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Synthesis of Novel Series of Phthalazine Derivatives as Potential Antitumor Agents

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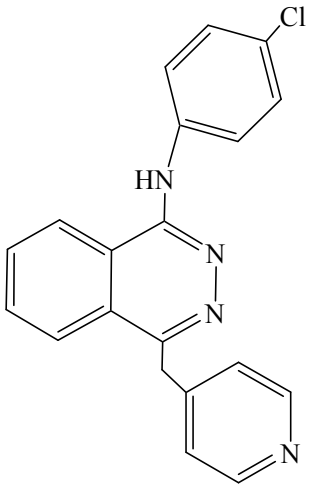
Abstract:
A novel series of phthalazine derivatives bearing isoindol-1,3-dione moiety were synthesized by treating 2-[4-(4-chlorophthalazine-1-yl)phenyl]-1*H*-isoindole-1,3-(2*H*)-dione **3** with various chemical reagents.The newly synthesi- zed compounds were characterized on the basis of their spectral (^{1}H NMR, ^{13}C NMR, Ms, IR) analyses. *In vitro*, most of the synthesized derivatives were screened for their antitumor activity against HepG2 cells using MTT assay. Compounds 11, 18 and 19c showed the most potent cytotoxic effect concluded from their IC_{50} values 217.4, 240.3 and 234.5 µg/ml respectively.
Key Words: Phthalazin-1-(2*H*)-One; chlorophthalazine; isoindol-1,3-dione; antitumor activity.

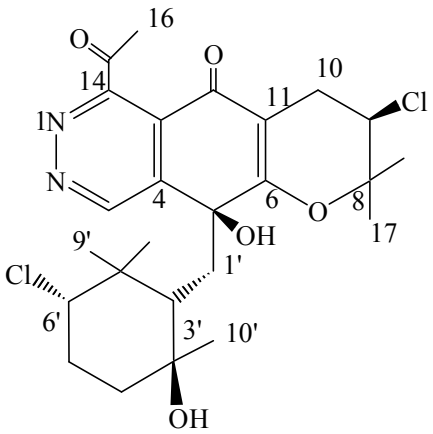
1. Introduction:

Nitrogen-containing heterocycles possesses diverse chemotherapeutic activities [1,2]. Among a large variety of nitrogen-containing heterocyclic compounds, phthalazin-1(2*H*)-ones, they were reported to be used as efficient antitumors [3] and therapeutic agents [4]. N-(4-Chlorophenyl)-4-(pyridin-4-ylmethyl)phthalazin-1-amine, also known as Vatalanib has been shown to serve as anticancer agent (Fig.1).

On the other hand, phthalazinones bearing a substituent at C-4 represent key intermediates in the synthesis of various compounds with highly interesting clinical applications and pharmacological properties, such as anti-heart disease [5], blood platelet aggregation inhibitors and cardiovascular antihypertensive agents [6-13].



Vatalanib (1)



Azamerone (2)

Fig. 1 Structures of Vatalanib and Azamerone.

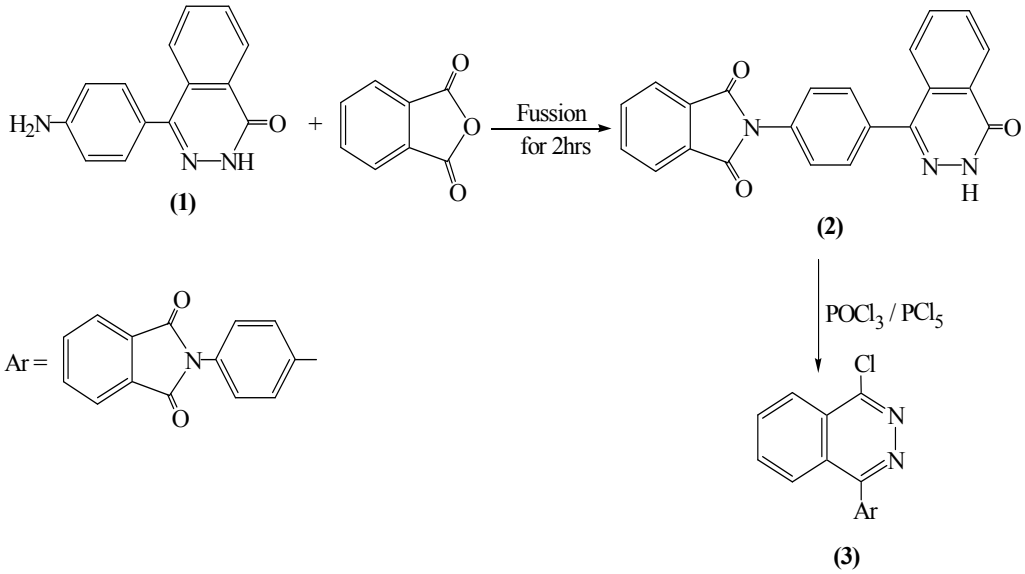
Furthermore, meroterpenoid Azamerone, isolated from the saline culture of a new marine-derived bacterium related to the genus Streptomyces represents example of this unique phthalazinone ring system as a natural product (Fig.1).

In addition, a number phthalazinone derivatives are well known to be active as potential anticonvulsant [14], cardiotonic[15], vasorelaxant [16], also used as anticancer agents and antitumor activity [17].

In view of the above mentioned facts and in continuation of our research interest for the synthesis of biologically active heterocycles [18-20], we report here, the synthesis of novel series of phthalazinones bearing isoindol-1,3-dione moiety followed by evaluation of their antitumor activities against HepG2 cells using MTT Cell Viability.

2. Results and Discussion:

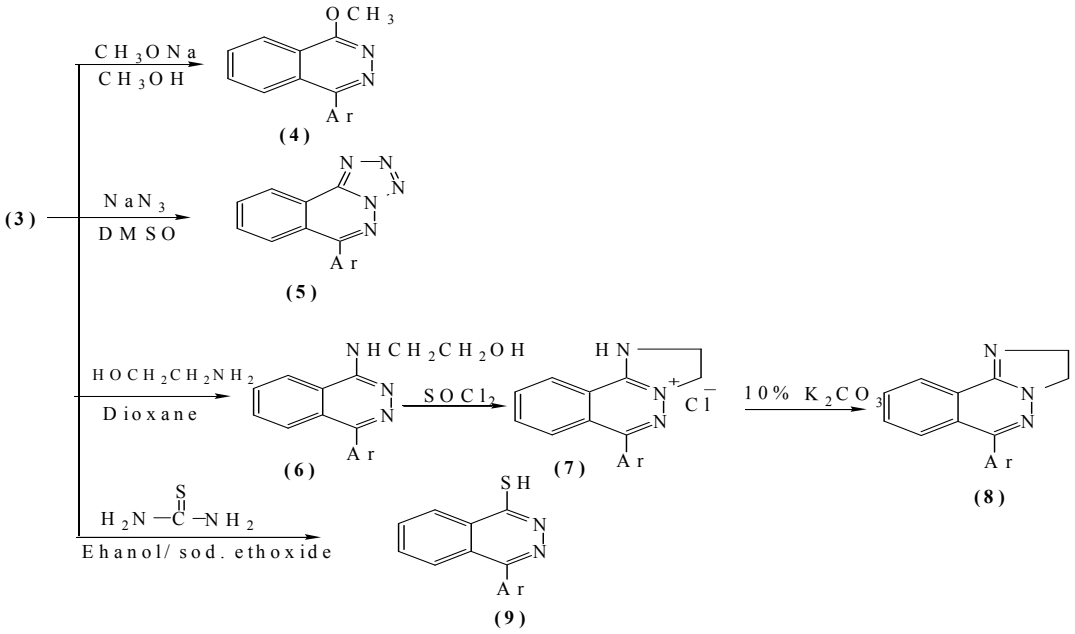
Synthesis of 2-[4-(4-oxo-3,4-dihydrophthalazin-1-yl)phenyl]-1*H*-isoindo- le-1,3-(2*H*)-dione **2** required as starting material was accomplished in high yield by fusion of phthalic anhydride with 4-(4-aminophenyl)-phthalazin-1- (2*H*)- one **1** (prepared from the reaction of γ-keto acids with hydrazine hydrate in ethanol [21-23]) (Scheme 1) .The structure of phthalazinone **2** was elucidated on the basis of spectral studies. ^{1}H NMR spectrum showed signal at δ 7.99 ppm corresponding to NH proton, 13.20 corresponding to OH proton exchangeable with D_2O, 7.40-7.93 ppm(m,12H, aromatic protons). Also, IR spectrum displayed absorption bands at ύ 3208-3085 cm^{-1}, 1746 – 1700 cm^{-1}, 1685 cm^{-1} due to NH↔OH, CO imidic, CO amidic, respectively. (Scheme 1)



Scheme 1

Treatment of phthalazinone **2** with phosphorus pentachloride and phosphorus oxychloride afforded 2-[4-(4-chlorophthalazin-1-yl)phenyl]-1*H*- isoindole-1,3(2*H*)-dione **3** which is used as a reactive key precursor for the synthesis of phthalazine series and their fused derivatives.

