Full Paper

Synthesis and Cytotoxic Evaluation of Some New Phthalazinylpiperazine Derivatives

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A new series of 1,4-disubstituted phthalazinylpiperazine derivatives **7a–f**, **12a–f** and **20a–f** were designed and synthesized in order to develop potent and selective antitumor agents. The target compounds were screened for their cytotoxic activities against A549, HT-29 and MDA-MB-231 cancer cell lines *in vitro*. Among them, compounds **7a–f** exhibited excellent selectivity for MDA-MB-231 with IC_{50} values ranging from 0.013 μM to 0.079 μM. The most promising compound, **7e** (IC_{50} = 2.19 μM, 2.19 μM, 0.013 μM), was 9.3, 10, and 4.9×10^3 times more active than vatalanib (IC_{50} = 20.27 μM, 21.96 μM, 63.90 μM), respectively.

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Introduction

The search for new anticancer chemotherapeutic agents continues to be an active area of research in many companies. During the last decade, many synthetic phthalazine derivatives have been reported as antitumor agents [1–5]. Among them, vatalanib (PTK-787), an anilinophthalazine compound, is currently undergoing phase III clinical trials for renal cell carcinoma cancer and glioblastoma. Moreover, several additional phase II studies are underway, such as myelodysplasia, meningioma and metastatic melanoma, *etc.* [6] Therefore, the broad spectrum and potent antitumor activity of vatalanib has attracted much attention in recent years.

In order to improve upon the antitumor efficacy of vatalanib, many new phthalazine derivatives were developed [7–9], such as compounds **I** and **II** (Figure 1) [7], which had been proved highly potent against human liver cancer *in vitro*. From these compounds, we could disclose that the introduction of a piperazinyl group between phthalzine and aniline was suitable, and the 4-phenyl group was generally well tolerated. So we combined vatalanib with the piperazinyl group in one structure, and inserted an acetyl-flexible linker into the structure to get the new chemical entities (Figure 1). Various substituents $\mathbf{R_1}$ were introduced into the aniline ring to investigate their influence on cytotoxic activities to obtain the first series of compounds **7a–f**. Furthermore, the pyridinyl group at position 4 of compounds **7a–f** was replaced by phenyl group to produce compounds **12a–f**. For further research on the influence of electrical effects in the phthalzine nucleus, we introduced two electron-donating methoxy groups into **12a–f** to get another series of derivatives **20a–f**. As a result, eighteen novel 1,4-disubstituted phthalazinylpiperazine derivatives (Figure 1) were designed and synthesized, and their cytotoxicities against A549, HT-29 and MDA-MB-231 cancer cell lines were tested.

Results and discussion

Chemistry

The synthesis of target compounds **7a–f** and **12a–f** was achieved using a convenient seven-step procedure starting from phthalic anhydride depicted in Scheme 1. The commercially available phthalic anhydride was reduced by $NaBH_4$ in dry tetrahydrofuran to give compound **1**, which was then reacted with 4-pyridine carboxaldehyde in methanol and

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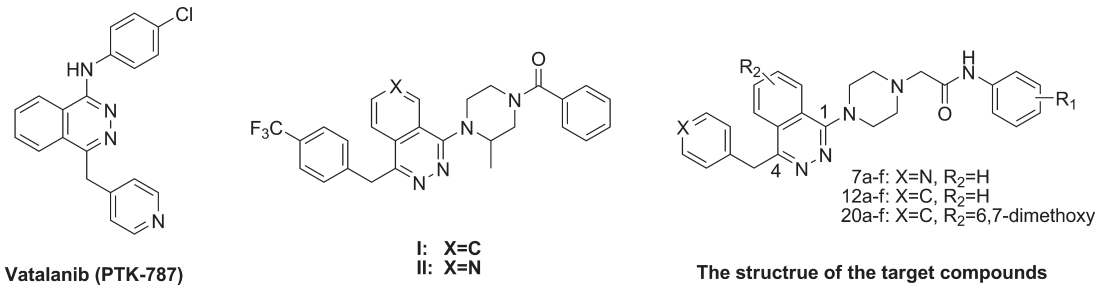
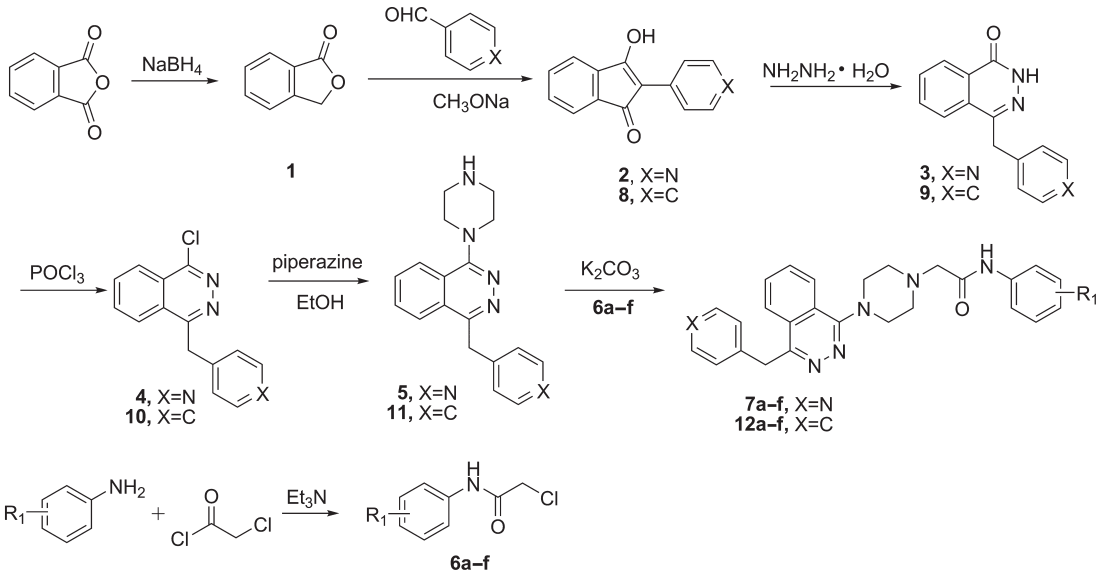


