SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF 3-(HALO SUBSTITUTED ANILINO) -5- (SUBSTITUTED ARYL)-ISOXAZOLES BY CONVENTIONAL HEATING AND MICROWAVE IRRADIATION METHOD

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ABSTRACT: ***Lipid bilayers are hydrolyzed using halogen atoms. Unfortunately, halogenated drugs have an unsavory tendency to accumulate in fatty tissues. The main of the study is to Synthesis, characterization, and biological evaluation of 3-(halo substituted anilino) -5- (substituted aryl)-isoxazoles by conventional heating and microwave irradiation method. To summarize, anthelmintic activity was shown by thiocarbonyl pyrazolines and strong antibacterial and antifungal activity by quinazolinone formazans among the synthesised compounds.***
Keywords: 3-(halo substituted anilino) -5- (substituted aryl)-isoxazoles, Conventional heating, Microwave irradiation, Athelmintic, Antifungal

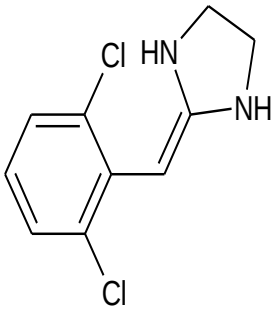
I. INTRODUCTION

The traditional approach to medicinal chemistry has been the synthesis of new molecules with altered known activities as its primary target. In the pursuit of better and safer medical therapies, one typical tactic is the creation and evaluation of physiologically active compounds. A review of the relevant literature indicates that the vast majority of pharmacologically active medicines are heterocyclic compounds.

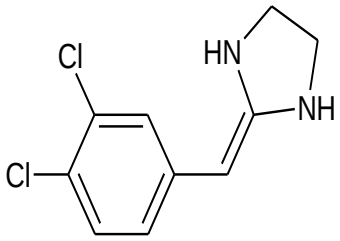
An estimated fifty percent of the organic chemistry literature is dedicated to the study of heterocyclic compounds, the most numerous and diverse class of organic molecules. When it comes to therapeutic medication molecules, heterocyclics make up about 70% of the total. This is likely because many heterocyclics adhere to Evans's suggested description of "privileged scaffolds" as ligands for various receptors, which allows them to be classified as such.

Introduction to halogenated heterocyclic Compounds:

Lipid bilayers are hydrolyzed using halogen atoms. Unfortunately, halogenated drugs have an unsavory tendency to accumulate in fatty tissues. The chemical reactivity of halogen atoms is affected by the kind of halogen and where they are attached to the lead. In contrast to their aliphatic halogen group counterparts, aromatic halogen groups are very chemically inert. The aromatic fluorine and chlorine groups, which are less reactive, are the most often used halogen substituents. But ring substituents that pull electrons out of the compound could make them too reactive. Placing a halogen or halogen-containing group's addition will change the compound's efficacy, much like other sorts of substituents. The antihypertensive clonidine, for example, works better with an o,o-chloro substitution than with its p,m-dichloro analogue



Clonidine
ED20 0.01mg kg^{-1}



ED20 3.00mg kg^{-1}

II. MATERIAL AND METHOD

Experimental Investigations

This research used microwave and traditional methods to create a number of novel halogenated isoxazoles, thiocarbamoyl pyrazolines, thiazolidinones, and Quinazolinone formazans.

General procedure for synthesis of -(halo substituted anilino) -5-(substituted aryl) isoxazoles

Synthesis of 3-(halo substituted anilino) -5-(substituted aryl)-isoxazoles by conventional heating method:

Step-1: The production of anilino-2-propene-1-ones and 3-substituted phenyl-1-anilino: Add 0.01 mole of aldehyde (or substituted aldehyde) in the presence of a 2% NaOH solution to a 25 ml ethanol solution containing acetanilide or its derivative compounds (0.01 mole). To ensure that the reaction was fully completed, the mixture was refluxed for 12 hours while being continuously watched by TLC. After filtering and purifying the combination using column chromatography, the heated reaction mixture was placed over crushed ice.

Step-2: Producing 3-[halosubstituted anilino]–-[substituted aryl]isoxazoles: Sodium acetate (0.08 mole), hydroxylamine hydrochloride (0.00014 mole), and chalcone (0.01 mole) were refluxed in ethanol for 6 hours to produce isoxazoles. The reaction was monitored attentively using TLC to ensure it completed successfully. After filtering and purifying the combination using column chromatography, the heated reaction mixture was placed over crushed ice.

Synthesis of 3-(halo substituted anilino) -5-(substituted aryl) isoxazoles by microwave irradiation:

Step-1: Make 3-phenyl or substituted phenyl-1 anilino or substituted anilino-2-propene-1-ones: In a 25 ml ethanol solution containing 0.01 mole of acetanilide or a compound having one or more of its derivatives, aldehyde or a substituted aldehyde with a concentration of 0.01 mole was added while a 2% NaOH solution was present. The reaction mixture was heated to 560W in a microwave synthesiser (Fig.3.1) for the duration given in Table No-3.1. Filtration and column chromatography were used to purify the heated reaction mixture after it was put onto broken ice.

Step-2: Producing 3-[halosubstituted anilino]–For the synthesis of 5-[substituted aryl]isoxazoles, chalcone (0.01 mole), hydroxylamine hydrochloride (0.00014 mole), and sodium acetate (0.08 mole) in ethanol were subjected to microwave irradiation (560W) for the duration given in Table No-3.1. Filtration and column chromatography were used to purify the heated reaction mixture after it was put onto broken ice.

