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ARTICLE

Synthesis of novel phthalazine derivatives as pharmacological activities

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Abstract

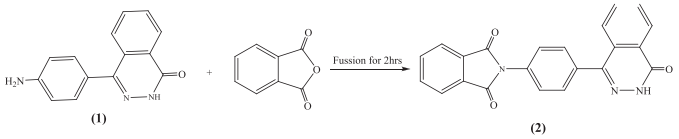
Phthalazine derivatives attached to amino acid derivatives were synthesized with high yields. The reaction of phthalazine derivatives with different phthalyl and tosylamino acids such as glycine, alanine, phenylalanine, valine, serine, and threonine in the presence of *N*,*N*-dicyclo hexylcarbodiimide (DCC) as a dehydrating agent reagent yielded high yields of the afforded compounds. Phthalylamino acids derivatives were obtained by deprotection of phthalazine derivatives, with the latter heating with hydrazine hydrate. The chemical structures of all phthalazine derivatives were affirmed by elemental analysis and spectral data (IR, MS, ^{1}HNMR, and ^{13}C NMR). Screening out and estimation of the synthesized derivatives for their cytotoxic and antioxidant activity were done, and most of them showed powerful activity in comparison with standard drugs.

1 | INTRODUCTION

Among the different nitrogen containing heterocycles, phthalazines play an important role in medicinal chemistry and subsequently have emerged as a pharmacophore. The phthalazine structural motifs have attracted a great deal of interest because of their ready accessibility, diverse chemical reactivity, and wide gamut of biological activities like antibacterial,[1] antifungal,[2] antitumor,[3–5] anti-inflammatory,[6–8] and anticonvulsant[9–11] activities. In organic synthesis, *N*-protected amino acids are essential key precursors and have been used in many areas, for example, peptide synthesis,[12,13] medicinal chemistry,[14–16] as chiral sources,[17–20] and polymer materials.[21,22] And in the recent years, they got interest because of their magnificent pharmacological and therapeutic properties,[23–25] and phthalazines linked amino acid derivatives as a new class of promising antimicrobial,[26–28] antitumor,[19–23] anihypertensive,[24,26] antidiabetic,[27,28]anti-inflammatory,[29,30] and vesarelaxant activities.[31] The integration of amino acid residues in various oxygen-,nitrogen-, and sulfur-containing heterocycles sometimes enhances the biological profile manifold over that of its parent nucleus.[32–35] According to these observations and in continuation of our work on synthesis heterocycles of pharmacological interest,[36–38] there was an interest in synthesizing new molecules involving phthalazine and amino acid moieties in a single molecular framework that possibly have antitumor, antioxidant, and DNA agents.

2 | RESULTS AND DISCUSSION

The starting molecule 2-[4-(4-oxophthalazin-1-yl)phenyl]-1*H*-isoindole-1,3-(2*H*)-dione (**2**) synthesis was achieved with high yield via fusion of phthalic anhydride with 4-(4-aminophenyl)-phthalazin-1-(2H)-one (**1**) (prepared by reacting γ-keto acids with hydrazine hydrate in ethanol)[39–41] (Scheme 1). Phthalazinone **2** structure was improved on the basis of its spectral data. ^{1}H NMR spectrum exhibited two signals (exchangeable) at δ 7.99 and 13.20 ppm corresponding to NH and OH protons that certify existence of lactam-lactim forms of phthalazinone **2**. Also, IR spectrum showed absorption bands at ύ 3208 to 3085 cm^{-1}, 1746 to 1700 cm^{-1}, and 1685 cm^{-1} due to NH↔OH, 3CO, respectively (Scheme 1).



SCHEME 1 Synthesis of phthalazinone derivative **2**

Synthesis of 2-(4-(4-aminophthalazin-1-yl)phenyl)isoindoline-1,3-dione (**4**) and 2-(4-(4-mercaptophthalazin-1-yl)phenyl)isoindoline-1,3-dione (**5**) required as starting material was accomplished with a good yield via reaction of phthalazinone derivative **2** with phosphorus pentachloride and phosphorus oxychloride to yield chlorophthalazine **3**, which in turn reacts with ammonium acetate and thiourea to give aminophthalazine **4** and mercaptophthalazine **5**, respectively. Aminophthalazine derivative **4** was confirmed by spectral studies. ^{1}H NMR spectrum showed signal at δ 8.13 (s, 2H, NH_2, exchangeable with D_2O), 8.06 to 7.33 ppm (m, 12H, aromatic protons). Also, IR spectrum displayed absorption bands at ύ 3469 to 3230 cm^{-1} and 1780 and 1735 cm^{-1} due to NH_2 and CO imidic, respectively. And the structure of mercaptophthalazine improved by IR spectrum (cm^{-1}) showed bands at 2590 (SH), 1788 to 1730 (CO imidic), 1640 (C═N), and 1330 (C═S); also, ^{1}H NMR spectrum showed signal at δ 11.50 (s,1H,SH), 8.20 to 7.70 ppm (m, 12H, aromatic protons) (Scheme 2).

Phthalazines **2**, **4**, and **5** were used as reactive key precursors to originate a group of amino acids derivatives by treating them with several *N*-protected amino acids followed by estimating their antitumor, antioxidant, and DNA activity.

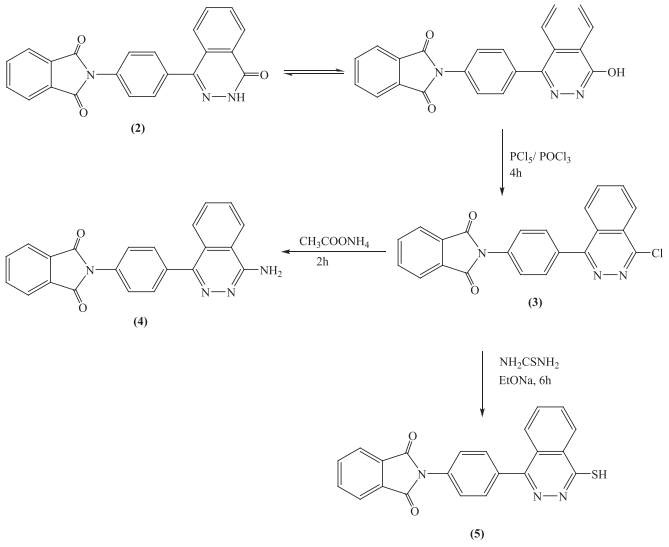
Consequently, phthalazines (**2**, **4**, and **5**) reacted with phthalyl derivatives of amino acids such as glycine, DL-alanine, DL-phenylalanine, L-valine, L-serine, and L-threonine as dehydrating agent to furnish the corresponding phthalazine derivatives **7** to **9a**-**f** (Scheme 3).

Deprotection of the amino group in 1-*N*-(arylaminoacycloxy)-2-[4-(4-oxo-phthalazin-1-yl)phenyl] isoindole-1,3-dione (**7a**-**f**), 1-*N*-(arylaminoacylamino)-2-[4-(4-oxo-phthalazin-1-yl)phenyl] isoindole-1,3-dione (**8a**-**f**), and 1-*N*-(arylaminoacylthio)-2-[4-(4-oxo-3,4-dihydrophthalazin-1-yl) phenyl]isoindole-1,3-dione (**9a**-**f**) was accomplished in good yield (70% to 75%) through their refluxing with ethanolic solution of hydrazine hydrate for 2 hours to afford unprotected aminoacyl derivatives (**10**-**12**)**a**-**f**, respectively (Scheme 3). Appearance of absorption band of amino group in compounds (**10**-**12**)**a**-**f** and disappearance of imidic group absorption confirm the deprotection process.

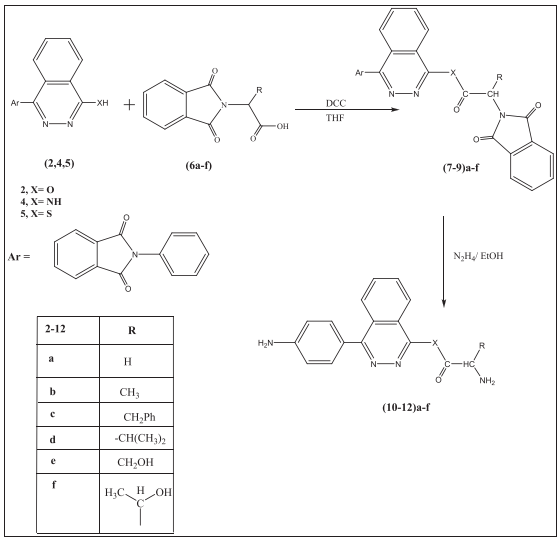
Similarly, phthalazines **2**, **4**, and **5** reacted with tosyl derivatives of the same amino acids to furnish phthalazine derivatives (**14**-**16**)**a**-**f** (Scheme 4).

