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Pyridazine and its related compounds: Part 32. Synthesis and antimicrobial evaluation of some 3-substituted amino-4,5,6-triphenylpyridazine derivatives

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ABSTRACT

3-Amino-4,5,6-triphenylpyridazine was subjected to some selected reactions with nitrous acid, formic acid/dimethylformamide, acetic anhydride, benzoyl chloride, chloroacetyl chloride, acetaldehyde, diethyl malonate, malonic acid/phosphoryl chloride, diethyl oxalate, ethyl cyanoacetate, ethyl acetoacetate, and ethyl benzoylacetate to give new 3-substituted aminopyridazine derivatives. A few sulfonamide derivatives (new) were also prepared. The structures of the synthesized compounds were proved by their infrared, mass spectra, ^{1}H NMR, and elemental analysis. The antimicrobial activity of the compounds obtained was examined against some selected microorganisms.

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1. Introduction

The pyridazine nucleus is a very common component in many compounds of biological interest manifested in several fields of medicinal chemistry, such as phosphodiesterase type 4 inhibitors for inflammatory diseases [1], as endothelin-A receptor antagonists [2], in the development of active compounds toward α1 and α2 adrenoceptors [3], as central analgesics and aldose reductose inhibitors [4]. The pyridazine derivatives are also used as analgesic [5], antibacterial [6], anti-inflammatory [7], anti-hypertensive [8], antihistaminic [9], antinociceptive agents [10], as well as platelet aggregation inhibitors [11]. They have a rapid systemic effect on the plants and are active at very low concentration. Some of the investigated pyridazine derivatives have chemical structures related to those of the phytohormones [12].

In addition, similar chemical structures occur in living cells, being involved in various biochemical reaction pathways. Some new synthesized pyridazine derivatives were used in many research fields due to their structure, stability, reactivity and their tendency to form stable derivatives with important biological properties. On the other hand, as a rule, a cytotoxic effect of higher concentrated compounds is correlated with their stimulation effect at lower concentrations [13]. Thus, due to this wide use of pyridazine based structures, great attention was paid to the development of different methods for the preparation of pyridazine and its functionalized derivatives.

2. Experimental

2.1. Instrumentation

All 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 Nicolet 6700 FT-IR (Thermo Scientific) as potassium bromide pellets and frequencies are reported in cm^{-1}.

The mass spectra were recorded on a GC-MS model Shimadzu Qp-2010 plus EI 70 eV.

The ^{1}H NMR spectra were recorded on a Varian Gemini-300 MHz NMR spectrometer and chemical shifts δ are in ppm relative to internal TMS. 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.

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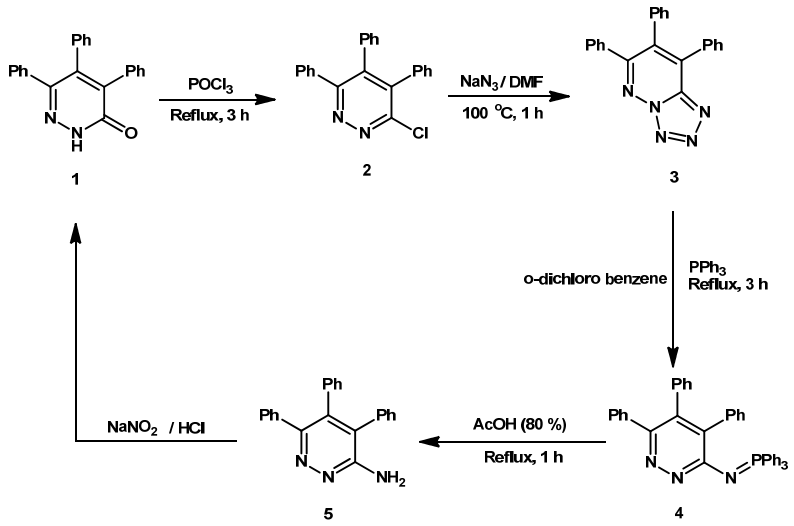


Scheme 1

2.2. Synthesis

2.2.1. Synthesis of 4,5,6-triphenylpyridazin-3(2H)-one (1)

4,5,6-Triphenylpyridazin-3(2*H*)-one (**1**) was prepared according to the synthetic pathway starting from benzilmono hydrazine and ethyl phenylacetate as described in literature (Scheme 1) [14].

2.2.2. Synthesis of 3-chloro-4,5,6-triphenylpyridazine (2)

A mixture of compound **1** (0.5 g, 1.54 mmol) and phosphoryl chloride (10 mL) was refluxed for 3 hours, the reaction mixture was cooled and poured into ice cooled water (100 mL). The solid product was filtered off, washed with water, dried and recrystallized from ethanol. M.p.: 170-172 °C (Scheme 1) [14].

2.2.3. Synthesis of 6,7,8-triphenyltetrazolo[1,5-b]pyridazine (3)

To a solution of compound **2** (0.5 g, 1.46 mmol) in dimethylformamide (10 mL) sodium azide (0.28 g, 4.38 mmol) was added. The reaction mixture was heated at 100 °C for 1 hour, the cooled reaction mixture was poured into water (100 mL), the solid product formed was filtered off, washed with water, dried and recrystallized from ethanol (Scheme 1). M.p.: 194-196 °C [15,16].

2.2.4. Synthesis of 4,5,6-triphenyl-N-(triphenylphosphoran ylidene)pyridazine-3-amine (4)

To a solution of compound **3** (0.5 g, 1.43 mmol) in *o*-dichlorobenzene (10 mL), triphenylphosphine (0.37 g, 1.43 mmol) was added. The reaction mixture was refluxed for 3 hours. The solvent was removed under reduced pressure; the residue was treated with diethyl ether (30 mL). The solid product was filtered off, dried and recrystallized from acetone similar to reported procedure (Scheme 1) [17]. Color: White. Yield: 83%. M.p.: 248-250 °C. FT-IR (KBr, ν, cm^{-1}): 3055-3023 (CH arom.), 1597 (C=N), 1537 (P-Ph), 1484, 1412 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.93-6.81 (m, 30H, 6Ph). MS (EI, *m/z* (%)): 583 (M^+, 100). Anal. calcd. for $C_{40}H_{30}N_3P$: C, 82.31; H, 5.18; N, 7.20. Found: C, 82.10; H, 5.00; N, 7.00%.

