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Research Article

STRUCTURE-ACTIVITY RELATIONSHIP STUDY: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL INVESTIGATION OF SCHIFF BASES DERIVED FROM 2-AMINOPHENOL AND 4-HALOACETOPHENONES

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ABSTRACT

Two biologically active Schiff bases (imines) **4-5** were synthesized by the reaction of 2-aminophenol **1** with 4-chloroacetophenone **2** or 4-hydroxyacetophenone **3** in the presence of conc. H_2SO_4. The characterization of Schiff bases were carried out by using spectroscopic techniques including IR, ^{1}H-NMR, EI-MS along with elemental analyses. The Schiff bases were checked for biological screening and found that the compound with -OH group to be more biologically active than the compound with halo (-X) group. The Schiff base **5** is a potent antioxidant agent as well as *α*-glucosidase inhibitor. The Schiff bases **4-5** also have excellent antibacterial activity for strains; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* while moderate for *Salmonella typhi* and *Pseudomonas aeruginosa,* against gentamicin as standard drug.

Keywords: Schiff Base, Structure-Activity Relationship, Potent Antioxidant Agent, *α*-Glucosidase Inhibitor, Lipoxygenase Inhibition Activity, Urease Inhibition Activity.

INTRODUCTION

Many Schiff bases are known to be medicinally important and used to design medicinal compounds [1]. Schiff bases are the compounds which possess an azomethine linkage (Fig. 1) and are found to attribute various pharmacological activities like antibacterial[2], antifungal[3], antimalarial[4], antimicrobial[5], antiviral[6], anticancer[7], antitubercular[8] and antiinflammatory activity. They also serve as a back bone for the synthesis of various heterocyclic compounds. The use of schiff bases as acid base indicators has also been observed. In this connection, sulfanilamide was condensed with *p*-dimethylaminobenzaldehyde and it was found that the reagent solution showed a reproducible change in its color upon the addition of base and acid[9]. A large number of reports on the preparation and reaction applications of such compounds are published every year[10]. As a result, a new series from this class of compounds (*i.e.* hydrazones) is utilized in metal coordination chemistry for the formation of metal complexes[11]. The enantioselective cyclopropanation of styrenes is carried out by using Schiff bases and their metal complexes[12]. The imine groups (-RC=N-) are usually achieved by the condensation of active carbonyl compounds with primary mono and diamine in methanol or any other suitable solvent. In addition to that, a large variety of transition metal complexes based on hydrazones have been prepared due to the involvement of hydrazone chemistry in the coordination complexes and their increased biological significance[13]. These complexes can behave as electroluminescent materials like schiff base complexes of Zn (II) which are being used these days[14]. It was found that the preparation of potentiometric sensors for determining cations and anions involves the use of Schiff bases as carrier compounds[15]. The use of schiff bases as corrosion inhibitors for mild steel and similarly copper, zinc and aluminum has also been observed[16]. Moreover, interesting photophysical properties are exhibited by Schiff base compounds and their complexes17. Schiff bases are also employed as protective groups in organic synthesis for amino groups18.

Antioxidant

A number of activated oxygen forms are produced during metabolism and include reactive oxygen species (ROS), like non free radical species such as H2O2, free radicals such as anion radicals (O2-), hydroxyl radical (OH•) and singlet oxygen (1O2) species19, and etiology and pathophysiology of human aging is associated with these species20 and is the major reason of, cancer, Alzheimer's disease, coronary heart disease21, cataracts and inflammation, neurodegenerative disorders, and atherosclerosis22. Antioxidants are the species which protect human body from oxidation by reacting with active oxygen species. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are among the most commonly used antioxidants.

