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Synthesis and Antimicrobial Activity of Azetidin-2-one Containing Pyrazoline Derivatives

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Abstract

Pyrazolines are well-known and important nitrogen containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis. A new series of 3-chloro-1-{4-[5-(Substitutedphenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-hydroxyphenyl) azetidin-2-one are synthesized by reacting 3-chloro-1-{4-[3-(Substituted phenyl)prop-2-enoyl]phenyl}-4-(4-hydroxyphenyl)azetidin-2-one with 99% hydrazine hydrate. All these compounds were characterized by means of their IR, ^{1}H NMR, Spectroscopic data and microanalysis. All the synthesized products were evaluated for their antimicrobial activity. All the compounds were tested for their antibacterial and antifungal activities by broth dilution method.

Keywords: Chalcones, 2-pyrazolines, azetidin-2-one, antimicrobial activity.

Introduction

Pyrazol belongs to the family of azoles**,** five membered heterocycles; pyrazolines have proved to be the most useful framework for biological activities. Pyrazolines have attracted attention of medicinal chemists for both with regard to heterocyclic chemistry and the pharmacological activities associated with them. The pharmaceutical importance of these compounds lies in the fact that they can be effectively utilized as antibacterial, antifungal, antiviral, antiparasitic, antitubercular and insecticidal agents. As evident from the literature, in recent years a significant portion of research work in heterocyclic chemistry has been devoted to pyrazolines containing different aryl groups as substituents.

2-Azetidinones, commonly known as beta-lactams, are well-known heterocyclic compounds among the organic and medicinal chemists[1] .The activity of the famous antibiotics such as penicillin, cephalosporin, monobactams and carbapenems are attributed to the presence of 2-azetidinone ring in them. Azetidin-2-ones can be prepared from Schiff's bases, which are the condensation products of aldehydes and amino compounds. They are considered significant owing to their wide range of biological application. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing 2-azetidinone ring. Such biological activities include antimicrobial, anti-tubercular, carbonic anhydrase inhibitors, local anesthetics, anti-inflammatory, anthelmintic, anticonvulsant, hypoglycemic activity[2-4].

2-pyrazolines are reported as antibacterial[5], antifungal[6-8], antimicrobial[9], antiviral[10], anti-arthritis[11] and anti-inflammatory[12] agents. Encouraged by these facts, we selected to work on Azetidin-2-one containing Pyrazoline with different substitutions on the phenyl ring.

In the present study we report the reaction of 3-chloro-1-{4-[3-(substituted phenyl) prop-2-enoyl] phenyl} - 4-(4-hydroxyphenyl) azetidin-2-one with 99% hydrazine hydrate to form pyrazoline (4a-j). The structures of the various synthesized compounds were assigned on the basis of IR, ^{1}H-NMR spectral data and elemental analysis (table-1). These compounds were also screened for their antimicrobial activity.

Material and Methods

The IR spectra were recorded on IR affinity-1, DRS-8000A, Shimadzu, Ptc. Ltd., Japan spectrophotometer. The ^{1}H-NMR was recorded in DMSO on Bruker Advance II 400 MHz spectrometer using TMS as an internal standard. Melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked by TLC-using Silica gel-G (Merck). Column chromatography was performed on silica gel. All the compounds were tested for their antibacterial and antifungal activities by broth dilution method.

Synthesis of 1-(4-{[(4-hydroxyphenyl) methylene] amino} phenyl) ethanone (1) : A mixture of 4-hydroxy benzaldehyde (0.01M), 1-(4-aminophenyl) ethanone (0.01M) and methanol (30ml) was heated for about 5 min. in a beaker (250 ml) to get a clear solution. The solution was kept overnight at room temperature to get the respective crude solid which was recrystallized from ethanol to obtain the pure crystals of 1-(4-{[(4-hydroxy phenyl)methylene]amino}phenyl) ethanone respectively. The yield of the product was 75% and the product melts at 195^{0}C. Found: C(75.28%) H(5.45%) N(5.82%) , Calcd.

for $C_{15}H_{13}NO_2$: C(75.30%) H(5.48%) N(5.85%). IR, cm^{-1}:3085 (-OH), 3040 (=C-H), 2920(-C-H), 1676(>C=O), 1647(>C=N-), 1606 (>C=C<), 1363(-CH_3, bend), 1314(-C-N<), 1284 (-C-O-), 1240(-C-CO-C-). ^{1}H-NMR (DMSO, δ, ppm): 2.5692 (3H, s, $COCH_3$), 6.5277-7.9774 (8H, m, Ar-H), 8.3820 (1H, s, -CH=N-), 9.6392 (1H, s, Ar-OH).

