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Research Article

EVALUATION OF ANTICANCER PROPERTY OF AMBLYONE FROM AMORPHOPHALLUS PAEONIIFOLIUS (ELEPHANT FOOT YAM) USING *IN SILICO* ANALYSIS

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ABSTRACT

The present work aims at evaluating the anticancer activity of Amblyone, a phytochemical present in the tuber of Amorphophallus Paeoniifolius (Elephant foot yam) and compare its activity with the commonly used antiapoptotic drug Tetrahydroisoquinoline amide substituted phenyl pyrazole derivative. Anti cancer activity is analyzed by docking the ligands against the antiapoptotic B-Cell Lymphoma-2 protein (Bcl-2) [PDB ID: 2w3l] using AutoDock Tool software. A docking score of -12.2 kcal/mol is obtained for the phytochemical Amblyone compared with-7.7 kcal/mol for Tetrahydroisoquinoline amide substituted phenyl pyrazole derivative. The better docking score is an indication that the amblyone can bind firmly on the groove of Bcl-2 protein which regulates the activity of pro-apoptotic proteins by direct binding and sequestration. The absorption, distribution, metabolism, excretion (ADME) and toxicity properties of the ligands are studied using Pre ADMET software. The *in silico* studies are further supported by *in-vitro* studies of the crude sample using A549 (Lung cancer) cells by direct observation of cells using Inverted phase contrast microscope followed by colorimetric assay method (MTT) and direct microscopic observations.

Keywords: Apoptosis, Docking, Amorphophallus Paeoniifolius, Amblyone.

1. INTRODUCTION

Cancer is a growing threat to the humans across the world. There are different types of cancer and most of them can be treated successfully using chemotherapy or radiation therapy if detected at an early stage. Most of the drugs are developed to enhance apoptosis. The major mechanism by which this cancer therapy occurs is through a p53-dependent pathway [1]. B-cell lymphoma-2 (Bcl-2) family members play a critical role in regulating and executing apoptosis [2]. Hence they are a promising target for anticancer drugs. The bcl-2 family of proteins consists of twenty five pro-apoptotic and anti- apoptotic (inhibiting) members, which interact to maintain a balance between newly forming cells and old dying cells. Any alteration in the expression of B-cell lymphoma-2 (Bcl-2) family members can led to development of resistance to cytotoxic antineoplastic drugs and delay apoptosis in response to radiation therapy [3], preventing the hope of cancer survivors by conventional cancer treatment methods.

Bcl-2 family proteins are integral membrane proteins mainly present on the outer membrane of mitochondria having a binding groove that binds the BH3 domain of pro-apoptotic family members [4].This binding process prevents the oligomerization of pro-apoptotic family members and the initiation of the apoptosis cascade [5]. Structural studies showed that the binding groove of Bcl-2 proteins can incorporate small molecules and such molecules facilitate cell death by preventing the anti-apoptotic effect of Bcl-2 family proteins. It is most successfully demonstrated with the small molecule ABT-737 by clinical trial [6].

This paper presents a phytochemical substitute which can interact with bcl-2 family of proteins with minimal side effects [7]. It is then compared with a known anticancer drug Tetrahydroisoquinoline amide substituted phenyl pyrazole molecule. The comparison study is based on docking procedure using Autodock Vina.

The selective Bcl-2 inhibitor activity of Tetrahydroiso-quinoline amide substituted phenyl pyrazoles was already established by high throughput screening

campaign [7]. For example: 3-((S)-3-(Azidomethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)-4-(5-butyl-3-(ethoxycarbonyl)-1-phenyl-1H-pyrazol-4-yl) benzoic acid,a derivative of Tetrahy-droisoquinoline amide substituted phenyl pyrazoles can act as an inhibitor of Bcl-2 family antiapoptotic proteins. The pubchem id of the ligand is 71086321. The 2D structure of the ligand is shown below (taken from pubchem).

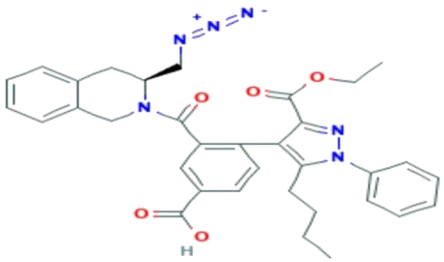

Fig.1: 3-((S)-3-(Azidomethyl)-1,2,3,4-tetrahydroisoquinoline-2-carbonyl)-4-(5-butyl-3-(ethoxycarbonyl)-1-phenyl-1H-pyrazol-4-yl) benzoicacid

The phytochemical amblyone from elephant foot yam (Amorphophallus paeoniifolious) is selected for this study. Elephant foot yam belongs to Araceae that have good culinary properties, medicinal properties, therapeutic and higher yield potential and hence referred to as the "king of tuber crops". Pharmacological studies proved that, Amorphophallus paeoniifolius possess, analgesic, anti- inflammatory [8], antimicrobial [9], anti-helminthic, hepatoprotetic, [9] cytotoxic and anti oxidant activities [10].

