



Synthesis and Biological Evaluation of New 1,2,4-Triazolo[1,5-*a*] pyridine and 1,2,4-Triazolo[1,5-*a*] isoquinoline Derivatives Bearing Diphenyl Sulfide Moiety as Antimicrobial Agents



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Abstract

Hydrazone derivative (**3**) was used as a precursor for the synthesis of novel [1,2,4]triazolo[1,5-*a*]pyridine derivatives via its reaction with some electrophilic reagents. Treatment of hydrazone derivative (**3**) with arylidenemalononitriles (**4**) in the presence of piperidine afforded the 1,2,4-triazolo[1,5-*a*]pyridine derivatives (**7a-d**). Ternary condensation of hydrazone (**3**), aliphatic aldehyde and malononitrile (1:1:1 molar ratio) in the presence of a basic catalyst furnished the novel 1,2,4-triazolo[1,5-*a*]pyridine derivatives (**8a,b**). Similarly, cyclization of hydrazone (**3**) with ethyl α -cyanocinnamates (**9**) (1:1 molar ratio) yields the corresponding 1,2,4-triazolo[1,5-*a*]pyridines (**10a-c**). The hydrazone (**3**) can be cyclized with appropriate arylazomalononitriles (**11**) to afford the corresponding 1,2,4-triazolo[1,5-*a*]pyridines (**14a,b**). The behavior of fused thiophene derivative (**15**) towards electron-poor olefins was investigated. It has been found that, 1,2,4-triazolo[1,5-*a*]isoquinoline derivative (**17**) was obtained by treatment of thiophene derivative (**15**) with dimethyl acetylenedicarboxylate (DMAD). Condensation of compound (**15**) with *N*-phenylmaleimide furnished pyrrolotriazoloisoquinoline derivative (**18**). Also, the triazoloisoquinoline derivative (**19**) was obtained by condensation of compound (**15**) with chalcone. All the newly synthesized compounds were characterized by analytical and spectral data and evaluated for their antibacterial and antifungal activities in vitro against two Gram-positive bacteria, two Gram negative bacteria as well as two fungi. In general, the newly synthesized compounds showed good antimicrobial activities.

Keywords: Cyanoacetic acid hydrazone, hydrazone, arylidenemalononitrile, antimicrobial activity; dye intermediates.

1. Introduction

The literature review revealed that, aryl thioether derivatives are valuable synthetic intermediates frequently found in biologically and pharmaceutically active molecules as well as in polymeric materials and also used as dye intermediates [1]. In particular, diaryl and aryl-heteroarylthioethers are essential components of numerous drugs with potential application in the treatment of inflammation, cancer, human immunodeficiency virus (HIV), asthma, Alzheimer's and Parkinson's diseases [2-8]. Furthermore, diarylthioethers are precursors to the

corresponding sulfoxides and sulfones that also exhibit important biological activities and are contained in antifungal and anti-cancer agents as well as in potential drug candidates for Alzheimer's disease or HIV [9-12]. It is well known in the literature that 1,2,4-triazolo[1,5-*a*]pyridine derivatives exhibit a variety of biological activities such as antihypertensive, bronchodilator, anti-inflammatory, analgesic and positive inotropic agents [13,14]. Amino-2-aryl-1,2,4-triazolo[1,5-*a*]pyridine-6-carboxylic amide derivatives were proved to the human adenosine 2a (hA2a) receptor [15] and substituted phenyl-1,2,4-triazolo[1,5-*a*]pyridines

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have been found to have antitumor activity. Also, some 1,2,4-triazolo[1,5-*a*]pyridines are known to exhibit pregnancy interceptive [16] and antifungal activity [17] and also used as dye intermediates. Prompted by the above facts and in continuation of our efforts in developing novel antimicrobial agents [18-20], we hereby report the synthesis and antimicrobial evaluation of some novel 1,2,4-triazolo[1,5-*a*]pyridine and 1,2,4-triazolo[1,5-*a*]isoquinoline derivatives bearing diphenyl sulfide moiety from inexpensive and readily available starting materials.

2. Experimental

Melting points were determined on a Gallenkamp apparatus and uncorrected. The purity of the compounds was checked by TLC. IR spectra were recorded in a Pye-Unicam SP300 instrument in potassium bromide discs. ¹H NMR spectra were recorded in a Varian Mercury VXR-300 spectrometer (300 MHz for ¹H NMR) in DMSO-*d*₆ and the chemical shifts were related to that of the solvent. Mass spectra were recorded in a GCMS-QP 1000 EX Shimadzu Spectrometer, the ionizing voltage was 70 eV. Elemental analyses were carried out in the Micro analytical Laboratory of Cairo University, Giza, Egypt. Arylidene malononitriles (**4**) [21], ethyl α -cyanocinnamates (**9**) and arylazomalononitriles (**11**) [22] were synthesized using methods previously published. Antimicrobial activities were carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

4-(Phenylthio)benzaldehyde (**2**).