2.2.5. Synthesis of 3-amino-4,5,6-triphenylpyridazine (5)

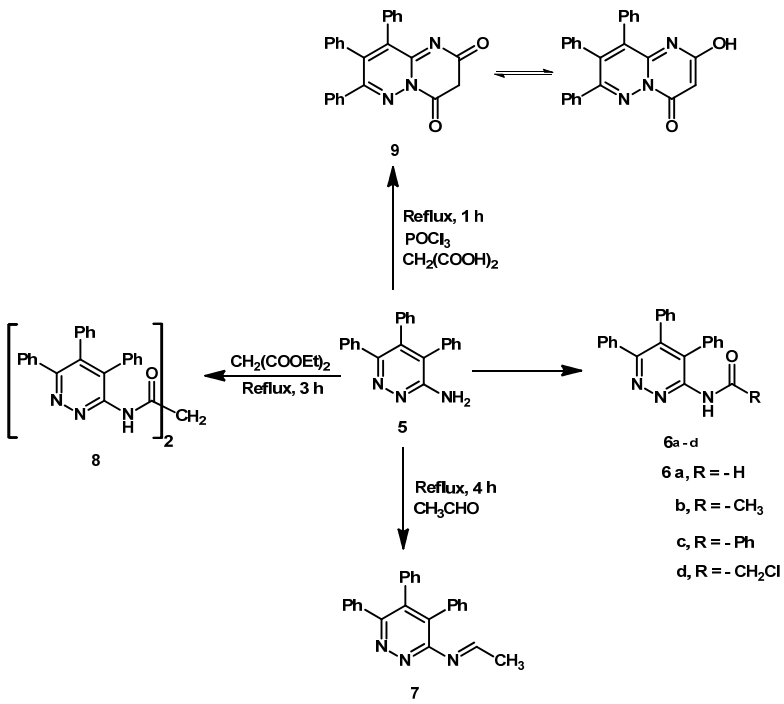
A solution of compound **4** (0.5 g, 0.86 mmol) in AcOH (80 %, 10 mL) was heated under reflux for 1 h. After cooling, the reaction mixture was poured into water (100 mL). The precipitate that formed was filtered off, washed with water, dried, and recrystallized from methanol (Scheme 1). Color: White. Yield: 86.61 %. M.p.: 260-262 °C. FT-IR (KBr, ν, cm^{-1}): 3470-3280 (NH_2), 3059 (CH Arom.), 1629 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 5.84 (s, 2H, NH_2), 6.87-7.62 (m, 15H, 3Ph). MS (EI, *m/z* (%)): 323([M]$^+$, 58.23), 322 ([M-1]$^+$ (100)), 246 (3.82), 178 (15.05), 146 (2.10), 117 (36.06), 103 (1.31), 77 (5.93). Anal.calcd. for $C_{22}H_{17}N_3$: C, 81.71; H, 5.30; N, 12.99. Found: C, 81.70; H 5.30; N 12.90%.

2.2.5.1. Reaction of compound 5 with nitrous acid

Compound **5** (0.5 g,1.55 mmol) was dissolved in concentrated HCl (5 mL), cooled to 0-5 °C in an ice bath, then a solution of $NaNO_2$ (0.22 g, 3.1 mmol) in H_2O (5 mL) was added drop by drop with stirring, keeping the temperature at 0-5 °C. The mixture was stirred for further 1 h at room temperature. The solid product was filtered off, washed with water, dried and recrystallized from benzene to give compound **1**, identical with that prepared from benzilmonohydrazone and ethyl phenyl acetate, according to reference (Scheme 1) [14] (M.p. and mixed m.p.). Yield: 0.43 g (85.66 %).

2.2.6. Synthesis of N-(4,5,6-triphenylpyridazin-3-yl) formamide (6a)

A mixture of compound **5** (0.5 g, 1.55 mmol) and formic acid (0.1 mL, 1.55 mmol) in dimethylformamide (10 mL) was refluxed for 7 h. After cooling, the reaction mixture was poured into ice water (100 mL). The precipitate that formed was filtered off, washed with water, dried, and recrystallized from ethanol (Scheme 2). Color: White. Yield: 92 %. M.p.: 237-238 °C. FT-IR (KBr, ν, cm^{-1}): 3279 (NH), 3058 (CH arom.), 1697 (C=O), 1629 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 5.78 (s, 1H, NH), 6.99-7.09 (m, 15H, 3Ph), 7.14 (s, 1H, CHO). MS (EI, *m/z* (%)): 351 ([M]$^+$, 2.37), 322 ([M-29]$^+$, 100), 246 (3.82), 178 (15.05), 146 (2.10), 117 (36.06), 103 (1.31). Anal. calcd. for $C_{23}H_{17}N_3O$: C, 78.61; H, 4.88; N, 11.96. Found: C, 78.30; H 5.00; N 12.20%.



Reagents and conditions for the products 6a-d: for 6a: HCOOH/DMF, reflux 7 h; for 6b: $(CH_3CO)_2O$, reflux 5 h; for 6c: PhCOCl/pyridine, reflux 7 h; for 6d: $ClCH_2COCl$/pyridine, stirring at r.t, 2 h.

Scheme 2

2.2.7. Synthesis of N-(4,5,6-triphenylpyridazin-3-yl) acetamide (6b)

A solution of compound **5** (0.5 g, 1.55 mmol) in acetic anhydride (10 mL) was heated under reflux for 5 h. The solvent was evaporated under reduced pressure; the solid residue was collected and recrystallized from ethanol (Scheme 2). Color: White. Yield: 90.26 %. M.p.: 206-208 °C. FT-IR (KBr, ν, cm^{-1}): 3425 (NH), 3027(CH arom.), 2935 (CH aliph.), 1723 (C=O), 1629 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.15 (s, 3H, CH_3), 7.02-7.39 (m, 15H, 3Ph), 7.40 (s, 1H, NH). MS (EI, *m/z*(%)): 365 ($[M]^+$, 71.47), 364 ($[M-1]^+$, 100), 350 (66.80), 322 (61.66), 307 (4.84), 288 (7.68), 263 (6.65). Anal. calcd. for $C_{24}H_{19}N_3O$: C, 78.88; H, 5.24; N, 11.50. Found: C, 79.10; H 5.10; N 11.20%.

2.2.8. Synthesis of N-(4,5,6-triphenylpyridazin-3-yl) benzamide (6c)

A mixture of compound **5** (0.5 g, 1.55 mmol) and benzoyl chloride (0.18 mL, 1.55 mmol) in pyridine (10 mL) was refluxed for 7 h. After cooling, the reaction mixture was poured into ice water (100 mL), the precipitate that formed was filtered off, washed with water, dried and recrystallized from ethanol (Scheme 2). Color: White. Yield: 74.13 %. M.p.: 227-229 °C. FT-IR (KBr, ν, cm^{-1}): 3348 (NH amide), 3059, 3031 (CH-Arom.), 1704 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.13-7.33 (m, 20H, 4Ph), 7.35 (s, 1H, NH). Anal. calcd. for $C_{29}H_{21}N_3O$: C, 81.48; H 4.95; N, 9.83. Found: C 81.70; H 4.50; N 10.10%.