Figure 1. Structures of vatalanib, compound I, II and the target compounds.



Scheme 1. Synthesis of target compounds 7a–f and 12a–f.

ethyl propionate under basic condition to obtain compound **2**. The key intermediate, pyridinylphthalazone **3**, was prepared from compound **2** in 80% hydrazine hydrate at 100°C for 5 h in 75% yield. Then, compound **3** was chlorinated using phosphoryl chloride in acetonitrile to produce compound **4** [10]. Next, compound **5** was synthesized by the reaction of compound **4** with excess piperazine in EtOH. Furthermore, the side chains **6a–f** were prepared *via* a series of substituted anilines with 2-chloroacetyl chloride in presence of Et_3N. Another phenylphthalazinylpiperazine derivative **11** was obtained according to the same method described for compound **5** except that 4-pyridine carboxaldehyde was replaced by benzaldehyde.

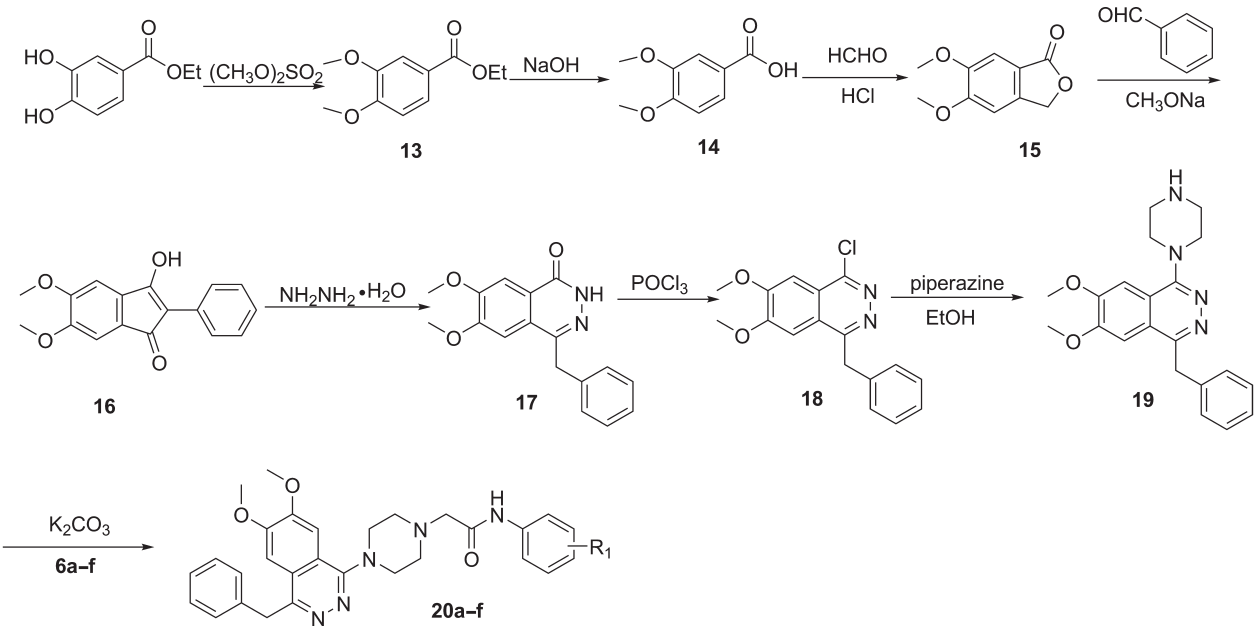
The synthesis of target compounds **20a–f** was summarized in Scheme 2. The starting material, ethyl 3,4-dimethoxybenzoate, was methylated with $(CH_3O)_2SO_2$ under basic condition to give compound **13**, which was subsequently hydrolyzed to get compound **14**. The 5,6-dimethoxyisobenzofuran-1(3*H*)-one **15** was prepared *via* cyclization of compound **14** with 37% formaldehyde solution in the presence of hydrogen chloride gas [11]. Similarly, the intermediate **19** was obtained as in the manner described for compound **5**, except that 4-pyridine carboxaldehyde was replaced with benzaldehyde.

