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Synthesis and antimicrobial evaluation of some annelated phthalazine derivatives and acyclo C-nucleosides from 1-chloro-4-(2,4,6-trimethylphenyl) phthalazine precursor

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A highly efficient and versatile synthetic approach to the synthesis of annelated phthalazine derivatives viz. 1,2,4-triazolo[3,4-a]phthalazine **11a,b, 14, 18, 19a,b, 29-31, 33,** 1,2,4-triazino[3,4-a]phthalazine **25a,b-28**, 1,3,5-triazino[4,3-a]phthalazine **22**, tetrazolo[5,1-a] phthalazine **23,** imidazophthalazine **9a,b,15**, and pyrimidinophthlazine **6, 10, 16, 17, 20** is presented. Moreover, acyclo C-nucleoside and double headed acyclo C-nucleoside of 1,2,4-triazolo[3,4-a]phthalazine **12, 13** were obtained via heterocyclization reaction of 1-chloro-4-(2,4,6-trimethylphenyl)phthalazine **(4)** with gluconic acid hydrazide and galactaric acid bis hydrazide, respectively. The new compounds were synthesized with the objective of studying their antimicrobial activity.

Key Words: Triazinophthalazine, pyrazolylphthalazine, triazolophthalazine, antimicrobial activity

Introduction

The synthesis of new compounds and testing their biological and pharmacological activities are the major goals of drug development projects. Nitrogen-containing heterocyclic compounds have received much attention as shown by the numerous studies published on their applicability in different areas, especially as drugs.[1,2] Phthalazines are examples of nitrogen heterocycles that possess exciting biological properties.[3–5] They form the structural profile for several biologically active compounds and hence they are considered

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important key elements. Several reports in the literature have focused on the pharmacology of phthalazine derivatives. These reports have resulted in a great number of contributions in diverse areas of interest.[6–11] Phthalazines have been reported to possess, anticonvulsant,[12] cardiotonic,[13] antimicrobial,[14] antitumor,[15–18] antihypertensive,[19,20] antithrombotic,[21] antidiabetic,[22,23] antitrypanosomal,[24] anti-inflammatory,[25–31] and vasorelaxant activities.[20,32] Additionally, phthalazines have recently been reported to potentially inhibit serotonin reuptake and are considered anti-depression agents.[33] Therefore, a number of methods have been reported for the synthesis of phthalazine derivatives.[12,34–40] Nevertheless, the development of new synthetic methods for the efficient preparation of heterocycles containing phthalazine ring fragment is an interesting challenge. In view of the aforementioned facts, it seemed most interesting to study the chemical behavior of 1-chloro-4-(2,4,6-trimethylphenyl) phthalazine **(2)** towards some nitrogen and carbon nucleophiles to produce new phthalazine derivatives with the aim to evaluate their antimicrobial activities.

Experimental

Melting points were determined on a MEL-TEMP II melting point apparatus in open glass capillaries and are uncorrected. The infrared spectra IR were recorded for potassium bromide disks on a Pye-Unicam SP1025 spectrophotometer. NMR spectra were obtained at ambient temperature (∼25 °C) with a Bruker AC-250 spectrometer or with a Varian Gemini 200 spectrometer at 250 MHz using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were obtained on a Hewlett-Packard 5995 gas chromatography-mass spectrometer system or on a Shimadzu GCMS-QP 1000 EX mass spectrometer. Homogeneity of the products and follow up of the reactions were checked by ascending thin-layer chromatography TLC on plates precoated with silica gel G (E. Merck; layer thickness 0.25 mm), used without pretreatment. Elemental microanalyses were performed at the Microanalytical Unit, Cairo University, Cairo, Egypt.

4-(2,4,6-Trimethylphenyl)lphthalazin-1(2*H*)-one (3)

Hydrazine hydrate (0.3 mL, 98%) was added to a solution of compound **2** (0.01 mol) in absolute ethanol and the reaction mixture was refluxed for 2 h. After cooling, the mixture to room temperature, a solid was obtained. This crude product was filtered off and recrystallized from ethanol to give **3**. Yield: 65%; mp 235-237 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3), 2.50 (s, 6H, $2CH_3$), 7.27-8.50 (m, 6H, Ar–H), 11.17 (s, 1H, NH, exchangeable with D_2O); IR (KBr) v: 3212 (NH), 1654 cm^{-1} (CO); MS (70 eV) m/z (%): 264 (M^+, 18). Anal. calcd. for $C_{17}H_{16}N_2O$: C 77.25, H 6.10, N 10.60; found C 77.16, H 6.18, N 10.70.

1-Chloro-4-(2,4,6-trimethylphenyl)phthalazine (4)

A suspension of compound **3** (0.01 mol) and PCl_5 (0.01 mol) in 8 mL of $POCl_3$ was heated under reflux on a water bath for 2 h. Then the mixture was cooled to room temperature and poured into crushed ice slowly. The solid formed was filtered off, washed with cold water, dried and recrystallized from benzene to give **4**. Yield: 70%; mp 160-161 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.40 (s, 3H, CH_3), 2.51 (s, 6H, $2CH_3$), 7.01-8.04 (m, 6H, Ar–H); IR (KBr) v: 1605 (C=N), 847 cm^{-1} (C-Cl); MS (70 eV) m/z (%): 284 (M^+Cl^{37}, 1), 282 (M^+Cl^{35}, 4). Anal. calcd. for $C_{17}H_{15}ClN_2$: C, 72.21; H, 5.35; N, 9.91; found C, 72.30; H, 5.41; N, 9.89.

5-(2,4,6-Trimethylphenyl)-8*H*-phthalazino[1,2-b]quinazolin-8-one (6)

In a fusion tube provided with an air condenser, a mixture of **4** (0.001 mol) and anthranilic acid (0.005 mol) was heated in an oil bath at 190-191 °C for 2 h. Then the mixture was cooled (to room temperature) and poured into 40 mL of cold water. The obtained solid product was collected and recrystallized from benzene to give **6**. Yield: 64%; mp. 220-223 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 2.45 (s, 6H, $2CH_3$), 6.98-8.13 (m, 10H, Ar–H); ^{13}C-NMR (DMSO-d_6) δ: 20.9, 23.5, 122.2, 125.8, 126.5, 127.1, 127.6, 127.9, 128.5, 128.9, 132.0, 132.6, 133.3, 134.9, 136.2, 140.6, 145.7, 146.3, 147.8, 155.5, 170.1; IR (KBr) v: 1670 (CO), 1614 cm^{-1} (C=N); %MS (70 eV) m/z (%): 365 (M^+, 8). Anal. calcd. for $C_{24}H_{19}N_3O$: C, 78.88; H, 5.24; N, 11.50; found C, 78.94; H, 5.19; N, 11.44.

General procedure for the synthesis of phthalazinylamino acids 7a,b and 8

The corresponding amino acid (0.01 mol) and sodium carbonate (0.005 mol) were dissolved in water (15 mL), and then adjusted to pH 9-9.5. Then compound **4** (0.005 mol) was added to it and refluxed at the same pH for 8 h. The reaction mixture was left overnight at room temperature, and then treated with cold hydrochloric acid (pH 0.5). The solid product obtained was filtered off, washed with water, and recrystallized from an appropriate solvent to give the target compound.

2-(4-(2,4,6-Trimethylphenyl)phthalazin-1-ylamino)acetic acid (7a)

Yield, 70% (dioxane); mp 243-244 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 2.42 (s, 6H, $2CH_3$), 4.61 (s, 2H, CH_2), 7.03-8.17 (m, 7H, ArH and NH), 10.81 (brs, 1H, OH, exchangeable with D_2O); IR (KBr) v: 3280-2510 (OH, NH), 1702 cm^{-1} (CO); MS (70 eV) m/z (%): 321 (M^+, 7). Anal. calcd. for $C_{19}H_{19}N_3O_2$: C, 71.01; H, 5.96; N, 13.08; found C, 71.10; H, 6.02; N, 13.00.

2-(4-(2,4,6-Trimethylphenyl)-1-ylamino)propanoic acid (7b)

Yield, 68% (dioxane); mp 233-234 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.53 (d, J = 10 Hz, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.51 (s, 6H, $2CH_3$), 4.50 (q, J = 10 Hz, 1H, CH), 7.11-8.12 (m, 7H, ArH and NH), 10.60 (brs, 1H, OH, exchangeable with D_2O); IR (KBr) v: 3240-2500 (OH, NH), 1694 (CO), 1605 cm^{-1} (C=N); MS (70 eV) m/z (%): 335 (M^+, 6). Anal. calcd. for $C_{20}H_{21}N_3O_2$: C, 71.62; H, 6.31; N, 12.53; found C, 71.57; H, 6.38; N, 12.60.

3-(4-(2,4,6-Trimethylphenyl)phthalazin-1-ylamino)propanoic acid (8)

Yield, 70% (ethanol); mp 244-245 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.44 (s, 6H, $2CH_3$), 2.81 (t, J = 10 Hz, 2H, NCH_2), 3.77 (t, J = 10 Hz, 2H, CH_2CO), 7.00-8.18 (m, 7H, ArH and NH), 11.00 (brs, 1H, OH, exchangeable with D_2O); IR (KBr) v: 3288-2511 (OH, NH), 1700 (CO), 1605 cm^{-1} (C=N); MS (70 eV) m/z (%): 335 (M^+, 4). Anal. calcd. for $C_{20}H_{21}N_3O_2$: C, 71.62; H, 6.31; N, 12.53; found C, 71.71; H, 6.23; N, 12.50.

