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Novel 2-chloro-4-anilino-quinazoline derivatives as EGFR and VEGFR-2 dual inhibitors

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ABSTRACT

Novel 2-chloro-4-anilino-quinazolines designed as EGFR and VEGFR-2 dual inhibitors were synthesized and evaluated for inhibitory effects. EGFR and VEGFR-2 are validated targets in cancer therapy and combined inhibition might be synergistic for both antitumor activity and resistance prevention. The biological data obtained proved the potential of 2-chloro-4-anilino-quinazoline derivatives as EGFR and VEGFR-2 dual inhibitors, highlighting compound **8o**, which was approximately 7-fold more potent on VEGFR-2 and approximately 11-fold more potent on EGFR compared to the prototype **7**. SAR and docking studies allowed the identification of pharmacophoric groups for both kinases and demonstrated the importance of a hydrogen bond donor at the *para* position of the aniline moiety for interaction with conserved **Glu** and **Asp** amino acids in EGFR and VEGFR-2 binding sites.

1. Introduction

Several cellular signaling pathways are involved in the evolution, aggressiveness and metastatic potential of malignant tumors. These diverse mechanisms generate heterogeneity, redundancy and the possibility for tumors to bypass a signal transduction blockade resulting in primary or acquired resistance [1]. Therefore, the multifactorial nature of cancer illustrates the need for multifunctional therapeutic tools, *e.g.* single compounds that can modulate different pathogenic pathways [2].

Protein kinases play important roles in the regulation of numerous cellular processes, including proliferation, differentiation and survival [3].

In particular, the epidermal growth factor receptor (EGFR, also called ErbB1, HER1) is a transmembrane receptor tyrosine kinase that belongs to the ErbB family [4], a four member family of tyrosine kinase growth factor receptors that include EGFR (HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4) [2]. Binding of a specific set of ligands/growth factors to the extracellular domain of ErbB receptors promotes dimerization and autophosphorylation, resulting in activation of the cytoplasmic tyrosine kinase domains and leading to downstream signaling pathways that regulate cell differentiation, growth, migration and apoptosis [4,5].

EGFR mediated signal transduction has been implicated in several human malignances by promoting growth, local invasion, angiogenesis and metastasis of tumor cells. EGFR overexpression and/or mutation are clinical features of numerous solid tumors and are usually associated with a poor prognosis [1,2,4]. EGFR signaling pathways also stimulate vascular endothelial growth factor (VEGF), which is the primary inducer of functional angiogenesis [6].

VEGF has also been described as the most common inducer of tumor angiogenesis. VEGF promotes endothelial cell activation, proliferation and migration and increases vascular permeability in

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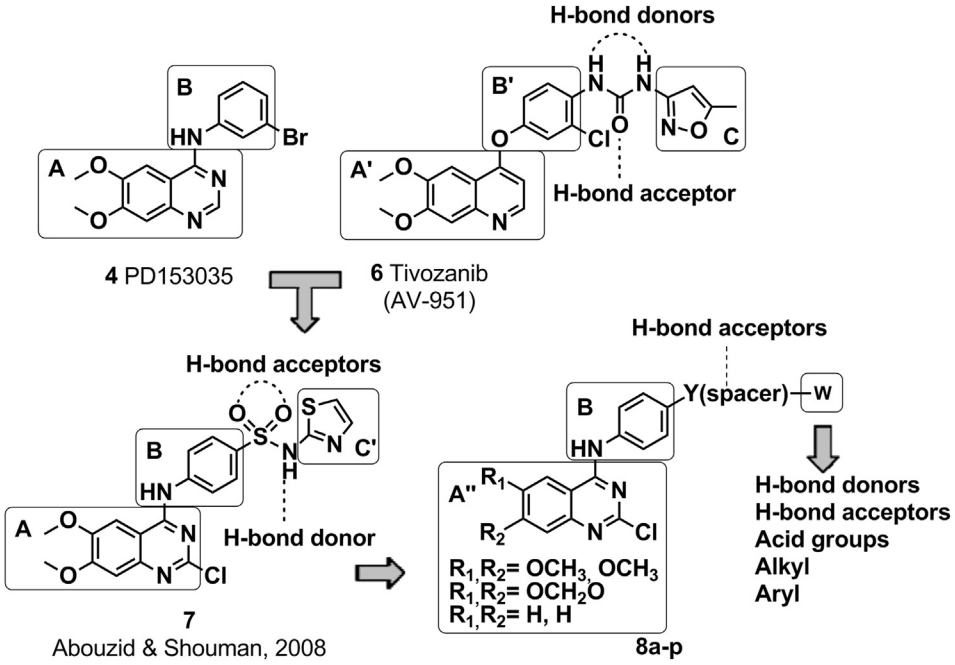

Fig. 1. Structural design of the target 2-chloro-4-anilino-quinazoline derivatives **8a**–**p**.

solid tumors. The key step in this process is mediated through a specific VEGF receptor, which is the kinase insert domain-containing receptor, also known as vascular endothelial growth factor receptor 2 (VEGFR-2) [7].

