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Antimicrobial investigations on synthetic *p*-tolylazo derivatives of thienopyrimidinone based on an ortho funtionalized thiophene nucleus

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ABSTRACT

New derivatives of thieno[2,3-*d*]pyrimidin-4-one, **4a-c**, and **6** were respectively synthesized based on thiophene derivative, **2c,** bearing *ortho*-disposed amino and ester groups. Carbohydrazide, **7,** was obtained by hydrazinolysis of 3-ethoxycabonylmethyl derivative, **4c**. Compounds **4b** and **7** were used to build up two series of novel 3-substituted-methylthieno[2,3-*d*]pyrimidin-4-ones, **7-11,** and their corresponding 3-substituted-amino analogues **12, 15a,b, 18**, which were of significant interest for biological study. The new thieno[2,3-*d*]pyrimidin-4-one derivatives, with various groups in position 3, were screened for their preliminary antimicrobial activity against a representative panel of Gram-positive and Gram-negative bacteria as well as fungi strains. The compounds tested displayed different levels of antibacterial and antifungal effects, with the assays carried out on six pathogenic bacteria and six pathogenic fungi. Of these compounds, the 3-unsubstituted-thieno[2,3-*d*]pyrimidin-4(3*H*)-one, **4a,** showed the lowest effect on pathogenic bacteria, while the corresponding 3-substituted analogues produced inhibitory effects against bacteria similar or superior to the reference drug Tetracycline. For those derivatives of thieno[2,3-*d*]pyrimidin-4-one in which the 3-position contains a methylene moiety, it has been observed that the antibacterial effect was in general found to be significantly higher than the corresponding analogues with a substituted-amino moiety in position 3. Despite promising *in vitro* antibacterial activity of the new thienopyrimidin-4-ones, only compounds **7, 10** and **11**, among the compounds tested, exhibited some kind of antifungal activity. The detailed synthesis and biological screening data were reported.

1. Introduction

The current interest in the development of new antimicrobial agents can be partially ascribed both to the increasing emergence of bacterial resistance to antibiotic therapy and to newly emerging pathogens [1,2]. Despite advances in antibacterial therapy, many problems remain to be solved for a new generation of antimicrobial agents. For example, in the hospital setting, the re-emergence of Gram-negative pathogens is of major concern. In fact, the most important cases of sepsis were caused by virulent Gram-negative bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Enterobacter spp.* [3,4]. Furthermore, emerging resistance among new pathogens such as *Acinetobacter baumannii*, and also the appearance of multidrug resistant Gram-positive bacteria, particularly, methicillin-resistant *Staphylococcus aureus (MRSA)* and vancomycin-resistant *Enterococci,* are causing a serious threat to public health. Therefore, the development of new and efficacious antimicrobial drugs is a very important goal, and most of the research efforts in this field are directed towards the design of new agents. A review of the recent literature shows that many effective antimicrobial agents contain a heterocyclic moiety within their structure [5,6] and, in particular, that substituted pyrimidinone derivatives have a wide spectrum of biological properties such as antibacterial, antifungal and antiviral activities [7,8]. Furthermore, thienopyrimidines are in general well known for their diverse pharmaceutical applications [9-11] with particular emphasis on the antimicrobial properties of their thieno[2,3-*d*]pyrimidine analogues [12-14].

Motivated by the significant antimicrobial activity recorded for some thienopyrimidines and as part of our ongoing studies in the development of new chemotherapeutic agents [15-17], we embarked upon the synthesis of a variety of novel thieno[2,3-*d*]pyrimidin-4-one derivatives having different groups in position 3 with the objective to investigate their antimicrobial properties in the search for new broad spectrum therapeutic agents.

2. Experimental

2.1. Instrumentation

Melting points are uncorrected. IR spectra were recorded (KBr) on a Pye Unicam SP-1000 spectrophotometer. NMR spectra were obtained on a Varian Gemini 300 MHz spectrometer in DMSO-d_6 as solvent and TMS as internal reference. Chemical shifts are expressed in δ ppm. The biological evaluation of the products was carried out at the

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Micro Analytical Center in Cairo University, Cario, Egypt. Compounds **1** [18] and **2a** [19] were prepared according to known methods.

2.2. Synthesis

2.2.1. General procedure for the synthesis of ethyl 5-arylazo-2-amino-4-phenylthiophene-3-carboxylates (2b, c)

A finely ground powder of the *p*-substituted aniline (0.002 mol) was dissolved in a mixture of concentrated hydrochloric acid (2 mL) and water (15 mL), and stirred overnight at room temperature. The solution was cooled to 0-5 °C, a well cooled saturated solution of sodium nitrite (0.0021 mol) was added portionwise at 5-10 °C, and the reaction content was stirred for a further 1 h at the same temperature. Excess nitrous acid was destroyed by the addition of urea, and the solution was cooled to 0-5 °C. The resulting clear diazonium salt solution was then added dropwise over 20 min with constant stirring and with frequent addition of ice to a cold (0-5 °C) stirred solution of coupling component aminoester **1** (0.0018 mol) dissolved in 20 mL of ethanol containing sodium acetate (0.01 mol) while maintaining temperature at 0-5 °C. After addition of the diazonium salt, the mixture was stirred for an additional 3 h at 5-10 °C. The precipitated product, in each case, separated upon dilution with cold water (30 mL) was filtered off, washed with hot water and with cold water, and dried. Recrystallization from the appropriate solvents gave the arylazo derivatives **2b** (0.40 g; 57%) and **2c** (0.51 g; 77%), respectively.

2.2.1.1. Ethyl 2-amino-5-(4-chlorophenylazo)-4-phenyl thiophene-3-carboxylate (2b)

This compound was obtained as a light brown solid (aqueous ethanol). M.p.: 170-171 °C. IR (ν/cm^{-1}): 3442, 3330 (NH_2), 3052 (arom CH), 2950 (aliph CH), 1675 (CO). ^{1}H NMR (δ ppm): 1.02 (t, 3H, *J* = 7.2 Hz, ester Me), 4.05 (q, 2H, *J* = 7.2 Hz, ester CH_2), 6.01 (s, br, 2H, NH_2, D_2O-exchangeable), 7.26-7.59 (m, 9H, PhH, ArH). *Anal.* Calcd. for $C_{19}H_{16}ClN_3O_2S$ (385.867): C, 59.14; H, 4.18; Cl, 9.19; N, 10.89; S, 8.31. Found: C, 58.93; H, 4.01; Cl, 8.97; N, 10.72; S, 8.16.

