In Silico Novel Identification of Anti-cancer Drugs using Density Functional Theory and Molecular Dynamics Simulation

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Introduction

The term tumour refers to the uncontrolled cell division or abnormal growth of cells occurring in any part of the living body, the part could be an organ, or a tissue or the bone. Classified according to the location of the occurrence and spreading mechanism, there are two distinct kinds of tumours, the first kind, that serves no purpose in the body, and its growth is limited to the emergent part of the body is known as benign tumour, whereas, the second kind of tumours that are not limited to the emergent location, and are able to get spread over any other part of the body to form secondary tumours are known as malignant tumours. The credit of naming the malignant tumour as cancer goes to Greek physician Hippocrates (460-370 BC), who used the terms *carcinos* and *carcinoma* to describe non-ulcer-forming and ulcer-forming tumours. The Roman physician, Celcus (28-50 BC), later translated the Greek term to *cancer* the Latin word used for crab. The loss of apoptotic nature by the cells in their metabolic pathways leads to cancer [1]. According to the World health organization (WHO) report on 2018, around 9.2 million deaths with the lung, prostate, colorectal, stomach and liver cancer in men, while breast, colorectal, lung cervix and thyroid cancer are the most commonly found in women [WHO]. Out of the reported statistics 3,00,000 new cases of cancer are diagnosed each year among children within the limit 0-19 years [1]. To tackle the emergence of the disease with no aftereffects, the advancement in the field of science has opened the doors for probing the herb and organic remedies for identifying the druggable targets [2, 3, 4]. Moreover, with time, resistance appeared in almost all the reported drugs due to revolutionized outcomes in cancers such as malignant melanoma, suffer from novel problems such as acquired resistance, idiosyncratic adverse effects and high costs [5, 6, 7]. Although it is still a controversy, whether all reported drugs kill cancer cells with 100% efficiency or sustains their survival under stressful conditions, more and more reports provide data to support that autophagy promotes cancer cell survival after chemotherapy or radiation therapy (X-rays, gamma rays, and charged particles radiations) [8]. Therefore, it becomes pressing need as per the present scenario, to search for the new

anticancer molecules that would not only prevent development of cancerous tumours, but also would save the healthy cells from any side-effects. As reports suggest, approximately 1,00,000 humans die each year due to the toxicity associated with the synthetic medicines/drugs [1]. The herbal and organic molecules that can be obtained from plants and/or animals are the best alternatives to the synthetic drugs/medicines owing to their sufficient availability, feasible extraction methods and last but most importantly non-toxic nature. Subsequently, numerous novel drug targets have been identified and validated to stop the generation of chromosomal translocations and fusion genes. [9, 10, 11]. Moreover, modification in glycosylation pattern often leads to malignant.

transformations among normal the cells [12, 13, 14]. In conjunction with upregulated sialyltransferases, it ultimately leads to hyper-sialylation at cell surface [15]. The drug target plays a significant role in successfully discovering the drugvia the assessment of the drug-target interaction. The promising drug target possesses specific properties viz. must be functional inside the pathogen, and inactive inside the humans; and for validating this, 3D structure of such targets should be available in the database. In this regard, the literature survey suggests that there are several essential and potent drug targets reported for the concerned cancers occurring within the human organism. The detailed study on the interaction and interaction mechanism of the anticancer drugs with respective targets aid in enhancing the drug discovery procedure. The present work shows the sheer use of various computational techniques for the identification and validation of the hit molecules. Considering the importance of computer-aided drug design (CADD), in the present study [16, 17], the defined saccharides were calculated to look for the affinity of inhibitors. Based on the validation, the screened molecules were prioritized by employing various computational quantum mechanical and classical treatments such as density functional theory (DFT) based on *first-principles calculations and molecular dynamics (MD) simulations* based on Newton's second law of motion.

