PYRIDAZINE AND ITS RELATED COMPOUNDS: PART 40. SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME NEW PYRIMIDINE FUSED WITH THIENOPYRIDAZINE DERIVATIVES

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In the present study we have investigated the behaviour of 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonitrile towards the hydrazine hydrate which gives 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carboximidohydrazide which in turn was subjected to subsequent reactions with benzaldehyde, acetic anhydride, acetyl acetone, formic acid, maleic anhydride and phthalic anhydride. The new synthesized compounds were confirmed by their IR spectra, mass spectrum, ^{1}H-NMR, and elemental analyses, and were screened for antimicrobial activity. Several compounds showed moderate to low activity against the examined microorganisms.

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Introduction

Literature survey revealed that pyridazine derivatives have diverse biological importance.[1,2] On the other hand, heterocyclic ring systems containing the thiophene ring fused to pyrimidine or pyridazine rings are interesting classes of compounds which are both chemically and biologically active, e.g., thienopyrimidines and thienopyridazines display significant chemical properties and exhibit a wide range of biological properties.[3-8] In view of these results and as an extension of our recent work concerned with the synthesis of heterocycles of interesting biological activity,[9] we decided to synthesis some new pyrimidothienopyridazine derivatives starting from the readily obtainable 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonytrile (**1**)[10] as a highly versatile and useful building block for the synthesis of a wide variety of pyrimido[4',5':4,5]thieno[2,3-c]pyridazine derivatives and to test their antimicrobial activity.

Experimental

Melting points were determined in open glass capillaries and are uncorrected. Elemental analyses (CHN) were carried out using a Perkin-Elmer 240 C Microanalyzer at the Microanalytical Laboratory, Cairo University. The IR spectra of compounds were recorded on a Perkin-Elmer spectrophotometer model 1430 as KBr pellets and frequencies are reported in cm^{-1}. The ^{1}H-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 70 ev. 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. **C**ompounds (**1**) and the corresponding carboximidohydrazide (**2**) were synthesized according to reported procedures.[10]

5-Amino-*N*'-benzylidene-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carboximidohydrazide (3)

To a solution of compound (**2**) (0.5 g, 1.39 mmol) in absolute ethanol (10 ml), benzaldehyde (0.15 g, 1.39 mmol) was added. The reaction mixture was refluxed for 10 h. The solvent was evaporated under reduced pressure, and the residue was washed with petroleum ether 40 - 60° C. the solid product was filtered off, dried, and recrystallized from ethanol to give (**3**). Yield: 0.31 g (49.82 %), m.p. 179 – 180 °C. IR: broad bands around 3378, 3168 (–NH_2, =NH, –NH), 1675 (C=N); MS (*m/z* %): 448 (M^+, 0.45 %), 178 (100 %). ^{1}H-NMR (DMSO-d_6): 8.76 (s, H, NH), 8.43 (s, 1H, N=CH), 7.94-7.31 (m, 15H, 3Ph), 5.69 (s, 1H, =NH), 4.56 (s, 2H, 5-NH_2). Anal Calcd. For $C_{26}H_{20}N_6S$: C, 69.62; H, 4.49; N, 18.74, Found C, 69.98; H, 4.57; N, 18.98 %.

7-Benzylideneamino-8-imino-6-methyl-3,4-diphenylpyrimido[4',5':4,5]thieno[2,3- *c*]pyridazine (4)

A solution of compound (**3**) (0.5 g, 1.12 mmol) in acetic anhydride (10 ml) was refluxed for 5 h. The solvent was evaporated under reduced pressure, and the solid product was recrystallized from acetic acid to give (**4**). Yield: 0.27 g (51.24 %), m.p. 184 – 185 °C. IR: 3422, 3168 (=NH), 2925(–CH_3), 1669 (C=N). MS (*m/z* %): 472 (M^+, 0.60 %), 327 (100). Anal Calcd. For $C_{28}H_{20}N_6S$: C 71.16, H 4.27, N 17.79, Found C, 71.49; H, 4.18; N, 17.51 %.

7-Amino-8-imino-3,4-diphenylpyrimido[4',5':4,5]thieno[2,3-*c*]-pyridazine (5)

Method A

A mixture of compound (**2**) (0.5 g, 1.39 mmol) and DMF (10 ml) was refluxed for 12 h. The reaction mixture was cooled to room temperature, and poured into ice water. The solid product was filtered off, washed with water, dried, and recrystallized from ethanol to give (**5**). Yield: 0.4 g (77.84 %), m.p. 230 – 231 °C. IR: 3300, 3157 (–NH_2, =NH), 1669 (C=N). MS (*m/z* %): 370 (M^+, 54.69 %), 77 (100 %). Anal: Calcd. For $C_{20}H_{14}N_6S$: C, 64.85; H, 3.81; N, 22.69; Found: C, 64.55; H, 3.73; N, 22.95 %.

