Synthesis, reactions and antimicrobial activity of thieno[2,3-*c*]pyridazine derivatives

Fatma El Mariah*

Department of Chemistry, Faculty of Girls, Ain Shams University, Cairo, Egypt

The reaction of N^1-(un)substituted 4-aminosulfonamide with 6-chloropyridothienopyridazine (**5**) and 8-chloro-pyrimidothienopyridazine (**14**) gave 6-substituted aminopyridothienopyridazine (**9**) and 8-substituted amino-pyrimidothienopyridazine (**16**) respectively. All of the derivatives have been characterised by analytical and spectroscopic studies and also tested for their *in vitro* antibacterial and antifungal activity against a variety of microorganisms.

Keywords: 6-substituted aminopyridothienopyridazine, 8-substituted pyrimidothienopyridazine, antimicrobial activity

The pyridazine[1] ring is a recurrent structural component of biologically active compounds.[2] Moreover, pyridazines are useful intermediates in the construction of several other heterocycles[3] and in physical organic chemistry.[4] Recently they have been explored as new α-helix mimetics.[5] On the other hand, thienopyridazines have also attracted attention because of their promising biological activities.[6,7] In connection with these facts, the utility of substituted thieno[2, 3-*c*]pyridazines as a synthon for the preparation of new substituted pyrido[2', 3':4, 5]thieno[2, 3-*c*]pyridazines and pyrimido[4', 5':4, 5]thieno-[2, 3-*c*]pyridazine is reported and an analysis of their biological activity is described. Amino ketone and aminocyano serve as good synthons for formation of a pyridine and pyrimidine rings.[3]

The precursor, 5-amino-6-benzoyl-3, 4-diphenylthieno [2, 3-*c*]pyridazine (**1**), was prepared from 4-cyano-5, 6-diphenyl-pyridazine-3(2H)thione and ω-chloroacetophenone using excess potassium carbonate in refluxing acetone.[1] Chloroacetylation of (**1**) with chloroacetyl chloride gave the 5-chloroacetyl-amino (**2**) which was obtained in analytically pure form directly from the reaction mixture in 85% yield.[8]

The reaction of a 1:1 mixture of compound (**2**) and sodium cyanide in ethanol afforded a solid for which the theoretically possible structure (**3**) was eliminated based on microanalytical and spectroscopic data. These data can only be intelligibly interpreted in terms of structure (**4**) (Scheme 1).

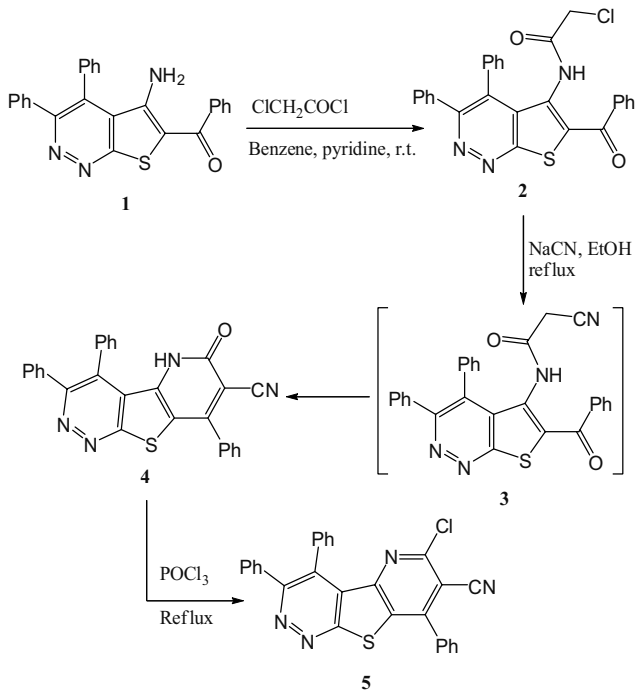
The structure of (**4**) is substantiated from micro analytical data and was confirmed by IR, ^{1}H NMR, and mass spectroscopy. Its IR spectrum revealed the absence of benzoyl ketone group in addition to the presence of 3423, 1602 (NH), 2339 (C≡N), 1679 (cyclic, C=O) cm^{-1}, molecular ion peak at *m/z* = 457 and ^{1}H NMR δ ppm: 7.2–7.82 (m, 15H, 3Ph), 8.10 (s, 1H, NH), as well as the disappearance of the methylene group.

The formation of (**4**) is assumed to proceed via the formation of the intermediate (**3**) which underwent smooth cyclisation to the corresponding fused pyridothienopyridazine.

Moreover independent chemical proof for compound (**4**) seemed necessary. Thus, the reaction of 7-cyano-3, 4, 8-triphenylpyrido[2', 3':4, 5]thieno[2, 3-*c*]pyridazin-6(5H)-one (**4**) with phosphorus oxy-chloride afforded the oxygen free compound which was identified to be the chloro derivative (**5**).

The chlorine atom of compound (**5**) showed the expected reactivity towards nucleophilic reagents. Treatment of 6-chloro derivative (**5**) with various nucleophiles such as alkoxide ions, amines and hydrazine led to normal halide displacement to the other 6-substituted derivatives (Scheme 2).

The reaction of compound (**5**) with alkoxide such as sodium methoxide and or sodium ethoxide afforded 6-methoxy (**6a**) and 6-ethoxy (**6b**) derivatives respectively. Moreover aniline and secondary amines such as diethyl amine, pyrrolidine,