Finally, the target compounds **7a–f**, **12a–f** and **20a–f** were successfully obtained *via* the reaction of the intermediate **5**, **11**, and **19** with **6a–f** in the presence of K_2CO_3 in acetone, respectively.

The structures and the data of ^{1}H-NMR, MS and elemental analysis were outlined in Table 1 and Table 2.

Evaluation of biological activity

The preliminary cytotoxic activities of compounds **7a–f**, **12a–f** and **20a–f** on A549 (human lung cancer cell lines), HT-29 (human colon cancer cell line) and MDA-MB-231 (human breast cancer cell line) were investigated *in vitro*. These compounds' properties are summarized in Table 3 and are compared to those of vatalanib.



Scheme 2. Synthesis of target compounds 20a–f.

The data in Table 3 indicated that the cytotoxic activities of compounds **7a–f**, **20a** and **20d** were more active than vatalanib against all three human cancer cell lines, and most of them were comparable to that of vatalanib.

Obviously, 4-pyridinylphthalazine derivatives (**7a–f**) were better than their 4-phenyl analogues (**12a–f**). As shown in Table 3, compounds **7a–f** displayed excellent cytotoxic activities for A549 and HT-29 cell lines with IC_{50} values in the single-digit μM. In particular, compounds **7a–f** showed IC_{50} values ranging from 0.013 μM to 0.079 μM against MDA-MB-231 cell line, which were 10^2 to 10^3-fold more active than those of the corresponding compounds **12a–f**.

As well, most of the target compounds exhibited better activities against MDA-MB-231 cell line than did vatalanib.

Table 1. The structures, MS, elemental analysis and physical data of compounds **7a–f**, **12a–f** and **20a–f**.

Compd.	X	R_1	m.p. (°C)	Elemental Analysis			$[MH^+]$ (*m*/*z*)	Yields (%)
				C%	H%	N%		
7a	N	4-CH_3	188–189	71.66(71.54)*	6.24 (6.19)	18.57 (18.66)	453.2	15%
7b	N	2-CF_3	149–150	64.02 (63.99)	4.97 (4.98)	16.59 (16.63)	507.1	47%
7c	N	3-Cl	76–77	66.03 (66.00)	5.33 (5.35)	17.77 (17.76)	473.1 475.1	38%
7d	N	4-OCF_3	76–77	62.06 (62.00)	4.82 (4.83)	16.08 (16.02)	523.3	44%
7e	N	4-F	215–216	68.40 (68.32)	5.52 (5.58)	18.41 (18.43)	457.5	55%
7f	N	3,5-diCF_3	80–81	58.54 (58.51)	4.21 (4.23)	14.63(14.60)	575.5	48%
12a	C	4-CH_3	165–167	74.47(74.43)	6.47 (6.51)	15.51 (15.54)	452.2	65%
12b	C	2-CF_3	154–155	66.52 (66.46)	5.18(5.19)	13.85 (13.89)	506.1	66%
12c	C	3-Cl	80–81	68.71 (68.66)	5.55 (5.59)	14.84 (14.74)	472.1 474.0	33%
12d	C	4-OCF_3	77–78	64.48 (64.55)	5.02 (4.94)	13.43 (13.45)	522.4	49%
12e	C	4-F	77–78	71.19 (71.22)	5.75 (5.64)	15.37 (15.45)	456.3	51%
12f	C	3,5-diCF_3	78–79	60.73 (60.77)	4.39 (4.44)	12.21 (12.12)	574.3	37%
20a	C	4-CH_3	90–92	70.43 (70.40)	6.50 (6.52)	13.69 (13.66)	512.3	25%
20b	C	2-CF_3	70–71	63.71 (63.66)	5.35 (5.39)	12.38 (12.40)	566.3	33%
20c	C	3-Cl	100–101	65.47(65.53)	5.68(5.62)	13.16 (13.10)	532.4 534.4	41%
20d	C	4-OCF_3	111–112	61.95(62.04)	5.20 (5.27)	12.04 (12.00)	582.4	36%
20e	C	4-F	106–107	67.56 (67.46)	5.86 (5.91)	13.58 (13.49)	516.3	45%
20f	C	3,5-diCF_3	92–93	58.77 (58.86)	4.61 (4.54)	11.05 (11.00)	634.5	41%

Table 2. The IR, ^{1}H-NMR data of compounds **7a–f**, **12a–f** and **20a–f**.

