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Research Article

# Discovery of Novel Inhibitors for HER2 from Natural Compounds Present in *Cayratia trifolia* (l.): An *In silico* Analysis

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**ABSTRACT**

HER2 is an established therapeutic target primarily in breast, gastric and many other type of cancers. Its Inhibition process suggests that, it could be a promising target for cancer. Therefore the main aim of the study is to identify the novel inhibitors for HER2 from the natural compounds present in *Cayratia trifolia.* The natural compounds were selected from GC-MS analysis of ethanolic extract of *Cayratia trifolia*.. Molecular docking studies were done to find the inhibitory activity angainst HER2 and ADME properties also were predicted to find pharmacokinetics and pharmacodynamics of the selected ligands. The docking analysis revealed that, the natural compounds of Ethyl Oleate, 4,8,12,16-Tetramethylheptadecan-4-olide and Heptacosanol has high inhibitory activities when compared with selected FDA approved drug and the ADME properties also were acceptable range. Thus, based on the results it can be concluded that these natural compounds may act as novel inhibitors for HER2 and its lead to drug development for variety of cancers.

**Keywords:** HER2; *Cayratia trifolia* (L.); Natural compounds; Molecular docking; ADME

## INTRODUCTION

Human epidermal growth factor 2 (HER2), is the second member of a family of transmembrane tyrosine kinase receptors involved in signal transduction pathways mediating tumour growth, differentiation and survival [(1)]. HER2 signaling promotes cellular proliferation through the RAS-mitogen-activated protein kinase (MAPK) pathway and inhibits cell death through the phosphatidylinositol 3-kinase (PI3K)-Akt pathway [(2)]. In addition, HER2 signaling upregulates the expression of vascular endothelial growth factor (VEGF), which is critical for tumor angiogenesis [(3)]. HER2 overexpression and amplification have central roles in the initiation, progression and metastasis of breast cancer and gastric cancer and are associated with poor prognosis [(4)]. Ligand independent homodimerisation or heterodimerisation occurs when HER2 is overexpressed with subsequent autophosphorylation of the intracellular tyrosine kinase domain and activation of the intracellular signalling cascade [(5)]. HER2 is an oncogenic driver and a therapeutic target in breast and gastric cancers. HER2 overexpression and/or amplification were demonstrated to occur in up to 30% of breast cancers associated with aggressive tumourorigenesis. A similar percentage of oesophago-gastric cancers were found to overexpress HER2 [(6)]. Variety of bioactive compounds are present in medicinal plants and they widely used against various diseases [(7)]. The demand for natural food constituents has resulted in broad research on naturally occurring bioactive compounds which are able to develop novel drug agents for many diseases [(8)]. Comprehension of the chemical constituents of medicinal plant is helpful in the discovery of therapeutic agents as well as new sources of economic materials like oil and gums [(9)]. *Cayratia trifolia* (L.) is the medicinal plant belongs to the family of Vitaceae, commonly known as *Fox grape* in English is native to India, Asia and Australia [(10)]. It is a perennial climber having trifoliated leaves with (2-3 cm), long petioles and ovate to oblong-ovate leaflets. Flowers are small greenish white brown in color. Fruits are fleshy, juicy, dark purple or black, nearly spherical, about 1 cm in diameter [(11)]. It has been reported to contain huge amount of bioactive compounds such as yellow waxy oil, steroids, terpenoids, flavonoids and tannins. Whole plant is used as diuretic in tumors, neuralgia and splenopathy [(12)]. The bark extract has been reported to have antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activities in animal models [(13)]. In the present research work aimed to identify the novel inhibitors for HER2 from the natural compounds present in the ethanolic extract of *Cayratia trifolia* using computational molecular analysis.

