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Synthesis and Reactions of Some New 2,6-Bis-Substituted Pyridine Derivatives as Antimicrobial Agents

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Abstract: A series of 2,6-bis-substituted pyridine derivatives 3-12 were synthesized by using 2,6-pyridinedicarbonylhydrazide (2) as starting material. The antimicrobial screening for the synthesized compounds showed that many of these compounds have good antibacterial activities comparable to Chloramphinicol^R and Fusidic acid as reference drugs. The detailed synthesis, spectroscopic data and pharmacological properties are reported.

Key words: Pyridine-2,6-dicarbohydrazide · Thiosemicarbazide · Thiadiazole · Antimicrobial agents

INTRODUCTION

In previous work we found that certain substituted pyridine derivatives show antimicrobial and antiinflammatory [1-6] and antitumer activities [7,8]. In addition, the biological and analgesic activities of many heterocyclic compounds containing a sulfur atom have been reviewed [9-11]. On the other hand, some thienopyrimidinone derivatives have been synthesized and tested as analgesic, anticonvulsant and antiparkinsonian activities [12]. Recently, the heterocyclic derivatives are used as potentantimicrobial, antiinflammatory agents [13,14]. In view of these observations and in continuation of our previous work in pyridine chemistry, we synthesized some heterocyclic compounds containing the pyridine moiety and tested their biological activities.

MATERIALS AND METHODS

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus. Elemental analyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Center, Cairo Egypt and were found within ±0.4% of the theoretical values. Infrared spectra were recorded on Carlzeise Spectrophotometer model "UR 10" spectrophotometer using the KBr disc technique (ν, cm^{-1}). ^{1}H-NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (DMSO-d_6) and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane as an internal standard. The mass spectra were measured using a Finnigan SSQ 7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany). The diethyl pyridine-2,6-dicarboxylate (1)[15-17] and pyridine-2,6-dicarbohydrazide (2) [18,19] were prepared according to the literature procedures.

Pyridine-2,6-dicarbothiosemicarbazide (3)

Method A: To a solution of 2 (1.95 g, 10 mmol) in methanol (50 ml), solution of potassium thiocyanate (0.79 g, 30 mmol) in hydrochloric acid (3 ml) was added with stirring. The reaction mixture was evaporated to dryness on a steam bath. The residue was heated for an additional one hour in methanol (50 ml). The resulting solid was filtered off, washed with water/ethanol, dried and crystallized from ethanol to afford compound (3), in 80% yield.

Method B: To a solution of 1 (6.69 g, 30 mmol) and thiosemicarbazide (5.82g, 60 mmol) in dioxane (30 ml), few drops of piperidine was added. The reaction mixture was

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refluxed for 10 h. then left to cool. The precipitated product was filtered off, washed with ethanol, dried and crystallized from ethanol to afford a product identical in all respects with that obtained by Method A, in 70 % yield. Mp: 300-302°C; IR: 3397, 3278 and 3250 (2NH, NH$_2$), 1690 (2C=O); ^{1}H-NMR: 4.43 (bs, 4H, 2NH$_2$, exchangeable with D$_2$O), 7.87 (d, 2H, pyrid-H), 8.10 (t, 1H, pyrid-H), 8.70 and 11.50 (2s, 4H, 4NH, exchangeable with D$_2$O); MS m/z (%): 313 (M$^+$, 15), 219 (20), 115 (18), 75 (100).

5-(6-(4,5-Dihydro-5-thioxo-1H-1,2,4-triazol-3-yl)pyridin-2-yl)-2H-1,2,4-triazole-3(4H)-thione (4): A suspension of 3 (0.31g, 1 mmol) in potassium hydroxide solution (10 ml, 7 %) was heated under reflux for 3 h. The reaction mixture was allowed to cool then acidified with hydrochloric acid (10%) to pH ~ 6. The precipitated solid was filtered off, washed with water, dried and crystallized from ethanol to afford triazolothiol derivative (4). Yield 68%, Mp: 320-322°C; IR: 3245, 3280 (4NH), 1660-1658 (3C=N), 1245 (2C=S); ^{1}H-NMR: 7.84 (d, 2H, pyrid-H), 8.20 (t, 1H, pyrid-H), 9.40, 12.10 (2s, 4H, 4NH, exchangeable with D$_2$O); MS m/z (%): 277 (M$^+$, 34), 205 (16), 195 (60), 75 (100).