Present Working scheme

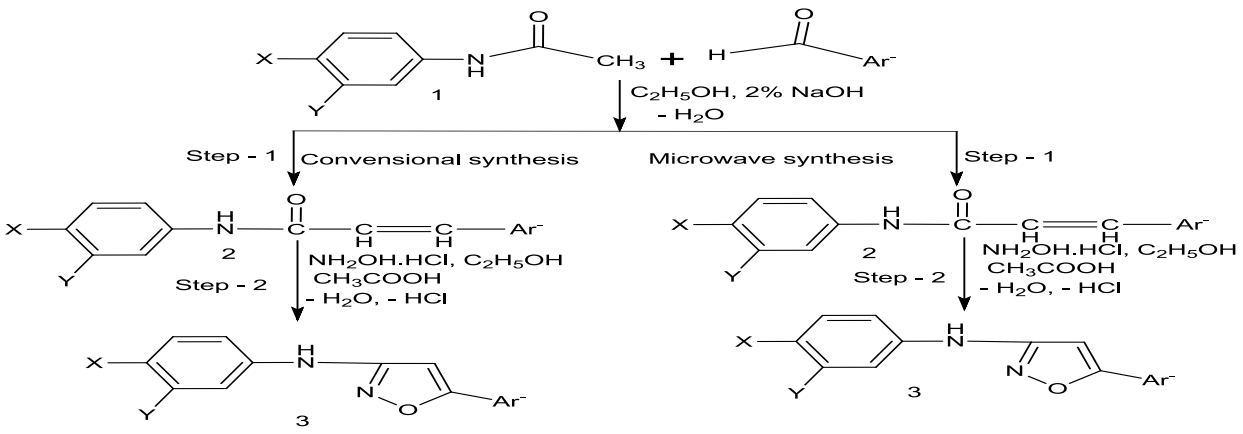


Table No. 3.1. synthesized compound

Sl. No.	Compound code	X	Y	Ar	IUPAC NAME	Reaction time (min)	
						Conventional	Microwave
1	HAI-1	Br	H	$C_6H_3(OH)(OCH_3)$	3-(4‟-Bromo anilino) -5-(3``-methoxy-4``-hydroxy phenyl) isoxazole	360	1.5
2	HAI-2	F	H	$C_6H_4N(CH_3)_2$	3-[4‟-Fluoroanilino]-5-[4-dimethylamino phenyl]-isoxazole	360	2
3	HAI-3	Cl	H	$C_6H_4N(CH_3)_2$	3-[4‟-Chloroanilino]-5-[4-dimethylamino phenyl]-isoxazole	360	3
4	HAI-4	Br	H	$C_6H_4(OCH_3)$	3-(4‟-Bromoanilino)-5-(4‟-methoxy phenyl) –isoxazole	360	3
5	HAI-5	F	H	C_6H_5	3-(4‟-Fluoro anilino)-5-phenylisoxazole	360	2.5

III. BIOLOGICAL EVALUATION

Screening for anti-bacterial, anti-fungal and anthelmintic activities

Anti- bacterial activity by Disc Diffusion Method:

In vitro testing with six bacterial strains using the paper disc technique confirmed the activity of all the compounds that were synthesised. Among these strains were both Gram-negative (Escherichia coli and Pseudomonas aeruginosa) and Gram-positive (Bacillus subtilis, Bacillus cereus, Staphylococcus aureus and Staphylococcus epidermidis) bacteria.

For sterility, a disc of grade-I filter paper with a diameter of 5 millimetres was autoclaved for 15 minutes at 121°C. These sterile discs have a variety of synthetic substances put into them. The synthetic chemicals were dissolved in a 10% DMSO/methanol solution at 30, 60, and 90 mcg/disc. The nutritional agar, measuring 20 m1, was placed in a petri dish with a flat base. Four cubic metres of a second nutrient solution containing test bacteria was evenly dispersed over the first layer at temperatures ranging from forty to forty-eight degrees Celsius. Once the second layer had solidified, the impregnated discs were carefully placed on top of the medium, ensuring they were spaced appropriately. In order to ensure complete diffusion, the plates were incubated at 5°C for 1 hour. After that, they were transferred to an incubator set at 37°C ±1°C for 18 to 24 hours. Traditional medicines (Cefotaxime, Gentamicin) and other synthetic compounds were compared to the inhibitory zones of the bacteria.

Anti-Fungal Activity by Paper Disc Diffusion Method

The in-vitro antifungal activity of all the synthesised compounds was tested against five distinct species using the paper disc technique: Aspergillus niger, Aspergillus foetidus, Saccharromyces cerevisiae, Candida albicans, and Candida glabrata. The quality of the individual's filter paperThe autoclave was programmed to sterilise a 5 mm diameter disc at 121°C and 15 lb/sq. inch for 15mm. These sterile discs have a variety of synthetic substances put into them.

Anthelmintic Activity:

Recent research has shown that helminth infections cause a lot of long-term sickness in people living in tropical regions. Over half of the global population suffers from worm diseases. The anthelmintic activity of the newly synthesised compounds was tested according to the method outlined by Kailashraj and Kurupa. Their resemblance to human intestinal roundworm parasites in terms of anatomy and physiology led to this action.

IV. RESULTS AND DISSCUSION

Table No. 3.2. Comparison of conventional heating and microwave irradiation reactions of 3-(halo substituted anilino) -5-(substituted aryl)-isoxazoles

Sr. No.	Compound	Conventional			Microwave		
		Reaction time (min)	% Yield	Appearance	Reaction time (min)	% Yield	Appearance
1	HAI – 1	360	59.2	Solid(amorphous) White	1.5	76.7	Solid(amorphous) White

2	HAI – 2	360	57.7	Solid(amorphous) light green	2.0	78	Solid(amorphous) light green
3	HAI – 3	360	71.5	Solid(amorphous) yellow	3.0	91	Solid(amorphous) yellow
4	HAI-4	360	56.2	Solid (crystal) White	3.0	82	Solid (crystal) White
5	HAI-5	360	26.7	Solid (amorphous) white	2.5	49	Solid (amorphous) white

Table No. 3.3. Physical characterization of 3-(halo substituted anilino) -5- (substituted aryl)-isoxazoles

S. No.	Compound	Mol. Formula	Mol. Wt	MP °C	Rf Value	Rm Value
1	HAI – 1	$C_{16}H_{13}N_2O_3Br$	361.18	226	0.596	-0.169
2	HAI – 2	$C_{16}H_{13}N_2O_3F$	300.28	143	0.596	-0.169
3	HAI – 3	$C_{17}H_{13}N_3OCl$	314.33	120	0.641	-0.251
4	HAI – 4	$C_{16}H_{13}N_2O_2Br$	345.18	149	0.724	-0.376
5	HAI – 5	$C_{14}H_{10}N_2O_3F$	273.24	140	0.689	-0.345

Table No. 3.4. Elemental analysis of 3-(halo substituted anilino) -5-(substituted aryl)-isoxazoles

Sr. No.	Compound	% Elemental analysis					
		Calculated			Found		
		C	H	N	C	H	N
1	HAI – 1	53.20	3.62	7.75	51.48	2.963	6.577
2	HAI – 2	67.01	4.36	9.33	66.01	4.634	8.798
3	HAI – 3	64.95	5.12	13.37	64.80	5.075	11.753
4	HAI – 4	55.16	3.79	8.11	56.1	3.297	8.016
5	HAI – 5	61.53	3.68	10.25	61.5	3.148	9.814

4.4. Spectral analysis of halogenated heterocyclic compounds Infra-Red spectral analysis

The structures of the compounds that were synthesised were revealed using the KBr-Pellet technique and a Perkin Elmer 1600 series Fourier Transformer-Infrared Spectrophotometer. Infrared (IR) readings are expressed as cm-1.

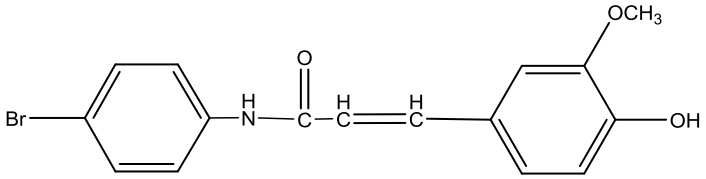
^{1}H NMR analysis

1H FT-NMR (BRUCKER MX 400 MHz) analysis using TMS as the internal standard was used to clarify the structures of the synthesised compounds. The materials are dissolved in either CDCl3 or DMSO, and the results are expressed as chemical values in delta (δ-ppm).

