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Regioselective synthesis and antimicrobial studies of novel bridgehead nitrogen heterocycles containing the thienopyrimidinone skeleton

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ABSTRACT

Versatile 2-(alkylthio)pyrimidine-type and 2-(phenacylamino)thiophene building blocks (**4a-d**) and **16** were obtained based on an ortho functionalized thiophene derivative **1**. A novel series of thieno[2,3-*d*]pyrimidin-4-one derivatives with annelated bridgehead nitrogen heterocycles was synthesized starting from precursors **4** and **16** through convenient methods. Cyclocondensation of 2-(phenacylthio)pyrimidinone derivative (**4b**) with sulfuric acid led to the tricyclic thiazole derivative **5**. Initial hydrazinolysis of 3-(carbethoxymethyl)pyrimidinone derivative (**4d**) followed by nitrous acid deamination of the formed *N*-aminolactam (**7**) to obtain a *N*-protodeamino analogue **8a**, which on further treatment with formaldehyde and piperidine yielded the respective Mannich-type base **8b**. On the other hand, initial hydrazinolysis of 3-unsubstituted pyrimidinone derivative **4a** and subsequent acetylation gave the condensed 3-methyltriazole derivative **12**, whereas the condensed pyrrole derivative **19** was obtained by heterocyclization of 2-phenacylamine derivative **16** with malononitrile. All newly-obtained thienopyrimidinones with annelated bridgehead nitrogen were screened for their antimicrobial activity against strains of a representative panel of Gram-positive and Gram-negative bacteria as well as fungi together with reference drugs. The compounds under investigation displayed generally good *in vitro* antibacterial and antifungal activities, with compound **8b** that has a *N*-piperidinylmethyl moiety showing essentially the highest inhibition in both assays. Despite promising antimicrobial activity of *N*-1-substituted imidazole derivative **8b**, the corresponding *N*-1-unsubstituted analogue **8a** displayed poor activity. The heteroannelation of a *N*-(piperidinylmethyl)imidazole or 3-methyltriazole moiety to the thienopyrimidinone scaffold could be considered as a potential strategy for the development of new therapeutic antimicrobial agents.

1. Introduction

There has been an increasing demand for new antimicrobial agents, as the fast development of microbial resistance to conventional drugs poses major difficulties in the treatment of microbial infections, especially for people with impaired immune systems such as patients affected with AIDS and cancer, and transplant patients [1]. In particular, antibiotic resistance among Gram-positive bacteria (*Staphylococci, Enterococci, and Streptococci*) is becoming increasingly serious [2]. It is known that the biochemical similarity of the human cell and fungi provides a significant obstacle to the discovery of therapeutic agents with selective activity, but the emergence of resistance is the main problem encountered in the development of safe and effective antifungals [3]. In order to overcome these emerging resistance problems, there is an urgent need to discover new antimicrobial agents with novel modes of action.

In the course of reviewing various structures which may be of use in the design of novel antimicrobial agents, azoles have attracted much of our attention because of their synthetic and biological importance. Among azoles, imidazole and triazole analogues are present in many effective antifungal drugs widely used for the treatment of topical or inner mycoses, in particular AIDS-related mycotic pathologies [4]. The main effect of antifungal azoles is to block fungal ergosterol biosynthesis by preventing the access of the natural substrate lanosterol to the active site of the cytochrome P-450-dependent enzyme 14α-lanosterol demethylase [4,5]. Over the past couple of decades, a number of antifungal imidazole agents were discovered and introduced in clinical practice such as clotrimazole, bifonazole and miconazole, whereas fluconazole was identified as one of the most important antifungal drugs in the triazole family [6]. Moreover, pyrrole and thiazole subunits are useful structural components in medicinal chemistry and have also found broad applications in antimicrobial drug developments. Pyrrolomycin B, used as an antibiotic, represents an example of a bioactive pyrrole [7]. Such medicines as sulfathiazole, phthalylsulfathiazole and related compounds are commonly used in medical practice as well [8]. Besides, the antimicrobial profile of condensed thiophenes, particularly, their thieno[2,3-*d*]pyrimidin-4-one analogues is also well categorized in the literature [9-11].

Based on these observations and as part of our ongoing studies in the development of new chemotherapeutic agents [12,13], we embarked upon the synthesis of a series of novel

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condensed tricyclic derivatives containing a thieno[2,3-*d*]pyrimidin-4-one core fused through N-3 with different heterocyclic fragments with the objective to develop new leads and to improve their efficacy.

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 Tetramethylsilane (TMS) as internal reference. Chemical shifts are expressed in δ ppm. EI mass spectra were recorded on a Shimadzu GC MS-QP 1000 EI mass spectrometer at 70 eV. Compound **1** was prepared by published procedure [14].

2.2. Synthesis

2.2.1. Synthesis of N-(3-carbethoxy-4-phenyl-5-phenyl azothien-2-yl)-N'-benzoylthiourea (2)

To a solution of ammonium thiocyanate (0.26 g, 0.0034 mol) in dry acetone (15 mL) benzoyl chloride (0.28 mL, 0.0024 mol) was added dropwise. To the resulting suspension, after being refluxed for 5 min, a solution of amino ester **1** (0.84 g, 0.0024 mol) in dry acetone (60 mL) was slowly added dropwise and with constant stirring. The reaction mixture was heated at reflux for 2 h, filtered while hot, left to cool to room temperature and poured onto iced water. The precipitate was collected by filtration, repeatedly washed with cold water and dried. The thienylthiourea **2** can be processed without recrystallization. An analytically pure sample can be obtained by recrytallization from ethanol to give pale yellow crystals of the title compound **2** (0.68 g; 55%). M.p.: 153-154 °C. IR (ν/cm^{-1}): 3312-3240 (2NH), 3041 (arom CH), 1677, 1672 (2CO), 1566 (N=N). ^{1}H NMR (δ ppm): 1.35 (t, 3H, *J* = 7.2 Hz, ester Me), 4.46 (q, 2H, *J* = 7.2 Hz, ester CH_2), 7.24-8.09 (m, 15H, 3PhH), 9.58 (s, 1H, NHCS, D_2O-exchangeable), 11.20 (s, br, 1H, NHCO, D_2O-exchangeable). MS (*m/z* (%)): 514 (M^+, 15%). Anal. Calcd. for $C_{27}H_{22}N_4O_3S_2$ (514.619): C, 63.02; H, 4.31; N, 10.89; S, 12.46. Found: C, 62.79; H, 4.20; N, 10.71; S, 12.21%.