Synthesis of Aryl Thiosemicarbazide Derivatives (5a,b): A mixture of 2 (1.95 g, 10 mmol) and phenyl or benzyl isothiocyanate (20 mmol) in absolute ethanol (40 ml) was refluxed for 3h. After cooling, the crystal that appeared was filtered off, dried and crystallized from dioxane to afford the corresponding aryl thiosemicarbazide derivatives (5a,b).

2,6-Bis-(Phenyl Thiosemicarbazide) Derivative (5a): Yield: 85 %, mp: 245-247°C; IR: 3370-3250 (6NH), 1680 (2C=O), 1660 (C=N), 1241 (2C=S); ^{1}H-NMR: 7.10-7.70 (m, 10H, Ar-H), 8.10 (d, 2H, pyrid-H), 8.50 (t, 1H, pyrid-H), 10.30, 10.80 and 11.50 (3s, 6H, 6NH, exchangeable with D$_2$O); MS m/z (%): 465 (M$^+$, 75), 310 (54), 195 (24), 77 (100).

2,6-Bis-(benzyl Thiosemicarbazide) Derivative (5b): Yield 88%, mp: 225-227°C; IR: 3350-2370 (6NH), 1685 (2C=O), 1662 (C=N), 1245 (2C=S); ^{1}H-NMR): 4.60 (s, 4H, 2CH$_2$), 7.10-7.50 (m, 10H, Ar-H), 7.90 (d, 2H, pyrid-H), 8.30 (t, 1H, pyrid-H), 9.60, 9.90 and 11.50 (3s, 6H, 6NH, exchangeable with D$_2$O); MS m/z (%): 493 (M$^+$, 60), 315 (25), 207 (46), 195 (100).

5-(6-(5-Mercapto-4-aryl-4H-1,2,4-triazol-3-yl)pyridin-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiols (6a,b): A solution of 5a,b (10 mmol) in potassium hydroxide solution (5%, 25 ml) was refluxed for 3 h. The resulting solution was treated with charcoal, filtered off and cooled. The filtrate was acidified with hydrochloric acid to pH ~ 5-6 and the formed solid was filtered off, dried and crystallized from dioxane to afford the triazolothiol derivatives (6a,b), respectively.

5-(6-(5-Mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)pyridin-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol (6a): Yield 65%, mp: 338-340°C; IR: 3350 (2NH), 1660-1655 (3C=N), 1247 (2C=S); ^{1}H-NMR: 7.10 (t, 6H, Ar-H), 7.40 (d, 4H, Ar-H), 7.80 (d, 2H, pyrid-H), 8.10 (t, 1H, pyrid-H), 11.16 (bs, 2H, 2NH, exchangeable with D$_2$O); MS m/z (%): 429 (M$^+$, 80), 280 (60), 149 (15.20), 77 (100).

5-(6-(5-Mercapto-4-benzyl-4H-1,2,4-triazol-3-yl)pyridin-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol (6b): Yield 70%, Mp: 260-262°C; IR: 3426 (2NH), 2924 (2CH$_2$), 1665-1660 (5C=N), 1252 (2C=S); ^{1}H-NMR: 5.22 (s, 4H, 2CH$_2$), 7.10 (t, 6H, Ar-H), 7.51 (d, 4H, Ar-H), 7.88 (d, 2H, pyrid-H), 8.06 (t, 1H, pyrid-H), 10.90 (bs, 2H, 2NH, exchangeable with D$_2$O); MS m/z (%): 457 (M$^+$, 21.70), 366 (11.10), 91 (100).

2,6-Bis(5-(methylthio)-4-aryl-4H-1,2,4-triazol-3-yl)pyridines (7a,b): To a solution of 6a,b (5 mmol) sodium ethoxide solution [prepared from sodium metal (0.11g) in ethanol (20 ml)] and then methyl iodide (0.65 g, 4 mmol) was added gradually with stirring. The reaction mixture was refluxed for 2 h., concentrated under reduced pressure, cooled, diluted with water. The obtained solid was filtered off, washed with water, dried and crystallized from dioxane/ethanol to give compounds (7a,b).

2,6-Bis(5-(methylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)pyridine (7a): Yield 70%, Mp: 342-344°C; IR: 1665-1658 (5C=N); ^{1}H-NMR: 2.50 (s, 6H, 2CH$_3$), 7.10-7.50 (m, 10H, Ar-H), 7.97 (d, 2H, pyrid-H), 8.21 (t, 1H, pyrid-H); MS m/z (%): 457 (M$^+$, 100), 442 (65), 244 (25), 91 (91), 77 (53).

