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. ^{1}H and ^{13}C NMR spectra were recorded in a Varian Mercury VXR-300 spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C 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 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^{0}C [Lit.53-54^{0}C] [30] .Anal.Calcd. For C_{13} H_{10} OS: 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 180–181ºC; IR (KBr; cm^{-1}): 3417, 3274, 3135 (NH_2/NH), 3030 (CH_{arom}), 2980 (CH_{aliph}), 1578 (C=N). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 6.23 (br, 2H, NH_2, exchangeable with D_2O)), 7.32 - 7.70 (m, 9H, Ar –H), , 8.17 (s, 1H, CH = N) and 11.40 ppm (s, 1H, NH, exchangeable with D_2O); MS(m/z) 60 (100%), 109 (25%), 211 (52.3%), 287 (21%), 288 (5.2%; M^+) . Anal. Calcd. for $C_{14}H_{13}N_3S_2$ (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-H_2O); mp 320–321ºC; IR (KBr; cm^{-1}): 3432 (NH), 3027 (CH_{arom}), 2986 (CH_{aliph}), 1639 (C=N). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 2.29 (s, 3H, CH_3), 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 D_2O)**.** ^{13}CNMR (75 MHz, DMSO-d_6, δ/ppm): 15.12 (CH_3), 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),

158.24 (C=N), 153.13 (C=N). MS (m/z): 114 (100%), 184 (18.2%), 325 (16%), 326 (8%: M+1). Anal. Calcd. for $C_{17}H_{15}N_3S_2$ (325.45): C, 62.74; H, 4.65; N, 12.91. Found: C, 62.90; H, 4.49; N, 12.80.

4-Phenyl-2-(2-(4-(phenylthio)benzylidene)hydrazinyl)thiazole **(6)**

Yield 75% as brown crystals (from 1,4-Dioxane-H_2O); mp 340–341ºC; IR (KBr; cm^{-1}): 3464 (NH), 3045 (CH_{arom}), 2976 (CH_{aliph}), 1638 (C=N). ^{1}HNMR (300 MHz, DMSO-d_6, 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 D_2O)**.** MS (m/z): 77 (100%), 132 (24%), 295 (26.3%), 387 (23%), 388 (5%;M^+). Anal. Calcd. for $C_{22}H_{17}N_3S_2$ (387.52): C, 68.19; H, 4.42; N, 10.84. Found: C, 68.10; H, 4.30; N, 10.90.

2-(2-(4-(Phenylthio)benzylidene)hydrazinyl)thiazol-4-amine **(7)**

Yield 78 % as yellow crystals; mp 310–311ºC; (KBr; cm^{-1}): 3434, 3320, 3110 (NH_2/NH), 3048 (CH_{arom}), 2982 (CH_{aliph}), 1638 (C=N). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 5.60 ppm (2H,br, NH_2, exchangeable with D_2O) , 6.20 (s, 1H, thiazole-H), 7.30-8.35 (m, 9H, Ar–H), 8.62 (s, 1H, CH=N) and 11.42 ppm (s, 1H, NH, exchangeable with D_2O). ^{13}CNMR (75 MHz, DMSO-d_6, 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 $C_{16}H_{14}N_4S_2$ (326.43): C, 58.87; H, 4.32; N, 17.16. Found: C, 58.76; H, 4.26; N, 17.10.

2-(4-Oxo-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 310–311ºC; (KBr; cm^{-1}): 3040 (CH_{arom}), 2950 (CH_{aliph}), 2200 (C≡N), 1695 (C=O). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 4.20 (s, 2H, CH_2CN), 7.33-7.78 ppm (m, 10H, Ar–H and methylidene-H). Anal. Calcd. for $C_{18}H_{12}N_2OS_2$ (336.43): C, 64.26; H, 3.60; N, 8.33. Found: C, 64.19; H, 3.49; N, 8.24.