2.2.9. Synthesis of 2-chloro-N-(4,5,6-triphenylpyridazin-3-yl)acetamide (6d)

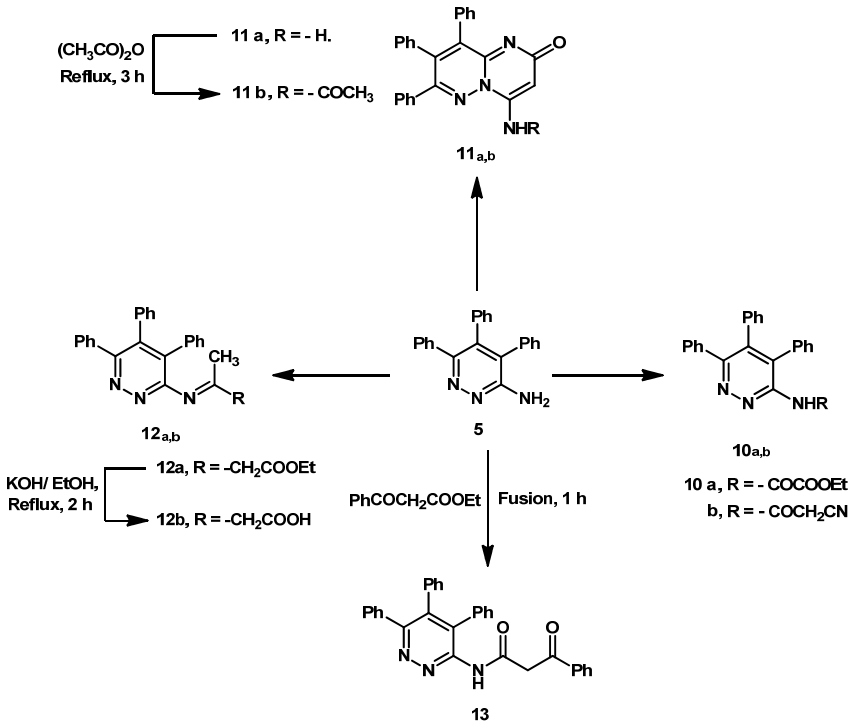
Chloroacetyl chloride (0.12 mL, 1.55 mmol) was added to a solution of compound **5** (0.5 g, 1.55 mmol) in pyridine (10 mL). The reaction mixture was stirred at room temperature for 2 h, poured into water (100 mL); the solid product that formed was filtered off, washed with water, dried and recrystallized from ethanol (Scheme 2). Color: White. Yield: 79.26 %. M.p.: 272-274 °C. FT-IR (KBr, ν, cm^{-1}): 3284 (NH amide), 3058 (CH-Arom.), 1646 (C=O amide). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.20 (s, 2H, CH_2), 6.93-7.68 (m, 15H, 3Ph), 7.88 (s, 1H, NH). Anal. calcd. for $C_{24}H_{18}ClN_3O$: C, 72.09; H, 4.54; N, 10.51. Found: C, 72.40; H, 4.80; N, 10.00%.

2.2.10. Synthesis of N-ethylidene-4,5,6-triphenylpyridazin-3-amine (7)

A mixture of compound **5** (0.5 g, 1.55 mmol) and acetaldehyde (0.1 mL, 1.55 mmol) was refluxed for 4 h, after cooling, the reaction mixture was treated with diethyl ether (50 mL). The solid product was filtered off, dried, and recrystallized from ethanol (Scheme 2). Color: Brown. Yield: 79.26 %. M.p.: 233-235 °C. FT-IR (KBr, ν, cm^{-1}): 3056, 3026 (CH Arom.), 2963, 2873 (CH aliph.), 1690 (C=N imino). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.80 (d, 3H, CH_3), 7.18-7.63 (m, 16H, 3Ph, =CH). Anal. calcd. for $C_{24}H_{19}N_3$: C, 82.49; H, 5.48; N, 12.03. Found: C, 82.10; H, 5.40; N, 12.3%.

2.2.11. Synthesis of N,N-bis(4,5,6-triphenylpyridazin-3-yl) propanediamide (8)

A mixture of compound **5** (0.5 g, 1.55 mmol) and diethyl malonate (0.24 g, 1.55 mmol) was heated under reflux for 3 h. After cooling, the reaction mixture was treated with diethyl ether (50 mL), and the solid product was filtered off,dried and recrystallized from dimethylformamide (Scheme 2). Color: White. Yield: 81.43 %. M.p.: 218-220 °C. FT-IR (KBr, ν, cm^{-1}): 3156 (NH amide), 3059, 3024 (CH Arom.), 2972, 2816 (CH Aliph.), 1698 (C=O amide). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.80 (s, 2H, CH_2), 6.87-7.31 (m, 30H, 6Ph), 10.23 (s, 2H, 2NH).



Reagents and conditions: for 10a: $(COOEt)_2$, reflux 3 h; for 10b: $CNCH_2CO_2Et$, reflux 1 h; for 11a:$CNCH_2CO_2Et$/ PPA/110 °C, stirring 6 h; for 12a: $CH_3COCH_2CO_2Et$, reflux 3 h.

Scheme 3

MS (EI, *m/z* (%)): 714 ([M]$^+$, 7.92), 658 (9.09), 406 (7.92), 363 (58.50), 322 (100). Anal. calcd. for $C_{47}H_{34}N_6O_2$: C, 78.97; H, 4.79; N, 11.67. Found: C 79.30; H, 4.69; N, 12.00%.

2.2.12. Synthesis of 7,8,9-triphenyl-2H-pyrimido[1,2-b] pyridazine-2,4(3H)-dione (9)

A mixture of compound **5** (0.5 g, 1.55 mmol), malonic acid (0.16 g, 1.55 mmol) and phosphoryl chloride (10 mL) was heated under reflux for 1 h. The reaction mixture was cooled to room temperature and poured into ice cooled water (200 mL). The solid product was filtered off, washed with water, dried and recrystallized from ethanol (Scheme 2). Color: Yellow. Yield: 72.70%. M.p.: 188-190 °C. FT-IR (KBr, ν, cm^{-1}): 3057 (CH Arom.), 2923 (CH Aliph.), 1703 (C=O), 1636 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.99 (s, 2H, CH_2), 6.78-7.65 (m, 15H, 3Ph). MS (EI, *m/z* (%)): 391 (M$^+$, 72.07), 363 (11.24), 349 (10.60), 335 (5.41), 321 (16.82), 314 (4.59), 288 (3.35), 213 (3.30), 178 (25.40), 103 (4.85), 69 (100). Anal. calcd. for $C_{25}H_{17}N_3O_2$: C, 76.71; H, 4.38; N, 10.74. Found: C, 76.50; H, 4.20; N, 10.60%.

