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Synthesis of N-(4,6-dimethylpyrimidin-2-yl)-4-((11R,15S)-12,14-dioxo- 9,10-dihydro-9,10-[3,4]epipyrroloanthracen-13-yl)benzenesulfonamide and proving as Anti-Breast Cancer

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Abstract

The N-(4,6-dimethylpyrimidin-2-yl)-4-((11R,15S)-12,14-dioxo-9,10-dihydro-9,10-[3,4] epipyrro-loanthracen-13-yl) benzene sulfonamide was synthesized, characterize, docking and experimentally evaluated as a breast cancer inhibitor. The prepared compound was identified by FT-IR, CHN, ^{1}HNMR, ^{13}CNMR and mass spectrometry. Investigated interactions with human epidermal growth factor receptor 2 HER2(PDB ID: 3PP0) by using virtual screening based on molecular docking to find potential compounds against HER2. 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 (HER2) (PDB ID:3PP0) showed that compound bind to the HER2. Binding involves two hydrogen bonds were formed between the studied candidate and the amino acids in important residues of the HER2 (PDB ID:3PP0) receptors, the hydrophobic interactions with 9 amino acid also were founds. The effect of the test titled compound on live breast cancer cells, and prepared compound was able to kill 66.932% of breast cancer cells, it was very high percentage. The results have shown that the prepared compound is very effective as an anti-breast cancer candidate.

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Keywords
Molecular docking, human epidermal growth factor receptor 2 (HER2), cyclic aimed, 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]. In this

study, we synthesized and investigated interactions between N-(4,6-dimethylpyrimidin-2-yl)-4-((9s,10s)-12,14-dioxo-9,10-dihydro-9,10- [3,4] epipyrrolo-anthracen-13-yl) benzene sulfonamide and HER2(3PP0) by using virtual screening based on molecular docking to find potential compounds against breast cancer. The effect of the test compound on live breast cancer cells and prepared compound was able to kill 66.932% of breast cancer cells, it was very high percentage.

The results revealed that the newly designed N-(4,6-dimethylpyrimidin-2-yl)-4-((11R,15S)-12,14-dioxo-9,10-dihydro-9,10- [3,4] epipyrroloanthracen-13-yl) benzene sulfonamide exhibited significant inhibition with HER2 exhibit anti breast cancer activity.

2. Methods

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 $DMSOd_6$ 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 compound were prepared as in the Fig. 1.