Synthesis of 1-(4-acetylphenyl)-3-chloro-4-(4-hydroxyphenyl) azetidin -2-one (2): In a 100ml Round bottom flask 1-(4-{[(4-hydroxyphenyl) methylene] amino} phenyl) ethanone (0.01M) in 70ml benzene was taken. Chloro acetyl chloride (0.01M) was added at room temperature with constant stirring and triethylamine 1ml was added and the reaction mixture was refluxed for 7 hours. After the completion of reaction, solvent was removed by vacuum distillation. The solid was filtered, dried and recrystallized from toluene. The yield of the product was 60% and the product melts at 119^0C. Found: C(64.64%) H(4.44%) N(4.42%), Calcd. for $C_{17}H_{14}ClNO_3$: C(64.67%) H(4.47%) N(4.44%). IR, cm^{-1}: 3300 (-OH), 3050(=C-H), 2950(-C-H), 1680(>C=O), 1600(>C=C<), 1375(-CH_3, bend), 1300(-C-N<), 1240(-C-CO-C-), 1220(-C-O), 560 (C-Cl). ^{1}H-NMR (DMSO, δ, ppm): 2.5392 (3H, s, $COCH_3$), 4.8954 (1H, d, >CH-Ar), 5.5151 (1H, d, >CH-Cl), 6.6720-8.0745 (8H, m, Ar-H), 9.7784 (1H, s, Ar-OH).

Synthesis of 3-chloro-1-{4-[3-(Substituted phenyl) prop-2-enoyl] phenyl}-4-(4-hydroxyphenyl) azetidin-2-one (3a-j): To the solution of 1-(4-acetylphenyl)-3-chloro-4-(4-hydroxyphenyl) azetidin-2-one (0.01M) in absolute ethanol (50 ml), substituted benzaldehyde (0.01M) and 2% NaOH were added and refluxed for 10 hours. After refluxing the reaction mixture was concentrated, cooled, filtered and neutralized with dil. HCl. The solid residue thus obtained was crystallized by absolute ethanol. IR(**3b**), cm^{-1}:3359(-OH), 3045(=C-H), 1728(>C=O), 1608(>C=C<), 1290(-C-N<), 1186 (-C-O-), 769(-C-Cl).^{1}H-NMR (**3c**-DMSO, δ, ppm): 3.8789 (6H, s, -OCH_3), 4.8613 (1H, d, >CH-Ar), 5.3413 (1H, d, >CH-Cl), 6.7340-7.8883 (11H, m, Ar-H), 7.9733 (2H, d, -CH=CH-), 9.8306 (1H, s, Ar-OH).

***Synthesis of 3-chloro-1-{4-[5-(Substituted phenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-hydroxyphenyl) azetidin-2-one*.(4a-j):** A mixture of 3-chloro-1-{4-[3-(Substituted phenyl) prop-2-enoyl] phenyl}-4-(4-hydroxyphenyl) azetidin-2-one (0.01M) and 99% hydrazine hydrate (0.015M) in ethanol (50ml) refluxed gently for 3 hours. Then the mixture was concentrated and allowed to cool. The resulting solid was filtered, washed with ethanol and recrystallized from ethanol to give a pale brown solid. IR(**4f**), cm^{-1}: 3317 (-OH), 3080 (=C-H), 1718(>C=O), 1658(>C=N-), 1544 (>C=C<), 1460(-CH_3, bend), 1324(-C-N<),1284 (-N-N), 1234 (-C-O),641 (-C-Cl-), 3463 (>NH). ^{1}H-NMR (**4h**-DMSO, δ, ppm): 3.1699 (6H, s, $N(CH_3)_2$), 3.9462 (2H, d, CH_2- of Pyrazol), 4.3000 (1H, t, >CH-Ar of Pyrazol), 4.8268 (1H, d, >CH-Ar of Azetidine), 5.3981 (1H, d, >CH-Cl of Azetidine), 6.6114-7.9986 (13H, m, Ar-H, -NH-), 9.5428 (1H, s, Ar-OH).