A mixture of 4-fluorobenzaldehyde (0.01 mol) and thiophenol (0.01 mol) in dimethyl sulfoxide (10 mL) was refluxed in the presence of anhydrous potassium carbonate (2 gm) for 1 hr, left to cool and poured into crushed ice. The obtained solid product was collected by filtration, washed with water and crystallized from ethanol to give (**2**) as colourless crystals. Yield 92%; m.p. 52-53°C [Lit. 53-54°C] [23]. Anal. Calcd. For C₁₃H₁₀OS: C, 72.87; H, 4.72; S, 14.96. Found: C, 72.97; H, 4.65; S, 14.85 %.

2-Cyano-N'-(4-(phenylthio)benzylidene)acetohydrazide (**3**).

A mixture of 4-(phenylthio)benzaldehyde (0.01 mol) and cyanoacetic acid hydrazide (0.01 mol) in ethanol (30 mL) was stirred room temperature for 1 h. The resulting solid product was collected by filtration and crystallized from ethanol to give (**3**). Yield 94 % as yellow crystals; mp 106-108 °C; IR (KBr; cm⁻¹): 3222 (NH), 2220 (C≡N), 1696 (C=O) and 1534 (C=N). ¹H NMR (300 MHz, DMSO-*d*₆, δ /ppm): 4.20

(s, 2H, CH₂), 7.11- 8.16 (m, 9H, Ar -H) 8.66 (s, 1H, methine-H), 11.98 (s, 1H, NH, exchangeable with D₂O). Anal. Calcd. For C₁₆H₁₃N₃OS: C, 65.08; H, 4.41; N, 14.23; S, 10.86. Found: C, 65.12; H, 4.41; N, 14.30; S, 10.90 %.

General Procedure for Synthesis of 1,2,4-triazolo[1,5-*a*]pyridines (**7a-d**)

Method (A): A mixture of compound (**3**) (0.01 mol) and arylidene malononitrile (**4**) (0.01 mol) in ethanol (30 mL) was treated with few drops of piperidine and refluxed for 3 hrs. A crystalline solid was obtained on cooling. It was crystallized from an appropriate solvent.

Method (B): A mixture of compound (**3**) (0.01 mol), aromatic aldehyde (0.01 mol) and malononitrile (0.01 mol) in ethanol (30 mL) was treated with few drops of piperidine and refluxed for 3 hrs. The resulting solid product was collected by filtration and crystallized to give (**7**).

7-(Furan-2-yl)-5-oxo-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (**7a**).

Yield 71 % as black crystals from Ethanol; mp 280-282 °C; IR (KBr; cm⁻¹): 3425 (NH), 2207 (C≡N), 1674 (C=O). ¹H NMR (300 MHz, DMSO-*d*₆, δ /ppm): 7.24-7.49 (m, 12H, Ar-H), 9.88 (s, 1H, NH, exchangeable with D₂O). Anal. Calcd. For C₂₄H₁₃N₅O₂S: C, 66.20; H, 3.01; N, 16.08; S, 7.36. Found: C, 66.31; H, 3.20; N, 16.21; S, 7.41 %.

5-Oxo-7-phenyl-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (**7b**).

Yield 73 % as yellow crystals from Ethanol; mp 183-185 °C; IR (KBr; cm⁻¹): 3377 (NH), 2200 (C≡N), 1682 (C=O). ¹H NMR (300 MHz, DMSO-*d*₆, δ /ppm): 6.91-7.61 (m, 14H, Ar-H), 9.68 (s, 1H, NH, exchangeable with D₂O). Anal. Calcd. For C₂₆H₁₅N₅O₂S: C, 70.10; H, 3.39; N, 15.72; S, 7.20. Found: C, 70.21; H, 3.42; N, 15.80; S, 7.31 %.

7-(4-Nitrophenyl)-5-oxo-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (**7c**).

Yield 58 % as buff crystals (from Ethanol); mp 170-172 °C; IR (KBr; cm⁻¹): 3301 (NH), 2195 (C≡N), 1650 (C=O). ¹H NMR (300 MHz, DMSO-*d*₆, δ /ppm): 7.20-7.61 (m, 13H, Ar-H), 9.65 (s, 1H, NH, exchangeable with D₂O). Anal. Calcd. For C₂₆H₁₄N₆O₃S: C, 63.67; H, 2.88; N, 17.13; S, 6.54. Found: C, 63.72; H, 2.90; N, 17.31; S, 6.60 %.

7-(3-Bromophenyl)-5-oxo-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (7d).

