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An efficient synthesis of some new 1,4-disubstituted phthalazine derivatives and their anticancer activity

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ABSTRACT

*A novel series of phthalazine derivatives bearing isoindol-1,3-dione moiety were synthesized by treating ethyl{4-[4-(1,3-dioxo-1,3-dihydroisoindole-2-yl)-phenyl] phthalazin-1-yloxy}acetate (**3**) and {4-[4-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)phenyl]phthalazin-1-yloxy} acetic acid hydrazide (**7a**) with various chemical reagents.The newly synthesized compounds were characterized on the basis of their spectral (^1H NMR, ^{13}C NMR, Ms, IR) analyses. In vitro, most of the synthesized derivatives were screened for their antitumor activity against MCF-7 cells using MTT assay. Compounds **16b**, **18**, **13**, **15**, **17** showed the most potent cytotoxic effect concluded from their IC_{50} values 50, 70, 150, 180 and 100 µg/ml respectively.*

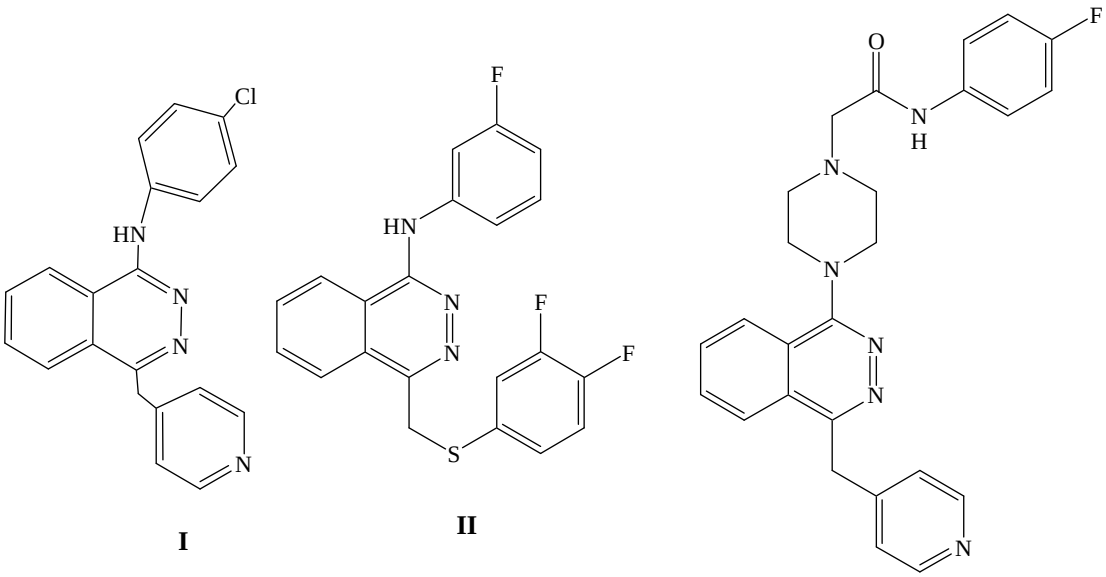
Key words: Phthalazin-1-(2H)-One; Acid hydrazide, Isoindol-1,3-dione; Antitumor activity.

INTRODUCTION

Breast cancer is the most common form of cancer and the second most frequent cause of cancer death among women [1]. Regardless of the use of surgical treatment and irradiation, chemotherapy still remains an important option for the treatment of solid cancers. Chemotherapeutic drugs should preferentially target tumor cells without harming normal cells or tissues. However, although new cytotoxic agents with unique mechanisms of action have been developed continuously, many of them have not been therapeutically useful due to low tumor selectivity and harsh side effects [2]. These facts prompted us to design and develop novel potent and selective anti-breast cancer agents. 1,4-Disubstituted phthalazines have received a considerable attention as antitumor agents in the past few years [3,4]. A successful example is N-(4-chlorophenyl)-4-(pyridin-4-ylmethyl)phthalazin-1-amine also known as Vatalanib (PTK-787) which is VEGFR (vascular endothelial growth factor receptor) inhibitor and is currently in Phase III clinical trials for metastatic colorectal cancer [5]. [4-(3,4-difluoro-phenylsulfanylmethyl)-phthalazin-1-yl]-(3-fluoro-phenyl)-amine II displayed excellent selectivity against MDA-MB-231 cell line [6]. Furthermore, N-(4-fluoro-phenyl)-2-[4-(4-pyridin-4-ylmethyl-phthalazin-1-yl)-piperazin-1-yl]-acetamide III has shown more potent cytotoxicity than cisplatin [7]. (Fig. 1)

On the other hand, nitrogen containing heterocyclic molecules constitutes the largest portion of chemical entities, which are part of many natural products, fine chemicals, and biologically active pharmaceuticals.

Phthalazin-1(2H)-ones is important building blocks in the construction of new molecular systems for biologically active molecules [8-10]. The development of new and efficient methodologies for synthesis of potentially bioactive phthalazin-1(2H)-one derivative is important.



I

Vatalanib (PTK-787)

II

III

Fig. 1 Examples of 1,4-disubstituted phthalazine lead compounds with anti-cancer activity, compounds I, II, and III

Phthalazin-1(2H)-one is of considerable interest due to their antidiabetic [11], antiallergic [12], Vasorelaxant [13], PDE4 inhibitors[14] , VEGF (vascular endothelial growth factor) receptor tyrosine kinases for the treatment of cancer [15, 16], antiasthmatic agents with dual activities of thromboxane A2 (TXA2) synthetase inhibition and bronchodialation [17], herbicidal [18], like activities. A number of established drug molecules like (N-(4-methylpent-3-en-2-ylideneamino)phthalazin-1-amine) is known as Budralazine [19, 20], ((RS)-4-[(4-chlorophenyl)methyl]-2-(1-methylazepan-4-yl)-phthalazin-1-one) known as Azelastine [21, 22] are prepared from the corresponding phthalazinones.

In view of the above mentioned facts and in continuation of our research interest for the synthesis of biologically active heterocycles [23-25], we report here, the synthesis of novel series of phthalazinones bearing isoindol-1,3-dione moiety followed by evaluation of their antitumor activities against MCF-7 cells using MTT Cell Viability.

MATERIALS AND METHODS

Melting points were determined by the capillary tube method, and the thermometer was uncorrected. Mass spectra were obtained on an Agilent 1100 HPLC-MS instrument. ^{1}H NMR spectra were run in DMSO-d_6, with TMS at the internal standard, on a Bruker ARX-300 instrument operating at 300 MHz. IR spectra (KBr disks) were recorded on a Bruker IFS 55 instrument. Elemental analysis was performed with a Carlo-Erba 1106 Elemental analysis instrument.

Experimental methods

*Synthesis of 4-(4-Aminophenyl)phthalazin-1-(2H)-one (**1**)*

To a solution of 2-(4-aminobenzoyl)-benzoic acid (2.37 gm,10 mmol) in absolute ethanol (15 mL) was added (12 gm, 240 mmol) hydrazine hydrate and the reaction mixture was refluxed for 6 h [21-23]. After cooling, the precipitated was filtered off and crystallized from dimethylformamide/ water to give brown crystals. M.p. 250-252 °C; Yield 50 %; IR (cm^{-1}) ν: 3295-3064 (OH ↔NH, NH_2),1685 (CO); Ms: m/z == 237 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 5.31 (s, 2H, NH_2 exchangeable), 6.50-8.19 (m, 8H, Ar-H), 7.99 (s,1H, NH↔OH exchangeable); Anal. Calcd.for $C_{14}H_{11}N_3O$ (237): C, 70.87; H, 4.67; N, 17.71; Found: C, 70.85; H, 4.69; N, 17.70%.

*2-[4-(4-Oxo-3,4-dihydrophthalazin-1-yl)-phenyl]-isoindole-1,3-dione (**2**)*

A mixture of 1 (2.37 gm,10 mmol) and phthalic anhydride (1.48 gm,10 mmol) is heated at 250 °C for 2 h , after cooling , the residue was triturated with H_2O and the solid that obtained was filtered off and recrystallized from ethanol to give yellow crystals. M.p. 305-307 °C; Yield 93 %; IR (cm^{-1}): 3490-3208 (OH ↔NH), 1746-1700 (CO), 1590 (C=N); Ms: m/z == 367 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.69-8.19 (m, 12H, Ar-H), 8.17 (s, 1H, NH exchangeable); ^{13}C NMR (DMSO-d_6) δ: 170.00, 163.22, 155.62, 140.51, 134.01, 132.31, 132.00, 130.93, 130.10, 129.21, 129.11, 127.42, 126.81, 120.50; Anal. Calcd. For $C_{22}H_{13}O_3N_3$ (367): C, 71.93; H, 3.57; N, 11.44, Found: C, 71.83; H, 3.60; N 11.40%.

*{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxy}-acetic acid ethyl ester (**3**)*
A solution of 2 (3.67 gm, 10 mmol), (5.52 gm, 40 mmol) anhydrous potassium carbonate and (4.9 gm, 40 mml) ethylchloroacetate in dry acetone (60 mL) were refluxed for 30h. After cooling, the reaction mixture was poured onto crushed ice. The solid that separated was filtered off, washed well for several times with water, dried and crystallized from ethanol to give yellow crystals. M.p. 160-162 °C, Yield 50 %; IR (cm^{-1}): 2922 (C-H alip.), 1734 (CO) ester, 1707-1698 (2CO), 1598 (C=N), 1074 (C-O-C); Ms: m/z == 453 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 1.22 (t, 2H, CH_3), 3.20-3.25 (t, 4H, $2CH_2$), 7.32 (s, 1H, NH, exchangeable), 7.46-8.15 (m, 12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 179.00, 171.00, 163.22, 144.01, 138.21, 133.31, 132.30, 132.11, 132.00, 128.21, 127.41, 127.20, 126.01, 121.50, 120.90, 119.00, 76.21, 59.51, 13.62; Anal. Calcd. For $C_{26}H_{19}N_3O_5$ (453): C, 68.87; H, 4.22; N, 9.27, Found: C, 68.77; H, 4.33; N, 9.37%.

