European Journal of Medicinal Chemistry 102 (2015) 115–131

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Molecular design and synthesis of certain new quinoline derivatives having potential anticancer activity

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ARTICLE INFO

Article history:
Received 6 December 2014
Received in revised form
15 July 2015
Accepted 16 July 2015
Available online 17 July 2015

Keywords:
4-anilinoquinoline-3-carboxamide
EGFR-TK inhibitors
Pharmacophore
Docking study
Binding energy
Anti-proliferative activity

ABSTRACT

EGFR, which plays a vital role as a regulator of cell growth, is one of the intensely studied TK targets of anticancer inhibitors. The most two common anticancer inhibitors are anilinoquiazolines and anilinoquinolines that inhibit EGFR kinase intracellularly. The present investigation dealt with design (pharmacophore, docking and binding energy) and synthesis of a new series of 4-anilinoquinoline-3-carboxamide derivatives as potential anticancer agents targeting EGFR. All the newly synthesized compounds were screened for their anticancer activity against MCF-7 and compounds **4f, 7a** and **7b** showed significant activity with IC_{50} values 13.96 μM, 2.16 μM and 3.46 μM, respectively. Most of the synthesized compounds were subjected to enzyme assay (EGFR TK) for measuring their inhibitory activity with the determination of IC_{50} values and the preliminary results revealed that compound **7b**, which had potent inhibitory activity in tumor growth and had potent activity on the EGFR TK enzyme with 67% inhibition compared to ATP would be a potential anticancer agent.

1. Introduction

Cancer is a fatal disease. It is an abnormal growth of cells caused by multiple changes in gene expression leading to dysregulated balance of cell proliferation and cell death and ultimately evolving into a population of cells that can invade tissues and metastasize to distant sites, causing significant morbidity and, if untreated, death of the host [1].

Cancer is caused by changes in a cell's DNA. Some of these changes may be inherited from our parents (genetic factors, 5–10%), while others may be caused by outside exposures, which are often referred to as environmental factors (90–95%) [2–4]. The biological properties of malignant tumor cells involve acquisition of sustained angiogenesis, ability to invade neighboring tissues, ability to build metastases at distant sites and self-sufficiency in growth signals, and loss of sensitivity to anti-growth signals, capacity for apoptosis, capacity for senescence and capacity to repair genetic errors [5]. The goals of cancer treatment methods fall into three categories: curative, control and palliative; the most common modalities are surgery, radiation, chemotherapy, hormonal therapy, and biotherapy [6].

Drug design in the cancer therapeutics is developing a trend toward more precise mechanisms of cancer cell destruction thereby minimizing adverse effects incurred during the course of cancer treatment (nausea, vomiting, hair loss, fatigue, organ toxicity, etc.). The key to selectively targeting cancer cells is to exploit some basic difference these cells have developed compared to their normal precursors. One such difference is the activity of the enzyme telomerase, topoisomerase and protein kinases [7–10].

The complexity and the number of the protein kinases (PKs) being used as molecular targets in drug discovery have greatly increased. The sequencing effort of the human genome project has revealed that ~600 PKs and ~130 protein phosphates are probably present in the human genome [7]. About 30% of human protein contains covalently bound phosphate. Protein phosphorylation is considered as one of the main post-translated mechanisms used by cells to finally tune their metabolic and regulatory pathways. PKs catalyze the phosphorylation of serine (Ser), threonine (Thr), and tyrosine (Tyr) residues of proteins using ATP or GTP as the phosphate donor, while phosphatases are responsible for dephosphorylation, the opposite reaction [11,12].

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http://dx.doi.org/10.1016/j.ejmech.2015.07.030
0223-5234/

Tyrosine kinases (TKs) are one of the most widely studied and important kinase families with respect to cancer biology. In humans, there are around 90 distinct TKs, which can broadly be divided into: (a) 58 receptor tyrosine kinases (RTKs), e.g. EGFR, PDGFR, FGFR and the VEGF and (b) 32 non-receptor tyrosine kinases (NRTKs) [13], e.g. SRC, ABL and FAK kinase. RTKs form a large superfamily of receptor molecules on the plasma membranes of eukaryotic cells. RTKs are specifically activated by growth factors, such as EGF, VEGF, FGF, PDGF and many others [14,15]. A typical member of RTKs is a single-membrane-spanning protein consisting of extracellular ligand binding domain, a short membrane spanning α helix, and a cytoplasmic domain with TK activity. The intracellular kinase domains of RTKs can be further divided into those containing a stretch of amino acids separating the kinase domain, e.g. VEGFR and PDGFR, and those in which the kinase domain is continuous, e.g. EGFR and HER2/neu, [14–16]. EGFR family of receptors consists of four structurally related receptors, HER1 (EGFR/ErbB1), HER2 (ErbB2), ErbB3, and ErbB4 [17], for which a variety of different ligands have been characterized [18]. In response to extracellular growth factors, these receptors combine to form 1 of 4 possible homodimers (EGFR/EGFR, HER2/HER2, ErbB3/ErbB3 and ErbB4/ErbB4) or 6 possible heterodimers (EGFR/HER2, EGFR/ErbB3, EGFR/ErbB4, ErbB3/HER2, ErbB4/HER2 and ErbB3/ErbB4) [17–20].

