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# Antimicrobial Potency and Molecular Mechanism of Benzothiazole Schiff Base Hybrids

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**Original Research Article**

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**Abstract:** Antimicrobial resistance reduces the effectiveness of the standard treatment and also increases the risk of spreading the disease to others. Therefore, there is an urgent need for the development of new class of antimicrobials. In such an attempt, benzothiazole schiff base hybrids synthesised were evaluated for antimicrobial potency against *E. coli, S. typhi, S. aureus, E. faecalis, K. pneumonia, P. aeruginosa, C. albicans, C. tropicalis*, and *C. krusei* and also an attempt was made to understand their molecular mechanism of action. Some of the benzothiazole schiff base hybrids have exhibited excellent antimicrobial activity with MIC of 3.91µg/ml against *S.aureus, P.aeruginosa* and *E.coli* in comparison with the standard drug ciprofloxacin. The molecular mechanism of action of benzothiazole schiff base hybrids was elucidated in both intact bacterial cells and plasmid DNA. The potent antimicrobial activity of the hybrids may be due to membrane perturbing mode of action explicated by membrane depolarization assay and fluorescent assisted cell cytometry (FACS) study on intact bacterial cell. However, the activity may also be due to the intracellular mode of action explicated by DNA cleavage study on plasmid DNA.

**Keywords:** Antimicrobials, Benzothiazole, Schiff base, Fluorescent assisted cell cytometer, DNA binding, Molecular Mechanism.

## INTRODUCTION

Research and development of new antimicrobial therapeutic agents is of paramount importance because of the innate ability of pathogens in developing resistance to existing drugs. The multidrug resistant strains pose a serious threat to public health.

In more specific, the extremely resistant organisms viz. penicillin-resistant *Streptococcus Pneumoniae,* methicillin-resistant *Stayphlococcus aureus* (MRSA) and vancomycin resistant *Enterococci* are accountable for an increased percentage of hospital acquired infections [1]. Advancements in the fields of genomics, molecular and structural biology, structural biochemistry and high throughput screening have led to essential standards in the pursuit at novel antimicrobial agents [2]. Thus, identification of novel drugs in treating infectious diseases are at the forefront of scientific research.

The biological significance of benzothiazole moiety has attracted continuing interest. Therefore, our research group is working on this benzothiazole scaffold [3-9]. Recently, we reported the synthesis of Benzothiazole-Schiff base hybrids [8] and it is worthwhile to screen them for antimicrobial potency and mode of action. So, we present here the antimicrobial potency, quantitative structure activity relationship and possible mode of action of our previously reported benzothiazole-Schiff base hybrids as antimicrobial agents. We also studied the ability of antimicrobial inhibitors, by studying their binding with Lipopolysaccharides (LPS), and depolarization of both the outer and cytoplasmic membranes. Further, their interaction with lipid monolayers, and killing of different bacterial strains were also studied. A simple and inexpensive method for the determination of intercalative and minor groove DNA binding properties was also developed for benzothiazole schiff base hybrids.

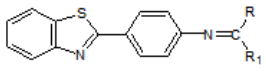
## MATERIALS AND METHODS

### Chemicals and reagents

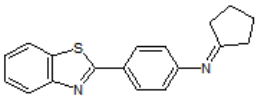
Plasmid DNA (pUC19) was purchased from Invitrogen. $DiSC_3$ (5) and Propidium iodide (PI) were

purchased from Aldrich. All other chemicals were of the highest grade purity commercially available and supplied either by Merck or Sigma. The benzothiazole Schiff base derivatives (S01-S20) (Table 1) were synthesized following our earlier reported protocol [8] and were used to evaluate their antimicrobial activity.

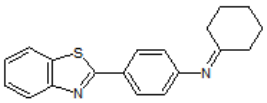
**Table-1: Chemical structure of the compounds (S01-S20) under study**



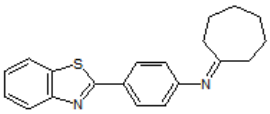
S01-17



S18



S19



S20

| Compound code | $R_1$ | R |
|---|---|---|
| S01 | 4-methoxy phenyl | H |
| S02 | 4-chloro phenyl | H |
| S03 | 2,4 dichloro phenyl | H |
| S04 | 4-pyridinyl | H |
| S05 | 2,4-dihydroxy phenyl | H |
| S06 | 3-bromo phenyl | H |
| S07 | 4-hydroxy phenyl | H |
| S08(template for QSAR) | N,N-dimethyl phenyl | H |
| S09 | Phenyl | H |
| S10 | 3-methyl phenyl | H |
| S11 | 3-nitro phenyl | H |
| S12 | 2-chloro phenyl | H |
| S13 | 3-hydroxy-4-methoxy phenyl | H |
| S14 | 2-nitro phenyl | H |
| S15 | 3,4,5-trimethoxy phenyl | H |
| S16 | 3-fluro phenyl | H |
| S17 | 3-indolyl | H |
| S18 | Cyclopentyl | - |
| S19 | Cyclohexyl | - |
| S20 | Cycloheptyl | - |