*Ethyl {4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]-1-oxo-1H-phthalazin-2-yl}acetate(**4**)*
A mixture of 2 (3.67 gm, 10 mmol), and (1.22 gm, 10 mml) ethylchloroacetate in least amount of pyridine (3 mL) was heated on a steam bath for 3 h. The reaction mixture poured onto cold H_2O/Hcl. The solid that separated was recrystallized from ethanol to give yellow crystals. M.p. 190-192 °C, Yield 55 %; IR (cm^{-1}): 2894 (C-H alip.), 1739 (CO) ester, 1700-1659 (2CO), 1601 (C=N); Ms: m/z == 454 ($M^{\cdot+}$+1); ^{1}H NMR (DMSO-d_6) δ: 1.32 (t, 2H, CH_3), 4.12 (q, 2H, CH_2), 4.16 (s, 2H, CH_2), 7.46-8.15 (m, 12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 179.00, 171.00, 163.22, 144.01, 138.21, 133.31, 132.30, 132.11, 132.00, 128.21, 127.41, 127.20, 126.01, 121.50, 120.90, 119.00, 76.21, 59.51, 13.62; Anal.Calcd. For $C_{26}H_{19}N_3O_5$ (453): C, 68.87; H, 4.22; N, 9.27, Found: C, 68.77; H, 4.33; N, 9.37%.

*2-[4-(3-Morpholin-4-ylmethyl-4-oxo-3,4-dihydro-phthalazin-1-yl)-phenyl]-isoindole-1,3-dione (**5**)*
A mixture of 3 (4.53 gm, 10 mmol) and (1.74 gm, 20 mmol) morpholine in formaldehyde (2 mL) was added to ethanol (30 mL) in conc. HCl (2 mL), the resulting reaction mixture was refluxed for 16 h. The solid that separated was filtered and recrystallized from benzene to give brown crystals. M.p. 170-172 °C, Yield 45 %; IR (cm^{-1}): 2894 (C-H alip.), 1700-1659 (2CO), 1589 (C=N), 1047 (C-O-C); Ms: m/z == 466 ($M^{\cdot+}$); ^{1}H NMR ($CDCl_3$) δ: 2.45 (s, 2H, CH_2-N), 3.72 (t, 2H, CH_2-O), 7.16-8.12 (m, 12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 168.00, 163.20, 155.61, 140.51, 134.01, 132.31, 132.00, 130.91, 130.00, 129.21, 129.11, 127.40, 126.80, 71.51, 70.1, 53.90; Anal.Calcd. For $C_{27}H_{22}N_4O_4$ (466): C, 68.77; H, 4.33; N, 9.37; Found: C, 68.87; H, 4.23; N, 9.39%.

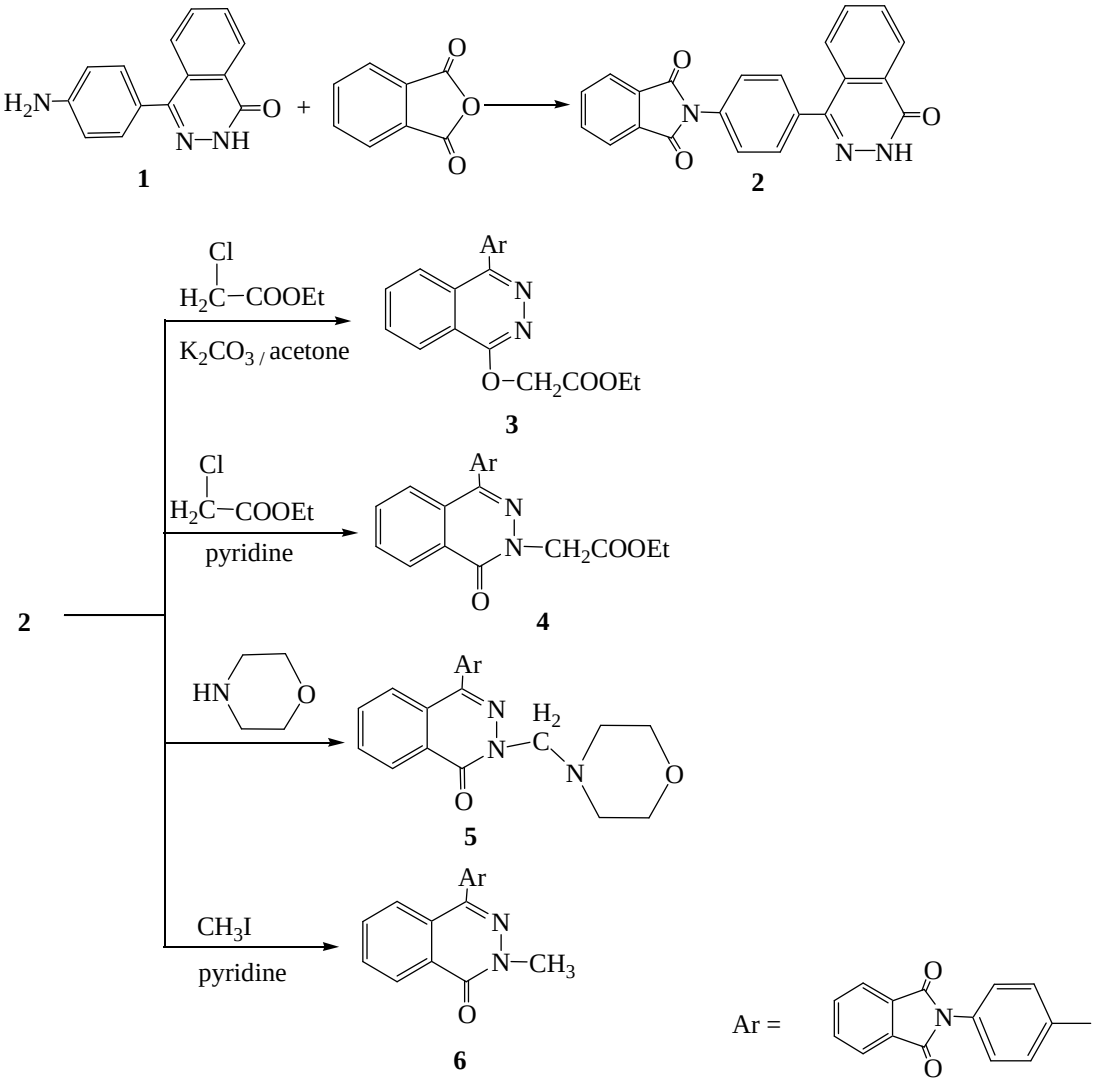
*2-[4-(3-Methyl-4-oxo-3,4-dihydro-phthalazin-1-yl)-phenyl]-isoindole-1,3-dione (**6**)*
A compound of 3 (4.53 gm, 10 mmol) and (2.82 gm, 20 mmol) methyl iodide in pyridine (3 mL) was heated on a steam bath for 3 h. The reaction mixture poured into cold H_2O/Hcl. The solid that separated was recrystallized from ethanol to give yellow crystals. M.p. 242-244 °C, Yield 39 %; IR (cm^{-1}): 2921 (C-H alip.), 1744-1707 (CO), 1599 (C=N); ^{1}H NMR ($CDCl_3$) δ: 2.75 (s, 2H, -N-CH_3), 7.61-8.13 (m, 12H, Ar-H); Ms: m/z == 381 ($M^{\cdot+}$); Anal. Calcd. For $C_{23}H_{15}N_3O_3$ (381): C, 72.43; H, 3.96; N, 11.02; Found: C, 72.33; H, 3.92; N, 11.42 %.

*General procedure for preparation of (**7a,b**)*
A mixture of 3 (4.53 gm, 10 mmol) and hydrazine hydrate or benzyl amine (0.5 gm, 10 mmol) in ethanol (50 mL) was heated under reflux for 3h. The solid that separated after concentration and cooling was recrystallized from proper solvent to give 7a,b.

*An alternative method for synthesis of **7a***
Firstly; synthesis of acid chloride 10 as shown in method (3.1.10) then take acid chloride 10 (4.43 gm, 10 mmol) and hydrazine hydrate (0.5 gm, 10 mmol) in dry benzene were heated on steam bath for 2 h. After evaporation of the dry benzene, the hydrazide 7a was formed as pale yellow crystals.

*{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxy}-acetic acid hydrazide (**7a**)*
M.p. 180 -182°C; Yield 80%; IR (cm^{-1}): 3421-3016 (NH, NH_2), 2900 (C-H alip.), 1730-1690 (2CO), 1659 (CO) carboxamide, 1562 (C=N), 1076 (C-O-C); Ms: m/z == 439 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 1.22 (t, 2H, CH_3), 4.10 (q, 3H, CH_2), 8.01 (s, 1H, NH,), 7.69-8.13 (m, 12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 179.01, 170.31, 163.21, 144.01, 138.21, 133.11, 132.30, 132.11, 132.01, 128.00, 127.41, 127.20, 126.00, 121.11, 120.91, 119.12, 78.41;Anal.Calcd. For $C_{24}H_{17}N_5O_4$ (439): C, 65.60; H, 3.90; N, 15.94, Found: C, 65.72; H, 3.85; N, 15.89%.

*N-Benzyl-2-{4-[4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-phenyl] phthalazin-1-yloxy}-acetamide (**7b**)*
Pale yellow crystals; M.p. 240-242°C; Yield 60%; IR (cm^{-1}): 3421 (NH_2), 2930 (C-H alip.), 1730-1690 (CO), 1659 (CO) carboxamide, 1599 (C=N), 1076 (C-O-C); ^{1}H NMR ($CDCl_3$) δ: 4.46 (s, 2H, O-CH_2), 4.46 (s, 2H, N-CH_2), 7.06-8.13 (m, 12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 179.01, 171.21, 163.21, 144.01, 142.41, 138.21, 133.00, 132.31, 132.11, 132.01, 128.31, 128.00, 127.40, 127.20, 127.10, 126.50, 126.00, 121.00, 120.9, 119.00, 76.70, 48.60; Ms: m/z == 514 ($M^{\cdot+}$); Anal.Calcd. For $C_{31}H_{22}N_4O_4$ (514): C, 72.36; H, 4.31; N, 10.89, Found: C, 72.39; H, 4.21; N, 10.95%.