2.2.2. Synthesis of 5-phenyl-6-phenylazo-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-one (3)

To a solution of potassium hydroxide (0.56 g, 0.01 mol) in aqueous ethanol (50%, 25 mL) was added the thienylthiourea **2** (2.57 g, 0.005 mol). The reaction content was refluxed under stirring for 2 h. After cooling, the reaction mixture was poured onto water, carefully acidified with hydrochloric acid and left under stirring at room temperature for 30 min. The precipitated crystals were collected by filtration, washed with cold water and air-dried to give by recrystallization from ethanol/dioxane the thienopyrimidinone derivative **3** as a yellow solid (0.71 g; 39%). M.p.: 191-192 °C. IR (ν/cm^{-1}): 3200, 3100 (2NH), 3038 (arom CH), 1670 (CO), 1552 (N=N), 1194 (CS). ^{1}H NMR (δ ppm): 7.16-7.51 (m, 10H, 2PhH), 12.67 (s, 1H, CONH, D_2O-exchangeable), 13.02 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ ppm): 116.9 (C-4a), 127.0, 127.5, 127.9, 128.3, 128.6, 129.4, 132.3, 135.2, 142.1, 152.5, 153.0, 159.7 (CO), 173.9 (CS). MS (*m/z* (%)): 364 (M^+, 21%). Anal. Calcd. for $C_{18}H_{12}N_4OS_2$ (364.444): C, 59.32; H, 3.32; N, 15.37; S, 17.60. Found: C, 59.11; H, 3.18; N, 15.24; S, 17.38%.

2.2.3. General procedure for the synthesis of 2-substituted-5-phenyl-6-(phenylazo)thieno[2,3-d]pyrimidin-4(3H)-ones 4a,b

A solution of compound **3** (1.82 g, 0.005 mol) in dimethylformamide (25 mL) was stirred with anhydrous potassium carbonate (1.04 g, 0.0075 mol) and then either methyl iodide or phenacyl bromide (0.005 mol) was added sequentially, while maintaining the temperature of the reaction mixture at 0-5 °C. Stirring was continued for 3 h at this temperature and continued for additional 2 h at room temperature. After treatment with water, the solid precipitates were collected by filtration, dried and recrystallized from the proper solvents to obtain the title compounds **4a** (0.76 g; 40%) and **4b** (0.75 g; 31%), respectively.

2.2.3.1. 2-Methylthio-5-phenyl-6-(phenylazo)thieno[2,3-d]pyrimidin-4(3H)-one (4a)

This compound was obtained as a canary yellow solid (ethanol). M.p.: 170-172 °C. IR (ν/cm^{-1}): 3170 (NH), 3047 (arom CH), 1662 (lactam CO), 1575 (N=N). ^{1}H NMR (δ ppm): 2.67 (s, 3H, SMe), 7.12-7.48 (m, 10H, 2PhH), 12.46 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ ppm): 13.3 (SMe), 117.2 (C-4a), 127.1, 127.5, 127.8, 128.2, 128.8, 129.4, 132.5, 135.2, 142.5, 152.6, 158.0, 161.6 (CO), 163.7 (C-2). Anal. Calcd. for $C_{19}H_{14}N_4OS_2$ (378.471): C, 60.30; H, 3.73; N, 14.80; S, 16.94. Found: C, 60.13; H, 3.57; N, 14.58; S, 16.74%.

2.2.3.2. 2-Phenacylthio-5-phenyl-6-(phenylazo)thieno[2,3-d]pyrimidin-4(3H)-one (4b)

This compound was obtained as a brown solid (dimethylformamide/water). M.p.: 211-212 °C. IR (ν/cm^{-1}): 3186 (NH), 3054 (arom CH), 1695, 1661 (2CO), 1570 (N=N). ^{1}H NMR (δ ppm): 5.06 (s, 2H, SCH_2), 7.22-7.79 (m, 15H, 3PhH), 12.60 (s, 1H, NH, D_2O-exchangeable). Anal. Calcd. for $C_{26}H_{18}N_4O_2S_2$ (482.577): C, 64.71; H, 3.76; N, 11.61; S, 13.29. Found: C, 64.47; H, 3.63; N, 11.41; S, 13.08%.

2.2.3.3. Synthesis of 3-methyl-2-methylthio-5-phenyl-6-(phenylazo)thieno[2,3-d]pyrimidin-4(3H)-one (4c)

To a mixture of compound **3** (1.09 g, 0.003 mol) and anhydrous potassium carbonate (0.88 g, 0.0064 mol) in dimethylformamide (15 mL), methyl iodide (0.37 mL, 0.006 mol) was added dropwise. The reaction mixture was stirred for 4 h at room temperature. Water was added to the mixture to form precipitate, which was collected by filtration, dried and purified by recrystallization from aqueous dimethylformamide to give light brown crystals of the title compound **4c** (0.61 g; 52%). M.p.: 203-204 °C. IR (ν/cm^{-1}): 3058 (arom CH), 1669 (CO), 1581 (N=N). ^{1}H NMR (δ ppm): 2.56 (s, 3H, SMe), 3.61 (s, 3H, NMe), 7.14-7.50 (m, 10H, 2PhH). ^{13}C NMR (δ ppm): 14.8 (SMe), 30.2 (NMe), 117.9, 127.1, 127.4, 127.8, 128.2, 128.9, 129.5, 132.6, 135.1, 142.3, 152.6, 156.7, 159.4 (C-2), 160.5 (CO). Anal. Calcd. for $C_{20}H_{16}N_4OS_2$ (392.497): C, 61.20; H, 4.11; N, 14.27; S, 16.34. Found: C, 60.95; H, 3.96; N, 14.10; S, 16.12%.

2.2.4. Synthesis of ethyl 2-(2-methylthio-4-oxo-5-phenyl-6-(phenylazo)thieno[2,3-d]pyrimidin-3(4H)-yl)acetate (4d)

To a solution of compound **4a** (1.14 g, 0.003 mol) in dimethylformamide (20 mL), anhydrous potassium carbonate (0.83 g, 0.006 mol) was added and the mixture was stirred at room temperature for 15 min, followed by the addition of ethyl chloroacetate (0.40 g, 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 precipitate, which was collected by filtration, washed with water, dried and purified by recrystallization from 1,4-dioxane to give white crystals of the title compound **4d** (0.68 g; 49%). M.p.: 159-160 °C. IR (ν/cm^{-1}): 3063 (arom CH), 1730 (ester CO), 1677 (pyrimidine CO), 1567 (N=N), 1211 (C-O-C). ^{1}H NMR (δ

ppm): 1.32 (t, 3H, *J* = 7.4 Hz, ester Me), 2.63 (s, 3H, SMe), 4.10 (q, 2H, *J* = 7.4 Hz, ester CH_2), 4.70 (s, 2H, NCH_2CO), 7.17-7.49 (m, 10H, 2PhH). Anal. Calcd. for $C_{23}H_{20}N_4O_3S_2$ (464.560): C, 59.46; H, 4.34; N, 12.06; S, 13.80. Found: C, 59.26; H, 4.25; N, 11.87; S, 13.62%.

