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Synthesis and Biological Activity of a New Schiff Base Ligand Pyridazine Based

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ABSTRACT: In this work 3, 6-bis (2-[2-sulfanyl-ethylimino)-methyl]-4-(4-nitro-phenylazo)-3-metoxy-phenol) pyridazine (2) was prepared and its antibacterial properties have studied. The structures of all the newly synthesized compounds have been comfirmed by elemental analysis, IR, 1H-NMR. Anti bacterial effects of compounds (1 and 2) have been studied. Following concentrations of materials were used: a=0.005 g/ml and b=0.01 M. Study was done on two strains of *S.aureus* and *E.coli* that experiments were done in Trypticase Soy Broth (TSB) and Nutrien Agar (TSB; Difco Laboratory). It is likely that these synthetic materials exert higher antibacterial effect on gram positive bacteria in comparison with gram negative.

KEYWORDS: Schiff base , Di-azo compound , Pyridazine , Antibiotic , O-vanilin

INTRODUCTION

Schiff bases are important intermediates for the synthesis of some bioactive compounds such as β-lactams [1, 3]. Furthermore, they are reported to show a variety of interesting biological actions, including antibacterial [4-9], antifungal [4-5, 10],anti mouse hepatitis virus (MHV0 [11],inhibition of herpes simplex virus type 1(HSV-1) and adenovirus type 5(Ad 5) [12], anticancer[13,16], antimosquito larvae [17] and herbicidal activities [18]. It is also known that the presence of a chlro or an azo moiety in different types of compounds can lead them to exhibit pesticidal activity [18]. Some azo compounds were synthesized by Jolly and co-workers have shown good antibacterial activity [19]. Both Schiff bases and azo compounds are important structures in the medicinal and pharmaceutical fields [20] and it has been suggested that the azomethine linkage might be responsible for the biological activities displayed by Schiff bases [16].Azo dyes are an important class of organic colorants which consist of at least a conjugated chromophore azo (-N=N-) group and the largest and most versatile class of dyes. It has been known for many years that azo compounds are the most widely used class of dyes due to their versatile application in various fields such as the dyeing of textile fiber and coloring of different materials, and for plastics, biological-medical studies, and advanced applications in organic synthesis [21,24]. Our attentions focused on nitrogen-containing heterocycle as nitrogen atoms [25]. Its ability to coordinate all of the divalent metal ions from Mn (II) to Zn (II) [as well as to Cu (II) and Pb (II)] has led to the isolation of a wide range of interesting dinuclear, doubly pyridazine-bridged complexes [26].

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The pyridazine skeletons are commonly found in compounds exhibiting a wide range of biological activity and metallic complexes [27]. In this work, we synthesized a new Schiff base pyridazine-based ligand (Figure 1) and its antibacterial properties are studied.

MATERIALS AND METHODS

Materials

All of the compounds were synthesized following previously published procedure [28]. All other chemicals and solvents were analytically graded and used without further purification.

Physical measurements

IR spectra of the compounds were recorded as KBr pellets on an Unicom Galaxy Series FT-IR 5000 spectrophotometer (200-4000 cm^{-1}). Microanalitycal data (C, H, and N) were collected on vario EI III elemental analyzer. The structures of all synthesized compounds were confirmed by ^{1}H NMR spectra, recorded on a Bruker AV 300 MHz spectrometer. Antibacterial studies were done on two strains of *S.aureus and E.coli* that have been isolated from blood and urinary tract infections of impatients of Shahid Madany hospital, Khorramabad. Experiments were done in Trypticase Soy Broth (TSB) and Nutrien Agar (TSB; Difco Laboratory).

Synthesis of compounds

2-hydroxy-3-methoxy-5(4-nitrophenylazo) Benz aldehyde (1)

Azo-coupled O-vanilline precursor (1) was prepared according to the well-known literature procedure [29, 30]. O-Vanillin (10mmol) was dissolved in water (20 ml) containing 10mmol of sodium hydroxide and 40mmol of sodium carbonate during the period of 30 min at 0 ºC. The resulting solution was added slowly to a solution of diazonium chloride (10mmol) in water at 0-5ºC. The reaction mixture was stirred for one h at 0ºC and then allowed to warm slowly to room temperature. The product was collected by filtration and washed with 100 ml of NaCl solution (10%) under vacuum. The obtained light red solid was dried. Under vacuum at 80ºC over night. Yield: 68 %, m.p.=210-212 ^{o}C. 1H NMR (CDCl3, ppm):4.01 (3H, s) ,7.07-8.26(6H, m), 10.08 (s,1H), 11.49 (s,1H). IR (KBr, cm^{-1}); 1653(CHO), 1516(N=N), 1458(NO_2), 1340(NO_2), 1269(C-O), 1130, 848 and 742. 3, 6-bis ((aminoethyl) thio) pyridazine (PTA) the method of preparation PTA was reported in previous paper [28].

