Original Research Article

BIOLOGICAL EVALUATION OF SOME NOVEL THIAZOLE, THIAZOLO[3,2-a]PYRIDINE AND THIAZOLO[3',2':1,6]PYRIDINE RERIVATIVES CONTAINING DIPHENYL MOIETY AS ANTIMICROBIAL AGENTS

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ABSTRACT

Purpose: The main objective of the present research study is to synthesize some novel thiazole, thiazolo[3,2-a]pyridine and thiazolo[3',2':1,6]pyrido[2,3-d]pyrimidine derivatives and evaluate them for their Antimicrobial effect.

Methods: Condensation of 4-phenylmercaptobenzaldehyde 2 with thiosemicarbazide afforded the new thiosemicarbazone derivative 3. Heterocyclization of thiosemicarbazone 3 with various α-halocarbonyl compounds furnished the novel thiazole derivatives 5, 6 and 7. Compound 2 was condensed with 2-cyanomethylene-4-thiazolidinone 8 to yield the 4-thiazolidinone derivative 9. Cyclocondensation of compound 9 with arylidenemalononitrile (1:1 molar ratio) afforded the novel thiazolo[3,2-a]pyridine derivatives 10a-c. Ternary condensation of compound 2, malononitrile and thioglycolic acid (2:2:1 molar ratio) yielded the thiazolo[3,2-a]pyridine derivative 12. Compound 12 was cyclized with formic acid and formamide to yield the thiazolo-[3',2':1,6]pyrido[2,3-d]pyrimidine derivatives 13 and 14, respectively. The structures of the newly synthesized compounds were confirmed on the basis of analytical and spectral data. Some of the prepared compounds 3, 5, 7, 9, 10a,b and 12 were tested in vitro against bacterial and fungal strains.

Results: The results of antimicrobial screening data revealed that most of the synthesized compounds showed varying degrees of inhibition against both bacteria and fungi.

Conclusion: New thiazole and thiazolo[3,2-a]pyridine derivatives were prepared from easily accessible starting materials. Some newly synthesized compounds were evaluated for their antibacterial and antifungal activities in vitro against four bacteria and two fungi.

Keywords: Thiosemicarbazone, 2-Cyanomethylene-4-thiazolidinone Thiazole, Thiazolo[3,2-a]pyridine, Antimicrobial agents.

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Running Title-Synthesis and antimicrobial screening of some novel thiazoles.

INTRODUCTION

Aryl sulfide derivatives are a common functionality found in numerous pharmaceutically active compounds. Indeed, a number of drugs in therapeutic areas such as diabetes and inflammatory, immune, Alzheimer’s, and Parkinson’s diseases contain the aryl sulfide functionality [1-12]. On the other hand, the heterocyclic scaffold comprising thiazole are present in compounds possessing a variety of biological activities such as antibacterial [13], antifungal [14], antioxidant [15], cytotoxic [16], analgesic, anti-inflammatory [17,18], anticonvulsant [19] anti YFV (yellow fever virus), anti-HIV [20] and anti-tubercular [21] and antiviral [22] activities. Also, our search of the literature revealed that, some
thiazolo[3,2-a]pyridine derivatives have been reported to possess antibacterial [23], Anti-virulence [24] and anti-hypertensive [25], antioxidant and cytotoxic [26] activities. Prompted by the above facts and in continuation of our efforts in the field of biologically active heterocyclic compounds [27-29], we hereby report the synthesis and antimicrobial evaluation of some novel thiazole and thiazolo[3,2-a]pyridine derivatives containing diphenyl sulfide moiety from easily accessible starting materials.

MATERIALS AND METHODS

General:

Melting points were determined on a Gallenkamp apparatus and are 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 and ¹³C NMR spectra were recorded in a Varian Mercury VXR-300 spectrometer (300 MHz for ¹H NMR and 75 MHz for ¹³C NMR) in DMSO-d₆ 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 Microanalytical Laboratory of Cairo University, Giza, Egypt. 2-Cyanomethylene-4-thiazolidinone [8] was synthesized using method previously published [30]. Antimicrobial activities were carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

Experimental:

a. 4-Phenylmercaptobenzaldehyde [2]

