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Synthesis and anticancer activity of new quinazoline derivatives

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ABSTRACT

In this study, a new series of quinazoline derivatives (**3**–**26**) was synthesized and characterized via physicochemical and spectral means. Treatment of 2-amino-5-methylbenzoic acid with butyl isothiocyanate resulted in the new 2-thioxoquinazolin-4-one (**3**). Alkylation and hydrazinolysis of the inherent thioxo group in (**1**–**3**) afforded the corresponding thioethers (**4**–**23**) and hydrazine derivatives (**24** and **25**), then **24** was further transformed into tricyclic derivative **26** via cyclocondensation reaction. Compounds **1** and **2,** which were previously synthesized, were found to exhibit anticancer activity. The cytotoxicity of all compounds was evaluated *in vitro* against the HeLa and MDA-MB231 cancer cell lines, including **1** and **2** for comparison, using MTT assay. The treatment of the cells was performed with the synthesized compounds and gefitinib at 0, 1, 5, 10, 25, and 50 μM and incubated for 24 h in 50% DMSO. The IC_{50} values of the target compounds were reported in μM, using gefitinib as a standard. Our results indicated that all compounds exhibited significant *in vitro* cytotoxicity against both cell lines. While compounds **1**–**3** showed good activity, compounds **21**–**23** were found to be more potent than gefitinib. Thus, compounds **21**–**23** may be potential anticancer agents, with IC_{50} values ranging from 1.85 to 2.81 μM in relation to gefitinib (IC_{50} = 4.3 and 28.3 μM against HeLa and MDA-MB231 cells, respectively).

1. Introduction

Although several chemotherapeutic agents are currently being used to treat human cancers, either alone or in combination, they have limited effectiveness and the response rates remain largely unimproved in clinical trials (Chandregowda et al., 2009; El-Messery et al., 2016). Despite major advances in chemotherapeutic management and cancer biology, cancer still poses a serious threat to human health globally (Al-Salahi et al., 2014a, 2015). Moreover, the great similarity between tumor and normal cells and diversity of tumor types are the main hurdles preventing the development of an ultimate anticancer therapy (El-Messery et al., 2012). Thus, persistent commitment to the arduous task of discovering and designing new anticancer agents remains critically essential.

The epidermal growth factor receptor (EGFR) plays a vital role in cell growth regulation and is considered one of the most intensely studied targets of tyrosine kinase (TK) inhibitors (El-Azab et al., 2010; Tiwari et al., 2015). Several TKs play important roles in cell proliferation, differentiation, metastasis and survival, and their unregulated activation through mechanisms such as point mutations can lead to a large percentage of clinical cancers (El-Azab et al., 2010; Tiwari et al., 2015; Al-Suwaidan et al., 2016). EGFR is overexpressed in numerous tumors, including brain, lung, bladder, ovarian, colon, breast, head, and prostate tumors (Fricker, 2006; Garofalo et al., 2008; Tiwari et al., 2015; Al-Suwaidan et al., 2016). Moreover, EGFR hyperactivation or aberrations in TKs have been implicated in other diseases including polycystic kidney disease, psoriasis, asthma, and diabetes.

Members of the erbB family of EGFR-TKs, which include erbB2 (HER2), erbB3 (HER3), and erbB4 (HER4), are overexpressed in a significant proportion of human tumors, and this overexpression is associated with poor prognosis of the disease (Meert et al., 2003; Ang et al., 2004; Chandregowda et al., 2009). Thus, inhibitors of erbB1 and erbB2 have been identified as potential anticancer drugs (Hynes and Lane, 2005; Chandregowda et al., 2009).

