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SYNTHESIS AND EVALUATION OF SUBSTITUTED AND UNSUBSTITUTED 3,6-DIMETHYLQUINOXALINE-2(1*H*)-THIOL DERIVATIVES FOR MRSA ACTIVITY

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ABSTRACT

When the viable solvents businesses launched penicillin, in the establishment of 19th century invented by Alexander Fleming, a major reduce in the number of deaths caused by bacterial infections has been confirmed. Optimists have even noticed an end to the period of bacterial diseases. However, too frequent, and frequently improper, applications of antibiotics have resulted in the development of drug resistant bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), WHO generated a list of priority pathogens to focus the development of new antibacterial drugs against; MRSA ranked as a high priority. Unfortunately, the development of new antibacterial drugs has progressively declined since the 1980s. In my research work novel derivatives of Quinoxaline obtained from ortho phenylene diamine and pyruvic acid gives 3,6-methyl quinoxaline-2(1H)-one [DR-I] it was treated with phosphorous pentasulphide gives 3,6-methyl quinoxaline-2(1H)-thiol [DR-II] which is converted into resultant compound derivatives of 2,6-methyl-3-[(3-nitropyridin-2-yl) sulfonyl] quinoxaline (DR-IIA-IIF). All the compounds synthesized were confirmed by spectral data and evaluated for their methicillin-resistant Staphylococcus aureus (MRSA) activity Vancomycin was used as standard. The compounds DR-IIA and DR-IID have shown good activity and remaining shows poor activity against bacteria.

KEYWORD: Quinoxalines, Antibacterial Activity, Vancomycin, Methicillin-Resistant Staphylococcus aureus (MRSA).

INTRODUCTION

Quinoxaline Another name is Benzopyrazine. It is a heterocyclic compound with benzene and pyrazine rings. Pyrazine is a colorless, stable compound that is soluble in water. Unlike pyridine, they are expensive and difficult to obtain, so they are rarely used as a starting material for the synthesis of their derivative. Quinoxaline is formed by fusing diazines with benzene rings. The pyrazine ring system, which is found in the fungal metabolite aspergilla acid and in dihydro form in Lucifer in a variety of battles including the fire fly, is responsible for this ostracod's chemiluminescence. Methoxy pyrazine is a key component of the aroma of many fruits and vegetables, including peas and capsicum peppers, as well as wines.[1] Quinoxaline derivatives in terms of structure-activity relationship (SAR). The residues defined the quinoxaline derivatives.[2]

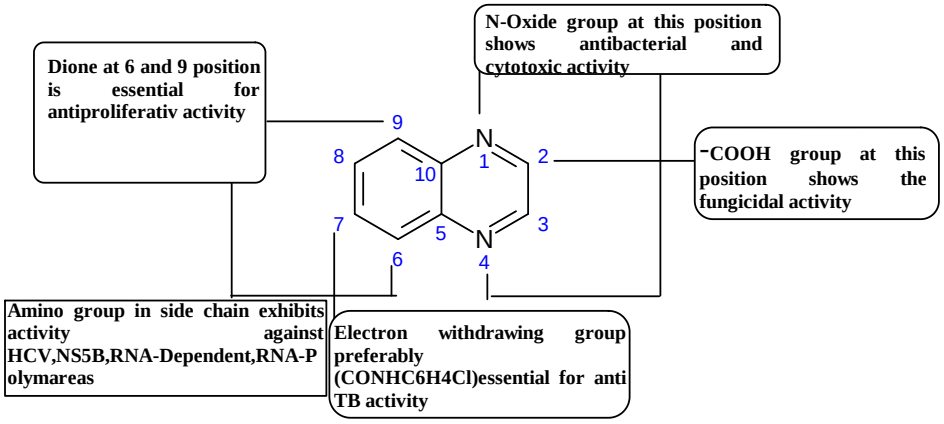


Fig. 1: SAR Quinoxaline compound.

Quinoxaline derivatives exhibit a broad spectrum of biological activity such as antibacterial, antifungal, antiviral, anticancer, anti-tubercular, antimalarial and anti-inflammatory. Quinoxaline is well known for its broad coverage in the field of medicine as well as for its application in the pharmaceuticals. Quinoxalines constitute an important class of compounds, of which some analog has synthesized and evaluated for antimicrobial activity. Many possess different biological activity such as insecticides, fungicides, herbicides, anthelmintic and antiviral.[3]

