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Docking of Three Oral Carcinoma Targets with Reserpine

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ABSTRACT
Reserpine is a drug that is used for the treatment of high blood pressure and for relief of psychotic symptoms. Reserpine is also known for its contribution in suppression of cancer cells. In this study, we are concerned about three oral carcinoma targets, namely, Caspar 3, Bcl-2 and p53. Two softwares were used for docking. ACD/ChemSketch is an advanced chemical drawing tool and contains tools for 2D structure cleaning, 3D optimization and viewing. GOLD is a program for calculating the docking modes of small molecules in protein binding sites and is provided as part of the *GOLD Suite*, a package of programs for structure visualisation and manipulation (Hermes), for protein-ligand docking (GOLD) and for post-processing (GoldMine) and visualisation of docking. Results show that Reserpine inhibits BCL-2 with the highest GOLD score of 68.4 when compared to the other targets. Hydrogen bond was high in reserpine-BCL-2 complex and was given the high gold score when compared with the other two targets. Docking is just the preliminary phase for any in vitro study. Since docking is high in reserpine with bcl-2, In-vitro study can be carried out and further binding capacity can be studied.

INTRODUCTION
Oral cancer is cancer that starts in the mouth or throat. Oral cancer is fairly common and very curable if found and treated at an early stage. A doctor or dentist usually finds oral cancer in its

early stages because the mouth can be easily examined. More than 90% of all oral cavity tumors are squamous cell carcinoma. Squamous cells make up the lining of the oral cavity (the mucosa). As cancer in the mouth's lining grows, it can spread deeper into the mouth's nearby tissues.
Verrucous carcinoma is another type of oral cancer. It's considered a type of squamous cell carcinoma, but this low-grade cancer rarely spreads to distant sites (metastasizes). It accounts for less than 5% of all diagnosed oral cancer.
Other much less common types of oral cancer include salivary gland tumors, including adenoid cystic carcinoma, adenocarcinoma, and other types of salivary gland cancer.

Resrpine is a drug that is used for the treatment of high blood pressure. It is usually taken along with a thiazide diuretic or vasodilator.[1] Large clinical trials have shown that combined treatment with reserpine plus a thiazide diuretic reduces mortality of people with hypertension.
The antihypertensive actions of reserpine are largely due to its antinoradrenergic effects, which are a result of its ability to deplete catecholamines (among other monoamine neurotransmitters) from peripheralsympathetic nerve endings. These substances are normally involved in controlling heart rate, force of cardiac contraction and peripheral vascular resistance.[2]
At doses of 0.05 to 0.2 mg per day, reserpine is well tolerated;the most common adverse effect being nasal stuffiness.
Reserpine has also been used for relief of psychotic symptoms. Reserpine is one of dozens of indolealkaloids isolated from the plant Rauvolfia serpentina. In the rauwolfia plant, tryptophan is the starting material in the biosynthetic pathway of reserpine, and is converted to tryptamine by tryptophan decarboxylase enzyme. Tryptamine is combined with secologanin in the presence of strictosidine synthetase enzyme and yields strictosidine. Various enzymatic conversion reactions lead to the synthesis of reserpine from strictosidine.[3] Reserpine is also known for its contribution in suppression of cancer cells.

In this study, we are concerned about three oral carcinoma targets, namely, Caspar 3, Bcl-2 and p53.
Caspases are crucial mediators of programmed cell death (apoptosis). Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins. However, the specific requirements of this (or any other) caspase in apoptosis have remained largely unknown until now. Pathways to caspase-3 activation have been identified that are either dependent on or independent of mitochondrial cytochrome c release and caspase-9 function. Caspase-3 is essential for normal brain development and is important or essential in other apoptotic scenarios in a remarkable tissue-, cell type- or death stimulus-specific manner.[4]Caspase-3 is also required for some typical hallmarks of apoptosis, and is indispensable for apoptotic chromatin condensation and DNA fragmentation in all cell types examined. Thus, caspase-3 is essential for certain processes associated with the dismantling of the cell and the formation of apoptotic bodies, but it may also function before or at the stage when commitment to loss of cell viability is made.[5]

Bcl-2 (**B-cell lymphoma 2**), encoded in humans by the ***BCL2***gene, is the founding member of the Bcl-2 family of regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis.[6]

p53 has been described as "the guardian of the genome" because of its role in conserving stability by preventing genome mutation.[7]

