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DESIGN, MICROWAVE ASSISTED SYNTHESIS AND ANTIBACTERIAL EVALUATION OF NEPHROTOXICITY FREE AMINOGLYCOSIDE SCAFFOLDS

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ABSTRACT

A series of some Neomycin derivatives were efficiently synthesized by react with Di-tert butyl dicarbonate (Boc), Triethylamine and methanol. This work aim is to synthesis some new potent antibacterial compounds of neomycin. The GLIDE score were obtained by using GLIDE module (version 9.1, Schrodinger, LLC, New York, 2010). We have designed and synthesis 20 compounds and docked with protein 4B3R (crystal structure of 30s ribosome of thermos thermophilus). Compound SP2, SP3 and SP9 have best Glide score as comparably Neomycin. *In-vitro* antibacterial evaluation of all synthesized compound (SP1-SP20) were performed by cup borer and well diffusion assay method. The compound SP3 shows good zone of inhibition, IC50 value 12.33mg/ml as compared to neomycin as standard. The characterizations of synthesized compounds were performed by TLC, Melting point and various spectroscopic techniques.

KEYWORDS

Antibacterial activity, Aminoglycoside, Drug Design, Microwave assisted synthesis and Toxicity.

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INTRODUCTON

Toxicity

Toxicity is defined as the sum of adverse effect or the degree of danger posed by a substance to any living organism. In other words toxicity is the degree to which a chemical substance or a particular mixture of substances can damage an organism. Toxicity can be referred to as the effect on complete organism, such as an animal, bacterium, or plant, also the effect on a substructure of the organism, like a cell (cytotoxicity) or on an organ such as the liver (hepatotoxicity).

Toxicity is expressed as a dose response relationship which involves the quantity of substance to which the organism is exposed and the route of exposure: skin (Absorption), mouth (Ingestion), and respiratory tract (Inhalation).

Types of Toxicity

Toxicity is generally classified according to the adverse effect produced by the substance in terms of exposure. There are 3 subclasses according to the criteria as mentioned below:

Acute Toxicity

Harmful effects produced through single or short term exposure.

Chronic Toxicity

Harmful effects produced through repeated or continuous exposure over an extended period.

Sub-chronic Toxicity

Harmful effects produced through repeated or continuous exposure over twelve months or more but less than the normal lifespan of the organism.

Drug Induced Toxicity

When any drug produces effects other than therapeutic response or any adverse effect after administration than the toxicity is termed as Drug Induced Toxicity. Drug induced toxicity is also referred as Adverse Drug Reactions. There are several types of drug induced toxicity; among them the main ones are namely:

- Cancer Formation (Carcinogenicity).
- Liver Toxicity (Hepatotoxicity).
- Kidney Toxicity (Nephrotoxicity).
- Skin Irritation/Inflammation.
- Cell toxicity (Cytotoxicity), etc.

Aminoglycosides

These are polycationic, hydrophilic, amine containing carbohydrates which are generally composed of three to five rings. Most of these aminoglycosides can be either natural products or may be the derivatives of soil actinomycetes.

They are often secreted by these actinomycetes as mixtures of closely related compounds. The chemical structure of aminoglycoside results in a binding with the anionic outer bacterial membrane and to anionic phospholipids present in the cell membranes of mammalian renal proximal tubular cells.

The bactericidal effects of these compounds is obtained from the former, while the latter binding is responsible for their toxicity. Because of their hydrophilicity, the transport of aminoglycosides across the hydrophobic lipid bi-layer of eukaryotic cell membranes is impeded.

The major clinically important aminoglycosides are amikacin (*Amikin*), gentamicin (*Garamycin*), kanamycin (*Kantrex*), netilmicin (*Netromycin*), neomycin (*Mycifradin*), streptomycin, and tobramycin (*Nebcin*).

The antibacterial action of these aminoglycosides involves two synergistic effects. Firstly, the binding of positively charged aminoglycoside to negatively charged sites present on the outer bacterial membrane, by which it disrupts membrane integrity. The rapid concentration dependent bactericidal effect of these compounds depends on the aminoglycoside induced bacterial outer membrane degradation.

Secondly, these aminoglycosides bind to various sites on bacterial 30S ribosomal subunits, interfering in the initiation of protein synthesis and producing errors in the translation of messenger RNA to peptides. These also bind at some sites on bacterial 50S ribosomal subunits, although its significance of binding is uncertain, also they have a post antibiotic effect which means they continue to suppress bacterial re-growth even after removal of the antibiotic from the bacterial microenvironment. The post antibiotic activity depends on ribosome disruption.

Structural Activity Relationship

Ring I and IV

Crucial for broad spectrum antibacterial activity.

Amino function at 6’ and 2’ are important for activity.

Methylation at 6’ carbon or 6’ amino confer resistance to enzymatic acetylation and do not lower antibacterial activity (e.g. gentamycin)

Removal of 3’ hydroxy or 4’ hydroxy or both confers resistance to phosphorylation and do NOT reduce antibacterial activity.

Phosphorylation of 3' hydroxyl reduces binding to 30S subunit.

Ring II

Modification at II ring generally results in LOSS of antibacterial activity, except

Acetylation at 1 of amino group does not alter antibacterial activity.

The ring may be ribose, Streptose (5 membered ring) or Streptamine (6 membered ring) which generally depends on type of aminoglycoside

Other than these, any substitution on any position results in total loss of antibacterial activity.

Ring III

Substitution at 2 position affects the antibacterial activity like

NH2 > OH > H

Toxicity of Amino Glycosides

Nephrotoxicity is also caused by Aminoglycosides, and their relative toxicity can be correlated with the number of constituent amine groups that each contains, for example neomycin is the most nephrotoxic and streptomycin is the least. Although their polycationic structure prevents their entry into most cells, aminoglycosides can diffuse from the tubular lumen across the apical membrane of proximal renal tubular cells following drug filtration through the glomerulus.

