*Section A-Research paper*

# Novel studies on Anti Apoptosis inhibitors as a Preventive Measure for Cancer

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**Abstract:**
Cancer is one of the most common diseases that affects a substantial proportion of the world's population. Apoptosis is a major contributor to the genesis and progression of cancer. The current issue is to transform new treatment prospects into knowledge gained about mechanisms of abnormal cell death control in malignancies. Basic research has highlighted the road to attaining this. Damage to the apoptosis pathway has been linked to the development of cancer. The apoptosis signalling pathway is a novel cancer drug target. Caspase-8 homologue cellular FLICE-like inhibitory protein (c-FLIP) is catalytically inactive. Vitamin B-17, also known as Amygdaline, is a natural chemotherapeutic drug present in over 1,200 plants. Dr. Krebs claims that we need at least 100 mg of vitamin B-17 each day to almost assure a cancer-free life. Vitamin B-17 is found in apricot seeds, cassava root, sorghum, and other foods.

We used the c-Flip protein in this study for homology modelling and additional ligand-based experiments to determine the best c-flip inhibitors. Because the structure of this protein was not accessible in the PDB, we modelled it and then docked it to locate the chemical. We also looked for it in a natural setting. After identifying a molecule with similar properties to Amygdaline, we chose Cassava and Sorghum as natural sources and processed them to extract the component.

**Keywords:** c-FLIP, Amygdaline, homology modeling, Cassava root.

## Introduction:

Cancer is one of the most common diseases that affect a substantial proportion of the world's population. Damage to the apoptosis pathway can result in the cells' continuing to develop, which can lead to cancer. Various researches on the apoptotic signaling system, which works as a novel therapeutic target for breast cancer, have been conducted recently. The death receptor-induced pathway and the mitochondria-mediated pathway are the two primary processes that cause cell apoptosis [1]. Death ligand binds to the death receptor in the Death receptor Induced Pathway, which aids in the creation of a death inducing signaling complex and Caspase-8 activation. Due to its involvement in the apoptosis pathway, tumor necrosis factor-related

apoptosis-inducing ligand (TRAIL) is gaining a lot of attention. At the moment, mutant TRAIL's are being employed as anti-apoptotic medicines in phase trials [2, 3].

