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Research paper

Structure-activity study of quinazoline derivatives leading to the discovery of potent EGFR-T790M inhibitors

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ABSTRACT

We have developed a series of 6, 7-disubstituted-4-(arylamino) quinazoline derivatives that functioned as irreversible EGFR inhibitors, and these compounds exhibited excellent enzyme inhibition potency. As compared with afatinib, some of them showed significantly enhanced activities towards H1975 cells (EGFR-T790M). Furthermore, the optimized compounds **7q** and **8f** also demonstrated good pharmacokinetic profiles, oral bioavailability as well as excellent *in vivo* efficacy in H1975 and HCC827 xenografts at a non-toxic dose. Based on the improved safety and efficacy against EGFR-T790M resistance, **7q** and **8f** are promising candidates for further studies.

1. Introduction

EGFR inhibitors, such as gefitinib and erlotinib, are effective clinical treatments for NSCLC patients whose tumors harbor activating mutations in EGFR [1–3]. However, all patients will ultimately manifest disease progression due to acquired resistance which in part is explained by a second-site T790M mutation in EGFR kinase domain. Although the second-generation irreversible EGFR inhibitors including dacomitinib [4], neratinib [5] and afatinib [6] have demonstrated activity in preclinical studies against T790M mutations, clinical trial data did not demonstrate distinctly improved efficacy. This was partially attributed to the dosage limitation imposed by the toxicity, which led to insufficient exposure level in plasma and remarkably decreased efficacy when used in patients with advanced lung tumor harboring the T790M acquired mutation [7–10]. Two kinds of compounds may be used to solve the above problem. The first class of compounds has less activity against EGFR-wt but keeping the high efficacy against EGFR-T790M. The third-generation irreversible EGFR inhibitors, such as AZD-9291 [11,12] and CO-1686 [13], are characterized by potently inhibiting EGFR phosphorylation in EGFRm+/T790M, whilst demonstrating much less activity against EGFR-wt. Avoiding the toxicity induced by the inhibition of EGFR-wt, AZD-9291 and CO-1686 are currently in human phase 2 clinical trials. The second class of compounds has comparable efficacy against EGFR-wt, but with greatly increased efficacy against EGFR-T790M in comparison with afatinib. Without needing the high dosage, these compounds can achieve safe and effective inhibiting concentration in *vivo* against EGFR-T790M resistance tumor. The designing of the second type of compounds has been proved to be quite challenging, and few reports exists.

Despite the rapid advances in EGFR oncology therapeutics over the past decade, substantial room for improvement remains. Most cancer patients do not respond to EGFR inhibitors therapy, which implied intrinsic resistance (e.g., over-expressing EGFR-wt or 'oncogenic shift') [14,15]. Although many papers or patents have been presented for quinazoline-based EGFR inhibitors [16–19], and afatinib has been discovered, it is still in high demand to develop safer and more effective irreversible EGFR inhibitors. As part of our

Abbreviations: EGFR, Epidermal growth factor receptor; NSCLC, Non-small cell lung cancer; SAR, Structure-activity relationship; DMF, Dimethylformamide; TLC, Thin layer chromatography; THF, Tetrahydrofuran; NMP, N-methyl-2-pyrrolidone; Rf, Retention factor; DMSO, Dimethyl sulphoxide; RPMI, Roswell Park Memorial Institute; GI_{50}, The concentration that causes 50% growth inhibition; PO, Oral administration; QD, One a day (from the Latin quaque die).

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ongoing research program in designing new irreversible inhibitors that overcome EGFR-over-expressing or EGFR-T790M resistance, a series of 6, 7-disubstituted-4-(arylamino) quinazoline derivatives were synthesized. We discovered that compounds with a primary amine-substituted center at the end of the Michael acceptor caused a significant improvement in cellular potency against T790M in comparison with afatinib. These efforts resulted in the identification of the novel quinazoline-based compounds **7q** and **8f** with greatly increased efficacy against H1975 (EGFR-T790M) tumors at a non-toxic dose in comparison with afatinib.

2. Results and discussion

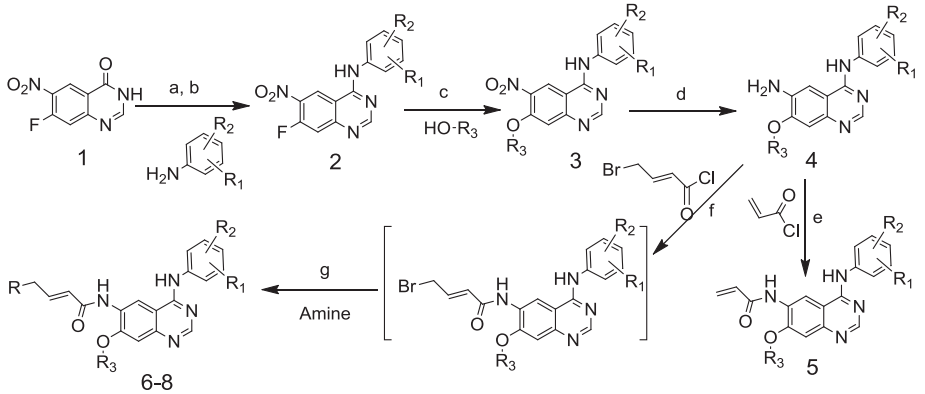
The aniline-substituted quinazoline motif (Intermediate **4**) was prepared in the following way: 7-fluoro-6-nitro-3*H*-quinazolin-4-one was firstly treated with thionyl chloride, and then reacted with an aromatic amine to give the intermediate **2**, which was further reacted with an alcohol of formula R_3OH in the presence of NaH to provide the intermediate **3**. Reduction of **3** gave the aniline-substituted quinazoline motif **4**. The Michael acceptor at the 6-position was constructed by reacting intermediate **4** with acryloyl chloride or (*E*)-4-bromobut-2-enoyl chloride which further reacted with various secondary amines or primary amines (Scheme 1).

