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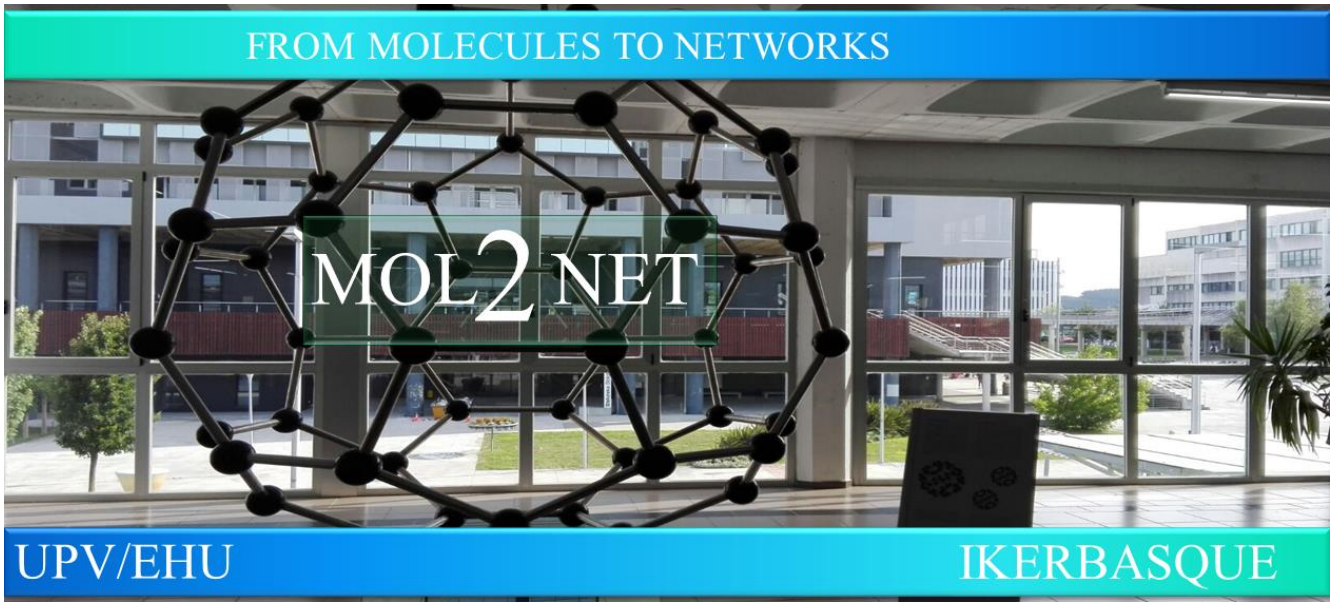

In Silico Insights into the Inhibitory Activity of Prodigiosin against Tumour Cells Targeting the Tyrosine Kinases Receptors

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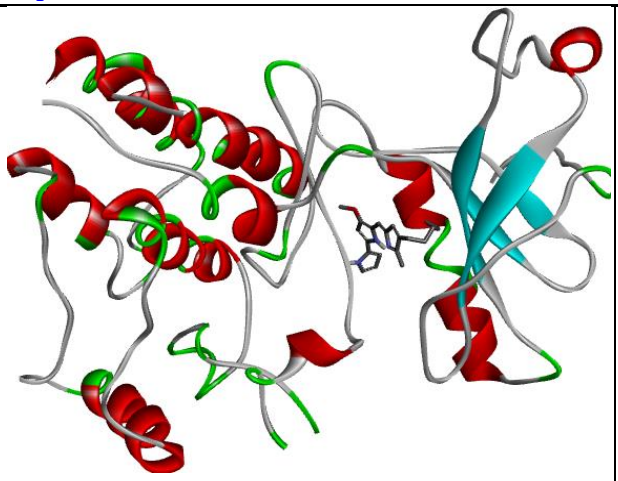

