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Research paper

Design, synthesis and structure-activity relationship studies of a focused library of pyrimidine moiety with anti-proliferative and anti-metastasis activities in triple negative breast cancer

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ABSTRACT

Triple-negative breast cancer (TNBC) is a clinical conundrum with distinct clinical and pathologic features, which is characterized by high aggression, poor prognosis, and lack of targeted therapies. In this study, based on the structural features of type II kinase inhibitors, we designed and synthesized a focused library of 41 pyrimidine derivatives possessing potent anti-proliferation activity, Y29 showed the most potent activity against MDA-MB-231 cells. Subsequently, we carried out target prediction, homology modeling, molecular docking, dynamics simulation and determination of enzymatic activity. The results suggested that PDGFR-β was its potential target. In vitro experiments revealed that Y29 attenuated metastasis by PDGFR-β inhibition-induced autophagy and could enhance autophagy-related cell death through AKT-MAPK feedback loop in MDA-MB-231 cells.

1. Introduction

Breast cancer is the most common malignancy in women, and one of the three most common cancers worldwide, along with lung and colon cancer [1]. Early breast cancer has been considered potentially curable with the development of therapeutic methods in the past years [2]. While Triple-negative breast cancer (TNBC), accounting for 10–17% of all breast carcinomas, a heterogeneous and aggressive disease, is still incurable because of its susceptible resistance and high metastatic properties [3]. TNBC is characterized by estrogen receptor (ER) negative, progesterone receptor (PR) negative and Receptor tyrosine-protein kinase erbB-2 (HER2) negative [4]. TNBC has distinct molecular features compared with other types of tumors. For instance, EGFR, c-Kit and p53 are usually overexpressed and TP53 gene mutations are also catholically observed in TNBC [5]. Additionally, p16, cyclin E and E2F3 are elevated in mRNA levels, while the levels of Rb and cyclin D1 are lower [6]. Currently, chemotherapy still plays a central role in the treatment of TNBC. In recent years, accumulating drugs have been exploited for the treatment of TNBC. Small molecules targeting aberrant DNA repair have been developed, such as PARP inhibitors (AZD2281; BSI-201) [7] and DNA transcription inhibitor (Trabectedin) [8]. Antiangiogenesis, Bevacizumab and Sunitinib have been applied to clinical [9]. EGFR targeting inhibitors showed potent antiproliferative activity, such as Cetuximab, Erlotinib [10,11]. Epigenetic modifications (Trichostatin A, Butyrate, Vorinostat) [12], Src inhibitor (Dasatinib) [13] and PI3K/Akt pathway (Everolimus) [14] also showed therapeutic potential. Despite the advanced development of abovementioned anticancer agents, since triple-negative breast cancer is susceptibly resistant to current targeting therapies and its highly metastatic property, clinical goals are mainly prolongation of survival and maintaining quality of life. TNBC is a clinical conundrum with distinct clinical and pathologic features, which is characterized by high aggression, poor prognosis, and lack of targeted therapies. It is considered as an opportunity to develop novel small molecule drugs with high anti-

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proliferative and metastatic activity for TNBC therapy.

Kinases have emerged as one of the most intensive classes of drug targets with approximately 30 various kinase targets being developed to the stage ready for clinical trials [15]. In recent years, a large number of small molecule kinase inhibitors with high anti-proliferation activity have been reported. Structurally, the pyrimidine ring was often used as a common skeleton in various kinase inhibitors (Fig. 1). Kinase inhibitors were generally divided into 4 types, Type I inhibitors, Type II inhibitors, allosteric inhibitors and covalent inhibitors. The type I inhibitors could recognize the active conformation of the kinases. Type I inhibitors typically have a heterocyclic ring system that occupies the purine binding site, which is commonly substituted by two hydrophobic groups that occupy the adjacent hydrophobic regions I and II. The type I inhibitors have poor selectivity and result in drug resistance. The type II kinase inhibitors could recognize the inactive conformation of the kinases. Movement of the activation loop to the DFG-out conformation exposes an additional hydrophobic binding site directly adjacent to the ATP binding site. The type II kinase inhibitors show low resistance and high selectivity, and have emerged as the focus of the development of kinase inhibitors, such as Imatinib, Lapatinib, sorafenib, Nilotinib and infigratinib [16]. The allosteric inhibitors only bind to the allosteric site, outside the ATP-binding site, which exhibit high degree of kinase selectivity. The covalent inhibitors can form an irreversible, covalent bond to the kinase active site, most frequently by reacting with cysteine residue. But the covalent inhibitors have special requirements on the structure of the target protein, which results in a small range of application.

