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Synthesis and Characterization of Dimeric Thio-Schiff Bases by Nano Cerium Oxide and Examination of Their Antimicrobial Activities

Aslıhan DALMAZ*[1], Sefa DURMUŞ[1], Gorkem DULGER[1], Başaran DÜLGER[1]

Abstract

In this work, firstly, CeO_2 nanoparticles, which can be used as catalysts in many reactions, were synthesized by preparing aqueous solution of cerium(III) nitrate hexahydrate in basic medium. In the second step, the synthesis of dimeric thio Schiff bases was carried out using two different methods. Effect of catalyst on some parameters such as reaction time and yield of product were investigated. The antimicrobial activities of the ligands have been screened in vitro against the organisms *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* (Gram negative bacteria), *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus cereus* (Gram positive bacteria) and *Candida albicans*, *C. tropicalis*, *C. guilliermondii*, *C. glabrata* by Disc Diffusion and Microdilution methods. At the same time, antimicrobial activities of ligands were compared to standard antibiotics (Cefotaxime, Amoxicillin/clavulanic acid, Posacanazole, Nystatin and Gentamicin). Generally, the results obtained in this research showed that all tested ligands exhibited more effect towards Gram positive bacteria and *Candida* species as compared to standard antibiotics.

Keywords: Anti-bacterial, Anti-candidal, Ceria, Disulphide-Schiff bases, Nano-catalyst

1. INTRODUCTION

One of the materials that has remarkable applications in the fields of catalysis, photochemistry and materials science is cerium oxide (CeO_2). [1,2]. Ceria has the ability to undergo a simple conversion between "IV" and "III" formal oxidation states. [3]. The reactions of various ligands containing metal ions and disulfide groups can be found in the literature. In the advancing years, disulphide have been important compounds in terms of both synthetic and biological [4,5]. Besides, different types of Schiff bases have been studied due to their remarkable aspects [6,7]. Moreover, these compounds exhibit antibacterial and antifungal

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activity as well as their photochromic properties. [8-11]. In recent years, antibiotic resistance has become a major problem. The World Health Organization (WHO) has said that the spread of deadly superbugs is now a reality. Bugs have developed and have become resistant to antibiotics and other drugs [12,13]. Therefore, in recent years, all scientists have been searching for new antibiotics.

In our study, thio-Schiff bases were synthesized both without catalyst and catalyst (with CeO_2 nanoparticles) through reaction of the 2,2'-diaminodiphenyl disulfide amine compound with various aldehydes having different substituent groups. The structures of thio-Schiff bases obtained were characterized by various spectroscopic methods. In addition, we have studied antibacterial and anti-Candidal activities of thio-Schiff bases. The antimicrobial activities of compound have been screened in vitro against the human pathogens by disc diffusion methods.

2. MATERIALS AND METHODS

2.1. General

The morphology and crystal specialties of synthesized CeO_2 nanocatalyst were characterized. Ligands structures were illustrated by Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance Spectroscopy (NMR) and Mass Spectroscopy (MS). Melting points were defined with the Stuart apparatus. FTIR results were recorded by Perkin Elmer spectrometer. The average of the wave numbers was taken in the spectrum range of 550-4000 cm^{-1}. The ^1H NMR spectra were recorded $CDCl_3$ on a Bruker spectrometer λ-400 MHz. The ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on Bruker spectrometer operating at 101 MHz. All chemical shifts were reported in δ (ppm) using TMS as an internal standard. Mass spectra were obtained in a AB SCIEX 4000 Q-TRAP LC-MS/MS instrument. Elemental analyses were carried out a Thermo Scientific Flash 2000.

The compounds 2-aminothiophenol, benzaldehyde, 2-thiophen carbaldehyde, 3-methyl-2-thiophen carbaldehyde, 5-methyl-2-thiophen carbaldehyde, sodium hydroxide and solvents were purchased from Merck(Germany), and cerium(III) nitrate hexahydrate, *o*-tolualdehyde, *p*-tolualdehyde were purchased from Acros (Acros Organics NJ, USA), and 2-hydroxy-1-naphthaldehyde was purchased from Sigma-Aldrich (Germany), and CeO_2 nanoparticles were prepared according to previous literatures [13].

2.2. Chemistry

Synthesis of 2,2'-diaminodiphenyl disulfide was carried out by exposure to 2-aminothiophenol oxidation **(1)** (method B). Ligands were synthesized according to previous studies [14,15]. It has been observed that the (-S-CH_2-CH_2-S-) disulfide compounds containing two atoms of carbon-carbon ethane between two sulfur atoms have been synthesized in previous studies [16-18]. But, in this study, nano-CeO_2 catalyst (method A) was used in the synthesis of dimeric thio-Schiff bases as a new method, different from the previous study.

*2,2'-disulfanediyldianiline **(2)***

Shiny yellow solid (EtOH), mp 90–92 °C. IR (ATR): ν_{max} 3375, 1471, 744, cm^{-1}. ^{1}HNMR (400 MHz, $CDCl_3$, δ): 7.19 – 7.07 (m, 2H), 6.66 (dd, 1H,*J* = 8.5, 1.3 Hz), 6.56 (td, 1H, *J* = 7.5, 1.2 Hz), 4.31 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$,δ): 148.7, 136.9, 131.7, 118.8, 118.2, 115. MS (*m/z)*: 249.3 $[M+H]^+$. Combustion analysis for $C_{12}H_{12}N_2S_2$: Calculated. C 58.03, H 4.87, N 11.28, S 25.82; found C 58.01, H4.69, N 11.14, S 26.16.

