Chapter – 4

Synthesis and study of antimicrobial and anticancer activities

of (E)-1-Aryl-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenones

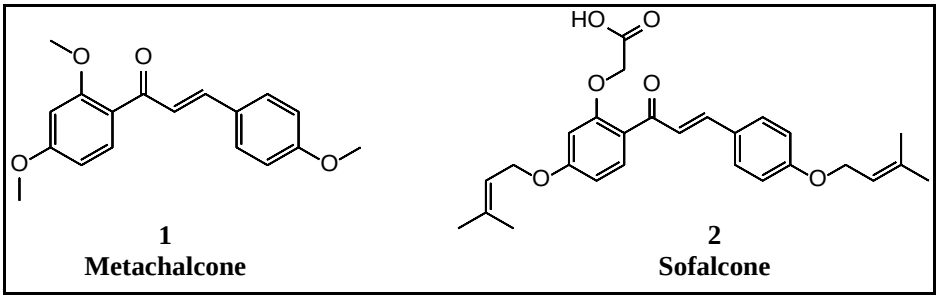
and

[5-Aryl- 3-{(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanones.

4.1 Introduction

Chalcone or 1,3-diphenyl-2-propenone is precursor in the biosynthesis of flavonoids and isoflavonoids and is the open chain intermediate in the synthesis of flavone.[1,2] The chalcone family has attracted much interest not only from the synthetic and biosynthetic perspectives but also due to its wide range of biological activities. Since many years the applications of chalcones as ingredients of plants and herbs to treat various ailments like cancer, inflammation, and diabetes are widely studied.[3–5] With a large number of papers on synthesis and study of chalcones' bioactivity, many mini reviews are published in past few years .[3–9]

Several chalcone-based compounds were approved for clinical use. For example, metachalcone (**1)** was once marketed as a choleretic drug, while sofalcone (**2)** was used as an antiulcer and mucoprotective drug.[4,5]



1
Metachalcone

2
Sofalcone

Figure 4.1

The privileged scaffold chalcone has been an attraction among chemists and biologist due to ease of synthesis, diversity of substituents they may possess, wide range of biological activities they show such as anticancer,[10,11] anti-diabetic,[12] anti-hypertensive,[13] anti-inflammatory,[14] anti-parasitic,[15] anti-malarial,[16] antioxidant,[17] anti-fungal,[18] anti-bacterial,[19] anti-platelet,[20] anti-retroviral,[21] anti-tubercular,[22] hypnotic,[23] anti-protozoal[24] activities. Chalcones are the core of many biologically important compounds from natural sources.[25] Some of the representative naturally occurring biologically active chalcone molecules are included in **Figure 4.2**.

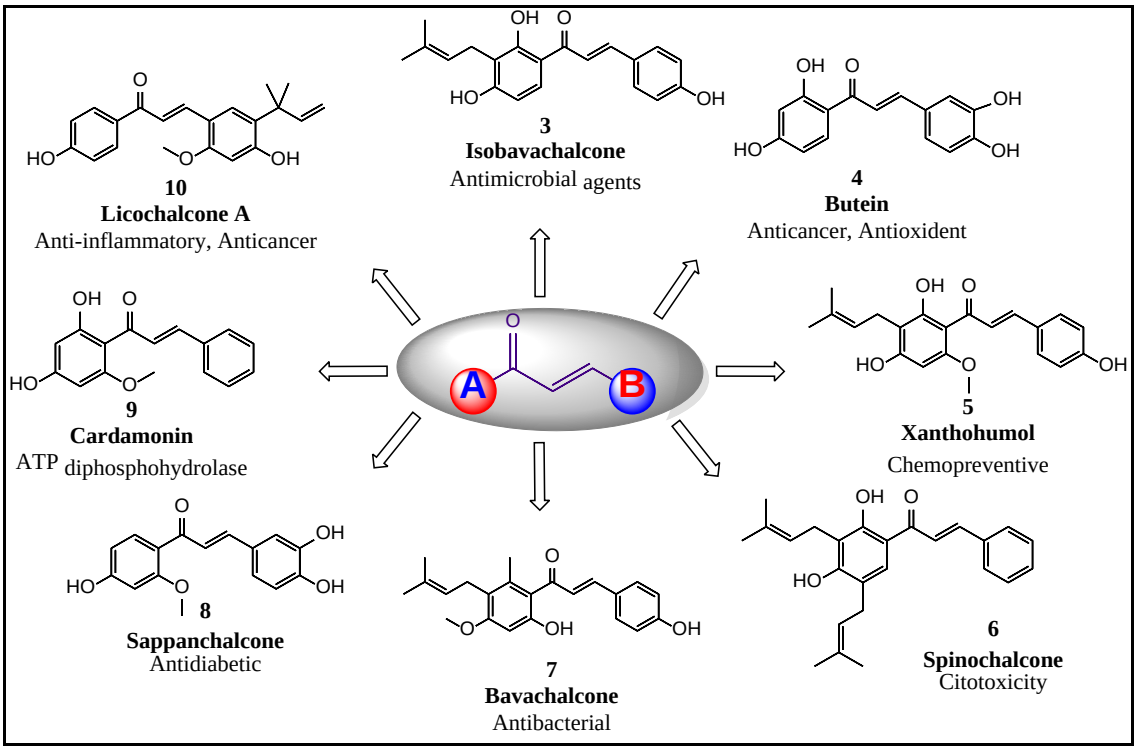


Figure 4.2 ***Naturally occurring biologically active chalcones.***

On the other hand, pyrazole (**11)** (**Figure 4.3**) is an aromatic heterocyclic compound that also belongs to the azole class of compounds. It has a five-membered heterocycle with two nitrogen atoms bound to each other along with three carbon atoms. Nitrogen atom **1** (**N1**) is ‘‘pyrrole-like” because its unshared electrons are conjugated with double bonds resulting in the aromatic system. Nitrogen atom **2** (**N2**) is ‘‘pyridine-like” since the unshared electrons are not compromised with resonance, similar to the pyridine system.

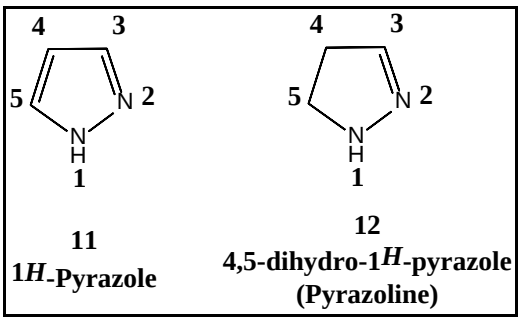


Figure 4.3

In medicinal chemistry, several compounds containing pyrazole rings exhibit various biological activities including anticancer,[26] antimicrobial,[27] anti-inflammatory,[28] anti-tubercular,[29,30] anti-leishmanial,[31] antiviral,[32] antihyperglycemic[33] activities.

With a desire to construct new molecules holding vibrant, versatile and advantageous properties, a member of the heterocyclic family called pyrazoline was found to be interesting for researchers. Pyrazoline (**12)** (**Figure 4.3**) is the 4,5-dihydro derivatives of pyrazole. Pyrazoline containing compounds widely occur in nature in the form of alkaloids, vitamins, pigments and as constituents of animal and plant cells.

Considerable attention has been focused on pyrazolines and substituted pyrazolines due to their interesting biological activities. A wide array of synthetic compounds containing pyrazoline moiety have been reported so far exhibiting diverse biological activities such as antimicrobial,[34] antiamoebic,[35] antitubercular,[36] anti-HIV,[37] anticancer,[38] antidepressant,[39] anticonvulsant,[40] anti-inflammatory,[41] and antimalarial[42] activities.

Several new compounds containing 1,3,5-trisubstituted pyrazoline framework (**13)** were prepared and reported as anticancer agents,[43] and a series of 3,5-disubstituted pyrazoline derivatives (**14)** (**Figure 4.4**) have been synthesized and evaluated for their *in vitro* cytotoxic activity against a panel of human cancer cell lines.[44]

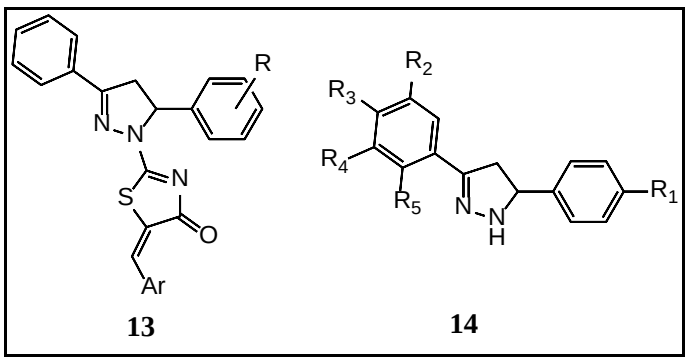
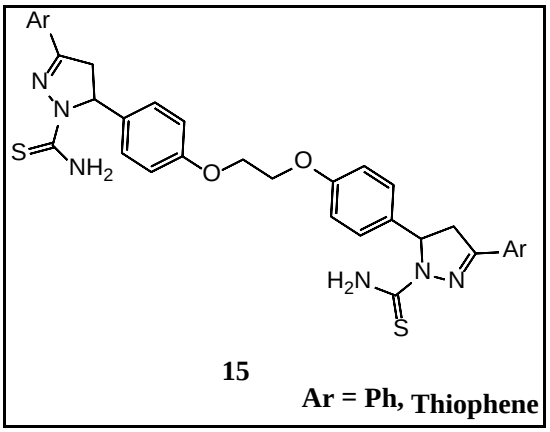


Figure 4.4

The bis(3-aryl-4,5-dihydro-1H-pyrazole-1-thiocarboxamide) derivatives (15**)** (**Figure 4.5**) were prepared and screened for their anti-inflammatory properties.[45]

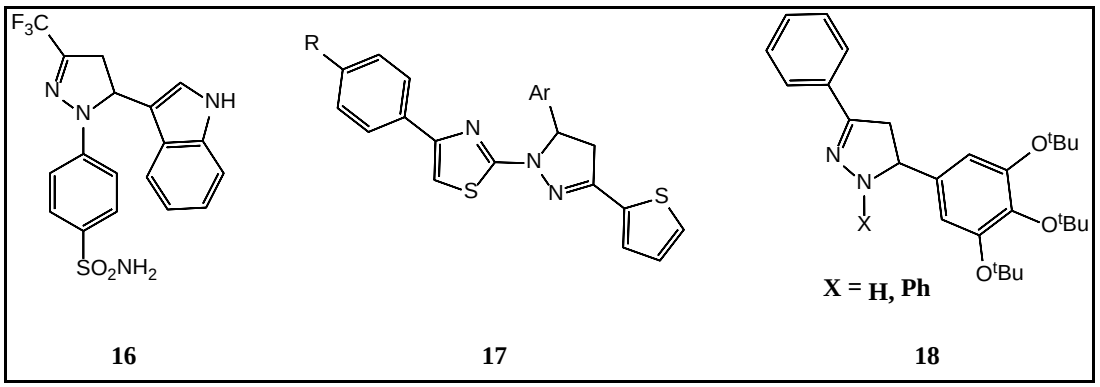


15

Ar = Ph, Thiophene

Figure 4.5

Some 5-indolylpyrazoline derivatives (**16)** (**Figure 4.6**) were prepared and screened for their anti-inflammatory activity as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) agents.[46] Several new 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives (**17)** (**Figure 4.6**) have been synthesized and evaluated for their antimicrobial activities.[47] Some new 1,3,5-aryl substituted pyrazolines (**18)** (**Figure 4.6**) were prepared and evaluated for their antimicrobial activities.[48]



16 **17** **18**

X = H, Ph

Figure 4.6

On the other hand pyridine is also an important heterocycle present in a large number of naturally occurring compounds. Pyridine derivatives are widely used as industrial, pharmaceutical and agricultural chemicals. Pyridine heterocyclic nucleus is an

essential part present in some synthetic bioactive agents such as rosiglitazone (**19)** (antidiabetic), pioglitazone (**20)** (antidiabetic), lansoprazole (**21)** (proton-pump inhibitor), and etoricoxib (**22)** (COX-2 selective inhibitor) (**Figure 4.7**).

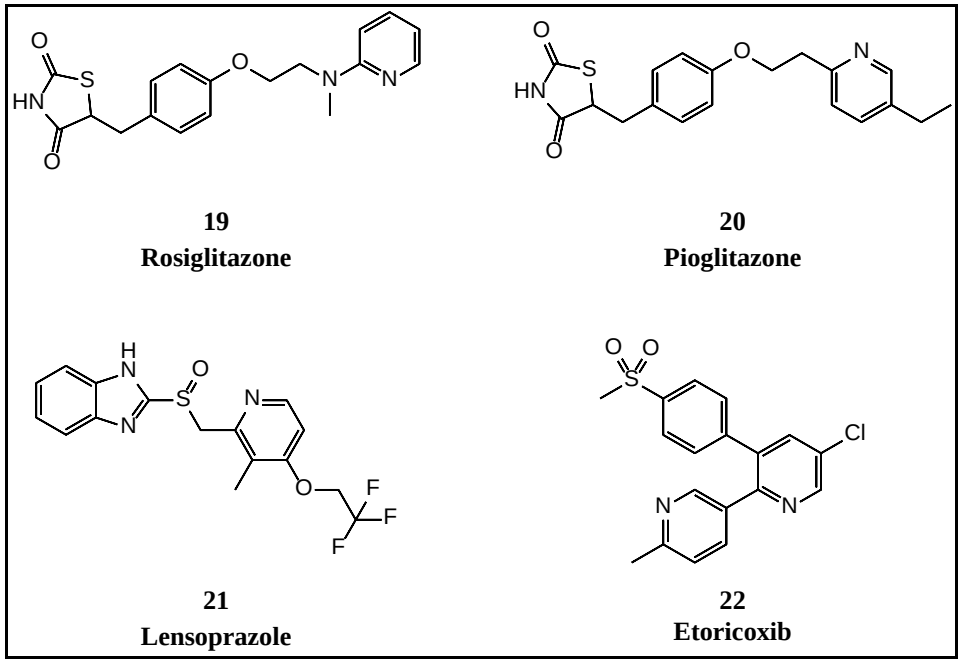


Figure 4.7

In addition to this, there are a number of reports on the compounds containing pyridine scaffold exhibiting a wide range of biological activities including antidiabetic,[49] antimicrobial,[50] anticancer,[51] anti-inflammatory,[52–54] anti-ulcer,[53] anti-viral,[55] analgesic,[54,56] anticonvulsant,[54] activities.

A series of 2-amino nicotinamide (**23)** (**Figure 4.8**) was synthesized and tested for *in vitro* antifungal activity against *C. albicans* and *A. fumigates* having MIC value comparable to the commonly used antifungal agents.[57]

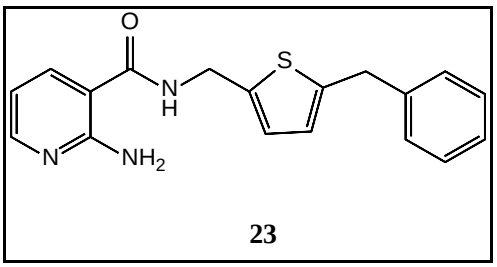


Figure 4.8

Some new hybrid pyrazolyl-4,5-dihydropyrazole and pyridine attached methanones (**24)** (**Figure 4.9**) were prepared and evaluated as antimicrobial agents.[58] A series of novel pyrazoline derivatives (**25)** (**Figure 4.9**) were synthesized and evaluated as antituberculer agents.[59] A series of new quinoline-isonicotinoyl-pyrazoline hybrid molecules (**26)** (**Figure 4.9**) are reported to possess a potent antimicrobial activity.[60]

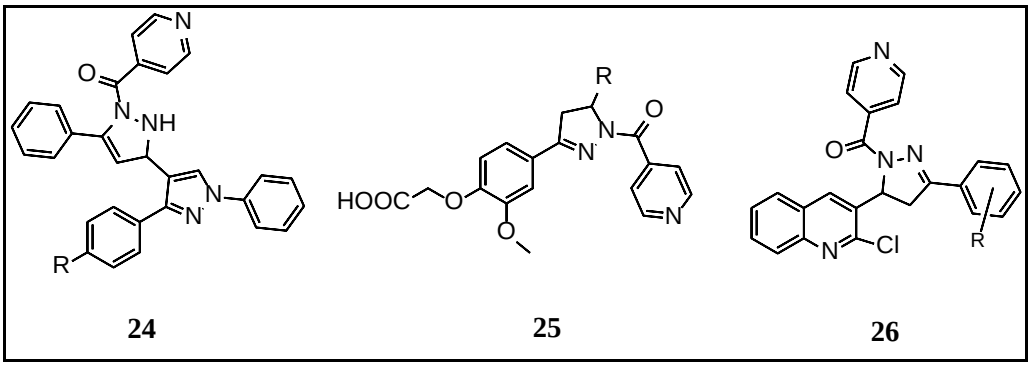
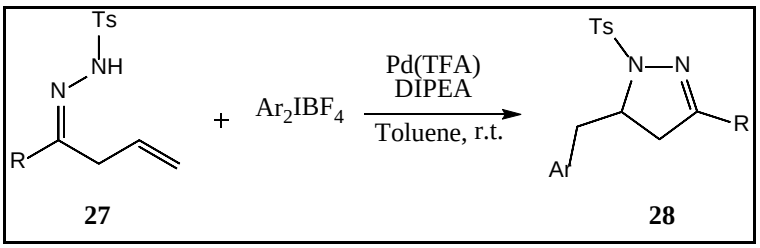


Figure 4.9

Synthesis of pyrazolines

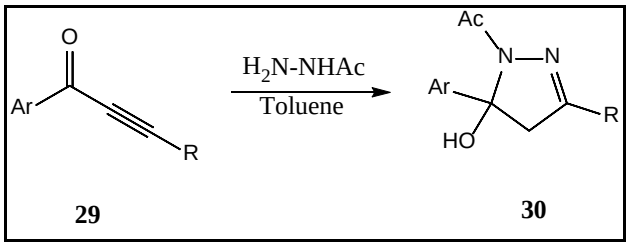
Pyrazolines can be prepared using several methods. Some of the recently reported methods are as described in the following content.

A variety of 3,5-disubstituted pyrazolines **28** were synthesized by a ligand-free, palladium-catalyzed aminoarylation of unactivated alkenes present in β,γ-unsaturated hydrazones **27** in good yields under mild reaction conditions[61] (**Scheme 4.1)**.



