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In-vivo anti bacterial activity of the novel series of quinazoline 2,3 dione derivatives

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Abstract
Aim and objective: The aim and object of the study is to analyse the invivo antibacterial effect of synthesized compound on albino swizz mice
Method: The novel series of quinazoline 2, 3 dione derivatives were synthesized, from the synthesized compounds 5 derivatives were evaluated for invivo antibacterial activity with standard drug ciprofloxacin at 50mg/kg dose. The derivatives were analysed for acute toxicity test and invivo antibacterial test (mouse protective test as per the guidelines). The test were carried out for two bacterial Stains such as S.aureus and E.coli.
Result: From the synthesized compound the compound T6 and A6 shows higher level of antibacterial activity and its was estimated that at a dose of 50mg/kg b.w the synthesized compound (T5, T6, A1,A5 and A6) shows no sign of toxicity so safety level was predicted at 50mg dose at the same time the compound T6 and A6 shows higher percentage of protection against S. aureus and E.coli with reference of standard drug ciprofloxacin at a dose of 25mg.
Conclusion: The above data reveals that the synthesized compound has antibacterial activity in a dose of 50mg. Compared to other derivatives compound T6 and A6 shows most prominent role in protection activity which means due to substitution of fluro and chloro compounds on the derivatives. In future if further derivatives were synthesized using this novel series it would be a most promising and lead molecule for many disease.

Keywords: quinaxaline 2, 3 dione derivatives, invivo antibacterial study, against s.aureus and e.coli,acute toxicity study, ir study

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 [2].

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], anti tubercular [15], cytotoxic [16], enzyme inhibitors [17], elastase inhibitors [18], and cholesterol absorption inhibitors [19].

Materials and Methods

General procedure for N1-substituted arylidene /heteroarylidene-4-(3-oxo3, 4dihydroquinoxaline-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 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 [21]. 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 recrystallized using absolute alcohol.

Synthesis of 2-azetidinones (A1-A6)

0.01 mol of Schiff bases (S1-S6) 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 chloroacetate chloride was added drop wise with continuous stirring in cold for 30 min and refluxed for 3 h [22]. 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 [23].

Evaluation of *In Vivo* Antimicrobial Activity

All the animal experimentation was performed according to the protocols and recommendation of the Institutional animal ethics committee.

IAEC /XXX/06/CLBNCP/2010 dated 22.09.2010.

Evaluation of Acute Oral Toxicity

Experimental Protocol (Acute toxic class method in mice)

In the present study the acute oral toxicity of the synthesized compounds (T5, T6, A1, A5, A6, T1, A5, and A6) were performed by acute toxic class method. In this methods the toxicity of the synthesized compounds were tested using a step wise procedure, each step using 3 mice of a single size. The mice were fasted prior to dosing (food but not water should be with held) for 3-4 hrs. Following the period of fasting the animal should be weighed and the synthesized compounds were administered orally at a dose of 2000mg/kg body weight [24]. Animals were observed for first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for total of 14 days. As the animals died, the dose of 300 mg/kg was administered orally and animals were observed carefully. At this dose level also the mortality was observed so the oral dose of 50mg/kg was administered to the animals and monitored them for 14 days as no mortality was observed at this level so based upon OECD guideline 423 a series of doses 50 and 100 mg/kg body weight were selected for the further pharmacological evaluation [25]. This test procedure with a starting dose of 2000mg/kg body weight as per OECD-423 Guideline was shown as follows.

***In Vivo* Anti-Bacterial Activity (Mouse Protection Test)**

Experimental protocol

The *in vivo* activity of synthesized compounds against systemic infection in mice was determined four week old male swiss albino mice weighing 18 to 22 gm were used for systemic infection model.

They were maintained in animal rooms kept at 23±2°c with 55% ± 20% relative humidity. Test organisms for infection were cultured in Hinton nutrient agar medium at 37°c for 18 hrs. For use as inoculate, staphylococcus aureus, E.coli was suspended in 0.9% saline solution containing 5% gastric mucin [26]. Mice were used in groups of six for each inoculum and were challenged intraperitoneally with a sigle 0.5 ml portion of the bacterial suspension, corresponding to an inoculums range of 10 to 100 times the minimal lethal dose of bacteria. Two dose levels were used for each synthesized drugs, depending on the invitro antimicrobial activity of the compound (i.e.) 50 and 100 mg/µg. Synthesized compounds at various dose regimens were orally administered to mice twice, at 1 and 4th hr. of past infection. Synthesized compounds were suspended in 1% carboxy methyl cellulose. Mortality was recorded for 7 days, and the median effective dose needed to protect 50% of the mice (ED50) was calculated by intrapolation among survival mice (% protection) in each group after a week. They express the total dose of compounds (mg/kg) required to protect 50% of the mice from an experimentally induced systemic infection of the indicated organism. The challenge inoculum was sufficient to kill 100% of the untreated control mice, which died within 48 hr post infection.

