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In-Vitro Anti-Bacterial and Anti-Fugal Activity of a Novel Series of Quinoxaline 2, 3-Dione Derivatives

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Abstract

Aim: The aim of the present study is to synthesis and evaluation of anti-microbial activity of novel series of quinoxaline 2, 3 Dione derivatives containing a 2-azetidinone (A1-A5) nucleus.

Method: The synthesis of azetidinones by reacting with chloroacetyl chloride in the presence of triethylamine. All the title compounds were tested for their anti-microbial activity against gram-positive (staphylococcus aureus, staphylococcus epidermidis, Bacillus cereus and pseudomonas aereginosa) and gram-negative (Escherichia coli and klebsiella pneumonia) bacteria, fungal activity against (Aspergillus Niger and Aspergillus fumigates) at a concentration of 100,200µg/ml.

Result: Antibacterial activity of synthesized compounds was tested against both gram positive and gram negative bacteria and the standard drug used for the study was ciprofloxacin. The chloro, fluro and bromo substitution in three derivatives showed significant antifungal activity when compared to standard. The hydroxy, nitro groups showed mild activity when compared to standard.

Conclution: A novel series of quinoxaline 2, 3-Dione derivatives has anti-microbial activity so supplementary exploration has been needed to establish other pharmacological performance of these derivatives and in future it would be a most promising and leading derivatives for several ailments.

Keywords: Quinoxaline 2; 3-Dione; Antimicrobial activity; Anti-fungal activity; Schiff bases reactions.

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Introduction

The aim of the present study is to synthesis and screening the anti-microbial activity of novel series of quinoxaline 2, 3 dione derivatives containing a 2-azetidinone (A1-A6) nucleus. Among the various classes of nitrogen-containing heterocyclic compounds, quinoxalines display a broad spectrum of biological activities. It known to possess antibacterial, antifungal, and cyto-toxic activities.[1] The chemistry of β-lactams has taken an important place in organic chemistry since the discovery of Penicillin by Sir Alexander Fleming in 1928 and shortly thereafter Cephalosporin which were both used as successful antibiotics. Even now the research in this area is stimulated because of development of bacterial resistance to widely used antibiotics of this type. There is a need for functionalized β-lactams or for new active principles in β-lactam series.[12] Azetidinones, commonly known as β-lactams are well known heterocyclic compounds among the organic and medicinal chemists. The activity of the famous antibiotics such as penicillins, cephalosporins, and carbapenems are attribute to the presence of 2-azetidinone ring in them. Azetidinones are very important class of

compounds possessing wide range of biological activities such as antibacterial[3], anti-inflammatory[4], antihyperlipidemic[5], CNS activity[6], tryptase inhibitory[7], human leukocyte elastase inhibitory[8], anti hyperglycemic[9], vasopressin v1a antagonist[10], anticancer activity[11], antimicrobial[12], pesticide[13], antitumor[14], antitubercular[15], cytotoxic[16], enzyme inhibitors[17], elastase inhibitors[18], and cholesterol absorption inhibitors.[19] They are also used in agriculture field as herbicides, fungicides and insecticides. In addition, quinoxaline derivatives are also useful in formation of dyes, efficient electron luminescent materials, organic semiconductor, cavitands and dehydroannulenes.

Because of wide range of variety and applications associated with quinoxaline moieties, their synthesis has remained the goal of many research groups over the years. Several kinds of synthetic routes towards quinoxaline have been developed, including condensation of aryl-1,2 diamines with a 1,2-diketones, bicatalyzed oxidative coupling of epoxides with ene-1,2-diamines, hetero annulations of nitro ketene N,S-aryl amino acetal with POCl3, cyclization of a-hydroxy ketone via a tandem oxidation process using Pd(OAc)2 or RuCl2 - (PPh3) 3 -TEMPO as well as MnO2 . But methods that have been established for preparation of quinoxaline derivatives are associated with one or more of the following drawbacks like low yield, long reaction time and harsh reaction conditions.

Materials and Methods

General procedure for N1-substituted arylidene / heteroarylidene-4-(3-oxo 3, 4 dihydroquinoxaline-2 (1H)-ylidenemino) benzene sulfonamide. A mixture of Schiff base sulphanilamide (0.01mol) and five different aromatic aldehydes (0.01 mol) were taken in separate RBF and reflux in ethanol for three h [20]. The reaction mixture was then poured in to crushed ice and the separated solid wash filtered and re-crystallizes using absolute alcohol.

Synthesis of 2-azetidinones (A1-A6):

0.01 mol of Schiff bases (S1-S5) were taken separately and (0.01 mol) of triethylamine in 25 ml dry dioxan was added to them to form a solution. To these solutions chloro acetyl chloride was added drop wise with continuous stirring in cold for 30 min and refluxed for 3 h.[21] The contents were then poured into crushed ice, filtered, and recrystallized using absolute alcohol.

The following experimental methods were used for the characterization of the synthesized compounds. Melting points of the synthesized compounds were determined in open capillary tubes (Thermonilic melting point apparatus) and were uncorrected. Thin layer chromatography was performed using precoated aluminium plates coated with silica gel GF254 [E. merk] of 0.25mm thickness, dichloromethane and methanol as the solvent system and UV chamber as the visualizing agent.

An IR spectrum was recorded on ABB BOMEM FTIR spectrometer using KBr pellets. 1H-NMR spectra of the compounds in DMSO was recorded on JEOL GSX 400 NMR spectrophotometers. A mass spectrum was recorded on JEOL GC MATE mass spectrometers.[22]

Experimental Work

Evaluation of in-vitro anti -bacterial and anti-fungal activity:

Bacteria are the most abundant prokaryotic organism that is vital to life of living things. Bacteria are ubiquitous, place a major positive role to the life of living things but some of them cause harmful diseases to the living things (humans. animals, plants, etc.). In nature bacteria can adopt any kind of living conditions than any other groups of organisms. Fungi are eukaryotic organism that is subdivided in to yeasts and moulds.[23] Yeasts are unicellular eukaryotic organisms which have size of large bacteria. The yeast mainly used in the fermentation of wine and beer, and in production of bread. Moulds are long chain cells often seen as fuzzy masses on bread and other acidic food products. Bacteria and fungi are the primary decomposers of organic matters in the world. As like bacteria some of the fungi cause harmful human diseases such as athlete's foot and thrush.

The following conditions must be accomplished for the determination of proper anti-microbial activity.[24]

- There should be intimate control between the test organism and substance to be evaluated
- Micro-organism should be provided with the required conditions for growth
- Measurement of activity should be done correctly
- Aseptic should be maintained
- Conditions should be maintained unchanged throughout the study

Antifungal Activity of the Synthesized Compounds

Micro organisms

The standard strains were procured from the American Type Culture Collection (ATCC), Rockville, USA, and the pathological strains were procured from the department of microbiology, CEEAL ANALYTICAL LAB, Chennai, India. The anti-microbial activity of the synthesized compounds was screened against the following fungi.

1. Aspergillusniger (ATCC9029)
2. Aspergillus fumigates (ATCC46645)

Preparation of Test Inoculum

(a) Subculture (preparation of seeded broth)

The strains of fungi were inoculated into test tubes containing 10 ml of Sabouraud dextrose broth; bacteria were inoculated into test tubes containing 10 ml of nutrient broth. One loopful of bacteria and fungi were transferred aseptically to each of the test tubes. The test tubes were incubated at 37°C for bacteria and 25°C for fungi for 24 h. This is referred to as seeded broth.[26]

(b) Standardization of seeded broth (viable count)

1 ml of the 24 h seeded broth of each strain was diluted with 99 mL of sterile normal saline containing 0.05% TWEEN 80 (8 drops of TWEEN 80 in 1000 mL of normal saline). From that 1 mL was further diluted to 10 mL with sterile normal saline. This was continued till 102, 104, 105 up to 1010 dilution of the seeded broth was obtained.

The dilutions were studied by inoculating 0.2 ml of each dilution on to the nutrient agar at 30 - 40°C. After inoculation it was transferred into Petri dish before it gets solidified. All the Petri dishes were incubated for 24 h at 37°C for bacteria and 25°C for fungi.[27] The number of well-formed colonies on the plates was counted. The seeded broth was then suitably diluted to contain between 107 –108 microorganisms/ml. This was designated as working stock, which was used for antimicrobial studies.

Zone of Inhibition of the Synthesized Compounds

Inoculate the previously prepared working stock appropriate to the assay with requisite quantity of suspension of the micro-organism, to the medium at a temperature between 40°C and 50°C and immediately pour the inoculated medium into Petri dishes to give a depth of 3 to 4 mm.The dishes were specially selected with bottoms and were placed on a level surface so as to ensure that there was a uniform thickness. The Petri dishes were allowed to be sterilized at 160°C – 170°C for 1 h.

