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Review Article

SYNTHESIS, CHARACTERISATION & ANTIFUNGAL ACTIVITY OF QUINOXALINE 2, 3-DIONE DERIVATIVES

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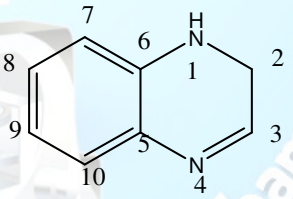
Abstract

A novel series of 15 new quinoxaline 2,3 dione derivatives containing a 4-thiazolidinone(T1-T5), 2-azetidinone(A1-A5),and Imidazolinones (I1-I5) nucleus. Imidazole is a planer five-membered heterocyclic ring system with three carbon and two nitrogen atoms in 1 and 3 positions. Oxo imidazoline, which is also known as imidazolinones is reported to exhibit a wide variety of therapeutic activities such as sedative, hypnotic, CNS depressant etc. The synthesis by reacting N'-substituted arylidene/heteroarylidene Quinoxaline 2,3 dione with thioglycollic acid in the presence of dry benzene, with chloroacetyl chloride in the presence of triethylamine and with 4-benzylidene-2-methyloxazole-5-one in the presence of pyridine respectively. All the title compounds were tested for their antibacterial 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. The compounds with chloro, hydroxyl and nitro substitution in the three derivatives showed better activity when compared to bromo and fluoro substitution. The synthesize compounds like thiazolidinones, azetidinones and imidazolinones derivatives of quinoxaline 2,3 dione from Schiff base as intermediate. The compounds T1-T5 are Thiazolidinones, compounds A1-A5 are azetidinones and compounds I1-I5 are imidazolinones. 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.

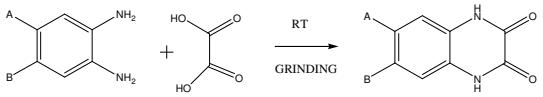
Keywords: Quinoxaline 2,3-Dione, Antimicrobial activity, Anti-fungal activity, Schiff bases reactions

Introduction

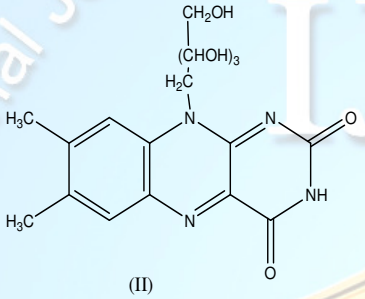
Quinoxaline, also known as Benzopyrazine (or) 1, 4-diazanapthalene. It is a heterocyclic compound containing a ring complex composed with a Benzene ring and pyrazine ring. These are isomeric with quinazolines [1]. It is used in the preparation of dyes, pharmaceuticals and antibiotics such as Echinomycin, Neomycin and Actinoleucin.



Quinoxaline analogs containing pyrazole moiety are reported to possess a wide spectrum of biological activities such as antimicrobial, anti-inflammatory and antitumor activities[3, 4]. By the spontaneous condensation of o-phenylenediamines with 1, 2-dicarbonyl compounds it gives the products Quinoxalines or benzopyrazines[2].

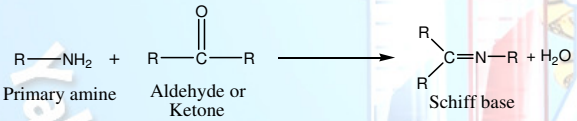


Since the preparations of quinoxalines are also carried out, when α-haloketones condense with o-phenylenediamine, the structure of these cyclic bases are obvious from the mode of formation and analytical data [3]. In nature the simple quinoxalines have not been found. The member of a more complex series of quinoxaline derivative is Riboflavin or vitamin B_2 (II), which are based on the pyrimido quinoxaline ring system.

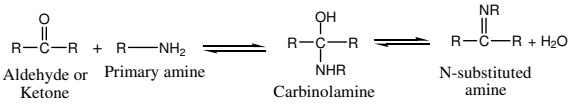


(II)

SCHIFF BASES: Schiff bases are also called as aldimines or azomethines. A Schiff base [19] consists of nitrogen analog of an aldehydes or ketone. Where C=O group is replaced by a C=N-R group [4]. The condensation of an aldehydes or ketone with primary amine normally gives Schiff base.

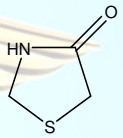


Here R may be represented as alkyl group. Schiff bases having aryl substituents are more stable and readily synthesized [20]. While those having alkyl substituent's are relatively unstable. Commonly, at high temperature and longer reaction times. Ketones react more slowly than aldehydes, Schiff bases of aromatic aldehydes have effective conjugation and are more stable and those of aliphatic aldehydes are relatively unstable. Polyhalogenated aldehydes, ketones to give stable imines. The formation of a Schiff base reaction is reversible [5] and it occurs under acid or base catalysis upon heating. The initial products would be expected to be hemiaminals and or amines. Based on pattern followed by analogous nucleophiles.



The formation is generally driven to completion by separation of the product or removal of water or both. The mechanism of Schiff base formation is a variation on the theme of nucleophilic addition to the carbonyl group. In this reaction the nucleophile is the amine. In the first part of the mechanism the amine reacts with the aldehydes or ketone to give an unstable addition compound called carbimolamine [5]. The carbionolamine (which is an alcohol) loses water by either acid or base catalyzed pathways.

Thiazolidinone: The derivatives of thiazolidine is 4-thiazolidinones [21]. In 4th position carbonyl group, substitution in the 2-,3-and 5 position may be varied .But the greatest difference in structure and properties is exerted by the group, when the carbon atom attached in the 2-position.



4-Thiazolidinones

Biological Activity of 4-thiazolidinones: 4-Thiazolidinones exhibit numerous activities. Such as Antimicrobial, Anticancer, Anti-tubercular, Anticonvulsant, Hypnotic, Respiratory, Anti-inflammatory, Antiproteolytic, Antihemolytic, Anthelmintic, Cardiovascular, Antiviral, Insecticidal and Herbicidal activity [6].

Azetidinones: Azetidine-2-one,which having acyclic lactam (β-lactam) skeleton was recognized as a useful building block for the synthesis of a large number of organic molecules by exploiting strain energy associated with it .Staudinger [7], before 1912, initiated work the chemistry of the azetidin-2-ones or β-lactams. Interest in these compounds was largely lost until 1943, when it was suggested that the penicillins might contain azetidinone rings. Since then a great deal of work has been done on these compounds, including Sheehan's practical synthesis of penicillin V. β-lactam antibiotics are used to treat a wide range of bacterial infections in both community and hospital environments.

Biological Activity of Azetidinones: The β-lactam drugs are still the most prescribed antibiotics used in medicine [19]. The azetidinones were tested as Antitubercular, Antimicrobial, Anesthetic, Anticonvulsant, Antidepressants, Anti-fungal activity, Anti-inflammatory, Antiviral, Hypolipidemic activity and also associated with Sedatives and Hypnotics. Recently 2-azetidinones have been assessed for Anti Parkinsonism, Herbicidal and Cholesterol Absorption inhibitors as they also function as an enzyme inhibitor and are effective on the central nervous system.

Materials and Methods: The synthesized Quinoxalne 2, 3-dione derivatives were characterized by the following experimental methods. Melting points of the synthesized compounds were taken in open capillary tubes and uncorrected. IR spectra were recorded on ABB BOMEM FTIR Spectrometer using potassium bromide pellets. The ^1H NMR and spectra of the synthesized compounds were recorded on a JOEL GSX 400 NMR spectrometer in DMSO. The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF_{254} 200 mesh) aluminum plates (E-merk) using benzene: ethyl acetate: pet. Ether as eluent and visualized in UV-chamber.

SYNTHETIC METHODS

Step-I Preparation of quinoxaline 2, 3 dione: This solvent-free method has an operationally simple procedure. In a typical experiment, a mixture of oxalic acid (1 mmol, 0.126 g) and *o*-Phenylene diamine (1 mmol, 0.108 g) was thoroughly ground with a pestle in a mortar at room temperature in an open atmosphere until the mixture turned into a melt. Then the melt was crystallized from water to get the pure product [8].

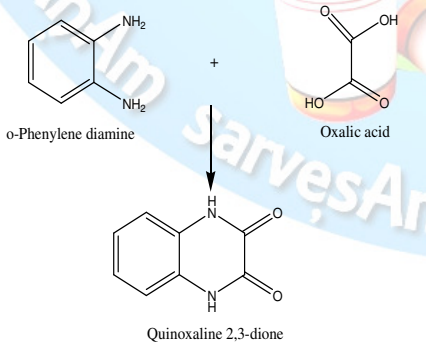


Figure No: 03 Quinoxaline 2, 3 dione

STEP-II: Preparation of (e)-4-(3-oxo-3,4-dihydroquinoxalin-2(1h)- ylideneamino) benzenesulphonamide.

3.42gm of Quinoxaline 2, 3 Dione and 3gm of sulphanilamide were taken in a RBF. To it 10ml of DMF and few drops of glacial acetic acid were added and the reaction mixture was refluxed for 3–5 h. The reaction mixture was poured on crushed ice or ice-cold water. The separated product was filtered out, washed and recrystallised from ethanol [9].

