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Research paper

Novel series of 6-(2-substitutedacetamido)-4-anilinoquinazolines as EGFR-ERK signal transduction inhibitors in MCF-7 breast cancer cells

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ABSTRACT

Epidermal growth factor receptor (EGFR) signaling pathway has been previously investigated for its significant role in the progression of different types of malignant tumors, where development of small molecules targeting EGFR is well known strategy for design of antitumor agents. Herein, we report the design and synthesis of two series of 6-(2-substitutedacetamido)-4-anilinoquinazolines (**6a-x** and **13a-d**) as EGFR inhibitors. All the newly synthesized quinazoline derivatives were *in vitro* evaluated for their anti-proliferative activity towards MCF-7 (Breast Cancer) and HepG2 (Hepatocellular carcinoma) cell lines. In particular, compound **6n** showed significant inhibitory activity against MCF-7 and HepG2 cell lines ($IC_{50} = 3$ and 16 μM, respectively), compared to that of Erlotinib ($IC_{50} = 20$ and 25 μM, respectively). Western blotting of **6n** at MCF-7 cell line revealed the dual inhibitory activity of **6n** towards diminishing the phosphorylated levels for EGFR and ERK. Also, ELISA assay confirmed the anti-EGFR activity of compound **6n** ($IC_{50} = 0.037$ μM). Finally, a molecular docking study showed the potential binding mode of **6n** within the ATP catalytic binding site of EGFR, exhibiting similar binding mode to EGFR inhibitor Erlotinib.

1. Introduction

Epidermal Growth Factor Receptor (EGFR) plays pivotal role in the regulation of the cellular functions such as cell growth, survival, proliferation, differentiation, and apoptosis [1]. EGFR activation occurs *via* binding of the endogenous ligands; epidermal growth factor (EGF), transforming growth factor-α (TGF-α), amphiregullin, betacellulin, heparin binding EGF, and epiregulin; resulting in the signaling transduction pathways [2]. EGFR is a member of tyrosine kinases, when ligand binds to the extracellular domain, conformational change happens, leading to EGFR activation *via* homodimerization or heterodimerization. EGFR dimerization activates its intrinsic intracellular protein-tyrosine kinase activity [3]. As a result, autophosphorylation of several tyrosine residues occured leading to downstream activation for Mitogen Activated Protein Kinase (MAPK) signaling transduction pathway *via* phosphorylation of a cascade of downstream proteins [3].

Different cancer types (as hepatocellular carcinoma, breast cancer, non-small cell lung cancer, pancreatic cancer, colorectal carcinoma, melanoma, and glioblastoma), have shown significant mutations in several members of MAPK pathway; including EGFR,

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RAF, MEK, and ERK, [4–6]. Emerging new chemical scaffolds as potential drug candidates targeting the phosphorylation cascade pathway of protein kinases created plausible rational for treatment of cancer *via* inhibition of cell growth and induction of apoptosis [7].

Development of kinase inhibitors was rationalized based on the fundamental understanding of the presence of ATP as the endogenous substrate binding to the kinases to aid in the phosphorylation process along the whole cascade. Hence, most of the small molecule kinase inhibitors have been emerged to be ATP competitive inhibitors binding to the ATP catalytic active site [7]. Different chemical classes were developed to be biologically evaluated for their potential to inhibit kinases in order of treatment of cancer [8]. 4-Anilinoquinazoline scaffold has emerged as an important leading one for developing effective EGFR inhibitors, exemplified by the clinically approved drugs; Gefitinib I, Erlotinib II, Lapatinib III, Afatinib IV and Vandetanib V [9–14] (Fig. 1).

Structure-Activity Relationship (SAR) studies for the 4-anilinoquinazoline-based EGFR inhibitors suggested that: The quinazoline heterocyclic moiety binds in the ATP-binding pocket and forms a hydrogen bond with the NH group of Met769 (Met793) in the hinge region. Erlotinib binds to the active conformation while Lapatinib binds to the displaced inactive αC-out conformation of EGFR. The anilino group of Gefitinib, Erlotinib, or Lapatinib extends into a hydrophobic region of the kinase domain of EGFR that is adjacent to the adenine-binding site (the back pocket), as shown in Fig. 2. This hydrophobic site extends only to the β5-sheet containing the Thr790 gate keeper residue and to Thr855 on top of the activation segment. The anilino moiety of Lapatinib contains a 3-fluorobenzyloxy extension that displaces the αC-helix to its out position. As a result of the larger size of Lapatinib, the hydrophobic pocket next to the adenine-binding site extends to the β4-sheet (Leu778), the αC-helix (Met766), the top of the β7-activation segment loop (Leu856), and the activation segment (Phe856) [15–19].

