Med Chem Res (2013) 22:2855–2861
DOI 10.1007/s00044-012-0283-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antitumor activity of novel 4-aminoquinoline derivatives

Jie Ren · Juan Zhao · Yong-Sheng Zhou · Xian-Hua Liu · Xin Chen · Kun Hu

Received: 27 March 2012 / Accepted: 15 October 2012 / Published online: 30 October 2012

Abstract A series of novel 4-aminoquinoline derivatives were synthesized as antitumor agents by reacting 4-chloroquinoline with the corresponding mono/dialkyl amines. The cytotoxicity of these compounds was evaluated in vitro against HCT-116, A549, DU-145, HepG2, and LN229 cell lines. The results showed that most of the synthesized compounds displayed excellent cytotoxicity, and 5,7-dimethoxy-2-phenyl-*N*-propylquinoline-4-amine (**6a**) displayed the most potent cytotoxicity against HCT-116 cells. Furthermore, **6a** could decrease VEGF protein expression.

Keywords 4-Aminoquinoline · Synthesis · Antitumor activity · VEGF

Introduction

Theoretically, antitumor agents could be developed to target each step in the tumor activation or inhibition process, and numerous new antitumor agents have been developed based on their inhibitory effects on tumor activation (Xia *et al.*, 2001). Inhibition of angiogenesis which is a fundamental process of tumor growth and metastasis has become one of the interesting areas in research and development of novel antitumor drugs. The vascular endothelial growth factor (VEGF), known as the most potent and specific angiogenetic factor, represents an important target for the discovery of antitumor inhibitors. Receptor tyrosine kinase (RTK) inhibitors which target VEGF receptor are one class of the most promising agents (Hennequin *et al.*, 1999).

Nitrogen-containing heterocycles are present in a variety of biologically active compounds that can be used in a wide range of therapeutic areas (Cantin *et al.*, 2007). 2-Phenylquinoline derivatives are the effective pharmacophore in medicinal chemistry as illustrated by its application in pharmaceutical agents as novel antitumor promoters (Kakadiya *et al.*, 2010; Sirisoma *et al.*, 2010; Zhang *et al.*, 2008; Chen *et al.*, 2006; Zhao *et al.*, 2005), antimalarial agents (Solomon *et al.*, 2005; Solomon *et al.*, 2010), and DNA-intercalator agents (Atwell *et al.*, 1989; Atwell *et al.*, 1988) according to their effective binding ability with DNA. 2-Phenylquinolines containing additional basic *N*-4-aminoalkyl groups would strongly increase the biological activity of immunostimulatory CpG-oligodeoxynucleotides (Strekowski *et al.*, 2003). Tacrine, which is used for treating Alzheimer's disease (Luo *et al.*, 2006), is a 4-amino-substituted quinolone derivative. Some of the 4-aminoquinoline derivatives had also been explored as ligands for the benzodiazepine binding site of brain $GABA_A$ receptors (Nilsson *et al.*, 2008).

According to the literature, 4-anilinoquinazoline could be potent inhibitors of VEGF receptor, and the quinoline derivative is almost equally potent as quinazoline (Hennequin *et al.*, 1999). The present work is a continuation of our ongoing efforts toward developing new and effective antitumor agents. We have designed and synthesized a series of novel 4-aminoquinoline derivatives, and their antitumor activity was evaluated against several tumor cell lines in vitro. Furthermore, the effect of one active compound on VEGF protein expression was also investigated.

J. Ren · J. Zhao · X.-H. Liu · X. Chen (✉) · K. Hu (✉)
School of Pharmaceutical Engineering & Life Science, Changzhou University, 1 Gehu Road, Changzhou 213164, Jiangsu, People's Republic of China
e-mail: xinchen@cczu.edu.cn

K. Hu
e-mail: hukun1979@163.com

Y.-S. Zhou
School of Petrochemical Engineering, Changzhou University, 1 Gehu Road, Changzhou 213164, Jiangsu, People's Republic of China

