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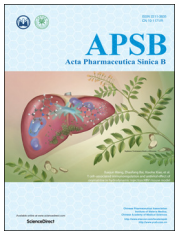

ORIGINAL ARTICLE

Inhibition of protein kinases by anticancer DNA intercalator, 4-butylaminopyrimido[4′,5′:4,5] thieno(2,3-*b*)quinoline

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Abstract Targeting protein kinases (PKs) has been a promising strategy in treating cancer, as PKs are key regulators of cell survival and proliferation. Here in this study, we studied the ability of pyrimido [4′,5′:4,5]thieno(2,3-*b*)quinolines (PTQ) to inhibit different PKs by performing computational docking and *in vitro* screening. Docking studies revealed that 4-butylaminopyrimido[4′,5′:4,5]thieno(2,3-*b*) quinoline (BPTQ) has a higher order of interaction with the kinase receptors than other PTQ derivatives. *In vitro* screening confirms that BPTQ inhibits VEGFR1 and CHK2, with the IC_{50} values of 0.54 and 1.70 μmol/L, respectively. Further, cytotoxicity of BPTQ was measured by trypan blue assay. Treatment with BPTQ decreased the proliferation of HL-60 cells with an IC_{50} value of 12 μmol/L and induces apoptosis, as explicated by the fall in the mitochondrial membrane potential, annexin V labeling and increased expression of caspase-3. Taken together, these data suggest that BPTQ possess ability to inhibit PKs and to induce cell death in human promyelocytic leukemia cells.

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1. Introduction

Cancer is the major cause of death worldwide. Mutations in the key regulatory genes like proto-oncogenes, tumor suppressor genes, and DNA repair genes are often lead to cancer[1–3]. The basic hallmarks of the cancer, such as the uncontrolled growth, survival, neo-vascularization, metastasis, and invasion, are mainly due to the perturbation of the regulatory signaling pathways[4]. The increasing understanding of molecular pathways altered in cancer diseases has resulted in the development of more specific anticancer agents, referred to as targeted therapies[5,6]. These targeted therapies include the use of monoclonal antibodies and small molecule inhibitors of protein kinases (PKs), which have emerged as promising targets for cancer therapy among several other novel targets identified[7,8].

PKs are key regulators of signal transduction pathways, mediating vital functions, such as cell proliferation, differentiation, and programmed cell death[9]. Also, a number of PKs are associated with different forms of cancers, and plays a prominent role in carcinogenesis. This occurs due to the aberrant activation of PKs, which induce anti-apoptotic effects and promote angiogenesis and metastasis[7,8]. Therefore, inhibitors of these oncogenic PKs are considered as potential anticancer molecules. One of the essential tools in the screening and development of these inhibitors is through structure-based drug design, using *in-silico* analysis and computational chemistry. Over the last several years, studies have yielded many small-molecule tyrosine kinase inhibitors, including numerous quinazoline (saracatinib) and pyrimidine (imatinib) derivatives[10,11]. These drugs act by interacting with their target kinases and blocking its catalytic activity, and are now being used as active candidates in the treatment of a wide range of malignancies like breast, colorectal, lung and pancreatic cancers, lymphoma, leukemia, and multiple myeloma[7,8,11]. Though these molecules possess effective antimalignant activity, we are still in dark to overcome all the tumor forms because of the acquirement of drug resistance. Hence, it is of higher importance to search for novel anticancer compounds targeting kinases to combat cancer.