Compd.	IR, $\bar{\nu}$/cm^{-1}	^{1}H-NMR (300 MHz, DMSO-d_6), δ
7a	3412.1 (υ_{NH}), 1675.8 ($\upsilon_{C=O}$), 1601.4, 1521.6, 1409.9 ($\upsilon_{C=C}$), 818.0, 779.1 ($\delta_{=CH}$)	9.78 (s, 1H, C***NH***C=O), 8.44 (d, J = 5.8 Hz, 2H, pyridinyl-***2H***,***6H***), 8.15 (m, 2H, phthalazine-***5H***,***8H***), 7.93 (m, 2H, phthalazine-***6H***,***7H***), 7.54 (d, J = 8.3 Hz, 2H, Ph-***2H***,***6H***), 7.31 (d, J = 5.6 Hz, 2H, pyridine-***3H***,***5H***), 7.13 (d, J = 8.3 Hz, 2H, Ph-***3H***,***5H***), 4.62 (s, 2H, C***CH$_2$***C), 3.46 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.26 (s, 2H, N***CH$_2$***C=O), 2.83 (s, 4H, CH$_2$N***(CH$_2$)$_2$***), 2.25 (s, 3H, C***CH$_3$***).
7b	3298.7 (υ_{NH}), 1698.7 ($\upsilon_{C=O}$), 1590.1, 1529.3 ($\upsilon_{C=C}$), 766.5 ($\delta_{=CH}$)	9.96 (s, 1H, C***NH***C=O), 8.44 (d, J = 5.6 Hz, 2H, pyridinyl-***2H***,***6H***), 8.27 (d, J = 8.1 Hz, 1H, Ph-***3H***), 8.18 (m, 2H, phthalazine-***5H***,***8H***), 7.94 (m, 2H, phthalazine-***6H***,***7H***), 7.72 (m, 2H, Ph-***4H***,***5H***), 7.34 (m, 3H, pyridinyl-***3H***,***5H*** + Ph-***6H***), 4.63 (s, 2H, C***CH$_2$***C), 3.47 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.34 (s, 2H, N***CH$_2$***C=O), 2.89 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
7c	3269.7 (υ_{NH}), 1686.5 ($\upsilon_{C=O}$), 1595.6, 1518.1 ($\upsilon_{C=C}$), 877.0, 778.6, 680.2 ($\delta_{=CH}$)	10.10 (s, 1H, C***NH***C=O), 8.44 (d, J = 5.6 Hz, 2H, pyridinyl-***2H***,***6H***), 8.16 (m, 2H, phthalazine-***5H***,***8H***), 7.93 (m, 3H, phthalazine-***6H***,***7H*** + Ph-***2H***), 7.58 (d, J = 7.7 Hz, 1H, Ph-***6H***), 7.34 (m, 3H, pyridinyl-***3H***,***5H*** + Ph-***5H***), 7.13 (d, J = 7.7 Hz, 1H, Ph-***4H***), 4.62 (s, 2H, C***CH$_2$***C), 3.47 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.30 (s, 2H, N***CH$_2$***C=O), 2.83 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
7d	3411.8 (υ_{NH}), 1688.3 ($\upsilon_{C=O}$), 1602.4, 1512.9, 1411.6 ($\upsilon_{C=C}$), 805.4, 778.5 ($\delta_{=CH}$)	10.10 (s, 1H, C***NH***C=O), 8.44 (d, J = 5.1 Hz, 2H, pyridinyl-***2H***,***6H***), 8.15 (m, 2H, phthalazine-***5H***,***8H***), 7.91 (m, 2H, phthalazine-***6H***,***7H***), 7.82 (d, J = 9.0 Hz, 2H, Ph-***2H***,***6H***), 7.32 (d, J = 7.9 Hz, 4H, pyridine-***3H***,***5H*** + Ph-***3H***,***5H***), 4.62 (s, 2H, C***CH$_2$***C), 3.47 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.30 (s, 2H, N***CH$_2$***C=O), 2.83 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
7e	3263.4 (υ_{NH}), 1671.0 ($\upsilon_{C=O}$), 1532.6, 1508.8 ($\upsilon_{C=C}$), 836.1, 779.4 ($\delta_{=CH}$)	9.93 (s, 1H, C***NH***C=O), 8.44 (d, J = 5.6 Hz, 2H, pyridinyl-***2H***,***6H***), 8.15 (m, 2H, phthalazine-***5H***,***8H***), 7.92 (m, 2H, phthalazine-***6H***,***7H***), 7.71 (m, 2H, Ph-***2H***,***6H***), 7.31 (d, J = 5.7 Hz, 2H, pyridine-***3H***,***5H***), 7.16 (t, J = 8.8 Hz, 2H, Ph-***3H***,***5H***), 4.62 (s, 2H, C***CH$_2$***C), 3.47 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.27 (s, 2H, N***CH$_2$***C=O), 2.83 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
7f	3422.1 (υ_{NH}), 1695.5 ($\upsilon_{C=O}$), 1601.6, 1532.4 ($\upsilon_{C=C}$), 778.3, 703.2, 680.8 ($\delta_{=CH}$)	10.55 (s, 1H, C***NH***C=O), 8.44 (m, 4H, pyridinyl-***2H***,***6H*** + phthalazine-***5H***,***8H***), 8.15 (m, 2H, Ph-***2H***,***6H***), 7.93 (m, 2H, phthalazine-***6H***,***7H***), 7.78 (s, 1H, Ph-***4H***), 7.32 (d, J = 5.8 Hz, 2H, Ph-***3H***,***5H***), 4.62 (s, 2H, C***CH$_2$***C), 3.49 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.36 (s, 2H, N***CH$_2$***C=O), 2.85 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