Results and discussion

Chemistry

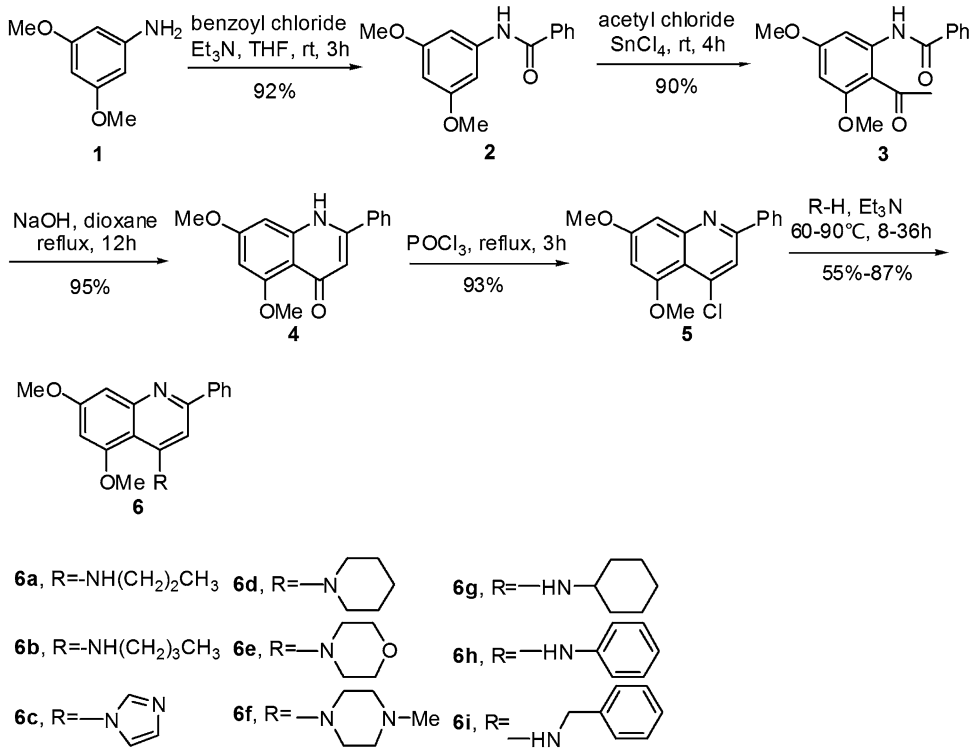
The synthetic route used to prepare the title compounds is outlined in Scheme 1. 3,5-Dimethoxybenzenamine **1** was acylated with benzoyl chloride to give *N*-(3,5-dimethoxyphenyl)benzamide **2**, which was then treated with acetyl chloride in the presence of tin (IV) chloride to afford *N*-(2-acetyl-3,5-dimethoxyphenyl) benzamide **3**. Upon treatment with NaOH in refluxing 1,4-dioxane, compound **3** was cyclized to generate 5,7-dimethoxy-2-phenylquinoline-4(1H)-one **4**. Chlorination of compound **4** with phosphoryl trichloride afforded 4-chloro-5,7-dimethoxy-2-phenylquinoline **5**. In the final step, a series of 4-aminoquinoline derivatives (**6a–6i)** were obtained through reacting 4-chloroquinoline **5** with various amines at 60–90 °C over 8–36 h in the presence of Et_3N. The structures of the new compounds were confirmed based on their ^1H NMR, ^{13}C NMR, MS, and IR spectral data.

Biological activity

The newly synthesized 4-aminoquinoline derivatives **6a–6i** were assayed for their cytotoxicity in vitro against five human tumor cell lines including HCT-116 (human colon cancer cell line), A549 (human lung cancer cell line), DU-145 (human prostate carcinoma cell line), HepG2 (human liver carcinoma cell line), LN229 (human glioblastoma cell line), and HEK293 (human embryonic kidney 293) by the standard MTT assay, and 5-FU (5-fluorouracil) was used as a positive control. Antitumor potency of the compounds was indicated by IC_{50} values (Table 1).

As shown in Table 1, most of the prepared compounds showed potent inhibitory effects on HCT-116 cell line. Compared to 5-FU, some compounds showed more potent cytotoxicity to certain types of cancer cell lines, such as **6a** to HCT-116, **6a**, **6b**, **6g**, and **6i** to A549 and HepG2. The cytotoxicity of most of the new synthesized compounds reflected certain selectivity for HCT-116 and LN229 cancer cell lines except for **6g** and **6i**.

