Kut et al., 2007; Salven et al., 1999). Tyrosine kinases, in recent times, have proven to be

significant mediators of growth factor signaling (Arora and Eric, 2005) as well as signal transduction process representing major portion of all oncoprotein that play a crucial role in a cornucopia of cancers (Paul and Mukhopadhyay, 2004; Scagliotti, 2007; Vlahovic and Crawford, 2003). Tyrosine kinase inhibitors include Imatinib (**20**) which mainly targets BCR-ABL TK (underlying chronic myelogenous leukemia), dasatinib and nilotinib (Cortes et al., 2007). Bortezomib (**21**) (Richardson et al., 2003), Carfilzomib (Kuhn et al., 2007), ONX0912 (Zhou et al., 2009) are some of the drugs that target and inhibit proteasome. Blocking proteasome triggers a series of reactions involving accumulation of various regulatory proteins which would ultimately result in apoptosis (Patrick, 2009). Nuclear Factor-κB (NF- κB), is a transcription factor critically implicated in the process of tumorigenesis in addition to the role in cancer and metastasis (Huber et al., 2004). Experimental evidences have also accredited NF- κB with initiation, promotion and progression of cancer (Karin, 2006) and also with activating transcription of the antiapoptotic factors in tumor cells resulting in enhanced cell survival (Straus, 2009), thus, making NF- κB an interesting target. Inhibitors of this transcriptional factor mainly include disulfiram (Wang et al., 2003), emetine, bithionol, sunitinib malate, narasim (Miller et al., 2010). Survivin, an inhibitor of apoptosis protein, has proven to be yet another not-so-much explored target. It was observed to mediate resistance to chemotherapy and/or recurrence of tumour, thus making it an attractive treatment strategy. The treatment plan mainly comprise of either suppressing the expression of survivin or antagonizing survivin function (Fukuda and Pelus, 2006). Another target which has caught the attention of researchers is the mTOR (mammalian target of rapamycin), a highly conserved serine-threonine kinase belonging to the family of PI-3K (Zaytseva et al., 2012), which also plays a crucial role in tumorigenesis (Zagouri et al., 2012). In cancer, their signaling is up regulated owing to the mutations in pathways related to mTOR (Zaytseva et al., 2012). The drugs are mainly intended to inhibit the mTOR/ PI-3K pathways (Azab, 2013) which include rapamycin (**23**), temsirolimus and everolimus (Villarreal-Garza et al., 2012; Zagouri et al., 2012) . HSP-90, molecular chaperon (Lu et al., 2012), is a leading therapeutic target (Workman et al., 2007) since quite a number of oncoprotein such as BRAF and EGFR are dependent on HSP-90 (Dias da

Rocha et al., 2005; Shimamura et al., 2005). These inhibitors act either additively or synergistically (Lu et al., 2012) with many other drugs. 17-allylamino, 17-dimthoxygeldanamycin (17AAG) is used in combination with trastuzumab in breast cancer (Modi et al., 2011) and with cisplatin in colon cancer cell lines (Vasilevskaya et al., 2003). Inhibitors used individually include VER 40990 (**24**) and PU24FCI. Recent evidences have shown stem cells to be responsible for the proliferation and metastasis of the neoplastic cells subsequent to the toxic exposure to chemotherapeutic and targeted therapy thus making this a consequential target (**25**) (Gilman and Goodman, 2011). Another appealing target in the anticancer therapy is the tumour suppressor gene p53 which is found to be mutated in several human cancers. p53, the genome guardian (Lane, 1992), lends its features for being the central participant in tumor suppression and also sensitizes the cancer cell towards chemo radiation. It has been suggested that the loss of p53 function decreases the efficacy of chemotherapy and radiotherapy (Chène, 2001; Wang and Sun, 2010). Topoisomerase enzymes are enzymes critical for DNA metabolism as they mediate the DNA unwinding, an important factor in the cellular processes of transcription and replication. The enzymatic mechanism creates transient nicks (type I) or breaks (type II) in the double stranded DNA polymer, thus allowing the DNA to unwind. Owing to the integral role played by topoisomerases in regulating DNA metabolism, these enzymes, are proven to be vital for cell survival thus making an interesting target, especially the topoisomerase II, for the targeted therapy (**26-27**) (Chikamori et al., 2010; Giles and Sharma, 2005; Kacprzak, 2013). DNA methylation and covalent modification of histone proteins comprises the epigenetic therapy. Tumor suppressor genes are inactivated when methylated and treatment aims at demethylating it using inhibitors (**28**). Similarly, deacetylation of histone can lead to cancer thus posing as an appealing target (**29**) (Peedicayil, 2006; Yoo and Jones, 2006)

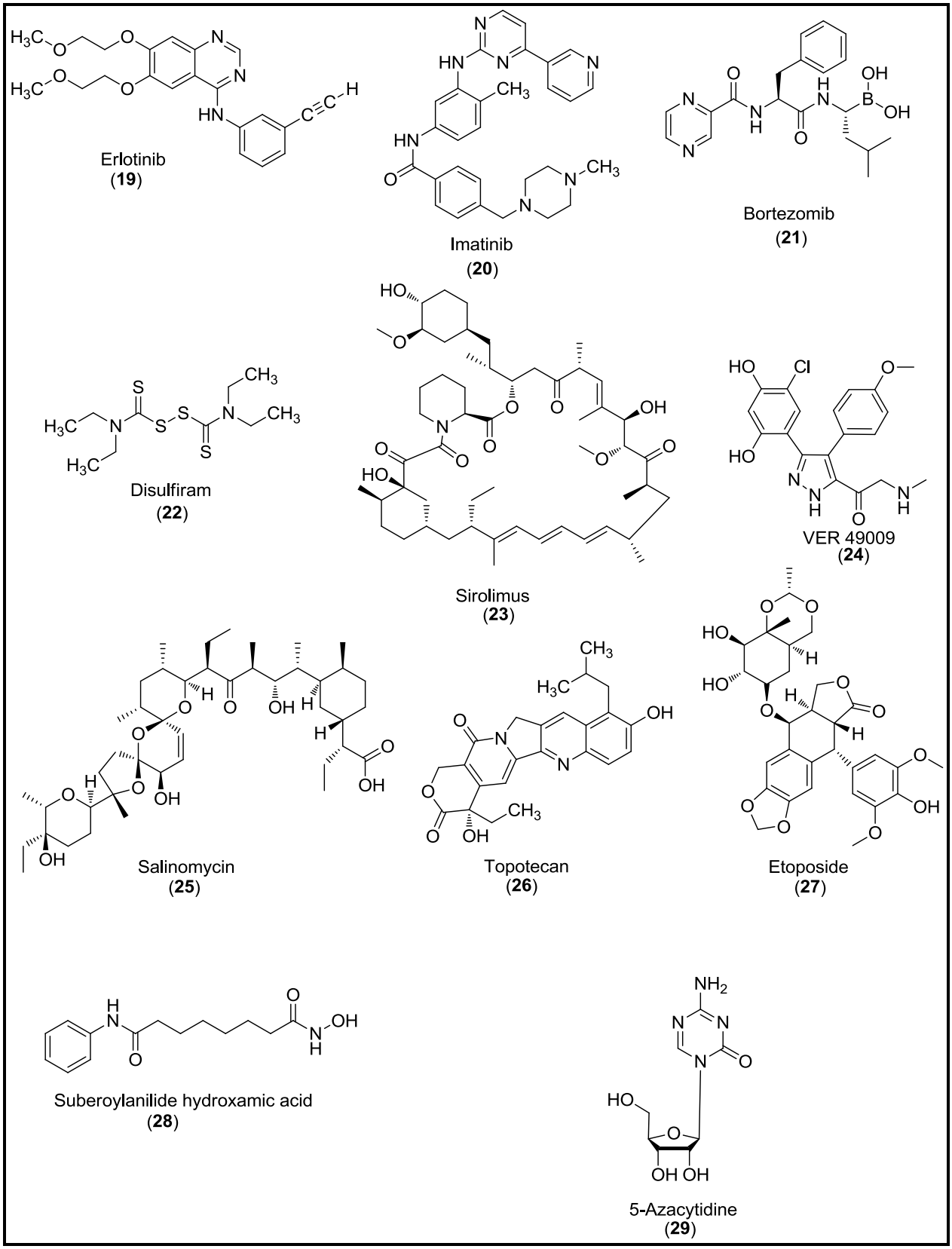


Figure 2.2 Compounds Used in Targeted Therapy

Although the idea of employing targeted therapy utilizing various drugs, gene therapy, monoclonal antibodies gave a fruitful result, it still wasn't successful in the complete eradication of this disease or the prevention of remission or drug resistance which so commonly ensues with these strategies. Thus, there was an ardent need to delve deeper into the physiology of cancer to find the missing link that makes the eradication of such an aggressive disease challenging and beyond reach and design & synthesize compounds that would circumvent the drawbacks accompanying the cancer treatment.

2.3 Heterocycles

Heterocycles constitute the class of organic compounds containing one or more rings in which one or more carbon atoms of the ring are replaced by heteroatoms. These heteroatoms are commonly oxygen, sulphur and nitrogen and rarely phosphorus, boron, silicon, tin, antimony etc (Dua et al., 2011; Gilchrist, 1992). Heterocycles have become indispensable in the recent years owing to the numerous functions that the compounds containing heterocycles help in carrying out. Functions carried out in the body on a day to day basis such as nerve impulse transmission, transferring hereditary information etc. find their identity in chemical reactions which make the presence of heterocyclic compounds such as vitamins, enzymes, coenzymes, ATP and serotonin irrefutable (Radin, 2008).

It is the ability of the heterocycles to manifest substituents around the core scaffold in a defined three dimensional representation that makes them so desirable to the drug industry. Moreover these heterocycles can easily alter drug properties such as potency, selectivity, lipophilicity, polarity and aqueous solubility. Heterocycles play a crucial part in design of therapeutic molecules owing to the properties that it can alter (Gomtsyan, 2012). The ability to manifest such properties may be attributed to its ability to undergo hydrogen bonding with target protein acting as either H-acceptor (heteroaromatic) or H-donor (N-heterocycles) (Dua et al., 2011; Gomtsyan, 2012). Wide number of drugs used contain heterocycles such as pyrrole, pyrimidine, indole, quinoline etc.in their pharmacophore structure. Effective treatments resulting from the use of synthetic drugs such as chlorpromazine, metronidazole, isoniazid,

captopril etc. have provided an incentive to the researchers to maneuver the research towards synthesis of compounds containing heterocycles. Among the many heterocycles commonly known pyrazole and 4, 5-dihydro pyrazole has managed to catch and keep the attention of the researchers owing to their numerous activities.

Pyrazolines

Pyrazoles (**1; Figure 2.3**), categorized as nitrogen-containing heterocycles, are well known for their interminable participation in the field of perpetual research and development of therapeutically active agents. As a consequence pyrazoles became an inevitable core of numerous drugs having diverse activities. The broad spectrum of activities portrayed by the pyrazoles instigated the researchers to modify the pyrazole ring as 4, 5-dihydro-1H-pyrazoles commonly known as 2-pyrazolines (**2; Figure 2.3**). Pyrazolines considered as cyclic hydrazine moiety (Gökhan-Kelekçi et al., 2009) possess an endocyclic double bond (Powers et al., 1998; Raj, 2010). These electron rich nitrogen heterocycles (Agrawal et al., 2012) can be subjected to reduction or oxidation (Wiley). On reduction 2-pyrazolines either yield pyrazolidines or undergo ring cleavage; and when oxidized they form blue or red colouring matter (Bardalai and Paneerselvam, 2012). The conjugated part of the ring (–N1–N2–C3–) includes an electron donating and electron withdrawing moieties within it. As observed from the X-ray analysis, all the atoms but C_5 of the pyrazoline ring adopt a planar system lof theory in heterocyclic chemistry (Rahman and Siddiqui, 2010; Sobhi et al., 2008). The pyrazolines find their application as alkaloids, vitamins, pigments, etc (Yusuf and Jain, 2011). Antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, **Figure 2.4**) was the first pyrazoline derivative used in the management of inflammation and pain (Dipankar et al., 2012).

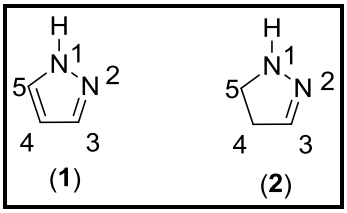


Figure 2.3 Pyrazole (**1**) and 4, 5-dihydro-1H-pyrazole (**2**)

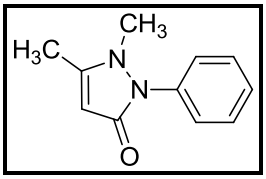


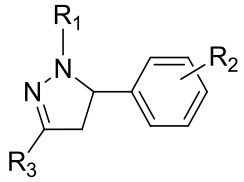
Figure 2.4 Antipyrine

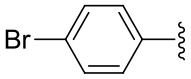
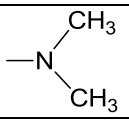
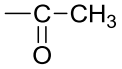
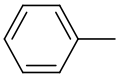
The presence of this moiety in several therapeutically active compounds encouraged the researchers in the direction of design and synthesis of novel pyrazoline derivatives possessing myriad activities. Some of the activities that pyrazolines exhibit are anticancer (Bhat et al., 2005; Lv et al., 2011), antitumor (Johnson et al., 2007), antiandrogenic (Abd et al., 2006), antioxidant (Babu et al., 2007), antimicrobial (Abdel-Wahab et al., 2009; Ali et al., 2012; Cetin et al., 2003), antiviral (Goodell et al., 2006), antitubercular (Kini et al., 2009 ; Taj et al., 2011), antimalarial (Wanare et al., 2010), antiamoebic (Bhat et al., 2009; Budakoti et al., 2008), COX-II (El-Sayed et al., 2012; Reddy et al., 2008), monoamine oxidase (Chimenti et al., 2008; Manna et al., 2002), xanthine oxidase (Nepali et al., 2011) and amine oxidase (Manna et al., 2002) inhibitory, etc. With an incentive to improve the existing activities several modifications are being done on this scaffold and many of which are proved to be successful. The most extensive modification in 2-pyrazoline was the substitution of diaryl/hetroaryl groups mainly at -3, 5 position (Nepali et al., 2011) since it was observed that heterocycles with functional groups greatly increased solubility in water (R. Brown et al., 1976; Durham et al., 1974).

Therapeutic application of pyrazoline containing compounds

Zeinab and her group (Ismaeil et al., 2011) synthesized and evaluated 3, 5-diaryl-Δ^{2}-pyrazoline derivatives (**Table 2.3**) for their anticancer activity. These compounds exhibited good activity against the human colon (HCT-116) and breast (MCF-7) cancer cell lines.

Table 2.3: Structural Modification on 2-Pyrazolines



Compound	R_1	R_2	R_3	IC_{50} (μM)	
				HCT-116	MCF-7
A1	H	F		-	3.43
A2				7.09	-
B1		*o*-Cl		6.8	12
C1		F		-	16.5
C2		NO_2		10.3	-

Bandaya et al. (Bandaya et al., 2010) synthesized 17-pyrazolinyl derivatives of pregnenolone (**Figure 2.5**) and evaluated the same against a panel of cancer cell lines - HT-29, HCT-15, 502713, HOP-62, A-545, MCF-7, SF-295. The compounds exhibited good activity against these cell lines.

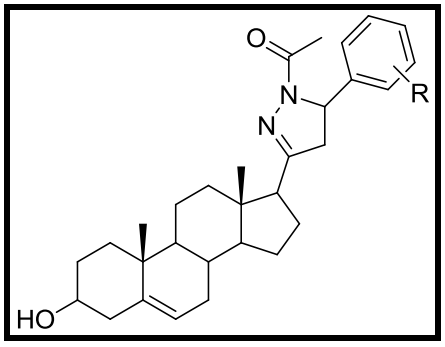


Figure 2.5 17-Pyrazolinyl Derivatives of Pregnenolone

Shaharyar et al. (Shaharyar et al., 2010) synthesized series of benzimidazole bearing 2-pyrazolines (**Figure 2.6**) and tested these compounds against various

cancer cell lines belonging to different panels such as renal, breast, colon, melanoma, prostate etc. Most active compound of the series was found to be 2-[5-(3, 4-dimethoxyphenyl) - 1-phenyl-4, 5-dihydro-1H-3-pyrazolyl]-1H-benzimidazole.

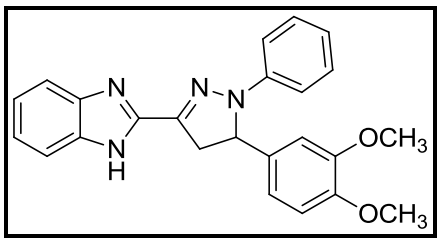


Figure 2.6 Benzimidazole Bearing 2-Pyrazolines

Fioravanti and the co-workers (Fioravanti et al., 2010) synthesized eighteen analogues of 1-N-substituted-3,5-diphenyl-2-pyrazoline derivatives (**Figure 2.7**) and evaluated the cyclooxygenase activity of these compounds. They synthesized compounds in which the N1 of pyrazoline was substituted with either acetyl moiety or thiocarbamoyl moiety.

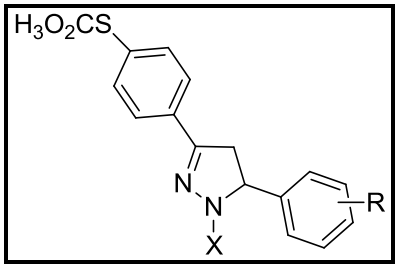
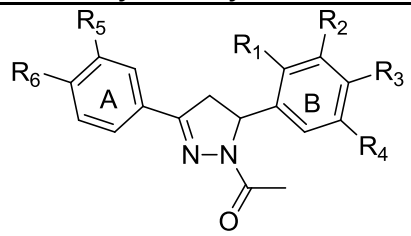
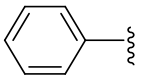
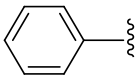
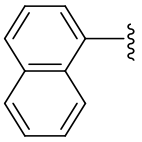
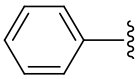
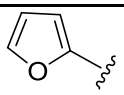
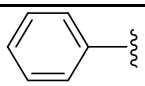
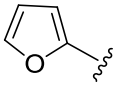
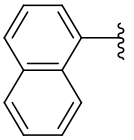
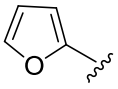
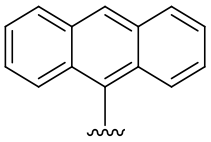


Figure 2.7 1-N-substituted-3, 5-diphenyl-2-pyrazoline derivatives

Our research group (Kumar et al., 2011; Nepali et al., 2011) synthesized fifty three analogues of 1-acetyl-3, 5-diaryl-4, 5-dihydro (1H) pyrazoles and evaluated their xanthine oxidase activity (**Table 2.4**) using bovine milk xanthine oxidase (XO) enzymatic assay. The synthesized compounds exhibited potent xanthine oxidase activity.

