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RESEARCH ARTICLE

Synthesis, Cytotoxicity, Antibacterial and Antioxidant Activity of New 2-Substituted Benzimidazole Containing 1,2,4-Triazoles

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Abstract:

The development of a potent new drug with high biological activity is a challenge in drug design and is of strategic importance. Due to the increasing resistance of pathogens on the available drugs there is always a demand for designing a potent drug with high biological activity. The pharmacological effects have been observed in compounds containing different moieties of pharmaceutical importance such as triazole, Schiff's base, benzimidazole. Triazole thione, Schiff's bases and two new compounds containing three pharmacophores *viz.* triazole, benzimidazole and Schiff base incorporated in a single compound were prepared by applying different synthetic reactions. Different spectroscopic methods, including FT-IR, UV-vis, ^{1}H-NMR, and ^{13}C-NMR, were used to confirm the structure of the prepared compounds. All compounds exhibited moderate antibacterial activity against *Staphylococcus aureus* (ATCC 6538P) and *Staphylococcus epidermidis* (ATCC 1228). Antioxidant activity was carried out by DPPH radical scavenging test and among the tested five compounds, the IC_{50} value of 4-amino-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione was found to be 32.364 µg.mL^{-1} which is closer to the ascorbic acid that was found to be 28.546 µgmL^{-1}. All tested compounds were toxic against brine shrimp where 2-(5-((1*H*-benzo[d]imidazol-2-yl)thio)-4-((4-chlorobenzylidene) amino)-4*H*-1,2,4-triazol-3-yl) was comparatively more toxic (LC_{50}= 26.827 µgmL^{-1}).

Keywords: *Heterocycles, Triazole, Schiff's base, Benzimidazole, Biological activity.*

Introduction

Heterocyclic compounds are a broader class of chemicals that display different biological activities. These compounds containing nitrogen in their structure are used widely as pharmaceuticals and agrochemicals; and show promising biological activity. They have a variety of applications as in herbicides [1], insecticides [2], photo-polymericadhesives [3], flavor and fragrances [4], pesticides [5], dyes [6] and drugs[7]. Nitrogenous compounds are of huge interest to the researchers around the world due to their vast area of the biological activity. The molecules that contain nitrogen are also a crucial component of natural products that possess significant pharmacological activity[8]. Pyridine, pyrroles, pyrans, imidazole, triazoles and benzimidazoles are some of the examples of the heterocyclic compounds containing nitrogen.

Two types of triazoles are in existence *viz.*1,2,3-triazole and 1,2,4-triazole each having tautomeric forms. Triazole nucleus has a prominent antifungal activity and has been part of different commercially available antifungal compounds such as Fluconazole, Posaconazole, Voriconazole, Itraconazole etc.[9] and a variety of therapeutically important medicines like Triazolam, Rizatriptan, Furacyclin, Alprazolam, Etizolam etc. [10]. Compounds containing triazole moiety are found to exhibit various biological activities like antibacterial [11], antifungal [12], antihistaminic [13], antiprotozoal [14], antioxidant [15], anticonvulsant [16], antimalarial [17], antiviral [18], anticancer [19] properties among many others.

Schiff's bases, first reported by Hugo Schiff, are condensation product of primary amine with carbonyl

compound and contain an imine or azomethine group. This group of organic compounds shares structural similarities with naturally occurring biological compounds and exhibits a wide range of biological and therapeutic benefits, including antimicrobial [20], antidyslipidemic [21], antioxidant [22], and other activities.

Benzimidazole is a heterocyclic molecule containing nitrogen. Hoebreaker first synthesized benzimidazole by reduction of 2-nitro-4-methylacetanilide followed by dehydration[23]. Albendazole, mebendazole and thiabendazole are some examples of antihelminthic medicines that contain benzimidazole ring[24]. The biological activity of benzimidazole containing compounds include antiprotozoal [25], antibacterial [26], antiviral [27], anticonvulsant [28], anticancer [29] properties among many others.

In the present work, 4-amino-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione have been synthesized starting with methyl salicylate as the precursor. By condensation of the triazole with *p*-chlorobenzaldehyde and furfuraldehyde, two Schiff bases were prepared and then their respective 2-substituted Benzimidazole derivatives. Thus, final compounds synthesized contain three biologically active moieties in a single molecule.

Materials and Methods

The analytical grade solvents and reagents *viz*. methyl salicylate (Fisher scientific), hydrazine monohydrate (Alfa Aeser), carbon disulphide (Fisher Scientific), ethyl alcohol (Changshu Hongsheng Fine Chemical Co. Ltd.), *p*-chlorobenzaldehyde (Himedia), furfuraldehyde (Himedia), *o*-phenylenediamine (Loba chemie), methyl alcohol (Fisher scientific), chloroacetic acid (Qualigens) were procured from chemical suppliers and were used as received. The reaction was carried out on a borosilicate R.B. flask and the advancement of the reaction was confirmed by TLC using Aluminum TLC plates coated with silica gel and the spots were visualized under ultraviolet lamp in a chamber. The melting point of each compound was determined on Optics Technology electro-thermal apparatus by using open capillary tube. UV spectra were recorded in methyl alcohol on double beam UV-Visible Spectrophotometer of Labtronics (Model LT-2802) in the region 1100-200 nm. FTIR spectral data were recorded on Perkin Elmer spectrum two spectrometer (version 10.6.2) in the region of 4000-500 cm^{-1} using KBr. NMR spectral data were obtained from Bruker AV III 500 MHz NMR spectrometer in the solvent DMSO-d_6. Chemical shifts are recorded in ppm relative to TMS and the signals obtained werere ported as singlet(s), doublet(d), triplet(t) and multiplet (m).

