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"Study of Ligand Based Virtual Screening Tools in Computer Aided Drug Designing For Oral Cancer"

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Abstract: *Insilico potential drug target identification involves chemical compounds for inhibiting or promoting chemical reactions in living organism computational methods are needed for screening through large database of these to identify a inhibitor for receptor 0ral Multi-AGC Kinase was employed for docking.We investigated the detailed pharmacology and antitumour activity of the novel clinical drugs and natural ligand. It is associated with Oral Multi-AGC kinase protein. The various ZINC analogs on basis of 99%, 95%, 90% similarity for best docking results with protein for predicting a ZINC analog which can be identified under the category of becoming a potential drug candidate in future. The best mol dock score were for ZINC analog ZINC ID 72131268 for Irinotecan is -169.087 kcl/mol, ZINC ID 04166028 for Teniposide is -178.235 kcl/mol, ZINC ID 30731084 for Topotecan is -166.939 kcl/mol and ZINC ID 00899824 for Curcumin is -141.537 kcl/mol.*
The two parameter for toxicity properties i.e. Oral LD50 and Mutagenicity were calculated. Oral LD50 and Mutagenicity values are found.

Keywords: *Computational methods screening, Database, Insilico, Drug target, Inhibitor, Receptor*.

I. Introduction

Cancer of the oral cavity and pharynx is the first and third commonest cancer in Indian men and women, respectively. Where as in most areas at high risk for cancer of the oral cavity other than India (e.g., central and Eastern Europe, South America),the ratios between male and female incidence rates range between and 10, in India the male-to-female ratio is approximately 1 (e.g.,Madras) or lower than 0.5 (Bangalore). Such very high incidence rates in Indian women reflect the persistent importance in India of paan chewing, a habit that is equally common in the 2 genders.(1)

Oral cancer most commonly occurs in middle-aged and older individuals, although a disturbing number of these malignancies is also being documented in younger adults in recent years from an epidemiological and clinic pathological perspective, "oral cancer" can be divided into three categories: Carcinomas of the oral cavity (Squamous-cell carcinoma of the tongue is the most common oral malignancy, accounting for 25-40% of all oral carcinomas. Characteristically, it appears as an indurated, nonhealing ulcer with elevated margins. Occasionally, the neoplasm may have a prominent exophytic as well as an endophytic growth pattern. Metastasis from the tongue toipsilateral lymph nodes of the neckis relatively common). Carcinomas of the lip Vermilion (Lip carcinomas account for 25-30% of all oral cancers. The slower growing, more common carcinoma of the lower lip has a better prognosis than upper-lip lesions. Lesions occur on the vermilion border and may appear as chronic nonhealing ulcers or exophytic lesions that are occasionally verrucous. Deeper invasion, clinically characterized by induration, occurs later in the course of the disease, and metastases to submental and submandibular lymph nodes are more likely with histologically less-differentiated lesions and more advanced lesions). Carcinomas arising in the oropharynx (The floor of the mouth is the second most common intraoral location for squamous-cell carcinomas, accounting for 15-20% of all cases. The usual appearance is a red or white, painless, nonhealing, indurated ulcer. Occasionally, the lesion may widely infiltrate the soft tissues of the floor of the mouth, causing decreased mobility of the tongue, characterized clinically by alteration of speech. Altered speech, persistent hoarseness or chronic cough with or without bloody sputum (hemoptysis) may indicate laryngeal metastasis or malignancy). Intraoral and oropharyngeal tumors are more common among men than women, with a male female ratio of over 2:1, However, the disparity in the malefemale ratio has become less pronounced over the past half century, probably because women have been more equally exposing themselves to known oral carcinogens such as tobacco and alcohol.Current research into chemotherapy and photodynamic therapy may provide additional modalities in the future. Verrucous carcinoma, a less common tumour, represents 4.5 - 9% of oral squamous-cell carcinomas. It typically presents as a slowly enlarging, gray or white, warty, exophytic growth on the buccal mucosa or gingiva of older men with an average age of 65 years at time of diagnosis. (2, 3)

Cancer evolves in a series of distinct steps, each characterized by the sequential accumulation of additional genetic defects followed by clonal expansion.1 It has been long established that smoking, alcohol consumption, as well as tobacco chewing are risk factors linked to the development of oral cancer. Other

suspected aetiological factors include viruses such as human papilloma virus and Epstein barr virus, as well as Candida albicans, areca (betel nut) chewing, diets low in carotenoids and vitamin A and several measures of poor oral hygiene, including frequency of tooth brush use. (4)