2.2.13. Synthesis of ethyl oxo[(4,5,6-triphenylpyridazin-3-yl)amino]acetate (10a)

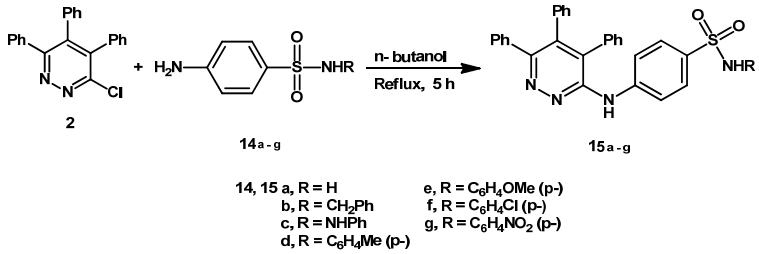
A mixture of compound **5** (0.5 g, 1.55 mmol) and diethyl oxalate (0.16 mL, 1.55 mmol) was heated under reflux for 3 h. After cooling, the reaction mixture was treated with diethyl ether (50 mL), and the solid product formed was filtered off, dried, and recrystallized from ethanol (Scheme 3). Color: Yellow. Yield: 80.92 %. M.p.: 217-219 °C. FT-IR (KBr, ν, cm^{-1}): 3425 (NH amide), 3059 (CH Arom.), 2976, 2933 (CH Aliph.), 1729 (C=O ester), 1682 (C=O amide), 1226 (C-O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.26 (t, *J* = 7.5 Hz, 3H, CH_3), 3.91 (q, 2H, CH_2), 6.99-7.62 (m, 15H, 3Ph), 7.86 (*s*, 1H, NH). MS (EI, *m/z* (%)): 423 (M$^+$, 6.77), 422 ([M-1]$^+$, 10.68), 378 (14.28), 350 (100), 322 (3.48), 307 (5.11), 206 (0.64), 178 (9.14), 77 (10.57), 76 (2.03). Anal. calcd. for $C_{26}H_{21}N_3O_3$: C, 73.74; H, 5.00; N, 9.92. Found: C 74.10; H, 5.40; N, 10.10%.

2.2.14. Synthesis of 2-cyano-N-(4,5,6-triphenylpyridazin-3-yl)acetamide (10b)

A mixture of compound **5** (0.5 g, 1.55 mmol) and ethyl cyanoacetate (0.16 mL, 1.55 mmol) was heated under reflux for 1 h. The cooled reaction mixture was treated with diethyl ether (50 mL). The solid product was filtered off, dried and recrystallized from ethanol (Scheme 3). Color: Brown. Yield: 79.51 %. M.p.: 178-180 °C. FT-IR (KBr, ν, cm^{-1}): 3184 (NH Amide), 3060 (CH Arom.), 2985 (CH Aliph.), 2204 (C≡N), 1658 (C=O amide). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.58 (s, 2H, CH_2), 6.51 (s, 1H, NH), 6.97-7.42 (m, 15H, 3Ph). MS (EI, *m/z* (%)): 390 (M$^+$, 79.96), 373 (12.58), 364 (5.13), 362 (11.96), 322 (100), 313 (12.83), 307 (20.20), 287 (25.24), 178 (30.92), 77 (42.05), 68 (71.96). Anal. calcd. for $C_{25}H_{18}N_4O$: C, 76.91; H, 4.65; N, 14.35. Found: C: 77.30; H, 4.90; N, 14.70%.

2.2.15. Synthesis of 4-amino-7,8,9-triphenyl-2H-pyrimido [1,2-b]pyridazin-2-one (11a)

A mixture of compound **5** (0.5 g, 1.55 mmol), ethyl cyanoacetate (0.16 mL, 1.55 mmol), and excess polyphosphoric acid (1 g) was heated at 110 °C for 6 h with stirring. The cooled mixture was poured into ice water (200 mL), and the solution was neutralised with NaOH (10 %). The solid product was filtered off, washed with water, dried and recrystallized from ethanol (Scheme 3). Color: Pale brown. Yield: 76.16 %. M.p.: 196-198 °C. FT-IR (KBr, ν, cm^{-1}): 3431, 3389 (NH_2), 3058 (CH Arom.), 1645 (C=O Amide).



Scheme 4

^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 5.49 (s, 1H, CH), 5.80 (s, 2H, NH_2), 6.89-7.63 (m, 15H, 3Ph). MS (EI, *m/z* (%)): 390 (M^+, 70.87), 350 (67.96), 347 (64.08), 335 (52.43), 312 (7.77), 271 (58.25), 246 (52.43), 236 (58.25), 204 (81.55), 161 (66.02), 103 (64.08), 79 (100), 77 (83.50), 76 (53.40). Anal. calcd. for $C_{25}H_{18}N_4O$: C, 76.91; H, 4.65; N, 14.35. Found: C, 77.40; H, 4.90; N, 14.70%.

2.2.16. Synthesis of 4-acetylamino-7,8,9-triphenyl-2H-pyrimido[1,2-b]pyridazin-2-one (11b)

A solution of compound **11a** (0.5 g, 1.28 mmol) in acetic anhydride (10 mL) was refluxed for 3 h. The solvent was then evaporated under reduced pressure; the solid product was collected and recrystallized from acetone (Scheme 3). Color: Dark brown. Yield: 79.57 %. M.p.: > 300 °C. FT-IR (KBr, ν, cm^{-1}): 3357 (NH Amide), 3059 (CH Arom.), 2979, 2932 (CH Aliph.), 1700 (C=O cyclic), 1634 (C=O amide). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.80 (s, 3H, CH_3), 6.56 (s, 1H, H-3), 7.70 (d, 1H, NH), 7.29-7.98 (m, 15H, 3Ph). MS (EI, *m/z* (%)):432 (M^+, 14.40), 377 (23.25), 372 (12.96), 353 (11.73), 350 (13.37), 328 (12.76), 314 (11.32), 305 (10.70), 227 (16.87), 205 (23.87), 125 (11.73), 117 (16.05), 102 (14.61), 84 (14.61), 80 (100), 59 (14.40), 53 (16.67). Anal. calcd. for $C_{27}H_{20}N_4O_2$: C, 74.98; H, 4.66; N, 12.95. Found: C, 74.70; H, 4.60; N, 12.70%.

