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ORIGINAL ARTICLE

Synthesis, characterization, and antimicrobial activity of Schiff bases derived from benzaldehydes and 3,3′-diaminodipropylamine

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Abstract Six Schiff bases were prepared by reacting 3,3′-diaminodipropylamine with different benzaldehyde derivatives. The structures of these compounds were confirmed through different spectroscopic methods such as ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. The prepared compounds were evaluated *in vitro* for their antimicrobial activity against a number of pathogenic Gram-positive and Gram-negative bacteria and Candida by the twofold serial dilution method. These compounds showed bacteriostatic rather than bactericidal activities against Gram positive and Gram-negative bacteria. In addition, compound **3c** exhibited significant anticandida activity with an MIC of 24 μg/ml and is, therefore, considered as a promising and potential antifungal agent; further modification can be done on the structure of the compound for a better drug candidate in the future.

1. Introduction

Nosocomial infections during the past decade have invaded hospitals worldwide by multi drug resistant Gram-positive and Gram-negative pathogens. Searching for novel antimicrobial agents and new microbial targets is in demand to intervene to avert the danger caused by these life-threatening infections (Rachakonda and Cartee, 2004). The treatment of nosocomial infections such as hospital acquired methicillin resistant *Staphylococcus aureus* (MRSA) and biofilm formers has become an important problem to deal with owing to their multidrug resistance (Chen et al., 2012; Maselli et al., 2012). Since the resistance towards the available antibiotics among pathogenic bacteria has grown rapidly, there is a clear need for the development of new and effective antimicrobial agents. Therefore, the success in designing antimicrobial agents which are distinct from those

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of the classical antibiotics is the key for treating such infectious diseases known for their chronicity and failure to treat with conventional antibiotics which will eventually lead to death.

Schiff bases have been found to possess pharmacological activities such as antibacterial (Venugopal and Jayashree, 2008), antifungal (Pandey et al., 2003), antitubercular (Bhat et al., 2005), antimicrobial (Wadher et al., 2009), antiviral (Karthikeyan et al., 2006), antimalarial (Li et al., 2003) and anticancer (Villar et al., 2004). They are important compound due to their wide range of biological activities and industrial application. They also serve as a back bone for the synthesis of various heterocyclic compounds (Wang et al., 2008). The condensing protein, (ketoacyl–acyl carrier protein synthase (KAS), is an essential target for a novel antibacterial drug design against multidrug resistant Gram positive pathogens (Lee et al., 2012), (Cheng et al., 2009), impressive efforts have lead to the synthesis of peptide and Schiff bases which can be potential antibiotic agents targeting KAS in Gram positive and Gram negative pathogens.

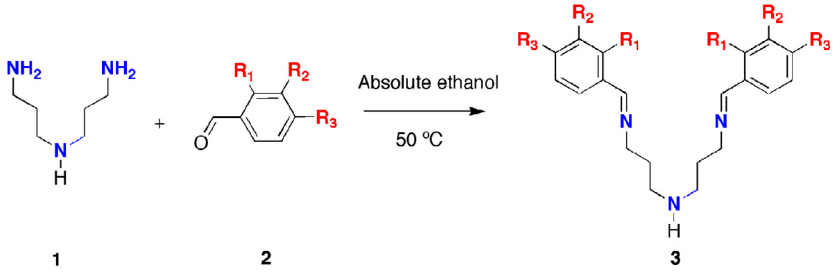
Moreover, Schiff compounds and their metal complexes have been of major interest for a long time because of their ability to bind oxygen to redox systems (Muhammad et al., 2011) exerting their ability to oxidize DNA (Landy, 1989). They showed significant antimicrobial activity due to the free radical scavenging ability of their metal complexes (Al-Amiery et al., 2012). Several research groups have been involved in the synthesis and biological screening of Schiff bases. Kriushnapriya et al. (2009) showed that the aldehyde Schiff base N-aryl thiosemicarbazones had stronger anti-MRSA potency, being effective at half the concentration of the vancomycin. Furthermore, preliminary results revealed that one of the thiazolidinedione-5-acetic acid amide derivatives exhibits promising antimicrobial activity (Shankar and Kallanagouda, 2011). Schiff bases derived from isatin derivatives and N[4-(4′-chlorophenyl)thiozole-2-yl] thiosemicarbazide, have already proved to be potent antimicrobial agents (Pandeya et al., 1999). Similarly, Khanam et al. (2002) discovered that 2,2′-diamino-1-azavinyl aminoamide can be used effectively against a number of both Gram-positive and Gram-negative bacteria. Quite recently (Sadeh et al., 2011), the synthesis and characterization of a number of new Schiff bases derived from metronidazole have been undertaken and their antigiardial and antimicrobial activities were evaluated.

