Design, synthesis, docking, and antimicrobial evaluation of some novel pyrazolo[1,5-\(a\)]pyrimidines and their corresponding cycloalkane ring-fused derivatives as purine analogs

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Background: Over the years, pyrazolopyrimidine derivatives have been recognized as having antimicrobial activities. Recently, we reported different synthetic methods to prepare pyrazolopyrimidine derivatives as anticancer and antimicrobial agents. The studies showed that our previously reported 5-aminopyrazoles 2 act as a building block for the preparation of a variety of interesting pyrazolopyrimidines as purine analogs.

Purpose: The objective of this study was to describe the direct new method for preparation of novel pyrazolo[1,5-\(a\)]pyrimidine derivatives and their corresponding cycloalkane ring-fused derivatives. Also, the new compounds were tested in vitro for their antibacterial and antifungal activity properties.

Methods: Pyrazolo[1,5-\(a\)]pyrimidine derivatives were prepared by the reaction of our previously reported 5-aminopyrazoles 2 with suitable sodium salts of (hydroxymethylene) cycloalkanones and sodium salts of unsaturated ketones.

Results: The structures of the new compounds were characterized according to their mass spectrometry, \(^1\)H NMR, IR and elemental analyses. Compounds 8b, 10e, 10i, and 10n were the most active compounds against Gram-positive and Gram-negative bacterial species. Compound 10i with two moieties of 4-Br-C\(_6\)H\(_4\) revealed increased reactivity compared with ampicillin as standard reference.

Conclusion: About twenty two novel pyrazolo[1,5-\(a\)]pyrimidine derivatives and their corresponding cycloalkane ring-fused derivatives were prepared through the reaction of 5-aminopyrazoles 2 with different sodium salts of (hydroxymethylene) cycloalkanones and sodium salts of unsaturated ketones. The antibacterial and antifungal activities of the newly synthesized compounds were evaluated and revealed that compounds 8b, 10c, 10i, and 10n were the most active compounds against Gram-positive and Gram-negative bacterial strains.

Keywords: pyrazolo[1,5-\(a\)]pyrimidines, 5-aminopyrazoles, 2-(hydroxymethylene)-1-cycloalkanones, 2-formylcycloalkanones, antibacterial, antifungal, docking studies

