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Synthesis and Characterization of Disulfide-Schiff Base Derivatives and *in vitro* Investigation of Their Antibacterial Activity Against Multidrug-Resistant *Acinetobacter baumannii* Isolates: A New Study[1]

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Abstract—In this study two different methods without a catalyst and with a CeO_2 nano catalyst were used for the synthesis of dimeric disulfide-Schiff bases. The dimeric disulfide-Schiff base derivatives were characterized by FT-IR, NMR, and MS spectra, and elemental analysis. The disulfide-Schiff bases and their derivatives **2–5c** were screened for *in vitro* antibacterial activity against 40 multidrug-resistant strains of *Acinetobacter baumannii*, and their minimum inhibitory concentrations were determined. Most of products exhibited high antibacterial activity against *Acinetobacter baumannii*.

Keywords: dimeric disulfide-Schiff bases, antibacterial activity, *Acinetobacter baumannii*, minimum inhibitory concentration (MIC), nanoceria, multidrug-resistant
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INTRODUCTION

Metal nanoparticles have attracted considerable attention in synthetic organic chemistry due to their high catalytic activity and reusability. However, nano CeO_2 catalyzed reactions are not common in organic synthesis [1]. Condensation of 2,2'-diaminodiphenyl 2,2'-disulfide with different aromatic aldehydes gives rise to a series of interesting Schiff bases of SNNS, $N_2S_2O_2$ donors, with potential applications in medicine. Steric and electronic properties of these compounds can be finely adjusted, thus making them attractive in coordination and inorganic chemistry [2, 3].

Over recent years, there have been many reports on applications of Schiff base compounds as antibacterial, antifungal, anticancer, antioxidant, anti-inflammatory, antimalarial, and antiviral agents [4, 5].

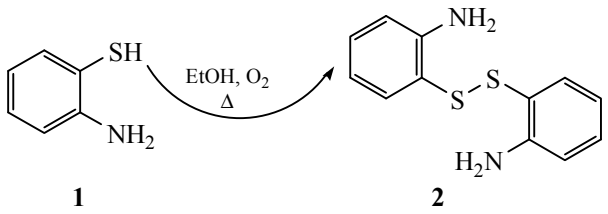
The *Acinetobacter* species are gram negative, non-motile, non-fermentative, opportunistic pathogens that are widely spread in soil and water and can colonize in hospitals [6]. Antimicrobial treatment of multidrug-resistant *Acinetobacter baumannii* (MDRAB) poses a significant problem at present time [7].

RESULTS AND DISCUSSION

In the current study, the title compounds were synthesized for the first time using a CeO_2 nano-catalyst, thus adding a new approach to the existing methods. Synthesis of the dimeric disulfide-Schiff bases **3–5c** was carried out by reacting compound **2** with several aldehydes including 2-hydroxy benzaldehyde, 2,3-dihydroxy benzaldehyde, 2,4-dihydroxy benzaldehyde, 2,5-dihydroxy benzaldehyde, 2-hydroxy-3-methoxy benzaldehyde, 2-hydroxy-4-methoxy benzaldehyde, and 2-hydroxy-5-methoxy benzaldehyde. Synthesis of these dimeric disulfide-Schiff bases was carried out in two different ways without catalysts and using CeO_2 nanocatalyst (Schemes 1 and 2). Both methods were determined to be efficient, however, the yield of the process catalyzed by CeO_2 nanocatalyst

[1] The text was submitted by the authors in English.

Scheme 1. Synthesis of 2,2'-disulfanediyldianiline (**2**).



1 **2**

was considerably higher. The reaction time of the catalyzed reaction was reduced from hours to minutes (Table 1). The structures of all synthesized compounds were characterized by FT-IR, ^{1}H, and ^{13}C NMR spectroscopic methods [8].

In IR spectra, except compounds **4a–4c**, the characteristic bands of the OCH_3 and OH groups were not clearly observed probably due to the effect of tautomerism [9–12]. The characteristic for Schiff bases C=N group was recorded by sharp stretching vibrations bands in the range of 1632–1606 cm^{-1} [13, 14]. Specific peaks of ν(C–C_{Ar}), ν(C–O_{Ar}), ν(C–S), and ν(S–S) were observed in the range of 1485–1439, 1278–1225, 758–729, and 577–553 cm^{-1}, respectively. Intensity of vibrations depended on the presence of different functional groups such as CH_3, OH, and NH_2.

