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Design, synthesis and antioxidant evaluation of certain new phthalazine derivatives

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ABSTRACT

*In this research, certain phthalazine scaffolds were designed and synthesized. Hyperidzation with pharmacophoric moieties possessing antioxidant activityincluding glycine hydrazides and derivatives(**10**, **11** and **12**), amide derivatives (**19**), hydrazone (**7**, **14** and **18**), Schiff's base derivatives(**6a-c**, **13a-c** and **20a-c**) and N-phtalimido derivatives(**8a-c**, **15a-c** and **21a-c**);was achieved.In addition, a synthetic comparison between O- and N-alkylation products of phthalazinone ring systemwas studied.All the newly synthesized compounds were screened for their in vitro antioxidant activity through ABTS antioxidant assay. Compound **5** showed the best activity, whereas compound **12** showed moderate activity. The rest of compounds showed weak to mild activity.*

Keywords: Phthalazines; ABTS; Antioxidant

INTRODUCTION

Phthalazines are unique well-known class of nitrogen-containing heterocyclic compounds, possessing versatile chemical, industrial and biological properties. Among the important pharmacological activities exhibited by phthalazine derivatives are: anti-inflammatory activity [1,3],PDE4 inhibitors [4-6], antihypertensive [7],vasodilator [8], cytotoxic [9],antitumor [10], anticancer [11],anticonvulsant [12],antiasthmatic [13], vasorelaxant [14],antibacterial [15], and antifungal activity [16].

In general, little efforts has been directed to evaluation of the effect of the 2,3-disubstituted-1-phthalazinones or 2,3-disubstituted-1,4-phthalazinediones on alteration of the pharmacological activity. Considerably less effort has been devoted to the modification of the benzene nucleus of the phthalazine in general [17-19].A library model for phthalazine essential skeleton for a variety of pharmacological activity is illustrated (**Figure 1**).

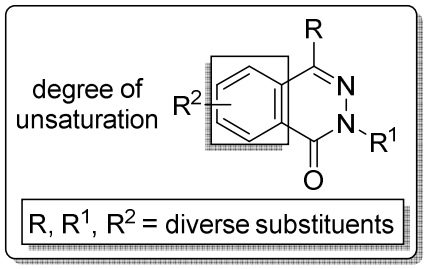


Figure 1.A library model for phthalazine scaffold

Antioxidants are molecules, natural, or synthetic, capable to interact with free radicals and stop their chain reactions before essential vital molecules are damaged. Owing to the contribution and implication of oxidative stress in many human diseases, antioxidants are intensively studied in medicinal chemistry and pharmacology. Their main use is for treatment, prevention, or protection against many disease conditions [20,21]. Diverse biological and pharmacological activities of phthalazines were reported by many workers. To the best of our knowledge, literature survey did not reveal antioxidant evaluation. Therefore, the first objective of this work to investigate and evaluate the potential antioxidant activity of this class of compounds via design, synthesis, and in vitro antioxidant screening of selective representatives of phthalazine derivatives bearing pharmacophoric moieties.

In this research, Introduction of a phenyl ring instead of R^1 in the library model in order to increase the lipophilicity, steric effect, and planarity, Modification of R by OR in **3** and by its isosteric NHR in **18** because of their polarity and their important crucial role in varying the pharmacological activity of such derivatives [22].

In addition, hybridization of the synthesized phthalazines scaffolds with potential pharmacophoric moieties possessing antioxidant activity, e.g. glycine and derivativesrs [23,24](**10**, **11** and **12**), Amide derivatives [25,26](**19**), Hydrazone [27,28](**7**, **14** and**18**), Schiff's base derivatives [29-31](**6a-c**, **13a-c** and **20a-c**), and *N*-Phtalimido derivatives [32]through ring expansion of *N*-phenylphthalimide into phthalazinedione derivative in **12** by incorporation of the glycine moiety, offering some flexibility to the heterocyclic ring (**8a-c**, **15a-c** and **21a-c**) was achieved to afford the designed target compounds.

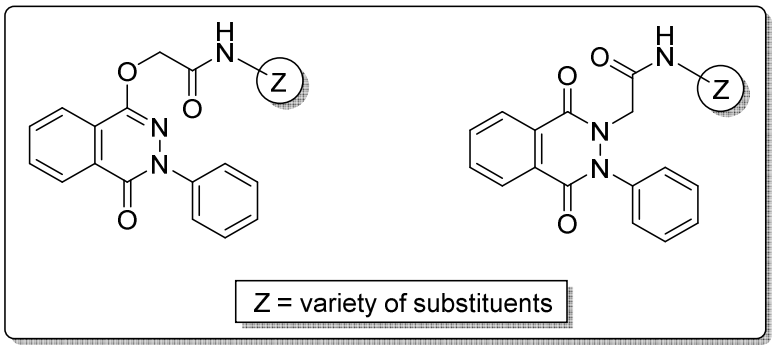


Figure 2.*O*- and *N*-Alkylation products of phthalazine

From a chemical point of view, some of the synthesized compounds were designed for the aim of comparison between *O*-alkylation and *N*-Alkylation products of phthalazine. Alkylation of 4-hydroxyphthalazin-1(2*H*)-one and its derivatives was studied sporadically, like the similar analogs of pyridone and quinolinone. Literature survey revealed that, alkylation of ambident tautomeric heterocyclic lactam-lactim or amide-imidic acid forms is a complex process. The alkylation product, either, *O*-alkylation, *N*-Alkylation, or a mix of *O*- and *N*-alkylation are varied according to (but without a well-defined rule): the more favored tautomeric form, the solvent and reagent used, the spatial demand of the alkylating agent, steric and/or electronic factors, and in favoring ring aromaticity.Therefore, the second aim of this work was to synthesize both *O*-alkylation (**Scheme 1,2**) and *N*-alkylation (**Scheme 3,4**) in the phthalazinone series. *O*-alkylation was accomplished*via* a direct method, whereas, a chemical evidence for *N*-alkylation was carried out through an indirect method (**Figure 2**).

MATERIALS AND METHODS

Chemistry

Melting points were determined on *Fisher-Johns* melting point apparatus and were uncorrected. Microanalyses were performed in the Micro Analytical Center, University of Cairo. IR spectra (KBr) were recorded on Mattson 5000 FT-IR spectrometer (ν in cm^{-1}), Micro Analytical Center, University of Cairo. ^1H NMR spectra were recorded on Bruker Ac 250 FT NMR spectrometer (250 MH_Z) in DMSO-d_6 or $CDCl_3$ using TMS as internal standard, chemical shifts in ppm were expressed in δ units, Micro Analytical Center, University of Cairo. MS analyses were performed on JEOL JMS-600H spectrometer, Micro Analytical Center, University of Cairo. Reaction times were determined by using TLC on Silica gel plates 60 F_{245} E. Merck, and the spots were visualized by U.V (366, 245 nm). Compounds: 4-hydrhoxy-2-phenylphthalazin-1(2*H*)-one (**1**) [33], 4-chloro-2-phenylphthalazin-1(2*H*)-one (**16**) [34] and 4-(1-hydrazonoethyl)aniline (**17**) [35] were prepared according to the published data.

Potassium 4-oxo-3-phenyl-3,4-dihydrophthalazin-1-olate (2)

Finely powdered potassium hydroxide (2.81 g, 50 mmol) was added to isopropyl alcohol (80 mL) and vigorously stirred until a clear solution is obtained. Compound **1** (11.91 g, 50 mmol) was then added in small portions with continuous stirring for 1 h. The formed precipitate of the potassium salt was then filtered, washed with ice-cold isopropyl alcohol (20 mL), and dried to afford 12.15 g of the titled compound. White crystals, mp >300 °C, yield 88%. Analysis for $C_{14}H_9KN_2O_2$ (276.33), Calc.: C, 60.85; H, 3.28; N, 10.14. Found: C, 60.73; H, 3.35; N, 10.31.