2.2.5. Synthesis of 3,6-diphenyl-7-phenylazo-5H-thiazolo [3,2-a]thieno[2,3-d]pyrimidin-5-one (5)

To 1.45 g (0.003 mol) of **4b** was added concentrated sulfuric acid (5 mL, 0.09 mol) dropwise. The mixture was stirred at room temperature for 72 h. The reaction mixture was poured over iced water with stirring. The resulting precipitate of solid product was collected by filtration, washed with sodium carbonate solution, followed by water and recrystallized from ethanol/dioxane mixture to obtain the corresponding 3-phenylthiazole derivative **5** as a dark brown solid (0.75 g; 54%). M.p.: 219-220 °C. IR (ν/cm^{-1}): 3060 (arom CH), 1704 (CO), 1556 (N=N). ^{1}H NMR (δ ppm): 6.97 (s, 1H, thiazole H-2), 7.20-7.63 (m, 15H, 3PhH). ^{13}C NMR (δ ppm): 111.3 (C-2), 117.5, 127.0, 127.3, 127.7, 127.9, 128.1, 128.4, 128.7, 129.2, 129.6, 132.4, 133.5, 135.3, 142.6, 146.2, 152.5, 156.8, 158.1, 161.8 (CO). MS (*m/z* (%)): 464 (M^+, 24%). Anal. Calcd. for $C_{26}H_{16}N_4OS_2$ (464.561): C, 67.22; H, 3.47; N, 12.06; S, 13.80. Found: C, 67.05; H, 3.32; N, 11.85; S, 13.61%.

2.2.6. Synthesis of 1-amino-6-phenyl-7-(phenylazo) imidazo[1,2-a]thieno[2,3-d]pyrimidine-2,5(1H,3H)-dione (7)

Compound **4d** (0.93 g, 0.002 mol) was mixed with hydrazine hydrate (8 mL, 0.16 mol) in absolute ethanol (20 mL) and the mixture was gently refluxed. The solid went into solution within 10 min. After 30 min, when the solid product started separating, heating was discontinued and the reaction mixture was allowed to cool to room temperature. The solid which separated was filtered off, washed with water and ethanol, and dried. Recrystallization from dimethylformamide gave yellowish white crystals of the N-amino compound **7** (0.60 g; 75%). M.p.: 233-236 °C. IR (ν/cm^{-1}): 3325, 3230 (NH_2), 3052 (arom CH), 1721, 1680 (2CO), 1572 (N=N). ^{1}H NMR (δ ppm): 4.80 (s, 2H, NH_2, D_2O-exchangeable), 4.92 (s, 2H, imidazole CH_2), 7.16-7.48 (m, 10H, 2PhH). Anal. Calcd. for $C_{20}H_{14}N_6O_2S$ (402.429): C, 59.69; H, 3.51; N, 20.88; S, 7.97. Found: C, 59.46; H, 3.32; N, 20.67; S, 7.81%.

2.2.7. Synthesis of 6-phenyl-7-(phenylazo)imidazo[1,2-a] thieno[2,3-d]pyrimidine-2,5(1H,3H)-dione (8a)

Compound **7** (1.21 g, 0.003 mol) was dissolved in acetic acid (20 mL), a small amount of insoluble material was filtered off, then the liquid was cooled in ice bath at 0-5 °C. The solution was stirred at this temperature and treated gradually with a cold saturated solution of sodium nitrite [1 g of sodium nitrite (0.015 mol) in water (10 mL)] over a period of 15 min. Stirring was continued for further 30 min, then the reaction mixture was left to stand at room temperature for 3 h. The resulting solid was filtered off, washed with water and dried. Recrystallization from ethanol gave the title compound **8a** as a colourless solid (0.80 g; 69%). M.p.: 254-255 °C. IR (ν/cm^{-1}): 3315 (NH), 3056 (arom CH), 1710, 1680 (2CO), 1561 (N=N). ^{1}H NMR (δ ppm): 4.89 (s, 2H, imidazole CH_2), 7.19-7.48 (m, 10H, 2PhH), 11.83 (s, 1H, NH, D_2O-exchangeable). MS (*m/z* (%)): 387 (M^+, 14%). Anal. Calcd. for $C_{20}H_{13}N_5O_2S$ (387.415): C, 62.00; H, 3.38; N, 18.08; S, 8.28. Found: C, 61.76; H, 3.21; N, 17.90; S, 8.14%.

2.2.8. Synthesis of 6-phenyl-7-phenylazo-1-(piperidin-1-ylmethyl)imidazo[1,2-a]thieno[2,3-d]pyrimidine-2,5 (1H,3H)-dione (8b)

To a stirred solution of compound **8a** (1.94 g, 0.005 mol) in methanol (10 mL), formaldehyde (37% aqueous solution, 1 mL) was added. A second methanolic solution (2.5 mL) of piperidine (0.50 mL, 0.005 mol) was added portionwise over the first solution. The mixture was stirred for 3 h at room temperature and left overnight in a refrigerator. The precipitate formed was filtered off, washed with petroleum ether, dried and recrystallized from ethanol/water to give the Mannich base **8b** as a dark brown solid (0.73 g; 30%). M.p.: 269-271 °C. IR (ν/cm^{-1}): 3062 (arom CH), 1724, 1682 (2CO), 1565 (N=N). ^{1}H NMR (δ ppm): 1.61 (m, 6H, $3CH_2$), 3.15 (t, 4H, $N[CH_2]_2$), 4.87 (s, 2H, imidazole CH_2), 4.98 (s, 2H, CH_2), 7.22-7.50 (m, 10H, 2PhH). MS(*m/z* (%)): 484 (M^+, 29%). Anal. Calcd. for $C_{26}H_{24}N_6O_2S$ (484.573): C, 64.44; H, 4.99; N, 17.34; S, 6.62. Found: C, 64.23; H, 4.81; N, 17.14; S, 6.51%.

2.2.9. Synthesis of 2-hydrazinyl-5-phenyl-6-(phenylazo) thieno[2,3-d]pyrimidin-4(3H)-one (9)

A mixture of compound **4a** (1.14 g, 0.003 mol) and hydrazine hydrate (1.5 mL, 0.03 mol) was refluxed in 15 mL of absolute ethanol for 4 h. The solid product, which precipitated during refluxing was collected by filtration and dried. Recrystallization from 1,4-dioxane gave the hydrazino derivative **9** as pale yellow crystals (0.71 g; 65%). M.p.: 240-241 °C. IR (ν/cm^{-1}): 3380-3237 (2NH, NH_2), 3065 (arom CH), 1660 (CO), 1577 (N=N). ^{1}H NMR (δ ppm): 3.75 (s, 2H, NH_2, D_2O-exchangeable), 7.14-7.44 (m, 10H, 2PhH), 9.23 (s, 1H, NH, D_2O-exchangeable), 12.51 (s, 1H, CONH, D_2O-exchangeable). ^{13}C NMR (δ ppm): 115.8, 127.2, 127.6, 127.9, 128.4, 128.8, 129.5, 132.5, 135.1, 142.5, 152.4, 156.5, 157.0, 160.9 (CO). Anal. Calcd. for $C_{18}H_{14}N_6OS$ (362.408): C, 59.65; H, 3.89; N, 23.19; S, 8.85. Found: C, 59.43; H, 3.75; N, 23.02; S, 8.65%.