Thus, the reaction of chlorophthalazine **3** with sodium methoxide furnish- ed 2-[4-(4-methoxyphthalazine-1-yl)-phenyl]isoindole-1,3-dione **4**.However, treatment of chlorophthalazine **3** with sodium azide in DMSO afforded tetrazolo phthalazine **5**, spectral data and elemental analysis confirmed the proposed structures.On the other hand, the reaction of chlorophthalazine **3** with ethanolamine in dioxane gave product **6** which in turn reacted with $SOCl_2$ in benzene to give phthalazinium chloride **7**. The corresponding free base **8** was formed by treating of compound **7** with 15% K_2CO_3 solution. Furthermore, reaction of chlorophthalazine **3** with thiourea in sodium ethoxide afforded phthalazine derivative **9**. (Scheme 2)



Scheme (2)

Formation of phthalazine **9** possibly proceeded according to mechanism described in Fig.2.

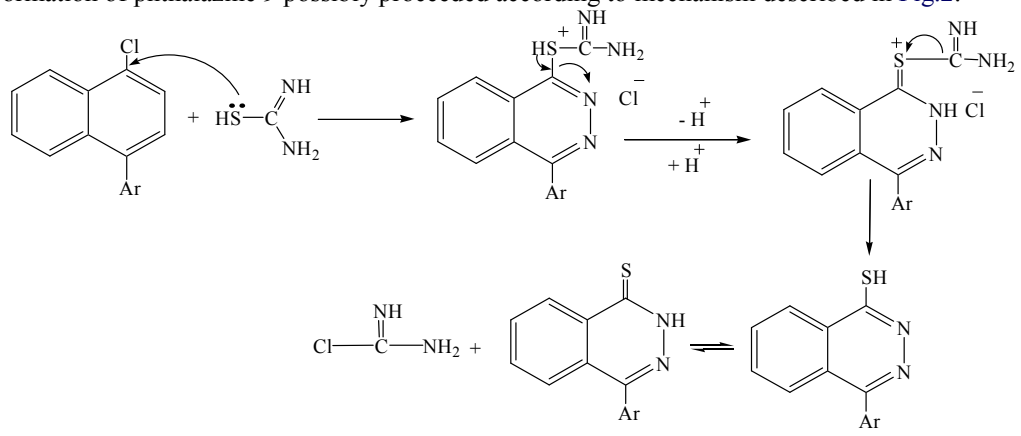
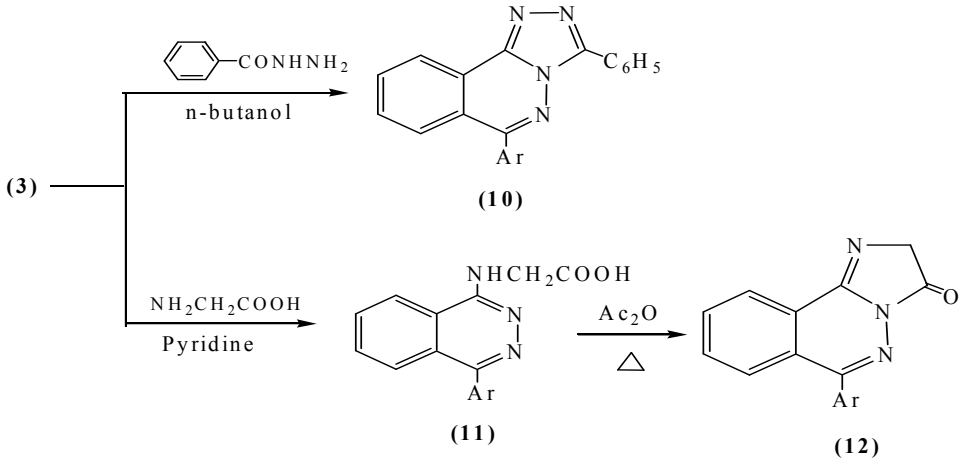


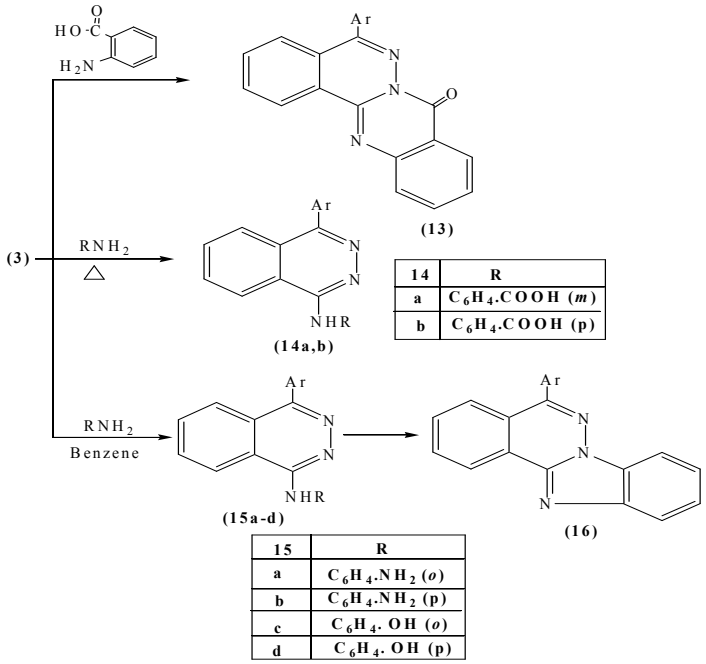
Fig. 2 Mechanism of the formation of compound 9

Also, refluxing of chlorophthalazine **3** with benzoylhydrazine in butanol furnished 2-[4-(3-phenyl-[1,2,4]triazolo[3,4-a]phthalazine-6-yl)-phenyl]-iso- indole-1,3-dione **10**. The reactivity of chlorophthalazine towards amino acids was also investigated .Thus; treatment of chlorophthalazine **3** with glycine in the presence of pyridine gave acid derivative **11.** ^{1}H NMR of acid **11** showed signals at 12.8 and 8.3 corresponding to OH and NH, respectively, singlet at 2.5 ppm corresponding to CH_2. However, the latter compound underwent ring closure by refluxing with acetic anhydride to afford 2-[4-(3-oxo-2,3-dihydro-imidazo[2,1-a]phthalazine-6-yl)-phenyl]-isoindole-1,3-dione **12**. (Scheme 3)



Scheme 3

On the other hand, anthranilic acid reacted with chlorophthalazine **3** to give tetracyclic system **13**. Whereas, *m*-amino benzoic acid and *p*-amino benzoic acid reacted with chlorophthalazine under the same reaction conditions to afford aminophthalazine derivatives **14a,b** respectively. Also, chlorophthalazine **3** was refluxed with aromatic amines such as *o*-phenylenediamine, *p*-phenylene diamine, *o*-aminophenol and *p*-aminophenol in benzene to afford phthalazine derivatives **15a-d** respectively. On the other hand, compounds **15a** and **15c** underwent thermal cyclization to furnish imidazophthalazine derivative **16**. (Scheme 4).