Yield 58 % as orange crystals (from Ethanol); mp 143–145 °C; IR (KBr; cm^{-1}): 3205 (NH), 2211 ($\text{C}\equiv\text{N}$), 1682 ($\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 6.68–7.73 (m, 13H, Ar-H), 9.92 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{26}\text{H}_{14}\text{BrN}_5\text{OS}$: C, 59.55; H, 2.69; Br, 15.24; N, 13.36; S, 6.11. Found: C, 59.62; H, 2.81; Br, 15.32; N, 13.50; S, 6.22 %.

General procedure for Synthesis of 1,2,4-triazolo[1,5-a]pyridines (8a,b)

A mixture of compound (3) (0.01 mol), aliphatic aldehyde (0.01 mol) and malononitrile (0.01 mol) in ethanol (30 mL) was treated with few drops of piperidine and refluxed for 3 hrs. The reaction was left to cool then triturated with water. The solid product, so formed was collected by filtration and crystallized to give (8).

5-Oxo-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (8a).

Yield 73 % as yellow crystals (from Ethanol); mp 180–181 °C; IR (KBr; cm^{-1}): 3308 (NH), 2188 ($\text{C}\equiv\text{N}$) and 1685 ($\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 5.22 (s, 1H, pyridine-H), 7.27–7.73 (m, 9H, Ar-H), 8.58 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{20}\text{H}_{11}\text{N}_5\text{OS}$: C, 65.03; H, 3.00; N, 18.96; S, 8.68. Found: C, 65.41; H, 3.21; N, 18.88; S, 8.72 %.

7-Methyl-5-oxo-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (8b).

Yield 70 % as yellow crystals (from Ethanol); mp 160–161 °C; IR (KBr; cm^{-1}): 3321 (NH), 2203 ($\text{C}\equiv\text{N}$) and 1700 ($\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 1.26 (s, 3H, CH_3), 7.26–7.93 (m, 9H, Ar-H), 8.42 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{21}\text{H}_{13}\text{N}_5\text{OS}$: C, 65.78; H, 3.42; N, 18.27; S, 8.36. Found: C, 65.85; H, 3.50; N, 18.40; S, 8.41 %.

General procedure for Synthesis 1,2,4-triazolo[1,5-a]pyridine (10a-c)

To a mixture of compound (3) (0.01 mol) and ethyl α -cyanocinnamate (9) (0.01 mol) in ethanol (30 mL), piperidine (0.1 mL) was added. The reaction was heated under reflux for 3 h. The solvent was removed by evaporation under reduced pressure and the remainder was left to cool. The solid product so formed was collected by filtration, washed with petroleum ether, and the crude product was crystallized from an appropriate solvent.

Ethyl 6-cyano-5-oxo-7-phenyl-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-8-carboxylate (10a).

Yield 63 % as orange crystals; mp 178–180 °C; IR (KBr; cm^{-1}): 3421 (NH), 2200 ($\text{C}\equiv\text{N}$) and 1680, 1665 ($\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 1.35 (t, 3H, CH_3), 4.36 (q, 2H, CH_2), 6.94–7.86 (m, 14H, Ar-H), 8.72 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{28}\text{H}_{20}\text{N}_4\text{O}_3\text{S}$: C, 68.28; H, 4.09; N, 11.37; S, 6.51. Found: C, 68.41; H, 4.23; N, 11.50; S, 6.60 %.

Ethyl 7-(4-chlorophenyl)-6-cyano-5-oxo-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-8-carboxylate (10b).

Yield 65 % as yellow crystals; mp 197–199 °C; IR (KBr; cm^{-1}): 3323 (NH), 2215 ($\text{C}\equiv\text{N}$) and 1705, 1662 ($\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 1.37 (t, 3H, CH_3), 4.34 (q, 2H, CH_2), 6.92–7.95 (m, 13H, Ar-H), 8.20 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{28}\text{H}_{19}\text{ClN}_4\text{O}_3\text{S}$: C, 63.81; H, 3.63; Cl, 6.73; N, 10.63; S, 6.08. Found: C, 63.90; H, 3.72; Cl, 6.82; N, 10.70; S, 6.20 %.

Ethyl 6-cyano-7-(3,4-dimethoxyphenyl)-5-oxo-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-8-carboxylate (10c).

Yield 72 % as yellow crystals; mp 187–189 °C; IR (KBr; cm^{-1}): 3304 (NH), 2215 ($\text{C}\equiv\text{N}$) and 1710, 1685 ($\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 1.39 (t, 3H, CH_3), 3.68, 3.96 (2s, 6H, 2OCH_3), 4.38 (q, 2H, CH_2), 6.92–7.95 (m, 12H, Ar-H), 8.15 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{30}\text{H}_{24}\text{N}_4\text{O}_5\text{S}$: C, 65.20; H, 4.38; N, 10.14; S, 5.80. Found: C, 65.40; H, 4.50; N, 10.30; S, 5.90 %.

