Int. J. Chem. Sci.: 14(4), 2016, 2535-2545
ISSN 0972-768X
www.sadgurupublications.com

ANTIBIOFILM ACTIVITY OF THIAZOLE SCHIFF BASES

NETAJI N. KARALE*

Department of Chemical sciences Vidya Pratishthan's Arts, Science and Commerce College, Baramati, PUNE – 413102 (M.S.) INDIA

ABSTARCT

Drug resistance is a growing problem of 21st century. Therefore, to curb the drug resistant microbial infections, in the present study, we synthesized Schiff base, N-(3,5 dichlorosalicylidene)-4-(*o*-methoxyphenyl)-2-aminothiazole, by reacting 4-(*o*-methoxyphenyl)-2-aminothiazole and 3,5 dichlorosalicylaldehyde by microwave irradiation (a green chemistry approach) and conventional method. The as-synthesized Schiff bases were characterized by Uv-Visible, IR, ^{1}H NMR, ^{13}C NMR and GC-MS. The Schiff bases have inhibited the biofilm formations in *Candida albicans* MTCC 227. An amount of 100 ug of Schiff bases was sufficient to bring about more than 70% inhibition of biofilm in *C. albicans*. The biofilm inhibition by the action of Schiff bases was further evident from optical and scanning electron microscopic observations. Our study is the first report of the use of schiff bases as an inhibitor of biofilm formation in *Candida albicans*. The antibiofilm activity shown by Schiff bases is significant in biomedical field in controlling biofilm formed by *C. albicanss* and similar organisms.

Key words: Aminothiazole, Schiff base, *Candida albicans*, Biofilm.

INTRODUCTION

The medical community is confronted with the challenges to treat and control emerging infectious diseases, and the increasing number of multi-drug resistant microbial pathogens.[1-5] In this instance, the infection caused by fungi, in particular, by *Candida albicans* is serious owing to physiological attributes (virulences) of *C.albicans* such as i) adhesion to human cells with subsequent invasion,[6] (ii) the ability to form biofilm on human mucosa, on artificial surfaces such as catheters[7,8] and dental devices[9,10] and (iii) the ability to switch from yeast to hyphal form.[11] Amongst these, the biofilm formation is an important factor in *C. albicans* pathogenesis since it is highly resistant to antifungal drugs. This resistance in *C. albicans* is multifactorial and complex, and involves: (i) limited drug penetration into the biofilm due to the viscous extracellular matrix, (ii) binding or adsorption of drug by the biofilm, (iii) over-expression of genes involved in drug resistance, (iv) and

*Author for correspondence; E-mail: nnkarale@gmail.com; Ph.: +91-2112 243 488

multidrug tolerance due to persistent cells.[12,13] The formation of *Candida* biofilms is clinically relevant because cells immobilized in biofilm are much more resistant to antifungal therapies as compared to their planktonic counterparts, and thus, are shielded from the host immune defenses.[14] Thus, the physiological outcome of biofilm, increased drug resistance and pathogenicity, emphasizes the need for new search of new antibiofilm agents that can inhibit biofilm formation or destroy preformed biofilm formed by *Candida* species, and in this context, Schiff bases of thiazoles can be considered as a new candidate for the control of biofilm in *C. albicans* and similar organisms.

Schiff bases (formed by the condensation of primary amines with carbonyl compounds) have been widely explored in the field of coordinated chemistry because of their synthetic flexibility, selectivity and sensitivity towards transition metals. A number reviews on studies of Schiff base metal complexes have been devoted to coordination chemistry.[15,16] In recent year Schiff received much attention in bioorganic chemistry for the synthesis of bioactive agents.[17-19] The bioactive potentials in Schiff bases is likely due to the coordination of imine nitrogen atom and other active centers available in thiazoles with metal ions like Cu (II), Co(II), Ni(II), or Zn(II),[20] resulting in the change of size, shape, charge density distribution, and redox potentials of Schiff bases.

Therefore, in continuation of our previous work[18,19] for the the search for the bioactive molecule, in the present study, we synthesized N-(3,5 dichlorosalicylidene) -4-(*o*-Methoxyphenyl)-2-aminothiazole, by reacting 4-(*o*-methoxyphenyl) 2-aminothiazole and 3,5 dichlorosalicylaldehyde by microwave irradiation (a green chemistry approach) and conventional method, and studied antibiofilm activity against *C. albicans*.

