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Elaboration and Evaluation of Diverse Fused Pyrano[2,3-*d*]pyrimidinones: Synthesis, Characterization, and Antimicrobial Efficacy Assessment

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Abstract

Herein, a facile metal-free approach for the annellation of a pyran ring to thiobarbituric acid was described, and pyrano[2,3-*d*]pyrimidine (**2**) was obtained in a good yield. Subsequently, the valuable bicyclic pyranopyrimidine derivative (**2**) was utilized as a versatile building block for constructing assorted and hitherto unreported heterocyclic compounds. The reactivity of various carbon and nitrogen nucleophiles toward pyranopyrimidine derivative (**2**) was thoroughly investigated, leading to the synthesis of novel heterocycles with promising antimicrobial activity. Comprehensive characterization of the proposed structures of the newly synthesized fused pyranopyrimidine derivatives was carried out using IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. Finally, the developed heterocycles were assessed in vitro for their antibacterial activity against selected bacterial strains. Additionally, the relationship between activity and structure was discussed. The results revealed very promising antibacterial properties for the majority of the synthesized heterocycles, indicating their potential as effective antibacterial agents.

Keywords: Pyrano[2,3-*d*]pyrimidine; Thiobarbituric acid; Knoevenagel condensation; Dimroth rearrangement; Antimicrobial

1. Introduction

Pyrano[2,3-*d*]pyrimidine analogs represent a significant class of heterocyclic compounds that display a broad scope of biological activities, making them privileged scaffolds in drug discovery and medicinal chemistry [1-3]. Furthermore, the annellation of a pyrimidine heterocycle in which the two nitrogen atoms of pyrimidine are located at the first and third positions of a pyran ring with an oxygen atom at the eighth position conferred pyrano[2,3-*d*]pyrimidine derivatives prominent merits in synthetic chemistry.

The diverse pharmacological activities and synthetic accessibility of pyrano[2,3-*d*]pyrimidinones have made them attractive targets for more studies. Pyrano[2,3-*d*]pyrimidines are powerful inhibitors of various enzymes that play crucial roles in cancer cell growth and proliferation, such as tyrosine kinases and topoisomerases [1, 4]. Additionally, they have been found to possess anti-inflammatory [5], antimicrobial, antibacterial, and anticancer properties [6-9], making them very appealing as potential therapeutic agents. Additionally, they exhibited antioxidant [10], antidiabetic [11], and cardiotonic [12] activities. Examples of some biologically active pyrano[2,3-*d*]pyrimidinones are presented in Figure 1.

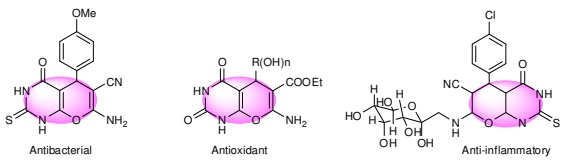

Figure 1: Examples of some biologically active pyrano[2,3-*d*]pyrimidinones.

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Moreover, pyrano[2,3-*d*]pyrimidinones possess potent inhibitory activity for the Hedgehog (Hh) signaling pathway [13], α-amylase, and α-glucosidase enzymes [14].

In the span of the preceding years, multidrug resistant (MDR) pathogens have emerged as one of the most conspicuous threats to public health. They are associated with community-acquired and nosocomial infections [15]. Thus, the World Health Organization (WHO) has identified antimicrobial resistance as one of the top 10 global public health threats endangering humanity [16]. Likewise, the Centers for Disease Control and Prevention (CDC) recognized multidrug resistant (MDR) pathogens as an urgent global public health threat that is directly connected to approximately 5 million fatalities worldwide per year [17]. Accordingly, without new classes of antibiotics to combat drug-resistant microbes, fatalities are expected to reach 10 million per year by 2050 [18]. Worldwide, there is a profound concern that the prevention and treatment of infectious diseases may be seriously jeopardized if new antibiotics capable of combating multidrug resistant pathogens are not integrated into clinical use.

Motivated by the aforementioned facts, diversified fused pyrazolo[3,4-*d*]pyrimidines were synthesized, aiming to promote their synthetic potential and screen their related antibacterial activity.

2. Experimental

***Synthesis of 7-amino-5-(benzo[d][1,3]dioxol-5-yl)-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d] pyrimidine -6-carbonitrile (2)* [19].**

Method A: An equimolar mixture of arylidene malononitrile **1** (1.98 g, 0.01 mol) and thiobarbituric acid (1.44 g, 0.01 mol) was heated under reflux for 8 hours in ethanol (30 mL) containing a few drops of triethylamine. After cooling, the produced precipitate was removed, dried, and recrystallized from ethanol as yellow crystals in 43% yield.

Method B: A mixture of equimolar amounts of arylidene malononitrile **1** (1.98 g, 0.01 mol) and thiobarbituric acid (1.44 g, 0.01 mol) was refluxed for 4 hours in sodium ethoxide solution (0.23 g of sodium metal (0.01 atom) in 25 mL absolute ethanol). To neutralize the reaction mixture, hydrochloric acid (2.0 N, 3.0 mL) was added after it had cooled and poured onto crushed ice. The product was obtained in 84% yield.

Method C: In 30 mL of ethanol containing a few drops of triethylamine, a mixture of piperonal (1.5 g, 0.01 mol), thiobarbituric acid (1.44 g, 0.01 mol), and malononitrile (0.66 g, 0.01 mol) was heated under reflux for 12 hours before cooling to room temperature. The product was obtained in 52% yield.

Synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-6-hydroxy-2-thioxo-1,2,3,5-tetrahydro-4H-pyrano[2,3-d:6,5-d']dipyrimidin-4-one (3).

A mixture of formic acid (40 mL) and pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) was refluxed for 24 h. After cooling, the crude solid product that precipitated out was filtered, dried, and recrystallized from ethanol as orange crystals in 82% yield; m.p. 314 °C; IR (KBr, cm^{-1}): 3421 (OH), 3216, 3170 (2NH), 3050 (CH aromatic), 2919 (CH aliphatic), 1654 (C=O), 1253 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 5.91 (s, 2H, 1,3-dioxole), 6.69 (s, 1H, methine), 7.13-7.21 (m, 3H, Ar-H), 8.98 (s, 1H, imine), 10.43, 11.20 (s, 2H, 2NH, D_2O-exchangeable), 12.43 (s, 1H, OH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 370 (M^+, 19.61), 369 (M^+-1, 12.44), 276 (100); Anal. cald for $C_{16}H_{10}N_4O_5S$ (370): C, 51.89; H, 2.72; N, 15.13; S, 8.66. Found: C, 52.11; H, 2.97; N, 15.05; S, 8.63.

Synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-8-methyl-2-thioxo-2,3,5,9-tetrahydro-4H-pyrano[2,3-d:6,5-d']dipyrimidine-4,6(1H)-dione (4).

Method A: A mixture of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) and acetic anhydride (5.0 mL) in glacial acetic acid (20 mL) was heated under reflux for 4 h. After cooling down, the produced precipitate was filtered and recrystallized using ethanol. The product was obtained as brown crystals in 79% yield.

Method B: Compound **2** (3.42 g, 0.01 mol) and acetyl chloride (0.7 mL, 0.01 mol) was heated under reflux in dioxane (30 mL) for 12 h. After cooling, the crude solid product that separated off was recovered by filtration, dried, and recrystallized from ethanol, resulting in the formation of brown crystals in 48% yield; m.p. 294 °C; IR (KBr, cm^{-1}): 3228, 3162 (3NH), 3050 (CH aromatic), 2900 (CH aliphatic), 1670 (C=O), 1253 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 1.91 (s, 3H, CH_3), 6.14 (s, 1H, methine), 6.20 (s, 2H, 1,3-dioxole), 7.04-7.10 (m, 3H, Ar-H), 8.29, 12.30, 12.39 (s, 3H, 3NH, D_2O-

exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz, ppm): δ: 21.5, 49.07, 96.45, 100.95, 102.79, 106.78, 109.06, 128.79, 129.03, 133.24, 145.07, 147.39, 148.81, 153.24, 163.52, 169.16, 173.24; MS (70 eV) *m/z* (%):385 (M^++1, 3.65), 384 (M^+, 9.44), 57 (100); Anal. cald for $C_{17}H_{12}N_4O_5S$ (384): C, 53.12; H, 3.15; N, 14.58; S, 8.34. Found: C, 53.01; H, 3.27; N, 14.45; S, 8.43.

Synthesis of 6-amino-5-(benzo[d][1,3]dioxol-5-yl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrano[2,3-d:6,5-d']dipyrimidin-4-one (5).

A formamide (30 mL) and pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) mixture was refluxed for 18 hours. After cooling, the reaction was poured onto crushed ice. The resulting product was then collected by filtration and recrystallized using acetic acid resulting in the formation of dark brown crystals with a yield of 78%; m.p. >360 °C; IR (KBr, cm^{-1}): 3367, 3170 (NH_2, 2NH), 3081 (CH aromatic), 2923 (CH aliphatic), 1658 (C=O), 1295 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 5.93 (s, 2H, 1,3-dioxole), 6.48 (s, 1H, methine), 6.82 (s, 2H, NH_2, D_2O-exchangeable), 7.92-8.00 (m, 3H, Ar-H), 8.82 (s, 1H, imine), 9.97, 13.04 (s, 2H, 2NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 370 (M^++1, 23.29), 369 (M^+, 100), 368 (M^+-1, 60.70); Anal. cald for $C_{16}H_{11}N_5O_4S$ (369): C, 52.03; H, 3.00; N, 18.96; S, 8.68. Found: C, 52.11; H, 2.87; N, 18.55; S, 8.83.