Abstract

Prodigiosin (PDG) is a linear derivative of pyrrolyl dipyrromethene with a 4-methoxy,2-2-bi-pyrrole ring system. It is produced by some species of bacteria and eubacteria and is reputed for its anticancer activity against breast, colon and lung cancers via induced cellular stress. The study investigated the PDG binding interaction with several co-crystallized receptor tyrosine kinases (rTKs) to estimate the binding energies (E) and inhibition constants (K*i*) of PDG. Prodigiosin was docked using AutoDockTools-1.5.6 against 20 co-crystallized rTKs selected from the protein data bank, PDB. The E, K*i*, RMSD, the number of H-bonds and the amino acids involved in the interactions of their best conformational poses were estimated and compared with those of doxorubicin, a potent cytotoxic agent. Comparatively, PDG interacted more efficiently with the collagen discoidin domain receptor subfamily 1 (DDR1) type II kinase protein (PDB: 4BKJ). A total of 16 amino acid residues were involved in hydrophobic (Val624, 2 Lys655, Glu672, Ile675, 2 Ile685, Met699, Thr701 and Asp784), hydrogen (2 Glu672, 3 Asp784) and π-stacking (Phe785) interactions with the DDR1 type II tyrosine kinase protein. A significant RMSD, E, K*i* of 60.071 A, -10.04 Kcal/mol and 43.90 nM respectively for the binding of PDG to the rTK were obtained *vis-a-viz* native ligand, imatinib (78.961 A, -14.20 Kcal/mol and 39.11 ρM) and doxorubicin control (52.52 A, -8.65 Kcal/mol and 457.29 nM) respectively. The significantly higher inhibition of the DDR1 type II kinase protein by PDG compared with doxorubicin provides vital insights into understanding the molecular basis of the mechanism of anticancer activity and its clinical application in the treatment of breast, colon and lung cancers.

Introduction

Kinases are important tyrosine or serine/threonine-specific drug targets with more than two thousand subunits already identified in the human genome. They play vital roles in the regulation of cellular activities, phosphorylation of proteins and cell signal transductions resulting in processes leading to cell survival [1]. Tyrosine kinase (TK), one of the most important kinase receptors, consists of an N-terminal extracellular ligand-binding domain (LBD), a transmembrane domain and a C-terminal

intracellular domain with tyrosine kinase and ATP-binding activities. Activation of TKs is known to regulate cell proliferation, reorganization and migration resulting in metastasis of tumours and other forms of cancers, thus their importance in anticancer drug development [2].

Cancer is one of the deadliest multifactorial diseases affecting many human organs. Lack of selectivity and specificity in addition to the possibility of relapse and multidrug resistance are known to hinder cancer chemotherapy, especially among the drugs targeting the DNA [3]. The current convention in the development of novel anticancer drugs involves targeting certain proteins such as the TKs as well as specific cell signaling pathways that are abnormally expressed in cancer cells. This multi-targeted approach in cancer eradication has also suffered setbacks in some malignancies prompting further and continuous search for a better alternative that natural products could provide [4].

Prodigiosin, a pyrrolyl dipyrromethene derivative with a 4-methoxy,2-2-bi-pyrrole ring system is reputed for its anticancer activity against breast, colon and lung cancers via induced cellular stress [5]. It is produced by *S. marcescens* and some species of bacteria such as *Streptoverticillium rubrireticuli* and *Vibrio psychoerythrus* and other eubacteria [5, 6]. It has diverse chemical functionalities existing as linear or cyclic forms as well as cis and trans isomeric forms [7,8]. Previous studies had attributed its anticancer activity to the inhibition of the proliferation of cancer cells via the cleavage of the active amine group into the intracellular cells resulting in apoptosis [6,9,10]. However, there was no reported attempt to identify its target or to understand its interactions with any known target. The study, therefore, proposed receptor tyrosine kinases as its target and the possible interactions with target proteins underlying its activity against breast, colon and lung cancers.

Materials and Methods

Preparation of receptors tyrosine kinases

Twenty 3D rTKs proteins, co-crystallized with different ligands with resolutions < 2.0 Å, were selected from the PDB. The water molecules and other co-crystallized non-essential moieties were removed followed by the repairs of missing amino acids [11]. The free proteins were protonated and charged using polar hydrogens and Kollman charges respectively.

Preparation of ligand molecules

The co-crystallized native ligands were downloaded from the PDB alongside the rTKs. The PDG and doxorubicin were drawn from the fragments in the MOE interface. The energy-minimized ligands were converted to the PDBQT format using the Open Babel GUI [12].