Results and Discussion

Synthesis

Two derivatives were syntheseized from the novel series of quinazoline derivatives which was shown in fig 1

IR study

In general, IR spectra of compounds (A1-A6) showed absorption bands ranging around 1759–1764cm– 1 indicating the presence of C=O groups in their structure (C=O group is in the cyclic ring) and also the NH stretching vibrations appeared between 3373–3383 cm–1. The result were shown in fig 2-7.

Acute toxicity study

Acute oral toxicity studies were performed according to the OECD guideline 423 method.

This method has been designed to evaluate the substance at the fixed doses and provides information both for hazard assessment and substance to be ranked for hazard classification purposes.

The synthesized compounds were administered initially at a starting dose of 2000 mg/kg b.w in 1% CMC (p.o) and observed 14 days mortality due to acute toxicity.

Careful observation were made at least twice a day for the effect on CNS, ANS, motor activity, salivation, skin colouration and other general signs of toxicity were also observed and recorded.

Since the mortality was observed at 2000mg/kg b.w. and 300 mg/kg b.w. the lower dose level of 50 mg/kg was selected.

Since no sign of toxicity observed at 50 mg/kg b.w. to the group of animals, the LD50 value of the title compounds(A1,A5,A6,,T5 and T6) expected to exceed 50 mg/kg b.w and represented as class 3 (50 mg/kg < LD50 < 300 mg/kg) From the toxicity studies the data revealed that all the synthesized compounds proved to be non-toxic at tested dose levels and well tolerated by the experimental animals as their LD50 cut of values >200 mg/kg b.w. the result was shown in table 1-2. From the synthesized derivatives, compounds A6 and T6 shows higher antibacterial activity against S. aureus and E.coli compare to other compounds which may be due to presence of chloro and fluro substitution in the derivatives. The result was expressed in fig 8-9. Schematic representation for the synthesized compound

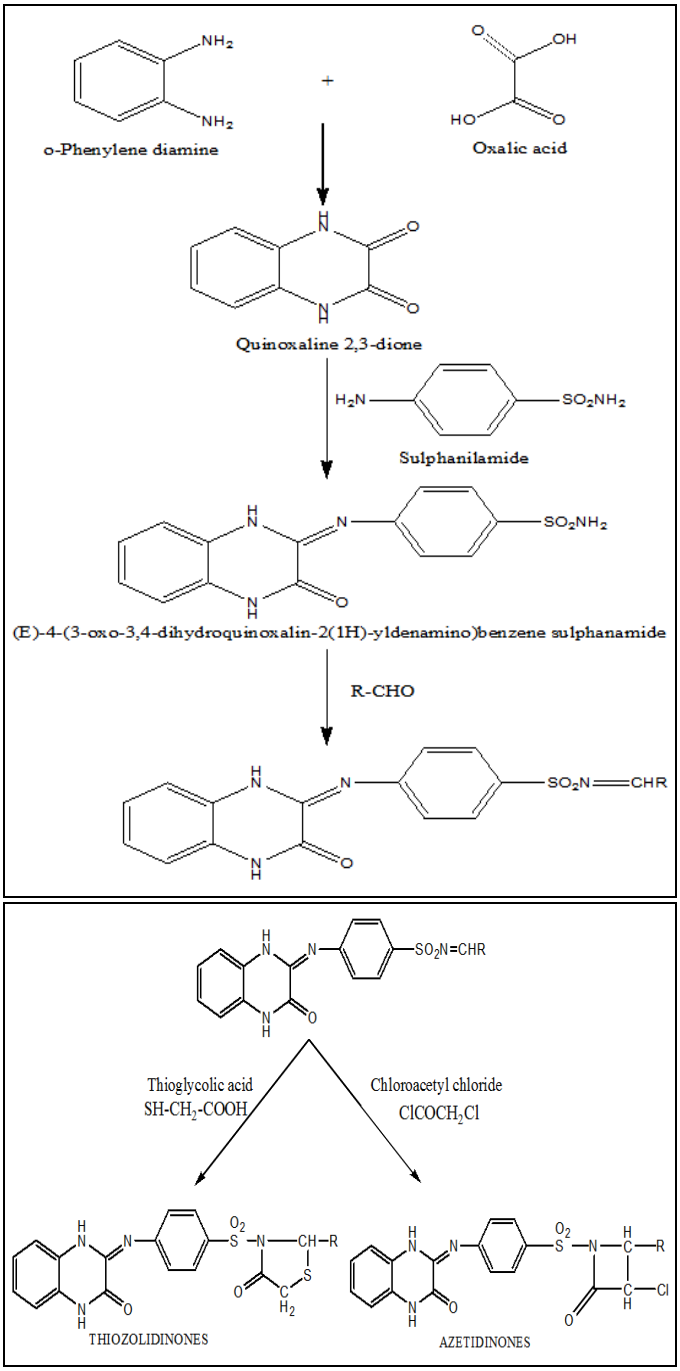
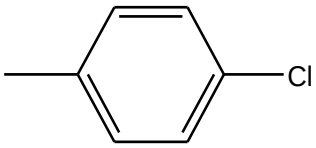
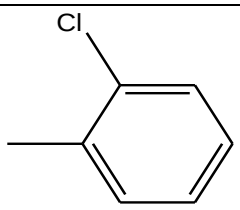
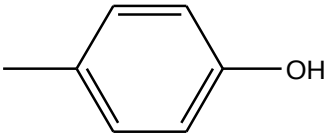
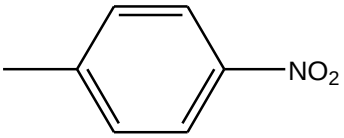
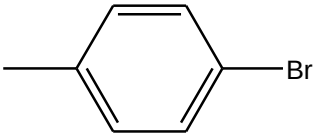
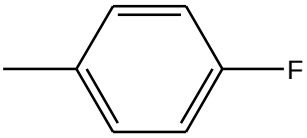