**Pharmacology**

Anti-bacterial activity of reported novel Schiff base benzothiazole hybrids (S01-S20) was evaluated against various pathogenic Gram-negative and Gram-positive bacterial strains viz., *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Salmonella typhi* (*S. typhi*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Enterococcus faecalis* (*E. faecalis*). Anti-fungal activity of the above compounds (S01-20) was evaluated against fungal strains viz. *Candida albicans* (*C. albicans*), *Candida tropicalis* (*C. tropicalis*) and *Candida krusei* (*C. krusei*). The activities were evaluated by agar disc diffusion method as per the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997) [10]. The solvent, DMSO, used for the preparation of solution, did not show inhibition against the tested microorganisms. The ciprofloxacin and fluconazole were used as positive controls in respective studies.

**Antimicrobial assay**

*In vitro* antimicrobial activity was measured by the standard serial dilution method as described previously [4]. Bacterial strains were cultured overnight at 37°C in Mueller-Hinton (MH) broth. Prior to assay, 100 µl of bacteria from overnight grown culture was resuspended in 5 ml of fresh MH broth (1/50 dilution) for an additional hour at 37°C to obtain a mid-log-phase culture. The minimum inhibitory concentration (MIC) value was determined using 96 well plate format. Two-fold serial dilutions of the test compounds were prepared to obtain the required drug concentrations ranging from 250-3.91 µg/ml. Each well was inoculated with 10 µl of the test compound and 90 µl aliquots of the cell suspension ($10^5$ CFU/ml). Further MH broth was added to make final volume of 200 µl. Inoculated plates were incubated at 37ºC for 24 h and the results were assessed by visible turbidity in each well. The minimum concentration of each sample required for a

visible inhibition of growth is defined as MICs. All readings were calculated in triplicates and the average was taken as final MICs.

**Quantitative structure activity relationship (QSAR)**

Antimicrobial activity (MIC) against *E.coli* was taken into account for Field-Based 3D-QSAR. The MICs were later converted into pMICs to construct a model for establishing relationship between the antimicrobial activity values and 3D characteristics of aligned benzothiazole-schiff bases (similar to CoMFA/CoMSIA) [11]. This model will allow us to assess qualitatively where to add or remove functional groups, and to predict activities for other molecules to development of lead compounds. First, the 3D structures of the ligands were fully prepared and properly aligned (Figure 1). Thereafter, the ligands were divided randomly into a training set (15 no's) and a test set (4 no's). Once the training and test sets were chosen, field based QSAR model was generated [12]. The predictive ability of the models is expressed by the predictive $r^2$ value, which is analogous to cross-validated $r^2$ ($q^2$) and was calculated using the formula $r^2_{pred} = (\frac{SD-PRESS}{SD})$.

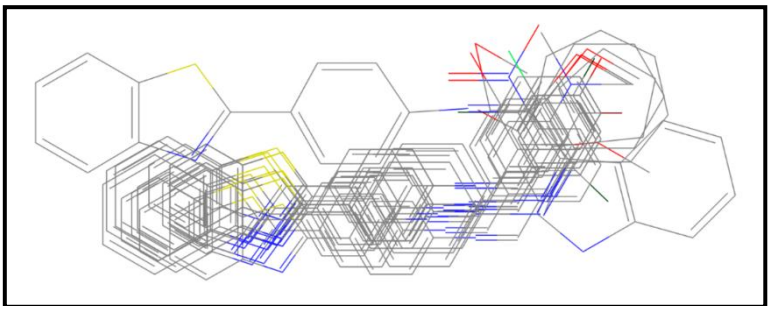


**Fig-1: Database alignment pattern for all the training & test set compounds**

**Bactericidal kinetics**

An overnight culture of *S. aureus* ATCC 25323 and *E.coli* ATCC 35218 was diluted in fresh MH Broth to the exponential phase (optical density of 0.6 at 600 nm). The killing activity of synthesized compounds with respect to time was determined using the 96-well plate reader as described previously [4]. The lead compounds with 4 times their MICs were added and the mixture was incubated at 37°C in a rotator shaker incubator set at 200 rpm. The absorbance of the plates incubated with compounds was recorded at 600 nm at regular time intervals i.e. 0, 1, 2, 3, 4 and 5 h. Each experiment was repeated on three dissimilar days and values are plotted as mean ± SD.

**Membrane depolarization assay**

The perturbation in the membrane potential was studied by employing fluorescence depolarization assay using the membrane potential-sensitive cyanine dye $DiSC_3(5)$ described previously [4]. The exponential-phase bacterial culture was harvested and resuspended in 5 mM HEPES–20 mM glucose buffer (pH 7.2) to an optical density of 0.06. Further, the cell suspension was incubated with 100 mM KCl (to equilibrate cytoplasmic and external $K^+$ concentration) and $DiSC_3(5)$ at a concentration of 1µM for 1h at room temperature. The excitation and emission wavelength used was 622 nm and 670 nm, respectively. All samples were measured in a 5 mm path length quartz cell at 25°C on Perkin Elmer Life Sciences LS 50-B spectrofluorimeter (Perkin-Elmer Corp., Norwalk, Conn.). Samples were stirred at a constant temperature 37°C during the experiment. Due to partitioning of samples into the membrane, an increase in fluorescence was recorded as a function of time till no further increase in intensity was observed.

