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Synthesis of Novel Phthalazine Derivatives as Potential Anticancer Agents from 1-Chloro-4-(4-phenoxyphenyl)phthalazine

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ABSTRACT

A new series phthalazine derivatives was synthesized from the reactionof 1-chloro-4-(4-phenoxyphenyl)phthalazine as a reactive starting material with different carbon, nitrogen, oxygen and sulfur nucleophiles. The Structural formula of all products were confirmed and characterized by elemental analyses and spectral data. Most of the synthesized derivatives were screened for their antitumor activity against fourhuman tumor cell lines using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. Compounds 1,20 and 25 showed the most potent cytotoxic effect concluded from their IC_{50}.

Keywords: Chlorophthalazine, Hydrazinylphthalazine, Anticancer activities, MTT assay

INTRODUCTION

Phthalazinederivatives are an important group of heterocycles which have been subject to extensive study in the past years. Among these phthalazines, 4-substituted phthalazines behave as effective antiinflammatory [1,2], antimicrobial [3-8], antitumor [9-12]. It was reported that, 1-chloropthalazine derivatives have occupied a unique position in the design of biologically active molecules like tetrazole, triazole, imidazole, pyrimidine, piperazine, triazine, pyrimidine etc., that exert remarkable anticancer [13-16], antimalarial [17], androgen receptor antagonists [18]. In addition, a number of 2,3-dihydrophthalazine-1,4-dione derivatives are well known to be active as potential anticonvulsant [19]. In view of the above mentioned facts and in continuation of our research interest for the synthesis of biologically active heterocycles [20-23], we report here, the synthesis of novel series of phthalazine derivatives bearing tetrazole, triazole, imidazole and triazine followed by evaluation of their anticancer activities against for human tumor cell lines namely Hepatocellular Carcinoma (Hep G2), Mammary Gland Breast Cancer (MCF-7), Human Prostate Cancer (PC-3) and Colorectal Carcinoma (HCT-116) using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay.

MATERIALS AND METHODS

The chemical reagents were purchased from Sigma-Aldrich. Solvents were commercially available from El-Nasr chemicals Co. in analytical grade and were used without further purification. TLC was conducted on precoated silica gel polyester sheets (Kieselgel 60 F254, 0.20 mm, Merck). Melting points are uncorrected and FT-IR spectra were recorded on a JASCO FT-IR 660 Plus spectrometer. NMR spectra were recorded on a Bruker Avance 400 (400 MHz) using DMSO and $CDCl_3$ as the solvents. Mass spectra were obtained using a Shimadzu GCMS-QP 1000 EX mass spectrometer.

Synthesis

Synthesis of 2-(4-phenoxybenzoyl)benzoic acid (1)

To a mixture of phthalic anhydride (0.01 mol) and diphenylether (0.01 mol) in tetrachloroethane (15 ml), anhydrous aluminum chloride (0.015 mol) was added within 15 min, with stirring in ice bath. The reaction mixture was continued stirring for 5 h., then left over night and poured onto ice/dilute hcl. The formed semisolid product was treated with petroleum ether and obtained solid was filtered off and crystallized from benzene.

Yield: 72%; M.p. 158-160°C. IR spectrum (kbr, ν, cm^{-1}): 3440-3350 (OH), 3060 (CH–Aromatic), 1686-1665 (2CO); ^{1}H NMR (DMSO-d_6) δ:11.25 (s,1H, OH, exchangeable), 7.01-7.99 (m, 13H, Ar-H); ^{13}CNMR, 186.42, 172.20 (2CO), 158.38 (C-O-C), 156.11, 132.67, 131.84, 129.34, 128.72, 128.37, 127.33, 126.44, 122.96, 121.50, 118.71, 118.28, 117.32 (aromatic carbons) Ms: m/z318 (M^{-+}); Anal. Calcd. For $C_{20}H_{14}O_4$ (318.33): C, 75.46; H, 4.43%. Found C, 75.39; H, 4.35%.

Synthesis of 4-(4-phenoxyphenyl)phthalazin-1(2H)-one (2)

To a solution of acid 1 (0.01 mol) in absolute ethanol (15 ml), hydrazine hydrate (0.01 mol) was added and the reaction mixture was refluxed for 4 h. After cooling, the precipitated solid was filtered off and crystallized from ethanol.

Yield: 85%; M.p. 219-221°C. IR spectrum (kbr, ν, cm^{-1}):3441-3250 (NH, OH),1671 (CO),1610 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 12.81 (s,1H, OH, exchangeable), 8.35-7.12 (m, 13H, Ar-H), 7.88 (s, 1H, NH, exchangeable); ^{13}C NMR 163.37 (CO), 157.18 (C-O-C), 156.21, 152.55, 133.33, 131.56, 131.13, 128.45, 127.30, 126.68, 121.15, 120.46, 118.65, 118.22 (aromatic carbons); Ms: m/z 314 ($M^{\cdot+}$); Anal. Calcd. For $C_{20}H_{14}N_2O_2$ (314.34): C, 76.42; H, 4.49; N, 8.91%. Found C, 76.32; H, 4.41; N, 8.85%.

Synthesis of 1-chloro-4-(4-phenoxyphenyl)phthalazine (3)

A mixture of phthalazinone 2 (0.01 mol), phosphorus pentachloride (0.01 mol) and phosphorusoxychloride (3ml) was refluxed for 4 h a steam bath. After cooling, the reaction mixture was poured carefully onto crushed ice. The solid that separated was filtered off, washed well for several times with water, dried and crystallized from ethanol. Yield: 79%; M.p. 130-132°C.IR spectrum (kbr, ν, cm^{-1}): 1662 (C=N), 835 (C-Cl);^{1}H NMR ($cdcl_3$, δ ppm): 8.31-7.03 (m, 13H, Ar-H); ^{13}C NMR 160.27 (C-O-C), 158.64 (C-Cl), 152.14, 134.75, 132.80, 128.26, 127.31, 126.86, 126.30, 124.50, 123.34, 121.11, 118.25, 117.229 (aromatic carbons); Ms:m/z:332($M^{\cdot+}$), 333 (M^{+1}); Anal. Calc. for $C_{20}H_{13}ClN_2O$ (332.79): C, 72.18; H, 3.94; N, 8.42%. Found 72.10; H, 3.85; N, 8.36%.

