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Research Article

CONVENIENT ONE-POT SYNTHESIS, ANTIOXIDANT AND UREASE INHIBITION ACTIVITIES OF SCHIFF BASES DERIVED FROM 2-AMINO-5-CHLOROBENZOPHEONONE AND MONOHALOBENZALDEHYDES

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Email: maslamchemist@hotmail.com. ***Dedicated to Dr. Zahra Noreen on the occasion of her birthday.***

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ABSTRACT

Two Schiff bases, {5-Chloro-2-[(2-chlorobenzylidene)-amino]phenyl}(phenyl)-methanone **(4)** and {2-[(3-Bromobenzylidene)amino]-5-chlorophenyl}(phenyl)methanone **(5)**, have been synthesized by the condensation of halobenzaldehydes **(1-2)** with 2-amino-5-chlorobenzopheonone **(3)**. Their structures were characterized by spectroscopic data including ^{1}H-NMR, IR, FAB-MS as well as elemental analyses. Compound **(5)** was further confirmed with X-ray crystallography. The products showed the moderate antioxidant and urease inhibition activities.

Keywords: Schiff base; Synthesis; ^{1}H-NMR; Antioxidant activity; Urease inhibition activity.

INTRODUCTION

The most intensifying and thriving division of science is the synthetic organic chemistry. During the past decades, it has been seen that the synthetic organic chemistry has gigantic growth, not only in terms of progress of unique methodologies for creation of carbon-carbon and carbon-hetero atom bonds but also in terms of improvement of latest reagents, strategies, catalysts, transformations [1] and expertise. The literature survey showed that Schiff bases have engaged in recreation and development of the synthetic chemistry. It is also well recognized that the azomethine functional group containing compounds have a broad range of biological activities such as anti-bacterial, anti-fungal [2], anticancer [3], anti-tuberculoses [4], anti-viral [5], anti-inflammatory [6], anti-HIV [7], insecticidal [8], anti-depressant [9], analgesic [10], anticonvulsant [11] and plant growth inhibitors [12] as well as have strong ability to form complexes. Due to good and versatile biological activities of Schiff bases, we have synthesized and reported a number of Schiff base compounds [13]. Herein, we report the synthesis and characterization of two new Schiff bases named {5-chloro-2-[(2-chlorobenzylidene)-amino]phenyl}(phenyl)-methanone (**4**) and {2-[(3-bromo-benzylidene)amino]-5-chlorophenyl}(phenyl)methanone (**5**). The target compounds showed the moderate antioxidant and urease inhibition activities.

MATERIALS AND METHODS

All the chemicals and solvents were purchased from E. Merck and used as obtained. TLC was performed on pre-coated silica gel G-25-UV254 plates (E. Merck), and detection was carried out at 254 and 366 nm. The IR spectra were recorded on Thermo Nicolet Avatar 320 FTIR spectrometer using KBr pellets. Melting points were recorded on a Gallenkamp apparatus and are uncorrected. Elemental analyses were performed on Perkin Elmer 2400 Series II elemental analyzer. The FAB mass spectra were recorded on JEOL SX102/DA-6000 mass spectrometer using glycerol as matrix and ions are given in *m/z* (%). The ^{1}H NMR spectra were recorded on a Bruker AMX-400 spectrometer in DMSO-d_6. The chemical shifts (δ) are given in ppm, relative to tetramethylsilane as an internal standard, and the scalar coupling constants (*J*) are reported in Hertz.

General Procedure for Synthesis of Schiff Bases (4-5)

The mixture of solutions of monohalobenzaldehyde (**1** or **2**) (0.01 mol in 50 ml ethanol) and 2-amino-5-chlorobenzophenone **(3)** (0.01 mol in 50 ml ethanol) along with 3-4 drops of conc. H_2SO_4 was refluxed with stirring at 70 ˚C for 5 hours. After cooling, the mixture was concentrated to one third of its volume under reduced pressure. In case of **(4)**, the concentrated mixture cold on ice water bath after adding acetone, kept at room temperature and yellow crystals were obtained after three days. In case of **(5)**, the concentrated mixture kept at room temperature for 5 days and orange red crystals were obtained. The crystalline product was collected, washed with methanol, dried and recrystallized with methanol. The recrystallized product was dried over anhydrous calcium hydroxide under the reduced pressure. The reaction was examined by TLC with time to time till completion.

{5-Chloro-2-[(2-chlorobenzylidene)-amino]phenyl}(phenyl) methanone (4)

Yellow crystalline solid; Yield: 79%; m.p: 103-105 ˚C; IR (KBr) ν_{max} cm^{-1}: 3061 (C-H), 1698 (C=O), 1653 (C=C), 1604 (C=N), 747, 700 (C-Cl); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.76 (1H, s, H-1'''), 7.70 (1H, d, *J* = 8.4 Hz, H-3), 7.68 (1H, dd, *J* = 8.0, 2.4 Hz, H-3''), 7.66 (1H, m, H-6''), 7.57 (1H, ddd, *J* = 8.4, 8.0, 2.4 Hz, H-4''), 7.54 (1H, d, *J* = 1.6 Hz, H-6), 7.50-7.43 (5H, m, H-2' - H-6'), 7.36 (1H, dd, *J* = 8.4, 1.6 Hz, H-4), 7.24 (1H, ddd, *J* = 8.4, 8.0, 2.4 Hz, H-5''); FAB-MS (+ve): *m/z* 354 $[M+H]^+$ (calcd for $C_{20}H_{14}Cl_2NO$, 354.0); Elemental analysis: found C 67.84, H 3.86, N 3.89; calcd C 67.81, H 3.70, N 3.95.

