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EFFICACY OF NATURAL INHIBITORS AGAINST PKC: AN *IN SILICO* APPROACH TO COMBAT CANCER

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Abstract

Protein Kinase C (PKC), a member of isozyme family plays an important role in cell growth regulation and differentiation and is being considered as a novel target of many anti-cancer drugs. In this study we have explored the affinity and interaction of some natural plant derived compounds against PKCα to find out some important active site amino acid residues that play an important role in the binding of inhibitors with the active site.

Keywords: Protein Kinase C, Natural inhibitors, Docking

Introduction

Protein kinase C is a member of an eleven mammalian isozyme family that plays an important role in cell growth regulation and differentiation. Protein kinases are enzymes which catalyses the transfer of phosphate from adenosine-5'-triphosphate to amino acids residues in certain proteins. Most protein kinase inhibitors are directed towards the ATP-binding site. Recent research reveals that these inhibitors must discriminate between ATP-binding sites of all protein kinases as well as other proteins that also utilize ATP. It is therefore beneficial to target protein kinases other than the ATP-binding site (Bogoyevitch *et al.*, 2005).

The central role of PKC in cellular signal transduction has made it an important therapeutic target for cancer especially prostate cancer. The targeting of PKC with some natural compounds like nimbolide, EGCG, curcumin, allicin, capsaicin, eugenol, cinnamaldehyde and farnesol may be an important step to provide an insight into its active site along with the efficacies of inhibitors. The aim of this work is to explore the affinity and interaction of some natural compounds against PKCα. In this study we have made an attempt to block diseases caused by abnormalities in these signaling pathways by using some selected natural inhibitors that are considered to be effective against cancer. The inhibitors taken for this study are well known natural compounds extracted from different plants.

Material and Methods

Natural Compounds used for Study: Nimbolide, Epigallocatechin Gallate (EGCG), Curcumin, Allicin, Capsaicin, Eugenol, Cinnamaldehyde and Farnesol.

Protein preparation: The crystal structure of PKCα taken in this study was extracted from protein data bank and all the HETATMS were removed. The protein was subjected to two steps energy minimization to remove the bad steric clashes using steepest descent and conjugate gradient methods for 1000 steps at RMS gradient of 0.1 and 0.05 respectively. During the energy minimization process the backbone were fixing the backbone.

Ligands preparations: The three dimensional structures of the natural inhibitors taken in this study were extracted from Pubchem compounds database. Char MM force field (Brooks *et al.,* 2009) was applied to them and further, was subjected to single step minimization using steepest descent method for 500 steps at RMS gradient of 0.01.

Docking: All the natural inhibitors taken for the study were subjected to dock within the active site of PKCα using LigandFit (Venkatachalam *et al.*, 2003) docking program available with DS2.5, Site 2 was selected consisting of 1156 points and partition level was set to 5. For docking Number of Monte Carlo steps was set to "2 500 120,4 1200 300,6 1500 350,10 2000 500,25 3000 750" with maximum 10 number of poses.

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Dock Analyses: All the docked inhibitors were analyzed on the basis of Dock score generated by LigandFit.

Docking Validation: To validate the docking program we again docked the inhibitor present within the active site of PKCα using the same parameters used for docking natural inhibitors into its active site.

Result and Discussion

Among all the natural inhibitors docked, EGCG was found to bind with the best efficacy. Curcumin and allicin were also effective. All the comparison was made on the basis of dock score given by LigandFit. The importance of natural inhibitors against cancer is well known. So, it was important to explore the mode of interaction of these inhibitors against the target enzyme. Epigallocatechin gallate (EGCG) a compound derived from green tea shows promising response and can be used as a potent inhibitor against PKCα for curing cancer. There are some other compounds revealed by this study that could be used as potent inhibitors against cancer. Most of the protein kinase inhibitors are directed towards the ATP-binding site. Recent research reveals that they must discriminate between ATP-binding sites of all protein kinases as well as the other proteins that also utilize ATP (Toledo *et al*., 1999 and Morin *et al*., 2000). It is therefore beneficial to target protein kinases other than the ATP-binding site (Bogoyevitch *et al*., 2005 and Mukherjee et *al*., 2009). Reports exist which indicates that the overexpression of PKC causes transformation of cancer cell lines (Mischak *et al*., 1993).

Some amino acid residues were found to play an important role in the binding of inhibitors within the active site of PKCα. M417 was involved in making hydrophobic contacts in all the cases, and is presumed to be a key player. The other amino acids that actively participated include A366, V420, A480 and D481. Among these V420 was making maximum number of hydrogen bonds with the inhibitors followed by D467, D481 and Q387 (Table-1). These residues along with others were also actively involved in forming hydrophobic contacts (Fig 1A-D).

To confirm the accuracy of the docking program as well as the parameters used for docking, the inhibitor from our parent molecule (pdb id: 3IW4) was extracted and again subjected to dock within the active site using the same parameters as that taken for docking the natural compounds. It was found that the binding orientation of the ligand was same as that in the parent molecule. This confirmed the accuracy of binding and docking program used. Fig 2 shows comparison between the binding modes of inhibitor as within the x-ray structure (shown in red) as well as found after the re-dock (shown in blue), both were found to bind in the same orientation.