General procedures for synthesis of compounds 4, 5

An equimolar amount of chlorophthalazine 3 (0.01 mol) and active methylene compounds (0.01 mol) namely malnonitrile, ethylcyanoacetate in ethanol containing sodium ethoxide was heated under reflux for 6 h, then the reaction mixture was poured into ice/water. The obtained solid product was collected and recrystallized from proper solvent to give 4, 5 respectively.

2-(4-(4-Phenoxyphenyl)phthalazin-1-yl)malononitrile (4)

Yield 70%; M.p. 122-4°C, IR spectrum (kbr, ν, cm^{-1}): 3065 (CH-aromatic), 2938, 2897 (CH- aliphatic), 2207 (C≡N), 1649 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 8.38-7.12 (m, 13H, Ar-H); 5.07 (s, 1H, CH), ^{13}C NMR, 158.34 (C-O-C), 154.46, 152.66, 133.28, 131.53, 131.29, 128.15, 127.36, 126.26, 121.15, 121.44, 118.47, 118.30, 116.44, 112.20, 22.32;Ms m/z: 362 (100.0%); Anal. Calcd. For $C_{23}H_{14}N_4O$ (362.39): C, 76.23; H, 3.89; N, 15.46%. Found 76.29; H, 3.96; N, 15.50%.

Ethyl 2-cyano-2-(4-(4-Phenoxyphenyl)phthalazin-1-yl)acetate (5)

Yield 74%; M.p. 143-5°C, IR spectrum (kbr, ν, cm^{-1}): 3064 (CH-aromatic), 2937, 2886 (CH-aliphatic), 2208 (C≡N), 1718 (CO), 1610 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 6.98-8.29 (m, 13H, Ar-H); 4.86 (s, 1H, CH), 3.42-3.46 (q, 2H, CH_2), 1.18 (t, 3H, CH_3); ^{13}CNMR, 167.87, 157.26, 156.55, 148.26, 132.37, 131.16, 131.29, 127.75, 127.26, 126.15, 121.85, 121.36, 118.39, 118.21, 117.20, 114.42, 63.38, 38.56, 15.10;Ms m/z: 409 ($M^{\cdot+}$ 56 %); Anal. Calcd. For $C_{25}H_{19}N_3O_3$ (409.45): C, 73.34; H, 4.68; N, 10.26%. Found C, 73.26; H, 4.57; N, 10.19%.

General procedures for synthesis of compounds 6, 7

A mixture of compounds 4, 5 (0.01 mol) and hydrazine hydrate (0.01 mol) in ethanol (20 ml) was heated under reflux for 6 h then allowed to cool. The precipitated solid was collected by filtration and recrystallized from proper solvent to give crystals of compounds 6, 7.

4-(4-(4-Phenoxyphenyl)phthalazin-1-yl)-4H-pyrazole-3,5-diamine (6)

Yield 73%; M.p. 156-8°C, IR spectrum (kbr, ν, cm^{-1}): 3365-3154 (NH_2), 3066 (CH-aromatic)2939 (CH-aliphatic), 1608 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 8.07-7.12 (m, 13H, Ar-H), 5.9 (s, 4H, $2NH_2$, exchangeable), 2.96 (s, 1H, CH); ^{13}C NMR 165.18, 160.14, 157.25, 154.23, 148.57, 133.36, 132.30, 131.41, 128.52, 127.28, 127.65, 121.10, 119.62, 118.72, 118.33, 116.44, 46.14; Ms: m/z 394 ($M^{\cdot+}$), 395 (M^{+1}), 396 (M^{+2}); Anal. Calcd. For $C_{23}H_{18}N_6O$ (Mol.Wt.394.44): C, 70.04; H, 4.60; N, 21.31%. Found C, 69.92; H, 4.48; N, 21.24%.

5-Amino-4-(4-(4-phenoxyphenyl)phthalazin-1-yl)-2,4-dihydro-3H-pyrazol-3-one (7)

Yield 71%; M.p. 173-5°C, IR spectrum (kbr, ν, cm^{-1}):3420-3156 (NH_2, NH),1697 (CO), 1625 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 9.32 (s, 1H, NH, exchangeable), 8.32-7.05 (m,13H, Ar-H), 5,72 (s, 2H, NH_2, exchangeable), 3.13 (s, 1H, CH); Ms: m/z=395 ($M^{\cdot+}$), 396 (M^{+1}); Anal. Calcd. For $C_{23}H_{17}N_5O_2$ (395.42): C, 69.86; H, 4.33; N, 17.71%.Found C, 69.78; H, 4.24; N, 17.62%.

5-(4-Phenoxyphenyl)benzo[4,5]imidazo[2,1-a]phthalazine (8)

A mixture of 3 (0.01 mol) and *o*-phenylendiamine or o-aminophenol (0.01 mol) was fused for 1h. After cooling, the solid residue was triturated with distilled water, filtered and recrystallized.

Yield 86%; M.p. 160-2°C, IR spectrum (kbr, ν, cm^{-1}): 3050 (CH-aromatic), 1610 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 8.43–6.96 (m, 17H, Ar-H);^{13}C NMR, 157.23 (C-O-C), 152.13, 147.36, 134.30, 133.72, 131.55, 129.18, 128.35, 128.11, 127.48, 126.23, 126.08, 115.49, 112.80, 111.37 (aromatic carbons); Ms: m/z=387 ($M^{\cdot+}$), 388 (M^{+1}); Anal. Calcd. For $C_{26}H_{17}N_3O$ (387.44): C, 80.60; H, 4.42; N, 10.85%. Found C, 80.51; H, 4.33; N, 10.72%.

5-(4-phenoxyphenyl)-8H-phthalazino[1,2-b]quinazolin-8-one (9)

In a fusion tube provided with an air condenser, a mixture of 3 (0.01 mol) and anthranilic acid (0.01 mol) was heated in an oil bath at 190-191°C for 2 h. Then the mixture was cooled and poured into 40 ml of cold water. The obtained solid product was collected and recrystallized from benzene to give yellow crystals.