Method B

A solution of compound (**3**) (0.5 g, 1.12 mmol) in DMF (10 ml) was refluxed for 8 h. The reaction mixture was cooled to room temperature, and then poured into ice water. The solid product was filtered off, washed with water, dried, and recrystallized from ethanol to give (**5**). It was identical with that prepared by method A (m.p and mixed m.p). Yield: 0.24 g (58.13 %).

7-Diacetylamino-8-imino-6-methyl-3,4-diphenylpyrimido-[4',5':4,5]thieno[2,3- *c*]pyridazine (6)

A solution of compound (**2**) (0.5 g, 1.39 mmol) in acetic anhydride (10 ml) was refluxed for 5 h. The solvent was evaporated under reduced pressure, and the solid product was recrystallized from acetic acid to give (**6**). Yield: 0.42 g (64.62 %). m.p. 180-182 °C. IR: 3422 (=NH), 2969, 2926 (–CH_3), 1722 (C=O), 1670 (C=N). MS (*m/z* %): 469 (M^++1, 2.56 %), 77 (100 %). ^{1}H-NMR (DMSO-d_6): 7.90-7.34 (m, 10H, 2Ph), 5.62 (s, 1H, =NH), 2.45 (s, 6H, 2-$COCH_3$), 2.15 (s, 3H,CH_3). Anal: Calcd. For $C_{25}H_{20}N_6O_2S$: C, 64.08; H, 4.31; N, 17.94; Found: C, 64.43; H, 4.38; N, 18.23 %.

7-Acetylamino-8-imino-6-methyl-3,4-diphenylpyrimido-[4',5':4,5]thieno[2,3-*c*]pyridazine (7)

Compound (**6**) (0.5 g, 1.07 mmol) was added to a solution of sodium hydroxide (1 g) in ethanol (10 mL). The reaction mixture was refluxed for 2 h. After cooling, it was poured into ice water, and neutralized with conc. HCl. The solid product was filtered off, washed with water, dried and recrystallised from ethanol to give (**7**). Yield: 0.3 g, (65.9 %), m.p. 220-222 °C. IR (cm^{-1}): 3422, 3157 (=NH, -NH amide), 2923 (-CH_3), 1652 (C=O amide), 1595 (C=N). MS (*m/z* %): 425 (M^+-1, 10.8), 383 (59.9), 178 (96.2), 77 (100 %); Anal: Calcd., for $C_{23}H_{18}N_6OS$ (426.48): C, 64.77; H, 4.25; N, 19.71; Found: C, 64.40; H, 4.30; N, 19.90 %.

5-Amino-6-[(3,5-dimethyl-1*H*-pyrazol-1-yl)(imino)methyl]-3,4-diphenylthieno[2,3-*c*]pyridazine (10)

To a solution of compound (**2**) (0.5 g, 1.39 mmol) in absolute ethanol (10 mL), acetylacetone (0.14 g, 1.39 mmol) was added. The reaction mixture was refluxed for 24 h. The solvent was evaporated under reduced pressure and the residue was washed with pet. ether 40-60 °C. The solid product was collected and recrystallized from ethanol. Yield: 0.52 g (88.3 %), mp 170-171°C; IR: 3318, 3178 (-NH_2, =NH), 2975, 2925 (2 Me group), 1674 (C=N). MS (m/z %): 422 (M^+-2, 6.8), 406 (4.0), 347 (7.0). ^{1}H-NMR (DMSO-d_6) δ ppm: 7.81-7.28 (*m*, 10H, 2Ph), 6.13 (*s*, 2H, NH_2), 5.69 (s, 1H, -CH pyrazole), 5.36 (s, 1H, =NH), 2.20 (s, 3H, CH_3-5), 2.15 (s, 3H, CH_3-3). Anal Calcd., for $C_{23}H_{20}N_6S$ (424.51): C, 67,90; H, 4.75; N, 19.80. Found: C, 67.50; H, 4.60; N, 19.50 %.

7-Formylamino-8-imino-3,4-diphenylpyrimido[4',5':4,5]thieno[2,3-*c*]pyridazine (11)

Method A

A mixture of compound (**2**) (0.5 g, 1.39 mmol) and formic acid (10 mL) was heated under reflux for 3 h. The cooled reaction mixture was poured into water (50 mL), the solid product was filtered, washed with water, dried and recrystallized from ethanol. Yield: 0.35 g (63.33 %), m.p. 190-191° C. IR (cm^{-1}): 3311, 3183 (=NH, -NH), 1670 (C=O), 1650 (C=N). MS (*m/z* %): 389 (M^+, 2.12), 396 (25.4), 307 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.17 (*s*, 1H, H-6), 8.06 (*s*, 1H, -CHO), 7.83-7.33 (*m*, 10H, 2Ph), 6.13 (*s*, 1H, -NHCO), 5.38 (*s*, 1H, =NH). Anal: Calcd., for $C_{21}H_{14}N_6OS$ (398.43): C, 63.30; H, 3.54; N, 21.09. Found: C, 63.60; H, 3.60; N, 21.3 %.