In designing of drug based on its structure, molecular docking is the most well-known strategy which has been broadly utilized as far back as the mid 1980s. Projects based on various calculations were produced to perform atomic docking examines, which have made docking an inexorably vital instrument in pharmaceutical research. Different astounding surveys on docking have been distributed previously and numerous correlation studies were directed to assess the overall accuracy of the studies.[8]

docking is the first step to any invitro study. The aim of molecular docking is to predict The binding capacity is first seen with the help of molecular docking software. If binding is more than 50%, then the study can be carried out in vitro where accurate results can be expected. If invitro is carried out directly without first trying docking and the results are not accurate then it's totally waste of money as intro vitro setup is much expensive. Docking is nothing but the study of lock and key mechanism. The orientation of binding of the ligand and receptor is studied in docking of drug to target. Docking can be done through two interrelated steps: first by sampling conformations of the ligand in the active site of the protein; then ranking these conformations with a scoring function. Hence in this study, the molecular structure of reserpine was identified through docking software and its binding capacity was seen with each of the oral carcinoma target i.e, caspase 3, Bcl-2 and p53.

Materials and methods

ACDlabsChemsketch

ACD/ChemSketch is an advanced chemical drawing tool and is the accepted interface for the industry's best NMR and molecular property predictions, nomenclature, and analytical data handling software.

ACD/ChemSketch is also available as freeware, with functionalities that are highly competitive with other popular commercial software packages. The freeware contains tools for 2D structure cleaning, 3D optimization and viewing, InChI generation and conversion, drawing of polymers, organometallics, and Markush structures—capabilities that are not even included in some of the commercial packages from other software producers. Also included is an IUPAC systematic naming capability for molecules with fewer than 50 atoms and 3 rings. The capabilities of ACD/ChemSketch can be further extended and customized by programming.

GOLD - Protein-Ligand Docking

GOLD is a program for calculating the docking modes of small molecules in protein binding sites and is provided as part of the *GOLD Suite*, a package of programs for structure visualisation and manipulation (Hermes), for protein-ligand docking (GOLD) and for post-processing (GoldMine) and visualisation of docking results. Hermes acts as a hub for many of CCDC's products, for more information please refer to the Hermes product page.

The product of a collaboration between the University of Sheffield, GlaxoSmithKline plc and CCDC, GOLD is very highly regarded within the molecular modelling community for its accuracy and reliability.

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GOLD features include:

• A genetic algorithm (GA) for protein-ligand docking
• An easy to use interface with interactive docking set-up via Hermes
• A comprehensive docking set-up wizard
• Full ligand flexibility
• Partial protein flexibility, including protein side chain and backbone flexibility for up to ten user-defined residues
• Energy functions partly based on conformational and non-bonded contact information from the CSD
• A variety of constraint options
• Improved flexible ring handling
• Automatic consideration of cavity bound water molecules
• Improved handling and control of metal coordination geometries
• Improved parameterisation for kinases and heme-containing proteins
• Automatic derivation of GA settings for particular ligands
• A choice of GoldScore, ChemScore, Astex Statistical Potential (ASP) or Piecewise Linear Potential (PLP) scoring functions
• Extensive options for customising or implementing new scoring functions through a Scoring Function Application Programming Interface, allowing users to modify the GOLD scoring-function mechanism in order to either: implement their own scoring function or enhance existing scoring functions; customise docking output
• A ChemScore Receptor Depth Scaling (RDS) rescore option so that the score attributed to hydrogen bonds is scaled depending on the depth in the binding pocket
• Automatic rescoring with an alternate scoring function at the end of a docking run.

GOLD's genetic algorithm parameters are optimised for virtual screening applications. GOLD is optimised for parallel execution on processor networks; a distributed version of GOLD is available for use on commercial PC GRID systems.

