Med Chem Res
DOI 10.1007/s00044-014-1011-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Pyridazine and its related compounds. Part 36. Synthesis and antimicrobial activity of some novel pyrimido[4′,5′:4,5]thieno[2,3-*c*]pyridazine derivatives

**Ali Deeb · Sebaey Mahgoub**

Received: 30 September 2013 / Accepted: 14 April 2014


**Abstract** Novel derivatives of pyrimidothienopyridazine were designed and synthesized through a versatile method. Some of the target compounds bearing the sulfonamide group were evaluated for their antimicrobial activity against representative Gram-positive bacteria, Gram-negative bacteria, and fungi by applying the agar plate diffusion technique. The results showed that derivatives **11a** have promising inhibitory activity against Gram-positive bacteria, and derivatives **11b** and **11e** have also potent inhibition against fungi. Rest of the compounds showed moderate to low activity against the examined microorganisms.

**Keywords** Thienopyridazine · Pyrimidothienopyridazine · Sulfonamide · Antimicrobial activity

## Introduction

Pyridazine and its fused heterocyclic derivatives have recently received much attention from their reactions, synthetic and effective biological importance. It have been reported to possess antimicrobial (Kandile *et al*., 2009; Asif *et al*., 2011), antituberculosis (Mangalagiu, 2011 and Mantu *et al*.,2010), antifungal (Drochioiu *et al*., 2007), anticancer (Butnariu *et al*., 2007), herbicidal (Han *et al*.,2010) activities, and plant growth regulators and crop protecting agents (Tucaliuc *et al.,* 2008). On the other hand, many fused compounds with thienopyrimidine fragment have received considerable interest because of their remarkable pharmacological properties (Chambhare *et al*., 2003).

Literature survey reveals that sulfonamides are important class of pharmaceutical compounds exhibiting a wide spectrum of biological activity (Hansch *et al*., 1990, Connor, 1998 and Hanson *et al*., 1999), and over 30 drugs containing this functionality are in clinical use, including, antibacterial agents, diuretics, anticonvulsants, hypoglycemic, and HIV protease inhibitors (Kleemann *et al*., 1999 and Deeb *et al*., 2014).

In the light of these facts, our interest was focused on synthesizing new heterocyclic compounds including pyrimidothienopyridazine moieties with suitable substituent of biological and pharmacological interest. The structures of the newly synthesized compounds were elucidated on the basis of elemental analysis and various spectroscopic methods and in some cases by comparison with samples previously prepared by unambiguous route. Some of the newly prepared compounds were preliminarily evaluated for their in vitro antibacterial activities against (*Micrococcus luteus* and *Staphylococcus aureus*) as representative examples of Gram-positive bacteria and (*Escherichia coli*) as an example of Gram-negative bacteria. They were evaluated for in vitro antifungal activity against (*Candida albicans*) as representative fungi.

## Materials and methods

### Chemistry

All melting points were determined in open-glass capillaries and are uncorrected. IR spectra were recorded on a

Taken in part from M.Sc. thesis of Sebaey Mahgoub, Zagazig University, 2013.

A. Deeb (✉) · S. Mahgoub
Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt
e-mail: dralideeb@hotmail.com

Published online: 15 May 2014

BRUKER Vector 22 Germany spectrometer (KBr). $^{1}$H-NMR spectra were recorded on Varian Gemini 200 MHz spectrometer and $^{13}$C-NMR spectra on JMS-AX500 (125 MHz), using tetramethylsilane as an internal reference. The Electron Impact mass spectra were obtained at 70 eV using Shimadzu QP-2010 Plus mass spectrometer. The reactions were followed up by thin-layer chromatography (TLC) on silica gel $F_{254}$ aluminum sheets (Merck), and the spots were detected by UV lamp at 254–365 nm. The synthesis of ethyl 5-amino-3,4-diphenylthieno[2,3-c]pyridazine-6-carboxylate **1** (Deeb *et al.,* 1990) was conducted according to known procedure.

Antimicrobial activity

The antimicrobial activity study was performed using standard cultures of *M. luteus* ATCC10240*, S. aureus* ATCC6538P*, E. coli* ATCC10536, and *C. albicans* ATCC2091. *M. luteus*, *S. aureus*, and *E. coli* cultures were incubated in Nutrient Broth (Difco), while *C. albicans* was incubated in Sabouraud Dextrose Broth (Difco), and dimethylformamide was used as a solvent for tested compounds. A blank disk impregnated with dimethylformamide followed by drying off was used as a negative control. Sulfadoxine, Sulfadimidine, and Nystatin at concentration of 400 μg/0.1 mL in dimethylformamide were used as positive control.

*Preparation of ethyl 5-[(ethoxymethylene)amino]-3,4-diphenylthieno[2,3-c]pyridazine-6-carboxylate (**2**)*

A stirred mixture of ethyl 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carboxylate **1** (1.0 g, 2.6 mmol) in triethyl orthoformate (20 mL) and acetic anhydride (1.0 mL) was refluxed for 6 h. The reaction mixture was evaporated under reduced pressure, and the product **2** was used directly in next steps without further purification.

*Preparation of 3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-8(7H)-one (**3**)*

A solution of compound **2** (1.0 g, 2.9 mmol) in concentrated ammonium hydroxide solution (20 mL) was stirred at 70 °C for 8 h. The reaction mixture was filtered; the filtrate was neutralized with conc. HCl. The precipitated product was filtered, washed with water and recrystallized from ethanol to give **3**, yellow crystals, yield 48.4 %; mp 188–190 °C; IR (KBr, $cm^{-1}$): 3165 (NH), 1655 (C=O); MS (*m/z, %*): 356 [$M^+$, 73.03 %], 314 [$M^+$–NCO, 87.64 %, $F_1$], 288 [$F_1$–HCN, 61.80 %, $F_2$], 232 [$F_2$–$SCH_2CH_2$, 60.67 %, $F_3$], 205 [$F_3$–$N_2$, 60.67 %, $F_4$]; $^{1}$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 8.8 (*s*, 1H, NH), 7.9 (*s*, 1H, H-6), 7.7–7.4 (*m*, 10H, 2Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.5 (C-9′), 159.0 (C=O), 155.5 (C-3), 148.3 (C-4″), 144.7 (C-6), 143.3 (C-8′), 135.9 (C-4), 134.5–126.0 (2Ph). Anal. Calcd for $C_{20}H_{12}N_4OS$ (356.4): C, 67.40; H, 3.39; N, 15.72. Found: C, 67.25; H, 3.20; N, 15.55.

*Preparation of 8-chloro-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazine (**4**)*

A mixture of compound **3** (1.0 g, 2.8 mmol) and phosphoryl chloride (15 mL) was heated at reflux temperature for 4 h. The reaction mixture was cooled and hydrolyzed by addition of crushed ice. The formed precipitate was filtered, washed with water, dried, and recrystallized from ethanol to give **4,** yield 66.6 %, mp 145–147 °C; IR (KBr, $cm^{-1}$): 1604 (C=N), 1071 (C–Cl); MS (*m/z, %)*: 374 [$M^+$, 13.1 %], 315 [$M^+$–N = C–Cl, 11.51 %], 57[100 %];$^{1}$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 8.2 (*s*, 1H, H-6), 7.6–7.3 (*m*, 10H, 2Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 161.9 (C-4″), 159.3 (C-9′), 156.7 (C-6), 155.5 (C-3), 154.1 (C–Cl), 135.9 (C-4), 134.5–126.3 (2Ph). Anal. Calcd for $C_{20}H_{11}ClN_4S$ (374.8): C, 64.08; H, 2.96; N, 14.95. Found: C, 63.90; H, 2.80; N, 14.85.

*Preparation of 7,8-diphenyltetrazolo[1′′,5′′:1′,6′]pyrimido[4′,5′:4,5]thieno[2,3-c]pyridazine (**5**)*

A solution of **4** (1.0 g, 2.6 mmol) and sodium azide (0.34 g, 5.2 mmol) in ethanol (25 mL) was refluxed for 4 h. The solvent was evaporated in vacuo, and the residue was washed with water, filtered, dried, and recrystallized from ethanol to give **5,** yellow crystals, yield 89.1 %; mp 248–250 °C; IR (KBr, $cm^{-1}$): 1635 (C=N); MS (*m/z, %*): 381 [$M^+$, 20.69 %], 357 [$M^+$–$N_2$, 24.14 %, $F_1$],327 [$F_1$–$N_2$, 21.38 %, $F_2$]; $^{1}$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 9.2 (*s*, 1H, H-6), 7.7–7.4 (*m*, 10H, 2Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 161.9 (C-4″), 159.3 (C-9′), 155.5 (C-3), 154.1 (C–Cl), 140.3 (C-6), 135.9 (C-4), 134.5–126.3 (2Ph). Anal. Calcd for $C_{20}H_{11}N_7S$ (381.4): C, 62.98; H, 2.91; N, 25.71. Found: C, 62.83; H, 2.80; N, 25.55.