**Flow Cytometry analysis**

The membrane rupture of *S. aureus* ATCC 25323 and *E.coli* ATCC 35218 was examined by flow cytometry after employing suitable fluorescent probes [4]. Briefly, the cells at exponential phase were incubated with the compounds A07a and A07b with constant shaking at 37 °C. The treated cells were further centrifuged, washed twice with Phosphate Buffer Saline (PBS) and incubated with propidium iodide (PI) at 4°C for 30 min, followed by removal of the unbound dye through washing with an excess of PBS and re-suspended in same buffer. These cells were then analyzed by flow cytometry in the form of dot plots with respect to the control without PI treatment; then with PI treated cells preincubated with test compounds and also with PI treated cells not preincubated with test compounds. The excitation and emission wavelength were 488 and 617nm, respectively for PI. The large number of dots in lower right quadrant fluorescing with PI after exposure to compounds might attribute the defective outer-membrane repair and consequent leakiness of more DNA and RNA.

**DNA binding assay**

DNA binding experiments were performed on synthesised compounds by agarose gel electrophoresis by monitoring the gel retardation as described previously [4]. Briefly, 200 ng of plasmid DNA (pUC19) was mixed with increasing amounts of lead

compounds in 20 µL of binding buffer (5% glycerol, 10 mM Tris- HCl, pH 8.0, 1 mM EDTA, 1 mM dithiothreitol, 20 mM KCl, and 50µg/ml bovine serum albumin). The reaction mixtures were incubated for 1h at 37°C. Consequently, each sample was analysed by running 1 % agarose gel electrophoresis at 60 V for 1h in 10 mM Tris-HCl buffer by adding loading buffer (containing 25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol). The DNA migration was detected by the fluorescence of ethidium bromide. The illuminated gel was photographed by Alpha Innotech Corporation Instrument.

## RESULTS AND DISCUSSIONS

The results of anti-microbial study of all the compounds against bacteria and fungi are presented in Table 2 and 3, respectively. Some of them inhibited the growth of bacteria with good to excellent MIC values ranging between 1 and 100 µg/ml, while few of them showed moderate to good anti-fungal activity with MICs between 15 and 65 µg/ml. According to the antimicrobial studies, all the compounds showed better antibacterial activity in comparison to their anti-fungal activity. Almost all the compounds showed activity against *E. coli* and *S. typhi* and only some of them showed activity on rest of the bacterial strains. In addition, compound S05 showed moderate activity against all bacterial strains, while compound S06 showed activity only against *E. coli.* Compounds S02, S08, S13, S15 and S19 showed good activity against all the bacterial strains. The comparative zone of inhibition of compounds S02, S08, S13, S15 and S19 against all the bacterial strains is represented in Figure 2.

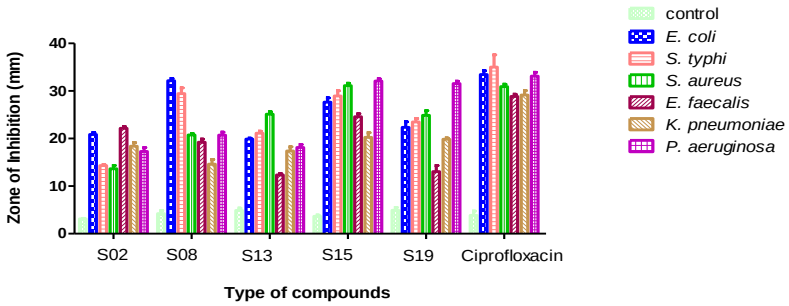



**Fig-2: Comparative zone of inhibition of compounds S02, S08, S13, S15 and S19**

Particularly, compound 4-((4-(benzo[d]thiazol-2-yl) phenylimino)methyl)-N,N-dimethyl benzenamine (S08) showed maximum activity (zone of inhibition up to 30-32 mm at concentration of 3.91µg/ml) against *E. coli*. Compounds N-(3,4,5-trimethoxybenzylidene)-4-(benzo[d]thiazol-2-yl)benzenamine (S15) and 4-(benzo[d]thiazol-2-yl)-N-cyclohexylidene benzenamine (S19) showed excellent activity (zone of inhibition up to 30–32 mm at concentration range of 3.91-7.81 µg/ml) against *S. aureus* and *P. aeruginosa.* Also compounds S11 and S14 had activity at higher concentration. In context to the anti-fungal screening, compounds S15 and S19 produced good activity at concentration of 15.6 µg/ml, against *C. krusei* and *C. albicans*, respectively. Compounds S01, S02, S05, S06, S08, S11, S14, S15, S18, S19 and S20 showed moderate activity against few fungal strains.