*(NZ,N'Z)-2,2'-disulfanediylbis(N-benzylidene aniline) **(3)***

Light yellow solid (EtOH), mp 133 °C. IR (ATR) ν_{max} 1617, 1459, 761, 556 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ): 8.47 (s, 1H), 7.99 – 7.96 (m, 2H), 7.66 (dd, 1H, *J* = 7.7, 1.5 Hz), 7.48 (d, 1H, *J* = 1.8 Hz), 7.17 (dd, 1H, *J* = 7.4, 1.6 Hz), 7.14 (dd, 1H, *J* = 7.6, 1.6 Hz), 7.02 (d, 1H, *J* = 1.5 Hz). ^{13}C NMR (101 MHz, $CDCl_3$,δ): 160.05, 148.96, 135.99, 132.06, 131.76, 129.16, 128.86, 126.96,

126.02, 117.26. MS (*m/z)*: 425.5 $[M+H]^+$. Combustion analysis for $C_{26}H_{20}N_2S_2$: Calculated. C 73.55, H 4.75, N 6.60, S 15.10; found C 73.71, H 4.59, N 6.47, S 15.23.

3,3'-((1Z,1'Z)-((disulfanediylbis(2,1-phenylene)) bis(azanylylidene))bis(methanylylidene))bis(nap hthalen-2-ol) ***(4)***

Bright orange solid (EtOH), mp 210 °C. IR (ATR)ν_{max} 1610, 1462, 1278, 747, 556 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ): 14.94 (s, 1H), 9.43 (s, 1H), 8.17 (d, 1H, *J* = 8.5 Hz), 7.87 (d, 1H, *J* = 9.0 Hz), 7.79 (d, 1H, *J* = 7.8 Hz), 7.71 (dd, 1H, *J* = 7.7, 1.0 Hz), 7.55 (t, 1H, *J* = 7.2 Hz), 7.42 – 7.35 (m, 1H), 7.24 – 7.21 (m, 1H), 7.21 – 7.18 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6,δ):164.8, 158.7, 145.1, 136.1, 132.6, 129.4, 128.9, 128.6, 128.0, 127.6, 127.4, 127.2, 123.7, 120.8, 119.9, 119.0, 109.4. MS (*m/z)*: 557.53 $[M+H]^+$. Combustion analysis for $C_{34}H_{24}N_2O_2S_2$: Calculated. C 73.35, H 4.35, N 5.03, O 5.75, S 11.52; found C73.17, H4.44, N 5.12,O 5.66, S 11.61.

(NZ,N'Z)-2,2'-disulfanediylbis(N-(thiophen-2-ylmethylene)aniline) ***(5a)***

Light brown solid (EtOH), mp 166 °C. IR (ATR) ν_{max} 1604, 1459, 749, 575 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$,δ): 8.55 (s, 1H), 7.65 (dd, 1H, *J* = 7.7, 1.5 Hz), 7.53 (d, 1H, *J* = 5.0 Hz), 7.51 (d, 1H, *J* = 3.6 Hz), 7.15 (dd, 2H, *J* = 7.4, 1.7 Hz), 7.12 (dd, 1H, *J* = 3.4, 1.4 Hz), 7.01 (dd, 1H, *J* = 7.4, 1.6 Hz). ^{13}C NMR (101 MHz, $CDCl_3$,δ):152.75, 148.48, 142.78, 132.57, 132.16, 131.22, 127.80, 126.91, 126.19, 117.30. MS (*m/z)*: 437.2 $[M+H]^+$. Combustion analysis for $C_{22}H_{16}N_2S_4$: Calculated. C 60.52, H 3.69, N 6.42, S 29.37; found C 60.39, H 3.82, N 6.35, S 29.44.

(NZ,N'Z)-2,2'-disulfanediylbis(N-((3-methylthio phen-2-yl)methylene)aniline) ***(5b)***

Shiny yellow solid (EtOH), mp 165 °C. IR (ATR) ν_{max} 2872, 1607, 1454, 751, 569 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$,δ): 8.60 (s, 1H), 7.65 (dd, 1H, *J* = 7.7, 1.4 Hz), 7.25 (dd, 2H, *J* = 10.9, 9.8 Hz), 7.20 (dd, 1H, *J* = 6.0, 1.6 Hz), 7.18 – 7.12 (m, 1H), 6.86 (t, 1H, *J* = 7.5 Hz), 2.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, δ):163.01, 159.49, 134.72, 131.62, 130.36, 127.64, 127.53, 126.52, 118.78, 118.45, 117.61, 15.68. MS (*m/z)*: 465.1 $[M+H]^+$. Combustion analysis for $C_{24}H_{20}N_2S_4$: Calculated. C 62.03, H 4.34, N 6.03, S 27.60; found C 62.11, H 4.26, N 6.13, S 27.50.

(NZ,N'Z)-2,2'-disulfanediylbis(N-((5-methylthio phen-2-yl)methylene)aniline) ***(5c)***

Shiny yellow solid (EtOH), mp 194 °C. IR (ATR)ν_{max} 2872,1608, 1454, 751, 569cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$,δ): 8.47 (s, 1H), 7.64 (dd, 1H, *J* = 7.7, 1.5 Hz), 7.32 (d, 1H, *J* = 3.6 Hz), 7.16 (td, 1H, *J* = 7.4, 1.5 Hz), 7.11 (td, 1H, *J* = 7.5, 1.5 Hz), 7.01 (dd, 1H, *J* = 7.5, 1.4 Hz), 6.81 (dd, 1H, *J* = 3.5, 0.8 Hz), 2.56 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, δ): 152.61, 148.61, 146.94, 140.76, 133.12, 132.10, 126.72, 126.61, 126.34, 125. 98, 117.19, 16.07. MS (*m/z)*: 465.1 $[M+H]^+$. Combustion analysis for $C_{24}H_{20}N_2S_4$: Calculated. C 62.03, H 4.34, N 6.03, S 27.60; found C 62.09, H 4.28, N 6.08, S 27.55.