3 | MATERIALS AND METHODS

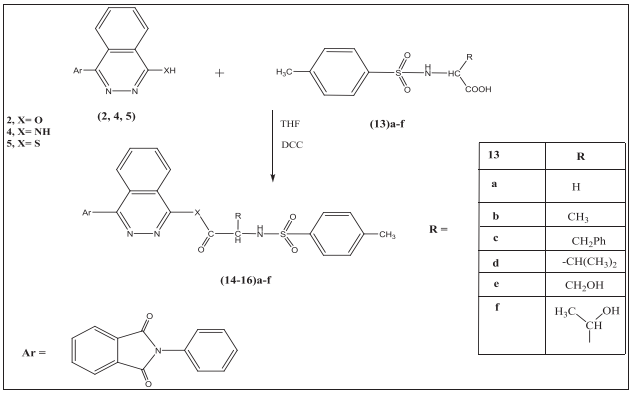
We got all chemical reagents from Sigma-Aldrich. Solvents were purchased from El-Nasr Chemicals in analytical grade. Also, we did thin-layer chromatography (TLC) on all products by using silica gel polyester sheets (Kieselgel 60 F254, 0.20 mm, Merck). All melting points are in degree centigrade (uncorrected). We recorded IR spectra (KBr) at the Microanalytical Center (Faculty of



SCHEME 2 Synthesis of phthalazines derivative (**4**,**5**)



SCHEME 3 Reactions of phthalazines with phthaloyl amino acids



SCHEME 4 Reaction of phthalazines with tosyl amino acids

Science; Mansoura University) on a Mattson 5000 FTIR Spectrophotometer. The ^{1}H NMR spectra were detected on a Varian Spectrophotometer at 300 MHz, using tetramethylsilane (TMS) as an internal reference and DMSO-d_6 as solvent at the Microanalytical Center (Faculty of Science, Ain Shams University).

Elemental analyses were performed on a CHN analyzer, and all compounds were within ±0.4 of the theoretical values. Pharmacological activities were carried in Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

4 | EXPERIMENTAL PROTOCOLS

4.1 | Chemistry

4.1.1 | Synthesis of 4-(4-aminophenyl) phthalazin-1-(2*H*)-one (1)

Ethanolic solution of hydrazine hydrate (0.01 mol) was added to a solution of 2-(4-aminobenzoyl)-benzoic acid (0.01 mol), and the solution was heated for 6 hours.[39–41] After cooling, we filtered off the precipitate and recrystallized dimethylformamide/water to produce the afforded compound **1**. Mp 250°C-252°C; yield 50%; IR (cm^{-1}) ν: 3295-3064 (NH_2, NH↔OH), 1685 (CO); Ms: *m/z* 237 (M^{+}); ^{1}H 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%.

4.1.2 | 2-[4-(4-Oxo-3,4-dihydrophthalazin-1-yl)-phenyl]-isoindole-1,3-dione(2)

Fusion of a mixture of compound **1** (0.01 mol) with phthalic anhydride (0.01 mol) at 250°C for 2 hours; after cooling, we added water to the residual product, and the solid that was obtained was filtered off and crystallized from DMF/H_2O to give yellow crystals.[17,18] Mp 305°C-307°C; yield 93%; IR (cm^{-1}): 3400-3200 (NH↔OH), 1780-1730 (2CO), 1600 (C═N); Ms: *m/z* 367 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 8.17 (s, 1H, NH exchangeable), 7.69-8.19 (m, 12H, Ar—H); ^{13}C NMR, 167.10, 165.02, 162.80, 159.40, 135.04, 134.71, 132.00, 131.10, 130.40, 129.45, 128.50, 127.51, 124.50, 123.70; 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%.

4.1.3 | Synthesis of 2-(4-(4-chlorophthalazin-1-yl)phenyl)isoindoline-1,3-dione (3)

A solution of phthalazinone **2** (0.01 mol) in phosphorus oxychloride (3 mL) and phosphorus pentachloride (0.01 mol) was heated for 4 hours in water bath. After cooling, pour the solution accurately into ice. The product was filtered, washed well with water, and crystallized from ethanol to give **3**. Yield, 80%; mp 130°C-132°C. IR spectrum (KBr, ν, cm^{-1}): 1780-1730 (CO imidic), 1662 (C═N), 835 (C—Cl); ^{1}H NMR ($CDCl_3$, δ, ppm): 8.31-7.03 (m, 12H, Ar—H); MS: *m/z* 385 (M^{+}), 386 (M^{+}); Anal. Calcd. for $C_{22}H_{12}ClN_3O_2$ (385.81): C, 68.49; H, 3.14; N, 10.89; Found: C, 68.40; H, 3.10; N, 10.82%.

Synthesis of 2-(4-(4-aminophthalazin-1-yl)phenyl) isoindoline-1,3-dione (4)

Fusion of an equimolar amount of chlorophthalazine **3** with ammonium acetate (0.01 mol) in oil bath for 2 hours. After cooling, add water to the formed solid, filter, dry, and crystallize from ethanol. Yield, 80%; mp 230°C-232°C. IR spectrum (cm^{-1}): 3469-3230 (NH_2), 3056 (CH— aromatic), 1780-1735 (CO imidic), 1704 (C═N); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.36 (s, 2H, NH_2, exchangeable), 8.30-7.88 (m, 12H,

Ar—H); ^{13}C NMR, 167.10, 166.02, 165.00, 152.32, 143.01, 133.01, 132.11, 131.8, 129.40, 128.50, 127.20, 126.20, 123.70, 119.30, 116.40; Anal. Calcd. for $C_{22}H_{14}N_4O_2$ (366.38): C, 72.12; H, 3.85; N, 15.29; Found: C, 72.01; H, 3.75; N, 15.20%.

Synthesis of 2-(4-(4-mercaptophthalazin-1-yl) phenyl)isoindoline-1,3-dione (5)

A solution of chlorophthalazine **3** (0.01 mol) in ethanol (20 mL) and thiourea (0.01 mol) containing sodium ethoxide was heated under reflux. After refluxing for 6 hours, pour the reaction mixture into ice cold water and then add acetic acid to acidify the product. The formed product was collected by filtration then dried and crystallized from ethanol to produce yellow crystals. Yield, 60%; mp 200°C-201°C. IR spectrum (cm^{-1}): 3460 (NH), 2590 (SH), 1788-1730 (CO imidic), 1640 (C═N), 1330 (C═S); ^{1}H NMR (DMSO-d_6, δ, ppm): 11.50 (s, 1H, NH↔SH, exchangeable), 8.20-7.70 (m, 12H, Ar—H); ^{13}C NMR, 172.73, 167.32, 165.33, 152.38, 143.34, 133.25, 132.69, 131.40, 129.40, 128.55, 127.90, 126.38, 123.42, 116.50, 56.50, 16.5; MS: *m/z* 383 (M^+); Anal. Calcd. for $C_{22}H_{13}N_3O_2S$ (383.43): C, 68.92; H, 3.42; N, 10.96; Found: C, 68.80; H, 3.32; N, 10.85%.

4.1.4 | General procedure for reaction of (2, 4, 5) with phthalylamino acids: Formation of (7-9)a-f

A well-stirred solution of phthalazine derivative **2**, amino phthalazine **4**, and mercaptophthalazine **5** (0.01 mol) in dry tetrahydrofurane (30 mL) and *N*-phthalayl amino acids, namely, glycine, DL-alanine, L-phenylalanine, DL-valine, L-serine, and threonine (0.01 mol), and dicyclohexylcarbodiimide (0.01 mol) were added at 0°C. for 24 hours at 0°C, the reaction mixture was stirred, and stirring was continued at room temperature for another 24 hours. The residual *N*,*N*-dicyclohexylurea was filtrated from the solution. The filterate was evaporated in vacuo, and the formed product dissolved in ethylacetate (25 mL), and the solution was filtrated again from the residual *N*,*N*-dicyclohexylurea. The products were attained by evaporation of filtrate in vacuo and recrystallized from proper solvent to give compounds (**7-9**)**a-f**.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl 2-(1,3-dioxoisoindolin-2-yl)acetate (7a)

Yield, 70%; mp 265°C-267°C; IR (cm^{-1}): υ_{max}: 1781-1733 (imidic CO), 1710 (C═O ester), 1591 (C═N); Ms: *m/z* 554 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.43-8.57 (m, 16H, Ar—H), 4.40 (s, 2H, CH_2); ^{13}C NMR, 171.20, 168.10, 167.02, 166.00, 165.12, 152.00, 133.51, 132.01, 129.40, 128.50, 127.50, 126.70,123.70, 119.71, 43.11; Anal. Calcd. for $C_{32}H_{18}N_4O_6$ (Mol.wt.554): C, 69.31; H, 3.27; N, 10.10; Found: C, 69.21; H, 3.20; N, 10.01%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl 2-(1,3-dioxoisoindolin-2-yl)propanoate (7b)

Yield, 60%; mp 252°C-254°C; IR (cm^{-1}): υ_{max}: 1780-1734 (imidic CO), 1704 (C═O of ester), 1591 (C═N); Ms: *m/z* 568 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.42-7.97 (m, 16H, Ar—H), 4.30 (q, 1H, *J* = 7.3, CH), 1.71 (d, 3H, *J* = 7.5, CH_3); ^{13}C NMR, 172.73, 167.32, 165.33, 152.38, 143.34, 133.25, 132.69, 131.40, 129.40, 128.55, 127.90, 126.38, 123.42, 116.50, 56.50, 16.50; Anal. Calcd. for $C_{33}H_{20}N_4O_6$ (Mol.wt.568): C, 69.72; H, 3.55; N, 9.85; Found: C, 69.62; H, 3.50; N, 9.81%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl 2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoate (7c)

Yield, 60%; mp 200°C-202°C; IR (cm^{-1}): υ_{max}: 1778-1730 (imidic CO), 1715 (C═O of ester), 1607 (C═N); Ms: *m/z* 630 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.43-7.97 (m, 20H, Ar—H), 4.47(t, 1H, *J* = 7.2, CH), 3.33 (d, 2H, *J* = 6.8, CH_2); Anal. Calcd. for $C_{39}H_{24}N_4O_6$ (Mol.wt.644): C, 72.66; H, 3.75; N, 8.69; Found: C, 72.66; H, 3.65; N, 8.60%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl 2-(1,3-dioxoisoindolin-2-yl)-3-methylbutanoate (7d)

