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# Synthesis And Molecular Docking Of Some Amic Acid Targeting Breast Cancer

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**Abstract**:

In this study, we synthesized and investigated interactions between three amic acid analogs and HER2( 3PP0) by using virtual screening based on molecular docking    to find potential compounds against HER2. The structures of the synthesized compounds were characterized based on a ^{1}HNMR, ^{13}CNMR, FT-IR and mass spectroscopy. The density function theory (DFT) calculation at the B3LYP method with 6-311++G(d,p) basis set are used to investigate the electronic structure and optimized geometrical structure of the mentioned compounds. Molecular docking against human epidermal growth factor receptor 2 (HER2) (PDB:3PP0) showed that compounds bind to the HER2. Binding involves hydrogen bonding for each compounds. The results revealed that the newly designed amic acid derivatives exhibited significant inhibition with HER2 exhibit anti breast cancer activity.

**Keywords:** Molecular docking; human epidermal growth factor receptor 2 (HER2); amic acid; Breast cancer

## 1- Introduction

Cancer is one of the serious threats to humans, causing deaths worldwide despite substantial advances in research for its diagnosis and treatment. Almost 20 million new cases are predicted by the year 2020 [1]. Breast cancer ranks as the second most common cancer for women and the most common cause of cancer deaths for women between ages 45–55 years old [2].

Several factors had been known for causing breast cancer including overexpressed of estrogen receptor-α (ERα) and human epidermal growth factor receptor 2 (HER2) [3]. 25–50 copies of the HER-2 gene can find in breast cancer and up to 40–100 times, resulting in 2 million receptors expressed in the tumor cell; the amplification is what defines a subtype of cancer, with a gene signature, and is maintained during the cancer progression [4]. Human epidermal growth factor receptor 2 (HER2) has an important role in cancer aggressiveness and poor prognosis. HER2 has been used as a drug target for cancers [5]. The human epidermal growth factor receptor (EGFR or HER) family consists of four closely related type 1 trans membrane tyrosine kinase receptors: EGFR, HER2, HER3 and HER4 [6].

During the past two decades, several quinazoline derivatives targeting these two tyrosine kinases have been approved by FDA as anticancer drugs, such as Gefitinib, Erlotinib, and Lapatinib [7]. HER2 is over expressed and gene amplified in human breast cancers. HER2 amplification and over expression have been linked to important tumor cell proliferation and survival pathways. HER-2 are biological target related to the development of an inhibitor could be a good strategy to design an effective drug of cancer [8]. The benefit of anti-HER2 therapies are one of the most promising molecules for targeted therapy [9].

Binding the protein with a ligand leading to a cascade of events that activate its tyrosine kinase domain and promoting the rapid cell growth, differentiation, survival and migration associated with HER-2 positive breast cancer [10]. The discovery of tyrosine kinase inhibitors targeting HER2 has provided a successful avenue of therapies in HER2-overexpressing breast cancer [11].

Computational biology and bioinformatics have the potential to speeding up the drug discovery and drug repurposing process, thus reducing the costs. Molecular docking is one important method of the drug molecule with the receptor [12]. Molecular docking has two essential requirements: the protein target of interest, and structural data, for candidate ligands and a procedure to estimate protein–ligand interaction [13].

On the other hand, amic acid is an organic acid produced by simple condensation reaction between anhydride and aliphatic or aromatic amines. amic acids, consisting of carboxylic acids and amides, are intermediates that can further undergo a dehydration−cyclization step to yield polymeric cyclic imides. amic acids containing heteorocyclic rings  have been used as protecting agents for amino sugars [14]. Amic acids can in principle provide multiple available interaction sites because of the proximal flexible hydrogen-bond donors and acceptors [15].

In this study, we synthesized and investigated interactions between three amic acid analogs and HER2( 3PP0) by using virtual screening based on molecular docking to find potential compounds against HER2. The results revealed that the newly designed amic acid derivatives exhibited significant inhibition with HER2 exhibit anti breast cancer activity.