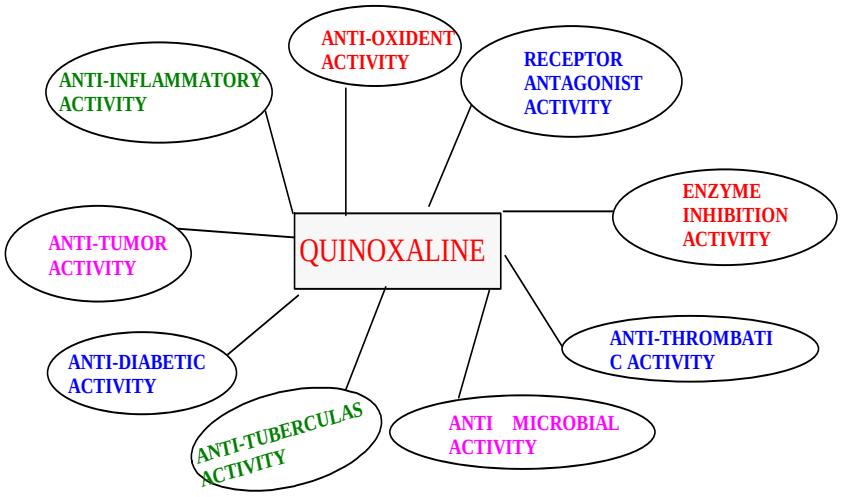
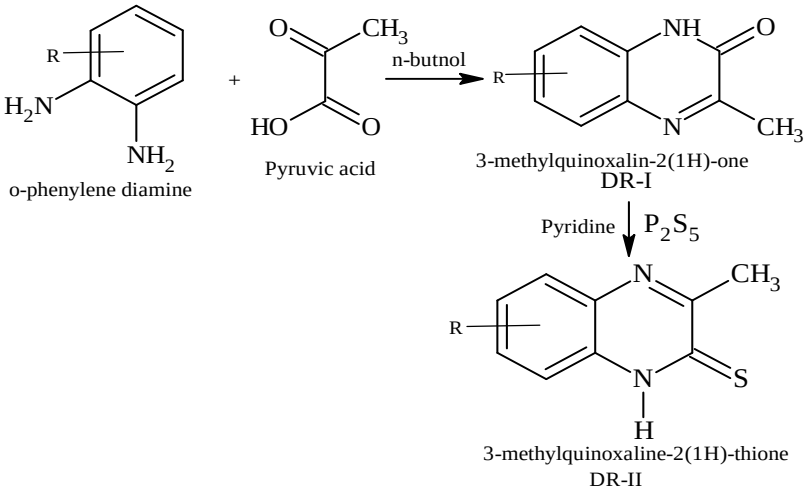


Fig. 2: Different activity of Quinoxaline compound.

Malaria is an infectious disease with an estimated 219 million cases and 660,000 deaths in 2010. Of these cases 86% correspond to children under 5 years old. In 2012, there were a total of 104 countries where malaria is considered to be endemic. Plasmodium falciparum is the most dangerous form of the malaria parasite and it is responsible for a very high percentage of clinical attacks.[4-5]

EXPRIMENTATION



❖ **PREPARATION OF COMPOUND 3,6-DIMETHYLQUINOXALINE-2(1H)-ONE (DR-I)**

O-Phenylene diamine (10.8g, 0.10 M) was dissolved in n-butanol (300 mL) with warming. Pyruvic acid (11.6 g, 15 mL, 0.10 M) was dissolved separately in n-butanol (100 mL) and added to the former solution with constant stirring. The solution was set aside for 30 min, and then it was heated for 1hour on a water bath. On cooling, the crystals that separated were filtered, washed with n-hexane and purified by recrystallization from ethanol to yield colorless, needle-shaped crystals of 2-hydroxy-3-methylquinoxaline.

❖ **PREPARATION OF COMPOUND 3,6-DIMETHYL QUINOXALINE-2(1H) THIOL[DR-II]**

The mixture of 3,6-methylquinoxaline-2(1*H*)-one [DR-I] (0.01mol) and Phosphorus Pentasulfide (0.01mol) as suspended in 40ml Pyridine. The reaction mixture was refluxed 5hr. then 200 ml of water was added to the content of flask. The precipitate was filtered off recrystallize to petroleum ether to afford corresponding compound.

❖ **PREPARATION OF DERIVATIVES OF COMPOUND3,6-METHYLEQUINOXALINE-2-(1H)-THIOL [DR-IIA-IIF]**

A mixture of 3,6-dimethylquinoxaline-2(1*H*) thiol [DR-II] (0.01 mol), Substituted halides (0.01 mol) and anhydrous potassium carbonate (2.0 g,0.01mol) in dimethyl Formamide (30 ml) was heated under reflux for 12 h. Solvent was evaporated *in* vacuum and the obtained residue was washed with water, dried and recrystallized from ethanol.

Table No. 01: Derivatives of 3, 6-Methylequinoxaline-2-(1H)-THIOL [DR-IIA – DR-IIF].