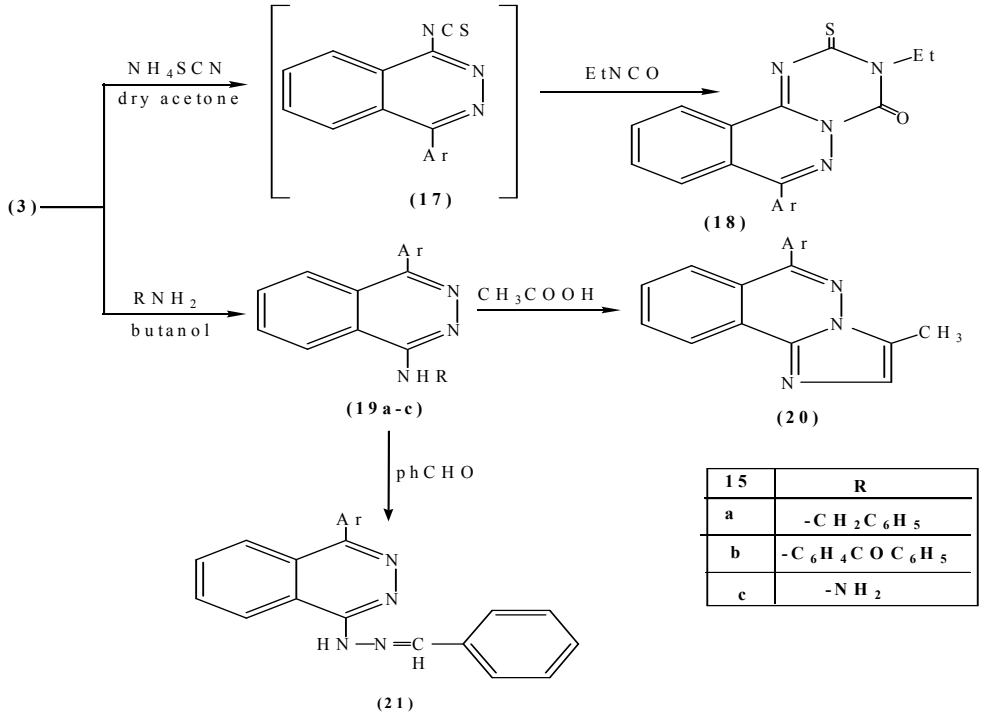


14	R
a	C_6H_4.COOH (*m*)
b	C_6H_4.COOH (p)

15	R
a	$C_6H_4.NH_2$ (*o*)
b	$C_6H_4.NH_2$ (p)
c	C_6H_4. OH (*o*)
d	C_6H_4. OH (p)

Scheme 4

However, reaction of chlorophthalazine **3** with ammonium thiocyanate lead to formation of **17** which in turn reacted with ethyl isocyanate in dry acetone to give 2-[4-(2-ethyl-1-oxo-3-thioxo-2,3-dihydro-1*H*-2,4,10,10a-tetraza- phenanthren-9-yl)-phenyl]-isoindole-1,3-dione **18**. Reactivity of chlorophthal- azine **3** towards primary amines namely, benzylamine, *p*-amino benzophenone and hydrazine hydrate in *n*-butanol afforded products **19a-c**, respectively. (Scheme 5).



15	R
a	-CH2C6H5
b	-C6H4COC6H5
c	-NH2

Scheme 5

Furthermore, boiling of hydrazide **19c** with acetic acid afforded imidazophthalazine derivative **20**. Finally, refluxing of **19c** with benzaldehyde in butanol lead to the formation of benzylidene hydrazone **21**. (Scheme 5)

3. Experimental protocols

3.1 Chemistry

All chemicals and solvents were of high analytical grade and were purchased from Sigma Chemical Company and all melting points were determined on Gallen Kamp and are uncorrected. IR spectra were measured with a Pye-Unicam spectrophotometer type 1200, using potassium bromide disk; results are given in cm^{-1}. Varian spectrometer was utilized for ^1H NMR spectra with TMS as internal reference and in the solvent DMSO-d_6. The chemical shifts are reported of frequency = 300 MHz (ppm) downfield from internal tetramethylsilane (TMS) and coupling constants (*J*) are given in Hz. Electron impact MS spectra were obtained on a Mat-711 spectrometer inlet temperature Ca, 200°C instrument at 70/eV.

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

To a solution of 2-(4-amino-benzoyl)-benzoic acid (2.37 gm,10 mmol) in absolute ethanol 15 mL, (12 gm, 240 mmol) was added 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 (NH_2,NH↔OH) ,1685 (CO); Ms: m/z 237 (M·+); ^1H NMR (DMSO-d_6) δ: 5.31 (s, 2H, NH_2 exchangeable),7.99 (s,1H, NH↔OH exchangeable), 6.50-8.19 (m, 8H, Ar-H); Anal. Calcd.for $C_{14}H_{11}N_3O$ (Mol.wt.237): C, 70.87; H, 4.67; N, 17.71; Found: C, 70.85; H, 4.69; N, 17.70%.

3.1.2. 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 hrs , 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 (NH↔OH), 1746-1700 (CO),1590 (C=N); Ms: m/z 367 (M^+); ^1H NMR (DMSO-d_6) δ: 8.17 (s, 1H, NH exchangeable), 7.69-8.19 (m, 12H, Ar-H); Anal. Calcd. For $C_{22}H_{13}O_3N_3$ (Mol.wt.367): C, 71.93; H, 3.57; N, 11.44, Found: C, 71.83; H, 3.60; N 11.40%.

3.1.3. 2-[4-(4-Chlorophthalazin-1-yl)-phenyl]-isoindole-1,3-dione (3)

A mixture of phthalazinone **2** (3.67 gm,10 mmol), phosphorus penta- chloride (2.8 gm,10 mmol) and phosphorus oxychloride (3.06 gm, 20 mmol) was refluxed for 4 hrs on 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 to give pale brown crystals.

M.p. 170-172 °C, yield 80 %; IR (cm^{-1}): 1741-1697 (2CO), 1590 (C=N), 710 (C-Cl); Ms: m/z 385 (M^{+}), 386 (M^{+}+1); ^{1}H NMR (DMSO-d_6) δ: 7.46-8.29 (m,12H, Ar-H); Anal. Calcd. For $C_{22}H_{12}ClN_3O_2$ (Mol.wt.385): C, 68.49; H, 3.14; N, 10.89, Found: C, 68.29; H, 3.11; N, 11.00%.

3.1.4. 2-[4-(4-Methoxyphthalazin-1-yl)-phenyl]-isoindole-1,3-dione (4)

A solution of Compound **3** (3.85 gm,10 mmol) in methanol (30 mL) containing sodium methoxide (0.54 gm,10 mmol) was refluxed for 6 hrs. After cooling, the reaction mixture was poured onto ice/HCl, the solid that separated was filtered off and recrystallized from ethanol to give pale yellow crystals.

M.p. 180-182 °C; yield 70 %; IR (cm^{-1}): 2947 (C–H aliphatic), 1740-1703 (2CO), 1595 (C=N); Ms: m/z 381 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 3.72 (s,1H,-OCH_3), 6.69-8.13 (m,12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 179.21, 163.22, 144.31, 138.20, 133.11,132.32,132.10, 132.01, 128.61,127.21, 127.42, 126.21, 121.01, 120.91,119.10, 56.41; Anal. Calcd. For $C_{23}H_{15}N_3O_3$ (Mol.wt.381): C 72.43, H 3.96, N 11.02, Found: C 72.33, H 3.93, N 11.12 %.

3.1.5. 2-[4-(Tetrazolo[5,1-a]phthalazin-6-yl)-phenyl]-isoindole-1,3-dione (5)

Sodium azide (0.65 gm,10 mmol) was added to a solution of chlorophtha- lazine **3** (3.85 gm,10 mmol) in DMSO (20 mL) and all was heated under reflux for 2 hrs .Then, the reaction mixture was cooled, poured onto ice.The separated solid was filtered off and recrystallized from ethanol to give pale yellow crystals.

M.p. 220-222 °C; yield 55%; IR (cm^{-1}): 1708-1698 (2CO), 1597(C=N); Ms: m/z 392 (M^{+}), 393 (M^{+1}); ^{1}H NMR (DMSO-d_6) δ: 7.69-8.13 (m, 12H, Ar-H); Anal. Calcd.For $C_{22}H_{12}N_6O_2$ (Mol.wt.392): C, 67.34; H, 3.08; N, 21.42; Found: C, 67.50; H, 3.01; N, 21.39%.

