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Chinese Chemical Letters 21 (2010) 1071–1074
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

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

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Received 9 January 2010

Abstract

A series of 1,4-substituted phthalazine derivatives were 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, compounds **7a**–**7h** showed excellent selectivity for MDA-MB-231 cell line with IC_{50} values from 1 nmol/L to 0.92 μmol/L. A preliminary SAR study of these derivatives was performed.

Keywords: 1,4-Substituted phthalazine; Synthesis; Cytotoxicity

Cancer is the leading cause of death in the world. Despite major breakthroughs in many areas of cancer therapies over the past few years, the successful treatment of cancer remains a significant challenge in the 21st century. In the last few years, a large number of phthalazine derivatives have been prepared and studied as to their antitumor potency [1–5]. Among them, Vatalanib (PTK-787), a phthalazine tyrosine kinase inhibitor, is currently in Phase III clinical trials for metastatic colorectal cancer [6]. Our research has been focused on the design of phthalazine derivatives with PTK-787 as the lead compound. In the continuation of previous research on synthesis and antitumor studies of substituted phthalazine agents [7–9], we combined the inherent antitumor agent PTK-787 and the 2-(piperazin-1-yl)-acetamide moiety in one structure. It should be emphasized that piperazine scaffold, a small and rigid heterocyclic backbone, could be found in a broad range of biological compounds displaying antitumor activities [10–12]. Thus, a series of 1,4-substitued phthalazine derivatives **7a**–**7h** and **12a**–**12d** containing piperazinyl group were synthesized in order to Q1 develop potent and selective antitumor agents (Fig. 1).

The target compounds were synthesized by a convenient six-step procedure as outlined in Scheme 1. The commercial available phthalic anhydride was reduced by $NaBH_4$ in THF at room temperature to afford **1**, which was then reacted with 4-pyridinecarboxaldehyde using CH_3ONa in methanol and ethyl propionate to give **2**. Subsequent condensation of **2** with 80% hydrazine hydrate led to phathalazone **3**. Next, chlorination of **3** in a solution of $POCl_3$ and CH_3CN gave **4** as a red solid [13]. Furthermore, the key important intermediate **5** was synthesized by the reaction of **4** with excess piperazine in EtOH at 60 °C. The side chains **6a**–**6j** was synthesized *via* a series of substituted aromatic

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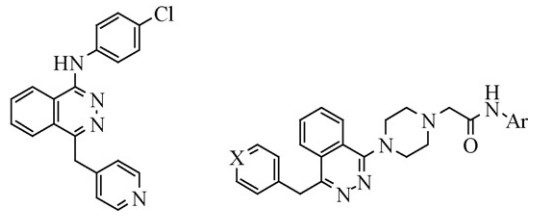
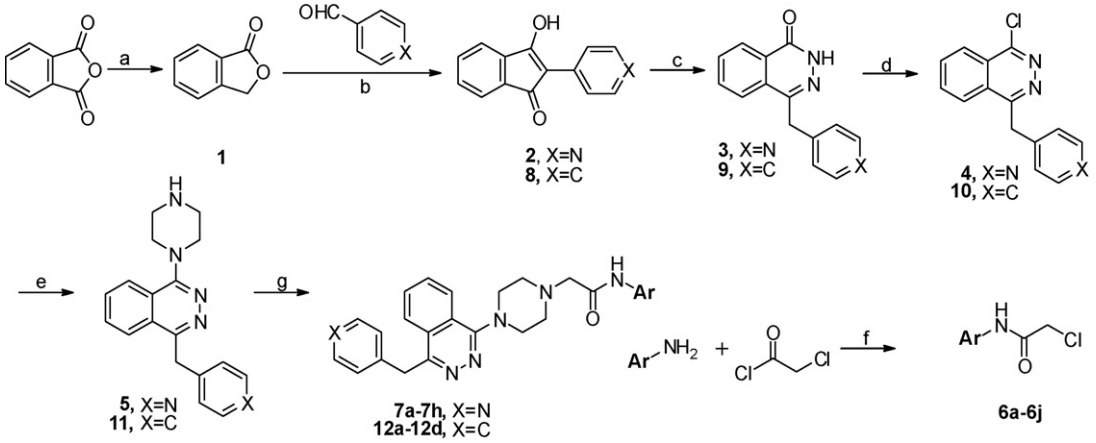
1001-8417/$ – see front matter
doi:10.1016/j.cclet.2010.04.004



Vatalanib (PTK-787) **7a-7h**: X=N, **12a-12d**: X=C

Fig. 1. The structure of Vatalanib and the target 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) $POCl_3$, CH_3CN, 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.

amines with 2-chloroacetyl chloride in the presence of Et_3N at room temperature. Another important intermediate **11** was obtained according to the same method described for **5** when 4-pyridinecarboxaldehyde was replaced by benzaldehyde. Finally, the target compounds **7a**–**7h** and **12a**–**12d** were successfully obtained *via* the reaction of **5** and **11** with **6a**–**6j** in the presence of K_2CO_3 in the refluxing acetone, respectively.