The paper disc (Whatman No.2) was cut down into small disc (6mm diameter) and sterilized at 180 o C for 30 min in hot air oven impregnated with the test and standard drug separately. The dried discs were placed on the surface of the medium [28]. After all the drugs are added Petri dishes were left standing for 1 to 4 h at room temperature, as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. All the Petri dishes were incubated for 24 h at the required temperatures, i.e., 37oC for bacteria and 25oC for fungi. After incubation the diameters of the circular inhibition zones were measured and from these values Minimum Inhibitory Concentration and biological activities were calculated.

Determination of Mic

Agar Streak Dilution Method[29]

MIC of the synthesized compound was determined by agar streak dilution method. A stock solution of the synthesized compound (100 µg mL-1) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for anti-fungal activity). A specified quantity of the medium (40-50°C) containing the compound was poured into a petridish to give a depth of 3-4 mm and allowed to solidify. Suspension of the microorganism were prepared to contain approximately 105 cfu mL-1 and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37°C for 24 h and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate.

Result and Discussion

In the present study attempt was made to synthesize the azetidinone derivatives of quinoxaline 2, 3 dione from Schiff base as intermediate. The compounds A1-A6 are azetidinones. All the compounds were

Schematic representation of Quinaxaline 2,3-dione derivatives-(32)

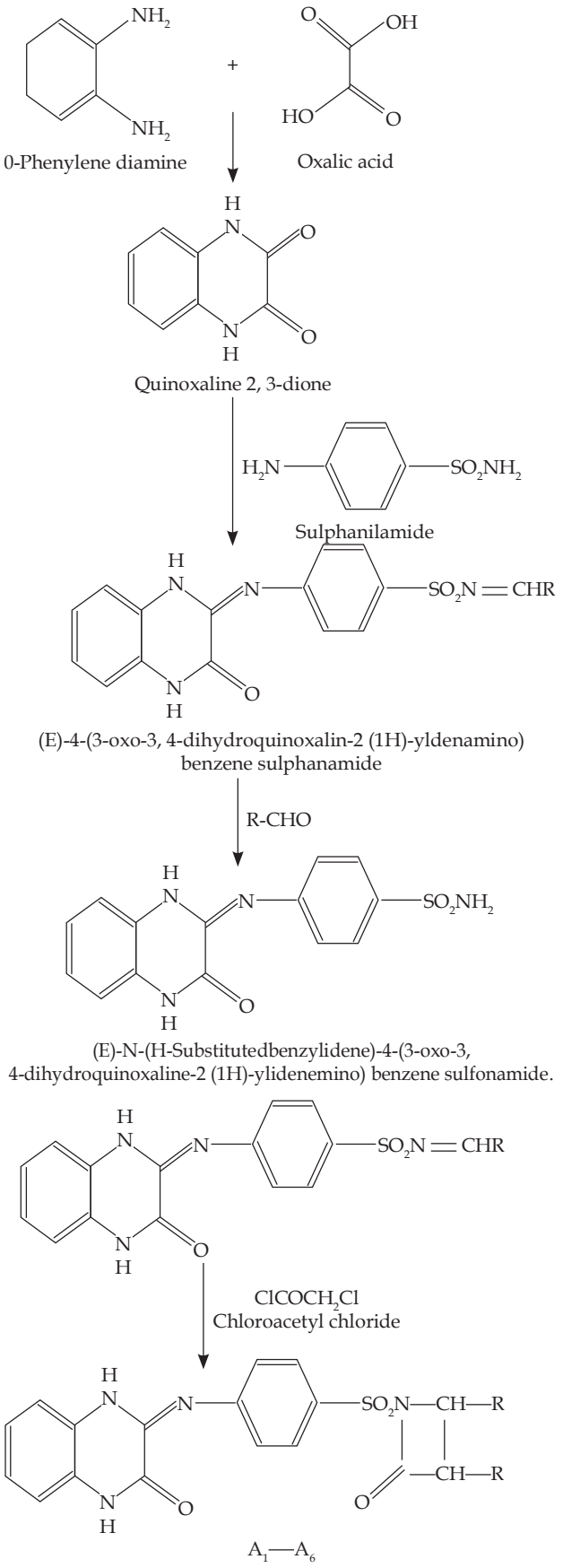
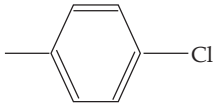
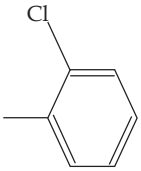
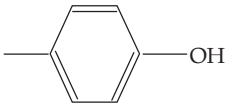
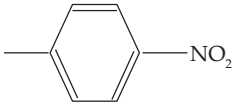
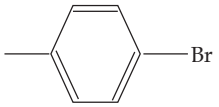
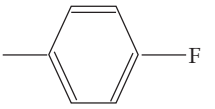


Fig 1: Scheme for the synthesis of compounds.

Table 1: Details of the –R Represented in the Synthetic Scheme-(33)

Compounds	-R
1	
2	
3	
4	
5	
6	

characterized and confirmed by H1 NMR, and Mass spectra.

The scheme for the synthesized derivatives were expressed in fig 1

The physical properties of an synthesized compound was comply with the standard so the result was shown in table 2

The ^{1}H NMR and spectra of the synthesized compounds were recorded on a JOEL GSX 400 NMR spectrometer in DMSO

In the nuclear magnetic resonance spectra (1H-NMR), the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities, and coupling constants. In particular, it must be pointed out that in compounds,the result was shown in table 3 and Fig 2-7

The mass spectral data was shown in table 5 and fig 8-13, the aim of the study is used to reveal the molecular structure and molecular weight of a synthesized compound. All the synthezised compound (A1-A6) shows the presence of molecular structure with suitable functional group so thereby it indicate the direct role in the activity of antibacterial and anti fungal.

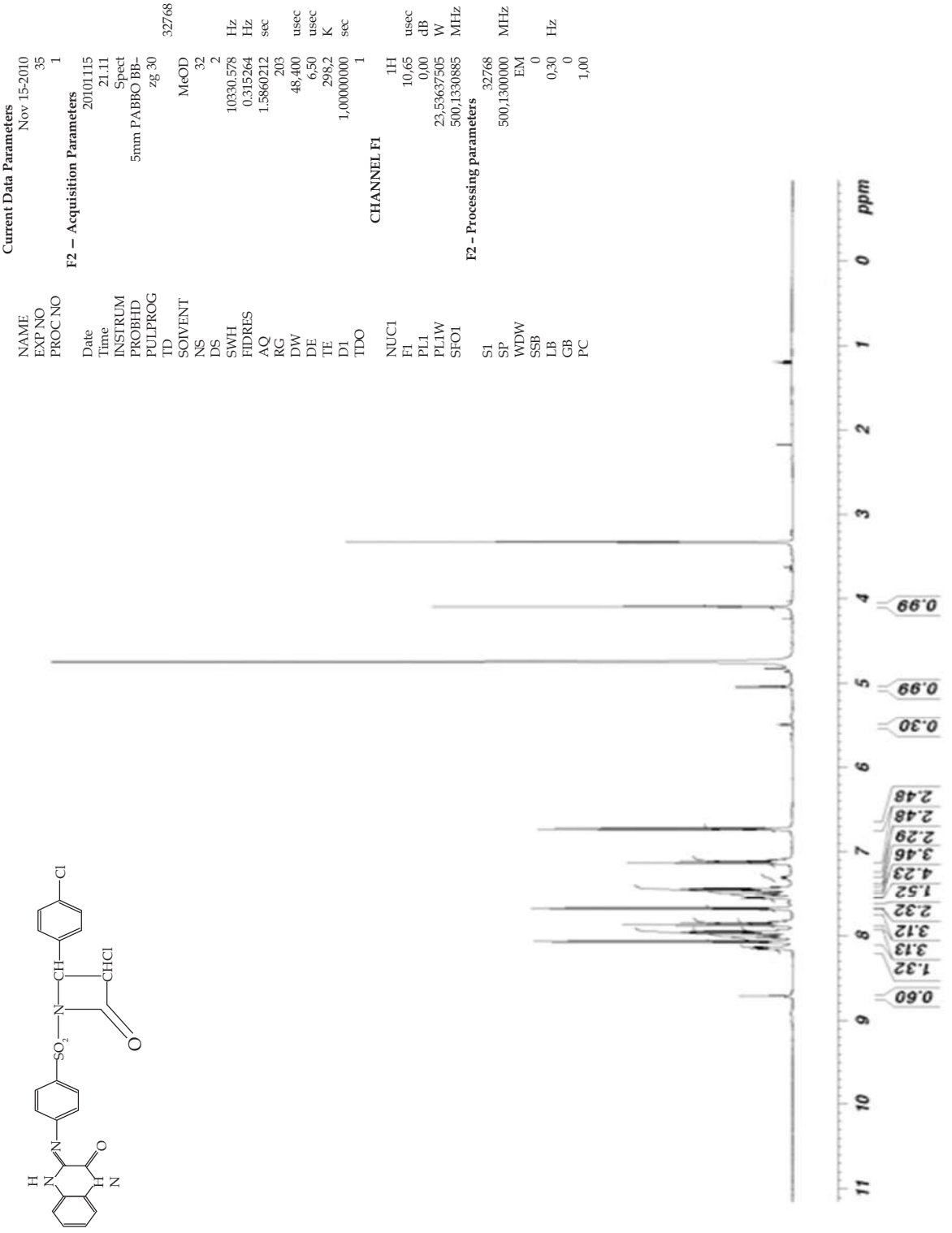


Fig. 2: NMR spectra of synthesized derivatives A1.

