Synthesis of pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5-ones as potential antimicrobial agents

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RESEARCH ARTICLE

Synthesis of pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-ones as potential antimicrobial agents

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Abstract Synthesis of new derivatives of pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one via reaction of 7-(4-bromophenyl)-1,2-dihydro-5-(4-fluorophenyl)-2-thioxopyrido[2,3-*d*] pyrimidin-4(3*H*)-one with hydrazonoyl chlorides or reaction of 2-hydrazino-pyrido[2,3-*d*]pyrimidin-4(3*H*)-one with different aldehydes followed by cyclization of the products. All the newly synthesized compounds were evaluated for their antimicrobial activities and also their minimum inhibitory concentration against most of test organisms was performed. Amongst the tested compounds displayed excellent activity against all the tested microorganisms except SR and PA.

Keywords Pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-ones · Hydrazonoyl chlorides · Antimicrobial activity

Introduction

Infections caused by multi-drug resistant bacteria are of major health concern worldwide. Particularly important are infections caused by the Gram-positive bacteria *Staphylococcus aureus* and species of the genus Enterococcus, due to increasing incidence of infections caused by these microorganisms in hospitals and communities, and their ability of developing antibiotic resistance to multiple antibiotics. Due to some serious side effects in newly introduced antibacterial agents such as semi-synthetic streptogramins quinupristin/dalfopristin, daptomycin, the development of a diversified series of antimicrobials still remains a necessity (Leclercq et al. 2009).

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Pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-ones are pharmacological scaffold that represent a wide range of biological activities (El-Gazzar et al. 2008, 2009; Abu-Zied et al. 2008). As a part of our ongoing research on the synthesis of biologically active heterocyclic compounds (Hassaneen et al. 2011; Gomha and Hassaneen 2011; Farghaly and Abdalla 2009; Abdel Hafez et al. 2010; Farghaly et al. 2010, 2012; Riyadh et al. 2010) herein we report an efficient methodology for synthesis of novel pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one derivatives and investigate their antimicrobial activities.

Materials and methods

Melting points were determined on an electrothermal Gallenkamp apparatus and are uncorrected. The IR spectra were recorded in KBr using a Pye Unicam SP-1000 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian EM-300 MHz spectrometer (^{1}H NMR 300 or 400 MHz) using DMSO-d_6, solvent with TMS as internal standard. ^{13}C NMR spectra were measured on a Varian EM-300 MHz spectrometer (100 MHz). Mass spectra were recorded on an AEI MS 30 mass spectrometer operating at 70e V. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Antimicrobial activities were carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

Synthesis of 7-(4-bromophenyl)-1,2-dihydro-5-(4-fluorophenyl)-2-thioxo-pyrido-[2,3-*d*]pyrimidin-4(3*H*)-one (**3**)

A solution of 6-amino-2,3-dihydro-2-thioxo-pyrimidin-4(1*H*)-one (**2**) (0.5 g, 3.5 mmol) and chalcone **1** (1.06 g, 3.5 mmol)

in 5 ml dimethylformamide was refluxed for 15 h. After the reaction completed (examined by TLC), the solution is cooled down to 5 °C and the precipitate formed is filtered off and crystallized from DMF to give compound **3** as yellow crystals; (80 % yield), mp 303–305 °C; IR (KBr): 3,150, 3,086 (2NH), 1,701 (C=O); ^{1}H NMR (DMSO-d_6): δ = 7.24 (d, J = 8 Hz, 2H, Ar–H), 7.51 (d, J = 8 Hz, 2H, Ar–H), 7.70 (s, 1H, pyridine-H), 7.75 (d, J = 8 Hz, 2H, Ar–H), 8.20 (d, J = 8 Hz, 2H, Ar–H), 12.30 (s, 1H, NH, D_2O-exchangeable), 13.0 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6): δ = 110.20, 114.54, 116.07, 121.05, 122.29, 123.02, 124.0, 130.97, 134.28, 143.20, 145.21, 158.82, 160.81, 161.64, 163.74. MS: m/z 430 (M^++2, 1), 429 (M^++ 1, 2), 428 (M^+, 2), 183 (3), 155 (1), 95 (2), 73 (100). Anal. calcd. For $C_{19}H_{11}BrFN_3OS$ (428.28) C, 53.28; H, 2.59; N, 9.81; Found: C, 53.14; H, 2.37; N, 9.74 %.

General procedure for the preparation of **7a**–**d**

Method A

To a mixture of equimolar amounts of compound **3** and the appropriate hydrazonoyl chlorides **4** (1 mmol of each) in dioxane (15 ml) was added triethylamine (0.14 ml, 1 mmol). The reaction mixture was refluxed till all of the starting materials have disappeared and hydrogen sulfide gas ceased to evolve (5 h, monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered off and recrystallized from dioxane to give products **7a**–**d**.

Method B

To a mixture of equimolar amounts of **9** and the appropriate hydrazonoyl chloride **4a** (1 mmol) in dioxane (15 ml) was added triethylamine (0.14 ml, 1 mmol). The reaction mixture was refluxed till all methanethiol gas ceased to evolve (10 h, monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered and recrystallized from dioxane to give product identical in all respects (mp, mixed mp and IR) with **7a**.