2.2.1.2. Ethyl 2-amino-4-phenyl-5-(p-tolylazo)thiophene-3-carboxylate (2c)

This compound was obtained as a yellow solid (ethanol), M.p.: 155-156 °C. IR (ν/cm^{-1}): 3465, 3341 (NH_2), 3056 (arom CH), 2940 (aliph CH), 1673 (CO). ^{1}H NMR (δ ppm): 0.98 (t, 3H, *J* = 7.2 Hz, ester Me), 2.30 (s, 3H, Ar-Me), 4.12 (q, 2H, *J* = 7.2 Hz, ester CH_2), 5.76 (s, br, 2H, NH_2, D_2O-exchangeable), 7.32-7.71 (m, 9H, PhH, ArH). ^{13}C NMR (δ ppm): 13.7 (ester Me), 21.6 (Ar-Me), 59.5 (ester CH_2), 107.9 (C-3), 125.4, 127.3, 128.4, 128.9, 129.6, 130.0, 131.5, 134.9, 138.4, 153.2, 162.1 (C-2), 165.7 (ester CO). *Anal.* Calcd. for $C_{20}H_{19}N_3O_2S$ (365.449): C, 65.73; H, 5.24; N, 11.50; S, 8.77. Found: C, 65.47; H, 5.09; N, 11.31; S, 8.61.

2.2.2. Synthesis of ethyl 2-[(dimethylamino methylene amino]-4-phenyl-5-(p-tolylazo)thiophene-3-carboxylate (3)

Compound **2c** (0.005 mol), in dry xylene (30 mL), was treated with DMFDMA (0.006 mol) portionwise. The reaction content was stirred under reflux for 6 h and then left to cool. Stirring was continued at room temperature for an additional 12 h. The solvent was removed by evaporation under vacuo to dryness. The residual semisolid was triturated with petroleum ether, whereby the solid product formed was filtered off, dried and recrystallized from 1,4-dioxane to give the amidine **3** as a pale yellow solid (0.84 g; 40%), M.p.: 178-179 °C. IR (ν/cm^{-1}): 3057 (arom CH), 2930-2865 (aliph CH), 1709 (CO). ^{1}H NMR (δ ppm): 1.25 (t, 3H, *J* = 7.2 Hz, ester Me), 2.21 (s, 3H, Ar-Me), 2.79, 2.89 (2s, 6H, NMe_2), 4.23 (q, 2H, *J* = 7.2 Hz, ester CH_2), 7.34-7.70 (m, 9H, PhH, ArH), 8.87 (s, 1H, amidine-H). ^{13}C NMR (δ ppm): 13.9 (ester Me), 21.5 (Ar-Me), 33.8, 38.3 (NMe_2), 59.8 (ester CH_2), 118.5 (C-3), 125.6, 127.6, 128.5, 128.9, 129.4, 129.8, 131.4, 134.8, 138.7, 152.4, 155.2 (amidine CH), 164.9 (C-2), 166.1 (ester CO). *Anal.* Calcd. for $C_{23}H_{24}N_4O_2S$ (420.527): C, 65.69; H, 5.75; N, 13.32; S, 7.62. Found: C, 65.54; H, 5.63; N, 13.09; S, 7.51.

2.2.3. Synthesis of 5-phenyl-6-(p-tolylazo)thieno[2,3-d] pyrimidin-4(3H)-one (4a)

2.2.3.1. Method A

A stirred suspension of glacial acetic acid (10 mL) and ammonium acetate (0.015 mol), was treated with compound **3** (0.005 mol). The mixture content was refluxed for 5 h after that it was cooled to room temperature and poured onto iced water. The material which separated upon cooling was isolated by filtration, washed with petroleum ether, dried and recrystallized from dimethylformamide to give the corresponding thienopyrimidine derivative **4a** as a golden yellow solid (0.76 g; 44%). M.p.: 221-223 °C. IR (ν/cm^{-1}): 3152 (NH), 3074 (arom CH), 2965, 2870 (aliph CH), 1667 (CO). ^{1}H NMR (δ ppm): 2.24 (s, 3H, Ar-Me), 7.31-7.75 (m, 9H, PhH, ArH), 8.30 (s, 1H, pyrimidine H-2), 12.53 (s, 1H, NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{19}H_{14}N_4OS$ (346.406): C, 65.88; H, 4.07; N, 16.17; S, 9.26. Found: C, 65.76; H, 3.90; N, 15.95; S, 9.07.

2.2.3.2. Method B

A solution of **2c** (0.002 mol) and formamide (20 mL) was refluxed for 4 h. The mixture was cooled and crude product was filtered off, washed with petroleum ether, dried and recrystallized from dimethylformamide to give a solid product, in 49% yield, identical in all aspects (mp, mixed mp and IR data) to that obtained above from method A.

2.2.4. Synthesis of 3-amino-5-phenyl-6-(p-tolylazo thieno [2,3-d]pyrimidin-4(3H)-one (4b)

A mixture of **3** (0.005 mol), hydrazine hydrate (0.05 mol, 2.5 mL) and absolute ethanol (12 mL) was refluxed for 8 h. The precipitate, formed after cooling overnight, was collected by filtration and washed with cold alcohol. Recrystallization from methanol gave the *N*-amino compound **4b** as an orange solid (0.74 g; 41%). M.p.: 202-204 °C. IR (ν/cm^{-1}): 3316-3204 (NH_2), 3065 (CH arom), 2960, 2872 (CH aliph), 1672 (CO). ^{1}H NMR (δ ppm): 2.19 (s, 3H, Ar-Me), 7.28-7.69 (m, 9H, PhH, ArH), 5.20 (s, 2H, NH_2, D_2O-exchangeable), 8.07 (s, 1H, pyrimidine H-2). *Anal.* Calcd. for $C_{19}H_{15}N_5OS$ (361.420): C, 63.14; H, 4.18; N, 19.38; S, 8.87. Found: C, 62.89; H, 4.01; N, 19.18; S, 8.72.

2.2.5. Synthesis of ethyl 2-[4-oxo-5-phenyl-6-(p-tolylazo) thieno[2,3-d]pyrimidin-3(4H)-yl]acetate (4c)

To a solution of compound **4a** (0.003 mol) in dimethylformamide (20 mL), anhydrous potassium carbonate (0.006 mol) was added and the mixture was stirred at room temperature for 15 min, followed by the addition of ethyl chloroacetate (0.0033 mol) in dimethylformamide (10 mL). Stirring was continued at room temperature overnight, according to thin layer chromatographic (TLC) analysis. And then ice/water mixture was added to the reaction mixture to form a precipitate, which was collected by filtration, washed with water, dried and recrystallized from ethanol to obtain compound **4c** as colorless needles (0.78 g; 60%). M.p.: 186 °C. IR (ν/cm^{-1}): 3060 (arom CH), 2934 (aliph CH), 1734 (ester CO), 1680 (pyrimidine CO). ^{1}H NMR (δ ppm): 1.30 (t, 3H, *J* = 7.5 Hz,

ester Me), 2.37 (s, 3H, Ar-Me), 4.13 (q, 2H, *J* = 7.5 Hz, ester CH_2), 4.83 (s, 2H, NCH_2CO), 7.30-7.75 (m, 9H, PhH, ArH), 8.49 (s, 1H, pyrimidine H-2). ^{13}C NMR (δ ppm): 14.3 (ester Me), 21.0 (Ar-Me), 45.3 (NCH_2), 61.4 (ester CH_2), 119.1 (C-4a), 125.3, 127.4, 128.2, 128.7, 129.5, 129.9, 132.3, 134.5, 138.5, 150.2 (C-2), 152.5, 157.6 (C-6, C-7a), 160.1 (pyrimidine CO), 168.6 (ester CO); *Anal.* Calcd. for $C_{23}H_{20}N_4O_3S$ (432.495): C, 63.87; H, 4.66; N, 12.95; S, 7.41. Found: C, 63.65; H, 4.49; N, 12.76; S, 7.20.