Synthesis

The syntheses of compounds were carried out as illustrated in the Figure 1.

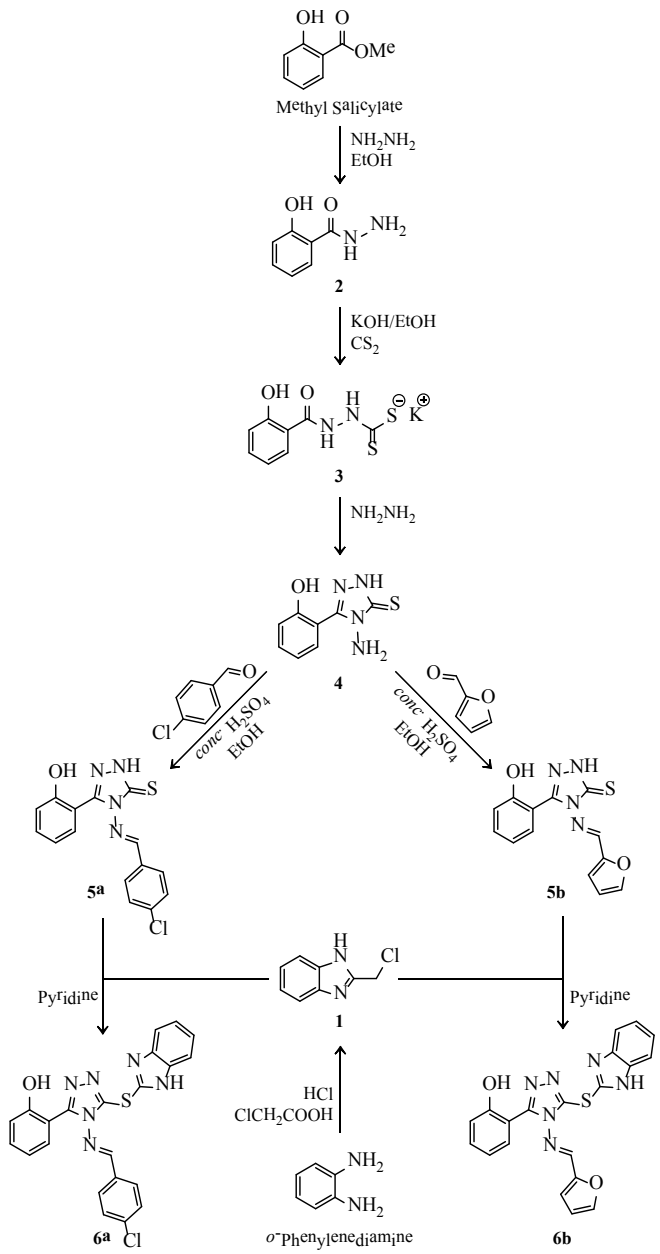

Figure1: *Scheme for the preparation of 1,2,4-triazole containing 2-substituted benzimidazole*

Synthesis of 2 - (chloromethyl) - 1 H-benzo[d] imidazole (1) [30]

2.162 g (0.020 mol) of *o*-phenylenediamine was dissolved in a 10 mL of HCl and 1.890 g (0.020 mol) chloroacetic acid was added. It was refluxed for 6-10 hrs. with constant stirring. After cooling the reaction mixture to 5 °C, $NaHCO_3$ solution was added to neutralize it. The resulting yellow solid after being separated by filtration under suction was recrystallized from hot ethyl alcohol.

Yield: 79%, Yellow crystal line solid, m.pt. 154° C, R*f*: 0.71 (*n*-hexane: ethyl acetate, 8:2). UV-Visible (MeOH) λ_{max}nm = 292,309,331. IR (KBr)cm^{-1} = 3061 (N-H, str, aromatic), 3042 (C-H, str, aromatic), 1622 (C=N, str), 1540 (NH, str) 1448 (C=C, str, aromatic), 1442 (C=C, str, aromatic), 735 (C-H bend), 698 (C-H, bend), 642(C-Cl, str).

Synthesis of 2-Hydroxybenzohydrazide (2)[31]

4.5 mL (0.090 mol) of hydrazine monohydrate was added slowly while being stirred constantly to the round bottom flask containing 9.129 g (0.060 mol) of methyl salicylate and refluxed for six hours.The excess solvent was evaporated on a hot water bath to reduce the volume of solution to half. The solution was cooled when white solid crystallized out. The solid after being separated by filtration was washed with cold ethyl alcohol, recrystallized from hot absolute ethyl alcohol and was dried at 50-60 °C.

Yield: 78%, white shining crystalline solid, m.p. 140° C, R*f*: 0.64 (ethyl acetate: *n*-hexane, 2:8)

Synthesis of Potassium 2-(2-hydroxybenzoyl) hydrazinecarbodithioate (3)[32]

4.565 g (0.030 mol) of acid hydrazide (**2**) was added to 1.683 g (0.030 mol) of KOH in 20 mL ice cold absolute ethyl alcohol. 1.8 mL (0.030 mol) of carbon disulphide was added dropwise to the mixture while being constantly stirred at a temperature not exceeding 30 °C and then stirred for 22 h at room temperature. After adding 20 mL of dry diethyl ether, potassium dithiocarbazinate that separated out was washed twice with diethyl ether. The solid was then dried at a temperature lower than 60 °C.

