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SYNTHESIS AND BIOLOGICAL EVALUATION OF SCHIFF BASE INVOLVING THIENO[2,3-D] PYRIMIDINE MOIETY AS ANTIMICROBIAL AGENTS

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ABSTRACT: A new series of thieno[2,3-*d*]pyrimidine derivatives were synthesized starting from ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate 1. The characterization of the newly synthesized compounds was established by IR, ^{1}H NMR and ^{13}C NMR. The synthesized compounds were screened for their antibacterial activity against Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, antifungal activity against *Candida albicans.* **Keywords:** Thieno[2,3-*d*]pyrimidine derivatives, Schiff base, Thiazolidinone, Antibacterial activity, Antifungal activity.

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1. INTRODUCTION

In the last few decades, the chemistry of pyrimidine and their fused heterocyclic derivatives has received considerable attention due to the influential role of pyrimidine unit in the functions of biologically important molecules [1]. Thienopyrimidine is an aromatic heterocyclic containing the fusion of thiophene and pyrimidine to consider as a bioisostere of quinazoline [2]. A large number of thieno[2,3-*d*]pyrimidine derivatives are reported to display analgesic [3-5], anti-inflammatory [6,7], antidiabetic [8-11], antioxidant [12,13], antimalarial, antiallergic [14], anticonvulsant[15], antiviral [16], anticancer [17-20], insecticidal[21], antibacterial[22-24] antimicrobial[25-27], adenosine receptor [28-30], aurora B kinase inhibitor [31, 32], human protein kinase CK2 inhibitor

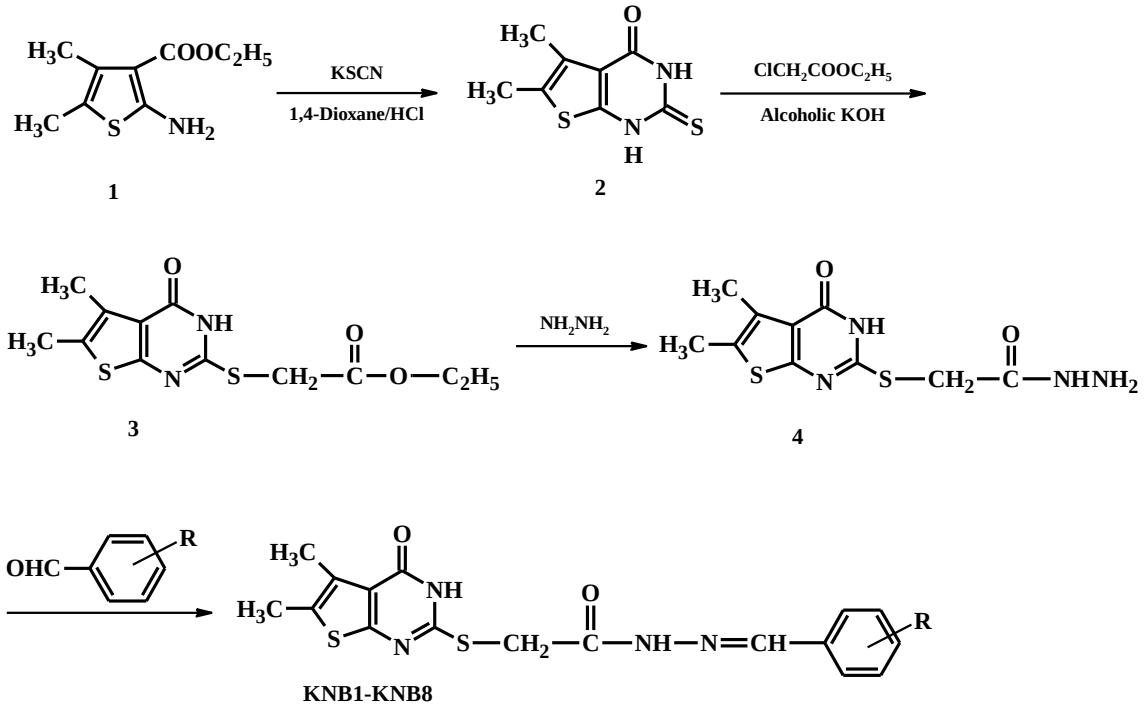
[33, 34] activity. In literature, it was documented that several compounds based on the thieno[2,3-*d*]derivatives have been synthesized and evaluated for antibacterial and antifungal activities against various strains.