2,6-Bis(5-(methylthio)-4-benzyl-4H-1,2,4-triazol-3-yl)pyridine (7b): Yield 67%, Mp: 267-269°C; IR: 2924 (2CH$_2$), 1662-1656 (5C=N); ^{1}H-NMR: 2.55 (s, 6H, 2 CH$_3$), 5.35 (s, 4H, 2CH$_2$), 7.10-7.60 (m, 10H, Ar-H), 7.87 (d, 2H, pyrid-H), 8.11 (t, 1H, pyrid-H); MS m/z (%): 485 (M$^+$, 100), 315(45), 169 (25), 77 (75).

N-Aryl-5-(6-(5-(arylamino)-1,3,4-oxadiazol-2-yl)pyridin-2-yl)-1,3,4-oxadiazol-2-amine (8a,b): A mixture of 5a,b (3 mmol) in pyridine (10 ml) was refluxed for 12 h. until all H$_2$S evolved. The reaction mixture was then standing overnight and poured onto cold water. The formed precipitate was filtered off, washed with water, dried and crystallized from dioxane/ethanol to give compounds (8a,b).

N-phenyl-5-(6-(5-(phenylamino)-1,3,4-oxadiazol-2-yl)pyridin-2-yl)-1,3,4-oxadiazol-2-amine (8a): Yield 55 %, Mp: 303-305°C; IR: 3276 (2NH), 1665-1654 (3C= N); ^{1}H-NMR: 7.05-7.45 (m, 10H, Ar-H), 8.15-8.35 (m, 3H, pyrid-H), 8.95 (bs, 2H, 2NH, exchangeable with D$_2$O); MS m/z (%): 398 (M$^+$+1, 17), 304 (11), 246 (14), 77 (35).

N-Benzyl-5-(6-(5-(benzylamino)-1,3,4-oxadiazol-2-yl)pyridin-2-yl)-1,3,4-oxadiazol-2-amine (8b): Yield 75%, mp: 240-242°C; IR: 3280 (2NH), 2945 (2CH$_2$), 1664-1660 (3C= N); H-NMR: 4.90 (s, 4H, 2CH$_2$), 7.20-7.70 (m, 10H, Ar-H), 7.80 (d, 2H, pyrid-H), 8.11 (t, 1H, pyrid-H), 9.90 (bs, 2H, 2NH, exchangeable with D$_2$O); MS m/z (%): 425 (M$^+$, 35), 256 (20), 149 (37), 91 (100).

N-Aryl-5-(6-(5-(arylamino)-1,3,4-thiadiazol-2-yl)pyridin-2-yl)-1,3,4-thiadiazol-2-amine (9a,b): A solution of 5a,b (3 mmol) in concentrated sulphuric acid (10 ml) was stirring for 4 h. The reaction mixture poured onto cold water, the precipitate that formed was filtered off, washed with water several times, dried and crystallized from ethanol to give compounds (9a,b).

N-phenyl-5-(6-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)pyridin-2-yl)-1,3,4-thiadiazol-2-amine (9a): Yield 80%, Mp: 325-327°C; IR: 3417-3255 (2NH), 1659-1654 (3C=N); ^{1}H-NMR: 6.93 (t, 6H, Ar-H), 7.68 (d, 4H, Ar-H), 8.07 (d, 2H, pyrid-H), 8.21 (t, 1H, pyrid-H), 10.64 (bs, 2H, 2NH, exchangeable with D$_2$O); MS m/z (%): 429 (M$^+$, 18), 150 (24), 64 (100).

N-benzyl-5-(6-(5-(benzylamino)-1,3,4-thiadiazol-2-yl)pyridin-2-yl)-1,3,4-thiadiazol-2-amine (9b): Yield 70%, Mp: 315-317°C; IR: 3295-3280 (2NH), 2969 (2CH$_2$), 1661-1655 (3C=N); ^{1}H-NMR: 4.99 (s, 4H, 2CH$_2$), 6.86 (t, 6H, Ar-H), 7.61 (d, 4H, Ar-H), 7.70 (d, 2H, pyrid-H), 8.26 (t, 1H, pyrid-H), 9.90 (bs, 2H, 2NH, exchangeable with D$_2$O); MS m/z (%): 457 (M$^+$, 70), 424 (12), 366 (65), 163 (34), 91 (100).

