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ORIGINAL RESEARCH

Synthesis and antimicrobial evaluation of novel *N*-substituted 4-ethylsulfanyl-2-pyridones and triazolopyridines

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Abstract

The design and development of new methods for the synthesis of antimicrobial drugs is an important goal currently for medicinal chemistry. Sixteen novel *N*-substituted-amino-, *N*-arylsulfonylamino-, and *N*-aryl-4-ethylsulfanyl-2-pyridones were synthesized. Antimicrobial activities of the compounds were evaluated against four fungal and eight bacterial strains. Antimicrobial results showed that compound **14b** had excellent activities as compared to all other newly synthesized compounds and a variety of standard ones against a variety of bacterial and fungal strains. Triazolo[1,5-*a*]pyridines **10c** and **10e** showed as well marked activities toward three of the tested Gram-negative bacteria.

Keywords 2-Pyridones · *N*-cyanoacetohydrazide · Cyanoaceto-*N*-phenylsulfonylhydrazide · Antimicrobial activity

Introduction

The resistance of the majority of microorganisms to antimicrobial drugs is a major problem affecting the health of people all around the world. According to WHO report on antimicrobial resistance, it is estimated that more than ten million people will suffer from multi-drug resistance infections with the number of human mortality is expected to rise as antimicrobial drug resistance increases (Li et al. 2000). Innovation, therefore, must be strengthened in this area of research activities related to the development of new and effective antimicrobial and antifungal drugs (Fassihi et al. 2009). *N*-Substituted 2-pyridones are important heterocycles that possess antimicrobial and antifungal activities making them ideal for a wide range of pharmaceutical applications (Desai et al. 2013, Maruza et al. 1992). As part of our program directed toward the preparation of potential antimetabolic agents (Elgemeie 2003; Elgemeie et al. 2017, 2018; Abu-Zaied et al. 2011), we have recently conducted numerous researches to develop different innovative synthetic methods for the preparation of *N*-sulfonylamino- and *N*-substituted-2-pyridones (Elgemeie and Jones 2002, 2016; Elgemeie et al. 2002), which have come into interest and application of NIH, NIAID and others as new forms of chemotherapeutic agents (https://pubchem.ncbi.nlm.nih.gov/compound/391346). The promising interest have led our research laboratory to continue this research to explore the new molecular mechanisms of these synthetic compounds and to use them as a promising chemotherapeutics. We have recently identified and reported different synthetic methods for preparing azoloazines using cyanoketene dithioacetals (Elgemeie et al. 2001, 2002, 2003, Elgemeie and Jones 2004). Derivatives of these ring systems are important as antimetabolic agents in biochemical reactions (Elgemeie et al. 2004, 2006). The present research deals with a novel synthesis of ***N*-**amino-, *N*-substituted-amino-, *N*-arylsulfonylamino-, and *N*-aryl-4-ethylsulfanyl-2-pyridones **6**, **12, 14**, and [1,2,4]triazolo[1,5-*a*]pyridines **10** by the reaction of cyanoketene dithioacetal **2** with substituted cyanohydrazides and the determination of their antimicrobial potency.

Materials and methods

Chemistry

All melting points were uncorrected on a Gallenkamp melting point apparatus. IR spectra (KBr discs) were recorded on a FTIR plus 460 or Pye unicam SP-1000 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were

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recorded on a Mercury-300BB (300 and 75 MHz, respectively), at Cairo University, in DMSO-d_6 as solvent using TMS [$Si(CH_3)_4$] as internal standard and chemical shifts are expressed as δ ppm. Elemental analyses were obtained from the Microanalytical Data Center at Cairo University, Egypt and were performed on Vario El III Elemental CHNS analyzer. Progress of the reactions was monitored by TLC using aluminum sheets coated with silica gel 60 F254 (Merck). Viewing under a short-wavelength UV lamp effected detection.

Synthesis of 1,6-diamino-4-(ethylthio)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (6)

To a solution of compound **1** (0.01 mol) in ethanol (30 mL), 2 equivalents of ethyl iodide were added (0.02 mol). The reaction mixture was refluxed for 3 h and then the solvent was evaporated under vacuum. The residue was dissolved in dioxane and added to a solution of cyanoacetohyrdazide **3** (0.01 mol) in dioxane (10 ml) containing (0.01 mol) of KOH. After stirring at room temperature for 24 h, the solid product formed was filtered, washed with absolute ethanol, dried and recrystallized from ethanol.

Yellow; yield 70%; mp 209 °C; IR (KBr) ν_{max} 3490, 3208, 2211, 1619 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.25$ (3H, t, $J = 7.2$ Hz, CH_3), 3.24 (2H, q, $J = 7.2$ Hz, CH_2), 5.54 (2H, s, NH_2), 8.40 (2H, br, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.1$ (CH_3), 29.2 (CH_2), 115.5, 116.3 (CN), 77.7, 90.2, 156.2, 156.7, 159.2 (ArC). Anal. calcd. for. $C_{15}H_{13}N_5O_3S_2$ (375.43): C, 45.95; H, 3.86; N, 29.77. Found: C, 44.87; H, 3.88; N, 28.44.

General procedure for the synthesis of 7-(Ethylthio)-5-oxo-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridines (10a–f)

To a solution of compound **1** (0.01 mol) in ethanol (30 mL), 2 equivalents of ethyl iodide were added (0.02 mol). The reaction mixture was refluxed for 3 h and then the solvent was evaporated under vaccum. The residue was dissolved in dioxane and added to a solution of 1-cyanoacetyl-4-arylidenesemicarbazide **7a–f** (0.01 mol) in dioxane contaning (0.01 mol) of KOH. After stirring at room temperature for 24 h, the solid product formed was filtered, washed with absolute ethanol, dried and recrystallized from ethanol.