^{1}H and ^{13}C NMR spectra of the synthesized compounds were measured in DMSO-d_6 and $CDCl_3$. The azomethine (H–C=N) proton was recorded as a singlet in the range of 8.54–9.04 ppm. Purity of compounds **2–5c** was determined by evaluating the melting point together with TLC and NMR spectra.

Microbiological assessment. Antibacterial activity of the some newly synthesized compounds was tested (Table 2). Meropenem was used as the standard antibiotic. Compounds **2**, **4a** and **5b** exhibited antibacterial effects against *A. baumannii* higher than the

Scheme 2. Synthesis of dimeric disulfide-Schiff base compounds **3–5c**.

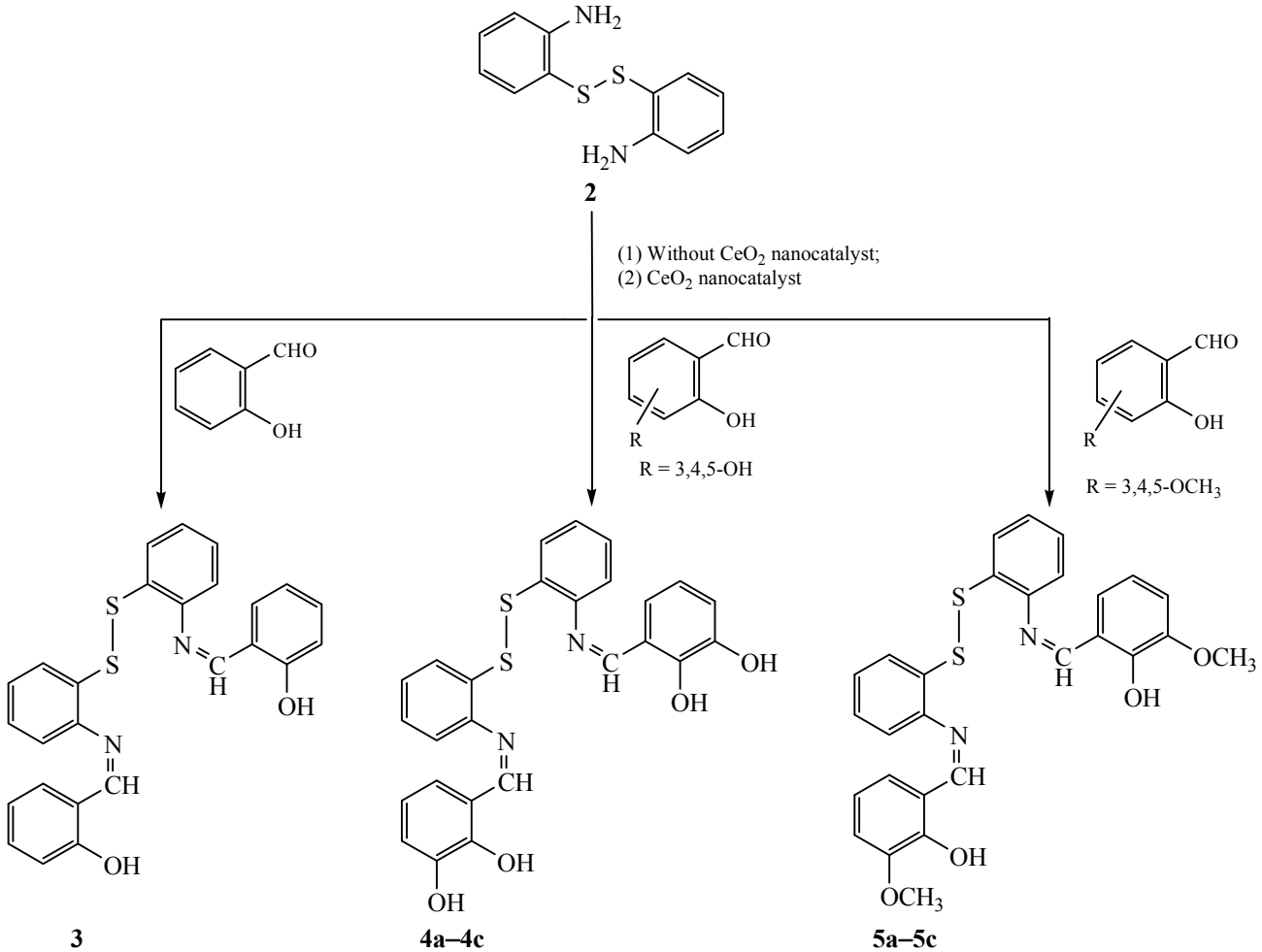


Table 1. Structures of synthesized dimeric disulfide-Schiff base compounds and reaction parameters

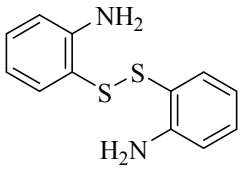
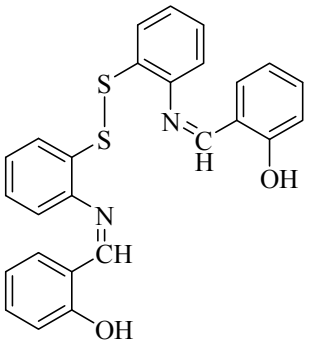
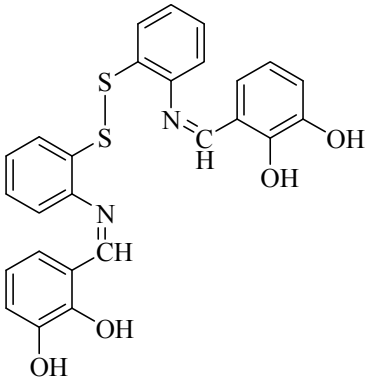
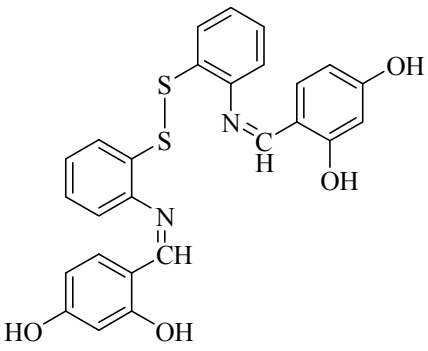
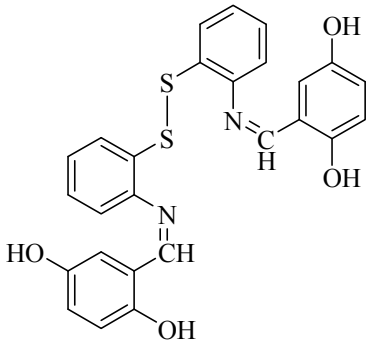
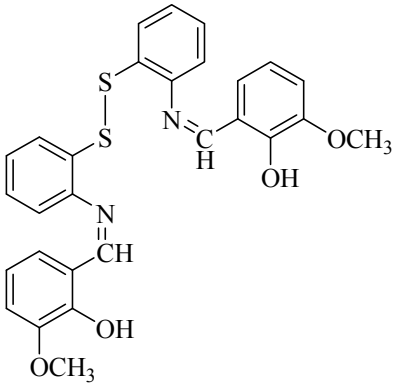
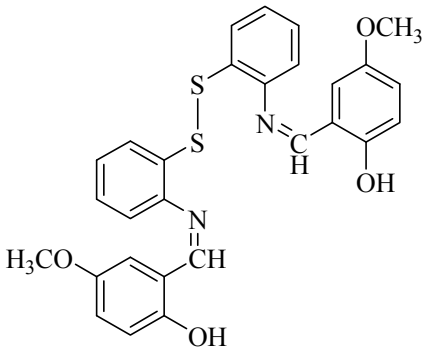
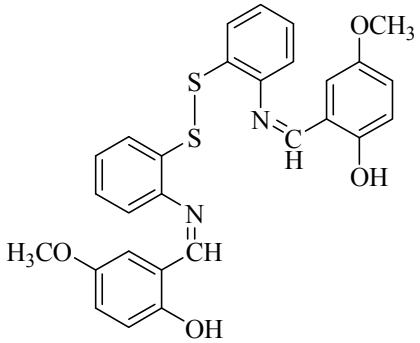
Comp. no.	Structure	Yield, %		Synthesis time	
		without CeO_2 nanocatalyst	CeO_2 nanocatalyst	without CeO_2 nanocatalyst, h	CeO_2 nanocatalyst, min
2		75	–	9	30
3		75	95	6	15
4a		80	95	6	15
4b		76	98	29	15