2.2.17. Synthesis of ethyl 3-[(4,5,6-triphenylpyridazin-3-yl) imino]butanoate (12a)

A mixture of compound **5** (0.5 g, 1.55 mmol) and ethyl acetoacetate (0.2 mL, 1.55 mmol) was heated under reflux for 3 h. After cooling, the reaction mixture was treated with diethyl ether (50 mL), and the solid product formed was filtered off, dried and recrystallized from ethanol (Scheme 3). Color: Brown. Yield: 77.22 %. M.p.: 216-218 °C. FT-IR (KBr, ν, cm^{-1}): 3057 (CH Arom.), 2977, 2927 (CH Aliph.), 1718 (C=O ester), 1650 (C=N imino), 1231 (C-O acetate). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.8 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.91 (s, 3H, CH_3C=N), 2.60 (s, 2H, CH_2CO), 3.83 (q, 2H, CH_2CH$_3$), 6.95-7.63 (m, 15H, 3Ph). MS (EI, *m/z* (%)): 435 (M^+, 23.09), 390 (44.86), 361 (17.00), 358 (19.57), 349 (22.15), 322 (52.64), 307 (52.67), 256 (13.28), 229 (13.43), 206 (49.44), 178 (18.91), 176 (100), 128 (29.51), 98 (8.46), 87 (26.46), 74 (22.10). Anal. calcd. for $C_{28}H_{25}N_3O_2$: C, 77.22; H, 5.79; N, 9.65. Found: C, 77.60; H, 6.00; N, 10.00%.

2.2.18. Synthesis of 3-[(4,5,6-triphenylpyridazin-3-yl)imino] butanoic acid (12b)

Compound **12a** (0.5 g, 1.15 mmol) was added to a solution of potassium hydroxide (1 g) in ethanol (10 mL), then the reaction mixture was heated under reflux for 2 h. After cooling, the reaction mixture was poured into ice water (100 mL), and neutralized with hydrochloric acid. The solid product was filtered off, washed with water, dried and recrystallized from ethanol (Scheme 3). Color: Dark brown. Yield: 81.20 %. M.p.: > 300 °C. FT-IR (KBr, ν, cm^{-1}): 3384 (OH acid), 3059 (CH Arom.), 2922, 2852 (CH Aliph.), 1714 (C=O acid). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 1.95 (s, 3H, CH_3), 2.71(s, 2H, CH_2), 7.41-7.79 (m, 15H, 3Ph) 11.03 (s, 1H, COOH). MS (EI, *m/z* (%)): 407 (M^+, 38.30), 392 (39.01), 379 (41.13), 361 (43.97), 348 (48.23), 307 (46.10), 303 (40.43), 267 (44.68), 141 (52.48), 104 (11.35), 100 (42.55), 58 (8.51), 57 (100). Anal. calcd. for $C_{26}H_{21}N_3O_2$: C, 76.64; H, 5.19; N, 10.31.Found: C, 76.90; H, 5.40; N, 10.50%.

2.2.19. Synthesis of N-(4,5,6-triphenylpyridazin-3-yl) benzoylacetamide (13)

A mixture of compound **5** (0.5 g, 1.55 mmol) and ethyl benzoylacetate (0.27 mL, 1.55 mmol) was heated under reflux for 1 h. after cooling, the reaction mixture was treated with diethyl ether (50 mL), and the solid product was filtered off , dried and recrystallized from ethanol (Scheme 3). Color: Dark brown. Yield: 68.87 %. M.p.: 190-192 °C. FT-IR (KBr, ν, cm^{-1}): 3429 (NH amide), 3058 (CH arom.), 2925 (CH aliph.), 1723 (C=O ketone), 1677 (C=O, amide). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.31 (s, 2H, CH_2), 7.11-7.82 (m, 20H, 4Ph), 7.97 (s, 1H, NH). MS (EI, *m/z* (%)): 469 (M^+, 4.09), 363 (3.21), 350 (4.94), 322 (5.34), 291 (11.45), 263 (5.51), 206 (4.12), 177 (5.60), 163 (4.32), 105 (89.44), 103 (7.46), 77 (100). Anal. calcd. for $C_{31}H_{23}N_3O_2$: C, 79.30; H, 4.94; N, 8.95. Found: C, 79.60; H, 5.10; N, 8.50%.

2.2.20. General procedure for the reaction of 3-chloro-4,5,6-triphenylpyridazine (2) with 4-aminobenzenesulfonamide derivatives (14a-g)

A mixture of compound **2** (0.5 g, 1.46 mmol) and 4-aminobenzenesulfonamide derivatives **14a-g** (1.46 mmol) in *n*-butanol (10 mL) was refluxed for 5 h, cooled to room temperature, and the separated solid was filtered off, washed with water, dried, and recrystallised from ethanol to givecompounds **15a-g** (Scheme 4).

4-[(4, 5, 6-Triphenylpyridazin-3-yl)amino]benzenesulfon amide (**15a**): Color: White. Yield: 81.66 %. M.p.: 216-218 °C. FT-IR (KBr, ν, cm^{-1}): 3369, 3294, 3214 (NH, NH_2), 3078 (CH arom.), 1328 (SO_2 asym.), 1156 (SO_2 sym.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.21 (s, 2H, NH_2), 7.70-7.76 (m, 19H, Ar-H), 10.25 (s, 1H, NH). MS (EI, *m/z* (%)): 478 (M^+, 25.09), 463 (21.91), 373 (18.37), 361 (18.37), 322 (19.08), 305 (21.20), 178 (33.57), 172 (100), 118 (8.13), 103 (3.89). Anal. calcd. for $C_{28}H_{22}N_4O_2S$: C, 70.27; H, 4.63; N, 11.71. Found: C, 70.50; H, 4.70; N, 11.40%.

N-Benzyl-4-[(4, 5, 6-triphenylpyridazin-3-yl)amino]benzene sulfonamide (**15b**): Color: White. Yield: 90.43 %. M.p.: 118-120 °C. FT-IR (KBr, ν, cm^{-1}): 3379, 3292 (2NH), 3064 (CH arom.), 2931, 2877 (CH aliph.), 1311 (SO_2, asym.), 1154 (SO_2, sym.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.87 (s, 2H, CH_2), 5.91 (s, 1H, $NHSO_2$), 6.58-7.61 (m, 24H, Ar - H), 7.63 (s, 1H, NH). MS (EI, *m/z* (%)): 567 ([M-1]$^+$, 48.86), 477 (3.41), 466 (37.50),

452 (29.55), 390 (34.09), 362 (35.23), 341 (11.93), 305 (31.25), 288 (37.50), 260 (47.73), 234 (52.27), 179(43.18), 170 (31.25), 116 (36.93), 106 (69.89), 103 (44.89), 93 (100), 91 (32.39), 77 (16.48). Anal. calcd. for $C_{35}H_{28}N_4O_2S$: C, 73.92; H, 4.96; N, 9.85.Found: C, 73.60; H, 4.80; N, 10.10%.