Table-1
Physical constant of 3-chloro-1-{4-[5-(Substituted phenyl)-4, 5-dihydro-pyrazol-3- yl] phenyl}-4-(4-hydroxyphenyl) azetidin-2-one

Comp	R	Molecular formula	Yield %	M.P. °C	Elemental Analysis		
					% C Found (Calcd)	% N Found (Calcd)	% H Found (Calcd)
4a	-2-Cl	$C_{24}H_{19}Cl_2N_3O_2$	68	173	63.70 (63.73)	9.26 (9.29)	4.19 (4.23)
4b	-2-OH	$C_{24}H_{20}ClN_3O_3$	70	182	66.42 (66.44)	9.62 (9.68)	4.61 (4.65)
4c	-3,4-$(OCH_3)_2$	$C_{26}H_{24}ClN_3O_4$	65	135	65.28 (65.34)	8.76 (8.79)	5.03 (5.06)
4d	-3-NO_2	$C_{24}H_{19}ClN_4O_4$	74	188	62.24 (62.27)	12.08 (12.10)	4.11 (4.14)
4e	-4-Cl	$C_{24}H_{19}Cl_2N_3O_2$	68	278	63.72 (63.73)	9.27 (9.29)	4.19 (4.23)
4f	-4-$N(C_2H_5)_2$	$C_{28}H_{29}ClN_4O_2$	64	138	68.74 (68.77)	11.43 (11.46)	5.94 (5.98)
4g	-4-OH	$C_{24}H_{20}ClN_3O_3$	72	178	66.40 (66.44)	9.63 (9.68)	4.60 (4.65)
4h	-4-$N(CH_3)_2$	$C_{26}H_{25}ClN_4O_2$	63	162	67.73 (67.75)	12.12 (12.15)	5.44 (5.47)
4i	CHO	$C_{24}H_{20}ClN_3O_2$	72	203	68.95 (68.98)	10.02 (10.06)	4.79 (4.82)
4j	-2-OH-3-OCH_3	$C_{25}H_{22}ClN_3O_4$	73	208	64.69 (64.72)	9.03 (9.06)	4.75 (4.78)

Results and Discussions

Antimicrobial activity: The MICs of synthesized compounds were carried out by broth micro dilution method as described by Rattan (2000). It is one of the non automated in vitro bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms.

The *in vitro* antimicrobial activity of test compounds were assessed against 24 hr cultures of several selected bacteria and fungi. The bacteria used were *E. coli, S.aureus, P. aeruginosa*, and *S. pyogenus*; the fungi used were *C. albicans, A. Niger, and A.clavatus.*

The antimicrobial activity was performed by broth dilution method in DMSO. Gentamycin, ampicilin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and greseofulvin were used as standard for the evaluation of antibacterial and antifungal activities respectively. The activity was reported by minimal inhibition concentration. The results are summarized in table-2

Table-2
Antimicrobial activities 3-chloro-1-{4-[5-(Substituted phenyl)-4, 5-dihydro-pyrazol-3-yl] phenyl}-4-(4-hydroxyphenyl) azetidin-2-one

Compound	R	Antibacterial activity Minimal Inhibition Concentration				Antifungal Activity Minimal Inhibition Concentration		
		E.COLI	***P.AERUGINOSA***	***S.AUREUS***	***S.PYOGENUS***	***C.ALBICANS***	***A.NIGER***	***A.CLAVATUS***
4a	-2-Cl	100	150	125	100	1000	1000	800
4b	-2-OH	175	200	175	250	800	700	700
4c	-3,4-$(OCH_3)_2$	225	225	150	200	>1000	800	600
4d	-3-NO_2	175	225	200	150	700	600	1000
4e	-4-Cl	100	175	100	175	700	>1000	1000
4f	-4-$N(C_2H_5)_2$	250	200	1250	200	500	1000	1000
4g	-4-OH	200	175	200	175	800	800	800
4h	-4-$N(CH_3)_2$	200	200	175	225	700	700	700
4i	CHO	175	225	200	125	750	600	800
4j	-2-OH-3-OCH_3	200	200	225	200	1000	800	>1000