Table 1. (Contd.)

Comp. no.	Structure	Yield, %		Synthesis time	
		without CeO_2 nanocatalyst	CeO_2 nanocatalyst	without CeO_2 nanocatalyst, h	CeO_2 nanocatalyst, min
4c		74	92	6	15
5a		70	96	5	15
5b		86	97	6	15
5c		90	96	5	15

other compounds and meropenem. This was particularly true for the compound **2**.

Probably difference in the MIC results was due to the presence of one or two OH groups in the structures of the compounds. The compounds **4a–4c** containing two OH groups on each aromatic cycle were more effective than the compound **3**, that contained one OH group. The presence of two hydroxy groups in the ortho positions in the structure of the compound **4a** made its efficiency higher than compounds **4b** and **4c** that contained the OH groups in meta and para positions. Among compounds **5a–5c**, the compound **5a** demonstrated the lowest activity probably due to ortho position of the hydroxy and methoxy groups.

Table 2. *In vitro* antibacterial activity of compounds against multi-drug resistant *A. baumannii*

Compound	Minimum inhibitory concentrations, mg/L	
	MIC_{50}[a]	MIC_{90}[b]
2	8	16
3	>64	>64
4a	16	16
4b	32	32
4c	32	32
5a	>64	>64
5b	16	32
5c	32	32
MEM[c]	>16	>16

[a] MIC_{50}: 50% minimum inhibitory dose. [b] MIC_{90}: 90% minimum inhibitory dose. [c] Meropenem (standard antibiotic).

EXPERIMENTAL

All reagents were commercially available and purchased from Merck and Acros. The CeO_2 nanoparticles were prepared in accordance with the earlier developed method [15–17].

2,2'-Diaminodiphenyl disulfide was synthesized by oxidation of 2-aminothiophenol. The ligands were synthesized according to the earlier developed method using the CeO_2 nanocatalyst and without a catalyst [15].

Fourier transform infrared-attenuated total reflection spectroscopy (FTIR-ATR) data were recorded on a Perkin Elmer spectrophotometer. ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ and DMSO-d_6 on a Bruker-400 spectrometer using TMS as an internal standard. Elemental analyses were carried out using a Thermo Scientific Flash 2000. Mass spectra were measured on an AB SCIEX 4000 Q-TRAP LC-MS/MS instrument.

2,2'-Disulfanediyldianiline (2). Shiny yellow solid, yield 75%, mp 90–92°C. IR spectrum, ν, cm^{-1}: 3375 (NH_2), 1471 (C=C), 744 (C–S). ^1H NMR spectrum, δ, ppm: 4.31 s (2H, NH_2), 6.56 t.d (1H, *J* = 7.5, 1.2 Hz, Ar-H), 6.56 t.d (1H, *J* = 7.5, 1.2 Hz, Ar-H), 6.66 d.d (1H, *J* = 8.5, 1.3 Hz, Ar-H), 7.07–7.19 m (2H, Ar-H). ^{13}C NMR spectrum, δ_C, ppm: 115.3, 118.2, 118.8, 131.7, 136.9, 148.7. Found, %: C 58.01; H 4.69; N 11.14; S 26.16. $C_{12}H_{12}N_2S_2$. Calculated %: C 58.03; H 4.87; N 11.28; S 25.82. *M* 249 $[M + H]^+$.

