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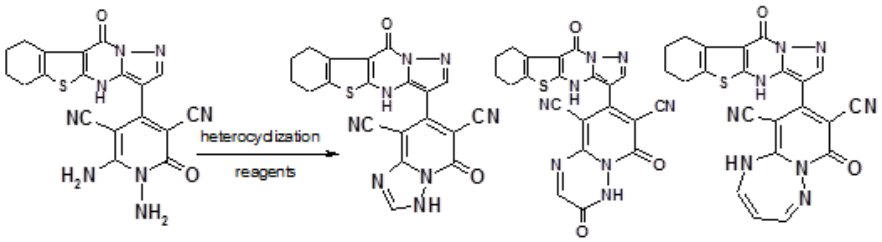
Synthesis and Antimicrobial Activities of some Novel Pyridines Carrying Pyrazolobenzothieno[2,3-d]pyrimidine Unit

Mohamed Abdel-Megid[1,2*], Azza M El-Kazak[1], Kamelia M EL-mahdy[1], Magdy Seada[1], Osama Farouk[1]

[1]*Department of Chemistry, Faculty of Education, Ain Shams University, Roxy, 11711, Cairo, Egypt*
[2]*Hurymilla College of Science and Humanities, Shaqra University, KSA, Saudi Arabia*

ABSTRACT

1,6-Diamino-2-oxo-4-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,2-dihydropyridine-3,5-dicarbonitrile (2) reacted with some mono electrophilic reagents such as acetic anhydride, carbon disulfide, chromone-3-carboxylic acid and anthranilic acid to give a novel series of triazolo[1,5-a] pyridines 3-6. Cyclocondensation of compound 6 with carbon disulphide and acetic anhydride afforded pyrido[1,2:2,3]triazolo[1,5-c]quinazolines 7,8. Also, the new pyrido[1,2-b][1,2,4]triazines 9-14 were yielded from interaction of, respectively. The effect of some α,γ-bifunctional electrophiles such as 2 compound 2 reacted with chloroacetyl chloride, oxalyl chloride, sodium pyruvate and/or benzyl -cyano-3,3-bis(methylthio)acrylonitrile, 2-cyano-3,3-bis(methylthio) prop-2-enamide,5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde, chromone-3-carbo-nitrile, 4-chlorobenzylidene malononitrile and/or ethyl 3-(4-chlorophenyl)-2-cyanoprop-2-enoate on compound 2 was also studied and gave some new pyrido[1,2-b][1,2,4]triazipines 15-21. The synthesized compounds were showed a low to high antimicrobial activity towards Gram- positive bacteria, Gram-negative bacteria, yeasts and the fungal strain.



GRAPHICAL ABSTRACT

Keywords: Bonzothienopyrimidines, Triazolopyridine, Pyridotriazine, Pyridotriazipine

INTRODUCTION

Nitrogenous heterocyclic compounds, with a sulfur atom are an important class of compounds in medicinal chemistry. Many condensed heterocyclic systems as thienopyrimidines play an important role as anti-inflammatory, antimicrobial and analgesic properties, antitumor, inhibition of cancer cell proliferation, prevention of cartilage destruction in particular diseases activities, antidepressant, antiplatelet, antihypertensive, herbicidal, and plant regulatory properties [1-22]. Also, pyrimidines and their ring-fused derivatives have a broad spectrum of biological activity; best known as the heterocyclic core of the nucleic acid bases. Furthermore, *o*-diaminopyrimidones are versatile substrates for building of various biologically active nitrogen bridgehead heterocyclic systems [23-26]. Motivated by the aforementioned biological and pharmacological importance of the title compounds, and as continuation with our previous work on thienopyrimidines [27,28], we report herein the use of 1,6-diamino-2-oxo-4-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo-[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1,2-dihydropyridine-3,5-dicarbonitrile 2 as a precursor to synthesize some novel series of biologically active nitrogen bridgehead systems including triazolo[1,5-*a*]pyridines**,** pyrido[1′,2′:2,3]triazolo[1,5-*c*]quinazolines, pyrido[1,2-*b*] [1,2,4]triazines and pyrido[1,2-*b*][1,2,4]triazipines and evaluate their antimicrobial activity.

EXPERIMENTAL SECTION

General

All the reported melting points were uncorrected. The IR spectra were recorded on FTIR Jasco 4100 spectrophotometer using KBr wafer technique. ^{1}H-NMR spectra were measured on Gemini-300BB spectrometer (300 MHz), using Deuterated Dimethyl Sulfoxide (DMSO-d_6) as a solvent and Tetramethylsilane (TMS) (δ) as an internal standard. Elemental microanalyses were recorded on a Perkin Elmer series II C, H, N, S analyzer 2400. Mass spectra were obtained using gas chromatography GC-MS qp-2010 and on a Shimadzu instrument mass spectrometer (70 eV). The purity of the synthesized compounds was checked by Thin Layer Chromatography (TLC).

Compound (1) has been prepared according to the reported method [27].

Synthesis of 1,6-diamino-2-oxo-4-(10-oxo-4,6,7,8,9,10-hexahydro-pyrazolo[1,5-a][1]benzo-thieno[2,3-d]pyrimidin-3-yl)-1,2-dihydropyridine-3,5-dicarbonitrile (2)

A mixture of compound 1 (0.321 g, 1 mmol) and cyanoacetohydrazide (0.099 g, 1 mmol) in DMF (10 ml) containing few drops of piperidine was heated under reflux for 3 h. The solid obtained was filtered off and recrystallized from dioxane to give compound 2 as brown crystals, yield 75%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3328-3172 (2NH_2, NH), 3059 ($CH_{arom.}$), 2930 ($CH_{aliph.}$), 2206, 2193 (2C≡N), 1666-1648 (2C=O), 1624 (C=N), 1545 cm^{-1} (C=C). Also, its ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.78-1.82 (m, 4H, 2CH_2), 2.73 (t, 2H, CH_2), 2.89 (t, 2H, CH_2), 3.43 (brs, 2H, *N*-NH_2 exchangeable with D_2O), 7.43 (s, 1H, NH exchangeable with D_2O), 7.95 (s, 1H, pyrazole-H2), 8.30 ppm (brs, 2H, *C*-NH_2 exchangeable with D_2O). MS (EI, *m/z*): 418 ($M^{+\cdot}$, 24.01%), 417 (M-1, 23.36), 390 (27.63), 352 (30.26), 305 (16.45), 291 (28.62), 265 (48.36), 261 (100%), 214 (15.79), 181 (19.41), 167 (26.64), 138 (23.36), 105 (32.24), 99 (52.6), 73 (16.8), 52 (49%). Analysis calculated for $C_{19}H_{14}N_8O_2S$ (418.44): C, 54.54; H, 3.37; N, 26.78; S, 7.66. Found: C, 54.73; H, 3.35; N, 26.59; S, 7.87.

Synthesis of 1-acetyl-2-methyl-5-oxo-7-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,5-dihydro[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbo-nitrile (3)

A mixture of compound 2 (0.418 g, 1 mmol) and acetic anhydride (10 ml) was heated under reflux for 2 h. The reaction mixture was cooled and poured gradually onto crushed. The solid obtained was filtered off and recrystallized from acetic acid to give compound 3 as brown crystals 53%, m.p. > 300°C. IR (KBr), (ν_{max}, cm^{-1}):3441 (NH), 3065 ($CH_{arom.}$), 2933 ($CH_{aliph.}$), 2212, 2179 (2C≡N), 1712, 1698, 1665 (3C=O), 1615 (C=N), 1559 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.80-1.82 (m, 4H, 2CH_2), 1.90 (s, 3H, CH_3), 2.49 (s, 3H, $COCH_3$), 2.73 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 7.82 (s, 1H, pyrazole-H2), 8.10 ppm (s, 1H, NH exchangeable with D_2O). MS (EI, *m/z*): 484 (($M^{+\cdot}$, 81%), 485 (M+1, 70), 434 (73), 376 (84), 359 (100%), 325 (72), 285 (45), 229 (36), 206 (48), 160 (55), 59 (78%). Analysis calculated for $C_{23}H_{16}N_8O_3S$ (484.50): C, 57.02; H, 3.33; N, 23.13; S, 6.62. Found: C, 57.31; H, 3.14; N, 23.52; S, 6.43.