8-(4-Bromophenyl)-6-(4-fluorophenyl)-1,3-diphenyl-pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]-pyrimidin-5-one (**7a**)

Yellow crystals; (72 % yield), mp 343–345 °C; IR (KBr): 1,705 (C=O); ^{1}H NMR (DMSO-d_6): δ = 6.98–7.84 (m, 18H, Ar–H), 7.66 (s, 1H, pyridine-H); ^{13}C NMR (DMSO-d_6): δ = 110.05, 118.55, 119.63, 120.25, 121.65, 123.32, 124.05, 126.53, 127.65, 129.37, 130.31, 131.78, 132.62, 133.85, 136.35, 138.16, 139.05, 147.08, 149.95, 151.28, 154.65, 158.23, 159.12, 162.32. MS: *m/z* 590 (M^++2, 9), 589 (M^++ 1, 25), 588 (M^+, 25), 587 (27), 586 (21), 220 (14), 103 (12), 91 (100), 75 (11). Anal. calcd. for $C_{32}H_{19}BrFN_5O$ (588.43) C, 65.32; H, 3.25; N, 11.90; Found: C, 65.30; H, 3.16; N, 11.85 %.

3-Acetyl-8-(4-bromophenyl)-6-(4-fluorophenyl)-1-phenyl-pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (**7b**)

Yellow crystals; (78 % yield), mp 292–294 °C; IR (KBr): 1,720, 1,674 (2C=O); ^{1}H NMR (DMSO-d_6): δ = 2.45 (s, 3H, CH_3CO), 7.12–7.86 (m, 13H, Ar–H), 7.68 (s, 1H, pyridine-H), ^{13}C NMR (DMSO-d_6): δ = 25.63, 110.54, 119.67, 120.28, 122.10, 123.68, 127.23, 129.63, 130.12, 132.13, 134.36, 136.54, 138.38, 138.78, 148.86, 150.31, 150.85, 151.11, 158.35, 158.22, 163.15, 190.32. MS: *m/z* 556 (M^++2, 23), 555 (M^++ 1, 77), 554 (M^+, 78), 553 (72), 485 (32), 158 (16), 91 (46), 77 (100). Anal. calcd. for $C_{28}H_{17}BrFN_5O_2$ (554.37) C, 60.66; H, 3.09; N, 12.63; Found: C, 60.51; H, 3.01; N, 12.53 %.

8-(4-Bromophenyl)-3-ethoxycarbonyl-6-(4-fluorophenyl)-1-phenyl-pyrido[2,3-*d*][1,2,4]-triazolo[4,3-*a*]pyrimidin-5-one (**7c**)

Yellow crystals; (74 % yield), mp 284–282 °C; IR (KBr): 1,751, 1,712 (2C=O); ^{1}H NMR (DMSO-d_6): δ = 1.30 (t, J = 7.2 Hz, 3H, CH_3), 4.43 (q, J = 7.2 Hz, 2H, CH_2), 6.97–7.87 (m, 13H, Ar–H), 7.66 (s, 1H, pyridine-H); ^{13}C NMR (DMSO-d_6): δ = 14.23, 58.66, 109.66, 119.88, 121.56, 124.09, 127.44, 129.56, 130.12, 131.54, 133.56, 136.55, 137.65, 138.54, 145.21, 148.23, 150.94, 151.72, 152.64, 153.08, 158.35, 160.62, 163.62. MS: *m/z* 586 (M^++2, 33), 585 (M^++ 1, 100), 584 (M^+, 96), 583 (94), 482 (33), 360 (19), 157 (22), 91 (99), 77 (99). Anal. calcd. For $C_{29}H_{19}BrFN_5O_3$ (584.40) C, 59.60; H, 3.28; N, 11.98; Found: C, 59.42; H, 3.16; N, 11.88 %.

8-(4-Bromophenyl)-6-(4-fluorophenyl)-1-phenyl-3-Phenylcarbamoyl-pyrido[2,3-*d*][1,2,4]-triazolo[4,3-*a*] pyrimidin-5-one (**7d**)

Yellow crystals; (70 % yield), mp 279–281 °C; IR (KBr): 3,063 (NH), 1,701, 1,680 (2C=O); ^{1}H NMR (DMSO-d_6): δ = 7.18–7.60 and 7.67–8.34 (m, 18H, Ar–H), 7.65 (s, 1H, pyridine-H), 12.42 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 111.14, 119.45, 118.33, 119.12, 120.16, 123.30, 124.48, 126.49, 126.99, 129.73, 131.20, 131.58, 132.60, 134.95, 136.15, 137.26, 139.44, 147.18, 149.0, 150.65, 153.95, 158.02, 158.69, 159.98, 161.47. MS: *m/z* 632 (M^++ 1, 11), 631 (M^+, 18), 554 (36), 485 (29), 158 (32), 118 (35), 93 (100), 77 (56). Anal. calcd. for $C_{33}H_{20}BrFN_6O_2$ (631.45) C,

62.77; H, 3.19; N, 13.31; Found: C, 62.58; H, 3.02; N, 13.22 %.

Synthesis of 7-(4-bromophenyl)-5-(4-fluorophenyl)-2-methylthio-pyrido[2,3-*d*]-pyrimidin-4(3*H*)-one (**9**)

To a stirred solution of compound **3** (2.14 g, 5 mmol) in dimethylformamide (20 ml) was added anhydrous potassium carbonate (0.70 g, 5 mmol) and methyl iodide (0.71 g, 5 mmol). The reaction mixture was stirred overnight at room temperature, then, the mixture was diluted with water. The solid formed was filtered off, washed with water, dried and crystallized from ethanol to give compound **9** as white solid; (76 % yield), mp 300–302 °C; IR (KBr): 3,074 (NH), 1,658 (2C=O); ^{1}H NMR (DMSO-d_6): δ = 2.72 (s, 3H, SCH_3), 7.24 (d, J = 8 Hz, 2H, Ar–H), 7.48 (d, J = 8 Hz, 2H, Ar–H), 7.73 (d, J = 8 Hz, 2H, Ar–H), 7.80 (s, 1H, pyridine-H), 8.24 (d, J = 8 Hz, 2H, Ar–H), 11.54 (s, 1H, NH, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6): δ = 12.05, 111.12, 113.21, 116.17, 120.47, 123.12, 123.11, 125.05, 129.19, 133.35, 143.54, 144.01, 156.28, 158.48, 159.78, 164.35. MS: *m/z* 444 (M^++2, 12), 443 (M^++ 1, 22), 442 (M^+, 62), 260 (23), 158 (13), 80 (12). Anal. calcd. for $C_{20}H_{13}BrFN_3OS$ (442.31) C, 54.31; H, 2.96; N, 9.50; Found: C, 54.21; H, 2.85; N, 9.64 %.