The cytotoxicities of the resulting 4-aminoquinoline derivatives appeared to be related to the nature of the substituent group at quinoline. It has been observed from Table 1 that most of the derivatives with aliphatic amino moieties have higher cytotoxicity than those with cyclic amino groups and aromatic amino groups. For example, the derivatives **6a**, **6b**, and **6g** bearing aliphatic amino moieties showed higher cytotoxicity than other derivatives, and derivatives **6d**, **6e**, and **6f** with cyclic amino groups possessed the most poor activity. For HCT-116 cell and LN229 cell lines, the derivative with cyclohexyl amino group (**6g**) had comparative activity with the derivative with phenyl amino group (**6h**). For DU-145 and HepG2



Scheme 1 Synthetic route for 4-aminoquinoline derivatives **6a–6i**

Table 1 The cytotoxicity of 4-aminoquinoline derivatives **6a–6i**

Compound	Cytotoxicity (IC_{50}, μM)[a]					
	HCT-116	A549	DU-145	HcpG2	LN229	HEK293
6a	0.97	13.14	9.77	5.21	1.82	2.00
6b	3.05	19.57	4.63	4.46	3.92	3.23
6c	42.39	>100	>100	58.64	55.29	ND
6d	31.99	>100	>100	>100	65.96	68.16
6e	28.05	>100	>100	>100	>100	>100
6f	7.43	>100	16.39	22.55	1.75	3.66
62	3.82	6.59	5.47	3.16	6.08	ND
6h	4.28	>100	22.25	36.39	5.90	13.16
6i	11.98	12.79	10.06	10.91	7.15	ND
5-FU	1.93	>100	2.95	19.20	ND	ND

ND not determined

[a] Data are the mean of three independent experiments

cell lines, **6g** showed better activity than **6h**. For the derivatives with aliphatic amino moieties, the activities appeared to be related to the size of substituent. For example, for most of the cell lines, the derivative with a propyl group (**6a**) showed more significant activity than that with the butyl group.

Among the tested compounds, **6a** displayed the most potent cytotoxicity against HCT-116 with IC_{50} values of 0.97 μM. The dose- and time-dependent curve of **6a** in HCT-116 cells was further observed (data not shown). Thus, **6a** was chose to further evaluate its anticancer activity in human colon cancer cell line (HCT-116), and study was carried out to determine whether or not its anticancer mechanism is related to VEGF.

In order to explore whether the novel 4-aminoquinoline derivatives target VEGF, we investigated the effect of **6a** on the VEGF protein expression (Fig. 1). VEGF protein expression was assessed by western blot in cells treated with different concentrations of **6a** (2.5, 5, and 10 μM) for 48 h (Fig. 1a), and the relative protein expression levels were measured by scanning densitometry of the band intensities (Fig. 1b). As shown in Fig. 1, **6a** significantly inhibited the protein expression of VEGF. The results indicated that **6a** could act as a VEGF inhibitor.

In summary, we have designed and synthesized nine novel 4-aminoquinoline derivatives, and evaluated their antitumor activities against five cell lines (HCT-116, A549, DU-145, HepG2, and LN229). Most of the new compounds displayed the selective cytotoxicity toward the tested cell lines. From the structure–activity relationship results we might conclude that the introduction of substituted amino groups at 4-position in quinoline is associated with enhanced cytotoxicity activity. When the quinoline contained aliphatic amino moieties, the effect was more obvious. The mechanism study showed that **6a** could effectively inhibit the

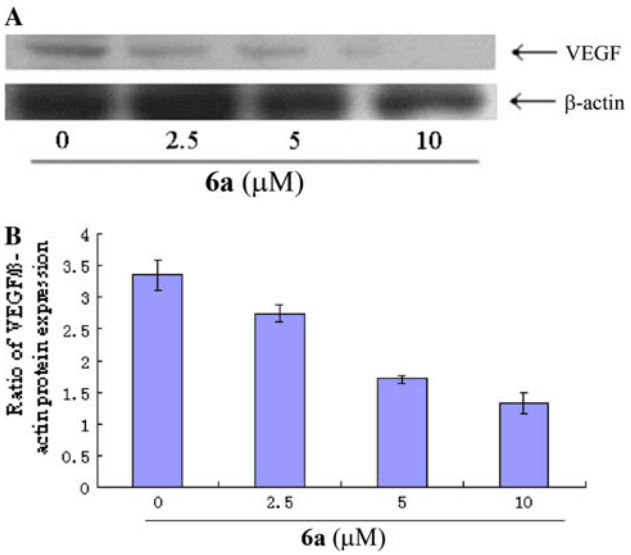


Fig. 1 Effect of compound **6a** on VEGF protein expression in HCT-116 cells. HCT-116 cells were treated with various concentrations of **6a** for 48 h. Control cells were incubated with vehicle. **a** The whole-cell lysates were analyzed by immunoblot analysis using various antibodies against VEGF and *β*-actin. The results presented are representative of three independent experiments. **b** The relative VEGF protein levels were measured by scanning densitometry of the band

protein expression of VEGF. This study may provide valuable insight and clues for designing and developing more potent antitumor agents. Further biological evaluation for the new 4-aminoquinoline derivatives is being undertaken to define their effects on tumor angiogenesis and action mechanism of antiproliferative activity.

