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Synthesis and antimicrobial evaluation of some new polyheterocyclic systems containing 1,2,4-triazine moiety

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ABSTRACT

7-(4-Chloro/3-nitrophenyl)-8-[5,6-diphenyl-4-(1,3-thiazol-2-yl)-1,4-dihydro-1,2,4-triazin-3-yl]-5-oxo-1,5-dihydro[1,2,4]triazolo[1,5-*a*]pyridine-6-carbonitrile (6a, b) was utilized as a key intermediate for the target polyheterocyclic systems. Reactions of 6a, b with halocarbonyl reagents followed by heterocyclization with bi-nitrogen nucleophiles gave some new nitrogen heterocycles (7-13). Structures of the new compounds were established by elemental analyses and spectral data. The synthesized compounds were screened for their antimicrobial activity.

1. Introduction

1,2,4-triazines and their derivatives occupy a pivotal position in modern medicinal chemistry, because of their high potential for biological activity. Various 1,2,4-triazine derivatives found application as pharmaceuticals, herbicides, pesticides and dyes [1-5]. Moreover, in recent years, 1,2,4-triazine compounds have been reported to possess biological activities as anti-AIDS [6], anticancer [7], anti-inflammatory [8], antihypertensive activities [9] and antimicrobial activities [10-11]. Prompted by the varied biological activities of 1,2,4-triazole moieties [12-13] that are bearing nitrogen heterocyclic system to study the biological activity. Derivatives of some new 1,2,4-triazines bearing nitrogen heterocyclic systems are generally prepared from 1,2-diamino pyridines [14-15]. In continuation of our work in the area of 1,2,4-triazine chemistry [16-19] and their heterocyclization via ring closure reactions with halocarbonyl reagents, the present work aimed to synthesize of some new 1,2,4-triazines bearing nitrogen heterocyclic systems, as well as their antimicrobial activity were screened.

2. Experimental

2.1. Instrumentation

Melting points were determined on a digital Stuart SMP-3 apparatus. Infrared spectra were recorded on FT-IR Bruker Vector 22 spectrophotometer using KBr wafer technique. ^{1}H NMR spectra were measured on Gemini spectrometer 200 MHz using DMSO-d_6 as solvent and TMS (Chemical sift in δ ppm) as an internal standard. Mass spectra were obtained through GCMS qp 1000 ex Schimadzu instrument mass spectrometer (70 eV). Elemental micro-analyses were performed at the Cairo University Microanalytical Center. The purity of the synthesized compounds was checked by thin layer chromatography on silica gel (silica gel, aluminum sheets 60 F254, Merck). Evaluation of antimicrobial activities was carried out by the Faculty of Agriculture for Girls Al-Azhar University, Nasr City, Cairo, Egypt. Ethyl 1,2-dimino-5-cyano-4-(4-chloro/3-nitrophenyl)-6-oxo-1,6-dihydro-pyridine-3-carboxylate (**2a,b**) were prepared according to the previous procedure [20].

2.2. Synthesis

2.2.1. Ethyl-7-(4-chloro/3-nitrophenyl)-6-cyano-5-oxo-1,5-dihydro[1,2,4]triazolo[1,5-a]pyridine-8-carboxylate (3a,b)

A mixture of compounds **2a, b** (0.01 mol) and triethyl orthoformate (0.02 mol) was refluxed for 3 h. The reaction mixture was cooled and the solid obtained was filtered off and crystallized to give compounds **3a**, **b**, respectively (Scheme 1).

Compound **3a** was crystallized from 1, 4-dioxane. Yield: 72%. M.p.: 238-240 °C. FT-IR (KBr) ν, (cm^{-1}): 3388 (NH), 2195 (C≡N), 1774, 1688 (2 C=O), 1614 (C=C), 1566 (C=N), 673 (C–Cl). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 1.45 (t, 3H, CH_3), 3.65 (q, 2H, CH_2CO), 7.64–8.31 (m, 5H, Ar-H), 6.39 (s, 1H, NH). Anal. Calcd. for $C_{16}H_{11}N_4O_3Cl$ (342.5): C, 56.05; H, 3.21; N, 16.35; Cl, 10.36. Found: C, 56.25; H, 3.52; N, 16.67; Cl, 10.13%.