(NZ,N'Z)-2,2'-disulfanediylbis(N-(2-methyl

benzylidene)aniline) ***(6a)***

Shiny, light-colored yellow solid (EtOH), mp 141-145 °C. IR (ATR) ν_{max} 2891, 1615, 1457, 754, 573cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$,δ):8.76 (s, 1H), 8.13 (dd, 1H, *J* = 7.6, 1.4 Hz), 7.67 (dd, 1H, *J* = 7.8, 1.4 Hz), 7.40 – 7.35 (m, 1H), 7.32 (t, 1H, *J* = 6.9 Hz), 7.23 (dd, 1H, *J* = 9.2, 4.7 Hz), 7.19 (dd, 1H, *J* = 7.5, 1.5 Hz), 7.14 (td, 1H, *J* = 7.6, 1.5 Hz), 7.03 (dd, 1H, *J* = 7.6, 1.4 Hz), 2.66 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$,δ): 159.25, 149.58, 139.08, 133.93, 132.04, 131.30, 131.21, 129.21, 126.95, 126.80, 126.41, 126.00, 117.26, 19.99. MS (*m/z)*: 453.3 $[M+H]^+$. Combustion analysis for $C_{28}H_{24}N_2S_2$: Calculated. C 74.30, H 5.34, N 6.19, S 14.17; found C 74.19, H 5.45, N 6.14, S 14.22.

(NZ,N'Z)-2,2'-disulfanediylbis(N-(4-methyl benzylidene)aniline) ***(6b)***

Shiny, light-colored yellow solid (EtOH), mp158-160°C. IR (ATR) ν_{max}2915, 1623, 1460, 753, 573cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, δ): 8.41 (s, 1H), 7.85 (d, 2H, *J* = 8.1 Hz), 7.65 (d, 1H, *J*= 7.8 Hz), 7.15 (dd, 1H, *J* =7.5, 1.5 Hz), 7.12 (dd, 1H, *J* = 7.6, 1.5 Hz), 7.01 (dd, 1H, *J* = 7.5, 1.4 Hz),

2.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, δ): 160.00, 158.20, 140.70, 138.00, 133.40, 130.70, 129.10, 127.50, 126.00, 117.10, 21.30. MS (*m/z)*: 453.3 $[M+H]^+$. Combustion analysis for $C_{28}H_{24}N_2S_2$: Calculated. C 74.30, H 5.34, N 6.19, S 14.17; found C 74.22, H 5.42, N 6.12, S 14.24.

2.3. Antimicrobial Screening

Test microorganisms

The *in vitro* antimicrobial studies were carried out with three Gram-negative bacteria (*Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae*), four Gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Bacillus cereus*) and four Candida species (*Candida albicans, C. tropicalis, C. guilliermondii, C. glabrata*) obtained from the Microbiology Research Laboratory of the Duzce University Department of Biology.

Disk diffusion method

In this study, test microorganisms isolated from patients admitted to Düzce University Medical Faculty Hospital were used. Antimicrobial susceptibility testing was performed using the disc diffusion method according to the protocol applied by CLSI (Clinical and Laboratory Standards Institute) [19,20]. For this purpose, sterilized antibiotic discs (6 mm) were used. Fresh stock solutions (30 μgL^{-1}) prepared from DMSO (dimethyl sulfoxide) of ligands (**2-6b**) were used. To eliminate the solvent effect on bacterial and fungal growth, a control test was performed with DMSO-assisted test medium containing the same procedures as the ligands. All test bacteria in the study were incubated for 24 hours at 30-35 °C in Brain Heart Infusion Water (BH). Inoculums containing $1.5x10^8$ cfu/mL bacterial cells or yeast cells were spread on Mueller Hinton Agar plates (1 mL inoculums for each plate). The discs were impregnated with 50 µL of each ligands solution. Discs were placed on agar. Incubated for bacteria and yeast at 35 °C (24 hours) and 25 °C (72 hours), respectively. Concurrently, the antibacterial and anti-candidal activities of ligands **2-6b** were compared to standard antibiotics. Cefotaxime and Amoxicillin/clavulanic acid for antibacterial activity were tested against the pathogenic bacteria and Posaconazole was tested against pathogenic yeasts. All experiments were repeated three times. Average values were recorded as the last reading.

Dilution method

Based on the procedure started out in the Clinical Microbial Handbook. Following the procedure in this Handbook, a clinical micro-dilution was prepared and a screening for antibacterial and antifungal activities was performed [21]. All bacteria were incubated and activated by inoculation for 24 hours at 30 °C, yeasts were incubated in Malt Extract for 48 hours. The ligands were dissolved in DMSO (2 $mgmL^{-1}$) and diluted using carefully adjusted Mueller Hinton Broth (Oxoid). Twice-fold serial concentrations of ligands were preferred to result MIC between 200 and 1.56$mgmL^{-1}$. Cultures were grown at 37 °C (20 h). The final inoculation (inoculums) was about 106 cfu mL^{-1}. Test cultures were incubated at 37 °C (24 h). The lowest concentrations of antimicrobial agents that result in complete inhibition of the micro-organisms were represented as MIC ($mgmL^{-1}$). In each case triplicate tests were performed. The results were expressed as means.

3. RESULTS AND DISCUSSION

3.1. Chemistry

As starting material **(2)** 2,2'-disulfanediyldianiline was selected to synthesize disulphide containing thio-Schiff bases. The synthesis of thio-Schiff bases **(3-6b)** were carried out by reacting ligand **2** with several different aldehydes called benzaldehyde, 2-hydroxy-1-naphthaldehyde, 2-thiophen carbaldehyde, 3-methyl-2-thiophen carbaldehyde, 5-methyl-2-thiophen carbaldehyde, 3-methyl benzaldehyde, 5-methyl benzaldehyde, respectively. At the same time, the syntheses of these thio-Schiff bases were carried out in two ways using no catalyst and using CeO_2 nanocatalyst. The methods used to obtain the targeted ligands are shown in (Figure 1 and 2). In the reaction performed in the presence

of nanocatalyst, it was determined that the reaction rate and efficiency increased. (Table 1).

In the determination of structures of the ligands, the vibrational spectra of the ligands synthesized with the literature data were compared [22]. Some of the thio-Schiff base ligands in this work have been functional groups such as -NH_2, -OH, -CH_3. The groups -CH_3 in the structures of ligands **(5b-5c)** and **(6a-6b)** are in different positions. The characteristic peaks of $\nu(CH_3)$ in the methyl group are seen in the range of 2915-2872 cm^{-1}. Also, the sharp stretching vibrations of group ν(C=N) which is the basis of Schiff bases, are observed in the range of 1623-1604 cm^{-1} [22].