Passage of the aminoglycosides across the apical membrane occurs via a saturable process of adsorption of polycationic aminoglycoside molecules to the proximal renal tubular lumen's anionic brush border and subsequent endocytosis and accumulation in lysosomes. Once the drug is within the lysosomes, it will bind to anionic phospholipids, inhibiting lysosomal phospholipase A2. This leads to lysosomal distension, rupture, and release of acid hydrolases and the aminoglycoside into the cytosol. Free aminoglycoside then binds to other cellular organelles.

The accumulation of Gentamicin in mitochondria displaces Ca, which leads to mitochondrial degeneration and cell necrosis. The necrotic cellular debris then sloughs off and is passed in the urine, leaving a denuded basement membrane. The development of toxicity depends upon the duration of aminoglycoside therapy and the mean trough blood plasma drug concentration. Nephrotoxicity is more prone in aminoglycoside- treated patients with gram-negative bacillary bacteremia than in those with staphylococcal bacteremia.

Nephrotoxicity is very usual and severe in patients with extrahepatic biliary obstruction, hepatitis, or cirrhosis. The severity of aminoglycoside nephrotoxicity is additive with that of vancomycin, polymixin, gallium, furosemide, enflurane, cisplatin, and cephalosporins. Aminoglycoside nephrotoxicity goes along with the toxicity associated with amphotericin B and cyclosporine. Even quite severe aminoglycoside-induced Nephrotoxicity is nearly always reversible upon prompt discontinuation of the drug. Verapamil and Ca can lessen the nephrotoxicity, but the latter may also inhibit the antibacterial effect of the aminoglycosides.

Aminoglycoside nephrotoxicity can be reduced by Polyaspartic acid which is a promising new agent, even though it may also partially inhibit the drug's antibacterial activity. Aminoglycosides accumulate in otolymph and can cause both vestibular and auditory ototoxicity, both of which can be irreversible. Uptake is driven by the concentration gradient between blood and the otolymph; this process is saturable. Sustained high concentrations in otolymph first destroy hair cells that are sensitive to high-frequency sounds. Streptomycin causes more of vestibular toxicity than ototoxicity. The co-administration of vancomycin, furosemide, bumetanide, and ethacrynic acid increases the severity of aminoglycoside induced ototoxicity. Ca may lessen the ototoxic effect.

Aminoglycosides can cause neuromuscular junction blockade by displacing Ca from the neuromuscular junction, inhibiting the Ca-dependent prejunctional release of acetylcholine and blocking postsynaptic acetylcholine receptor binding. This is usually clinically significant only in patients with myasthenia gravis, hypocalcemia, or hypermagnesemia or when the aminoglycoside is given shortly after the use of a neuromuscular blocking agent. The neuromuscular blockade can be reversed by administration of intravenous calcium.

Nephrotoxicity results in increased serum creatinine level of reduced creatinine clearance.

Drug Design

Drug Discovery is a multidisciplinary, and acumen concentrated process. Present day tranquilize outline procedures can make medicate disclosure process more productive and sane.

Drug design and configuration is an iterative procedure, which starts when a chemists recognizes an aggravation that show an intriguing biological profile and ends when both the action profile and the chemical synthesis blends of new chemical entity (NCE) are streamlined.

Drug design is a method that is utilized for designing and inventing new molecules based on the knowledge of the desired biological target. It is a novel method that focuses on the molecule design and binding of the molecule towards its target protein.

Type of Drug Design

Structure-based Drug Design

This is a process of drug designing based on knowledge of three dimensional structure of the active site. This mainly involves Docking, which is defined as the computational methods that is used to predict the binding efficiency and ability of a molecule to a defined or specified target. High-throughput docking has become increasingly important in the field of drug discovery process.

Regardless of the technical problems in efficient prediction of the binding of a molecule and its binding efficiency to other molecules has yielded a significant success as compared to the random screening of the active molecules. A variety of software's are readily available for docking, selection of which depends on the scoring, binding energies and requirement of flexible or rigid docking. Examples: De novo drug design, docking, etc.

Ligand-based Drug Design

Ligand based drug design is an approach used in the absence of the receptor 3D information and it relies on knowledge of molecules that bind to the biological target of interest. 3D quantitative structure activity relationships (3D QSAR) and pharmacophore modeling are the most important and widely used tools in ligand based drug design. They can provide predictive models suitable for lead identification and optimization.

Docking

Docking is a computational method that is used for estimation defines structure binding in the dynamic active site of the target. This joins choosing the presentation of escalate, its conformational geometry and the scoring. Score is a coupling imperativeness, free imperativeness, or a subjective numerical measure. By one means or another, each docking figuring subsequently put molecule in various presentations and adjustments in dynamic site, and a while later procedures a score for each. A couple of ventures store data for most of the attempted presentations, yet most simply keep some with best docking scores. Docking might be used to dismember tens or an immense number of blends through the traverse of a multiyear configuration.

Types of Docking

There are 2 types of docking that are utilized in drug design:

Rigid Docking

Rigid dock include the geometry of ligand and receptor as constant and then docking is performed.

Flexible Docking

A list on the turns of one of the particles (normally smaller one) is performed. In each revolution the surface cell inhabitancy and vitality is computed, later the most ideal posture is chosen.

Major Steps in Molecular Docking

Building the Receptor

Protein's 3D structure is taken form protein data bank; later the accessible structure should to be handled. This incorporate expulsion of the water particles from the depression (cavities), balancing out the charges, filling the missing build-ups, era of the side chains and so on as per the parameters accessible. The receptor should be in an organically dynamic and stable state.