## MATERIALS AND METHOD

*Computational molecular analysis*

*Ligand selection preparation*

Based on the our previous studies 18 natural compounds were selected from GC-MS analysis of ethanolic extract of *Cayratia trifolia* (L.) [(14)] and FDA approved drug of

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Table 1: GlideScores and GlidEnergies of various ligands with HER2 (the natural compounds are identified from ethanolic extract of *Cayratia trifolia* (L.) and Cyclophosphamide is a FDA approved drug) complexes are given as calculated from the molecular docking studies.

| S. No | Compounds | Glide Score | Glide Energy |
|---|---|---|---|
| 1 | Cyclopentadecane | -4.682 | -18.953 |
| 2 | 9-Borabicyclo [3.3.1]nonane, 9-(2-propen-1-yloxy)-.1 | -4.365 | -21.400 |
| 3 | 3-Octadecyne | 0.713 | -29.482 |
| 4 | 2-(Octadecyloxy)ethanol | -4.298 | -38.717 |
| 5 | 9-Octadecyne | -0.794 | -31.696 |
| 6 | citronellyl formate | -1.017 | -25.611 |
| 7 | Hexadecanoic acid | -0.830 | -32.308 |
| 8 | Phytol | -2.519 | -32.769 |
| 9 | Trans-13-Octadecenoic acid | -1.404 | -33.010 |
| 10 | Linoleic Acid | -1.383 | -31.843 |
| 11 | Ethyl Oleate | -5.778 | -37.514 |
| 12 | Stearic acid | -0.766 | -33.400 |
| 13 | 3-Eicosene | 0.256 | -31.826 |
| 14 | 4,8,12,16-Tetramethylheptadecan-4- olide | -5.653 | -32.855 |
| 15 | Hentriacontane | -4.082 | -41.863 |
| 16 | Heptadecane | 0.249 | -27.814 |
| 17 | Oxirane | -4.949 | -13.434 |
| 18 | Heptacosanol | -5.876 | -42.427 |
| 19 | Cyclophosphamide (FDA drug) | -5.496 | -30.241 |

Cyclophosphamide (standard drug for comparison) also were prepared using the LigPrep 2.4 [15] for molecular docking analysis. The structure of each ligands were optimized by means of the OPLS 2005 force field using a default setting.

*Preparation of protein structure*

The 3D structure of HER2 (PDB ID: 3BE1) was retrieved from Protein Data Bank (www.rcsb.org) and the protein was processed for missing atoms/hydrogens, optimized for sample water orientations and energy was minimized using (standard methods) RMSD 0.30Å and OPLS (2005) force field by protein preparation wizards module of Schrodinger suite [16].

*Active site prediction*

The active site (binding pockets) and functional residues of HER2 were identified and characterized by SiteMap 5.5 module from Schrodinger package [17]. SiteMap calculation initiateswith 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 generated.Hydrogen binding possibilities, hydrophilic maps, hydrophobic are may guide the protein-ligand docking analysis.

*Molecular docking analysis*

All docking analysis were performed by using the standard precision (SP) which is Standard mode of Glide 5.6 (Gridbased Ligand Docking with Energetic) module from Schrodinger 2012 [18]. All selected natural compounds were docked in to the binding site of HER2 using Glide modiule. The scaling Vander Waals radii were 1.0 in the receptor grid generation. Grid was prepared with the bounding box set on 20Aº. The co-ordinates of this enclosing box with the help of the active site residues to be set default. The force field is using for the docking protocol is OPLS_2005. The docked lowest-energy complexes were found in the majority of similar docking conformations.

*ADME properties prediction*

The HER2 ligands were checked for their ADME properties using QikProp 2.3 module [19]. It helps to analyze the pharmacokinetics and pharmacodynamics of the ligands by accessing the drug like properties. The significant ADME properties such as Molecular weight (MW), H-Bond donor, HBond acceptor and log P (O/W) were predicted.