Experimental protocols

General

Chemical reagents were obtained from commercial suppliers, and were dried and purified by standard methods when necessary. The progress of the reaction was monitored by TLC using silica gel plates. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 500 Hz spectrometer using $CDCl_3$ or DMSO-d_6 as solvent. Tetramethylsilane (δ 0.0 ppm) was used as an internal standard. IR spectra (cm^{-1}) were recorded on a Bruker Optics TENSOR27 infrared spectrometer using the KBr disk technique. Mass spectra were recorded on a Shimadzu VG-Autospec-3000 mass spectrometer.

Synthetic procedure for *N*-(3,5-dimethoxyphenyl)benzamide (**2**)

To a solution of 3,5-dimethoxybenzenamine **1** (765 mg, 5 mmol) in dry THF (15 mL) chilled with an ice bath,

triethylamine (1.04 mL, 7.5 mmol) was added dropwise. Then the mixture was stirred for 15 min, followed by the dropwise addition of benzoyl chloride (0.87 mL, 7.5 mmol) over 20 min. After the suspension was stirred at room temperature for 3 h, the reaction mixture was poured into ice water. The resulting mixture was extracted with ethyl acetate (3 × 15 mL). The organic layers were dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was crystallized by appropriate solvent to give compound **2** (92 % yield) as white needle. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.82 (6H, s, $-OCH_3$), 6.29 (1H, t, $J = 2.1$ Hz, –NH), 6.90 (2H, d, $J = 2.0$ Hz, 2′,6′-2H), 7.51 (2H, d, $J = 8.8$ Hz, 3,5-H), 7.57 (1H, t, $J = 7.4$ Hz, 4-H), 7.76 (1H, s, 4′-H), 7.86 (2H, d, $J = 7.6$ Hz, 2,6-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.4 ($-OCH_3$), 97.1 (4′-C), 98.5 (2′,6′-2C), 127.0 (3,5-2C), 128.8 (2,6-2C), 131.8 (4-C), 134.9 (1-C), 139.8 (1′-C), 161.1 (3′,5′-2C), 165.9 (–CO–); IR (KBr disk): ν 3248 (–CONH–), 1653 (C=O), 1532 (C=C), 1319 (C–N), 1057 (C–O); ESI-MS: m/z 258 (M + 1).

Synthetic procedure for *N*-(2-acetyl-3, 5-dimethoxyphenyl) benzamide (**3**)

To a cooled solution of *N*-(3,5-dimethoxyphenyl)benzamide **2** (645 mg, 5 mmol) in dry DCM (15 mL), $SnCl_4$ (0.58 mL, 10 mmol) and acetyl chloride (0.25 mL, 7.5 mmol) were added dropwise. After stirring for 4 h at room temperature, the reaction mixture was poured into ice water (10 mL), and then extracted with ethyl acetate (3 × 15 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. The residual solid was purified by crystallization to give compound **3** (90 % yield) as white needle. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.64 (1H, s, $-COCH_3$), 3.92 (6H, s, $-OCH_3$), 6.25 (1H, d, $J = 2.25$ Hz, 6′-H), 7.55 (3H, m, 3,4,5-H), 8.04 (2H, m, 2,6-H), 8.27 (1H, d, $J = 2.25$ Hz, 4′-H), 12.90 (1H, s, –NH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 34.2 ($-CH_3$), 55.6 ($-OCH_3$), 55.7 ($-OCH_3$), 94.4 (4′-C), 96.8 (6′-C), 108.7 (2′-C), 127.5 (3,5-2C), 128.8 (2,6-2C), 131.9 (4-C), 134.9 (1-C), 143.9 (1′-C), 162.7 (3′-C), 164.8 (5′-C), 166.3 (–CO–NH), 202.9 (–CO–); IR (KBr disk): ν 3136 (–CONH–), 1672 (C=O), 1517 (C=C), 1309 (C–N), 1025 (C–O); ESI-MS: m/z 290 (M + 1).