EGFR and VEGFR-2 are closely linked, sharing common downstream signal transduction pathways and playing key roles in tumor growth and angiogenesis [1]. Their functional relationship in cancer therapy is well known, *i.e.* inhibition of VEGFR-2 signaling pathway contributes to the antitumoral effect of EGFR inhibitors; whereas activation of VEGF expression independent of EGFR signaling is thought to be one of the resistance mechanisms to anti-EGFR therapy [1,8].

Therefore, tyrosine kinases EGFR and VEGFR-2 are validated targets in cancer therapy and several inhibitors have been approved by the FDA for clinical use in EGFR and/or VEGFR-2 overexpressing solid tumors, including the ATP-mimetic tyrosine kinase inhibitors (TKIs) (Chart 1) gefitinib (**2**) and lapatinib (**3**) for EGFR [2,3,5,6] and sorafenib (**5**) [9] for VEGFR-2.

Secondary resistance following the initial benefits of treatment with approved EGFR inhibitors remains a challenge in cancer therapy and demonstrates the need for the development of novel therapeutic alternatives [10]. Therefore, dual inhibition of EGFR and VEGFR-2 represents a promising approach to cancer treatment because VEGFR-2 inhibition increases the efficacy of EGFR TKIs by exerting a synergistic effect [8]. Combined VEGFR-2 and EGFR inhibition is currently being evaluated in several preclinical and clinical trials [8,10–12] and this novel therapeutic approach can be exemplified by the dual tyrosine kinase inhibitor vandetanib (**1**) (Chart 1), targeting EGFR and VEGFR-2, which was recently approved in April 2011 by FDA for treatment of late-stage metastatic medullary thyroid cancer [13].

Among EGFR TKIs either already approved by the FDA or currently in clinical trials, the 4-anilinoquinazoline class of compounds stands out. Many 4-anilinoquinazoline TKIs have been described in the literature and generally show a potent inhibitory effect *in vitro* at nanomolar concentrations. However, many of these compounds show limited activity *in vivo*. Therefore, several analogs of PD153035 (**4**), the most potent known inhibitor of EGFR (IC_{50} = 25 pM), have been developed through the insertion of solubilizing groups in an attempt to improve their aqueous solubility and, consequently, their oral bioavailability [4].

Aiming to identify novel inhibitors with different structural features, Abouzid and Shouman [14] recently described the synthesis of a new class of 2-chloro-4-anilino-quinazoline compounds designed as EGFR inhibitors and selected by virtual screening. In particular, compound **7** (Fig. 1) exhibited a potent anti-proliferative effect on the MCF-7 human breast carcinoma cell line over-expressing EGFR (IC_{50} = 0.13 nM). However, no inhibition data for EGFR was reported. We evaluated compound **7** and found it to be only a weak inhibitor of EGFR (Table 1).

Compound **7** differs from the first generation of EGFR inhibitors, including those commercially available (**2** and **3**), by the presence of a 2-chloro-quinazoline core containing a *para*-sulfonamide group at the aniline moiety with a heterocyclic substituent instead of ordinary halogenated rings. This provides several additional interaction points for binding to the therapeutic target, *e.g.* hydrogen bond donor and acceptors.

Hydrogen bonds involving *para*-substituents in the aniline moiety of EGFR inhibitors with the amino acids in the ATP binding site of this tyrosine kinase have not been extensively explored. However, this type of binding is known as an important feature for the selectivity and potency of several urea VEGFR-2 TKIs [7,9,15], such as sorafenib (**5**) and tivozanib (**6**) [9] (Chart 1), which form a hydrogen bond between the urea NH groups and the side chains of amino acids **Glu855** and **Asp1046** in the binding site of VEGFR-2.

Therefore, considering the great interest in associating EGFR and VEGFR-2 inhibition for cancer therapy and noticing the presence of structural features previously described as favorable for the interaction with VEGFR-2 binding site in compound **7** (Fig. 1) [14], this compound was selected to evaluate this structural pattern as a basis for the design of novel dual inhibitors of the tyrosine kinases EGFR and VEGFR-2, which are structurally and clinically related.

Starting from the structure of compound **7**, the 2-chloro-4-anilino-quinazoline scaffold was preserved in the design concept