2-(4-Oxo-3-phenyl-3,4-dihydrophthalazin-1-yloxy)acetic acid (3)

A mixture of **2** (2.76 g, 10 mmol), chloroacetic acid (0.95 g, 10 mmol), and absolute ethanol (25 mL) was refluxed with continuous stirring for 10 h. The reaction mixture was filtered while hot and allowed to cool to rt, and then cooled in ice-water. The precipitated solid was filtered, air-dried, and recrystallized from aqueous ethanol. White crystals, mp 153-155 °C, yield 60%. Analysis for $C_{16}H_{12}N_2O_4$ (296.28), Calc.: C, 64.86; H, 4.08; N, 9.46. Found: C, 64.65; H, 4.33; N, 9.25. IR (KBr): 3300-2500 (-COOH), 3017 (CH, aromatic), 2980 (CH, aliphatic), 1661 (-COOH), 1601 (N-C=O), 1080 (O=C-O). ^1H NMR (DMSO-d_6): δ 4.62 (s, 2H, OCH_2), 7.23-8.16 (m, 9H, Ar-H).

Ethyl 2-(4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yloxy)acetate (4)

A mixture of **3** (2.96 g, 10 mmol), absolute ethanol (50 mL), and concentrated sulfuric acid (1 mL) was refluxed for 24 h then allowed to cool to rt and further in ice-cold water. A cold solution of sodium bicarbonate (5%) was added gradually until effervescence ceased. The solid obtained was collected by filtration, suspended in sodium bicarbonate solution (5%, 20 mL) and stirred for 10 min to remove unreacted acid. The precipitate was collected by filtration, washed thoroughly with cold water, dried, and recrystallized from ethyl acetate to give 2.43 g. white crystals, mp 90-92 °C, yield 60%. Analysis (%) for $C_{18}H_{16}N_2O_4$ (324.33), Calc.: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.97; H, 4.58; N, 8.62. IR (KBr): 3061 (CH, aromatic), 2987 (CH, aliphatic), 1735 (O=C-O), 1660 (N-C=O), 1229, (O=C-O), 1180 (C-O-C). ^1H NMR ($CDCl_3$): δ 1.15 (t, 3H, $OCH_2C\underline{H_3}$), 4.15 (q, 2H, $OC\underline{H_2}CH_3$), 4.82 (s, 2H, OCH_2CO), 7.25-8.36 (m, 9H, Ar-H).

2-(4-Oxo-3-phenyl-3,4-dihydrophthalazin-1-yloxy)acetohydrazide (5)

A mixture of **4** (3.24 g, 10 mmol) and hydrazine hydrate (5 mL, 99%) in ethanol (25 mL) was refluxed for 8 h. The formed precipitate was collected by filtration, washed with ethanol, crystallized from isopropyl alcohol, and dried to afford 2.64 g. White crystals, mp 180-182 °C, yield 85%. Analysis for $C_{16}H_{14}N_4O_3$ (310.31), Calc.: C, 61.93; H, 4.55; N, 18.06. Found: C, 62.17; H, 4.33; N, 17.79. ^1H NMR (DMSO-d_6): δ 4.44 (s, 2H, OCH_2), 7.21-8.24 (m, 9H, Ar-H), 9.50 (s, 1H, NH, D_2O exchangeable), NH_2 protons seemed to be exchanged by the solvent. MS (m/z): 311 (M^++1, 4.70), 279 (11.04), 238 (11.17), 237 (11.55), 221 (12.69), 145 (7.61), 104 (37.06), 77 (14.34).

2-(4-Oxo-3-phenyl-3,4-dihydrophthalazin-1-yloxy)-N'-(4-(un)substituted benzylidene)acetohydrazides (6a-c)

A mixture of **5** (0.31 g, 1 mmol) and the appropriate 4-(un)substituted benzaldehyde (1 mmol) in absolute ethanol (25 mL) was refluxed for 5 h, cooled to rt. The separated crystalline solid was collected by filtration, dried and recrystallized from absolute ethanol to afford the titled products.

N'-Benzylidene-2-((4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)oxy)acetohydrazide ***(6a)***

Yellowish white crystals, mp 176-178 °C, yield 85%. Analysis for $C_{23}H_{18}N_4O_3$ (398.41), Calc.: C, 69.34; H, 4.55; N, 14.06. Found: C, 69.50; H, 4.29; N, 14.45. IR (KBr): 3215 (NHCO), 3060 (CH, aromatic), 2966 (CH, aliphatic), 1704 (CH_2CONH-), 1647 (N-C=O), 1587 (N-N=C-R), 1103 (C-O-C). MS (m/z): 398 (M^+, 55.77), 279 (47.43), 251

(13.17), 238 (100), 237 (17.40), 221 (34.22), 119 (5.78), 104 (19.51), 91 (18.47), 77 (21.43). ^{1}H NMR (DMSO-d_6): δ 4.52 (s, 2H, OCH_2), 7.23-8.14 (m, 14H, Ar-H), 8.37 (s, 1H, N=CH), 10.33 (s, 1H, NH, D_2O exchangeable).

*N'-(4-Chlorobenzylidene)-2-((4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)oxy)acetohydrazide (**6b**)*
Yellowish white crystals, mp 207-209 °C, yield 85%. Analysis for $C_{23}H_{17}ClN_4O_3$ (432.86), Calc.: C, 63.82; H, 3.96; N, 12.94. Found: C, 63.57; H, 4.19; N, 12.61. IR (KBr): 3223 (NHCO), 3078 (CH, aromatic), 2958 (CH, aliphatic), 1704 (CH_2CONH), 1644 (N-C=O), 1620 (N-N=C-R), 1103 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 4.51 (s, 2H, OCH_2), 7.25-8.06 (m, 14H, Ar-H), 8.24 (s, 1H, N=CH), 9.77 (s, 1H, NH, D_2O exchangeable).

*N'-(4-hydroxybenzylidene)-2-((4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)oxy)acetohydrazide (**6c**)*
Yellowish white crystals, mp 233-235 °C, yield 75%. Analysis for $C_{23}H_{18}N_4O_4$ (414.41), Calc.: C, 66.66; H, 4.38; N, 13.52. Found: C, 66.84; H, 4.71; N, 12.61. MS (m/z): 414 (M, 7.17), 279 (25.52), 251 (6.67), 238 (100), 237 (19.17), 221 (21.27), 104 (14.86), 91 (23.56), 77 (17.27). ^{1}H NMR (DMSO-d_6): δ 4.59 (s, 2H, OCH_2), 6.79-8.10 (m, 13H, Ar-H), 8.39 (s, 1H, N=CH), 8.71 (s, 1H, OH, D_2O exchangeable), 9.65 (s, 1H, NH, D_2O exchangeable).

2-(4-Oxo-3-phenyl-3,4-dihydrophthalazin-1-yloxy)-N'-(1-phenylethylidene)acetohydrazide (7)
A mixture of **5** (0.31 g, 1 mmol) and acetophenone (0.12 g, 1 mmol) in glacial acetic acid (20 mL) was refluxed for 5 h, cooled to rt, and then poured over crushed ice and allowed to stand overnight. The separated solid was collected by filtration, dried and recrystallized from DMF/water to give the titled product, Yellowish-white crystals, mp 191-193 °C, yield 75%. Analysis (%) for $C_{24}H_{20}N_4O_3$ (412.44), calc.: C, 69.89; H, 4.89; N, 13.58. Found: C, 70.12; H, 4.63; N, 13.27. IR (KBr): 3200 (NHCO), 3074 (CH, aromatic), 2967 (CH, aliphatic), 1698 (CH_2CONH-), 1663 (N-C=O), 1624 (N-N=C-R), 1108 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 2.53 (s, 3H, CH_3), 4.57 (s, 2H, OCH_2), 7.23-8.16 (m, 14H, Ar-H), 9.77 (s, 1H, NH, D_2O exchangeable).