Identification of the Active Site

The receptor is augmented; the dynamic site inside the receptor ought to be distinguished. Receptor has numerous dynamic destinations however the one of the interest should be chosen. Water molecules and other atoms are excluded if present.

Ligand Preparation

Ligands are taken from databases such as Zink, Pub Chem or can be portrayed utilizing Chemsketch. While choosing the ligand, it's necessary that Lipinski's Rule of 5 ought to be claimed. The administer is critical for tranquilize advancement where a pharmacologically dynamic lead structure is upgraded stepwise for expanded movement and selectivity, and additionally drug like properties as portrayed.

Docking

This is the last pace, where the ligand is docked onto the receptor and the various associations are checked. The scoring capacity creates score depending upon which the best fit ligand is chosen.

Microwave Assisted Synthesis

The bottleneck of conventional synthesis is typically the optimization, i.e. finding the optimum conditions for a specific reaction to obtain the desired products in good yields and purities. Since many synthesis reactions require at least one or more heating steps for long time periods, these optimizations are often difficult and time-consuming. Microwave-assisted heating under controlled conditions has been shown to be a valuable technology for any application that requires heating of a reaction mixture, since it often dramatically reduces reaction times – typically from days or hours to minutes or even seconds. Compounds can therefore be rapidly synthesized in either a parallel or (automated) sequential way using this new promising technology.

Most pharmaceutical, agrochemical, biotechnology and material science companies are already heavily using microwave synthesis as major methodology in their chemical laboratories. They have realized the ability of microwave technology to speed up chemical reactions and therefore their whole production process.

MATERIAL AND METHODS

Reagents and chemicals

The research work has been done in pharmaceutical chemistry lab, Department of Pharmacy SGSITS Indore. All the solvent and chemicals have been used from departmental chemical store. Neomycin (Boc) have been used from Sigma

METHODOLOGY

Molecular Docking Study using Schrödinger LLC

Structure-based docking studies was carried out using the poses predicted by docking using the Glide module v5.6 program (Schrodinger LLC., New York, USA; http://www.schrodinger.com). All the substituted neomycin structures were docked against Crystal structure of the 30S ribosome of Thermusthermophilus (PDB ID: 4B3R). Molecular docking studies involve mainly ligand preparation, protein selection and preparation, receptor grid generation, docking and further analysis of docking studies. Schrodinger software was mainly used for all the above steps.

The steps involved in docking are as follows

Ligand Preparation

All compound structures were built with Chem Draw Ultra v8.0 (Cambridge Soft Corporation, Cambrigde, MA, USA; http://www.cambridgesoft.com), and their 3D structures were further minimized with the Lig Prep v2.4 program (Schrodinger LLC, New York, USA; http://www.schrodinger.com), using the OPLS 2005 force field at pH 7.0 to generate single low energy 3-D structure for each input structure and the rest of the parameter values by default.

Protein Selection and Preparation

Protein with accession number was selected and downloaded from Protein Data Bank (PDB). This protein is reported to bind with drug. The protein was imported, optimized, minimized by removing unwanted molecules and other defects reported by the software. Protein is a crystal structure of 30S ribosome of Thermusthermophilus. For the purpose of studies, chain A was retained and other was deleted and water molecules near the ligands were retained. Finally a low energy minimized protein structure was obtained and used for further docking studies.

Grid Generation

Minimized protein was used for grid generation which involves selected ligand as the reference as it

signifies the binding sites of drug with respect to the target. The generated grid was used for further docking of new molecules.

Docking Studies

Standard Precision (SP) docking was selected for screening the ligands. Flexible docking mode was selected in which the Glide program generates conformations internally during the docking process. No request was made for any constraint for docking. Extra-precision docking was also done. This generates favorable ligand poses which are further screened through filters to examine spatial fit of the ligand in the active site. Ligand poses which pass through initial screening are subjected to evaluation and minimization of grid approximation. Scoring was then carried on energy minimized poses to generate Glide score.

Chemistry

Synthesis of Boc protected Neomycin

Triethylamine (TEA) (3.5ml) and methanol (5ml) was added to a stirred solution of neomycin (1g) in water (5ml). Once a clear solution is obtained Boc (2.5g) was then added, and the resultant reaction mixture was taken in G30 vial. Vial was then placed in the reaction cavity of Anton Paar Monowave 300. Reaction mixture was stirred at 600 rpm at elevated temperature and pressure (65°C, 7 bar) for 1 hr. Vial was taken out after completion of reaction. Methanol was removed by evaporation and the residue was partitioned between ethyl acetate (100ml) and water (50ml). The aqueous layer was extracted with fresh ethyl acetate (2 X 25ml) and the combined organic layer was dried by sodium sulphate (Na_2SO_4). Organic layer was then evaporated in vacuum to obtain slightly cream colored crystals of Boc protected neomycin.

Synthesis of Substituted Neomycin

A solution of Boc protected neomycin (1g, 0.82mmol) and substituting compound (0.82mmol) in dry pyridine (20ml). The reaction mixture was then transferred to G30 vial. Vial was then placed in the reaction cavity of Anton Paar Monowave 300. Reaction mixture was stirred at 600 rpm at elevated temperature and pressure (40°C, 7 bar) for 0.5 hr. After completion of reaction the vial was taken out. Pyridine was removed by co-evaporation with toluene. The crude residue was then dissolved in ethyl acetate (100ml) and washed with water (2 X 50ml). The organic layer was dried by sodium sulphate (Na_2SO_4). Organic layer was evaporated in vacuum to obtain crystals of substituted neomycin.