*Preparation of 3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazine-8(7H)-thione (**6**)*

A solution of **4** (1.0 g, 2.6 mmol) and thiourea (1.0 g, 1.3 mmol) in ethanol (30 mL) was refluxed for 4 h, the solvent was evaporated on vacuo, and the residue was heated in sodium hydroxide solution (50 mL, 2.5 N) for 30 min and then filtered while hot. The filtrate was cooled and acidified with hydrochloric acid (pH 2), the yellow precipitate obtained was filtered, washed with water, dried, and recrystallized from ethanol to give **6,** yield 80.8 %, mp 230–232 °C; IR (KBr, $cm^{-1}$): 3390 (NH), 1665 (C=N), 1531 (C=C), 1194 (C=S); MS (*m/z, %*): 372 [$M^+$,

47.24 %], 85 [100 %]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 12.2 (*s*, 1H, NH), 8.1 (*s*, 1H, H-6), 7.8–7.4 (*m*, 10H, 2Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 175.7 (C=S), 155.5 (C-3), 152.3 (C-9′), 142.3 (C-6), 138.3 (C-4″), 135.9 (C-4), 133.5–125.3 (2Ph). Anal. Calcd for $C_{20}H_{12}N_4S_2$ (372.4): C, 64.49; H, 3.25; N, 15.04. Found: C, 64.24; H, 3.09; N, 14.90.

*Preparation of 7-amino-3,4-diphenylpyrimido[4′,5′:4,5] thieno[2,3-c]pyridazin-8(7H)-one (**7**)*

To solution of compound **2** (1.0 g, 2.9 mmol) in glacial acetic acid (10 mL), hydrazine hydrate 85 % (2.0 mL, 19.0 mmol) was added. The reaction mixture was refluxed for 8 h, left to cool at room temperature and the precipitated product was filtered, washed with water, and recrystallized from ethanol to give **7,** yellow crystals, yield 34.9 %; mp 180–182 °C; IR (KBr, cm$^{-1}$): 3414, 3150 ($NH_2$), 3057 ($CH_{arom}$), 1676 (C=O), 1594 (C=N); MS (*m/z*, %): 371 [$M^+$, 9.95 %], 342 [$M^+$–$N_2$, 2.21 %, $F_1$], 289[$F_1$–NCO, 1.70 %, $F_2$], 233 [$F_2$–$CH_2CH_2SH$, 3.74 %, $F_3$],204 [$F_3$–$N_2$, 1.96 %, $F_4$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 8.1 (*s*, 1H, H-6), 7.7–7.5 (*m*, 10H, 2Ph), 2.3 (*s*, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.7 (C-9′), 159.5 (C = O), 155.5 (C-3), 148.3 (C-4″), 145.8 (C-6), 135.9 (C-4), 134.5–127.3 (2Ph). Anal. Calcd for $C_{20}H_{13}N_5OS$ (371.4): C, 64.68; H, 3.53; N, 18.86. Found: C, 64.50; H, 3.43; N, 18.70.

*General procedure for the synthesis of 7-(arylideneamino)-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-8 (7H)-one derivatives (**8a–c**)*

A mixture of compound **7** (1.0 g, 2.6 mmol) and the appropriate aldehyde, namely benzaldehyde, *p*-methoxybenzadehyde, and *p*-nitrobenzaldehyde (2.6 mmol), was refluxed in ethanol (20 mL) for 4 h. The solvent was evaporated under reduced pressure, and the residue was triturated with diethyl ether (20 mL). The separated solid was filtered, dried, and recrystallized from ethanol to give **8a–c.**

*7-(Benzylideneamino)-3,4-diphenylpyrimido[4′,5′:4,5] thieno[2,3-c]pyridazin-8(7H)-one (**8a**)*

Brown crystals; yield 81.3 %; mp 205–207 °C; IR (KBr, cm$^{-1}$): 3056 ($CH_{arom}$), 1687 (C=O), 1630, 1620 (C=N); MS (*m/z*, %): 459 [$M^+$, 49.09 %], 357 [$M^+$–PhCN, 56.36 %, $F_1$], 317 [$F_1$–NCO, 69.09 %, $F_2$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 9.1 (*s*, 1H, N = CHPh), 8.2 (*s*, 1H, H-6), 7.89–7.42 (*m*, 15H, 3Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.5 (C-9′), 161.5 (C=O), 155.5 (C-3), 153.3 (CH=NPh), 148.3 (C-4″), 146.9 (C-6), 136.9 (C-4), 133.7–127.5 (3Ph). Anal. Calcd for $C_{27}H_{17}N_5OS$ (459.5): C, 70.57; H, 3.73; N, 15.24. Found: C, 70.40; H, 3.63; N, 15.10.

*7-((4-Methoxybenzylidene)amino)-3,4-diphenylpyrimido [4′,5′:4,5]thieno[2,3-c]pyridazin-8(7H)-one (**8b**)*

Brown crystals; yield 76.3 %; mp 170–172 °C; IR (KBr, cm$^{-1}$): 3054 ($CH_{arom}$), 2990 ($CH_{aliph}$), 1665 (C=O), 1630 (C=N), 1079 ($OCH_3$); MS (*m/z*, %): 489 [$M^+$, 17.21 %], 458 [$M^+$–$OCH_3$, 17.80 %, $F_1$], 384 [$F_1$–Ph, 18.40 %, $F_2$], 342 [$F_2$–$CH_2N_2$, 23.44 %, $F_3$], 288 [$F_3$–NCO, 22.55 %, $F_4$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 9.1 (s, 1H, N=CHPh), 8.2 (*s*, 1H, H-6), 7.89–7.05 (*m*, 15H, 3Ph), 3.5 (*s*, 1H, CH3); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.5 (C-9′), 161.9 (C–O),160.9 (C=O) 155.5 (C-3), 153.1 (N=CHPh), 148.3 (C-4″), 145.8 (C-6), 135.9 (C-4), 133.7–114.5 (3Ph) 55 ($CH_3$). Anal. Calcd for $C_{28}H_{19}N_5O_2S$ (489.5): C, 68.70; H, 3.91; N, 14.31. Found: C, 68.55; H, 3.75; N, 14.21.

*7-((4-Nitrobenzylidene)amino)-3,4-diphenylpyrimido [4′,5′:4,5]thieno[2,3-c]pyridazin-8(7H)-one (**8c**)*

Brown crystals; yield 74.0 %; mp 125–127 °C; IR (KBr, cm$^{-1}$): 3056 ($CH_{arom}$), 1658 (C=O), 1519, 1437 ($NO_2$); MS (*m/z*, %): 504 [$M^+$, 15.47 %], 460 [$M^+$–$NO_2$, 21.07 %, $F_1$], 383 [$F_1$ - Ph, 18.67 %, $F_2$], 341 [$F_2$ - $CH_2N_2$, 16.80 %, $F_3$],288 [$F_3$–NCO, 14.40 %, $F_4$], 260 [$F_4$–$N_2$, 19.47 %, $F_5$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 9.01 (*s*, 1H, N = CHPh), 8.2 (*s*, 1H, H-6), 7.95–7.42 (*m*, 14H, 3Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.3 (C-9′), 161.5 (C=O), 156.1 (C-3), 153.8 (N=CHPh), 159.8 (C-$NO_2$), 149.1 (C-4″), 146.1 (C-6), 136.7 (C-4), 129.7–124.5 (3Ph). Anal. Calcd for $C_{27}H_{16}N_6O_3S$ (504.5): C, 64.28; H, 3.20; N, 16.66. Found: C, 64.10; H, 3.06; N, 16.50.

*General procedure for the preparation of 7-N-substituted 3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-8(7H)-one (**9a–d**)*

To a solution of compound **2** (1.0 g, 2.9 mmol) in glacial acetic acid (10 mL), substituted amines (2.9 mmol) were added, the reaction mixture was refluxed for 8 h, cooled and the formed precipitate was filtered, washed with water, and recrystallized from appropriate solvent to give **9a–d.**

*7-Benzyl-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-8(7H)-one (**9a**)*

Yellow crystals from chloroform, yield 48.5 %; mp 283–285 °C. IR (KBr, cm$^{-1}$): 3058 ($CH_{arom}$), 2916 ($CH_{aliph}$), 1676 (C=O), 1597 (C=N); MS (*m/z*): 446 [$M^+$, 2.67 %], 370 [$M^+$–$C_6H_5$, 1.32 %, $F_1$], 341 [$F_1$–$NCH_3$,

1.69 %, $F_2$], 288 [$F_2$–N = CHCHO, 1.76 %, $F_3$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 8.2 (*s*, 1H, H-6), 7.69–7.26 (*m*, 15H, 3Ph), 4.2 (s, 2H, $CH_2$Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.5 (C-9′), 160.3 (C = O), 156.5 (C-3), 147.3 (C-6), 136.9 (C-4), 135.7–126.5 (3Ph), 47.1 ($CH_2$Ph). Anal. Calcd for $C_{27}H_{18}N_4OS$ (446.5): C, 72.63; H, 4.06; N, 12.55. Found: C, 72.48; H, 3.90; N, 12.41.