Table 2.4 XO Inhibitory Activity of N1- Acetyl Pyrazolines



Compound	Ring A	Ring B	R	XO Inhibitory Activity IC_{50} (μM)
A			H	61.4
B			H	41.3
C			H	26.4
D			R_2 =-OCH_3	21.3
E			R_2 =NO_2	13.1
F			-	91.4
G			-	Not tested

N-substituted thiocarbamoyl-3-(2-furyl)-5-phenyl/(2-furyl)-2-pyrazoline derivative (**Figure 2.8**) was synthesized by Z. Ozdemir and his team (Ozdemir et al., 2007) proved their potential as antidepressants and anticonvulsants by Porsolt's behavioral despair and maximal electroshock (MES) & subcutaneous pentylenetetrazole (scMet), respectively.

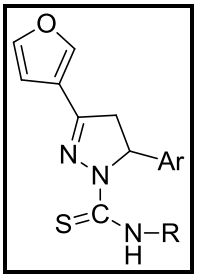


Figure 2.8 N-substituted thiocarbamoyl-3-(2-furyl)-5-phenyl/(2-furyl)-2- pyrazoline derivative

N-substituted-3-[(2'-hydroxy-4'-prenyloxy)-phenyl]-5-phenyl-4,5-dihydro-(1H)-pyrazolines (**Figure 2.9**) in particular pyrazoline N-substituted with either thiocarbamoyl or acetyl group, synthesized and evaluated by Fioravanti et al. (Fioravanti et al., 2010) for their potential to inhibit monoamine oxidase. Significant activity was shown by the compounds bearing acetyl substitution at N1.

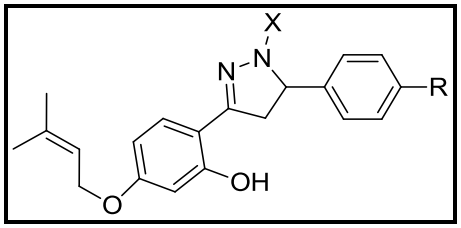


Figure 2.9 N-substituted-3-[(2'-hydroxy-4'-prenyloxy)-phenyl]-5-phenyl-4, 5-dihydro-(1H)-pyrazolines

Revanasiddappa et al. (Revanasiddappa et al., 2010) synthesized and determined the pyrazoline derivatives for their in vitro antimicrobial activity against different gram negative and gram positive bacterial strains as well as fungal strains. Although these compounds showed comparable antibacterial and antifungal activity, the activity was seen mainly due to the presence of electron withdrawing groups at R_1 on the C2 of the phenyl ring (**Figure 2.10**).

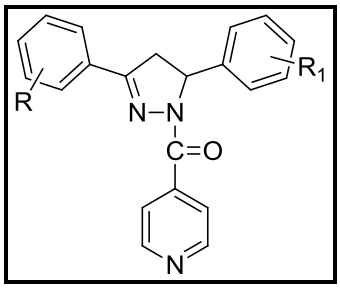
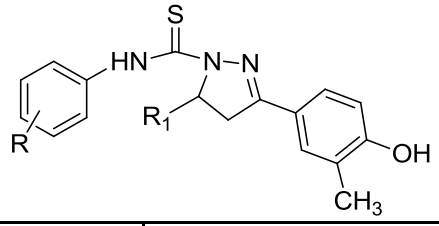


Figure 2.10 Nicotinoyl Substituted Pyrazolines

Ali et al. (Ali et al., 2007) synthesized pyrazoline derivatives and tested their anti-tubercular activity against Mycobacterium tuberculosis $H_{37}Rv$. Compound with 2,6-dichloro group substitution [anilino-3-(4-hydroxy-3-methylphenyl)-5-(2,6-dichlorophenyl)-4,5-dihydro-1H-1-pyrazolylmethanethione] produced highest efficacy and exhibited >90% inhibition at very low concentration (**Table 2.5**).

Table 2.5: MIC Values for Antituberculer Activity



Compound	R	R_1	Antituberculer activity MIC (µg/mL)
A	OCH3	2,6-Dichloro phenyl-	1.66
B	OCH_3	3-Nitro phenyl-	-
C	OCH_3	3,4-Dimethoxy phenyl-	5.67
D	OCH_3	3,4,5-Trimethoxy phenyl-	-
E	CH_3	3,4,5-Trimethoxy phenyl-	-

Wani et al. (Wani et al., 2012) synthesized and subjected 1, 3, 5-trisubstituted pyrazoline derivatives (**Figure 2.11**) to in vitro antiamoebic screening to assess their potential against growth of Entamoeba histolytica. The information drawn from SAR studies revealed that compounds having methyl groups at R_1 and R_2 possessed the highest activity.

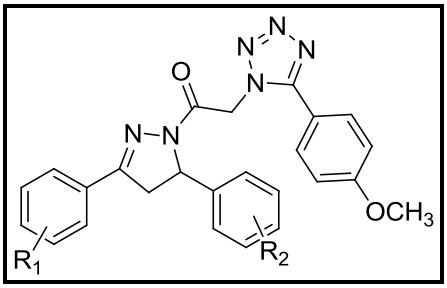


Figure 2.11 1, 3, 5-Trisubstituted Pyrazoline Derivatives

Mohamed A. Ali and his group (Ali et al., 2007) synthesized and assessed nicotinoyl substituted pyrazolines (**Table 2.12**) for their activity against HIV strains IIIB and ROD. Among the series, the compound having a phenyl ring bearing electron donating groups at C5 of the pyrazoline showed the highest activity.

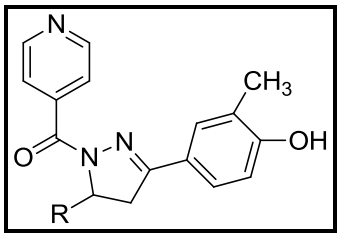


Figure 2.12 Nicotinoyl Substituted Pyrazolines

Acharya et al. (Acharya et al., 2010) synthesized and evaluated a series of nicotinoyl substituted pyrazolines (**Figure 2.13**) for their potential as antimalarials against chloroquine sensitive (MRC-02) and chloroquine resistant (RKL 9) strains of Plasmodium falciparum. Among these derivatives, the SAR studies assisted in inferring that the compounds possessing electron withdrawing groups at either ortho or para or both showed commendable activity against both the strains.

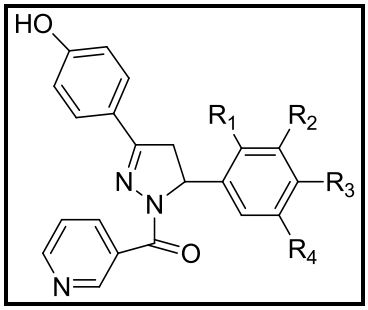
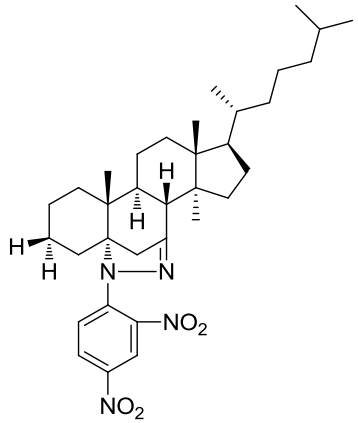


Figure 2.13 Nicotinoyl Substituted Pyrazolines

Shamsuzzaman and his coworkers (Khanam et al., 2012) synthesized and screened 2'-(2", 4"-dinitrophenyl)-5a-cholestano [5, 7-c d] to assess their antimicrobial

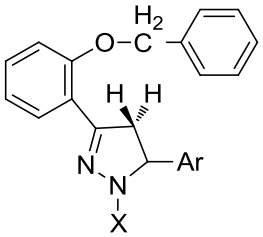
activity against different bacterial strains (Corynebacterium xerosis, Staphylococcus epidermidis and Escherichia coli) method and fungal strains (Mucor azygosporus, Claviceps purpurea and Aspergillus niger) employing the broth dilution and agar diffusion method, respectively. The promising antifungal and antibacterial activity shown by the compound was attributed to the dinitrophenyl ring substituted at the N1 of the pyrazoline ring (**Table 2.6**).

Table 2.6: Antimicrobial Activity of Steroidal Pyrazolines

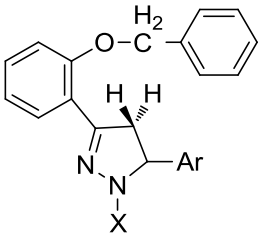
Compound	**MIC (Antibacterial)**		
	E. coli	***C. xerosis***	***S. epidermidis***
	3.125	0.781	0.195
	MIC (Antifungal)		
	M. azygosporus	***C. purpurea***	***A.niger***
2´-(2´´,4´´-dinitrophenyl)-5a-cholestano [5,7-c d] pyrazolines	3.125	6.250	1.562

Mamta Rani and her research group (Rani and Mohamad, 2011) synthesized three series of pyrazoline derivatives (**Table 2.7**) with the same basic framework but different substituent and assessed their antibacterial activity against strains of Aeromonas hydrophila, Yersinia enterocolitica, Listeria monocytogenes, and Staphylococcus aureus with the help of Halo Zone Test (Moon et al., 2003) and also compared their activity by measuring the zone of inhibition.

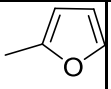
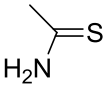
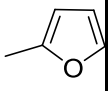
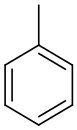
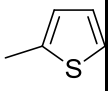
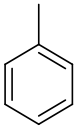
Table 2.7 Antimicrobial Activity



A



B

Comp-ound	Ar	X	Halo Zone Test (unit,mm) Microorganisms			
			A.hydrophila	***Y.enterocolitica***	***L.monocytogenes***	***S.aureus***
Genta-mycin			21	-	-	17
A1			21.5	24.4	21.3	22.8
B1			25.3	18.6	20.8	24.5
A2			22.3	21.4	24.3	22.8
B2			21.7	25.2	19.4	23.6
A3			20.5	22.4	24.3	22.8
B3			21.7	18.4	21.2	19.4

Bano et al. (Bano et al., 2011) synthesized 2-pyrazolines bearing benzene sulphonamides (**Figure 2.14**) and evaluated these compounds for their anti-inflammatory activity. The benzene sulphonamides substituted at the N1 of the pyrazoline ring attributed to these compounds their activity.

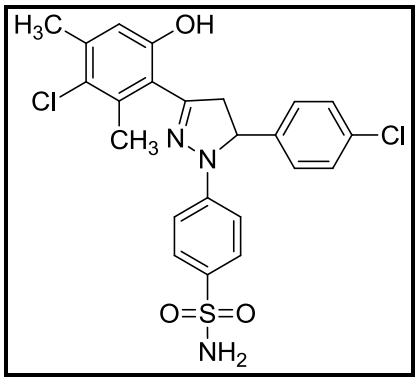


Figure 2.14 2-Pyrazolines Bearing Benzene sulphonamides

P. M. Sivakumar and his team (Sivakumar et al., 2010) synthesized 1, 3, 5-triphenyl-2-pyrazolines (**Figure 2.15**) and screened these compounds for their anti-infective activities against Mycobacterium tuberculosis $H_{37}Rv$, bacterial and fungal strains. It was observed that sulfonylmethyl substitution increased the activity towards the $H_{37}Rv$ strain since it was observed to bring the log P value to 3 (ideal for penetration through mycobacterial cell). Compounds having thiomethyl substitution in the A-ring resulted in higher activity against the bacterial and the fungal strains.

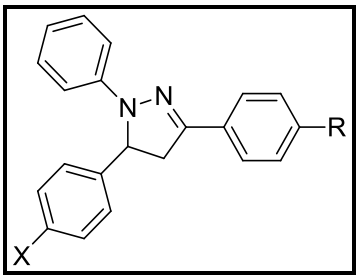


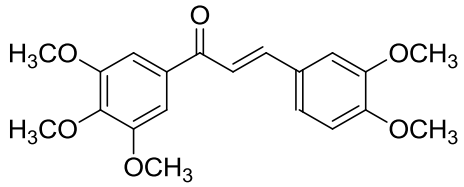
Figure 2.15 1, 3, 5-Triphenyl-2-Pyrazolines

CHAPTER- III

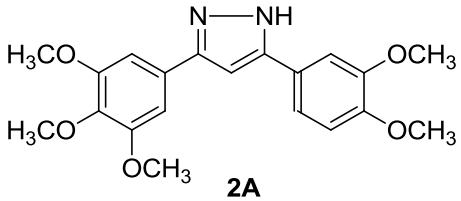
RATIONALE OF THE PROPOSAL

Pyrazolines are indebted to 1, 3-diaryl propenones for their basic synthon from which they are formed. 1, 3-diaryl propenones or commonly called chalcones are known to possess myriad biological activities (Katsori and Hadjipavlou-Litina, 2011; Ni et al., 2004) in addition to anticancer activity. A large number of their analogues have been synthesized which claim to exhibit anticancer potential through several mechanisms namely inhibition of EGFR TK, activation of caspase cascade, suppression of bcl-2 expression (Ni et al., 2004). They are also evidenced of possessing cytotoxic and apoptotic properties towards tumors and in cell lines (Manna et al., 2005). Owing to the structural similarity with colchicine with respect to the two aryl rings, 1,3-diaryl propenones show high affinity towards the colchicine binding site, thereby acting as antimitotic agents (Bhat et al., 2005; Boumendjel et al., 2008). Realizations of the numerous activities they possessed prompted the researchers to carry out modifications in the basic framework of 1, 3 diaryl propenones with an aim to design agents with enhanced activity. One such prominent modification was the addition of the pyrazole ring which drastically increased the anticancer potential as is clear from the IC_{50} values (**Table 3.1**) (Bhat et al., 2005).

Table 3.1 Comparison of IC_{50} values of 1, 3-Diaryl Propenones and their Corresponding Pyrazoles



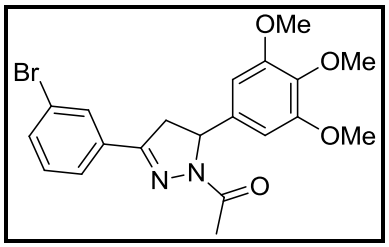
1A



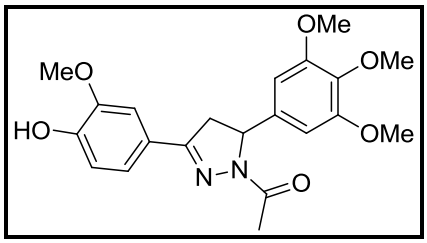
2A

COMPOUNDS	**CANCER CELL LINES (μM)**		
	HT-29 (**Human colorectal adenocarcinoma**)	**HCT-15** (**Colon carcinoma**)	**SW-620** (**Colon carcinoma**)
1A	6.64	7.95	5.80
2A	0.47	0.25	0.23

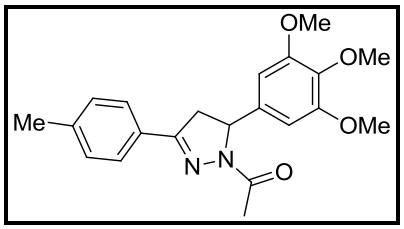
SAR studies eventually led to the introduction of a pyrazole nucleus between the two aryl rings which retaining the conformation of the two aryl rings, enhanced the rigidity besides polarity and solubility of the molecule which contributed significantly towards increasing the anticancer potential. Further modification done by the addition of N-acetyl group also showed an enhanced activity (Bhat et al., 2005) which could be attributed to the twisted structure which showed a better interaction with the biological target (Congiu et al., 2010) as shown in **Figure 3.1**.



NCI-H460: 0.35 ± 0.01 µM



NCI-H460: 0.21 ± 0.005µM



NCI-H460: 0.69 ± 0.02 µM

Figure 3.1: N-Acetyl Pyrazoline Derivatives Tested for their Anticancer Activity on NCI-H460 (Human Non-Small Cell Lung Carcinoma)

Further modifications have also been reported in which the substitution of the heterocyclic ring for the aryl ring led to a greater activity (Ahasan and Islam, 2007). The increase in activity seen with the heterocycles was attributed to either an increase in solubility or in other cases, to the reversible distortion to the membrane structure (Brown et al., 1976).

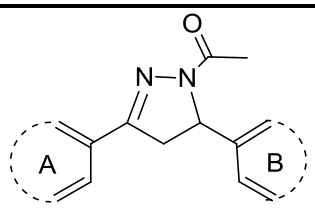
The increase in biological activities as a consequence of structural modification provided the necessary impetus to synthesize pyrazoline derivatives with either/both C3 or C5 substituted with a heterocycle and the N1 of the pyrazoline moiety acetylated.

CHAPTER IV

OBJECTIVES

The plethora of information obtained from the literature survey encouraged us to propose the research project with the following aims:

1. To design and synthesize N-acetyl pyrazoline derivative of 1, 3-dia heteroaryl propenones having the structure shown below:



Both Ring A and Ring B can be either aromatic or heteroaromatic or just one of them heteroaromatic

2. To evaluate the synthesized compounds for their in vitro anticancer activity using MTT assay.

CHAPTER V

MATERIALS AND METHODS

5.1 Synthesis

5.1.1 General

1. The reagents were purchased from Sigma-Aldrich, Loba- Chemie Pvt. Ltd., S.D. Fine Chemicals, Sisco Research Laboratory and used without further purification.
2. The progress of the reaction was determined by thin layer chromatography (TLC) carried out on pre-coated silica plates. Ethyl acetate: toluene (1:5), Ethyl acetate: n-hexane (1:10) were used commonly as the eluents. Spots were then visualized under UV light.
3. UV-VIS Spectrophotometer, Shimadzu was used for measuring absorbance and λ_{max}.
4. Melting points were recorded on Stuart SMP-30 melting point apparatus with open glass capillary tube and were uncorrected.
5. Infrared spectra of compounds were recorded on Bruker IR spectrophotometer.
6. The ^{1}H and ^{13}C NMR of the compounds were recorded on Bruker Advance II instrument at 400 MHz and 100 MHz frequencies respectively, in $CDCl_3$ and TMS (δ=0) as internal standard at SAIF, Panjab University, Chandigarh.