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

A mixture of compound **9** (0.01 mol) and arylidenemalononitrile (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).**

Yield 72% as orange crystals (from Ethanol); mp 214–215ºC; IR (KBr; cm^{-1}): 3346, 3221 (NH_2), 3059 (CH_{arom}), 2927 (CH_{aliph}), 2210 (C≡N), 1725 (C=O). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 5.6o (s, 1H, pyridine-H), 6.70 (br, 2H, NH_2, exchangeable with D_2O) 7.12-7.48 (m, 14H, Ar-H and methylidene-H). ^{13}CNMR (75 MHz, DMSO-d_6, δ/ppm): 35.25 (pyridine-C4), 56.14, 76.36, 124.92, 127.45, 128.15, 128.66, 129.51, 131.16, 131.76, 133.26, 133.52, 135.23, 135.64, 143.36, 149.55, 158.19 (Ar-C and Ar-CH), 116.65, 118.75 (2CN), 162.48 (C=O). Anal. Calcd. for $C_{28}H_{18}N_4S_2O$ (490.60): C, 68.55; H, 3.70; N, 11.42. Found: C, 68.41; H, 3.84; N, 11.28.

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 228–229ºC; IR (KBr; cm^{-1}): 3400, 3340 (NH_2), 3042 (CH_{arom}), 2951 (CH_{aliph}), 2215, 2209 (2C≡N), 1719 (C=O). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 5.40 (s, 1H, pyridine-H), 6.50 (br, 2H, NH_2, exchangeable with D_2O), 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 $C_{28}H_{17}N_5S_2O_3$ (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-a]pyridine-6,8-dicarbonitrile **(10c)**

Yield 65% as yellow crystals (from Ethanol); mp 216–217ºC; IR (KBr; cm^{-1}): 3401, 3364 (NH_2) 3054 (CH_{arom}), 2937 (CH_{aliph}), 2200 (C≡N), 1690 (C=O). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 2.36 (s, 3H, CH_3), 5.30 (s, 1H, pyridine-H), 6.80 (br, 2H, NH_2, exchangeable with D_2O),7.12-7.48 (m, 14H, Ar–H,and methylidene-H)**.** Anal. Calcd. for $C_{29}H_{20}N_4OS_2$ (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-a]pyridine-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 1**2**. Yield 80% as orange crystals; mp 237–238ºC; IR (KBr; cm^{-1}): 3383, 3200 (NH_2), 3035 (CH_{arom}), 2950 (CH_{aliph}), 2211, 2197 (2C≡N), 1716 (C=O). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 5.62 (s, 1H, H-pyridine) 6.40 (br, 2H, NH_2, exchangeable with D_2O) and 7.30 - 7.78 ppm (m, 19H, Ar-H, and methylidene-H). . Anal. Calcd. for $C_{34}H_{22}N_4S_3O$ (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 (CH_{arom}), 2215 (C≡N), 1710, 1659 (2C=O). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 5.61 (s, 1H, pyridine-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 D_2O). Anal. Calcd. for $C_{35}H_{22}N_4S_3O_2$ (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)**

