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A convenient synthesis of some new fused pyridine and pyrimidine derivatives of antimicrobial profiles

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Abstract Some novel triazolo[1,5-*a*]pyridine (**4–6**), thiazolo[3,2-*a*]pyridine (**7**), thiazolo[3,2-*a*]pyrimidines (**9**, **11**), oxoimidazo[1,2-*a*]pyrimidine (**10**), and pyrimido[2,1-*b*]quinazoline (**12**) have been synthesized. The structures of target compounds were confirmed by elemental analyses and spectral data. The antimicrobial activity of some of the target synthesized compounds were tested against various microorganisms such as *Salmonella typhimurium*, *Pseudomonas aeruginos* and *Staphylococcus aureus* (bacteria), *Aspergillus flavus* (fungus), and *Candida albicans* (yeast fungus) by the disc diffusion method. In general, the novel synthesized compounds showed a good antimicrobial activity against these microorganisms.

Keywords Triazolo[1,5-*a*]pyridine · Thioxopyrimidine derivatives · Antimicrobial activities

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Introduction

The treatment of bacterial infections still remains an important and challenging therapeutic problem because of factors that include emerging infectious diseases and the increasing number of multi-drug-resistant microbial pathogens. In spite of the large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic-resistant bacterial strains in recent decades constitutes a substantial need for new classes of antibacterial agents [1]. Condensed heterocyclic systems containing triazolo[1,5-*a*]pyridine are characterized by significant pharmacological activity. These types of compounds exhibit antitumor, antioxidant, analgesic, anti-inflammatory, antibacterial, antifungal activity, etc. [2–7]. In addition to the previously mentioned properties, thiazolo[3,2-*a*]pyridines, also containing two fused heterocyclic moieties in one molecule, are found in a broad range of biologically active compounds and have many important bioactivities such as inhibitors of beta-amyloid production [8], CDK2-cyclin-A [9], and α-glucosidase [10], potential uterus stimulants [11, 12], and antibacterial and antifungal activities [13], and are found to exhibit a broad spectrum of potent anticancer activity and are useful for chemotherapy of various cancers, such as leukemia, lung cancer, and melanoma [14]. On the other hand, certain pyrimidine derivatives are also known to exhibit diverse pharmacological properties such as effective bactericides and fungicides [15], as antimalarial [16], antioxidant [17, 18], and antihypertensive agents, as adrenergic and neuropeptide antagonists [19] and having anti-HIV activities [20, 21]. Along with the varied biological activities of pyrimidine, other heterocycles fused with pyrimidines play an essential role in several biological processes and have a considerable chemical and pharmacological importance [22, 23]. In the view of these facts and as part of our efforts to discover potentially active new agents, we have synthesized some new triazolo[1,5-*a*]pyridine, thiazolo[3,2-*a*]pyridine, and dihydropyrimidinecarbonitrile, and their fused derivatives. The novel derivatives were characterized by spectral data and elemental analysis and tested for their antimicrobial activities.

Experimental

All melting points were uncorrected and were taken on a Boetius melting point microscope. The infrared (IR) spectra were recorded on a Bruker-Vector 22, Germany, using KBr discs at the microanalytical center, Cairo University. ^{1}HNMR spectra were performed on a Varian Gemini 300 MHz spectrometer and JEOL EX-270 MHz at the Central Services Laboratory, National Research Centre, Cairo, Egypt, using tetramethylsilane (TMS) as internal standard. All chemical shifts are quoted in δ values using parts per million scale (ppm) downfield from TMS. Mass Spectra were recorded on a Hewlett-Packard 5988 A (1,000 Hz) instrument and Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV at Cairo University. Elemental analysis were performed by the Microanalytical unit at Cairo University and the results were within ±0.4 of the theoretical values. All reactions were

monitored by TLC using precoated aluminum sheet silica gel Merck $60F_{254}$ plates, and detection of the components was made by short and long UV light.