Figure 1 (A-D): 2D representation of the top four docked ligands within the active site of PKCα. Fig 1(A) Binding orientation of EGCG within the active site of PKCα. Fig 1(B) Binding orientation of Curcumin within the active site of PKCα. Fig 1(C) Binding orientation of Allicin within the active site of PKCα. Fig 1(D) Binding orientation of Capsaicin within the active site of PKCα

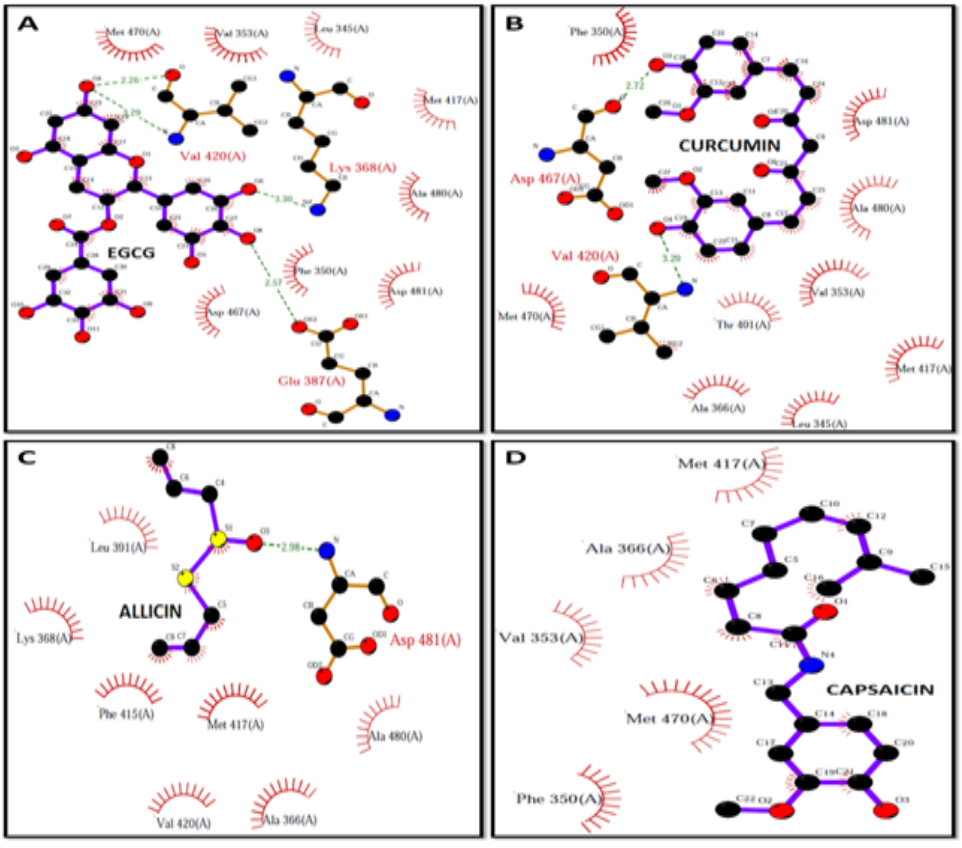

Figure 2: Binding orientation of the inhibitor already present within the active site of PKCα (in red) and that after re-docking the same with LigandFit docking program (Blue)

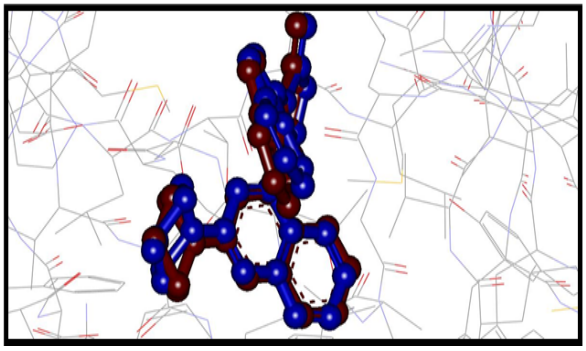

Table1: Dock Scores of natural compounds with target enzyme PKC along with details of the residues involved in binding

Natural Inhibitor	Dock Score	H-bonding Residues	Residues involved in making hydrophobic contacts
EGCG	64.192	K368, E387, V420	L345, F350, V353, M417, D467, M470, A480, D481
Curcumin	48.451	V420, D467	L345, F350, V353, A366, T401, M417
Allicin	46.014	D481	A366, K368, L391, F415, M417, V420A480
Capsicum	45.832	No H-Bond Formed	L345, F350, A366, M417, D467, M470
Eugenol	42.574	E387	V353, A366, K368, L391, T401, F415, M417, A480
Farnesol	41.987	T401, D481	F350, V353, A366, L391, T401, M417, M470, D481
Nimbolide	37.625	No H-Bond Formed	F350, V353, T401, M417, D424,
Cinnameldehyde	34.486	No H-Bond Formed	A366, T401, M417, V420, D424, D467, N468, M470, A480, D481

Conclusion

This study shows the effectiveness of natural compounds that can further be studied as potential anticancerous drugs. It also shows the scope of other natural compounds that have not yet been explored. The role of some crucial amino acids involved in proper binding of inhibitors with the active site of PKCα can be useful for designing better drugs to combat cancer.

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