Scheme 1

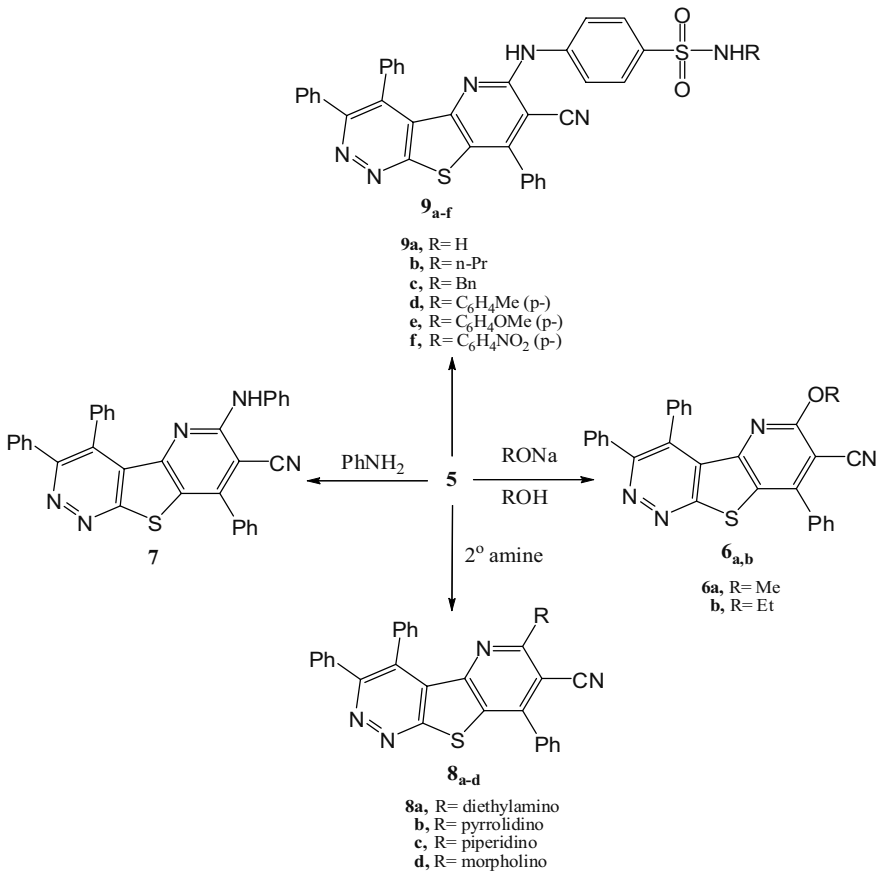
piperidine, and morpholine reacted with (**5**) in refluxing benzene to furnish 6-substituted aminopyridothienopyridazine derivatives (**7**) and (**8a–d**) respectively.

The investigations were extended to include the behaviour of (**5**) towards aromatic amines containing sulfonamide moiety in the *para* positions for antimicrobial screening. Thus, treatment of (**5**) with N^1-(un) substituted 4-aminobenzenesulfonamides under reflux in benzene yielded the corresponding 6-substituted aminopyridothienopyridazines (**9a–f**) (Scheme 2). The structure of the synthesised compounds was established on the basis of their IR, mass and ^{1}H NMR spectral studies.

To expand the use of chloro derivative (**5**), when reacted with ethanolic thiouronium salt in 85% yield was obtained which, on hydrolysis with 2.5 N sodium hydroxide followed by acidification with hydrochloric acid (pH 6), gave 7-cyano-3, 4, 8-triphenylpyrido[2', 3':4, 5]thieno[2, 3-*c*]pyridazine-6(5H)-thione (**10**).The structure of (**10**) was determined from micro analytical and spectroscopic data.

Furthermore, treatment of (**5**) with hydrazine hydrate in refluxing ethanol resulted in the formation of novel aminopyrazolopyridothienopyridazine derivative (**11**) in high yield. The structure of (**11**) was established on the basis of analytical and spectral data. Its IR spectrum revealed the absence of cyano group and the presence of amino group. Also, the mass spectrum showed a molecular ion

* Correspondent. E-mail: fatma_elmariah@yahoo.com

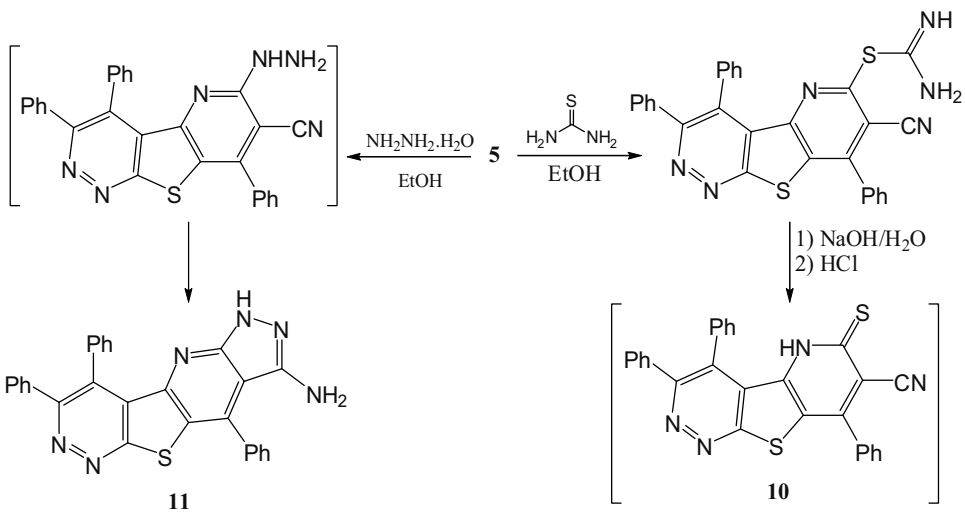


Scheme 2

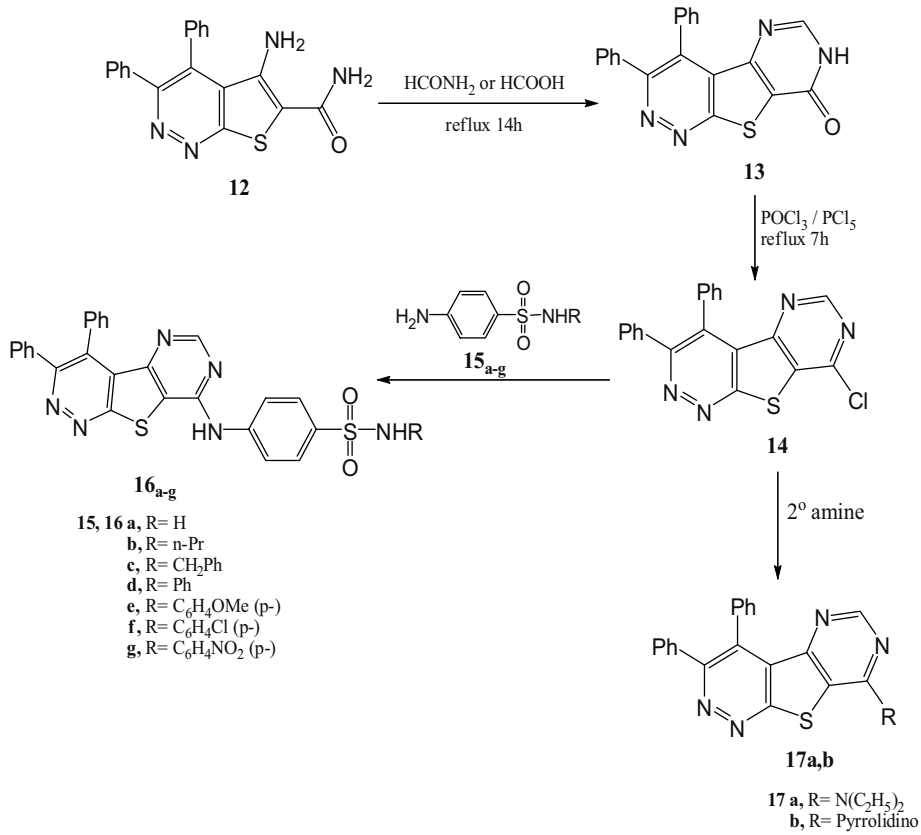
peak at *m/z* = 472. The formation of (**11**) is assumed to proceed via the formation of intermediate followed by intramolecular cyclisation of amino group to the cyano group (Scheme 3).