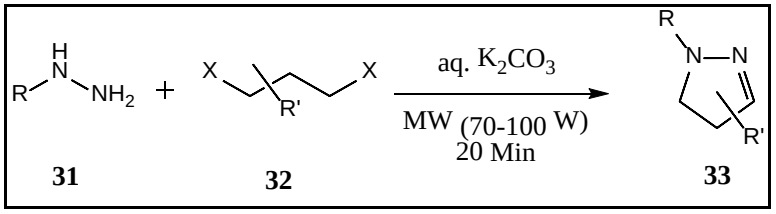
Scheme 4.1

Several functionally substituted 1-acyl-5-hydroxy-pyrazolines **30** have been prepared from the corresponding 2-alkyn-1-ones **29** in moderate to excellent yields[62] (**Scheme 4.2**).



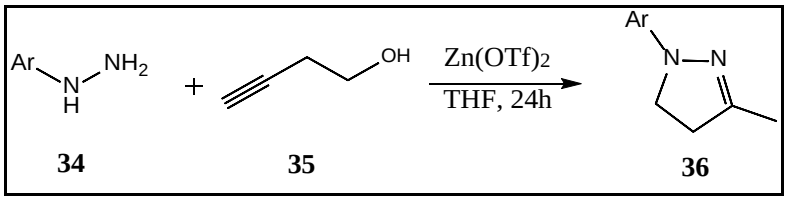
Scheme 4.2

Pyrazolines **33** could be synthesized from hydrazines or primary amines **31** and alkyl dihalides **32** and under microwave irradiation via cyclocondensation in an alkaline aqueous medium[63] (**Scheme 4.3**).



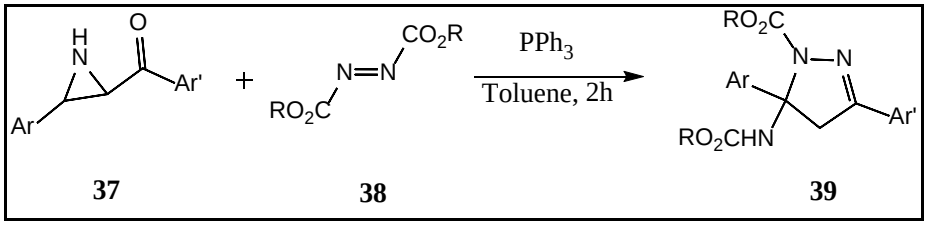
Scheme 4.3

The regeoselective reaction of arylhydrazines **34** with 3-butynol **35** in the presence of a catalytic amount of zinc triflate yielded aryl-substituted pyrazolines **36**[64] (**Scheme 4.4**).



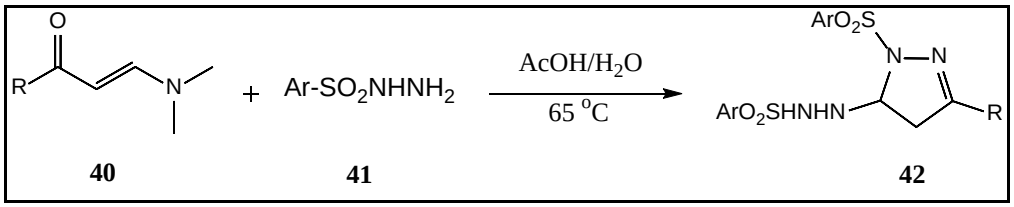
Scheme 4.4

Substituted pyrazolines **39** were synthesized via a novel and efficient domino reaction of 2-acylaziridines **37** with the Huisgen zwitterions generated from dialkyl azodicarboxylate **38** and PPh_3 furnished 2-pyrazolines[65] (**Scheme 4.5**).



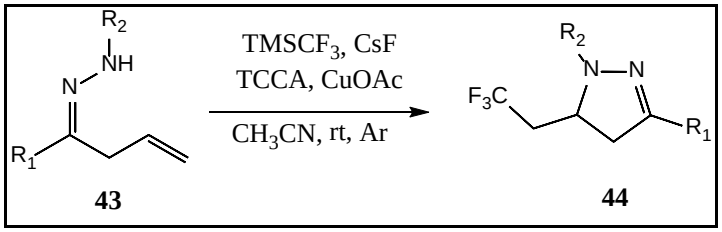
Scheme 4.5

A novel synthesis of pyrazoline **42** involved domino reactions between enaminones **40** and sulfonyl hydrazines **41** in water as a reaction medium and acetic acid as an additive[66] (**Scheme 4.6**).



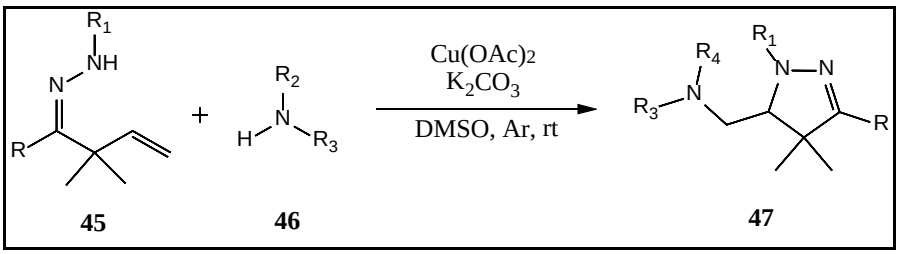
Scheme 4.6

A novel and efficient method for the preparation of trifluoroethyl subtituted pyrazolines **44** by cyclization reaction of β,γ-unsaturated hydrazones **43** using trichloroisocyanuric acid as a promoter and $TMSCF_3$ (Trimethyl-(trifluoromethyl)silane) as the trifluoromethylating reagent[67] (**Scheme 4.7**).



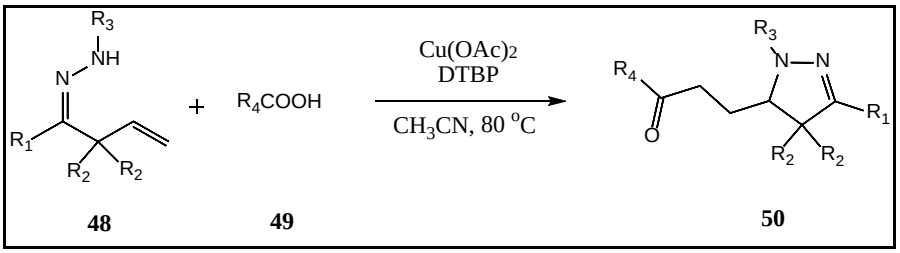
Scheme 4.7

Another convenient methodology for the preparation of pyrazolines **47** under mild reaction conditions involved copper-catalyzed intra-/intermolecular diamination of β,γ-unsaturated hydrazones **45** with simple amines **46** as external amine sources[68] (**Scheme 4.8**).



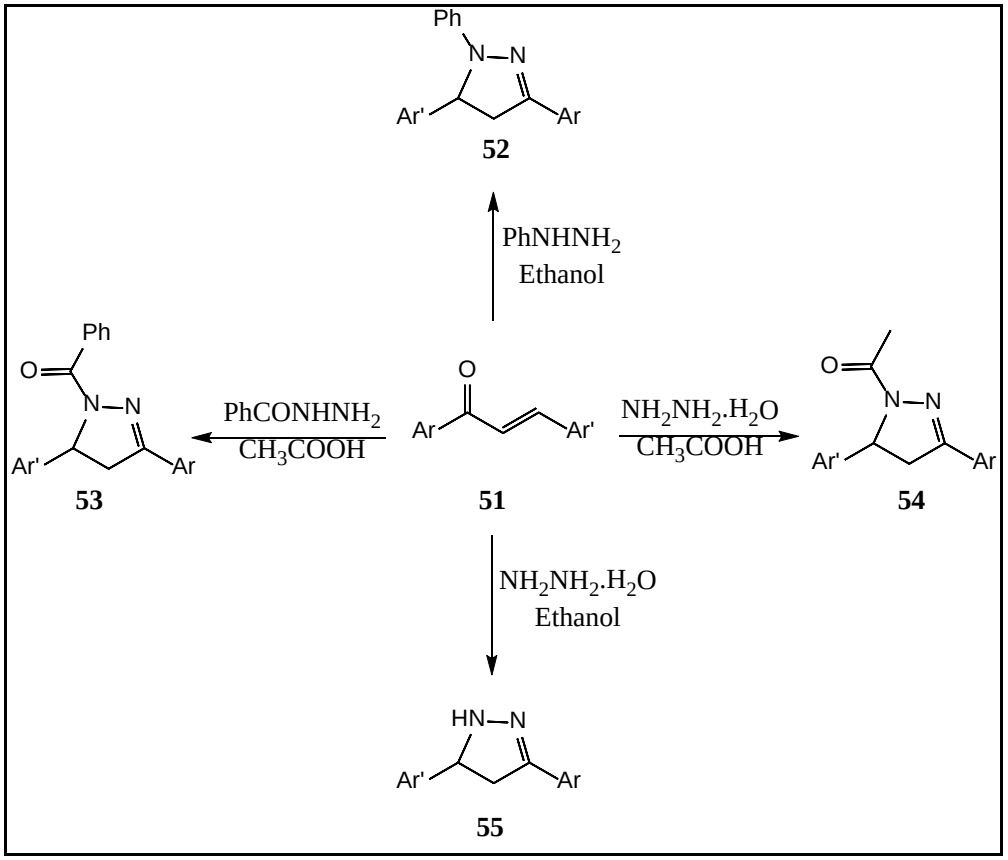
Scheme 4.8

A number of structurally diverse acyloxyl-substituted pyrazolines **50** were synthesized from unactivated alkenes of unsaturated hydrazones **48** by a copper-catalyzed aminoacyloxylation using DTBP (di-tert-butyl peroxide) as an oxidant and carboxylic acids **49** as the acyloxylating reagents[69] (**Scheme 4.9**).



Scheme 4.9

Various substituted pyrazolines can be synthesized from the α,β-unsaturated carbonyl compounds (**51)** as shown below[70–73] (**Scheme 4.10**).



Scheme 4.10

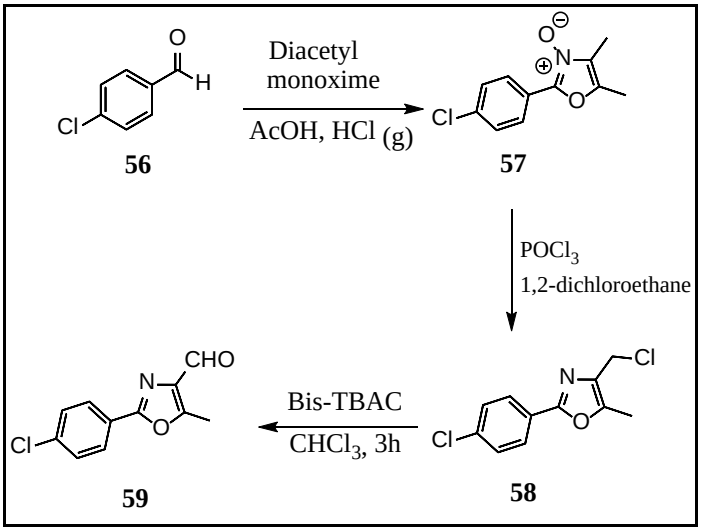
4.2 Results and discussion

Looking at the biological importance of the chalcones, pyrazolines and pyridine possessing compounds, some new compounds having α,β-unsaturated linkage were synthesized. Employing these α,β-unsaturated compounds, several new pyrazoline hybrid compounds containing oxazole and pyridine heterocycles were prepared. All the new compounds were characterized and were studied for their antimicrobial and anticancer activities.

The synthesis of 2-aryl-4-formyl oxazoles using Vilsmeier reagent requires preparation of aryl acyl halides which after conversion to the corresponding azides on cyclization result in the corresponding oxazoles. The preparation is novel involving an unprecedented rearrangement[74] but some of the final products are having low yields. Yields for the same oxazole aldehydes also fluctuate.

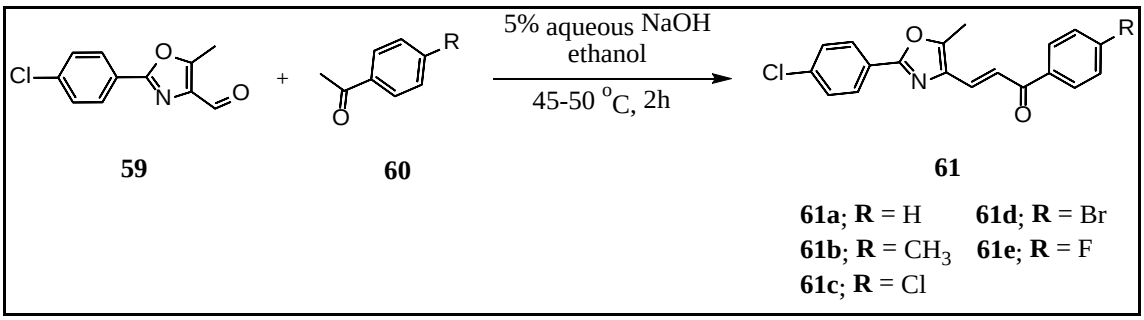
To overcome the difficulty in the preparation and handling of aromatic acyl halides and fluctuation in the yields of the formyl oxazoles, another synthesis route was explored. The alternate synthesis was leading to 5-methyl derivatives of 2-aryl-4-formyl-1,3-oxazoles. Thus 4-chlorobenzaldehyde **56** was reacted with diaceyl monoxime in glacial acetic acid to give 4-(chloromethyl)-2-(4-chlorophenyl)-5-methyloxazole 3-oxide[75,76] **57** which was converted to 4-(chloromethyl)-2-(4-chlorophenyl)-5-methyl-1,3-oxazole **58** on heating with $POCl_3$ in dichloroethane.[75,76] The 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** were obtained by reacting 4-(chloromethyl)-2-(4-chlorophenyl)-5-methyl-1,3-oxazole **57** with bis-TBAC (Bis-Tetrabutylammonium dichromate) in chloroform[77] (**Scheme 4.11**).

*For the application in the present chapter 4-chlorobenzaldehyde **56** was employed for the synthesis of 1,3-oxazole **59**. Similar 2-aryl-5-methyl-1,3-oxazole-4-carbaldehydes were reported earlier using some other synthetic routs as patents and were not fully characterized.[78–80]



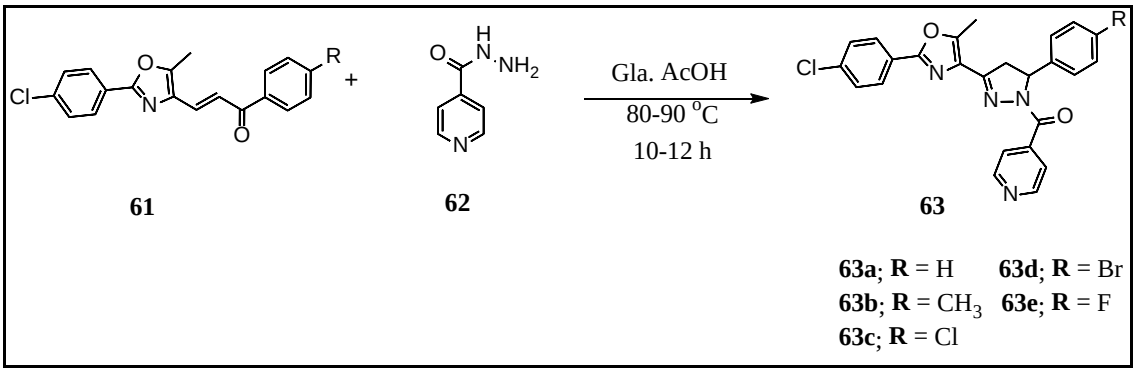
Scheme 4.11 ***Synthesis of 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde 59.***

For the synthesis of 1-aryl-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenones **61** the Claisen-Schmidt reaction between 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** and various 4-substituted acetophenones **60** was carried out[81] in ethanol using aqueous alkali at 45-50 ^{o}C to afford the heteroaryl chalcones **61** in good yields (**Scheme 4.12**).



Scheme 4.12 ***Synthesis of chalcones 61.***

Further, employing the 1-aryl-oxazolyl-3-aryl-propenones **61a-e,** five new derivatives of oxazolyl-pyrazolyl-pyridinyl-methanones **63a-e** were prepared[36,60] by reacting an equimolar quantities of 1-aryl-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenones **61a-e** and isoniazid **62** in glacial acetic acid to afford the corresponding [5-aryl-3-{(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-4,5-dihydro-1*H*-pyrazol-1-yl)] (pyridin-4-yl)methanones **63a-e** in good yields (**Scheme 4.13**).



Scheme 4.13 ***Synthesis of substituted 4,5-dihydro-1H-pyrazoles 63.***

4.2.1 Spectral Characterization

All the synthesized compounds 1-aryl-3-(aryloxazolyl)-propenones **61a-e** and (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **63a-e** were characterized by various spectro analytical techniques.

In the IR spectrum of 1,3-oxazole-4-carbaldehyde **59,** an aromatic C-H stretching are observed at ~3049 cm^{-1} and formyl C-H stretching band is observed at ~2850 cm^{-1} . The carbonyl stretching is observed at 1690 cm^{-1}. In the IR spectra of 3-aryl-oxazolyl-1-aryl-propenones **61a-e** aromatic C-H stretching are observed at ~3055 cm^{-1}. A characteristic band is observed at ~1670-1660 cm^{-1} for >C=O stretching as it is a part of extended conjugation. The aromatic C=C stretching bands around ~1581-1480 cm^{-1} with variable intensity are observed in the IR of compounds **61a-e**. In the IR spectrum of compound **61d (**R = Br**)** aromatic C-Br stretching is observed at ~560 cm^{-1}. In the IR spectra of (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **63a-e,** C-H stretching is observed at ~2925 cm^{-1}. An IR band for the carbonyl >C=O stretching for N-acyl group in pyrazolines **63a-e** is observed at ~1615 cm^{-1}. As all the compounds having Cl attached to the phenyl ring, an aromatic C-Cl stretching is observed between ~755-739 cm^{-1}. All the major values observed in IR are included in the experimental section and spectra are included in the spectral data section.

Nuclear Magnetic Resonance (NMR) spectroscopy study for all these compounds was carried out using modern NMR techniques on a 400 MHz NMR instrument.