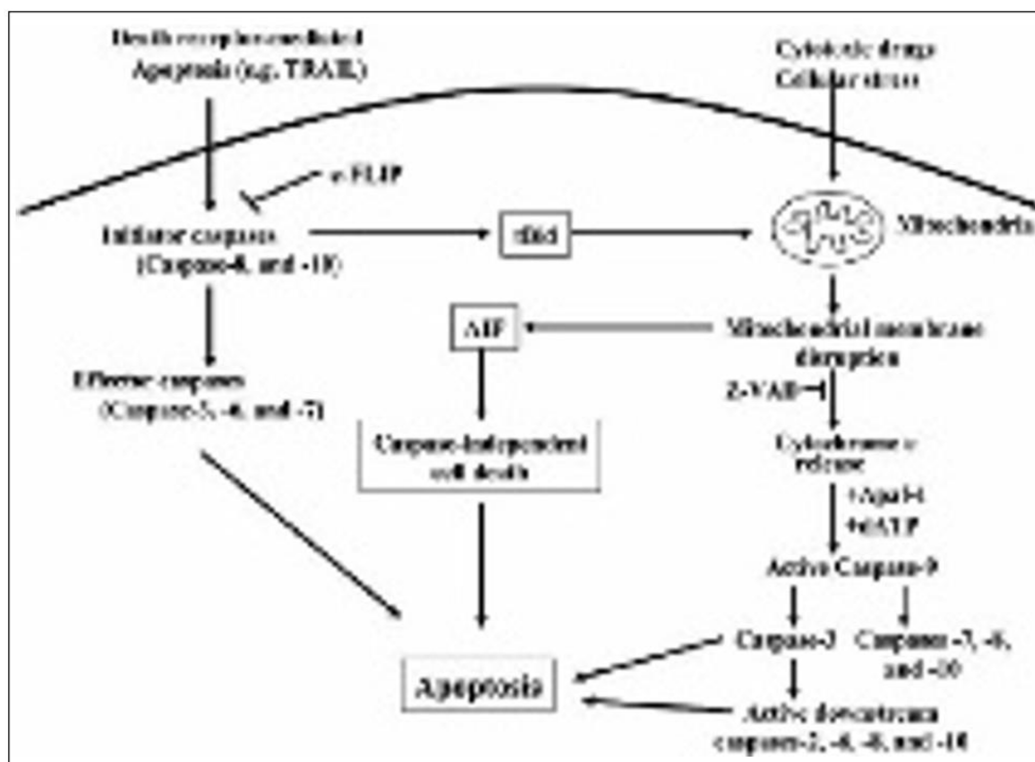



**Fig 1: Mitochondrial Death Pathway**

c-FLIP (cellular FLICE-like inhibitory protein) is a catalytically inactive Caspase-8 homologue that inhibits death receptor-mediated apoptosis by blocking Caspase-8 from attaching to the death inducing signalling complex [4,5]. c-FLIP is made up of several variations, the most well-known of which are c-FLIPL and c-FLIPS. Two death effector domains (DED) are present in these two versions [6, 7, 8, 9]. Overexpression of c-FLIP is caused by an increase in resistance to apoptosis, which is mediated by TRAIL and FAS [10]. By interacting with the DISC and blocking Caspase-8 and Caspase-10 activation, the two proteins short form and long form (FLIPL and c-FLIPS) play a vital role in death receptor mediated apoptosis [11]. TRAIL and FAS-mediated apoptosis can be sensitised by down-regulating c-FLIP activity, according to several studies [12, 13, 14, 15]. Various studies have shown that various chemical and natural chemicals that block or regulate the activity of the protein molecule can down-regulate c-FLIP [16, 17, 18].

Various synthetic and natural chemicals have been investigated for their ability to inhibit the c-FLIP protein, which is one of the most studied therapeutic targets in the death receptor-mediated apoptosis pathway. It has also been discovered that some naturally occurring plant extracts not only suppress the function of c-FLIP, but also the proliferation of specific cancer cells [19, 20, 21, 22, 23, 24].

The Aim of this research work is to study the natural cflip inhibitors and its processing method for extracting the required entity. In this study we have considered c-Flip protein for homology modeling and further ligand based studies to find out best c-flip inhibitors. Further we searched it

in a natural source. After finding the compound same feature like Amygdalin we selected its natural source Cassava and Sorghum then processed it for extracting the same.

**Methodology:**

1. **Flow Chart of Protocol Followed for Cflip Protein In-silico Study**

**Fig 2: Flow Chart of Protocol**

2. **Selection of Natural Plant product from structural output.**

3. **Extraction of desired anti apoptosis chemical entities from natural sources.**

**Formation of nutritional food products from selected natural sources.1.**

1. **<u>Cflip Protein Insilico Study</u>**

**Selection of protein molecule:** The swissprot database is used to select protein molecules. The Swissprot database was used to verify the existence of 3D structures and to investigate the functional domains of protein molecules.

**Template selection and alignment of the sequence:** The NCBI Blast algorithm is used to find structures that are comparable to the protein. In which the structure with the highest resemblance is chosen. The protein's 3D structure as well as the fasta format were obtained and used. The sequence alignment algorithm in Discovery Studio Software was used to align the template and protein sequences.

**Protein homology modelling and model verification:** The protein molecule's structure is modelled in the Discovery studio software using the Build Homology model procedure in the parameters file, utilising the template specified and the alignment file. After the structure of the protein has been modelled, it is confirmed using several model verification services such as Procheck, prosa, and RMSD.

**Preparation of proteins and energy conservation:** After that, the modelled protein molecule is cleaned and the CHARMm forcefields are applied to it. The potential energy of the synthesised protein molecule is reduced utilising various algorithms such as steepest descent and conjugate gradient approaches, which reduce the potential energy of the protein molecule [25-26].

**Ligand sketching and preparation:** All the ligand molecules were sketched using the chemsketch software and then the preparation of ligand molecules is done by prepare ligands protocol in discovery studio.