2,2'-{(1*Z*,1'*Z*)-[(Disulfanediylbis(2,1-phenylene)]-bis(azanylylidene)bis(methanylylidene)}diphenol (3). Straw yellow solid, yield 95%, mp 167–169°C. IR spectrum, ν, cm^{-1}: 1610 (C=N), 1462 (C=C), 1278 (C–O), 747 (C–S), 556 (S–S). ^1H NMR spectrum, δ, ppm: 6.94 t (1H, *J* = 7.1 Hz, Ar-H), 7.04 d (1H, *J* = 8.4 Hz, Ar-H), 7.09–7.14 m (1H, Ar-H), 7.15–7.26 m (2H, Ar-H), 7.39 d.d (2H, *J* = 5.7, 1.7 Hz, Ar-H), 7.62–7.67 m (1H, Ar-H), 8.59 s (1H, H–C=N), 12.87 s (1H, Ar-OH). ^{13}C NMR spectrum, δ_C, ppm: 117.5, 117.7, 119.2, 119.3, 127.2, 127.3, 127.7, 127.8, 131.6, 132.7, 133.7, 161.2, 162.8. Found, %: C 68.45; H 4.35; N 6.09; S 14.08. $C_{26}H_{20}N_2O_2S_2$. Calculated %: C 68.40; H 4.42; N 6.14; S 14.05. *M* 457 $[M + H]^+$.

3,3'-{(1*Z*,1'*Z*)-[(Disulfanediylbis(2,1-phenylene)]-bis(azanylylidene)bis(methanylylidene)}bis(benzene-1,2-diol) (4a). Shiny scarlet solid, yield 95%, mp 205–210°C. IR spectrum, ν, cm^{-1}: 3306 (O–H), 1611 (C=N), 1456 (C=C), 1244 (C–O), 749 (C–S), 574 (S–S). ^1H NMR spectrum, δ, ppm: 6.87 t (1H, *J* = 7.8 Hz, Ar-H), 7.04 d (1H, *J* = 7.8 Hz, Ar-H), 7.20 d (1H, *J* = 7.8 Hz, Ar-H), 7.32 t (1H, *J* = 7.6 Hz, Ar-H), 7.39 t (1H, *J* = 7.5 Hz, Ar-H), 7.56 d (1H, *J* = 7.8 Hz, Ar-H), 7.60 d (1H, *J* = 7.8 Hz, Ar-H), 9.04 s (1H, H–C=N), 9.39 s (1H, O–H), 12.78 s (1H, O–H). ^{13}C NMR spectrum, δ_C, ppm: 118.40, 119.16, 119.47, 119.64, 123.08, 125.97, 127.96, 128.11, 130.32, 145.66, 148.97, 151.17, 164.14. Found, %: C 63.81; H 4.18; N 5.79; S 13.06. $C_{26}H_{20}N_2O_4S_2$. Calculated %: C 63.92; H 4.13; N 5.73; S 13.13. *M* 489 $[M + H]^+$.

4,4'-{(1*Z*,1'*Z*)-[(Disulfanediylbis(2,1-phenylene)]-bis(azanylylidene)bis(methanylylidene)}bis(benzene-1,3-diol) (4b). Carmine solid, yield 98%, mp 237°C.

IR spectrum, ν, cm^{-1}: 3100 (O–H), 1606 (C=N), 1456 (C=C), 1225 (C–O), 746 (C–S), 559 (S–S). ^{1}H NMR spectrum, δ, ppm: 6.87 t (1H, *J* = 7.8 Hz, Ar-H), 6,99 d (1H, *J* = 7.8 Hz, Ar-H), 7.19 d (1H, *J* = 7.8 Hz, Ar-H), 7.29 t (1H, *J* = 7.6 Hz, Ar-H), 7.38 t (1H, *J* = 7.5 Hz, Ar-H), 7.54 d (1H, *J* = 7.8 Hz, Ar-H), 7.48 d (1H, *J* = 7.8 Hz, Ar-H), 9.01 s (1H, H–C=N), 9.28 s (1H, O–H), 12.81 s (1H, O–H). ^{13}C NMR spectrum, δ_C, ppm: 101.57, 108.47, 112.25, 114.23, 120.98, 126.64, 127.55, 127.82, 132.27, 145.37, 148.67, 156.85, 166.81. Found, %: C 63.99; H 4.10; N 5.69; S 13.04. $C_{26}H_{20}N_2O_4S_2$. Calculated %: C 63.92; H 4.13; N 5.73; S 13.13. *M* 489 $[M + H]^+$.

