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Design, synthesis and anticancer studies of novel aminobenzazolyl pyrimidines as tyrosine kinase inhibitors

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ABSTRACT

Abnormal signalling from the Protein tyrosine kinases (PTKs) like receptor tyrosine kinases and intracellular tyrosine kinases can lead to diseases such as cancer especially non-small cell lung cancer, chronic myeloid leukaemia and gastrointestinal stromal tumours. Various Protein tyrosine kinase inhibitors are available but face poor bioavailability, severe toxicities and recent cases of drug-resistant cancers prompts for development of better drug molecules. In this study we report the design and development of a novel Protein Tyrosine Kinase (PTK) inhibitor on the basis of pharmacophore modelling. Compound 2-(benzo[*d*]oxazol-2-ylamino)-*N*-(2-chloro-4-fluorophenyl)-4-methyl-6-(3-nitrophenyl) pyrimidine-5-carboxamide **31** was obtained containing essential pharmacophore structural features. This compound exhibited highest activity against leukaemia cell line (RPMI-8226) at 0.7244 μM, renal cancer cell line (A498) at 0.8511 μM and prostate cancer cell line (PC-3) at 0.7932 μM on the NCI five dose assay test. The PTK assay provides promising activity at IC_{50} of 0.07 μM in the human breast cancer cell line MDA-MB-468. Compound **31** had good intermolecular interaction with PTK in the molecular docking studies, this ligand-enzyme complex was found to stable in the MM-PBSA study over 100 ns. It had 54.22% oral bioavailability with T_{max} of 0.60 h which is higher compared to the dasatinib with bioavailability and T_{max} of 14–34% and 1–1.42 h respectively. Anticancer action of **31** was found to be impressive in pharmacokinetic studies making it a potential lead molecule.

1. Introduction

Protein tyrosine kinases (PTKs) are responsible for the regulation of proliferation, differentiation and signalling processes in cells of the immune system. These are classified broadly into transmembrane receptor linked kinases and cytoplasmic kinases [1]. Any abnormal signalling from receptor tyrosine kinases and intracellular tyrosine kinases can lead to diseases such as cancer especially non-small cell lung cancer, chronic myeloid leukaemia and gastrointestinal stromal tumours [2]. This enzyme plays a very crucial role in cancer aetiology and hence has attracted major attention since a long time. Recent reports on the development of resistance to chemotherapeutic agents due to single point mutations, altered metabolism, induction and expression of membrane transporters causing pumping out of drug from intracellular domain, changes in glutathione system calls for the development of more efficient inhibitors with less toxicity [3,4]. Inhibitors that blocks the activity of PTK and its activated unregulated signalling pathways can provides a useful basis for designing new drug candidates [5].

Drugs such as nilotinib **9**, dasatinib **7** are used as kinase inhibitors (Fig. 1), they act by irreversibly inhibiting the human epidermal growth factor receptor 2 (Her2) and epidermal growth factor receptor (EGFR) kinases [6]. PTK inhibitors (PTKIs) have several toxicities other than classical ones like diarrhoea and myelotoxicity. Gefitinib **4** and Erlotinib **5** show skin toxicity, rashes and eruptions. Imatinib **8** has severe toxic effect on the heart and other cardiac toxicities, Sunitinib **6** leads to disturbance in the metabolic haemostasis of heart. Many of the PTKIs suffers from issues like variable bioavailability due to poor or erratic absorption, poor aqueous solubility, drug interaction, membrane transport and tissue permeability [7]. Thus, development of newer drug molecules with lower toxicity and better oral bioavailability is needed for this target. To overcome these drawbacks, it would be clever to discover such anticancer agents that Fcan act selectively, have better

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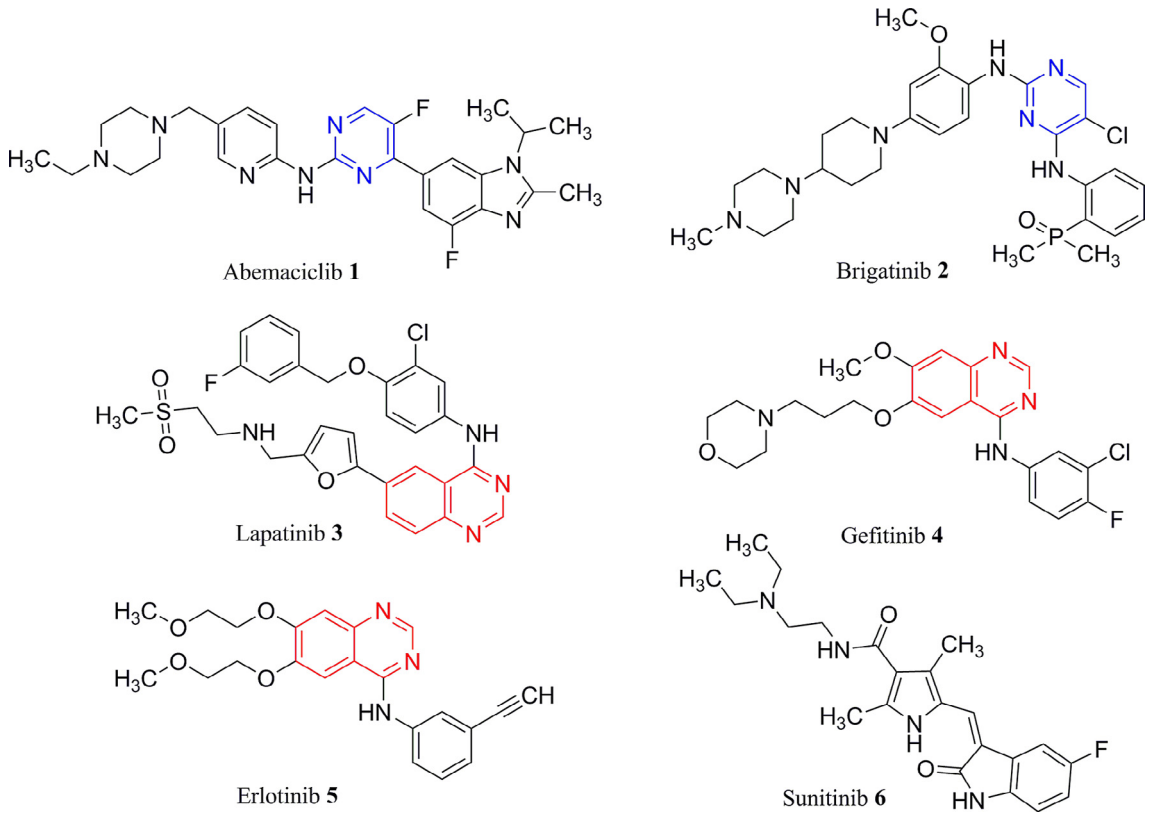