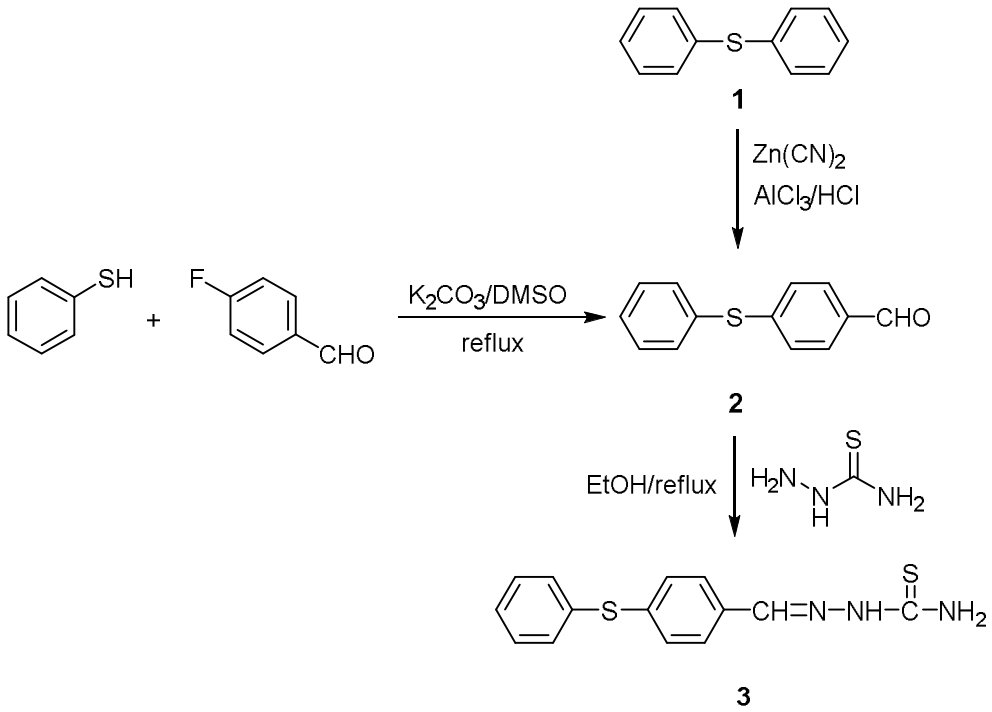
A mixture of **12** (0.01 mol) in absolute ethanol (20 mL) and formamide (5 mL) was refluxed for 5 hrs and poured into ice -water. The solid product was collected by filtration, washed with water and crystallized from ethanol to give **14**. Yield 68% brown solid; mp 220–221ºC; IR (KBr; cm^{-1}): 3420, 3407 (NH_2), 3068 (CH_{arom}), 2929 (CH_{aliph}), 2211 (C≡N), 1700 (C=O). ^{1}HNMR (300 MHz, DMSO-d_6, δ/ppm): 5.63 (s, 1H, pyridine-H), 6.40 (s, 2H, NH_2, exchangeable with D_2O), 7.20-8.02 ppm (m, 19H, Ar–H and methylidene-H), 8.43 (s, 1H, pyrimidine-H). Anal. Calcd. for $C_{35}H_{23}N_5S_3O$ (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** ,**10a**,**b** , and **12** were tested *in vitro* against four bacterial strains, *Bacillus cereus*, *Micrococcus luteus , Escherischia 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 l 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

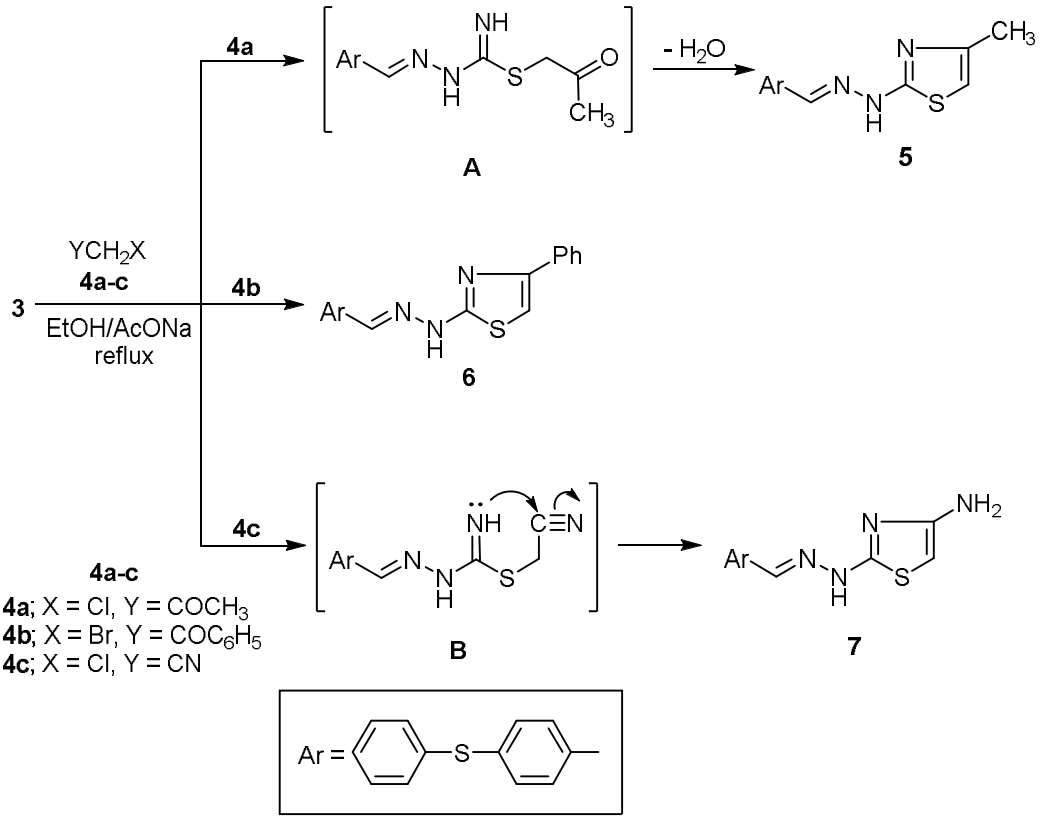
Szmant and co-works [30] have performed the synthesis of 4-phenylmercaptobenzaldehyde 2 from the reaction of diphenyl sulfide 1 with zinc cyanide in presence of aluminum chloride and stream hydrogen chloride in low yield (45.7%). In our lab, we synthesize 4-phenylmercaptobenzaldehyde 2 via nucleophilic 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 ^1H 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 $C_{14}H_{13}N_3S_2$ 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**