A mixture of 4-fluorobenzaldehyde (0.01 mol) and thiophenol (0.01 mol) in dimethyl sulfoxide (20 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 acetic acid / water to give 2 as colorless crystals. Yield 92%; m.p. 52-53°C [Lit. 53-54°C] [30]. Anal. Calcd. For C₁₃H₁₀OS: C, 72.87; H, 4.72; S, 14.96. Found: C, 72.97; H, 4.65; S, 14.91.

b. 2-(4-(Phenylthio)benzylidene)hydrazine-1-carbothioamide [3]

A mixture of 4-phenylmercaptobenzaldehyde [2] (0.01 mol) and thiosemicarbazide (0.01 mol) in ethanol (30 mL) was heated under reflux for 1 h. A crystalline solid was obtained on cooling. It was crystallized from ethanol. Yield 83% as yellow crystals; mp 180 – 181°C; IR (KBr; cm⁻¹): 3417, 3274, 3135 (NH₂/NH), 3030 (CH_arom), 2980 (CH_aliph), 1578 (C=N). ¹HNMR (300 MHz, DMSO-d₆, δ/ppm): 6.23 (br, 2H, NH₂, exchangeable with D₂O), 7.32 - 7.70 (m, 9H, Ar-H), 8.17 (s, 1H, CH = N) and 11.40 ppm (s, 1H, NH, exchangeable with D₂O); MS (m/z): 60 (100%), 109 (25%), 211 (52.3%), 287 (21%), 288 (5.2%; M⁺). Anal. Calcd. for C₁₄H₁₃N₃S₂ (287.40): C, 58.51; H, 4.56; N, 14.62. Found: C, 58.30; H, 4.60; N, 14.80.

c. General Procedure for the Synthesis of compounds [5, 6 and 7]

A mixture of compound [3] (0.01 mol) and α-halocarbonyl reagents (0.01 mol) [4a-c] and fused sodium acetate (2 gm) in ethanol (30 mL) was refluxed for 3 hrs, left to cool and poured into crushed ice. The obtained solid product was collected by filtration, washed with water and crystallized from the proper solvents.

4-Methyl-2-(2-(4-(phenylthio)benzylidene)hydrazinyl)thiazole [5]

Yield 73% as gray crystals (from 1,4-Dioxane-H₂O); mp 320 – 321°C; IR (KBr; cm⁻¹): 3432 (NH), 3027 (CH_arom), 2986 (CH_aliph), 1639 (C=N). ¹HNMR (300 MHz, DMSO-d₆, δ/ppm): ¹HNMR (300 MHz, DMSO-d₆, δ/ppm): 2.29 (s, 3H, CH₃), 6.18 (s, 1H, thiazole-H), 7.27-7.56 (m, 9H, Ar-H), 7.83 (s, 1H, CH=N) and 8.56 ppm (s 1H,NH, exchangeable with D₂O). ¹³CNMR (75 MHz, DMSO-d₆, δ/ppm): 15.12 (CH₃), 141.16, 138.11, 135.14, 132.34, 131.15, 130.57, 129.25, 129.44, 127.33, 108.15 (Ar-C and Ar-CH),
Yield 75% as brown crystals (from 1,4-Dioxane-H₂O); mp 340–341°C; IR (KBr; cm⁻¹): 3464 (NH), 3045 (CHₑ₀₉ᵣₒ₉), 2976 (CHₑ₀₉ᵝᵢ₉ᵢ), 1638 (C=N). ¹H NMR (300 MHz, DMSO-d₆, δ/ppm): 6.52 (s, 1H, thiazolo-H), 7.14-7.81 (m, 14 H, Ar-H), 8.7 (s, 1H, CH=N) and 8.96 ppm (s 1H,NH, exchangeable with D₂O). MS (m/z): 77 (100%), 132 (24%), 295 (26.3%), 387 (23%), 388 (5%; M⁺). Anal. Calcd. for C₂₂H₁₇N₃S₂ (387.52): C, 68.19; H, 4.42; N, 10.84. Found: C, 68.10; H, 4.30; N, 10.90.