Yield, 65%; mp 210°C-212°C; IR (cm^{-1}): υ_{max}: 1780-1727 (imidic CO), 1710 (C═O of ester), 1591 (C═N); Ms: *m/z* 596 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.27-8.27 (m, 16H, Ar—H), 4.32 (m, 1H, CH), 1.09 (d, 6H, *J* = 6.8, $2CH_3$); ^{13}C NMR, 168.10, 167.19, 166.00, 152.40, 150.04, 133.11, 132.00, 129.45, 128.50, 127.51, 126.70, 123.70, 66.4, 27.8, 18.9; Anal. Calcd. for $C_{35}H_{24}N_4O_6$ (Mol.wt.596): C, 70.46; H, 4.05; N, 9.39; Found: C, 70.36; H, 4.00; N, 9.28%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl 2-(1,3-dioxoisoindolin-2-yl)-3-hydroxypropanoate (7e)

Yield, 70%; mp 230°C-232°C; IR (cm^{-1}): υ_{max}: 3468 (OH), 1780-1733 (imidic CO), 1700 (C═O of ester), 1607 (C═N); Ms: *m/z* 584 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 5.55 (s, 1H, OH, exchangeable with D_2O), 7.43-7.95 (m, 16H, Ar—H), 4.43 (q, 1H, *J* = 7.1, CH), 4.10 (d, 2H, *J* = 7.5, CH); ^{13}C NMR, 197.60, 193.01, 165.33, 167.10, 165.00, 152.05, 132.01, 129.40, 128.00, 127.70, 126.60, 133.10, 123.70, 72.90, 59.30; Anal. Calcd. for $C_{33}H_{20}N_4O_7$ (Mol.wt.584): C, 67.81; H, 3.45; N, 9.58; Found: C, 67.71; H, 3.40; N, 9.50%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl 2-(1,3-dioxoisoindolin-2-yl)-3-hydroxybutanoate (7f)

Yield, 65%; mp 253°C-255°C; IR (cm^{-1}): υ_{max}: 3377 (OH), 1775-1735 (imidic CO), 1701 (C═O of ester), 1608 (C═N);

Ms: *m/z* 598 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 5.59 (s, 1H, OH), 7.42-7.97 (m, 16H, Ar—H), 4.95 (q, 1H, *J* = 6.8, CH), 4.30 (d, 1H, *J* = 7.0, CH), 1.22 (d, 3H, *J* = 6.8, CH_3); Anal. Calcd. for $C_{34}H_{22}N_4O_7$ (Mol.wt.598): C, 68.22; H, 3.70; N, 9.36; Found: C, 68.15; H, 3.60; N, 9.30%.

2-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-(4-(1,3-dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl) acetamide (8a)

Yield, 60%; mp 250°C-252°C; IR (cm^{-1}): υ_{max}: 3350-3250 (NH), 1780-1735 (imidic CO), 1701 (C═O of amide), 1608 (C═N); Ms: *m/z* 553 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 9.11 (s,1H, NH, exchangeable), 7.60-7.97 (m, 16H, Ar—H), 3.83 (s, 2H, CH_2); ^{13}C NMR, 168.50, 167.12, 166.00, 165.10, 152.00, 143.51, 133.1, 132.00, 131.81, 129.41, 128.50, 127.20, 126.90, 123.70, 119.71, 116.40, 47.17; Anal. Calcd. for $C_{32}H_{19}N_5O_5$ (Mol.wt.553): C, 69.44; H, 3.46; N, 12.65; Found: C, 69.34; H, 3.40; N, 12.55%.

2-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-(4-(1,3-dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl) propanamide (8b)

Yield, 65%; mp 230°C-232°C; IR (cm^{-1}): υ_{max}: 3450-3200 (NH), 1780-1735 (imidic CO), 1701 (C═O of amide), 1608 (C═N); Ms: *m/z* 567 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 10.22 (s,1H, NH, exchangeable), 7.60-7.97 (m, 16H, Ar—H), 3.53 (q, 1H, *J* = 7.5, CH), 1.81(d, 3H, *J* = 7, CH_3); ^{13}C NMR, 172.73, 167.17, 166.20, 143.69, 132.20, 131.80, 129.40, 128.65, 127.70, 126.92, 123.71, 119.30, 116.40, 56.80, 16.50; Anal. Calcd. for $C_{33}H_{21}N_5O_5$ (Mol.wt.567): C, 69.84; H, 3.73; N, 12.34; Found: C, 69.74; H, 3.63; N, 12.23%.

2-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-(4-(1,3-dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl)-3-phenylpropanamide (8c)

Yield, 70%; mp 210°C-212°C; IR (cm^{-1}): υ_{max}: 3380-3220 (NH), 1780-1735 (imidic CO), 1701 (C═O of amide), 1608 (C═N); Ms: *m/z* 630 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 8.23 (s,1H, NH, exchangeable), 7.40-7.87 (m, 16H, Ar—H), 4,40 (t, 1H, *J* = 6.5, CH), 3.43 (d, 2H, *J* = 8.1, CH_2); ^{13}C NMR, 171.12, 170.00, 168.20, 167.11, 166.02, 165.45, 152.78, 150.44, 135.91, 133.43, 132.54, 131.85, 129.40, 128.50, 127.51, 126.72, 119.71, 62.84, 34.70; Anal. Calcd. for $C_{39}H_{25}N_5O_5$ (Mol.wt.643): C, 72.78; H, 3.92; N, 10.88; Found: C, 72.70; H, 3.81; N, 10.80%.

2-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-(4-(1,3-dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl)-3-methylbutanamide (8d)

Yield, 75%; mp 240°C-242°C; IR (cm^{-1}): υ_{max}: 3400-3200 (NH), 1780-1735 (imidic CO), 1701 (C═O of amide), 1608 (C═N); Ms: *m/z* 595 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 9.23 (s,1H, NH, exchangeable), 7.40-7.87 (m, 16H, Ar—H), 4.50 (d, 1H, *J* = 7.8, CH), 2.87 (m, 1H, CH), 1.22 (d, 6H, *J* = 6.8, 7.3, $2CH_3$); Anal. Calcd. for $C_{35}H_{25}N_5O_5$ (Mol. wt.595): C, 70.58; H, 4.23; N, 11.76; Found: C, 70.50; H, 4.13; N, 11.64%.

2-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-(4-(1,3-dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl)-3-hydroxypropanamide (8e)

Yield, 70%; mp 201°C-203°C; IR (cm^{-1}): υ_{max}: 3420-3260 (NH, OH), 1780-1735 (imidic CO), 1701 (C═O of amide), 1608 (C═N); Ms: *m/z* 583 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 10.55 (s,1H, NH, exchangeable), 7.40-7.87 (m, 16H, Ar—H), 4.95 (s, 1H, OH), 4.32 (t, 1H, *J* = 6.5, CH), 3.43 (d, 2H, *J* = 8.2, CH_2); Anal. Calcd. for $C_{33}H_{21}N_5O_6$ (Mol. wt.583): C, 67.92; H, 3.63; N, 12.00; Found: C, 67.82; H, 3.53; N, 11.95%.

2-(1,3-Dioxoisoindolin-2-yl)-*N*-(4-(4-(1,3-dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl)-3-hydroxybutanamide (8f)

Yield, 70%; mp 201°C-203°C; IR (cm^{-1}): υ_{max}: 3420-3260 (NH, OH), 1780-1735 (imidic CO), 1701 (C═O of amide), 1608 (C═N); Ms: *m/z* 597 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 8.80 (s,1H, NH), 7.40-7.87 (m, 16H, Ar—H), 5.50 (s, 1H, OH), 4.52 (d, 1H, *J* = 8.5, CH), 2.23 (q, 1H, *J* = 7.3, CH), 1.20 (d, 3H, *J* = 6.8, CH_3); Anal. Calcd. for $C_{34}H_{23}N_5O_6$ (Mol.wt.597): C, 68.34; H, 3.88; N, 11.72; Found: C, 68.25; H, 3.80; N, 11.62%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 2-(1,3-dioxoisoindolin-2-yl) ethanethioate (9a)

Yield, 60%; mp 230°C-232°C; IR (cm^{-1}): υ_{max}: 1760-1730 (imidic CO), 1720 (C═O of ester), 1660 (C═N); Ms: *m/z* 570 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 7.40-7.87 (m, 16H, Ar—H), 4.52 (s, 2H, CH_2); ^{13}C NMR, 196.53, 193.02, 167.01, 165.02, 152.11,133.14, 132.05, 129.40, 128.50, 127.70, 126.60, 123.75, 57.40; Anal. Calcd. for $C_{32}H_{19}N_4O_5S$ (Mol.wt.570): C, 67.36; H, 3.18; N, 9.82; Found: C, 67.30; H, 3.10; N, 9.73%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 2-(1,3-dioxoisoindolin-2-yl) propanethioate (9b)

Yield, 65%; mp 210°C-212°C; IR (cm^{-1}): υ_{max}: 1770-1735 (imidic CO), 1710 (C═O of ester), 1600 (C═N); Ms: *m/z* 584 (M^{+}); ^{1}H NMR (DMSO-d_6) δ: 7.40-8.12 (m, 16H, Ar—H), 4.47 (q, 1H, *J* = 6.3, CH), 1.18 (d, 3H, *J* = 6.8, CH_3); Anal. Calcd. for $C_{33}H_{20}N_4O_5S$ (Mol.wt.584): C,

67.80; H, 3.45; N, 9.58; Found: C, 67.71; H, 3.36; N, 9.48%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanethioate (9c)