EXPERIMENTAL

Materials and methods

All the chemicals used in present study were of A. R. Grade. 4-(o-methoxyphenyl)-2-aminothiazole was synthesized according to the procedure available in literature[21]. The solvents were dried according to the standard procedures and distilled before use. UV-Visible spectra were recorded in ethanol on Shimadzu A 600UV-Visible 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 300MHz 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.

Synthesis of thiazole Schiff base (conventional method)

A solution of 3,5-dichlorosalicylaldehyde in ethanol was added to the ethanolic solution of 4-(*o*-methoxyphenyl)-2-aminothiazole in equimolar quantity. The mixture was refluxed in a water bath for 30 min. The Schiff bases, thus formed, N-(3,5 dichlorosalicylidene)-4-(*o* methoxyphenyl)-2-aminothiazole was filtered at suction, recrystallized form ethanol and dried under vacuum. Purity of Schiff bases was checked by molecular weight determination, elemental analysis and TLC.

Synthesis of thiazole Schiff base (microwave assisted)

4-(*o*-Methoxyphenyl)-2-aminothiazole (1 mmole) and 3,5 dichlorosalicylaldehyde (1 mmole) were mixed with each other in mortar-pestle and the reaction mixture was placed in small conical flask at room temperature, then 1 mL alcohol was added. The mixture was then exposed to microwave irradiation at 10% power for 10-20 sec. (Scheme 1). Completion of the reaction was tested by TLC. The reaction mixture was then cooled to room temperature. The yellow colored Schiff base was obtained, which was recrystallized from ethanol and dried under reduced pressure.

Antibiofilm activity

In short, 100 uL of 10^7 cells/mL of *C. albicans* (grown overnight in yeast peptone dextrose broth) in water were allowed to adher to the surface of 96 well polystyrene plate. The un-adhered cells were gently washed with phosphate buffer saline (PBS). 100 uL of Roswell Park Memorial Institute (RPMI) medium containing of different concentrations (0-500 ug/L) of Schiff bases were added to each well, and incubated for 24 hrs at 37^{o}C. After 24 hrs of incubations, the medium supernatant were removed, and the biofilm formed was gently washed with PBS and further incubated with 100 uL of 5 mg/mL 3-(4,5-Dimethylthiazol-2-yl)-2,5 Diphenyltetrazolium Bromide) (MTT) solution. After 6 hrs of incubations, 100 uL of dimethyl sulfoxide were added to each well and optical density was recorded on 540 nm (Multimode Plate Reader, Thermo-Fisher, India). For, scanning electron microscopy (SEM), the washed biofilm was incubated with 4% glutaraldehyde solution for overnight at 4^{o}C, and dehydrated in a series of ethanol (10-100%, each 15 mins), and observed for the biofilm at 1000X magnification under SEM (Joel, JSM).

RESULTS AND DISCUSSION

Synthesis of Schiff base

The Schiff base was synthesized by reacting equimolar amounts of 4-(*o*-methoxyphenyl)-2-aminothiazole and 3,5 dichlorosalicylaldehyde under microwave

irradiation. Schiff base was yellow crystalline solids having sharp melting points 190 ^{o}C. It is soluble in common organic solvent like acetone, alcohol, chloroform, carbon tetrachloride, etc. The Schiff base is having a molecular formula of $C_{17}H_{12}N_2O_2SCl_2$ and gave satisfactory data of elemental (C, H and N) analysis. The reaction time, percentage yield and melting points of the Schiff bases were given in Table 1. We found that microwave irradiated synthesis of Schiff bases is a convenient and rapid synthetic method resulting in good yield of the expected product and is 180 times faster than the conventional method of the synthesis which requires 20 to 30 min heating on water bath. The reaction time, percentage yield and melting points of the Schiff bases were given in Table 1.