**Results and discussion:**

**Protein molecule selection:** The protein molecule with the Accession number O15519 was chosen from the Swissprot database.The protein sequence is extracted in FASTA format from amino acids 1-376 that comprise DED1 and DED2 functional domains, and the FASTA format is sent to protein blast to produce a structure that is similar to the protein sequence.

**Template selection:** The template is chosen using the PBlast search engine. The template sequence is 3H11, which has a 99 percent identity. 3H11 is a combination of Zymogene Caspase-8 and c-Flip protease domains. The template's structure is extracted from the PDB database and imported into Discovery studio.

Select: All None Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

| Description | Max score | Total score | Query cover | E value | Ident | Accession |
|---|---|---|---|---|---|---|
| Chain A, Zymogen Caspase-8:c-Flipl Protease Domain Complex >pdb\|3H13\|A Chain A, C-Flipl Prc | 353 | 353 | 44% | 3e-120 | 99% | 3H11_A |
| Chain A, Crystal Structure Of Mc159 Reveals Molecular Mechanism Of Disc Assembly And Vflip Inh | 85.9 | 85.9 | 61% | 4e-19 | 30% | 2BBZ_A |
| Chain A, Crystal Structure Of A Viral Flip Mc159 | 82.4 | 82.4 | 45% | 3e-18 | 32% | 2F1S_A |
| Chain A, Crystal Structure Of Mc159 Reveals Molecular Mechanism Of Disc Assembly And Vflip Inh | 82.4 | 82.4 | 48% | 4e-18 | 32% | 2BBR_A |
| Chain A, Crystal Structure Of A Vflip-Ikkgamma Complex: Insights Into Viral Activation Of The Ikk Sig | 75.1 | 75.1 | 44% | 1e-15 | 34% | 3CL3_A |
| Chain B, Crystal Structure Of The Caspase-8/p35 Complex >pdb\|2FUN\|B Chain B, Alternative P35- | 75.1 | 75.1 | 35% | 3e-15 | 33% | 1I4E_B |
| Chain A, Solution Structure Of The Catalytic Domain Of Procaspase-8 | 75.1 | 75.1 | 35% | 3e-15 | 33% | 2K7Z_A |
| Chain A, Caspase-3 Specific Unnatural Amino Acid-based Peptides | 75.1 | 75.1 | 35% | 4e-15 | 33% | 4JJ7_A |

Fig 3: BLAST results at NCBI server, showing that the 'A'chain of 3H11 protein molecules has the highest identity with the predicted protein structure.

**Alignment of Sequences:** In the Discovery Studio software, the protein and template sequences were aligned with a sequence identity of 33.9 percent

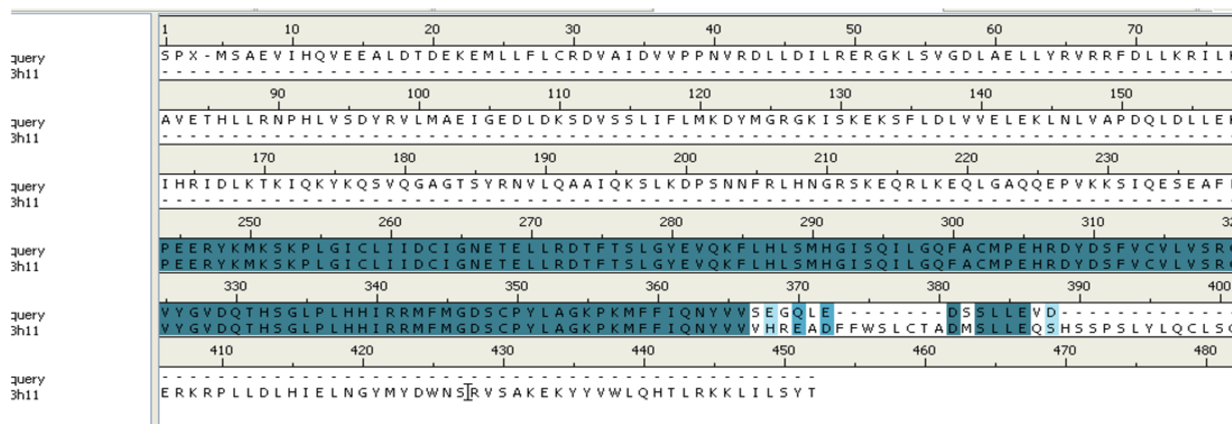


Fig. 4: In Discovery studio software, the sequence alignment of C-FLIP and 3H11 is shown, with the shaded regions in the picture representing similar amino acids in the two sequences.