Synthesis of 5-oxo-7-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyramidin-3-yl)-2-thioxo-1,2,3,5-tetrahydro[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (4)

A mixture of compound 2 (0.418 g, 1 mmol) and carbon disulfide (0.076 ml, 1 mmol) in pyridine (10 ml) was heated under reflux for 8 h. The reaction mixture was cooled and poured gradually onto crushed ice and neutralized with HCl. The solid obtained was filtered off and recrystallized from methanol to give compound 4 as brown crystals, yield 69%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3447-3200 (3NH), 3058 ($CH_{arom.}$), 2922 ($CH_{aliph.}$), 2207, 2195 (2C≡N), 1653, 1636 (2C=O), 1618 (C=N), 1522 (C=C), 1181 cm^{-1} (C=S). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.77-1.80 (m, 4H, 2CH_2), 2.72 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 8.14 (s, 1H, NH exchangeable with D_2O), 8.20 (s, 1H, NH exchangeable with D_2O), 8.46 (s, 1H, pyrazole H2), 8.86 ppm (s, 1H, NH exchangeable with D_2O). MS (EI, *m/z*): 460 ($M^{+\cdot}$, 0.1%), 345 (0.1), 277 (0.24), 272 (0.43), 255 (1.42), 191 (2.16), 159 (3.33), 127 (3.44), 79 (100%), 64 (27.44), 52 (68.66%). Analysis calculated for $C_{20}H_{12}N_8O_2S_2$ (460.50): C, 52.17; H, 2.63; N, 24.33; S, 13.93. Found: C, 52.06; H, 2.74; N, 24.12; S, 13.54.

Synthesis of 5-oxo-2-(4-oxo-4H-chromen-3-yl)-7-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo [1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,5-dihydro[1,2,4]triazolo[1,5-a]pyridine-6,8-di carbonitrile (5)

A mixture of compound 2 (0.418 g, 1 mmol) and chromone-3-carboxylic acid (0.19 g, 1 mmol) in phosphorus oxychloride (5 ml) was heated under reflux on a water bath for 4 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from ethanol to give compound 5 as pale brown crystals, yield 58%, m.p. 156-158°C. IR (KBr), (ν_{max}, cm^{-1}): 3447-3391 (2NH), 3050 ($CH_{arom.}$), 2925 ($CH_{aliph.}$), 2220, 2215 (2C≡N), 1684, 1653, 1636 (3C=O), 1617 (C=N), 1559 cm^{-1} (C=C). MS (EI, *m/z*): 572 ($M^{+\cdot}$, 6.81%), 573 (M+1, 4.36), 533 (6.27), 336 (43.53), 269 (22.57), 149 (86.69), 97 (48.89), 83 (33.89), 70 (20.35%), 57 (100%). Analysis calculated for $C_{29}H_{16}N_8O_4S$ (572.57): C, 60.84; H, 2.82; N, 19.57; S, 5.60. Found: C, 60.63; H, 2.71; N, 19.78; S, 5.84.

Synthesis of 2-(2-aminophenyl)-5-oxo-7-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1] benzothieno[2,3-d]pyrimidin-3-yl)-3,5-dihydro[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbo-nitrile (6)

A mixture of compound 2 (0.418 g, 1 mmol) and anthranilic acid (0.135 g, 1 mmol) in $POCl_3$ (5 ml) was heated under reflux on a water bath for 4 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from DMF to give compound 6 as pale brown crystals, yield 56%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3442-3172 (NH_2, 2NH), 3010 ($CH_{arom.}$), 2930 ($CH_{aliph.}$), 2213, 2205 (2C≡N), 1680, 1651 (2C=O), 1620 (C=N), 1557 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.78-1.82 (m, 4H, 2CH_2), 2.72 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 4.69 (brs, 2H, NH_2 exchangeable with D_2O), 7.31 (s, 1H, NH exchangeable with D_2O), 7.75-8.14 (m, 4H, Ar-H), 8.28 (s, 1H, pyrazole-H2), 9.84 ppm (s, 1H, NH exchangeable with D_2O). MS (EI, *m/z*): 519 ($M^{+\cdot}$, 0.63%), 440 (3.96), 339 (12.58), 310 (4.77), 266 (7.47), 245 (100%), 238 (12.24), 217 (56.24), 146 (12.69), 120 (27.28), 77 (29.69%). Analysis calculated for $C_{26}H_{17}N_9O_2S$ (519.55): C, 60.11; H, 3.30; N, 24.26; S, 6.17. Found: C, 60.05; H, 3.11; N, 24.65; S, 6.48.

Synthesis of 2-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d] pyrimidin-3-yl)-4-oxo-7-thioxo-4,7-dihydro-8H-pyrido[1$^\backslash$,2$^\backslash$:2,3][1,2,4]triazolo[1,5-c] quinazoline-1,3-dicarbonitrile (7)

A mixture of compound 6 (0.43 g, 1 mmol) and carbon disulfide (0.076 ml, 1 mmol) in pyridine (10 ml) was heated under reflux for 8 h. The reaction mixture was cooled and poured gradually onto crushed ice and neutralized with HCl. The solid obtained was filtered off and recrystallized from dioxane to give compound 7 as brown crystals, yield 48%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3422-3348 (2NH), 3058

($CH_{arom.}$), 2927 ($CH_{aliph.}$), 2213, 2191 (2C≡N), 1684, 1653 (2C=O), 1617 (C=N), 1559 (C=C), 1196 cm^{-1} (C=S). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=8.78 and 8.80 ppm corresponding to two NH protons, in addition to signals at δ=1.78-1.82 (m, 4H, $2CH_2$), 2.69 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 7.93-8.49 ppm (m, 5H, Ar-H and pyrazole-H2). MS (EI, *m/z*): 561 ($M^{+\cdot}$, 35.19%), 526 (29.32), 496 (27.78), 378 (29.94), 344 (26.54), 261 (31.17), 246 (25.62), 171 (28.70), 133 (29.32), 78 (14.20), 67 (40.12%), 51 (100%). Analysis calculated for $C_{27}H_{15}N_9O_2S_2$ (561.61): C, 57.75; H, 2.69; N, 22.45; S, 11.42. Found: C, 57.52; H, 2.57; N, 22.74; S, 11.73.

Synthesis of 2-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimi-din-3-yl)-7-methyl-4-oxo-4H-pyrido[1\',2\':2,3][1,2,4]triazolo[1,5-c]quinazoline-1,3-dicarbo- nitrile (8)

A mixture of compound 6 (0.43 g, 1 mmol) and acetic anhydride (10 ml) was heated under reflux for 3 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from DMF to give compound 8 as yellow crystals, yield 53%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3443 (NH), 3020 ($CH_{arom.}$), 2926 ($CH_{aliph.}$), 2220, 2193 (2C≡N), 1672, 1647 (2C=O), 1628 (C=N), 1556 cm^{-1} (C=C). MS (EI, *m/z*): 543 ($M^{+\cdot}$, 40.84%), 544 (M+1, 41.36), 507 (46.60), 463 (46.60), 391 (53.93), 336 (51.83), 297 (51.83), 239 (92.67), 147 (68.06), 119 (59.69), 95 (100%), 58 (47.64%). Analysis calculated for $C_{28}H_{17}N_9O_2S$ (543.57): C, 61.87; H, 3.15; N, 23.19; S, 5.90. Found: C, 61.96; H, 3.36; N, 23.08; S, 5.64.