2-(2-(2-(4-(Phenylthio)benzylidene)hydrazinyl)benzothiazol-4-amine (7)

A mixture of 2-cyanomethylene-4-thiazolidinone 8 (0.01 mol) with 4-phenylmercaptobenzaldehyde 2 (0.01 mol) in ethanol (30 mL) and a few drops of piperidine was refluxed for 1 hour. After cooling, the resulting solid product was collected by filtration, washed with water and the crude product crystallized from ethanol to give 9. Yield 68% as orange crystals; mp 310–311°C (KBr; cm⁻¹): 3434, 3320, 3110 (NH₃NH₂), 3048 (CHₑ₀₉ᵣₒ₉), 2982 (CHₑ₀₉ᵝᵢ₉ᵢ), 1638 (C=N). ¹H NMR (300 MHz, DMSO-d₆, δ/ppm): 5.60 ppm (2H,br, NH₂, exchangeable with D₂O), 6.20 (s, 1H, thiazolo-H), 7.30-8.35 (m, 9H, Ar-H), 8.62 (s, 1H, CH=N) and 11.42 ppm (s, 1H, NH, exchangeable with D₂O). ¹³CNMR (75 MHz, DMSO-d₆, δ/ppm): 139.25, 137.45, 134.26, 132.71, 131.30, 130.15, 129.56, 128.69, 127.53, 110.65 (Ar-C and Ar-CH), 156.13 (C=N), 152.28 (C=N). Anal. Calcd. for C₁₅H₁₄N₄S₂ (326.43): C, 58.87; H, 4.32; N, 17.16. Found: C, 58.76; H, 4.26; N, 17.10.

2-(4-Oxoy-5-(4-(phenylthio)benzylidene)-4,5-dihydrothiazol-2-yl)-acetonitrile (9)

A mixture of 2-cyanomethylene-4-thiazolidinone 8 (0.01 mol) with 4-phenylmercaptobenzaldehyde 2 (0.01 mol) in ethanol (30 mL) and a few drops of piperidine was refluxed for 1 hour. After cooling, the resulting solid product was collected by filtration, washed with water and the crude product crystallized from ethanol to give 9. Yield 68% as orange crystals; mp 310–311°C (KBr; cm⁻¹): 3434, 3320, 3110 (NH₃NH₂), 3048 (CHₑ₀₉ᵣₒ₉), 2982 (CHₑ₀₉ᵝᵢ₉ᵢ), 1638 (C=N). ¹H NMR (300 MHz, DMSO-d₆, δ/ppm): 5.60 ppm (2H,br, NH₂, exchangeable with D₂O), 6.20 (s, 1H, thiazolo-H), 7.30-8.35 (m, 9H, Ar-H), 8.62 (s, 1H, CH=N) and 11.42 ppm (s, 1H, NH, exchangeable with D₂O). ¹³CNMR (75 MHz, DMSO-d₆, δ/ppm): 139.25, 137.45, 134.26, 132.71, 131.30, 130.15, 129.56, 128.69, 127.53, 110.65 (Ar-C and Ar-CH), 156.13 (C=N), 152.28 (C=N). Anal. Calcd. for C₁₅H₁₄N₄S₂ (326.43): C, 58.87; H, 4.32; N, 17.16. Found: C, 58.76; H, 4.26; N, 17.10.

d. General Procedure for the Synthesis of thiazolo[3,2-a]-pyridines (10a-c)

A mixture of compound 9 (0.01 mol) and aryldenemalononitrile (0.01 mol) in ethanol (30 mL) was treated with a few drops of piperidine and refluxed for 3 hrs. A crystalline solid was obtained on cooling. It was crystallized from an appropriate solvent.

5-Amino-3-oxo-7-phenyl-2-(4-(phenylthio)benzylidene)-2,3-dihydro-7H-thiazolo[3,2-a]pyridine-6,8-dicarbonitrile (10a).

5-Amino-7-(4-nitrophenyl)-3-oxo-2-(4-(phenylthio)benzylidene)-2,3-dihydro-7H-thiazolo[3,2-a]pyridine-6,8-dicarbonitrile (10b).