Targets: Caspase 3 , BCL-2 , P53

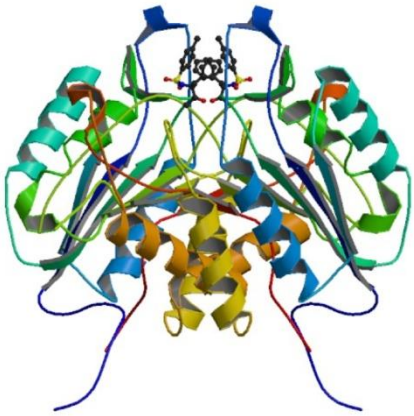

Fig 1- CRYSTAL STRUCTURE OF CASPASE 3(2XYG)

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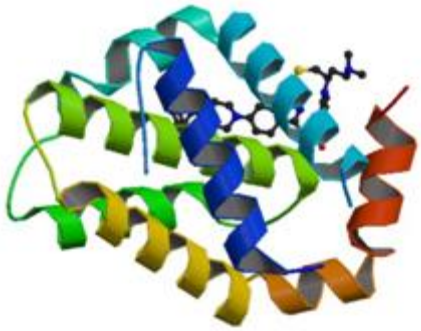

Fig 2- CRYSTAL STRUCTURE OF Bcl-2(4IEH)

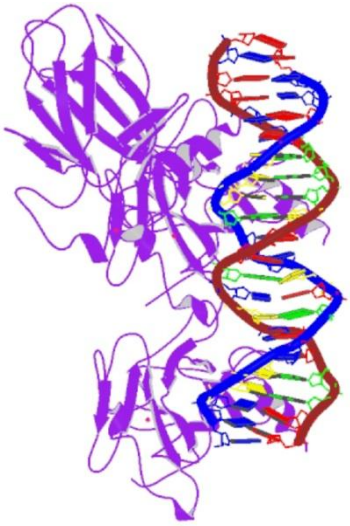

Fig 3- CRYSTAL STRUCTURE OF P53 (1TSR)

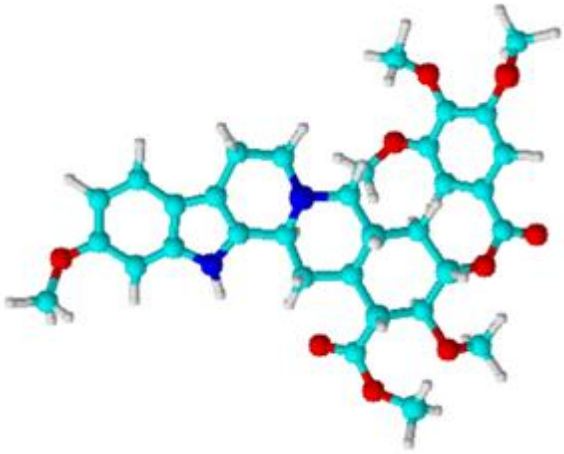

Fig 4- STRUCTURE OF RESERPINE

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ACTIVE SITE OF CASPASE 3

MET39A , CYS 163A , GLY 165A , TYR 204B , HIS 121A , GLY 122A , MET 61A , THR 62A , GLY 60A , PHE 128A , THR 59A , THR 166A , GLU 123A , SER 205B , TYR83 ,PHE114.

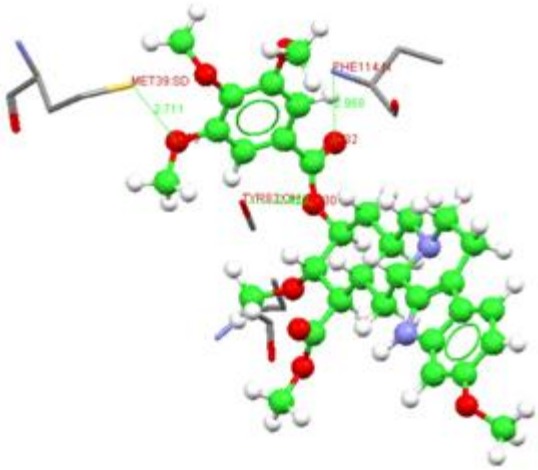

Fig 5- RESPIRINE IN COMPLEX WITH CASPASE -3

ACTIVE SITE OF BCL-2

TYR 161A , ARG 66A , ASP 62A, ALA 59A , PHE 63A , VAL 107A , GLY 104A , LEU 96A , VAL 92A , GLU 95A , MET 74A , ALA 108A , PHE 112A , TYR 67A , ARG 105A , PHE 71A , VAL 115A , PHE 109A