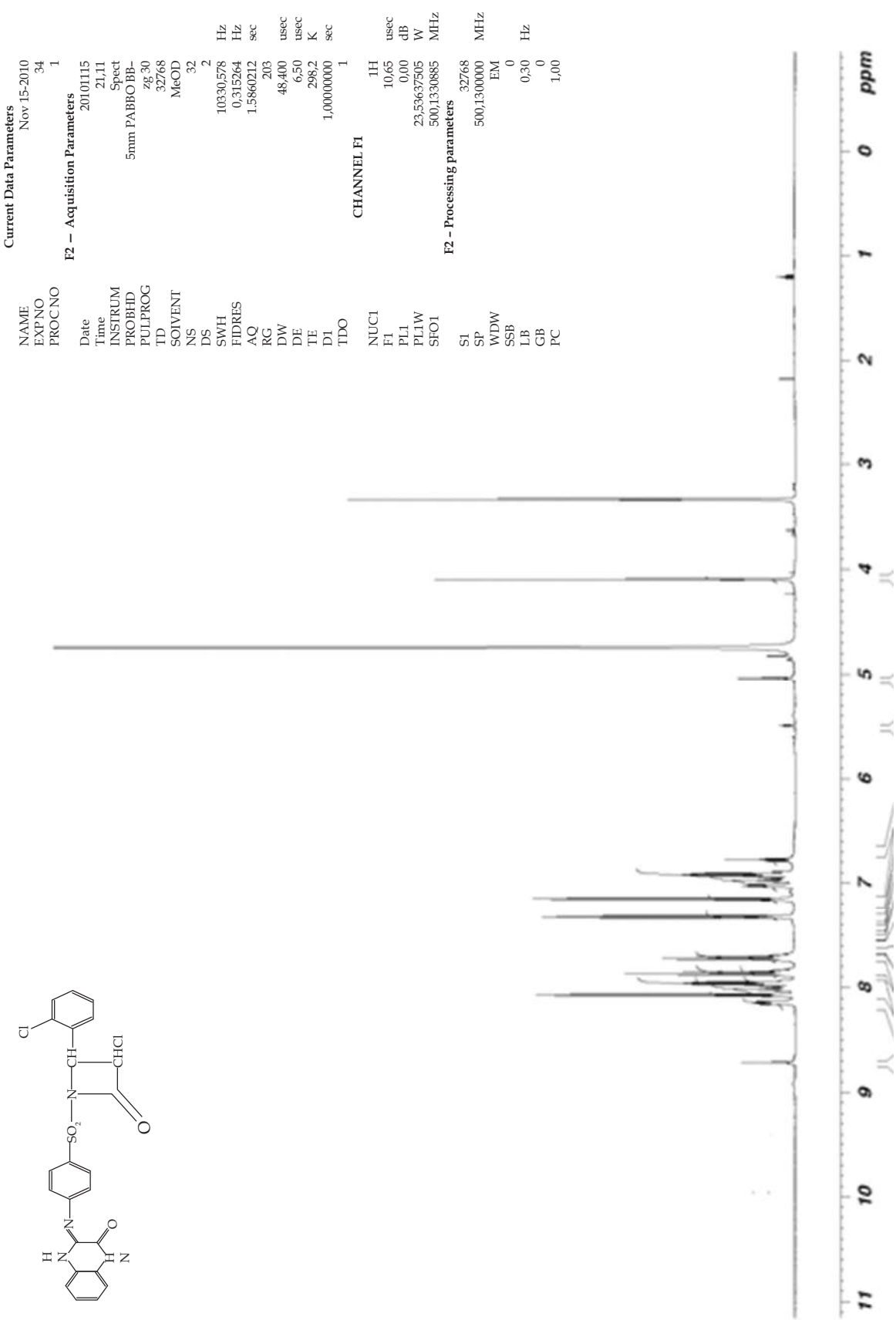


Fig. 3: NMR spectra of synthesized derivatives A2.

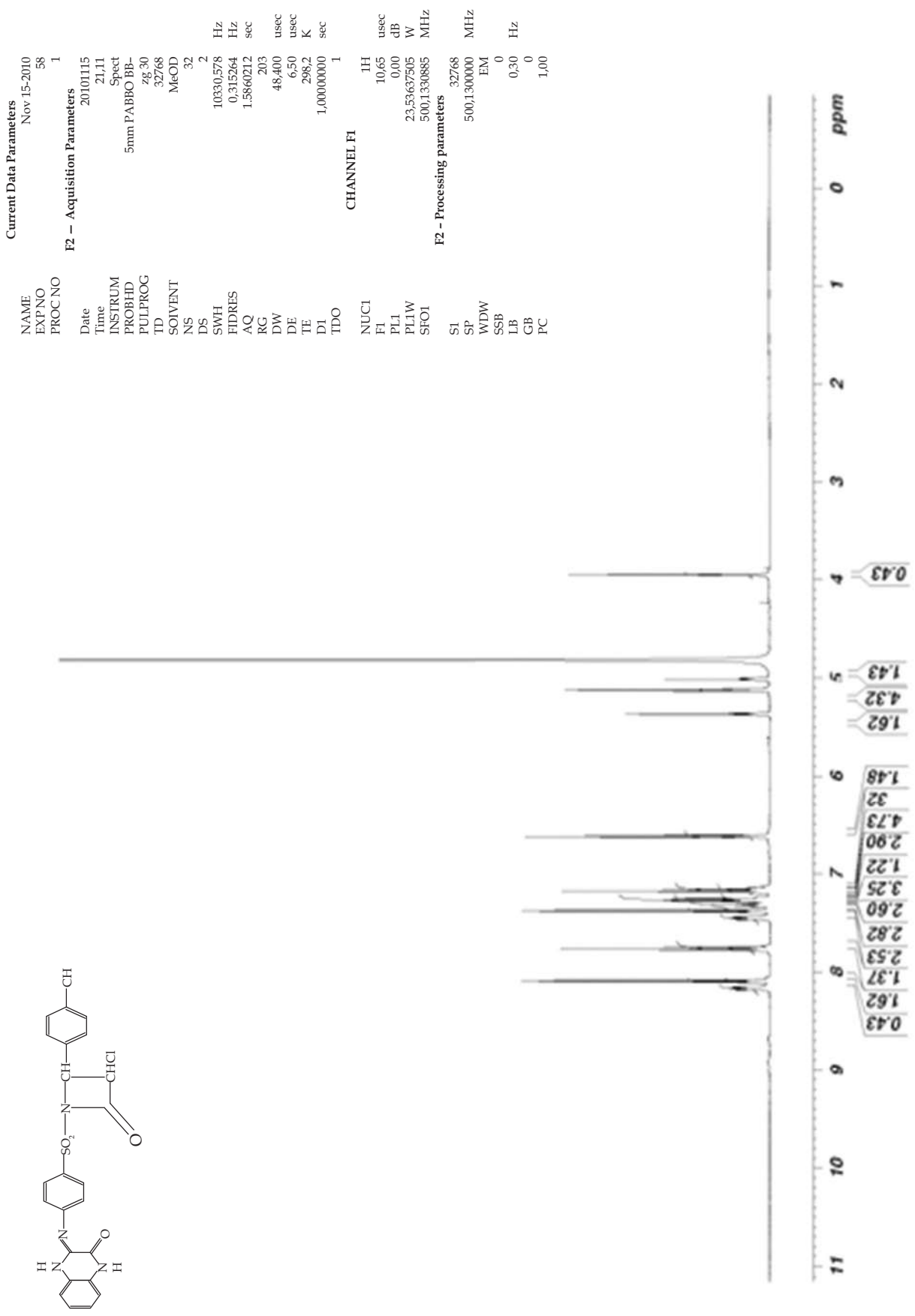


Fig. 4: NMR spectra of synthesized derivatives A3.