Yield, 68%; mp 240°C-245°C; IR (cm^{-1}): υ_{max}: 1780-1730 (imidic CO), 1715 (C═O of ester), 1610 (C═N); Ms: *m/z* 646 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.45-8.22 (m, 16H, Ar─H), 3.40 (t, 1H, J = 7.2,CH), 2.89(d, 2H, J = 7.49, CH_2); ^{13}C NMR, 197.62, 167.10, 152.08, 136.64, 133.61, 132.70, 129.40, 128.60, 127.74, 126.62, 125.90, 123.01, 72.04, 35.30; Anal. Calcd. for $C_{39}H_{24}N_4O_5S$ (Mol.wt.660): C, 70.90; H, 3.66; N, 8.48; Found: C, 70.80; H, 3.60; N, 8.44%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 2-(1,3-dioxoisoindolin-2-yl)-3-methylbutanethioate (9d)

Yield, 70%; mp 240°C-245°C; IR (cm^{-1}): υ_{max}: 1780-1730 (imidic CO), 1715 (C═O of ester), 1610 (C═N); Ms: *m/z* 612 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.45-8.22 (m, 16H, Ar─H), 3.40 (s, 1H, CH), 2.55(m, 1H, CH), 1.19 (d, 6H, J = 8.45, $2CH_3$); ^{13}C NMR, 197.62, 167.90, 152.00, 133.11, 132.21, 129.40, 127.71, 126.62, 123.70, 76.80, 29.00, 19.45; Anal. Calcd. for $C_{35}H_{24}N_4O_5S$ (Mol.wt.612): C, 68.62; H, 3.95; N, 9.15; Found: C, 68.52; H, 3.85; N, 9.08%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 2-(1,3-dioxoisoindolin-2-yl)-3-hydroxypropanethio-ate (9e)

Yield, 60%; mp 230°C-235°C; IR (cm^{-1}): υ_{max}: 1780-1730 (imidic CO), 1715 (C═O of ester), 1610 (C═N); Ms: *m/z* 600 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.45-8.22 (m, 16H, Ar─H), 4.94 (s, 1H, OH), 4.40 (t, 1H, J = 7.5, CH), 3.92 (d, 2H, J = 7.1, CH_2); Anal. Calcd. for $C_{33}H_{20}N_4O_6S$ (Mol. wt.600): C, 65.99; H, 3.36; N, 9.33; Found: C, 65.89; H, 3.30; N, 9.23%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 2-(1,3-dioxoisoindolin-2-yl)-3-hydroxybutanethioate (9f)

Yield, 60%; mp 250°C-252°C; IR (cm^{-1}): υ_{max}: 1780-1730 (imidic CO), 1715 (C═O of ester), 1610 (C═N); Ms: *m/z* 614 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.45-8.22 (m, 16H, Ar─H), 5.56 (s, 1H, OH), 4.40 (d, 1H, J = 6.9, CH), 2.75(m, 1H, CH), 1.18 (d, 3H, J = 7.5, CH_3); ^{13}C NMR, 197.62, 167.10, 152.00, 133.11, 132.21, 129.40, 128.60, 127.71, 126.61, 123.70, 77.50, 65.50, 19.50; Anal. Calcd. for $C_{34}H_{22}N_4O_6S$ (Mol.wt.614): C, 66.44; H, 3.61; N, 9.12; Found: C, 66.32; H, 3.52; N, 9.01%.

4.1.5 | General procedure for reaction of (7, 8, 9)a-f with hydrazine hydrate: Formation of compounds (10-12)a-f

To a solution of compounds (**7**, **8**, **9**)**a**-**f** (0.01 mol) in absolute ethanol (20 mL), hydrazine hydrate (0.01 mol) was added and refluxed for 2 hours, then left for 24 hours at room temperature. Evaporation of the solvent in vacuo resulted in a solid material, which in return was added to water (10 mL) and the solution acidified with acetic acid till (pH = 6), then heated for 1 hour on steam bath; the suspension was diluted with water (15 mL) and filtrated off. The filtrate was concentrated and cooled to produce the solid, which crystallized from proper solvent to obtain (**10**-**12**)**a**-**f**.

4-(4-Aminophenyl)phthalazin-1-yl glycinate (10a)

Yield, 75%; mp 240°C-242°C; IR (cm^{-1}): υ_{max}: 3360-3210 ($2NH_2$), 1730 (C═O of ester), 1610 (C═N). The vanishing of bands corresponding to CO of cyclic imide at 1780, 1730, was highly informative and established the accomplishment of deprotection process; Ms: *m/z* 294 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 8.50 (s, 2H, NH_2), 7.43-7.97 (m, 8H, Ar─H), 4.53 (s, 2H, CH_2); ^{13}C NMR, 171.00, 168.11, 150.00, 145.60, 133.51, 131.81, 128.30, 127.40, 126.60, 123.00, 119.10, 115.92, 39.90; Anal. Calcd. for $C_{16}H_{14}N_4O_2$ (Mol.wt.294); C, 65.30; H, 4.79; N, 19.04; Found: C, 65.22; H, 4.70; N, 18.00%.

4-(4-Aminophenyl)phthalazin-1-yl alaninate (10b)

Yield, 69%; mp 235°C-238°C; IR (cm^{-1}): υ_{max}: 3310-3100 ($2NH_2$), 1730 (C═O of ester), 1600 (C═N); Ms: *m/z* 308 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 8.76 (s, 2H, NH_2), 7.50-7.93 (m, 8H, Ar─H), 5.52 (s, 2H, NH_2), 3.53 (q, 1H, J = 7.2, CH), 1.33 (d, 3H, J = 6.8, CH_3); ^{13}C NMR, 171.70, 150.31, 145.61, 143.00, 132.80, 131.80, 128.30, 127.60, 126.70, 123.02, 119.70, 116.40, 115.11, 49.51, 16.80; Anal. Calcd. for $C_{17}H_{16}N_4O_2$ (Mol.wt.308); C, 66.22; H, 5.23; N, 18.17; Found: C, 66.20; H, 5.20; N, 18.00%.

4-(4-Aminophenyl)phthalazin-1-yl 2-amino-2-phenyla-cetate (10c)

Yield, 75%; mp 239°C-241°C; IR (cm^{-1}): υ_{max}: 3450-3200 ($2NH_2$), 1730 (C═O of ester), 1600 (C═N); Ms: *m/z* 500 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 8.40 (s, 2H, NH_2), 7.50-7.80 (m, 12H, Ar─H), 4.53 (t, 1H, J = 6.5, CH), 2.46 (d, 2H, J = 7.5, CH_2); Anal. Calcd. for $C_{22}H_{18}N_4O_2$ (Mol. wt.370); C, 71.34; H, 4.90; N, 15.13; Found: C, 71.30; H, 4.80; N, 15.10%.

4-(4-Aminophenyl)phthalazin-1-yl valinate (10d)

Yield, 70%; mp 230°C-232°C; IR (cm^{-1}): υ_{max}: 3380-3150 ($2NH_2$), 1730 (C═O of ester), 1600 (C═N); Ms: *m/z* 466 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 8.11 (s, 2H, NH_2),

7.43-7.80 (m, 12H, Ar—H), 4.53 (d, 1H, J = 8.32, CH), 2.15 (m, 1H, CH), 1.46 (d, 6H, J = 7.5, CH_3); ^{13}C NMR, 171.00, 168.30, 167.10, 166.01, 165.01, 152.01,133.50, 132.00, 129.40, 128.50, 127.70, 126.70, 119.70, 59.00, 30.40, 18.90; Anal. Calcd. for $C_{19}H_{20}N_4O_2$ (Mol. wt.336); C, 67.84; H, 5.99; N, 16.66; Found: C, 67.80; H, 5.90; N, 16.60%.

4-(4-Aminophenyl)phthalazin-1-yl serinate (10e)

Yield, 70%; mp 229°C-231°C; IR (cm^{-1}): υ_{max}: 3400-3050 ($2NH_2$), 1730 (C═O of ester), 1600 (C═N); ^{1}H NMR (DMSO-d_6) δ: 8.52 (s, 2H, NH_2), 7.39-7.95 (m, 12H, Ar—H), 5.45 (s, 1H, OH), 4.53 (t, 1H, J = 7.5, CH), 3.47 (d, 2H, J = 6.8, CH_2); Ms: m/z 324 ($M^{\cdot+}$); Anal. Calcd. for $C_{17}H_{16}N_4O_3$ (Mol.wt.324); C, 62.95; H, 4.97; N, 17.27; Found: C, 62.90; H, 4.90; N, 17.20%.

4-(4-Aminophenyl)phthalazin-1-yl 2-amino-3-hydroxy-butanoate (10f)

Yield, 78%; mp 201°C-203°C; IR (cm^{-1}): υ_{max}: 3300-3180 ($2NH_2$), 1730 (C═O of ester), 1600 (C═N); ^{1}H NMR (DMSO-d_6) δ: 8.96 (s,2H,NH_2), 7.32-7.93 (m, 12H, Ar—H), 5.37 (s, 1H, OH), 4.28 (d, 1H, J = 7, CH), 3.36 (q, 1H, J = 7, CH), 1.16 (d, 3H, J = 7.5, CH_2); ^{13}C NMR, 171.00, 168.30, 167.10, 150.01,133.50, 132.00, 129.40, 128.50, 127.70, 123.70, 119.70, 67.40, 60.50, 19.42; Ms: m/z 338 ($M^{\cdot+}$); Anal. Calcd. for $C_{18}H_{18}N_4O_3$ (Mol.wt.338.14); C, 63.89; H, 5.36; N, 16.56; Found: C, 63.80; H, 5.30; N, 16.50%.