5.1.2A General Procedure for the Synthesis of 1, 3-Diaryl Propenones (JA 01-17)

An aryl or heteroaryl ketone was allowed to react with an aryl or heteroaryl aldehyde in the presence of sodium hydroxide (5%, 4 mL). To this mixture, methanol was added in quantity sufficient to dissolve the reactants. The reaction mixture was subjected to stirring on a magnetic stirrer at room temperature for about 4-5 h. Solid was obtained after filtration and was recrystallized from methanol to afford the pure 1, 3-diaryl propenones. The completion of reaction was ensured using thin layer chromatography (TLC).

5.1.2B Characterizations of Synthesized 1, 3-Diaryl Propenones

(*E*)-1-(4-Bromophenyl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-1)

Yellow solid; yield: 86%; m.p.: 112-114˚C; λ_{max} (MeOH): 348 nm

IR (KBr cm^{-1}): 1650 (C=O), 1590 (C=C aromatic), 1130 (C-S stretch), 708 (C-Br)

(*E*)-3-(Thiophen-2-yl)-1-(*p*-tolyl) prop-2-en-1-one (JA-2)

Yellowish brown solid; yield: 62%; m.p.: 70-72˚C; λ_{max} (MeOH): 346 nm

IR (KBr cm^{-1}): 1658 (C=O), 1592 (C=C aromatic), 1177 (C-S stretch).

(*E*)-1-(4-Methoxyphenyl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-3)

Yellow solid; yield: 96%; m.p.: 95-98˚C; λ_{max} (MeOH): 348 nm

IR (KBr cm^{-1}): 1650 (C=O), 1587 (C=C aromatic), 1255 (C-O stretch), 1174 (C-S stretch).

(*E*)-1-(4-Chlorophenyl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-4)

Light yellow solid; yield: 88%; m.p.: 114-116˚C; λ_{max} (MeOH): 350 nm

IR (KBr cm^{-1}): 1651 (C=O), 1591(C=C aromatic), 1100 (C-S stretch), 707 (C-Cl).

(*E*)-1-(2, 4-Dichlorophenyl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-5)

Yellow solid; yield: 80%; m.p.: 60-62˚C; λ_{max} (MeOH): 352 nm

IR (KBr cm^{-1}): 1650 (C=O), 1590 (C=C aromatic), 1130 (C-S stretch), 707 (C-Br).

(*E*)-1-Phenyl-3-(thiophen-2-yl) prop-2-en-1-one (JA-6)

Yellow solid; yield: 20%; m.p.: 61-63˚C; λ_{max} (MeOH): 346 nm

IR (KBr cm^{-1}): 1657 (C=O), 1573 (C=C aromatic), 1130 (C-S stretch).

(*E*)-1-(4-Chlorophenyl)-3-(furan-2-yl) prop-2-en-1-one (JA-7)

Bright yellow solid; yield: 60%; m.p.: 65-66˚C; λ_{max} (MeOH): 357 nm

IR (KBr cm^{-1}): 1656 (C=O), 1598 (C=C aromatic), 1284 (C-O stretch), 638 (C-Cl).

(*E*)-1-(2, 4-Dichlorophenyl)-3-(furan-2-yl) prop-2-en-1-one (JA-8)

Yellow solid; yield: 86%; m.p.: 70-72˚C; λ_{max} (MeOH): 343 nm

IR (KBr cm^{-1}): 1660 (C=O), 1587 (C=C aromatic), 1281 (C-O stretch), 753 (C-Cl).

(*E*)-3-(Furan-2-yl)-1-(*p*-tolyl) prop-2-en-1-one (JA-9)

Yellowish brown solid; yield: 90%; m.p.: 49-51˚C; λ_{max} (MeOH): 358 nm

IR (KBr cm^{-1}): 1655 (C=O), 1596 (C=C aromatic), 1282 (C-O stretch).

(*E*)-3-(Furan-2-yl)-1-(4-methoxyphenyl)- prop-2-en-1-one (JA-10)

Dark brown solid; yield: 67%; m.p.: 61-63˚C; λ_{max} (MeOH): 354 nm
IR (KBr cm^{-1}): 1656 (C=O), 1653 (C=C aromatic), 1282 (C-O stretch).

(*E*)-1-(4-Bromophenyl)-3-(furan-2-yl) prop-2-en-1-one (JA-11)

Dark brown solid; yield: 86%; m.p.: 65-67˚C; λ_{max} (MeOH): 359 nm

IR (KBr cm^{-1}): 1656 (C=O), 1603 (C=C aromatic), 1282 (C-O stretch), 751 (C-Br).

(*E*)-3-(2, 5-Dimethoxyphenyl)-1-(pyridin-2-yl) prop-2-en-1-one (JA-12)

Yellow solid; yield: 68%; m.p.: 77-78˚C; λ_{max} (MeOH): 353 nm
IR (KBr cm^{-1}): 1688 (C=O), 1598 (C=C aromatic), 1278 (C-O stretch).

(*E*)-3-(Furan-2-yl)-1-(pyridin-3-yl) prop-2-en-1-one (JA-13)

Light brown solid; yield: 87%; m.p.: 71-73˚C; λ_{max} (MeOH): 346 nm
IR (KBr cm^{-1}): 1656 (C=O), 1597 (C=N), 1549 (C=C aromatic), 1284 (C-O stretch).

(*E*)-3-(4-Chlorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one (JA-14)

Peach coloured solid; yield: 67%; m.p.: 110-113˚C; λ_{max} (MeOH): 355 nm
IR (KBr cm^{-1}): 1644 (C=O), 1585 (C=C aromatic), 639 (C-Cl)

(*E*)-3-Phenyl-1-(thiophen-2-yl) prop-2-en-1-one (JA-15)

Yellow solid; yield: 97%; m.p.: 67-69˚C; λ_{max} (MeOH): 348 nm
IR (KBr cm^{-1}): 1651 (C=O), 1591(C=C aromatic), 1141 (C-S stretch).

(*E*)-3-Phenyl-1-(pyridin-2-yl) prop-2-en-1-one (JA-16)

Yellow solid; yield: 74%; m.p.: 66-68˚C; λ_{max} (MeOH): 350 nm
IR (KBr cm^{-1}): 1670 (C=O), 1575 (C=C aromatic).

(*E*)-1-(Pyridin-2-yl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-17)

Yellow solid; yield: 84%; m.p.: 73-75˚C; λ_{max} (MeOH): 350 nm
IR (KBr cm^{-1}): 1665 (C=O), 1573 (C=C aromatic), 1148 (C-S stretch).

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0) δ: 8.74 (1H, m), 8.17 (2H, m), 7.24 (1H, s), 7.84-7.88 (1H, m), 7.46-7.49 (1H, m), 7.44-7.40 (2H, m), 7.09 (1H, m).

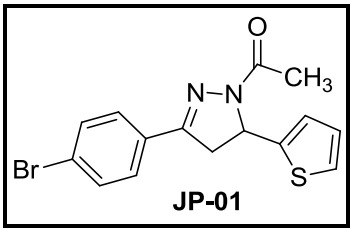
^{13}C NMR (100 MHz, $CDCl_3$, TMS = 0) δ: 189.1, 154.1, 148.8, 140.8, 137.1, 137.0, 132.2, 129.2, 128.2, 126.8, 122.8, 119.8.

5.1.3A General Procedure for the Preparation of 1-Acetyl-3,5-diaryl-4,5-dihydro-(1*H*)- pyrazoles (JP 01-17)

To a solution of an appropriate 1, 3-diaryl propenones **(JA 01-17)** in 15-20 mL acetic acid, hydrazine hydrate 80% (1.5 mmol) was added in a 50 mL round bottom flask. Then the mixture was refluxed for about 3- 6 h. After the completion of the reaction as determined by thin layer chromatography (TLC), the mixture was poured into ice water mixture to get crude pyrazole derivatives, which was then purified by recrystallization from methanol to afford the pure compound **(JP 01-17)**.

5.1.3B Synthesis and Characterizations of Synthesized 1-Acetyl-3,5-diaryl-4,5-dihydro-(1*H*)- pyrazoles

1-(3-(4-Bromophenyl)-5-(thiophen-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-01)



JP-01

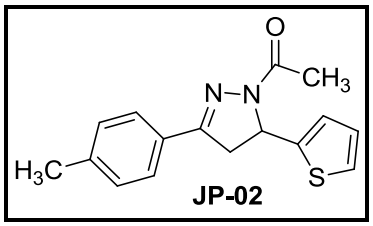
To a solution of (E)-1-(4-bromophenyl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-01; 500 mg, 1 mmol) in 10 mL of acetic acid in a 50 mL round bottom flask, hydrazine hydrate 80% (1.5 mmol) was added. The mixture was then allowed to reflux for 3h. After the completion of the reaction, the reaction mixture was poured into ice water mixture to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-01**).

Light yellow solid; yield: 65%; m.p.: 113-115˚C; λ_{max} (MeOH): 325 nm

IR (KBr cm^{-1}): 3014 (C-H), 1660 (C=O), 1591 (C=N stretch), 1246 (C-N stretch), 1136 (C-S stretch).

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0) δ: 7.61(1H, d, J = 8.64 Hz), 7.57 (1H, d, J = 8.62 Hz), 7.55 (1H, d, J = 6.64 Hz), 7.18 (1H, dd, J = 1.04 and 5.08 Hz), 7.01 (1H, d, J = 3.36 Hz), 6.92 (1H, dd, J = 3.56 and 5.08 Hz), 5.91 (1H, dd, J = 4.04 and 11.48 Hz), 3.70 (1H, dd, J =11.48 and 17.6 Hz), 3.31 (1H, dd, J = 4.08 and 17.6 Hz), 2.38 (3H, s).

1-(5-(Thiophen-2-yl)-3-(*p*-tolyl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-02)



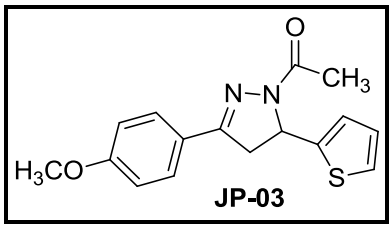
JP-02

(E)-3-(thiophen-2-yl)-1-(p-tolyl) prop-2-en-1-one (JA-02; 170 mg, 1 mmol) was first dissolved in 12 mL of acetic acid taken in a 50 mL round bottom flask. Hydrazine hydrate 80% (1.5 mmol) was added to this solution and was allowed to reflux for 4h. After the completion of the reaction, the reaction mixture was poured into ice water mixture to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-02**).

Yellow solid; yield: 52%; m.p.: 114-117˚C; λ_{max} (MeOH): 304 nm
IR (KBr cm^{-1}): 3007 (C-H stretch), 1660 (C=O), 1609 (C=N stretch), 1587 (C=C aromatic), 1176 (C-N stretch), 1014 (C-S stretch).

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0) δ: 7.65 (1H, s), 7.63 (1H, s), 7.26 (1H, s), 7.24 (1H, s), 7.17 (1H, dd, J = 1.0 and 5.04 Hz), 7.01 (1H, d, J = 3.36 Hz), 6.91 (1H, m), 5.89 (1H, dd, J = 3.9 and 11.4 Hz), 3.70 (1H, dd, J =11.4 and 17.56 Hz), 3.33 (1H, dd, J = 3.9 and 17.56 Hz), 2.40 (3H, s), 2.39 (3H, s).

1-(3-(4-Methoxyphenyl)-5-(thiophen-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-03)



JP-03

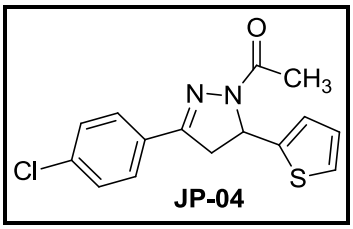
To a solution of (E)-1-(4-methoxyphenyl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-03; 150 mg, 1mmol) in 15 mL of acetic acid in a 50 mL round bottom flask, hydrazine hydrate 80% (1.5 mmol) was added. The mixture was then allowed to reflux for 4h. After the completion of the reaction, the reaction mixture was poured into ice water mixture to afford the crude pyrazole, which was then purified by employing recrystallization from methanol to afford the pure compound (**JP-03**).

Light brown; yield: 50%; m.p.: 89-90 °C; λ_{max} (MeOH): 315 nm

IR (KBr cm^{-1}): 3007 (C-H stretch), 1657 (C=O), 1608 (C=N stretch), 1517 (C=C aromatic), 1113 (C-S stretch), 1253 (C-O stretch), 1216 (C-N stretch).

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0) δ: 7.7 (1H, d, J = 9.64 Hz), 7.69 (1H, d, J = 2.0 Hz), 7.22 (1H, d, J = 5.08 Hz), 7.16 (1H, dd, J = 1.2 Hz), 6.90 -6.94 (2H, m), 6.95 (1H, s), 5.89 (1H, dd, J = 3.88 and 11.36 Hz), 3.69 (1H, dd, J = 11.36 and 17.44 Hz), 3.31 (1H, dd, J = 3.96 and 17.48 Hz), 3.85 (3H, s) 2.38 (3H, s).

1-(3-(4-Chlorophenyl)-5-(thiophen-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-04)



JP-04

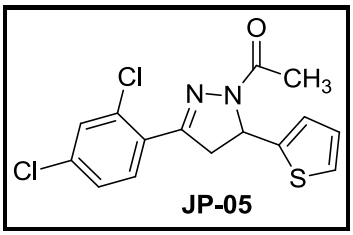
Hydrazine hydrate 80% (1.5 mmol) was added to a solution of (E)-1-(4-chlorophenyl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-04; 200 mg, 1mmol) in 20 mL of acetic acid in a 50 mL round bottom flask. The mixture was then allowed to reflux for 3h. When the reaction was complete as determined from TLC, crude pyrazole was obtained when the reaction mixture was poured into ice water mixture, which was then purified using recrystallization from methanol to afford the pure compound (**JP-04**).

Yellow solid; yield: 67%; m.p.: 125-127˚C; λ_{max} (MeOH): 307 nm

IR (KBr cm^{-1}): 3010 (C-H stretch), 1660 (C=O), 1593 (C=N stretch), 1215 (C-N stretch), 1142 (C-S), 702 (C-Cl stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 7.69 (1H, d, J = 1.80 Hz), 7.68 (1H, s), 7.41 (1H, d, J = 1.84), 7.39 (1H, s), 7.18 (1H, dd, J = 1.0 and 5.08 Hz), 7.01 (1H, d, J = 3.36 Hz), 6.94 (1H, m), 5.92 (1H, dd, J = 4.0 and 11.44 Hz), 3.70 (1H, dd, J = 11.48 and 17.6 Hz), 3.31 (1H, dd, J = 4.0 and 17.56 Hz), 2.39 (3H, s).

1-(3-(2,4-Dichlorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-05)



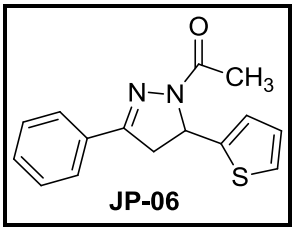
JP-05

To a solution of (E)-1-(2,4-dichlorophenyl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-05; 200 mg, 1 mmol) in 16 mL of acetic acid in a 50 mL round bottom flask, hydrazine hydrate 80% (1.5 mmol) was added. The mixture was then allowed to reflux for 3h. After the completion of the reaction, the reaction mixture was poured into ice water mixture to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-05**).

Yellow solid; yield: 85%; m.p.: 115-116˚C; λ_{max} (MeOH): 293 nm
IR (KBr cm^{-1}): 3012 (C-H stretch), 1658 (C=O), 1609 (C=N stretch), 1589 (C=C aromatic), 1176 (C-S stretch), 1215 (C-N), 702 (C-Cl stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 7.7 (1H, d, J = 8.48 Hz), 7.46 (1H, s), 7.3 (1H, s), 7.19 (1H, dd, J = 1.08 and 5.04 Hz), 7.03 (1H, d, J = 3.2 Hz), 6.93 (1H, m), 5.91 (1H, dd, J = 3.76 and 11.44 Hz), 3.91 (1H, dd, J = 11.48 and 18.08 Hz), 3.49 (1H, dd, J = 3.80 and 18.04 Hz), 2.37 (3H, s).

1-(3-Phenyl-5-(thiophen-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-06)



JP-06

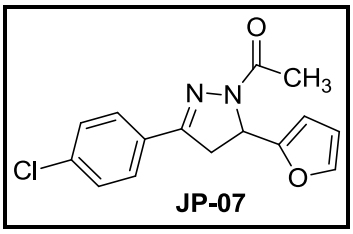
(E)-1-phenyl-3-(thiophen-2-yl) prop-2-en-1-one (JA-06; 40 mg, 1 mmol) was allowed to solubilize in 10 mL of acetic acid in a 50 mL round bottom flask; subsequent to which hydrazine hydrate 80% (1.5 mmol) was added. The mixture was then allowed to reflux for 4h which was followed by pouring the reaction mixture into ice water mixture to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-06**).

Dark brown solid; yield: 24%; m.p.: 110-112˚C; λ_{max} (MeOH): 249 nm

IR (KBr cm^{-1}): 2925 (C-H stretch), 1658 (C=O), 1620 (C=N stretch), 1523 (C=C aromatic), 1232 (C-N stretch), 1090 (C-S stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 7.70 (1H, d, J = 2.32 Hz), 7.46 (1H, s), 7.30 (1H, s), 7.19 (1H, dd, J = 1.12 and 5.08 Hz), 7.03 (1H, d, J = 3.52 Hz), 6.93 (1H, dd, J = 3.52 and 5.04 Hz), 5.9 (1H, dd, J = 3.76 and 11.44 Hz), 3.9 (1H, dd, J = 11.48 and 18.08 Hz), 3.5 (1H, dd, J = 3.8 and 18.04 Hz), 2.3 (3H, s).