2.2.6. Synthesis of ethyl 2-acetamido-4-phenyl-5-(p-tolylazo) thiophene-3-carboxylate (5)

A mixture of *ortho*-aminoester **2c** (0.005 mol) and acetic anhydride (2 mL) was refluxed for 1 h with constant stirring in acetic acid (5 mL). The mixture was cooled and poured over iced water. The resulting precipitate was collected by filtration, washed with water and dried. The residue was purified by recrystallization from acetic acid to obtain the acetamido ester **5** as a canary yellow solid (0.63 g; 31%). M.p.: 191-192 °C. IR (ν/cm^{-1}): 3264 (NH), 3056 (arom CH), 2952-2871 (aliph CH), 1674, 1665 (2CO). ^1H NMR (δ ppm): 1.18 (t, 3H, *J* = 7.2 Hz, ester Me), 2.27 (s, 3H, Me), 2.39 (s, 3H, Me), 4.20 (q, 2H, *J* = 7.2 Hz, ester CH_2), 7.37-7.76 (m, 9H, PhH, ArH), 12.42 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ ppm): 14.0 (ester Me), 21.6 (Ar-Me), 23.5 (amide Me), 59.4 (ester CH_2), 109.0, 125.1, 127.5, 128.3, 128.7, 129.3, 130.1, 131.2, 134.7, 138.6, 149.2, 152.8 (C-2, C-5), 166.3 (ester CO), 168.1 (amide CO). *Anal.* Calcd. for $C_{22}H_{21}N_3O_3S$ (407.485): C, 64.85; H, 5.19; N, 10.31; S, 7.87. Found: C, 64.62; H, 4.99; N, 10.08; S, 7.71.

2.2.7. Synthesis of 3-amino-2-methyl-5-phenyl-6-(p-tolylazo) thieno[2,3-d]pyrimidin-4(3H)-one (6)

This compound was synthesized from *N*-acetyl derivative **5** (0.002 mol) and hydrazine hydrate (0.02 mol, 1 mL) in a manner similar to that described for the synthesis of **4b**. It was purified by recrystallization from a mixture of ethanol and water (4:1) to obtain compound **6** as a dark brown solid (0.44 g; 59%). M.p.: 241-242 °C. IR (ν/cm^{-1}): 3321, 3200 (NH_2), 3036 (CH arom), 2940-2861 (CH aliph), 1679 (CO); ^1H NMR (δ ppm): 2.26, 2.45 (2s, 6H, 2Me), 5.02 (s, br, 2H, NH_2, D_2O-exchangeable), 7.41-7.78 (m, 9H, PhH, ArH). *Anal.* Calcd. for $C_{20}H_{17}N_5OS$ (375.447): C, 63.98; H, 4.56; N, 18.65; S, 8.54. Found: C, 63.76; H, 4.40; N, 18.45; S, 8.43.

2.2.8. Synthesis of 2-[4-oxo-5-phenyl-6-(p-tolylazo thieno [2,3-d]pyrimidin-3(4H)-yl]acetohydrazide (7)

Hydrazine hydrate (0.015 mol) was added to a solution of compound **4c** (0.003 mol) in absolute ethanol (25 mL). The reaction mixture was refluxed for 4 h, concentrated *in vacuo*, cooled and diluted with water. The obtained precipitate was collected by filtration, washed with cold water, dried and purified by recrystallization from an ethanol and water mixture to give yellowish white crystals of the title compound **7** (0.82 g; 65%), M.p.: 210-212 °C. IR (ν/cm^{-1}): 3361-3158 ($NHNH_2$), 3062 (arom CH), 2920 (aliph CH), 1681 (pyrimidine CO), 1657 (hydrazide CO). ^1H NMR (δ ppm): 2.32 (s, 3H, Ar-Me), 4.45 (s, 2H, NH_2, D_2O-exchangeable), 4.64 (s, 2H, NCH_2CO), 7.33-7.71 (m, 9H, PhH, ArH), 8.43 (s, 1H, pyrimidine H-2), 9.50 (s, 1H, NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{21}H_{18}N_6O_2S$ (418.472): C, 60.27; H, 4.34; N, 20.08; S, 7.66. Found: C, 60.03; H, 4.15; N, 19.86; S, 7.52.

2.2.9. Synthesis of 2-{2-[4-oxo-5-phenyl-6-(p-tolylazo)thieno [2,3-d]pyrimidin-3(4H)-yl]acetyl}-N-phenylhydrazine carbothioamide (8)

A solution containing carbohydrazide **7** (0.003 mol) and phenyl isothiocyanate (0.003 mol), in ethanol (20 mL), was heated at reflux for 2 h and then cooled at room temperature. The formed precipitate was collected by filtration, washed with water, dried. Recrystallization from ethanol gave the thiosemicarbazide **8** as a reddish brown solid (0.85 g; 51%), M.p.: 232-233 °C. IR (ν/cm^{-1}): 3300-3175 (3NH), 3062 (arom CH), 2926 (aliph CH), 1681 (pyrimidine CO), 1667 (thiosemicarbazide CO). ^1H NMR (δ ppm): 2.31 (s, 3H, Ar-Me), 4.71 (s, 2H, NCH_2CO), 6.97-7.62 (m, 14H, 2PhH, ArH), 8.45 (s, 1H, pyrimidine H-2), 9.65, 9.80 (2s, 2H, NHCSNH, D_2O-exchangeable), 10.61 (s, 1H, CONH, D_2O-exchangeable). *Anal.* Calcd. for $C_{28}H_{23}N_7O_2S_2$ (553.658): C, 60.74; H, 4.19; N, 17.71; S, 11.58. Found: C, 60.49; H, 3.96; N, 17.52; S, 11.42.