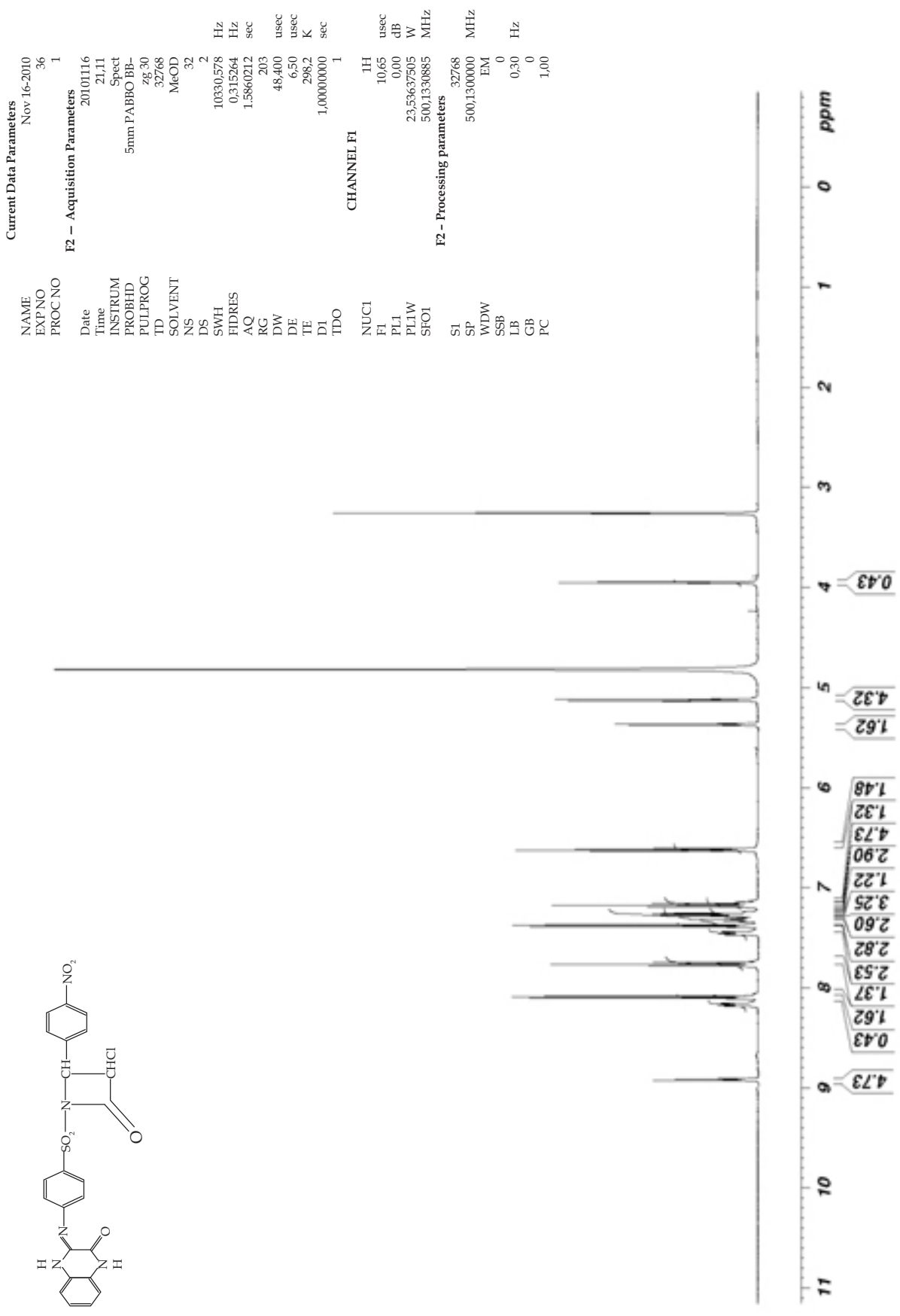


Fig. 5: NMR spectra of synthesized derivatives A4.

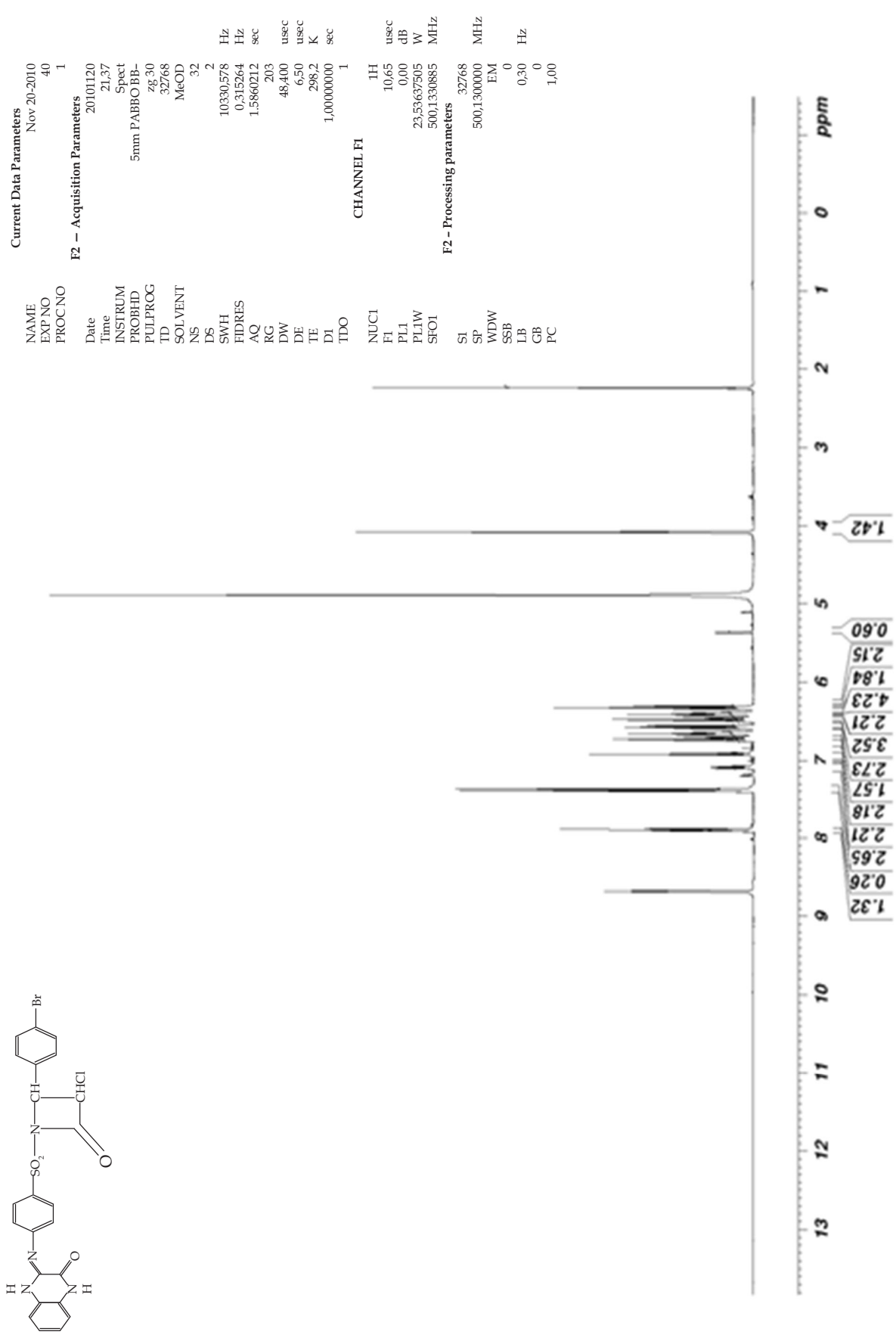


Fig. 6: NMR spectra of synthesized derivatives A5.

