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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of anti-proliferative activity of 1,4-disubstituted phthalazines

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Abstract A series of new phthalazine derivatives **3a–i** and **4a–c** were synthesized via the reaction of 1-chlorophthalazine derivative **2** with either *N*-substituted-piperazines, primary or their secondary amines. The structure of the synthesized, new compounds were characterized by spectral data. The anti-proliferative activity on human breast cancer cell line MCF-7 of the synthesized compounds was determined. The results showed that six of the test compounds (**3a**, **3g**, **3i**, and **4a–c**) displayed potent cytotoxic activity ranging from 1.4 to 2.3 μmol.

Keywords Breast cancer · Anti-proliferative activity · Phthalazine · Phthalazinone

Introduction

Breast cancer is the most common form of cancer and the second most frequent cause of cancer death among women (Wang *et al*., 2004; Albrand and Terret, 2008)**.** Regardless of the use of surgical treatment and irradiation, chemotherapy still remains an important option for the treatment of solid cancers. Chemotherapeutic drugs should preferentially target tumor cells without harming normal cells or tissues. However, although new cytotoxic agents with unique mechanisms of action have been developed continuously, many of them have not been therapeutically useful due to low tumor selectivity and harsh side effects (Chari, 2008). These facts prompted us to design and develop novel potent and selective anti-breast cancer agents.

1,4-Disubstituted phthalazines have received a considerable attention as antitumor agents in the past few years (Menear *et al.*, 2008a; Menear *et al.*, 2008b; Miller-Moslin *et al.*, 2009; Vasiliou *et al.*, 2009). A successful example is Vatalanib (PTK-787), (Fig. 1), which is VEGFR (vascular endothelial growth factor receptor) inhibitor and is currently in Phase III clinical trials for metastatic colorectal cancer (Scott *et al.*, 2007). Growing interest in the potential of 1,4-disubstituted phthalazines as anticancer agents led to the synthesis of phthalazine derivatives with *N*-arylpiperazine side chains (Zhang *et al.*, 2010). The piperazinyl moiety, a small and rigid heterocyclic backbone, has been an attractive pharmacological scaffold present in several antitumor drugs (Zhang *et al.*, 2010; Hennequin *et al.*, 2006; Pollard and Mortimore, 2009). Among them, certain compounds, II, displayed excellent selectivity against MDA-MB-231 cell line (Zhang *et al.*, 2010). Furthermore, the 1,4-disubstituted phthalazine derivatives III has shown more potent cytotoxicity than cisplatin (Xin *et al.*, 2008) (Fig. 1).

In an effort to discover and develop potent cytotoxic agent, we synthesized a series of novel 1,4-disubstituted phthalazine derivatives bearing piperazinyl, primary, or secondary amino functions in position (1) of phthalazine pharmacophore and a 4-bromophenyl group in the (4) position. In addition, we screened their cytotoxic activities against MCF-7 cancer cell line in vitro as potent anti-breast cancer agents. Some of these compounds showed promising cytotoxic effect (Fig. 1).