In view of the wide interest in the activity and profile of Schiff bases derived from benzaldehydes due to their pharmacological interest, we described herein the synthesis and characterization of six Schiff bases derived from benzaldehydes and 3,3′-diaminodipropylamine which, some of them are to the best of our knowledge, have not previously been described in the literature. The antimicrobial activity of the synthesized compounds was evaluated. Also, published articles concerning antimicrobial activity sometimes lack containing information about the cytotoxicity of such compounds.

2. Result and discussion

2.1. Chemistry

The Schiff bases **3a–e** were prepared by reacting 3,3′-diaminodipropylamine with different benzaldehyde derivatives in ethanol as shown in Scheme 1. The prepared compounds were checked for purity by TLC using glass plates precoated with silica gel 60 GF254, supplied by Fluka as stationary phase and suitable solvent system as mobile phase. The structures of the prepared compounds were confirmed by NMR, and mass spectrometry. The ^{1}H and ^{13}C-NMR spectra of all prepared compounds are in total agreement with the suggested structures. DEPT experiments were employed to differentiate secondary and quaternary carbons from primary and tertiary ones. Additional supports of the proposed structures come



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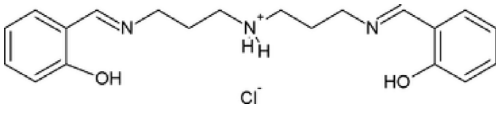
	R_1	R_2	R_3
3a	OH	H	H
3b	OCH_3	H	H
3c	OH	H	OH
3d	H	H	H
3e	H	H	NO_2
4b	(AlDamen and Haddad, 2012)		



Scheme 1 Synthesis of compounds **3a–e**.

from mass spectral data; mass spectra of the prepared compounds showed the correct molecular ions as suggested by their molecular formulae. Compound **3e** is soluble in water, compound **3d** is insoluble, and compounds **3a–c** are partially soluble in water. However, all of them are soluble in DMSO and DMF and are also soluble in acidic media. Compound **3a** is known in the literature while compounds **3b–e** were prepared as new compounds

3. Biological study

3.1. Antimicrobial activity

The antimicrobial activity of the compounds against human pathogenic Gram positive, Gram-negative bacteria and Candida of **3** derivatives was measured by measuring the zone of inhibition in disc diffusion method. Test sample per disc was 250 μg/disc.

In Table 2, measured zones of inhibition for the compounds ranged from 0.9 to 2 cm (**3a**), 0.8 to 1.5 cm (**4a**), 1.3 to 2.1 cm (**3b**), 1.1 to 2.4 cm (**3c**), 1.5 to 2.4 cm (**3d**) and 1.9 to 3 cm for (**3e**) for Gram positive bacteria. Furthermore, the zone of inhibition for the compounds ranged from 0.7 to 1.3 cm (**3a**), 0.9 to 1.1 cm (**4a**), 1.0 to 1.3 cm (**3b**), 1.0 to 2.0 cm (**3c**), 1.0 to 1.8 cm (**3d**) and 1.9 to 2.5 cm (**3e**) for Gram-negative bacteria. However, the inhibition zone for the compounds against Candida ranged from 0.6 to 0.8 cm (**3b**), 2.8 to 3.2 cm (**3c**), 1.7 to 2.3 cm (**3d**) and finally **3e** ranged from 2 to 2.4 cm. Based on zones of inhibition results, the compounds showed better activity against Gram positive bacteria than the activities against Gram negative and Candida. The potency has increased with a moiety of NO_2 at R3 position in **3e** when compared to H and OH in the other compounds.

The previous results were in consistent with the results obtained in micro dilution methods for assessing the antimicrobial activities for the studied compounds. The median MIC results in Table 3 and Figure 1 showed that the median MIC for the **3e** compound had the best bacteriostatic and fungistatic activity among the assessed compounds. The compound 3e showed promising activity against *S. aureus* strains, which are methicillin sensitive and biofilm producers. The MIC ranged (24–49 μg/ml).

The compound **3c** derivative had a significant activity against Candida (24 μg/ml) when compared to its activity on bacterial strains irrespectively to its Gram stain. The activity was due to OH moiety at position R3 that indicates evidence of selectivity of the ligand to the target in Candida.