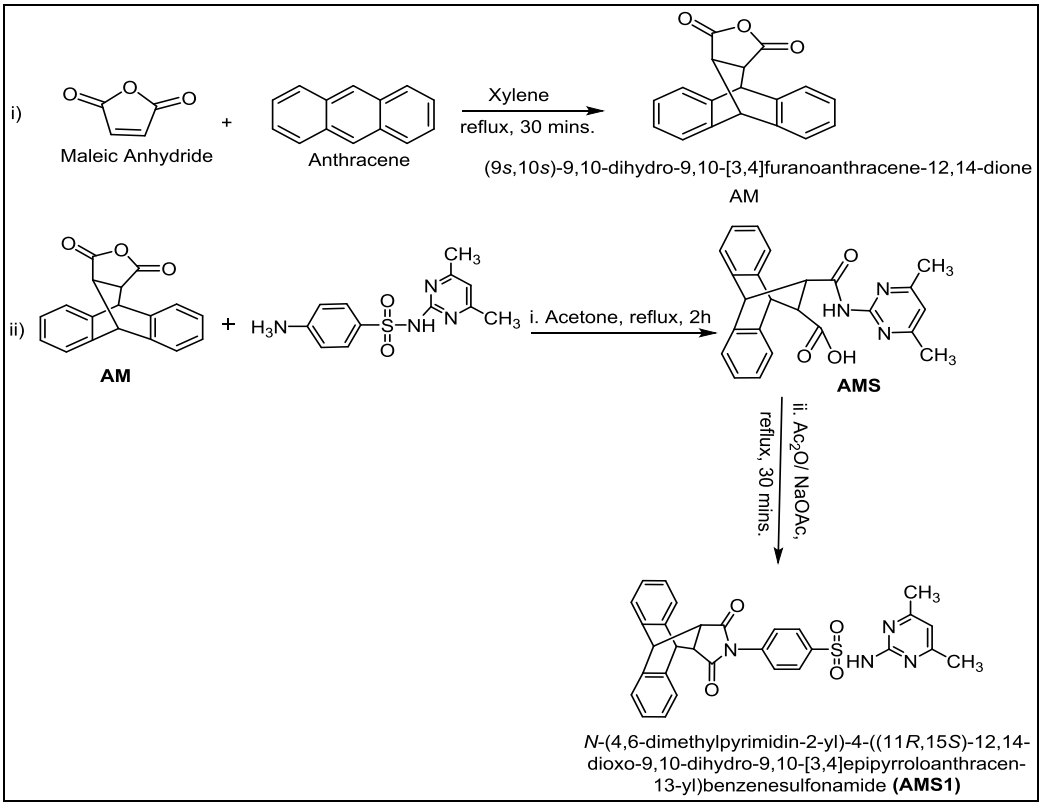

Fig. 1- path way reaction to synthesis of compound AMS1

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.
IR(KBr) υ(cm^{-1}): 3076-3050 (υ Ar-H), 2970-2900 (υ C-H), 1782 (υ C=O), 1070 (υ C-O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.48-7.46 (m, 2H), 7.35 (m, 2H), 4.88 (d, J = 1.8 Hz, 2H), 3.67 (d, J = 1.8 Hz, 2H). MS (EI, m/z (%)): $M^+[C_{18}H_{12}O_3]$, 276(44%), M+1 277(8.3%), 203(38%), 178(100%)

2.2.2.2. Synthesis of (11S,12R)-11-((4,6-dimethylpyrimidin-2-yl)carbamoyl)-9,10-dihydro-9,10-ethanoanthracene-12-carboxylic acid (AMS):

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) υ(cm^{-1}): 3390 (υ NH amide), 3336(υ OH, C=O acid), 1724 (υ C=O acid), 1627 (υ C=O amide), 1211 (υ C-O), 1242 (υ C-N). ^{1}H NMR (500 MHz, DMSO-d_6) 10.27 (s, 1H), 9.52 (s, 1H), 7.72-7.47(m, 4H), 7.34-7.16 (m, 4H), 6.74 (s, 1H), 4.86 (d, 2H), 3.65 (d, 2H), 2.3 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) δ 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(Alip. C).

2.2.2.3. N-(4,6-dimethylpyrimidin-2-yl)-4-((9s,10s)-12,14-dioxo-9,10-dihydro-9,10- [3,4] epipyrrolo- anthracen-13-yl) benzene sulfonamide (AMS1):

Mix the (0.001 mol) of compound prepared in the second step (AMS) with 5 mL of acetic anhydride and (0.0013 mol, 0.1066 g) of sodium acetate in a circular reaction flask, stirring the mixture with a magnetic stirrer and a reflux for half an hour, cooled to room temperature, then iced distilled water was added to it and the contents left for 1 hour to form a precipitate that was collected by filtration, and it was re-crystallized from acetone to give a pale white powder whose. yield of 89%, m.wt. 536.61, m. p. (225-226 ^{o}C). The chemical reaction was followed up by using the thin layer chromatography (TLC) technique using a mixture (hexane: ethyl acetate) with a ratio (3:7).
IR(KBr) υ(cm^{-1}): 3344 (υ NH amide), 3070-3050 (υ Ar-H), 2968-2800 (υ C-H), 1784 (υ C=O), 1072 (υ C-O), 1400 (υ C-N), 1159, 1321 (υ S=O). ^{1}H NMR (500 MHz, DMSO-d_6) 10.27 (s, 1H), 7.72-7.47(m, 4H), 7.34-7.16 (m, 4H), 6.74 (s, 1H), 4.86 (d, 2H), 3.65 (d, 2H), 2.24 (s, 6H).^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) δ 172.05, 168.67(C=O), 141.37(C-N), 139.09(C-S), 138.44**,** 127.79**,** 126.54, 117.81(Ar-C), 24 (CH_3). MS (EI, m/z (%)): $M^{+}[C_{30}H_{24}N_4O_4S]$, 536(11.9%), 276(45.2%), 256(22.6%), 178(100%).

3. Results and Discussion

3-1 Characterization of compounds

Fig. 2 show the mechanism of . the mechanism of N-(4,6-dimethylpyrimidin-2-yl)-4-((9s,10s)-12,14-dioxo-9,10-dihydro-9,10- [3,4] epipyrrolo- anthracen-13-yl) benzene sulfonamide (AMS1), and figures (3-8) show FT-IR, ^{1}HNMR, and Mass spectra of AM and AMS1 respectively.