2,2'-{(1*Z*,1'*Z*)-[(Disulfanediylbis(2,1-phenylene)]-bis(azanylylidene)bis(methanylylidene)}bis(benzene-1,4-diol) (4c). Shiny brown solid, yield 92%, mp 225°C. IR spectrum, ν, cm^{-1}: 3372 (O–H), 1617 (C=N), 1439 (C=C), 1252 (C–O), 758 (C–S), 575 (S–S). ^{1}H NMR spectrum, δ, ppm: 6.88 d (1H, *J* = 8.8 Hz, Ar-H), 6.96 d.d (1H, *J* = 8.8, 2.9 Hz, Ar-H), 7.17 d (1H, *J* = 2.9 Hz, Ar-H), 7.28–7.33 m (1H, Ar-H), 7.37 t.d (1H, *J* = 7.6, 1.3 Hz, Ar-H), 7.52 d.d (1H, *J* = 7.8, 0.9 Hz, Ar-H), 7.58 d.d (1H, *J* = 7.8, 1.2 Hz, Ar-H), 8.96 s (1H, H–C=N), 9.23 s (1H, O–H), 11.83 s (1H, O–H). ^{13}C NMR spectrum, δ_C, ppm: 116.79, 117.37, 118.34, 119.36, 121.84, 125.70, 127.75, 127.92, 130.39, 146.17, 149.78, 153.06, 163.02. Found, %: C 63.80; H 4.19; N 5.79; S 13.02. $C_{26}H_{20}N_2O_4S_2$. Calculated %: C 63.92; H 4.13; N 5.73; S 13.13. *M* 489 $[M + H]^+$.

6,6'-{(1*Z*,1'*Z*)-[(Disulfanediylbis(2,1-phenylene)]-bis(azanylylidene)bis(methanylylidene)}bis(2-methoxyphenol) (5a). Bright orange solid, yield 96%, mp 174°C. IR spectrum, ν, cm^{-1}: 2839 (OCH_3), 1610 (C=N), 1455 (C=C), 1247 (C–O), 729 (C–S), 559 (S–S). ^{1}H NMR spectrum, δ, ppm: 3.96 s (3H, OCH_3), 6.98 d (3H, *J* = 51.5 Hz, Ar-H), 7.19 d (3H, *J* = 10.2 Hz, Ar-H), 7.68 s (1H, Ar-H), 8.66 s (1H, H–C=N), 13.26 s (1H, O–H). ^{13}C NMR spectrum, δ_C, ppm: 56.39, 116.52, 118.93, 119.38, 119.84, 124.47, 126.68, 128.41, 128.62, 130.88, 146.36, 148.44, 150.78, 164.23. Found, %: C 65.13; H 4.65; N 5.49; S 12.38. $C_{28}H_{24}N_2O_4S_2$. Calculated %: C 65.09; H 4.68; N 5.42; S 12.41. *M* 517 $[M + H]^+$.

6,6'-{(1*Z*,1'*Z*)-[(Disulfanediylbis(2,1-phenylene)]-bis(azanylylidene)bis(methanylylidene)}bis(3-methoxyphenol) (5b). Bright orange solid, yield 97%, mp 198°C. IR spectrum, ν, cm^{-1}: 2839 (OCH_3), 1632 (C=N), 1461 (C=C), 1243 (C–O), 757 (C–S), 553 (S–S). ^{1}H NMR spectrum, δ, ppm: 3.86 s (3H, OCH_3), 6.48–6.59 m (2H, Ar-H), 7.13 d.d (1H, *J* = 7.7, 1.2 Hz, Ar-H), 7.17 d.d (1H, *J* = 7.7, 1.4 Hz, Ar-H), 7.23 t.d (1H, *J* = 7.4, 1.3 Hz, Ar-H), 7.30 d (1H, *J* = 8.5 Hz, Ar-H), 7.65 d.d (1H, *J* = 7.8, 1.3 Hz, Ar-H), 8.54 s (1H, H–C=N), 13.27 s (1H, O–H). ^{13}C NMR spectrum, δ_C, ppm: 55.55, 101.13, 107.42, 113.19, 117.52, 126.97, 127.19, 127.47, 131.39, 133.88, 146.48, 161.79, 163.61, 164.33. Found, %: C 65.25; H 4.65; N 5.38; S 12.37. $C_{28}H_{24}N_2O_4S_2$. Calculated %: C 65.09; H 4.68; N 5.42; S 12.41. *M* 517 $[M + H]^+$.

