European Journal of Medicinal Chemistry 89 (2015) 826–834

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original articles

Design, synthesis and biological evaluation of 6-(nitroimidazole-1*H*-alkyloxyl)-4-anilinoquinazolines as efficient EGFR inhibitors exerting cytotoxic effects both under normoxia and hypoxia

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ARTICLE INFO

Article history:
Received 5 August 2014
Received in revised form
27 October 2014
Accepted 5 November 2014
Available online 6 November 2014

Keywords:
EGFR inhibitor
4-Anilinoquinazoline
Nitroimidazole
Normoxia
Hypoxia

ABSTRACT

A series of novel 6-(nitroimidazole-1*H*-alkyloxyl)-4-anilinoquinazoline derivatives (**15a**–**15r**) were designed, synthesized and evaluated as efficient EGFR inhibitors through introduction of hypoxia activated nitroimidazole moiety into the quinazoline scaffold of EGFR inhibitors. The majority of these newly synthesized compounds exhibited comparable EGFR inhibitory activities to gefitinib and moderate to excellent anti-proliferative activities against HT-29 cells under normoxia and hypoxia. The most promising compound **15c** displayed the IC_{50} value of 0.47 nM against EGFR kinase and excellent cytotoxic effect against HT-29 cells under normoxia and hypoxia with the IC_{50} values of 2.21 μM and 1.62 μM, respectively. The mimic reductive activation study revealed that compound **15c** exerted reductive activation properties under hypoxia, which were consistent with the *in vitro* metabolic study, wherein **15c** was easily reductive activated under hypoxia and much more stable under normoxia. All these results suggested that **15c** was a potential cancer therapeutic agent both under normoxia and hypoxia and was worth of further development.

1. Introduction

As a member of the HER family, epidermal growth factor receptor (EGFR) plays a vital role in the cellular signaling transduction processes [1]. The over-expression of EGFR has been observed in many solid tumors, such as colon [2], ovarian [3], and non-small cell lung cancer (NSCLC) [4]. Therefore, EGFR inhibition has been developed as one of the most efficient strategies for cancer therapy. In the past few years, more than 20 EGFR inhibitors have been advanced into clinical trials, and four 4-anilinoquinazoline derivatives, termed as gefitinib (**1**), erlotinib (**2**), lapatinib (**3**), and afatinib (**4**), have been approved by FDA for the treatment of NSCLC, pancreatic cancer and advanced breast cancer (Fig. 1) [5].

As an inevitable circumstance in most solid tumors, hypoxia is responsible for the resistance to radiotherapy and chemotherapy and presents a tremendous challenge to cancer therapy [6]. Especially, the expression of EGFR is up-regulated when tumors are under hypoxia, which is closely related to tumor survival [7,8]. Therefore, design and development of EGFR inhibitors with potency to overcome EGFR up-regulation under hypoxia may exert better effects for cancer therapy.

The specific hypoxic micro-environment of tumor cells also makes it as an attractive and exploitable therapeutic target. 2-Nitroimidazole derivatives have been developed as hypoxia activated radio-sensitization and chemotherapy agents since 1960s [9]. Under hypoxia, the 2-nitroimidazole moiety was reduced by nitroreductase to yield reactive radicals, which could deplete tumor endogenous protective agents such as glutathione (GSH) to make tumors be sensitive to radiotherapy [10]. Moreover, the irreversibly binding of reactive radicals to the cellular protein and nucleic acids makes them accumulate therein and exert cytotoxic effects [11]. Up to now, several radioactive 2-nitroimidazole derivatives have been advanced into clinical trials for the noninvasive assessment of hypoxia in cancer, including [^{18}F]FMISO (**5**), [^{18}F]FAZA (**6**) and [^{18}F]HX4 (**7**) (Fig. 1) [12,13]. Based on the above reports, we envisioned that EGFR inhibitors containing hypoxia activated pharmacophore may exert efficient antitumor activities both under normoxia and hypoxia.

The crystal structures of 4-anilinoquinazoline–EGFR complexes reveal that the quinazoline scaffold confers an H-bond interaction to the hinge domain of the kinase and the aniline moiety inserts into the hydrophobic pocket, which are crucial for EGFR inhibitory

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http://dx.doi.org/10.1016/j.ejmech.2014.11.010

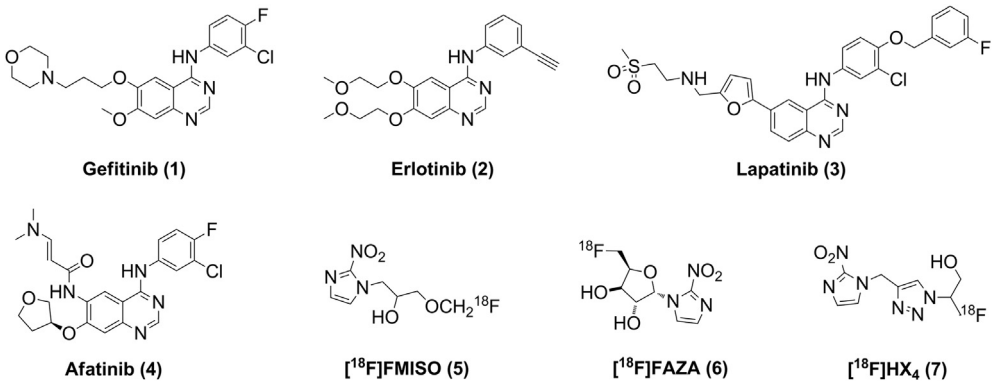

Fig. 1. Structures of selected EGFR inhibitors and 2-nitroimidazole derivatives.

