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Antibacterial and antifungal activities of tobramycin, gentamycin, neomycin and amikacin derivatives, derived from vanillin

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ABSTRACT

Vanillin was used to synthesis a number of derivatives of Tobramycin, Gentamycin, Neomycin, and Amikacin. The creation of the imine bond and its reduction with NaBH4 were accomplished. The imine bond was confirmed by stretching band 1650cm-1 between 1625cm^{-1} and 1660cm^{-1} by FTIR, which displayed absorption at 400nm wave length in UV-Visible Spectroscopy. Antibacterial and antifungal activities were tested on *S. Aureus, Bacillus Sb, E.Coli, and Salmonella Sb*, and it was shown to be two times that of parent medicines while mild antifungal activities were found.

Key Words; - Aminoglycosides, Derivatives, Vanillin, Synthesis, Antibacterial and antifungal

1. INTRODUCTION

Antibiotics are medicines that inhibit the growth or destroy microorganisms; these are also called antibacterial or antimicrobial drugs [1, 16], and used in treatment and prevention of infections created by bacteria. The searching of new antibiotics is very important due to extensive drug resistant strains of microorganisms in throughout the world [2]. This serious health issue demands to discover or develop new class of antibiotic compounds to overcome the resistance of bacteria and fungi [3].

Aminoglycosides are very important group of antibiotics which are natural or semi synthetic derived from actinomycetes [4]. These are among the first antibiotics which were used clinically. Amino glycoside synergizes with a variety of other antibacterial classes to treat multi resistant bacterial strain infections [5]. Amino glycoside antibiotics are used in the treatment of severe infections of the abdomen, eyes and urinary tract infections, they act by the way of protein inhibition mechanism on bacteria [6]. Some selected amino glycoside antibiotics are shown in Fig. 1.

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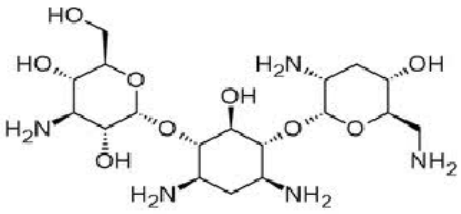

Fig 1a; - Tobramycin

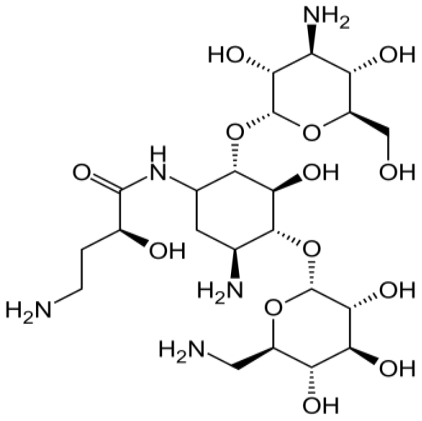

Fig 1b;- Amikacin

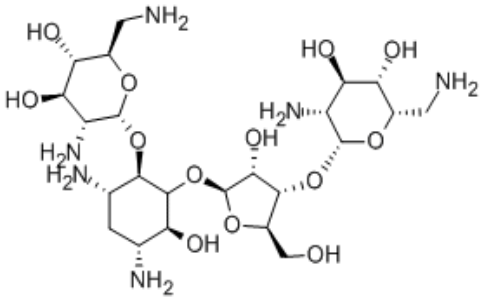

Fig 1c; - Neomycin

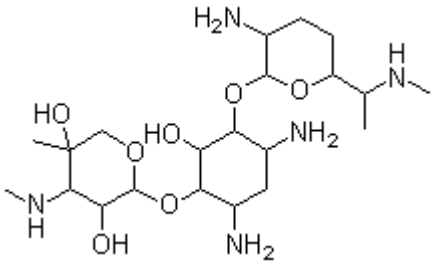

Fig 1d; - Gentamycin

In this research study the imine intermediates of amino glycoside were synthesized and the bacterial activity was found two fold then original drugs [7].

