BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2016; 11: 545-551
ISSN: 1991-0088

Assessment of dual inhibitory activity of epifriedelanol isolated from *Cayratia trifolia* against ovarian cancer

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Article Info

Received: 8 September 2015
Accepted: 8 January 2016
Available Online: 11 May 2016

DOI: 10.3329/bjp.v11i2.24933

Cite this article:
Perumal PC, Sowmya S, Velmurugan D, Sivaraman T, Gopalakrishnan VK. Assessment of dual inhibitory activity of epifriedelanol isolated from *Cayratia trifolia* against ovarian cancer. Bangladesh J Pharmacol. 2016; 11: 545 -51.

Abstract

Cayratia trifolia is used as diuretic, in tumors, neuralgia and splenopathy. However, compounds depicting anti-ovarian cancer activities from this plant source have not yet been identified and structurally characterized till date. In the present study, X-ray structure of epifriedelanol, a bioactive compound, isolated from the ethanolic extract of the *C. trifolia* and its binding affinities against a few proteins (HER2, EGFR and CXCR4) that are reported to get overexpressed under ovarian cancer had been thoroughly studied by using molecular docking means. Binding affinities of the compound vis-à-vis that of carboplatin, a FDA approved drug to the ovarian cancer, to interact with the protein targets are quite impressive. The drug-likeness properties of the epifriedelanol and scope to develop the compound as a potent anti-ovarian cancer drug are discussed in this paper.

Introduction

Ovarian cancer accounts for the highest tumor related mortality among gynecologic malignancies and is the fifth most frequent cause of cancer related death. In 2014, the incidence rate for women in developed countries was about 9.4 per 100,000 compared to 5.0 per 100,000 in developing countries. However, approximately 25% of cases are diagnosed between ages 35 and 54 (Jayson et al., 2014). Above 70% of women were diagnosed with late stage III and IV disease.

Although not withstanding great advancements have been made in the treatments and management control of the cancer progression. A number of undesired adverse effects, sometimes, occurs during chemotherapy (Desai et al., 2008). Research reports suggest that compounds from natural sources are superior to synthetic compounds in terms of pharmacokinetic and pharmacodynamics properties.

In general, usage of medicinal compounds is always superior to the synthetic compounds. So, the recent research has been focused towards the plant compound isolation and compounds production at large scale (Fortes et al., 2012).

A large proportion of the World population depends on the traditional medicine because of the shortage and high expenses of orthodox medicine (Dutta and Maharia, 2012; Li et al., 2011; Sultana et al., 2011). Natural products play a central role in the development of novel drug for the treatment and prevention of diseases (Khan et al., 2015; Sharma et al., 2015; Yu et al., 2013; Dhanamani et al., 2011).

Cayratia trifolia (L.) is commonly known as Fox grape in English, Kattuppirantai in Tamil, Amlabel and Ramchana in Hindi and Amlavetash in Sanskrit (Perumal et al., 2014; Perumal et al., 2015). It has been reported to contain huge amount of bioactive compounds such as

yellow waxy oil, steroids, terpenoids, flavonoids and tannins (Singh et al., 2012). The whole plant is used as a diuretic, in tumors, neuralgia and splenopathy. The bark extract has been reported to have antiviral, antibacterial, anti-protozoal, hypoglycemic, anti-cancer and diuretic activities in animal models (Gupta et al., 2012). So far against ovarian cancer, the bioactive compounds have not been isolated from this plant. Therefore, the purpose of the present study is to isolate and identify the potent anti-ovarian cancer compound from *C. trifolia* by using experimental models and to establish the possible real value of these kinds of compounds as anti-ovarian cancer agents. The steps of fractionation, purification and structure elucidation are basically required to characterize the plant based compounds. Computational molecular simulation studies will be conducted to study the molecular interactions of bioactive compound (ligand) with ovarian cancer target proteins and to validate the potential binding mode. These studies provide excellent prediction on possible mechanism of action of the experimentally studied compound.

Materials and Methods

Collection of plant and authentication

The whole plant of *C. trifolia* was collected from in and around the area of Poonthottam (Kumbakonam Town), Tamil Nadu, India and it was authenticated by Dr. P. Sathyanarayanan, Botanical Survey of India, TNAU Campus, Coimbatore. The voucher number of the plant was BSI/SRC/5/23/2010-2011/Tech.1527. The fresh material of the whole plant was washed under the running tap-water, dipped in saline overnight, air dried and then pulverized. The fine powder obtained from the procedure was used in the present study (Perumal et al., 2012).