7-(Ethylthio)-5-oxo-2-phenyl-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (10a)

White; yield 70%; mp 222 °C; IR (KBr) ν_{max} 3413, 3205, 2212, 1635 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.25 (3H, t, $J = 7.5$ Hz, CH_3), 3.16 (2H, q, $J = 7.5$ Hz, CH_2), 7.04–7.07 (2H, m, C_6H_5), 8.06–8.10 (3H, m, C_6H_5). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.4$ (CH_3), 29.5 (CH_2), 116.9, 118.0 (CN), 83.3, 88.4, 128.5, 129.5, 130.3, 133.6, 149.2, 154.1, 156.0, 161.4 (ArC). Anal. calcd. for. $C_{16}H_{11}N_5OS$ (321.36): C, 59.80; H, 3.45; N, 21.79. Found: C, 59.77; H, 3.43; N, 21.67

2-(4-Chlorophenyl)-7-(ethylthio)-5-oxo-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]-pyridine-6,8-dicarbonitrile (10b)

Beige; yield 85%; mp 317 °C; IR (KBr) ν_{max} 3495, 3418, 2216, 1628 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.24$ (3H, t, $J = 7.2$ Hz, CH_3), 3.18 (2H, q, $J = 7.2$ Hz, CH_2), 7.57 (2H, d, $J = 9$ Hz, C_6H_4), 8.15 (2H, d, $J = 9$ Hz, C_6H_4); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.1$ (CH_3), 29.7 (CH_2), 116.7, 118.2 (CN), 83.1, 88.0, 129.0, 129.4, 129.8, 135.2, 148.8, 153.9, 156.2, 161.6 (ArC). Anal. calcd. for. $C_{16}H_{10}ClN_5OS$ (355.80): C, 54.01; H, 2.83; N, 19.68. Found: C, 53.76; H, 2.80; N, 18.98.

7-(Ethylthio)-2-(4-methoxyphenyl)-5-oxo-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]-pyridine-6,8-dicarbonitrile (10c)

Pale yellow; yield 73%; mp 214 °C; IR (KBr) ν_{max} 3397, 3297, 2210, 1645 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 1.27$ (3H, t, $J = 7.2$ Hz, CH_3), 3.26 (2H, q, $J = 7.2$ Hz, CH_2), 3.87 (3H, s, °CH_3), 7.00-7.96 (4H, m, C_6H_4); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.2$ (CH_3), 29.3 (CH_2), 55.7 (OCH_3), 114.7, 116.5 (CN), 78.2, 83.4, 115.5, 129.0, 132.3, 144.7, 153.2, 155.0, 156.1, 161.3 Anal. calcd. for. $C_{17}H_{13}N_5O_2S$ (351.38): C, 58.11; H, 3.73; N, 19.93. Found**:** C, 58.42; H, 3.77; N, 19.40.

7-(Ethylthio)-5-oxo-2-*p*-tolyl-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (10d)

Buff; yield 70%; mp 268 °C; IR (KBr) ν_{max} 3423, 3215, 2212, 1632 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.23 (3H, t, $J = 7.5$ Hz, ethyl CH_3), 2.31 (3H, s, CH_3), 3.14 (2H, q, $J = 7.5$ Hz, CH_2), 7.06 (2H, d, $J = 9$ Hz, C_6H_4), 8.08 (2H, d, $J = 9$ Hz, C_6H_4). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.1$ (ethyl CH_3, 21.4 (CH_3), 29.1 (CH_2), 116.1, 117.0 (CN), 76.8, 91.0, 126.5, 128.5, 139.4, 145.1, 153.2, 158.5, 159.0 (ArC); Anal. calcd. for. $C_{17}H_{13}N_5OS$ (335.38): C, 60.88; H, 3.91; N, 20.88. Found: C, 60.46; H, 3.89; N, 20.79.

7-(Ethylthio)-2-(4-fluorophenyl)-5-oxo-3,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (10e)

Beige; yield 82%; mp 287 °C; IR (KBr) ν_{max} 3545, 3447, 2223, 1632 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.24 (3H, t, $J = 7.5$ Hz, CH_3), 3.18 (2H, q, $J = 7.5$ Hz, CH_2), 7.30–7.36 (2H, m, C_6H_4), 8.16–8.21 (2H, m, C_6H_4);

^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.1$ (CH_3), 29.1 (CH_2), 116.1, 117.0 (CN), 83.2, 87.9, 126.4, 128.0, 129.9, 135.2, 147.9, 153.2, 158.5, 159.0 (ArC); Anal. calcd. for. $C_{16}H_{10}FN_5OS$ (339.35): C, 56.63; H, 2.97; N, 20.64. Found: C, 55.99; H, 2.86; N, 20.44.

7-(Ethylthio)-2-(3-nitrophenyl)-5-oxo-3,5-dihydro-[1,2,4] triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (10f)

Yellowish brown; yield 82%; mp 223 °C; IR (KBr) ν_{max} 3437, 3199, 2214, 1628 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.24$ (3H, t, $J = 7.2$ Hz, CH_3), 3.19 (2H, q, $J = 7.2$ Hz, CH_2), 7.80–8.87 (4H, m, C_6H_4); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.4$ (CH_3), 28.8 (CH_2), 116.3, 117.0 (CN), 83.5, 87.9, 118.2, 124.0, 128.2, 129.0, 133.2, 147.4, 148.2, 153.6, 158.9, 161.2 (ArC); Anal. calcd. for. $C_{16}H_{10}N_6O_3S$ (366.35): C, 52.46; H, 2.75; N, 22.94. Found: C, 52.40; H, 2.74; N, 22.74.