General procedure for the synthesis of imidazophthalazines 9a,b and 10

A mixture of **7a, 7b,** or **8** (0.01 mol), acetic anhydride (30 mL), and anhydrous sodium acetate (0.82 g, 0.01 mol) was heated under reflux for 3 h. The solvent was removed under reduced pressure and the residue washed with water, filtered, dried, and crystallized from appropriate solvent to give **9a,b** and **10**.

6-(2,4,6-Trimethylphenyl)imidazo[2,1-a]phthalazin-3(2*H*)-one (9a)

Yield, 60% (DMF); mp 179-180 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.40 (s, 6H, $2CH_3$), 5.03 (s, 2H, CH_2), 6.98-8.05 (m, 6H, ArH); IR (KBr) v: 1685 (CO), 1610 cm^{-1} (C=N); MS (70 eV) m/z (%): 303 (M^+, 19). Anal. calcd. for $C_{19}H_{17}N_3O$: C, 75.23; H, 5.65; N, 13.85; found C, 75.19; H, 5.71; N, 13.92.

6-(2,4,6-Trimethylphenyl)-2-methylimidazo[2,1-a]phthalazin-3(2*H*)-one (9b)

Yield, 63% (benzene); mp 170-171 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.64 (d, J = 10 Hz, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.44 (s, 6H, $2CH_3$), 4.90 (q, J= 10 Hz, 1H, methine proton), 7.12-8.13 (m, 6H, ArH); ^{13}C-NMR (DMSO-d_6, 300 MHz) δ: 19.3, 20.4, 23.0, 66.2, 127.0, 127.8, 129.2, 129.9, 130.4, 132.0, 132.5, 132.9, 137.1, 139.2, 144.0, 158.3, 180.5; IR (KBr) v: 1670 (CO), 1604 cm^{-1} (C=N). Anal. calcd. for $C_{20}H_{19}N_3O$: C, 75.69; H, 6.03; N, 13.24; found C, 75.77; H, 6.10; N, 13.14.

7-(2,4,6-Trimethylphenyl)-2*H*-pyrimido[2,1-a]phthalazin-4(3*H*)-one (10)

Yield, 65% (ethanol); mp 199-200 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 2.46 (s, 6H, $2CH_3$), 3.04 (t, J = 10 Hz, 2H, CH_2), 4.02 (t, J= 10 Hz, 2H, CH_2), 6.99-7.90 (m, 6H, ArH); IR (KBr) v: 1675 (CO), 1610 cm^{-1} (C=N); MS (70 eV) m/z (%): 317 (M^+, 20). Anal. calcd. for $C_{20}H_{19}N_3O$: C, 75.69; H, 6.03; N, 13.24; found C, 75.60; H, 6.11; N, 13.19.

Reaction of 1-chloro-4-(2,4,6-trimethylphenyl)phthalazine (4) with some acid hydrazides

General procedure

A mixture of chlorophthalazine **4** (2.82 g, 0.01 mol) and appropriate acid hydrazides (0.01 mol) namely acetic acid hydrazide, benzoic acetic acid hydrazide and/or gluconic acid hydrazide in ethanol (40 mL) was heated under reflux for 4 h. The solid separated after concentrating and cooling, and recrystallized from the appropriate solvent to give **11a,b** and **12**

6-(2,4,6-Trimethylphenyl)-3-methyl-[1,2,4]triazolo[3,4-a]phthalazine (11a)

Yield, 70% (ethanol); mp 189-190 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.44 (s, 6H, $2CH_3$), 2.48 (s, 3H, CH_3), 7.03-7.97 (m, 6H, ArH); ^{13}C-NMR (DMSO-d_6) δ: 10.1, 20.3, 22.2, 122.0, 126.1, 126.8, 127.2, 128.2, 129.0, 134.1, 134.9, 137.0, 139.2, 139.8, 149.0, 155.1; IR (KBr) v: 1611 cm^{-1} (C=N); MS (70 eV) m/z (%): 302 (M^+, 16). Anal. calcd. for $C_{19}H_{18}N_4$:C 75.47, H 6.00, N 18.53; found C 75.50, H 5.91, N 18.45.

6-(2,4,6-Trimethylphenyl)-3-phenyl-[1,2,4]triazolo[3,4-a]phthalazine (11b)

Yield, 73% (ethanol); mp 201-202 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.42 (s, 6H, $2CH_3$), 6.98-8.12 (m, 11H, ArH); IR (KBr) v: 1609 cm^{-1} (C=N); MS (70 eV) m/z (%): 364 (M^+, 20). Anal. calcd. for $C_{24}H_{20}N_4$: C 79.10, H 5.53, N 15.37; found C 79.08, H 5.55, N 15.39.

1-[6-(2,4,6-Trimethylphenyl)]-[1,2,4]triazolo[3,4-a]phthalazin-3-yl)pentane-1,2,3,4,5-pentaol (12)

Yield, 60% (ethanol); mp 280-281 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.44 (s, 6H, $2CH_3$), 3.19-3.64 (m, 4H, 4CH), 3.70-3.84 (m, 2H, CH_2OH), 4.41-5.11 (m, 5H, 5OH, D_2O exchangeable), 6.98-8.00 (m, 6H, ArH); IR (KBr) v: 3425-3222 (OH), 1608 cm^{-1} (C=N). Anal. calcd. for $C_{23}H_{26}N_4O_5$: C, 63.00; H, 5.98; N, 12.78; found C, 64.01; H, 6.03; N, 12.69.

1,4-Bis{6-(2,4,6-trimethylphenyl)-[1,2,4]triazolo[3,4-a]phthalazin-3-yl}butane-1,2,3,4-tetraol (13)

A mixture of **4** (2.82 g, 0.01 mol) and galactaric acid bishydrazide (0.005 mol) in 20 mL of absolute ethanol containing a few drops of acetic acid was refluxed for 5 h. The solid separated after concentrating and cooling, and recrystallized from H_2O-EtOH to give compound **13** in 71% yield; mp 291-292 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.33 (s, 6H, $2CH_3$), 2.48 (s, 12H, $4CH_3$), 3.79-4.84 (2d, 4H, 4CH), 5.10-5.45 (2d exchangeable, 4H, 4OH), 6.99- 8.08 (m, 12H, ArH); ^{13}C-NMR (DMSO-d_6) δ: 20.0, 23.2, 70.93, 71.23, 122.3, 127.1, 127.7, 128.0, 128.6, 129.5, 135.0, 136.2, 136.9, 137.4, 150.4, 153.1, 156.2; IR (KBr) v: 3488-3210 (OH), 1610 cm^{-1} (C=N); MS (70 eV) m/z (%): 694 (M^+, 0.55). Anal. calcd. for $C_{40}H_{38}N_8O_4$: C, 69.15; H, 5.51; N, 16.13; found C, 69.24; H, 5.41; N, 16.17.

Bis{6-(2,4,6-Trimethylphenyl)-[1,2,4]triazolo[3,4-a]phthalazin-3-yl}methane (14)

A solution of malonic acid dihydrazide (0.005 mol) in benzene (10 mL) was added to the solution of **4** (0.01 mol) in 40 mL of benzene and the mixture was heated under reflux for 8 h. The product that separated upon cooling was filtered, washed with benzene, and crystallized from chloroform to give **14** in 67% yield; mp 300-301 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.32 (s, 6H, $2CH_3$), 2.46 (s, 12H, $4CH_3$), 5.03 (s, 2H, CH_2), 7.02-8.04 (m, 12H, ArH); IR (KBr) v: 1602 cm^{-1} (C=N); MS (70 eV) m/z (%): 588 (M^+, 7). Anal. calcd. for $C_{37}H_{32}N_8$: C, 75.49; H, 5.48; N, 19.03; found C, 75.57; H, 5.40; N, 19.10.

5-(2,4,6-Trimethylphenyl)benzo[4,5]imidazo[2,1-a]phthalazine (15)

A mixture of compound **4** (2.8 g, 0.01 mol) and *o*-phenylenediamine (1.0 g, 0.01 mol) was heated in a fusion tube provided with an air condenser in an oil bath at 170-180 °C for 2 h. After cooling (to room temperature), the reaction mixture was poured into cold water (80 mL). The solid obtained was filtered off and recrystallized from n-butanol to give **15** in 64% yield; mp 204-205 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 2.48 (s, 6H, $2CH_3$), 7.01- 8.10 (m, 10H, ArH); IR (KBr) v: 1610 cm^{-1} (C=N); MS (70 eV) m/z (%): 337 (M^+, 37). Anal. calcd. for $C_{23}H_{19}N_3$: C, 81.87; H, 5.68; N, 12.45; found C, 81.94; H, 5.60; N, 12.51.

