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Original Article

Structure-based approach for drug discovery: *In-silico* molecular modelling and docking studies of tumor suppressor protein p53 with berberine, gallic acid, rutin and mangiferin

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ABSTRACT

p53, a tumor suppressor protein has a prominent role in forestalling tumor development and advancement through its involvement in cell division control and initiation of apoptosis. Hence p53 is an attractive drug target. Some alkaloids, xanthones and nutraceuticals have anticancer activities. Herein, berberine, gallic acid, rutin and mangiferin were evaluated for their binding efficiency and identification of active drug binding sites. p53 plays an important role in cell cycle regulation. Berberine, gallic acid, rutin and mangiferin were screened for binding residues and possible interaction with p53 (PDB ID-2VUK). The MOL2 form of selected structures was developed by running Open Babel software. Depending on the binding affinity values the models were selected and executed.

KEYWORDS: p53, berberine, gallic acid, rutin, mangiferin.

INTRODUCTION

Tumor protein p53 is also known as p53 (393 aa). This homolog is necessary for multicellular organisms, in which it prohibits tumor development and hence, works as a tumor suppressor. p53 is known to protect the cell integrity and preserve the genome by preventing mutation [1]. p53 is inactivated due to mutation in most of the cancers. In most types of cancer, overexpression of murine double minute 2 (MDM2) often leads to inactivation of p53 [2]. Generally, one-third of the alterations decreases the melting temperature of the protein molecule, contributing to its accelerated denaturation. Natural products binding to mutants will increase the stability of p53 and could be potent anticancer lead molecules. Natural products represent a large class of anticancer compounds, ranging from complex molecules like paclitaxel and vinblastine to simple molecules like berberine [3]. Here in this study we selected rutin, berberine, galic acid and mangiferin, to evaluate the interactions with p53.

MATERIALS AND METHODS

AutoDock Vina (v.1.1.2) was used for docking studies. Molecular interactions were calculated using protein-ligand interaction profiler (PLIP) accessible through http://www.projects.biotec.tu-dresden.de and surface area specifications were calculated by the PDBePISA server (http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver). Pymol (www.pymol.org, PyMol-Version 1.6. Schro¨dinger, LLC.) was used to generate the structural models.

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RESULTS AND DISCUSSION

A surface cavity formed at the site destabilizes the protein (4 kcal/mol), which is a cause of Y220C mutation, which has been observed in more than 75,000 cases related to cancer [4]. p53 mutants will be stabilized by the binding of small molecules and this can be shown by a DNA double-strand [5], heparin [6], or a peptide bound to p53 protein structure [7]. The selected natural products are targeted on the p53 core domain, hence the p53 core domain should also bind to all other mutant forms of p53. Berberine, mangiferin, gallic and rutin have been suggested to stabilize the p53 core domain in folded state., e.g., CP-31398 [8] and do not bind reversibly to the core domain of p53 [9, 10]; this provides another way to inhibit cancer other than stabilising the p53 [11]. A simple binding model of PhiKan083 was obtained by fitting the kinetics of denaturation p53C-Y220C at 37 °C. Protein half-life was increased from 3.8 min to 15.7 min. PhiKan083 binds to Y220C with plausible affinity [12]. The study of hydrophobic packing interactions indicates the significant molecular dynamics to understand the binding moieties. Two alternative conformation were adopted by Cys-220. The ethyl moiety is in close proximity to sulfhydryl moiety of Cys-220 and is also close to the side chains of hydrophobic aminoacids (Phe-109, Leu-145, Val-147, and Leu-257). These amino acid moieties are identified as binding ligands to the pocket of p53. The hydrophobic side chains of Pro-222 along with Pro-223 on one side of the cleft and Val- 147 along with pro-151 on the other side of the cleft sandwiches the planar carbazole ring. The ring nitrogen is positioned near to the location of the hydroxyl moiety of the tyrosine residue in the unmutated form of p53. (1.0-Å distance). The *N*-methyl methanamine moiety forms a hydrogen bond with the main-chain carbonyl of Asp-228 (2.7-Å distance). Ligand binding to the protein causes a small structural shift. The amino acid residues 109, 145-147, 150, 151, 220-223, 228-230, and 257 are within 5 Å of PhiKan083 which is superimposed with rmsd of 0.3 Å. The most significant shift is observed for the side chain of Thr-150, which is displaced by up to 1.4 Å upon binding, thus widening the entrance of the pocket, at the site of mutation in the oncogenic forms of p53. Hence, tumor protein p53 dynamics is an important target for anticancer drug discovery. Docking studies have been carried out with berberine, gallic acid, mangiferin, and rutin (Figure 1) with major binding site of p53 human protein. The structure of the ligand molecules - berberine, gallic acid, mangiferin, and rutin in SDF format were downloaded. The MOL2 format of these structures was generated using Open Babel software [13]. Protein data bank (PDB) has seventeen p53 protein structure complexes with Phikan083 (PDB IDs: 2VUK, 2WGX, 2X0U, 2X0V, 2X0W, 2XWR, 2YBG, 2YDR, 4AGL, 4AGM, 4AGN, 4AGO, 4AGP, 4AGQ, 5G4M, 5G4N, 5G4O). High-resolution tubulin crystal structures complexed with Phikan 083 (PDB ID: 2VUK) were selected from PDB for further studies. Input files necessary for docking studies were prepared by removing the bound ligands, ions and water, and later polar hydrogen atoms were added to the protein molecule using AutoDockTools (v.1.5.6) [14]. The grid maps were created for both receptor and ligands based on the corresponding ligand binding sites. The docking studies were performed using AutoDock Vina (v.1.1.2) [15] and the docking parameters were kept as default values. Molecular interactions were calculated using protein-ligand interaction profiler (PLIP) accessible through http://www.projects.biotec.tu-dresden.de and surface area specifications were calculated by the PDBePISA server (http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver). The structural models were generated by Pymol (www.pymol.org, PyMol-Version 1.6. Schro¨dinger, LLC.).

