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Short communication

Synthesis and antitumor activities of novel 1,4-disubstituted phthalazine derivatives

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ABSTRACT

In an attempt to develop potent and selective antitumor agents, a series of novel 1,4-disubstituted phthalazine derivatives was designed and synthesized. All the prepared compounds were screened for their cytotoxic activities against A549, HT-29 and MDA-MB-231 cell lines *in vitro*. Among them, seven compounds (**7a**–**7e**, **7j** and **7i**) displayed excellent selectivity for MDA-MB-231 cells with IC_{50} values in the nM range, a desirable range for pharmacological testing. The most promising compound, 7a (IC_{50} = 3.79 μM, 2.32 μM, 0.84 nM), was 5.6-, 10.8- and 6.9 × 10^4- times more active than PTK-787 (IC_{50} = 21.16 μM, 22.11 μM, 57.72 μM), respectively.

1. Introduction

Cancer is the major leading causes of death in the world. Therefore, the design of new antitumor agents is one of the most urgent research areas in medicinal chemistry. In the past few years, a large number of phthalazine derivatives have been prepared and studied for their antitumor activities [1–5]. Among them, Vatalanib (PTK-787), a 1,4-disubstituted phthalazine VEGFR (vascular endothelial growth factor receptor) inhibitor, is currently in Phase III clinical trials for metastatic colorectal cancer [6]. Based on the structure of PTK-787, it has been suggested that the structure activity relationships (SARs) for 1,4-disubstituted phthalazines and their analogues include the following: (i) a [6,6]-fused (or related) aromatic system, (ii) a 4- or 3,4-disubstituted aniline function in the position 1 of phthalazine, and (iii) a hydrogen-bond acceptor (Lewis' base: lone pair(s) of a nitrogen- or oxygen atom(s)) attached to the position 4 *via* an appropriate linker (aryl or fused aryl group) [7,8]. In this paper, our research interests have focused on the synthesis and evaluation of the 1,4-disubstituted phthalazine derivatives containing a piperazinyl group.

The piperazinyl group, a small and rigid heterocyclic backbone, has been an attractive pharmacological scaffold present in several antitumor drugs [9–13]. From the viewpoint of molecular design, the combination of two active molecules or pharmacophores with a linker is a well-known approach for the build-up of drug-like molecules, which allows us to find more potent agents. To provide further insight into the cytotoxic activities of 1,4-disubstituted phthalazine derivatives, we combined the inherent antitumor agent PTK-787 and the piperazinyl group into one structure, and introduced an acetyl-flexible linker into the structure to develop more active agents. Therefore, we designed and synthesized a series of novel 1,4-disubstituted phthalazine derivatives containing piperazinyl groups. In addition, we screened their cytotoxic activities against different cancer cell lines *in vitro* to find potent and selective agents. Some of these compounds showed promising cytotoxicity against cancer cell lines (Fig. 1).

2. Chemistry

The synthetic routes of the target compounds 7a–7m and 12a–12m are illustrated in Scheme 1. Isobenzofuran-1(3*H*)-one (**1**) was obtained by reduction of the phthalic anhydride starting material in the presence of $NaBH_4$ in dry THF. Next, compound **1** was treated with 4-pyridine carboxaldehyde using CH_3ONa in methanol and ethyl propionate to give the corresponding 3-hydroxy-2-(pyridin-4-yl)-1*H*-inden-1-one (**2**), which was then reacted with 80% hydrazine hydrate to give 4-(pyridin-4-ylmethyl) phthalazin-1(2*H*)-one (**3**) as a white solid. Furthermore,

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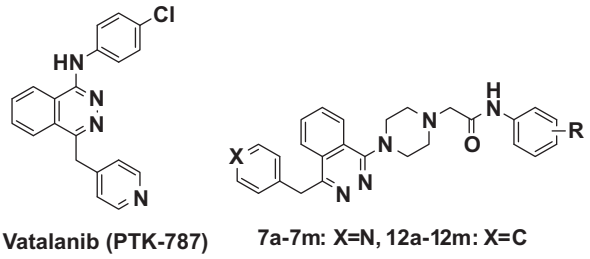


Fig. 1. The structures of PTK-787 and the target compounds.

chlorination of compound **3** with a solution of phosphoryl chloride and acetonitrile formed 1-chloro-4-(pyridin-4-ylmethyl)phthalazine (**4**) [7].

Thereafter, the key important intermediate (compound **5**) was synthesized by the reaction of compound **4** with an excess of piperazine in ethanol at 60 °C. Another important intermediate (compound **11**) was obtained according to the same method as described for compound **5** except that 4-pyridine carboxaldehyde was replaced by benzaldehyde. The side chains **6a**–**6m** were synthesized *via* a series of substituted anilines with 2-chloroacetyl chloride in the presence of Et_3N in acetone at room temperature.

Finally, the target compounds **7a**–**7m** and **12a**–**12m** were successfully obtained *via* the reaction of intermediate 5 and 11 with **6a**–**6m** in the presence of K_2CO_3 in refluxing acetone, respectively. The products obtained were purified by column chromatography on silica gel. The chemical structures of these novel compounds were confirmed by ^1H NMR, ^{13}C NMR, IR and MS spectra.

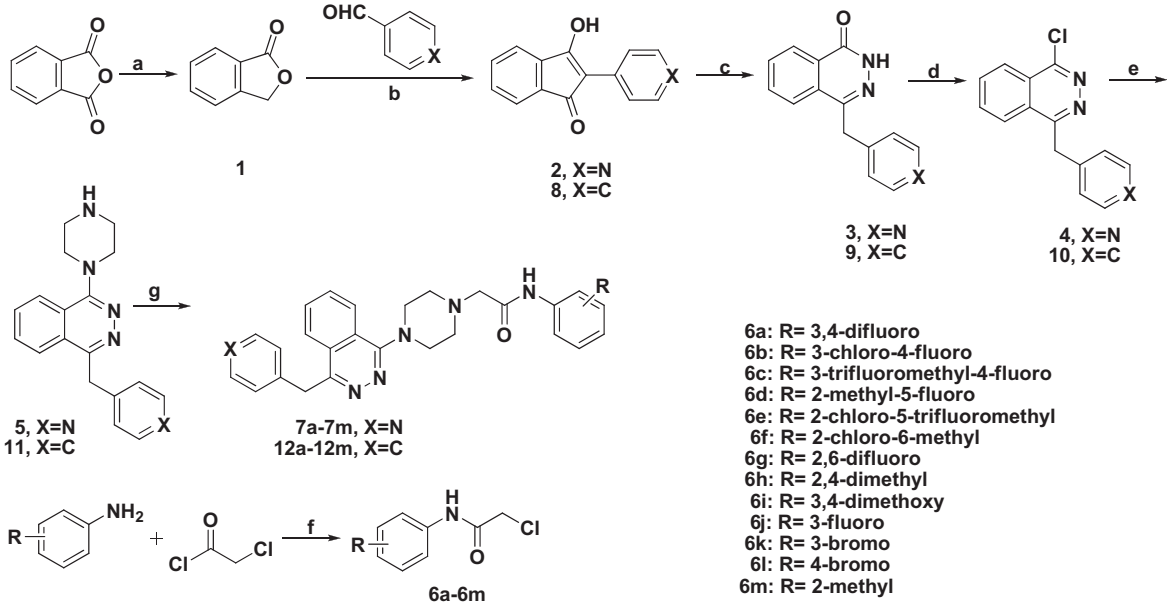
3. Biological evaluation

All the compounds **7a**–**7m** and **12a**–**12m** were evaluated for their cytotoxic activities on VEGFR overexpressing non-small-cell lung cancer cell line (A549) [14], breast cancer cell line (MDA-MB-231) [15] and human colorectal cancer cell line (HT-29) [16] by the MTT assay, using PTK-787 as a standard. And the results expressed as IC_{50} were summarized in Table 1. The IC_{50} values are the average of at least two independent experiments.