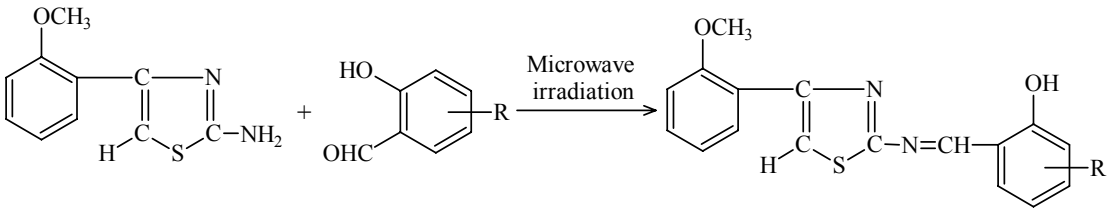
Table 1: Comparative account of synthesis of Schiff base by conventional and microwave methods

S. No.	Conventional method		Microwave method		Melting Pt. (^{o}C)
	Yield (%)	Time (min.)	Yield (%)	Time (sec.)	
1	90	30	98	10	190

We found that microwave irradiated synthesis of Schiff bases is a convenient and rapid synthetic method resulting in good yield of the expected product and is 180 times faster than the conventional method of the synthesis which requires 20 to 30 min heating on water bath.

Structural characterization of Schiff base

The UV–Visible spectrum of Schiff base exhibits λ_{max} at 396 nm. UV-Vis spectra of the Schiff bases exhibit an intense band at ~400 nm. The UV-Vis spectrum of 2-aminothiazole exhibits an intense band at ~275 nm and other aromatic amino compounds with comparable structures exhibit absorption[22,23] 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.[24] The IR spectrum of Schiff base in KBr shows IR (cm^{-1}): ν(O-H) 3456, ν(C=N) 1630, ν(C-O) 1236, ν(C-S-C) 675 and phenyl and thiazole ring vibrations 1578, 1480, 1347,1165. The IR spectra of the Schiff bases exhibit ν(O-H), ν(C=N), ν(C-O) and ν(C-S-C) modes at ~ 3430 - ~3460, ~1640, ~ 1280 and ~660 cm^{-1} respectively, and these values are in accordance with the earlier reports.[21] 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.[25]



Scheme 1: Reaction of N-(3,5 dichlorosalicylidene)-4-(*o*-methoxyphenyl)-2-aminothiazole

The ^{1}H NMR spectrum of Schiff base shows signals at ($CDCl_3$, TMS, δ ppm) 3.94 (3H, s, Ar-OCH_3), 7.15 (1H, s, H-thiazole), 7.15 to 8.12 (7H, m, Ar-H), 8.12 (1H, s, benzylidenimin), 9.40 (1H, s, Ar-OH). The assignments of NMR signals show close resemblance with the earlier results (Silverstein et al, 1991). The ^{13}C NMR spectrum of Schiff base shows signals at ($CDCl_3$, TMS, δppm) 206.77, 192.71, 170.41, 163.71, 156.96, 133.40, 129.60, 121.01, 118.50, 111.69, 60.00, 54.73, 47.68, 39.51, 29.79, 20.67, 14.03. Mass spectra of Schiff base represented by m/z (relative intensity %) is described as: 378/380* (60.97/41.46) (M^+ peak) (Molecular Formula: $C_{17}H_{12}N_2O_2SCl_2$), 345 (14.63), 232 (14.63), 215 (17.07), 189 (39.02), 164 (14.34), 146 (36.58), 132 (100), 121 (39.02), 102 (19.51), 91 (17.07), 77 (26.82), 63 (7.31), 45 (9.75), The peaks marked with (*) are isotopic peaks.

Antibiofilm activity

The major clinical concern in fungi-related infections arise due to the ability of such fungi to form fungal biofilm, and are a major clinical concern as these structured microbial communities present in biofilm are characterized by increased resistance to antifungal therapy.[7] This intrinsic increased tolerance of biofilm to antimycotics renders current treatment options for fungal biofilm insufficient.

Therefore, confronted with this problem, in present study we attempted biological application of Schiff bases, N-(3,5 dichlorosalicylidene)-4-(*o*-methoxyphenyl)-2-aminothiazole, as an antibiofilm agent. When the adhered cells of *C. albicans* was subjected to various concentrations of Schiff bases, and observed for the formation of biofilm. It was observed that Schiff bases inhibit biofilm formations in a concentration dependent manner. Whereas the biofilm formed by *C. albicans* was 100% in control (0 ug/mL), it's formation decreased with increasing concentrations (Fig. 1). A drastic decrease in biofilm formations (approximately 75%) was observed at 125 ug/mL of Schiff bases.