Introduction

Antimicrobial resistance threatens prevention and treatment of diseases caused by fungi, bacteria, viruses, and parasites. Diseases caused by infection over time are a growing threat to the overall health of people worldwide. Urgent and preventive action must be taken in all societies.\(^1\) Antimicrobial resistance occurs when microscopic organisms such as viruses, bacteria, parasites, and fungi change, when treated with antimicrobial agents such as fungicides, antivirals, and antibiotics. Over time, microorganisms promote and acquire antimicrobial resistance. In consequence, most drugs become virtually ineffective in treatment, and diseases and infections
will be persistent in the human body, leading to increased and evolving risk of proliferation in communities and threats to our actual ability to treat infectious and common diseases that are known to lead to death.\textsuperscript{2} It is clear that antimicrobial resistance occurs naturally and spontaneously over time, usually through genetic changes. Antimicrobial resistance is naturally generated by natural selection from random mutations. When the new gene is made, bacteria can convert genetic information in a horizontal way. If the bacteria carry several resistant genes, they are called multi-resistance bacteria. The effect of antimicrobial resistance is the environmental stress on bacteria, but the mutations that appear in some bacterial cells make them escape the antimicrobial resistance effect. Next, this feature moves to the next offspring, which is characterized as a generation with full antimicrobial resistance. Poor ability to control infection, lack of adequate hygienic conditions, and inadequate proper handling of all types of foods lead to increased prevalence of resistance to all antimicrobials. Patients with an infection caused by drug-resistant bacteria are always at increased risk of poor clinical outcomes and acute death as they consume more medicines and medical resources than other infected patients with non-resistant strains of the same microbes and bacteria.\textsuperscript{3} Many pyrazolopyrimidines are known to possess antimicrobial and antifungal activities;\textsuperscript{4} we have recently reported different innovative synthetic methods to prepare pyrazolopyrimidine derivatives that found application and appeared to constitute new classes of anticancer and antimicrobial agents.\textsuperscript{5,6} A series of one of our previously reported novel 5-aminopyrazoles \textsuperscript{1a–b,7–11} (Figure 1) was used recently by other research groups as a starting material for the construction of pyrazolopyrimidines.\textsuperscript{12–19} The studies demonstrated that our aminopyrazoles act as a building block for a variety of interesting pyrazolopyrimidines as purine analogs. In another study conducted, our previously reported 5-aminopyrazoles \textsuperscript{2a,b,20,21} (Figure 1) were proven as a good starting synthetic material for the preparation of a variety of interesting pyrazolopyrimidines.\textsuperscript{22,23} We have reported that 5-aminopyrazoles, aminotriazoles, aminotetrazole, and aminobenzimidazole reacted with sodium salts of (hydroxymethylene) cycloalkanones and sodium salts of unsaturated keto compounds to give the corresponding angular azolopyrimidine derivatives.\textsuperscript{24–31} These promising results have motivated our research group to continue this work exploring novel molecular mechanisms of these synthetic compounds and their use as chemotherapeutic agents. In view of these findings and as a part of our program directed toward the preparation of potential antimetabolic agents,\textsuperscript{32} we have recently reported different synthetic methods for the preparation of azoloazines using activated nitriles.\textsuperscript{33} Many derivatives of these ring systems are considered important as antimetabolites in most biochemical reactions.\textsuperscript{34} In the light of these reports and in the continuing results of our previous research into the synthesis of biologically active heterocyclic compounds,\textsuperscript{35} the present research reports a new preparation of cycloalkane ring-fused pyrazolo[1,5-\textit{d}]pyrimidines \textsuperscript{8a–f} (Scheme 1) and substituted pyrazolo[1,5-\textit{d}]pyrimidines \textsuperscript{10a–n} (Scheme 2) by the reaction of our previously reported 5-aminopyrazole \textsuperscript{2} with suitable sodium salts of (hydroxymethylene)-cycloalkanones \textsuperscript{7a–d} and sodium salts of unsaturated keto compounds \textsuperscript{9a–h}. The synthesized heterocycles were tested and evaluated for their antifungal and antibacterial activities.

### Materials and methods

The melting points were determined on a Gallenkamp melting point apparatus and were uncorrected. Infrared (IR) spectra (KBr discs) were recorded on a Fourier-transform infrared (FTIR) plus 460 IR spectrophotometer (Shimadzu, Japan). \textsuperscript{1}H NMR spectra were recorded on a BRUKER-400 spectrometer operating at 400 MHz in DMSO-\textit{d}_6 with Si(CH\textsubscript{3})\textsubscript{4} as an internal standard at the Faculty of Pharmacy, Ain Shams University, Egypt. Shifts were given in ppm and the abbreviations were as follows: s (singlet), d (doublet), t (triplet), and m (multiplet). The mass spectra were run in the Microanalytical Center at Cairo University. The reagents and solvents were purchased in commercially available grade purity. 5-Aminopyrazoles \textsuperscript{2} were prepared following our previously reported method.\textsuperscript{20}

### Synthetic procedures

#### General procedure for the synthesis of (8a–h)

To a solution of any of \textsuperscript{2b} (2.17 g, 0.01 mol) or \textsuperscript{2e} (2.96 g, 0.01 mol), the sodium salt of \textsuperscript{7a} (1.34 g, 0.01 mol), \textsuperscript{7b} (1.48 g, 0.01 mol), \textsuperscript{7c} (1.62 g, 0.01 mol), or \textsuperscript{7d} (1.76 g, 0.01 mol) and piperidine acetate (1 mL; prepared from 4.2 mL glacial acetic

![Figure 1] Structure of our previously reported 5-aminopyrazoles 1a,b\textsuperscript{7–11} and 2a,b\textsuperscript{20,21}

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acid, 10 mL water, and 7.2 mL piperidine) were refluxed in water (50 mL) for 10 min. Acetic acid (1.5 mL) was added to the hot solution and refluxing was continued for about 15 min. The reaction mixture was allowed to cool to room temperature. The precipitate, in each case, was collected by filtration and crystallized from ethanol.