Compound **3b** was crystallized from methanol. Yield: 76%. M.p.: 275- 278 °C. FT- IR (KBr) ν, (cm^{-1}): 3321 (NH), 2184 (C≡N), 1720, 1680 (2 C=O), 1610 (C=C), 1550 (C=N). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 2.52 (t, 3H, *J*=8.83Hz, CH_3), 3.43 (q, *J*=32.01 Hz, 2H, CH_2CO), 7.36–7.70 (m, 5H, ArH), 6.33 (s, 1H, NH). Anal.Calcd for $C_{16}H_{11}N_5O_5$ (353): C, 54.39; H, 3.11; N, 19.83. Found: C, 54.62; H, 3.32; N, 20.15%.

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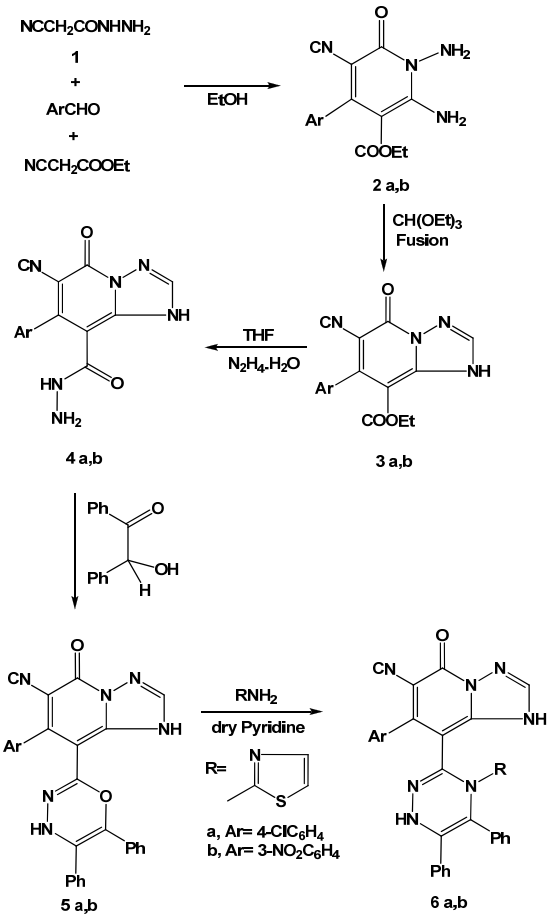


Scheme 1

2.2.2. 7-(4-Chloro/3-nitrophenyl)-6-cyano-5-oxo-1,5-dihydro[1,2,4]triazolo[1,5-a]pyridine-8-carbohydrazide (4a,b)

A mixture of compounds **3a, b** (0.01 mol) and hydrazine hydrate 98% (0.015 mol) in tetrahydrofuran (20 cm^3) was heated under reflux for 5 h and then allowed to cool. The precipitate that formed was filtered off, dried and crystallized to give compounds **4a, b**, respectively (Scheme 1).

Compound **4a** was crystallized from a mixture of methanol and DMF (1:1).Yield: 65%. M.p.: 187-189 °C. FT- IR (KBr) ν, (cm^{-1}): 3412, 3341, 3262, 31800 (NH_2, 2NH), 2213 (C≡N), 1736, 1676 (2C=O), 1626 (C=C), 1576 (C=N), 721 (C-Cl). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 4.36 (s, br, 2H, NH_2), 7.28–7.91 (m, 5H, ArH), 5.75 and 8.75 (2s, 2H, 2NH). Anal. Calcd. for $C_{14}H_9N_6O_2Cl$ (328.5): C, 51.14; H, 2.73; N, 25.57; Cl, 10.80. Found: C, 51.43; H, 3.01; N, 25.93; Cl, 10.45%.