2. MATERIALS AND METHODS

The structures of the synthesized compounds were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance and Fourier transform infrared. ^{1}H NMR spectra were recorded with NMR spectrometer varian-400 MHz at Centre of Excellence, Vapi, in DMSO-d_6 using TMS as internal standard and chemical shifts are expressed in δ ppm. ^{13}C NMR spectra of the compounds were recorded with NMR spectrometer varian-400 MHz at Centre of Excellence, Vapi. The IR spectra were recorded on FTIR spectrometer PerkinElmer using KBr disc. The progress of reactions and the purity of synthesized compounds were checked by TLC on E-Merck precoated 60 F_{254} plates and the spotes were examined under short-wave UV light.

Experimental procedure

General synthetic procedure for compounds (KNB1-KNB8) was performed according to Scheme 1. Ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (1), used as the key intermediate for further synthesis, was prepared in good yield as per the reported procedure [35]. Compound (1) was treated with potassium thiocyanate in dioxane in the presence of concentrated hydrochloric acid, it furnished a single product identified as 5,6-dimethyl-2-thioxo-2,3-dihydrothieno[2,3-*d*] pyrimidin-4(1*H*)-one (2), which was established on its spectral data. Then reaction between compound (2) and ethyl chloroacetate in alcoholic KOH to form ethyl((5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio)acetate (3). Hydrazinolysis of compound (3) in ethanol afforded the corresponding hydrazino derivative (4). compound (4) and aromatic aldehyde were refluxed to afford 2-((5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio)-*N*′-(substitutedbenzylidene) acetohydrazide [KNB1-KNB8]. The entire synthesized compound also confirmed by ^{1}H NMR and ^{13}C NMR analysis.



R=2-Cl, 4-F, 4-NO_2, 4-CH_3, 4-OCH_3, 2-OH, -H, 3-OPh

Synthesis of ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate [1]

To a mixture of 2-butanone 144.23 g (2.0 mol), ethyl-2-cyano acetate 113.13 g (1.0 mol), powdered elemental sulfur 32.06 g (1.0 mol) in ethanol (150 mL) was added stirring morpholine 87.13 g (1.0 mol) over a period of 30 min at room temperature. The reaction mixture was gradually heated to 60 °C in the water bath. The sulphur dissolves gradually; the temperature of the reaction mixture was maintained for 3 hrs. The reaction was monitored by TLC (R_f: 0.8 in 10% ethyl acetate in *n*-hexane). After 3 hrs, once the reaction was completed, it was allowed to cool at room temperature and concentrated under reduce pressure maintaining the water bath temperature 40 °C. The obtained residue was poured on mixture of ice and water, the mixture was filtered off with suction and crystallized from a little ethanol to get compound 1 with 70 % yield.

Mol. Formula: $C_9H_{13}NO_2S$, Mol. Wt:) 199.27, M. P. 88-92 °C, IR: (KBr) ν (cm^{-1}): 3404, 3301 (NH), 2938, 2929, 2902 (CH_3), 1648 (COO); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.21 (3H, s, CH_3), 2.03 (3H, s, CH_3), 2.04 (3H, s, CH_3), 4.13 (2H, q, NH), 7.10 (2H, s, NH).

Synthesis of 5,6-dimethyl-2-thioxo-2,3-dihydrothieno[2,3-*d*]pyrimidin-4(1*H*)-one [2]

A mixture of ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate 130 g (0.654 mol) and potassium thiocyanate 191 g (1.963.0 mol) in 1,4-doxane (325 ml) and 10 % HCl (325 ml) was reflux for 4 hrs. The reaction was monitored by TLC (R_f: 0.45 in 10% ethyl acetate in n-hexane). After the completion of reaction, the mixture was cooled to room temperature during which compound was precipitated which was then poured into ice cold water with stirring. The separated solid was filtered off, washed with water, dried, recrystallized from ethanol and to get compound 2 with ~58 % Yield.

Mol. Formula: $C_8H_8N_2OS_2$, Mol. Wt: 212.29, M. P. 268-270 °C, IR: (KBr) ν (cm^{-1}): 3389, 3304

(2NH), 3199-2979 (CH_3), 1655 (CO); ^{1}H NMR (DMSO-d_6): δ (ppm) 2.12 (3H, s, CH_3), 2.18 (3H, s, CH_3), 9.45 (1H, s, NH), 13.78 (1H, s, NH).