2-Amino-*N*-(4-(4-aminophenyl)phthalazin-1-yl) acetamide (11a)

Yield, 78%; mp 201°C-203°C; IR (cm^{-1}): υ_{max}: 3400-3250 (NH, $2NH_2$), 1680 (C═O amide), 1600 (C═N); ^{1}H NMR (DMSO-d_6) δ: 8.60 (s, 1H, NH, exchangeable), 7.50 (s, 2H, NH_2, exchangeable), 7.60-7.95 (m, 12H, Ar—H), 4.41 (s, 2H, CH_2); Ms: m/z 468 ($M^{\cdot+}$); Anal. Calcd. for $C_{16}H_{15}N_5O$ (Mol.wt.293); C, 65.52; H, 5.15; N, 23.88; Found: C, 65.41; H, 5.10; N, 23.80%.

2-Amino-*N*-(4-(4-aminophenyl)phthalazin-1-yl) propanamide (11b)

Yield, 80%; mp 198°C-200°C; IR (cm^{-1}): υ_{max}: 3360-3010 (NH, $2NH_2$), 1660 (C═O amide), 1620 (C═N); ^{1}H NMR (DMSO-d_6) δ: 9.50 (s, 1H, NH, exchangeable), 7.42 (s, 2H, NH_2, exchangeable), 7.43-8.11 (m, 12H, Ar—H), 4.47 (q, 1H, J = 7.3, CH), 1.25 (d, 3H, J = 7, CH_3); ^{13}C NMR, 172.70, 167.10, 166.10, 143.01, 132.21, 131.80, 129.40, 128.60, 127.70, 123.70, 119.70, 116.40, 49.50, 16.80; Ms: m/z 437 ($M^{\cdot+}$); Anal. Calcd. for $C_{17}H_{17}N_5O$ (Mol. wt.307); C, 66.43; H, 5.58; N, 22.79; Found: C, 66.40; H, 5.50; N, 22.70%.

2-Amino-*N*-(4-(4-aminophenyl)phthalazin-1-yl)-3-phenyl propanamide (11c)

Yield, 70%; mp 240°C-242°C; IR (cm^{-1}): υ_{max}: 3350-3100 (NH, $2NH_2$), 1700 (C═O amide), 1620 (C═N); ^{1}H NMR (DMSO-d_6) δ: 10.61 (s, 1H, NH, exchangeable), 8.86 (s, 2H, NH_2, exchangeable), 7.14-7.86 (m, 17H, Ar—H), 4.53 (t, 1H, J = 6.8, CH), 3.16 (d, 2H, J = 6.8, CH_2); Ms: m/z 500 ($M^{\cdot+}$); Anal. Calcd. for $C_{22}H_{19}N_5O$ (Mol.wt.369); C, 71.53; H, 5.18; N, 18.96; Found: C, 71.50; H, 5.10; N, 18.90%.

2-Amino-*N*-(4-(4-aminophenyl)phthalazin-1-yl)-3-methylbutanamide (11d)

Yield, 78%; mp 250°C-254°C; IR (cm^{-1}): υ_{max}: 3400-3200 (NH, $2NH_2$), 1690 (C═O amide), 1620 (C═N); ^{1}H NMR (DMSO-d_6) δ: 10.21 (s, 1H, NH), 8.33 (s, 2H, NH_2), 7.33-7.80 (m, 12H, Ar—H), 4.43 (d, 1H, J = 7.5, CH), 2.30 (m, 1H, CH), 1.26 (d, 6H, J = 6.5, CH_3); Ms: m/z 335 ($M^{\cdot+}$); Anal. Calcd. for $C_{19}H_{21}N_5O$ (Mol.wt.335); C, 68.04; H, 6.31; N, 20.88; Found: C, 68.00; H, 6.21; N, 20.80%.

2-Amino-*N*-(4-(4-aminophenyl)phthalazin-1-yl)-3-hydroxypropanamide (11e)

Yield, 80%; mp 210°C-212°C; IR (cm^{-1}): υ_{max}: 3350 (NH, $2NH_2$), 3100 (C—H aromatic), 2940 (C—H aliphatic), 1698 (C═O amide), 1620 (C═N); Ms: m/z 323 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 10.43(s, 1H, NH, exchangeable), 8.50 (s, 2H, NH_2, exchangeable), 7.43-7.97 (m, 12H, Ar—H), 4.53 (t, 1H, J = 6.5, CH), 3.16 (d, 2H, J = 6.8, CH_2); Anal. Calcd. for $C_{17}H_{17}N_5O_2$ (Mol.wt.323); C, 63.15; H, 5.30; N, 21.66; Found: C, 63.10; H, 5.20; N, 21.60%.

2-Amino-*N*-(4-(4-aminophenyl)phthalazin-1-yl)-3-hydroxybutanamide (11f)

Yield, 70%; mp 210°C-212°C; IR (cm^{-1}): υ_{max}: 3350 (NH, $2NH_2$), 3100 (C—H aromatic), 2940 (C—H aliphatic), 1680 (C═O of amide), 1620 (C═N); Ms: m/z 467 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 9.43 (s, 1H, NH), 8.87 (s, 2H, NH_2), 7.43-7.80 (m, 12H, Ar—H), 4.53 (d, 1H, J = 7.5, CH), 3.36 (m, 1H, CH), 1.20 (d, 3H, J = 6.8, CH_3); Anal. Calcd. for $C_{18}H_{19}N_5O_2$ (Mol.wt.337); C, 64.08; H, 5.68; N, 20.76; Found: C, 64.00; H, 5.60; N, 20.70%.

S-(4-(4-Aminophenyl)phthalazin-1-yl) 2-aminoethanethioate (12a)

Yield, 60%; mp 301°C-303°C; IR (cm^{-1}): υ_{max}: 3350-3020 ($2NH_2$), 1710 (C═O), 1620 (C═N); Ms: m/z 440 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 8.50 (s, 2H, NH_2), 7.43-7.97 (m, 12H, Ar—H), 4.53 (s, 2H, CH_2); Anal. Calcd. for $C_{16}H_{14}N_4OS$ (Mol.wt.310); C, 61.92; H, 4.55; N, 18.05; Found: C, 61.87; H, 4.50; N, 18.00%.

S-(4-(4-Aminophenyl)phthalazin-1-yl) 2-aminopropanethioate (12b)

Yield, 65%; mp 312°C-314°C; IR (cm^{-1}): υ_{max}: 3400-3150 ($2NH_2$), 1700 (C═O), 1620 (C═N); Ms: *m/z* 454 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 8.50 (s, 2H, NH_2, exchangeable), 7.40-8.15 (m, 12H, Ar—H), 3.97 (q, 1H, *J* = 7.1, CH), 1.09 (d, 3H, *J* = 6.8, CH_3); Anal. Calcd. for $C_{17}H_{16}N_4OS$ (Mol. wt.324); C, 62.94; H, 4.97; N, 17.27; Found: C, 62.90; H, 4.90; N, 17.17%.

S-(4-(4-Aminophenyl)phthalazin-1-yl) 2-amino-3-methylbutanethioate (12c)

Yield, 69%; mp 210°C-212°C; IR (cm^{-1}): υ_{max}: 3380-3100 ($2NH_2$), 3100 (C—H aromatic), 1690 (C═O), 1620 (C═N); ^1H NMR (DMSO-d_6) δ: 8.11 (s, 2H, NH_2, exchangeable), 7.10-7.78 (m, 17H, Ar—H), 4.53 (t, 1H, *J* = 7.1, CH), 3.16 (d, 2H, *J* = 6.5, CH_2); Ms: *m/z* 352 ($M^{\cdot+}$); Anal. Calcd. for $C_{19}H_{20}N_4OS$ (Mol.wt.352); C, 64.75; H, 5.72; N, 15.90; Found: C, 64.70; H, 5.62; N, 15.80%.

S-(4-(4-Aminophenyl)phthalazin-1-yl) 2-amino-3-me-thylbutanethioate (12d)

Yield, 45%; mp 330°C-332°C; IR (cm^{-1}): υ_{max}: 3300-3280 ($2NH_2$), 3100 (C—H aromatic), 2940 (C—H aliphatic), 1705 (C═O), 1620 (C═N); Ms: *m/z* 352 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 7.50 (s, 2H, NH_2), 7.43-7.97 (m, 16H, Ar—H), 4.67 (d, 1H, *J* = 6.2, CH), 2.46 (m, 1H, CH), 1.01 (d, 6H, *J* = 7.5, $2CH_3$); Anal. Calcd. for $C_{19}H_{20}N_4OS$ (Mol.wt.352); C, 64.75; H, 5.72; N, 15.90; Found: C, 64.70; H, 5.62; N, 15.80%.

S-(4-(4-Aminophenyl)phthalazin-1-yl) 2-amino-3-hydro-xypropanethioate (12e)

Yield, 50%; mp 338°C-340°C; IR (cm^{-1}): υ_{max}: 3390-3250 ($2NH_2$), 1730 (C═O of ester), 1620 (C═N); Ms: *m/z* 340 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 7.20 (s, 2H, NH_2), 7.55-7.87 (m, 12H, Ar—H), 4.42 (t, 1H, *J* = 7, CH), 2.46 (d, 2H, *J* = 6.8, CH_2), 5.50 (s, 1H, OH); ^{13}C NMR, 197.60, 167.30, 152.01,133.10, 132.20, 129.40, 128.50, 127.70, 126.40, 123.70, 71.10, 64.70; Anal. Calcd. for $C_{17}H_{16}N_4O_2S$ (Mol.wt.340); C, 59.98; H, 4.74; N, 16.46; Found: C, 59.90; H, 4.70; N, 16.40%.