Yield: 87%, White shining crystalline solid, m.pt. 241° C, R*f:* 0.58 (*n*-hexane : ethyl acetate, 8:2)

Synthesis of 4-amino-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione(TZ, 4)[33]

In 5 mL of distilled water, 4.680 g (0.018 mol) of potassium dithiocarbazinate (**3**) and 1.5 mL of hydrazine monohydrate were suspended. The resulting mixture was refluxed for five hours to ensure that no more hydrogen sulphide gas was released (checked with $K_2Cr_2O_7$). Once the reaction mixture had reached to room temperature it was diluted with 100 mL of cold water that contained some crushed ice (made from distilled water) and acidified by adding *conc.* HCl. The resulting triazole after being separated by filtration waswashed twice with 30 mL cold and recrystallizedfrom absolute ethyl alcohol.

Yield: 84%, White crystalline solid, m.pt. 178 °C, R*f*: 0.43 (*n*-hexane : ethyl acetate, 8:2).

Syntheses of Schiff's Bases

Required Schiff's bases were obtained by condensation reaction between triazole thione and desired aldehydes.

***Synthesis of 4-((4-chlorobenzylidene)amino)-3-(2-hydroxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (5a, SBPC)*[20]**

In a little amount of heated ethyl alcohol 4.217 g (0.030 mol) of *p*-chlorobenzaldehyde and 6.247 g (0.030 mol) of triazole thione (**4**) were added.After heating the suspension until it turned into clear solution it was treated with5 drops of *conc.* H_2SO_4. The mixture was refluxed for 5h. On cooling a solid precipitated out which after being separated by filtration was washed with cold ethylalcohol and recrystallized from hot ethyl alcohol.

Yield 72%, White cottony solid, m. pt. 215 ˚C, R*f*: 0.78 (*n*-hexane : ethyl acetate, 8:2).

UV-Visible (MeOH) λ_{max}nm = 302,309,331,340, 370. IR (KBr) cm^{-1} = 3482(O-H, str, aromatic), 3218(N-H, str, aromatic), 3092(C-H, str, aromatic), 1620(C=N, str), 1583(NH, str), 1508(C=C, str,

aromatic), 1244(C-O, str, aromatic), 1015 (N-N, bend, aromatic), 941(C=S, str), 737(C-H bend), 687 (C-H, bend). ^{1}H-NMR (500MHz, DMSO-d_6)ppm. =14.13 (br s, 1H, SH), 10.09(s, 1H, OH), 9.64(s, 1H, N=CH), 7.79 (d, *J*=8.55, 2H, Ar-H), 7.57 (d, *J* = 7.93 Hz, 2H, Ar-H),7.33-7.42 (m, 2H Ar-H), 6.88-6.95 (m, 2H, Ar-H) ^{13}C-NMR (100MHz,DMSO-d_6)=164.59 (triazole-C5), 162.24(Ali-C), 156.62 (Ar-C),148.88 (triazole-C3), 137.76 (Ar-C), 132.91 (Ar-C), 131.59 (Ar-C), 131.55(Ar-C), 130.65(Ar-C) , 129.79(Ar-C) , 119.42(Ar-C) , 116.43(Ar-C) , 113.28(Ar-C).

Synthesis of 4-((furan-2-ylmethylene)amino)-3-(2-hydroxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (5b, SBFF)[20]

In a little amount of heated ethyl alcohol 2.7 mL (0.030 mol) of furfuraldehyde and 6.247 g (0.030 mol) of triazole thione (**4**) were added. After heating the suspension until it turned into clear solution it was treated with 5 drops of *conc.* H_2SO_4. The mixture was refluxed for 5h. On cooling a solid precipitated out which after being separated by filtration was washed with cold ethyl alcohol and recrystallized from hot ethyl alcohol.

Yield 70.9%, White cottony solid, m. pt. 215 ˚C, R*f*: 0.78 (*n*-hexane: ethylacetate, 8:2).

UV-Visible (MeOH) λ_{max}nm = 302, 309, 331, 340, 370. IR (KBr) cm^{-1} = 3443 (O-H, str, aromatic), 3221 (N-H, str, aromatic), 3092 (C-H, str, aromatic), 1615 (C=N, str), 1583 (NH, str) 1542 (C=C, str, aromatic), 1243 (C-O, str, aromatic), 1002 (N-N, str, aromatic), 943 (C=S, str), 756 (C-H bend), 687 (C-H, bend). 1H-NMR (500 MHz, DMSO-d6) ppm = 14.09(br s, 1H, SH),10.06(s, 1H, OH), 9.41(s, 1H, N=CH), 7.97 (br, s, 1H, Ar-H), 7.34-7.45(m, 2H, Ar-H), 7.30 (d, *J*=3.66, 1H, Ar-H), 6.87-6.96 (m, 2H, Ar-H), 6.73 (dd, *J*=3.05, 1.83Hz, 1H). 13C-NMR (100 MHz, DMSO-d_6) = 162.16 (triazole-C5), 156.56 (Ali-C), 154.42 (Ar-C), 148.82 (triazole-C3), 148.34 (Ar-C), 147.66 (Ar-C), 132.82 (Ar-C), 131.59 (Ar-C), 120.89 (Ar- C), 119.39 (Ar-C), 116.47 (Ar-C), 115.30 (Ar-C),113.39(Ar-C).