General procedure for the synthesis of *N*-arylsulfonylamino-2-pyridones (12a, b)

To a solution of compound **1** (0.01 mol) in ethanol (30 mL), 2 equivalents of ethyl iodide were added (0.02 mol). The reaction mixture was refluxed for 3 h and then the solvent was evaporated under vacuum. The residue was dissolved in dioxane and added to a solution of *N*-cyanoacetoarylsulfonylhydrazides **11a, b** (0.01 mol) in dioxane containing (0.01 mol) of KOH. After stirring at room temperature for 24 h, the solid product formed was filtered, washed with absolute ethanol, dried, and recrystallized from ethanol.

N-(6-Amino-3,5-dicyano-4-(ethylthio)-2-oxopyridin-1(2*H*)-yl) benzene-sulfonamide (12a)

White; yield 77%; mp 273 °C; IR (KBr) ν_{max} 3429, 3396, 2214, 1628 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.24 (3H, t, $J = 7.5$ Hz, CH_3), 3.16 (2H, q, $J = 7.5$ Hz, CH_2), 7.34–7.71 (5H, m, C_6H_5), 7.72 (1H, br, NH); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.1$ (CH_3), 29.7 (CH_2), 116.7, 118.2 (CN), 75.8, 91.0, 116.1, 127.4, 129.7, 148.7, 153.8, 156.3, 161.8 (ArC); Anal. calcd. for. $C_{15}H_{13}N_5O_3S_2$ (375.43): C, 47.99; H, 3.49; N, 18.65. Found: C, 47.86; H, 3.40; N, 18.62.

N-(6-Amino-3,5-dicyano-4-(ethylthio)-2-oxopyridin-1(2*H*)-yl)-4-methylbenzene-sulfonamide (12b)

Beige; yield 83%; mp 292 °C; IR (KBr) ν_{max} 3409, 3283, 2214, 1609 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.24 (3H, t, $J = 7.2$ Hz, ethyl CH_3), 2.32 (3H, s, CH_3), 3.17 (2H, q, $J = 7.2$ Hz, CH_2), 7.15 (2H, d, $J = 8.1$ Hz, C_6H_4), 7.58 (2H, d, $J = 8.1$, C_6H_4), 7.82 (1H, br, NH); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.2$ (ethyl CH_3), 21.6 (CH_3), 30.0 (CH_2), 116.6, 118.0 (CN), 75.9, 91.2, 118.7, 127.7, 132.4, 150.0, 153.9, 156.8, 160.5 (ArC); Anal. calcd. for. $C_{16}H_{15}N_5O_3S_2$ (389.45): C, 49.34; H, 3.88; N, 17.98. Found: C, 48.98; H, 3.69; N, 16.97.

General procedure for the synthesis of *N*-aryl-2-pyridones (14a–f)

To a solution of compound **1** (0.01 mol) in ethanol (30 mL), 2 equivalents of ethyliodide were added (0.02 mol). The reaction mixture was refluxed for 3 h and then the solvent was evaporated under vacuum. The residue was dissolved in dioxane and added to a solution of arylcyanoacetamide **13a–f** (0.01 mol) in dioxane containing (0.01 mol) of KOH. After stirring at room temperature for 3 h, the solid product thus formed was filtered, washed with absolute ethanol, dried, and recrystallized from ethanol.

6-Amino-4-(ethylthio)-2-oxo-1-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile (14a)

White; yield 88%; mp 281 °C; IR (KBr) ν_{max} 3409, 3296, 2205, 1608 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.33 (3H, t, $J = 7.5$ Hz, CH_3), 3.31 (2H, q, $J = 7.5$ Hz, CH_2), 7.32–7.59 (5H, m, C_6H_5), 7.69 (2H, br, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 14.8$ (CH_3), 28.7 (CH_2), 115.4, 116.0 (CN), 78.1, 90.5, 128.5, 129.8, 130.2, 133.8, 156.6, 157.7, 158.9 (ArC); Anal. calcd. for. $C_{15}H_{12}N_4OS$ (296.35): C, 60.79; H, 4.08; N, 18.91. Found**:** C, 60.11; H, 4.10; N, 18.34.

6-Amino-1-(4-chlorophenyl)-4-(ethylthio)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (14b)

Beige; yield 87%; mp 296 °C; IR (KBr) ν_{max} 3405, 3301, 2209, 1608 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.32 (3H, t, $J = 7.5$ Hz, CH_3), 3.30 (2H, q, $J = 7.5$ Hz, CH_2), 7.40 (2H, d, $J = 9$ Hz, C_6H_4), 7.61 (2H, d, $J = 9$ Hz, C_6H_4), 7.86 (2H, br, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 14.8 (CH_3), 28.7 (CH_2), 115.2, 116.0 (CN), 78.0, 90.7, 130.3, 130.6, 132.7, 134.7, 156.6, 158.0, 158.8 (ArC); Anal. calcd. for. $C_{15}H_{11}ClN_4OS$ (330.79): C, 54.46; H, 3.35; N, 16.94. Found: C, 54.28; H, 3.31; N, 16.94.