Proton NMR spectra of 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** show singlet at δ 2.7 ppm due to CH_3 protons. An aldehyde proton is observed as a singlet at δ 10.0 ppm. Aromatic protons are observed between δ 7.4-8.0 ppm.

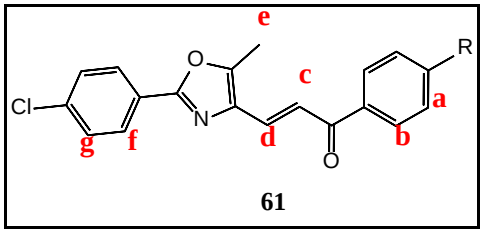


Figure 4.10A ***Compounds 61 with protons labelled.***

Proton NMR spectra of 1-aryl-3-aryloxazolyl-propenones **61a-e** (refer **fig. 4.10A** for proton labels) show a singlet at δ 2.56 ppm due to the CH_3 group protons **e** on the oxazole ring. For the compound **61b (**R = CH_3**)** another singlet is observed at δ 2.45 ppm. The protons **c** and **d** of **61a-e** (**Figure-4.10A**) of the double bond appear at δ 7.72 ppm and 7.84 ppm as doublets with vicinal coupling constant J = 15 Hz confirming *trans* stereochemistry.

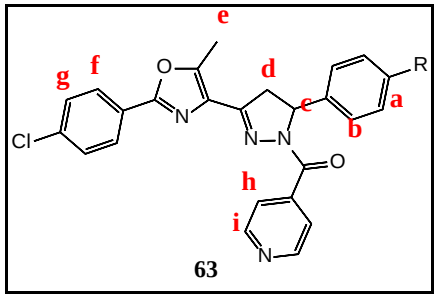


Figure 4.10B ***Compounds 63 with protons labelled.***

In the proton NMR spectra of (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **63a-e** (refer **Fig. 4.10B** for proton labels) the CH_3 protons **e** are observed at δ 2.59 ppm. For compound **63b (**R = CH_3**)** another singlet observed at δ 2.42 ppm. The -CH_2 protons **d** are observed at δ 3.68-3.69 ppm as a doublet of doublets with germinal coupling constant ^1J = 6.4 Hz and vicinal coupling constant ^2J = 10.8 Hz as both the protons couple with the proton **c** present on carbinol carbon. The -CH proton **c** attached to the stereogenic carbinol carbon is observed at δ 5.74 ppm as a doublet of doublets with ^1J = ^2J = 10.8 Hz as it couples differently with two diastereotopic vicinal protons **d** situated

of the methylene group. The most downfield signals for protons **i** of pyridine ring are observed at δ 8.74 ppm.

In carbon 13 NMR of 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** the CH_3 carbon is observed at δ 11.8 ppm and the carbonyl carbon is observed at δ 185.3 ppm**.** All other carbons are observed between δ 124-160 ppm**.**

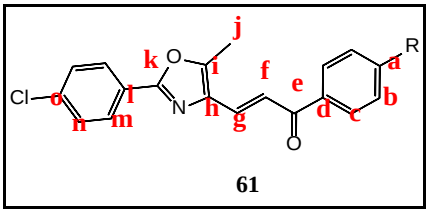


Figure 4.11A ***Compound 61 with labelled carbons.***

The carbon NMR of 1-aryl-3-aryl-oxazolyl-propenones **61a-e (Figure 4.11A** with carbon labelled**)** shows methyl carbon **j** at δ 10.7 ppm. Carbonyl >C=O carbon **e** of all five compounds appear at δ ~189 ppm. All the sp2 carbons appear in between δ 120-160 ppm.

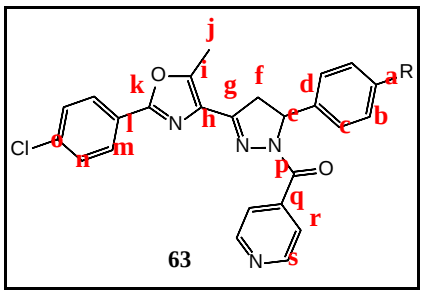


Figure 4.11B ***Compound 61 with labelled carbons.***

In ^{13}C NMR of (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **63a-e** (**Figure 4.11B** with carbon labelled) methyl carbon **j** is observed at δ 10.6 ppm. Carbon **e** and **f** appear at δ 53.1 and δ 37.9 ppm respectively. Carbonyl carbon **p** is observed at slightly lower δ value (upfield) at δ 164.7 ppm.

4.2.2 Single crystal X-ray diffraction study

Single crystals of one of the oxazole aldehyde **59 (**R = Cl**)** and one of the α,β-unsaturated compounds **61c** (R = Cl) were developed and studied for single crystal X–ray diffraction characteristics. The ORTEP diagrams (**Figure 4.12**) and unit cell packing (**Figure 4.13**) of monoclinic (space group P21/c) crystal of the oxazole aldehyde **59 (**R = Cl**)** are as presented**.**

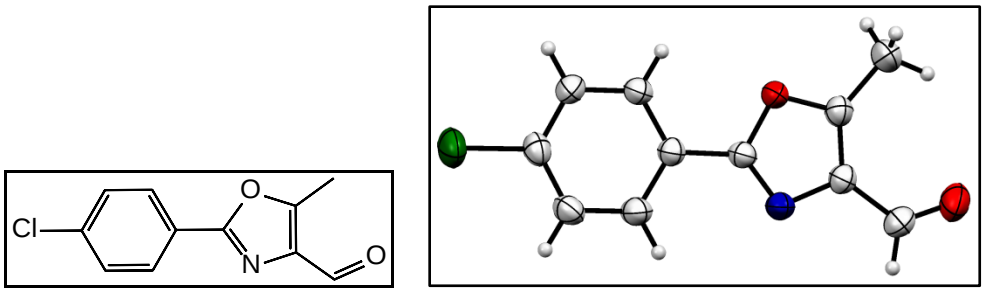


Figure 4.12 ***Structure and ORTEP diagram of 59.***

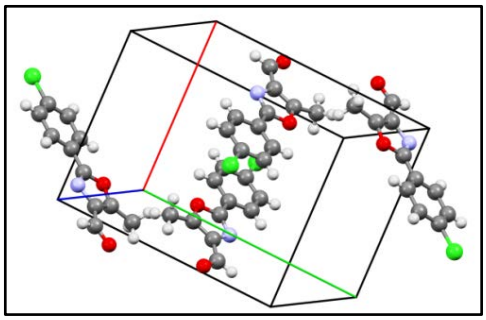


Figure 4.13 ***Unit cell of 59.***

In case of α,β-unsaturated ketone **61b** (R = CH_3) the monoclinic (space group P21/n) crystals, ORTEP diagram and packing in unit cell are included in **Figure 4.14** and **4.15** respectively. The intermolecular π-π stacking interactions observed between C1---C19 (3.372 Å). There are three sets of the intermolecular CH---π interactions observed between C19---H6 (2.894 Å), C19---H20 (2.866 Å) and C24---H16 (2.885 Å). Two sets of the intermolecular CH---Cl interactions observed with distance 2.903 Å and distance 2.942 Å forming 1D linear chain. The intermolecular interactions between O15---H2 (2.508 Å) are also observed. The 1D chain is further linked to form 2D molecular packing pattern as presented in **Figure 4.16**.

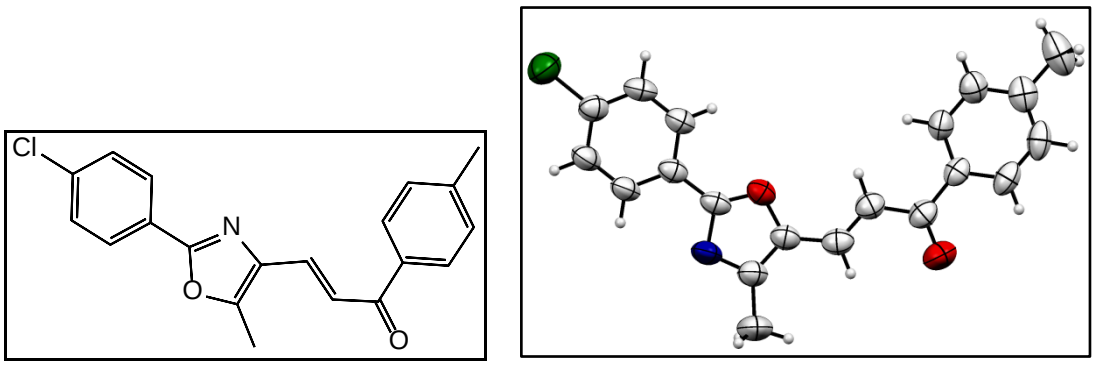


Figure 4.14 ***Structure and ORTEP diagram of 61b.***

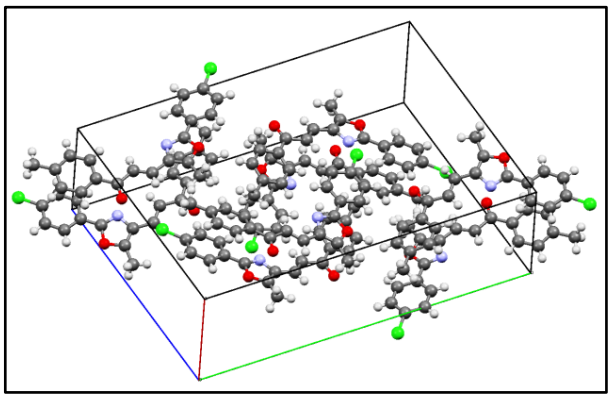


Figure 4.15 ***Unit cell of 61b.***

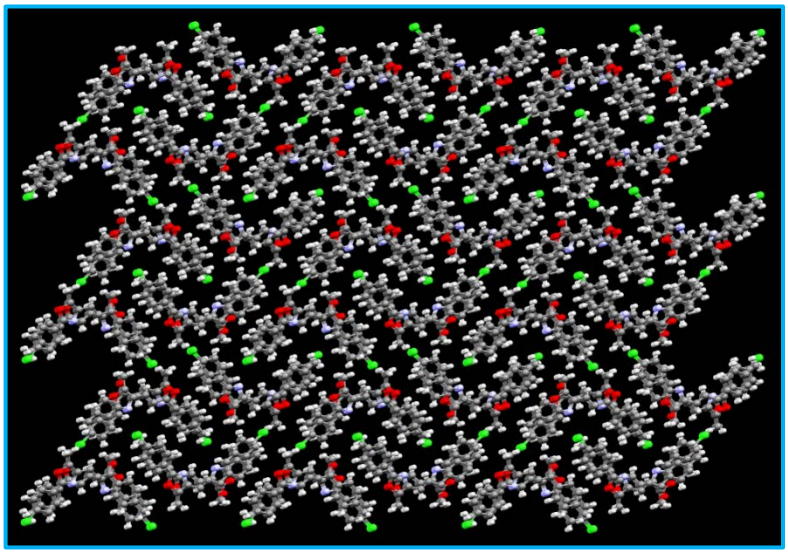
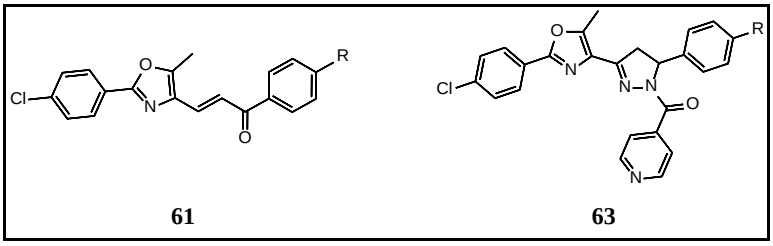


Figure 4.16 ***Molecular packing pattern of 61b.***

The substitution pattern, yield and melting point of all the newly synthesized compounds are summarized in **Table 4.1.**

Table 4.1 ***Physical data (Yield, mp) of newly synthesized compounds.***



ID	Substituent (R)	Molecular formula	Isolated Yield	mp
61a	-H	$C_{19}H_{14}ClNO_2$	79%	148 ^oC
61b	-CH_3	$C_{20}H_{16}ClNO_2$	76%	136 ^oC
61c	-Cl	$C_{19}H_{13}Cl_2NO_2$	80%	152 ^oC
61d	-Br	$C_{19}H_{13}ClBrNO_2$	76%	147 ^oC
61e	-F	$C_{19}H_{13}ClFNO_2$	81%	160 ^oC
63a	-H	$C_{25}H_{19}ClN_4O_2$	73%	142 ^oC
63b	-CH_3	$C_{26}H_{21}ClN_4O_2$	75%	148 ^oC
63c	-Cl	$C_{25}H_{18}Cl_2N_4O_2$	74%	154 ^oC
63d	-Br	$C_{25}H_{18}Cl_2N_4O_2$	71%	150 ^oC
63e	-F	$C_{25}H_{18}ClFN_4O_2$	72%	144 ^oC

4.2.3 Antimicrobial activity study

All the newly synthesized compounds (**61a-e** and **63a-e**) were screened for antibacterial activity against (*Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 619) as a Gram positive bacteria and *Escherichia coli* (MTCC 739) and *Pseudomonas aeruginosa* (MTCC 741) as Gram negative bacteria. The study antifungal activity was carried out against *Aspergillus niger* (MTCC 282) and *Candida albicans* (MTCC 183). Both the studies were carried out at Microcare Laboratory, Surat, Gujarat. The activity is compared with ciprofloxacin, an effective antibiotic taken as a reference compound. The detailed experimental procedure is included in the experimental section of this chapter. Results of the antibacterial and antifungal activities are summarized in **Table 4.2** and graphically presented as shown **Figures 4.17** for the activity results against Gram +ve bacteria, **Figure 4.18** for the activity results against Gram –ve bacteria and **Figure 4.19** representing antifungal activity results.

Table 4.2 ***Antimicrobial activity results.***

ID	Zone of inhibition in mm and (MIC in µg/mL)											
	Gram(+ve) bacteria				Gram(-ve) bacteria				Fungi			
	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*		*C. albicans*		*A. niger*	
	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)	Zone (mm)	MIC (µg/mL)
61a	19	125	20	100	22	50	23	25	18	125	20	100
61b	**26**	**12.5**	**26**	**12.5**	**28**	**6.25**	**26**	**12.5**	24	25	23	25
61c	21	100	22	50	24	25	23	25	**25**	**12.5**	**26**	**12.5**
61d	23	25	**25**	**12.5**	17	125	22	50	22	100	21	100
61e	21	100	18	125	24	25	**25**	**12.5**	**25**	**12.5**	22	100
63a	20	100	22	50	21	100	18	125	20	100	23	25
63b	**45**	**6.25**	**25**	**12.5**	**26**	**12.5**	23	25	24	25	**25**	**12.5**
63c	**26**	**12.5**	23	25	**25**	**12.5**	**26**	**12.5**	**25**	**12.5**	20	100
63d	24	25	**26**	**6.25**	**25**	**12.5**	24	25	**28**	**6.25**	**26**	**12.5**
63e	**25**	**12.5**	24	25	22	50	**25**	**12.5**	21	100	**25**	**12.5**
Ciprofloxacin	**30**	**<3.12**	**31**	**<3.12**	**33**	**<3.12**	**32**	**<3.12**	-----		----	
Griseo fulvin	----		----		----		----		**33**	**<3.12**	**30**	**<3.12**
DMSO	----		----		----		----		----		----	

