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Newly synthesized Olaparib Analogues: Clinical Activity Evaluation against the MCF-7 Breast Carcinoma Cell Lines

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Abstract

Breast cancer is malignant tissue cells and has grown to be one of the world's most serious medical issues, as well as the cause of death in women. One of the most effective treatments for breast cancer is the use of poly (ADP-ribose) polymerase-1 (PARP) inhibitors. These two Olaparib analogs were tested on the Michigan Cancer Foundation-7 (MCF-7) and Madin Darby Canine Kidney (MDCK) cell lines in earlier unpublished work and found to be successful. One of these analogs, for example, is more active versus MCF-7 cell lines (IC50: 64.517 µg/mL versus 68.951 µg/mL for Olaparib) and has a better cytotoxic profile when tested on MDCK cell lines (IC50: 5.975 mg/mL versus 6.648 mg/mL for Olaparib). The obtained data concluded that two newly synthesized compounds had inhibitory activity against MCF-7, and they were close to the approved drug Olaparib. There is a good agreement between our docked results and the experimental results (In vitro study) of two compounds gave the high docking results that showed a promising cytotoxic activity among the tested compounds.

Keywords: Breast Cancer ; Olaparib analog s; PARP-1 inhibitors ; IC50 ; MCF-7 ; MDCK ; Cell Lines.

1. Introduction

Breast cancer is an issue for women all over the world. Breast cancer is the most prevalent cancer among women in the United States, and it is also the second leading cause of cancer mortality [1]. In women, breast cancer is the most usually diagnosed cancer, and it is currently the leading cause of cancer mortality. It accounts for about a quarter of all cancer diagnoses and 14% of all cancer deaths globally. That equates to over half a million women dying from breast cancer in 2008, and incidence rates in most countries are still growing [2]. One of the successful approaches in the treatment of breast cancer is the employment of PARP inhibitors. That is considered a potential treatment of cancers with specific defective DNA-repairing mechanisms [3]. Breast cancer cell lines might supply a limitless source of homogeneous self-replicating materials utilizing simple standard conditions and techniques, a substantial percentage of existing information on breast carcinomas was generated from in vivo and in vitro investigations employing breast cancer cell lines [4]. In order to develop an effective and economically viable medication, a multidisciplinary effort is required throughout the drug discovery process. The DNA repair process of the cell may hold significant clues to the solution to the issue described above. PARP (poly (ADP-ribose) polymerases-1) is an enzyme involved in the DNA damage repair process. PARP-1 inhibitors have the potential to be used as a therapy for malignancies that lack BRCA1 or BRCA2 genes due to PARP inhibitory ability, which may lead to the induction of apoptosis [5]. Olaparib is the little chemical that can induce synthesis lethality in BRCA defective cells, an orally active PARP-i inhibitor [6, 7]. The United States Food and Drug Administration approved Olaparib in 2018 for patients to treatment who had previously undergone chemotherapy and had germline BRCA-mutated HER 2-negative

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metastatic cancer [8, 9]. It was created as a stand-alone treatment as well as in conjunction with radiation and chemotherapy. For several prominent journal publishers, the biological study of freshly synthesized possible anticancer candidates is a requirement in order to publish their work in their journals [10]. In this study, cytotoxic, apoptotic activities, and computational studies of synthesized novel PARP-1 bioactive compounds were carried out. These compounds were successfully designed, synthesized, and characterized with superior activity and reduced toxicity which might lead to potential management of breast carcinoma.

2. Experimental

2.1. Materials

Benzyl Sulfonyl Chloride, Dioxane Dimethylformamide DMF, Dry Benzene, Ethanol 99.9%, Thionyl Chloride (BDH, England). Cyclopropane Sulfonyl Chloride 95%, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2- Methyl Piperazine, Methylsulfamoyl Chloride, Olaparib 98% (Sigma Aldrich, Germany). Dimethyl Sulphoxide 99% (CDH, India), 96-well microtiter plate (CELLTREAT Scientific Products, USA).