Method B

A mixture of compound (**5**) (0.5 g, 1.35 mmol) and formic acid (10 mL) was refluxed for 2 h. The cooled reaction mixture was poured into water (50 mL). The solid product was filtered off, washed with water, dried and recrystallized from ethanol to give (**11**)**.** It was identical with that obtained by method A (m.p. and mixed m.p.). Yield: 0.38 g (70.66 %).

6-Imino-10,11-diphenylpyridazino[4'',3'',4',5']-thieno[3',2':4,5]-pyrimido[1,2-*b*]pyridazin- 3(4H)-one (12)

To a solution of compound (**2**) (0.5 g, 1.39 mmol) in DMF (10 mL), malic anhydride (1.36 g, 1.39 mmol) was added and the reaction mixture was refluxed for 5 h. The cooled reaction mixture was poured into water (50 mL). The solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.39 g (66.53 %), m.p. 193-195° C. IR (cm^{-1}): 3420, 3162 (=NH, -NH), 1692 (C=O amide), 1595 (C=N). MS (*m/z* %): 422 (M^+, 3.83), 421 (10.31), 149 (100). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.41 (*s*, 1H, NH amide), 7.80-6.60 (*m*, 12H, Ar-H), 5.05 (*s*, 1H, =NH). Anal: Calcd., for $C_{23}H_{14}N_6OS$ (422.45): C, 65.39; H, 3.34; N, 19.89. Found: C, 65.70; H, 3.40; N, 20.15 %.

8-Imino-12,13-diphenylpyridazino[4'',3'':4',5'] thieno-[3',2':4,5]pyrimido[2,1-*a*]phthalazin-5(6*H*)-one (13)

To a solution of compound (**2**) **(**0.5 g, 1.39 mmol) in DMF (10 mL), phthalic anhydride (0.21 g, 1.39 mmol) was added and the reaction mixture was refluxed for 5 h. The cooled

reaction mixture was poured into water (50 mL). The solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.49 g (74.75 %), m.p. 209-210^{o} C. IR (cm^{-1}): 3400, 3155 (=NH), 1742 (C=O amide), 1674 (C=N). MS (*m/z* %): 472 (M^{+}, 64.59), 473 (100), 444 (2.03). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.15 (*s*, 1H, NH amide), 8.10-7.33 (*m*, 14H, Ar-H), 5.39 (*s*, 1H,=NH). Anal: Calcd., for $C_{27}H_{16}N_6OS$ (472.51): C, 68.63; H, 3.41; N, 17.79. Found: C, 68.91; H, 3.47; N, 17.60 %.

3-Chloro-6-imino-10,11-diphenylpyridazino[4'',3'':4',5']thieno [3',2':4,5]pyrimido[1,2-*b*]pyridazine (14)

A mixture of compound (**12**) (0.5 g, 1.18 mmol) and phosphoryl chloride (10 mL) was refluxed for 3 h. The cooled reaction mixture was poured into iced water (50 mL), the solid product was filtered, washed with water, dried and recrystallized from ethanol. Yield: 0.38 g (76.28 %), m.p. 184-186 ^{o}C. IR (cm^{-1}): 3422 (=NH), 1599 (C=N). MS (*m/z* %): 441 (M^{+}, 0.26), 443 (M^{+}+2, 0.22), 414 (0.22), 149 (100). Anal: Calcd., for $C_{23}H_{13}ClN_6S$ (440.90): C, 62.65; H, 2.97; N, 19.06. Found: C, 62.80; H, 3.00; N, 19.22 %.

6-Imino-10,11-diphenylpyridazino[4'',3'':4',5']thieno-[3',2':4,5]pyrimido[1,2-*b*]tetrazolo[5,1-*f*]pyridazine (15)

To a solution of compound (**14**) (0.5 g, 1.13 mmol) in ethanol (10 mL), sodium azide (0.22 g, 3.39 mmol) was added and the reaction mixture was heated under reflux for 7 h. The solvent was evaporated under reduced pressure and the residue was treated with water. The solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.40 g (78.82 %), m.p. 270-272 ^{o}C. IR (cm^{-1}): 3394, (=NH), 1627 (C=N), 1559 (N=N). MS (*m/z* %): 447 (M^{+}, 3.21), 423 (2.01), 178 (100). Anal: Calcd., for $C_{23}H_{13}N_9S$ (447.46): C, 61.73; H, 2.93; N, 28.17. Found: C, 62.00; H, 3.00; N, 28.30 %.