Synthesis of De-protected Substituted Neomycin

Boc substituted neomycin in methanol (10ml) was dissolved in a solution of HCL in methanol (2M). The reaction mixture was then transferred to G30 vial. Vial was then placed in reaction cavity of Anton Parr Monowave 300. The reaction was stirred at elevated temperature and pressure (35°C, 6 bar) for 0.5 hr. After completion of reaction the vial was taken out and pH was adjusted to 8-9 with NaOH (2M). Reaction mixture was dissolved in ethyl acetate (100ml) and was washed with water (2 X 50ml). The organic layer was dried by sodium sulphate (Na_2SO_4). Organic layer was evaporated in vacuum to obtain pure crystals of product.

RESULTS AND DISCUSSION

Characterization of Synthesized Compounds

Thin Layer Chromatography

Thin Layer Chromatography is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. While observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. Thin Layer Chromatography was carried out with silica gel plates (silica gel 60 F_{254}), and DCM: Methanol: Chloroform (10:1:3) was used as mobile phase. (Table No.5).

Melting Point

The melting point determination was done in melting point apparatus and is uncorrected. (Table No.5)

Clog P Values

The Clog P values, the indicative of hydrophobicity, were predicted for all the derivatives using CS Chem Office-2004 version 8.0. (Table No.5).

IR Spectroscopy

The Infrared spectroscopy of all the synthesized compounds were recorded on IR AFFINITY-1 1400

using KBr pellet technique were carried out from Indian Institute of Science and Research, Bhopal and are expressed in cm^{-1}.

Mass Spectroscopy

Mass spectra analysis was recorded using a mass spectrometer with an ESI source as m/z fragmentation pattern for molecular ion peak determination Indian Institute of Science and Research, Bhopal.

^{1}H NMR Spectroscopy

^{1}H NMR spectra were recorded using Bruker Advance II 300 MHz NMR spectrometers using Methanol-d6 as solvent at Indian Institute of Science and Research, Bhopal.

Biological evaluation

The anti-bacterial activity was performed by Cup Borer or Well Diffusion methods on human pathogenic bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*).

In assaying antibiotic by this technique the test organism is grown on a suitable complete agar medium in Petri dishes. "Cups" cut out of the agar are filled with appropriate dilutions of a standard compound solution and of the test compound. After incubation the cups are found to be surrounded by circular zones of inhibition. The zone of inhibition was then measured in terms of area in mm shown in Table No.6 and compared with the area of whole quadrant to find out the inhibition concentration 50% compared with the standard neomycin.

Table No.1: Docking score, Glide emodel energy and RMSD of (SP1-SP20) on Crystal Structure of 30S Ribosome of Thermusthermophilus (PDB ID: 4B3R)

S.No	Compound Code	Docking Score	Glide emodel	RMSD
1	SP 1	-6.6359	-34.299455	0.003514
2	SP 2	-8.9344	-36.503485	0.013627
3	SP 3	-8.1679	-31.981321	0.031968
4	SP 4	-7.8553	-39.733893	0.004822
5	SP 5	-7.1496	-41.594859	0.005643
6	SP 6	-4.0831	-40.02639	0.001676
7	SP 7	-7.9742	-38.283785	0.003188
8	SP 8	-6.3910	-33.636859	0.042798
9	SP 9	-8.9942	-28.769052	0.012978
10	SP 10	-6.6459	-26.75219	0.012151
11	SP 11	-6.3581	-38.719811	0.003107
12	SP 12	-7.1483	-39.648192	0.015184
13	SP 13	-6.0937	-26.172395	0.027509
14	SP 14	-3.7490	-42.574835	0.01317
15	SP 15	-8.2679	-41.049514	0.022274
16	SP 16	-7.7792	-37.709187	0.018373
17	SP 17	-5.8652	-28.268165	0.006275
18	SP 18	-8.2359	-43.868133	0.047954
19	SP 19	-8.2668	-44.575075	0.001505
20	SP 20	-6.1278	-37.01711	0.019348
21	NEOMYCIN	-6.7893	-31.834681	0.023428

Table No.2: Name and Structure of Substitutions

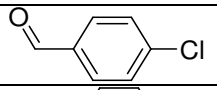
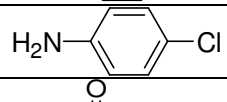
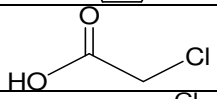
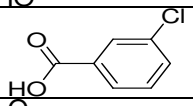
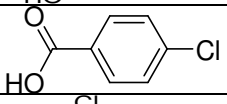
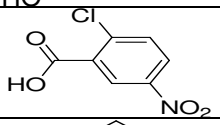
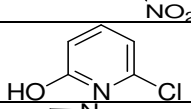
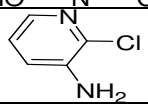
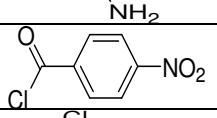
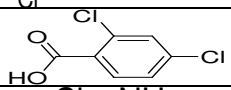
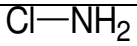
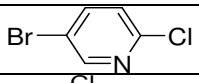
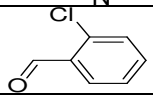
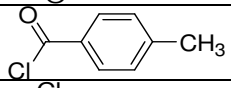
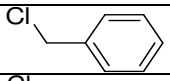
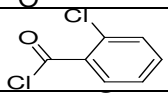
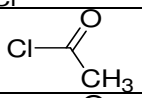
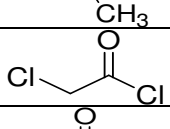
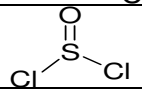
S.No	COMPOUND CODE	Structure	Name
1	SP 1		4-Chlorobenzaldehyde
2	SP 2		4-Chloroaniline
3	SP 3		Chloroacetic Acid
4	SP 4		3-Chlorobenzoic Acid
5	SP 5		4-Chlorobenzoic Acid
6	SP 6		2-Chloro-5-nitro Benzoic Acid
7	SP 7		6-Chloro-2-hydroxy Pyridine
8	SP 8		3-Amino-2-chloro Pyridine
9	SP 9		4-Nitro Benzoyl Chloride
10	SP 10		2,4-Dichloro Benzoic Acid
11	SP 11		Ammonium Chloride
12	SP 12		5-Bromo-2-chloro Pyridine
13	SP 13		o-Chlorobenzaldehyde
14	SP 14		4-Methyl Benzoyl Chloride
15	SP 15		Benzyl Chloride
16	SP 16		Benzoyl Chloride
17	SP 17		2-Chloro Benzoyl Chloride
18	SP 18		Acetyl Chloride
19	SP 19		Chloroacetyl Chloride
20	SP 20		Thionyl Chloride

