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Research Article

SYNTHESIS, CHARACTERISATION AND IN SILICO ANALYSIS OF 4-(4- (DIMETHYLAMINO)PHENYL)-6-ETHYL-1,3-DIPHENYLPIPERIDIN 2-ONE AS AN INHIBITOR OF COLON CANCER

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ABSTRACT

Aim: To evaluate the potential of the currently synthesized molecule for its biological significance with reference to anticancer activity. Cancer is one of the most feared and deadliest diseases in the world. The drugs for this disease are still being framed for different cases. CDK5 is found to have significant role in various diseases like neuronal cell survival and death, migration and also cancer. Small molecules as ligands of natural origin and those synthesized form laboratories are on the rise. Methods: The present work deals with the computational analysis of a synthetic compound as a ligand with anticancer activity. The molecule is analysed for its druggable property and biological significance using several softwares. The molecule was docked with the receptor protein bearing the PDB ID 1UNG. The pharmacophore features are also analysed. Results: The computational analysis show that the molecule possesses anticancer activity and the work has been discussed.

Keywords: CDK5, piperidone, inhibitor, docking, pharmacophore.

INTRODUCTION

Cyclin-dependent kinase is found in Saccharomyces cerevisia to human they are highly expressed in Mitotic cells. In human there are 13 different CDKs CDK1, CDK2- CDK 13. Cyclin-dependent kinase 5 (CDK5) is a proline-directed serine/threonine kinase, that are important for mitotic cell division.[1] CDK5 is not directly involved in cell cycle regulation. It plays a major role in cytoskeletal dynamics, signalling cascade, gene expression and cell survival etc. Among the cyclin-dependent kinase (CDK) family, CDK5 is an unusual member with specific functions. Though CDK5 is ubiquitously expressed, previous studies about CDK5 were mainly focused on neuronal origin. Unlike other mitotic CDKs, CDK5 is activated by binding to P35 or P39.[2] In the central nervous system, CDK5 has been proved as a key regulator of neuronal migration, synaptic activity and neuronal cell survival and death.[3,4,5] Over the past decade, an increasing body of evidence has suggested that CDK5 may also have a significant role in the tumorigenesis of multiple organs, such as breast cancer, pancreatic cancer and neuroendocrine thyroid carcinoma.[6,7] However, the knowledge on the role and underlying mechanism of CDK5 in CRC remains poorly unknown. Inhibition of cyclin-dependent kinase 5 (CDK5) activities has recently been suggested as potential new therapeutic target in several malignancies. Small molecules playing the role if inhibitors has been discussed of late(8-12). In addition, the primary cause of chemotherapy failure and is the reason for the continued high mortality rate, especially of advanced-stage, metastatic cancers.[8] Many mechanisms have been identified that cause or contribute to cancer MDR. One of the best known and most extensively studied is the active efflux of anticancer drugs out of tumor cells by promiscuous transmembrane proteins called ATP-binding cassette (ABC) transporters.[9] CUR can inhibit the activity of P-gp, BCRP and MRP1, and has been shown to also inhibit MRP5, a transporter implicated in pancreatic cancer resistance to gemcitabine and 5-fluorouracil therapy.[10,11] It is reported that a high level of EGFR kinase enzyme is overexpressed in several tumours such as those in colon, prostate, breast, HeLa, HepG2, and non-small lung cancers.[12,13,14]

In this arena, synthetic small molecules also find their way as inhibitors of the activity of proteins and macromolecules.[15] The small molecule visualized by us to play the role of inhibitor is a new tri phenyl piperidinone derivative, was synthesized, characterized and subjected to the in silico studies. This paper discusses new strategies for targeting Cdk5 and its downstream mechanisms as anti-cancer treatments.

MATERIALS & METHODS

Mechanism of Preparing the Compound 4-(4-(Dimethylamino)Phenyl)-6-Ethyl-1,3-Diphenylpiperidin -2-One

Tumor targeting tracers are on the rise with the advances in molecular biology and biotechnology.[15-20] Many tumor types are being inhibited by synthetic ligands. Hence, the synthesis of compounds with structure similar to the drugs in use is one of the area of research in pharmaceutical chemistry.[21-23]

(*E*)-3-(4-(Dimethylamino)Phenyl)-1-Phenylprop-2-En-1-One (3)

A mixture of Dimethylaminobenzaldehyde**(2)** (14.9g, 0.1 mole) and butan-2-one (**1**)(7.2.mL, 0.1 mole) in dry ethanol (25 mL) was refluxed with added NaOH (1.0 g), monitoring the progress of reaction by TLC. The reaction was stopped at the appropriate

point 6 h, the reaction mixture was worked up and subjected to column chromatography over silica gel (60-120 mesh) using 02:98% ethyl acetate in petroleum ether as eluent.