Structure-activity relationship study has revealed that the conversion of amino group to azomethine group via Schiff base formation is responsible for the enhanced activity. The greater antimicrobial activity of S02, S08, S13, S15 and S19 may be attributed to the presence of electron donating substituents such as methoxy, hydroxyl on phenyl ring. Schiff bases of cyclic ketones were found to show more antibacterial action as compared to antifungal action. The presence of electronegative atom such as chloro group at para position also enhanced the antimicrobial effect.

**Table-2: MIC values (µg/ml) of the synthesized schiff bases against the tested bacteria**

| Compound code | *E. coli* (ATCC 35218) | *S. typhi* (MTCC 3216) | *S. aureus* (ATCC 25323) | *E. faecalis* (Clinical isolate) | *K. pneumonia* (ATCC 31488) | *P. aeruginosa* (ATCC 27893) |
|---|---|---|---|---|---|---|
| S01 | 15.6 | 31.2 | 31.2 | 31.2 | - | - |
| S02 | 15.6 | 31.2 | 31.2 | 7.81 | 15.6 | 15.6 |
| S03 | 31.2 | - | 62.5 | 15.6 | - | - |
| S04 | 62.5 | 15.6 | - | 15.6 | - | 7.81 |
| S05 | 62.5 | 15.6 | 31.2 | 62.5 | 31.2 | 31.2 |
| S06 | 62.5 | - | - | - | - | - |
| S07 | 31.2 | 31.2 | - | - | - | - |
| S08 | 3.91 | 7.81 | 15.6 | 15.6 | 31.2 | 7.81 |
| S09 | 62.5 | 31.2 | 31.2 | - | - | - |
| S10 | 31.2 | 15.6 | - | 31.2 | 62.5 | - |
| S11 | 62.5 | - | >100 | - | >100 | >100 |
| S12 | 31.2 | - | 31.2 | - | 62.5 | 31.2 |
| S13 | 15.6 | 15.6 | 7.81 | 31.2 | 15.6 | 15.6 |
| S14 | >100 | >100 | >100 | >100 | >100 | >100 |
| S15 | 7.81 | 7.81 | 3.91 | 7.81 | 15.6 | 3.91 |
| S16 | 7.81 | 62.5 | - | - | 31.2 | 62.5 |
| S17 | 62.5 | 62.5 | 31.2 | 62.5 | 31.2 | 62.5 |
| S18 | 15.6 | 31.2 | - | 31.2 | 31.2 | 15.6 |
| S19 | 15.6 | 15.6 | 7.81 | 31.2 | 15.6 | 3.91 |
| S20 | 15.6 | 31.2 | 15.6 | 31.2 | - | 15.6 |
| Ciprofloxacin | ≥6.25 | ≥6.25 | ≥6.25 | ≥6.25 | ≥6.25 | ≥3.12 |

The value of each compound consisted of 'zone of inhibition range (MIC)' of 03 replicates. Level of significance $p < 0.05$

**Table-3: MIC values (µg/ml) of the synthesized schiff bases against the tested fungi**

| Compound code | *C. albicans* (ATCC 90028) | *C. tropicalis* (ATCC 750) | *C. krusei* (ATCC 6268) |
|---|---|---|---|
| S01 | 62.5 | 62.5 | - |
| S02 | - | 62.5 | - |
| S03 | - | - | - |
| S04 | - | - | - |
| S05 | 31.2 | - | - |
| S06 | - | 62.5 | - |
| S07 | - | - | - |
| S08 | 31.2 | - | 62.5 |
| S09 | - | - | - |
| S10 | - | - | - |
| S11 | - | 62.5 | 62.5 |
| S12 | - | - | - |
| S13 | - | - | - |
| S14 | 62.5 | - | - |
| S15 | 31.2 | 31.2 | 15.6 |
| S16 | - | - | - |
| S17 | - | - | - |
| S18 | - | 31.2 | |
| S19 | 15.6 | 31.2 | - |
| S20 | - | - | 62.5 |
| Fluconazole | ≥6.25 | ≥6.25 | ≥6.25 |

The value of each compound consisted of 'zone of inhibition range (MIC)' of 03 replicates. Level of significance $p < 0.05$

A statistically significant 3D-QSAR model was built for training set compounds via partial least-squares (PLS) analysis; later the developed model was validated with test set compounds. The predicted activity and residual value of the training and test set compounds were shown in Table 4. Predicted values are

the calculated activities of the equation and the residual values are the difference between the observed antimicrobial activity (MIC) against *E. coli* and the calculated/predicted activity. The residual values were found to be low. The result of field-based 3D-QSAR model was presented in Table 5. It revealed that the correlation coefficient ($r^2$) based on training set compounds was 0.71 and the correlation coefficient ($q^2$) based on test set compounds was 0.65, and the pearson R was 0.9, which is the measure of strength of a linear relationship between two variables. The Scatter plot for training set and test set compounds are represented in Figure 3. The contour map analysis shows that the observed and predicted activities are very close to each other (Table 4), thus indicating the validity and predictive power of the QSAR model. The pattern of substitution required for better biological activity around the pharmacophore was exhaustively studied (Figure 4). On the 4'-phenyl substituted ring, there is blue contour map indicating that electron donating group around the ring (*o/m/p* position) can increase the activity and maximum activity may be achieved by placing substitution at the *p* position. Compound S8 containing *N*,*N*-dimethyl group at *p* position showed most impressive MIC against *E.coli* (Table 4). The red contour map around the benzothiazole ring and imine linkage illustrates the requirement of electron withdrawing group at the respective position. Most of our synthesized compounds possess the electron withdrawing group in the desired red counter map region. According to hydrophobic and hydrophilic counter map, the desired hydrophobic area lies on the 2-phenyl substituted ring.