Extract preparation

Based on the previous studies, 300 g of dried plant powder was extracted in 1,500 mL of ethanol in a sporadic shaker for 72 hours at room temperature. The extract was collected and kept concentrated at 40°C under reduced pressure using rotary evaporator. The dried extract was stored at 4°C until further compound isolation process.

Compound isolation

Ethanolic extract of the *C. trifolia* (5 g) was subjected to purification on silica gel column (3 × 30 cm) and various fractions were successfully eluted with petroleum ether (100%) followed by a ratio of petroleum ether: chloroform (8:2, 6:4, 4:4, 2:8 v/v). The column fractions were collected in 20 mL test tubes. Totally 52 fractions were collected from the silica gel column and these fractions were loaded on the activated silica gel TLC plates (20 × 20 cm). The plates were developed using petroleum ether: chloroform (90:10), chloroform: ethyl acetate (90:10) and ethyl acetate: methanol (90:10) solvents. The single spot was located by exposing the plate to iodine fumes.

Structure elucidation

The isolated bioactive compound was crystallized in alcoholic medium using slow evaporation method at room temperature. The intensity data were collected using Bruker Smart apex II single crystal X-Ray diffractometer equipped with CCD available in CAS in Crystallography and Biophysics, The University of Madras. The structures were solved by direct methods using the program SHELXS-97 (Cremer and Pople, 1975) and refined by full-matrix least-squares procedures using the program SHELXL-97 inbuilt in WinGX software (Sheldrick, 2008). The geo-metrical parameters were calculated using the program PARST and graphical plots were drawn using the programs ORTEP-3 (Farrugia, 2012) and PLATON (Spek, 2009).

In silico studies on chemical molecules – protein targets interactions

Preparation of protein structure

The 3D structure of HER2, EGFR and CXCR4 were retrieved from the protein data bank (www.rcsb.org) and these proteins were prepared by protein preparation wizards (standard methods) that are available in grid-based ligand docking with energetics. Proteins were optimized using sample water orientation and minimized by using RMSD 0.30 Å and OPLS (2005) force field (Protein Preparation Wizard).

Active site prediction

The active sites (binding pockets) and functional residues of HER2, EGFR and CXCR4 were identified and characterized by Site-Map module from Schrodinger package (SiteMap version 3.3). SiteMap calculation begins with an initial search step that identifies or characterizes through the use of grid points- one or more regions on the protein surface that may be suitable for binding ligands to the receptor. Contour maps were then generated, produced hydrophobic, hydrophilic maps hydrogen binding possibilities which may guide the protein-ligand docking analysis.

Ligand preparation

Epifriedelanol and carboplatin were used in molecular docking studies. These ligands were prepared using LigPrep version 3.3.. The structure of each ligands were optimized by means of the OPLS 2005 force field using a default setting.

Molecular docking analysis

All docking analyses were performed by using the standard precision (SP) which is standard mode of

Glide (Grid-based ligand docking with energetic) module from Glide version 6.6. Epifriedelanol and carboplatin ligands were docked to in the binding site of HER2, EGFR and CXCR4 using GLIDE. The scaling Vander Waals radii were 1.0 in the receptor grid generation. Grid was prepared with the bounding box set on 20A°. The co-ordinates of this enclosing box with the help of the active site residues to be set default. The force field is used for the docking protocol is OPLS_2005. The lowest-energy docked complexes were found in the majority of similar docking conformations (Srinivasan et al., 2014).

ADME properties prediction

Epifriedelanol and carboplatin (existing FDA approved drugs for ovarian cancer) were checked for their ADME properties using QikProp 4.3 module. QikProp helps in analyzing the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties. Predicted significant ADME properties are viz, Molecular weight, H-bond donor, H-bond acceptor and log P (o/w) (QikProp, version 4.3).

Results

Concentrated crude extract (5 g) was collected from the ethanolic extract of *C. trifolia* and it was fractionated with column chromatography. Through the column, 52 fractions were collected and analyzed by TLC plates. Out of 52 fractions, 13th fraction showed single spot and it's formed as crystal (yielded crystal weight was 15 mg). The isolated crystal data retrieved from X-ray crystallography method (Table I). In the crystal structure, all the five cyclohexane rings adopt a chair conformation (Figure 1).