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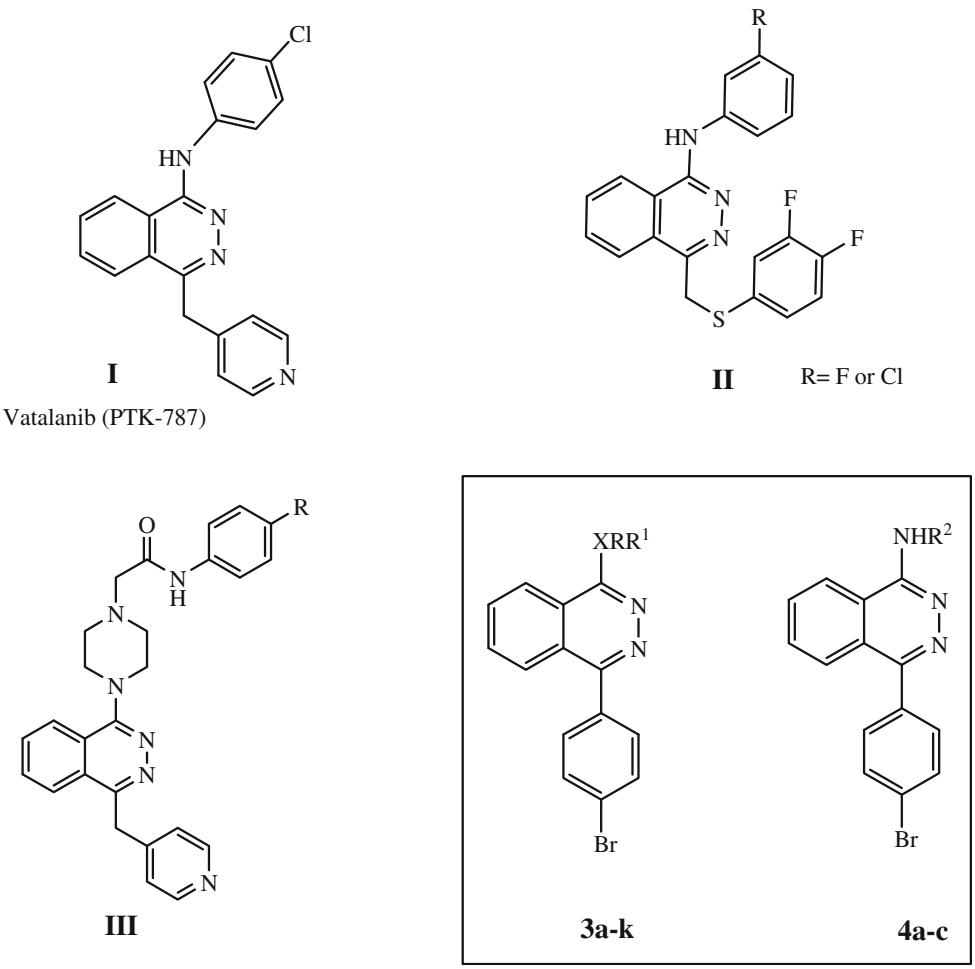


Fig. 1 Examples of 1,4-disubstituted phthalazine lead compounds with anti-cancer activity, compounds **I**, **II**, and III, and the synthesized target compounds **3a–i** and **4a–c**

