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Chemical synthesis, Spectral Characterization, and Biological Potency Evaluation of imine-based Compounds

K. Savithri[1], C. M. Shivaprasad[2], H. K. Vivek[3], B. C. Vasanthkumar[4], B. Jayalakshmi[5], M. Jyothipriya[1], K. Shiva Prasad[6] and H. D. Revanasiddappa[1]*

1. Department of Studies in Chemistry, University of Mysore, Mysuru-570 006, Karnataka, **INDIA**
2. Department of Chemistry, Government First Grade College for Women, Vijaynagar, Mysuru-570 032, Karnataka, **INDIA**
3. Centre for Research and Innovation, School of Natural Sciences, Adichunchanagiri University, BGSIT campus, B.G. Nagara-571448, Karnataka, **INDIA**
4. Department of Chemistry, DRM Science College, Davangere-577 004, Karnataka, **INDIA**
5. Department of Botany, Government First Grade College for Women, Mandya-571401, Karnataka, **INDIA**
6. Department of Sciences, Amrita School of Arts and Sciences, Amrita Vishwa Vidyapeetham, Mysuru Campus, Mysuru-570 026, **INDIA**

Email: revanasiddappa1066@gmail.com

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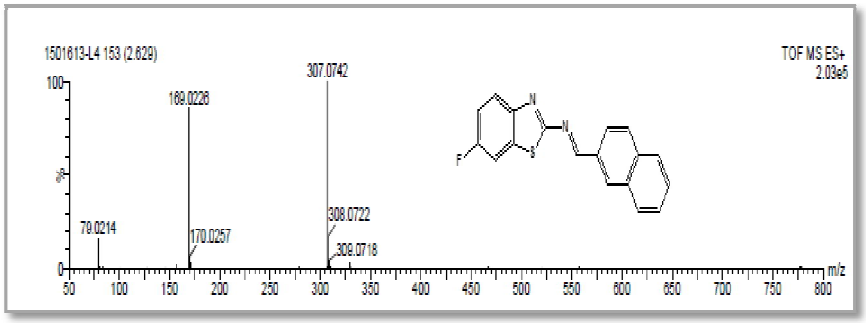
ABSTRACT

The facile, conventional and green syntheses of a series of fluorobenzothiazole derived imine based compounds such as 4-fluoro-2-{(E)-[(6-fluoro-1,benzothiazol-2-yl) imino] methyl}phenol (SL_1), (E)-1-(2H-1,3-benzodioxol-5-yl)-N-(6-fluoro-1,3-benzothiazol-2-yl) methanimine (SL_2) and (E)-N-(6-fluoro-1,3-benzothiazol-2-yl)-1-(naphthalen-1-yl) methanimine (SL_3) have been explored in this research. The synthesized compounds have primarily been characterized by physical methods (melting point, TLC and elemental analysis), and structurally elucidated by spectral studies. Also, these compounds were tested for their antimicrobial (disc diffusion method), antioxidant ($DPPH^{\cdot}$) and anti-inflammatory (by carrageen an edema model) potentialities.

Graphical Abstract



ESI-MS spectra of imine bases SL_3.

Keywords: Imine-based compounds, Spectral studies, Anti-inflammatory, Antimicrobial.

INTRODUCTION

Imines are resourceful novel compounds, which are eminently used due to their flexible entanglement, remarkable adaptability and facile synthesis [**1**]. They are broadly employed for their better yield and are commonly prepared by the suitable combination of the carbonyl compounds with an aromatic amine were condensed under variable circumstances of pH, temperature and solvents. The presence of azomethine (imine) linkage (−C=N) has gained interest for their stability, chelating property, striking biological properties, novel structural and medicinal efficacies, crystallographic features, etc. [**2, 3**].

Structure-activity-based drug design has been predicted as an efficacious platform for more cost-efficient and faster lead discovery of potent medications in the field of therapeutic science in contrast to the traditional method [**4**]. Now-a-days, various protocols are under extensive research for the refinement of such prototypes in which a molecular hybridization process has emerged as a powerful and advanced approach for rational designing of ligand scaffolds, which depend on the pharmacophoric entities present in the compound framework [**5**]. Thus, it leads to the formation of a new context bears intrinsic properties of the initial array and exhibits an innovative formulation with enhanced affinity and efficacy in comparison to the parent drug.

2-Aminobenzothiazoles are highly reactive synthons since the amino group assists the reaction with electrophiles and constitutes many fused heterocyclic compounds [**6, 7**], 6-fluoro benzothiazole derivatives known to have a wide range of chemical reactivity and bioactivity owing to their basic functionalities. Substituted benzothiazole derivatives with fluorine substituted molecules have received huge consideration for their potential biological efficacies [**8, 9**]. 2-Aminobenzothiazole was intensively studied as the unique and versatile scaffold in one of the privileged structure in experimental drug design and is endowed with abundant bioactivities [**10, 11**]. The approach towards the development of organic compounds as chemotherapeutic probes with immense biological properties like antimicrobial and anti-inflammatory activity plays a vital role in protecting the body from oxidative damage.

The drug-protein interaction makes a stabilized drug-protein complex, which consequently makes an effect on drug distribution and its metabolism in the blood. The distribution of drugs is generally taken up by HSA as mainly drugs move in the plasma and achieve the targeted tissues through binding to albumin, hence functionalizing as a most preferential substitute for the purpose of drug delivery [**12**].

Nowadays, imine-based compounds with mixed donor atoms (N/O) are preferred as their versatile derivatives and have the ability to form high nuclear composites. Imine bases derived from hydroxy naphthaldehyde have been broadly examined because of their substantial uses in the therapeutic field. Hydroxy naphthaldehyde exhibits strong steric hindrance and high conjugating properties because of the naphthol ring, thereby may form imine-based metal complexes with more coordination sites and higher stability [**13**].