3.1.6. 2-{4-[4-[(2-Hydroxy-ethylamino)phthalazin-1-yl]-phenyl}-isoindole-1,3-dione (6)

Ethanol amine (0.61 gm, 10 mmol) was added to a solution of compound **3** (3.85 gm, 10 mmol) in dioxane (40 mL). Whole mixture was heated under reflux for 20 hrs. The solid that separated after concentration was triturated with water, filtered off and crystallized from ethanol to give yellow crystals.

M.p. 260-262 °C, yield 60 % ; IR (cm^{-1}): 3461-3154 (OH,NH), 2995 (C–H aliphatic), 1706-1668 (2CO) ,1594 (C=N); Ms: m/z 410 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 2.19 (s, 1H, OH exchangeable), 3.20-3.25 (t, 2H, CH_2), 4.9 (s, 1H, NH), 7.46-8.15 (m, 12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 166.01, 163.21, 143.11, 138.2, 133.12, 132.01,132.21, 132.30, 127.41, 127.22 , 127.01, 120.91, 119.01, 116.01, 64.51, 54.91;Anal. Calcd. For $C_{24}H_{18}N_4O_3$ (Mol.wt.410): C, 70.23; H, 4.42; N, 13.65, Found: C, 70.33; H, 4.32; N, 13.69%.

3.1.7.6-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]-2,3-dihydro-1H-imi-dazo[2,1-a]phthalazin-4-ylium chloride (7)

A mixture of compound **6** (4.1 gm, 1o mmol) and $SOCl_2$ (1.18 gm,10 mmol) was refluxed for 2 hr in the presence of dry benzene. The solid that separated upon cooling was filtered off and recrystallized from ethanol to give pale yellow crystals.

M.p. 280-282 °C; yield 50 %; IR (cm^{-1}): 3250 cm^{-1} (NH),1747-1665 (2CO),1596 (C=N); Ms: m/z 428 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 6.32 (s, 1H, NH exchangeable), 3.17-3.23 (t, 2H, CH_2), 7.46-8.13 (m,12H, Ar-H); Anal. Calcd. For $C_{24}H_{17}ClN_4O_2$ (Mol.wt.428): C, 67.21; H, 4.00; N, 13.06, Found: C, 67.31; H, 4.31; N 12.99%.

3.1.8. 2-[4-(2,3-Dihydro-imidazo[2,1-a]phthalazin-6-yl)-phenyl]-isoindole-1,3-dione (8)

A compound **7** (4.28 gm, 10 mmol) was dissolved in 10% K_2CO_3 then extracted the solution with chloroform. Evaporation of the solvent gave free base **8** which was recrystallized from ethanol to give light brown crystals.

M.p. 300-302 °C; yield 70 %; IR (cm^{-1}): 1747-1665 (2CO), 1602 (C=N); Ms: m/z 392 (M·+); Anal. Calcd. For $C_{24}H_{16}N_4O_2$ (Mol.wt.392): C, 73.46; H, 4.11; N, 14.28, Found: C, 73.51; H, 4.12; N 14.38%.

3.1.9 2-[4-(4-Mercaptophthalazin-1-yl)-phenyl]-isoindole-1,3-dione (9)

An equimolar amount of **3** (3.85 gm,10 mmol) and thiourea (0.76 gm,10 mmol) in ethanol (20 mL) containing 10 mmol of sodium ethoxide was heated under reflux for 6 hrs. The reaction mixture was poured onto ice/water, then, acidified with acetic acid.The solid product which formed was collected by filteration, dried and crystallized from ethanol to give yellow crystals. M.p. 230-232 °C; yield 56 %; IR (cm^{-1}): 2660 cm^{-1} (SH), 1747-1665 (2CO), 1596 (C=N); Ms: m/z 384 (M^{+}+1); ^{1}H NMR (DMSO-d_6) δ: 9.81 (s, 1H, SH, exchangeable), 7.46-8.13 (m, 12H, Ar-H); Anal. Calcd. For $C_{22}H_{13}N_3O_2S$ (Mol.wt.383): C, 68.91; H, 3.42; N, 10.96, Found: C, 68.81; H, 3.49; N 10.99%.

3.1.10. 2-[4-(3-Phenyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)-phenyl]-isoindole-1,3-dione (10)

A mixture of **3** (3.85 gm,10 mmol) and benzoylhydrazine (1.36 gm ,10 mmol) in *n*-butanol (30 mL) was refluxed for 50 hrs. The solid that separated after concentration and cooling was filtered off, and crystallized from ethanol to give yellow crystals. M.p. 230-232 °C; yield 65 %; IR (cm^{-1}): 1704-1668 (2CO), 1597 (C=N); Ms: m/z 468 (M^{+}+1); Anal. Calcd. For $C_{29}H_{17}N_5O_2$ (Mol.wt.467): C, 74.52; H, 3.67; N, 14.98, Found: C, 74.81; H, 3.70; N, 15.01%.

3.1.11. {4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-yl amino}-acetic acid (11)

Compound **3** (3.85 gm,10 mmol) and glycine (0.75 gm,10 mmol) in pyridine containing few drops of water (4

mL) was heated under reflux for 3 hrs. The reaction mixture poured onto ice/HCl. The solid that separated was filtered off, washed with water and crystallized from acetic acid to give pale yellow crystals.
M.p. 235-237 °C, yield 65 %; IR (cm^{-1}): 3462-3151 (OH, NH), 2996 (C-H aliphatic) ,1703-1669 (CO), 1594 (C=N); Ms: m/z 425 (M^++1); ^{1}H NMR (DMSO-d_6) δ 12.84 (s, 1H, OH exchangeable), 8.29 (s, 1H, NH exchangeable), 2.5(s, 2H, CH_2), 7.28-8.27 (m, 12H, Ar-H); Anal. Calcd. For $C_{24}H_{16}N_4O_4$ (Mol.wt.424): C, 67.92; H, 3.80, N, 13.20; Found: C, 67.99; H, 3.75; N 13.30%.

3.1.12. 2-[4-[3-Oxo-2,3-dihydro-imidazo[2,1-a]phthalazin-6-yl)-phenyl]-isoindole-1,3-dione (12)

Acid **11** (4.24 gm, 10 mmol) in acetic anhydride (15 ml) was refluxed for 2 hrs. After cooling, the reaction mixture poured onto ice. The solid that separated filtered off, washed with water and crystallized from ethanol to give pale yellow crystals. M.p. 190-192 °C; yield 50 %; IR (cm^{-1}): 1708 -1700 (2C=O), 1596 (C=N); Ms: m/z 407 (M^++1); Anal. Calcd. For $C_{24}H_{14}N_4O_3$ (Mol.wt.406): C, 70.93; H, 3.47; N, 13.79, Found: C, 71.01; H, 3.37; N 13.19%.

3.1.13. 2-[4-(7-Oxo-7H-6,6a,12-triaza-benzo[a]anthracen-5-yl)-phenyl]-isoindole-1,3-dione (13)

In a fusion tube provided with an air condenser, a mixture of **3** (3.85 gm,10 mmol) and anthranilic acid (1.37 gm ,10 mmol) was heated in an oil bath at 190-191°C for 2 h. Then the mixture was cooled (to room temperature) and poured into 40 mL of cold water. The obtained solid product was collected and recrystallized from benzene to give yellow crystals.
M.p. 220-222 °C; yield 69 %; IR (cm^{-1}): 1720-1700 (2CO), 1594 (C=N); Ms: m/z 468 (M^{-+}); ^{1}H NMR (DMSO-d_6) δ 7.44-7.99 (m, 16H, Ar-H); ^{13}C-NMR (DMSO-d_6) δ: 170.23, 164.32, 163.21, 155.62, 152.32, 140.52, 133.41, 133.21, 132.31, 132.01, 130.91, 130.01, 129.20, 129.10, 128.81, 128.62, 127.41, 127.11, 127.01, 126.82, 126.01, 122.12, 120.51; Anal. Calcd.For $C_{29}H_{16}N_4O_3$ (Mol.wt.468): C, 74.35; H, 3.44; N, 11.96, Found: C, 74.45; H, 3.59; N 11.82%.