**Table-4: Predicted activity and residual values of the developed model**

| Ligand Name | QSAR Set | Observed Activity MIC (µM) | Predicted Activity | Residual |
|---|---|---|---|---|
| S1 | test | 7.343 | 7.35254 | 0.00954 |
| S2 | training | 7.349 | 7.36865 | 0.01965 |
| S3 | training | 7.089 | 7.12959 | 0.04059 |
| S4 | test | 6.702 | 6.95132 | 0.24932 |
| S5 | training | 6.743 | 7.03847 | 0.29547 |
| S6 | test | 6.798 | 7.03181 | 0.23381 |
| S7 | training | 7.024 | 7.08831 | 0.06431 |
| S8 | training | 7.961 | 7.70554 | 0.25546 |
| S9 | training | 6.701 | 6.86442 | 0.16342 |
| S10 | training | 7.022 | 7.16705 | 0.14505 |
| S11 | training | 6.759 | 6.94736 | 0.18836 |
| S12 | training | 7.048 | 6.87068 | 0.17732 |
| S13 | training | 7.363 | 7.504 | 0.141 |
| S15 | training | 7.714 | 7.6735 | -0.0405 |
| S16 | training | 7.628 | 7.1226 | -0.5054 |
| S17 | training | 6.752 | 6.54189 | 0.21011 |
| S18 | training | 7.272 | 7.27362 | 0.00162 |
| S19 | test | 7.293 | 7.30689 | 0.01389 |
| S20 | training | 7.312 | 7.44132 | 0.12932 |

**Table-5: Results of field-based 3D-QSAR model**

| S. No | $R^2$ | $Q^2$ | Pearson R | RMSE | P | Stability | F | SD |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.71 | 0.65 | 0.99 | 0.17 | 0.0001 | -0.0798 | 30.4 | 0.21 |

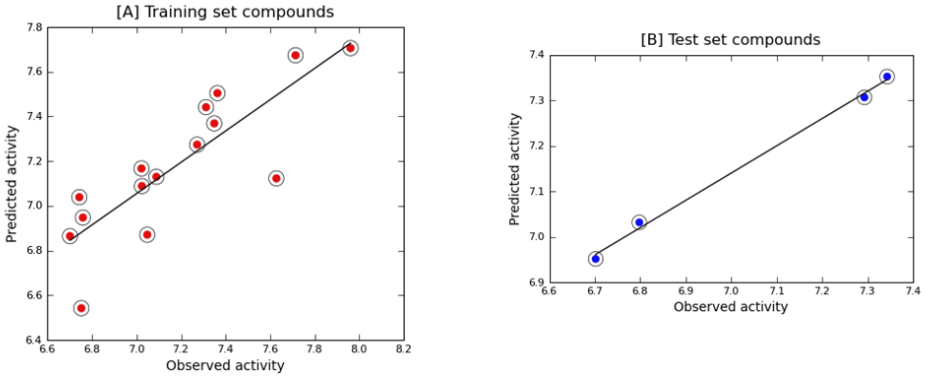



**Fig-3: Scatter plot for training and test set compounds**

A: Scatter plot for training set compounds with the best fit line y = 0.70x + 2.15 (R2 = 0.71)
B: Scatter plot for test set compounds with best fit line y = 0.60x + 2.94 (R2 = 1.00)

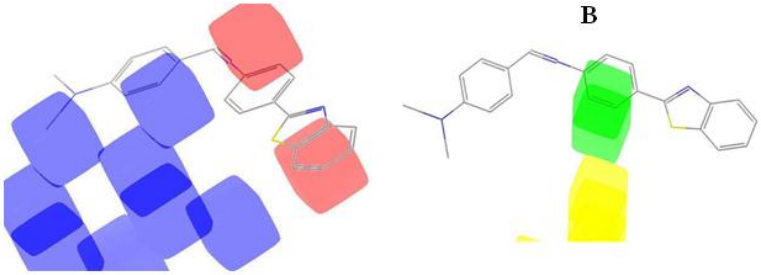



**Fig-4: Contour map analysis with 2Å grid spacing (compound S8)**

A: Electron withdrawing contour map. Blue contours refer to the region where electron donating substituents are favoured; red contours indicate the regions where electron withdrawing substituents are favoured
B: Hydrophobic contour map. Green contour refers to hydrophobic regions; yellow contour indicates hydrophilic areas.