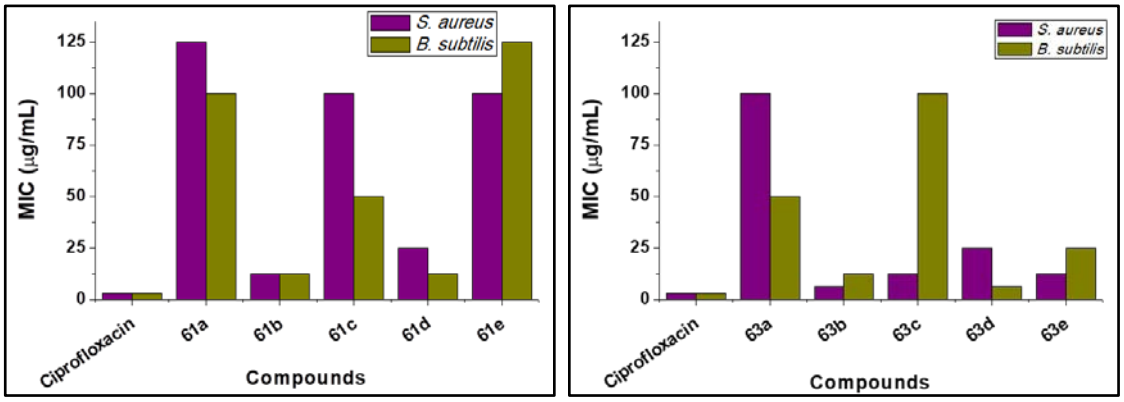


Figure 4.17 ***Antibacterial (Gram+ve) activity (MIC) results for 61 and 63.***

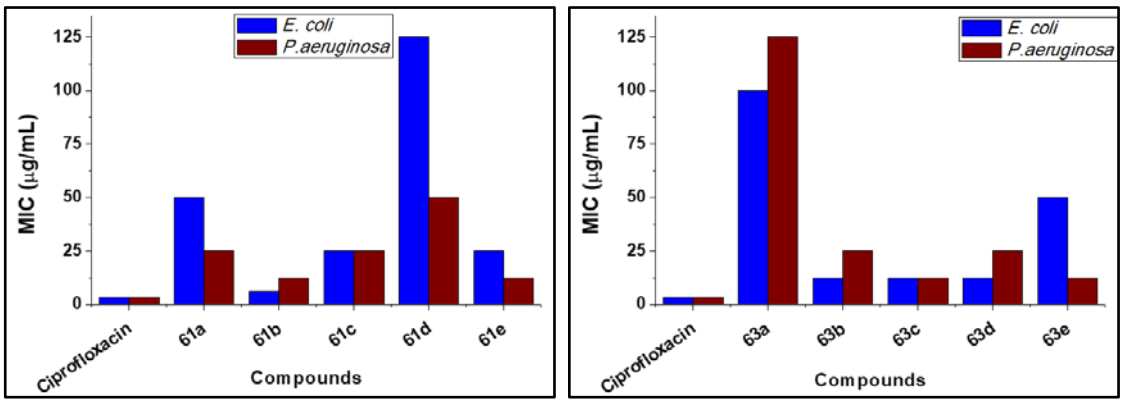


Figure 4.18 ***Antibacterial (Gram-ve) activity (MIC) results for 61 and 63.***

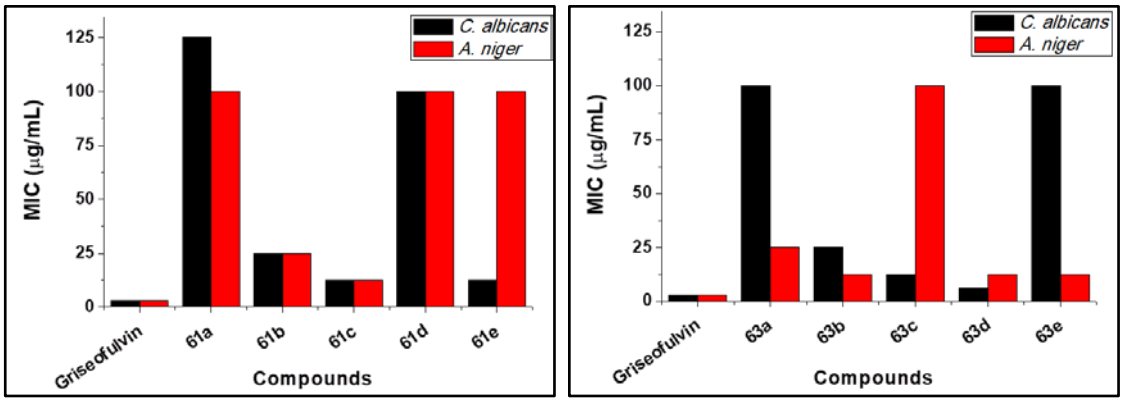


Figure 4.19 ***Antifungal activity (MIC) results 61 and 63.***

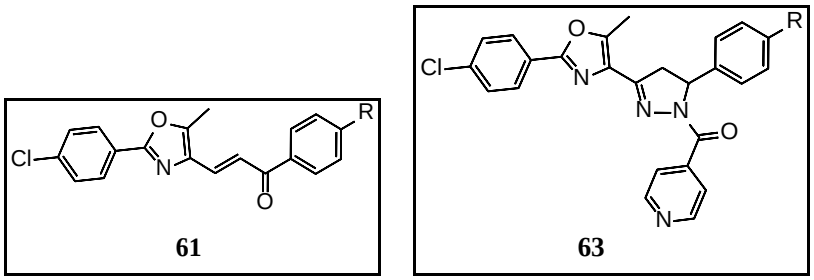


Figure 4.21 ***General structures of compound 61 and 63.***

Amongst all the newly synthesized compounds, several compounds exhibited good to excellent antimicrobial activity. From aryl-oxazolyl-propenones **61a-e** Compounds **61b** (R = -CH_3), **61d** (R = -Br) displayed good inhibition potency against *S. aureus* bacterial strain with MIC of 12.5 µg/ml and 25 µg/ml while compounds **61b** (R = -CH_3) and **61d** (R = -Br) showed good inhibition with MIC = 12.5 µg/ml against *B. subtilis* bacterial strain (**Figure 4.17**).

In case of Gram negative bacteria, compound **61b** (R = -CH_3) showed excellent inhibition (MIC = 6.25 µg/ml) against *E. coli*, while compounds **61b** (R = -CH_3) and **61e** (R = -F) displayed inhibition at the concentration 12.5 µg/ml against *P. aeruginosa* (**Figure 4.18**). Antifungal activity of these compounds show compounds **61c** (R = -Cl) and **61e** (R = -F) displayed good inhibition (MIC = 12.5 µg/ml) against fungal strain *C. albicans* (**Figure 4.19**). While compound **61c** also exhibit good inhibition potency with MIC = 12.5 µg/ml (**Figure 4.19**) against *A. niger* fungal strain.

In case of (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **63a-e,** compound **63b** (R = -CH_3) showed greater inhibition with MIC = 6.25 µg/ml against *S. aureus* as

compared to that of the parent chalcone **61b** (**Figure 4.17**). Compound **63e** (R = -F) displayed good inhibition potency against the same bacterial strain with MIC of 12.5 µg/ml. Highest inhibition was observed by compound **63d** (R = -Br) with MIC = 6.25 µg/ml against *B. subtilis* as compared to that of chalcone **61d** (12.5 µg/ml) (**Figure 4.18**). While compound **63b** (R = $-CH_3$) showed good inhibition with MIC = 12.5 µg/ml against the same bacterial strain which is same as that of the chalcone **61b** (**Figure 4.18**).

In case of Gram negative bacteria compound **63b** (R = $-CH_3$), **63c** (R = -Cl), **63d** (R = -Br) showed noteworthy inhibition against *E.coli* with MIC = 12.5 µg/ml which are higher than that of parent oxazolyl-propenones shows the higher potency of pyrazolyl-methanone **63d** as compared to that of oxazolyl propenones **61d** (**Figure 4.19**). Compounds **63c** (R = -Cl), **63e** (R = -F) displayed inhibition at the concentration 12.5 µg/ml against *P. aeruginosa* which comparable as that of the oxazolyl chalcones **61c** and **61e** (**Figure 4.19**).

Antifungal activity of pyrazolyl-methanones **63a-e** shows that compound **63d** (R = -Br) exhibit excellent inhibition with MIC = 6.25 µg/ml against *C. albicans* as compared to that of **61d** (MIC = 100 µg/ml) while compound **63c** (R = -Cl) displayed good inhibition (MIC = 12.5 µg/ml) against the same fungal strain which is comparable as that of **61c** (**Figure 4.19**). Compounds **63b** (R = $-CH_3$), **63d** (R = -Br), **63e** (R = -F) are found to inhibit growth at MIC = 12.5 µg/ml of *A. niger* fungal strain (**Figure 4.19**). Against *A. niger*, compounds **63d** and **63e** (MIC = 12.5 µg/ml) showed greater potency than that of the parent chalcones **61d** and **61e** (MIC = 100 µg/ml) (**Figure 4.19**). The remaining compounds of the series possessed noteworthy antimicrobial activity.

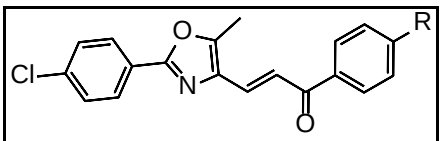
From the antimicrobial activity results revealed that oxazolyl-pyrazolyl-pyridinyl-methanones **63** show greater potency against bacterial and fungal strains then that of oxazolyl-propenones **61**. On comparing the effect of the substitutions on aromatic ring it was observed that halogen substituted compounds showed greater antimicrobial activity.

4.2.4 Anticancer activity study

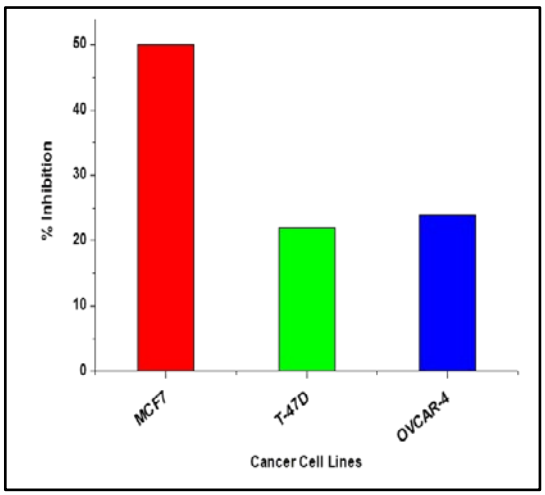
The newly synthesized compounds were also studied for their anticancer activities with the support from project of Development Therapeutic Program (DTP), at National Cancer Institute (NCI), Chemotherapeutic Research division, United States of America (USA) against 60 cancerous cell line panel as per their protocol. All the newly synthesized compounds were submitted and all of them were selected for *in-vitro* anticancer assay at NCI for one dose 60 cancer cell line screening. Results of each test compound are reported as percentage growth of the treated cells when compared with untreated control cells.

The results of the single dose anticancer screening of all the ten selected compounds in form of one dose mean graphs are included in appendix at the end of the thesis (**Sheet 11-20**).

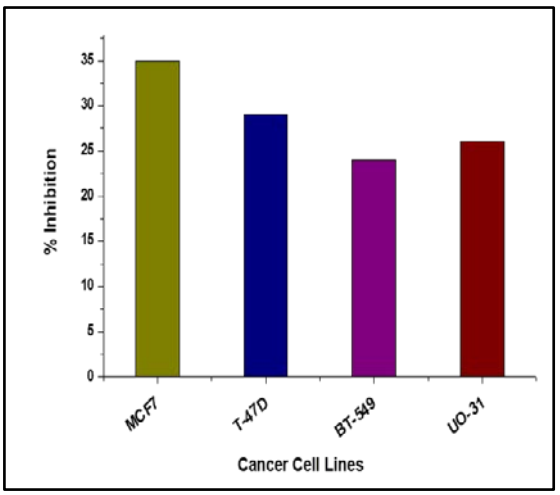
The summary of the significant results of single dose 60 cancer cell lines for **61a-e** are presented in graphical form (**Figure 4.20**). For a particular compound the cell lines with greater than 20% growth inhibition are included in the graphs.



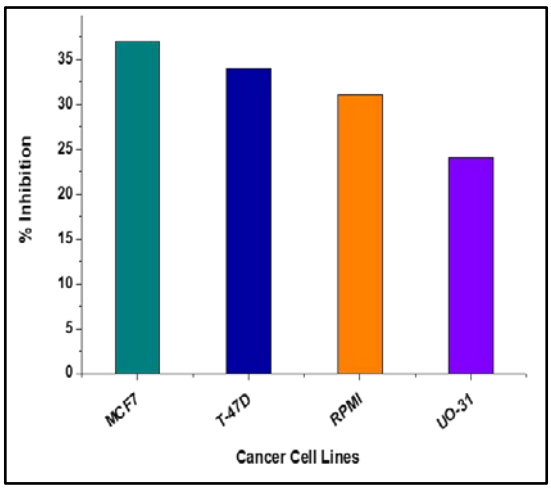
61



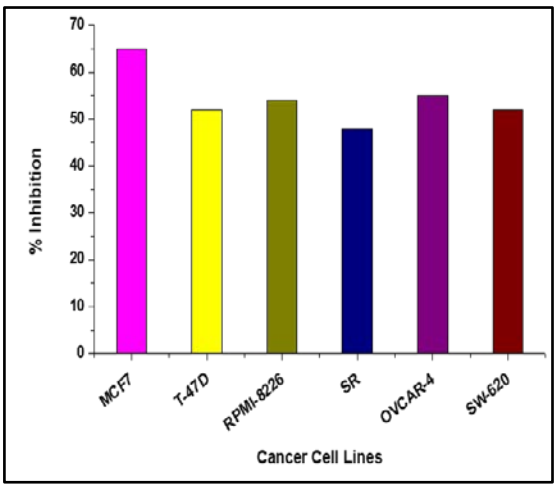
Compound 61a, R = H



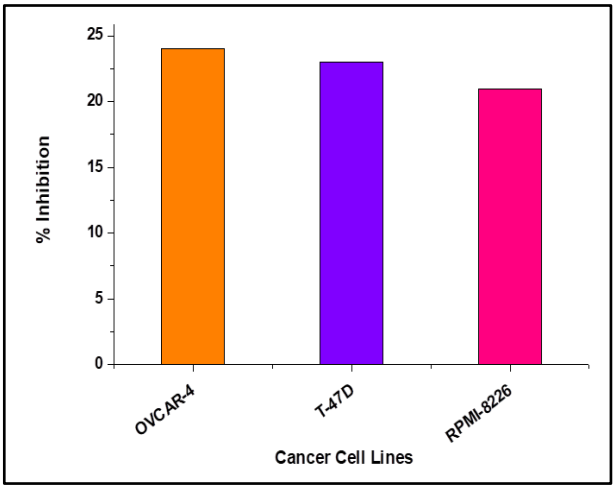
Compound 61b, R = CH_3



Compound 61c, R = Cl



Compound 61d, R = Br

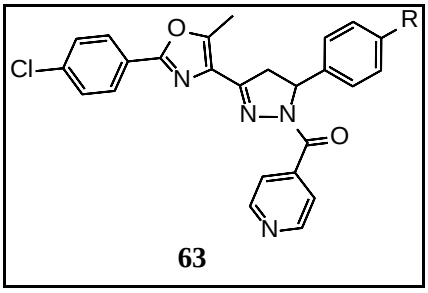


Compound 61e, R = F

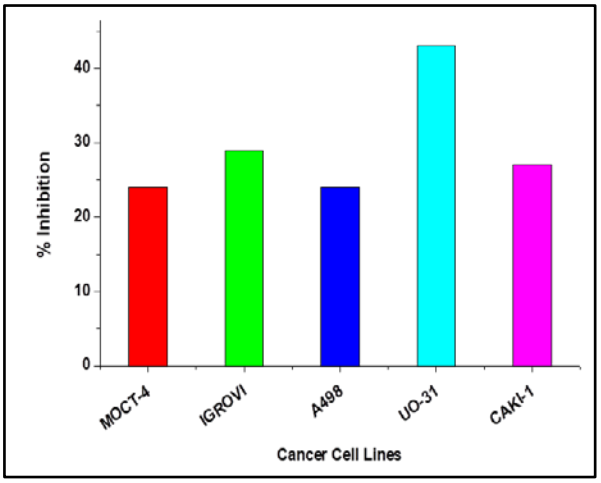
Figure 4.20 ***Results of single dose anticancer screening from 61 at NCI.***

The anticancer activity study of 3-aryl-5-methyloxazolyl-1-aryl-propenone **61a-e** show noteworthy results against only few of the cancerous cell lines as shown (**Figure 4.20**). Compound **61a (**R = H**), 61b (**R = CH_3**), 61c (**R = Cl**) and 61d (**R = Br**)** showed 50%, 35%, 37% and 65% inhibition against Breast cancer (MCF7) cell line respectively. The chalcone with Fluoro substitution is showing poor inhibition compared to the other chalcones. This indicates that there is no significant inductive effect of the substitution. Bromo substitution is causing highest inhibition. Compound **61d (**R = Br**)** showed inhibition of 55% against Ovarian cancer (OVCAR-4) cell line, 61% against Breast cancer (T47D) cell line, 54% against Leukemia (RPMI-8226) cell line and 52% inhibition against Colon cancer (SW-620) cell line (**Figure 4.20**). While the heteroaryl chalcone with methyl substitution does not showed significant enhancement in the anticancer activity.

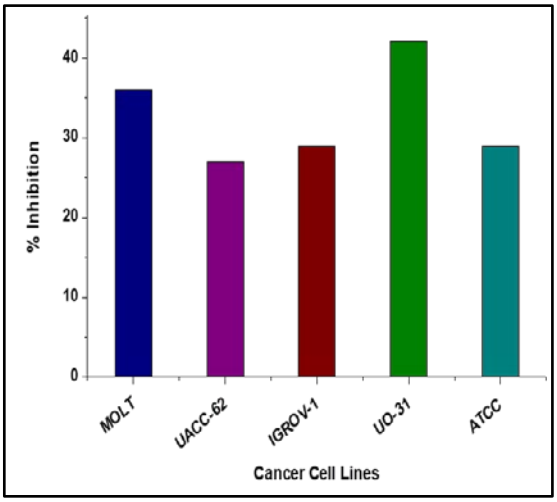
The results of single dose 60 cancer cell lines for all selected compounds **63a-e** are presented in graphical form (**Figure 4.21**). For a particular compound the cell lines with greater than 20% growth inhibition are included in the graphs.



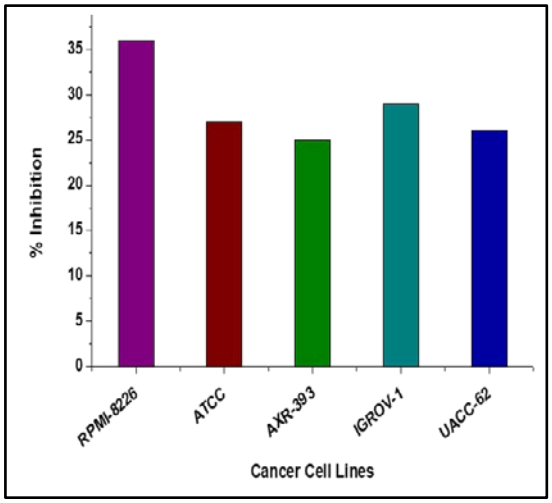
63



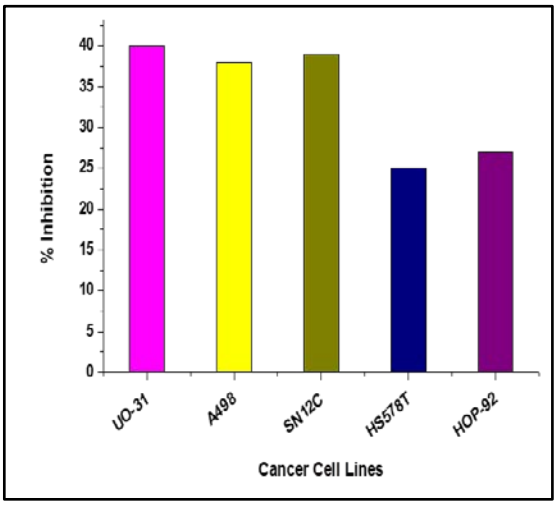
Compound 63a, R = H



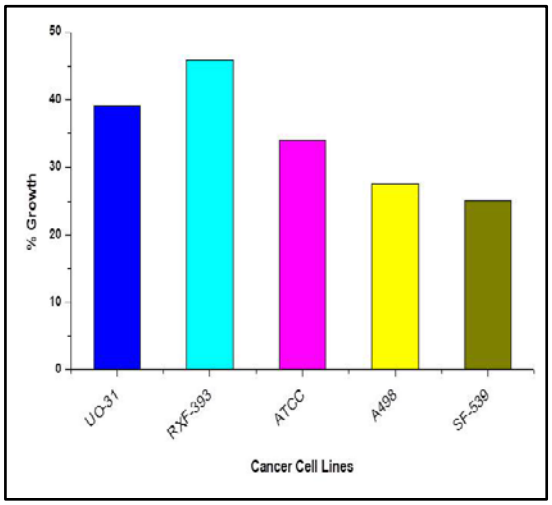
Compound 63b, R = CH_3



Compound 63c, R = Cl



Compound 63d, R = Br



Compound 63e, R = F

Figure 4.21 ***Results of single dose anticancer screening from 63 at NCI.***

The anticancer activity study of (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **63a-e** show greater anticancer activity as shown (**Figure 4.21**). Compound **63a (**R = H**)** and **63b (**R = CH_3**)** displayed 43% and 42% inhibition against renal cancer (UO-310) cell line respectively (**Figure 4.21**). Compound **63d (**R = Br**)** showed about 40% inhibition for renal cancer (UO-31) cell line, breast cancer (A498) cell line and renal cancer (SN12C) cell lines (**Figure 4.21**). From the anticancer results the highest activity was observed for compound **63d (**R = Br**)** against breast cancer (MCF7) cell line and Compound **63d (**R = Br**)** showed 55% inhibition against ovarian cancer (OVCAR-4) cell line and 63% inhibition against breast cancer (T47D) cell line (**Figure 4.21**).