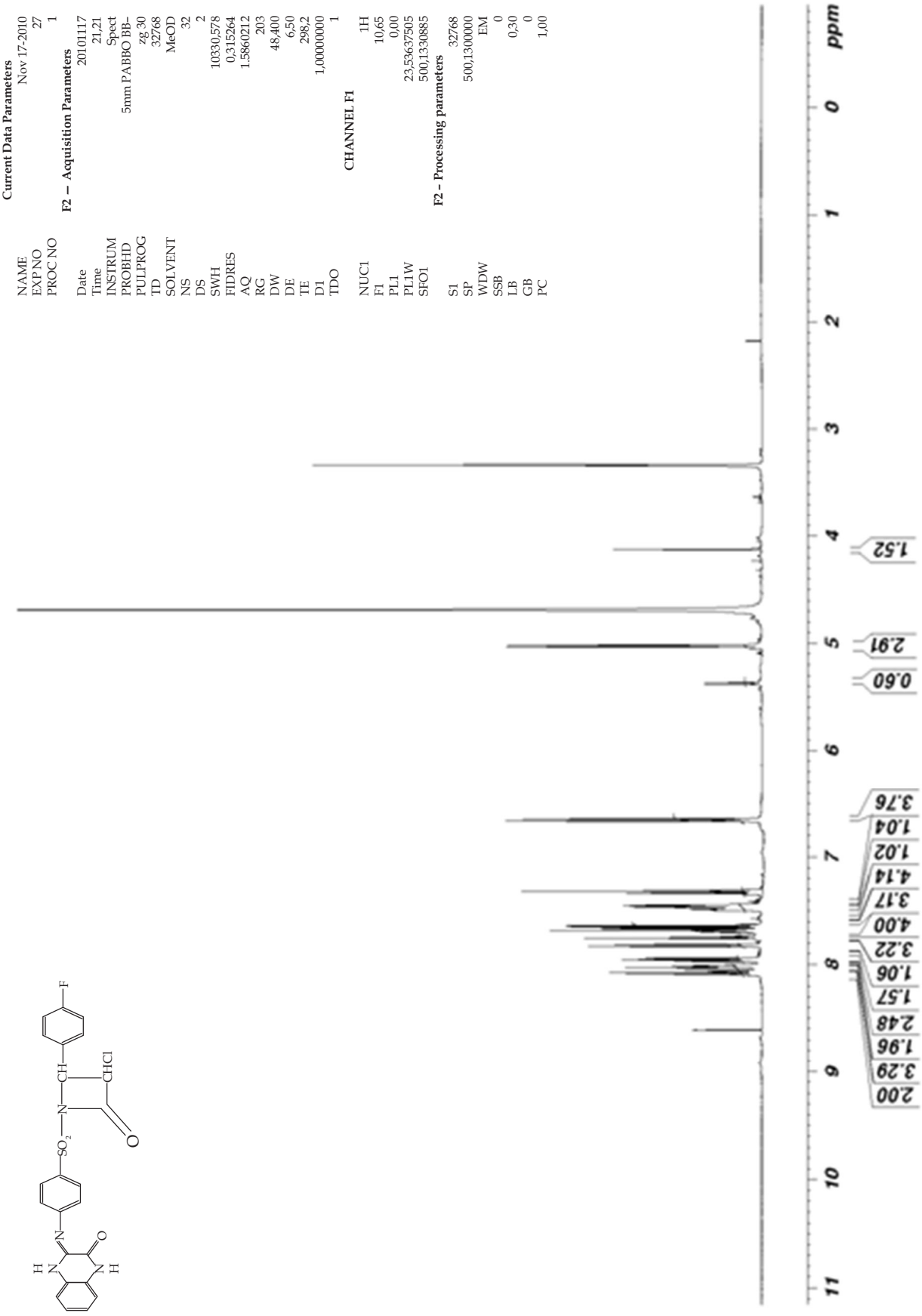


Fig. 7: NMR spectra of synthesized derivatives A6.

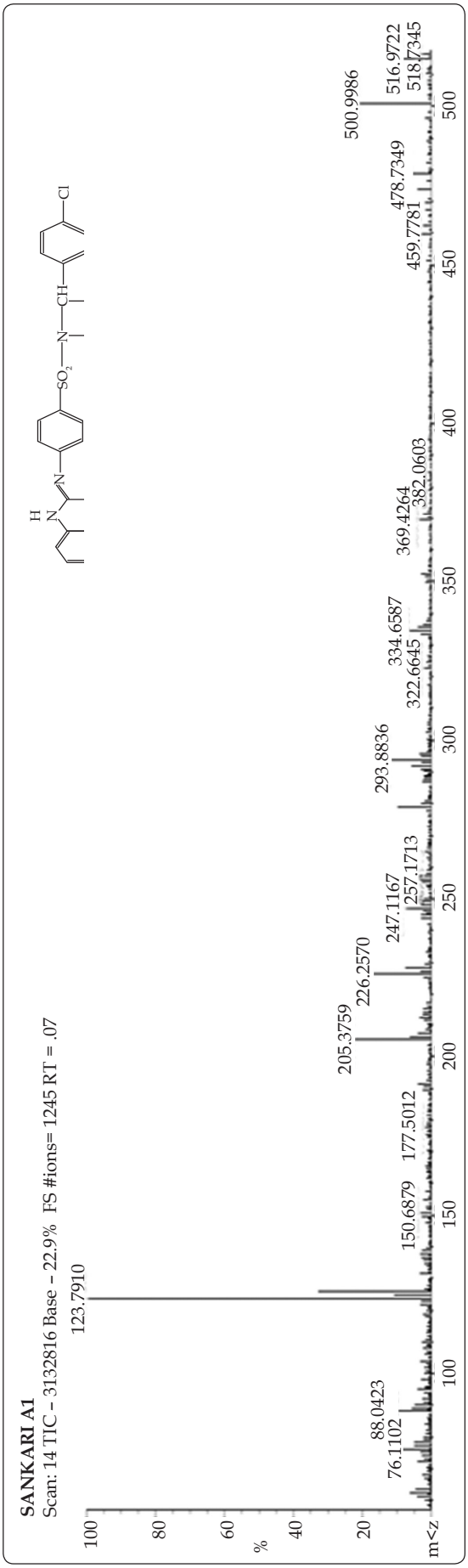


Fig. 8: Mass spectra of synthesized compound A1

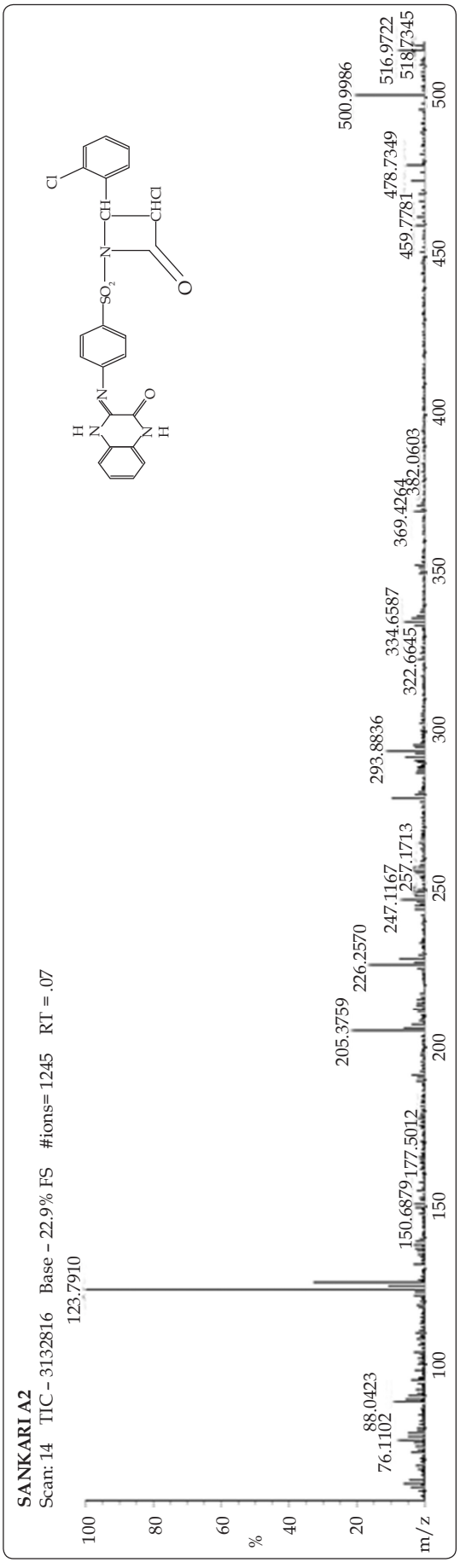


Fig. 9: Mass spectra of synthesized compound A2.