12a	3391.2 (υ_{NH}), 1671.6 ($\upsilon_{C=O}$), 1521.5, 1407.7 ($\upsilon_{C=C}$), 818.6, 763.2, 713.6 ($\delta_{=CH}$)	9.78 (s, 1H, C***NH***C=O), 8.18 (m, 2H, phthalazine-***5H***,***8H***), 7.90 (m, 2H, phthalazine-***6H***,***7H***), 7.54 (d, J = 8.4 Hz, 2H, aniline-***2H***,***6H***), 7.31 (m, 4H, Ph-***2H***,***3H***,***5H***,***6H***), 7.10 (m, 3H, Ph-***4H***, aniline-***3H***,***5H***), 4.58 (s, 2H, C***CH$_2$***C), 3.46 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.26 (s, 2H, N***CH$_2$***C=O), 2.83 (s, 4H, CH$_2$N***(CH$_2$)$_2$***), 2.25 (s, 3H, C***CH$_3$***).
12b	3264.2 (υ_{NH}), 1698.4 ($\upsilon_{C=O}$), 1588.1, 1532.7 ($\upsilon_{C=C}$), 768.5 ($\delta_{=CH}$)	9.97 (s, 1H, C***NH***C=O), 8.18 (m, 3H, aniline-***3H***, phthalazine-***5H***,***8H***), 7.88 (m, 2H, phthalazine-***6H***,***7H***), 7.91 (m, 2H, aniline-***4H***,***5H***), 7.32 (m, 5H, Ph-***2H***,***3H***,***5H***,***6H*** + aniline-***6H***), 7.18 (t, J = 7.2 Hz, 1H, Ph-***4H***), 4.59 (s, 2H, C***CH$_2$***C), 3.46 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.34 (s, 2H, N***CH$_2$***C=O), 2.89 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
12c	3276.2 (υ_{NH}), 1687.4 ($\upsilon_{C=O}$), 1594.4, 1516.9 ($\upsilon_{C=C}$), 778.8, 713.8, 675.1 ($\delta_{=CH}$)	10.10 (s, 1H, C***NH***C=O), 8.17 (m, 2H, phthalazine-***5H***,***8H***), 7.92 (m, 3H, phthalazine-***6H***,***7H*** + aniline-***2H***), 7.61 (d, J = 8.3 Hz, 1H, aniline-***6H***), 7.31 (m, 5H, Ph-***2H***,***3H***,***5H***,***6H*** + aniline-***5H***), 7.13 (m, 2H, Ph-***4H***, aniline-***4H***), 4.58 (s, 2H, C***CH$_2$***C), 3.46 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.30 (s, 2H, N***CH$_2$***C=O), 2.83 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
12d	3303.0 (υ_{NH}), 1688.4 ($\upsilon_{C=O}$), 1513.8, 1410.8 ($\upsilon_{C=C}$), 846.7, 762.8, 712.8 ($\delta_{=CH}$)	10.11 (s, 1H, C***NH***C=O), 8.18 (m, 2H, phthalazine-***5H***,***8H***), 7.89 (m, 2H, phthalazine-***6H***,***7H***), 7.82 (d, J = 8.6 Hz, 2H, aniline-***2H***,***6H***), 7.29 (m, 6H, Ph-***2H***,***3H***,***5H***,***6H*** + aniline-***3H***,***5H***), 7.14 (t, J = 7.1 Hz, 1H, Ph-***4H***), 4.58 (s, 2H, C***CH$_2$***C), 3.46 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.31 (s, 2H, N***CH$_2$***C=O), 2.84 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
12e	3282.3 (υ_{NH}), 1695.8 ($\upsilon_{C=O}$), 1506.3 ($\upsilon_{C=C}$), 824.4, 762.9 ($\delta_{=CH}$)	9.93 (s, 1H, C***NH***C=O), 8.17 (m, 2H, phthalazine-***5H***,***8H***), 7.93 (m, 2H, phthalazine-***6H***,***7H***), 7.70 (m, 2H, aniline-***2H***,***6H***), 7.31 (m, 4H, Ph-***2H***,***3H***,***5H***,***6H***), 7.16 (t, J = 8.9 Hz, 3H, Ph-***4H***, aniline-***3H***,***5H***), 4.58 (s, 2H, C***CH$_2$***C), 3.46 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.27 (s, 2H, N***CH$_2$***C=O), 2.83 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
12f	3282.8 (υ_{NH}), 1696.7 ($\upsilon_{C=O}$), 1532.4 ($\upsilon_{C=C}$), 762.4, 700.4, 680.9 ($\delta_{=CH}$)	10.58 (s, 1H, C***NH***C=O), 8.47 (s, 2H, aniline-***2H***,***6H***), 8.17 (m, 2H, phthalazine-***5H***,***8H***), 7.89 (m, 2H, phthalazine-***6H***,***7H***), 7.78 (s, 1H, aniline-***4H***), 7.26 (m, 4H, Ph-***2H***,***3H***,***5H***,***6H***), 7.16 (t, J = 6.9 Hz, 1H, Ph-***4H***), 4.58 (s, 2H, C***CH$_2$***C), 3.48 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.36 (s, 2H, N***CH$_2$***C=O), 2.85 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
20a	3419.7 (υ_{NH}), 1690.2 ($\upsilon_{C=O}$), 1515.0, 1464.7, 1420.7 ($\upsilon_{C=C}$), 817.4 ($\delta_{=CH}$).	9.76 (s, 1H, C***NH***C=O), 7.54 (d, J = 8.2 Hz, 2H, aniline-***2H***,***6H***), 7.34 (m, 6H, phthalazine-***5H***,***8H*** + Ph-***2H***,***3H***,***5H***,***6H***), 7.13 (m, 3H, aniline-***3H***,***5H*** + Ph-***4H***), 4.55 (s, 2H, C***CH$_2$***C), 3.94 (s, 3H, O***CH$_3$***), 3.87 (s, 3H, O***CH$_3$***), 3.40 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.25 (s, 2H, N***CH$_2$***C=O), 2.82 (s, 4H, CH$_2$N***(CH$_2$)$_2$***), 2.25 (s, 3H, C***CH$_3$***).