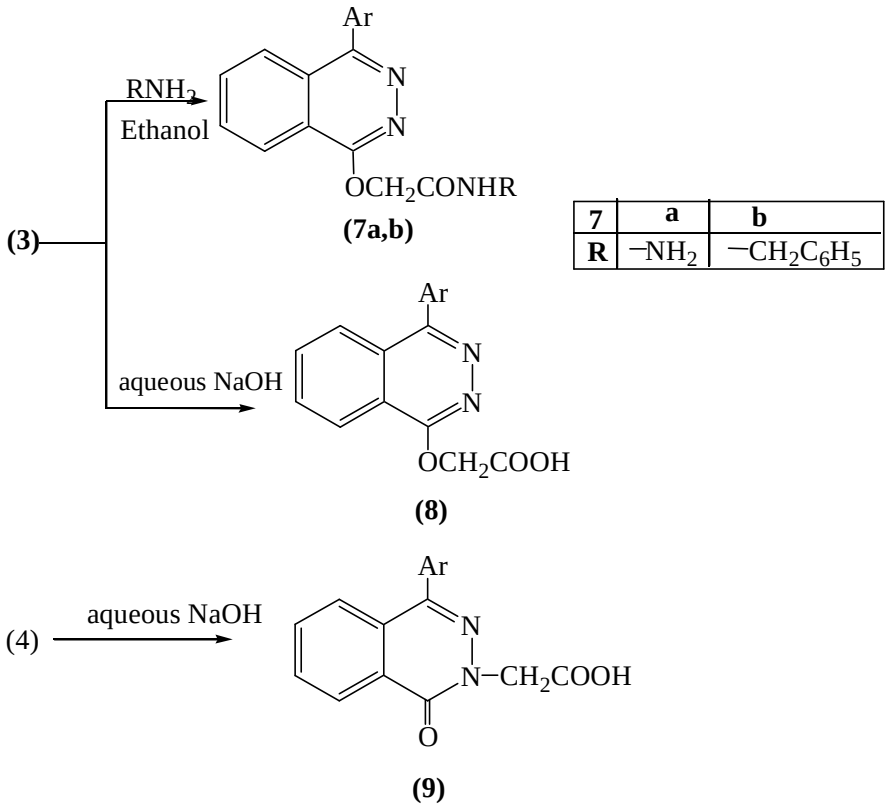


Scheme 1: Reaction of phthalazinone derivative 2 with some reagents

*{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxy}-acetic acid (**8**)*
The ester 3 was refluxed with 10% aqueous sodium hydroxide solution (10mL per 1gm of ester) for 3h. The alkaline solution was acidified with HCl and extracted with ether. Evaporation of ether gave 8 which was recrystallized from ethanol to give yellow crystals.

*An alternative method for synthesis of **8***
A solution of the ester 3 (3.67 gm, 10 mmol), chloro acetic acid (0.94 gm, 10 mmol), (20 mL) ethanol and (15 mL) of 10% sodium Hydroxide was added were refluxed fo 3h on steam bath. Then cooled, filtered, the filtrate was acidified by cold H_2O/dil HCl. The precipitate that formed was washed with water, dried and recrystallized from ethanol to give yellow crystals 8. M.p. 204-206 °C; Yield 50 %; IR (cm^{-1}): 3163 (OH), 1725-1658 (2CO), 1705 (CO) due to carboxylic group, 1594 (C=N); Ms: m/z = 425 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 2.49 (s, 2H, OCH_2), 7. 06-8.19 (m, 12H, Ar-H), 10.29 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ: 179.01, 176.31, 163.21, 144.01, 138.21, 133.00, 132.30, 132.11, 132.01, 128.00, 127.41, 127.20, 126.00, 121.00, 120.91, 119.00, 78.21; Anal. Calcd.For $C_{24}H_{15}N_3O_5$ (425): C, 67.76; H, 3.55; N, 9.88; Found: C, 67.86; H, 3.45; N, 9.98%.

*{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]-1-oxo-1H-phthalazin-2-yl}-acetic acid (**9**)*
The ester 4 was refluxed with 10% aqueous sodium hydroxide solution (10mL per 1gm of ester) for 3h. The alkaline solution was acidified with HCl and extracted with ether. Evaporation of ether gave 9 which was recrystallized from ethanol to give yellow crystals. M.p. 280-282 °C; Yield 60 %; IR (cm^{-1}): 3163 (OH), 1715-1698 (CO), 1710 (CO) due to carboxylic group, 1594 (C=N); Ms: m/z == 427 ($M^{\cdot+}$+2); ^{1}H NMR (DMSO-d_6) δ: 2.51 (s, 2H, CH_2)), 7.54-8.14 (m, 12H, Ar-H), 12.95 (s, 1H, OH; ^{13}C NMR (DMSO-d_6) δ: 179.01, 176.31, 163.21, 144.01, 138.21, 133.00, 132.30, 132.11, 132.01, 128.00, 127.41, 127.20, 126.00, 121.00, 120.91, 119.00, 78.21; Anal. Calcd.for $C_{24}H_{15}N_3O_5$ (425): C, 67.76; H, 3.55; N, 9.88; Found: C, 67.86; H, 3.45; N, 9.98%.



7	a	b
R	−NH_2	−$CH_2C_6H_5$

Scheme 2: Reaction of Ester 3 with primary Ar. Amines and Hydrolysis of 3 and 4

*{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxy}-acetyl chloride (**10**)*
A mixture of the acid 8 (4.25 gm, 10 mmol) and PCl_5 (3.3 gm, 15 mmol) was grinding together in a mortar for five minutes. The acid chloride 10 was formed as yellow paste which can be taken up in dry pyridine (25 mL) and used as it is for the proceeding reaction:

*An alternative method for synthesis of acid chloride **10***
A solution of the acid 8 (4.25 gm, 10 mmol) and $SOCl_2$ (10 mL) was heated under reflux for 3h. After evaporation of the thionyl chloride, the acid chloride 10 was formed as yellow paste which can be taken up in dry pyridine (25 mL) and used as it is for the proceeding reaction:

*2-(2-{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthala- zin-1-yloxy}acetylamino)benzoic acid (**11**)*
To a solution of anthranilic acid (1.37 gm, 10 mmol) in pyridine (30 mL), the acid chloride 10 (8.87 gm, 20 mmol) was added. The mixture was shaken for five minutes and set aside at room temperature for an hour with occasional shaking. The reaction mixture was poured into cold water and the precipitate was filtered off. The residue was washed from pyridine with cold water and then crystallized from ethanol to give pale yellow crystals 11. M.p. 180-182 °C; Yield 60 %; IR (cm^{-1}): 2934 (C-H alip.),1705-1657 (2CO), 1594 (C=N); Ms: m/z == 544 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 2.45 (s, 2H, OCH_2), 7.99 (s, 1H, NH), 6.50-8.19 (m, 16H, Ar-H), 10.12 (s, 1H, OH); Anal. Calcd.for $C_{31}H_{20}N_4O_6$ (544): C, 68.37; H, 3.70; N, 10.29; Found: C, 68.42; H, 3.82; N, 10.39%.

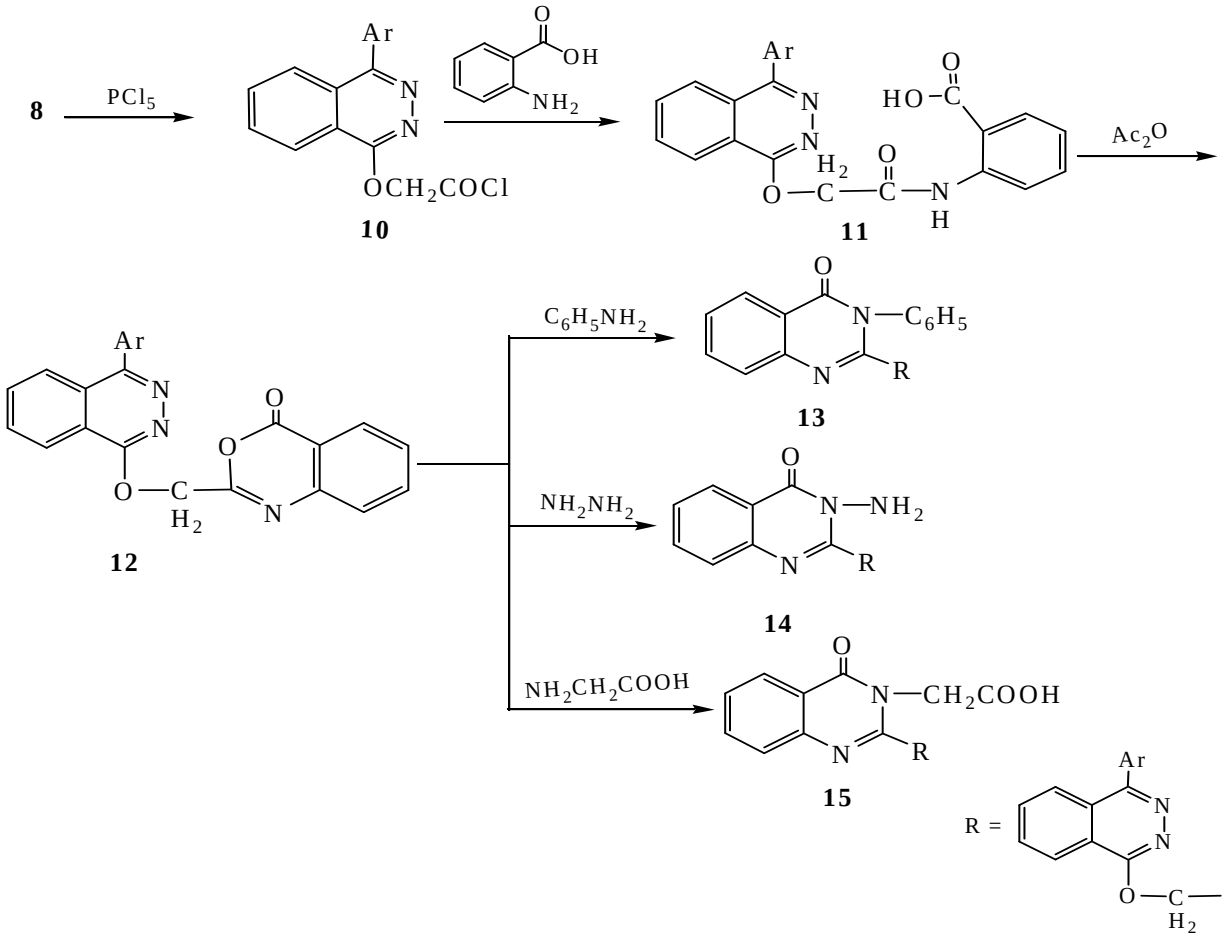
*2-{4-[4-(4-Oxo-4H-benzo[d][1,3]oxazin-2-ylmethoxy)-phthalazin -1-yl]-phenyl}-isoindole-1,3-dione (**12**)*
A mixture of 11 (5.44 gm, 10 mmol) and Ac_2O (2 mL for 1 gm of 7) was heated on a steam bath for 3 h. After cooling, the reaction mixture was poured onto crushed ice. The solid that separated was filtered off, washed well for several times with water, dried and crystallized from methanol to give brown crystals 12. M.p. 158-160 °C; Yield 65 %; IR (cm^{-1}): 3407-3026 (OH, NH), 1705-1657 (2CO); Ms: m/z == 526 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 4.01 (s, 2H, CH_2),7.46-8.13 (m, 16H, Ar-H);^{13}C NMR (DMSO-d_6) δ: 179.01, 172.00, 164.21, 163.20, 150.61, 144.00, 138.21, 135.00, 133.12, 132.32, 132.11, 132.00, 131.31, 128.00, 127.43, 127.20, 127.11, 126.90, 126.00, 124.11, 121.91, 121.01, 120.91, 74.72; Anal. Calcd.for $C_{31}H_{18}N_4O_5$ (526): C, 70.72; H, 3.45; N, 10.64; Found: C, 70.62; H, 3.55; N, 10.74%.