Synthesis of 2-substituted Benzimidazole derivatives

2-Substituted benzimidazole derivatives were prepared by reacting Schiff's bases with 2-(chloromethyl)-1*H*-benzo[d]imidazole,

Synthesis of 2-(5-((1H-benzo[d]imidazol-2-yl) thio)-4-((4-chlorobenzylidene)amino)-4H-1,2,4-triazol-3-yl)phenol (6a, FCPC)

In 10 mL of pyridine 1.666 g (0.010 mol) of compound **1** and 3.308 g (0.01 mol) of compound **5a** were added. After refluxing for 6-10 h, it was poured to 100 mL ice cold water. Thus, obtained yellow-green solid after being separated by filtration was washed with cold ethyl alcohol and recrystallized from hot ethyl alcohol.

Yield 78%, Dark green solid, m. pt. 175 ˚C, R*f*:0.77(*n*-hexane : ethyl acetate, 8:2).

UV-Visible (MeOH) λ_{max}nm = 302,309,331,340, 370.IR (KBr) cm^{-1} = 3261(O-H, str, aromatic), 3075(NH, str), 3022 (C-H, str, aromatic), 1620(C = N, str), 1584(NH, str), 1485 (C = C, str, aromatic), 1247(C-O, str, aromatic), 1015 (N-N, str, aromatic), 744(C-H bend), 696 (C-H, bend). ^{1}H-NMR (500MHz, DMSO-d_6)ppm = 12.69 (br s, 1H, NH),10.09(br s, 1H, OH), 9.66(s, 1H, N = CH), 7.77-7.83 (m, 2H, Ar-H) 7.71(d, *J* = 7.32 Hz, 2H, Ar-H), 7.58 (d, *J* = 8.55 Hz, 2H, Ar-H), 7.45-7.55 (m, 2H, Ar-H), 7.34-7.4 (m, 2H, Ar-H), 6.88-7.05 (m, 2H, Ar-H). ^{13}C-NMR (100MHz,DMSO-d_6) = 164.52 (Triazole-C5), 162.25(Ali-C), 156.75 (Benzimidazole-C), 156.65 (Ar-C), 148.89 (Triazole-C3), 143.62 (Benzimidazole-C), 137.74 (Ar-C), 132.88 (Ar-C), 131.58 (Ar-C), 131.50(Ar-C), 130.65(Ar-C), 129.79(Ar-C), 129.40(Ar-C), 119.40 (Ar-C), 119.40(Ar-C), 116.46(Ar-C), 113.29(Ar-C).

Synthesis of 2-(5-((1H-benzo[d]imidazol-2-yl) thio)-4-((furan-2-ylmethylene)amino)-4H-1,2,4-triazol-3-yl)phenol (6b, FCFF)

In 10 mL of pyridine 1.666 g (0.010 mol) of compound **1** and 2.863 g (0.01 mol) of compound **5b** were added. After refluxing for 6-10 hr., it was poured to 100 mL ice cold water. Thus, obtained dark brown solid after

being separated by filtration waswashedwith cold ethyl and recrystallized from hot ethyl alcohol.

Yield 80%, Dark brown solid, m. pt. 161˚C, R*f*: 0.74 (*n*-hexane : ethyl acetate, 8:2).

UV-Visible (MeOH) λ_{max}nm=302,309,331,340, 370.IR (KBr) cm^{-1}=3261 (O-H, str, aromatic), 3101(NH, str),3022(C-H, str, aromatic),1620(C=N, str),1584(NH, str), 1486(C=C, str, aromatic), 1247(C-O, str, aromatic), 1015 (N-N, str, aromatic), 740(C-H bend), 702(C-H, bend).^{1}H-NMR(500MHz, DMSO-d_6)ppm = 12.70(s, 1H, NH),10.09(br s, 1H, OH), 9.41(s, 1H, N=CH), 7.97(br s, 1H, Ar-H), 7.72-7.88(m , 2H, Ar-H), 7.54-7.63 (m, 2H, Ar-H),7.36-7.40 (m, 2H, Ar-H), 7.30 (d, *J*=3.66, 1H, Ar-H), 6.88-6.95 (m, 2H, Ar-H), 6.73(dd, *J*=3.66, 1.83Hz, 1H). ^{13}C-NMR(100MHz,DMSO-d_6)=162.16(Triazole-C5),156.75(Ali-C), 156.66(Benzimidazole-C), 151.41(Triazole-C3), 148.83 (Ar-C), 148.33 (Benzimidazole-C), 147.68 (Ar-C), 143.62 (Ar-C), 132.82(Ar-C), 131.58(Ar-C), 129.41(Ar-C), 120.89(Ar-C), 119.58(Ar-C), 119.40(Ar-C), 116.49(Ar-C), 115.30(Ar-C), 113.39(Ar-C).

Antibacterial activity

The antibacterial activity of compounds **5a**, **5b**, **6a** and **6b** were tested at 100µg.mL^{-1} concentration against *Staphylococcus aureus* (ATCC 6538P) and *Staphylococcus epidermidis* (ATCC 1228) by Agar well diffusion method. Bacterial culture drawn from the respective inoculums equivalent to 0.5 McFarland standard turbidity was uniformly distributed over Mueller-Hinton Agar (MHA) medium with a sterile swab. Swabbing was repeated twice and after each swabbing, the plate was rotated through a 60-degree angle. After drying for maximum of 15 minutes, four wells each of 6 mm diameter were created in the inoculated plates with a sterile cork borer. Using a micropipette, the test solutions for the four samples and the DMSO negative control were added to each well. The test solutions were allowed to diffuse into the media by standing the plates in upright condition with lids closed for 30 minutes. After incubation in an inverted position at 35±2 °C for 18-24 hours, the plates were examined for the growth of organisms and zone of inhibition (ZOI) was measured. Azithromycin was used as the positive control and the ZOI was compared.