1,6-Diamino-2-oxo-4-(5-methylfuran-2-yl)-1,2-dihydropyridine-3,5-dicarbonitrile (**3**)

A mixture of freshly prepared cyanohydrazide (0.02 mol) and 2-((5-methylfuran-2-yl)methylene)malononitrile (**1**) (0.01 mol) in absolute ethanol (25 mL) containing a catalytic amount of piperidine was allowed to stir for 5 h at room temperature. The resulting precipitate was filtered off and washed several times with ethanol and crystallized from methanol to give **3** in 90 % yield; m.p. 290–292 °C; IR (KBr, cm^{-1}) ν_{max}: 3,381, 3,260 (2 NH_2), 2,205 (CN), 1,638 (CO); ^{1}HNMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 5.61 (s, 2H, NH_2), 6.45 (d, 1H, CH), 7.27 (d, 1H, CH), 8.31 (s, 2H, NH_2); MS: *m*/*z* 255 (M^+) consistent with the molecular formula ($C_{12}H_{19}N_5O_2$).

2-Aryl-3,5-dihydro-7-(5-methylfuran-2-yl)-5-oxo-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (**4a**–**e**)

A mixture of equimolar amounts of **3** and the appropriate aromatic or heterocyclic aldehydes, namely, 4-bromobenzaldehyde, *p*-tolualdehyde, 3-indol aldehyde, 3-methoxybenzaldehyde and D-galactose, in ethanol was allowed to reflux for 8 h. The resulting precipitate was filtered off, washed several times with ethanol, and crystallized from glacial acetic acid.

*2-(4-Bromophenyl)-3,5-dihydro-7-(5-methylfuran-2-yl)-5-oxo-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (**4a**)*

Yield 78 %; m.p. 240–241 °C; IR (KBr, cm^{-1}) ν_{max}: 3,432 (NH), 2,210 (CN); ^{1}HNMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 2.55 (s, 1H, NH), 6.38 (d, 1H, CH), 7.12 (d, 1H, CH), 7.71, 8.09 (2dd, 4H, ArH); MS: *m*/*z* 421 (M+1) consistent with the molecular formula ($C_{19}H_{10}BrN_5O_2$).

*2-(P-Tolyl)-3,5-dihydro-7-(5-methylfuran-2-yl)-5-oxo-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (**4b**)*

Yield 78 %; m.p. 230–232 °C; IR (KBr, cm^{-1}) ν_{max}: 3,417 (NH), 2,210 (CN); ^{1}HNMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 6.38 (d, 1H, CH), 7.24 (d, 1H, CH), 7.37, 8.06 (2 dd, 4H, ArH), 8.30 (s, 1H, NH); MS: *m*/*z* 358 (M+2) consistent with the molecular formula ($C_{20}H_{13}N_5O_2$).

*2-(1H-Indol-3-yl)-3,5-dihydro-7-(5-methylfuran-2-yl)-5-oxo-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (**4c**)*

Yield 80 %; m.p. 263–265 °C; IR (KBr, cm^{-1}) ν_{max}: 3,290, 3,205 (2 NH), 2,212 (CN), 1,621 (CO); ^{1}HNMR (DMSO-d_6) δ: 2.42 (s, 3H, CH_3), 6.48 (d, 1H, CH), 7.27

(d, 1H, CH), 7.52, 8.15 (2 m, 4H, ArH), 8.79 (s, 1H, NH), 12.10 (s, 1H, NH); MS: *m*/*z* 316 (M−64) consistent with the molecular formula ($C_{21}H_{12}BrN_6O_2$).

*2-(3-Methoxyphenyl)-3,5-dihydro-7-(5-methylfuran-2-yl)-5-oxo-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (**4d**)*

Yield 80 %; m.p. 213–215 °C; ^{1}HNMR (DMSO-d_6) δ: 2.40 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 6.40 (d, 1H, CH), 7.06 (d, 1H, CH), 7.10 (m, 4H, ArH), 8.12 (s, 1H, NH).

*2-(Galactosyl)-3,5-dihydro-7-(5-methylfuran-2-yl)-5-oxo-[1,2,4]triazolo[1,5-a]pyridine-6,8-dicarbonitrile (**4e**)*

Yield 70 %; m.p. 240–241 °C; IR (KBr, cm^{-1}) ν_{max}: 3,386 (OH), 3,279 (NH), 2,208 (CN), 1,635 (CO); ^{1}HNMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 2.86–3.31 (m, 2H, 6′-H, 6″-H), 3.52 (m, 2H, 5′-H, 4′-H), 3.56 (t, 1H, 6′-OH), 4.21–4.73 (m, 6H, 5′-OH, 4′-OH, 2′-H, 3′-H, 3′-OH, 2′-OH), 5.60 (s, 1H, NH), 6.45 (d, 1H, CH), 7.28 (d, 1H, CH).