Synthesis of 3,6-dioxo-8-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,3,4,6-tetrahydro-2H-pyrido[1,2-b][1,2,4]triazine-7,9-dicarbonitrile (9)

A mixture of compound 2 (0.418 g, 1 mmol) and chloroacetyl chloride (0.11 ml, 1 mmol) in DMF (10 ml) containing few drops of triethylamine was heated under reflux for 4 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from DMF/H_2O to give compound 9 as pale brown crystals, yield 69%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3441-3338 (3NH), 3073 ($CH_{arom.}$), 2931 ($CH_{aliph.}$), 2220, 2205 (2C≡N), 1660-1636 (3C=O), 1600 (C=N), 1525 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ= 1.77-1.82 (m, 4H, $2CH_2$), 2.72 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 4.16 (s, 2H, CH_2), 7.40 (s, 1H, NH exchangeable with D_2O), 7.94 (s, 1H, pyrazole-H2), 8.13 (s, 1H, NH exchangeable with D_2O), 8.42 ppm (s, 1H, NH exchangeable with D_2O). MS (EI, *m/z*): 458 ($M^{+\cdot}$, 17.30%), 459 (M+1, 13.74), 420 (17.3), 391 (23.22), 320 (17.54), 251 (22.27), 142 (95.26), 113 (35.05), 98 (100%), 70 (54.74), 55 (48.34%). Analysis calculated for $C_{21}H_{14}N_8O_3S$ (458.46): C, 55.02; H, 3.08; N, 24.44; S, 6.99. Found: C, 55.31; H, 3.19; N, 24.83; S, 6.58.

Synthesis of 2,3,6-trioxo-8-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno [2,3-d]pyrimidin-3-yl)-1,3,4,6-tetrahydro-2H-pyrido[1,2-b][1,2,4]triazine-7,9-dicarbonitrile (10)

A mixture of compound 2 (0.418 g, 1 mmol) and oxalyl chloride (0.126 ml, 1 mmol) in DMF (10 ml) containing few drops of triethylamine was heated under reflux for 2 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from dioxane to give compound 10 as brown crystals, yield 58%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3446-3335 (3NH), 3068 ($CH_{arom.}$), 2925 ($CH_{aliph.}$), 2207, 2192 (2C≡N), 1691-1640 (4C=O), 1624 (C=N), 1540 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.79-1.82 (m, 4H, $2CH_2$), 2.73 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 7.45 (s, 1H, NH exchangeable with D_2O), 7.95 (s, 1H, pyrazole-H2), 8.11 (s, 1H, NH exchangeable with D_2O), 8.28 ppm (s, 1H, NH exchangeable with D_2O). MS (EI, *m/z*): 472 ($M^{+\cdot}$, 1.53%), 473 (M+1, 1.35), 374 (2), 351 (65.51), 334 (70.25), 270 (18.58), 239 (8.24), 212 (100%), 169 (30.90), 95 (41.45), 81 (32.79), 69 (21.22%). Analysis calculated for $C_{21}H_{12}N_8O_4S$ (472.44): C, 53.39; H, 2.56; N, 23.72; S, 6.79. Found: C, 53.08; H, 2.37; N, 23.43; S, 6.98.

Synthesis of 3-methyl-2,6-dioxo-8-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzo- thieno[2,3-d]pyrimidin-3-yl)-1,6-dihydro-2H-pyrido[1,2-b][1,2,4]triazine-7,9-dicarbonitrile (11)

A mixture of compound 2 (0.418 g, 1 mmol) and sodium pyruvate (0.11 g, 1 mmol) in glacial acetic acid (10 ml) containing freshly fused sodium acetate (0.1 g) was heated under reflux for 3 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from ethanol to give compound 11 as pale brown crystals, yield 61%, m.p. > 300°C. IR (KBr), (ν_{max}, cm^{-1}): 3423-3309 (2NH), 3060 ($CH_{arom.}$), 2934 ($CH_{aliph.}$), 2214, 2194 (2C≡N), 1674-1653 (3C=O), 1624 (C=N), 1518 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.79-1.90 (m, 4H, $2CH_2$), 2.20 (s, 3H, CH_3), 2.73 (t, 2H, CH_2), 2.89 (t, 2H, CH_2), 7.25 (s, 1H, NH exchangeable with D_2O), 8.59 (s, 1H, pyrazole-H2), 8.70 ppm (s, 1H, NH exchangeable with D_2O). MS (EI, *m/z*): 470 ($M^{+\cdot}$, 12.13%), 471 (M+1, 7.67), 423 (13.11), 377 (11.99), 332 (10.18), 270 (4.88), 168 (11.16), 149 (15.06), 73 (27.06%), 57 (100%). Analysis calculated for $C_{22}H_{14}N_8O_3S$ (470.47): C, 56.17; H, 3.00; N, 23.82; S, 6.82. Found: C, 56.06; H, 3.19; N, 23.41; S, 6.94.

Synthesis of 2,3-diphenyl-6-oxo-8-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzo-thieno[2,3-d]pyrimidin-3-yl)-6H-pyrido[1,2-b][1,2,4]triazine-7,9-dicarbonitrile (12)

A mixture of compound 2 (0.418 g, 1 mmol) and benzil (0.21 g, 1 mmol) in glacial acetic acid (10 ml) containing freshly fused sodium acetate (0.1 g) was heated under reflux for 4 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from acetic acid to give compound 12 as brown crystals, yield 55%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3447 (NH), 3058 ($CH_{arom.}$), 2933 ($CH_{aliph.}$), 2210, 2190 (2C≡N), 1674, 1661 (2C=O), 1594 (C=N), 1579 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.79-1.82 (m, 4H, $2CH_2$), 2.73 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 7.92 (s, 1H, NH exchangeable with D_2O), 7.61-7.94 ppm (m, 11H, Ar-H and pyrazole-H2). MS (EI, *m/z*): 592 ($M^{+\cdot}$, 0.24%), 439 (0.42), 322 (0.65), 300 (0.94), 276 (1.56), 267 (2.87), 225 (4.83), 179 (8.08), 149 (3.98), 105 (100%), 97 (5.85), 80 (63.98), 64(45.54%). Analysis calculated for $C_{33}H_{20}N_8O_2S$ (592.64): C, 66.88; H, 3.40; N, 18.91; S, 5.41. Found: C, 66.57; H, 3.31; N, 18.66; S, 5.72.

Synthesis of 8-oxo-10-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,8-dihydropyrido[1,2-b]indolo[2,3-e][1,2,4]triazine-9,11-dicarbonitrile (13)

A mixture of compound 2 (0.418 g, 1 mmol) and isatin (0.147 g, 1 mmol) in glacial acetic acid (10 ml) containing freshly fused sodium acetate (0.1 g) was heated under reflux for 4 h. The solid obtained during heating was filtered off and recrystallized from DMF to give compound 13 as pale brown crystals, yield 59%, m.p. > 300°C. IR (KBr), (ν_{max}, cm^{-1}): 3404-3349 (2NH), 3058 ($CH_{arom.}$), 2933 ($CH_{aliph.}$), 2230, 2211 (2C≡N), 1683, 1649 (2C=O), 1617 (C=N), 1590 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.80-1.82 (m, 4H, $2CH_2$), 2.74 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 6.90-7.61 (m, 4H, Ar-H), 7.96 (s, 1H, pyrazole-H2), 8.75 (s, 1H, NH exchangeable with D_2O), 11.03 ppm (s, 1H, NH exchangeable with

D_2O). MS (EI, *m/z*): 531 (M+2, 6.87%), 494 (8.63), 465 (7.40), 418 (8.37), 345 (9.07), 251 (8.99), 147 (29.96), 119 (40.09), 96 (46.96), 73 (38.59%), 55 (100%). Analysis Calculated for $C_{27}H_{15}N_9O_2S$ (529.54): C, 61.24; H, 2.86; N, 23.81; S, 6.06. Found: C, 61.55; H, 2.65; N, 23.42; S, 6.35.