Mass spectroscopic analysis:

The compound's mass spectra were captured using an MDS spiex AP 12000 LC-MS mass spectrometer. At m/z, the compound's mass spectra revealed a molecular ion.

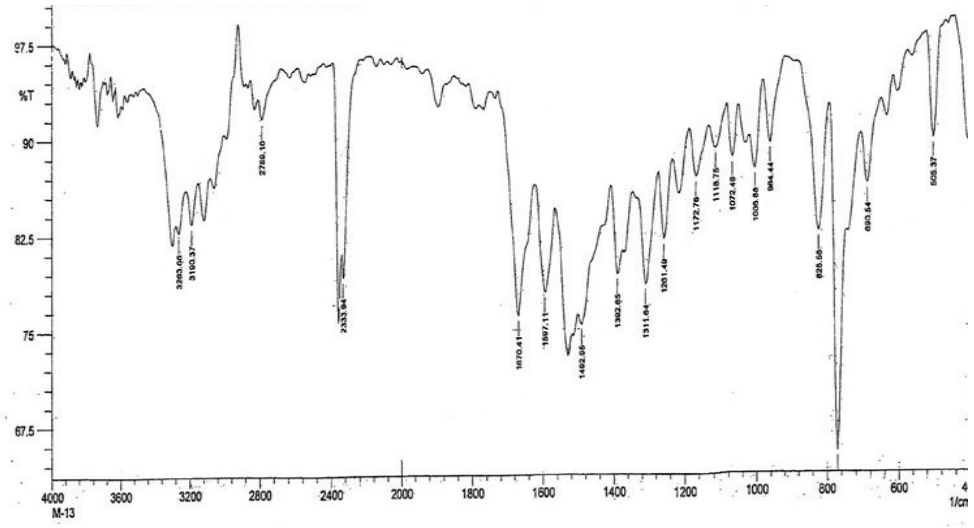
Intermediate Compounds-1:3-[3-Methoxy-4-hydroxy phenyl]-1-[4-bromo aniline]-2- propen-1-one



IR (KBr, cm4) : »3263.66(N-H str); 3267.52 (OH str); 1670.41 (C=0 str);825.58 (Ar-CH str)

H-NMR (8 ppm) : 3.345 (NH); 6.940 (CH=CH); 3.810 (OCH3);7.552 (ArH)