Compound **4b** was crystallized from ethanol. Yield: 67 %. M.p.: 177-179 °C. FT- IR (KBr) ν, (cm^{-1}): 3423, 3356, 3272, 2289 (NH_2, 2NH), 1729, 1696 (2C=O), 1624 (C=C), 2218 (C≡N), 1556 (C=N). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 5.42 (s, br, 2H, NH_2), 6.95–7.76 (m, 5H, Ar-H), 6.76 (s, 1H, 1NH), 8.85 (s, 1H, 1NH). Anal. Calcd. for $C_{14}H_9N_7O_4$ (339): C, 49.55; H, 2.65; N, 28.90. Found: C, 50.03; H, 2.94; N, 28.55%.

2.2.3. 7-(4-Chloro/3-nitrophenyl)-8-(5,6-diphenyl-4H-1,3,4-oxadiazin-2-yl)-5-oxo-1,5-dihydro[1,2,4]triazolo[1,5-a]pyridine-6-carbonitrile (5a,b)

A mixture of compounds **4a, b** (0.01 mol) and 2-hydroxy-1,2-diphenylethanone (0.01 mol) in DMF (20 cm^3) was refluxed for 8 h, and then allowed to cool. The reaction mixture was poured onto ice-water. The solid product that formed was filtered off, dried and crystallized to give compounds **5a**, **b**, respectively (Scheme 1).

Compound **5a** was crystallized from isopropanol. Yield: 62 %. M.p.: 210–212 °C. FT-IR (KBr) ν, (cm^{-1}): 3344, 3206 (2NH), 1784 (C=O), 2214 (C≡N), 1621 (C=C), 1587 (C=N), 721 (C–Cl), ^{1}H NMR (200 MHz, DMSO-d_6), δ: 7.28–7.98 (m, 15H, Ar-H), 5.71 (s, 1H, NH), 8.71 (s, 1H, NH). Anal. Calcd. for $C_{28}H_{17}N_6O_2Cl$ (504.5): C, 66.60; H, 3.36; N, 16.65; Cl, 7.03. Found: C, 66.35; H, 3.17; N, 16.29; Cl, 6.74%.

Compound **5b** was crystallized from isopropanol. Yield: 71 %. M.p.: 203–205 °C. FT- IR (KBr) ν, (cm^{-1}): 3354, 3276 (2NH), 1695 (C =O), 2219 (C≡N), 1632 (C=C), 1540 (C=N), 727 (C–Cl). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 7.29–7.96 (m, 15H, Ar-H), 8.83 (s, 1H, NH), 11.23 (s, 1H, NH). Anal. Calcd. for $C_{28}H_{17}N_7O_4$ (515): C, 65.24; H, 3.30; N, 19.02. Found: C, 65.69; H, 3.66; N, 18.65%.

2.2.4. 7-(4-Chloro/3-nitrophenyl)-8-[5,6-diphenyl-4-(1,3-thiazol-2-yl)-1,4-dihydro-1,2, 4-triazin-3-yl]-5-oxo-1,5-dihydro[1,2,4]triazolo[1,5-a]pyridine-6-carbonitrile (6a,b)

A mixture of compounds **5a, b** (0.01 mol) and 2-aminothiozole (0.01 mol) in absolute ethanol (20 cm^3) containing piperidine (0.3 cm^3) was heated under reflux for 5 h and then allowed to cool. The precipitate that formed was filtered off, dried and crystallized to give compounds **6a, b**, respectively (Scheme 1).

Compound **6a** was crystallized from ethanol. Yield: 56%. M.p.: 194-196 °C. FT- IR (KBr) ν, (cm^{-1}): 3383, 3263 (2NH), 2213 (C≡N), 1688 (C=O), 1597(C=N), 791 (C–Cl). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 7.41–7.62 (m, 17H, Ar-H), 6.08 (s, 1H, NH), 8.73 (s, 1H, NH). Anal. Calcd. for $C_{31}H_{19}N_8OSCl$ (586.5): C, 63.42; H, 3.23; N, 19.09; S, 5.45; Cl, 6.05. Found: C, 63.05; H, 2.91; N, 18.75; S, 5.12; Cl, 6.16%.