2.2. Instrument

Electronic Sensitive Balance (Mettler, Germany). Elisa-Washer SAGA (Linear, Spain), Elisa-Reader GEA (Linear, Spain). Fourier-transform infrared spectroscopy (FT-IR) and Mass Spectroscopy (Shimadzu, Japan). Nuclear magnetic resonance spectroscopy (1H-NMR) (Bruker, Germany). Incubator 37˚C (Memmert, Germany). Inverted Microscope (Zeiss, Germany). Melting point (Stuart, Germany). Magnetic stirrer with a hot plate and Safety Cabinet Class II (LabTech, Korea).

2.3. Synthesis of Compounds

i. 2-fluoro-5-[(4-oxo-3,4-dihydrophthalazin-1-yl) methyl] benzoyl chloride (compound A)

At room temperature 25˚C, a specified amount of 2-fluoro-5-((4-oxo3,4-dihydrophthalazin-1-yl) methyl) benzoic acid 2.98 gm, (0.01 mole) thionyl chloride was added to a stirring solution 15 mL inside work hood and a volume of 10 mL of Dry Benzene, and the reaction mixture was kept at a constant temperature for 7 hours then cooled and put the round bottom to ice and pour it into a watch glass. Consequently, a solid precipitate was isolated dried the precipitate 2-fluoro-5-[(4-oxo-3,4-dihydrophthalazin-1-yl) methyl] benzoyl chloride (Compound A) was yellowish-brown powder, (yield=98%, Melting Point (M.P.) =226-229˚ C) as shown in figure 1 [11].

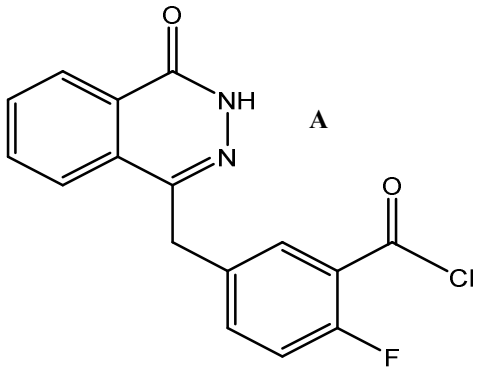


Fig.1: The chemical structure of compound **A**.

ii. 4-(4-fluoro-3-(piperazine-1-carbonyl) benzyl) phthalazin-1(2H)-one (compound B), and 4-(4-fluoro-3-(3-methylpiperazine-1-carbonyl) benzyl) phthalazin-1(2H)-one (compound C)

To a solution of 2-fluoro-5-[(4-oxo-3,4-dihydrophthalazin-1-yl) methyl] benzoyl chloride (Compound A), a weight of 3.16gm, (0.01 moles) was dissolved in 15 mL DMF, a volume of 5 mL of Piperazine or 2-methyl Piperazine was added drop by drop, then the reaction mixture was stirred on a hot plate until it was complete. Then, let the mixture be refluxed for 7 hours when the time of the refluxed is finished drops of cold water are put into the reaction mixture drop by drop 10 mL and stored in the refrigerator at - 4^0 C until the next day. As a result, by filtering, a solid precipitate was isolated. The precipitate that formed dried by oven at 60^0 C then recrystallized from Dioxane. White powder for

compound A and white fluffy for compound B. The 65% yield, 70% yield. M.P. 323-325˚C and 312-315˚C respectively as shown in figure 2.