Molecular docking

The algorithms of AutoDockTools-1.5.6 were used in the docking. The ligands were assigned with torsions using default settings. The potential grid maps were executed using the AutoGrid module with 50 hybrid GA-LS runs and a population size of 300, 2.5 million energy evaluations and 27000 generations [13]. A root mean square deviation of 2.0 Å was set to group the clusters while other parameters were at default. The docking protocol was validated by re-docking the native ligands into the rTKs proteins using the Lamarckian Genetic Search algorithms. The binding poses visualization was performed using PyMol and protein-ligand interaction profiler webserver.

Results and Discussion

Many potential drug targets have been described for the treatment of cancer and many have been found ineffective due to issues related to toxicity or efficacy. Over 30% of anticancer discovery studies are

targeted on receptor kinases due to the vital roles they play in the pathogenesis of cancer and their overexpression in cancer cells. Prodigiosin, a pigmented natural compound has demonstrated activity against breast, colon and lung cancers with no understanding of the molecular basis [7].

Docking scoring and validation

Virtual screening protocol was adopted in the molecular docking of all the ligands to their respective rTKs with appropriate grid box points and spacing which reproduced the RMSD (RMSD < 2.0 Å) of the co-crystalized ligands. Further validation was based on the replication of most interactions when the co-crystalized ligands were re-docked in their respective targets. Of the 20 rTKs, the RMSD and the interactions could not be replicated with one protein. The binding free energies (E), inhibition constants (K_i) and the amino acids involved in the interactions of PDG with some of the rTks are shown in Table 1. Notably, Ki is the theoretical inhibition constant and like binding energy, lower values indicate more favorable interaction with protein [14]

Table 1. Binding parameters of PDG to DDR1 type II kinase protein

Ligands	RMSD	E (Kcal/mol	K_i (nM)	H-bonds	Amino acids involved
Imatinib	78.961	-14.20	0.03911	4	Val624, Lys655, Met676, Ile685, Thr701, Tyr703, Leu773, Leu773, Leu679, Asp784 & Phe785
PDG	60.071	-10.04	43.80	5	Val624, Lys655, Lys655, Glu672, Ile675, Ile685, Ile685, Met699, Thr701 & Asp784
Doxo	52.520	-8.65	457.29	6	Ile 675, Ile685, Leu679, Phe762, Phe762, Asp784

Doxorubicin (doxo); root mean square deviation (RMSD); number of hydrogen, drug-enzyme (H-bond); Prodigiosin (PDG); binding energy (E); inhibition constant (K_i)

Analysis of binding poses

Analysis of the binding pose of the best conformer using the protein-ligand interaction profiler tools revealed three important types of PDG interaction with the protein. The two hydrogens of the 2,2-bipyrrole system of PDG interacted with the oxygen of carboxylate groups of Glu672 and Asp784 with favourably hydrogen-acceptor and donor-acceptor bond lengths while the oxygen of the 4-methoxy substituent in PDG formed a hydrogen bonding with the amine of Asp784 (Figure 1A). The second interaction observed was hydrophobic bonding involving eight hydrophobic carbons of amino acids and carbon chains of PDG, especially the methyl and n-pentyl groups of PDG (Figure 2). The aromatic 2,2-bipyrrole ring systems of PDG show a perpendicular $\pi - \pi$ interaction with the aromatic ring of Phe785