Amblyone present in the tuber of Amorphophallus paeoniifolius belongs to the family of triterpenoid (six isoprene unit) with a molecular formula of $C_{27}H_{42}O_4$ [9]. The pubchem Id of the ligand is 101921672. The 2D structure of the ligand is shown below (taken from pubchem).

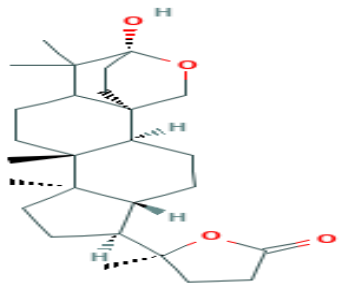

Fig 2: 2D structure of Amblyone

The IUPAC name of Amblyone is (5S)-5-[(1S,2S,5R,6S,9R,10R,15S)-15-hydroxy-9,10,14,14-tetramethyl-16-oxapentacyclo[13.2.2.01,13.02,10.05,9]nonadecan-6-y l]-5-methyloxolan-2-one.

2. MATERIAL AND METHODS

2.1. *In-silico* study

The antipoptotic protein belong to the Bcl-2 family with PDB ID: 2w3l is selected as the target for the docking study. The major materials used for carrying out this computational study are Protein Data Bank, PubChem, PyMOL molecular graphic system version 1.5.0.3, Auto Dock Tool Vina, Open Babel 2.3.2 version, Discovey Studio 2017 Client, preADMET.

The 3D structure of the target antiapoptotic Bcl-2 protein (PDB ID: 2w3l) was taken from RCSB protein data bank [http://www.rcsb.org/]. The 2D structres of the ligands Amblyone and the derivative of tetrahydroisoquinoline amide substituted phenyl pyrazole were downloaded from PubChem [https://pubchemncbi.nlm.nih.gov]. Then the protein and ligand preparations were done by Py Mol and Open Babel softwares. Using Autodock Tool software the prepared protein and the ligand in the ". pdb" file is converted to ".pdbqt" file. Then the docking was done and the docking output were visualised using Discovery Studio client 2017. The absorption, distribution, metabolism, excretion and toxicity properties were obtained using PreADMET software.

2.2. *In-vitro* anticancer study

The in-vitro anticancer study of the crude sample obtained from the tuber of Amorphophallus Paeoniifolius was carried out using A549 (Lung Cancer) cells. A549 cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigma Aldrich, USA). The cell line was cultured was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator.

Preparation of compound stock:1mg of sample was weighed and dissolved in 1mL DMEM using a cyclomixer. The sample solution was filtered through 0.22 µm millipore syringe filter to ensure the sterility.

Anticancer Activity: After 24 hours the growth medium was removed, freshly prepared compounds in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of 5% DMEM) and 100µl of each concentration were

added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator. Non treated control cells were also maintained.

2.2.1. *Anticancer assay by direct microscopic observation*

Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

2.2.2. *Anticancer assay by MTT method*

Fifteen mg of tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT ,Sigma, M-5655) was reconstituted in 3 ml phosphate-buffered saline (PBS) until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT solubilization solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico *et al.*).

The percentage of growth inhibition was calculated using the formula:

% of viability = (Mean Optical Density of Samples/ Mean Optical Density of control group) x 100

3. RESULTS AND DISCUSSION

3.1. *In-silico* study

Docking of the ligands Amblyone, phytochemical of Amorphophallus Paeoniifolius and the derivative of Tetrahydroisoquinoline amide substituted phenyl pyrazole, an antiapoptotic drug were done at the binding site of the target protein belonging to Bcl-2 family (PDB ID 2w3l)where its own natural ligand is present. The grid box was created at that place and docking procedure was done using AutoDock Tool software.