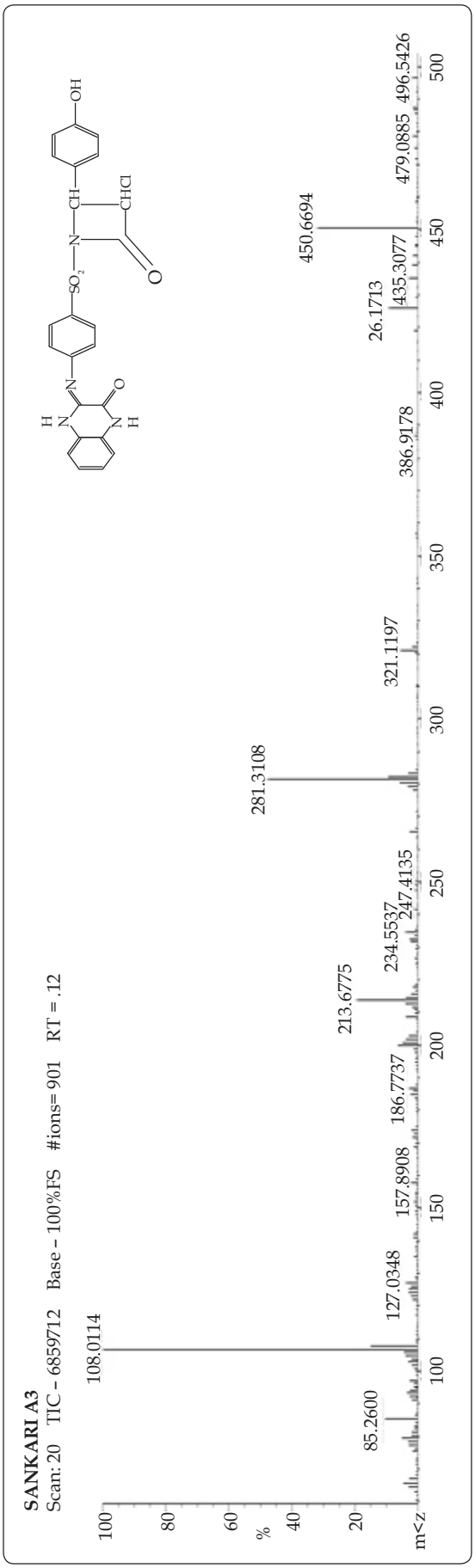


Fig. 10: Mass spectra of synthesized compound A3.

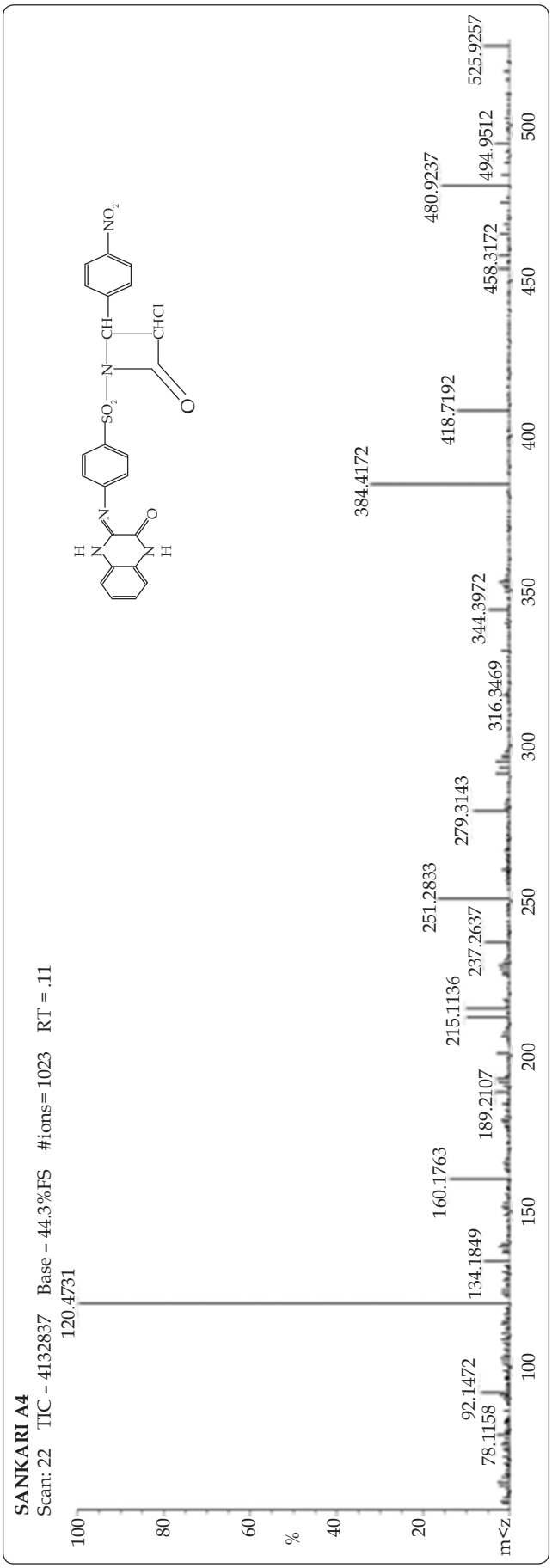


Fig. 11: Mass spectra of synthesized compound A4.

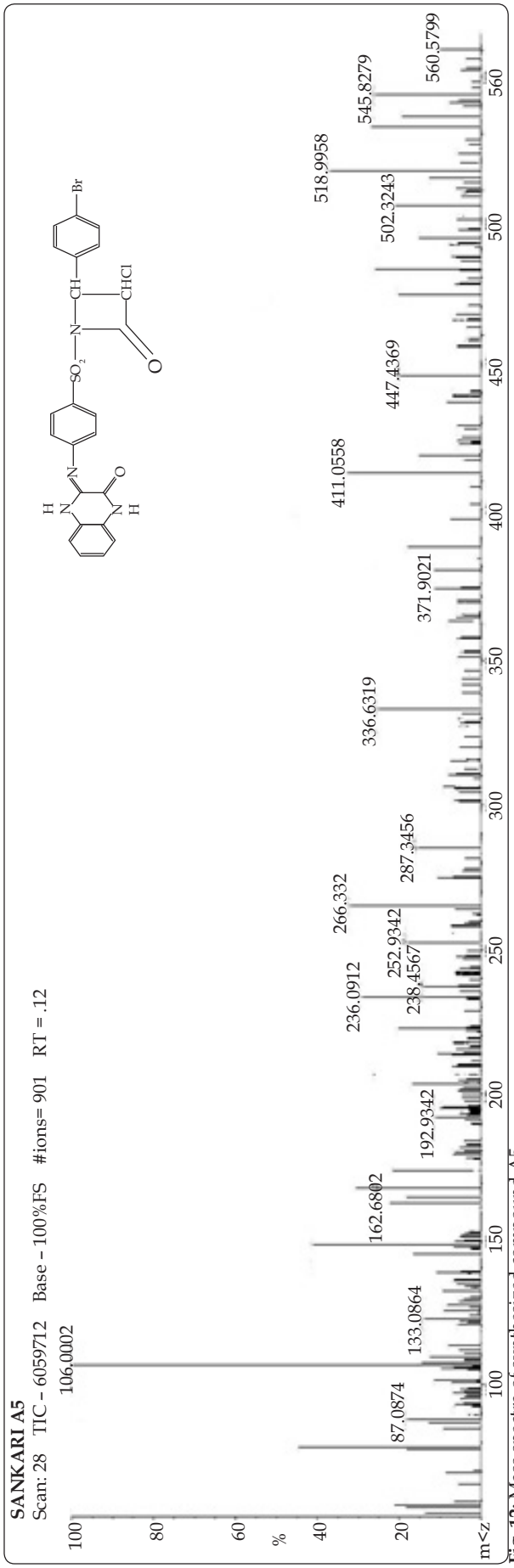


Fig. 12: Mass spectra of synthesized compound A5.

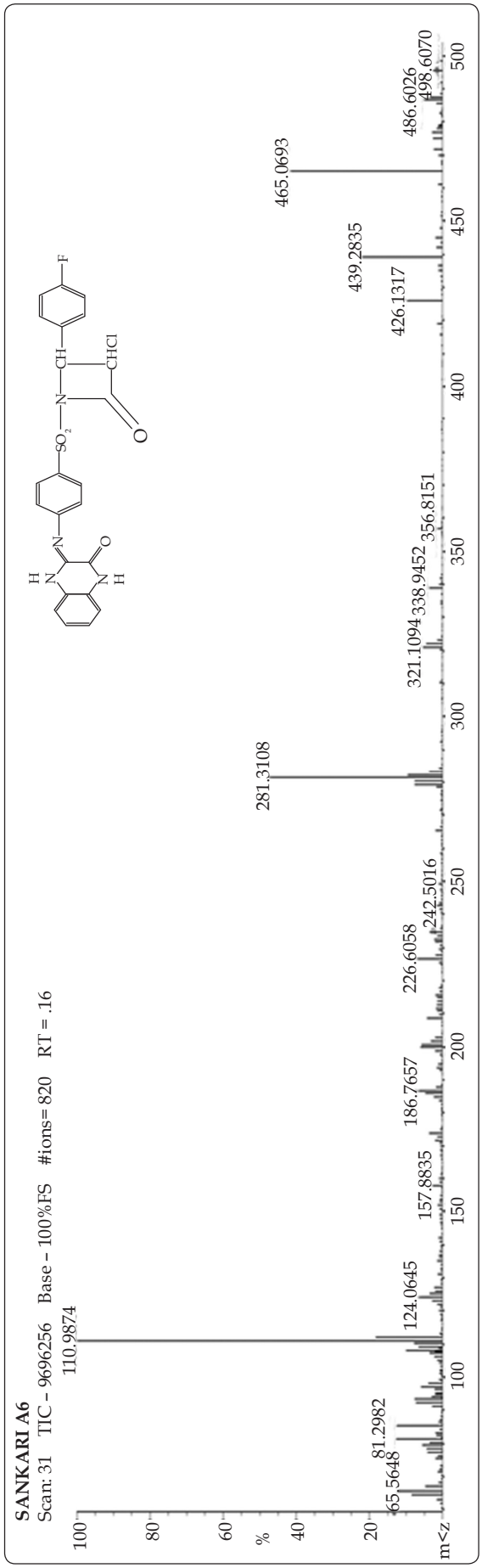


Fig. 13: Mass spectra of synthesized compound A6.