Synthesis of ethyl((5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl) thio)acetate [3]

To a warmed ethanolic KOH solution [prepared by dissolving KOH 30.22g (0.458 mol) in ethanol (150 mL)] was added compound 2, 80.5 g (0.382 mol), the heating was continued for 30 min and the mixture was allowed to cooled to room temperature, and the ethyl-2-chloroacetate (56.11 g, 0.458 mol) was added. The mixture was stirred under reflux for 2hrs. The reaction was monitored by TLC (R_f: ~0.65 in 10 % ethyl acetate in n-hexane). Once the reaction was completed, it was cool to room temperature and poured into cold water (600 mL). The obtained solid was filtered off, washed with water, dried and crystallized from ethanol to obtain compound 3 with ~58 % Yield.

Mol. Formula: $C_{13}H_{16}N_2O_3S_2$, Mol. Wt: 312.40, M. P. 180-182 °C. IR: (KBr) ν (cm^{-1}): 3428 (NH), 2980-2842 (CH_3), 1738 (COO), 1660 (CO); ^{1}H NMR (DMSO-d_6): δ (ppm) 1.34 (3H, t, CH_3) 2.12 (3H, s, CH_3), 2.18 (3H, s, CH_3), 3.22 (2H, q, CH_2), 3.64 (2H, s, CH_2), 9.40 (1H, s, NH).

Synthesis of 2-[(5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio]aceto hydrazide [4]

A mixture of compound 3, 80.2g (0.269 mol) and 85% hydrazine hydrate17.7g (0.282 mol) in ethanol (400 mL) was stirred reflux for 1 h. The reaction was monitored by TLC (R_f: ~0.3 in 10 % ethyl acetate in *n*-hexane). Once the reaction was completed, the reaction mixture was allowed to cool to 0°C, filtered off, washed with chilled methanol, and dried to obtain compound 4 with 85 % Yield.

Mol. Formula: $C_{10}H_{12}N_4O_2S_2$, Mol. Wt: 284.35, M. P. 188-190 °C. IR: (KBr) ν (cm^{-1}): 3420-3302 (NH_2, NH), 2988-2842 (CH_3), 1672 (CO); ^{1}H NMR (DMSO-d_6): δ (ppm) 2.24 (3H, s, CH_3), 2.30 (3H, s, CH_3), 3.33 (2H, s, NH), 3.61 (2H, s, CH_2), 6.34 (1H, s, NH), 9.34 (1H, s, NH).

Synthesis of 2-((5,6-dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio) -*N*′-(substitutedbenzylidene) acetohydrazide [KNB1-KNB8]

A mixture of compound 4, (1.0 mol), substituted Aromatic aldehyde (1.05 mol) and 2-3 drops of glacial acetic acid in methanol (5 mL) was heated to reflux for 3 hrs. The reaction was monitored by TLC (R_f: ~0.35 in 40% ethyl acetate in *n*-hexane). Once the reaction was completed, the reaction mixture was allowed to cool to 0°C, filtered off, washed with chilled methanol, and dried to obtain compound KNB1 to KNB8.

KNB1: *N*'-(2-chlorobenzylidene)-2-((5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin -2-yl)thio)acetohydrazide

75 % Yield, Mol. Formula: $C_{17}H_{15}ClN_4O_2S_2$, Mol. Wt: 406.90, M. P. 189-191 °C; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.25 (3H, s, CH_3), 2.30 (3H, s, CH_3), 4.37 (2H, s, CH_2), 7.19-7.47 (4H, m, Ar-H), 8.14 (1H, s, CH), 11.67 (1H, s, NH), 12.57 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 12.85, 13.16,

32.43, 120.28, 127.85, 128.86, 129.43, 129.51, 129.66, 129.75, 131.13, 132.75, 146.19, 155.46, 158.74, 164.78, 169.19.

KNB2:2-((5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio)-*N*'-(4-fluoro benzylidene) acetohydrazide

78 % Yield, Mol. Formula: $C_{17}H_{15}FN_4O_2S_2$, Mol. Wt: 390.45, M. P. 188-190 °C; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.26 (3H, s, CH_3), 2.30 (3H, s, CH_3), 4.41 (2H, s, CH_2), 7.20-8.01 (4H, m, Ar-H), 8.21 (1H, s, CH), 11.91 (1H, s, NH), 12.61 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 12.81, 13.10, 32.44, 116.18, 120.22, 127.85, 128.86, 131.10, 132.85, 146.10, 155.47, 158.70, 162.30, 164.73, 169.29

KNB3:2-((5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio)-*N*'-(4-nitro benzylidene)acetohydrazide

58 % Yield, Mol. Formula: $C_{17}H_{15}N_5O_4S_2$, Mol. Wt: 417.46, M. P. 288-290 °C; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.26 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 4.39 (s, 2H, CH_2), 7.42-8.00 (m, 4H, Ar-H), 8.18 (s, 1H, CH), 11.87 (s, 1H, NH), 12.71 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 12.80, 13.22, 32.48, 120.33, 123.89, 12700, 127.98, 128.99, 138.81, 146.12, 147.99, 155.40, 158.73, 164.72, 169.13.