2. MATERIAL AND METHOD

Reagents and Media

Antibiotic agar, Sabouraud dextrose agar, S. Aureus ATTC 6538 Bacillus Sb, ATTC 6633, Salmonella Sb ATTC 14028, and candida Albicans ATTC 10231 from Mosa Jee Adam (Pvt) Ltd Karachi. Vanillin 99.0% from Sigma Aldrich. Merck (Pvt) Ltd provided sodium phosphate monobasic and sodium phosphate Dibasic in a 99.0% percent assay, and it was used without further purification. Ophth Pharma Private Ltd provided samples of selected aminoglycosides such as Tobramycin Gentamycin, Neomycin and Amikacin etc.

Instrumentals

The UV-Visible spectra were recorded on a Shimadzu spectrophotometer 1601 with a quartz cell of 1.0 cm path length. FTIR Agilent technologies were used to capture infrared spectra in the 4000 – 400 cm-1 range. The ^{1}HNMR spectrum was recorded at 400MHz using tetramethylsilane as the internal standard. All materials were weighed on a semi-microelectronic balance (Shimadzu Japan Libror AEX 120).

Preparation of reagents and solutions

Vanillin 2.0% Solution;-

Weighed 2.0gm vanillin into a 100ml volumetric flask, added 10 ml methanol to increase solubility, and filled with water to make up the volume.

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Stock solution of Tobramycin, Gentamycin, Neomycin and Amikacin Sulfate;-

50mg of each antibiotic were accurately weighted in an electronic balance and transferred into 50ml volumetric flasks.

Phosphate Buffer pH 7.04;-

It was taken 6.8 gm sodium phosphate dibasic and 2.5 gram sodium phosphate mono basic and carefully transferred into 1000 ml flask and water was used as solvent. The final concentration of solution was made 0.05M. The pH of solution was maintained by HCl and NaoH up to 7.04 ± 0.1.

Nutrient Agar Preparation;-

SDA and Antibiotic Agar media were accurately weighed at 15.0 gram each and transported to separate media bottles, which were then diluted with distilled water. The media was autoclaved for 15 minutes at 121°C and 1.5 psi pressure.

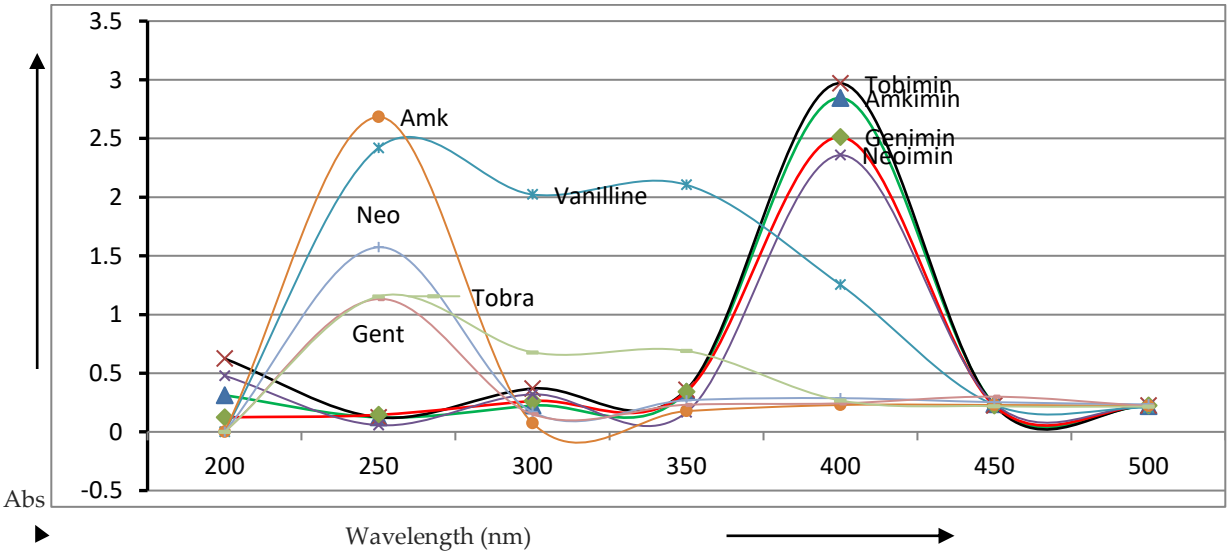
Preparation of Tobramycin, Gentamycin, Neomycin, and Amikacin imine intermediates (Schiff bases)