2.2.10. Synthesis of 5-phenyl-3-[(5-phenylamino-1,3,4-thiadiazol-2-yl)methyl]-6-(p-tolylazo)thieno[2,3-d]pyrimidin-4(3H)-one (9)

To 0.003 mol of thiosemicarbazide, **8**, concentrated sulfuric acid (5 mL) was added dropwise. The mixture was stirred at room temperature for 30 min. The reaction mixture was poured over ice/water mixture with stirring. The precipitated solid was filtered off, washed with sodium carbonate solution, followed by water and recrystallized from ethanol to obtain compound **9** as a yellow solid (0.85 g; 53%), M.p.: 265 °C. IR (ν/cm^{-1}): 3240 (NH), 3076 (arom CH), 2924 (aliph CH), 1680 (CO). ^1H NMR (δ ppm): 2.28 (s, 3H, Ar-Me), 4.99 (s, 2H, NCH_2), 6.95-7.58 (m, 14H, 2PhH, ArH), 8.50 (s, 1H, pyrimidine H-2), 10.52 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ ppm): 20.9 (Ar-Me), 45.7 (NCH_2), 117.5, 118.9, 122.8, 125.2, 127.7, 128.1, 128.6, 129.0, 129.6, 130.1, 132.5, 134.6, 138.5, 140.2, 149.8, 152.4 (C-2, C-6), 155.1 (thiadiazole C-2), 157.5 (C-7a), 160.9 (pyrimidine CO), 165.0 (thiadiazole C-5). *Anal.* Calcd. for $C_{28}H_{21}N_7OS_2$ (535.643): C, 62.78; H, 3.95; N, 18.30; S, 11.97. Found: C, 62.54; H, 3.81; N, 18.08; S, 11.78.

2.2.11. Synthesis of 5-phenyl-3-[(4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]-6-(p-tolylazo)thieno[2,3-d]pyrimidin-4(3H)-one (10)

Compound **8** (0.005 mol) was dissolved in ethanolic solution of sodium hydroxide (20 mL, 4%) and stirred under reflux for 5 h. The resulting solution was cooled and filtered. The filtrate was acidified with dilute hydrochloric acid and was kept aside for 1 h. The separated solid product was collected by filtration, washed repeatedly with water, dried and recrystallized from a mixture of dimethylformamide and water to obtain brown crystals of the thione derivative **10** (0.80 g; 30%). M.p.: 249-250 °C. IR (ν/cm^{-1}): 3192 (NH), 3072 (arom CH), 2924 (aliph CH), 1680 (CO), 1186 (CS). ^1H NMR (δ ppm): 2.28 (s, 3H, Ar-Me), 5.08 (s, 2H, NCH_2), 6.64-7.56 (m, 14H, 2PhH, ArH), 8.47 (s, 1H, pyrimidine H-2), 13.75 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ ppm): 21.2 (Ar-Me), 48.6 (NCH_2), 119.2, 125.4, 127.6, 127.9, 128.4, 128.8, 129.2, 129.7, 130.0, 132.3, 133.2, 134.0, 134.8, 138.7, 148.0 (triazoline C-3), 150.1 (C-2), 152.5, 157.3 (C-6, C-7a), 160.5 (pyrimidine CO), 171.6 (triazoline CS). *Anal.* Calcd. for $C_{28}H_{21}N_7OS_2$ (535.643): C, 62.78; H, 3.95; N, 18.30; S, 11.97. Found: C, 62.61; H, 3.74; N, 18.12; S, 11.74.

2.2.12. Synthesis of 3-{5-(N-acetyl-N-phenylamino)-1,3,4-thiadiazol-2-yl]methyl}-5-phenyl-6-(p-tolylazo)thieno[2,3-d]pyrimidin-4(3H)-one (11)

A mixture of thiadiazole **9** (0.001 mol) in 5 mL acetic anhydride was heated at reflux for 1 h. The mixture was diluted with 30 mL of cold water, and the precipitate was filtered off, washed with water and dried. The residue was purified by recrystallization from 1,4-dioxane to obtain *N*-phenylacetamide derivative **11** as a brown solid (0.47 g; 82%). M.p.: 227-228 °C. IR (ν/cm^{-1}): 3075 (arom CH), 2940-2865 (aliph CH), 1686 (amide CO), 1679 (pyrimidine CO). ^{1}H NMR (δ ppm): 2.25, 2.51 (2s, 6H, 2Me), 5.05 (s, 2H, NCH_2), 7.35-7.67 (m, 14H, 2PhH, ArH), 8.47 (s, 1H, pyrimidine H-2). *Anal.* Calcd. for $C_{30}H_{23}N_7O_2S_2$ (577.679): C, 62.37; H, 4.01; N, 16.97; S, 11.10. Found: C, 62.13; H, 3.79; N, 16.82; S, 10.92.

2.2.13. Synthesis of 3-(benzylideneamino)-5-phenyl-6-(p-tolylazo)thieno[2,3-d]pyrimidin-4(3H)-one (12)

2.2.13.1. Method A

To a solution of equimolar amounts (0.003 mol) of compound **4b** and benzaldehyde in absolute ethanol (15 mL), two drops of glacial acetic acid were added. The reaction content was heated at reflux for 4 h. After cooling to room temperature, the resulting solid product was collected by filtration, dried and recrystallized from a mixture of ethanol and dimethylformamide (2:1) to give the respective condensation product **12** as a dark brown solid (0.61 g; 45%), M.p.: > 300 °C. IR (ν/cm^{-1}): 3048 (arom CH), 2950 (aliph CH), 1673 (CO). ^{1}H NMR (δ ppm): 2.30 (s, 3H, Ar-Me), 7.32-7.76 (m, 14H, 2PhH, ArH), 8.29 (s, 1H, pyrimidine H-2), 8.75 (s, 1H, N=CH). *Anal.* Calcd. for $C_{26}H_{19}N_5OS$ (449.527): C, 69.47; H, 4.26; N, 15.58; S, 7.13. Found: C, 69.30; H, 4.02; N, 15.36; S, 6.95.

2.2.13.2. Method B

The same product was synthesized from compound **14** (0.001 mol) and DMFDMA (0.0012 mol) in a manner similar to that described before for the synthesis of compound **3**. It was recrystallized from an ethanol and dimethylformamide mixture (2:1) to give a solid product, in 79% yield, identical in all aspects (mp, mixed mp and IR data) to that obtained above from method A.

2.2.14. Synthesis of 2-amino-4-phenyl-5-(p-tolylazo) thiophene-3-carbohydrazide (13)

The same experimental procedure described before for the synthesis of compound **7** was followed except using compound **2c** (0.003 mol) instead of **4c**. Recrystallization from ethanol gave the corresponding aminocarbohydrazide **13** as a reddish brown crystals (0.79 g; 75%). M.p.: 174-175 °C. IR (ν/cm^{-1}): 3410-3182 (NH, NH_2), 3035 (arom CH), 2922 (aliph CH), 1659 (CO). ^{1}H NMR (δ ppm): 2.27 (s, 3H, Ar-Me), 4.30 (s, 2H, NH_2 carbohydrazide, D_2O-exchangeable), 6.92 (s, 2H, NH_2, D_2O-exchangeable), 7.35-7.70 (m, 9H, PhH, ArH), 9.17 (s, 1H, NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{18}H_{17}N_5OS$ (351.425): C, 61.52; H, 4.88; N, 19.93; S, 9.12. Found: C, 61.27; H, 4.74; N, 19.69; S, 9.01.