Specific peaks of $\nu(C\text{-}C_{Ar})$, $\nu(C\text{-}O_{Ar})$, ν(C-S) and ν(S-S) are observed in the range of 1471-1457, 1278, 761-744, 575-556 cm^{-1}, respectively. It is seen that the vibration values change depending on the presence of different groups such as -CH_3, -OH, -NH_2 in the structures of the ligands and the increase of the aromatics. In addition to having sharp peaks, there are differences in the intensity of functional groups depending on their ortho and para orientation. ^{1}H NMR spectra of the ligands synthesized were recorded using DMSO-d_6, $CDCl_3$ solvents, and the peaks of these solvents were determined as 7.25 and 2.51 ppm. When the ^{1}H NMR spectra of synthesized ligands are examined, there are some specific proton signals that are noted. From these, the signals of the azomethine (H-C = N) proton were observed as a proton singlet in the range of 8.41-9.43 ppm [23,24]. The protons of the hydroxy and methyl groups in the structure of some ligands were found as a singlet with chemical shifts of 14.94 ppm and 2.33-2.66 ppm, respectively. The protons belonging to the aromatic ring were observed as dublets, triplets and multiplets between 8.17 and 6.56 ppm. When the ^{13}C NMR spectra of the ligands synthesized were examined, it was found that the aromatic carbons were in the region of 110.02-159.49 ppm depending on the substituent groups [22,25]. It was also found that the carbon atoms bound to the azomethine group were found in the region 152.61-163.01 ppm, and the methyl group carbons were found in the region 15.68-21.30 ppm.

Although all the compounds were obtained with high efficiency in both methods, the yields were increased highly with nanocatalyst method, and the reaction time for several hours decreased to the minute. The authenticities of (**2-6b**) ligands were checked with mixing TLC, melting point and NMR spectrum values.

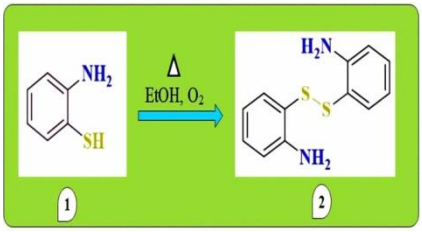


Figure 1 Demonstration of compound 2 synthesis

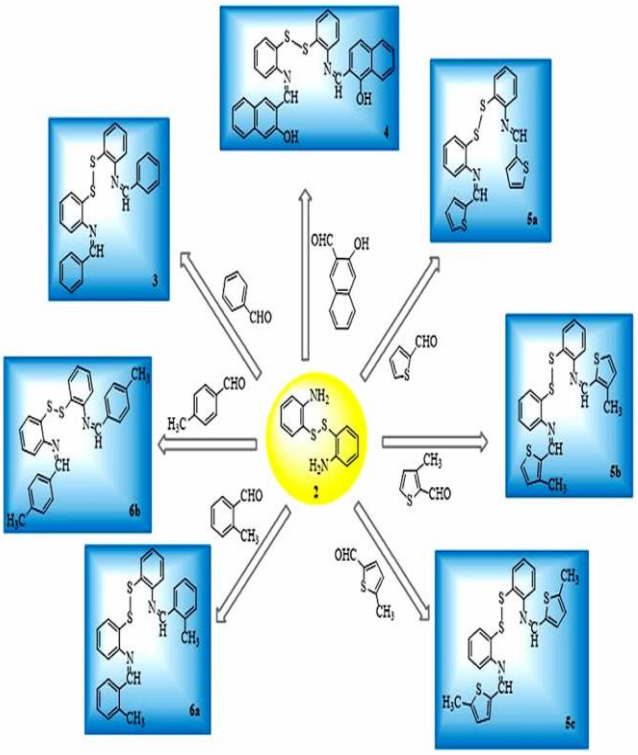
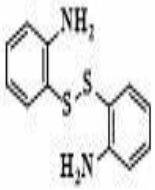
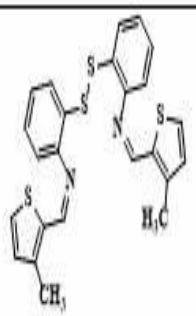
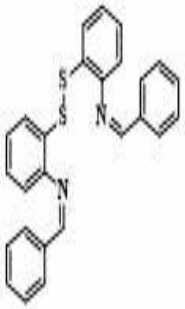
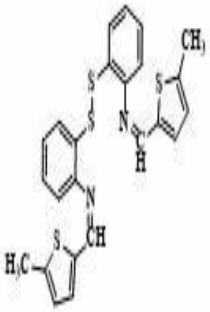
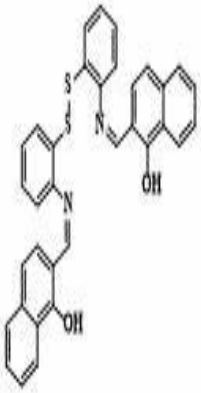
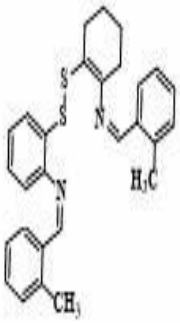
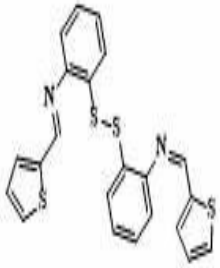
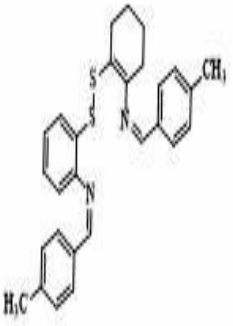


Figure 2 Synthesis of disulphide Schiff base ligand derivatives

Table 1 Structures of synthesized disulphide-Schiff base ligands and reaction parameters (synthesis time, yield)

Entry	Structure	Yield (%) Method (A)	Yield (%) Method (B)	Time (min.) Method (A)	Time (h.) Method (B)	Entry	Structure	Yield (%) Method (A)	Yield (%) Method (B)	Time (min.) Method (A)	Time (h.) Method (B)
2		-	75	-	9	5b		94	85	15	6
3		88	65	15	6	5c		97	80	15	6
4		98	87	15	4	6a		95	80	15	5
5a		92	84	15	17	6b		98	86	15	6

3.2. Biological Activity

Antibacterial and anti-Candidal screening

The results for in vitro antibacterial and anti-Candidal activities of ligands together with the inhibition zone values and MIC values of standard drugs are summarized in (Table 2-3 and Figure 3-6). Researchers have been reported that aromatic substituent at different positions in some Schiff compounds show different electronic characteristics and biological activity [12].