Overall the results show that the introduction of pyrazoline and isonicotinoyl moieties have found to enhance no increment of the anticancer activities as compared to their parent chalcones. Decreased solubility and cell permeability could be resulting their decreased bioavailability. Their conversion to water soluble salts may result in increase in bioactivity.

4.3 Conclusion

In the present study, chloroaryl derivative of 5-methyl-1,3-oxazolyl-4-carbaldehyde is prepared. Employing this formyl oxazole some new α,β-unsaturated ketones are synthesized employing well known Claisen-Smidth condensation. These resulting chalcones have been utilized to prepare the corresponding pyridine containing pyrazoline hybrids. All the new multi heterocyclic compounds are characterized by various spectroscopic methods and analytical data of all the compounds are well in accordance with the structures proposed. The single crystal X-ray diffraction study of compounds **59** and **61c** gives further insight on the conformation and crystal packing of these compounds. The new compounds are studied for their *in vitro* antibacterial and antifungal activities against the selected bacterial and fungal strains. Antimicrobial activity reveals that some of the compounds exhibit excellent activity. Compounds (oxazolyl-pyrazolyl)-(pyridinyl)-methanones **63a-e** showed better antimicrobial activity as compare to 1-ary-3-aryl-5-methyl-1,3-oxazolyl-propenones **61a-e.** Greater activity was observed for the halogenated compounds. Anticancer activity of all the compounds was carried out. All the synthesized compounds displayed a moderate anticancer activity against some of the cancer cell lines. The highest anticancer activity was observed for compound **61d (**R = Br**)** against the breast cancer (MCF7) cell line and the Compound **61d (**R = Br**)** showes 55% inhibition against the ovarian cancer (OVCAR-4) cell line and 63% inhibition against the breast cancer (T47D) cell line.

4.4 Experimental

General

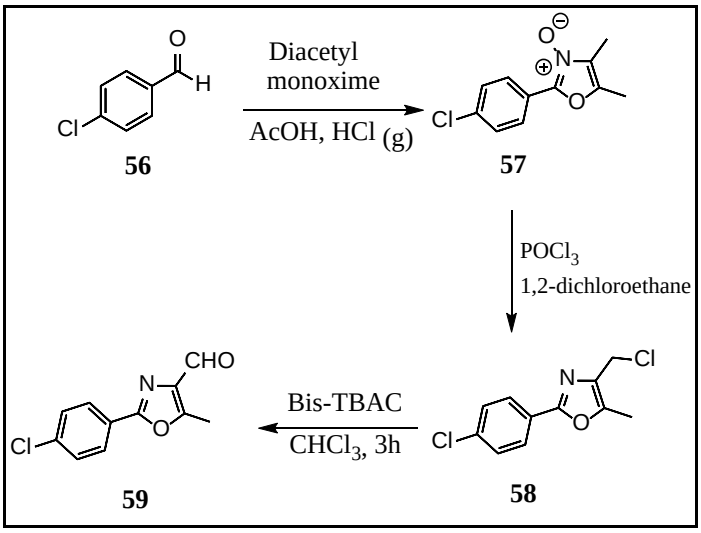
The chemicals were used as received from local companies without further purification. Organic solvents were purified by distillation prior to use.

Column chromatography was carried out using silica gel (60-120 mesh). Thin layer chromatography was performed on the pre-coated silica gel 60 F_{254} aluminium sheets. Melting points are determined in open capillary and are uncorrected.

FT-IR spectra were recorded on Perkin Elmer FTIR spectrometer between 4000-400 cm^{-1} in solid state as KBr discs. The NMR spectra were recorded on 400 MHz Bruker Avance-III instrument and chemical shifts are given in parts per million. In the NMR data for ^{19}F decoupled ^{1}H NMR experiments, the data for the affected signals only are included. ^{19}F chemical shift values are of ^{1}H decoupled ^{19}F signals.

Mass spectra were recorded on Waters' Xevo G2-XS QToF at the Zydus Research Centre, Ahmedabad. X-ray diffraction data for the compounds were collected at room temperature using a Bruker Smart Apex CCD diffractometer.

Synthesis of 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde 59.[75–77]



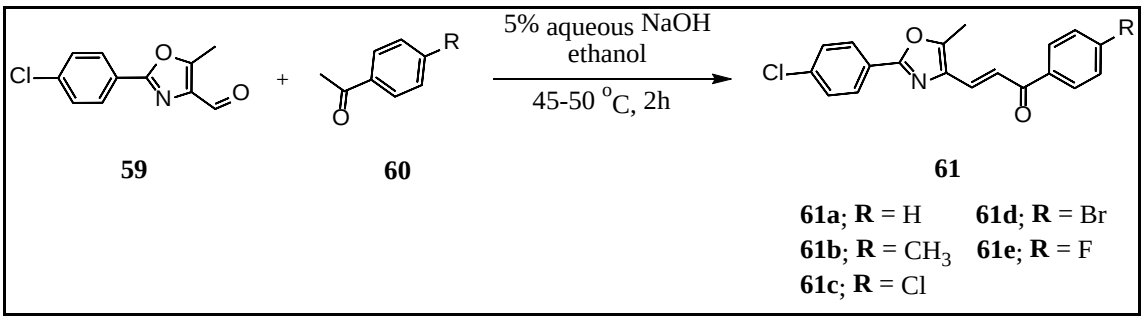
2-(4-Chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** was prepared from 4-chlorobenzaldehyde and diacetyl monoxime. To an ice-cold mixture of 4-chloro benzaldehyde **56** (142 mmol, 1 eq) and diacetyl monoxime (142 mmol, 1 eq) in acetic acid (30 ml, 3 fold), dry HCl gas was passed for 3h at 0 ^{o}C. The reaction mixture was than diluted with diethyl ether (70 ml, 6 fold). Separated solid was filtered, washed with diethyl ether and dried under vacuum to obtain 2-(4-chlorophenyl)-4,5-dimethyl-1,3-oxazole N-oxide **57** as a white solid. Yield = 71%.

To an ice cold suspension of 2-(4-chlorophenyl)-4,5-dimethyl-1,3-oxazole N-oxide **57** (45 mmol, 1 eq) in dichloro ethane (DCE) (35ml, 5 fold) was added $POCl_3$ (49 mmol, 1.1 eq) dropwise over a period of 2h at 10 ^{o}C. The reaction mixture was slowly heated to 60 ^{o}C and stirred at that temperature for 3h. The reaction mixture was cooled to room temperature, poured into ice cold water and extracted with DCE. The combined organic extracts were washed with water, dried over $CaCl_2$ and concentrated under vacuum to furnish 4-(chloromethyl)-2-(4-chlorophenyl)-5-methyl-1,3-oxazole **58** with excellent yield. Yield = 74%.

Homogeneous solution of 4-(chloromethyl)-2-(4-chlorophenyl)-5-methyl-1,3-oxazole **58** (10 mmol, 1eq) and **b**is-tetrabutyl ammonium dichromate[75–77] (6 mmol, 0.6 eq) in chloroform (7.5 ml) was heated under reflux for 3h. The crude product was filtered through silica gel to eliminate the TBA salt. The silica was then washed with diethyl ether (100 ml). Evaporation of the combined organic layer afforded the desired

2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59**. Yield: 1.32 g, 59%; white solid. **IR (KBr) cm^{-1}**: 2956, 1687, 1596, 1066, 829; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.73 (3H, s, -CH_3 protons), 7.46 (2H, d, *J* = 6.8 Hz, Ar-H), 7.90 (2H, d, *J* = 6.8 Hz, Ar-H), 10.02 (1H, s, -CHO proton); **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 11.8 (-**C**H_3), 124.8, 127.3, 127.8, 129.0, 129.2, 135.9, 137.4, 156.7, 159.5, 185.3 (-**C**=O). **ESI-MASS**: (m/z) 221.95 $(M+H)^+$ for M = $C_{11}H_8ClNO_2$.

General procedure for the synthesis of 1-aryl-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)- propenones 61.[81]

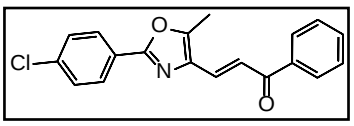


59 **60** **61**

61a; **R** = H **61d**; **R** = Br
61b; **R** = CH_3 **61e**; **R** = F
61c; **R** = Cl

To a magnetically stirred mixture of 4-substituted acetophenones **60** (0.01 mol) in ethanol (95%, 80 ml) and NaOH (0.012 mol) in10 ml water in a 250 ml round bottom flask, a solution of 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** (0.01 mol) in 20 ml ethanol was added drop wise using addition funnel during 20-30 minutes at room temperature. The reaction mixture was heated in water bath at 45-50 °C and the reaction was continued for further 2 hours to complete the reaction (TLC: 10% E.A.). The reaction mixture was then poured into ice cold water to precipitate the product as yellow solid, which was then filtered, dried and crystallized from ethanol. Yield: 76-81%.

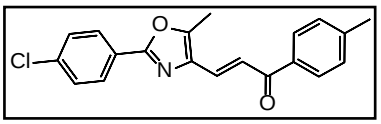
3-(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-1-phenyl-propenone 61a.



Compound **61a** was prepared following the general procedure described above by treating 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** (0.5g, 2.25 mmol) in ethanol with acetophenone **60** (0.45g, 2.25 mmol) and NaOH (0.1g, 2.7 mmol) in 10 ml water. Yield = 0.57g, 79%; Light Yellow Solid; M.P. = 148 °C; **IR (KBr) cm^{-1}**: 3057, 1659,

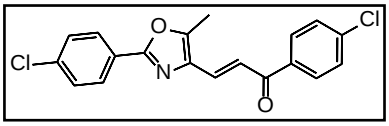
1626, 1568, 1110, 738; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.56 (3H, s, -CH_3), 7.47 (2H, m, Ar-H), 7.54 (2H, d, *J* = 8 Hz, Ar-H), 7.61 (1H, m), 7.72 (1H, d, -CH=CH-, *J* = 14.8 Hz), 7.84 (1H, d, -CH=CH-, *J* = 14.8 Hz), 8.03 (2H, m, Ar-H), 8.13 (2H, t(b), Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.7 (-**C**H_3), 121.8, 125.4, 145.8, 128.6, 128.6, 128.1, 131.8, 132.9, 134.2, 136.7, 138.0, 151.5, 159.6, 189.9 (-**C**=O); **Mass (TOF MS ES+)**: m/z 324.0 $(M+H)^+$ for M = $C_{19}H_{14}ClNO_2$.

3-(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-1-(4-methylphenyl)-propenone 61b.



Compound **61b** was prepared following the general procedure described above by treating 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** (0.5g, 2.25 mmol) in ethanol with 4-methyl acetophenone **60** (0.3g, 2.25 mmol) and NaOH (0.1g, 2.7 mmol) in 10 ml water. Yield = 0.58g, 76%; Light Yellow Solid; M.P. = 136 °C; **IR (KBr) cm^{-1}**: 3055, 1662, 1616, 1581, 1117, 739; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.45 (3H, s, -CH_3), 2.55 (3H, s, -CH_3), 7.32 (2H, d, *J* = 8 Hz, Ar-H), 7.46 (2H, t, Ar-H), 7.70 (1H, d, -CH=CH-, *J* = 15.2 Hz), 7.84 (1H, d, -CH=CH-, *J* = 15.2 Hz), 8.03 (4H, m, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.7 (-**C**H_3), 21.7 (-**C**H_3), 121.8, 125.4, 145.7, 128.7, 128.1, 147.4, 131.4, 134.2, 135.4, 136.7, 143.8, 151.3, 159.5, 189.4 (-**C**=O); **Mass (TOF MS ES+)**: m/z 338.0 $(M+H)^+$ for M = $C_{20}H_{16}ClNO_2$. 337.0, found: (m/z).

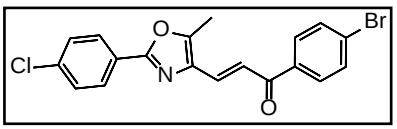
1-(4-Chlorophenyl)-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenone 61c.



Compound **61c** was prepared following the general procedure described above by treating 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** (0.5g, 2.25 mmol) in ethanol with 4-chloro acetophenone **60** (0.34g, 2.25 mmol) and NaOH (0.1g, 2.7 mmol) in 10 ml water. Yield = 0.64g, 80%; Light Yellow Solid; M.P. = 152 °C; **IR (KBr) cm^{-1}**: 3045, 1652, 1618, 1571, 1017, 729; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.56 (3H, s, -CH_3), 7.46-7.50 (4H, m, Ar-H), 7.71 (1H, d, -CH=CH-, *J* = 15.2 Hz), 7.78 (1H, d, -CH=CH-, *J* = 15.2 Hz), 8.05 (4H, m, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.7 (-**C**H_3), 121.1, 125.3, 145.7, 128.7, 128.9, 128.1, 130.0, 132.3, 134.1, 136.3,

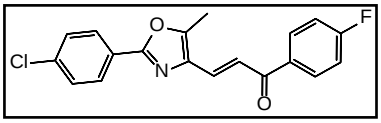
136.8, 139.3, 151.8, 159.6, 188.5 (-**C**=O); **Mass (TOF MS ES+)**: m/z 358.0 $(M+H)^+$ for M = $C_{19}H_{13}Cl_2NO_2$.

1-(4-Bromophenyl)-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenone 61d.



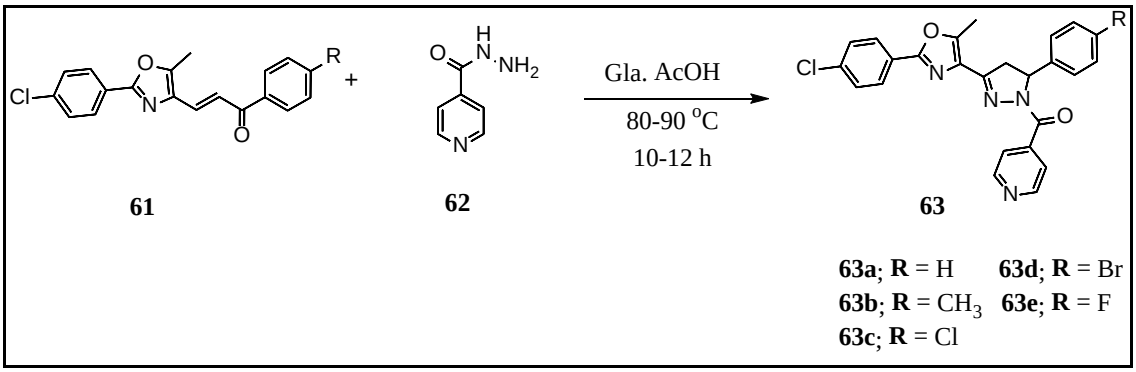
Compound **61d** was prepared following the general procedure described above by treating 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** (0.5g, 2.25 mmol) in ethanol with 4-bromo acetophenone **60** (0.44g, 2.25 mmol) and NaOH (0.1g, 2.7 mmol) in 10 ml water. Yield = 0.69g, 76%; Light Yellow Solid; M.P. = 146 °C; **IR (KBr) cm^{-1}**: 3050, 1660, 1610, 1580, 1127, 739; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.57 (3H, s, -CH_3), 7.47 (2H, m, Ar-H), 7.67 (2H, m, Ar-H), 7.72 (1H, d, -CH=CH-, *J* = 14.8 Hz), 7.78 (1H, d, -CH=CH-, *J* = 14.8 Hz), 7.99 (2H, m, Ar-H), 8.04 (2H, m, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.8 (-**C**H3), 121.1, 125.3, 145.7, 128.0, 128.2, 130.1, 131.9, 132.3, 134.1, 136.7, 136.8, 151.8, 159.6, 188.7 (-**C**=O); **Mass (TOF MS ES+)**: m/z 403.7 $(M+H)^+$ for M = $C_{19}H_{13}ClBrNO_2$.

3-(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-1-(4-fluorophenyl)-propenone 61e.



Compound **61e** was prepared following the general procedure described above by treating 2-(4-chlorophenyl)-5-methyl-1,3-oxazole-4-carbaldehyde **59** (0.5g, 2.25 mmol) in ethanol with 4-fluoro acetophenone **60** (0.31g, 2.25 mmol) and NaOH (0.1g, 2.7 mmol) in 10 ml water. Yield = 0.62g, 81%; Light Yellow Solid; M.P. = 160 °C; **IR (KBr) cm^{-1}**: 3025, 1642, 1636, 1561, 1017, 730; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.56 (3H, s, -CH3), 7.19 (2H, m, Ar-H), 7.47 (2H, dd, *J* = 6.8 Hz), 7.70 (1H, d, -CH=CH-, *J* = 14.8 Hz), 7.80 (1H, d, -CH=CH-, *J* = 14.8 Hz), 8.03 (2H, d, *J* = 6.8 Hz, Ar-H), 8.16 (2H, m, Ar-H); **^{19}F NMR (376 MHz, $CDCl_3$, δ ppm)** : -105; **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.7 (-**C**H_3), 115.7 (d, $^2J_{CF}$ = 22 Hz), 121.3, 125.3, 125.7, 128.1, 131.1 (d, $^3J_{CF}$ = 9 Hz), 132.0, 134.1, 134.3 (d, $^4J_{CF}$ = 3 Hz), 136.8, 151.6, 159.6, 165.6 (d, $^1J_{CF}$ = 250 Hz), 188.2 (-**C**=O); **Mass (TOF MS ES+)**: m/z 342.08 $(M+H)^+$ for M = $C_{19}H_{13}ClFNO_2$.