In the second track study, the pyrimido[4', 5':4, 5]thieno [2, 3-*c*]pyridazine derivative (**13**) was prepared according to previously described procedure.[9,10] Starting from 5-amino-3, 4-diphenylthieno[2, 3-*c*]pyridazine-6-carboxamide (**12**) and formamide or formic acid. Upon treatment with phosphorus oxychloride, (**13**) afforded the 8-chloro derivative (**14**),[10] which exhibited a remarkable reactivity of its 8-chloro substituent towards nucleophilic agents, thus affording the new 8-substituted pyrimido[4', 5':4, 5]-thieno[2, 3-*c*] pyridazine derivatives (**16a–g**) and (**17a,b**) (Scheme 4).

The N^1-(un)substituted 4-aminobenzenesulfonamides (**15**) were prepared using a previously described method.[7,11] The nucleophilic substitution of the 8-chloro group of compound (**14**) yielded the corresponding 8-substituted aminopyrimido [4', 5':4, 5]thieno-[2, 3-*c*]pyridazine derivatives (**16a–g**) and (**17a,b**). The structure of the synthesised compounds was established on the basis of their IR, MS and ^{1}H NMR spectral studies. Compound (**16**) and (**17**) showed a characteristic singlet between δ 7.36 ppm and δ 9.86 ppm for H-6 in the ^{1}H NMR spectra.



Scheme 3



Scheme 4

Screening for antimicrobial activities

Applying the agar plate diffusion technique[12] the newly synthesised compounds were screened *in vitro* for antimicrobial activity against Gram positive bacteria *(Staphylococcus aureus, Bacillus subtilis),* Gram negative bacteria *(Escherichia coli, Pseudomonas aeruginosa)*, yeast *(Candida albicans)* and fungi *(Aspergillus niger)*. In this method a standard 5 mm sterilised filter paper disc impregnated with the compound (0.3 mg/ 0.1 mL of dimethyl formamide) was placed on an agar plate seeded with the test organism. The plates were incubated for 24 h at 37 °C for bacteria and 28 °C for fungi. The inhibition zone of bacterial and fungal growth around the disc was determined. The screening results are given in (Table 1).

The antimicrobial activity of the compounds against examined Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* varied from one compound to another. Six synthesised compounds showed high antimicrobial activity against one or both Gram positive examined bacteria *Staphylococcus aureus* (compounds **5, 6a, 6b, 9b, 9c** and **9f**) and/or *Bacillus subtilis* (compounds **6a, 9b, 9f** and **16f**), whereas 16 compounds showed moderate antimicrobial activity against one/or both examined Gram positive bacteria. On the other hand, 15 compounds showed moderate antimicrobial activity against one or both examined gram negative bacteria *Escherichia coli* and/or *Pseudomonas aeruginosa***.** Only four synthesised compounds showed antimicrobial activity against both Gram negative examined bacteria (compounds **5, 6b, 9b** and **9f**). It is clear from this results that four compounds showed high or moderate antibacterial activity against both Gram positive and Gram negative bacteria (compound **5, 6b, 9b** and **9f**).

Furthermore, (**6b**) and (**11**) synthesised compounds showed very high antifungal activity against both examined fungi *Candida albicans* (yeast) and *Aspergillus niger*. Only seven compounds showed very high or high antifungal activity against the both examined fungi *Candida albicans* and *Aspergillus niger* (compounds **5, 6a, 6b, 8a, 8b, 9c** and **11**). In addition, compound **10** showed very high antifungal activity against one of examined fungi.

It should be mentioned here that three compounds (**5, 6b** and **9f**) showed very high, high or moderate antimicrobial activity against all examined bacteria and fungi.

In conclusion, results of antimicrobial activity revealed that synthesised compounds showed high and/or very high antimicrobial activity against bacteria and fungi respectively. It could be concluded from these results that the biologically active compounds are nearly as active as standard antibacteria Ciprofloxacin against the tested both Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa***.** On the other hand, the biologically active of synthesised compounds are active as standard Fungicide Nystin against the both tested fungi *Candida albicans* and *Aspergillus niger*.

Experimental

Melting points were determined in open glass capillaries and are uncorrected. Elemental analyses were carried out in the micro analytical laboratory of the Faculty of Science, Cairo University. The IR spectra of compounds were recorded on a Perkin-Elmer spectrophotometer model 1430 as potassium bromide pellets and frequencies are reported in cm^{-1}. The ^1H NMR spectra were recorded on a Perkin-Elmer R12B spectrometer 200 MHz and chemical shifts δ are in ppm relative to internal TMS, and mass spectra were recorded on a mass spectrometer HP model MS 5988 El 70ev. Reactions were routinely followed by thin layer chromatography (TLC) on silica gel; F_{254} aluminum sheets (Merck). The spots were detected by UV irradiation at 254–365 nm.