LC-MS (m/z,) : 347.9 (M1- +1)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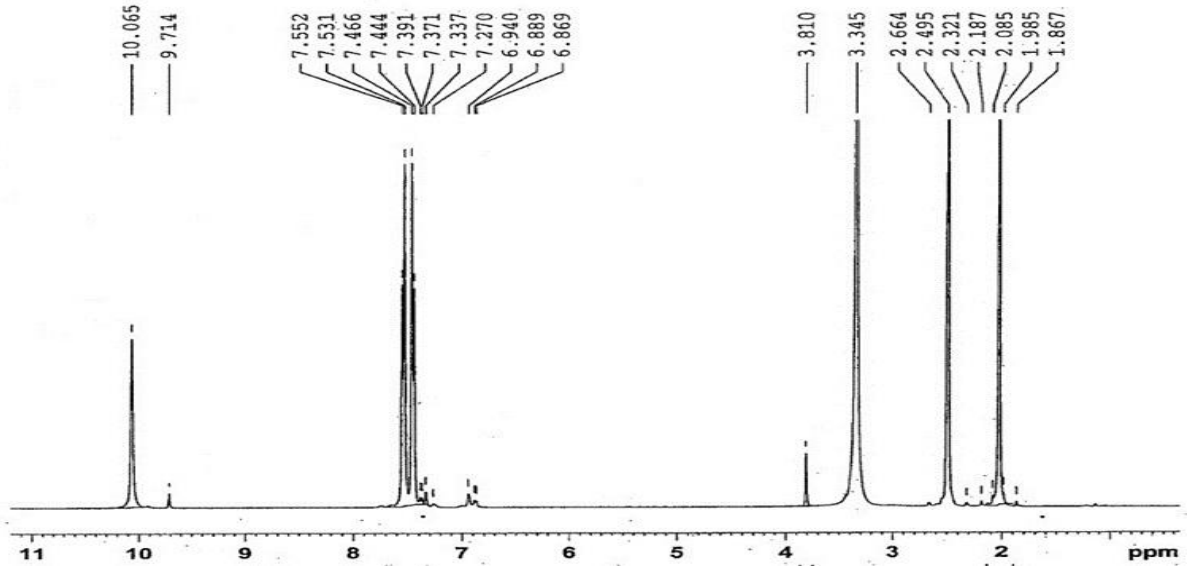
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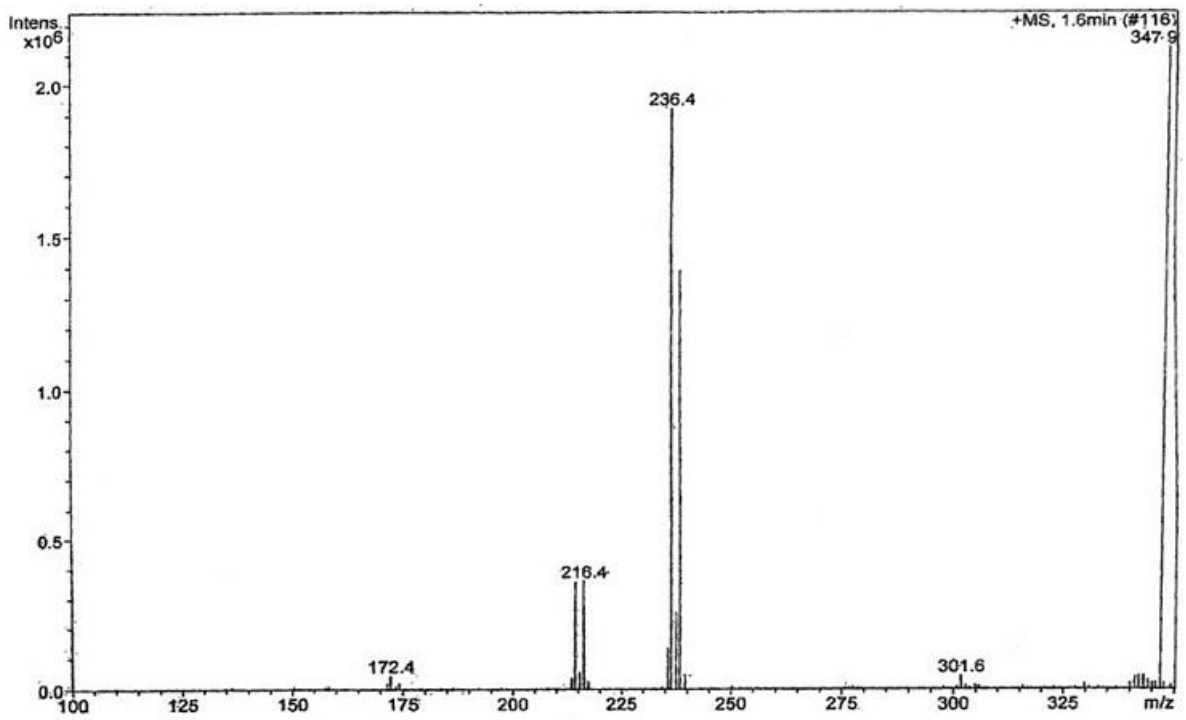
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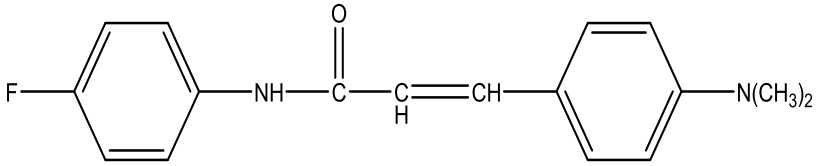
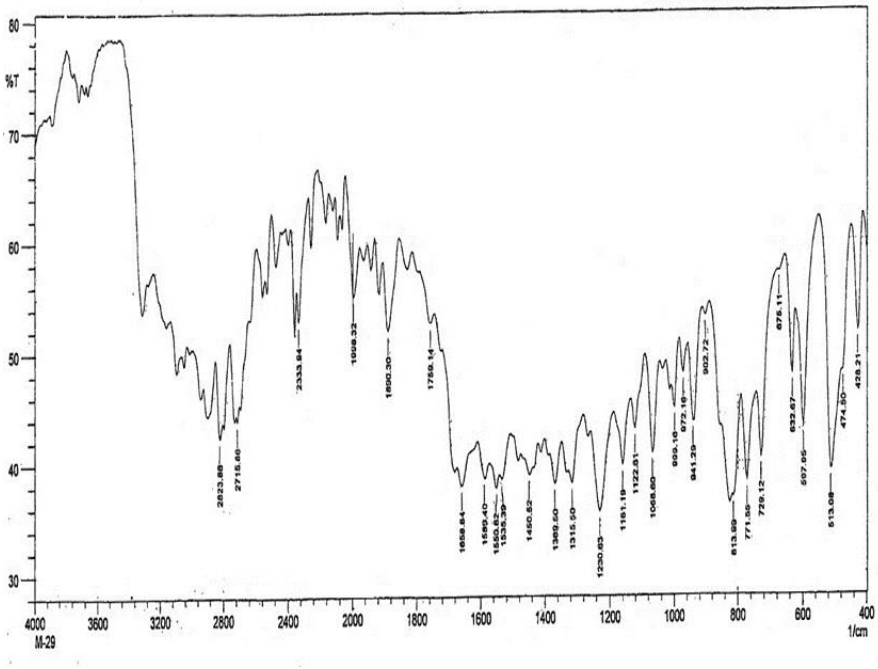
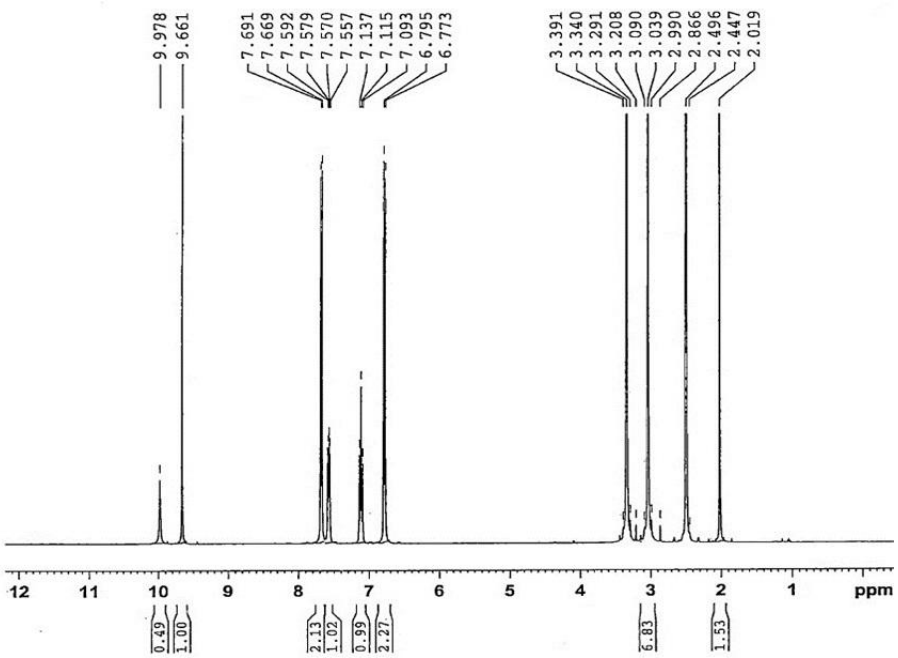
Intermediate Compounds-2:3-(4-Dimethyl amino)-l-(4-fluoro anilino)-2-propen-l-one



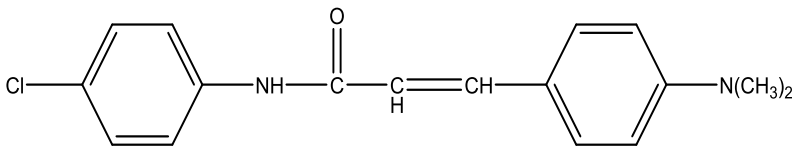
IR (KBr, cm4):3250.55 (N-H str); 1658.84 (C=0 str);1680.00 (CH=CH Str); 771.55 (Ar-CH str);

H-NMR (8 ppm):3.391 (NH); 6.795 (CH=CH); 3.090 (NCH3); 7.691 (ArH);