6-Amino-4-(ethylthio)-1-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (14c)

White; yield 85%; mp 262-263 °C; IR (KBr) ν_{max} 3402, 3307, 2206, 1648 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ $= 1.33$ (3H, t, $J = 7.2$ Hz, CH_3), 3.30 (2H, q, $J = 7.2$ Hz, CH_2), 3.82 (3H, s, °CH_3), 7.09 (2H, d, $J = 8.1$ Hz, C_6H_4),

Table 1 Antibacterial activities (gram positive bacteria) of the synthesized compounds

Sample	Gram positive bacteria			
	S. pneumoniae	*S. aureus*	*B. subtilis*	*E. faecalis*
10a	21.2 ± 1.5	19.3 ± 0.63	15.6 ± 0.36	13.9 ± 0.21
10b	10.6 ± 0.58	12.3 ± 0.58	10.7 ± 0.24	9.9 ± 0.34
10c	18.3 ± 1.2	21.2 ± 0.58	23.1 ± 0.47	13.5 ± 0.34
10d	NA[a]	NA	NA	NA
10e	22.4 ± 0.44	20.6 ± 0.63	21.4 ± 0.52	19.7 ± 0.56
12a	16.7 ± 0.19	18.2 ± 0.58	17.6 ± 0.58	15.4 ± 0.38
12b	13.3 ± 0.19	13.5 ± 0.36	9.8 ± 0.34	11.3 ± 0.39
14a	NA	NA	NA	NA
14b	21.4 ± 0.58	22.3 ± 0.63	16.1 ± 0.53	17.9 ± 0.48
14c	NA	NA	NA	NA
14d	12.3 ± 0.52	10.6 ± 0.63	10.2 ± 0.31	8.8 ± 0.24
14e	15.2 ± 0.63	12.3 ± 0.58	15.3 ± 0.55	13.4 ± 0.35
14f	19.3 ± 1.5	18.2 ± 0.53	15.9 ± 0.59	14.5 ± 0.62
Ampicillin	24.3 ± 0.86	27.4 ± 0.72	32.4 ± 0.67	25.9 ± 0.54
Ciprofloxacin	24.2 ± 0.86	30 ± 0.67	NT[b]	25 ± 0.72

[a]NA: No observed activity

[b]NT: Not tested

7.25 (2H, d, $J = 8.1$ Hz, C_6H_4), 7.71 (2H, br, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 14.8$ (CH_3), 28.7 (CH_2), 55.4 (OCH_3), 115.3, 116.0 (CN), 77.8, 90.9, 115.5, 126.0, 129.7, 156.9, 157.7, 159.0, 160.0 (ArC); Anal. calcd. for. $C_{16}H_{14}N_4O_2S$ (326.37): C, 58.88; H, 4.32; N, 17.17. Found: C, 58.80; H, 4.29; N, 17.15

6-Amino-4-(ethylthio)-2-oxo-1-*p*-tolyl-1,2-dihydropyridine-3,5-dicarbonitrile (14d)

White; yield 89%; mp 273 °C; IR (KBr) ν_{max} 3405, 3299, 2207, 1607 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.33 (3H, t, $J = 7.2$ Hz, ethyl CH_3), 2.38 (3H, s, CH_3), 3.30 (2H, q, $J = 7.2$ Hz, CH_2), 7.21 (2H, d, $J = 7.8$ Hz, C_6H_4), 7.36 (2H, d, $J = 8.1$ Hz, C_6H_4), 7.69 (2H, br, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.3$ (ethyl CH_3), 21.4 (CH_3), 29.2 (CH_2), 115.7, 116.5 (CN), 78.3, 91.5, 128.7, 131.3, 131.5, 139.9, 157.2, 158.3, 159.4 (ArC); Anal. calcd. for. $C_{16}H_{14}N_4OS$ (310.37): C, 61.92; H, 4.55; N, 18.05. Found: C, 62.99; H, 4.54; N, 17.39.

6-Amino-4-(ethylthio)-2-oxo-1-*o*-tolyl-1,2-dihydropyridine-3,5-dicarbonitrile (14e)

Yellow; yield 84%; mp 194 °C; IR (KBr) ν_{max} 3423, 3307, 2211, 1626 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.33 (3H, t, $J = 7.2$ Hz, ethyl CH_3), 2.01 (3H, s, CH_3), 3.33 (2H, q, $J = 7.2$ Hz, CH_2), 7.26–7.44 (4H, m, C_6H_4), 7.80 (2H, br, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.2$ (ethyl CH_3), 17.0 (CH_3), 29.3 (CH_2), 115.6, 116.3 (CN), 76.1, 91.2, 128.4, 128.9, 130.7, 132.1, 133.0, 136.2, 156.6, 158.7, 158.9 (ArC); Anal. calcd. for. $C_{16}H_{14}N_4OS$ (310.37): C, 61.92; H, 4.55; N, 18.05. Found: C, 61.90; H, 4.53; N, 18.00.

6-Amino-4-(ethylthio)-2-oxo-1-*m*-tolyl-1,2-dihydropyridine-3,5-dicarbonitrile (14f)

Buff; yield 85%; mp 219 °C; IR (KBr) ν_{max} 3409, 3324, 2205, 1650 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta =$ 1.33 (3H, t, $J = 7.2$ Hz, ethyl CH_3), 2.36 (3H, s, CH_3), 3.31 (2H, q, $J = 7.2$ Hz, CH_2), 7.11-7-47 (4H, m, C_6H_4), 7.69 (2H, br, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 15.2$ ethyl CH_3), 21.3 (CH_3), 29.2 (CH_2), 115.7, 116.5 (CN), 78.4, 91.4, 125.9, 129.3, 130.5, 131.0, 134.0, 140.3, 157.0, 158.3, 159.3 (ArC); Anal. calcd. for. $C_{16}H_{14}N_4OS$ (310.37): C, 61.92; H, 4.55; N, 18.05. Found: C, 61.88; H, 4.51; N, 18.02.

3-Amino-7-(3-nitrophenyl)-4-oxo-4,6-dihydro-1*H*-pyrazolo[3,4-d][1,2,4]-triazolo[1,5-a]pyridine-9-carbonitrile (15)

A mixture of **10f** (0.001 mol) and hydrazine hydrate (0.01 mol) was dissolved in ethanol (20 mL) and few drops of piperidine were added. The mixture was refluxed for 1 h. The resulting precipitated solid was filtered off and recrystallized from ethanol.

