SYNTHESIS AND CHARACTERIZATION OF PYRAZOLE AND PERIMIDINE CONJUGATES AS ANTICANCER AGENTS

A

Thesis submitted

In the partial fulfilment of the requirement for the degree of

MASTERS OF SCIENCE

IN

CHEMISTRY

Submitted By

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UNDER THE SUPERVISION OF

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PATIALA 147004

2018

CERTIFICATE

This is to certify that the thesis entitled "Synthesis and characterization of Pyrazole and Perimidine conjugates as Anticancer Agents" submitted by **Ms. Jasleen Kaur** in the partial fulfillment of the requirements for the degree of **Master of Science in Chemistry** from **Thapar Institute of Engineering and Technology, Patiala** is a bonafied piece of work carried out under the guidance and supervision of **Dr. Kamaldeep Paul**, Associate Professor, School of Chemistry and Biochemistry, Thapar Institute of Engineering & Technology, Patiala and no part of this project has been submitted for award of any other degree in this or any other university.

Jasleen Kaur

DATE: 29/6/2018 **JASLEEN KAUR**

This is to certify the above statement made by student concerned is correct and true to the best of my knowledge.

Kamaldeep Paul

(Dr. Kamaldeep Paul)
Associate Professor (Supervisor),
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SELF DECLARATION

The work embodied in the project entitled **"Synthesis and characterization of Pyrazole and Perimidine conjugates as Anticancer Agents"** has been done by me in the partial fulfillment of requirement for the award of degree of **Masters of Science in Chemistry,** submitted in the **School of Chemistry and Biochemistry, Thapar Institute of Engineering & Technology, Patiala,** is an authentic record of my own carried out under the supervision and guidance of **Dr. Kamaldeep Paul** Associate Professor, School Of Chemistry and Biochemistry, Thapar Institute of Engineering & Technology, Patiala. All the ideas and references have been duly acknowledged.

Date: 29/6/2018

Place: Patiala

Jasleen Kaur
JASLEEN KAUR

This is to certify the above statement made by student concerned is correct and true to the best of my knowledge.

ACKNOWLEDGEMENT

This six month training is of substantial academic record and is significant for all the M.sc students who have to join PhD or industries with theoretical understanding; this practical knowledge helps us to better understand the scientific concepts.

I have put in my extra effort in this project work, it would not have achievable without the hospitable support of my mentor and supervisor.

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At last but not the least, appreciation goes to my parents for their never-ending love and care, for economical support and for being there at all times when I need their advice the most.

Jasleen Kaur

Place: Patiala

Jasleen Kaur

ABSTRACT

Despite the development of many anticancer drugs, cancer is still most evident cause of death. So, now instead of using single-target therapy for the treatment of cancer, molecular hybridization methodology is useful which involves grouping of different pharmacophoric moieties to generate a new and effective anticancer hybrid drug. Heterocyclic moieties show significant role in treatment of cancer. We have synthesized a series of pyrazole-perimidine hybrids as potential anticancer agents. These compounds were further characterized by ^{1}H and ^{13}C NMR spectrometry. Evaluation of these hybrids for the anticancer activity is in progress.

TABLE OF CONTENTS

1. INTRODUCTION

Cancer is a prominent reason for deaths worldwide and it is increasing day by day. Cancer is recognized by loss of control over propagation of cells, leading perpetually to death.[1] Cancer causes uncontrollable cell division and growth and thus destroys nearby healthy body tissues. A normal human cell divides and grows to form new cells and when cells are damaged or aged, they died, and new cells replace them. But cancer affects this orderly process and these cells become abnormal and old or damaged cells survive instead of being eliminated, and new cells form when they are not needed. These excessive cells can divide uncontrollably and may form massive growth called "Tumors". These tumors can undergo a process called Metastasis and thus causes a life threatening condition until eliminated from body as they can grow and interfere with other physiological functions of body.[2]

World Health Organization (WHO) reported that about 8.8 million individuals passed away because of cancer worldwide in 2015. According to recent analysis, the main reason for lung cancer is smoking that resulted in nearly 33% of 595,690 deaths in USA. Moreover, 20% of all cancers detected are related to physical activity, poor nutrition, excess consumption of alcohol and obesity while some cancers are associated with infections triggered by human papilloma virus (HPV). In India, a woman dies every 8 minute due to untreatable cancer especially, cervical cancer and breast cancer.[3] European Code Against Cancer (ECAC) recommended 12 lifestyle behaviors, therefore, advice not to smoke, to maintain ideal body weight, active routine and balanced diet, to minimize alcohol consumption, to avoid exposure to ultraviolet radiation (especially for children), to participate in vaccination programs of HPV (for girls) etc. Even now, more than half of the adults are fat, more than a quarter still smokes and many fail to meet guidelines of aerobic activities.[4]

There are many methods available for treatments of cancer. These types of treatments are based upon their overall advancements. Treatments of cancer such as surgery, chemotherapy, targeted therapy, or hormone therapy are available. A very comprehensive study and research is being carried out in order to control the outbreak of cancer at genetic level. But as the history signifies the failed attempts, the success at a clinical level is very low.[5] A number of drugs has been synthesized and optimized on molecular level and their anticancer activities were studied against various cancer cell lines, some of them are successful at various clinical trials. But there is need to develop much effective drug which provides a relief at an early diagnostic stage. The most effective anticancer remedy is chemotherapy. Drug resistance and toxicities limits the use of available chemotherapeutics.

Chemotherapy is resisted by increase in the creation of the target protein, unusual metabolic paths and mutations that block the drug binding to target sites. These drugs show cytotoxic behavior to cancer as well as normal cells and are not selective. Thus, more selective and less toxic anticancer drugs are being required by the effect of design of agents on the target sites. Molecular hybridization is an innovative idea in the area of drug designing and discovery, grounded on the fact that grouping of different pharmacophoric moieties of biologically active frames to discover a new and effective hybrid compound. Also, this approach can results in compounds, presenting an improved selectively profile and less toxic effects.[6]

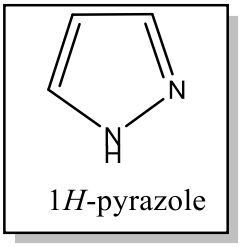
In the past few years, pyrazole derivatives have gradually become more significant. They are recognized as exceptionally beneficial intermediates for the creation of innovative biological materials. The pyrazole ring exists in plentiful of pharmacologically essential compounds.[7] Perimidine derivatives also comprise of a vital class of natural and non-natural products, numerous of which reveal valuable biological activity.[8]

2. REVIEW OF LITERATURE

A number of heterocyclic compounds like pyrazole, perimidine, benzimidazole, coumarin, indole, naphthalimide etc. have been found an effective role in medicinal and pharmaceutical applications. These heterocyclic centers have found their applications in oncology as an effective response to the targets. Many drugs are designed for the treatment of cancer with heterocyclic moieties as their basis. Thus, research is being going on to produce efficient hybridized moieties as drug contender and to check the anticancer activities of these moieties.

2.1 Pyrazole and its biological activity

Pyrazole comprises ring of five-members having two nitrogen atoms and three atoms of carbon in neighbouring positions as denoted by molecular formula $C_3H_4N_2$. Ludwig Knorr in 1833 first coined the term pyrazole. They are categorized as alkaloids due to its conformation and pharmacological effects on human beings. First natural pyrazole was 1-pyrazolyl-alanine that was isolated from watermelon seeds in the year 1959.



1*H*-pyrazole

Pyrazoles possess wide range of biological activities like anticancer, anti-microbial, anti-fungal, anti-tubercular, estrogen receptor (ER) ligand activity, etc. Several derivatives of pyrazole have also found their applications as nonsteroidal anti-inflammatory drugs, like phenazone (anaesthetic and antipyretic), metamizole (antipyretic and anaesthetic), aminophenazone (antipyretic, anaesthetic, and anti-inflammatory), phenylbutazone (anti-inflammatory and antipyreticused in rheumatoid arthritis, reiter's disease, osteoarthritis, spondylitis), oxyphenbutazole (anti-inflammatory, antipyretic, mild uricosuric and analgesic), and sulfinpyrazone (chronic gout).[9]

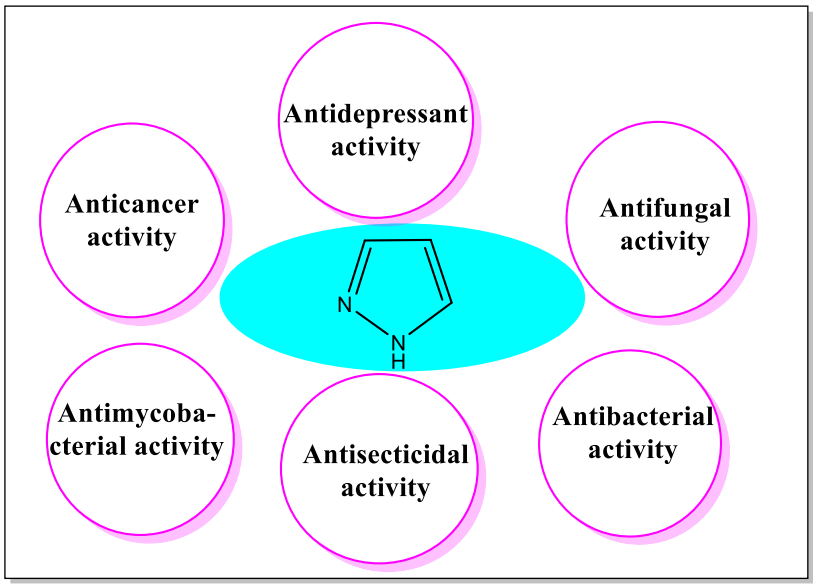
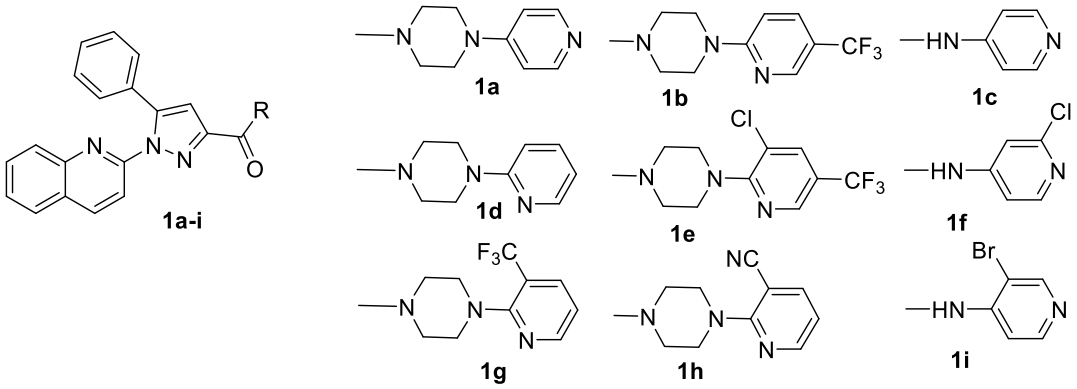
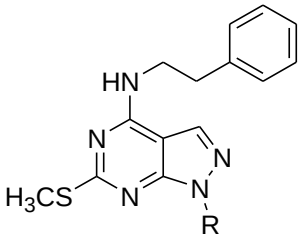