After docking has been completed it was found that the two ligands selected were exactly bounded at the binding site. Among them Ambylone has high docking score, -12.2 kcal/mol and it has a better ligand interaction with the target than 3-((S)-3-(Azidomethyl)-1,2,3,4- tetrahydroisoquinoline-2-carbonyl)-4-(5-butyl-3-(ethoxycarbonyl)-1-phenyl-1H-pyrazol-4-yl) benzoic acid, the Tetrahydroiso-quinoline amide substituted phenyl pyrazole derivative. Since it has a docking score of only-7.7 kcal/mol. The docking interaction of the Ambylone and 3-((S)-3-(Azidomethyl)-1,2,3,4- tetrahydroisoqui-noline-2-carbonyl)-4-(5-butyl-3-(ethoxycarbonyl)-1-phenyl-1H- pyrazol-4-yl) benzoic acid with the target protein 2w3l with their 3D structures are shown below:

The Fig. 3 shows that the ligand amblyone properly binds on the binding pocket of the 2w3l protein as indicated by the good docking score of -12.2 kcal/mol. Fig. 4 shows the nature of interaction between the ligand amblyone and the protein. Here the ligand forms pi-sigma bond with PHE 112 , hydrogen bond with four amino acids PHE 89, ARG 88, GLU 95, LEU 96 and covalent bond with THR 91 and VAL 93in the protein chain. The Fig. 5 shows that the ligand properly binds on the binding pocket of the 2w3l protein as indicated by the good docking score of -7.7 kcal/mol. The Fig.6 indicates the type of interaction between the ligand and the protein. The ligand forms pi-pi stacking interactions with PHE 63, PHE 71and TYR 67. Pi-sigma interaction of ligand is with LEU 96, and Hydrogen bonding is with ARG 105 and TYR 67. Besides, there is an unfavourable positive-positive interaction with ARG 66.

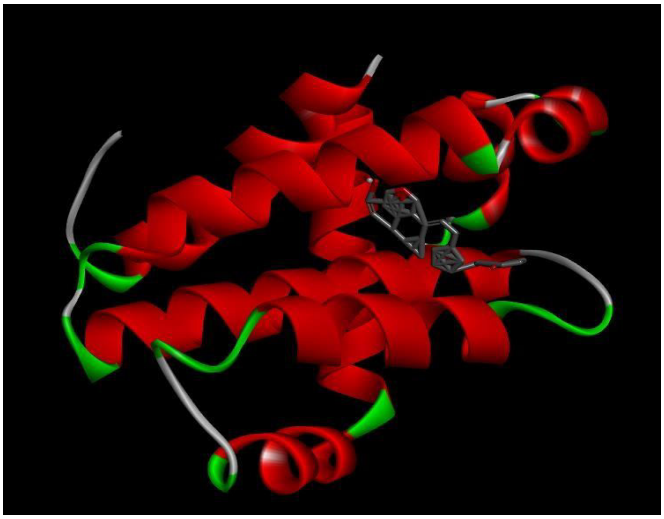

Fig 3: Ambylone with target protein 2w3l

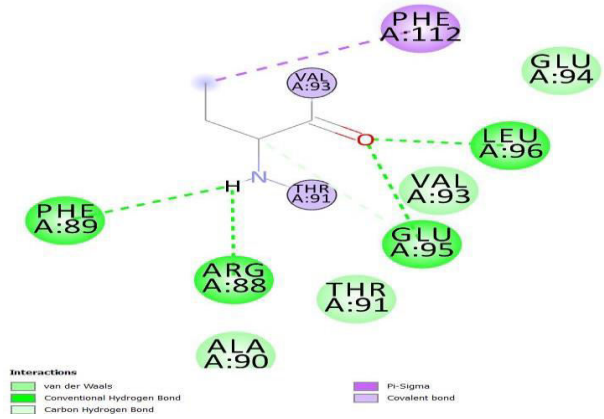

Fig.4: 2D diagram of receptor-ligand interaction

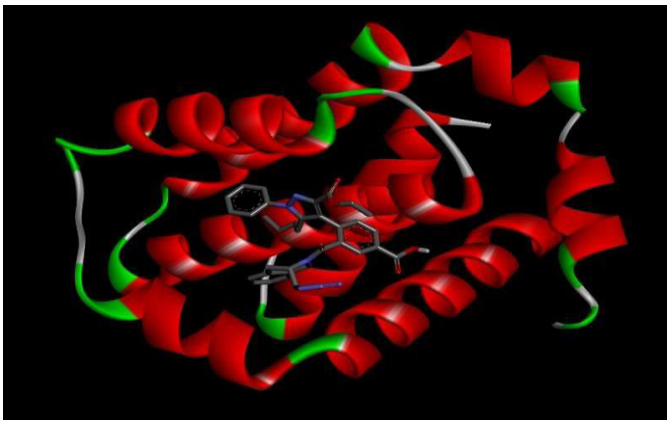

Fig.5: Tetrahydroisoquinoline amide substituted phenyl pyrazole derivative with target protein 2w3l