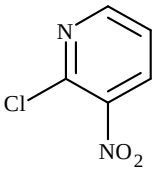
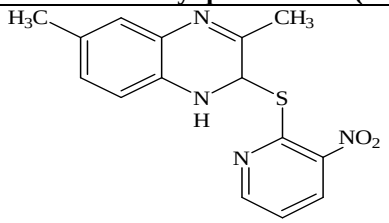
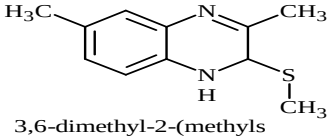
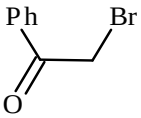
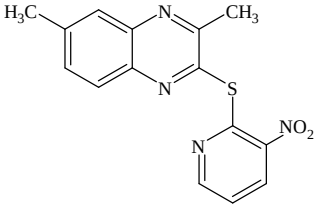
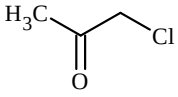
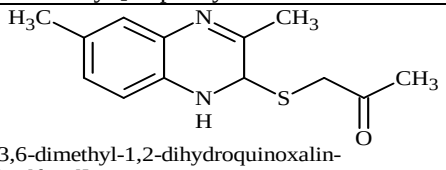
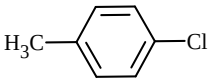
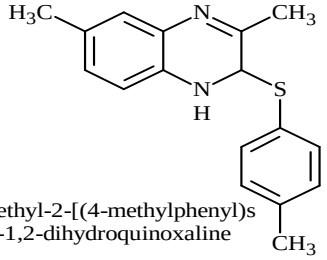
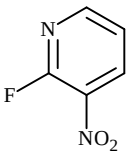
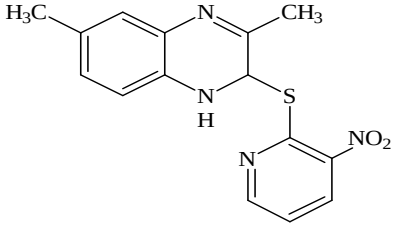
Compound Code	Substituted Halides	Derivatives of 3- Methylquinoxaline-2(1H)-Thiol
DR-IIA	 2-chloro-3-nitropyridine	 3,6-dimethyl-2-[(3-nitropyridin-2-yl) sulfanyl]-1,2-dihydroquinoxaline
DR-IIB	CH_3I Methyl iodide	 3,6-dimethyl-2-(methylsulfanyl)-1,2-dihydroquinoxaline
DR-IIC	 2-bromo-1-phenylethan-1-one	 2-[(3,6-dimethyl-1,2-dihydroquino xalin-2-yl) sulfany1]-1-phenylethan-1-one
DR-IID	 1-chloropropan-2-one	 1-[(3,6-dimethyl-1,2-dihydroquinoxalin-2-yl)sulfanyl]propan-2-one
DR-IIE	 1-chloro-4-methylbenzene	 3,6-dimethyl-2-[(4-methylphenyl)sulfanyl]-1,2-dihydroquinoxaline
DR-IIF	 2-fluoro-3-nitropyridine	 3,6-dimethyl-2-[(3-nitropyridin-2-yl)sulfanyl]-1,2-dihydroquinoxaline

Table No-02: Physicochemical Properties of Derivatives of Compound 3, 6-Methyl Quinoxaline-2(1h) Thiol Derivatives [DR-IIA-IIC]

Sl. No	Parameter	DR-IIA	DR-IIB	DR-IIC
1	Molecular Formula	$C_{15}H_{12}N_4O_2S$	$C_{11}H_{14}N_2S$	$C_{18}H_{18}N_2OS$
2	Molecular weight	312.34	206.30	310.41
3	Theoretical yield	2.23gm	2.23gm	2.23gm
4	Practical yield	2.14gm	1.4gm	2.18gm
5	% Yield	95.96%	63.00%	97.75%
6	Melting point	240-245° C	286^0-289° C	305-308° C
7	Recrystallization	Ethanol	Ethanol	Ethanol
8	TLC	Benzene: Chloroform 5:1	Benzene: Chloroform5:1	Benzene: Chloroform5:1
9	R_f Value	0.85	0.96	0.90

Table No-03: Physicochemical Properties of Derivatives of Compound 3, 6-Methyl Quinoxaline-2(1h) Thiol Derivatives [DR-IID-DR-IIF]

Sl. No	Parameter	DR-IID	DR-IIE	DR-IIF
1	Molecular Formula	$C_{13}H_{16}N_2OS$	$C_{17}H_{18}N_2S$	$C_{15}H_{14}N_4O_2S$
2	Molecular weight	248.34	282.40	314.36
3	Theoretical yield	2.23gm	2.23gm	2.23gm
4	Practical yield	1.02gm	1.5gm	1.8gm
5	% Yield	45.73%	67.26%	80.71%
6	Melting point	310-312° C	288-290° C	240-245° C
7	Recrystallization	Ethanol	Ethanol	Ethanol
8	TLC	Benzene: Chloroform5:1	Benzene: Chloroform5:1	Benzene: Chloroform5:1
9	R_f Value	0.84	0.92	0.84