activity. The side chains at the *C*-6 and *C*-7 positions are projected to the solvent portion which exerts good compatibility for long ether chains or other moieties [14–17] (Fig. 2). Therefore, a series of novel EGFR inhibitors were designed and synthesized through incorporating 2-nitroimidazole and its analogue 2-methyl-5-nitroimidazole into the *C*-6 position of the 4-anilinoquinazoline scaffold. Different kinds of anilines from gefitinib, erlotinib, lapatinib and other advanced EGFR inhibitor were employed to the *C*-4 position of the quinazoline template, as well as the length of linker between the nitroimidazole and quinazoline was probed for the SAR study (Fig. 2).

2. Results and discussion

2.1. Chemistry

The synthetic route to compounds **15a**–**15r** was displayed in Scheme 1. Compounds **9a**–**9e** were prepared via alkylation of 2-nitroimidazole (**8**) with α, ω-dibromoalkane in the presence of potassium carbonate, and compounds **11a**–**11c** were obtained in a similar way by using 2-methyl-5-nitroimidazole (**10**) as the start material. Condensation of compound **12** with substituted anilines afforded 4-anilinoquinazoline derivatives **13a**–**13d** [18], followed by de-acetylation with aqueous ammonia provided **14a**–**14d** as the key intermediates. Finally, reaction of **14a**–**14d** with **9a**–**9e** or **11a**–**11c** in the presence of potassium carbonate furnished title compounds **15a**–**15r**.

2.2. In vitro EGFR inhibitory activity

The EGFR inhibitory activities of compounds **15a**–**15r** were evaluated using a well established Z′-Lyte assay and gefitinib was employed as the positive control [19]. As shown in Table 1, most of the tested compounds (**15b**–**15j**, **15q**, and **15r**) exhibited potent EGFR inhibitory activities with the IC_{50} values ranging from 0.32 to 1.18 nM, which were comparable to that of gefitinib (IC_{50} = 0.45 nM). The introduction of the bulky substituents on aniline resulted in a dramatic decrease in activity. For instance, compounds **15k**–**15n** only demonstrated EGFR inhibition with the IC_{50} values ranging from 15.1 to 51.2 nM, which were more than 30 fold less potent than gefitinib. Other compounds with small substituents on aniline demonstrated favorable inhibitory activity with the IC_{50} values ranging from 0.32 to 4.90 nM, indicating that bulky moiety on aniline may prevent the aniline from adapting the hydrophobic pocket of EGFR kinase. The length of linker between the imidazole and quinazoline also significantly affected the activities. The compromised EGFR inhibitory activities of compound **15a**, **15b**, **15o** and **15p** compared to **15c** and **15q** indicated that longer linkers (n = 3–5) were more favorable than shorter ones (n = 1–2).

2.3. In vitro anti-proliferative activity

The human colorectal adenocarcinoma HT-29 cells were widely used to evaluate the biological activities of the hypoxia-activated drugs, and this kind of cells also expressed high levels of EGFR [20–23]. Therefore, the anti-proliferative activities of compounds **15a**–**15r** were evaluated against HT-29 cells under normoxia and hypoxia. As shown in Table 1, most of these compounds demonstrated moderate to excellent anti-proliferative activities. Especially, five compounds (**15c**, **15i**, **15j**, **15l**, and **15m**) were more potent than gefitinib both under normoxia (IC_{50} values: 1.87–3.55 μM) and hypoxia (IC_{50} values: 1.36–3.46 μM). Gefitinib exhibited less potent anti-proliferative activity under hypoxia (IC_{50} = 5.21 μM) than normoxia (IC_{50} = 3.63 μM), probably because the over-expression of EGFR made tumors be more resistant under hypoxia. For the newly synthesized compounds, most of them (**15a**–**15i** and **15m**–**15p**) exhibited comparable or more potent anti-proliferative activities under hypoxia than under normoxia. We deduced that the cytotoxicities contributed by the reductive activation of these compounds were partly offset by the increased

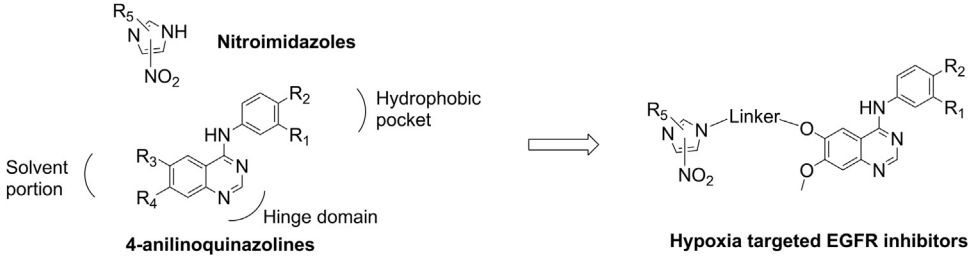

Fig. 2. Design of new EGFR inhibitors containing hypoxia activated pharmacophores.