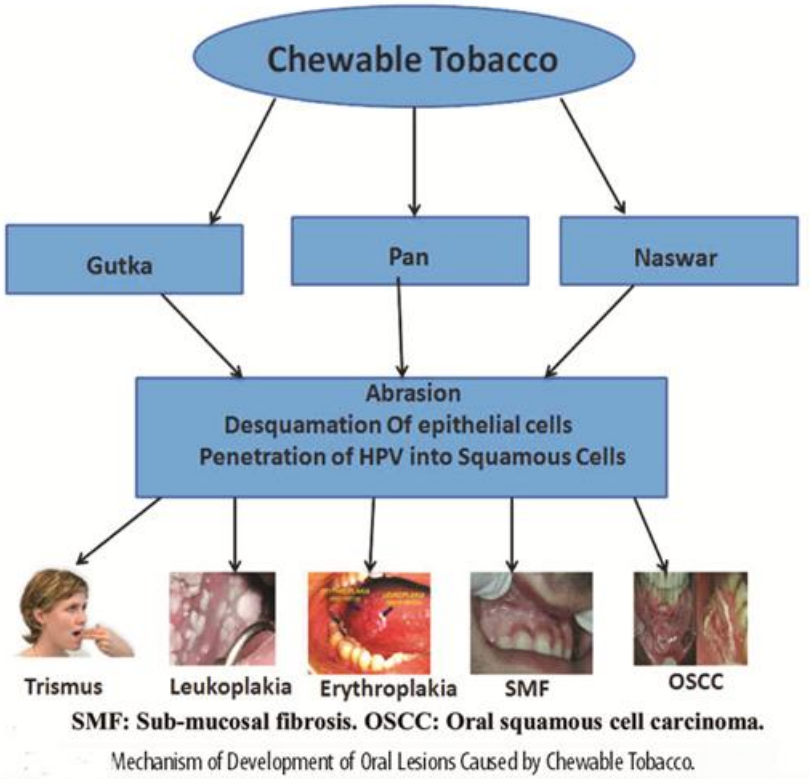


Figure 1: (REF:www.intechopen.com)

Role of protein Oral Multi-AGC Kinase-

The class I phosphoinositide 3-kinases (PI3K) are key mediators of intracellular signaling between the membrane bound receptor tyrosine kinases (RTKs) and down stream effector molecules, which control many vital cellular functions ,including survival,growth, proliferation, and motility (8,9) Downstream of these PI3Ks lies a network of serine/threonine kinases, including several members of the AGC kinase family, such as AKT, also known as protein kinase B(PKB), phosphoinositide-dependent kinase 1 (PDK1),p70S6 kinase (p70S6K), p90 ribosomal S6 kinase (RSK),serum- and glucocorticoid-induced kinase (SGK), and Rho kinase (10,11). The PI3K-AKT axis of this signaling network is hyperactivated in multiple cancers through different mechanisms, including the deregulation of upstreamRTKs, for example, insulin-like growth factor-1 receptor(IGF-1R), and genetic alterations of PIK3CA, PTEN, or AKT genes,AKT1, 2, and 3 (8,9). Thus, pharmacologic inhibition of this pathway is an area of great therapeutic interest (12).

Several drugs targeting the PI3K-AKT pathway are currentlyin clinical development, including inhibitors of PI3K, AKT, and mTORC1/2 (12,13) . However, inhibiting PI3K-AKTsignaling at a single node has shown relatively limitedclinical efficacy to date. There are several possible explanations for this. First, AKT inhibition has been shown torelieve feedback suppression of RTK expression and activity,which may attenuate antitumor activity (14). Second, PI3Kderegulation may promote cancer through both AKT- dependent and AKT-independent mechanisms, the latter involving the AGC kinases PDK1 and SGK (15). Third,inhibition of a single node such as PI3K or AKT may allow clinical resistance as reported for the selective BRAF inhibitor vemurafenib (15).Furthermore ,concurrent blockade of multiple components of the PI3K network may have greater therapeutic value than inhibition of any single target . Thus, the simultaneous inhibitionof several essential nodes of the PI3K signaling network may provide greater overall suppression of key pathways, with the potential for improved therapeutic efficacy across abroader range of cancer types with less opportunity forresistance to develop.(16)

Human Papillomavirus life cycle for Oral Cancer –

Papillomaviruses infect epithelial cells, and depend on epithelial differentiation for completion of their life cycle.The expression of viralgene products is closely regulated as the infected basal cell migrates towards

the epithelial surface. Expression of E6 and E7 in the lower epithelial layers drives cells into S-phase, which creates an environment that is conducive for viral genome replication and cell proliferation.

