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3D-QSAR CoMFA analysis of C_5 substituted pyrrolotriazines as HER2 (ErbB2) inhibitors

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Abstract

Human cancers are characterized by an up-regulation of some of the RTKs (EGFR and HER2) and have been clinically validated as targets for cancer therapy. C4 and C5 substituted pyrrolotriazines showed dual inhibition of HER2 and EGFR protein tyrosine kinases. To explore the relationship between the structures of the aforementioned classes of molecules and their HER2 inhibition, 3D-QSAR CoMFA analysis have been performed. The developed CoMFA model showed statistically significant results with good predictive ability.

Keywords: Receptor tyrosine kinase; EGFR; Human epidermal growth factor receptor; CoMFA

1. Introduction

Despite improvements in survival rates, cancer remains the second leading cause of death world wide. The epidermal growth factor receptor (EGFR, ErbB1 or HER1) and the human epidermal growth factor receptor2 (HER2, ErbB2) are members of the ErbB family of receptor tyrosine kinases (RTKs). HER2, like other EGFR members, is a transmembranous glycoprotein with intrinsic tyrosine kinase activity encoded by the HER2 protooncogene located on the long arm of chromosome 17. In normal cells, activation of this RTK family triggers a rich network of signaling pathways, which control normal cell growth, differentiation, motility, and adhesion in several cell lineages [1]. Many human cancers are characterized by an up-regulation of some of these RTKs and have been clinically validated as targets for cancer therapy. These receptors (except that of HER3) share the same molecular structure with an extracellular cysteine rich ligand binding domain, a single alpha-helix transmembrane domain, and an intracellular domain with tyrosine kinase (TK) activity at the carboxy-terminal tail [2]. The TK domains of HER2 and HER4 showed an 80% homology with that of EGFR members. Ligand binding induces EGFR homodimerization, as well as heterodimerization with other types of HER proteins. EGFR/EGFR homodimers are unstable, whereas EGFR/HER2 heterodimers are stable, and recycle more rapidly to the cell surface [3]. The dimerization activates the RTKs, which phosphorylate special tyrosine residues on proteins. These phosphorylated tyrosine residues initiate downstream multiple signaling pathways associated with cell growth (or differentiation) mainly including the Ras/MAP kinase pathway and PKB/Akt pathway. HER2 over expression is identified on many tumor cells and the relationship between HER2 status and clinicopathological characteristics in breast cancer has been well investigated [4]. Statistically, over expression of HER2 occurs in a number of human cancers, including 25–30% of breast cancer, 5–28% of pulmonary adenocarcinoma and 17% of colorectal aden carcinoma [5]. Its up-regulated expression is also related to rapid disease progression, chemoresistance, accelerated relapse as well as poor prognosis and mortality. Consequently, they have become targets of intense drug discovery efforts to identify novel anti-cancer agents. The frequent co-expression of HER2 and EGFR in variety of tumor types and their capacity to form heterodimers with other members of the ErbB family provided a strong rationale for simultaneously targeting both of these receptors. There are currently several small molecules of dual EGFR and HER2

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kinase inhibitors in clinical development which include: lapatinib (GW572016), AEE-788, 4 and BMS-599626. The latter utilizes the bicyclic pyrrolotriazine ring system as a scaffold for the construction of an ATP mimic [6]. Its lipophilic C_4 substituent provides potent and selective kinase inhibition while its C_6 solubilizing side chain imparts good potency and pharmacokinetics.

Review of literature shown *in silico* studies to understand the mode of action and the relationship between physico-chemical properties and the inhibitory activities of different kind of EGFR/HER2 inhibitors. Shi et al. performed QSAR analysis of tyrosine kinase inhibitors on 4-(3-bromoanilino)-6,7-dimethoxyquinazoline series using modified ant colony optimization and multiple linear regression method [7]. A receptor-guided alignment-based comparative 3D-QSAR studies was carried out by Kamath and Buolamwini on benzylidene malonitrile tyrphostins as EGFR and HER2 kinase inhibitors [8].