## 2. Experimental

### 2.1. Apparatus

FT-IR spectra were recorded on Shimadzu FT-IR 8400S spectrometer in the range 4000-500 cm$^{-1}$ using KBr disc. $^1$H and $^{13}$C NMR spectra were recorded on Varian 500 (and 125 MHz for $^{13}$C NMR) spectrometer( using DMSO$d_6$ as a solvent and TMS as an internal reference. Mass spectrum was recorded on Agilent Technologies-5975C (EI, 70 eV).

### 2.2 Materials and methods

Starting materials were obtained from commercial suppliers and used without further purification. Melting points were determined in open glass capillaries on a Fisher–Johns melting point apparatus and are uncorrected. All the reactions were monitored by Thin-layer chromatography (TLC) on Silica Gel 60 F254 plates (VWR, Darmstadt); visualization by UV detection at 254 nm.

### 2.2.2. Synthesis

The studied compounds were prepared as in the scheme 1.
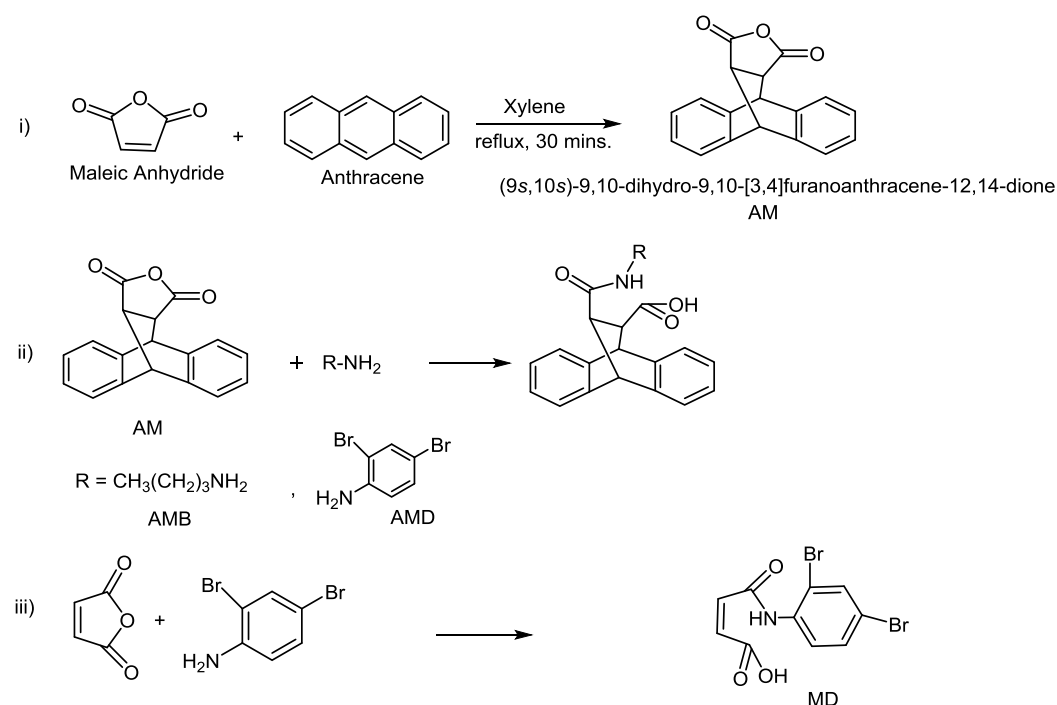


scheme 1

#### 2.2.2.1. Synthesis of 9,10,11,15-tetrahydro-9,10-[3,4]-furanoanthracene-12,14-dione AM:

A mixture of maleic anhydride (0.5gm, 0.005mole) and anthracene (1gm, 0.005mole) in 20ml of xylene was refluxed for about one hours in a round bottom flask on a sand bath. White crystalline product was obtained on cooling. Yield 2.45gm (89%), m.p: 2620C., M.W. 276.29gm/mole.