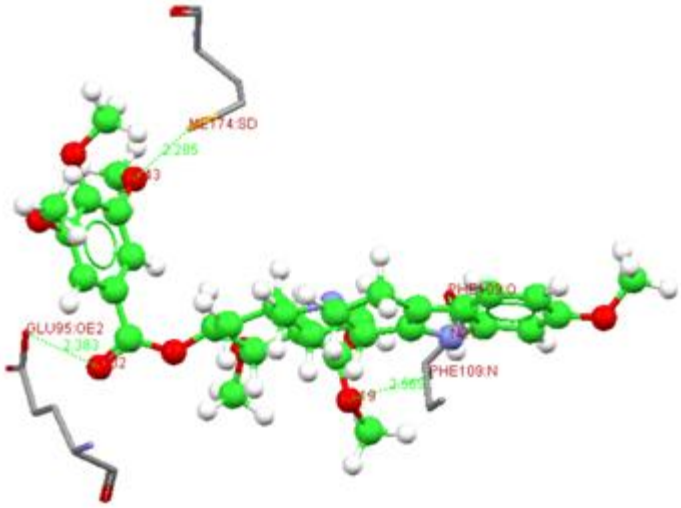

Fig 6- RESPIRINE IN COMPLEX WITH BCL-2

ACTIVE SITE OF P53

SER95 , SER96 , VAL97 , PRO98 , SER99 , SER121 , THR123 , ALA138 , LYS139 , THR140 , ARG158 , MET160 , SER166 , HIS168 , MET169 , THR170 , GLU171 , VAL172 , ARG174 , CYS176 , PRO177 , HIS178 , HIS179 , ARG181 , CYS182 , SER185 , ASP186 , LEU188 , ARG196 , GLU198 , GLY199 , ASN200 , LEU201 , ARG202 , LEU206 , ASP208 , THR211 , PHE212 , ARG213 , VAL218 , GLU221 , PRO222 , PRO223 , GLU224 , VAL225 , THR230 , THR231 , HIS233 , ASN235 , ASN239 , SER240 , SER241 , CYS242 , MET243 , ASN247 ,

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ARG248 , ARG249 , THR256 , GLU258 , GLY262 , ASN263 , LEU264 , ARG267 , ARG273 , VAL274 , CYS275

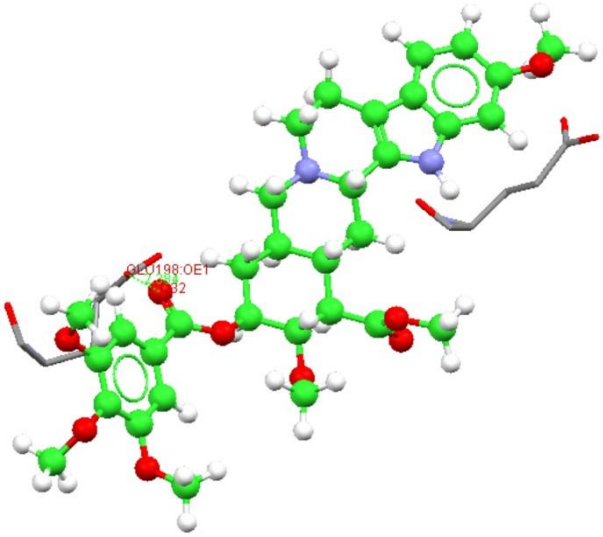

Fig 7- RESERPINE IN COMPLEX WITH P53

RESULTS-

INTERACTIONS OF RESERPINE WITH THE ORAL CARCINOMA TARGETS

Protein	Atom in Ligand	Atom in Protein	H-Bond Distance	Score
CASPASE 3	O29	MET39:SD	2.711	48.14
	O30	TYR83:OH	2.429	
	O32	PHE114:N	2.968	
BCL-2	O43	MET74:SD	2.285	68.4
	O32	GLU95:OE2	2.383	
	O19	PHE109:N	2.584	
	N21	PHE109:O	1.451	
P53	O32	GLU198:OE1	2.384	45.98

Reserpine inhibits BCL-2 with the highest GOLD score of 68.4 when compared to the other targets.

DISCUSSION-

There are three types of chemical bonds namely covalent bond, hydrogen bond and vanderwaal bond. Among these three, vanderwaal bond is the weakest[9], so the drug will detach easily. Covalent bond is the strongest[10] but to break the bond for deactivation is very difficult. Hence only hydrogen bonds were evaluated in this study as they are neither too strong nor too weak[11]. So the hydrogen bond was high in reserpine-BCL-2 complex and was given the high gold score when compared with the other two targets.

CONCLUSION-

Docking is just the preliminary phase for any in vitro study. Since docking is high in reserpine with bcl-2, In-vitro study can be carried out and further binding capacity can be studied.

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