General procedure for Synthesis 1,2,4-triazolo[1,5-a]pyridine (14a,b)

A mixture of compound (3) (0.01 mol) and arylazomalononitrile (11) and piperidine (0.1 mL) in ethanol (30 mL) was heated under reflux for 5 h. The reaction was left to cool and then poured to ice cooled water (100 mL). The solid product was filtered off dried well and crystallized from an appropriate solvent.

7-Amino-5-oxo-8-(phenyldiazenyl)-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-6-carbonitrile (14a).

Yield 70 % as brown crystals; mp 172–174 °C; IR (KBr; cm^{-1}): 3452, 3420 (NH_2), 2220 ($\text{C}\equiv\text{N}$) and 1691 ($\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 4.39 (br, 2H, NH_2 , exchangeable with D_2O), 7.20–7.83 (m, 14H, Ar-H), 8.58 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{25}\text{H}_{17}\text{N}_7\text{OS}$: C, 64.78; H, 3.70; N, 21.15; S, 6.92. Found: C, 64.80; H, 3.69; N, 21.40; S, 6.80 %.

7-Amino-8-((4-chlorophenyl)diazanyl)-5-oxo-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-a]pyridine-6-carbonitrile (14b).

Yield 73 % as brown crystals; mp 185–187 °C; IR (KBr; cm^{-1}): 3315, 3300 (NH_2), 2200 ($\text{C}\equiv\text{N}$) and 1685 ($\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 4.47 (br, 2H, NH_2 , exchangeable with D_2O), 6.92 - 7.73 (m, 13H, Ar -H), 8.59 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{25}\text{H}_{16}\text{ClN}_7\text{OS}$: C, 60.30; H, 3.24; Cl, 7.12; N, 19.69; S, 6.44. Found: C, 60.40; H, 3.50; Cl, 7.22; N, 19.80; S, 6.53 %.

6-amino-5-oxo-2-(4-(phenylthio)phenyl)-3,5-dihydrothieno[3,4-d][1,2,4]triazolo [1,5-a]pyridine-9-carbonitrile (15).

To a solution of compound (8b) (0.01 mol) in absolute ethanol (30 mL) containing triethylamine (0.1 mL), elemental sulfur (0.01 mol) was added. The reaction mixture was refluxed under reflux for 4 h. then cooled, poured onto ice cold water. The solid product so formed was collected by filtration and crystallized. Yield 70 % as orange crystals (from ethanol/DMF); mp 203–204 °C; IR (KBr; cm^{-1}): 3313, 3190, 3100 (NH/NH_2), 2210 ($\text{C}\equiv\text{N}$) and 1650 ($\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 6.90 (br, 2H, NH_2 , exchangeable with D_2O), 7.21-7.28 (m, 10H, Ar -H), 8.62 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{21}\text{H}_{13}\text{N}_5\text{OS}_2$: C, 60.71; H, 3.15; N, 16.86; S, 15.43. Found: C, 60.80; H, 3.30; N, 16.90; S, 15.50 %.

General procedure for Synthesis 1,2,4- triazolo[1,5-a]isoquinolines(17),(18) and (19)

A mixture of compound (15) (0.01 mol) and electron-poor olefin (0.01 mol) and in dry pyridine (10 mL) was heated under reflux for 5 h. The reaction was left to cool and then poured to ice cooled water (100 mL). The solid product that filtered off dried well and crystallized from an appropriate solvent.

Diethyl-6-amino-10-cyano-5-oxo-2-(4-(phenylthio)phenyl)-3,5-dihydro-[1,2,4]triazolo[1,5-b]isoquinoline-7,8-dicarboxylate (17).

Yield 68 % as brown crystals (from ethanol/DMF); mp 247–249 °C; IR (KBr; cm^{-1}): 3335, 3319, and 3100 (NH/NH_2), 2212 ($\text{C}\equiv\text{N}$), 1745, 1710, and 1645 ($3\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 1.54, 2.25 (2s, 6H, 2 CH_3), 4.04 (br, 2H, NH_2 , exchangeable with D_2O), 7.21 - 7.73 (m, 10H, Ar -H), 12.06 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_5\text{S}$: C, 62.92; H, 4.19; N, 12.65; S, 5.79. Found: C, 62.80; H, 4.30; N, 12.70; S, 5.80 %.

6-Amino-5,7,9-trioxo-8-phenyl-2-(4-(phenylthio)phenyl)-5,7,8,9-tetrahydro-1H-

pyrrolo[3,4-g][1,2,4]triazolo[1,5-b]isoquinoline-11-carbonitrile (18).

Yield 73 % as yellow crystals (from ethanol/DMF); mp 226–228 °C; IR (KBr; cm^{-1}): 3330, 3100 (NH_2), 2212 ($\text{C}\equiv\text{N}$), 1710, 1650 ($2\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 4.21 (br, 2H, NH_2 , exchangeable with D_2O), 7.33 - 7.96 (m, 15H, Ar -H), 8.27 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{31}\text{H}_{18}\text{N}_6\text{O}_3\text{S}$: C, 67.14; H, 3.27; N, 15.15; S, 5.78. Found: C, 67.30; H, 3.40; N, 15.50; S, 5.80 %.

