



Original Research Article

Novel unsymmetric diimines: Synthesis and biological evaluation against pathogenic microorganisms

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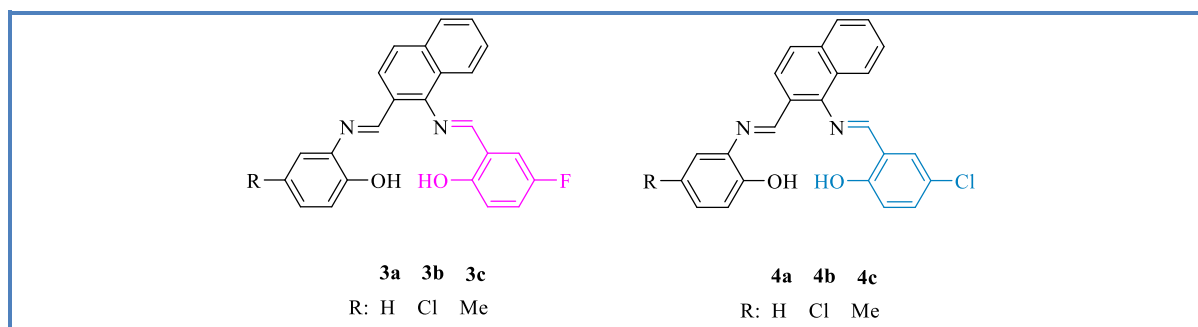
ABSTRACT

In this research study, three new unsymmetric diimines (**3a-3c**) were prepared using a two-step process. The synthesized unsymmetric tetradentate diimines were elucidated by FT-IR, ¹H-NMR, LC-MS, elemental analysis techniques. The antimicrobial and the antifungal properties of newly synthesized unsymmetrical diimines (**3a-3c**) and previously reported by one of us unsymmetrical diimines (**4a-4c**) were evaluated against *L. monocytogenes 4b*, *B. cereus sp.*, *S. typhi H*, *Br. abortus*, *S. epidermis sp.*, *M. Luteus sp.*, *E. coli*, *P. putida sp. Sh. dys. typ. 7* ve *C. albicans*. **3b**, **3c**, **4a** and **4c** are showed the highest antimicrobial activity against *B.cereus sp.* **3a** and **4b** are exhibited the highest activity against *S.epidermis sp.* and *Br. abortus*, respectively. In addition, all unsymmetrical diimines are showed high antifungal activity against *C. albicans*.

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Graphical Abstract



Introduction

Schiff bases are also called as imine or azomethine and are obtained from the reaction of amines and aldehyde or a ketone [1]. They have been widely investigated due to their potential applications in many fields. Imines and their metal complexes can act as catalysts, pigments, dyes, intermediates and cation carriers in various systems, such as, biological, biochemical, enzymatic, organic, and potentiometric sensor [2-6]. Some imine complexes show plant growth regulator activity and insecticidal activity [7]. Most of the imines have antimicrobial and antifungal efficacy against different microorganisms [8, 9]. Schiff bases have a broad range of biological efficacy as they include azomethine [10, 11]. They have some features such as antimalarial, antimicrobial, antitumor, antiinflammatory, and antiviral. They have also anticancer, antioxidant, antidepressant, and antitubercular activities [12-17]. Symmetrical Schiff bases (i.e. $-C_aH=N_a\text{-aryl-N}_b=C_bH-$ or $-N_a=HC_a\text{-aryl-C}_bH=N_b-$) could be directly by condensation reaction of aromatic diamines/or dialdehydes and aldehydes/or amines. However, the symmetrical Schiff bases (i.e. $-C_aH=N_a\text{-aryl-C}_bH=N_b-$) cannot be obtained directly due to a reaction formed between $-NH_2$ and $-CHO$ groups in the same aromatic ring. These type unsymmetrical ligands were first reported by one of us proposing a novel two-stage procedure [18]. Hereafter, studies on these type Schiff bases containing unsymmetric imines on the same aromatic ring were followed due to their special structures [19-23]. In this study, we report the preparation and elucidate of novel three unsymmetrical diimines. Previously, synthesis of different three unsymmetric ligands was reported by one of us [24]. We also investigated the biological efficacy of six

unsymmetrical imines against selected pathogenic microorganisms with yeast.