2-(4-Oxo-3-phenyl-3,4-dihydrophthalazin-1-yloxy)-N-(un)substituted-1,3-dioxo-2H-1,3-dihydroisoindol-2-yl)acetamides (8a-c)
A mixture of **5** (0.31 g, 1 mmol) and the appropriate (un)substituted phthalic acid anhydride (1 mmol) in glacial acetic acid (20 mL) was heated under reflux for 5 h, allowed to cool to rt, and then poured over ice-water. The separated solid was collected by filtration, dried and recrystallized from glacial acetic acid to give pure products.

*N-(1,3-Dioxoisoindolin-2-yl)-2-((4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)oxy)acetamide (**8a**)*
Yellowish white crystals, mp 159-161 °C, yield 85%. Analysis for $C_{24}H_{16}N_4O_5$ (440.41), Calc.: C, 65.45; H, 3.66; N, 12.72. Found: C, 65.11; H, 3.91; N, 12.46. IR (KBr): 3217 (NHCO), 3006 (CH, aromatic), 1797, 1740 two (O=C-N-C=O), 1658 (CH_2CONH), 1626 (N-C=O), 1187 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 4.49 (s, 2H, OCH_2), 7.24-8.16 (m, 13H, Ar-H), 9.77 (s, 1H, NH, D_2O exchangeable).

*1,3-Dioxo-2-(2-((4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)oxy)acetamido)isoindoline-5-carboxylic acid (**8b**)*
Yellowish white crystals, mp 182-184 °C, yield 65%. Analysis for $C_{25}H_{16}N_4O_7$ (484.42), Calc.: C, 61.99; H, 3.33; N, 11.57. Found: C, 62.27; H, 3.42; N, 11.31. IR (KBr): 3500-2750 (COOH), 3211 (NHCO), 3008 (CH, aromatic), 1797, 1743 two (O=C-N-C=O), 1700 (COOH), 1654 (CH_2CONH), 1625 (N-C=O), 1195 (C-O-C), 1103 (O=C-O). MS (m/z): 484 (M^+, 12.44), 439 (6.04), 295 (100), 279 (21.02), 251 (21.44), 221 (26.75), 119 (2.19), 104 (28.44), 91 (38.66), 77 (38.98). ^{1}H NMR (DMSO-d_6): δ 4.50 (s, 2H, OCH_2), 7.21-8.02 (m, 12H, Ar-H), 9.75 (s, 1H, NH, D_2O exchangeable), COOH proton seemed to be exchanged by the sovent.

*2-((4-Oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)oxy)-N-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)acetamide (**8c**)*
Yellowish white crystals, mp 147-149 °C, yield 85%. Analysis for $C_{24}H_{12}Cl_4N_4O_5$ (578.18), Calc.: C, 49.86; H, 2.09; N, 9.69. Found: C, 50.14; H, 1.88; N, 9.85. ^{1}H NMR (DMSO-d_6): δ 4.53 (s, 2H, OCH_2), 7.23-8.15 (m, 9H, Ar-H), 9.77 (s, 1H, NH, D_2O exchangeable).

2-(1,4-Dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetic acid (10)
An ice-cooled solution of chloroacetic acid (4.73 g, 50 mmol) in water (50 mL) was neutralized by dropwise addition of 10% sodium hydroxide solution. Phenyl hydrazine (5.41 g, 50 mmol) was then added dropwise, and the reaction mixture was heated under reflux for 3 h. A solution of phthalic acid anhydride (6.67 g, 45 mmol) in glacial acetic acid (30 mL) was then added in small portions over a period of 1 h followed by addition of hydrochloric acid (30 mL, 36%) and reflux was continued for 24 h. The formed precipitate was filtered, washed thoroughly with water, dried, and recrystallized from acetic acid to afford 8 g of the titled compound. Off-white crystals, mp 192-194

°C, yield 60%. Analysis for $C_{16}H_{12}N_2O_4$ (296.28), Calc.: C, 64.86; H, 4.08; N, 9.46. Found: C, 65.03; H, 4.04; N, 9.66. IR (KBr): 3250-2500 (COOH), 3015 (CH, aromatic), 2980 (CH, aliphatic), 1723 (COOH), 1659-1624 (O=C-N-N-C=O), 1078 (O-C=O). ^{1}H NMR (DMSO-d_6): δ 4.23 (s, 2H, NCH_2), 6.88-8.34 (m, 9H, Ar-H), COOH proton seemed to be exchanged by the solvent.

Ethyl 2-(1,4-dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetate (11)

A mixture of **10** (2.96 g, 10 mmol), absolute ethanol (50 mL), and concentrated sulfuric acid (1 mL) was heated under reflux for 24 h then concentrated and allowed to cool overnight. The precipitated crystalline product was filtered off, washed with sodium bicarbonate solution (5%, 20 mL) and finally with water, dried, and recrystallized from absolute ethanol to give 2.27 g. Yellowish white crystals, mp 119-121 °C, yield 70%. Analysis for $C_{18}H_{16}N_2O_4$ (324.33), Calc.: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.51; H, 5.22; N, 8.55. IR (KBr): 3061 (CH, aromatic), 2987 (CH, aliphatic), 1735 (COOEt), 1660-1624 (O=C-N-N-C=O), 1229 (COOEt). ^{1}H NMR ($CDCl_3$): δ 1.35 (t, 3H, $OCH_2C\underline{H}_3$), 4.18 (q, 2H, $OC\underline{H}_2CH_3$), 4.26 (s, 2H, NCH_2CO), 6.89-8.32 (m, 9H, Ar-H).

2-(1,4-Dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetohydrazide (12)

A mixture of **11** (3.24 g, 10 mmol) and hydrazine hydrate (5 mL, 99%) in ethanol (25 mL) was heated under reflux for 12 h. The formed precipitate was collected by filtration, washed with ethanol, recrystallized from acetic acid, and dried to afford 2.26 g. Yellow crystals, mp 262-264 °C, yield 72%. Analysis for $C_{16}H_{14}N_4O_3$ (310.31), Calc.: C, 61.93; H, 4.55; N, 18.06. Found: C, 62.11; H, 4.49; N, 18.22. IR (KBr): 3336 ($CH_2CONHNH_2$), 3180 (-$CH_2CONHNH_2$), 3039 (CH, aromatic), 2922 (CH, aliphatic), 1662 (CH_2CONH), 1650-1627 (O=C-N-N-C=O). ^{1}H NMR (DMSO-d_6): δ 4.32 (s, 2H, NCH_2), 6.89-8.32 (m, 9H, Ar-H), 11.50 (s, 1H, NH, D_2O exchangeable), NH_2 protons seemed to be exchanged by the solvent.

2-(1,4-Dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)-N'-((un)4-substituted benzylidene)acetohydrazides (13a-c)

A mixture of **12** (0.31 g, 1 mmol) and the appropriate 4-(un)substituted benzaldehyde (1 mmol) in absolute ethanol (25 mL) was heated under reflux for 5 h, allowed to cool to rt. The separated crystalline product was filtered off, dried, and recrystallized from absolute ethanol to afford the titled compounds.

N'-Benzylidene-2-(1,4-dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetohydrazide ***(13a)***

Yellow crystals, mp 247-249 °C, yield 68%. Analysis for $C_{23}H_{18}N_4O_3$ (398.41), Calc.: C, 69.34; H, 4.55; N, 14.06. Found: C, 69.50; H, 4.40; N, 13.88. ^{1}H NMR (DMSO-d_6): δ 4.02 (s, 2H, NCH_2), 6.84-8.34 (m, 14H, Ar-H), 8.45 (s, 1H, N=CH), 11.07 (s, 1H, NH, D_2O exchangeable).

N'-(4-Chlorobenzylidene)-2-(1,4-dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetohydrazide ***(13b)***

Yellow crystals, mp 198-200 °C, yield 78%. Analysis for $C_{23}H_{17}ClN_4O_3$ (432.86), Calc.: C, 63.82; H, 3.96; N, 12.94. Found: C, 63.76; H, 3.88; N, 12.90. ^{1}H NMR (DMSO-d_6): δ 3.81 (s, 2H, NCH_2), 6.81-8.07 (m, 13H, Ar-H), 8.22 (s, 1H, N=CH), 11.91 (s, 1H, NH, D_2O exchangeable).