Figure-1. Biological activities of pyrazole derivative

Pirol *et al.* have worked on hybrid of pyrazole and quinoline (**1**) and evaluated for anti-proliferative activities against various cancer cell lines such as breast, colon carcinoma, and human liver. Compound **1f** (IC_{50}= 1.1 µM (HCT116), 1.6 µM (Huh7) and 3.3 µM (MCF7)) showed notable anticancer activity against three cancer cell lines.[10]



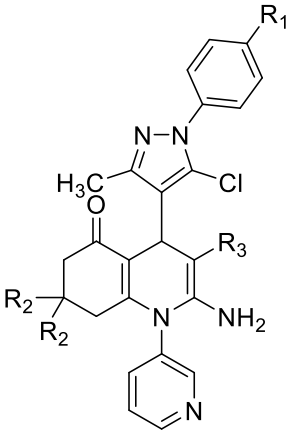
Adriano *et al.* synthesized pyrazolo[3,4-*d*]pyrimidines (**2**) and evaluated against prostate carcinoma cell line, PC3. Both the inhibitors reduced the growth of PC3 cells (IC_{50} = 50 µM).[11]



2

SI35, R = CH_2-CHCl-C_6H_5
SI40, R = CH=CH-C_6H_5

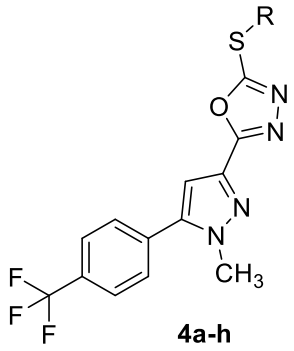
Sangani *et al.* have designed and synthesized a series of pyrazole derivatives with quinolone ring at C4-position and *in vitro* antibacterial and anticancer activities were tested for all these compounds. Most of the derivatives exhibited antitumor and antibacterial activity against various cancer cell lines and strains, respectively. Compounds **3b** (IC_{50}= 3.1 µM) and **3k** (IC_{50} = 0.05 µM) also exhibited highest potent inhibition activity against Epidermal Growth Factor Receptor (EGFR) in comparison with other members of the series.[12]



3a-l

Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3
3a	H	H	CN	**3g**	H	H	COOEt
3b	CH_3	H	CN	**3h**	CH_3	H	COOEt
3c	Cl	H	CN	**3i**	Cl	H	COOEt
3d	H	CH_3	CN	**3j**	H	CH_3	COOEt
3e	CH_3	CH_3	CN	**3k**	CH_3	CH_3	COOEt
3f	Cl	CH_3	CN	**3l**	Cl	CH_3	COOEt

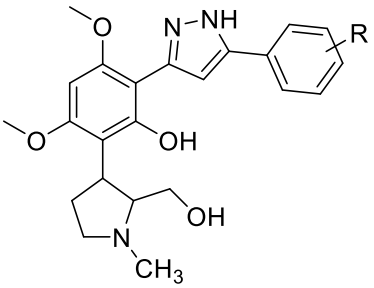
Puthiyapurayil *et al.* worked on the series of pyrazole moieties (**4**) with 1,3,4-oxadiazole at C-3 position and tested for anti-proliferative activity against various cancer cell lines. Compound **4e** has been found to be the most promising anticancer activity against MCF-7 cancer cell lines with IC_{50} value of 15.54 µM.[13]



4a-h

Compound	R	Compound	R
4a	2,6-F_2-C_6H_3-CH_2-	**4e**	4-CF_2- C_6H_4-CH_2-
4b	2-F- C_6H_4-O-CH_2-CH_2-	**4f**	4-F- C_6H_4-CH_2- CH_2-
4c	2,3- Cl_2-C_6H_3-CH_2-	**4g**	3-NO_2-C_6H_4-CH_2-
4d	3,4- Cl_2-C_6H_3-CH_2-	**4h**	4-Cl-2-F-C_6H_3-CH_2-

Bandgar *et al.* have designed and synthesized a series of derivatives of 2-(2-(hydroxymethyl)-1-methylpyrrolidin-3-yl)-3,5-dimethoxy-6-(5-phenyl-1*H*-pyrazol-3-yl)phenol (**5**). These synthesized derivatives were tested for their antitumor activity towards five cancer cell lines (prostate cancer cell line, breast cell line, lung cell line, promyelocytic leukemia cell line, and colon cancer cell line). Derivatives **5a** and **5d** also showed noticeable anticancer activity against 60 human cancer cell lines with growth inhibition of 61-73% at 10 µM concentration.[14]

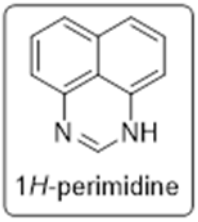


5a-f

5a = 2-Cl
5b = 2-Br
5c = 2,4-Cl
5d = 2-Cl, 4-NO_2
5e = 2-F
5f = 2-CH_3

2.2 Perimidine and its biological activity

Perimidine is a significant class of heterocyclic systems because of their pharmaceutical and biological activity. Antitumor potential and DNA binding affinity of perimidine derivatives have been well evaluated in literature in addition to antimicrobial, antiulcer, and antifungal properties. For synthesizing complex polyheterocycles, derivatives of perimidine proved to be effective starting substance.



1*H*-perimidine

Perimidine comprises of a tricyclic ring system with uncommon electronic properties among azines, in which the lone pair of pyrrole-like nitrogen contributes in the π-system of the molecule. Perimidine shows electron density transfer to naphthalene moiety from the heterocyclic moiety. Hence, it shows the features of both π-excessive and π-deficient systems.

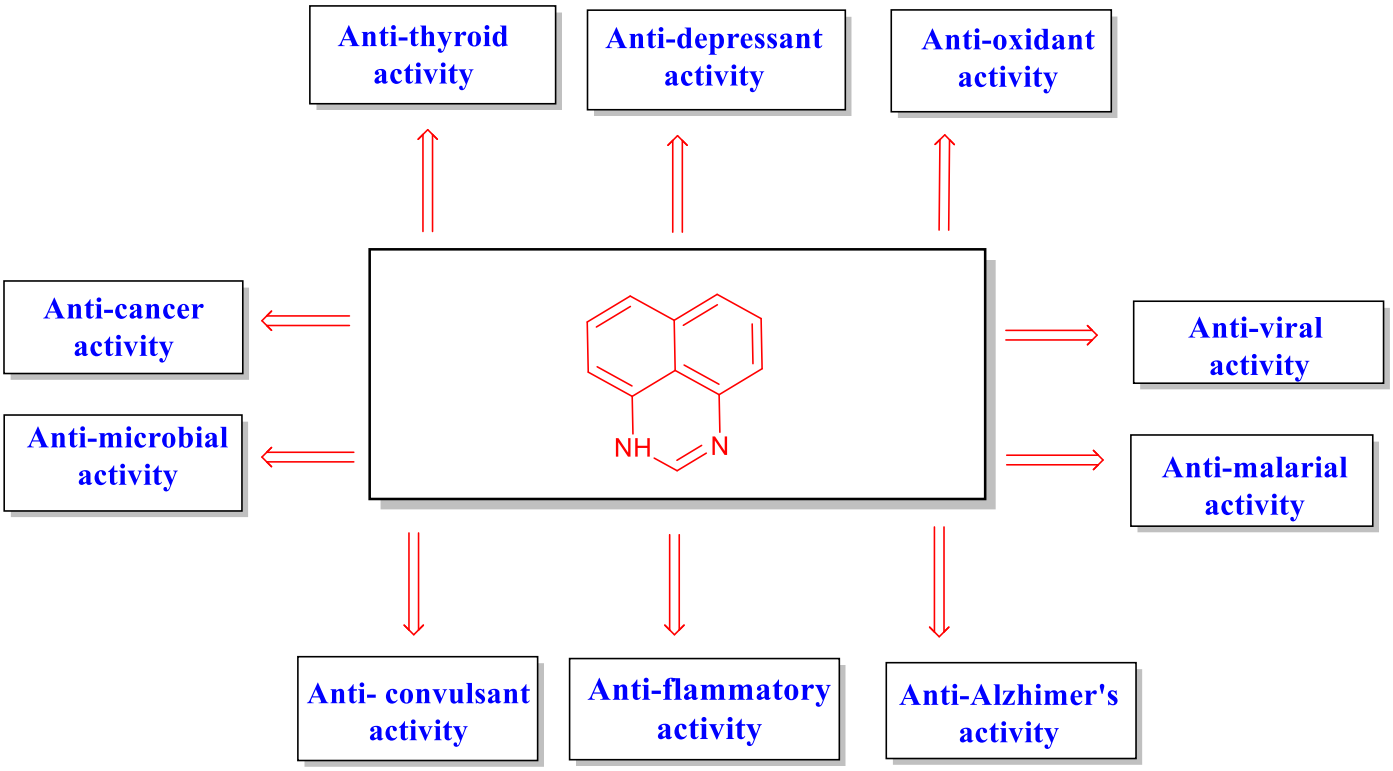
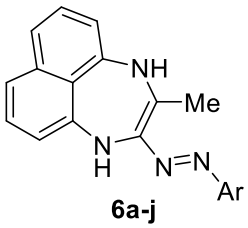