7-(2,4,6-Trimethylphenyl)-2,3-dimethyl-4*H*-thieno-[2',3':4,5]-pyrimido[2,3-a]pyridazin-4-one (16)

To a solution of **4** (0.01 mol) in absolute ethanol (50 ml), ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (0.01 mol) was added and the reaction mixture was refluxed for 5 h. The solid obtained upon cooling was collected by filtration, dried, and crystallized from ethanol to give **16** in 69% yield; mp 311-312 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.83 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.48 (s, 6H, $2CH_3$), 6.94-8.02 (m, 6H, ArH); IR (KBr) v: 1695(CO), 1614 cm^{-1} (C=N); MS (70 eV) m/z (%): 399 (M^+, 43). Anal. calcd. for $C_{24}H_{21}N_3OS$: C, 72.15; H, 5.30; N, 10.52; S, 8.03; found C, 72.22; H, 5.21; N, 10.57; S, 8.10.

2-(2,4,6-Trimethylphenyl)-10,11,12,13-tetrahydro-14*H*-[1]-benzothieno-[2',3':4,5]-pyrimido [2,3-a] pyridazin-14-one (17)

A mixture of **4** (0.001 mol) and ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (0.005 mol) was heated in a fusion tube provided with an air condenser in an oil bath at 180-182 °C for 2 h. Then the reaction mixture was cooled to room temperature and added to 50 mL of cold water. The solid product obtained was collected and recrystallized from benzene to give **17** in 63% yield; mp 325-326 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.21-2.26 (m, 4H, $2CH_2$), 2.30-2.34 (m, 4H, $2CH_2$), 2.41 (s, 3H, CH_3), 2.50 (s, 6H, $2CH_3$), 7.00-8.05 (m, 6H, Ar–H); ^{13}C-NMR (DMSO-d_6) δ: 20.2, 22.5, 24.1, 24.9, 26.3, 27.4, 119.0, 126.3, 126.9, 128.1, 128.5, 129.0, 130.4, 133.2, 134.2, 136.7, 136.9, 139.8, 142.2, 142.9, 153.1, 158.3, 165.6; IR (KBr) v: 1711 (CO), 1622 cm^{-1} (C=N); MS (70 eV) m/z (%): 425 (M^+, 3). Anal. calcd. for $C_{26}H_{23}N_3OS$: C, 73.38; H, 5.45; N, 9.87; S, 7.54; found C, 73.27; H, 5.55; N, 9.90; S, 7.60.

6-(2,4,6-Trimethylphenyl)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (18)

A mixture of compound **4** (2.82 g, 0.01 mol) and thiosemicarbazide (0.01 mol) in absolute ethanol was refluxed for 8 h. The solid product obtained upon cooling was filtered off, dried, and crystallized from ethanol to give **18** in 73% yield; mp 239-240 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 2.45 (s, 6H, $2CH_3$), 6.24 (s, 2H, NH_2, exchangeable with D_2O), 7.3-8.01 (m, 6H, ArH); IR (KBr) v: 3422, 3380 (NH_2), 1605 cm^{-1} (C=N). Anal. calcd. for $C_{18}H_{17}N_5$: C, 71.27; H, 5.65; N, 23.09; found C, 71.31; H, 5.74; N, 23.00.

Reaction of 6-(2,4,6-trimethylphenyl)-3-amino-[1,2,4]triazolo[3,4-a]phthalazine (18) with some aromatic aldehydes

General procedure

A mixture of **18** (0.001 mol) and an appropriate aromatic aldehyde, namely benzaldehyde or p-chlorobenzaldehyde (0.001 mol), was refluxed in 20 mL of absolute ethanol for 7 h. The solid obtained upon cooling was collected by filtration, dried, and crystallized from the appropriate solvent to give the title compounds **19a,b,** respectively.

***N*-Benzylidene-6-(2,4,6-trimethylphenyl)-[1,2,4]triazolo[3,4-a]phthalazin-3-amine (19a)**

Yield, 70% (ethanol); mp 210-211 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 2.47 (s, 6H, $2CH_3$), 7.00-8.10 (m, 11H, ArH), 8.51 (s, 1H, CH); IR (KBr) v: 1618 cm^{-1} (C=N); MS (70 eV) m/z (%): 391 (M^+, 10). Anal. calcd. for $C_{25}H_{21}N_5$: C, 76.70; H, 5.41; N, 17.89; found C, 76.63; H, 5.37; N, 17.96.

_N_-(4-Chlorobenzylidene)-6-(2,4,6-trimethylphenyl)-[1,2,4]triazolo[3,4-a]phthalazin-3-amine (19b)

Yield, 73% (ethanol); mp 200-201 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3), 2.49 (s, 6H, $2CH_3$), 6.98-8.01 (m, 10H, ArH), 9.05 (s, 1H, CH); IR (KBr) v: 1621 cm^{-1} (C=N). Anal. calcd. for $C_{25}H_{20}ClN_5$: C, 70.50; H, 4.73; N, 16.44; found C, 70.66; H, 4.70; N, 16.51.

7-(2,4,6-Trimethylphenyl)-13*H*-phthalazino[2,1-a]quinazoline (20)

A mixture of compound **4** (2.82 g, 0.01 mol) and *o*-chlorobenzylamine (0.01 mol) in pyridine (20 mL) was refluxed for 7 h. After cooling, the reaction mixture was poured on ice water (80 mL). The solid obtained was filtered off and recrystallized from ethanol to give **20** in 63% yield; mp 185-186 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.37 (s, 3H, CH_3), 2.49 (s, 6H, $2CH_3$), 4.90 (s, 2H, CH_2 quinazoline), 6.97-8.14 (m, 10H, ArH); IR (KBr) v: 1610 cm^{-1} (C=N). Anal. calcd. for $C_{24}H_{21}N_3$: C, 82.02; H, 6.02; N, 11.96; found C, 82.10; H, 6.11; N, 11.90.

7-(2,4,6-Trimethylphenyl)-3-phenyl-2-thioxo-2,3-dihydro-[1,3,5]triazino[2,1-a] phthalazin-4-one (22)

The solution of ammonium thiocyanate (0.005 mol) in dry acetone was added to a stirred solution of chlorophthalazine **4** (0.005 mol) in dry acetone. The reaction mixture was stirred for 1 h at room temperature. Ammonium chloride was precipitated during the progress of the reaction. After filtration of the ammonium chloride, phenyl isocyanate (0.6 g, 0.005 mol) was added to the filtrate. The reaction mixture was heated under reflux for 30 min. The solid product that separated after cooling was crystallized from ethanol to give **22** in 55% yield; mp 239-240 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 2.46 (s, 6H, $2CH_3$), 6.99-8.12 (m, 11H, ArH); ^{13}C-NMR (DMSO-d_6) δ: 20.1, 23.2, 125.6, 126.9, 127.8, 128.5, 128.8, 129.9, 130.7, 132.0, 132.7, 134.1, 134.8, 136.0, 136.8, 139.0, 144.2, 155.8, 157.0, 180.5; IR (KBr) v: 1675 (CO), 1618 (C=N), 1265 cm^{-1} (C=S). Anal. calcd. for $C_{25}H_{20}N_4OS$: C, 70.73; H, 4.75; N, 13.20; S, 7.55; found C, 70.77; H, 4.69; N, 13.27; S, 7.61.

6-(2,4,6-Trimethylphenyl)-tetrazolo[5,1-a]phthalazine (23)

A mixture of compound **4** (2.82 g, 0.01 mol) and sodium azide (0.01 mol) in acetic acid (30 mL) was refluxed for 5 h. After cooling, the reaction mixture was poured on ice water (80 mL). The solid obtained was filtered off and recrystallized from ethanol to give **23** in 70% yield; mp 175-176 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 2.40 (s, 6H, $2CH_3$), 7.01- 8.00 (m, 6H, ArH); IR (KBr) v: 1607 cm^{-1} (C=N); MS (70 eV) m/z (%): 289 (M^+, 39). Anal. calcd. for $C_{17}H_{15}N_5$: C, 70.57; H, 5.23; N, 24.21; found C, 70.66; H, 5.20; N, 24.30.

1-[(2,4,6-Trimethylphenyl)-1,2-dihydrophthalazin-1yl]hydrazine (24)

To a solution of **4** (0.01 mol) in 15 mL of absolute ethanol was added 0.3 mL of hydrazine hydrate (98%) and the reaction mixture was refluxed for 5 h. After cooling, the obtained solid was filtered off and crystallized from ethanol to give **24** in 71% yield; mp 266-267 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 2.45 (s, 6H, $2CH_3$), 5.31 (s, 2H, NH_2 exchangeable with D_2O), 7.27-8.50 (m, 6H, Ar–H), 9.55 (s, 1H, NH exchangeable with D_2O); IR (KBr) v: 3390-3112 cm^{-1} ($NHNH_2$); MS (70 eV) m/z (%): 278 (M^+, 21). Anal. calcd. for $C_{17}H_{18}N_4$: C, 73.35; H, 6.52; N, 20.13; found C, 73.44; H, 6.43; N, 20.10.

7-(2,4,6-Trimethylphenyl)-3-methyl/phenyl-2*H*-[1,2,4]triazino[3,4-a]phthalazine (25a,b)

A mixture of **24** (2.78 g, 0.01 mol) and α-haloketones (0.01 mol) (viz. chloroacetone and phenacyl bromide) in dry xylene (40 mL) was heated under reflux for 8 h. The solid that separated upon cooling was filtered off and recrystallized from the appropriate solvent to give **25a,b**.