3.1.14. 3- or 4-{4-[4-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-phenyl]phthalazin-1-ylamino}-benzoic acid (14a,b)

A compound **3** (3.85 gm,10 mmol) and amino acid (1.37 gm ,10 mmol) of namely, *m*-amino benzoic acid or *p*-amino benzoic acid were fused into oil bath at 175 °C for 4 hrs. After cooling, water was added and the solid obtained filtered off and recrystallized from proper solvent to give **14a,b.**
M.p. for 14a, 14b 275°C, 200 °C respectively; yield 70, 81 %; IR (cm^{-1}): 3492-3117 (OH, NH), 1721-1696 (2CO), 1603 (C=N); Ms: 488 (M^++2); ^{1}H NMR (DMSO-d_6) δ: 12.85 (s, 1H, OH exchangeable), 7.96 (s, 1*H*, NH exchangeable), 7.41-8.06 (m, 16H, Ar-H); Anal. Calcd. For $C_{29}H_{18}N_4O_4$: (Mol.wt.486) C, 71.60; H, 3.73; N, 11.52, Found: C, 71.52; H, 3.63; N, 11.72%.

3.1.15. General procedure for synthesis of (15a-d)

An equimolar amount of chlorophthalazine **3** (3.85 gm,10 mmol) and *o*-phenylene diamine, *p*-phenylene diamine, *o*-amino phenol or *p*-amino phenol in benzene (40mL) was heated under reflux for 5 hrs; then the reaction mixture was concentrated by evaporation . The solid that obtained was crystallized from ethanol to give (15a-d) respectively

3.1.15.1. 2-{4-[4-(2-Aminophenylamino)phthalazin-1-yl]-phenyl}-isoindole-1,3 -dione (15a)

Pale yellow crystals; M.p. 230-232 °C; yield 70 %; IR (cm^{-1}): 3405-3158 (NH, NH_2), 1707-1700(2CO), 1595 (C=N); ^{1}H NMR (DMSO-d_6) δ: 12.85 (s, 1H, NH exchangeable), 8.30 (s, 1H, NH_2 exchangeable), 6.40-8.27 (m,16H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 166.01, 163.21, 143.01, 138.21, 133.31, 133.01, 132.31, 132.11,132.01,127.41,127.21, 127.01, 120.90, 119.30, 119.01, 116.01, 115.90; Ms: m/z 458 (M^++1); Anal. Calcd. For $C_{28}H_{19}N_5O_2$ (Mol.wt.457): C, 73.51; H, 4.19; N, 15.31; Found: C, 73.61; H, 4.27; N, 14.98%.

3.1.15.2. 2-{4-[4-(4-Amino-phenylamino)phthalazin-1-yl]-phenyl}-isoindole-1,3-dione (15b)

Pale yellow crystals; M.p. 210-212°C; yield 65 %; IR (cm^{-1}): 3396-3155 (NH, NH_2), 1705-1698 (2CO), 1593 (C=N); Ms: 458 (M^++1); Anal. Calcd. For $C_{28}H_{19}N_5O_2$ (Mol.wt.457): C, 73.51; H, 4.19; N, 15.31, Found : C, 73.70; H, 4.22; N, 15.11%.

3.1.15.3. 2-{4-[4-(2-Hydroxyl-phenylamino)phthalazin-1-yl]-phenyl}-isoindole -1,3-dione (15c)

Brown crystals; M.p. 280-282 °C; yield 68 %; IR (cm^{-1}): 3374-3202 (NH, OH), 1743-1705(2CO), 1596 (C=N); Ms: m/z 458 (M^{-+}); ^{1}H NMR (DMSO-d_6) δ: 5.21 (s, 1H, OH phenol exchangeable),4.32 (s, 1H, NH exchangeable), 7.41-8.06 (m, 16H, Ar-H); Anal. Calcd. For $C_{28}H_{18}N_4O_3$ (Mol.wt.458): C, 73.35; H, 3.96; N, 12.22 Found : C, 73.45; H, 3.99; N, 12.42%.

3.1.15.4. 2-{4-[4-(4-Hydroxyl-phenylamino)phthalazin-1-yl]-phenyl}-isoindole -1,3-dione (15d)

Brown crystals; M.p. 230-232 °C ; yield 75 %; IR (cm^{-1}): 3374-3102 (NH, OH), 1743-1705(2CO), 1596 (C=N); Ms: m/z 458 (M^{-+}); Anal. Calcd. For $C_{28}H_{18}N_4O_3$ (Mol.wt.458): C, 73.35; H, 3.96; N, 12.22, Found: C, 73.46; H, 3.85; N, 12.41%.

3.1.16 2-(4-Benzo[4,5]imidazo[2,1-a]phthalazin-5-yl-phenyl)-isoindole-1,3-dione (16)

In a fusion tube provided with an air condenser, Compound **15a** and **15c** was heated in sand bath at 285-290ºC for 2

h. Then, it was cooled (to room temperature) and 30 mL of cold water was added. The obtained solid product was collected and recrystallized from ethanol to give yellow crystals. M.p. 300-302 °C; yield 75 %; IR (cm^{-1}): 1743-1705 (2CO), 1601 (C=N); Ms: m/z 441 (M^{+}+1); Anal. Calcd. For $C_{28}H_{16}N_4O_2$ (Mol.wt.440): C, 76.35; H, 3.66; N, 12.72, Found: C, 76.44; H, 3.76; N, 12.64%.

3.1.17. 2-[4-(2-Ethyl-1-oxo-3-thioxo-2,3-dihydro-1H-2,4,10,10a-tetrazaph enanthren-9-yl)-phenyl]-isoindole-1,3-dione (18)

A mmoniumthiocyanate (0.71 gm, 10 mmol) in dry acetone was added to a stirred solution of chlorophthalazine **3** (3.85 gm, 10 mmol) in dry acetone. The reaction mixture was stirred for 1 h at room temperature. Ammonium chloride was precipitated during the progress of the reaction. After filtration of the ammonium chloride, ethyl isocyanate 10 mmol was added to the filtrate. The reaction mixture was heated under reflux for 30 min. The solid product that separated after cooling was crystallized from ethanol to give yellow crystals.

M.p. 120-122 °C, yield 56 %; IR (cm^{-1}): 2950 (C–H aliphatic), 1710 -1699 (2CO); Ms: m/z 479 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 1.32 (t, 3H, CH_3), 3.22 (q, 2H, CH_2), 7.59-8.13 (m, 12H, Ar-H); Anal. Calcd. For $C_{26}H_{17}N_5O_3S$ (Mol.wt.479): C, 65.12; H, 3.57; N, 14.61, Found: C, 65.01; H, 3.65; N 14.81%.

3.1.18. General procedure for synthesis of (19a-c)

A solution of **3** (3.85 gm,10 mmol) and primary amines (10 mmol) namely, benzylamine, *p*-amino benzophenone or hydrazine hydrate in *n*-butanol (40 mL) was heated under reflux for 6hrs. The solid that separated after concentration was recrystallized from proper solvent to give **19a-c**.

3.1.18.1. 2-[4-(4-Benzylaminophthalazin-1-yl)-phenyl]-isoindole-1,3-dione (19a)

Yellow crystals; M.p. 160-162 °C ; yield 60 %; IR (cm^{-1}): 3422 (NH), 2930 (C–H aliphatic), 1743-1704 (2CO), 1599 (C=N); Ms: m/z 458 (M^{+}+2); ^{1}H NMR (DMSO-d_6) δ: 7.96 (s, 1H, NH exchangeable), 7.06-8.13 (m, 16H, Ar-H); Anal. Calcd. For $C_{29}H_{20}N_4O_2$ (Mol.wt.456): C, 76.30; H, 4.42; N, 12.27, Found: C, 76.01; H, 4.62; N 12.41%.