The bactericidal activity of lead compounds S08 and S15 was carried out against *S. aureus* and *E. coli* strains in order to confirm the potency. It was determined at fixed time intervals to see the potential killing effects of bacterial cells at concentration four fold of MIC's (4 × MIC). Time-kill studies demonstrated that compounds S08 and S15 were rapid in killing with 90 to 99% lethality within 2 to 5 h (Figure 5). The inhibition kinetics in *S. aureus* showed that the compound S15 was more effective than compound S08 even up to 5 h and vice versa in *E. coli* strain. At concentration of 4 × MIC, both the compounds inhibited bacterial growth from 2 h onwards, until 5 h.

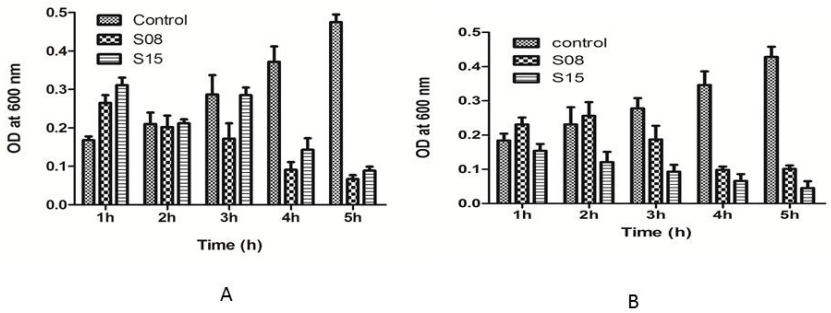



**Fig-5: Time dependent killing study of compounds S08 and S15**

(A) *S. aureus* upon treatment with compounds S08 and S15 at 4 × MIC
(B) *E. coli* upon treatment with compounds S08 and S15 at 4 × MIC

To learn the mechanism of antimicrobial activity of designed analogues, compound-induced permeability of *S. aureus* and *E. coli* was examined by membrane depolarization assay. Cationic membrane potential-sensitive cyanine dye [3, 3'-dipropyl-thiadicarbocyanine iodide $DiSC_3$ (5)] was used to determine the depolarization ability of cytoplasmic membrane. In this experiment, an increase in fluorescence intensity of dye was observed for any analogues altering the membrane potential due to pore formation/ membrane destabilization. This ultimately leads to the loss of the membrane potential gradient, causing the dye to be released into the medium resulting in membrane depolarization [20]. Triton (2%) was found to depolarize the bacterial membrane and was used as a positive control. At the maximum concentration (i.e., 4 x MIC) of designed analogues S08 and S15 tested, the level of depolarization reached to saturation from about 2 to 5 mins of time. Compounds S08 and S15 have completely depolarized the membrane at concentrations lower than those other compounds. All compounds showed similar profile with respect to positive control (Figure 6).

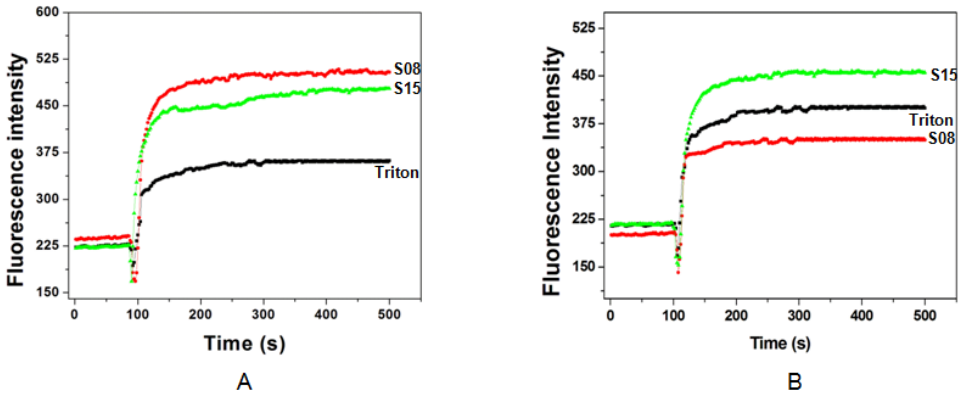



**Fig-6: Membrane depolarization ability of compounds S08 and S15**
(A) *E. coli* treated with 4xMIC concentration of compounds S08 and S15
(B) *S. aureus* treated with 4xMIC concentration of compounds S08 and S15

Cell viability was examined using Fuorescence assisted cell cytometer (FACS) by quantifying the amount of DNA released in terms of Propidium Iodide (PI) after the incubating the test compounds with *S. aureus* and *E. coli*. The results were consistent with the outer membrane depolarization assay of bacteria. The compound S08 induced maximum damages to the membrane organization of *E. coli*, whereas compound S15 leads to more pore formation in *S. aureus* as compared to *E. coli*, indicated by PI staining of the cells (Figure 7). However, both compounds S08 and S15 induced reasonable damage of bacterial membrane in both strains.