As shown in Table 1, most of the prepared compounds showed moderate to excellent cytotoxic activities against different cancer cell lines. Indeed, the cytotoxic activities of compounds **7a**–**7m** were more active than the reference drug PTK-787 against all three human cancer cell lines, and most of compounds **12a**–**12m** exhibited better activities against MDA-MB-231 cells than PTK-787. Interestingly, the pharmacological results indicated that the cytotoxicities of **7a**–**70m** and **12a**–**12m** against MDA-MB-231 cells was higher than against A549 and HT-29 cells, which reflects excellent selectivity for a particular human breast cancer cell type.

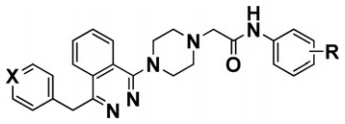
In most cases, the antitumor activities of **7a**–**7m** were much more potent than those of the corresponding compounds **12a**–**12m** against all three human cancer cell lines. As shown in Table 1, compounds **7a**–**7m** displayed excellent cytotoxic activities against A549 and HT-29 cell lines with IC_{50} values in the single-digit μM range. In particular, seven of the compounds **7a**–**7m** (**7a**–**7e**, **7j** and **7i**) showed IC_{50} values in the nM range against MDA-MB-231 cells, which were 10^2–10^3-fold more active than those of the corresponding compounds **12a**–**12m** (**12a**–**12e**, **12j** and **12i**). It was noteworthy that the most promising compound, 7a (IC_{50} = 3.79 μM, 2.32 μM, 0.84 nM), was 5.6-, 10.8- and 6.9 × 10^4-times more active than PTK-787 (IC_{50} = 21.16 μM, 22.11 μM, 57.72 μM), respectively. The preliminary SAR showed that the pyridinyl group at position 4 of the phthalazine scaffold plays an important role in enhancing their antitumor activities of these compounds.

We also studied these compounds and compared different substituted groups of benzene ring were studied. Among compounds **7a**–**7m**, the cytotoxicity of compounds **7f**–**7i** and **7m** were less active than those of the other compounds against all three different cancer cell lines. It can be concluded that the introduction of halogen groups into the target compounds was beneficial for enhancing their antitumor activities. Indeed, halogen groups at the meta and para positions of the benzene ring facilitated the increase of their cytotoxic activities of these compounds.



Scheme 1. Reagents and conditions: a) $NaBH_4$/MeOH/THF, r.t. 3 h; b) CH_3ONa/MeOH/ethyl propionate, r.f. 1 h; c) 80% $NH_2NH_2 \cdot H_2O$, 100 °C, 5 h; d) $POCl_3$, 90 °C, 3 h; e) piperazine/EtOH, 60 °C, 5 h; f) Et_3N/acetone, r.t. 4–8 h; g) K_2CO_3/acetone, r.f. 7–12 h.

Table 1
Cytotoxicity of the tested compounds against A549, HT-29 and MDA-MB-231 cell lines *in vitro*.



Compd.	X	R	IC_{50}(μM)[a]		
			A549[b]	HT-29[b]	MDA-MB-231[b]
7a	N	3,4-difluoro	3.79 ± 0.01	2.32 ± 0.38	***0.00084 ± 0.00004***
7b	N	3-chloro-4-fluoro	12.28 ± 0.27	0.88 ± 0.15	0.0040 ± 0.0010
7c	N	3-trifluoromethyl-4-fluoro	16.01 ± 0.31	1.11 ± 0.11	0.0019 ± 0.0080
7d	N	2-methyl-5-fluoro	38.89 ± 5.00	0.47 ± 0.14	0.0064 ± 0.0002
7e	N	2-chloro-5-trifluoromethyl	1.85 ± 0.52	4.81 ± 0.22	0.0037 ± 0.0012
7f	N	2-chloro-6-methyl	7.19 ± 1.02	22.59 ± 2.53	2.67 ± 0.23
7g	N	2,6-difluoro	54.37 ± 2.24	28.69 ± 0.78	1.90 ± 0.31
7h	N	2,4-dimethyl	45.01 ± 1.31	1.93 ± 0.23	0.24 ± 0.11
7i	N	3,4-dimethoxy	26.48 ± 1.32	40.11 ± 1.44	0.10 ± 0.02
7j	N	3-fluoro	4.60 ± 0.41	6.13 ± 0.72	0.0044 ± 0.0003
7k	N	3-bromo	1.53 ± 0.21	1.35 ± 0.17	0.0017 ± 0.0002
7l	N	4-bromo	2.71 ± 1.16	1.99 ± 0.34	0.0190 ± 0.0030
7m	N	2-methyl	43.09 ± 1.35	15.03 ± 3.1	0.20 ± 0.012
12a	C	3,4-difluoro	10.14 ± 0.62	9.51 ± 0.34	2.75 ± 0.26
12b	C	3-chloro-4-fluoro	NA[c]	13.29 ± 0.24	7.36 ± 1.25
12c	C	3-trifluoromethyl-4-fluoro	2.48 ± 0.12	4.59 ± 0.31	0.76 ± 0.17
12d	C	2-methyl-5-fluoro	NA[c]	170.50 ± 21.30	19.18 ± 2.54
12e	C	2-chloro-5-trifluoromethyl	89.03 ± 3.55	66.77 ± 5.40	19.47 ± 1.35
12f	C	2-chloro-6-methyl	7.42 ± 0.52	53.59 ± 1.78	3.92 ± 0.41
12g	C	2,6-difluoro	84.53 ± 4.45	19.65 ± 2.43	5.49 ± 0.71
12h	C	2,4-dimethyl	NA[c]	NA[c]	180.50 ± 15.80
12i	C	3,4-dimethoxy	NA[c]	144.80 ± 35.90	32.18 ± 0.79
12j	C	3-fluoro	129.60 ± 7.12	94.96 ± 4.45	3.73 ± 0.36
12k	C	3-bromo	NA[c]	13.39 ± 0.56	3.88 ± 0.21
12l	C	4-bromo	2.00 ± 0.25	60.18 ± 7.10	12.42 ± 0.47
12m	C	2-methyl	NA[c]	113.0 ± 23.80	17.29 ± 0.88
PTK-78[d]			21.16 ± 0.89	22.11 ± 0.15	57.72 ± 6.18

[a] IC_{50}: concentration of the compound (μM) producing 50% cell growth inhibition after 72 h of drug exposure, as determined by the MTT assay. Each experiment was run at least two times, and the results are presented as average values ± standard deviation.
[b] A549, non-small-cell lung adenocarcinoma cell line; HT-29, human colon cancer cell line; MDA-MB-231, human breast cancer cell line.
[c] NA: Compounds having IC_{50} value > 200 μM.
[d] Used as a positive control.