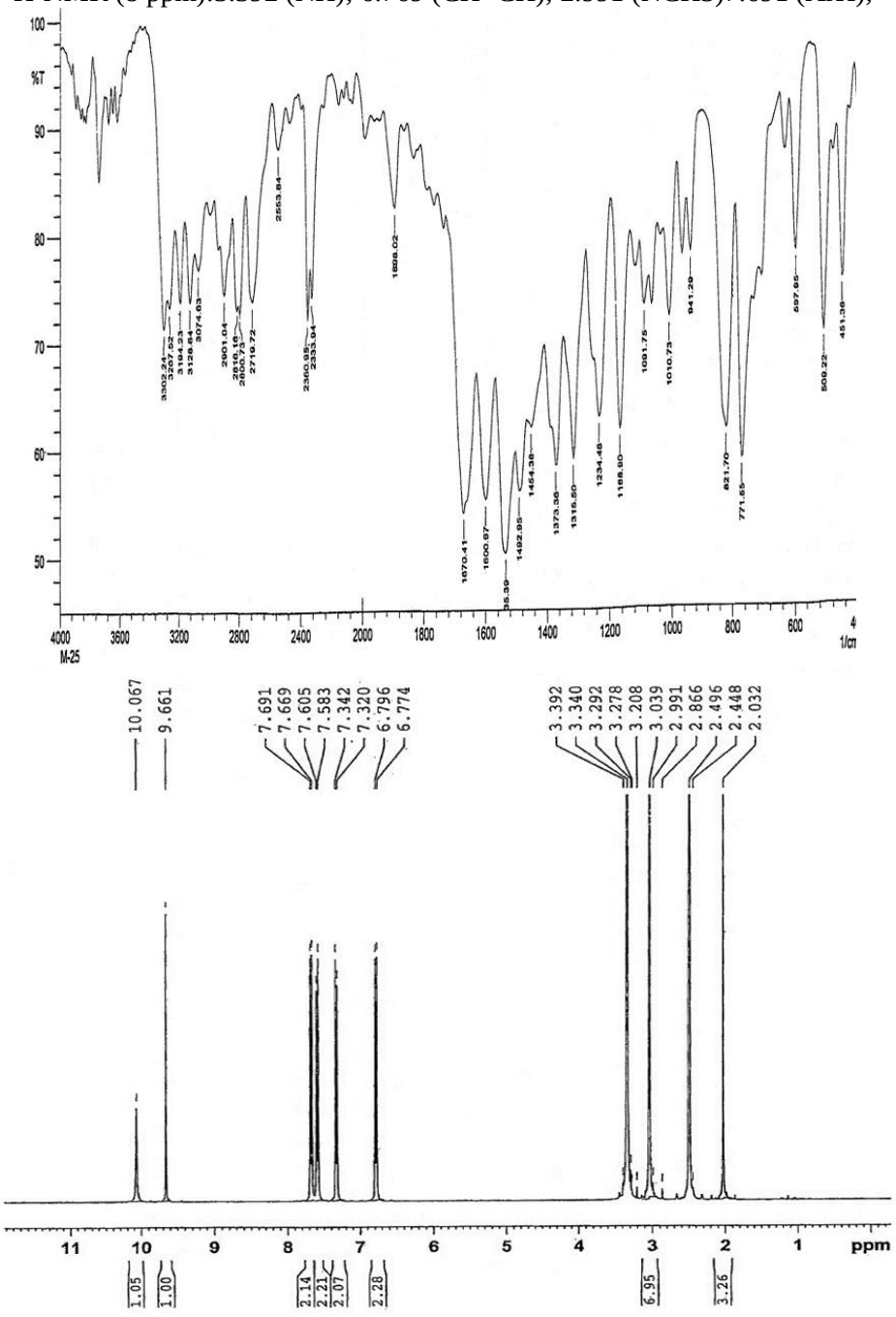


Intermediate Compounds-3:3-[4-Dimethyl amino phenyl]- l-[4-chloro anilino]-2-propen-l-one

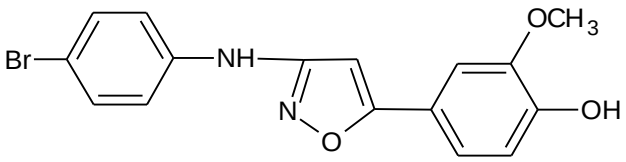


IR (KBr, cm’1):3302.24 (N-H str); 1670.41 (C=0 str); 771.55 (Ar-CH str); 1680.0 (CH=CH str)

'H-NMR (8 ppm):3.392 (NH); 6.769 (CH=CH); 2.991 (NCH3)7.691 (ArH);