*7-(2-Chlorobenzyl)-3,4-diphenylpyrimido[4′,5′:4,5]thieno [2,3-c]pyridazin-8(7H)-one (**9b**)*

Yellow crystals from chloroform; yield 54.05 %; mp 275–277 °C; IR (KBr, cm$^{-1}$): 3060 ($CH_{arom}$), 2917 ($CH_{aliph}$), 1677 (C=O), 1597 (C=N); MS (*m/z*): 480 [$M^+$, 0.89 %], 446 [$M^+$–Cl, 0.17 %, $F_1$], 355 [$F_1$–$PhCH_3$, 0.58 %, $F_2$],313 [$F_2$–NCO, 4.93 %, $F_3$], 287 [$F_3$–$N_2$, 0.01 %, $F_4$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 8.3 (*s*, 1H, H-6), 7.81–7.26 (*m*, 14H, 3Ph), 4.91 (*s*, 2H, $CH_2$Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.1 (C-9′), 160.1 (C=O), 156.4 (C-3), 148.1 (C-6), 137.2 (C-4), 135.4–127.3 (3Ph), 42.3 ($CH_2$Ph). Anal. Calcd for $C_{27}H_{17}ClN_4OS$ (480.9): C, 67.42; H, 3.56; N, 11.65. Found: C, 67.27; H, 3.40; N, 11.50.

*3,4-Diphenyl-7-(phenylamino)pyrimido[4′,5′:4,5]thieno [2,3-c]pyridazin-8(7H)-one (**9c**)*

Yellow crystals from chloroform; yield 57.8 %; mp 290–292 °C; IR (KBr, cm$^{-1}$): 3390 (NH), 3059 ($CH_{arom}$), 1677 (C=O), 1596 (C=N); MS (*m/z*, %): 447 [$M^+$, 0.58 %], 370 [$M^+$–Ph, 3.65 %, $F_1$], 342 [$F_1$–$N_2$, 0.45 %, $F_2$], 287 [$F_2$–NCO, 0.38 %, $F_3$], 260[$F_3$–$N_2$, 1.15 %, $F_4$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 8.4 (*s*, 1H, H-6), 7.81–6.5 (*m*, 15H, 3Ph), 4.3 (*s*, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.9 (C-9′), 159.9 (C=O), 155.9 (C-3), 147.9 (C-6), 136.89 (C-4), 135.4–127.0 (3Ph). Anal. Calcd for $C_{26}H_{17}N_5OS$ (447.5): C, 69.78; H, 3.83; N, 15.65. Found: C, 69.63; H, 3.68; N, 15.52.

*7-(4-Hydroxyphenethyl)-3,4-diphenylpyrimido[4′,5′:4,5] thieno[2,3-c]pyridazin-8(7H)-one (**9d**)*

Yellow crystals from chloroform, yield 46.5 %; mp 296–298 °C; IR (KBr, cm$^{-1}$): 3425 (OH), 3058 ($CH_{arom}$), 2916 ($CH_{aliph}$), 1676 (C=O), 1597 (C=N); MS (*m/z*, %): 476 [$M^+$, 0.27 %], 461 [$M^+$–OH, 0.54 %, $F_1$], 384 [$F_1$–Ph, 0.90 %, $F_2$], 356 [$F_2$–$CH_2CH_2$, 6.81 %, $F_3$], 329 [$F_3$–CN, 0.72 %, $F_4$], 288 [$F_4$–NCO, 0.72 %, $F_5$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 8.6 (*s*, 1H, H-6), 7.81–6.3 (*m*, 14H, 3Ph), 5.9 (*s*, 1H, OH), 3.5 (*t*, 2H, $CH_2$N), 2.9 (*t*, 2H, $CH_2$Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.5 (C-9′), 160.5 (C=O), 156.7 (C-3), 153.6 (C–O), 146.9 (C-6), 133.4–115.0 (3Ph), 48.2 ($CH_2$N), 40.1 ($CH_2$Ph). Anal. Calcd for $C_{28}H_{20}N_4O_2S$ (476.55): C, 70.57; H, 4.23; N, 11.76. Found: C, 70.41; H, 4.08; N, 11.60.

*General procedure for the preparation of 7-substituted 3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-8(7H)-one (**11a–g**)*

To a solution of compound **2** (1.0 g, 2.9 mmol) in glacial acetic acid (10 mL), N‘-substituted 4-aminobenzenesulfonamides $\mathbf{10_{a–g}}$ (2.9 mmol) were added, the reaction mixture was refluxed for 8 h, cooled and the formed precipitate was filtered, washed with water, dried, and recrystallized from appropriate solvent to give pure **11a–g**.

*4-(8-Oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)benzenesulfonamide (**11a**)*

Yellow crystals, yield 59.3 %; mp 180–182 °C; IR (KBr, cm$^{-1}$): 3390, 3150 ($NH_2$), 3054 ($CH_{arom}$), 1664 (C=O), 1566 (C=N), 1337, 1134 ($SO_2$); MS (*m/z*, %): 512 [$M^+$ + 1, 4.56 %], 448 [$M^+$–$SO_2$, 0.85 %, $F_1$], 432 [$F_1$–$NH_2$, 1.07 %, $F_2$], 356 [$F_2$–Ph, 0.35 %, $F_3$], 312 [$F_3$–NCO, 3.68 %, $F_4$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 8.5 (*s*, 1H, H-6), 8.1–7.52 (*m*, 14H, 3Ph), 2.3 (*s*, 2H, $NH_2$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.4 (C-9′), 161.2 (C=O), 156.2 (C-3), 147.5 (C-6), 136.5 (C–S), 133.4–121.3 (3Ph). Anal. Calcd for $C_{26}H_{17}N_5O_3S_2$ (511.5): C, 61.04; H, 3.35; N, 13.69. Found: C, 60.90; H, 3.20; N, 13.50.

*4-(8-Oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)-N-phenylbenzene sulfonamide (**11b**)*

Yellow crystals from chloroform; yield 51.4 %, mp 207–209 °C; IR (KBr, cm$^{-1}$): 3115 (NH), 3061 ($CH_{arom}$), 1672 (C=O), 1592 (C=N), 1324, 1163 ($SO_2$); MS (*m/z*, %): 587 [$M^+$, 0.03 %], 523 [$M^+$–$SO_2$, 0.08 %, $F_1$], 432 [$F_1$–$PhNH_2$, 0.06 %, $F_2$], 313 [$F_2$–PhNCO, 0.05 %, $F_3$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 10.2 (*s*, 1H, NH), 8.5 (*s*, 1H, H-6), 8.0–6.52 (*m*, 19H, 4Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.1 (C-9′), 159.2 (C=O), 155.7 (C-3), 146.6 (C-6), 133.4–119.3 (4Ph). Anal. Calcd for $C_{32}H_{21}N_5O_3S_2$ (587.6): C, 65.40; H, 3.60; N, 11.92. Found: C, 65.26; H, 3.44; N, 11.76.

*N-(4-Chlorophenyl)-4-(8-oxo-3,4-diphenylpyrimido[4′,5′:4,5] thieno[2,3-c]pyridazin-7(8H)-yl) benzenesulfonamide (**11c**)*

Yellow crystals from chloroform; yield 34.7 %, mp 235–237 °C; IR (KBr, cm$^{-1}$): 3259 (NH), 3062 ($CH_{arom}$), 1670 (C=O), 1592 (C=N), 1325, 1164 ($SO_2$); MS (*m/z*, %): 621 [$M^+$, 1.34 %], 511 [$M^+$–PhCl, 0.64 %, $F_1$], 448 [$F_1$–$SO_2$, 0.61 %, $F_2$], 356 [$F_2$–$PhNH_2$, 0.35 %, $F_3$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 10.3 (*s*, 1H, NH), 8.4 (*s*, 1H, H-6),

7.8–7.22 (*m*, 18H, 4Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.9 (C-9′), 158.9 (C = O), 156.2 (C-3), 145.9 (C-6), 134.1–122.3 (4Ph). Anal. Calcd for $C_{32}H_{20}ClN_5O_3S_2$ (621.1): C, 61.78; H, 3.24; N, 11.26. Found: C, 61.63; H, 3.10; N, 11.11.

