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

Maher Abdel Aziz EL-HASHASH¹, Ahmed Youssef SOLIMAN², Ibrahim Essam EL-SHAMY³,*

¹Chemistry Department, Faculty of Science, Ain Shams University, Cairo-EGYPT
²Chemistry Department, College of Science, King Khalid University, Abha-SAUDI ARABIA
³Chemistry Department, Faculty of Science, Fayoum University, Fayoum-EGYPT

e-mail: iei00@fayoum.edu.eg

Received: 30.11.2011

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 pyrimidinophthalazine 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

*Corresponding author
Synthesis and antimicrobial evaluation of some annelated phthalazine..., M. A. A. EL-HASHASH, et al.

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,\(^{22,23}\) antitypanosomal,\(^{24}\) 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 (\(\sim 25 \degree C\)) 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)phthalazin-1(2H)-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 \degree C; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 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\(_2\)O); IR (KBr) \(\nu\): 3212 (NH), 1654 cm\(^{-1}\) (CO); MS (70 eV) \(m/z\) (%): 264 (M\(^+\), 18). Anal. calcd. for C\(_{17}\)H\(_{16}\)N\(_2\)O: 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 \degree C; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 2.40 (s, 3H, CH\(_3\)), 2.51 (s, 6H, 2CH\(_3\)), 7.01-8.04 (m, 6H, Ar–H); IR (KBr) \(\nu\): 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)-8H-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; 1H-NMR (DMSO-d6) δ: 2.38 (s, 3H, CH3), 2.45 (s, 6H, 2CH3), 6.98-8.13 (m, 10H, Ar–H); 13C-NMR (DMSO-d6) 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) ν: 1670 (CO), 1614 cm⁻¹ (C=N); %MS (70 eV) m/z (%): 365 (M⁺, 8). Anal. calcd. for C24H19N3O: 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; 1H-NMR (DMSO-d6) δ: 2.33 (s, 3H, CH3), 2.42 (s, 6H, 2CH3), 4.61 (s, 2H, CH2), 7.03-8.17 (m, 7H, ArH and NH), 10.81 (brs, 1H, OH, exchangeable with D2O); IR (KBr) ν: 3280-2510 (OH, NH), 1702 cm⁻¹ (CO); MS (70 eV) m/z (%): 321 (M⁺, 7). Anal. calcd. for C19H19N3O2: 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; 1H-NMR (DMSO-d6) δ: 1.53 (d, J = 10 Hz, 3H, CH3), 2.39 (s, 3H, CH3), 2.51 (s, 6H, 2CH3), 4.50 (q, J = 10 Hz, 1H, CH), 7.11-8.12 (m, 7H, ArH and NH), 10.60 (brs, 1H, OH, exchangeable with D2O); IR (KBr) ν: 3240-2500 (OH, NH), 1694 (CO), 1605 cm⁻¹ (C=N); MS (70 eV) m/z (%): 335 (M⁺, 6). Anal. calcd. for C20H21N3O2: 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; 1H-NMR (DMSO-d6) δ: 2.30 (s, 3H, CH3), 2.44 (s, 6H, 2CH3), 2.81 (t, J = 10 Hz, 2H, NCH2), 3.77 (t, J = 10 Hz, 2H, CH2CO), 7.00-8.18 (m, 7H, ArH and NH), 11.00 (brs, 1H, OH, exchangeable with D2O); IR (KBr) ν: 3288-2511 (OH, NH), 1700 (CO), 1605 cm⁻¹ (C=N); MS (70 eV) m/z (%): 335 (M⁺, 4). Anal. calcd. for C20H21N3O2: 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(2H)-one (9a)

Yield, 60% (DMF); mp 179-180 °C; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 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\(_3\)O: 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(2H)-one (9b)

Yield, 63% (benzene); mp 170-171 °C; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 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) \(\delta\): 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\(_3\)O: C, 75.69; H, 6.03; N, 13.24; found C, 75.77; H, 6.10; N, 13.14.

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

Yield, 65% (ethanol); mp 199-200 °C; \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\): 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\(_2\)), 6.99-7.97 (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\(_4\)O: 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-\(d_6\)) \(\delta\): 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-\(d_6\)) \(\delta\): 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$_6$) $\delta$: 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$_6$) $\delta$: 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$_2$OH), 4.41-5.11 (m, 5H, D$_2$O 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$_4$O$_5$: 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$_2$O-EtOH to give compound 13 in 71% yield; mp 291-292 °C; $^1$H-NMR (DMSO-d$_6$) $\delta$: 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$_6$) $\delta$: 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$_8$O$_4$: 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$_6$) $\delta$: 2.32 (s, 6H, 2CH$_3$), 2.48 (s, 12H, 4CH$_3$), 5.03 (s, 2H, CH$_2$), 7.02-8.04 (m, 12H, ArH); $^{13}$C-NMR (DMSO-d$_6$) $\delta$: 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 (%): 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.