Table-3
Antibacterial Activity: Minimal Inhibition Concentration (The Standard Drugs)

Drug	E.Coli	P. Aeruginosa	S. Aureus	S. Pyogenus
-	MTCC 443	MTCC 1688	MTCC 96	MTCC 442
(Microgramme/ml)				
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	--	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

Table-4
Antifungal Activity: Minimal Inhibition Concentration (The Standard Drugs)

DRUG	C.ALBICANS	A.NIGER	A.CLAVATUS
-	MTCC 227	MTCC 282	MTCC 1323
(Microgramme/ml)			
Nystatin	100	100	100
Greseofulvin	500	100	100

Biological screening result of activities 3-chloro-1-{4-[5-(substituted phenyl)-4, 5-dihydro-pyrazol-3-yl] phenyl}-4-(4-hydroxyphenyl) azetidin-2-one based derivatives shows that compound (**4a**) have shown better activity against E. coli and S. pyogenus, while (**4e**) have Shawn better activity against E.coli, while rest of all compound possessed good activity against S.aureus in the range of 100-225 µg/ml. Compounds with substitution 2-Chloro (**4a**), shown good antibacterial activity against S. pyogenus , while rest of all derivatives possessed good activity against S. pyogenus in the range of 125-250 µg/ml. Compound (**4f**) is found to be good antifungal activity against C. albicans, against standard drugs Greseofulvin. While rest of all derivatives are poor against A. Niger and A. clavatus

Conclusion

The Main focus of this research work was to synthesize, characterize and evaluate antimicrobial activities of the newly synthesized pyrazoline derivatives, structures of synthesized compounds were confirmed and characterized with the help of analytical data's such as IR and ^{1}H-NMR. In summary, we have described the synthesis and antimicrobial activity of some new 3-chloro-1-{4-[5-(substituted phenyl)-4, 5-dihydro-pyrazol-3-yl] phenyl}-4-(4-hydroxyphenyl) azetidin-2-one MIC values revealed that amongst newly synthesized compound having chlorophenyl type linkage has shown good activity against the bacterial strains..