*4-(8-Oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)-N-(o-tolyl)benzene sulfonamide (**11d**)*

Yellow crystals from acetic acid; yield 50.4 %, mp 295–297 °C; IR (KBr, cm$^{-1}$): 3130 (NH), 3057 ($CH_{arom}$), 2919 ($CH_{aliph}$), 1676 (C=O), 1596 (C=N), 1319, 1170 ($SO_2$); MS (*m/z*, %): 601 [$M^+$, 8.54 %], 587 [$M^+$–$CH_3$, 5.92 %, $F_1$], 523 [$F_1$–$SO_2$, 1.52 %, $F_2$], 431 [$F_2$–$PhNH_2$, 9.09 %, $F_3$],312 [$F_3$–PhNCO, 16.67 %, $F_4$], 284 [$F_4$–$N_2$, 5.37 %, $F_5$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 10.1 (*s*, 1H, NH), $\delta$ 8.3 (*s*, 1H, H-6) $\delta$ 7.8–7.22 (*m*, 18H, 4Ph), $\delta$ 2.9 (*s*, 3H, $CH_3$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.1 (C-9′), 157.9 (C=O), 155.8 (C-3), 147.1 (C-6), 131.1–126.3 (4Ph), 18.1 ($CH_3$). Anal. Calcd for $C_{33}H_{23}N_5O_3S_2$ (601.7): C, 65.87; H, 3.85; N, 11.64. Found: C, 65.72; H, 3.70; N, 11.50.

*4-(8-Oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)-N-(m-tolyl)benzene sulfonamide (**11e**)*

Yellow crystals from ethanol; yield 43.2 %, mp 296–298 °C; IR (KBr, cm$^{-1}$): 3145 (NH), 3058 ($CH_{arom}$), 2915 ($CH_{aliph}$), 1676 (C=O), 1596 (C=N), 1319, 1172 ($SO_2$); MS (*m/z*, %): 602 [$M^+$ + 1, 1.80 %], 587 [$M^+$–$CH_3$, 1.99 %, F1], 495 [$F_1$–$PhNH_2$, 0.38 %, $F_2$], 431 [$F_2$–$SO_2$, 0.26 %, $F_3$],313 [$F_3$–PhNCO, 1.90 %, $F_4$], 285 [$F_4$–$N_2$, 0.17 %, $F_5$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 10.3 (*s*, 1H, NH), $\delta$ 8.2 (*s*, 1H, H-6) $\delta$ 8.8 – 6.45 (*m*, 18H, 4Ph), $\delta$ 2.85 (*s*, 3H, $CH_3$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.1 (C-9′), 157.9 (C=O), 155.8 (C-3), 147.1 (C-6), 131.1–126.3 (4Ph), 22.3 ($CH_3$). Anal. Calcd for $C_{33}H_{23}N_5O_3S_2$ (601.7): C, 65.87; H, 3.85; N, 11.64. Found: C, 65.74; H, 3.75; N, 11.48.

*N-Benzyl-4-(8-oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)benzene sulfonamide (**11f**)*

Yellow crystals from ethanol; yield 35.9 %, mp 290–292 °C; IR (KBr, cm$^{-1}$): 3155 (NH), 3058 ($CH_{arom}$), 2916 ($CH_{aliph}$), 1677 (C=O), 1596 (C=N), 1318, 1173 ($SO_2$); MS (*m/z*, %): 602 [$M^+$ + 1, 3.85 %], 537 [$M^+$–$SO_2$, 6.24 %, $F_1$], 447 [$F_1$–$PhCH_2$, 1.70 %, $F_2$], 356 [$F_2$–$PhNH_2$, 1.80 %, $F_3$], 313 [$F_3$–NCO, 1.98 %, $F_4$], 285 [$F_4$–$N_2$, 14.14 %, $F_5$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) = 10.5 (*s*, 1H, NH), $\delta$ 8.4 (*s*, 1H, H-6) $\delta$ 7.68–7.35 (*m*, 19H, 4Ph), $\delta$ 3.38 (*s*, 2H, $CH_2$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.1 (C-9′), 157.9 (C=O), 155.8 (C-3), 147.1 (C-6), 141.5–126.9 (4Ph), 45.3 ($CH_2$). Anal. Calcd for $C_{33}H_{23}N_5O_3S_2$ (601.7): C, 65.87; H, 3.85; N, 11.64. Found: C, 65.77; H, 3.72; N, 11.50.

*N′-(2,4-Dinitrophenyl)-4-(8-oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl) benzenesulfonohydrazide (**11g**)*

Yellow crystals from ethanol; yield 37.5 %, mp 254–256 °C; IR (KBr, cm$^{-1}$): 3390, 3170 (2NH), 3059 ($CH_{arom}$), 1677 (C=O), 1596 (C=N), 1490, 1444 ($NO_2$), 1322, 1174 ($SO_2$); MS (*m/z*, %): 694 [$M^+$ + 2, 2.97 %], 604 [$M^+$ –$2NO_2$, 3.76 %, $F_1$], 511 [$F_1$–$PhNH_2$, 1.03 %, $F_2$], 433 [$F_2$–$SO_2$, 3.84 %, $F_3$], 313 [$F_3$–PhNCO, 4.34 %, $F_4$], 285 [$F_4$–$N_2$, 5.63 %, $F_5$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) : 10.1 (*s*, 1H, NH), 8.2 (*s*, 1H, H-6), 8.68–7.15 (*m*, 17H, 4Ph), 3.38 (*s*, 2H, $CH_2$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 162.8 (C-9′), 158.1 (C=O), 155.2 (C-3), 147.1 (C-6), 141.5–118.2 (4Ph). Anal. Calcd for $C_{32}H_{20}N_8O_7S_2$ (692.6): C, 55.49; H, 2.91; N, 16.18.Found: C, 55.34; H, 2.80; N, 16.03.

*Ethyl ((4-(8-oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)phenyl)sulfonyl) carbamate (**12**)*

A mixture of **11a** (1.0 g, 1.9 mmol), ethyl chloroformate (0.21 g, 1.9 mmol), and anhydrous potassium carbonate (2.0 g) in dry acetone (100 mL) was refluxed for 5 h. The solvent was removed under reduced pressure, the residue was dissolved in water (100 mL) and neutralized with acetic acid. The product separated out was filtered, washed with water, dried, and recrystallized from ethanol to give **12.** Yellow crystals, yield 70.1 %, mp 175–177 °C; IR (KBr, cm$^{-1}$): 3350 (NH), 3059 ($CH_{arom}$), 1721 (C=O, amide), 1670 (C=O, cyclic), 1600 (C=N), 1375, 1172 ($SO_2$); MS (*m/z*, %): 583 [$M^+$, 27.20 %], 537 [$M^+$–$CH_3CH_2OH$, 36.40 %, $F_1$],432 [$F_1$–$SO_2NCO$, 26.36 %, $F_2$], 356 [$F_2$–Ph, 23.01 %, $F_3$], 330 [$F_3$–$N_2$, 6.69 %, $F_4$], 287 [$F_4$–NCO, 22.59 %, $F_5$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) : 10.26 (*s*, 1H, NH), 8.7 (*s*, 1H, H-6), 7.7–7.2 (*m*, 14H, 3Ph), 3.3 (*q*, 2H, $CH_2$), 2.07 (*t*, 3H, $CH_3$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.8 (C-9′), 158.5 (2C=O), 156.2 (C-3), 148.1 (C-6), 140.5–121.2 (3Ph), 63.5 ($CH_2$), 15.8 ($CH_3$). Anal. Calcd for $C_{29}H_{21}N_5O_5S_2$ (583.64): C, 59.68; H, 3.63; N, 12.00. Found: C, 59.50; H, 3.47; N, 11.85.

*General procedure for the preparation of substituted 4-(8-oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno [2,3-c]pyridazin-7 (8H)-yl)benzenesulfonylurea (**13a–e**)*

To a solution of **12** (1.0 g, 1.7 mmol) in boiling toluene (30 mL), the desired primary amines were added (1.7 mmol) dropwise. The mixture was subsequently refluxed for further 6 h, and the solvent was evaporated. The residue was washed with diethyl ether and recrystallized from ethanol.

*N-(Benzylcarbamoyl)-4-(8-oxo-3,4-diphenylpyrimido [4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)benzenesulfonamide (**13a**)*

Yellow crystals, yield 63.6 %, mp 177–179 °C; IR (KBr, $cm^{-1}$): 3351, 3102 (2NH), 3055 ($CH_{arom}$), 2923 ($CH_{aliph}$), 1725 (cyclic C=O), 1646 (amide C=O), 1605 (C=N), 1333, 1140 ($SO_2$); MS (*m/z*, %): 643 [$M^+$–1, 0.46 %], 581 [$M^+$–$SO_2$, 0.44 %, $F_1$], 490 [$F_1$–$PhCH_2$, 0.66 %, $F_2$], 431 [$F_2$–NHCONH, 0.55 %, $F_3$], 356 [$F_3$–Ph, 0.66 %, $F_4$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm) : 10.2 (*s*, 2H, NHCONH), 8.4 (*s*, 1H, H-6), 8.1–7.23 (*m*, 19H, 3Ph), 4.3 (*s*, 2H, $CH_2$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.2 (C-9′), 161.8 (C=O, amide), 157.5 (C=O, cyclic), 156.9 (C-3), 147.1 (C-6), 140.5–126.2 (4Ph), 45.5 ($CH_2$). Anal. Calcd for $C_{34}H_{24}N_6O_4S_2$ (644.72): C, 63.34; H, 3.75; N, 13.04.Found: C, 63.19; H, 3.59; N, 12.88.