KNB4:2-((5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio)-*N*'-(4-methyl benzylidene)acetohydrazide

70 % Yield, Mol. Formula: $C_{18}H_{18}N_4O_2S_2$, Mol. Wt: 386.49, M. P. 163-165 °C; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.19 (3H, s, CH_3), 2.23 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 4.42 (s, 2H, CH_2), 7.16-7.37 (m, 4H, Ar-H), 8.14 (s, 1H, CH), 11.64 (s, 1H, NH), 12.50 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 12.78, 13.22, 21.11, 32.55, 120.33, 127.70, 127.23, 128.88, 129.48, 131.31, 138.88, 146.54, 155.62, 158.64, 164.86, 169.15.

KNB5:2-((5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio)-*N*'-(4-methoxy benzylidene)acetohydrazide

74 % Yield, Mol. Formula: $C_{18}H_{18}N_4O_3S_2$, Mol. Wt: 402.49, M. P. 154-156 °C; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.23 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 4.35 (2H, s, CH_2), 6.82-7.33 (m, 4H, Ar-H), 8.17 (s, 1H, CH), 11.64 (s, 1H, NH), 12.50 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 2.75, 13.10, 32.83, 55.77, 114.41, 120.40, 127.00, 127.97, 128.63, 129.74146.67, 155.67, 158.43, 160.02, 164.66, 169.40.

KNB6:2-((5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio)-*N*'-(2-hydroxy benzylidene)acetohydrazide

79 % Yield, Mol. Formula: $C_{17}H_{16}N_4O_3S_2$, Mol. Wt: 388.46, M. P. 194-196 °C; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.27 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 4.01 (s, 2H, CH_2), 4.12 (1H, s, OH), 6.80-7.65 (m, 4H, Ar-H), 8.30 (s, 1H, CH), 10.96 (s, 1H, NH), 12.11 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 12.57, 13.35, 32.47, 116.64, 120.30, 120.50, 120.89, 127.65, 127.86, 128.45, 129.61, 146.87,

155.60, 158.67, 158.90, 164.70, 169.34.

KNB7:*N*'-benzylidene-2-((5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio) acetohydrazide

62 % Yield, Mol. Formula: $C_{17}H_{16}N_4O_2S_2$, Mol. Wt: 372.46, M. P. 144-146 °C; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.29 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 4.02 (s, 2H, CH_2), 7.02-7.70 (m, 5H, Ar-H), 8.22 (s, 1H, CH), 11.68 (s, 1H, NH), 12.63 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ (ppm) 12.60, 13.34, 32.49, 120.78, 127.54, 127.70, 128.00, 128.86, 130.50, 136.56, 146.28, 155.40, 158.64, 164.70, 169.21.

KNB8:2-((5,6-Dimethyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-2-yl)thio)-*N*'-(3-phenoxy benzylidene)acetohydrazide

60 % Yield, Mol. Formula: $C_{23}H_{20}N_4O_3S_2$, Mol. Wt: 464.55, M. P. 126-128 °C; ^{1}H NMR (DMSO-d_6): δ (ppm) 2.26 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.99 (s, 2H, CH_2), 7.37-7.91 (m, 9H, Ar-H), 8.21 (s, 1H, CH), 11.63 (s, 1H, NH), 12.00 (s, 1H, NH). ^{13}C NMR (DMSO-d6): δ (ppm) 12.78, 13.11, 32.33, 120.26, 120.38, 120.83, 127.29, 127.43, 127.65, 127.78, 128.86, 129.21, 129.33, 134.54, 146.22, 155.40, 155.80, 158.71, 158.91, 164.65, 169.10.