**Modeling:** Homology modelling of the protein molecule is carried out with the help of Discovery studio software, which is used to create homology models in the procedures.

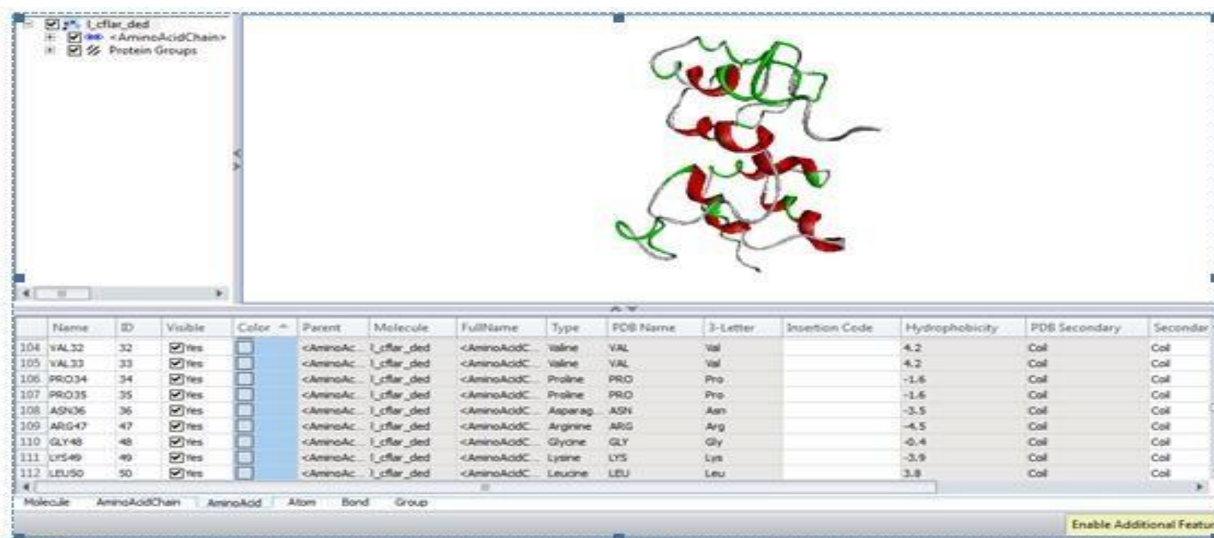


Fig5: In the discovery studio, the modelled structure of the protein molecule is displayed in a solid ribbon fashion.

The quality of the modelled protein molecule is checked utilising multiple servers during model verification.

