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ORIGINAL PAPER

Pyrimidines as block units in heterocycles: novel synthesis of pyrimidines and condensed pyrimidine derivatives

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Abstract
Compound **1** condensed with benzaldehyde to produce styryl pyrimidine **2**. Pyridopyrimidines **5**, **8** and **10** resulted from [4 + 2] cycloaddition (condensation) of **1** with malononitrile, ethyl cyanoacetate and/or ethyl acetoacetate. Compound **1** was also concerted to pyridopyrimidine **14** through multistep reaction (hydrolysis, chlorination, isothiocyanate formation and intramolecular cyclization). Bromopyrimidine **15** transformed into thiazolopyrimidine **17**. Cyclization of bromomethyl pyrimidine **15** resulted in imidazolopyrimidine **19**. Upon reacting the compound **15** with ammonium acetate resulted in amination affording the amino derivative **20**. Oxazolopyrimidine **21** was obtained as the result of the reaction of compound **1** with chloroacetic acid and *p*-nitrobenzaldehyde. Compound **21** was transformed into pyridopyrimidine **23** and pyridopyrimidine of type **25** when reacted with cyanoacetamide and/or phenylhydrazine, respectively. All of the tested compounds showed good microbial activity against pathogenic microorganisms especially pyridopyrimidine derivative **21**.

Keywords Pyridopyrimidines · Thiazolopyrimidine · Bromopyrimidine · Oxazolopyrimidine

Introduction

Pyrimidines are heterocyclic aromatic products containing two nitrogen atoms and consider as a great interest because they constitute an important class of natural and non-natural products, Pyrimidines are a standout among the most vital classes of biologically active [1–4]. It is an essential part of DNA and RNA thus broadly conveyed in living beings [5]. Because of their inclusion as bases in DNA and RNA, they have turned out to be imperative in the world of manufactured organic chemistry. Over the last decades, condensed pyrimidine compounds have been reported as antitumor [6, 7], analgesic, antiviral, anti-inflammatory [8, 9], anticancer [10–14], antimicrobial [15, 16], anti-HIV [17, 18], antineoplastic [19], antitubercular [20, 21], diuretic [22], antagonists of the human A_2A adenosine receptor [23, 24] and calcium-sensing receptor antagonists [25].

Because of the wide applicability of these heterocycles, compounds and its novel derivatives encouraged and enhance the chemists to contribute and synthesize a large number of biologically active novel drugs and introduce some easy and efficient methods. From this point, we will discuss and study new methods of synthesis of new condensed pyrimidine derivatives.

Experimental

Chemistry

Melting points were measured using an Electrothermal IA 9100 equipment with an open capillary tube and were uncorrected. All experiments were done using dry solvents. TLC was performed on Merck Silica Gel 60F254 with detection by way of UV light. The formed compounds had been purified using recrystallization. The IR spectra (KBr disk) were recorded on a Pye Unicam Sp-3-300 or a Shimadzu FTIR 8101 PC infrared spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were measured on a JEOL-JNM-LA 400 MHz spectrometer using DMSO-d6 as a solvent. All chemical shifts had been expressed on the δ (ppm) scale using TMS as an internal well-known reference. The coupling constant

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(*J*) values are given in Hz. Analytical information was acquired from the Microanalysis center at Cairo University, Giza, Egypt.

Ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1) A mixture of ethyl acetoacetate (1.30 g, 10 mmol), benzaldehyde (1.06 g, 10 mmol), urea (0.60 g, 10 mmol) and 4 drop conc. HCl in ethanol (20 ml) was refluxed for 8 h. After cooling at room temperature, the formed precipitate filtered off, then collected and recrystallized from ethanol to afford a pure white powder [26]; yield, 81%; MP: 204–205 °C; IR (KBr, cm^{-1}): 3244 (NH), 3113 (NH), 1724 (C=O ester), 1701 (C=O pyrimidine ring), 1219 (C−O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.11 (3H, t, $J=8$ Hz, $CH_2\underline{CH_3}$), 2.25 (3H, s, CH_3), 4.00 (2H, q, $J=8$ Hz, $\underline{CH_2}CH_3$), 5.15 (1H, s, Ph−CH), 7.22−7.34 (5H, m, ArH's), 7.73 (1H, s, NH exch. with D_2O), 9.19 (1H, s, NH exch. with D_2O). Elemental anal. calcd. for $C_{14}H_{16}N_2O_3$ (260.29): C, 64.60; H, 6.20; N, 10.76%. Found C, 64.48; H, 6.07; N, 10.71%.

Ethyl 2-oxo-4-phenyl-6-styryl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2) A mixture of compound **1** (2.78 g, 10 mmol), benzaldehyde (1.06 g, 10 mmol) and catalyst (6.48 g ferric chloride powder) in acetonitrile (20 ml) was refluxed for 16 h. After cooling at room temperature, the formed precipitate filtered off, then collected and recrystallized from ethanol/water (50:50) to afford a pure beige powder [27]; yield 88%; MP: 189–190 °C; IR (KBr, cm^{-1}): 3402 (NH), 3244 (2NH), 1685 (C=O), 1639 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz δ: 1.18 (3H, t, $J=8$ Hz, $CH_2\underline{CH_3}$), 4.07 (2H, q, $J=8$ Hz, $\underline{CH_2}CH_3$), 5.27 (1H, s, Ph−CH), 7.27−7.92 (12H, m, ArH's, olefinic proton), 7.96 (1H, s, NH exch. with D_2O), 9.23 (1H, s, NH exch. with D_2O). Elemental anal. calcd. for $C_{21}H_{20}N_2O_3$ (348.40): C, 72.40; H, 5.79; N, 8.04%. Found: C, 72.28; H, 5.66; N, 8.10%. ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 165.7, 153.0, 144.9, 144.6, 136.3, 135.2, 129.6, 129.4, 129.0, 128.8, 128.7, 127.6, 126.7, 102.5, 60.3, 54.4 and 14.4.