Synthetic procedure for 5,7-dimethoxy-2-phenylquinoline-4(1H)-one (**4**)

A mixture of compound **3** (1.45 g, 5 mmol) and NaOH (0.7 g, 17.5 mmol) in 1,4-dioxane (20 mL) was refluxed for 12 h. After returning to room temperature, the mixture was concentrated and then poured into 20 mL of ice water. The precipitate was collected and crystallized from ethyl acetate to give compound **4** (95 % yield) as pale yellow powder. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.83 (6H, s, $-OCH_3$), 6.11 (1H, s, 3-H), 6.33 (1H, s, 6-H), 6.81 (1H, s, 8-H), 7.55 (3H, m, 3′,4′,5′-H), 7.79 (2H, m, 2′,6′-2H), 11.24 (1H, s, –NH–); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 55.2 (7-OCH_3), 55.7 (5-OCH_3), 94.9 (6-C), 98.9 (8-C), 108.4 (3-C), 109.9 (10-C), 127.0 (3′,5′-2C), 128.8 (2′,6′-2C), 130.0 (4′-C), 145.5 (1′-C), 160.3 (2-C), 160.6 (9-C), 161.8 (5-C), 164.8 (7-C), 170.3 (4-C); IR (KBr disk): ν 3448 (NH), 1616 (C=O), 1507 (C=C), 1311 (C–N), 1041 (C–O); ESI-MS: m/z 282 (M + 1).

Synthetic procedure for 4-chloro-5,7-dimethoxy-2-phenylquinoline (**5**)

A solution of compound **4** (2.81 g, 10 mmol) in $POCl_3$ was refluxed for 3 h, and then the reaction mixture was cooled to room temperature, and poured into ice water. The precipitate was collected and crystallized from ethyl acetate to afford compound **5** (93 % yield) as yellow powder. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.83 (6H, s, $-OCH_3$), 6.11 (1H, s, 3-H), 6.33 (1H, s, 6-H), 6.81 (1H, s, 8-H), 7.55 (3H, m, 3′,4′,5′-H), 7.79 (2H, m, 2′,6′-H),11.24 (1H, s, -NH-); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.2 (7-OCH_3), 55.7 (5-OCH_3), 94.9 (6-C), 98.9 (8-C), 108.4 (3-C), 109.9 (10-C), 127.0 (3′,5′-2C), 128.8 (2′,6′-2C), 130.0 (4′-C), 145.5 (1′-C), 160.3 (2-C), 160.6 (9-C), 161.8 (5-C), 164.8 (7-C), 170.3 (4-C); IR (KBr disk): ν 1634 (C=N), 1576 (C=C), 1317 (C–N), 1050 (C–O); ESI-MS: m/z 300 (M + 1).

General procedure for preparation of 4-aminoquinoline derivatives (**6a–6i**)

To a solution of compound **5** (300 mg, 1 mmol) in amine (4 mL) Et_3N (1 mmol) was added. The reaction mixture was maintained at 60–90 °C over 8–36 h while stirring, and then distilled water (20 mL) was added, extracted with DCM (3 × 15 mL), and the combined DCM extracts were dried over anhydrous $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel to afford **6a–6i** as light yellow or white solid.

*5,7-Dimethoxy-2-phenyl-N-propylquinoline-4-amine (**6a**)*

Yield 61 %; Mp = 142 °C; ^{1}H NMR($CDCl_3$, 500 MHz): δ 1.09 (3H, t, $J = 7.4$ Hz, $-CH_3$), 1.81 (2H, m, $-CH_2$), 1.74 (2H, m, CH_2), 3.92 (3H, s, $-OCH_3$), 3.95 (3H, s, $-OCH_3$), 6.37 (1H, d, $J = 22.5$ Hz, 6-H), 6.62 (1H, s, 3-H), 7.04 (1H, d, $J = 22.5$ Hz, 8-H), 7.41 (1H, m, 4′-H), 7.47 (1H, m, 3′,5′-H), 8.03 (2H, d, $J = 21.5$ Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.9 (CH_3), 20.4 (CH_2), 30.9 (CH_2), 42.7 (CH_2), 53.3 ($2CH_2$), 55.5 (7-OCH_3), 55.9 (5-OCH_3),

95.3 (6-C), 96.6 (8-C), 101.3 (3-C), 104.8 (10-C), 127.5 (3′,5′-2C), 128.5 (2′,6′-2C), 128.8 (4′-C), 140.7 (1′-C), 152.3 (4-C), 153.0 (9-C), 157.5 (5-C), 158.4 (7-C), 160.2 (2-C); IR (KBr disk): *v*3432 (NH), 1630 (C=N), 1594 (C=C), 1167 (C–N), 1039 (C–O); ESI-MS: *m/z* 323 (M + 1).