Synthesis of 7-(4-bromophenyl)-5-(4-fluorophenyl)-2-hydrazino-pyrido[2,3-*d*]-pyrimidin-4(3*H*)-one (**11**)

To thione **3** or its methylthio analogue **9** (5 mmol) in ethanol (20 ml) was added hydrazine hydrate (10 ml, 80 %). The reaction mixture was refluxed till all H_2S (30 h) or MeSH (5 h) ceased to evolve, and then cooled. The solid that precipitated was filtered off and crystallized from dilute dioxane to give compound **11** as white solid; (80 % yield), mp 276 °C; IR (KBr): 3,200, 3,150, 3,100 (NH_2, 2NH) in IR 3310 cm^{-1}, 1,689 (C=O); ^{1}H NMR (DMSO-d_6): δ = 2.0 (br.s, 2H, HNS), 6.13 (br., 1H, NH), 7.24–8.19 (m, 9H, Ar–H) 10.56 (br., 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 111.0, 114.68, 119.88, 120.18, 123.91, 124.26, 128.35, 130.55, 134.94, 135.41, 145.20, 147.70, 158.71, 159.69, 164.35. MS: *m/z* 428 (M^++2, 5), 427 (M^++ 1, 2), 426 (M^+, 16), 395 (12), 156 (34), 121 (52), 96 (18), 76 (100). Anal. calcd. for $C_{19}H_{13}BrFN_5O$ (426.24) C, 53.54; H, 3.07; N, 16.43; Found: C, 53.35; H, 3.05; N, 16.28 %.

General procedure for the preparation of **12a–c**

A mixture of hydrazine **11** (1.07 g, 2.5 mmol) and the appropriate aldehydes (2.5 mmol) in acetic acid (20 ml) and few drops of conc. hydrochloric acid (≈1 ml) was heated under reflux for 3 h. The reaction mixture was then cooled and diluted with water. The so-formed solid product was then collected by filtration, dried and recrystallized from dimethylformamide to afford the corresponding hydrazones **12a–c**

7-(4-Bromophenyl)-5-(4-fluorophenyl)-2-[(phenylmethylene)hydrazino]-3*H*-pyrido[2,3-*d*]pyrimidin-4-one (**12a**)

Pale Yellow solid; (76 % yield), mp 336–338 °C; IR (KBr): 3,336, 3,100 (2NH), 1,681 (C=O); ^{1}H NMR (DMSO-d_6): δ = 7.23–7.27 and 7.50–7.53 (m, 9H, Ar–H), 7.41 (s, 1H, =CH), 7.71 (d, J = 8 Hz, 2H, Ar–H), 7.97 (s, 1H, pyridine-H), 8.15 (d, J = 8 Hz, 2H, Ar–H), 12.25 (br.s, 2H, 2NH, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6): δ = 112.26, 118.21, 120.05, 121.57, 122.99, 127.53, 129.15, 131.78, 131.28, 133.12, 134.50, 137.12, 138.95, 147.54, 150.17, 154.36, 155.01, 158.29, 159.45, 161.45. MS: *m/z* 515 (M^++ 1, 54), 514 (M^+, 42), 513 (50), 437 (54), 201 (50), 199 (69), 158 (27), 105 (58), 104 (62), 89 (50), 77 (46), 76 (100). Anal. calcd. for $C_{26}H_{17}BrFN_5O$ (514.35) C, 60.71; H, 3.33; N, 13.62; Found: C, 60.49; H, 3.26; N, 13.47 %.

7-(4-Bromophenyl)-5-(4-fluorophenyl)-2-[(4-florophenylmethylene)hydrazino]-3*H*-pyrido[2,3-*d*]pyrimidin-4-one (**12b**)

Yellow solid; (80 % yield), mp 340–342 °C; IR (KBr): 3,200, 3,143 (2NH), 1,666 (C=O); ^{1}H NMR (DMSO-d_6): δ = 7.24–8.09 (m, 12H, Ar–H), 8.14 (s, 1H, pyridine-H), 8.33 (s, 1H, = CH), 12.25 (br.s, 2H, 2NH, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6): δ = 110.89, 113.53, 114.17, 120.65, 121.44, 123.14, 127.35, 131.54, 131.97, 133.58, 137.23, 137.96, 147.58, 150.19, 153.70, 158.06, 159.15, 159.94, 160.24, 161.58. MS: *m/z* 533 (M^++ 1, 2), 532 (M^+, 3), 89 (25), 76 (11). Anal. calcd. for $C_{26}H_{16}BrF_2N_5O$ (532.34) C, 58.66; H, 3.03; N, 13.16; Found: C, 58.44; H, 3.14; N, 13.08 %.