Fig 1: Scheme for the synthesis of compounds

Table 1: Details of The–R Represented In the Synthetic Scheme

Compounds	-R
1	
2	

3	
4	
5	
6	

IR spectra were recorded on ABB BOMEM FTIR Spectrometer using potassium bromide pellets.

Table 2: Infra-Red Spectral Data of Synthesized Compounds.

Compounds	Ar- CH Stretch	CH(S)	C=O	C=N	C-S	C-N	CH(b)	N-H	C-Cl	Ar-OH	Ar-NO2	Ar-Br	Ar-F
A1	3057	2865	1759 1598	1519	-	1324	1403	3373	748	-	-	-	-
A2	3057	2865	1759 1598	1519	-	1324	1403	3373	748	-	-	-	-
A3	3067	2853	1746 1621	1516	-	1319	1406	3379	758	3397	-	-	-
A4	3087	2840	1732 1712	1518	-	1349	1413	3378	753	-	1540	-	-
A5	3086	2874	1711 1656	1510	-	1326	1408	3348	745	-	-	548	-
A6	3067	2865	1764 1602	1519	-	1317	1415	3383	746	-	-	-	1285

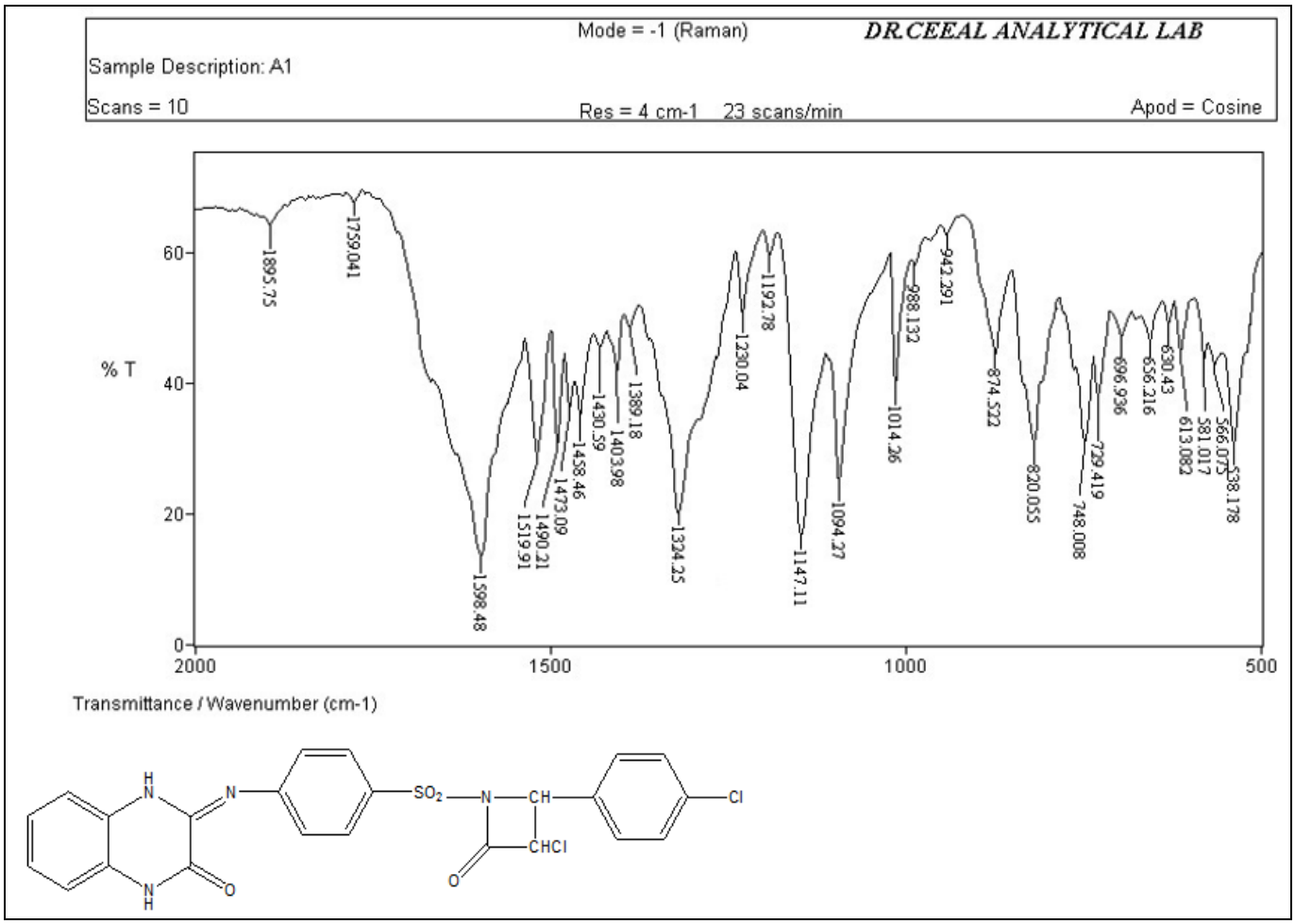


Fig 2: IR spectrumof compound A1

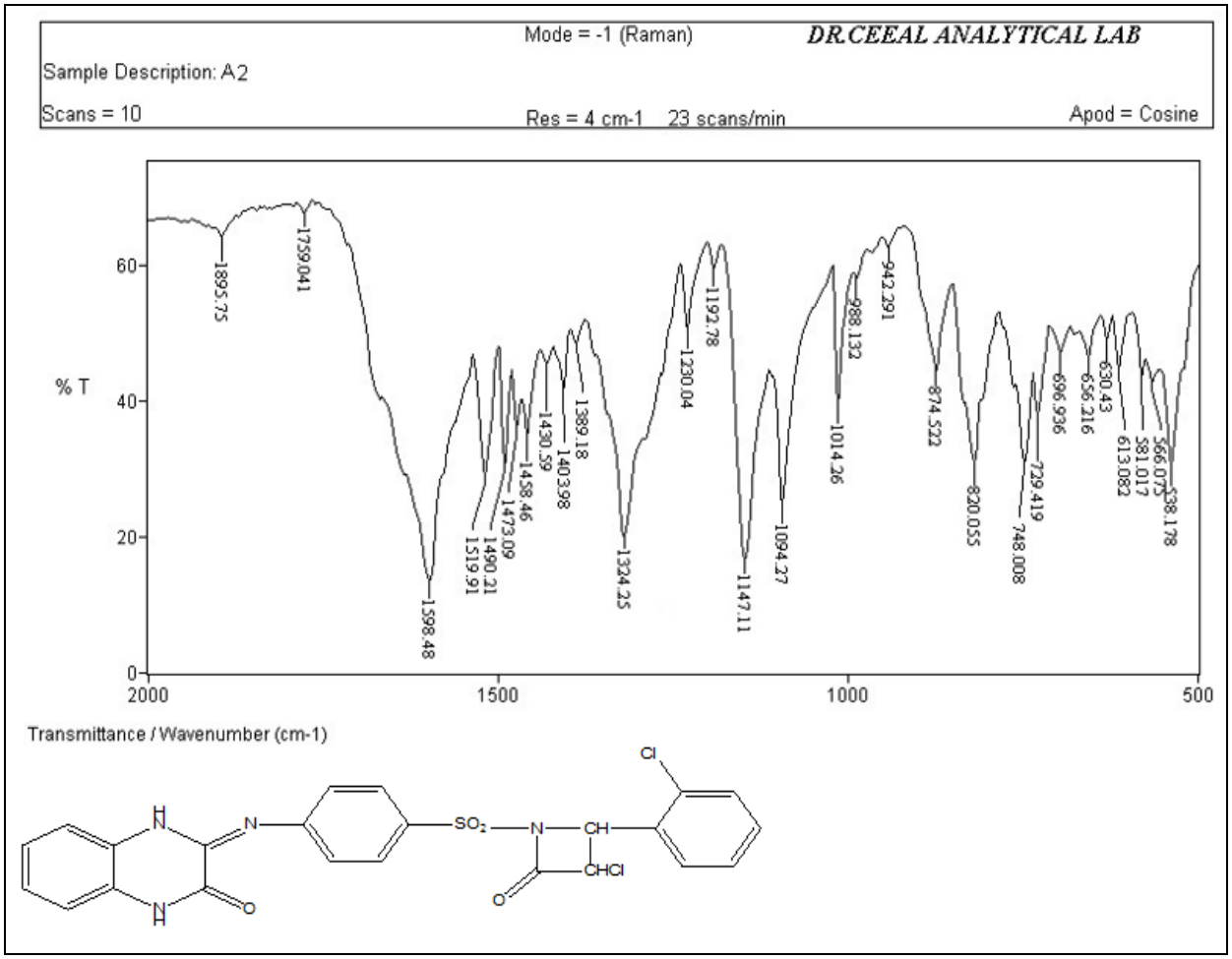


Fig 3: IR spectrumof compound A2

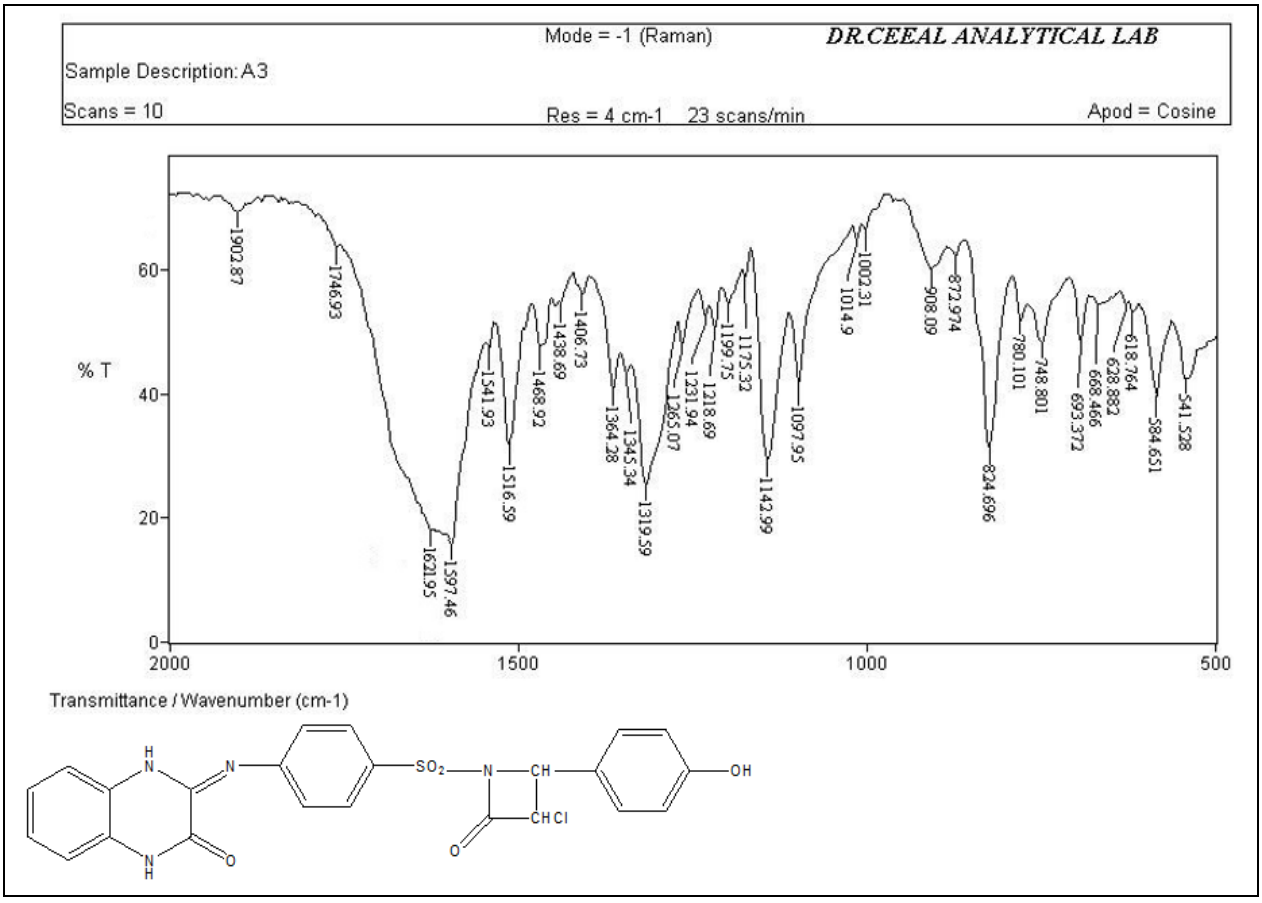


Fig 4: IR spectrumof compound A3

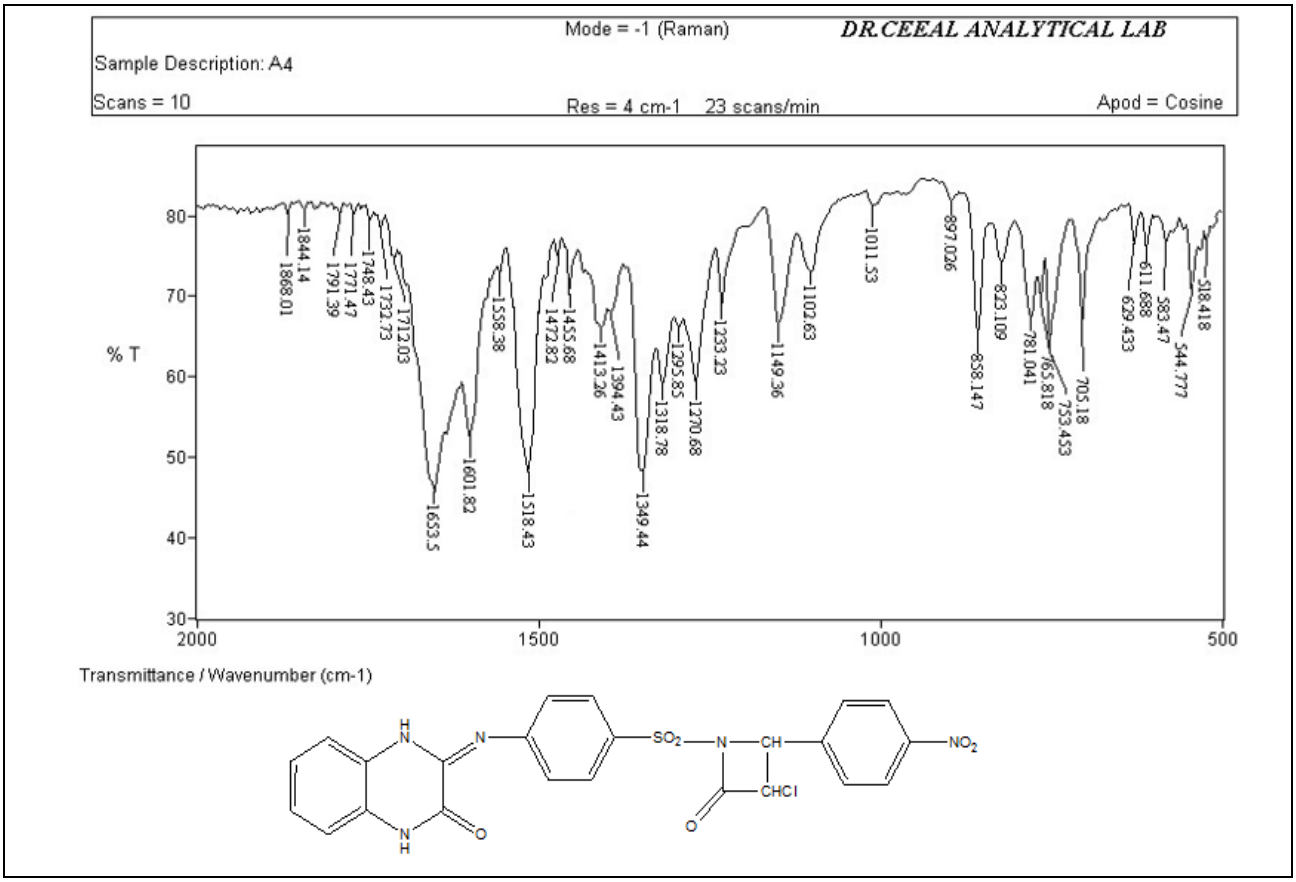