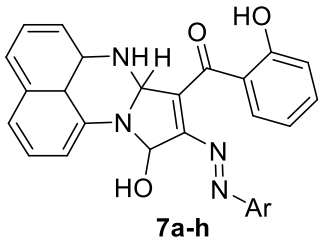
Figure-2. Biological activities of perimidine derivative

Farghaly *et al.* prepared two biological active series of naphtho[1,8-*ef*][1,4]diazepines (**6**) and pyrrolo[1,2-*a*]perimidines (**7**). These compounds were evaluated for anticancer activity towards three human cancer cell lines, UACC-62 (melanoma), TK-10 (renal cancer) and MCF-7 (breast cancer). Compound **6i** showed inhibition towards TK-10 (GI_{50}= 96.73 μM), MCF-7 (GI_{50} = 81.29 μM) and UACC-62 (GI_{50} =41.97 μM) cell lines. Compounds **6d** and **6f** showed excellent anti-proliferative activity against MCF-7 and TK-10 cells, respectively. Compound **7c** exhibited stronger inhibition to TK-10 and MCF-7 cell lines and showed inhibition (TGI) = 25.28 μM and 21.87 μM, for TK-10 and MCF-7 cell lines respectively.[15]



6a-j

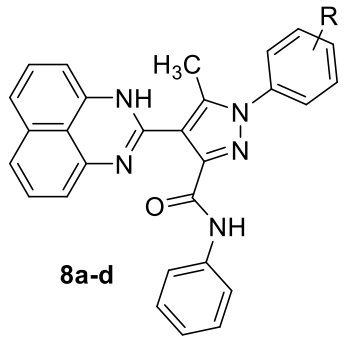
a: Ar = 4-$OMeC_6H_4$ f: Ar = 4-ClC_6H_4
b: Ar = 4-MeC_6H_4 g: Ar = 3-$NO_2C_6H_4$
c: Ar = 3-MeC_6H_4 h: Ar = 4-$NO_2C_6H_4$
d: Ar = C_6H_5 i: Ar = 4-$COMeC_6H_4$
e: Ar = 3-ClC_6H_4 j: Ar = 4-$CO_2EtC_6H_4$



7a-h

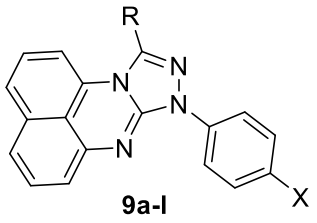
a: Ar = 4-MeC_6H_4 e: Ar = 4-BrC_6H_4
b: Ar = 3-MeC_6H_4 f: Ar = 3-$NO_2C_6H_4$
c: Ar = C_6H_5 g: Ar = 4-$NO_2C_6H_4$
d: Ar = 3-ClC_6H_4 h: Ar = 4-$COMeC_6H_4$

Farghaly *et al.* have also worked on another series of perimidine rings (**8** and **9**) and evaluated against various human cancer cell lines. Compound **8b** had shown higher antitumor property towards the two cancer cell lines used (IC_{50} = 0.49 (MCF-7) and 0.59 (HEPG-2) µM), compared to doxorubicin (IC_{50} = 0.42 (MCF-7) and 0.46 (HEPG-2) µM). Similarly, compounds **9f** and **9k** also showed good antitumor activity towards HEPG-2 (liver cancer) and MCF-7 (breast cancer) cancer cell lines, respectively.[16]



8a-d

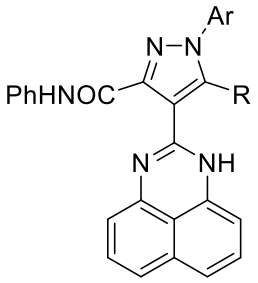
a; R = H, **b;** R **=** 4-Me, **c**; R = 4-Cl, **d;** R **=** 4-NO_2



9a-l

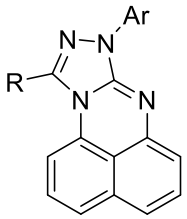
	R	X
a	CONHPh	H
b	CONHPh	Me
c	CONHPh	Cl
d	CONHPh	NO_2
e	COOEt	H
f	COOEt	Me
k	COMe	Cl
l	COMe	NO_2

Eldeab *et al.* have synthesized two series of compounds *viz.*, 4-(1*H*-perimidin-2-yl)-1*H*-pyrazole-3-carboxamides (**10**) and 8*H*-[1,2,4]triazolo[4,3-*a*]perimidine (**11**). Synthesized compounds showed good growth inhibition activity against cancer cell lines from 0.59 to >50 µg/mL concentrations towards the HepG2 cell line. These compounds also showed activity towards MCF-7 (breast cancer) cancer cell lines with growth inhibition of 0.49 to >50 µg/mL. Amongst all the tested derivatives, compounds **10c**, **11a**, and **11g** showed greatest anticancer activity against the two tested cancer cell lines (HepG2 and MCF-7).[17]



10a-f

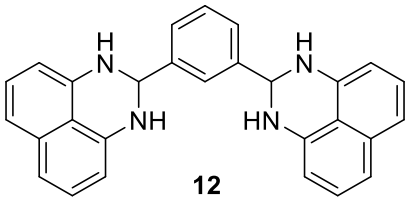
	R	Ar		R	Ar
10a	CH_3	C_6H_5	**10d**	CH_3	$4\text{-}NO_2C_6H_4$
10b	CH_3	$4\text{-}FC_6H_4$	**10e**	C_6H_5	C_6H_5
10c	CH_3	$4\text{-}CH_3C_6H_4$	**10f**	C_6H_5	$4\text{-}CH_3C_6H_4$



11a-h

	R	Ar		R	Ar
11a	$COCH_3$	$4\text{-}FC_6H_4$	**11e**	$CONHC_6H_5$	$4\text{-}CH_3C_6H_4$
11b	$COCH_3$	$4\text{-}NO_2C_6H_4$	**11f**	$CONHC_6H_5$	$4\text{-}NO_2C_6H_4$
11c	$CONHC_6H_5$	C_6H_5	**11g**	$COOC_2H_5$	$4\text{-}CH_3C_6H_4$
11d	$CONHC_6H_5$	$4\text{-}FC_6H_4$	**11h**	$COOC_2H_5$	$4\text{-}NO_2C_6H_4$

Roy *et al.* have synthesized a new perimidine based compound; 1,3-bis(2,3-dihydro-1*H*-perimidin-2-yl)benzene (**12**). Compound **12** has been shown 6-times better antioxidant activity than *L*-ascorbic acid.[18]

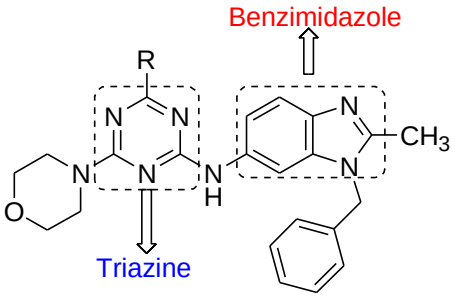


12

2.3 Molecular hybridization

The molecular hybridization methodology is one of the most important structural modification tool beneficial for the detection of ligands and prototypes, either enhanced affinity for one bioreceptor or the ability to regulate more than one bioreceptor related to the target disease. The growing attempts to synthesize hybrid drugs resulting from the mixture of two or more pharmacophoric moieties of dissimilar known lead compounds, have brought a new confidence for the remedies of multifactorial diseases in current years.[19]

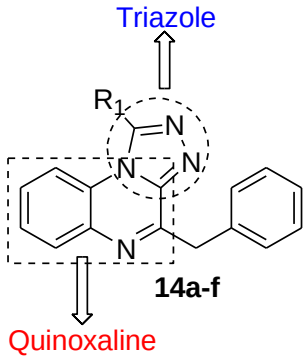
Paul *et al.* have designed and synthesized a series of hybrids of benzimidazole-triazine and tested all the synthesized hybrids against nine tumor cell panels. Three hybrids **13a**, **13b** and **13c** exhibited noticeable cell growth inhibitory activity. Hybrid **13a** displayed good activity of antitumor with growth inhibition (GI_{50}) values ranging between 3.56-19.0 µM against all selected nine cancer cell panel and having the mean graph mid-point (MG-MID) value of 9.78µM.[20]



13a-f

13a; R = chloro
13b; R = morpholine
13c; R = piperidine
13d; R = pyrrolidine
13e; R = *N*-methylpiperazine
13f; R = 1,2-ethanolamine

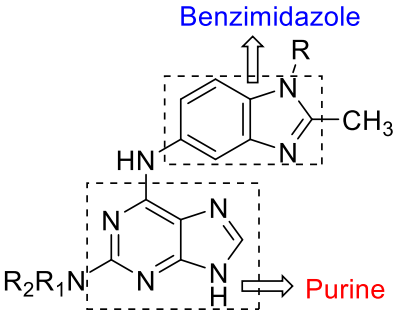
Issa *et al.* designed and synthesized a series of hybrids of 1,2,4-triazolo-quinoxaline analogues (**14**) and evaluated for anticancer activity against the NCI-60 cancer cell subpanel. Compound **14c** showed noticeable anti-proliferative activity against A498 (renal cancer), HOP-92 (non-small cell lung cancer), PC-3 (prostate cancer), MDA-MB-468 (breast cancer), HCT-15 (leukemia SR), HCT-116 (colon cancer) and U251 (CNS cancer) cell lines with GI_{50} values in the range of 1.80-5.55 µM.[21]