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$_6$) $\delta$: 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-4H-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; 1H-NMR (DMSO- 6) δ: 1.83 (s, 3H, CH3), 2.30 (s, 3H, CH3), 2.39 (s, 3H, CH3), 2.48 (s, 6H, 2CH3), 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 C24H21N3O: 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-14H-[1]-benzothieno-[2’,3’:4,5]-pyrimido[2,3-a]pyridazin-14-one (17)

A mixture of compound 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; 1H-NMR (DMSO-d 6) δ: 2.21-2.26 (m, 4H, 2CH2), 2.30-2.34 (m, 4H, 2CH2), 2.41 (s, 3H, CH3), 2.50 (s, 6H, 2CH3), 7.00-8.05 (m, 6H, Ar–H); 13C-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 C26H23N3OS: 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; 1H-NMR (DMSO-d 6) δ: 2.34 (s, 3H, CH3), 2.45 (s, 6H, 2CH3), 6.24 (s, 2H, NH2, exchangeable with D2O), 7.3-8.01 (m, 6H, ArH); IR (KBr) v: 3422, 3380 (NH2), 1605 cm −1 (C=N). Anal. calcd. for C18H17N5: 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; 1H-NMR (DMSO-d 6) δ: 2.36 (s, 3H, CH3), 2.47 (s, 6H, 2CH3), 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 C25H21N5: 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; 1H-NMR (DMSO-d6) δ: 2.39 (s, 3H, CH3), 2.49 (s, 6H, 2CH3), 6.98-8.01 (m, 10H, ArH); IR (KBr) v: 1621 cm⁻¹ (C=N). Anal. calcd. for C25H20ClN5: C, 70.50; H, 4.73; N, 16.44; found C, 70.66; H, 4.70; N, 16.51.

7-(2,4,6-Trimethylphenyl)-13H-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; 1H-NMR (DMSO-d6) δ: 2.37 (s, 3H, CH3), 2.49 (s, 6H, 2CH3), 4.90 (s, 2H, CH2 quinazoline), 6.97-8.14 (m, 10H, ArH); IR (KBr) v: 1610 cm⁻¹ (C=N). Anal. calcd. for C24H21N3: 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; 1H-NMR (DMSO-d6) δ: 2.33 (s, 3H, CH3), 2.46 (s, 6H, 2CH3), 6.99-8.12 (m, 11H, ArH); 13C-NMR (DMSO-d6) 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 C25H20N4OS: 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; 1H-NMR (DMSO-d6) δ: 2.33 (s, 3H, CH3), 2.40 (s, 6H, 2CH3), 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 C17H15N5: 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-1-yl]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; 1H-NMR (DMSO-d6) δ: 2.33 (s, 3H, CH3), 2.45 (s, 6H, 2CH3), 5.31 (s, 2H, NH2 exchangeable with D2O), 7.37-8.50 (m, 6H, Ar–H), 9.55 (s, 1H, NH exchangeable with D2O); IR (KBr) v: 3390-3112 cm⁻¹ (NHNH2); MS (70 eV) m/z (%): 278 (M⁺, 21). Anal. calcd. for C17H18N4: 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-2H-[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; 1H-NMR (DMSO-d6) δ: 2.11 (s, 3H, CH3), 2.36 (s, 3H, CH3), 2.50 (s, 6H, 2CH3), 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 C20H20N4: 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; 1H-NMR (DMSO-d6) δ: 2.30 (s, 3H, CH3), 2.47 (s, 6H, 2CH3), 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 C25H22N4O: C, 79.34; H, 5.86; N, 14.80; found C, 79.29; H, 5.90; N, 14.88.

7-(2,4,6-Trimethylphenyl)-2H-[1,2,4]triazino[3,4-a]phthalazin-3(4H)-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; 1H-NMR (DMSO-d6) δ: 2.29 (s, 3H, CH3), 2.44 (s, 6H, 2CH3), 4.57 (s, 2H, CH2 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 C19H18N4O: C, 71.68; H, 5.70; N, 17.60; found C, 71.57; H, 5.77; N, 17.55.

7-(2,4,6-Trimethylphenyl)-2H-[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; 1H-NMR (DMSO-d6) δ: 2.33 (s, 3H, CH3), 2.47 (s, 6H, 2CH3), 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 C19H16N4O2: 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-4H-[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; 1H-NMR (DMSO-d6) δ: 2.01 (s, 3H, CH3), 2.28 (s, 3H, CH3), 2.43 (s, 6H, 2CH3), 7.01-8.00 (m, 6H, ArH); 13C-NMR (DMSO-d6) 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 C20H18N4O: 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; 1H-NMR (DMSO-d6) δ: 2.30 (s, 3H, CH3), 2.48 (s, 6H, 2CH3), 6.98-7.89 (m, 6H, ArH), 9.57 (s, 1H, =CH triazol); IR (KBr) ν: 1601 cm⁻¹ (C=N). Anal. calcd. for C18H16N4: 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(2H)-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; 1H-NMR (DMSO-d6) δ: 2.35 (s, 3H, CH3), 2.50 (s, 6H, 2CH3), 7.01-7.90 (m, 6H, ArH), 9.32 (s, 1H, NH exchangeable with D2O); IR (KBr) ν: 3218 (NH), 1601 cm⁻¹ (C=N); MS (70 eV) m/z (%): 320 (M+ +, 10). Anal. calcd. for C18H16N4S: 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(2H)-one (31)