Table 2. (*continued*)

Compd.	IR, $\overline{\nu}/cm^{-1}$	^{1}H-NMR (300 MHz, DMSO-d_6), δ
20b	3317.8 (υ_{NH}), 1697.1 ($\upsilon_{C=O}$), 1514.1, 1457.1 ($\upsilon_{C=C}$), 766.4 ($\delta_{=CH}$).	9.97 (s, 1H, C***NH***C=O), 8.26 (d, J = 8.3 Hz, 1H, aniline-***3H***), 7.85 (t, J = 7.3 Hz, 1H, aniline-***4H***), 7.74 (m, 2H, aniline-***5H***,***6H***), 7.35 (m, 7H, phthalazine-***5H***,***8H*** + Ph-***2H***,***3H***,***4H***,***5H***,***6H***), 4.56 (s, 2H, C***H$_2$***C), 3.95 (s, 3H, O***CH$_3$***), 3.88 (s, 3H, O***CH$_3$***), 3.38 (m, 6H, (CH$_2$***CH$_2$***)$_2$N + N***CH$_2$***C=O), 2.88 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
20c	3299.8 (υ_{NH}), 1687.3 ($\upsilon_{C=O}$), 1513.2, 1421.0 ($\upsilon_{C=C}$), 817.7, 778.7 ($\delta_{=CH}$).	10.06 (s, 1H, C***NH***C=O), 7.90 (s, 1H, aniline-***2H***), 7.59 (d, J = 8.2 Hz, 1H, aniline-***6H***), 7.37 (m, 7H, phthalazine-***5H***,***8H*** + Ph-***2H***,***3H***,***5H***,***6H*** + aniline-***5H***), 7.24 (m, 2H, Ph-***4H*** + aniline-***4H***), 4.55 (s, 2H, C***H$_2$***C), 3.94 (s, 3H, O***CH$_3$***), 3.87 (s, 3H, O***CH$_3$***), 3.41 (m, 6H, (CH$_2$***CH$_2$***)$_2$N + N***CH$_2$***C=O), 2.82 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
20d	3411.5 (υ_{NH}), 1690.9 ($\upsilon_{C=O}$), 1513.4, 1421.7 ($\upsilon_{C=C}$), 817.3 ($\delta_{=CH}$).	10.07 (s, 1H, C***NH***C=O), 7.78 (d, J = 8.8 Hz, 2H, aniline-***2H***,***6H***), 7.34 (m, 8H, phthalazine-***5H***,***8H*** + Ph-***2H***,***3H***,***5H***,***6H*** + aniline-***3H***,***5H***), 7.18 (t, J = 7.4 Hz, 1H, Ph-***4H***), 4.55 (s, 2H, C***H$_2$***C), 3.96 (s, 3H, O***CH$_3$***), 3.88 (s, 3H, O***CH$_3$***), 3.34 (m, 6H, (CH$_2$***CH$_2$***)$_2$N + N***CH$_2$***C=O), 2.82 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
20e	3426.1 (υ_{NH}), 1687.5 ($\upsilon_{C=O}$), 1511.2, 1421.6 ($\upsilon_{C=C}$), 836.1 ($\delta_{=CH}$).	10.30 (brs, 1H, C***NH***C=O), 7.70 (dd, J = 8.7 Hz, 2H, aniline-***2H***,***6H***), 7.37 (m, 6H, phthalazine-***5H***,***8H*** + Ph-***2H***,***3H***,***5H***,***6H***), 7.17 (m, 3H, Ph-***4H*** + aniline-***3H***,***5H***), 4.57 (s, 2H, C***H$_2$***C), 3.96 (s, 3H, O***CH$_3$***), 3.88 (s, 3H, O***CH$_3$***), 3.49 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.37 (s, 2H, N***CH$_2$***C=O), 3.03 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).
20f	3398.5 (υ_{NH}), 1699.5 ($\upsilon_{C=O}$), 1514.1, 1470.3 ($\upsilon_{C=C}$), 818.2 ($\delta_{=CH}$).	11.17 (brs, 1H, C***NH***C=O), 8.44 (s, 2H, aniline-***2H***,***6H***), 7.81 (s, 1H, aniline-***4H***), 7.38 (m, 6H, phthalazine-***5H***,***8H*** + Ph-***2H***,***3H***,***5H***,***6H***), 7.18 (t, J = 7.2 Hz, 3H, Ph-***4H*** + aniline-***3H***,***5H***), 4.61 (s, 2H, C***H$_2$***C), 3.98 (s, 3H, O***CH$_3$***), 3.90 (s, 3H, O***CH$_3$***), 3.56 (s, 4H, (CH$_2$***CH$_2$***)$_2$N), 3.15 (s, 2H, N***CH$_2$***C=O), 2.84 (s, 4H, CH$_2$N***(CH$_2$)$_2$***).