Yield 68% as yellow crystals (from Ethanol); mp 214–215°C; IR (KBr; cm⁻¹): 3400, 3340 (NH₂), 3042 (CHₑ₀₉ᵣₒ₉), 2951 (CHₑ₀₉ᵝᵢ₉ᵢ), 2215, 2209 (2CN), 1719 (C=O). ¹H NMR (300 MHz, DMSO-d₆, δ/ppm): 5.40 (s, 1H, pyridine-H), 6.50 (br, 2H, NH₂, exchangeable with D₂O ), 7.23-7.52 (m, 14H, Ar-H and methylidene-H). MS (m/z): 66 (100%), 109 (43%), 242 (19.5%), 360 (26%), 535 (20%), 536 (8.3%; M⁺). Anal. Calcd. for C₂₉H₁₇N₅S₃O₃ (535.60): C, 62.79; H, 3.20; N, 13.08. Found: C, 62.63; H, 3.26; N, 12.87.
5-Amino-7-(4-methoxyphenyl)-3-oxo-2-(4-(phenylthio)-benzylidene)-2,3-dihydro-7H-thiazolo[3,2-alpyridine-6,8-dicarbonitrile (10c)

Yield 65% as yellow crystals (from Ethanol); mp 216–217ºC; IR (KBr; cm⁻¹): 3401, 3364 (NH₂) 3054 (CH₃), 2937 (CH₃), 2211, 2197 (C=O). ¹HNMR (300 MHz, DMSO-d₆, δ/ppm): 2.36 (s, 3H, CH₃), 5.30 (s, 1H, pyridine-H), 6.80 (br, 2H, NH₂, exchangeable with D₂O), 7.12-7.48 (m, 14H, Ar-H, and methylidene-H). Anal. Calcd. for C₂₉H₂₅N₂O₄S₂ (504.63): C, 69.03; H, 4.00; N, 11.10. Found: C, 68.92; H, 3.84; N, 11.15.

e.5-Amino-3-oxo-2-(4-(phenylthio)benzylidene)-7-(4-(phenylthio)phenyl)-2,3-dihydro-7H-thiazolo[3,2-alpyridine-6,8-dicarbonitrile (12)

A mixture of 4-phenylmercaptobenzaldehyde 2 (0.02 mol), malononitrile (0.02 mol) and thioglycolic acid (0.01 mol) in ethanol (30 mL) was treated with a few drops of piperidine and refluxed for 3 hrs. The solid product so formed was collected by filtration and crystallized from ethanol to give 12. Yield 62% as brown crystals; mp 210–211ºC; IR (KBr; cm⁻¹): 3383, 3200 (NH₂), 3035 (CH₃), 2950 (CH₃), 2211, 2197 (C=O). ¹HNMR (300 MHz, DMSO-d₆, δ/ppm): 5.62 (s, 1H, H-pyridine) 6.40 (br, 2H, NH₂, exchangeable with D₂O) and 7.30 - 7.78 ppm (m, 19H, Ar-H, and methylidene-H). Anal. Calcd. for C₃₉H₂₂N₂S₂O (598.76): C, 68.20; H, 3.70; N, 9.36. Found: C, 68.11; H, 3.52; N, 9.19.

f.4,9-Dioxo-8-(4-(phenylthio)benzylidene)-5-(4-(phenylthio)phenyl)-3,5,8,9-tetrahydro-4H-thiazolo[3',2':1,6]pyrido[2,3-d]pyrimidine-6-carbonitrile (13)

A mixture of 12 (0.01 mol) in absolute ethanol (30 mL) and formic acid (5 mL) was refluxed for 5 hrs. After cooling, the solid product was collected by filtration and crystallized from ethanol to give 13. Yield 62% as brown crystals; mp 210–211 ºC; IR (KBr; cm⁻¹): 3444 (NH), 3059 (CH₃), 2215 (C=O), 1710, 1659 (2C=O). ¹HNMR (300 MHz, DMSO-d₆, δ/ppm): 5.61 (s, 1H, pyrimidine-H), 6.91-7.67 (m, 19H, Ar-H and methylidene-H), 7.90 (s, 1H, pyrimidine-H) and 11.6 (s, 1H, NH, exchangeable with D₂O). Anal. Calcd. for C₃₉H₂₂N₂S₂O (626.77): C, 67.07; H, 3.54; N, 8.94. Found: C, 66.93; H, 3.39; N, 8.68.

g.4-Amino-9-oxo-8-(4-(phenylthio)benzylidene)-5-(4-(phenylthio)phenyl)-8,9-dihydro-5H-thiazolo[3',2',1,6]pyrido[2,3-d]pyrimidine-6-carbonitrile (14)