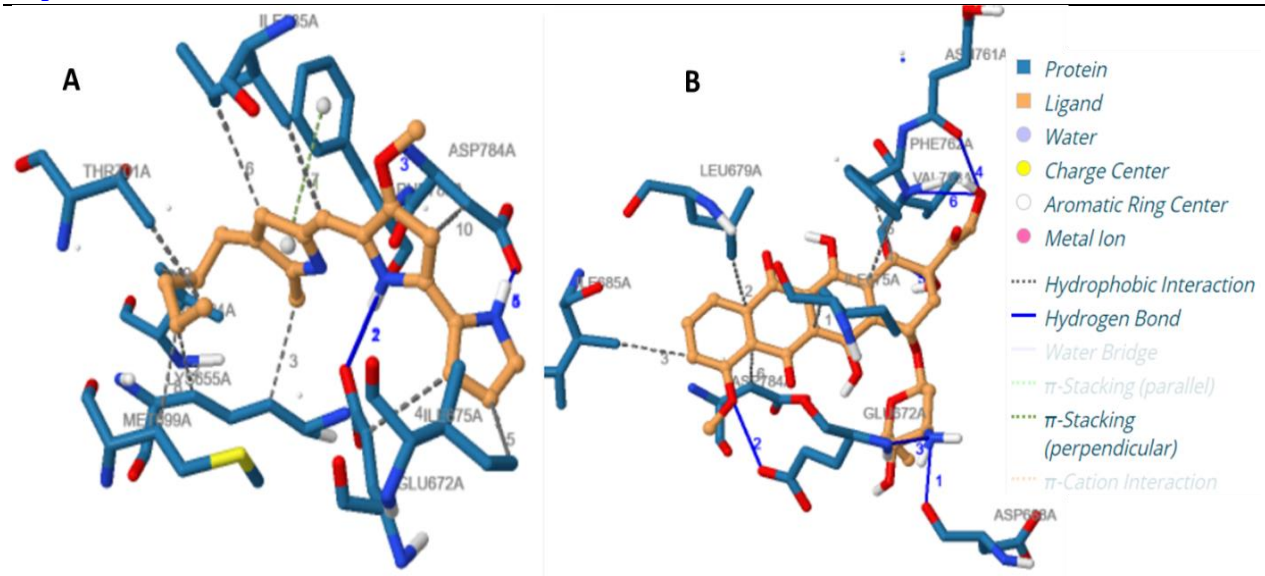

Figure 1. Theoretical 3D binding poses for DDR1 type II kinase protein- (A) PDG and (B) doxorubicin. The protein residue and ligand are represented in thick line format. Various interactions are color coded as shown on the right panel. The background represents the protein's molecular surface

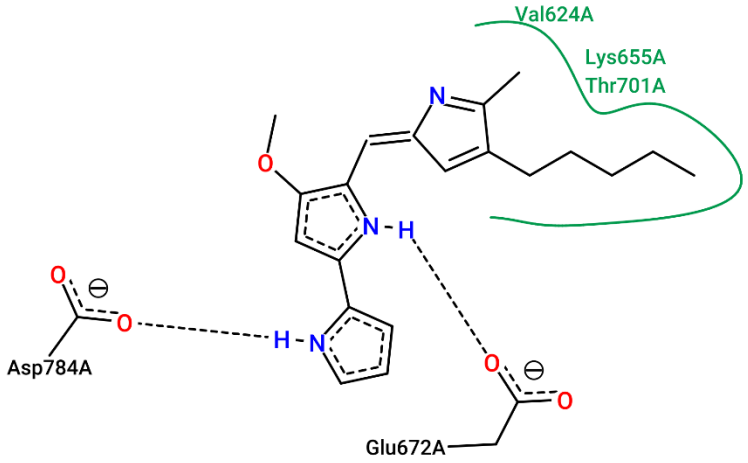

Figure 2. 2D representation of some hydrogen and hydrophobic interaction of PDG

Atherosclerosis, fibrotic disorders and cancer are some of the human diseases whose progression has been linked to the DDR kinases [15]. The potential of DDR kinases as drug targets has been demonstrated by gene knockdown or overexpression of DDR1 and DDR2, especially in breast, colon and lung cancer. A DDR1 kinase comprises extracellular (N-terminal DS and a DS-like) and intracellular (C-terminal kinase) receptor domains linked by a cytoplasmic domain [16]. The best conformational pose of protein-PDG interactions showed interesting binding characteristics. The pyrrole ring with its methyl and *n*-pentyl moieties was found pocked in the N-terminal lobe of DDR1 where hydrophobic interactions were favourable, similar to imatinib. The other pyrrole rings were docked in the P-lope, activation segment and the C-terminal lobe where considerably favourable hydrogen bonds were predominant [17].

As anticipated, four out of six hydrogen bonds (Glu672, Asp784 and Arg789 amino acid residues) were replicated in the best protein-imatinib conformation in the ATP pocket of the protein. In this study, however, the N-atom of the three pyrrole rings and the oxygen of the OMe formed five hydrogen bonds; all with Glu672 and Asp784 amino acid residues. The favourable hydrophobic and π

–π stacking interactions also contributed significantly to stronger binding energy and inhibition constant of PDG compared with doxorubicin, a known cytotoxic agent.

Conclusion

In conclusion, with the favourable physicochemical features (molar weight < 500 Da, topological polar surface area < 60, number of rotatable bonds < 5, logP < 5) and strong binding to the DDR1 type II tyrosine kinase protein, prodigiosin has shown potential for further development as tyrosine inhibitor targeting breast, colon and lung cancer.

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