Results and discussion

Chemistry

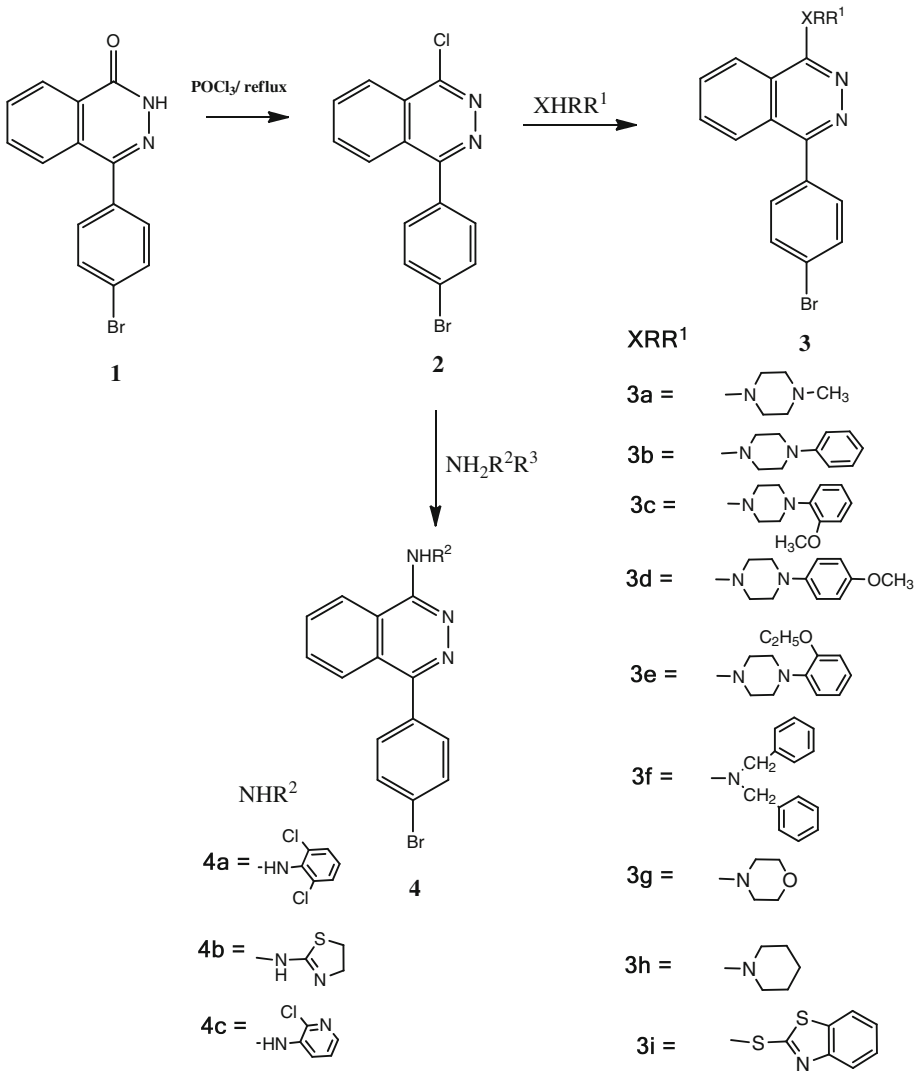
The synthetic pathways leading to the 1,4-disubstituted-phthalazine derivatives **3a–i** and **4a–c** are outlined in Scheme 1. 4-(4-Bromophenyl)phthalazinone (**1**) was synthesized by cyclization of 2-(4-bromobenzoyl)benzoic acid with hydrazine hydrate according to a reported method Yamaguchi *et al.*, 1993). Compound 1 was treated with phosphorus oxychloride to give 1-chlorophthalazine derivative **2** (Colotta *et al.*, 1994). Reacting **2** with *N*-substituted-piperazines, secondary amines or 2-mercaptobenzothiazole in n-butanol afforded **3a–i**. Furthermore, reaction of 1-chlorophthalazine derivative **2** with different primary amines in n-butanol, yielded **4a–c**. The structures of all the newly synthesized compounds were elucidated using IR, ^{1}HNMR, MS, and element analyses. ^{1}HNMR spectra of the compounds **3a–e** displayed two characteristic signals corresponding to piperazine protons in the range of δ 2.49–3.63 ppm and δ 3.32–3.68 ppm. Compound **3f** showed in its ^{1}HNMR spectrum a sharp singlet signal due to four equivalent benzylic protons at 3.66 ppm. ^{1}HNMR spectra of **4b** exhibited two triplets of two CH_2 protons of thiazoline at 3.62 and 4.59 ppm. All the other aromatic and aliphatic protons were observed at the expected regions.

Biological evaluation

In vitro antitumor activity for compounds **3a–i** and **4a–c** was determined by measurement of their cytotoxic properties against MCF-7 (human breast adenocarcinoma cell line) by SRB assay using doxorubicin as a standard. The results were expressed as IC_{50} and summarized in Table 1. The IC_{50} values are the average of three independent experiments.

As shown in Table 1 and Fig. 2, most of the prepared compounds showed good-to-excellent cytotoxic activities against MCF-7 cancer cell line. In general, the cytotoxic activities of compounds **4a–c** were more active than

Scheme 1 Synthesis of 1-(4-bromophenyl)phthalazine-4-amine derivatives



3a–i series. Six of the tested compounds **3a**, **3g**, **3i**, and **4a–c** demonstrated higher cytotoxic activity than the reference drug doxorubicin. Preliminary structure–activity relationship (SAR) showed that among the substituted-piperazine derivatives, **3a–e**, N-methylpiperazine, **3a**, exhibited the most potent antiproliferative activity ($IC_{50} = 1.5$ μM). On the other hand, the unsubstituted and ortho-substituted N-phenylpiperazine derivatives showed more potent effect than the para–substituted derivative. On the other hand, replacement of piperazine with morpholine or piperidine reduced the potency drastically. Furthermore, the cytotoxicity of the S-substituted compound **3i** was found to be twice more potent than the *N*,*N*-dibenzyl derivative **3f**. Moving our attention back to the compounds, **4a–c**, the pharmacological results indicated that the phthalazine derivatives that contain NH (compounds **4a–c**) displayed excellent cytotoxicity compared to phthalazines attached to tertiary amino functions, (compounds **3a–h**).