2-(1,4-Dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)-N'-(4-hydroxybenzylidene)acetohydrazide ***(13c)***

Yellow crystals, mp 211-213 °C, yield 73%. Analysis for $C_{23}H_{18}N_4O_4$ (414.41), Calc.: C, 66.66; H, 4.38; N, 13.52. Found: C, 66.30; H, 4.46; N, 13.67. IR (KBr): 3346 (OH), 3209 (NH), 3067 (CH, aromatic), 2957 (CH, aliphatic), 1680 (-CH_2CONH-), 1643 (O=C-N-N-C=O), 1597 (HN-N=C). ^{1}H NMR (DMSO-d_6): δ 4.11 (s, 2H, NCH_2), 6.77-8.03 (m, 13H, Ar-H), 8.25 (s, 1H, N=CH), 8.65 (s, 1H, OH, D_2O exchangeable), 11.80 (s, 1H, NH, D_2O exchangeable),

2-(1,4-Dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)-N'-(1-phenylethylidene)acetohydrazide (14)

A mixture of **12** (0.31 g, 1 mmol) and acetophenone (0.12 g, 1 mmol) in glacial acetic acid (20 mL) was heated refluxed for 10 h, cooled to rt, poured over crushed ice, and allowed to stand overnight. The separated solid was collected by filtration, dried and recrystallized from glacial acetic acid to afford pure products. Off-white crystals, mp 253-255 °C, yield 63%. Analysis for $C_{24}H_{20}N_4O_3$ (412.44), Calc.: C, 69.89; H, 4.89; N, 13.58. Found: C, 69.81; H, 4.80; N, 13.35. IR (KBr): 3271 (NH), 3062 (CH, aromatic), 2957 (CH, aliphatic), 1676 (-CH_2CONH-), 1660-1627 (O=C-N-N-C=O), 1593 (H_3C-C=N-N). ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, H_3C), 4.25 (s, 2H, CH_2), 7.12-8.37 (m, 14H, Ar-H), 10.81 (s, 1H, CONH, D_2O exchangeable).

2-(1,4-Dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)-N-((un)substituted-1,3-dioxo-2H-1,3-dihydroisoindol-2-yl)acetamides (15a-c)

A mixture of **12** (0.31 g, 1 mmol) and the appropriate (un)substituted phthalic acid anhydride (1 mmol) in glacial acetic acid (20 mL) was heated under reflux for 8 h, cooled to rt, and then poured over ice-water. The separated solid was collected by filtration, dried, and recrystallized from glacial acetic acid to give pure products.

2-(1,4-Dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)-N-(1,3-dioxoisoindolin-2-yl)acetamide ***(15a)***

Yellow crystals, mp 205-207 °C, yield 78%. Analysis for $C_{24}H_{16}N_4O_5$ (440.41), Calc.: C, 65.45; H, 3.66; N, 12.72. Found: C, 65.69; H, 3.46; N, 12.50. ^{1}H NMR (DMSO-d_6): δ 3.94 (s, 2H, CH_2), 6.86-8.29 (m, 13H, Ar-H), 10.90 (s, 1H, CONH, D_2O exchangeable). MS (m/z): 440 (M, 60.97), 279 (86.22), 251 (34.18), 238 (100), 237 (40.56), 221 (79.59), 104 (60.20), 91 (48.47), 77 (29.59).

2-(2-(1,4-Dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)acetamido)-1,3-dioxoisoindoline-5-carboxylic acid ***(15b)***

Yellow crystals, mp 247-249 °C, yield 62%. Analysis for $C_{25}H_{16}N_4O_7$ (484.42), Calc.: C, 61.99; H, 3.33; N, 11.57. Found: C, 62.16; H, 3.57; N, 11.85. ^{1}H NMR (DMSO-d_6): δ 4.09 (s, 2H, CH_2), 6.84-8.16 (m, 12H, Ar-H), 10.82 (s, 1H, CONH, D_2O exchangeable), 11.72 (s, 1H, COOH, D_2O exchangeable).

2-(1,4-Dioxo-3-phenyl-3,4-dihydrophthalazin-2(1H)-yl)-N-(4,5,6,7-tetrachloro-1,3-dioxoisoindolin-2-yl)acetamide ***(15c)***

Yellow crystals, mp 260-262 °C, yield 85%. Analysis for $C_{24}H_{12}Cl_4N_4O_5$ (578.19), Calc.: C, 49.86; H, 2.09; N, 9.69. Found: C, 50.04; H, 2.23; N, 9.44. IR (KBr): 3213 (NH), 1800, 1748 two (O=C-N-C=O), 1654 (N-NHCOCH$_2$), 1625 (O=C-N-N-C=O), 734 (C-Cl). ^{1}H NMR (DMSO-d_6): δ 4.08 (s, 2H, CH_2), δ 6.86-8.29 (m, 9H, Ar-H), δ 10.82 (s, 1H, CONH, D_2O exchangeable).

4-(2-(1-(4-Aminophenyl)ethylidene)hydrazinyl)-2-phenylphthalazin-1(2H)-one (18)

A mixture of **16** (2.57 g, 10 mmol), **17** (1.49 g, 10 mmol) and anhydrous sodium acetate (0.82 g, 10 mmol) in absolute ethanol (25 mL) was refluxed for 5 h. The formed precipitate was collected by filtration, air-dried, and recrystallized from aqueous ethanol. Fluffy yellow crystals, mp 106-108 °C, yield 81%. Analysis for $C_{22}H_{19}N_5O$(369.42), Calc.: C, 71.53; H, 5.18; N, 18.96. Found: C, 71.69; H, 4.95; N, 19.23. ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 3H, H_3C), 7.12-8.37 (m, 13H, Ar-H), 10.81 (s, 1H, CONH, D_2O exchangeable), NH_2 protons seemed to be exchanged by the solvent. MS (m/z): 369 (M, 9.44), 251 (26.11), 119 (50.00), 118 (100.00), 104 (18.89), 91 (43.89).

N-(4-(1-(2-(4-Oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)hydrazono)ethyl)phenyl)acetamide (19)

Compound **18** (0.37 g, 1 mmol) was refluxed in acetic anhydride (10 mL) for 5 h, cooled to rt, and poured over ice-water (30 mL). The formed crystalline product was filtered, dried, and recrystallized from aqueous ethanol to give 0.26 g. White crystals, mp 135-137 °C, yield 65%. Analysis for $C_{24}H_{21}N_5O_2$ (411.46), Calc.: C, 70.06; H, 5.14; N, 17.02. Found: C, 70.28; H, 5.39; N, 16.88. IR (KBr): 3389, 3345 (H_3C-C=N-NH and CH_3CONH), 3071 (CH, aromatic), 2925 (CH, aliphatic), 1681 (CH_3CONH), 1643 (N-C=O), 1611 (H_3C-C=N-NH). ^{1}H NMR (DMSO-d_6): δ 2.15 (s, 3H, COH_3C), δ 2.65 (s, 3H, N=C-CH_3), 7.23-8.13 (m, 13H, Ar-H), 9.17 (s, 1H, NHCO, D_2O exchangeable), =N-NH proton seemed to be exchanged by the solvent.

4-(2-(1-(4-(4-(un)Substituted benzylideneamino)phenyl)ethylidene)hydrazinyl)-2-phenylphthalazin-1(2H)-ones (20a-c)

A mixture of **18** (0.37 g, 1mmol) and the appropriate 4-(un)substituted benzaldehyde (1 mmol) in absolute ethanol (25 mL) was heated under reflux for 5 h, allowed to cool to rt. The separated crystalline product was filtered off, dried, and recrystallized from absolute ethanol to afford the titled compounds.