1-(3-(4-Chlorophenyl)-5-(furan-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-07)



JP-07

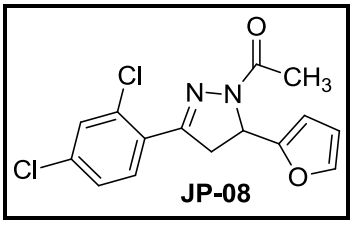
Hydrazine hydrate 80% (1.5 mmol), was added to a solution of (E)-1-(4-chlorophenyl)-3-(furan-2-yl) prop-2-en-1-one (JA-07; 170 mg, 1 mmol) in 15 mL of acetic acid in a 50 mL round bottom flask. The mixture was subjected to reflux for 3h. After the completion of the reaction, the reaction mixture was poured into ice cold water to afford the crude pyrazole, which was then purified by utilizing the recrystallization method from methanol to afford the pure compound (**JP-07**).

Whitish yellow solid; yield: 72%; m.p.: 156-157˚C; λ_{max} (MeOH): 297 nm

IR (KBr cm^{-1}): 3010 (C-H stretch), 1663 (C=O), 1596 (C=N stretch), 1502 (C=C aromatic), 1252 (C-O stretch), 1215 (C-N stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 7.70 (1H, d, J = 2.36 Hz), 7.68 (1H, d, J = 1.96 Hz), 7.41 (1H, s), 7.39 (1H, d, J =2.4 Hz), 7.30 (1H, m), 6.33 (1H, s), 6.31 (1H, t), 5.69 (1H, dd, J = 4.84 and 11.72 Hz), 3.56 (1H, dd, J = 11.72 and 17.52 Hz), 3.41 (1H, dd, J = 4.84 and 17.52 Hz), 2.32 (3H, s).

1-(3-(2, 4-Dichlorophenyl)-5-(furan-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-08)



JP-08

To a solution of (E)-1-(2,4-dichlorophenyl)-3-(furan-2-yl) prop-2-en-1-one (JA-08; 120 mg, 1 mmol) in 13 mL of acetic acid in a 50 mL round bottom flask, hydrazine

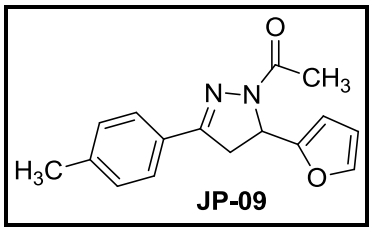
hydrate 80% (1.5 mmol) was added. The mixture was then allowed to reflux for 3h. Then the reaction mixture was poured into ice water mixture to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-08**).

Yellow solid; yield: 64%; m.p.: 115-116˚C; λ_{max} (MeOH): 295 nm

IR (KBr cm^{-1}): 3013 (C-H stretch), 1711 (C=O), 1665 (C=N aromatic), 1586 (C=C aromatic), 1257 (C-O stretch), 1215 (C-N stretch), 751 (C-Cl stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 7.75 (1H, d, J = 8.48 Hz), 7.46 (1H, s), 7.32-7.29 (2H, m), 6.33 (1H, s), 6.32 (1H, m), 5.67 (1H, dd, J = 4.56 and 11.8 Hz), 3.79 (1H, dd, J = 11.8 and 17.96 Hz), 3.57 (1H, dd, J = 4.96 and 17.92 Hz), 2.36 (3H, s).

1-(5-(Furan-2-yl)-3-(*p*-tolyl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-09)



JP-09

Required amount of (E)-3-(furan-2-yl)-1-(p-tolyl) prop-2-en-1-one (JA-09; 200 mg, 1 mmol) was allowed to dissolve in sufficient quantity of acetic acid (15 mL) in a 50 mL round bottom flask; followed by the addition of hydrazine hydrate 80% (1.5 mmol). The mixture was then allowed to reflux for 5h. After the completion of the reaction as determined by TLC, the reaction mixture was poured into cold water to afford the crude pyrazole, which was then purified using the recrystallization technique from methanol to afford the pure compound (**JP-09**).

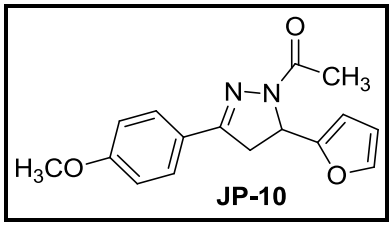
Light brown solid; yield: 70%; m.p.: 120-121˚C; λ_{max} (MeOH): 297 nm

IR (KBr cm^{-1}): 2923 (C-H stretch), 1661 (C=O), 1640 (C=N stretch), 1591 (C=C aromatic), 1250 (C-O stretch), 1210 (C-N stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 7.66 (1H, s), 7.63 (1H, s), 7.29 (1H, s), 7.24 (1H, s), 7.25 (1H, s), 6.31 (1H, s), 6.30 (1H, d, J = 1.4 Hz), 5.68 (1H, dd, J = 4.72 and

11.64 Hz), 3.56 (1H, dd, J = 11.64 and 17.48 Hz), 3.42 (1H, dd, J = 4.76 and 17.48 Hz), 2.40 (3H, s), 2.38 (3H, s).

1-(5-(Furan-2-yl)-3-(4-methoxyphenyl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-10)



JP-10

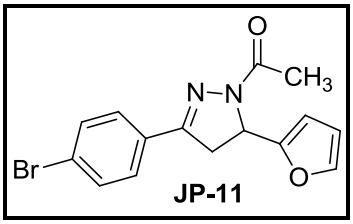
(E)-3-(furan-2-yl)-1-(4-methoxyphenyl)- prop-2-en-1-one (JA-10; 200 mg, 1 mmol) was allowed to dissolve in 20 mL of acetic acid in a 50 mL round bottom flask, hydrazine hydrate 80% (1.5 mmol) was added to this solution. The mixture was then allowed to reflux for 4h. After the completion of the reaction, the reaction mixture was poured into ice water mixture to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-10**).

Dark brown solid; yield: 38%; m.p.: 122-124˚C; λ_{max} (MeOH): 301 nm

IR (KBr cm^{-1}): 3005 (C-H stretch), 1659 (C=O), 1606 (C=N stretch), 1596 (C=C aromatic), 1250 (C-O stretch), 1223 (C-N stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 7.71 (1H, d, J = 2.0 Hz), 7.69 (1H, d, J = 2.04 Hz), 7.29 (1H, m), 6.95 (1H, d, J = 2.0 Hz), 6.93 (1H, d, J = 2.0 Hz), 6.31 (1H, d, J = 1.48 Hz), 6.30 (1H, d, J = 1.44 Hz), 5.67 (1H, dd, J = 4.68 and 11.6 Hz), 3.85 (3H, s), 3.55 (1H, dd, J = 11.56 and 17.36 Hz), 3.41 (1H, dd, J = 4.68 and 17.36 Hz), 2.38 (3H, s).

1-(3-(4-Bromophenyl)-5-(furan-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-11)



JP-11

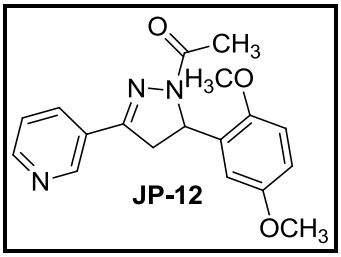
To a solution of (E)-1-(4-bromophenyl)-3-(furan-2-yl) prop-2-en-1-one (JA-11; 200 mg, 1 mmol) in 20 mL of acetic acid in a 50 mL round bottom flask, hydrazine hydrate 80% (1.5 mmol) was added. The mixture was then allowed to reflux for 3h. After the completion of the reaction, the reaction mixture was poured into ice water mixture to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-11**).

Light brown solid; yield: 68%; m.p.: 141-143˚C; λ_{max} (MeOH): 393 nm

IR (KBr cm^{-1}): 3011 (C-H stretch), 1659 (C=O), 1594 (C=N stretch), 1532 (C=C aromatic), 1252 (C-O stretch), 1215 (C-N stretch), 754 (C-Br stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 7.62 (1H, s), 7.61 (1H, s), 7.56 (1H, s), 7.55 (1H, s), 7.29 (1H, m), 6.32 (1H, s), 6.31 (1H, s), 5.69 (1H, dd, J = 4.84 and 11.76 Hz), 3.56 (1H, dd, J = 11.72 and 17.44 Hz), 3.41 (1H, dd, J = 4.88 and 17.48 Hz), 2.38 (3H, s).

1-(5-(2, 5-Dimethoxy)-3-phenyl-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-12)



JP-12

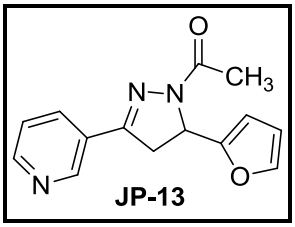
(E)-3-(2,5-dimethoxyphenyl)-1-(pyridin-2-yl) prop-2-en-1-one (JA-12; 58 mg, 1 mmol) was allowed to dissolve in 10 mL of acetic acid in a 50 mL round bottom flask. This was followed by the addition of hydrazine hydrate 80% (1.5 mmol) to the reaction mixture. The mixture was then allowed to reflux for 4h. After the completion of the reaction, the reaction mixture was poured into ice cold water to afford the crude pyrazole, which was then recrystallized from methanol to afford the pure compound (**JP-12**).

Yellow solid; yield: 74%; m.p.: 112-114˚C; λ_{max} (MeOH): 328 nm

IR (KBr cm^{-1}): 3015 (C-H stretch), 1660 (C=O), 1583(C=N stretch), 1215 (C-N stretch).

^1H NMR (400 MHz, $CDCl_3$, TMS = 0) δ: 8.86 (1H, d, J = 1.68 Hz), 8.64 (1H, dd, J =1.56 and 4.80 Hz), 8.09-8.06 (m, 1H), 7.36-7.33 (1H, m), 6.82 (1H, d, J = 8 Hz), 6.75-6.73 (m, 1H), 6.58 (1H, d, J = 2 Hz), 5.83 (1H, dd, J = 4.68 and 11.84 Hz), 3.04 (1H, dd, J = 4.72 and 17.72 Hz), 3.41 (1H, dd, J = 11.92 and 17.32 Hz), 3.81 (3H, s), 3.72 (3H, s), 2.46 (3H, s).

1-(5-(Furan-2-yl)-3-(pyridin-3-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-13)



JP-13

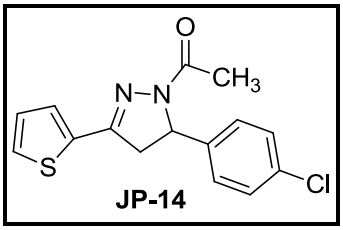
To 10 mL of acetic acid in a 50 mL round bottom flask (E)-3-(furan-2-yl)-1-(pyridin-3-yl) prop-2-en-1-one (JA-13; 100 mg, 1 mmol) was added. To this mixture hydrazine hydrate 80% (1.5 mmol) was added prior to subjecting it to reflux. The reaction was refluxed for 4h. On completion of the reaction, the reaction mixture was poured into ice water mixture to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-13**).

Yellow solid; yield: 50%; m.p.: 95-97˚C; λ_{max} (MeOH): 301 nm

IR (KBr cm^{-1}): 3011 (C-H stretch), 1662 (C=O), 1595 (C=N stretch), 1553 (C=C aromatic), 1256 (C-O stretch), 1215 (C-N stretch).

^1H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 8.92 (1H, d, J = 1.64), 8.67 (1H, dd, J =1.48 and 4.76 Hz), 8.10 (1H, t, J = 1.84 Hz), 7.40-7.36 (1H, m), 7.30 (1H, m), 6.35 (1H, m), 6.32 (1H, m), 5.72 (1H, dd, J = 4.92 and 11.76 Hz), 3.61 (1H, dd, J = 11.76 and 17.56 Hz), 3.47 (1H, dd, J = 4.88 and 11.76 Hz), 2.39 (3H, s).

1-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-14)



JP-14

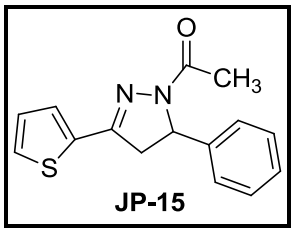
To a solution of (E)-3-(4-chlorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one (JA-14; 220 mg, 1 mmol) in 17 mL of acetic acid in a 50 mL round bottom flask, hydrazine hydrate 80% (1.5 mmol) was added. The mixture was then allowed to reflux for 3h. After the completion of the reaction, the reaction mixture was poured into ice cold water to afford the crude pyrazole, which was subjected to recrystallization from methanol to afford the pure compound (**JP-14**).

Yellow solid; yield: 77%; m.p.: 107-109˚C; λ_{max} (MeOH): 317 nm

IR (KBr cm^{-1}): 3007 (C-H stretch), 1657 (C=O), 1632 (C=N stretch), 1523 (C=C aromatic), 1232 (C-N stretch), 1089 (C-S stretch), 709 (C-Cl stretch).

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0) δ: 7.44 (1H, dd, J = 1.08 and 5.08 Hz), 7.28 (1H, s), 7.21 (1H, dd, J = 1.04 and 3.68 Hz), 7.18 (1H, s), 7.16 (1H, s), 7.16 (1H, s), 7.07 (1H, dd, J = 3.68 and 5.0), 5.55 (1H, dd, J = 4.68 and 11.84 Hz), 3.76 (1H, dd, J = 11.84 and 17.48 Hz), 3.18 (1H, dd, J = 4.72 and 17.52 Hz), 2.38 (3H, s).

1-(5-Phenyl-3-(thiophen-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-15)



JP-15

Hydrazine hydrate 80% (1.5 mmol) was added to a solution of (E)-3-phenyl-1-(thiophen-2-yl) prop-2-en-1-one (**JA-15**; 150 mg, 1 mmol) in sufficient quantity of acetic acid (15 mL) taken in a 50 mL round bottom flask,. This reaction mixture was subjected to reflux for 3h. The reaction mixture was poured into ice water, after the

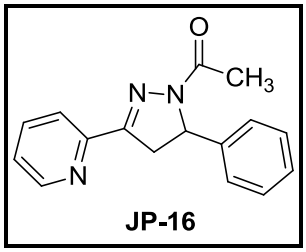
completion of the reaction, to afford the crude pyrazole. Pure compound (**JP-15**) was obtained by recrystallization of the crude product from methanol.

Yellow solid; yield: 56%; m.p.: 118-120˚C; λ_{max} (MeOH): 311 nm

IR (KBr cm^{-1}): 3013 (C-H stretch), 1655 (C=O), 1590 (C=N stretch), 1523 (C=C aromatic), 1141 (C-S stretch), 1215 (C-N stretch).

^{1}H NMR (400 MHz, $CDCl_3$, TMS = 0) δ: 7.43 (1H, dd, J = 1.04 and 5.04 Hz), 7.32 (1H, d, J =1.16), 7.30 (1H, d, J = 1.28 Hz), 7.26 (1H, s), 7.24-7.20 (3H, m), 7.06 (1H, dd, J = 3.68 and 5.08 Hz), 5.59 (1H, dd, J = 4.56 and 11.76 Hz), 3.76 (1H, dd, J = 11.84 and 17.48 Hz), 3.15 (1H, dd, J = 4.60 and 11.44 Hz), 2.39 (3H, s).

1-(5-Phenyl-3-(pyridin-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-16)



JP-16

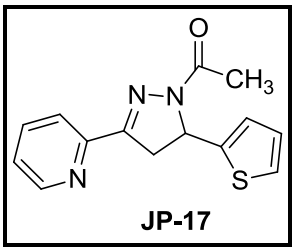
To a solution of (E)-3-phenyl-1-(pyridin-2-yl) prop-2-en-1-one (JA-16; 1g, 1 mmol) in 20 mL of acetic acid in a 50 mL round bottom flask, hydrazine hydrate 80% (1.5 mmol) was added. The mixture was then allowed to reflux for 5h. After the completion of the reaction, the reaction mixture was poured into ice water mixture to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-16**).

Yellow solid; yield: 47%; m.p.: 135-137˚C; λ_{max} (MeOH): 304 nm

IR (KBr cm^{-1}): 3007 (C-H stretch), 1664 (C=O), 1581 (C=N stretch), 1561 (C=C aromatic), 1253 (C-N stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 8.61 (1H, m), 8.10 (1H, d, J = 7.92), 7.78-7.4 (1H, m), 7.33 (1H, d, J = 2.16 Hz), 7.31 (1H, d, J = 1.56 Hz), 7.29 (1H, s), 7.25 (1H, s), 7.23 (1H, d, J = 6.28 Hz), 7.22 (1H, s), 5.61 (1H, dd, J = 4.76 and 12 Hz), 3.86 (1H, dd, J = 11.96 and 18.56 Hz), 3.38 (1H, dd, J = 4.76 and 18.6 Hz), 2.44 (3H, s).

1-(3-(Pyridin-2-yl)-5-(thiophen-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl) ethanone (JP-17)



JP-17

(E)-1-(pyridin-2-yl)-3-(thiophen-2-yl) prop-2-en-1-one (JA-17; 1g, 1 mmol) was allowed to dissolve in 20 mL of acetic acid taken in a 50 mL round bottom flask. To this solution, hydrazine hydrate 80% (1.5 mmol) was added. The mixture was then allowed to reflux for 5h. After the completion of the reaction, the reaction mixture was poured into ice water to afford the crude pyrazole, which was then purified via recrystallization from methanol to afford the pure compound (**JP-17**).

Yellow solid; yield: 24%; m.p.: 113-114˚C; λ_{max} (MeOH): 304 nm

IR (KBr cm^{-1}): 3013 (C-H stretch), 1657 (C=O), 1585 (C=N stretch), 1522 (C=C aromatic), 1148 (C-S stretch), 1215 (C-N stretch).

^{1}H NMR (400MHz, $CDCl_3$, TMS = 0) δ: 8.63-8.61 (1H, m), 8.09 (1H, d, J = 8.0 Hz), 7.70 (1H, d, J = Hz 7.72), 7.34-7.30 (1H, m), 7.17 (1H, d, J = 6.2 Hz), 7.03 (1H, s), 6.91 (1H, d, J = 5.04 Hz), 5.92 (1H, dd, J = 4.20 Hz and 11.52 Hz), 3.82 (1H, dd, J = 11.56 Hz and 18.52 Hz), 3.59 (1H, dd, J = 4.20 Hz and 18.52 Hz), 2.41 (3H, s).