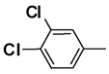
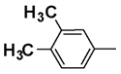
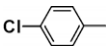
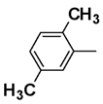
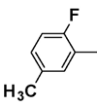
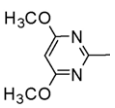
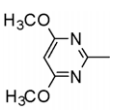
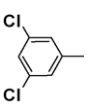
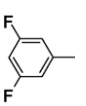
With general procedures above, compound **7a**–**7h** and **12a**–**12d** were prepared, and their structures and spectral data of ^1H NMR and MS were outlined in Table 1.

1. Antitumor activities

The cytotoxicity of compounds **7a**–**7h** and **12a**–**12d** were evaluated with three human cancer cell lines (A549: human lung carcinoma cell line; HT-29: human colon cancer cell line; MDA-MB-231: human breast cancer cell line) by the standard MTT assay *in vitro*, with PTK-787 as the positive control, and the results expressed as IC_{50} were summarized in Table 2.

The data indicated that all the prepared compounds showed excellent moderate to cytotoxic activities against different cancer cell lines. Among them, cytotoxicity of compounds **7c** (IC_{50} = 6.43 μmol/L, 5.06 μmol/L, 0.014 μmol/L) and **7g** (IC_{50} = 4.20 μmol/L, 2.08 μmol/L, 0.50 μmol/L) were more active than the reference drug PTK-787 (IC_{50} = 20.27 μmol/L, 21.96 μmol/L, 63.90 μmol/L) against all the three human cancer cell lines, and other compounds exhibited better activities against one or two cancer cell lines superior to PTK-787. Meanwhile, the pharmacological results indicated that the cytotoxicity of the prepared compounds against MDA-MB-231 cell line were higher than A549 and HT-29 cell lines, reflecting excellent selectivity for a particular human breast cancer type.

Table 1
The substituents, melting points, ^{1}H NMR and MS data of compounds **7a–7h** and **12a–12d**.