A mixture of 12 (0.01 mol) in absolute ethanol (20 mL) and formamide (5 mL) was refluxed for 5 hrs and poured into ice-water. The solid product was collected by filtration, washed with water and crystallized from ethanol to give 14. Yield 68% brown solid; mp 220–221 ºC; IR (KBr; cm⁻¹): 3420, 3407 (NH₂), 3068 (CH₃), 2929 (CH₃), 2211 (C=O), 1700 (C=O). ¹HNMR (300 MHz, DMSO-d₆, δ/ppm): 5.63 (s, 1H, pyridine-H), 6.40 (s, 2H, NH₂, exchangeable with D₂O), 7.20-8.02 ppm (m, 19H, Ar–H and methylidene-H), 8.43 (s, 1H, pyrimidine-H). Anal. Calcd. for C₃₉H₂₃N₃S₃O (625.78): C, 67.18; H, 3.70; N, 11.19. Found: C, 67.10; H, 3.56; N, 11.07.

**Antimicrobial screening**

Some of the prepared compounds 3, 5, 7, 9, 10ab, and 12 were tested in vitro against four bacterial strains, *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli* and *Serratia marcesens* and two strains of fungi, *Candida albicans* and *Aspergillus niger* using the agar cup diffusion (8 mm diameter) assay against the microbial organisms, listed in Table 1 [31]. A 1 mg/mL solution in DMSO was used. The bacteria and fungi were maintained on nutrient agar and Czapek’s-Dox agar media, respectively. DMSO showed no inhibition zones. The agar media were inoculated with different microorganism cultures tested. After 24h of incubation at 30 ºC for bacteria and 48 h of incubation at 28ºC for fungi. Zones of inhibition were recorded in millimeters. Ampicillin in a concentration 25 µg mL⁻¹ and Mycostatine (30 µg mL⁻¹) used as a reference for antibacterial and antifungal activities, respectively. The minimal inhibitory concentration (MIC) of some of the tested compounds was measured by a serial plate dilution method [31].
DISCUSSION

Szmant and co-workers [30] have performed the synthesis of 4-phenylmercaptobenzaldehyde 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 synthesize 4-phenylmercaptobenzaldehyde 2 via nucleophilic substitution reaction of 4-fluorobenzaldehyde with thiophenol in refluxing dimethyl sulfoxide in the presence of anhydrous potassium carbonate. The product was isolated in 92% yield (Scheme 1). Thiosemicarbazone derivative 3 was obtained by condensation of 4-phenylmercaptobenzaldehyde 2 with thiosemicarbazide in refluxing ethanol. The molecular structure of thiosemicarbazone 3 was readily established by analytical and spectral data. The infrared spectrum of 3 showed the presence of NH$_2$ group stretching at 3417, 3274 cm$^{-1}$ as well as C=N group at 1578 cm$^{-1}$. The $^1$H NMR spectrum was also in accordance with the proposed structure. Also, the mass spectrum of compound 3 showed a molecular ion peak at m/z 288 (5.2%; M$^+$) corresponding to the molecular formula C$_{14}$H$_{13}$N$_3$S$_2$ and the base peak was found in the spectrum at m/z 60 (100%) characteristic for the thiocarbamoyl moiety.

![Scheme 1 Synthesis of 4-phenylmercaptobenzaldehyde 2 and thiosemicarbazone 3](image-url)