^{13}C NMR (100 MHz, $CDCl_3$, TMS = 0) δ = 169.1, 155.4, 150.6, 149.4, 144.1, 136.3, 126.7, 124.8, 124.6, 124.4, 121.2, 55.5, 41.7, 22.0

5.2 Biological Experiments

5.2.1 General

5.2.1.1 Chemicals

1. Media used for culture of the cancer cell lines - RPMI 1640 and DMEM, Penicillin/ Streptomycin antibiotic solution, phosphate buffer saline and fetal bovine serum were purchased from HiMedia.
2. MTT dye employed for MTT assay was purchased from HiMedia.
3. DMSO, extrapure AR was purchase from SRL.
4. H_2DCFDA (from Molecular Probes).

5.2.1.2 Instruments

1. Cells were counted on the automated cell counter, Countess from Invitrogen.
2. Cells were incubated in the presence of carbon dioxide at 37˚C in incubator – Galaxy, New Brunswick.
3. Centrifugation was done in refrigerated Centrifuge 5430 R from Eppendorf, Germany was used.
4. Magnus, Olympus inverted microscope was used for observing the cancer cells.
5. 6 well/24 well/96 well plate Costar 3590, Corning Incorporated were used.
6. All experiments involving cell culture was carried out in aseptic condition under laminar air flow fitted with UV lamps and HEPA filters, purchased from Macro Scientific Works, New Delhi.
7. UV-VIS Spectrophotometer, Shimadzu was used for measuring absorption in antioxidant assay.
8. MTT reading was taken on the microplate reader at G S Diagnostics, Bathinda.

5.2.2 A. Cell lines under study

Four cancer cell lines – MCF-7, T-47D, A-549 and H-460 – used for the purpose of evaluation of anticancer assay were procured from National Cell Repository at National Centre for Cell Sciences, Pune.

A-549, non-small cell lung cancer, first derived from the human alveolar carcinoma (Giard et al., 1973) was observed to contain short and small microvilli on its surface in addition to multilamellar cytoplasmic inclusion bodies which are characteristic of those found in type II alveolar epithelial cells of the lung (Lieber et al., 1976). These spherical shaped cells (Jiang et al., 2010) having a modal chromosome number of 66 could synthesize lecithin with high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway (A.T.C.C.; Lieber et al., 1976).

H-460, human alveolar basal epithelial adherent cells derived from pleural effusion of lung carcinoma is a large cell lung cancer cell line. It has a modal chromosome number of 57 (A.T.C.C.) and are known to contain a subset of stem like cells responsible for its anchorage-independent floating spheres, great proliferative potential exhibiting enhanced tumorigenicity and self-renewal capacity (Shi et al., 2012).

MCF-7, the first breast cancer line observed to be hormone responsive, was derived from pleural effusion from a patient suffering from metastatic breast cancer and were observed to retain several characteristics of differentiated mammary epithelium, including the cytoplasmic estrogen receptor (Soule et al., 1973). Experimental observations revealing the presence of numerous receptors as well as biological responses to a variety of hormones such as estrogen, androgen, progesterone, glucocorticoids, EGFR, IGF etc., make the MCF-7 an ideal in vitro model for exploring the relationship between binding and biological action of these hormones especially in case of tumor response to endocrine therapy (Horwitz et al., 1975; Levenson and Jordan, 1997; Osborne et al., 1987). These cells with a population doubling time of 38 h and showing 88 modal chromosomes (Horwitz et al., 1975) express the WNT7B oncogene (Huguet et al., 1994).

T-47D are estrogen responsive cell line derived from the infiltrating ductal carcinoma of the human breast epithelial adherent cells. These genetically unstable cells express high levels of progesterone receptors. They pose as ideal in vitro models for investigating the cellular and molecular events which lead to hormonal and antihormonal resistance in breast cancer cells (Fernandez et al., 1998). These cells having a modal chromosome number of 65 (A.T.C.C.) also express WNT7B oncogene (Huguet et al., 1994).

B. Control cells used in study

Human peripheral blood lymphocyte cells were used for the purpose of evaluation of antioxidant activity of some compounds.

C. Dye used for antioxidant assay

The dye employed for the purpose of quantitating the free radicals in the antioxidant assay is the cell permeant dihydrodichloro flurouscien (H_2DCFDA) dye. This free radical sensor, also known as dichlorofluores cin diacetate, is mainly the chemically reduced form of fluorescein. It is a general oxidative stress indicator which within the cell undergoes oxidation to give a colored compound which is read spectrophotometrically at excitation wavelength of 485 nm and emission wavelength of 530 nm. Their carboxy and carboxy methyl analogues are also available (Abcam; Life Technologies).

5.2.3 Routine assay in cell culture laboratory

5.2.3.1 Culturing of the cell lines

Cancer cell lines were passaged in the appropriate medium (DMEM). As the cancer cell lines are adherent cells, trypsin is added to detach them from the surface in a process known as trypsinization. After detachment of cells, the action of trypsin was inactivated by the addition of 1mL of media containing serum. Cells were harvested by centrifugation at 1200 rpm at 37˚C for 10 minutes. The supernatant was discarded and the cell pellet was resuspended in 2 mL of the media. The cell number

was counted using automated cell counter. The cells were transferred to fresh media every three days.

5.2.3.2 Maintenance and sub-culturing of cell lines

The cancer cell lines were cultured and maintained in 25 cm^2 or 75 cm^2 flasks containing DMEM medium supplemented with 10% fetal Bovine serum (FBS), 1X antibiotic solution and thereafter incubated at 37˚C in a humidified atmosphere containing 5% CO_2 and 95% humidity.

Sub-culturing was done in 25 cm^2 flasks and becomes important when the cell lines have attained 70-80% growth. The reagents required for the purpose must be placed in water bath maintained at 37˚C for 10-15 mins prior to the sub-culturing. During sub-culturing, trypsin is added for the purpose of detaching these adherent cells from the surface. After 5 mins during which the trypsin would have acted, 1 ml of media containing serum is added in order to terminate the action of trypsin. Cells were transferred to 15 mL centrifuge tubes and centrifuged for 10 mins at 1200 rpm. The supernatant is discarded and the pellet is again resuspended in complete media. The cell lines are transferred to fresh media every three days.

5.2.4 Evaluation of anticancer activity of the synthesized compounds

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) Assay:

MTT assay is a colorimetric assay (Mirzayans et al., 2007; Mosmann, 1983) which is a cell proliferation or cell viability assay. MTT, a yellow tetrazole, is 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium salt of bromine which is reduced to purple formazan in living cells. Mitochondrial reductase (succinate dehydrogenase) has the ability to reduce the MTT to a purple colored formazan product. This conversion is achieved only in the metabolically active cells and not otherwise.

The underlying principle of MTT assay involves the tetrazole dye entering the cells. If the cells are metabolically active then they are reduced by succinate dehydrogenase to the formazon product (**Figure 5.2.1**). This formazan is not easily solubilized; thus either dimethyl sulfoxide, an acidified ethanol solution or a solution of

detergent SDS in dil. HCl is added. The absorbance of the colored product is read spectrophotometrically at wavelength between 500-600nm.

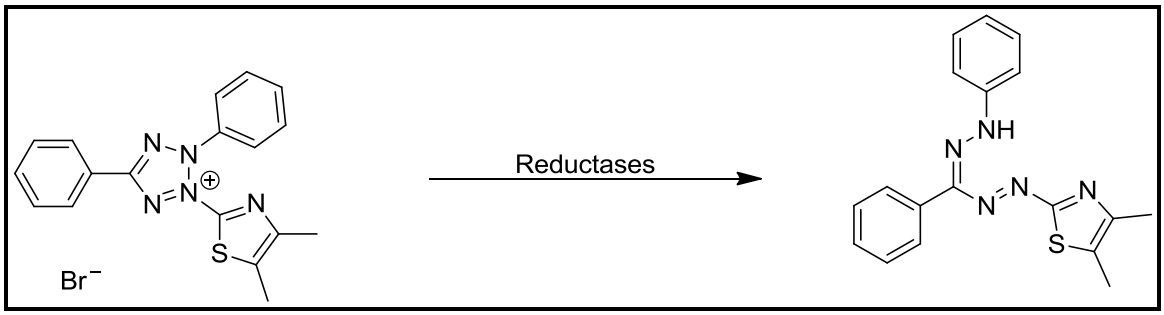


Figure 5.2.1 Reduction of MTT by Reductases

The cell from the cancer cell line – MCF-7, T-47D, A-549 and H-460 were counted on the automated cell counter. Approximately 8,000-10,000 cells were seeded in each well of the 96 well plate (**Figure 5.2.2**). This plate was incubated in incubator maintained at 5% CO_2 at 37˚C for 24 h. At the end of this time duration, treatment were given to the cells in triplicate concentrations of 5 µM, 25 µM and 50 µM and incubated for 48 h. The media is removed from each well and replaced with MTT solution (5 mg/10mL) and incubated in the dark for 4 h. At the end of 4 h, the MTT solution was discarded from each well and the intracellular precipitate was dissolved in DMSO solution and the absorbance of the violet color formed as consequence of DMSO addition is read on the microplate reader at 570 nm. The data is expressed as % inhibition (Mean ± S.D.).

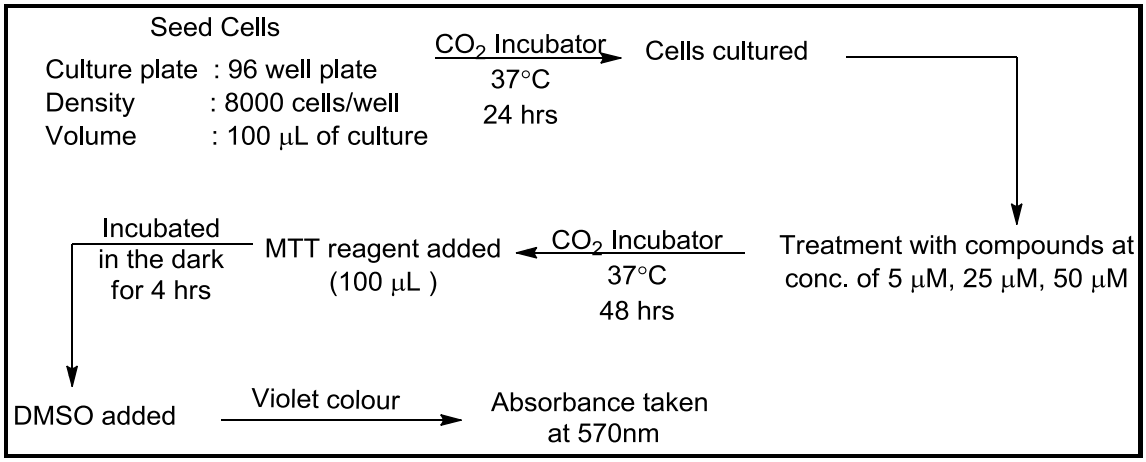


Figure 5.2.2 Schematic Representation of MTT Assay Protocol

5.2.5 Antioxidant assay

Free radicals are the highly reactive species produced within the body in response to various stress which may be environmental, physical or mental. Reactive oxygen species (ROS) encompasses reactive species such as superoxide; hydrogen peroxide; hydroxyl radical; hydroxyl ion; and nitric oxide. ROS are produced mainly as byproducts during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes and metal catalyzed oxidation and possess the potential to stimulate a cascade of undesirable and fatal events, thus warranting its detection and prevention.

Antioxidants play an indispensable role in preventing such free radicals from affecting the cells thus, protecting them.

The underlying principle for antioxidant assay (**Figure 5.2.3**) was based on the fact that oxidation of 2', 7' dichlorofluorescin (H_2DCF) takes place within the cell to give 2'-7'dichlorofluorescein (DCF) which is coloured and the absorbance of which can be measured spectrometrically. Oxidation of H_2DCF by ROS converts the molecule to 2', 7' dichlorodihydrofluorescein (DCF), which is highly fluorescent.

In the experiment performed, hydrogen peroxide was employed as the initiator of free radicals and dihydro dichlorofluroscien diacetate (H_2DCFDA) was used for the detection and quantification of reactive oxygen species. The absorbance was read at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

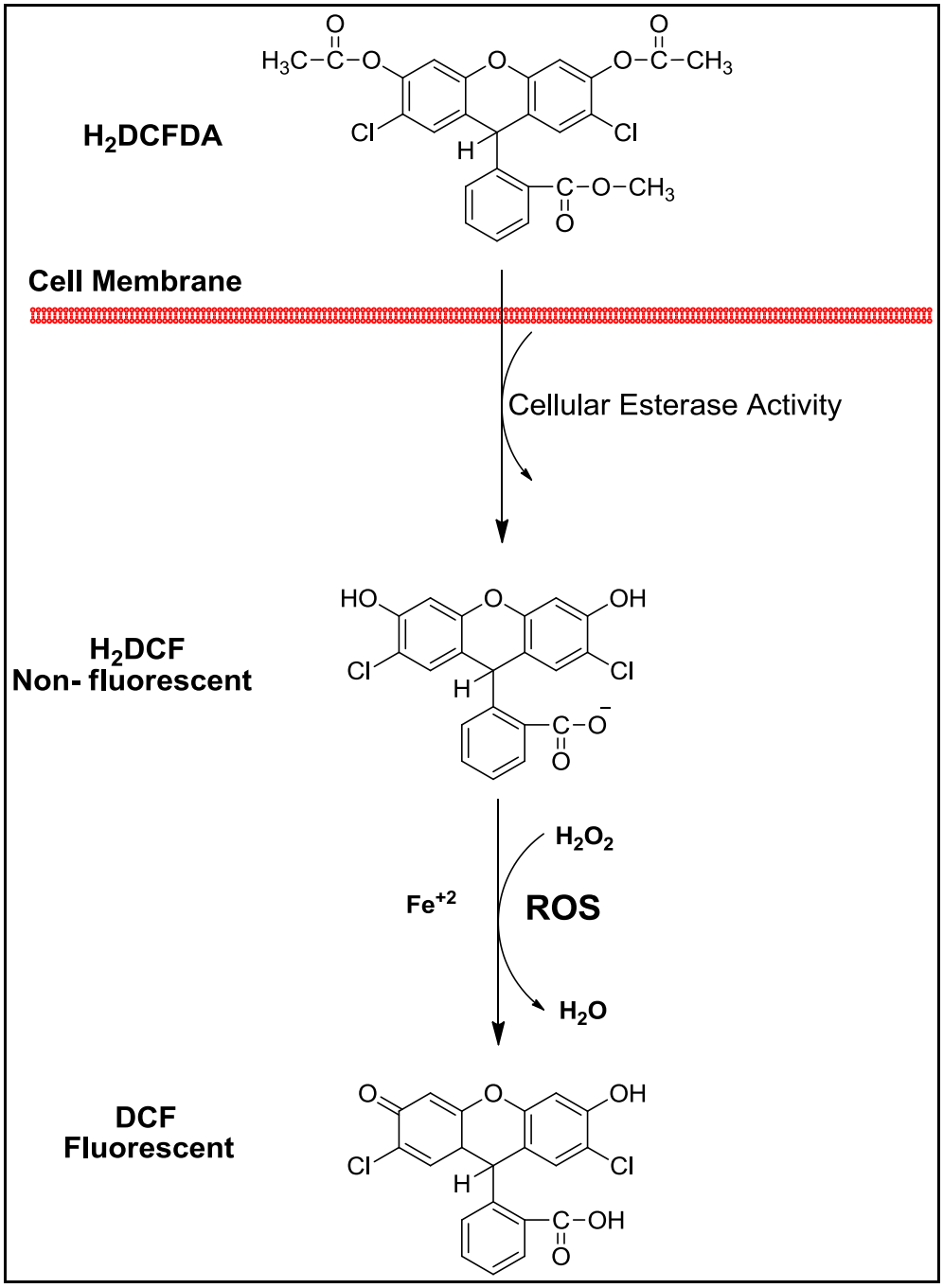


Figure 5.2.3 Conversion of H_2DCFDA to DCF within the Cell

Four compounds – **JP- 5**, **8**, **12** and **14** were tested for their antioxidant potential of which only one was observed to possess good antioxidant activity and the other compounds tested showed no significant decrease in the level of free radicals.

5.2.5.1 Antioxidant Assay Protocol

5 mL of human blood was taken and processed with RBC lysis buffer to get the lymphocytes and counting was done using trypan blue assay in the automated cell counter, 10,000 cells were seeded into 12 wells of the microtitre plate. The compounds to be evaluated were added in triplicate to the respective cells in concentration such that the final concentration of each well would be 5 µM. Various treatment strategies were used in the experiments as detailed in **Figure 3.2.4**.

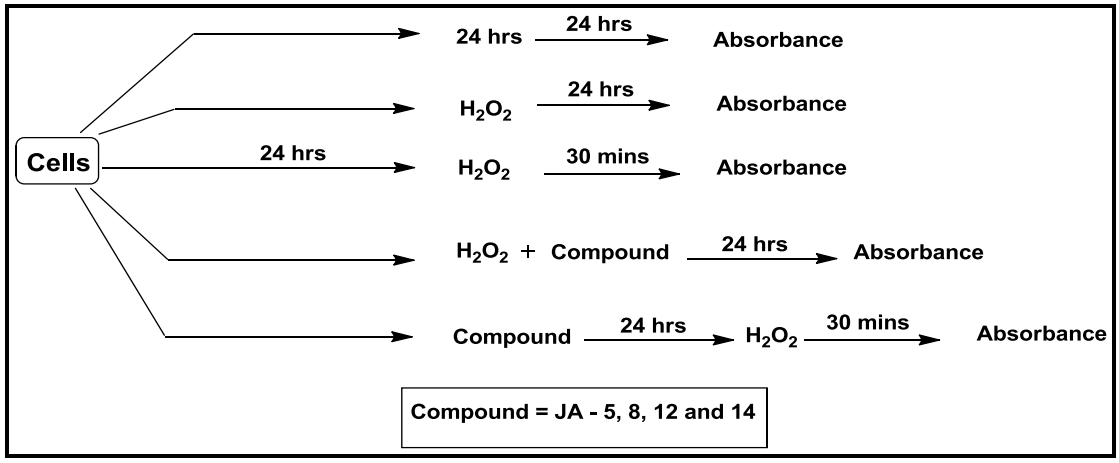


Figure 5.2.4 Treatment Plan Employed in Antioxidant Assay

Briefly cells were incubated for 24 hours followed by treatment either with compounds alone, compounds + H_2O_2 or H_2O_2 alone for 24h followed by other treatments as shown in the **Figure 5.2.4**. After the treatments, 4 µL of H_2DCFDA dye was added in the cultured cells and kept in dark for 30 minutes followed by absorbance at emission wavelength of 530 nm. The all the treatments were done in triplicates.