*2-{4-[4-(4-Oxo-3-phenyl-3,4-dihydro-quinazolin-2-ylmethoxy)-phthalazin-1-yl]-phenyl}-isoindole-1,3-dione (**13**)*
A solution of 12 (5.26 gm, 10 mmol) and aniline (0.93 gm, 10 mmol) in ethanol (50 mL) was heated under reflux for 3 h. The solid that separated after concentration and cooling was recrystallized from ethanol to give yellow crystals

13. M.p. 205-207 °C; Yield 73 %; IR (cm^{-1}):1657-1705 (2CO), 1703 (CO) amide, 1598 (C=N); Ms: m/z == 601 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 4.01 (s, 2H, CH_2), 6.50-8.13 (m, 16H, Ar-H); Anal. Calcd.for $C_{37}H_{23}N_5O_4$ (601): C, 73.87; H, 3.85; N, 11.64; Found: C, 73.92; H, 3.75; N, 11.64%.

*2-{4-[4-(3-Amino-4-oxo-3,4-dihydro-quinazolin-2-ylmethoxy)phthalazin-1-yl]-phenyl}-isoindole-1,3-dione (**14**)*
A mixture of 12 (5.26 gm, 10 mmol) and hydrazine hydrate (0.5 gm, 10 mmol) in ethanol (50 mL) was heated under reflux for 6h. The solid that separated after concentration and cooling was recrystallized from ethanol to give pale yellow crystals 14. M.p. 212-214 °C; Yield 78 %; IR (cm^{-1}): 3427 (NH_2), 2920 (C-H alip.),1745-1657 (CO), 1074 (C-O-C), 1560 (C=N); Ms: m/z == 542 (M^{+}+2); ^{1}H NMR (DMSO-d_6) δ: 4.01 (s, 2H, CH_2), 5.01 (s, 2H, NH_2 exchangeable), 6.50-8.19 (m,12H, Ar-H); Anal. Calcd.for $C_{31}H_{20}N_6O_4$ (540): C, 68.88; H, 3.73; N, 15.55; Found: C, 68.91; H, 3.83; N, 15.45%.

*(2-{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxymethyl}-4-oxo-4H-quinazolin-3-yl)-acetic acid (**15**)*
A mixture of 12 (5.26 gm, 10 mmol) and glycine (0.75 gm, 10 mmol) in a solution of equal volume of pyridine and water (50 mL) was heated under reflux for 6h. The reaction mixture poured into ice/dil HCl. The solid that separated filtered off and recrystallized from acetic acid to give yellow crystals 15. M.p. 262-264 °C; Yield 75 %; IR (cm^{-1}): 3404 (OH), 1708- 1630 (2CO); Ms: m/z == 583 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 4.01 (s, 2H, CH_2), 6.50-8.19 (m, 16H, Ar-H), 10.21 (s, 1H, OH exchangeable); Anal. Calcd.for $C_{33}H_{21}N_5O_6$ (583): C, 67.92; H, 3.63; N, 12.00; Found: C, 67.99; H, 3.73; N, 12.22%.



Scheme 3: Reaction of Benzoxazinone with Aniline, Hydrazine hydrate and Glycine

*General procedure for preparation of (**16a,b**)*
An equimolar amounts of 7a (4.39 gm, 10 mmol) and aromatic aldehydes such as benzaldehyde and p-chlorobenzaldehyde in ethanol (30ml) was heated under reflux for 3h. The solid that separated after concentration and cooling was recrystallized from dimethylformamide /water to give 16a,b respectively.

*{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxy}-acetic acid benzylidene-hydrazide (**16a**)*
Pale yellow crystals; M.p. 220-222 °C; Yield 65 %; IR (cm^{-1}): 3163-3016 (NH) , 1705-1687 (2CO), 1659 (CONH), 1605 (C=N); Ms: m/z == 527 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 4.81 (s, 2H, O-CH_2), 6.38-8.13 (m,12H, Ar-H) , 7.99

(s,1H, NH), 9.01 (s, 1H, CH); ^{13}C NMR (DMSO-d_6) δ: 179.00, 173.11, 163.21, 154.71, 144.00, 138.20, 133.00, 132.31, 132.12, 131.22, 132.00, 130.80, 129.00, 128.61, 128.00, 127.41, 127.21, 126.11, 121.00, 120.9, 119.00, 78.7; Anal. Calcd.For $C_{31}H_{21}N_5O_4$ (527): C, 70.58; H, 4.01; N, 13.28; Found: C, 70.68; H, 3.96; N, 13.38%.

{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxy} -acetic acid (7-chloro-hepta- 2,4,6-triynylidene)-hydrazide
(16b)
Yellow crystals; M.p. 163-165 °C; Yield 75 %; IR (cm^{-1}): 3162-3016 (NH), 1730-1659 (CO), 1599 (C=N), 708 (C-Cl); Ms: m/z == 561 ($M^{\cdot+}$), 562 ($M^{\cdot+}$+1); ^{1}H NMR (DMSO-d_6) δ: 4.83 (s, 2H, O-CH_2), 7.00 (s, 1H, NH), 7.46-8.13 (m,12H, Ar-H) , 7.99 (s,1H, NH), 9.01 (s, 1H, CH); Anal. Calcd.for $C_{31}H_{20}ClN_5O_4$ (561): C, 66.25; H, 3.59; N, 12.46; Found: C, 66.38; H, 3.63; N, 12.48%.

General procedure for preparation of ***(17a-c)***
A mixture of 7a (4.39 gm, 10 mmol) and aromatic acids namely benzoic acid, p-hydroxybenzoic acid and p-nitrobenzoic acid in $POCl_3$ (30 mL) was refluxed on steam bath for 5 h. 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 the proper solvent to give 17-c respectively.

2-{4-[4-(5-Phenyl-[1,3,4]oxadiazol-2-ylmethoxy)phthalazin-1-yl]-phenyl}-isoindole-1,3-dione ***(17a)***
Orange crystals; M.p. 185-187 °C; Yield 85 %; IR (cm^{-1}): 1742-1703 (2CO), 1595 (C=N); Ms: m/z == 525 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 4.83 (s, 2H, CH_2), 6.50-8.19 (m, 12H, Ar-H); Anal. Calcd.for $C_{31}H_{19}N_5O_4$ (525): C, 70.85; H, 3.64; N, 13.33; Found: C, 70.96; H, 3.54; N, 13.53%.

