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Design, synthesis and molecular modeling studies of few chalcone analogues of benzimidazole for epidermal growth factor receptor inhibitor in search of useful anticancer agent

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ABSTRACT

In the present investigation, few 3-(substitutedphenyl)-1-[2-(1-hydroxy-ethyl)]-1*H*-benzimidazol-1-yl) prop-2-en-1-ones are EGFR antagonist are designed, by molecular docking analysis. The synthesized compounds were tested for their *in vitro* anticancer activity by *propidium iodide fluorescent assay* and *Trypan blue viability assay* against colorectal cancer cell lines (HCT116) and non-small cell lung cancer cell lines (H460). Human Epithelial Kidney cell lines (HEK) are used as normal cell lines for studying effect of drug on non-cancerous cells within human body. Evaluation of cytotoxic studies of synthesized compounds CHL(1–8) reveal that compound CHL1 [IC_{50} = 7.31 and 10.16 μM against HCT116 and H460 cell lines respectively, by PI assay] and CHL8 [IC_{50} = 12.52 and 6.83 against HCT116 and H460 μM cell lines respectively] possess promising cytotoxic activity.

1. Introduction

Tyrosine kinase is one of the subtypes in enzyme family of protein kinases. It catalyzes the transfer of phosphate groups from adenosine triphosphate (ATP) to amino acid residues in proteins. Enzyme tyrosine kinases transfer phosphate groups from amino acid, *i.e.* only tyrosine, to amino acid residues—threonine and serine. Phosphorylation of proteins is a basis of signal transduction, and is known to control cellular functions, including cell division too. Thus, Tyrosine kinase serves as "on/off" button in many functions of the cell. Protein tyrosine kinase, if happen to be mutated, is fixed in the 'on' state and results in uncontrolled cell growth and thus develops cancers, particularly colon cancer, breast cancer, lung cancer and bladder cancers (Bhuva and Kini, 2010).

Tyrosine kinases can be classified into two classes: receptor tyrosine kinases (Transmembrane) and non-receptor tyrosine kinases (cytoplasmic proteins). The epidermal growth factor receptor (EGFR: Her1 and ErbB1) is the receptor tyrosine kinase and is a validated target for the therapy of non-small-cell lung cancer (NSCLC), the gastrointestinal stromal tumor (GIST), colon cancer and breast cancer. Inhibiting the intracellular tyrosine kinase domain of the receptors by small molecules posit a working strategy for EGFR kinases-targeted anticancer drug design (Zhong et al., 2009a).

Hence, trials to selective inhibition of EGFR protein tyrosine kinase would lead to molecular structures which would further provide a feeble approach for developing newer cytotoxic agents.

Past decade saw the discovery and emergence of the 4-anilinoquinazolines as most developed class of potent and selective ATP-competitive inhibitors of EGFR. Gefitinib, Erlotinib and Imatinib are such small-molecule tyrosine kinase inhibitors that inhibit the EGFR for the treatment of NSCLC, and colon cancer (Gambacorti, 2008). These drugs are clinically used and their analogs are being studied extensively to circumvent the resistance generated by prolonged usage of these drugs. The study furthers to provide opportunities for improving drug efficacy, reducing safety concerns; new drug candidates in this class have already reached various phases of clinical trials (Deininger and Druker, 2003; Vigneri and Wang, 2001; Weisberg et al., 2007).

Chalcones are secondary metabolite precursors of flavonoids and isoflavonoids, which are commonly found in edible plants. These comprise one of the main classes of naturally occuring small

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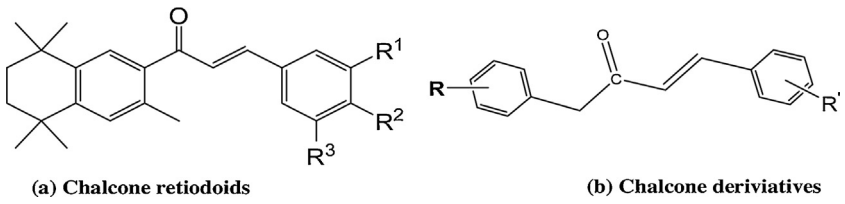

Fig. 1. Structures of chalcones derivatives having anticancer activity.

molecules with very promising anticancer activity. Several research groups have focused on the antitumor activity of this class of compounds. There are a number of reports on the activity of chalcones against several cell lines including prostate 1 and breast cancer 2 in low nanomolar concentrations (Lawrence and McGown, 2005; Zhou et al., 2009; Modzelewska et al., 2006).

Novel compounds of chalcone- retinoids (Fig. 1(a)), have been synthesized and evaluated for their cytotoxic activity against HT-29, a colon cancer cell-lines (Shiby et al., 2010; Ducki, 2007). Chalcone analogues consisting of substituted benzene with groups such as methoxy, halogens, and hydroxyl, result in molecules with potent anticancer activity against colon cancer cell lines exhibiting IC_{50} value below 1 μM (Cassia et al., 2010).

Another research group reported compounds carrying hydroxyl substituent that reduced ascites tumour in animals (Anto et al., 1995; Vogel et al., 2008).