Experimental

All the substances were obtained from Sigma-Aldrich and Merck. Elemental analysis was performed using the Leco CHNS-932 spectrometer. Infrared spectroscopy analysis was obtained using a Mattson-5000 FT-IR spectrophotometer. 1H -NMR spectra were performed using a Bruker Avance 500 MHz spectrometer. Mass spectra were acquired with an Agilent Technologies 6410 Triple Quad.

General procedure for synthesis of unsymmetric diimines 3a-3c

The starting Schiff bases **1a** (**1b** or **1c**) were obtained by reacting 50 mmol of 2-aminophenol (or 2-amino-4-chlorophenol or 2-amino-4-methylphenol) with 1-nitro-2-naphthaldehyde (50 mmol) in ethyl alcohol (100 mL) and heated for 1 h at 60 °C as we described previously [24]. The unsymmetric diimines **3a-3c** were synthesized with two-stage procedure (Figure 1). Firstly, the starting Schiff base **1a** (or **1b** or **1c**) (2 mmol) was solved in 100 mL ethyl alcohol-water mixture (1v:1v) at 70 °C. To obtain the amino derivative of the unsymmetric diimines, reduction proceeding was done using 5 mmol sodium dithionite ($Na_2S_2O_4$). At this stage, the solid $Na_2S_2O_4$ was added to the mixture in 1 h. The boiling and stirring process was continued for a further 1 h at 45 °C. Secondly, to prepared the unsymmetrical diimines **3a-3c**, 5-fluoro-2-hydroxybenzaldehyde (2 mmol) was dissolved in ethyl alcohol (25 mL) and added to the solution and was heated for 2 h at 55 °C under reflux. The solution was cooled at room temperature for 1 day and the orange solid was obtained.

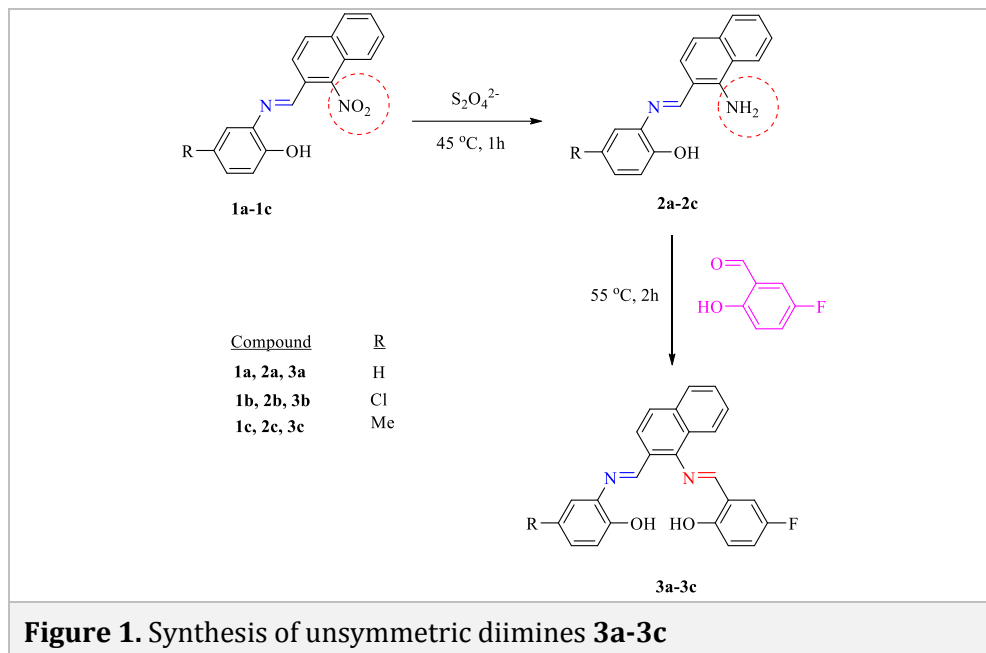


Figure 1. Synthesis of unsymmetric diimines **3a-3c**

Compound **3a**:

It was obtained as orange product. Yield: 65%, m.p: 187 °C; IR (KBr, ν cm^{-1}): 3316 (OH), 3051 (CH)_{arom.}, 1629, 1609 (C=N), 1594-1459 ($\text{C}=\text{C}$)_{ring}; $^1\text{H-NMR}$ (500 MHz, DMSO-d_6 , δ ppm): 13.42 (s, 1H, OH), 10.20 (s, 1H, OH), 9.89 (s, 1H, CH=N), 8.95 (s, 1H, CH=N), 6.30-8.48 (m, arom.-H); MS m/z : 386.3 [$\text{M}+2\text{H}$]⁺. Anal. calcd. for $\text{C}_{24}\text{H}_{17}\text{N}_2\text{O}_2\text{F}$: C, 75.00, H, 4.43, N, 7.29; found: C, 73.26, H, 4.22, N, 6.82.