3. RESULTS AND DISCUSSION

The structures of synthesized compounds were in agreement with IR, ^{1}H NMR and ^{13}C NMR Spectral analysis. The final compounds were tested for *in-vitro* antimicrobial activity by modified Kirby Bauer method. The newly synthesized compounds were screened for their antibacterial activity against Gram positive bacteria Staphylococcus aureus and Bacillus subtilis, Gram negative bacteria Escherichia coli and Pseudomonas aeruginosa, antifungal activity against Candida albicans. The Ciprofloxacin and Fluconazole used as standard drug. The percentage of inhibition was calculated using the statistical analysis. The antibacterial results show that all compounds found weak to moderate activity. The antifungal results of newly synthesized compound show that all compounds found weak to moderate active against *Candida albicans*.

Antibacterial activity

Determination of minimal inhibitory concentration by modified Kirby Bauer method. All the synthesized compounds were used for antibacterial test. Mueller Hinton medium being used for the Kirby Bauer test is very high in protein. Swab a Mueller Hinton plate with only two of the bacteria. Inoculate the plates by dipping a sterile swab into the inoculum. Remove excess inoculum by pressing and rotating the swab firmly against the side of the tube above the level of the liquid. Streak the swab all over the surface of the medium three times, rotating the plate through an angle of 90 degree. Allow the surface to dry for a few minutes at room temperature before placing antibiotics disks on the agar. Pair of sterile forceps is used for the Antimicrobial discs can be placed on the inoculated plates. Disks should not be placed closer than 24 mm on the Muller Hinton agar plate. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by

spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37˚C overnight. The tubes are then incubated overnight. The MIC of the control organism is read to check the accuracy of the drug concentrations. Antibacterial and antifungal results of final compounds are given in Table 1.

Table 1: Antimicrobial activity of the synthesized compounds

Standard antibacterial agent								
Ciprofloxacin								
Strain	**5 µg**	**10 µg**	**15 µg**	**20µg**	**30 µg**	**35 µg**	**40 µg**	**MIC**
Pseudomonas aeruginosa	10 mm	13 mm	16 mm	18 mm	23 mm	29 mm	35 mm	10 µg
Escherichia coli	10 mm	12 mm	15 mm	18 mm	20 mm	23 mm	26 mm	10 µg
Staphylococcus aureus	10 mm	10 mm	10 mm	12 mm	17 mm	20 mm	23 mm	20 µg
Bacillus subtilis	13 mm	18 mm	23 mm	26 mm	30 mm	33 mm	37 mm	05 µg

Standard antifungal agent								
Fluconazole								
Strain	**1 µg**	**2 µg**	**5 µg**	**10 µg**	**20 µg**	**30 µg**	**40 µg**	**MIC**
Candida albicans	10 mm	10 mm	10 mm	13 mm	15 mm	18 mm	22 mm	10 µg

Gram Negative Bacteria														
Pseudomonas aeruginosa								***Escherichia coli***						
Compounds	**10 µg**	**20 µg**	**40 µg**	**80 µg**	**160 µg**	**320 µg**	**MIC µg**	**10 µg**	**20 µg**	**40 µg**	**80 µg**	**160 µg**	**320 µg**	**MIC µg**
KNB1	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
KNB2	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
KNB3	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
KNB4	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
KNB5	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
KNB6	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
Gram Positive Bacteria														
Staphylococcus aureus								***Bacillus subtilis***						
Compounds	**10 µg**	**20 µg**	**40 µg**	**80 µg**	**160 µg**	**320 µg**	**MIC µg**	**10 µg**	**20 µg**	**40 µg**	**80 µg**	**160 µg**	**320 µg**	**MIC µg**
KNB1	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
KNB2	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
KNB3	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320

KNB4	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
KNB5	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320
KNB6	10	10	10	10	10	10	>320	10	10	10	10	10	10	>320

Fungi							
Candida albicans							
Compounds	**10 µg**	**20 µg**	**40 µg**	**80 µg**	**160 µg**	**320 µg**	**MIC µg**
KNB1	10	10	10	10	10	10	>320
KNB2	10	10	10	10	10	10	>320
KNB3	10	10	10	10	10	10	>320
KNB4	10	10	10	10	10	10	>320
KNB5	10	10	10	10	10	10	>320
KNB6	10	10	10	10	10	10	>320

4. CONCLUSION

The newly synthesized compounds showed weak to moderate antimicrobial activities against *Staphylococcus aureus* and *Bacillus subtilis* as Gram positive bacteria and *Escherichia coli* and *Pseudomonas aeruginosa* as Gram negative bacteria, antifungal activity against *Candida albicans*.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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