3,5-Dihydro-7-(5-methylfuran-2-yl)-5-oxo-2-(2-oxopropyl)-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (**5**)

A mixture of equimolar amounts of **3** and ethylacetoacetate was refluxed for 3 h in ethanol (25 mL). The precipitate formed was filtered off, washed several times with ethanol, and crystallized from aqueous DMF to give compound **5** in 86 % yield; m.p. 225–227 °C; ^{1}HNMR (DMSO-d_6) δ: 2.37 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 4.26 (s, 2H, CH_2), 6.37 (s, 1H, CH), 7.10 (d, 1H, CH).

3,5-Dihydro-2-methyl-7-(5-methylfuran-2-yl)-5-oxo-[1,2,4]triazolo[1,5-*a*]pyridine-6,8-dicarbonitrile (**6**)

A solution of (**3**) (0.002 mol) in freshly distilled acetic anhydride and acetic acid (1:1) was reflux for 10 h. The precipitate formed on cooling to room temperature was filtered off and crystallized from DMF to give compound (**6**) in 86 % yield; m.p. 270–273 °C; IR (KBr, cm^{-1}) ν_{max}: 3483 (NH), 2,214 (CN), 1,676 (CO); ^{1}HNMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.37 (s, 1H, CH), 7.24 (d, 1H, CH),7.94 (s, 1H, NH).

(2*Z*)-5-Amino-3,7-dihydro-7-(5-methylfuran-2-yl)-2-((5-methylfuran-2-yl)methylene)-3-oxo-2*H*-thiazolo[3,2-*a*]pyridine-6,8-dicarbonitrile (**7**)

A solution of **1** (0.02 mol) and thioglycolic acid (0.01 mol) in ethanol (50 mL) and a catalytic amount of piperidine was refluxed for 5 h. The solvent was concentrated and the solid product was collected by filtration and recrystallized from ethanol to give the title compound (**7**) in 91 % yield; m.p. 210–212 °C; IR (KBr, cm^{-1}) ν_{max}: 3,396 (NH), 2,197 (CN), 1,700 (CO); ^{1}HNMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3),

4.74 (s, 1H, CH), 6.05 (s, 1H, CH), 6.26 (d, 1H, CH), 6.46 (d, 1H, CH), 7.11 (d, 1H, CH), 7.55 (s, 2H, NH_2), 7.62 (s, 1H, CH); MS: *m/z* 390 consistent with the molecular formula ($C_{20}H_{14}N_4O_3S$).

1,2,3,4-Tetrahydro-6-(5-methylfuran-2-yl)-4-oxo-2-thioxopyrimidine-5-carbonitrile (**8**)

A mixture of ethyl cyanoacetate (0.1 mol), thiourea (0.1 mol) and 5-methylfurfural (0.1 mol) in 50 mL sodium ethoxide (2.3 g, 0.1 mol) was stirred for 24 h at room temperature and poured onto ice-water, and acidified with diluted HCl to give precipitates, which were filtered off, dried, and crystallized from methanol to give compound **8** in 76 % yield; m.p. 296–298 °C; IR (KBr, cm^{-1}) ν_{max}: 3,424, 3,169 (2 NH), 2,216 (CN), 1,723 (CO); ^{1}HNMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3), 6.41 (d, 1H, CH), 7.40 (d, 1H, CH), 10.32 (s, 1H, NH, D_2O exchangeable), 11.72 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (DMSO): 15.17, 82.50, 117.43, 110.65, 1125.78, 155.86, 156.29, 167.48, 178.13, 179.62; MS: *m/z* 235 (M^++2) consistent with the molecular formula ($C_{10}H_7N_3O_2S$).

7-(5-Methylfuran-2-yl)-5-oxo-3-phenyl-5*H*-thiazolo[3,2-*a*]pyrimidine-6-carbonitrile (**9**)

A mixture of carbonitrile **8** (0.01 mol) and phenacyl bromide (0.01 mol) was refluxed in ethanol for 5 h. After completion of the reaction, the precipitate formed on hot was filtered, washed with ethanol, and dried to give compound **9** in yield of 69 %; m.p. 229–231 °C; IR (KBr, cm^{-1}) ν_{max}: 2,206 (CN), 1,715 (CO); ^{1}HNMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 5. 23 (s, 1H, CH), 6.28 (d, 1H, CH), 7.12–7.39 (m, 5H, ArH), 7.47 (d, 1H, CH); ($C_{18}H_{11}N_3O_2S$). ^{13}CNMR (DMSO): 15.23, 98.36, 109.76, 110.22, 112.51, 116.80, 127.03, 129.12, 129.87, 135.45, 143.19, 135.93, 155.37, 163.58, 163.95, 170.62; MS: *m/z* 333 (M^+) consistent with the molecular formula ($C_{18}H_{11}N_3O_2S$).