Cytotoxicity assay

The brine shrimp assay was used for the toxicity test. The compounds shown in Table 1 were dissolved in 1 L of distilled water to make artificial sea water and its pH was maintained to 8±0.2.

Table 1: *Chemical composition of sea water*

S.N.	Composition	Amount ($g.L^{-1}$)
1.	NaCl	23.5000
2.	Na_2SO_4	4.0000
3.	KCl	0.6800
4.	H_3BO_3	0.0270
5.	$MgCl_2.2H_2O$	10.6800
6.	$CaCl_2.2H_2O$	1.4800
7.	$NaHCO_3$	0.1970
8.	Na_2EDTA	0.0003

One full spatula eggs of brine shrimp were sprinkled on the artificial sea water taken in a plastic container. The container was illuminated for 48 h using a 60 Watt bulb at room temperature. The eggs hatching container was adjusted by passin g heat, light and air. After 48 h, large number of tiny larvae (nauplii) can be seen swimming on the container. The nauplii are then counted and transferred to test tubes for toxicity assay[34].

Sample preparation

Due to the correlation between toxicity and anticancer properties, the brine shrimp assay provides a quick, low-cost, and reliable method for determining lethality of compound [35]. For the sample preparation, Meyer's brine shrimp bioassay method [36] was slightly modified. Three different concentrations 1000 ppm, 100 ppm and 10 ppm of the compounds **5a**, **5b**, **6a** and **6b** in methyl alcohol were prepared from their respective stock solutions. Three experiments were carried out for each concentration. 10 brine-shrimp nauplii were counted for each of the test solutions. After complete evaporation of the solvents in the test solution, the nauplii were added to the test tube with 5 mL sea water making total

volume of 10 mL, illuminated, kept for 24 h and then disposable pipettes were used to count the number of survivors.

Data analysis for toxicity assay

The lethal concentration of dose necessary to kill 50% of the organisms utilized in a bioassay is expressed as LC_{50} value.

If '*n*' is the number of replicates (in this experiment three), '*x*' is the log of the concentration of the solution in $\mu g.mL^{-1}$ (log10, log100, log1000 here) and '*y*' is the probit for average survivors for all replicates, then we have,

$$\alpha = \frac{[\sum y - \beta \sum x]}{n} \quad (1)$$

$$\beta = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sum x^2 - \frac{(\sum x)^2}{n}} \quad (2)$$

Now, from the probit regression,

$$y = \alpha + \beta x \quad (3)$$

$$x = \frac{y - \alpha}{\beta} \quad (4)$$

Where *y* is constant having value 5 for calculating LC_{50} value.

Hence,

$$LC_{50} = \text{Antilog}\,(x) \quad (5)$$

The calculation of the data for probit regression, calculation of LC_{50} values and plots were done inMATLAB software and plots were visualized in Orange data mining software Version 3.32.

Antioxidant Screening

Any substance which considerably slows down or stops a substrate from oxidizing, even when present at low concentrations compared to an oxidizable substrate, is referred to be an antioxidant. The indication of antioxidant activity is also important in other sciences such as medicine, biology, health and nutrition, epidemiology etc. Antioxidants are important class of chemical compounds and have various health benefits as well as used widely in the food industries as lipid peroxidation inhibitors[37].

The DPPH assay technique is employed to investigate the antioxidant activity of freshly prepared compounds and to ascertain their radical scavenging capability. The equation below demonstrates that the DPPH radical is a stable radical whose scavenging activity is measured by the drop in absorbance at 517 nm as a result of reduction by the antioxidant or interaction with the radical species.

$$DPPH^{\cdot} + R^{\cdot} \longrightarrow DPPH\text{-}R$$

The radical scavenging activity of any extract or compound can be determined by;

$$\%\,RSA = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (6)$$

After plotting the %RSA *vs* concentration graph, using a four-parameter logistic regression model the IC_{50} is calculated using the Quest graph™ IC_{50} calculator at aatbio.com. The equation used to determine IC_{50} regression is as follows:

$$y = \frac{\text{Max-Min}}{1 + \left(\frac{x}{IC_{50}}\right)^{H}} \quad (7)$$

Where *y*= Final IC_{50} value, Max= maximum value, Min= minimum value, H= Hill coefficient.

Results and Discussion

Chemistry involved

Methyl salicylate was treated with hydrazine monohydrate to produce acid hydrazide. The ethanolic solution of acid hydrazide was reacted with carbon disulphide to obtain potassium dithiocarbazinate. Thus, formed dithiocarbazinate was refluxed with hydrazine monohydrate to obtain 1,2,4- triazole thione. The triazole thus obtained was condensed subsequently with two aldehydes *viz.* *p*-chlorobenzaldehyde and furfuraldehyde to obtain Schiff's bases **5a** and **5b**. *o*-phenylenediamine was treated with chloroacetic acid to obtain 2-chloromethyl benzimidazole. Thus, formed benzimidazole was condensed with Schiff's bases 6a and 6b to obtain

Table 2: *Zones of inhibition displaying the antibacterial activities of Schiff bases, benzimidazole derivatives, and the +ve control*