Earlier in our laboratory, we have studied the ability of pyrimido [4′,5′:4,5]thieno(2,3-*b*)quinoline (PTQ) with amino (APTQ), morpholino (Morpho-PTQ), methoxy (Methoxy-PTQ), oxo (OPTQ) and butylamino (BPTQ) substitutions (Fig. 1) to interact with DNA, and to inhibit cancer cell proliferation[12–16]. Our studies clearly showed that these PTQ derivatives are effective DNA intercalators. Some of the DNA-intercalating anticancer compounds, such as mitoxantrone and anthraquinone derivatives, have displayed kinase inhibitory activity, paving the way for new targets of DNA intercalators[17,18]. It is reported that kinase inhibitory ability will contribute to increase the therapeutic efficacy of DNA binders[17]. Therefore, in this report, we sought to analyze these PTQ derivatives for their ability to inhibit different PKs by performing molecular docking and *in vitro* kinase screening. Results suggest that, 4-butylaminopyrimido[4′,5′:4,5] thieno(2,3-*b*)quinoline (BPTQ) possessed higher interactive ability with the kinases among all the molecules. *In vitro* screening of BPTQ on ten different PKs confirmed that the BPTQ possess inhibitory activity against VEGFR1 and CHK2. Further, cytotoxicity studies revealed that BPTQ induces mitochondrial mediated apoptosis in human promyelocytic leukemia HL-60 cells.

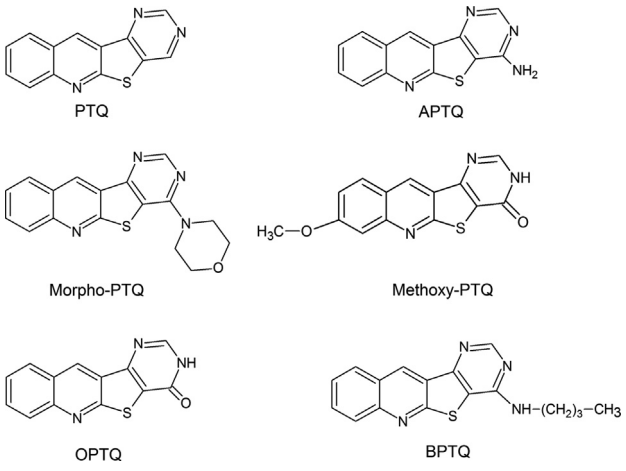

Figure 1 Structures of test compounds used for the study.

2. Materials and methods

2.1. Ligand and receptor preparation

To perform docking studies, ten different kinases receptors (Table 1) were retrieved from the Protein Data Bank (http://www.rcsb.org/). Ligands and water molecules were removed using PyMol molecular visualization software from the retrieved receptor. The three dimen sional chemical structure of standard drug, staurosporine was retrieved from the PubChem (https://pubchem.ncbi.nlm.nih.gov/). The two-dimensional (2D) structure of the test molecules (ligands) was drawn using the software ACD/Chemsketch. This was further followed by hydrogen addition and conversion to 3D structure. All the obtained sdf and mol format files were converted into pdb format files using the Open Babel software.

2.2. Virtual screening by molecular docking

Docking was performed using Hex 6.3 software[19] between the kinases (receptor) and test or standard drugs. To identify the interaction between the receptor and drug, the best solution binding mode is selected out of 2000 possible solutions. Following are the parameters which are used in the controls to perform docking: correlation type: shape + electrostatics; FFT mode: 3D; post processing: MM minimization; grid dimension: 0.4; solutions: 2000; receptor range: 180; step size: 7.5; ligand range: 180; step size: 7.5; twist range: 360; step size: 5.5; distance range: 40; scan step: 0.8; steric scan: 18; final search: 25. After completion of docking calculations, respective energy values were noted and complex structures of both receptor and drug were saved, and visualized in the PyMol molecular visualization software for interactions.

2.3. Reagents

All chemicals unless otherwise mentioned were purchased from Sigma–Aldrich (USA). Fetal bovine serum was obtained from Gibco (USA). Penicillin, streptomycin, annexin V–fluorescein isothiocyanate (FITC) apoptosis detection kit, and propidium iodide were purchased from Invitrogen (USA).

2.4. In vitro primary kinase profiling and IC_{50} determinations

In vitro kinase assays were carried out by performing a radioactive filter binding assay using [γ-^{33}P] ATP in a 96-well format[20,21]. The inhibition potential of BPTQ was tested on ten different PKs, such as aurora A, aurora B, checkpoint kinase 1 (CHK1), checkpoint kinase 2