*N-Butyl-5,7-dimethoxy-2-phenylquinoline-4-amine (**6b**)*

Yield 57 %; Mp = 115 °C; ^{1}H NMR($CDCl_3$, 500 MHz): δ 1.01 (3H, t, J = 5.9 Hz, -CH_3), 1.51 (2H, m, CH_2), 1.74 (2H, m, CH_2), 3.31 (2H, m, CH_2), 3.92 (3H, s, –OCH_3), 3.96 (3H, s, –OCH_3), 6.37 (1H, d, J = 2.0 Hz, 6-H), 6.62 (1H, s, 8-H), 7.04 (1H, br, 3-H), 7.41 (1H, m, 4′-H), 7.47 (2H, m, 3′,5′-H), 8.03 (2H, d, J = 21.5 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.9 (CH_3), 20.4 (CH_2), 30.9 (CH_2), 42.7 (CH_2), 55.5 (7-OCH_3), 55.9 (5-OCH_3), 95.3 (6-C), 96.6 (8-C), 101.3 (3-C), 104.8 (10-C), 127.5 (3′,5′-2C), 128.5 (2′,6′-2C), 128.8 (4′-C), 140.7 (1′-C), 152.3 (4-C), 153.0 (9-C), 157.5 (5-C), 158.4 (7-C), 160.2 (2-C); IR (KBr disk): *v*3437 (NH), 1629 (C=N), 1594 (C=C), 1133 (C–N), 1037 (C–O); ESI-MS: *m/z* 337 (M + 1).

*4-(1H-imidazole-1-yl)-5,7-dimethoxy-2-phenylquinoline (**6c**)*

Yield 87 %; Mp = 199 °C; ^{1}H NMR($CDCl_3$, 500 MHz): δ 3.64 (3H, s, −OCH_3), 3.98 (3H, s, −OCH_3), 6.52 (1H, d, J = 2.0 Hz, 6-H), 7.18-7.23 (3H, m, 3,8,4″-H), 7.48–7.54 (4H, m, 3′,4′,5′,5″-H), 7.69 (1H, S, 2″-H), 8.06 (2H, d, J = 21.5 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.8 (7-OCH_3), 55.9 (5-OCH_3), 100.0 (6-C), 101.2 (8-C), 110.7 (3-C), 115.3 (10-C), 121.6 (5″-C), 127.4 (3′,5′-2C), 128.6 (4″-C), 129.0 (2′,6′-2C), 130.0 (4′-C), 138.4 (2″-C), 138.5 (1′-C), 142.2 (4-C), 152.9 (9-C), 156.1 (5-C), 158.0 (7-C), 161.9 (2-C); IR (KBr disk): *v*1617 (C=N), 1590 (C=C), 1163 (C–N), 1048 (C–O); ESI-MS: *m/z* 332 (M + 1).

*5,7-Dimethoxy-2-phenyl-4-(piperidin-1-yl)quinoline (**6d**)*

Yield 56 %; Mp = 154 °C; ^{1}H NMR ($CDCl_3$, 500 MHz):δ 1.82 (2H, s, –CH_2), 2.74 (4H, br, 2CH_2), 3.55 (4H, br, CH_2), 3.94 (6H, d, –OCH_3), 6.48 (1H, d, J = 22.5 Hz, 6-H), 7.10 (1H, s, 3-H), 7.11 (1H, d, J = 22.5 Hz, 8-H), 7.42 (1H, m, 4′-H), 7.48 (2H, m, 3′,5′-H), 8.06 (2H, d, J = 21.5 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.5 (CH_2), 26.1 (2CH_2), 54.3 (2CH_2), 55.5 (7-OCH_3), 56.1 (5-OCH_3), 98.3 (6-C), 101.6 (8-C), 104.7 (3-C), 109.8 (10-C), 127.5 (3′,5′-2C), 128.6 (2′,6′-2C), 128.9 (4′-C), 140.4 (1′-C), 153.5 (4-C), 156.9 (9-C), 158.2 (5-C), 158.6 (7-C), 160.3 (2-C); IR (KBr disk): *v*1612 (C=N), 1579 (C=C), 1160 (C–N), 1044 (C–O); ESI-MS: *m/z* 349 (M + 1).