Vanillin was grafted onto the primary amino groups of Tobramycin, Gentamycin, Neomycin, and Amikacin, resulting in the creation of Schiff bases. In separate beakers for each antibiotic, the vanillin (20.0mmol) and the antibiotics (10.0mmol) were combined. After 30 minutes of heating the reaction mixtures in a water bath at 90-100°C, a pale yellow tinted product was generated. Sodium Borohydride was used to reduce the imine bond (NaBH4).

3. RESULTS AND DISCUSSION

Maximum wave length (λ – max)

The maxima and minima of the reaction mixture were determined by using a UV-Visible spectrophotometer and scanning from 700nm to 200 nm. The new bands were discovered in the ultraviolet-visible spectra of imine intermediates. These bands are not produced by aminoglycosides or vanillin on their own. The formation of imine intermediates causes the appearance of a longer wavelength absorption band in the visible region of UV-Visible spectra. Figure 02 depicts the UV-Visible spectra of reagents and intermediates. The presence of imine bond formation between vanillin and aminoglycosides is clearly identified by the stretching band 1650 cm-[1] between 1660-1624 cm-[1].



Tobimine;- imine intermediate of Tobramycin, Amkimine;- Imine intermediate of Amikacin, Genimie;- I imine intermediate of gentamycin, Neoimine;- Imine intermediate of Neomycin

Fig 02;- Spectrograms of Intermediates, Vanillin and aminoglycoside antibiotic

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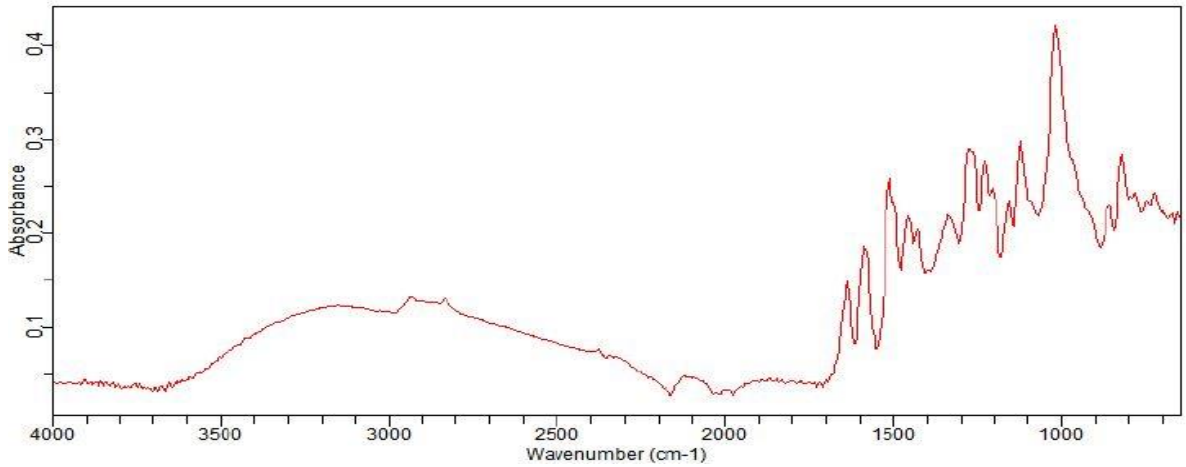