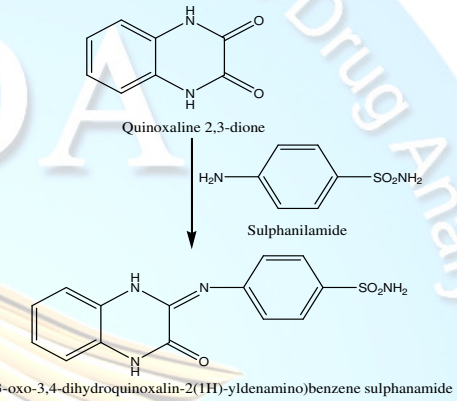


Figure No: 04 (e)-4-(3-oxo-3,4-dihydroquinoxalin-2(1h)- ylideneamino) benzenesulphonamide

STEP-III: Preparation of (e)-n-(4-substitutedbenzylidene)-4-(3-oxo- 3, 4-dihydroquinoxaline-2(1h)-ylidenemino) benzene sulfonamide: (S1-S6) A mixture of Schiff base of sulphanilamide (0.01moles) and five different aromatic aldehydes (0.01 moles) were taken in separate RBF and refluxed in ethanol for three hours. The reaction mixture was then poured in to crushed ice and the separated solid wash filtered and re-crystallizes using absolute alcohol [10].

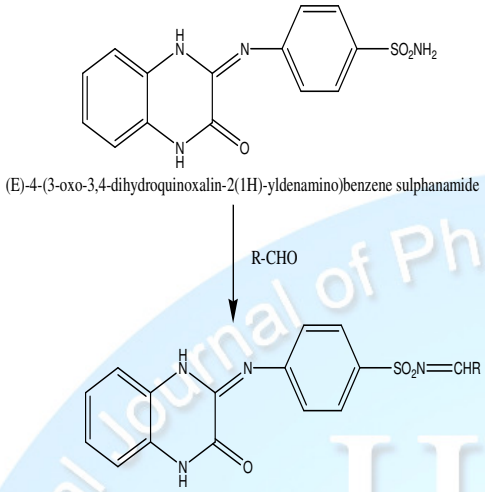


Figure No: 05 (E)-N-(H-Substitutedbenzylidene)-4-(3-oxo-3,4-dihydroquinoxaline-2(1H)-ylidenemino) benzene sulfonamide

Step-IV: Synthesis of 4-thiazolidinones (t_1-t_6): The above synthesized different Schiff bases (0.01 moles), S_1-S_6 and thioglycollic acid (0.01moles) in 25ml dioxin were taken in separate round bottom flasks. To these mixtures a pinch of anhydrous zinc chloride was added and refluxed for 8 hours. The reaction mixtures were cooled, poured into crushed ice, filtered and recrystallised using absolute alcohol[11].

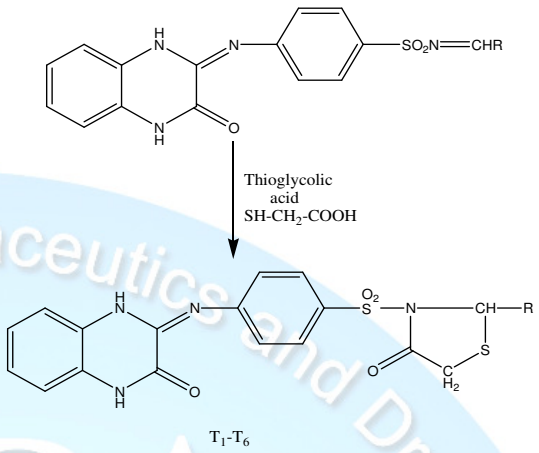


Figure No: 06 4-thiazolidinones

SYNTHESIS OF 2-AZETIDINONES (A_1-A_6): 0.01 moles of Schiff bases (S_1-S_6) were taken separately and (0.01 moles) of triethylamine in 25 ml dry dioxan was added to them to form a solution. To these solutions chloroacetate chloride was added drop wise with continuous stirring in cold for 30 minutes and refluxed for 3 hours. The contents were then poured into crushed ice, filtered, and recrystallised using absolute alcohol [18].

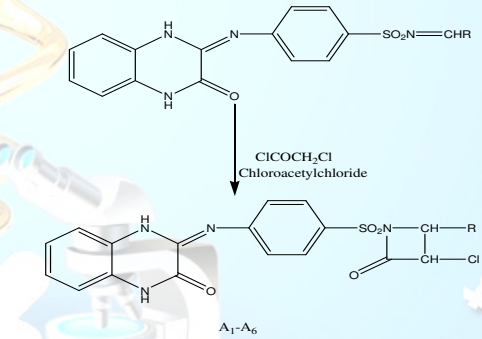


Figure No: 07 OF 2-AZETIDINONES

Table no: 01 Infra red spectral data of synthesized compounds

COMPOUNDS	Ar- CH Stretch	CH(S)	C=O	C=N	C-S	C-N	CH(b)
T1	3056	2863	1712 1598	1518	630	1320	1406
T2	3056	2863	1712 1598	1518	630	1320	1406
T3	3068	2857	1732 1615	1513	668	1317	1408
T4	3070	2855	1748 1653	1515	629	1347	1410
T5	3078	2938	1755 1650	1511	641	1321	1407
T6	3069	2865	1762 1599	1508	691	1338	1415
A1	3057	2865	1759 1598	1519	-	1324	1403
A2	3057	2865	1759 1598	1519	-	1324	1403
A3	3067	2853	1746 1621	1516	-	1319	1406
A4	3087	2840	1732 1712	1518	-	1349	1413
A5	3086	2874	1711 1656	1510	-	1326	1408
A6	3067	2865	1764 1602	1519	-	1317	1415

Table no: 02 Physical properties of the synthesized compounds

Compounds	Molecular formula	Mol. Wight	M.P. (ºC)	Yield (%)	Solvent system	R_f value
T_1	$C_{23}H_{17}ClN_4O_4S_2$	512	250-255	**60.78**	Benzene: Ethyl Acetate: Pet ether (8:4:1)	0.82
T_2	$C_{23}H_{17}ClN_4O_4S_2$	512	248-254	**57.63**		0.58
T_3	$C_{23}H_{18}N_4O_5S_2$	494	230-236	**71.8**		0.68
T_4	$C_{23}H_{17}N_5O_6S_2$	523	237-240	**63.7**		0.75
T_5	$C_{23}H_{17}BrN_4O_4S_2$	558	260-264	**65.7**		0.81
T_6	$C_{23}H_{17}FN_4O_4S_2$	496	230-233	**70.4**		0.75
A_1	$C_{23}H_{16}Cl_2N_4O_4S$	516	219-222	**65.2**		0.69
A_2	$C_{23}H_{16}Cl_2N_4O_4S$	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

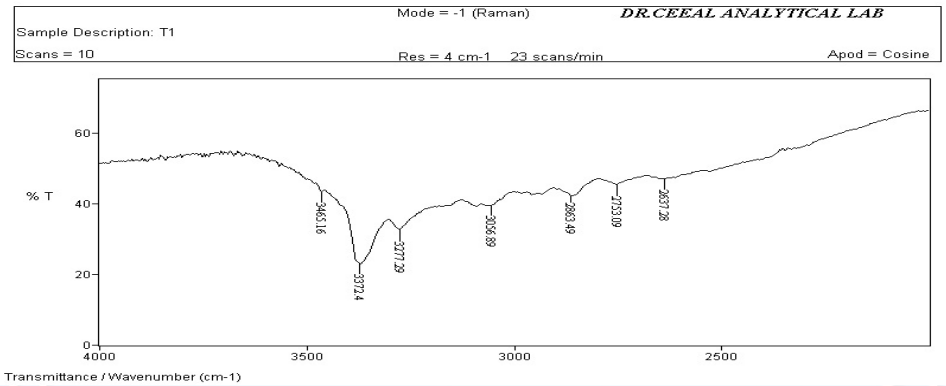


Figure No: 08 FTIR Sample T1 (E)-3-(4-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3,4-dihydroquinoxqline-2(1H)-one

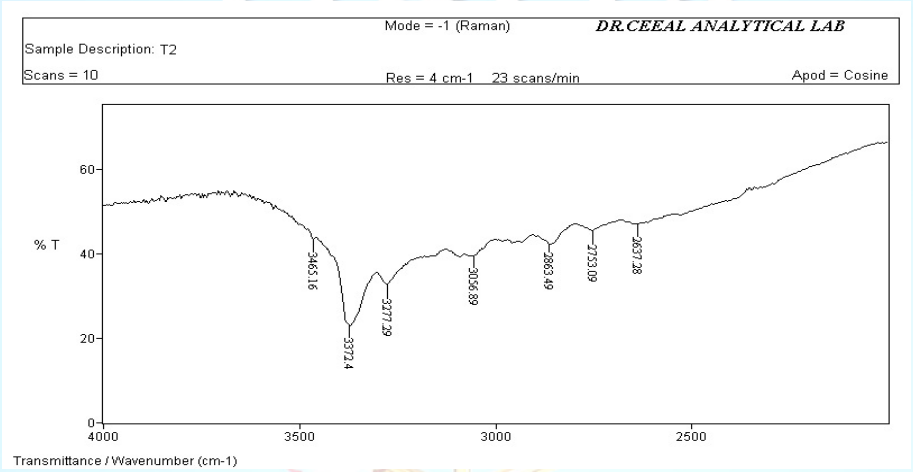


Figure No: 09 FTIR Sample T2 (E)-3-(4-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3,4-dihydroquinoxqline-2(1H)-one.

