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MEDICINAL CHEMISTRY RESEARCH

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Synthesis and anti-biofilm activity of thiazole Schiff bases

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Abstract A series of thiazole Schiff bases (SB-1 to SB-7) have been synthesized by reacting 4-(*o*-methoxyphenyl)-2-aminothiazole and R substituted salicylaldehyde (R = H, 3-Me, 4-Me, 5-Me, 3-OMe and 5-Br) or 2-hydroxy-1-naphthaldehyde under microwave irradiation (a green chemistry approach). The compounds were characterized by spectral (UV–Vis, IR, ^{1}H NMR, ^{13}C NMR and GC–MS) and thermal analyses, and tested for the evaluation of anti-biofilm activity against *Pseudomonas aeruginosa* and anti-bacterial activity against Gram positive (*Bacillus subtilis* NCIM 2063) and Gram negative (*Escherichia coli* NCIM 2931) bacteria. The scanning electron microscopic images of the bacterial surfaces have shown that the Schiff bases have impeded the biofilm formation at 50–100 μg/mL concentration, without affecting the growth of the cells (and thus behave as antiquorum sensing agents). Confocal laser scanning microscopy has also confirmed the biofilm inhibition. The anti-biofilm and anti-bacterial activities of the Schiff bases are promising in the design and bio-fabrication of medical devices to combat the biofilm-forming pathogenic organisms.

Keywords Quorum sensing · Biofilm · Schiff Bases · Anti-bacterial

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Introduction

Treatment of emerging infectious diseases and the increasing number of multidrug resistant microbial pathogens are the taunting tasks in the medical community (Dessen *et al.*, 2001; Tenover and McDonald, 2005; Muroi *et al.*, 2004; Pfeltz and Wilkinson, 2004; Roberts, 2004). The search for an effective compound to combat bacterial pathogenicity is the need of an hour. Thiazoles are well known as biologically active compounds and exhibit several biological activities such as anti-hypertensive, anti-inflammatory, anti-bacterial, anti-HIV, anti-tumor and cytotoxic activity (Kashyap *et al.*, 2012). Schiff bases derived from aminothiazoles have been shown to possess anti-bacterial and anti-fungal activities (More *et al.*, 2001).

The anti-bacterial activity of different types of Schiff bases is also well reported. Karthikeyan *et al.* (2006) have synthesized Schiff bases (with a 2,4-dichloro-5-fluorophenyl moiety) exhibiting anti-microbial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. Shi *et al.* (2007) have synthesized a series of Schiff bases by reacting 5-chloro-salicylaldehyde and primary amines, and evaluated their anti-bacterial activity against *Bacillus subtilis*, *E. coli*, *P. fluorescence* and *S. aureus*. Sinha *et al.* (2008) have synthesized eight novel heterocyclic Schiff bases derived from the condensation reactions of indole 3-carboxaldehyde with different l-amino acids (histidine, glutamic acid, aspartic acid, leucine, valine) as well as with some aminophenols, and these compounds were found to exhibit anti-bacterial activity against *B. subtilis*, *P. fluorescence*, *S. aureus*. Kamaria *et al*, (2011) have reported a series of Schiff bases (derived from indole-3-aldehyde by microwave-assisted synthesis), which were active against *S. aureus* and *B. subtilis*. However, synthesis of present

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series of Schiff bases derived from aminothiazoles, and evaluation of their bioactivities are not yet reported.

The biofilm synthesized by pathogenic organism has been one of the reasons for the ineffectiveness of antibiotics and development of antibiotics resistance. The formation of biofilm is QS mediated phenomenon. The QS is an inter-cell communication system aided by released chemical signals when cell density reaches a critical concentration. Bacteria utilize QS to control a wide variety of processes, including the biofilm (Dickschat, 2010). A number of pathogenic bacteria employ QS virulence determinants, for example, biofilm formation in *P. aeruginosa* (Stewart and Costerton, 2001; Irie and Parsek, 2008; Hentzer *et al.*, 2003; de Kievit, 2009; Bjarnsholt *et al.*, 2011). Biofilms are defined as conglomerations of bacterial cells protected by a self-synthesized extrapolymeric substance (EPS) (Parsek and Singh, 2003). As microorganisms are protected inside the biofilm, they show an increased resistance to anti-microbial agents including antibiotics compared to free-floating cells. In the medical sector, biofilms have been implicated as the cause of serious infections and up to 60 % of all the human infections are caused by biofilms (Spoering and Lewis, 2001). Bacterial biofilm formation also causes significant economic loss in industrial sectors (VanHoudt and Michiels, 2010). In industry, biofilms have been implicated in the contamination of installations in food industry, decreased passage through pipelines by colonization of the interior of the pipes, and resistance of vessels by initiation of 'biofouling' on the vessel hulls. The yearly economic loss caused by 'biofouling' in the marine industry is estimated at $ 6.5 billion (Yebra *et al.*, 2004).

Antibiotics act by inhibiting the growth (microbiostatic) or killing of the microorganisms (microbiocidal). They act by inhibiting the bacterial functions (such as cell wall synthesis, DNA replication, RNA transcription and protein synthesis) that are essential for growth. However, the actions of antibiotics have been shown to impose a selective pressure that fosters the growth of antibiotic-resistant strains (Sharma *et al.*, 2009). Therefore, antimicrobial therapies that are neither bacteriostatic nor bacteriocidal (i.e. that reduce selective pressure) need to be explored to curb the menace of antibiotic-resistant pathogens.

