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Original Article

Synthesis Characterization of Sulphadiazine Scaffold: *In silico* and *in vitro* Antibacterial Activity

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ABSTRACT

Aim: The present study involves the synthesis, characterization, and evaluation of six novel Sulphadiazine derivatives (SV1-SS6) using Schiff base reactions with various aromatic aldehydes. **Materials and Methods:** The compounds were characterized by melting point analysis, TLC, FTIR, and ^{1}H NMR spectroscopy. *In silico* studies using Molinspiration, ChemSpider, and Protox-III predicted good drug-likeness, favourable absorption, and low toxicity (Class IV) for all derivatives. **Results:** Antibacterial activity was assessed against Pseudomonas aeruginosa and Bacillus subtilis using the agar well diffusion method. Among the compounds, SD3 and SS6 showed the most potent activity, particularly against *P. aeruginosa*, even at lower concentrations. **Conclusion:** The study highlights the potential of Sulphadiazine derivatives as promising candidates for further development of antibacterial agents.

Keywords: *In silico* studies, Sulphadiazine Scaffold, Schiff base reaction, Molinspiration, Protox-III.

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INTRODUCTION

Sulphadiazine, a sulfonamide antibiotic, is widely studied for its therapeutic applications due to its anti-microbial, anti-malarial, anti-cancer and anti-inflammatory properties. The modification of the basic sulphadiazine structure leads to derivatives with improved biological activity and pharmacokinetics. Sulphadiazine is a derivative of sulfanilamide where the amide group is linked to a pyrimidine ring. The key sites for derivatization include the sulfonamide Nitrogen (N1), the pyrimidine ring and the aromatic amine (para-NH_2 group).[1]

Sulfadiazine is used to treat or prevent infections in many different parts of the body. It belongs to the group of medicines known as sulfonamide antibiotics. It works by preventing the growth of bacteria. However, this medicine will not work for colds, flu, or other virus infections. Sulfadiazine is an antibiotic, used together with pyrimethamine, a dihydrofolate reductase inhibitor; it is the treatment of choice for toxoplasmosis, which is caused by a protozoan parasite.[2]

Sulphadiazine is a sulfonamide antibiotic. It is used as anti-bacterial, anti-cancer, anti-microbial, anti-inflammatory, antioxidant, anti-pyretic agents. It is used to treat many different kinds of bacterial infections, like those of the brain, ears, and urinary tract**.** Sulfadiazine is a competitive inhibitor of bacterial Para-Aminobenzoic Acid (PABA), a substrate of the enzyme dihydropteroate synthetase. The inhibited reaction is necessary in these organisms for the synthesis of folic acid and very limited penetration through the skin. Dihydropteroate synthase (DHPS) is an enzyme classified under EC 2.5.1.15. It Produces dihydropteroate in bacteria, but it is not expressed in most eukaryotes including humans. This makes it a useful target for sulfonamide antibiotics, which compete with the PABA precursor.[3,4]

In silico studies

The term *in silico* is a modern word that usually means experimentation performed by computer and is related to more commonly known biological terms *in vivo* and *in vitro. In silico* approaches are intended to identify thresholds of toxicology concern below which no toxicity is expected or provided. Database to build QSAR's structural alerts for given endpoints. *In silico* methods provide a platform for screening the activity of potential therapeutics against the molecular targets. *In silico* studies are one of the techniques used in computer aided drug development. *In silico* studies means the use of software techniques in creation of computation models to predict and provide the discoveries or advances in medicine and therapeutics,[5-7] Softwares used are Molinspiration, Protox-III, Chemdraw, Chemspider.

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Antibacterial drug resistance

Antibacterial resistance happens when bacteria develop the ability to defeat the drugs designed to kill them. Antibacterial resistance does not mean our body is resistant to antibiotics, it means the bacteria causing the infection are resistant to the antibiotic or antibacterial treatment. The development of resistance to an antibacterial is complex. Susceptible bacteria can become resistant by acquiring resistant genes from other bacteria or through mutations in their own genetic material-DNA. Once acquired, the resistance characteristic is passed on to the future generations and sometimes to other bacterial species.[8,9]

The present research work aims to synthesise sulphadiazine derivatives and further characterization includes finding properties and spectral data. Applications of different *in silico* tools and generate molecular properties then antibacterial evaluation using well plate method and analysing the results.