General procedure for the synthesis of [5-aryl-3-(2-{(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone 63.[36,60]

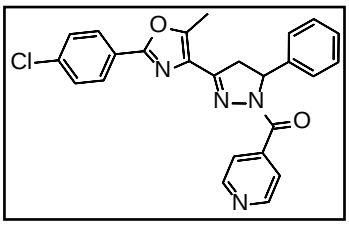


61 **62** **63**

63a; **R** = H **63d**; **R** = Br
63b; **R** = CH_3 **63e**; **R** = F
63c; **R** = Cl

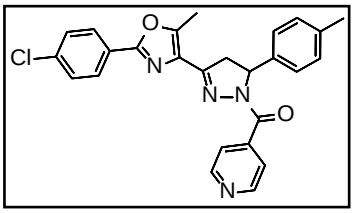
An equimolar quantities of 1-aryl-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenones **61** (1.47 mmol) and isoniazid **62** (1.47 mmol) were heated at 80-90 °C in glacial acetic acid (15 mL) for 10–12 h. After the completion of the reaction (TLC), the reaction mixture was cooled, poured on to crushed ice and neutralized with dilute ammonia solution. A solid so obtained was filtered, washed and subjected to column chromatography to furnish the corresponding compounds **63**. Yield: 70-75%.

[(3-{(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone 63a.



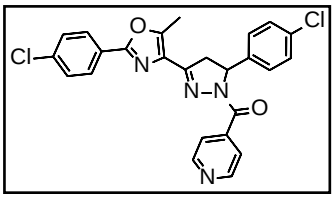
Compound **63a** was prepared following the general procedure described above by treating 3-(2-(4 chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-1-phenyl-propenone **61a** (0.47g, 1.47 mmol) and isoniazid **62** (0.2g, 1.47 mmol) in glacial acetic acid. Yield = 0.47g, 73%; Light Yellow Solid; M.P. = 142 °C; **IR (KBr) cm^{-1}**: 2924, 1636, 1445, 1091, 835, 755; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.59 (3H, s, -CH3), 3.69 (2H, dd, ^1J = 6.4, Hz, ^2J = 10.8), 5.74 (1H, dd, ^1J = ^2J = 10.8 Hz), 7.37 (2H, d, J = 6.8 Hz, Ar-H), 7.47 (3H, m, Ar-H), 7.76 (2H, m, Ar-H), 7.83 (2H, d, J = 6.8 Hz, Ar-H),7.88 (2H, d, J = 6.8 Hz, Ar-H), 8.73 (2H, d, J = 6.0 Hz, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.6 (-**C**H_3), 37.9 (-**C**H_2-), 53.1 (-**C**H-),123.8, 125.9, 126.8, 127.4, 128.1, 128.7, 128.8, 128.9, 130.7, 130.9, 133.5, 136.0, 141.9, 146.9, 149.3, 156.6, 159.0, 164.6 (-**C**=O); **Mass (TOF MS ES+)**: m/z 443.13 $(M+H)^+$ for M = $C_{25}H_{19}ClN_4O_2$.

[(3-{(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-5-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone 63b.



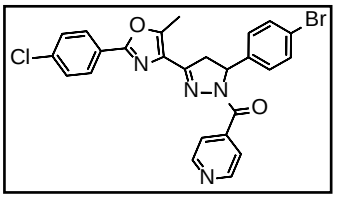
Compound **63b** was prepared following the general procedure described above by treating 3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-1-(4-methylphen-yl)-propenone **61b** (0.5g, 1.47 mmol) and isoniazid **62** (0.2g, 1.47 mmol) in glacial acetic acid. Yield = 0.50g, 74%; Light Yellow Solid; M.P. = 148 °C; **IR (KBr) cm^{-1}**: 2937, 1640, 1430, 1090, 837, 745; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.42 (3H, s, -CH_3), 2.59 (3H, s, -CH_3), 3.67 (2H, dd, ^1J = 6.4, Hz, ^2J = 10.8), 5.72 (1H, dd, ^1J = ^2J = 10.8 Hz), 7.26 (2H, d(b), J = 8 Hz, Ar-H), 7.36 (2H, m, Ar-H), 7.65 (2H, d(b), J = 8.4 Hz, Ar-H), 7.87 (4H, m, Ar-H), 8.73 (2H, d, J = 5.2 Hz, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.6 (-**C**H_3), 21.6 (-**C**H_3), 38.0 (-**C**H_2-), 53.0 (-**C**H-),124.0, 125.9, 126.9, 127.4, 128.1, 128.7, 128.9, 129.5, 133.6, 136.0, 141.2, 142.3, 146.8, 148.9, 156.8, 159.0, 164.3 (-**C**=O); **Mass (TOF MS ES+)**: m/z 457.15 $(M)^+$ for M = $C_{26}H_{21}ClN_4O_2$.

[(5-(4-Chlorophenyl)-3-{(2-(4-chlorophenyl)-5-methyloxazol-4-yl)}-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone 63c.



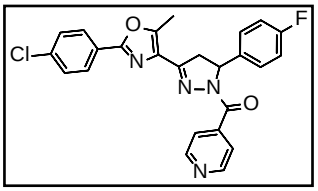
Compound **63c** was prepared following the general procedure described above by treating 1-(4-chlorophenyl)-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenone **61c** (0.52g, 1.47 mmol) and isoniazid **62** (0.2g, 1.47 mmol) in glacial acetic acid. Yield = 0.52g, 75%; Light Yellow Solid; M.P. = 154 °C; **IR (KBr) cm^{-1}**: 2925, 1645, 1447, 1120, 833, 742; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.6 (3H, s, -CH_3), 3.66 (2H, dd, ^1J = 6.4, Hz, ^2J = 10.8), 5.74 (1H, dd, ^1J = ^2J = 10.8 Hz), 7.37 (2H, m, Ar-H), 7.43 (2H, m, Ar-H), 7.69 (2H, m, Ar-H), 7.79 (2H, m, Ar-H), 7.89 (2H, m, Ar-H), 8.73 (2H, d, J = 6 Hz, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.6 (-**C**H_3), 37.8 (-**C**H_2-), 53.2 (-**C**H-), 123.6, 125.9, 126.9, 127.4, 128.2, 128.9, 129.1, 129.5, 133.4, 136.0, 136.7, 141.6, 147.0, 149.6, 155.4, 159.1, 164.7 (-**C**=O); **Mass (TOF MS ES+)**: m/z 476.8 $(M+H)^+$ for M = $C_{25}H_{18}Cl_2N_4O_2$.

[(5-(4-Bromophenyl)-3-{(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone 63d.



Compound **63d** was prepared following the general procedure described above by treating 1-(4-bromophenyl)-3-(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-propenone **61d** (0.59g, 1.47 mmol) and isoniazid **62** (0.2g, 1.47 mmol) in glacial acetic acid. Yield = 0.54g, 71%; Light Yellow Solid; M.P. = 150 ^{o}C; **IR (KBr) cm^{-1}**: 2926, 1645, 1428, 1090, 830, 744; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.6 (3H, s, -CH_3), 3.66 (2H, dd, ^{1}J = 6.4, Hz, ^{2}J = 10.8), 5.74 (1H, dd, ^{1}J = ^{2}J = 10.8 Hz), 7.37 (2H, d, *J* = 6.8 Hz, Ar-H), 7.61 (4H, m, Ar-H), 7.79 (2H, dd, *J* = 4.8 Hz, Ar-H), 7.88 (2H, d, *J* = 6.8 Hz, Ar-H), 8.74 (2H, d, *J* = 6.0 Hz, Ar-H); **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.6 (-**C**H_3), 37.7 (-**C**H_2-), 53.2 (-**C**H-), 123.6, 125.1, 125.9, 127.4, 128.4, 128.9, 129.9, 132.0, 133.4, 136.0, 141.6, 147.0, 149.6, 155.5, 159.1, 164.7 (-**C**=O); **Mass (TOF MS ES+)**: m/z 522.74 $(M+H)^+$ for M = $C_{25}H_{18}Cl_2N_4O_2$.

[(3-{(2-(4-Chlorophenyl)-5-methyl-1,3-oxazol-4-yl)}-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)](pyridin-4-yl)methanone 63e.



Compound **63e** was prepared following the general procedure described above by treating 3(2-(4-chlorophenyl)-5-methyl-1,3-oxazol-4-yl)-1-(4-fluorophenyl)-propenone **61e** (0.50g, 1.47 mmol) and isoniazid **62** (0.2g, 1.47 mmol) in glacial acetic acid. Yield = 0.49g, 72%; Light Yellow Solid; M.P. = 144 ^{o}C; **IR (KBr) cm^{-1}**: 2945, 1635, 1433, 1091, 830, 782; **^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)**: 2.5 (3H, s, -CH3), 3.64 (2H, dd, ^{1}J = 6.4, Hz, ^{2}J = 10.8), 5.74 (1H, dd, ^{1}J = ^{2}J = 10.8 Hz), 7.15 (2H, m, Ar-H), 7.37 (2H, m, Ar-H), 7.76 (4H, m, Ar-H), 7.88 (2H, d, *J* = 6.8 Hz, Ar-H), 8.73 (2H, d, *J* = 6.0 Hz, Ar-H); **^{19}F NMR (376 MHz, $CDCl_3$, δ ppm)** : -108; **^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)**: 10.5 (-**C**H_3), 37.9 (-**C**H_2-), 53.2 (-**C**H-), 115.8, 116.0, 123.6, 125.9, 127.4, 128.9, 129.0, 133.5, 136.0, 141.7, 146.9, 149.6, 155.4, 159.0, 164.7 (-**C**=O); **Mass (TOF MS ES+)**: m/z 460.85 $(M)^+$ for M = $C_{25}H_{18}ClFN_4O_2$.

Experimental procedure for antimicrobial activity.

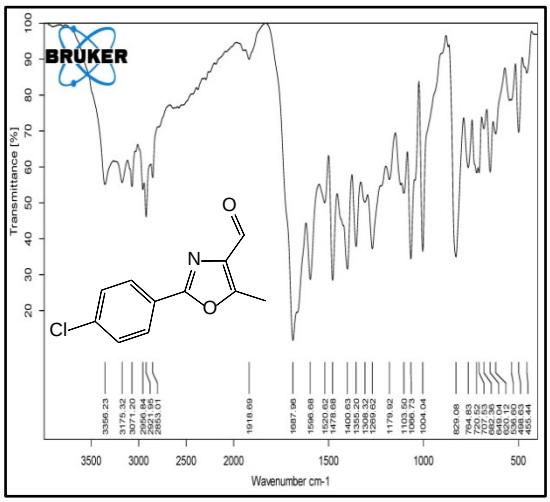
The synthesized pyrazoline-oxazole-pyridine hybrid final compounds were examined for antimicrobial activity against four bacterial and two fungal species using paper disc diffusion technique[82]. The Mueller-Hinton agar medium was sterilized (autoclaved at 120°C for 30 min), poured at uniform depth of 5 mm and allowed to solidify. The microbial suspension (105 CFU/mL; 0.5 McFarland Nephelometery Standards) was streaked over the surface of media using a sterile cotton swab to ensure even growth of the organisms.

The test compounds were dissolved in dimethyl sulfoxide to give solutions of 3.12–125 μg/mL. Sterile filter paper discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper), previously soaked in a known concentration of the respective test compound in dimethyl sulfoxide were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganism and the plates were incubated for 24 h at (37±1) °C. A control disc impregnated with an equivalent amount of dimethyl sulfoxide without any sample was also used and did not produce any inhibition. Ciprofloxacin and Gresiofulvin (100 μg/disc) were used as control drugs for antibacterial and antifungal activity, respectively.

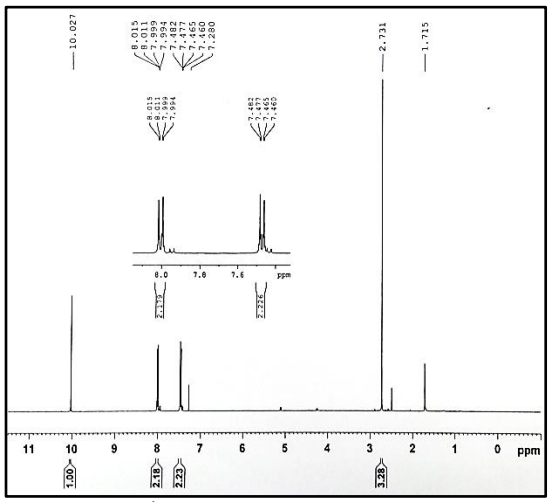
MIC of the test compound was determined by agar streak dilution method[83]. A stock solution of the newly prepared compounds (100 μg/mL) in dimethyl sulfoxide was prepared and classified amounts of the test compounds were incorporated in a specified quantity of molten sterile agar, that is nutrient agar for evaluation of antibacterial and sabouraud dextrose agar for antifungal activity, respectively. The medium containing the test compound was poured into a petri dish at a depth of 4–5 mm and allowed to solidify under sterile conditions. A suspension of the respective microorganism of nearly 105 CFU/mL was prepared and applied to plates with successively diluted compounds with concentrations in the range of 3.12-125 μg/mL in dimethyl sulfoxide and incubated at (37±1) °C for 24 h (bacteria) or 48 h (fungi). The lowest concentration of the substance that prevents the development of visible growth is considered to be the MIC value.