3-Ethoxy-6-imino-10,11-diphenylpyridazino[4'',3'':4',5']thieno [3',2':4,5]pyrimido[1,2-*b*]pyridazine (16)

To a solution of sodium ethoxide (0.1 g Na in absolute 10 mL ethanol), compound (**14**) (0.5 g, 1.13 mmol) was added and the reaction mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure and the residue was treated with water/HCl. The solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.35 g (68.51 %), m.p. > 300 ^{o}C. IR (cm^{-1}): 3384, (=NH), 2922, 2853, 1444 (-OEt), 1622 (C=N). MS (*m/z* %): 449 (M^{+}-1, 1.02), 420 (1.15), 63 (100). Anal: Calcd., for $C_{25}H_{18}N_6OS$ (450.50): C, 66.65; H, 4.03; N, 18.66. Found: C, 66.90; H, 4.10; N, 18.90 %.

6-Imino-N-(4-methoxyphenyl)-10,11-diphenylpyridazino-[4'',3'':4',5']thieno[3',2':4,5]pyrimido[1,2-*b*]pyridazin-3-amine (17)

To a solution of compound (**14**) (0.5 g, 1.13 mmol) in ethanol / THF (10 mL, 1:4), p-anisidine (0.14 g 1.13 mmol) was added, the reaction mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure and the residue was treated with water. The solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.46 g (76.88 %), m.p. 148-149 ^{o} C. IR (cm^{-1}): broad band 3371, (=NH, -NH), 1612 (C=N), 2843, 1443 (-OMe). MS (*m/z* %): 527 (M^{+}, 0.26), 496 (0.31), 63 (100). Anal Calcd., for $C_{30}H_{21}N_7OS$ (527.58): C, 68.29; H, 4.01; N, 18.58. Found: C, 68.60; H, 4.10; N, 18.30 %.

4-[(6-Imino-10,11-diphenylpyridazino[4'',3'':4',5'] thieno-[3',2':4,5]pyrimido[1,2- *b*]pyridazin-3-yl)amino]-*N*-phenyl-benzenesulphonamide (18)

A mixture of compound (**14**) (0.5 g, 1.18 mmol) and 4-amino-N-phenylbenzenesulphonamide (0.28 g, 1.13 mmol) in ethanol / THF (10 mL 1:4) was refluxed for 3 h. The solvent was evaporated under reduced pressure, the residue was triturated with ethanol and the solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.44 g (59.44 %), m.p. 174-176 ^{o}C. IR (cm^{-1}): 3420, 3349, 3295 (=NH, -NH groups), 1636 (C=N), 1317 (SO_2, asym.), 1150 (SO_2, sym.). MS (*m/z* %): 652 (M^{+}, 0.03), 576 (0.01), 474 (0.05), 247 (50.00). Anal: Calcd., for $C_{35}H_{24}N_8O_2S_2$ (652.72): C, 64.40; H, 3.71; N, 17.17. Found: C, 64.71; H, 3.80; N, 16.90 %.

5-Chloro-8-imino-12,13-diphenylpyridazino[4'',3'':4',5']thieno[3',2':4,5]pyrimido[2,1-*a*]phthalazine (19)

A mixture of compound (**13**) (0.5 g, 1.06 mmol) and phosphoryl chloride (10 mL) was refluxed for 5 h. The cooled reaction mixture was poured into ice water (50 mL), the solid product was filtered, washed with water, dried and recrystallized from ethanol. Yield: 0.39 g (75.10 %), m.p. 216-217 ^{o}C. IR (cm^{-1}): 3419 (=NH), 1627 (C=N). MS (*m/z* %): 491 (M^{+}, 4.82), 493 (M^{+}+2, 2.58), 455 (1.49), 289 (100). Anal: Calcd., for $C_{27}H_{15}ClN_6S$ (490.96): C, 66.05; H, 3.08; N, 17.12. Found: C, 66.30; H, 3.10; N, 17.30 %.

6-Imino-10,11-diphenylpyridazino[4'',3'':4',5']thieno [3',2':4,5]pyrimido[2,1-*a*]tetrazolo[1,5-c] phthalazine (20)

To a solution of compound (**19**) (0.5 g, 1.02 mmol) in ethanol (10 mL), sodium azide (0.20 g 3.06 mmol) was added, the reaction mixture was refluxed for 7 h. The solvent was evaporated under reduced pressure and the residue was treated with water. The solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.40 g (78.88 %), m.p. 221-222 ^{o}C. IR (cm^{-1}): 3421, (=NH), 1622 (C=N), 1576 (N=N). MS (*m/z* %): 497 (M^{+}, 0.61), 469 (0.75), 57 (100). Anal: Calcd., for $C_{27}H_{12}N_9S$ (497.52): C, 65.18; H, 3.04; N, 25.34. Found: C, 65.50; H, 3.10; N, 25.09 %.