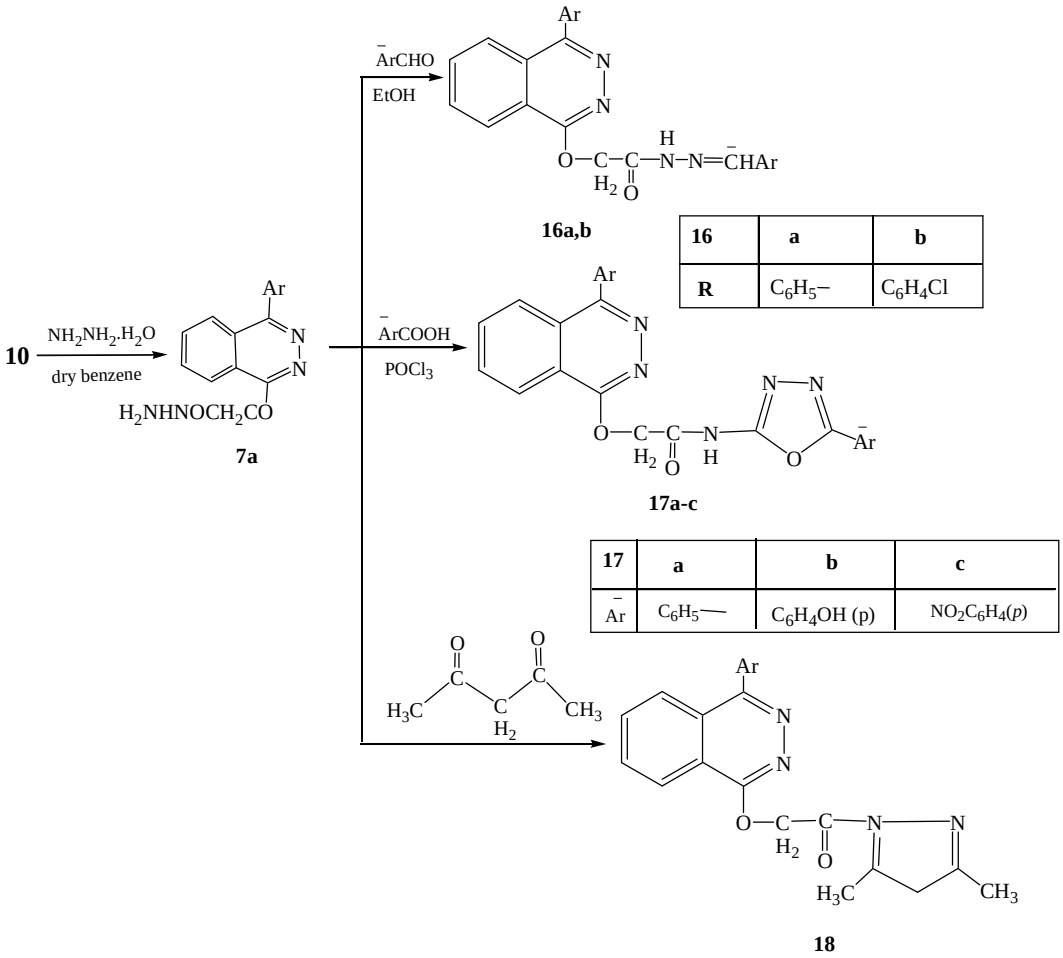
2-(4-{4-[5-(4-Hydroxyphenyl)-[1,3,4]oxadiazol-2-ylmethoxy] phthalazin-1-yl}-phenyl)-isoindole-1,3-dione ***(17b)***
Brown crystals; M.p. 220-222 °C; Yield 85 %; IR (cm^{-1}): 3350 (OH), 1735-1698 (2CO), 1595 (C=N); Ms: m/z == 541 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 2.51 (s, 1H, OH exchangeable), 3.82 (s, 2H, CH_2), 7.50-8.13 (m, 12H, Ar-H); Anal. Calcd.for $C_{31}H_{19}N_5O_6$ (541): C, 68.76; H, 3.54; N, 12.93; Found: C, 68.83; H, 3.60; N, 12.98%.

2-(4-{4-[5-(4-Nitro-phenyl)-[1,3,4]oxadiazol-2-ylmethoxy] phthalazin-1-yl}-phenyl)-isoindole-1,3-dione ***(17c)***
Yellow crystals; M.p. 168-170 °C; Yield 85 %; IR (cm^{-1}): 1735-1698 (2CO), 1606 (C=N), 1496 (NO_2); Ms: m/z == 570 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 5.20 (s, 2H, -O-CH_2), 7.46-8.13 (m, 12H, Ar-H); Anal. Calcd.for $C_{31}H_{18}N_6O_6$ (570): C, 65.26; H, 3.18; N, 14.73; Found: C, 65.36; H, 3.06; N, 14.83%.

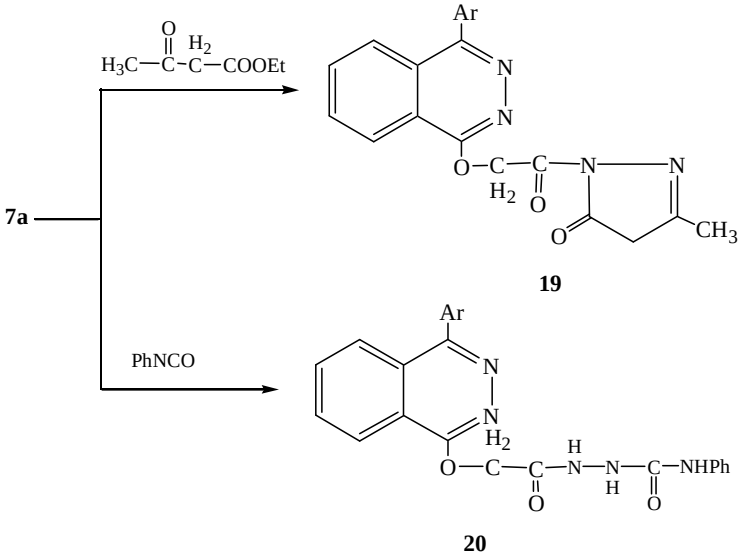
2-(4-{4-[2-(3-Methyl-5-oxo-4,5-dihydro-pyrazol-1-yl)-2-oxo-ethoxy]phthalazin-1-yl}-phenyl)-isoindole-1,3-dione ***(18)***
A solution of 7a (4.39 gm, 10 mmol) and ethylacetoacetate (1.3 gm, 10 mmol) was heated under reflux in ethanol (30 mL) for 5h. The solid that separated after concentration and cooling was recrystallized from ethanol to give yellow crystals 19. M.p. 195-197 °C; Yield 75 %; IR (cm^{-1}): 1740-1658 (CO), 1604 (C=N), 1074 (C-O-C); Ms: m/z == 505 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 1.2 (s, 3H, CH_3), 5.82-4.83 (s, 4H, 2CH_2), 7.19-8.13 (m,12H, Ar-H); Anal. Calcd.for $C_{28}H_{19}N_5O_5$ (505): C, 66.53; H, 3.79; N, 13.85; Found: C, 66.63; H, 3.89; N, 13.75%.

2-(4-{4-[2-(3,5-Dimethyl-pyrazol-1-yl)-2-oxo-ethoxy]-phthalazin-1-yl}-phenyl)-isoindole-1,3-dione ***(19)***
A compound 7a (4.39 gm, 10 mmol) and acetylacetone (1 gm, 10 mmol) were refluxed in ethanol (30 mL) for 5h. The solid that separated after concentration and cooling was recrystallized from ethanol to give pale yellow crystals 18. M.p. 180-182 °C; Yield 85 %; IR (cm^{-1}): 2897 (C-H aliphatic), 1709-1658 (CO), 1604 (C=N); Ms: m/z == 504 ($M^{\cdot+}$+1); ^{1}H NMR (DMSO-d_6) δ: 3.42 (s, 6H, 2CH_3), 5.05 (s, 2H, CH_2), 5.82 (s, 1H, CH), 7.45-8.13 (m,12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 200.00, 179.11, 163.21, 147.10, 144.01, 138.21, 133.00, 132.31, 132.10, 132.00, 128.00, 127.40, 127.21, 126.00, 121.00, 120.91, 119.00, 106.11, 72.31, 13.80, 7.11 Anal. Calcd.for $C_{29}H_{21}N_5O_4$ (503): C, 69.18; H, 4.20; N, 13.91; Found: C, 69.28; H, 4.23; N, 13.85%.

2-{2-[4-(4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl) phthalazin-1-yloxy] acetyl}-N-phenyl hydrazine carbothioamide (***20)***
A mixture of 7a (4.39 gm, 10 mmol) phenylisocyanate (0.17 gm, 10 mmol) in ethanol (30 mL) was heated under reflux for 4 h. The solid that separated after concentration and cooling was recrystallized from ethanol to give yellow crystals 20. M.p. 240-242 °C; Yield 75 %; IR (cm^{-1}): 3162 (NH), 1705-1659 (CO), 1604 (C=N); Ms: m/z == 558($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 4.83 (s, 2H, CH_2), 7.46-8.13 (m, 12H, Ar-H), 8.01-7.93 (s, 3H, 3NH); Anal. Calcd.for $C_{31}H_{22}N_6O_5$ (558): C, 66.66; H, 3.97; N, 15.05; Found: C, 66.75; H, 3.90; N, 15.15 %.



Scheme 4: Reaction of hydrazide 7 with Ar. Aldehyde, Benzoic acid and Acetylacetone



Scheme 5: Reaction of 7 with ethylacetoacetate and ethylisocyanate

{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxy}-acetic acid (2,3,4,5,6-pentahydroxy-hexylidene)-hydrazide ***(21 a,b)***

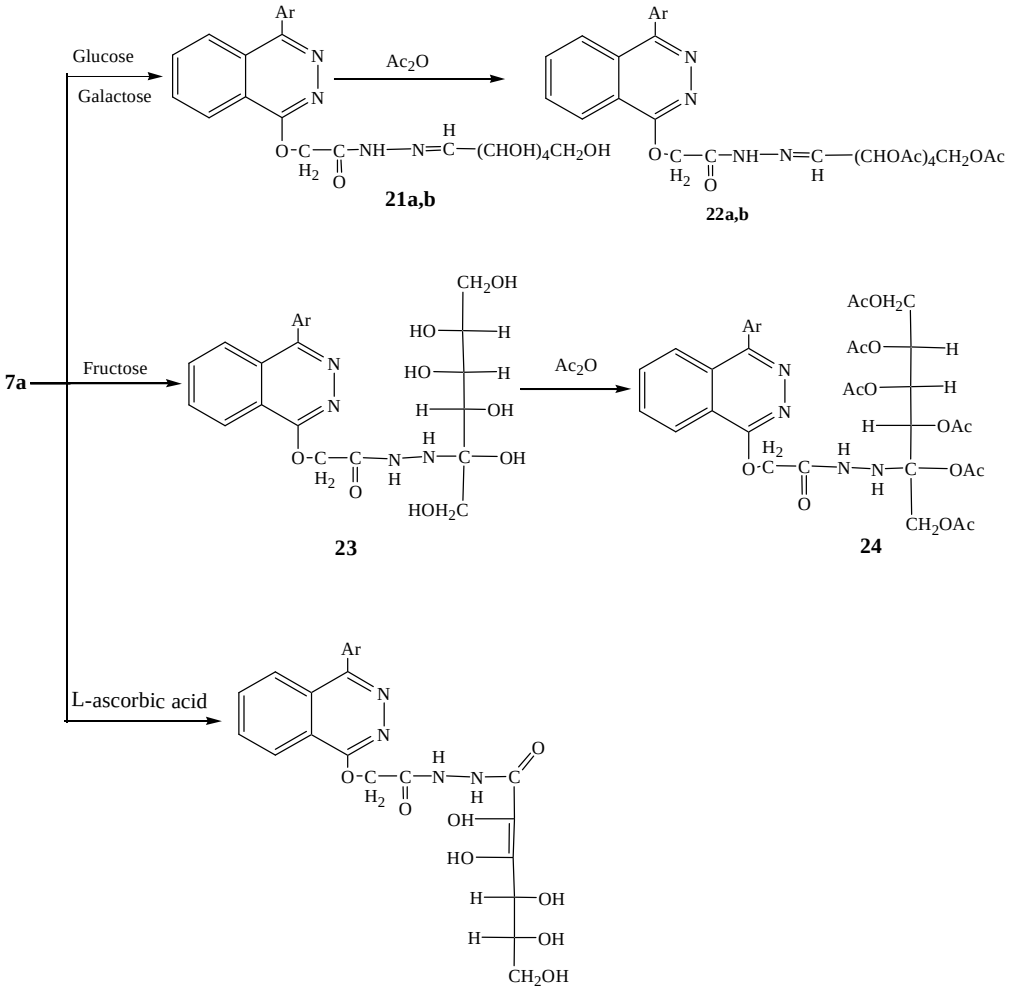
A compound of 7a (4.39 gm, 10 mmol) and carbohydrates such as glucose and galactose (1.8 gm, 10 mmol) in absolute ethanol (30ml) was heated under reflux for 3 h. The mixture was dried and crystallized from ethanol to afford 21a,b. The compound was obtained as brown crystals. M.p. 185-187 °C; Yield 70 %; IR (cm^{-1}): 3415-3018 (OH, NH) , 1712-1658 (CO), 1604 (C=N); Ms: m/z == 601 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 3.37 (s,5H, 5CH), 3.86 (s,2H, CH_2), 5.67 (s, 5H, 5OH), 7.46-8.13 (m,12H, Ar-H); Anal. Calcd.for $C_{30}H_{27}N_5O_9$ (601): C, 59.90; H, 4.52; N, 11.64; Found: C, 60.10; H, 4.42; N, 11.74%.