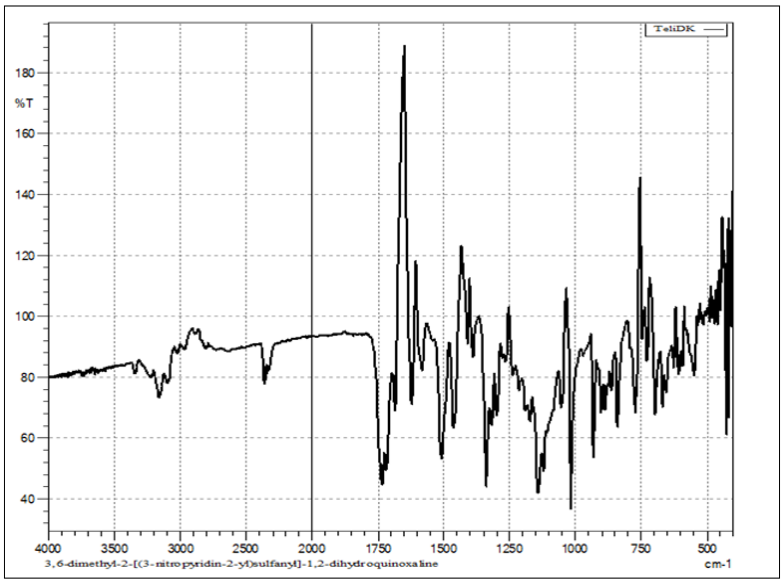


Fig. 03: FT-IR Spectrum data of compound 3,6-dimethyl-2-[(3-nitropyridin-2-yl) sulfonyl]-1,2-dihydroquinoxaline.

Table No-04: FT-IR Spectrum data of compound 3, 6-dimethyl-2-[(3-nitropyridin-2-yl) sulfonyl]-1,2-dihydroquinoxaline.

Types of Vibrations	Group frequency in Wavenumber (cm^{-1})
-NH Stretching	3300-3390 cm^{-1}
Aromatic -CH stretching	3150-3250cm^{-1}
Aliphatic-CH	2910-3100cm^{-1}
$-CH_3$Stretching	1010 cm^{-1}
-C-S stretching	780 cm^{-1}

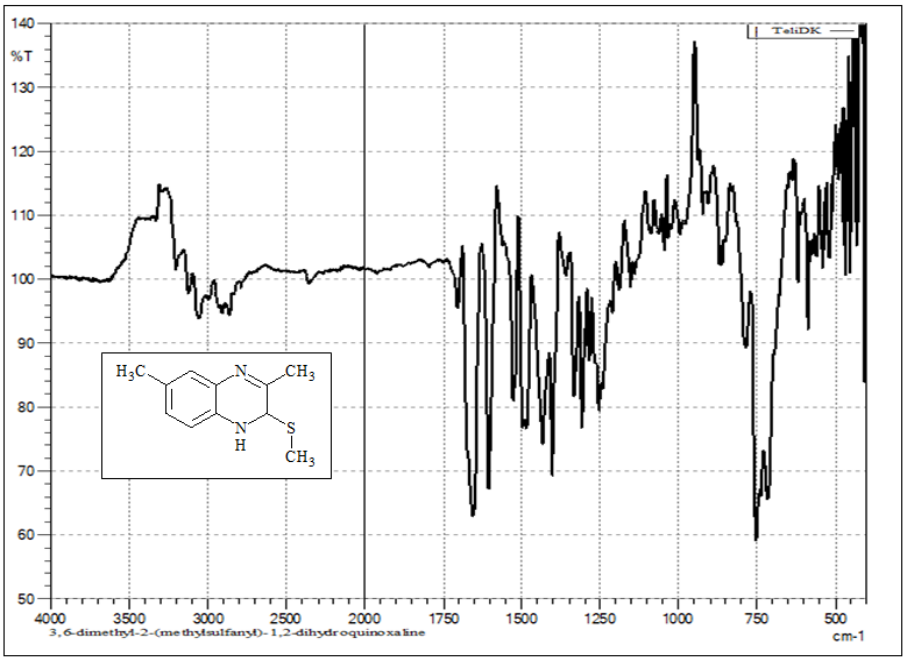


Fig. 04: FT-IR Spectrum data of compound 3, 6-dimethyl-2-(methylsulfanyl)-1, 2-dihydroquinoxaline.

Table No.05: FT-IR Spectrum data of compound 3, 6-dimethyl-2-(methylsulfanyl)-1,2-dihydroquinoxaline.