4.5 Spectral Data

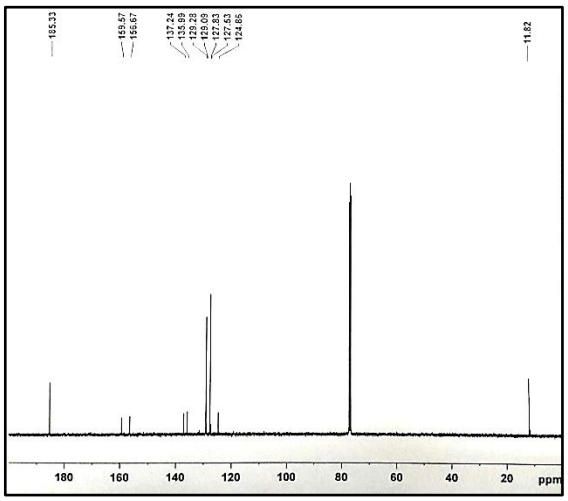
Compound 59



Spectrum 1. IR of compound 59

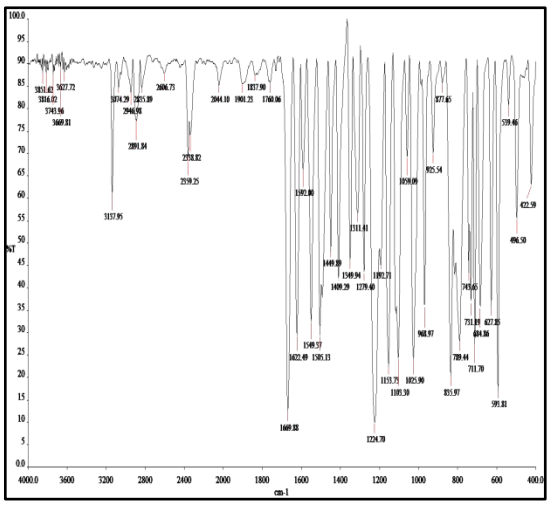


Spectrum 2. ^{1}H NMR of compound 59

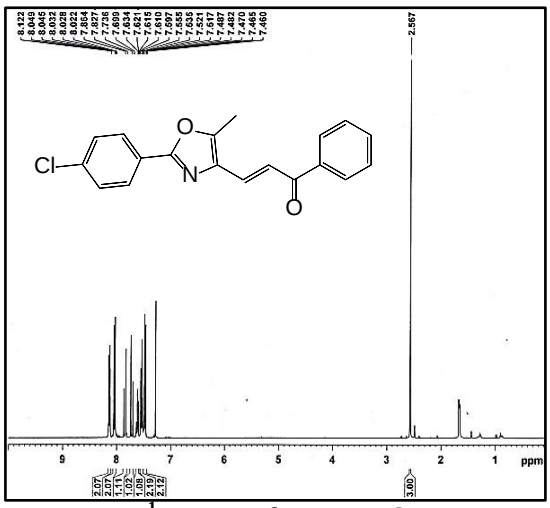


Spectrum 3. 13C NMR of compound 59

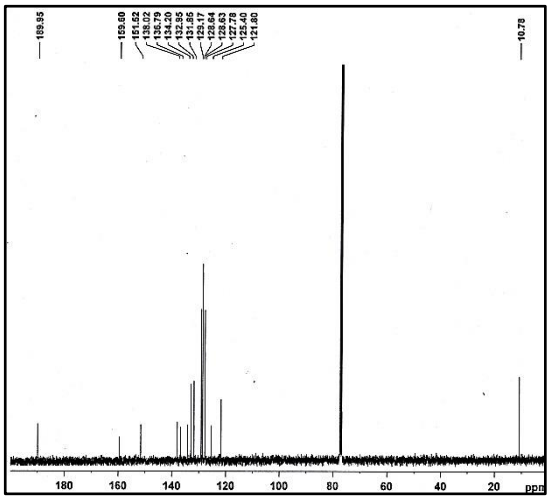
Compound 61a



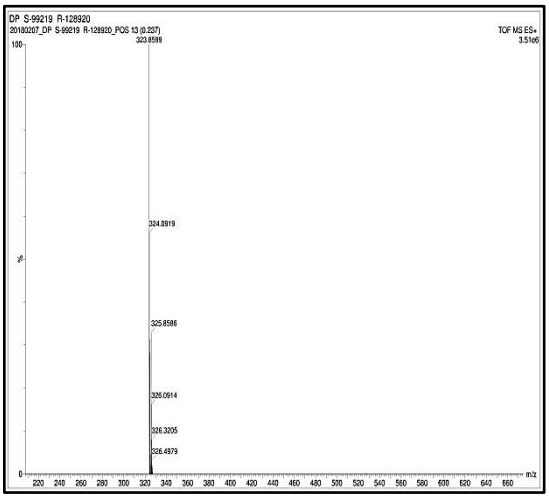
Spectrum 4. IR of compound 61a



Spectrum 5. ^{1}H NMR of compound 61a

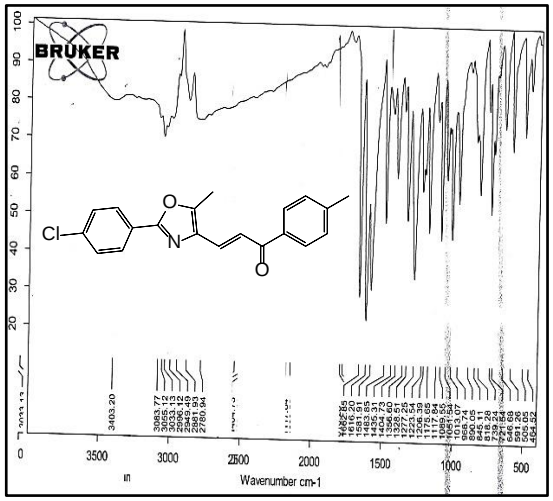


Spectrum 6. 13C NMR of compound 61a

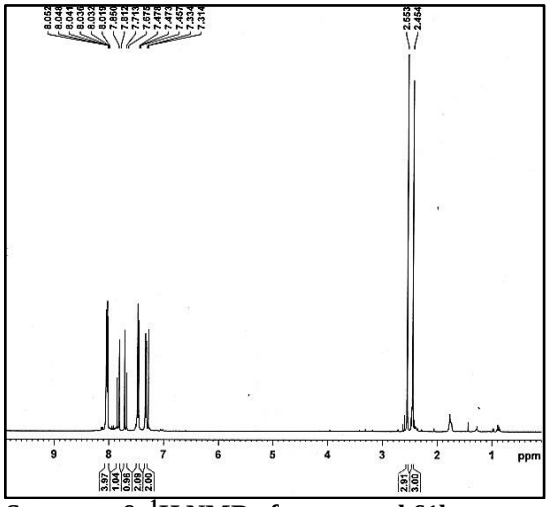


Spectrum 7. MASS of compound 61a

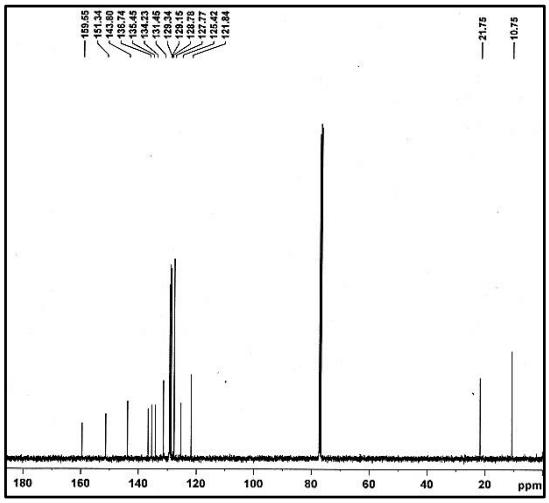
Compound 61b



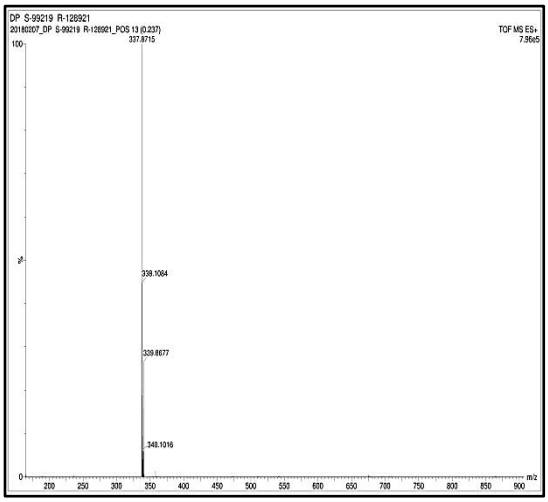
Spectrum 8. IR of compound 61b



Spectrum 9. ^{1}H NMR of compound 61b

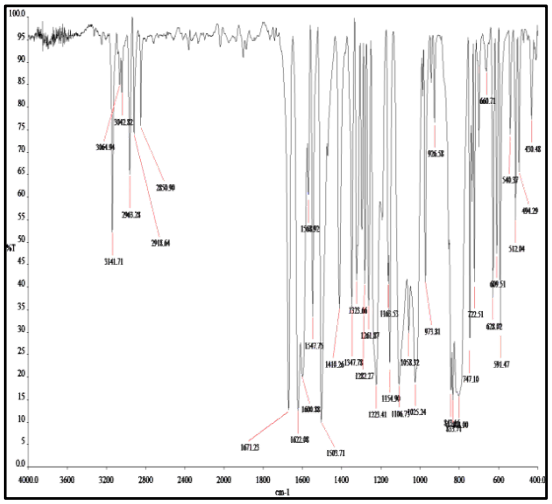


Spectrum 10. 13C NMR of compound 61b

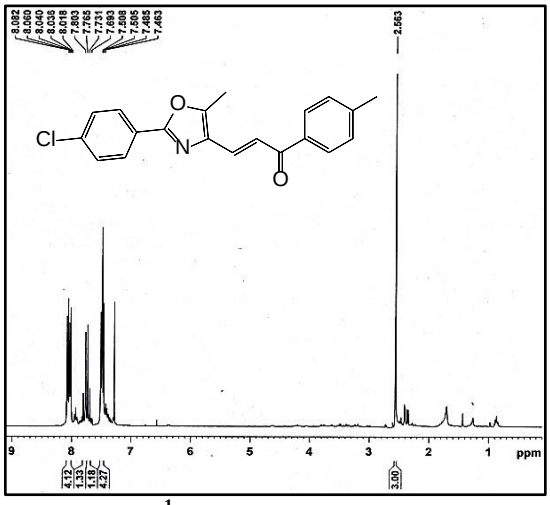


Spectrum 11. MASS of compound 61b

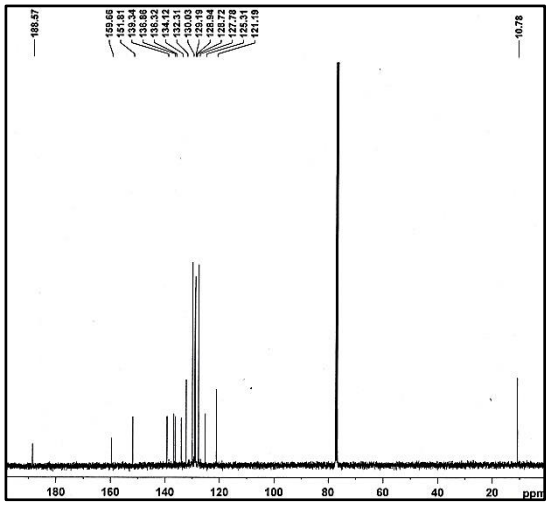
Compound 61c



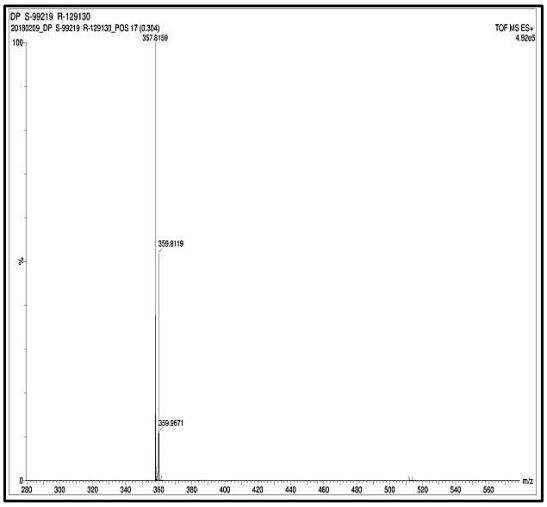
Spectrum 12. IR of compound 61c



Spectrum 13. ^{1}H NMR of compound 61c

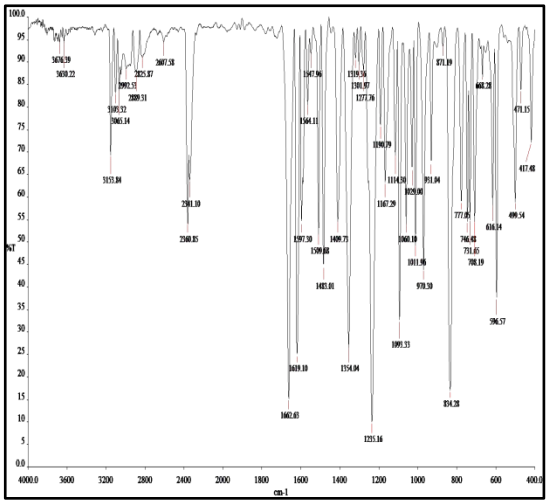


Spectrum 14. 13C NMR of compound 61c

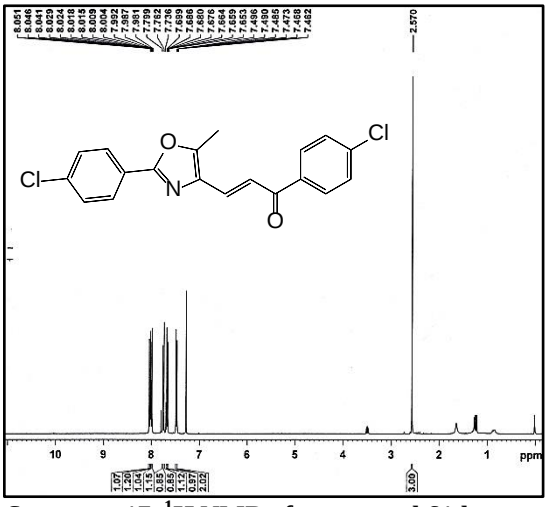


Spectrum 15. MASS of compound 61c

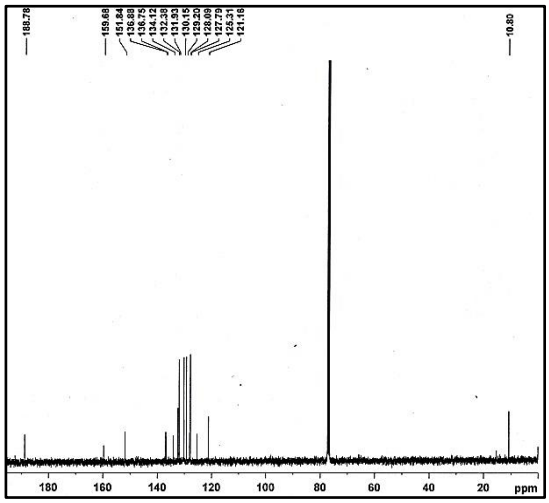
Compound 61d



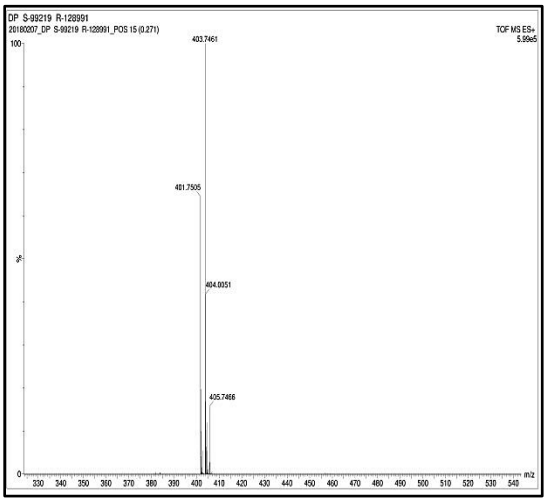
Spectrum 16. IR of compound 61d



Spectrum 17. ^{1}H NMR of compound 61d

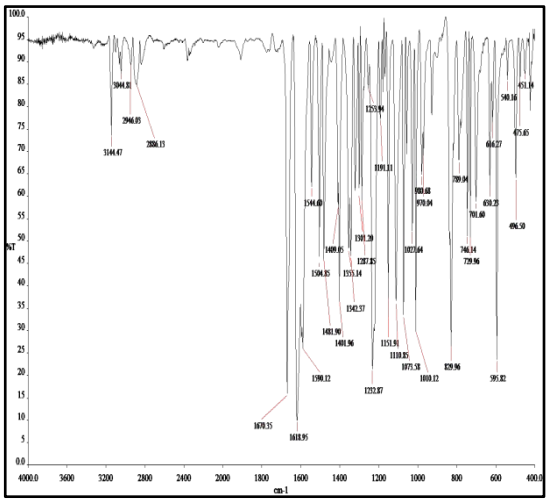


Spectrum 18. 13C NMR of compound 61d

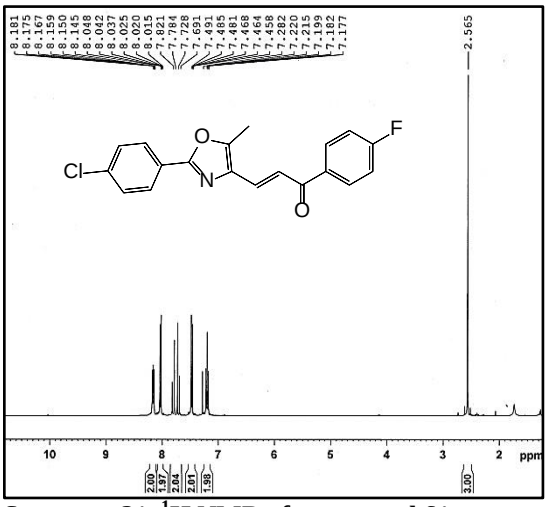


Spectrum 19. MASS of compound 61d

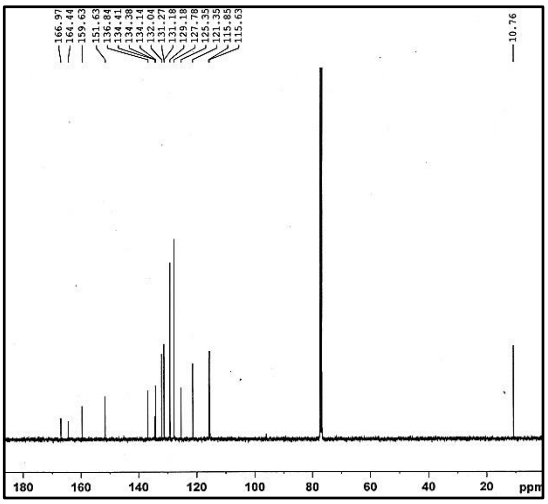
Compound 61e



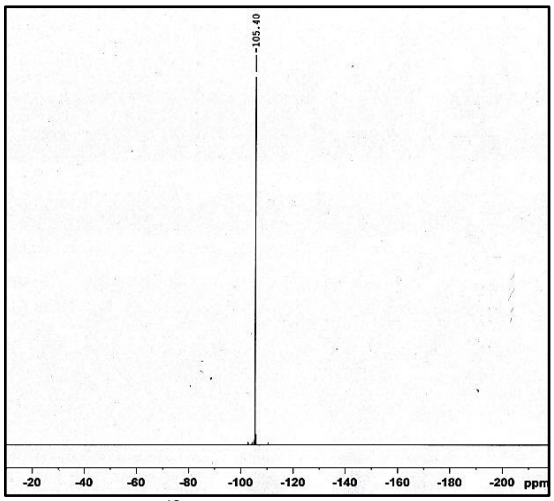
Spectrum 20. IR of compound 61e



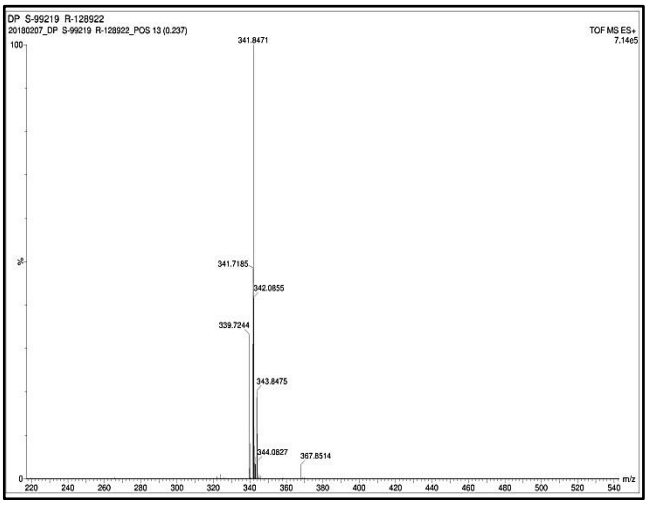
Spectrum 21. ^{1}H NMR of compound 61e



Spectrum 22. 13C NMR of compound 61e