*Acetic acid 2,3,4,5-tetraacetoxy-6-[(2-{4-[4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-phenyl] phthalazin-1-yloxy}-acetyl)-hydrazono]-hexyl ester (**22a,b**)*

A mixture of compounds 21a,b and acetic anhydride (5 mL) were refluxed for 3 h. Then, the reaction mixture was poured onto cold water. The precipitate was filtered off and recrystallized from ethanol to afford 22a,b. The compound was obtained as yellow crystals. M.p. 172-175 °C; Yield 75 %; IR (cm^{-1}): 3458-3058 (NH, aromatic ring), 1734-1698 (CO), 1597 (C=N); Ms: m/z == 811 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 4.01 (s, 2H, NH_2), 6.50-8.19 (m,8H, Ar-H),7.99 (s,1H, NH); Anal. Calcd.for $C_{40}H_{37}N_5O_{14}$ (811): C, 59.18; H, 4.59; N, 8.63; Found: C, 59.28; H, 4.49; N, 8.73%.

*{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxy}-acetic acid N'-(1,2,3,4,5-pentahydroxy-1-hydroxymethyl-pentyl)-hydrazide (**23**)*

A mixture of 7a (4.39 gm, 10 mmol) and fructose (1.76 gm, 10 mmol) in absolute ethanol (30 mL) was heated under reflux for 3h. The mixture was dried and crystallized from ethanol to afford 23. The compound was obtained as brown crystals. M.p. 159-161 °C; Yield 70 %; IR (cm^{-1}): 3462-3016 (OH, NH), 1705-1659 (CO), 1602 (C=N); Ms: m/z == 621 ($M^{\cdot+}$+2); ^{1}H NMR (DMSO-d_6) δ: 2.01 (s, 1H, -OH, -NH), 4.83 (s, 2H, -O-CH_2), 8.00 (s, 1H, -N-), 6.50-8.13 (m,8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 179.11, 170.30, 163.21, 144.01, 138.21, 133.00, 132.31, 132.10, 132.00, 128.00, 127.40, 127.21, 126.00, 121.00, 120.91, 119.00, 85.80, 78.70, 77.00, 74.21, 67.41, 67.20, 64.71; Anal. Calcd.for $C_{30}H_{29}N_5O_{10}$ (619): C, 58.16; H, 4.72; N, 11.30; Found: C, 58.26; H, 4.62; N, 11.22%.



Scheme 6: Reaction of 7 with some aldohexoses and ketohexose

*Acetic acid 2,3,4-triacetoxy-1-{1,2-diacetoxy-1-[N'-(2-{4-[4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-phenyl] phthalazin-1-yloxy}-acetyl)-hydrazino]-ethyl}-butyl ester (**24**)*

A mixture of 23 and acetic anhydride (5 mL) were refluxed for 3 h. Then, the reaction mixture was poured onto cold water. The precipitate was filtered off and recrystallized from ethanol to afford 24. The compound was obtained as yellow crystals. M.p. 195-197 °C; Yield 65 %; IR (cm^{-1}): 3460-3064 (NH, aromatic ring), 1734-1698 (CO), 1596 (C=N); Ms: m/z == 871 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 2.01 (s, 3H, -CH_3) , 2.28 (s, 1H, -NH), 4.54 (s, 2H, -CH_2),

8.00 (s, 1H, -N-), 6.50-8.13 (m,8H, Ar-H); Anal. Calcd.for $C_{42}H_{41}N_5O_{16}$ (871): C, 57.86; H, 4.74; N, 8.03; Found: C, 57.67; H, 4.84; N, 8.13%.

*{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]-phthalazin-1-yloxy}-acetic acid (2,3,4,5-tetra hydroxyl-pentylidene)-hydrazide (**25**)*

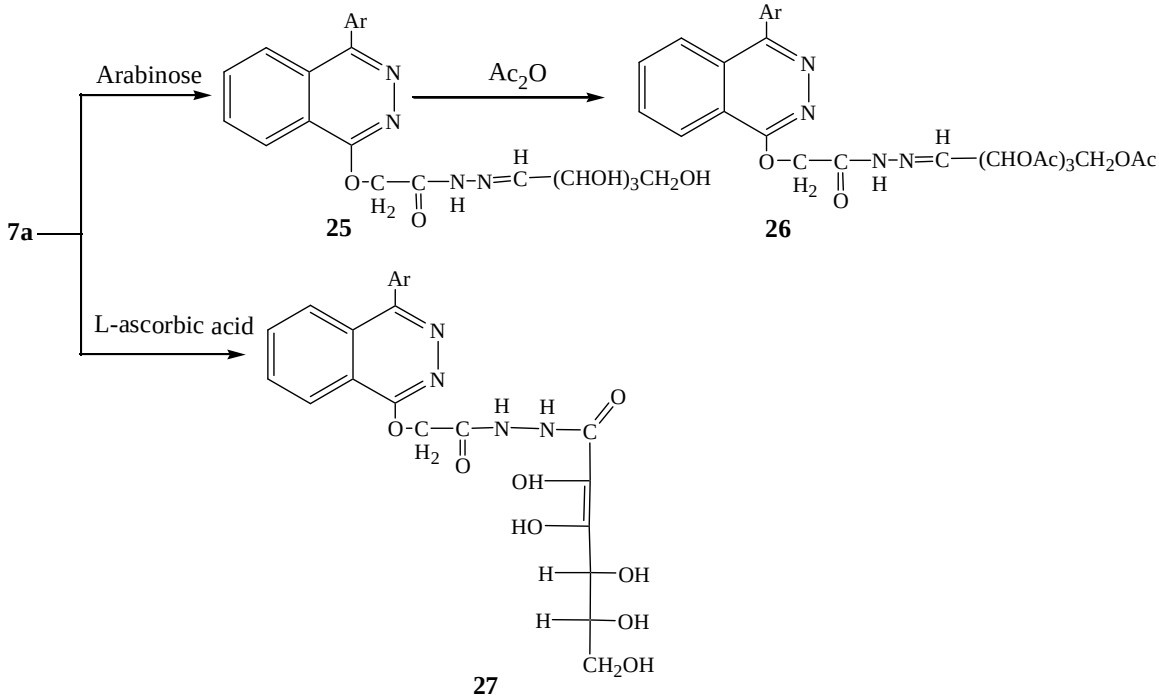
A compound 7a (4.39 gm, 10 mmol) and arabinose (1.50 gm, 10 mmol) in absolute ethanol (30 mL) was heated under reflux for 3 h. The mixture was dried and crystallized from ethanol to afford 25. The compound was obtained as yellow crystals. M.p. 188-190 °C; Yield 56 %; 3462-3016 (NH, OH, aromatic ring), 1715-1698 (CO), 1602 (C=N); Ms: m/z == 571 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 2.01 (s, 1H, -OH), 3.31- 3.42 (s, 1H, -CH), 4.83 (s, 2H, -O-CH_2), 7.00 (s, 1H, -N-), 7.50 (s, 1H, N=CH), 6.50-8.13 (m,8H, Ar-H); Anal. Calcd.for $C_{29}H_{25}N_5O_8$ (571): C, 60.94; H, 4. 41; N, 12.25; Found: 60.99; H, 4. 61; N, 12.13%.

*Acetic acid 2,3,4-triacetoxy-5-[(2-{4-[4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yloxy}-acetyl)-hydrazono]-pentyl ester (**26**)*

A mixture of compound 25 and acetic anhydride (5 mL) were refluxed for 3 h. Then, the reaction mixture was poured onto cold water. The precipitate was filtered off and recrystallized from ethanol to afford 26. The compound was obtained as yellow crystals. M.p. 146-148 °C; Yield 65 %; IR (cm^{-1}): 3460-3064 (NH, aromatic ring), 1734-1699 (CO), 1596 (C=N) Ms: m/z == 739 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 2.01 (s, 3H, -CH_3), 5.14 (s, 1H, CH), 4.83(s, 1H, -OCH_2),7.00 (s, 1H, -N-), 7.50 (s, 1H. N=CH), 6.50-8.13 (m,8H, Ar-H); Anal. Calcd.for $C_{37}H_{33}N_5O_{12}$ (739): C, 60.08; H, 4.50; N, 9.47; Found: C, 60.18; H, 4.49; N, 9.37%.