Microwave-assisted synthesis of organic compounds is an efficient and eco-friendly synthetic strategy and has now become a powerful tool in green chemistry. Microwave-assisted organic reaction is advantageous over the conventional heating owing to shorter reaction time, experimental simplicity, selectivity of products, and easy working up procedures (Caddick, 1995; Verma, 1999; Mavandadi and Lidström, 2004). Schiff bases have been widely explored in the field of coordinated chemistry because of their synthetic flexibility, selectivity and sensitivity towards transition metals (Yamada, 1999). In view of the importance of Schiff bases, and as part of our ongoing project for the synthesis of new antimicrobial agents (Lawand *et al.*, 2008, 2011), we report herein microwave-assisted synthesis of thiazole Schiff bases, their characterization (elemental, spectral and thermal analyses) and exploration of novel functionality as anti-bacterial and anti-biofilm agents.

Results and discussion

Chemistry

All the Schiff bases (SB-1 to SB-7) were yellow crystalline solids having sharp melting points and gave satisfactory data of elemental (C, H and N) analyses.

Two representatives Schiff bases, SB-1 and SB-5, were selected for thermal studies. The thermogravimetric (TG) curves of SB-1 and SB-5 are shown in Figs. 1 and 2, respectively. No weight loss was observed upon heating till 190 °C, and thus, ruling out the presence of water molecules. SB-1 undergoes decomposition in two stages (stage I: 213.05–317.89 °C, weight loss 34.90 % and stage II: 512.18–708.74 °C, weight loss 23.43 %), SB-5 also undergoes decomposition in two stages (stage I: 226.50–470.54 °C, weight loss 38.88 % and stage II 527.39–732.14 °C, weight loss 32.53 %). The kinetic parameters, viz., E (energy of activation), *n* (order of reaction), Z (pre exponential factor), ΔS (entropy of activation) and G (free energy change) have been calculated using Coats Redfern, MacCallum–Tanner and Horowitz–Metzger methods (Horowitz and Metzger, 1963; Coats and Redfern, 1964; MacCallum and Tanner, 1970).

The values of *n*, E, Z, ΔS and G calculated by the three methods are in accordance with each other (Table 1). The values of E for SB-1 (lying in the range 33–39 kcal mol^{-1}

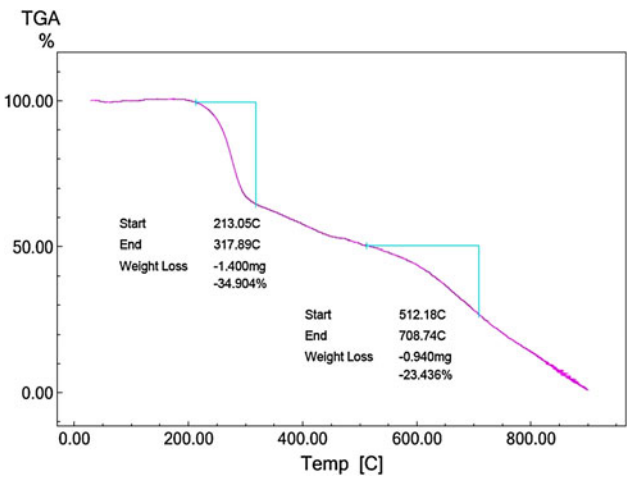

Fig. 1 Thermogrametric curve of SB-1

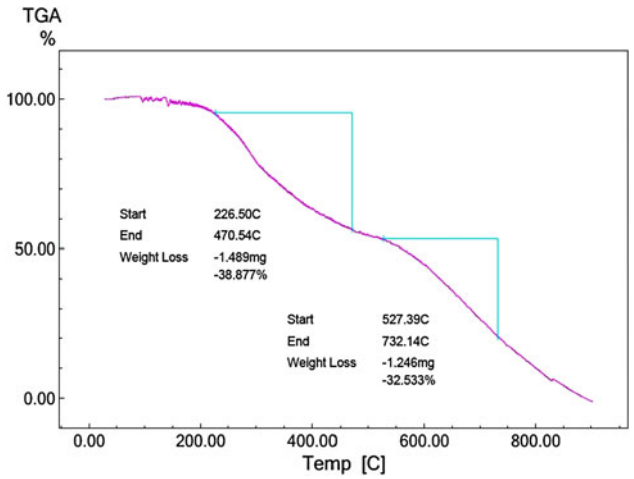

Fig. 2 Thermogravemetric curve of SB-5

for stage I and 34–45 kcal mol^{-1} for stage II) and for SB-5 (20–25 kcal mol^{-1} for stage I and 37–45 kcal mol^{-1} for stage-II) are sufficiently high and indicate that the compounds are thermally stable.

The reaction time, percentage yields and melting points data are given in Table 2. The microwave irradiated synthesis of Schiff bases is convenient and rapid, and resulting in good yield of the expected product. The conventional method of the synthesis of Schiff bases require 20–30 min heating on water bath, whereas the microwave method in the present study requires only 30 s heating in microwave oven (180 times faster than the conventional method of synthesis).