3, 6-bis (2-[2-sulfanyl-ethylimino)-methyl] - 4 - (4-nitro-phenylazo) -3-metoxy-phenol) pyridazine (2)

A solution of 3, 6-bis ((aminoethyl)thio) pyridazine (PTA) (1 mmol) in absolute EtOH (10 ml) was added to a stirring solution of compound 1 (2 mmol) in absolute EtOH during a period of 30 min at 50 ºC. The solution was heated in water bath for 2 h at 80 ºC with stirring, then cooled and let to stand at ambient temperature. The resulted dark red product was collected by filtration, washed successively with diethyl ether and dried in air. Yield: 61 %, m.p. =175 ^{o}C. ^{1}H NMR (d6-DMSO, ppm): δ 3.34 (t,4H, J= 3.37 Hz), 3.75 (t,4H, J= 6.88 Hz), 7.29 (s, 2H), 7.52 (s, 2H), 7.69 (s, 2H), 7.87 (d,4H, J=7.269), 8.30(d, 4H, J=8.44 Hz), 8.63 (s,2H), 13.26 (br,2H). IR (KBr, cm^{-1}); 1643(C=N), 1514(N=N), 1454(NO_2), 1336, 1261(C-O), 1132, 1103 and 856. Anal. Calcd for $C_{36}H_{32}N_{10}S_2O_8$:C, 54.26 ; H, 4.05 ; N, 17.58 . Found: C, 54.1 ; H, 3.9 ; N, 17.8.

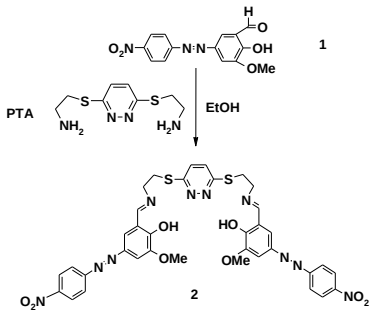

Figure 1. Structure of the synthetic co mpound

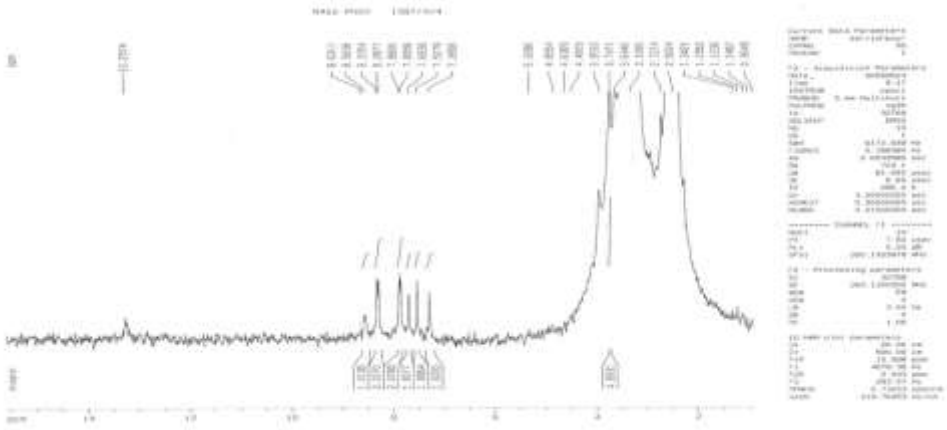

Figure 2. NMR spectera of 3, 6-bis (2-[2-sulfanyl-ethylimino)-methyl]-4-(4-nitro-phenylazo)-3-metoxy-phenol) pyridazine