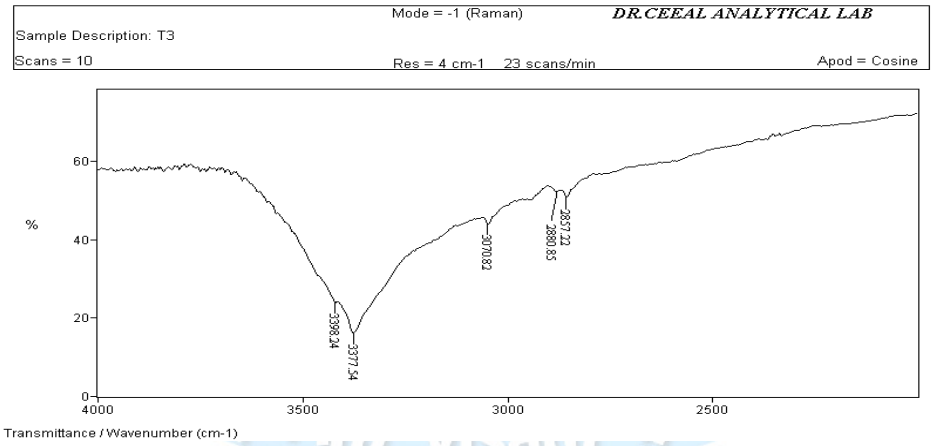


Figure No: 10 FTIR Sample T3 (E) – 3 - (4 - (2 - (4 – hydroxy phenyl) – 4 – oxothiazolidin – 3 –Sulfonyl) phenylimino)-3,4-dihydroquinoxqline-2(1H)-one.

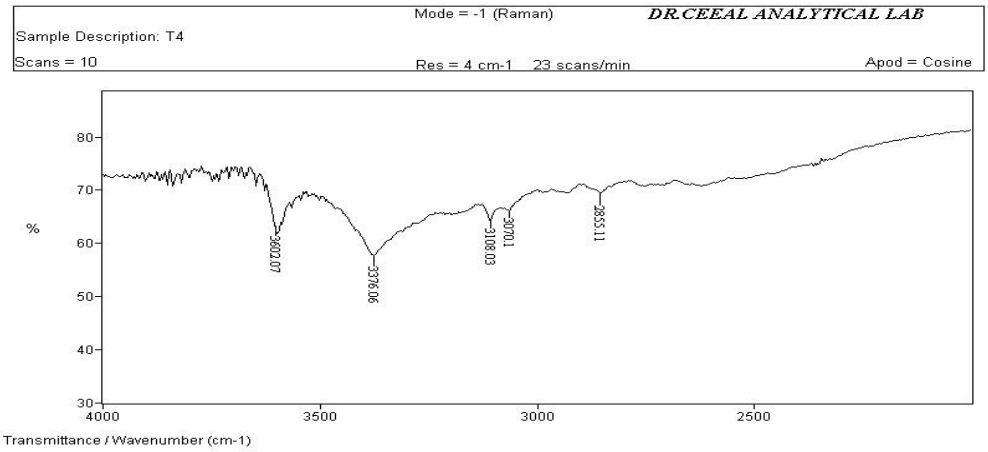


Figure No: 11 FTIR Sample T4 (E)-3-(4-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxqline-2(1H)-one

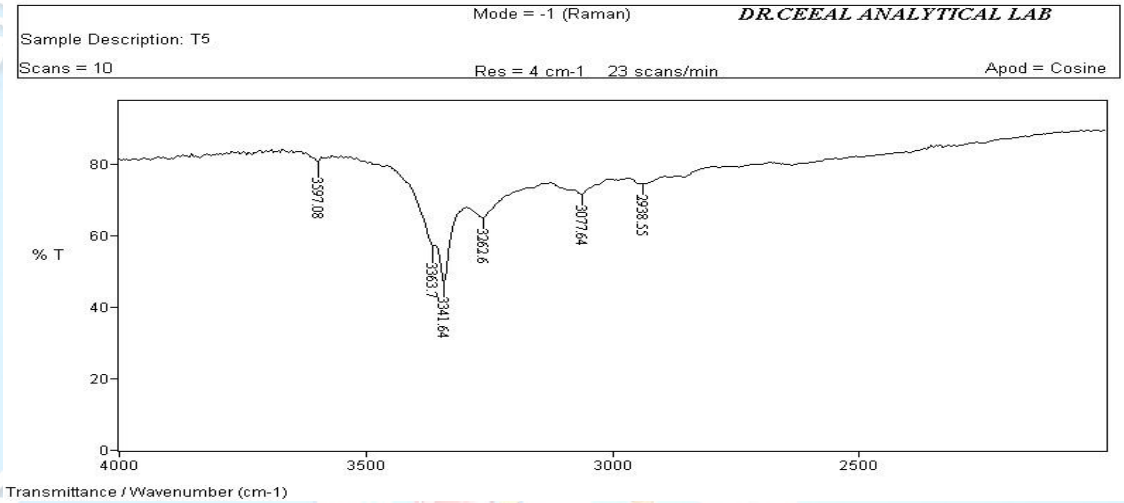


Figure No: 12 FTIR Sample T5 (E)-3-(4-(2-(4-bromophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxqline-2(1H)-one

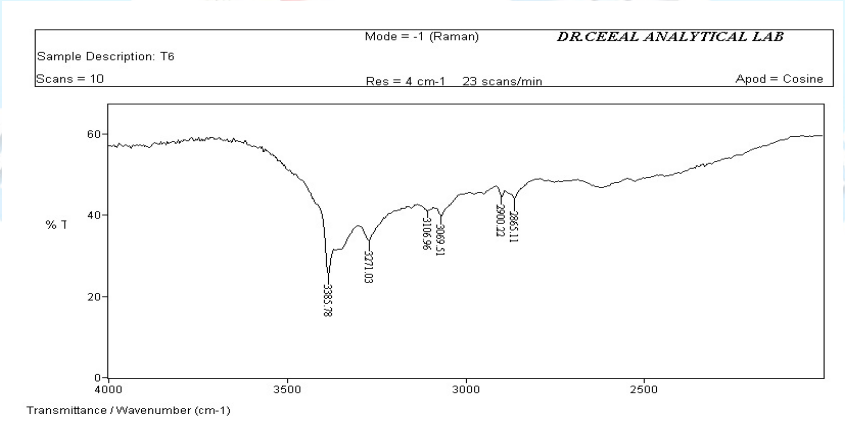


Figure No: 13 FTIR Sample T6 (E)-3-(4-(2-(4-flurophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3,4-dihydroquinoxqline-2(1H)-one

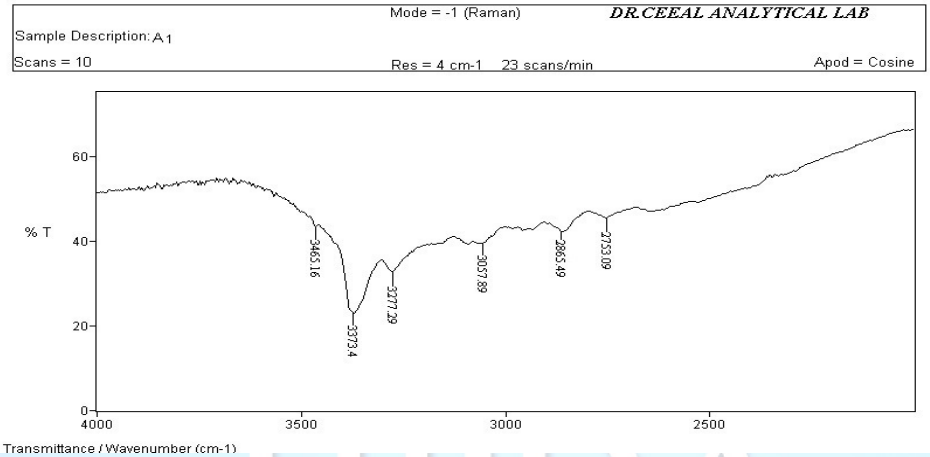


Figure No: 14 FTIR Sample A1 (E)-3-(4-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

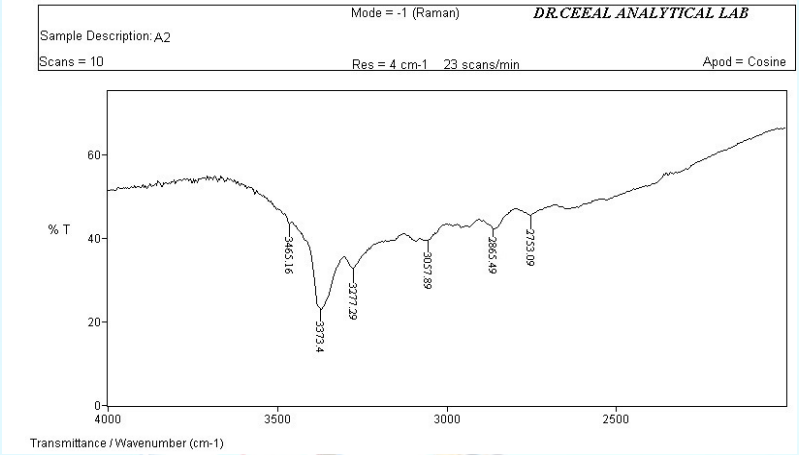


Figure No: 15 FTIR Sample A2 (E)-3-(4-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