Synthesis of N-{2-[7,9-dicyano-3,6-dioxo-8-(10-oxo-4,6,7,8,9,10-hexa-hydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-3,6-dihydro-4H-pyrido[1,2-b][1,2,4]triazin-2-yl] phenyl} acetamide (14)

A mixture of compound 2 (0.418 g, 1 mmol) and *N*-acetylisatin (0.19 g, 1 mmol) in glacial acetic acid (10 ml) was heated under reflux for 4 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from ethanol to give compound 14 as yellow crystals, yield 64%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3446-3225 (3NH), 3050 ($CH_{arom.}$), 2934 ($CH_{aliph.}$), 2206, 2194 (2C≡N), 1701, 1691, 1653, 1647 (4C=O), 1617 (C=N), 1559 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.78-1.80 (m, 4H, $2CH_2$), 2.08 (s, 3H, $COCH_3$), 2.72 (t, 2H, CH_2), 2.88 (t, 2H, CH_2), 6.91-7.66 (m, 4H, Ar-H), 7.26 (s, 1H, NH exchangeable with D_2O), 7.66 (s, 1H, NH exchangeable with D_2O), 7.94 (s, 1H, pyrazole-H2), 10.60 ppm (s, 1H, NH exchangeable with D_2O). MS (EI, *m/z*): 589 ($M^{\ddagger}$, 31.29%), 536 (38.04), 467 (33.74), 359 (37.12), 316 (41.10), 234 (100%), 157 (33.74), 133 (47.85), 121 (68.10), 77 (29.45), 64 (81.29%). Analysis calculated for $C_{29}H_{19}N_9O_4S$ (589.60): C, 59.08; H, 3.25; N, 21.38; S, 5.44. Found: C, 59.27; H, 3.06; N, 21.19; S, 5.73.

Synthesis of 2-amino-4-(methylthio)-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,7-dihydropyrido[1,2-b][1,2,4]triazepine-3,8,10-tri- carbonitrile (15)

A mixture of compound 2 (0.418 g, 1 mmol) and 2-cyano-3, 3-bis(methylthio)acrylonitrile (0.17 g, 1 mmol) in DMF (10 ml) containing few drops of triethylamine was heated under reflux for 4 h. The solid obtained was filtered off and recrystallized from DMF/EtOH to give compound 15 as brown crystals, yield 58%, m.p. >300°C. IR (KBr), (ν_{max}, cm^{-1}): 3444-3200 (NH_2, 2NH), 3028 ($CH_{arom.}$), 2947 ($CH_{aliph.}$), 2202, 2191, 2180 (3C≡N), 1676, 1659 (2C=O), 1625 (C=N), 1533 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.79-1.82 (m, 4H, $2CH_2$), 1.66 (s, 3H, SCH_3), 2.73 (t, 2H, CH_2), 2.93 (t, 2H, CH_2), 3.71 (brs, 2H, NH_2 exchangeable with D_2O), 7.95 (s, 1H, pyrazole-H2), 8.10 (s, 1H, NH exchangeable with D_2O), 9.90 ppm (s, 1H, NH exchangeable with D_2O). MS (EI, *m/z*): 540 ($M^{\ddagger}$, 0.33%), 378 (0.43), 320 (1.72), 300 (1.85), 273 (3.16), 207 (50.54), 192 (37.45), 167 (40.57), 133 (32.14), 109 (81.98), 94 (55.12), 84 (100%), 69 (63.24), 55 (64.14%). Analysis calculated for $C_{24}H_{16}N_{10}O_2S_2$ (540.59): C, 53.32; H, 2.98; N, 25.91; S, 11.86. Found: C, 53.11; H, 2.87; N, 25.62; S, 11.47.

Synthesis of 2-amino-8,10-dicyano-4-(methylthio)-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexa-hydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,7-dihydropyrido[1,2-b][1,2,4] triazepine-3-carboxamide (16)

A mixture of compound 2 (0.418 g, 1 mmol) and 2-cyano-3,3-bis(methylthio)-prop-2-enamide (0.188 g, 1 mmol) in DMF (10 ml) containing few drops of triethylamine was heated under reflux for 4 h. The solid obtained was filtered off and recrystallized from DMF to give compound 16 as yellow crystals, yield 61%, m.p. > 300°C. IR (KBr), (ν_{max}, cm^{-1}): 3446-3200 ($2NH_2$, 2NH), 3063 ($CH_{arom.}$), 2928 ($CH_{aliph.}$), 2201, 2183 (2C≡N), 1700, 1684, 1653 (3C=O), 1630 (C=N), 1559 cm^{-1} (C=C). MS (EI, *m/z*): 558 ($M^{\ddagger}$, 2.53%), 523 (16.43), 4.88 (7.78), 392 (29.90), 369 (35.93), 313 (23.51), 236 (36.01), 121 (30.33), 109 (22.20), 83 (100%), 73 (41.78), 55 (80.42%). Analysis calculated for $C_{24}H_{18}N_{10}O_3S_2$ (558.60): C, 51.60; H, 3.25; N, 25.07; S, 11.48. Found: C, 51.39; H, 3.04; N, 25.48; S, 11.69.

Synthesis of 3-methyl-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzo-thieno[2,3-d]pyrimidin-3-yl)-1-phenyl-7,11-dihydro-1H-pyrazolo[3,4-e]pyrido[1,2-b][1,2,4]triazepine-8,10-dicarbonitrile (17)

A mixture of compound 2 (0.418 g, 1 mmol) and 5-chloro-3-methyl-1-phenyl-pyrazole-4-carbaldehyde (0.22 g, 1 mmol) in DMF (10 ml) containing few drops of triethylamine was heated under reflux for 4 h. The solid obtained was filtered off and recrystallized from DMF to give compound 17 as brown crystals, yield 66%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3421-3312 (2NH), 3020 ($CH_{arom.}$), 2929 ($CH_{aliph.}$), 2201, 2185 (2C≡N), 1653, 1647 (2C=O), 1620 (C=N), 1559 cm^{-1} (C=C). While, that of compound 17 showed absorption bands at 3420-3200 (NH_2, 2NH, OH), 3050 ($CH_{arom.}$), 2933 ($CH_{aliph.}$), 2215, 2209 (2C≡N), 1664, 1654, 1647 (3C=O), 1624 (C=N) and 1522 cm^{-1} (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ=1.80-1.82 (m, 4H, $2CH_2$), 2.26 (s, 3H, CH_3), 2.73 (t, 2H, CH_2), 2.89 (t, 2H, CH_2), 7.40 (s, 1H, NH exchangeable with D_2O), 7.50 (s, 1H, NH exchangeable with D_2O), 7.53-7.55 (m, 5H, Ar-H), 7.96 (s, 1H, pyrazole-H2), 9.95 ppm (s, 1H, triazepine-H4). MS (EI, *m/z*): 584 ($M^{\ddagger}$, 11.14%), 585 (M+1, 6.54%), 533 (13.68), 424 (10.41), 385 (11.50), 369 (12.59), 349 (20.58), 334 (100%), 287 (10.29), 247 (16.46), 212 (42.25), 141 (18.40), 77 (4.48%). Analysis calculated for $C_{30}H_{20}N_{10}O_2S$ (584.62): C, 61.64; H, 3.45; N, 23.96; S, 5.48. Found: C, 61.85; H, 3.64; N, 23.67; S, 5.37.