4-(2-(1-(4-(Benzylideneamino)phenyl)ethylidene)hydrazinyl)-2-phenylphthalazin-1(2H)-one ***(20a)***

Yellow crystals, mp 181-183 °C, yield 87%. Analysis for $C_{29}H_{23}N_5O$ (457.53), Calc.: C, 76.13; H, 5.07; N, 15.31. Found: C, 76.31; H, 4.85; N, 15.20. ^{1}H NMR (DMSO-d_6): δ 2.33 (s, 3H, H_3C-C=N-), 7.20-8.14 (m, 18H, Ar-H), 8.61 (s, 1H, -N=CH), 10.76 (s, 1H, NH, D_2O exchangeable).

4-(2-(1-(4-((4-Chlorobenzylidene)amino)phenyl)ethylidene)hydrazinyl)-2-phenylphthalazin-1(2H)-one ***(20b)***

Yellow crystals, mp 254-256 °C, yield 71%. Analysis for $C_{29}H_{22}ClN_5O$ (491.97), Calc.: C, 70.80; H, 4.51; N, 14.24. Found: C, 71.08; H, 4.75; N, 14.06. IR (KBr): 3390 (HN-N=C), 3070 (CH, aromatic), 2924 (CH, aliphatic), 1675 (N-C=O), 1621, 1583 (H_3C-C=N-NH and ph-C=N-ph). ^{1}H NMR (DMSO-d_6): δ 2.38 (s, 3H, H_3C-C=N-), 7.22-8.20

(m, 17H, Ar-H), 8.60 (s, 1H, -N=CH), 10.78 (s, 1H, NH, D_2O exchangeable).

*4-(2-(1-(4-((4-Hydroxybenzylidene)amino)phenyl)ethylidene)hydrazinyl)-2-phenylphthalazin-1(2H)-one (**20c**)*
Yellow crystals, mp 119-121 °C, yield 64%. Analysis for $C_{29}H_{23}N_5O_2$ (473.53), Calc.: C, 73.56; H, 4.90; N, 14.79. Found: C, 73.20; H, 5.18; N, 14.62. ^{1}H NMR (DMSO-d_6): δ 2.42 (s, 3H, H_3C-C=N-), 7.18-8.14 (m, 17H, Ar-H), 8.53 (s, 1H, -N=CH), 9.75 (s, 1H, NH, D_2O exchangeable), 10.92 (s, 1H, NH, D_2O exchangeable).

2-(4-(1-(2-(4-Oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)hydrazono)ethyl)phenyl)(un)substituted-2H-1,3-dihydroisoindole-1,3-diones (21a-c)
A mixture of **18** (0.37 g, 1 mmol) and the appropriate (un)substituted phthalic acid anhydride (1 mmol) in glacial acetic acid (20 mL) was heated under reflux for 8 h, cooled to rt, and then poured over ice-water. The separated solid was collected by filtration, dried, and recrystallized from glacial acetic acid to give pure products.

*2-(4-(1-(2-(4-Oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)hydrazono)ethyl)phenyl)isoindoline-1,3-dione (**21a**)*
Yellow crystals, mp 137-139 °C, yield 70%. Analysis for $C_{30}H_{21}N_5O_3$ (499.52), Calc.: C, 72.13; H, 4.24; N, 14.02. Found: C, 71.85; H, 4.41; N, 14.35. IR (KBr): 3423 (NH), 3070 (CH, aromatic), 2925 (CH, aliphatic), 1779, 1719 two (O=C-N-C=O), 1680 (N-C=O), 1584 (H_3C-C=N-NH). ^{1}H NMR (DMSO-d_6): δ 2.55 (s, 3H, H_3C-C=N-), 7.21-7.89 (m, 17H, Ar-H), 10.59 (s, 1H, NH, D_2O exchangeable).

*1,3-Dioxo-2-(4-(1-(2-(4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)hydrazono)ethyl)phenyl)isoindoline-5-carboxylic acid (**20b**)*
Yellow crystals, mp 257-259 °C, yield 74%. Analysis for $C_{31}H_{21}N_5O_5$ (543.53), Calc.: C, 68.50; H, 3.89; N, 12.88. Found: C, 68.77; H, 4.14; N, 12.62. IR (KBr): 3426 (NH), 3250-2500 (-CO**OH**), 3065 (CH, aromatic), 2925 (CH, aliphatic), 1783, 1723 (O=C-N-C=O), 1681 (-COOH), 1653 (N-C=O), 1601 (H_3C-C=N-NH). ^{1}H NMR (DMSO-d_6): δ 2.58 (s, 3H, H_3C-C=N-), 7.23-8.61 (m, 16H, Ar-H), 10.31 (s, 1H, NH, D_2O exchangeable), COOH proton seemed to be exchanged by the solvent.

*4,5,6,7-Tetrachloro-2-(4-(1-(2-(4-oxo-3-phenyl-3,4-dihydrophthalazin-1-yl)hydrazono)ethyl)phenyl)isoindoline-1,3-dione (**20c**)*
Yellow crystals, mp 283-285 °C, yield 70%. Analysis for $C_{30}H_{17}C_{l4}N_5O_3$ (637.30), Calc.: C, 56.54; H, 2.69; N, 10.99. Found: C, 56.69; H, 2.48; N, 11.24. ^{1}H NMR (DMSO-d_6): δ 2.67 (s, 3H, CH_3), δ 7.21-8.14 (m, 13H, Ar-H), 10.75 (s, 1H, NH, D_2O exchangeable).

Antioxidant Screening
Preparation of reagents
ABTS solution was prepared as 0.1 g/100 mL. MnO_2 solution (25 mg/mL) was used instead of potassium persulfate. All reagents were prepared in phosphate buffer (pH 7, 0.1 M). The two reagents ABTS/MnO_2 (2:3) were mixed and centrifuged. The supernatant was obtained as green-blue solution ($ABTS^{\bullet+}$ radical solution). This color remained stable for more than 1 h. The absorbance was adjusted at about 0.2 at 734 nm. *L*-Ascorbic acid solution was prepared as 2% solution: 1 g/50 mL distilled water. Each test sample was used at a concentration of 0.01 mg/mL in methanol/phosphate buffer (1:1).