2-(Phenylamino)-7,8-dihydro-6H-cycloalk[e]pyrazolo[1,5-a]pyrimidine-3-carboxamide (8a)

Canary yellow crystals; yield: 75% (2.21 g), melting point (mp): 310°C–315°C; IR (KBr, ν cm⁻¹): 3,378, 3,301 (NH₂), 3,145 (NH), 3,050 (CH-aromatic), 2,955, 2,918 (CH₂), 1,651 (C=O), and 1,596, 1,449 (C=C). ¹H-NMR (400 MHz

Scheme 1 Synthesis of 7,8-dihydro-6H-cycloalk[e]pyrazolo[1,5-a]pyrimidine-3-carboxamide derivatives 8a–h.
DMSO-\(d_6\) \(\delta\): 2.52–2.50 (m, 2H, CH\(_2\)), 3.06–3.33 (m, 4H, 2CH\(_2\)), 6.94–7.36, 7.68–7.70 (m, 5H, C\(_6\)H\(_5\)), 7.48–7.57 (s, 2H, NH\(_2\)), 8.54 (s, 1H, NH). Analysis calculated (Anal. Calcd.) for C\(_{16}\)H\(_{15}\)N\(_5\)O (293.32): C, 65.52; H, 5.15; N, 23.88. Found: C, 65.69; H, 4.90; N, 24.01.

**2-((4-Bromophenyl)amino)-7,8-dihydro-6H-cyclopenta[e]pyrazolo-[1,5-a]pyrimidine-3-carboxamide (8b)**

Faint yellow crystals; yield: 80% (2.96), mp: 282°C–290°C; IR (KBr, \(\nu\) cm\(^{-1}\)): 3,405, 3,266 (NH\(_2\)), 3,168 (NH), 3,040 (CH-aromatic), 2,954, 2,855 (CH\(_2\)), 1,651 (C=O) and 1,593, 1,450

**Scheme 2** Synthesis of 7-substituted-pyrazolo[1,5-a]pyrimidine-3-carboxamide derivatives 10a–n.
2-(Phenylamino)-6,7,8,9-tetrahydropyrazolo[1,5-a]quinoxaline-3-carboxamide (8c)
White crystals; yield: 99% (3.04 g), mp: 263°C–266°C; IR (KBr, ν cm⁻¹): 3,393, 3,306 (NH), 3,184 (NH), 3,050 (CH-aromatic), 2,936, 2,862 (CH), 1,650 (C=O) and 1,595, 1,455 (C=C). ¹H-NMR (DMSO-d₆) δ: 2.17–2.26 (m, 2H, CH₂), 2.93–3.04 (m, 4H, 2CH₂), 7.45–7.49 (s, 2H, NH₂), 7.60–7.67 (m, 4H, C₆H₄), 8.52 (s, 1H, CH pyrimidine), 9.75 (s, 1H, NH). Anal. Calcd. for C₂₆H₂₂N₂O (372.22): C, 51.63; H, 4.10; N, 18.82. Found: C, 51.89; H, 4.01; N, 18.99.

2-((4-Bromophenyl)amino)-6,7,8,9-tetrahydropyrazolo[1,5-a]quinoxaline-3-carboxamide (8d)
Off white crystals; yield: 96% (3.70 g), mp: 280°C–285°C; IR (KBr, ν cm⁻¹): 3,393, 3,392 (NH), 3,272 (NH), 3,159 (CH-aromatic), 2,933 (CH₃), 1,650 (C=O) and 1,592, 1,453 (C=C). ¹H-NMR (DMSO-d₆) δ: 1.77–1.94 (m, 4H, 2CH₂), 2.75–3.11 (m, 4H, 2CH₂), 6.94–7.36, 7.65–7.71 (m, 5H, C₆H₄), 7.51–7.53 (s, 2H, NH₂), 8.87 (s, 1H, CH pyrimidine), 9.59 (s, 1H, NH). Anal. Calcd. for C₂₇H₂₃N₂O (307.35): C, 66.43; H, 5.58; N, 22.79. Found: C, 66.77; H, 5.31; N, 22.98.

2-(Phenylamino)-6,7,8,9,10-pentahydropyrazolo[1,5-a]quinoxaline-3-carboxamide (8e)
White crystals; yield: 70% (2.24 g), mp: 222°C–224°C; IR (KBr, ν cm⁻¹): 3,350, 3,300 (NH₂, NH), 3,020 (CH-aromatic), 2,960 (CH₃), 1,670 (C=O). ¹H-NMR (DMSO-d₆) δ: 1.60–2.00 (m, 6H, 3CH₂), 2.65–3.20 (m, 4H, 2CH₂), 6.90–7.80 (m, 5H, C₆H₄), 7.80–7.83 (s, 2H, NH₂), 8.89 (s, 1H, CH pyrimidine), 10.11 (s, 1H, NH). Anal. Calcd. for C₂₉H₂₄N₂O (321.38): C, 67.27; H, 5.96; N, 21.79. Found: C, 67.00; H, 5.70; N, 22.00.