Fig 5: IR spectrumof compound A4

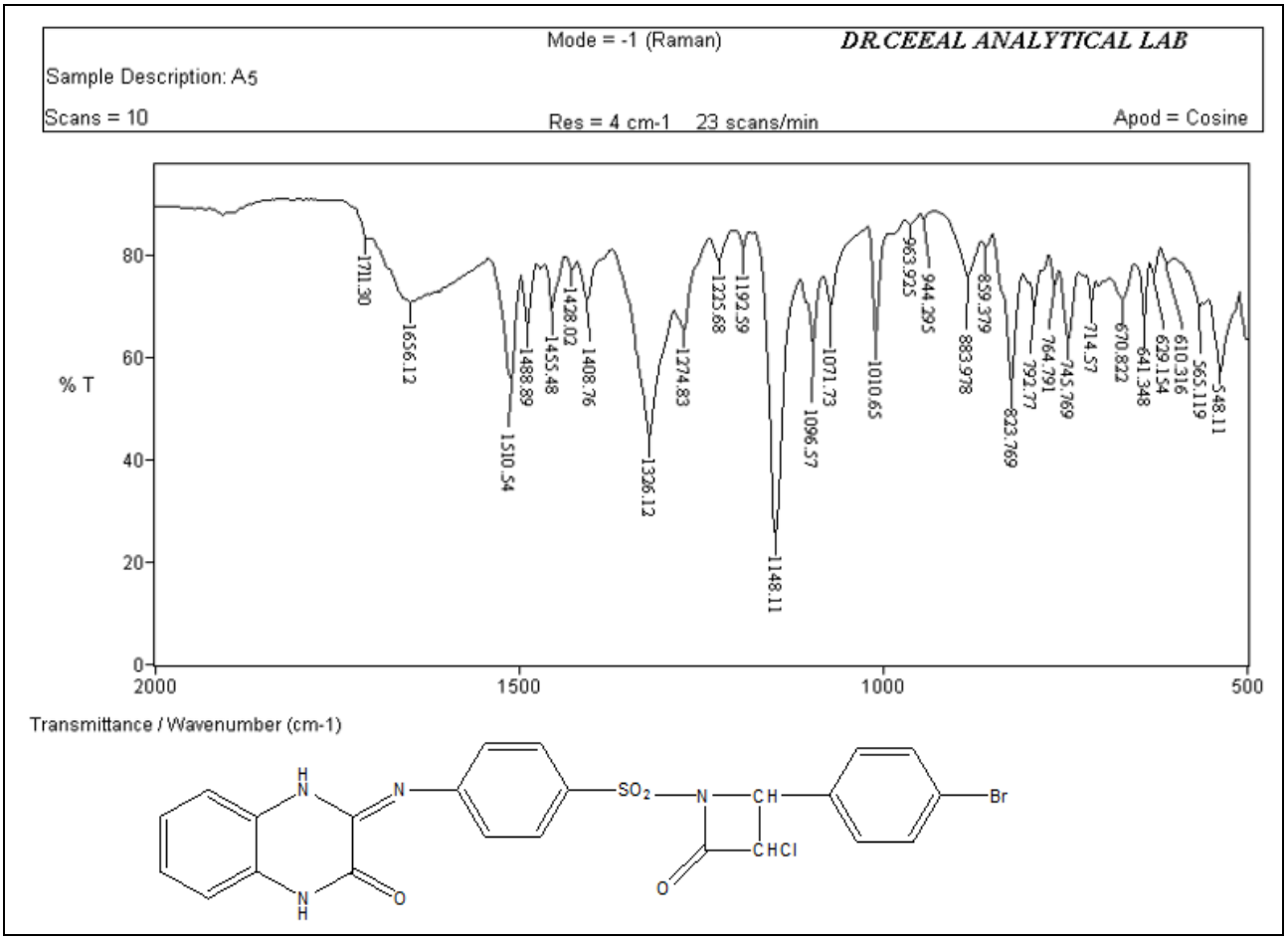


Fig 6: IR spectrumof compound A5

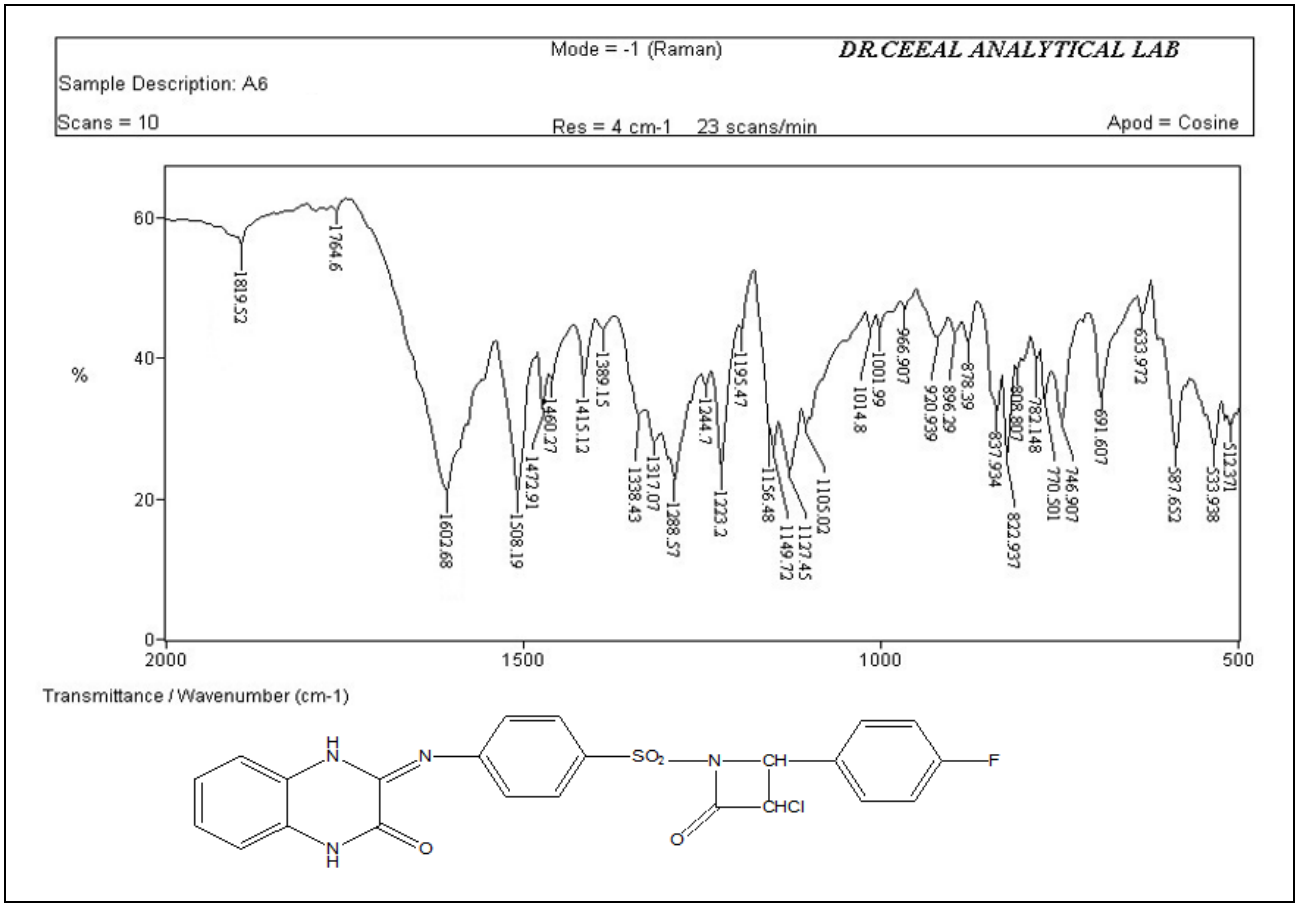


Fig 7: IR spectrumof compound A6

Table 3: Invivo Results of the Synthesized Compounds against *Staphylococcus aureus*

S.no	Compounds	Dose	Percentage Protection	$ED_{50\ mg/kg}$
1	T_5	50 mg/kg	66.6%	37mg/kg
		100mg/kg	83.3%	
2	T_6	50 mg/kg	83.3 %	30mg /kg
		100mg/kg	100 %	
3	A_1	50 mg/kg	50.0%	50mg/kg
		100mg/kg	66.6%	