### 2.2.2.2. Synthesis of (12S)-12-(butylcarbamoyl)-9,10-dihydro -9,10-ethanoanthracene-11-carboxylic acid AMB:

To a well stirred solution of (0.002 mole, 0.552g) 9,10,11,15-tetrahydro-9,10-[3,4]-furanoanthracene-12,14-dione 1 (adduct) in 15 ml acetone in round bottom flask, a solution of (0.002 mole, 0.146 g) of butylamine in 10 ml acetone was added portion wise with constant stirring within 30 minutes. The products were filtered, washed with acetone and vacuum dried, giving white to colourless microcrystals. Yield 73%, m.p: 168-169 $^{o}$C., M.W. 349.3 gm/mole.

IR(KBr) $\upsilon$(cm$^{-1}$): 3390 ($\upsilon$ NH amide), 3336($\upsilon$ OH, C=O acid), 1724 ($\upsilon$ C=O acid), 1627 ($\upsilon$ C=O amide), 1211 ($\upsilon$ C-O), 1242 ($\upsilon$ C-N). $^1$H NMR (500 MHz, DMSO-$d_6$, $\delta$, ppm ) $\delta$ 7.54 (t, $J$ = 7.1 Hz, 1H), 7.25 (dd, $J$ = 7.0, 2.0, Hz, 4H), 7.09 (dd, $J$ = 7.3, 4.7 Hz, 4H), 4.82 – 4.58 (m, 2H), 3.66 (t, $J$ = 7.0 Hz, 1H), 3.47 (t, $J$ = 7.0 Hz, 1H), 3.21 – 2.97 (m, 2H), 1.65 – 1.39 (m, 2H), 1.39 – 1.18 (m, 2H), 0.92 (t, $J$ = 7.9 Hz, 3H).$^{13}$C NMR (125 MHz, DMSO-$d_6$, $\delta$, ppm) $\delta$ 174.47, 172.87(C=O), 137.04, 136.94, 127.13, 126.54 (Ar C), 45.41, 44.49, 41.79, 41.42, 39.94, 30.88, 20.34, 13.71(Aliphatic C).

### 2.2.2.3. Synthesis of (9S,10S,12R)-11-(2,4-dibromophenyl)carbamoyl)-9,10-dihydro -9,10-ethano anthracene-12-carboxylic acid AMD:

To a well stirred solution of (0.0012 mole, 0.338g) 9,10,11,15-tetrahydro-9,10-[3,4]-furanoanthracene-12,14-dione 1 (adduct) in 10 ml acetone in round bottom flask, a solution of (0.0012 mole, 0.301 g) 2,4-di bromo aniline in 20 ml acetone was added portion wise with constant stirring. The mixture was heated under reflux for 2h with continuous stirring. The brawn precipitate which separated filtered and washed with acetone. The crude product was recrystallized from ethanol. Yield 82%, m.p: 76-78 $^{o}$C., M.W. 527.21 gm/mole.

IR(KBr) $\upsilon$(cm$^{-1}$): 3402 ($\upsilon$ NH amide), 3304-3000 ($\upsilon$ OH, C=O acid), 1784 ($\upsilon$ C=O acid), 1622 ($\upsilon$ C=O amide), 1228 ($\upsilon$ C-O), 1290 ($\upsilon$ C-N). $^1$H NMR (500 MHz, DMSO-$d_6$, $\delta$, ppm ) $\delta$ 9.33 (s, 1H), 7.81 (d, $J$ = 7.5 Hz, 1H), 7.77 (d, $J$ = 1.5 Hz, 1H), 7.44 (dd, $J$ = 7.5, 1.7 Hz, 1H), 7.25 (dt, $J$ = 7.2, 1.2 Hz, 4H), 7.12 – 7.01 (m, 4H), 4.78 – 4.72 (m, 2H), 3.68 (t, $J$ = 7.0 Hz, 1H), 3.61 (t, $J$ = 7.0 Hz, 1H).$^{13}$C NMR (125 MHz, DMSO-$d_6$, $\delta$, ppm) $\delta$ 174.60, 171.97(C=O), 137.15, 136.50, 134.13, 132.11, 127.11, 126.46, 122.93, 118.57, 115.76(Ar C), 44.98, 44.45, 41.85, 41.34(Aliphatic C).