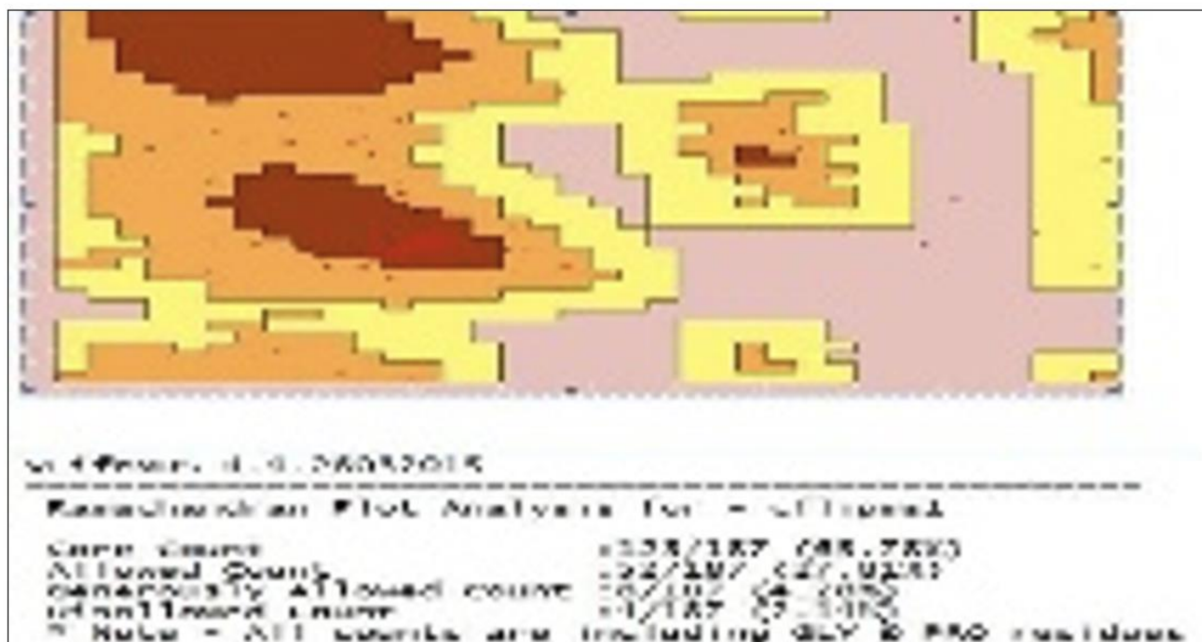


Fig 6: Model verification
Identification of binding site

Fig 7: identification of binding