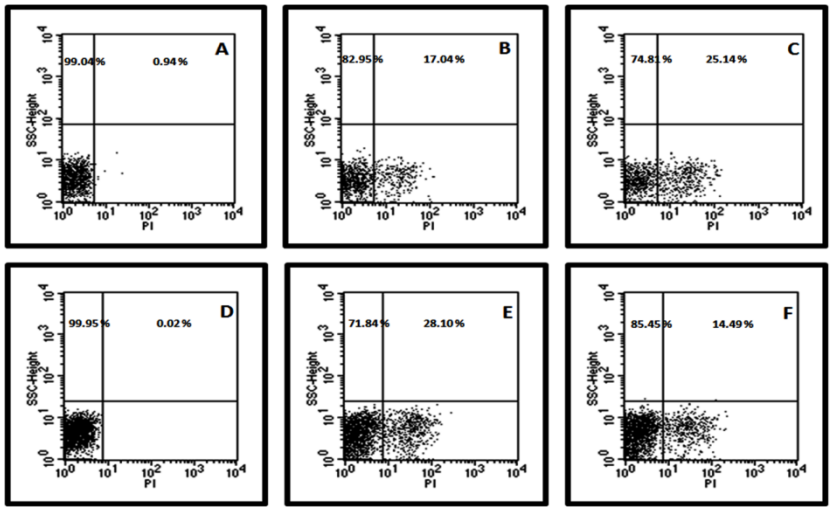



**Fig-7: Compound-induced membrane damage of S. aureus and E. coli (A–F).**
PI staining of *S. aureus* (A) control without PI treatment; (B) S08 (C) S15 and on *E. coli* (D) control without PI treatment; (E) S08; (F) S15

By interpreting the results of membrane permeabilization assay and FACS analysis, we found that Benzothiazole Schiff base hybrids could extricate the integrity of plasma membrane, leading to leakage of bacterial cell contents. Also, the test compounds unavoidably enter into the cytoplasm, but the effect of these test compounds in the cytoplasm is absolutely unknown. Thus, we determined the DNA-binding propensity of test compounds S08 and S15 by analyzing the electrophoretic mobility of plasmid DNA at different concentrations. Compound S15 shows prominent binding with pUC19 plasmid DNA (200ng) causing retardation at 31.2 µg/ml, whereas the compound S08 shows no retardation even at all concentration range used (Figure 8).

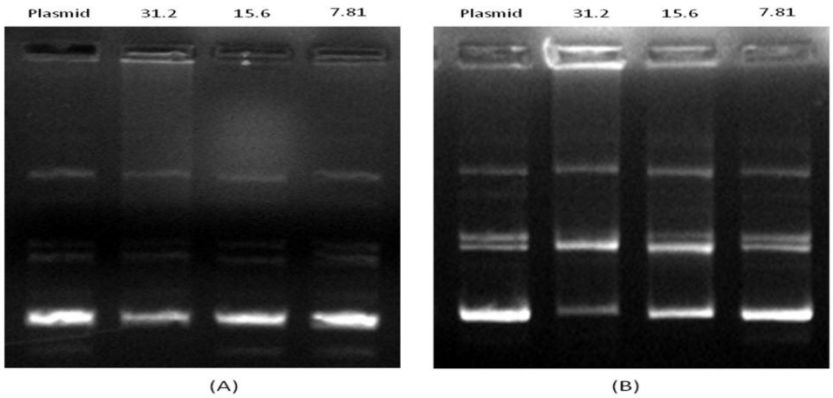



**Fig-8: Agarose gel electrophoresis patterns of pUC19 (200 ng)**

(A) Cleaved by S08 (31.2–7.81 µg/ml), after 1 h incubation time (concentration dependent) Lane 1: control; Lane 2: 31.2 µg/ml S08 + DNA; Lane 3: 15.6 µg/ml S08 + DNA; Lane 4: 7.81 µg/ml S08 + DNA

(B) Cleaved by S15 (31.2–7.81 µg/ml), after 1 h incubation time (concentration dependent) Lane 1: control; Lane 2: 31.2 µg/ml S15 + DNA; Lane 3: 15.6 µg/ml S15 + DNA; Lane 4: 7.81 µg/ml S15 + DNA, in buffer (5 mM Tris–HCl/50 mM NaCl, pH 7.2 at 25 °C)

However, it was interesting that DNA binding was predisposed with the whole structure of the compound, i.e. tri-methoxy containing compound S15 showed most potent DNA binding ability in comparison with compound S08. Overall, the study reveals that the lead compounds (S08 and S15) and related compounds disrupts the membrane potential which was exploited for cellular energy production and membrane damage and, somehow showed binding interactions with plasmid DNA.

To explore the actual mechanism of preferential binding with major and minor groove regions of DNA, the MG/DAPI staining techniques were used. The DNA was treated with DAPI or MG, prior to the addition of compounds. When DAPI (minor groove binder) was added to the reaction mixture containing compound S15, significant inhibition in the cleavage pattern was observed. But, in presence of methyl green (major groove binder), the cleavage was not suppressed (Figure 9). Thus electrophoretic pattern demonstrated that compound S15 showed specific binding affinity towards the major groove and partial affinity towards the minor groove.