1,2,3,5-Tetrahydro-7-(5-methylfuran-2-yl)-5-oxoimidazo[1,2-*a*]pyrimidine-6-carbonitrile (**10**)

Equamolar mixture of thioxopyrimidine **8** (0.1 mol), and ethanolamine were heated to reflux for 8 h. The reaction mixture was concentrated under reduced pressure, the separated solid was collected by filtration and crystallized from glacial acetic acid to afford compound **10** in 45 % yield, m.p. 251–253 °C; IR (KBr, cm^{-1}) ν_{max}: 2,234 (CN), 1,714 (CO); ^{1}HNMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 3.02 (t, 2H, CH_2), 3.76 (t, 2H, CH_2), 6.43 (d, 1H, CH), 7.40 (d, 1H, CH), 10.32 (s, 1H, NH, D_2O exchangeable), 11.72 (s, 1H, NH, D_2O exchangeable); ^{13}CNMR (DMSO): 14.89, 40.09, 41.27, 98.84, 109.54, 112.33, 117.01, 153.41, 154.79, 159.50, 163.17, 170.85; MS: *m/z* 242 (M+) consistent with the molecular formula ($C_{12}H_{10}N_4O_2$).

(2*Z*)-2-(Substitutedbenzylidene)-3,5-dihydro-7-(5-methylfuran-2-yl)-3,5-dioxo-2*H*-thiazolo-[3,2-*a*]pyrimidine-6-carbonitrile (**11a**, **b**)

General method

A mixture of compound **6** (0.01 mol), chloroacetic acid (0.01 mol), required aromatic aldehydes namely, 2,4,6-trimethyl benzaldehyde and 4-cyano benzaldehyde (0.01 mol) in a mixing solvent of acetic anhydride and acetic acid (10/20 mL) in the presence of fused sodium acetate (0.5 g) was refluxed for 2 h. The solid obtained was crystallized from proper solvent to give **11a**, **b**, respectively.

*(2Z)-2-(2,4,6-Trimethyl benzylidene)-3,5-dihydro-7-(5-methylfuran-2-yl)-3,5-dioxo-2H-thiazolo[3,2-a]pyrimidine-6-carbonitrile (**11a**)*

Yield 68 %; m.p. 243–246 °C; IR (KBr, cm^{-1}) ν_{max}: 3,436, 3,173 (2NH), 2,219 (CN); ^{1}HNMR (DMSO-d_6) δ: 2.24 (s, 3H, CH_3), 2.37 (s, 9H, 3 CH_3), 6.56 (d, 1H, ArH), 7.29 (d, 1H, ArH), 7.94 (s, 1H, =CH); ^{13}CNMR (DMSO): 15.04, 18.25, 24.13, 98.65, 109.22, 112.47, 116.37, 117.21, 127.80, 127.96, 135.06, 135.67, 135.82, 136.72, 143.58, 153.11, 154.61, 165.48, 165.34, 167.26, 171.18; MS: *m/z* 386 (M^+) consistent with the molecular formula ($C_{20}H_{10}N_4O_3S$).

*(2Z)-2-(4-Cyanobenzylidene)-3,5-dihydro-7-(5-methylfuran-2-yl)-3,5-dioxo-2H-thiazolo[3,2-a]pyrimidine-6-carbonitrile (**11b**)*

Yield 68 %; m.p. 213–2125 °C; IR (KBr, cm^{-1}) ν_{max}: 3,423, 3,119 (2NH), 2,209 (CN); ^{1}HNMR (DMSO-d_6) δ: 2.24 (s, 3H, CH_3), 6.67 (d, 2 H, ArH), 7.41 (d, 2 H, ArH), 8.01 (s, 1H, =CH); ^{13}CNMR (DMSO): 14.36, 98.16, 109.76, 111.43, 112.28, 116.95, 117.08, 117.54, 127.32, 134.15, 138.89, 147.35, 153.27, 154.88, 165.68, 166.05, 167.69, 171.41; MS: *m/z* 403 (M^+) consistent with the molecular formula ($C_{22}H_{17}N_3O_3S$).