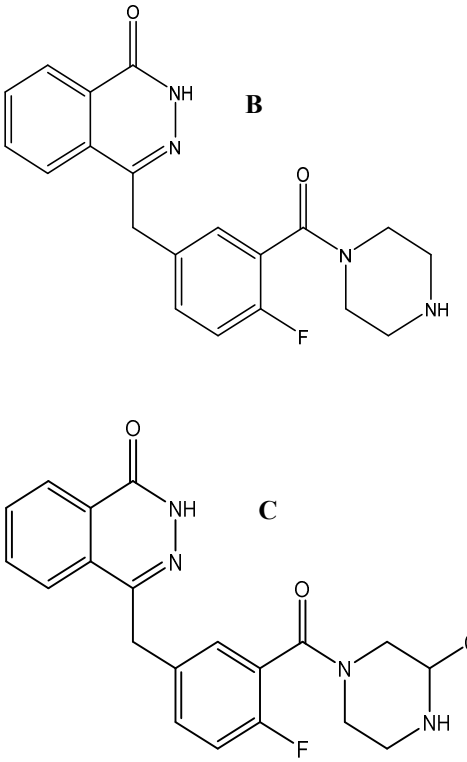


Fig. 2: The chemical structures of compound B and C

iii. 4-(4-fluoro-3-(3-methyl-4-(phenylsulfonyl) piperazine-1-carbonyl)benzyl) phthalazin-1 (2H)-one (Compound D1) and 4-(4-fluoro-3-(4-(phenylsulfonyl) piperazine-1-carbonyl)benzyl) phthalazin-1(2H)-one (Compound D2**)**

At room temperature (25˚C), chloro sulfonyl derivatives (0.005 moles) in 10 mL dioxane were added dropwise to stirred solution of compounds B or C in 15 mL of dioxane. After that, the mixture was allowed to reflux for 5 hours, after which it was put into a beaker containing ice water. The precipitate was filtered and dried before being recrystallized from water: dioxane (7:3). Compound D1: (Yellowish- brown, yield =85%, M.P. = (167-170˚C). Compound D2: (Yellowish- brown, yield =83%, M.P. = (300-303˚C) as shown in figure 3.

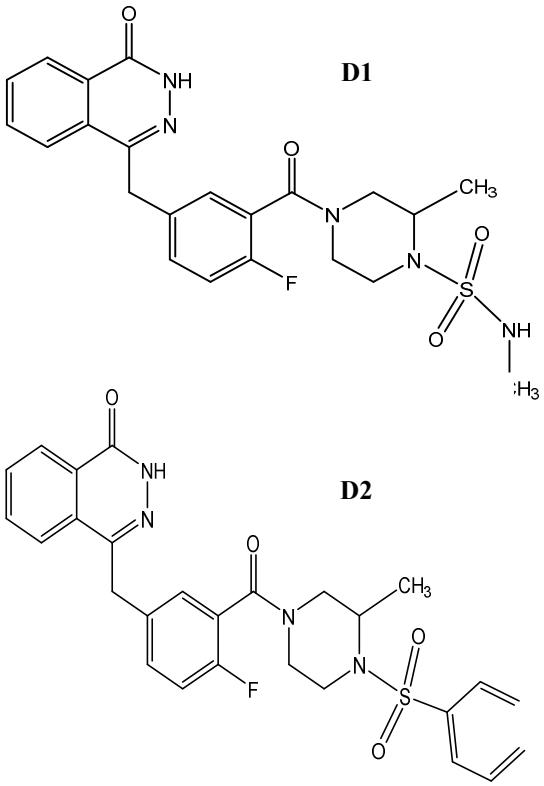
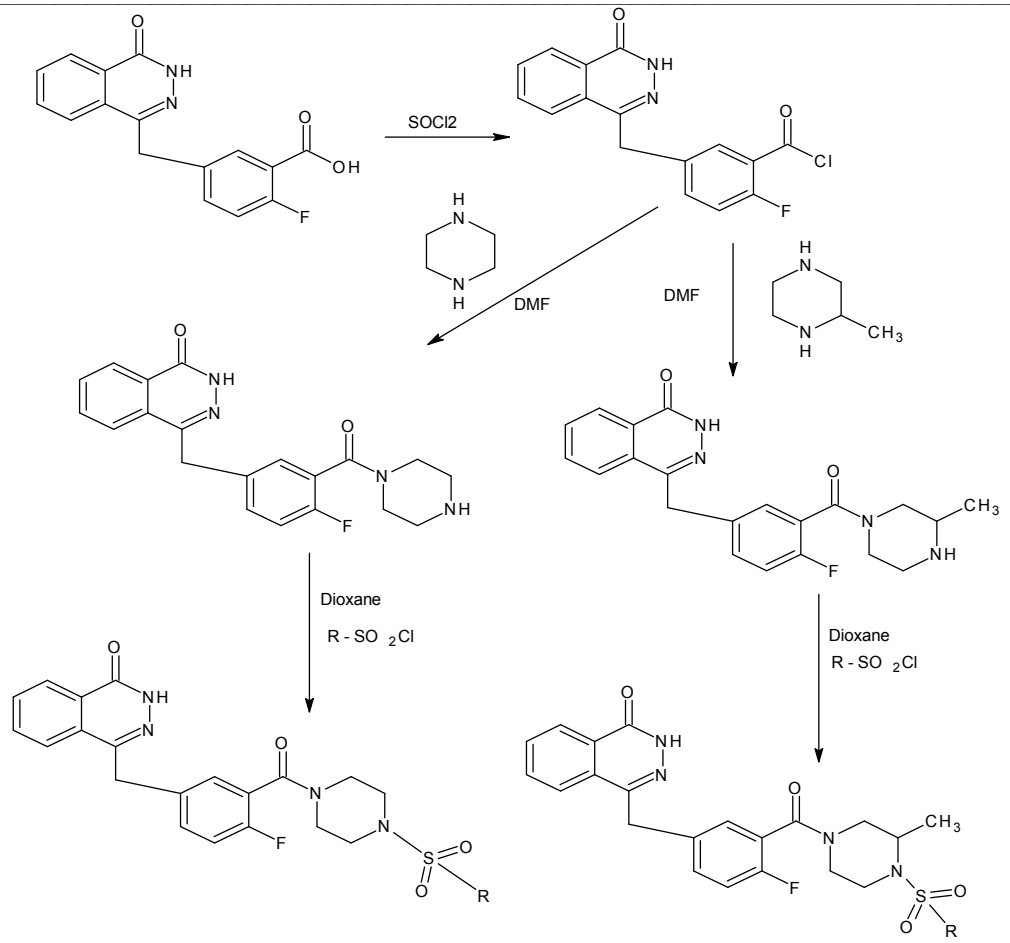