Biological applications

Anti-bacterial studies

We have studied anti-bacterial action of Schiff base by determining the minimum inhibitory concentration (MIC) by dye reduction method and minimum bactericidal concentration (MBC) by counting the number of viable organisms [colony forming units per millilitre (CFU/mL)], which respectively are the lowest concentrations of a compound that can inhibit bacterial growth or kill 99.9 % of organisms (3log), in comparison to the control. We also have performed antimicrobial activities by agar diffusion test. The MIC determination by dye reduction method uses a redox dye, resazurin, which shows a deep blue colour in its oxidized state and a purple colour in its reduced state. When the bacterial cells are viable and metabolically active, i.e. in growing stage, oxidative enzymes present inside the cells reduced the dye to pink colour, however, when the cells are metabolically inactive, the deep blue colour of dye remains unchanged. During the determination of MIC, the cells of the test organism were subjected to Schiff bases in Muller Hinton (MH) broth and incubated for 12 h. Schiff bases SB-6 and SB-7 exhibited anti-bacterial activity whereas other Schiff bases found to be inactive. SB-6 has shown anti-bacterial activity against *B. subtilis*, whereas SB-7 has shown anti-bacterial activity against *B. subtilis* and *E. coli*. The MIC values of SB-6 and SB-7 lie in the range 25–50 μg/mL for *B. subtilis* and 100 μg/mL for *E. coli* respectively. The MIC determination gives information of the compound as a bacteriostatic agent; however, it does not give any idea about the compound as bactericidal agent, as well. The bactericidal property of compounds is far better over bacteriostatic as former property irreversibly inhibit the growth of bacteria. To know the bactericidal property of the Schiff base, the determination of the minimum bactericidal concentration (MBC) was carried out by broth dilution and agar diffusion methods. When an aliquot from all MIC-wells were spread on the MH agar plate, and incubated for 24 h to develop colony, if any, it was found that SB-6 and SB-7 showed a reduction in the number of viable cells, and the rate of reduction was concentration dependent (Fig. 3). The MBC value of SB-6 was found to be 50 μg/mL against *B. subtilis* and that of SB-7 was found to be 100 μg/mL against *E. coli* (Fig. 3). Agar diffusion test has confirmed the MBC

Table 1 Calculation of kinetic parameters by Coats-Redfern (C.R.), MacCallum-Tanner (M.T.) and Horowitz-Metzger (H.M.) methods

Schiff bases	K.E	Stage-I			Stage-II		
		C.R	M.T	H.M	C.R	M.T	H.M
SB-1	*n*	1.17	1.77	1.38	1.30	1.285	1.65
	E	33.13	37.57	39.02	34.60	40.30	44.45
	Z	9.81	7.64	12.34	4.45	2.62	8.99
	ΔS	−7.46	−12.46	−1.60	−20.32	−24.52	−14.46
	G	37.19	44.35	39.89	53.13	62.66	57.63
SB-5	*n*	2.35	2.23	3.0	1.35	1.32	1.52
	E	20.80	24.45	25.03	37.82	43.35	45.27
	Z	4.14	2.07	6.07	5.19	3.23	7.08
	ΔS	−20.56	−25.33	−16.17	−18.66	−23.14	−14.26
	G	32.45	38.40	34.19	54.96	64.60	58.36

E = kcal mol^{-1}, Z = s^{-1}, ΔS = J K^{-1} mol^{-1}, G = kcal mol^{-1}

Table 2 Comparative account of synthesis of Schiff bases by conventional and microwave methods

Sr No.	Comp	Conventional method		Microwave method		M P (°C)
		Yield (%)	Time (Min)	Yield (%)	Time (Sec)	
1	SB-1	95	30	98	10	133
2	SB-2	90	30	95	20	156
3	SB-3	92	30	99	10	139
4	SB-4	90	30	98	10	157
5	SB-5	89	30	95	10	168
6	SB-6	88	30	95	10	209
7	SB-7	91	30	97	10	184

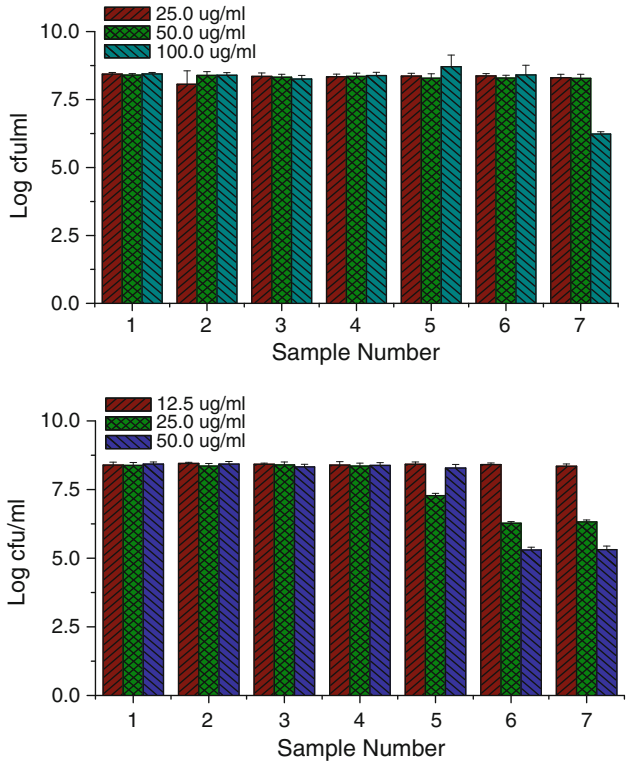

Fig. 3 Determination of minimum bactericidal concentration (MIC) of Schiff bases (SB1–SB7) against *B. subtilis* and *E. coli*. Cells of test organisms subjected to various conc. of Schiff bases showed a reduction in number of viable cells. Only SB-6 and SB-7 at 25 µg/mL showed a 3log reduction against *B. subtilis* (*upper*) while SB-7 at 100 µg/mL showed 2log reductions against *E. coli* (*lower*). Note very less reduction of cells (1log reduction) in the presence of SB-5 against *B. subtilis*. *Error bars* represents the standard deviation ($n = 3$)