The minimal bactericidal concentration for the compounds found in Table 4 showed that the compound 3e had static activity rather than cidal against Gram positive and Gram-negative pathogens. These results are illustrated as no MBC for the compound 3e. Nevertheless, MBC for 3e against one strain of *S. aureus ATCC 25923* was 48 μg/ml. This strain is used as a laboratory standard and it is known for its susceptibility to many antibiotics according to NCCLS (2003, 2006).

Finally, cytotoxicity of these compounds shows that 3a and 4a have IC_{50} (μg/ml) ± SD of 132 ± 1.22 and 113 ± 2.03,

Table 1 Microorganism used in this study.

	Origin
Gram-positive bacteria	
Methicillin resistant *Staphylococcus aureus* (MRSA) 1168	Clinical isolate
Methicillin resistant *Staphylococcus aureus* (MRSA) 1052	Clinical isolate
Methicillin resistant *Staphylococcus aureus* (MRSA) 755	Clinical isolate
Methicillin resistant *Staphylococcus aureus* (MRSA) 1164	Clinical isolate
Staphylococcus epidermidis Tu 3298	Biofilm negative
Staphylococcus aureus (MSSA)	Methicillin susceptible *S. aureus*
Staphylococcus aureus ATCC 25923	Control for antibiotic susceptibility testing
Staphylococcus aureus ATCC 6538	Methicillin sensitive
Staphylococcus aureus v329	Biofilm positive
Staphylococcus epidermidis ATCC 12228	Control for antibiotic susceptibility testing
Staphylococcus aureus ATCC 25923	Methicillin susceptible
Staphylococcus aureus Newman	Biofilm positive
Methicillin resistant *Staphylococcus aureus* small colony variants (MRSA/SCV) 1204	Clinical isolate
Methicillin resistant Staphylococcus aureus wild type (MRSA/Wild) 1204	Clinical isolate
Staphylococcus epidermidis RP62A	Biofilm positive
Staphylococcus epidermidis	Clinical isolate
Streptococcus pneumoniae	Clinical isolate
Bacillus cereus ATCC10987	Control for antibiotic susceptibility testing
Gram-Negative Bacteria	
Klebsiella pneumoniae ATCC 10031	Control for antibiotic susceptibility testing
Proteus spp	Clinical isolate urinary tract infections
Serratia spp	Clinical isolate
Escherichia coli ATCC 8739	Control for antibiotic susceptibility testing
Salmonella typhi ATCC 14026	Control for antibiotic susceptibility testing
Yeast	
Candida albicans ATCC 10231	Control for susceptibility testing

Table 2 Antimicrobial activity of various chemical compounds by disc diffusion method.