MATERIALS AND METHODS

Synthesis procedure

0.01 mole of sulphadiazine and 0.01 mole of different aromatic aldehydes were taken in conical flask, to this add 3 drops of HCl and 20 mL of water stirring by using magnetic stirrer for 1 hr at room temperature, after reaction completion solid precipitates.[10,11] Then wash the filtrate and dry the product under room temperature for 24 hr to get Schiff base product and reaction was given in Figure 1.

Identification and characterization

Identification, characterization of the synthesized derivatives are performed by the following procedures to determine all the synthesized derivatives had varying chemical nature compared to that of the parent compounds. We used melting point apparatus to perform melting points of new derivatives. The solubility of new compounds are all insoluble in chloroform, ethanol, water, ethyl acetoacetate and all compounds soluble in DMSO. TLC is a quick, easy, and inexpensive method that requires small amounts of sample and does not require sophisticated equipment. Mobile phase is Chloroform [8.5]: Methanol [1.5]: Ammonia hydroxide Solution [33%v/v] [0.1]. TLC plate is Pre-coated silica gel G F 254. It was detected using UV chamber.[12]

Infra-Red Studies

It is one of the most common and widely used spectroscopic techniques employed mainly by inorganic and organic chemists due to its usefulness in determining the structures of compounds and identifying them.

The method or technique of infrared spectroscopy is conducted with an instrument called an infrared spectrometer (or spectrophotometer) to produce an infrared spectrum.

Infrared spectrometers, similar in principle to other spectrometer, permit chemists to obtain absorption spectra of compounds that are a unique reflection of their molecular structure.

The fundamental measurement obtained in infrared spectroscopy is an infrared spectrum, which is a plot of measured infrared intensity versus wavelength of light.

IR Spectroscopy measures the vibrations of atoms, and based on this it is possible to determine the functional groups.

Generally, stronger bonds and light atoms will vibrate at a high stretching frequency (wavenumber).[13]

^{1}H-NMR Studies

^{1}H-NMR, or proton nuclear magnetic resonance spectroscopy, is a powerful analytical technique used to determine the structure of organic compounds by analysing the behaviour of hydrogen nuclei in a magnetic field.

In ^{1}H-NMR, a sample is placed in a strong magnetic field, causing the hydrogen nuclei to align either parallel or antiparallel to the field. When Radiofrequency (RF) energy is applied, the nuclei absorb energy and transition between energy states.

As they return to their original state, they emit RF energy, which is detected by a receiver coil. The resulting spectrum, known as a ^{1}H-NMR spectrum, provides information about the chemical environment of hydrogen nuclei in the molecule.

Key features of ^{1}H-NMR spectra include chemical shift, which is the position of signals along the horizontal axis (measured in parts per million, ppm), and signal intensity, which corresponds to the number of hydrogen atoms giving rise to each signal. The chemical shift is influenced by factors such as the electronic environment and neighbouring atoms.

Additionally, coupling between hydrogen nuclei, known as spin-spin coupling, results in the splitting of signals into multiplets. The number of peaks in a multiplet and their relative intensities provide information about the number and type of hydrogen atoms interacting with the nucleus under observation.

Overall, ^{1}H-NMR spectroscopy is widely used in organic chemistry for structure elucidation, compound identification, and quantitative analysis due to its high sensitivity, non-destructive nature, and ability to provide detailed information about molecular structure and connectivity.

Antibacterial evaluation

An antibacterial agent is a therapeutic substance that kill (bactericide) or inhibit (bacteriostatic) the growth of bacteria by reducing the metabolic activity of bacteria. Antibacterial chemicals can be grouped into three broad categories like antibacterial drugs, anticancer and disinfectants.

Bacteria are a microscopic single celled organism that lives in and around us. Bacteria may be helpful, but in certain condition they may cause illness such as strep throat, food poisoning, skin, ear sinus infections. Christian Gram proposed a technique called as gram staining in order to distinguish between types of bacteria based on the difference in their cell wall structures for the ability to hold the gram stain.

Gram positive such as *Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes, Clostridium difficile, C. perfringens, Listeria monocytogenes*. Gram-negative bacteria such as *E. coli*, Salmonella, Shigella, Pseudomonas, Moraxella, Helicobacter, Stenotrophomonas.