The cyclohexane ring 1 (C1/C2/C3/C20/C21/C22) made a dihedral angle of 13.86 (1)° with the cyclohexane ring 2 (C3/C4/C5/C6/C19/C20); a dihedral angle of 28.22(1)° with the cyclohexane ring 3 (C6/C7/C16/C17/C18/C19); a dihedral angle of 30.72(1)° with the cyclohexane ring 4 (C7/C8/C9/C10/C15/C16); a dihedral angle of 35.72 (1)° with the cyclohexane ring 5 (C10/C11/C12/C13/C14/C15). The cyclohexane ring 2 made a dihedral angle of 14.75(1)° with the cyclohexane ring 3; a dihedral angle of 18.12(1)° with the cyclohexane ring 4; a dihedral angle of 22.04 (1)° with the cyclohexane ring 5. The cyclohexane ring 3 made a dihedral angle of 14.31 (1)°; a dihedral angle of 11.82 (1)° with the cyclohexane ring 5. The dihedral angle between the cyclohexane ring 4 and 5 was 8.03 (1)°. The methyl carbon atoms C23 and C24 attached with the cyclohexane ring 1 deviated by -0.0971 (3)Å and -1.8200 (3) Å, respectively. The methyl carbon atom C25 attached with the cyclohexane ring 2 deviated by -1.7663 (3) Å. The methyl carbon atoms C26 and C27 attached with the cyclohexane ring 4 deviated by 1.9114 (3) Å and -1.8341 (3) Å, respectively. The methyl carbon atoms C28, C29 and C30 attached with the cyclohexane ring 5 deviated by -1.6445 (4) Å , 0.4093 (5) Å and -1.7176 (4) Å, respectively. The hydroxyl oxygen atom O1 attached with the cyclohexane ring 1 deviated by -1.3138 (5) Å. No significant hydrogen bond was found in the structure. Thus, based on the X-ray crystallo-

Table I

Crystallographic data

Parameter	Parameters
Empirical formula	C_{30} H_{52} O_1
Formula weight	428.72
Temperature (K)	293 (2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	C2
Unit cell dimensions	
a (Å)	13.4113 (5)
b (Å)	6.4226 (2)
c (Å)	29.5869 (10)
α (°)	90.00
β (°)	91.848 (2)
γ (°)	90.00
Volume (Å^3)	2547.15 (2)
Z, D_{cal} (Mgm^{-3})	4, 1.118
Absorption coefficient (mm^{-1})	0.064
F(000)	960
Crystal size (mm)	0.30 × 0.25 × 0.20
Theta range for data collection (°)	0.69 to 28.33
Limiting indices	-17<=h<=16, -7<=k<=8, -34<=l<=38
Reflections collected / unique	12293 / 5563
R(int)	0.022
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5563 / 1 / 282
Goodness-of-fit on F^2	1.06
Final R indices [I>2σ(I)]	R1 = 0.054, wR2 = 0.153
R indices (all data)	R1 = 0.0596, wR2 =0.1454
Largest diff. peak and hole (e.Å^{-3})	0.32 and −0.25

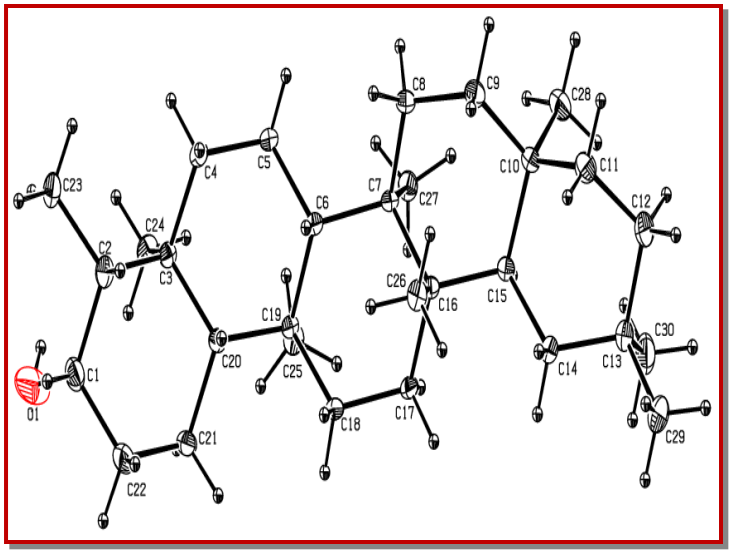

Figure 1: Crystal structure of epifriedelanol showed the atom position on the structure