Strain	Gentamicin 10 μg	**3a**	**4a**	**3b**	**3c**	**3d**	**3e**
Diameter (cm)							
MRSA 1168	NI	1.2 ± 0.2	1.0 ± 0.1	1.4 ± 0.2	2.0 ± 0.2	2.1 ± 0.3	2.3 ± 0.4
MRSA 1052	NI	1.2 ± 0.4	0.8 ± 0.3	1.5 ± 0.2	2.0 ± 0.1	2.1 ± 0.3	2.5 ± 0.2
MRSA 755	NI	1.5 ± 0.2	0.8 ± 0.4	1.3 ± 0.3	1.9 ± 0.2	2.2 ± 0.3	2.5 ± 0.2
MRSA 1164	NI	1.3 ± 0.2	0.8 ± 0.2	1.3 ± 0.2	2.0 ± 0.1	2.2 ± 0.4	2.5 ± 0.1
S.epidermidis Tu 3298	3.2 ± 0.2	1.6 ± 0.2	1.0 ± 0.4	1.2 ± 0.3	2.1 ± 0.2	2.1 ± 0.3	3.0 ± 0.2
MSSA	1.8 ± 0 .1	1.6 ± 0.5	0.7 ± 0.3	2.1 ± 0.2	2.1 ± 0.3	2.2 ± 0.3	3.0 ± 0.4
S.aureus ATCC 25923	2.6 ± 0.3	2.3 ± 0.3	0.6 ± 0.4	2.1 ± 0.2	2.3 ± 0.3	2.3 ± 0.2	2.7 ± 0.2
S.aureus ATCC 6538	2.3 ± 0.1	2.3 ± 0.4	1.0 ± 0.3	2.2 ± 0.2	2.4 ± 0.2	2.2 ± 0.3	2.4 ± 0.1
S. aureus v329	2.0 ± 0.4	2.0 ± 0.2	1.2 ± 0.1	2.1 ± 0.1	2.4 ± 0.1	2.2 ± 0.3	2.5 ± 0.1
S.epidermidis ATCC 12228	3.4 ± 0.2	1.7 ± 0.4	1.5 ± 0.2	1.6 ± 0.3	1.6 ± 0.1	1.6 ± 0.2	2.0 ± 0.1
S.aureus ATCC 25923	2.4 ± 0.4	1.5 ± 0.3	1.3 ± 0.4	2.1 ± 0.2	2.3 ± 0.3	2.4 ± 0.1	2.4 ± 0.3
S. aureus Newman	2.0 ± 0.4	0.7 ± 0.3	1.2 ± 0.3	1.8 ± 0.4	1.9 ± 0.3	1.9 ± 0.1	2.5 ± 0.3
MRSA SCV 1204	NI	1.0 ± 0.4	1.0 ± 0.4	1.5 ± 0.2	1.5 ± 0.2	1.8 ± 0.2	1.9 ± 0.2
MRSA/Wild 1204	NI	1.0 ± 0.1	1.0 ± 0.3	1.6 ± 0.3	1.8 ± 0.1	1.8 ± 0.2	2.0 ± 0.2
S. epidermidis RP62A	1.5 ± 0.4	0.9 ± 0.4	1.5 ± 0.3	1.6 ± 0.2	1.6 ± 0.1	1.6 ± 0.3	2.0 ± 0.2
S. epidermidis	1.0 ± 0.3	1.0 ± 0.2	1.5 ± 0.3	1.5 ± 0.2	1.6 ± 0.2	1.6 ± 0.3	2.4 ± 0.3
S. pneumoniae	1.5 ± 0.2	2.0 ± 0.2	1.0 ± 0.1	1.6 ± 0.2	1.6 ± 0.2	1.9 ± 0.3	2.0 ± 0.3
B.cereus ATCC 10987	NI	1.0 ± 0.1	1.0 ± 0.3	1.3 ± 0.2	1.1 ± 0.2	1.5 ± 0.1	2.0 ± 0.2
K. pneumoniae ATCC 10031	NI	1.2 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	1.3 ± 0.1	1.7 ± 0.2	1.9 ± 0.2
Proteus spp	1.6 ± 0.1	1.2 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	1.9 ± 0.2
Serratia spp	2.0 ± 0.3	0.7 ± 0.4	1.0 ± 0.1	1.1 ± 0.2	1.0 ± 0.2	1.3 ± 0.2	2.0 ± 0.1
E. coli ATCC 8739	2.0 ± 0.3	1.3 ± 0.3	1.1 ± 0.1	1.3 ± 0.3	2.0 ± 0.1	1.8 ± 0.3	2.5 ± 0.3
S. typhi ATCC 14026	2.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.3	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	2.0 ± 0.2
C. albicans	1.5 ± 0.4[a]	0.6 ± 0.1	NI	0.7 ± 0.1	3.0 ± 0.2	2.0 ± 0.3	2.2 ± 0.2

The results are the mean ± SD (n = 3).
[a] This is the diameter of Nystatin disc (25 μg) against Candida. NI, No inhibition zone; DMSO, NI.

Table 3 Minimum inhibition concentration (MIC) for the compounds against different organisms.

Strains	Gentamicin (10 μg)	**3a**	**4a**	**3b**	**3c**	**3d**	**3e**
MIC (μg/ml)							
MRSA 1168	500	800	1250	1500	3100	1500	200
MRSA 1052	500	800	1250	1500	3100	1500	200
MRSA 755	500	800	1250	1500	3100	1500	200
MRSA 1164	500	800	1250	1500	3100	1500	200
S.epidermidis Tu 3298	0.5	400	1250	800	1500	800	24
MSSA	0.5	400	700	1500	200	1900	24
S.aureus ATCC 25923	0.5	400	700	1500	1500	1500	24
S.aureus ATCC 6538	0.5	800	2500	800	800	800	97
S. aureus v329	0.5	200	13800	15600	1500	1500	49
S.epidermidis ATCC 12228	0.5	400	700	900	400	800	24
S.aureus ATCC 25923	0.5	800	1250	1500	400	1500	48
S. aureus Newman	0.5	800	2500	1500	200	1500	48
MRSA SCV 1204	2500	1250	1270	1500	3100	1500	400
MRSA/Wild 1204	600	1250	1270	1500	3100	1500	200
S. epidermidis RP62A	0.5	400	700	800	200	1500	24
S. epidermidis	0.5	400	700	800	200	800	49
S. pneumoniae	0.5	800	1250	200	200	200	98
B.cereus ATCC 10987	ND	700	800	800	800	800	400
K. pneumoniae ATCC 10031	ND	800	1250	1500	1500	800	400
Proteus spp	1.3	800	1200	800	3120	1500	200
Serratia spp	1.6	400	3400	1500	3120	1500	200
E. coli ATCC 8739	1	200	1250	1500	400	1500	24
S. typhi ATCC 14026	1	400	2750	1500	3120	1500	200
C. albicans	6[a]	NA	NA	4000	24	400	97