Objectives

The present work aims to study the interaction and interaction mechanism of selected herbal and organic molecules with respective target species that are responsible for cancer under the join association of density functional theory and molecular dynamics simulations. The proposed properties have to be computed as a function of temperature, pH and aqueous medium for painting a complete picture of the interaction between the molecules that act as cancer inhibitors and the target. The unique combination of quantum and classical mechanical treatments to the proposed systems would aid in drawing fruitful insight to the dominating factors together with firm conclusions that would help in drug discover and designing. To achieve this goal, following objectives were formulated and fulfilled for, the analysis of response of drugs against the target for a given time span.

1. To find the structure-based inhibitors against the selected target and analyse the dynamical behaviour of the inhibitors as a function of time.
2. To check the capacity of strengthening from drug-target interactions; compare the Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (ROG) and Hydrogen bond analysis of solid bodies.
3. To calculate the Binding Energy (B.E) between druggable targets and leads.
4. To verify physiochemical properties of selected drug molecules.

Summary of Research Work

Cancer has emerged as one of the major chronic yet curable diseases, imposing compulsion on the researchers and eminent scientists to design and develop novel non-toxic drugs that can fight against cancer causing conditions. Recently, computational design, discovery and study of

the molecules that can not only stop growth of cancerous cells but also have the property not to harm normal cells. Hence, it has become mandatory to generate database covering basic properties of drug molecules to their interaction mechanism with the targets, which is only possible with incorporation of high-throughput techniques like DFT and MD. These methods are reliable and have been proven for reproducing the experimental results, of course with lower accuracy, yet providing deep insight to the underlying mechanism. Apart from this, these techniques are eco-friendly, economic and more preferable than the direct experimental techniques owing to chemical free, dry nature. Henceforth, the *in-silico* approach stands out to be unique and necessary step before directly going for experimental synthesis and validation. The present thesis is organized in the following manner. The chapter wise description of the research work carried out is summarized as under:

Chapter 1 presents the expression of cancer cells in accordance with the respective organs and their representation in human body. The overview of the proposed study with detailed information is incorporated to justify the motivation of proposing the study. Cell surface proteins are elaborated with covalently attached complex array of N-linked glycans. Glycans offer correct folding of the protein, and provides resistance to proteases and facilitate its interaction with ligands. The dynamical behaviours and glycosylation changes often show the malignant transformation. This is characterized by an increased branching of N-linked glycans thus creating additional sites for terminal sialic acid (SA) residues [12, 13, 14]. Further, it is shown that over-expression of SA on malignant colonic cells and tissues, in vitro, correlates with the metastatic stage [18]. Moreover, the challenge now a day is to directly pass the macromolecules to cell membrane without any active process. It is found that the herbal drug paclitaxel (PTX) is dynamic against a wide range of cancers that are considered to be intractable to conventional chemotherapy. This has led to the regulatory approval of PTX in the palliative therapy of patient with breast cancer [19], ovarian cancer [20], lung cancer [21], pancreatic cancer [22] and many more. Unfortunately, Cremophor EL itself is toxic, which makes finding a suitable alternative with high priority. Therefore, the development of

novel techniques for introducing bioactive molecules inside the living cells is an active area of research. Finally, the study shows the newly predicted drug and drug carrier to control the malignant changes with respect of body environment, different physiological variables such as pH and temperature.

Theoretical description of computational methodology used throughout the work is presented in **Chapter 2**. In this chapter, the theoretical concepts which are the basis of density functional theory (DFT) based on *first-principles* approach and Molecular dynamics (MD) simulations based on Newton's second law of motion are presented and discussed. In particular, all quantities which help to calculate the electronic, structural and dynamical properties of the complexes on the basis of the DFT and MD simulation are discussed respectively. The present study covers the assessment of all the properties considering the body environment and chemotherapeutic conditions. To get insight to the dynamical behaviour, the GROMOS 53a6 [23] and OPLS_2005 force fields which are the enhanced versions for all atom force field developed by Schrödinger [24] and to keep the temperature and pressure constant, the Nose-Hoover thermostat and Berendsen barostat have been utilized respectively. Moreover, energy-minimization of the system is performed using the leap-frog algorithm in the NVT canonical and NPT isothermic ensemble, respectively.

