

Article

Synthesis and Biological Screening of 4-Benzyl-2H-phthalazine Derivatives

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Received: 20 June 2011; in revised form: 13 July 2011 / Accepted: 13 July 2011 /

Published: 17 August 2011

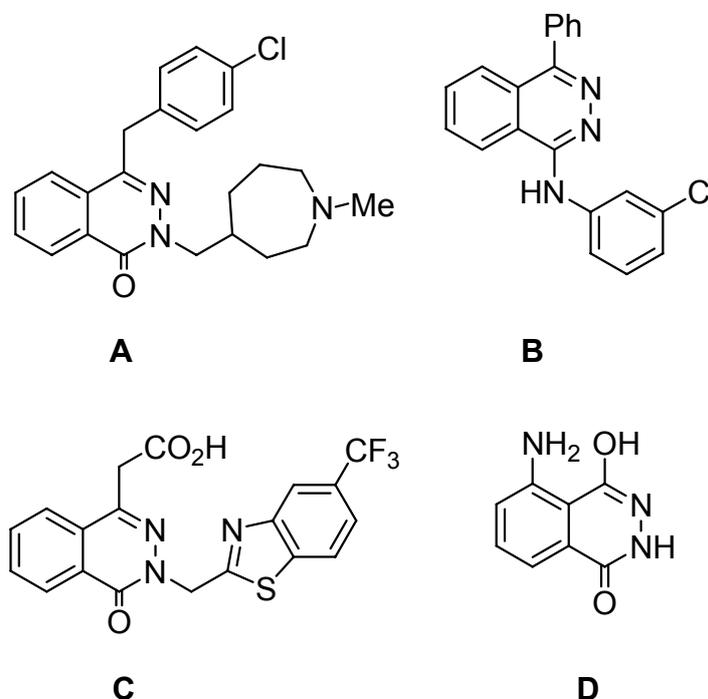
Abstract: Preparation of 4-benzyl-2-substituted phthalazin-1-one derivatives **2-8** is reported. Condensation of 4-benzyl-1-chlorophthalazine (**9**) with a series of different nucleophiles gave 4-benzylphthalazin-1-ylamino derivatives (**10-13** and **16**) and 4-amino-2-[N-(4-benzylphthalazin-1-yl)-hydrazino]-6-arylpyrimidine-5-carbonitriles (**14a,b**). Interaction of **9** with ambident anions was also studied. 5-Benzyl-6,6a,12-triazobenzo[*a*]-anthracen-7-one (**15**) is obtained from **9** and anthranilic acid derivatives. Treatment of **16** with (EtO)₃CH/Ac₂O under reflux afforded the corresponding ethoxymethylene derivative **17**, while aqueous ammonium hydroxide treatment afforded carboxamide derivative **18**. The structures of the newly synthesized derivatives were confirmed by their elemental analysis, IR, ¹H NMR, ¹³C NMR and mass spectral studies. Antimicrobial activities of some selected compounds were also studied and some of these were found to exhibit promising effects against Gram-positive and Gram-negative bacteria and fungi.

Keywords: phthalazine derivatives; anthracene derivatives; synthesis; antimicrobial activity

1. Introduction

The phthalazine derivative azelastine (**A**, Figure 1) is an antihistamine used in the treatment of allergic rhinitis [1]. Newer agents are more selective inhibitors of the cGMP-inhibited phosphodiesterase (PDE) and can be exemplified by phthalazine derivatives like MY5445 (**B**, Figure 1) [2-5]. Zopolrestat (**C**, Figure 1) is a phthalazinone derivative that has been in clinical trials; it inhibits aldose reductase and has potential use in the prevention of retinopathy, neuropathy, and cataract formation in diabetes [6]. The chemiluminescence reactions of luminol (**D**, Figure 1) and related phthalazines have found analytical applications, particularly in biological systems where the inherent signal strength and low signal noise ratio contribute to sensitivity. The hydrogen peroxide/luminol system has been used for the on-line determination by chemiluminescence of nitric oxide in isolated organ perfusates [7-10].

Figure 1. Phthalazine derivatives (azelastin, MY 5445, zopolrestat and luminol).



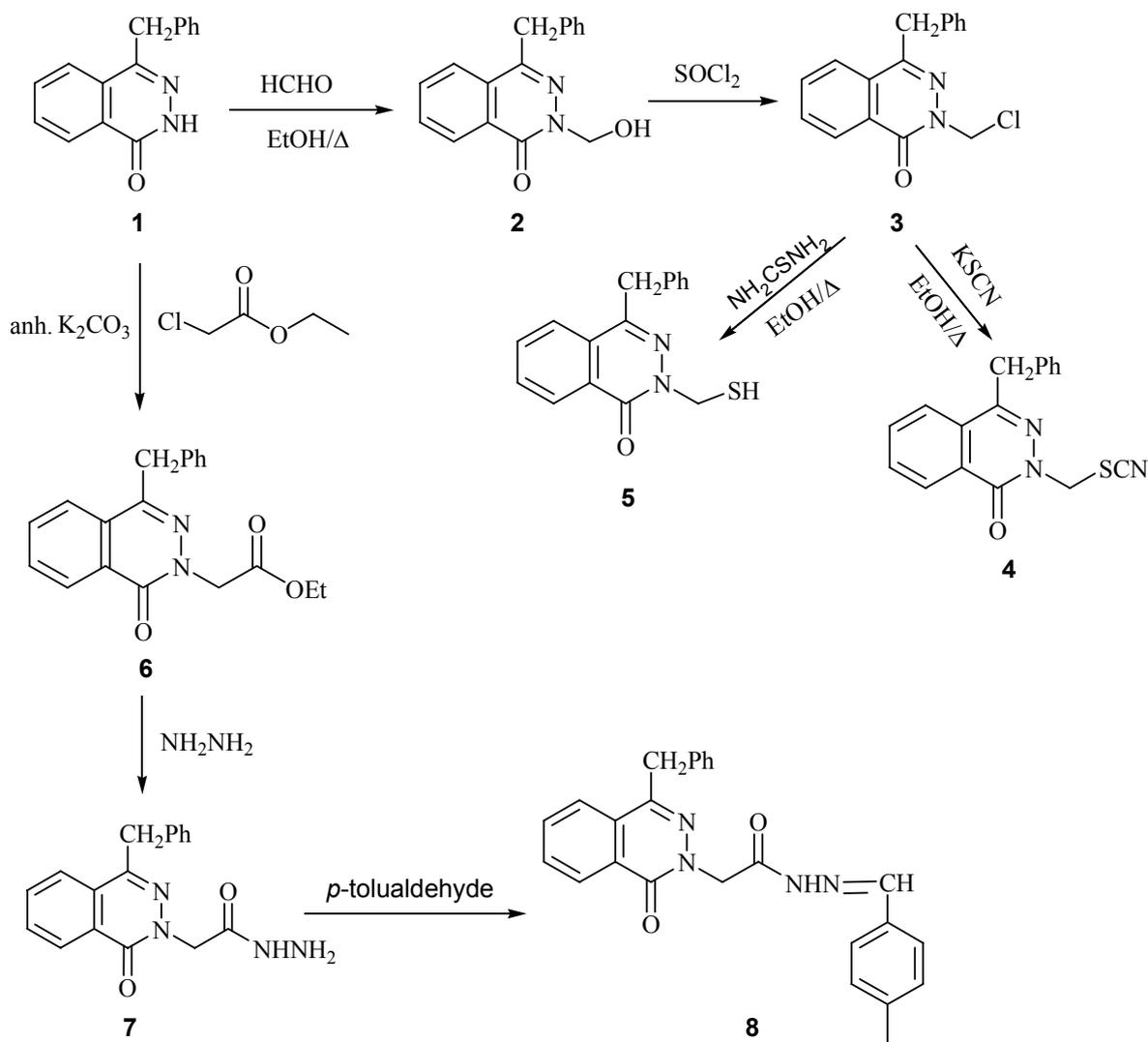
Phthalazine derivatives have been widely applied as therapeutic agents due to their anticonvulsant, cardiotoxic, vasorelaxant and anti-inflammatory properties [11-17] in addition to having antimicrobial activity [18].