Genome amplification, which is necessary for the production of infectious virions, is prevented until the levels of viral replication protein arise, and depends on the co-expression of several viral proteins. Virus capsid proteins are expressed in cells that also express E4 as the infected

cell enters the upper epithelial layers. The timing of these events varies depending on the infecting papillomavirus, and in the case of the high-risk human papilloma viruses (HPVs), on the severity of neoplasia. Viruses that are evolutionarily related, such as HPV1 and canine oral

papillomavirus (COPV), generally organize their productive cycle in a similar way, despite infecting different hosts and epithelial sites. In some instances, such as following HPV16 infection of the cervix or cottontail rabbit papillomavirus (CRPV) infection of domestic rabbits papillomaviruses can undergo abortive infections in which the productive cycle of the virus is not completed. As with other DNA tumourviruses, such abortive infections can predispose to cancer.(17)

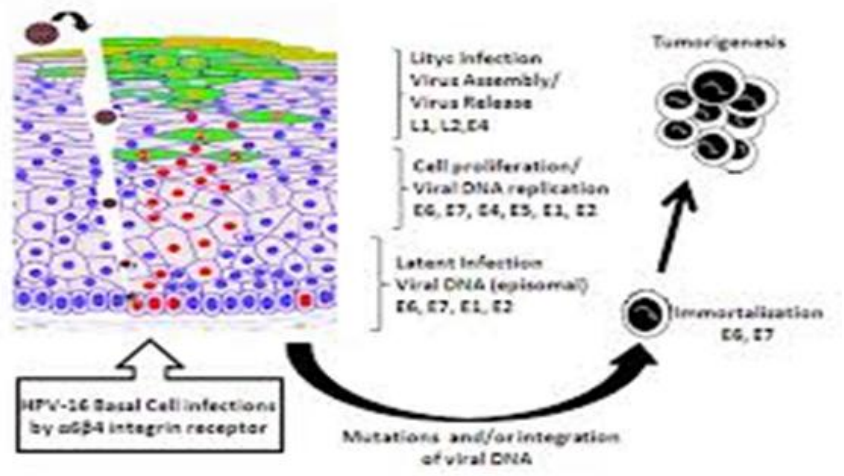


Figure2: (REF:Human Papilloma Virus — role in precancerous and cancerous oral. jpma.org.pk)

II. Methodology

Retrieval of the target protein

The amino acid sequence of protein Oral Multi-AGC Kinase PDB ID 4AXA was retrieved from RCSB (http://www.rcsb.org) FASTA sequence of Oral Multi-AGC Kinase Protein structures obtained from RCSB, PDB ID 4AXA of protein Oral Multi-AGC Kinase has 351 residues of amino acids in length.