Table No.3: Substitution

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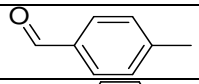
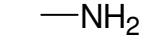
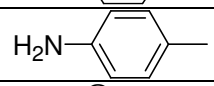
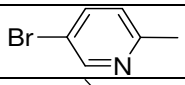
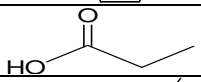
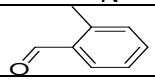
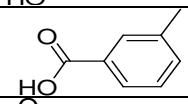
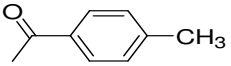
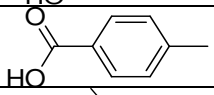
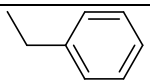
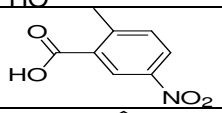
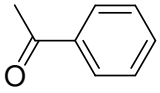
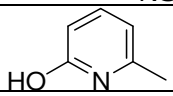
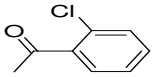
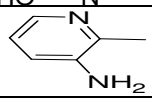
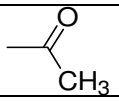
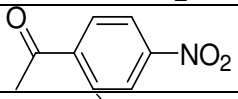
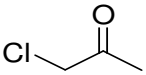
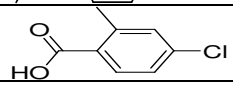
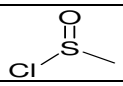
1	SP 1		SP 11	
2	SP 2		SP 12	
3	SP 3		SP 13	
4	SP 4		SP 14	
5	SP 5		SP 15	
6	SP 6		SP 16	
7	SP 7		SP 17	
8	SP 8		SP 18	
9	SP 9		SP 19	
10	SP 10		SP 20	

Table No.4: IUPAC Name of Neomycin Derivatives (SP 1-SP 20)

S.No	Compound Code	IUPAC Name
1	SP 1	4-{3-(3-Amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethoxy}-benzaldehyde
2	SP 2	5-amino-2-(aminomethyl)-6-[4, 6-diamino-2-[4-[3-amino-6-(aminomethyl)-4, 5-dihydroxyoxan-2-yl]oxy-3-hydroxy-5-(4-aminophenoxy methyl)oxolan-2-yl]oxy-3-hydroxycyclohexyl]oxyoxane-3, 4-diol
3	SP 3	{3-(3-Amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethoxy}-acetic acid
4	SP 4	3-{3-(3-Amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethoxy}-benzoic acid
5	SP 5	4-{3-(3-Amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethoxy}-benzoic acid
6	SP 6	2-{3-(3-Amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethoxy}-5-nitro-benzoic acid

7	SP 7	5-amino-2-(aminomethyl)-6-({4, 6-diamino-2-[(4-{[3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy}-3-hydroxy-5-{[(6-hydroxypyridin-2-yl)oxy]methyl}oxolan-2-yl)oxy]-3-hydroxycyclohexyl}oxy)oxane-3, 4-diol
8	SP 8	5-amino-2-(aminomethyl)-6-({4,6-diamino-2-[(4-{[3-amino-6-(aminomethyl)-4, 5-dihydroxyoxan-2-yl]oxy}-5-{[(3-aminopyridin-2-yl)oxy]methyl}-3-hydroxyoxolan-2-yl)oxy]-3-hydroxycyclohexyl}oxy)oxane-3, 4-diol
9	SP 9	4-Nitro-benzoic acid 3-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethyl ester
10	SP 10	2-{3-(3-Amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethoxy}-4-chloro-benzoic acid
11	SP 11	5-amino-2-(aminomethyl)-6-({4, 6-diamino-2-[(4-{[3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy}-5-[(aminooxy)methyl]-3-hydroxyoxolan-2-yl)oxy]-3-hydroxycyclohexyl}oxy)oxane-3, 4-diol
12	SP 12	5-amino-2-(aminomethyl)-6-({4, 6-diamino-2-[(4-{[3-amino-6-(aminomethyl)-4, 5-dihydroxyoxan-2-yl]oxy}-5-{[(5-bromopyridin-2-yl)oxy]methyl}-3-hydroxyoxolan-2-yl)oxy]-3-hydroxycyclohexyl}oxy)oxane-3, 4-diol
13	SP 13	2-{3-(3-Amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethoxy}-benzaldehyde
14	SP 14	4-Methyl-benzoic acid 3-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethyl ester
15	SP 15	5-amino-2-(aminomethyl)-6-[4, 6-diamino-2-[4-[3-amino-6-(aminomethyl)-4, 5-dihydroxyoxan-2-yl]oxy-3-hydroxy-5-(benzyloxymethyl)oxolan-2-yl]oxy-3-hydroxycyclohexyl]oxyoxane-3, 4-diol
16	SP 16	Benzoic acid 3-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethyl ester
17	SP 17	(3-{[3-amino-6-(aminomethyl)-4, 5-dihydroxyoxan-2-yl]oxy}-5-[(3, 5-diamino-2-{[3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy}-6-hydroxycyclohexyl)oxy]-4-hydroxyoxolan-2-yl)methyl 2-chlorobenzoate
18	SP 18	Acetic acid 3-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethyl ester
19	SP 19	Chloro-acetic acid 3-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethyl ester
20	SP 20	Chlorosulfurous acid 3-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-5-[3, 5-diamino-2-(3-amino-6-aminomethyl-4, 5-dihydroxy-tetrahydro-pyran-2-yloxy)-6-hydroxy-cyclohexyloxy]-4-hydroxy-tetrahydro-furan-2-ylmethyl ester