Types of Vibrations	Group frequency in Wavenumber (cm^{-1})
-NH Stretching	3050-3190 cm^{-1}
Aromatic -CH stretching	2950-3020cm^{-1}
Aliphatic- CH	2800-2930cm^{-1}
$-CH_3$ Stretching	860 cm^{-1}
-C-S stretching	750 cm^{-1}

BIOLOGICAL ACTIVITY

Antimicrobial susceptibilities were determined using the cup-plate method, as recommended by of vancomycin were used. Multidrug resistance was defined as resistance to class of antimicrobials. *S. aureus* was used as a control strain.

STAPHAYLOCOCCUS AUREUS

Staphylococcus aureus is a Gram-positive, round-shaped bacterium, a member of the Firmicutes, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin.

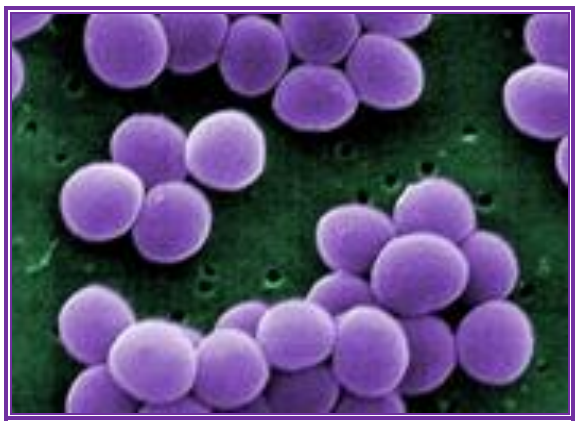


Fig. 05: Microscopic *Organism* of *S. aureus.*

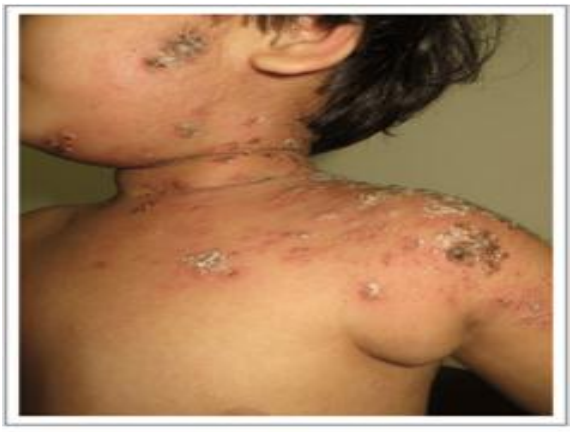


Fig. 06: Symptoms of S. aureus.

MECHANISM OF *S. AUREUS* ANTIMICROBIAL RESISTANCE

Generally, bacteria acquire resistance against antibiotics via different molecular mechanisms, including enzymatic inactivation of antibiotics, alteration of antibiotics target(s) leading to decreased affinity for the antibiotics, removing antibiotics via efflux pumps and changing membrane permeability. *S. aureus* is known to resist all the clinically approved antibiotics using various resistance mechanisms mentioned above. The detailed resistance mechanisms for important antibiotics,

including penicillin and methicillin, are discussed below.[6]

PENICILLIN RESISTANCE

Penicillin was first isolated from a soil fungus, *Penicillium* the 1940s. This antibiotic was once thought to be a miracle drug as it could cure previously fatal infections. However, few years after its introduction, penicillin resistance including penicillin-resistant *S. aureus* was isolated from hospitals. Penicillin resistance of *S. aureus* is highly prevalent with up to 86% of clinical *S. aureus* isolates being resistant to the antibiotic in the US. Meantime, far way in Australia, a similar observation was made as 80% of *S. aureus* isolates were resistant to penicillin. Penicillin resistance in staphylococci is mediated by the production of enzyme penicillinase or beta-lactamase encoded by the *blaZ* gene. This enzyme inactivates the antibiotic by hydrolysis of the beta-lactam ring of the antibiotic. Studies show that penicillin's genes can be present on either plasmid of the chromosome of *S. aureus*.[6]