6-amino-7-benzoyl-5-oxo-8-phenyl-2-(4-(phenylthio)phenyl)-1,5-dihydro-[1,2,4]triazolo[1,5-b]isoquinoline-10-carbonitrile (19).

Yield 70 % as yellow crystals (from ethanol/DMF); mp 287–289 °C; IR (KBr; cm^{-1}): 3361, 3390, and 3210 (NH/NH_2), 2210 ($\text{C}\equiv\text{N}$), 1680, 1650 ($2\text{C}=\text{O}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$, δ/ppm): 5.20 (br, 2H, NH_2 , exchangeable with D_2O), 7.39 - 7.93 (m, 20H, Ar -H), 10.13 (s, 1H, NH, exchangeable with D_2O). Anal. Calcd. For $\text{C}_{36}\text{H}_{23}\text{N}_5\text{O}_2\text{S}$: C, 73.33; H, 3.93; N, 11.88; S, 5.44. Found: C, 73.40; H, 3.80; N, 11.90; S, 5.50 %.

In vitro antimicrobial activity

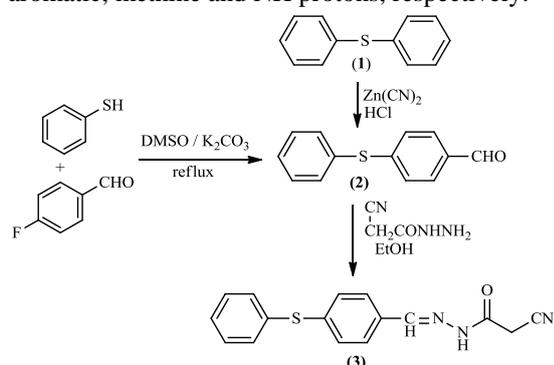
The *in vitro* antimicrobial activities of all the synthesized compounds were evaluated for two Gram-positive bacteria viz. *Bacillus Cereus*, *Micrococcus luteus* and two Gram-negative organisms viz. *Escherichia coli*, *Serratiamarcesens*, as well as two fungi viz. *Candida albicans* and *Aspergillusniger* by a modified twofold serial dilution method [23,24]. All target compounds were evaluated at the concentrations ranging from 0.45 to 250 $\mu\text{g mL}^{-1}$ and scored for minimum inhibitory concentrations (MICs, $\mu\text{g mL}^{-1}$) that were defined as the lowest concentrations of the compound at which microbial growth was inhibited. Ampicillin 25 $\mu\text{g mL}^{-1}$ and Mycostatine 30 $\mu\text{g mL}^{-1}$ were co-assayed as positive control against tested bacteria and fungi, respectively.

3. Results and Discussion

Chemistry

Szmantet *al.* [25] have reported the synthesis of 4-(phenylthio)benzaldehyde (2) from the reaction of diphenyl sulfide (1) with zinc cyanide in presence of aluminum chloride and stream hydrogen chloride in low yield (45.7%). In our lab, we prepare, 4-(phenylthio)benzaldehyde (2) *via* nucleophilic substitution reaction of 4-fluorobenzaldehyde with thiophenol in refluxing dimethyl sulfoxide in the presence of anhydrous potassium carbonate. The

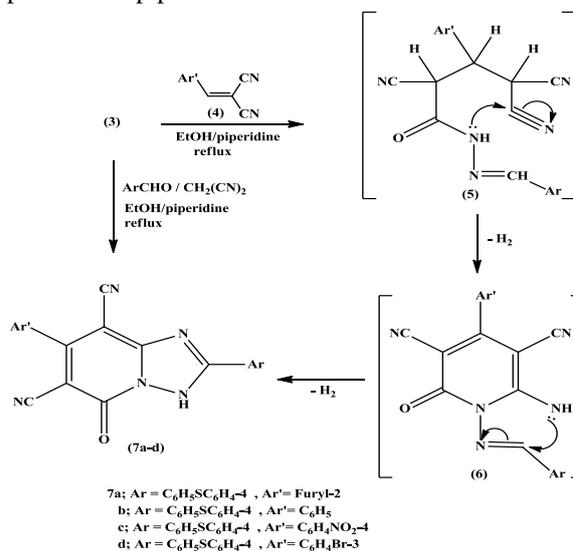
product was formed in excellent yield (92%) (Scheme 1). Treatment of 4-(phenylthio)benzaldehyde (**2**) with cyanoacetic acid hydrazide in absolute ethanol at room temperature furnished the novel 2-cyano-*N*-(4-phenylsulfanylbenzylidene) acetamide (**3**) in good yield (94 %) as yellow crystals, (Scheme 1). The structure of (**3**) was readily established by analytical and spectral data. Its infrared spectrum exhibited absorption bands at 3222, 2220 and 1696 cm^{-1} due to stretching vibrations of NH, $\text{C}\equiv\text{N}$ and $\text{C}=\text{O}$ groups. The ^1H NMR spectrum ($\text{DMSO}-d_6$) displayed signals at δ 4.20, 7.11-8.16, 8.66 and 11.98 ppm which were readily assigned to the methylene, aromatic, methine and NH protons, respectively.