Table No.5: Melting point, R_f value and Clog P of Synthesized Compounds

S.No	Compound Code	Melting Point Range	R_f Value	Clog P
1	SP 1	144-147ºC	0.68	-6.37
2	SP 2	58-60 ºC	0.76	-6.91
3	SP 3	128-132ºC	0.60	-4.69
4	SP 4	112-118ºC	0.76	-9.12
5	SP 5	124-130ºC	0.78	-9.12
6	SP 6	139-141ºC	0.83	-9.18
7	SP 7	119-126ºC	0.67	-6.63
8	SP 8	135-138ºC	0.87	-7.53
9	SP 9	118-123ºC	0.72	-5.98
10	SP 10	109-115ºC	0.75	-8.52
11	SP 11	129-137ºC	0.73	-8.26
12	SP 12	97-104ºC	0.70	-5.94
13	SP 13	148-151ºC	0.61	-6.37
14	SP 14	133-139ºC	0.88	-5.41
15	SP 15	147-151ºC	0.59	-5.75
16	SP 16	127-133ºC	0.85	-5.92
17	SP 17	126-129ºC	0.80	-5.32
18	SP 18	103-107ºC	0.91	-6.97
19	SP 19	97-104ºC	0.60	-7.44
20	SP 20	126-131ºC	0.87	-8.05
21	NEOMYCIN	130-135ºC	0.26	-8.42

Table No.6: Zone of Inhibition Diameter

S.No	Compound Code	*Staphylococcus aureus* Gram (+)	*Pseudomanasaeruginosa* Gram (-)	*Escherichia coli* Gram (-)
1	SP 1	15 mm	13 mm	-
2	SP 2	23 mm	21 mm	19 mm
3	SP 3	23 mm	29 mm	24 mm
4	SP 4	-	9 mm	10 mm
5	SP 5	9 mm	-	-
6	SP 6	16 mm	11 mm	13 mm
7	SP 7	12 mm	7 mm	11 mm
8	SP 8	14 mm	10 mm	-
9	SP 9	19 mm	22 mm	23 mm
10	SP 10	8 mm	-	11 mm
11	SP 11	18 mm	19 mm	17 mm
12	SP 12	-	16 mm	13 mm
13	SP 13	17 mm	8 mm	16 mm
14	SP 14	10 mm	14 mm	-
15	SP 15	21 mm	26 mm	23 mm
16	SP 16	9 mm	12 mm	11 mm
17	SP 17	-	9 mm	-
18	SP 18	20 mm	22 mm	19 mm
19	SP 19	21 mm	25 mm	23 mm
20	SP 20	-	-	17 mm
21	Neomycin	14 mm	12 mm	16 mm