Urease

The study of enzyme inhibition is the most significant area of pharmaceutical research because a number of useful drug discoveries for a variety of physiological conditions were based on such studies. The activity of enzymes is blocked by the interaction of various enzyme inhibitors. The potent antiulcer drugs are based upon enzyme inhibitors in recent days. Sardonically, the first crystallized enzyme was urease but no clear picture of its mechanism of action was reported in literature. The broad classification of urease inhibitors includes two categories such as: (i) Inhibitors directed by active site (substrate-like), (ii) Inhibitors directed by mechanism. Only few inhibitors having binding mode analogous to urea can inhibit urease due to its high substrate (urea) specificity. The enzyme-inhibitor complex is stabilized by various non-covalent interactions like hydrogen bonding and hydrophobic interactions of inhibitors with the enzyme. The functional groups having electronegative atoms like nitrogen, oxygen and sulfur etc. are among the reported functional groups which serve as bidentate in most of the cases and tridentate in few cases, or as ligand-chelator to form distorted octahedral complexes with nickel ion in the enzyme. The presence of bulky groups in the the pharmacophore causes a decrease in the activity of inhibitors and the absence of bulky groups results in easy entry of urease inhibitors into the substrate binding pockets of enzyme. Moreover, the lack of bulky groups also decreases the chances of unfavourable steric interaction of inhibitor with the amino acid residues of enzyme23.

Lipoxygenase

A family of non-haeme iron-containing dioxygenases, called lipoxygenases (LOXs) which have been considered to serve a major role in the pathophysiology of several inflammatory and allergic diseases, are the key enzymes involved in the biosynthesis of leukotrienes. The oxygenation catalyzed by LOXs produces some moities like hydroperoxyeicosatetraenoic acids (HPETE), hydroxyeicosatetraenoic acids (HETE), leukotrienes and lipoxins , which are apparently taking part in rheumatoid arthritis, psoriasis, asthmatic responses and glomerular nephritis24.

α-Glucosidase

This enzyme has a crucial role in diabetes, viral infection and cancer and involved in the synthesis and breakdown of carbohydrates. The diverse bioactivities of α-glucosidase enable this enzyme as a preferred drug target in the field of pharmaceuticals. A large variety of α-glucosidase inhibitors have been known so far and different aspects have been studied. The routinely used antidiabetics cause a delay in the hydrolysis of carbohydrates and increase postprandial hyperglycemia because the clinically used anti-diabetic agents like acarbose25, voglibose and miglitol26, are the major cause of competitive α-glucosidase inhibition in the brush border of the small intestine. The adverse effects, such as abdominal discomfort, diarrhoea, flatulence27, and hepatotoxicity28 have been reported as adverse effects of the continuous administration of such agents. Therefore, the need for discovering and developing novel α-glucosidase inhibitors is still prevailing. The therapeutic challenges of type 2 diabetes mellitus are very significant in this regard29.

Antibacterial

The abundantly found Gram-positive organism, Bacillus subtilis present in soil and vegetation is considered as a very significant cause of ropiness. Another Gram-positive, facultative anaerobic coagulase-positive catalase-positive potential pathogen Staphylococcus aureus is found in soft tissue, skin, endovascular, bone joint, and wound infections. Life-threatening diseases like pneumonia, toxic shock syndrome and sepsis are also caused by Staphylococcus aureus along with a number of illnesses as minor skin infections like cellulitis folliculitis and scalded skin syndrome. The greatly feared strain is methicillin-resistant S. aureus which has caused resistance against most widely used antibiotics. The Gram-negative non-sporulating bacterium Escherichia coli is frequently found in warm-blooded organisms, especially in the area of lower intestine. Several serotypes are the major cause of bacterial infections including cholecystitis, pneumonia, cholangitis, bacteremia, urinary tract infection (UTI), bacteremia,vomiting, bloody diarrhea and food poisoning but most of them are harmless. Salmonella typhi is a Gram-negative, facultative anaerobic pathogen and causes the typhoid or enteric fever, which causes 60 thousands deaths and affects 17 million people annually in the world. The gram-negative rod shaped Pseudomonas aeruginosa is facultative anaerobic pathogen and causes lungs infection and other infections associated with urinary tract, and kidneys.

MATERIALS AND METHODS

Reagent grade solvents and chemicals were purchased from Merck which were used without further purification. Washing of all the glassware during the reaction was accomplished by using distilled water and drying was carried out at 110 °C.