2. Results and Discussion

4-Benzyl-2*H*-phthalazin-1-one (**1**) [19] was used as a starting material for this investigation. Reaction of compound **1** with formalin in ethanol afforded 4-benzyl-2-hydroxymethyl-2*H*-phthalazin-1-one (**2**). Treatment of **2** with thionyl chloride afforded the corresponding 4-benzyl-2-chloromethyl-2*H*-phthalazin-1-one (**3**). Interaction of **3** with alcoholic potassium thiocyanate yield the corresponding 4-benzyl-2-thiocyanatomethyl-2*H*-phthalazin-1-one (**4**), while with ethanolic thiourea it afforded 4-benzyl-2-mercaptomethyl-2*H*-phthalazin-1-one (**5**). Treatment of **1** with ethyl chloroacetate in the presence of anhydrous K_2CO_3 afforded the corresponding ethyl (2-(4-benzyl-1-oxo-1*H*-phthalazin-2-yl)acetate (**6**).

Reaction of **6** with hydrazine hydrate gave the corresponding (4-benzyl-1-oxo-1*H*-phthalazin-2-yl)acetic acid hydrazide (**7**) which was reacted with *p*-tolualdehyde to give the corresponding (4-benzyl-1-oxo-1*H*-phthalazin-2-yl) acetic acid (4-methylbenzylidene) hydrazide (**8**) (Scheme 1).

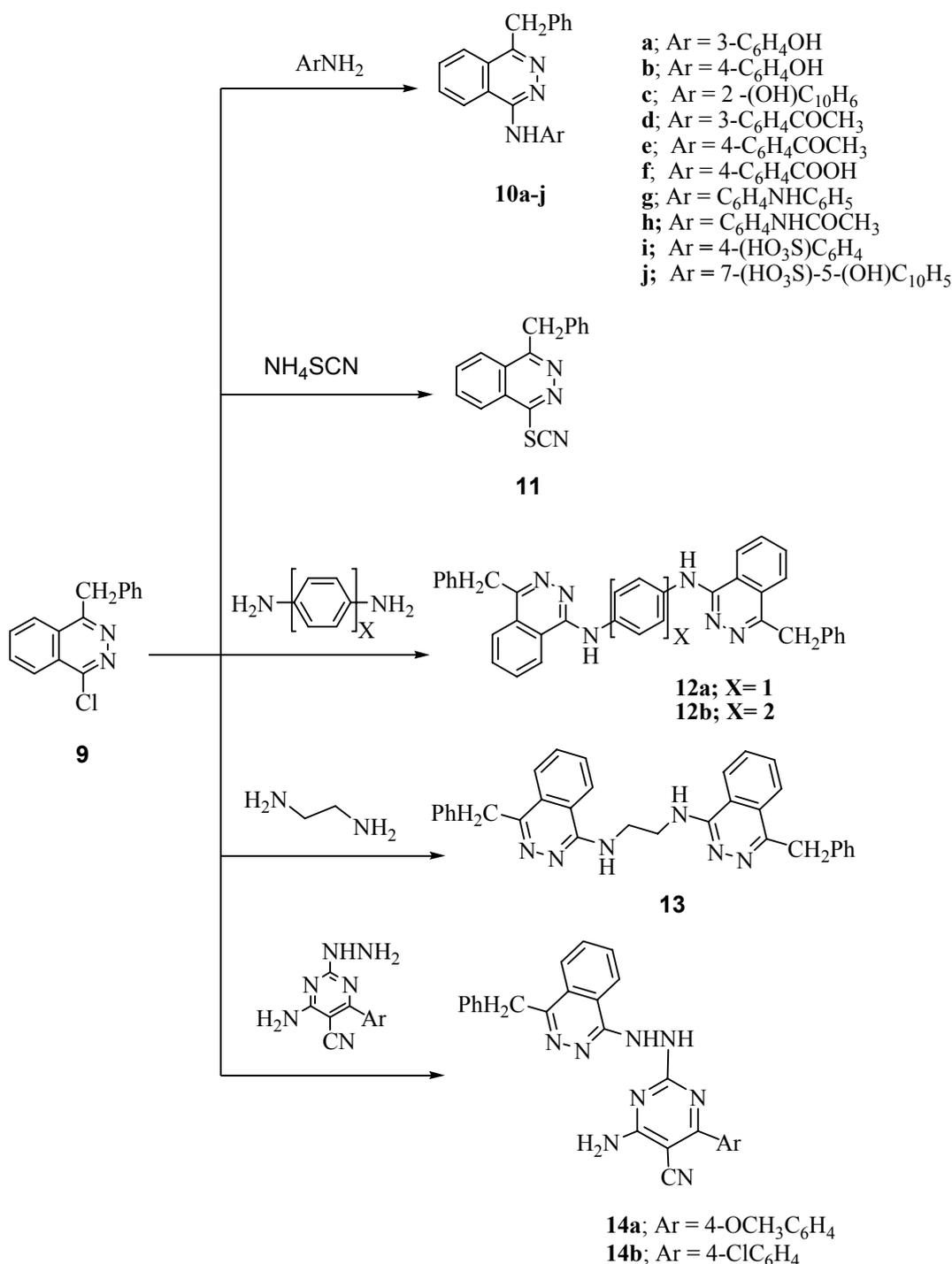
Scheme 1. Synthesis of phthalazine-1-one derivatives.