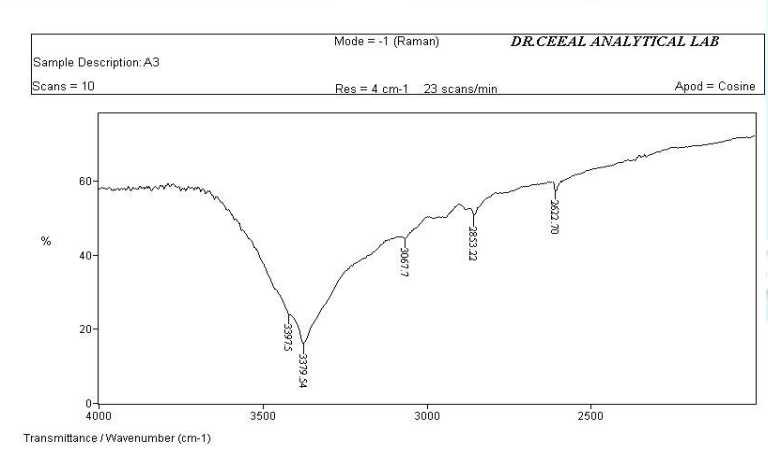


Figure No: 16 FTIR Sample A3 (E)-3-(4-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

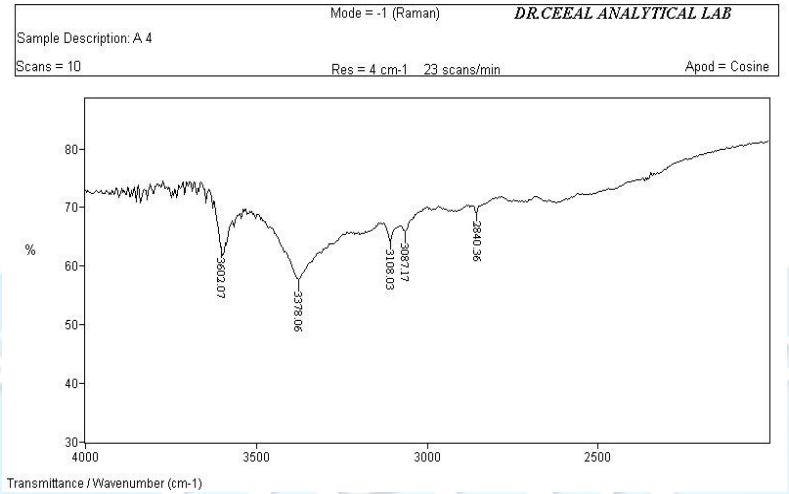


Figure No: 17 FTIR Sample A4 (E)-3-(4-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

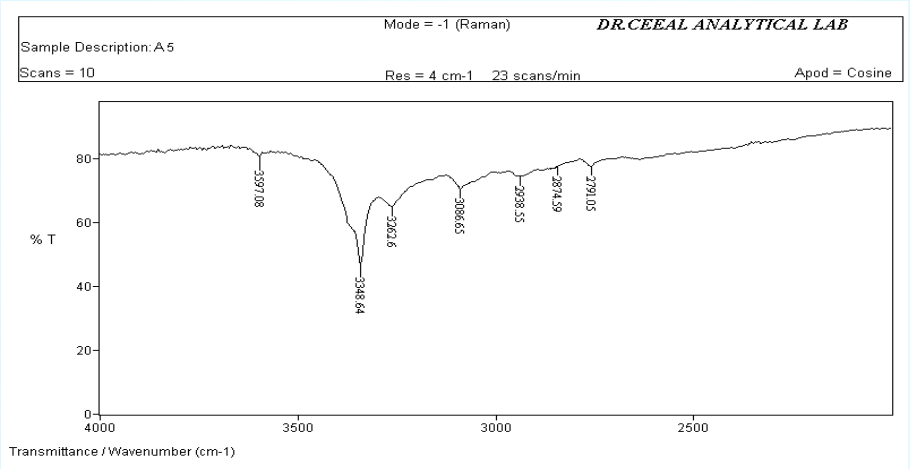


Figure No: 18 FTIR Sample A5 (E)-3-(4-(3-chloro-2-(4-bromophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

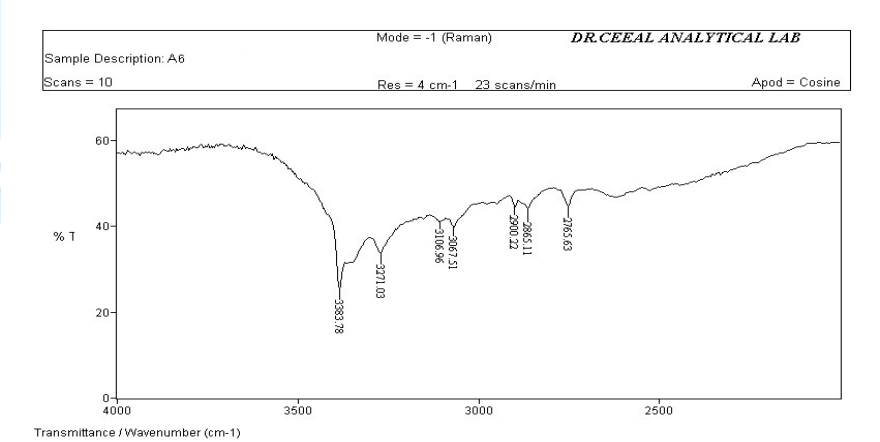


Figure No: 19 FTIR Sample A6 (E)-3-(4-(3-chloro-2-(4-flurophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-o

Table no: 3 ^{1}H NMR of the synthesized compounds

COMPOUNDS	Ar Pro-ton	NH	THIAZOLIDINONE		Ar-OH	AZETIDINONE	
			CH-S	CH_2-S		N-CH	CH-Cl
T1	6.5-8.2 (12 H)	3.8 (1H)	4.4 (1H)	4.2 (2H)	-	-	-
T2	6.4-7.9 (12 H)	3.9 (1H)	4.4 (1H)	4.7 (2H)	-	-	-
T3	6.3-7.9 (12)	4.0 (1H)	4.3 (1H)	4.7 (1H)	5.1 (1H)	-	-
T4	6.4-8.0 (12 H)	4.0 (1H)	4.3 (1H)	4.1 (2H)	-	-	-
T5	6.5-8.1 (1H)	3.8 (1H)	4.4 (1H)	4.3 (1H)	-	-	-
T6	6.6-8.3 (12 H)	4.1 (1H)	4.9 (1H)	5.0 (2H)	-	-	-
A1	6.7-8.2 (12H)	4.1 (1H)	-	-	-	5.0-5.5 (1H)	8.7 (1H
A2	6.7-8.2 (12H)	4.1 (1H)	-	-	-	5.0-5.5 (1H)	8.8 (1H)
A3	6.6-8.2 (12H)	4.0 (1H)	-	-	5.1 (1H)	5.2-5.3 (1H)	8.2 (1H)
A4	6.6-8.2 (12H)	4.0 (1H)	-	-	-	5.1-5.4 (1H).	9.0 (1H)
A5	6.3-7.9 (12H)	4.1 (1H)	-	-	-	5.4 (1H)	8.7 (1H)
A6	6.7-8.1 (12H)	4.1 (1H)	-	-	-	5.0-5.4 (1H)	8.6 (1H)

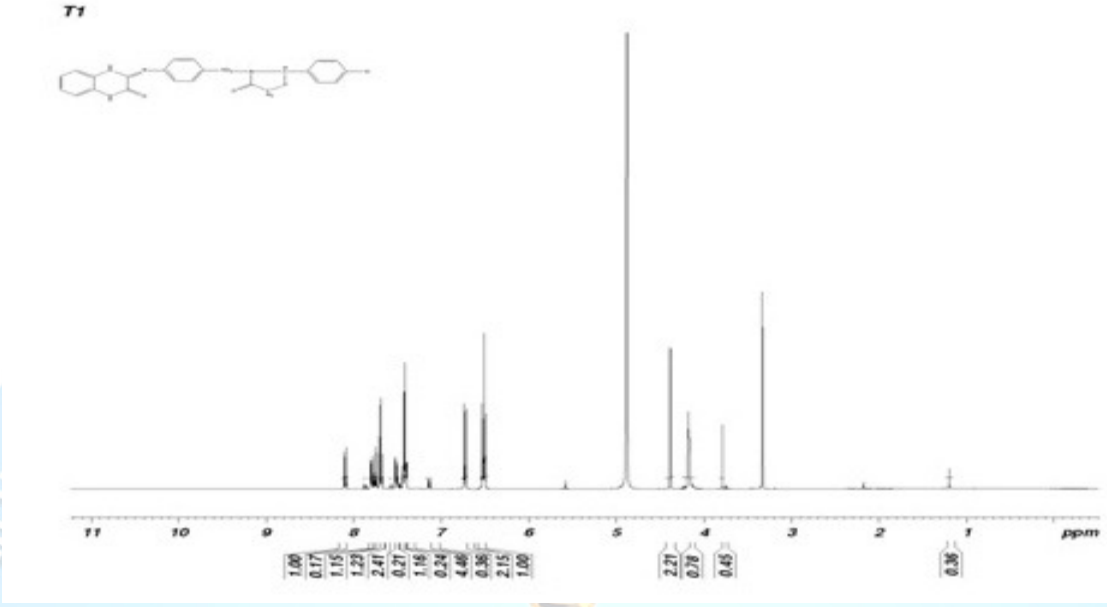


Figure No: 21 ^{1}HNMR of Sample T1: (E)-3-(4-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3,4-dihydroquinoxqline-2(1H)-one