4. Conlusion

In summary, we have designed and synthesized twenty-six 1,4-disubstituted phthalazine derivatives, and evaluated their cytotoxic activities against all three cancer cell lines (A549, HT-29 and MDA-MB-231). Most of the prepared compounds displayed moderate to excellent cytotoxic activity against all three different cancer cell lines. In particular, seven compounds (**7a**–**7e**, **7j** and **7i**) showed excellent selectivity for MDA-MB-231 cells with IC_{50} values in the nM range, a desirable range for pharmacological testing. From the structure activity relationships (SARs) we may conclude that the introduction of the pyridinyl group at position 4 of the phthalazine scaffold plays an important role in enhancing their antitumor activities of these compounds. Further studies are in progress in our laboratories and will be reported upon in the future.

5. Experimental protocols

5.1. Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). Proton (1H) nuclear magnetic resonance spectroscopy were performed using Bruker ARX-300, 300 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. IR spectra (KBr disks) were recorded with a Bruker IFS 55 instrument (Bruker). Unless otherwise noted, all the materials were obtained from commercially available sources and were used without further purification.

5.2. Isobenzofuran-1(3H)-one (*1*)

$NaBH_4$ (126.2 g, 3.32 mol) was added slowly to a stirred solution of phthalic anhydride (123 g, 0.83 mol) in dry THF (750 mL) at 0–10 °C. Anhydrous methanol was added to the reaction mixture drop-wise at 0–10 °C, and the reaction mixture was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure. The residue was poured into a 10% hydrochloric acid aqueous solution (1000 mL), stirred intensively for 3 h and separated by filtration. The solid was alkalized with a 10% Na_2CO_3 aqueous solution (1000 mL), stirred for 1 h and separated by filtration to give compound **1** as a white crystal (78 g, 70%). m.p.: 72–73 °C. MS[MH^+](m/z): 135.1($M + 1$).·^{1}H NMR·(300 MHz, DMSO) δ: 7.85 (d, $J = 7.6$ Hz, 1H), 7.78 (t, $J = 7.4$ Hz, 1H), 7.68 (d, $J = 7.7$ Hz, 1H), 7.59 (t, $J = 7.4$ Hz, 1H), 5.42 (s, 2H). $C_8H_6O_2$ (134.13).

5.3. 3-Hydroxy-2-(pyridin-4-yl)-1H-inden-1-one (*2*)

A solution of **1** 50 g (0.37 mol) and 4-pyridine carboxaldehyde 42 g (0.39 mol) in ethyl propionate (200 mL) was portion-wise added to a stirred solution of CH_3ONa (82 g, 1.49 mol) in MeOH

(800 mL) at 0–10 °C. The reaction mixture was stirred at room temperature for 1 h, heated to reflux for 1 h, and then concentrated under reduced pressure. The residue was poured into ice water (1000 mL), acidified with glacial acetic acid to pH 2–3 and separated by filtration to give compound **2** as an orange solid (43.8 g, 52.6%). m.p.: >300 °C. MS[MH^+](m/z): 224.3 (M + 1). ^{1}H NMR (300 MHz, DMSO) δ: 13.23 (s, 1H), 8.72 (d, J = 7.0 Hz, 2H), 8.18 (d, J = 7.0 Hz, 2H), 7.51 (m, 4H). $C_{14}H_9NO_2$ (223.23).

5.4. 4-(Pyridin-4-ylmethyl)phthalazin-1(2H)-one (**3**)

A solution of 80% hydrazine hydrate ($NH_2NH_2 \cdot H_2O$) (306.6 mL, 4.9 mol) and compound **2** (43.8 g, 0.20 mol) was stirred at 100 °C for 5 h. The mixture was cooled, separated by filtration and washed with EtOH to give compound **3** as a light yellow crystal (34.9 g, 75%). m.p.: 210–211 °C. MS[MH^+](m/z): 238.3 (M + 1). ^{1}H NMR (300 MHz, DMSO) δ: 12.64 (s, 1H), 8.46 (d, J = 4.3 Hz, 2H), 8.26 (d, J = 7.4 Hz, 1H), 8.00–7.77 (m, 3H), 7.32 (d, J = 5.2 Hz, 2H), 4.34 (s, 2H). $C_{14}H_{11}N_3O$ (237.26).

5.5. 1-Chloro-4-(pyridin-4-ylmethyl)phthalazine (**4**)

Compound **3** (17.5 g, 0.07 mol) was added to a stirred solution of $POCl_3$ (170 mL) and CH_3CN (80 mL) at room temperature, and then 3 drops of DMF were added to the mixture. The reaction mixture was heated to 90 °C for 3 h. The mixture was concentrated under reduced pressure. The residue was poured into ice water (500 mL), alkalized with a 10% Na_2CO_3 aqueous solution to pH 7–8 and separated by filtration to give compound **4** as a red solid (13.5 g, 71.6%). m.p.: 180–181 °C. MS[MH^+](m/z): 255.8 (Cl = 35), 257.8 (Cl = 37). ^{1}H NMR (300 MHz, DMSO) δ: 8.46 (d, J = 4.3 Hz, 2H), 8.35 (m, 2H), 8.13 (m, 2H), 7.33 (d, J = 5.2 Hz, 2H), 4.76 (s, 2H). $C_{14}H_{10}ClN_3$ (255.70).

5.6. 1-(Piperazin-1-yl)-4-(pyridin-4-ylmethyl)phthalazine (**5**)

Compound **4** (13.5 g, 0.05 mol) was added portion-wise to a stirred solution of piperazine (30.4 g, 0.4 mol) in anhydrous ethanol at room temperature. The mixture was heated to 60 °C for 5 h, and then concentrated under reduced pressure. The residue was poured into ice water (200 mL), stirred intensively for 1 h and separated by filtration to give compound **5** as a light yellow solid (11.45 g, 71%). m.p.: 209–210 °C. MS[MH^+](m/z): 306.4 (M + 1). $C_{18}H_{19}N_5$ (305.38).

5.7. General procedure for preparation of 2-chloro-N-(substituted-phenyl)acetamide (**6a**–**6m**)

Triethylamine (36.7 mL, 0.3 mol) was added to a solution of substituted aniline (0.2 mol) in acetone (250 mL), and then 2-chloroacetyl chloride (21.4 mL, 0.26 mol) was added drop-wise to the reaction mixture at 0–10 °C. The reaction mixture was stirred at room temperature for 4–8 h. The mixture was concentrated under reduced pressure. The residue was poured into ice water (200 mL), stirred at room temperature for 1 h and separated by filtration to obtain **6a**–**6m**.

5.8. General procedure for preparation of compound (**7a**–**7m**)

K_2CO_3 (0.23 g, 1.64 mmol) was added to a stirred solution of compound **5** (0.2 g, 0.65 mmol) and 2-chloro-*N*-(substituted-phenyl)acetamide (0.72 mmol) in acetone (2 mL). The reaction mixture was heated to reflux for 7–12 h. The mixture was cooled, separated by filtration. The filter liquor was concentrated under reduced pressure, and purified by chromatography on silica gel using MeOH/CH_2Cl_2 to obtain **7a**–**7m** as light yellow crystals.