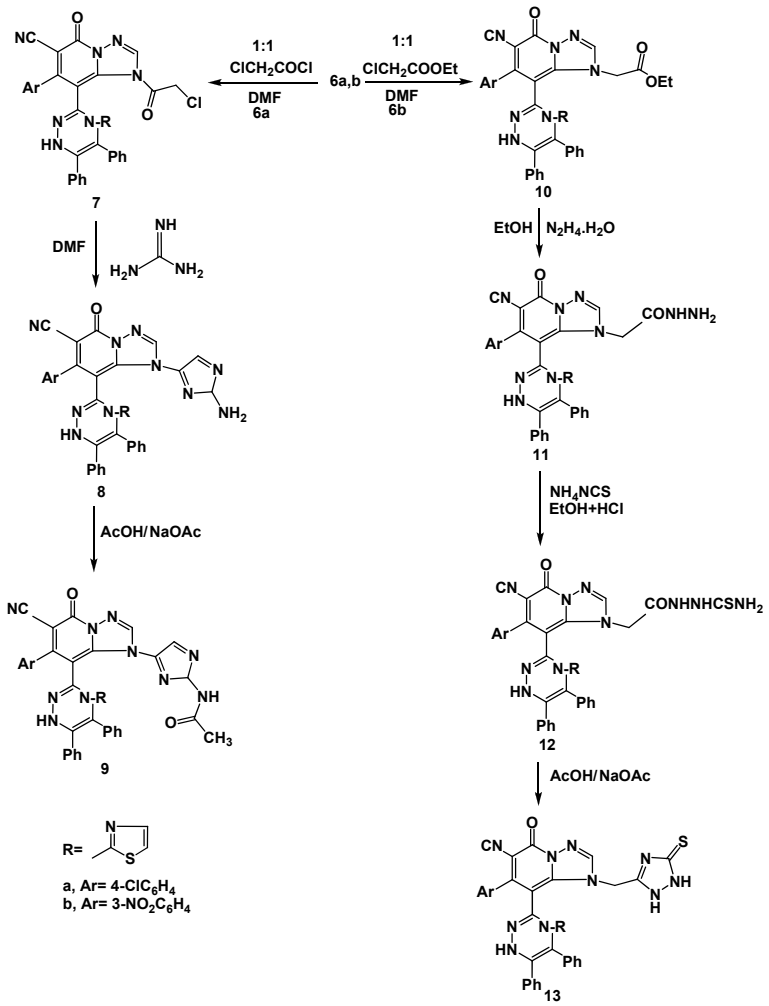
Compound **6b** was crystallized from isopropanol. Yield: 64 %. M.p.: 187-188 °C. FT- IR (KBr) ν, (cm^{-1}): 3374, 3263 (2NH), 2216 (C≡N), 1687 (C=O), 1567 (C=N). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 7.21–7.98 (m, 17H, Ar-H), 6.49 (s, 1H, NH), 8.67 (s, 1H, NH). MS (m/z, I %): 599 (M+2, 6%), 443 (4), 321 (17), 293 (7), 235 (13), 216 (6), 206 (10), 192 (13), 165 (17), 164 (12), 115 (13), 105 (100), 57 (14). Anal. Calcd. for $C_{31}H_{19}N_9O_3S$ (597): C, 62.31; H, 3.18; N, 21.10; S, 5.36. Found: C, 61.97; H, 2.89; N, 20.76; S, 5.11%.

2.2.5. 8-[5,6-Diphenyl-4-(1,3-thiazol-2-yl)-1H-1,2,4-triazin-3-yl]-7-(3-nitrophenyl)-5-oxo-1-(chloroacetyl)[1,2,4]triazolo [1,5-a]pyridine-6-carbonitrile (7)

An equimolar mixture of compound **6b** (0.01 mol) and chloroacetyl chloride (0.01 mol) in DMF (20 cm^3) was refluxed for 2h, and then allowed to cool. The reaction mixture was poured onto ice water. The solid product that formed was filtered off, dried and crystallized from a mixture of methanol and water (1:1) (Scheme 2) .Yield: 80%. M.p.: 252-255 °C. FT-IR (KBr) ν, (cm^{-1}): 3265 (NH), 2985 (CH_2), 2214 (C≡N), 1654, 1686 (2C=O), 1594 (C=N). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 2.89 (s, 2H, CH_2), 7.52-8.3, (m, 17H, Ar-H), 9.76 (s, 1H, NH). Anal. Calcd. for $C_{33}H_{20}N_9O_4SCl$ (673.5): C, 58.79; H, 2.96; N, 18.70; S, 4.75; Cl, 5.27. Found: C, 58.45; H, 2.60; N, 18.41; S, 4.46; Cl, 5.03 %.