The interaction of p53 human protein with natural products that act as anticancer agents was studied to identify the drug binding sites. p53 acts as a tumor suppressor in many tumor types; it induces growth arrest or apoptosis depending on the physiological circumstances and cell type. p53 is involved in cell cycle regulation as a trans-activator, that is known to negatively regulate cell division by controlling a set of genes. In order to investigate the extent of interactions involved between the most active compound berberine and the known active binding sites of p53 human protein, molecular docking studies were carried out. Initial docking was performed with the control molecules of C_{16} H_{18} N_2 (P83) (Figure 2)

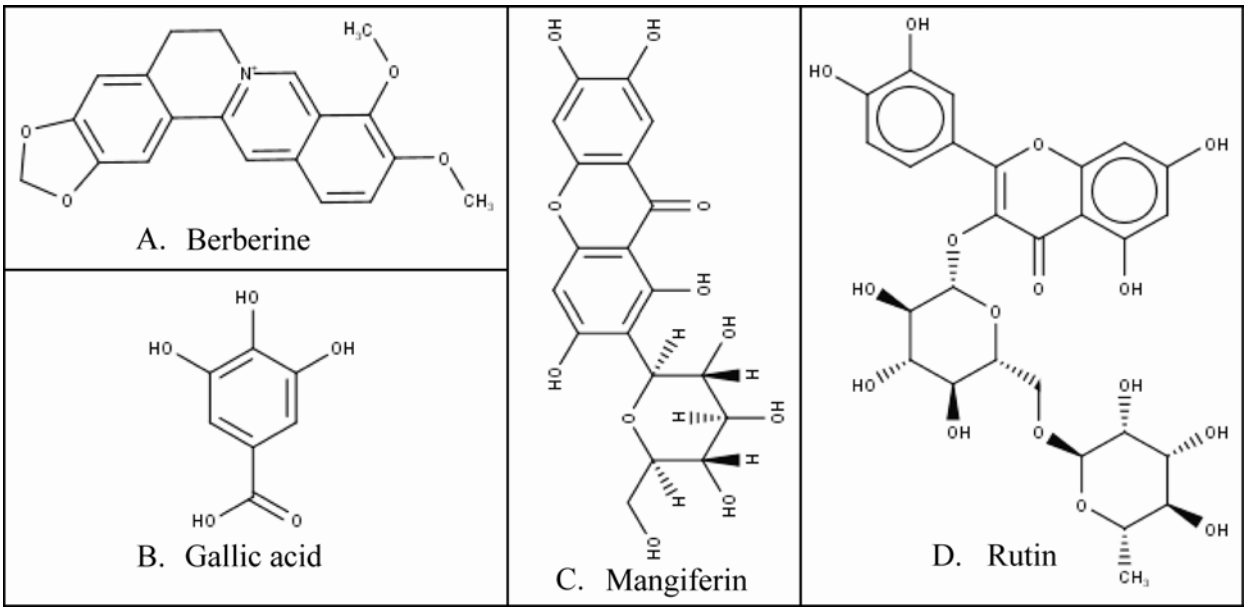

Figure 1. Chemical structures of p53 human protein bound to ligand: (A) berberine, (B) gallic acid, (C) mangiferin and (D) rutin.