5.8.1. N-(3,4-Difluorophenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7a**)

Yield: 36%. m.p.: 207–208 °C. MS[MH^+](m/z): 475.1 (M + 1). IR (KBr) cm^{-1}: 3263.3, 3056.8, 2932.0, 2846.7, 1676.1, 1604.5, 1517.5, 1411.9, 1368.3, 1264.0, 1204.7, 1150.4, 1134.7, 1015.6, 777.9. ^{1}H NMR (300 MHz, DMSO) δ: 10.12 (s, 1H), 8.45 (d, J = 5.8 Hz, 2H), 8.22–8.14 (m, 1H), 8.14–8.07 (m, 1H), 8.01–7.82 (m, 3H), 7.51–7.36 (m, 2H), 7.31 (d, J = 5.7 Hz, 2H), 4.62 (s, 2H), 3.47 (s, 4H), 3.29 (s, 2H), 2.83 (s, 4H). ^{13}C NMR (150.9 MHz, DMSO) δ: 168.77, 159.43, 149.77, 148.01, 135.88, 132.27, 132.01, 126.85, 125.26, 124.88, 124.29, 120.95, 117.55, 117.44, 115.97, 108.70, 61.79, 52.75, 50.85, 37.66. $C_{26}H_{24}F_2N_6O$ (474.51).

5.8.2. N-(3-Chloro-4-fluorophenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7b**)

Yield: 33%. m.p.:169–170 °C. MS[MH^+](m/z): 491.1 (Cl = 35), 493.1 (Cl = 37). IR (KBr) cm^{-1}: 3421.8, 2923.4, 2851.8, 1681.0, 1601.3, 1524.2, 1499.6, 1411.6, 1386.3, 1264.8, 1013.2. ^{1}H NMR (300 MHz, DMSO) δ: 10.11 (s, 1H), 8.45 (d, J = 6.0 Hz, 2H), 8.14 (m, 2H), 8.02 (m, 1H), 7.96–7.88 (m, 2H), 7.70–7.59 (m, 1H), 7.38 (t, J = 9.1 Hz, 1H), 7.31 (d, J = 5.9 Hz, 2H), 4.62 (s, 2H), 3.47 (s, 4H), 3.29 (s, 2H), 2.83 (s, 4H). ^{13}C NMR (75.5 MHz, DMSO) δ: 168.78, 159.41, 154.13, 149.75, 148.00, 136.14, 132.24, 131.98, 126.83, 125.23, 124.86,124.27, 121.07, 119.91, 117.08, 61.71, 52.71, 50.83, 37.64. $C_{26}H_{24}ClFN_6O$ (490.96).

5.8.3. N-(4-Fluoro-3-(trifluoromethyl)phenyl)-2-(4-(4-(pyridin-4-ylmethyl)phthalazin-1-yl)piperazin-1-yl) acetamide (**7c**)

Yield: 22%. m.p.: 121–122 °C. MS[MH^+](m/z): 525.4 (M + 1). IR (KBr) cm^{-1}: 3412.3, 2922.1, 2849.2, 1692.2, 1601.1, 1506.9, 1450.2, 1411.6, 1370.7, 1217.6, 1194.4, 1164.1, 1131.0, 1012.6. ^{1}H NMR (300 MHz, DMSO) δ: 10.22 (s, 1H), 8.45 (s, 2H), 8.18 (m, 3H), 7.93 (m, 3H), 7.49 (m, 1H), 7.31 (s, 2H), 4.62 (s, 2H), 3.47 (s, 4H), 3.31–3.27 (m, 2H), 2.83 (s, 4H). $C_{27}H_{24}F_4N_6O$ (524.51).

5.8.4. N-(5-Fluoro-2-methylphenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7d**)

Yield: 21%. m.p.: 192–193 °C. MS[MH^+](m/z): 471.4 (M + 1). IR (KBr) cm^{-1}: 3311.6, 2919.0, 2838.9, 1685.4, 1600.8, 1523.2, 1450.2, 1413.0, 1394.8, 1149.4, 1131.2, 1270.1, 1013.2, 778.8. ^{1}H NMR (300 MHz, DMSO) δ: 9.61 (s, 1H), 8.45 (d, J = 5.5 Hz, 2H), 8.21–8.16 (m, 1H), 8.13 (m, 1H), 7.93 (m, 2H), 7.82 (dd, J = 11.3, 2.6 Hz, 1H), 7.32 (d, J = 5.7 Hz, 2H), 7.30–7.23 (m, 1H), 6.88 (t, J = 8.4 Hz, 1H), 4.63 (s, 2H), 3.48 (s, 4H), 3.31 (s, 2H), 2.89 (s, 4H), 2.26 (s, 3H). ^{13}C NMR (75.5 MHz, DMSO) δ: 168.36, 159.32, 154.21, 149.70, 147.92, 137.38, 132.23, 131.97, 131.49, 126.82, 125.20, 124.77, 124.25, 123.86, 120.91, 110.25, 107.71, 61.43, 52.79, 51.22, 37.62. $C_{27}H_{27}FN_6O$ (470.54).

5.8.5. N-(2-Chloro-5-(trifluoromethyl)phenyl)-2-(4-(4-(pyridin-4-ylmethyl)phthalazin-1-yl)piperazin-1-yl) acetamide (**7e**)

Yield: 24%. m.p.: 189–190 °C. MS[MH^+](m/z): 541.2 (Cl = 35), 543.1 (Cl = 37). IR (KBr) cm^{-1}: 3252.3, 2919.2, 2846.2, 1700.1, 1601.6, 1588.2, 1526.4, 1430.3, 1331.4, 1262.5, 1169.6, 1129.2, 1079.2, 1012.1, 778.8. ^{1}H NMR (300 MHz, DMSO) δ: 10.23 (s, 1H), 8.69 (s, 1H), 8.45 (d, J = 5.6 Hz, 2H), 8.23–8.16 (m, 1H), 8.13 (m, 1H), 7.99–7.86 (m, 2H), 7.82 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 5.6 Hz, 2H), 4.63 (s, 2H), 3.50 (s, 4H), 3.38 (s, 2H), 2.91 (s, 4H). $C_{27}H_{24}ClF_3N_6O$ (540.97).

5.8.6. N-(2-Chloro-6-methylphenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7f**)

Yield: 26%. m.p.: 85–86 °C. MS[MH^+](m/z): 487.1 (Cl = 35), 489.2 (Cl = 37). IR (KBr) cm^{-1}: 3418.2, 2920.2, 2846.0, 1682.6,

1598.9, 1496.1, 1411.9, 1266.0, 1178.0, 1150.8, 1132.1, 1012.5, 777.4. ^{1}H NMR (300 MHz, DMSO) δ: 9.54 (s, 1H), 8.45 (d, J = 5.8 Hz, 2H), 8.15 (m, 2H), 7.92 (m, 2H), 7.40–7.28 (m, 3H), 7.22 (m, 2H), 4.62 (s, 2H), 3.50 (s, 4H), 3.30 (s, 2H), 2.89 (s, 4H), 2.21 (s, 3H). $C_{27}H_{27}ClN_6O$ (487.00).