EGFR can bind to several ligands including epidermal growth factor (EGF), transforming growth factor-α (TGF-α), betacellulin (BTC), epiregulin (EPR), heparin-binding EGF like growth factor (HB-EGF) and amphiregulin (AR) [21,22]. In absence of ligand, EGFR exist as monomers on the cell surface, while binding of ligand to EGFR leads to the formation of receptor homo- and heterodimers, depending on whether EGFR is dimerized with another EGFR or with other ErbB family members, respectively [22,23]. Two different EGFR dimer structures occur, “back-to-back” configuration, in which the two receptors are linked by the dimerization loops so that the associated ligands are located at opposite sites on the dimer, and “head-to-head” configuration, in which subdomain I of each receptor interacts with subdomain III of its dimeric counterpart, so that the ligands are located at the center of the dimer. The back-to-back dimer has better conformational symmetry, a wider interface between the receptors, and a more conserved amino acid sequence at the dimer interface than the alternative head-to-head dimer. Therefore, the back-to-back dimer is favored as the biologically relevant conformation [15].

Lapatinib, Anilinoquinazolines, also known as (GW-2016) had approved by FDA in 2007, as a dual inhibitor of EGFR and the closely related receptor ErbB2 (HER2). The latter receptor has been identified as an important therapeutic target in a number of cancers as it is overexpressed in around 20–30% of patients with aggressive breast cancer and other tumors. For this reason, Lapatinib is under clinical assays for several solid tumors [24,25]. Recently, many of anilinoquinazolines had been discovered as EGFR inhibitors, e.g. Refs. [26], and Allitinib [27,28].

Some of the anilinoquinolines **(I)** [29] act as anti-tumor agents by inhibiting CSF-1 kinase while few 3-cyanoquinolines **(II)** [30] developed as inhibitors of insulin like growth factor receptors (IGF−1R). A few 4-anilinoquinolines **(III)** [31] have been found to be TK inhibitors. HIK-272 [32] and EKB-569 [33] are also cyanoquinoline derivatives that inhibit irreversibly EGFR [34,35] [Fig. 1].

In continuing our work [36,37] strategy to develop new and potent antitumor agents, herein we carried out design, synthesis and biological evaluation of new class of quinoline-3-carboxamide derivatives as potent EGFR inhibitors with remarkable antitumor effect.

2. Result and discussion

2.1. Rationale and design

In this investigation, Lapatinib (IC_{50} = 10.8 nM) [38], and the biological active 3, 4, 6-trisubstituted quinoline, compound **(IV)** (IC_{50} = 0.65 μM) [39] were used as a reference compounds. The design of targeted compounds was derived from the structure optimization of these reference compounds, which depends on the reported SAR of 4-anilinoquinazolines and the molecular modeling studies.

2.1.1. The reported SAR of the proposed compounds

Quinazoline and quinoline-3-carbonitrile of Lapatinib and compound **(IV)**, were bioisosterically replaced with cyanoacrylamide in compound **4** or quinoline-3-carboxamide in compounds **6**, **7**, **8** and **9** to occupy the adenine region of ATP binding pocket. The H-bond formed via N3 of quinazoline ring in Lapatinib and the mediated water molecule was replaced by an H-bond between the cyano [40] in compound **4** or carboxamide substituent in the other compounds and the amino acid (THR 854) in the ATP binding site. The anilino moiety at position-4 was retained and a small hydrophobic group at its *p*-position was added to occupy the hydrophobic region, which not occupied by ATP. Different extended side chains at position-6 were designed to mimic that of reference compounds to occupy the sugar/phosphate region**.** The design included the incorporation of isoxazole or thiazole ring in some targeted compounds to mimic the furan ring of Lapatinib and the other strategy was the introduction of α, β-unsaturated ketone to other targeted compounds hoping to form covalent bond with EGFR TK enzyme [Fig. 2].

2.2. Molecular modeling studies

2.2.1. Field analysis

We carried out molecular field analysis to estimate the similarities between our proposed compounds and the reference ones. Field analysis had approved that there is a high similarities between the 4-anilinoquinazoline, 4-anilinoquinoline-3-carbonitrile and 4-anilinoquinoline-3-carboxamide moieties of Lapatinib**,** compound **(IV)** and our proposed compounds, respectively [Fig. 3].

2.2.2. Pharmacophore model development

The goal of this account is to develop 3D pharmacophore models based on the known EGFR inhibitors, which can correctly reflect the SAR of the existing EGFR inhibitors. Then, this model will be used as 3D search queries for searching the proposed compounds to identify new inhibitors of EGFR. The hit compounds will be subsequently subjected to filtering by Lipinski's rule of five [41], docking studies and binding energy calculations to refine the retrieved hits. Finally, the promising compounds will be synthesized and will be subjected to an *in-vitro* inhibitory assay against EGFR protein kinase and antitumor inhibitory activity [42].

2.2.2.1. Training set selection and conformational analysis. A set of 25 EGFR inhibitors were collected from different literature resources and were carefully chosen to form a training set which was based on the principles of structural diversity and wide coverage of activity range. The IC_{50} values of the inhibitors in the training set span a range of five orders of magnitude or more (IC_{50} values range from 29 pM to 2.7 μM) [Fig. 4]. All compounds were built in 2D/3D Visualizer within CATALYST4.1 and minimized to the closest local minimum using the CHARMm-like force field incorporated in the CATALYST program. A series of energetically reasonable conformational models, which represent the flexibility of each compound