The organisms that retain the primary colour (crystal violet dye) and appear purple brown under a microscope is Gram-positive organisms. The organisms that do not take up primary stain appear red under a microscope and are Gram-negative organisms.[14]

The best method for determining the antibacterial (antimicrobial) susceptibility of microorganisms involves careful estimation of antibacterials Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentrations (MBC). This determination can be performed with liquid or solid media.

Antibacterial activity by agar well diffusion method

We have performed Anti-bacterial activity using Agar well method. We have noted response of organism to the new compounds. The anti-bacterial activity area shows its effect were measured and compared with the standard and calculated the zone of inhibition.

Antibacterial procedure

The test compounds were dissolved in 5% DMSO to get a stock concentration of 5 mg/mL (5 mg dissolved in 1 mL DMSO).

The test organisms *Esch. coli*, Staphylococcus aureus and *Bacillus subtilis* were revived from stock culture by plating on blood agar medium to get isolated colonies.

The individual colonies of each species were emulsified in sterile phosphate buffered saline (pH 7.2) to get a turbidity of 0.5 McFarland standard (0.5x108 organisms/mL).

The test compounds were subjected to two-fold dilution in a 48-well tissue culture plate. Five wells were selected for each compound. To each well 200 µL of BHI (Brain art Infusion) broth was added. Two hundred µL of stock solution (0.5 mg/mL) was added to the first well, mixed properly and 200 µL was transferred to the second well. This procedure was repeated up to the fifth well from which 200 µL was discarded. The drug concentrations after this dilution in the five wells were 50%, 25%, 12.5%, 6.25% and 3.13% respectively.

The bacterial suspension prepared as mentioned earlier was plated on to the sterile plates with cotton swabs. Mueller Hinton agar plates were used to determine the antimicrobial activity of the test compounds. A sterile cotton swab was dipped into the BHI broth containing organism suspension and was inoculated onto the surface of the agar plate. Then, five wells of 8 mm were cut into each agar plate with a plastic template. Into each well, 50 µL of the different dilutions of the drug were inoculated and appropriately labelled. The plates were incubated at 37°C for 24 hr. The zone of inhibition if any was noted around each test compound measured with a scale and recorded.[15]

Molinspiration

Molinspiration is software used for calculation of important molecular properties such as log P, polar surface area, number of hydrogen bond donors and acceptors and others. Virtual screening is a computational lead identification method. It analyses number of molecules predicts their bio physiochemical properties including the biological activity potential. This helps in identification of hits and leads. This method is more efficient. Molinspiration is a web-based virtual screening tool. This analysis provides the receptor affinity data and the three-dimensional visualization of the molecules.[16,17]

Lipinski Rule of 5 properties

It is set of simple molecular descriptors used by Lipinski in formulating his "Rule of 5". The rule states, that most "drug-like" molecules have log *p*<= 5, molecular weight <= 500, number of hydrogen bond acceptors <= 10, and number of hydrogen bond donors <= 5.

Protox-III

It is designed for multiple input formats for chemicals either by generic name, structure, or chemical formula and classifies the chemical based on the programs reference database. This particular model is assessing the predictive "oral" toxicity in rodents and will produce multiple potential results. *In silico* toxicity screening by using Prorox-III. It is an online web resource that will give toxic parameters like hepatotoxicity, carcinogenicity, immunogenicity, mutagenicity, cytotoxicity and LD_{50} values. Protox-III webserver includes both chemical and molecular target knowledge. A novelty of the Protox-III webserver is that the prediction scheme is classified into different levels of toxicity such oral toxicity, organ toxicity (hepatotoxicity), toxicological endpoints (such as mutagenicity, carcinogencity, cytotoxicity and immunotoxicity), toxicological pathways (AOPs) and toxicity targets thereby providing insights into the possible molecular mechanism behind such toxic response.[18,19]

Chemspider

From the ChemSpider results, search results are zero, as we conclude all compounds are new and not previously reported.