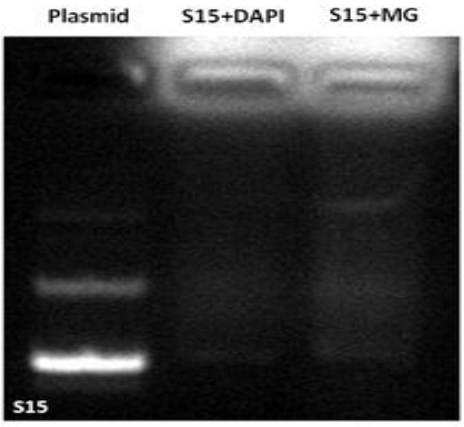



**Fig-9: Agarose gel electrophoresis pattern for the cleavage of supercoiled DNA (200 ng) by compound S15 in the presence of DAPI and Methyl Green**

## CONCLUSION

The antimicrobial screening of a series of benzothiazole Schiff base hybrids has led to the identification of compounds S02, S08, S13, S15 and S19 as potential candidates worthy of further structural modification and pharmacological evaluation. It may be concluded that, compounds having electron donating substituents such as methoxy, hydroxyl on phenyl ring as well as schiff bases of cyclic ketones have pronounced activity which was also supported by the 3D-QSAR study. Further, the compounds containing electron withdrawing substituent on phenyl ring (fluro, bromo, nitro) do not produce significant anti-bacterial activity. Compounds S08 and S15 acts by membrane perturbing mode of action at concentration higher than their MICs. Compound S15 was also able to alter the electrophoretic mobility of DNA. Although DNA binding capability was not directly related to antibacterial activity but may be responsible for further enhanced potency of this compound due to intracellular mode of action. On further optimization of lead compounds obtained from this study may result in efficacious antimicrobial therapeutic agents.

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## REFERENCES

1. Martins, M., McCusker, M., Amaral, L., & Fanning, S. (2011). Mechanisms of Antibiotic Resistance in Salmonella: Efflux Pumps, Genetics, Quorum Sensing and Biofilm Formation. *Lett Drug Des Discov*, *8*(2), 114-123.
2. Lerner, C. G., & Beutel, B. A. (2002). Antibacterial drug discovery in the post-genomics era. *Curr Drug Targets Infect Disord*, *2*(2), 109-119.
3. Singh, M., & Singh, S. K. (2014). Benzothiazoles: how relevant in cancer drug design strategy? *Anti-Cancer Agents in Medicinal Chemistry*, *14*(1), 127-146.
4. Singh, M., Singh, S. K., Gangwar, M., Nath, G., & Singh, S. K. (2014). Design, synthesis and mode of action of some benzothiazole derivatives bearing an amide moiety as antibacterial agents. *RSC Adv*, *4*(36), 19013-19023.
5. Singh, M., Gangwar, M., Nath, G., & Singh S. K. (2014). Synthesis, DNA cleavage and antimicrobial activity of 4-thiazolidinones-benzothiazole conjugates. *Indian J Exp Biol. 52*(11), 1062-1070.
6. Singh, M., Singh, S. K., Gangwar, M., Sellamuthu, S., Nath, G., & Singh, S. K. (2016). Design, Synthesis and Mode of Action of some new 2-(4′-aminophenyl) benzothiazole Derivatives as Potent Antimicrobial Agents. *Lett Drug Des Discov*, *13*(5), 429-437.
7. Singh, M., Singh, S. K., Gangwar, M., Nath, G., & Singh, S. K. (2016). Design, synthesis and mode of action of novel 2-(4-aminophenyl) benzothiazole derivatives bearing semicarbazone and thiosemicarbazone moiety as potent antimicrobial agents. *Med Chem Res*, *25*(2), 263-282.
8. Singh, M., Singh, S., Thakur, B., & Ray, P. (2016). Design and Synthesis of Novel Schiff base-benzothiazole hybrids as potential Epidermal Growth Factor Receptor (EGFR) Inhibitors. *Anti-cancer agents in medicinal chemistry*, *16*(6), 722-739.
9. Singh, M., Modi, A., Narayan, G., & Singh, S. K. (2016). Benzothiazole derivatives bearing amide moiety: potential cytotoxic and apoptosis-inducing agents against cervical cancer. *Anticancer Drugs*, *27*(6), 519-532.
10. Wayne, P. (1997). National committee for clinical laboratory standards. NCCLS Approved standard M27-A, USA.
11. Joshi, S. D., Kumar, D., More, U. A., & Aminabhavi, T. M. (2014). Docking, CoMFA, and CoMSIA analyses of phenoxy triazole derivatives as enoyl-ACP reductase inhibitors for *Escherichia coli*. *Med Chem Res*, *23*(11), 4932-55.
12. Gao, L., Zu, M., Wu, S., Liu, A. L., & Du, G. H. (2011). 3D QSAR and docking study of flavone derivatives as potent inhibitors of influenza H1N1 virus neuraminidase. *Bioorg Med Chem Lett*, *21*(19), 5964-5970.