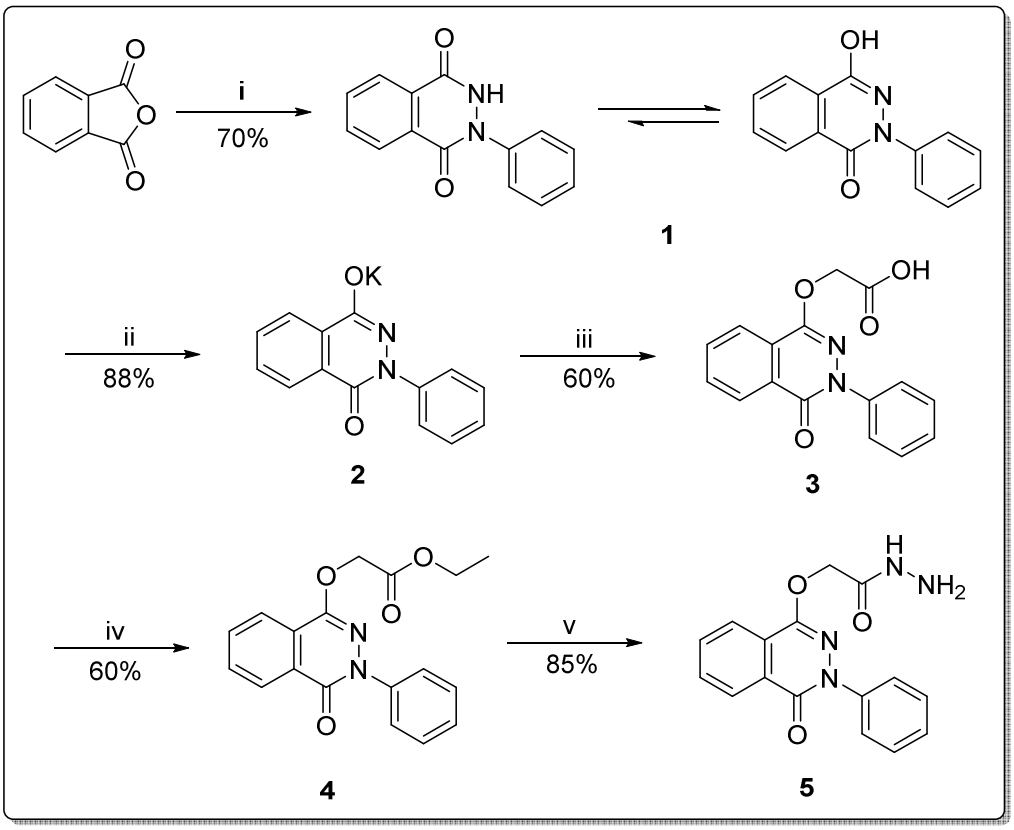
Starting assay
Of ABTS/MnO_2, 900 mL was added to a spectrophotometer cuvette (SPEKOL 11), and the absorbance was measured at 734 nm against a blank made of methanol/phosphate buffer (1:1). Of the ABTS/MnO_2 mixture, 900 mL was added to 100 mL standard L-ascorbic acid, and the absorbance was measured against the blank: methanol/phosphate buffer (1:1) + 100 mL of *L*-ascorbic acid. 900 µL of the ABTS/MnO_2 mixture was added to 100 mL of sample, and the absorbance was measured against the blank: methanol/phosphate buffer (1:1) + 100 mL of sample. The % inhibition of absorbance for each of the tested compounds was calculated from the equation: $\%\ \text{Inhibition} = (\text{Abs of control} - \text{Abs of test})/(\text{Abs of control}) \text{x } 100$.

RESULTS AND DISCUSSION

Chemistry
The starting compound 4-hydroxy-2-phenylphthalazin-1(2*H*)-one (**1**) was converted to its potassium salt by a salting out with KOH in isopropyl alcohol. This salt formation suggests the existence of phthalhydrazide-

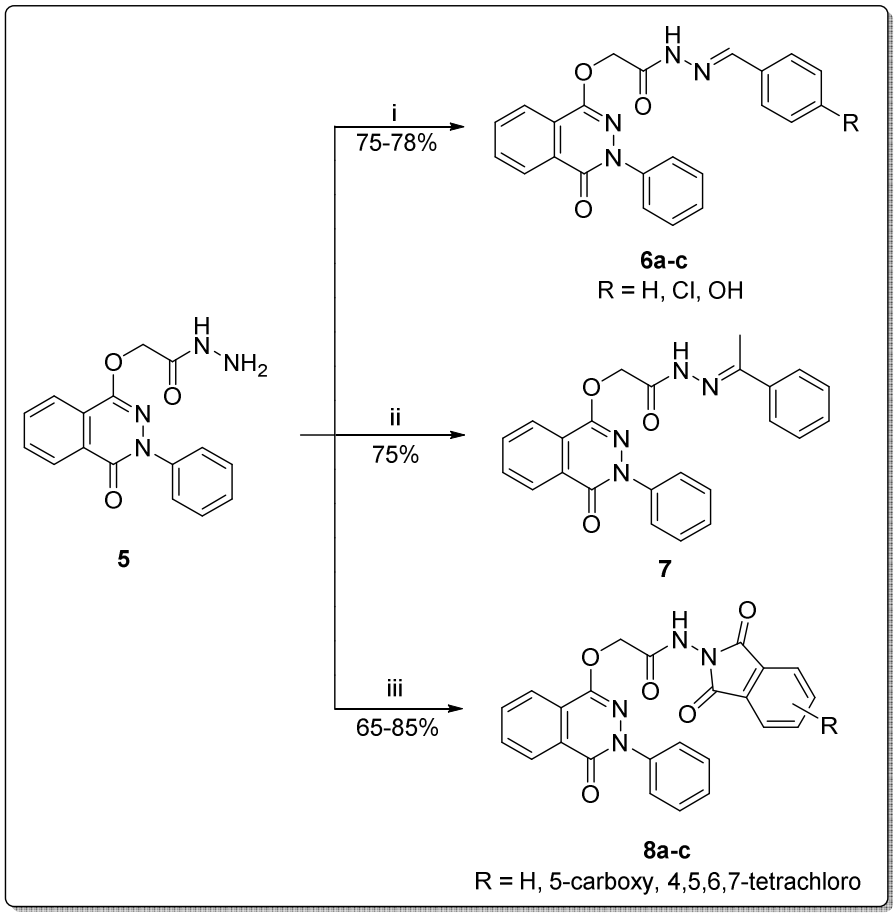
phthalazinoneequilibrium (lactam-lactimtautomeric forms) which predominatessufficiently to cause acidic charactersand favor more stable ring structure [36].Alkylation of **2** with chloroacetic acid via Williamson reaction [37] afforded compound**3**. The corresponding ester **4** was prepared via Fischer esterification [38],through refluxing the acid derivative **3** and absolute ethanol in the presence of catalytic amount of sulfuric acid.The acid hydrazide **5** was prepared by refluxing a mixture of the ester **4** and hydrazine hydrate in ethanol(**Scheme 1**).

Compounds **6a-c**were prepared by condensation of **5** and the appropriate aromatic aldehyde in ethanol as a solvent. The acetophenonehydrazone**7** was prepared by refluxing a mixture of **5** and acetophenone in glacial acetic acid, whereas the acetamide derivatives **8a-c**were synthesized through refluxing **5** with the appropriate phthalic acid anhydride in glacial acetic acid (**Scheme 2**).

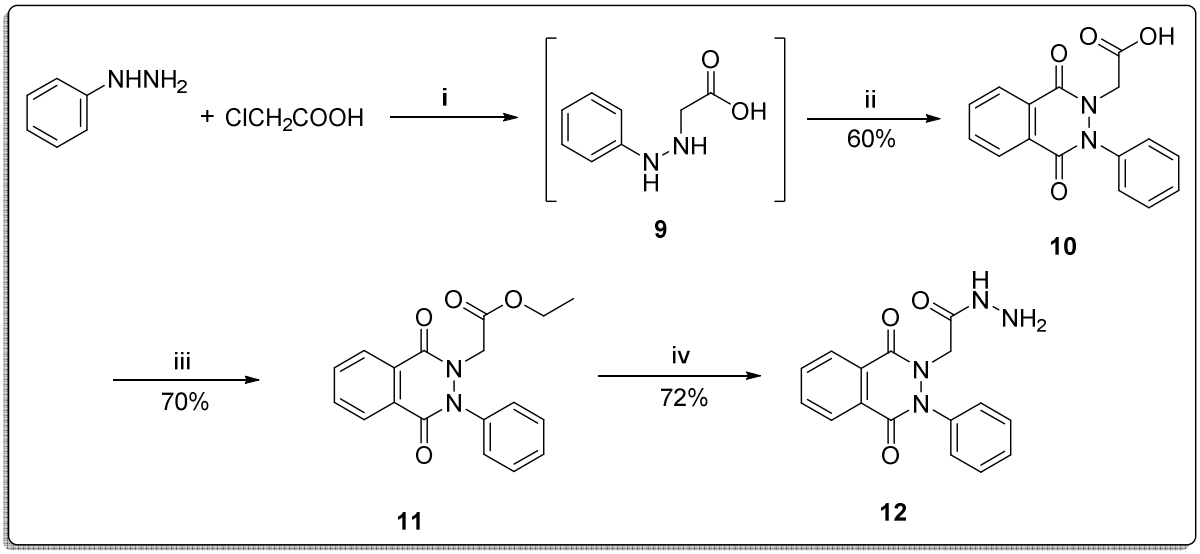


Scheme 1.Synthesis of compounds 1-5. Reagents and conditions: (i) $PhNHNH_2$, H_2O, CH_3COOH, HCl, reflux, 10 h; (ii) KOH, isopropyl alc., stirring, 1 h; (iii) $ClCH_2COOH$, ethanol, reflux, 10 h; (iv) ethanol, H_2SO_4, reflux, 24 h; (v) $NH_2NH_2.H_2O$, ethanol, reflux, 8 h