Conclusion

In summary, we have synthesized a series of 1,4-disubstitutedphthalazine derivatives, and evaluated their cytotoxic activities against MCF-7 (human breast adenocarcinoma cell line). Most of the prepared compounds displayed good to excellent cytotoxic activity, in particular, six compounds (**3a**, **3g**, **3i**, and **4a–c**) with IC_{50} values ranging from 1.4 to 2.3 μM. From the structure–activity relationships (SARs), we may conclude that the introduction of the primary amines to the 4-substitutedphthalazine scaffold plays an important role in enhancing antitumor activities of these compounds.

Experimental

Melting points were determined on Griffin apparatus and the values given are uncorrected. IR spectra were

Table 1 In vitro cytotoxic activity of the test compounds against human breast cancer cell line (MCF-7)

Compound	IC_{50} (μM)[a,b]
Doxorubicin	2.97
3a	1.5
3b	9.7
3c	8.6
3d	17.5
3e	4.77
3f	4.8
3g	1.9
3h	9.1
3i	2.3
4a	1.7
4b	1.4
4c	1.7

[a] IC_{50} dose of the compound which inhibit tumor cell proliferation by 50%

[b] Values are means of three experiments

determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}). ^{1}H-NMR spectra were carried out using a Varian Gemini 200 MHz Spectrophotometer and Varian Mercury-300 (300 MHz) Spectrophotometer using TMS as internal standard. Chemical shift values are recorded in ppm on δ scale, Microanalytical Center, Cairo University, Egypt. Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Microanalytical Center, Cairo University, Egypt. Progress of the reactions was monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254 using acetone/benzene (1: 9) and were visualized using UV lamp.

All chemicals were obtained from Aldrich, Fluka, or Merck chemicals.

4-(Bromophenyl)phthalazin-1(2H)-one (Yamaguchi *et al.*, 1993) and 1-chloro-4-(4-methoxyphenyl) phthalazine (Colotta *et al.*, 1994) were prepared according to reported procedures.

1-(4-Substitutedpiperazin-1-yl) 4-(4-methoxyphenyl)phthalazines (**3a–i**), (**4a–c**)

To a solution of 1-chloro-4-(4-bromophenyl)phthalazine (2) (0.318 gm, 0.001 mol) in *n*-butanol (20 ml), the appropriate *N*-substituted-piperazine or amine (0.001 mol) was added, and the mixture was heated under reflux for 5 h. The reaction mixture was filtered while hot, the filtrate was evaporated to half its volume, and cooled. The separated solid was filtered, washed by sodium carbonate solution, and crystallized from isopropanol.

*1-(4-Bromophenyl)-4-(4-methylpiperazin-1-yl)phthalazine (**3a**)*

Yield 65%; mp 150–151°C; IR (υ cm^{-1}): 3074 (C–H aromatic), 1605 (C=N); ^{1}HNMR (DMSO–d_6): 2.30 (s, 3H, N–CH_3), 2.63–2.65 (br m, 4H, piperazine), 3.45–3.48 (br m, 4H, piperazine), 7.61 (dd, 2H, Ar–H), 7.70 (dd, 2H, Ar–H), 7.79–8.40 (m, 4H, Ar–H); MS (EI) m/z (% rel. Int.): 99 (10.14), 283 (11.63), 285 (7.53), 382 (M^{+1}, 0.31), 384 ($M + 2^{+1}$, 0.30). Anal. Calcd for $C_{19}H_{19}BrN_4$: C, 59.54; H, 5.00; N, 14.62. Found: C, 59.30; H, 5.20; N, 14.55.