14a-f

14a; R = H
14b; R = CH_3
14c; R = 2-Cl-C_6H_4
14d; R = 4-NO_2-C_6H_4
14e; R = CH_2CH_2COOH
14f; R = COOEt

Paul *et al.* have also synthesized hybrids of benzimidazole and purine and tested against NCI-60 cell panel for anti-proliferative activity. Compound **15a** revealed significant inhibitory activity against colon cancer, ovarian cancer, and CNS cancer cell lines with GI_{50} values of 3.16, 1.34 and 2.00 µM, respectively. Moreover, derivative **15a** showed excellent inhibitory activity with MG-MID value of 18.12 µM at five dose concentration levels.[22]

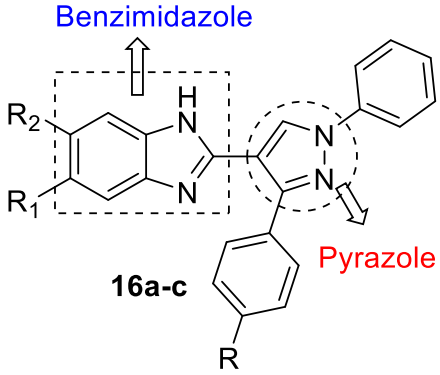


15a-e

	R	NR_1R_2
15a	Allyl	pyrrolidin-1-yl,
15b	Allyl	morpholin-4-yl
15c	Allyl	piperidin-1-yl
15d	butyl	pyrrolidin-1-yl,
15e	butyl	morpholin-4-yl

Reddy *et al.* have designed and synthesized hybrids of pyrazole and benzimidazole moieties, and evaluated the derivatives for anticancer activity against three cancer cell lines

A-549 (lung cancer), HeLa (cervical cancer), and MCF-7 (breast cancer). Hybrids **16a**, **16b** and **16c** showed growth inhibition, IC_{50} values lies between 0.84-1.82 µM against the selected cancer cell lines. The most effective hybrid **16a** (IC_{50} = 0.84 µM) exhibited the maximum anticancer activity towards breast cancer cell lines MCF-7.[23]



	R	R_1	R_2
16a	H	H	Br
16b	F	F	H
16c	Cl	Cl	H

3. RESEARCH GAPS AND OBJECTIVES

Literature survey suggests that individual moieties such as pyrazole and perimidine showed potent anticancer properties but lack an efficient activity values against most of the human cancer cell lines. According to the best of our knowledge, no report has been given in literature till now using the combination of these two biological active pharmacophores for hybridized compounds. To achieve these gaps, our main objective is to adopt the methodology of drug hybridization to synthesize new hybrid molecules by the combination of two biological active moieties *viz.*, pyrazole and perimidine as drug candidates and characterize these synthesized molecules using NMR spectroscopic techniques. These hybridized compounds will be used for anticancer activity towards appropriate cancer cell lines. The mechanism of action of these compounds will also be studied through DNA interactions.

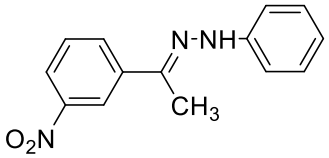
4. EXPERIMENTAL

4.1 Chemistry

All the reactions occurred in oven-dried glasswares. All the solvents and chemicals used were of commercial grade and were used without purification. They were provided by Aldrich, Loba, Spectrochemicals. Melting points (^oC) were calculated in open capillaries. Jeol-ECS 400 MHz and 100 MHz NMR spectrometer were used for recording ^1H and ^{13}C NMR spectra, respectively in $CDCl_3$ and DMSO-d_6 as solvents. The chemical shifts were indicated as ppm using tetramethylsilane (TMS) which is used as an internal reference and *J* values are

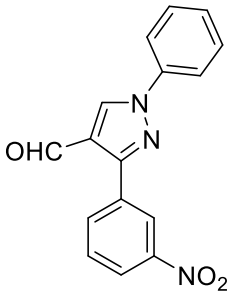
donated by Hz. Plates coated with silica gel HF-254 were used for monitoring the reactions and column chromatography was accomplished using silica gel 60-120 mesh. Hexane:ethyl acetate was the accepted solvent system for TLC and coloumn chromatopgraphy.

4.2. Procedure for synthesis of (*E*)-1-(1-(3-nitrophenyl)ethylidene)-2-phenylhydrazine (3):



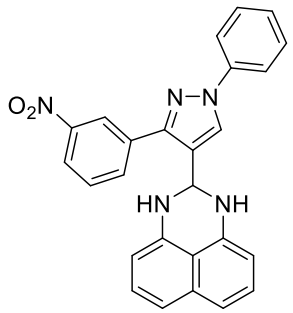
3-Nitroacetophenone (**1**) (2.0 g, 1 mmol) and phenyl hydrazine (**2**) (1.3 g, 1 mmol) in acetic acid (20 ml) were charged in 100 ml round bottom flask. The reaction was stirred at room temperature for 30 min. The reaction was examined by thin layer chromatography. On the consumption of reactants, 250 ml water was added to the reaction mixture. Precipitates were formed, which was further filtered and dried. Yield: 89%; colour: orange-yellow; m.pt.: 80-84 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.59 (t, *J* = 2.32 Hz, 1H, ArH), 8.18-816 (m, 1H, ArH), 8.15-8.13 (m, 1H, ArH), 7.56 (t, *J* = 8.28 Hz, 1H, ArH), 7.36 (d, *J* = 7.32 Hz, 1H, ArH), 7.33 (d, *J* = 7.36 Hz, 1H, ArH), 7.24-7.21 (m, 3H, ArH & NH), 6.97 (d, *J* = 7.32 Hz, 1H, ArH), 2.29 (s, 3H, CH_3).

4.2.1. Procedure for synthesis of 3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (4):



(*E*)-1-(1-(3-Nitrophenyl)ethylindene)-2-phenylhydrazine (**3**) (1.0 g, 3.4 mmoles) in DMF (10) was charged in round bottom flask. Then, $POCl_3$ (50 ml) was added dropwise at room temperature for 15 min. Thin layer chromatography was used to detect the product formation. On completion of reaction, reaction mixture was poured into crushed ice. The precipitates formed were filtered and washed with water and then dried. Yield: 74%; colour: grey; m.pt.: 200–203 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.09 (s, 1H, CHO), 8.86 (t, *J* = 1.84 Hz, 1H, ArH), 8.58 (s, 1H, ArH), 8.34 (t, *J* = 8.24 Hz, 2H, ArH), 7.82 (d, *J* = 7.80 Hz, 2H, ArH), 7.70 (t, *J* = 8.24 Hz, 1H, ArH), 7.57 (t, *J* = 7.80 Hz, 2H, ArH), 7.46 (t, *J* = 7.32 Hz, 1H, ArH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 183.5 (CHO), 151.2, 148.3, 138.6, 134.7, 133.5, 133.0, 129.8, 129.5, 128.3, 123.8, 123.7, 122.6, 119.6 (ArC).

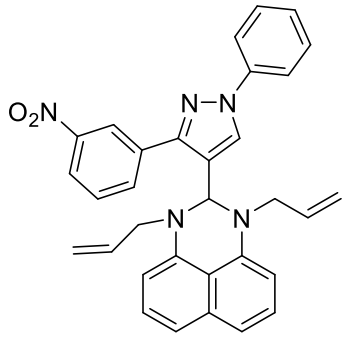
4.2.2. Procedure for synthesis of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (6):



3-(3-Nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**4**) (1 g, 3.4 mmol) has been charged in 100 ml oven dried round bottom flask. Then, 1,8-diaminonaphthalene (**5**) (0.593 g, 6 mmol) in nitrobenzene was added, and heated at 70 ºC for 6h. On completion of reaction, 50 ml hexane was added to the reaction mixture. The precipitates formed were filtered and washed with cold hexane. The precipitates were dried to get the crude product. The product was purified by column chromatography using hexane: ethyl acetate as eluents.Yield: 80%; colour: yellow; m.pt.: 228-231 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.96 (s, 1H, ArH), 8.90 (s, 1H, ArH), 8.53 (d, *J* = 7.80 Hz, 1H, ArH), 8.20 (d, *J* = 8.28 Hz, 1H, ArH), 8.00 (d, *J* = 7.80 Hz, 2H, ArH), 7.72 (t, *J* = 7.80 Hz, 1H, ArH), 7.58 (t, *J* = 7.80 Hz, 2H, ArH), 7.39 (t, *J* = 7.56 Hz, 1H, ArH), 7.18 (t, *J* = 8.00 Hz, 2H, ArH), 7.04 (d, *J* = 7.80 Hz, 2H, ArH), 6.91 (s, 2H, NH), 6.50 (d, *J* = 7.32 Hz, 2H, ArH), 5.58 (s, 1H, CH-perimidine); ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 148.8, 147.8, 143.3, 139.2, 135.0, 134.5, 130.7, 129.9, 129.8, 126.9, 126.8, 123.3, 122.9, 122.7, 121.9, 118.5, 115.8, 112.9, 104.8 (ArC), 59.8 (CH-perimidine).

4.2.3. Procedure for synthesis of 1,3-di/1-mono alkyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (8-10): Mixture of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine **(6)** (1 mmol) and allyl/propagyl/butyl bromide (**7a-c**) (1.5 mmol) and potassium carbonate (1.5 mmol) in DMF (5 ml) was heated at 110 ºC for 12-20 h. Reaction completion was detected by thin layer chromatography. 500 ml water was added to reaction mixture and product was extracted with ethyl acetate (50 ml × 3). Mixture of hexane and ethylacetate was used as solvent in column chromatography to obtain pure product.