2.2.15. Synthesis of amino-N'-benzylidene-4-phenyl-5-(p-tolylazo)thiophene-3-carbohydrazide (14)

Synthesis of compound **14** was carried out by the same method as described before for compound **12**. Recrystallization from acetic acid gave the corresponding benzylidene carbohydrazide **14** as a yellow solid (0.83 g; 63%). M.p.: 256-258 °C. IR (ν/cm^{-1}): 3453-3178 (NH, NH_2), 3044 (arom CH), 2936 (aliph CH), 1668 (CO). ^{1}H NMR (δ ppm): 2.27 (s, 3H, Ar-Me), 6.76 (s, 2H, NH_2, D_2O-exchangeable), 7.32-7.79 (m, 14H, 2PhH, ArH), 8.10 (s, 1H, N=CH), 11.06 (s, 1H, NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{25}H_{21}N_5OS$ (439.532): C, 68.32; H, 4.82; N, 15.93; S, 7.30. Found: C, 68.09; H, 4.64; N, 15.73; S, 7.22.

2.2.16. Synthesis of 3-[(dimethylamino)methyleneamino]-5-phenyl-6-(p-tolylazo)thieno[2,3-d]pyrimidin-4(3H)-one (15a)

This compound was obtained using compound **4b** in a manner similar to that described before for the synthesis of compound **3**. It was recrystallized from dimethylformamide to give the corresponding amidine **15a** (0.94 g, 45%). Alternatively, the same product was obtained, in 49% yield, from aminocarbohydrazide **13** (0.001 mol) and DMF-DMA (0.0024 mol) following the same procedure as that of **3**. This compound was obtained as an orange solid, M.p.: 181-182 °C. IR (ν/cm^{-1}): 3040 (arom CH), 2942-2860 (aliph CH), 1674 (CO). ^{1}H NMR (δ ppm): 2.32 (s, 3H, Ar-Me), 2.96, 3.03 (2s, 6H, NMe_2), 7.40-7.73 (m, 9H, PhH, ArH), 7.94 (s, 1H, amidine-H), 8.27 (s, 1H, pyrimidine H-2); *Anal.* Calcd. for $C_{22}H_{20}N_6OS$ (416.499): C, 63.44; H, 4.84; N, 20.18; S, 7.70. Found: C, 63.24; H, 4.71; N, 19.97; S, 7.61.

2.2.17. Synthesis of 3-[(Ethoxymethylene)amino]-5-phenyl-6-(p-tolylazo)thieno[2,3-d]pyrimidin-4(3H)-one (15b)

A mixture of compound **4b** (0.003 mol) and triethyl orthoformate (1.5 mL, 0.009 mol), was heated at reflux in acetic anhydride (10 mL) for 6 h. The reaction mixture was evaporated under reduced pressure and the viscous mass was treated with ether or petroleum ether (5 mL). The precipitated solid product was collected by filtration and dried. Recrystallization from ethanol gave pale brown crystals of the *N*-ethoxymethylene derivative **15b** (0.63 g; 50%). M.p.: 150 °C. IR (ν/cm^{-1}): 3040 (arom CH), 2957-2874 (aliph CH), 1675 (CO). ^{1}H NMR (δ ppm): 1.20 (t, 3H, *J* = 7.3 Hz, ethoxy Me), 2.31 (s, 3H, Ar-Me), 4.33 (q, 2H, *J* = 7.3 Hz, OCH_2), 7.37-7.73 (m, 9H, PhH, ArH), 8.15 (s, 1H, methylenic CH), 8.31 (s, 1H, pyrimidine H-2). *Anal.* Calcd. for $C_{22}H_{19}N_5O_2S$ (417.484): C, 63.29; H, 4.59; N, 16.78; S, 7.68. Found: C, 63.07; H, 4.40; N, 16.62; S, 7.53.

2.2.18. Synthesis of 3-(formylamino)-5-phenyl-6-(p-tolylazo) thieno[2,3-d]pyrimidin-4(3H)-one (18)

Compound **4b** (0.002 mol) was heated at reflux in formic acid (10 mL) for 7 h. The reaction mixture was then diluted with cold water and allowed to stand overnight. The resulting precipitate was filtered off, washed with ethanol (20 mL), dried and recrystallized from a mixture of 1,4-dioxane and dimethylformamide (3:1) to give the formyl derivative **18** (0.56 g; 72%). Alternatively, the same product was obtained, in a comparable yield, using compound **13** instead of **4b**. This compound was obtained as a dark brown solid, M.p.: > 300 °C. IR (ν/cm^{-1}): 3300 (NH), 3061 (CH arom), 2953 (CH aliph), 1720 (HCO), 1680 (CO ring). ^{1}H NMR (δ ppm): 2.21 (s, 3H, Ar-Me), 7.29-7.68 (m, 9H, PhH, ArH), 8.07 (s, 1H, pyrimidine H-2), 8.46 (s, 1H, CHO), 10.95 (s, 1H, NH, D_2O-exchangeable). *Anal.* Calcd. for $C_{20}H_{15}N_5O_2S$ (389.430): C, 61.68; H, 3.88; N, 17.98; S, 8.23. Found: C, 61.49; H, 3.67; N, 17.84; S, 8.06.

2.2.19. Reaction of 15a,b with hydrazine hydrate

To a solution of either **15a** or **15b** (0.001 mol), in methanol (20 mL), hydrazine hydrate (0.0025 mol) was added and the reaction content was then stirred under reflux for 2-4 h. The reaction mixture was cooled and diluted with a three-fold amount of water and the precipitate was filtered off, dried and

recrystallized from methanol to give, in each case, a solid product in 69-77% yield, identical in all respects (mp, mixed mp and IR data) to the *N*-amino compound **4a**.

2.3. Antimicrobial Assay

The preliminary antimicrobial activity was investigated on a dozen of newly synthesized thienopyrimidin-4-one derivatives having different groups in position 3 in order to increase the selectivity of these derivatives towards test microorganisms. The antimicrobial profile was tested against three Gram-positive bacteria species (*Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis*), three Gram-negative bacteria species (*Escherichia coli, Neisseria gonorrhoeae, Pseudomonas aeruginosa*), two moulds (*Aspergillus flavus, Aspergillus niger*) and four yeasts (*Candida albicans, Candida parapsilosis, Candida tropicalis, Saccharomyces cerevisiae*) using a modified Kirby-Bauer disc diffusion method [20]. Briefly, 100 µl of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 108 cells/mL for bacteria or 105 cells/mL for fungi [21]. One hundred µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained.

Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method [22]. Of the many media available, National Committee for Clinical Laboratory Standards (NCCLS) recommends Mueller-Hinton agar as it results in good batch-to-batch reproducibility. Disc diffusion method for filamentous fungi was tested by using approved standard method (M38-A) developed by the NCCLS [23] for evaluating the susceptibilities of filamentous fungi to antifungal agents. Disc diffusion method for yeasts was developed by using approved standard method (M44-P) according to reference [24]. The plates were incubated at 25 °C for 48 h for fungi such as *Aspergillus flavus* and at 35-37 °C for 24-48 h for bacteria such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeuroginosa*, whereas for yeast such as *Candida albicans* they were incubated at 30 °C for 24-48 h. The resulting inhibition zone diameters (IZDs) were measured in millimeters and used as criterion for the antimicrobial activity [20]. The size of the clear zone is proportional to the inhibitory action of the compound under investigation.