[a] Nystatin used as control. NA, no activity; ND, not determined. Bold font indicates significant results.

respectively. However, all other compounds are nontoxic with IC_{50} greater than 200 μg/ml.

Finally, further study should be done on the mode of action of these compounds to elucidate the structure–function

Table 4 Minimum bactericidal/fungicidal concentration (MBC/MFC in μg/ml) for the compounds against different organisms.

Strains	Gentamicin	**3a**	**4a**	**3b**	**3c**	**3d**	**3e**
MBC/MFC in (μg/ml)							
MRSA 1168	2500	1560	2500	3000	NO MBC	1560	NO MBC
MRSA 1052	2500	1560	5000	2500	NO MBC	NO MBC	NO MBC
MRSA 755	2500	1560	2500	6250	6250	1560	1560
MRSA 1164	2500	1560	2500	3120	6250	1560	NO MBC
S.epidermidis Tu 3298	2	800	2500	3120	3120	1560	NO MBC
MSSA	2	800	1200	1560	NO MBC	1500	1500
S.aureus ATCC 25923	2	800	13800	31200	3120	3120	3120
S.aureus ATCC 6538	2	800	2500	800	NO MBC	1560	400
S. aureus v329	2	400	NO MBC	3120	3120	3120	NO MBC
S.epidermidis ATCC 12228	2	800	1200	1560	800	1560	NO MBC
S.aureus ATCC 25923	2	1560	2500	1560	800	NO MBC	48
S. aureus Newman	2	1560	2500	1500	NO MBC	1560	NO MBC
MRSA SCV 1204	>2500	800	1560	1560	NO MBC	1560	NO MBC
MRSA/Wild 1204	2500	400	1560	1560	1560	1560	NO MBC
S. epidermidis RP62A	2	800	1200	3120	400	3100	NO MBC
S. epidermidis	2	3000	1200	1560	800	1560	NO MBC
S. pneumoniae	2	1500	2500	400	400	NO MBC	400
B.cereus ATCC 10987	ND[c]	1560	2500	1560	3125	3125	800
K. pneumoniae ATCC 10031	ND	1500	5000	1560	1560	1560	800
Proteus spp	7	1500	2500	NO MBC	6000	3120	400
Serratia spp	6	1500	5000	NO MBC	12500	3120	NO MBC
E. coli ATCC 8739	5	400	2500	6200	800	NO MBC	NO MBC
S. typhi ATCC 14026	5	400	5000	NO MBC	12500	3120	NO MBC
C. albicans	30[a]	NA[b]	NA	NO MBC	200	800	400

[a] Nystatin used as a control.
[b] NA, no activity.
[c] ND, not determined.

relationship. Based on a study published on some Schiff base complexes (Joseyphus and Nair, 2008), it was suggested that the mode of action may involve various targets in microorganisms. These mechanisms can be classified into four points: (1) the interference with the cell wall synthesis as a result the cell permeability may be altered or they may disorganize the lipoproteins leading to cell death, (2) Deactivate various cellular enzymes which are important in the microorganism's metabolic pathways, (3) Formation of a hydrogen bond through the azomethine group with the active centres of cell constituents resulting in interfering with the normal cell processes, (4) Denaturation of one or more proteins of the cell, as a result of which the normal cellular processes are impaired.

Due to the similarities in structures with the substances studied by (Joseyphus and Nair, 2008), we might speculate that one of the previously suggested targets could be considered for our compounds. But our speculation should be supported by biochemical studies in the future.

4. Conclusion

In conclusion, four Schiff bases were synthesized by reacting 3,3′-diaminodipropylamine with benzaldehyde and some other derivatives. Three of the prepared compounds were prepared for the first time. The structures of the prepared compounds were confirmed by different spectroscopic methods and the antimicrobial activity was assessed. The compounds were tested against a large number of pathogenic strains of Gram-positive, Gram-negative bacteria and Candida. Compound **3e** exhibited good growth inhibition activity against pathogenic microorganisms and Candida and is promising to act as a potential antimicrobial agent. Results also revealed that compound **3c** showed moderate activity against Candida; further structural modifications could lead to a promising anti-candida agent. Based on the results obtained, slight modifications of the structures might produce potent and potential compounds which could be used as anticandida drugs, especially if further work proves that cytotoxicity of the prepared Schiff bases is less than the drug, Nystatin.