6,11-Dihydro-2-(5-methylfuran-2-yl)-4,6-dioxo-4*H*-pyrimido[2,1-*b*]quinazoline-3-carbonitrile (**12**)

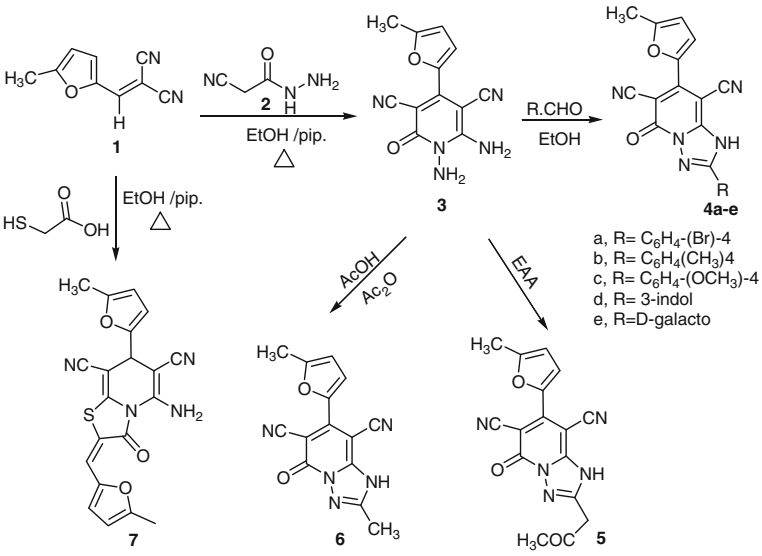
A mixture of the thiouracil derivative **8** (0.01 mol) and anthranilic acid (0.01 mol) were refluxed in sodium ethoxide solution (0.3 g sodium metal in 100 mL absolute ethanol) for 12 h, cooled, and poured onto acidified ice-water. The obtained solid was filtered off, washed with water, dried, and crystallized from DMF/water to give compound **12**, in yield of 57 %; mp. 268–269 °C; IR (KBr, cm^{-1}) ν_{max}: 3217 (NH), 2220 (CN), 1719 (CO); ^{1}HNMR (DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 6.28 (d, 1H, CH), 7.10–7.82 (m, 5H, CH + ArH), 9.59 (s, 1H, NH, D_2O exchangeable); MS: *m/z* 318 (M^+) consistent with the molecular formula ($C_{17}H_{10}N_4O_3$).

Results and discussion

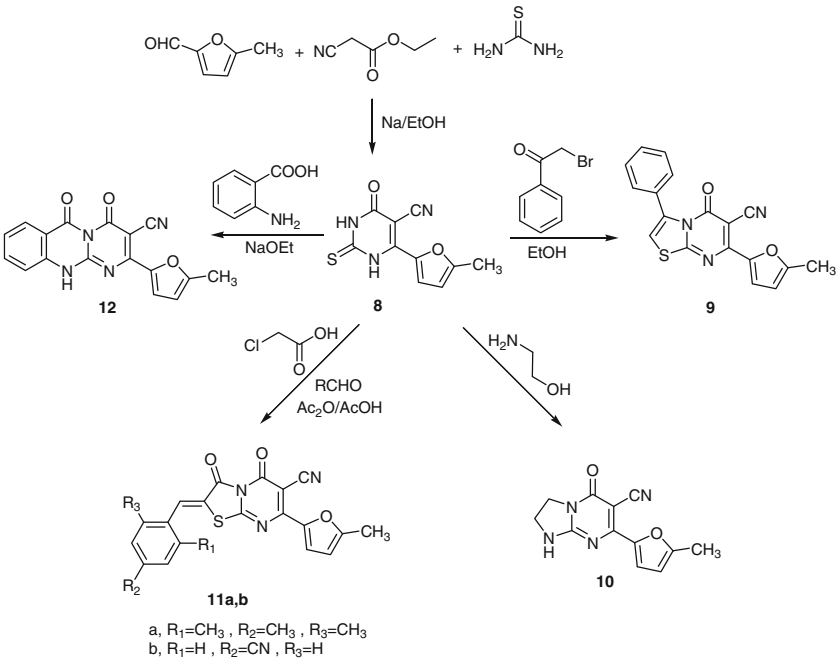
1,6-Diamino-3,5-dicyano-4-(5-methylfuran-2-yl)-2-pyridone (**3**), [15] was prepared in a good yield by reacting 2-((5-methylfuran-2-yl)methylene)malononitrile (**1**) with cyanoacetohydrazide (**2**) in ethanol at room temperature in the presence of a catalytic amount of piperidine. Formation of triazolo[1,5-*a*]pyridine derivatives **4a–e**, **5** and **6** were prepared by reaction of the key intermediate **3** with appropriate aromatic and/or sugar aldehydes, ethyl acetoacetate and acetic acid/acetic anhydride, respectively. Also, reaction of the starting material **1** with thioglycolic acid in a molar ratio of 2:1 in ethanol in the presence of a catalytic amount of piperidine gave (2*Z*)-5-amino-3,7-dihydro-7-(5-methylfuran-2-yl)-2-((5-methylfuran-2-yl)methylene)-3-oxo-2*H*-thiazolo[3,2-*a*]pyridine-6,8-di-carbonitrile (**7**) (Scheme 1).