2.2.10. Synthesis of 3-acetyl-2-methylthio-5-phenyl-6-(phenylazo)thieno[2,3-d]pyrimidin-4(3H)-one (13)

A solution of compound **4a** (1.89 g, 0.005 mol) in 7.5 ml (0.079 mol) of acetic anhydride was heated under reflux for 1 h. The solution was then poured onto 75 mL ice/water and stirred for several hours until crystallization was complete. The material which separated out was isolated by filtration, washed with water and dried. It was recrystallized from ethanol to give the corresponding *N*-acetyl derivative **13** as reddish brown crystals (0.84 g; 40%). M.p.: 227-229 °C. IR (ν/cm^{-1}): 3066 (arom CH), 1690, 1681 (2CO), 1550 (N=N). ^{1}H NMR (δ ppm): 2.47, 2.65 (2s, 6H, 2Me), 7.20-7.51 (m, 10H, 2PhH). ^{13}C NMR (δ ppm): 15.1 (SMe), 24.9 (acetyl Me), 117.3, 127.0, 127.3, 127.8, 128.1, 128.7, 129.1, 132.7, 135.4, 142.5, 152.6, 157.3, 158.4, 159.8 (pyrimidine CO), 167.2 (acetyl CO). Anal. Calcd. for $C_{21}H_{16}N_4O_2S_2$ (420.507): C, 59.98; H, 3.84; N, 13.32; S, 15.25. Found: C, 59.73; H, 3.68; N, 13.11; S, 15.06%.

2.2.11. Synthesis of 3-methyl-6-phenyl-7-(phenylazo) thieno[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one (12)

2.2.11.1. Method A

This compound was synthesized from hydrazino compound **9** in a manner similar to that described before for the synthesis of compound **13** (reaction time: 4 h). It was recrystallized from acetic acid to give the respective 3-methyltriazole derivative **12** as a brown solid (0.68 g; 35%). M.p.: > 300 °C. IR (ν/cm^{-1}): 3140 (NH), 3059 (arom CH), 1698 (CO), 1566 (N=N). ^{1}H NMR

(δ ppm): 2.36 (s, 3H, Me), 7.18-7.51 (m, 10H, 2PhH), 10.57 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (δ ppm): 19.1 (Me), 117.5, 127.3, 127.6, 128.0, 128.4, 128.9, 129.2, 132.6, 135.4, 142.3, 148.5, 151.8, 152.4, 157.0, 162.7 (CO). MS (*m/z* (%)): 386 (M^+, 17%). Anal. Calcd. for $C_{20}H_{14}N_6OS$ (386.430): C, 62.16; H, 3.65; N, 21.75; S, 8.30. Found: C, 61.98; H, 3.50; N, 21.52; S, 8.11%.

2.2.11.2. Method B

A mixture of compound **13** (0.84 g, 0.002 mol), hydrazine hydrate (1 mL, 0.02 mol) in absolute ethanol (8 mL) was refluxed for 8 h. After cooling overnight and dilution with water, the separated solid product was collected by filtration, dried and purified by recrystallization from acetic acid to give a solid product, in 55% yield, identical in every respect (Melting point, mixed melting point and IR data) to that obtained above from method A.

2.2.11.3. Method C

A solution of hydrazino compound **9** (0.72 g, 0.002 mol) and acetylacetone (0.002 mol) in ethanol (20 mL) was refluxed for 4 h. Most of the solvent was removed under reduced pressure and the residue was purified by recrystallization from acetic acid to give a solid product in 49% yield. Again, this product was identified as **12**.

2.2.12. Synthesis of ethyl 2-(2-oxo-2-phenylethylamino)-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (16)

A mixture of phenacyl bromide (1 g, 0.005 mol) in 15 mL of dimethylformamide and triethylamine (two drops) was stirred until homogeneity. Amino ester **1** (1.76 g, 0.005 mol) was added to the solution and then the reaction content was refluxed under stirring for 4 h. Upon cooling, a solid was obtained which was collected by filtration, washed with water, dried and purified by recrystallization from ethanol to obtain the title compound **16** as white crystals (0.87 g; 37%). M.p.: 142-143 °C. IR (ν/cm^{-1}): 3163 (NH), 3045 (arom CH), 1679, 1671 (2CO), 1574 (N=N). ^1H NMR (δ ppm): 1.31 (t, 3H, *J* = 7.2 Hz, ester Me), 4.45 (q, 2H, *J* = 7.2 Hz, ester CH_2), 4.91 (d, 2H, CH_2), 7.28-7.84 (m, 15H, 3PhH), 8.25 (s, br, 1H, NH, D_2O-exchangeable). MS (*m/z* (%)): 469 (M^+, 19%). Anal. Calcd. for $C_{27}H_{23}N_3O_3S$ (469.555): C, 69.06; H, 4.94; N, 8.95; S, 6.83. Found: C, 68.82; H, 4.77; N, 8.76; S, 6.67%.

2.2.13. Synthesis of ethyl 2-(2-amino-3-cyano-4-phenyl-1H-pyrrol-1-yl)-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (18)

Compound **16** (5.87 g, 0.0125 mol) was mixed with an equivalent amount of malononitrile (0.83 g, 0.0125 mol) in ethanol (10 mL) containing a catalytic amount of piperidine (0.25 mL). The reaction content was heated at reflux for 1 h, concentrated and then held at room temperature for 30 min. After cooling and dilution with water, the obtained precipitate was filtered off, dried and purified by recrystallization from an ethanol and dimethylformamide mixture to give orange crystals of the enaminonitrile derivative **18** (1.94 g; 30%). M.p.: 183-184 °C. IR (ν/cm^{-1}): 3412, 3325 (NH_2), 3070 (arom CH), 2206 (CN), 1730 (CO), 1590 (N=N). ^1H NMR (δ ppm): 1.27 (t, 3H, *J* = 7.2 Hz, ester Me), 4.39 (q, 2H, *J* = 7.2 Hz, ester CH_2), 6.31 (s, br, 2H, NH_2, D_2O-exchangeable), 6.98 (s, 1H, pyrrole H-5), 7.11-7.52 (m, 15H, 3PhH). Anal. Calcd. for $C_{30}H_{23}N_5O_2S$ (517.601): C, 69.61; H, 4.48; N, 13.53; S, 6.19. Found: C, 69.42; H, 4.33; N, 13.28; S, 6.07%.