2.2.6. 8-[5,6-diphenyl-4-(1,3-thiazol-2-yl)-1H-1,2,4-triazin-3-yl]-7-(3-nitrophenyl)-5-oxo-1-(2-Amino-4H-imidazol-5-yl)[1,2,4]triazolo[1,5-a]pyridine-6-carbonitrile (8)

A mixture of **7** (0.01 mol) and guanidine hydrochloride (0.01 mol), dissolved in a little amount of water (3 cm^3), in DMF (30 cm^3) was heated under reflux for 10 h, and then allowed to cool. The reaction mixture was poured onto ice water. The solid



Scheme 2

product that formed was filtered off, dried and crystallized from methanol (Scheme 2). Yield: 60%. M.p.: 227- 230 °C. FT-IR (KBr) ν, (cm^{-1}): 3395, 2930(NH_2), 1487 (*def.* CH_2), 2213 (C≡N), 1686 (C=O), 1578 (C=N). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 6.55 (s, br, 2H, NH_2), 7.64–8.53 (m, 20H, Ar-H), 8.98 (s, 1H, NH). Ms, m/z (I %): 677 (M^+, 10%), 597 (11), 421 (13), 317 (100), 227 (3), 216 (6), 115 (21), 57 (11). Anal. Calcd. for $C_{34}H_{21}N_{12}O_3S$ (677): C, 60.26; H, 3.21; N, 24.81; S, 4.72. Found: C, 59.93; H, 3.43; N, 24.45; S, 4.37%.

2.2.7. 8-[5,6-diphenyl-4-(1,3-thiazol-2-yl)-1H-1,2,4-triazin-3-yl]3-[6-cyano-7-(3-nitro-phenyl-5-oxo-[1,2,4]triazolo[1,5-a]pyridine-8-yl]-1-(4H-imidazol-2-yl)acetamide (9)

A suspension of **8** (0.01 mol) in glacial acetic acid (30 cm^3) with fused sodium acetate (3 g) was heated under reflux for 4 h , cooled and poured onto crushed ice. The precipitate that formed was filtered off, dried and crystallized from benzene (Scheme 2). Yield: 59%. M.p.: 221-223 °C. FT- IR (KBr) ν, (cm^{-1}): 3278 (NH), 2982 (CH_3), 2845 (CH_2), 2217 (C≡N), 1676, 1662 (2 C=O), 1594 (C=N). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 2.68 (s, 3H, CH_3), 7.36–8.28, (m, 22H, Ar-H), 8.78 (s, 1H, NH), 9.37 (s, 1H, NH). Ms, m/z (I %): 719 (M^+, 11%), 597 (19), 443 (15), 363 (11), 252 (31), 216 (38) 178 (63), 158 (32), 77 (100), 57(21). Anal. Calcd. for $C_{36}H_{23}N_{12}O_4S$ (719): C, 58.41; H, 3.19; N, 23.36; S, 4.45. Found: C, 58.64; H, 3.59; N, 23.61; S, 4.66%.

2.2.8. Ethyl-8-[5,6-Diphenyl-4-(1,3-thiazol-2-yl)-1H-1,2,4-triazin-3-yl]-3[7-(4-chloro-phenyl)-6-cyano-5-oxo-[1,2,4]triazolo[1,5-a]pyridine-8-yl]-1-acetate (10)

A mixture of compound **6a** (0.01 mol) and ethyl chloroacetate (0.01 mol) in DMF (20 cm^3) was refluxed for 2 h, the reaction mixture was poured onto crushed ice and the separated solid was filtered off, dried and crystallized from ethanol (Scheme 2). Yield: 74 %. M.p.: 189-192 °C. FT- IR (KBr) ν, (cm^{-1}): 3434 (NH), 2982 (CH_2), 2214 (C≡N), 1685, 1670 (2 C=O) 1488 (def. CH_2), 1556 (C=N), 696 (C–Cl). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 1.55 (t, 3H, CH_3), 3.97 (s, 2H, CH_2), 7.25–7.96 (m, 17H, Ar-H), 9.78 (s, 1H, NH). Anal. Calcd. for $C_{35}H_{25}N_8O_3SCl$ (672.5): C, 62.45; H, 3.71; N, 16.65; S, 4.75; Cl, 5.27. Found: C, 62.09; H, 3.51; N, 16.75; S, 4.51; Cl, 5.15%.