In this perspective, the current study is structured to provide a series of three novel hybrid structures of 6-fluoro benzothiazole substituted derivatives incorporated both from benzothiazole moiety and different aromatic aldehydes through imine linkage at 2nd position of benzothiazole to extend the significance of fluoro-substituted benzothiazole with simple aromatic aldehydes containing –OH, groups as potential antimicrobials, antioxidants and anti-inflammatory agents.

MATERIALS AND METHODS

Analytical and physical measurements: Commercial reagent grade chemicals and solvents were used throughout the experiments without further purification. The melting point of allthe compounds was determined on an ELICO-3210 apparatus in the open capillary tube using a precision digi melting

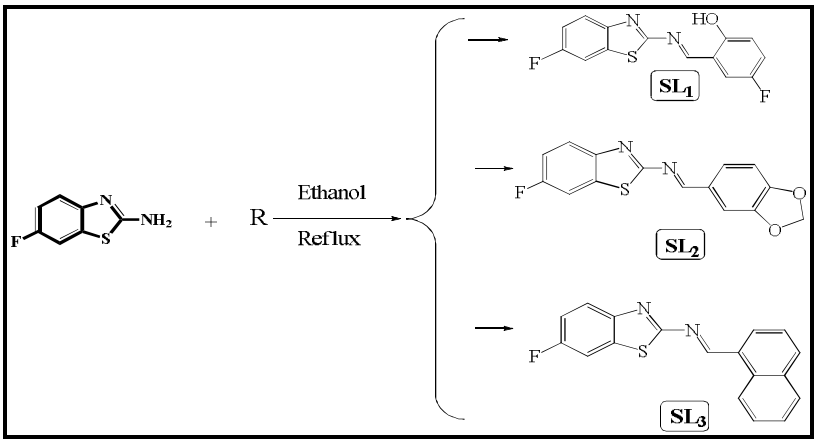
point apparatus and was uncorrected. Elemental analysis (CHNO) was performed with a model 240 Perkin Elmer elemental analyzer.ESI (positive) mass spectra of synthesized compounds in DMSO were run in a Synapt G2 HDMS spectrometer equipped with an electrospray ion (ESI) source.IR spectra of newly synthesized molecules were recorded in 4000-400 cm^{-1}range using a Perkin–Elmer spectrophotometer version 10.03.09.*via* FT-ATR. UV-Vis spectrophotometer (DU 730 'Life sciences' M/S Beckman coulter, USA) was used to record the absorption spectra of the prepared compounds lie in the range 200-800 nm.

For the structural identification, Agilent-NMR spectrophotometer (Model 400MR DD 22) was used to record ^{1}H and^{13}C spectra in d_6-DMSO solvent using TMS as an internal reference at ambient temperature.

Synthetic protocol for imine-based compounds

Classical method: The synthetic route of a series of anils (SL_1-SL_3) is displayed in scheme 1 and each of these was prepared by the condensation of an equimolar ratio of substituted aldehydes [5-fluorosalicyladehyde (SL_1), piperonal (SL_2) and 1-naphthal (SL_3)] and 6-fluoro-2-aminobenzothiazole dissolved in absolute hot ethanol. The resulting reaction mixture was stirred vigorously at ambient temperature (25°C) and refluxed at 80°C for 6-7 h. The yellow solid precipitate of imine-based compounds was formed, which were then filtered, washed with cold ethanol, and dried in a desiccator. TLC plates in n-hexane:ethyl acetate (8:2) were used intermittently to access the reaction and purity of synthesized compounds obtained from continuous recrystallization.

Microwave method: In this method, each compound (SL_1-SL_3) was synthesized by an equimolar ratio of the mixture of 2-amino-6-fluoro benzothiazole and the corresponding aldehydes were thoroughly mixed in a grinder. The reaction flask sealed with Teflon was then irradiated in a microwave oven, connected to a pressure gauge outside the oven and heated for 7 min at 140°C with a power level of 7 (560 W) using dry methanol (10 mL) as a solvent. TLC was used to assess the completion of the reaction progress and to preliminary check on the purity of the product. The reaction mixture was allowed to attain room temperature (25±1°C). The colored solid was obtained after recrystallization from methanol.



Where R = SL_1: 5-flourosalicyldehyde, SL_2: Piperonal, SL_3: Naphthal

Scheme 1. Schematic diagram for the synthesis of imine based compounds

Bioassay studies

Antimicrobial screening: *In vitro* antimicrobial activity of the imine based compounds was determined by the disc diffusion method [**14**] against bacterial species namely, *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) under Gram –*ve* and *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus*(MTCC 96) under Gram +*ve* bacteria and pathogenic fungi such as

Candida albicans (ATCC 10231) and *Aspergillus niger*(ATCC 6275). A sterile filter paper disc dipped in DMSO is served as a negative control. Chloramphenicol and fluconazole are the standards for antibacterial and antifungal activities, respectively. To get the required concentrations of test solution, the compounds were prepared in DMSO. The compounds which show significant activities were selected to determine the minimum inhibitory concentration (MIC) using well diffusion technique. The experiments were run in triplicate and the results were recorded as the average diameter of inhibition zones (mm) of microbial growth around the disc. This is further subjected to MIC evaluation.

The biocidal activity was assessed by measuring minimum inhibitory concentrations (MIC). The MIC value of each compound was determined at its lowest concentration (highest dilution) required to arrest the microbial growth by standard broth dilution assay [**15**]. A compound showing significant inhibition of zonal growth of microbes acts as a promising antimicrobial agent was selected for MIC studies. A suspension of 1-2×10^4 CFU mL^{-1} has been prepared for each organism was inoculated to the petriplates with serially diluted test compounds as well as a standard drug solution. Then the plates were kept incubated at 37°C for a day. The measured inhibitory zone diameter helped to determine the MIC value.