Physical measurements

The melting points of the synthesized compounds were determined by Gallenkamp melting point apparatus and are uncorrected. Elemental composition was determined by Perkin-Elmer 2400 Series II elemental analyzer. For IR spectra, Thermo Nicolet Avatar 320 FT-IR spectrometer within 400-4000 cm^{-1} range was used by employing KBr disc method. The recording of El-MS spectra was conducted by electron impact mode on Finnigan MAT-112 spectrometer (Finnigan, Waltham, MA, USA) and *m/z* (%) of $[M]^+$ ions reported. Pre-coated silica gel G-25-UV_{254} plates (E-Merck) were utilized for checking the purity of compounds by TLC method. The compounds were dissolved in DMSO-d_6 for the measurement of ^{1}H-NMR spectra on Bruker AMX-400 spectrometer. The values for chemical shift (δ) are given in ppm, while employing TMS as internal standard and the data of scalar coupling constants (*J*) is presented in Hertz.

Synthesis of Schiff bases 4-5

2-Aminophenol **1** (0.01 mol in 50 mL EtOH) was mixed with 4-chloroacetophenon **2** (0.01 mol in 50 mL EtOH) or 4-hydroxyacetophenone **3** (0.01 mol in 50 mL EtOH) and the reaction mixture was refluxed for 3 h with stirring at 70 °C after adding 3-4 drops of conc. H_2SO_4. Then the mixture was concentrated and excess solvent was evaporated for diminution of its volume to one third, by using rotary evaporator. The solid products were obtained by placing the reaction mixture at ambient temperature. The products, thus obtained, were washed with cooled methanol after filtration and recrystallized with absolute methanol after drying. Anhydrous calcium hydroxide at reduced pressure was used for drying purpose. The completion of reaction was monitored by taking TLC after certain intervals of time.

2-[1-(4-Chlorophenyl)ethylideneamino]phenol 4

Black solid; yield, 72.87 %; mp, 173 ºC; IR (KBr) ν_{max} (cm^{-1}): 3356 (C-OH), 3066 (C-H), 1686 (C=N), 1589 (C=C), 827 (C-Cl); ^{1}H-NMR (DMSO-$_6$*d*, 400 MHz) δ: 7.95 (2H, d, *J* = 8.4 Hz, H-10, -14), 7.58 (2H, d, *J* = 8.4 Hz, H-11, -13), 6.71-6.75 (2H, m, H-3, -5), 6.58-6.62 (2H, m, H-2, -4), 2.49 (3H, s, H-15); EI-MS *m/z*: 245.3 $[M]^+$; Anal. Calcd. for $C_{14}H_{12}ClNO$: C, 68.44; H, 4.92; N, 5.70. Found: C, 68.30; H, 5.03; N, 5.78.

2-[1-(4-Hydroxyphenyl)ethylideneamino]phenol 5

Dark black solid; yield, 69.02 %; mp, 169 ºC; IR (KBr) ν_{max} (cm^{-1}): 3304, 3376 (C-OH), 3057 (C-H), 1601 (C=N), 1511 (C=C); ^{1}H-NMR (DMSO-$_6$*d*, 400 MHz) δ: 7.80 (2H, d, *J* = 8.4 Hz, H-10, -14), 6.93 (2H, d, *J* = 8.4 Hz, H-11, -13), 6.72-6.77 (2H, m, H-3, -5), 6.62-6.65 (2H, m, H-2, -4), 2.23 (3H, s, H-15); EI-MS *m/z*: 227.5 $[M]^+$; Anal. Calcd. for $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16. Found: C, 74.14; H, 5.91; N, 6.19.