S-(4-(4-Aminophenyl)phthalazin-1-yl) 2-amino-3-hydro-xybutanethioate (12f)

Yield, 70%; mp 338°C-340°C; IR (cm^{-1}): υ_{max}: 3300-3260 ($2NH_2$), 1730 (C═O), 1620 (C═N); Ms: *m/z* 484 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 7.44 (s, 2H, NH_2), 7.43-7.97 (m, 12H, Ar—H), 4.03 (d, 1H, *J* = 8, CH), 2.11 (m, 1H, CH), 5.22 (s, 1H, OH); Anal. Calcd. for $C_{18}H_{18}N_4O_2S$ (Mol.wt.354); C, 61.00; H, 5.12; N, 15.81; Found: C, 60.90; H, 5.02; N, 15.71%.

4.1.6 | General procedure for reaction of (2, 4, 5) with *N*-tosyl-amino acids: Formation of (14-16)a-f

Similarly, phthalazine-1(1*H*)-one **2**, aminophthalazine **4**, and mercaptophthalazine **5** reacted with *N*-tosylamino acids **13a-f** in presence of DCCI under the same reaction conditions to give to afforded compounds (**14-16**)**a-f**.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl tosylglycinate (14a)

Yield, 75%; mp 230°C-232°C; IR (cm^{-1}): υ_{max}: 3430 (NH), 1781-1700 (3CO), 1593 (C═N), 1380 (SO_2); Ms: *m/z* 578 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 7.81 (s, 1H, NH), 7.32-7.95 (m, 16H, Ar—H), 4.34 (s, 2H, CH_2), 2.35 (s, 3H, CH_3); ^{13}C NMR, 171.00, 168.30, 167.10, 150.01,136.30, 133.50, 132.00, 129.40, 128.50, 127.70, 126.62, 123.70, 119.70, 43.80; Anal. Calcd. for $C_{31}H_{22}N_4O_6S$ (Mol.wt.578); C, 64.35; H, 3.83; N, 9.68; Found: C, 64.25; H, 3.80; N, 9.62%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl tosylalaninate (14b)

Yield, 70%; mp 235°C-237°C; IR (cm^{-1}): υ_{max}: 3368 (NH), 1781-1701 (3CO), 1607 (C═N), 1381 (SO_2); Ms: *m/z* 592 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 10.90 (s, 1H, NH), 7.03-7.76 (m, 16H, Ar—H), 4.57 (s, 1H, CH), 1.31 (q, 3H, *J* = 7.2, CH_3), 2.38 (s, 3H, CH_3—Ar); Anal. Calcd. for $C_{32}H_{24}N_4O_6S$ (Mol.wt.592); C, 64.86; H, 4.08; N, 9.45; Found: C, 64.80; H, 4.01; N, 9.40%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl tosylphenylalaninate (14c)

Yield, 65%; mp 240°C-242°C; IR (cm^{-1}): υ_{max}: 3300 (NH), 1781-1735 (3CO), 1589 (C═N), 1382 (SO_2); Ms: *m/z* 668 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 11.44 (s,1H, NH), 7.02-8.28 (m, 16H, Ar—H), 4.89 (t, 1H, *J* = 8.2, CH), 2.68 (d, 2H, *J* = 7.5, CH_2), 2.28 (s, 3H, CH_3); ^{13}C NMR, 171.00, 168.30, 167.10, 150.01,136.30, 141.50, 137.50, 133.50, 132.00, 129.40, 128.50, 127.70, 126.62, 123.70, 119.70, 58.30, 35.82, 21.30; Anal. Calcd. for $C_{38}H_{28}N_4O_6S$ (Mol.wt.668); C, 68.25; H, 4.22; N, 8.38; Found: C, 68.20; H, 4.21; N, 8.30%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl-tosylvalinate (14d)

Yield, 70%; mp 215°C-217°C; IR (cm^{-1}): υ_{max}: 3328 (NH),1785-1700 (3CO), 1575 (C═N), 1329 (SO_2); ^1H NMR (DMSO-d_6) δ: 10.10 (s, 2H, NH_2), 7.18-7.30 (m, 16H, Ar—H), 5.12 (d, 1H, *J* = 6.3, CH), 3.13-3.46 (m, 1H, CH), 2.45 (s, 3H, CH_3—Ar), 1.01 (d, 6H, *J* = 7.2, $2CH_3$); Anal. Calcd. for $C_{34}H_{28}N_4O_6S$ (Mol.wt.620); Found: C, 65.79; H, 4.55; N, 9.03; O, 15.47; S, 5.17%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl-tosylserinate (14e)

Yield, 75%; mp 220°C-222°C; IR (cm^{-1}): υ_{max}: 3330 (NH, OH), 3050 (C—H aromatic), 2950 (C—H aliphatic), 1780-1700 (3C═O), 1600 (C═N), 1330 (SO_2); ^1H NMR (DMSO-d_6) δ: 8.99 (s,1H, NH), 7.43-7.96 (m, 16H, Ar—H), 5.01(s,1H, OH), 2.34 (d, 2H, J = 6.4, CH_2), 4.22 (t, 1H, J = 6.8, CH), 2.80 (s, 3H, CH_3); Anal. Calcd. for $C_{32}H_{24}N_4O_7S$ (Mol.wt.608); C, 63.15; H, 3.97; N, 9.21; Found: C, 63.10; H, 3.91; N, 9.11%.

4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl)phthalazin-1-yl 3-hydroxy-2-((4-methylphenyl) sulfonamido) butanoate (14f)

Yield, 70%; mp 226°C-228°C; IR (cm^{-1}): υ_{max}: 3400-3100 (NH, OH), 3080 (C—H aromatic), 2970 (C—H aliphatic), 1780-1700 (3CO), 1600 (C═N), 1330 (SO_2); Ms: m/z 622 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 8.42 (s,1H,NH), 7.13-7.90 (m, 16H, Ar—H), 4.90 (s, 1H, OH), 4.34 (d, 1H, J = 7.5, CH), 2.34 (m,1H, CH), 1.20 (d, 3H, J = 6.8, CH_3), 3.55 (s, 3H, CH_3—Ar); ^{13}C NMR, 171.00, 168.30, 167.10, 150.01,136.30, 133.50, 132.00, 129.40, 128.50, 127.70, 126.62, 123.70, 119.70, 43.80; Anal. Calcd. for $C_{32}H_{24}N_4O_7S$ (Mol.wt.622); C, 63.66; H, 4.21; N, 9.00; Found: C, 63.60; H, 4.11; N, 9.12%.

***N*-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl)-2-((4-methylphenyl) sulfonamido)acetamide (15a)**

Yield, 60%; mp 203°C-205°C; IR (cm^{-1}): υ_{max}: 3400 (NH), 1780-1700 (3CO), 1710 (C═O), 1600 (C═N); Ms: m/z 577 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 10.70 (s, 1H, NH, exchangeable with D_2O), 7.13-7.90 (m, 16H, Ar—H), 3.45 (s, 2H, CH_2), 2.12 (s, 3H, CH_3); ^{13}C NMR, 171.00, 168.30, 167.10, 150.01,136.30, 133.50, 132.00, 129.40, 128.50, 127.70, 126.62, 123.70, 119.70, 43.80, 21.36; Anal. Calcd. for $C_{31}H_{23}N_5O_5S$ (Mol.wt.577); C, 64.46; H, 4.01; N, 12.12; Found: C, 64.40; H, 4.00; N, 12.02%.

***N*-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl)-2-((4-methylphenyl) sulfonamido)propanamide (15b)**

Yield, 68%; mp 190°C-192°C; IR (cm^{-1}): υ_{max}: 3320 (NH), 1780-1710 (3CO), 1710, 1600 (C═N); Ms: m/z 591 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 8.34 (s, 1H, NH, exchangeable with D_2O), 7.03-7.26 (m, 16H, Ar—H), 4.22 (q, 1H, J = 6.7, CH), 2.35 (s, 3H, CH_3—Ar), 1.29 (d, H, J = 7.1, CH_3); ^{13}C NMR, 171.00, 168.30, 167.10, 150.01, 136.30, 133.50, 132.00, 129.40, 128.50, 127.70, 126.62, 123.70, 119.70, 53.40, 21.30, 17.00; Anal. Calcd. for $C_{32}H_{25}N_5O_5S$ (Mol.wt.591); C, 64.96; H, 4.26; N, 11.84; Found: C, 64.90; H, 4.22; N, 11.72%.

N-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl)-2-((4-methylphenyl) sulfonamido)-3-phenylpropa-namide (15c)

Yield, 70%; mp 200°C-202°C; IR (cm^{-1}): υ_{max}: 3380 (NH), 1780-1700 (3CO), 1600 (C═N); Ms: m/z 653 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 11.92 (s, 1H, NH, exchangeable with D_2O), 7.15-8.32 (m, 16H, Ar—H), 4.22 (t,1H, J = 7.5, CH), 2.70 (d, 2H, J = 6.3, CH_2), 3.12 (s, 3H, CH_3); Anal. Calcd. for $C_{38}H_{29}N_5O_5S$ (Mol.wt.667); C, 68.35; H, 4.38; N, 10.49; Found: C, 68.30; H, 4.30; N, 10.39%.

***N*-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl)-3-methyl-2-((4-methylphenyl) sulfonamido)butana-mide (15d)**

Yield, 55%; mp 220°C-222°C; IR (cm^{-1}): υ_{max}: 3400 (NH, CONH), 1780-1700 (3CO), 1600 (C═N); Ms: m/z 619 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 10.23 (s, 1H, NH, exchangeable with D_2O), 7.14-7.86 (m, 16H, Ar—H), 5.22 (d,1H, J = 6.4, CH), 3.55-3.65 (m, 1H, CH), 2.75 (d, 6H, J = 7.8, $2CH_3$), 1.01 (s, 3H, CH_3—Ar); Anal. Calcd. for $C_{34}H_{29}N_5O_5S$ (Mol.wt.619); C, 65.90; H, 4.72; N, 11.30; Found: C, 65.81; H, 4.61; N, 11.23%.