7-(4-Bromophenyl)-5-(4-fluorophenyl)-2-[(4-nitrophenylmethylene)hydrazino]-3*H*-pyrido[2,3-*d*]pyrimidin-4-one (**12c**)

Yellow solid; (80 % yield), mp 328–330 °C; IR (KBr): 3,375, 3,100 (2NH), 1,701 (C=O); ^{1}H NMR (DMSO-d_6): δ = 7.18–7.69 (m, 12H, Ar–H), 7.71 (s, 1H, pyridine-H), 8.21 (s, 1H, = CH), 11.80 (br.s, 2H, 2NH, D_2O-exchangeable); ^{13}C NMR (DMSO-d_6): δ = 111.27, 120.48, 122.88, 123.0, 126.34, 127.50, 129.58, 131.02, 131.47, 135.48, 137.48, 138.04, 148.25, 148.98, 151.75, 155.45, 158.78, 159.61, 162.01, 164.42. MS: *m/z* 560 (M^++2, 1), 559 (M^++ 1, 1), 558 (M^+, 2), 531 (100), 436 (21), 109 (64), 95 (58), 76 (15). Anal. calcd. for $C_{26}H_{16}BrFN_6O_3$ (559.35) C,

55.83; H, 2.88; N, 15.02; Found: C, 55.72; H, 2.81; N, 15.21 %.

General procedure for the preparation of **13a–c**

Method A

Bromine (0.052 g, 1 mmol) in acetic acid (5 ml) in presence of sodium acetate (1 mmol) was added drop wise to a stirred solution of the appropriate hydrazone **12a–c** (1 mmol of each) in acetic acid (10 ml). The reaction mixture was then poured onto ice cold water, and the solid that precipitated was filtered off, washed with sodium bicarbonate solution and then with water, dried and crystallized from the dimethylformamide to give the respective compounds **13a**, **c**.

Method B

A mixture of 2-hydrazinopyrido[2,3-*d*]pyrimidine **11** (1 mmol) and aroyl chloride (1.5 mmol) in dry pyridine (8 ml) was heated under reflux for 20 h. the reaction mixture was cooled and the solid formed was filtered, washed with ethanol, dried and ethanol and crystallized from the appropriate solvent to give the respective compounds **13a**, **c**.

8-(4-Bromophenyl)-6-(4-fluorophenyl)-3-phenyl-pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]-pyrimidin-5 (1*H*)-one (**13a**)

Yellow solid; (68 % yield), mp 326–328 °C; IR (KBr): 3,313 (NH), 1,708 (C=O); ^{1}H NMR (DMSO-d_6): δ = 6.98–7.98 (m, 13H, Ar–H), 8.05 (s, 1H, pyridine-H), 12.08 (br. 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6): δ = 110.33, 119.45, 120.34, 122.26, 123.19, 126.90, 129.68, 130.06, 132.35, 132.52, 134.48, 137.32, 138.68, 148.36, 151.98, 154.28, 156.21, 158.27, 160.53, 162.94. MS: *m/z* 512 (M^+, 12), 511 (29), 106 (21), 104 (44), 97 (47), 91 (27), 84 (47), 77 (52), 55 (100). Anal. calcd. for $C_{26}H_{15}BrFN_5O$ (512.33) C, 60.95; H, 2.95; N, 13.67; Found: C, 60.76; H, 3.57; N, 13.52 %.

8-(4-Bromophenyl)-6-(4-fluorophenyl)-3-(4-florophenyl)-pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**13b**)

Yellow solid; (65 % yield, mp 328–330 °C; IR (KBr): 3,282 (NH), 1,701 (C=O); ^{1}H NMR (DMSO-d_6): δ = 7.16–8.18 (m, 12H, Ar–H), 8.09 (s, 1H, pyridine-H), 12.11 (br. 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6): δ = 110.33, 114.14, 115.81, 120.32, 122.34, 123.22, 129.82, 130.11, 132.15, 134.38, 137.37, 138.86, 148.28, 151.97, 154.35, 158.57, 160.54, 162.95, 164.24, 167.06. MS: *m/z* 534 (M^++4, 18), 533 (M^++ 3, 78), 532, (M^++ 2, 81), 531 (M^++ 1, 86), 530 (M^+, 100), 529 (59), 122 (25), 108 (91), 75 (25). Anal. calcd. for $C_{26}H_{14}BrF_2N_5O$ (530.32) C, 58.88; H, 2.66; N, 13.21; Found: C, 58.74; H, 2.48; N, 13.11 %.

8-(4-Bromophenyl)-6-(4-fluorophenyl)-3-(4-nitrophenyl)-pyrido[2,3-*d*][1,2,4]-triazolo[4,3-*a*]pyrimidin-5(1*H*)-one (**13c**)

Yellow solid; (66 % yield), mp 338–340 °C; IR (KBr): 3,200 (NH), 1,697 (C=O); ^{1}H NMR (DMSO-d_6): δ = 7.19–8.05 (m, 12H, Ar–H), 8.04 (s, 1H, pyridine-H), 12.14 (br. 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6): δ = 110.34, 120.34, 122.27, 123.23, 124.08, 126.23, 129.76, 130.15, 131.43, 134.33, 137.41, 138.64, 148.19, 148.85, 151.88, 154.29, 158.28, 160.63, 162.99, 164.46. MS: *m/z* 559, (M^++ 2, 12), 558 (M^++ 1, 15), 557 (M^+, 40), 122 (16), 95 (25), 91 (86), 89 (58), 77 (100). Anal. calcd. for $C_{26}H_{14}BrFN_6O_3$ (557.33) C, 56.03; H, 2.53; N, 15.08; Found: C, 55.94; H, 2.45; N, 15.01.