Synthesis of ethyl (E)-N-(5-(benzo[d][1,3]dioxol-5-yl)-6-cyano-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidin-7-yl)formimidate (6).

Triethyl orthoformate (10 mL) was added to a solution of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) in absolute ethanol (30 mL). Then, with continuous stirring, the reaction mixture was heated under reflux for 8 hours. Upon cooling to ambient temperature, the separated solid was filtered off, dried, and subsequently recrystallized from methanol, resulting in the formation of brown crystals with a yield of 79%; m.p. 205 °C; IR (KBr, cm^{-1}): 3355, 3174 (2NH), 3045 (CH aromatic), 2923 (CH aliphatic), 2213 (C≡N), 1643 (C=O), 1257 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 1.22 (t, J = 7.2, 3H, CH_3), 3.76 (q, J = 7.1, 2H, CH_2), 6.01 (s, 2H, 1,3-dioxole), 6.09 (s, 1H, methine), 6.48-6.61 (m, 3H, Ar-H), 7.26 (s, 1H, imidate), 9.53, 12.28 (s, 2H, 2NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 398 (M^+, 0.8), 395 (M^+-3, 2.09), 294 (100); Anal. Calcd for $C_{18}H_{14}N_4O_5S$ (398): C, 54.27; H, 3.54; N, 14.06; S, 8.05. Found: C, 54.48; H, 3.68; N, 13.97; S, 8.16.

Synthesis of 7-amino-5-(benzo[d][1,3]dioxol-5-yl)-6-imino-2-thioxo-1,2,3,5,6,7-hexahydro-4H-pyrano[2,3-d:6,5-d']dipyrimidin-4-one (7).

To a solution of formimidate derivative **6** (3.98 g, 0.01 mol) in absolute ethanol (40 mL), hydrazine hydrate (3.0 mL, excess) was added, and then the reaction was heated under reflux for 8 hours. The reaction mixture was placed onto ice-cold water after it had cooled. The resulting product was separated through filtration, dried, and subsequently recrystallized from ethanol, resulting in the formation of brown crystals with a yield of 59%; m.p. 269 °C; IR (KBr, cm^{-1}): 3351, 3332, 3212 (NH_2, 3NH), 3055 (CH aromatic), 2923 (CH aliphatic), 1697 (C=O), 1249 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 4.98 (s, 2H, NH_2, D_2O-exchangeable), 6.00 (s, 1H, methine), 6.13 (s, 2H, 1,3-dioxole), 6.76-6.93 (m, 3H, Ar-H), 8.31 (s, 1H, imine), 9.48, 12.07, 12.39 (s, 3H, 3NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 385 (M^++1, 20.18), 384 (M^+, 24.38), 43 (100); Anal. Calcd for $C_{16}H_{12}N_6O_4S$ (384): C, 50.00; H, 3.15; N, 21.86; S, 8.34. Found: C, 50.28; H, 3.08; N, 21.93; S, 8.50.

Synthesis of 1-(5-(benzo[d][1,3]dioxol-5-yl)-6-cyano-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidin-7-yl)-3-phenylthiourea (8).

Pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) and phenyl isothiocyanate (1.2 mL, 0.01 mol) were mixed together and subjected to reflux for 12 hours. Thereafter, the reaction was cooled down to ambient temperature and triturated with ethanol. The separated solid product was recovered through filtration, after which it was dried and subsequently recrystallized from dioxane, resulting in the formation of brown crystals with a yield of 76%; m.p. 213 °C; IR (KBr, cm^{-1}): 3367, 3214 (4NH), 3065 (CH aromatic), 2919 (CH aliphatic), 2213 (C≡N), 1631 (C=O), 1249 (C=S); MS (70 eV) *m/z* (%): 478 (M^++1, 2.19), 77 (100); Anal. cald for $C_{22}H_{15}N_5O_4S_2$ (477): C, 55.34; H, 3.17; N, 14.67; S, 13.43. Found: C, 55.22; H, 3.32; N, 14.59; S, 13.52.

Synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-3-phenyl-2,8-dithioxo-2,3,5,7,8,9-hexahydro-4H-pyrano[2,3-d:6,5-d']dipyrimidine-4,6(1H)-dione (9).

Phenylthiourea derivative **8** (4.77 g, 0.01 mol) was dissolved in 20 mL of absolute pyridine and refluxed for 12 hours. The solution was cooled, and the separated precipitate was then collected *via* filtration, dried, and recrystallized from ethanol, resulting in the formation of brown crystals with a yield of 80%; m.p.

245 °C; IR (KBr, cm^{-1}): 3359, 3193 (3NH), 3045 (CH aromatic), 2919 (CH aliphatic), 1635 (C=O), 1249 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 6.14 (s, 2H, 1,3-dioxole), 6.18 (s, 1H, methine), 6.72-6.78 (m, 3H, Ar-H), 7.73-7.78 (m, 5H, Ar-H), 9.81, 10.61, 12.58 (s, 3H, 3NH, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz, ppm): δ: 49.07, 87.42, 96.43, 100.93, 102.98, 106.77, 108.89, 112.96, 116.44, 119.73, 127.37, 129.02, 131.97, 134.87, 145.04, 147.89, 152.93, 156.45, 160.44, 162.58, 173.26, 178.71. MS (70 eV) *m/z* (%): 478 (M^+, 13.50), 476 (M^+-2, 20.43), 206 (100); Anal. cald for $C_{22}H_{14}N_4O_5S_2$ (478): C, 55.22; H, 2.95; N, 11.71; S, 13.40. Found: C, 55.08; H, 3.11; N, 11.58; S, 13.56.

Synthesis of (5-(benzo[d][1,3]dioxol-5-yl)-6-cyano-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidin-7-yl)carbamodithioic acid (10).

Pyranopyrimidine derivative **2** (3.42 g, 0.01 mol), carbon disulfide (1.0 mL), and potassium hydroxide (0.56 g, 0.01 mol) were mixed together in ethanol (20 mL). The mixture was then heated in a water bath for 8 hours. Subsequently, it was allowed to cool and poured into crushed ice, and dilute hydrochloric acid was used to neutralize the reaction mixture. The resulting solid product was obtained, dried, and ethanol was used in recrystallizing, resulting in the formation of yellow crystals with a yield of 63%; m.p. 320 °C; IR (KBr, cm^{-1}): 3355, 3234, 3150 (3NH), 3078 (CH aromatic), 2919 (CH aliphatic), 2217 (C≡N), 1643 (C=O), 1249 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 5.95 (s, 2H, 1,3-dioxole), 6.05 (s, 1H, methine), 6.15 (s, 1H, SH), 6.79-6.90 (m, 3H, Ar-H), 10.76, 11.49, 12.49 (s, 3H, 3NH, D_2O-exchangeable); Anal. cald for $C_{16}H_{10}N_4O_4S_3$ (418): C, 45.92; H, 2.41; N, 13.39; S, 22.98. Found: C, 46.21; H, 2.31; N, 13.43; S, 23.11.

Synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-2,6,8-trithioxo-1,2,3,5,6,7,8,9-octahydro-4H-pyrano[2,3-d:6,5-d']dipyrimidin-4-one (11).

Method A: Carbamodithioic acid derivative **10** (4.18 g, 0.01 mol) was heated under reflux for 8 hours with sodium ethoxide solution (0.23 g of sodium metal (0.01 atom) in 25 mL absolute ethanol). After the reaction had cooled, it was poured into crushed ice, and the medium was acidified *via* hydrochloric acid (2.0 N, 3.0 mL). The resulting product was separated through filtration, dried, and subsequently recrystallized from ethanol, resulting in the formation of orange crystals with a yield of 78%.

Method B: A mixture of carbon disulfide (2.0 mL) in pyridine (20 mL) and pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) was heated in a water bath for 24 hours. After cooling and being poured over ice, hydrochloric acid was used to neutralize the reaction mixture. The resulting solid product was collected *via* filtration, dried, and recrystallized from ethanol, resulting in the formation of orange crystals with a yield of 81%; m.p. 230 °C; IR (KBr, cm^{-1}): 3413, 3224, 3178 (4NH), 3089 (CH aromatic), 2946 (CH aliphatic), 1662 (C=O), 1253 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 5.50 (s, 1H, methine), 5.90 (s, 2H, 1,3-dioxole), 6.40-6.54 (m, 3H, Ar-H), 10.81, 11.55, 12.06, 13.27 (s, 4H, 4NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 419 (M^++1, 13.86), 418 (M^+, 37.34), 417 (M^+-1, 28.92), 180 (100); Anal. cald for $C_{16}H_{10}N_4O_4S_3$ (418): C, 45.92; H, 2.41; N, 13.39; S, 22.99. Found: C, 46.11; H, 2.37; N, 13.55; S, 23.08.

Synthesis of 6-amino-5-(benzo[d][1,3]dioxol-5-yl)-2,8-dithioxo-1,2,3,5,8,9-hexahydro-4H-pyrano[2,3-d:6,5-d']dipyrimidin-4-one (12).