2-((4-Bromophenyl)amino)-6,7,8,9,10-pentahydropyrazolo[1,5-a]quinoxaline-3-carboxamide (8f)
White powder; yield: 60% (2.40 g), mp: 241°C–245°C; IR (KBr, ν cm⁻¹): 3,400, 3,300 (NH₂, NH), 3,130 (CH-aromatic), 2,900 (CH₃), 1,660 (C=O). ¹H-NMR (DMSO-d₆) δ: 1.37–1.90 (m, 6H, 3CH₂), 2.40–2.90 (m, 4H, 2CH₂), 7.00 (s, 2H, NH₂), 7.20–7.90 (m, 4H, C₆H₄), 8.50 (s, 1H, CH pyrimidine), 9.22 (s, 1H, NH). Anal. Calcd. for C₂₈H₂₃N₂OBr (400.27): C, 54.01; H, 4.53; N, 17.50. Found: C, 54.00; H, 4.22; N, 17.20.

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7-Ethyl-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (10b)

Off white crystals; yield: 78% (2.21 g), mp: 215°C–219°C; IR (KBr, ν cm⁻¹): 3,395, 3,273 (NH₂), 3,166 (NH), 3,040 (CH-aromatic), 2,925 (CH₂, CH₃), 1,653 (C=O), and 1,595, 1,449 (C=C). ¹H-NMR (DMSO-d₆) δ: 1.35–1.39 (t, 3H, CH₃), 3.11–3.17 (q, 2H, CH₂), 6.95–7.37, 7.60–7.71 (m, 5H, C₆H₅), 7.44–7.52 (s, br, 2H, NH₂), 8.52 (s, 1H, CH pyrimidine), 8.83 (s, 1H, CH pyrimidine), 9.59 (s, 1H, NH). Anal. Calcd. for C₁₅H₁₃N₅O (281.31): C, 64.04; H, 5.37; N, 24.90. Found: C, 64.33; H, 5.61; N, 25.20.

2-((4-Bromophenyl)amino)-7-ethylpyrazolo[1,5-a]pyrimidine-3-carboxamide (10c)

Faint yellow crystals; yield: 97% (3.48 g), mp: 217°C–220°C; IR (KBr, ν cm⁻¹): 3,423, 3,315 (NH₂), 3,264–3,178 (NH), 3,012 (CH-aromatic), 2,973, 2,919 (CH₂, CH₃), 1,659 (C=O) and 1,591, 1,455 (C=C). ¹H-NMR (DMSO-d₆) δ: 1.37–1.41 (t, 3H, CH₃), 3.14–3.20 (q, 2H, CH₂), 7.06–7.51, 7.63–7.71 (m, 4H, C₆H₅), 7.57 (s, br, 2H, NH₂), 8.58 (s, 1H, CH pyrimidine), 8.91 (s, 1H, CH pyrimidine), 9.63 (s, 1H, NH). Anal. Calcd. for C₁₅H₁₃N₅OBr (360.21): C, 50.02; H, 3.92, N, 19.44. Found: C, 50.22; H, 4.02; N, 19.20.

7-Phenyl-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (10d)

Canary yellow crystals; yield: 97% (3.51 g), mp: 237°C–240°C; IR (KBr, ν cm⁻¹): 3,376, 3,312 (NH₂), 3,269 (NH), 3,149 (CH-aromatic), 1,654 (C=O) and 1,596, 1,444 (C=C). ¹H-NMR (DMSO-d₆) δ: 6.92–7.67 (m, 10H, 2CH₂), 8.19–8.20 (s, 2H, NH₂), 8.65 (s, 1H, CH pyrimidine), 8.66 (s, 1H, CH pyrimidine), 9.66 (s, 1H, NH). Anal. Calcd. for C₁₅H₁₃N₅O (329.36): C, 69.29; H, 4.59; N, 21.26. Found: C, 69.45; H, 4.70; N, 21.44.