Biological assay

Agar diffusion well method to determine the antimicrobial activity

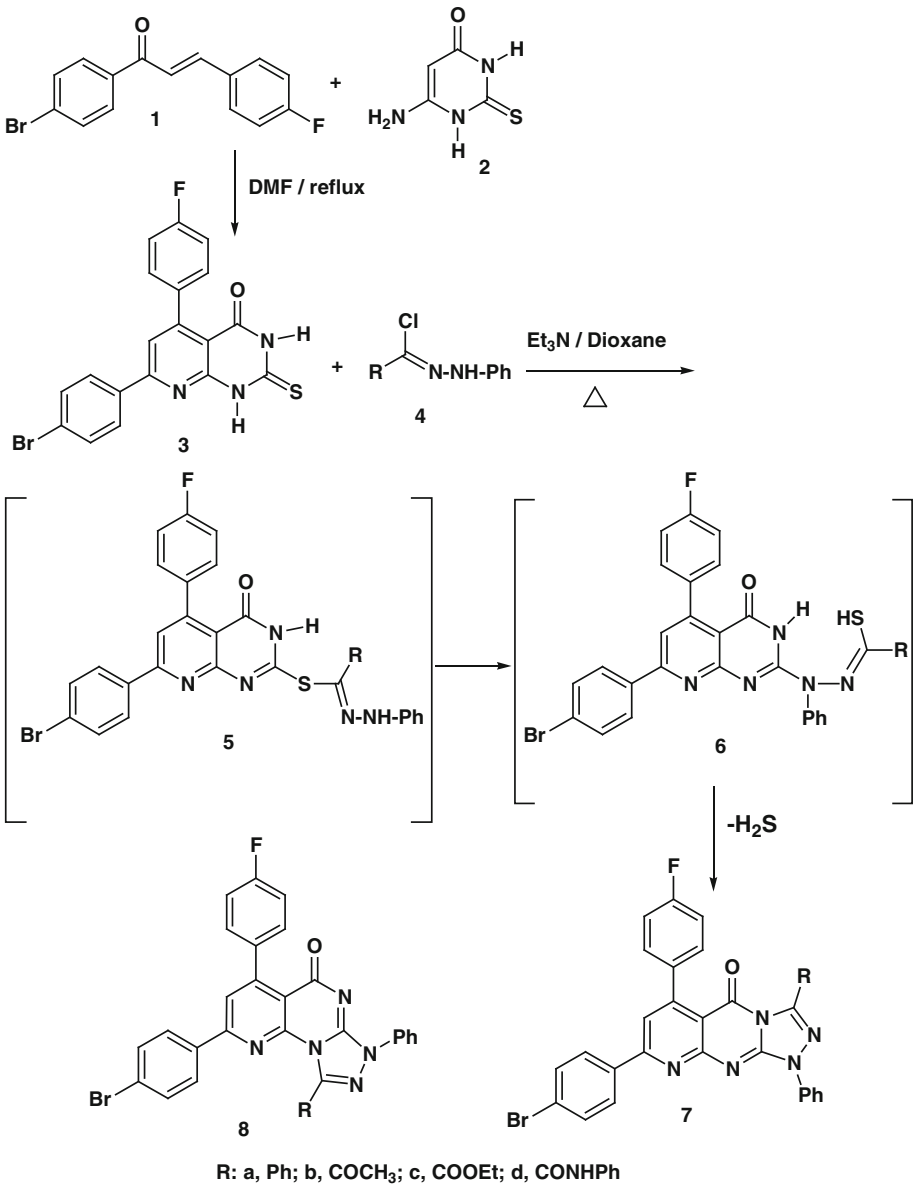
The microorganism inoculums were uniformly spread using sterile cotton swab on a sterile Petri dish containing Malt extract agar (for fungi) and nutrient agar (for bacteria). Each sample (100 µl) was added to each well (6 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24–48 h at 37 °C (for bacteria) and at 28 °C (for fungi). After incubation, microorganism growth was observed. Inhibition of the bacterial and fungal growth were measured in mm. Tests were performed in triplicate (Smania et al. 1999).

Results and discussion

The starting 7-(4-bromophenyl)-1,2-dihydro-5-(4-fluorophenyl)-2-thioxo-pyrido-[2,3-*d*] pyrimidin-4(3*H*)-one **3** was prepared by refluxing 6-amino-2-thiouracil **2** and 3-(4-fluorophenyl)-1-(4-bromophenyl)-2-propen-1-one **1** in dimethylformamide according to the procedure reported previously (Quiroga et al. 1992) (Scheme 1). The structure of compound **3** was established on the bases of spectral data (Ms, IR, ^{1}H NMR and ^{13}C NMR) and elemental analysis (see Experimental part).

Reaction of compound **3** with hydrazonoyl chlorides **4a–d** in dioxane in the presence of triethylamine was yielded one isolable product in each case. Both spectroscopic

Scheme 1 8-(4-Bromophenyl)-6-(4-fluorophenyl)-1,3-aryl-pyrido[2,3-*d*][1,2,4]triazolo-[4,3-*a*]pyrimidin-5-one **7**



data and elemental analyses were consistent with structure **7** or **8** (Scheme 1). An immediate distinction between these two structures was reached by comparison of the ^{13}C NMR spectra with those of similar annulated pyrimidinones. Literature report (Reiter et al. 1987) has shown that the chemical shift for the carbonyl carbon in 4-pyrimidinone derivatives is markedly affected by the nature of the adjacent nitrogen (N3) (pyrrole type in structure **7** and pyridine type as in structure **8**). For example, the ^{13}C NMR spectra of **7b** and **7c** taken as typical examples of the series prepared, revealed the signals of the carbonyl carbon of the pyrimidinone ring residue at δ 163.15 and 163.62 ppm, respectively. Such chemical shift values are similar to those of annulated pyrimidines with N3 pyrrole type rather than those of N3 pyridine type. On the basis of this similarity, the isolated products were assigned structure **7** and the isomeric structure **8** was excluded.