Yield 84%; M.p. 177-9°C, IR spectrum (KBr, ν, cm^{-1}): 3053 (CH-aromatic), 1679 (CO), 1587 (C=N);^{1}H NMR (DMSO-d_6, δ ppm): (m, 17H, Ar-H); ^{13}C NMR, 168.45 (CO), 157.67 (C-O-C), 155.14, 145.74, 133.63, 133.19, 131.88, 133.20, 131.52, 128.27, 128.08, 127.36, 126.17, 123.45, 118.60, 118.46, 114.21 (aromatic carbons); Ms: m/z=415 ($M^{\cdot+}$); Anal. Calcd. For $C_{27}H_{17}N_3O_2$ (415.45): C, 78.06; H, 4.12; N, 10.11%. Found C, 78.13; H, 4.15; N, 10.1%.

(4-(4-phenoxyphenyl)phthalazin-1-yl)glycine(10)

To asolution of 3 (3.32 g,10 mmol) in pyridine containing few drops of water (4 ml), glycine (0.75 g, 10 mmol) was added, then heated under reflux for 3 h. The reaction mixture poured onto ice/HCl, filtered, washed with water then acidified with acetic anhydride and crystallized from proper solvent.

Yield 70%; M.p. 185-7°C, IR spectrum (KBr, ν, cm^{-1}): 3440-3156 (OH, NH), 3066 (CH-aromatic), 2939, 2858 (CH- aliphatic), 1685 (CO), 1630(C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 11.52 (s, 1H, OH, exchangeable), 9.35 (s, 1H, NH, exchangeable), 7.05-8.31 (m, 13H, Ar-H), 3.73 (s, 2H, CH_2); ^{13}C NMR 171.54 (CO), 162.20 (CNH), 156.20 (C-O-C), 152.10, 132.57, 132.15, 128.30, 127.52, 126.35, 121.46, 118.65, 118.12, 116.70 (aromatic carbons), 42.21 (CH_2); Ms: m/z 371 ($M^{\cdot+}$), 372 (M^{+1}); Anal. Calcd. For $C_{22}H_{17}N_3O_3$ (371.40): C, 71.15; H, 4.61; N, 11.31%. Found C, 71.05; H, 4.57; N, 11.25%.

6-(4-phenoxyphenyl)imidazo[2,1-a] phthalazin-3(2H)-one (11)

Acetic anhydride (15 ml) was added to acid 14 (0.01 mol) and heated under reflux for 3 h. After cooling, the precipitated solid was filtered, dried and crystallized from ethanol.

Yield 78%; M.p. 164-6°C, IR spectrum (kbr, ν, cm^{-1}): 3064 (CH-aromatic), 2939, 2886 (CH-aliphatic), 1690 (co), 1610 (C=N); m^{1}H NMR (DMSO-d_6, δ ppm): 8.37-7.15 (m, 13H, Ar-H) 3.88 (s, 2H, CH_2); Ms: m/z =353 ($M^{\cdot+}$); Anal. Calcd. For $C_{22}H_{15}N_3O_2$ (353.38): C, 74.78; H, 4.28; N, 11.89%. Found C, 74.95; H, 4.16; N, 11.75%.

2-((4-(4-phenoxyphenyl)phthalazin-1-yl)amino)ethan-1-ol (12)

A mixture of ethanol amine (0.01 mol) and chlorophthalazine 3 (0.01 mol) in dioxan was heated under reflux for 12 h then the reaction mixture cooled, filtrated and washed with water. The solid that separated was recrystallized from ethanol.

Yield: 60%; M.p. 260-262°C, IR spectrum (kbr, ν, cm^{-1}): 3437-3157 (OH, NH), 3067 (C–H aromatic), 2919-2855 (C–H aliphatic), 1645 (CO),1640 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 8.27-7.31 (m, 13H, Ar-H), 3.10-3.39 (m, 4H, $2CH_2$), 5.72 (s, 1H, NH, exchangeable), 2.9 (s, 1H, OH, exchangeable); Ms: m/z=357 ($M^{\cdot+}$), 358 (M^{+1}); Anal. Calcd. For $C_{22}H_{19}N_3O_2$ (357.41): C, 73.93; H, 5.36; N, 11.76%. Found C, 73.85; H, 5.27; N, 11.64%.

6-(4-phenoxyphenyl)-2,3-dihydroimidazo[2,1-a]phthalazine (14)

A mixture of 12 (0.01 mol) and thionylchloride (2 ml) in dry benzene (30 ml) was refluxed for 2 h, then evaporated *in vacuo*. The solid residue was dissolved in 10 percent potassium carbonate (20 cm^3), then the solution was extracted with chloroform. The solvent was evaporated in vacuo then the solid residue crystallized from methanol.

Yield: 66%; M.p. 210-12°C, IR spectrum (kbr, ν, cm^{-1}): 3050 (CH-aromatic), 2944, 2865 (C–H aliphatic), 1649 (C=N); ^{1}HNMR (DMSO-d_6, δ ppm): 8.34-7.08 (m, 13H, Ar-H), 3.72-3.57 (m, 4H, $2CH_2$); Anal. Calcd. For $C_{22}H_{17}N_3O$ (339.40): C, 77.86; H, 5.05; N, 12.38%. Found C, 77.75; H, 5.16; N, 12.34%.

6-(4-Phenoxyphenyl)-3-phenyl-[1,2,4]triazolo[3,4-a]phthalazine (15)

A solution of chlorophthalazine 3 (0.01 mol) and benzoylhydrazine (0.01 mol) in *n*-butanol (30 ml) was refluxed for 24 h. The solid that separated after concentration and cooling was filtered off and crystallized from ethanol to give yellow crystals of 19.