5. Experimental section

5.1. Chemistry

The following chemicals, used in this study, were purchased from Acros and were used as received: 3,3′-diaminodipropylamine, benzaldehyde, 2-hydroxybenzaldehyde, 2,4-dihydroxybenzaldehyde, 2,3,4-trihydroxybenzaldehyde, 4-nitrobenzal dehyde and 2-methoxybenzaldehyde. Silica gel for column chromatography was purchased from Macherey–Nagel GmbH & Co (Germany). Melting points (uncorrected) were determined on a Stuart scientific melting point apparatus in open capillary tubes. ^{1}H- and ^{13}C-NMR spectra were recorded on a 300 MHz spectrometer (Bruker DPX-300) with *TMS* as the internal standard. Chemical shifts are expressed in δ units; *J*-values for ^{1}H–^{1}H coupling constant are given in Hertz. High-resolution mass spectra (HRMS) were acquired (in positive or negative mode) using electrospray ion trap (ESI) technique by collision-induced dissociation with the aid of a Bruker APEX-4 (7-Tesla) instrument. The samples were dissolved in

acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1% formic acid) and infused using a syringe pump with a flow rate of 2 μL/min. External calibration was conducted using arginine cluster in a mass range *m*/*z* 175–871.

5.1.1. General procedure for preparation of **compounds 3a–e**

Compounds **3a–e** were prepared according to a published procedure that involved adding 3,3′-diaminodipropylamine (3.0 ml, 21 mmol), drop wise, to a solution of the corresponding aldehyde (42 mmol) in absolute ethanol (25 ml). The mixture was refluxed for 4 h. Evaporation of the solvent under reduced pressure afforded the desired product in 80–93% yield. The product was washed with water, dried and purified on preparative silica gel TLC plates. In case of **4a**, the synthesis of such salts is difficult, we obtained this salt by gently heating one equivalent of cyanuric acid with three equivalents of **3a** in EtOH and the salt was characterized as monochloride. Recently, AlDamen and Haddad (AlDamen and Haddad, 2012) published the crystal structure of this compound.

5.1.2. 2,2′-{iminobis[(1E)prop-3-yl-1-ylidene(E)azanylylidene]}diphenol (**3a**)

Yield = 90%, yellow oil. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.99 (m, 4H, $\underline{CH_2}CH_2NH$), 2.42 (m, 1H, NH), 2.95 (m, 4H, $\underline{CH_2}NH$), 3.63 (m, 4H, CH═N$\underline{CH_2}$), 6.85 (m, 4H, H-3 + H-5), 7.28 (t, J = 7.8 Hz, 2H, H-4), 7.39 (d, J = 7.5 Hz, 2H, H-6), 8.56 (s, 2H, CH═N), 8.71 (br m, 2H, OH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 31.51 ($CH_2\underline{CH_2}CH_2$), 45.3 (NH–C), 55.9 (═N–C), 116.9 (C-3), 119.1 (C-5), 123.9 (C-1), 132.2 (C-6), 132.9 (C-4), 160.6 (HC═N), 167.0 (C-2). HRMS (ESI) *m*/*z*: Calcd for $C_{20}H_{26}N_3O_2$ $[M + H]^+$ 340.20250; found 340.20293.

5.1.3. (3E)-3-[(2-methoxyphenyl)imino]-N-{(3E)-3-[(2-methoxyphenyl)imino]propyl}propan-1-amine (**3b**)

Yield = 80%, pale yellow oil. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.79 (m, 4H, $\underline{CH_2}CH_2NH$), 2.46 (m, 4H, $\underline{CH_2}NH$), 3.45 (m, 4H, CH═N$\underline{CH_2}$), 3.87 (s, 3H, OCH_3), 6.91 (m, 2H, H-5), 7.03 (d, J = 8.1 Hz, 2H, H-3), 7.38 (m, 2H, H-4), 7.58 (d, J = 7.7 Hz, 2H, H-6), 8.45 (s, 1H, HC═N). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 31.1 ($CH_2\underline{CH_2}CH_2$), 47.0 (NH–CH_2), 54.5 (═N–CH_2), 110.4 (C-3), 119.7 (C-5), 120.0 (C-1), 126.1 (C-6), 131.0 (C-4), 158.2 (C-2) 149.6 (C-4), 158.8 (HC═N). HRMS (ESI) *m*/*z*: Calcd for $C_{22}H_{30}N_3O$ $[M + H]^+$ 368.23380; found 368.23325.