5-Ethoxy-8-imino-12,13-diphenylpyridazino[4'',3'':4',5']thieno [3',2':4,5]pyrimido[2,1-*a*]phthalazine (21)

To a solution of sodium ethoxide (0.1 g Na in absolute ethanol 10 mL) compound (**19**) (0.5 g, 1.01 mmol) was added and the reaction mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure and the residue was treated with water / HCl. The solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.30 g (58.79 %), m.p. 253-254 ^{o}C. IR

(cm^{-1}): 3389, (=NH), 2919, 2850, 1443 (-OEt), 1600 (C=N). MS (*m/z* %): 500 (M^+, 0.78), 471 (0.35), 57 (100). Anal: Calcd., for $C_{29}H_{20}N_6OS$ (500.56): C, 69.58; H, 4.03; N, 16.79. Found: C, 69.93; H, 4.12; N, 16.51 %.

8-imino-N-(4-methoxyphenyl)-12,13-diphenylpyridazino-[4'',3'':4',5']thieno-[3',2':4,5]pyrimido[2,1-*a*]phthalazin-5-amine (22)

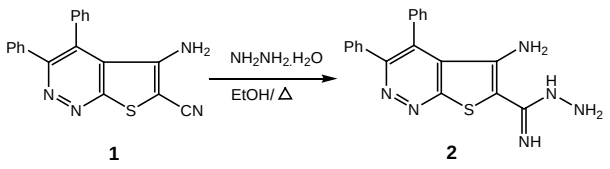
To a solution of compound (**19**) **(**0.5 g, 1.02 mmol) in ethanol / THF (10 mL, 1:4), p-anisidine (0.13 g 1.02 mmol) was added, the reaction mixture was heated under reflux for 3 h. The solvent was evaporated under reduced pressure and the residue was treated with water. The solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.41 g (69.63 %), m.p. 166-167 ^oC. IR (cm^{-1}): broad band 3421, (=NH, -NH), 1617 (C=N), 2917, 1443 (-OMe). MS (*m/z* %): 577 (M^+, 0.04), 562 (0.04), 108 (100); ^{1}H-NMR (DMSO-d_6) δ ppm: 7.90-6.85 (*m*, 18H, Ar-H), 5.52 (*s*, 1H,=NH), 3,90 (*s*, 1H, -NH), 3.71 (*s*, 3H, -OCH_3). Anal: Calcd., for $C_{34}H_{23}N_7OS$ (577.63): C, 70.69; H, 4.01; N, 16.97. Found: C, 70.99; H, 4.09; N, 16.70 %.

4-[(8-Imino-12,13-diphenylpyridazino[4'',3'':4',5']thieno-[3',2':4,5]pyrimido[2,1-*a*]phthalazin-5-yl)amino]-N-phenyl-benzenesulphonamide (23)

A mixture of compound (**19**) (0.5 g, 1.01 mmol) and 4-amino-*N*-phenylbenzenesulphonamide (0.25 g, 1.02 mmol) in ethanol / THF (10 mL 1:4) was refluxed for 3 h. The solvent was evaporated under reduced pressure, the residue was triturated with ethanol and the solid product was filtered off, washed with water, dried and recrystallized from ethanol. Yield: 0.38 g (53 %), mp.. 204-206^o C. IR (cm^{-1}): 3378, 3250 (=NH, -NH groups), 1627 (C=N). 1304 (SO_2, asym.), 1151 (SO_2, sym.). MS (*m/z* %):702 (M^+, 24.68), 651 (22.08), 610 (14.72), 216 (100). Anal: Calcd., for $C_{39}H_{26}N_8O_2S_2$ (702.78): C, 66.65; H, 3.73; N, 15.94. Found: C, 66.90; H, 3.80; N, 15.70 %.

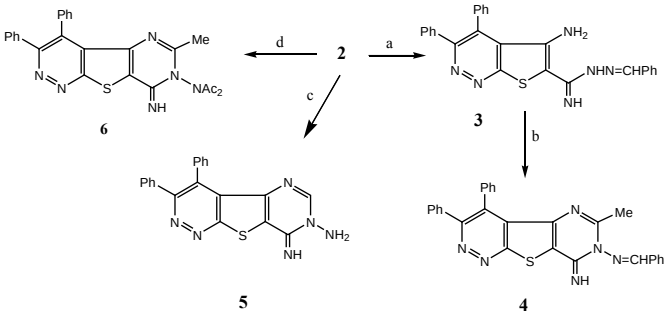
Results and Discussion

The carboxamidohydrazide derivative (**2**) was the starting material for syntheses carried out. This compound was prepared by refluxing 5-Amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonitrile (**1**) with hydrazine hydrate in ethanol for 2 h according to the method reported by us previously.[10]