25a; Yield, 61%; mp 200-201 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.11 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.50 (s, 6H, $2CH_3$), 5.88 (s, 1H, CH of triazine), 7.10-8.28 (m, 7H, ArH and NH of triazine); IR (KBr) v: 3190 (NH), 1615 cm^{-1} (C=N); Anal. calcd. for $C_{20}H_{20}N_4$: C, 75.92; H, 6.37; N, 17.71; found C, 76.01; H, 6.40; N, 17.66.

25b; Yield, 66%; mp 212-213 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.47 (s, 6H, $2CH_3$), 6.50 (s, 1H, CH of triazine), 7.03-8.31 (m, 12H, ArH and NH of triazine); IR (KBr) v: 3222 (NH), 1610 cm^{-1} (C=N); MS (70 eV) m/z (%): 378 (M^+, 11). Anal. calcd. for $C_{25}H_{22}N_4$: C, 79.34; H, 5.86; N, 14.80; found C, 79.29; H, 5.90; N, 14.88.

7-(2,4,6-Trimethylphenyl)-2*H*-[1,2,4]triazino[3,4-a]phthalazin-3(4*H*)-one (26)

A mixture of **24** (2.78 g, 0.01 mol) and ethyl bromoacetate (0.03 mol) in absolute ethanol (30 mL) was heated under reflux for 15 h. The solid that separated after cooling and recrystallization from ethanol gave **26** in 53% yield; mp 246-247 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.44 (s, 6H, $2CH_3$), 4.57 (s, 2H, CH_2 of triazine), 6.97-8.25 (m, 7H, ArH and NH of triazine); IR (KBr) v: 3198 (NH), 1677 (CO), 1613 cm^{-1} (C=N); MS (70 eV) m/z (%): 318 (M^+, 5). Anal. calcd. for $C_{19}H_{18}N_4O$: C, 71.68; H, 5.70; N, 17.60; found C, 71.57; H, 5.77; N, 17.55.

7-(2,4,6-Trimethylphenyl)-2*H*-[1,2,4]triazino[3,4-a]phthalazine-3,4-dione (27)

A mixture of **24** (2.78 g, 0.01 mol) and diethyl oxalate (0.01 mol) in absolute ethanol (40 mL) was heated under reflux for 18 h. After cooling, the separated solid produced was collected and recrystallized from ethanol to give **27** in 65% yield; mp 259-260 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 2.47 (s, 6H, $2CH_3$), 7.02-8.32 (m, 6H, ArH and NH of triazine); IR (KBr) v: 3228 (NH), 1691-1680 (CO), 1607 cm^{-1} (C=N); MS (70 eV) m/z (%): 332 (M^+, 1.5). Anal. calcd. for $C_{19}H_{16}N_4O_2$: C, 68.66; H, 4.85; N, 16.86; found C, 68.75; H, 4.90; N, 16.79.

7-(2,4,6-Trimethylphenyl)-3-methyl-4*H*-[1,2,4]triazino[3,4-a]phthalazin-4-one(28)

A mixture of **24** (2.78 g, 0.01 mol) and pyruvic acid (0.01 mol) was heated in oil bath at 180 °C for 1 h. After cooling, the formed precipitate was heated with ethanol and the precipitate was collected and crystallized from acetic acid to give **28** in 47% yield; mp 269-270 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.01 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.43 (s, 6H, $2CH_3$), 7.01-8.00 (m, 6H, ArH); ^{13}C-NMR (DMSO-d_6) δ: 16.8, 20.7, 22.4, 126.0, 126.7, 129.0, 129.6, 130.8, 130.9, 133.1, 133.9, 135.0, 140.2, 143.3, 158.1, 158.9, 166.0; IR (KBr) v: 1679 (CO), 1603 cm^{-1} (C=N); MS (70 eV) m/z (%): 330 (M^+, 15). Anal. calcd. for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96; found C, 72.75; H, 5.57; N, 16.88.

6-(2,4,6-Trimethylphenyl)-[1,2,4]triazolo[3,4-a]phthalazine (29)

A solution of **24** (2.78 g, 0.01 mol) in formic acid (20 mL) was heated under reflux for 15 h. After cooling and dilution with water, the formed precipitate was collected and crystallized from dioxane to give **29** in 66% yield; mp 220-221 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.48 (s, 6H, $2CH_3$), 6.98-7.89 (m, 6H, ArH), 9.57 (s, 1H, =CH triazol); IR (KBr) v: 1601 cm^{-1} (C=N). Anal. calcd. for $C_{18}H_{16}N_4$: C, 74.98; H, 5.59; N, 19.43; found C, 75.10; H, 5.57; N, 19.38.

6-(2,4,6-Trimethylphenyl)-[1,2,4]triazolo[3,4-a]phthalazine-3(2*H*)-thione (30)

To an ice cooled solution of **24** (2.78 g, 0.01 mol) in absolute ethanol (10 mL) containing potassium hydroxide (0.01 mol), carbon disulfide (2 mL) was added dropwise with stirring. The mixture was diluted with absolute ethanol (20 mL) and refluxed for 8 h. The reaction mixture was filtered, concentrated, diluted with water, and neutralized with acetic acid. The precipitated product was crystallized from dioxane to give **30** in 60% yield; mp 310-311 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 2.50 (s, 6H, $2CH_3$), 7.01-7.90 (m, 6H, ArH), 9.32 (s, 1H, NH exchangeable with D_2O); IR (KBr) v: 3218(NH), 1601 cm^{-1} (C=N); MS (70 eV) m/z (%): 320 (M^+, 10). Anal. calcd. for $C_{18}H_{16}N_4S$: C, 67.47; H, 5.03; N, 17.49; S, 10.01; found C, 67.56; H, 5.10; N, 17.38; S, 10.00.

6-(2,4,6-Trimethylphenyl)-[1,2,4]triazolo[3,4-a]phthalazin-3(2*H*)-one (31)

A mixture of **24** (2.78 g, 0.01 mol) and ethylchloroformate (0.02 mol) in 20 mL of pyridine was heated on a water bath for 12 h. After cooling, the reaction mixture was poured onto ice water. The precipitated solid was collected by filtration, dried and crystallized from ethanol to give **31** in 61% yield; mp 320-321 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 2.48 (s, 6H, $2CH_3$), 6.98-7.94 (m, 6H, ArH), 8.78 (s, 1H, NH exchangeable with D_2O); IR (KBr) v: 3211 (NH), 1664 (CO), 1608 cm^{-1} (C=N); MS (70 eV) m/z (%): 304 (M^+, 16). Anal. calcd. for $C_{18}H_{16}N_4O$: C, 71.04; H, 5.30; N, 18.41; found C, 71.11; H, 5.36; N, 18.32.

2-(4-(2,4,6-Trimethylphenyl)phthalazin-1-yl)-*N*-phenylhydrazinecarbothioamide (32)

A mixture of **24** (2.78 g, 0.01 mol) and phenyl isothiocyanate (0.01 mol) in absolute ethanol (30 mL) was heated under reflux for 8 h. After cooling, the separated solid produced was collected and recrystallized from ethanol to give **32** in 73% yield; mp 177-176 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.96 (s, 1H, CSNH, exchangeable with D_2O), 2.33 (s, 3H, CH_3), 2.44 (s, 6H, $2CH_3$), 6.99-8.00 (m, 11H, ArH), 12.52 (s, 1H, NHPh, exchangeable with D_2O); IR (KBr) v: 3211 cm^{-1} (NH); MS (70 eV) m/z (%): 413 (M^+, 6). Anal. calcd. for $C_{24}H_{23}N_5S$: C, 69.71; H, 5.61; N, 16.94; S, 7.75; found C, 69.60; H, 5.70; N, 16.91; S, 7.81.

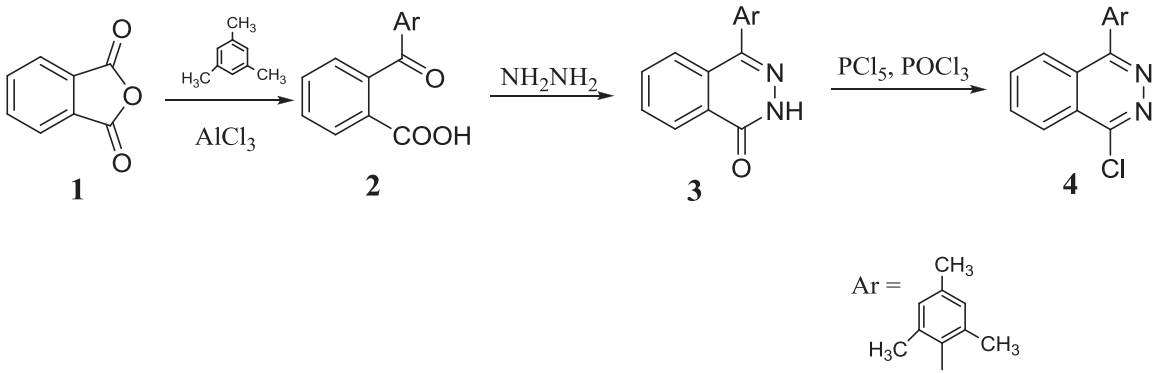
6-(2,4,6-Trimethylphenyl)-*N*-phenyl-[1,2,4]triazolo[3,4-a]phthalazin-3-amine (33)

A mixture of **32** (0.01 mol) and 3 molar equivalents of mercuric chloride in dry chloroform (50 mL) was heated under reflux, while stirring, for 24 h. Chloroform was evaporated, and the residue boiled with ethanol (20 mL) containing 10% aqueous hydrochloric acid solution (30 mL) and saturated with hydrogen sulfide gas. The mercuric sulfide formed was filtered, and the filtrate evaporated until all alcohol was removed. After cooling, it

was extracted with benzene; the benzene was then removed to give uncyclized products. The acidic layer was made distinctly alkaline with sodium hydrogen carbonate to separate compound **33** in 50% yield; mp 163-164 °C; ^{1}H-NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 2.48 (s, 6H, $2CH_3$), 7.01-8.10 (m, 11H, ArH), 9.25 (s, 1H, NHPh, exchangeable with D_2O); IR (KBr) v: 3211 cm^{-1} (NH); MS (70 eV) m/z (%): 379 (M^+, 6). Anal. calcd. for $C_{24}H_{21}N_5$: C, 75.97; H, 5.58; N, 18.46; found C, 76.10; H, 6.00; N, 18.38.