Utilizing the reported key binding features between the EGFR and 4-anilinoquinazoline template and as a part of our ongoing efforts to assemble novel small molecules targeting kinases [20–24], herein we present two new series of 6-substituted-4-anilinoquinazolines (**6a-x** and **13a-d**) as promising candidates for EGFR inhibition. Our strategy is directed towards designing a variety of ligands with diverse chemical properties hypothesizing that the potency and selectivity of these molecules might be enhanced by attaching new moieties at C6 of substituted 4-anilinoquinazolines and/or by varying the aniline substitution pattern, Fig. 3.

Gefitinib, Erlotinib and Lapatinib were used as lead compounds. Initially, the 4-anilinoquinazoline core was retained, followed by introduction of three different modifications. The first modification focused on introducing variety of hydrophilic solubilizing moieties (as pyrrolidino, piperidino, morpholino, *N*-methylpiperazino) to the C-6 position of the quinazoline core through acetamide spacer (-CH_2-CONH-) to elucidate their impact on the activity. The second modification included introduction of different electron withdrawing groups (as CF_3, Cl, or Br) to 3′- and/or 4′-positions of the 4-anilino moiety (**6a-x**). The third modification focused on extending the anilino moiety of the first series **6a-x** with a benzyloxy tail to afford the second series **13a-d**, in an attempt to increase the hydrophobic interaction with EGFR hydrophobic back pocket in analogy to Lapatinib.

The newly synthesized 4-anilinoquinazoline analogs were evaluated in enzyme-based and cell-based assays for their ability to inhibit EGFR and the proliferation against MCF-7 and HepG2 cancer cell lines expressing EGFR. In addition we also investigated their effect on the activation of EGFR by western blot.

2. Results and discussion

2.1. Chemistry

The synthetic pathways adopted for the preparation of the intermediates and target 4-anilinoquinazolines are depicted in Schemes 1 and 2. Synthesis was initiated in Scheme 1 by reacting 5-nitroanthranilonitrile **1** with DMF-DMA to afford the formamidine intermediate **2**, which cyclized upon reaction with different anilines in refluxing glacial acetic acid into 6-nitro-4-anilinoquinazolines **3a-f** through Dimroth rearrangement. Reduction of the latter using sodium dithionite/ammonia mixture yielded the 6-amino-4-anilinoquinazolines **4a-f**, which was subsequently acylated with 2-chloroacetyl chloride in dioxane to afford the corresponding 2-chloroacetamido derivatives **5a-f**. The target compounds **6a-x** were prepared through a nucleophilic substitution on **5a-f** with the appropriate heterocyclic secondary amines in dry acetonitrile in the presence of anhydrous potassium carbonate and a catalytic amount of potassium iodide (Scheme 1).

In Scheme 2, the *N*-(4-arayloxyphenyl)acetamides **8a** and **b** were prepared by *o*-alkylation of paracetamol **7** with appropriate benzyl halide. This was followed by hydrolysis of the protected amino group of compounds **8a**, **b** using NaOH to give the benzyloxyanilines **9a**, **b** as white solid and an oily residue, respectively, which used directly in cyclization of the formamidine **2** in refluxing glacial acetic acid to afford 6-nitro-4-(benzyloxyanilino)quinazolines **10a**, **b**. Then, the target compounds **13a-d** were obtained adopting the general synthetic pathway for synthesis of **6a-x**. The

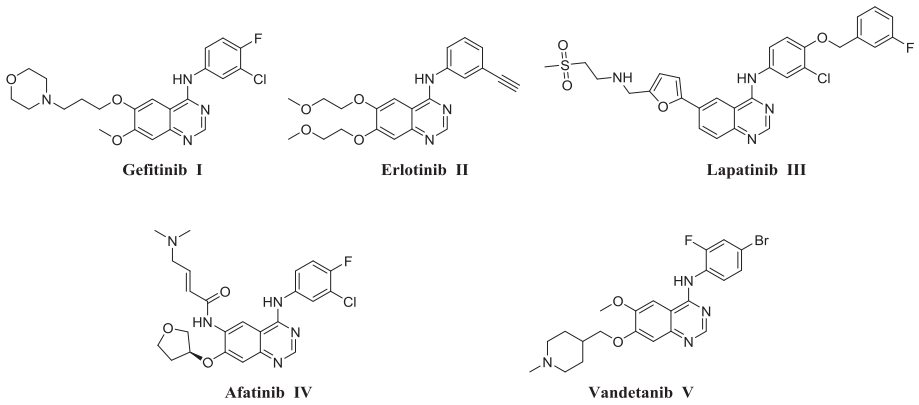

Fig. 1. Chemical structure for the clinically approved 4-anilinoquinazoline EGFR inhibitors **I-V**.