Scheme 1

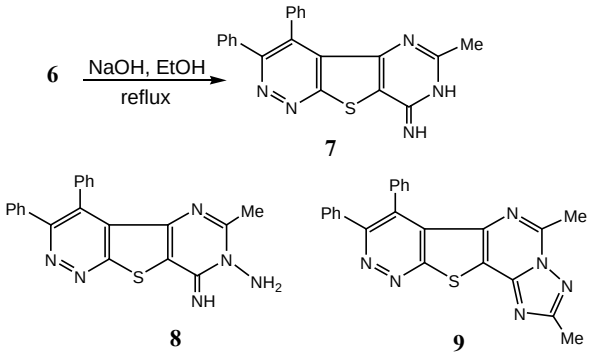
The condensation of 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carboxamidohydrazide (**2**) with benzaldehyde was carried out in 1:1 molar ratio with elimination of one molecule of water producing *N*-benzylidene-6-carboximidohydrazide derivative (**3**), which can be cyclized on boiling with acetic anhydride to give 7-benzylideneamino-8-imino-6-methyl-3,4-diphenylpyrimido-[4,5:4,5]thieno[2,3-*c*]pyridazine (**4**) in good yield. On heating compound (**2**) in DMF at refluxing temperature for 12 h, a single product **5** in 77.8% yield was obtained. The isolated product was proven to be 7-amino-8-imino-3,4-diphenylpyrimido[4,5:4,5] thieno[2,3-c]pyridazine. On the other hand, compound (**3**) on refluxing with DMF underwent formylation and cyclisation, followed by hydrolysis with elimination of a benzaldehyde molecule to furnish (**5**)



Reagents: a, PhCHO, ethanol, reflux; b, acetic anhydride reflux; c, DMF, reflux; d, acetic anhydride, reflux.

Scheme 2

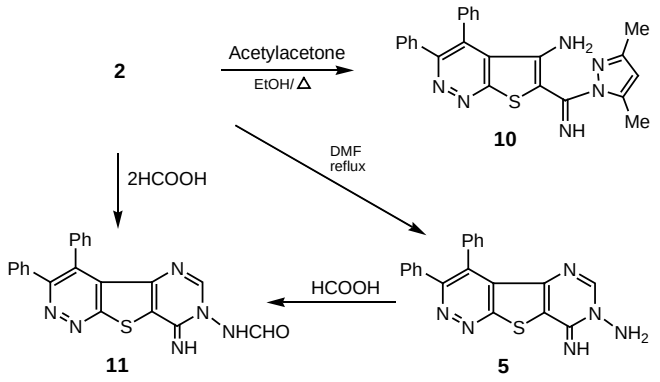
Refluxing compound (**2**) with acetic anhydride for 5 h produced the corresponding monoacetyl derivative, which underwent smooth cyclization to the corresponding fused primidothienopyridazine followed by diacetylation of the amino group to give (**6**) which was proven to be 7-diacetylamino-8-imino-6-methyl-3,4-diphenylpyrimido [4,5:4,5]thieno[2,3-*c*] pyridazine. Solvolysis of compound (**6**) in boiling 2N ethanolic sodium hydroxide followed by cooling and neutralizing with hydrochloric acid leads to formation of crystals, which was identified by the analytical and spectral data as 7-acetylamino-8-imino-6-methyl-3,4-diphenylpyrimido [4,5:4,5]thieno[2,3-*c*]pyridazine (**7**) as proved by the analytical and spectral data. Neither the expected 7-amino-8-imino derivative (**8**) nor the tetracyclic triazolopyrimido thienopyridazine derivative (**9**) is formed.



Scheme 3

When an ethanolic solution of (**2**) is treated with acetylacetone at refluxing temperature, it easily afforded the corresponding pyrazolyl derivative (**10**)**.** The structure of prazolyl derivative (**10**) was proved from its analytical and spectral data to be 5-amino-6-[(3,5-dimethyl-1*H*-pyrazol-1-yl)(imino)-methyl]-3,4-diphenylthieno[2,3-*c*]pyridazine.

The expected 7-formylamino derivative (**11**) was obtained in moderate yield by refluxing (**2**) with formic acid. The structure of compound **11** was elucidated by elemental analysis and spectral data. The structure of (**11**) was confirmed by comparison with an authentic sample also (m.p, mixed m.p and super imposable IR) prepared by formylation of compound (**5**) with formic acid at reflux temperature (**Scheme 4**).