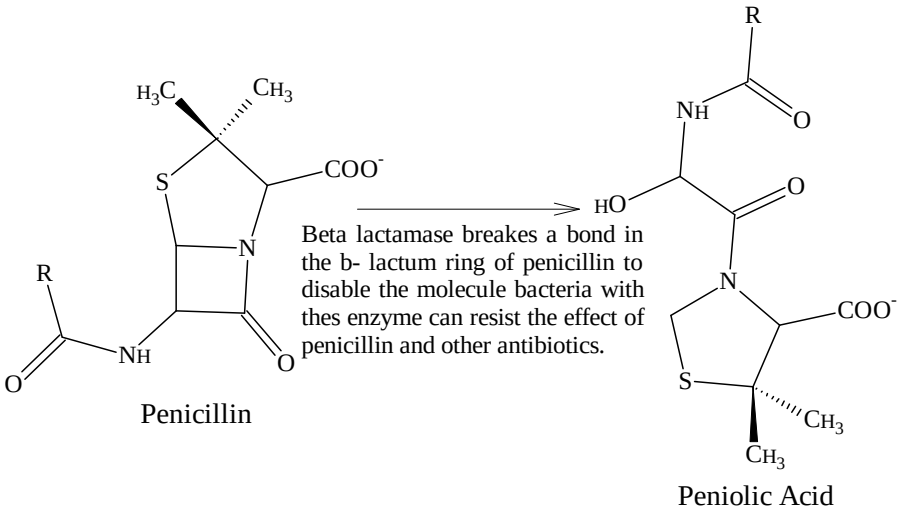


Fig. 07: Metabolism of Penicillin Resistances.

METHICILLIN RESISTANCE

Methicillin is a penicillinase-resistant beta-lactam. It was first introduced in 1950s and prescribed for *S. aureus* infection. The first MRSA was documented in 1961 in the UK while the first MRSA in the US was first reported in 1968. Since then, many MRSA clones spread to every corner of the globe. Methicillin resistance is usually encoded by *mecA* gene that is located in a mobile genetic element of *S. aureus*, known as the Staphylococcal Chromosomal Cassette *mec* (SCC*mec*). *MecA* is responsible for the synthesis of low-affinity PBP2a which leads to decreased methicillin binding. Methicillin resistance confers broad spectrum of activity generally to the entire beta-lactam class of antibiotics including penicillin's and cephalosporins. The origin of SCC *mec* is thought to be originated from coagulase-negative staphylococcal species as there is no homologue of *mecA* present in methicillin-susceptible staphylococci. In recent years, a novel *mecA* homologue, *mecC* has been identified in both livestock and human in European Countries Similar to *mecA*, *mecC* codes for PBP2a with reduced affinity for methicillin and oxacillin, making them MRSA.[6]

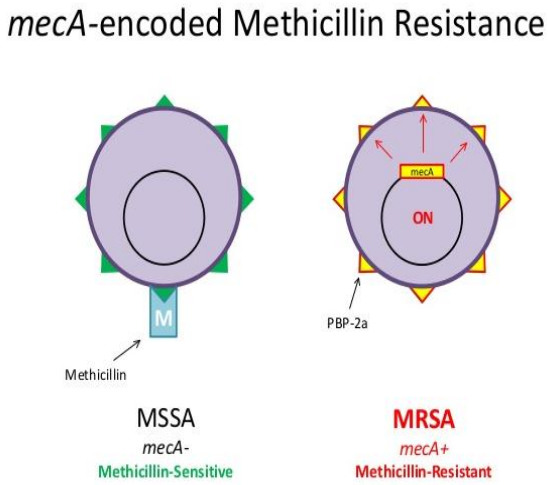


Fig. 08: Methicillin Resistance mecA- and mecA+.

BACTERIAL TARGETS OF S. AUREUS BY NATURAL ON BODY

We discussed the anti-staphylococcal activities by various natural products alone and in combination with multiple types of antibiotics. As some of the mechanisms of antibiotic resistance have already been studied and reported, such as enzymes inactivation, antibiotics trapping, and efflux pumps, this information enables the anti-staphylococcal molecular targets of the natural products to be elucidated. These particular section

summaries the molecular targets of natural products against drug resistant *S. aureus* such as bacterial cell wall and membrane, cell division protein Fts Z, pyruvate kinase, DNA topoisomerase, efflux pump proteins, and PBP2a. The reported pharmacological targets are depicted.

MATERIAL AND METHODS

Test Organisms

- *Staphylococcus Aureus* (Gram positive Organism)

All the synthesized compounds were screened for MRSA activity against the above-mentioned strains by cup-plate method. The following materials were used for the testing.

- Nutrient agar.
- Sterilized petri dishes, pipette sand beakers.
- Sterilized 6mm corkborer and tuberculin syringes.
- 18-24 hold growth culture in nutrient broth.
- Sterilized test tubes containing solution of test compounds in desired concentration

PREPARATION OF NUTRIENT AGAR MEDIA

Nutrient agar (40g), bacteriological peptone (1g), beef extract (5g) and sodium chloride (5g) were dissolved in distilled water (1000 ml). The pH of the solution was adjusted to 7 to 7.4 by using sodium hydroxide solution (40%, approximately 0.25 ml for 100 ml of nutrient broth) and then sterilized for 30 min. at 15 lbs. pressure in anautoclave.[7]

PREPARATION OF SUB CULTURE

One day prior to test the microorganisms were inoculated in to the sterilized nutrient broth and incubated at 37^0C for 24 hr. On the day of testing the organisms ware sub-cultured into sterile nutrient broth. After incubating for 3 hr., the growth thus obtained was used as inoculums forthetest.[7]

STERILIZATION OF MEDIA AND GLASS WARES

The media used in the present study, nutrient agar and nutrient broth were sterilized in a conical flask of suitable capacity by autoclaving the same at 15 lbs. pressure for 20 min. The cork borer, petri dishes, test tubes and pipettes, were sterilized by employing hot air oven at 160^0C for1hr.