Table 2: Physical Properties of the Synthesized Compounds-(34)

Compounds	Molecular formula	Mol. Wight	M P (°C)	Yield (%)	Solvent system	R_f value
A_1	$C_{23}H_{16}C_{l2}N_4O_4S$	516	219-222	65.2	Benzene: Ethyl Acetate: Pet ether (8:4:1)	0.69
A_2	$C_{23}H_{16}C_{l2}N_4O_4S$	516	224-230	64.8		0.58
A_3	$C_{23}H_{17}ClN_4O_5S$	496	229-233	66.5		0.54
A_4	$C_{23}H_{16}ClN_5O_6S$	525	231-233	68.4		0.75
A_5	$C_{23}H_{16}BrClN_4O_4S$	560	232-239	67.9		0.77
A_6	$C_{23}H_{16}FClN_4O_4S$	498	240-245	72.2		0.62

The structure of all newly synthesized 4-thiazolidinone derivatives of Quinoxaline 2, 3dione were confirmed on the basis of analytical and spectral data.

Table-3: ^{1}H NMR of the Synthesized Compounds-(35).

Compounds	Ar Proton	NH	Ar-OH	Azetidinone	
				N-CH	CH-Cl
A1	6.7-8.2	4.1		5.0-5.5	8.7
	(12H)	(1H)	–	(1H)	(1H)
A2	6.7-8.2	4.1		5.0-5.5	8.8
	(12H)	(1H)	–	(1H)	(1H)
A3	6.6-8.2	4.0	5.1	5.2-5.3	8.2
	(12H)	(1H)		(1H)	(1H)
A4	6.6-8.2	4.0		5.1-5.4	9.0
	(12H)	(1H)	–	(1H).	(1H).
A5	6.3-7.9	4.1	–	5.4	8.7
	(12H)	(1H)		(1H)	(1H)
A6	6.7-8.1	4.1		5.0-5.4	8.6
	(12H)	(1H)	–	(1H)	(1H)

Table 5: Mass Spectral Data Of Synthesized Compounds-(36).

Compounds	Molecular formula	Molecular weight	m/e
A1	$C_{23}H_{16}Cl_2N_4O_4S$	516	516 (M+) (3%), 518 (M+2) (20%), 500 (22%), 293 (12%), 226 (17%), 205 (23%), 123 (100%), 88 (10%)
A2	$C_{23}H_{16}Cl_2N_4O_4S$	514	514 (M+) (3%), 516 (M+2) (20%), 500 (22%), 293 (12%), 226 (17%), 205 (23%), 123 (100%), 88 (10%)
A3	$C_{23}H_{17}ClN_4O_5S$	496	496 (M+) (3%), 450 (33%), 261 (10%), 321 (9%),
A4	$C_{23}H_{16}ClN_5O_6S$	525	525 (M+) (6%), 480 (16%), 384 (37%), 251 (17%), 215 (10%), 160 (15%), 281 (48%), 213 (20%), 108 (100%). 120 (100%), 78 (4%)
A5	$C_{23}H_{16}BrClN_4O_4S$	560	560 (M+) (12%), 545 (22%), 518 (37%), 266 (33%), 236 (30%), 148 (42%), 106 (100%).
A6	$C_{23}H_{16}FClN_4O_4S$	498	498 (M+) (1%), 486 (6%), 465 (42%), 439 (23%), 281 (47%), 226 (7%), 157 (3%), 110 (100%), 81 (13%)

Table 6: Zone of Inhibition of the Synthesized Compounds-(37).

Compounds	Zone of inhibition (in mm)											
	S.aureus		S.epidermis		B.cerus		K.pneumonia		P.aeroginosa		E .coli	
	100	200	100	200	100	200	100	200	100	200	100	200
A1	25	29	25	28	24	31	20	25	22	29	22	25
A2	20	21	18	25	20	28	18	21	20	23	18	20
A3	21	23	19	22	19	25	17	20	21	24	19	21
A4	19	25	18	24	20	26	18	23	20	26	20	24
A5	23	27	26	31	23	31	19	24	28	33	23	29
A6	21	26	24	32	23	29	19	23	24	32	24	26
Ciprofloxacin 100µg/ml	38		39		38		37		38		37	
Control	–		–		–		–		–		–	

Table 7: Zone of Inhibition of the synthesized compounds-(38).

Compounds	Zone of inhibition (in mm) Concentration(µg/ml)			
	A.niger		A.fumigatus	
	100	200	100	200
A1	22	29	21	24
A2	21	28	22	23
A3	24	31	24	21
A4	20	28	18	20
A5	28	33	25	31
A6	25	32	27	33
Ketoconazole (100µg/ml)	38		37	
Control	–	–	–	–

Table 8: Minimum inhibitory concentration of synthesized compounds and standard drug against gram positive and gram negative bacteria (µg/ML)-(39).

Sr. No.	Organism	A1	A2	A3	A4	A5	A6	Ciprofloxacin
1	S.aureus	1.4	2.4	2.3	2.7	1.4	1.6	0.5-2.5
2	S.epidermis	2.6	2.6	2.9	3.4	2.5	2.4	0.125-0.25
3	B.cerus	1.2	1.9	1.6	1.8	1.1	1.5	0.25-0.5
4	K.pneumonia	2.6	2.9	3.8	3.7	2.2	2.0	<0.06-0.125
5	P.aeroginosa	1.6	1.6	1.8	1.9	1.4	1.2	0.06-1.5
6	E.coli	1.3	1.7	1.6	1.7	1.4	1.5	<0.06

Table 9: Minimum inhibitory concentration of synthesized compound against fungi-(40-41).

Compounds	Minimum Inhibitory Concentration Concentration (µg/ML)	
	A.niger	A.fumigatus
A1	13.9	15.9
A2	15.3	14.9
A3	14.1	15.4
A4	14.9	15.6
A5	13.2	13.4
A6	13.6	13.2
Ketoconazole (100µg/ml)	10.9	11.3

Anti bacterial activity against Staphylococcus aureus

Compounds : A1-A6

Concentration : 100, 200µg/ml

Control : DMSO

Standard : Ciprofloxacin (100µg/ml)

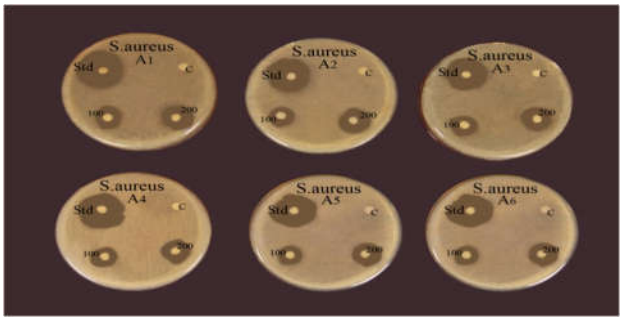


Fig. 14: Anti bacterial activity against Staphylococcus aureus

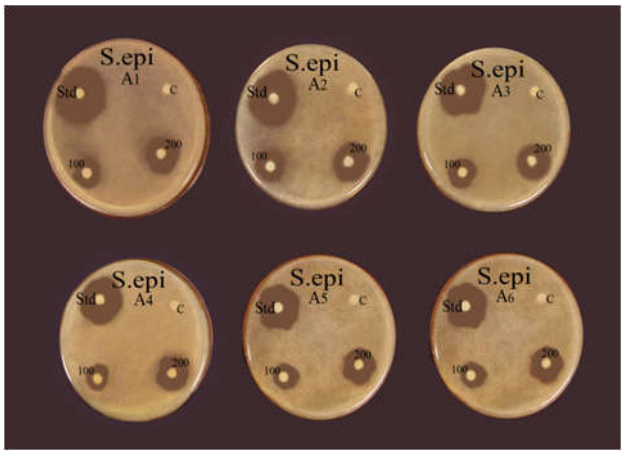


Fig. 15: Anti bacterial activity against Staphylococcus epidermidis.