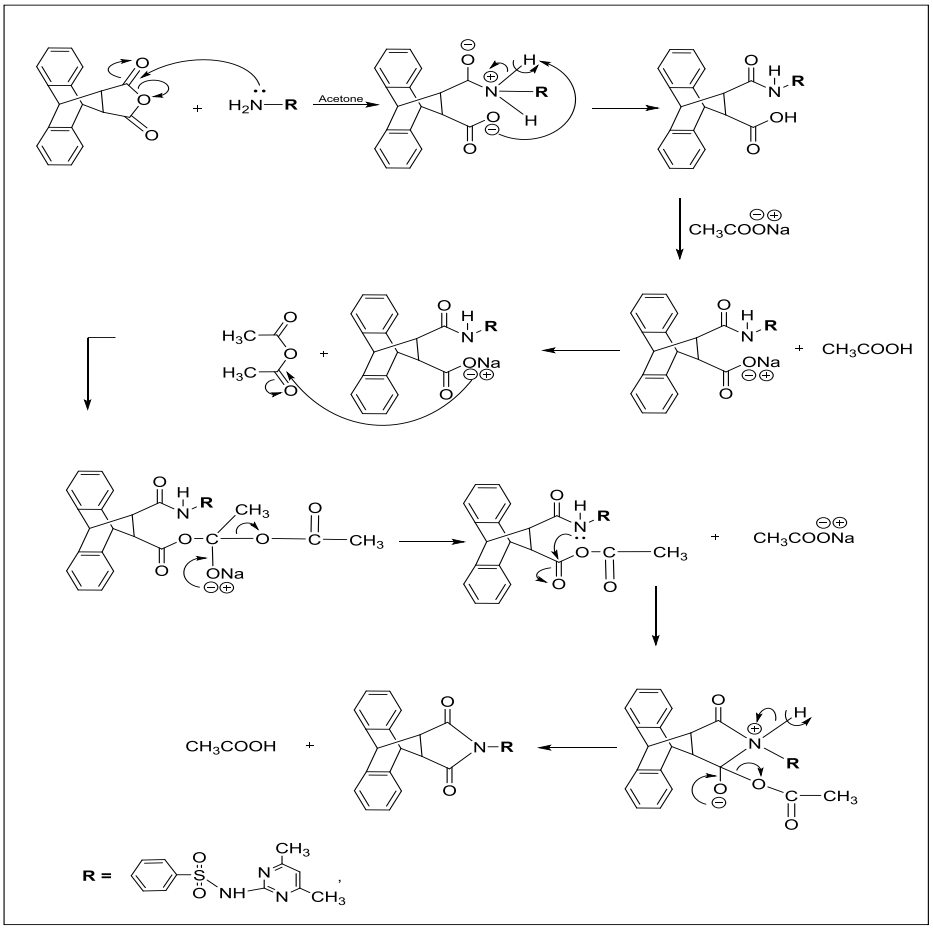

Fig. 2- Mechanism of N-(4,6-dimethylpyrimidin-2-yl)-4-((9s,10s)-12,14-dioxo-9,10-dihydro-9,10- [3,4] epipyrrolo- anthracen-13-yl) benzene sulfonamide (AMS1)

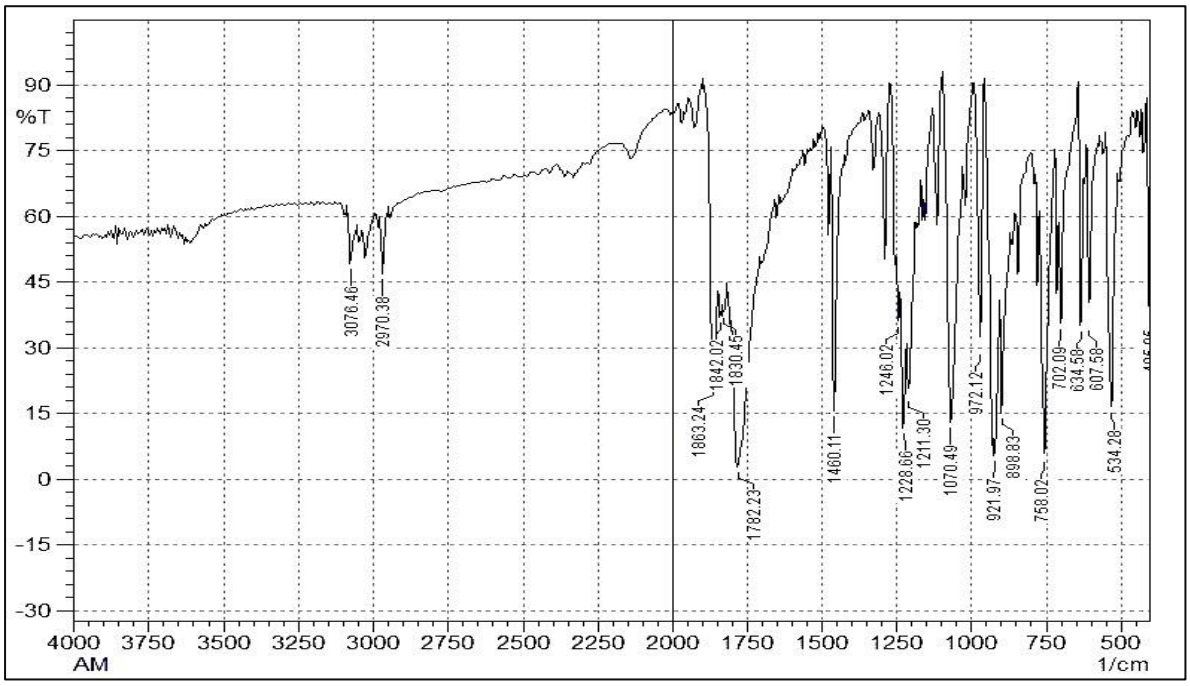

Fig. 3- FT-IR spectrum of 9,10,11,15-tetrahydro-9,10-[3,4]-furanoanthracene-12,14-dione (AM).