8-Amino-4-(ethoxycarbonyl)-1-oxo-3,6-diphenyl-2,3-dihydro-1H-pyrido[1,2-c]pyrimidine-7-carboxylic acid (5) A mixture of compound **2** (3.48 g, 10 mmol), malononitrile (0.66 g, 10 mmol), sodium acetate (1.23 g, 15 mmol) and 3 ml of Ac_2O in acetic acid (25 ml) was refluxed for 16 h. After cooling at room temperature, the formed precipitate filtered off, then collected and recrystallized from ethanol/water (50:50) to afford a pure beige powder; yield, 90%; MP: 177–178 °C; IR (KBr, cm^{-1}): 3394−3086 br. (OH, NH and NH_2), 1685 (C=O), 1639 (C=O), 1507 (acid C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.18 (3H, t, $J=8$ Hz, $CH_2\underline{CH_3}$), 4.07 (2H, q, $J=8$ Hz, $\underline{CH_2}CH_3$), 5.27 (1H, s, Ph−CH), 7.29−7.92 (11H, m, ArH's, olefinic proton), 7.97 (2H, s, NH_2 exch. with D_2O), 9.23 (1H, s, NH exch. with D_2O), 12.44 (1H, s, OH exch. with D_2O). Elemental anal. calcd. for $C_{24}H_{21}N_3O_5$ (431.45): C, 66.81; H, 4.91; N, 9.74%. Found: C, 66.72; H, 4.86; N, 9.67%.

Ethyl 8-amino-1-oxo-3,6-diphenyl-2,3-dihydro-1H-pyrido[1,2-c]pyrimidine-4-carboxylate (8) A mixture of compound **2** (3.48 g, 10 mmol), ethyl cyanoacetate (1.13 g, 10 mmol), sodium acetate (1.23 g, 15 mmol) and 3 ml of Ac_2O in acetic acid (25 ml) was refluxed for 16 h. After cooling at room temperature, the formed precipitate filtered off, then collected and recrystallized from ethanol/water (50:50) to afford a pure light brown; yield, 90%; MP: 178–179 °C; IR (KBr, cm^{-1}): 3236 (NH), 3086 (NH_2), 1685 (C=O), 1639 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.18 (3H, t, $J=8$ Hz, $CH_2\underline{CH_3}$), 4.07 (2H, q, $J=8$ Hz, $\underline{CH_2}CH_3$), 5.27 (1H, s, Ph−CH), 7.27−7.92 (11H, m, ArH's, olefinic proton), 7.96 (2H, s, NH_2 exch. with D_2O), 9.23 (1H, s, NH exch. with D_2O). Elemental anal. calcd. for $C_{23}H_{21}N_3O_3$ (387.44): C, 71.30; H, 5.46; N, 10.85%. Found: C, 71.18; H, 5.39; N, 10.78%. ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 165.6, 153.0, 145.0, 144.7, 136.3, 135.2, 133.9, 131.2, 129.6, 129.4, 129.0, 127.9, 127.6, 126.7, 120.0, 102.5, 60.3, 54.4 and 14.4.

Ethyl 7-acetyl-8-hydroxy-1-oxo-3,6-diphenyl-2,3-dihydro-1H-pyrido[1,2-c]pyrimidine-4-carboxylate (10) A mixture of compound **2** (3.48 g, 10 mmol), ethyl acetoacetate (1.30 g, 10 mmol), sodium acetate (1.23 g, 15 mmol) and 3 ml of Ac_2O in acetic acid (25 ml) was refluxed for 16 h. After cooling at room temperature, the formed precipitate filtered off, then collected and recrystallized from ethanol/water (50:50) to afford a pure white powder; yield, 88%; MP: 165–166 °C; IR (KBr, cm^{-1}): 3304−3232 br. (OH & NH), 1685 (C=O), 1639 (C=O), 1597 (acid C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.18 (3H, t, $J=8$ Hz, $CH_2\underline{CH_3}$), 2.51 (3H, s, CH_3), 4.07 (2H, q, $J=8$ Hz, $\underline{CH_2}CH_3$), 5.27 (1H, s, Ph−CH), 7.27−7.96 (11H, m, ArH's, olefinic proton), 9.23 (1H, s, NH), 12.34 (1H, s, OH). Elemental anal. calcd. for $C_{25}H_{22}N_2O_5$ (430.46): C, 69.76; H, 5.15; N, 6.51%. Found: C, 69.64; H, 5.11; N, 6.43%.

4-Phenyl-7-thioxo-4,6,7,8-tetrahydropyrido[4,3-d]pyrimidine-2,5(1H,3H)-dione (14) 6-Methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro pyrimidine-5-carboxylic acid (2.32 gm, 10 mol) and 16 ml of thionyl chloride were left on reflux for 35 mints on a water bath [28–30]. The mixture was heated on a water bath to remove the unreacted thionyl chloride. The formed acid chloride was treated with ammonium isothiocyanate (0.76 gm, 10 mol) in dioxane (25 ml) and then was refluxed for 6 h. After cooling at room temperature, the formed precipitate filtered off, then collected and recrystallized from acetone to afford compound **14**. Light

Brown powder; yield, 73%; MP: 289–290 °C; IR (KBr, cm^{-1}): 3390−3244 3(NH), 1693 br. 2 (C = O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 2.51 (2H, s, CH_2), 5.54 (1H, s, CH Ph), 7.29−7.40 (5H, m, ArH's), 8.00 (1H, s, NH), 8.10 (1H, s, NH), 8.25 (1H, s, NH). Elemental anal. calcd. for $C_{13}H_{11}N_3O_2S$ (273.31): C, 57.13; H, 4.06; N, 15.37%. Found: C, 57.06; H, 4.01; N, 15.29; %.

Ethyl 6-(bromomethyl)-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (15) The compound **1** (2.78 g, 10 mmol) with 20 ml of glacial acetic acid was placed in a 250-ml flask, and then, 1.59 g (10 mmol, 0.498 ml) of bromine in 10 ml glacial acetic acid was added slowly to the flask under stirring. The temperature was maintained to be from 0 to 5 C. After cooling at room temperature, the mixture was poured into 450 ml water. The formed precipitate filtered off, then collected and recrystallized from ethanol to afford the target compound [31]. White powder; yield, 85%; MP: 149–150 °C; IR (KBr, cm^{-1}): 3379 (NH), 3213 (NH), 1685 (C=O), 1635 (C=O). ^{1}H-NMR (DMSO-d6, 400 MHz) δ: 1.14 (3H, t, $J = 8$ Hz, $CH_2\underline{CH_3}$), 4.07 (2H, q, $J = 8$ Hz, $\underline{CH_2}CH_3$), 4.66 (2H, d–d, J = 8 Hz, J = 8 Hz, CH_2), 5.19 (1H, s, Ph−CH), 7.23−8.01 (5H, m, ArH's,), 9.46 (1H, s, NH), 9.77 (1H, s, NH). Elemental anal. calcd. for $C_{14}H_{15}BrN_2O_3$ (339.19): C, 49.58; H, 4.46; N, 8.26%. Found: C, 49.48; H, 4.37; N, 8.17.