RESULTS

A pure compound Sulphadiazine and other aldehyde compounds and ethanol used in the synthetic reaction. The reagents used were obtained from PES University, Bengaluru and are of AR grade. The ^{1}H NMR spectra were recorded on an instrument 600 MHz, Chemical shifts are reported in δ ppm using TMS as an internal standard. Abbreviations indicating multiplicity were used as follows: s = singlet, bs = broad singlet, d = doublet, t=triplet, q=quartet, and m=multiplet. Melting points were measured on the melting point apparatus. ^{1}H NMR was carried out at IISC Bengaluru, Anti-bacterial activities were carried at Maratha Mandal Central Research Laboratory, Belagavi. Sulphadiazine was procured from PES University, Bengaluru. Six Compounds were prepared and reported. Color observation of the derivatives revealed distinct appearances, which may be attributed to the electronic effects of the substituents used given in Table 1.[20]

Formula for percentage absorption: % ABS =109-0.345×TPSA

Molinspiration result analysis

According to the data, substances exhibit the partition coefficient indicated by the log *p*-value. Every chemical exhibits TPSA below 120 A^0, which suggests that the plasma membrane has good drug permeability.[11] Good intestinal absorption is indicated by percentage Absorption (%ABS) between 69.74 and 79.71% was mentioned in Table 2.

Spectral Analysis of new compounds

(E)-4-(4-hydroxy-3-methoxybenzylideneamino)-N-(pyrimidin-2-yl) benzenesulfonamide

IR 3354 (br, O-H, phenol), 3200-3250 (br, N-H, sulfonamide), 1610 (C=N, azomethine), 1576 (C=C, aromatic), 1323 (C-O, aryl ether / S=O sym), 1150-1092 (S=O asym) ^{1}H-NMR: (400 MHz,

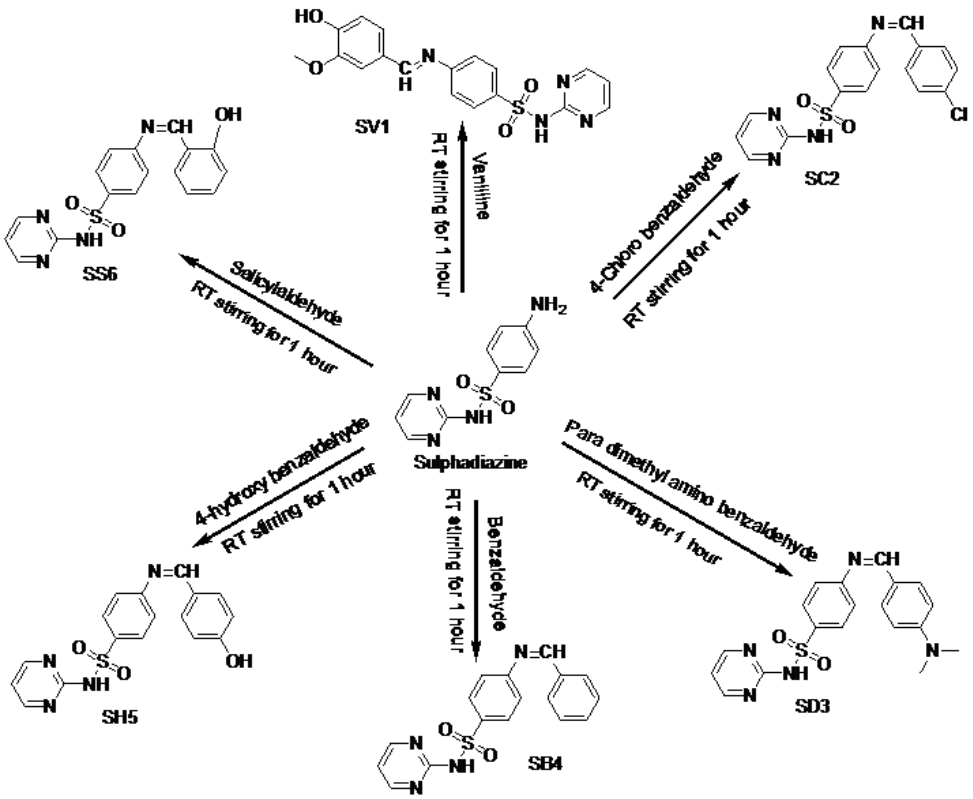

Figure 1: Synthesis scheme.

Table 1: Physico-chemical properties of synthesized compounds.