Buff; yield 77%; mp 317 °C; IR (KBr) ν_{max} 3387, 3260, 2196, 1660 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta =$ 5.18 (2H, br, NH_2), 7.76–8.85 (4H, m, C_6H_4), 11.40 (1H,

Table 2 Antibacterial activities (gram negative bacteria) of the synthesized compounds

Sample	Gram negative bacteria			
	P. aeruginosa	*E. coli*	*S. marcescens*	*S. typhimurium*
10a	NA[a]	21.9 ± 1.2	14.1 ± 0.65	13.2 ± 0.58
10b	NA	8.3 ± 0.58	12.6 ± 0.54	11.2 ± 0.44
10c	NA	23.5 ± 0.63	21.5 ± 0.88	20.2 ± 0.73
10d	NA	NA	NA	NA
10e	NA	23.2 ± 0.19	24.3 ± 0.42	19.5 ± 0.35
12a	NA	15.4 ± 0.44	15.7 ± 0.47	16.6 ± 0.62
12b	NA	11.4 ± 0.36	11.5 ± 0.43	10.8 ± 0.46
14a	NA	NA	NA	NA
14b	NA	23.2 ± 1.5	28.1 ± 0.76	23.4 ± 0.77
14c	NA	NA	NA	NA
14d	NA	10.6 ± 0.62	12.6 ± 0.38	9.7 ± 0.37
14e	NA	13.3 ± 1.2	12.9 ± 0.63	13.2 ± 0.58
14f	NA	20.3 ± 1.2	15.7 ± 0.45	17.4 ± 0.61
Ciprofloxacin	20.6 ± 0.73	23.4 ± 0.61	19.3 ± 0.42	24.1 ± 0.51

[a]NA: No observed activity

br, NH); Anal. calcd. for. $C_{14}H_8N_8O_3$ (336): C, 50.01; H, 2.40; N, 33.32. Found: C, 50.00; H, 2.44; N, 33.58.

Antimicrobial evaluation

Antimicrobial tests were carried out by diffusion agar technique. A bottomless cylinder containing a measured quantity of the sample (100 µL, 5 mg/mL) is placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium (Sabouraud dextrose agar), which has been heavily seeded with a spore suspension of the tested organism (1×10^8 CFU/mL of tested bacteria on nutrient agar broth and 1×10^6 CFU/mL of fungi on Sabouraud dextrose agar media). After incubation for 24 h for bacteria and 5 days for fungi, the diameter of the clear zone of inhibition surrounding the sample was measured and used to indicate the inhibitory power of the sample against a particular tested organism. The solvent used was DMSO. Ampicillin and Ciprofloxacin were used as the standard for antibacterial activity and Amphotericin B was used as the standard for antifungal activity. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms and compared with that of the standard as summarized in Tables 1–3.

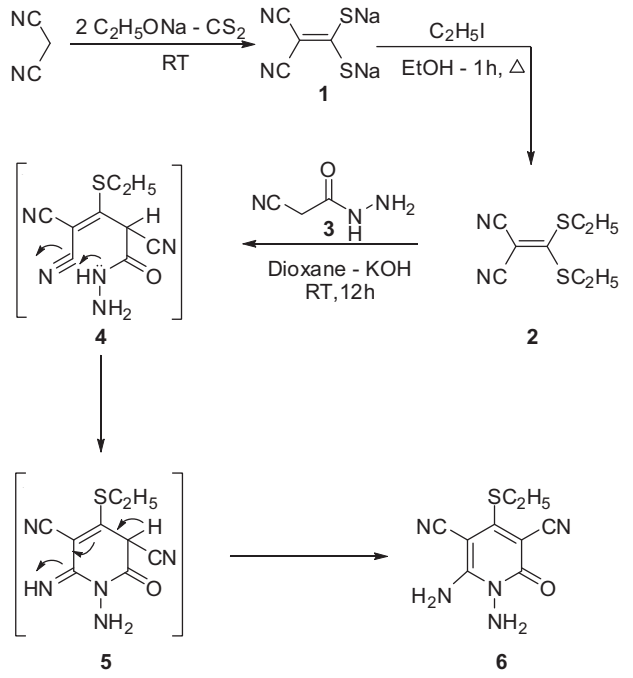
Minimum inhibition concentration (MIC) measurement

For each strain, three to five isolated colonies were selected from the fresh agar plate and were transferred into a tube containing 3–4 mL of sterile broth medium. The bacterial suspension was mixed well and incubated at 35–37 °C for 2–6 h. The turbidity of the bacterial suspension should be equal to or greater than the turbidity of a McFarland Standard 0.5. After that, 1 mg of the each tested compound (antimicrobial agent) was dissolved in 1 mL DMSO and two-fold serial dilution was done using broth medium. A fixed volume of the prepared bacterial inoculum was added to each tube and incubated for at 37 °C 16–20 h. The MIC is defined as the lowest concentration of the antimicrobial agent that inhibits visible growth of the tested isolate as observed with the unaided eye (Wiegand et al. 2008).