3.1.18.2. 2-[4-(4-Benzoylphenylaminophthalazin-1-yl)-phenyl]-isoindole-1,3-dione (19b)

Yellow crystals; M.p. 200-202 °C; yield 66 %; IR (cm^{-1}): 3396 (NH), 1705-1689 (2CO), 1595 (C=N); Ms: m/z 546 (M^{+}); ^{1}HNMR (DMSO-d_6) δ 7.32 (d, 1H, NH, exchangeable), 7.06-8.13 (m, 16H, Ar-H); Anal. Calcd.For $C_{35}H_{22}N_4O_3$ (Mol.wt.546): C, 76.91; H, 4.06; N, 10.25, Found : C, 76.99; H, 4.23; N, 10.15% .

3.1.18.3. 2-[4-(4-Hydrazinophthalazin-1-yl)-phenyl]-isoindole-1,3-dione (19c)

Pale yellow crystals ; M.p. 170-172 °C, yield 70 % ; IR (cm^{-1}): 3424 -3159 (NH, NH_2), 17851-1664 (2CO) ,1596 (C=N); Ms: m/z 384(M^{+}+3); ^{1}H NMR (DMSO-d_6) δ: 7.85 (d, 1H, NH exchangeable), 8.30 (s, 1H, NH_2 exchangeable),7.06-8.13 (m, 12H, Ar-H); Anal. Calcd. For $C_{22}H_{15}N_5O_2$ (Mol.wt.381): C, 69.28; H, 3.96; N, 18.36, Found: C, 69.48; H, 3.86; N, 18.56%.

3.1.19. 2-[4-(3- methyl-[1,2,4]triazolo-[3,4-a]phthalazin-6-yl) phenyl]- isoindole -1,3-dione (20)

Hydrazinophthalazine **18c** (3.81 gm,10 mmol) in AcOH (30 mL) was heated under reflux for 8 hrs.The solid that separated after concentration and cooling was recrystallized from ethanol to give yellow crystals. M.p.200-202 °C; yield 65 %; IR (cm^{-1}): 2950 (C–H aliphatic), 1764-1704 (2CO), 1596 (C=N); Ms: m/z 406 (M^{+}+1); ^{1}H NMR (DMSO-d_6) δ: 2.35 (m, 3H, CH_3), 7.46-8.13 (m, 12H, Ar-H); Anal. Calcd. For $C_{24}H_{15}N_5O_2$ (Mol.wt.405): C, 71.10; H, 3.73; N, 17.27, Found : C, 71.32; H, 3.63; N, 17.47%.

3.1.20. 2-{4-[4-(N-Benzylidene-hydrazino)phthalazin-1-yl]-phenyl}-isoindole-1,3-dione (21)

A mixture of **18c** (3.81 gm,10 mmol) and benzaldehyde (1.06 gm,15 mmol) was heated under reflux for 4 hrs. in n-butanol (40 mL) . The solid that separated after concentration and cooling was recrystallized from dimethylform- amide/water to give pale yellow crystals.

M.p.210-212 °C; yield 69 %; IR (cm^{-1}): 3437 (NH), 1750-1664 (2CO); Ms: m/z 469 (M^{+}); Anal. Calcd. For $C_{29}H_{19}N_5O_2$ (Mol.wt.469): C, 74.19; H, 4.08; N, 14.92, Found: C, 74.23; H, 4.11; N, 14.85%.

3.2. Pharmacology

Material and methods

3.2.1. Cell Culture

Human hepatocarcinoma cell line (HepG2), purchased from ATCC, USA, was 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 mg/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 samples were dissolved in dimethyl sulphoxide (DMSO), and then diluted serially 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.

3.2.2. Anti-tumor activity

Cytotoxicity of tested samples was measured against HepG2 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 (Hansen et al, 1989).

3.2.2.1 Reagents preparation:

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

3.2.2.2 Procedure

Cells ($0.5X10^5$ cells/ well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20µl of different concentrations of the tested sample for 48 h at 37 ºC, 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 [24].

3.2.2.3 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.

3.2.2.4 Results

Anti-tumor activity

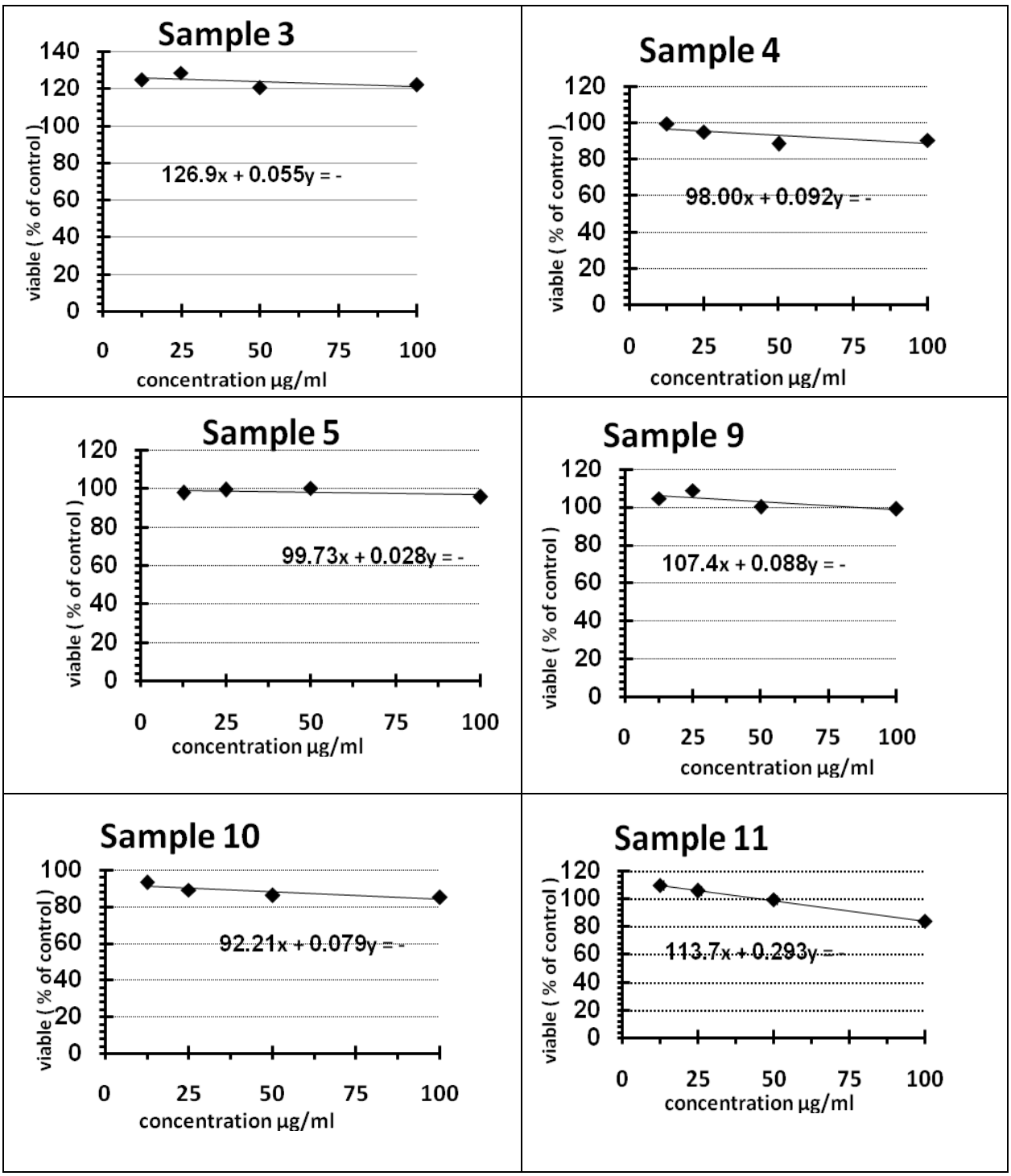
Using MTT assay, the effect of the samples on the proliferation of Hep-G2 cells were studied after 48 h of incubation. As shown in figure 1, the treatment of Hep-G2 cells with the samples showed different cytotoxic effect against Hep-G2.