**Pharmacophore Identification**

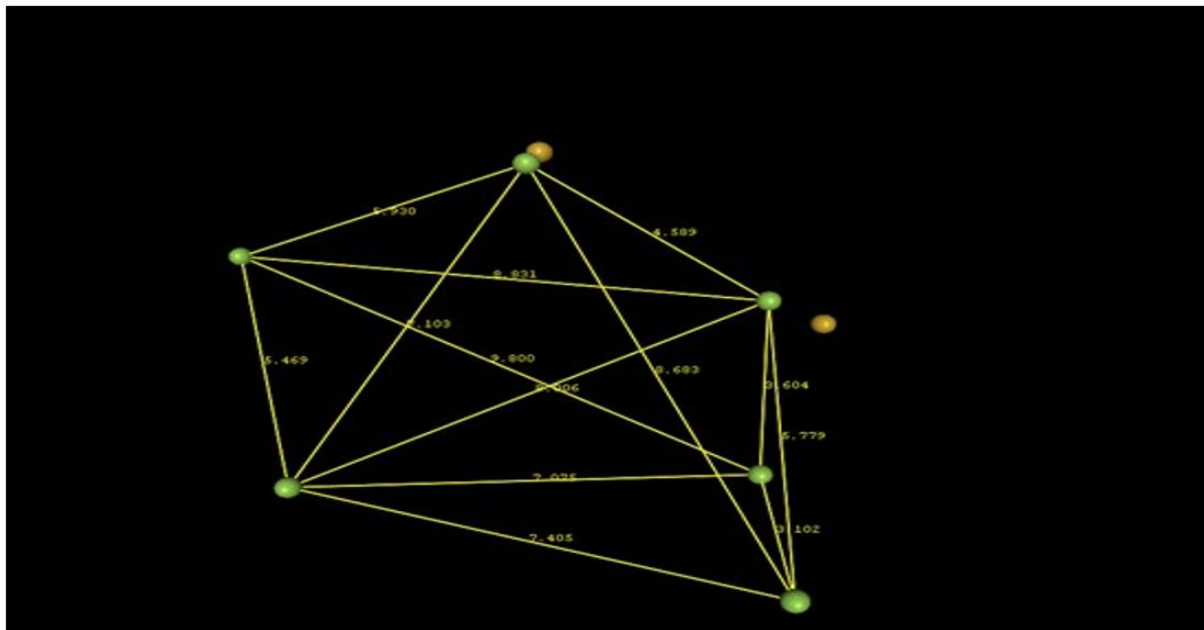
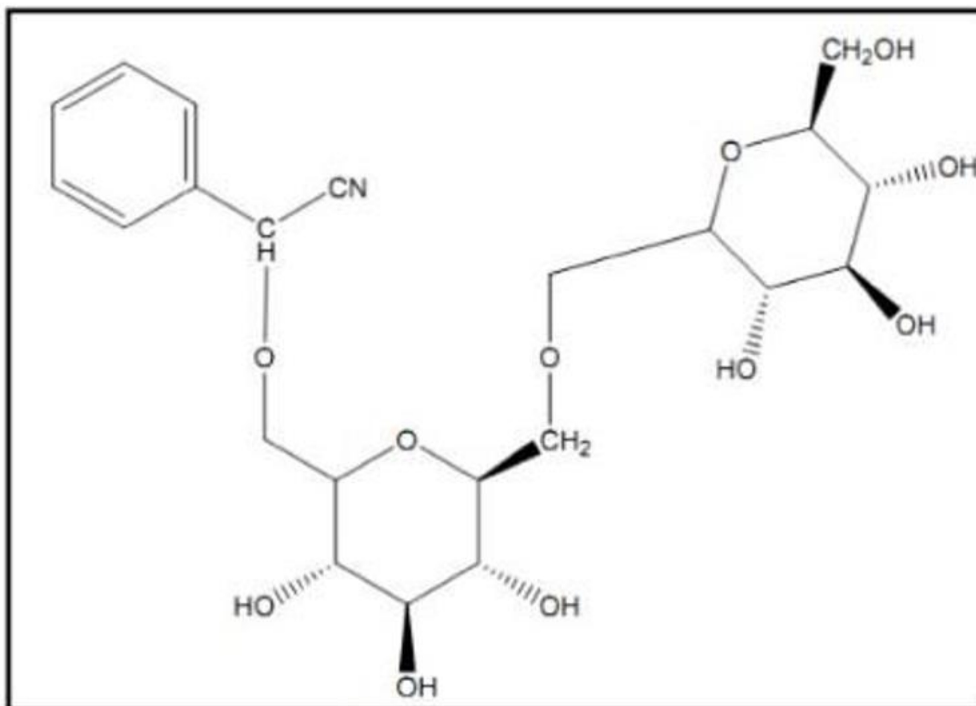
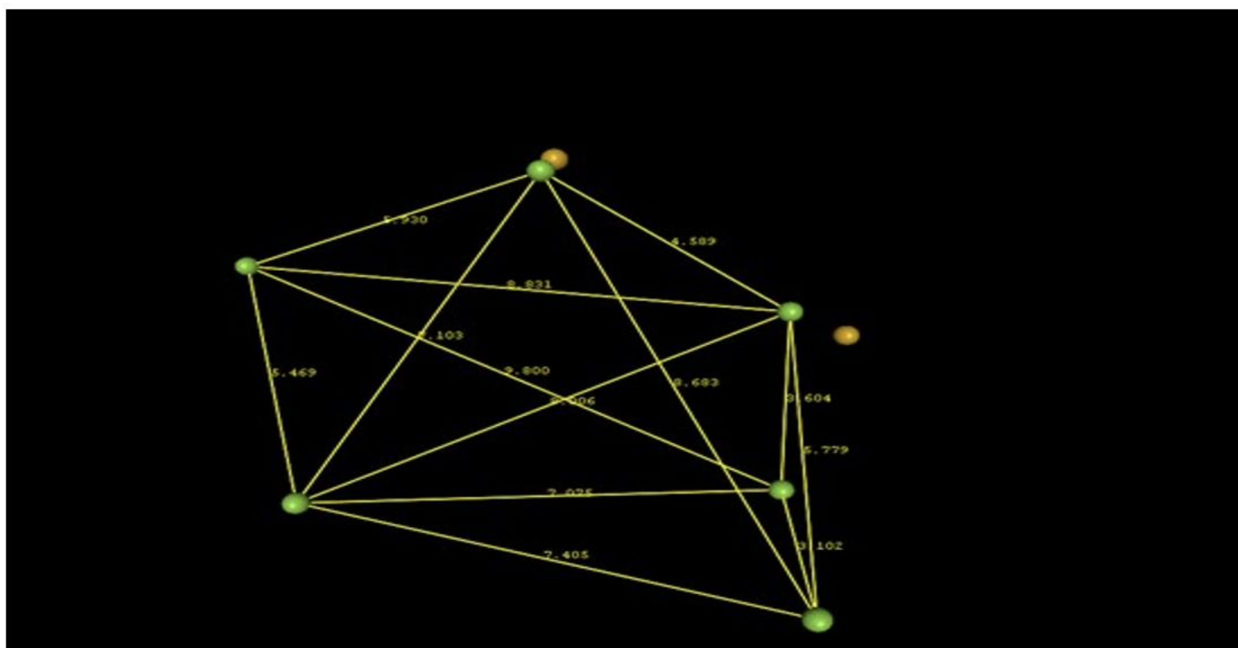