*1-(4-Bromophenyl)-4-(4-phenylylpiperazin-1-yl)phthalazine (**3b**)*

Yield 70%; mp 169–170°C; IR (υ cm^{-1}): 3051 (C–H aromatic), 1597 (C=N); ^{1}HNMR (DMSO–d_6): 3.40–3.45 (br m, 4H, piperazine), 3.60–3.62 (br m, 4H, piperazine), 6.79–8.28 (m, 13H, Ar–H); MS (EI) m/z (% rel. Int.): 161 (3.34), 162 (37.77), 283 (0.21), 285 (0.33), 311 (0.09), 444 (M^{+1}, 0.00). Anal. Calcd for $C_{24}H_{21}BrN_4$: C, 64.73; H, 4.75; N, 12.58. Found: C, 64.50; H, 4.80; N, 12.60.

*1-(4-Bromophenyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)phthalazine (**3c**)*

Yield 72%; mp 110–111°C; IR (υ cm^{-1}): 3051 (C–H aromatic), 1597 (C=N); ^{1}HNMR (DMSO–d_6): 3.30–3.32 (br m, 4H, piperazine), 3.36 (s, 3H, OCH_3), 3.42–3.45 (br

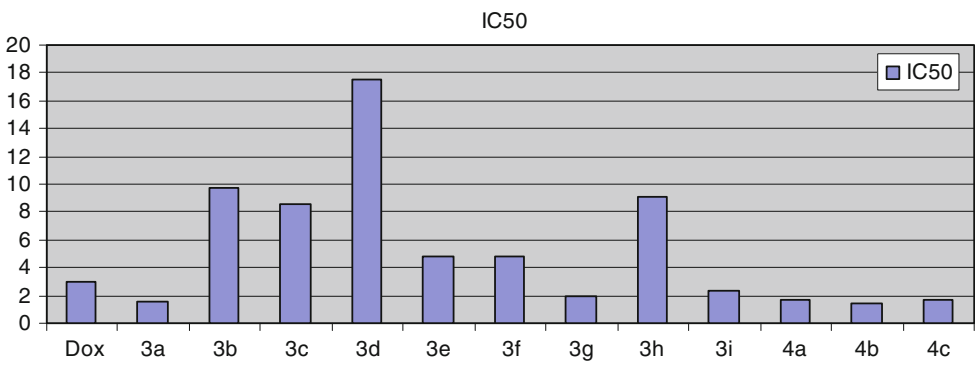


Fig. 2 IC_{50} values of test compounds **3a–i**, **4a–c** and doxorubicin against MCF-7

m, 4H, piperazine), 7.60 (dd, 2H, Ar–H), 7.76 (dd, 2H, Ar–H), 7.79–8.27 (m, 8H, Ar–H); MS (EI) m/z (% rel. Int.): 443 (9.79), 476 ($M + 2^{-1+}$, 24.39). Anal. Calcd for $C_{25}H_{23}BrN_4O$: C, 63.16; H, 4.88; N, 11.79. Found: C, 63.30; H, 4.75; N, 11.66.

*1-(4-Bromophenyl)-4-(4-(4-methoxyphenyl)piperazin-1-yl)phthalazine (**3d**)*

Yield 75%; mp 183–184°C; IR (υ cm^{-1}): 3070 (C–H aromatic), 1616 (C=N); ^{1}HNMR (DMSO–d_6): 3.60–3.63 (br m, 4H, piperazine), 3.66–3.68 (br m, 4H, piperazine), 3.71 (s, 3H, OCH_3), 6.86 (dd, 2H, Ar–H), 6.99 (dd, 2H, Ar–H), 7.64 (dd, 2H, Ar–H), 7.80 (dd, 2H, Ar–H), 7.93–8.25 (m, 4H, Ar–H); MS (EI) m/z (% rel. Int.): 163 (100), 191 (2.38), 283 (7.23), 2.85 (7.07), 311 (1.85), 313 (11.91), 474 (M^{-1+}, 6.37), 476 ($M + 2^{-1+}$, 6.18). Anal. Calcd for $C_{25}H_{23}BrN_4O$: C, 63.16; H, 4.88; N, 11.79. Found: C, 63.40; H, 4.65; N, 11.70.