*2,3,4,5,6-Pentahydroxy-hex-2-enoic acid N'-(2-{4-[4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]-phthalazin-1-yloxy}-acetyl)-hydrazide (**27**)*

A compound of 7a (4.39 gm, 10 mmol) and L-ascorbic acid (1.76 gm, 10 mmol) in absolute ethanol (30 mL) was heated under reflux for 3 h. The mixture was dried and crystallized from ethanol to afford 27. The compound was obtained as brown crystals. M.p. 185-187 °C; Yield 70 %; IR (cm^{-1}): 3462-3016 (OH, NH), 1715-1656 (CO), 1602 (C=N); Ms: m/z == 616 ($M^{\cdot+}$+1); ^{1}H NMR (DMSO-d_6) δ: 2.01 (s, 1H, -OH), 3.31- 3.42 (s, 1H, -CH), 4.83 (s, 2H, -O-CH_2), 7.00 (s, 1H, -N-), 7.50 (s, 1H, N=CH), 6.50-8.13 (m,8H, Ar-H); Anal. Calcd.for $C_{30}H_{25}N_5O_{10}$ (615): C, 58.54; H, 4.09; N, 11.38; Found: C, 58.64; H, 4.29; N, 11.18%.



Scheme 7: Reaction of 7 with aldopentoses and L-ascorbic acid

RESULTS AND DISCUSSION

Pharmacology

Cell Culture

Human breast adenocarcinoma cell line (MCF-7) which was purchased from ATCC, USA, were used to evaluate the cytotoxic effect of the tested extracts. Cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's

Medium), which was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 100 units/ml streptomycin sulphate, and 250 ng/ml amphotericin B. Cells were maintained at sub-confluency at 37ºC in humidified air containing 5% CO_2. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested extracts were dissolved in dimethyl sulphoxide (DMSO), and then diluted thousand times in the assay to begin with the mentioned concentration. All cell culture material was obtained from Cambrex BioScience (Copenhagen, Denmark). All chemicals were from Sigma/Aldrich, USA, except mentioned. All experiments were repeated three times, unless mentioned.

Anti-tumor activity

Cytotoxicity of tested drug was measured against MCF-7 cells using the MTT Cell Viability Assay. MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals which is largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm.

Reagents preparation:

MTT solution: 5mg/ml of MTT in 0.9%NaCl.
Acidified isoprpanol: 0.04 N HCl in absolute isopropanol.

Procedure

Cells (0.5X105 cells/ well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20 µl of different concentrations of the tested extracts for 48 h at 37º C, in a humidified 5% CO_2 atmosphere. After incubation, media were removed and 40 µl MTT solution / well were added and Incubated for an additional 4 h. MTT crystals were solubilized by adding 180 µl of acidified isopropanol / well and plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability [29].

Calculation

Percentage of relative viability was calculated using the following equation:

[Absorbance of treated cells/ Absorbance of control cells)] X 100

Then the half maximal inhibitory concentration (IC_{50}) was calculated from the equation of the dose response curve.

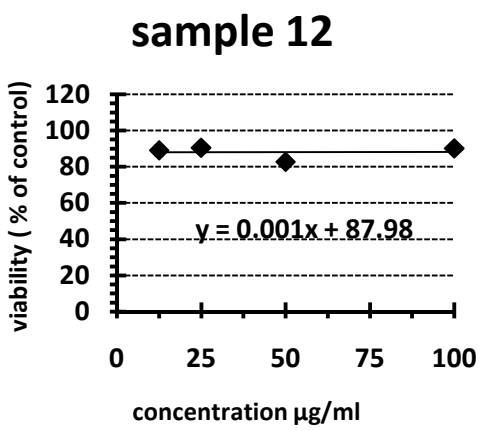
Results

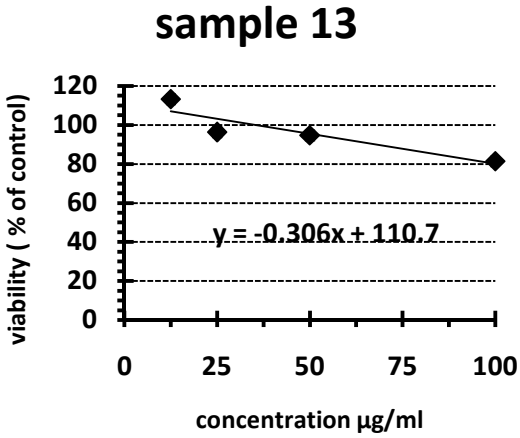
Anti-tumor activity

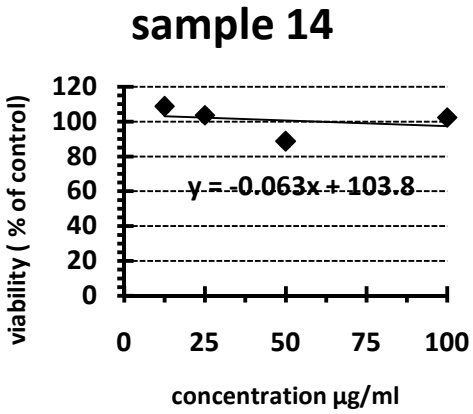
Using MTT assay, the effect of the samples on the proliferation of MCF-7 cells was studied after 48 h of incubation. The effect of the samples was variable.

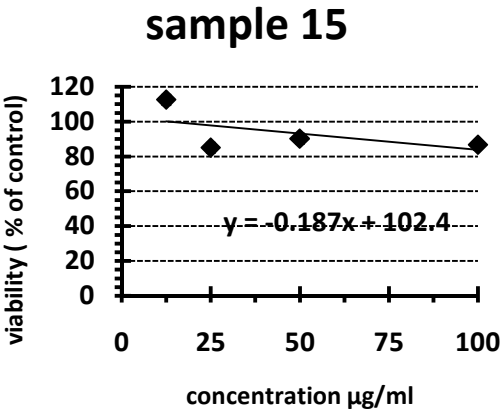
Samples **16b** and **18** showed strong cytotoxic effect against MCF-7, as concluded from their IC_{50} values 50, 70 µg/ml respectively.Samples **13**, **15** and **17** showed moderate cytotoxic effect against MCF-7, as concluded from their IC_{50} values 150, 180, 100 µg/ml respectively, while samples **19** and **22a** showed weak cytotoxic effect concluded from their IC50 values 255.1 and 287 µg/ml respectively, on the other hand, samples **14**, **16a** and **24** showed very weak cytotoxic effect concluded from their very high IC_{50} values 853.9, 1399.4 and 1716.9 µg/ml respectively. Controversially treatment with samples **1**, **7a**, **21a**, **23** and **25** did not show cytotoxic effect as all of them increased proliferation of the cell as shown in figure 2 and figure 3.

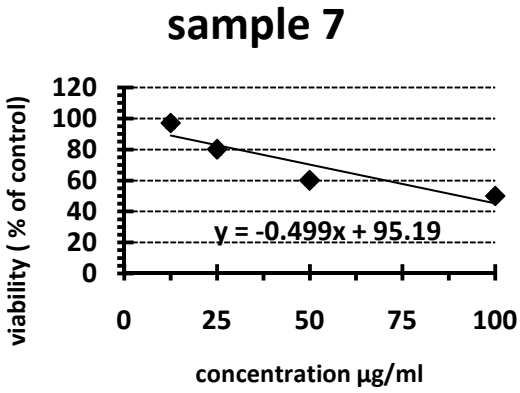
In figure 2, calculated IC_{50} was plotted to compare the efficacy of the active samples to each other.

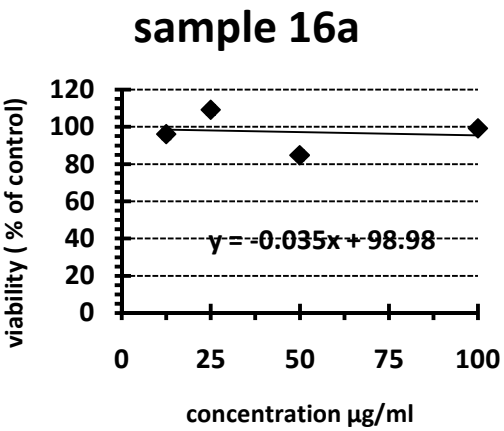

sample 12
viability (% of control)
120
100
80
60
40
20
0
y = 0.001x + 87.98
0
25
50
75
100
concentration µg/ml

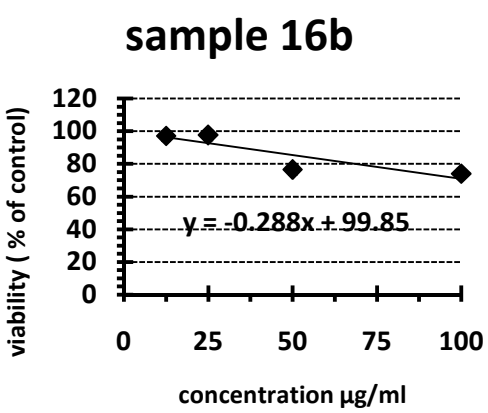

sample 13
viability (% of control)
120
100
80
60
40
20
0
y = -0.306x + 110.7
0
25
50
75
100
concentration µg/ml

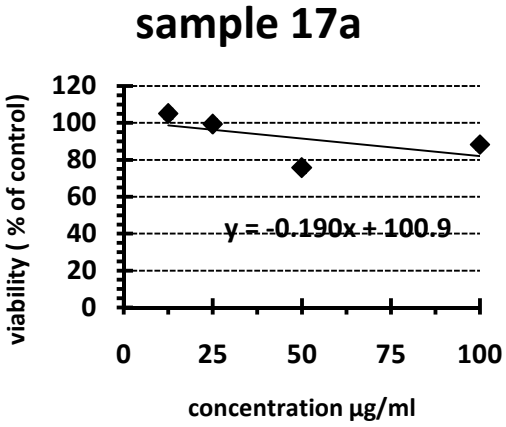

sample 14
viability (% of control)
120
100
80
60
40
20
0
y = -0.063x + 103.8
0
25
50
75
100
concentration µg/ml