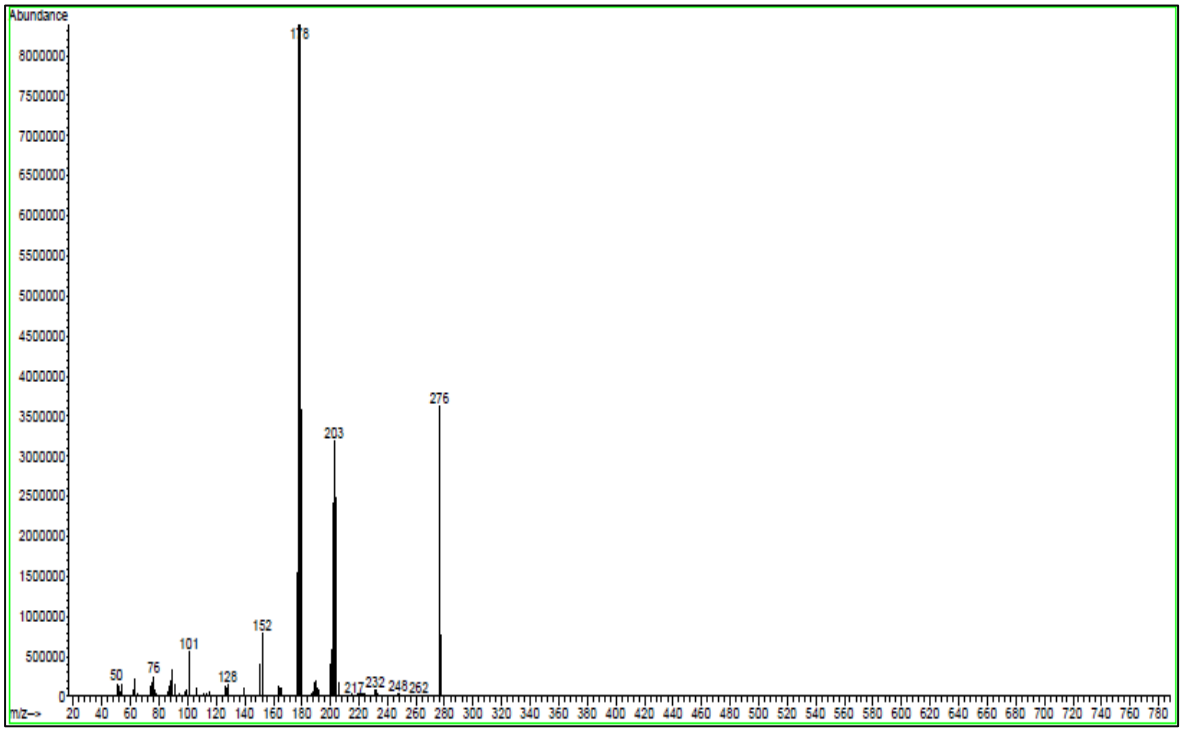

Fig. 4- Mass spectrumof 9,10,11,15-tetrahydro-9,10-[3,4]-furanoanthracene-12,14-dione (AM).