Table 3. The antitumor activities of compounds **7a–f**, **12a–f** and **20a–f**.

Compd.	IC_{50}[a] (μM)			Compd.	IC_{50} (μM)		
	A549	HT-29	MDA-MB-231		A549	HT-29	MDA-MB-231
7a	11.05	2.32	0.044	**12e**	20.87	26.36	18.01
7b	1.58	2.57	0.079	**12f**	NA[b]	10.82	127.3
7c	1.75	2.22	0.021	**20a**	3.71	3.71	4.50
7d	3.44	2.49	0.038	**20b**	49.51	93.71	68.95
7e	2.19	2.19	0.013	**20c**	114.6	4.70	20.68
7f	4.53	0.82	0.052	**20d**	13.93	4.30	39.55
12a	68.70	59.84	17.29	**20e**	25.02	NA[a]	NA
12b	NA[a]	31.67	15.24	**20f**	162.6	26.26	75.76
12c	23.98	11.46	4.24				
12d	NA[a]	26.86	19.19	vatalanib	20.27	21.96	63.90

[a] IC_{50}: The IC_{50} was the average of at least two independent experiments.
[b] NA: Compounds having IC_{50} value > 200 μM.

Moreover, the pharmacological results indicated that the cytotoxicity of compounds **7a–f** against MDA-MB-231 cell line were 10 to 10^2-fold higher than A549 and HT-29 cell lines, reflecting excellent selectivity for a particular type of human breast cancer.

Compounds **20a–f** were chosen as an example to study the effect of electron-donating groups on antitumor activities. Among the six compounds bearing 6,7-dimethoxy groups, the most promising compound, **20a** (IC_{50} = 3.71 μM, 3.71 μM, 4.50 μM), was 5.5, 5.5, and 14-fold more active than vatalanib (IC_{50} = 20.27 μM, 21.96 μM, 63.90 μM), respectively. However, it was disappointing to note that the potencies of compounds **20a–f** were much lower than compounds **7a–f**.

The cytotoxic activities were not influenced by the introduction of different groups with various substituted position, spatial and electrical effects on aniline moiety.

Experimental

Chemistry

Melting points were measured with a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. Mass spectra (MS) were taken in ESI mode on an

Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). Proton (^{1}H), nuclear magnetic resonance spectroscopy were performed using Bruker ARX-300, 300MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Elemental analysis were conducted with a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). Infrared spectra (KBr disks) were recorded with a Bruker IFS 55 instrument (Bruker). Unless otherwise noted, all solvents and reagents were commercially available and used without further purification.

*Synthesis of 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 (750mL) at 0–10°C, and then anhydrous methanol was added drop-wise to the reaction mixture at 0–10°C. The reaction mixture was stirred at room temperature for 3 h, and concentrated under vacuum. The residue was poured into a 10% hydrochloric acid aqueous solution (1000 mL), stirred vigorously 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 **1** as a white crystal (78 g, 70%).