Time-dependent bacterial killing studies: Time-kill kinetic study is the rate at which a microorganism is subjected to kill by the drug as a function of survival data recorded at enough exposure time points such that a graphical model is constructed where the decline in population over time to an extinction point. The *in vitro* assay of the compounds against *E. coli* bacterium was carried out at 0.5× 1× and 2× the MIC, and an initial stock culture was 1×10^6 CFU $well^{-1}$ concentration. The inoculated plates were kept for incubation at 37°C for 24 h at the time interval of every 6 h. The absorbance was recorded at 625 nm after the period of 6 h incubation at different MICs of the compounds. The medium with the only bacterial inoculum (without test extract) was treated as a control. Three replicates were done for the same. The bactericidal rate was stated as mean colony count data (log_{10} CFU mL^{-1}) against time [**16**].

Antioxidant activity: Stoichiometric approaches to predict the antioxidant activity of the prepared compounds were made by α,α-Diphenyl-β-picrylhydrazyl (DPPH, $C_{18}H_{12}N_5O_6$) radical assay where the rate of change of free radical is measured with the spectroscopic absorbance. A natural antioxidant ascorbic acid is taken as a reference. A spectroscopic blank consists of 90% aq. methanol without DPPH was used to have a null adjustment initially. In parallel, a methanolic $DPPH^{\cdot}$ solution without served samples was kept as a control. Test solutions were prepared in a range of 25-100 µg mL^{-1} in methanol. Volume of 1 mL of each sample at different concentrations of the prospective compound is mixed with the DPPH solution (7.88 mg of 0.2 mM DPPH in 1L CH_3OH) and incubated at 25°C for about half an hour. Then the absorbance was recorded at 518 nm. As the name free radical scavenging activity implies the measurement of radical abstraction capacity of the compounds or in terms of hydrogen donating capacity [**17**]. With the same view, the method was developed by Blois [**18**] in a like manner using a persistent $DPPH^{\cdot}$ by the virtue of the delocalization of an electron over the molecule as a whole. An unpaired electron lying on the nitrogen of DPPH shows π-π* transitions which cause two bands in the visible region at the extinction coefficient 11700 $M^{-1}cm^{-1}$ (~518 nm absorption), which is responsible for the purple color of DPPH solution. The $DPPH^{\cdot}$ was then reduced to DPPH-H (diphenylhydrazine) molecule in the presence of anti-oxidants makes the loss of visible band; consequently, the decrease in absorbance as a result of decolorization from violet to pale yellow for the number of electrons captured, which can be monitored by UV-Vis spectroscopy. The tests were replicated thrice and evaluated the percentage of DPPH radical quenched after reaching a steady-state plateau by applying the mean ± standard deviation (S.D.) in equation 1.

$$I\ (\%) = (A_{control} - A_{sample}/\ A_{control}) \times 100 \qquad (1)$$

***In vivo* anti-inflammatory assay:** Effective anti-inflammatory compounds were determined by Winter method using carrageenan-induced mouse paw edema model [19]. In the carrageenan model, adult male albino mice (weight range 120–150 g) were treated with a subcutaneous injection of 0.10 mL of 1% w/v carrageenan-λ in normal physiological saline into the sub-plantar section of the right hind paw of each animal. The extracts of each compound (100 mg kg^{-1}) in 0.3% sodium carboxymethyl cellulose and each of these were administered one hour after the injection of carrageenan-induced in the sub-plantar region of the paw followed by other groups of control and standard. Mean normal paw volume was measured 30 min before carrageenan injection using standard fluid displacement procedure plethysmographically (plethysmometer-model 7150, Ugo Basile, Italy). Mean increase in the paw volume for the control group (post-carrageen an injection) and treated group was measured at an hourly interval of 1-4 h. The mean percent reduction of indomethacin (an internal standard anti-inflammatory agent) and test compounds at 10 mg kg^{-1} concentrations were compared with control using student's t-test and p-values with replicate measurements. The data obtained were expressed as standard error of mean:

$$\% \text{ inhibition} = \frac{t - t_0}{t} \times 100$$

t = mean edema of the control group, t_0= mean edema of the test compound
*The above studies were as per recognized guidelines on animal experimentation.

RESULTS AND DISCUSSION

Simple preparative accessibility protocols and optimized reaction conditions would pave for facile synthesis of imine-based compoundsSL_1-$SL_3$and microwave-assisted methods have been employed for the convenient and reproducible synthesis of imine base compounds were structurally elucidated by microanalysis and spectral data. The prepared compounds were stable at atmospheric conditions, non-hygroscopic and soluble in all common organic solvents. The purity of the formed products was often checked by TLC with a suitable solvent system.

IUPAC name, analytical data and detailed spectral data obtained from UV-Vis, IR, NMR and mass spectroscopy's for the compounds SL_1-$SL_3$are given systematically as follows.

SL_1:*4-fluoro-2-{(E)-[(6-fluoro-1,3-benzothiazol-2-yl)imino]methyl}phenol,* $C_{14}H_8F_2N_2OS$; **Color** Yellow crystalline solid; Yield **(%)** and R_f value69 (conv.), 82 (MW) and 0.65;M.p.**(°C)**182-184; Elemental analysis **Found (calc.)** C 57.93 (57.26), H 2.78 (2.61), N 9.65 (9.25); FT IR **(υ_{max}./cm^{-1})**(υOH) 3178, (HC=N) azomethine 1675; ^{1}H NMR **(295 K/ δ in ppm/ DMSO-d_6 400 MHz):** HC=N 9.28 (1H, s); phenolic OH 6.87 (1H, dd, J=8.0, 0.4 Hz); Ar-H 7.17-7.91 (6H, m) [7.17 (1H, dd, J=8.0, 1.4 Hz), 7.36 (1H, dd, J=9.1, 2.0 Hz), 7.69 (1H, dd, J=2.0, 0.4 Hz), 7.80 (1H, dd, J=1.4, 0.4 Hz), 7.91 (1H, dd, J=9.1, 0.4 Hz)];^{13}C NMR **(295 K/ δ(ppm)/ DMSO-$d_6$100 MHz):**164.246, 161.285, 158.670, 157.898, 149.424, 135.610, 135.443, 128.358, 124.494, 124.494, 124.513, 123.780, 121.386, 118.773, 115.713, 115.451, 109.423, 109.150; Mass (ESI MS) m/z 290.28; UV-vis. ($\lambda_{max.}$, nm)320;