Fig 8: Pharmacophore Identification

**After docking studies, we got core structure having the structural similarities as that of the Laetrile.**






**Docking of c- flip with amygdalin**

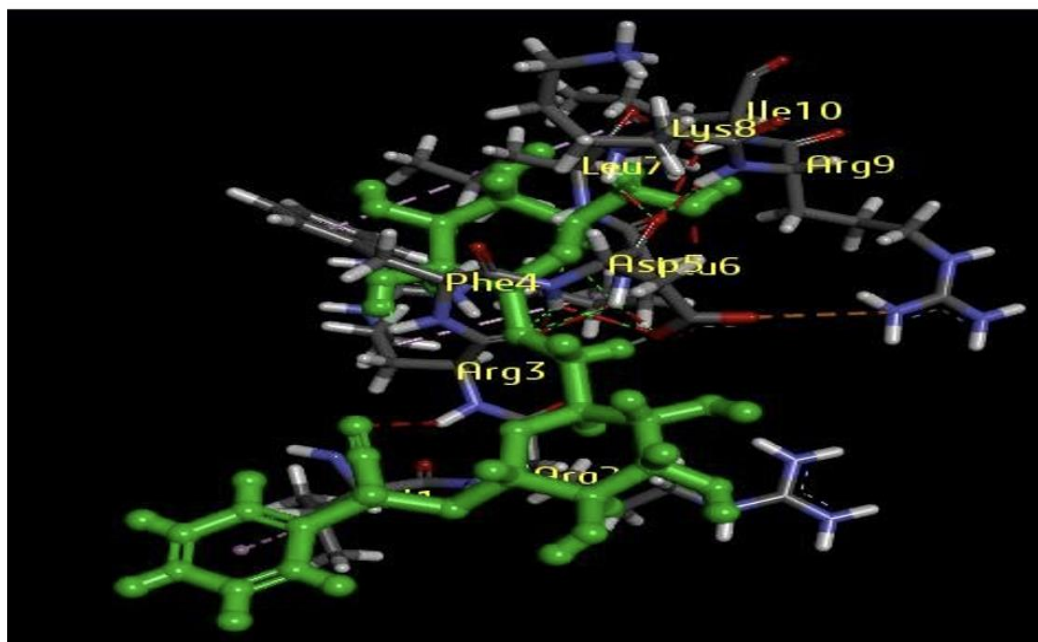



Fig 10: Docking of c- flip with amygdalin

**MOA of Amygdaline**

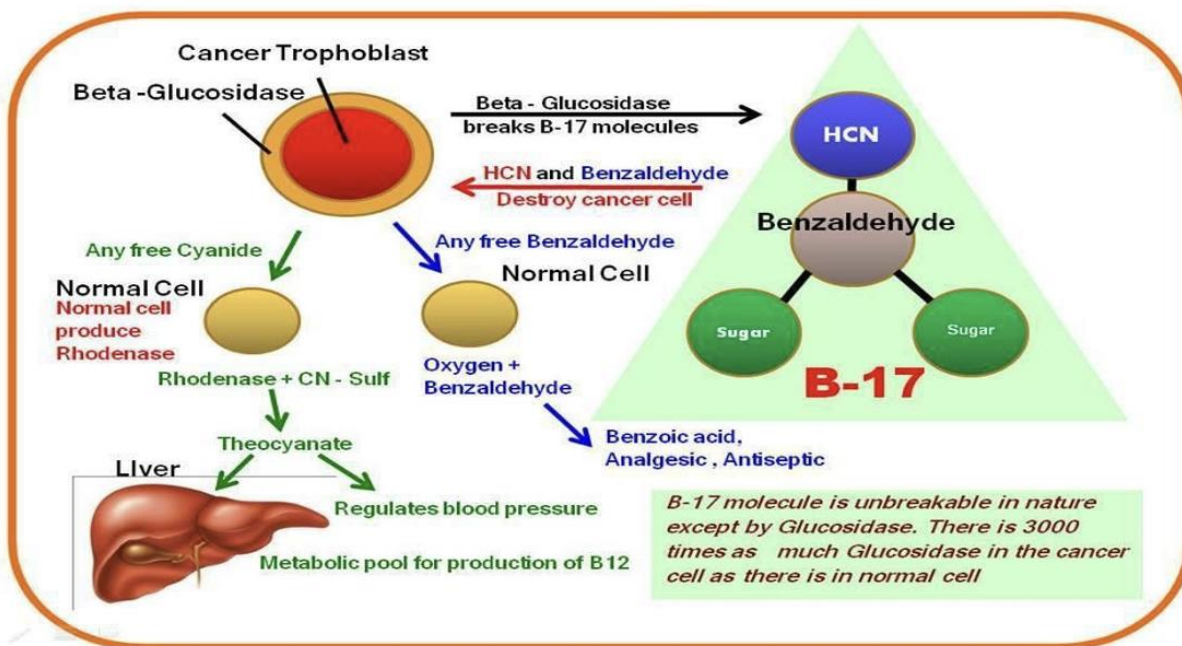



Fig 11: MOA of amygdaline

**Selection of Natural Plant product from structural output.**

**Fig 12: Cassava Root**

**Fig 13: Sorghum**

**Selected Natural Sources of Laetrile /Amygdalin**

**1.Extraction of desired anti apoptosis chemical entities from natural sources.**

**Processing on Cassava Root**

1. Collection and authentication of plant.

2. Cassava Roots (15kg).

3. Peeling and Cleaning.

4. Soaking in water for 4 days-Fermentation.

5. Sun drying and cabinet drying at $75^0$C.

6. Milling and collecting in an airtight container.

7. Determination of Amygdalin content by UV spectrophotometer at 256nm.

8. Amygdalin content -near about13-14mg/g.

**Processing on Sorghum:**

1. Collection and authentication of plant.

2. Sorghum sample (200gm) Cleaning

3. Soaking in a plastic bucket containing 300ml of water for 3 days at room temperature (28-$30^0$C).

4. Steeped water decanting and steeped grains wet-milling.

5. Milled slurry sieving for removing over tails.

6. Collecting the troughs for further fermentation for 2 days at room temperature.

7. Decanting souring water and collecting slurry into muslin cloth for getting the cake for drying at 30-$40^0$C