Biological Assays

Antioxidant: DPPH radical scavenging assay[31]

The solution of DPPH (0.3 mM) was prepared in ethanol. 5 µL methanol solution of each sample of different concentration (5-500 µg) was mixed with 95 µL of DPPH solution in ethanol. The mixture was then dispersed in 96 well plate and incubated at 37 °C for 30 min, then absorbance was measured at 515 nm by microtitre plate reader (Spectramax plus 384 Molecular Device, U.S.A.). BHA is used as standard. The percent radical scavenging activity was determined in comparison to the methanol treated control with the following formula:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Absorbance of control (DMSO treated)} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The IC_{50} value of the compounds was determined by monitoring the effect of different concentrations (1-1000 µM). The IC_{50} of the compounds were calculated using EZ-fit enzyme kinetic program (Pellera Scientific Inc. Amherst, U.S.A).

Urease inhibition assay

The urease enzyme solution was prepared by taking 0.125 units in each well in phosphate buffer ($K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Each well was filled with 80 µL of 0.05 M potassium phosphate buffer (pH 8.2), 10 µL of the test compound (concentration range 5 - 500 µM), contents were mixed and incubated for 15 min at 30 °C. 40 µL of substrate solution (urea) (50 mM) was added in each well for initiating reaction. Then, 70 µL alkaline reagent (0.5 % NaOH and 0.1 % active NaOCl) and 40 µL of phenol reagent (1% phenol and 0.005 % w/v sodium nitroprusside) were introduced to each well. The reaction mixture containing well plates were incubated for 50 minutes and absorbance was recorded at 630 nm. IC_{50} values were determined by monitoring the effect of increasing concentrations of test compounds on extent of inhibition[31].

Lipoxygenase inhibition assay[32]

All the chemicals including linoleic acid and lipoxygenase (EC 1.13.11.12) purchased from Sigma (St. Louis, Missouri, USA). 160 µL

of 100 mM sodium phosphate buffer (pH 8.0) and 10 µL of test compound solution in methanol (of concentrations 5-500 µM) was added in each well. 20 µL of lipoxygenase (LOX) solution (enzyme 130 units per well) was added, mixed and incubated for 10 min at 25 ˚C. The reaction was then initiated by the addition of 10 µL substrate solution (linoleic acid, 0.5 mM, 0.12 % w/v tween-20 in ratio of 1:2) in each well. The absorption changed with the formation of (9*Z*,11*E*)-13*S*)-13-hydroperoxyo-ctadeca-9,11-dienoate and was measured after 15 min at 234 nm. Baicalein was used as standard and IC_{50} values were determined by EZ-fit enzyme kinetic program (Pellera Scientific Inc. Amherst, U.S.A).

α-Glucosidase inhibition assay

The inhibitory activity of α-glucosidase was determined by modification of the previously reported method[33]. The inhibition assay was performed in 96 well microplate in a total volume of 100 µL. Standard solutions of the inhibitors were prepared in methanol. α-Glucosidase (from *Saccharomyces cerevisiae*) and *p*-nitrophenyl α-D-glucopyranoside (pNPG) as substrate were prepared in 0.07 M phosphate buffer (pH 6.8). The assay mixture was initially comprised of inhibitor solution (10 µL), buffer (70 µL) and 0.25 unit/mL enzyme solution (10 µL). This mixture was pre-incubated at 37 °C for 5 min. After pre-incubation, *p*-nitrophenyl glucopyranoside (pNPG) (10 µL) was added as a substrate at 1 mM end concentration start the enzymatic reaction. The reaction mixture was incubated at 37 °C for 30 min and enzymatic reaction was stopped by adding 80 µL of 0.2 M Na_2CO_3. Negative control contained 10 µL of methanol instead of inhibitor. Acarbose was used as a positive control. The α-glucosidase activity was determined by measuring the amount of *p*-nitrophenol released from pNPG at 405 nm. The % inhibition was calculated by following equation:

$$\% \text{ Inhibition} = \left[1 - \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right)\right] \times 100$$

IC_{50} values were calculated using non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA). The IC_{50} value was defined as the concentration of α-glucosidase inhibitor that inhibited 50% of α-glucosidase activity.