2.2.9. 8-[5,6-Diphenyl-4-(1,3-thiazol-2-yl)-1H-1,2,4-triazin-3yl]-3-[7-(4-chlorophenyl)-6-cyano-5-oxo[1,2,4]triazolo[1,5-a]pyridine-8-yl)]1-carbohydrazide (11)

A mixture of compound **10** (0.01 mol) and hydrazine hydrate 98 % (0.01 mol) in absolute ethanol (25 cm^3) was refluxed for 2 h, the reaction mixture was poured onto crushed ice and the separated solid was filtered off, dried and crystallized from a mixture of DMF and water (1:1) (Scheme 2). Yield: 69%. M.p. > 280 °C. FT- IR (KBr) ν, (cm^{-1}): 3327, 3290 (NH_2), 3229 (NH), 3209 (NH), 3080 (CH_2), 2219 (C≡N), 1695, 1663 (2C=O), 776 (C–Cl). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 2.82

(s, 2H, CH_2CO), 5.72 (s, br, 2H, NH_2), 8.78 (s, 1H, NH), 9.25 (s, 1H, NH). Anal. Calcd. for $C_{33}H_{23}N_{10}O_2SCl$ (658.5): C, 60.13; H, 3.49; N, 21.26; S, 4.85; Cl, 5.39. Found: C, 60.48; H, 3.84; N, 21.52; S, 5.21; Cl, 5.64%.

2.2.10. 8-[5,6-Diphenyl-4-(1,3-thiazol-2-yl)-1,2,4-triazin-3-yl]-3-(7-(4-chloro-phenyl)-6-cyano-5-oxo[1,2,4]triazolo[1,5-a]pyridine-8-yl)-1-carbonyl-methylhydrazinecarbo thioamide (12)

A mixture of compound **11** (0.01 mol) and ammonium thiocyanate (0.01 mol) in ethanol (20 cm^3) and HCl (3 cm^3) was refluxed for 4 h and then allowed to cool. The precipitate that formed was filtered off, dried and crystallized from DMF (Scheme 2). Yield: 80%. M.p.: 257-259 °C. FT- IR (KBr) ν, (cm^{-1}): 3385, 3289 (NH_2), 3266, 3184, 3147 (3 NH), 2991 (CH_2), 2214 (C≡N), 1686, 1653 (2C=O), 1623 (def. NH_2), 1590 (C=N), 1484 (def. NH_2), 1237 (C=S), 765 (C–Cl). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 3.87 (s, 2H, CH_2CO), 4.94 (s, br, 2H, NH_2), 7.25–8.35 (m, 22H, Ar-H), 7.98 (s, 1H, NH), 8.88 (s, 1H, NH), 9.12 (s, 1H, NH). Anal.Calcd. for $C_{34}H_{24}N_{11}O_2S_2Cl$ (717.5): C, 56.86; H, 3.34; N, 21.46; S, 8.91 Cl, 4.94. Found: C, 56.52; H, 3.59; N, 21.80; S, 8.49; Cl, 4.70%.