PREPARATION OF SOLUTION OF TEST COMPOUND

The test compound (10mg each) was dissolved in freshly distilled DMF (10 ml) in serially labeled sterile test tubes, thusgivingafinalconcentrationof100µgm/0.1ml; similarly, 150µgm/0.1mlconcentrationswere also prepared.

PREPARATION OF STANDARD SOLUTION

The standard compound Vancomycin (250 mg) was dissolved in freshly distilled water (10 ml) in serially labeled sterile test tubes, thus giving a final concentration of 100µgm/0.1 ml; similarly, 150µgm/0.1 ml concentration was also prepared.[7]

METHOD OF TESTING

The method depends on the diffusion of an antibiotic from a cavity through the solidified agar layer in a petri dish to an extent such that growth of the added microorganisms is prevented entirely in a circular area or zone around the cavity containing a solution of test compounds. About 15-20 ml of molten nutrient agar was poured into each of the sterile petri dishes. The cups were made by scooping out nutrient agar with a sterile corkborer. The agar plates so prepared were divided into different set and each set of the plates were inoculated with the suspension of particular organism by spread plate technique. The cups of inoculated plates were then filled with 0.1 ml of the test solution; the plates were then incubated at 370C for 24 hours. The zone of inhibition (diameter in mm) developed, if any, was then measured for the particular compound with each organism. The solvent DMSO was used as negative-control to know the activity of the solvent. The results of MRSA testing are summarized in the following Table. The tested compounds are then compared with that of standard drug used i.e., Vancomycin to measure the activity of the compounds.

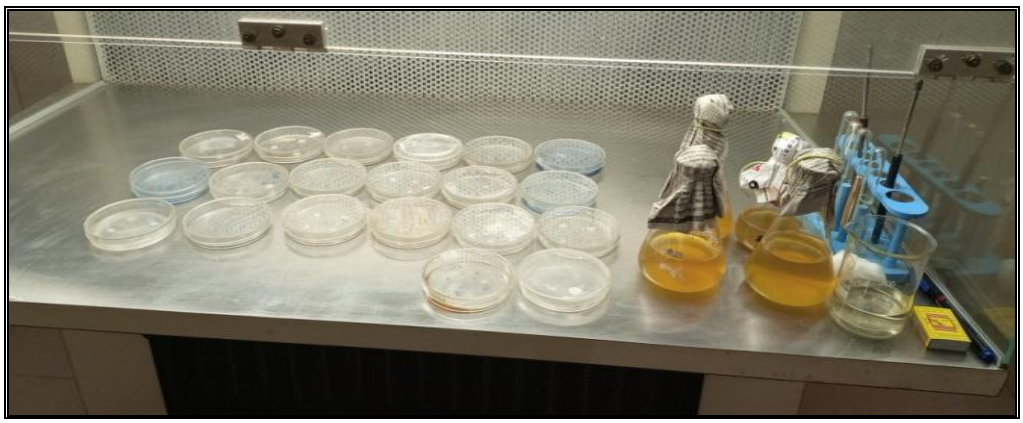


Fig. 08: Inoculation of bacteria and drugs (STD and sample) into Agar media.

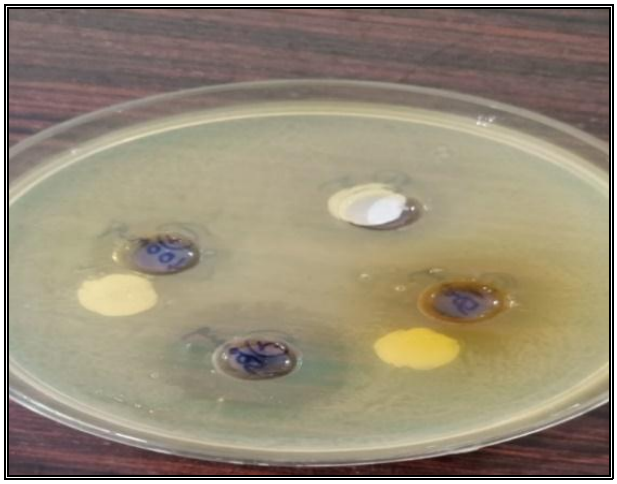


Fig. 09: Zone of inhibition in DR-IIA.