Compound		*S. aureus*			*S. epidermidis*		
No.	Code	ZOI (mm)	MIC ($mg.mL^{-1}$)	MMC between ($mg.mL^{-1}$)	ZOI (mm)	MIC ($mg.mL^{-1}$)	MMC between ($mg.mL^{-1}$)
5a	**SBPC**	14.20	12.5	12.5-25	16.12	12.5	12.5-25
5b	**SBFF**	0.00	-	-	15.50	12.5	12.5-25
6a	**FCPC**	9.74	12.5	12.5-25	0.0	-	-
6b	**FCFF**	9.12	12.5	12.5-25	0.0	-	-
Azithromycin		30.18			29.72		

* MIC = Minimum inhibitory concentration, MMC=Minimum microbicidal concentration

respective benzimidazole derivatives. Good yields of the compounds were achieved. The compounds are easily soluble in solvents like DMSO and methyl alcohol, and are stable at room temperature. By using different spectroscopic techniques including UV-Vis, FT-IR, ^{1}H-NMR, and ^{13}C-NMR, the structure of compounds wasconfirmed.

Antibacterial evaluation

The results of the screening of Schiff's bases (5a and 5b) and Benzimidazole derivatives (6a and 6b) against bacterial strains *Staphylococcus aureus* (ATCC 6538P) and *Staphylococcus epidermidis* (ATCC 1228) are shown in table 2. Diameters of zone of inhibition indicates that all four compounds **5a**, **5b**, **6a&6b** exhibited some degrees of activity against both the tested microbial specimens.

The synthesized compounds exhibited moderate activitya gainst *S. aureus* and *S. epidermidis* using Azithromycin as standard (positive controls). Schiff's bases SBPC and SBFF showed more activity than Benzimidazole derivatives. For the organism, *S. aureus*, SBPC had a ZOI of 14.20 mm, FCPC with 9.74 mm and FCFF with 9.12 mm whereas Azithromycin had a ZOI of 30.18 mm. For the organism, *S. epidermidis*, SBPC had a ZOI of 16.12 mm, SBFF with 15.5 mm azithromycin had a ZOI of 29.72 mm. The Schiff base SBPC had MMC of 12.5–25$mg.mL^{-1}$ for the organisms, *S. aureus* and *S. epidermidis*. Compound SBFF had MMC of 12.5–

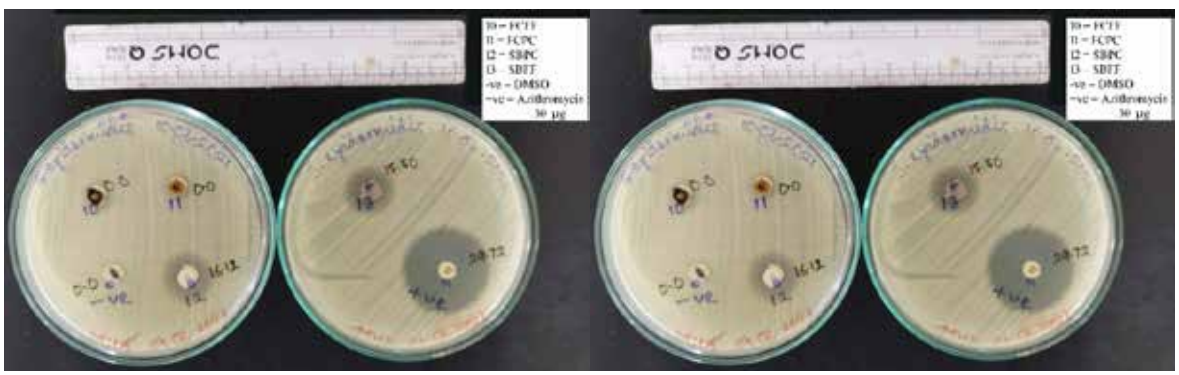

Figure 2: *ZOI of synthesized compounds with S. Aureus (left), S. epidermidis(right)*

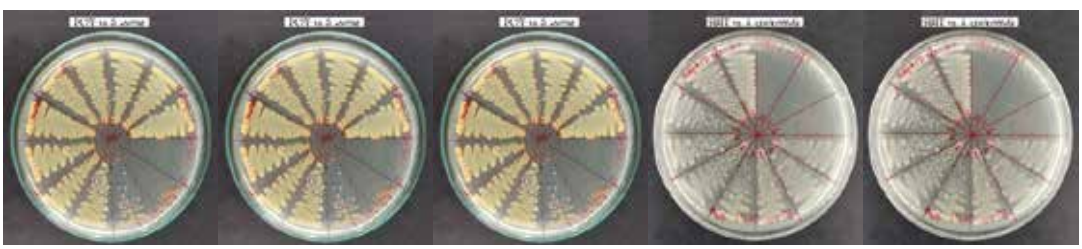

Figure 3: *MIC and MMC of synthesized compounds against S. Aureus and S. epidermidis*

Table 3: Toxicity assay of Schiff bases and benzimidazole derivatives

Compound		Conc^n in µg/mL (z)	No. of Nauplii Added	Alive(y)	Death	Death %	No of replicates	x = log(z)	xy	x^2
No.	Code									
		1000	10	4	6	60	3	3	12	9
5a	SBPC	100	10	8	2	20	3	2	16	4
		10	10	9	1	10	3	1	9	1
		1000	10	4	6	60	3	3	12	9
5b	SBFF	100	10	7	3	30	3	2	14	4
		10	10	10	0	0	3	1	10	1
		1000	10	1	9	90	3	3	3	9
6a	FCPC	100	10	6	4	40	3	2	12	4
		10	10	8	2	20	3	1	8	1
		1000	10	4	6	60	3	3	12	9
6b	FCFF	100	10	6	4	40	3	2	12	4
		10	10	9	1	10	3	1	9	1