*5,7-Dimethoxy-4-morpholino-2-phenylquinoline (**6e**)*

Yield 74 %; Mp = 169 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ3.28 (4H, br, –CH_2), 3.96 (10H, s, 2-OCH_2–, 2-OCH_3), 6.50 (1H, d, J = 22.5 Hz, 6-H), 7.09 (1H, s, 3-H), 7.13 (1H, d, J = 22.5 Hz, 8-H), 7.43 (1H, m, 4′-H), 7.49 (2H, m, 3′,5′-H), 8.05 (2H, d, J = 21.5 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 53.3 (2CH_2), 55.5 (7-OCH_3), 56.1 (5-OCH_3), 67.0 (2CH_2), 98.9 (6-C), 101.8 (8-C), 104.5 (3-C), 109.5 (10-C), 127.5 (3′,5′-2C), 128.7 (2′,6′-2C), 129.1 (4′-C), 140.1 (1′-C), 153.5 (4-C), 156.6 (9-C), 157.5 (5-C), 158.3 (7-C), 160.5 (2-C); IR (KBr disk): *v*1613 (C=N), 1581 (C=C), 1162 (C–N), 1049 (C–O); ESI-MS: *m/z* 351 (M + 1).

*5,7-Dimethoxy-4-(4-methylpiperazin-1-yl)-2-phenylquinoline (**6f**)*

Yield 64 %; Mp = 154 °C; ^{1}H NMR($CDCl_3$, 500 MHz): δ 2.41 (3H, s, –CH_3), 2.96 (4H, br, 2CH_2), 3.52 (4H, br, 2CH_2), 3.95 (6H, d, 2-OCH_3), 6.49 (1H, d, J = 22.5 Hz, 6-H), 7.09 (1H, s, 3-H), 7.12 (1H, d, J = 22.5 Hz, 8-H), 7.43 (1H, m, 4′-H), 7.49 (2H, m, 3′,5′-H), 8.06 (2H, d, J = 21.5 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ46.2 (CH_3), 52.7 (2CH_2), 55.2 (2CH_2), 55.5 (7-OCH_3), 56.1 (5-OCH_3), 98.6 (6-C), 101.7 (8-C), 104.7 (3-C), 109.6 (10-C), 127.5 (3′,5′-2C), 128.7 (2′,6′-2C), 129.0 (4′-C), 140.3 (1′-C), 153.5 (4-C), 156.7 (9-C), 157.5 (5-C), 158.3 (7-C), 160.4 (2-C); IR (KBr disk): *v*1613 (C=N), 1578 (C=C), 1162 (C–N), 1054 (C–O); ESI-MS: *m/z* 364 (M + 1).

*N-Cyclohexyl-5,7-dimethoxy-2-phenylquinoline-4-amine (**6g**)*

Yield 55 %; Mp = 123 °C; ^{1}H NMR($CDCl_3$, 500 MHz): δ 1.24 (2H, m, CH_2), 1.44 (2H, m, CH_2),1.50 (2H, m, CH_2), 1.66 (2H, m, CH_2), 1.77 (2H, m, CH_2), 2.10 (1H, m, CH), 3.56 (1H, br, NH), 3.92 (3H, s, –OCH_3), 3.96 (3H, s, –OCH_3), 6.36 (1H, d, J = 22.5 Hz, 6-H), 6.62 (1H, s, 3-H), 7.00 (1H, s, 8-H), 7.40 (1H, t, 4′-H), 7.47 (2H, t, 3′,5′-H), 8.00 (2H, d, J = 21.5 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.6 (4″-C), 25.9 (3″,5″-C), 32.4 (2″,6″-C), 55.5 (7-OCH_3), 56.1 (5-OCH_3), 67.0 (2CH_2), 95.8 (6-C), 96.5 (8-C), 101.5 (3-C), 104.9 (10-C), 127.5 (3′,5′-2C), 128.5 (2′,6′-2C), 128.7 (4′-C), 141.2 (1′-C), 151.9 (4-C), 152.9 (9-C), 158.5 (5-C), 158.7 (7-C), 160.1 (2-C); IR (KBr disk): *v*3396 (NH), 1618 (C=N), 1590 (C=C), 1132 (C–N), 1041 (C–O); ESI–MS: *m/z* 363 (M + 1).