Ethyl 3,5-dioxo-7-phenyl-5,6,7,8-tetrahydro-3H-thiazolo[3,4-c]pyrimidine-8-carboxylate (17) A mixture of compound **15** (3.39 g, 10 mmol) and ammonium thiocyanate (0.76 g, 10 mmol) in butanol (25 ml) was refluxed for 8 h. After cooling, the mixture was poured into water. The formed precipitate filtered off, then collected and recrystallized from ethanol/water (50:50) to afford a pure light yellow powder; yield, 67%; MP: 161–163 °C; IR (KBr, cm^{-1}): 3309 (NH), 1701 (C=O), 1685 (C=O), 1639 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.28 (3H, t, $J = 8$ Hz, $CH_2\underline{CH_3}$), 4.16 (2H, q, $J = 8$ Hz, $\underline{CH_2}CH_3$), 5.16 (1H, d, $J = 4$ Hz, CH pyrimidine ring), 5.21 (1H, d, $J = 4$ Hz, Ph−CH), 7.17−7.79 (6H, m, ArH's, olefinic proton), 9.18 (1H, s, NH). Elemental anal. calcd. for $C_{15}H_{14}N_2O_4S$ (318.35): C, 56.59; H, 4.43; N, 8.80%. Found: C, 56.49; H, 4.37; N, 8.75%.

Ethyl 3,5-dioxo-7-phenyl-2,3,5,6,7,8-hexahydroimidazo-[1,5-c]pyrimidine-8-carboxylate (19) A mixture of compound **15** (3.39 g, 10 mmol) and potassium cyanate (0.81 g, 10 mmol) in butanol (25 ml) was refluxed for 8 h. After cooling, the mixture was poured into water. The formed precipitate filtered off, then collected and recrystallized from ethanol/water (50:50) to afford a pure white powder; yield, 65%; MP: 207–208 °C; IR (KBr, cm^{-1}): 3397 (NH), 3240 (NH), 1701 (C=O), 1685 (C=O), 1639 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.14 (3H, t, $J = 8$ Hz, $CH_2\underline{CH_3}$),, 4.06 (2H, q, $J = 8$ Hz, $\underline{CH_2}CH_3$), 5.18 (1H, d, $J = 4$ Hz, CH pyrimidine ring), 5.22 (1H, d, J = 4 Hz, Ph−CH), 7.30−7.86 (6H, m, ArH's, olefinic proton), 9.23 (1H, s, NH), 9.32 (1H, s, NH). Elemental anal. calcd. for $C_{15}H_{15}N_3O_4$ (301.30): C, 59.80; H, 5.02; N, 13.95%. Found: C, 59.73; H, 5.06; N, 13.90%.

Ethyl 6-(aminomethyl)-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (20) A mixture of compound **15** (3.39 g, 10 mmol) and ammonium acetate (0.77 g, 10 mmol) in ethanol (20 ml) was refluxed for 8 h. After cooling, the mixture was poured into water. The formed precipitate filtered off, then collected and recrystallized from ethanol to afford a pure white powder; yield, 73%; MP: 180–181 °C; IR (KBr, cm^{-1}): 3371 (NH), 3205 (NH), 3097 (NH_2), 1701 (C=O), 1685 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.11 (3H, t, $J = 8$ Hz, $CH_2\underline{CH_3}$), 2.5 (2H, s, CH_2),4.07 (2H, q, $J = 8$ Hz, $\underline{CH_2}CH_3$), 5.18 (1H, s, Ph−CH), 7.23−7.94 (7H, m, ArH's, NH_2 proton), 8.01 (1H, s, NH exch. with D_2O), 9.77 (1H, s, NH exch. with D_2O). Elemental anal. calcd. for $C_{14}H_{17}N_3O_3$ (275.31): C, 61.08; H, 6.22; N, 15.26%. Found: C, 61.01; H, 6.16; N, 15.19%. ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 164.6, 152.3, 146.8, 143.8, 129.1, 128.3, 126.6, 99.1, 61.22, 54.0, 32.0 and 14.22.

Ethyl 3-hydroxy-7-(4-nitrostyryl)-5-phenyl-5H-oxazolo[3,2-a]pyrimidine-6-carboxylate (21) A mixture of compound **1** (2.78 g, 10 mmol), *p*-nitrobenzaldehyde (1.55 g, 10 mmol), chloroacetic acid (0.94 g, 10 mmol), sodium acetate (1.23 g, 15 mmol) and 3 ml of Ac_2O in acetic acid (25 ml) was refluxed for 12 h. After cooling, the mixture was poured into water. The formed precipitate filtered off, then collected and recrystallized from ethanol to afford a pure light yellow powder; yield, 79%; MP: 192–194 °C; IR (KBr, cm^{-1}): 3244 br. (OH), 1724 (C=O) 1222 (C−O). ^{1}H-NMR (DMSO-d6, 400 MHz) δ: 1.11 (3H, t, $J = 8$ Hz, $CH_2\underline{CH_3}$), 4.00 (2H, q, $J = 8$ Hz, $\underline{CH_2}CH_3$), 5.14 (1H, s, Ph−CH), 7.23−7.72 (12H, m, ArH's, Olefinic 3 protons), 9.18 (1H, s, OH exch. with D_2O). Elemental anal. calcd. for $C_{23}H_{19}N_3O_6$ (433.42): C, 63.74; H, 4.42; N, 9.70. Found: C, 63.71; H, 4.32; N, 9.65%. ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 165.8, 152.5, 148.8, 145.3, 128.8, 128,6, 128.0, 127.9, 127.7, 127.0, 126.7, 126.5, 126.0, 119.1, 99.7, 59.6, 54.4, 18.2 and 14.5.