4	A_5	50 mg/kg	33.3%	100mg/kg
		100mg/kg	50.0%	
5	A_6	50 mg/kg	83.3 %	30mg /kg
		100mg/kg	100 %	
Std.	Ciprofloxacin	50mg/kg	100%	25mg/kg

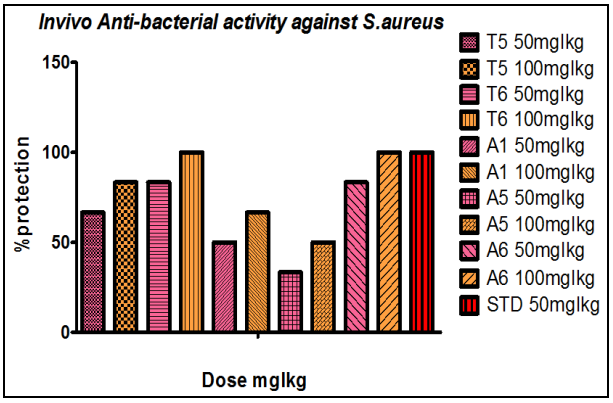


Fig 8: In-vivo antibacterial activity of S. aureus

Table 4: *In Vivo* Results of the Synthesized Compounds against *Escherichia coli*

S.no	Compounds	Dose	Percentage Protection	$ED_{50\ mg/kg}$
1	T_5	50 mg/kg	50.0%	50mg/kg
		100mg/kg	66.6%	
2	T_6	50 mg/kg	83.3 %	30mg /kg
		100mg/kg	100 %	
3	A_1	50 mg/kg	66.6%	37mg/kg