*5,7-Dimethoxy-N,2-diphenylquinoline-4-amine (**6h**)*

Yield 75 %; Mp = 190 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.95 (3H, s, $-OCH_3$), 4.01 (3H, s, $-OCH_3$), 6.49 (1H, d, J = 22.5 Hz, 6-H), 7.06 (1H, s, 3-H), 7.19 (1H, m, 8-H), 7.22 (1H, m, Ar–H), 7.40–7.45 (7H, m, 3′,4′, 5′-H, Ar–H), 7.94 (2H, d, J = 21.5 Hz, 2′,6′-H), 9.05 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 55.6 (7-OCH_3), 56.2 (5-OCH_3), 97.2 (6-C), 97.7 (8-C), 101.7 (3-C), 105.3 (10-C), 124.0 (2Ar–C), 124.8 (1Ar–C), 127.4 (3′,5′-2C), 128.5 (2′,6′-2C), 129.1 (4′-C), 130.9 (2Ar–C), 139.9 (1Ar–C), 140.4 (1′-C), 150.7 (4-C), 153.1 (9-C), 158.0 (5-C), 158.4 (7-C), 160.3 (2-C); IR (KBr disk): *v*3385 (NH), 1608 (C=N), 1584 (C=C), 1156 (C–N), 1043 (C–O); ESI-MS: *m*/*z* 357 (M + 1).

*N-Benzyl-5,7-dimethoxy-2-phenylquinoline-4-amine (**6i**)*

Yield 63 %; Mp = 160 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.91 (3H, s, $-OCH_3$), 3.92 (3H, s, $-OCH_3$), 6.39 (1H, d, J = 2.5 Hz, 6-H), 7.64 (1H, s, 3-H), 7.04 (1H, d, J = 2.5 Hz, 8-H), 7.30 (1H, m, Ar–H), 7.36–7.44 (7H, m, 3′,4′,5′-H, Ar–H), 7.94 (2H, d, J = 1.5 Hz, 2′,6′-H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 47.3 (CH_2), 55.5 (7-OCH_3), 56.0 (5-OCH_3), 96.0 (6-C), 96.8 (8-C), 101.6 (3-C), 104.9 (10-C), 127.2 (2Ar–C), 127.4 (1Ar–C), 127.5 (3′,5′-2C), 128.5 (2′,6′-2C), 128.7 (4′-C), 128.9 (2Ar–C), 138.2 (1Ar–C), 140.8 (1′-C), 152.5 (4-C), 152.7 (9-C), 158.4 (5-C), 158.6 (7-C), 160.2 (2-C); IR (KBr disk): *v* 3431 (NH), 1614 (C=N), 1589 (C=C), 1165 (C–N), 1055 (C–O); ESI-MS: *m*/*z* 371 (M + 1).

Cell lines and culture conditions

HCT-116 (human colon cancer cell line), A549 (human lung cancer cell line), DU-145 (human prostate carcinoma cell line), HepG2 (human hepatoma cell line), LN229 (human glioblastoma cell line), and HEK293 (human embryonic kidney 293) were kindly provided by Shanghai Jiao-Tong University. The cell was routinely cultured in RPMI-1640 medium or DMEM medium, supplemented with 10 % neonatal bovine serum (NBS) or 10 % fetal bovine serum (FBS). The culture was maintained at 37 °C with a gas mixture of 5 % CO_2/95 % air. All media were supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin.

Cell viability assay

The cytotoxic activity in vitro was measured using the MTT assay. The MTT solution (10.0 μL/well) was added in culture media after cells were treated with various concentrations of drugs for 72 h, and cells were incubated for further 4 h at 37 °C. The purple formazan crystals were dissolved in 100 μL DMSO. After 10 min, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) at 570 and 630 nm. Assays were performed in triplicate on three independent experiments. The concentration required for 50 % inhibition of cell viability (IC_{50}) was calculated using the software "Dose–Effect Analysis with Microcomputers." The tumor cell lines panel consisted of HCT-116, A549, DU-145, HepG2, and LN229. In all of these experiments, three replicate wells were used to determine each point.

Western blot analysis

HCT-116 cells were treated with compound **6a** (2.5, 5, and 10 μM) for 48 h. Proteins were extracted with cell lysis buffer for Western and IP (Beyotime Institute of Biotechnology, Haimen, China). Equal amounts (40 μg/lane) of protein were separated on 12 % SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride (PVDF) membranes (Millipore Corporation, Bedford, MA, USA), and blocked at room temperature for 1 h in 3 % (w/v) non-fat milk in TBST. The blots were incubated overnight at 4 °C with the VEGF and β-actin primary antibodies (1:500 or 1,000) diluted in TBST buffer. After washing with TBST, the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies (1:5,000), and visualized with the ECL detection kit (Thermo, USA) according to the manufacturer's instructions.

Acknowledgments We sincerely thank the financial supports from the National Natural Science Foundation of China (Grant No. 21202012), 2010 Industry for Attracting Ph.D. Scientists Program of Jiangsu Province and Changzhou Key Technology R&D Program (Society Development).

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