Standard discs of the bactericide Tetracycline and the fungicide Amphotericin B served as positive controls for antimicrobial activity but filter discs impregnated with 10 µl of solvent (distilled water, chloroform, DMSO) were used as a negative control. The agar used is Meuller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated with 10µl of the tested concentration of the stock solutions. When a filter paper disc impregnated with a tested chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "Zone of inhibition" or "Clear zone". For the disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards [22]. Agar-based methods such as Etest and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods [25].

Solvent controls (DMF) were included in every experiment as negative controls. DMF was used for dissolving the crude extracts and showed no inhibition zones, confirming that it has no influence on growth of the tested microoganisms.

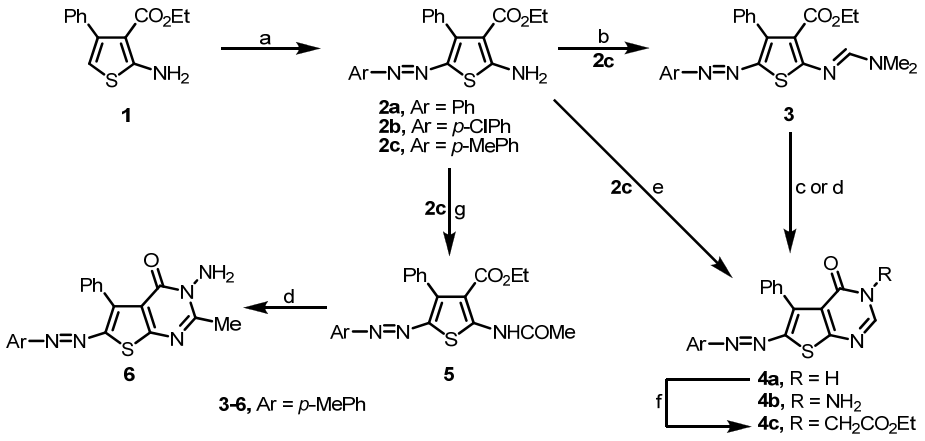
3. Results and Discussion

3.1. Chemistry

The starting material ethyl 2-amino-4-phenylthiophene-3-carboxylate (**1**) was obtained according to the *Gewald* method [18]. Coupling reaction of compound **1** with diazotized aniline, *p*-chloroaniline and *p*-toluidine, respectively, led to the corresponding arylazo derivatives, **2a-c**. Aminoformylation of *p*-tolylazo derivative, **2c**, with DMF dimethylacetal (DMF-DMA) provided the desired *N,N*-dimethylformamidine, **3**, whose structure was confirmed from its ^{1}H NMR spectrum. Thus, the spectrum of **3** displayed the anticipated signals from sp^3 carbons of the ethyl ester group at δ_H 1.25 and 4.23 ppm, and signals from dimethylamino group at δ_H 2.79 and 2.89 ppm. In the downfield region of the spectrum, the resonance at δ_H 8.87 ppm was due to a formamidinic proton. The ^{13}C NMR spectrum of the reaction product provided further evidence for the proposed structure, where sp^3 carbons of the ester group resonated at δ_C 13.9 and 59.8 ppm as well as signals for amidine and ester carbonyl carbons, detected at δ_C 155.2 and 166.1 ppm, respectively. The remaining carbons were also observed at the expected regions. Compound **3** was readily cyclized to the corresponding thieno[2,3-*d*]pyrimidin-4-one derivative, **4a**, on reflux in a mixture of ammonium acetate and glacial acetic acid. It is worth mentioning that compound **4a** could be also obtained directly by reacting *ortho*-aminoester, **2c**, with formamide. Compound **4a** prepared by the latter route was found to be identical to that obtained by the former method as evidenced by TLC analysis, m.p., mixed m.p. and IR data. Treating amidine, **3**, with hydrazine hydrate led to closure of the pyrimidine ring and accordingly led to the formation of anothor bicyclic thieno[2,3-*d*]pyrimidine, **4b** (Scheme 1).

Typical for the established alkylation of 3-unsubstituted-thienopyrimidin-4-ones [26-28], compound **4a** reacted readily with ethyl chloroacetate in DMF in the presence of potassium carbonate to afford the target *N*-alkylated product, **4c**. All spectroscopic data fitted perfectly with the proposed structure **4** (see Experimental section). Acylation of the amino group in compound **2c** with acetic anhydride yielded the acetamidoester, **5**. Heterocyclization to the thienopyrimidine ring system was carried out by heating **5** at reflux in an alcoholic solution of hydrazine hydrate to afford the 2-methylpyrimidin-4-one derivative **6** (Scheme 1).

Treatment of compound **4c** with excess hydrazine hydrate resulted in the formation of the acetohydrazide, **7** (Scheme 2). When compound **7** was treated with phenyl isothiocyanate, it produced the acetyl-*N*-phenylhydrazinecarbothioamide derivative, **8**. Cyclodehydration of the 3-side chain of compound **8** with concentrated sulfuric acid led to a single product with the molecular formula of $C_{28}H_{21}N_7OS_2$, which may be formulated as thiadiazole, **9**, or triazoline, **10**. The CO band, present in the IR spectrum of **8**, was not detectable for the isolated product, demonstrating the disappearance of this group on ring closure. Both elemental analysis and molecular ion peak data are not supportive for discrimination between the two isomeric structures **9** and **10**. However, the structure of the isolated product was considered to be thiadiazole structure, **9**, rather than the other possible isomeric structure **10** as evidenced by spectroscopic investigations, beside a chemical proof. In the ^{1}H NMR spectrum of the reaction product, the signal associated with the NH proton appeared at a higher field,



Reagents and conditions: (a) ArN_2Cl/EtOH/AcONa/0-5 °C; (b) $(MeO)_2CHNMe_2$/xylene/△; (c) $AcONH_4$/gl AcOH/△; (d) $H_2NNH_2.H_2O$/ab EtOH/△; (e) $HCONH_2$/△; (f) $ClCH_2CO_2Et$/DMF/K_2CO_3/r.t.; (g) Ac_2O/AcOH/△ r.t. = room temperature