Synthesis of compounds 10-12: To a mixture of 2 (1.95 g, 10 mmol) and ethyl benzoylacetate, ethyl acetoacetate or acetylaceton (20 mmol) in ethanol (20 ml), one drop of pipredine was added. The reaction mixture was refluxed for 8 h., after cooling, the precipitated solid was filtered off, dried and crystallized from dioxane to afford the corresponding compounds 10-12.

2,6-Bis(1-carbonyl-3-phenyl-1H-pyrazol-5(4H)-one)pyridine (10): Yield 95%, Mp: 369-370°C; IR: 1724, 1680 (4C=O), 1662-1660 (3C=N); MS m/z (%): 451 (M$^+$, 42), 418 (25), 346 (98), 185 (43), 77 (81).

2,6-Bis(1-carbonyl-3-methyl-1H-pyrazol-5(4H)-one) pyridine (11): Yield 90%, Mp: 350-352°C; IR: 2936 (2CH$_2$), 1699, 1675 (4C=O), 1662-1658 (3C=N); ^{1}H-NMR: 2.52 (s, 6H, 2CH$_3$), 3.80 (s, 4H, 2CH$_2$), 7.95-8.22 (m, 3H, pyrid-H); MS m/z (%): 327 (M$^+$, 35) 164 (100), 77 (13.90).

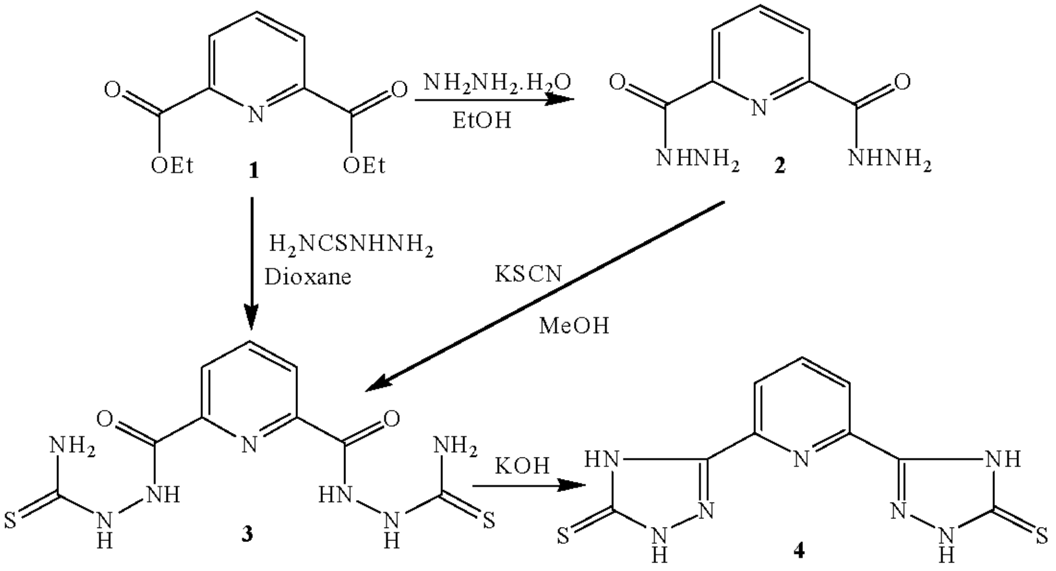
2,6-Bis[1-carbonyl-3,5-(dimethyl)-1H-pyrazolo]pyridine (12): Yield 95%, Mp: 320-322°C; IR: 1682 (2C=O), 1662-1660 (3C=N); ^{1}H-NMR: 2.35 (s, 6H, 2CH$_3$), 2.52 (s, 6H, 2CH$_3$), 3.41 (s, 4H, 2CH$_2$), 5.82 (s, 2H, 2CH), 8.05-8.25 (m, 3H, pyrid-H); MS m/z (%): 324 (M$^+$+1, 33), 284 (100), 228 (15), 106 (30).

Antimicrobial Assay: The antimicrobial activity was measured at a concentration 50 ã/mL of the tested compounds using the bioassay sensitivity technique of antibiotics specified in the US Pharmacopoeia [20]. The degree of inhibition is measured in comparison with that of Chloramphinicol® taken as standard.