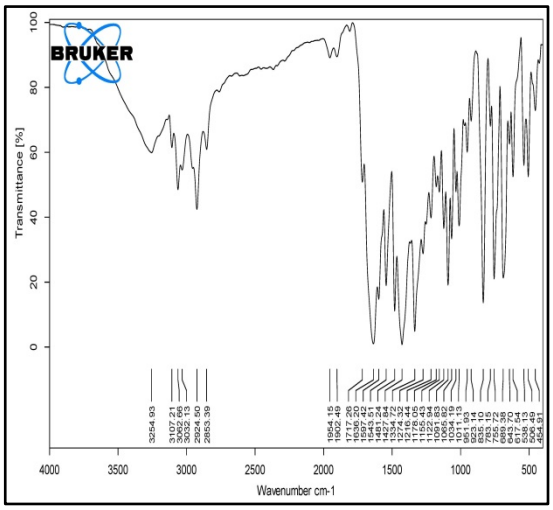


Spectrum 23. ^{19}F NMR of compound 61e

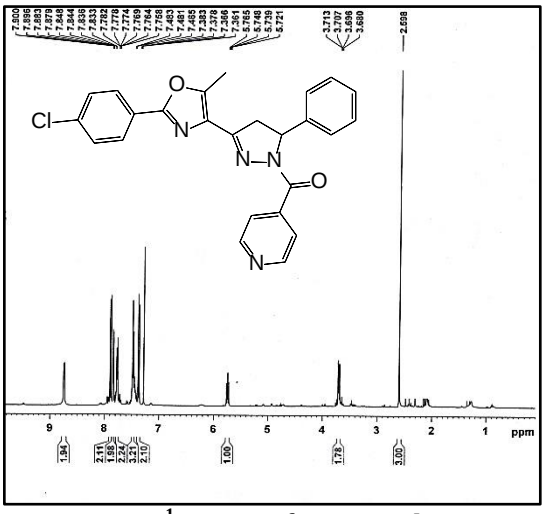


Spectrum 24. MASS of compound 61e

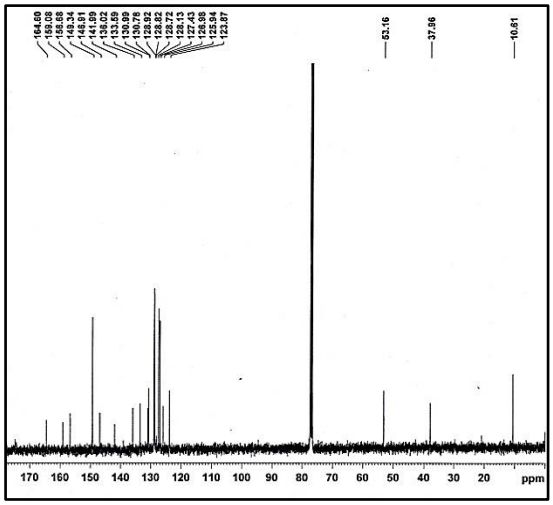
Compound 63a



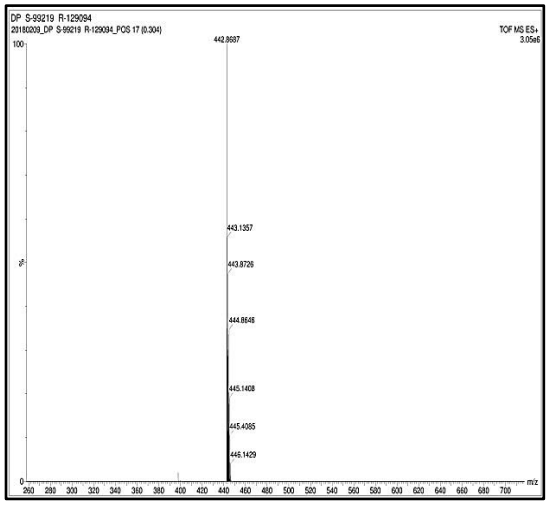
Spectrum 25. IR of compound 63a



Spectrum 26. ^{1}H NMR of compound 63a

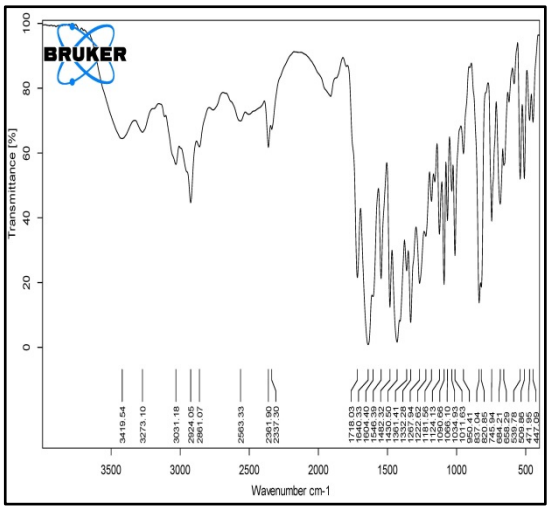


Spectrum 27. 13C NMR of compound 63a

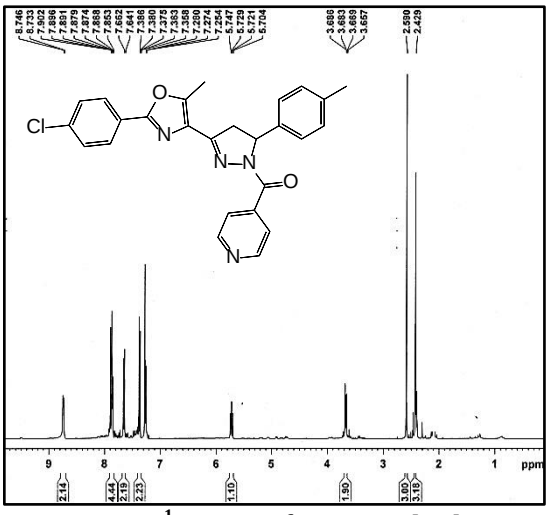


Spectrum 28. MASS of compound 63a

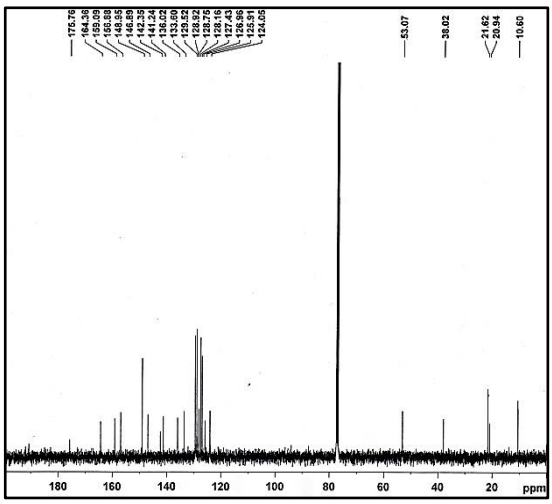
Compound 63b



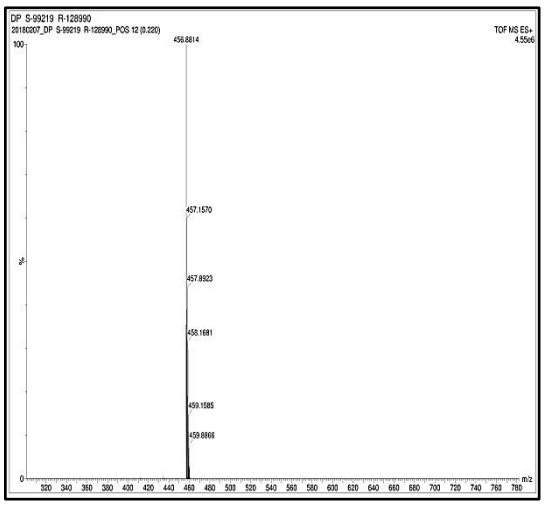
Spectrum 29. IR of compound 63b



Spectrum 30. ^{1}H NMR of compound 63b

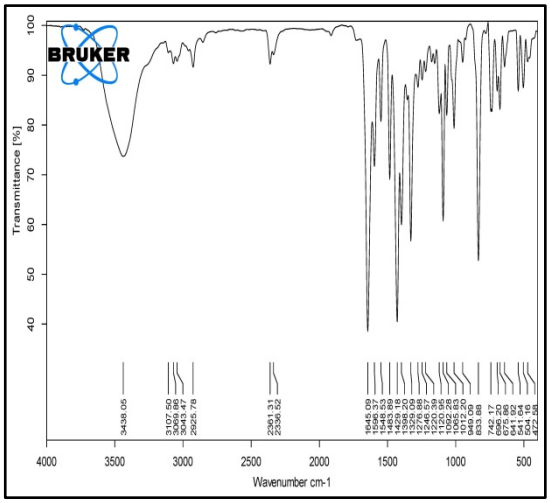


Spectrum 31. 13C NMR of compound 63b

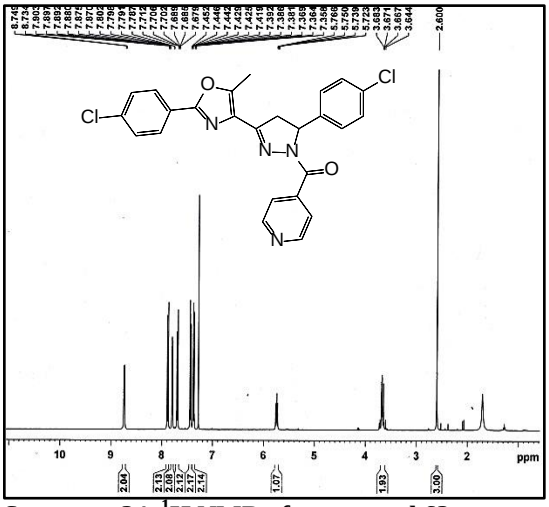


Spectrum 32. MASS of compound 63b

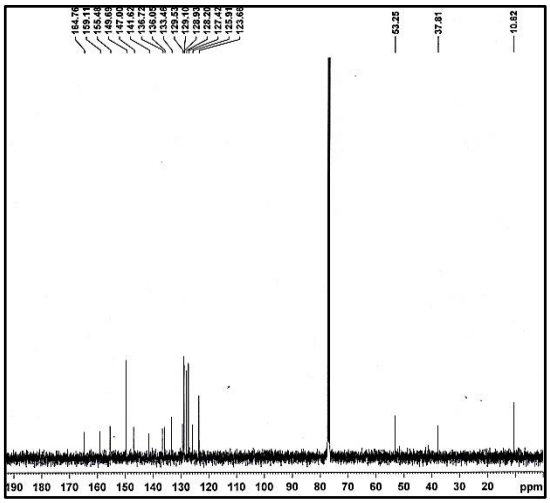
Compound 63c



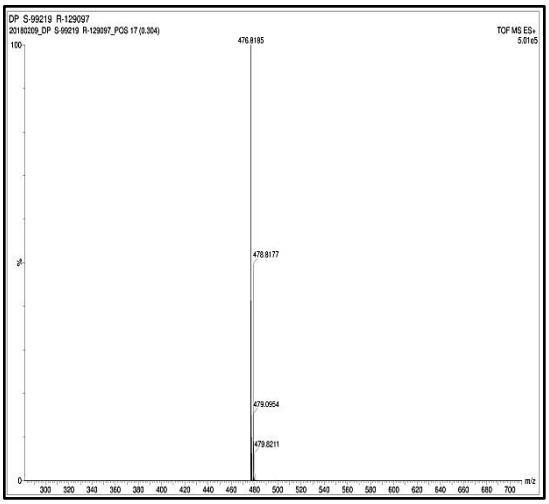
Spectrum 33. IR of compound 63c



Spectrum 34. ^{1}H NMR of compound 63c

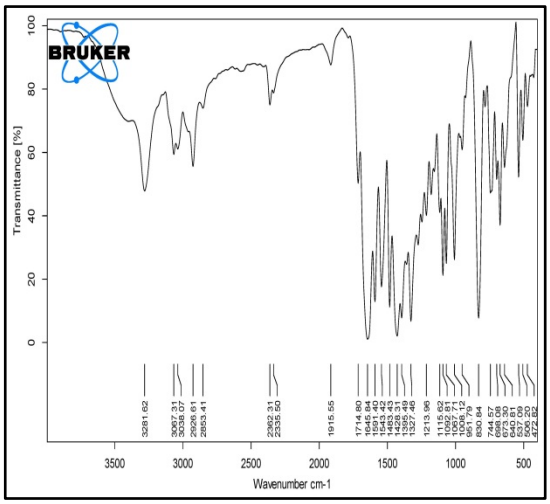


Spectrum 35. 13C NMR of compound 63c

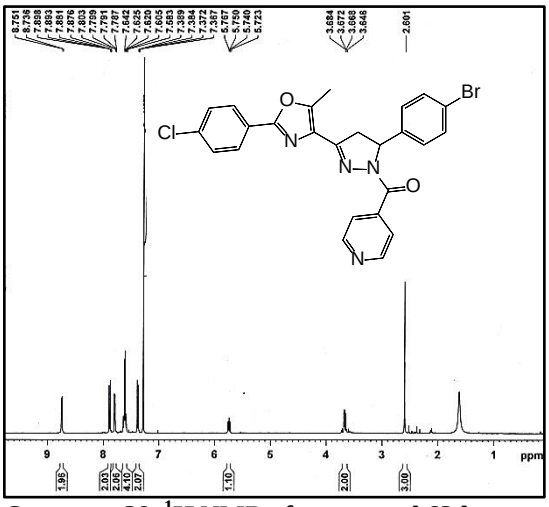


Spectrum 36. MASS of compound 63c

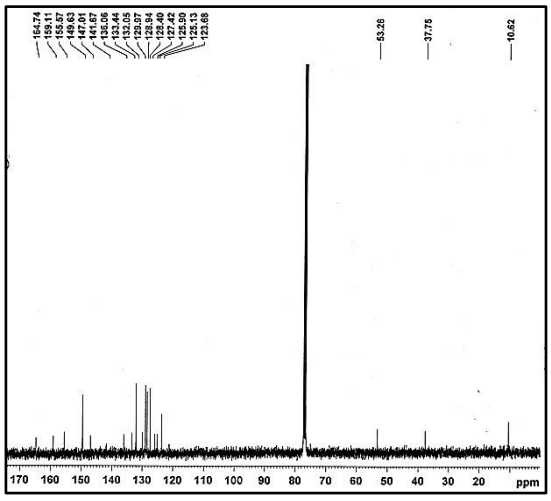
Compound 63d



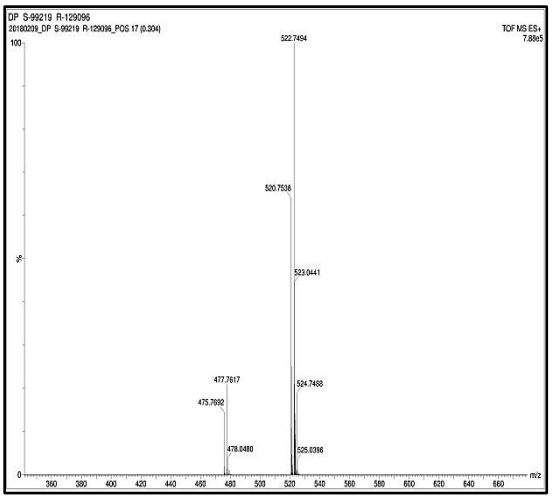
Spectrum 37. IR of compound 63d



Spectrum 38. ^{1}H NMR of compound 63d

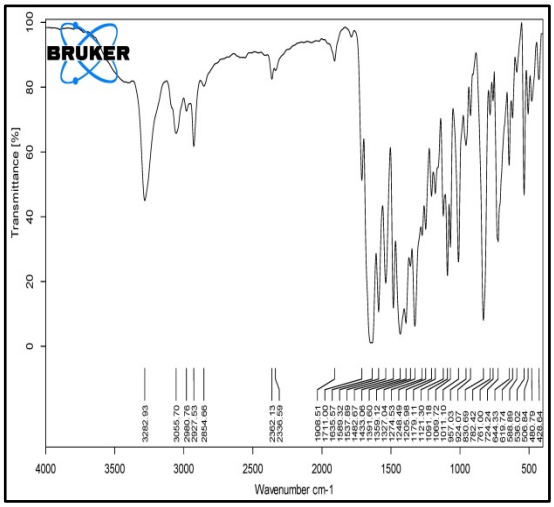


Spectrum 39. 13C NMR of compound 63d

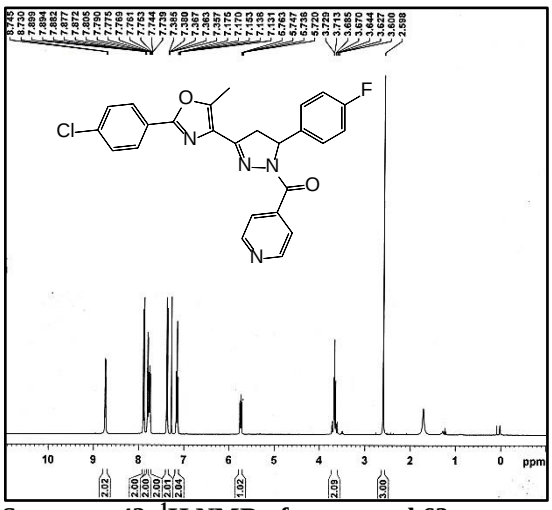


Spectrum 40. MASS of compound 63d

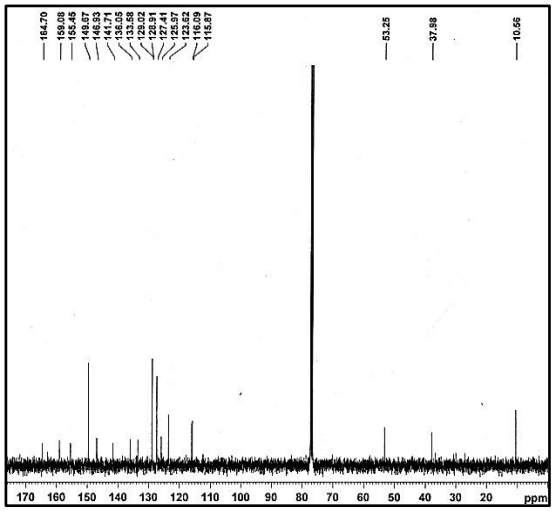
Compound 63e



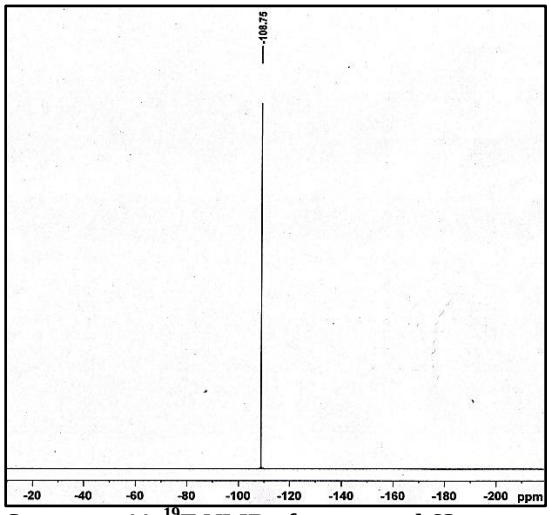
Spectrum 41. IR of compound 63e



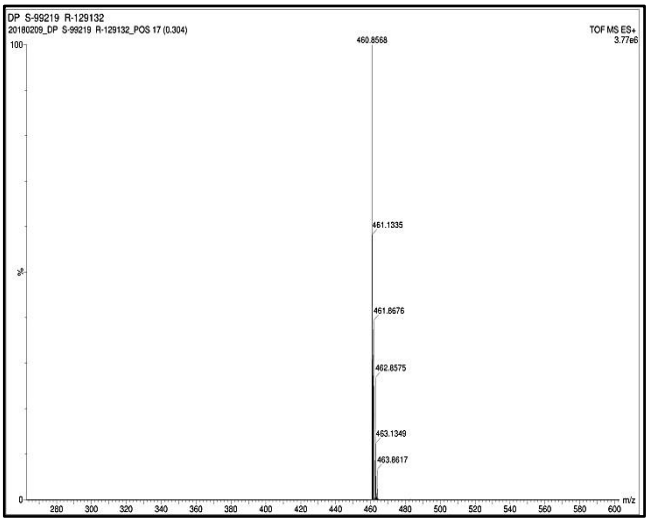
Spectrum 42. ^{1}H NMR of compound 63e



Spectrum 43. 13C NMR of compound 63e



Spectrum 44. ^{19}F NMR of compound 63e



Spectrum 45. MASS of compound 63e

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