2.2.14. Synthesis of 4-oxo-3,7-diphenyl-2-phenylazo-4,5-dihydropyrrolo[1,2-a]thieno[3,2-e]pyrimidine-6-carbonitrile (19)

2.2.14.1. Method A

Compound **18** (1.04 g, 0.002 mol) was dissolved in a solution of sodium ethoxide (from 0.05 g, 0.0022 mol of sodium metal and 10 mL of absolute ethanol) and the solution was heated at reflux for 2 h. The sodium salt was collected by filtration, then dissolved in water and neutralized with hydrochloric acid. The product which separated was filtered off, washed with ethanol, dried and purified by recrystallization from dimethylformamide to give brown crystals of the pyrrole derivative **19** (0.62 g; 66%). Mp.: 276-278 °C. IR (ν/cm^{-1}): 3195 (NH), 3068 (arom CH), 2210 (CN), 1667 (CO), 1585 (N=N). ^1H NMR (δ ppm): 6.87 (s, 1H, pyrrole H-8), 7.13-7.49 (m, 15H, 3PhH), 12.35 (s, 1H, NH, D_2O-exchangeable). MS (*m/z* (%)): 471 (M^+, 23%). Anal. Calcd. for $C_{28}H_{17}N_5OS$ (471.532): C, 71.32; H, 3.63; N, 14.85; S, 6.80. Found: C, 71.09; H, 3.50; N, 14.63; S, 6.69%.

2.2.14.2. Method B

To a solution of sodium ethoxide (from 0.0022 mol of sodium metal and 10 mL of absolute ethanol) each of compound **16** (0.94 g, 0.002 mol) and malononitrile (0.13 g, 0.002 mol) were added. The reaction mixture was processed as described above in method A (reaction time: 5 h). The material obtained after recrystallization from dimethylformamide proved to be **19** (59% yield).

2.3. Microbiology

2.3.1. Antimicrobial activity

The antimicrobial activity was investigated on six newly-obtained condensed thiophenes containing different *N*-heterocyclic moieties. The antimicrobial profile was evaluated by measuring the inhibitory effects and the potency of such compounds against Gram-positive, Gram-negative bacteria and fungi using the agar diffusion technique [15,16]. Screening results are summarized in Tables 1 and 2.

2.3.2. Organisms and maintenance

Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and *Aspergillus fumigatus* were used against the tested compounds and were obtained from Faculty of Agriculture, Ain Shams University. Chloramphenicol and fluconazole were used as reference drugs and were also obtained from the pharmaceutical factories in Egypt.

The bacterial strains were cultured on nutrient agar, *C. albicans* was maintained on Yeast-malt extracts medium (YM), while *A. fumigatus* was maintained on Czapeck-Dox medium.

2.3.3. Method

2.3.3.1. Preparation of bacterial suspensions

Suspension of the above mentioned bacterial strains was prepared by inoculating fresh stock cultures into separate broth tubes, each containing 7 mL of nutrient broth (*pepton*, 0.3%) beef extract (0.3%). The inoculated tubes were incubated at 37±2°C for 24 h.

2.3.3.2. Preparation of solutions of the tested compounds and reference drugs

Solutions of the tested compounds and reference drugs were prepared by dissolving 0.5 g of the compound in dimethyl sulfoxide (10 mL).

Table 1. Antibacterial activity of test compounds and reference drug[a].

Sample	IZD[b] (mm)			
	G +ve		G -ve	
	S. aureus	Potency	*P. aeruginosa*	Potency
Control	0	0.0	0	0.0
Standard[c]	22±0.5	1.0	21±0.2	1.0
5	23±0.4	1.05	19±0.6	0.90
7	12±0.1	0.55	11±0.2	0.53
8a	11±0.3	0.50	9±0.4	0.43
8b	20±0.7	0.91	22±0.8	1.05
12	15±0.2	0.68	16±0.7	0.76
19	14±0.1	0.64	15±0.5	0.71

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

Table 2. Antifungal activity of test compounds and reference drug[a]

Sample	IZD[b] (mm)			
	Mould		Yeast	
	A. fumigatus	Potency	*C. albicans*	Potency
Control	0	0.0	0	0.0
Standard[c]	13±0.2	1.0	16±0.3	1.0
5	13±0.4	1.0	17±0.1	1.06
7	6±0.7	0.46	11±0.1	0.69
8a	4±0.01	0.30	12±0.5	0.75
8b	10±0.6	0.77	17±0.2	1.06
12	9±0.1	0.69	18±0.4	1.13
19	6±0.04	0.46	11±0.08	0.69

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

2.3.3.3. Determination of minimum inhibitory concentration (MIC)

Determination of the MIC of compound **12** against *C. albicans* and compound **8b** against *P. aeruginosa* was achieved using a 96-well microbioassay system [17]. MIC value was given in μg/ml and was compared to MIC value for relevant standard drug in each case (Table 3).

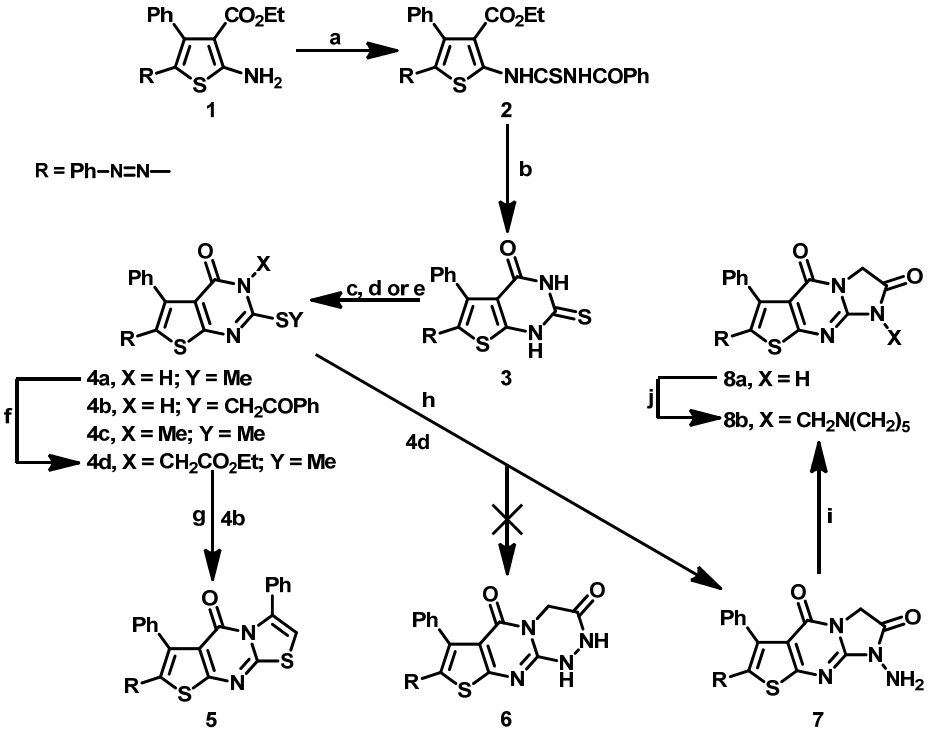
Table 3. MIC (μg/mL) of compound **8b** and chloramphenicol against *P. aeruginosa*, and compound **12** and fluconazole against *C. albicans*.