## RESULTS AND DISCUSSION

The selected bioactive compounds posses many biological activities such as anti-cancer, anti-inflammatory, anti-microbial, anti-diabetic etc., [20-23]. The best active site (binding pocket/site) was preferred based on the site score and hydrophobic/hydrophilic areas, which holds better binding cavity [24]. The binding site residues of HER2 were predicted and it may be involved in the binding of substrate and small molecule. Thus, all these residues were confirmed as HER2 active site residues and picked to generate grid in the cen roid of these residues for molecular docking approach. 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 [25]. The docking results of 18 natural compounds and FDA approved drug of Cyclophosphamide also formed complexwith HER2 protein shown in Table 1. Among these compounds Ethyl Oleate, 4,8,12,16-Tetramethylheptadecan-4-olide and Heptacosanol had good affinity when compared with the FDA drug. The HER2/ Ethyl Oleate complex (Figure 1a) possesses comparable good Glide score of -5.77 and Glide energy of -37.514 kcal/mol, when compared to HER2/ cyclophosphamide complex (Figure 1d) which has Glide score of -5.496 and Glide energy of -30.241 kcal/mol. The Ethyl Oleate compound strongly binds in hydrophobic region of HER2 at GLY442 and ASN466 residue (Figure 2a). In the same way HER2/4,8,12,16-Tetramethylheptadecan-4-olide (Figure 1b) complex also has comparable good Glide score of -5.653 and Glide energy of -32.855 kcal/mol, when compared with standard drug. The natural compound of 4,8,12,16-Tetramethylheptadecan-4-olide binds in hydrophobic region of HER2 at GLY442 and ASN466 residue (Figure