Equimolar amounts of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) and thiourea (0.76 g, 0.01 mol) were added to glacial acetic acid (20 mL) containing HCl (1.0 mL) and the reaction was refluxed for 12 hours. The mixture was poured into cold water and neutralized with an ammonia solution once it had cooled to ambient temperature. The precipitate was filtered out, dried, and recrystallized from methanol, resulting in the formation of orange crystals with a 71% yield; m.p. 253 °C; IR (KBr, cm^{-1}): 3417, 3228, 3162 (NH_2, 3NH), 3080 (CH aromatic), 2908 (CH aliphatic), 1670 (C=O), 1249 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 6.01 (s, 1H, methine), 6.04 (s, 2H, 1,3-dioxole), 6.82-6.94 (m, 3H, Ar-H), 7.12 (s, 2H, NH_2, D_2O-exchangeable), 8.59, 8.77, 9.81 (s, 3H, 3NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 401 (M^+, 35.16), 397 (M^+-4, 39.27), 127 (100); Anal. cald for $C_{16}H_{11}N_5O_4S_2$ (401): C, 47.87; H, 2.76; N, 17.45; S, 15.98. Found: C, 48.01; H, 2.87; N, 17.39; S, 15.83.

Synthesis of 6,8-diamino-5-(benzo[d][1,3]dioxol-5-yl)-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2H-pyrido[3',2':5,6]pyrano[2,3-d]pyrimidine-7-carbonitrile (13).

To a solution of malononitrile (0.66 g, 0.01 mol) in absolute ethanol (40 mL) containing a few drops of piperidine, pyranopyrimidine derivative **2** (3.42 g,

0.01 mol) was placed, and the reaction was allowed to reflux for 12 hours. After the reaction mixture cooled to room temperature, the separated solid product was extracted by filtration and dried. Recrystallization took place using methanol and the product was acquired as gray crystals in 65% yield; m.p. 304 °C; IR (KBr, cm^{-1}): 3413, 3336, 3243 ($2NH_2$, 2NH), 3054 (CH aromatic), 2915 (CH aliphatic), 2213 (C≡N), 1650 (C=O), 1257 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 3.59 (s, 2H, NH_2, D_2O-exchangeable), 5.16 (s, 2H, NH_2, D_2O-exchangeable), 5.89 (s, 2H, 1,3-dioxole), 6.59 (s, 1H, methine), 7.00-7.14 (m, 3H, Ar-H), 10.41, 10.46 (s, 2H, 2NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 410 (M^++2, 27.45), 408 (M^+, 68.31), 164 (100); Anal. Calcd for $C_{18}H_{12}N_6O_4S$ (408): C, 52.94; H, 2.96; N, 20.58; S, 7.85. Found: C, 52.83; H, 2.67; N, 20.65; S, 7.71.

Synthesis of ethyl 6-amino-5-(benzo[d][1,3]dioxol-5-yl)-4,8-dioxo-2-thioxo-1,3,4,5,8,9-hexahydro-2H-pyrido[3',2':5,6]pyrano[2,3-d]pyrimidine-7-carboxylate (14).

To a solution of diethyl malonate (1.52 mL, 0.01 mol) in absolute ethanol (30 mL) containing a few drops of piperidine, pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) was placed, and the reaction was refluxed for 12 hours. After cooling to room temperature, the separated solid was filtered and dried. Ethanol was used in recrystallization, resulting in the formation of brown crystals in 80% yield; m.p. 271 °C; IR (KBr, cm^{-1}): 3436, 3208, 3118 (NH_2, 3NH), 3097 (CH aromatic), 2923 (CH aliphatic), 1727 (C=O, ester carbonyl group), 1682, 1666 (2C=O, cyclic amide), 1253 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 1.24 (t, J = 7.3, 3H, CH_3), 3.65 (q, J = 7.1, 2H, CH_2), 6.02 (s, 2H, 1,3-dioxole), 6.18 (s, 1H, methine), 6.57-6.71 (m, 3H, Ar-H), 6.92 (s, 2H, NH_2, D_2O-exchangeable), 9.82, 10.49, 12.79 (s, 3H, 3NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 456 (M^+, 33.15), 275 (100); Anal. Calcd for $C_{20}H_{16}N_4O_7S$ (456): C, 52.63; H, 3.53; N, 12.28; S, 7.03. Found: C, 52.44; H, 3.67; N, 12.05; S, 7.21.

Synthesis of 11-(benzo[d][1,3]dioxol-5-yl)-8-thioxo-1,2,7,8,9,11-hexahydropyrazolo[3'',4'':4',5']pyrido[3',2':5,6]pyrano[2,3-d]pyrimidine-3,4,10(5H)-trione (15).

Hydrazine hydrate (3.0 mL, excess) was added to a solution of pyranopyrimidine carboxylate derivative **14** (4.56 g, 0.01 mol) in absolute ethanol (40 mL) and refluxed for 18 hours. The reaction mixture was poured into ice-cold water after it had cooled. The separated solid product was recovered through filtration, after which it was dried and subsequently recrystallized from dioxane, resulting in the formation of brown crystals with a yield of 80%; m.p. 289 °C; IR (KBr, cm^{-1}): 3158, 3118 (5NH), 3065 (CH aromatic), 2962 (CH aliphatic), 1697 (C=O), 1241 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 5.92 (s, 2H, 1,3-dioxole), 6.49 (s, 1H, methine), 6.64-6.71 (m, 3H, Ar-H), 7.54, 7.79, 9.81, 11.56, 12.39 (s, 5H, 5NH, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz, ppm): δ: 41.85, 89.04, 97.58, 99.62, 102.22, 108.94, 109.59, 115.88, 116.72, 122.92, 128.53, 147.67, 149.28, 154.92, 157.14, 160.35, 162.53, 191.50. MS (70 eV) *m/z* (%): 425 (M^+, 42.47), 423 (M^+-2, 7.60), 42 (100); Anal. Calcd for $C_{18}H_{11}N_5O_6S$ (425): C, 50.83; H, 2.61; N, 16.46; S, 7.54. Found: C, 50.22; H, 2.68; N, 16.74; S, 7.50.

Synthesis of ethyl 6-amino-5-(benzo[d][1,3]dioxol-5-yl)-4-oxo-2-thioxo-1,2,3,4,5,8-hexahydropyrrolo[3',2':5,6]pyrano[2,3-d]pyrimidine-7-carboxylate (16).

To a solution of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) in dry acetone (40 mL) containing anhydrous potassium carbonate (1.38 g, 0.01 mol), ethyl bromoacetate (1.10 mL, 0.01 mol) was added, and the reaction was refluxed with continuous stirring for 10 hours. The reaction was brought to ambient temperature and poured into ice-cold water, and then the separated solid was filtered. After being dried, washed with water, and crystallized from ethanol, yellow crystals were separated in 69% yield; m.p. 220 °C; IR (KBr, cm^{-1}): 3436, 3355, 3205 (NH_2, 3NH), 3048 (CH aromatic), 2923 (CH aliphatic), 1731 (C=O, ester carbonyl group), 1666 (C=O, cyclic amide), 1253 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 1.29 (t, J = 7.3, 3H, CH_3), 4.44 (q, J = 7.1, 2H, CH_2), 5.99 (s, 2H, 1,3-dioxole), 6.58 (s, 2H, NH_2, D_2O-exchangeable), 6.61 (s, 1H, methine), 6.78-6.88 (m, 3H, Ar-H), 10.57, 10.84, 11.20 (s, 3H, 3NH, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz, ppm): δ: 21.44, 49.06, 55.19, 83.85, 91.13, 95.66, 102.26, 108.97, 109.32, 115.57, 123.36, 128.01, 132.42, 137.39, 147.78, 149.34, 160.78, 161.63, 166.24; MS (70 eV) *m/z* (%): 428 (M^+, 2.99), 294 (100); Anal. Calcd for $C_{19}H_{16}N_4O_6S$ (428): C, 53.27; H, 3.76; N, 13.08; S, 7.48. Found: C, 53.34; H, 3.87; N, 13.25; S, 7.24.

Synthesis of 6-amino-5-(benzo[d][1,3]dioxol-5-yl)-7-benzoyl-2-thioxo-2,3,5,8-tetrahydropyrrolo[3',2':5,6]pyrano[2,3-d]pyrimidin-4(1H)-one (17).

Phenacyl bromide (1.99 g, 0.01 mol) was added to a solution of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) in dry acetone (40 mL) containing anhydrous K_2CO_3 (1.38 g, 0.01 mol). The reaction was refluxed with continuous stirring for 12 hours and then brought to ambient temperature and poured into ice-cold water. Finally, the solid precipitate was filtered, dried, washed with water, and crystallized from ethanol. Yellow crystals were separated in 83% yield; m.p. 168 °C; IR (KBr, cm^{-1}): 3444, 3355, 3239 (NH_2, 3NH), 3027 (CH aromatic), 2919 (CH aliphatic), 1727 (C=O, COPh), 1697 (C=O, cyclic amide), 1249 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 5.91 (s, 2H, 1,3-dioxole), 6.49 (s, 2H, NH_2, D_2O-exchangeable), 6.69 (s, 1H, methine), 6.94-7.05 (m, 3H, Ar-H), 7.34-7.40 (m, 5H, Ar-H), 10.61, 11.48, 12.36 (s, 3H, 3NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 462 (M^++2, 17.91), 460 (M^+, 36.18), 459 (M^+-1, 21.58), 119 (100); Anal. Calcd for $C_{23}H_{16}N_4O_5S$ (460): C, 59.99; H, 3.50; N, 12.17; S, 6.96. Found: C, 59.82; H, 3.67; N, 12.29; S, 7.14.