SL_2:*(E)-1-(2H-1,3-benzodioxol-5-yl)-N-(6-fluoro-1,3-benzothiazol-2-yl)methanimine,* $C_{15}H_9FN_2O_2S$; **Color** Yellow crystalline solid; Yield **(%)** and R_f value68 (conv.), 80 (MW) and 0.44;M.p.**(°C)**150-154; Elemental analysis **Found (calc.)** C 59.13 (58.93), H 3.19 (3.31), N 9.20 (9.28); FT IR **(υ_{max}./cm^{-1})**(Ar-CH) 3065, (HC=N) azomethine 1617; ^{1}H NMR **(295 K/ δ in ppm/ DMSO-d_6 400 MHz):** HC=N 9.77 (1H, s); -CH_2 6.14-6.16 (2H, d, J=12.0 Hz); Ar-H 6.99-8.21 (6H, m) [6.99 (1H, dd, J=8.4, 0.4 Hz), 7.01(1H, dd, J=8.3, 1.4 Hz), 7.14 (1H, dd, J=8.3, 0.5 Hz), 7.30-7.34 (2H, 7.33 (dd, J=1.7, 0.4 Hz), 7.31 (dd, J=1.4, 0.5 Hz)), 7.61 (1H, dd, J=8.4, 1.7 Hz)];^{13}C NMR **(295 K/ δ(ppm)/ DMSO-$d_6$100 MHz):**170.831, 168.501, 159.433, 159.106, 156.898, 155.851, 155.494, 142.624, 133.267, 121.747, 117.255, 115.828, 115.608, 106.699, 105.288; Mass (ESI MS) m/z 301.38 [M+1]; UV-vis. ($\lambda_{max.}$, nm)315;

SL_3:*(E)-N-(6-fluoro-1,3-benzothiazol-2-yl)-1-(naphthalen-1-yl)methanimine*, $C_{18}H_{11}FN_2S$; **Color** Orange solid; Yield **(%)** and R_f value71(conv.), 82 (MW) and 0.65;M.p.**(°C)**178-180; Elemental analysis **Found (calc.)**C 70.09 (70.12), H 3.62 (3.96), N 9.01 (9.10); FT IR **(υ_{max}./cm^{-1})**(Ar-CH) 3124, (HC=N) azomethine 1610; ^{1}H NMR **(295 K/ δ in ppm/DMSO-d_6 400 MHz):**HC=N 8.99 (1H, s); Ar-H 7.4-8.20 (10H, m) [7.46 (1H, dd, J=7.2, 2.0 Hz), 7.53-7.79 (5H, 7.62 (ddd, *J* = 8.4, 1.8, 0.5 Hz), 7.56 (ddq, *J* = 8.4, 2.6, 0.5 Hz), 7.70 (dddd, *J* = 7.9, 7.3, 1.9, 0.5 Hz), 7.75 (dddt, *J* = 7.7, 1.9, 1.7, 0.5 Hz), 7.72 (dddd, *J* = 7.7, 7.3, 2.4, 0.4 Hz)), 7.82 (1H, dd, *J* = 2.0, 0.5 Hz), 8.00 (1H, dd, *J* = 7.2, 0.5 Hz), 8.10-8.20 (2H, 8.14 (dddq, *J* = 7.9, 2.6, 2.4, 0.5 Hz), 8.19 (ddq, *J* = 1.8, 1.7, 0.4 Hz)), 8.99 (1H, s)]; ^{13}C NMR **(295 K/ δ(ppm)/DMSO-$d_6$100 MHz):**175.035, 174.268, 152.481, 147.458, 142.973, 138.526, 136.689, 136.454, 135.855, 133.153, 129.389, 129.245, 127.970, 123.667, 119.547, 114.735, 109.560; Mass (ESI MS) m/z 307.14 [M+1]; UV-vis. ($\lambda_{max.}$, nm)336;

Spectral features

NMR spectroscopy: ^{1}H-NMR of the compounds SL_1-SL_2 (Figure 1) showed the presence of a high-resolution singlet peak at ~9.2–10.22 ppm corresponds to -CH=N, which confirmed the condensation between the amino group and the aldehyde group. The ^{1}H-NMR spectrum of SL_1 shows higher field singlet at δ ~5-6.5 ppm (s, 1H, -OH). In compound SL_2, an additional resonance was assigned to the –CH_2 (δ6.14 ppm, 2H) was observed. The protonation constant of all the compounds was determined in the d_6-DMSO solvent, which is observed at ~2ppm.

The ^{13}C NMR spectra of compounds display a common characteristic signal at 163.26-168.501 ppm is attributed to the imino carbon. The signals at ~160.176 and 159.63 correspond to C-N, 158.848 for C-S, 59.06 for C-OCH_3 and other aromatic carbon lies in the range 148.414-105.423 [**20**].