The behavior of thiosemicarbazone derivative **3** toward some α-halocarbonyl reagents was investigated with respect to the synthesis of highly substituted thiazoles . Heterocyclization of thioacarbamoyl 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 thiocabamoyl group absorption band, indicating the formation of thiazole derivative **5**. In addition, the ^1H NMR spectrum (DMSO-d_6) of compound **5** revealed singlet signal at δ 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 δ 7.27-7.56 ppm (9H) for aromatic protons,while the NH proton appears as a broad singlet at δ 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 $C_{17}H_{15}N_3S_2$. The formation of thiazole derivative **5**

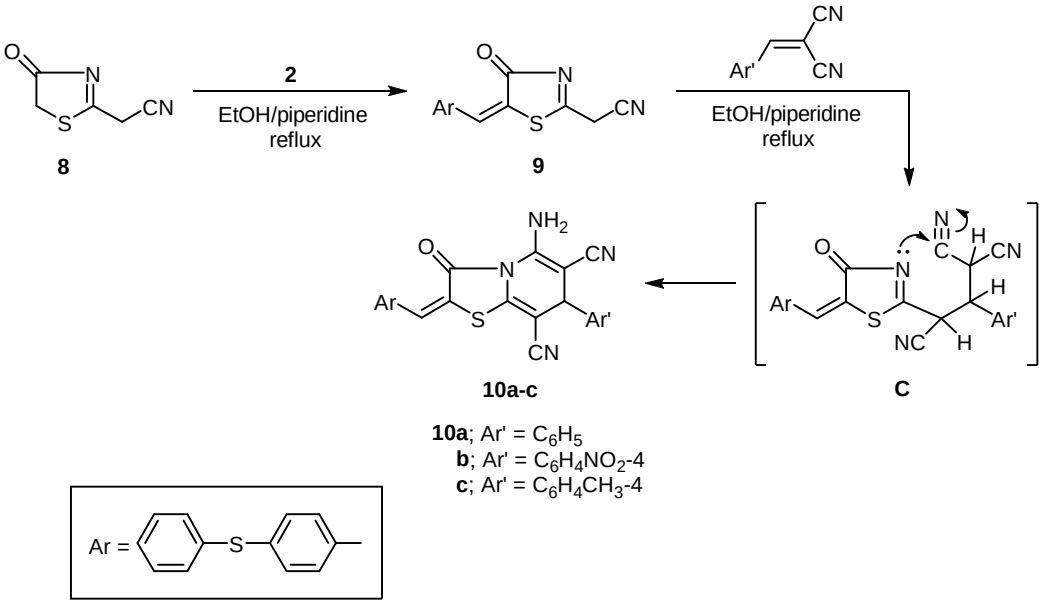
is assumed to proceed through initial alkylation by loss of sodium chloride to form intermediate **A**, followed by intramolecular cyclization via elimination of water [28]. In a similar manner, cyclocondensation of thiocarbamoyl functional group in compound **3** with phenacyl bromide **4b** in refluxing 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 $C_{22}H_{17}N_3S_2$ 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 δ 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**.

Condesation 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 δ 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 piperdine 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, ^{1}H NMR, ^{13}C NMR and MS). The infrared spectra of compounds **10a-c** showed the presence of the NH_2, C≡N and C=O absorption bands. The ^{1}H NMR spectrum (DMSO-d_6) 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 NH_2 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 $C_{28}H_{17}N_5S_2O_3$ 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].