Scheme 1

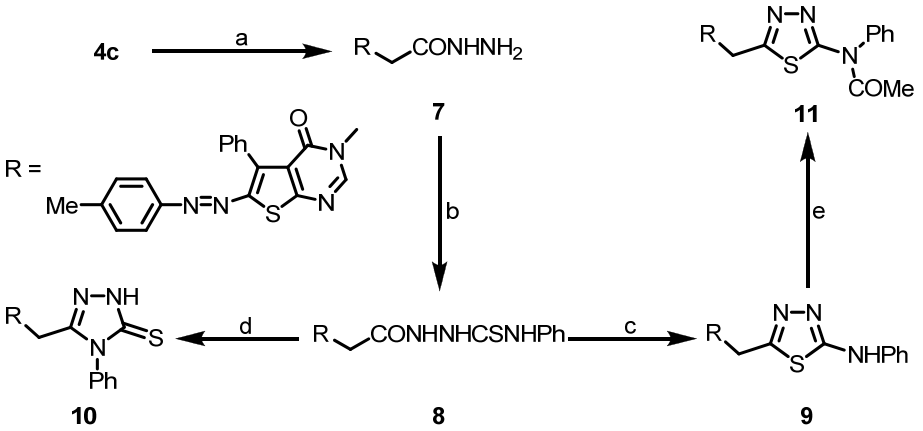
typically at δ_H 10.52 ppm as a singlet which disappeared upon addition of deuterium oxide. Such an upfield NH resonance is in complete agreement with the thiadiazole structure **9** rather than with the triazoline structure **10**, for which the thioamide NH proton would be expected to be deshielded due to the adjacent thione group. As a result, the isomeric structure **10**, if isolated, would be expected to display a downfield shift for the thioamide NH proton, typically in the range of δ_H *ca.* 13.00-14.00 ppm as reported by different authors in similar protocols [29-36]. The lack of signals arising from the thione function in the downfield region at δ_C value above 169 ppm [36-39], in the ^{13}C NMR spectrum of the product isolated from the studied reaction, is also evidence in favor of the structure **9**. On the basis of such data, it is not unreasonable to conclude that the studied reaction is completely regioselective and the structure of the isolated product is thiadiazole **9**; the alternative cyclization direction to the corresponding triazoline **10** is therefore discarded. The preferred formation of thiadiazole ring under such acidic conditions could be explained by the loss of nucleophilicity of N-4 of the carbothioamide moiety as a result of its protonation or by the higher nucleophilicity of the sulfur atom in this *N*-phenylthiourea-type group and proceeds by nucleophilic attack by the sulfur atom on the acyclic carbonyl carbon, followed by elimination of water [40]. Therefore, 5-phenylaminothiadiazole **9** was isolated as the sole product (Scheme 2).

In support of this hypothesis, the alternative structure **10** could be successfully obtained from the cyclodehydration of acetylhydrazinecarbothioamide, **8**, in boiling ethanolic sodium hydroxide solution at reflux. The configuration of the resulting triazoline derivative, **10**, is worth a special mention. Both IR and NMR spectra were informative in establishing structure **10**. Its IR spectrum have important absorption bands at ν 3192 and 1186 cm^{-1} belonging to stretching vibrations of NH and CS groups, respectively. The absorption bands associated with other functional groups appeared in the expected regions. In the ^1H NMR spectrum of **10**, a signal for the triazoline CS-NH proton was observed to be shifted downfield to 13.75 ppm. This downfield shift of NH proton in this reaction product as compared to the corresponding NH proton in compound **9** can be rationalized on the basis of the deshielding effect of the adjacent CS group, thus establishing the thione structure, **10**, [29-35]. Conclusive evidence for the proposed structure **10** was provided by ^{13}C NMR spectrum of the isolated product, in which an important signal at δ_C 171.6 ppm was detected. Such a lowfield signal can be assigned only to the thioamide CS carbon atom, which was reported to appear around that chemical shift value [37-39]. It is worthwhile to report here that compound **10** was observed to exist mainly in the thione form as its IR spectrum revealed no absorption band at around ν 2600-2550 cm^{-1}, which is indicative of thiol form [32-34, 41]. In addition, the absence of the signal due to the SH proton that resonates in the range of 1.6-4.0 ppm, in the ^1H NMR spectrum of **10**, provided confirmatory evidence for thione formation [32, 36]. This alternative mode of cyclization into a triazoline ring under such alkaline conditions may be due to the enhanced nucleophilicity of N-4 of the carbothioamide moiety towards the attack on the carbonyl carbon and subsequent dehydration to give thione derivative **10** as the sole isolated product [40].

Compound **9** was well illustrated as it afforded the acetylated thiadiazole derivative, **11**, by a nucleophilic acetylation reaction of 5-aminophenyl derivative, **9**, with acetic anhydride. This chemical transformation gives added proof for the proposed structure, besides the spectroscopic evidence. Notably, the latter products, **9-11**, presented in Scheme 2 seem to be of significant interest for antimicrobial investigations, since the antimicrobial profile of 1,2,4-triazoline-3-thione [31-35,41-43] and 1,3,4-thiadiazole [32,43-47] derivatives is well explored in the literature.

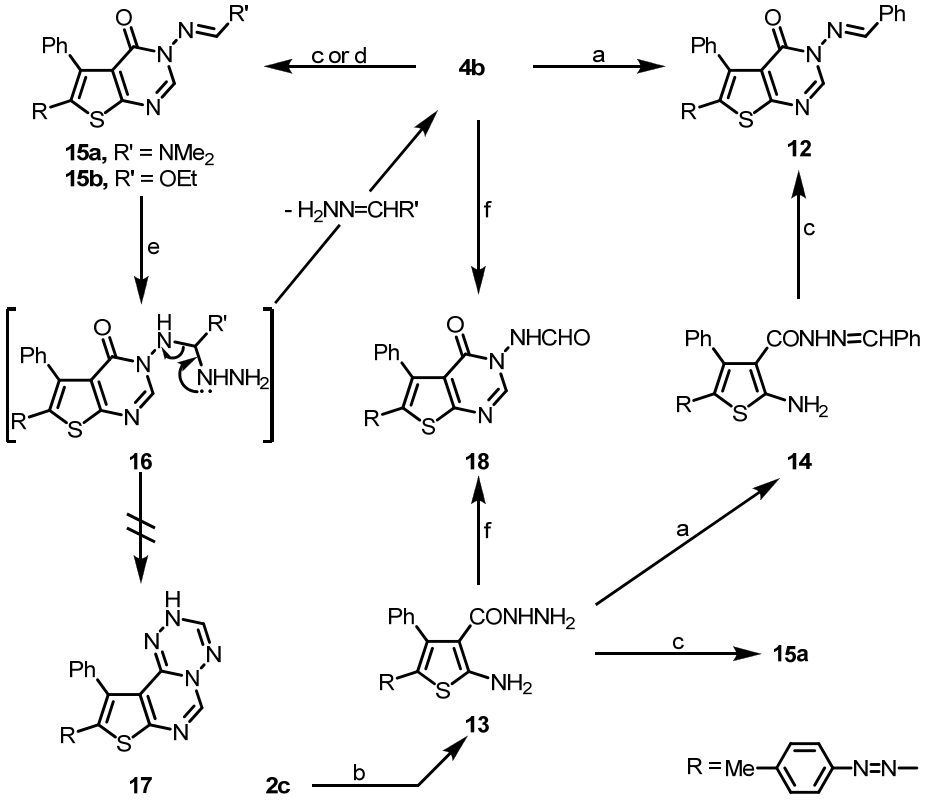
As expected, condensation of the *N*-amino compound **4b** with benzaldehyde furnished the corresponding benzylideneamino derivative, **12** (Scheme 3). Another synthesis of **12** was achieved *via* an alternative synthetic route involving the reaction of aminoester, **2c**, with hydrazine hydrate to give the aminocarbohydrazide **13**, which on subsequent treatment with benzaldehyde yielded the acyclic condensation product **14**. A further reaction of **14** with DMF-DMA led to a product (79% yield) that was identified as **12** according to TLC analysis, m.p., mixed m.p. and IR data of the isolated material. Also, amidation reaction of compound **4b** with DMF-DMA gave the corresponding amidine **15a** while with triethyl orthoformate, the *N*-ethoxymethylene derivative, **15b**, was formed. Alternatively, compound **15a** could be also obtained by reacting aminocarbohydrazide, **13**, with an excess of DMF-DMA. Elucidation of structures for the latter products was established on the basis of elemental and spectroscopic analyses in each case (see Experimental section).