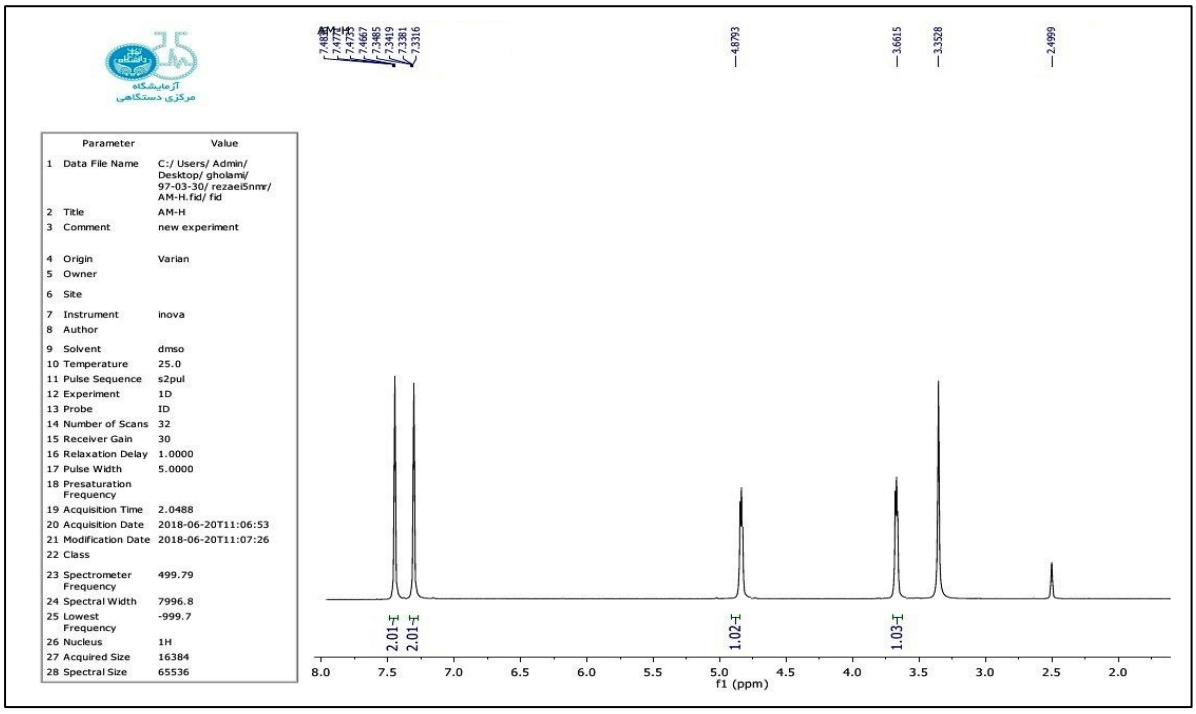

Fig. 5- ^{1}HNMR spectrum of 9,10,11,15-tetrahydro-9,10-[3,4]-furanoanthracene-12,14-dione (AM).

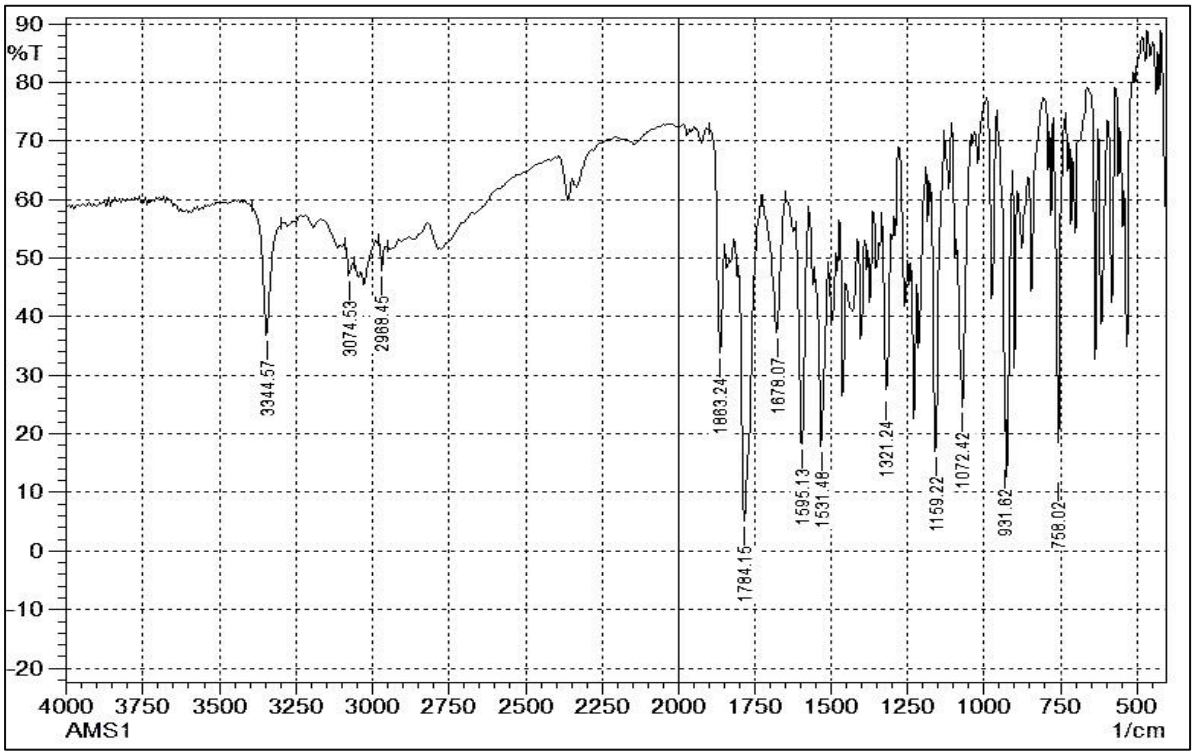

Fig. 6- FT-IR spectrum of N-(4,6-dimethylpyrimidin-2-yl)-4-((9s,10s)-12,14-dioxo-9,10-dihydro-9,10- [3,4] epipyrrolo- anthracen-13-yl) benzene sulfonamide (AMS1).