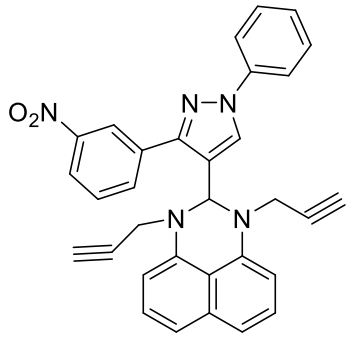
4.2.4 Spectral data of 1,3-diallyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (8):



Yield: 75%; colour: light brown; m.pt.: 237-240 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.68 (t, *J* = 2.28 Hz, 1H, ArH), 8.28 (dd, ^{2}J = 8.36 Hz, ^{3}J = 1.36 Hz, 1H, ArH), 8.09 (d, *J* = 7.80 Hz, 1H, ArH), 7.64 (t, *J* = 7.80 Hz, 1H, ArH), 7.36-7.29 (m, 7H, ArH), 7.25 (d, *J* = 2.76 Hz, 1H, ArH), 7.22 (t, *J* = 6.88 Hz, 1H, ArH), 7.10 (s, 1H, ArH), 6.64 (d, *J* = 7.32 Hz, 2H, ArH), 5.93-5.84 (m, 2H, 2×CH-allyl), 5.54 (s, 1H, CH-

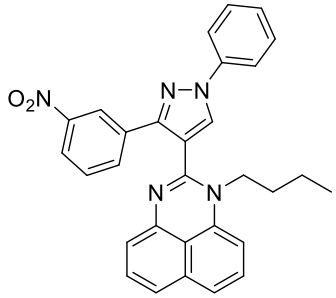
perimidine), 5.18-5.11 (m, 4H, 2×CH_2-allyl), 4.09 (dd, ^2J = 15.56 Hz, ^3J = 5.48 Hz, 2H, CH_2-allyl), 3.67 (dd, ^2J = 15.60 Hz, ^3J = 7.16 Hz, 2H, CH_2-allyl). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 149.8, 148.1, 140.9, 139.2, 135.2, 134.8, 134.3, 134.0, 129.3, 129.2, 127.0, 126.7, 125.9, 123.8, 122.8, 120.2, 118.9, 118.6, 117.8, 116.4, 107.5 (ArC), 67.7 (CH-perimidine), 53.8 (Allyl).

4.2.5. Spectral data of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1,3-di(prop-2-yn-1-yl)-2,3-dihydro-1*H*-perimidine (9):



Yield: 71%; colour: dark brown; m.pt. 231-234 ºC: ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.92 (t, *J* = 1.84 Hz, 1H, ArH), 8.37 (d, *J* = 7.80 Hz, 1H, ArH), 8.23 (dd, ^2J = 8.24 Hz, ^3J = 1.40 Hz, 1H, ArH), 7.58 (t, *J* = 8.24 Hz, 1H, ArH), 7.51 (t, *J* = 7.80 Hz, 3H, ArH), 7.41-7.34 (m, 6H, ArH), 7.25 (t, *J* = 4.56 Hz, 1H, ArH), 6.78 (d, *J* = 6.44 Hz, 2H, ArH), 5.90 (s, 1H, CH-perimidine), 4.22 (dd, ^2J = 17.88 Hz, ^3J = 2.32 Hz, 2H, CH_2-propargyl), 3.94 (dd, ^2J = 17.88 Hz, ^3J = 2.32 Hz, 2H, CH_2-propargyl), 2.14 (t, *J* = 2.04 Hz, 2H, 2×CH-propargyl); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 149.9, 148.1, 140.0, 135.2, 134.7, 134.3, 129.3, 129.2, 127.7, 126.9, 123.9, 122.8, 119.6, 118.9, 118.5, 116.2, 107.7 (ArC), 78.4 (CH-propargyl), 73.0 (CH-propargyl), 68.5 (CH-perimidine), 39.1 (*N*-CH_2-propargyl).

4.2.6. Spectral data of 1-butyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-yl)-1*H*-perimidine (10):



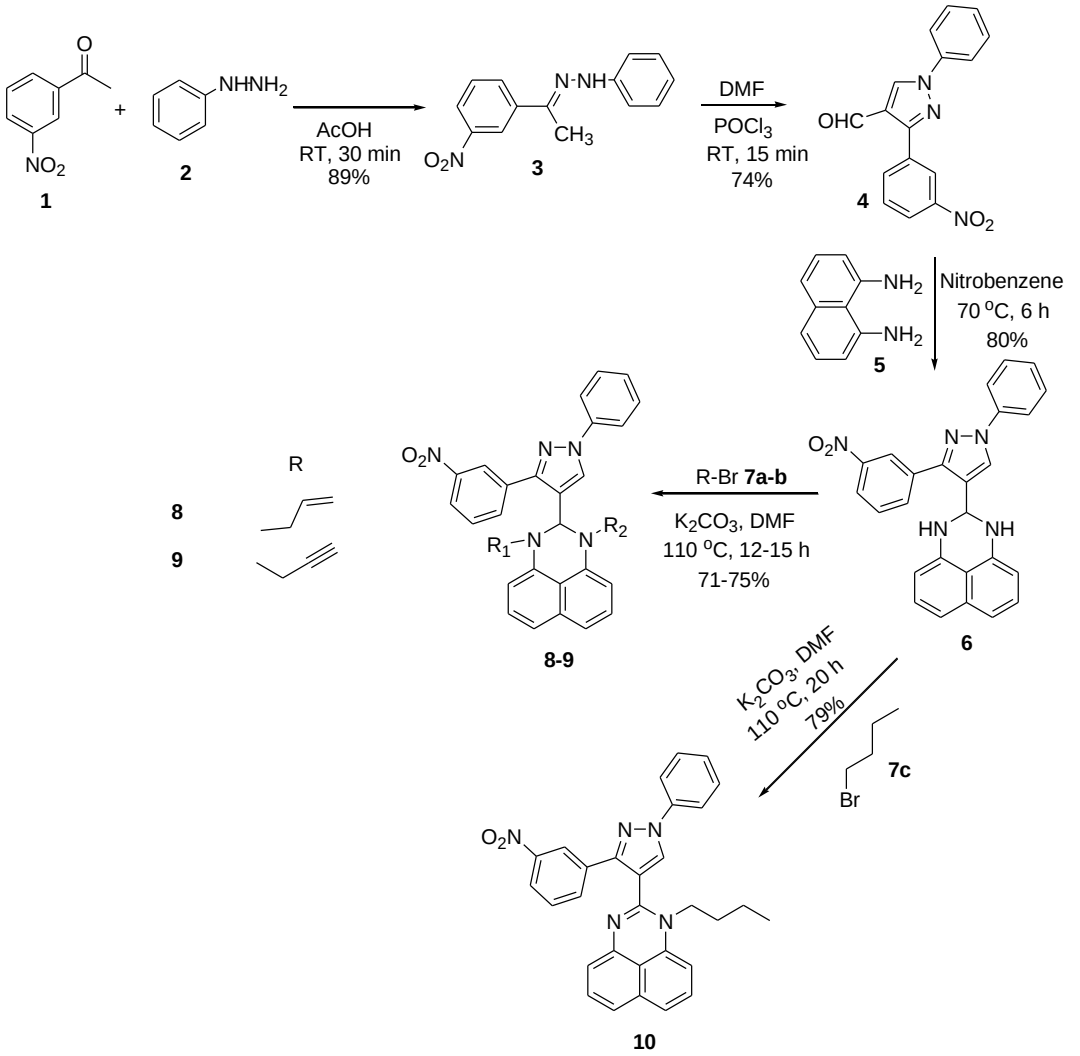
Yield: 79%; colour: black; m.pt.: 245-248 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.93 (t, *J* = 3.64 Hz, 1H, ArH), 8.27 (s, 1H, ArH), 8.19-8.16 (m, 2H, ArH), 7.83 (d, *J* = 7.80 Hz, 3H, ArH), 7.55 (t, *J* = 6.44 Hz, 4H, ArH), 7.41 (t, *J* = 7.32 Hz, 1H, ArH), 7.33 (t, *J* = 7.36 Hz, 1H, ArH), 7.19 (d, *J* = 2.72 Hz, 1H, ArH), 6.93 (dd, ^2J = 7.32 Hz, ^3J = 0.92 Hz, 1H, ArH), 6.23 (d, *J* = 7.32 Hz, 1H, ArH), 3.48 (t, *J* = 7.76 Hz, 2H, CH_2-butyl), 3.48 (t, *J* = 7.76 Hz, 2H, CH_2-butyl), 1.50 (m, 2H, CH_2-butyl), 1.21 (m, 2H, CH_2-butyl), 0.75 (t, *J* = 7.32 Hz, 3H, CH_3-butyl); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 150.1, 148.6, 147.5, 142.6 139.2, 138.2, 135.4, 133.9, 132.6, 129.8, 129.7, 129.6, 128.9, 128.8, 127.5, 123.0, 122.8, 121.9, 120.8, 119.6, 119.3, 117.3, 115.3, 102.3 (ArC), 46.8 (*N*-CH_2-butyl), 27.5 (CH_2-butyl), 19.6 (CH_2-butyl), 13.4 (CH_3-butyl).