Ethyl 10-amino-3-hydroxy-8-(4-nitrophenyl)-5-phenyl-5H,11aH-oxazolo[3,2-a]pyrido[1,2-c]pyrimidine-6-carboxylate (23) A mixture of compound **21** (4.33 g, 10 mmol), cyanoacetamide (0.84 g, 10 mmol) and sodium hydroxide (0.39 g, 10 mmol) ethanol (25 ml) was refluxed for 8 h. After cooling, the mixture was poured into HCl/H_2O (1:10). The formed precipitate filtered off, then collected and recrystallized from ethanol/water (50:50) to afford a pure light yellow

powder; yield, 60%; m.p.: 214–216 °C; IR (KBr, cm^{-1}): 3244 br. (OH), 3113 (NH_2), 1701 (C=O) 1222 (C−O). ^{1}H-NMR (DMSO-d6, 400 MHz) δ: 1.11 (3H, t, $J=8$ Hz, $CH_2\underline{CH_3}$), 4.00 (2H, q, $J=8$ Hz, $\underline{CH_2}CH_3$), 5.15 (1H, s, Ph−<u>CH</u>), 5.3 (1H, s, pyrimidine ring proton), 7.23−7.34 (12H, m, ArH's, Olefinic 3 protons), 7.73 (2H, s, NH_2 exch. with D_2O), 9.18 (1H, s, OH exch. with D_2O). Elemental anal. calcd. for $C_{25}H_{22}N_4O_6$ (474.47): C, 63.29; H, 4.67; N, 11.81. Found: C, 63.19; H, 4.61; N, 11.71%. ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 165.8, 165.7, 152.6, 148.7, 145.1, 144.6, 136.3, 135.2, 129.4, 129.0, 128.8, 128.0, 127.6, 126.6, 102.5, 99.8, 60.3, 59.7, 54.4, 18.1 and 14.4.

3-Hydroxy-8-(4-nitrophenyl)-5-phenyl-7-(phenylamino)-5,7-dihydro-6H-oxazolo[3,2-a]pyrido[4,3-d]pyrimidin-6-one (25) A mixture of compound **21** (4.33 g, 10 mmol), phenylhydrazine (1.08 g, 10 mmol), sodium acetate (1.23 g, 15 mmol) and 3 ml of Ac_2O in acetic acid (25 ml) was refluxed for 8 h. After cooling, the mixture was poured into water. The formed precipitate filtered off, then collected and recrystallized from ethanol/water (50:50) to afford a pure light yellow powder; yield, 80%; m.p.: 228–230 °C; IR (KBr, cm^{-1}): 3444 br. (OH), 3298 (NH), 1693 (C=O) 1265 (C−O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 5.15 (1H, s, Ph−<u>CH</u>), 6.82−8.24 (16H, m, ArH's, Olefinic 2 protons), 10.33 (H, s, NH exch. with D_2O), 10.89 (1H, s, OH exch. with D_2O). Elemental anal. calcd. for $C_{27}H_{19}N_5O_5$ (493.48): C, 65.72; H, 3.88; N, 14.19. Found: C, 65.65; H, 3.81; N, 14.12%. ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 146.4, 145.7, 144.8, 143.0, 136.9, 136.2, 134.1, 129.1, 128.8, 128.0, 127.6, 127.0, 126.4, 126.0, 124.5, 120.4, 119.2, 113.0, 112.4, 18.2 and 14.5.

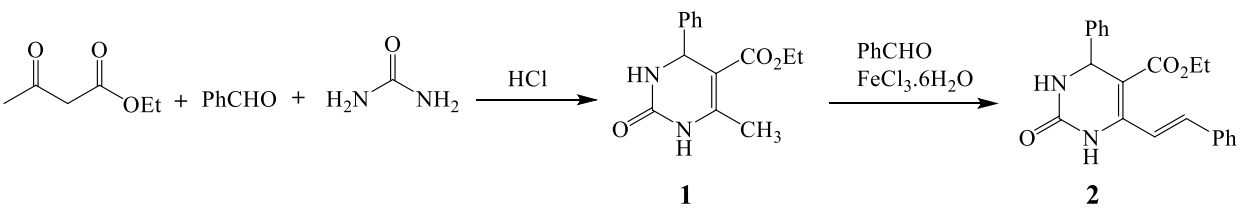
In vitro antimicrobial activities

Antimicrobial activity of the prepared compounds was investigated by a previously reported modified method of Beecher and Wong [32], against different bacterial and fungal species, such as *E. Coli* ATCC11229, listeria ATCC8729, *S. aureus* ATCC6538, *Salmonella typhi* ATCC14028 and *Aspergillus niger* OC10. The tested microorganisms were isolated from Egyptian soil and identified according to the standard mycological and bacteriological keys for identification of bacteria as stock cultures in the microbiology laboratory, Faculty of Science, Zagazig University. The nutrient agar for antibacterial was Müller–Hington agar (30.0% beef extract, 1.75% casein hydrolysate, 0.15% starch and 1.7% agar) for antifungal (3% sucrose, 0.3% $NaNO_3$, 0.1% K_2HPO_4, 0.05% KCl, 0.001% $FeSO_4$, 2% agar–agar) [33] were prepared and then cooled to 47 °C and seeded with tested microorganisms. After solidification, 5 mm diameter holes were punched by a sterile cork borer. The investigated compounds, i.e., ligands and their complexes, were introduced in holes (only 100 µL) after being dissolved in DMSO at 10–3 M. These culture plates were then incubated at 37 °C for 20 h for bacteria and for 5 days at 30 °C for fungi. The activity was determined by measuring the diameter of the inhibition zone (in mm). Microbial growth inhibition was calculated with reference to the positive control, i.e., cefotaxime and amoxicillin/clavulanic. The micro-dilution broth susceptibility assay was used for the evaluation of the minimal inhibitory concentration (MIC). After incubation at 37 °C for 24 h, the first tube without turbidity was determined as the MIC.

Results and discussion

The present work shows an efficient route to synthesize condensed pyrimidines from pyrimidine of activated methyl function. The synthesis of methylpyrimidine of type **1** was carried out using a multicomponent reaction of Biginelli-typed of benzaldehyde, urea and ethyl acetoacetate (Scheme 1) [26]. Compound **1** showed absorption bands at 3244, 3113 cm^{-1}, 1724 cm^{-1} and 1701 cm^{-1} corresponding to 2NH and two C=O groups, respectively. Its ^{1}H NMR spectrum revealed the presence of two downfield (D_2O exchangeable) signals at 9.19 ppm and 7.73 ppm for the 2NH groups in addition to a signal at 5.16 ppm for the PhCH−CH system (pyrimidine ring proton).