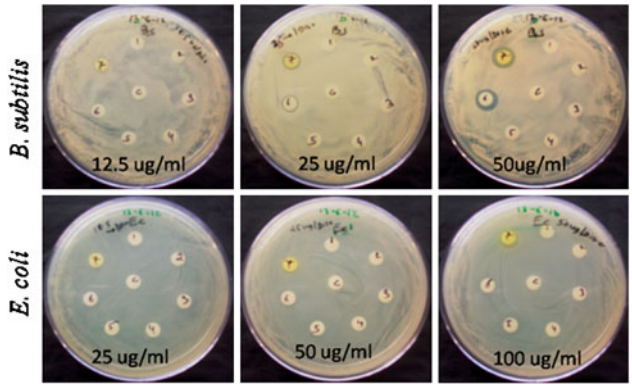

Fig. 4 Determination of minimum bactericidal concentration (MIC) of Schiff bases against *B. subtilis* and *E. coli*. A zone of inhibition was observed around disc against *B. subtilis* for sample 6 and 7, while SB-7 only showed a zone of inhibition in *E. coli*

values (Fig. 4). A zone of inhibition was observed around the disc of SB-6 and SB-7 against *B. subtilis* (25 and 50 µg/mL), while a similar zone of inhibition was observed around the disc of SB-7 against *E. coli* (100 µg/mL). Thus, the characterized Schiff bases showed an anti-bacterial property. Literature survey reveals that Schiff bases have been shown to possess antimicrobial activities (Walsh *et al.*, 1996; Sridhar *et al.*, 2001; Mladenova *et al.*, 2002; Panneerselvam *et al.*, 2005; Bharti *et al.*, 2010; Desai et al. 2013; Pandey *et al.*, 2012), however, evaluation of Schiff bases synthesized by microwave-assisted methods are rare (Kamaria *et al.*, 2011; Pandey *et al.*, 2012; Venkatesan *et al.*, 2012) and that too by reacting amine with salicylaldehyde are very rare (Pandey *et al.*, 2012). Shi *et al.* 2007 have evaluated the anti-microbial activity of a series of Schiff bases derived from the condensation of 5-chloro-salicylaldehyde and primary amines, and showed that the compounds with aromatic rings were more active than compounds with aliphatic chains. Infact, it seems that the order of anti-bacterial activity against bacterial strains increases with an increase in the complexities of the R-substituent of salicylaldehyde.

Anti-biofilm

Most of the bacteria acquire the resistance to antibiotics by virtue of the synthesis of an extracellular matrix (biofilm), a QS trait. So, an anti-biofilm (anti QS) approach is the promising to tackle the aforementioned problem (Hammer and Bassler, 2003; Cvitkovitch *et al.*, 2003). Current efforts towards small molecule-based strategies to control biofilm formation have focused almost exclusively on inhibiting QS, a signalling cascade that is critical for bacterial communication (Smith *et al.*, 2003; Geske *et al.*, 2005). However,

Schiff bases were not yet explored as anti-biofilm agents. So, we have taken up a study to explore a possibility role of Schiff bases in inhibiting/impeding the formation of biofilm in *P. aeruginosa*.

Biofilm and SEM

As we were interested to study the QS mediated biofilm inhibition in *P. aeruginosa*, we have selected only those Schiff bases that showed an anti-bacterial activity. Only SB-5 and SB-6 showed an anti-bacterial activity against *P. aeruginosa*. When the cells of *P. aeruginosa* were subjected to sub-inhibitory concentration of Schiff bases, there was a prominent decrease in the biofilm formation; the enmeshed uncovered cells were observed i.e. cells without the biofilm. However, in absence of Schiff bases, cells of *P. aeruginosa* were seen as enmeshed-covered structures, and cells were enclosed, a typical of biofilm structures (Fig. 5). An enmesh observed under SEM is a complex of polysaccharides, synthesized by growing cells in response to QS; however, in presence of Schiff bases QS was disturbed, and hence an enmesh of the structures was not observed under SEM. More importantly, the numbers of the planktonic cells (cells in suspension) were not affected, suggesting that the inhibition of the biofilm in *P. aeruginosa* is QS mediated.

Biofilm and CLSM

The biofilm is a complex mixture of DNAs, proteins and majorly polysaccharides. The Concanavalin A is a fluorescent molecule with a high affinity towards glucosyl-mannosyl residue of polysaccharide of biofilm. When the biofilm of the *P. aeruginosa* was treated with Con A alexafluor 488 fluorescent dye and observed under CLSM at 10 X magnification, a continuous and green fluorescence was observed. However, in presence of the Schiff base, while the green fluorescence was diminished in presence of SB-5, SB-6, it decreased in presence of SB-7. The fluorescence intensity of the biofilm in presence of SB-1, SB-2, SB3 and SB-4 was unaffected (Fig. 6), thus further confirming the results of SEM.