Synthesis of (5-(benzo[d][1,3]dioxol-5-yl)-6-cyano-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidin-7-yl)glycinoyl chloride (18).

Method A: To a solution of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) in dioxane (30 mL) containing a few drops of piperidine, chloroacetyl chloride (0.79 mL, 0.01 mol) was added, and the reaction mixture was refluxed with continuous stirring for 4 hours. Thereafter, the reaction was brought to ambient temperature and poured onto ice-cold water. The solid precipitate was collected by filtration, washed with water and dried. A mixture of *N*,*N*-dimethylformamide:ethanol (1:1) was used in recrystallization, resulting in the formation of brown crystals with a yield of 76%.

Method B: To a solution of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) in dry acetone (40 mL) containing anhydrous K_2CO_3 (1.38 g, 0.01 mol), chloroacetyl chloride (0.79 mL, 0.01 mol) was added, and the reaction mixture was refluxed with continuous stirring for 10 hours. The reaction was brought to room temperature and poured into crushed ice, and then the precipitate was collected by filtration. After being dried, the solid precipitate was washed with water, and a mixture of *N*,*N*-dimethylformamide:ethanol (1:1) was used in recrystallization, resulting in the formation of brown crystals in 69% yield; m.p. 238-240 °C; IR (KBr, cm^{-1}): 3355, 3235 (3NH), 3038 (CH aromatic), 2923 (CH aliphatic), 2213 (C≡N), 1727 (COCl), 1643 (C=O, cyclic amide), 1249 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 4.26 (s, 2H, CH_2), 5.89 (s, 2H, 1,3-dioxole), 5.95 (s, 1H, methine), 6.74-6.81 (m, 3H, Ar-H), 7.45, 9.56, 11.21 (s, 3H, 3NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 420.5 (M^++2, 3.61), 418.5 (M^+, 8.62), 120 (100); Anal. Calcd for $C_{17}H_{11}ClN_4O_5S$ (418.5): C, 48.75; H, 2.65; Cl, 8.47; N, 13.38; S, 7.66. Found: C, 49.02; H, 2.57; Cl, 8.55; N, 13.29; S, 7.80.

Synthesis of 5-(benzo[d][1,3]dioxol-5-yl)-8-phenyl-2-thioxo-2,3,5,7-tetrahydro-4H-pyrano[2,3-d:6,5-d']dipyrimidine-4,6(1H)-dione (20).

To a solution of pyranopyrimidine derivative 2 (3.42 g, 0.01 mol) in dry acetone (40 mL) containing anhydrous K_2CO_3 (1.38 g, 0.01 mol), benzoyl chloride (1.2 mL, 0.01 mol) was added, and the mixture was refluxed with continuous stirring for 15 hours. Thereafter, the mixture was brought to ambient temperature and poured onto ice-cold water. The formed precipitate was collected by filtration, washed with water and dried. Acetic acid was used for recrystallizing, resulting in the formation of yellow crystals with a yield of 89%; m.p. >360 °C; IR (KBr, cm^{-1}): 3343, 3197 (3NH), 3050 (CH aromatic), 2919 (CH aliphatic), 1689, 1670 (2C=O, cyclic amide), 1253 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 5.84 (s, 2H, 1,3-dioxole), 6.15 (s, 1H, methine), 6.70-6.82 (m, 3H, Ar-H), 7.80-7.95 (m, 5H, Ar-H), 9.47, 11.22, 12.05 (s, 3H, 3NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 446 (M^+, 25.60), 441 (M^+-5, 21.55), 55 (100); Anal. Calcd for $C_{22}H_{14}N_4O_5S$ (446): C, 59.19; H, 3.16; N, 12.55; S, 7.18. Found: C, 59.30; H, 3.22; N, 12.47; S, 7.25.

Synthesis of 3-amino-4-(benzo[d][1,3]dioxol-5-yl)-7-thioxo-4,6,7,8-tetrahydro-pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5(1H)-one (21).

Method A: To a solution of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) in glacial acetic acid (30 mL) containing anhydrous sodium acetate (0.2 g), hydroxylamine hydrochloride (0.695 g, 0.01 mol) was added, and the reaction mixture was refluxed for 12 hours. Subsequently, the mixture was allowed to

cool down to ambient temperature and then poured into ice-cold water. The separated crude product was filtered, washed with water, dried, and recrystallized from methanol, resulting in the formation of yellow crystals with a yield of 88%.

Method B: Hydrazine hydrate (5.0 mL, excess) was added to a solution of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) in absolute ethanol (20 mL) and the reaction was refluxed for 6 hours. Thereafter, the reaction mixture was placed into ice-cold water after it had cooled. The separated crude product was filtered, dried, and recrystallized from methanol, resulting in the formation of colorless crystals with a 92% yield; m.p. > 360 °C; IR (KBr, cm^{-1}): 3424, 3313 (NH_2, 3NH), 3035 (CH aromatic), 2888 (CH aliphatic), 1654 (C=O, cyclic amide), 1253 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 4.74 (s, 2H, NH_2, D_2O-exchangeable), 5.96 (s, 2H, 1,3-dioxole), 6.52 (s, 1H, methine), 6.93-7.16 (m, 3H, Ar-H), 8.07, 9.57, 11.71 (s, 3H, 3NH, D_2O-exchangeable); MS (70 eV) *m/z* (%): 359 (M^++2, 11.85), 357 (M^+, 37.22), 65 (100); Anal. Calcd for $C_{15}H_{11}N_5O_4S$ (357): C, 50.42; H, 3.10; N, 19.60; S, 8.97. Found: C, 50.35; H, 3.18; N, 19.49; S, 8.85.

Synthesis of methyl 9-amino-7-(benzo[d][1,3]dioxol-5-yl)-8-cyano-4,6-dioxo-4H,6H,7H-pyrano[2',3':4,5]pyrimido[2,1-b][1,3]thiazine-2-carboxylate (22).

Dimethyl acetylenedicarboxylate (1.22 mL, 0.01 mol) was added to a solution of pyranopyrimidine derivative **2** (3.42 g, 0.01 mol) in ethanol (30 mL) and the reaction was refluxed for 12 h. The separated crude product was filtered, dried, and recrystallized from methanol, resulting in the formation of red crystals with a 70% yield; m.p. 258 °C; IR (KBr, cm^{-1}): 3440, 3336 (NH_2), 3056 (CH aromatic), 2919 (CH aliphatic), 2179 (C≡N), 1724 (C=O, ester carbonyl gp), 1697, 1643 (2C=O, cyclic amide), 1631 (C=N); ^{1}H NMR (DMSO-d_6, 400 MHz, ppm): δ: 3.79 (s, 3H, CH_3), 5.62 (s, 1H, methine), 6.02 (s, 2H, 1, 3-dioxole), 6.78 (s, 1H, ethylene), 6.80-6.90 (m, 3H, Ar-H), 6.93 (s, 2H, NH_2, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6, 100 MHz, ppm): δ: 49.07, 52.40, 57.92, 100.93, 102.98, 106.77, 109.06, 112.96, 116.44, 119.73, 127.37, 129.02, 131.97, 134.87, 147.89, 152.93, 156.45, 160.44, 162.58, 163.48. MS (70 eV) *m/z* (%): 454 (M^++2, 2.61), 452 (M^+, 5.69), 263 (100); Anal. Calcd for $C_{20}H_{12}N_4O_7S$ (452): C, 53.10; H, 2.67; N, 12.38; S, 7.09. Found: C, 53.21; H, 2.59; N, 12.46; S, 7.15.

Biological assays

To assess the antibacterial properties of the novel synthesized heterocycles against precise bacterial strains, the disc diffusion method was employed. Sterile Whatman-No.5 filter paper discs with a diameter of 11 mm were used in this screening. The heterocyclic compounds under investigation were dissolved in DMSO and a concentration of 0.01 g/0.5 mL was prepared. A total of 50 µL of this solution was loaded onto each filter paper disc and allowed to completely dry under a hot air stream.

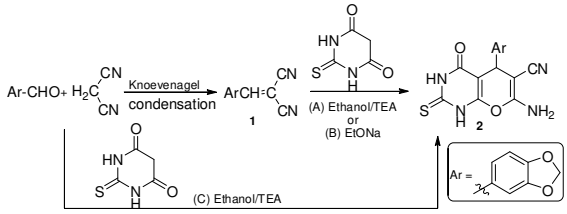
For the preparation of the test plates, 10 mL of Muller-Hinton agar medium was poured into each plate and inoculated with the target bacterial strain (3×10^8 CFU/mL). The dried discs were carefully placed on the surface of the agar plates. The plates were then incubated at 5 °C for 1 hour to ensure proper diffusion. Subsequently, all the plates were further incubated at 37 °C for 24 hours. The growth of the bacteria was observed and recorded.

The experiment was conducted in triplicate, and the average diameter of the inhibition zones was measured in millimeters as a standard for assessing the antibacterial activity. The size of the clear zone observed indicates the inhibitory effect of the investigated heterocycles. To serve as controls, a disc containing only the pure solvent (negative control) and a disc containing the standard drug, amoxicillin, (positive control) for comparison of antibacterial activity were included in all experiments.