7-(4-Chlorophenyl)-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (10f)

Canary yellow crystals; yield: 96% (3.47 g), mp: 272°C–275°C; IR (KBr, ν cm⁻¹): 3,381, 3,317 (NH₂), 3,271 (NH), 3,169 (CH-aromatic), 1,659 (C=O) and 1,597, 1,452 (C=C). ¹H-NMR (DMSO-d₆) δ: 6.94–7.78 (m, 9H, C₆H₅, C₆H₅), 8.26–8.82 (s, 2H, NH₂), 8.34 (s, 1H, CH pyrimidine), 8.69 (s, 1H, CH pyrimidine), 9.75 (s, 1H, NH). MS (EI): m/z (%) 366 [M+2]+ (4.49), 365 [M+1]+ (20.13), 364 [M]+ (13.72), 363 [M–1] (16.83), 362 [M–2]+ (1.09), 346 (100.00). Anal. Calcd. for C₁₅H₁₃N₅OCl (363.80): C, 62.73; H, 3.88; N, 19.25. Found: C, 62.99; H, 4.01; N, 19.40.

2-((4-Bromophenyl)amino)-7-(4-chlorophenyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (10g)

Canary yellow crystals; yield: 94% (4.14 g), mp: 272°C–275°C; IR (KBr, ν cm⁻¹): 3,409, 3,220 (NH₂, NH), 3,050 (CH-aromatic), 1,636 (C=O) and 1,586, 1,468 (C=C). ¹H-NMR (DMSO-d₆) δ: 6.14–7.94 (m, 8H, 2CH₂), 7.97–7.99 (s, 2H, NH₂), 8.11 (s, 1H, CH pyrimidine), 8.25 (s, 1H, CH pyrimidine), 9.72 (s, 1H, NH). Anal. Calcd. for C₁₅H₁₃N₅OBrCl (442.70): C, 51.55; H, 2.96; N, 15.82. Found: C, 51.69; H, 3.01; N, 15.97.

7-(4-Bromophenyl)-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (10h)

Canary yellow crystals; yield: 99% (4.04 g), mp: 275°C–278°C; IR (KBr, ν cm⁻¹): 3,400, 3,378 (NH₂), 3,271 (NH), 3,168 (CH-aromatic), 1,656 (C=O) and 1,596, 1,450 (C=C). ¹H-NMR (DMSO-d₆) δ: 6.94–7.92 (m, 9H, C₆H₅, C₆H₅), 8.18–8.20 (s, 2H, NH₂), 8.69 (s, 1H, CH pyrimidine), 8.70 (s, 1H, CH pyrimidine), 9.67 (s, 1H, NH). MS (EI): m/z (%) 410 [M+2]+ (13.48), 409 [M+1]+ (61.29), 408 [M]+ (15.20), 407 [M–1] (60.21), 406 [M–2]+ (1.25), 390 (100.00). Anal. Calcd. for C₁₅H₁₃N₅OBr (408.25): C, 55.90; H, 3.46; N, 17.15. Found: C, 56.10; H, 3.80; N, 17.30.

7-(4-Bromophenyl)-2-((4-bromophenyl)amino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (10i)

Yellow crystals; yield: 85% (4.14 g), mp: 197°C–200°C; IR (KBr, ν cm⁻¹): 3,406, 3,350 (NH₂), 3,270 (NH), 3,169 (CH-aromatic), 1,635 (C=O) and 1,588, 1,468 (C=C). ¹H-NMR (DMSO-d₆) δ: 6.13–7.79 (m, 8H, 2CH₂), 7.89–7.92 (s, 2H, NH₂), 8.70 (s, 1H, CH pyrimidine), 8.81 (s, 1H, CH pyrimidine), 9.73 (s, 1H, NH). Anal. Calcd. for C₁₅H₁₃N₅OBrCl (501.87): C, 54.70; H, 3.46; N, 17.15. Found: C, 54.49; H, 3.92; N, 17.35.
7-(4-Methoxyphenyl)-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (10k)