Compounds (**1**, **2**, **12**, **13**, **14** and **15**) were prepared by the reported methods.[7,8,9,10,11]

7-Cyano-3,4,8-triphenylpyrido[2',3': 4,5]thieno[2,3-c]pyridazin-6(5H)-one (**4**): To a solution of compound (**2**) (0.48 g, 1.0 mmol) in

Table 1 Antibacterial and antifungal activity

Sample no.	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
4	-	+ +	+ +	-	+ + + +	+ +
5	+ + +	+ +	+ +	+ +	+ + + +	+ + +
6a	+ + +	+ + +	+ +	-	+ + +	+ + +
6b	+ + +	+ +	+ +	+ +	+ + + +	+ + + +
8a	+ +	-	+ +	-	+ + +	+ + +
8b	+ +	+ +	+ +	-	+ + +	+ + + +
8c	+ +	+ +	+ +	-	+ + +	+ +
8d	-	+ +	-	-	+ + +	-
9a	-	-	-	-	+ + +	+ +
9b	+ + +	+ + +	+ +	+ +	+ + +	-
9c	+ + +	+ +	-	-	+ + + +	+ + +
9d	+ +	+ +	-	+ +	+ + + +	-
9e	+ +	+ +	+ +	-	+ + +	-
9f	+ + +	+ + +	+ +	+ +	+ + +	+ +
10	+ +	+ +	+ +	-	+ + + +	-
11	+ +	+	+ +	+	+ + + +	+ + + +
16a	+	-	-	+ +	-	+ +
16b	+ +	+	-	-	-	+ +
16c	-	-	-	-	-	+ +
16d	+ +	+ +	+ +	-	+ +	+ + +
16e	+	-	-	-	-	+ +
16f	+	+ + +	-	-	+ + +	+ +
16g	+	+	-	-	-	+
17a	+ +	-	-	-	-	+ +
17b	+ +	-	-	-	-	+ +
Ciprofloxacin	+ + + +	+ + + +	+ + + +	+ + + +	-	-
Fungicide Nystin	_	_	_	_	**+ + + +**	**+ + + +**

The concentration of all synthesised compounds and two references compounds was 0.30 mg/0.10 mL of dimethyl formamide. Zone of inhibition: + = < 15 mm; + + = 15 – 24 mm; + + + = 25 – 34 mm; + + + + = 35 – 44 mm; – = no inhibition.

ethanol (20 mL), sodium cyanide (0.074 g, 1.5 mmol) was added. The reaction mixture was heated under reflux for 3 h, and then the solvent was concentrated. The solid product was collected, dried and recrystallised from ethanol to give 0.35 g (73%) of **4** as red crystals, m.p. 164–165 °C; IR(cm^{-1}): 3423, 1602 (NH), 2339 (C≡N), 1679 (cyclic C=O) cm^{-1}; MS (*m/z*%): 457 ($M^{+,}$ 4), 431 (4), 403 (6), 326 (8), 286 (7), 226 (100), 231 (5), 171 (6), 93 (17), 81 (14); ^{1}H NMR (DMSO – d_6) δ ppm: 7.20–7.82 (m, 15H, 3Ph), 8.1 (s, 1H, NH). Anal. Calcd for $C_{28}H_{16}N_4OS$: C, 73.66; H, 3.53; N, 12.27. Found: C, 73.40; H, 3.60; N, 12.50%.

6-Chloro-7-cyano-3,4,8-triphenylpyrido[2',3': 4,5]thieno[2,3-c] pyridazine (**5**): A mixture of 7-cyano-3, 4, 8-triphenylpyrido[2', 3' : 4, 5] thieno[2, 3-*c*] pyridazin-6(5H)-one (**4**) (0.46 g, 1.0 mmol) in excess phosphorus oxychloride (20 mL) was heated for 5 h. The excess phosphorus oxychloride was evaporated under reduced pressure, ice-cold water was added. The separated solid product was filtered off, washed with water, dried and recrystallised from ethanol to give 0.38 g (83%) of **5** as pale brown crystals, m.p. 152–153 °C. IR(cm^{-1}): 2229 (C≡N), 1599 (C=N) cm^{-1}; MS (*m/z*%): 475 (M^+, 3), 477(M^+ + 2, 5), 394 (4), 368 (6), 286 (6), 185 (15), 109 (18), 96 (51), 89 (5), 84 (40), 77 (36), 57 (100). Anal. Calcd for $C_{28}H_{15}ClN_4S$: C, 70.80; H, 3.18; N, 11.80. Found: C, 71.10; H, 3.30; N, 11.50%.

6-Substituted derivatives **6a**, **b**; *general procedure*
A mixture of 6-chloro-7-cyano-3, 4, 8-triphenylpyrido[2', 3' : 4, 5] thieno[2, 3-*c*]pyridazine (**5**) (1.0 mmol) and sodium metal (1.5 mmol) in dry methanol, absolute ethanol (10 mL) respectively was refluxed for 3 h. The excess solvent was evaporated and water was added. The separated solid product was filtered off, washed with water, dried and recrystallised from ethanol to give (**6a**, **b**) respectively.

7-Cyano-6-methoxy-3,4,8-triphenylpyrido[2',3': 4,5]thieno[2,3-c] pyridazine (**6a**)**:** Yield (0.38 g, 81%), m.p. 158–159 °C; IR(cm^{-1}): 2193 (C≡N), 1593 (C=N) $cm^{-1;}$ MS (*m/z*%): 470 (M^+, 0.2), 393 (0.6), 286 (1), 184 (0.70), 139 (11), 127 (11), 101(6), 95 (29), 89 (8), 77 (49), 69 (54), 57(100). Anal. Calcd for $C_{29}H_{18}N_4OS$: C, 74.02; H, 3.86; N, 11.91. Found: C, 74.30; H, 4.00; N, 11.70%.

7-Cyano-6-ethoxy-3,4,8-triphenylpyrido[2',3': 4,5]thieno[2,3-c] pyridazine (**6b**): Yield (0.40 g, 83%), m.p. 140–141 °C; IR(cm^{-1}): 2193 (C≡N), 1595 (C=N) $cm^{-1;}$ MS (*m/z*%): 484 (M^+, 5), 458 (10), 401 (10), 363 (10), 286 (7), 231 (100), 198 (34), 127 (18), 101 (11), 89 (13), 77 (17); ^{1}H NMR (DMSO – d_6) δ ppm: 1.22 (t, 3H, CH_3), 3.79 (q, 2H, CH_2), 7.04–7.98 (m, 15H, 3Ph). Anal. Calcd for $C_{30}H_{20}N_4OS$: C, 74.36; H, 4.16; N, 11.56. Found: C, 74.60; H, 4.30; N, 11.80%.