N-Phenyl-4-[(4, 5, 6-triphenylpyridazin-3-yl)amino]benzene sulfonamide (**15c**): Color: White. Yield: 80.35 %. M.p.: 191-193 °C. FT-IR (KBr, ν, cm^{-1}): 3349, 3247 (2NH), 3068 (CH arom.), 1318 (SO_2 asym.), 1154 (SO_2 sym.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 5.94 (s, 1H, NH), 6.51-7.39 (m, 24H, Ar-H), 9.82 (s, 1H, $NHSO_2$). MS (EI, *m/z* (%)): 554 (M^+, 22.10), 462 (25.21), 399 (4.53), 376 (15.58), 350 (20.96), 326 (19.83), 306 (17.56), 245 (16.43), 206 (18.41), 178 (12.18), 154 (18.13), 129 (7.65), 107 (100). Anal. calcd. for $C_{34}H_{26}N_4O_2S$: C, 73.62; H, 4.72; N, 10.10. Found: C, 73.90; H, 4.90; N, 9.80%.

N-4-Tolyl-4-[(4, 5, 6-triphenylpyridazin-3-yl)amino]benzene sulfonamide (**15d**): Color: White. Yield: 84.4 %. M.p.: 188-190 °C. FT-IR (KBr, ν, cm^{-1}): 3344, 3128 (2NH), 3064 (CH arom.), 2918, 2857 (CH aliph.), 1320 (SO_2 asym.), 1152 (SO_2 sym.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.17 (s, 3H, CH_3), 5.91 (s, 1H, NH), 6.49-7.37 (m, 23H, Ar-H), 9.64 (s, 1H, $NHSO_2$). MS (EI, *m/z* (%)): 568 (M^+, 74.00), 540 (54.00), 526 (65.00), 477 (65.00), 450 (84.00), 435 (84.00), 401 (100), 399 (58.00), 388 (54.00), 312 (55.00), 308 (57.00), 279 (6.00), 261 (71.00), 169 (78.00), 105 (57.00), 76 (82.00). Anal. calcd. for $C_{35}H_{28}N_4O_2S$: C, 73.92; H, 4.96; N, 9.85. Found: C, 73.60; H, 5.10; N, 9.60%.

N-(4-Methoxyphenyl)-4-[(4, 5, 6-triphenylpyridazin-3-yl) amino]benzenesulfonamide (**15e**): Color: White. Yield: 86.75 %. M.p.: 198-200 °C. FT-IR (KBr, ν, cm^{-1}): 3388, 3282 (2 NH), 3074 (CH arom.), 2957, 2835 (CH aliph.), 1310 (SO_2 asym.), 1252 (C–O), 1148 (SO_2, sym,). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.66 (s, 3H, CH_3), 5.90 (s, 1H, NH), 6.48-7.32 (m, 23H, Ar-H), 9.43 (s, 1H, $NHSO_2$). MS (EI, *m/z* (%)): 584 (M^+, 44.78), 554 (41.79), 508 (28.86), 478 (28.36), 463 (25.87), 406 (41.79), 398 (33.83), 377 (26.87), 321 (28.36), 262 (41.79), 206 (34.83), 186 (28.36), 179 (29.85), 123 (32.34), 107 (80.10), 106 (100), 77 (42.79). Anal. calcd. for $C_{35}H_{28}N_4O_3S$: C, 71.90; H, 4.83; N, 9.58. Found: C, 71.50; H, 4.90; N, 9.30%.

N-(4-Chlorophenyl)-4-[(4, 5, 6-triphenylpyridazin-3-yl) amino]benzenesulfonamide (**15f**): Color: White. Yield: 87.3 %. M.p.: 193-195 °C. FT-IR (KBr, ν, cm^{-1}): 3414, 3347 (2NH), 3056 (CH aomr.), 1316 (SO_2 asym.), 1153 (SO_2 sym.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 5.98 (s, 1H, NH), 6.51-7.40 (m, 23H, Ar-H), 9.98 (s, 1H, $NHSO_2$). MS (EI, *m/z* (%)): 590.5 (M^++2, 0.60), 588.5 (M^+, 2.87), 486 (3.08), 477 (3.63), 396 (2.41), 282 (45.14), 156 (100), 155 (19.51), 127 (17.78). Anal. calcd. for $C_{34}H_{25}ClN_4O_2S$: C, 69.32; H, 4.28; N, 9.51. Found: C, 69.64; H, 4.20; N, 9.78%.

N-(4-Nitrophenyl)-4-[(4,5,6-triphenylpyridazin-3-yl) amino] benzenesulfonamide (**15g**): Color: White. Yield: 91.43 %. M.p.: 208-210 °C. FT-IR (KBr, ν, cm^{-1}): 3389, 3342 (2NH), 3061 (CH arom.), 1528 (NO_2 asym.), 1348 (NO_2 sym.), 1317 (SO_2 asym.), 1152 (SO_2 sym.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.05 (s, 1H, NH), 6.53-6.92 (m, 23H, Ar-H), 10.49 (s, 1H, $NHSO_2$). MS (EI, *m/z* (%)): 599 (M^+, 16.40), 554 (8.84), 479 (10.13), 422 (11.25), 407 (4.02), 395 (9.97), 371 (8.84), 323 (4.82), 293 (27.33), 277 (18.17), 206 (10.61), 202 (2.73), 191 (8.36), 178 (7.72), 138 (38.26), 92 (100). Anal. calcd. for $C_{34}H_{25}N_5O_4S$: C, 68.10; H, 4.20; N, 11.68. Found: C, 68.40; H, 4.10; N, 11.9%.

2.3. Antimicrobial activity

Applying the agar plate diffusion technique [18], the newly synthesised compounds were screened for their antimicrobial activity against some Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), yeast (*Candida albicans*), and fungi (*Aspergillus niger*). In this method, a standard 5 mm sterilised filter paper disk impregnated with the compound (0.3 mg in 0.1 mL of dimethylformamide) was placed on agar plate seeded with the tested organism. The plates were incubated for 24 h at 37 °C for bacteria, and for four days at 28 °C for fungi. The inhibition zone of bacteria and fungi growth around the disk were determined.

