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Abstract—Cyclooxygenase enzyme has recently garnered a great deal of attention as a promising molecular target for cancer therapy. Present study was aimed to find out cyclooxygenase inhibiting compounds from Phellinus adamantinus - a wood rot mushroom. 3D structures of nine compounds were retrieved from the protein data bank (PDB). Some 3000 - 4000 plant samples representing 2000 species were screened each year for antitumor activity under the auspices of United States National Cancer Institute (NCI). The compounds analyzed exhibited antiviral, antibacterial, antineoplastic, anti-diabetic and anti-inflammatory properties. The present investigation is limited to only one mechanism of action of antineoplastic which gave high Pa (probability of activity) value for all the compounds tested. Molecular interaction of nine compounds with cyclooxygenase enzyme was performed using Discovery studio® – ligand fit docking analysis. Among the nine phytochemical molecules docked against the Cyclooxygenase enzyme the phytochemical-4 methyl benzyl azide was found to be the most effective one when compared to commercial drug, Aspirin. ADME/Tox analysis was also performed. In vitro cytotoxic studies on EAC and DLC cell lines have confirmed the anticancerous activity of methanol extract of P. adamantinus. Thus phytochemicals from wood rot mushroom were proved to be potent anticancerous drug molecules.

Keywords—Cyclooxygenase; Anticancer; Phellinus adamantinus; in silico; PASS; ADME/Tox.

I. INTRODUCTION

Cancer is a leading cause of death in developed and developing countries. The current research in cancer is focussed on the identification of new and unequivocal target for the development of novel anticancer agents. When Richard M. Nixon signed the National Cancer Act in 1971, he committed the US to a war on cancer. Major research programs are being undertaken in a number of laboratories in various parts of the world for screening plant extracts for antitumor activity under the auspices of United States National Cancer Institute (NCI). Some 3000 - 4000 plant samples representing 2000 species are screened each year for antitumor property. Mushrooms are also undergoing scientific analysis and development to prevent and treat cancer. Mushrooms usefulness against cancer is known in China, Russia, Japan, Korea, United States and Canada [1]. The complementary and alternative medicines have disseminated much of the current awareness regarding the utility of mushrooms. Cyclooxygenase (COX) enzymes are widely used to determine the anticancer effects of potential therapeutic products. The present study was carried out to investigate the in silico and in vitro therapeutic potential of bioactive compounds from wood rot mushroom namely Phellinus adamantinus belonging to Basidioymycetes, Aphyllophorales, Polyporaceae.

II. MATERIALS AND METHODS

A. Ligand Preparation

The compounds reported in Gas Chromatography and Mass Spectrum (GCMS) analysis of methanolic extract of P. adamantinus were used in the present study. The structures of the nine compounds (ligands) were generated using Chemdraw ultra 10.0 version of Cambridge University. Their 3D atomic coordinates were created using the ACD/Labs – Chemsketch 12.0 software.

B. Prediction of activity spectra for biologically active substances (PASS)

PASS, a web server which predicts more than 300 pharmacological effects and biochemical mechanisms on the basis of the structural formula of a substance, was efficiently used to find new targets (mechanisms) for all nine ligands. The biological activity spectrums of all nine ligands were obtained one by one proceeding via the internet by submitting a standard .mol file. The prediction result is returned in the form of a table containing the list of biological activity with appropriate probability values i.e. probable activity (Pa) and probable inactivity (Pi).

C. Docking analysis

The three dimensional structure of human COX - 1 and COX-2 enzymes were retrieved from the protein databank (PDB) (Source:www.rcsb.org/pdb/). The native auto inducer and all water molecules were removed. Hydrogen were added using the templates for the protein residues. The ligands were then loaded in the Discovery Studio® (http: www.accelrys.com/ product/ dstudio/) software (Accelrys® software corporation, San Diego, USA). The ligands were docked into the active site using the ‘Ligand Fit’ option. The docked poses with low energy was recorded and validated.
Protocol for receptor-ligand interactions
Receptor structure was loaded in discovery studio

Ligand / Aspirin (standard known ligand) were loaded in discovery studio

Hydrogen atoms were added to the receptor

Binding site specified

Ligand was docked with receptor

Results and dock score with low energy was saved

D. Analysis of absorption, Distribution, metabolism excretion and toxicity properties

Drug discovery needs the study of pharmacodynamics, curing the disease without causing harmful side effects and pharmacokinetics, namely ADMET. The nine compounds predicted from methanolic extracts of *Phellinus adamantinus* were studied for ADMET properties using Discovery Studio 2.0-ADME/Tox software. By using this software Blood - Brain Barrier penetration (BBB), Human intestinal absorption (HIA), aqueous solubility and Plasma protein binding (PPB) were analysed.

E. Cytotoxicity screening

The *in vitro* short term cytotoxicity of methanolic extract of *P.adamantinus* was assessed using EAC (Ehrlich Ascites Carcinoma) and DLA (Daltons Lymphoma Ascites) tumor cell lines by Trypan blue exclusion method [2]. The cell lines were obtained from Amala Cancer Research Institute, Thrissur, Kerala, India.