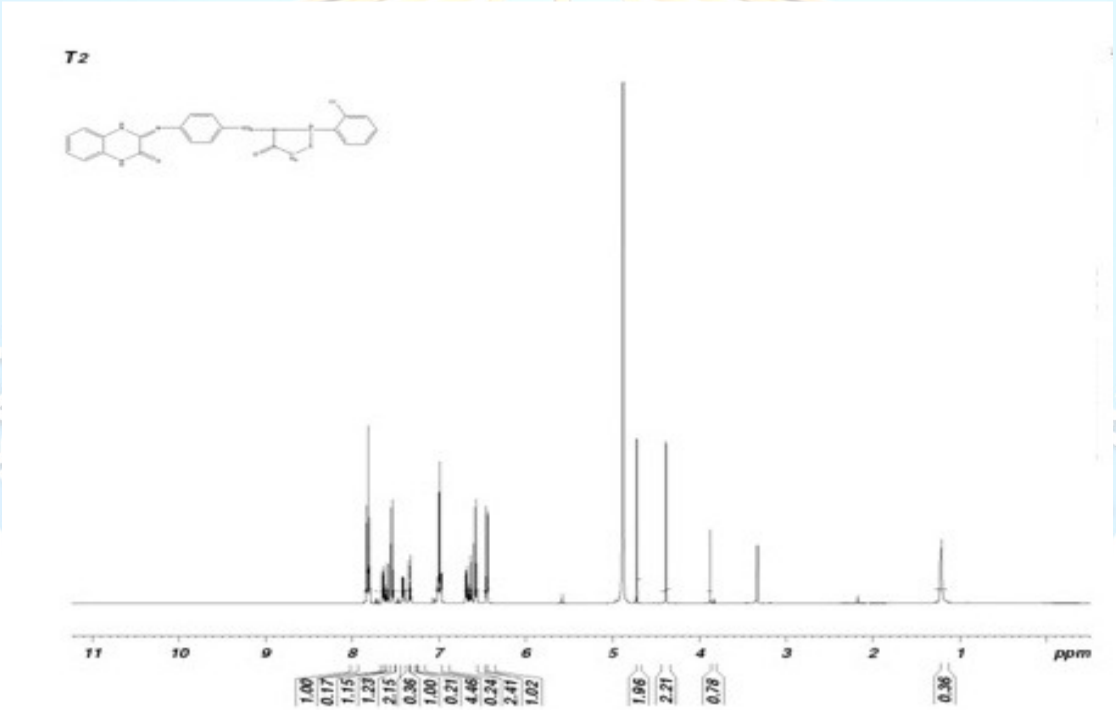


Figure No: 22 ^{1}HNMR of Sample T2: (E)-3-(4-(2-(2-chlorophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3,4-dihydroquinoxqline-2(1H)-one

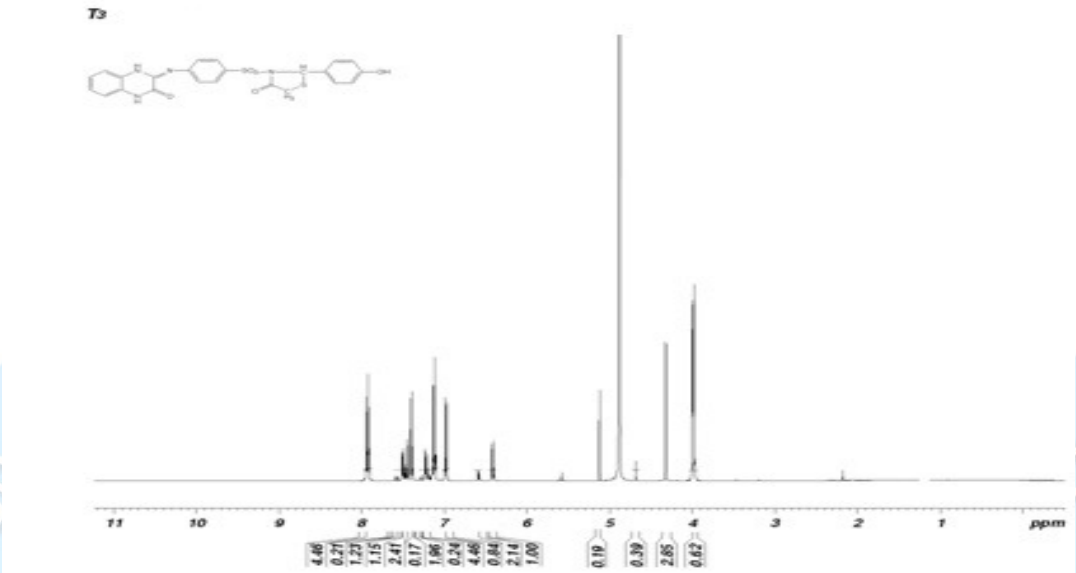


Figure No: 23 [1]HNMR of Sample T3: (E)-3-(4-(2-(4-hydroxyphenyl)-4-oxothiazolidin-3-ylSulfonyl)phenylimino)-3,4-dihydroquinoxqline-2(1H)-one

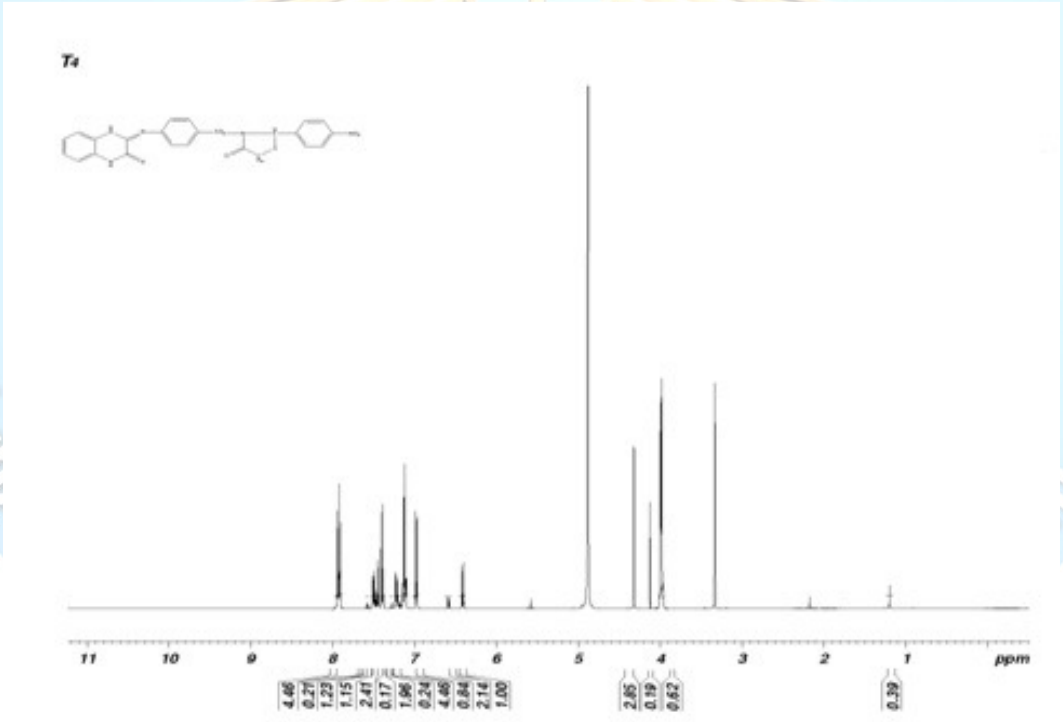


Figure No: 24 [1]HNMR of Sample T4: (E)-3-(4-(2-(4-nitrophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxqline-2(1H)-one

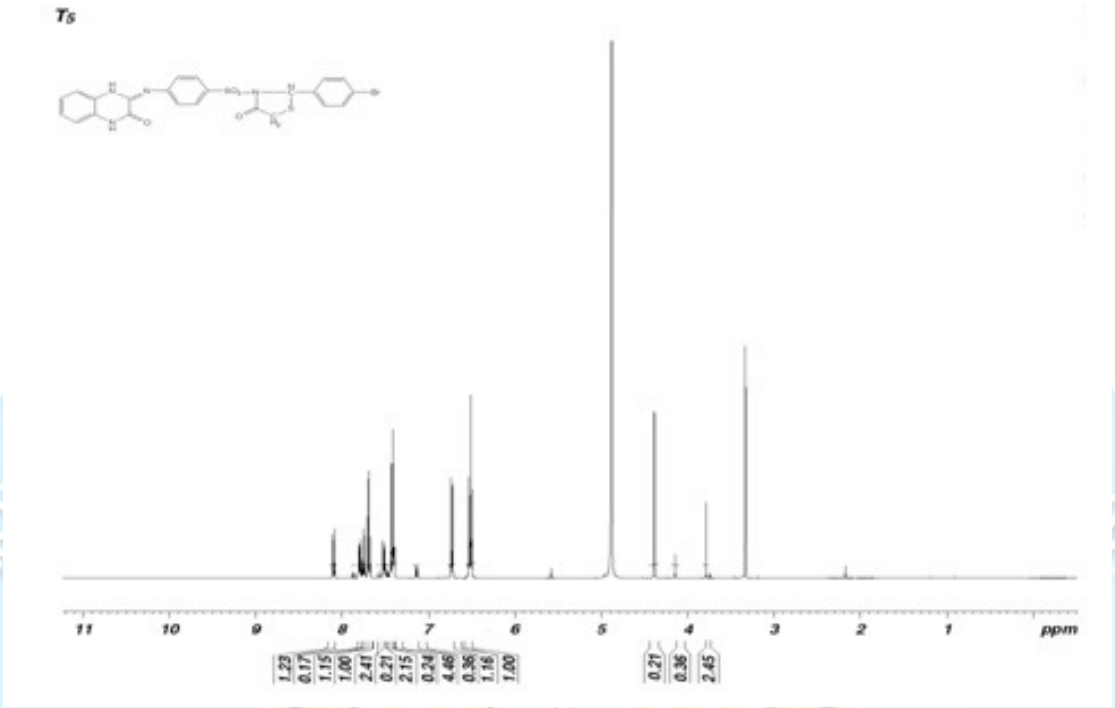


Figure No: 25 [1]HNMR of Sample T5: (E)-3-(4-(2-(4-bromophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxqline-2(1H)-one