Of several candidate compounds that have been synthesized and tested, gefitinib (Fig. 1a) is the most potent and selective EGFR-TK inhibitor reported to date that, along with erlotinib

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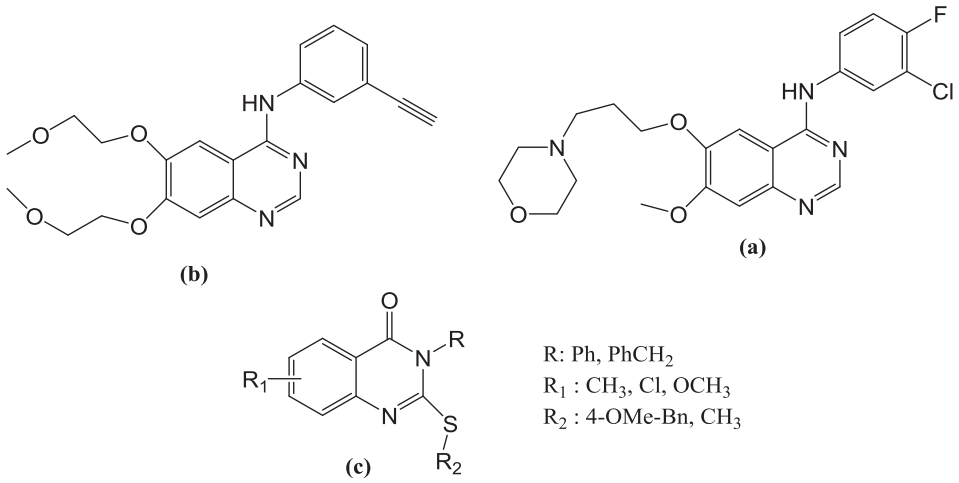

Fig. 1. Chemical structure of gefitinib, erlotinib, and quinazolines.

(Fig. 1b), has been approved by the United States Food and Drug Administration (US FDA) for the treatment of non-small cell lung cancer (NSCLC) (Arteaga and Johnson, 2001; Barker et al., 2001; Barlési et al., 2005). Gefitinib, which belongs to a new class of quinazolines, inhibits EGFR-TK overexpression through its effect on EGFR autophosphorylation and EGF-stimulated signal transduction. Hence, designing compounds with EGFR-TK inhibitory activity is an attractive chemotherapeutic strategy against malignant and nonmalignant epithelial diseases. Recently somatic mutations in the TK domain of erbB1 have been observed in a subgroup of gefitinib- and erlotinib-treated NSCLC patients (Lynch et al., 2004; Fukui et al., 2010). The focus is now on developing molecules with multiple kinase inhibition, particularly erbB1 and erbB2 inhibition (Barker and Johnstone, 1997).

Owing to the current interest on quinazolines (Fig. 1c) as antitumor agents (Al-Rashood et al., 2006; Alafeefy et al., 2014, 2015; El-Messery et al., 2016) and our ongoing studies on the quinazoline scaffold that are aimed at finding new leads with potential cytotoxic effects, we synthesized several new quinazoline derivatives (**3**–**16**, **24,** and **26**) containing a butyl group with different fragments and evaluated their *in vitro* cytotoxicity against HeLa and MDA-MB231 cancer cells. Additionally, we synthesized some derivatives (**17**–**23** and **25**) containing a benzyl group from the previously prepared parent compounds (**1**and **2**). In the present study, various functional groups were specifically incorporated at positions 3, 6, and 8 of the quinazoline scaffold to investigate the effect of various electronic environments on the cytotoxicity of the target molecules.

2. Materials and methods

2.1. Chemistry

The NMR spectra were measured on a Bruker AMX 500 spectrometer (Bruker, Billerica, MA, USA) in deuterated dimethyl sulfoxide (DMSO-d_6) and reported as δ (ppm) values relative to tetramethylsilane (TMS) at 500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively. *J* values were recorded in Hz. The electrospray ionization mass spectrometry (ESI-MS) spectra were recorded using a Micromass Quattro micro™ triple-quadruple tandem mass spectrometer (Waters Corp., Milford, MA, USA). The X-ray data were collected on a Bruker APEX-II D8 Venture area diffractometer using graphite monochromatic Mo Kα radiation (λ = 0.71073 Å) at 100(2) K. The uncorrected melting point (mp) values were determined using a Stuart SMP10 melting point apparatus with open glass capillaries. The reactions were followed, and the product purity was checked by thin layer chromatography (TLC) on a DC-Mikrokarten Polygram® SIL G/UV$_{254}$, TLC plate (Thickness: 0.25 mm; Macherey-Nagel, Düren, Germany).