Most of the anti-biofilm studies explored natural products or its derivatives. The best-studied examples of the natural products as anti-biofilm agents are (1) halogenated furanones, which were originally isolated from the seaweed Deliseapulchra, (2) analogues of the homoserine lactone signalling molecules and (3) analogues of the sponge-derived marine natural alkaloids oroidin and bromoageliferin (Stowe *et al.*, 2011). However, examples of synthetic compounds as anti-biofilm agents are few. The scaffolds of the chemical structures explored till date to study the anti-biofilm activities are aminothiazoles, substituted pyrimidinium salts, substituted imidazoles, TAGE-triazole conjugates and 4-Thiazolidinones derivatives (Huigens *et al.*, 2009; Steenackers *et al.*, 2010; More et al. 2012; Rane *et al.*, 2012; Tello *et al.*, 2013). Thus, vast reportier of the chemical scaffolds are yet unexplored as an anti-biofilm agent. Because expression of many virulence factors and establishment of chronic infections take an advantage of biofilm (regulated by QS) [Van Delden and Iglewski, 1998], the disruption of biofilm is expected to reduce the pathogenicity of many biofilm forming pathogens.

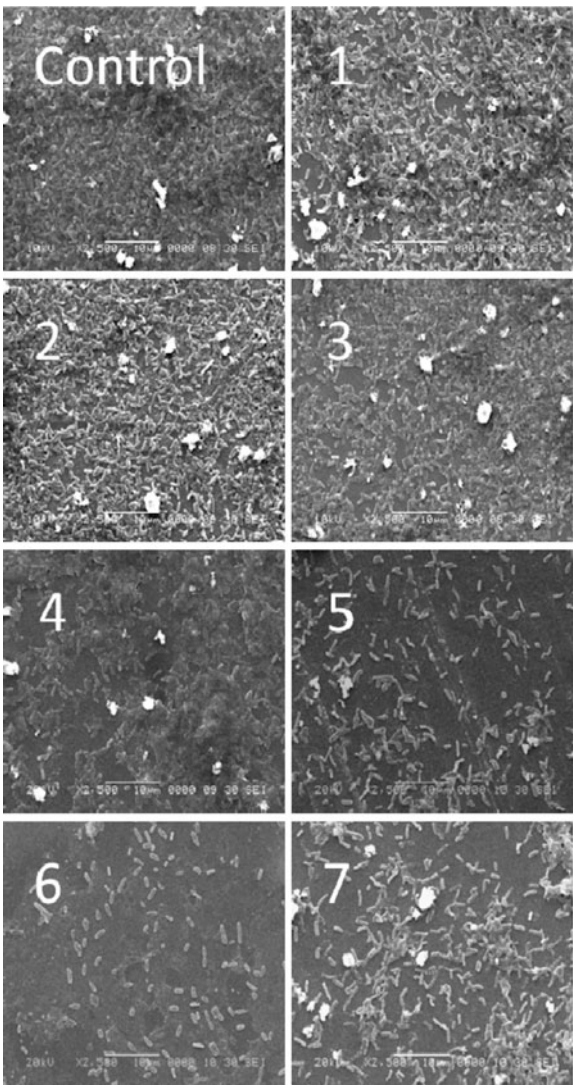

Fig. 5 Scanning electron microscope analysis of Biofilm *P. aeruginosa* shows dense mass of cells surrounded by biofilm. However, in presence of Schiff bases, 5, 6 and 7, individual cells were observed, indicating an inhibition of biofilm formation

Conclusion

The microwave-assisted method successfully synthesized 4-(*o*-methoxyphenyl)-2-aminothiazole by green chemistry approach. The as-synthesized compound shows a thermal

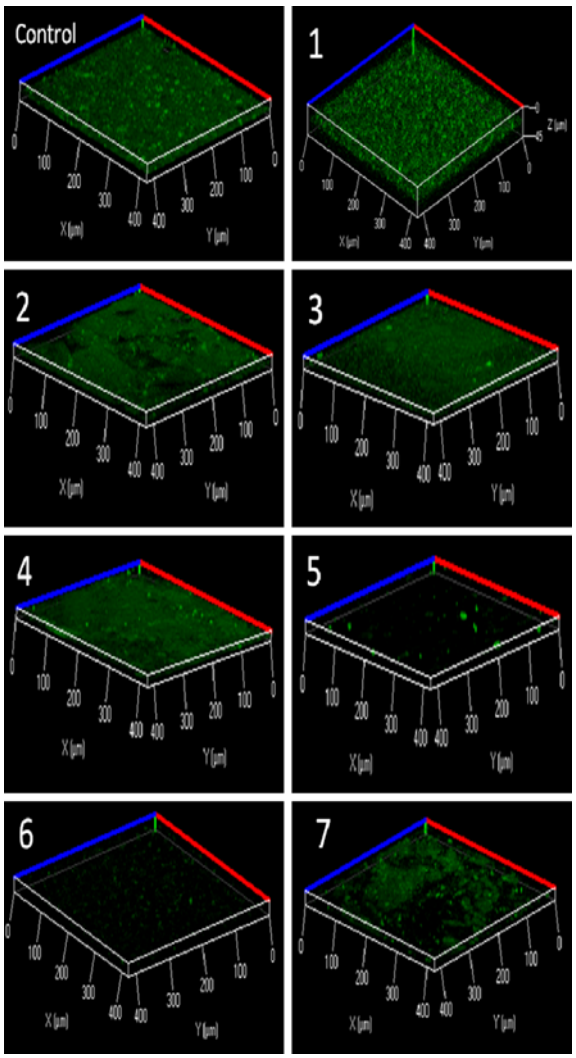

Fig.. 6 Confocal laser scanning microscope analysis of biofilm. *Pseudomonas aeruginosa* shows the patches of fluorescence in presence of Schiff bases, SB-5, SB-6 and SB-7, indicated a truncated formation of biofilm. However, in absence of Schiff bases (control), fluorescence was regular and entire, thus indicating an intact biofilm