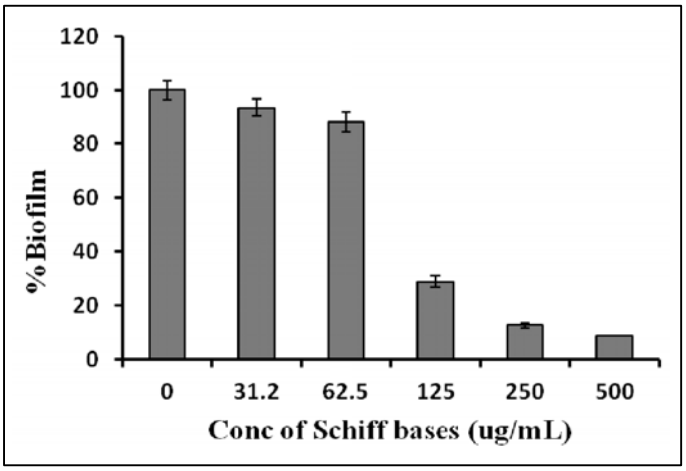


Fig. 1: Biofilm formation in *Candida albicans* at different concentration of Schiff bases

When the structures of biofilm at 25 ug/mL of Schiff bases under SEM was analyzed, we observed circular to oval shaped of *C. albicans*, indicating inhibition of biofilm formation (Fig. 2). Under controlled conditions, when cell of *C. albicans*, were allowed to adhere to the surface of polystyrene plate, they are usually circular to oval shaped. When these cells acquired nutrients present in the RPMI medium, they divided and re-divided, and ultimately formed entangled mycelia structures with polysaccharide matrix around (biofilm). However, due to inhibitory action of the Schiff bases, cells of *C. albicans* did not divide, and were observed as circular to oval shaped, thus indicating biofilm inhibitions in *C. albicans*.

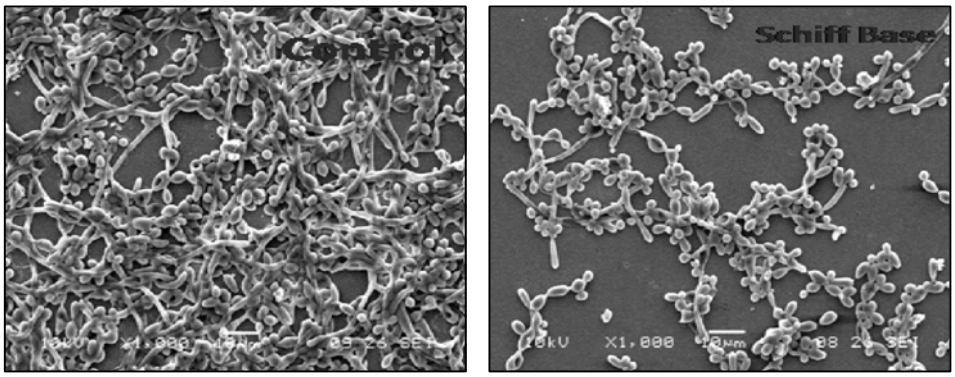


Fig. 2: Effect of Schiff bases on biofilm of *C. albicans*

The exact reason for the antibiofilm action of Schiff bases is subject of further research, but a hypothetical reason, based on current literature on biofilm of *C. albicans*,

suggests disruptions of lipid metabolism responsible for the biosynthesis of plasma membrane.[26] When we surveyed the literature, we found that there are numerous reports of the anti-biofilm studies exploring natural products or its derivatives.[27] 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 conju-gates and 4-thiazolidinones derivatives.[27-32] Thus, vast reportier of the chemical scaffolds are yet unexplored as an anti-biofilm agent. As mentioned earlier, the establishment of chronic infections takes an advantage of biofilm, the disruption of biofilm is expected to reduce the pathogenicity of many biofilm forming pathogens. When we reviewed literature survey for the use of Schiff bases for antibiofilm activity, we were surprised to find that there are no reports on the antibiofilm activity of Schiff bases on *C. albicans*, instead there are few reports on the antifungal activity as discussed following. Rehman et al. (2004)[33] showed that a concentration of 500 ppm of N-(Salicylidene)-2-hydroxyaniline is required to inhibit the activity of phytopathogenic fungi, *Alternaria brassicae* and *A. brassicicola*, Guo et al.[34], showed antifungal activity against *Botrytis cinerea* and *Colletotrichum lagenarium* by 26-33% and 35-38% at 1000 ppm of Schiff bases, chitosan-derived Schiff bases, Karthikeyan et al.[35] showed antifungal activity against *Aspergillus fumigatus*, *A. flavus*, *Trichophyton mentagrophytes*, and *Penicillium marneffei* by 6.3-12.5 ug/mL of 2,4-dichloro-5-fluorophenyl Schiff bases, Echevarria et al.[36] showed antifungal activity against *Trichophyton rubrum* and *Epidermophyton floccosum* respectively at 820-980 uM and 200-930 uM of Piperonyl-derived Schiff bases, Pandeya et al.[37] reported antifungal activity against *Microsporum audouinii* and *M. gypseum* respectively at 2.4 to 9.7 and 1.2 to 9.7 ug/mL of Isatin-derived Schiff bases. In present study, we also attempted microwave-assisted synthesis of Schiff bases since it is eco-friendly and has advantageous over the conventional heating method owing to shorter reaction time, experimental simplicity, selectivity of products, and easy working up procedures.[38-40]