As depicted in Scheme 1, the reaction proceeded through *S*-alkylation (Geis et al. 1991), to give *S*-alkylated products **5** followed by Smiles rearrangement (Elliott et al. 1975), afforded intermediate **6** which consumed in situ via elimination of hydrogen sulfide gas to give the desired products **7a**–**d**. The assignment for the structure products and reaction mechanism can be manifested by alternate synthesis. Thus, refluxing of methylthio-derivative **9** (prepared by the reaction of **3** with methyl iodide in dimethylformamide in presence of anhydrous potassium carbonate) with hydrazonoyl chloride **4a** under the same reaction conditions afforded product **7a** through the intermediate **10** with concurrent elimination of methanethiol.

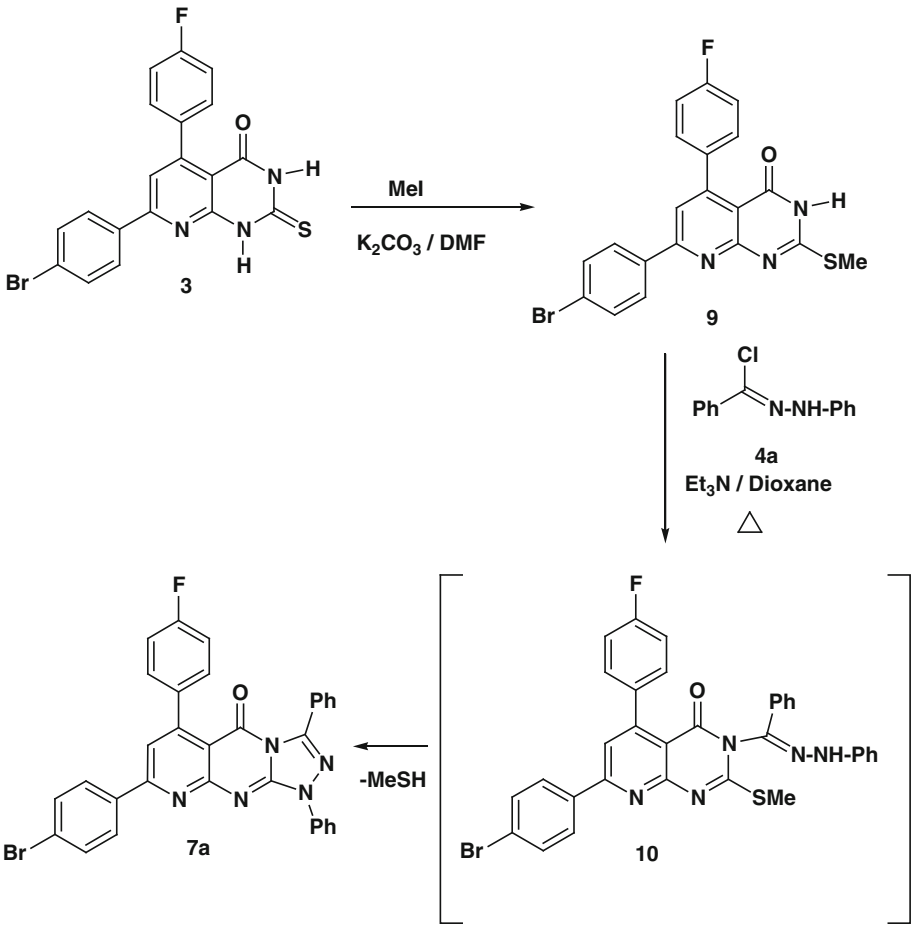
The product **7a** found to be identical in all respects (mp, mixed mp and IR) with the product produced from the reaction of **3** with **4a** (Scheme 2).

Reacting compound **3** or **9** with hydrazine hydrate in absolute ethanol afforded 2-hydrazino-pyrido[2,3-*d*]pyrimidin-4(3*H*)-one **11**. The structure of **11** was evidenced by its spectra and elemental analysis (see experimental section). Condensation of the hydrazine derivative **11** with appropriate aldehydes afforded the required 2-(*N*-arylidene-hydrazino)-7-(4-bromophenyl)-5-(4-fluoro-phenyl)-3*H*-pyrido[2,3-*d*]pyrimidin-4-one derivatives **12** (Scheme 3). All such hydrazones have not been reported hitherto. Their structures were confirmed by their elemental analyses and spectral (Ms, IR and ^{1}H NMR) data. For example, their ^{1}H NMR spectra revealed in each case, a characteristic singlet signal in region 7.41–8.33 ppm assignable to the –N=CH- proton. Their IR spectra showed the characteristic band for the N–H stretch of the hydrazone group in region 3,375–3,100 cm^{-1}. The conversion of **12** into **13** is reminiscent of the oxidative cycloaddition of aldehyde *N*-heteroaryl hydrazone with bromine, which have been reported to proceed via generation of respective nitilimines, with undergo in situ 1,5-electrocyclization to give the respective fused heterocycles (Shawali 2010).

Thus, in our hand, treatment of hydrazone **12** with equiv. quantity of bromine in acetic acid in the presence of sodium acetate gave, in each case, a single product as evidenced by TLC analysis. Elemental analyses and mass spectra reveled that each such isolated product has two hydrogens less than the respective hydrazone. This finding was confirmed by ^{1}H NMR spectra, which indicated the absence of the –N=CH- proton. On the basis of this finding the isolated products were pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one derivatives **13**. The assigned structure of compounds **13** was further confirmed by chemical transformation. Thus, reaction of **11** with aroyl chloride in dry pyridine led to formation of products which are identical in all respects (mp, mixed mp and IR) with the products produced from the reaction of **12** with bromine/acetic acid (Scheme 3).