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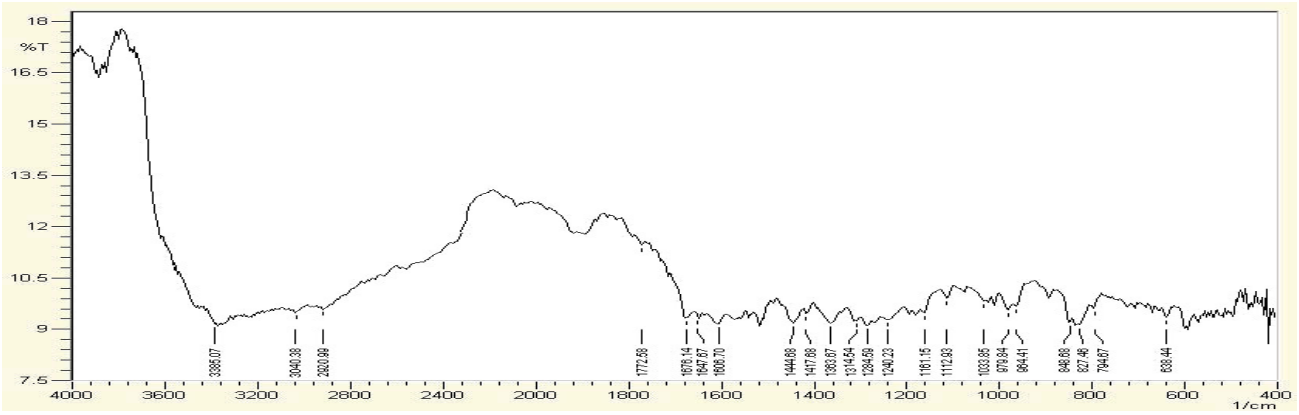
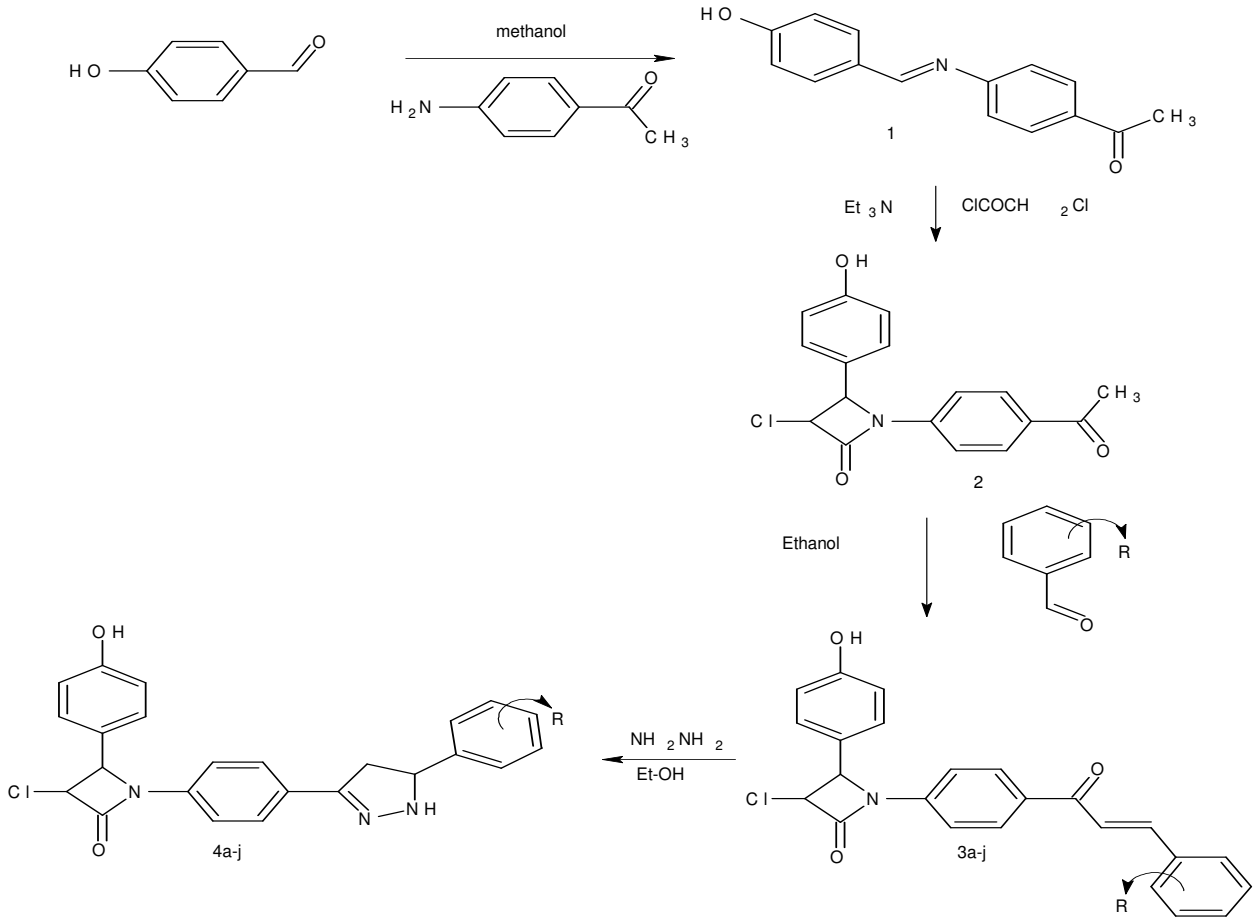


Figure-1
IR Spectra of (1)

