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Original article

Synthesis and anticancer activities of 5,6,7-trimethoxy-*N*-phenyl(ethyl)-4-aminoquinazoline derivatives

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ABSTRACT

A series of 5,6,7-trimethoxy-*N*-phenyl(ethyl)-4-aminoquinazoline compounds was prepared by microwave irradiation and conventional heating methods. Compounds **6p**, **6q**, and **6x** strongly inhibited extracellular regulated kinase1/2 (ERK1/2) phosphorylation induced by epidermal growth factor (EGF) at 1.28 μM in PC3 cells. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay showed that all compounds had certain anticancer activities, and the IC_{50} values of **6x** were 6.2 ± 0.9, 3.2 ± 0.1, and 3.1 ± 0.1 μM against PC3, BGC823, and Bcap37 cells, respectively. Acridine orange/ethidium bromide staining, Hoechst 33258 staining, DNA ladder, and flow cytometry analyses revealed that **6x** induced cell apoptosis in PC3 cells, with apoptosis ratios of 11.6% at 1 μM and 31.8% at 10 μM after 72 h.

1. Introduction

Protein kinases play diverse and important roles in regulating cellular processes such as cell proliferation, cell cycle, metabolism, survival, apoptosis, and DNA damage/repair. Thus, protein kinases are extensively targeted for the discovery of inhibitors as potential cancer-treatment drugs [1–4]. Epidermal growth factor receptor (EGFR) protein tyrosine kinase is one of the most important kinases that play a fundamental role in signal transduction pathways. The tyrosine kinase activity of EGFR is activated by ligand binding to the receptor, and an inhibitor of EGFR tyrosine kinase may compete with a ligand for EGFR binding or may directly interfere with the catalytic site of EGFR [5]. Fry et al. [6] first discovered that the 4-anilinoquinazoline derivativePD153035 possesses specific inhibitory activity against EGFR tyrosine kinase. Since then, various 4-anilinoquinazoline derivatives have been synthesized based on the 4-anilinoquinazoline framework [7–11]. Modification of the quinazoline structure has been performed in many anticancer studies, such as against NSCLC, PC3, BTC, and MCF-7 cells [12–20]. Gefitinib (Iressa, ZD-1839) [21,22] and erlotinib (OSI-774, Tarceva) [23,24], which are first-generation EGFR-targeting 4-anilino quinazoline chemotherapeutics, have been approved for the treatment of non-small-cell lung cancer. These two small compounds directly act on the ATP binding area of EGFR, interfering with the binding of ATP to EGFR and inhibiting the activity of EGFR-TK. The second-generation EGFR-targeting chemotherapeuticBIB W2992, which is an excellent non-irreversible EGFR inhibitor [25], has also been approved for Phase-II clinical trials against lung cancer [26].

Trimethoxyphenyl is a crucial pharmacophoric group for analogs of the antitumor natural product CA-4((*Z*)-2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenol) [27]. In our study on PD153035 (the specific inhibitor for EGFR-TK) as the leading compound, we have successfully produced 6,7,8-trimethoxy-*N*-aryl-4-aminoquina zoline compounds that are potent inhibitors of PC3, Bcap37, BGC823, and A431 cells. We found that methoxy-substituted

Abbreviations: AO/EB, acridine orange/ethidium bromide; ^{13}C NMR, ^{13}C nuclear magnetic resonance; CH, conventional heating; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK1/2, extracellular regulated kinase1/2; ESI-MS, electrospray ionization mass spectrometry; HCPT, 10-hydroxyl camptothecine; ^{1}H NMR, proton nuclear magnetic resonance; IR, infra-red; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MW, microwave irradiation.

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quinazoline compounds possess potent antitumor activity but not antiphosphorylation inhibitory activity, suggesting that cytotoxicity may not result from inhibiting EGFR [28]. Thus, a series of new quinazoline compounds **6a**–**6z** was designed and synthesized with three methoxy (5,6,7-OCH_3) groups on the quinazoline ring to study further the effect of the substitution position of quinazoline methoxy groups on anticancer and antiphosphorylation activities. Twenty-six new 5,6,7-trimethoxy-*N*-aryl-4-aminoquinazoline derivatives were synthesized from 2,3,4-trimethoxybenzoic acid by the synthesis route shown in Scheme 1. The structures of the title compounds were characterized by infrared (IR), ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and elemental analyses. The microwave irradiation (MW) synthesis conditions for the target compounds were also optimized. The antiproliferation activities of the title compounds against PC3, Bcap-37, andBGC823 cells *in vitro* were evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. The results showed that title compounds **6a**–**6z** possessed weak to strong anticancer activities, and most of the compounds exerted an inhibitory effect on EGF-induced ERK1/2 phosphorylation in PC3 cells.