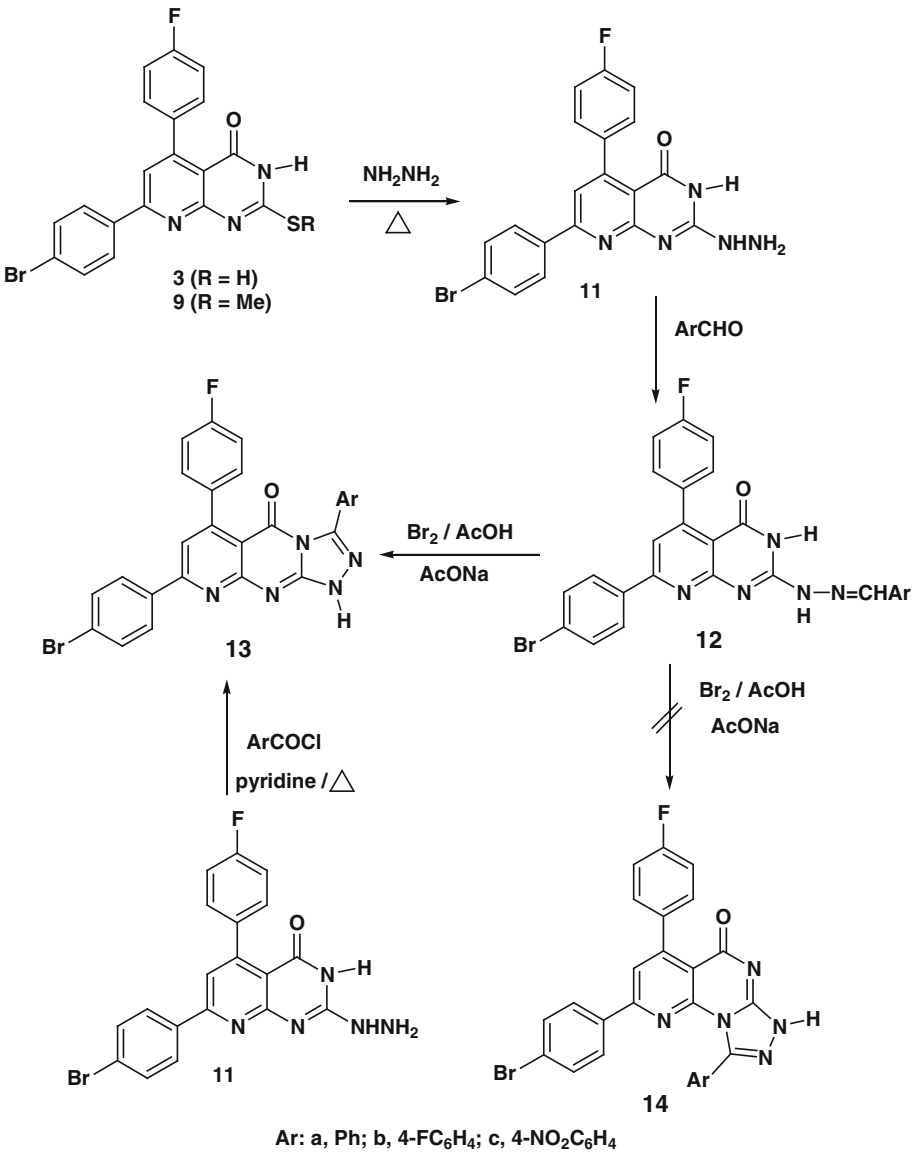
Antimicrobial activity

In vitro antimicrobial screening of compounds prepared in this study was carried out using cultures of four fungal



Scheme 2 Alternative synthesis of compound **7**

Scheme 3 Synthesis of compound **13**



strains, including *Aspergillus fumigatus* (RCMB 002003) (AF), *Syncephalastrum racemosum* (RCMB 005003) (SR), *Geotrichum candidum* (RB 052006) (GC), and *Candida albicans* (RCMB 005002) (CA) as well as four bacteria species, namely, Gram positive Bacteria, *S. aureus* (RCMB 000106) (SA) and *Bacillus subtilis* (RCMB 000107) (BS), Gram negative Bacteria, *Pseudomonas aeruginosa* (RCMB 000102) (PA) and *Escherichia coli* (RCMB 000103) (EC). Amphotericin B as an antifungal agent, Ampicillin as an antibacterial agent for gram (+) bacteria and Gentamicin as an antibacterial agent for gram (−) bacteria were used as references to evaluate the potency of the tested compounds under the same conditions. Most of newly synthesized compounds showed excellent with respect to control drugs. The results of antimicrobial activities were shown in Table 1. Data in Table 1 revealed that most of compounds have superior significant antibacterial potency to antifungal potency. Compounds **3**, **7a**, **9**, **11**, **12a** and **13c** exhibited the highest potency against all tested organisms with respect to reference drugs except *SR* and *PA*. The other derivatives showed moderate activity against the microorganisms used.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the synthesized compounds against highly inhibited organism is reported in Table 2.

Compound **7a** revealed high MIC 500 μg/ml against *Aspergillus fumigatus* (RCMB 002003), *Candida albicans* (RCMB 005002) and *Escherichia coli* (RCMB 000103) respectively. Compound **13c** exhibited low MIC 0.12 μg/ml against *Aspergillus fumigatus* (RCMB 002003) and also 0.24 μg/ml against *S. aureus* (RCMB 000106).