Thus, the Schiff base, N-(3,5 dichlorosalicylidene)-4-(*o*-Methoxyphenyl)-2-aminothiazole has shown a new bioactive applications as an agent inhibiting biofilm formations in *C. albicans*. This is important in the view of the antimicrobial resistance shown by the cells residing inside biofilm. The as-synthesized Schiff base can also be viewed as an antibiofilm agent against other biofilm forming organisms. Our study is the first report of the use of schiff bases as an inhibitor of biofilm formation in *Candida albicans*.

CONCLUSION

The conventional and microwave-assisted method successfully synthesized Schiff base, N-(3,5 dichlorosalicylidene)-4-(*o*-Methoxyphenyl)-2-aminothiazole. However, the

microwave-assisted method for Schiff bases synthesis is feasible over the conventional method. The as-synthesized compound shows the typical characteristics of Schiff bases. The as-synthesized Schiff bases showed an antibiofilm activity against *C. albicans*, which is significant against the organisms residing inside the biofilm. The SEM analysis further validated the biofilm inhibitions in *C. albicans* by N-(3,5 dichlorosalicylidene)-4-(*o*-methoxyphenyl)-2-aminothiazole.

ACKNOWLEDGEMENT

Netaji N. Karale acknowledges the funding agency, Board of College and University Department, Savitribai Phule Pune University for financial assistance to this work. Netaji N. Karale also acknowledges the assistance from Department of Chemical Sciences, Vidya Pratishthan's Arts, Science and Commerce College, Baramati, Pune.

REFERENCES

1. A. Dessen, A. M. Di Guilmi, T. Vernet and O. Dideberg, Molecular Mechanisms of Antibiotic Resistance in Gram-Positive Pathogens, Curr. Drug Targets Infect. Disord., **1**, 63-77 (2001).

2. H. Muroi, K. Nihei, K. Tsujimoto and I. Kubo, Synergistic Effects of Anacardic Acids and Methicillin against Methicillin Resistant *Staphylococcus Aureus*, Bioorg. Med. Chem., **12**, 583-587 (2004).

3. R. F. Pfeltz and B. J. Wilkinson, The Escalating Challenge of Vancomycin Resistance in *Staphylococcus Aureus*, Curr Drug Targets Infect Disord, **4**, 273–294 (2004).

4. M. C. Roberts, Distribution of Macrolide, Lincosamide, Streptogramin, Ketolide and Oxazolidinone (MLSKO) Resistance Genes in Gram-Negative Bacteria, Curr. Drug Targets Infect. Disord., **4**, 207-215 (2004).

5. F. C. Tenover and L. C. McDonald, Vancomycin-Resistant Staphylococci and Enterococci: Epidemiology and Control, Curr. Opin. Infect. Dis., **18**, 300-305 (2005).

6. B. Wachtler, D. Wilson, K. Haedicke, F. Dalle and B. Hube, From Attachment to Damage: Defined Genes of Candida Albicans Mediate Adhesion, Invasion and Damage During Interaction with Oral Epithelial Cells, PLoS One, **6**, e17046 (2011).

7. G. Ramage, J. P. Martinez and J. L. Lopez-Ribot, Candida Biofilms on Implanted Biomaterials: A Clinically Significant Problem, FEMS Yeast Res, **6**, 979-986 (2006).