The ligands **2-6b** were tested against Gram negative bacteria (*A. baumannii*, *E. coli* and *K. pneumaniae*) and Gram positive bacteria (*S. aureus, S. epidermidis, E. faecalis* and *B. cereus*) at concentrations 30 μg/mL. Generally, the disc diffusion results showed that all tested ligands exhibited more effect towards Gram positive bacteria than Gram negative bacteria (Table 2). Ligand **2** was found to be more effective than all ligands (**3**, **4**, **5a**, **5b**, **5c**, **6a**, **6b**) and standard antibiotics (CTX30, AMC30). In a previous study, researchers reported that the compound 2,2'-disulfanedidyldianiline, which contains sulfur and nitrogen [25], has a toxic effect on bacteria [8]. Similarly, it was determined that the compound obtained in our study showed antimicrobial activity against bacteria and yeast. Ligand **3**, **5a** and **5c** have shown a strong antibacterial effect than those of the standard antibiotics CTX30 and AMC30 against *S. epidermidis* and *E. faecalis*. Antibacterial effects of ligand **5a** and **5b** are equivalent to AMC30 against *E. faecalis*. In addition, ligand **4**, **5a** and **5 c**are more potent to CTX30 against *B. cereus*. Ligand **5a** has no effect against *A. baumannii*. It has significant activity against *E. coli* and *K. pneumaniae* as compared to CTX30 but it has low effect against the same bacteria as compared to AMC30. Ligand **6a** and **6b** have a low effect against *A. baumannii* than those of AMC30 and this effect higher than those of CTX30. The same ligands were also found to be more effective against other tested bacteria as compared to CTX30. But these ligands have been less effective than AMC30 against all test bacteria.

For instance, ligand **2** showed high activity against all Gram negative bacteria (1.56 $mgmL^{-1}$), than did the reference Gentamycin on the same bacteria and Gram positive bacteria such as *S. epidermidis* and *E. faecalis* (1.56 $mgmL^{-1}$). Against Gram-positive bacteria *Staphylococcus aureus* and *B. cereus,* ligand **2** has shown a potential antibacterial activity (3.13 $mgmL^{-1}$), as compared to standard antibiotic Gentamycin. Besides, ligand **3** (3.13 $mgmL^{-1}$), **4** (6.25 $mgmL^{-1}$), **5a** (6.25 $mgmL^{-1}$) have more potent effects than those of the standard reference antibiotic Gentamycin against *S. epidermidis*, while ligand **3**, **4**, **5a**, **5b** and **5c** equivalent to Gentamycin effects (6.25 $mgmL^{-1}$). Notably, Ligand **6a** and **6b** (3.13 $mgmL^{-1}$) have shown a strong antibacterial activity as compared to Gentamycin (6.25 $mgmL^{-1}$). Similarly, the same ligand shows a superior antifungal activity against *C. Guilliermondii* (0.78 $mgmL^{-1}$) than occurred with the reference Nystatin (Table 3). Against *C. albicans* and *C. tropicalis* (1.56 $mgmL^{-1}$), *C. Glabrata* (3.13 $mgmL^{-1}$), ligand **2** is equivalent to the reference antifungal antibiotic Nystatin. The other ligands were far below than the reference antifungal antibiotics.

Presence of a strong electron donor methyl group attached to the benzene ring is thought to increase both antibacterial [27] and anti-Candidal activity. It has been determined that groups with para position have antibacterial effect against *S. aureus* [13]. In our study, thio-Schiff bases (**5b**, **5c**, **6a**, **6b**) containing methyl groups have been para position and ortho position are showed same effect with standard antibiotic against *S. aureus*. [28].

According to the anti-Candidal activity results, ligand **2** show improved anti-Candidal activity compared to the other test ligands on all test yeast, particularly against *C. guilliermondii*. This ligand exhibit about the same antifungal activity against *C. tropicalis* and *C. albicans*. Other test ligands tested in this study were compared to Posaconazole (PCZ 5 μg) under the experimental conditions given. Interestingly, it is either very weakly effective or has no effect on Candida species.

Table 2 The in vitro antibacterial and anti-Candidal activity of ligands **2-6b**

	Inhibition zones (mm)*										
Microorganisms	**Ligands**								**Antibiotics**		
(Gram negative bacteria)	**2**	**3**	**4**	**5a**	**5b**	**5c**	**6a**	**6b**	**CTX30**	**AMC30**	**PCZ5**
Acinetobacter baumannii	20.0	11.0	7.0	6.0	8.0	7.0	9.0	9.0	7.0	11.0	-
Escherichia coli	20.0	8.0	10.0	7.0	7.0	7.0	10.0	9.0	6.0	13.0	-
Klebsiella pneumaniae	21.0	13.0	11.0	10.0	8.0	9.0	7.0	6.0	6.0	13.0	-
(Gram positive bacteria)											
Staphylococcus aureus	17.0	12.0	7.0	9.0	10.0	9.0	8.0	7.0	8.0	10.0	-
Staphylococcus epidermidis	28.0	19.0	17.0	18.0	15.0	13.0	10.0	12.0	15.0	17.0	-
Enterococcus faecalis	25.0	17.0	17.0	18.0	18.0	19.0	17.0	16.0	18.0	18.0	-
Bacillus cereus	21.0	12.0	15.0	13.0	15.0	16.0	6.0	9.0	14.0	18.0	-
***(Candida* species)**											
Candida tropicalis	20.0	6.0	8.0	8.0	8.0	7.0	6.0	6.0	-	-	12.0
Candida guilliermondii	25.0	10.0	8.0	7.0	6.0	9.0	10.0	10.0	-	-	13.0
Candida albicans	20.0	10.0	9.0	10.0	7.0	10.0	9.0	9.0	-	-	20.0
Candida glabrata	17.0	11.0	6.0	7.0	7.0	10.0	11.0	8.0	-	-	19.0