25 mg.mL^{-1} for *S. epidermidis*. The benzimidazole derivatives FCPC and FCFF had MMC of 12.5–25 mg.mL^{-1} for bacterium *S. aureus*. The results show that the bacterium *S. aureus* is resistant to the compounds SBPC, FCPC and FCFF with MIC at 12.5 mg.mL^{-1} (>200 µg/mL) and completely resistant to SBFF. Similarly, the bacterium *S. epidermidis* is resistant to the Schiff's bases SBPC, SBFF with MIC at 12.5 mg.mL^{-1} and completely resistant to the benzimidazole derivatives FCPC and FCFF [38].

Brine Shrimp assay

The number of nauplii alive, dead, in different concentrations with supplementary data is presented in Table 3.

A compound is considered to be pharmacologically active and toxic if its LC_{50} value is less than 1000 µg.mL^{-1}. Table 4 clearly shows that all the synthesized compounds are toxic against brine shrimp causing lethality at different concentrations. LC_{50} values for the compounds SBPC, SBFF, and FCFF indicate that they are moderately toxic with LC_{50} values of 630.957 µg.mL^{-1}, 215.443 µg.mL^{-1}, and 341.455 µg.mL^{-1}, respectively, whereas the compound FCPC is substantially more toxic with an LC_{50} value of 26.827 µg.mL^{-1}.

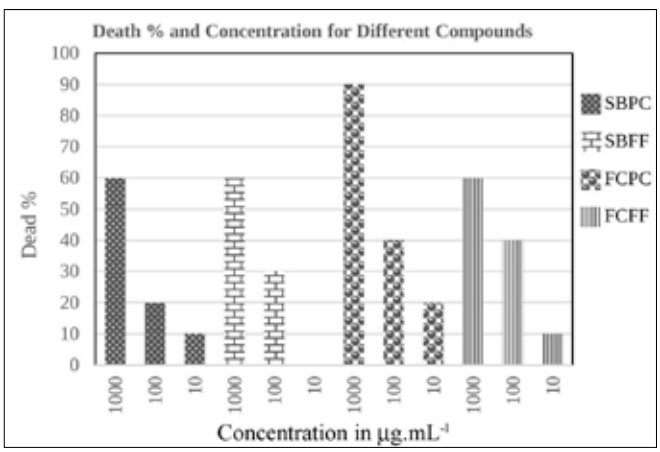

Figure 4: *Death percentage of nauplii after 24 hr. at different concentrations.*

Figure 4 shows the death percentage of nauplii at three different concentrations where the death percentage of nauplii is seen highest for the compound FCPC.

Table 4: Calculated toxicity of the synthesized compounds

Compound		β	α	x	LC_{50} (µg.mL^{-1})
No.	Code				
5a	SBPC	-2.5	12.00	2.800	630.957
5b	SBFF	-3.0	12.00	2.333	215.443
6a	FCPC	-3.0	10.00	1.428	26.870
6b	FCFF	-2.5	11.33	2.533	341.455

Table 5: Radical scavenging percentage and IC_{50} values of synthesized compounds and Ascorbic Acid.

Concentration ($\mu g.mL^{-1}$)	% Radical Scavenging Activity					
	TZ (4)	SBPC (5a)	SBFF (5b)	FCPC (6a)	FCFF (6b)	Ascorbic acid (Standard)
20	30.801	15.195	28.131	2.464	2.053	43.326
40	58.932	27.926	52.772	6.571	28.337	59.959
60	72.485	47.228	66.735	14.784	40.246	74.127
80	83.984	66.530	78.645	17.454	69.199	89.938
100	87.680	77.823	93.018	25.462	78.645	99.589
$IC_{50}(\mu g.mL^{-1})$	32.364	62.137	38.941	206.367	64.110	28.546

Other tested compounds showed similar death percentage of nauplii at 1000 $\mu g.mL^{-1}$concentration.

Antioxidant data

Antioxidant activity was studied from DPPH assay of synthesized compounds with reference to ascorbic acid. The absorbance values of different concentration of SBPC, SBFF, FCFF, FCPC of 10, 20, 40, 60, 80, and 100 ppm were measured at 517 nm. These values were used to calculate percentage inhibition of DPPH radicals against the sample. The antioxidant activity was estimated by plotting free radical scavenging percentage *vs* concentration and IC_{50} value of respective synthesized compounds. The data for percentage radical scavenging activity and IC_{50} values reported for the compounds 5a, 5b, 6a and 6b are given in Table 5.