*5.8.7. N-(2,6-Difluorophenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7g**)*

Yield: 46%. m.p.: 74–75 °C. MS[MH$^+$](m/z): 475.3 (M + 1). IR (KBr) cm^{-1}: 3218.7, 2938.8, 2822.9, 1692.2, 1598.6, 1511.5, 1467.0, 1416.0, 1304.1, 1283.7, 1267.1, 1242.5, 1009.5, 780.1. ^{1}H NMR (300 MHz, DMSO) δ: 9.60 (s, 1H), 8.45 (d, J = 5.7 Hz, 2H), 8.17 (m, 1H), 8.15–8.09 (m, 1H), 7.92 (m, 2H), 7.38 (m, 1H), 7.31 (d, J = 5.8 Hz, 2H), 7.17 (t, J = 8.1 Hz, 2H), 4.62 (s, 2H), 3.49 (s, 4H), 3.33 (s, 2H), 2.85 (s, 4H). $C_{26}H_{24}F_2N_6O$ (474.51).

*5.8.8. N-(2,4-Dimethylphenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7h**)*

Yield: 33%. m.p.: 158–159 °C. MS[MH$^+$](m/z): 467.1 (M + 1). IR (KBr) cm^{-1}: 3340.7, 2922.5, 2842.4, 1681.9, 1598.6, 1511.7, 1451.3, 1412.2, 1394.8, 1268.0, 1128.3, 1014.7. ^{1}H NMR (300 MHz, DMSO) δ: 9.39 (s, 1H), 8.45 (d, J = 5.9 Hz, 2H), 8.17 (m, 1H), 8.13 (m, 1H), 8.01–7.84 (m, 2H), 7.65 (d, J = 8.1 Hz, 1H), 7.32 (d, J = 5.7 Hz, 2H), 7.04 (s, 1H), 6.99 (d, J = 8.1 Hz, 1H), 4.63 (s, 2H), 3.48 (s, 4H), 3.27 (s, 2H), 2.88 (s, 4H), 2.24 (s, 3H), 2.23 (s, 3H). $C_{28}H_{30}N_6O$ (466.58).

*5.8.9. N-(3,4-Dimethoxyphenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7i**)*

Yield: 32%. m.p.: 81–82 °C. MS[MH$^+$](m/z): 499.4 (M + 1). IR (KBr) cm^{-1}: 3408.3, 2913.8, 2832.7, 1675.7, 1602.0, 1514.6, 1412.2, 1263.6, 1234.0, 1133.7, 1025.0, 806.1, 780.0, 755.4. ^{1}H NMR (300 MHz, DMSO) δ: 9.67 (s, 1H), 8.45 (d, J = 5.5 Hz, 2H), 8.16 (m, 1H), 8.11 (m, 1H), 7.93 (m, 2H), 7.42 (d, J = 4.0 Hz, 1H), 7.39 (s, 1H), 7.31 (d, J = 5.5 Hz, 2H), 7.05 (d, J = 8.1 Hz, 1H), 4.62 (s, 2H), 3.46 (s, 4H), 3.24 (s, 2H), 2.82 (s, 4H), 2.19 (s, 3H), 2.16 (s, 3H). ^{13}C NMR (75.5 MHz, DMSO) δ: 167.87, 159.38, 149.71, 148.61, 147.97, 145.01, 132.41, 132.20, 131.93, 126.81, 125.19, 124.83, 124.23, 120.91, 112.08, 111.44, 104.77, 61.83, 55.83, 55.51, 52.75, 50.87, 37.63. $C_{28}H_{30}N_6O_3$ (498.58).

*5.8.10. N-(3-Fluorophenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7j**)*

Yield: 37%. m.p.: 74–75 °C. MS[MH$^+$](m/z): 457.2 (M + 1). IR (KBr) cm^{-1}: 3280.9, 2920.2, 2826.8, 1687.8, 1600, 1528.6, 1493.4, 1443.0, 1414.7, 1265.6, 1134.2, 1013.4, 777.9. ^{1}H NMR (300 MHz, DMSO) δ: 10.09 (s, 1H), 8.45 (d, J = 5.4 Hz, 2H), 8.17 (m, 1H), 8.12 (m, 1H), 8.00–7.84 (m, 2H), 7.68 (d, J = 11.8 Hz, 1H), 7.44 (d, J = 8.3 Hz, 1H), 7.40–7.33 (m, 1H), 7.31 (d, J = 5.4 Hz, 2H), 6.89 (t, J = 7.2 Hz, 1H), 4.62 (s, 2H), 3.47 (s, 4H), 3.30 (s, 2H), 2.83 (s, 4H). ^{13}C NMR (150.9 MHz, DMSO) δ: 168.77, 162.96, 161.37, 159.35, 154.03, 149.68, 147.93, 140.59, 140.51, 132.15, 131.88, 130.34, 130.28, 126.79, 125.14, 124.78, 124.21, 120.89, 115.28, 109.91, 109.77, 106.37, 106.19, 61.71, 52.65, 50.82, 37.61. $C_{26}H_{25}FN_6O$ (456.51).

*5.8.11. N-(3-Bromophenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7k**)*

Yield: 52%. m.p.: 74–75 °C. MS[MH$^+$](m/z): 517.0 (Br = 79), 519.0 (Br = 81). IR (KBr) cm^{-1}: 3418.0, 2919.0, 2846.6, 1686.2, 1593.0, 1516.8, 1477.7, 1415.3, 1307.3, 1266.0, 1012.7, 777.9. ^{1}H NMR (300 MHz, DMSO) δ: 10.05 (s, 1H), 8.45 (d, J = 5.7 Hz, 2H), 8.17 (m, 1H), 8.12 (m, 1H), 8.04 (s, 1H), 7.99–7.86 (m, 2H), 7.64 (d, J = 7.3 Hz, 1H), 7.31 (d, J = 5.6 Hz, 2H), 7.26 (m, 2H), 4.62 (s, 2H), 3.47 (s, 4H), 3.29 (s, 2H), 2.83 (s, 4H). ^{13}C NMR (75.5 MHz, DMSO) δ: 168.84, 159.37, 154.05, 149.70, 147.96, 140.45, 132.18. 131.92, 130.71, 126.81, 126.02, 125.17, 124.81, 124.24, 121.89, 121.54, 120.90, 118.34, 61.68, 52.65, 50.84, 37.63. $C_{26}H_{25}BrN_6O$ (517.42).

*5.8.12. N-(4-Bromophenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7l**)*

Yield: 28%. m.p.: 78–79 °C. MS[MH$^+$](m/z): 517.1 (Br = 79), 519.1 (Br = 81). IR (KBr) cm^{-1}: 3407.6, 2919.9, 2845.5, 1688.8, 1600.6, 1514.0, 1412.2, 1395.0, 1306.9, 1266.7, 1010.5, 826.7, 778.2. ^{1}H NMR (300 MHz, DMSO) δ: 10.00 (s, 1H), 8.45 (d, J = 5.8 Hz, 2H), 8.20–8.14 (m, 1H), 8.12 (m, 1H), 8.03–7.82 (m, 2H), 7.67 (d, J = 8.9 Hz, 2H), 7.59–7.38 (d, J = 8.7 Hz, 2H), 7.31 (d, J = 5.9 Hz, 2H), 4.62 (s, 2H), 3.46 (s, 4H), 3.28 (s, 2H), 2.83 (s, 4H). $C_{26}H_{25}BrN_6O$ (517.42).