Reaction Scheme



Scheme

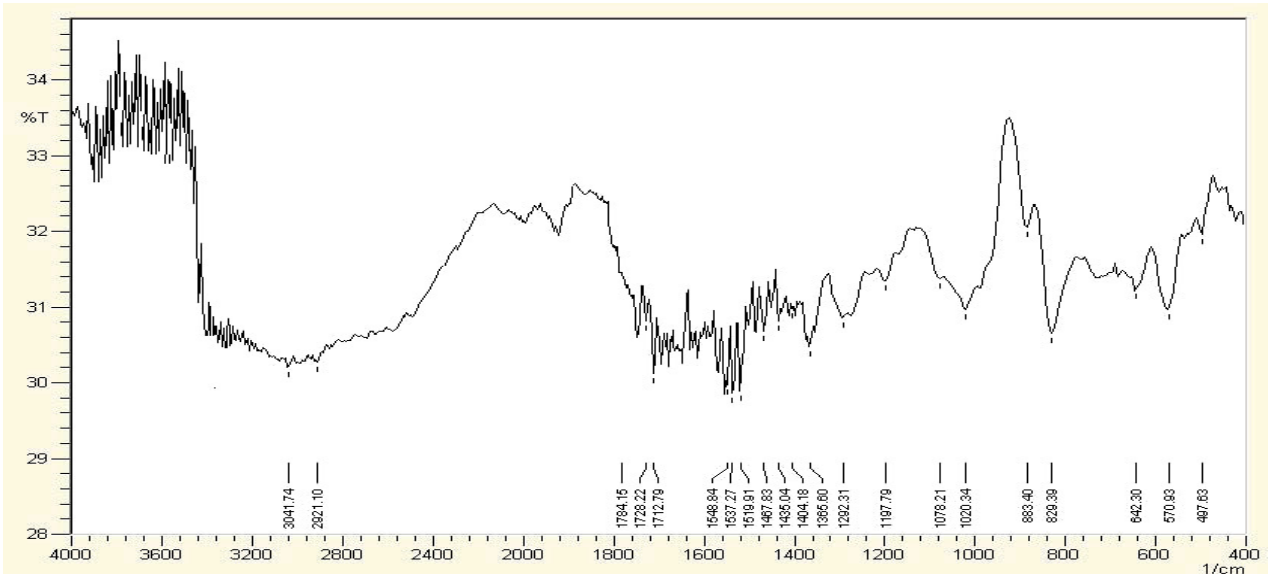


Figure-2
IR Spectra of (2)