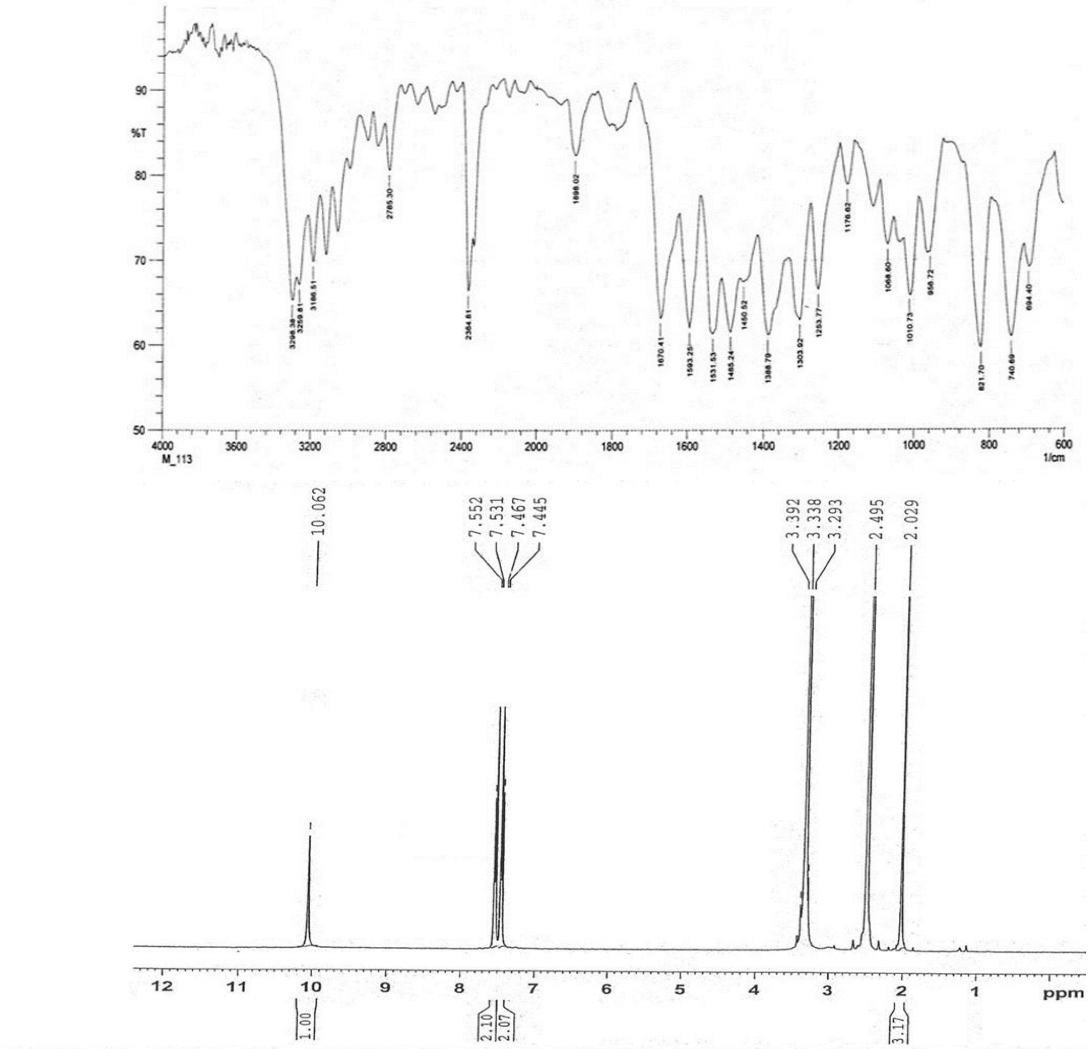


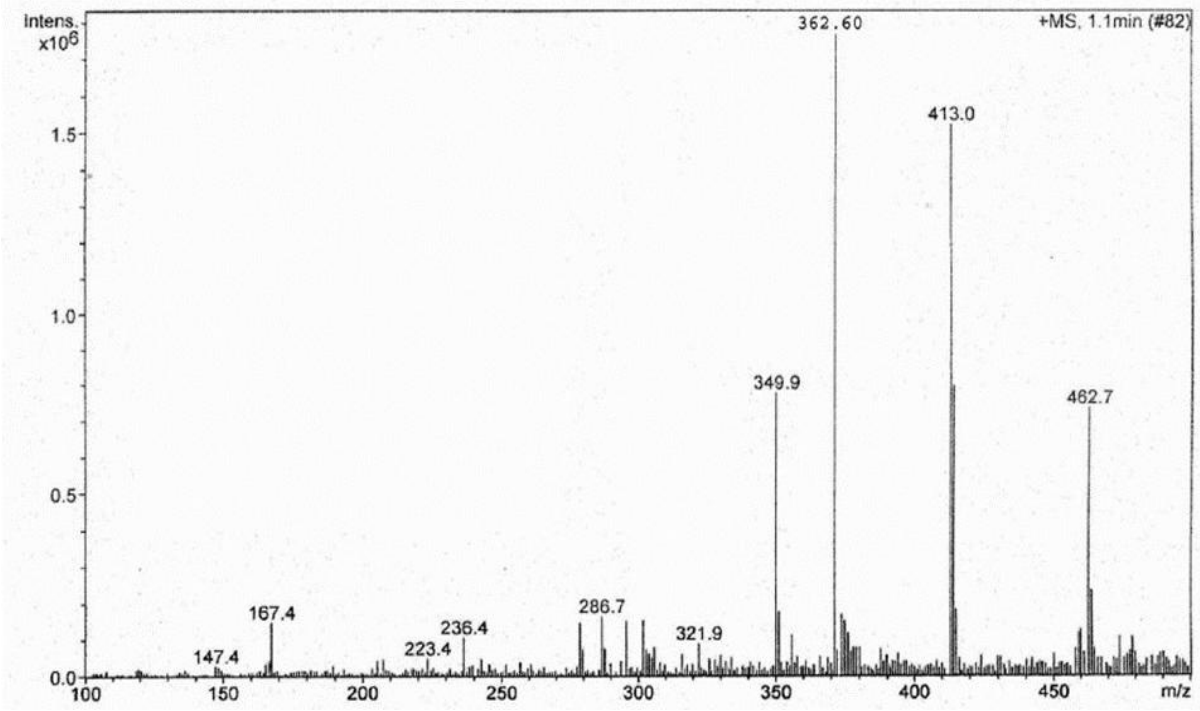
Compound HAI-1: 3-(4-Bromoanilino)-5-(3-methoxy-4-hydroxy phenyl) isoxazole



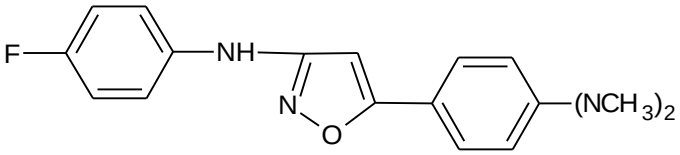
IR (KBr, cm^{-1}): 3229.38(N-H str); 1670.41(CH=CH str); 3259.81(OH str); 740.69(Ar-CH str)

^{1}H-NMR (δ ppm): 3.392 (NH); 7.552(ArH); 10.062(ArOH)
LC-MS (m/z): 362.60 (M^{+} +1)