*5.8.13. N-(2-Methylphenyl)-2-(4-(4-(pyridin-4-ylmethyl) phthalazin-1-yl)piperazin-1-yl)acetamide (**7m**)*

Yield: 36%. m.p.: 183–184 °C. MS[MH$^+$](m/z): 453.3 (M + 1). IR (KBr) cm^{-1}: 3303.2, 2919.0, 2831.0, 1687.7, 1599.7, 1526.8, 1456.5, 1411.6, 1266.2, 1178.1, 1152.0, 1132.4, 1012.2, 755.0. ^{1}H NMR (300 MHz, DMSO) δ: 9.48 (s, 1H), 8.45 (d, J = 5.3 Hz, 2H), 8.18 (m, 1H), 8.13 (m, 1H), 7.99–7.88 (m, 2H), 7.82 (d, J = 7.7 Hz, 1H), 7.32 (d, J = 5.6 Hz, 2H), 7.21 (m, 2H), 7.05 (t, J = 7.2 Hz, 1H), 4.63 (s, 2H), 3.49 (s, 4H), 3.29 (s, 2H), 2.89 (s, 4H), 2.27 (s, 3H). $C_{27}H_{28}N_6O$ (452.55).

*5.9. 3-Hydroxy-2-phenyl-1H-inden-1-one (**8**)*

Prepared in a similar procedure as described for compound 2, prepared from isobenzofuran-1(3H)-one 1 (30 g, 0.22 mol) and benzaldehyde (23.7 mL, 0.23 mol) to give compound 8 as a light yellow solid (34 g, 68.4%). m.p.: 147–148 °C. MS[MH$^+$](m/z): 223.2 (M + 1). $C_{15}H_{10}O_2$ (222.24).

*5.10. 4-Benzylphthalazin-1-ol (**9**)*

Prepared in a similar procedure as described for compound **3**, prepared from compound **8** (10 g, 0.045 mol) to give compound **9** as a white crystal (7.8 g, 73.6%). m.p.: 200–201 °C. MS[MH$^+$](m/z): 237.1 (M + 1). ^{1}H NMR (300 MHz, DMSO) δ: 12.60 (s, 1H), 8.25 (d, J = 7.7 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.90–7.76 (m, 2H), 7.37–7.23 (m, 4H), 7.19 (t, J = 6.5 Hz, 1H), 4.30 (s, 2H). $C_{15}H_{12}N_2O$ (236.27).

*5.11. 1-Chloro-4-benzylphthalazine (**10**)*

Prepared in a similar procedure as described for compound **4**, prepared from compound **9** (7.8 g, 33 mmol) to give compound **10** as a milk white solid (8.3 g, 99%). m.p.: 145–146 °C. MS[MH$^+$](m/z): 255.0 (Cl = 35), 257.0 (Cl = 37). ^{1}H NMR (300 MHz, DMSO) δ: 8.38 (dd, J = 6.2, 3.1 Hz, 1H), 8.31 (dd, J = 6.3, 3.1 Hz, 1H), 8.17–8.06 (m, 2H), 7.37–7.24 (m, 4H), 7.19 (t, J = 7.1 Hz, 1H), 4.72 (s, 2H). $C_{15}H_{11}ClN_2$ (254.71).

*5.12. 1-(Piperazin-1-yl)-4-benzylphthalazine (**11**)*

Prepared in a similar procedure as described for compound **5**, prepared from compound **10** (7.5 g, 29.4 mmol) to give compound **11** as a white solid (5.5 g, 61%). m.p.: 77–78 °C. MS[MH$^+$](m/z): 305.3 (M + 1). $C_{19}H_{20}N_4$ (304.39).

*5.13. General procedure for preparation of compound (**12a**–**12m**)*

Prepared in a similar procedure as described for **7a**–**7m**.

*5.13.1. N-(3,4-Difluorophenyl)-2-(4-(4-benzylphthalazin-1-yl) piperazin-1-yl)acetamide (**12a**)*

Yield: 44%. m.p.: 73–75 °C. MS[MH$^+$](m/z): 474.4 (M + 1). IR (KBr) cm^{-1}: 3283.8, 3061.2, 2921.0, 2827.3, 1689.4, 1615.5, 1517.2, 1411.2, 1264.9, 1205.4, 1132.6, 1013.4, 763.1. ^{1}H NMR (300 MHz, DMSO) δ: 10.24 (s, 1H), 8.18 (m, 1H), 8.09 (m, 1H), 7.88 (m, 3H),

7.51–7.36 (m, 2H), 7.36–7.21 (m, 4H), 7.16 (t, J = 7.1 Hz, 1H), 4.58 (s, 2H), 3.47 (s, 4H), 3.37 (s, 2H), 2.88 (s, 4H). ^{13}C NMR (75.5 MHz, DMSO) δ: 168.72, 159.28, 155.17, 139.10, 131.98, 131.71, 128.63, 128.55, 126.82, 126.35, 125.44, 124.70, 121.00, 117.50, 117.26, 115.93, 108.73, 108.45, 61.72, 52.71, 50.82, 38.48. $C_{27}H_{25}F_2N_5O$ (473.20).

*5.13.2. N-(3-Chloro-4-fluorophenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12b**)*

Yield: 41%. m.p.: 77–79 °C. MS[MH$^+$](m/z): 490.3 (Cl = 35), 492.3 (Cl = 37). IR (KBr) cm^{-1}: 3277.0, 3028.3, 2918.6, 2826.3, 1692.0, 1602.6, 1498.7, 1400.3, 1263.8, 1210.5, 1131.6, 1013.0, 819.9, 762.5. ^{1}H NMR (300 MHz, DMSO) δ: 10.12 (s, 1H), 8.18 (m, 1H), 8.10 (m, 1H), 8.03 (dd, J = 6.9, 2.6 Hz, 1H), 7.90 (m, 2H), 7.64 (m, 1H), 7.40 (d, J = 9.1 Hz, 1H), 7.30 (m, 4H), 7.17 (t, J = 7.1 Hz, 1H), 4.58 (s, 2H), 3.46 (s, 4H), 3.29 (s, 2H), 2.83 (s, 4H). ^{13}C NMR (75.5 MHz, DMSO) δ: 169.42, 159.96, 155.86, 139.77, 132.67, 132.40, 129.30, 129.23, 127.47, 127.03, 126.13, 125.38, 121.69, 120.54, 117.70, 62.35, 53.37, 51.48, 39.14. $C_{27}H_{25}ClFN_5O$ (489.17).