2. Results and discussion

2.1. Chemistry

The starting material 2,3,4-trimethoxybenzoic acid was nitrated with70% nitric acid, esterified with methanol in the presence of 98% sulfuric acid, hydrogenated with Pd/C as catalyst in EtOH, cyclized with formamide, and finally chlorinated with phosphorus oxychloride to give the key intermediate 4-choloro-5,6,7-trimethoxyquinazoline. The target compounds **6a**–**6z** were obtained by the substitution reaction of 4-chloro-5,6,7-trimethoxyquinazoline with amine to yield 5,6,7-trimethoxyl-*N*-aryl-4-aminequinazoline (Scheme 1). The yields of the title compounds were relatively low under conventional heating (CH) method. MW method was used to improve the yields of the title compounds, and the reaction conditions under MW were optimized using **6o** as the model compound. Table 1 shows that when the power of MW was optimized from 40 W to 100 W, the yield of **6o** increased from 73.1% to 80.7% (Table 1, entries 1–4) within 30 min at 80 °C. Then, the effect of reaction time on yield was investigated. With increased reaction time from 10 min to 30 min, the yield increased from 72.3% to 80.7% within 30 min under an MW power 100 W (Table 1, entries 4–6). However, the yield did not obviously increase when the reaction time was 40 min (Table 1, entry 7). Thus, the reaction conditions for MW irradiation were as follows: temperature, 80 °C; reactant molar ratio, 1:1; MW power, 100 W; and reaction time, 30 min. For comparison, the yield of **6o** was found to be only 45.0% after 8 h of reaction (Table 1, entry 8).

All title compounds were synthesized under both CH and MW methods, and the results are listed in Table 2. The yields of the title compounds increased from 30.1% to 71.6% under CH to 80.1%–90.0% under MW with decreased reaction time from 2 to 12 h to 30 min. Interestingly, **6f** and **6r** were obtained only under CH method, whereas **6g** and **6m** were obtained only under MW irradiation.

Table 1
Effects of reaction time and MW power on the yield of **6o**.[a]

Entry	Reaction time	Power (Watt)	Yield (%)
1	30 min.	40	73.1
2	30 min.	60	78.5
3	30 min.	80	79.4
4	30 min.	100	80.7
5	10 min.	100	72.3
6	20 min.	100	78.7
7	40 min	100	77.3
8[b]	8 h	0	45.0

[a] Reaction conditions: reactants molar ratio, 1:1; reflux temperature of isopropanol, 80 °C.
[b] Conventional heating.

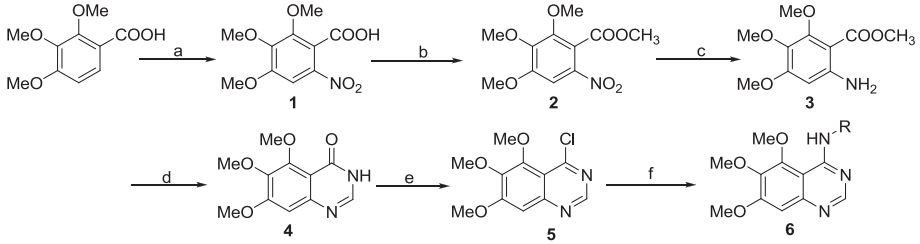
2.2. Inhibition activity of title compounds against ERK1/2 phosphorylation induced by EGF

Western blot analysis demonstrated that the title compounds had significant inhibitory activities against EGF-induced ERK1/2 phosphorylation in PC3 cells. As shown in Fig. 1, **6b**, **6d**–**6g** (Fig. 1A), **6i**, **6m**–**6q** (Fig. 1B), **6r** (Fig. 1C), and **6x** (Fig. 1D) inhibited ERK1/2 phosphorylatin in PC3 cells induced by EGF at 10 μM. The 4-phenyl ring containing a fluorine atom or trifluoromethyl group generally had good antiphosphorylation activities (**6e**, **6f**, **6i**, **6m**, **6o**–**6q**, and **6x**).

Further dose experiments were carried out on **6d**, **6e**, **6f**, **6p**, **6q**, and **6x**. Fig. 2 shows that the inhibition activities of **6d**–**6f** against ERK phosphorylation were weak to moderate at 5.12 μM but strong at 10 μM (Fig. 2A–C). However, **6p**, **6q**, and **6x** showed strong inhibition activities against ERK phosphorylation using low concentration of 1.28 μM (Fig. 2D–F). Furthermore, the 4-aniline moiety bearing a 3- or 4-trifluoromethyl group showed very strong inhibition activities against ERK1/2 phosphorylation in PC3 cells. Interestingly, 4-phenylethylamine substitution also showed very strong inhibition activities against ERK phosphorylation in PC3 cells.

2.3. Antiproliferation activities of title compounds against PC3, BGC823, and Bcap-37 cells

The antiproliferation activities of title compounds **6a**–**6z** were evaluated against PC3, BGC823, and Bcap-37 cells using PD153035as a positive control. As shown in Table 3, the antiproliferation activity of **6z** against PC3 cells at 10 μM was 61.75% ± 12.5%, similar to that of PD153035. The antiproliferation activities of **6l**, **6v**, **6w**, and **6z** against BGC823 cells at 10 μM were



Scheme 1. Reagents and conditions of the synthesis of title compounds **6**: (a) nitric acid, 0 °C, 2 h; (b) CH_3OH, 98% H_2SO_4, reflux, 12 h; (c) H_2, Pd/C, 95% C_2H_5OH, reflux, 12 h; (d) DMF, formamide, CH_3OH, CH_3ONa, 150 °C, 24 h; (e) N_2, toluene, $POCl_3$, reflux, 3.5 h; (f) aryl amine, 2-propanol, MW, 100 W, 80 °C, 30 min to obtain **6a**–**6z**; aryl amine, 2-propanol, reflux, 2–12 h to obtain **6a**–**6z**.