2.2.11. 7-(4-Chlorophenyl)-8-[5,6-diphenyl-4-(1,3-thiazol-2-yl)-1H-1,2,4-triazin-3-yl]-1-[(5-thioxo-2,5-dihydro-1H-1,2,4-trazolo-3-yl)methyl]-5-oxo[1,2,4]triazolo[1,5-a] pyridine-6-carbonitrile (13)

A suspension of **12** (0.01 mol) in glacial acetic acid (30 cm^3) and fused sodium acetate (3 g) was heated under reflux for 4h, cooled and poured onto crushed ice. The formed solid product was collected by filtration, dried and crystallized from methanol (Scheme 2). Yield: 60%. M.p.: 259-262 °C. FT- IR (KBr) ν, (cm^{-1}): 3212, 3209, 3184 (3 NH), 2945 (CH_2), 2216 (C≡N), 1674 (C=O), 1565 (C=N), 1285 (C=S), 768 (C–Cl). ^{1}H NMR (200 MHz, DMSO-d_6), δ: 4.12 (s, 2H, CH_2), 7.26–8.38 (m, 20H, Ar-H), 8.96 (s, 1H, NH), 9.57 (s, 1H, NH), 9.89 (s, 1H, NH). Ms, m/z (I %): 699.5 (M^+, 3%), 570 (13), 119 (4), 114 (3), 105 (100), 70 (13), 41 (17). Anal. Calcd. for $C_{34}H_{22}N_{11}OS_2Cl$ (699.5): C, 58.32; H, 3.14; N, 22.01; S, 9.14 Cl, 5.07. Found: C, 58.66; H, 3.43; N, 22.35; S, 9.35; Cl, 5.31%.

2.3. Antimicrobial Screening

Representative compounds of the synthesized products were screened *in vitro* for their antimicrobial activities against two strains of bacteria *Bacillus subtilis* and *Escherichia coli* and two strains of fungi *Aspergillus fumigatus and Candida albicans* by the agar diffusion method [21–23]. The dishes were incubated at 37 °C for 48 hr (for bacteria) and at 30 °C for 72 hr (for fungi), where clear or inhibition zones were detected around each hole. Each 0.1 mL of DMF alone was used as a control under the same conditions for each microorganism and by subtracting the diameter of inhibition zone resulting with DMF alone from that obtained from that obtained in each case as a mean of three replicates (Table 1). Terbinafin used as a standard antifungal agent and Chloramphenicol used as a standard antibacterial agent.

3. Results and Discussion

3.1. Synthesis

The reaction of ethyl 1,2-diamino-5-cyano-4-(4-chloro/3-nitrophenyl)-6-oxo-1,6-dihydropyridine-3-carboxylate **(2a,b)** with triethylorthoformate followed by hydrazinolysis of compound **3a, b** in boiling ethanol afforded the corresponding carbohydrazide derivatives **4a,b** (Scheme 1). Cyclocondensation of **4a, b** with 2-hydroxy-1,2-diphenylethanone in DMF afforded the required starting compound**s** 7-aryl 8-oxadiazinyltriazolopyridine derivatives **5a,b**. Structures of the compounds **5a,b** were based on analytical and spectral data. The ^{1}H NMR spectrum of **5a** revealed the appearance of characteristic singlet at δ 5.71 and 8.77 ppm assigned to NH protons of 1,3,4-oxadiazione moieties.

The compounds **5a, b** were used for the synthesis of 1,2,4-triazine systems. Thus, cyclocondensation of **5a,b** with 2-amino-1,3-thiazole in dry pyridine [24] gave 7-(4-chloro/3-nitrophenyl)-8-[5,6-diphenyl-4-(1,3-thiazol-2-yl)-1,4-dihydro-1,2,4-triazin-3-yl]-5-oxo-1,5-dihydro[1,2,4]triazolo[1,5-*a*]pyridine-6-carbonitrile (**6a,b**) (Scheme 1). Structures of compounds **6a**, **b** were elucidated on the basis of elemental analysis and spectral data (see Experimental section).