Antibacterial assay

Antibacterial activities of compounds **4-5** was carried out against Gram-positive and Gram-negative bacteria viz. *B. subtilis, S. aureus, E. coli, S. typhi* and *P. aeruginosa* bacteria by modified agar well diffusion method using Mueller Hinton agar medium[34]. Each compound (200 mg) was dissolved in 10 ml 99.9 % dimethyl sulfoxide (DMSO) to get the concentration of 20 mg/ml. Test organism were grown individually in tryptic Soya broth for overnight and subsequently mixed with physiological saline until turbidity standard 10^8 Colony Forming Unit (CFU) per ml was achieved. Molton Mueller Hinton agar medium was seeded for individual organism with 10 ml of prepared inoculums (inoculum size was 10^8 cells/ml as per McFarland standard) and after proper homogenization, it was poured into 20×100 mm petri dishes. After solidification, required numbers of wells were made in the seeded plates with help of a sterile crock-borer (8.0 mm). The test compound (100 µl) was introduced into respective well. Positive control (gentamicin 0.3%) and negative control (DMSO) was also applied in each plate then all the plates were incubated at 37 ˚C for 24 h. Antibacterial activity was determined by measuring the diameter of the zone inhibition.

RESULTS AND DISCUSSION

Chemistry and characterization

The condensation of 2-aminophenol **1** with 4-chloroacetophenone **2** or 4-hydroxyacetophenone **3** (Fig. 2) resulted in the formation of Schiff bases **4-5**. The success of reaction was monitored by taking TLC periodically. The sharp melting points of synthesized Schiff bases **4-5** and their stability in air indicated that they are almost pure. The molecular formula of synthesized compounds **4-5** was found in complete harmony with that of elemental analysis data for the Schiff bases **4**-**5**. The spectrometric techniques like IR, ^{1}H-NMR and EI-MS were employed for the assignment of structures to the Schiff bases **4**-**5**.

Infrared spectroscopy

The IR spectra of compounds **4**-**5** showed two peaks at 1686 and 1601 cm^{-1} which were attributed to the presence of azomethine (-C=N-) linkage, respectively and confirmed the formation of schiff bases **4-5**.

^{1}H-NMR spectroscopy

The proton NMR spectrum of the compound **4** showed a doublet of two protons at 7.95 ppm for H-11 and H-13 which was due to the presence of H-10 and H-14 at ortho position and are reflected by the doublet at 7.58 ppm. Two multiplets were observed at 6.71-6.75 and 6.58-6.62 ppm due to H-2, H-3 and H-4, H-5, respectively. The presence of methyl group on the carbon atom of azomethine linkage was observed at 2.49 ppm as a singlet. ^{1}H-NMR spectrum of compound **5** was analogous to compound **4** and it showed a doublet of two protons at 7.80 ppm for H-11 and H-13 which was due to the presence of H-10 and H-14 at ortho position and are reflected by the doublet at 6.93 ppm. Two multiplets were observed at 6.72-6.77 and 6.62-6.65 ppm due to H-2, H-3 and H-4, H-5, respectively. The presence of methyl group on the carbon atom of azomethine linkage was observed at 2.23 ppm as a singlet.

Mass spectroscopy

EI-MS spectra of Schiff bases **4**-**5** showed two peaks at *m/z* 245.3 and 227.5 which represents the molecular ion peak of compounds **4-5**, respectively. The data was in complete concurrence with the theoretical masses of compounds **4**-**5**.

Microanalyses

The elemental analyses data was also supported the molecular weight of Schiff bases **4-5** and confirmed the structures of Schiff bases **4**-**5**, being elucidated.

Biological studies

The biological studies like antioxidant, urease, lipoxygenase and α-glucosidase inhibition activities were studied for the compounds **4-5**. The outcome of biological studies is presented in Table 1.

Antioxidant study

The radical scavenging abilities of compounds was determined making use of a stable radical, 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$). This test is representative of the ability of compounds to scavenge free radicals independently from any enzymatic activity.