Synthesis of 2-amino-3-(2-hydroxybenzoyl)-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyra- zolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,7-dihydropyrido[1,2-b][1,2,4]triazepine-8,10-dicarbonitrile (18)

A mixture of compound 2 (0.418 g, 1 mmol) and chromone-3-carbonitrile (0.17 g, 1 mmol) in DMF (10 ml) was heated under reflux for 4 h. The solid obtained was filtered off and recrystallized from DMF to give compound 18 as pale brown crystals, yield 69%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3420-3200 (NH_2, 2NH, OH), 3050 ($CH_{arom.}$), 2933 ($CH_{aliph.}$), 2215, 2209 (2C≡N), 1664, 1654, 1647 (3C=O), 1624 (C=N), 1522 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.81-1.84 (m, 4H, $2CH_2$), 2.74 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 3.46 (brs, 2H, NH_2 exchangeable with D_2O), 7.43-7.72 (m, 4H, Ar-H), 7.78, 7.80 (s, 2H, 2NH exchangeable with D_2O), 7.96 (s, 1H, pyrazole-H2), 8.20 (s, 1H, OH exchangeable with D_2O), 9.55 ppm (s, 1H, triazepine-H4). MS (EI, *m/z*): 589 (($M^{\ddagger}$, 51.39%), 591 (M+2, 48.61), 590 (M+1, 45.14), 547 (43.06), 394 (43.75), 361 (62.50), 349 (81.25), 311 (36.11), 266 (40.28), 210 (74.31), 177 (45.83), 150 (22.92), 129 (100%), 95 (83.33), 77 (29.86), 60 (46.53%). Analysis calculated for $C_{29}H_{19}N_9O_4S$ (589.60): C, 59.08; H, 3.25; N, 21.38; S, 5.44. Found: C, 59.39; H, 3.04; N, 21.67; S, 5.15.

Synthesis of 2-amino-4-(4-chlorophenyl)-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,7-dihydropyrido[1,2-b][1,2,4]triazepine-3,8,10-tricarbonitrile (19)

A mixture of compound 2 (0.418 g, 1 mmol) and 4-chlorobenzylidene malononitrile (0.189 g, 1 mmol) in DMF (10 ml) containing few drops of triethylamine was heated under reflux for 4 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from DMF/EtOH to give compound 19 as brown crystals, yield 56%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3447-3280 (NH_2, 2NH), 3063 ($CH_{arom.}$), 2933 ($CH_{aliph.}$), 2207, 2200, 2186 (3C≡N), 1662, 1654 (2C=O), 1636 (C=N), 1559 cm^{-1} (C=C). MS (EI, *m/z*): 605 ($M^{\ddagger}$, 0.12%), 607 (M+2, 0.12), 606 (M+1, 0.11), 572 (0.1), 270 (2.15), 246 (54.30), 217 (5.28), 155 (16.14), 128 (11.32), 113 (26.60), 80

(94.77), 64 (100%), 55 (26.26%). Analysis calculated for $C_{29}H_{17}ClN_{10}O_2S$ (605.04): C, 57.57; H, 2.83; Cl, 5.86; N, 23.15; S, 5.30. Found: C, 57.28;

H, 2.72; Cl, 5.67; N, 23.54; S, 5.61.

Synthesis of ethyl 2-amino-4-(4-chlorophenyl)-8,10-dicyano-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-a][1]benzothieno[2,3-d]pyrimidin-3-yl)-1,7-dihydropyrido[1,2-b] [1,2,4]triazepine-3-carboxylate (21)

A mixture of compound 2 (0.418 g, 1 mmol) and ethyl 2-cyano-3-(4-chlorophenyl)prop-2-enoate (0.236 g, 1 mmol) in DMF (10 ml) containing few drops of triethylamine was heated under reflux for 4 h. The reaction mixture was cooled and poured gradually onto crushed ice. The solid obtained was filtered off and recrystallized from DMF/H_2O to give compound 21 as pale brown crystals, yield 75%, m.p.>300°C. IR (KBr), (ν_{max}, cm^{-1}): 3423-3170 (NH_2, 2NH), 3010 ($CH_{arom.}$), 2927 ($CH_{aliph.}$), 2207, 2189 (2C≡N), 1710, 1680, 1662 (3C=O), 1630 (C=N), 1574 cm^{-1} (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ=1.06 (t, 3H, *J*=6.9 Hz, CH_2CH_3), 1.79-1.82 (m, 4H, $2CH_2$), 2.74 (t, 2H, CH_2), 2.89 (t, 2H, CH_2), 3.42 (q, 2H, *J*=6.9 Hz, CH_2CH_3), 4.23 (brs, 2H, NH_2 exchangeable with D_2O), 7.32-7.95 (m, 4H, Ar-H), 8.02 (s, 1H, pyrazole-H2), 8.21 (s, 1H, NH exchangeable with D_2O), 8.52 ppm (s, 1H, NH exchangeable with D_2O). MS (EI, *m/z*): 652 ($M^{+\cdot}$, 7.33%), 612 (8.85), 543 (10.62), 467 (8.34), 345 (10.62), 307 (10.37), 185 (12.14), 160 (35.52), 111 (25.28), 73 (68.14%), 60 (100%). Analysis calculated for $C_{31}H_{22}ClN_9O_4S$ (652.10): C, 57.10; H, 3.40; Cl, 5.44; N, 19.33; S, 4.92. Found: C, 57.42; H, 3.19; Cl, 5.65; N, 19.61; S, 4.64.

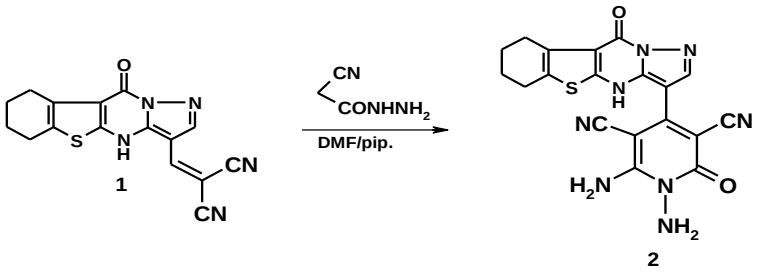
Antimicrobial evaluation

All the newly synthesized compounds were evaluated *in vitro* for their antibacterial activities against *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6635), as representatives of Gram-positive bacteria and *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028) as examples of Gram-negative bacteria. They were also examined against *Candida albicans* (ATCC 10231) and *Aspergillus fumigatus* as fungi. Agar diffusion technique was used for the determination of the preliminary antibacterial and antifungal activities. The compounds were dissolved in DMSO which has no inhibition activity to get concentration of 100 $\mu g/ml^{-1}$. The test was performed on medium Potato Dextrose Agars (PDA) which contains infusion of 200 g potatoes, 6 g dextrose and 15 g agar. Uniform size filter paper disks (3 disks per compound) were impregnated by equal volume (10 µl) from the specific concentration of dissolved tested compounds and carefully placed on inoculated agar surface. After incubation for 36 h at 27°C in the case of bacteria and for 48 h at 24°C in the case of fungi, inhibition of the organisms was measured and used to calculate mean of inhibition zones.

RESULTS AND DISCUSSION

Chemistry

The key compound, 1,6-Diaminopyridone derivative 2 was synthesized by refluxing of arylidene malononitrile derivative 1 [27] with cyanoacetohydrazide in dimethylformamide containing a catalytic amount of piperidine [29,30] (Scheme 1).



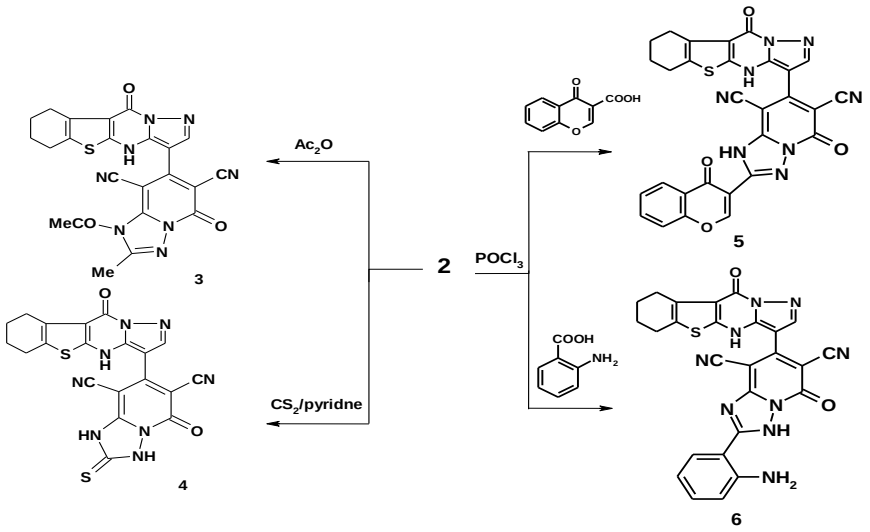
Scheme 1: Synthesis of 1,6-diaminopyridone derivative 2

The structure of compound 2 was confirmed on the basis of its elemental analysis and spectroscopic measurements, The IR spectrum of compound 2 displayed absorption bands corresponding to 2C≡N, NH and $2NH_2$ functions. Its ^{1}H-NMR spectrum revealed two singlets at δ=3.43 and 8.30 ppm exchangeable with D_2O each integrating for two protons, corresponding to $2NH_2$. Its mass spectrum showed the molecular ion peak at *m/z* 418 (M^+, 24.01%) which agree well with the molecular weight and supports the identity of its structure.