8. Determination of Amygdalin content by UV spectrophotometer at 256nm.

9. Amygdalin content -near about 44-45mg/g.

**Formation of nutritional food products from selected natural sources.**

Fig 14: Prepared Food Product from Sorghum and Cassava Root

**Conclusion:**

In the case of TRAIL and drug/chemotherapy resistant cell lines, the anti-apoptotic protein c-FLIP is one of the most important therapeutic targets. C-FLIP has become increasingly important in cancer treatment; inhibiting c-FLIP may aid in boosting cancer cell death. The interaction of c-FLIP with natural and synthetic inhibitors that block c-FLIP activity was investigated in this study. C-FLIP comprises two death effector regions (DED1, DED2) that have the function of inactivating c-FLIP. We modelled the c-FLIP protein molecule using 3H11 as the template structure in Discovery studio. The Ramachandran plot analysis is used to validate the modelled protein structure in order to forecast its quality. After finding the compound same feature like Amygdaline we selected its natural source Cassava and Sorghum then processed it for extracting the same. C-flip protein modelling and its inhibitor study is new pathway for various anticancer studies. Natural c-flip inhibitors like Cassava and Sorghum are having dual advantage as a nutrients and as a anticancer agent or useful for cancer prevention. Prevention is better than cure, the reported sources of Amygdalin/ Vit. B 17 is an ideal food for cancer prevention.

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