		100mg/kg	83.3%	
4	A_5	50 mg/kg	33.3%	100mg/kg
		100mg/kg	50.0%	
5	A_6	50 mg/kg	83.3 %	30mg /kg
		100mg/kg	100 %	
Std.	Ciprofloxacin	100mg/kg	100%	25mg/kg

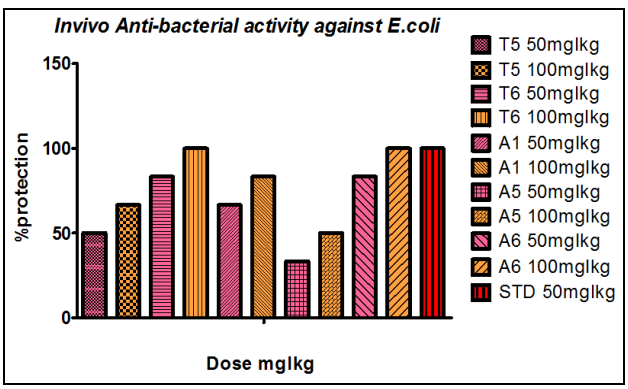


Fig 9: *In vivo* antibacterial activity of E. coli

Conclusion

The novel series of quinaxaline 2, 3 Dione derivatives were synthesized and analysed for *invivo* antibacterial activity. From the synthesized compound the two most promising derivatives such as A6 and T6 shows higher level of protection againt S. aureus and E. coli. Respectively with standard drug ciprofloxacin. Even more number of derivatives can be synthesized from the same compound and which may have a potency to cure many Disease's. But in future lot of research should be needed to prove the various activity of the synthesized derivatives.

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Conflict of Interest

No conflict of interest from authors

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