Yield: 65%; M.p. 230-232 °C, IR spectrum (KBr, ν, cm^{-1}):3046 (CH-aromatic), (1642 (C=N); Ms: m/z 414 (M^+); ^{1}H NMR (DMSO-d_6, δ ppm): 8,40-7.12 (m, 18H, Ar-H);^{13}C NMR, 157.15, 153.41, 150.85, 147.26, 133.38, 133.20, 131.74, 129.66, 128.93, 127.69, 127.40, 126.57, 121.60, 118.84, 118.30, 112,65, Anal. Calcd. For $C_{27}H_{18}N_4O$ (414.47): C, 78.24; H, 4.38; N, 13.52%. Found C, 78.13; H, 4.27; N, 13.45%.

6-(4-Phenoxyphenyl)tetrazolo[5,1-a]phthalazine (16)

A mixture of sodium azide (0.01 mol) and chlorophthalazine 3 (0.01 mol) in acetic acid (20 ml) was heated under reflux for 6 h. Then, the reaction mixture was cooled, poured onto ice. The separated solid was filtered off and recrystallized from ethanol to give pale yellow crystals.

Yield: 63%; M.p. 182-4°C, IR spectrum (KBr, ν, cm^{-1}): 3060 (CH-aromatic), 1635 (C=N); Ms: m/z 339 ($M^{\cdot+}$), 340 (M^{+1}); Anal. Calcd. For $C_{20}H_{13}N_5O$ (339.36): C, 70.79; H, 3.86; N, 20.64%. C, 70.70.60; H, 3.79; N, 20.52%.

N-(4-Methoxyphenyl)-4-(4-phenoxyphenyl)phthalazin-1-amine (17)

An equimolar amount of chlorophthalazine 3 (0.01 mol) and *p*-ansidine in n-butanol (30 ml) was heated under reflux for 6 h, then the reaction mixture was concentrated by evaporation. The solid that obtained was crystallized from butanol l to give 21a,b respectively.

Yield: 75%; M.p. 215-7°C, IR spectrum (KBr, ν, cm^{-1}): 3432 (NH), 3043 (CH-aromatic), 2935, 2896 (C–H aliphatic), 1650 (C=N); ^{1}H NMR (DMSO-d_6) δ: 7.97-6.85 (m, 17H, Ar-H), 6.39 (s, 1H, NH exchangeable), 3.75 (s, 3H, OCH_3); ^{13}C NMR, 157.35, 151.37, 147.46, 133.79, 133.36, 131.25, 129.18, 128.50, 128.32, 127.77, 127.52, 126.12, 121.51, 118.29, 118.03, 115.34,52.38, Ms: m/z=419 ($M^{\cdot+}$), 420 (M^{+1}); Anal. Calcd. For. $C_{27}H_{21}N_3O_2$ (419.48): C, 77.31; H, 5.05; N, 10.02%. Found C, 77.25; H, 4.89; N, 9.91%.

4-(4-Phenoxyphenyl)phthalazin-1-amine (18)

An equimolar amount of chlorophthalazine 3 and ammonium acetate (0.01 mol) was heated for 2 h. After cooling, the solid residue was triturated with water, filtered, dried and crystallized from ethanol/water.

Yield 79%; M.p. 224-6°C, IR (cm^{-1}): 3427-3250 (NH_2), 3058 (CH-aromatic), 1649 (C=N); ^{1}HNMR (DMSO-d_6) δ: 8.36-6.97 (m,13H,Ar-H); Anal. Calcd. For $C_{20}H_{15}N_3O$ (313.36): C, 76.66; H, 4.83; N, 13.41%. Found C, 76.57; H, 4.74; N, 13.30%.

6-(4-Phenoxyphenyl)-[1,2,4]triazolo[3,4-a]phthalazin-3-amine (19)

A mixture of chlorophthalazine 3 (0.01 mol) and thisemicarbazide (0.01 mol) in ethanol (30 ml) was heated under reflux for 6h. The separated solid after cooling was collected by filtration, dried and recrystallized from ethanol to give pure crystals of 19.

Yield 87%; M.p. 171-3°C, IR (cm^{-1}): 3415-3270 (NH2), 3053 (CH-aromatic), 1610 (C=N); ^{1}H NMR (DMSO-d_6) δ: 8.34-7.29 (m, 13H, Ar-H), 6.2 (s, 2H, NH_2 exchangeable), Anal. Calcd. For $C_{21}H_{15}N_5O$ (353.39): C, 71.38; H, 4.28; N, 19.82%. Found C, 71.31; H, 4.22; N, 19.71%.

Synthesis of 1-hydrazinyl-4-(4-phenoxyphenyl)phthalazine (20)

A mixture of equimolar amounts of chlorophthalazine 3 and hydrazine hydrate (0.01 mol) in n-butanol was heated under reflux for 6 h. After cooling, the precipitated product was filtered off, dried and crystallized from n-butanol as pale yellow crystals.

Yield 70%; M.p. 170-172°C, IR spectrum (KBr, ν, cm^{-1}): 3418-3383 (NH, NH_2), 3037 (CH- aromatic), 1613 (C=N); ^{1}H NMR (DMSO-d_6) δ:11.83 (s, 1H, NH exchangeable), 7.23 (s, 2H, NH_2 exchangeable), 7.13-8.83 (m, 13H, Ar-H);^{13}C NMR 159.73 (C-O-C), 156.32, 146.33, 133.38, 131.34, 128.25, 127.69, 127.40, 126.55, 123.90, 122.38, 121.42, 118.50, 118.31 (aromatic carbons); Ms: m/z=328 (M^+); Anal. Calcd.For$C_{20}H_{16}N_4O$ (328.38) C, 73.15; H, 4.91; N, 17.06%. Found C, 72.98; H, 4.80; N, 16.97%.

General procedures for synthesis of 21a,b

A mixture of hydrazinylphthalazine 20 (0.01 mol) and aromatic aldehydes namely thiophene-2-carbaldhyde and p-chlorobenzaldhyde in n-butanol was heated under reflux for 8 h. Then the reaction mixture was concentrated by evaporation, cooled, filtrated off and crystallized to give 23a,b respectively.