CHAPTER VI
RESULTS AND DISCUSSION

6.1 Synthesis:

Research over the years has led to the development and introduction of several synthetic routes for the efficient synthesis of pyrazolines. Mostly the starting materials required to prepare pyrazoline compounds are obtained either via Claisen-Schmidt condensation or through synthesis of 1,3-dicarbonyl compounds. Some of the retro-synthetic approaches for the construction of pyrazolines have been sketched in **Figure 6.1.1**. One of the strategies involved is the parallel solution phase synthesis of **1** which involves the use of polymer bound bases A and B as depicted in route **A** (Bauer et al., 2000). Although routes **B** and **G** use the same reactants **3**, route **B** follows microwave irradiation (Kumari et al., 2012) unlike route **G** in which the reactants are subjected to heating at 120 ºC. Route **B** employed an efficient, rapid, and green synthesis under solvent free conditions in the presence of scandium triflate [$Sc(OTf)_3$] resulting pyrazoline (**1)** in yield ranging from 74-92% in 5 min and silica chloride (route **G**) catalyzed one pot cyclocondensation (Jawale et al., 2011) afforded yield of 80% in 2h. Suzuki-Miyaura reaction (Khera et al., 2011) exploiting $Pd(OAc)_2$ (5 mol %) as a catalyst in the presence of SPhos (10 mol %) in an aqueous solution of K_2CO_3 (2 M) (route **C**) afforded pyrazoline in 60-66% yield in 8h. Yet another strategy employed included solvent-free synthesis (Suryakiran et al., 2006) as in route **D** which used equivimolar concentration of methanesulphonic acid and **5** at 80 º C giving a yield of 95% in 45 min. Route **E** followed asymmetric synthesis (Mahé et al., 2012) through an enantioselective phase transfer organocatalytic addition of N-Boc hydrazine to **2** followed by a transprotection sequence allowing N-Boc transformation into N-Ac or other functional groups resulting in **1**. Route **F** followed a simple yet highly efficient and environment friendly one-pot condensation reaction (Wen et al., 2011) of **6** with tosylhydrazide in water yielding 74-92%. Route **H** followed a regioselective synthesis of **1** (Tinarelli et al., 2011) by acylation of N-Boc-N-methylhydrazones followed by TFA giving a yield of 95-98%. However, we have followed the simple route as shown in

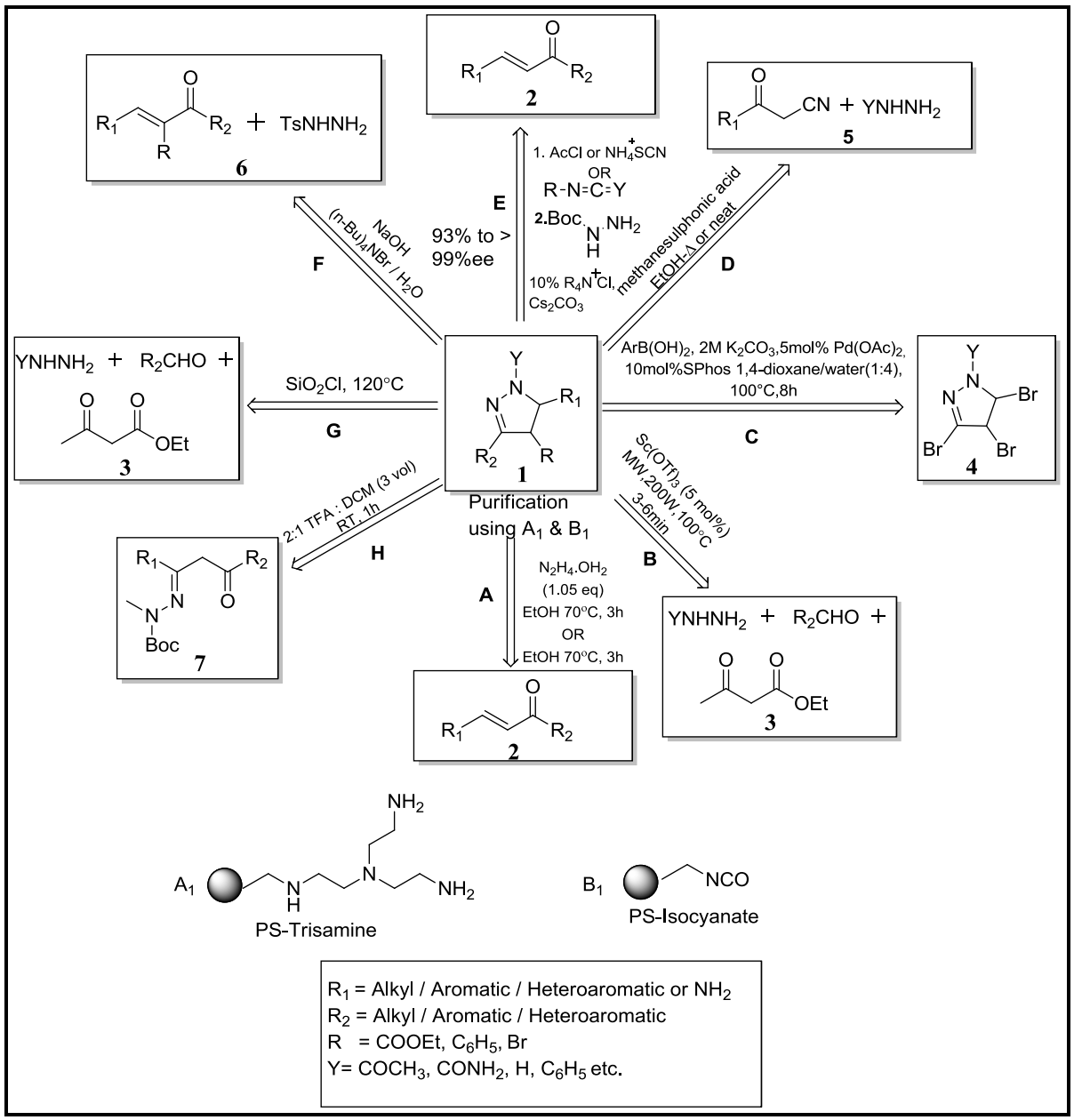
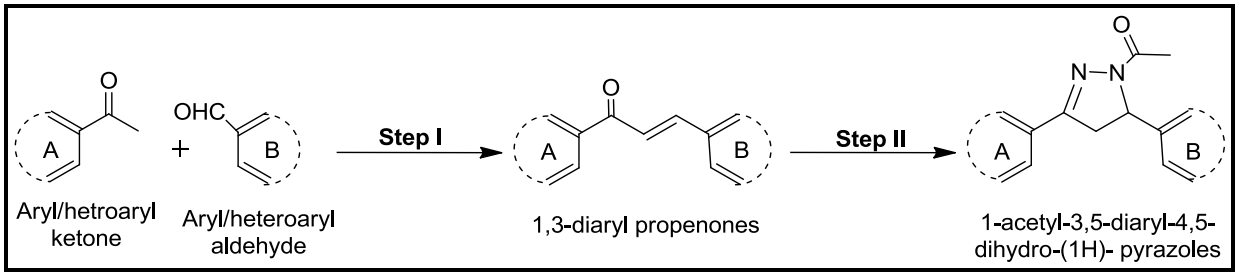


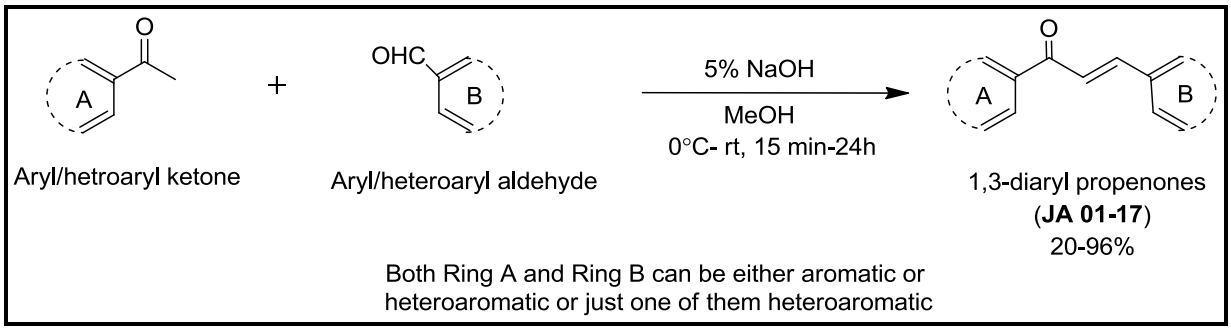
Figure 6.1.1: Retrosynthetic Approaches for the Synthesis of Pyrazoline Derivatives



Scheme 6.1.1: General Route for the Synthesis of the Pyrazoline Derivatives

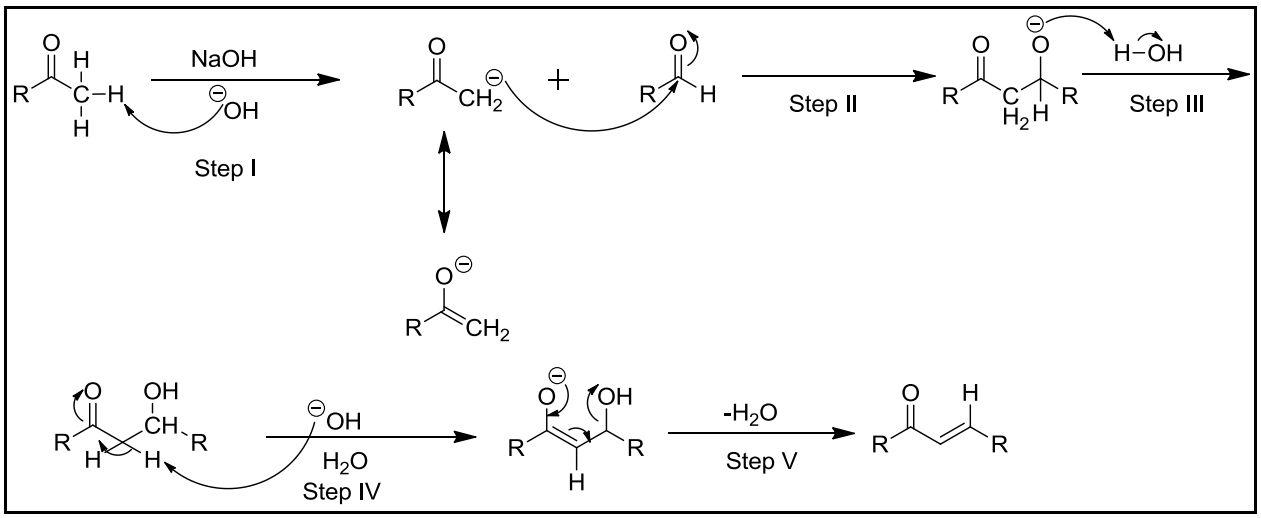
6.1.1 Synthesis of 1, 3-diaryl propenones (JA 01-17)

In the first step synthesis of 1, 3-diaryl propenones**,** commonly known as chalcones was carried out by Claisen-Schmidt condensation of various substituted aryl aldehydes with aryl ketones at room temperature in the presence of sodium hydroxide as the base and methanol as the solvent as shown in **Scheme 6.1.2.**



Scheme 6.1.2: Synthesis of 1, 3-Diaryl Propenones

Mechanistically, base catalyzed Claisen-Schmidt condensation is characterized by the formation of an enolate ion of the ketone. As represented in **Scheme 6.1.3**, formation of enolate ion (Step I) takes place as the consequence of hydrogen abstraction (alpha to the carbonyl moiety) from the ketone by the base (NaOH). This enolate ion acting as the nucleophile attacks at the electrophilic carbon of the aldehyde (Step II) in the process rendering the oxygen electron rich which takes up the proton from aqueous solution (Step III). The final step of the reaction is dehydration (Step V) which affords the 1, 3-diaryl propenones (**JA 1-17**).



Scheme 6.1.3: Plausible Mechanism for Formation of 1, 3-Diaryl Propenones

During the synthetic process, it was observed that the time taken for the completion of the reaction varied from compound to compound. The reaction time was less in case of compounds where electron withdrawing groups were present on the aryl aldehyde or ketone (**JA - 4, 5, 7, 8 and 14**) as compared those containing electron releasing group (**JA - 2, 3, 9 and 10**). Although, most of reactions occurred at room temperature, in certain cases the reaction occurred only in cold condition. The crude 1, 3-diaryl propenones (**JA-1-17**; **Figure 6.1.2**) thus obtained were purified by recrystallization from methanol.

In all the cases, the completion of the reaction was ascertained primarily by TLC. The compounds were characterized by their melting point values, FT-IR and NMR Spectra. UV spectroscopy also assisted in confirming the formation of 1,3 diaryl propenones since the λ_{max} obtained were in high range of 342-359 nm which can be attributed to the presence of the α, β-unsaturated system in conjugation with the carbonyl compounds. Moreover, in compounds with the substitution of auxochromes such as —Cl, —Br, —OCH_3 the λ_{max} values were seen to undergo bathochromic shift (353-359 nm).

IR spectra showed the peaks for carbonyl group of the 1, 3-diaryl propenones in the range of 1668-1644 cm^{-1}, which were much lower than the normal value of 1710 cm^{-1}. This lower than expected value for the carbonyl group, believed to be due to conjugation of α, β double bond with the carbonyl compound, confirms the formation of 1, 3-diaryl propenones. Spectrum also represented other peaks at 1177-1100 cm^{-1} (C-S stretch) in case of thiophene, 1284-1281 cm^{-1} (C-O stretch) of furan, 1653-1585 cm^{-1} (C=C aromatic).

^{1}H NMR spectrum of the representative compound showed the formation of C=C bond which was determined by calculating the coupling constant for the vicinal protons (J = 14.9 Hz) which appeared in the range of 12-18 Hz, confirming the formation of the trans isomer. Chemical shift values for these protons appeared at 7.09 ppm and 7.84 ppm and the J value for these protons was calculated to be 14.9 Hz. ^{13}C spectra confirmed that the product formation by indicating the peak for the carbonyl carbon at a downfield value of 189.13 ppm. Other characteristic peaks which

appeared in the spectra were that of the aromatic ring carbons at a range of 119-148 ppm and that of the thiophene ring appeared at 122.8 -137.02 ppm. The peak obtained for C=C at 129.2 ppm and 132.2 ppm again confirmed the formation of 1, 3-diaryl propenones. The melting point for these compounds were found to be in the range of 60-98˚C with the exception of some compounds which showed melting point in the range of 112-116˚C.

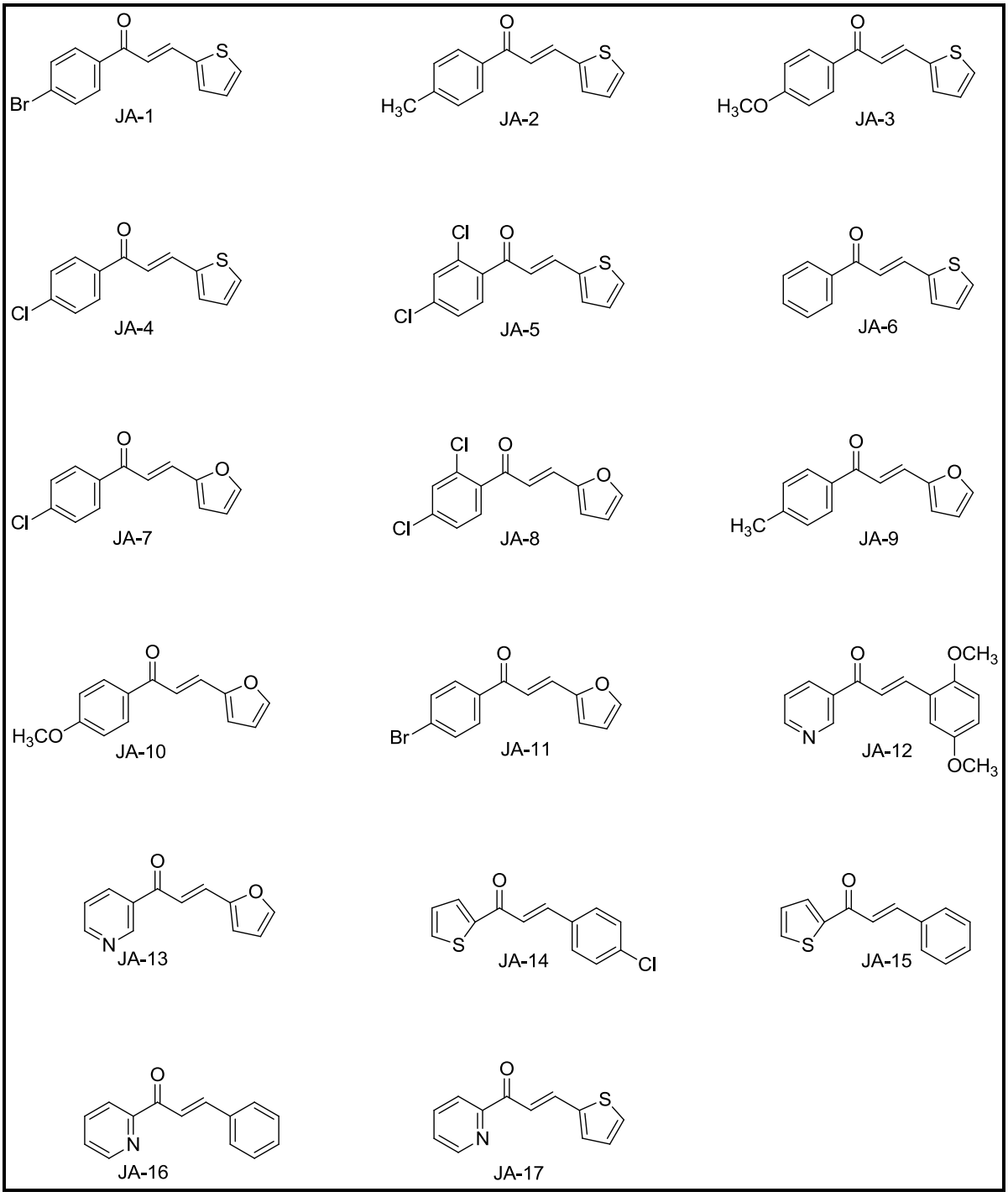
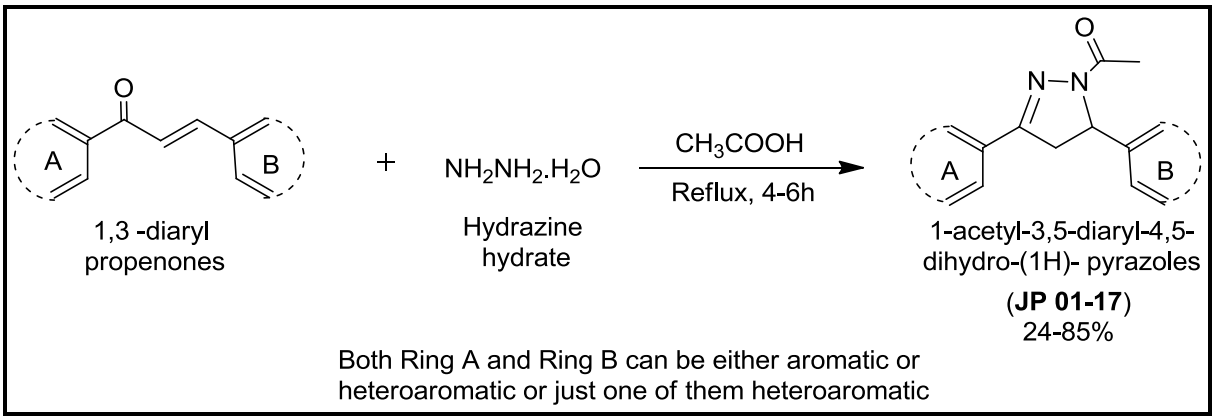


Figure 6.1.2 Chemical Structures of Synthesized 1, 3-Diaryl Propenones

6.1.2 Synthesis of 1-acetyl-3,5-diaryl-4,5-dihydro-(1*H*)- pyrazoles (JP 01-17)

In the second step, synthesis of 1-acetyl-3,5-diaryl-4,5-dihydro-(1H)-pyrazoles or 2H-pyrazolines (**Scheme 6.1.4**) was carried out by Michael condensation of

hydrazine hydrate with 1, 3- diaryl propenones followed by cyclisation (via imine bond formation) and N- acetylation in the presence of acetic acid under reflux.