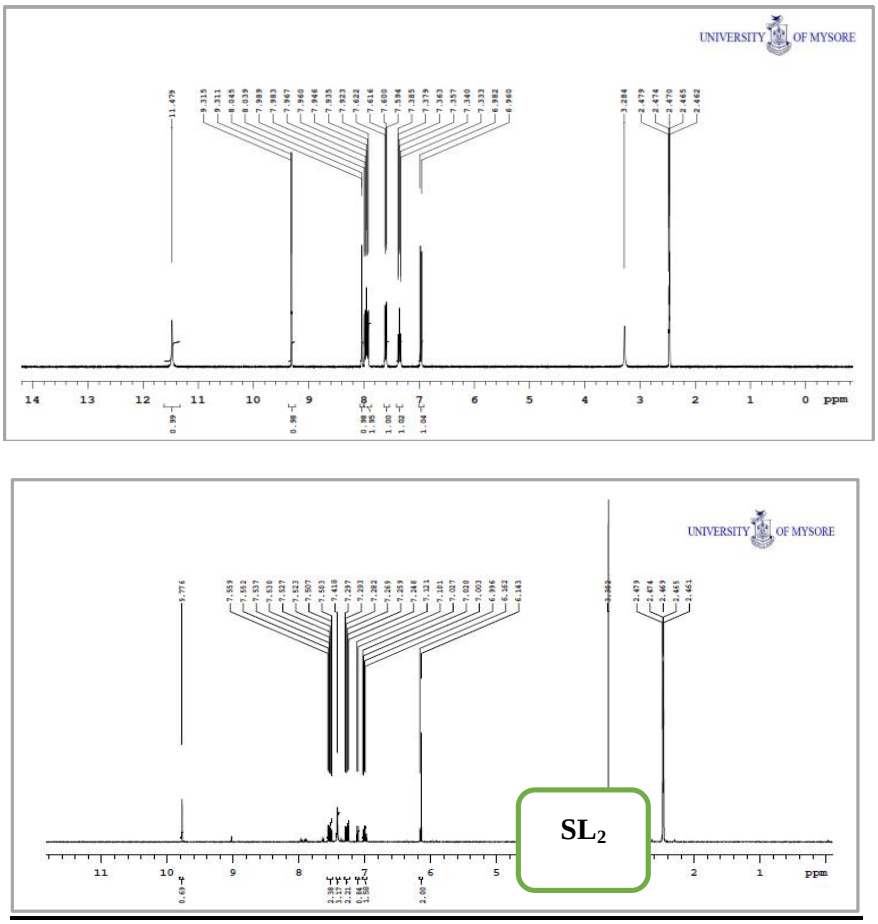


Figure 1.^{1}H NMR spectra of SL_1 and SL_2.

FT IR spectroscopy: The Fourier Transform Infrared Spectra (Figure 2) of the compounds exhibits a sharp band at 1662-1557 cm^{-1} due to azomethine group vibration. The compounds have typical characteristic bands at 1562 cm^{-1} for υC=N of thiazole ring and 748 cm^{-1} for υC-S-C [21]. The -OH group involved in intramolecular hydrogen bonding for SL_1-L_3 indicated by a shallow/broadband extends within 3253-2700 cm^{-1} gives clue for the presence of enol form. The existence of the aromatic rings was demonstrated by the following bands: aromatic carbon-H stretching υC−H (3045−3040 cm^{-1}) and phenyl ring stretching υC−C (1575−1500 cm^{-1}) in synthesized compounds confirm the aromatic stretching vibrations and the appearance of a medium to strong absorption bands above 1600 cm^{-1}due to a stretching vibration of the imine (C=N) bond formation in synthesized compounds *via* condensation.

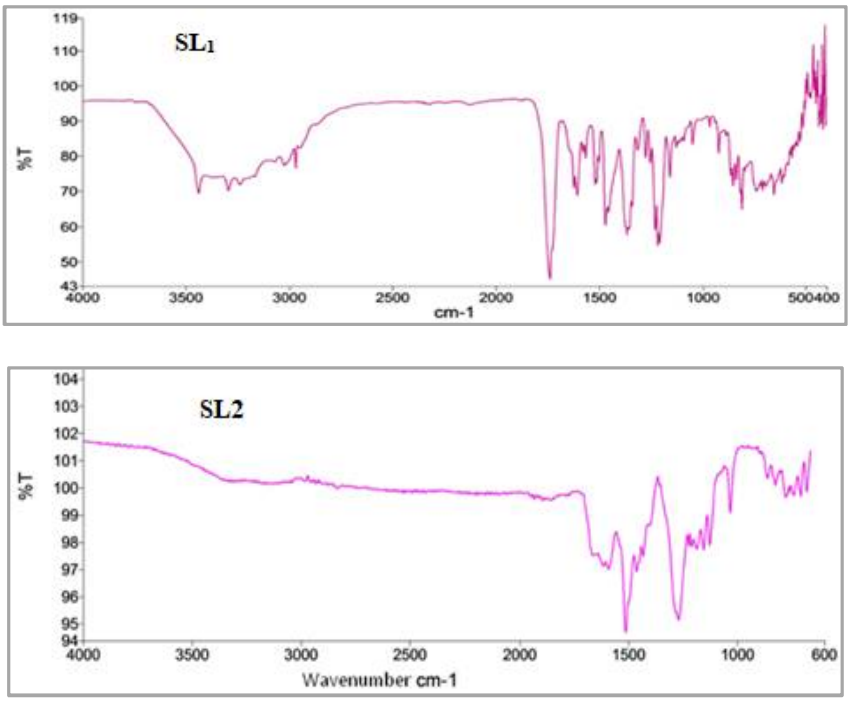


Figure 2. FT IR spectra of SL_1 and SL_2.

Mass Spectroscopy: The mass spectra of the imine bases show the molecular ion peak [M^+] at their m/z values shows the molecular ion peak corresponding to their formulation. Mass spectrum of SL_3 shows the molecular ion peak [M+1] at m/z 307, where this molecular ion undergoes two major fragmentation pathways. The observed first ion-peak at m/z 169 is due to the cleavage of 6-fluoro benzothiazole moiety. This fragment ion undergoes further fragmentation to give ion peak at m/z 79 (base peak) of phenyl ion radical. The m/z values were found to be 290.28, 301.38 and 307.14 for SL_1, $SL_2$and SL_3, respectively (SL_3 in Figure 3).