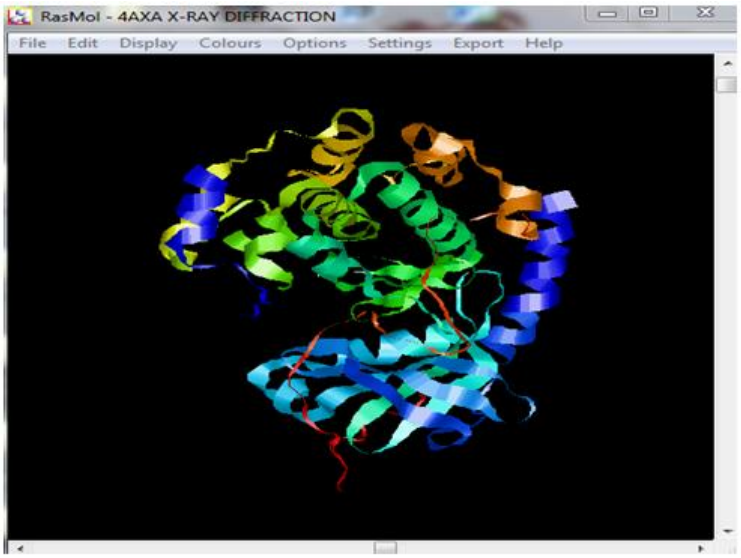


Figure 3:- Image of PDB ID 4AXA of protein Oral Multi-AGC Kinase .GEFTINIB and HISTONE DEATYLASE compound still in clinical trials and natural ligand CURCUMIN and drugs IRINOTECAN, TENIPOSIDE, TOPOTECAN, AMIFOSTINE, BISPHOSPHONATE selected into the ligands binding site detected by ACTIVE SITE PREDICTION www.scfbio-iitd.res.in/dock/ActiveSite.jsp

MOLEGRO VIRTUAL DOCKER was then used to perform virtual screening on the PDB ID 4AXA of protein Oral Multi-AGC Kinase using a screening set from the ZINC DATABASE (http://zinc.docking.org/) that contained 90%,95%,99% molecules for each of the inhibitors, the compounds in clinical trials and the natural ligands.

Preparations of the target protein and screening set

Compound set were prepared from the ZINC DATABASE in January 2013 based on two criteria: firstly, compounds were selected according to their (99%, 95%, 90%) similarity with the drugs, compounds for clinical trials and natural ligands selected and secondly, excluding compounds with multiple components. Eventually a set of structures was obtained that consisted of 700 compounds.

To reduce the complexity and running time of the computational program, the compounds were separated into number of different sets according to the extent of similarity on basis of percentage.

MOLEGRO VIRTUAL DOCKER then docked each compound in the screening set against this binding cavity and ranked each compound by the docked energy of the docked conformation.

The docked conformation of the selected compounds from ZINC DATABASE with the lowest scoring value was compared with docking values of the drugs, clinical trial compounds and the natural ligands selected and along with this the molecular recognition of the protein was also investigated to determine the constraints of the ligand during the virtual screening.

III. Result Analysis And Discussion

Recent progress in bioinformatics has brought about with it many protein structures for virtual screening as drug targets. **Cellular toxicity** further complicates biological activity assays as well as bioinformatics. Here, in taking the devise as less resourced demanding for screening process, given emphasis on computational approaches that are solely based on the structures of a pictorial region of the target.Oral Multi-AGC kinase protein.Then we performed virtual screening on a set of medical drugs because we recognized that using medical drugs could potentially minimize cellular toxicity.

The inhibitors of Oral Multi-AGC kinase protein were having high toxicity and side effects so, the computer aided insilico drug target for selection of potent inhibitors for Oral Cancer protein was performed. The basis of selection of a potent inhibitors for Oral Multi-AGC kinase protein was to select a particular ZINC analog (Which is similar to the drugs and natural ligands up to 99% ,95% ,90% in structure with lesser toxicity and much higher stability with receptor as compared of drug and natural Ligands, after the comparison of the docking result of ZINC analog with the respective drugs and natural ligands, Irinotecan, Teniposide, Topotecan and Curcumin gave the best docking result among all.

Analysis Pdb Id 4AXA Of Oral Multi-AGC Kinase

Table 1:- Analysis PDB ID 4AXA of protein Oral Multi-AGC Kinase

S.No	Inhibitors Name	Zinc Analogs (Id)	MolDock Score	Compounds selected
1.	Geftinib	ZINC22056257	-134.312	
2.	**Irinotecan**	**ZINC72131268**	**-169.087**	**ZINC72131268**
3.	**Teniposide**	**ZINC04166028**	**-178.235**	**ZINC04166028**
4.	Bisphosphonate	ZINC19632883_1	-61.8548	
5.	**Topotecan**	**ZINC30731084**	**-166.939**	**ZINC30731084**
6.	Amifostine	ZINC34856648	-98.2773	
7.	**Curcumin**	**ZINC00899824**	**-141.537**	**ZINC00899824**