In this study, based on the structural features of type II inhibitors (Fig. 1), we designed and synthesized a series of different pyrimidine derivatives to inhibit the anti-proliferation of triple negative breast cancer cells (Fig. 2). Firstly, the pyrimidine moiety was utilized as a scaffold to initiate the interactions with kinase hinge as adenine did. Then a substituted aromatic ring was introduced to the 2-or 4-position to occupy the hydrophobic pocket I and a

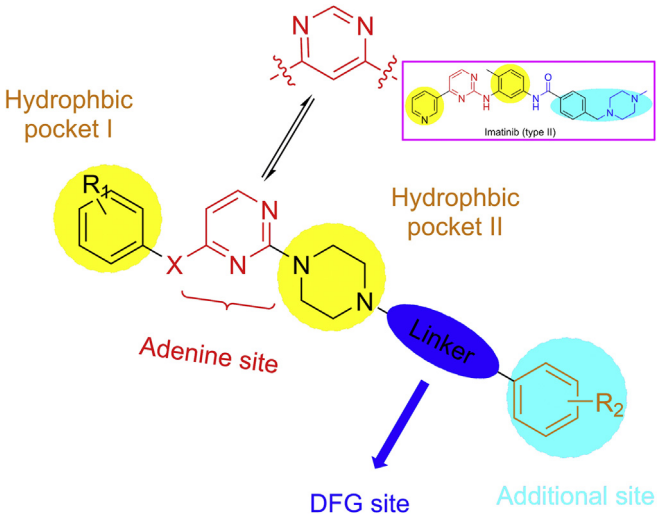

Fig. 2. Design of Type II Kinase inhibitors with pyrimidine moiety.

piperazine group was incorporated into the pyrimidine scaffold to occupy the hydrophobic pocket II. Next, a urea or amide group was used as a linker to interact with the conformation of DFG out, resulting in exposing an additional hydrophobic binding site. Finally, a substituted aromatic ring was induced to occupy the additional hydrophobic binding site. After several rounds of structural optimization based on anti-proliferative activity, a focused library of 41 pyrimidine derivatives was synthesized, which possessed medium to potent anti-proliferation activity, **Y29** showed the most potent activity against MDA-MB-231 cells. Subsequently, we carried out the target prediction, homology modeling, molecular docking, dynamics simulation and determination of enzymatic activity. The results suggested that PDGFR-β was its potential target. The results suggested that PDGFR-β was

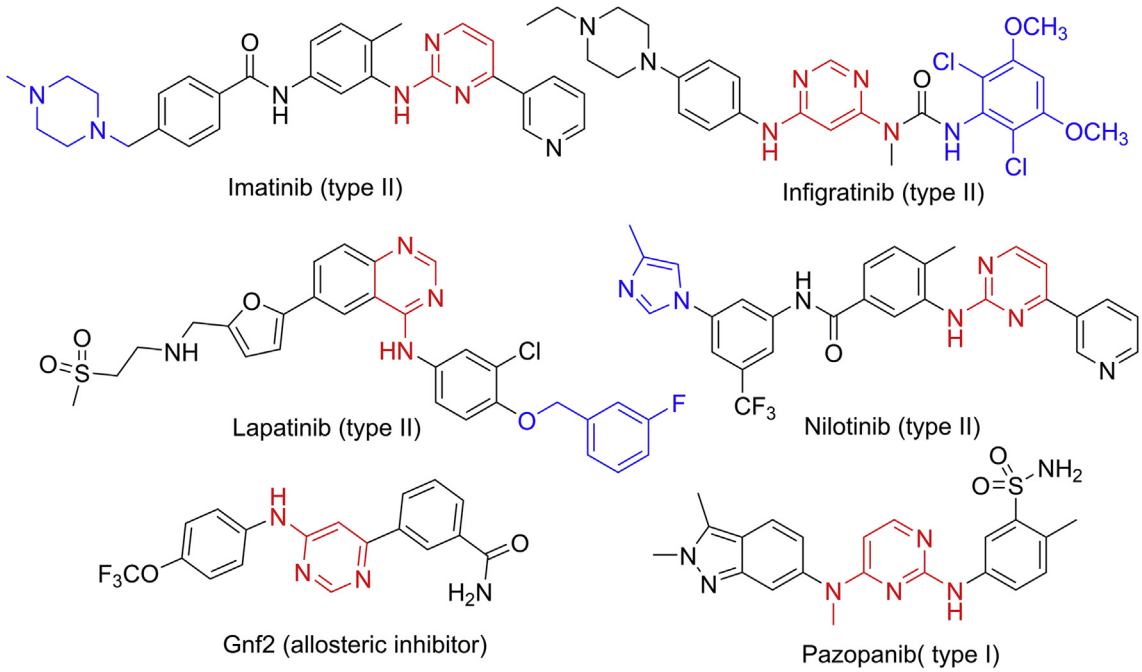

Fig. 1. The structures of several classic anticancer agents with a pyrimidine moiety. The pyrimidine moiety was marked by red, which served as a scaffold binding to the kinase hinge. Compared to type I inhibitors, type II inhibitors have an additional hydrophobic group (marked by blue) that occupy the additional binding site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)