5.1.4. 4,4′-{iminobis[(1E)prop-3-yl-1-ylidene(E)azanylylidene]}dibenzene-1,3-diol (**3c**)

Yield = 84%, m.p. = 335–338 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.67 (m, 4H, $\underline{CH_2}CH_2NH$), 2.40 (br m, 1H, NH), 2.48 (m, 4H, $\underline{CH_2}NH$), 3.46 (m, 4H, CH═N$\underline{CH_2}$), 4.51 (br s, 4H, OH). 6.09 (d, J = 2.0 Hz, 2H, H-3), 6.17 (dd, J = 9.0, 2.0 Hz, 2H, H-5) 7.09 (d, J = 9.0 Hz, 2H, H-4), 8.21 (s, 2H, CH═N). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 31.0 ($\underline{CH_2}CH_2CH_2$), 47.2 (NH–C), 54.6 (═N–C), 103.3 (C-3), 107.2 (C-5), 111.5 (C-1), 133.8 (C-6), 162.8 (HC═N), 164.8 (C-4), 166.6 (C-2). HRMS (ESI) *m*/*z*: Calcd for $C_{20}H_{26}N_3O_4$ $[M + H]^+$ 372.19233; found 372.19178.

5.1.5. (3E)-3-(phenylimino)-N-[(3E)-3-(phenylimino)propyl]propan-1-amine (**3d**)

Yield = 93%, m.p. = 62–64 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.76 (m, 4H, $\underline{CH_2}CH_2NH$), 2.40 (m, 4H, $\underline{CH_2}NH$), 2.42 (m, 1H, NH), 3.47 (m, 4H, CH═N$\underline{CH_2}$), 7.55 (m, 6H, H-3 + H-4 + H-5), 7.75 (d, J = 7.7 Hz, 4H, H-2 + H-6), 8.25 (s, 2H, CH═N). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 31.3 ($CH_2\underline{CH_2}CH_2$), 45.4 (NH–C), 58.0 (═N–C), 128.3 (C-3/C-5), 129.2 (C-2/C-6), 131.4 (C-4) 137.6 (C-1), 159.9 (HC═N). HRMS (ESI) *m*/*z*: Calcd for $C_{20}H_{26}N_3$ $[M + H]^+$: 308.21267; found 308.21306.

5.1.6. (3E)-3-[(4-nitrophenyl)imino]-N-{(3E)-3-[(4-nitrophenyl)imino]propyl}propan-1-amine (**3e**)

Yield = 83%, m.p. = 86–88 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.76 (m, 4H, $\underline{CH_2}CH_2NH$), 2.32 (m, 4H, $\underline{CH_2}NH$), 2.35 (br s, 1H, NH), 3.43 (m, 4H, CH═NCH_2), 7.58 (d, J = 8.7 Hz, 2H, H-2 + H-6), 7.70 (d, J = 8.7 Hz, 2H, H-3 + H-5), 8.21 (s, 1H, HC═N). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 27.8 ($CH_2\underline{CH_2}CH_2$), 45.5 (NH–C), 59.2 (═N–C), 123.8 (C-3/C-5), 128.6 (C-2/C-6), 141.5 (C-1) 149.6 (C-4), 158.8 (HC═N). HRMS (ESI) *m*/*z*: Calcd for $C_{22}H_{30}N_3O$ $[M + H]^+$ 368.23380; found 368.23325. HRMS (ESI) *m*/*z*: Calcd for $C_{20}H_{24}N_5O_4$ $[M + H]^+$ 398.18283; found 398.18198.

5.2. Microbiological procedures

5.2.1. Bacterial culture

About 24 bacterial strains included in this study were from American Type Culture Collection (ATCC) (Rockville, MD and USA), clinical isolates were obtained from Asem A. Shehabi (Professor of Microbiology, University of Jordan) and staphylococcal biofilm mutant strains from José R. Penadés Senior Researcher at the Consejo Superior de Investigaciones Científicas, Spain. There was one Candida strain (ATCC) included in this study was a gift from Basem Jaber (Assistant professor of mycology, The University of Jordan). All the strains were confirmed by cultural and biochemical characteristics and maintained in slants for further use. The bacterial strains were either Gram positive or Gram negative. These organisms are found in Table 1.