Compound 2 is a useful precursor for the synthesis of some new triazolopyridones by its reaction with some mono-electrophilic. Thus, heterocyclization of compound 2 with acetic anhydride under reflux afforded 1-acetyl-2-methyl-5-oxo-7-(10-oxo-4,6,7,8,9,10-hexa-hydropyrazolo[1,5-*a*][1]benzo-thieno[2,3-*d*]pyrimidin-3-yl)-1,5-dihydro[1,2,4]triazolo[1,5-*a*] pyridine-6,8-dicarbonitrile (3). While, treatment of diaminopyridone 2 with carbon disulfide in pyridine under reflux yielded 5-oxo-7-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-2-thioxo-1,2,3,5-tetrahydro[1,2,4]triazolo[1,5-*a*]pyrid- ine-6,8-dicarbonitrile (4). Furthermore, the chemical behavior of diaminopyridone 2 was studied towards chromone-3-carboxylic acid [31] in $POCl_3$ and produced 5-oxo-2-(4-oxo-4*H*-chromen-3-yl)-7-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*] pyramidin-3-yl)-1,5-dihydro-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (5). Also, 2-(2-aminophenyl)-5-oxo-7-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-3,5-dihydro[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (6) was prepared via condensation of compound 2 with anthranilic acid under the same reaction conditions (Scheme 2).

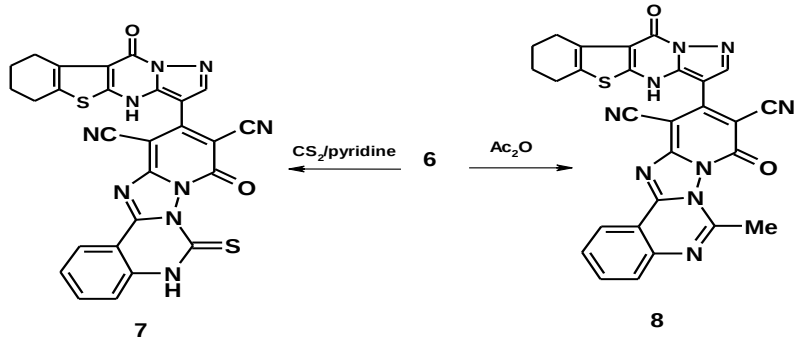
The mass spectrum of compounds 3 and 4 showed the molecular ion peak at *m/z* 484 (81%) and 460 (0.1%) corresponding to the molecular formulas $C_{23}H_{16}N_8O_3S$ and $C_{20}H_{12}N_8O_2S_2$ respectively, which agree well with the molecular weight and supports the identity of their structures. The IR spectrum of compound 3 displayed absorption bands corresponding to 3C=O, 2C≡N and NH functions, respectively. Whereas its ^{1}H-NMR spectrum exhibited signals at δ=1.90, 2.49 ppm assigned to CH_3, $COCH_3$ protons. The ^{1}H-NMR spectrum of compound 4 showed signals at δ=8.14, 8.20 and 8.86 ppm assigned to three NH protons which also showed absorption bands at 3447-3200 cm^{-1} in its IR corresponding to 3NH. The IR spectrum of compound 5 exhibited absorption bands at 1636, 1653 and 1684 cm^{-1} corresponding to 3C=O functions. Its mass spectrum exhibited the molecular ion peak at *m/z* 572 ($M^{+\cdot}$, 6.81%). Whereas, the ^{1}H-NMR spectrum of compound 6 showed signals at δ=4.69

ppm assigned to NH_2 exchangeable with D_2O.



Scheme 2: Formation of triazolopyridines 3-6

Compound 6 was used as a precursor for the synthesis of pyridotriazoloquinazoline derivatives *via* the reaction with mono-electrophilic reagents. Thus, cyclocondensation of compound 6 with carbon disulphide and acetic anhydride afforded 2-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-4-oxo-7-thioxo-4,7-dihydro-8*H*-pyrido[1',2':2,3][1,2,4]triazolo[1,5-*c*]quinazoline-1,3-dicarbonitrile (7) and2-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-7-methyl-4-oxo-4*H*-pyrido[1',2':2,3][1,2,4]triazolo[1,5-*c*]quinazoline-1,3-dicarbonitrile(8), respectively (Scheme 3).

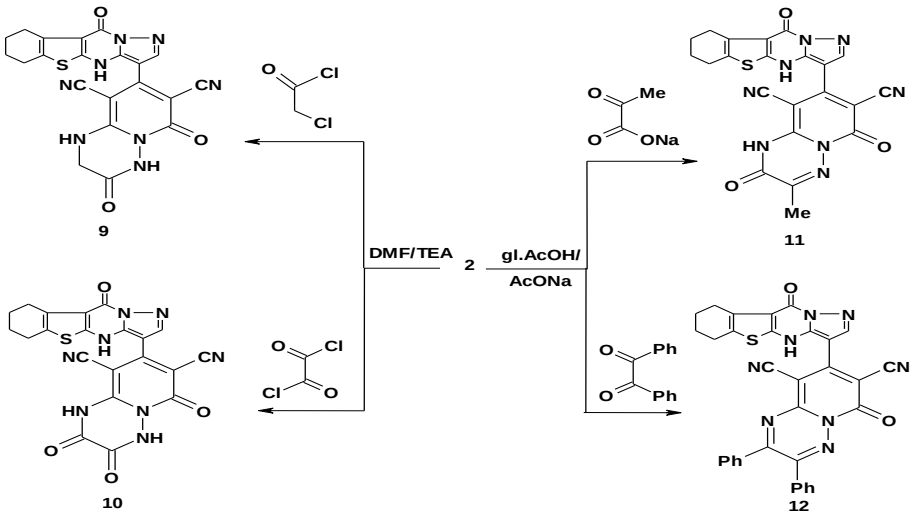


Scheme 3: Synthesis of pyridotriazoloquinazoline 7, 8

The structures of compounds 7 and 8 were characterized from their spectroscopic as well as elemental analytical data. ^{1}H-NMR spectrum of compound 7 showed two D_2O exchangeable singlet signals at δ=8.78 and 8.80 ppm corresponding to two NH protons. Also, it's the mass spectrum of which revealed the molecular ion peak at *m/z* 561 ($M^{+\cdot}$, 35.19%). Moreover, the structure of compound 8 was also supported from its mass spectrum which showed the molecular ion peak at *m/z* 543 ($M^{+\cdot}$, 40.84%).

Diaminopyridone 2 is very active substrate for building of various nitrogen bridgehead pyrido[1,2-*b*][1,2,4]triazines *via* the reaction of compound 2 with α,β-bifunctional electrophiles. Thus, cyclocondensation of compound 2 with chloroacetyl chloride and/or oxalyl chloride gave 3,6-dioxo-8-(10-oxo-4,6,7,8,9,10-hexahydro-pyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1,3,4,6-tetrahydro-2*H*-pyrido[1,2-*b*] [1,2,4]triazine-7,9-dicarbonitrile (9) and 2,3,6-trioxo-8-(10-oxo-4,6,7,8,9,10-hexahydro- pyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1,3,4,6-tetrahydro-2*H*-pyrido[1,2-*b*][1,2,4]triazine-7,9-dicarbonitrile (10), respectively (Scheme 4).