Fig 3a;- FTIR Spectra of Product

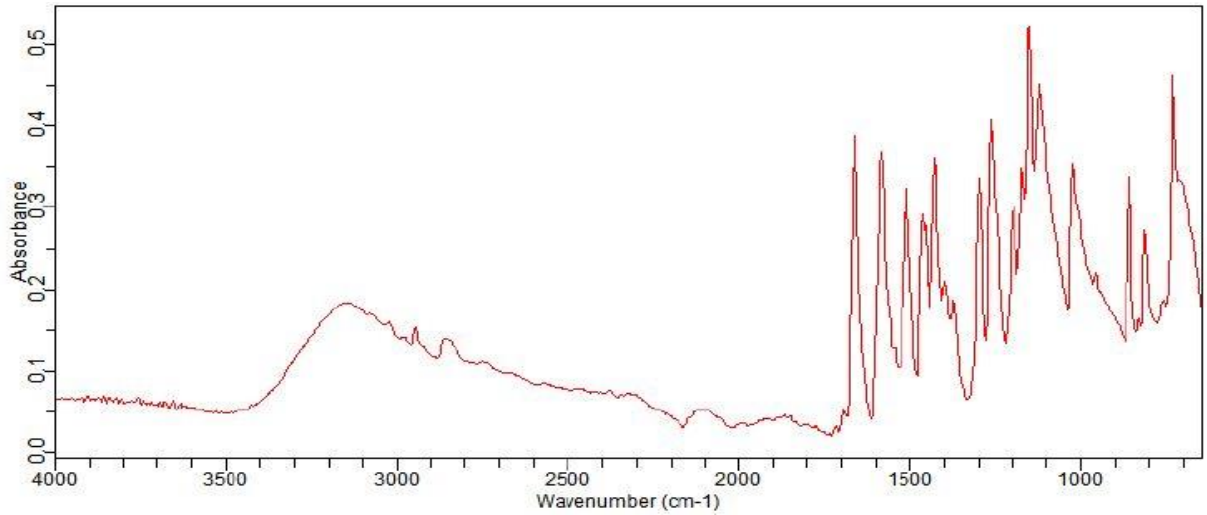

Fig 3b;- FTIR Spectra of Vanillin

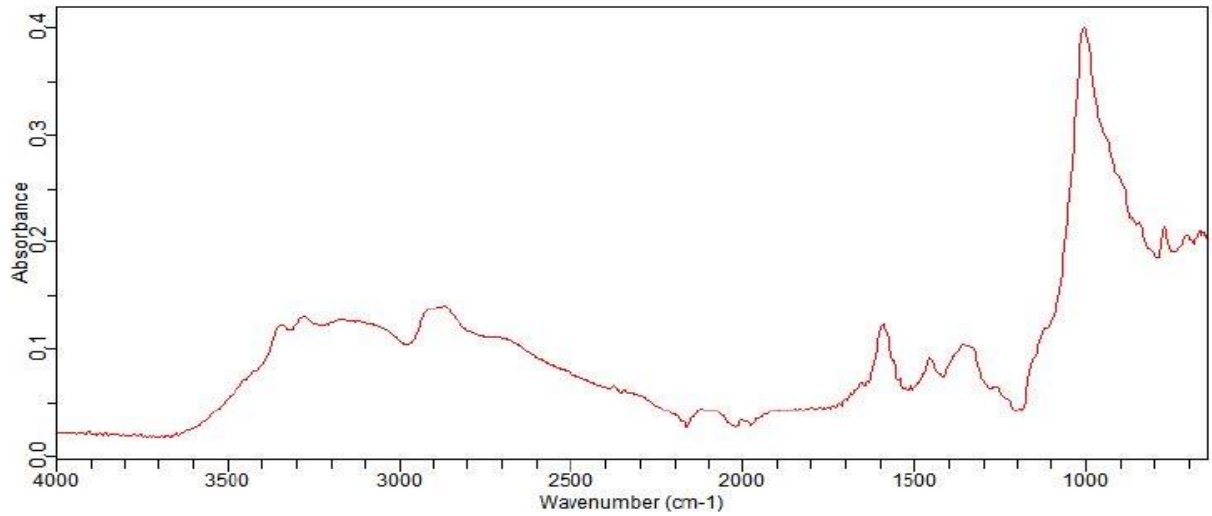

Fig 3c;- FTIR Spectra of Tobramycin

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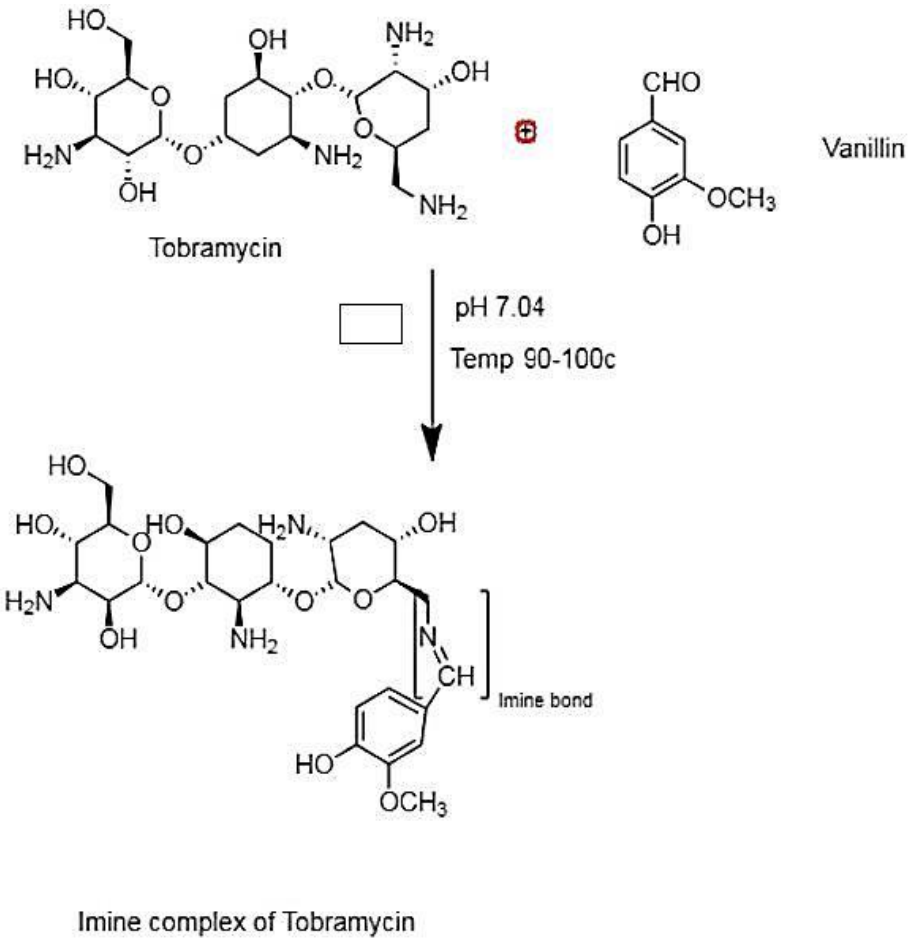
Suppose reaction for imine;-



Imine complex of Tobramycin

Fig 3d;- Zone Of inhibitions of imine intermediates in S. Aureus and E.coli

Determination of Mole Ratio between Selected aminoglycoside antibiotics and Vanillin;-

The mole ratio between Vanillin and Tobramycin, Amikacin, Neomycin and Gentamycin were determined by using the mole ratio method. The experimentally determine mole ratio is 1:2.The obtained representation was shown in Fig 4;-