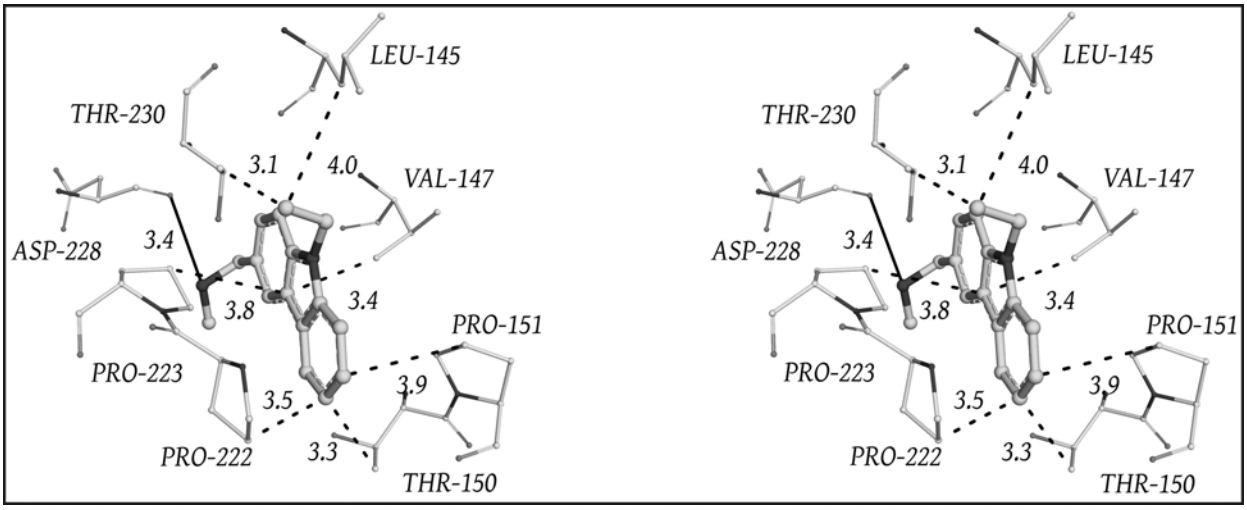

Figure 2. The wall-eyed stereo visualization of P83 interaction with p53 human protein (PDB ID-2VUK): (A) Ball and stick model of P83 interaction with p53 human protein.

to check the accuracy of molecular docking studies. Later, berberine, gallic acid, mangiferin and rutin were docked into same binding sites (Figure 3). Autodock Vina yielded different conformations for active binding sites. Selection of the final models was done based on the binding affinity values. The respective binding energies and computational molecular interaction details for each of the ligands are presented in Table 1.

CONCLUSION

The large set of species-specific p53 target genes contains multiple transcription factors which likely contribute to the species-specific p53-dependent