Scheme 1: Synthesis of 4-(phenylthio)benzaldehyde (**2**) and 2-cyano-*N*-(4-phenylsulfanylbenzylidene) acetamide (**3**).

Hydrazone derivative (**3**) was used as a precursor for the synthesis of novel [1,2,4]triazolo[1,5-*a*]pyridine derivatives *via* its reaction with some electrophilic reagents. It has been found that, hydrazone derivative (**3**) reacted readily with arylidenemalononitriles (**4**) at reflux temperature in absolute ethanol in the presence of piperidine to give 1,2,4-triazolo[1,5-*a*]pyridine derivatives (**7a-d**) (Scheme 2). The structures of (**7**) were confirmed based on the elemental analyses and spectral data. The infrared spectra of compounds (**7a-d**) showed the presence of the NH, $\text{C}\equiv\text{N}$ and $\text{C}=\text{O}$ absorption bands. For example, the ^1H NMR spectrum ($\text{DMSO}-d_6$) of compound (**7a**) revealed a multiplet at δ 7.24-7.49 ppm assigned to the aromatic protons and downfield singlet at δ 9.88 ppm assigned to the NH proton. The mass spectrum of compound (**7a**) showed a molecular ion peak at m/z 435 (22.3 %) corresponding to the molecular formula $\text{C}_{24}\text{H}_{13}\text{N}_5\text{O}_2\text{S}$. Compounds **7** were assumed to be formed *via* addition of the active methylene group in (**3**) to the activated double bond in (**4**) to give the Michael adducts (**5**) which cyclized to give the

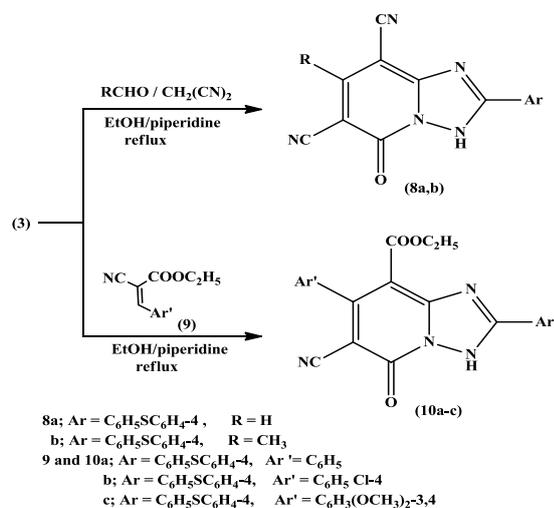
intermediates (**6**), followed by intramolecular cyclization and subsequent oxidation under the reaction conditions [26] (Scheme 2). Also, the structure of (**7**) was established by an independent synthesis *via* three component condensation of compound (**7**), aromatic aldehyde and malononitrile (1:1:1 molar ratio) in refluxing ethanol in the presence of piperidine.



Scheme 2: Synthesis of [1,2,4]triazolo[1,5-*a*]pyridines (**7a-d**).

As an extension of such synthetic route, ternary condensation of hydrazone (**3**), aliphatic aldehyde and malononitrile (1:1:1 molar ratio) in refluxing ethanol in the presence of piperidine furnished the novel 1,2,4-triazolo[1,5-*a*]pyridine derivatives (**8a,b**) (Scheme 3). The structures of compounds (**8a,b**) were established on the basis of their elemental analysis and spectral data. The infrared spectra of compounds (**8a,b**) indicated the presence of the NH, $\text{C}\equiv\text{N}$ and $\text{C}=\text{O}$ groups. The ^1H NMR spectrum of compound (**8b**) (CDCl_3) showed singlet signal at 1.62 ppm assigned to the methyl protons, a multiplet at δ 7.26-7.93 ppm assigned to the aromatic protons and downfield singlet at δ 8.42 ppm assigned to the NH proton. Moreover, the mass spectrum of the reaction product (**8a**) exhibited a molecular ion peak at m/z 369 (12.9%). By a similar route, the 1,2,4-triazolo[1,5-*a*]pyridine derivatives (**10a-c**) were obtained in good yields by condensation of hydrazone (**3**) with ethyl α -cyanocinnamates (**9**) (1:1 molar ratio) in refluxing ethanol in the presence of piperidine. Elemental and spectral data are compatible with triazolopyridines