CTX30: Cefotaxime 30 µg; AMC30: Amoxicillin/clavulanicacid 30 µg; PCZ5: Posacanazole 5 µg

(*): The figures on the scale show the inhibition diameters.

Table 3 The i*n vitro* antimicrobial activity (MIC, mgmL^{-1}) of the of ligands **2-6b**

Microorganism	**Ligands**								**Antibiotics**	
(Gram negative bacteria)	**2**	**3**	**4**	**5a**	**5b**	**5c**	**6a**	**6b**	**GEN**	**NYS**
Acinetobacter baumannii	1.56	25.0	25.0	25.0	25.0	25.0	25.0	25.0	6.25	-
Escherichia coli	1.56	25.0	25.0	25.0	25.0	25.0	12.5	25.0	6.25	-
Klebsiella pneumaniae	1.56	12.5	25.0	25.0	25.0	25.0	25.0	25.0	6.25	-
(Gram positive bacteria)										
Staphylococcus aureus	3.13	12.5	25.0	25.0	25.0	25.0	25.0	25.0	25.0	-
Staphylococcus epidermidis	1.56	3.13	6.25	6.25	12.5	25.0	25.0	25.0	12.5	-
Enterococcus faecalis	1.56	6.25	6.25	6.25	6.25	6.25	3.13	3.13	6.25	-
Bacillus cereus	3.13	25.0	12.5	25.0	12.5	12.5	25.0	25.0	6.25	-
***Candida* species**										
Candida tropicalis	1.56	25.0	25.0	25.0	25.0	25.0	25.0	25.0	-	1.56
Candida guilliermondii	0.78	25.0	25.0	25.0	25.0	25.0	25.0	25.0	-	3.13
Candida albicans	1.56	25.0	25.0	25.0	25.0	25.0	25.0	25.0	-	1.56
Candida glabrata	3.13	25.0	25.0	25.0	25.0	25.0	25.0	25.0	-	3.13