1-(4-Phenoxyphenyl)-4-(2-thiophen-2ylmethylene)hydrazinyl)phthalazine (21a)

Yield 83% ; M.p. 206-8°C, IR spectrum (KBr, ν, cm^{-1}): 3156 (NH), 3055 (CH aromatic), 2941, 2898 (CH-aliphtic), 1660 (C=N); ^{1}H NMR (DMSO-d_6) δ: 12.84 (s, 1H, NH exchangeable), 8.36 (s, 1H, CH=N), 8.34-7.14 (m, 16H, Ar-H); Ms: m/z=422 (M^+); Anal. Calcd. For $C_{25}H_{18}N_4OS$ (422.51): C, 71.07; H, 4.29; N, 13.26%. Found C, 71.14; H, 4.33; N, 13.31%.

1-(2-(4-Chlorobenzylidene)hydrazinyl)-4-(4-phenoxyphenyl)phthalazine (21b)

Yield 86% ; M.p. 218-20°C, IR spectrum (KBr, ν, cm^{-1}): 3296-3154 (NH), 3065 (CH aromatic), 2939, 2897 (CH aliphatic), 1661 (C=N); ^{1}H NMR (DMSO-d_6) δ: 12.81 (s, 1H, NH exchangeable), 8.70 (s, 1H, CH=N), 8.36-7.12 (m, 17H, Ar-H); 13C NMR 161.32, 159.70, 158.00, 156.51, 146.33, 134.08, 132.09, 131.62, 130.68, 130.49, 129.56, 128.38, 127.07, 126.57, 124.51, 119.77, 118.56 Ms: m/z=450 (M^+); Anal. Calcd. For $C_{27}H_{19}ClN_4O$ (450.93) C, 71.92; H, 4.25; N, 7.86%. Found C, 71.97; H, 4.32; N, 7.78%.

7-(4-Phenoxyphenyl)-2H-[1,2,4]triazino[3,4-a]phthalazine-3,4-dione (22)

Diethyloxalate (0.01 mol) was added to hydrazinylphthalazine 22 (0.01 mol) in ethanol (30 ml) and the mixture was refluxed for 6 h, the obtained solid after cooling was filtered off and crystallized.

Yield 88% ; M.p. 211-13°C, IR spectrum (KBr, ν, cm^{-1}):): 3441-3156(NH), 3063 (CH aromatic), 1687-1675 (2CO), 1598 (C=N); ^{1}H NMR (DMSO-d_6) δ: 12.82 (s, 1H, NH exchangeable), 8.36-7.12 (m, 13H, Ar-H); Ms: m/z=382 (M^+); Anal. Calcd. For $C_{22}H_{14}N_4O_3$ (382.38): C, 69.10; H, 3.69; N, 14.65%. Found C, 68.96; H, 3.60; N, 14.54%.

7-(4-Phenoxyphenyl)-2H-[1,2,4]triazino[3,4-a]phthalazin-3(4H)-one (23)

A mixture of ethyl chloroacetate (0.01 mol) and hydrazinylphthalazine 20 (0.01 mol) in ethanol (30 ml) was refluxed for 6 h, the obtained solid after cooling was filtered off and recrystallized from ethanol.

Yield 80%; M.p. 185-7°C, IR (cm^{-1}): 3439-3200 (NH), 3040 (CH-aromatic), 2913 (CH–aliphatic), 1675 (CO), 1605 (C=N) Anal. Calcd. For Anal.Calcd. For $C_{22}H_{16}N_4O_2$ (368.40): C, 71.73; H, 4.38; N, 15.21%. Found C, 71.66; H, 4.29; N, 15.14%.

1-Methoxy-4-(4-phenoxyphenyl)phthalazine (24)

A solution of chlorophthalazine 3 (0.01 mol) in methanol containing sodium was refluxed for 6 h. After cooling, the reaction mixture was poured onto crushed ice/HCl then filtrated and crystallized from ethanol to give crystals of compound 24.

Yield 83%; M.p. 203-205°C, IR spectrum (KBr, ν, cm^{-1}): 3030 (CH-aromatic), 2930, 2895 (C–H aliphatic), 1640 (C=N), 1236 (C-O-C); ^{1}H NMR (DMSO-d_6) δ: (m, H, Ar-H), (s, 3H, OCH_3); Ms: m/z=328 (M^+); Anal. Calcd. For Anal. Calcd. For $C_{21}H_{16}N_2O_2$ (328.37): C, 76.81; H, 4.91; N, 8.53%. Found C, 76.69; H, 4.83; N, 8.42%.

Synthesis of 4-(4-phenoxyphenyl)phthalazin-1-thiol (25)

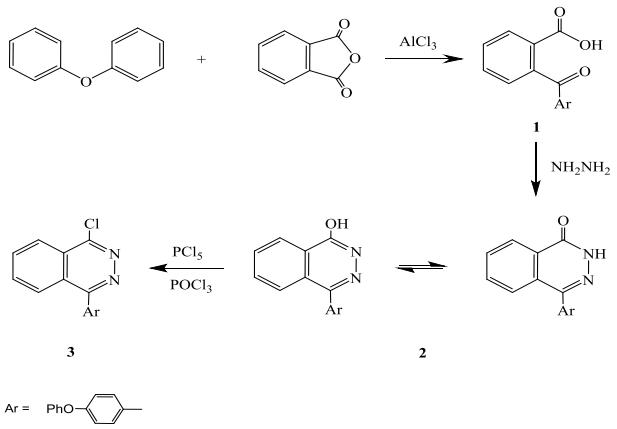
A mixture of chlorophthalazine 3 (0.01 mol) and thiourea (0.01 mol) in ethanol (20 ml) containing sodium ethoxide was heated under reflux for 6 h. The reaction mixture was poured onto ice/water then acidified with acetic acid. The solid product which formed was collected by filtration, dried and crystallized from ethanol to give yellow crystals.