4-Benzyl-1-chlorophthalazine (**9**) [19] was used as starting material from preparation of new 1,4-disubstituted phthalazine derivatives. Thus, interaction of **9** with equimolar amount of aminophenols, aminoacetophenones and *p*-aminobenzoic acid gave the corresponding 4-benzylphthalazin-1-ylamino derivatives (**10a-f**). Compound **9** also reacted with *N*-(4-aminophenyl)acetanilide under reflux to give the corresponding 4-(4-benzylphthalazin-1-ylamino)acetanilide (**10g**), in the case of *p*-phenylene-diamine and/or benzidine but in the case of *p*-aminodiphenyl amine the only isolable product is 4-(4-benzylphthalazin-1-ylamino)diphenylamine (**10h**). Treatment of **9** with sulfanilic acid and/or *J*-acid (2-amino-5-naphthol-7-sulfonic acid) gave the corresponding 4-(4-benzylphthalazin-1-ylamino)benzenesulfonic acid (**10i**) and/or 7-(4-benzylphthalazin-1-ylamino)-4-hydroxynaphthalene-2-sulfonic acid (**10j**), while **9** with ammonium thiocyanate gave the corresponding 4-benzylphthalazin-1-ylthiocyanate (**11**). Interaction of **9** with dibasic aromatic amines in a 1:2 ratio under fusion conditions afforded the corresponding *N,N'*-bis(4-benzyl-phthalazin-1-yl)-1,4-phenylenediamine

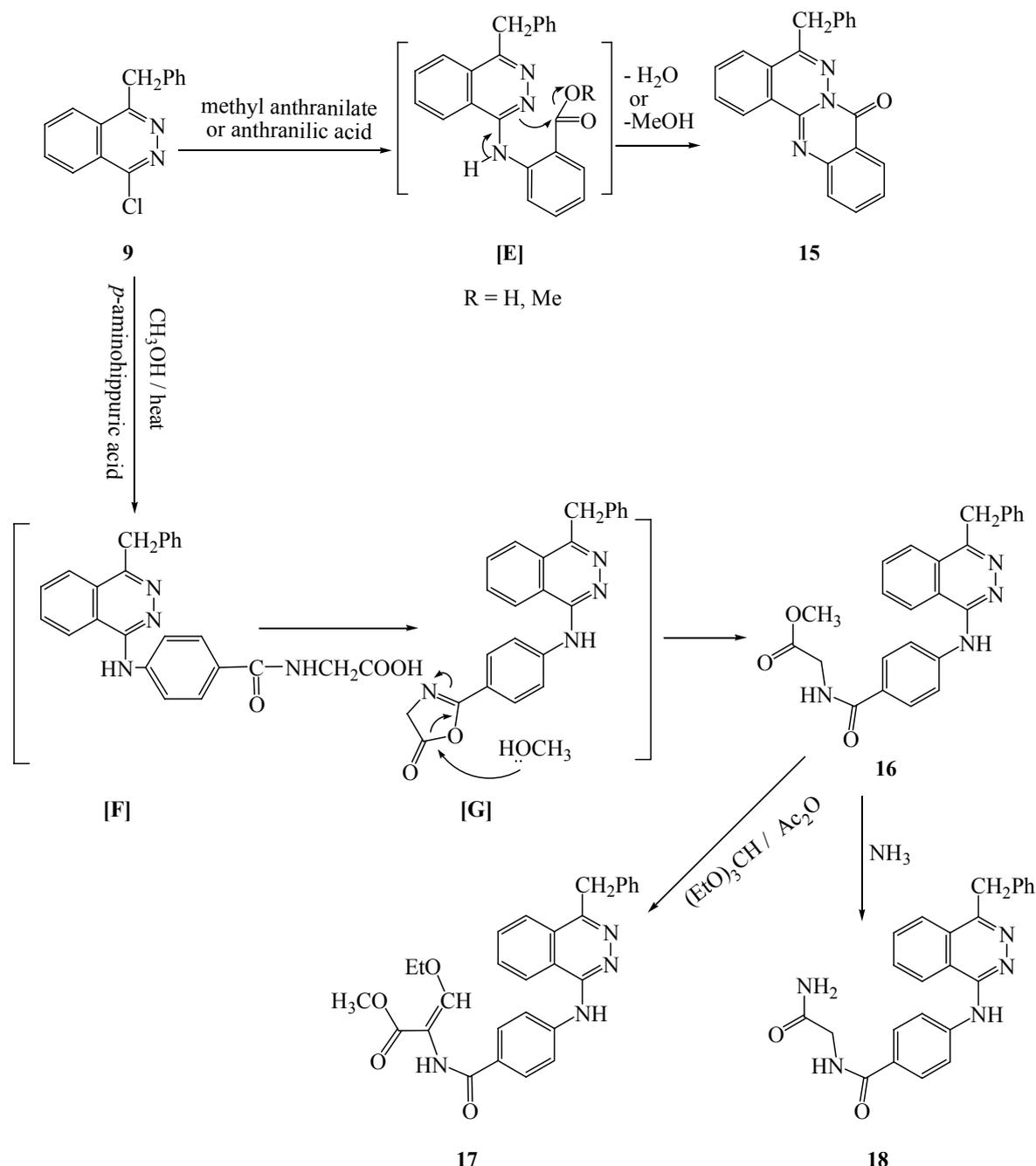
and/or *N,N'*-bis(4-benzylphthalazin-1-yl)biphenyl-4,4'-diamines (**12a,b**). Also, **9** was reacted with ethylenediamine in boiling ethanol to give *N,N'*-bis(4-benzylphthalazin-1-yl)ethane-1,2-diamine (**13**). However, interaction of **9** with 2-hydrazino-4-amino-5-cyano-6-arylpyrimidine gave the corresponding pyrimidine-5-carbonitrile derivatives **14a,b** respectively (Scheme 2). Interaction of **9** with methyl anthranilate and/or anthranilic acid gave anthracen-7-one derivative **15** as the only isolable product. The formation of **15** was explained by the formation of intermediate [E] which undergoes intramolecular ring closure to the final product **15** (Scheme 3).