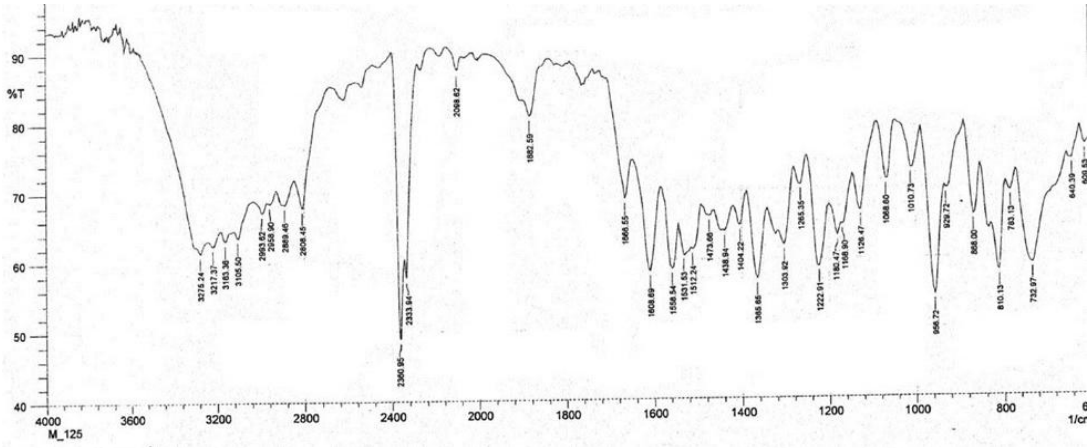


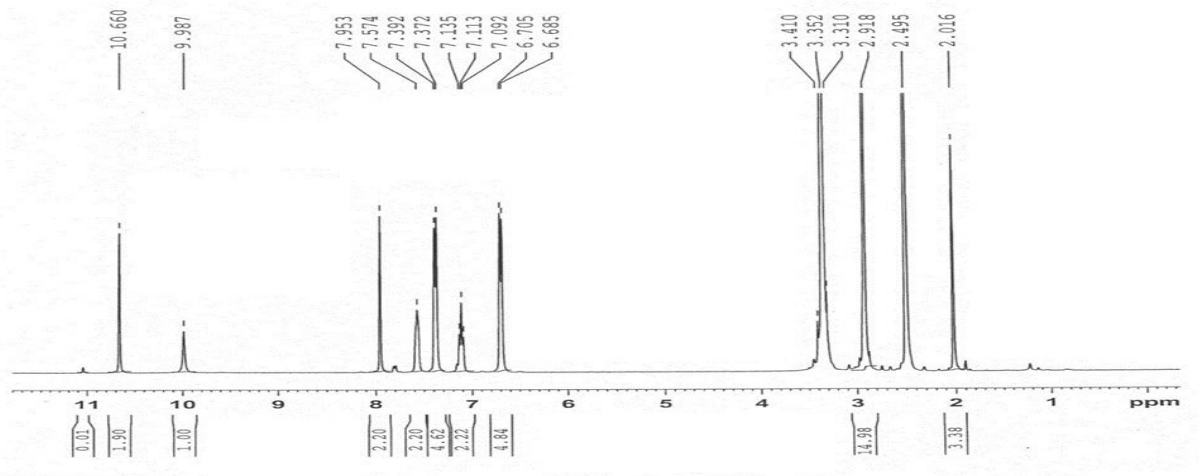


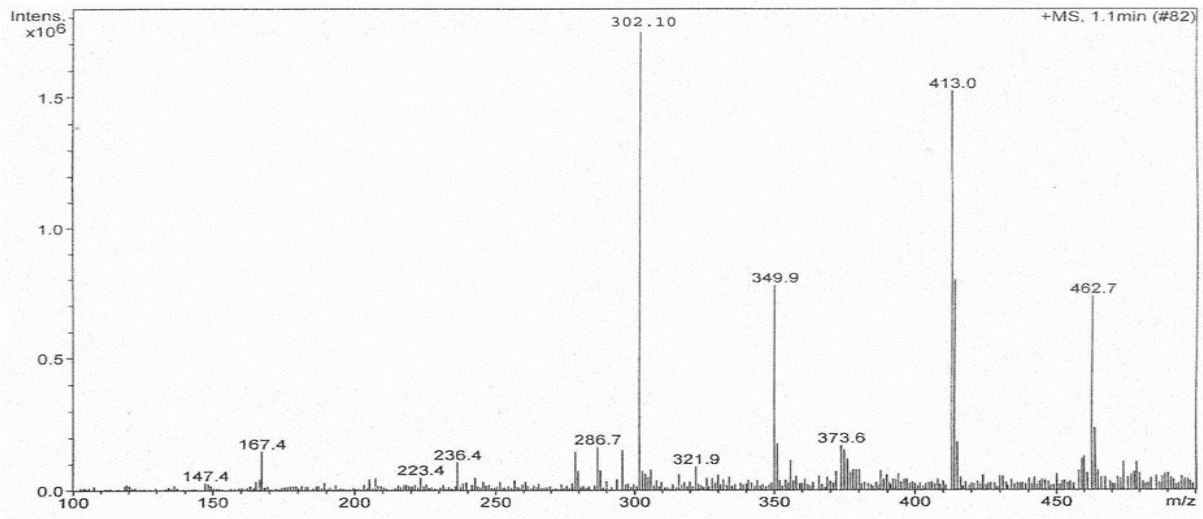
Compound HAI-2: 3-(4'-Fluoro anilino)-5-(4''-dimethylamino phenyl) isoxazole



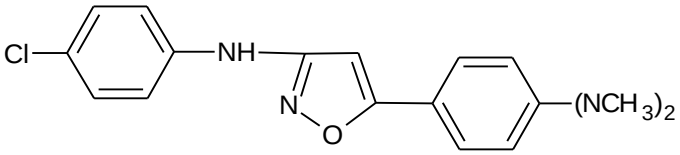
IR (KBr, cm^{-1}): 3275.38(N-H str); 1660.55(CH=CH str); 732.97(Ar-H str); 1365.65(N=C)
^{1}H-NMR (δ ppm): 3.140 (NH); 6.705(CH=CH); 7.953(ArH) 2.918(N-CH)
LC-MS (m/z): 302.10 (M^+ +1)