### 2.2.2.4. Synthesis of (9S,10S,12R)-11-(2,4-dibromophenyl)carbamoyl)-9,10-dihydro -9,10-ethano anthracene-12-carboxylic acid MD:

(0.0055 mol, 1.4g) 2,4-dibromoanline in (10 mL) acetone was added dropwise into maleic anhydride (0.0055 mol) was dissolved in acetone (25 mL) with stirred to 30

min. The products were filtered, washed with acetone and dried, giving white powder. The product was recrystallized from methanol. Yield 47.14%, m.p: 165-168$^o$C., M.W. 348.98 gm/mole.

IR(KBr) υ(cm$^{-1}$): 3421 (υ NH amide), 3296 (υ OH, C=O acid), 1697 (υ C=O acid), 1627 (υ C=O amide), 1062 (υ C-O), 1273 (υ C-N). $^1$H NMR (500 MHz, DMSO-$d_6$, δ, ppm ) δ 9.58 (s, 3H), 7.80 (d, $J$ = 7.5 Hz, 4H), 7.75 (d, $J$ = 1.5 Hz, 4H), 7.47 (dd, $J$ = 7.5, 1.5 Hz, 4H), 6.50 (d, $J$ = 11.0 Hz, 3H), 6.31 (d, $J$ = 10.8 Hz, 3H). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ, ppm) δ 168.26, 165.08(C=O), 136.05, 134.02, 131.75, 128.20, 124.85, 123.40(Ar C), 118.03, 115.11 (Olefin C) .

### 3. Computational results

### 3.1. Geometrical optimization

The density function theory (DFT) calculation at the B3LYP method with 6-311++G(d,p) basis set are used to investigate the electronic structure and optimized geometrical structure of the mentioned compounds as in Figure 1.

Creating the correct optimization structure for the studied compound is important and very necessary to properly perform the docking calculations. In order to achieve the best optimization for the prepared compounds, Gaussian 09W Where the process of preparing the calculation was performed by selecting the appropriate calculation method and Basis Set using the Density Functional Theory method(DFT) with B3LYP and 6-311 + G (d, p) level were chosen after performing a series of calculations with different basis set of a molecule MD and AM and after comparing theoretically calculated results with the measured results of these and similar molecules using X-ray single crystal measurements obtained from the literature [16,17]. The basis set that gave values for the lengths and angles of the synergy were adopted as closely as possible of the experimental results.