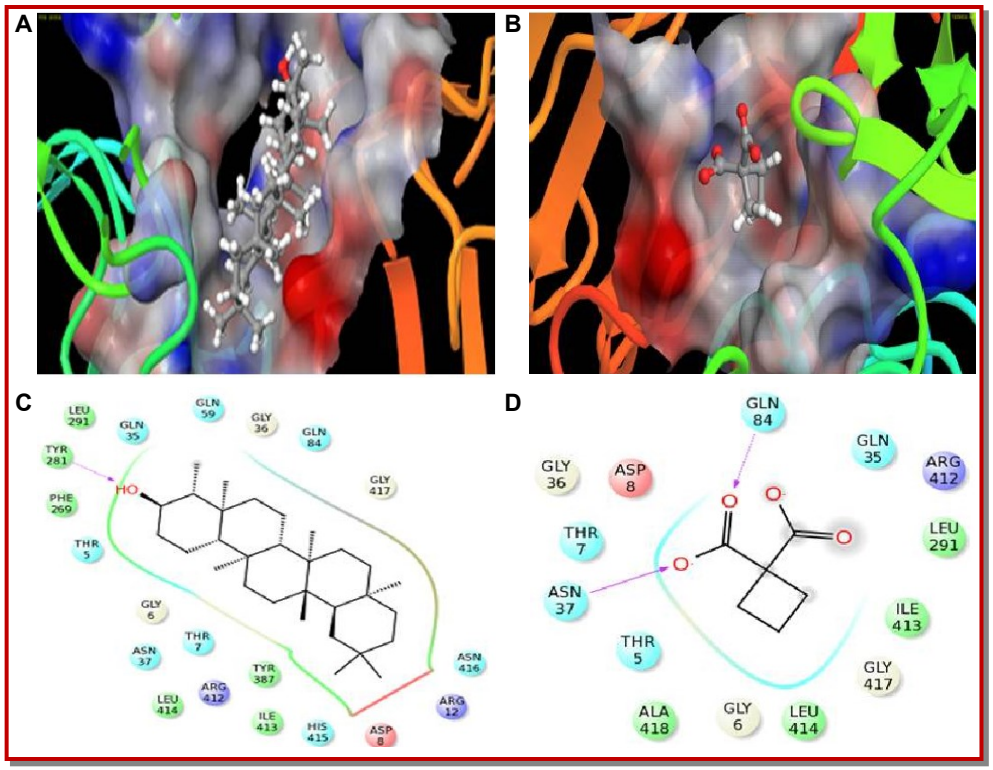

Figure 2: Docking complexes of HER2 with (A) epifriedelanol and (B) carboplatin generated by using Glide-XP module of Schrodinger suite are shown in this figure. The proteins, ligands and binding pockets are represented in ribbon, sticks and surface models, respectively. Residues of the HER2 that are within 4 Å proximities to the (C) epifriedelanol and (D) carboplatin are illustrated in 2D graphics. Dotted lines denote 'Hydrogen bonds' between the corresponding atoms

graphy data, the isolated crystal structure was identified as epifriedelanol and it had been reported (NMR structure) to have *in vitro* antitumor properties in a potato disc bioassay study (Kundu et al., 2000).

In docking results, the HER2-epifriedelanol complex had comparable good Glide score of -4.808 and Glide energy of -36.178 kcal/mol, when compared with HER2 -carboplatin complex which had Glide score of -4.802 and Glide energy of -17.098 kcal/mol. HER2-epifriedelanol complex had good affinity because of strong hydrogen bond formed between H atom and residue TYR281 within the complex. On the other hand, HER2-carboplatin complex also had comparable affinity through the hydrogen bonds between residue and atom of ASN37-O and GLN84-O respectively (Figure 2).