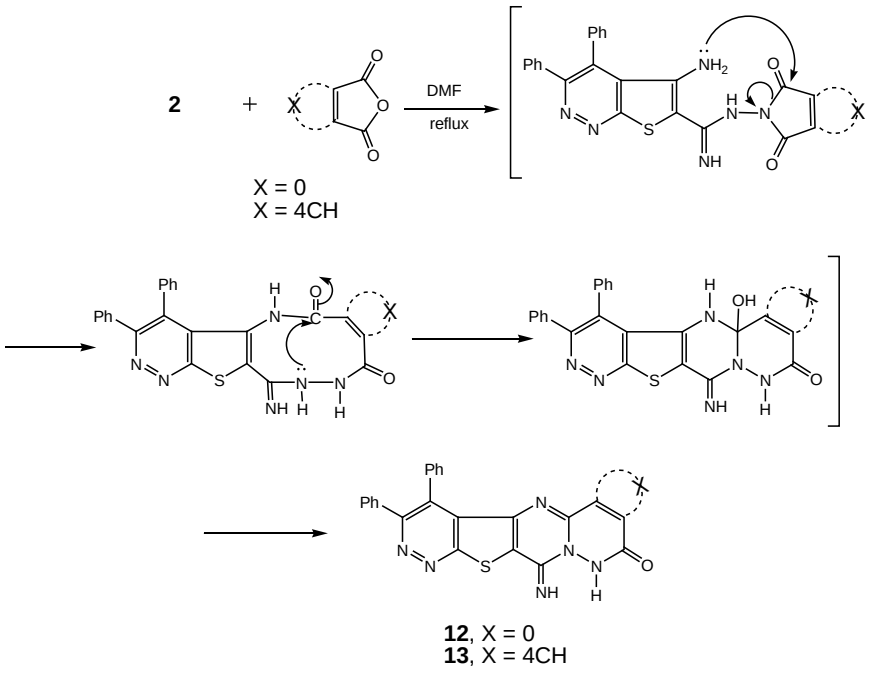
Scheme 4

Heating a mixture of carboximidohydrazide (**2**) and maleic anhydride and/or phthalic anhydride in DMF at reflux temperature afforded a single product.

The isolated product was proven to be 6-imino-10,11-diphenyl-pyridazino[4’’,3’’:4’,5’]thieno-[3’,2’:4,5]pyrimido[1,2-*b*]-pyridazine-3(4*H*)-one (**12**) or pentacyclic compound (**13**). The structure of compound (**12**) was assigned by its spectral and elemental data. Mechanistically, the formation of the tetracyclic compound (**12**) involves the initial formation of a maleimide derivative, which undergoes intramolecular nucleophilic attack of the thiophene amino group on the maleimide carbonyl group with elimination of one molecule of water. Similarly, formation of compound (**13**) takes place through the initial formation of a cyclic isoindol derivative, which undergoes immediate intramolecular nucleophilic attack of the thiophene amino group on the isoindol carbonyl group with elimination of one molecule of water (**Scheme 5**). The structure of 8-imino-12,13-diphenyl-pyridazino[4’’,3’’:4’,5’]thieno-[3’,2’:4,5]pyrimido[2,1-*a*]-phthalazin-5(6H)-one (**13**) was proved by its spectral and elemental data.

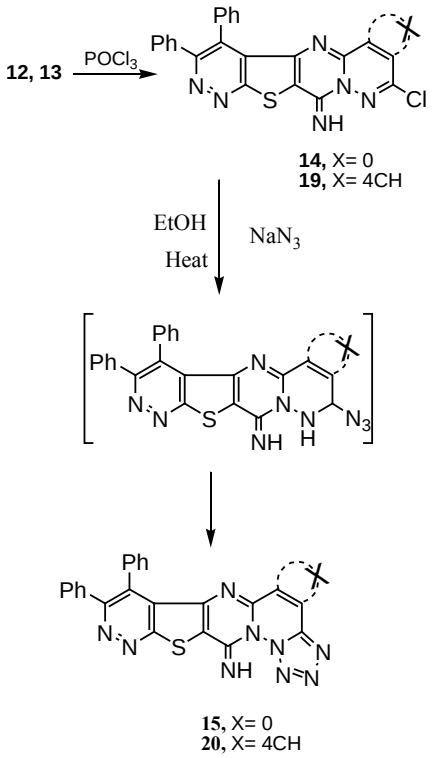
Compounds (**12**) and (**13**) on reaction with phosphoryl chloride at reflux temperature gave the corresponding 3-chloro derivatives (**14**) and **(19**) (Scheme 6). The infrared spectrum of compound (**14**) showed absorption band at 1599 (C=N) cm^{-1} and there is no band in the carbonyl region.

Since the reactivity of the chlorine atom was expected, the chloro compound (**14**) was subjected to nucleophilic substitution reactions to obtain the newer derivatives of the ring systems, it was reacted with sodium azide in ethanol at reflux temperature giving 6-imino-10,11-diphenyl-pyridazino[4’’,3’’:4’,5’]thieno-[3’,2’:4,5]pyrimido[1,2-*b*]-tetrazolo[5,1-*f*]pyridazines (**15**) and (**20**), wherein 3-azido derivative was formed in the first step of this reaction , then intramolecular cyclization to the tetrazolo derivative occurred immediately.