5. RESULTS AND DISCUSSION

Pyrazole-perimidine hybridized compounds **8-10** were synthesized by commercially available 3-nitroacetophenone (**1**) and phenyl hydrazine (**2**) as starting materials as shown in **Scheme 1**. 3-Nitroacetophenone (**1**) was reacted with phenylhydrazine (**2**) in the presence of acetic acid for 30 minutes at room temperature to get orange-yellow coloured compound of (*E*)-1-(1-(3-nitrophenyl)ethylindene)-2-phenylhydrazine (**3**) in 89% yield (m.pt. = 80-84 ^{o}C). Compound **3** was further reacted with DMF and $POCl_3$ at room temperature for 15 minutes in Vilsmeier-Haack reaction, gave grey coloured compound of 3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**4**) in 74% yield (m.pt. = 200-203 ^{o}C). Compound **4** on reaction with 1,8-diaminonaphthalene (**5**) in nitrobenzene at 70 ^{o}C for 6h, gave 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**6**) as yellow coloured compound in 80 % yield (m.pt. = 228-231 ^{o}C). For the synthesis of target compounds, intermediate **6** was further reacted with allyl and propargyl bromide **(7a-b)** in the presence of K_2CO_3 in DMF at 110 ^{o}C for 12-15 hrs. On work up with water and ethyl acetate, 1,3-diallyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**8**) and 1,3-dipropagyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**9**) were obtained in 75% (m.pt. = 237-240 ^{o}C) and 71% (m.pt. = 231-234 ^{o}C) yields, respectively.

Interestingly, on heating compound **6** with butyl bromide (**7c**) in the same reaction conditions gave monoalkylated product 1-butyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-yl)-1*H*-perimidine (**10**) as black coloured compound in 79% yield (m.pt. = 245-248 ^{o}C). The formation of monoalkylated product is due to the low reactivity of butyl bromide for the substitution reaction and thus favoured for the formation of C=N bond instead of alkylation. All the intermediates and targeted compounds were well characterized by ^{1}H and ^{13}C NMR spectroscopy methods.

^{1}H NMR spectrum of (*E*)-1-(1-(3-nitrophenyl)ethylidene)-2-phenylhydrazine (**3**) showed wide range of splitting pattern ranging from δ 8.59-6.97 ppm corresponding to ten aromatic protons with one NH proton. Compound **3** also showed one singlet at δ 2.29 ppm of three protons corresponding to CH_3 group. Appearance of ten aromatic protons and one NH proton in ^{1}H NMR spectrum confirmed the formation of (*E*)-1-(1-(3-nitrophenyl)ethylindene)-2-phenylhydrazine **(3) (Figure-3).**



Scheme 1: Synthesis of 1,3-di/1-mono alkyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**8-10**)

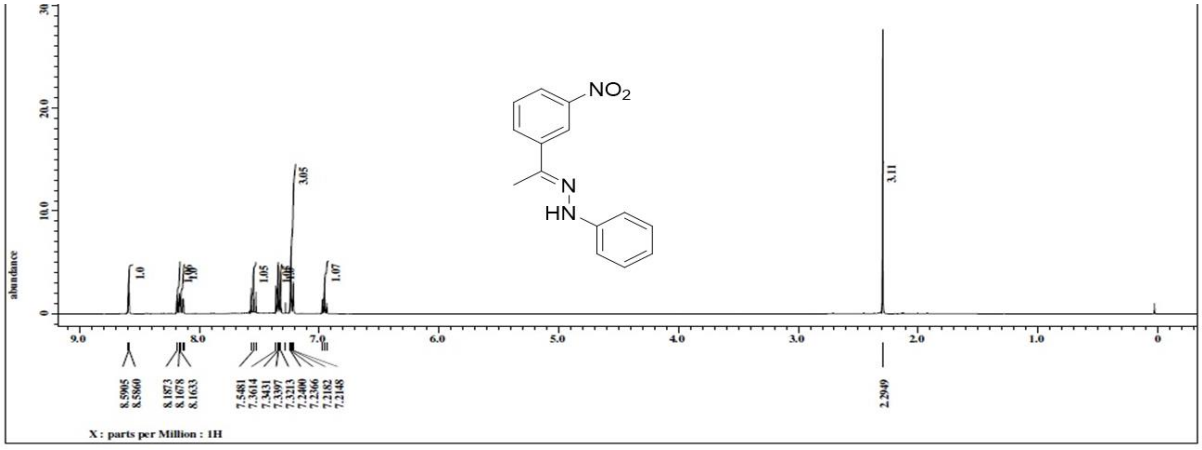


Figure-3. ^{1}H NMR spectrum of (*E*)-1-(1-(3-nitrophenyl)ethylidene)-2-phenylhydrazine **(3)**

^{1}H NMR spectrum of compound **4** indicated singlet of one proton at δ 10.09 ppm corresponding to CHO group (**Figures 4** and **5**). Aromatic protons of 3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**4**) showed wide range of splitting pattern ranging from δ 8.86-7.46 ppm corresponding to ten protons. ^{13}C NMR spectrum showed the distinctive –CHO peak at δ 183.5 ppm along with other aromatic signals at δ 151.2, 148.3, 138.6, 134.7, 133.5, 133.0, 129.8, 129.5, 128.3, 123.8, 123.7, 122.6, 119.6 (ArC). Appearance of new signals at δ 10.09 and 183.5 ppm in respective ^{1}H and ^{13}C NMR spectra, confirmed the formation of 3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**4**).

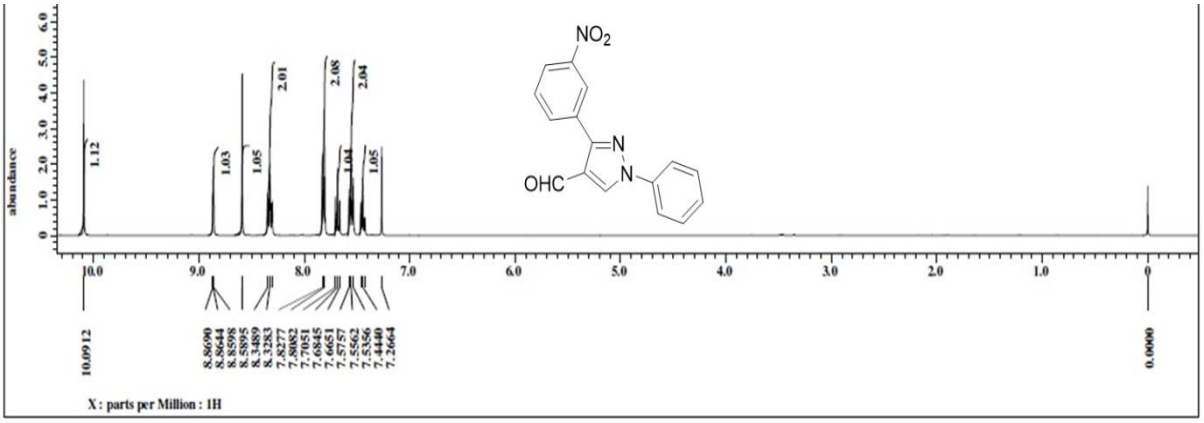


Figure-4. ^{1}H NMR spectrum of 3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**4**)

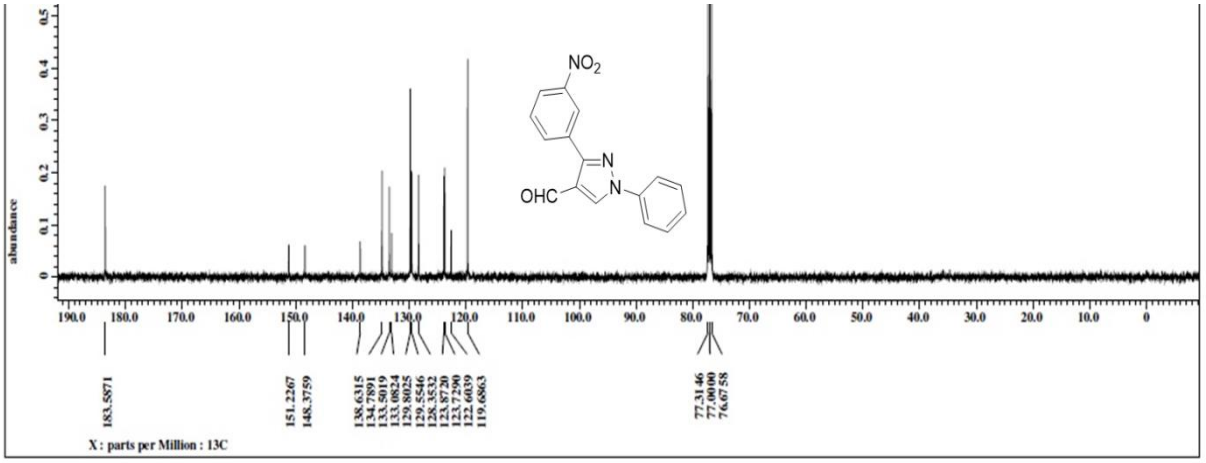


Figure-5. ^{13}C NMR spectrum of 3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**4**)

^{1}H NMR spectrum of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**6**) showed one singlet at δ 6.91 ppm corresponding to two protons of NH of perimidine ring and singlet at δ 5.58 ppm corresponding to CH of perimidine ring. Aromatic

protons of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine showed wide range of splitting pattern ranging from δ 8.96–6.50 ppm corresponding to sixteen protons. Disappearance of peak at δ 10.09 ppm of CHO proton and appearance of another six aromatic protons and two NH protons, confirmed the formation of compound **6**. ^{13}C NMR spectrum showed the signals at δ 148.8, 147.8, 143.3, 139.2, 135.0, 134.5, 134.5, 130.7, 129.9, 129.8, 126.9, 126.8, 123.3, 122.9, 122.7, 121.9, 118.5, 115.8, 112.9, 104.8 of aromatic carbons and signals at δ 59.8 ppm corresponding to CH of perimidine ring. Disappearance of signal at δ 183.5 ppm of CHO and appearance of new signal at δ 59.8 ppm due to CH group also confirmed the formation of compound **6**.So, ^{1}H and ^{13}C NMR spectral study confirmed the formation of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine **(6)** (**Figures 6** and **7**).