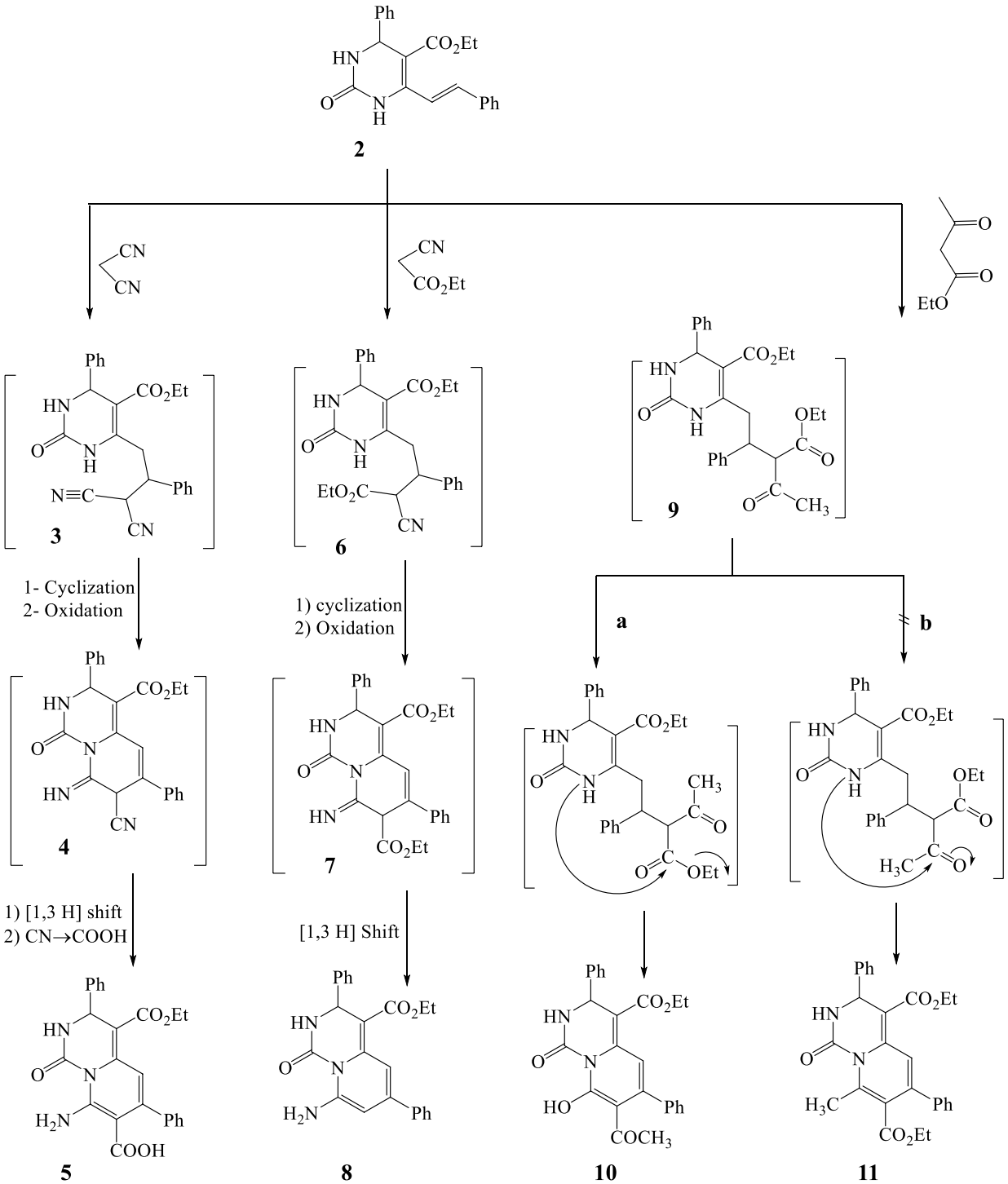
The compound **1** condensed with benzaldehyde in the presence of Lewis acid (iron-catalyzed) to form styryl pyrimidine **4** (Scheme 1) [27]. Compound **4** showed a stretching frequency at 3244 cm^{-1} for NH in addition 1685 cm^{-1} and 1639 cm^{-1} due to the two C=O of a different environment. ^{1}H NMR analysis revealed the absence of H_5 of pyrimidine in addition to the appearance of two (Exchangeable) signals



Scheme 1 Preparation of styryl pyrimidine-5-carboxylate derivative

of 2NH. ^{13}C revealed two downfield signals at 165 and 153 ppm for the two carbonyl groups.

Malononitrile added its nucleophile's methylene to the exocyclic polarized double bond of **2** to furnish pyridopyrimidine **5**. Compound **5** formed from **2** and malononitrile through the formation of non-isolable Michael adduct **3**, intramolecular cyclization via the addition of cyclic imino group to cyano function, oxidation followed by cyanohydrolysis and subsequent [1,3H] shift (Scheme 2). Compound **5** produced stretching frequencies at 3236, 1685, 1639 cm^{-1} for NH and two C=O groups. The ^{1}H NMR of



Scheme 2 Heterocyclization of styryl pyrimidine 2 by [4+2] cycloaddition in acidic medium

target **5** contained three downfield (D_2O Exchangeable) signals for COOH, NH and NH_2 protons.

As depicted in (Scheme 2), ethyl cyanoacetate and compounds **2** underwent [4 + 2] cycloaddition in acidic medium to furnish compound **8**. Compound **8** showed characteristic stretching frequencies at 3236, 1681 and 1639 for NH and the two C=O groups. ^{1}H NMR analysis shows (D_2O Exchangeable) signals at δ 9.23 ppm and 7.96 ppm for NH and NH_2 protons.

Acidic-mediated cyclocondensation of **2** and ethyl acetoacetate produced hydroxyl pyridopyrimidine **10** via pathway **a** while rout **b** not detected (Scheme 2). IR spectrum of **10** displayed characteristic frequencies at 3394 cm^{-1} broad (OH), 1685 cm^{-1} and 1635 cm^{-1} due to the two C=O groups. Its ^{1}H NMR provides two downfield signals at 12.34 ppm and 9.23 ppm for OH and NH groups, respectively.

Upon hydrolysis treatment of **1** with sodium hydroxide and subjected the product to thionyl chloride followed by ammonium thiocyanate resulted in hydrolysis chlorination, followed by formation of isothiocyanate and subsequent addition of CH_3 to heteroallene system leading to pyridopyrimidine **14** (Scheme 3).

Compound **14** provides ^{1}H NMR spectrum involving downfield signals for 3NH. Also, the IR spectrum showed a broad peak at 1693 cm^{-1} for carbonyl groups.

Brominating of pyrimidine **1** resulted in bromomethyl pyrimidine **15** (Scheme 4) [31]. Bromomethyl pyrimidine undergoes substitution reaction with NH_4SCN in butanol to produce thiazole-cyclization **17** in addition to the transesterification. ^{1}H NMR of **17** revealed signals for NH and (PhCH−CH) system. IR spectrum produces a broadband at 1685 cm^{-1} due to C=O groups.