2.2. Preparation of 3-butyl-2,3-dihydro-6-methyl-2-thioxoquinazolin-4(1H)-one (**3**)

A mixture of butyl isothiocyanate (5 mmol) and 2-amino-5-methylbenzoic acid (5 mmol) in ethanol (15 mL) or *N,N*-dimethylformamide (DMF) (10 mL) was refluxed in the presence of triethylamine (Et_3N, 2.4 mmol) for 2 h. The mixture was then cooled and poured into ice/water (Al-Salahi et al., 2015). The resulting solid was filtered, washed with water, and dried. Yield: 90%; mp: 230–231 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 12.85 (s, 1H, —NH—), 7.75 (br s, 1H, H-5), 7.56 (br d, *J* = 8 Hz, 1H, H-7), 7.29 (d, *J* = 8.5 Hz, 1H, H-8), 4.39 (t, *J* = 7.5 Hz, 2H, H-1′), 2.36 (s, 3H, Ar—$\underline{CH_3}$), 1.66 (quintuplet, *J* = 7.5 Hz, 2H, H-2′), 1.34 (m, 2H, H-3′), 0.93 (t, *J* = 7.5 Hz, 3H, H-4′); ^{13}C NMR (DMSO-d_6) δ (ppm): 174.9 (C-2), 159.7 (C-4), 137.5 (C-4b), 136.9 (C-6), 134.5 (C-7), 127.0 (C-5), 116.0 (C-8), 115.8 (C-4a), 45.9 (C-1′), 28.9 (C-2′), 20.9 (Ar—$\underline{CH_3}$), 20.1 (C-3′), 14.2 (C-4′); ESI-MS (*m*/*z*): 247.3 $[M-H]^-$ (negative mode) for molecular weight (MW) = 248.34.

2.3. General procedure for the Preparation of compounds **4**–**23**

Potassium carbonate (1.2 mmol) was added portion-wise over a period of 5 min to a mixture of compounds **1**, **2** or **3** (1 mmol) in DMF (8 mL) at room temperature. The appropriate alkyl halide (1.5 mmol) was then added, and the reaction mixture was stirred at 90 °C for 18 h. The mixture was poured into ice/water, and the precipitate was filtered off, washed with water, and dried (Al-Salahi et al., 2015).

2.3.1. 3-Butyl-2-(ethylthio)-6-methylquinazolin-4(3H)-one (**4**)

White amorphous powder; yield: 77%; mp: 89–90 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 7.86 (br s, 1H, H-5), 7.59 (br d, *J* = 8 Hz, 1H, H-7), 7.44 (d, *J* = 8.5 Hz, 1H, H-8), 4.02 (t, *J* = 7.5 Hz, 2H, H-1″), 3.26 (q, *J* = 7.5 Hz, 2H, H-1′), 2.42 (s, 3H, Ar—CH_3), 1.65 (quintuplet, *J* = 7.5 Hz, 2H, H-2″), 1.37 (m, 5H, H-2′, H-3″), 0.93 (t, *J* = 7.5 Hz, 3H, H-4″); ^{13}C NMR (DMSO-d_6) δ (ppm): 160.9 (C-4), 155.8 (C-2), 145.5 (C-4b), 136.3 (C-7), 135.8 (C-6), 126.2 (C-5), 126.1 (C-8), 118.9 (C-4a), 44.1 (C-1″), 30.1 (C-2″), 26.3 (C-1′), 21.2 (Ar—CH_3), 20.1 (C-3″), 14.4 (C-2′), 14.1 (C-4″); ESI-MS (*m*/*z*): 275.4 $[M-H]^-$ (negative mode) for MW = 276.40.

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