Chemical reactivity of interesting functionally substituted 1,2,4-triazines **6a,b** have received considerable attention. Thus, acylation of **b** using chloroacetyl chloride in dry DMF afforded 8-[5,6-diphenyl-4-(1,3-thiazol-2-yl)-1H-1,2,4-triazin-3-yl]-7-(3-nitrophenyl)-5-oxo-1-(chloroacetyl)[1,2,4]triazolo[1,5-*a*]pyridine-6-carbonit-rile (**7**) which underwent cyclocondensation reaction with guanidine hydrochloride in the presence of DMF to give 8-triazinyltriazolopyridine derivative **8** (Scheme 2). Disappearance of only NH triazole proton in the ^{1}H NMR spectrum of **7** supported that acylation process occurred on NH triazole and not NH triazine. Also, the presence of amino group in compound **8** has been confirmed by acylation with acetic acid in the presence of fused sodium acetate to give the acetamide derivative **9** (Scheme 2). The IR spectrum of **9** showed two characteristic absorption bands at 3278 and 2982 cm^{-1} due to NH and methyl group in addition to the carbonyl absorption band at 1662 cm^{-1}. Its ^{1}H NMR spectrum revealed two characteristic signals at 8.78 and 9.37 ppm assigned to two NH protons. Also, its mass spectrum showed the molecular ion peak at *m/z* 719 [M^+] and the base peak at *m/z* 77.

On the other hand, alkylation of compound **6a** with ethyl chloroacetate in the presence of DMF produced **10**, which on refluxing with hydrazine hydrate in absolute ethanol yielded the carbohydrazide derivative **11**. Treatment of the latter compound with ammonium thiocyanate afforded acylthio semicarbazide derivative **12**. An intramolecular cyclocondensation of **12** was carried out by refluxing it in glacial acetic acid in the presence of fused sodium acetate to yield 5-thioxo-1,2,4-trazolo pyridine derivative **13** (Scheme 2).

The structures of compounds **10-13** were confirmed from elemental analysis and spectral data. The IR spectrum data of **10** showed disappearance of the absorption band due to NH group with the presence of new absorption band at 1685 cm^{-1} for C=O ester group, while that of **11** showed absorption bands at 3327, 3229 and 1663 cm^{-1} due to NH_2, NH and C=O groups, respectively.

On the other hand, the structure of **13** was elucidated on the basis of elemental analysis and spectral data. Its IR spectrum showed absorption bands at 3212, 3209 and 3184 cm^{-1} due to three (NH) groups, with absence of both NH_2 and C=O bands which revealed that cyclocondensation has occurred between NH_2 and C=O in the side chain of acylthio-semicarbazide **12**. Its ^{1}H NMR spectrum revealed the appearance of singlets at 8.96, 9.57, 9.89 ppm due to three different NH protons.

3.2. Antimicrobial activities

The results of the biological studies are summarized in Table 1. From the data it is clear that most of the synthesized compounds were found to possess various antimicrobial activities towards all the microorganisms tested. The antimicrobial activities are carried out against two bacterial strains, *B. subtilis* and *E. coli* and two fungal strains, namely *A.*

fumigates and *C. albicans*. In general, most of the synthesized compounds showed a greater inhibitory effect against both the bacterial and fungal strains compared to the starting material **3** which confirmed improving biological properties. However, compound **10** showed maximum antimicrobial activity comparable to the standard drugs. We can conclude form the preliminary antimicrobial screening that the compounds **5a**, **6a**, **7**, **9**, **10** and **13** enhance the biological properties due to the presence of bioactive moieties as 1,2,4-triazol and 1,2,4-triazine.

Table 1. Antibacterial and antifungal activity data*.

Compound No	Antibacterial activity		Antifungal activity	
	Gram +	Gram -		
	Bacillus subtilis	*Escherichia coli*	*Aspergillus fumigatus*	*Candida albicans*
2a	++	++	+	+
3a	+	+	+	+
5a	+	++	+	++
5b	+	+	+	+
6a	++	+	++	++
6b	++	++	++	++
7	+	+	+	+
8	++	+	++	+
9	+	+	+	+
10	+++	++	++	+
11	+	+	+	+
12	++	+	+	+
13	+	+	++	++
Chloramphenicol	+++	+++	-	-
Terbianfin	-	-	+++	++
Solvent Control (DMF)	-	-	-	-

* The test done using the diffusion agar technique: Well diameter = 0.06 cm; Inhibition values = 0.1–0.5 cm beyond control = + (less active); Inhibition values = 0.6–1.0 cm beyond control = ++ (moderate active); Inhibition values =1.1–1.5 cm beyond control= +++ (highly active); solvent control: DMF for antibacterial; Chloramphenicol for antibacterial: Terbianfin for antifungal.

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