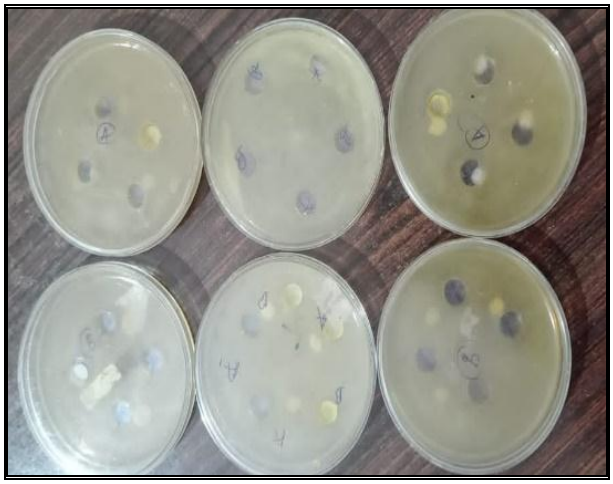


Fig. 10: Zone of inhibition in all plates.

Table No. 07: Zone of Inhibition of Methicillin resistant antibacterial activity.

Compound Code	Inhibition zone diameter in mm (Average triplicate± Standard deviation)	
	S. Aureus	
	100 µgm.	150 µgm.
DR-IIA	16	20
DR-IIB	09	14
DR-IIC	12	15
DR-IID	15	19
DR-IIE	13	16
DR-IIF	15	18
Vancomycin	19	24

- 0-15mm Zone of inhibition Average Methicillin resistant antibacterial activity
- 15-20 mm Zone of inhibition Good Methicillin resistant antibacterial activity
- 20-25mm Zone of inhibition significant Methicillin resistant antibacterial activity

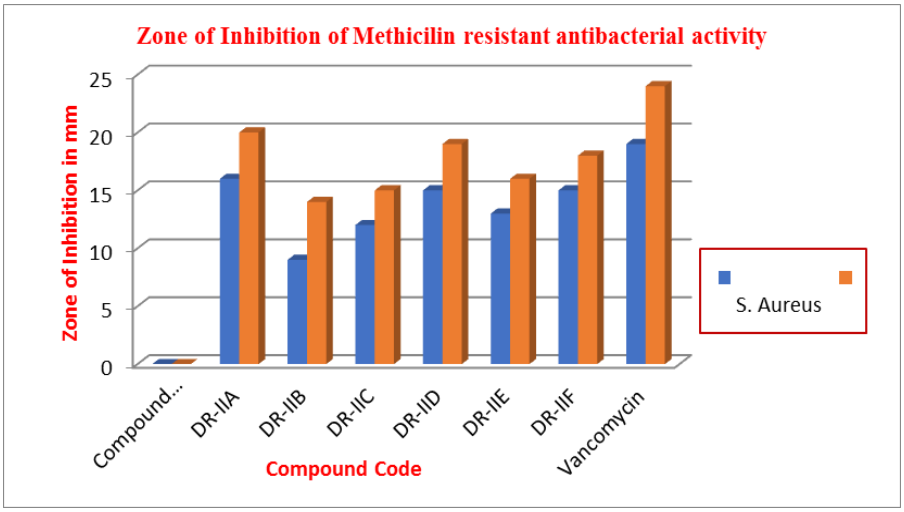


Fig. 11: Zone of Inhibition of Methicillin resistant antibacterial activity.

RESULT AND DISCUSSION

From the literature survey it reveals that the substituted quinoxaline has been reported for number of pharmacological activities and some molecules have shown significant activities and some compounds shows moderate and good activities. Here we have synthesized some novel derivatives of 3,6-methyl quinoxaline-2(1H)-thiol [DR-II] analogues and screened them for their antibacterial activities.

The purity and homogeneity of the synthesized compounds were preliminary checked by M P, TLC and FT-IR Spectroscopy. The final compounds were found to be soluble in organic solvents. These compounds were subjected to FT-IR spectra 710-840 cm^{-1}(-C-S) stretching proves formation of title compound and these derivatives (DR-IIA-IIF) carried out for biological evaluation.

BIOLOGICAL EVALUATION

All the newly synthesized derivatives of compounds 3,6-methyl quinoxaline-2(1H)-thiol [DR-IIA-IIF] were assayed in vitro for their antibacterial activity against *Staphylococcus aureus* (Gram-positive bacteria. The minimum inhibitory concentration (MIC) value for antibacterial activity of compounds was determined by the cup plate method by using nutrient agar media (NAM). For comparison, Vancomycin was used as the reference antibacterial agents. The compounds DR-IIA and DR-IID have shown good activity and remaining shows poor activity against bacteria.

Literature survey reveals that electrons-withdrawing or donating groups amend the Lipophilicity of the test compounds, which in turn alters permeability across the bacterial cell membrane. Further, the presence of electron-withdrawing groups showed maximum antimicrobial activity.

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