III. RESULTS AND DISCUSSION

For millennia, mushrooms have been valued by mankind as an edible and medicinal source. Attempts have been made in many parts of the world to explore the mushrooms and their metabolites for the treatment of a variety of human ailments. But still a vast wealth of mushrooms has not been explored which may contain active medicinal properties of curing a number of diseases. Therefore, the biological screening studies should be concentrated on the unexplored mushrooms for their potential values. The present investigation was carried out by *in silico* and *in vitro* analysis to characterize the wood rot mushroom *Phellinus adamantinus* as important raw drug material.

![Figure 1. Probable structures of compounds in methanol extract of *P. adamantinus* fruit body as reported by GCMS analysis](image)

A. Rendering of 3D atomic coordinates & PASS Prediction

The 2D structures of nine compounds present in *P. adamantinus* by GCMS analysis were retrieved from Chemdraw ultra10.0 version of Cambridge University. Their structures were cleaned, optimized and energy minimized by charm forcefield and converted to 3D atomic coordinates using ACD/Labs Chemsketch 12.0. (Fig.1)

B. Prediction of activity spectra for biologically active substances (PASS)

The activity spectra of compounds of *P. adamantinus* were predicted using online server PASS. All the compounds analyzed exhibited the following activities: antibacterial, antiviral, antineoplastic, antidiabetic, antipyretic, anti-inflammatory etc. with varying mechanisms of action. The actual PASS output of 4-methyl benzyl azide is presented as screen shot in Fig.2.

This compound 4-methyl benzyl azide contains many types of activities. The present investigation is limited to only one mechanism of action of antineoplasty which gave high Pa (probability of activity) value [3] for all nine compounds tested. The mechanisms of action of nine compounds were found to inhibit cyclooxygenase enzyme (Fig.5).
C. Crystal structure of target Cyclooxygenase (COX) 1&2

The crystal structure of Cyclooxygenase 1 (Fig. 3a) and 2 (Fig. 3b) were retrieved from Protein Data Bank (PDB). The pharmacological inhibition of COX is an effective target to fight against cancer [4]. COX-2 expression was increased in certain types of solid tumors such as gastric cancer [5], pancreatic cancer [6] and oesophageal cancer [7]. Although the exact mechanism of COX-2 in carcinogenesis is not completely understood, it was suggested that it is due to up-regulation of prostaglandin-induced angiogenesis [8]. Therefore, new generation of COX-2 inhibitors are anticipated. Hence the present investigation was carried out to find new drug molecules which potentially inhibit COX-1 and 2.

The docking energies for the cyclooxygenase 1 (PDB id: 1CQE) and cyclooxygenase 2 (PDB id: 6COX) showed that the ligands from methanol extract of \textit{P. adamantinus} have selectivity for COX - 2 over COX - 1. The ligands such as 4-methyl benzyl azide, 2,3 – epoxy – 5,8 – hexadecadien – 1 – ol and 3 – methyl – 2 – (2 – oxo propyl) furan (Fig. 4) showed excellent binding with COX – 2 when compared to aspirin which is evident from the docking poses.

Table I presents the energy minimization for the best docked poses and the tightest binding ligand for COX -2 as revealed in the present study was 4 – methyl benzyl azide (-30.97 kcal mol\(^{-1}\)) and for COX - 1 was also the same ligand (-24.07 kcal mol\(^{-1}\)) but the standard commercial drug aspirin showed less binding energy (-19.32 kcal/mol).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Binding energies of the best poses (Kcal Mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COX - 1</td>
</tr>
<tr>
<td>2-Isobutyl-3-methyl furan</td>
<td>- 8.96</td>
</tr>
<tr>
<td>2-Tert-butyl-4-(2,4,4-trimethyl pentan-2-yl) phenol</td>
<td>- 16.32</td>
</tr>
<tr>
<td>3-Methyl-2-(2-methylallyl) furan</td>
<td>- 12.73</td>
</tr>
<tr>
<td>Tetradecan-1-ol</td>
<td>- 9.73</td>
</tr>
<tr>
<td>Bicycle[3.1.1]hept-2-ene-carbaldehyde</td>
<td>- 6.22</td>
</tr>
<tr>
<td>3-Methyl-2-(2-methylbut-2-ethyl)furan</td>
<td>- 18.68</td>
</tr>
<tr>
<td>4-Methyl benzyl azide</td>
<td>- 24.07</td>
</tr>
<tr>
<td>2,3-Epoxy-5,8-hexadecadien-1-ol</td>
<td>- 14.43</td>
</tr>
<tr>
<td>3-Methyl-2-(2-oxo(propyl)) furan</td>
<td>- 9.05</td>
</tr>
<tr>
<td>Aspirin</td>
<td>- 19.32</td>
</tr>
</tbody>
</table>
Figure 5. Screenshot of 4-methybenzyl azide’s PASS result showing Cyclooxygenase inhibition as mechanism of action

Figure 6. Cytotoxic effects of methanol extracts of Phellinus adamantinus fruit body on EAC & DLA cell lines.