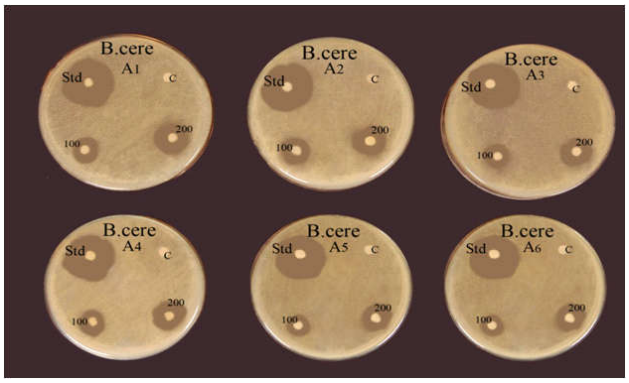


Fig. 16: Anti bacterial activity against Bacillus cereus.

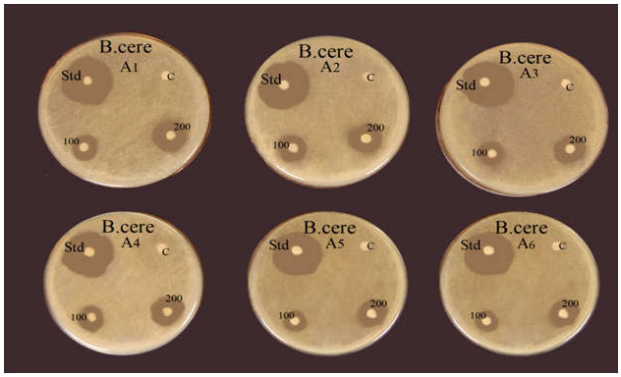


Fig. 17: Anti bacterial activity against Klebsiellapneumonia.

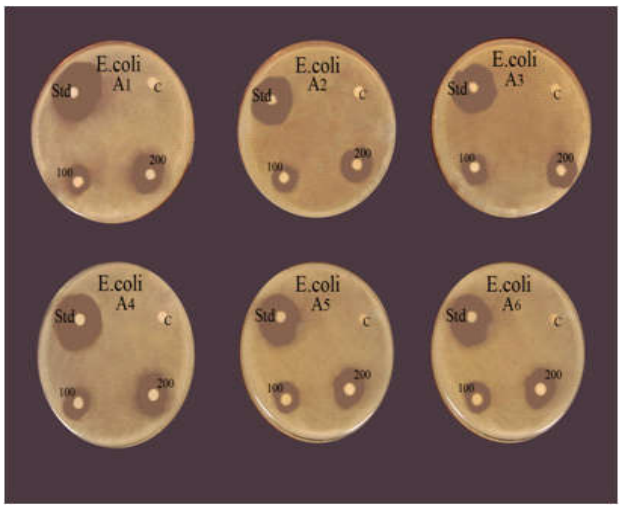


Fig. 18: Anti bacterial activity against Escherichia Coli.

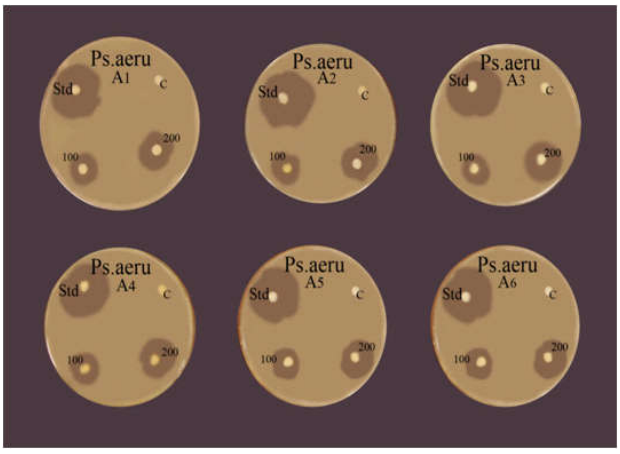


Fig. 19: Anti bacterial activity against Pseudomonas aeruginosa.

Anti fungal activity

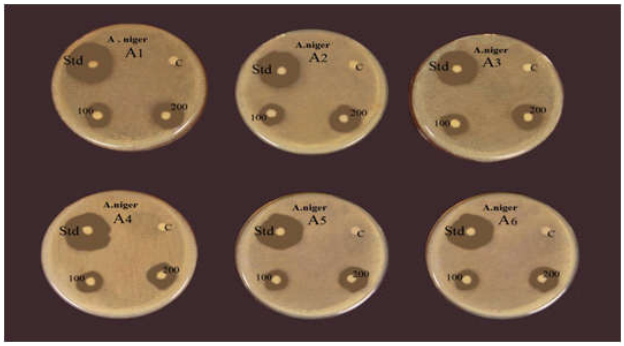


Fig. 20: Anti fungal activity against Aspergillusniger

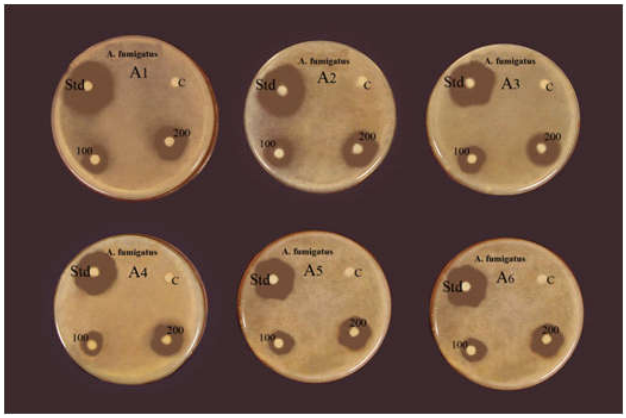


Fig. 21: Anti fungal activity against Aspergillusfumigatus.

Synthesized compounds were (50 and 100µg/ml-1) screened for invitro anti-microbial activity by paper disc diffusion method.

From the data shown in table- the observation were made as follows, Most of the synthesized compounds exhibited moderate to good anti-microbial activity against the tested microorganisms.

Antibacterial activity

Antibacterial activity of synthesized compounds was tested against both gram positive and gram negative bacteria and the standard drug used for the study was ciprofloxacin.

When compared to standard drug (Ciprofloxacin) compounds containing Chloro, Bromo, Fluro substitution in 4th of the Azetidinone (A1,A5,A6) ring system were found to exhibit good anti-bacterial activity. The result was shown in fig 14-19

The compounds with chloro (A1), hydroxyl (A3) and nitro (A4) substitution in the three derivatives showed better activity when compared to bromo (A5) and fluro (A6) substitution.

The MIC of the synthesized compounds were determined by agar streak method from the MIC data shown in table- the observation were made as follows

All the synthesized compounds were active against all the tested micro-organisms with the range of MIC values for S.aureus (2.7-1.2 µg/ml), S.epidermidis (3.9-2.3µg/ml), B.cereus (2.2-1.1µg/ml), K.pneumonia (3.9-2.1µg/ml), P.aeroginosa (2.6-1.2 µg/ml), E.coli (2.9-0.8 µg/ml).

Antifungal acticity

The antifungal activity of the synthesized compounds was tested against Aspergillus Niger and Aspergillus fumigates and the standard drug used for the study was Ketoconazole.

When compared to standard drug (Ketoconazole) compounds containing Chloro, Bromo, Fluro substitution in 4th of the compounds (A5, A6) ring system were found to exhibit good anti-fungal activity .The result was shown in fig 20-21

The chloro (A1), fluro (A6) and bromo (A5) substitution in three derivatives showed significant antifungal activity when compared to standard. The hydroxy (A3), nitro (A4) groups showed mild activity when compared to standard.

The MIC of the synthesized compounds were determined by agar streak method from the MIC data shown in table- the observation were made as follows

All the synthesized compounds were active against all the tested micro-organisms with the range of MIC values for A.niger(14.9-13.0µg/ml),A. fumigatus(15.9-12.8 µg/ml)

Conclusion

The literature survey shows that quinoxaline nucleus has lot of biological activity. In this paper we described the scheme of quinoxaline derivatives .those derivatives were analyzed for structural determination by IR and Mass study. The quinaxaline derivatives have antibacterial activity ,anti fungal activity , anti-tuberculosis activity, anticancer activity and antidepressant activity which are the most encouraging activity for pharmacists. Due their wide application in pharmaceutical field, these compounds have greet attention in connection with their synthesis. from the above data it was revealed that the synthesized derivatives has potent antibacterial and antifungal activity against gram positive , gram negative organism when compared with the standard drug ciprofloxacin and ketoconazole, so it can be

concluded that lot of quinoxaline derivatives will be developed in future for treatment of several disease with low toxicity and high safety.

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