6-Substituted aminopyridothienopyridazine derivatives (**7**) *and* (**8a-d**)**;** *general procedure*
A mixture of 6-chloro-7-cyano-3, 4, 8-triphenylpyrido[2', 3' : 4, 5] thieno[2, 3-*c*]pyridazine (**5**) (1.0 mmol) aniline, and the secondary amines, namely diethyl amine, pyrrolidine, piperidine and morpholine respectively (1.0 mmol) were refluxed in benzene (20 mL) for 5 h, then the solvent was concentrated. The solid product was collected, dried and recrystallised from benzene to give (**7**) and (**8a–d**)**.**

6-Anilino-7-cyano-3,4,8-triphenylpyrido[2',3': 4,5]thieno[2,3-c] pyridazine (**7**): Yield (0.49 g, 92%), m.p. 148–150 °C; IR(cm^{-1}): 3387 (NH), 2126 (C≡N), 1583 (C=N) cm^{-1}; MS (*m/z*%): 533 (M^+ + 1, 11), 446 (18), 414 (13), 275 (21), 169 (28), 128 (15), 92 (100), 77 (25); ^{1}H NMR (DMSO – d6) δ ppm: 3.98 (s, 1H, NH), 6.63–7.07 (m, 5H, Ph-N), 7.14–7.95 (m, 15H, 3Ph). Anal. Calcd for $C_{34}H_{21}N_5S$: C, 76.81; H, 3.98; N, 13.18. Found: C, 76.60; H, 4.10; N, 12.90%.

7-Cyano-6-diethylamino-3,4,8-triphenylpyrido[2',3': 4,5]thieno [2,3-c]pyridazine (**8a**): Yield (0.46 g, 90%), m.p. 96–97 °C; IR(cm^{-1}): 2228 (C≡N), 1661 (C=N), 1341 (3°-amine) cm^{-1}; MS (*m/z*%): 511 (M^+, 3), 408 (11), 375 (10), 286 (7), 225 (5), 77 (100), 72 (34); ^{1}H NMR (DMSO – d_6) δ ppm: 1.16 (t, 3H, CH_3), 3.52 (q, 2H, CH_2), 7.27–7.92 (m, 15H, 3Ph). Anal. Calcd for $C_{32}H_{25}N_5S$: C, 75.12; H, 4.93; N, 13.69. Found: C, 75.40; H, 4.80; N, 13.90%.

7-Cyano-6-pyrrolidino-3,4,8-triphenylpyrido[2',3': 4,5]thieno [2,3-c]pyridazine (**8b**): Yield (0.43 g, 85%), m.p. 146–147 °C; IR(cm^{-1}): 2218 (C≡N), 1634 (C=N), 1339 (3°-amine) cm^{-1}; MS (*m/z*%): 509 (M^+, 2), 483 (8), 363 (28), 286 (3), 134 (12), 101 (8), 89 (100). Anal. Calcd for $C_{32}H_{23}N_5S$: C, 75.42; H, 4.55; N, 13.74. Found: C, 75.70; H, 4.70; N, 13.50%.

7-Cyano-6-piperidino-3,4,8-triphenylpyrido[2',3': 4,5]thieno [2,3-c]pyridazine (**8c**): Yield (0.42 g, 80%), m.p. 117–118 °C; IR(cm^{-1}): 2229 (C≡N), 1660 (C=N), 1378 (3°-amine) cm^{-1}; MS (*m/z*%): 523 (M^+, 8), 408(11), 375 (39), 300 (5), 237 (2), 84 (100). Anal. Calcd for $C_{33}H_{25}N_5S$: C, 75.69; H, 4.81; N, 13.38. Found: C, 75.40; H, 4.90; N, 13.10%.

7-Cyano-6-morpholino-3,4,8-triphenylpyrido[2',3': 4,5]thieno [2,3-c]pyridazine (**8d**): Yield (0.43 g, 82%), m.p. 82–83 °C; IR(cm^{-1}): 2228 (C≡N), 1654 (C=N), 1341 (3°-amine) cm^{-1}; MS (*m/z*%): 525 (M^+, 0.4), 448 (4), 375 (20), 286 (5), 139 (8), 127 (12), 89 (100). Anal. Calcd for $C_{32}H_{23}N_5OS$: C, 73.12; H, 4.41; N, 13.33. Found: C, 73.40; H, 4.50; N, 13.60%.

6-Substituted aminopyridothienopyridazine derivatives **9a–f**; *general procedure*

A mixture of 6-chloro-7-cyano-3, 4, 8-triphenylpyrido[2', 3':4, 5] thieno[2, 3-*c*]pyridazine (**5**) (1.0 mmol) and 4-aminobenzenesulfonamide derivatives (**15a–f**) (1.0 mmol) was refluxed in benzene (20 mL) for 5 h. The reaction mixture was cooled to room temperature and the separated solid was filtered off, washed with water, dried, and recrystallised from ethanol to give (**9a–f**)**.**

4-[(7-Cyano-3,4,8-triphenylpyrido[2',3': 4,5]thieno[2,3-c] pyridazin-6-yl)amino]benzenesulfonamide (**9a**): Yield (0.55 g, 90%), m.p. 158–159 °C; IR(cm^{-1}): 3369, 3294 (NH, SO_2NH_2), 2251 (C≡N), 1658 (C=N), 1329 (SO_2, asym), 1156 (SO_2, sym) cm^{-1}; MS (*m/z*%): 611 (M^+, 2), 508 (7), 407 (100), 376 (50), 101 (47). Anal. Calcd for $C_{34}H_{22}N_6O_2S_2$: C, 66.87; H, 3.63; N, 13.76. Found: C, 67.10; H, 3.80; N, 13.50%.