Analysis Of Selected Compounds

Compound 1

- **Inhibitor :-** Irinotecan
- **Zinc ID:-** ZINC ID 72131268

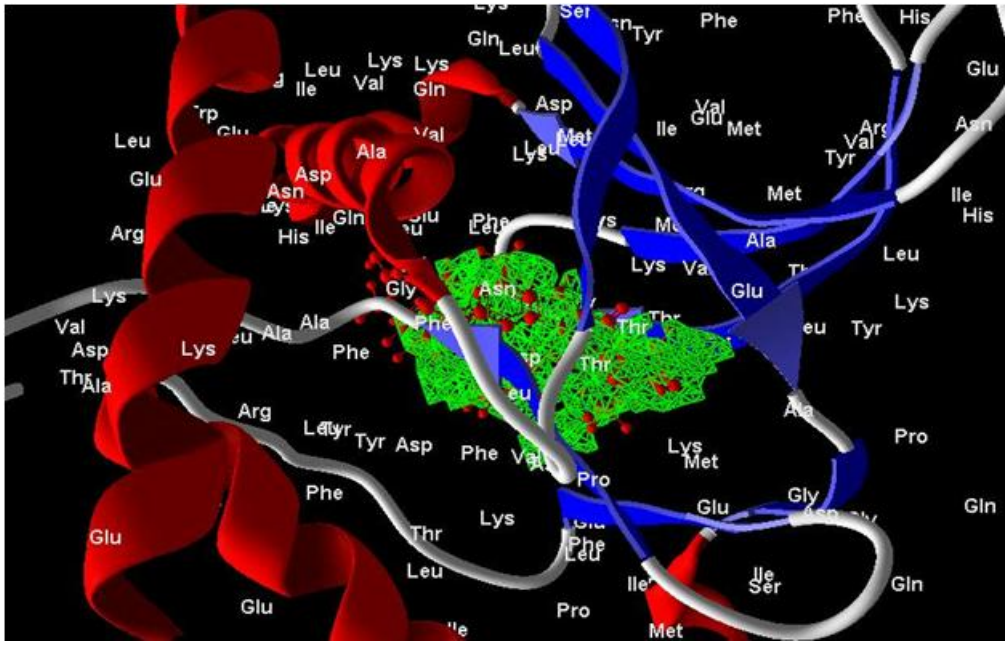


Fig :- 4 Docking image of PDB Id **4AXA**Of protein **OralMulti-AGC Kinase** and**ZINCID 72131268,** Moldock score-**169.087Kcal/mol .**Amino Acid residues at ligand binding site are **Asn, Thr ,Asn ,Gly, Phe**

Compound 2

- **Inhibitor :-**Teniposide
- **Zinc ID:-** ZINC ID 04166028

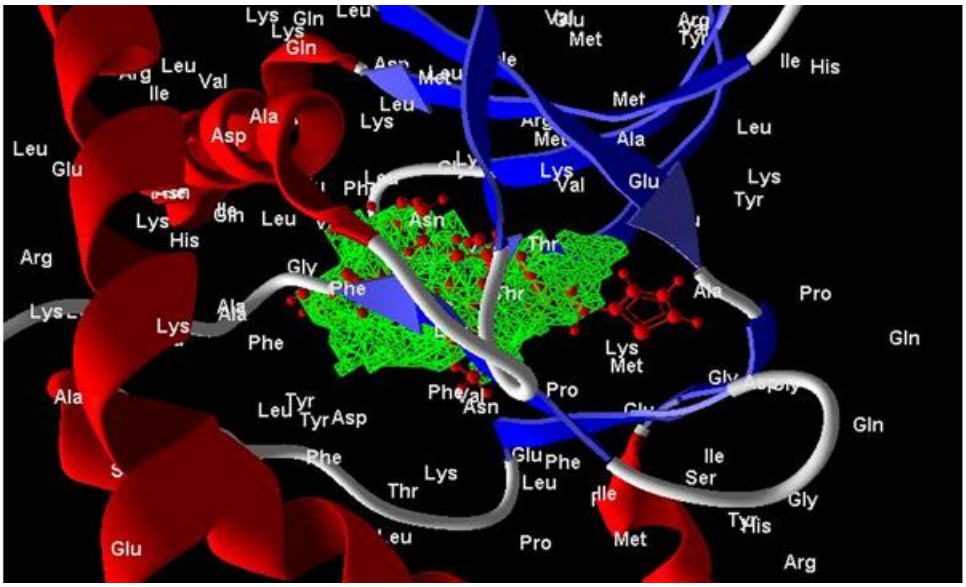


Fig 5:- Docking image of PDB ID **4AXA** Of protein **OralMulti-AGC Kinase** and **ZINC ID** 04166028.Moldock score-178.235**Kcal/mo .** Amino Acid residues at ligand binding site are**Asn, Thr ,Asn ,Gly, Phe**