2,2'-{(1*Z*,1'*Z*)-[(Disulfanediylbis(2,1-phenylene)]-bis(azanylylidene)bis(methanylylidene)}bis(4-methoxyphenol) (5c). Bright orange solid, yield 96%, mp 170°C. IR spectrum, ν, cm^{-1}: 2832 (OCH_3), 1614 (C=N), 1485 (C=C), 1271 (C–O), 748 (C–S), 577 (S–S). ^{1}H NMR spectrum, δ, ppm: 3.82 s (3H, OCH_3), 6.91 d (1H, *J* = 2.7 Hz, Ar-H), 7.02 d.t (2H, *J* = 14.0, 5.8 Hz, Ar-H), 7.16 t.d (1H, *J* = 7.3, 1.4 Hz, Ar-H), 7.20 d.d (1H, *J* = 7.6, 1.6 Hz, Ar-H), 7.24 d.d (1H, *J* = 7.5, 1.6 Hz, Ar-H), 7.66 d.d (1H, *J* = 7.7, 1.5 Hz, Ar-H), 8.59 s (1H, H–C=N), 12.43 s (1H, O–H). ^{13}C NMR spectrum, δ_C, ppm: 55.98, 115.45, 117.63, 118.32, 118.76, 121.15, 127.22, 127.64, 127.77, 131.67, 146.34, 152.36, 155.50, 162.51. Found, %: C 65.17; H 4.64; N 5.38; S 12.38. $C_{28}H_{24}N_2O_4S_2$. Calculated %: C 65.09; H 4.68; N 5.42; S 12.41. *M* 517 $[M + H]^+$.

Antimicrobial susceptibility patterns of *Acinetobacter baumannii*. Multiple-resistant *A. baumannii* isolates were obtained from various clinical samples (Medical Microbiology Laboratory of Duzce University Medical Faculty). Identification of *A. baumannii* isolates was performed by conventional microbiological methods (Gram staining, oxidase, three sugar iron broth and motion test) and an automatized-bacterial identification system (Vitek 2, bioMerieux, France).

Meropenem and other antibiotic susceptibilities of *A. baumannii* isolates were determined by the Vitek 2 system and evaluated according to CLSI [18]. Detection of resistance to typical antibiotics belonging to at least three antibiotic classes was defined as multiple antibiotic resistance. Antibacterial activities of all the compounds were determined by the broth microdilution method [18]. The compounds were dissolved in DMSO to a final concentration of 60 μg/mL. The isolates were adjusted to McFarland's 0.5 standard and 100 μL of the Mueller Hinton broth was placed in each 96-well plate. Afterwards, serial dilutions of the solutions were carried out. MIC_{50} and MIC_{90} values of

all compounds **2–5c** against *A. baumannii* isolates were compared with standard antibiotic meropenem MIC values.

CONCLUSIONS

All compounds were synthesized with high yield. However, in the new method that involved the CeO_2 nanocatalyst, the yield was increased. The catalyzed reactions were complete within minutes. The CeO_2 nanocatalyst could be used repeatedly, making the processes less costly.

Several synthesized compounds demonstrated high antibacterial activity. The present study exhibited that disulfide-Schiff base compounds were partially responsible for antibacterial activity against multi-drug-resistant *A. baumannii*, thus indicating that these can be used in advanced pharmacological studies for treatment of multi-drug-resistant bacterial strains.

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