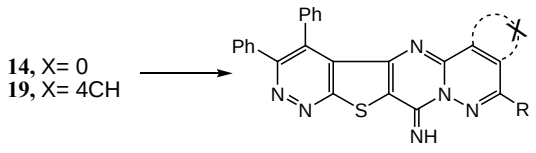


Scheme 5

The predomination formation of the tetrazolo derivatives (**15**) and (**20**) were supported by the infrared spectral data , which exhibited no absorption bands around 2200 cm^{-1} due to azido group, and there is absorption bands at 1627(C=N) and 1559(N=N).



Scheme 6



16, X = 0 and **21**, X = 4CH, R = OEt
17, X = 0 and **22**, X = 4CH, R = $NHC_6H_4OMe(p\text{-})$
18, X = 0 and **23**, X = 4CH, R = $NHC_6H_4SO_2NHPh$

Scheme 7

In continuation of the substitution of the 3-chloro group in (**14**) and (**19**), we found that refluxing (**14**) and (**19**) with sodium ethoxide in absolute ethanol afforded a good yield of 3-ethoxy 6-imino-10,11-diphenylpyridazino[4'',3'':4',5']-thieno-[3',2':4,5]pyrimido[1,2-*b*]pyridazine (**16**) and (**21**).

Heating the 3-chloro derivatives, (**14**) and (**19**), with equimolar amount of p-anisidine in EtOH/THF (1:4) at reflux temperature gives the expected product (**17**) and (**22**), which were proven to be 6-imino-*N*-(4-methoxyphenyl)-10,11-diphenylpyridazino[4'',3'':4',5']thieno-[3',2':4,5]pyrimido[1,2-*b*]pyridazin-3-amine (**17**) and 8-amino-N-(4-methoxyphenyl)-12,13-diphenylpyridazino[4'',3'':4',5']thieno-[3',2':4,5]pyrimido[2,1-*a*]phthalazin-5-amine (**22**). Reaction of equimolar amounts of the 3-chloro derivativs, (**14**) and (**19**), with 4-amino-N-benzenesulphonamide in EtOH/THF (1:4) at reflux temperature afforded a single product each. The isolated products are shown to be 4-[6-imino-10,11-diphenylpyridazino[4'',3'':4',5']thieno-[3',2':4,5]pyrimido-[1,2-*b*]pyridazin-3-yl)amino]-*N*-phenyl-benzenesulphonami-de (**18**) and 4-[8-imino-12,13-diphenylpyridazino[4'',3'':4',5']thieno-[3',2':4,5]pyrimido-[2,1-*a*]phthalazin-5-yl)amino]-N-phenylbenzenesulphon-amide (**23**) (Scheme 7).

The newly synthesized compounds were characterized by the IR, NMR, and mass spectrum as well as the elemental analysis listed in experimental section. The spectral analyses were in accordance with the assigned structures.

Screening for antimicrobial activities

Applying the agar plate diffusion technique,[11] the newly synthesized compounds were screened *in vitro* for antimicrobial activity against Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), yeast (*Candida albicans*), and a fungus (*Aspergillus niger*). In this method, a standard 5 mm sterilized filter paper disk impregnated with the compound (0.3 mg / 0.1 ml of DMF) was placed on an agar plate seeded with the tested organism. The plates were incubated for 24 h at 37 °C for bacteria and 28 °C for fungi. The inhibition zones of bacteria and fungi growth around the discs were determined. The screening results are given in Table 1.

The results indicated that seven synthesized compounds (**3**), (**10**), (**12**), (**14**), (**18**) and (**23**) showed moderate antimicrobial activity against the examined Gram positive bacteria *Staphylococcus aureus*. In addition, compound (**12**) showed very high antifungal activity against the examined yeast, *Candida albicans* and a fungus, *Aspergillus niger*.

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

Table 1 Anti-microbial activity of synthesized compounds.

Compd.	*Staphylococcus Aurus*	*Bacillus Subtitles*	*E.coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillums Niger*
3	+++	++	-	++	++	++
4	++	++	-	++	++	+++
6	++	++	-	++	++	++
10	+++	++	-	+	++	++
11	++	++	+	-	++	++
12	+++	++	-	-	++++	++++
13	++	++	++	+	++	+
14	+++	+	-	-	++	++
15	++	++	-	++	++	++
17	++	++	-	++	++	-
18	+++	++	-	++	++	++
19	++	+	-	+	+++	++
20	++	+	-	+	++	+
22	++	+	-	++	++	++
23	+++	++	-	-	++	+++
DMF	-		-	-	-	-
Nystin	-	-	-	-	++++	++++
Ciprofloxacin	++++	++++	++++	++++	-	-

The concentration of all synthesised compounds and the two references was 0.30 mg 0.10 mL^{-1} of dimethylformamide. Zone of inhibition: + = < 15 mm; ++ = 15-24 mm; +++ = 25-34 mm; ++++ = 35-44 mm; - = no inhibition.

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