*Synthesis of 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 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 1h, and concentrated under vacuum. The residue was poured into ice water (1000 mL), acidified with glacial acetic acid to pH 2–3, and separated by filtration to give **2** as an orange solid (43.8 g, 52.6%).

*Synthesis of 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 **3** as a light yellow crystal (34.9 g, 75%).

*Synthesis of 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 at 90°C for 3 h and concentrated under vacuum. 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 **4** as a red solid (13.5 g, 71.6%).

*Synthesis of 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 at 60°C for 5 h and concentrated under vacuum. The residue was poured into ice water (200 mL), stirred vigorously for 1 h, and separated by filtration to give **5** as a light yellow solid (11.45 g, 71%).

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

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

*General procedure for preparation of compound (**7a–f**)*

A mixture of compound **5** (0.2 g, 0.65 mmol), 2-chloro-*N*-(substituted-phenyl)acetamide (0.72 mmol), K_2CO_3 (0.23 g, 1.64 mmol), and acetone (2 mL) was stirred at reflux for 7–12 h and then cooled to room temperature. The liquid was collected by filtration and evaporated. The crude product was purified by chromatography on silica gel using MeOH/CH_2Cl_2 to obtain **7a-f** as light yellow crystals.

*Synthesis of 3-hydroxy-2-phenyl-1H-inden-1-one (**8**)*

Prepared in a similar procedure as described for **2** (68.4% yield).

*Synthesis of 4-benzylphthalazin-1-ol (**9**)*

Prepared in a similar procedure as described for **3** (73.6% yield).

*Synthesis of 1-chloro- 4-benzylphthalazine (**10**)*

Prepared in a similar procedure as described for 4 (99% yield).

*Synthesis of 1-(piperazin-1-yl-)4-benzylphthalazine (**11**)*

Prepared in a similar procedure as described for **5** (61% yield).

*General procedure for preparation of compound (**12a–f**)*

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

*Synthesis of Ethyl 3,4-dimethoxybenzoate (**13**)*

A mixture of ethyl 3,4-dimethoxybenzoate (50g, 0.28 mol), K_2CO_3 (110 g, 0.80 mol), and acetone (300 mL) was stirred at room temperature for 0.5 h. And then $(CH_3O)_2SO_2$ (57 mL, 0.60 mol) was added drop-wise to the reaction. The reaction mixture was heated at 50°C for 5 h and concentrated under vacuum. The residue was poured into a 10% hydrochloric acid aqueous solution (1000 mL), stirred intensively for 3 h, and then dissolved in EtOAc (700 mL). The resulting solution was washed with brine and water. The organic phase was dried (Na_2SO_4) and evaporated to yield compound 13 as a light yellow oil (54.5 g, 94.5%).

*Synthesis of 3,4-dimethoxybenzoic acid (**14**)*

Compound **13** (154.5 g, 0.26 mol) was added to a stirred solution of NaOH (20.5 g, 0.513 mol) in water (150 mL) at 0–10°C. The mixture was heated at 70°C for 2 h. After cooling to room temperature, the resulting mixture was poured into water (100 mL). Then the mixture was acidified with concentrated hydrochloric acid to pH 2–3, stirred at room temperature for 1h and separated by filtration to give **14** as a white crystal (43.5 g, 92.2%).

*Synthesis of 5,6-dimethoxyisobenzofuran-1(3H)-one (**15**)*

Hydrogen chloride gas was gradually passed into the 37% formaldehyde solution (250 ml, 3.31 mol) for 0.5 h at room temperature, and then compound **14** (33.5 g, 0.184 mol) was added to the reaction. The mixture was heated to 65°C for 14 h and concentrated under vacuum. The residue was poured into water

(250 mL) and alkalized with a 10% sodium hydroxide aqueous solution, stirred for 1h at room temperature, and separated by filtration to give **15** as a light purple powder, which was recrystallized from ethanol to obtain a white crystal (23 g, 50%).

*Synthesis of 3-hydroxy-5,6-dimethoxy-2-phenyl-1H-inden-1-one (**16**)*

Prepared in a similar procedure as described for **2** (73.9% yield).

*Synthesis of 4-benzyl-6,7-dimethoxyphthalazin-1(2H)-one (**17**)*

Prepared in a similar procedure as described for 3 (64.6% yield).

*Synthesis of 1-chloro-4-benzyl-6,7-dimethoxyphthalazine (**18**)*

Prepared in a similar procedure as described for **4** (99% yield).

*Synthesis of 1-(piperazin-1-yl)-4-benzyl-6,7-dimethoxyphthalazine (**19**)*

Prepared in a similar procedure as described for **5** (85.7% yield).

*General procedure for preparation of compound (**20a–f**)*

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

Evaluation of biological activity

The cytotoxic activities of compounds **7a–f**, **12a–f** and **20a–f** were evaluated with A549, HT-29 and MDA-MB-231 cell lines by the standard MTT assay *in vitro* [12], with vatalanib 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 using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

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