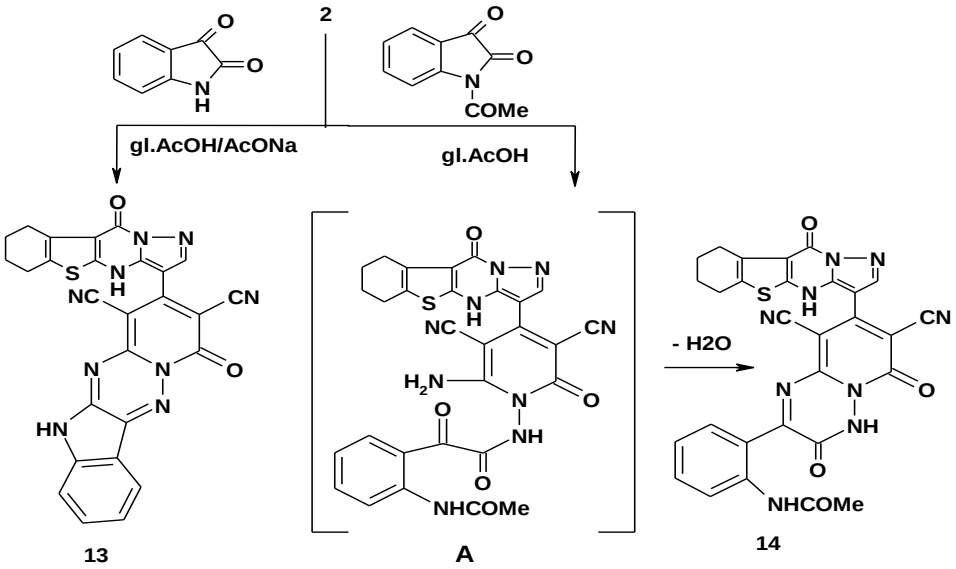
Also, reaction of compound 2 with sodium pyruvate and/or benzil in glacial acetic acid containing freshly fused sodium acetate afforded 3-methyl-2,6-dioxo-8-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1,6-dihydro-2*H*-pyrido[1,2-*b*][1,2,4]triazine-7,9-dicarbonitrile (11) and 2,3-diphenyl-6-oxo-8-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-6*H*-pyrido[1,2-*b*] [1,2,4]triazine -7,9-dicarbonitrile (12), respectively (Scheme 4).



Scheme 4: Formation of pyridotriazines 9-12

The IR spectrum of compound 9 showed absorption bands corresponding to 2C≡N and 3NH functions. Its ^{1}H-NMR spectrum showed exchangeable signals at δ=7.40, 8.13 and 8.42 ppm assigned to the 3NH protons. The structure of compound 10 was supported from its mass spectrum which showed the molecular ion peak at *m/z* 472 (1.53%) corresponding to the molecular formula $C_{21}H_{12}N_8O_4S$, which agree well with the molecular weight (472.43) and supports the identity of the structure and the base peak at m/z 212 (100%). Also, the IR spectrum of compound 11 exhibited absorption bands corresponding to 3C=O and 2NH functions. Its ^{1}H-NMR spectrum showed characteristic signals at δ=2.20, 7.25 and 8.70 ppm assigned to the CH_3 and 2NH protons respectively. Whereas, ^{1}H-NMR of compound 12 exhibited signals at δ=7.92 assigned to the NH proton exchangeable with D_2O and 7.61-7.94 ppm assigned to the Ar-H and pyrazole-H2.

Heating 2 with isatin in glacial acetic acid containing freshly fused sodium acetate afforded 8-oxo-10-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1,8-dihydro-pyrido[1,2-*b*]indolo[2,3-*e*][1,2,4]triazine-9,11-dicarbonitrile (13). While, *N*-acetylisatin showed a different behavior [31]. Reaction of diaminopyridone 2 with *N*-acetylisatin in glacial acetic acid led to *N*-{2-[7,9-dicyano-3,6-dioxo-8-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-3,6-dihydro-4*H*-pyrido[1,2-*b*][1,2,4]triazin-2-yl]phenyl}acetamide (14) through not isolated intermediate (A) (Scheme 5).



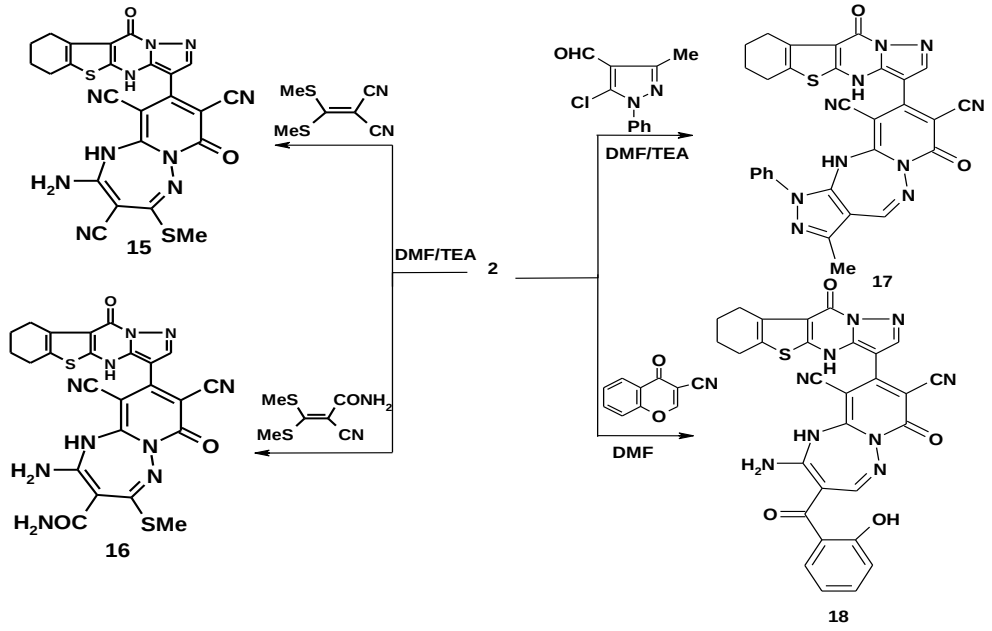
Scheme 5: Reaction of 2 with isatin and/or *N*-acetylisatin

The IR spectrum of compound 13 displayed absorption bands corresponding to 2C=O, 2C≡N and 2NH functions. The ^{1}H-NMR spectrum of compound 13 showed an exchangeable signal at δ=8.75 and 11.03 ppm attributed to the 2NH protons. While, that of compound 14 showed signals at δ=7.26, 7.66 and 10.60 ppm assigned to the 3NH protons. The structure of compound 14 was also supported from its mass spectrum which showed the molecular ion peak at *m/z* 589 (M^+, 31.29%) and the base peak at *m/z* 234 (100%). Treatment of compound 2 with some α,γ-bifunctional electrophiles such as 2-cyano-3,3-bis(methylthio)acrylonitrile and/or 2-cyano-3,3-bis(methylthio)prop-2-enamide in DMF containing few drops of triethylamine afforded 2-amino-4-(methylthio)-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1,7-dihydro-pyrido[1,2-*b*][1,2,4]triazepine-3,8,10-tricarbonitrile (15) and 2-amino-8,10-dicyano-4-(methylthio)-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*] pyrimidin-3-yl)-1,7-dihydropyrido[1,2-

b][1,2,4]triazepine-3-carboxamide (16), respectively (Scheme 6).

The behavior of diaminopyridone 2 towards heterocyclic *o*-chloroaldehydes was investigated [32]. Thus, condensation of compound 2 with 5-

chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde [33] in DMF containing few drops of triethylamine afforded 3-methyl-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1-phenyl-7,11-dihydro-1*H*-pyrazolo[3,4-*e*]pyrido[1,2-*b*][1,2,4]triazepine-8,10-dicarbonitrile (17), while, treatment of compound 2 with chromone-3-carbonitrile [34] in DMF gave 2-amino-3-(2-hydroxybenzoyl)-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1] benzothieno[2,3-*d*]pyrimidin-3-yl)-1,7-dihydropyrido[1,2-*b*][1,2,4]triazepine-8,10-dicarbonitrile (18) (Scheme 6).