Scheme 2. Reactions of 4-benzyl-1-chlorophthalazine with amines derivatives.



Treatment of **9** with *p*-aminohippuric acid in boiling methanol/TEA, gave the corresponding methyl ester derivative **16** as the only isolable product. The formation of **16** can be explained by the nucleophilic transformation into benzylphthalazin-1-ylamino derivative [**F**] as intermediate which undergoes intramolecular cyclization into oxazolone intermediate [**G**] then reacted with a molecule of methanol to give the methyl ester derivative **16** as the final product (Scheme 3).

Scheme 3. Synthesis of triazobenzobenzo[*a*]anthracen-7-one, methyl ester and carboxamide derivatives.



Treatment of **16** with $(\text{EtO})_3\text{CH}/\text{Ac}_2\text{O}$ under reflux afforded the corresponding ethoxymethylene derivative **17**. Treatment of **16** with aqueous ammonium hydroxide under reflux in dioxane in the presence of triethylamine afforded the corresponding carboxamide derivative **18** respectively (Scheme 3).

3. Experimental

3.1. General

Melting points were determined on a Stuart melting point apparatus and are uncorrected. IR spectra were recorded in KBr using a FT-IR 5300 spectrometer and Perkin Elmer spectrum RXIFT-IR system (ν , cm^{-1}). The ^1H NMR at (300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded in CDCl_3 or $\text{DMSO-}d_6$ on a Varian Mercury VX-300 NMR spectrometer. Chemical shifts (δ) are related to that of the solvent. Mass spectra were measured on a Shimadzu GMMS-QP-1000 EX mass spectrometer at 70 eV. The elemental analyses were performed at the Microanalytical Center, Cairo University, Cairo (Egypt).

4-Benzyl-2-hydroxymethyl-2H-phthalazin-1-one (2). A mixture of compound **1** (0.23 g, 1.0 mmol) and formaldehyde solution 38% (0.8 mL) in ethanol (30 mL) was refluxed for 3 hours. The solvent was evaporated under vacuum, and then water (25 mL) was added. The solid obtained was filtered off and recrystallized from ethanol. Colourless crystals, 90%, 0.19 g. mp 115-116 °C; IR (ν_{max} , cm^{-1}): 3334 (OH), 1640 (CO). ^1H NMR (CDCl_3): δ_{H} 4.13 (t, $J = 8.4$ Hz, 1H, OH), 4.31 (s, 2H, CH_2Ph), 5.69 (d, $J = 8.4$ Hz, 2H, CH_2OH), 7.30 (m, 5H, Ph-H), 7.71 (m, 3H, phthalazinyl-3H), 8.45 (m, 1H, phthalazinyl-1H); the singlet at 4.134 was cancelled by D_2O . ^{13}C NMR ($\text{DMSO-}d_6$) δ 37.3 (CH_2), 60.1 (CH_2OH), 125.7 ($\text{C}_4\text{-Ar}$), 128 (C_5), 128.3 (C_8), 128.8 ($\text{C}_3\text{-Ar}$, $\text{C}_5\text{-Ar}$), 129.1 ($\text{C}_2\text{-Ar}$, $\text{C}_6\text{-Ar}$), 130.2 (C_{5a}), 130.3 (C_{8a}), 131.2 (C_7), 132.3 (C_6), 137.2 ($\text{C}_1\text{-Ar}$), 155.1 (C_4) and 160.1 (CO). MS, m/z (%) = 266 (M^+ , 10.25), 235 (100) together with other peaks at 207 (5.74), 178 (20.38), 149 (15.82), 130 (9.19), 91 (12.03), 57 (17.77); Anal. Calcd for $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_2$ (266.10): C, 72.16; H, 5.30; N, 10.52%. Found: C, 71.89; H, 5.02; N, 9.96%.

4-Benzyl-2-chloromethyl-2H-phthalazin-1-one (3). A mixture of compound **2** (0.26 g, 1.0 mmol) and thionyl chloride (5 mL) was refluxed for 1 hour. The solid obtained was filtered off and recrystallized from benzene. Colourless crystals, 87%, 0.12 g. mp 125-127 °C; IR (ν_{max} , cm^{-1}): 3046 (C-H aromatic), 1662 (CO). MS, m/z (%) = 284 (M^+ , 43.4), $\text{M}+2$ (12.7), 249 (100) together with other peaks at 220 (34.9), 178 (17.9), 130 (29.2), 91 (80.7), 51 (28.9); Anal. Calcd for $\text{C}_{16}\text{H}_{13}\text{ClN}_2\text{O}$ (284.07): C, 67.49; H, 4.60; N, 9.84%. Found: C, 66.90; H, 4.08; N, 9.11%.

4-Benzyl-2-thiocyanatomethyl-2H-phthalazin-1-one (4). A mixture of compound **3** (0.28 g, 1.0 mmol) and potassium thiocyanate (1.0 mmol) in ethanol (30 mL) was refluxed for 3 hours. The solvent was evaporated under vacuum. The solid obtained was filtered off and recrystallized from ethanol. White crystals, 85%, 0.17 g. mp 155-156 °C; IR (ν_{max} , cm^{-1}): 2158 (SCN), 1664 (CO). MS, m/z (%) = 307 (M^+ , 0.7), 249 (56.3), 130 (12.8) and 91 (100); Anal. Calcd for $\text{C}_{17}\text{H}_{13}\text{N}_3\text{OS}$ (307.36): C, 66.43; H, 4.26; N, 13.67%. Found: C, 66.22; H, 3.98; N, 13.50%.