Scheme 6.1.4 Synthesis of **JP 1-17**

Time taken for the reaction to reach completion varied with the substituents present; reaction with 1, 3-diaryl propenones consisting of electron withdrawing group occurred relatively faster than those bearing electron releasing group. The synthesis of pyrazoline containing pyridine ring was also a time consuming reaction taking about 5 h to reach completion (**Figure 6.1.3**).

The completion of the reaction was determined primarily by TLC and confirmed by melting point and various spectroscopic techniques such as UV, IR and NMR spectroscopy. UV spectroscopy confirmed the complete conversion of 1, 3-diaryl propenones into pyrazolines as indicated by the lower λ_{max} value of 297-328 nm as compared to former. This is mainly due to absence of conjugation as was seen in case of 1, 3-diaryl propenones.

IR spectra showed the carbonyl absorptions due to N-acetyl group in the range 1664-1657 cm^{-1} (C=O), which were much lower than the normal value of 1710 cm^{-1}, owing to conjugation between the nitrogen lone pair and the carbonyl double bond. Other absorptions that confirmed the formation of pyrazoline is the absorption appearing in the range of 1632-1590 cm^{-1} for C=N of the pyrazoline ring and that appearing in the range of 1223-1176 cm^{-1} for C-N of the pyrazoline ring. Spectrum also represented other peaks at 1176-1014 cm^{-1} (C-S stretch) in case of thiophene, 1250-1256 cm^{-1} (C-O stretch) of furan.

^{1}H NMR spectra of the pyrazoline derivatives showed the characteristic ABX system with three double doublets out of which one double doublet was sufficiently deshielded and appeared more downfield than the other two at a chemical shift (δ) value of 5.55-5.91 ppm with the J values appearing in the range of 3.76-12 Hz. The chemical shift (δ) values for the other two proton appeared in the range of 3.15-1.47 and 3.56-3.86 ppm with their J values in the range of 3.90 and 18 Hz; 11.36 and 18.56 Hz, respectively. These three double doublets represent the three protons of the pyrazoline nucleus; confirming its formation. The protons of the benzene ring appeared in the range of 7.24-7.78 ppm, varying according to the substitution on the ring; such as in case of electronegative atom the peak would appear more downfield at a value of 7.62-7.7 ppm. Likewise, for pyridine, the peaks for protons appeared at 8.92-7.2 ppm owing to high electronegativity. The methyl and methoxy protons showed a sharp singlet in the range of 2.30-2.40 and 3.75-3.80 ppm, respectively. The protons of furan appeared in the range of 6.30-7.30; on the contrary the peaks for the protons of the thiophene nucleus appeared at 6.91-7.45 ppm.

^{13}C NMR spectra obtained for the representative pyrazoline compound showed characteristic peaks like for carbonyl carbon of the acetyl moiety, C-N, CH_2, CH_3 at 169.1, 55.5, 41 and 22 ppm, respectively. The melting points for these compounds were found to be in the range of 107-157˚C with the exception of two compounds for which melting point appeared in the range of 89-98˚C.

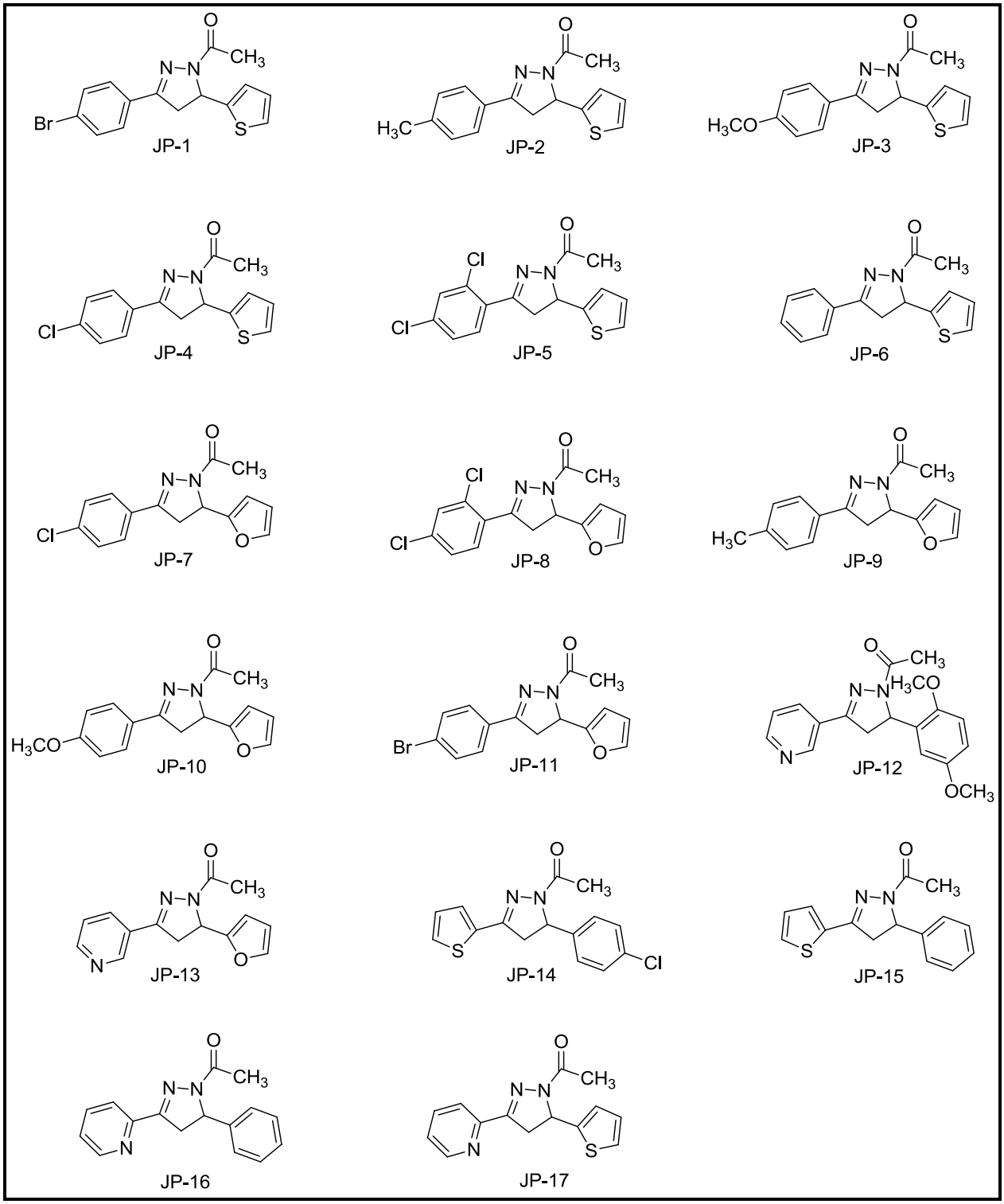


Figure 6.1.3 Chemical Structures of Synthesized 1-Acetyl-3,5-diaryl-4,5-dihydro-(1H)-pyrazoles

6.2 Biological Experiments

6.2.1 Evaluation of anticancer properties of the compounds:

With an aim to the test the anticancer potential of the newly synthesized compounds (**JP-1**-**17**), MTT assay was carried out with breast (MCF-7 and T-47D) and lung (A-549 and H-460) cancer cell lines. Approximately 8,000-10,000 cells were seeded per well of 96 well plate, overnight and treatments as indicated in the experimental design were given in triplicates. The results highlighted compounds- **JP - 1, 3, 4, 5, 7, 14** and **15** which showed significant growth inhibition in breast cancer cells with little or no effect on the lung cancer cells A-549. MTT assay carried out with another lung cancer cell line- H-460 showed very little or no activity, thus, confirming the insensitivity of the compounds against lung cancer cells.

Anticancer potential of certain compounds against the MCF-7 cell lines encouraged us to evaluate the anticancer activity of these compounds against another breast cancer cell line - T-47D. However, the MTT results indicated that there was little or no effect of the compounds on these breast cancer cells. MCF7 cells are originated from non-metastatic fibro-adenoma of the breast while T-47D is derived from IDC (Infiltrating Ductal Carcinoma) of the breast which is highly metastatic in nature in addition to possessing proteins such as G1/S-specific cyclin-D3 and prohibitin which stimulate cell growth, anti-apoptosis and carcinogenesis (Aka and Lin, 2012). Our results thus indicated that due to strong defense against apoptosis and aggressive nature, the T-47D cells are resistant to the anticancer compounds. On the other hands MCF-7 showed significant sensitivity towards the compounds. When tested on normal human cells, these compounds showed no effect on cell viability (results not shown).

Four cell lines- MCF-7, A-549, H-460 and T-47D were seeded in a 96 well plate separately and subsequently treated with the compounds at different concentrations and allowed to incubate for 48h. The absorbance was measured on the microplate reader at 570 nm. The values obtained as a result of three

independent experiment in triplicates were expressed as % inhibition (Mean ± S.D.) and graphs for the percent inhibition were plotted.

All the compounds were tested against MCF-7 and A-549 cancer cell lines but selected compounds were further tested against H-460 and T-47D cancer cell line. Detailed below are the MTT assay results against different cell lines:

1. **MCF-7:** As evident from **Figure 6.2.1** and **Table 6.2.1**, it was observed that some of the compounds especially **JP-1, 3, 4, 5, 7, 14 and 15** showed significant antiproliferative activity against the MCF-7 cell line. The compounds achieved good inhibitory potential mainly at concentrations of 25 µM and 50 µM although one or two compounds were observed to exhibit antiproliferative activity at 5 µM also.

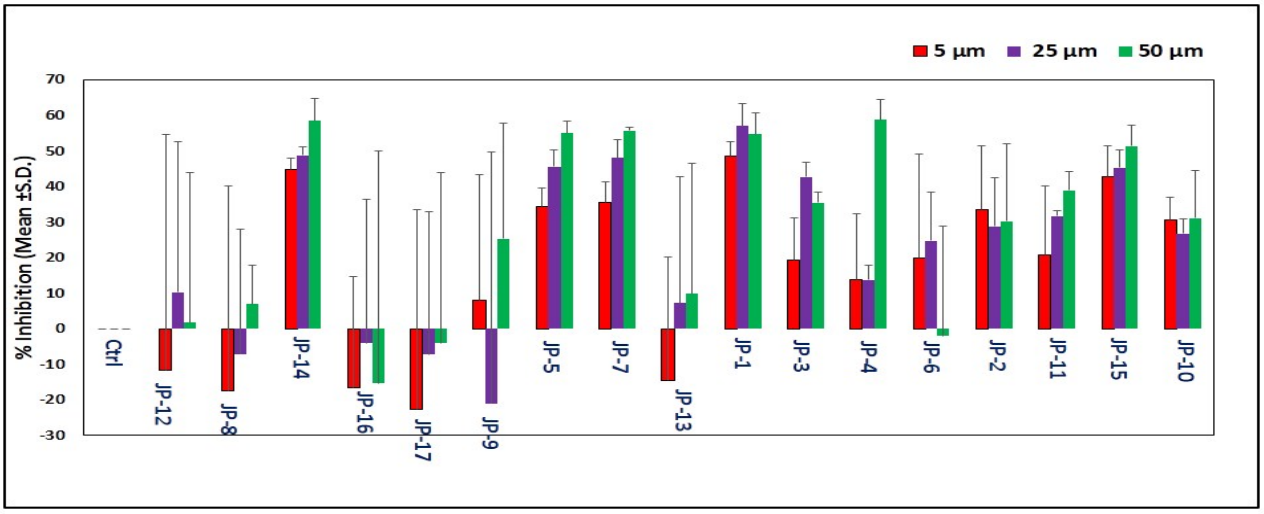


Figure 6.2.1 Percent inhibition of MCF-7 in response to treatment with synthesized compounds at concentrations of 5 µM, 25 µM and 50 µM for a time duration of 48 hrs. Data is expressed as mean values ± S.D. of three independent experiments.

Table 6.2.1 Compounds Exhibiting Antiproliferative Activity against MCF-7 Cell Line

Compound	% Inhibition		
	5 µM	25 µM	50 µM
JP-1	48.4	56.9	54.5
JP-2	33.5	28.6	30.2
JP-3	19.2	42.5	35.3
JP-4	13.8	13.7	58.7
JP-5	34.2	45.5	54.8
JP-6	19.7	24.6	-2.1
JP-7	35.4	47.9	55.7
JP-10	30.4	26.7	31.0
JP-11	20.7	31.6	38.8
JP-14	44.7	48.6	58.4
JP-15	42.8	45.2	51.3

Percent inhibition of MCF-7 in response to treatment with synthesized compounds at concentrations of 5 µM, 25 µM and 50 µM for a time duration of 48 hrs. Data is expressed as % inhibition (Mean values ± S.D.)

2. **T-47D:** Similar assay was performed in another breast cancer cell line, T-47D and the results are summarized in **Figure 6.2.2** and **Table 6.2.2**. As compared to MCF-7 cells, though the inhibitory potential shown by the compounds is low. It can be thus observed that most of the compound showing good activity towards MCF-7 also showed activity against T-47 D cell line. The data obtained can assist in concluding that since MCF-7 is a non-metastatic cancer as compared to T-47D, the compounds may be helpful in inhibiting the MCF-7 cancer cells in the initial stages of cancer progression.

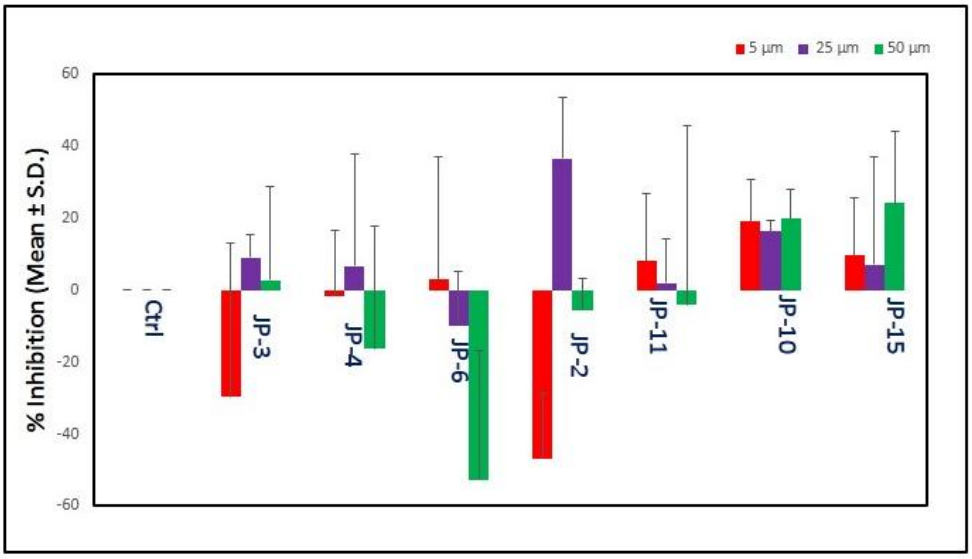


Figure 6.2.2 Percent inhibition of T-47D in response to treatment with synthesized compounds at concentrations of 5 µM, 25 µM and 50 µM for a time duration of 48 hrs. Data is expressed as mean values ± S.D. of three independent experiments.

Table 6.2.2 Compounds Exhibiting Antiproliferative Activity against T-47D Cell Line

Compound	Average		
	5 µM	**25 µM**	**50 µM**
JP-3	-29.7	8.9	2.6
JP-4	-1.7	6.5	-16.3
JP-6	2.8	-9.9	-52.6
JP-2	-46.8	36	-5.4
JP-11	8.0	1.9	-4.1
JP-10	18.0	16.2	19.7
JP-15	9.6	7	24.0

Percent inhibition of T-47D in response to treatment with synthesized compounds at concentrations of 5 µM, 25 µM and 50 µM for a time duration of 48 hrs. Data is expressed as % inhibition.
(Mean values ± S.D.)

3. **A-549: Figure 6.2.3** represents the inhibitory potential of the compounds tested against A-549 cancer cell line. As observed from the graph, the compounds supported the cancer cell lines rather than inhibiting them as evident from the negative values of the inhibitory potential.