The optimization of aromatic amine groups at the 4-position was firstly investigated, and the results are shown in Table 1. These data indicated that 3-chloro-4-fluoroaniline, 3-ethynylaniline, 4-(pyridin-2-ylmethoxy)aniline or 3-chloro-4-(pyridin-2-ylmethoxy)aniline group gratifyingly afforded a number of analogs (e.g., **5b**, **5n**, **6b**) with high activities against EGFR. 3-Chloro-4-(3-fluorobenzyloxy)aniline, 4-chloro-3-(trifluoromethyl) aniline, 5-amino-2-fluorobenzonitrile and *N*-(4-amino-2-(trifluoromethyl)phenyl)acrylamide were not ideal pharmacophore groups, and compounds containing those groups (e.g., **6a**, **6e**, **6p**) couldn't effectively inhibit EGFR kinase. The presence of linear chain or heterocyclic groups at the 7-position of the quinazoline scaffold was well tolerated and often had little effect on its' *in vitro* activities. The introduction of a dimethylamine group at the end of the Michael acceptor led to a modest increase in potency, most compounds of series 6 in Table 1 were more active in comparison with the corresponding compounds of series 5. Although with excellent *in vitro* activities, compounds containing the 4-(pyridin-2-ylmethoxy)aniline or 3-chloro-4-(pyridin-2-ylmethoxy)aniline group (e.g., **6g**, **6m** and **6n**) exhibited weak activities against A431 (human epidermoid carcinoma) xenografts in nude mice, which might be attributed to their large molecular weight and low permeability. Hence, the ideal groups at the 4-position of the quinazoline were 3-chloro-4-fluoroaniline and 3-ethynylaniline.

Considering that the terminal dialkylamino group of the crotonamide Michael acceptor affected the compound's physicochemical properties, water-solubility as well as the activity in both kinase and cell proliferation assays [20], further optimizing the Michael acceptor side chains were done. Tables 2 and 3 show compounds where the terminal alkylamino group of the crotonamide Michael acceptor was varied. It was evident that *N*-piperidinyl, cyclopropanamine, *N*-methylcyclopropanamine and propan-2-amine groups were very well tolerated, and for most of these analogs, the kinase and cellular activities were minimally affected by the choice of these alkylamino groups. One exception was the dicyclopropylamine group that greatly reduced potency of compounds **7k**–**7n**, which implied that incorporation of some steric bulk at the end of the Michael acceptor might be unfavorable and hinder the intramolecular base catalysis of the Michael addition to form a covalent adduct. Compound **7g**, where the Michael acceptor was terminated with a 3, 3-difluoroazetidine group, showed reduced activity in the kinase assay, and this loss of activity was likely attributed to the reduced basicity [21]. However, the diminished activity of piperidin-4-one-*N*-substituted analog **7c** or thiomorpholine-1-oxide-*N*-substituted analogs **7h**–**7j** may be due to the acyl group which interrupted the intramolecular base-catalyzed Michael addition. As expected, when the Michael acceptor side chains containing piperidinyl, cyclopropanamine, *N*-methylcyclopropanamine or propan-2-amine group were introduced into 4-(3-ethynylaniline)-substituted quinazoline analogs, compounds **8a**–**8i** also showed high *in vitro* efficacy. There was no major difference in activities between the 3-chloro-4-fluoroaniline-substituted derivatives and the 3-ethynylaniline -substituted derivatives (Tables 2 and 3).

In addition, H1975 cell growth inhibition assay was used to demonstrate the superior cell-based efficacy of those analogs against the resistant EGFR mutants (EGFR-T790M). The primary amine-substituted analogs **7o**–**7q** showed the highest potency and were at least 5-fold more effective than afatinib in abolishing survival of H1975 cells (Table 2). Similar results were also observed in the 3-ethynylaniline-substituted derivatives **8f**–**8i** (Table 3).

After the promising *in vitro* results were observed, further *in vivo* antitumor activities of the primary amine-substituted analogs **7o**–**7q**, **7r**, **7u** and **8e**–**8i** were evaluated first using a H1975 xenograft model (Supplementary information). Because the afatinib free base was difficult to dissolve at a high dose, and resulted in uneven dispersion, afatinib dimaleate was prepared for *in vivo* evaluation. Except for **7u** and **8i,** reductions of tumor size were observed versus controls in H1975 xenograft (Fig. 1). Intriguingly, although with high potency *in vitro*, compound **7u** or **8i** at 50 mg/kg/day dose level didn't show any activities in H1975 xenograft.



Scheme 1. Synthesis of 6, 7-disubstituted-4-anilino quinazoline derivatives (series **5**–**8**). Reaction and conditions: (a) thionyl chloride, DMF, reflux; (b) CH_3CN, reflux; (c) NaH, anhydrous THF, 0 °C to rt; (d) Fe, NH_4Cl, ethanol/water, 60 °C; (e) CH_3CN, NMP, −5 °C; (f) anhydrous THF, triethylamine, 0 °C; (g) K_2CO_3, KI, DMF, 40 °C.