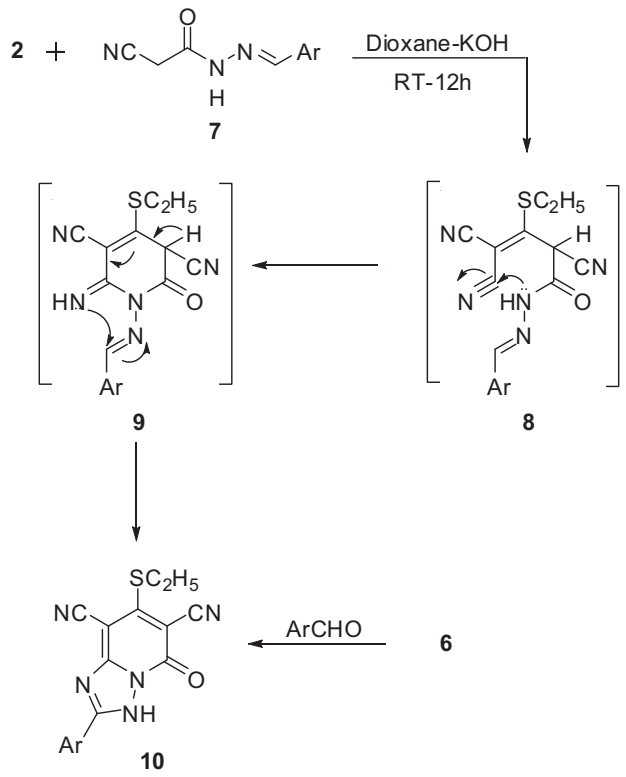
Results and discussion

Chemistry

Reaction of 2,2-dicyanoethene-1,1-bis(ethylthiolate) **2**, prepared by malononitrile, carbon disulfide, and ethyl iodide in the presence of sodium ethoxide, with *N*-cyanoacetohydrazide **3** at room temperature for 24 h in the presence of KOH-dioxane produced the corresponding *N*-[4-(ethyllthio)-2-oxopyridin **6**, Scheme 1. The structure of **6** was established by IR spectrum and revealed absorption band by cm^{-1} at 3490 and 3208 for NH_2, 2211 for CN and 1619 for C=O groups. Its ^1H NMR spectrum showed by δ triplet peak at 1.25 ppm and quartet at 3.24 ppm for CH_3 and CH_2 of the SCH_2CH_3 group, respectively, and two broad peaks at 5.54 ppm and 8.40 ppm for two NH_2 groups. Furthermore, the reaction of **2** with *N*'-[(aryl)-methylene]-2-cyanoacetohydrazides **7a–f**, was carried out by treating **7a–f** with one equivalent of compound **2** in KOH-dioxane at room temperature for 24 h and obtained the corresponding 7-(ethylthio)-3,5-dihydro[1,2,4]triazolo[1,5-*a*]pyridines



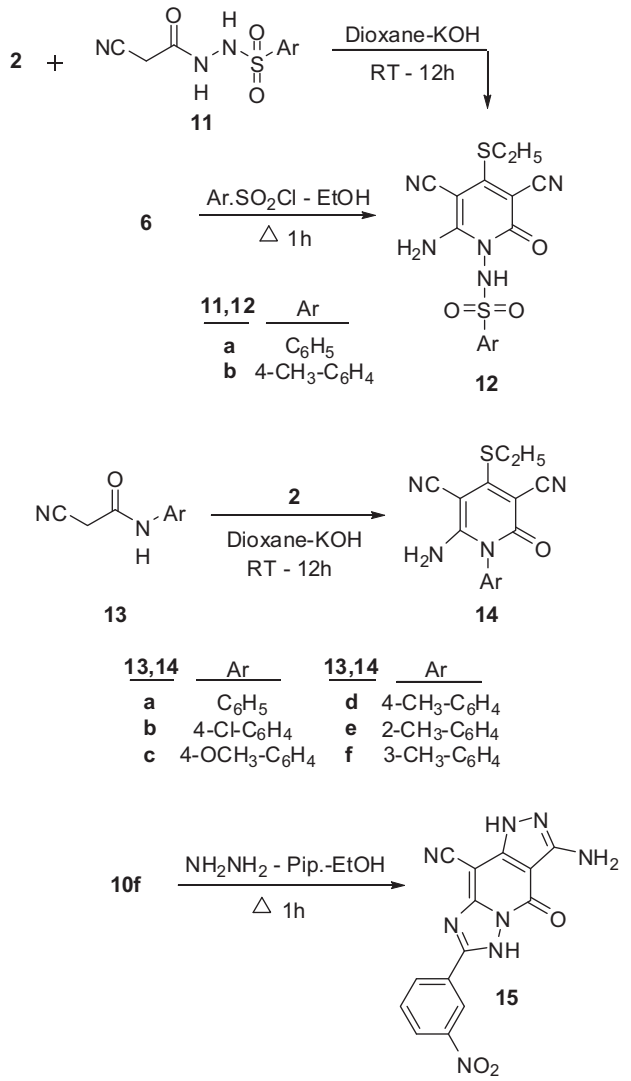
Scheme 1 A synthetic pathway for *N*-amino-4-(ethylthi)-pyridine-2-one **(6)**



7,10	Ar	7,10	Ar
a	C_6H_5	d	4-CH_3-C_6H_4
b	4-Cl-C_6H_4	e	4-F-C_6H_4
c	4-OCH_3-C_6H_4	f	3-NO_2-C_6H_4

Scheme 2 Synthetic pathways for 7-(Ethylthio)-5-oxo-[1,2,4]triazolo [1,5-*a*]pyridines **(10a–f)**