EGFR–epifriedelanol complex possessed comparable

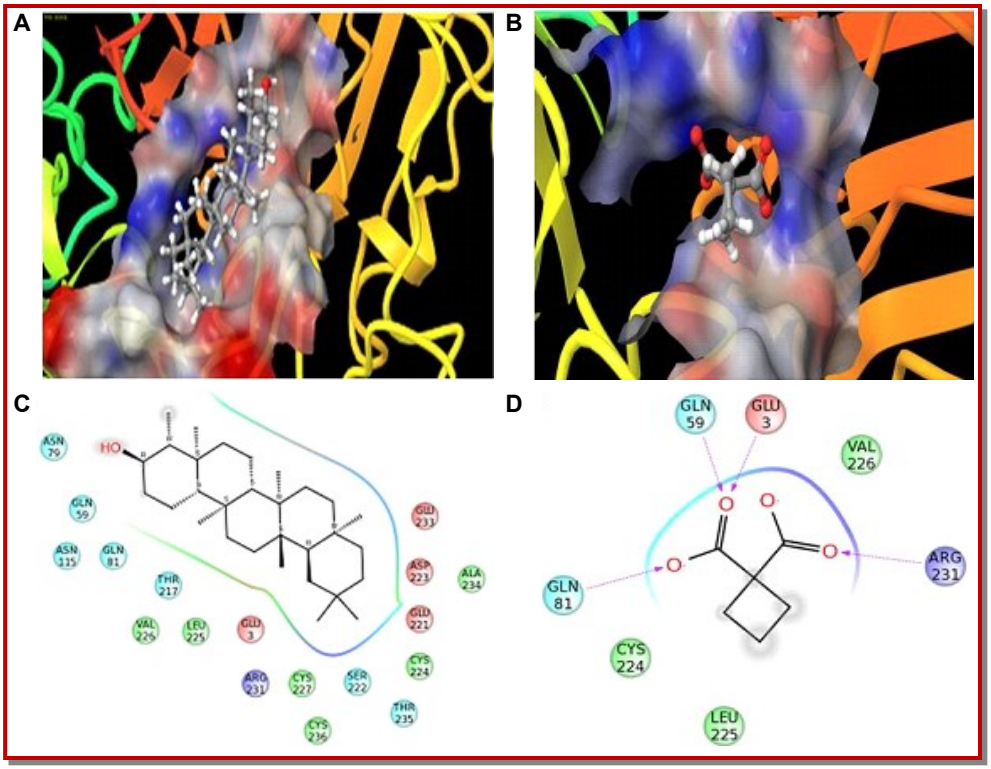

Figure 3: Docking complexes of EGFR protein with (A) epifriedelanol and (B) carboplatin generated by using Glide-XP module of Schrodinger suite are shown in this figure. The proteins, ligands and binding pockets are represented in ribbon, sticks and surface models, respectively. Residues of the EGFR that are within 4 Å proximities to the (C) epifriedelanol and (D) carboplatin are illustrated in 2D graphics. Dotted lines denote 'Hydrogen bonds' between the corresponding atoms

Glide score of -3.953 and good Glide energy of -27.068 kcal/mol when compared with EGFR-carboplatin complex which had Glide score of -4.152 and Glide energy of -11.852 kcal/mol. The epifriedelanol compound strongly binded in hydrophobic region of EGFR. In the same way, EGFR-carboplatin complex also possess-ed good affinity, because of strong hydrogen bond that was formed between residues and atoms of C -O-GLN-81, GLU3, GLN59 and ARG231-C=O respectively (Figure 3).

CXCR4–epifriedelanol complex possessed good Glide score of -4.935 and Glide energy of -40.795 when compared with CXCR4-carboplatin complex whose Glide score and Glide energy were -4.540 and -16.907 kcal/mol respectively. The epifriedelanol compound binded in hydrophobic region of CXCR4. On the other hand, in carboplatin compound C-O atom strongly bind with CXCR4 residue of TYR121 by the hydrogen bond (Figure 4). The ADME properties prediction result of epifriedelanol and carboplatin were under acceptable range.

Discussion

The phytochemical constituents of the plant extracts are the major basis of pharmacological activities of medicinal plants whereas flavonoids are anti-oxidants and minerals play significant roles in many processes taking place in living systems (Starlin et al., 2012).