4-Benzyl-2-mercaptopmethyl-2H-phthalazin-1-one (5). A mixture of compound **3** (0.28 g, 1.0 mmol) and thiourea (1.0 mmol) in ethanol (30 mL) was refluxed for 3 h hours. The solvent was evaporated under vacuum. The solid obtained was filtered off and recrystallized from ethanol. White crystals, 80%, 0.10 g. mp 145-146 °C; IR (ν_{max} , cm^{-1}): 2750 (SH), 1650 (CO). MS, m/z (%) = 282 (M^+ , 17.2),

249 (78.2), 235 (18.1), 132 (27.3), 130 (23.5), 91 (100) 51 (32.8); Anal. Calcd for $C_{16}H_{14}N_2OS$ (282.36): C, 68.06; H, 5.00; N, 9.92%. Found: C, 67.45; H, 4.67; N, 9.22%.

Ethyl (4-benzyl-1-oxo-1H-phthalazin-2-yl)acetate (6). A mixture of compound **1** (0.23 g, 10 mmol) and ethyl chloroacetate (2 mL) and anhydrous K_2CO_3 (0.13 g, 1.0 mmol) was refluxed for 4 hours. The solvent was evaporated under vacuum, then water (50 mL) was added. The solid obtained was filtered off and recrystallized from pet. ether and chloroform, respectively. Violet crystals, 65%, 0.13 g. mp 76-78 °C; IR (ν_{max} , cm^{-1}): 2978 (CH aliphatic), 1744, 1648 (CO). MS, m/z (%) = 322 (M^+ , 37.9) and base peak at 248 together with other peak at 249 (97.9), 221 (8.2), 220 (21.5), 219 (24.3), 193 (4.6), 102 (14.6), 91 (98.4), 76 (11.4); Anal. Calcd for $C_{19}H_{18}N_2O_3$ (322.12): C, 70.79; H, 5.63; N, 8.69%. Found: C, 70.36; H, 5.44; N, 8.52%.

(4-Benzyl-1-oxo-1H-phthalazin-2-yl)acetic acid hydrazide (7). A mixture of compound **6** (0.32 g, 10 mmol) and hydrazine hydrate (0.8 mL) in ethanol (30 mL) was refluxed for 3 hours. The solvent was evaporated under vacuum, then water (25 mL) was added. The solid obtained was filtered off and recrystallized from ethanol. White crystals, 86%, 0.18 g. mp 130-132 °C; IR (ν_{max} , cm^{-1}): 3326 (NH), 1684 (CO, carboxylic acid hydrazide), 1642 (CO, phthalazinyl). 1H NMR ($CDCl_3$): δ_H 14.29 (s, 2H, CH_2Ph), 4.75 (s, 2H, NCH_2CO), 7.29 (m, 5H, Ar-H), 7.81 (m, 5H, phthalazinyl-3H and NH_2), 8.25 (m, 1H, phthalazinyl-H) and 9.30 (s, 1H, CONH; cancelled by D_2O). MS, m/z (%) = 308 (M^+ , 0), 278 (7.2), 277 (23.4), 249 (44.9), 130 (15.3), 91 (100); Anal. Calcd for $C_{17}H_{16}N_4O$ (308.33): C, 66.22; H, 5.23; N, 18.17%. Found: C, 66.09; H, 5.12; N, 17.89%.

3.2. General Procedure for the Synthesis of Benzylphthalazin-1-ylamino Derivatives **10a-h**

A mixture of compound **9** (0.25g, 10 mmol) and aromatic amine (10 mmol) in ethanol (30 mL) was refluxed for 3 hours. The solvent was evaporated under vacuum. The solid obtained was filtered off to give crude products

(4-Benzylphthalazin-1-ylamino)phenols (10a,b). Compound **10a**: Yellow crystals, 90%, 0.17 g. mp 240-242 °C; IR (ν_{max} , cm^{-1}): 3258 (NH and OH phenolic) as broad peak. MS, m/z (%) = 307 327 (M^+ , 42.2) and the base peak at 326 together with other peaks at 325 (84.3), 91 (62.6); Anal. Calcd for $C_{21}H_{17}N_3O$ (327.10): C, 77.04; H, 5.23; N, 12.84%. Found: C, 76.83; H, 5.02; N, 12.56%. Compound **10b**: Yellow crystals, 80%, 0.12 g. mp 236-237 °C; IR (ν_{max} , cm^{-1}): 3268 (NH and OH phenolic) as broad peak. MS, m/z (%) = 327 (M^+ , 40.0) and the base peak at 326 together with other peaks at 325 (86.7), 91 (50.8); Anal. Calcd for $C_{21}H_{17}N_3O$ (327.10): C, 77.04; H, 5.23; N, 12.84%. Found: C, 76.75; H, 5.01; N, 12.59%.

7-(4-Benzylphthalazin-1-ylamino)naphthalen-2-ol (10c). Green crystals, 70%, 0.09 g. mp 242-243 °C; IR (ν_{max} , cm^{-1}): 3272 (OH and NH) as broad peak. 1H NMR ($DMSO-d_6$): δ_H 24.65 (s, 2H, CH_2Ph), 7.08-7.33, 7.55-7.58, 8.09-8.11 (ms, 9H, Ar-H), 7.38 (d, $J = 7.8$ Hz, 1H, Ar-H), 7.78 (d, $J = 9$ Hz, 1H, Ar-H), 7.86 (d, $J = 9$ Hz, 1H, Ar-H), 8.14 (d, $J = 1.8$ Hz, 1H, Ar-H), 8.16 (s, 1H, NH, exchangeable with D_2O), 8.33 (d, $J = 1.8$ Hz, 1H, Ar-H), 9.04 (d, $J = 7.8$ Hz, 1H, Ar-H) and 9.96 (s, 1H,

phenolic-OH, exchangeable with D₂O).; Anal. Calcd for C₂₅H₁₉N₃O (377.13): C, 79.55; H, 5.07; N, 11.13%. Found: C, 79.32; H, 4.87; N, 10.92%.

1-[(4-Benzylphthalazin-1-ylamino)phenyl]ethanones (10d,e). Compound **10d**: White crystals, 75%, 0.11 g. mp 225-226 °C; IR (ν_{\max} , cm⁻¹): 3390 (NH), 1662 (CO). MS, m/z (%) = 353 (73.56) and the base peak at 352 together with other peaks at 311 (3.7), 310 (11.97), 262 (10.56), 220 (5.37), 219 (4.82) and 91 (54.14); Anal. Calcd for C₂₃H₁₉N₃O (353.05): C, 78.16; H, 5.42; N, 11.89%. Found: C, 77.88; H, 5.23; N, 11.64%. Compound **10e**: Yellow crystals, 80%, 0.13 g. mp 260-261 °C; IR (ν_{\max} , cm⁻¹): 3312 (NH), 1670 (CO). ¹H NMR (DMSO-*d*₆): δ_{H} 2.57 (s, 3H, CH₃CO), 4.75 (s, 2H, CH₂Ph), 7.21-7.27 (m, 2H, Ar-H), 7.31 and 7.40 (AB-q, $J = 7.2$ Hz, 4H, Ar-H), 8.01 (s, 1H, NH), 8.10-8.18 (m, 5H, Ar-H) and 8.39, 8.94 (2d, $J = 7.8$ Hz, 2H, Ar-H); Anal. Calcd for C₂₃H₁₉N₃O (353.23): C, 78.18; H, 5.42; N, 11.89%. Found: C, 77.97; H, 5.18; N, 11.69%.