3. Results and Discussion

To further advance our endeavors in assembling novel fused heterocycles [20-22], pyrano[2,3-*d*]pyrimidinone derivatives were accessible through various feasible, metal-free, and straightforward synthetic pathways. Although the utilization of enaminonitriles is one of the most common routes to synthesize pyranopyrimidine derivatives [23, 24], arylidenes were chosen as our key starting compound. Initially, arylidene derivative **1** was obtained through a piperidine-catalyzed Knoevenagel condensation reaction according to the reported procedure [25]. Later, the annellation of a pyran ring to the thiobarbituric heterocycle took place *via* a basic-catalyzed thermal cyclization reaction with arylidene malononitrile **1** to obtain our key compound, 7-amino-5-(benzo[*d*][1,3]dioxol-5-yl)-4-

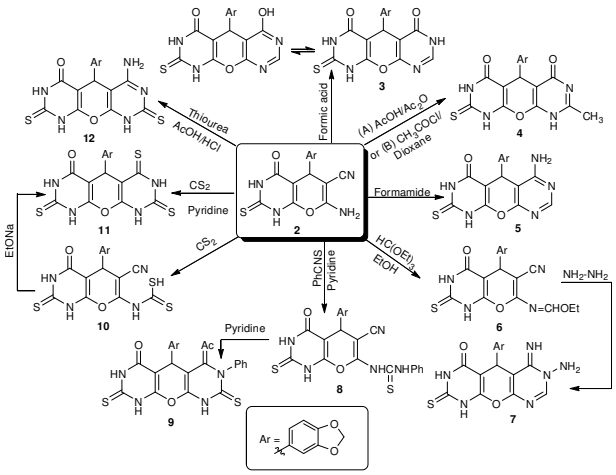
oxo-2-thioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyramid-ine-6-carbonitrile (**2**) (Scheme 1).



Scheme 1: Various attempts to assemble pyrano[2,3-*d*]pyrimidine derivative **2**.

Bhardwaj and coworkers successfully synthesized pyrano[2,3-*d*]pyrimidine derivative **2** in 81% yield [19]. However, to further improve the yield, different approaches and reaction conditions were examined. Various bases were explored in which arylidene malononitrile **1** was reacted with thiobarbituric acid to give pyrano[2,3-*d*]pyrimidine derivative **2** either in the presence of ethanol and a catalytic amount of triethylamine or in a strongly alkaline sodium ethoxide solution (Scheme 1). Additionally, since multicomponent reactions (MCRs) have emerged as a very distinct and powerful strategy for the synthesis of bicyclic pyranopyrimidine nuclei [26-28], a new endeavor employing a one-pot multicomponent reaction was also explored. This involved refluxing piperonal, malononitrile, and thiobarbituric acid in the presence of ethanol and a few drops of triethylamine to afford pyrano[2,3-*d*]pyrimidine derivative **2**. Interestingly, among all the explored methods, refluxing a mixture of arylidine malononitrie **1** with thiobarbituric acid in the presence of a strongly alkaline sodium ethoxide solution was found to be the most convenient method with the highest yield. Unfortunately, despite the extensive efforts made, the enhancement in yield was minimal, with only a slight improvement resulting in an attainable yield of 84%.

Prompted by the high functionality of our key pyrano[2,3-*d*]pyrimidine derivative **2**, the influence of diversified carbon- and nitrogen-based nucleophiles under various reaction conditions was examined. Thus, the annellation of a new pyrimidine ring to the pyran ring present in pyrano[2,3-*d*]pyrimidine **2** was achieved *via* refluxing a mixture of starting compound **2** with formic acid to afford 5-(benzo[*d*][1,3]dioxol-5-yl)-6-hydroxy-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrano[2,3-*d*:6,5-*d'*]dipyrimidin-4-one (**3**) (Scheme 2).



Scheme 2: Reactions of pyrano[2,3-*d*]pyrimidine derivative **2**.

Pyranodipyrimidinone derivative **3** was characterized and established based on its spectral and analytical data. Along with the disappearance of the nitrile group vibration frequency, the IR spectrum of pyranodipyrimidinone **3** revealed the existence of a new broad hydroxyl group band at $\bar{\upsilon}$ 3421 cm^{-1}, along with 2NH group absorption bands at $\bar{\upsilon}$ 3216 and 3170 cm^{-1}, and an intense cyclic amide carbonyl group absorption band at 1654 cm^{-1}. Furthermore, the ^1H NMR spectrum displayed a singlet peak at δ 8.98 ppm due to the imine proton (-C*H*=N-) and three D_2O-exchangeable singlet peaks at δ 10.43, 11.20, and 12.43 ppm corresponding to 2NH protons and the OH proton, respectively. Compound **3** exhibited the molecular ion peak at *m/z* = 370, equivalent to $C_{16}H_{10}N_4O_5S$.

Likewise, refluxing pyrano[2,3-*d*]pyrimidine derivative **2** with acetic anhydride and glacial acetic acid gave rise to another new pyranodipyrimidinone heterocycle **4** (Scheme 2). The formation of 5-(benzo[*d*][1,3]dioxol-5-yl)-8-methyl-2-thioxo-2,3,5,9-tetrahydro-4*H*-pyrano[2,3-*d*:6,5-*d'*]dipyrimidine-4,6(1*H*)-dione (**4**) was also chemically confirmed *via* assembly utilizing an alternative route in which pyrano[2,3-*d*]pyrimidine derivative **2** was allowed to reflux with acetyl chloride in dioxane (Scheme 2). Elemental analysis and spectral data of pyranodipyrimidinone **4** were in full agreement with the suggested structure. The IR spectrum showed the disappearance of the cyclic enaminonitrile group absorption and the presence of absorption bands at $\bar{\upsilon}$ 3228 and 3162 cm^{-1} characteristic of 3NH groups, in addition to an

intense absorption band at ῡ 1670 cm^{-1} characteristic of cyclic amide carbonyl groups. Additionally, a novel singlet signal at δ 1.91 ppm, corresponding to the methyl protons, was recorded in the ^1H NMR spectrum, in addition to three slightly broad D_2O-exchangeable signals at δ 8.29, 12.30, and 12.39 ppm representing the 3NH protons. Furthermore, a new methyl group carbon was observed in the ^{13}C NMR at δ 21.50 ppm. Finally, the derivative **4** molecular ion peak was detected in the mass spectrum at *m/z* = 384.

In the same context, more fused pyranodipyrimidinones were attainable *via* refluxing pyrano[2,3-*d*]pyrimidine derivative **2** with formamide (Scheme 2). Examination of spectral and analytical data asserted the proposed structure of 6-amino-5-(benzo[*d*][1,3]dioxol-5-yl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrano[2,3-*d*:6,5-*d*'] dipyrimidin-4-one (**5**), in which the IR spectrum not only revealed no absorption for the nitrile group but also exhibited intense absorption bands at ῡ 3367 and 3170 cm^{-1} corresponding to NH_2 and 2NH groups and at ῡ 1658 cm^{-1} due to the cyclic amide carbonyl group. In addition, the ^1H NMR spectrum of pyranodipyrimidinone derivative **5** showed slightly broad D_2O-exchangeable signals at δ 6.82, 9.97, and 13.04 ppm for the NH_2 and 2NH protons, respectively. Additionally, a singlet peak for the imine proton (-C*H*=N-) was recorded at δ 8.82 ppm. Another piece of evidence for the suggested structure of derivative **5** was acquired from the mass spectrum, in which the molecular ion peak (M^+) was detected at *m/z* = 369.

In our attempts to assemble more fused pyranodipyrimidinone heterocycles, a two-step reaction was disclosed in which pyrano[2,3-*d*]pyrimidine derivative **2** and triethyl orthoformate were refluxed in ethanol to yield the acyclic imidate ester derivative **6**, which was submitted to a subsequent 6-exo-dig cyclization reaction upon refluxing with hydrazine hydrate to afford 7-amino-5-(benzo[*d*][1,3]dioxol-5-yl)-6-imino-2-thioxo-1,2,3,5,6,7-hexahydro-4*H*-pyrano[2,3-*d*:6,5-*d*']dipyrimidin-4-one (**7**) (Scheme 2). The proposed structures were completely consistent with the spectral data and elemental analyses of derivatives **6** and **7**. The IR spectrum of imidate ester derivative **6** showed absorptions at ῡ 3355 and 3174 cm^{-1} corresponding to 2NH groups in addition to the nitrile group characteristic band at 2213 cm^{-1}, which completely disappeared in the IR spectrum of pyranodipyrimidinone derivative **7**. The ^1H NMR data of formimidate **6** displayed the ethoxy group's triplet-quartet pattern at δ 1.22 and 3.76 ppm. Also, it showed a characteristic band at δ 7.26 ppm for the imidate proton (-N=C*H*OR) and slightly broad D_2O-exchangeable absorption bands at δ 9.53 and 12.28 ppm due to 2NH protons. Furthermore, the ^1H NMR spectrum of pyranodipyrimidinone derivative **7** displayed four D_2O-exchangeable singlet signals at δ 4.98, 9.48, 12.07, and 12.39 ppm, corresponding to the protons of NH_2 and 3NH. Finally, the molecular ion peaks of formimidate derivative **6** and pyranodipyrimidinone derivative **7** were detected at *m/z* = 398 and 384, respectively.