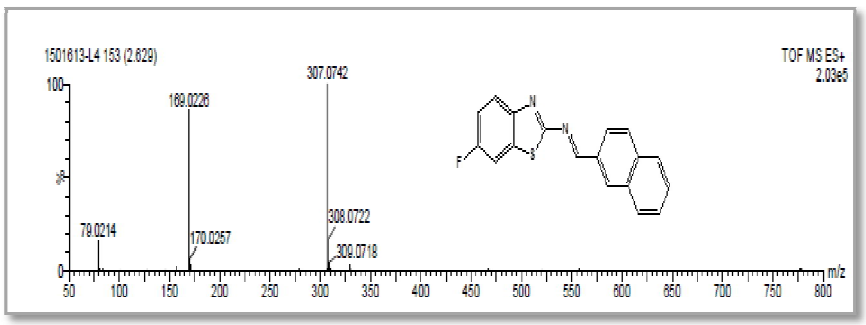


Figure 3. ESI-MS spectra of imine bases SL_3.

UV-Vis spectroscopy: The electronic absorption spectra (Figure 4**)** of compounds display high energy bands in the UV region at ~266-270 nm corresponds to $\pi \rightarrow \pi^*$ transitions of the aromatic ring and within C=N group *i.e.*, electronic transitions between HOMO and LUMO. The absorption peak at the longer wavelength at ~340-374 nm is due to $n \rightarrow \pi^*$ intra-ligand charge transfer band with the involvement of imine group. The characteristic charge transfer bands found to be at320, 315 and 336 nm correspondingly for SL_1-SL_3.

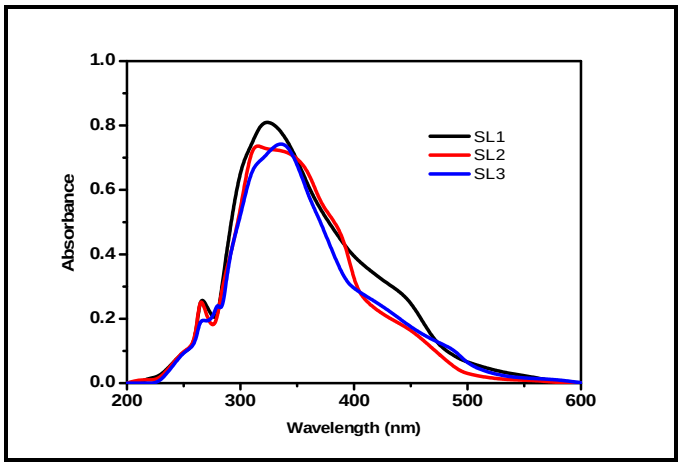


Figure 4. Electronic spectra of compounds SL_1-SL_3.

Pharmacology

Micro bactericidal results: The synthesized benzothiazole derivatives were screened for their antimicrobial potency and the relevant data of *in vitro* activity with commercial standard drugs are listed in table 1. In the antibacterial sensibility test (anti biogram), pathogenic bacterial strains displayed higher susceptibility to compounds SL_2showing excellent inhibition halo in the range of 14.78-13.89 mm ± standard deviation (SD), while the compounds SL_1 and SL_3 showed moderate/weak activity.

Table 1. Antimicrobial screening data of synthesized compounds

Compound (30 µg disc^{-1})	Diameter of Zone of inhibition (mm) ±SD*					
	Gram -ve		Gram +ve		Fungi	
	E. coli	*P.aeruginosa*	*B. subtilis*	*S. aureus*	*C. albicans*	*A. niger*
SL_1	8.1±0.1	8.3±0.1	12±0.5	16±0.1	8.7±0.7	15±0.1
SL_2	14±0.2	6.45±0.1	10±0.4	14±0.2	10±0.4	15±0.2
SL_3	13±0.4	9.3±0.2	8.4±0.3	9.5±0.2	11±0.1	15±0.2
Ciprofloxacin	20±0.6	10±0.4	14.7±0.4	15±0.3	--	--
Fluconazole	--	--	--	--	17±0.4	15±0.5

**Bioassays were performed in triplicate.*

6-fluoro benzothiazole itself being a well-known antimicrobial agent; the imine-based compounds owning a characteristic CH=N group is responsible for the greater antibacterial and antifungal activities [22]. The MIC values were determined and are presented in table 2. Fluoro benzothiazole derivatives showed antimicrobial action against tested bacteria are in the order of SL_2>SL_1>SL_3while the fungicidal action shown by ligands are in the order SL_2>SL_1>SL_3.

The compounds in the series possessing electron-withdrawing substituent's are more effective in inhibiting pathogenic fungi rather than methoxy with -OH group and un-substituent's. The compound SL_2 produces good antimicrobial activity by possessing dioxy ring. The electron-withdrawing group in

SL_1 enhances maximum fungal growth inhibition; the compound SL_3 is weakly active, and this may be due to the absence of any substituents attached.

Table 2. Minimum inhibitory concentration (µg mL^{-1}) of compounds against microbial strains

Probes	Gram –ve bacteria		Gram +vebacteria		Fungi	
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*C. albicans*	*A. niger*
SL_1	15	22	30	15	5.5	10
SL_2	5.5	10	20	15	20	22
SL_3	30	25	22	20	30	20

The above structure-activity correlation study provides a substantial effect on the antimicrobial activity influenced by electronic factors in benzothiazole ring, nature of the linkage, substituent's on the aromatic ring [23], and the lone pair electrons present in sp^2 hybridized orbital of imine nitrogen atom [24].