The behavior of thiosemicarbazone derivative 3 toward some α-halocarbonyl reagents was investigated with respect to the synthesis of highly substituted thiazoles. Heterocyclization of thiocarbamoyl functional group in compound 3 with chloroacetone 4a at reflux temperature in the presence of fused sodium acetate furnished the 4-methylthiazole derivative 5 (Scheme 2). The structure of compound 5 was confirmed on the basis of its elemental analysis and spectral data. The infrared spectrum of compound 5 indicated the absence of the thiocarbamoyl group absorption band, indicating the formation of thiazole derivative 5. In addition, the $^1$H NMR spectrum (DMSO-$d_6$) of compound 5 revealed singlet signal at $\delta$ 2.29 ppm (3H) for methyl protons, 6.18 ppm (1H) corresponding to thiazole proton at 5-position, 7.83 ppm (1H) attributable to the methine proton and the presence of multiplet signal at $\delta$ 7.27-7.56 ppm (9H) for aromatic protons, while the NH proton appears as a broad singlet at $\delta$ 8.56 ppm. Also, the mass spectrum of compound 5 showed a molecular ion peak at m/z 326 which is in agreement with the molecular formula C$_{17}$H$_{15}$N$_3$S$_2$. The formation of thiazole derivative 5...
is assumed to proceed through initial alkylation by loss of sodium chloride to form intermediate A, followed by intramolecular cyclization via elimination of water [28]. In a similar manner, cyclocondensation of thiocarbamoyl functional group in compound 3 with phenacyl bromide 4b in refluxing ethanol in the presence of fused sodium acetate afforded 4-phenylthiazole derivative 6 (Scheme 2). The mass spectrum of 6 is in accordance with the proposed structure which showed a molecular ion peak at m/z 388 (5%) corresponding to the molecular formula C_{22}H_{17}N_{3}S_{2} and the base peak was found in the spectrum at m/z 77 (phenyl moiety). Cycloalkylation of thiocarbamoyl functional group in compound 3 with chloro acetonitrile 4c by refluxing in ethanol in the presence of fused sodium acetate [28] furnished 4-aminothiazole derivative 7. The infrared spectrum of the reaction product showed the characteristic absorption bands at 3434, 3320, 3110 cm\(^{-1}\) for the NH\(_2\)/NH groups and 1578 cm\(^{-1}\) for the C=N group. The \(^1\)H NMR spectrum (DMSO-d\(_6\)) showed a broad singlet at \(\delta\) 5.60 ppm (br, 2H) assigned to the NH\(_2\) protons, 6.2 (s, 1H), 7.30-8.35 (m,9H), 8.62 (s, 1H) and 11.42 (s, 1H) ppm which were assigned to the thiazole proton at 5-position, aromatic, methine, and NH protons, respectively. The formation of 7 is assumed to proceed via initial alkylation to form the intermediate B followed by nucleophilic addition to the cyano group (Scheme 2).

Scheme 2  Synthesis of thiazole derivatives 5, 6 and 8.

Condensation of 2-cyanomethylene-4-thiazolidinone 8 with 4-phenyl-mercaptobenzaldehyde 2 in ethanolic piperidine and reflux yielded 2-(4-oxo-5-(4-(phenylthio)benzylidene)-4,5-dihydrothiazol-2-yl)acetonitrile 9, (Scheme 3). The molecular structure of 9 was elucidated on the basis of analytical and spectral data. The infrared spectrum showed the appearance of absorption bands at 2200 and 1695 cm\(^{-1}\) for C≡N and C=O (4-thiazolidinone), respectively. Its \(^1\)HNMR spectrum (DMSO-d\(_6\)) showed the appearance of a signal for methylene protons at \(\delta\) 4.20 ppm and 7.33-7.78 ppm for aromatic and methylidene protons. Cyclization of 4-thiazolidinone derivative 9 with arylidenemalononitrile (1:1 molar ratio) in ethanol containing a catalytic amount of piperidine afforded the novel thiazolo[3,2-a]pyridine.
derivatives 10a-c (Scheme 3). The structures of 10 were established on the basis of their elemental analysis and spectral data (IR, 1H NMR, 13C NMR and MS). The infrared spectra of compounds 10a-c showed the presence of the NH2, C≡N and C=O absorption bands. The 1H NMR spectrum (DMSO-d6) of compound 10a revealed a singlet at δ 5.60 ppm which was assigned to the proton at the 7-position of the thiazolo[3,2-a]pyridine ring, a broad singlet at δ 6.70 ppm assigned to the NH2 protons and a multiplet at δ 7.12-7.48 (m, 14H) assigned to the aromatic and methylidene protons. Moreover, the mass spectrum for the thiazolo[3,2-a]pyridine 10b showed a molecular ion peak at m/z 536 (8.3%) corresponding to the molecular formula C28H17N5S2O3 with base peak at m/z 66 (malononitrile fragment) The formation of thiazolopyridine 10 is assumed to proceed via Michael addition of methylene functional group in compound 9 to the benzylidene moiety to yield Michael adduct C (Scheme 3), followed by intramolecular cyclization at the cyano group and aromatization[23].