4-(4-Benzylphthalazin-1-ylamino)benzoic acid (10f). Yellow crystals, 85%, 0.16 g. mp 280-281 °C; IR (ν_{\max} , cm⁻¹): 3224 (NH and OH), 1676 (CO). MS, m/z (%) = 355 (38.4) and base peak at 354 together with other peaks at 310 (3), 234 (5.3), 204 (9.9), 219 (6.9), 204 (9.9), 178 (10.1), 102 (15.9), 91 (68.3), 90 (60.2), 77 (30.6) and 76 (29.7); Anal. Calcd for C₂₂H₁₇N₃O₂ (355.16): C, 74.35; H, 4.82; N, 11.82%. Found: C, 74.14; H, 4.57; N, 11.60%.

4-(4-Benzylphthalazin-1-ylamino)diphenyl amine (10g). Green crystals, 70%, 0.13 g. mp 215-217 °C; IR (ν_{\max} , cm⁻¹): 3276 (NH). MS, m/z (%) = 402 (M⁺, 46.5), 117 (88.7), 92 (60.32), 76 (28.37), 91 (18.88), 57 (87.49), 56 (29.88) & 55 (100); Anal. Calcd for C₂₇H₂₂N₄ (402.02): C, 80.57; H, 5.51; N, 13.92%. Found: C, 80.44; H, 5.49; N, 13.75%.

4-(4-benzylphthalazin-1-ylamino)acetanilide (10h). Yellow crystals, 80%, 0.18 g. mp > 340 °C; IR (ν_{\max} , cm⁻¹): 3428 (NH), 1620 (CO). MS, m/z (%) = 368 (M⁺, 12.94), 235 (10.25), 149 (88.35), 117 (78.7), 92 (63.32), 76 (28.37), 91 (18.88), 57 (87.49), 56 (29.88) and 55 (100); Anal. Calcd for C₂₃H₂₀N₄O (368.17): C, 74.98; H, 5.47; N, 15.21%. Found: C, 74.80; H, 5.32; N, 15.12%.

3.3. General Method for the Synthesis of Sulfonic Acid Derivatives **10i, j**

A mixture of compound **9** (0.25 g, 1.0 mmol) in ethanol (30 mL) sulfanilic acid or (2-amino-5-naphthol-7-sulfonic acid) (1.0 mmol) and 2 drops of triethylamine (TEA) was refluxed for 3 hours. The solvent was evaporated under vacuum. The solid obtained was filtered off and recrystallized from dioxane.

4-(4-Benzylphthalazin-1-ylamino)benzenesulfonic acid (10i). Yellow crystals, 80%, 0.17 g. mp > 340 °C; IR (ν_{\max} , cm⁻¹): 3402 (OH), 3256 (NH). MS, m/z (%) = 391 (M⁺, 4.7), 327 (18.12), 311 (72.15), 310 (95.64), 235 (40.60), 219 (89.60), 193 (100), 92 (57.72) and 91 (16.78). Anal. Calcd for C₂₁H₁₇N₃O₃S (391.09): C, 64.43; H, 4.38; N, 10.73%. Found: C, 64.36; H, 4.28; N, 10.65%.

7-(4-benzylphthalazin-1-ylamino)-4-hydroxynaphthalene-2-sulfonic acid (10j). Yellow crystals, 65%, 0.13 g. mp > 340 °C; IR (ν_{\max} , cm⁻¹): 3308 (OH), 3066 (NH). MS, m/z (%) = 457 (M⁺, 5.3), 455

(naphthalsaltone cationic radical, 59.1), 222 (5.1), 235 (100), 203 (6.4), 193 (3.6) and 178 (15.8); Anal. Calcd for C₂₅H₁₉N₃O₄S (457): C, 65.63; H, 4.19; N, 9.18%. Found: C, 65.54; H, 3.97; N, 9.10%.

Synthesis of N,N'-bis(4-benzylphthalazin-1-yl)-1,4-phenylenediamine (11) and N,N'-bis(4-benzylphthalazin-1-yl)biphenyl-4,4'-diamine (12). A mixture of compound **9** (0.50 g, 20 mmol) and dibasic aromatic amines (10 mmol) in ethanol (30 mL) was refluxed for 3 hours. The solvent was evaporated under vacuum. The solid obtained was filtered off and recrystallized from dioxane. Compound **11** Yellow crystals, 70%, 0.13 g. mp 250-251 °C; IR (ν_{max}, cm⁻¹): 3344 (NH). MS, *m/z* (%) = 544 (M⁺, 0.74) and base peak at 325 together with other peaks at 234 (5.7), 219 (3.29), 106 (1.74), 91 (13.66), 76 (1.25); Anal. Calcd for C₃₆H₂₈N₆ (544.27): C, 79.39; H, 5.18; N, 15.43%. Found: C, 79.08; H, 5.06; N, 15.22%. Compound **12**: Yellow crystals, 70%, 0.12 g. mp > 340 °C; IR (ν_{max}, cm⁻¹): 3277 (NH). MS, *m/z* (%) = 620 (M⁺, O), M/2 (310, 9.3); the base peak at 401 and other peaks at 102 (1.8), 91 (17.1); Anal. Calcd for C₄₂H₃₂N₆ (620.13): C, 81.27; H, 5.20; N, 13.54%. Found: C, 80.96; H, 5.04; N, 13.30%.