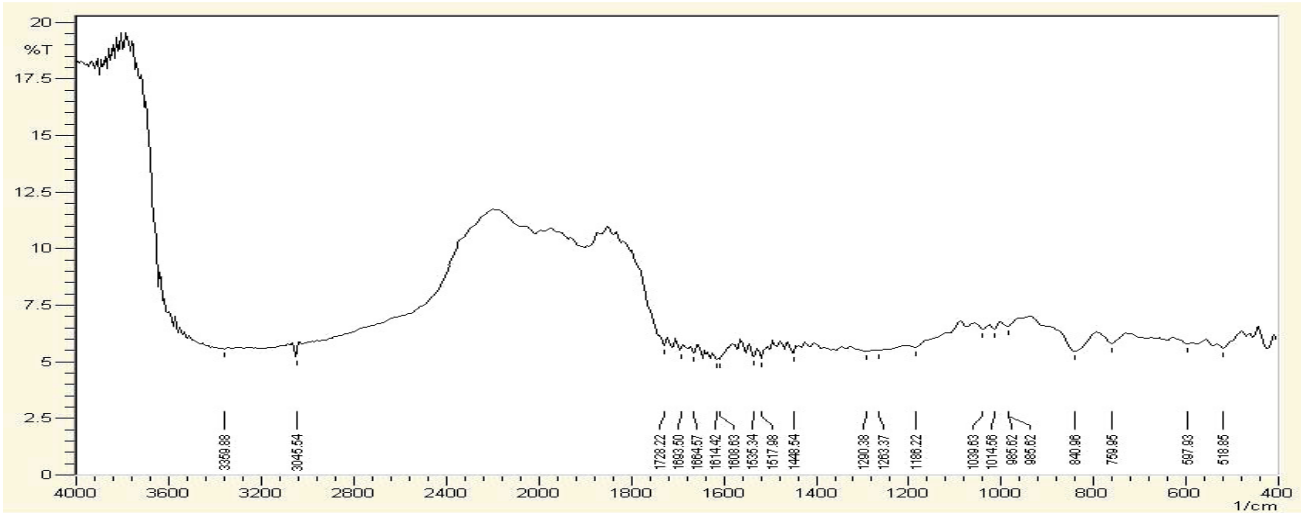


Figure-3
IR Spectra of 3b

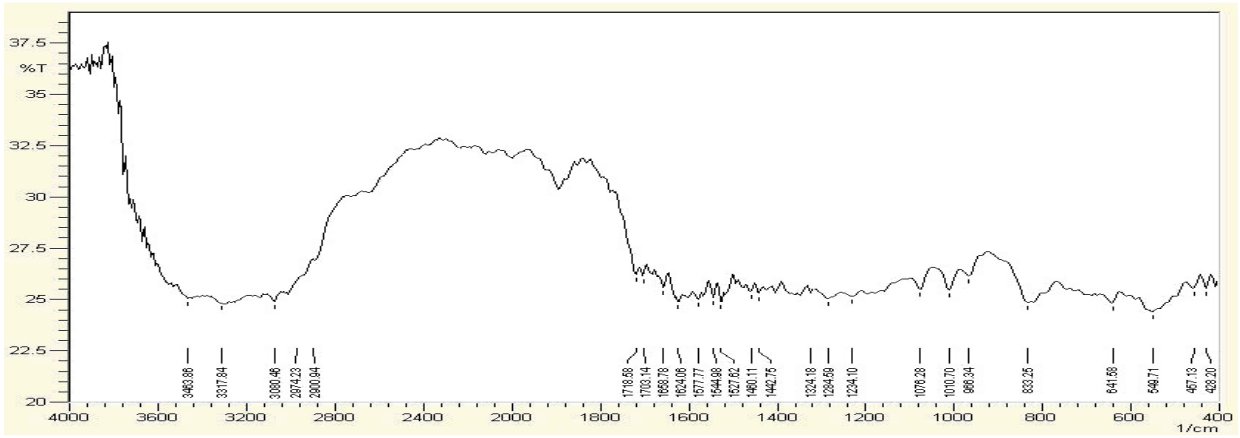


Figure-4
IR Spectra of 4f

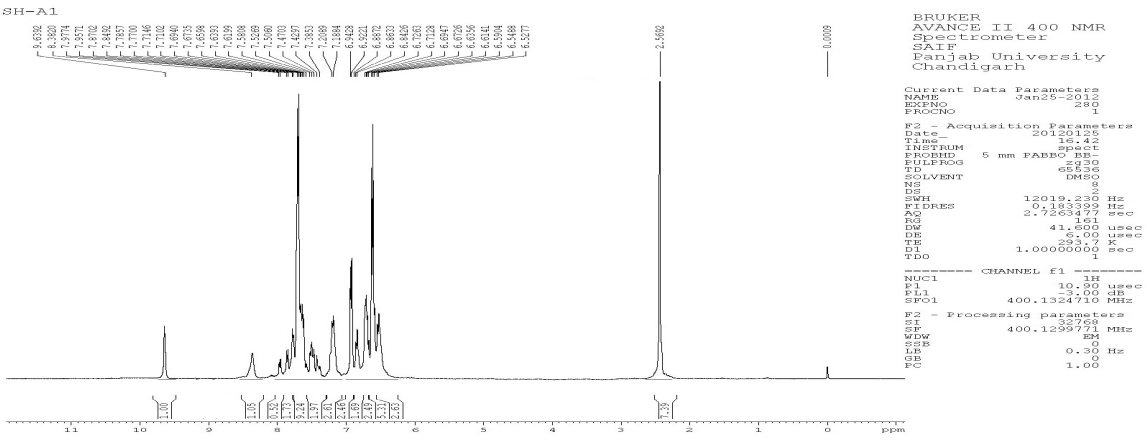


Figure-5
NMR Spectra of (1)