Benzimidazole scaffold is a useful structural motif for displaying potent biological activities, like antimicrobial activity (Pawar et al., 2004), antifungal activity (Sparatore et al., 1968) antihistamine activity (Ryuichi et al., 1987; Iemura and Kawashima, 1986) antiprotozoal activity(Gabriel et al., 2006; Andrzejewskaa et al., 2004). *Antivira* (Brude and Bukharin, 1969) antiamoebic (Sondhi et al., 2002), hypotensive activity (Frinkelstein and Kromer, 1960), antipyretic, analgesics, anti-inflammatory activities (Sham et al., 2002; Thimme et al., 2009) antitumor (Nour and Mansour, 2011). Several derivatives of benzimidazole are synthesized and tested for evaluating their anticancer activity (Fig. 2) (Luo et al., 2011; Zhu et al., 2008).

Owing to the utility of chalcones and benzimidazole as cytotoxic, described in preceding description, it was decided to use these pharmacophore to develop a new series of compounds that would possess similar activities with better tolerance.

2. Results and discussion

2.1. In silico molecular docking simulations for design of novel EGFR kinase inhibitors

X-ray crystallographic studies reveal that imatinib binds EGFR kinase receptor *via* six hydrogen bond interactions. This stabilizes the imatinib complex and prevents ATP from reaching its binding site. The hydrogen bonds involve the pyridine-N and backbone-NH of Met-318, the aminopyrimidine and side chain hydroxyl of Thr-315, the amide-NH and side chain carboxylate of Glu-285, the carbonyl and backbone-NH of Asp-381, the protonated methyl-piperazine with the backbone-carbonyl atoms of Ile-360 and His-361. Additionally, a number of *van der Waals* interactions contribute to binding. A hydrophobic pocket is formed by the aromatic rings in amino acid residues Ile-293, Leu-298, Leu-354 and Val-379 around the phenyl ring adjacent to the piperazinyl-methyl group in imatinib (Zhong et al., 2009b) Fig. 3.

With a desire in mind to synthesize molecules capable in ameliorating pains suffered by cancer patients, compounds having functionality to display above described interactions with specific sites on the causative enzyme were designed. These were conceived to possess the elements responsible for the observed interactions. Accordingly, several benzimidazole analogues were tested for their *in silico* affinity for EGFR tyrosine kinases. Molecular docking simulations were performed in to the active site of EGFR kinases by using Auto-dock Vina (version). Ligands which bind at similar pharmacophoric points, as those are bound by established inhibitor imatinib, having smaller scoring functions (binding energy) and also synthetically feasible [CHL(1–8)] were selected for synthesis and anticancer testing.

Crystal structure of EGFR kinase was selected from the Protein Data Bank (PDB ID: 2HYY) "Human Abl kinase domain in complex with imatinib" with a resolution of 2.40 A° (Cowan-Jacob et al., 2007). The protein preparation step involved removal of bound ligand and water molecules. The protein was energy minimized using steepest descent method and simulated under water body solvent system in GROMACS for 01 ns (Pronk et al., 2016). The active site was generated from hydrogen-containing protein pdb file by keeping the default parameters in Discovery Studio Visualizer 4.1 (Accelrys Software Inc., 2013) and validated by Auto-dock 4.2 (Morris et al., 2009) and CastP server (Liang et al., 1998). The ligands were first drawn in Chem draw and optimize using Universal Force Field (Rappe et al., 1992). Finally the molecular docking exercise is carried out using evolutionary genetic algorithm in Auto-dock Vina version (Trott and Olson, 2010; Blair et al., 2007). The obtained binding energies (Dock scores) are represented in *KJ/mol*; smaller dock scores represent strong affinity for the receptor.

The Cleaned, energy minimized and simulated structure under the Gromacs at 01 nano second revealed a potential energy of −139340 kj/mol (Fig. 4a and b).

The validated active site revealed the following residue with a volume of 1774 A area and 2798.1 A volume (Fig. 5) with the following amino acid residues in it.

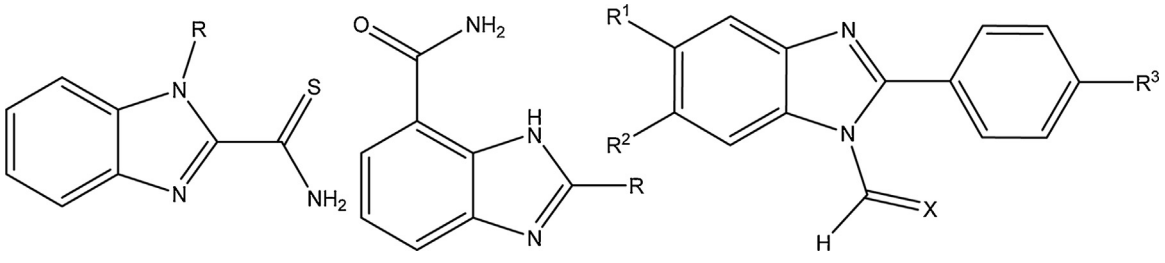

Fig. 2. Structures of benzimidazole analogues having anticancer activity.