Fig. 1. Protein kinase inhibitors in therapy, these are representative compounds therapeutically used and FDA approved anticancer agents; Abemaciclib **1**, brigatinib **2**, lapatinib **3**, gefitinib **4**, erlotinib **5**, sunitinib **6**. Protein kinase inhibitors generally consist of pyrimidine (blue coloured) and quinazoline (red coloured) scaffolds.

oral bioavailability and should be less toxic. In pursuit of these goals we tried to develop certain novel aminobenzazolyl pyrimidines using pharmacophore modelling and structure based drug design methods. Aminobenzazoles such as aminobenzothiazole, aminobenzoxazoles and benzimidazoles in conjugation or as hybrids with pyrimidines have been reported as aurora B kinase inhibitors [8], as Rho Kinase inhibitors [9,10] and Bcr-Abl kinase inhibitors [11].

2. Results and discussion

To achieve our goal of developing a PTKI, we planned this investigation consisting of structure based design, synthesis, *in vitro* and *in vivo* studies. The structure based design included computational study with various steps starting with retrieval of crystal data. On the basis of reported crystal structures of PTK inhibitor a 3D pharmacophore model describing major chemical features was generated. New hypothesis was obtained by merging pharmacophore models which helped further in designing a synthetic scheme. On the basis of pharmacophoric features and manual observations, several molecules were designed, synthesized and evaluated for their anticancer activity.

2.1. Generation of structure based pharmacophore model

The generation of structure based pharmacophore model was initiated with the help of LigandScout software [12–14]. It is a tool that enables to create pharmacophore models from available crystal structures and structure-ligand complexes. It allows identifying excluded volume spheres, inaccessible areas and possible steric restriction. The ligand bound PTK crystal structures were used as input for pharmacophore generation. Pyrimidine containing compounds bound to tyrosine kinase were selected (Fig. 2); dasatinib **7** (PDB: 2GQG) [15], imatinib **8** (PDB: 4CSV) [16] and nilotinib **9** (PDB: 3CS9) [17] for development of the structure based pharmacophore. All the three drugs mentioned above have specificity for the Bcr-Abl tyrosine kinase. They are specifically used for the treatment of chronic myelogenous kinase inhibitors and Philadelphia chromosome-positive acute lymphoblastic leukaemia. A 3D pharmacophore hypothesis representing main interactions between inhibitor and the enzyme were obtained by automated structure based model generation. These interactions are explained in Fig. 2-panel 7a, b and c, it shows dasatinib, pharmacophore model and 2D representation respectively. It consists of four hydrogen bond features; (i) two hydrogen bond acceptor binding to residue Met318 and water molecule HOH623, (ii) two hydrogen bond donors pointed towards Thr315 and Met318. It also has two hydrophobic pockets shown as faint yellow spheres, first pocket formed by the chloro substituted phenyl ring and it was lined by residues Ala380, Val299, Met290, Ile313 and Val270. The second pocket was formed by pyrimidine ring lined by residues Phe317 and Leu248.

Fig. 2-Panel 8a, b and c show imatinib, pharmacophore model and 2D representation respectively. It has five hydrogen bond features; (i) three hydrogen bond acceptors pointed towards Met84, Asp147 and HOH 1014, (ii) two hydrogen bond donor points at Thr81 and Asp147. In case of imatinib the pharmacophore shows four hydrophobic pockets for pyridine, pyrimidine and two phenyl rings lined by Ala35, Met84, Leu14, Tyr83, Leu136, Phe148, Val22, Thr81 and Ile55. Fig. 2-Panel 9a, b and c shows nilotinib, pharmacophore model and 2D representation respectively. It exhibits four hydrogen bond features; (i) three hydrogen bond acceptor pointed