Sl. No.	Codes	Melting point	Molecular formula	Molecular weight	Colour	State	% yield	R_f
1.	SV1	264°C	$C_{18}H_{16}N_4O_4S$	384.41	Pale cream	Solid	68.3	0.38
2.	SC2	273°C	$C_{17}H_{13}ClN_4O_2S$	372.83	Light cream	Solid	15.4	0.98
3.	SD3	224°C	$C_{19}H_{19}N_5O_2S$	381.45	Light yellowish cream	Solid	23.4	050
4.	SB4	233°C	$C_{17}H_{14}N_4O_2S$	338.38	Light brown	Solid	50.2	0.82
5.	SH5	247°C	$C_{17}H_{14}N_4O_3S$	354.38	Pale orange	Solid	8.50	0.47
6.	SS6	250°C	$C_{17}H_{14}N_4O_3S$	354.38	Yellow	Solid	62.5	0.87

Table 2: Calculation of molecular descriptors.

Code	Mi Log P	TPSA	M.W.	N. Atoms	Noh	nOH NH	N. Violations	nrot b	Volume	%ABS
SV1	1.75	113.78	384.42	27	8	2	0	6	319.21	69.74
SC2	3.09	84.32	372.84	25	6	1	0	5	299.18	79.71
SD3	2.52	87.56	381.46	27	7	1	0	6	331.55	78.19
SB4	2.41	84.32	338.39	24	6	1	0	5	285.64	79.71
SH5	1.93	104.55	354.39	25	7	2	0	5	293.66	72.93
SS6	2.35	104.55	354.39	25	7	2	0	5	293.66	72.93
Sulphadiazine	-0.04	97.98	250.28	17	6	3	0	3	202.26	75.20

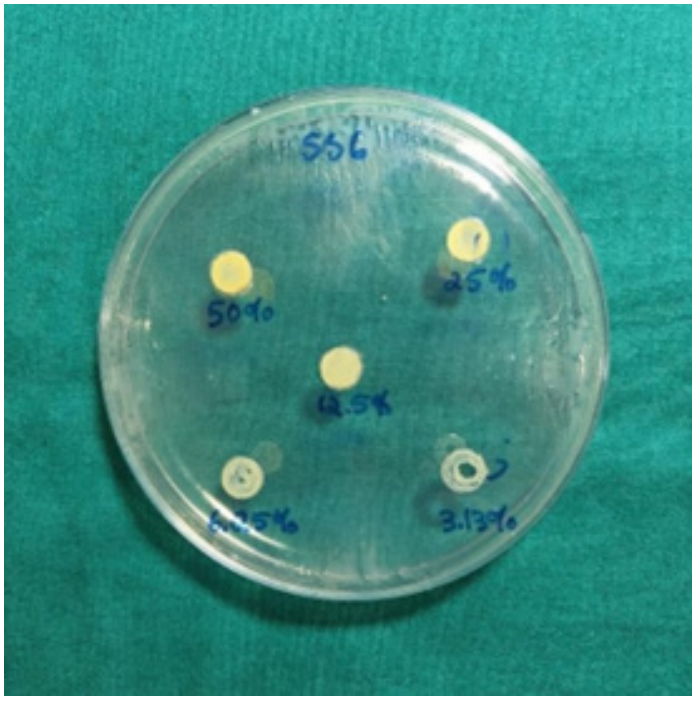

Figure 2: *Bacillus subtilis* is sensitive to SS6 at 50%, 25%, 12.5%.

DMSO-d_6, δ ppm) 8.23 (1H,s, azomethine),8.08 (1H,d, J=5.2 Hz, Pyrimidine), 7.99 (1H,d, J=2.2 Hz, anisole ring), 7.89 (2H, d,J=8.6 Hz, sulphonamide ring), 7.60 (2H,d,J=8.6 Hz sulphonamide ring), 7.42 (1H,m,J=8.4 Hz anisole ring), 7.01 (1H,d,J=8.4 Hz Anisole ring), 6.60 (1H,d, J=5.2 Hz, pyrimidine), 5.10(IH,s, OH), 9.8-10.5 (1H,s,NH, sulphonamide), 3.73(3H,s, OCH3),; SV1