*N-((2-Chlorobenzyl)carbamoyl)-4-(8-oxo-3,4diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)benzenesulfonamide (**13b**)*

Yellow crystals, yield 60.3 %, mp 168–170 °C; IR (KBr, $cm^{-1}$): 3341, 3190 (2NH), 3057 ($CH_{arom}$), 2981 ($CH_{aliph}$), 1720 (cyclic C=O), 1675 (amide C=O), 1603 (C=N), 1366, 1149 ($SO_2$); MS (*m/z*, %): 679 [$M^+$, 20 %], 567 [$M^+$–PhCl, 13.84 %, $F_1$], 511 [$F_1$–$CH_3NCO$, 13.84 %, $F_2$], 431 [$F_2$–$SO_2NH_2$, 5.25 %, $F_3$], 404 [$F_3$–$N_2$, 10.26 %, $F_4$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm): 10.2 (*s*, 2H, NHCONH), 8.4 (*s*, 1H, H-6), 8.1–7.23 (*m*, 18H, 4Ph), 4.5 (*s*, 2H, $CH_2$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.2 (C-9′), 161.4 (C=O, amide), 157.1 (C=O, cyclic), 156.9 (C-3), 147.1 (C-6), 142.5–126.6 (4Ph), 43.5 ($CH_2$). Anal. Calcd for $C_{34}H_{23}ClN_6O_4S_2$ (679.17): C, 60.13; H, 5.22; N, 12.37. Found: C, 60.00; H, 3.25; N, 12.20.

*4-(8-Oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)-N-(o-tolylcarbamoyl) benzenesulfonamide (**13c**)*

Yellow crystals, yield 54.5 %, mp 125–127 °C; IR (KBr, $cm^{-1}$): 3344, 3280 (2NH), 3057 ($CH_{arom}$), 2980 ($CH_{aliph}$), 1720 (cyclic C=O), 1674 (amide C=O), 1599 (C=N), 1372, 1151 ($SO_2$); MS (*m/z*, %): 644 [$M^+$, 30.0 %], 630 [$M^+$–$CH_3$, 28.5 %, $F_1$], 554 [$F_1$–Ph, 33.0 %, $F_2$], 431 [$F_2$–$SO_2NHCONH$, 43.0 %, $F_3$], 315 [$F_3$–PhNCO, 32.5 %, $F_4$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm): 10.1 (*s*, 2H, NHCONH), $\delta$ 8.4 (*s*, 1H, H-6) $\delta$ 8.1–7.4 (*m*, 18H, 4Ph),$\delta$ 3.2 (*s*, 3H, $CH_3$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.1 (C-9′), 161.0 (C=O, amide), 157.2 (C=O, cyclic), 156.3 (C-3), 147.1 (C-6), 140.5–115.6 (4Ph), 18.5 ($CH_3$). Anal. Calcd for $C_{34}H_{24}N_6O_4S_2$ (644.7): C, 63.34; H, 3.75; N, 13.04. Found: C, 63.20; H, 3.62; N, 12.90.

*4-(8-Oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)-N-(m-tolylcarbamoyl) benzenesulfonamide (**13d**)*

Yellow crystals, yield 63.6 %, mp 130–132 °C; IR (KBr, $cm^{-1}$): 3343, 3275 (2NH), 3056 ($CH_{arom}$), 2980 ($CH_{aliph}$), 1720 (cyclic C=O), 1674 (amide C=O), 1602 (C=N), 1371, 1151 ($SO_2$); MS (*m/z*, %): 644 [$M^+$, 7.51 %], 631 [$M^+$–$CH_3$, 5.20 %, $F_1$], 554 [$F_1$–Ph, 7.80 %, $F_2$], 496 [$F_2$–NHCONH, 0.19 %, $F_3$], 355 [$F_3$–$PhSO_2$, 5.49 %, $F_4$],330 [$F_4$–$N_2$, 5.0 %, $F_5$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm): 10.1 (*s*, 2H, NHCONH), 8.4 (*s*, 1H, H-6), 8.1–7.4 (*m*, 18H, 4Ph), 2.9 (*s*, 3H, $CH_3$); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.1 (C-9′), 161.0 (C=O, amide), 157.2 (C=O, cyclic), 156.3 (C-3), 147.1 (C-6), 140.5–115.6 (4Ph), 26.5 ($CH_3$). Anal. Calcd for $C_{34}H_{24}N_6O_4S_2$ (644.7): C, 63.34; H, 3.75; N, 13.04. Found: C, 63.18; H, 3.60; N, 12.88.

*N-((4-Chlorophenyl)carbamoyl)-4-(8-oxo-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)benzenesulfonamide (**13e**)*

Yellow crystals, yield 61.9 %, mp 115–117 °C; IR (KBr, $cm^{-1}$): 3351, 3290 (2NH), 3056 ($CH_{arom}$), 1720 (cyclic C=O), 1676 (amide C=O), 1596 (C=N), 1372, 1153 ($SO_2$); MS (*m/z*, %): 665 [$M^+$, 0.14 %], 553 [$M^+$–$C_6H_4Cl$, 0.19 %, $F_1$], 495 [$F_1$–NHCONH, 0.31 %, $F_2$], 432 [$F_2$–$SO_2$, 0.18 %, $F_3$], 315 [$F_3$–PhNCO, 0.88 %, $F_4$], 287 [$F_4$–$N_2$, 5.83 %, $F_5$]; $^1$H-NMR (DMSO-$d_6$): $\delta$ (ppm): 10.6 (*s*, 2H, NHCONH), 8.5 (*s*, 1H, H-6), 8.4–7.4 (*m*, 18H, 4Ph); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ (ppm): 163.1 (C-9′), 161.0 (C=O, amide), 157.2 (C=O, cyclic), 156.3 (C-3), 147.1 (C-6), 140.5–120.6 (4Ph). Anal. Calcd for $C_{33}H_{21}ClN_6O_4S_2$ (665.1): C, 59.59; H, 3.18; N, 12.63. Found: C, 59.41; H, 3.01; N, 12.50.

## Results and discussion

The condensation of ethyl 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carboxylate **1** with triethyl orthoformate in the presence of acetic anhydride as a catalyst give the corresponding ethoxymethyleneamino intermediate **2**. This was reacted directly without purification. The intermediate **2** was allowed to react with ammonium hydroxide at 70 °C affording the 3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-*c*]pyridazin-8(7*H*)-one **3** in a good yield. The structure of compound **3** was proved by its infrared, mass spectrum, and elemental analysis. The IR spectrum showed absorption bands at 3,165 $cm^{-1}$ corresponding to NH, 1,655 $cm^{-1}$ corresponding to C=O.

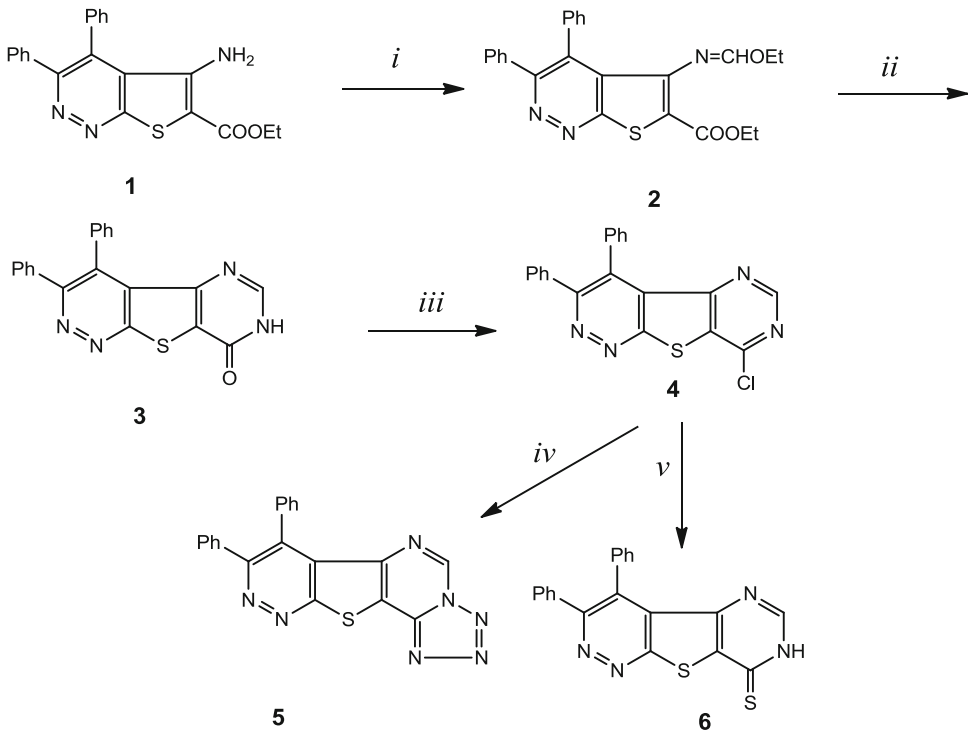
Heating compound **3** with phosphoryl chloride gave the corresponding 8-chloro derivative **4** in a good yield. Its

structure was proved by elemental analysis and spectral data, IR spectrum showed absorption band at 1,604 $cm^{-1}$ corresponding to C=N, 1,071 $cm^{-1}$ corresponding to C–Cl, and there are no absorption bands in the carbonyl region.