A mixture of 24 (2.78 g, 0.01 mol) and ethyl chloroformate (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; 1H-NMR (DMSO-d6) δ: 2.36 (s, 3H, CH3), 2.48 (s, 6H, 2CH3), 6.98-7.94 (m, 6H, ArH), 8.78 (s, 1H, NH exchangeable with D2O); IR (KBr) ν: 3211 (NH), 1664 (CO), 1608 cm⁻¹ (C=N); MS (70 eV) m/z (%): 304 (M+ +, 16). Anal. calcd. for C18H16N4O: C, 71.04; H, 5.30; N, 17.49; found C, 71.11; H, 5.36; N, 18.32.

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

A solution 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 reaction mixture was poured onto ice water. The precipitated solid was collected by filtration, dried and crystallized from ethanol to give 32 in 73% yield; mp 177-176 °C; 1H-NMR (DMSO-d6) δ: 2.33 (s, 3H, CH3), 2.44 (s, 6H, 2CH3), 6.99-8.00 (m, 11H, ArH), 12.52 (s, 1H, NHPh, exchangeable with D2O); IR (KBr) ν: 3211 (NH), 1664 (CO), 1608 cm⁻¹ (C=N); MS (70 eV) m/z (%): 413 (M+ +, 6). Anal. calcd. for C24H23N5S: 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 24 (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
Synthesis and antimicrobial evaluation of some annelated phthalazine..., M. A. A. EL-HASHASH, et al.

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; 1H-NMR (DMSO-d6) δ: 2.36 (s, 3H, CH3), 2.48 (s, 6H, 2CH3), 7.01-8.10 (m, 11H, ArH), 9.25 (s, 1H, NHPH, exchangeable with D2O); IR (KBr) ν: 3211 cm⁻¹ (NH); MS (70 eV) m/z (%): 379 (M⁺, 6). Anal. calcd. for C24H21N5: 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)-2H-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 1H-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).](image)

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 13C-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 phthalazinylamino 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 \(\delta\) 4.61, and exchangeable NH and OH protons as singlet signals at \(\delta\) 8.01 and 10.81, respectively. The \(^1\)H-NMR spectrum of compound 7b displayed a CH proton as a quartet signal at \(\delta\) 4.50 and CH\(_3\) protons as a doublet signal at \(\delta\) 1.53. The \(^1\)H-NMR spectrum of compound 8 showed a triplet signal at \(\delta\) 2.81 assigned for NCH\(_2\) and a triplet signal at 3.77 assigned for CH\(_2\)CO beside NH and OH protons. The amino acid derivatives 7a,b and 8 were easily cyclized 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 acyclic 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 bis(hydrazide) 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 \(\delta\) 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 \(\delta\) 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-carboxethoxy 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 \(\delta\) 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 \(\delta\) 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

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$_2$O) and 9.55 for assigned NH (exchangeable with D$_2$O). Cyclo-condensation of compound 24 with different $\alpha$-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.
to acyl carbon of the ester group through a tetrahedral mechanism followed by $S_N^2$-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$_2$O). Condensation of compound 24 with pyruvic acid by heating at 180 $^\circ$C gave the corresponding triazino derivat-
Synthesis and antimicrobial evaluation of some anellated phthalazine..., M. A. A. EL-HASHASH, et al.

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 ethyl chloroformate 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 D₂O) and 12.52 (s, 1H, NHPh, exchangeable with D₂O) ppm. The ¹H-NMR spectrum of compound 33 showed δ 9.26 assigned for NHPh (exchangeable with D₂O).

**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 *Aspergillus 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 μ g 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.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Minimum inhibitory concentration (MIC) in μg/mL</th>
<th>Bacterial strains</th>
<th>Fungal strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
<td>B. subtilis</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td>9a</td>
<td></td>
<td>200</td>
<td>-</td>
</tr>
<tr>
<td>9b</td>
<td></td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>11a</td>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>11b</td>
<td></td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>-</td>
<td>500</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>200</td>
<td>500</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>19a</td>
<td></td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>19b</td>
<td></td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>25a</td>
<td></td>
<td>250</td>
<td>-</td>
</tr>
<tr>
<td>25b</td>
<td></td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>26</td>
<td></td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>200</td>
<td>50</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>31</td>
<td></td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>33</td>
<td></td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td></td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

363
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.

References

Synthesis and antimicrobial evaluation of some annelated phthalazine..., M. A. A. EL-HASHASH, et al.


Synthesis and antimicrobial evaluation of some annelated phthalazine..., M. A. A. EL-HASHASH, et al.