Compound **3b**:

It was obtained as orange product. Yield: 61%, m.p: 196 °C; IR (KBr, ν cm^{-1}): 3451 (OH), 3057 (CH)_{arom.}, 1603, 1588 (C=N), 1547-1460 ($\text{C}=\text{C}$)_{ring}; $^1\text{H-NMR}$ (500 MHz, DMSO-d_6 , δ ppm): 13.04 (s, 1H, OH), 10.03 (s, 1H, OH), 9.54 (s, 1H, CH=N), 8.80 (s, 1H, CH=N), 6.36-8.43 (m, arom.-H); MS m/z : 421.0 [$\text{M}+3\text{H}$]⁺. Anal. calcd. for $\text{C}_{24}\text{H}_{16}\text{N}_2\text{O}_2\text{ClF}$: C, 68.82, H, 3.82, N, 6.69; found: C, 70.15, H, 3.14, N, 7.21.

Compound **3c**:

It was obtained as orange product. Yield: 59%, m.p: 204 °C; IR (KBr, ν cm^{-1}): 3310 (OH),

3056 (CH)_{arom.}, 1610, 1594 (C=N), 1545-1465 ($\text{C}=\text{C}$)_{ring}; $^1\text{H-NMR}$ (500 MHz, DMSO-d_6 , δ ppm): 13.50 (s, 1H, OH), 10.18 (s, 1H, OH), 9.88 (s, 1H, CH=N), 9.69 (s, 1H, CH=N), 6.52-8.48 (m, arom.-H), 2.50 (s, CH_3); MS m/z : 400.0 [$\text{M}+2\text{H}$]⁺. Anal. calcd. for $\text{C}_{25}\text{H}_{19}\text{N}_2\text{O}_2\text{F}$: C, 75.38, H, 4.77, N, 7.04; found: C, 74.77, H, 4.52, N, 6.26.

General procedure for synthesis of unsymmetric diimines **4a-4c**

As we described earlier, the unsymmetrical diimines **4a-4c** were synthesized and demonstrated in Figure 2 [24]. Primarily, the starting Schiff bases **1a** (**1b** or **1c**) were obtained by reacting 50 mmol of 2-aminophenol or its derivatives (2-amino-4-chlorophenol, 2-amino-4-methylphenol) with 1-nitro-2-naphthaldehyde (50 mmol) in ethyl alcohol (100 mL). To obtain amino derivative of the unsymmetric diimines, reduction proceeding was done using 5 mmol $\text{Na}_2\text{S}_2\text{O}_4$. Lastly, to prepared the unsymmetrical diimines **4a-4c**, 2-hydroxy-5-chlorobenzaldehyde (2 mmol) was dissolved in ethyl alcohol (25 mL) and added to the solution.

Spectroscopic data for compound **4a-4c** were reported [24].