Upon refluxing the compound **15** and KNC=O resulted in imidazole cyclization affording imidazolopyrimidine **19** (Scheme 4). Compound **19** contained two downfield signals for 2NH in addition to (PhCH−CH) protons. IR spectrum displayed stretching frequency broadband at 1685 cm^{-1} due to C=O groups.

Upon dealing bromomethyl pyrimidine **15** with ammonium acetate in refluxing ethanol resulted in debromination **20,** and none of pyrrolopyrimidine was obtained [may be due to the involvement of carbonyl in ring resonance] (Scheme 4). Compound **20** produced two downfield (D_2O Exchangeable) signals at 9.77 ppm and 8.01 ppm due to 2NH protons.

The synthesis of oxazolopyrimidine derivative **21** was performed by reaction of three components at the one-pot reaction of compound **1**, chloroacetic acid and *p*-nitrobenzaldehyde in presence of Ac_2O/CH_3COONa mixture using acetic acid as a solvent (Scheme 5). Compound **21** resonate at high δ value (D_2O Exchangeable) due to OH group. IR showed characteristic peaks at 3244 cm^{-1} and 1647 cm^{-1} due to OH and C=O groups. ^{13}C provided a downfield signal at *δ* 165 ppm and 152 ppm due to carbonyl carbons of the different electronic environment.

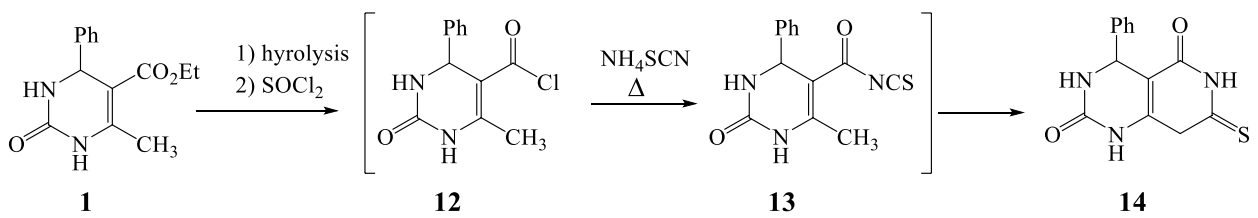
[4 + 2] cycloaddition of cyanoacetamide and **21** generated pyridopyrimidine **23** (Scheme 5). Compound **23** contained a downfield two (D_2O exchangeable) signals at 7.18 ppm and 7.73 ppm due to OH and NH_2 protons. IR spectrum showed a stretching frequency at 3244 cm^{-1}, 1705 cm^{-1} and 1647 cm^{-1} for NH, CO and C=N groups. ^{13}C produced a downfield signal at 165 ppm for C=O group.

Pyrimidine of type **21** was subjected to react with phenylhydrazine to produce pyridopyrimidine **25**. The chemical shift of protons was located at 10.89 ppm, 10.33 (D_2O exchangeable) for OH and NH groups. Also, the disappearance of ester protons potentiates the proposed structure. IR spectrum showed a signal at 1639 cm^{-1} for C=O group. ^{13}C produced a signal at 196 ppm for CO group.

Screening of antimicrobial activity

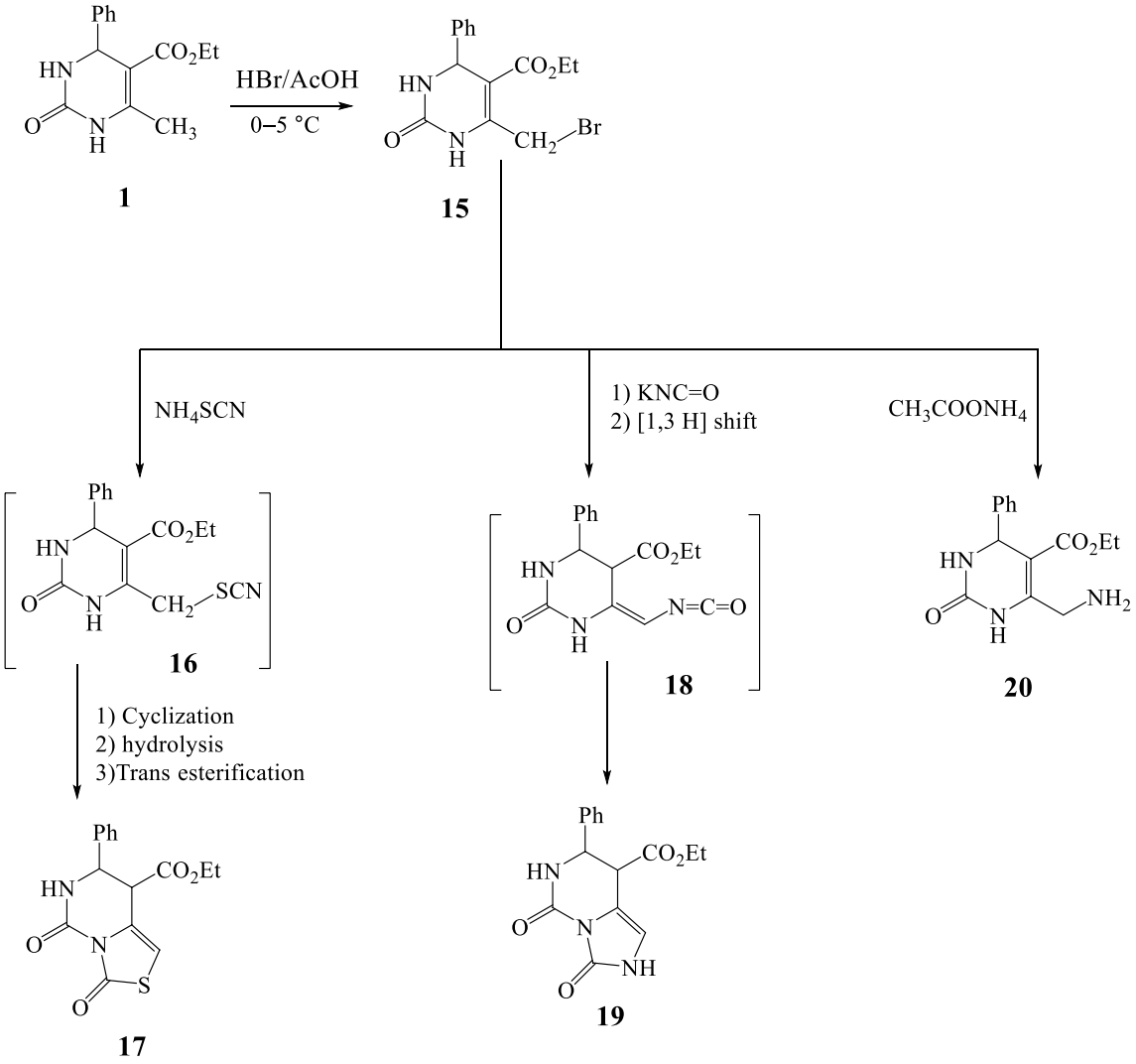
Some synthesized compounds were assessed by screening them in vitro growth inhibitory activity contrary to the variety of strains of bacteria and fungi. The susceptibility of certain strains of bacterium, such as, *E. Coli* ATCC11229, *Listrea* ATCC8729, *S. aureus* ATCC6538, and *Salmonella typhi* ATCC14028 and antifungal screening was studied against, *Aspergillus niger* screening were studied against toward compounds **1**, **2**, **8**, **15**, **20** and **21** and judged by measuring size of the inhibitions diameter (Table 1). The results of the antibacterial study of these compounds have inhibitory action against all types of bacteria and fungi (Fig. 1).