8. E. M. Kojic and R. O. Darouiche, Candida Infections of Medical Devices, Clin. Microbiol. Rev., **17**, 255-267 (2004).

9. L. Li, M. B. Finnegan, S. Ozkan, Y. Kim, P. B. Lillehoj, C. M. Ho, R. Lux, R. Mito, Z. Loewy and W. Shi, *In Vitro* Study of Biofilm Formation and Effectiveness of Antimicrobial Treatment on Various Dental Material Surfaces, Mol Oral Microbiol, **25**, 384-390 (2010).

10. C. J. Nobile, J. E. Nett, D. R. Andes and A. P. Mitchell, Function of *Candida Albicans* Adhesin Hwp1 in Biofilm Formation, Eukaryot Cell, **5**, 1604-1610 (2006).

11. T. Umeyama, A. Kaneko, Y. Nagai, N. Hanaoka, K. Tanabe, Y. Takano, M. Niimi and Y. Uehara, *Candida Albicans* Protein Kinase CaHsl1p Regulates Cell Elongation and Virulence. Mol Microbiol, **55**, 381-395 (2005).

12. G. Ramage, S. P. Saville, D. P. Thomas and J. L. Lopez-Ribot, Candida Biofilms: An Update, Eukaryot Cell, **4**, 633-638 (2005).

13. G. Ramage, R. Rajendran, L. Sherry and C. Williams, Fungal Biofilm Resistance, Int. J. Microbiol, 528521 (2012).

14. C. d'Enfert, Biofilms and their Role in the Resistance of Pathogenic Candida to Antifungal Agents, Curr. Drug Targets, **7**, 465-470 (2006).

15. W. Qin, S. Long, M. Panunzio, B. Stefano, Schiff Bases: A Short Survey on an Evergreen Chemistry Tool, Molecules, **18**, 12264-12289 (2013).

16. S. Kumar, D. N. Dhar and P. N. Saxena, Applications of Metal Complexes of Schiff Bases - A Review, J. Sci. Ind. Res., **68**, 181-187 (2009).

17. M. da Silva Cleiton, L. da Silva Daniel, V. Modolo Luzia, B. Alves Rosemeire, A. de Resende Maria, V. B. Martins Cleide and de Fátima Ângelo, Schiff bases: A short Review of their Antimicrobial Activities, J. Adv. Res., **2**, 1-8 (2011).

18. P. G. More, N. N. Karale, A. S. Lawanda, N. Narang and R. H. Patil, Synthesis and Anti-Biofilm Activity of Thiazole Schiff Bases, Med. Chem. Res., **23**, 790-799 (2014).

19. N. N. Karale and R. H. Patil, Spectral, Magnetic and Biological Studies of Zn^{II} Complexes of Schiff Bases Derived From 4-(O-Methoxyphenyl)-2-Aminothiazole, J. App. Pharm. Sci., **5**, 041-044 (2015).

20. S. J. Kashyap, V. K. Garg, P. K. Sharma, N. Kumar, R. Dudhe and J. K. Gupta, Thiazoles: Having Diverse Biological Activities, Med. Chem. Res., **21**, 2123-2132 (2012).

21. R. M. Silverstein, G. C. Basler and C. T. Morrill, Spectrometric Identification of Organic Compounds, 5th Ed. Wiley, New York (1991) p. 220.

22. W. Wilson and R. Woodger, 4′: 5′-Dihydroglyoxalino (2′: 1′- 2: 3) Thiazolium Salts, and the Ultraviolet Light Absorption of Thiazole Derivatives, J. Chem. Soc., 2943-2948 (1955).

23. R. A. Mathes and J. T. Gregory, Reactions of 3-Thiocyano-2-Butanone. II.1 The Preparation of 2-Arylamino- and 2-Alkylamino-4,5-Dimethylthiazoles, J. Am. Chem. Soc., **74**, 3867-3868 (1952).

24. S. Patai, The Chemistry of Carbon-Nitrogen Double Bond, Interscience, London (1970) p. 181.

25. J. E. Kovacic, The CN Stretching Frequency in the Infrared Spectra of Schiff's Base Complexes-I, Copper Complexes of Salicylidene Anilines, Spectrochim. Acta, **23**, 183-187 (1967).