Yield 60%; M.p. 198-200°C, IR (cm^{-1}): 3427 (NH), 3050 (CH-aromatic), 2593 (SH), 1645 (C=N),1334 (C=S); ^{1}H NMR (DMSO-d_6) δ: 14.53 (s, 1H, NH, exchangeable), 7.12-8.84 (m, 13H, Ar-H), 3.26 (s, 1H, SH, exchangeable); ^{13}C NMR174.56, 157.27, 155.35, 151.43, 133.45, 132.22, 129.21, 128.51, 127.12, 127.53, 126.50, 121.65, 118.72, 117.33; Ms: m/z=330 (M^++1); Anal. Calcd.For $C_{20}H_{14}N_2OS$ (330.41): C, 72.70; H, 4.27; N, 8.48%. Found C, 72.57; H, 4.15; N, 8.39%.

s

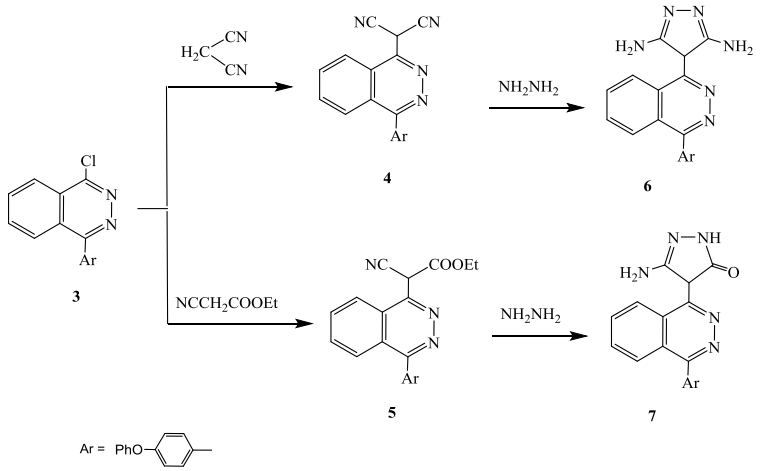
RESULTS AND DISCUSSION

Hydranzinolysis of acid 1 [prepared from acylation of diphenylether with phthalic anhydride in the presence of catalytic amount of anhydrous aluminium chloride via Fridel-Crafts reaction] with hydrazine hydrate in ethanol furnished 4-(4-phenoxyphenyl)phthalazin-1(2H)-one (2). The structural formula of phthalazine (2) was established in two tautomeric forms on the basis of its IR spectrum that showed the presence absorption bands of NH, OH groups at 3250-3441 cm^{-1} and CO at 1671 cm^{-1}. Also, H^1 NMR spectrum showed signals attributed to NH at 7.88 ppm and OH at 12.81 ppm. Treatment of phthalazinone 2 with a mixture of phosphorous oxychloride and phosphorous pentchloride afforded 1-chloro-4-(4-phenoxyphenyl)phthalazine (3) (Scheme 1). The latter product was used as a reactive key precursor to synthesize a new series of substituted Phthalazine and fused phthalazine derivatives through its reactivity towards carbon, nitrogen, oxygen and sulfur nucleophiles.



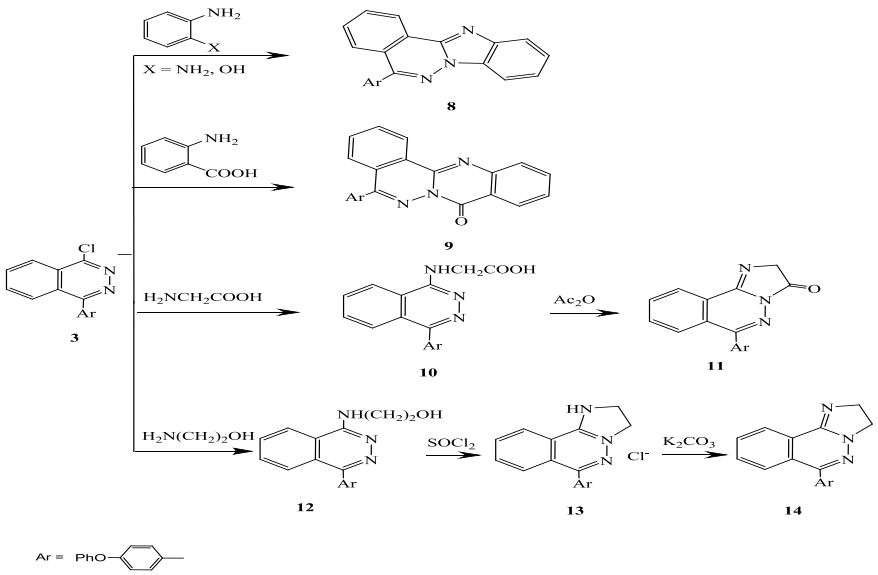
Scheme 1: Synthesis of 1-chloro-4-(4-phenoxyphenyl) phthalazine (3)

Treatment of chlorophthalazine 3 with active methylene compounds namely malononitrile and ethyl cyanoacetate in the presence of sodium ethoxide as carbon nucleophile Safford phthalazine derivatives 4 and 5. Cyclization of derivatives 4 and 5 with hydrazine hydrate in ethanol afforded pyrazoles 6 and 7. The structural formulas of the products were confirmed on the basis of their spectral data and elemental analyses (Scheme 2).



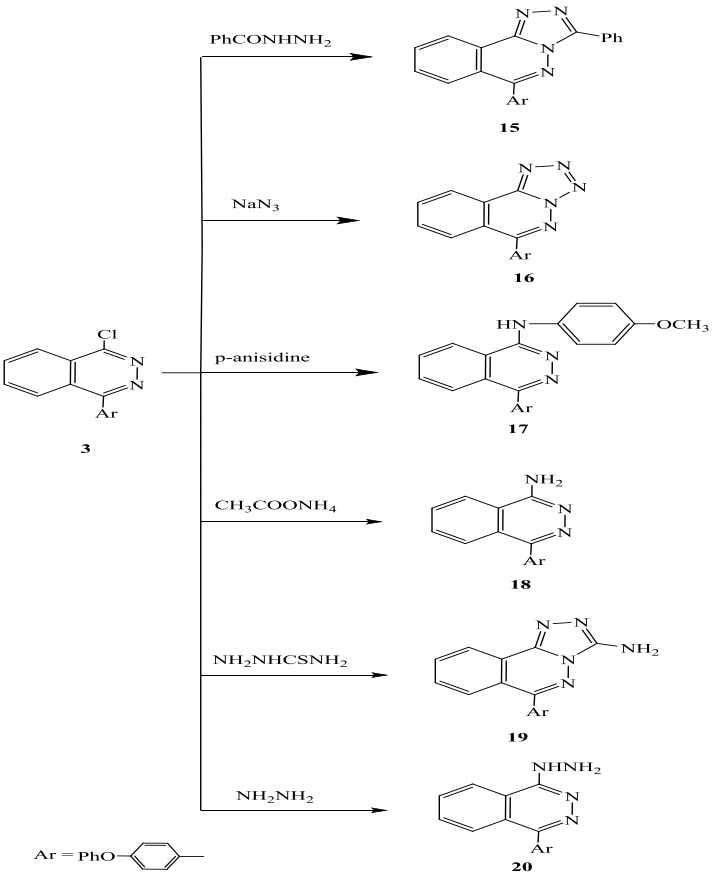
Scheme 2: Reaction of 1-chloro-4-(4-phenoxyphenyl)phthalazine (3) with active methylene compounds