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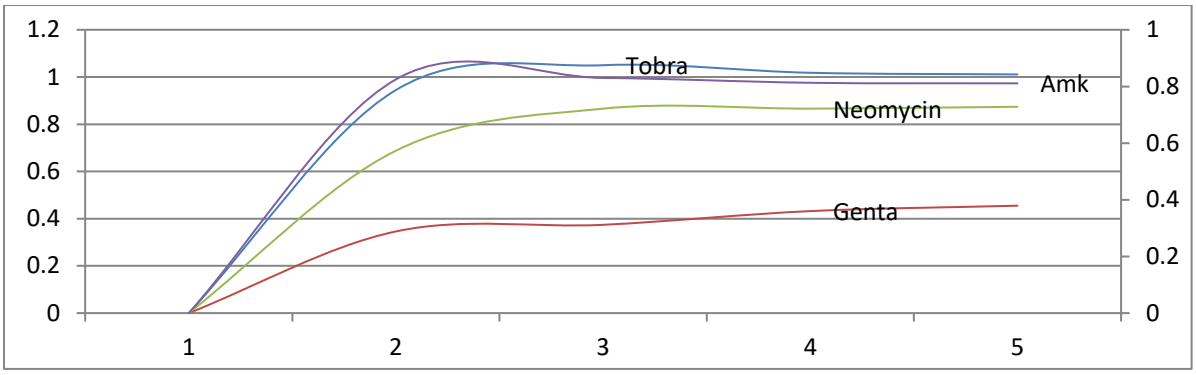

Fig 4;- Spectra for Mole Ratio

Antibacterial Activity of synthesized imine intermediates as to generic drug

The antibiotic activity of Tobramycin, Gentamycin, Neomycin, and Amikacin synthesized imine intermediates against gram positive (S. Aureus ATTC 6538 Bacillus Sb ATTC 6633) and gram negative (E.Coli Attc 8739 and Salmonella Sb Attc14028) bacteria was determined using the disc diffusion method on nutrient Agar medium. 1 ml of previously prepared inoculum of each bacterial strain was poured into sterile Nutrient Agar medium and mixed vigorously at 40-45 c°. In 90 cm petri plates, 15 ml media was poured. The 10 mm diameter containing bore was used to drill holes in Agar medium, and 100 µl of standard and imine intermediate solution was injected into the holes. The media plates were covered and incubated at 37 ± 2°C for 24 hours to determine the size of any zones of inhibition, which were tabulated in Table 01.

The antifungal activity of the produced compounds against Candida Albicans was tested using the poisoned food technique. The Sabouraud Dextrose Agar (SDA) media was prepared, and 15 ml was put into 90 mm petri plates, with the 7-day-old fungus culture smeared evenly throughout. Bore was used to create the 10 mm holes, and the sample was then injected. The petri plates were incubated for 7 days at 26°C. The zone was measured after incubation, and replicates for each treatment were kept.

Table 1 Antibacterial and antifungal Activities (Zone of inhibitions)

Strain type	*Tobimine (mm)	*Amkimine (mm)	*Neoimine (mm)	*Genimie (mm)	Tob (mm)	Amk (mm)	Genta (mm)	Neomycin (mm)
S. Aureus	25.2	30.2	27.4	25.6	16.4	17.6	13.7	15.2
Bacillus	21.9	23.25	26.1	25.8	17.0	16.8	11.9	12.3
E.Coli	19.6	25.2	20.5	19.5	16.8	16.5	14.4	10.2
Salmonella	25.5	20.9	22.3	27.1	17.1	16.2	13.2	10.5
Candida Albicans	9.4	-	-	10.1	-	-	-	-

*Tobimine;- imine intermediate of Tobramycin, Amkimine;- Imine intermediate of Amikacin, Genimie;- I imine intermediate of gentamycin, Neoimine;- Imine intermediate of Neomycin

4. CONCLUSION

In the future, the synthesized vanillin derivative could play a vital role as a novel antibiotic molecule, perhaps saving lives. Their primary purpose is to inhibit gram positive and gram negative bacteria, and their use as a medicinal product may meet the demand for novel antibiotics to combat resistant microbes.

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Ethical approval

Not applicable.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

Data and materials availability:

All data associated with this study are present in the paper.

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