stability and is bioactive. The reduction in the number of viable cells in comparison to the control shows an antibacterial nature of the compound. Reduction of the polysaccharides (a matrix) in presence of Schiff bases shows impedance in the QS mediated biofilm formation. *The anti-QS approach is highly attractive because it does not impose harsh selective pressure for the development of resistance with antibiotics because QS is not directly involved in the processes essential for growth of bacteria.* This is important because when the growth is not affected, there is no selective pressure for the development of resistant bacteria. Inhibition of bacterial QS, rather than bactericidal or bacteriostatic strategies, may find application in many different fields, such as medicine, agriculture and food technology.

Experimental

All the chemicals used were of A. R. Grade. 4-(*o*-methoxyphenyl)-2-aminothiazole was synthesized according to the procedure available in literature (Patai, 1970). The solvents were dried according to the standard procedures and distilled before use. UV–Vis spectra were recorded in ethanol on Shimadzu A 600 UV–Vis spectrometer. IR spectra were recorded in KBr pellets on Shimadzu FT-IR 8400 spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ using TMS as the standard on Varian 300 MHz spectrometer. ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as the standard on Varian 300 MHz spectrometer. GC–MS were recorded on Shimadzu GC–MS QP 5050 mass spectrometer. Microwave mediated reactions were carried out in Onida-power conventional 25 DLX microwave oven.

Microwave-assisted synthesis of Schiff bases (SB-1 to SB-7)

4-(*o*-Methoxyphenyl)-2-aminothiazole (1 mol) and *o*-hydroxyaldehyde (1 mol) were mixed with each other in mortar-pestle, and the reaction mixture was placed in small conical flask at room temperature. The mixture was then added with 1 mL alcohol exposed to microwave irradiation at 10 % power for 10–20 s. (Fig. 7). Completion of the reaction was tested by TLC. The reaction mixture was cooled to room temperature. The yellow coloured Schiff base was further recrystallized from ethanol and dried under reduced pressure.

Spectral analysis

UV–Vis spectra of the Schiff bases exhibit an intense band at ~400 nm. The UV–Vis spectrum of 2-aminothiazole

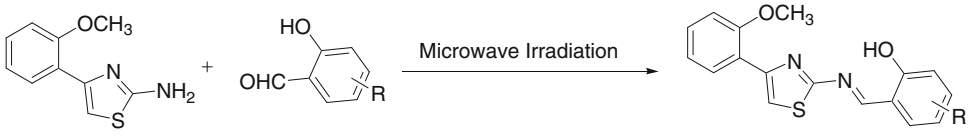

Fig. 7 Thiazole Schiff base SB-1: (R = H), SB-2: (R = 3-CH_3), SB-3: (R = 4-CH_3), SB-4: (R = 5-CH_3), SB-5: (R = 3-OCH_3), SB-6: (R = 5-Br), SB-7: (R = 2-H_1-N)

exhibits an intense band at ~275 nm and other aromatic amino compounds with comparable structures exhibit absorption (Conover and Tarbell, 1950; Mathes and Gregory, 1952; Wilson and Woodger, 1955) at ~300 nm. The shifting of absorption band towards higher wavelength (~400 nm) in the present Schiff bases may be due to extended conjugation in the molecule (Patai, 1970). The IR spectra of the Schiff bases exhibit ν(O–H), ν(C=N), ν(C–O) and ν(C–S–C) modes at ~3,430 to ~3,460, ~1,640, ~1,280 and ~660 cm^{-1} respectively, and these values are in accordance with the earlier reports (Silverstein *et al.*, 2005). The ν(OH) mode is broad and weak and this may due to hydrogen bonding between phenolic OH and nitrogen of the azomethine group forming a six membered ring (Kovacic, 1967). The ^1H NMR spectra of the Schiff bases were recorded. The assignments of NMR signals show close resemblance with the earlier results (Silverstein *et al.*, 2005). The spectral analyses data confirm the structure of Schiff bases as represented in Fig. 1. The spectral data is represented below.

Sb-1

UV–Vis: λ_{max} 381 nm; IR(cm^{-1}): ν(O–H) 3454, ν(C=N) 1622, ν(C–O) 1276, ν(C–S–C) 665 and phenyl and thiazole ring vibrations 1597, 1487, 1159; ^1H NMR: ($CDCl_3$, TMS, δppm), 3.93 (3H, s, Ar-OCH_3), 6.93 (1H, s, H-thiazole), 6.95–7.86 (8H, m, Ar–H), 8.25 (1H, s, benzylidenimin), 12.38 (1H, s, Ar–OH). GC–MS: m/z (relative intensity, %): 310 (99.21) (M^+ peak) (Molecular formula: $C_{17}H_{14}N_2O_2S$), 293 (30.69), 277 (70.29), 191 (27.72), 164 (29.70), 147 (56.43), 132(100), 121 (54.45), 104 (24.75), 91 (33.66), 77 (72.27), 63 (19.80), 51 (44.55).