(Z)-4-(4-chlorobenzylideneamino)-N-(pyrimidin-2-yl) benzenesulfonamide

IR 3350 (br, N-H, sulfonamide, H-bonded), 3100 (w, aromatic C-H), 1580 (C=N, azomethine), 1350-1320 (m, S=O, νsym/νas overlap), 1090 (s, S=O), 1058 (s, C-Cl, aryl-Cl). ^{1}H-NMR : (400 MHz, DMSO-d_6 δ ppm) 11.24 (1H,s, NH sulphonamide), 8.48 (1H,d, J=4.8 Hz pyrimidine), 8.35 (1H,s,=CH-N azomethine) 7.93 (2H,d, J=8.6 Hz benzenesulphonamide), 7.53 (2H,d,J=8.6 Hz, benzenesulphonamide), 7.18 (2H,d, 4-chlorophenyl), 7.04 (2H,d, 4-chlorophenyl), 6.56 (1H,d, J=4.8 Hz pyrimidine); SC2

(Z)-4-(4-(dimethylamino)benzylideneamino)-N-(pyrimidin-2-yl) benzenesulfonamide

IR 3300 (br, N-H, sulfonamide), 3101 (aromatic C-H), 1576.7 (C=N, imine), 1405 (aromatic C-C / C-N contribution), 1310 (S=O, as.), 1140 (S=O, sym.), 1092 (S-O / C-N). ^{1}HNMR :(400 MHz, DMSO-d_6 δ ppm) 10.20 (s,1H,NH,sulphonamide) 8.39 (s,1H,CH=N, imine), 8.16(m, 2H, pyrimidine), 7.90 (d, J=8.8Hz, 2H, Ar-SO_2) 7.62 (d, J=8.8Hz, 2H, Ar-SO_2), 7.44-7.50 (m,2H, Aromatic) 6.62(d, J=9.0 Hz, 2H, 4-dimethylamino phenyl) 6.58(m,1H,Pyrimidine), 2.85(s,6H, dimethyl amino methyl)); SD3

(Z)-4-(benzylideneamino)-N-(pyrimidin-2-yl) benzenesulfonamide

IR 3300 (br, N-H, sulfonamide), 3101 (aromatic C-H), 1576 (C=N, imine), 1310 (S=O, asym), 1140 (S=O, sym), 1092 (S-O stretch). ^{1}H-NMR: (400 MHz, DMSO-d_6 δ ppm) 10.20(s,1H,NH, sulphonamide) 8.39 (s,1H, CH=N, imine),8.15-7.55(m, 6H,Aromatic protons), 7.29 (t, J=7.6 Hz, 3H,aromatic protons), 6.58 (t, J=4.8 Hz, 1H, Pyrimidine); SB4

(E)-4-(4-hydroxybenzylideneamino)-N-(pyrimidin-2-yl)benzenesulfonamide

IR 3355 (O-H, phenol, broad), 3270 (N-H, sulfonamide), 3100 (aromatic C-H), 1577 (C=N, imine), 1439 (C=C, aromatic), 1310 (S=O, asym), 1140 (S=O, sym), 1088 (S-O) ^{1}H-NMR :(400 MHz, DMSO-d_6 δ ppm) 10.15 (s, 1H, NH, sulphonamide), 8.38 (m,2H,pyrimidine),8.10-7.5(m, 6H, Aromatic), 6.76(d, J=8.8 Hz, 2H, 4-hydroxyphenyl), 6.58 (t, J=4.8 Hz, 1H, Pyrimidine), 5.0 (s,1H,OH, phenolic); SH5

(Z)-4-(2-hydroxybenzylideneamino)-N-(pyrimidin-2-yl)benzenesulfonamide

IR 3350 (O-H, phenol, broad), 3270 (N-H, sulfonamide), 3079 (Ar-H), 1580 (C=N, imine), 1410 (C=C, aromatic), 1310 (S=O, asym), 1140 (S=O, sym), 1088 (S-O). ^{1}H-NMR :(400 MHz, DMSO-d_6 δ ppm) 10.2 (s,1H, NH, sulphonamide), 8.38 (m,2H,pyrimidine),8.10-7.15(m, 4H, benzenesulphonyl),

7.12-6.76 (m,3H, 2-hydroxyphenyl), 6.58 (t,J=4.8 Hz, 1H, Pyrimidine), 5.0 (s,1H,OH, phenolic); SS6

Antibacterial Activity

The two microorganisms used *Bacillus subtilis* (Gram +ve) and *Pseudomonas aeruginosa* (Gram -ve). The zone of inhibition for gentamycin was 17 mm for *S. aureus*, and 22mm for Bacillus subtilis (12mm and above is considered sensitive) was given in Table 3. The samples have good antibacterial potential against *P. aeruginosa*, especially SD3 and SS6, maintaining activity even at low concentrations. The decreasing trend confirms a concentration-dependent response. Bacillus is less susceptible to the tested agents. Most samples lose activity below 25%, suggesting limited or no effectiveness at low concentrations. The antibacterial effect is mild and significantly less than that against *P. aeruginosa* was given in Figures 2 and 3.