5.3. Antimicrobial activity

5.3.1. Disc diffusion method

The synthetic compound **3** was tested *in vitro* for their antimicrobial activity against Gram positive and Gram-negative organisms and Candida at 0.02 g/ml, respectively by Kirby–Bauer agar diffusion method (Bauer et al., 1966). The current NCCLS (CLSI document M2-A9, 2006)guidelines recommend using Mueller–Hinton agar medium for bacteria and Sabouraud dextrose agar medium for Candida (NCCLS-M27-A 1997).

Briefly, 100 μl of the test bacteria/Candida was grown in 10 ml of fresh media until they reached a count of ca. 10^8 cells/ml for bacteria (0.5 McFarland standards), One hundred microlitre (100 μl) of microbial suspension was spread onto the Mueller–Hinton agar medium.

The compounds were tested using 6 mm sterilized filter paper discs (antibiotic assay discs, Whatman-model 2017-006) as mentioned before (Antonio-Velmonte et al., 1988). Discs were impregnated with 25 μL of 0.02 g/ml of the compound and allowed to dry and placed onto inoculated plates. The plates

were allowed to stand at 4 °C for 2 h before incubation with the test microbial agents. Inoculated plates with different pathogenic bacteria and Candida were incubated at 37 °C for 24 h, and then the diameters of the inhibition zones were measured in centimetres. Each antimicrobial assay was performed in triplicate and mean values were reported. Standard antibiotics, gentamicin (10 μg/disc), and nystatin 25 μg/disc served as positive controls for antimicrobial and Candida activity, respectively. Filter discs impregnated with 10 μl of distilled water were used as a negative control. Solvent control disc (DMSO) was also placed with the test as a negative control. The inhibition zone diameters were measured. The inhibitory effects of the synthetic compounds against these organisms are given in Table 2.

5.3.2. Serial dilution method (broth microdilution assay)

A broth microdilution method was employed to determine the minimum inhibition concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) according to the National Committee for Clinical Laboratory Standards (NCCLS, Document M100–S11, 2003). The inocula of the bacterial strains or Candida were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. A serial doubling dilution of the compounds was prepared in a 96/well microtiter plate. A double strength of Mueller Hinton broth was used as a diluent. The concentrations were in range of 20–0.006 mg/ml for the compounds. Bacterial strains and Candida tested were inoculated in Mueller Hinton broth and inoculated into wells (the final concentration in each well adjusted to 2.0×10^6 CFU/ml for bacteria and 2.0×10^5 of Candida strains). The plate was incubated for 24 h at 37 °C. A control well containing the growth medium and the bacteria or Candida was set-up. Gentamicin and Nystatin served as positive controls, while the solvent (DMSO) was used as a negative control. MIC was defined as the lowest concentration of compound at which micro organisms show no visible growth as shown in Table 3. To determine MBC/MFC broth was taken from each well and inoculated on Mueller Hinton agar for 24 h at 37 °C for bacteria or in Sabouraud dextrose agar for Candida strains. The results were obtained for MBC/MFC as the lowest concentration of antibiotic required to kill the organism as indicated in Table 4.

5.3.3. Cytotoxicity study

The Vero cell line was used to determine the cytotoxicity of compounds included in this investigation. Vero cells were grown in Minimum Essential Medium Eagle (MEM) (Gibco, UK) supplemented with 10% heat inactivated fetal bovine serum (Gibco, UK), 29 μg/ml L-glutamine, and 40 μg/ml gentamicin and were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. The cytotoxicity of compounds was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Promega, USA). The assay detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product, which reflects the normal function of mitochondria and cell viability (Lau et al., 2004). Eight concentrations (200, 150, 100, 50, 25, 12, 6, and 3 μg/ml) were prepared from each compound. Exponentially growing Vero cells were washed and seeded at 17000 cells/well (in 200 μl of growth medium) in 96 well microplates (Nunc, Denmark). After 24 h incubation, a partial monolayer was formed then the medium was removed and 200 μl of the medium containing the compound (initially dissolved in DMSO) was added and re-incubated for 48 h. Then 100 μl of the medium was aspirated and 15 μl of the MTT solution was added to the remaining medium (100 μl) in each well. After 4 h contact with the MTT solution, blue crystals were formed. 100 μl of the stop solution was added and incubated further for 1 h. Reduced MTT was assayed at 650 nm using a microplate reader (Das, Italy). Control groups received the same amount of DMSO (0.1%). Untreated cells were used as a negative control.

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