Sb-2

UV–Vis: λ_{max} 384 nm; IR(cm^{-1}): ν(O–H) 3455, ν(C=N) 1640, ν(C–O) 1235, ν(C–S–C) 665 and phenyl and thiazole ring vibrations 1590, 1560, 1400, 1100; ^1H NMR: ($CDCl_3$, TMS, δppm) 2.30 (3H, s, Ar–CH_3), 3.92 (3H, s, Ar–OCH_3), 6.88 (1H, s, H-thiazole), 7.04–8.27 (7H, m, Ar–H), 9.22 (1H, s, benzylidenimin), 12.60 (1H, s, Ar–OH); ^{13}C NMR: ($CDCl_3$, TMS, δppm) 167.4, 164.6, 159.8, 156.9, 149.5, 135.4, 131.2, 130.0, 129.0, 126.3, 122.8, 120.8, 119.0, 117.7, 116.9, 111.0, 55.3, 15.4. GC–MS: m/z (relative intensity, %): 324 (100) (M^+ peak) (Molecular formula: $C_{18}H_{16}N_2O_2S$), 291 (50.49), 191 (20.79), 161 (17.82), 146 (21.78), 132 (80.19), 121 (43.56), 91 (33.66), 77 (40.59), 65 (22.77), 51 (17.82).

Sb-3

UV–Vis: λ_{max} 383 nm; IR(cm^{-1}): ν(O–H) 3470, ν(C=N) 1630, ν(C–O) 1236, ν(C–S–C) 662 and phenyl and thiazole ring vibrations 1591, 1485, 1157, 1130; ^1H NMR: ($CDCl_3$, TMS, δppm) 2.37 (3H, s, Ar–CH_3), 3.95 (3H, s, Ar–OCH_3), 6.85 (1H, s, H-thiazole), 7.01–8.27 (7H, m, Ar–H), 9.24 (1H, s, benzylidenimin), 12.35 (1H, s, Ar–OH). GC–MS: m/z (relative intensity, %): 324 (86.73) (M^+ peak) (Molecular formula: $C_{18}H_{16}N_2O_2S$), 307 (34.69), 291 (51.02), 191 (23.46), 178 (16.32), 161 (23.46), 146 (29.59), 132 (100), 121 (37.75), 91 (38.77), 77 (30.61), 65 (22.44), 51 (13.26.)

Sb-4

UV–Vis: λ_{max} 383 nm; IR(cm^{-1}): ν(O–H) 3455, ν(C=N) 1626, ν(C–O) 1271, ν(C–S–C) 667 and phenyl and thiazole ring vibrations 1570, 1356, 1182, 1146; ^1H NMR: ($CDCl_3$, TMS, δppm) 2.30 (3H, s, Ar–CH_3), 3.94 (3H, s, Ar–OCH_3), 6.94 (1H, s, H-thiazole), 7.04–8.20 (7H, m, Ar–H), 9.21 (1H, s, benzylidenimin), 12.20 (1H, s, Ar–OH). GC–MS: m/z (relative intensity, %): 324 (97.02) (M^+ peak) (Molecular formula: $C_{18}H_{16}N_2O_2S$), 307 (65.34), 291(94.35), 192 (18.81), 161 (21.78), 146 (23.74), 132 (100), 121 (42.57), 91 (33.66), 77 (39.60), 65 (21.78), 51 (17.82).

Sb-5

UV–Vis: λ_{max} 382 nm; IR(cm^{-1}): ν(O–H) 3435, ν(C=N) 1627, ν(C–O) 1258, ν(C–S–C) 661 and phenyl and thiazole ring vibrations 1588, 1485, 1465, 1175; ^1H NMR: ($CDCl_3$, TMS, δppm) 3.94 (3H, s, Ar–OCH_3 of thiazole), 3.96 (3H, s, Ar–OCH_3 of aldehyde), 6.92 (1H, s, H-thiazole), 6.99–8.28 (7H, m, Ar–H), 9.32 (1H, s, benzylidenimin), 12.63 (1H, s, Ar-OH); ^{13}C NMR: ($CDCl_3$, TMS, δppm) 167.2, 164.5,156.9, 151.5, 149.5, 148.3, 130.0, 129.0, 124.9, 122.7, 120.7, 119.0, 118.4, 117.1, 115.8, 111.0, 56.1, 55.3. GC–MS: m/z (relative intensity, %): 340 (100) (M^+ peak) (Molecular formula: $C_{18}H_{16}N_2O_3S$), 323 (5.88), 307 (16.66), 282 (6.82), 264 (18.63), 205 (23.52), 192 (31.37), 177 (27.45), 162 (46.07), 150 (44.11), 132 (88.23),121 (46.07), 105 (21.56), 91 (34.31), 77 (58.82), 63 (48.03), 45 (48.03), 40 (70.58).