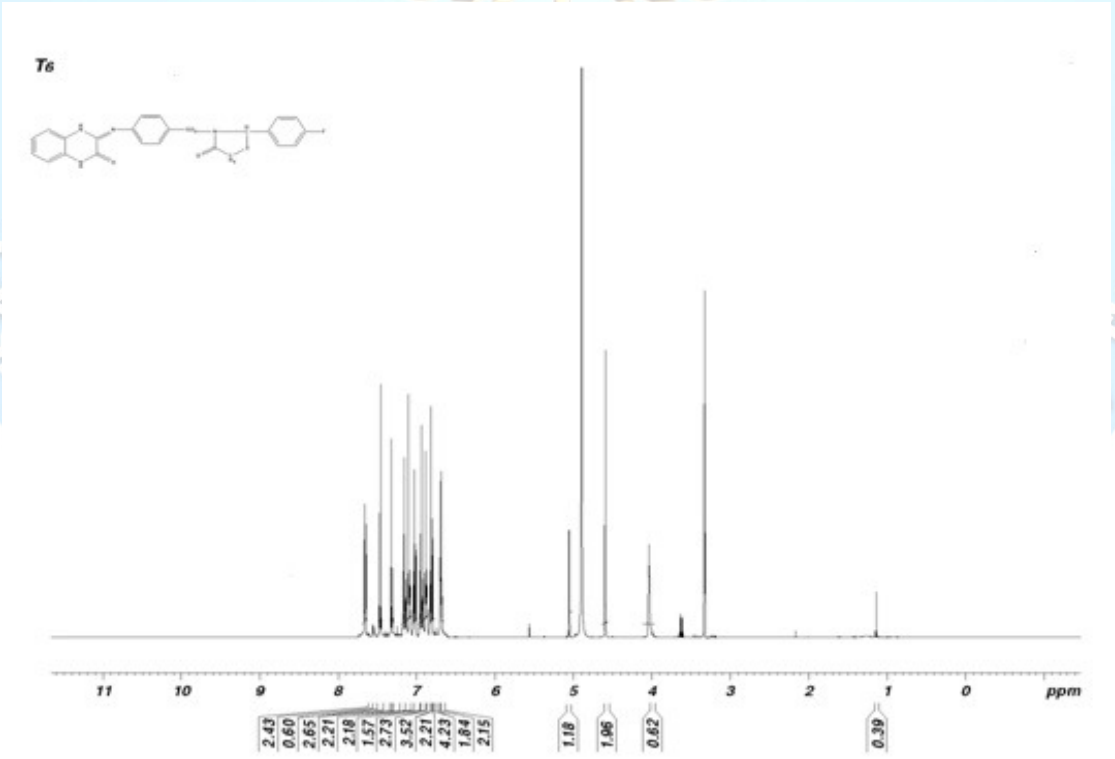


Figure No: 26 [1]HNMR of Sample T6: (E)-3-(4-(2-(4-flurophenyl)-4-oxothiazolidin-3-yl Sulfonyl) phenylimino)-3,4-dihydroquinoxqline-2(1H)-one

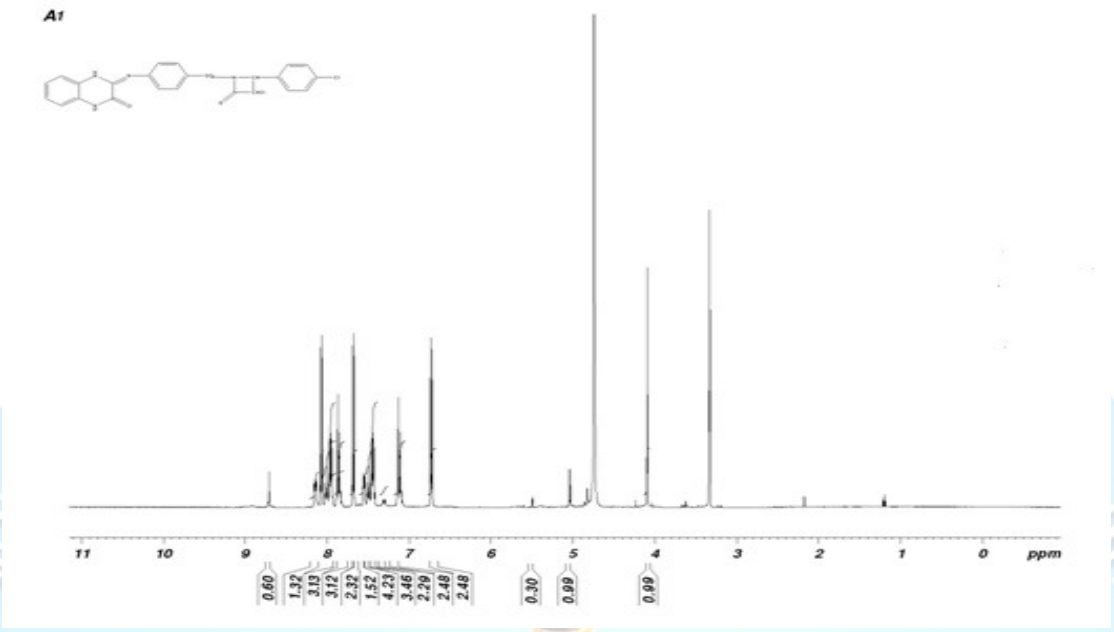


Figure No: 27 ^{1}HNMR of Sample A1: (E)-3-(4-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

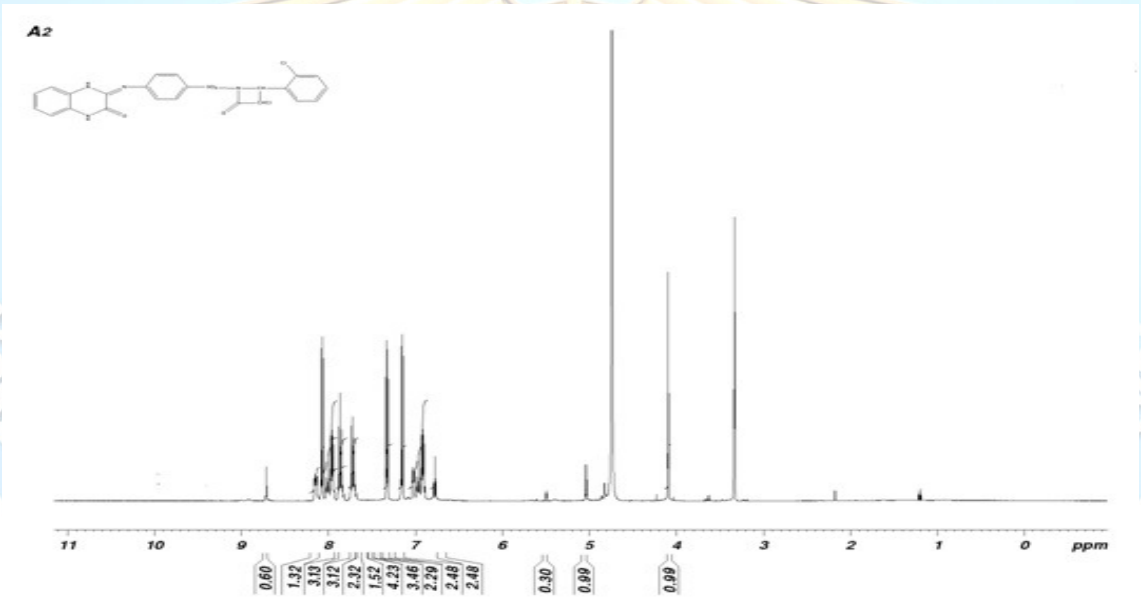


Figure No: 28 ^{1}HNMR of Sample A2: (E)-3-(4-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