The *N,N*-disubstituted phthalazine **10** was synthesized by refluxing phthalic anhydride with the *in situ*-formed 2-(2-phenylhydrazinyl)acetic acid (**9**) [39] in a strong acidic mixture of acetic acid and hydrochloric acid. This is a widely used synthetic approach for pyridazines or phthalazines synthesis, in which a reaction between hydrazine or a substituted hydrazine and 1,4-dicarbonyl compounds, such as 1,4-diketones, 1,4-ketoaldehydes, 1,4-dialdehydes, 1,4-ketoacids, 1,4-dicarboxylic acids, and their derivatives, in particular their acid anhydrides was performed. Generally, the reaction may proceed in a single step or *via* the corresponding intermediate hydrazones. It is customary to perform the condensation of 1,4-dicarbonyl compound with hydrazines in the presence of mineral acid to avoid the formation of *N*-aminopyrole derivatives [40]. Esterification of **10** with ethanol afforded the corresponding ester **11**, while condensation of the ester **11** with hydrazine hydrate afforded the corresponding acid hydrazide **12**. The ester **11** and the hydrazide **12** derivatives were referred to in a publication [41], applying different method for preparation of the ester **11**. Due to the absence of more detailed data, these compounds were partially characterized (**Scheme 3**).



Scheme 2.Synthesis of compounds 6a-c, 7 and 8a-c. Reagents and conditions: (i) appropriate benzaldehyde, ethanol, reflux, 5 h; (ii) acetophenone, CH_3COOH, reflux, 5 h; (iii) appropriate phthalic anhydride, CH_3COOH, reflux, 5 h



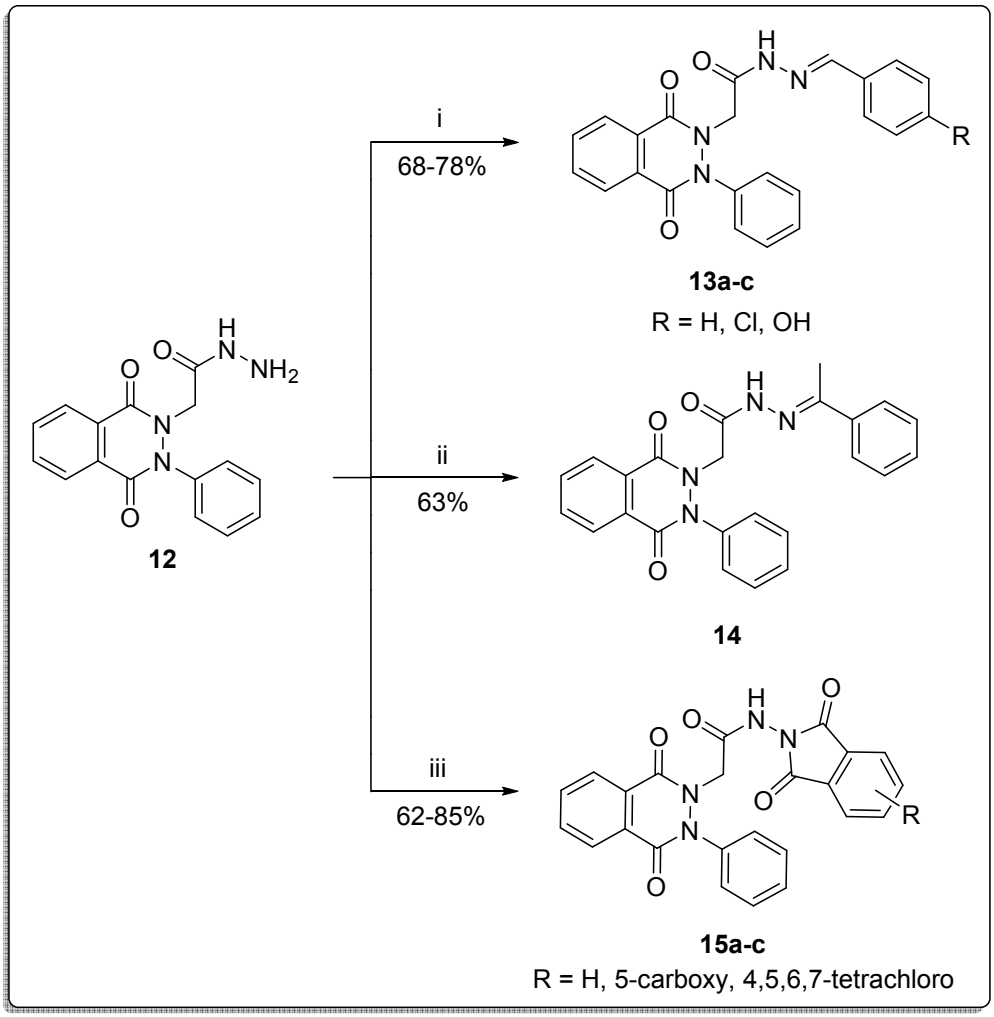
Scheme 3.Synthesis of compounds 10-12. Reagents and conditions: (i) 10% NaOH, reflux, 3 h; (ii) phthalic anhydride, CH_3COOH, HCl, reflux, 24 h; (iii) ethanol, H_2SO_4, reflux, 24 h; (iv) $NH_2NH_2.H_2O$, ethanol, reflux, 12 h

The target products 13a-c, 14 and 15a-c were prepared in a similar manner to Scheme 2*via*condensation of the acid hydrazide derivative 12 with the appropriate aromatic aldehyde, acetophenone and the appropriate phthalic anhydride, respectively (Scheme 4).

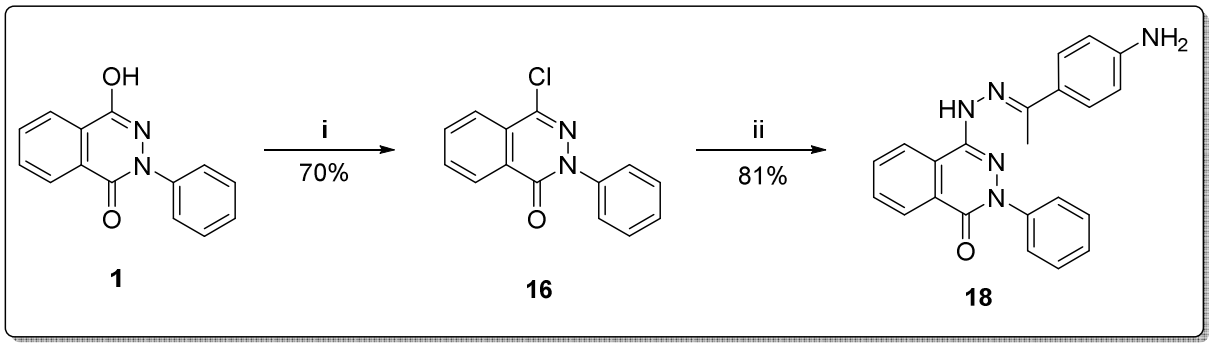
Compound **18** was prepared *via* alkylation of 4-(1-hydrazonoethyl)aniline (**17**) [35] with 4-chloro-2-phenylphthalazin-1(2*H*)-one (**16**) [34] in presence of catalytic anhydrous sodium acetate in refluxing ethanol (**Scheme 5**).

The acetamido derivative **19** was synthesized by acetylation of the corresponding amine **18** in acetic anhydride.Schiff's bases **20a-c** target products were prepared by condensation of **18** with the appropriate aromatic aldehyde in refluxing ethanol. The phthaloyl derivatives **21a-c**were synthesized by refluxing the amine **18** and the appropriate phthalic acid anhydride in glacial acetic acid (**Scheme 6**).