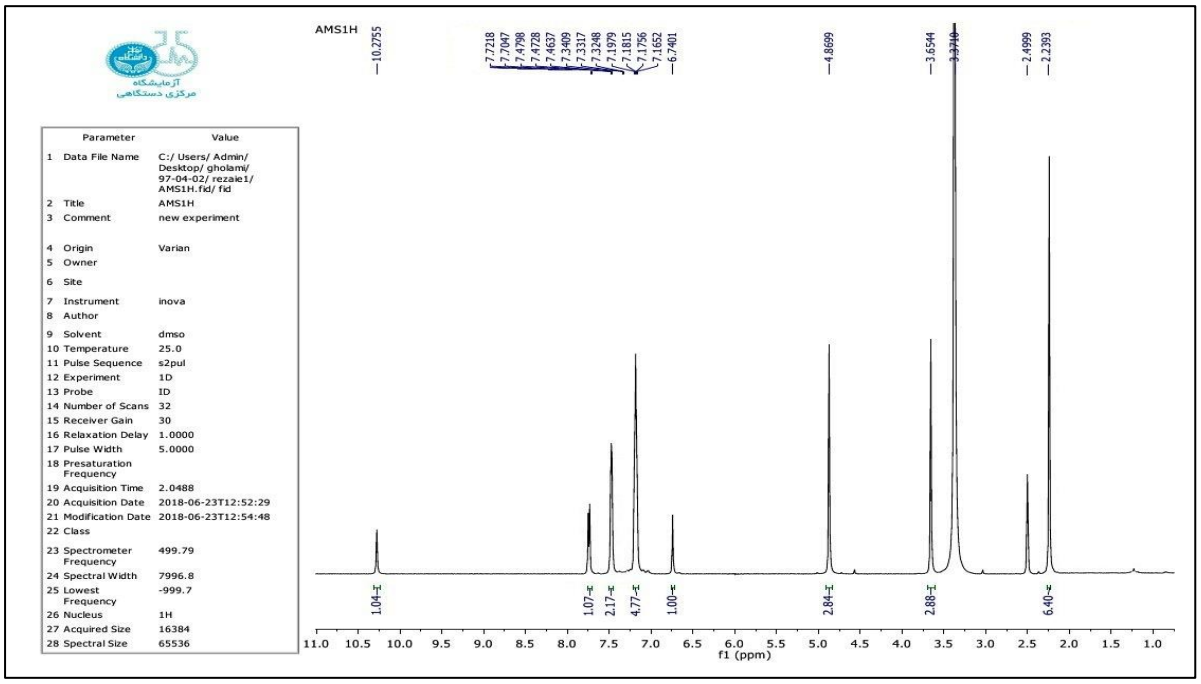

Fig. 7- ^{1}HNMR spectrum of N-(4,6-dimethylpyrimidin-2-yl)-4-((9s,10s)-12,14-dioxo-9,10-dihydro-9,10- [3,4] epipyrrolo- anthracen-13-yl) benzene sulfonamide (AMS1).

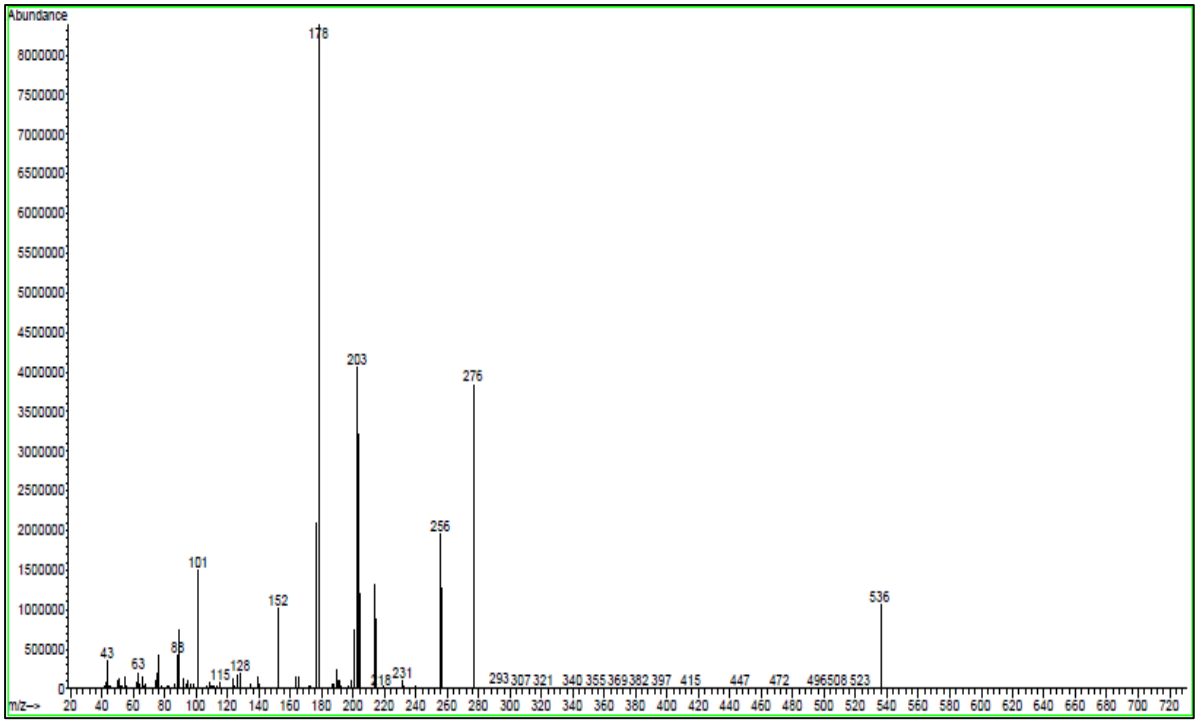

Fig. 8- Mass spectrum of N-(4,6-dimethylpyrimidin-2-yl)-4-((9s,10s)-12,14-dioxo-9,10-dihydro-9,10- [3,4] epipyrrolo- anthracen-13-yl) benzene sulfonamide (AMS1).