*5.13.3. N-(4-Fluoro-3-(trifluoromethyl)phenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12c**)*

Yield: 33%. m.p.: 77–79 °C. MS[MH$^+$](m/z): 524.1 (M + 1). IR (KBr) cm^{-1}: 3284.6, 3063.4, 2931.8, 2827.8, 1695.6, 1606.0, 1506.4, 1424.3, 1327.3 1267.7, 1247.2, 1132.1, 1053.7, 1013.6, 899.3, 824.2. ^{1}H NMR (300 MHz, DMSO) δ: 10.25 (s, 1H), 8.19 (m, 2H), 8.09 (d, J = 6.8 Hz, 1H), 7.99 (m, 1H), 7.95–7.83 (m, 2H), 7.49 (t, J = 9.8 Hz, 1H), 7.39–7.21 (m, 4H), 7.18 (t, J = 6.8 Hz, 1H), 4.58 (s, 2H), 3.46 (s, 4H), 3.31 (s, 2H), 2.83 (s, 4H). ^{13}C NMR (150.9 MHz, DMSO) δ: 168.98, 159.28, 155.14, 139.09, 135.65, 131.94, 131.67, 128.61, 128.52, 126.81, 126.32, 125.67, 125.62, 125.40, 124.68, 121.00, 117.61, 117.46, 61.66, 52.69, 50.80, 38.47. $C_{28}H_{25}F_4N_5O$ (523.20).

*5.13.4. N-(5-Fluoro-2-methylphenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12d**)*

Yield: 33%. m.p.: 191–192 °C. MS[MH$^+$](m/z): 470.1 (M + 1). IR (KBr) cm^{-1}: 3305.0, 2920.2, 2827.8, 1692.6, 1602.0, 1527.7, 1494.9, 1453.0, 1410.2, 1266.7, 1010.8, 728.6. ^{1}H NMR (300 MHz, DMSO) δ: 9.61 (s, 1H), 8.18 (d, J = 7.8 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 7.99–7.87 (m, 2H), 7.83 (d, J = 11.5 Hz, 1H), 7.44–7.22 (m, 5H), 7.18 (t, J = 6.7 Hz, 1H), 6.89 (t, J = 7.2 Hz, 1H), 4.59 (s, 2H), 3.47 (s, 4H), 3.31 (s, 2H), 2.89 (s, 4H), 2.27 (s, 3H). ^{13}C NMR (75.5 MHz, DMSO) δ: 168.38, 159.25, 155.33, 139.08, 132.06, 131.79, 131.39, 128.66, 128.56, 126.84, 126.37, 125.49, 124.69, 121.01, 110.26, 108.05, 61.45, 52.82, 51.25, 38.49. $C_{28}H_{28}FN_5O$ (469.23).

*5.13.5. N-(2-Chloro-5-(trifluoromethyl)phenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12e**)*

Yield: 24%. m.p.: 168–169 °C. MS[MH$^+$](m/z): 540.3 (Cl = 35), 542.3 (Cl = 37). IR (KBr) cm^{-1}: 3271.8, 3029.9, 2922.1, 2885.3, 2824.1, 1702.2, 1585.1, 1523.3, 1455.0, 1415.1, 1331.6, 1263.6, 1162.7, 1127.5, 1082.0, 1013.3, 903.2, 824.5, 732.3. ^{1}H NMR (300 MHz, DMSO) δ: 10.23 (s, 1H), 8.69 (s, 1H), 8.20 (d, J = 7.8 Hz, 1H), 8.11 (d, J = 6.8 Hz, 1H), 8.00–7.87 (m, 2H), 7.82 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.37–7.22 (m, 4H), 7.18 (t, J = 7.1 Hz, 1H), 4.59 (s, 2H), 3.49 (s, 4H), 3.38 (s, 2H), 2.91 (s, 4H). ^{13}C NMR (75.5 MHz, DMSO) δ: 169.21, 159.20, 155.35, 139.07, 135.36, 132.07, 131.80, 130.62, 128.66, 128.56, 126.85, 126.37, 125.49, 124.68, 121.40, 121.01, 117.28, 61.24, 52.76, 51.25, 38.49. $C_{28}H_{25}ClF_3N_5O$ (539.17).

*5.13.6. N-(2-Chloro-6-methylphenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12f**)*

Yield: 42%. m.p.: 80–81 °C. MS[MH$^+$](m/z): 486.4 (Cl = 35), 488.4 (Cl = 37). IR (KBr) cm^{-1}: 3300.8, 2921.0, 2827.7, 1698.0, 1494.3, 1410.1, 1266.0, 1177.2, 1132.1, 1012.7, 766.5. ^{1}H NMR (300 MHz, DMSO) δ: 9.55 (s, 1H), 8.18 (d, J = 8.7 Hz, 1H), 8.11 (d, J = 6.8 Hz, 1H), 7.97–7.82 (m, 2H), 7.38–7.30 (m, 3H), 7.25 (m, 4H), 7.17 (t, J = 6.7 Hz, 1H), 4.58 (s, 2H), 3.49 (s, 4H), 3.30 (s, 2H), 2.89 (s, 4H), 2.22 (s, 3H). ^{13}C NMR (75.5 MHz, DMSO) δ: 168.30, 159.26, 155.13, 139.09, 138.36, 133.80, 131.95, 131.77, 131.68, 129.01, 128.61, 128.53, 127.87, 126.90, 126.82, 126.32, 125.41, 124.71, 120.99, 61.29, 52.94, 50.82, 38.47. $C_{28}H_{28}ClN_5O$ (485.20).

*5.13.7. N-(2,6-Difluorophenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12g**)*

Yield: 32%. m.p.: 177–178 °C. MS[MH$^+$](m/z): 474.3 (M + 1). IR (KBr) cm^{-1}: 3417.9, 2920.9, 2849.4, 1701.5, 1598.8, 1512.4, 1466.7, 1412.9, 1266.9, 1132.4, 1178.7, 1152.3, 1132.4, 1010.2, 779.7. ^{1}H NMR (300 MHz, DMSO) δ: 9.61 (s, 1H), 8.17 (m, 1H), 8.09 (m, 1H), 7.94–7.83 (m, 2H), 7.41–7.22 (m, 5H), 7.16 (m, 3H), 4.57 (s, 2H), 3.46 (s, 4H), 3.31 (s, 2H), 2.83 (s, 4H). ^{13}C NMR (75.5 MHz, DMSO) δ: 168.87, 159.28, 155.17, 139.10, 131.99, 131.73, 128.63, 128.55, 126.82, 126.35, 125.44, 124.71, 121.01, 111.98, 111.68, 60.98, 52.72, 50.78, 38.49. $C_{27}H_{25}F_2N_5O$ (473.20).

*5.13.8. N-(2,4-Dimethylphenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12h**)*

Yield: 31%. m.p.: 176–178 °C. MS[MH$^+$](m/z): 466.2 (M + 1). IR (KBr) cm^{-1}: 3323.7, 3027.1, 2919.8, 2844.7, 1693.0, 1590.7, 1521.6, 1409.4, 1265.8, 1178.4, 1151.4, 1130.5, 1013.7, 824.0, 765.2. ^{1}H NMR (300 MHz, DMSO) δ: 9.40 (s, 1H), 8.19 (d, J = 8.5 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.97–7.85 (m, 2H), 7.65 (d, J = 8.0 Hz, 1H), 7.37–7.23 (m, 4H), 7.18 (t, J = 6.9 Hz, 1H), 7.04 (s, 1H), 6.99 (d, J = 8.4 Hz, 1H), 4.58 (s, 2H), 3.47 (s, 4H), 3.26 (s, 2H), 2.87 (s, 4H), 2.23 (d, J = 4.4 Hz, 6H). $C_{29}H_{31}N_5O$ (465.25).