*1-(4-Bromophenyl)-4-(4-(2-ethoxyphenyl)piperazin-1-yl)phthalazine (**3e**)*

Yield 60%; mp 112–113°C; IR (υ cm^{-1}): 3050 (C–H aromatic), 1581 (C=N); ^{1}HNMR (DMSO–d_6): 2.49–2.52 (br m, 4H, piperazine), 2.90 (t, 3H, OCH_2CH_3), 3.32–3.35 (br m, 4H, piperazine), 4.65 (q, 2H, OCH_2CH_3), 7.60 (dd, 2H, Ar–H), 7.76 (dd, 2H, Ar–H), 7.79-8.27 (m, 8H, Ar–H); MS (EI) m/z (% rel. Int.): 176 (100), 177 (65.84), 312 (39.82), 314 (35.57), 488(M^{-1+}, 2.09), 490 ($M + 2^{-1+}$, 6.06). Anal. Calcd for $C_{26}H_{25}BrN_4O$: C, 63.81; H, 5.15; N, 11.45. Found: C, 63.70; H, 5.50; N,11.50.

*1-(4-Bromophenyl)-4-(N,N-dibenzylamino)phthalazine (**3f**)*

Yield 64%; mp 100–101°C; IR (υ cm^{-1}): 3040 (C–H aromatic), 1581 (C=N); ^{1}HNMR (DMSO–d_6): 3.66 (s, 4H, $2CH_2$), 7.29–8.26 (m, 18H, Ar–H); MS (EI) m/z (% rel. Int.): 283 (2.46), 285 (2.95), 300 (100), 302 (94.83), 479 (M^{-1}+, 0.01), 481 ($M + 2^{-1+}$, 0.01). Anal. Calcd for $C_{28}H_{22}BrN_3$: C, 70.00; H, 4.62; N, 8.75. Found: C, 69.90; H, 4.50; N, 8.65.

*1-(4-Bromophenyl)-4-(morpholin-1-yl)phthalazine (**3g**)*

Yield 75%; mp 139–140°C; IR (υ cm^{-1}): 3050 (C–H aromatic), 1605 (C=N); ^{1}HNMR (DMSO–d_6): 3.44–3.45 (m, 4H, $2CH_2$–N), 3.88–3.90 (m, 4H, $2CH_2$–O), 7.62 (dd, 2H, Ar–H), 7.79 (dd, 2H, Ar–H), 7.89–8.22 (m, 4H, Ar–H); MS (EI) m/z (% rel. Int.): 86 (100), 283 (19.77), 285 (23.46), 369 (M^{-1}+, 55.38), 371 ($M + 2^{-1+}$, 44.54). Anal. Calcd for $C_{18}H_{16}BrN_3O$: C, 58.39; H, 4.36; N, 11.35. Found: C, 58.50; H, 4.20; N, 11.50.

*1-(4-Bromophenyl)-4-(piperidin-1-yl)phthalazine (**3h**)*

Yield 70%; mp 152–153°C; IR (υ cm^{-1}): 3050 (C–H aromatic), 1605 (C=N); ^{1}HNMR (DMSO–d_6): 1.67–1.69 (m, 2H, CH_2 piperidinyl), 1.80–1.85 (m, 4H, $2CH_2$ piperidinyl), 3.39–3.41 (m, 4H, $2CH_2$ piperidinyl), 7.62 (dd, 2H, Ar–H), 7.78 (dd, 2H, Ar–H), 7.87–8.13 (m, 4H, Ar–H); MS (EI) m/z (% rel. Int.): 84 (100), 283 (11.13), 285 (31.96), 367 (M^{-1+}, 25.96), 369 ($M + 2^{-1+}$, 24.37). Anal. Calcd for $C_{19}H_{18}BrN_3$: C, 61.97; H, 4.93; N, 11.41. Found: C, 61.59; H, 4.80; N, 11.60.