Time-dependent killing kinetics: Killing kinetics has exhibited a marked effect made by compounds on bacterial strain chosen (Figure 5). In general, the lag phase of growth was extended at the sub-MIC levels; the growth rate and final cell density were reduced with increasing concentrations of compounds. *E.coli* was tested in the time-kill study showing a maximum reduction in the number of CFU mL^{-1} at 24 h on exposure to 2× MIC. The control showed no noticeable bactericidal growth

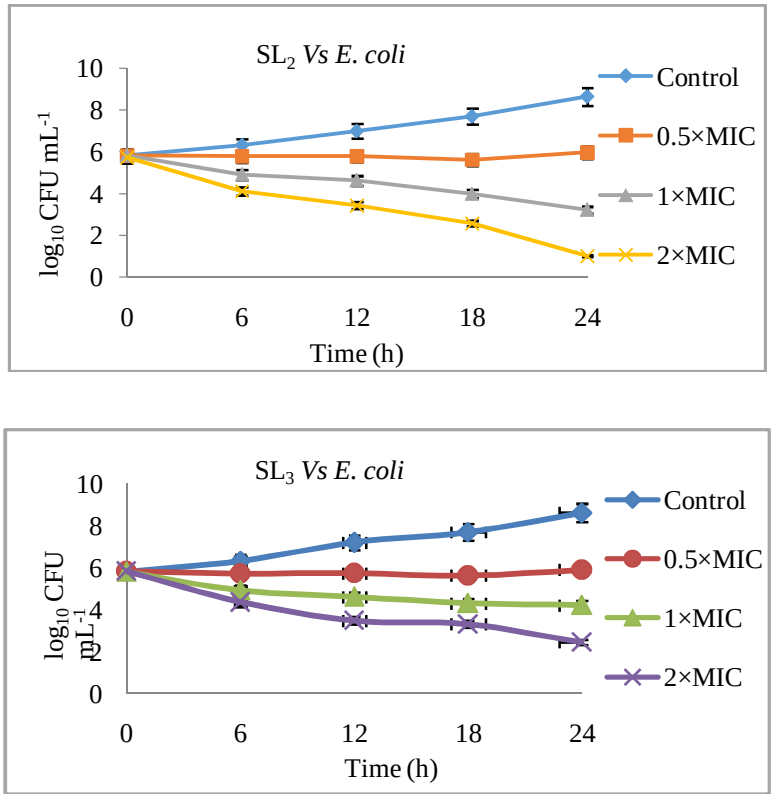


Figure 5. Time course kinetics of the bactericidal activity of SL_2 and SL_3 on *E.coli* after exposure to 0.5×, 1× and 2× the MIC and the control group treated with normal saline.

throughout 24 h incubation for all the compounds performed. The compound SL_1 at 0.5× MIC showed no significant effect on *E. coli* up to 18 h after that it showed a slow decline of up to 24 h. At 1× and

2× the MICs, the rate of killing is increased with increasing incubation time and reached ~1× 10^3 and ~1× 10^2 CFU mL^{-1}, respectively. Time-kill curve for SL_2 and SL_3 displayed in figure 5 against *E. coli*, where a mild activity was observed at 0.5×, the rate of killing was gradually increased with time and complete bacterial killing occurred at 2× the MIC. The compound SL_2 have a more or less similar kind of killing kinetics showed modest activity at 1× and 2× the MIC. A higher rate of killing was observed in the ligands with substituents rather than unsubstituted.

Antioxidant assay: The DPPH radical test was proposed for quantitating relative antiradical action of the synthesized compounds. The percentage of scavenging activity of compounds was calculated with the absorption at different concentrations. Visual pattern of reaction curves for different concentrations of methanolic solutions of SL_1 and ascorbic acid are depicted in figure 6**,** where the derivatives SL_1 showed remarkable scavenging activity when compared to the reference. The rating of radical quenching activity among benzothiazole analogs is markedly reliant on the presence of phenolic content –OH (or other H donating) than non-phenolic compounds (SL_2 and SL_3), relative concentrations of test antioxidants and their redox potentials, solvent polarity and hydrogen bonding strength [25]. The activity of compounds that tends to quench DPPH by both electron and hydrogen atom transfer under specific conditions of pH, time and solvent used. The rudimentary laboratory recording DPPH quenching displays a marked difference in the reaction curves for different antioxidants at various concentrations.

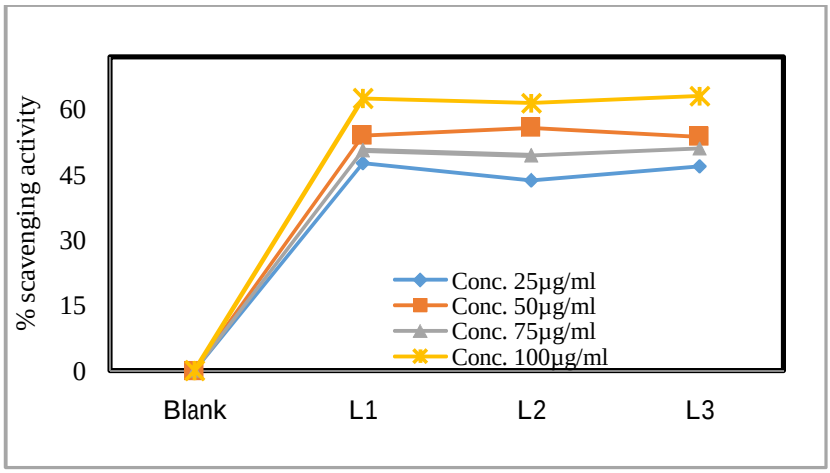


Figure 6. Graphical representation of % Scavenging activity of the benzothiazole derivatives at different concentrations with standard ascorbic acid (AA).

Anti-inflammatory activity: All the synthesized compounds were assessed for their potential *in vivo* anti-inflammatory (AI) effects; carrageenan has been chosen as a suitable agent since edemas of this type are relatively sensitive to non-steroidal anti-inflammatory drugs. Rat paw edema assay, one of the well-established acute inflammatory *in vivo* models where in the test samples were orally administered at molar equivalent doses of indomethacin (0.01g kg^{-1}, p.o). The AI was assessed as survival rates after induction at hourly intervals of 1–4 h. Table 3 compiles the mean paw volume (mL) and the percentage anti-inflammatory activity. Benzothiazole derivatives induced substantial anti-inflammatory activity relative to untreated, control and standard indomethacin.