GEN: Gentamycin; NYS: Nystatin

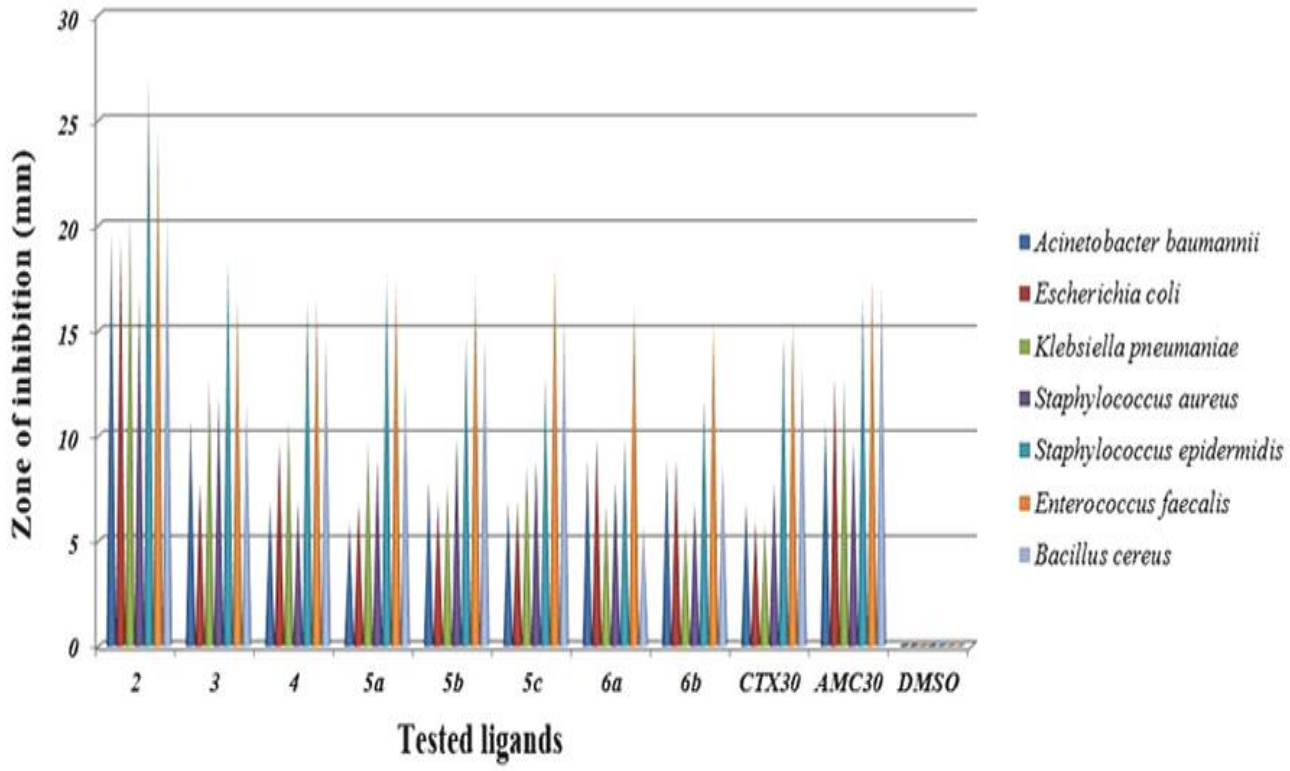


Figure 3 The *in vitro* antibacterial activity of ligands **(2-6b)**

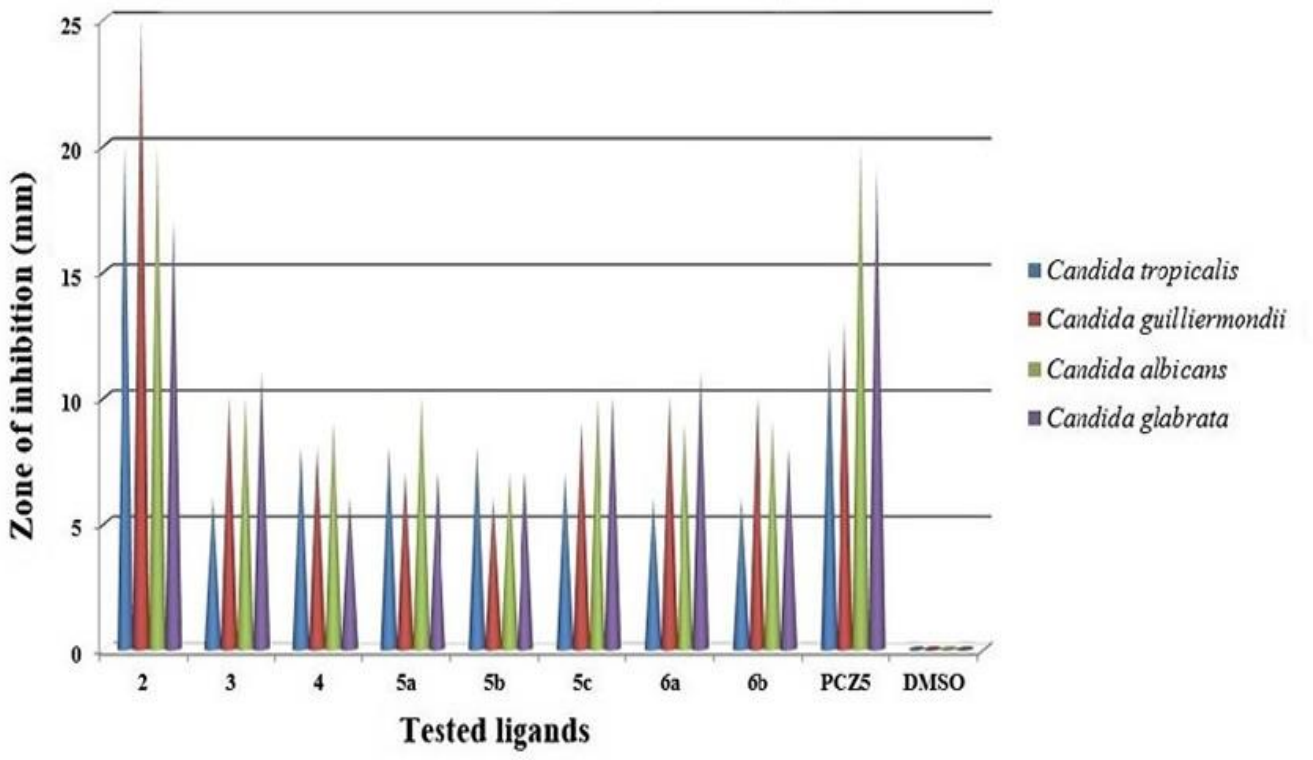


Figure 4 The *in vitro* anti-Candidal activity of ligands **(2-6b)**

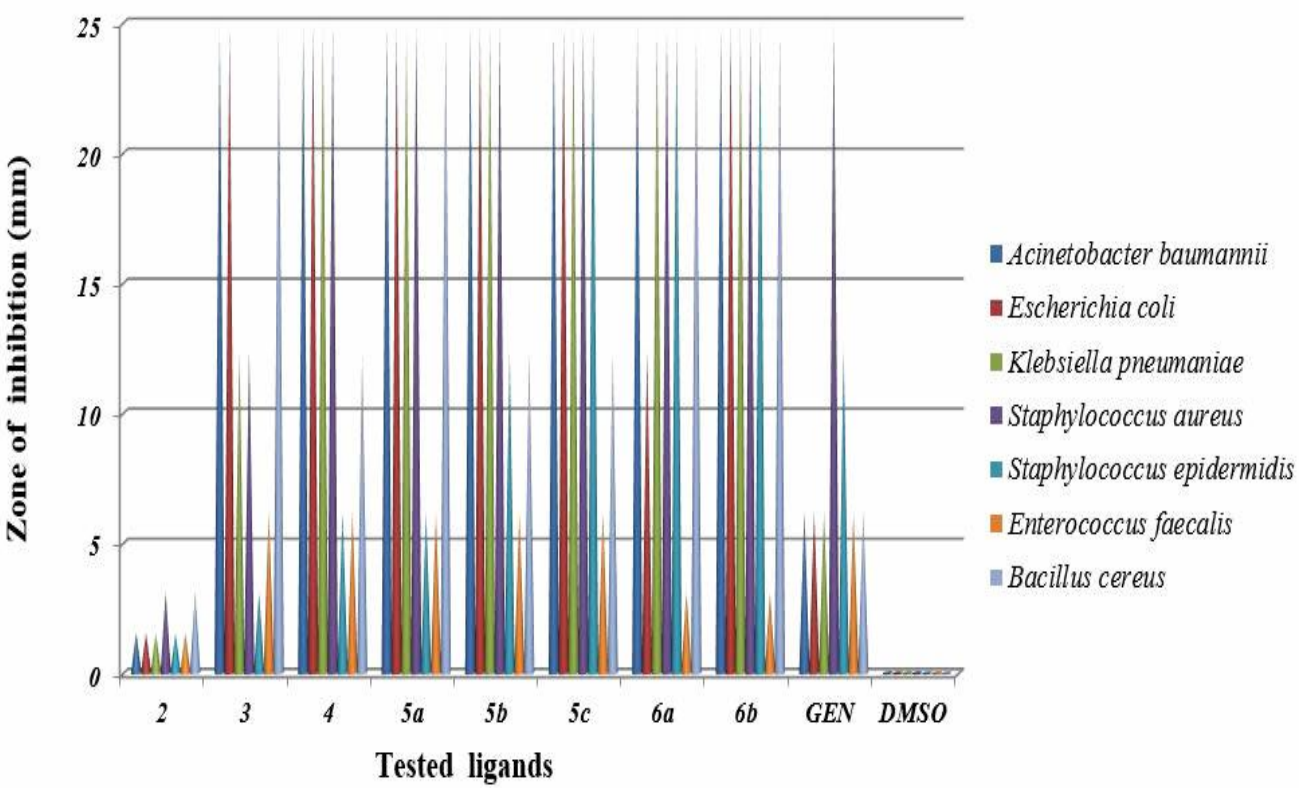


Figure 5 The *in vitro* antibacterial activity (MIC, $mgmL^{-1}$) of ligands **(2-6b)**

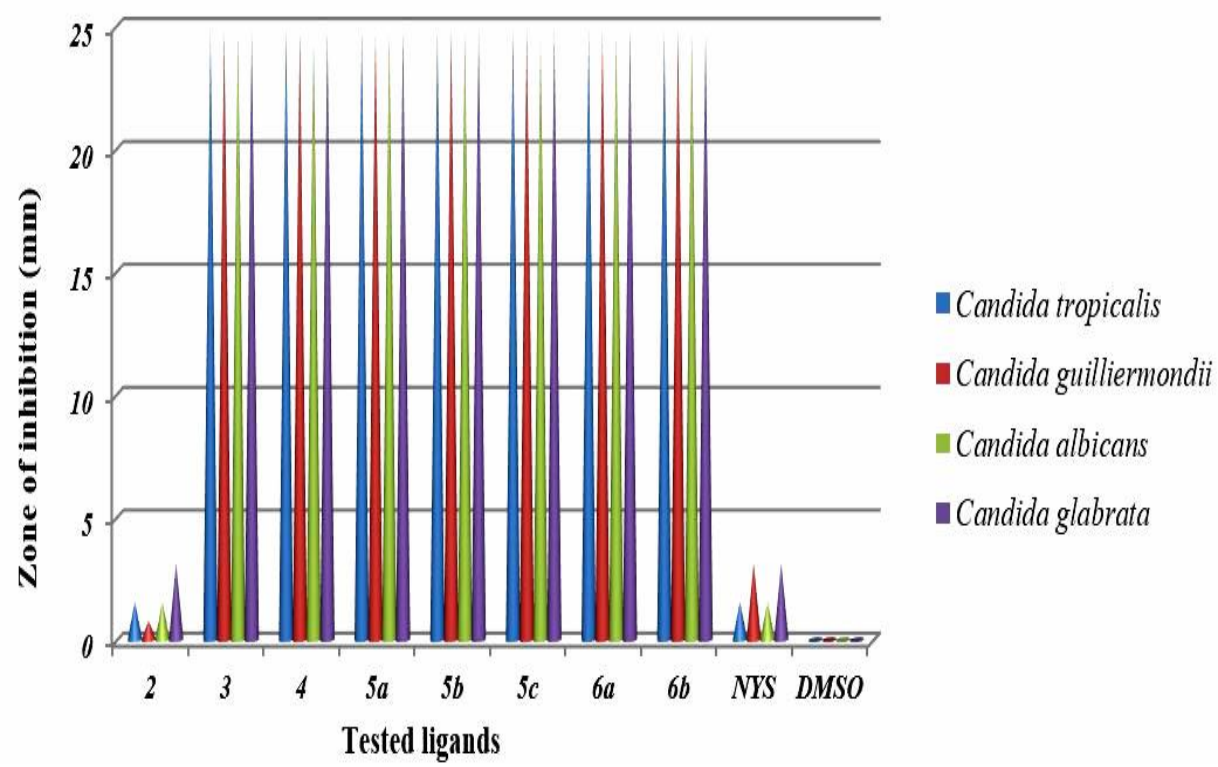


Figure 6 The *in vitro* anti-Candidal activity (MIC, $mgmL^{-1}$) of ligands **(2-6b)**

4. CONCLUSIONS

In short, a dimeric amine and seven dimeric thio-Schiff bases were successfully obtained by two different methods (without catalyst and using CeO_2 nanocatalyst). In addition, the catalytic effect of the prepared CeO_2 nanocatalyst on the synthesis of dimeric thio-Schiff bases was investigated in this work and found to be an efficient and reversible catalyst. Additively, we were studied antibacterial and anti-Candidal activities of dimeric thio-Schiff bases. At the same time, antimicrobial activities of ligands **2-6b** were compared to standard antibiotics. Overall, ligand **2** was found to be more efficient than other all ligands (**3**, **4**, **5a**, **5b**, **5c**, **6a**, **6b**) and standard antibiotics (CTX30, AMC30, PCZ5). The short reaction time of the second process, being environmentally friendly, non-hazardous, reusability of the catalyst, ease of preparation and applicability to a wide variety of ligands make this method preferable. The results of our study showed that dimeric thio-Schiff bases will be useful when producing new and different metabolites. The ligands exhibiting especially antimicrobial activity against Gram positive bacteria and *Candida* species could result in the discovery of novel antibacterial and antifungal agents, showing demonstrating broad spectrum activities. This study may help in the discovery of new chemical class antibiotics used in the treatment of infectious diseases in future pharmacological investigations.

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

Authors' Contribution

Aslıhan DALMAZ and Görkem DÜLGER conceived and designed the experiments, analyzed and interpreted data and performed experiments; Sefa DURMUŞ and Başaran DÜLGER conceived and designed the experiments, analyzed and interpreted data, revised the manuscript. All authors discussed the results and contributed to the final manuscript.

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The authors declare that this document does not require an ethics committee approval or any special permission.

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