![Scheme 3 Synthesis of 4-thiazolidinone 9 and thiazolo[3,2-a]pyridines 10a-c](image)

Ternary condensation of 4-phenylmercaptobenzaldehyde 2, malononitrile and thioglycolic acid (2: 2: 1 molar ratio) in ethanol at reflux temperature in the presence of piperidine yielded thiazolo[3,2-a]pyridine derivative 12 (Scheme 4). The assignment of the structure 12 was based on elemental analysis and spectral data. The infrared spectrum of 12 displayed stretching bands at 3383, 3200 cm⁻¹ for the formed amino group and two absorption bands for two C≡N groups of 2211, 2197 cm⁻¹, in addition to the presence of absorption at 1716 cm⁻¹ for the C=O group. Its 1H NMR spectrum (DMSO-d6) showed appearance of a signal at δ 5.62 ppm for the proton at the 7-position of the thiazolo[3,2-a]pyridine ring and broad signal at δ 5.62 ppm for new formed amino protons, in addition to broad signal at δ 7.30-7.78 ppm for aromatic and methylidene protons. The formation of thiazolopyridine derivative 12 can be explained by the reaction pathway depicted in Scheme 5.

Thiazolo[3′,2′:1,6]pyrido[2,3-d] pyrimidine derivative 13 was obtained by refluxing of compound 12 with formic acid [32] (Scheme 6). The structure of compound 13 was confirmed by its analyses and spectral data. The infrared spectrum displayed absorption bands for the NH, C≡N and two carbonyl functions at 3444, 2215, 1710 and 1659 cm⁻¹, respectively. Its 1H NMR spectrum (DMSO-d6) revealed a singlet signal at δ 5.61 ppm for the proton at the 5-position of the thiazolopyridopyrimidine ring, a multiplet at δ 6.91-7.67 ppm assigned to the aromatic and methylidene protons, a singlet signal at δ 7.90 ppm assigned to the proton at the 2-position of the pyrimidine ring while the NH was
appeared at δ 11.6 ppm. The formation of 13 was assumed to take place via the intramolecular cyclization of the intermediate E with formic acid (Scheme 1). Cyclocondensation of compound 12 with formamide under reflux furnished the corresponding thiazolo-[3',2':1,6]pyrido[2,3-d]pyrimidine derivative 14 (Scheme 6). The structure of this product was established on the basis of its elemental analysis and spectroscopic data.

Scheme 4 One-pot synthesis of thiazolo[3,2-a]pyridine 12

The infrared spectrum of the products showed absorption bands corresponding to NH$_2$, nitrile and carbonyl groups. The $^1$H NMR spectrum was also in accordance with the proposed structure. The
Formation of 14 was assumed to proceed through initial formation of the intermediate F followed by intramolecular cyclization via nucleophilic addition of amino group to the cyano group [23] (Scheme 6).

![Scheme 6 Synthesis of thiazolo[2,3':6,1]pyrido[2,3-d]-pyrimidines 13&14](image)

**Table 1: Antimicrobial activity of some of the newly synthesized compounds.**

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
<th>Fungi</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Bacillus Cereus</td>
<td>Micrococcus luteus</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>10b</td>
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<td>++</td>
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<td>10c</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>12</td>
<td>++</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Standard</td>
<td>++++</td>
<td>++++</td>
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</tr>
</tbody>
</table>

+ : Less active (0.1-0.5 cm)
++ : Moderately active (0.6-1.4 cm)
+++ : Highly active (1.5-3.0 cm)
++++ : Very highly active (over 3.0 cm)

Standard: For Gram positive and Gram negative bacteria: Ampicillin 25 μg mL⁻¹; for fungi: Mycostatine 30 μg mL⁻¹.
Antimicrobial screening

The antibacterial screening revealed that some of the tested compounds showed good inhibition against various tested microbial strains. The result indicated that among the synthesized compounds, 3 and 5 showed good activity against *Serratia marcesens*. Compound 7 also possessed good antibacterial activity against *Bacillus Cereus*. The remaining compounds showed moderated activity against the tested bacterial strains.

The in vitro antifungal data indicated that the compound 10b exhibited good antifungal activity against the fungi tested *Candida albicans*. Also, compound 9 with a diphenyl sulfide moiety showed excellent activity against *Aspergillus niger*. Compounds 10b,c were found to be weakly active against both *Micrococcus luteus* and *Aspergillus niger*. The results of the antimicrobial screening of selected new compounds are summarized in Table 1.

REFERENCES