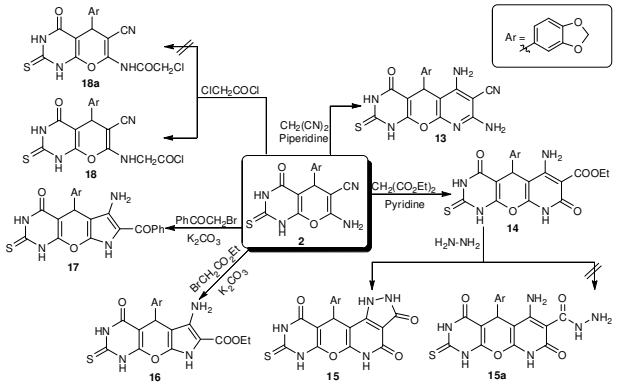
In continuation of our attempts to have access to various annulated bioactive pyrano[2,3-*d*]pyrimidines, different approaches were examined to construct new pyrimidinethione rings fused to our pyranopyrimidine-based scaffold. Thus, refluxing pyrano[2,3-*d*]pyrimidines with phenyl isothiocyanate in pyridine for 12 h gave rise to the acyclic phenylthiourea derivative **8**, which exhibited an intense IR absorption band at ῡ 2213 cm^{-1}, assuring the presence of the nitrile group (Scheme 2). As we aimed to have access to cyclic dithioxopyrano[2,3-*d*:6,5-*d*']dipyrimidinedione derivative **9**, the isolable acyclic phenylthiourea intermediate **8** was further refluxed in pyridine for extra 12 h (Scheme 2). The structure of derivative **9** was determined by means of spectroscopic data. The IR spectrum revealed the absence of the nitrile group absorption band in addition to the appearance of a stretching absorption band at ῡ 1635 cm^{-1} attributed to the cyclic amide carbonyl groups. Furthermore, the ^1H NMR spectrum of dithioxopyrano[2,3-*d*:6,5-*d*']dipyrimidinedione derivative **9** showed three D_2O-exchangeable peaks at δ 9.81, 10.61, and 12.58 ppm corresponding to 3NH protons, while the two thione carbon (C=S) signals were recorded in ^{13}C NMR at δ 173.26 and 178.71 ppm. Finally compound **9** revealed , the molecular ion peak at *m/z* = 478.

Another fusion of a novel pyrimidinethione heterocycle to our pyranopyrimidine nucleus was achieved through a two-step reaction in which pyrano[2,3-*d*]pyrimidine derivative **2** was first reacted with carbon disulfide in ethanolic KOH to furnish the acyclic carbamodithioic acid derivative **10**. Then the isolable intermediate **10** was submitted to a subsequent cyclization, utilizing strongly alkaline

reaction conditions, to afford the cyclic 5-(benzo[*d*][1,3]dioxol-5-yl)-2,6,8-trithioxo-1,2,3,5,6,7,8,9-octahydro-4*H*-pyrano[2,3-*d*:6,5-*d*']dipyrimidin-4-one (**11**) (Scheme 2). Interestingly, derivative **11** was chemically proven by assembling it through an alternative straightforward synthetic approach, including a direct condensation reaction between pyranopyrimidine derivative **2** and carbon disulfide in pyridine (Scheme 2). As the IR spectrum of acyclic intermediate **10** exhibited an intense absorption at ύ 2217 cm^{-1}, indicating the presence of the nitrile group, compound **11** lacked any absorption bands corresponding to the nitrile group but revealed strong absorption bands at ύ 3413, 3224, and 3178 cm^{-1} corresponding to 4NH groups, in addition to the characteristic cyclic amide carbonyl absorption at ύ 1662 cm^{-1}. The ^{1}H NMR spectrum of trithioxopyrano[2,3-*d*:6,5-*d*']dipyrimidinone derivative **11** exhibited four D_2O-exchangeable singlet signals at δ 10.81, 11.55, 12.06, and 13.27 ppm corresponding to 4NH protons, while the molecular ion peak of **11** was detected in the mass spectrum at *m/z* = 418.

Additionally, the reaction of compound **2** with an equimolar amount of thiourea in glacial acetic acid gave rise to a new pyrimidinethione heterocycle and thioxopyrano[2,3-*d*:6,5-*d*']dipyrimidinedione derivative **12** was acquired (Scheme 2). The structure of 6-amino-5-(benzo[*d*][1,3]dioxol-5-yl)-2,8-dithioxo-1,2,3,5,8,9-hexahydro-4*H*-pyrano[2,3-*d*:6,5-*d*']dipyrimidin-4-one (**12**) was confirmed by its spectroscopic data; thus, the IR spectrum revealed no absorption bands corresponding to the nitrile group but showed absorption bands around ύ 3417, 3228, and 3162 cm^{-1} corresponding to NH_2 and 3NH groups. The ^{1}H NMR spectrum of thioxopyrano[2,3-*d*:6,5-*d*']dipyrimidinedione derivative **12** showed a singlet D_2O-exchangeable signal at δ 7.12 ppm due to NH_2 protons, beside another three D_2O-exchangeable signals at δ 8.59, 8.77, and 9.81 ppm corresponding to 3NH protons. The molecular ion peak of compound **12** was recorded in the mass spectrum at *m/z* = 401.

Prompted by the high functionality of pyrano[2,3-*d*]pyrimidine derivative **2**, the influence of diversified active methylene compounds and alkylating agents was thoroughly probed. Thus, treatment of pyranopyrimidine derivative **2** with malononitrile and a catalytic amount of piperidine gave rise to 6,8-diamino-5-(benzo[*d*][1,3]dioxol-5-yl)-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2*H*-pyrido[3',2':5,6]pyrano[2,3-*d*]pyrimidine-7-carbonitrile (**13**) (Scheme 3).



Scheme 3: Reactions of pyrano[2,3-*d*]pyrimidine derivative **2**.

Fusion of a new pyridine ring to our pyrano[2,3-*d*]pyrimidine nucleus in compound **13** was substantiated based on spectroscopic data; for instance, the IR spectrum exhibited absorptions at ύ 3413, 3336, and 3243 cm^{-1} due to $2NH_2$ and 2NH groups, 2213 cm^{-1} corresponding to the nitrile group, and at ύ 1650 cm^{-1} owing to the cyclic amide carbonyl group. Additionally, the protons of the two D_2O-exchangeable amino groups were observed in the ^{1}H NMR spectrum at δ 3.59 and 5.16 ppm, the mass spectrum of compound **13** was in full agreement with the suggested structure, and the molecular ion peak was recorded at *m/z* = 408.

Furthermore, the assembly of another polyfunctionally fused pyridine ring was achieved *via* the treatment of compound **2** with diethyl malonate to afford derivative **14** (Scheme 3). The IR spectrum of ethyl 6-amino-5-(benzo[*d*][1,3]dioxol-5-yl)-4,8-dioxo-2-thioxo-1,3,4,5,8,9-hexahydro-2*H*-pyrido[3',2':5,6]pyrano[2,3-*d*]pyrimidine-7-carboxylate (**14**) not only exhibited the disappearance of the nitrile group absorption band but also exhibited absorptions at ύ 3436, 3208, and 3118 cm^{-1} for NH_2 and NH groups, an intense absorption band for ester carbonyl group at ύ 1727 cm^{-1}, and for cyclic amide carbonyl groups at ύ 1682 and 1666 cm^{-1}. The ^{1}H NMR spectrum of derivative **14** showed a new triplet signal at δ 1.24 ppm, corresponding to the CH_3 group, and a new quartet signal at δ 3.65 ppm due to the CH_2 group. Finally the molecular ion peak of compound **14** was detected at *m/z* = 456.

Subsequently, refluxing derivative **14** with hydrazine hydrate and ethanol was expected to afford carbohydrazide derivative **15a**, but in a stark contrast to the suggested structure, a cyclization reaction took place and 11-(benzo[*d*][1,3]dioxol-5-yl)-8-thioxo-1,2,7,8,9,11-hexahydropyrazolo[3'',4'':4',5']pyrido[3',2':5,6]pyrano [2,3-*d*] pyrimidine-3,4,10(5*H*)-trione (**15**) was the sole product obtained (Scheme 3). The IR spectrum of compound **15** showed no absorption bands for the ester carbonyl group but characteristic bands at 3158 and 3118 cm^{-1} for NH groups were observed. The ^1H NMR spectrum of compound **15** revealed five D_2O-exchangeable singlet peaks at δ 7.54, 7.79, 9.81, 11.56, and 12.39 ppm corresponding to 5NH protons; however, ^{13}C NMR displayed three peaks at δ 157.14, 160.35, and 162.53 ppm attributed to the three carbonyl groups. Finally, the molecular ion peak of compound **15** was obtained in the mass spectrum at *m/z* = 425, equivalent to $C_{18}H_{11}N_5O_6S$.

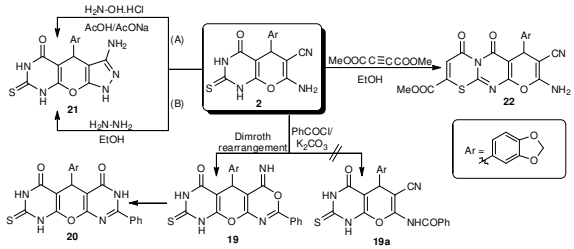
Additionally, a new system comprising a pyrrole heterocycle fused to our key pyrano[2,3-*d*]pyrimidine motif was accessible *via* an alkylation reaction of pyranopyrimidine derivative **2** with α-haloacetic ester followed by a subsequent K_2CO_3-catalyzed cyclization reaction of the alkylated product (Scheme 3). The aforementioned reaction afforded ethyl 6-amino-5-(benzo[*d*][1,3]dioxol-5-yl)-4-oxo-2-thioxo-1,2,3,4,5,8-hexahydro-pyrrolo[3',2':5,6]pyrano[2,3-*d*]pyrimidine-7-carboxylate (**16**) in good yield, and the structure was confirmed based on its spectroscopic data. The IR spectrum of compound **16** was devoid of any nitrile group absorption bands; instead, a new absorption band corresponding to the ester carbonyl group was observed at ύ 1731 cm^{-1}. Furthermore, NH_2 and NH group stretching frequencies were observed in the IR at ύ 3436, 3355, and 3205 cm^{-1}. Moreover, the distinct triplet-quartet pattern of the ethyl group was recorded in ^1H NMR at δ 1.29 and 4.44 ppm, while ^{13}C NMR exhibited the new methyl group carbon at δ 21.44 ppm and the methylene carbon at δ 55.19 ppm. The mass spectrum of derivative **16** exhibited the molecular ion peak at *m/z* = 428, corresponding to $C_{19}H_{16}N_4O_6S$.