All the newly synthesized compounds were characterized by physical analytical and spectral data and found to be in agreement with calculated and predicted values.



Scheme 4.Synthesis of compounds 13a-c, 14 and 15a-c. Reagents and conditions: (i) appropriate benzaldehyde, ethanol, reflux, 5 h; (ii) acetophenone, CH_3COOH, reflux, 5 h; (iii) appropriate phthalic anhydride, CH_3COOH, reflux, 5 h



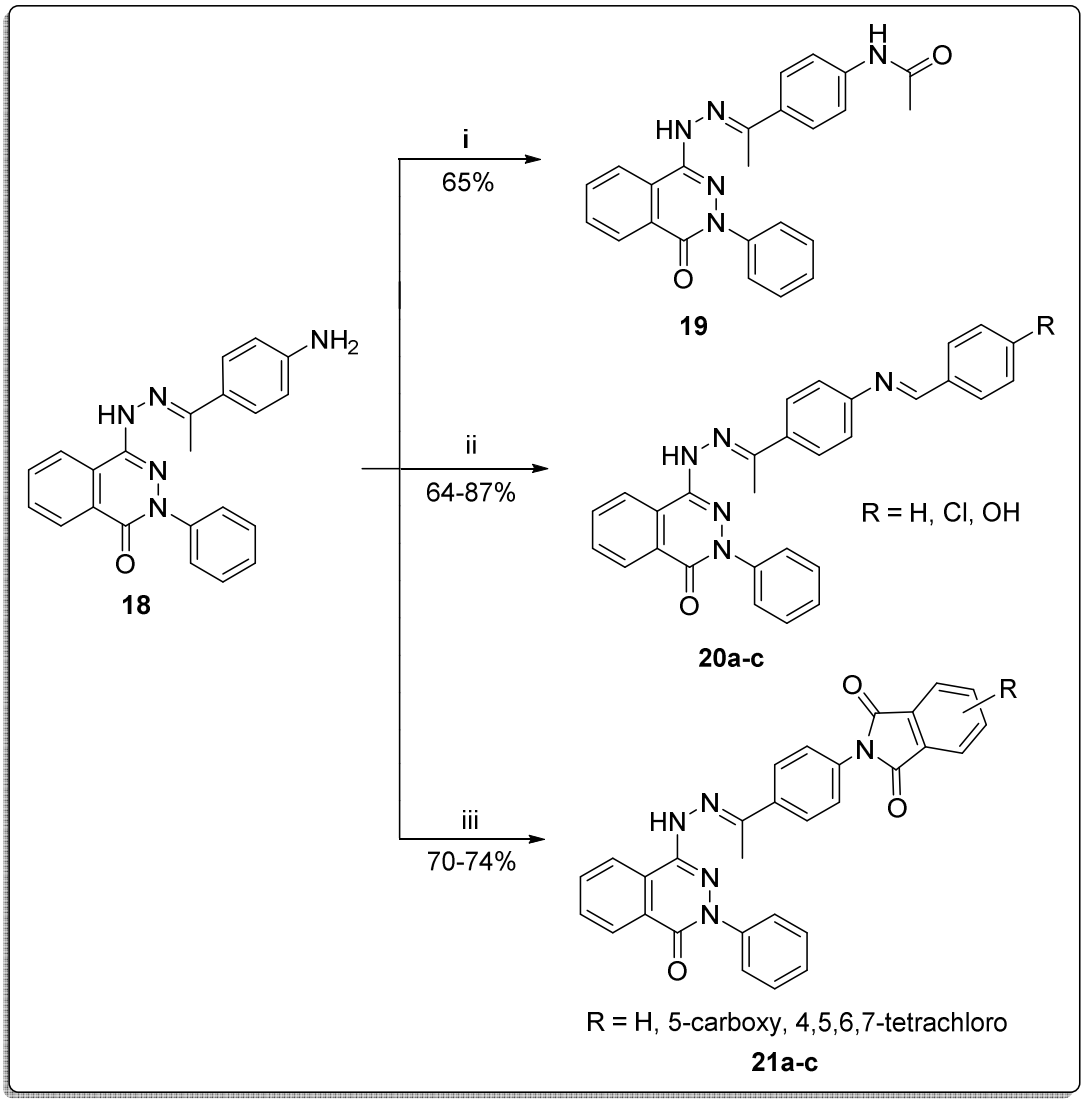
Scheme 5.Synthesis of compound 18. Reagents and conditions: (i) $POCl_3$, reflux, 5 h; (ii) 17, CH_3COONa, reflux, 5 h

Antioxidant Screening

All the newly synthesized compounds have been subjected for preliminary screening for their *in vitro* anti-inflammatory activity through measuring of the antioxidant activity. Superoxide dismutase enzyme is an important class of antioxidant defense system (free radical scavengers). It was reported that very low levels of circulating

superoxide dismutase were observed in inflammatory conditions. These levels were significantly improved with NSAIDs therapy [42]. On the other hand; free radicals and reactive oxygen species (ROS) were found to be implicated in different inflammatory conditions, so that inhibition of these species helped in controlling the inflammatory conditions [43,44].

ABTS, (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt), radical cation decolonization test and is also a spectrophotometic method widely used for the assessment of antioxidant activity of various substances [45]. It is applicable for both lipophilic and hydrophilic compounds. The radical cation ($ABTS^{\bullet+}$) was generated by oxidation of ABTS with potassium persulfate. The absorbance is bleached by antioxidants due to their capacity to reduce the preformed radical. The results of the preliminary qualitative antioxidant screening (scavenger activity) of all the tested compounds are listed (**Table 1**). Compound **5** showed the best activity, whereas compound **12** showed moderate activity. Both **5** and **12** are carrying hydrazide moiety and are *O*-substituted for **5** and *N*-substituted for **12**, respectively. The rest of compounds showed weak to mild activity.



Scheme 6.Synthesis of compounds 19, 20a-c and 21a-c. Reagents and conditions: (i) $(CH_3CO)_2O$, reflux, 5 h; (ii) appropriate benzaldehyde, ethanol, reflux, 5 h; (iii) appropriate phthalic anhydride, CH_3COOH, reflux, 8 h

Table 1: Results of ABTS assay

Comp. No.	Absorbance (mean)	% Inhibition	Comp. No.	Absorbance (mean)	% Inhibition
Control	0.54	0	**13a**	0.44	18.50
L-Ascorbic acid	0.05	90.70	**13b**	0.43	20.30
3	0.39	27.70	**14a**	0.47	12.90
4	0.44	18.50	**14b**	0.43	20.30
5	0.15	72.20	**15a**	0.45	16.60
6a	0.44	18.50	**15b**	0.53	1.80
6b	0.37	31.40	**15c**	0.38	29.60
6c	0.41	24.00	**18**	0.43	20.30
7	0.47	12.90	**19**	0.43	20.30
8a	0.45	16.60	**20a**	0.44	18.50
8b	0.37	31.40	**20b**	0.46	14.80
8c	0.37	31.40	**20c**	0.40	25.90
10	0.45	16.60	**21a**	0.48	11.10
11	0.50	7.40	**21b**	0.46	14.80
12	0.26	51.80	**21c**	0.45	16.60

CONCLUSION

From a chemical point of view, synthesis of targeted phthalazine derivatives were achieved effective and simple chemical pathways. Both*O*- and *N*-substituted phthalazine derivatives can be served as versatile heterocyclic building blocks for different bioactive molecules. Biologically, antioxidant screening datarevealed that compound **5** showed the best activity, whereas, compound **12** showed moderate activity. Both **5** and **12** are carrying a hydrazide moiety hybridized with phthalazine ring and are *O*-substituted for **5** and *N*-substituted for **12**, respectively. The rest of compounds showed weak to mild activity.Further structural modification of functionalities is required for improvement of biological activity.

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