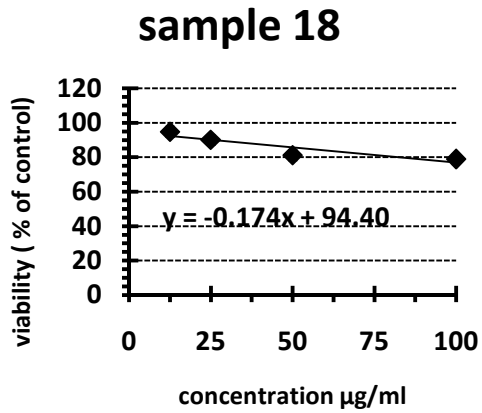

sample 15
viability (% of control)
120
100
80
60
40
20
0
y = -0.187x + 102.4
0
25
50
75
100
concentration µg/ml

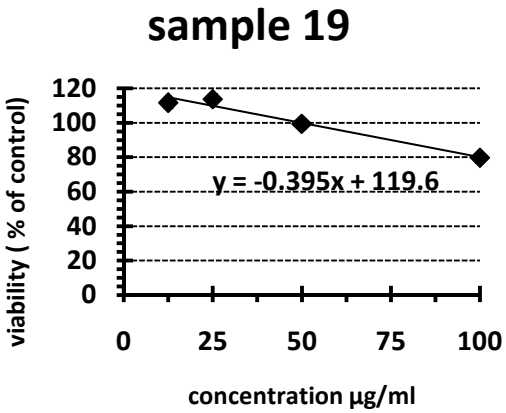

sample 7
viability (% of control)
120
100
80
60
40
20
0
y = -0.499x + 95.19
0
25
50
75
100
concentration µg/ml

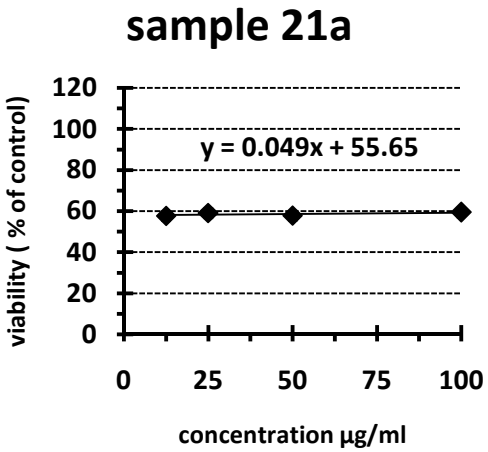

sample 16a
viability (% of control)
120
100
80
60
40
20
0
y = -0.035x + 98.98
0
25
50
75
100
concentration µg/ml

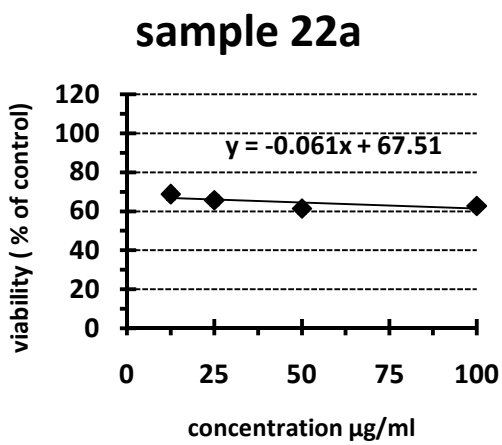

sample 16b
viability (% of control)
120
100
80
60
40
20
0
y = -0.288x + 99.85
0
25
50
75
100
concentration µg/ml


sample 17a
viability (% of control)
120
100
80
60
40
20
0
y = -0.190x + 100.9
0
25
50
75
100
concentration µg/ml


sample 18
viability (% of control)
120
100
80
60
40
20
0
y = -0.174x + 94.40
0
25
50
75
100
concentration µg/ml


sample 19
viability (% of control)
120
100
80
60
40
20
0
y = -0.395x + 119.6
0
25
50
75
100
concentration µg/ml


sample 21a
viability (% of control)
120
100
80
60
40
20
0
y = 0.049x + 55.65
0
25
50
75
100
concentration µg/ml


sample 22a
viability (% of control)
120
100
80
60
40
20
0
y = -0.061x + 67.51
0
25
50
75
100
concentration µg/ml

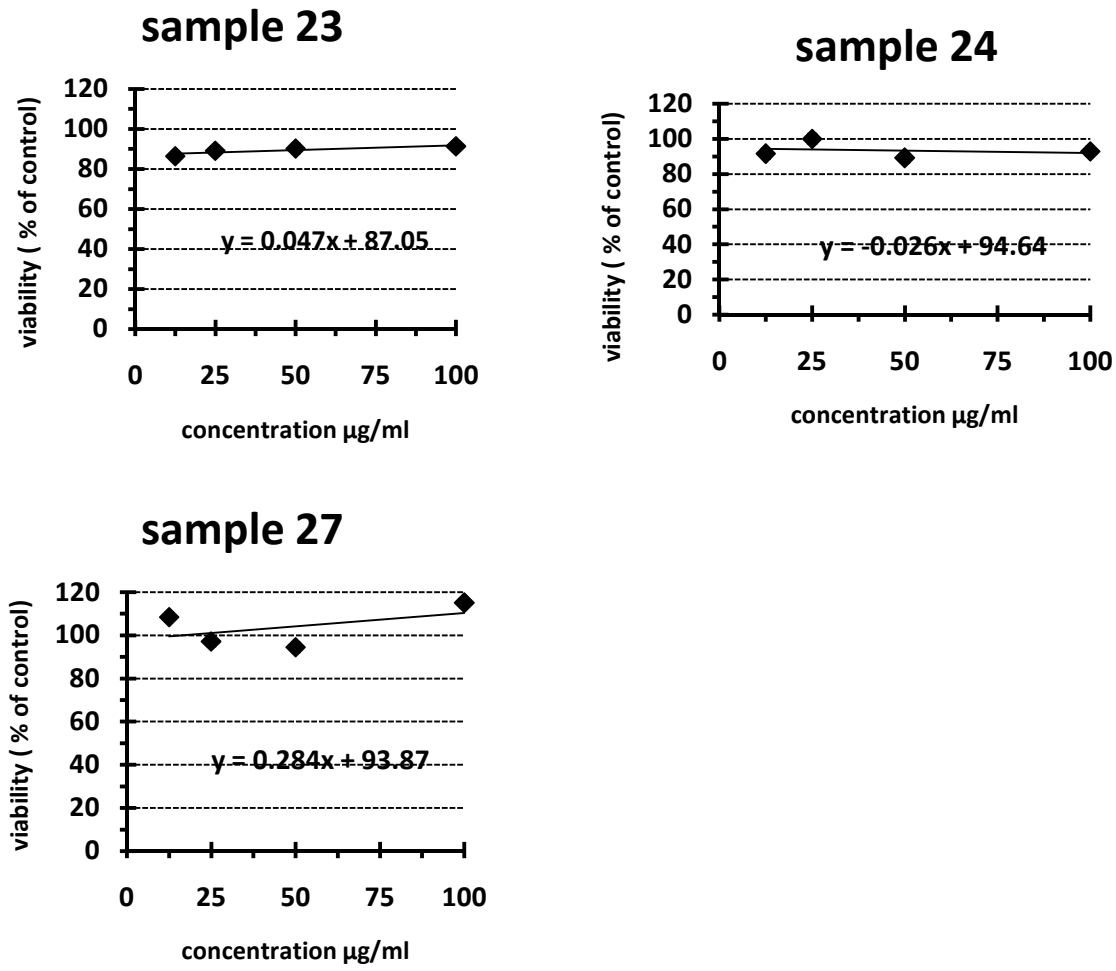


Fig.2: Cytotoxic effect of tested samples against MCF-7 cells using MTT assay (n=4), data expressed as the mean value of cell viability (% of control) ± S.d.

Calculated IC_{50} of the active samples

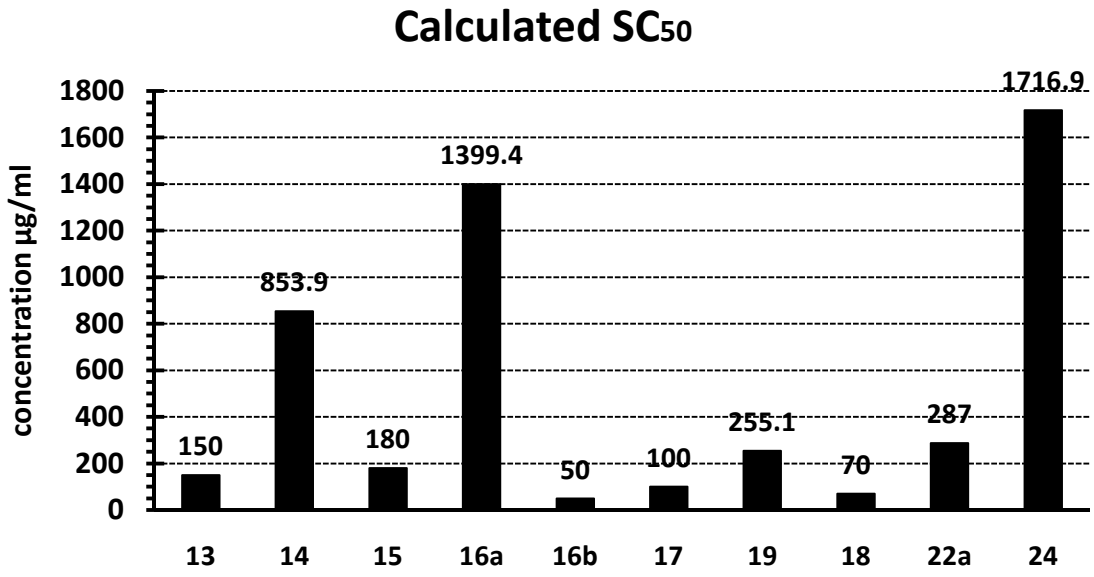


Fig.3: Calculated IC_{50} for effective samples.

CONCLUSION

We have synthesized new phthalazinone derivatives (1-27) from commercially available 2-(4-aminobenzoyl)-benzoic acid and screened for the antitumor activity against human breast adenocarcinoma cell line (MCF-7). All of the screened compounds exhibited good to excellent activity when compared to the standard drug cisplatin and (IC_{50} = 100µM). It's observed that the compounds 16b, 17, 18, 13 exhibited excellent anticancer activity against MCF-7.

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