10a–f with 70–82% yield, Scheme 2. The formation of **10a–f** from **2** and **7a–f** is assumed to proceed *via* intermediate Michael adducts **8** and **9**, which cyclized to yield the novel triazolopyridine derivatives **10**. The structure of **10a–f** was confirmed by ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis. For example, ^{1}H NMR spectrum of compound **10b**, showed a triplet peak at 1.24 ppm for CH_2, quartet at 3.18 ppm for CH_3, and two doublets at 7.57 ppm and 8.15 ppm for the benzene ring. The absence of proton of –CH=N– group in ^{1}H NMR spectrum proved the cyclization of adduct **9** and the formation of triazolopyridine derivatives **10**. Compounds **10** can also be prepared by the reaction of the corresponding *N*-[4-(ethyllthio)-2-oxopyridin-1 **6** with substituted aromatic aldehydes. In order to explore the reactivity of cyanoketene dithioacetal **2** with other classes of substituted cyanoacetohydrazide, compound **2** reacted with cyanoaceto-*N*-phenylsulfonyl-hydrazide **11a, b** at room temperature for 24 h in the presence of KOH-dioxane to give the corresponding *N*-[4-(ethylthio)-2-oxopyridin-1(2*H*)-yl]benzene-sulfonamide **12a, b**, Scheme 3. The structures of compounds **12a, b** were established on the basis of spectroscopic data and elemental analysis, Scheme 3. IR of **12b** revealed absorption band at 3409 and 3283 cm^{-1} for NH_2, 2214 cm^{-1} for CN and 1609 cm^{-1} for C=O groups. Its ^{1}H NMR spectrum displayed triplet peak at 1.24 ppm for CH_2, singlet peak at 2.32 ppm for CH_3 of tosyl group, quartet peak at 3.17 ppm for CH_3, two doublet peaks at 7.15 ppm and 7.58 ppm for the benzene ring and a broad singlet peak at 7.82 ppm for NH. Compounds **12** can also be prepared by the reaction of the corresponding 1,6-diamino-2-oxo-4-(ethylthio)-1,2-dihydropyridine-3-carbonitriles **6** with benzene sulfonyl chloride in KOH-dioxane. When compound **2** is treated with cyanoacetanilides **13** at room temperature for 24 h in the presence of KOH-dioxane, **14a–f** were obtained in excellent yield, Scheme 3. Compound **10f** reacted with hydrazine in refluxing ethanol in presence of catalytic amount of piperidine to give the corresponding pyrazolo[4,3-*c*][1,2,4]triazolo[1,5-*a*]pyridines **15**. The structure of compound **15** was established on the basis of spectral data and elemental analysis as outlined in Scheme 3. Its IR spectrum showed absorption band at 3387 and 3260 cm^{-1} for NH_2, band at 2196 cm^{-1} for CN and band at 1660 cm^{-1} for C=O groups. Furthermore, the formation of compound **15** was confirmed by ^{1}H NMR, two broad signals at 5.18 ppm and 11.40 ppm for NH_2 and NH, respectively, and multiplet at 7.76–8.85 ppm for the benzene ring. The disappearance of triplet and quartet peaks for CH_3 and CH_2, respectively, and the presence of broad peaks at 5.18 ppm for NH_2 and 11.40 ppm for NH groups in ^{1}H NMR spectrum proved the formation of pyrazol ring.



Scheme 3 Synthetic pathways for *N*-arylsulfonylamino-2-pyridones (**12a, b**), *N*-aryl-2-pyridones (**14a–f**) and pyrazolo[3,4-d][1,2,4]-triazolo[1,5-a]pyridine-9-carbonitrile (**15**)

Antimicrobial evaluations

Some bacteria and fungi have shown resistance to existing antimicrobial agents. Pyridone derivatives are important scaffolds which exhibit promising antimicrobial activities (Darwish et al. 2014, Shah et al. 2013). This promoted us to develop new pyridone derivatives as antibacterial and antifungal agents. The aim of our work is to synthesize different series of pyridine bearing an ethylsulfanyl group at C4 with different substitutes at N1. Additionally, we studied the anti (bacterial and fungal) activities of these derivatives and the influence of the various substituents at N1 position.

The synthesized pyridone derivatives **10a–e**, **12a, b** and **14a–f** bearing fused triazole, *N*-sulfonylamino and *N*-aryl moieties, respectively, were individually tested against (i) *Aspergillus fumigatus* (RCMB 02568), *Candida albicans* (RCMB 05036), *Geotrichum candidum* (RCMB 052006) and *Syncephalastrum racemosum* (RCMB 005003) strains of fungi, (ii) *Streptococcus pneumoniae* (RCMB 010010), *Staphylococcus aureus* (RCMB 010028), *Bacillis subtilis* (RCMB 010067) and *Enterococcus faecalis* (RCMB 010068) strains of Gram-positive bacteria, and (iii) *Pseudomonas aeruginosa* (RCMB 010043), *Escherichia coli* (RCMB 010052), *Serratia marcescens* (RCMB 010071) and *Salmonella typhimurium* (RCMB 010072) strains as gram-negative bacteria. Antimicrobial tests were carried out at the Regional Center for Mycology and Biotechnology, Antimicrobial Activity Unit, Al-Azhar University, Cairo, Egypt. Amphotericin B, Ampicillin and Ciprofloxacin were used as standard. The results were recorded for each tested compound as inhibition diameter zones in millimeters (mm) ± standard deviation and are summarized in Tables 1–3. The MIC measurements were determined for the most active compounds **10c**, **10e**, and **14b** by using twofold serial dilution method and the results were all summarized in Table 4.