Compd.	X	Ar	mp (°C)	^{1}H NMR (300 MHz, DMSO-d_6) δ	MS *m/z*
7a	N		191–192	9.72 (s, 1H), 8.45 (d, 2H, *J* = 5.2 Hz), 8.17 (m, 1H), 8.13 (m, 1H), 8.02 (m, 1H), 7.98–7.88 (m, 2H), 7.34–7.25 (m, 3H), 7.24–7.13 (m, 2H), 4.63 (s, 2H), 3.47 (s, 4H), 3.33 (s, 2H), 2.87 (s, 4H)	457.3
7b	N		192–193	10.21 (s, 1H), 8.45 (d, 2H, *J* = 5.5 Hz), 8.20–8.14 (m, 1H), 8.11 (m, 2H), 7.99–7.86 (m, 2H), 7.67 (dd, 1H, *J* = 8.8, 2.4 Hz), 7.58 (d, 1H, *J* = 8.8 Hz), 7.31 (d, 2H, *J* = 5.8 Hz), 4.62 (s, 2H), 3.47 (s, 4H), 3.31 (s, 2H), 2.83 (s, 4H)	507.3 509.3
7c	N		78–79	9.67 (s, 1H), 8.45 (d, 2H, *J* = 5.5 Hz), 8.16 (m, 1H), 8.11 (m, 1H), 7.93 (m, 2H), 7.47–7.35 (m, 2H), 7.31 (d, 2H, *J* = 5.5 Hz), 7.05 (d, 1H, *J* = 8.1 Hz), 4.62 (s, 2H), 3.46 (s, 4H), 3.24 (s, 2H), 2.82 (s, 4H), 2.19 (s, 3H), 2.16 (s, 3H)	467.4
7d	N		207–208	10.00 (s, 1H), 8.45 (d, *J* = 5.9 Hz, 2H), 8.16 (m, 1H), 8.12 (m, 1H), 7.93 (m, 2H), 7.72 (d, 2H, *J* = 8.8 Hz), 7.37 (d, 2H, *J* = 8.9 Hz), 7.31 (d, 2H, *J* = 5.8 Hz), 4.62 (s, 2H), 3.47 (s, 4H), 3.29 (s, 2H), 2.83 (s, 4H)	473.1 475.1
7e	N		177–178	9.41 (s, 1H), 8.45(d, 2H, *J* = 5.5 Hz), 8.15 (m, 2H), 7.93 (m, 2H), 7.66 (s, 1H), 7.31 (s, 2H), 7.10 (d, 1H, *J* = 8.1 Hz), 6.87 (d, 1H, *J* = 7.6 Hz), 4.62 (s, 2H), 3.48 (s, 4H), 3.27 (s, 2H), 2.88 (s, 4H), 2.25 (s, 3H), 2.21 (s, 3H)	467.4
7f	N		192–193	9.66 (s, 1H), 8.45 (d, 2H, *J* = 5.0 Hz), 8.21–8.15 (m, 1H), 8.14–8.08 (m, 1H), 7.92 (m, 2H), 7.85 (d, 1H, *J* = 6.9 Hz), 7.32 (d, 2H, *J* = 5.2 Hz), 7.15 (dd, 1H, *J* = 10.8, 8.5 Hz), 6.95 (s, 1H), 4.63 (s, 2H), 3.46 (s, 4H), 3.32 (s, 2H), 2.86 (s, 4H), 2.28 (s, 3H)	471.3
7g	N		109–110	10.14 (s, 1H), 8.45 (d, 2H, *J* = 5.9 Hz), 8.22–8.14 (m, 1H), 8.14–8.08 (m, 1H), 8.00–7.84 (m, 2H), 7.31 (d, 2H, *J* = 5.9 Hz), 5.94 (s, 1H), 4.62 (s, 2H), 3.88 (s, 6H), 3.47 (s, 2H), 3.42 (s, 4H), 2.86 (s, 4H)	501.2
7h	N		175–176	10.17 (s, 1H), 8.93 (dd, 1H, *J* = 4.2, 1.5 Hz), 8.50–8.43 (m, 2H), 8.39 (d, 1H, *J* = 8.1 Hz), 8.22–8.09 (m, 2H), 7.94 (m, 2H), 7.88 (m, 2H), 7.81–7.72 (m, 1H), 7.61 (m, 1H), 7.32 (d, 2H, *J* = 6.0 Hz), 4.63 (s, 2H), 3.54 (s, 4H), 3.44 (s, 2H), 2.94 (s, 4H)	490.2
12a	C		76–77	10.14 (s, 1H), 8.17 (m, 1H), 8.08 (m, 1H), 7.97–7.81 (m, 2H), 7.40–7.21 (m, 4H), 7.18 (t, 1H, *J* = 7.0 Hz), 5.94 (s, 1H), 4.58 (s, 2H), 3.88 (s, 6H), 3.47 (s, 2H), 3.42 (s, 4H), 2.86 (s, 4H)	500.4
12b	C		124–125	10.18 (s, 1H), 8.93 (dd, 1H, *J* = 4.2, 1.6 Hz), 8.40 (d, 1H, *J* = 8.3 Hz), 8.20 (m, 1H), 8.16–8.10 (m, 1H), 7.90 (m, 4H), 7.83–7.72 (m, 1H), 7.61 (m, 1H), 7.37–7.23 (m, 4H), 7.17 (t, 1H, *J* = 7.1 Hz), 4.59 (s, 2H), 3.53 (s, 4H), 3.44 (s, 2H), 2.94 (s, 4H)	489.3
12c	C		92–93	10.24 (s, 1H), 8.18 (d, 1H, *J* = 6.9 Hz), 8.09 (d, 1H, *J* = 6.6 Hz), 7.91 (m, 2H), 7.83 (m, 2H), 7.34–7.24 (m, 5H), 7.18 (d, 1H, *J* = 6.8 Hz), 4.58 (s, 2H), 3.48 (s, 4H), 3.34 (s, 2H), 2.86 (s, 4H)	506.1 508.1
12d	C		94–95	10.37 (s, 1H), 8.18 (d, 1H, *J* = 9.0 Hz), 8.09 (d, 1H, *J* = 6.6 Hz), 7.96–7.84 (m, 2H), 7.50 (d, 2H, *J* = 7.8 Hz), 7.37–7.21 (m, 4H), 7.16 (t, 1H, *J* = 7.0 Hz), 6.92 (t, 1H, *J* = 9.4 Hz), 4.58 (s, 2H), 3.45 (s, 4H), 3.32 (s, 2H), 2.89–2.70 (m, 4H)	474.4

Besides, the antitumor activities of **7a–7h** were much more potent than those of compounds **12a–12d** against the three human cancer cell lines in most cases. As shown in Table 2, compounds **12a–12d** displayed moderate cytotoxic activities against HT-29 and MDA-MB-231 cell lines, but not to A549 cell line. While, compounds **7a–7h** displayed excellent selectivity for MDA-MB-231 cell line with IC_{50} values from 1 nmol/L to 0.92 μmol/L. In particular, compounds **7b** and **7e** showed IC_{50} values in the single-digit nmol/L range against MDA-MB-231 cell line. The preliminary structure–activity relationship (SAR) showed that the pyridyl group at position-4 of phthalazine scaffold plays an important role in enhancing their cytotoxic activities.

Table 2
The cytotoxicity of the target compounds against A549, HT-29 and MDA-MB-231 cell lines.

Compd.	IC_{50} (μmol/L)			Compd.	IC_{50} (μmol/L)		
	A549	HT-29	MDA-MB-231		A549	HT-29	MDA-MB-231
7a	29.75	47.97	0.92	**7g**	4.20	2.08	0.50
7b	35.73	10.09	0.001	**7h**	60.26	4.49	0.31
7c	6.43	5.06	0.014	**12a**	>100	30.69	17.19
7d	9.94	76.11	0.08	**12b**	>100	59.40	9.42
7e	32.15	0.19	0.0086	**12c**	>100	22.77	24.55
7f	5.53	31.88	0.13	**12d**	11.62	1.06	2.53
PTK-787	20.27	21.96	63.90				

The conclusions above were made just preliminarily, further studies are in progress in our laboratories and will be reported upon in the future.

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