N,N'-bis(4-benzylphthalazin-1-yl)ethane-1,2-diamine (13). A mixture of compound **9** (0.50 g, 20 mmol) and ethylenediamine (10 mmol) in absolute ethanol (30 mL) anhydrous K₂CO₃ was refluxed for 4 hours. The obtained was cooled to room temperature, then diluted with water. The solid obtained was filtered off and recrystallized from ethanol. White crystals, 60%, 0.21 g. mp 234-235 °C; IR (ν_{max}, cm⁻¹): 3316, 3260 (NH) 2930, 2870 (C-H aliphatic). ¹H NMR (DMSO-*d*₆): δ_H 3.93 (s, 4H, CH₂-CH₂), 4.48 (s, 4H, 2CH₂Ph), 7.14-7.32 (m, 10H, Ar-H), 7.78-7.28 (m, 4H, Ar-H), 7.93-7.90 (br s, 2H, NH; exchangeable with D₂O), 8.00-8.021(m, 2H, Ar-H) and 8.26-8.29 (m, 2H, Ar-H); Anal. Calcd for C₃₂H₂₈N₆ (496.60): C, 77.39; H, 5.68; N, 16.92%. Found: C, 76.29; H, 5.45; N, 16.65%.

3.4. General Method for the Synthesis of Hydrazino Pyrimidine Derivatives **14a,b**

A mixture of compound **9** (0.25 g, 10 mmol) and 2-hydrazino-4-amino-5-cyano-6-arylpyrimidine (10 mmol) in *n*-butanol (30 mL) was refluxed for 3 hours. The solvent was evaporated under vacuum. The solid obtained was filtered off and recrystallized from dioxane.

4-Amino-2-[N'-(4-benzylphthalazin-1-yl)hydrazino]-6-(4-methoxyphenyl)-pyrimidine-5-carbonitrile (14a). White crystals, 75%, 0.16 g. mp 294-295 °C; IR (ν_{max}, cm⁻¹): 3450, 3210 (NH), 2206 (CN), 1634 (C=N). ¹H NMR (DMSO-*d*₆): δ_H 3.84 (s, 3H, OCH₃), 4.58 (s, 2H, CH₂Ph), 7.10 and 7.13 (2d, *J* = 8.4 Hz), 2H, Ar-H), 7.17 and 7.22 (2d, *J* = 7.8 Hz, 2H, Ar-H), 7.37 and 7.57 (2d, *J* = 7.8 Hz, 4H, Ar-H), 8.18 and 8.46 (2d, *J* = 8.1 Hz, 2H, Ar-H), 8.684 (s, 1H, Ar-H), 6.99 and 7.88 (m, 2H, Ar-H), 6.250 (br s, 2H, NH₂), 8.90 (br s, 2H, 2-NH); Anal. Calcd for C₂₇H₂₂N₈ (474.02): C, 68.34; H, 4.67; N, 23.612%. Found: C, 68.34; H, 4.55; N, 23.54%.

4-Amino-2-[N'-(4-benzylphthalazin-1-yl)hydrazino]-6-(4-chlorophenyl)pyrimidine-5-carbonitrile (14b). White crystals, 80%, 0.19 g. mp 274-275 °C; IR (ν_{max}, cm⁻¹): 3380, 3270 (NH), 2192 (CN), 1638 (C=N). MS, *m/z* (%) = 478 (M⁺, and M+2), 462 (19.22), 368 (14.61), 236 (12.68), 275 (75.31), 138 (36.35), 128 (46.12), 111 (29.49), 91(100); Anal. Calcd for C₂₆H₁₉ClN₈ (478.01): C, 65.20; H, 4.00; N, 23.40%. Found: C, 65.08; H, 3.87; N, 23.29%.

5-Benzyl-6,6a,12-triazobenzo[a]anthracen-7-one (15). A mixture of compound **9** (0.25 g, 10 mmol) and methyl anthranilate or anthranilic acid (10 mmol) in absolute ethanol (30 mL) and 2 drops of triethylamine (TEA) was refluxed for 3 hours. The solvent was evaporated under vacuum. The solid obtained was filtered off and recrystallized from dioxane. Yellow crystals, 80%, 0.16 g. mp 176-178 °C; IR (ν_{\max} , cm^{-1}): 1704 (CO); MS, m/z (%) = 337 (M^+ , 66.92), 336 (100) and 91 (13.09); Anal. Calcd for $\text{C}_{22}\text{H}_{15}\text{N}_3\text{O}$ (337.12): C, 78.32; H, 4.48; N, 12.46%. Found: C, 78.19; H, 4.37; N, 12.30%.

[4-(4-Benzylphthalazin-1-ylamino)benzoylamino]acetic acid methyl ester (16). A mixture of compound **9** (0.25 g, 10 mmol) and *p*-aminohippuric acid (10 mmol) in methanol (30 mL) and three drops of TEA was refluxed for 3 hours. The solvent was evaporated under vacuum. The solid obtained was filtered off and recrystallized from dioxane. Yellow crystals, 90%, 0.18 g. mp 165-167 °C; IR (ν_{\max} , cm^{-1}): 3110, 3328 (NH), 1752 (CO), 1642 (CONH). ^1H NMR (DMSO- d_6): δ_{H} 3.67 (s, 3H, OCH_3), 3.95 (d, $J = 5.4$ Hz), 1H, non equivalent CH_2 protons), 4.04 (d, $J = 5.4$ Hz), 1H, non equivalent CH_2 protons), 4.73 (s, 2H, PhCH_2), 7.88 and 8.006 (2d, $J = 8.7$ Hz, 2H, Ar-H), 8.44 (d, $J = 7.8$ Hz, 1H, Ar-H), three sets of multiplets at (7.24-7.30), (8.14-8.28), (8.93-9.01) (6H, Ar-H and NH), 7.33 and 7.41 (AB-q, $J = 7.2$ Hz, 4H, Ar-H), 10.80 (hump, 1H, NH, exchangeable by D_2O); MS, m/z (%) = 426 (M^+ , 48.2), 425 (100), 338 (27.8), 310 (5.4), 219 (2.7), 128 (5.4) and 91 (37.2); Anal. Calcd for $\text{C}_{15}\text{H}_{22}\text{N}_4\text{O}_3$ (426.46): C, 70.41; H, 5.20; N, 13.14%. Found: C, 70.25; H, 5.07; N, 12.87%.