On the other hand, the thioxopyrimidine heterocyclic core were constructed according to Biginelli reaction by a cyclo-condensation of equivalent molar quantities of 5-methylfurfural, thiourea, and ethyl cyanoacetate in sodium ethoxide solution. After neutralization with acid, the desired 5-cyano-4-oxo-6-(5-methylfuran-2-yl)-2-thioxo-1,2,3,4-tetrahydro-pyrimidine derivative (**8**) was obtained.

Treatment of **8** with phenacyl bromide and 2-ethanolamine in refluxing ethanol afforded the fused rings bicyclic thiazolo[3,2-*a*]pyrimidines **9** and oxoimidazo[1,2-*a*]pyrimidine **10**, respectively. Syntheses of 2-arylmethylene derivatives **11a**, **b** were achieved in a single step by treating thioxopyrimidine derivative **8** with chloroacetic acid and aromatic aldehydes in the presence of sodium acetate, acetic acid and acetic anhydride. Compound **8** also reacted with anthranilic acid in sodium ethoxide to yield 6,11-dihydro-2-(5-methylfuran-2-yl)-4,6-dioxo-4*H*-pyrimido[2,1-*b*]quinazoline-3 carbonitrile (**12**) (Scheme 2).



Scheme 1 Synthetic route for compounds **3**–**7**



Scheme 2 Synthetic route for compounds **8–12**

Antimicrobial assay

Preparation of microbial suspensions

Seven compounds were tested for their antimicrobial activities against highly pathogenic strains; one Gram-positive bacteria (*Staphylococcus aureus*), one Gram-negative bacteria (*Escherichia coli O157*) isolated from minced meat, and two mycotic strain (*Candida albicans*, *Aspergillus flavus*) isolated from mastitic milk. Agar disk diffusion (qualitative method) and minimum inhibitory concentration (MIC) (quantitative method) were used in this study. A suspension of bacterial and mycotic isolates were freshly prepared by inoculating fresh stock culture from each strain into separate broth tubes, each containing 7 mL of Muller Hinton Broth for bacterial strains and Sabaroud Dextrose broth for mycotic strain. The inoculated tubes were incubated at 37 and 28 °C for 24 h, respectively. Serial dilutions were carried out for each strain; the dilution matching with 0.5 McFarland (about 1×10^8 cells/mL) was selected for screening of antimicrobial activities. Ciprofloxacin 100 μg/mL and fluconazole 100 μg/mL were used as reference drugs (Oxoid). DMSO was used as control negative.

Determination of antimicrobial activity by disc-diffusion method [24]

Muller Hinton and Sabaroud dextrose agar plates were prepared. Bacterial and fungal strains matching with 0.5 McFarland were spread onto the surface of the agar

plates using sterile cotton swabs. For evaluation of antibacterial activities, Whatman no. 1 filter paper disks were saturated with 50 μL of the compound dissolved in DMSO (100 μg of the tested compound dissolved in 1 mL DMSO), others were saturated with 50 μL ciprofloxacin (100 μg/mL), and others 50 μL DMSO as control negative. The same method was used for evaluation of antimycotic activities using fluconazole (100 μg/mL). Disks were dried and then placed onto inoculated agar plates and left for 1 h at 25 °C to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were re-incubated at 37 and 28 °C for 24 h for bacterial and mycotic isolates, respectively. After incubation, plates were observed for antimicrobial activities by determining the diameters of the zones of inhibition for each of the samples. For an accurate analysis, tests were run in triplicate for each strain to avoid any error.