4-(4-(Dimethylamino) Phenyl)-1,3,6-Triphenylpiperidin-2-One (5)

Conventional Method: (*E*)-1-(4-(dimethylamino)phenyl)pent-1-en-3-one **(3)** (2.04g, 0.01 mole) in dry toluene, sodium hydride (0.1 molar equiv) and N,2-diphenylacetamide **(4)** (2.12g. 0.01 mole) were added. The resultant mixture was stirred at 90-100°C for 5h, cooled and then the reaction mixture was added to a large amount of water. The precipitate was filtered and purified by re-crystallization from ethanol.

The following figure- a shows the mechanism of arriving of the compound 4-(4- (dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin -2-one.

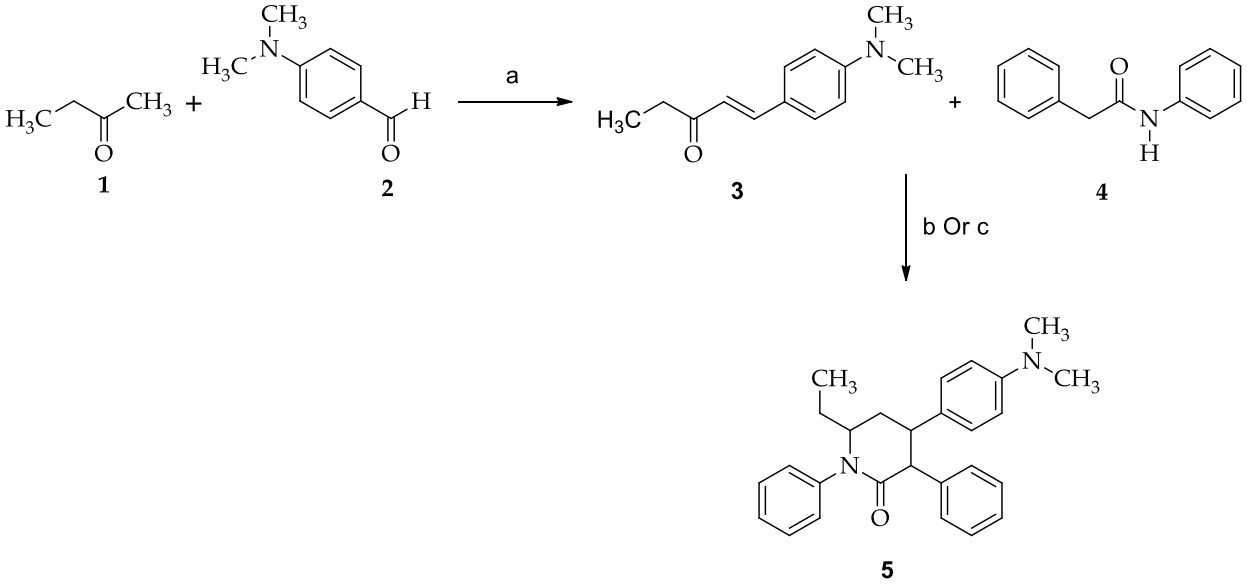

Fig. 1: Mechanism of arriving of the compound 4-(4- (dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin -2-one . (a)NaOH, EtOH, RT, 5h; (b) NaH, EtOH, 80°C,6h; (c) NaH, DMF, 10min 200W in mw.

Microwave Method:(2.04g, 0.01 mole) of (E)-1-(4-(dimethylamino)phenyl)pent-1-en-3-one **(3)** and N,2-diphenylacetamide **(4)** (2.21g, 0.01mole) in small amount of DMF and a catalytic amount of sodium hydride is heated for 10 minutes at 140°C in 250 watts. The yellow coloured product stirred with 1000 mL cold water, neutralized with dilute HCl, filtered, dried and washed with 200 mL of ethyl acetate. It was recrystallized from ethanol to give a pure yellow crystalline powder with good yield. The physical data obtained exactly matched with the product formed in the conventional method. **Conventional**: Yield 75%; **Microwave**: Yield 90%; m.p. 130-131 °C; 82% yield [24].

Retrieval of the Structure of the Protein

The structure of the drug target protein Cyclin-dependent kinase 5 and its Xray crystallographic structure with 2.3Å was retrieved from protein data bank. Protein identification number 1UNG, commonly known as PDB ID (http://www.rcsb.org/pdb/).

Protein Preparation

The raw protein from the protein databank with the PDB ID 1UNG human protein Cyclin-dependent kinase 5 is further prepared for docking studies. Initially, all other chemical moieties, present in protein are removed. All water molecules were removed and on the final stage hydrogen atoms were added to the target protein molecule.

Preparation of the Ligand

The ligand molecule 4-(4- (dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin -2-one was analysed for its pharmacophore features. The analysis was done using Accelrys Discovery Studio 2.0.