Table 1 Antimicrobial activity expressed as inhibition diameter zones in millimeter (mm) of chemical compounds against the pathological strains based on well diffusion as assay

Compound no.	Fungi				Gram positive bacteria		Gram negative bacteria	
	A. fumigatus	*S. racemosum*	*G. candidum*	*Candida albicans*	*Staphylococcus aureus*	*B. subtilis*	*Pseudomonas aeruginosa*	*E. coli*
3	18.7	N.A.	19.3	15.2	16.2	19.4	N.A.	16.2
7a	21.9	N.A.	24.3	19.3	20.4	22.8	N.A.	18.9
7b	9.3	N.A.	10.4	8.4	10.0	12.3	N.A.	7.4
7c	N.A.	N.A.	N.A.	N.A.	10.2	12.1	N.A.	6.4
7d	16.2	N.A.	17.6	16.0	17.3	18.7	N.A.	17.9
9	18.9	N.A.	19.5	15.9	16.8	19.7	N.A.	17.4
11	19.0	N.A.	19.8	16.3	17.1	19.9	N.A.	18.2
12a	19.9	N.A.	21.3	18.2	20.0	21.2	N.A.	17.2
12b	12.8	N.A.	14.2	11.7	15.8	18.6	N.A.	12.3
12c	17.2	N.A.	19.8	16.1	18.8	20.3	N.A.	18.0
13a	15.4	N.A.	18.3	13.5	17.8	19.3	N.A.	15.7
13b	11.3	N.A.	12.8	10.2	13.4	15.3	N.A.	9.1
13c	21.3	N.A.	26.4	24.2	22.0	21.4	N.A.	18.9
Amphotericin B	23.7	19.7	28.7	25.4	N.A.	N.A.	N.A.	N.A.
Ampicillin	N.A.	N.A.	N.A.	N.A.	23.8	32.4	N.A.	N.A.
Gentamicin	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	17.3	19.9

The experiment was carried out in triplicate and average zone of inhibition was calculated; (N.A. = no activity)

Table 2 Minimum inhibitory concentration (μg/ml) against the pathological strains

Compound no.	Fungi				Gram positive bacteria		Gram negative bacteria	
	A. fumigatus	*S. racemosum*	*G. candidum*	*Candida albicans*	*Staphylococcus aureus*	*B.subtilis*	*Pseudomonas aeruginosa*	*E. coli*
7a	15.63	N.A.	62.5	62.5	62.5	7.82	N.A.	15.63
11	500	N.A.	250	500	250	125	N.A.	500
12a	7.82	N.A.	3.91	15.63	3.91	3.91	N.A.	31.25
12c	250	N.A.	125	250	125	62.5	N.A.	500
13c	0.12	N.A.	0.04	5.63	0.24	0.02	N.A.	2.01
Amphotericin B	0.12	1.95	0.03	0.06	N.A.	N.A.	N.A.	N.A.
Ampicillin	N.A.	N.A.	N.A.	N.A.	0.24	0.015	N.A.	N.A.
Gentamicin	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	7.82	1.95

Structure activity relationship

From the results depicted in Tables 1 and 2 we found that the parent pyridopyrimidine derivative **3** showed promising activity against the tested organisms. This encouraged derivatization of compound **3** hopping to improve the antimicrobial effect. Here, the chemistry represented two classes, the first one constituted pyridopyrimidine nucleus linked to different Schiff bases **12a–c**, while the second class the Schiff base included in linear cyclic structure forming triazolopyridopyrimidines **7a–d, 13a–c**. They are tested against the previously mentioned microorganisms comparatively with the parent compound **3** and standard drug for each microbial class. Recorded data clarified that, the importance of hydrophobic moieties for the antimicrobial activity. Replacing the –SH group in compound **3** with –SMe or $–NHNH_2$ groups in compounds **9** and **11** respectively, produced positive impact on the activity. On the other hand, Schiff base bicyclic systems **12a–c** showed negative effect on the activity except compound **12a**. While in triazolopyridopyrimidine system, direct linking of hydrophobic phenyl moiety at C3 in **7a** explored reliable antimicrobial activity that was dramatically diminished upon introducing CONH moiety as spacer in **7d.** The activity was almost abolished in **7b, c** upon replacing the phenyl group with $COCH_3$ and COOEt in **7b, c**

respectively. Moreover, compounds **13a–c** showed the vital role of *p*-NO_2 group in **13c.** Compound **13c** displayed strong antimicrobial activity which was diminished in phenyl and *p*-F phenyl analogues **13a, b**. Compared to the reference drug, triazolo derivatives **7a** and **13c** were the most effective compounds. They explored similar antimicrobial activity proving that substitution at C3 dramatically affects the activity rather than at N1. Compound **13c** carries H atom at N1 which was replaced by phenyl group in **7a**. Simultaneously, substituent at C3 in **7a** was phenyl group that replaced by *p*-NO_2phenyl group in **13c**. All the tested compounds are fully inactive against SR and PA organism.

Conclusion

In conclusion, we reported herein a simple and convenient routes for the synthesis of new derivatives of pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one via two different methods. All the newly synthesized compounds were evaluated for antimicrobial activity and the result showed that compounds **3**, **7a**, **9**, **11**, **12a** and **13c** have excellent activity against all the tested microorganisms except *SR* and *PA*. The most effective derivative was compound **13c** which includes pharmacologically active 4-NO_2, 4-F, and 4-Br groups attached to phenyl ring at position 3, 6 and 8.

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