3. Results and discussion

3.1. Synthesis

During a study of the synthesis of pyridazine derivatives, 3-aminopyridazine (**5**) was selected as an ideal starting material for our purpose. Its preparation was accomplished *via* the classical Staudinger reaction [17]. Tetrazolopyridazine (**3**) was reduced to the corresponding aminopyridazine **5** *via* formation of phosphazene intermediate (**4**), as depicted in (Scheme 1). 3-Chloro-4, 5, 6-triphenylpyridazine (**2**) was obtained by treatment of 4,5,6-triphenylpyridazin-3(2*H*)-one(**1**) with phosphoryl chloride at refluxing temperature. Azidation of compound **2** with sodium azide in dimethyl formamide at 100 °C gave the corresponding tetrazolo[1,5-*b*]pyridazine, **3** [15,16]. Refluxing the latter compound with one equivalent of triphenylphosphine in 1,2-dichlorobenzene for 3 hours yielded 4,5,6-triphenyl-*N*-(triphenylphosphanylidene)pyridazin-3-amine (**4**) [17]. Subsequent acid hydrolysis of compound **4** with a mixture of acetic acid: water (4:1, *v*:*v*) provided the corresponding 3-aminopyridazine intermediate **5**, in 86% yield. The structure of compound **5** was established by the presence of stretching absorption bands at 3473 and 3280 cm^{-1} in the IR spectrum due to NH_2 group. It was also supported by the presence of D_2O exchangeable broad singlet band due to the NH_2 protons at 5.80 ppm in ^{1}H NMR. In addition, the mass spectrum showed the molecular ion peak M^+ at *m/z* = 323, which was in agreement with the calculated molecular weight ($C_{22}H_{17}N_3$).Treatment of compound **5**with hydrochloric acid/sodium nitrite solution, carried out at low temperature, gave a single product obtained from the diazonium ion, which was generated as an intermediate followed by ultimate hydroxylation. The isolated product was found to be identical with the authentic sample **1** (M.p., mixed m.p. and identical IR spectra), which was synthesized according to method A [14] (Scheme 1). Formylation, acetylation, and benzoylation of the amino group in compound **5** were achieved successfully by heating with formic acid/ dimethylformamide, acetic anhydride, and benzoyl chloride/ pyridine at refluxing temperature to afford *N*-(4,5,6-triphenyl pyridazin-3-yl)formamide **6a**, *N*-(4,5,6-triphenylpyridazin-3-yl)acetamide **6b** and *N*-(4,5,6-triphenylpyridazin-3-yl)benzamide **6c**, respectively.

Furthermore, reaction of compound **5** with chloroacetyl chloride in pyridine at room temperature yielded 2-chloro-*N*-(4,5,6-triphenylpyridazin-3-yl)acetamide **6d** (Scheme 2). The structures of compounds **6a-d** were confirmed on the basis of spectroscopic data (IR, Mass, and^{1}H NMR spectra) and elemental analysis as described in the experimental part. Reaction of the 3-aminopyridazine(**5**) with aromatic aldehydes under different reaction conditions did not produce the arylidene amino derivatives, but led only to the recovery of the starting material, possibly owing to steric hindrance of the bulky phenyl group in position four, and the electronic effect of sp^2 cyclic nitrogen atom. On the other hand, refluxing compound **5** with little excess of acetaldehyde for 4 hours afforded *N*-ethylidene-4,5,6-triphenylpyridazin-3-amine (**7**) (Scheme 2), as proved by the disappearance of NH_2 bands of the starting material in IR and ^{1}H NMR spectra. The reaction of compound **5** with active methylene compoundswas investigated, such as diethyl malonate at refluxing temperature for 3 hours gave the dimeric structure **8** (Scheme 2) as indicated from elemental analysis and spectral data (IR, Mass, and ^{1}H NMR spectra).

On the other hand, reaction of compound **5** with malonic acid in presence of phosphoryl chloride at refluxing temperature for 1 hour afforded 7,8,9-triphenyl-2*H*-pyrimido[1,2-*b*]pyridazine-2,4(3*H*)-dione (**9**) in good yield.

Table 1. Antimicrobial activity of the prepared compounds *.

Compound	Zone of inhibition					
	Staphylococcus Aureus (G^+)	*Bacillus Subtilis* (G^+)	*Escherichia coli* (G^-)	*Pseudomonas aeruginosa* (G^-)	*Candida albicans*	*Aspergillus niger*
5	+++++	+	++	++	-	-
6b	+	-	++	+++	++	++
9	++	++	+++	+++	++	+++
11a	++	++	++++	+++	++	+++
15a	++	+	-	-	++	+++
15b	++	+	+++	++	++	+++
15c	++	++	+	++	+++	+++++
15d	++	+++	+++	++	++	+++++
15e	+++	++++	++	+++	-	-
15f	+	+++	+++	++	++	+++++
15g	+++++	++	++	++	-	-
Septrin D.S.	++++	++++	+++	++++	-	-
Fungistatin	-	-	-	-	++++	++++

* The concentration ofthe all synthesised compounds and the two references was 0.30 mg in 0.10 mL of dimethylformamide. Zone of inhibition: + = < 15 mm; ++ = 15-24 mm; +++ = 25-34 mm; ++++ = 35-44 mm; +++++ = 45-54 mm, - = no inhibition.

However, when compound **5**was refluxed in diethyl oxalate, a product was isolated in 80% yield, which was proved to be ethyl oxo[(4,5,6-triphenylpyridazin-3-yl) amino]acetate, **10a** (Scheme 3) on the basis of elemental and spectral data. Refluxing compound **5** with ethyl cyanoacetate produced 2-cyano-*N*-(4,5,6-triphenyl pyridazin-3-yl) acetamide **10b** (Scheme 3), as proved by the presence of cyano group at 2204 cm^{-1} and amide carbonyl group at 1658 cm^{-1}, and the disappearance of the NH_2 bands in the IR spectrum. On the other hand, heating equimolar amounts of compound **5** and ethyl cyanoacetate in polyphosphoric acid at 110 °C for 6 hours, quenching the reaction mixture with water, and neutralizing with 10% sodium hydroxide, yielding 4-amino-7, 8, 9-triphenyl-2*H*-pyrimido[1,2-*b*]pyridazin-2-one (**11a**), which was identified on the basis of elemental analysis and spectral data.

The reaction of the 4-aminopyrimidopyridazine**11a** with acetic anhydride produced the corresponding 4-acetylamino derivative **11b** (Scheme 3), which affords a further evidence for the structure of the compound **11a**.

Fusion of the 3-aminopyridazine derivative **5** with ethyl acetoacetate was found to proceed steadily to give the condensation product **12a** (Scheme 3). The IR spectrum of compound **12a** showed the presence of ester carbonyl group at 1718 cm^{-1}, C=N imino group at 1650 cm^{-1}, and the disappearance of the NH_2 bands. The mass spectrum showed the molecular ion peak M^+ at *m/z*435. Further evidence for the structure of compound **12a** was obtained by hydrolysis of the latter compound in boiling ethanolic potassium hydroxide to yield the corresponding carboxylic acid **12b**. However, fusion of compound **5** with ethyl benzoylacetate produced *N*-(4,5,6-triphenylpyridazin-3-yl)benzoylacetamide, **13** (Scheme 3), as proved by IR spectrum showing absorption bands at 3429 (NH, amide), 1723(C=O, ketone) and 1677 cm^{-1} (C=O, amide). The mass spectrum revealed the molecular ion peak M^+ at *m/z*469.The study was also extended to prepare some sulfonamide derivatives containing the pyridazine nucleus with the aim to compare the antimicrobial activity relative to the systems prepared earlier [18]. When 3-chloropyridazine derivative **2** reacted with N^1-unsubstituted 4-aminobenzene sulfonamides **14a**, and N^1-substituted 4-aminobenzenesulfon amides **14b-g**, N^1-4-[(4, 5, 6-triphenylpyridzin-3-yl)amino] benzenesulfonamide, **15a-g** were produced (Scheme 4). The structure of the latter compounds was established on the basis of elemental analysis, IR, Mass, ^1H NMR spectra. The sulfonamide derivatives **14a-g** was prepared using a previously described method [19].