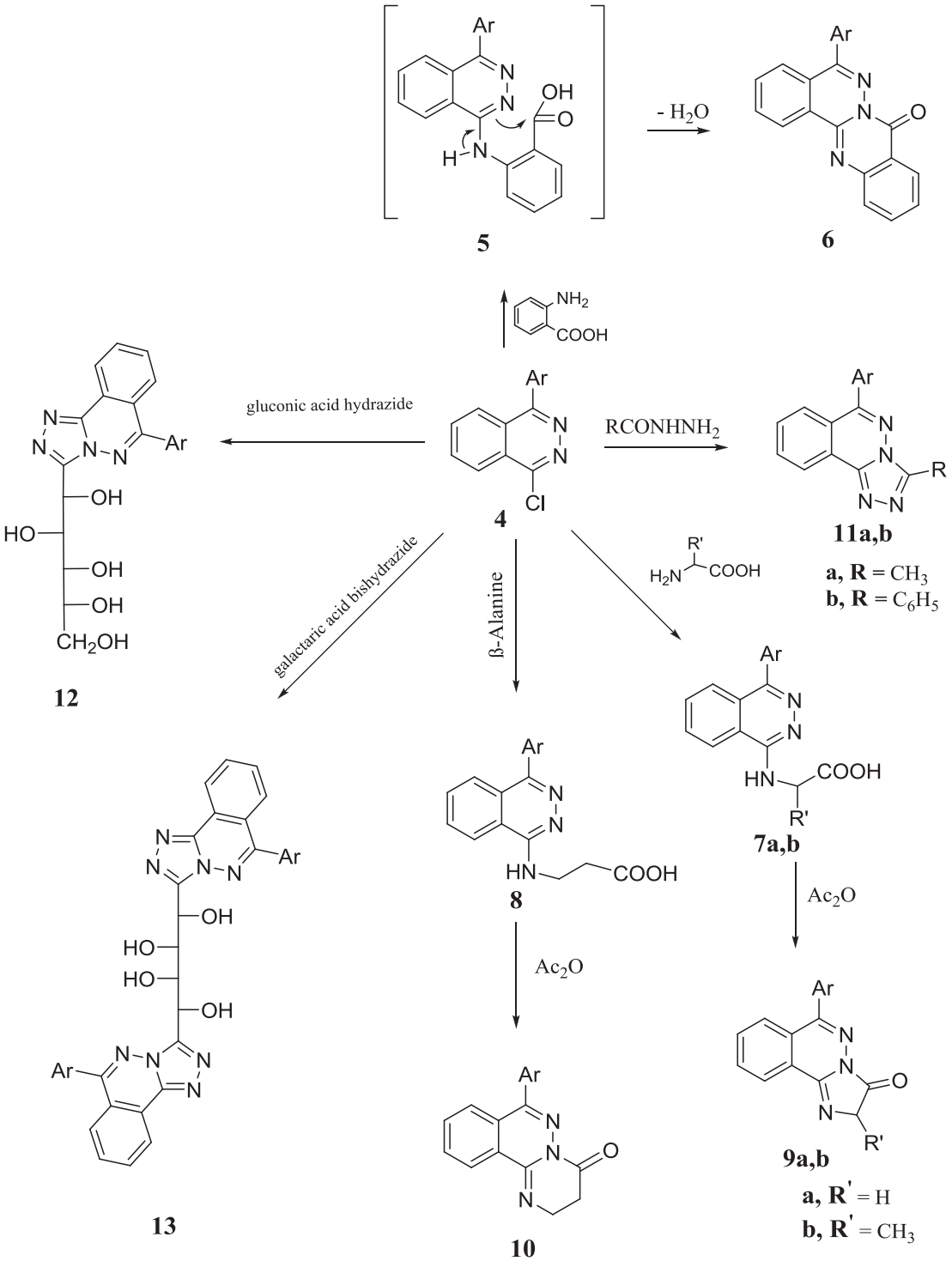
Results and discussion

Aroylation of an aromatic system by reaction with phthalic anhydride under Friedel Craft's conditions yields *o*-aroylbenzoic acid.[41,42] Thus, reaction of mesitylene with phthalic anhydride in the presence of anhydrous aluminum chloride was carried out to produce 2-(2,4,6-trimethyl benzoyl)benzoic acid **(2)**. Merchant et al.[43] prepared phthalazin-l-ones via the condensation of the aroyl benzoic acid with hydrazine hydrate in boiling ethanol. Accordingly, adopting the Merchant et al. procedure, condensation of benzoic acid derivative **2** with hydrazine hydrate in boiling ethanol afforded the 4-(2,4,6-trimethyl phenyl)-2*H*-phthalazin-l-one **(3)** in 65% yield. The IR spectrum showed a characteristic absorption band at 1654 cm^{-1} corresponding to the CO group. The ^{1}H-NMR spectrum of compound **3** showed a NH signal at 11.17 ppm. Compound **3** was treated with a mixture of phosphorous oxychloride and phosphorous pentachloride to afford the corresponding chlorophthalazine **4**, which is a promising intermediate for the synthesis of diverse annelated phthalazine derivatives (Scheme 1). The structure of compound **4** was confirmed on the basis of its elemental analysis and spectral data. The IR spectrum revealed no absorption for NH and CO groups.



Scheme 1. Synthesis of 1-chloro-4-(2,4,6-trimethylphenyl)phthalazine **(4)**.

The reactivity of chlorophthalazine **4** towards some nitrogen nucleophiles was investigated for the construction of a novel heteroaromatic system. Thus, interaction of chlorophthalazine **4** with anthranilic acid under fusion conditions afforded the corresponding quinazolinone derivative **6** as the only isolable product. The formation of **6** was explained by the formation of intermediate **5,** which undergoes intramolecular ring closure to form the final product **6**. The IR spectrum of compound **6** showed an absorption band at 1670 cm^{-1} assigned to the CO group. The ^{13}C-NMR spectrum of **6** exhibited the expected number of signals for the aromatic carbons as well as 3 methyl signals and a carbonyl signal at 20.9, 23.5 and 170.1 ppm. Treatment of compound **4** with the sodium salt of various amino acids, namely glycine, alanine, and β-alanine under reflux conditions