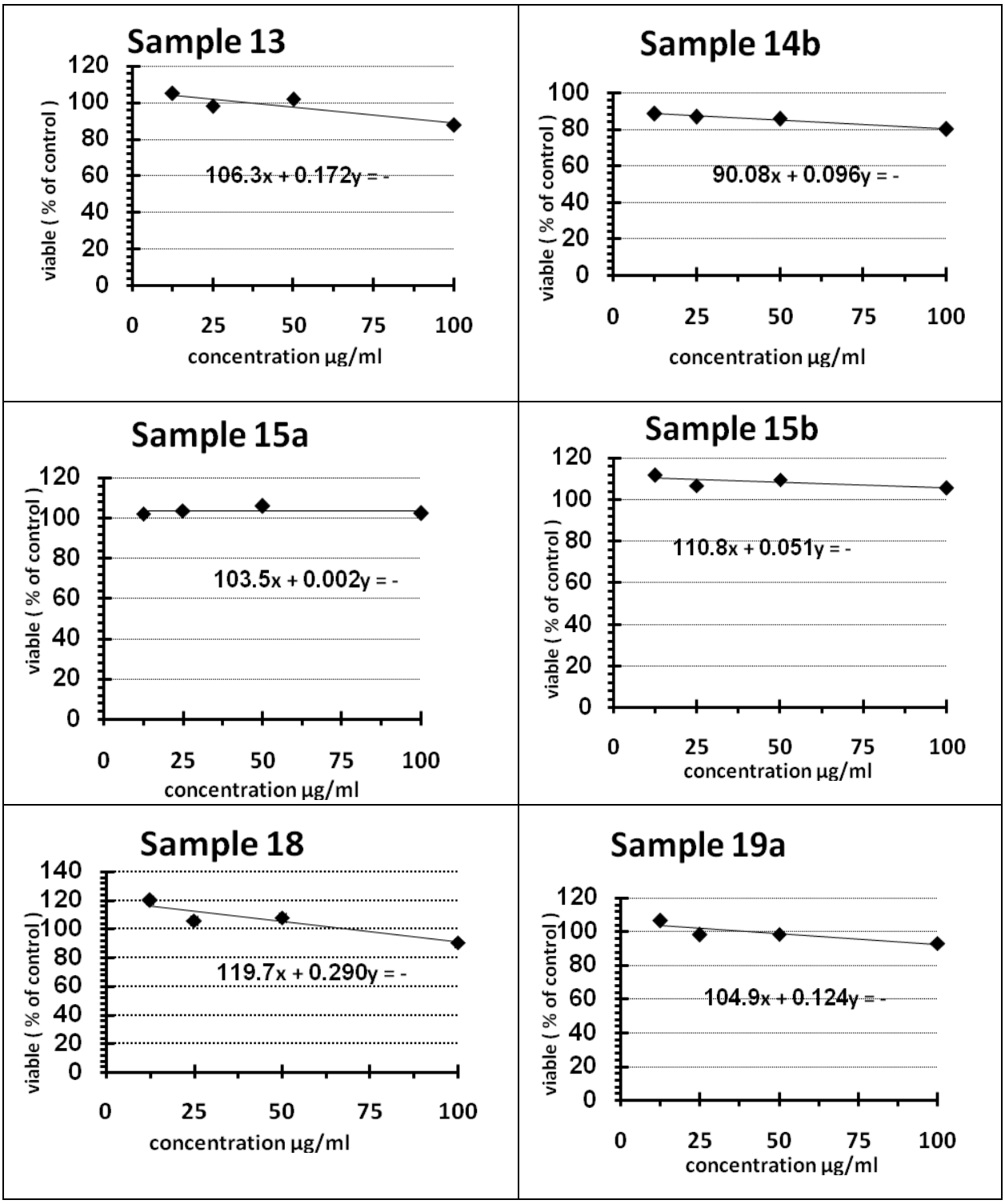
Presence of amino acid, triazine and hydrazine moieties attached to phthalazine enhances the antitumor activity.

So for samples **11**, **18** and **19c** they showed the most potent cytotoxic effect concluded from their IC_{50} values 217.4, 240.3 and 234.5 µg/ml respectively. For samples **13**, **14b**, **19a**, **19b** and **21** they showed weak cytotoxic effect concluded from their IC50 values of 327.3, 417.5, 442.7, 440.7 and 314.2 µg/ml respectively.

For all other samples **3**, **4**, **5**, **9**, **10**, **15a** and **15b** they did not show any cytotoxic effect concluded from their IC50 values > 500 µg/ml. Fig.3: showed cytotoxic effect of different samples against Hep-G2 cells using MTT assay (n=4), data expressed as the mean value of cell viability (% of control) ± S.E.

And Fig. 4 showed a comparison of the IC50 values between different samples.


Sample 3
viable (% of control)
126.9x + 0.055y = -
concentration µg/ml
Sample 4
viable (% of control)
98.00x + 0.092y = -
concentration µg/ml
Sample 5
viable (% of control)
99.73x + 0.028y = -
concentration µg/ml
Sample 9
viable (% of control)
107.4x + 0.088y = -
concentration µg/ml
Sample 10
viable (% of control)
92.21x + 0.079y = -
concentration µg/ml
Sample 11
viable (% of control)
113.7x + 0.293y = -
concentration µg/ml


Sample 13
viable (% of control)
120
100
80
60
40
20
0
106.3x + 0.172y = -
0
25
50
75
100
concentration µg/ml
Sample 14b
viable (% of control)
100
80
60
40
20
0
90.08x + 0.096y = -
0
25
50
75
100
concentration µg/ml
Sample 15a
viable (% of control)
120
100
80
60
40
20
0
103.5x + 0.002y = -
0
25
50
75
100
concentration µg/ml
Sample 15b
viable (% of control)
120
100
80
60
40
20
0
110.8x + 0.051y = -
0
25
50
75
100
concentration µg/ml
Sample 18
viable (% of control)
140
120
100
80
60
40
20
0
119.7x + 0.290y = -
0
25
50
75
100
concentration µg/ml
Sample 19a
viable (% of control)
120
100
80
60
40
20
0
104.9x + 0.124y = -
0
25
50
75
100
concentration µg/ml