N-Propyl-4-[(7-cyano-3,4,8-triphenylpyrido[2',3': 4,5]thieno[2,3-c] pyridazin-6-yl)amino]benzenesulfonamide (**9b**): Yield (0.57 g, 88%), m.p. 122–123 °C; IR(cm^{-1}): 3380, 3201 (NH, SO_2NHR), 2229 (C≡N), 1670 (C=N), 1336 (SO_2, asym), 1161 (SO_2, sym) cm^{-1}; MS (*m/z*%): 652 (M^+, 0.42), 439 (30), 366 (73), 334 (100), 58 (40); ^{1}H NMR (DMSO – d6) δ ppm: 0.6–0.8 (t, 3H, CH3), 0.95–1.51 (m, 2H, CH_2CH_3), 2.49–2.82 (m, 2H, $NHCH_2$), 2.77–2.95 (t, 1H, NH), 3.48 (s, 1H, NH), 6.63–7.12 (m, 4H, Ar), 7.15–7.95 (m, 15H, 3Ph). Anal. Calcd for $C_{37}H_{28}N_6O_2S_2$: C, 68.08; H, 4.32; N, 12.88. Found: C, 68.30; H, 4.20; N, 13.10%.

N-Benzyl-4-[(7-cyano-3,4,8-triphenylpyrido[2',3': 4,5]thieno[2,3-c] pyridazin-6-yl)amino]benzenesulfonamide (**9c**): Yield (0.60 g, 85%), m.p. 92–93 °C; IR(cm^{-1}): 3316, 3213 (NH, SO_2NHR), 2240 (C≡N), 1678 (C=N), 1313 (SO_2, asym), 1153 (SO_2, sym) cm^{-1}; MS (*m/z*%): 702 (M^+ + 1, 47), 624 (62), 598 (43), 338 (100), 286 (43), 184 (41), 169 (46); ^{1}H NMR (DMSO – d6) δ ppm: 3.98–4.04 (d, 2H, CH_2), 2.49–2.83 (t, 1H, NH), 4.0 (s, 1H, SO_2NH), 6.62–6.65 (m, 4H, Ar), 7.22–7.94 (m, 20H, 4Ph). Anal. Calcd for $C_{41}H_{28}N_6O_2S_2$: C, 70.26; H, 4.03; N, 11.99. Found: C, 70.00; H, 4.20; N, 11.70%.

N-(4-Methylphenyl)-4-[(7-cyano-3,4,8-triphenylpyrido[2',3': 4,5] thieno[2,3-c]pyridazin-6-yl)amino]benzenesulfonamide (**9d**): Yield (0.56 g, 80%), m.p. 142–143 °C; IR(cm^{-1}): 3313, 3202 (NH, SO_2NHR), 2233 (C≡N), 1681(C=N), 1324 (SO_2, asym), 1151 (SO_2, sym) cm^{-1}; MS (*m/z*%): 700 (M^+, 0.01), 439 (2), 375 (19), 359 (100), 101 (12). Anal. Calcd for $C_{41}H_{28}N_6O_2S_2$: C, 70.26; H, 4.03; N, 11.99. Found: C, 70.50; H, 3.90; N, 12.30%.

N-(4-Methoxyphenyl)-4-[(7-cyano-3,4,8-triphenylpyrido[2',3': 4,5] thieno[2,3-c]pyridazin-6-yl)amino]benzenesulfonamide (**9e**): Yield (0.61 g, 85%), m.p. 120–121 °C; IR(cm^{-1}): 3372, 3211 (NH, SO_2NHR), 2232 (C≡N), 1670 (C=N), 1321 (SO_2, asym), 1152 (SO_2, sym) cm^{-1}; MS (*m/z*%): 716 (M^+, 2), 627 (3), 589 (2), 439 (83), 375 (100), 336 (26); ^{1}H NMR (DMSO – d6) δ ppm: 2.50 (s, 1H, NH), 2.76 (s, 1H, SO_2NH), 3.62 (s, 3H, CH_3), 6.51–6.78 (m, 4H, Ar), 6.93–7.09 (m, 4H, Ar-SO_2), 7.11–7.96 (m, 15H, 3Ph). Anal. Calcd for $C_{41}H_{28}N_6O_3S_2$: C, 68.70; H, 3.94; N, 11.73. Found: C, 68.40; H, 3.80; N, 11.50%.

N-(4-Nitrophenyl)-4-[(7-cyano-3,4,8-triphenylpyrido[2',3': 4,5] thieno[2,3-c]pyridazin-6-yl)amino]benzenesulfonamide (**9f**): Yield (0.47 g, 65%), m.p. 146–147 °C; IR(cm^{-1}): 3315, 3199 (NH, SO_2NHR), 2222 (C≡N), 1675 (C=N), 1523 (NO_2, asym), 1392 (NO_2, sym), 1340 (SO_2, asym), 1153 (SO_2, sym) cm^{-1}; MS (*m/z*%): 732 (M^+, 0.1), 629 (3), 446 (5), 357 (34), 102 (61), 90 (54), 64 (43), 77 (100). Anal. Calcd for $C_{40}H_{25}N_7O_4S_2$: C, 65.65; H, 3.44; N, 13.40. Found: C, 65.90; H, 3.30; N, 13.10%.

7-Cyano-3,4,8-triphenylpyrido[2',3': 4,5]thieno[2,3-c]pyridazin-6(5H)-thione (**10**): A mixture of 6-chloro-7-cyano-3, 4, 8-triphenylpyrido[2', 3':4, 5]thieno[2, 3-*c*]pyridazin (**5**) (1 g) and thiourea (1 g) in ethyl alcohol (20 mL) was refluxed for 2 h. The solvent was evaporated and the residue was dissolved in 2.5N sodium hydroxide solution followed by refluxing for half an hour. The solution was filtered while on hot, allowed to cool and acidified with hydrochloric acid (pH 6). The separated solid product was filtered off, washed with water, dried and recrystallised from ethanol to give 0.85 g (85%) of (**10**)**,** m.p. 145–146 °C as yellow crystals; IR(cm^{-1}): 2225 (C≡N), 1647 (C=N), 697 (strong band, C–S) cm^{-1}; MS (*m/z*%): 473 (M^+, 1), 265 (2), 254 (4), 187 (1), 173 (2), 140 (1), 128 (4), 110 (2), 98 (3), 90 (1), 77 (3), 64 (100). Anal. Calcd for $C_{28}H_{16}N_4S_2$: C, 71.16; H, 3.41; N, 11.86; Found: C, 71.40; H, 3.50; N, 11.60%.