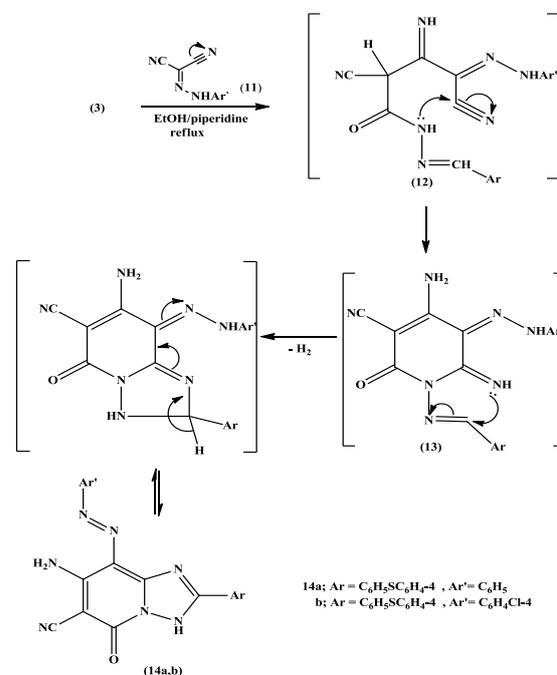
(10a-c). The infrared spectra of compounds (10a-c) showed the characteristic absorption bands for the NH group, cyano and two carbonyl groups. The ^1H NMR spectrum (DMSO- d_6) of compound (10b) showed a triplet at δ 1.37 ppm and a quartet δ 4.34 ppm assigned to the ethyl protons and a multiplet at δ 6.92-7.95 assigned to the aromatic protons as well as downfield singlet at δ 8.20 ppm assigned to the NH proton. In addition, the mass spectrum of compound 10c showed a molecular ion peak at m/z 552 (16 %) corresponding to the molecular formula $\text{C}_{30}\text{H}_{24}\text{N}_4\text{O}_5\text{S}$.



Scheme 3: Synthesis of [1,2,4]triazolo[1,5-a] pyridines (8a,b) and (10a-c).

The behaviour of hydrazone derivative (3) toward arylazomalononitriles (11) was also examined. Thus, compound (3) reacted with arylazomalononitriles (11) in ethanol containing a catalytic amount of piperidine to yield triazolopyridine derivatives (14a,b) (Scheme 4). Assignment of structure (14) was confirmed on the basis of analytical and spectral data. Thus, the infrared spectrum of compound (14a) showed the presence of NH₂ group stretching at 3452, 3420 cm^{-1} and C≡N group stretching at 2220 cm^{-1} as well as C=O at 1685 cm^{-1} . The ^1H NMR spectrum of compound (14b) (CDCl₃) showed singlet signal at δ 1.62 ppm assigned to the NH₂ protons, a multiplet at δ 6.92-7.73 ppm assigned to the aromatic protons and downfield singlet at δ 8.59 ppm assigned to the NH proton. The mass spectrum of compound (14b) showed a molecular ion peak at m/z 498 (42%) corresponding to the molecular formula $\text{C}_{25}\text{H}_{16}\text{N}_7\text{OClS}$. The formation (14) is assumed to

proceed *via* the addition of the active methylene group in (3) to the cyano group in (11) to give (12), which cyclized into the intermediate (13) followed by intramolecular cyclization and subsequent oxidation under the reaction conditions, (Scheme 4).

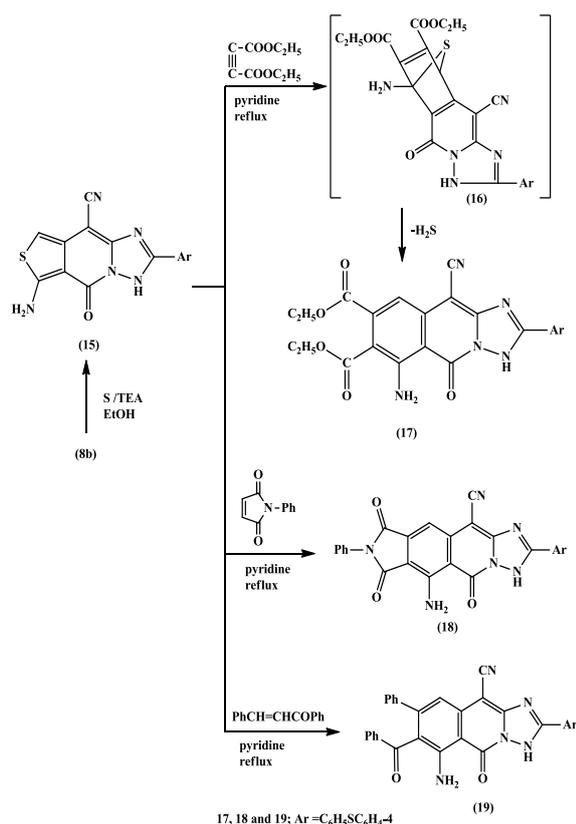


Scheme 4: Synthesis of [1,2,4]triazolo[1,5-a] pyridines (14a,b).