Sample	MIC (μg/mL)
Compound **8b**	15.5±0.06
Chloramphenicol	18.0±0.03
Compound **12**	13.5±0.03
Fluconazole	17.5±0.04

3. Results and discussion

3.1. Chemistry

The starting ethyl 2-amino-4-phenyl-5-(phenylazo)thiophene-3-carboxylate (**1**) [14] reacted with benzoyl isothiocyanate, *in situ* prepared [18], in acetone to give the corresponding *N'*-benzoylthiourea derivative **2** (Scheme 1). Treatment of the latter product with an aqueous alcoholic (1:1) solution of potassium hydroxide caused an intramolecular cyclization to afford the 2-thioxothienopyrimidin-4-one derivative **3**. The regioselective alkylation of **3** was achieved by slow addition of equivalent amounts of each of methyl iodide and phenacyl bromide in alkaline medium leading to the S-alkylated products **4a**,**b**, respectively, which are unsubstituted at the N-3 position as indicated by spectroscopic studies. In the ^{1}H NMR spectra, the NH protons were deshielded and appeared in the downfield region (δ_H 12.46-12.60 ppm) due to the deshielding effects of the neighbouring carbonyl groups. These data are in accordance with literature assignments, that predict these NH protons would resonate in the downfield region (δ_H ~ 12 ppm) by analogy with related lactam species [19-22]. Similarly, the IR spectra were also informative in establishing the structure of 3-unsubstituted pyrimidin-4-ones **4a**,**b**. The spectra were well characterized by the presence of absorption bands belonging to stretching vibrations of amidic carbonyl groups at the typical low frequencies (ν 1661-1662 cm^{-1}) and of cyclic secondary amides (ν 3170-3186 cm^{-1}). Similar stretching frequencies have been reported on closely related heterocyclic amide systems [23-25]. Moreover, the ^{13}C NMR spectrum of **4a** displayed distinct shifts of nineteen carbon atoms. Among these, three important shifts at δ_C 13.3, 161.6 and 163.7 ppm were characteristic for the SMe, lactam CO and C-2 carbons, respectively, thus lending additional support to the structural assignments. The prevalence of the lactam (oxo) form in compounds **4a**,**b** was further verified on the basis of a direct comparison of the ^{13}C NMR spectra of the monomethyl derivative **4a** and the corresponding dimethylated analogue **4c**, which was obtained from the methylation of 2-thiouracil derivative **3** with two equivalent amounts of methyl iodide. It can be seen that the ^{13}C chemical shift of the C-4 resonance in compound **4c** (δ_C 160.5 ppm) is close, with a slight upfield shift, to the corresponding signal in parent compound **4a**, thus indicating the presence of a lactam CO in **4c**. Also, the appearance of a methyl carbon signal at the typical upfield shift (δ_C 30.2 ppm) characteristic for N-3-methyl resonance position [26] provided strong evidence in support of lactam formation. O-Methylation should lead to downfield shifts at both C-4 and Me resonance positions [26,27]. Furthermore, a relatively deshielded SMe signal (δ_C 14.8 ppm) and a large shielded C-2 signal (δ_C 159.4 ppm) were also observed in the spectrum of N-3-methylated pyrimidinone **4c** as compared with that of the corresponding N-3-protodemethylated analogue **4a**. Similar assignments have been reported on closely related N-3-methyllactams [26]. These observations led us to conclude that the most stable form in the solvent for the 2-(alkylthio) thienopyrimidin-4-ones **4** is the lactam (oxo) form rather than the lactim (enol) form. In a similar fashion, the structure assigned to lactams **4** is supported by the ^{1}H NMR spectrum of **4c**. The spectrum showed the disappearance of the NH proton and the appearance of a diagnostic singlet signal at δ_H 3.61 ppm arising from the N-methyl protons.



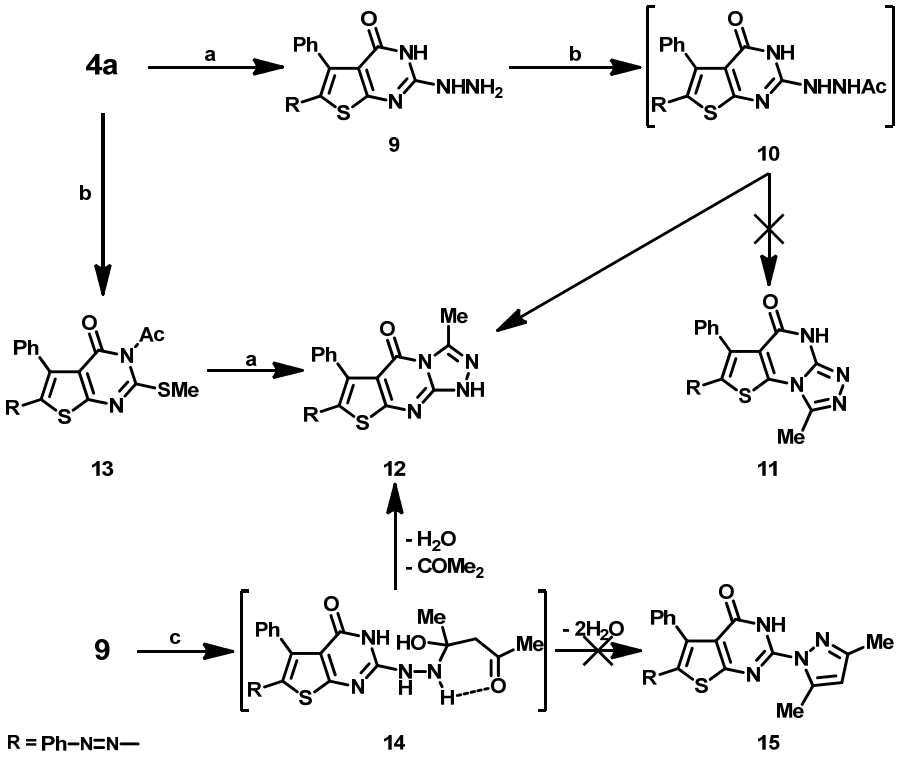
Reagents and conditions: (a) PhCONCS, Me_2CO, reflux; (b) KOH, EtOH, reflux; (c) MeI, K_2CO_3, DMF, 0-5 °C; (d) $PhCOCH_2Br$, K_2CO_3, DMF, 0-5 °C; (e) 2MeI, K_2CO_3, DMF, r.t.; (f) $ClCH_2CO_2Et$, EtOH, reflux; (g) H_2SO_4, r.t.; (h) $N_2H_4.H_2O$, EtOH, reflux; (i) $NaNO_2$, AcOH, 0-5 °C; (j) HCHO, $HN(CH_2)_5$, MeOH, r.t.
r.t.= room temperature

Scheme 1

This shift is characteristic for a N-3-methyl group, which has been reported by Ram *et al.* [28], and by others [29-31], to experience a further downfield shift to around $\delta_H \sim 3.5$ ppm due to the additional deshielding effect of the adjacent carbonyl moiety. The remaining resonances were also observed at the expected frequencies (see Experimental section). For the isomeric N-1-methylated analogue, the corresponding shift of N-1-Me moves upfield to around $\delta_H \sim 3.2$ ppm [28,29], whereas O-methylation produces a downfield shift of the methyl resonance, which is consistently found at δ_H *ca.* 4.0 ppm [29,32]. The prevalence of the 3*H*- rather than the 1*H*-form in a number of 2-(alkylthio)uracils is well documented [20-22,28,33-35]. Consequently, it is not unreasonable to conclude that our studied alkylation reactions take place with complete selectivity, since the site of alkylation is initially the sulfur rather than the nitrogen and in the case of the disubstituted products the N-3 is the second position for alkylation.