All the tested compounds have antibacterial activity against Gram-negative (*Escherichia coli*) and Gram-positive



1 **12** **13** **14**

Scheme 3 Formation of pyridopyrimidine 14



Scheme 4 Synthesis of fused pyrimidine derivatives

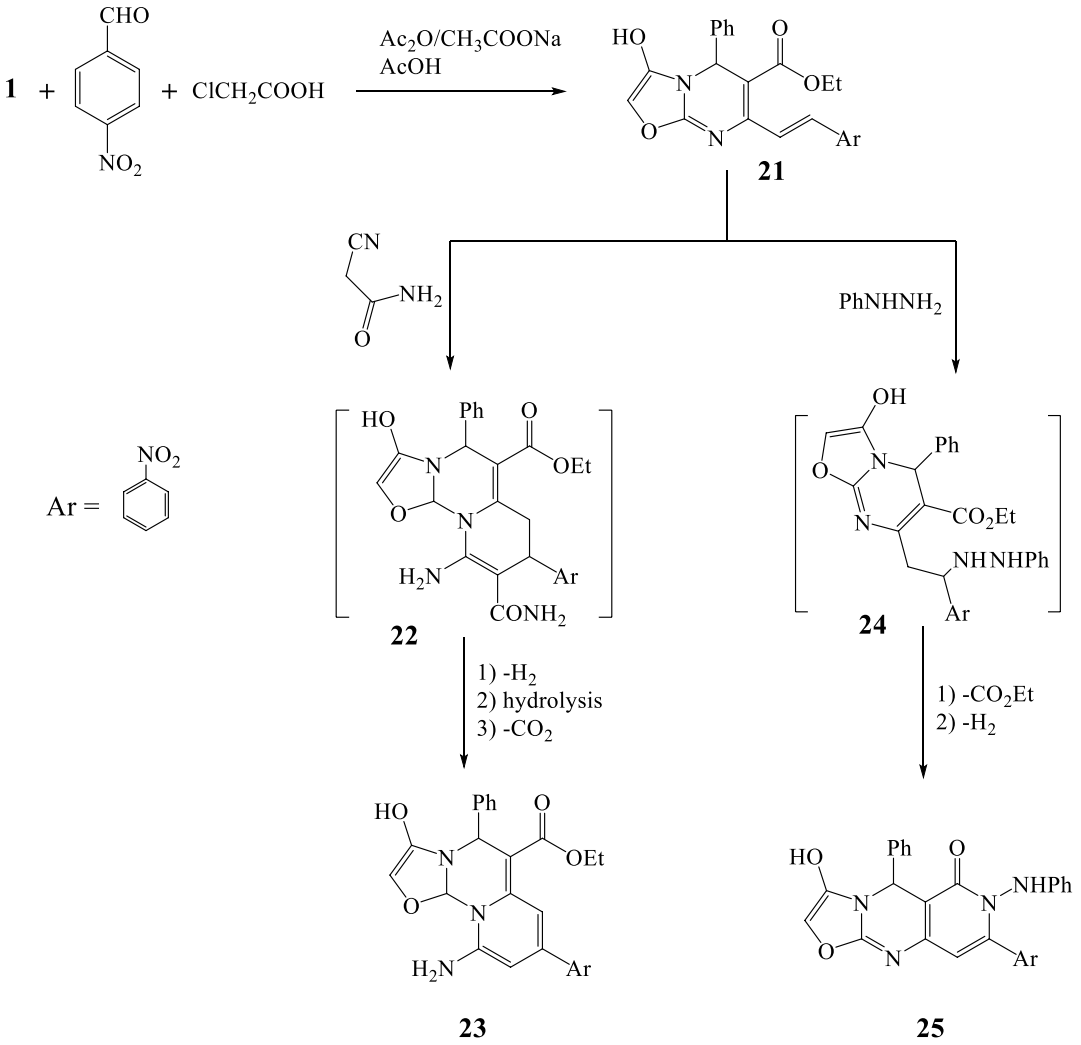
(*Staphylococcus aureus*) bacteria except compound **20** which had antibacterial activity against Gram-negative bacteria only. Compound **21** showed the highest antibacterial activity. All the tested compounds had no antifungal activity.

Determination of MIC for the most sensitive organisms

The antimicrobial potency of the synthetic compounds was determined against the most sensitive bacteria and fungi (Table 2 and Fig. 2). The lowest MIC against these compounds recorded the data in (Tables 2, 3, 4, 5, 6). These results ensured that the activity of synthetic compound **21** on the pathogenic bacteria and fungi showed the most sensitive pathogens dedicated the minimum inhibitory concentration (MIC).

Conclusion

Our present investigation is centered on the studies of synthesis, reactions, spectral analysis and biological activities of condensed pyrimidine derivatives. The procedure proved more beneficial than those previously reported in the literature. Compound **21** exhibited the most potent antibacterial. The findings demonstrate the potential for condensed pyrimidine derivatives to serve as lead compounds for further development as medicinal agents.