Determination of minimum inhibitory concentration (MIC) [25]

Microtiter dilution plate quantitative method, i.e. the MIC was used for evaluation of the antimicrobial activity of tested compounds. Determination of MIC of extract against tested strains was achieved using 96-well sterile micro-plates. The first well contain the concentrated form of the tested compound used in the agar disk diffusion method (100 μg of the tested compound dissolved in 1 mL DMSO), then two-fold serial dilutions was carried out for the tested compounds, reference drugs (ciprofloxacin and fluconazole), and DMSO, Then, the wells were inoculated with 100 μL of tested isolates (0.5 McFarland, about 1×10^8 cells/mL) and incubated at 37–28 °C for 24 h for bacterial and fungal strains, respectively. After incubation, plates were examined visually for bacterial or fungal growth precipitation. The experiment was repeated three times. The lowest concentration that showed complete hindrance of growth was taken as MIC.

Table 1 Agar well diffusion method showing antimicrobial activities of the tested compounds compared with reference drugs, results given in (mm)

Compounds	Gram-negative bacteria	Gram-positive bacteria	Fungi	
	E. coli 0157	*S. aureus*	*C. albicans*	*A. flavus*
3	8	10	16	17
4a	9	12	18	10
4b	Negative	Negative	13	10
4e	Negative	Negative	19	16
6	7	13	17	12
7	Negative	Negative	9	12
8	Negative	Negative	Negative	Negative
DMSO	Negative	Negative	Negative	Negative
Fluconazole 100 μg/L	ND	ND	32	31
Ciprofloxacin 100 μg/L	42	39	ND	ND

Table 2 The antimicrobial activity of tested compounds against bacterial and fungal strains isolated from animal byproduct origin using minimum inhibitory concentration test

Compounds	*C. albicans*	*A. flavus*
3	1.56	1.56
4a	0.78	100
4b	12.5	100
4e	0.19	3.125
6	1.56	25
7	100	25
Fluconazole 100 mg/mL	0.097	0.097

Biological evaluation

All the newly synthesized compounds were screened for their in vitro antibacterial activity against Gram-positive bacteria (*S. aureus*), and Gram-negative bacteria (*E. coli*) using ciprofloxacin as the standard drug (100 μg/mL). They were also evaluated for their in vitro antifungal activity against the mycotic isolates (*C. albicans*, *A. flavus*) using fluconazole as a standard antifungal drug (100 μg/mL). The agar well diffusion method [20] was used in this investigation for determination of the preliminary antibacterial and antifungal activity and the results were recorded for each tested compound as the average diameter (in mm) of inhibition zones (IZ) of bacterial or fungal growth around the discs (Table 1). The minimum inhibitory concentrations (MIC) were recorded for compounds that showed promising growth inhibition using the two-fold serial dilution method [21]. The MIC (μg/mL) values against the tested bacterial and fungal isolates were recorded in (Table 2). The agar well diffusion method showed very weak activity of the compounds (**3**), (**4a**) and (**6**) against bacterial isolates comparing with reference drugs, while other compounds showing no hindrance of the tested compounds for the bacterial strains, The antimycotic activity were more promising for the compounds (**3**) and (**4e**) with mean zones of inhabitation equal to 16, 17, and 19 mm and 16 mm against *C. albicans* and *A. flavus*, respectively, followed by compound (**6**) which showed a zone of inhibition equaling 17 and 12 mm, then compounds (**4a**) and (**4b**) which showed zones of hindrance against *C. albicans* equalling 18 and 13 mm compared with reference drug (fluconozole) which give activity of 25 and 24 mm against *C. albicans* and *A. flavus*, respectively. MIC was carried out against mycotic strains only, and results against *C. albicans* showed that compound (**4e**) has the highest hindrance capability at dilution 0.19 followed by compounds (**3**) and (**6**) with MIC equal 1.56 against *A. flavus*. Compound (**3**) was the best with MIC 1.56 followed by compound (**4e**) with MIC equal to 3.125 compared with fluconazole which gave MIC equal to 0.097 for both tested strains.

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