Reaction of 8-chloro derivative **4** with sodium azide in ethanol at refluxing temperature afforded 7,8-diphenyltetrazolo[1″,5″:1′,6′]pyrimido[4′,5′:4,5]thieno[2,3-*c*]pyridazine **4,** wherein 8-azido derivative was formed at the first step, then intramolecular cyclization to the tetrazolo derivative **4** occurred immediately. The predominant existence of the tetrazolo derivative **5** was supported by infrared spectral data, which exhibited no absorption bands around 2,200 $cm^{-1}$ due to azido group, and there is absorption bands at 1,635 $cm^{-1}$ corresponding to C=N. The 8-chloro derivative **4** on treatment with thiourea in absolute ethanol gave thiouronium salt which on hydrolysis with 2.5 N sodium hydroxide followed by acidification with hydrochloric acid (pH 2) gave 3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-*c*]pyridazine-8(7*H*)-thione **6** in 48 % yield. Structure of compound **6** was confirmed by its elemental analysis and spectral data. The IR spectrum showed absorption bands at 3,390 $cm^{-1}$ corresponding to NH, 1,665 $cm^{-1}$ corresponding to C=N, 1,531 $cm^{-1}$ corresponding to C=C and 1,194 $cm^{-1}$ corresponding to C=S (Scheme 1).

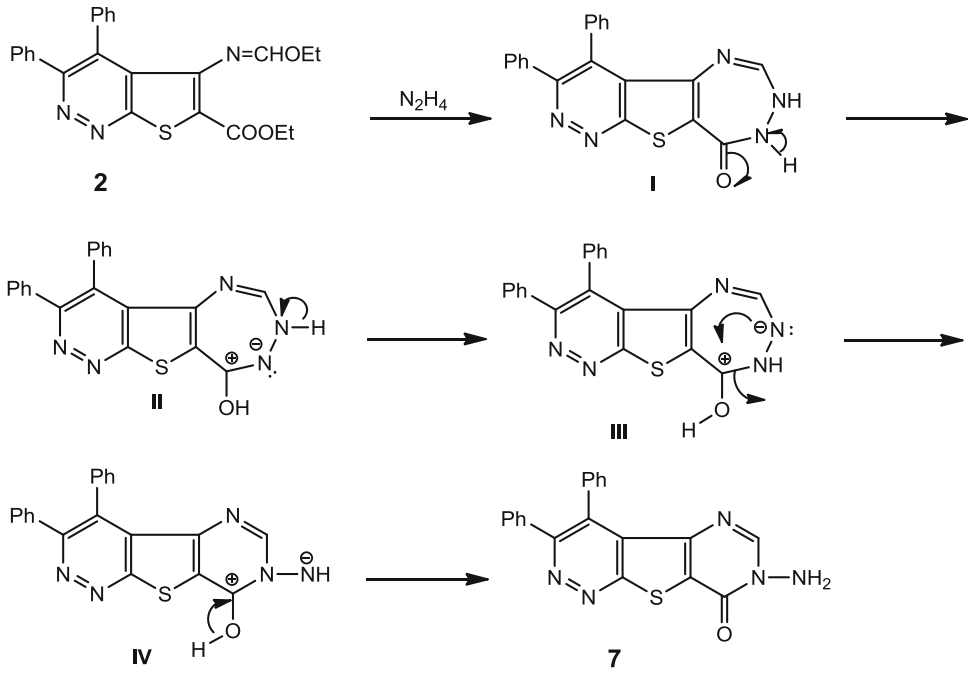
When the ethoxymethyleneamino derivative **2** was heated with hydrazine hydrate in acetic acid at refluxing temperature, afforded 7-amino-3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-*c*]pyridazin-8(7*H*)-one **7.** The assignment of structure of **7** was based on an analytical and spectral data. The IR spectrum showed the presence of $NH_2$ group at 3,414 and 3,150 $cm^{-1}$, carbonyl group at 1,676 $cm^{-1}$, and C=N at 1,594 $cm^{-1}$. Mechanistically, the formation of tricyclic compound **7** from **2** and hydrazine hydrate in involved the initial formation of acid hydrazide which undergoes immediate intramolecular nucleophilic attack with elimination of ethanol molecule, forms 1,2,4-triazepine derivative **I**, which, as a result of transfer of the hydrogen atom from NH group to the oxygen atom, forms a hybrid **II**. The hybrid, following a translocation of the hydrogen atom from the nitrogen atom at position 3, changes into a transient structure **III**, whereas at position 3, an electron is still present as a result of hydrogen bonding accompanying the nonbonding pair of electrons of the nitrogen atom. That strongly electronegative nitrogen atom, in the reaction of intermolecular nucleophilic rearrangement, forms a link with the carbonyl carbon atom, releasing the C–N bond. Structure **IV**, formed as result of that rearrangement, is not stable and, after translocation of the hydrogen atom from the carbonyl oxygen atom, transforms into compound **7** which is the product of this reaction chain (Scheme 2).

Alternatively, compound **7** was obtained upon heating of 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbohydrazide in dimethyl formamide as previously reported (Deeb and El-Abbasy, 2006). Then, condensation of 7-amino derivative **7** with aromatic aldehydes namely benzaldehyde, *p*-methoxybenzadehyde, and *p*-nitrobenzaldehyde was carried out in (1:1) molar ratio with




**Scheme 1** Synthesis of derivatives **1, 2, 3, 4, 5**, and **6**. Reagents and conditions *i* $CH(OEt)_3/Ac_2O$/reflux, *ii* $NH_4OH$, *iii* $POCl_3$/reflux, *iv* $NaN_3$/EtOH, *v* $NH_2CSNH_2$/EtOH, NaOH, and then HCl

**Scheme 2** .




elimination of water producing 7-arylideneamino derivatives **8a–c.** The structures of compounds **8a–c** were supported by their elemental analysis and spectral data. IR spectrum revealed the presence of absorption bands at 3,056–3,054 $cm^{-1}$ due to $CH_{arom}$, 1,687–1,665 $cm^{-1}$ corresponding to carbonyl group, and 1,630–1,620 $cm^{-1}$corresponding to C=N, further more IR spectrum of compound **8b** showed the presence of $CH_{aliph}$ at 2,990 $cm^{-1}$ and $OCH_3$ at 1,079 $cm^{-1}$. Also, IR spectrum of compound **8c** showed the presence $NO_2$ at 1,519, 1,437 $cm^{-1}$.

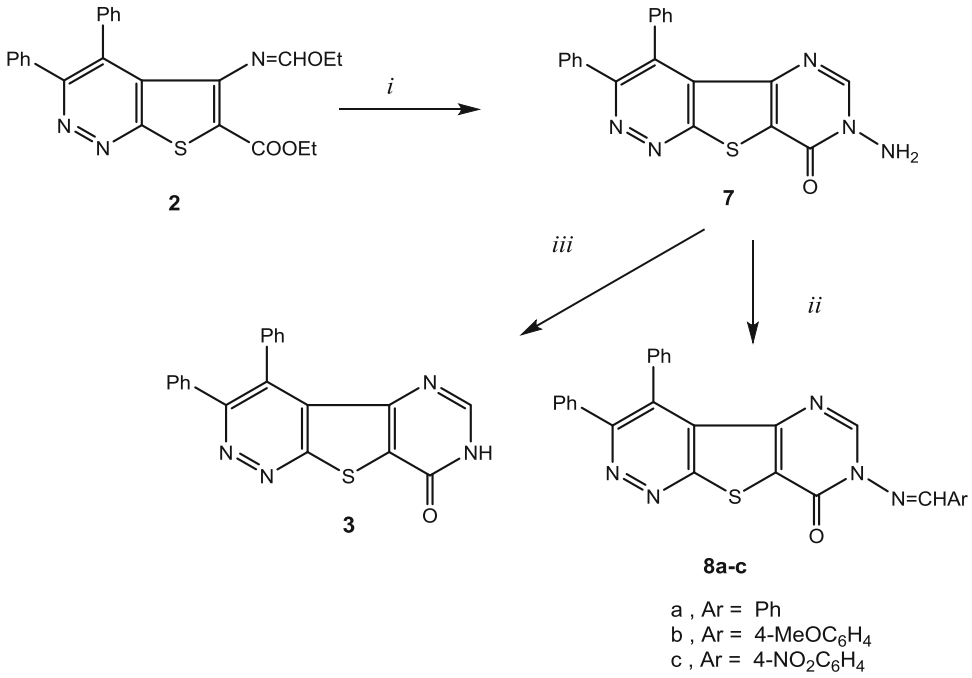
Since the primary amino group when is directly attached to a nitrogen of the heteroaromatic nucleus, an *N*-diazonium ion is generated as an intermediate which decomposes with ultimate deamination and formation of dinitrogen monoxide (Doyle *et al.,* 1974). It was of interest that the reaction of 7-amino derivative **7** with nitrous acid yields the anticipated 3,4-diphenylpyrimido[4′,5′:4,5]thieno[2,3-*c*]pyridazin-8(7*H*)-one **3.** Compound **3** was identical (mp, mixed mp, and superimposable IR) with an authentic sample synthesized in this work from 5-ethoxymethyleneamino derivative **2** which underwent smooth cyclization to **3** in the presence of ammonium hydroxide (Scheme 3).