Recently synthesized compound **5** was found as a potent antioxidant agent (IC_{50} 45.3) but compound **4** was found to show antioxidant activity to a significant level (IC_{50} 109.2) as compared to standard, BHA (IC_{50} 44.2). The reason for the antioxidant capacity of the compound **4-5** can be explained by looking into the structure of compounds **4-5**. It is well known that the compounds with structures containing one or more functional groups such as –OH, –SH, –COOH, –N, –S–, –O– can show antioxidant activity. The potent activity of the new synthesized compound **5** is believed to be due to two hydroxyl groups as compared to compound **4**, which has chloro group at C-12 as a replacement for hydroxyl. The results clearly pointed out that the active hydroxyl group appears to induce the activity of the compound **5** and it act as good radical scavenger.

Urease inhibition study

The urease (EC 3.5.1.5) is a protein and found in bacteria, yeast, higher plants and exceptional in *Helicobacter pylori*. Many gastrointestinal or urinary tract pathogens also produce urease. It is a nickel-enzyme, which catalyzes the hydrolysis of urea to ammonia and carbamate, which decomposes to ammonia and carbonic acid, consequently pH is increased. It causes the gastric ulceration, urinary stone formation, pyelonephritis and other dysfunctions[35]. The urease inhibition activity was found to be non-significant for Schiff bases **4-5**.

Lipoxygenase inhibition study

The lipoxygenase (EC 1.13.11.12) is an iron-enzyme that catalysis many reactions of xenobiotic metabolism and also catalyze the polyunsaturated fatty acid or lipids into leukotrienes, which plays an important role in the pathophysiology of several inflammatory

diseases. Many compounds have been identified which inhibit the biosynthesis of leukotrienes. Lipoxygenases (LOX's) are sensitive to antioxidants and the most of their action may consist in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipidperoxy-radicals formed in course of enzymic peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX. The results obtained showed, the significant lipoxygenase inhibition for compound **5** (IC_{50} 79.2) and moderate for compounds **4** (IC_{50} 79.2) in contrast to Baicalein, a standard lipoxygenase.

α-Glucosidase inhibition study

α-Glucosidase (EC 3.2.1.20) hydrolyzes terminal non-reducing 1-4 linked α-glucose residues to release a single α-glucose molecule. The hydrolysis of glucosidic linkage catalyzed by every carbohydrate-hydrolase is a reaction in which the product retains or inverts the anomeric configuration of the substrate. The substrate selectivity of α-glucosidase is due to subsite affinities of the enzyme's active site. α-Glucosidases were suggested to be grouped into two families by their primary structures. The catalytic reaction mechanisms of carbohydrate-hydrolases were discussed in the two significant models of a nucleophilic displacement mechanism and an oxocarbenium ion intermediate mechanism. The synthetic analog with dihydroxyl groups exhibited much higher inhibitory activity with lower IC_{50} values than others, the inhibitory effects of compound **5** (IC_{50} 30.0) was lower than that of standard acarbose (IC_{50} 39.0). Comparison among compounds **4-5**, the introduction of chloro group at the C-12 position led to the lower activity than that of hydroxyl group. From the results, it was confirmed that the hydroxyl groups could form a more tight interaction with α-glucosidase to exert more potential inhibitory activities as compared to halo groups[36].

Anti-bacterial study

The anti-bacterial activity of synthesized compounds was determined by employing the bacterial strains as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* and taking gentamicin drug as a standard (Table 2). The significant anti-bacterial activity of compounds **4-5** were observed against the *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* which was attributed to binding of hydroxyl group with bacteria[37], however, moderate activity was observed for the *Salmonella typhi* and *Pseudomonas aeruginosa.* The variation in the effectiveness of these compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells. Although the exact biochemical mechanism is not completely understood, the mode of action may involve various targets in the microorganisms. These targets include the following: (i) Interference with the synthesis of cellular walls, causing damage that can lead to altered cell permeability characteristics or disorganized lipoprotein arrangements, ultimately resulting in cell death. (ii) Deactivation of various cellular enzymes that play a vital role in the metabolic pathways of these microorganisms. (iii) Denaturation of one or more cellular proteins, causing the normal cellular processes to be impaired. (iv) Formation of a hydrogen bond through the azomethine group with the active centers of various cellular constituents, resulting in interference with normal cellular processes[38].