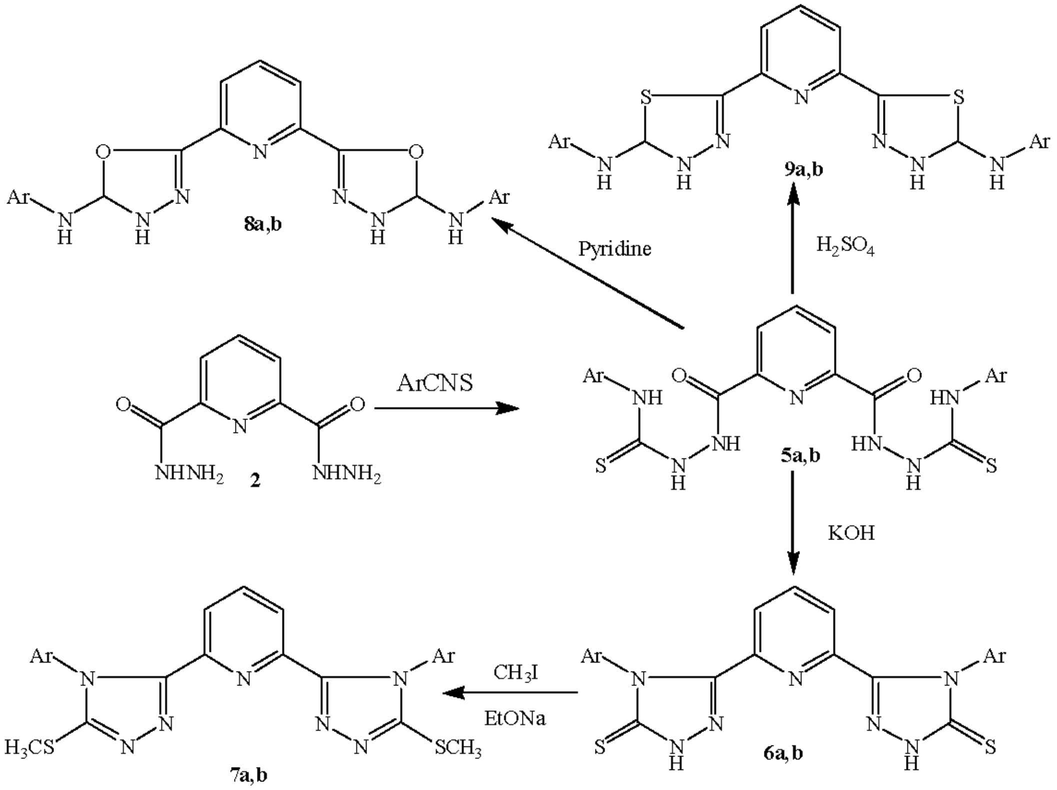
RESULTS AND DISCUSSION

In the course of our investigation, we have found that diethyl pyridine-2,6-dicarboxylate (1) is an excellent building block for the synthesis of a numerous heterocyclic ring systems. Thus, when diethyl pyridine-2,6-dicarboxylate (1) was treated with hydrazine hydrate in ethanol, it afforded pyridine-2,6-dicarbohydrazide (2). Treatment of pyridine-2,6-dicarbohydrazide (2) with potassium thiocyanate and hydrochloric acid under reflux afforded the thiosemicarbazide derivative (3). The structure of compound (3) was further confirmed by its alternate synthesis from the reaction of diethyl pyridine-2,6-dicarboxylate (1) with thiosemicarbazide in refluxing dioxane. When the pyridine thiosemicarbazide derivative 3 was treated with potassium hydroxide in refluxing ethanol, it afforded 5,5'-(pyridine-2,6-diyl)bis(4H-1,2,4-triazole-3-thiol) (4) (Scheme 1).

Treatment of the pyridine-2,6-dicarbohydrazide (2) with aryl isothiocynate in ethanol under reflux afforded aryl thiosemicarbazide derivatives (5a,b). Heating of 5a,b with sodium hydroxide solution afforded the corresponding 5-(6-(5-mercapto-4-aryl-4H-1,2,4-triazol-