3.2. Computational results

3.2.1. 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 N-(4,6-dimethylpyrimidin-2-yl)-4-((9s,10s)-12,14-dioxo-9,10-dihydro-9,10- [3,4] epipyrrolo- anthracen-13-yl) benzene sulfonamide (AMS1). 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 compound 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 compound 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 component. 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 0.987 . Fig. 9 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.

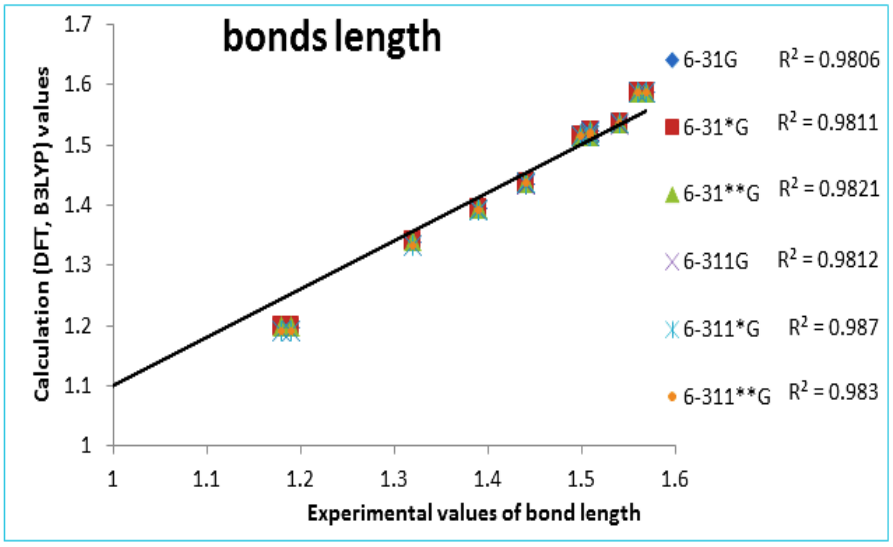

Fig. 9- The linear relationship between the experimental and theoretical results calculated with different basis set for the lengths of the bonds in the AM compound and the values r^2

3.2.2 Molecular Docking Studies

Molecular docking against human epidermal growth factor receptor 2 (HER2) (PDB ID:3PP0) showed that prepared compound AMS1 interacted with the HER2 by binding. Binding involves two hydrogen bonds. The results revealed that the newly designed candidate 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 calculation of synthesized compound AMS1 was performed with Autodck Vina [18, 19] in order to identify binding interactions with the target protein HER2 (PDB ID: 3PP0). The binding energies – 8.9 kcal mol^{-1} were calculated for AMS1 compound. 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 compound could interact with Arg849(A) and Ser728(A) residues in the (PDB ID:3PP0) by two hydrogen bonds with distances 2.69Å and 2.83 Å, other close interactions the hydrophobic interaction residues with nine amino acid residues, Phe918, Gly919, Lys921, Ala920, Asp924, Trp913, Pro942, Tyr923 and Glu939. The interaction of the synthesized compounds with (PDB ID:3PP0) protein is shown in the Figures (10-12). The docking results using PyMol program indicates [20, 21] a abundant number of interactions are offered by the active site residues of 3pp0 protein with the titled compound AMS1 (Figures 8-10). These observations clearly indicate that the synthesized compound 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 compound AMS1

Comp.	Binding energy (Affinity) (kcal/mol)	Protein ligand interaction			
		No. of H-bond	Distance (Å)	Amino acid residues	Hydrophobic interaction residues
AMS1	-8.9	2	3.19 2.98	Arg418(A) Arg418(A)	Phe918, Gly919, Lys921, Ala920, Asp924, Trp913, Pro942, Tyr923 , Glu939

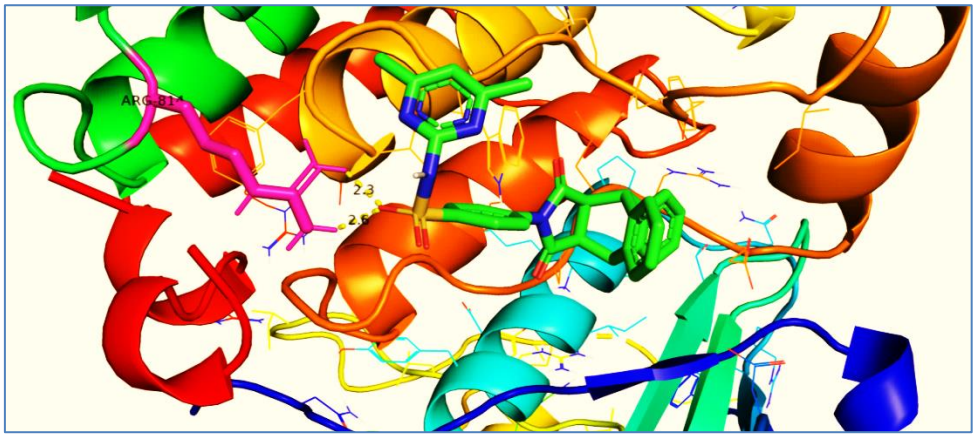

Fig. 10- The 3D carton protein (PDB ID: 3PP0) stereo mode in PyMOL of compound AMS1