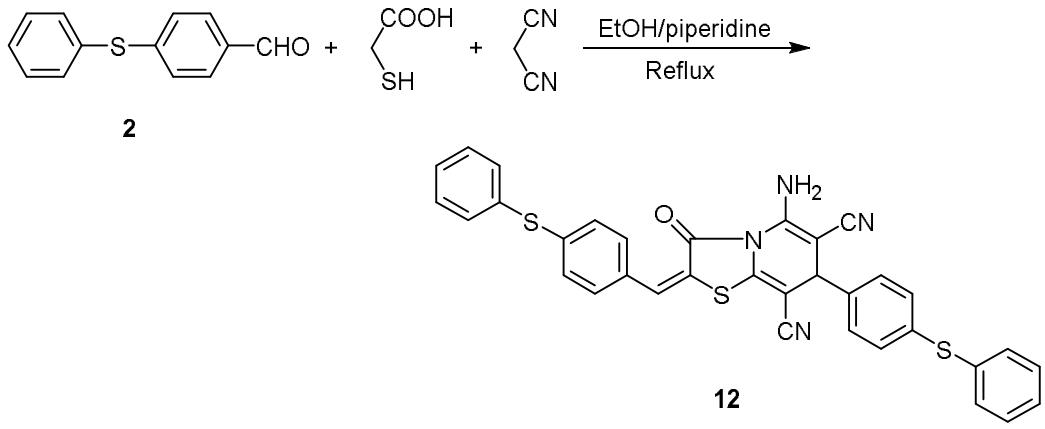


Scheme 3 Synthesis of 4-thiazolidinone **9** and thiazolo[3,2-*a*]pyridines **10a-c**

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, 2197cm^{-1}, in addition to the presence of absorption at 1716 cm^{-1} for the C=O group. Its ^{1}HNMR spectrum (DMSO-d_6) 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 methylidine protons. The formation of thiazolopyridine derivative **12** can be explained by the reaction pathway depicted in Scheme 5.

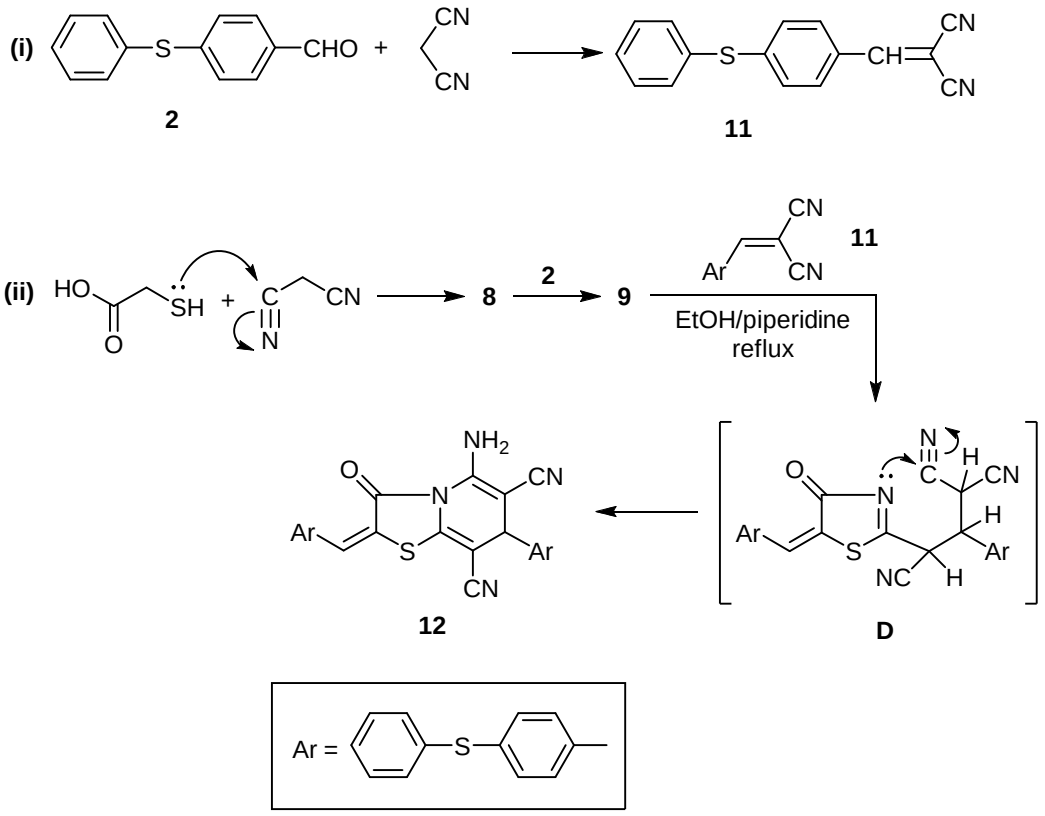
Thiazolo[3',2':1,6]pyrido[2,3-*d*] pyrimidine derivative **13** was obtained by refluxing of compound **12** with formic acid [32] (Scheme 6). The structure of compound **13** was confirmed by its analyses and spectral data. The infrared spectrum displayed absorption bands for the NH, C≡N and two carbonyl functions at 3444, 2215, 1710 and 1659 cm^{-1}, respectively. Its ^{1}H NMR spectrum (DMSO-d_6) 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.