Pharmacophore Generation of the Ligand

The prepared ligand was analysed for its pharmacophore groups. This facilitates to analyze the interaction between the ligand and the protein in terms of the various group characteristics.

RESULTS AND DISCUSSION

The molecule of interest, 4-(4- (dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin -2-one was synthesised and characterized by physico chemical procedures. The details of synthesis and spectral assignment of the compound is already discussed.[24] The molecule, 4-(4- (dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin -2-one was analysed for Lipinski;'s rulle of 5 and was found to hold good to serve as a ligand. The logP value of the synthetic ligand was found to be 4.07. It was also analysed for the ADMET analysis (Fig- 1). The role of CDK5 in central nervous system is well characterised. The implication of CDK5 in the progression of a variety of cancers has been recently established.[25] The view of CDK5 in tumorigenesis of multiple organs has been suggested over the past decade.[26-29]

The ADMET properties of the synthesized molecule was also analysed using Discovery Studio 2.0. The pharmacophore feature of the compound 4-(4- (dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin -2-one was also analyzed using Accelrys Discovery Studio 2.0 and was found to have five hydrophobic groups and one hydrogen bond acceptor (Fig. 2).

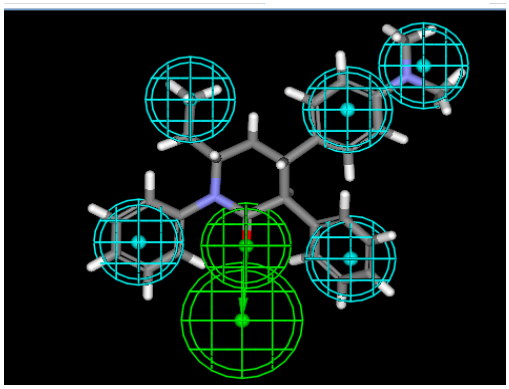

Fig. 2: Pharmacophore features of compound 4-(4-(dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin -2-one

Structure based drug design is the method of performing docking using the known protein docking with known ligands. This has been the frequently used method of analyzing the receptor-ligand interactions between the active site of the protein and the chemical molecules. In this current study the PDB ID 1UNG Cyclin-dependent kinase 5 is docked with 4-(4-(dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin 2-one, using ligandfit protocol available through Acclerys Discovery Studio 2.1.

Any target protein should have the potential to be druggable, so that it possesses binding sites which favours interactions with small molecules.[30-32] The key results in a docking log are the docked structures found at the end of each run, the energies of these docked structures and their similarities to each other. The similarity of docked structures is measured by Internal energy, Dock Score.

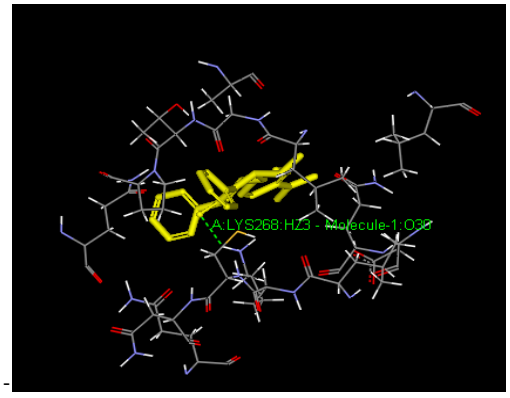

Fig. 3: Receptor-Ligand Interaction of compound 4-(4-(dimethylamino)phenyl)-6-ethyl-1,3- diphenylpiperidin -2-one

The ligand is found to interact with the CDK5 and hence inhibit its activity (Fig.3). Accelrys Discovery Studio 2.0 is the commercial software used to study the interaction between molecules. In our present study, the inhibition of the protein 1UNG by the synthetic ligand 4-(4- (dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin -2-one has been analyzed using it. The protein structure of CDK5 is 1UNG, which was retrieved from PDB. The structure of the protein has been solved by x-ray diffraction with a resolution of 2.3 A^{o} units. The force field CharmM was applied to the protein. The figure 3 shows the interaction of the ligand with the protein. The free energy of the protein is -17981.68931 K cal/mol after minimisation. It can be seen that the HZ3 of the amino acid lysine forms a hydrogen bond with the O 38 of the ligand. The interaction has a dock score of 52 and has energy of -10.644. The higher dock score with lower energy implies that the molecule forms a stable complex with the protein.

CONCLUSION

The ligand has been synthesized by using piperidone(4-(4-(dimethylamino)phenyl)-6-ethyl-1,3-diphenylpiperidin -2-one. The biological importance of the synthesized compound has been analyzed by using computational approach. It has been found that the ligand could be a potential lead compound in the inhibition of the CDK5 protein, which needs verification from the other studies. It is extremely important to validate the efficacy and safety of these drugs in clinical trials.

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