Upon treatment of 5-ethoxymethyleneamino derivative **2** with amines such as benzyl amine, *o*-chlorobenzyl amine, tyramine, and phenylhydrazine, finally afforded 7-N-substituted pyrimidothienopyridazines **9a–d.** The infrared spectra of **9a–d** revealed the presence of absorption bands at 3425 due to OH, 3,390 $cm^{-1}$ corresponding to NH, 3,060–3,058 $cm^{-1}$ corresponding to $CH_{arom}$, 2,917–2,916 $cm^{-1}$ corresponding to $CH_{aliph}$, 1,677–1,676 $cm^{-1}$ corresponding to C=O, and absorption bands at 1,597–1,596 $cm^{-1}$ corresponding to C=N.

The *N*′-(un)substituted 4-aminobenzenesulfonamides **10** were prepared using a previously described method (Vogel *et al*, 1980). The nucleophilic substitution of the 4-amino group of **10** with 5-ethoxymethyleneamino-6-carboxylate **2** yielded the corresponding 7-*N*-substituted pyrimido thienopyridazines **11a–g**. The structure of the synthesized compounds was established on the basis of their IR, $^1$H-NMR, and mass spectral studies. The infrared spectra of **11a–g** showed absorption bands at 3,390–3,115 $cm^{-1}$ corresponding to NH groups, 3,062–3,054 $cm^{-1}$ corresponding to $CH_{arom}$, 1,676–1,664 $cm^{-1}$ corresponding to C=O, 1,592–1,566 $cm^{-1}$ corresponding to C=N and bands at 1,337–1,324, and 1,170–1,134 $cm^{-1}$ corresponding to the symmetrical and asymmetrical vibrations of the $SO_2$ group. The $^1$H-NMR spectrum of **11c** showed signals at $\delta$ (ppm) = 10.3 corresponding to ($SO_2NH$) and at $\delta = 8.4$ corresponding to (H-6). Furthermore, the $^1$H-NMR spectrum of **11f** showed signals at $\delta$ 10.5 corresponding to ($SO_2NH$), at $\delta = 8.4$ corresponding to (H-6) and at $\delta = 3.38$ corresponding to ($CH_2$) (Scheme 4).

On the other hand, we have prepared the interesting sulfonylurea derivatives starting from the 7-(4-sulfamoylphenyl)pyrimidothienopyridazine **11a** on treatment with ethyl chloroformate in refluxing acetone containing anhydrous potassium carbonate yielded ethyl carbamate derivative **12**. The resulting carbamate was subsequently condensed with different substituted amines such as benzyl amine, *o*-chlorobenzyl amine, *o*-toluidine, *m*-toluidine, and *p*-chloroaniline to give novel benzene sulfonylurea

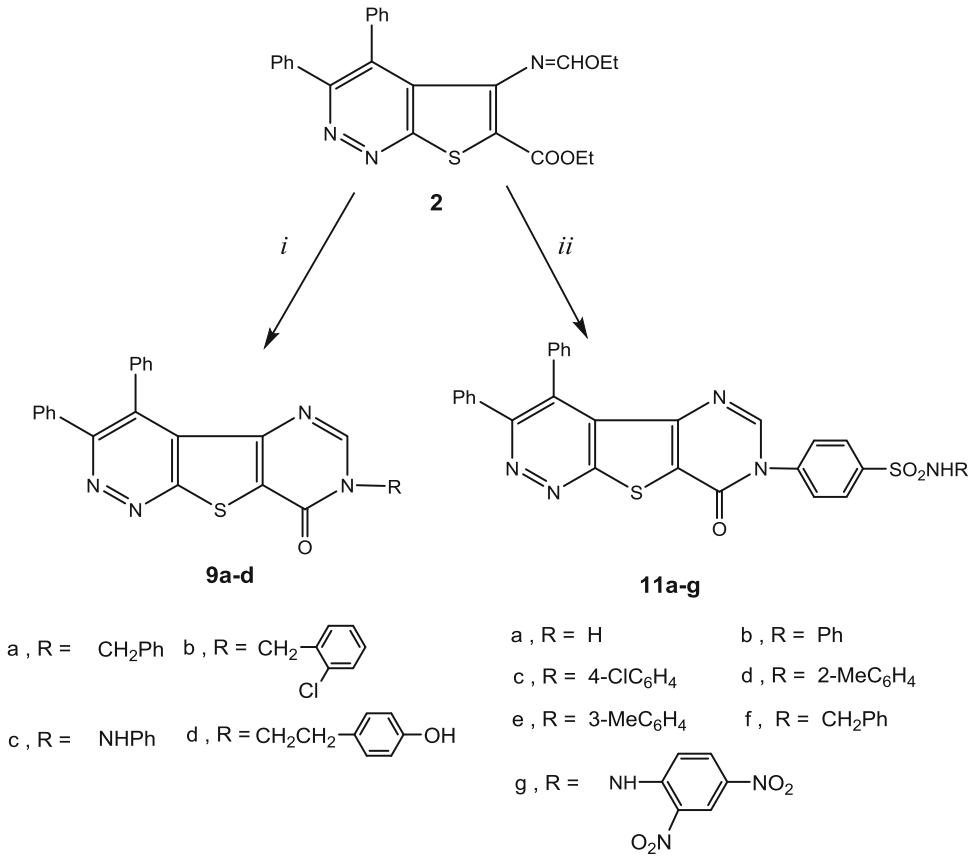
**Scheme 3** Synthesis of derivatives **7** and **8a–c**. Reagents and conditions *i* $NH_2NH_2.H_2O$/AcOH/reflux, *ii* ArCHO/EtOH/reflux, *iii* $HNO_2$/HCl



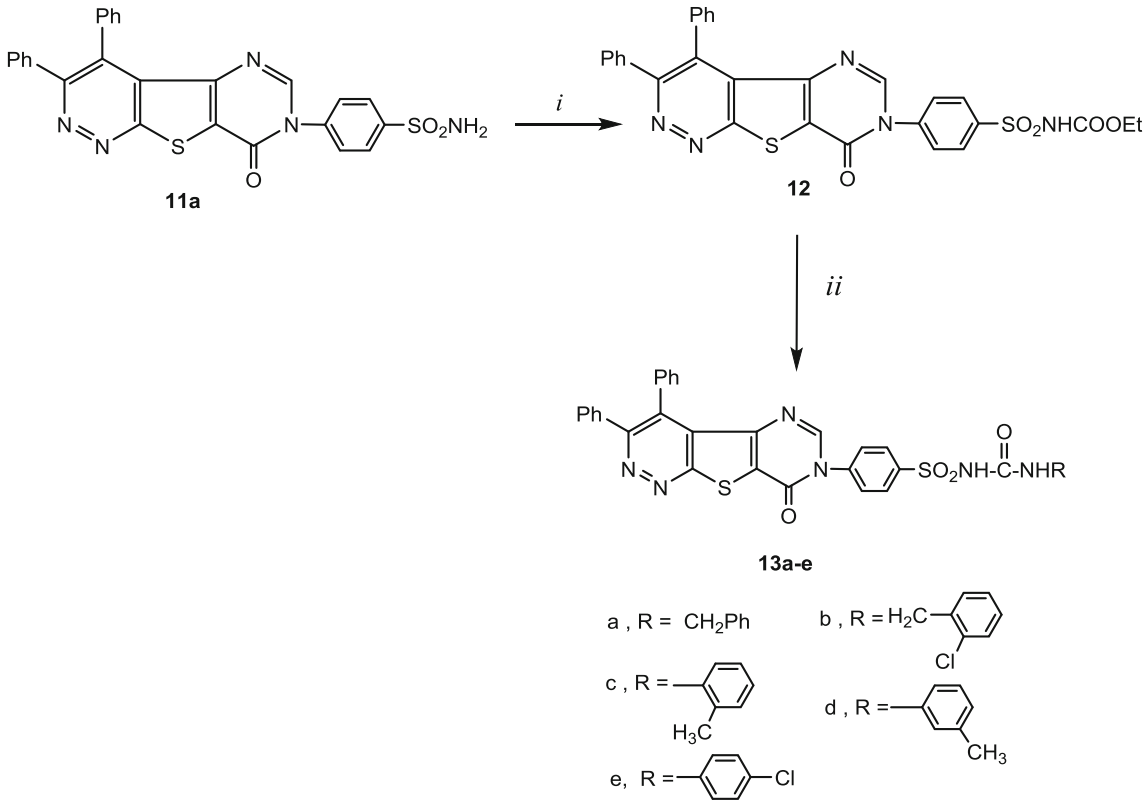

**Scheme 4** Synthesis of derivatives **9a–d** and **11a–g**. Reagents and conditions *i* R-$NH_2$/AcOH/reflux, *ii*

*ii* $H_2N$–$C_6H_4$–$SO_2NHR$

**(10a–g),** AcOH/reflux




derivatives **13a–e**. The structures of compound **12** as well as the final products **13a–e** were confirmed on the basis of both analytical and spectroscopic data. The IR spectrum of compound **12** showed absorption band at 3,350 $cm^{-1}$ corresponding to NH group, 3,059 $cm^{-1}$ corresponding to $CH_{arom}$, 1,721 $cm^{-1}$ corresponding to (cyclic C=O),




