Date: 24-01-2022

Certification of Collaboration for research

This is to acknowledge that Drug design and Cheminformatics laboratory (PI: Dr. Achintya Saha, Professor, Department of Chemical Technology, University of Calcutta) has been in active research support and collaboration from 2015 with Dr. Souvik Basak, Associate Professor, Division of Pharmaceutical Chemistry, Dr. B. C. Roy College of Pharmacy & Allied Health Sciences, Durgapur, WB, India. The collaboration is mainly to exchange of research in Computational Chemistry, Biology, Phytochemistry and Contraceptive field whereas Dr. Souvik Basak has extended his support in experimental design, formulation, bioanalytical and *in vivo* studies and some part of computational chemistry as well as biology. As an outcome, several papers have been published in internationally acclaimed journals such as Computational Biology and Chemistry, Journal of Pharmacy and Pharmacology, Nanomedicine- Nanotechnology, Biology and Medicine, Acta Tropica, Current Analytical Chemistry and Others. Furthermore, as a part of current research, the collaborative research groups are engaged in certain anticancer drug design, the first part of which has been accomplished and communicated. The groups are hopeful to extend this support and collaboration in future as well.

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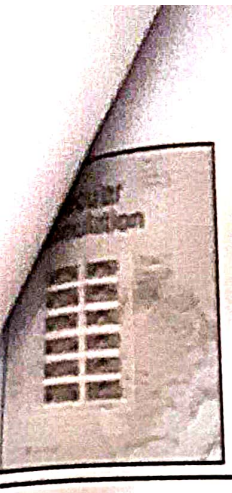

Molecular Simulation

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Genetic algorithm-*de novo*, molecular dynamics and MMGBSA based modelling of a novel Benz-pyrazole based anticancer ligand to functionally revert mutant P53 into wild type P53

Ashik Chhetri, Moloy Roy, Puja Mishra, Amit Kumar Halder, Souvik Basak, Aditi Gangopadhyay, Achintya Saha & Plaban Bhattacharya

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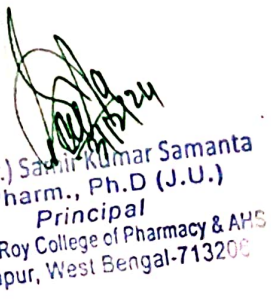

MOLECULAR SIMULATION
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Genetic algorithm-*de novo*, molecular dynamics and MMGBSA based modelling of a novel Benz-pyrazole based anticancer ligand to functionally revert mutant P53 into wild type P53

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ABSTRACT

Mutations in P53 cause a loop unfolding, resulting in loss of activity and finally leading to cancer. One strategically reported way to arrest such oncogenesis is the restoration of tertiary structure as well as the function of mutant P53. In this attempt, we have designed a benzo-pyrazole-based novel ligand starting from a carbazole compound (EYB or PK9324) reported earlier to reinstate such function in mutant P53 (Y220C mutant, PDB: 6GGD). Assuming PK9324 as the template scaffold, *de novo* technique (Genetic algorithm, eLEA3D) was adopted within the binding pocket of 6GGD and our ligand **DLIG1** was designed after several rounds of mutations. Docking and molecular dynamics (MD) simulation revealed significant interactions with key amino acid residues such as Cys220, Asp228, Leu145, Trp146, Val147, Thr150, Pro151, Pro152, Pro222, Pro223, Asp228, and Thr230. Along with sufficient binding stability, the MMGBSA analysis revealed its comparable binding free energy with other reported reference ligands (i.e. PK9324 and PK9318). Similar to these reference ligands, **DLIG1** exhibited specificity in binding towards the Y220C mutant rather than towards wild-type P53. Finally, **DLIG1** displayed a reorientation of a hydrophobic cavity in Y220C that hinted restoration of electrostatic interactions within the key loops of P53 favoring regain of its function.

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KEYWORDS
Mutant P53; docking; molecular dynamics; MMGBSA; hydrophobic cavity

1. Introduction

The P53-mediated apoptotic pathway is a major pathway that is involved in the progression of cancer, with approximately 50% of human cancers arising due to mutations in the *p53* gene [1]. The P53 protein plays a significant role in suppressing unregulated cell division and is therefore referred to as a tumour suppressor [2,3]. In humans, the P53 phosphoprotein comprises 393 amino acids and has a molecular mass of 53 kDa, and is coded by the human *p53* gene located on chromosome number 17. The P53 protein typically comprises three functional domains, including an NH_2-terminal, transactivation domain (TA; residues 1–63), a DNA-binding domain (DB; residues 102–292), and a COOH-terminal oligomerisation domain (OD; residues 319–359) [3,4].