As discussed above, N-3-alkylated lactam **4d** could also be obtained in a selective manner by reacting compound **4a** with ethyl chloroacetate in dimethylformamide in the presence of potassium carbonate. All spectroscopic data fitted perfectly with the proposed structure **4d**. In its IR spectrum, the ester carbonyl stretching frequency was observed at ν 1730 cm^{-1}. Also, its ^{1}H NMR spectrum showed the presence of a pattern corresponding to an ethyl group as a triplet and quartet at 1.32 and 4.10 ppm, respectively. Other resonances were observed as expected and are recorded in the experimental section. Cyclodehydration of **4b** in sulfuric acid at room temperature occurred readily with the formation of the corresponding condensed thiazole derivative **5**, whose IR spectrum lacks bands belonging to both NH and CO functions, supporting the formation of the thiazole ring. In the ^{1}H NMR spectrum of compound **5**, the absence of signals corresponding to the NH and CH_2 protons, present in the parent S-alkylated derivative **4b**, also verified ring closure through cyclodehydration. The structure assigned to the reaction product was further supported by its ^{13}C NMR spectrum (see Experimental section).

On reaction with hydrazine hydrate, the N-3-alkylated pyrimidinone **4d** gave a product which might be assigned the structure of a 1,2,4-triazinone **6** or aminoimidazolidinone **7**. The signals for the protons of the ethyl ester and methylthio groups, present in the ^{1}H NMR spectrum of **4d**, were not detectable for the isolated product, demonstrating the disappearance of these groups on ring closure. Elemental analysis data could not discriminate between the two isomeric structures **6** and **7** as both structures had the composition $C_{20}H_{14}N_6O_2S$. However, the structure of the isolated product was considered to be the *N*-aminolactam **7** rather than the alternative triazinone **6** as evidenced by spectroscopic investigations as well as a chemical transformation. In the ^{1}H NMR spectrum of the reaction product, the presence of only one D_2O-exchangeable singlet signal provided confirmatory evidence for *N*-aminolactam formation. In support of this hypothesis, the possibility of formation of the triazinone **6** was ruled out chemically on the basis of the successful deamination of isolated product, where its treatment with sodium nitrite and acetic acid led to the respective 1-protodeamino analogue **8a**. This observation is in complete agreement with previous literature reports on the well established nitrous acid deamination of the exocyclic amino group in related cyclic *N*-aminoamides [36-39]. Compound **8a** displayed a molecular ion peak, which confirmed its molecular formula. The constitution of compound **8a** was also well supported by its conversion to the corresponding Mannich-type base **8b** on treatment with formaldehyde and piperidine as a secondary amine (Scheme 1). This condensation reaction demonstrated the presence of an acidic imide hydrogen in **8a**, which was replaced by a substituted aminomethyl group, in accordance with the findings of related reports [39-41].



Reagents and conditions: (a) $N_2H_4.H_2O$, EtOH, reflux; (b) Ac_2O, reflux; (c) Ac_2CH_2, EtOH, reflux.

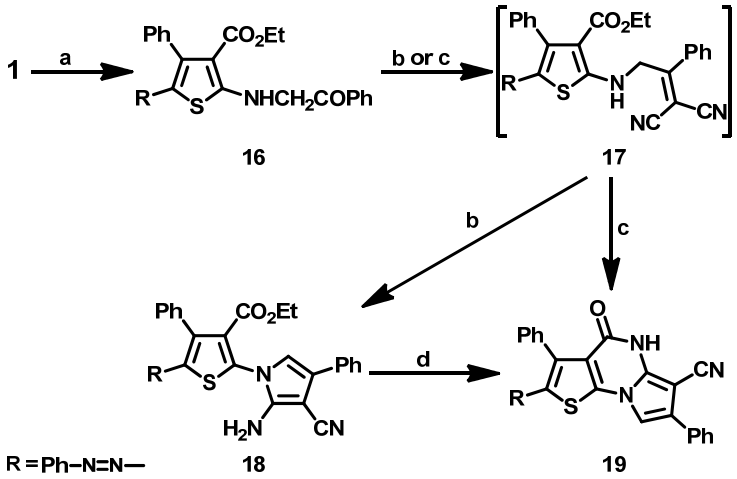
Scheme 2

The mass spectrum of **8b** showed a molecular ion peak at *m/z* 484, which is indicative of an increase of 97 from the parent **8a**, confirming the assigned structure.

Hydrazinolysis of the *S*-methylated thienopyrimidinone **4a** in ethanol provided the desired 2-hydrazino compound **9** (Scheme 2). By reaction of the latter product with acetic anhydride we obtained a product of dehydrative acetylation for which two isomeric structures **11** and **12** might be formulated. However, the structure of the isolated product was considered to be of the 5-one type **12** rather than the corresponding 4-one **11** based on the established selective functionalization of N-3 for 2-thiouracil systems over derivatization at N-1 [20,28,33,34,42-47]. This selectivity can be explained by the enhanced nucleophilicity of N-3 as compared with N-1 position and therefore the N-3, being richer in electron density, is more reactive towards electrophiles and produces products of exclusive functionalization at N-3. This fact led to the conclusion that a dehydrative cyclization, involving the participation of N-3 and not N-1, occurred in our studied reaction with the regioselective formation of the N-3 ring closed product **12** rather than the alternative 1-cyclized analogue **11**. The IR and ^{13}C NMR spectra provided further evidence for the proposed structure by comparison of these spectra with those of similar annelated pyrimidinones. The IR spectrum of the isolated product showed an important absorption of the carbonyl group of the pyrimidinone ring at ν 1698 cm^{-1}. This high CO stretching frequency is in favour of the pyrimidin-5-one formulation **12** [33,43]. It has been reported [33,48] that the most diagnostic signal, in the ^{13}C NMR spectrum of pyrimidin-4-one systems, is due to the carbonyl, as this signal is markedly affected by the nature of the adjacent nitrogen (N-3) (pyrrole type in our structure **12** and pyridine type as in structure **11**). Thus, the ^{13}C NMR spectrum could give unequivocal proof for the structure of the isolated product by the observation of a carbonyl carbon signal appearing at the chemical shift of 162.7 ppm. Such an upfield shift is consistent with pyrimidin-5-one structure **12** rather than with pyrimidin-4-one **11**, for which an IR carbonyl absorption would be expected to appear at around $\nu \sim 1660$ cm^{-1} and the ^{13}C NMR chemical shift of the carbonyl carbon would be expected to resonate in the lower field region ($\delta_C \sim 170$ ppm), in line with prior observations of spectra of closely related compounds [33,49]. Accordingly, it can be concluded that the studied reaction is completely regioselective and the linear pyrimidin-5-one structure **12** of the obtained product is preferred over the respective angular 4-one structure **11**. It is believed that the formation of compound **12** proceeds through the intermediacy of the acetylhydrazino **10** followed by intramolecular cyclodehydration under the applied reaction conditions (Scheme 2).