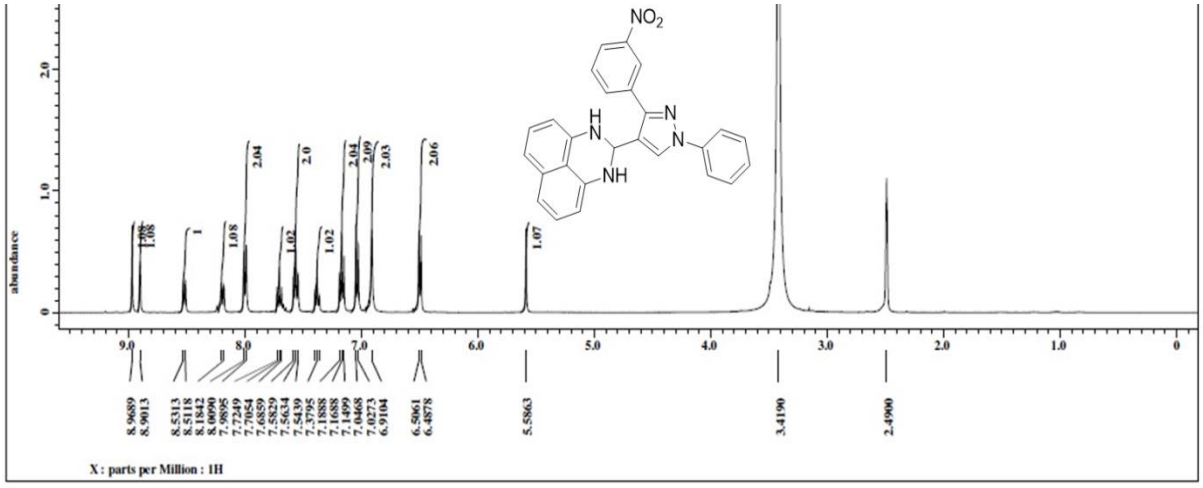


Figure-6. ^{1}H NMR spectrum of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**6**)

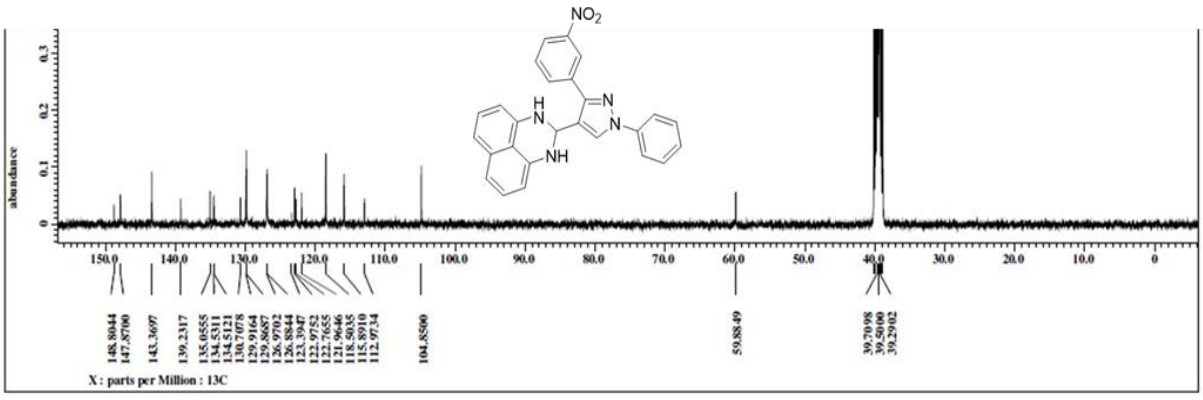


Figure-7. ^{13}C NMR spectrum of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**6**)

^{1}H NMR spectrum of 1,3-diallyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**8)** showed wide range of splitting pattern ranging from δ 8.68–6.64

ppm corresponding to sixteenaromatic protons and a singlet at δ 5.54 ppm corresponding to one proton of CH of perimidine ring. Disappearance of peak at δ 6.91 ppm of two NH protons of perimidine ring and appearance of characteristic peaks of two allyl groups (two multiplets of two protons each at δ 5.93-5.84 and 5.18-5.11 ppm corresponding to two CH_2 of allyl, doublet of doublet of two protons at δ 4.09 ppm corresponding to CH of allyl and doublet of doublet of two proton at δ 3.67 ppm corresponding to CH_2 of allyl) in aliphatic region confirmed the formation of compound **8** having two allyl substitution at NH of perimidine ring (**Figure 8**). ^{13}C NMR spectrum showed the signals at δ 149.8, 148.1, 140.9, 139.2, 135.2, 134.8, 134.3, 134.0, 129.3, 129.2, 127.0, 126.7, 125.9, 123.8, 122.8, 120.2, 118.9, 118.6, 117.8, 116.4, 107.5 (ArC), 67.7 (CH-perimidine), 53.8 (Allyl) of aromatic carbons and signals at δ 57.7 and 53.8 corresponding to allyl groups (**Figure-9**). Appearance of new signal at δ 57.7 and 53.8 ppm due to allyl groups also confirmed the formation of compound **8.**

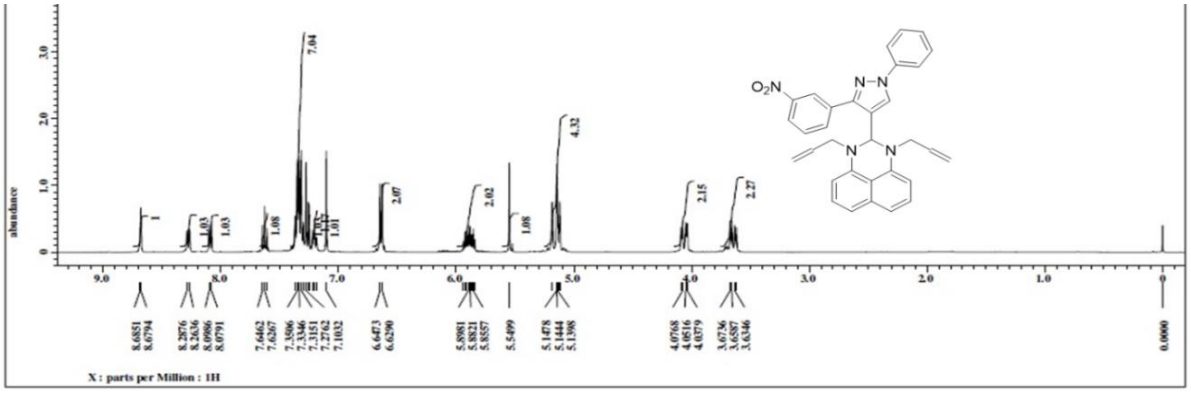


Figure-8. ^{1}H NMR spectrum of 1,3-diallyl-2-(3-(3-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)2,3-dihydro-1H-perimidine (**8**)

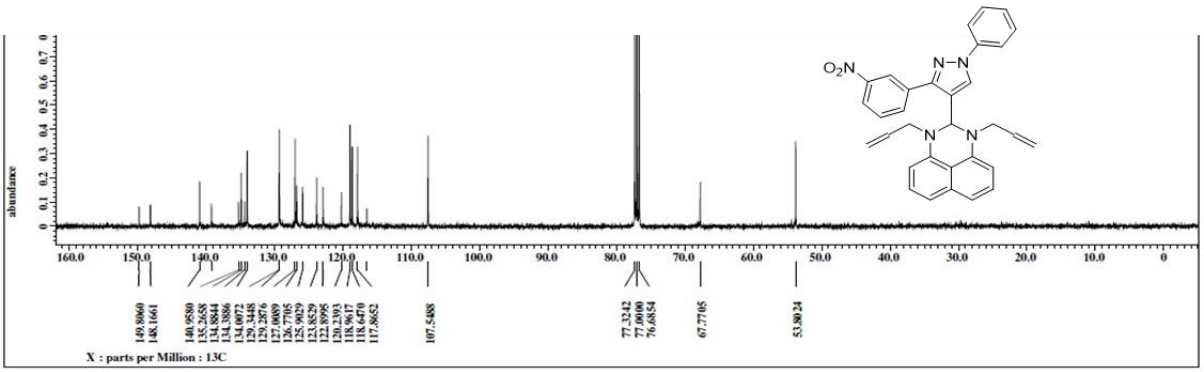


Figure-9. ^{13}C NMR spectrum of 1,3-diallyl-2-(3-(3-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,3-dihydro-1H-perimidine (**8**)

^{1}H NMR spectrum of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1,3-di(prop-2-yn-1-yl)-2,3-dihydro-1*H*-perimidine (**9)** showed wide range of splitting pattern ranging from δ 8.92–6.78 ppm corresponding to sixteen aromatic protons and a singlet at δ 5.90 ppm corresponding to one proton of CH of perimidine ring. Disappearance of peak at δ 6.91 ppm of two NH groups protons of perimidine ring and appearance of characteristic peaks of propargyl group (doublet of doublet of two protons at δ 4.22 ppm and δ 3.94 ppm corresponding to two CH_2 groups of propargyl, triplet of two protons with weak coupling constant at δ 2.14 ppm, corresponding to CH of propargyl) in aliphatic region confirmed the formation of compound **9** having two propargyl substitution at NH of perimidine ring. ^{13}C NMR spectrum showed the signals at δ 149.9, 148.1, 140.0, 135.2, 134.7, 134.3, 129.3, 129.2, 127.7, 126.9, 123.9, 122.8, 119.6, 118.9, 118.5, 116.2, 107.7 ppm of aromatic carbons,