***N*-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl)-3-hydroxy-2-((4-methylphenyl) sulfonamido)propanamide (15e)**

Yield, 65%; mp 225°C-227°C; IR (cm^{-1}): υ_{max}: 3298-3280 (NH, OH), 1780-1710 (3CO), 1600 (C═N); Ms: m/z 607 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 10.55 (s, 1H, NH, exchangeable with D_2O), 7.40-7.86 (m, 16H, Ar—H), 5.50 (s, 1H, OH), 4.52 (d, 1H, J = 6.8, CH), 3.22 (d, 2H, J = 7.5, CH_2), 2.75 (s, 3H, CH_3—Ar); ^{13}C NMR, 172.00, 168.30, 167.10, 150.01,136.30, 133.50, 132.00, 129.40, 128.50, 127.70, 126.62, 123.70, 119.70, 60.50, 59.81, 21.43; Anal. Calcd. for $C_{32}H_{25}N_5O_6S$ (Mol.wt.607); C, 63.25; H, 4.15; N, 11.53; Found: C, 63.12; H, 4.10; N, 11.42%.

***N*-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl)-3-hydroxy-2-((4-methylphenyl) sulfonamido)butanamide (15f)**

Yield, 70%; mp 242°C-244°C; IR (cm^{-1}): υ_{max}: 3300-3180 (NH, OH), 1780-1730 (3CO), 1600 (C═N); Ms: m/z 621 ($M^{\cdot+}$); ^1H NMR (DMSO-d_6) δ: 9.12 (s, 1H, NH, exchangeable with D_2O), 7.20-7.56 (m, 16H, Ar—H), 5.20 (s, 1H, OH), 4.52 (d, 1H, J = 7, CH), 3.22 (q, 1H, J = 7.2, CH), 2.58 (s, 3H, CH_3—Ar), 1.32 (d, 3H, J = 7, CH_3); Anal. Calcd. for $C_{33}H_{27}N_5O_6S$ (Mol.wt.621); C, 63.76; H, 4.38; N, 11.27; Found: C, 63.71; H, 4.30; N, 11.15%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 2-((4-methylphenyl) sulfonamido)ethanethioate (16a)
Yield, 60%; mp 233°C-235°C; IR (cm^{-1}): υ_{max}: 3295 (NH), 1780-1700 (3CO), 1600 (C═N), 1350 (SO_2); Ms: *m/z* 594 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 8.51 (s, 1H, NH, exchangeable with D_2O), 7.40-7.91 (m, 16H, Ar─H), 4.32 (s, 2H, CH_2), 2.12 (s, 3H, CH_3); ^{13}C NMR, 196.50, 168.30, 167.10, 150.01,136.30, 133.50, 132.00, 129.40, 128.50, 127.70, 126.62, 123.70, 119.70, 54.00, 21.33; Anal. Calcd. for $C_{31}H_{22}N_4O_5S_2$ (Mol.wt.594); C, 62.61; H, 3.73; N, 9.42; Found: C, 62.51; H, 3.62; N, 9.31%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 2-((4-methylphenyl) sulfonamido)propanethioate (16b)
Yield, 50%; mp 243°C-245°C; IR (cm^{-1}): υ_{max}: 3390 (NH), 3080 (C─H aromatic), 1780-1730 (3CO), 1600 (C═N), 1350 (SO_2); Ms: *m/z* 608 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ:7.85 (s, 1H, NH, exchangeable with D_2O), 7.43-8.30 (m, 16H, Ar─H), 4.25 (q, 1H, CH), 2.36 (s, 3H, CH_3─Ar), 1.36 (d, 3H, $J = 7$, CH_3); Anal. Calcd. for $C_{32}H_{24}N_4O_5S_2$ (Mol.wt.608); C, 63.14; H, 3.97; N, 9.20; Found: C, 63.10; H, 3.90; N, 9.10%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 2-((4-methylphenyl) sulfonamido)-3-phenylpropane-thioate (16c)
Yield, 55%; mp 240°C-242°C; IR (cm^{-1}): υ_{max}: 3750 (NH), 1780-1705 (3CO), 1600 (C═N), 1350 (SO_2); Ms: *m/z* 670 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 8.22 (s,1H,NH, exchangeable with D_2O), 7.04-7.66 (m, 16H, Ar─H), 4.29 (t, 1H, $J = 7.5$, CH), 2.88 (d, 2H, $J = 6.9$, CH_2), 2.35 (s, 3H, CH_3); Anal. Calcd. for $C_{38}H_{28}N_4O_5S_2$ (Mol. wt.684); C, 66.65; H, 4.12; N, 8.18; Found: C, 66.60; H, 4.08; N, 8.10%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 3-methyl-2-((4-methylphenyl) sulfonamido)butane-thioate (16d)
Yield, 70%; mp 205°C-207°C; IR (cm^{-1}): υ_{max}: 3360 (NH), 1780-1700 (3CO), 1600 (C═N), 1350 (SO_2); Ms: *m/z* 636 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.31 (s, 1H, NH, exchangeable with D_2O), 7.23-7.46 (m, 16H, Ar─H), 4.50 (d, 1H, $J = 7$, CH), 3.35-3.13 (m, 1H, CH), 2.49 (s, 3H, CH_3), 1.30 (d, 6H, $J = 6.8$, $2CH_3$); ^{13}C NMR, 197.60, 168.30, 167.10, 150.01,136.30, 133.50, 132.00, 129.40, 128.50, 127.70, 126.62, 123.70, 119.70, 69.40, 61.10, 21.33; Anal. Calcd. for $C_{34}H_{28}N_4O_5S_2$ (Mol.wt.636); C, 64.14; H, 4.43; N, 8.80; Found: C, 64.10; H, 4.33; N, 8.71%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 3-hydroxy-2-((4-methylphenyl) sulfonamido)propa-nethioate (16e)
Yield, 65%; mp 240°C-242°C; IR (cm^{-1}): υ_{max}: 3390 (NH), 3080 (C─H aromatic), 2970 (C─H aliphatic), 1780-1690 (3CO), 1600 (C═N), 1350 (SO_2); Ms: *m/z* 624 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 8.75 (s, 1H, NH, exchangeable with D_2O), 7.20-7.99 (m, 16H, Ar─H), 5.53(s, 1H, OH), 4.12 (t, 1H, $J = 7.5$, CH), 2.85 (d, 2H, $J = 7$, CH_2), 2.40 (s, 3H, CH_3); Anal. Calcd. for $C_{32}H_{24}N_4O_6S_2$ (Mol.wt.624); C, 61.53; H, 3.87; N, 8.97; Found: C, 61.41; H, 3.80; N, 8.91%.

S-(4-(4-(1,3-Dioxoisoindolin-2-yl)phenyl) phthalazin-1-yl) 3-hydroxy-2-((4-methyl phenyl) sulfonamido)butane-thioate (16f)
Yield, 68%; mp 210°C-212°C; IR (cm^{-1}): υ_{max}: 3320 (NH), 1780-1680 (3CO), 1600 (C═N), 1350 (SO_2); Ms: *m/z* 638 ($M^{\cdot+}$); ^{1}H NMR (DMSO-d_6) δ: 7.85 (s, 1H, NH, exchangeable with D_2O), 7.52-8.21 (m, 16H, Ar─H), 5.03(s, 1H, OH), 4.42 (d, 1H, $J = 6.8$, CH), 2.11 (q, 1H, $J = 6.8$, CH), 1.12 (d, 3H, $J = 7.4$, CH_3), 2.58 (s, 3H, CH_3─Ar); ^{13}C NMR, 197.76, 168.30, 167.10, 150.01,136.30, 133.50, 132.00, 129.40, 128.50, 127.70, 126.62, 123.70, 119.70, 74.11, 67.30, 19.51; Anal. Calcd. for $C_{33}H_{26}N_4O_6S_2$ (Mol.wt.638); C, 62.06; H, 4.10; N, 8.77; Found: C, 62.00; H, 4.00; N, 8.70%.

5 | PHARMACOLOGICAL

5.1 | Cytotoxicity and anticancer evaluation

Different human cancer cell lines, namely, breast cancer (MCF-7), hepatic cancer (HePG-2), human prostate cancer (PC3), and colon cancer (HCT-116) of some novel synthesized compounds were measured via standard MTT assay in vitro.[42–44] This colorimetric assay is relying on the changing of the yellow tetrazolium bromide to a purple formazan derivative by mitochondrial succinate dehydrogenase enzyme. The synthesized compounds showed different degrees of inhibitory activity toward the tested human tumor cell lines compared with the standard doxorubicin. Compounds **8c**, **10a**, **10d**, **10f**, **10e**, **11d, 11e**, **11b**, and **12e** revealed the highest cytotoxic activity among the studied series toward HePG-2,MCF-7, PC3, and HCT-116, with IC_{50} ranges between 4 and 10 mg/mL. The highest cytotoxic activity against PC3 was shown by compounds **7f**, **10a**, **10d, 10f**, **11d**, **11e**, **11b**, and **12e** with IC_{50} values 9.72, 5.43, 8.33, 6.22, 6.51, 5.55, 7.22, and 9.05 mg/mL, respectively. Powerful inhibitory activity was also established by compounds **10a**, **11d**, **11e**,

and **11b** toward MCF-7. At the other side, other compounds showed cytotoxic activity that ranges from moderate to weak toward the human cancer cell lines. It is concluded from the given data in Table 1 that the existence of amino acid moieties attached to phthalazines improves their cytotoxic effect in comparison with starting phthalazines **2**, **4**, and **5.** Also, combination of phthaloyl phenyl alanine and phthaloyl L-threonine in derivatives **8c** and **7f** upgraded their activity toward HepG-2 and HCT-116. (Table 1).