In **Chapter 3**, the results on systematically investigated pH and temperature dependent glycosylation pattern are presented. The results verify the dual approach of cationization and attachment of identification of peptide in targeting the colon cancer cells, exhibiting metastatic-stage dependent expression of SA. The computation of electronic and surface/morphological properties using density functional theory (DFT) based on the *first-principles* calculations demonstrates that the carriers decorated single antennary saccharides and mimic exhibit high affinity towards over-expressed SA and galectin residues on cancer cell surface. We have also included the pH with two types of state physiological temperatures (37˚C)- the body temperature and (42 ˚C)-the chemotherapeutic temperature to obtain the accurate absorption

energy. The calculations demonstrate a stronger D- galactose- SA interaction at tumour-relevant low pH and hyper thermic conditions. Furthermore, basis set superposition error (BSSE) of intermolecular potential function was corrected by Boys-Bernardi counterpoise method [25]. We found that the D-galactose at 6.0pH and 42 ˚C temperature have 1.67 Å distance from SA with 0.22297eV energy gap and -26.52 eV interaction energy. In a nut-shell, the measurements reveal that; (i) increasing temperature and decreasing pH, in general, have a favourable effect on binding affinity of complex, and (ii) induction of hyperthermia at tumour-relevant pH offers pronounced enhancement in the binding affinity.

The next chapter, **Chapter 4** presents the comparative study of single and bi-antennary saccharides and mimics with extra cellular SA using DFT and MD simulations to evaluate the quantum mechanical and classical mechanical based properties respectively. Bi-antennary phenyl boronic acid (2PBA) domain is the primary region of interest for probing the impact of SA activators, and binding affinities of this ligand to cellular SA domain. The binding of three complexes have been compared amongst themselves. Where, 2PBA displays the ability to form reversible covalent interaction with SA. Further we have employed computations with different time slots to investigate the pathway of interaction and predict the properties of complex under the physiological environment such as, 1 bar pressure and 37 ˚C temperature. The OPLS_2005 force fields [24] have been adopted for the proposed computations. The time course of the SA-ligand interaction has been investigated for 5, 15 and 25 ns, respectively. The results indicate that with the presence of strong interaction energy, bi-antennary PBA molecules spontaneously moves towards the SA. The structure for 15ns time slot quickly interacts with SA with 7.8596eV binding energy with 1.579(Å) distance between the PBA and SA. Finally, the conclusion is that after 15ns, in the presence of strong interaction energy, bi-antennary PBA molecules would spontaneously move towards the SA showing high sensitivity of SA for 2PBA.

In the last chapter, **Chapter 5,** the potential drug delivery systems are discussed that are used to free the drugs on the surface of target, due to longer circulation time, higher drug uptake and selectively, lower dosage and better therapeutic efficiency [26]. To understand the protonation and diameter effects on drug loading and releasing processes, the PTX loaded with three armchair chirality (n,n) such, as (12, 12), (13, 13) and (15, 15) sized single wall carbon nanotubes (SWCNTs). Literature reveals a report by Wong and Xu have investigated the interaction mechanism of DOX and SWCNT to understand the protonation and stated that encapsulation is much stronger than the adsorption of doxorubicin on the sidewall of CNTs also they have confirmed the diameter effects on drug loading and releasing process [26]. We have chosen the drug loading instead of adsorbing the drug on the side wall of CNT, to know the temperature effect on PTX loaded SWCNTs with different diameter. The physiological temperature and chemotherapeutic temperature are incorporated to check the structural and dynamical properties with solvent accessibility. Here, higher pair distribution function of the complex per atom and interaction energy at 42 ˚C indicates strong interaction between (15, 15) armchair SWCNT and PTX. 20.53 Å diameter is found optimal for the encapsulation of PTX at 42 ˚C temperature for drug delivery time of 440ps.

The detailed systematic investigation of our results on electronic, dynamical, mechanical and structural properties of complexes using DFT and MD has been summarized in **Chapter 6**. Further we have included the target oriented possible drugs, their derivations and carriers which can show better confirmative results under biological conditions. Finally, the concluding remarks of the thesis with a brief discussion on future scope have been presented.

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