The structure–activity relationship of 5-methyl pyrrolotriazines with different C_4 substituents showed analogs with small C_4 anilines or bicyclic heterocycles and are selective EGFR inhibitors [9]. Appending a lipophilic benzyl group to the C_4 aniline increased HER2 kinase inhibition without reducing EGFR inhibition. To further explore the relationship between the structure of the a fore mentioned classes of molecules and their HER2 inhibitor activity, three dimensional quantitative structure–activity relationship (3D-QSAR) studies using comparative molecular field analysis (CoMFA) were performed.

2. Methodology

Comparative molecular field analysis (CoMFA): 3D-QSAR method CoMFA was introduced by Cramer et al. [10], in which an assumption is made that the interaction between an inhibitor and its molecular target is primarily non-covalent in nature and shape dependant. Therefore, QSAR may be derived by sampling the steric and electrostatic fields surrounding a set of ligands and correlating the difference in these fields to biological activity. CoMFA calculate steric field using Lennard–Jones potential and electrostatic field using Coulomb potential.

3. Computational details

3.1. Dataset for analysis

In vitro biological data of C_5 substituted pyrrolotriazine dual inhibitors of HER2 and EGFR protein tyrosine kinases (in human tumor xenograft models) reported by Mastalerz et al. was used [9] to construct CoMFA model and for analysis of physico-chemical features. The structure and experimental value of activity for the 32 molecules used in this study are shown in Table 1. The molecules under study were built using SYBYL7.1 [11] molecular modeling package installed on a Silicon Graphics Fuel Work station running IRIX 6.5. Since crystal structure of tyrosine kinase domain of HER2 is not available, and we presumed that its homology model may not be good enough for docking analysis, in order to obtain accurate bioactive conformation of the various ligands under study, in the present CoMFA analysis. Therefore, bioactive conformation of the molecules are computed by selecting, the basic skeleton and conformation of most active molecule **11** (IC_{50} value 0.018 μm), to which Gastaiger–Huckel charges were applied and systematic search were carried out to obtain low energy conformer in gaseous phase, which is subsequently energy minimized by Powell method using Tripos force field with 0.05 kcal/mol energy gradient convergence criterion. The rest of the molecules were built by changing required substitution on template molecule **11** and energy minimized as stated previously. These molecules were then used to construct 3D-QSAR model. The IC_{50} value in micro molar (μM) range were converted into molar (M) range and then its logarithmic scale (pIC_{50}, M) were then used for subsequent QSAR analysis (Eq. (1)) as the response variable

$$pIC_{50} = -\log IC_{50} \quad (1)$$

3.2. Molecular alignment

Molecular alignment is the most sensitive parameter in 3D-QSAR analysis. This renders the spatial alignment of molecules under study as one of the most sensitive and determining factors in obtaining robust and meaningful models. In the present study geometry optimized molecules were aligned on the template molecule **11** (Supplementary Fig. S1) by common substructure alignment using ALIGN DATABSE command in SYBYL. The common substructure used for alignment and superimposed structure after alignment is presented in Fig. 1.

3.3. CoMFA interaction energies

CoMFA steric and electrostatic potential fields were calculated at each lattice intersection on a regularly spaced grid of 2.0 Å units in *x*, *y*, and *z* directions on the aligned dataset. The pattern of 3D cubic lattice generated automatically by SYBYL/CoMFA routine, extended at least 4.0 Å beyond the volumes of all investigated molecules along these axes. The van der Waals potential and Coulombic terms, which represent

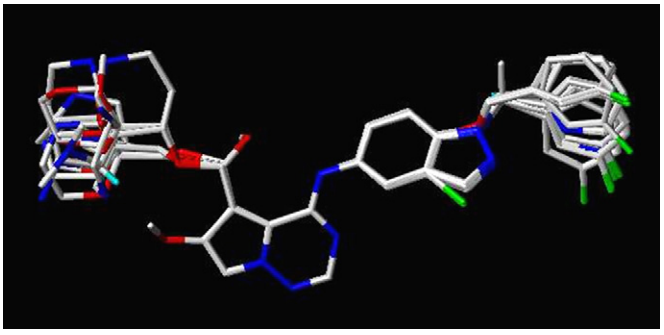

Fig. 1. Alignment of molecules.