Nevertheless, a proof for the proposed structure **12** was accomplished chemically by using an independent route for the synthesis of compound **12** involving initial acetylation of **4a** with acetic anhydride and subsequent treatment of the formed *N*-acetyl derivative **13** with hydrazine hydrate, yielding a product (55%) that proved to be identical to the previous product **12** as confirmed by TLC analysis, melting point, IR data and undepressed mixed melting point with the previously isolated material.

It is remarkable to report here that an unexpected reaction took place on reacting **9** with acetylacetone in an attempt to obtain the dimethylpyrazolyl derivative **15**. To our surprise, this reaction did not give the expected product **15** and instead the methyltriazole derivative **12** was again obtained, the structure of which was unambiguously confirmed by comparison of TLC analysis, m.p., mixed m.p. and IR data with those of the previously isolated sample **12**.



Reagents and conditions: (a) $PhCOCH_2Br$, DMF, Et_3N, reflux; (b) $CH_2(CN)_2$, EtOH, pip, reflux; (c) $CH_2(CN)_2$, NaOEt, EtOH, reflux; (d) NaOEt, EtOH, reflux.

Scheme 3

This result can be explained by assuming the reaction occurs initially with formation of a *α*-hydroxyhydrazine **14**. Subsequent loss of water and acetone, respectively, leads eventually to the final methyltriazole derivative **12**. This hypothesis is consistent with similar observations [50-52].

This approach was also suitable for the synthesis of a condensed pyrrole system, where reaction of 2-aminothiophene **1** with phenacyl bromide afforded the respective phenacylamine derivative **16** *via* the nucleophilic displacement of the bromine by the heterocyclic amine. Condensation of **16** with malononitrile in ethanol in the presence of piperidine produced the enaminonitrile derivative **18** through the intermediate formation of **17** followed by subsequent nucleophilic cyclization under the applied reaction conditions. Heteroannelation of **18** in boiling ethanolic sodium ethoxide solution occurred with the formation of the tricyclic pyrrole derivative **19**, *via* loss of ethanol (Scheme 3). It is worth mentioning that compound **19** could be also obtained directly in a reaction of phenacylamine derivative **16** with malononitrile in boiling ethanolic sodium ethoxide solution. Compound **19** prepared by the latter route was found to be identical to that obtained by the former method. Elucidation of structure for the latter product was established on the basis of elemental and spectroscopic analyses (see Experimental section).

3.2. Antimicrobial Evaluation

The biological evaluation of antibacterial and antifungal effects are summarized in Tables 1 and 2. As shown by these results, the new bridgehead nitrogen heterocycles under investigation displayed variable *in vitro* antibacterial and antifungal actions. In general, the chemical structure, comprising the nature of the heterocyclic system as well as the substituted function present in the heterocyclic ring, has a pronounced effect on antimicrobial activity. In particular, it was found that the attachment of a *N*-(piperidinylmethyl)imidazole pharmacophore to the thienopyrimidinone core favoured antibacterial activity especially against the Gram-negative strain, as observed for compound **8b**, whereas the annelation of a 3-phenylthiazole moiety to the same core produced an inhibitory effect against Gram-positive bacteria superior to the reference drug chloramphenicol as observed for compound **5** (Table 1). As for antifungal action, compound **12**, which incorporated a 3-methyltriazole fragment, exhibited the highest effect on yeast (*C. albicans*) with potency 1.13, whilst the most prominent and consistent antifungal activity was obtained with 3-phenylthiazole derivative **5**, which produced inhibitory effects similar or superior to the reference drug fluconazole with potency 1.0 (Table 2). On the contrary, there was no essential *in vitro* antimicrobial profile of compound **8a** with an N-1-unsubstituted triazole moiety.

Based on the biological evaluation, compounds **8b** and **12** were selected for further assessment. The minimum inhibitory concentration (MIC) of *N*-(piperidinylmethyl)imidazole derivative **8b** against *P. aeruginosa* was 15.5 μg/mL, which is less than that of chloramphenicol as a reference antibacterial drug as depicted in Table 3. Also, the MIC of 3-methyltriazole derivative **12** against *C. albicans* was 13.5 μg/mL, which is less than that of fluconazole as a reference antifungal drug (Table 3). From the structure-activity relationship (SAR), we can conclude that the *N*-piperidinylmethyl pharmacophore is important for antibacterial activity, especially against *P. aeruginosa* as observed for compound **8b**. In addition, heteroannelation of a 3-methyltriazole moiety to the thienopyrimidinone scaffold can improve anti-*Candida* activity as observed for compound **12**, which can be considered as a lead compound in this field. Further studies are in progress on both compounds to increase their efficacy and understand their QSAR.

4. Conclusion

In conclusion, we have demonstrated that the heterocyclization of 2-(alkylthio)pyrimidine-type building blocks **4** and its parent *ortho*-aminoester **1** provided easy and versatile access to a variety of bridgehead nitrogen heterocycles, which were of significant interest for biological investigations. The biological potential of all new *N*-heterocyclic derivatives was further investigated by screening their antimicrobial activity against two pathogenic bacteria and two pathogenic fungi. Biological study of the compounds under investigation indicated that the highest anti-*Candida* activity was observed for compound **12** with a methyl group attached to the triazole ring at the 3-position. Its MIC value (13.5 μg/mL) towards *C. albicans* very significant. Compound **8b** showed an appreciable broad spectrum of action against both Gram-positive and Gram-negative bacteria. Its MIC value (15.5 μg/mL) towards *P. aeruginosa* is very interesting. In light of the results presented in this work and taking into account that this preliminary study does not produce conclusive evidence regarding structure-antimicrobial relationships, we have

focused our attention on the most promising compounds **8b** and **12** as an interesting starting point for the development of a new class of antimicrobial agents. Further structural modifications might lead to the discovery of more potent antimicrobial agents and this work is in progress. 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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