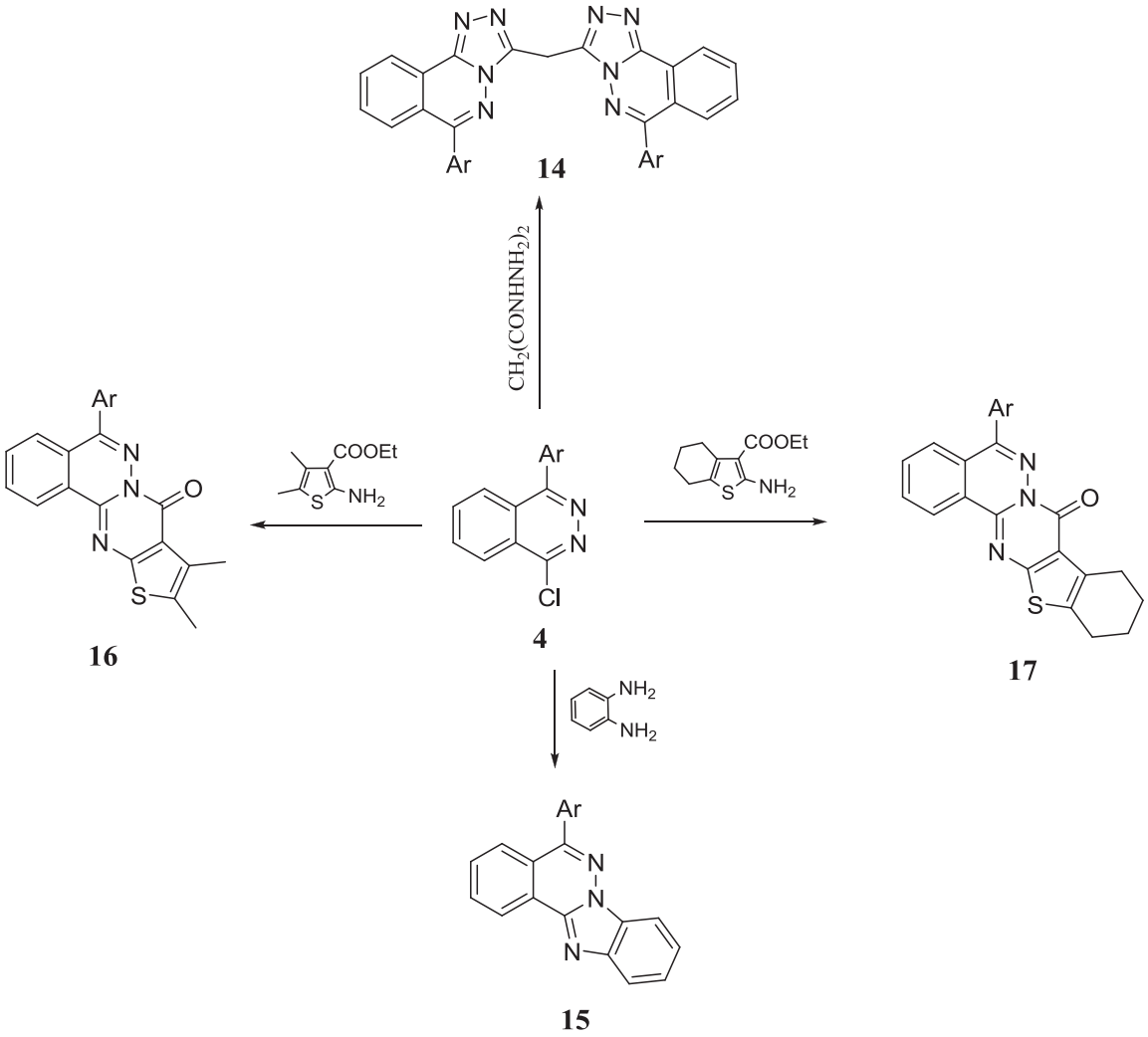


Scheme 2. Synthesis of acyclo *C*-nucleosides **12**, **13** and some annelated phthalazine derivatives.

produced the corresponding phthalazinaylamino acids (**7a,b** and **8**). The IR spectrum of compounds **7a,b** and **8** revealed the presence of a CO group at 1694-1702 cm^{-1}. The ^{1}H-NMR spectrum of compound **7a** displayed CH_2 protons as a singlet signal at δ 4.61, and exchangeable NH and OH protons as singlet signals at δ 8.01 and 10.81, respectively. The ^{1}H-NMR spectrum of compound **7b** displayed a CH proton as a quartet signal at δ 4.50 and CH_3 protons as a doublet signal at δ 1.53. The ^{1}H-NMR spectrum of compound **8** showed a triplet signal at δ 2.81 assigned for NCH_2 and a triplet signal at 3.77 assigned for CH_2CO beside NH and OH protons. The amino acid derivatives **7a,b** and **8** were easily cyclized[44] via 1,3-tautomerism in boiling acetic anhydride in the presence of anhydrous sodium acetate to yield imidazophthalazine derivatives **9a,b** and pyrimidinophthalazine **10**. The ^{1}H-NMR spectrum of compounds **9a,b** and **10** showed the absence of NH and OH signals. The reaction of chlorophthalazine **4** with different acid hydrazide, namely acetic acid hydrazide and benzoic acid hydrazide afforded 1,2,4-triazolo[3,4-a]phthalazine derivatives **11a,b**, respectively. The ^{13}C-NMR spectrum of **11a** exhibited the expected number of signals for the aromatic carbons as well as 4 methyl signals at 10.1, 20.3 and 22.2 ppm. Treatment of compound **4** with gluconic acid hydrazide afforded 1,2,4-triazolo[3,4-a]phthalazine acyclo *C*-nucleoside derivative **12**. The IR spectrum of compound **12** revealed a broad absorption band at 3425-3222 cm^{-1} attributable to OH groups. The ^{1}H-NMR spectrum showed the presence of the sugar protons. On the other hand, treatment of 2 equivalents of **4** with galactaric acid bishydrazide and malonic acid dihydrazide yielded double headed 1,2,4-triazolo[3,4-a]phthalazine acyclo *C*-nucleoside derivative **13** and bis{6-(2,4,6-trimethylphenyl)-[1,2,4]triazolo[3,4-a]phthalazin-3-yl}methane (**14**), respectively (Scheme 2). The ^{1}H-NMR spectrum of compound **13** displayed tetritolyl 4 exchangeable OH protons as 2 doublet signals at δ 5.45 and 5.10 and tetritolyl 4 CH protons as 2 doublet signals at 4.84 and 3.79 ppm. The ^{13}C-NMR spectrum of compound **13** gave characteristic signals in accordance with the assigned structure. The ^{1}H-NMR spectrum of compound **14** showed δ 5.03 ppm for assigned CH_2 and aromatic protons.

Fusion of chlorophthalazine **4** with *o*-phenylenediamine afforded benzo[4,5]imidazo[2,1-a]phthalazine derivative **15**. Interaction of chlorophthalazine **4** with 2-amino-3-carboxy-4,5-dimethylthiophene afforded the corresponding dimethylthienopyrimidinone derivative **16**. Similarly, **4** reacted with 2-amino-3-carbethoxy tetrahydrobenzothiophene to afford tetrahydrobenzothienopyrimidinone derivative **17**. The IR spectrum of compound **16** and **17** revealed the presence of a CO group at 1695 and 1711 cm^{-1}, respectively (Scheme 3). The ^{13}C-NMR spectrum of compound **17** gave characteristic signals in accordance with the assigned structure.

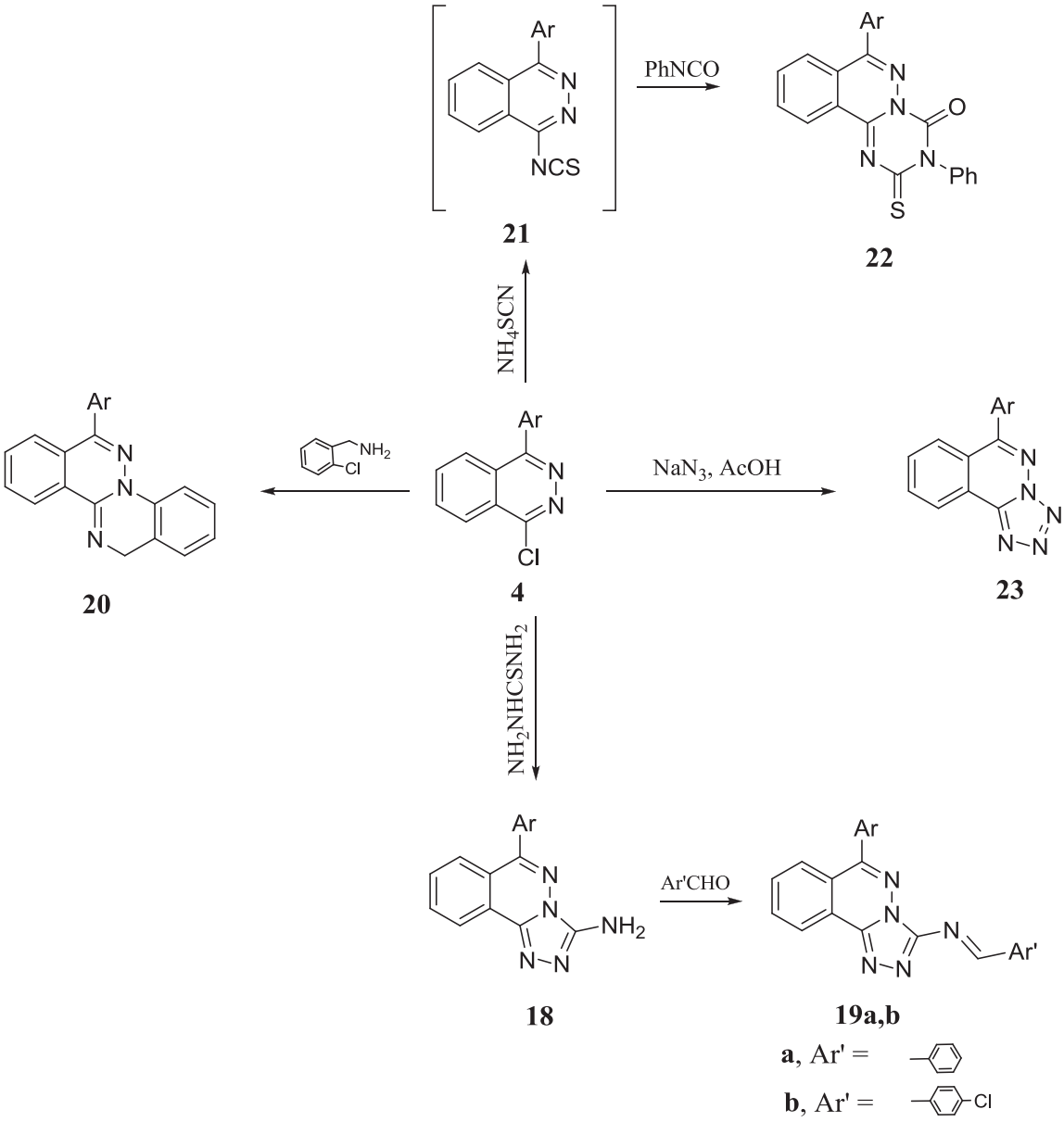
Treatment of chlorophthalazine **4** with thiosemicarbazide in boiling ethanol afforded triazolo derivative **18**. The ^{1}H-NMR spectrum of compound **18** displayed 2 exchangeable NH_2 protons as a singlet signal at 6.24 ppm. Condensation of **18** with different aromatic aldehydes, namely benzaldehyde and *p*-chlorobenzaldehyde in absolute ethanol afforded the corresponding Schiff bases **19a,b**, respectively. The IR spectrum of compounds **19a,b** showed a characteristic absorption band at 1618 and 1621 cm^{-1} corresponding to the C=N group, respectively. The ^{1}H-NMR spectra of compounds **19a,b** showed the presence of azomethin (CH=N) at δ 8.51 and 9.05 ppm, respectively. Interaction of compound **4** with *o*-chlorobenzylamine in pyridine afforded phthalazino[2,1-a]quinazoline derivative **20**. The ^{1}H-NMR spectrum of compound **20** showed δ 2.37 (s, 3H, CH_3), 2.49 (s, 6H, $2CH_3$), 4.90 (s, 2H, CH_2 quinazoline), and aromatic protons. Furthermore, the reaction of chlorophthalazine **4** with ammonium thiocyanate in dry acetone afforded the nonisolable intermediate **21** that reacted in situ with phenyl isocyanate via 2+4 cycloaddition reaction to yield triazinophthalazine derivative **22**. The IR spectrum of compound **22** revealed the presence of a CO group at 1675 cm^{-1}. The ^{13}C-NMR spectrum



Scheme 3. Synthesis of annelated phthalazine derivatives **14-17**.