The P53 gets immobilised by mutation in major cancer types with the point mutation of tyrosine in the 220th residue with cysteine (Y220C) affecting its DNA binding ability. The destabilisation of the DNA-binding domain leads to unfolding and aggregation due to the loss of thermostability and hydrophobic interactions [1,2,5–7]. In various cancer cases, tumour suppressor P53 gets inactivated by mutation and its function could be restored by binding to small molecule stabilisers [8]. These small molecules could effectively bind to Y220C pocket and reactivate the mutant to provide a promising strategy for new anti-cancer therapeutics [5,6,8].

A literature survey suggests that the N-ethyl carbazole compound PK083 could be a potential stabiliser of the P53 mutant protein [1,2,5,7]. Similar promising mutant P53 stabilising activities have been reported for pyrrole-substituted pyrazole derivative PK7088 [5]. Carbazole derivatives substituted with 5-membered heterocycle have been acknowledged to increase binding affinity with P53 mutant by two folds resulting in restoration of its active tertiary structure and gain of function as guardian of the cell [1,5]. Oxazole-substituted carbazole (PK9318) is also reported to be a potent binder with Y220C variant of P53 and it was subsequently acknowledged to induce cell restoration activity in the liver cancer cell line. Similarly, thiophene substitution in carbazole resulted in another compound (PK9324) that also exhibited significant thermo-stabilisation of the mutant protein.

Various *in silico* methods such as fragment-based drug design and structure-guided molecular dynamics (MD) may help in designing such small molecule stabilisers. Thus, an in-depth analysis of Y220C binding site flexibility is performed first [9] and the 3D conformation of P53 protein is then utilised for the fragment-based design of potent small molecule. Herein, a carbazole-based fragment was selected as a template for *de novo* design of the lead molecule, the binding affinity of which was subsequently confirmed using robust computational methods, such as molecular docking (both flexible

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ORIGINAL RESEARCH PAPER

Analytical method development for exploring pharmacokinetic profile of ursolic acid in rat tissues by high-performance thin-layer chromatography

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Abstract
A simple and rapid high-performance thin-layer chromatographic (HPTLC) determination of ursolic acid (UA) in tissue is necessary for its utilization in therapeutic survey. In the present work, an attempt has been made to develop a method for the estimation of UA disposited in the liver, heart, spleen, lung, kidney, brain, stomach, intestine, ovary and uterine tissues at various requisite time intervals after oral administration. The biosamples were prepared based on protein precipitation by methanol with a recovery ranged from 91.38% to 98.47%. The mobile phase with the best resolution was achieved with toluene–ethyl acetate–methanol (4:1:1, *V/V*) and toluene–ethyl acetate–methanol–formic acid (4:1:0.7:1, *V/V*) solvent systems for normal and amine-coated plates, respectively. The mobile phase resolved UA efficiently from other constituents with R_F of 0.60 and 0.50 in normal and amine-coated TLC plates. The method exhibited satisfactory linearity ($r^2 > 0.99$) in the range of 9.6 to 38.4 µg/mL, i.e., 96 to 384 ng/spot. It was observed that UA mainly disposited in liver and uterine tissues indicating biliary extraction. This assay range is adequate for analyzing disposited UA after a single oral dose (40 mg/kg) administration for which the lower limit of quantification (LLOQ) values in different biological matrices are in the range of 2.63–7.88 nmol/mL. For routine quality control analysis, this method showed reliability by a rapid, reproducible, cheap and eco-friendly manner.

Keywords High-performance thin-layer chromatography (HPTLC) · Ursolic acid · Tissue disposition

1 Introduction

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) (UA), a triterpenoid compound, is present ubiquitously in herbal formulations and it is an integral part of the human diet and cosmetics preparations [1], possessing a number of pharmacological effects [2–10], like hepatoprotective, anti-inflammatory, hypoglycemic, anti-tumor, anti-HIV, antimicrobial, antifungal, anti-ulcer, gastroprotective, hypolipidemic and estrogenic activities. With the growing significance of a potential beneficial role of UA in human health, there is an increasing demand for analyzing it in vivo. Quantification of drug in the target organ is essentially required for its therapeutic survey and is based on the proposition that the concentrations in the target tissues are directly proportional to the intensity of the effect. It is not sufficient to ascertain the concentration of UA in different tissues from the data of its concentration in the blood. But due to the high lipophilicity of UA, it might be stored in different tissues for a long time. There is a report on bioavailability/quantification of tissue disposited UA by high-performance liquid chromatography (HPLC) after intravenous administration of UA-phospholipid nanoparticle [11], without any validation data. As UA has nutraceutical values and absorbed by intra-gastric route, an attempt has been made to develop a simple and rapid method to estimate UA disposited in the different target tissues after oral administration.

In the present work, modern thin-layer chromatography (TLC) is chosen as it is a simple, reproducible, rapid, economic and eco-friendly tool of analysis. Several samples can be analyzed upon one TLC plate, using a small amount of mobile phase and in a small analysis time. With the

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