*5.13.9. N-(3,4-Dimethoxyphenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12i**)*

Yield: 34%. m.p.: 97–99 °C. MS[MH$^+$](m/z): 498.4 (M + 1). IR (KBr) cm^{-1}: 3461.5, 2935.2, 2834.4, 1686.1, 1610.1, 1516.7, 1453.6, 1409.1, 1262.9, 1236.0, 1133.1, 1028.0, 774.8. ^{1}H NMR (300 MHz, DMSO) δ: 9.73 (s, 1H), 8.18 (d, J = 9.1 Hz, 1H), 8.09 (d, J = 6.6 Hz, 1H), 7.88 (m, 2H), 7.38 (d, J = 2.0 Hz, 1H), 7.36–7.22 (m, 5H), 7.21–7.14 (m, 1H), 6.89 (d, J = 8.8 Hz, 1H), 4.58 (s, 2H), 3.73 (s, 3H), 3.71 (s, 3H), 3.46 (s, 4H), 3.26 (s, 2H), 2.84 (s, 4H). ^{13}C NMR (75.5 MHz, DMSO) δ: 167.89, 159.29, 155.15, 148.61, 144.99, 139.10, 132.47, 131.97, 131.70, 128.62, 128.54, 126.81, 126.34, 125.41, 124.69, 121.00, 112.10, 111.45, 104.82, 61.78, 55.84, 55.52, 52.75, 50.88, 38.48. $C_{29}H_{31}N_5O_3$ (497.24).

*5.13.10. N-(3-Fluorophenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12j**)*

Yield: 44%. m.p.: 77–78 °C. MS[MH$^+$](m/z): 456.4 (M + 1). IR (KBr) cm^{-1}: 3282.2, 3062.3, 2920.1, 2826.8, 1687.4, 1612.4, 1524.4, 1493.4, 1442.7, 1415.2, 1308.6, 1265.4, 1134.4, 1013.5, 868.5, 778.1. ^{1}H NMR (300 MHz, DMSO) δ: 10.20 (s, 1H), 8.18 (d, J = 6.2 Hz, 1H), 8.09 (d, J = 6.0 Hz, 1H), 7.89 (d, J = 3.7 Hz, 2H), 7.70 (d, J = 11.7 Hz, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.39–7.23 (m, 5H), 7.17 (t, J = 6.8 Hz, 1H), 6.88 (t, J = 7.2 Hz, 1H), 4.58 (s, 2H), 3.46 (s, 4H), 3.33 (s, 2H), 2.85 (s, 4H). $C_{27}H_{26}FN_5O$ (455.21).

*5.13.11. N-(3-Bromophenyl)-2-(4-(4-benzylphthalazin-1-yl)piperazin-1-yl)acetamide (**12k**)*

Yield: 44%. m.p.: 80–81 °C. MS[MH$^+$](m/z): 516.3 (Br = 79), 518.3 (Br = 81). IR (KBr) cm^{-1}: 3282.1, 3060.9, 2919.8, 2825.3, 1687.4, 1591.4, 1516.0, 1415.4, 1306.7, 1265.6, 1177.2, 1131.8, 1012.7, 778.0. ^{1}H NMR (300 MHz, DMSO) δ: 10.11 (s, 1H), 8.25–8.14 (m, 1H), 8.14–8.02 (m, 2H), 7.96–7.82 (m, 2H), 7.65 (d, J = 7.2 Hz, 1H), 7.28 (m, 6H), 7.16 (t, J = 6.9 Hz, 1H), 4.58 (s, 2H), 3.45 (s, 4H), 3.30 (s, 2H), 2.83 (s, 4H). $C_{27}H_{26}BrN_5O$ (515.13).

*5.13.12. N-(4-Bromophenyl)-2-(4-(4-benzylphthalazin-1-yl) piperazin-1-yl)acetamide (**12l**)*

Yield: 21%. m.p.: 192–193 °C. MS[MH^+](m/z): 516.1 (Br = 79), 518.1 (Br = 81). IR (KBr) cm^{-1}: 3226.2, 3056.5, 2932.2, 2823.4, 1670.3, 1587.0, 1505.0, 1393.3, 1306.1, 1267.7, 1176.4, 1132.5, 1011.7821.0, 762.4, 711.9. ^1H NMR (300 MHz, DMSO) δ: 10.03 (s, 1H), 8.23–8.14 (m, 1H), 8.14–8.04 (m, 1H), 7.90 (m, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.50 (d, J = 8.7 Hz, 2H), 7.29 (m, 4H), 7.17 (t, J = 7.0 Hz, 1H), 4.58 (s, 2H), 3.45 (s, 4H), 3.29 (s, 2H), 2.83 (s, 4H). ^{13}C NMR (75.5 MHz, DMSO) δ: 168.62, 159.27, 155.15, 139.10, 138.26, 131.97, 131.70, 131.48, 128.62, 128.54, 126.81, 126.34, 125.43, 124.69, 121.51, 120.99, 114.98, 61.70, 52.68, 50.87, 38.49. $C_{27}H_{26}BrN_5O$ (515.13).

*5.13.13. N-(2-Methylphenyl)-2-(4-(4-benzylphthalazin-1-yl) piperazin-1-yl)acetamide (**12m**)*

Yield: 35%. m.p.: 170–171 °C. MS[MH^+](m/z): 452.3 (M + 1). IR (KBr) cm^{-1}: 3296.9, 2919.2, 2824.5, 1688.4, 1585.5, 1526.7, 1454.8, 1412.6, 1386.8, 1262.9, 1012.9, 762.8. ^1H NMR (300 MHz, DMSO) δ: 9.49 (s, 1H), 8.19 (d, J = 8.1 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 7.98–7.86 (m, 2H), 7.83 (d, J = 7.8 Hz, 1H), 7.28 (m, 5H), 7.18 (t, J = 7.5 Hz, 2H), 7.05 (t, J = 7.3 Hz, 1H), 4.59 (s, 2H), 3.48 (s, 4H), 3.29 (s, 2H), 2.89 (s, 4H), 2.28 (s, 3H). ^{13}C NMR (150.9 MHz, DMSO) δ: 167.93, 159.21, 155.23, 139.05, 136.16, 131.99, 131.71, 130.31, 129.00, 128.51, 126.82, 126.32, 126.28, 125.42, 124.65, 124.40, 122.18, 120.99, 61.54, 52.84, 51.14, 38.46. $C_{28}H_{29}N_5O$ (451.24).

5.14. Cytotoxicity assay in vitro

The cytotoxic activities of compounds **7a**–**7m** and **12a**–**12m** were evaluated with A549, HT-29 and MDA-MB-231 cell lines (A549: non-small-cell lung cancer cell line, HT-29: human colon cancer cell line, MDA-MB-231: human breast cancer cell line) by the standard MTT assay [17] *in vitro*, with PTK-787 as the positive control. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS).

Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The test compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested twice in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of two determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

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