In studying the structure-activity relationship (SAR) between the activity and synthesized analogs, compounds having electron donating groups (OH and CH_3) presents on the compounds (**8c**, **10**(**a**, **d**, **f**, **e**), **11** (**d**, **e**, **f**) and **12e**) were found to be the most potent anticancer activity. The biological activities also depend

TABLE 1 Cytotoxic activity of some compounds against human tumor cells

	In Vitro Cytotoxicity IC_{50} (μM)[a]			
Compounds	**HePG-2**	**MCF-7**	**PC3**	**HCT-116**
DOX[b]	4.50 ± 0.2	4.17 ± 0.2	8.87 ± 0.6	5.23 ± 0.3
7a	14.46 ± 1.3	26.28 ± 2.1	28.43 ± 2.3	13.19 ± 1.4
7b	32.37 ± 2.4	42.91 ± 3.4	48.83 ± 3.5	34.37 ± 2.6
7d	12.48 ± 1.2	17.62 ± 1.7	19.40 ± 1.8	10.25 ± 1.0
7f	7.30 ± 0.5	15.83 ± 1.4	9.72 ± 1.0	9.13 ± 0.8
8b	58.53 ± 3.6	70.52 ± 4.3	79.28 ± 4.2	89.98 ± 4.9
8c	9.16 ± 0.8	11.50 ± 1.2	13.89 ± 1.3	7.50 ± 0.6
8e	52.87 ± 3.4	46.66 ± 3.6	51.71 ± 3.5	43.14 ± 2.9
8f	20.68 ± 1.9	30.51 ± 2.4	38.97 ± 2.7	16.63 ± 1.5
9a	30.71 ± 2.1	50.16 ± 2.5	40.83 ± 2.9	60.40 ± 4.7
9b	20.37 ± 2.8	32.81 ± 3.2	50.83 ± 3.5	25.37 ± 2.5
9e	15.44 ± 1.8	30.41 ± 2.0	22.05 ± 2.5	25.55 ± 1.9
10a	7.90 ± 1.3	10.14 ± 2.1	5.43 ± 1.9	9.10 ± 1.4
10d	6.90 ± 0.3	11.03 ± 1.1	8.33 ± 0.9	5.91 ± 0.4
10e	4.85 ± 0.4	12.50 ± 1.5	10.15 ± 0.7	5.10 ± 0.7
10f	2.95 ± 0.2	9.64 ± 0.8	6.22 ± 0.5	4.03 ± 0.3
11b	9.95 ± 0.2	6.64 ± 0.8	7.22 ± 0.5	5.03 ± 0.3
11d	5.27 ± 3.8	9.15 ± 2.5	6.51 ± 1.9	7.51 ± 1.0
11e	7.11 ± 1.7	9.50 ± 2.1	5.55 ± 2.7	10.56 ± 1.9
12a	12.11 ± 1.5	15.50 ± 3.01	10.05 ± 2.05	7.56 ± 1.9
12b	11.24 ± 1.6	13.51 ± 3.0	16.05 ± 2.5	22.56 ± 2.9
12e	10.11 ± 3.7	12.50 ± 1.5	9.05 ± 2.8	7.56 ± 2.0
14c	37.10 ± 2.6	42.03 ± 3.2	44.98 ± 3.1	28.38 ± 2.3
14d	82.48 ± 4.7	>100	>100	>100
14e	91.47 ± 5.1	57.87 ± 3.9	70.98 ± 3.8	48.78 ± 3.5
15a	40.71 ± 3.1	66.78 ± 4.0	62.93 ± 3.7	81.40 ± 4.7
15b	10.16 ± 1.0	18.71 ± 1.9	15.48 ± 1.5	12.28 ± 1.3
15d	29.50 ± 2.2	32.65 ± 2.6	23.12 ± 1.9	18.22 ± 1.7
15e	18.24 ± 1.6	40.51 ± 3.0	32.05 ± 2.5	22.56 ± 1.9
16a	50.60 ± 4.1	60.87 ± 3.0	42.82 ± 4.0	71.31 ± 3.7
16d	30.58 ± 1.9	30.45 ± 1.6	27.02 ± 2.2	19.42 ± 2.6
16e	20.51 ± 2.6	30.61 ± 3.3	35.15 ± 2.8	20.46 ± 1.5

[a]IC_{50} (μM): 1-10 (very strong); 11-20 (strong); 21-50 (moderate); 51-100 (weak) and above 100 (non-cytotoxic).
[b]DOX, doxorubicin.

on the nature of amino acids presents on the heterocyclic moieties. On the basis of these previous results, amino acids were playing a crucial role in increasing biological activities. Some of the amino acids played a key factor for increasing the anticancer activity. Amino acids containing long-chain aliphatic hydrocarbons showed more potent anticancer agents compared with other conjugated amino acids such as glycine and alanine derivatives. This fact may be explained on the basis of aromaticity and hydrophobicity of phenylalanine, valine, serine, and threonine possessing good anticancer properties. The other two amino acid derivatives (glycine and alanine) were also moderately active, which may be due to their simple side chain functionalities and steric hindrance of aliphatic side chains. While the number of electron-donating groups of amino acids attached to phthalazine derivatives (**8c**, **10**(**a**, **d**, **f**, **e**), **11** (**d**, **e**, **f**) and **12e**) increases, the anticancer activity also increases compared with the minimum number of electron-donating groups present on the amino acids attached to

TABLE 2 Results of ABTS assay

Compound No.	Absorbance, mean	% Inhibition
Control	0.510	0
***L*-absorbance**	0.056	89.0
7a	0.378	25.9
7b	0.415	18.6
7d	0.357	30.0
7f	0.327	35.9
8b	0.450	11.8
8c	0.332	34.9
8e	0.428	16.1
8f	0.396	22.3
9a	0.314	38.4
9b	0.471	7.6
9e	0.462	9.4
10a	0.042	91.8
10d	0.302	40.8
10e	0.115	77.5
10f	0.288	43.5
11b	0.105	97.4
11d	0.345	32.4
11e	0.452	11.4
12a	0.276	45.9
12b	0.476	6.7
12e	0.309	39.4
14c	0.406	20.4
14d	0.489	4.1
14e	0.441	13.5
15a	0.434	14.9
15b	0.349	31.6
15d	0.361	29.2
15e	0.385	24.5
16a	0.476	6.6
16d	0.129	74.7
16e	0.411	19.4

TABLE 3 Results of bleomycin-dependent DNA damage assay on some selected compounds

Compounds	Absorbance of Samples
Ascorbic acid	0.072
7a	0.089
7b	0.106
7d	0.086
7f	0.074
8b	0.127
8c	0.078
8e	0.123
8f	0.097
9a	0.145
9b	0.139
9e	0.154
10a	0.058
10d	0.069
10f	0.067
10e	0.064
11b	0.074
11d	0.066
11e	0.072
12a	0.063
12b	0.053
12e	0.061
14c	0.111
14d	0.153
14e	0.132
15a	0.089
15b	0.081
15d	0.104
15e	0.093
16a	0192
16d	0.119
16e	0.105

phthalazine rings. The presence of strong electron-donating groups (OH and CH_3) on the aromatic ring increases the hydrophobicity of the molecules and is responsible for the enhanced anticancer activity. This fact suggests the importance of the electron-withdrawing groups for anticancer activity of this series.

5.2 | Antioxidant screening (ABTS)

ABTS, (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid diammonium salt), radical cation decolonization test is a spectrophotometric method greatly used in evaluating the antioxidant activity of different substances.[45] It can be applied for both lipophilic and hydrophilic compounds. Oxidation of ABTS gives a radical cation ($ABTS^{\cdot+}$) in presence of potassium persulfate. Because of their capacity to reduce the preformed radical, the absorbance is bleached by antioxidants. The preliminary qualitative antioxidant screening results (scavenger activity) of all the tested compounds are recorded (Table 2). This in comparison with standard ascorbic acid; compounds **10a**, **10e**, and **11b** showed a potent activity, while compounds **10d** and **12a** displayed moderate activity. The rest of the compounds showed weak to mild activity.

5.3 | Bleomycin-dependent DNA damage

Bleomycin is used routinely as antitumor agents; it is a family of glycopeptide antibiotics. Bleomycin assay has been used for evaluating the pro-oxidant effects of food antioxidants. Bleomycin binds iron ions and DNA. The bleomycin iron complex degrades DNA, which, upon heating with thiobarbituric acid (TBA), yields a pink chromogen. Upon the addition of suitable reducing agents, antioxidants compete with DNA and decrease chromogen formation.[46–48] To show the mechanism of action of our strongly synthesized compounds, their protective activity against DNA damage induced by the bleomycin iron complex were evaluated. The results in Table 3 displayed that compounds **7a**, **7d**, **7f**, **8c**, **8f**, **15a**, **15b**, and **15e** showed high protection against DNA damage induced by the bleomycin iron complex, therefore reducing chromogen formation between the damaged DNA and TBA molecules.

6 | CONCLUSION

In summary, we designed and synthesized a novel series of amino acids conjugated with phthalazine analogs screened for their in vitro anticancer properties using the MTT method and antioxidant and DNA binding studies. It was detected that a lot of the synthesized derivatives displayed potent cytotoxic activity from their IC_{50} values. In studying the SAR, hydrophobic and aromatic amino acids phenylalanine, valine, serine, and threonine, containing electron-donating groups, were found to most favor anticancer activity. Our results presented here could be used as a preliminary stage for the development of powerful phthalazine conjugated amino acids as anticancer therapies.

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