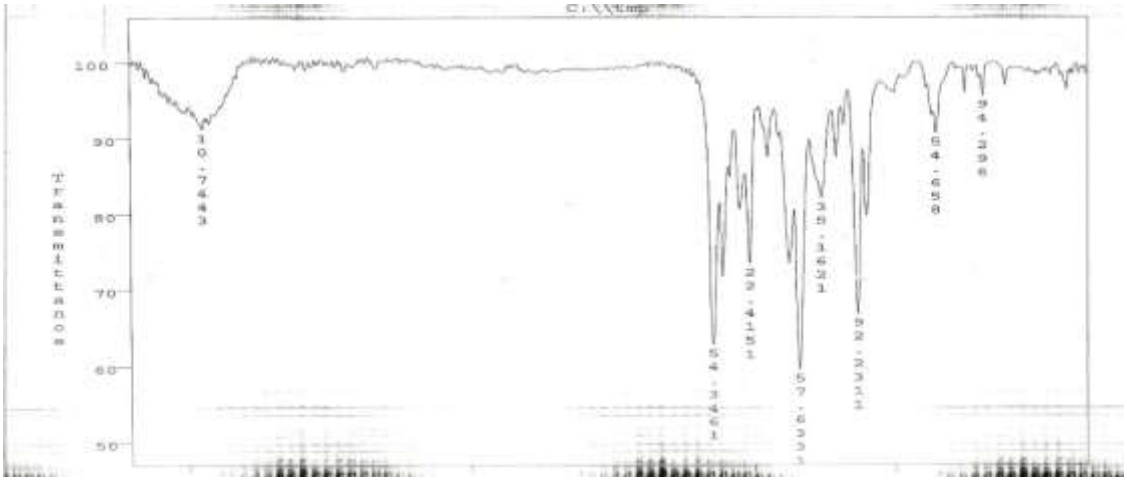

Figure 3. IR spectera of 3, 6-bis (2-[2-sulfanyl-ethylimino)-methyl]-4-(4-nitro-phenylazo)-3-metoxy-phenol) pyridazine

RESULTS AND DISCUSSION

Anti bacterial effects of compounds (1 and 2) have been studied. Following concentrations of materials were used: a=0.005 g/ml and b=0.01 M. Study was done on two strains of *S.aureus* and *E.coli* that have been isolated from blood and urinary tract infections of impatients of Shahid Madany hospital, Khorramabad. Experiments were done in Trypticase Soy Broth (TSB) and Nutrien Agar (TSB; Difco Laboratory).

Determination of MIC and MBC Bacteria were cultured in TSB, for 18 hours at 37^{o}C. A freshly 3 h culture was prepared in TSB in second day and the suspensions diluted to 0.5 McFarland turbidity. 180 µl of bacterial suspensions were inoculated in each well of microtiter plate (7 wells for each of substances). Then 20 µl of each dilution of substances (each of the substances two-fold diluted from 1, 1/2 to 1/64) were poured in serial wells. Microplates were incubated in 37^{o}C and growth of bacteria in treated dilutions was observed exactly in front of a light source (Table 1).

Based on bacteriological standard methods, the highest dilution of antibacterial that inhibited the growth of bacteria, considered as Minimum Inhibitory concentration (MIC) against corresponding bacterium. For determination of MBC, 0.01 ml of microplate wells suspension was cultured on nutrient agar for inspection of microbial growth [31] (Table 2).

Findings among 2 substances of 1 and 2 indicated MBC effect on S.aureus in its stock concentration (0.01 M). Compound of 1 had not antibacterial effect on E.coli but 2 had an effect near to MIC against E.coli in its stock concentration. Dilutions of 1=1/2 and 2=1/8 had antibacterial effects equivalent to MIC against S.aureus. Compound of 2 had higher antibacterial effect than 1. It is likely that these synthetic materials exerted higher antibacterial effect on S.aureus in comparision with E.coli. This hypothesis could be evaluated on different kinds gram positive and gram negative bacteria.

Table 1. Effect of new azo Schiff base on the growth of tested bacteria

Compound	**1**		**2**	
Bacteria **Dilution**	Gram positive S. aureus	Gram negative. E. Coli	Gram positive S. aureus	Gram negative E. Coli
1	-	-	-	-
2	-	+	-	+
3	+	+	-	+
4	+	+	-	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+

Table 2. Results of growth 0.01 M from substances for determination of MBC

Compound	**1**		**2**	
Bacteria **Dilutio**	Gram positive S. aureus	Gram negative. E. Coli	Gram positive S. aureus	Gram negative E. Coli
1	100 Coloni	300	1	300
1/2	200-300	·<100	40	·<100
1/4	High	·<100	40	·<100
1/8	High	·<100	high	·<100
1/16	High	·<100	high	·<100

The newly synthesized Schiff base ligand was very stable at room temperature in the solid state. The ligand is generally soluble inTHF, DMF and DMSO. Based on the existence of drug resistant bacteria in hospitals and economic burden of related infections on countries, production of new generation of antibacterials could be one of the important subjects in hospital acquired infection (HAI) control programs. In addition of destructive economic effects, HAIs play an important role in mortality and morbidity of hospitalized patients [32, 33]. Only in the United State of America, drug resistant bacteria infections cause 105 human deaths annually. That is equal to sum of deaths causing from 3 devastating diseases including AIDS, cancers, and heart diseases. In the future production of synthetic antibacterials could be help to infection professional control to fight against drug resistant and emerging infections. It is noteworthy that synthetic antibacterials could be producted with low cost and easy methods in comparsion with current methods.

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