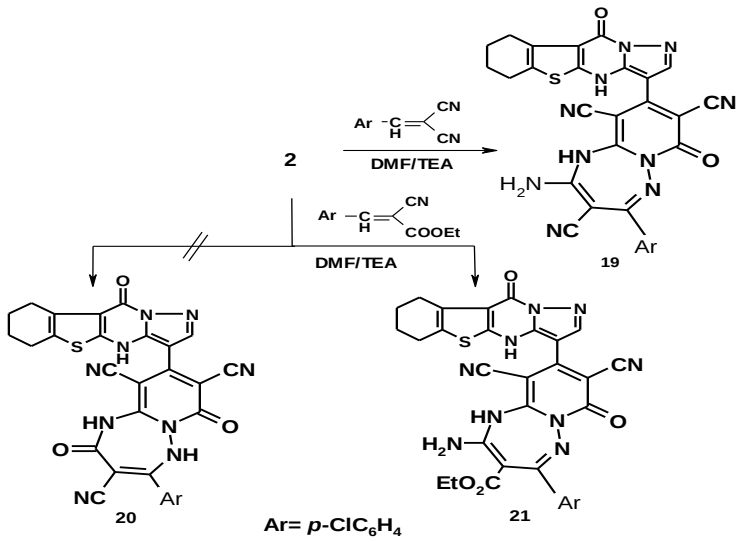


Scheme 6: Formation of pyridotriazepines 15-18

The IR spectrum of compound 15 exhibited absorption bands at 2180, 2191 and 2202 cm^{-1} attributed to 3C≡N functions. While, its ^{1}H-NMR spectrum showed a characteristic an exchangeable singlet signals at δ=1.66 3.71, 8.10, 9.90 ppm attributed to SCH_3, NH_2 and 2NH protons, respectively. While, the structure of compound 16 supported from its mass spectrum which showed the molecular ion peak at *m/z* 558 ($M^{+\cdot}$, 2.53%).

The IR spectrum of compound 17 exhibited absorption bands for 2C=O and 2NH. While, that of compound 18 showed absorption band a corresponding to 3C=O functions. Also, the ^{1}H-NMR spectrum of compound 17 showed signals at δ=7.96 and 9.95 attributed to pyrazole-H2 and triazepine-H4 respectively. Whereas, that of compound 18 exhibited exchangeable signals at δ=3.46, 8.20 ppm assigned to NH_2 and OH protons.

Treating of diaminopyridone 2 with 4-chlorobenzylidene malononitrile in DMF containing few drops of triethylamine gave 2-amino-4-(4-chlorophenyl)-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydro-pyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1,7-dihydro- pyrido[1,2-*b*] [1,2,4]triazepine-3,8,10-tricarbonitrile (19). Also, cyclocondensation of compound 2 with ethyl 3-(4-chlorophenyl)-2-cyanoprop-2-enoate under the same reaction conditions yielded ethyl 2-amino-4-(4-chloro-phenyl)-8,10-dicyano-7-oxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1,7-dihydro -pyrido[1,2-*b*][1,2,4] triazepine-3-carboxylate (21) and not the other possible product 4-(4-chlorophenyl)-2,7-dioxo-9-(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*]pyrimidin-3-yl)-1,2,5,7-tetrahydropyrido[1,2-*b*][1,2,4]triazepine-3,8,10-tricarbonitrile (20) (Scheme 7).



Scheme 7: Formation of pyridotriazepines 19-21

The IR spectrum of compound 19 exhibited absorption bands attributed to 3C≡N functions. While, that of compound 21 showed absorption bands at corresponding to 2C≡N functions. Also, the ^{1}H-NMR spectrum of compound 21 was characterized by the appearance of CH_3 and CH_2 protons of ethoxycarbonyl group and singlet signals at δ=4.23, 8.21 and 8.52 ppm attributed to NH_2 and 2 NH protons respectively. Moreover, the structure of compound 21 was also supported from its mass spectrum which showed the molecular ion peak at *m/z* 652 (7.33%) which agree well with the molecular weight and supports the identity of the structure [35,36].

Biological activities

The standardized disc agar diffusion method was followed to determine the activity of the synthesized compounds against the sensitive organisms *Staphylococcus aureus* and *Bacillus subtilis* as Gram-positive bacteria, *Salmonella typhimurium* and *Escherichia coli* as Gram-negative bacteria and *Candida albicans and Aspergillus fumigatus* as yeasts and fungus strain. The results depicted in Table 1, showed various activities against all species of microorganisms which suggest that the variations in the structures effect on the growth of the microorganisms. Thus, we can conclude from these results that, some of the prepared compounds showed a low to high antimicrobial activity towards Gram positive bacteria, Gram -negative bacteria, Yeasts and the fungal strain (Table 1).

Table 1: The antimicrobial activity of the synthesized compounds

Organism	Mean* of zone diameter, nearest whole mm											
	Gram-positive bacteria				Gram-negative bacteria				Yeasts and fungi**			
	Staphylococcus aureus (ATCC 25923)		*Bacillus subtilis* (ATCC 6635)		*Salmonella typhimurium* (ATCC 14028)		*Escherichia coli* (ATCC 25922)		*Candida albicans* (ATCC 10231)		*Aspergillus fumigatus*	
Concentration / Sample	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml
2	-	-	-	-	16	14	-	-	21	16	-	-
3	10	6	-	-	9	10	-	-	12	7	-	-
4	9	7	14	20	-	-	16	11	-	-	-	-
5	14	11	8	7	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	14	12	-	-	-	-
7	-	-	-	-	-	-	-	-	21	17	-	-
8	15	14	-	-	-	-	-	-	-	-	17	11
9	-	-	9	7	-	-	-	-	28	14	-	-
10	10	7	-	-	14	12	-	-	15	13	-	-
11	9	6	-	-	15	19	-	-	-	-	18	12
12	11	7	8	7	-	-	-	-	14	10	-	-
13	16	10	-	-	13	14	-	-	-	-	9	10
14	-	-	-	-	23	18	-	-	-	-	-	-
15	11	18	10	7	-	-	20	1	-	-	-	-
16	14	12	9	7	-	-	-	-	14	11	-	-
17	17	14	-	-	10	8	-	-	-	-	19	15
18	-	-	25	13	-	-	-	-	24	11	10	7
19	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	17	14	-	-
Control #	35	26	35	25	36	28	38	27	35	28	27	26

*=Calculate from 3 values; **=Identified on the basis of routine cultural, morphological and microscopical characteristics; - = No effect; Low activity=Mean of zone diameter ≤ 1/3 of mean zone diameter of control; Intermediate activity=Mean of zone diameter ≤ 2/3 of mean zone diameter of control; High activity=Mean of zone diameter > 2/3 of mean zone diameter of control; #: Chloramphenicol in the case of Gram-positive bacteria, cephalothin in the case of Gram-negative bacteria and cycloheximide in the case of fungi

CONCLUSIONS

In the present work, a novel series of polynuclear triazolo[1,5-*a*] pyridines 3-6, pyrido[1',2':2,3] triazolo[1,5-*c*]quinazolines 7,8, pyrido[1,2-*b*][1,2,4]triazines 9,14 and pyrido[1,2-*b*][1,2,4]triazipines 15-21 were efficiently synthesized *via* the key intermediate 1,6-Diamino-2-oxo-4-[(10-oxo-4,6,7,8,9,10-hexahydropyrazolo[1,5-*a*][1]benzothieno[2,3-*d*] pyrimidin-3-yl)-1,2-dihydropyridine-3,5-dicarbonitrile (2). The synthesized compounds were screened for their antimicrobial activity.

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