3.2. Antimicrobial activity bioassay

The results (Table 1) indicated that two synthesized compounds **5** and **15g**inhibited the growth of the Gram positive bacteria *Staphylococcus aureus* completely, while compound **15e** showed relatively very high antimicrobial activity against *Bacillus subtilis*. On the other hand, the latter compound **15e**; as well as compounds **15d** and **15f** showed high activity against both *Staphylococcus aureus* and *Bacillus subtilis*, respectively. Compounds **9**, **11a**, **15a**, **15b, 15c** and **15d** possessed moderate activity against *Staphylococcus aureus*, while compounds **9**, **11a**, **15c** and **15g** showed moderate inhibition of *Bacillus subtilis*. Only compounds **9**, **11a** and **15c** showed moderate antimicrobial activity against both examined *Staphylococcus aureus* and *Bacillus subtilis*. On the other hand, compound **11a** showed relatively very high antimicrobial activity against *Escherichia coli*. In addition, compounds **9**, **15b**, **15d** and **15f**; and compounds **6b**, **9**, **11_a** and **15e** possessed high activity against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. Compound **9** was highlyactive against both *Escherichia coli* and *Pseudomonas aeruginosa*. Also, the results indicated thatcompounds **5**, **6b**, **15e** and **15g**; and compounds **5**, **15b**, **15c**, **15d**, **15f** and **15g** showed moderate activity against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. Compounds **5** and **15g** were moderate active against both *Escherichia coli* and *Pseudomonas aeruginosa*. The results revealed that compounds **15c**, **15d** and **15f** showed complete inhibition for the growth of *Aspergillus niger*. In addition, compound **15c**; as well as compounds **9**, **11a**, **15a** and **15b** were highly active against *Candida albicans* and *Aspergillus niger*, respectively. Also, the results indicatedthat compounds **6b**, **9**, **11a**, **15a**, **15b**, **15d** and **15f**; as well as compound **6b** showed moderate activity against *Candida albicans* and *Aspergillus niger*, respectively. Compound **6b** showed moderateinhibition of both *Candida albicans* and *Aspergillus niger*. In general, the synthesised compounds showed moderate to high antimicrobial activity against the microorganisms under test comparable with the standard Septrin D.S. and Fungistatin.

4. Conclusion

A new series of aminopyridazine bearing sulfonamide moiety and pyrimidopyridazine were synthesized and biological evaluated. Compounds **5** and **15a-g** showed promising antibacterial activities, compared with the reference drugs.

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References

[1]. VanDerMey, M.; Boss, H.; Hatzelmann, A.; VanDerLaan, I. J.; Sterk, G. I.; Timmerman, H. *J. Med. Chem.* **2002**, *45*, 2520-2523.
[2]. Bradbury, R. H.; Bath, C.; Butlin, R. J.; Dennis, M.; Heys, C.; Hunt, S. J.; James, R.; Mortlock, A. A.; Sumner, N. F.; Tang, E. K.; Telford, B.; Whiting, E.; Wilson, C. *J. Med. Chem.* **1997**, *40*, 996-1004.
[3]. Barbara, R.; Betti, L.; Botta, M.; Corelli, F.; Giannaccini, G.; Maccari, L.; Manetti, F. G.; Strappahetti, G.; Corsano, S. *J. Med. Chem.* **2001**, *44*, 2118-3132.
[4]. Cignarella, G.; Barlocco, D. *J. Heterocycl. Chem.* **2002**, *39*, 545-550.
[5]. Mann, E.; Moisan, L.; Hou, J.; Rebek, J. *Tetrahedron Lett.* **2008**, *49*, 903-905.
[6]. Tucker, J. A.; Allwine, D. A.; Grega, K. C.; Barbachyn, M. R.; Klock, J. L.; Adamski, J. L.; Brickner, S.; Hutchinson, S. J.; Ford, D. K.; Zurenko, C. W.; Conradi, G. E.; Burton, R. A.; Jensen, P. R. M. *J. Med. Chem*. **1998**, *41*, 3727-3735.
[7]. Tamayo, N.; Liao, L.; Goldberg, M.; Powers, D.; Tudor, Y. Y.; Yu, V.; Wong, L. M.; Henkle, B.; Middleton, S.; Syed, R.; Harvey, T.; Jang, G.; Hungate, R.; Dominguez, C. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 2409-2413.
[8]. Benson, S. C.; Palabrica, C. A.; Snyder, J. K. *J. Org. Chem*. **1987**, *52*, 4610-4614.
[9]. Gyoten, M.; Nagaya, H.; Fukuda, S.; Ashida, Y.; Kawano, Y. *Chem. Pharm. Bull*. **2003**, *51*, 122-133.
[10]. Piaz, V. D.; Vergelli, C.; Giovannoni, M. P.; Scheideler, A. M. A.; Petrone, G.; Zaratin, P. *Il Farmaco* **2003**, *58*, 1063-1071.
[11]. Crespo, A.; Meyers, C.; Coelho, A.; Sotelo, E.; Fraiz, N.; Sotelo, E.; Maes, B. U. W.; Laguna, R.; Cano, E.; Lemière, G. L. F.; Ravina, E. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 1080-1083.
[12]. Drochioiu, G.; Sunel, V.; Onisco, C.; Basu, C.; Murariu, M. *Roum. Biotechnol. Lett.* **2001**, *6*, 155-165.
[13]. Tucaliuc, R. B.; Risca, I. M.; Drochioiu, G.; Mangalagiu, I. *Roum. Biotechnol. Lett.* **2008**, *13*, 3837-3842.
[14]. Schmidt, P.; Druey, J. *Helv. Chim. Acta* **1954**, *37*, 134-140.
[15]. Deeb, A.; Sterk, H.; Kappe, T. *Liebigs. Ann. Chem*. **1991**, *11*, 1225-1227.
[16]. Staudinger, H.; Meyer, J. *Helv. Chim. Acta*. **1919**, *2*, 635-646.
[17]. Deeb, A.; Said, S. A. *Collect. Czech. Chem. Commun.* **1990**, *55*, 2795-2799.
[18]. Bauer, A. W.; Mkriby, M. D. K.; Sherris, J. C.; Turck, M. *Am. J. Clin. Pathol.* **1966**, *45,* 493-496.
[19]. El-Mariah, F.; Nassar, E.; Hosny, M.; Deeb, A. *Phosphorus Sulfur* **2008**, *184*, 92-102.
[20]. Vogel, A. I.; Furniss, A.; Brian, S. Vogel's Textbook of Practical Organic Chemistry. 4th ed. Longman, 1980, pp. 651.