26. G. Pappenberger, T. Schulz-Gasch, E. Kusznir, F. Müller and M. Hennig, Structure-Assisted Discovery of an Aminothiazole Derivative as a Lead Molecule for Inhibition of Bacterial Fatty-Acid Synthesis, Acta Crystallogr. D. Biol. Crystallogr., **63**, 1208-1216 (2007).

27. S. D. Stowe, J. J. Richards, A. T. Tucker, R. Thompson, C. Melander and J. Cavanagh, Anti-Biofilm Compounds Derived from Marine Sponges, Mar. Drugs, **9**, 2010-2035 (2011)

28. R. W. Huigens, S. A. Rogers, A. T. Steinhauer and C. Melander, Inhibition of *Acinetobacter Baumannii*, *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* Biofilm Formation with a Class of TAGE-Triazole Conjugates, Org. Biomol. Chem., **7**, 794-802 (2009)

29. H. P. L. Steenackers, D. S. Ermolat'ev, B. Savaliya, A. De Weerdt, D. De Coster, A. Shah, E. V. Van der Eycken, D. E. De Vos, J. Vanderleyden and S. C. J. De Keersmaecker, Structure−Activity Relationship of 4(5)-Aryl-2-Amino-1H-Imidazoles, N1-Substituted 2-Aminoimidazoles and Imidazo[1,2-a]pyrimidinium Salts as Inhibitors of Biofilm Formation by *Salmonella Typhimurium* and *Pseudomonas aeruginosa*, J. Med. Chem., **54**, 472-484 (2010).

30. P. G. More, N. N. Karale, A. S. Lawanda, S. V. Rajmane, S. V. Pawar and R. H. Patil, A 4-(o-Methoxyphenyl)-2-Aminothiazole: An Anti-Quorum Sensing Compound, Med. Chem. Res., **22**, 4183-4191 (2013).

31. R. A. Rane, N. U. Sahu and C. P. Shah, Synthesis and Antibiofilm Activity of Marine Natural Product-Based 4-Thiazolidinones Derivatives, Bioorg. Med. Chem. Lett., **22**, 7131-7134 (2012).

32. E. Tello, L. Castellanos and C. Duque, Synthesis of Cembranoid Analogues and Evaluation of their Potential as Quorum Sensing Inhibitors, Bioorg. Med. Chem., **21**, 242-256 (2013).

33. W. Rehman, M. K. Baloch, B. Muhammad, A. Badshah and K. M. Khan, Characteristic Spectral Studies and *In Vitro* Antifungal Activity of some Schiff Bases and their Organotin (IV) Complexes, Chin. Sci. Bull., **49**, 119-122 (2004).

34. Z. Guo, R. Xing, S. Liu, Z. Zhong, X. Ji, L. Wang and P. Li, Antifungal Properties of Schiff Bases of Chitosan, N -Substituted Chitosan and Quaternized Chitosan, Carbohydr. Polym, **71**, 694-697 (2008).

35. M. S. Karthikeyan, D. J. Prasad, B. Poojary, K. S. Bhat, B. S. Holla and N. S. Kumari, Synthesis and Biological Activity of Schiff and Mannich Bases Bearing 2,4-Dichloro-5-Fluorophenyl Moiety, Bioorg. Med. Chem., **15**, 7482-7489 (2006).

36. A. Echevarria, M. G. Nascimento, V. Gerônimo, J. Miller and A. Giesbrecht, NMR Spectroscopy, Hammett Correlations and Biological Activity of some Schiff Bases Derived from Piperonal, J. Braz. Chem. Soc., **10**, 60-64 (1999).

37. S. N. Pandeya, D. Sriram, G. Nath and E. de Clercq, Synthesis and Antimicrobial Activity of Schiff and Mannich Bases of Isatin and its Derivatives with Pyrimidine, IL Farmaco, **54**, 624-628 (1999).

38. S. Caddick, Microwave Assisted Organic Reactions, Tetrahedron, **51**, 10403-10432 (1995).

39. R. S. Verma, Solvent-Free Organic Syntheses using Supported Reagents and Microwave Irradiation, Green Chem., **1**, 43-55 (1999).

40. F. Mavandadi and P. Lidström, Microwave-Assisted Chemistry in Drug Discovery, Curr. Top Med. Chem., **4**, 773-792 (2004).

Revised : 18.10.2016 *Accepted : 21.10.2016*