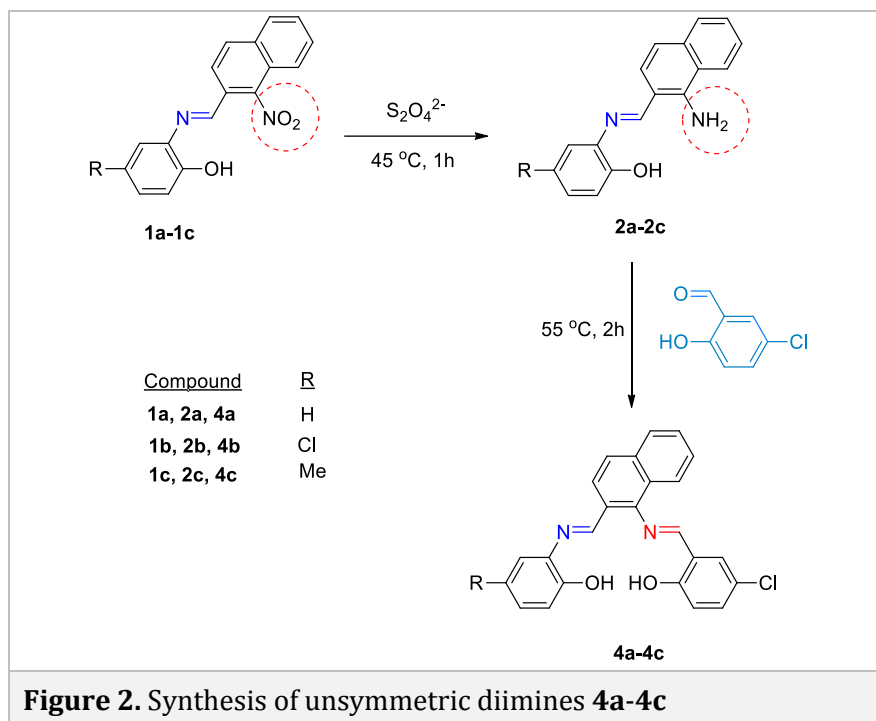


Figure 2. Synthesis of unsymmetric diimines **4a-4c**

Detection of antimicrobial activity

The antimicrobial and antifungal efficacy of the unsymmetric diimines were studied using well-diffusion method against *Sh. dys. typ. 7* (NCTC-9363), *L. monocytogenes 4b* (ATCC 19115), *E. coli* (ATCC 1230), *S. typhi H* (NCTC 901.8394), *S. epidermis sp.* (ATCC 12228), *Br. abortus* (RSKK-03026), *M. Luteus sp.* (ATCC 93419), *B. cereus sp.*, *P. putida sp.* and *C. albicans* (Y-1200-NIH).

In this screening, the dimethylformamide (DMF) was used as solvent control. It was found to have no antimicrobial activity against any of the tested organisms. All unsymmetrical diimines were kept dry at room temperature and solved (3.5 µg/mL) in DMF 1% (v/v) of a 24-h broth culture including 106 CFU/mL was poured into sterile Petri dishes. Molten nutrient agar was studied for culturing the test bacteria and it was stored at ca. 45 °C. The molten agar

was poured into sterile petri dishes and was left for solidification. Later, holes of 6 mm diameter were pierced with sterile cork borer and the test solutions were filled into each of the bores. As the last stage, the plates were incubated at 37 °C for 24 h. Average value determined for all the holes were used to compute the zone of inhibition growth. Pathogenic bacterial cultures and yeast were tested for resistance to five antibiotics (produced by Oxoid Ltd., Basingstoke, UK): kanamycin, sulphamethoxazol, ampicillin, amoxicillin, and nystatin. Their properties are as follows: kanamycin (used in the treatment of infections susceptible to microorganisms), sulphamethoxazol (it affects the synthesis of folic acid in sensitive bacteria), ampicillin (it inhibits the growth of gram (-) bacteria), amoxicillin (it is a penicillin effective against gram (+) and gram (-) microorganisms and it is a broad spectrum antibiotic with bactericidal

effect), nystatin (it binds with the sterols in the cell membrane of fungus and changes the membrane permeability).

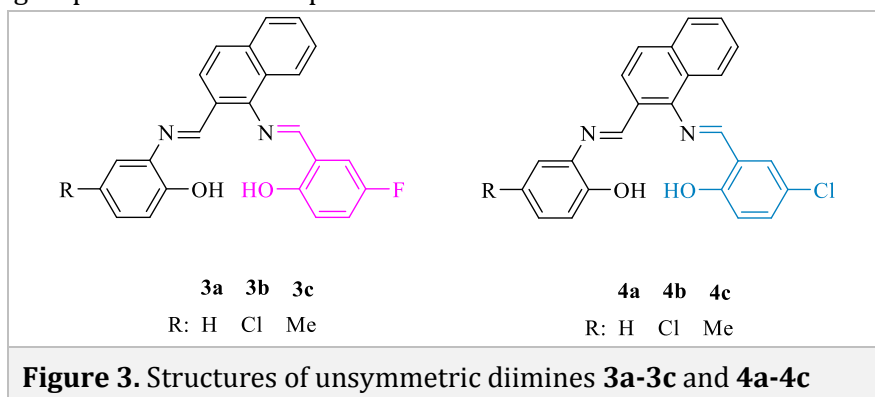
Results and Discussion

Chemistry

The ligands **3a-3c** reported herein are unsymmetric in terms of the 'location' of the azomethine bond ($-C_aH=N_a-aryl-C_bH=N_b-$) and in terms of the groups on the terminal phenolic

residues. **3a**, **3b** and **3c** are N_2O_2 type tetradentate ligands with two imine nitrogens and two phenolic oxygens (Figure 3). These unsymmetric diimines are less soluble in polar solvents and insoluble in water and are stable at room temperature, insoluble in water, and slightly soluble in polar organic solvents. The spectral data of these unsymmetric diimines **3a-3c** is presented in Table 1-3.