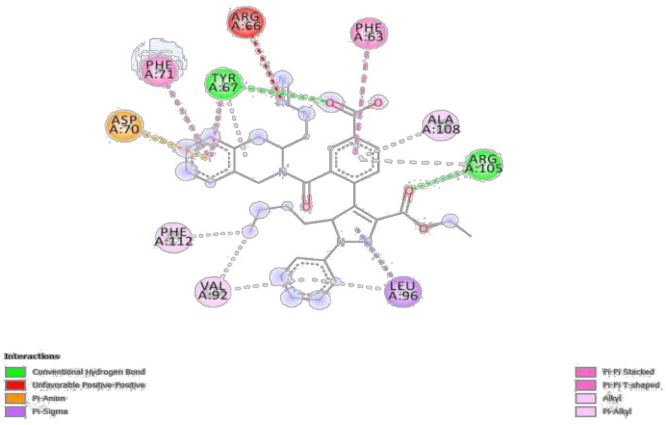

Fig. 6: 2D Diagram of receptor-ligand interaction.

3.2. Pre-ADMET study of ligands

The ADME and toxicity properties of both the ligands are studied with the help of PreADMET Software . The properties obtained are listed below

Table 1: ADME properties of the ligands under study

ADME properties	Ambylone	Drug
BBB	2.41588	1.02868
Caco2	37.6132	20.6608
CYP_2C19_inhibition	non	non
CYP_2D6_inhibition	non	inhibitor
CYP_2D6_substrate	non	non
HIA	95.742302	99.087118
SKlogP_value	4.419110	8.242300
SKlogS_buffer	-3.204670	-3.207770

The blood brain barrier (BBB) of both ligands lie in middle absorption range, hence it will not affect central nervous system activity. In the case of intestinal cell permeability, as indicated by Caco2 value, amblyone has better score than drug [11]. The ligand amblyone is found to be a non-inhibitor to cytochrome (CYP) isoforms namely CYP 2C19 and CYP 2D6 which are actually involved in drug metabolism. But the drug is a CYP 2D6 inhibitor. Both the ligands, amblyone and drug show HIA value near to hundred percentage showing good intestinal absorption, which is essential for a drug. The SKlogP_value (partition co-efficient in 1-octanol /H_2O) which determines the lipophilicity is 4.4(<5 as per Lipinski rule) for amblyone while it is 8.2 for the drug (violating Lipinski rule). For the oral bioavailability of the dug, the SKlogS should lies between -6 and -0.5, which is satisfied by the phytochemical amblyone.

Table 2: Toxicity properties of the ligands

Toxicity Properties	Amblyone	Drug
Ames_test	non-mutagen	mutagen
Carcino_Mouse	negative	negative
Carcino_Rat	positive	negative
hERG_inhibition	Low risk	Low risk
TA100_10RLI	negative	negative
TA100_NA	negative	negative
TA1535_10RLI	negative	negative
TA1535_NA	negative	negative

The preliminary toxicity studies of amblyone using preADMET software, shows negative values for Ames (uses bacteria to test whether a given chemical can cause mutations in the DNA of the test), salmonella mutagenity test (TA100_10RLI, TA100_NA etc) and carcino mouse test value, indicating that the phytochemical is non-mutagen and non- carcinogen.

The lower risk value of amblyone for hERG inhibition shows that it has no cardiac side effects [11]. The ADME and toxicity values of the ligand obtained using preADMET software support its druggable character.

3.3. *In-vitro* anticancer study of crude sample

The *in-vitro* anticancer study of crude sample is done on A549 (Lung cancer) cells .The viability of cell is evaluated by direct observation using inverted phase contrast microscope and MTT assay method and also by Direct Microscopic observation.

3.3.1. Colorimetric assay method (MTT method)

Evaluation of viability of cell by Inverted phase contrast microscope followed by MTT assay method is discussed below.