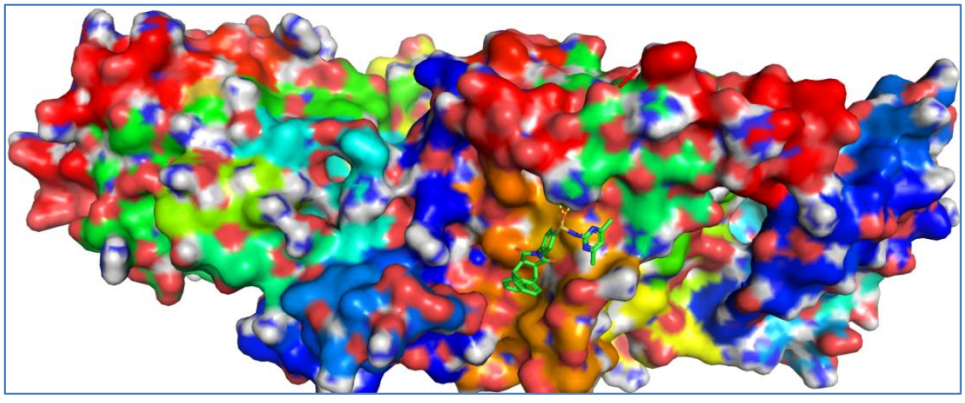

Fig. 11- Surface carton protein (PDB: 3PP0) stereo mode in PyMOL of compound AMS1

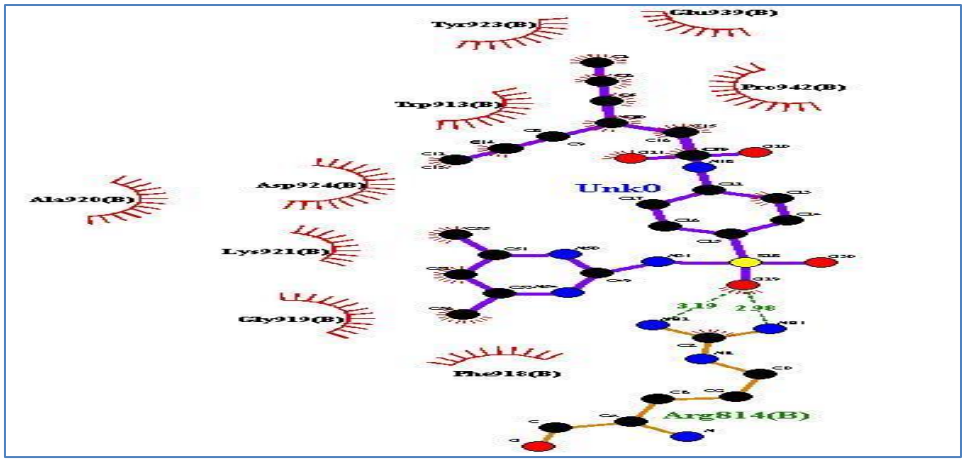

Fig. 12- The 2D interaction of receptor 3PP0 with compound AMS1 using LigPlus+ program

3. 3. Evaluation of the prepared compound as an inhibitor of live breast cancer cells

The efficacy of compound S against live breast cancer cells was tested in the Cell and Molecular Biology Lab, Department of Zoology and Cytology, Government College University, Faisalabad, Pakistan.

The results of practical measurements showed that the prepared compound AMS1 gave very high efficacy to inhibit the growth or killing of live breast cancer cells. Table (2) includes the values of measurements of the effect of the prepared compound on inhibiting the growth or killing of live breast cancer cells. Where it is noticed from the repeated measurement for three times that the percentage of cells that remained alive after being treated with our compound under study is 0.249 out of the reference value of 0.897 in the first experiment, and that the percentage of remaining living cells was 33.0677%, meaning that the compound was able to kill 66.932%, which is a very high percentage. Thus, the percentage of live cells in the second and third experiments was 42.7307% and 48.8054%, respectively, after increasing the concentration of live cells in the treated samples. The prepared compound is new and it has proven very highly effective against live breast cancer cells in killing and inhibiting the growth of more than half of the cells under test.

Table 2: Measures effected of the prepared compounds AMS1 on inhibiting the growth and killing of live breast cancer cells

absorbance	1	2	3	% viability	% viability	% viability
Control values	0.897	0.968	0.979	100	100	100
AMS1	0.249	0.338	0.429	33.06773	42.73072	48.80546

Acknowledgments

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