The spectroscopic data of unsymmetrical ligands **4a-4c** were presented [24].



The elemental analyses and MS data of unsymmetric diimines are presented in Table 1. The elemental analyses are compatible with the chemical formulas of the unsymmetric diimines. The molecular ion peaks and the fragmentation products are also compatible with the suggested structures of unsymmetric ligands.

The molecular ion peaks are determined at the foreseen values of m/z : 386.3 $[M+2H]^+$ **3a**, 421.0 $[M+3H]^+$ **3b** and 400.0 $[M+2H]^+$ **3c**. The highest intensity peak for **3a** at m/z : 232.1, m/z : 283.0 for **3b** and m/z : 329.1 for **3c**, respectively, are assigned to the loss of the different fragmentation pathways.

Table 1. Elemental analyses and mass spectral data for unsymmetric diimines							
Compound	Elemental analysis (calc.) %			Mass spectrum			
	Formula Colour	C	H	N	m/z	%	Peak
3a	$C_{24}H_{17}N_2O_2F$ Orange	73.26 (75.00)	4.22 (4.43)	6.82 (7.29)	386.3	17.5	$[M+2H]^+$
3b	$C_{24}H_{16}N_2O_2ClF$ Orange	70.15 (68.82)	3.14 (3.82)	7.21 (6.69)	421.0	3.9	$[M+3H]^+$
3c	$C_{25}H_{19}N_2O_2F$ Orange	74.77 (75.38)	4.52 (4.77)	6.26 (7.04)	400.0	15.0	$[M+2H]^+$

The characteristic peak of the unsymmetric diimines **3a-3c** are presented in Table 2. The IR spectra of all diimines indicate two strong bands in the region 1588-1629 cm^{-1} , associated to $\nu\text{C}=\text{N}$. These peaks are observed due to the

two unsymmetrical imine groups. The $\nu\text{C}=\text{C}$ bands of all ligands are observed in the ranges 1459-1594 cm^{-1} . The νOH and $\nu\text{CH}_{(\text{arom.})}$ are appeared in the 3310-3451 cm^{-1} with 3051-3057 cm^{-1} regions, respectively.

Table 2. Mean Values of Characterized FCT Formulated by Wet Granulation Based on US Pharmacopeia.

Compound	IR				$^1\text{H-NMR}$			
	$\nu(\text{OH})$	$\nu(\text{CH}_{\text{arom.}})$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}=\text{C})_{\text{ring}}$	OH	CH=N	Arom.-H	CH_3
3a	3316	3051	1629, 1609	1594- 1459	13.42(s) 1H 10.20(s) 1H	9.89(s) 1H 8.95(s) 1H	6.30- 8.48 (m)	-
3b	3451	3057	1603, 1588	1547- 1460	13.04(s) 1H 10.03(s) 1H	9.54(s) 1H 8.80(s) 1H	6.36- 8.43 (m)	-
3c	3310	3056	1610, 1594	1545- 1465	13.50(s) 1H 10.18(s) 1H	9.88(s) 1H 9.69(s) 1H	6.52- 8.48 (m)	2.50 (s)

The $^1\text{H-NMR}$ spectra for unsymmetric diimines **3a-3c** are presented in Table 2. Due to the different chemical environments of the asymmetric imines, the $^1\text{H-NMR}$ spectra of all ligands display two signals in the range 8.80-9.69 ppm and 9.54-9.89 ppm. The two phenolic protons are observed in the region 10.03-10.20 ppm and 13.04-13.50 ppm for all ligands. The aromatic protons are revealed in the ranges 6.30-8.48 ppm. Furthermore, the peak attributable to CH_3 in **3c** is appeared at 2.50 ppm.