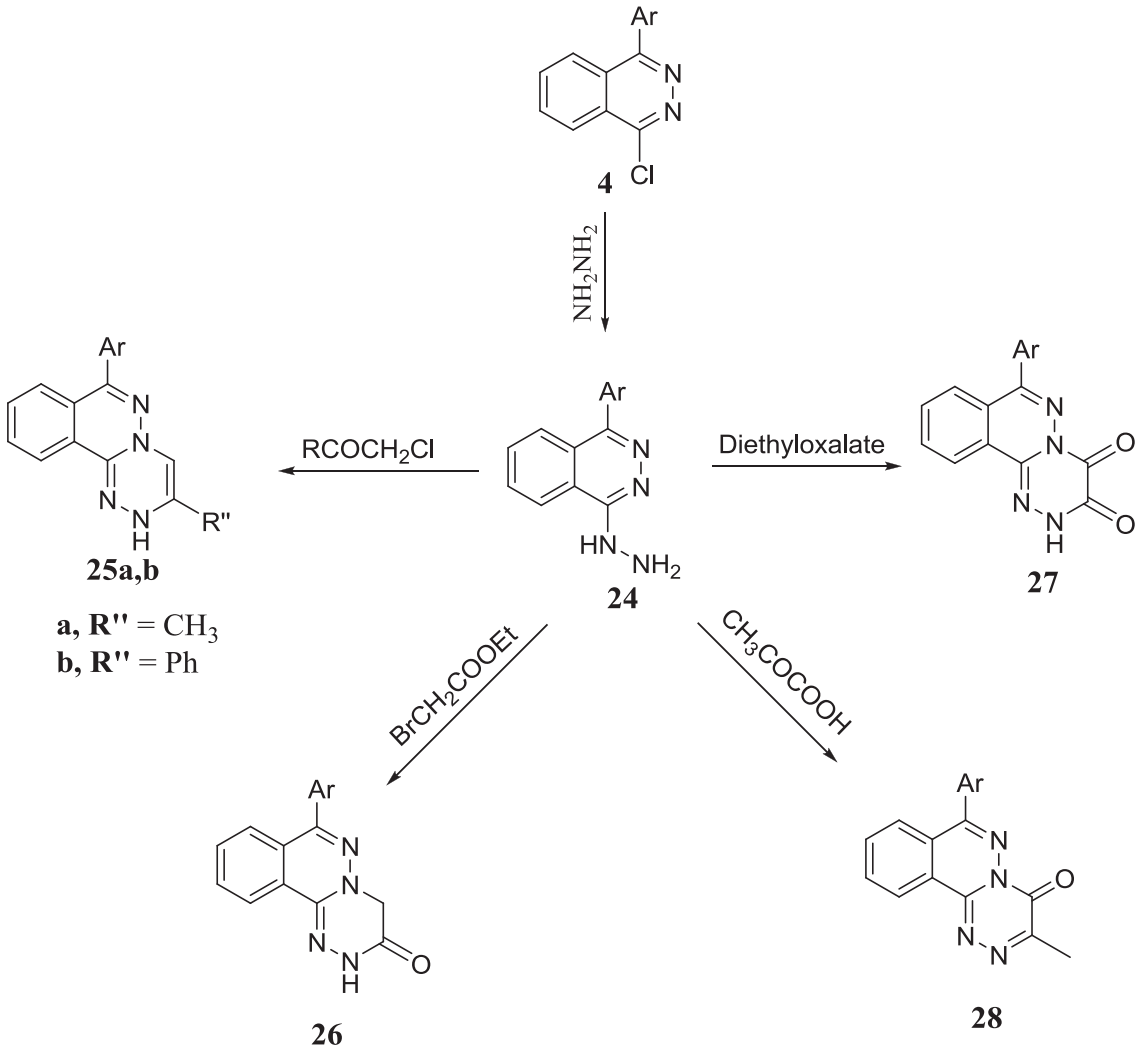
of **22** exhibited the expected number of signals for the aromatic carbons as well as 3 methyl signals, carbonyl and thiocarbonyl signals at 20.1, 23.2, 155.8, and 180.5 ppm. Treatment of chlorophthalazine **4** with sodium azide in boiling acetic acid afforded tetrazolo derivative **23** (Scheme 4). The mass spectrum of **23** showed a peak corresponding to its molecular ion at m/z 289.

Treatment of chlorophthalazine **4** with hydrazine hydrate in boiling ethanol afforded hydrazine derivative **24**. The ^{1}H-NMR spectrum of **24** showed signals at 5.31 for assigned NH_2 (exchangeable with D_2O) and 9.55 for assigned NH (exchangeable with D_2O). Cyclo-condensation of compound **24** with different α-haloketons, namely chloroacetone and phenacylbromide in dry xylene afforded the corresponding hydrazone, which underwent 1,3-tautomerism followed by ring closure to give 1,2,4-triazino[3,4-a]phthalazine derivatives **25a,b**. The IR spectra of compounds **25a,b** showed a characteristic band at 1615 and 1610 cm^{-1} corresponding to the C=N group. The ^{1}H-NMR spectrum of compound **25a** showed the presence of a NH signal at 7.92 ppm. Moreover, the reaction of compound **4** with ethylbromoacetate yielded 7-(2,4,6-trimethylphenyl)-2*H*-[1,2,4]triazino[3,4-a]phthalazin-3(4*H*)-one **(26)**. Compound **26** was obtained by nucleophilic attack of NH_2 of hydrazino moiety



Scheme 4. Synthesis of annelated phthalazine derivatives **18-23**.

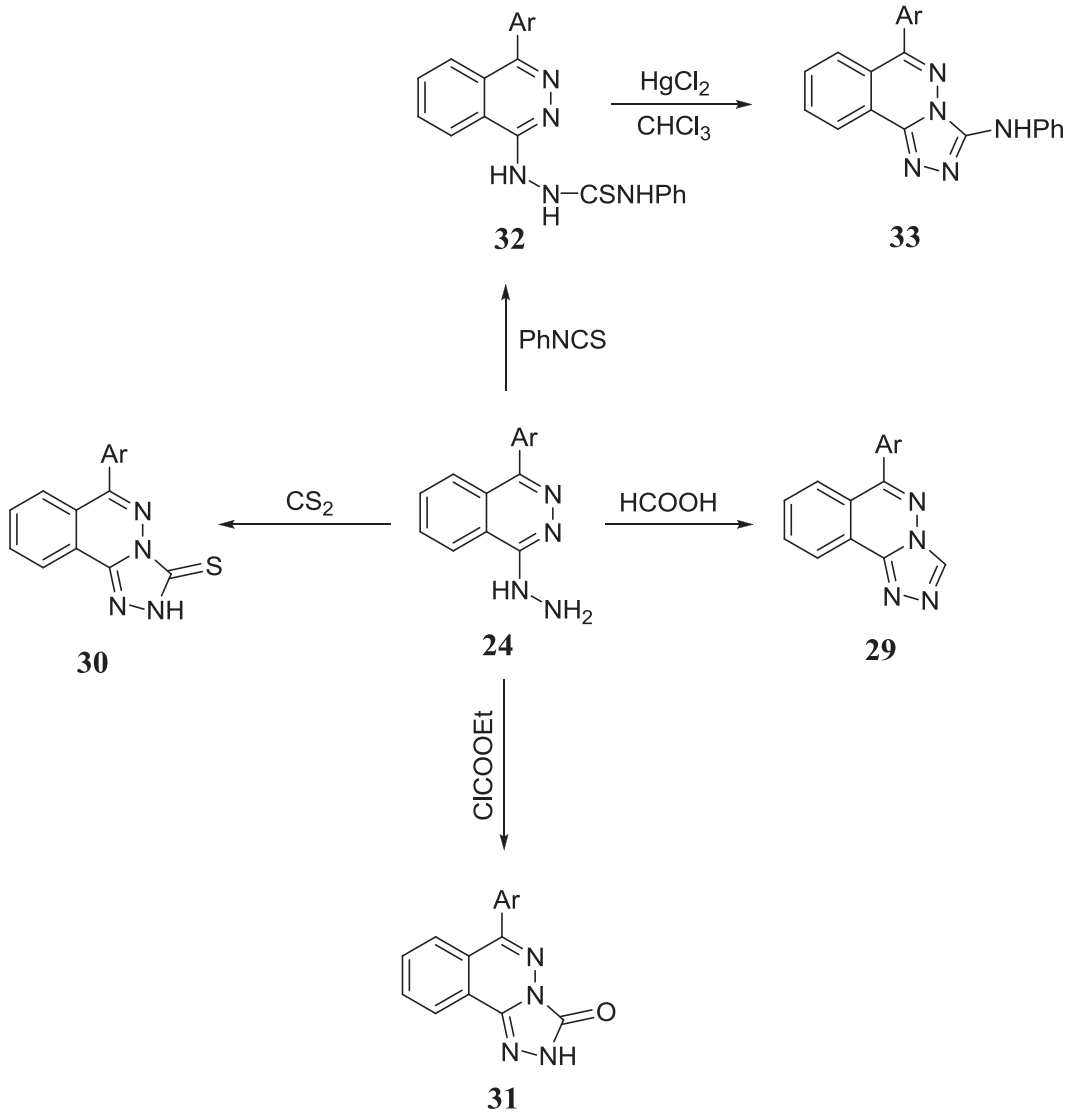
to acyl carbon of the ester group through a tetrahedral mechanism followed by 1,3-tautomerism and ring closure via a S_N^2 mechanism. The IR spectrum of compound **26** showed the presence of an absorption band for CO group at 1677 cm^{-1}. The ^{1}H-NMR spectrum of compound **26** showed a singlet signal at 4.57 for assigned CH_2 (triazine moiety). Moreover, 1,2,4-triazino[4,3-c]phthalazine **27** was obtained from the reaction of compound **4** with diethyl oxalate in absolute ethanol by a ring closure happening via consecutive 2 tetrahedral mechanisms. The IR spectrum of compounds **27** showed the presence of absorption bands for CO groups at 1680-1691 cm^{-1}. The ^{1}H-NMR spectrum of compound **27** showed a signal at 8.32 for assigned NH (exchangeable with D_2O). Condensation of compound **24** with pyruvic acid by heating at 180 °C gave the corresponding triazino deriva-