Unexpectedly, the attempted cyclization of **15a,b** with hydrazine hydrate did not give the target tetrazine, **17**, but instead the *N*-amino compound, **4b**, was isolated as indicated from TLC analysis, m.p., mixed m.p. and IR data of the reaction product. This result could be explained by assuming the formation of adduct **16** as a first step. Subsequent elimination of ethyl formohydrazonate or *N,N*-dimethylformohydrazon amide gives back the original amino derivative, **4b**. This observation is supported by previous reports in the recent



Reagents and conditions: (a) $N_2H_4.H_2O$/ab EtOH/Δ; (b) PhNCS/EtOH/Δ; (c) conc H_2SO_4/r.t.; (d) NaOH/EtOH/Δ then HCl; (e) Ac_2O/Δ
r.t. = room temperature

Scheme 2



Reagents and conditions: (a) PhCHO/ab EtOH/gl AcOH/D; (b) $N_2H_4.H_2O$/ab EtOH/D; (c) $(MeO)_2CHNMe_2$/xylene/D; (d) $HC(OEt)_3/Ac_2O$/D; (e) $N_2H_4.H_2O$/MeOH/D; (f) HCO_2H/D

Scheme 3

literature concerning similar transamination of the amidine [48,49] and imino ether [50-52] moieties. Reaction of compound **4b** with formic acid provided a reaction product for which the 3-formylamino structure **18** was established on the basis of its analytical and spectroscopic data. The structure of the product **18** was further established by an independent synthesis of the same product *via* heating aminocarbohydrazide, **13**, with formic acid (Scheme 3).

3.2. Antimicrobial Evaluation

The results of testing for antibacterial and antifungal effects are summarized in Tables 1 and 2. As shown by these results, the new thienopyrimidinones tested displayed variable *in vitro* antibacterial and antifungal actions. In general, the chemical structure of the whole molecule, comprising the nature of the heterocyclic system as well as the type of the substituted function present in the heterocyclic ring structure, has a pronounced effect on antimicrobial activity. In particular, it has

Table 1. *In vitro* antibacterial activity of test compounds.[a]

Compound	IZD[b] (mm)					
	Gram-positive			Gram-negative		
	B. subtilis	*S. aureus*	*S. faecalis*	*E. coli*	*N. gonorrhoeae*	*P. aeruginosa*
4a	13	9	15	0.0	13	12
4b	36	33	24	33	25	34
4c	33	33	24	36	29	34
7	37	48	28	36	30	38
8	41	45	32	43	28	40
9	38	51	30	36	31	36
10	40	39	28	38	30	40
11	34	39	25	38	26	30
12	37	35	29	38	30	36
15a	36	33	24	34	25	37
15b	39	31	29	34	28	34
18	36	38	31	38	32	36
Standard[c]	32	27	33	32	34	33

[a] DMF has no antibacterial activity at the concentration used to dissolve the test compounds.
[b] IZD: inhibition zone diameter.
[c] Standard for bacteria: Tetracycline.

Table 2. *In vitro* antifungal activity of test compounds.[a]

Compound	IZD[b] (mm)					
	A. flavus	*A. niger*	*C. albicans*	*C. parapsilosis*	*C. tropicalis*	*S. cerevisiae*
4a	0.0	0.0	0.0	0.0	0.0	0.0
4b	0.0	0.0	0.0	0.0	0.0	0.0
4c	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	10.0	0.0	9.0	0.0	9.0
8	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	10.0	0.0	13.0	9.0
11	0.0	0.0	15.0	0.0	0.0	14.0
12	0.0	0.0	0.0	0.0	0.0	0.0
15a	0.0	0.0	0.0	0.0	0.0	0.0
15b	0.0	0.0	0.0	0.0	0.0	0.0
18	0.0	0.0	0.0	0.0	0.0	0.0
Standard[c]	16.0	12.0	18.0	13.0	12.0	10.0

[a] DMF has no antifungal activity at the concentration used to dissolve the test compounds.
[b] IZD: inhibition zone diameter.
[c] Standard for fungi: Amphotericin B.

been found that antimicrobial activity was relatively dependent on the type of substituent in the 3-position of pyrimidine ring. It was observed that the synthesized compounds substituted with a methylene moiety in the 3-position of the heterocyclic nucleus favored the antibacterial activity especially against the Gram-positive strains. Replacement of the methylene moiety by a substituted amino moiety diminished the antibacterial activity slightly. On the contrary, there was no essential *in vitro* antifungal profile of the test compounds against moulds and yeasts. Only a few thienopyrimidin-4-ones with a methylene moiety (compounds **7, 10** and **11**) revealed poor activity against the test fungi isolates. Based on the biological evaluation, compounds **8** and **9** may be considered promising for the development of new antibacterial agents.

4. Conclusion

In summary, we have demonstrated in the present study that reactions of 3-acetylhydrazine, **7**, and *N*-amino compound **4b** provide an easy and versatile access to functionalized thienopyrimidin-4-ones of significant interest for biological study. The biological potential of the new thienopyrimidin-4-one derivatives was further investigated by screening their antimicrobial activity against six pathogenic bacteria and six pathogenic fungi. Biological study of the compounds under investigation indicated that the most prominent and consistent antimicrobial activity was obtained with compound **9** carrying a methylene attached to thiadiazole moiety in the 3-position of the heterocycle. Its IZD value (51 mm) toward *S. aureus* is very significant. Compound **8** showed an appreciable broad spectrum of action against both Gram-positive and Gram-negative bacteria. In light of the results presented in this work and taking into account that this preliminary study does not produce conclusive evidence regarding a structure-antimicrobial activity relationship, we have focused our attention on the most promising compounds **8** and **9** as an interesting starting point for the development of a new class of antibacterial agents. Further optimization and structure-activity relationships (SAR) of these type of heterocycles are well underway. We believe that research in this direction should be encouraged in order to broaden the applicability of these new heterocyclic frameworks to serve as leads for designing novel chemotherapeutic agents.

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