Compound 3

- **Inhibitor :-**Curcumin
- **Zinc ID:-** ZINC ID00899824

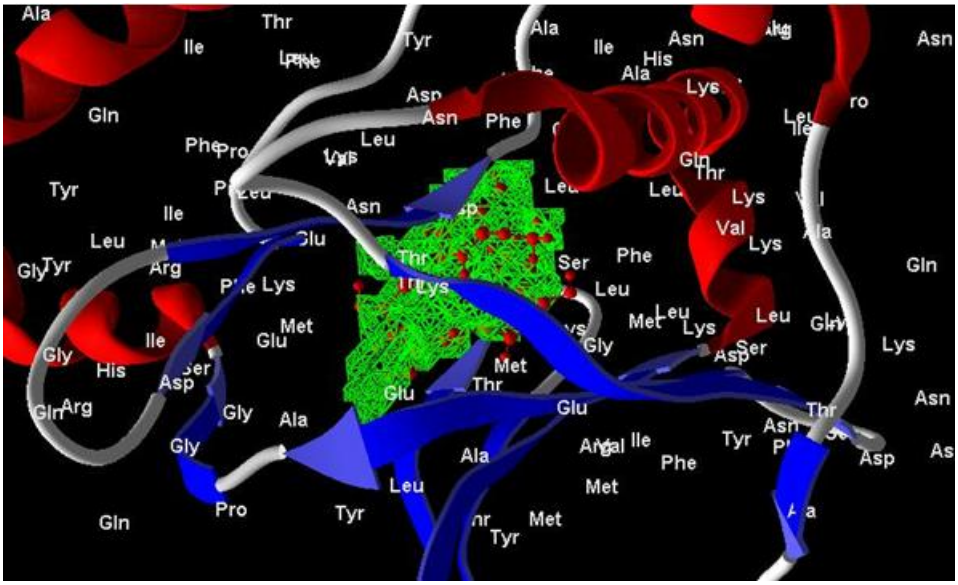


Fig 6:- Docking image of **PDB ID 4AXA** Of protein **Oral Multi-AGC Kinase** and **ZINC ID** 00899824. Moldock score-**141.537 Kcal/mol.** Amino Acid residues at ligand binding site are **Asp, Thr ,Asp Lys, Phe ,Glu**