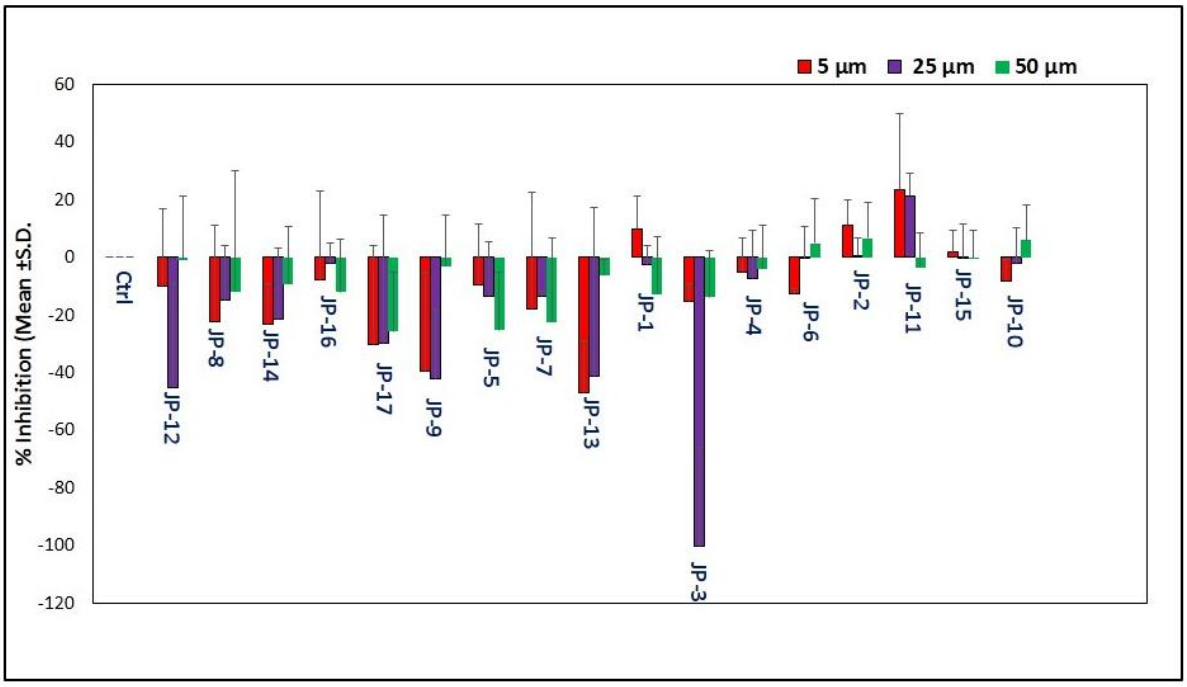


Figure 6.2.3 Percent inhibition of A-549 in response to treatment with synthesized compounds at concentrations of 5 µM, 25 µM and 50 µM for a time duration of 48 hrs. Data is expressed as mean values ± S.D. of three independent experiments.

4. **H-460:** Similar to H-460 results, **Figure 6.2.4** further confirms the unresponsiveness of the H-460 cell lines towards the compounds. In the **Figures 6.2.3 and 6.2.4**, poor efficacy of the compounds towards the lung cancer cell line is highlighted.

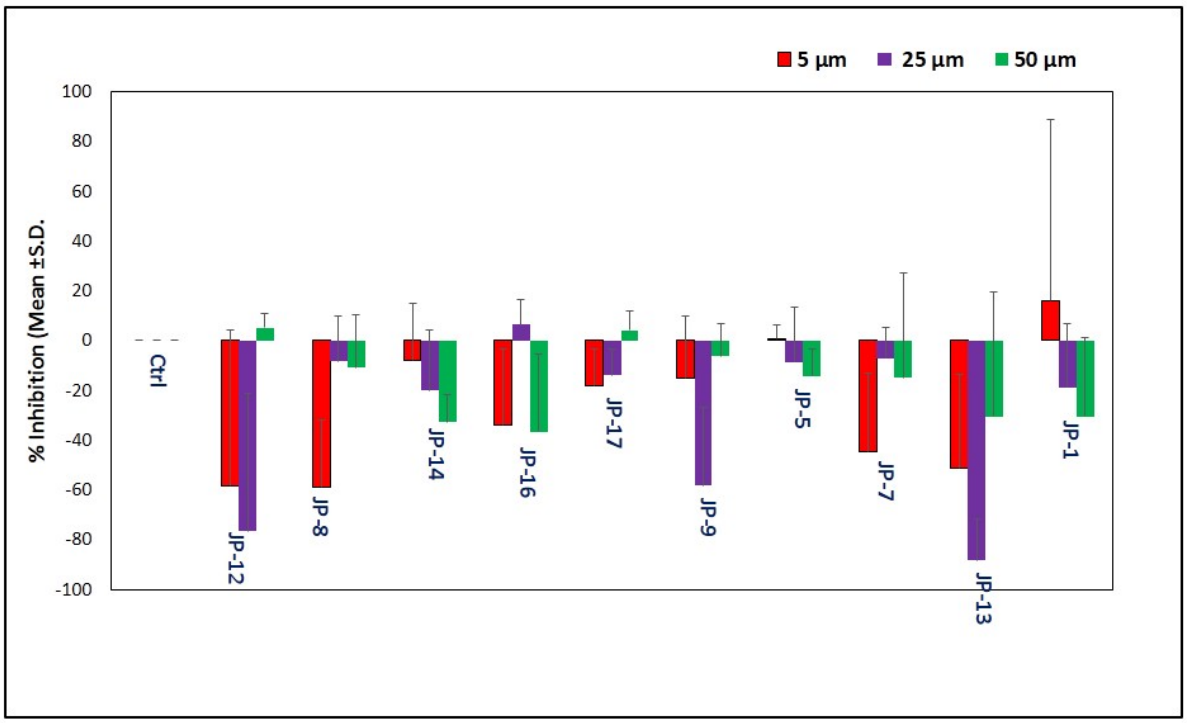


Figure 6.2.4 Percent inhibition of H-460 in response to treatment with synthesized compounds at concentrations of 5 µM, 25 µM and 50 µM for a time duration of 48 hrs. Data is expressed as mean values ± S.D.

6.2.2 Assessing Antioxidant Activity of the Synthesized Compounds

After ascertaining the anticancer potential of the compound, the antioxidant activity of these compounds was also assessed (**Table 6.2.3 and Figure 6.2.5**). We developed an assay whereby free radicals formation was first induced in primary human cells and then their scavenging by the compounds was evaluated. H_2O_2 was served as the free radicals inducer while free radical production was studied using fluorescent H_2DCFDA stain by spectrophotometry. The treatment design is shown in **Figure 5.2.4**. The results indicate that compounds **JP- 5** showed significant antioxidant properties while compound **JP- 8, 12 and 14** did not show any effect. When pretreated with H_2O_2, compound **JP- 5** (when hydrogen peroxide is added for only 30 min rather than 24h) showed significant antioxidant activity.

6.2.2.1 Results for antioxidant assay

Table 6.2.3 Antioxidant assay results for compounds **JP- 5**, **8**, **12** and **14**

S.No.	Treatment	1	2	3	Average
1.	Cells only	0.16	0.16	0.17	0.17
2.	Cells + H_2O_2 (24 h)	0.23	0.24	0.24	0.24
3.	Cells + T1 (24 h)		0.56	0.50	0.54
4.	Cells + T1 + H_2O_2 (24 h)	0.89	0.66	0.79	0.78
5.	Cells + T1 (24 h) + H_2O_2 (30 mins)	0.52	0.49	0.60	0.54
6.	Cells + T2 (24 h)		0.83	0.52	0.68
7.	Cells + T2 + H_2O_2 (24 h)	0.58	0.42	0.47	0.49
8.	Cells + T_2 (24 h) + H_2O_2 (30 mins)	0.47			0.47
9.	Cells + T3 (24 h)		0.51	0.58	0.55
10.	Cells + T3 + H_2O_2 (24 h)	0.50	0.49	0.47	0.48
11.	Cells + T3 (24 h) + H_2O_2 (30 mins)	0.85			0.84
12.	Cells + T4 (24 h)		0.33		0.33
13.	Cells + T4 + H_2O_2 (24 h)	0.49	0.68	0.60	0.59
14.	Cells + T4 (24 h) + H_2O_2 (30 mins)	0.61			0.61

T1, T2, T3 and T4 are compounds JP- 5, 8, 12 and 14 respectively which were tested for their antioxidant activity on blood cells.

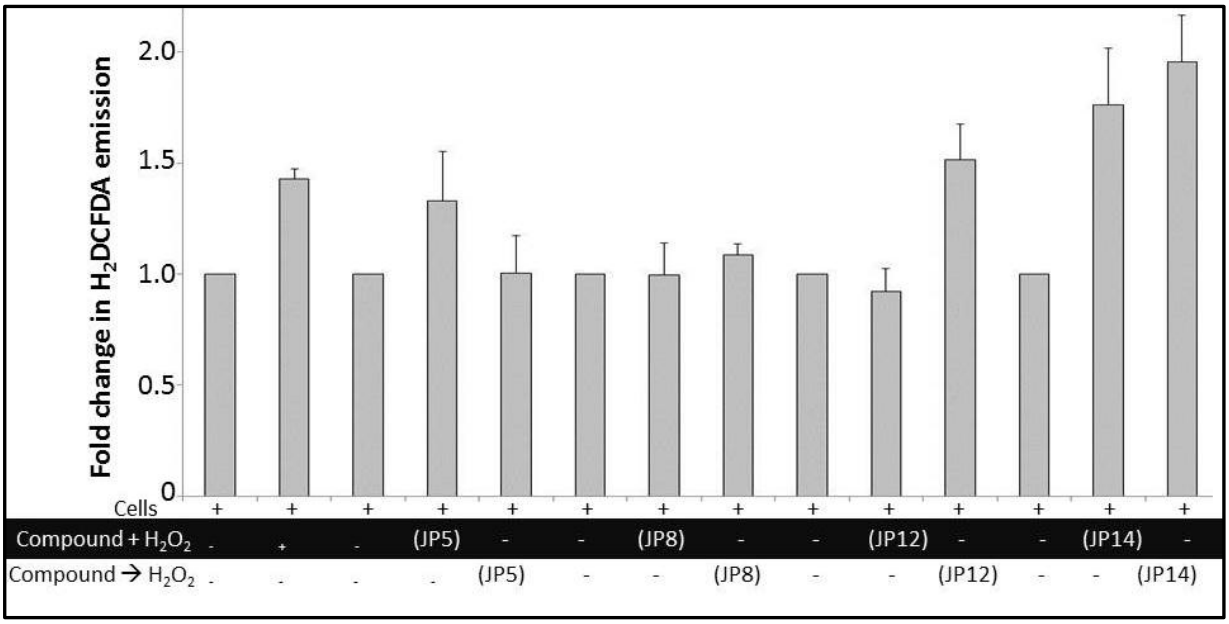


Figure 6.2.5 Graphical representation of the absorbance of the blood cells in response to the compounds JP- 5, 8, 12 and 14. Antioxidant action of the compound in case of pre as well as post treatment with either the compounds or H_2O_2.

Taken together it may be concluded that compounds **JP-1, 3, 4, 5, 7, 14** and **15** show significant anticancer activity selectively against breast fibroadenoma cell line, MCF-7. Though the compounds show significant antioxidant properties as well, but it may not be the primary mechanism of their anticancer action, otherwise proliferation of metabolically hyperactive cell lines like T47D, A549 and H460 would also have been inhibited by the compounds. Thus, further studies are needed to characterize the mechanism of action of these compounds. Such studies will also give insights to the selective action of the compounds against non-metastatic breast cancer cells. Furthermore the results will help us modify the existing compounds to achieve better anticancer actions.

6.2.2 Structural Activity Relationship (SAR) emerged from the biological studies

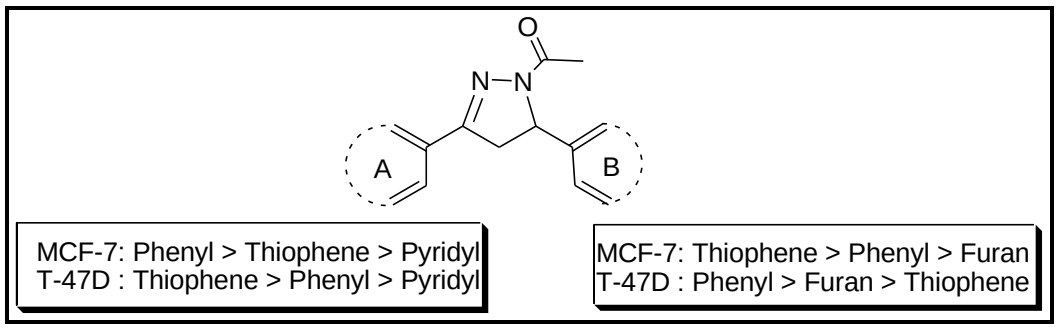


Figure 6.2.6 SAR for Pyrazoline Derivatives

The information about the inhibitory potential obtained from the MTT assay results of MCF-7 (**Figure 6.2.1**), T-47D (**Figure 6.2.2**), A-549 (**Figure 6.2.3**) and H-460 (**Figure 6.2.4**) assisted us in outlining the structure activity relationship for the pyrazoline derivatives (**Figure 6.2.6**). As revealed from these results the pyrazoline derivatives exhibited activity against only two cancer cell lines – MCF-7 and T-47D. Important structural activity relationships emerged from these data which highlighted that role played by ring A and ring B modifications as depicted in the **Figure 6.2.6**.

Modifications on the ring A

Significant inhibitory potential was observed against the MCF-7 cells with phenyl ring (**JP-1-11**) which was also comparable to compounds bearing thiophene ring (**JP-14 and 15**) whereas in case of T-47D the thiophene bearing compound (**JP-15)** was seen to be more active. On the contrary, the phenyl substitution failed to show activity against T-47D (**JP-6**), The compounds with pyridyl ring as ring A were observed to be completely devoid of activity in both MCF-7 as well as T-47D (**JP-12, 13, 16 and 17**).

Modifications on the ring B

Among the active compounds, comparing **JP-1, 2, 3, 4, 5** with **11, 9, 10, 7** and **8** respectively it can be concluded that the thiophene bearing compounds exhibited excellent inhibitory potential against the MCF-7 cells as compared to furan containing compounds. However, analogue containing phenyl ring (**JP-14 and 15**) exhibited better activity than furan containing compounds. In case of T-47D cells, significant

activity is exhibited for derivatives with furan, but to a lesser extent in case of phenyl (**JP-12**) and completely devoid of activity in pyrazolines with thiophene as ring B.

Effect of substituents

Among the thiophene containing derivatives the activity was better for bromo substitution (**JP-1**) than for chloro (**JP- 4** and **5**). Furan containing derivative with chloro substitution (**JP-7**) showed appreciable activity although only at higher concentration of 50 µM. Among the derivatives bearing electron releasing group, $-CH_3$ bearing thiophene derivative (**JP-2**) showed almost twice the activity seen with $-OCH_3$ (**JP-3**) but in case of furan containing compounds, $-OCH_3$ (**JP-10**) exhibited potent activity whereas $-CH_3$ (**JP-9**) exhibited no inhibitory potential.

On the contrary, the MTT results obtained for the T-47D cell line suggested that the compounds bearing electron releasing group especially in $-CH_3$ bearing furan derivative (**JP-9**) exhibited better inhibitory potential than the electron withdrawing group (**JP-7** and **11**). **JP-12,** a pyridine analogue also showed comparable activity. Thiophene containing compounds bearing any electron withdrawing or releasing group exhibited no activity except for an unsubstituted analogue, **JP- 15** which was observed to show potent inhibitory potential against both the breast cancer cell lines.

Antioxidant assay results obtained outlined the compounds bearing electron withdrawing groups especially dichloro substitution (**JP-5**) to be more potent antioxidant than monochloro substituted pyrazoline derivative (**JP-14**) which was completely devoid of activity.

CHAPTER VII

CONCLUSION

Rational and perpetual research in the field of drug design and discovery has been unsuccessful time and again in completely eradicating the most life-threatening of all diseases- cancer. Despite this, unceasing research is being carried to achieve the breakthrough required to resolve the issue. A large number of chemotherapeutically active compounds are known which owe their anticancer potential to the heterocycle ring present in the core structure. Taking cue from such compounds, we also made an effort to synthesize seventeen compounds having pyrazoline ring as the core structure. Furthermore, an effort was also made to substitute either/both C3 and C5 with heterocycle along with acetylation the N1of the pyrazoline. The progression and the subsequent completion of the reaction for each of 17 pyrazoline derivatives of 1, 3-diaryl propenones was confirmed by TLC. The purity of all the compounds were ascertained by melting point, UV spectroscopy, IR spectroscopy and NMR spectroscopy. An attempt was also made to evaluate the compounds for their biological activity- anticancer and antioxidant.

The anticancer potential of the compounds were assessed against a panel of four cancer cell lines viz MCF-7, T-47D, H-460 and A-549 at different concentrations of 5 µM, 25 µM and 50 µM. Some of the compounds showed promising activity against the breast cancer cell line, MCF-7 and among these compounds, some also showed activity against another breast cancer cell line T-47D, although to a very small extent. On the contrary, the pyrazoline derivatives failed to show any significant activity against the lung cancer cell line-H-460 and A-549. Four pyrazoline derivatives were evaluated for their potential to exhibit antioxidant activity especially against reactive oxygen species. Only one of the four compound tested showed significant antioxidant property. In addition, the information about inhibitory potential of the compounds acquired from the MTT assay results helped us to explore the structural relationship studies and highlight the modifications which led to increase in the activity.

In future, tracing the mechanism of action of the active compounds may further assist in design and synthesis of more potent anticancer agents through lead optimization or structure based drug design.

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APPENDIX

MANUSCRIPTS

1. **Alex, J. M.**, & Kumar, R. (2013). 4, 5-Dihydro-1 H-pyrazole: an indispensable scaffold. Journal of Enzyme Inhibition and Medicinal Chemistry, In press: 1-16.

2. **Alex, J. M.**, Singh, S. & Kumar, R. (2013). Anticancer Potential of New N-acetyl Pyrazoline Derivatives of 1, 3-Diaryl/Heteroaryl Propenones: Synthesis and Evaluation. Manuscript under preparation.

3. ^{1}H and ^{13}C and FT-IR Spectrum for compound JA-17 and JP-17.