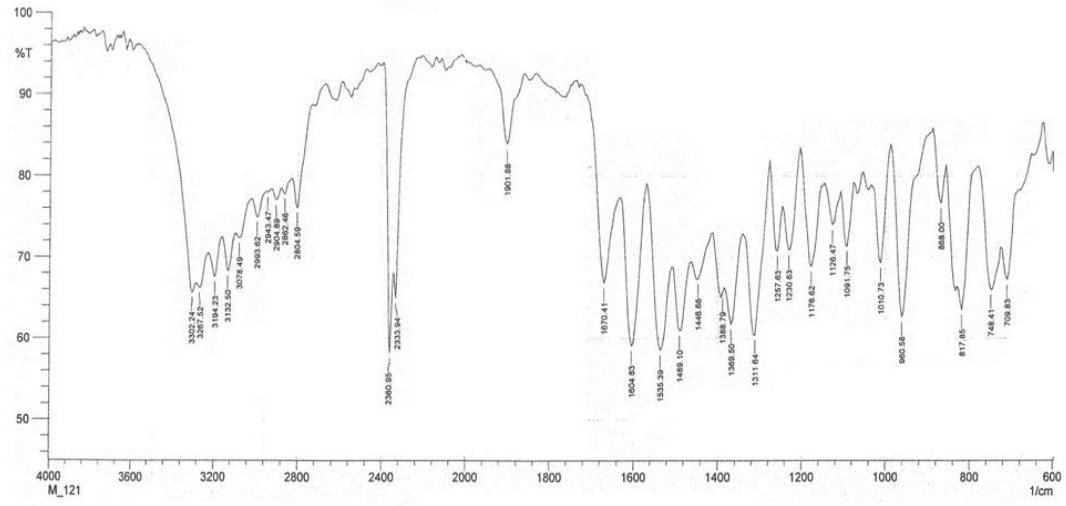


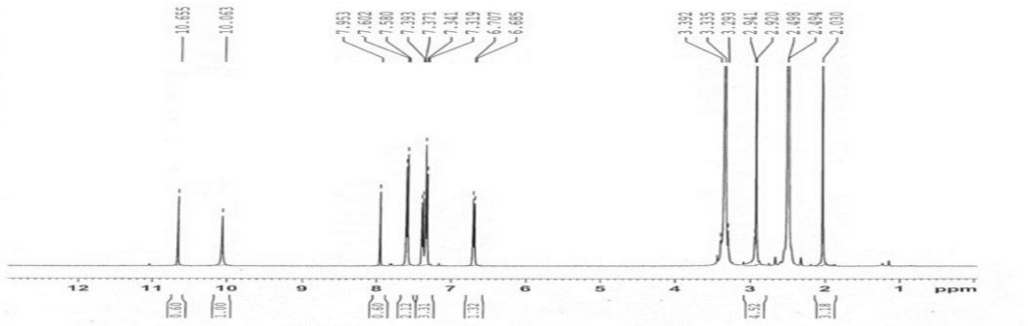


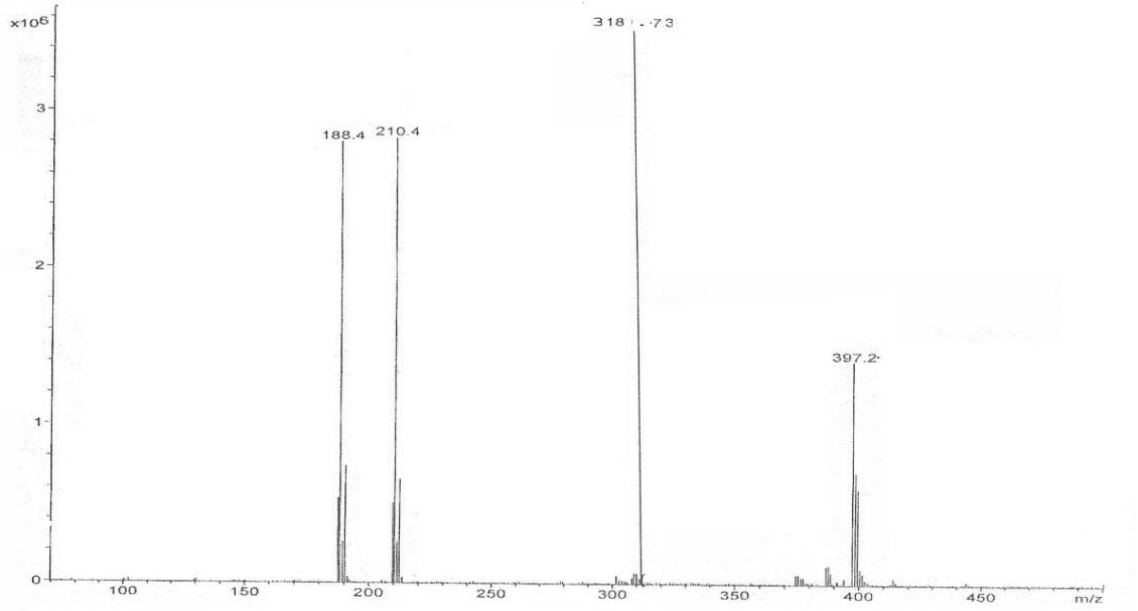
Compound HAI-3: 3-(4'-Chloro anilino)-5-(4''-dimethyl amino phenyl) isoxazole



IR (KBr, cm^{-1}): 3302.24(N-H str); 1670.41(CH=CH str); 748.41(Ar-H str); 1369.50(N=CH str)
^{1}H-NMR (δ ppm): 3.392 (N-H); 6.707(CH=CH); 7.602(Ar-H) 2.941(N-CH)
LC-MS (m/z): 318.73 (M^+ +1)







V. BIOLOGICAL EVALUATION

Table No. 4.1. Antibacterial activity of 3-(halo substituted anilino) -5-(substitutedaryl)-isoxazoles

S. No.	Compound	Zone of inhibition (in mm)																	
		B. subtilis			*B. cereus*			*E. coli*			*S. aureus*			*S. epidermidis*			*P. aeruginosa*		
		Drug concentration (µg/disc)																	
		30	60	90	30	60	90	30	60	90	30	60	90	30	60	90	30	60	90
1	HAI-1	6	10	13	5	9	6	5	7	12									
2	HAI-2	5	6	8										5	7	9			
3	HAI-3	5	6	8															
	Cefotaxime	10	16	21	9	13	16	12	17	22	10	14	19				6	8	11
	Gentamicin	15	19	24	14	18	25	12	15	18	11	15	19	15	19	26	7	9	12

Table NO. 4.2: Anti-fungal activity of 3-(halo substituted anilino) -5-(substituted aryl)-isoxazoles

S. No.	Compound	Zone of inhibition (in mm)														
		A. niger			*A. foetidus*			*S. cerevisiae*			*C. albicans*			*C. glabrata*		
		Drug concentration (µg/disc)														
		30	60	90	30	60	90	30	60	90	30	60	90	30	60	90
1	HAI-1							6	7	8	7	11	16			
2	HAI-2				6	8	8	9	12	15				5	7	9
3	HAI-3				7	9	10	9	16	19						
4	HAI-4				5	7	7	8	11	14				5	6	8
5	HAI-5				6	8	9	8	11	13						
	Fluconazole	9	11	15	11	13	16	8	10	13	12	16	22	12	17	21
	Clotrimazole				9	15	20	8	11	15	13	16	21	11	16	20

Table No 4.3.: Anthelmintic Activity of 3-(halo substituted anilino) -5- (substituted aryl)-isoxazoles

S. No.	Compound	Paralytic (sec)	Lethal (sec)
1	HAI-1	20 ± 0.42	48 ± 0.33
2	HAI-2	18 ± 0.52	45 ± 0.691
3	HAI-3	25 ± 0.35	59 ± 0.632
4	HAI-4	27 ± 0.52	47 ± 0.691
5	HAI-5	24 ± 0.35	60 ± 0.632
	Albendazole	13 ± 0.014	34 ± 0.233
	Mebendazole	9 ± 0.1	28 ± 0.11

VI. DISCUSSION

Screening of synthesized compounds for antibacterial activities: All synthesized compounds were screened for antibacterial, antifungal and anthelmintic activities. **Antibactrial activity of 3-(halosubstituted anilino) -5-(substituted aryl)-isoxazoles:** All title compounds were evaluated for antibacterial activity. The antibacterial activity of newer halogen containing isoxazoles were given in Table No. 4.1. Compound HIA-1 was active against *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* whereas compounds 31J was exhibited antibacterial activity against *Bacillus subtilis* and *Staphylococcus epidermidis*.

Antifungal activity of 1-thiocarbamoyl-3-(halosubstituted anilino) –5-(substituted aryl)-2-pyrazolines: The title compounds were evaluated for antifungal activity. The results of Table No. 4.2. indicated that compounds HAI-2 and HAI-3 were active against *Aspergillus niger* and *Aspergillus.*

Anthelmintic activity of 3-(halosubstituted anilino) -5-(substituted aryl)-isoxazoles: All title compounds were evaluated for anthelmintic activity. The results were given in Table No 4.3. Compounds HAI-2 exhibited good anthelmintic activity.

VII. SUMMARY AND CONCLUSION

Rapid exploration and expansion in the variety of organic molecules may be achieved using microwave aided organic synthesis. Aromatic and heterocyclic molecule synthesis is a breeze. Instantaneous and highly targeted heating is possible without ever touching the reaction vessel to the energy source. Both the reaction time and the method's impact on the environment are little affected. The main advantages of microwave assisted synthesis over the old method were a shorter reaction time, higher yields, milder reaction conditions, an easier experimental work-up procedure, and environmental acceptability.

In this study, halogen-containing isoxazoles, thiocarbamoylpyrazoles, thiozolidonones, and quinazolinone formazans were synthesised and studied utilising microwave irradiation and conventional heating methods.

Tables show the results of in-vitro tests for antibacterial, antifungal, and anthelmintic activity performed on all of the synthesised compounds. The compounds' activities are dependent on the kind and location of the aryl moiety's substituents. Attached to the phenyl ring, certain substituents, such as methyl, methoxy, chloro, fluoro, and bromo groups, significantly increased the activity. To summarise, anthelmintic activity was shown by thiocarbanoyl pyrazolines and strong antibacterial and antifungal activity by quinozolinone formazans among the synthesised compounds.

Finally, halogen-containing heterocyclic molecules that are crucial in pharmacology may be synthesised simply, quickly, cheaply, and safely using microwave irradiation.

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