COMPOUND 4

- **Inhibitor :-**Topotecan
- **Zinc ID:-** ZINC ID 30731084

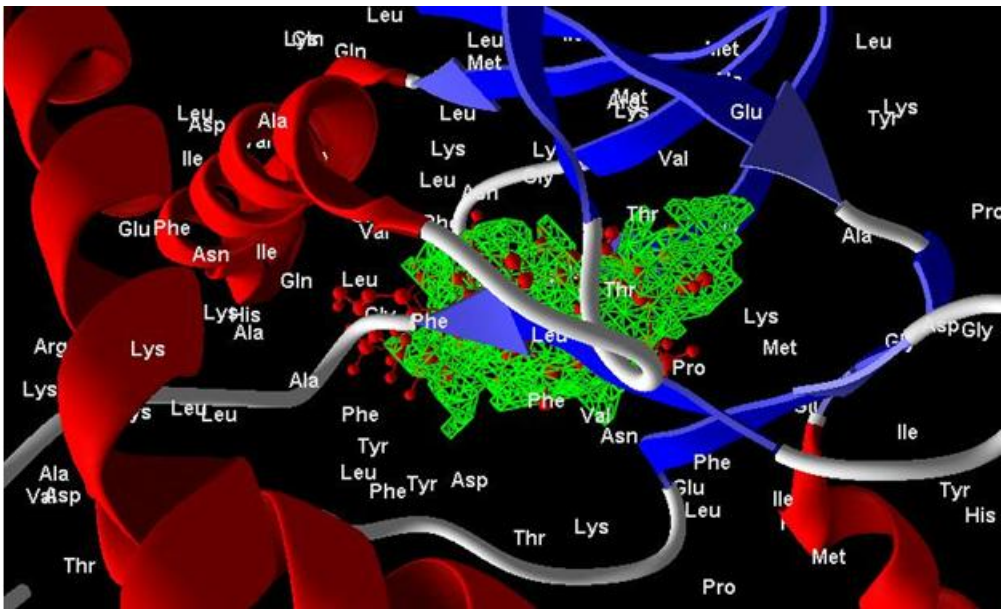


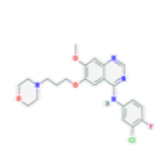
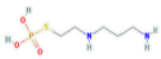
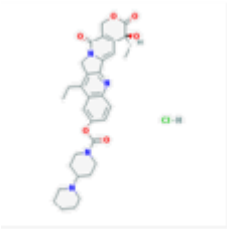
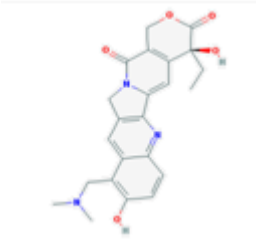
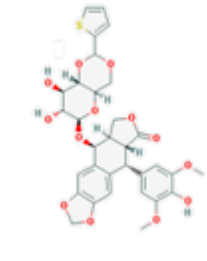
Fig 7:- Docking image of PDB ID **4AXA** Of protein **Oral Multi-AGC Kinase** and**ZINC ID 30731084**.Moldock score-**166.939Kcal/mol.** Amino Acid residues at ligand binding site are**Thr, Leu, Phe, Val, Gly**

Toxicity Prediction

Extent of the toxic effects of the drugs and the natural ligands were predicted using the software T.E.S.T (Toxicity Estimation Software Tool)(http://www.epa.gov/nrmrl/std/qsar/qsar.html).

The two properties of the drugs and the natural compounds were calculated i.e. Predicted Oral rat LD50 and the mutagenicity properties.

Table 2:-Predicted oral rat LD50 for drugs and natural ligands

S.No	Molecule	Prediction results			Individual predictions	
		End point	Experimental value	Predicted value	Method	Predicted value - Log10 (mol/kg)
1.	 GEFTINIB	Oral rat LD_{50} - Log10(mol/kg)	N/A	2.78	Hierarchical clustering	2.73
					FDA	3.25
		Oral rat LD_{50} mg/kg	N/A	742.92	Nearest neighbor	2.36
2.	 AMIFOSTINE	Oral rat LD_{50} - Log10(mol/kg)	N/A	N/A	Hierarchical clustering	N/A
		Oral rat LD_{50}mg/kg	N/A	N/A	FDA	N/A
					Nearest neighbor	N/A
3.	 IRINOTECAN	Oral rat LD_{50} - Log10(mol/kg)	N/A	N/A	Hierarchical clustering	N/A
		Oral rat LD_{50} mg/kg	N/A	N/A	FDA	N/A
					Nearest neighbor	N/A
4.	 TOPOTECAN	Oral rat LD_{50} - Log10(mol/kg)	N/A	3.49	Hierarchical clustering	2.62
		Oral rat LD_{50}mg/kg	N/A	136.60	FDA	3.58
					Nearest neighbor	4.27
5.	 TENIPOSIDE	Oral rat LD_{50} - Log10(mol/kg)	N/A	N/A	Hierarchical clustering	N/A
		Oral rat LD_{50} mg/kg	N/A	N/A	FDA	N/A
					Nearest neighbor	1.63
6.		Oral rat LD_{50} - Log10(mol/kg)	N/A	2.56	Hierarchical clustering	2.63
					FDA	2.45