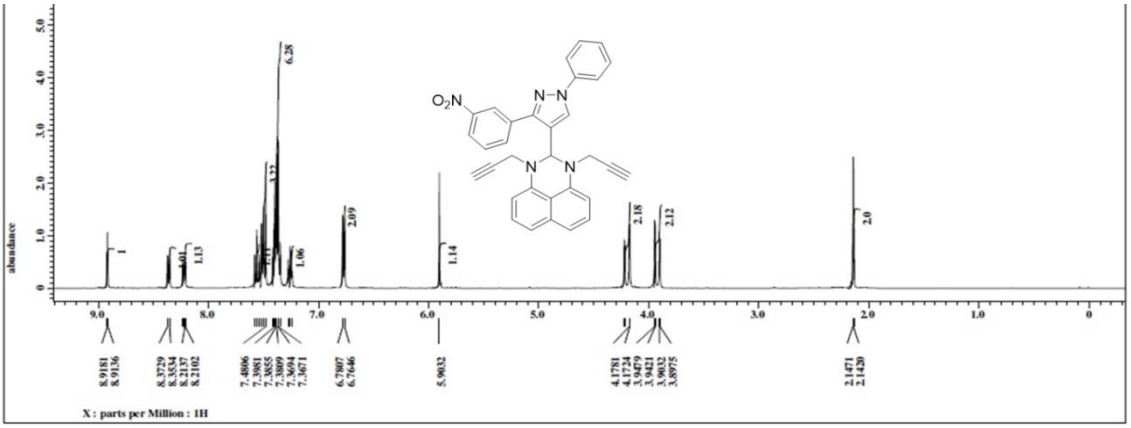


Figure-10.^{1}H NMR spectrum of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1,3-di(prop-2-yn-1-yl)-2,3-dihydro-1*H*-perimidine (**9**)

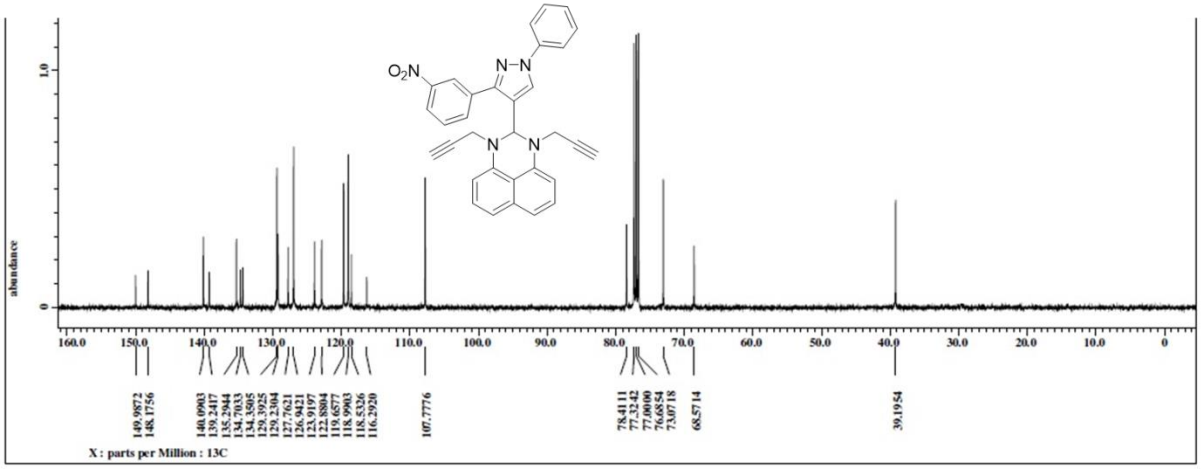


Figure-11. ^{13}C NMR spectrum of 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1,3-di(prop-2-yn-1-yl)-2,3-dihydro-1*H*-perimidine (**9**)

two signals at δ 78.4 and 73.0 corresponding to two propargyl groups, signal at δ 68.5 corresponding to CH-perimidine and signal at δ 39.1 corresponding to CH_2-propargyl. So, ^{1}H

and ^{13}C NMR spectral analysis confirmed the formation 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1,3-di(prop-2-yn-1-yl)-2,3-dihydro-1*H*-perimidine (**9**) (**Figures 10** and **11**).

^{1}H NMR spectrum of 1-butyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**10)** showed wide range of splitting pattern ranging from δ 8.93–6.23 ppm corresponding to sixteen aromatic protons. Appearance of characteristic peaks of *n*-butyl group (triplet of two protons at δ 3.48 ppm corresponding to CH_2 of butyl, multiplet of two protons at δ 1.50 ppm corresponding to CH_2 of butyl, multiplet of two protons at δ 1.21 ppm corresponding to CH_2 of butyl and triplet of three protons at δ 0.75 ppm corresponding to CH_3 of butyl) in aliphatic region confirmed the formation of single substitution of butyl group to NH of perimidine. ^{13}C NMR spectrum showed the signals at δ 150.1, 148.6, 147.5, 142.6 139.2, 138.2, 135.4, 133.9, 132.6, 129.8, 129.7, 129.6, 128.9, 128.8, 127.5, 123.0, 122.8, 121.9, 120.8, 119.6, 119.3, 117.3, 115.3, 102.3 of aromatic carbons, signal at δ 46.8 of N-CH_2-butyl, signal at δ 27.5 of CH_2-butyl, signal at δ 19.6 of CH_2-butyl and signal at δ 13.4 of CH_3-butyl group. Thus, the appearance of one butyl group at perimidine ring and absence of CH of perimidine at δ 5.58 ppm in ^{1}H NMR spectrum and absence of signal at δ 59.8 ppm of CH of perimidine in ^{13}C NMR spectrum confirmed the formation of monoalkylated 1-butyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-yl)-1*H*-perimidine **(10)** (**Figures-12** and **13**).

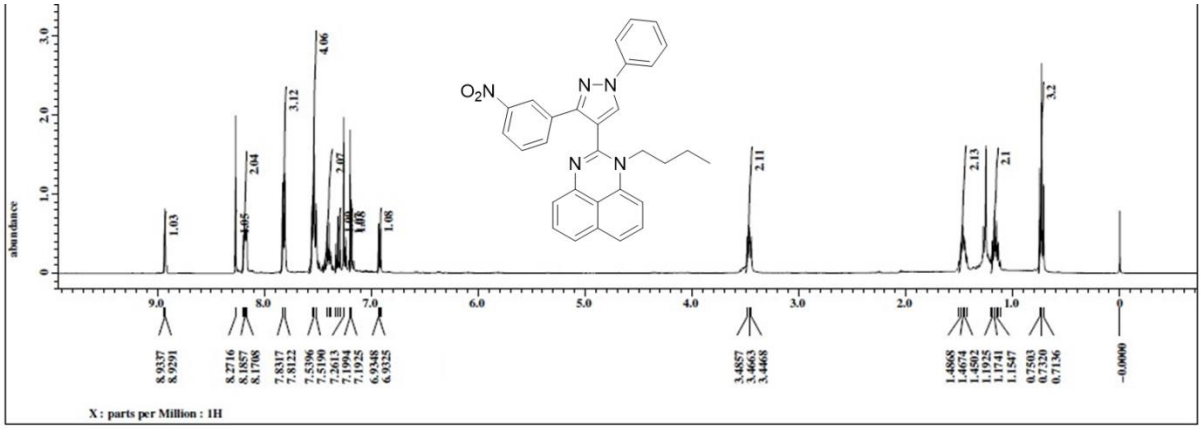


Figure-12. ^{1}H NMR spectrum of 1-butyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-yl)-1*H*-perimidine (**10**)

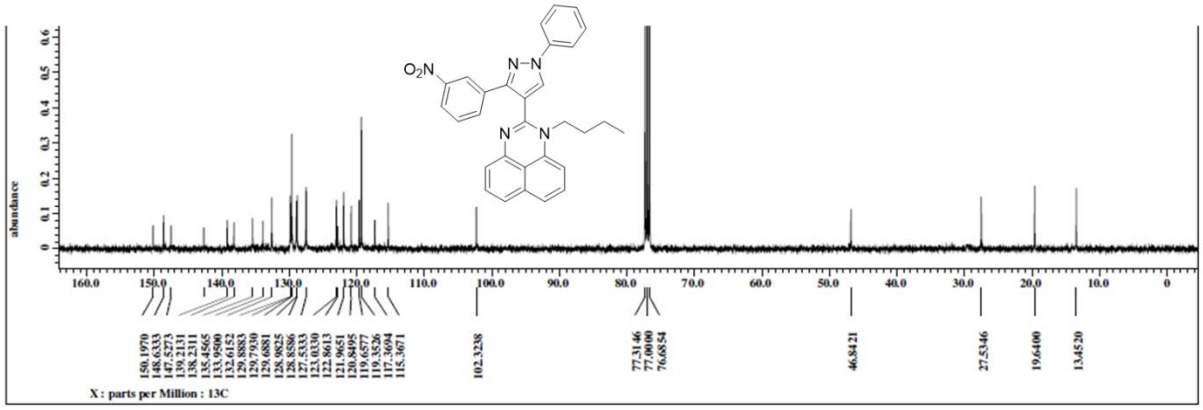


Figure-13. ^{13}C NMR spectrum of 1-butyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-yl)-1*H*-perimidine (**10**)

6. CONCLUSIONS

- Intermediates such as (*E*)-1-(1-(3-nitrophenyl)ethylidene)-2-phenylhydrazine (**3**), 3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**4**) and 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**6**) etc. were synthesized in moderate to good yields.
- Two dialkylated products 1,3-diallyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-perimidine (**8**) and 2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-1,3-di(prop-2-yn-1-yl)-2,3-dihydro-1*H*-perimidine (**9**) and one monoalkylated compound 1-butyl-2-(3-(3-nitrophenyl)-1-phenyl-1*H*-pyrazole-4-yl)-1*H*-perimidine (**10**) were synthesized in good yields. These compounds were well characterized by ^{1}H and ^{13}C NMR spectrometry. Further synthesis and characterization of pyrazole-perimidine conjugates are in progress.
- The final compounds will further be used for their biological activities as anti-cancer agents as well as DNA interactions.

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