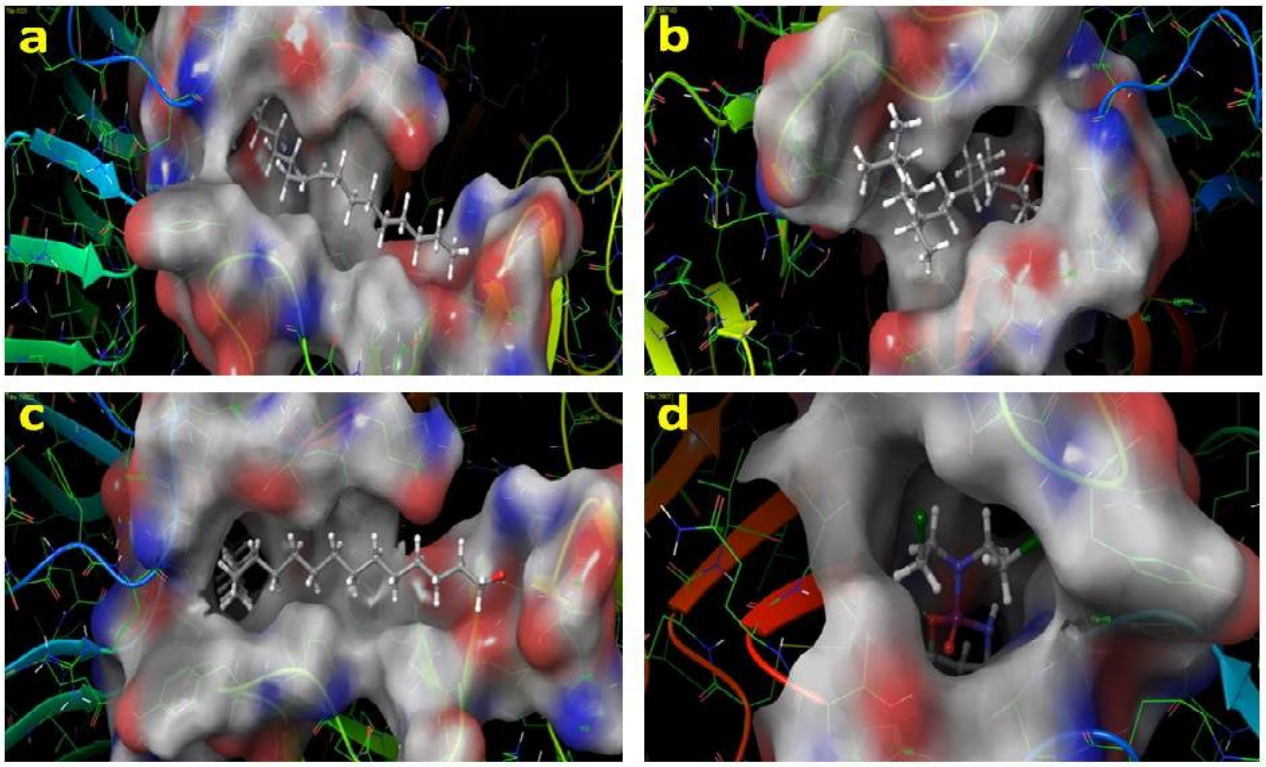


**Figure 1: Docking complex of HER2 with (a) Ethyl Oleate, (b) 4,8,12,16-Tetramethylheptadecan-4-olide, (c) Heptacosanol and (d) Cyclophosphamide generated by using Glide-SP module of Schrodinger suite are shown in this figure. The proteins, ligands and binding pockets are represented in ribbon, sticks and surface models, respectively.**

2b). The docking complex of HER/ Heptacosanol (Figure 1c) hold high Glide score of -5.876 and Glide energy of -42.427 kcal/mol when compared with all the docked complexes, because the Heptacosanol makes strong hydrogen bond with ASN237 residue of HER2 (Figure 2c). Functional characterization of cancer-associated genetic alterations has led to new therapeutic approaches that have dramatically improved patients' therapeutic outcome [(26)]. Cancer genome-sequencing studies are identifying novel cancer-associated genetic alterations at an unprecedented rate, but the functional consequences of these genetic alterations are known only in a few cases [(27)]. If these HER2 somatic mutations are driver events in breast cancer, then these patients may benefit from existing HER2-targeted drugs. The sensitivity of these mutations to several HER2-targeted drugs weretested to provide critical preclinical data for HER2 sequencing-directed breast cancer clinical trials [(28)]. HER2 gene amplification is a major therapeutic target in breast cancer and it is the clinical criterion for the use of U.S. Food and Drug Administration-approved, HER2-targeted drugs [(29)]. The ADME properties predicts these compounds were under acceptable range. The limitations of ADME properties are: not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptor, molecular mass less than 500 daltons, an octanol- water partition coefficient log P not greater than 5 (Table 2).

## CONCLUSION

In the present study , the natural compounds were selected (from ethanolic extract of *Cayratia trifolia*) for identification of novel HER2 inhibitors by computational molecular analysis. This study showed that, the natural compounds of Ethyl Oleate, 4,8,12,16-Tetramethylheptadecan-4-olide and Heptacosanol has good docking with HER2 molecule and had a acceptable score and complex energy when compared toFDA approved drug ofcyclophosphamide. The ADME properties of these compounds were under an acceptable range. Therefore it can be concluded that, Ethyl Oleate, 4,8,12,16-Tetramethylheptadecan-4-olide and Heptacosanol compounds may work as novel inhibitors against HER2 and it may lead to development of drug agents for variety of cancers. However, the molecular simulation studies alone cannot completely support to control the HER2 expression. The combined *in silico* approach has to be translated into *in vitro* and *in vivo* molecular studies which have provided *in silico* results against HER2 overexpression.

## CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests regarding the publication of this paper.

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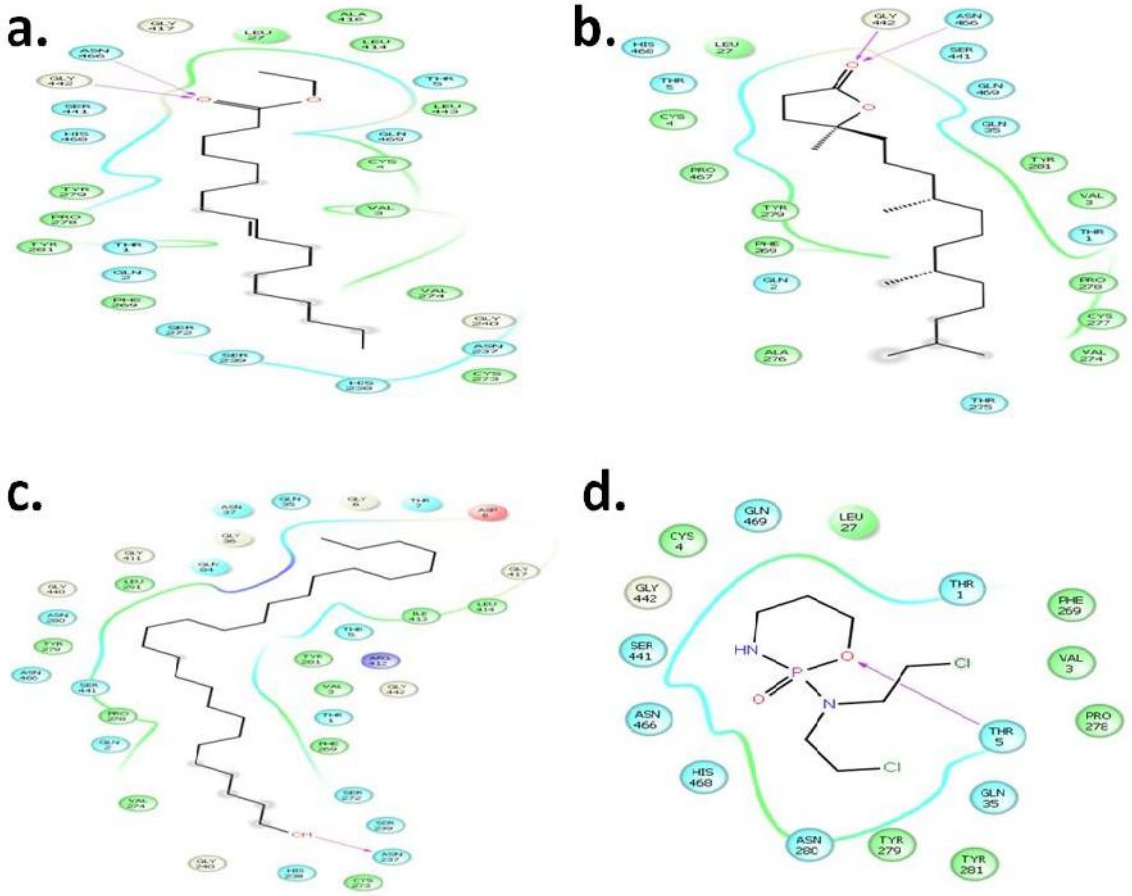


Figure 2: Residues of the HER2 that are within 4 Å proximities to (a) Ethyl Oleate, (b) 4,8,12,16-Tetramethylheptadecan-4-olide, (c) Heptacosanol and (d) Cyclophosphamide are illustrated in 2D graphics. Dotted arrow lines denote 'Hydrogen bonds' between the corresponding atoms.

Table 2: ADME properties of selected compounds and FDA drug as predicted by using QikProp module of Schrodinger suite are listed.

| S. No | Ligands | Molecular Weight (g/mol) | H-Bond donor | H-Bond acceptor | Log*P* O/W |
|---|---|---|---|---|---|
| 1 | Ethyl Oleate | 310.51 | 0 | 2 | 8 |
| 2 | 4,8,12,16-Tetramethylheptadecan-4-olide | 324.546 | 0 | 3 | 6.03 |
| 3 | Heptacosanol | 396.73 | 1 | 1 | 5.32 |
| 4 | Cyclophosphamide FDA drug | 261.08 | 1 | 4 | 0.6 |

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