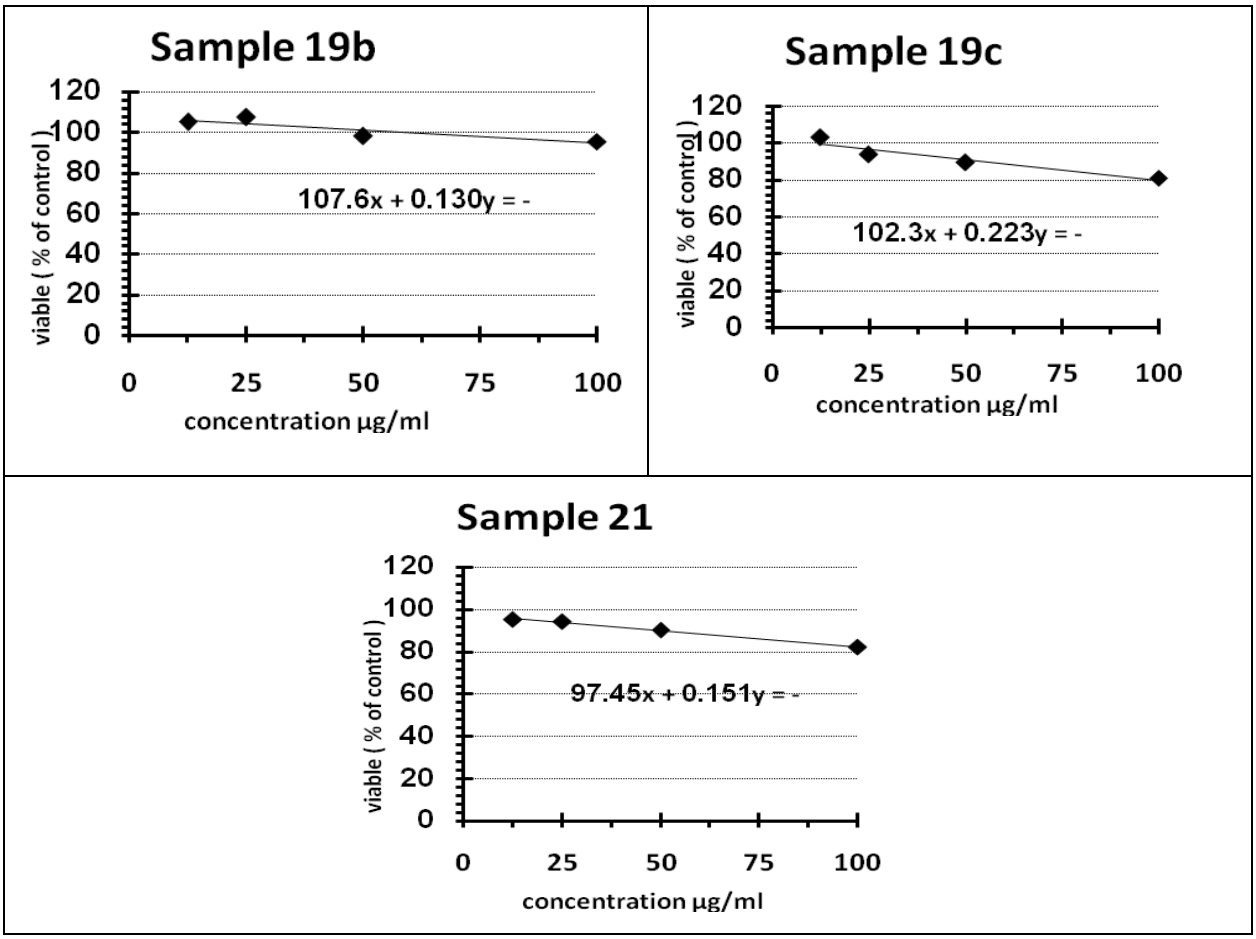


Fig.3: Cytotoxic effect of different samples against Hep-G2 cells using MTT assay (n=4), data expressed as the mean value of cell viability (% of control) ± S.E.

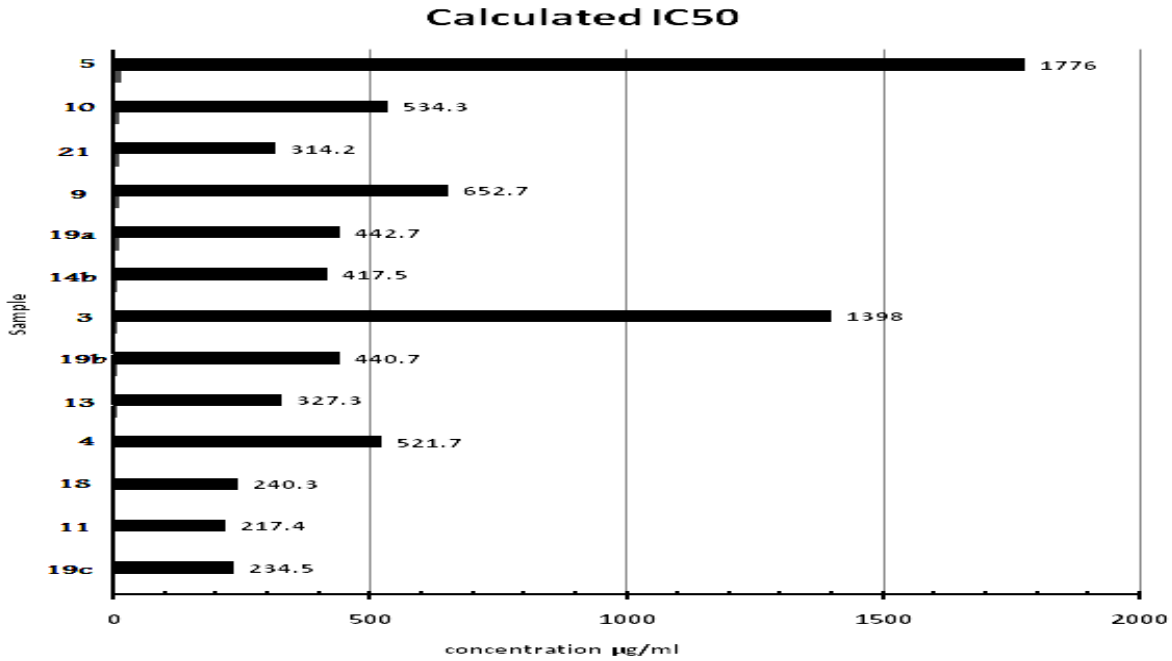


Fig.4: Calculated IC50 of different samples against Hep-G2 cells using MTT assay.

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References:

[1] F. Al-Assar, K. N. Zelenin, E. E. Lesiovskaya, I. P. Bezhan, B. A. Chakchir, Pharm. Chem. J. 36 (2002) 598–603.
[2] R. P. Jain, J. C. Vederas, Bioorg. Med. Chem. Lett. 14 (2004) 3655–3658.
[3] J. S. Kim, H. J. Lee, M. E. Suh, H. Y. Park Choo, S. K. Lee, H. J. Park, C. Kim, S. W. Park, C. O. Lee, Bioorg.Med. Chem. 14(2004) 3683.
[4] A. E. Porter, A.R. Katritky, D. Bartond, W.D. Ollis, Comprehensive Organic Chemistry. Oxford, (1979) pp. 7–85.
[5] K. Moro, O. Karomaru, M. Takage, 91(1991) 246,286.
[6] A.I. Sugimoto, Y. Eguchi, H. Tanaka, Y. Takashima, M. Ishikawa,
Inst. Med. Chem. Chim. Ther. 19(1984) 223–228.
[7] Y. Eguchi, M. Ishikawa, Chem. Pharm. Bull. 39 (1991) 1846–1848.
[8] E. Del-Olmo, B. Barboza, M. Ybarra, J. Ló pez-Pérez, R. Carrón, M. Sevilla, C. Boselli, A. San-Feliciano, Bio Med. Chem. Lett 16 (2006) 2786-2787.
[9] E. N. Scott, G .Meinhardt, C. Jacques et al , Expert Opin Investig. Drugs 16 (2007)367–379.
[10] S. Tanaka, M. Tanaka, A. Akashi, Stroke 20 (1989) 1724-1729.
[11] R. Moroi, K. Ono, T. Saito, T. Akimoto, M. Sano, Chem. Pharm. Bull. 25 (1977) 830-835.
[12] J.P. Kemp, E.O. Meltzer, H.A. Orgel, M.J. Welch, G.A. Bucholtz, E. Middleton, S.L. Spector, J.J. Newton, J.L. Perhach Jr., J. Allergy. Clin. Immunol. 79 (1987) 893-899.
[13] G. Scheffler, J. Engel, B. Kutscher, W.S. Sheldrick, P. Bell, Archiv. der. Pharmazie.321 (1988) 205-208.
[14] S. Grasso, G. De Sarro, A. De Sarro, N. Micale, M. Zappala, G. Puia, M. Baraldi, C. De Micheli, J. Med. Chem. 43(2000) 2851-2859.
[15] Y. Nomoto, H. Obase, H. Takai, M. Teranishi, J. Nakamura, K. Kubo, Chem. Pharm. Bull. (Tokyo) 38 (1990) 2179–2182.
[16] N. Watanabe, Y. Kabasawa, Y. Takase, M. Matsukura, K. Miyazaki, H. Ishihara, K.Kodama, H. Adachi, J. Med. Chem. 41 (1998) 3367–3372.
[17] M. Yamaguchi, K. Kamei, T. Koga, N. Ohi. J.Med. Chem. 36 (1993) 4052–4060.
[18] A.A. Aly, A.A.F. Wasfy, Indian J. Chem., 43B (2004) 629-635.
[19] R. El-sayed, A.A. Wasfy, A.A. Aly, J. Heterocyclic Chem. 42 (2005) 125-130.
[20] M. S. Behalo, J. Sulf. Chem. 2010, 31(4) 287–297
[21] M. Tishler, B. Stanovnik, Adv. Heterocyclic Chem. 9 (1968) 121-125.
[22] M. Tishler, B. Stanovnik, Adv. Heterocyclic Chem. 24 (1979) 363-365.
[23] M. Tishler, B. Stanovnik, Adv. Heterocyclic Chem. 49 (1990) 385-389.
[24] Hansen MB, Nielsen SE and Berg K: Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J. Immunol. Methods 1989; 119:203-10.

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