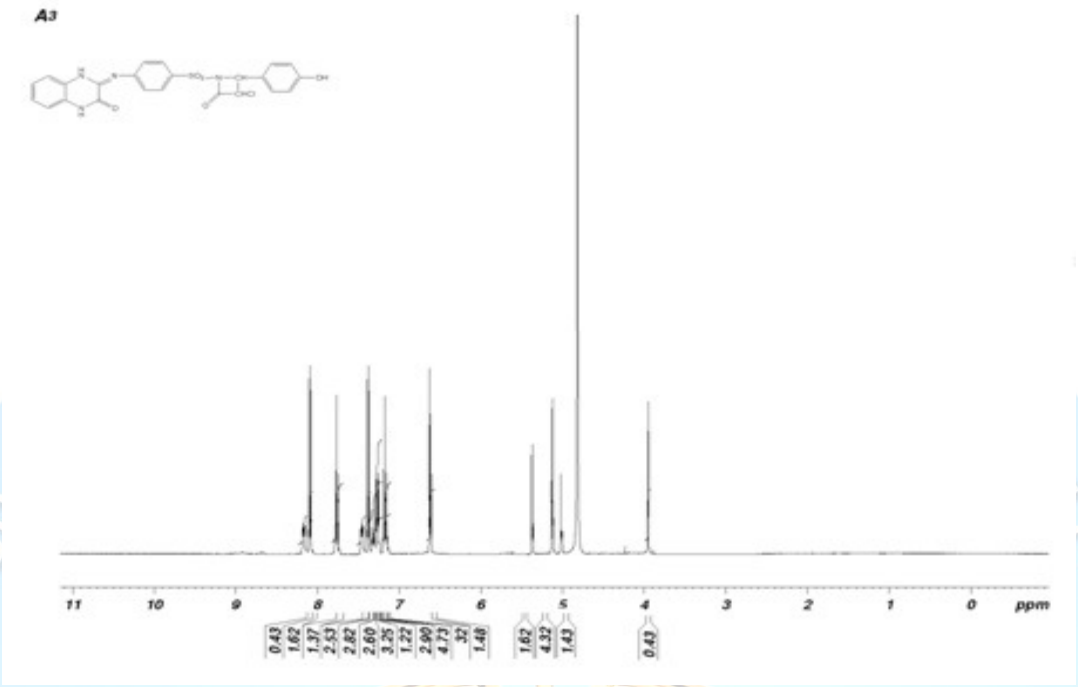


Figure No: 29 [1]HNMR of Sample A3: (E)-3-(4-(3-chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

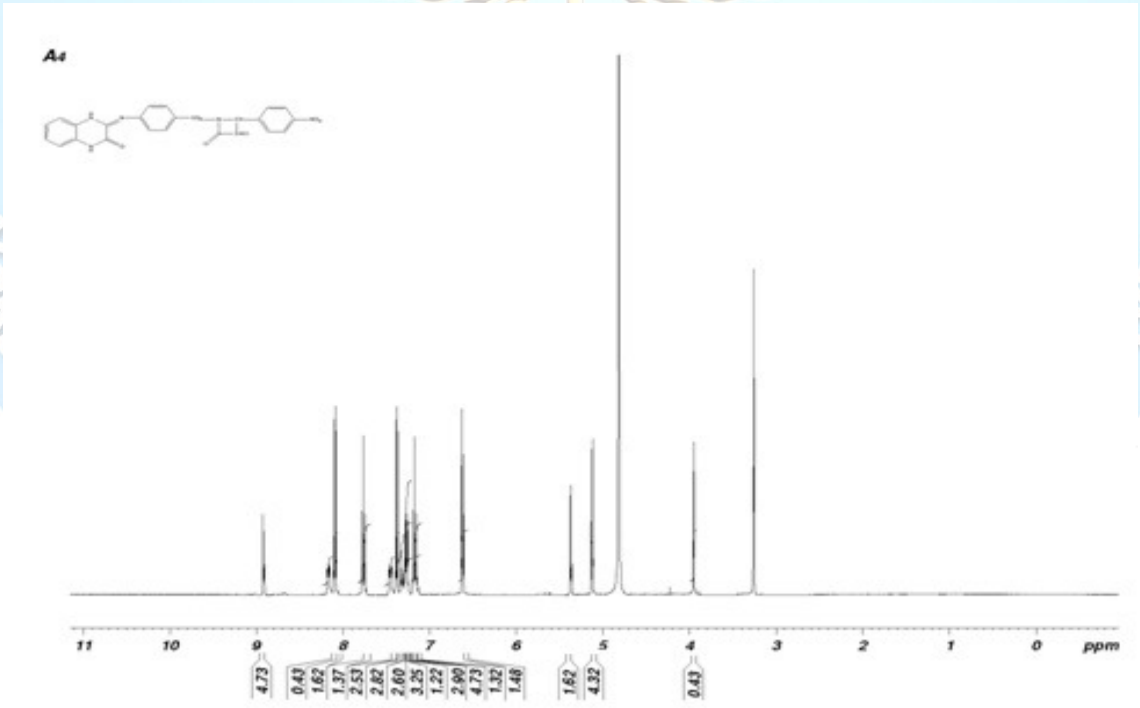


Figure No: 30 [1]HNMR of Sample A4: (E)-3-(4-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

Figure No: 31 ^{1}HNMR of Sample A5: (E)-3-(4-(3-chloro-2-(4-bromophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

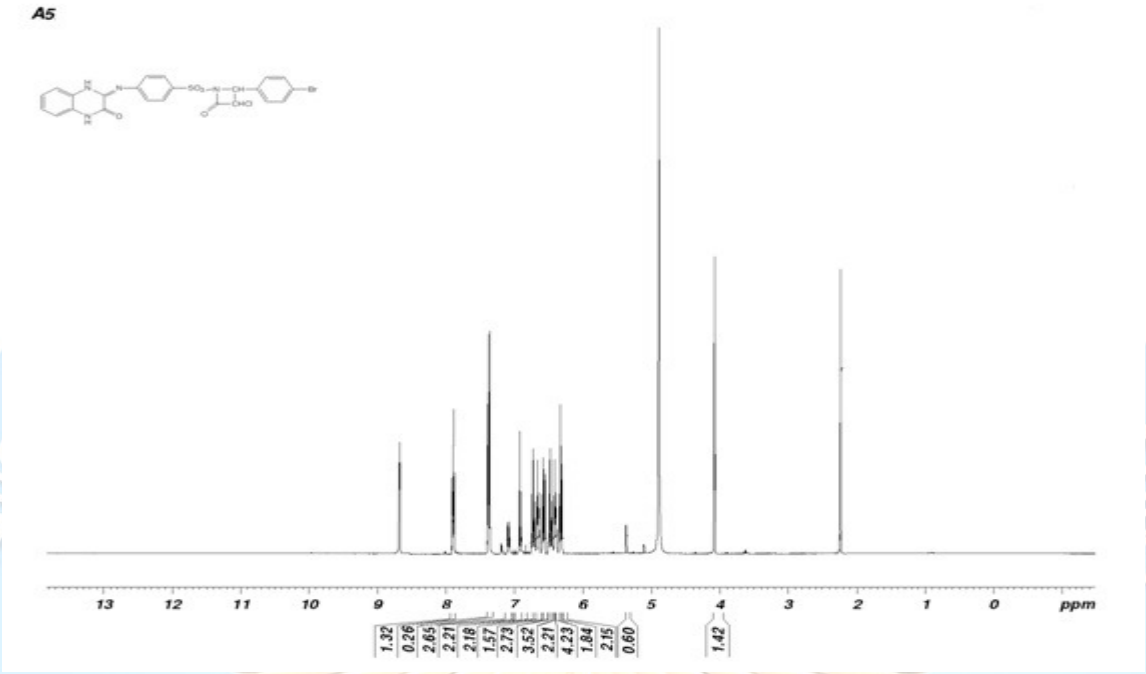


Figure No: 32 ^{1}HNMR of Sample A5: (E)-3-(4-(3-chloro-2-(4-bromophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

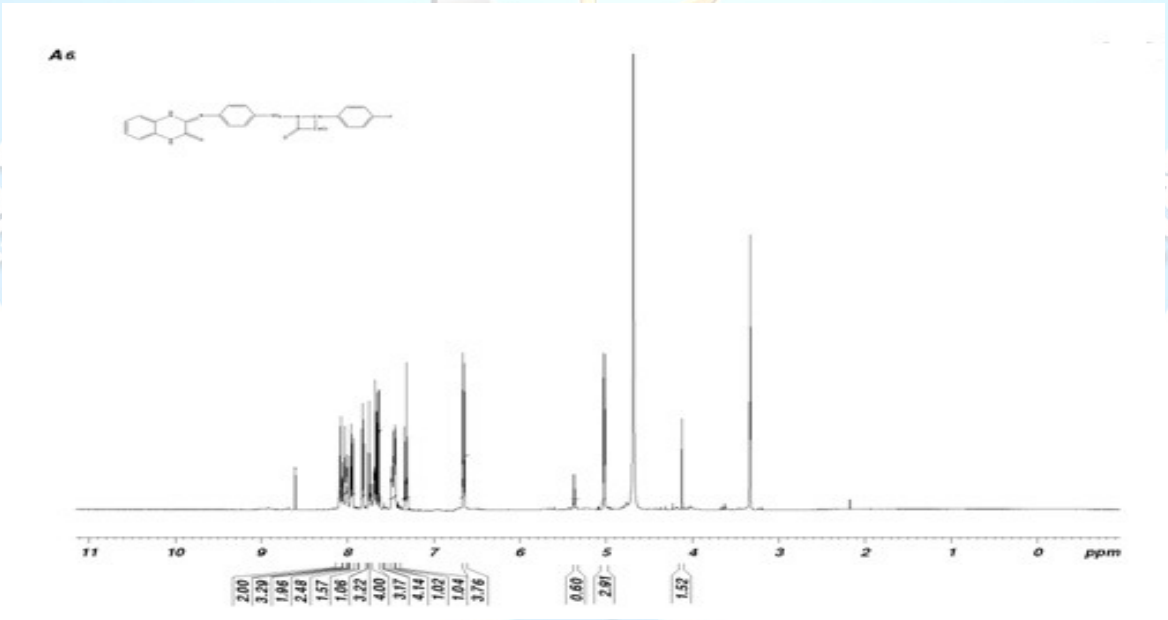


Figure No: 33 ^{1}HNMR of Sample A6: (E)-3-(4-(3-chloro-2-(4-flurophenyl)-4-oxoazetidin-1-yl Sulfonyl) phenylimino)-3, 4-dihydroquinoxalin-2(1H)-one

Evaluation of *invitro* anti microbial activity: The microbial world comprises of micro-organism which are microscopic in size. But these microscopic organisms have several features that are common to higher organism [12]. Bacteria, fungi (yeast and moulds) and microscopic algae are some of micro-organism. This organism can be distinguished into two broad groups such as prokaryotes and the eukaryotes. Eukaryotes contain nucleus and organelles (such as endoplasmic reticulum, Golgi bodies, lysosome, mitochondrion and chloroplast) where as prokaryotes lacks above features.