Methyl 2-(4-(4-benzylphthalazin-1-ylamino)benzamido)-3-ethoxyacrylate (17). A mixture of compound **16** (0.42 g, 1.0 mmol) and triethyl orthoformate (0.14 g, 1.0 mmol) in acetic anhydride (10 mL) was refluxed for 5 hours, and then allowed to cool at room temperature and diluted with water (20 mL). The solid obtained was filtered off and recrystallized from dioxane. Yellow crystals, 60%, 0.17 g. mp 175-177 °C; IR (ν_{\max} , cm^{-1}): 3060 (C-H olefinic), 3366 (NH), 2942 (C-H aliphatic), 1752, 1666 (CO). ^1H NMR (DMSO- d_6): δ_{H} 2.08 (t, 3H, CH_2CH_3), 3.64 (s, 3H, COOCH_3), 4.01 (q, 2H, CH_2CH_3), 4.75 (s, 2H, CH_2Ph), (7.17-8.38) (sets of multiplets, 15H, Ar-H and 2NH), 8.97 (s, 1H, =CH); Anal. Calcd for $\text{C}_{28}\text{H}_{26}\text{N}_4\text{O}_4$ (482.52): C, 69.70; H, 5.43; N, 11.49%. Found: C, 69.63; H, 5.23; N, 11.49%.

2-(4-(4-Benzylphthalazin-1-ylamino)benzamido)acetamide (18). To compound **16** (0.42 g, 1.0 mmol) and distilled water and/or ammonium hydroxide, two drops of triethylamine (TEA) were added, and the mixture was refluxed in dioxane (15 mL) for 2 hours. The solvent was evaporated under vacuum. The solid obtained was filtered off and recrystallized from dioxane. Yellow crystals, 75%, 0.19 g. mp 240-242 °C; IR (ν_{\max} , cm^{-1}): 3272, 3354 (NH), 1734, 1640 (CO); MS, m/z (%) = 411 (55.9), 394 (20.7), 355 (59), 354 (100), 338 (48.0), 246 (32.8); Anal. Calcd for $\text{C}_{24}\text{H}_{21}\text{N}_5\text{O}_2$ (411): C, 70.06; H, 5.14; N, 17.02%. Found: C, 69.89; H, 5.07; N, 16.93%.

3.5. Antibacterial Activity

The newly synthesized compounds were screened for their antimicrobial activities *in vitro* against two species of Gram-negative bacteria *Pseudomonas aeruginosa* (MTCC 741); *Escherichia coli* (NCTC-10410); and four Gram-positive bacteria, *Bacillus cereus* (ATGG 14579); *Bacillus subtilis* (MTCC 441); *Bacillus sphaericus* (MTCC 11); *Staphylococcus* (MTCC 96); and two fungus, *Aspergillus ochraceus* Wilhelm (AUCC-230); *Penicillium chrysogenum* Thom (AUCC-530). The

activities of these compounds were tested using the disc diffusion method [20]. For bacteria and the paper disk diffusion method [21] for fungi. The area of zone of inhibition was measured using Ampicillin; tetracycline and norfloxacin ($30 \mu\text{g mL}^{-1}$) as standard antibiotic and mycostatin ($30 \mu\text{g mL}^{-1}$) was used as a reference antifungal. The tested compounds were dissolved in *N,N*-dimethylformamide (DMF) to give a solution of 1 mg mL^{-1} . The inhibition zones were measured in millimeters at the end of an incubation period of 48 hours at $28 \text{ }^\circ\text{C}$. *N,N*-dimethylformamide (DMF) showed no inhibition zone. Test results are shown in Table 1

Table 1. Antimicrobial activity of the new compounds.

Compd. No. ^a	Inhibition zone diameter in mm							
	Gram-negative bacteria		Gram-positive bacteria				Fungi	
	PA	EC	BC	BS	BS	SC	AOW	PCT
1	10	12	10	14	12	10	11	13
2	14	10	12	11	10	13	10	11
3	10	10	14	12	11	10	14	10
4	13	10	12	13	10	12	10	12
5	11	14	10	13	14	13	12	13
6	14	10	13	10	12	14	11	14
7	10	12	15	12	10	11	10	11
8	12	13	10	14	10	14	10	10
10a	16	15	14	15	15	16	17	15
10b	18	19	18	17	15	19	14	17
10c	16	16	19	15	18	14	17	18
10d	15	17	16	19	17	15	16	14
10e	17	16	14	17	19	16	19	19
10f	16	15	17	18	17	14	15	17
10g	18	15	15	16	16	17	18	16
10h	19	18	14	15	17	15	14	19
10i	17	16	17	19	18	15	17	15
10j	18	15	19	16	18	15	16	14
11	12	10	14	12	10	14	10	11
12a	20	22	20	19	20	22	20	20
12b	20	23	22	24	24	20	20	20
13	22	20	23	22	23	20	24	22
14a	23	22	24	23	22	24	23	23
14b	24	20	20	20	22	24	20	22
15	19	22	23	22	24	20	20	21
16	18	17	18	17	19	18	16	17
17	17	19	16	19	17	19	15	16
18	15	18	15	17	18	16	18	15
Ampicillin	22	22	22	22	22	22	-	-
Tetracycline	20	20	20	20	20	20	-	-
Norfloxacin	25	25	25	25	25	25	-	-
Mycostatin	-	-	-	-	-	-	20	20

^a $c = 1 \text{ mg mL}^{-1}$ of new compounds in DMF. Microorganisms: *Pseudomonas aeruginosa* (MTCC 741); *Escherichia coli* (NCTC-10410); *Bacillus cereus* (ATGG 14579); *Bacillus subtilis* (MTCC 441); *Bacillus sphaericus* (MTCC 11); *Staphylococcus* (MTCC 96); *Aspergillus ochraceus* Wilhelm (AUCC-230); *Penicillium chrysogenum* Thom (AUCC-530).

3.6. Antibacterial Activities

The screening results indicate that compounds **2–8** and **11** show weaker inhibitory activity than the standard drugs, while compounds **15–18** are moderately inhibitory, compared to the standard drugs. Compounds **10a–j**, **12**, **13** and **14a,b** showed nearly the same inhibition activity as standard antibacterial activity (Table 1). It is clear that decomposition of chloride atom at C-1 of 4-benzyl-1-chlorophthalazine with N-nucleophiles is responsible for the antimicrobial activities.

4. Conclusions

The results from this screening demonstrated that replacement of the hydrogen atom attached to the phthalazine nucleus at N-1 with amino derivatives (compounds **10a–j**), diimino derivatives (compounds **12a,b–13**) and pyrimidine derivatives (**14a,b**) resulted in spectrum of moderate antibacterial activity against all tested Gram positive and Gram negative fungi.

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