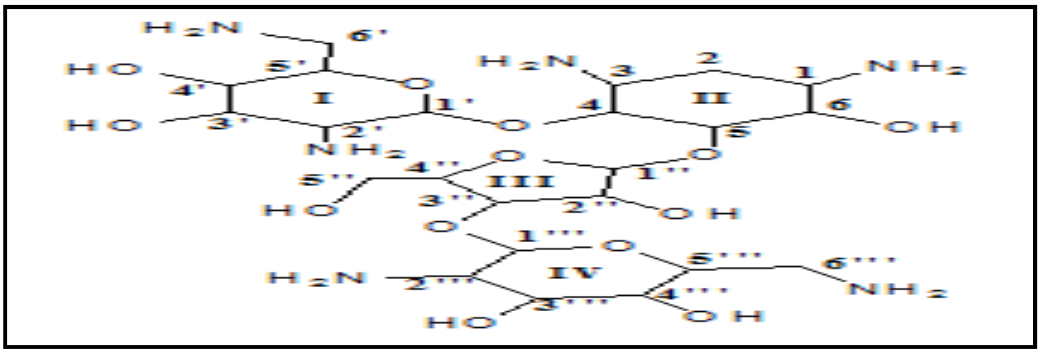
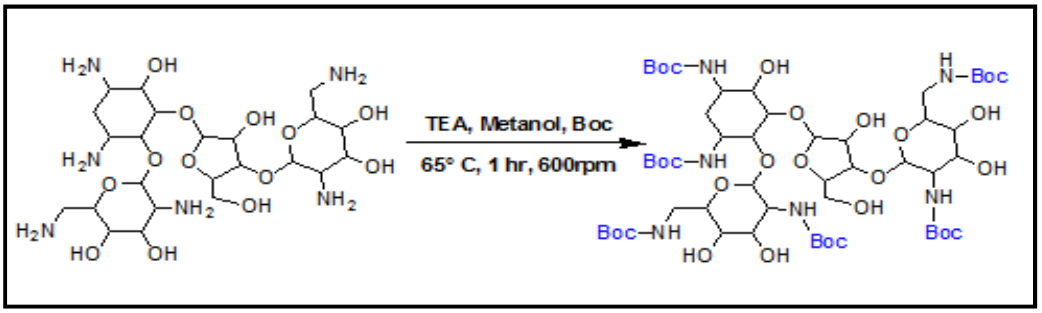
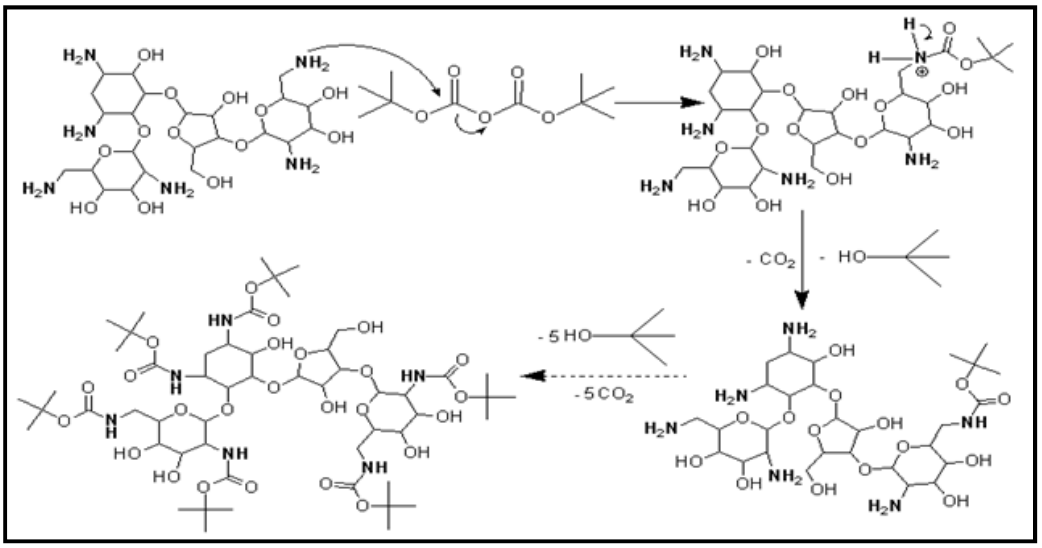