On the other hand, chlorophthalazine 3 was employed in the reaction with some nitrogen nucleophiles to construct a new series of heterocyclic derivatives that exhibited remarkable antitumor and antioxidant activities. 5-(4-phenoxyphenyl)benzo[4,5]imidazo[2,1-a]phthalazine (8) can be formulated through fusion of chlorophthalazine 3 with *o*-phenylediamine or *o*-aminophenol while fusion with anthranilic acid afforded 5-(4-phenoxyphenyl)-8*H*-phthalazino[1,2-b]quinazolin-8-one (9). Treatment of chlorophthalazine 3 with glycine in pyridine and few drops of water gave amino acid 10, cyclization of the latter with acetic anhydride furnished imidazolidinone derivative 11 (Scheme 3).



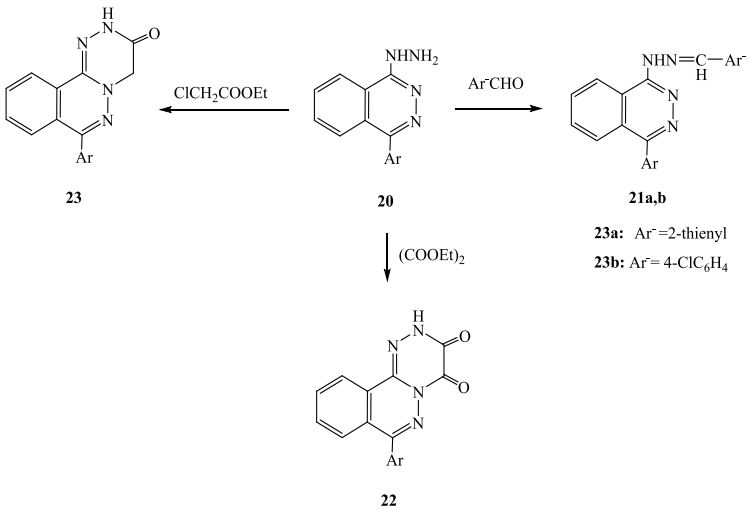
Scheme 3: Reaction of 1-chloro-4-(4-phenoxyphenyl)phthalazine (3) with nitrogen nucleophiles

Also, chlorophthalazine 3 reacts with ethanol aminetogive2-((4-(4-phenoxyphenyl)phthalazin-1-yl)amino)ethan-1-ol (12). Cyclization of the latter was achieved through its heating with thionyl chloride in dry benzene to give phthalazinium chloride 13 which in turn converted into 6-(4-phenoxyphenyl)-2,3-dihydroimidazo[2,1-a]phthalazine (14) after treatment with potassium carbonate solution (Scheme 4).

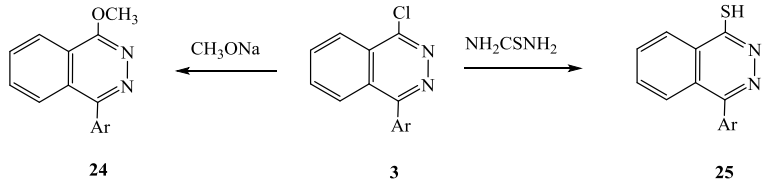


Scheme 4: synthesis of phthalazine derivatives 19-24

The reaction of chlorophthalazine 3 with benzoylhydrazine under reflux in n-butanolafforded6-(4-phenoxyphenyl)-3-phenyl-[1,2,4]triazolo[3,4-a]phthalazine (15) and its treatment with sodium azide furnished tetrazole 16. On the other hand, treatment of chlorophthalazine3 with *p*-anisidine gave phthalazine derivative 17. Fusion of chlorophthalazine with ammonium acetate resulted in formation of aminophthalazine 18. On the other hand, treatment of compound 3 with thiosemicarbazone due to formulation of aminotriazolopthalazine 19. In addition, chlorophthalazine3 was allowed to react with hydrazine hydrate in ethanol to afford1-hydrazinyl-4-(4-phenoxyphenyl)phthalazine (20). The structural formula of 20 was inferred by its spectral data, ^{1}H NMR spectrum showed singlet signals of NH_2 and NH at 11.83, 7.23 ppm and IR spectrum exhibited absorption bands at 3418-3383 cm^{-1} corresponding to absorption of NH_2 and NH groups. Also, the structure of hydrazinophthalazine 20 was confirmed chemically through its reaction with aromatic aldehyde namely 2-thiophene carbaldahyde and *p*-chlorobenzaldahyde to afford arylidene derivatives 21(a,b) that showed signals attributed to methine protons at 8.36, 8.72 ppm respectively in their ^{1}H NMR spectra. Heating of hydrazinophthalazine 20 with diethyloxalate and ethyl chloroacetatein ethanol under reflux furnished triazine derivatives 22 and 23 respectively.