Calculations of the optimization of the AM and MD molecules were performed using the density function theory, B3LYP method, and different basis set (6-31, 6-31 +, 6-31 ++, 6-311 +, 6-311 ++, and 6-311 ++) and when Compare the lengths of the computed bonds using different base elements with those practically measured values for the AM and MD components. The basis set  6-311 + G (d, p) gave the best results, as the basis set 6-311 + G (d, p) gave the best value for $r^2$ equal to 0.975 , Figure 2 shows the linear relationship between the experimental and theoretical results calculated with different basis set for the lengths of the bonds in the AM molecule and the values of $r^2$.
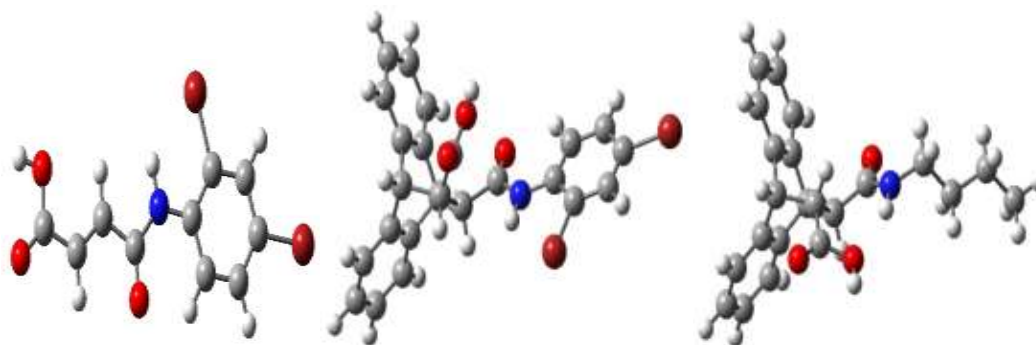
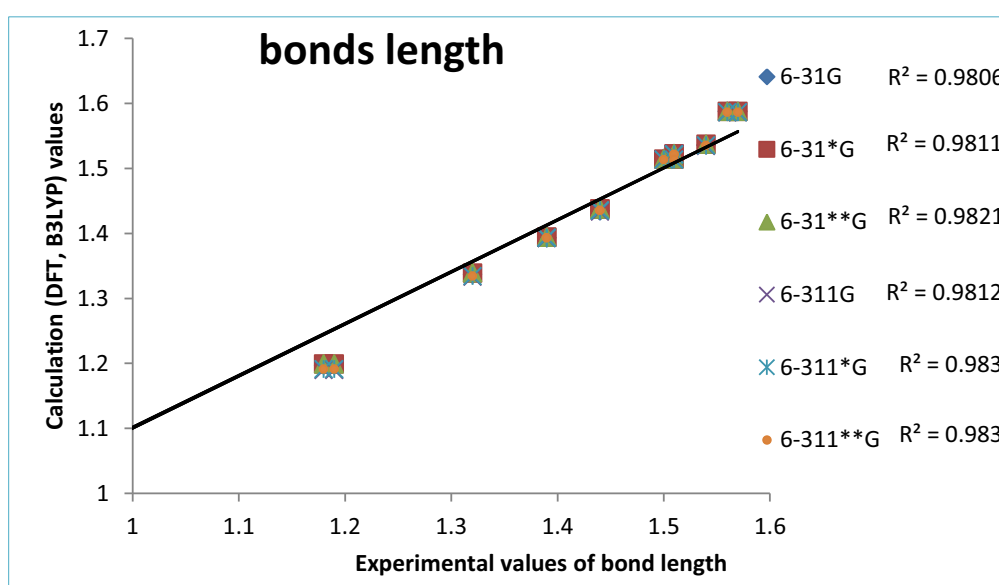Figure 1: The optimized geometrical structure of the mentioned compounds as in



Figure 2: The linear relationship between the experimental  and theoretical results calculated with different basis set for the lengths of the bonds in the AM molecule and the values of $r^2$

### 3.2 Molecular Docking Studies

Molecular docking against human epidermal growth factor receptor 2 (HER2) (PDB:3PP0) showed that prepared compounds (MD, AMD and AMB) interacted with the HER2 by binding. Binding involves hydrogen bonding for each compounds. The results revealed that the newly designed amic acid derivatives exhibited significant inhibition with HER2 exhibit anti breast cancer activity.Autodock Vina and AutoDock 4 package was used for molecular docking. Autodock Vina uses an advanced docking algorithm and scoring function of protein ligand interactions.

The molecular docking calculations of synthesized compounds were performed with Autodck Vina [18, 19] in order to identify binding interactions with the target protein HER2 (PDB ID: 3PP0). The binding energies $-$ 6.2, $-$ 7.6 and  -6.3 kcal mol$^{-1}$ were calculated for MD, AMD and AMB compounds respectively. A good selectivity for binding to an active site that indicate of the protein HER2. Binding energy and hydrogen bond  shown in Table **1**. The molecular docking results revealed that

synthesized 2 compound could interact with Arg868, Lys753, Ser760 and Ser760 residues in the HER2 by four hydrogen bonds. The compounds **AMD** and **AMB** are well binding site of human HER2 (ID: 3PP0) by hydrogen bonds and other close interactions. The interaction of the synthesized compounds with HER2 protein and is shown in the Figures 3-5.

A Van der Waals interactions are found between prepared compounds and the Amino acid residues of receptor 3PP0. Numbers of Van der Waals forces and Hydrogen bonds interaction with the receptor  indicate good docking results to study any inhibiter candid[20].