Protox-III Result analysis

For all substances, the projected LD_{50} is 2500 mg/kg. All chemicals have a projected toxicity of class 4. According to the toxicity model study, all chemicals' toxicity is inert against cytotoxicity, carcinogenicity, and mutagenicity, but they are all active against hepatotoxicity and immunogenicity. Based on the data below, we can conclude that the anticipated toxicity of synthesised chemicals is zero. *In silico* toxicity prediction is extremely important for toxicologists, medicinal chemists, computational chemists, and regulatory authorities. It saves time, the necessity for animal testing, and the accompanying costs given Table 4.

DISCUSSION

The synthesized derivatives of Sulphadiazine (SV1 to SS6) were successfully obtained using various substituted aldehydes via Schiff base reactions. The physico-chemical characterization using melting point determination, TLC, IR, and ^{1}H-NMR spectroscopy confirmed the successful formation and purity of each compound. All derivatives were found to be soluble in DMSO and insoluble in common organic solvents like ethanol and chloroform.[21,22]

In silico studies using tools such as Molinspiration, SwissADME, and Protox-III suggested favorable drug-like properties for all synthesized compounds, including acceptable Lipinski's Rule of 5 parameters, moderate to high predicted absorption, and toxicity class IV with LD_{50} values indicating relative safety. Antibacterial activity screening against *Pseudomonas aeruginosa* (Gram-negative) and Bacillus subtilis (Gram-positive) showed that the compounds had significantly higher efficacy against *P. aeruginosa*. Notably, SD3 and SS6 exhibited the highest zones of inhibition, indicating strong antibacterial potential, with SD3 showing up to 46 mm at 50% concentration and maintaining activity down to 3.13%. Conversely, activity against Bacillus subtilis was modest, with inhibition zones decreasing sharply