*1-(4-Bromophenyl)-4-[(benzothioazol-2-yl)thio]phthalazine (**3i**)*

Yield 74%; mp 115–116°C; IR (υ cm^{-1}): 3082 (C–H aromatic), 1581 (C=N); ^{1}HNMR (DMSO–d_6): 7.57 (dd, 2H, Ar–H), 7.63 (dd, 2H, Ar–H), 7.76–8.27 (m, 8H, Ar–H). MS (EI) m/z (% rel. Int.): 166 (8.1), 283 (2.63), 285 (3.15), 449 (M^{-1+}, 0.01), 451 ($M + 2^{-1+}$, 0.01). Anal. Calcd for $C_{21}H_{12}BrN_3S_2$: C, 56.00; H, 2.69; N, 9.33. Found: C, 56.10; H, 2.75; N, 9.50.

*1-(4-Bromophenyl)-4-(2,6-dichloroanilino-1-yl) phthalazine (**4a**)*

Yield 75%; mp 125–126°C; IR (υ cm^{-1}): 3082 (C–H aromatic), 1581 (C=N); ^{1}HNMR (DMSO–d_6): 7.56 (dd, 2H, Ar–H), 7.63 (dd, 2H, Ar–H), 7.93–8.26 (m, 7H, Ar–H); MS (EI) m/z (% rel. Int.): 283 (2.58), 285 (3.08), 298 (6.67%), 300 (100), 443 (M^{-1+}, 0.00). Anal. Calcd for $C_{20}H_{12}BrCl_2N_3$: C, 53.96; H, 2.72; N, 9.44. Found: C, 53.70; H, 2.85; N, 9.50.

*1-(4-Bromophenyl)-4-(4,5-dihydrothiazol-2-yl)aminophthalazine (**4b**)*

1-(4-bromophenyl)-4-(4,5-dihydrothiazol-2-yl) aminophthalazine Yield 70%; mp 114–115°C; IR (υ cm^{-1}), 3082 (C–H aromatic), 1581 (C=N), ^{1}HNMR (DMSO–d_6): 3.62 (t, 2H, CH_2 thiazoline), 4.59 (t, 2H, CH_2 thiazoline), 7.59 (dd, 2H, Ar–H), 7.62 (dd, 2H, Ar–H), 7.92–8.25 (m, 4H, Ar–H); MS (EI) m/z (% rel. Int.): 102 (8.22), 285 (19.75), 384 (M^{-1+}, 35.75), 386 ($M + 2^{-1+}$, 30.47). Anal. Calcd for $C_{17}H_{13}BrN_4S$: C, 53.00; H, 3.40; N, 14.54. Found: C, 52.90; H, 3.75; N, 14.50.

*1-(4-Bromophenyl)4-(2-chloropyridin-3-yl)aminophthalazine (**4c**)*

Yield 73%; mp 138–139°C; IR (υ cm^{-1}): 3050 (C–H aromatic), 1605 (C=N); ^{1}HNMR (DMSO-d_6): 7.67 (dd, 2H, Ar–H), 7.82 (dd, 2H, Ar–H), 8.03-8.38 (m, 7H, Ar–H); MS

(EI) m/z (% rel. Int.): 127 (2.12), 283 (9.15), 285 (2.48), 410 ($M^{\cdot 1+}$, 0.12), 412 ($M + 2^{\cdot 1+}$, 0.13). Anal. Calcd for $C_{19}H_{12}BrClN_4$: C, 55.43; H, 2.94; N, 13.61. Found: C, 55.60; H, 2.75; N, 13.50.

Cytotoxicity assessment against MCF-7 breast adenocarcinoma cell line

Methodology

The cytotoxicity of the synthesized compounds were tested against MCF-7 cells by SRB assay as reported (Skehan *et al.,* 1990). Exponentially growing cells were collected using 0.25% Trypsin–EDTA and plated in 96-well plates at 1,000–2,000 cells/well. Cells were exposed to test compounds for 72 h and subsequently fixed with TCA (10%) for 1 h at 4°C. After several washings, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris–HCl was used to dissolve the SRB-stained cells, and color intensity was measured at 540 nm.