The fused thiophene derivative (15) was prepared by react the compound (8b) with elemental sulfur in ethanol under reflux in the presence of a base, via thiolation of the methyl group with elemental sulfur with the subsequent ring closure [27].

It is well established that, thienoheteroaromatics have been extensively investigated as novel dienes in Diels–Alder reactions [28, 29].

Thus, the behaviour of (15) towards electron-poor olefins was investigated. It has been found that, the reaction of thiophene derivative (15) with dimethyl acetylenedicarboxylate (DMAD) in dry pyridine, afforded 1,2,4-triazolo[1,5-a]isoquinoline derivative (17) (Scheme 5).



Scheme 5: Synthesis of compounds (17), (18) and (19).

The structure of (17) was established by examining analytical and spectral data. The infrared spectrum indicated the presence of absorption bands at 3335, 3319, 3100, 2212, 1745, 1710 and 1645 cm⁻¹ corresponding to NH, NH₂, C≡N and three carbonyl function groups, respectively.

Its ¹H NMR spectrum (DMSO-*d*₆) revealed two singlet signals at δ 1.54, 2.25 ppm which were readily assigned to the two methyl protons, a broad singlet at δ 4.10 ppm assigned to the NH₂ protons, a multiplet at δ 7.21-7.73 ppm assigned to the aromatic protons

Table 1 Antimicrobial activity of some of the newly synthesized compounds

Comp. no.	Gram positive bacteria		Gram negative bacteria		Fungi	
	<i>Bacillus Cereus</i>	<i>Micrococcus luteus</i>	<i>Escherichia coli</i>	<i>Serratia marcescens</i>	<i>Aspergillusnige</i> <i>r</i>	<i>Candida albicans</i>
3	++	+	+	+	++	+
7a	+	++	+	++	++	++
7b	+	+++	++	+	-	+
7c	++	++	++	+	++	+
7d	+++	+++	+	+++	+	+
8a	+++	+	+	++	+	+
8b	+	++	+++	+	+	+

and downfield singlet at δ 12.06 ppm assigned to the NH proton.

The formation of compound (17) is assumed to proceed via an initially formed cycloadducts (16), followed by hydrogen sulfide elimination under the reaction conditions [39,40].

Under similar reaction conditions, cyclization of compound (15) with *N*-phenylmaleimide furnished pyrrolo-triazoloisoquinoline derivative (18). Its infrared spectrum exhibited absorption bands 3330, 3120, 2213, 1710 and 1650 cm⁻¹ corresponding to NH₂, C≡N and two carbonyl function groups, respectively.

Moreover, its ¹H NMR spectrum (DMSO-*d*₆) showed a broad singlet at δ 4.21 ppm assigned to the NH₂ protons, a multiplet at δ 7.33-7.96 ppm assigned to the aromatic protons and downfield singlet at δ 8.27 ppm assigned to the NH proton. The triazoloisoquinoline derivative (19) was obtained by cyclocondensation of compound (15) with chalcone in dry pyridine.

The ¹H NMR spectrum (DMSO-*d*₆) of (19) showed a broad singlet at δ 5.20 ppm assigned to the NH₂ protons, a multiplet at δ 7.39-7.93 ppm assigned to the aromatic protons and downfield singlet at δ 10.13 ppm assigned to the NH proton.

Antimicrobial screening

The results of antimicrobial screening data revealed that most of the synthesized compounds showed varying degrees of inhibition against both bacteria and fungi. The antibacterial and antifungal data were depicted in Table 1.

10a	++	-	++	++	+	+
10b	++	+	+	++	++	++
10c	+	++	+++	+	+	++
14a	+++	+	++	+	++	+
14b	++	++	+	+	++	+
15	+	++	+++	++	+	++
17	++	+	++	+	++	-
18	++	+++	+	-	+	++
19	+++	++	++	+	++	+
Standard	++++	++++	++++	++++	++++	++++

+: Less active (0.1-0.5 cm), ++: Moderately active (0.6-1.4 cm), +++: Highly active (1.5-3 cm) and ++++ : Very highly activity (over 3.0 cm). Standard: For Gram positive and Gram negative bacteria: Ampicillin 25 µg mL⁻¹; for fungi: Mycostatine 30 µg mL⁻¹

4. Conclusions

New 1,2,4-triazolo[1,5-*a*]pyridine and 1,2,4-triazolo[1,5-*a*]isoquinolinederivatives were prepared from easily accessible starting materials. All the newly synthesized compounds were evaluated for their antibacterial and antifungal activities *in vitro* against four bacteria and two fungi.

5. Conflicts of interest

There are no conflicts to declare.

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