Yellow crystals; yield: 76% (2.60 g), mp: 257°C–260°C; IR (KBr, v cm⁻¹): 3,407, 3,315 (NH), 3,322 (NH), 3,215 (CH-amine), 1,653 (C=O) and 1,599, 1,450 (C=C). ¹H-NMR (DMSO-d₆) δ: 3.92 (s, 3H, CH₃), 8.62 (s, 1H, CH pyrimidine), 7.83 (s, 1H, CH pyrimidine), 7.92 (s, 1H, NH). MS (EI): m/z (%) 345 [M+2]⁺ (2.17), 344 [M+1]⁺ (17.27), 343 [M⁺] (70.81), 325 [M-2]⁻ (0.12), 324 (100.00). Anal. Calcd. for C₂₀H₁₅NO₂ (343.38): C, 69.96; H, 4.99; N, 20.04. Found: C, 69.71; H, 4.67; N, 20.73.

2-((4-Bromophenyl)amino)-7-(p-tolyl)pyrazolo[1,5-a]pyrimidine-3-carboxamide (10m)

Yellow crystals; yield: 99% (4.20 g), mp: 217°C–220°C; IR (KBr, v cm⁻¹): 3,400, 3,315 (NH), 3,260 (NH), 3,156 (CH-amine), 1,644 (C=O) and 1,590, 1,470 (C=C). ¹H-NMR (DMSO-d₆) δ: 3.12 (s, 3H, CH₃), 6.11–7.65 (m, 8H, 2C₆H₅), 8.12–8.14 (s, 2H, NH₂), 8.64 (s, 1H, CH pyrimidine), 8.66 (s, 1H, CH pyrimidine), 9.70 (s, 1H, NH). Anal. Calcd. for C₂₀H₁₅NO₂Br (422.28): C, 56.89; H, 3.82; N, 16.58. Found: C, 57.14; H, 4.02; N, 16.70.

7-(2-Hydroxyphenyl)-2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide (10n)

Faint brown crystals; yield: 65% (2.26 g), mp: 207°C–210°C; IR (KBr, v cm⁻¹): 3,442, 3,415 (NH), 3,351 (NH), 3,159 (CH-amine), 1,662 (C=O) and 1,583, 1,447 (C=C). ¹H-NMR (DMSO-d₆) δ: 6.70–7.20 (m, 9H, C₆H₅), 7.32–7.33 (s, 2H, NH₂), 8.88 (s, 1H, CH pyrimidine), 8.99 (s, 1H, CH pyrimidine), 8.98 (s, 1H, NH), 11.01 (s, 1H, OH). Anal. Calcd. for C₁₉H₁₄NO₂ (353.35): C, 66.08; H, 4.38; N, 20.28. Found: C, 66.22; H, 4.01; N, 20.45.

Docking studies and structure–activity relationship

In the absence of a crystal structure, homology models of *Bacillus subtilis* (Bsu) MurC (Accession No:NP_390857), *Pseudomonas aeruginosa* (Pae) MurC (Accession No: B7UZI9), and *Staphylococcus aureus* (Sau) MurC (Accession No:A6U2K6) were built based on the published cocrystal structure of *Escherichia coli* (Eco) MurC (pdb ID: 2F00; Figure 2). The sequence identities between *Eco* MurC and *Bst* MurC, *Pae* MurC, and *Sau* MurC were found to be 26%, 56%, and 25%, respectively. The *E. coli* ATP binding site (126-GTHGKTSTT₁³₂) was conserved by >85% with *B. subtilis* (126-GAHGTKTSTT₁³⁴), *P. aeruginosa* (₁²₂-GTHGKTSTT₁³₂), and *S. aureus* (₁₀⁶-GAHGKTSTT₁³⁶). The model was validated using protein Preparation Wizard and minimized prior to docking. Docking of a set of pyrazolopyrimidines was carried out in the ATP binding site using Schrodinger 16.4 software Glide (XP) extra precision module from Schrodinger. The best Docking Score is obtained as the most negative value for the active ligands. All the compounds were constructed using the fragment library of Maestro 9.2, and all compounds were prepared by using the LigPrep 2.9. Glide docking parameters were set to the default hard potential function. No constraints were applied for all the docking studies. Structure–activity relationship (SAR) analysis was performed using R-Group Analysis.