Scheme 5. Synthesis of 1,2,4-triazino[3,4-a]phthalazine **25a,b-28**.

tive **28** in 47% yield (Scheme 5). The IR spectrum of compound **28** revealed the presence of a CO group at 1679 cm^{-1}. The ^{13}C-NMR spectrum of **28** exhibited the expected number of signals for the aromatic carbons as well as 4 methyl signals and a carbonyl signal at 16.8, 20.7, 22.4, and 166.0 ppm.

Cyclocondensation of compound **24** with formic acid gave *s*-triazolo derivative **29**. Its IR revealed no absorption for $NHNH_2$. The ^{1}H-NMR spectrum of compound **29** showed a CH signal of the triazole ring at 9.57 ppm. Cyclization of **24** using carbon disulfide in alcoholic potassium hydroxide gave the corresponding triazolo derivative **30**. The IR spectrum of compound **30** revealed the presence of a NH group at 3218 cm^{-1}. On the other hand, cyclization of **24** using ethylchloroformate in pyridine gave the corresponding triazolo derivative **31**. The IR spectrum of compound **31** revealed the presence of a CO group at 1664 cm^{-1}. The ^{1}H-NMR spectrum of compound **31** showed a NH signal at 8.78 ppm. Refluxing of compound **24** with phenyl isothiocyanate in absolute ethanol afforded the thiocarbamate derivative **32**. Cyclodesulfurization reaction of compound **32** with mercuric chloride afforded the phenylamino derivative **33** (Scheme 6). The ^{1}H-NMR spectrum of compound



Scheme 6. Synthesis of 1,2,4-triazolo[3,4-a]phthalazines **29- 31** and **33**.

32 showed δ 1.96 (s, 1H, CSNH, exchangeable with D_2O) and 12.52 (s, 1H, NHPh, exchangeable with D_2O) ppm. The ^{1}H-NMR spectrum of compound **33** showed δ 9.26 assigned for NHPh (exchangeable with D_2O).

Antimicrobial assay

The antimicrobial activity of the newly synthesized compounds **6**, **9-23** and **25-31, 33** were evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi* bacterial strains and *Aspergillums niger* and *Candida albicans* fungal strains by disk diffusion method. Amoxicillin and Ketoconazole were used as standard drugs for the bacteria and fungi, respectively. Preliminary screening of phthalazine-derivatives and standard drugs was performed at fixed concentrations of 500 μg/mL. Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria and 72 h for fungi. Each experiment was repeated

twice. Based on the results of zone of inhibition, the minimum inhibitory concentration (MIC) of compounds **6**, **9-23** and **25-31, 33** against all bacterial and fungal strains was determined by liquid dilution method. Stock solutions of tested compounds with 500, 250, 200, 100, 50, 25, 12.5, and 6.25 μg mL^{-1} concentrations were prepared with DMSO solvent. The solutions of standard drugs, Amoxicillin and Ketoconazole, were prepared

Table. Antimicrobial activity of compounds **6**, **9-23** and **25-31, 33.**

Compounds	Minimum inhibitory concentration (MIC) in μg/mL					
	Bacterial strains				Fungal strains	
	S. aureus	*B. subtilis*	*S. typhi*	*E. coli*	*A. niger*	*C. albican*
6	500	200	-	500	-	500
9a	200	-	250	500	250	500
9b	250	500	200	50	250	500
10	500	250	250	100	500	250
11a	50	100	50	50	125	125
11b	25	50	50	25	62.5	100
12	25	50	25	50	62.5	125
13	50	25	50	25	62.5	62.5
14	200	200	250	200	-	250
15	-	500	250	500	-	500
16	200	250	500	250	250	250
17	200	500	250	250	500	250
18	50	50	100	50	125	125
19a	25	25	25	50	125	125
19b	25	50	25	25	125	62.5
20	500	200	250	200	500	250
22	25	50	25	25	125	62.5
23	25	25	25	25	62.5	62.5
25a	250	-	500	500	-	-
25b	100	50	50	200	250	500
26	500	250	-	-	500	-
27	200	50	100	100	250	500
28	200	250	100	200	500	250
29	50	50	50	25	250	125
30	25	50	25	100	62.5	125
31	100	200	50	50	125	250
33	100	50	50	50	250	125
Amoxicillin	6.25	6.25	6.25	6.25	-	-
Ketoconazole	-	-	-	-	31.25	31.25

in the same concentrations. Inoculums of the bacterial and fungal culture were also prepared. To a series of tubes containing 1 mL each of phthalazine compound solution with different concentrations and 0.2 mL of the inoculums was added. Further 3.8 mL of sterile water was added to each of the test tubes. These test tubes were incubated for 24 h at 37 °C and observed for the presence of turbidity. This method was repeated by changing phthalazine compounds with standard drugs Amoxicillin and Ketoconazole for comparison. The minimum inhibitory concentration at which no growth was observed was taken as the MIC value (Table). The comparison of the MICs (in μg/mL) of potent compounds and standard drugs against tested strains are presented in the Table.

Investigation of the antibacterial screening data (Table) showed that some of the compounds were active against 4 pathogenic bacteria. 1,2,4-Triazolo[3,4-a]phthalazine derivatives **11b, 12, 19a,b, 30**, 1,3,5-triazino[4,3-a]phthalazine **22**, and tetrazolo[5,1-a] phthalazine **23** exhibited good activity against *S. aureus.* Similarly 1,2,4-triazolo[3,4-a]phthalazine derivatives **13, 19a**, and tetrazolo[5,1-a] phthalazine **23** exhibited good activity against *B. subtilis.* 1,2,4-Triazolo[3,4-a]phthalazine derivatives **12, 19a,b, 30**, 1,3,5-triazino[4,3-a]phthalazine **22**, and tetrazolo[5,1-a] phthalazine **23** exhibited good activity against *S. typhi.* 1,2,4-Triazolo[3,4-a]phthalazine derivatives **11b, 13, 19b, 29**, 1,3,5-triazino[4,3-a]phthalazine **22**, and tetrazolo[5,1-a] phthalazine **23** exhibited good activity against *E. coli.*

The antifungal results (Table) revealed that the synthesized compounds showed variable degrees of inhibition against the tested fungi. Compounds **11b, 12, 13, 19b, 22, 23,** and **30** possessed good antifungal activity against *A. niger* and *C. albican.* From the results it was concluded that the 1,2,4-triazolo[3,4-a]phthalazine derivatives, 1,3,5-triazino[4,3-a]phthalazine **22**, and tetrazolo[5,1-a] phthalazine **23** showed better activity.

Conclusions

In this article we report the synthesis of annelated phthalazine derivatives and acyclo C-nucleoside starting from 1-chloro-4-(2,4,6-trimethylphenyl)phthalazine **(4)**. Investigation of their antimicrobial activity revealed that 1,2,4-triazolo[3,4-a]phthalazine derivatives, 1,3,5-triazino[4,3-a]phthalazine **22**, and tetrazolo[5,1-a] phthalazine **23** were the most active compounds although the activity was significantly less than that of the positive control.

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