Scheme 5 The synthesis of oxazolopyrimidine and pyridopyrimidine derivatives

Table 1 Averages of inhibition growth diameter (mm) of the tested compounds and standards against selected Gram-positive, Gram-negative bacteria and one fungi strain

Compounds	Microorganisms				
	Fungi	Gram-negative bacteria		Gram-positive bacteria	
	A. niger	*Listeria*	*E. coli*	*S. auruas*	*S. typhi*
1	NA	$5^{+3} \pm 0.12$	$3^{+1} \pm 0.14$	$9^{+3} \pm 0.28$	NA
15	NA	$7.5^{+3} \pm 0.5$	$6^{+3} \pm 0.24$	$7^{+3} \pm 0.24$	NA
21	NA	$5^{+3} \pm 0.21$	$4.5^{+2} \pm 0.19$	$3^{+1} \pm 0.11$	$2^{+1} \pm 0.1$
8	NA	$4^{+2} \pm 0.13$	$3^{+1} \pm 0.16$	$7.5^{+3} \pm 0.23$	NA
20	NA	NA	$4^{+2} \pm 0.13$	0	NA
2	NA	$3^{+1} \pm 0.11$	$5^{+3} \pm 0.18$	$9^{+3} \pm 0.3$	NA
Amoxicillin/clavulanic	NA	1.7^{NS}	1^{NS}	NA	1.4^{NS}
Cefotaxime	NA	NA	NA	NA	NA

NA no activity, data are expressed in the form of mean ± standard deviation (SD). Statistical significance P^{NS} *P* not significant, $P < 0.05$; P^{+1} *P* significant, $P > 0.05$; P^{+2} *P* highly significant, $P > 0.01$; P^{+3} *P* very highly significant, $P > 0.001$; student's *t*-test (Paired)

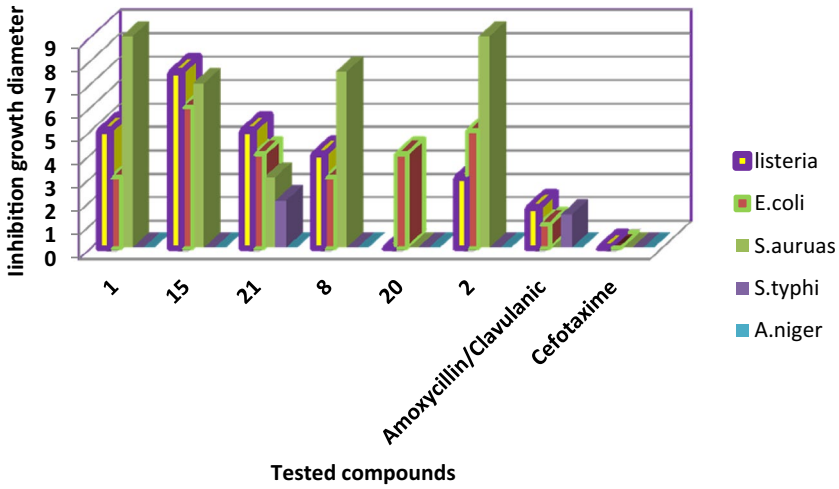


Fig. 1 Statistical representation for biological activity of the prepared compounds

Table 2 Determination of MIC for the most sensitive organisms

Compounds	*Listeria*	*E. coli*	*S. typhi*	*S. auraus*
1	0.16 ± 0.012	0.16 ± 0.02	0	0.16 ± 0.012
15	0.08 ± 0.011	0.08 ± 0.01	0	0.12 ± 0.011
21	0.16 ± 0.025	0.16 ± 0.025	0.24 ± 0.031	0.16 ± 0.025
8	0.16 ± 0.033	0.16 ± 0.03	0	0.16 ± 0.033
20	0	0.16 ± 0.01	0	0
2	0.08 ± 0.01	0.08 ± 0.02	0	0.12 ± 0.01

Table 3 Of one-way ANOVA: *E. coli* versus samples

Compounds	Mean	Grouping
2	0.08	A
15	0.08	A
1	0.16	B
8	0.16	B
20	0.16	B
21	0.16	B

Means that do not share a letter are significantly different

Grouping information using the Fisher LSD method and 95% confidence interval

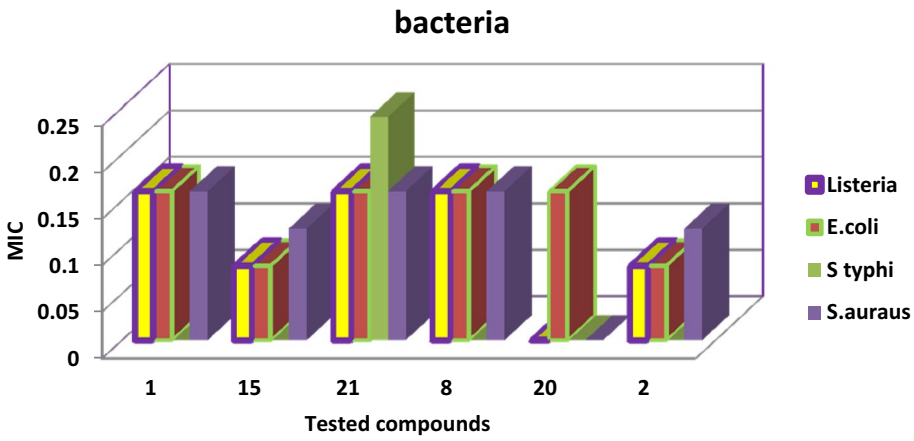


Fig. 2 MIC for the most sensitive bacteria of the tested compounds

Table 4 Of one-way ANOVA: Listeria versus samples

Compounds	Mean	Grouping
2	0.08	A
15	0.08	A
1	0.16	B
8	0.16	B
21	0.16	B
20	0.00	C

Means that do not share a letter are significantly different

Grouping information using the Fisher LSD method and 95% confidence interval

Table 5 Of one-way ANOVA: *S. typhi* versus samples

Compounds	Mean	Grouping
21	0.24	A
20	0.00	B
15	0.00	B
8	0.00	B
2	0.00	B
1	0.00	B

Means that do not share a letter are significantly different

Grouping information using the Fisher LSD method and 95% confidence interval

Table 6 Of one-way ANOVA: *S. auraus* versus samples

Compounds	Mean	Grouping
2	0.12	A
15	0.12	A
1	0.16	B
8	0.16	B
21	0.16	B
20	0.00	C

Means that do not share a letter are significantly different

Grouping information using the Fisher LSD method and 95% confidence interval

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Compliance with ethical standards

Conflict of interest This research holds no conflict of interest and is not funded through any source.

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