Data analysis

The dose-response curve of compounds was analyzed using $E_{\max}$ model.

$$\%\ \text{Cell viability} = (100 - R) \times \left(1 - \frac{[D]^m}{K_d^m + [D]^m}\right) + R$$

where R is the residual unaffected fraction (the resistance fraction), $[D]$ is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate, and m is a Hill-type coefficient. IC_{50} was defined as the drug concentration required to reduce fluorescence to 50% of that of the control (i.e., $K_d = IC_{50}$ when $R = 0$ and $E_{\max} = 100 - R$).

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References

Albrand G, Terret C (2008) Early breast cancer in the elderly: assessment and management considerations. Drugs Aging 25: 35–45

Chari RV (2008) Targeted cancer therapy: conferring specificity to cytotoxic drugs. Acc Chem Res 41:98–107

Colotta V, Cecchi L, Catarzi D, Filacchioni G, Galli A, Mori F (1994) Synthesis and structure-activity relationships of 1-aminophthalazinium salts as $GABA_A$ receptor antagonists. Eur J Med Chem 29:95–105

Hennequin LF, Allen J, Breed J et al (2006) *N*-(5-chloro-1,3-benzodioxol-4-yl)-7-[2-(4-methylpiperazin-1-yl)ethoxy]-5-(tetrahydro-2H-pyran-4-yloxy)quinazolin-4-amine, a novel, highly selective, orally available, dual-specific c-Src/Abl kinase inhibitor. J Med Chem 49:6465–6488

Menear KA, Adcock C, Alonso FC et al (2008a) Novel alkoxybenzamide inhibitors of poly(ADP-ribose) polymerase. Bioorg Med Chem Lett 18:3942–3945

Menear KA, Adcock C, Boulter R et al (2008b) 4-[3-(4-Cyclopropanecarbonylpiperazine-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one: a novel bioavailable inhibitor of poly(ADP-ribose) polymerase-1. J Med Chem 51:6581–6591

Miller-Moslin K, Peukert S, Jain RK et al (2009) 1-Amino-4-benzylphthalazines as orally bioavailable smoothened antagonists with antitumor activity. J Med Chem 52:3954–3968

Pollard JR, Mortimore M (2009) Discovery and development of aurora kinase inhibitors as anticancer agents. J Med Chem 52:2629–2651

Scott EN, Meinhardt G, Jacques C et al (2007) Vatalanib: the clinical development of a tyrosine kinase inhibitor of angiogenesis in solid tumours. Expert Opin Investig Drugs 16:367–379

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 82:1107–1112

Vasiliou S, Castaner R, Bolos J et al (2009) SNS-032-cyclin-dependent kinase inhibitor oncolytic. Drugs of the Future 34:101–105

Wang X, Bastow KF, Sun CM, Lin YL, Yu HJ, Don MJ, Wu TS, Nakamura S, Lee KH (2004) Antitumor agents. 239. Isolation, structure elucidation, total synthesis, and anti-breast cancer activity of neo-tanshinlactone from Salvia miltiorrhiza. J Med Chem 47:5816–5819

Xin Z, Juan L, Lei H, Su Z, Yao Bin Z, Ping G (2008) Synthesis and in vitro cytotoxicity of novel 1,4-disubstituted phthalazines. Chinese Chem Lett 19(1):29–32

Yamaguchi M, Kamei K, Koga T, Akima M, Kuroki T, Ohi N (1993) Novel antiasthmatic agents with dual activities of thromboxane A2 synthetase inhibition and bronchodilation. 1,2-[2-(1-imidazolyl)alkyl]-1(2H)-phthalazinones. J Med Chem 36:4052–4060

Zhang S, Zhao Y, Liu Y, Chen D, Lan W, Zhao Q, Dong C, Xia L, Gong P (2010) Synthesis and antitumor activities of novel 1,4-disubstituted phthalazine derivatives. Eur J Med Chem 45: 3504–3510