The active site (binding pocket/site) was preferred based on the site score and hydrophobic/hydrophilic areas, which holds better binding cavity. The binding site residues of HER2, EGFR and CXCR2 produced hydrophobic, hydrophilic maps and hydrogen binding possibilities which may guide the protein-ligand docking analysis (Tripathi et al., 2012). In order to rationalize the described structure characterization, protein preparation and site map prediction results, molecular docking studies were undertaken on epifriedelanol (isolated compound) and carboplatin (FDA approved drug for ovarian cancer) complexes with X-Ray crystal structure of HER2, EGFR and CXCR2 (ovarian cancer targets). The molecular docking is frequently used to predict the binding orientation of small molecule drug candidate to their protein targets in order to predict the affinity and activity of the small molecule (Pratibha et al., 2014). The molecular docking performance indicated that, compared with FDA approved drug the isolated bioactive compound of epifriedelanol has better affinity with ovarian cancer targets. The limitations of ADME properties are: Not more than 5 hydrogen bond donors, Not more than 10 hydrogen bond acceptor, A molecular mass less than 500 daltons, An octanol- water partition coefficient log P not greater than 5. Thus, the ADME

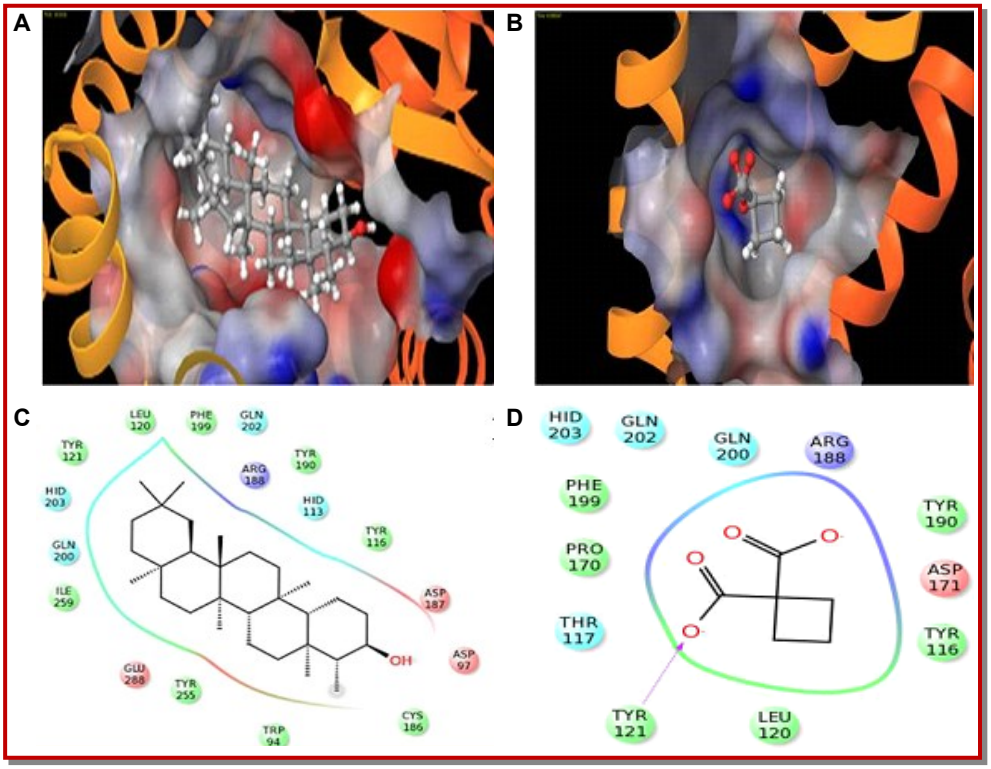

Figure 4: Docking complexes of CXCR4 protein with (A) epifriedelanol and (B) carboplatin generated by using Glide-XP module of Schrodinger suite are shown in this figure. The proteins, ligands and binding pockets are represented in ribbon, sticks and surface models, respectively. Residues of the CXCR4 that are within 4 Å proximities to the (C) epifriedelanol and (D) carboplatin are illustrated in 2D graphics. Dotted lines denote 'Hydrogen bonds' between the corresponding atoms

properties prediction of epifriedelanol and carboplatin are under acceptable range.

Conclusion

The isolated bioactive compound, epifriedelanol might act as a good inhibitor against ovarian cancer targets.

Financial Support

Indian Council of Medical Research (ICMR), New Delhi for providing the financial support (File No. BIC/11(19)/2013)

Conflict of Interest

Authors declare no conflict of interest

Acknowledgement

The authors are thankful to our Academic and Administrative authorities of Karpagam University for providing facilities and encouragement. We extend our deepest thanks to SASTRA University for providing us an opportunity to use Schrodinger Suite (*in silico* analysis). We express our sincere thanks to CAS in Crystallography and Biophysics, University of Madras for crystallography studies.

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