Antimicrobial evaluation

The antimicrobial activities of the samples tested were studied on Mueller–Hinton agar plates by the disc diffusion technique against Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strain. Ampicillin (AM 20 µg) was used as the standard antibacterial agent obtained from Bioanalyse® Ltd. (Ankara, Turkey). Sterile Whatman filter paper discs (6 mm) were individually impregnated with 10 µL of solvent (distilled water, chloroform, DMSO) containing 20 µg concentration of each sample at a pH value of 6. All the discs were dried aseptically and placed on the surface of Mueller–Hinton agar plates.
agar plates seeded with $1.8 \times 10^8$ cfu/mL (0.5 OD$_{600}$) of the test bacteria. Following 24 h incubation at 37°C, plates were examined for the presence of inhibition zones. The inhibition zones surrounding the disks were measured (mm) considering only halos $>6$ mm. Inhibition zones obtained are the mean of three replicates for each experiment.

**Results and discussion**

**Chemistry**

In this study, 5-aminopyrazoles 2b was found to react with the sodium salts of (hydroxymethylene)-cycloalkanones 7a–d in acetic acid-piperidine acetate to give adduct for which structure 8a–h is set. The reaction starts with an initial nucleophilic attack from the external amino group to the formyl group followed by cyclization and then removal of one molecule of water to produce angular three-ring compounds 8a–h. This requires that in the presence of acidic medium, it occurs by first protonation of the ring nitrogen which is the most nucleophilic center in the compound 2 and directs the exocyclic amino group to attack the unhindered formyl group of 4 to give compounds 8a–h (Scheme 1).

The structures of later compounds were confirmed by the spectral data and elemental analysis. Thus, the IR spectrum of compound 8a, as an example of this series, revealed the presence of three bands at $\nu$ 3,378, 3,301, and 3,145 cm$^{-1}$ for NH$_2$ and NH groups and a characteristic C=O band at $\nu$ 1,651 cm$^{-1}$. Moreover, $^1$H NMR of 8a showed the existence of a signal at $\delta$ 8.54 ppm assigned for pyrimidine-H proton, two multiplets at a range of $\delta$ 2.52–3.33 ppm assigned for three CH$_2$ groups, and two broad singlets at $\delta$ 7.48–7.57 and 9.69 ppm assigned for NH$_2$ and NH groups. The behavior of the 5-aminopyrazoles 2b toward sodium salts of unsaturated keto compounds 9a–h was also studied: the pyrazolopyrimidine compounds 10a–n were obtained by cyclic condensation of 2b with 9a–h in acetic acid-piperidine acetate (Scheme 2). The structure of the 10a–n reaction products was confirmed by spectral data and elemental analysis (IR, $^1$H NMR, MS). Thus, analytical data were revealed for 10a molecular formula C$_{14}$H$_{13}$N$_5$O (M$^+$ 267). The IR spectrum of compound 10a revealed the presence of three bands at $\nu$ 3,395, 3,271, and 3,161 cm$^{-1}$ for NH$_2$ and NH groups and a characteristic C=O band at $\nu$ 1,651 cm$^{-1}$. Also, $^1$H NMR...
revealed a multiplet at δ 7.38–7.58 ppm assignable to the aromatic protons and two signals at δ 8.52 and 8.98 ppm assignable for two pyrimidine CH protons. These results obtained in this study, when combined with our previous results, show that the reaction of 5-aminopyrazoles with sodium salts of (hydroxymethylene)-cycloalkanones and sodium salts of unsaturated aliphatic ketones can be used as a new and effective method in the preparation of many important pyrazolo[1,5-a]pyrimidine derivatives and their cycloalkane ring-fused derivatives.

Antimicrobial evaluation and the structure–activity relationship

Mur ligases play a vital role in the bio-bacterial peptidoglycans. Mur ligases play an important role in the biosynthesis of the cell wall peptidoglycan. Many enzymes stimulate the early stages of the pathogenesis of the peptidoglycan named MurA to MurF. MurC, the third enzyme in Mur ligases of the peptidoglycan pathway, initiates the synthesis of pentapeptide precursor where the L-alanine binds to the UDP-N-acetylmuramic acid converting to UDP-N-acetylμuramic acid-L-alanine. The reactivity of all the newly synthesized products against bacterial and fungi species was evaluated through Table 1. Pyrazolopyrimidine compounds possess bactericidal activity against both Gram-negative and Gram-positive MurC enzymes.