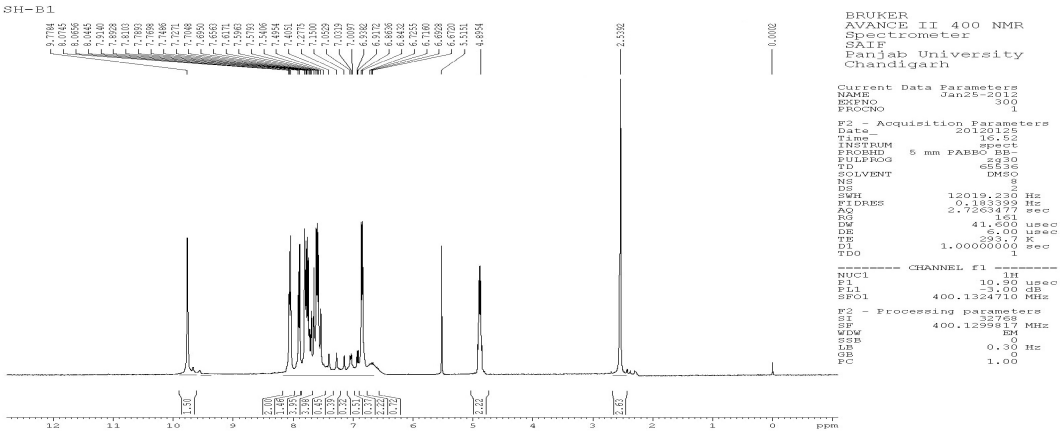


Figure-6
NMR Spectra of (2)

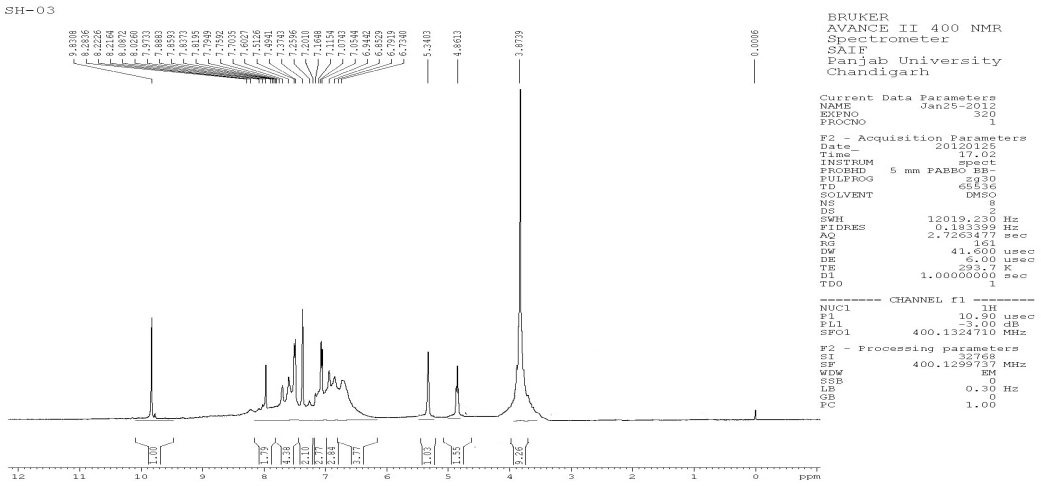


Figure-7
NMR Spectra of 3c

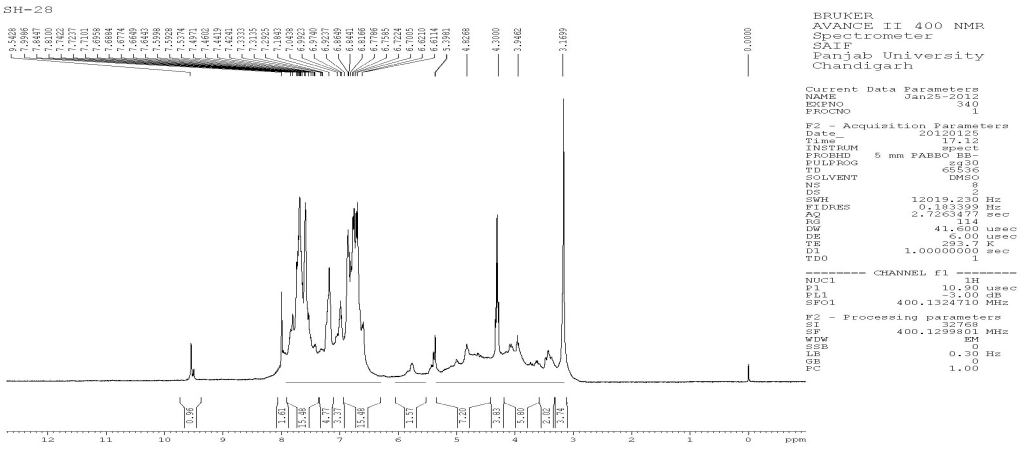


Figure-8
NMR Spectra of 4h