2 **12**

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

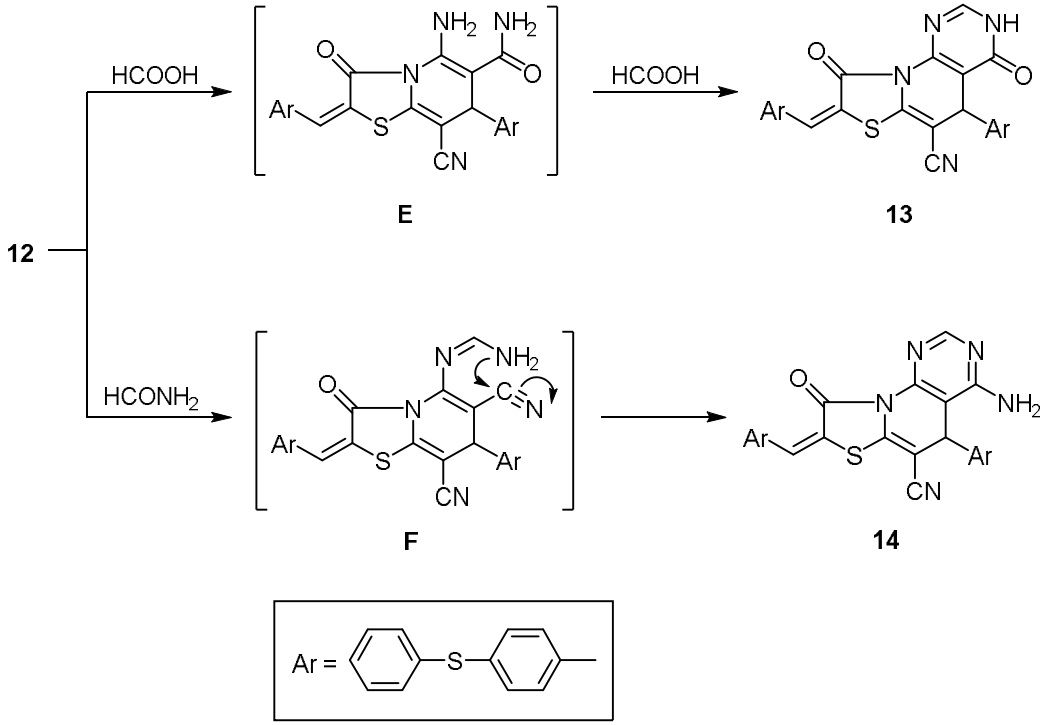


2 **11** **8** **9** **D** **12**

Scheme 5: Possible mechanism for 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 ^1H 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**

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

	Gram positive bacteria		*Gram negative bacteria*		*Fungi*	
Comp. no.	*Bacillus Cereus*	*Micrococcus luteus*	*Escherischia coli*	*Serratia marcesens*	*Candida albicans*	*Aspergillus niger*
3	++	++	++	+++	+	+
5	++	++	++	+++	+	+
7	+++	+	++	+	+	+
9	+	+	++	+	+	+++
10b	+	-	++	+	+	-
10c	+	-	++	++	++	-
12	++	+	++	++	++	+
Standard	++++	++++	++++	++++	++++	++++

+ : Less active (0.1-0.5 cm)

++ : Moderately active (0.6-1.4 cm)

+++ : Highly active (1.5-3.0 cm)

++++ : 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}.

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 nig*er. The results of the antimicrobial screening of selected new compounds are summarized in Table 1.

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