Likewise, fusion of a pyrrole ring to pyrano[2,3-*d*]pyrimidine scaffold **2** was readily accessible through an alkylation reaction of derivative **2** with phenacyl bromide, then boiling the product in dry acetone containing anhydrous potassium carbonate to afford 6-amino-5-(benzo[*d*][1,3]dioxol-5-yl)-7-benzoyl-2-thioxo-2,3,5,8-tetrahydropyrrolo[3',2':5,6]pyrano[2,3-*d*]pyrimidin-4(1*H*)-one (**17**) (Scheme 3). Derivative **17** revealed no absorption bands for the nitrile group in the IR spectrum but rather exhibited intense absorption bands at ύ 1697 cm^{-1} and 1727 cm^{-1} due to cyclic amide carbonyl and ketone carbonyl (COPh), respectively. The ^1H NMR data revealed four D_2O-exchangeable singlet signals at δ 6.49, 10.61, 11.48, and 12.36 ppm attributed to NH_2 and 3NH protons, respectively. The mass spectrum of **17** showed the molecular ion peak at *m/z* = 460, equivalent to $C_{23}H_{16}N_4O_5S$.

In continuation of our endeavors to assess the impact of alkylating agents on pyrano[2,3-*d*]pyrimidine derivative **2**, chloroacetyl chloride reacted with **2** under diversified reaction conditions. The acetylated chloroacetamide derivative **18a** was expected to be the only product, but interestingly, the alkylated product (5-(benzo[*d*][1,3]dioxol-5-yl)-6-cyano-4-oxo-2-thioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidin-7-yl)- glycinoyl chloride **18** was acquired instead (Scheme 3). The elemental analysis and spectral data were compatible with the structure of alkylated product **18**. In addition to the cyclic amide carbonyl stretching frequency at ύ 1643 cm^{-1}, the IR spectrum revealed a new stretching frequency at ύ 1727 cm^{-1} characteristic of (COCl) carbonyl. The ^1H NMR spectrum of glycinoyl chloride derivative **18** revealed a new singlet peak at δ 4.26 ppm corresponding to CH_2 protons and three slightly broad D_2O-exchangeable peaks at δ 7.45, 9.56, and 11.21 ppm due to 3NH protons. Additionally, the molecular ion peak was recorded in the mass spectrum at *m/z* = 418.5.

Notably, acyclic *N*-benzoylated product **19a** was expected to be the only outcome of the basic-catalyzed benzoylation reaction of compound **2** with benzoyl chloride, but interestingly, the IR spectrum of the product showed no absorption band corresponding to the nitrile group, and a new cyclic amide carbonyl was recorded. Further investigations revealed that a Dimroth rearrangement reaction occurred *via* the oxazine intermediate **19** to afford the corresponding 5-(benzo[*d*][1,3]dioxol-5-yl)-8-phenyl-2-thioxo-2,3,5,7-tetrahydro-4*H*-pyrano[2,3-*d*:6,5-*d*']dipyrimidine-4,6(1*H*)-dione (**20**) (Scheme 4). The Dimroth rearrangement involved a heterocycle isomerization, wherein oxygen and nitrogen

heteroatoms relocated through a ring opening and closure process, as observed in this reaction [29-31].



Scheme 4: Reactions of pyrano[2,3-d]pyrimidine derivative 2.

The spectral data were in full agreement with tricyclic derivative **20**. The ^{1}H NMR spectrum showed slightly broad D_2O-exchangeable singlet signals at δ 9.47, 11.22, and 12.05 ppm attributed to 3NH protons, and the mass spectrum showed the molecular ion peak at *m/z* = 446.

Furthermore, a new pyrazole ring fused to our key pyrano[2,3-*d*]pyrimidine nucleus was attainable *via* two different synthetic approaches (Scheme 4). As depicted in Scheme 4,3-amino-4-(benzo[*d*][1,3]dioxol-5-yl)-7-thioxo-4,6,7,8-tetrahydro-pyrazolo [4',3':5,6]pyrano[2,3-*d*]pyrimidin-5(1*H*)-one (**21**) was assembled by refluxing a mixture of pyranopyrimidine derivative **2** either with hydroxylamine hydrochloride or hydrazine hydrate. It is worth mentioning that excellent reaction profiles and yields were obtained when utilizing hydrazine hydrate. Characterization of the proposed structure **21** was achieved through various spectroscopic and analytical data. The IR spectrum was devoid of any absorption for the cyano group. Also, it exhibited characteristic bands for the NH_2 and NH groups at ύ 3424 and 3313 cm^{-1}, and an intense absorption band for the cyclic amide carbonyl group at 1654 cm^{-1}. Another piece of evidence for the proposed compound was gained from the ^{1}H NMR data that showed a D_2O-exchangeable singlet peak at δ 4.74 ppm for NH_2 protons and the molecular ion peak was recorded in the mass spectrum at m/z = 357, supporting the molecular formula $C_{15}H_{11}N_5O_4S$.

Finally, the reaction of pyranopyrimidine derivative 2 with dimethyl acetylenedicarboxylate in refluxing ethanol was investigated and methyl 9-amino-7-(benzo[d][1,3]dioxol-5-yl)-8-cyano-4,6-dioxo-4H,6H,7H-pyrano[2',3':4,5] pyrimido[2,1-b][1,3]thiazine-2-carboxylate (22) was the sole product isolated (Scheme 4). The structure of tricyclic derivative 22 was asserted from its spectroscopic data, such as the IR spectrum, which exhibited the presence of cyclic amide carbonyl groups indicated by the absorption bands at ύ 1697 and 1643 cm^{-1}, ester carbonyl at ύ 1724 cm^{-1}, and a nitrile group characteristic band at ύ 2179 cm^{-1}. Additional support for the proposed structure was obtained through the analysis of ^{1}H NMR data that showed a singlet peak at δ 3.79 ppm integrated for three protons of the methyl group and a peak at δ 6.78 ppm characteristic of the thiazinone proton. Furthermore, ^{13}C NMR exhibited a new methyl carbon signal at δ 52.40 ppm and three carbonyl carbons at δ 160.44, 162.58, and 163.48 ppm, while the mass spectrum showed the molecular ion at m/z = 452.

Biological Activity

Pyrimidine is an essential constituent of DNA and RNA. Various pyrimidine-containing heterocycles play a crucial role in drug discovery [32-34]. Additionally, annulated pyrano[2,3-*d*]pyrimidine/uracil heterocycles are outstanding candidates for drug resistance adversity in clinically applied therapies [9]. The effectiveness of pyrano[2,3-*d*]pyrimidines as chemotherapeutic agents is attributed to their capacity to hinder crucial enzymes involved in DNA production, like thymidylate synthetase (TSase), reverse transcriptase (RTase), thymidine phosphorylase (TPase), and dihydrofolate reductase (DHFR) [3].

In studying the interaction of pyrano[2,3-*d*]pyrimidine skeletons with antimicrobial targets, Akhter and coworkers illustrated that the existence of heteroaryl rings, amino, and cyano groups on the pyran moiety increases the basic nature of pyrano[2,3-*d*]pyrimidines, which in turn boosts their affinity to penetrate the bacterial cell wall (Figure 2) [3].

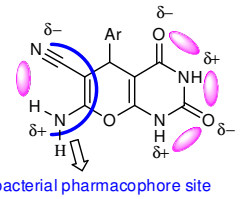


Figure 2: Molecular structure and antibacterial ($NH^{\delta+}$, $N^{\delta-}$) pharmacophore sites based on POM theory [3].

Moreover, Elhady *et al.* [8] defined four fundamental structural characteristics that confer

annulated pyrano[2,3-*d*]pyrimidines high antimicrobial potency: i) a planar pyrano[2,3-*d*]pyrimidine platform (chromophore), ii) an aromatic ring at position-5, iii) imine, cyano, and/or carbonyl groups at position-6, and iv) 1^{ry}, 2^{ry}, and/or 3^{ry} amino groups at position-7. Elhady *et al.* attributed the bactericidal effect of pyrano[2,3-*d*]pyrimidines to their impact on the formation of bacterial cell walls, which can alter cell size and shape and trigger stress responses that ultimately lead to cell lysis.

Inspired by the research conducted by Akhter and coworkers [3] that used POM (Petra/Osiris/Molinspiration) theory to identify the antibacterial pharmacophore sites of pyrano[2,3-*d*]pyrimidine derivatives (Figure 2) and according to Elhady *etal*. findings [8], our novel assembled pyrano[2,3-*d*]pyrimidine-based pharmacophores were designed and screened for their antibacterial efficacy.