All the test compounds have exhibited a significant reduction in rat paw edema reflecting their anti-inflammatory action. The percent inhibition had reached its remarkable value at the fourth hour (after the third hour) with the compounds$SL_2$and SL_3. Among the series of compounds tested, benzothiazole entities with electron-donating group SL_1 and SL_2 had shown excellent activity as compared to group SL_1 ,has a moderate or weak effect on the anti-inflammatory activity. The above results were following the observations claiming that the halogen compounds show a sensible activity [26].

Table 2. Percent change in rat paw edema after carrageen an sub-planter injection

Compound (100 mg kg^{-1})	Percentage change in paw volume (mean ± S.E.M)			
	1 h	2 h	3 h	4 h
Control	0.75±0.05	0.82±0.02	0.94±0.09	1.21±0.1
SL_1	0.59±0.06	0.56±0.07	0.54±0.05	0.50±0.05
SL_2	0.48±0.08	0.45±0.02	0.40±0.06	0.39±0.01
SL_3	0.44±0.01	0.45±0.03	0.43±0.06	0.42±0.03
Indomethacin (10 mg kg^{-1})	0.51±0.02	0.47±0.06	0.42±0.08	0.38±0.01

All the values are expressed as mean ± Standard error of mean (SEM) (n = 7); p< 0.01,p< 0.001, Statistically significant compared to the control and reference drug (indomethacin).

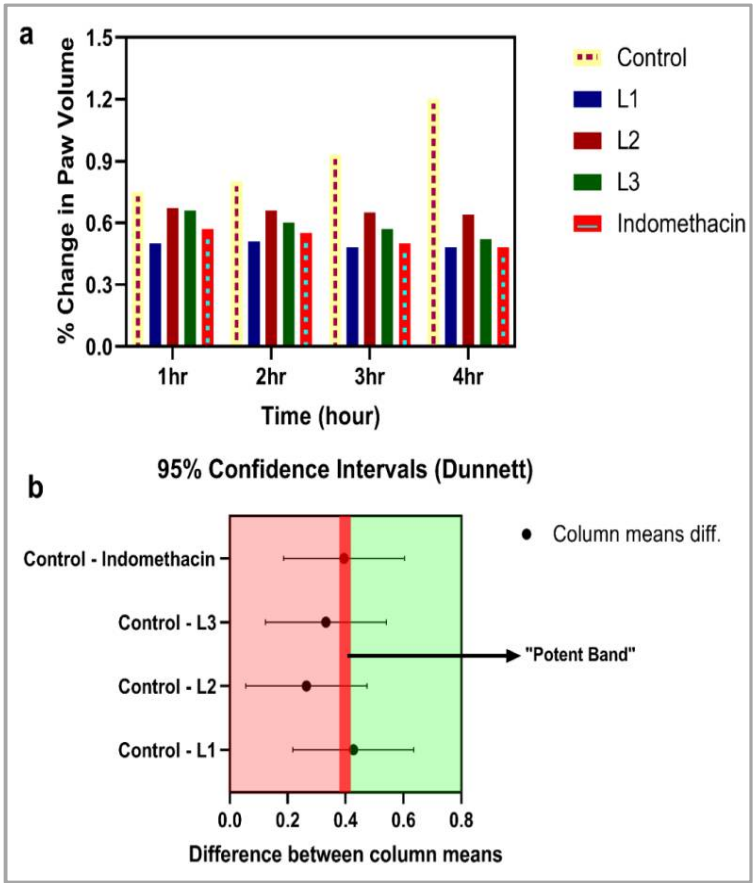


Figure 7. Neutralization of edema-inducing activity of $sPLA_2$ by compounds (SL_1-SL_3) in mice which received $sPLA_2$ (Control), treated group $sPLA_2$ (100 mg kg^{-1}) and standard drug Indomethacin **(a)** Multi-comparison followed by Dunnett's honest significant difference test with 95% confidence intervals revealing the band of significance ("Potent band") when compared to control and standard. **(b)** All the values represent mean ± SEM statistically significant compared to the control and reference drug (indomethacin).

Statistically computed by multi-comparison followed by Tukey's honest significant difference test with the confidence intervals of 95%, and it is found that these potent compoundsSL_2 and SL_3 mark the band of potency which is highlighted by a red color band in Figure 7(A) and 7(B). When compared with control, all the ligands where the band of potency will move towards left-hand side from the difference between the groups mean as calculated by Tukey's test to validate *in vitro* as well as statistical data. The results suggest that too much higher electronegative will hamper the activity and hence moderate electronegative along with hydrophobicity is essential to ligand bind at the active site of hydrophobic core; thus methoxy substituent's show higher activity. All the data values represent mean ± SEM statistically significant compared to the control and reference drug.

APPLICATION

This method would be useful for the medicinal chemists to develop more potent Schiff base ligands for various medical applications.

CONCLUSION

The sequences of imine based benzothiazole analogs (SL_1-SL_3) were prepared conveniently under green synthetic method; the prepared compounds have well characterized by various techniques and supported for the proposed composition.SAR has become a useful tool to study the molecular determinants leading to the bioactivity of synthesized analogs towards clinically important pathogens and was identified as viable leads for further studies. Antiradical potency existed in the compounds was quantified by known DPPH assay compared to standard. Subsequently, all the compounds were subjected to the evaluation of *in vivo* anti-inflammatory assay and thereby helped to design novel potent inhibitor using the Carrageen an-induced rat paw edema method with reference to the indomethacin. The results indicated that phenolic form of the test compounds could be the potent antioxidants and inflammation inhibitors as showed mild to moderate activity.

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