Biological evaluation

The antimicrobial activities for all unsymmetric diimines **3a-3c** and **4a-4c** are presented in Table 3. The unsymmetrical diimines were screened for antimicrobial activity against gram (+) *L. monocytogenes* 4b, *S. epidermis*, *M. luteus* sp., *B. cereus* sp., gram (-) *Sh. dys. typ. 7*, *E. coli*, *S. typhi* H, *Br. abortus*, *P. putida* sp. and the fungus *C. albicans* in DMF solvent

control. All unsymmetric diimines and antibiotics are shown varying degree of inhibitory influences on the growth of varied selected strains. It can be said that the functional substituents (H, F and Cl) on the benzene ring a very important factor in influencing the biological efficacy of the unsymmetric ligands [25]. **3b**, **3c**, **4a** and **4c** are exhibited the highest antimicrobial activity against *B.cereus* sp. The bacteria is known as opportunist pathogens, a food-poisoning organism, and common contaminating organisms [26]. **3a** and **4b** are showed a significant activity against *B.cereus* sp. At the same time, **3a** and **4b** are showed the highest activity against *S.epidermis* sp. and *Br. abortus*, respectively. *S.epidermis* sp. is an opportunistic microorganism. It is major skin associated bacteria. The bacteria is regarded as one of the main cause of nosocomial infections [27]. *Br. abortus*, is an intracellular pathogen leading to bacteremia and chronic infections in humans

[28]. Additionally, **4b** is exhibited a significant activity against *S.epidermis sp.*, too. The compounds (**3b**, **3c**, **4b** and **4c**) that have electron-withdrawing groups F and Cl are more active than the compounds (**3a** and **4a**) that including H atom for *B.cereus*, *S.epidermis* and *Br. abortus* (except **4c** for *Br. abortus*). **3a** and **4c** are inactive against *Sh.dys. typ 7* and *Br. abortus*, respectively. All unsymmetrical ligands are showed high antifungal activity against *C. albicans*. It is formed various infections in humans and animals for antifungal activity [29]. In general, the results of antimicrobial screening point out that **4a-4c** groups showed

more activity than the other **3a-3c** groups. As a result, it can be said that all unsymmetric diimines are pharmacologically active compounds. According to the results, it can be said that researches of such unsymmetric Schiff bases can be important. Additionally, the antimicrobial activity of the unsymmetric diimines was also check against five commercial antibiotics, so-called Kanamycin, Sulfamethoxazol, Ampicillin, Amoxycillin and Nystatin. The obtained the unsymmetrical diimines were found to be as effective as the indicated antibiotics.

Table 3. Antimicrobial activities of unsymmetric diimines (diameter of zone of inhibition (mm))

Microorganisms	Compound						
	3a	3b	3c	4a	4b	4c	
<i>S.aureus</i>	14	14	12	19	16	16	
<i>Sh.dys. typ 7</i>	-	22	22	15	20	20	
<i>L.monocytogenes</i>	13	15	20	19	19	24	
<i>4b</i>							
<i>E.coli</i>	17	15	14	23	20	20	
<i>S.typhi H</i>	13	13	14	20	15	20	
<i>S.epidermis sp.</i>	21	25	23	25	28	30	
<i>Br. abortus</i>	12	20	13	25	30	-	
<i>M.luteus sp.</i>	16	25	19	24	22	30	
<i>B.cereus sp.</i>	19	28	24	26	28	33	
<i>P.putida sp.</i>	18	18	16	25	23	28	
<i>Candida albicans</i> (Fungus)	25	25	22	26	29	25	
DMF (solvent control)	-	-	-	-	-	-	
Positive Control		<i>L.monocytogenes</i>	<i>E.coli</i>	<i>S.typhi H</i>	<i>Br.abortus</i>	<i>P.putida</i>	<i>C.albicans</i>
K30		15	25	20	-	14	-
SXT25		11	18	17	-	18	-
AMP10		16	10	11	-	8	-
AMC30		22	14	19	-	15	-
NYS100		-	-	-	-	-	20

Standart reagents: K30 Kanamycin 30 µg, SXT25 Sulfamethoxazol 25 µg, AMP10 Ampicillin 10 µg, AMC30 Amoxycillin 30 µg, NYS100 Nystatin 100 µg.

Conclusions

Novel unsymmetric diimines **3a-3c** were obtained using a two-stage method. Herein, particularly these type ligands were selected

due to the unsymmetrical nature of the imine bond. The synthesized compounds were structurally identified using the spectral analysis. In addition, antibacterial and antifungal activities of the unsymmetric diimines **3a-3c** and **4a-4c** were researched against some pathogenic microorganisms. According to these antimicrobial results, it can be stated that these unsymmetric diimines might be used in potential biomedical applications owing to their antimicrobial properties.

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Conflict of interest

We have no conflicts of interest to disclose.

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