Most of the synthesized compounds except **10d**, **14a**, and **14c** were shown to possess moderate to good antimicrobial activity against most of the tested organisms except *P. aeruginosa* as compared to Amphotericin B and Ciprofloxacin. The most active compound was found to be **14b**, which has 4-chlorobenzene at N1 showing interesting antibacterial and antifungal activities. It was shown to have close activity to Amphotericin B against *A. fumigatus* (Inhibition Zone 22.3 ± 0.44 mm; MIC 125 µg/mL), similar activity to Ciprofloxacin against *E. coli* (Inhibition Zone 23.2 ± 1.5 mm; MIC 250 µg/mL) and *S. typhimurium* (Inhibition Zone 23.4 ± 0.77 mm; MIC 250 µg/mL) and higher activity than Ciprofloxacin against *S. marcescens* (Inhibition Zone 28.1 ± 0.76 mm; MIC 62.5 µg/mL) as clearly shown in Tables 1–3. Alternatively, pyridone derivatives fused with triazol moiety such as **10c** and **10e** showed marked activities toward Gram-negative bacteria, Table 2, as compared to Ciprofloxacin. Both **10c** and **10e** have higher activity than Ciprofloxacin against *S. marcescens* with inhibition zone 21.5 ± 0.88 and 24.3 ± 0.42 mm, respectively, but lower than **14b**, and similar activity to Ciprofloxacin against *E. coli*. Comparing both **10c** and **10e** to Amphotericin B, Table 3, showed slightly lower activities for the former against *A. fumigatus* with inhibition zone 21.3 ± 1.5 and 21.3 ± 0.58 mm, respectively. Despite the fact that several drugs such as sulfamethoxazole and sulfadimidine, which contain sulfonamide group are well known as antimicrobial agents, the synthesized pyridone derivatives containing the *N*-sulfonylamino group, **12a** and **12b**, surprisingly showed low activities against all tested organisms as compared to Amphotericin B and Ciprofloxacin standard.

Table 3 Antifungal activities of the synthesized compounds

Sample	Fungi			
	A. fumigatus	*C. albicans*	*G. candidum*	*S. racemosum*
10a	20.6 ± 1.2	18.3 ± 0.58	10.3 ± 0.32	10.9 ± 0.28
10b	9.3 ± 0.58	11.2 ± 0.58	11.7 ± 0.32	10.3 ± 0.34
10c	21.3 ± 1.5	20.2 ± 1.2	12.2 ± 0.33	13.9 ± 0.52
10d	NA[a]	NA	NA	NA
10e	21.3 ± 0.58	20.3 ± 0.58	20.8 ± 0.54	22.3 ± 0.64
12a	16.3 ± 0.44	18.2 ± 0.73	15.8 ± 0.52	18.5 ± 0.58
12b	14.9 ± 0.25	14.7 ± 0.58	9.7 ± 0.42	8.9 ± 0.31
14a	NA	NA	NA	NA
14b	22.3 ± 0.44	20.3 ± 0.19	17.6 ± 0.58	15.4 ± 0.38
14c	NA	NA	NA	NA
14d	13.3 ± 0.62	15.2 ± 0.53	11.9 ± 0.47	10.1 ± 0.42
14e	12.3 ± 1.2	13.2 ± 0.58	10.5 ± 0.32	10.8 ± 0.23
14f	17.8 ± 0.58	16.3 ± 1.2	16.3 ± 0.52	19.6 ± 0.58
Amphotericin B	23.7 ± 1.2	25.4 ± 0.58	28.7 ± 0.27	26.3 ± 0.34

[a]NA: No observed activity

Table 4 The minimum inhibitory concentration (MIC, μg/mL) of the most active compounds **10c, 10e**, and **14b**

Sample	Gram positive bacteria			
	S. pneumoniae	*S. aureus*	*B. subtilis*	*E. faecalis*
10c	1000	500	500	1000
10e	250	1000	1000	1000
14b	500	500	1000	1000
Ampicillin	31.25	62.5	31.25	31.25
	Gram Negative Bacteria			
	P. aeruginosa	*E. coli*	*S. marcescens*	*S. typhimurium*
10c	NA	250	125	500
10e	NA	250	125	1000
14b	NA	250	62.5	250
Ciprofloxacin	125	31.25	31.25	31.25
	Fungi			
	A. fumigatus	*C. albicans*	*G. candidum*	*S. racemosum*
10c	500	500	500	1000
10e	500	1000	500	1000
14b	125	1000	500	1000
Amphotericin B	31.25	31.25	31.25	31.25

Structural–activity relationship (SAR) of the synthesized compounds and their antimicrobial activities showed quite interesting observations. Firstly, the variation of the substituents on the aryl moieties of the triazol ring system fused with the pyridone derivatives had a profound effect on the compounds, **10a–e**, activities as shown in Tables 1–3. It was noticed that the presence of electron withdrawing groups such as fluoride, **10e**, exhibited activities close to that of the standard. The presence of electron donating group such as methyl group on the benzene ring **10d** did not result in noticeable activity of the analog against all organisms while unsubstituted benzene ring **10a** resulted in moderate activity against *A. fumigatus*, *S. pneumonia* and *E. coli* and low activity toward other organisms. Secondly, similar observation was also noticed regarding the presence of methyl group on the benzene ring of pyridone derivatives with *N*-sulfonylamino, **12b**. It lowered the activity toward all organisms as compared to the case of unsubstituted

benzene ring, **12a**. Thirdly, the presence of electron withdrawing group such as Cl on the aryl moiety of compound **14b** showed the highest antibacterial activity of all compounds toward three of the tested Gram-negative bacteria. Finally, in case of pryidone derivatives **14d–f**, the position of the methyl group on the aryl moiety varied the activity of the corresponding derivative. Compound **14f** with methyl group on the *meta* position showed higher antimicrobial activity than both **14d** and **14e** with methyl group on the *para* and *ortho* positions, respectively. In addition and specifically in case of the tested fungi, the presence of methyl group on the *para* position of the benzene ring caused the increase in the activity of compound **14d** as compared to that of the same compound with *ortho*-substituted methyl group, **14e**, Table 3. However, in case of the tested Gram-positive and Gram-negative bacteria, the activity of **14d** showed opposite behavior, Tables 1, 2.

Conclusion

In summary, new series of *N*-substituted derivatives of 4-ethylsulfanyl-2-pyridones and triazolopyridines were synthesized, characterized by spectral analysis and evaluated for their in vitro antimicrobial activities. The antimicrobial results showed that three compounds (**10c**, **10e**, and **14b**) were the most active antimicrobial agents in this study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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