PREPARATION OF TEST INOCULUM:

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 [13]. The test tubes were incubated at 37°C for bacteria and 25°C for fungi for 24 hr. This is referred to as seeded broth.

Standardization of seeded broth (viable count): 1 mL of the 24 hr 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 10^2, 10^4, 10^5 up to 10^{10} 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 [14]. All the Petri dishes were incubated for 24 h at 37°C for bacteria and 25°C for fungi. The number of well-formed colonies on the plates was counted. The seeded broth was then suitably diluted to contain between 10^7-10^8 micro-organisms/mL. This was designated as working stock, which was used for antimicrobial studies [17].

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 petridishes 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 160-170°C for 1 hr, before use. The antimicrobial of compound was evaluated by filter paper disc method. This method is based on the diffusion of an antibiotic from a filter paper disc through the solidified culture media of a petridishes [15]. Growth of inoculated micro-organism is inhibited entirely in a circular area called zone of inhibition around the filter paper disc containing a solution of the antibiotic. After all the drugs are added Petri dishes were left standing for 1 to 4 hr at room temperature, as a period of pre-incubation diffusion to minimize the effects of variation in time between the application of different solutions. All the Petri dishes were incubated for 24 hr at the required temperatures, i.e., 37°C for bacteria and 25°C 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. **Antifungal activity of the synthesized compounds**: Amphotericin B and Flucytosine are systemically active drugs against fungi they lead to a variety of side effects with renal toxicity and urinary tract infection [16]. Till now more research works are carried out to reduce or complete abolition of these effects. In that way the study of anti fungal activity of the synthesized compounds were important.

Result and Discussion: The synthesized Quinoxaline 2, 3-diones were subjected to in vitro antimicrobial activity. The zone of inhibition at various concentrations against bacteria and fungi were measured. The minimum inhibitory concentration of the synthesized compounds against bacteria and fungi were determined by agar streat dilution method. From the *invitro* antibacterial evalution it was revealed that few compounds are showed activity against various *gram positive bacteria* and *gram negative bacteria* and fungi. Among the synthesized compounds T_4, T_5, A_1, A_4, A_5, Showed potent activity against Staphylococcus aureus and E.coli

The compounds were found to be effective against aspergillosis-induced mortality in immune compromised mice. It has been already shown the di-

rect antifungal activity of the compounds against the fungus under *invitro* conditions. Therefore, the observed protection from aspergillosis induced mortality is likely to be due to the antifungal activity of the Title compounds.

Synthesized compounds were (50 and 100µg/ml^{-1}) screened for invitro anti-microbial activity by paper disc diffusion method. Most of the synthesized compounds exhibited moderate to good anti-microbial activity against the tested microorganisms. When compared to standard drug (Ciprofloxacin) compounds containing Bromo, Fluro substitution in 4th position of the Thiazolidinone (T_5,T_6) and Chloro, Bromo, Fluro substitution in 4th of the Azetidinone (A_1,A_5,A_6) ring system were found to exhibit good anti-bacterial activity. When compared to standard drug (Ketoconazole) compounds containing Chloro, Bromo, Fluro substitution in 4th of the compounds (T_1, A_5, A_6) ring system were found to exhibit good anti-fungal activity. 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). 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 (15.9-12.8µg/ml)

Summary and conclusion: In the present study an attempt was made to synthesize 2, 3 substituted Quinoxaline dione derivatives. All the synthesized compounds of present study were characterized and confirmed by IR and ^{1}H NMR. The newly synthesized 2, 3-substituted Quinoxaline dione derivatives were subjected to preliminary pharmacological screening. Anti-microbial studies were performed for all the synthesized compounds. The zone of inhibition and minimum inhibitory concentration of synthesized compounds against various bacteria and fungi were determined. Anti-bacterial activity of synthesized compounds were tested against *S.Aureus*, *S.Epidermidis,B.Cereus E.Coli, Pse.Aureus* and *K.Pneumoniae.* The compounds showed potent to moderately potent anti-bacterial activity. Among the series the compounds T_5, T_6, A_1, A_5 and A_6 showed potent Anti- bacterial activity. Anti-fungal activities of synthesized compounds were tested against *A.Fumigates* and *A.Niger.* Among the series the compounds T_1, A_5 and A_6 showed potent anti-fungal activity. While compounds T_1, A_5 and A_6 were screened for the antifungal activity. Antifungal activity of the test compounds was comparable to the standard drug Ketoconazole. The present study reveals that the quinoxaline nucleus varying in Thiazolidinone and Azetidinone substructures showed anti-bacterial and antifungal activity. Therefore may serve as lead molecule for further modification to obtain therapeutically useful novel entities in this series.

Table no: 4 Zone of inhibition of the synthesized compounds

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
T_1	19	24	19	24	18	23	15	20	23	29	19	22
T_2	20	21	17	22	17	26	16	21	19	25	18	23
T_3	17	24	18	23	19	24	17	21	20	26	18	21
T_4	18	22	19	22	18	27	16	23	25	31	17	21
T_5	19	25	20	23	20	28	20	24	24	32	19	24
T_6	18	26	19	25	21	29	18	23	26	31	20	25
A_1	25	29	25	28	24	31	20	25	22	29	22	25
A_2	20	21	18	25	20	28	18	21	20	23	18	20
A_3	21	23	19	22	19	25	17	20	21	24	19	21
A_4	19	25	18	24	20	26	18	23	20	26	20	24
A_5	23	27	26	31	23	31	19	24	28	33	23	29
A_6	21	26	24	32	23	29	19	23	24	32	24	26
Ciprofloxacin 100µg/ml	38		39		38		37		38		37	
Control	-		-		-		-		-		-	

Table No: 5 Minimum Inhibitory Concentration of Synthesized Compounds and Standard drug against gram positive and gram negative bacteria (µg/ml)

Sr.No	Organ-ism	T_1	T_2	T_3	T_4	T_5	T_6	A_1	A_2	A_3	A_4	A_5	A_6	Ciprofloxacin
1	S.aureus	2.4	2.2	2.0	2.1	1.9	1.3	1.4	2.4	2.3	2.7	1.4	1.6	0.5-2.5
2	S .epidermis	3.8	2.1	3.7	3.9	2.8	2.5	2.6	2.6	2.9	3.4	2.5	2.4	0.125-0.25
3	B .cerus	1.9	2.0	1.8	2.2	1.6	1.4	1.2	1.9	1.6	1.8	1.1	1.5	0.25-0.5
4	K .pneumonia	3.1	2.7	2.7	2.9	2.6	2.5	2.6	2.9	3.8	3.7	2.2	2.0	<0.06-0.125
5	P .aeroginosa	1.6	1.9	1.3	2.2	1.2	2.4	1.6	1.6	1.8	1.9	1.4	1.2	0.06-1.5
6	E .coli	2.7	2.1	2.6	2.9	1.4	1.2	1.3	1.7	1.6	1.7	1.4	1.5	<0.06

Table no: 6 Zone of inhibition of the synthesized compounds

Compounds	Zone of inhibition (in mm)			
	Concentration(µg/ml)			
	A.niger		A.fumigatus	
	100	200	100	200
T_1	**20**	**25**	**21**	**24**
T_2	17	22	18	22
T_3	19	21	19	21
T_4	20	24	18	20
T_5	18	21	20	24
T_6	19	22	19	22
A_1	22	29	21	24
A_2	21	28	22	23
A_3	24	31	24	21
A_4	20	28	18	20
A_5	28	33	25	31
A_6	25	32	27	33
Ketoconazole (100µg/ml)	38		37	
control	-	-	-	-

Table no: 7 Minimum inhibitory concentration of synthesized compound

Compounds	Minimum Inhibitory Concentration	
	Concentration(µg/ml)	
	A.niger	A.fumigatus
T_1	13.8	14.2
T_2	15.0	15.8
T_3	14.8	14.6
T_4	14.6	15.8
T_5	14.7	15.7
T_6	14.9	14.6
A_1	13.9	15.9
A_2	15.3	14.9
A_3	14.1	15.4
A_4	14.9	15.6
A_5	13.2	13.4
A_6	13.6	13.2
Ketoconazole (100µg/ml)	10.9	11.3

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