The percentage of growth inhibition was calculated using the formula:

% of viability = (Mean OD Samples/Mean OD of control group) x 100

At sample concentration 6.25 µg/mLthe percentage viability is decreased from 100% of control (non treated cell) to 88.99 % and for 12.5 µg/mL, 25 µg/mL, 50 µg/mL percentage viability decreases to 80.24%, 75.57%, 65.58% respectively. When the sample concentration is increased to 100 µg/mL the percentage viability decreases to 51.92 % (half of the cell is affected), *i.e.* as the sample concentration increases the percentage viability decreases.

Table 5: Percentage viability of lung cancer cell lines with increase in sample concentration

Sample Concentration (µg/mL)	OD value I	OD value II	OD value III	Average OD	Percentage Viability
Control	1.1106	1.1127	1.1836	1.1356	100.00
Sample name: AMP0102					
6.25	1.0356	0.9975	0.9986	1.0106	88.99
12.5	0.9115	0.9128	0.9093	0.9112	80.24
25	0.8521	0.8617	0.8608	0.8582	75.57
50	0.7690	0.7629	0.7705	0.7675	65.58
100	0.5702	0.5734	0.6253	0.5896	51.92

3.3.2. Direct microscopic observation method

In this method the entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable change in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

The above images obtained from the direct microscopic method shows the effect of sample at different concentrations on A549 (Lung cancer) cells.

At different sample concentrations say; 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL ,the cell were found to be affected by the action of sample and at 100 µg/mL half of the cells were affected as indicated by the detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells.

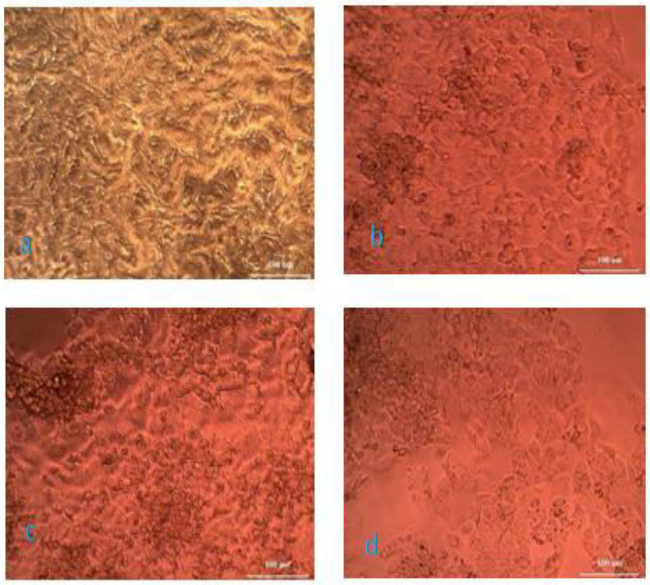

Fig. 7: (a) Control (A549 Lung Cancer cell) (b) 6.25 µg/mL of sample on A549 cell (c) 12.5 µg/mL of sample on A549 cell (d) 25 µg/mL of sample on A549 cell

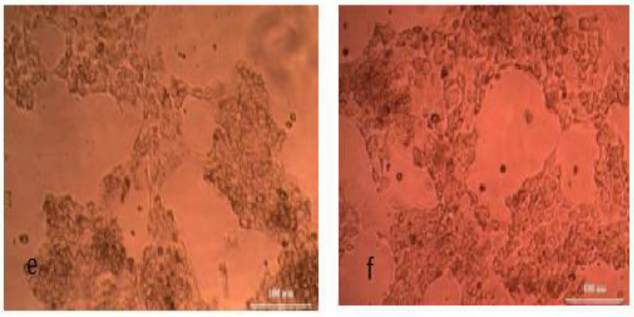

Fig. 8: (e) 50 µg/mL of sample on A549 cell (f) 100 µg/mL of sample on A549 cell

4. CONCLUSION

The docking study showed that the phytochemical amblyone from the tuber of elephant foot yam can effectively interact with the anti apoptotic Bcl-2 protein and was found to be better than the anticancer drug , as indicated by its high docking score of -12.2kcal/mol.

The in-vitro anticancer study of the crude sample (tuber of Amorphophallus paeoniifolius) showed that the sample has potent anticancer activity. The presence of the phytochemical amblyone in the tuber may be responsible for its anticancer property as indicated by its high docking score when docked with antiapoptotic target protein 2w3l. The good docking score is an indication that the amblyone can bind firmly on the groove of Bcl-2 protein which regulates the activity of pro-apoptotic proteins by direct binding and sequestration. The ADME and toxicity values of the ligand obtained using preADMET software also support its druggable character.

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