3-Amino-4,8,9-triphenyl-1H-pyrazolo[3',4': 2,3]pyrido[5',6': 5,4] thieno[2,3-c]pyridazin (**11**): A mixture of compound (**5**) (0.48 g, 1.0 mmol) and hydrazine hydrate (0.05 g, 99%, 1.0 mmol) in *n*-butanol (10 mL) was refluxed for 3 h. The solvent was removed under reduced pressure. The solid product was collected and recrystallised from ethanol to give 0.40 g (76%) of (**11**), m.p. 116–117 °C as brown crystals; IR(cm^{-1}): 3588, 3444(NH_2), 3322(NH), 1611 (C=N), 1364 (3°-amine) cm^{-1}; MS (*m/z*%): 472 (M^+ + 1, 0.03), 383 (0.43), 286 (100), 254 (3), 230 (9), 202 (6), 190 (16), 103 (31), 87 (13), 77 (54). Anal. Calcd for $C_{28}H_{18}N_6S$: C, 71.47; H, 3.86; N, 17.86. Found: C, 71.70; H, 3.90; N, 17.60%.

8-Substituted 3,4-diphenylpyrimido[4',5': 4,5]thieno[2,3-c]pyridazins (**16a–g**) *and* (**17a,b**); *general procedure*

A mixture of 8-chloro-3, 4-diphenylpyrimido[4', 5':4, 5]thieno[2, 3-*c*] pyridazin (**14**) (1.0 mmol) and 4-aminobenzenesulfonamide derivatives (**15a–g**) and 2°- amine (diethyl amine and pyrrolidine) (1.0 mmol) was refluxed in *n*-butanol (10 mL) or ethanol/THF (10 mL 1:4 v/v) for 3 h. The reaction mixture was cooled to room temperature. The separated solid was filtered off, washed with water, dried and recrystallised from ethanol.

4-[(3,4-Diphenylpyrimido[4',5': 4,5]thieno[2,3-c]pyridazin-8-yl) amino]benzenesulfonamide (**16a**): Yield (0.43 g, 85%), m.p. 190–191 °C; IR(cm^{-1}): 3369, 3293 (NH, SO_2NH_2), 1656 (C=N), 1330 (SO2, asym), 1155 (SO2, sym) cm^{-1}; MS (*m/z*%): 512 (M^+ + 1, 0.03), 286 (30), 242 (14), 172 (100), 155 (57), 101 (45), 91 (54), 64 (50), 76 (30). Anal. Calcd for $C_{26}H_{18}N_6O_2S_2$: C, 61.16; H, 3.55; N, 16.46. Found: C, 61.40; H, 3.50; N, 16.20%.

N-Propyl-4-[(3,4-diphenylpyrimido[4',5': 4,5]thieno[2,3-c] pyridazin-8-yl)amino]benzenesulfonamide (**16b**): Yield (0.50 g, 90%), m.p. 154–155 °C; IR(cm^{-1}): 3436, 3363 (NH, SO_2NHR), 1596 (C=N), 1338 (SO_2, asym), 1157 (SO_2, sym) cm^{-1}; MS (*m/z*%): 555 (M^+ + 2, 4), 286 (7), 242 (11), 213 (8), 170 (1), 64 (100); ^{1}H NMR (DMSO – d_6) δ ppm: 1.09 (t, 3H, CH_3), 1.52 (m, 2H, CH_2), 2.78 (s, 1H, NH), 3.37 (m, 2H, CH_2), 4.45 (t, 1H, NH), 6.26–7.32 (m, 14H, ArH), 7.36 (s, 6-H, 1H). Anal. Calcd for $C_{29}H_{24}N_6O_2S_2$: C, 63.02; H, 4.38; N, 15.21. Found: C, 63.30; H, 4.20; N, 15.50%.

N-Benzyl-4-[(3,4-diphenylpyrimido[4',5': 4,5]thieno[2,3-c] pyridazin-8-yl)amino]benzenesulfonamide (**16c**): Yield (0.49 g, 82%), m.p. 133–134 °C; IR(cm^{-1}): 3369, 3261 (NH, SO_2NHR), 1596 (C=N), 1361 (SO_2, asym), 1157 (SO_2, sym) cm^{-1}; MS (*m/z*%): 600 (M^+, 0.10), 532 (0.1), 300 (1), 254 (1), 101 (1), 77 (100), 53 (7); ^{1}H NMR (DMSO – d_6) δ ppm: 3.38 (t, 1H, NH), 3.88 (s, 1H, NH), 4.34 (d, 2H, CH_2), 6.27–7.60 (m, 19H, ArH), 7.65(s, 6-H, 1H). Anal. Calcd for $C_{33}H_{24}N_6O_2S_2$: C, 65.98; H, 4.03; N, 13.99. Found: C, 65.70; H, 4.20; N, 13.70%.

N-Phenyl-4-[(3,4-diphenylpyrimido[4',5': 4,5]thieno[2,3-c] pyridazin-8-yl)amino]benzenesulfonamide (**16d**): Yield (0.50 g, 85%), m.p. 172–173 °C; IR(cm^{-1}): 3349, 3241 (NH, SO_2NHR), 1594 (C=N), 1311 (SO_2, asym), 1153 (SO_2, sym) cm^{-1}; MS (*m/z*%): 587 (M^+, 0.42), 531 (4), 248 (34), 156 (68), 92 (69), 77 (26), 64 (56), 60 (100); ^{1}H NMR (DMSO – d_6) δ ppm:4.78 (s, br, 1H, NH), 5.02 (s, 1H, NH), 6.52–7.36 (m, 19H, ArH), 9.86 (s, 6-H, 1H). Anal. Calcd for $C_{32}H_{22}N_6O_2S_2$: C, 65.51; H, 3.78; N, 14.33. Found: C, 65.80; H, 3.70; N, 14.60%.

N-(4-Methoxyphenyl)-4-[(3,4-diphenylpyrimido[4',5': 4,5]thieno [2,3-c]pyridazin-8-yl)amino]benzenesulfonamide (**16e**): Yield (0.50 g, 80%), m.p. 176–177 °C; IR(cm^{-1}): 3282, 3264 (NH, SO_2NHR), 1594 (C=N), 1308 (SO_2, asym), 1149 (SO_2, sym) cm^{-1}; MS (*m/z*%): 616 (M^+, 0), 277 (33), 186 (0.10), 170 (0.30), 122 (100), 107 (7), 91 (8), 64 (13), 52 (7); ^{1}H NMR (DMSO – d_6) δ ppm: 3.66 (s, 3H, CH_3), 5.73 (s, br, 1H, NH), 6.50–7.36 (m, 18H, ArH), 9.46 (s, 6-H, 1H). Anal. Calcd for $C_{33}H_{24}N_6O_3S_2$: C, 64.27; H, 3.92; N, 13.63. Found: C, 64.00; H, 3.80; N, 13.90%.