Figure No.1: Structure of Neomycin

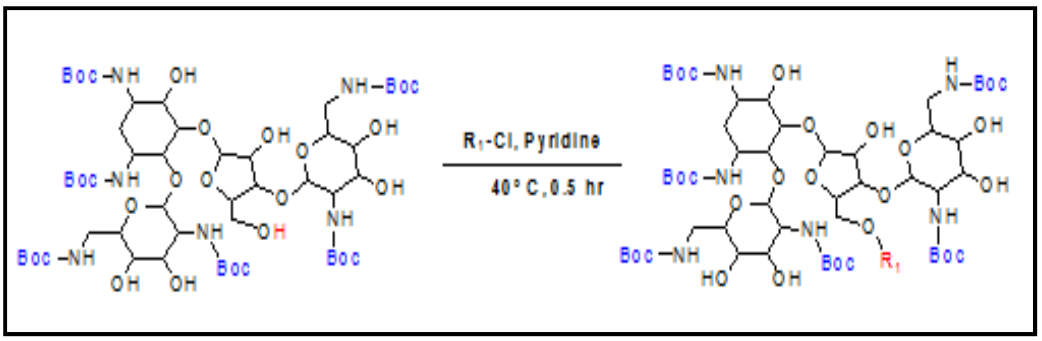
Reaction Scheme



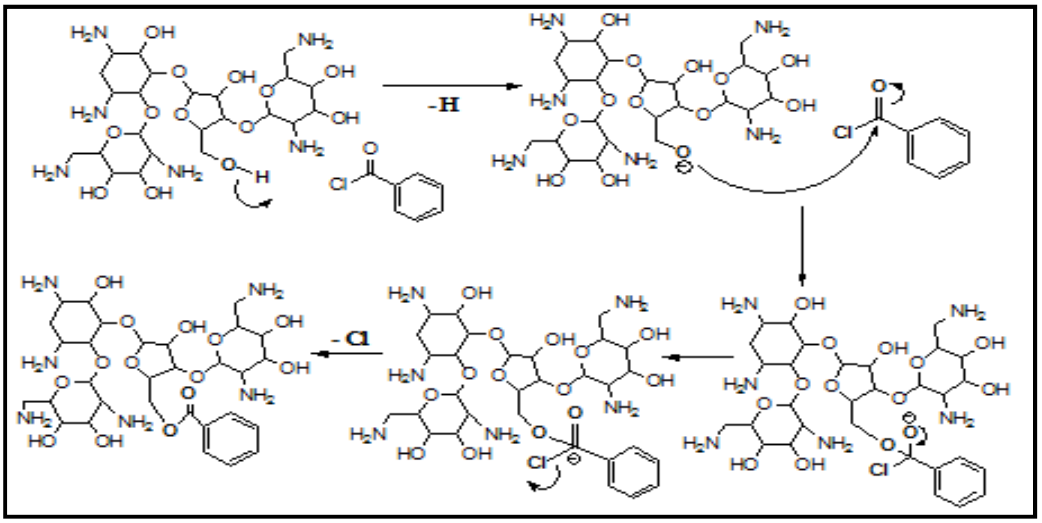
Reaction Mechanism



Reaction Scheme



Reaction mechanism



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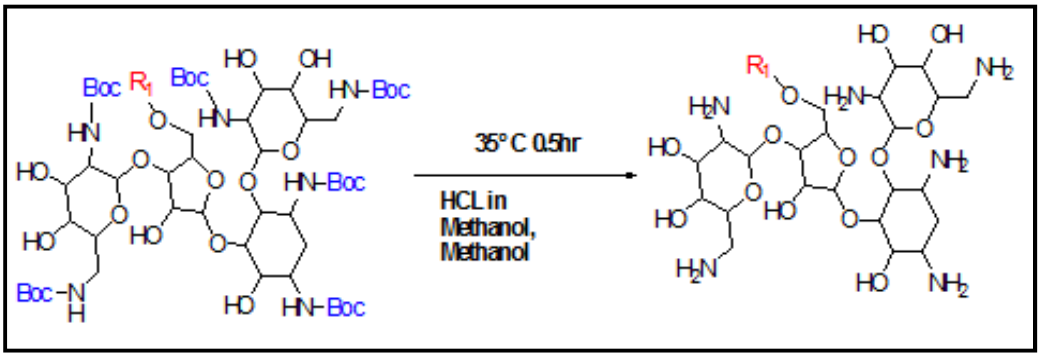

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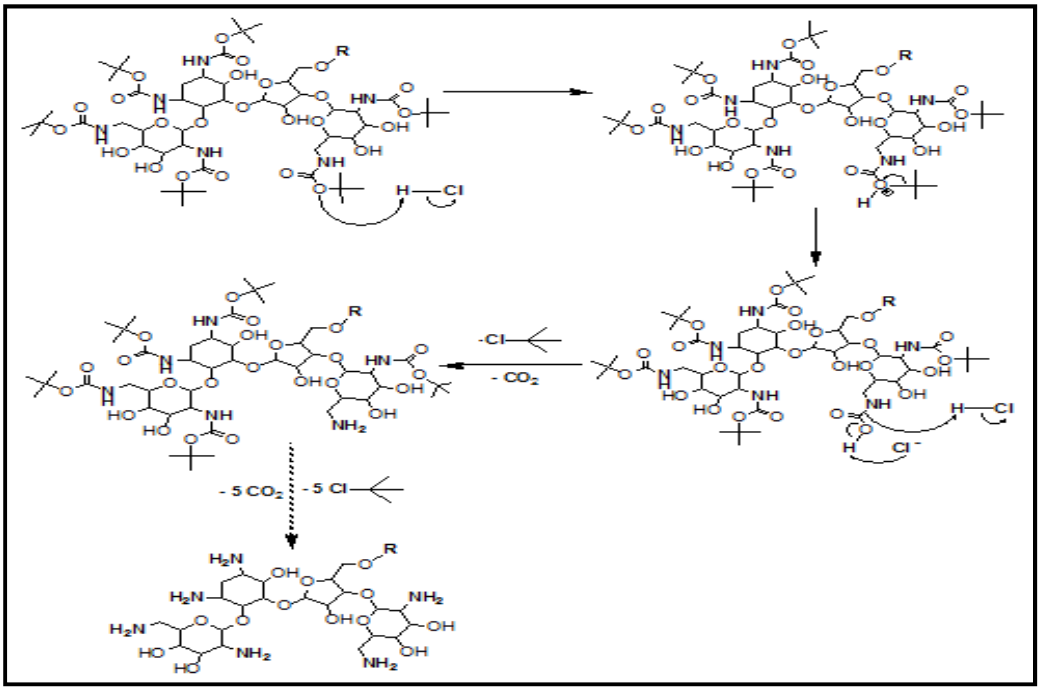

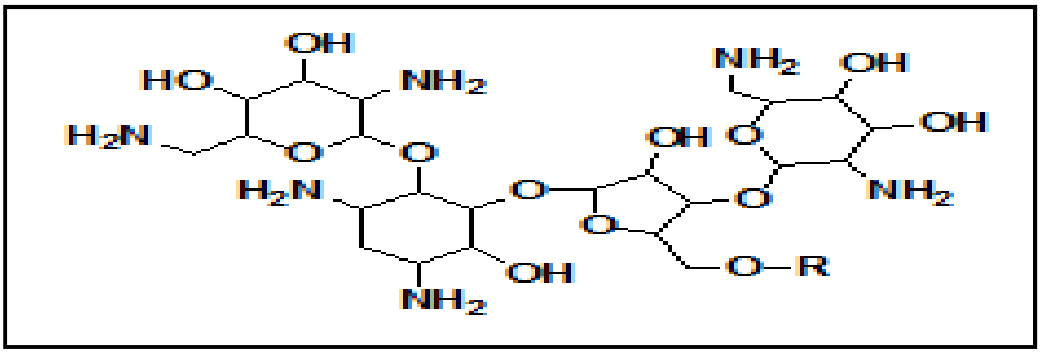

Figure No.2: Structure of Compound

CONCLUSION

All the designed compounds were synthesized by microwave assisted synthesis. Compounds namely SP2, SP3, SP9, SP15, SP18 and SP19 are found to be good Glide score against the standard neomycin. Among them SP3 is best compound as it has good values regarding docking score, emodel, RMSD. When evaluated for its antibacterial activity it also shows good zone of inhibition value along with good average IC50 value (i.e. 12.33mg/ml) as compared to parent neomycin (i.e. 56.33mg/ml). It also has maximum LD_{50} value among all the synthesized compounds (i.e. 782mg/kg) as well as its LD50 value is much more than parent molecule neomycin (i.e. 305mg/kg). After evaluating all the parameters SP3 is considered to be best derivative of neomycin in every aspect. The field is further open for extensive evaluation and further studies.

ACKNOWLEDGEMENT

I'm very thankful to Department of pharmacy SGSITS Indore, Faculty of Pharmacy, I would also like to thank the Management, for providing the necessary facilities to carry out this work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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