 Analysis of docking results with PyMol program indicates [21] a sufficient number of interactions are offered by the active site residues of 3pp0 protein  to the selected compounds (Figures 1-3). These observations clearly indicate that the synthesized compounds possess high binding affinity for the 3pp0 protein and specifically bind to the active site residues, which may significantly anti breast cancer inhibit .

Table 1: Molecular docking results showing binding energy and interacting residues from the active site of 3pp0 protein  with prepared compounds MD, AMD and AMB.

| Binding energy (kcal/mol) | Compounds | Protein ligand interaction by H-bonds | | | Protein ligand interaction by Van der Waals forces | |
|---|---|---|---|---|---|---|
| | | No. of H-bonds | Amino acid residues | Distance ($A^o$) | **Amino acid residues** | **No. amino acid residues** |
| MD | -6.3 | 3 | Thr862 Asp863 Ser783 | 3.18 2.94 3.12 | Ala430, Phe864 Val734, Asp863 Leu852, | 5 |
| AMB | -6.3 | 4 | Lys762 Arg868 Ser760 Ser760 | 3.10 3.18 3.06 3.33 | Phe731, Gly865, Ala763, Glu766 | 4 |
| AMD | -6.5 | 2 | Ser760 Arg868 | 2.94 2.92 | Ala763, Lys762, Glu766, Gly865, Phe731, Gly882 | 6 |
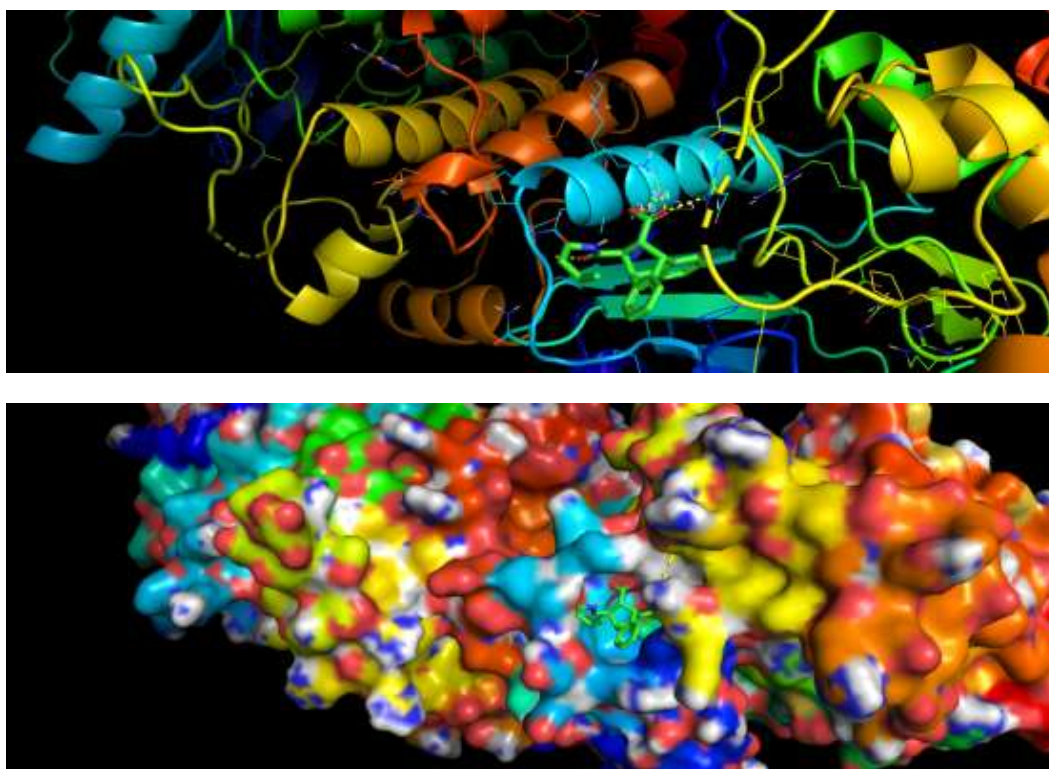
**Figure 3.** 2D, 3D and surface carton protein (PDB: 3PP0) stereo mode in PyMOL of compound **AMB**
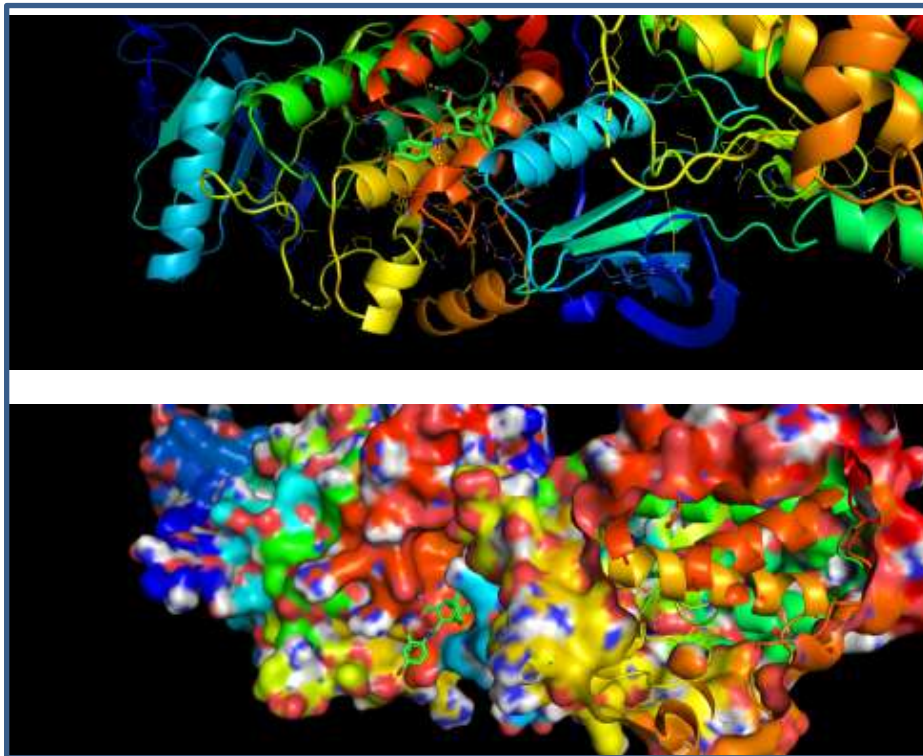
**Figure 4.** 3D and surface carton protein (PDB: 3PP0) stereo mode in PyMOL of compound **AMD**
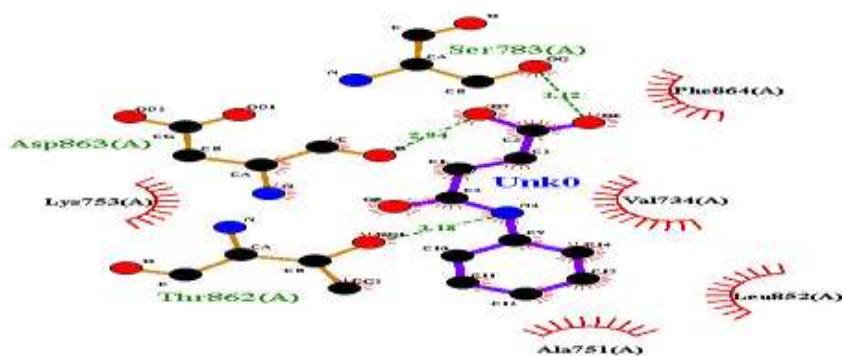
**Figure 5.** 2D and 3D carton protein (PDB:3PP0) stereo mode in PyMOL of compound **MD**

**Conclusion**

The molecular docking study revealed that amic acid derivatives showed good interacting with many amino acid residues. Thus, the in silico method adopted in the present study helped in identifying the lead molecules and also may partly explain their beneficial effect in in vitro and in vivo study. Synthesized amic acid compounds indicated  exerted HER2 inhibition as anti-breast cancer.

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