Compounds 8b, 10e, 10i, and 10n were found to be the most active compounds against Gram-positive and Gram-negative bacterial species (Figure 3). The presence of the two moieties of 4-Br-C₂H₄ in compound 10i increased the reactivity of the compound when comparing with ampicillin as a standard reference.

The effects of substituents (R₁, R₂, and R₃) in the pyrazolopyrimidine-3-carboxamide region were investigated, and a series of derivatives are summarized in Table 2 and Figure 4. Compound 7-(4-bromophenyl)-pyrazolopyrimidine-3-carboxamide (10i) with 17-Br and 13-bromophenyl substituents exhibited most inhibitory effect for MurC ligase of Gram-negative and Gram-positive bacteria (Table 2).

To investigate the binding mode of 10i, it was docked into the active site of MurC ligase of Bacillus subtilis. As shown in Figure 5A, compound 10i interacted with extended conformation. The bromophenyl group formed face-to-face π–π interactions with His263. The carbonyl oxygen atom formed hydrogen bonding interaction with a side chain of Ser275. However, compound 10i formed hydrogen bonding interaction with Gly205 in the case of S. aureus (Figure 5B). MurC ligase of E. coli formed hydrogen bonding interaction

Table 1 Reactivity of the newly synthesized products against bacterial and fungi species

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<th>Compd number</th>
<th>Inhibition zone diameter (mm/mg sample)</th>
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<td>Amphtericin B</td>
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Note: Solvent used: DMSO solutions.
**Figure 3** The most active synthesized products against Gram-positive and Gram-negative bacterial species.

**Abbreviations:** G+, Gram positive; G−, Gram negative; B. S., Bacillus subtilis; S. A., Staphylococcus aureus; E. C., Escherichia coli; P. A., Pseudomonas aeruginosa.

**Table 2** SAR activates for MurC ligase of 2-(phenylamino)pyrazolo[1,5-a]pyrimidine-3-carboxamide moiety

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(Continued)
Table 2 (Continued)

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Abbreviation: SAR, structure–activity relationship.

with Asn 194 (Figure 5C). *P. aeruginosa* MurC ligase formed 3 H-bonding interactions with Gln 318, Gln 325, and Val 326 (Figure 5D). However, 7-(4-bromophenyl)-pyrazolopyrimidine-3-carboxamide (10h) showed only inhibitory activities against *Bacillus subtilis*. 7-(2-hydroxyphenyl)-pyrazolopyrimidine-3-carboxamide (10n) containing 2-hydroxyphenyl possessed good inhibitory activities for all MurC ligases of Gram-positive and Gram-negative bacteria (Table 2). Compounds bearing R3 Br-substituents of the 2-(phenylamino)-pyrazolopyrimidine-3-carboxamide displayed better potency for the MurC ligase than those without substituents at the same positions. However, introducing Br-substituents to 2-((4-bromophenyl)amino)-7-(p-tolyl)pyrazolopyrimidine-3-
Carboxamide (10m) removed its inhibitory activities in comparison with 2-(phenylamino)-7-(4-methylphenyl) pyrazolopyrimidine-3-carboxamide (10l; Table 2). Compounds 8c and 8g did not show any inhibitory activities for MurC ligase of Gram-negative and Gram-positive bacteria (Table 2). However, Br-substitution of compounds 8d and 8h evidently increased their inhibitory effects for MurC ligase (Table 2).

**Figure 5** Binding mode analysis of (10i) with MurC ligase (A) Bacillus subtilis, (B) Escherichia coli K12, (C) Pseudomonas aeruginosa, and (D) Staphylococcus aureus.
Conclusion
The conclusion of this study was summarized through the reaction of 5-aminopyrazoles with different sodium salts of (hydroxymethylene) cycloalkanones and sodium salts of unsaturated ketones to obtain the novel pyrazolo[1,5-a] pyrimidine derivatives and their corresponding cycloalkane ring-fused derivatives. The newly synthesized compounds were evaluated according to their antibacterial and antifungal activities. The evaluations showed that compounds 8b, 10e, 10i, and 10n were the most active compounds against Gram-positive and Gram-negative bacterial strains.

Author contributions
GHE and AEMA conceived, designed, and performed the experiments; GHE and AEMA analyzed the data, contributed reagents/materials/analysis tools; GHE and AEMA wrote and approved the final manuscript.

Disclosure
The authors report no conflicts of interest in this work.

References