**Scheme 5** Synthesis of derivatives **12** and **13a–e**. Reagents and conditions *i* ClCOOEt/$K_2CO_3$/Acetone/reflux, *ii* $RNH_2$/Toluene/reflux

**Table 1** Antimicrobial activity of some synthesized compounds

| Compound | *Micrococcus luteus* | *Staphylococcus aureus* | *Escherichia coli* | *Candida albicans* |
|---|---|---|---|---|
| **11a** | ++++ | ++++ | +++ | +++ |
| **11b** | ++ | ++ | +++ | ++++ |
| **11c** | + | + | +++ | +++ |
| **11d** | ++ | ++ | +++ | +++ |
| **11e** | + | + | ++ | ++++ |
| **11f** | ++ | ++ | +++ | +++ |
| **11g** | +++ | +++ | +++ | ++ |
| **11f** | ++ | ++ | +++ | +++ |
| DMF | – | – | – | – |
| Sulfadoxine | +++ | +++ | ++ | +++ |
| Sulfadimidine | +++ | +++ | ++ | +++ |
| Nystatin | – | – | – | ++++ |

Values are mean inhibition zone (mm) of two replicates, 25–35 mm = ++++, 18–24 mm = +++, 11–16 mm = ++, ≤10 mm = +, – = negative inhibition

1,670 $cm^{-1}$ corresponding to (amide C=O), 1,600 $cm^{-1}$ corresponding to C=N and absorption band at 1,375, and 1,172 $cm^{-1}$ corresponding to the symmetrical and asymmetrical vibrations of the $SO_2$ group. The $^1$H-NMR spectrum showed signals at $\delta$ (ppm) = 10.26 corresponding to ($SO_2NH$), and at $\delta$ = 3.3 and 2.07 corresponding to $CH_2$ and $CH_3$, respectively. The IR spectra of compounds **13a–e** showed absorption bands at 3,351–3,102 $cm^{-1}$ corresponding to NH groups, 3,057–3,055 $cm^{-1}$ corresponding to $CH_{arom}$, 2,980–2,923 $cm^{-1}$ corresponding to $CH_{aliph}$, 1,725–1,720 $cm^{-1}$ corresponding to (cyclic C=O), 1,674–1,646 $cm^{-1}$ corresponding to (amide C=O), 1,605-

1,599 $cm^{-1}$ corresponding to C=N, and absorption bands at 1,372–1,333 and 1,151–1,140 $cm^{-1}$ corresponding to the symmetrical and asymmetrical vibrations of the $SO_2$ groups (Scheme 5).

## Antimicrobial activity

Applying the agar plate diffusion technique (Bauer *et al.*, 1966), the newly synthesized sulfonamide derivatives were screened in vitro for antimicrobial activity against Gram-positive bacteria (*S. aureus* and *M. luteus*), Gram-negative bacteria (*E. coli*), and fungi (*C. albicans*). In this method, a standard 5 mm diameter sterilized filter paper dis impregnated with the compounds (400 μ/0.1 mL of dimethylformamide) was placed on the agar plates which had previously been inoculated with the above organisms. The petri dishes were left at 4 °C for 2 h.; then the injected plates with bacteria were incubated at 37 ± 0.1 °C for 24 h., and those inoculated with fungi incubated at 25 ± 0.1 °C for 48 h. At the end of the period, diameter of inhibition zones was measured in mm; these studies were performed in two replicates. The screening results given in (Table 1) indicated that all the compounds exhibited antimicrobial activity against all the test organisms.

## Conclusion

In summary, we have developed and synthesized novel derivatives of pyrimidothienopyridazine. Some of the target compounds bearing the sulfonamide group were evaluated for their antimicrobial activity, and the following was concluded: derivative **11a** has promising inhibitory activity against Gram-positive bacteria. Derivatives **11b** and **11e** have remarkable inhibitory activity against fungi. Rest of the compounds showed moderate to low activity against the examined micro-organisms.

## References


Asif M, Singh A, Ratnakar L (2011) Antimicrobial agents: brief study of pyridazine derivatives against some phathogenic microorganisms. J Pharm Res 4(3):664–667

Bauer AW, Kirby MDK, Sherries JC, Turck M (1966) Antibiotic susceptibility testing by standard single disc diffusion method. Am J Clin Pathol 45:493–496

Butnariu RM, Caprosu MD, Bejan V, Mangalagiu II, Ungureanu M, Poiata A, Tuchilus C, Florescu M (2007) Pyridazine and phthalazine derivatives with potential antimicrobial activity. J Hetrocycl Chem 44(5):1149–1152

Chambhare RV, Khadse BG, Bobde AS (2003) Synthesis and preliminary evaluation of some N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-carboxamide and 3-substituted-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-ones as antimicrobial agents. Euro J Med Chem 38(1):89–100

Connor EE (1998) Sulfonamide Antibiotics. Prim Care Update Ob Gyns 5(1):32–35

Deeb A, El-Abbasy M (2006) Pyridazine derivatives and related compounds, part 20:1 synthesis of different heterocycles from 5-Aminothieno[2,3-c]pyridazine-6-carbohydrazide. Phosphorus Sulfur Silicon 181:397–404

Deeb A, Said SA, Hamed MM, Yasin F (1990) Pyridazine derivatives and related compounds, part 1. Some reactions with 4-cyano-5,6-diphenyl-2,3-dihydropyridazine-3-one. J Chin Chem Soc 37:287–294

Deeb A, El-Eraky W, El-Awdan S, Mahgoub S (2014) Pyridazine and its related compounds, Part 34: hypoglycemic and hypolipidemic activity of some novel condensed pyridazine sulfonamides. Med Chem Res 23:34–41

Doyle MP, Kalmbacher JG, Wierenga W, Deboer JE (1974) Nitrosative cleavage of benzalazine and related aldehyde azines. Production, decomposition and trapping of iminodiazonium ions. Tetrahedron Lett 15(15):1455–1458

Drochioiu G, Sunel V, Oniscu C, Basu C, Murariu M (2007) The breakdown of plant biostructure followed by amino acids determination. Rom Biotechnol Lett 6(2):155–165

Han X, Hong YH, You Quan ZHU, Mao LIU, Bim ZX, Fang Zhong HU, Zheng YH (2010) Synthesis and herbicidal activities of novel 4-(3-trifluoromethylphenyl)-2H-pyridazin-3-one derivatives. Sci China Chem 53(1):157–166

Hansch C, Sammes PG, Taylor JB (1990) Comprehensive medicinal chemistry. Pergamon Press, Oxford, pp 255–270

Hanson PR, Probst DA, Robinson RE, Yau M (1999) Cyclic sulfonamides via the ring-closing metathesis reaction. Tetrahedron Lett 40:4761–4764

Kandile NG, Mohamed MI, Zaky H, Mohamed HM (2009) Novel pyridazine derivatives: synthesis and antimicrobial activity evaluation. Eur J Med Chem 44(5):1989–1996

Kleemann A, Engel J, Kutscher B, Reichert D (1999) Pharmaceutical substance: syntheses, patents, applications, 2nd edn. Thieme, Stuttgart

Mangalagiu II (2011) Recent achievements in the chemistry of 1,2-diazines. Curr Org Chem 15(5):730–752

Mantu D, Luca MC, Moldoveanu C, Zbancioc G, Mangalagiu II (2010) Synthesis and antituberculosis activity of some new pyridazine derivatives. Part II. Eur Med Chem 45(11):5164–5168

Tucaliuc RB, Risca IM, Drochioiu G, Mangalagiu I (2008) Biological effect of some new pyridazine derivatives on wheat in germination experiments. Rom Biotechnol Lett 13(4):3837–3842

Vogel AI, Furniss Brian S (1980) Vogel's textbook of practical organic chemistry, 4th edn. Longman Group Limited, London, p 651