N-(4-Chlorophenyl)-4-[(3,4-diphenylpyrimido[4',5': 4,5]thieno [2,3-c]pyridazin-8-yl)amino]benzenesulfonamide (**16f**): Yield (0.47 g, 75%), m.p. 168–169 °C; IR(cm^{-1}): 3345, 3056 (NH, SO_2NHR), 1594 (C=N), 1317 (SO_2, asym), 1153 (SO_2, sym) cm^{-1}; MS (*m/z*%): 621 (M^+, 0.1), 623 (M^+ + 2, 0.1), 281 (21), 155 (67), 126 (45), 64 (34), 91 (46), 77 (100). Anal. Calcd for $C_{32}H_{21}ClN_6O_2S_2$: C, 61.88; H, 3.41; N, 13.53. Found: C, 61.60; H, 3.50; N, 13.30%.

N-(4-Nitrophenyl)-4-[(3,4-diphenylpyrimido[4',5': 4,5]thieno [2,3-c]pyridazin-8-yl)amino]benzenesulfonamide (**16g**): Yield (0.44 g, 70%), m.p. 200–201 °C; IR(cm^{-1}): 3390, 3100 (NH, SO_2NHR), 1594 (C=N), 1527 (NO_2, asym), 1400 (NO_2, sym), 1344 (SO_2, asym), 1157 (SO_2, sym) cm^{-1}; MS (*m/z*%): 633 (M^+ + 1, 0.1), 478 (0.60), 346 (6), 210 (4), 201 (2), 170 (4), 146 (2), 103 (100), 85 (3), 69 (11), 56 (11). Anal. Calcd for $C_{32}H_{21}N_7O_4S_2$: C, 60.84; H, 3.35; N, 15.52. Found: C, 61.10; H, 3.20; N, 15.80%.

8-Diethylamino-3,4-diphenylpyrimido[4',5': 4,5]thieno[2,3-c] pyridazin (**17a**): Yield (0.33 g, 80%), m.p. 146–147 °C; IR(cm^{-1}): 1598 (C=N), 1375 (3°-amine) cm^{-1}; MS (*m/z*%): 411 (M^+, 8), 382 (10), 339 (4), 300 (29), 289 (91), 237 (11), 127 (16), 98 (26), 72 (42), 58 (100); ^{1}H NMR (DMSO – d6) δ ppm: 1.18 (t, 3H, $CH_2\underline{CH_3}$), 2.84 (q, 2H, $\underline{CH_2}CH_3$), 6.91–7.29 (m, 10H, 2Ph), 7.40 (s, 6-H, 1H). Anal.

Calcd for $C_{24}H_{21}N_5S$: C, 70.04; H, 5.14; N, 17.02. Found: C, 69.80; H, 5.00; N, 17.30%.

3,4-Diphenyl-8-(*pyrrolidin-1-yl*)*pyrimido[4',5' : 4,5]thieno[2,3-c] pyridazin* (**17b**): Yield (0.31 g, 75%), m.p. 300–302 °C; IR(cm^{-1}): 1590 (C=N), 1378 (3°-amine) cm^{-1}; MS (*m/z*%): 409 (M^+, 23), 339 (3), 327 (11), 300 (38), 286 (5), 173 (4), 159 (2), 146 (100), 132 (4), 109 (7), 96 (13); ^{1}H NMR (DMSO – d6) δ ppm: 1.28 (t, 4H, $2CH_2$, β-pyrrolidine protons), 1.81 (t, 4H, $2CH_2$, α-pyrrolidine protons), 7.07–7.26 (m, 10H, 2Ph), 7.70 (s, 6-H, 1H). Anal. Calcd for $C_{24}H_{19}N_5S$: C, 70.39; H, 4.68; N, 17.10. Found: C, 70.60; H, 4.50; N, 16.80%.

The author acknowledges the help of Quality Control and Propagation of Plants, Department of Botany, Faculty of Girls, Ain Shams University for carrying out the antimicrobial activity.

Received 23 June 2009; accepted 11 August 2009
Paper 09/0652 doi: 10.3184/030823409X12508790019612
Published online: 8 October 2009

References

1 J. Sauer, *Comprehensive, heterocyclic chemistry II*; Pergamon, London, 1996, Vol. 6; pp 901.
2 J.A. Tucker, D.A. Allwine, K.C. Grega, M.R. Barbachyn, J.L. Klock, J.L. Adamski, S.J. Brickner, D.K. Hulchinson, C.W. Ford, G.E. Zurenko, R.A. Conradi, P.S. Burton and R.M. Jensen, *J. Med. Chem*., 1998, **41**, 3723.
3 G.P. Ellis, *Synthesis of fused feterocycles*; John Wiley and Sons Inc.: New York, 1987, pp. 226.
4 Z.X. Yu, Q. Dang and Y.D. Wu, *J. Org. Chem*., 2001, **66**, 6029.
5 A. Volonterio, L. Moisan and J. Rebec, *J. Org. Lett*., 2007, **9**, 3733.
6 R.S. Vartanyan, Zh. V. Kazaryan, M.A. Sheriranyan, A.S. Agaranyan and N.O. Stepanyan, *Khim, Farm., Zh*., 1996, **30**, 29.
7 F. El-Mariah, E. Nassar, M. Hosny and A. Deeb, *Phosphorus, Sulfur, Silicon,* 2009, **184**, 92.
8 F. El – Mariah, *Phosphorus, Sulfur, Silicon*, 2008, **183**, 2795.
9 A. Deeb, A. Essawy, F. Yasine and R. Fikry, *Z. Naturforsch*, 1991**, 46b**, 835.
10 J.M. Quintela, M.C. Veiga, R. Alvarez-Sarandes, L. González and C. Peinador, *Monatsh. Chem.*, 1996**, 127**, 1037.
11 *Vogel's textbook of practical organic chemistry*, Longman Group Limited, London, 1980, **4**th edn., pp. 651.
12 A.W. Bauer, W.W. Mkriby, J.C. Sherris and M. Turck, *Am. J. Clin. Pathol*., 1966, **45**, 493.