Thus the molecular docking studies reveal that the ligands from methanol extract of P. adamantinus fruit bodies have strong binding affinities for the cancer-relevant molecular targets and are shown to inhibit these enzymatic targets (Table I).

The docking energies for flavonoid ligands of Lonchocarpus in the binding sites of COX-1 and COX-2 presented excellent binding [9]. These docked orientations were slightly different than those observed for flavonoids docking to COX-1 [10].

D. ADME/Tox analysis

In ADMET intestinal absorption, aqueous solubility, blood brain barriers and protein binding percentage properties of the ligands were analyzed. The intestinal absorption was good in all the ligands tested. The aqueous solubility was optimal in 3-methyl-2-(2-oxopropyl) furan. The blood brain barrier ratio was very high in 2-tert-butyl-4(2, 4, 4-trimethyl pentan-2-yl) phenol, tetradecan-1-ol and 2,3-epoxy-5,8-hexadecadien-1-ol. The plasma protein binding percentage was more than 90% for 2-tert-butyl-4 (2, 4, 4-trimethyl pentan-2-yl) phenol, tetradecan-1-ol, 4-methyl benzyl azide and 2, 3 - epoxy-5,8-hexadecadien-1-ol (Table II).

Flavonoids have been effective at inhibiting cyclooxygenase enzymes [11]. The same is predicted in the present study also, where the most effective molecule from P. adamantinus is 4-methyl benzyl azide. This drug also exhibited good ADMET properties indicating scope for further studies towards developing this compound into a potent drug candidate.

E. Cytotoxicity study

The trypan blue exclusion method was used to study the cytotoxicity when the EAC and DLA cells from the carrier mice were treated with different concentrations of the extract for 3h, the relative cell survival progressively decreased in a dose dependent manner (Fig.6). The strong cytotoxic effect of methanolic extract of P. adamantinus against EAC cells implies that the methanol extract may have a potent anticancer effect on cancer cells. The activity of the extract of the macro fungi against DLA cell lines is relatively poor. This is in accordance with the report on Pleurotus florida which did not exhibit cytotoxicity against DLA cell lines up

<table>
<thead>
<tr>
<th>Name of the ligand</th>
<th>Absorption</th>
<th>Solubility</th>
<th>Brain - Blood ratio</th>
<th>Plasma protein binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Isobutyl-3-methyl furan</td>
<td>Good</td>
<td>Low</td>
<td>High</td>
<td>&lt; 90%</td>
</tr>
<tr>
<td>2-Tert-butyl-4 (2,4,4-trimethyl pentan-2-yl) phenol</td>
<td>Moderate</td>
<td>Good</td>
<td>Very high</td>
<td>&gt;=95%</td>
</tr>
<tr>
<td>3-Methyl-1-2- (2-methyl allyl) furan</td>
<td>Good</td>
<td>Good</td>
<td>High</td>
<td>&lt; 90%</td>
</tr>
<tr>
<td>Tetradecon-1-ol</td>
<td>Good</td>
<td>Good</td>
<td>Very high</td>
<td>&gt;=95%</td>
</tr>
<tr>
<td>Bicyclo[3.1.1]hept-2-ene-carbaldehyde</td>
<td>Good</td>
<td>Good</td>
<td>High</td>
<td>&lt; 90%</td>
</tr>
<tr>
<td>3-Methyl-1-2- (2-methyl but-2-enyl) furan</td>
<td>Good</td>
<td>Good</td>
<td>High</td>
<td>&lt; 90%</td>
</tr>
<tr>
<td>4-Methyl benzyl azide</td>
<td>Good</td>
<td>Good</td>
<td>High</td>
<td>&gt;=95%</td>
</tr>
<tr>
<td>2,3-Epoxy-5,8-hexadecadien-1-ol</td>
<td>Good</td>
<td>Good</td>
<td>Very high</td>
<td>&gt;=90%</td>
</tr>
<tr>
<td>3-Methyl-1-2- (2-oxopropyl) furan</td>
<td>Good</td>
<td>Optimal</td>
<td>Medium</td>
<td>&lt; 90%</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Very low</td>
<td>Optimal</td>
<td>Very high</td>
<td>&lt; 90%</td>
</tr>
</tbody>
</table>
to a concentration of 1mg/mL in a short-term bioassay [12]. Nonetheless, strong cytotoxic activity of ethyl acetate and methanol extracts of *Phellinus rimosus* against EAC and DLA cell lines which partially explains its significant antitumor activity against ascites and solid tumor [13].

### IV. CONCLUSION

This study extends the understanding on the molecular mechanism underlying the diverse biological activities of the medicinal mushroom *P. adamantinus*. Further, it supports the pharmacological basis that these macro fungi have been used as traditional medicine for the treatment of cancer.

### REFERENCES