The radical scavenging activity is expressed in the form of IC_{50}. Ascorbic acid had IC_{50}value of 28.546 $\mu g.mL^{-1}$. The IC_{50} values of tested compounds SBPC (62.137 µg.mL-1), SBFF (38.941 µg.mL-1), FCPC

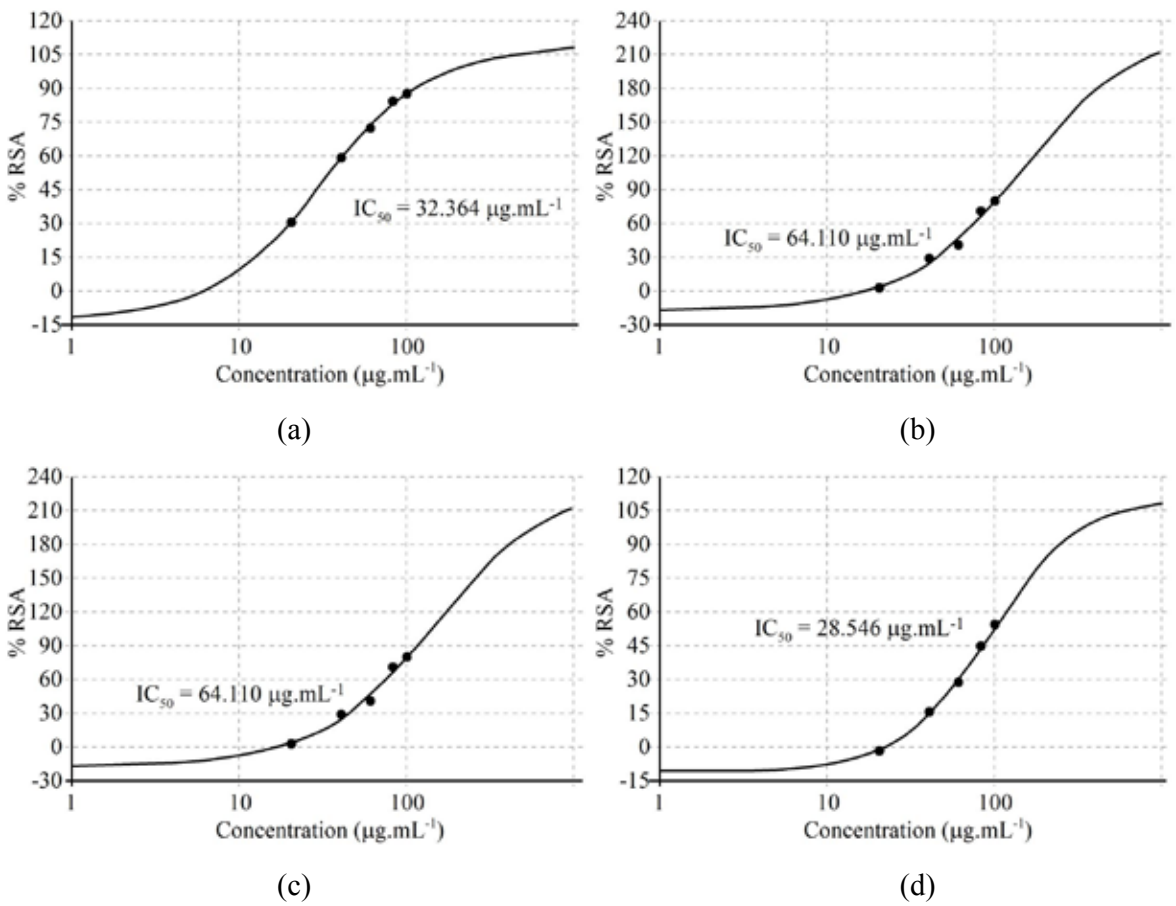

Figure 5: Radical Scavenging Activity of (a) TZ, (b) SBFF, (c) FCFF and (d) Ascorbic acid

(206.367 µg.mL-1), and FCFF (64.110 µg.mL-1) indicated their considerable radical scavenging action against DPPH. Among the tested compounds, the IC_{50} value of triazole thione (TZ) was found to be 32.364 $\mu g.mL^{-1}$ which is closer to the ascorbic acid sample.

Conclusion

The Schiff's bases and benzimidazole derivatives containing 1,2,4-triazole moiety has centered itself in the field of research in recent times because of their potential utility in medicinal chemistry, agriculture, corrosion science, complex chemistry and chemical synthesis. The Schiff's bases 4 - ((4 - chlorobenzylidene)amino) - 3 - (2 - hydroxyphenyl) - 1*H* - 1, 2, 4 - triazole - 5(4*H*) - thione**(5a)** and 4 - ((furan - 2 - ylmethylene)amino) - 3 - (2 - hydroxyphenyl) - 1*H* - 1, 2, 4 - triazole -5(4*H*) - thione**(5b)** as well as the benzimidazole derivatives 2 - (5 - ((1*H* - benzo[d] imidazol - 2 - yl) thio) - 4 - ((4 - chlorobenzylidene) amino) - 4*H* -1, 2, 4 - triazol - 3 - yl) phenol **(6a)** and 2 - (5 - ((1H - benzo[d]imidazol - 2 - yl) thio) - 4 - ((furan - 2 - ylmethylene) amino) - 4*H* - 1, 2, 4 - triazol - 3 - yl) phenol **(6b)** were successfully prepared in lab. The compounds were characterized by spectroscopic methods such as UV-Vis, IR, ^{1}H-NMR and ^{13}C- NMR. Moderate activity was displayed by the compounds against the tested bacterial strains.The Schiff's base **5a** was more effective than compound **5b** against tested bacterial strains.

The compound 4-amino-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazole-5(4*H*)-thione(**4**) showed good antioxidant property with IC_{50} value of 32.364 $\mu g.mL^{-1}$ which is comparable to that of Ascorbic acid (28.546 $\mu g.mL^{-1}$). Schiff's bases and benzimidazole derivatives showed good to moderate antioxidant properties. This is due to the presence of -OH group in the triazole derivative. For the toxicity assay, all compounds were found toxic against the brine shrimp larvae. Among them, compound FCPC is found comparatively more toxic with LC_{50} value of 26.827 $\mu g.mL^{-1}$ and highest death percentage.

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