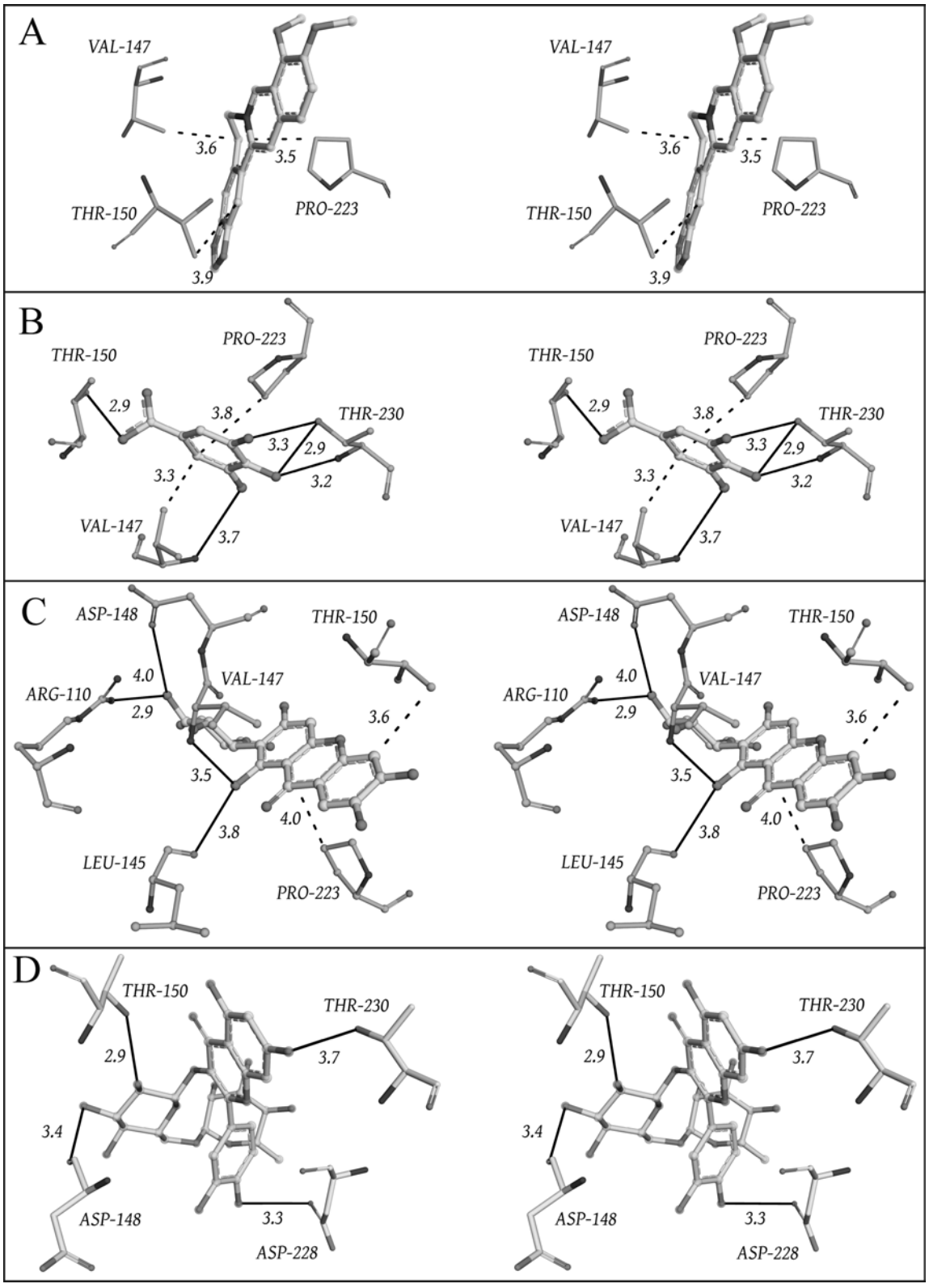

Figure 3. The wall-eyed stereo representation and molecular interactions of targeted ligands (A) berberine, (B) gallic acid, (C) mangiferin and (D) rutin with p53 human protein (PDB ID-2VUK).

Table 1. Molecular docking results of ligands berberine, gallic acid, mangiferin, and rutin with binding site of p53 human protein.

Molecular docking studies of tumor suppressor protein p53 using AutoDock-Vina					
Standard drug molecules					
Chemical properties	**P83 (Carbazole derivative)**	**Berberine**	**Gallic acid**	**Mangiferin**	**Rutin**
Molecular formula	C_{16} H_{18} N_2	$C_{20}H_{18}NO_4$+	$C_7H_6O_5$	$C_{19}H_{18}O_{11}$	$C_{27}H_{30}O_{16}$
Molecular weight (g/mol)	238.328	336.3612	170.12	422.33	610.52
Over all solvent-accessible area, (A° 2)	451.1	10202.9	10202.9	10202.9	10202.9
Binding affinity (kcal/mol)	-5.7	-7.2	-5.5	-6.6	-5.8
Hydrogen bonding of p53 (amino acids)	Asp228	-	Val147, Thr150, Thr230	Arg110, Leu145, Val147, Asp148	Asp148, Thr150, Asp228, Thr230
Hydrophobic bonding of p53 (amino acids)	Leu145, Val147, Thr150, Pro151, Pro222, Pro223, Thr230	Val147, Thr150, Pro223	Val147, Pro223	Thr150, Pro223	-
Solvent-accessible area, interface, area of protein (A° 2)	359.3	108.2	78.9	58.0	7.9

regulation of more than a thousand protein-coding genes. Natural products were the source of the drugs used by mankind in the past. Natural products are also the source of new drugs and drug lead molecules. The cell cycle thus offers several targets for therapeutic intervention, and several of the proteins involved either directly or indirectly in controlling this cycle are the targets of some important anticancer agents. The current study gives an overview of possible binding sites of p53 for berberine, gallic acid, rutin and mangiferin.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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