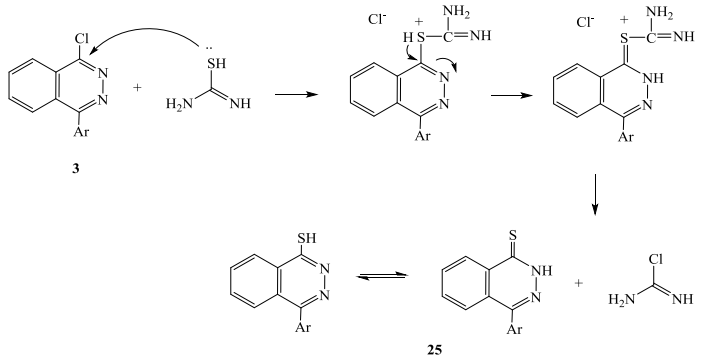


Finally, chlorophthalazine 3 was also employed in the reaction with oxygen and sulfur nucleophiles to afford new bioactive molecules. Thus, treatment of chlorophthalazine 3 with sodium methoxide and thiourea gave phthalazine derivatives 1-methoxy-4-(4-phenoxyphenyl)phthalazine (24) and 4-(4-phenoxyphenyl)phthalazin-1-thiol (25) respectively (Scheme 5).



Scheme 5: reaction of 1-chloro-4-(4-phenoxyphenyl)phthalazine (3) with oxygen and sulfur nucleophiles

The reaction of compound 3 with thiourea probably takes place via the following mechanism [24].



Cytotoxicity and anticancer evaluation

Some of the newly synthesized compounds were evaluated for their *in vitro* anticancer effect using the MTT assay [25,26] against four human tumor cell lines namely; Hepatocellular Carcinoma (HePG-2), Mammary Gland Breast Cancer (MCF-7), Human Prostate Cancer (PC3) and Colorectal Carcinoma (HCT-116). This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Doxorubicin was used as a standard anticancer drug for comparison. The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Table 1: Relative viability of cells (%)

	IC50 (mg/ml)*			
Compounds	**HePG-2**	**MCF-7**	**PC-3**	**HCT-116**
Dox µg				
100	6.3	6.2	8.8	7.1
50	11.2	10.9	16.3	13.9
25	14.1	14.3	21.7	18.7
12.5	28.3	26.9	38.9	31.4
6.25	45.8	41.5	59.2	47.9
3.125	57.6	58.4	73.6	60.5
1.56	71.2	69.1	95.3	73.8
1				
100	13.6	25.3	23.1	14.7
50	20.3	36.4	31.7	22.1
25	24.7	47.2	42.5	25.9
12.5	39.8	60.1	54.6	41.3
6.25	68.5	76.8	70.3	69.6
3.125	78.1	95.5	86.4	73.5
1.56	97.2	100	100	98.2
4				
100	32.6	32.8	29.5	28.2
50	46.8	43	41.5	40.1
25	57.1	55.2	53.8	52.4
12.5	70.3	67.1	63.9	61.6
6.25	83.9	89.3	78.1	76.5
3.125	99.5	100	97.2	95.8
1.56	100	100	100	100
20				
100	8.1	19.3	15.2	7.8
50	15.3	31.2	24.8	16.4
25	22.5	37.4	34.7	24.7
12.5	37.2	48.1	42.6	39.1

6.25	58	65.7	61.3	56.9
3.125	74.6	87.2	79.1	81.3
1.56	95.4	100	100	96.6
25				
100	23.8	18.6	10.6	21.2
50	30.7	25.8	18.1	29
25	45.2	34.1	24.9	38.3
12.5	57.1	45.3	39.2	50.5
6.25	69.3	63.7	58.7	74.1
3.125	95.5	85.6	73.5	91.4
1.56	100	100	95.3	100
2				
100	45.9	39.6	48.1	48.1
50	58.2	55.7	60.3	61.2
25	72.3	70.2	70.7	76.3
12.5	84.6	91.9	82.5	87.4
6.25	97.5	100	98.8	99.7
3.125	100	100	100	100
1.56	100	100	100	100
23b				
100	33.4	38.3	36.1	31.2
50	46.1	52.7	49.2	41.9
25	62.8	65.1	61.6	54.7
12.5	75.2	77.2	76.3	65.8
6.25	94.7	98.8	95.5	77.9
3.125	100	100	100	98.1
1.56	100	100	100	100

IC_{50}, (mg/ml): 1-10 (very strong); 11-25 (strong); 26-50 (moderate); 51-100(weak), above 100 (non-cytotoxic), Dox (Doxorubicin, standard cytotoxic agent)

It was observed from the obtained results in Table 1, the synthesized compounds exhibited varying degrees of inhibitory activity toward the tested human tumor cell lines in comparison with the standard doxorubicin. Compound 20 showed the highest cytotoxic activity against both HePG-2 and HCT-116 concluded from their percentage viability IC_{50} at 8.1 and 7.8 mg/ml respectively. Strong inhibitory activity was also demonstrated by compounds 20, 25 towards both MCF-7 and PC-3.

On the other hand, compound 1 reveled strong cytotoxic activities towards HePG-2, PC-3, HCT-116 cell lines and moderate activity against MCF-7 that have IC_{50} at 13.6, 23.1, 14.7 and 25.3 mg/ml respectively. The remaining compounds showed varying degrees from moderate to weak cytotoxic activities towards the tested cell lines.

Table 2: Antioxidant assay (ABTS)

ABTS		**Method**	**Compound No.**
(Abs(control)-Abs(test)/Abs(control) × 100			
% inhibition	**Absorbance of samples**	**Compounds**	
0%	0.505	Control of ABTS	
89.30%	0.054	Ascorbic-acid	
30.20%	0.212	1	1
18.60%	0.411	4	2
73.60%	0.045	20	3
46.50%	0.11	25	4
26.10%	0.373	2	5
17.00%	0.419	23b	**6**

Antioxidant activity

It was observed from the data given in Table 2 that among the investigated compounds, only compound 20 exhibited higher antioxidant activity while compounds1,25and 2 showed moderate activities in comparison with standard ascorbic acid.

CONCLUSION

In summary, we have reported a novel and highly efficient synthesis of phthalazine derivatives starting from 1-chloro-4-(4-phenoxyphenyl)phthalazine as reactive key precursor and variety of nucleophilic reagents. In addition, some of the synthesized products were investigated for their antitumor activity against four human tumor cell lines using MTT assay and it was observed that compounds 1, 20 and 25 showed the most potent cytotoxic effect concluded from their IC_{50} values.

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