Sb-6

UV–Vis: λ_{max} 390 nm; IR(cm^{-1}): ν(O–H) 3455, ν(C=N) 1614, ν(C–O) 1271, ν(C–S–C) 679 and phenyl and thiazole ring vibrations 1550, 1487, 1350, 1155; ^1H NMR: ($CDCl_3$, TMS, δppm) 3.97 (3H, s, Ar–OCH_3), 6.95 (1H, s, H-thiazole), 7.00–8.27 (7H, m, Ar–H), 9.24 (1H, s, benzylidenimin), 12.39 (1H, s, Ar–OH); ^{13}C NMR: ($CDCl_3$, TMS, δppm) 166.8, 163.0, 160.4, 157.0, 149.8, 136.9, 135.3, 130.1, 129.2, 122.7, 120.9, 119.1, 119.4, 117.6, 116.1, 111.0, 55.4. GC–MS: m/z (relative intensity, %): 388/390* (35.29/32.35) (M^+ peak) (Molecular formula: $C_{17}H_{13}$ Br N_2O_2S),

371/373* (11.76/12 .74), 355/357* (13.23/13.72), 199 (10.29), 191 (26.47), 164 (19.60), 146 (26.47), 132 (100), 121 (47.05), 102 (22.54), 89 (27.45), 77 (50.00), 63 (35.29), 51 (29.41). The peaks marked with (*) are isotopic peaks.

Sb-7

UV–Vis: λ_{max} 395 nm; IR(cm^{-1}): ν(O–H) 3410, ν(C=N) 1620, ν(C–O) 1242, ν(C–S–C) 661 and phenyl and thiazole ring vibrations 1554, 1456, 1155; ^{1}H NMR: ($CDCl_3$, TMS, δppm) 3.97 (3H, s, Ar–OCH_3), 7.00 (1H, s, H-thiazole), 7.10–8.35 (10H, m, Ar–H), 10.18 (1H, s, benzylidenimin), 14.42 (1H, s, Ar–OH). GC–MS: m/z (relative intensity, %): 360 (100) (M^+ peak) (Molecular formula: $C_{21}H_{16}N_2O_2S$), 343 (59.80), 327 (19.60), 192 (55.88), 169 (27.45), 164 (21.56), 149 (19.60), 132 (99.01), 121 (45.09), 89 (23.52), 77 (51.96), 63 (21.56), 45 (28.43).

Anti-bacterial study

Determination of MIC and MBC values

An anti-bacterial study was carried out by broth dilution method using two different organisms, Gram positive, *B. subtilis* NCIM 2063 and Gram negative, *E. coli* NCIM 2931. The anti-bacterial study was carried out by dye reduction method. In short, a 24-well microtitre plates containing 1 mL MH broth, with various Schiff bases, in the concentration range of 12.5–100 μg/mL, were inoculated with the test strains (approximate final cell density of 1×10^6 CFU/mL), and incubated at 37 °C for 15 h. The lowest concentration of compound showing no change in colour (no growth) was considered as MIC. The MBC was measured by preparing serial dilutions from the MIC assay and plating the dilutions on MH agar plates. The MBC is defined as the minimum concentration at which there was a 3 log reduction in the CFU. The data was recorded as survival rates (CFU/mL), based on 100 % survival for the untreated control. All MIC and MBC values reported were based on three experimental repeats. The anti-bacterial activity was also studied by disc diffusion methods. In short, a 100 μL of overnight culture adjusted to 1.0 O.D (600 nm) was spread on 90 mm MH gar plate and antibiotics discs with 2.5–100 μg of the respective compound were impregnated and incubated at 37 °C for 15 h, and observed for zone of inhibition around discs.

Antiquorum sensing

SEM analysis of biofilm

Pseudomonas aeruginosa was grown overnight in LB medium at 37 °C with agitation. After growth, the culture was diluted with LB medium (OD_{600} 0.02), and 50 μL of the diluted culture was added to 950 μL of LB medium supplemented with 25 μg/mL of Schiff bases and were incubated statically for 18 h at 37 °C in 8-well glass chamber slide. After incubation, planktonic bacteria were discarded, and the biofilms were washed three times with cocdylate buffer (0.1 M, pH 7.4). Biofilms formed on glass plates were fixed in 2 % glutaraldehyde in 0.1 M cocdylate buffer (pH 7.4) for 4 h at 4 °C. After thorough washing with cocadylate buffer, samples were dehydrated in a series of ethanol solutions (10–100 %). The samples were dried, mounted on aluminium stubs with conductive carbon cement and then coated with a gold film. Samples were observed with a Joel, JSM, 6760A SEM at 20 kV accelerating voltage.

CLSM analysis of biofilm

Pseudomonas aeruginosa was grown overnight in LB medium at 37 °C with agitation. After growth, the culture was diluted with LB medium (OD_{600} 0.02), and 50 μL of the diluted culture was added to 950 μL of LB medium supplemented with 25 μg/mL of Schiff bases (SB-1 to SB-7) and were incubated statically for 18 h at 37 °C in 8-well glass chamber slide. After incubation, planktonic bacteria were discarded, and the biofilms were washed three times with cocdylate buffer (0.1 M, pH 7.4). Biofilms formed on glass plates was covered with 50 μL of 10 μg/mL of dye, Concanavalin A Alexafluor 488 and incubated for 30 min at 4 °C. The biofilm was finally washed with cocdylate buffer of 0.1 M, pH 7.4 and observed under fluorescence microscopy (Zeiss, Germany) with an excitation of wavelength of 488 nm and emission at 519 nm.

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