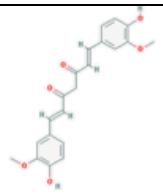
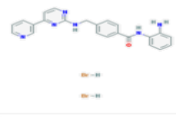
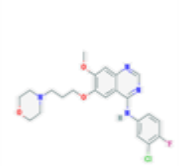
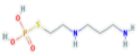
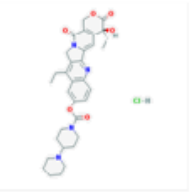
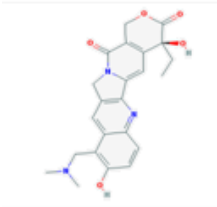
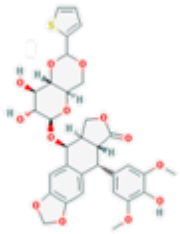
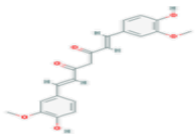
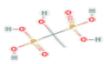
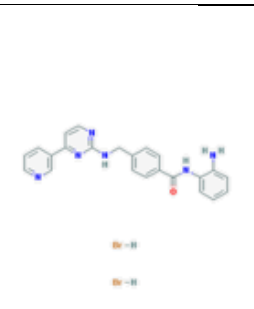
	 **CURCUMIN**	Oral rat LD_{50} mg/kg	N/A	1015.00	Nearest ighbor	2.60
7.	 **BISPHOSPHONATE**	Oral rat LD_{50} - Log10(mol/kg)	N/A	N/A	Hierarchical clustering	N/A
		Oral rat LD_{50}mg/kg	N/A	N/A	FDA	2.56
					Nearest neighbor	N/A
8.	 **Histone deactylase**	Oral rat LD_{50} - Log10(mol/kg)	N/A	2.21	Hierarchical clustering	N/A
		Oral rat LD_{50} mg/kg	N/A	2445.66	FDA	2.36
					Nearest neighbor	2.06

Table 3:-Mutagenicity properties for drugs and natural ligands

S.No	Molecule	Prediction results			Individual predictions	
		End point	Experimental value	Predicted value	Method	Predicted value - Log10 (mol/kg)
1.	 **GEFTINIB**	Mutagenicity value	N/A	0.43	Hierarchical clustering	0.65
		Mutagenicity result	N/A	Mutagenicity Negative	FDA	-0.03
					Nearest neighbor	0.67
2.	 **AMIFOSTINE**	Mutagenicity value	N/A	N/A	Hierarchical clustering	N/A
		Mutagenicity result	N/A	N/A	FDA	N/A
					Nearest neighbor	N/A
3.	 **IRINOTECAN**	Mutagenicity value	N/A	N/A	Hierarchical clustering	N/A
		Mutagenicity result	N/A	N/A	FDA	N/A
					Nearest neighbor	N/A

4.	 **TOPOTECAN**	Mutagenicity value	N/A	0.49	Hierarchical clustering	0.13
		Mutagenicity result	N/A	Mutagenicity Negative	FDA	0.69
					Nearest neighbor	0.67
5.	 **TENIPOSIDE**	Mutagenicity value	N/A	N/A	Hierarchical clustering	N/A
		Mutagenicity result	N/A	N/A	FDA	N/A
					Nearest neighbor	0.00
6.	 **CURCUMIN**	Mutagenicity value	0.00	0.09	Hierarchical clustering	0.13
		Mutagenicity result	Mutagenicity Negative	Mutagenicity Negative	FDA	0.12
					Nearest neighbor	0.00
7.	 **BISPHOSPHONATE**	Mutagenicity value	N/A	N/A	Hierarchical clustering	N/A
		Mutagenicity result	N/A	N/A	FDA	1.08
					Nearest neighbor	N/A
8.	 **HISTONE DEACTYLASE**	Mutagenicity value	N/A	0.88	Hierarchical clustering	0.56
		Mutagenicity result	N/A	Mutagenicity Negative	FDA	1.40
					Nearest neighbor	0.67

IV. Conclusion

Docking was performed of PDB ID 4AXA of Oral Multi-AGC kinase protein among known inhibitors Irinotecan ,Teniposide , Topotecan , Curcumin has shown, quite good docking scores for PDB ID 4AXA of protein Oral Multi-AGC kinase protein Inhibitors of lower docking score were used for similarity search from ZINC database. Obtained molecules were further screened to find more potent inhibitor of Oral Multi-AGC kinase.

Among all the screened analog ZINC ID 72131268 for Irinotecan , ZINC ID 04166028 for Teniposide , ZINC ID 30731084 for Topotecan, ZINC ID 00899824 for Curcumin has given the best docking scores i.e. -169.087 kcl/mol , -178.235 kcl/mol ,-166.939 kcl/mol ,-141.537 kcl/mol based on the obtained data these molecules could be used for development of a potent inhibitor of PDB ID 4AXA of Oral Multi-AGC kinase protein.

Thus the receptor PDB ID 4AXA is considered to be more active participant in Oral cancer, therefore it can positively be used for development of potent inhibitor for treatment of Antitumor activity in Oral cancer.

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