{2-[(3-Bromobenzylidene)amino]-5-chlorophenyl}(phenyl) methanone (5)

Orange red crystalline solid; Yield: 87%; m.p: 152-154 ˚C; IR (KBr) ν_{max} cm^{-1}: 3058 (C-H), 1697 (C=O), 1634 (C=C), 1583 (C=N), 768 (C-Br), 672 (C-Cl); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.56 (1H, s, H-1'''), 8.07 (1H, t, *J* = 1.6 Hz, H-2''), 7.90 (1H, dd, *J* = 8.0, 1.6 Hz, H-6''), 7.52-7.60 (7H, m, H-2' - 6', -4'', -5''), 7.31 (1H, dd, *J* = 8.8, 2.4 Hz, H-4), 7. 16 (1H, d, *J* = 2.4 Hz, H-6), 6.89 (1H, d, *J* = 8.8 Hz, H-3); FAB-MS (+ve): *m/z* 398 $[M+H]^+$ (calcd for $C_{20}H_{14}BrClNO$, 398.0); Elemental analysis: found C 60.54, H 3.32, N 3.62; calcd C 60.25, H 3.29, N 3.51.

Assays of Biological Studies

Antioxidant: DPPH Radical Scavenging Activity

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 with 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 IC50 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 0.01M $LiCl_2$). 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 except B enzyme for initiating reaction. Then, 70 µL alkaline reagent (0.5 % NaOH and 0.1 % active NaOCl) and 40 µL of phenol reagent (1% Phenol & 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} was determined by monitoring the effect of increasing concentrations of test compounds on extent of inhibition [14].

RESULTS AND DISCUSSION

The Schiff bases **(4)** and **(5)** were synthesized by refluxing of 2-chlorobenzaldehyde (**1**) and 3-bromobenzaldehyde (**2**) with 2-amino-5-chlorobenzophenone (**3**) at 70 ˚C in ethanol followed by few drops of conc. sulfuric acid, respectively (Figure 1). The products were obtained as crystalline solids and fairly stable in air. The progress of the reactions and the purity of the products were monitored by TLC. In the IR spectra of Schiff bases **(4)** and **(5)**, the absence of absorption bands of free amine and aldehydic functionalities and the presence of frequencies of imines at 1604 and 1583 cm^{-1}, revealing the formation of Schiff bases. In the ^{1}H-NMR spectra, the presence of singlets at δ 8.76 and 8.56 of azomethine protons confirming the formation of required products. The structure of compound **(5)** was also further confirmed by X-ray crystallography [13f] (Figure 2). All the spectral data and elemental analyses were in complete agreement to the product structures (**4-5)** (Figure 1).

Biological Studies

Antioxidant: DPPH Radical Scavenging Activity

Reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals play an important role in the etiology and pathophysiology of human aging [15] and diseases such as cancer, coronary heart disease, Alzheimer's disease [16], neurodegenerative disorders, atherosclerosis, cataracts and inflammation [17]. Antioxidants fate the active oxygen and resultantly protected to oxidize the cells. Commonly, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as antioxidants.

The antioxidant activity of compounds **(4-5)** was carried out with DPPH by well diffusion method. Both compounds showed moderate activity against DPPH (Table 1).

Urease inhibition activity

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 that catalyzes the hydrolysis of urea to ammonia and carbamate, which decomposes to ammonia and carbonic acid, resulting the elevation of pH. It causes the gastric ulceration, urinary stone formation, pyelonephritis, and other dysfunctions [18]. Both the target compounds **(4-5)** showed moderate urease inhibition activity (Table 1).

Fig. 1: Synthetic reaction of the Schiff bases (4-5).

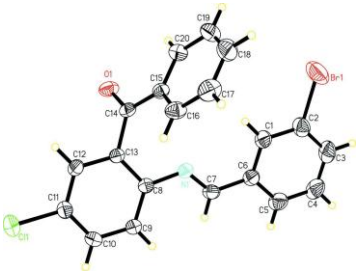

Fig. 2: X-ray crystal structure of Schiff base (5)

Table 1: Antioxidant and urease inhibition activities of Schiff bases (4-5).

Compounds	DPPH Scavenging Activity IC_{50} (µM)	Urease Inhibition Activity IC_{50} (µM)
4	142	183
5	102	156
Thiourea[a]	-	21.6
BHA[b]	44.2	-

[a]Thiourea standard for urease inhibition activity

[b]Butylated hydroxyanisole (BHA) standard for antioxidant activity

CONCLUSION

The newly synthesized Schiff bases **(4-5)** were found to have moderate antioxidant and urease inhibition activities.

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