The antibacterial activity of the compounds under investigation was evaluated *in vitro* using four different types of bacteria. These included two pathogenic Gram-positive bacterial strains, *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*), as well as two pathogenic Gram-negative bacterial strains, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*). The antibacterial activity of the examined heterocycles against the selected pathogenic microbes was measured as inhibitory diameter zones in millimeters (mm) (Table) using Norfloxacin as a control criterion owing to its broad-spectrum ability to effectively target both Gram-positive and Gram-negative bacterial strains. The tested pyrano[2,3-*d*]pyrimidine derivatives were recrystallized twice and tested in their ultrapure form, as confirmed by TLC.

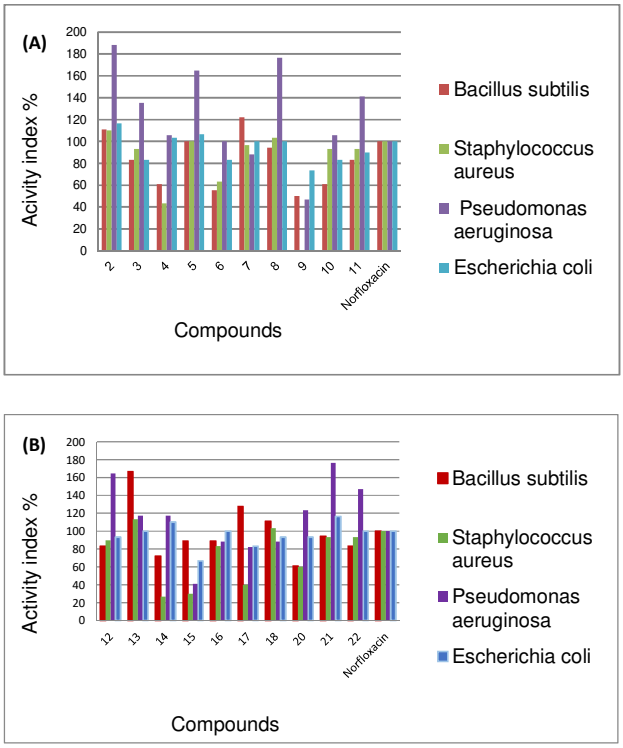


Figure 3: Activity indexes of annulated pyrano[2,3-*d*]pyrimidine-based pharmacophores.

As depicted in Table and Figure 3, nearly all the tested pyrano[2,3-*d*]pyrimidine-based pharmacophores were active against the selected bacterial strains except for compound **9**, which revealed no biological activity against *S. aureus* (Table; entry 8).

Table: *In vitro* antibacterial efficacy of the assembled pyrano[2,3-*d*]pyrimidine-based pharmacophores

Entry	Compound	Gram (+Ve) bacteria				Gram (-Ve) bacteria			
		B. subtilis		*S. aureus*		*P. aeruginosa*		*E. coli*	
		I.Z.±S.D.*	% Activity index	I.Z.±S.D.*	% Activity index	I.Z.±S.D.*	% Activity index	I.Z.±S.D.*	% Activity index
1	**2**	20± 0.29	111.1	33±1.00	110	32±0.58	188.2	35±0.76	116.6
2	**3**	15±0.29	83.3	28±0.29	93.3	23±0.58	135.2	25±0.29	83.3
3	**4**	11±0.50	61.1	13±0.29	43.3	18±029	105.8	31±0.50	103.3
4	**5**	18±0.29	100	30±0.58	100	28±0.29	164.7	32±0.58	106.6
5	**6**	10±0.50	55.5	19±0.29	63.3	17±1.26	100	25±0.29	83.3
6	**7**	22±0.58	122.2	29±0.50	96.6	15±0.50	88.2	30±0.58	100
7	**8**	17±0.29	94.4	31±1.00	103.3	30±0.58	176.4	30±0.76	100

8	**9**	9±1.00	50	NA	-	8±0.29	47	22±0.29	73.3
9	**10**	11±0.29	61.1	28±0.58	93.3	18±0.58	105.8	25±1.00	83.3
10	**11**	15±0.29	83.3	28±0.29	93.3	24±1.00	141.1	27±0.29	90
11	**12**	15±0.76	83.3	27±0.50	90	28±0.29	164.7	28±0.50	93.3
12	**13**	30±0.76	166.6	34±1.26	113.3	20±0.58	117.6	30±0.50	100
13	**14**	13±0.58	72.2	8±1.00	26.6	20±0.58	117.6	33±1.04	110
14	**15**	16±0.29	88.8	9±0.58	30	7±0.76	41.1	20±0.50	66.6
15	**16**	16±0.29	88.8	25±0.58	83.3	15±0.76	88.2	30±0.58	100
16	**17**	23±0.50	127.7	12±0.29	40	14±0.29	82.3	25±0.29	83.3
17	**18**	20±0.50	111.1	31±0.58	103.3	15±0.58	88.2	28±0.29	93.3
18	**20**	11±0.50	61.1	18±0.29	60	21±0.29	123.5	28±0.29	93.3
19	**21**	17±0.50	94.4	28±0.29	93.3	30±0.76	176.4	35±0.76	116.6
20	**22**	15±0.58	83.3	28±0.76	93.3	25±0.50	147	30±1.26	100
21	**Norfloxacin**	18	100	30	100	17	100	30	100

[*]I.Z. Inhibition diameter zones expressed in millimeters (mm), S.D. Standard Deviation

[a)] NA: No antimicrobial activity was observed.

Pyrano[2,3-*d*]pyrimidine derivative **2** revealed the strongest broad-spectrum inhibition activity against our tested Gram-negative bacteria, *P. aeruginosa* (188.2%) and *E. coli* (116.6%) (Table; entry 1); however, pyrano[2,3-*d*]pyrimidine derivative **13** exhibited the strongest antagonistic properties against the selected Gram-positive bacteria, *B. subtilis* (166.6%) and *S. aureus* (113.3%) (Table; entry 12).

As illustrated in Figure 4, the annulated pyrano[2,3-*d*]pyrimidine derivative **2** not only fulfilled the four fundamental structural characteristics proposed by Elhady *etal*., but also comprises the more nucleophilic and basic thione group, which exerted a potent influence on the antibacterial activity of compound **2** as it will be more able to effectively penetrate the bacterial cell wall (Table, Figure 4) [3].

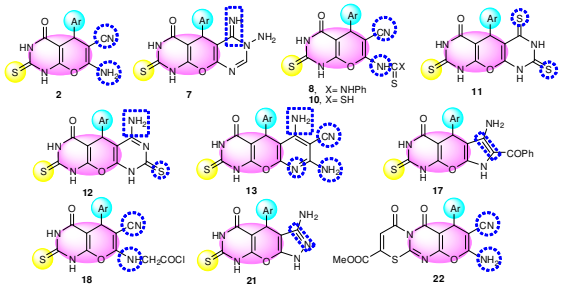


Figure 4: Promising pharmacophoric features of assembled pyrano[2,3-*d*]pyrimidine derivatives.

For derivative **13**, fusion of a pyridine ring to the pyranopyrimidine skeleton inherited derivative **13**, which has broad antibacterial activity against the microbial strains under investigation. Additionally, the presence of the amino and cyano groups attached to the pyrimidine ring significantly enhanced the inhibitory activity of **13** (Table, Figure 4).

In addition, for *B. subtilis*, compounds **2**, **7**, **17**, and **18** revealed very promising antibacterial activities that exceeded the criterion drug activity index (Table; entries 1, 6, 16, and 17, respectively). Furthermore, activity indexes exceeding Norfloxacin activity were measured with *S. aureus* for heterocycles **2**, **8**, and **18** (Table; entries 1, 7, and 17, respectively). Noteworthy, in the case of *P. aeruginosa* bacteria, most of the tested pyrano[2,3-*d*]pyrimidine derivatives revealed very potent antibacterial activity; however, in the case of *E. coli* bacteria, the inhibition activity was diminished but still exceeded the criterion drug's antibacterial efficacy (Table).

As illustrated in Figure 4, all the promising candidates comprise various basic centers, imine, cyano, carbonyl, and/or amino groups.

4. Conclusions

In summary, the present work demonstrated versatile and effective approaches for synthesizing

pyrano[2,3-*d*]pyrimidine derivatives using readily available starting materials. The impact of various nucleophiles, including nitrogen, carbon, and active methylene nucleophiles, on our valuable pyrano[2,3-*d*]pyrimidine precursor **2** was investigated to produce unprecedented annulated pyrano[2,3-*d*]pyrimidines. Simple and straightforward reaction routes were employed to achieve good yields and reaction profiles. Furthermore, the primary antibacterial activity of the newly developed heterocyclic compounds was evaluated *in vitro* against two Gram-positive bacterial strains (*B. subtilis* and *S. aureus*) and two Gram-negative bacterial strains, (*P. aeruginosa* and *E. coli*). All compounds exhibited good to excellent antibacterial activity against the selected pathogens except for compound **9**, which was inactive against *S. aureus*. The excellent activity indexes, which frequently exceeded the criterion drug activity index, indicate the promising potential of the newly synthesized pyrano[2,3-*d*]pyrimidines as antibacterial candidates for future drug development. Finally, spectroscopic analyses were utilized to assert the newly-reported pyrano[2,3-*d*]pyrimidines.

5. Conflict of interest:

The authors have no conflict of interest to declare that are relevant to the content of this article.

6. Funding

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8. Ethical Approval

Not applicable.

9. References

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