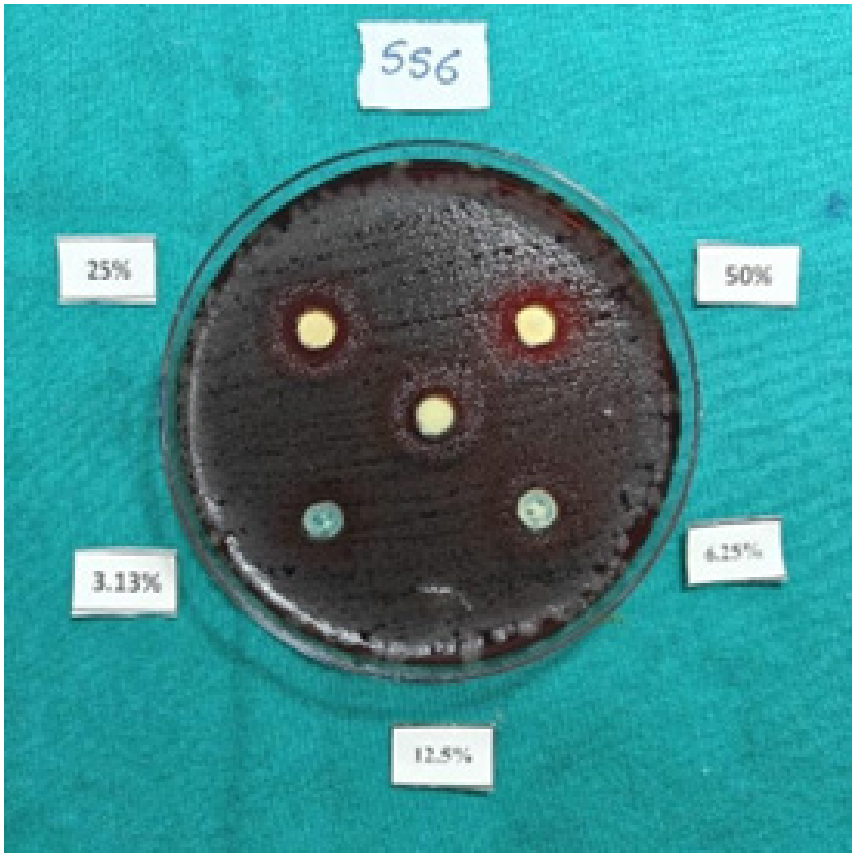

Figure 3: *Pseudomonas aeruginosa* is sensitive to SS6 at all different concentration.

Table 3: Anti-bacterial activity of newly synthesized compounds.

Pseudomonas aeruginosa (Gram -ve)	Code	50%	25%	12.50%	6.25%	3.13%
	SV1	42 mm	38 mm	35 mm	32 mm	30 mm
	SC2	42 mm	38 mm	36 mm	34 mm	32 mm
	SD3	46 mm	39 mm	38 mm	35 mm	32 mm
	SB4	39 mm	37 mm	34 mm	32 mm	31 mm
	SH5	42 mm	36 mm	33 mm	32 mm	31 mm
	SS6	45 mm	42 mm	40 mm	38 mm	37 mm
Bacillis subtillis (Gram +ve)		50%	25%	12.50%	6.25%	3.13%
	SV1	14 mm	12 mm	R	R	R
	SC2	15 mm	11 mm	R	R	R
	SD3	13 mm	11 mm	R	R	R
	SB4	16 mm	13 mm	R	R	R
	SH5	14 mm	12 mm	R	R	R
	SS6	15 mm	13 mm	12 mm	R	R

Table 4: Toxicity model report, A for Active, I for Inactive.

Classification	Target	SV1	SC2	SD3	SB4	SH5	SS6
Organ Toxicity	Hepatotoxicity	I	I	A	I	I	I
Toxicity end points	Carcinogenicity	A	A	A	A	A	A
Toxicity end points	Immunogenicity	I	I	I	I	I	I
Toxicity end points	Mutagenicity	I	I	I	I	I	I
Toxicity end points	Cytotoxicity	I	I	I	I	I	I

below 25% concentration, and most samples showing resistance at lower dilutions.[23-29]

CONCLUSION

Biological evaluation demonstrated strong antibacterial activity, particularly against the Gram- negative bacterium *Pseudomonas aeruginosa*, with SD3 and SS6 showing the highest zones of inhibition across all tested concentrations. Activity against Bacillus subtilis (Gram-positive) was moderate and concentration-dependent, with limited efficacy at lower doses. These findings underscore the importance of structural modifications on the sulphadiazine scaffold to enhance selective antibacterial properties.

Overall, the study demonstrates that Schiff base derivatives of sulphadiazine have significant potential as antimicrobial agents. The combination of strong *in vitro* activity, favorable pharmacokinetic predictions, and low toxicity suggests that these compounds, especially SS6, were being further investigation for development as antibacterial therapeutics.

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ABBREVIATIONS

IR: Infrared spectroscopy; **^{1}H-NMR:** Proton Nuclear magnetic resonance; **DMSO:** Dimethyl sulfoxide; **%ABS:** Percentage absorption, ppm Parts per Million; **δ:** Chemical shift; **MIC:** Minimum Inhibitory concentration; **MBC:** Minimum bacterial concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The designed schemes utilized sulphadiazine as hydrophobic scaffold and different aldehydes were used as pharmacophore. The molecules were obtained through "Schiff base" using conventional synthesis. There were totally 6 compounds synthesized and screened for *in silico* studies. The compounds were designed by using chemdraw, a sketching tool. The *in silico* of compounds was assessed against freely available softwares like molinspiration and

Protox, ChemSpider. All compounds Molinspiration properties were predicted and calculated the percentage absorption in the gut. Compound shows good absorption in the intestine. All the new compounds were confirmed by melting point, TLC and structures were confirmed by spectral characterization like IR, ^{1}H NMR spectra. The *in silico* studies showed high potent activity when compared to standard drugs. The *in vitro* antibacterial activity was carried agar well diffusion method and got good results on *Pseudomonas aeruginosa* bacteria.

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