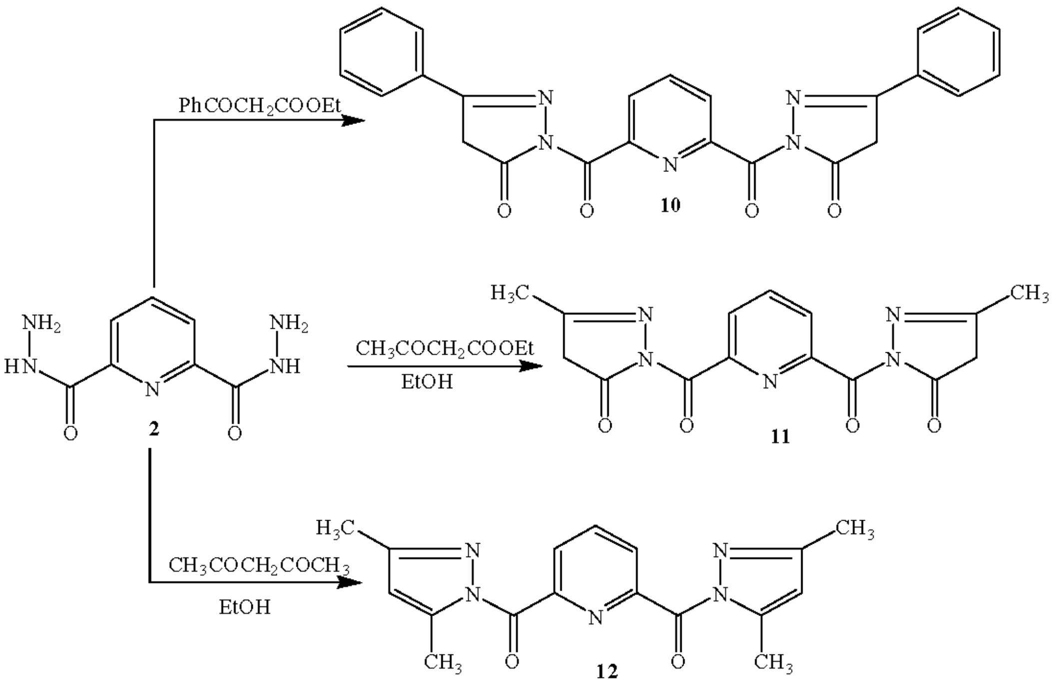


Scheme 1



a, Ar = Ph; **b**, Ar = CH_2Ph

Scheme 2



Scheme 3

Table 1: Antimicrobial activities of some newly synthesized compounds

	Inhibition zone (cm)					
	Gram + ve Bacteria			Gram - ve Bacteria	Yeast	Fungi
Compound No.	*B. subtilis*	*B. aureus*	*Staph. aureus*	*E. coli*	*C. albicans*	*A. niger*
3	1.72	1.50	1.98	0.58	-	1.75
4	1.95	1.98	1.90	0.96	1.18	2.00
5a	1.54	1.34	1.45	0.68	-	1.65
5b	1.65	1.05	1.55	0.64	-	1.75
6a	1.38	1.82	1.58	0.68	-	1.95
7a	1.82	1.52	1.58	0.89	1.00	1.95
9a	1.80	1.92	1.85	0.78	0.94	1.48
9b	1.68	1.65	1.78	0.82	1.00	1.88
17	1.55	1.78	1.65	0.68	-	1.58
18	1.84	2.00	1.99	0.92	0.98	2.04
19	1.50	1.84	1.60	0.72	1.05	1.62
Chlora-mphenicol®	2.00	2.10	2.00	0.95	-	-
Fusidic acid	-	-	-	-	1.9	1.9

3-yl)pyridin-2-yl)-4-phenyl-4H-1,2,4-triazole-3-thiols (6a,b) Treatment of 6a,b with sodium ethoxide solution followed by addition of methyl iodide under reflux, afforded 2,6-bis(5-(methylthio)- 4-aryl-4H - 1,2,4-triazol-3-yl)pyridines (7a,b). When thiosemicarbazide derivatives (5a,b) were refluxed in pyridine or sulphuric acid, it afforded the corresponding N-aryl-5-(6-(5-(arylamino)-1,3,4-oxadiazol-2-yl)pyridin-2-yl)-1,3,4- oxadiazol-2-amines (8a,b) and N-aryl-5-(6-(5-(arylamino)-1,3,4-thiadiazol-2-yl)pyridin-2-yl)-1,3,4-thiadiazol-2-amine derivatives (9a,b), respectively (Scheme 2).

The reaction of 2 with dicarbonyl compounds, namely, ethyl benzoylacetate, ethyl acetoacetate or acetyl acetone afforded the corresponding pyrazolo compounds 10-12, respectively (Scheme 3).

Antimicrobial Activity: The newly synthesized compounds were tested for their preliminary antimicrobial activity against different microorganisms representing Gram-positive bacteria (*Bacillus subtilis*, *Bacillus aureus* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), yeast (*Candida albican*) and fungi (*Aspergillus niger*).The results are summarized in Table 1.

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