Effect of azomethine (-C=N-) group

The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group (>C=N-) with the active centers of cell constituents, resulting in interferences with the normal cell process[39].

Structure-Activity Relationship

The results of biological studies revealed that the compounds with hydroxyl groups as a substituent, biologically more active as compared to compounds having substituent of halogens (-X). In the present study, the compounds **5** has two hydroxyls groups (*i.e.* at C-1 and C-12) while compound **4** has one hydroxyl at C-1 and one chloro at C-12. Hence, compound **5** is more biologically active in relation to compound **4** as well as standards.

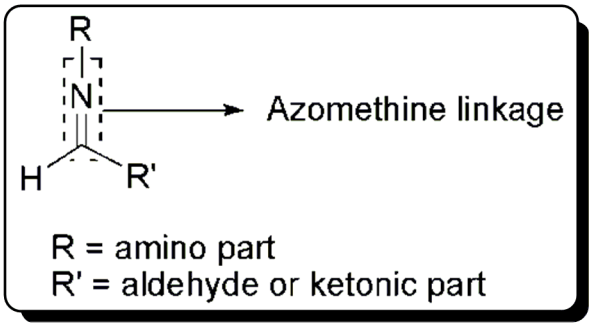

Fig. 1: General representation of the structure of a Schiff base.

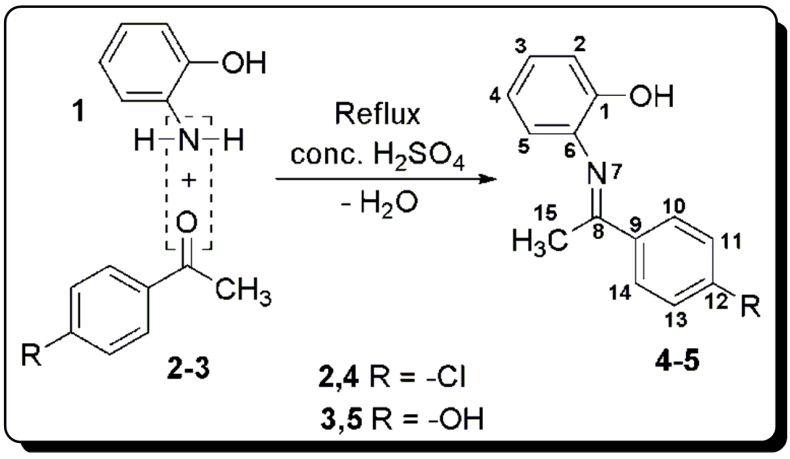

Fig. 2: The condensation reaction for synthesis of Schiff bases 4-5.

Table 1: IC_{50} (µM) values for Schiff bases 4-5 in the antioxidant, urease, lipoxygenase and α-glucosidase assays.

Compound	DPPH Scavenging Activity IC_{50} (µM)	Urease Inhibition Activity IC_{50} (µM)	Lipoxygenase Inhibition Activity IC_{50} (µM)	α-Glucosidase Inhibition Activity IC_{50} (µM)
4	109.2	+	121.5	+
5	45.3	+	79.2	30.0
BHA	44.2	-	-	-
Baicalein	-	-	22.6	-
Thiourea	-	21.6	-	-
Acarbose	-	-	-	39.0

(+, Non-significant)

Table 2: % Inhibition values for Schiff base 4-5 in the antibacterial assay.

Bacteria	Gentamicin (0.3%)	4	5
	Zone inhibition (mm)	Zone inhibition (mm)	Zone inhibition (mm)
B. subtilis	36	26	32
S. aureus	25	16	17
E. coli	22	11	16
S. typhi	27	13	15
P. aeruginosa	30	13	14

CONCLUSION

From the above mentioned discussions, it was found that the compounds with -OH group to be more biologically active than the compounds with halo (-X) group. The Schiff base **5** is a potent antioxidant agent as well as *α*-glucosidase inhibitor and the Schiff bases **4-5** also have great antibacterial activity for strains; *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* using gentamicin as standard drug.

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