Fig. 3: The chemical structures of compound D1 and D2

2.4. Cell Lines

In this study, we used two types of cell lines (Human Breast cancer MCF-7 cell line and Madin-Darby Canine Kidney MDCK cell line) [12, 13].



Scheme 1: Chemical target syntheses and reaction conditions (D1 and D2). Synthesis of target compounds, 2-fluoro-5-((4-oxo 3,4-dihydrophthalazin-1-yl) methyl) benzoic acid, 2-fluoro-5-[(4-oxo-3,4-dihydrophthalazin-1-yl) methyl] benzoyl chloride compound (A), 4-(4-fluoro-3-(piperazine-1-carbonyl)benzyl) phthalazin-1(2H)-one, C: 4-(4-fluoro-3-(3-methylpiperazine-1-carbonyl) benzyl) phthalazin-1(2H)-one (B), 4-(4-fluoro-3-(3-methyl-4-(phenylsulfonyl) piperazine-1-carbonyl) benzyl)phthalazin-1(2H)-one (D1) and 4-(4-fluoro-3-(4-(phenylsulfonyl) piperazine-1-carbonyl)benzyl)phthalazin-1(2H)-one (D2)

2.5. Cell Lines Preparation

Both the MCF-7 and MDCK cell lines were cultured in medium 1640 with 10% heat-inactivated fetal bovine serum (FBS) (RPMI-1640, Gibco-BRL) to get the desired results (Gibco). Cell culture plates (Celltreat®) 96 wells were uses in the experiment, and the cell line was assigned and incubated at 37°C for growth. The cell culture period reduced from 72 to 48 hours, which seemed to be optimal, and all processes involved in this study [14] are below:

i. MTT Stock solution preparation

To achieve the concentration of 5 mg/mL, a precisely weighed quantity of each manufactured chemical component was dissolved in pure DMSO. The solutions were filtered using a sterile filter of 0.2 μm after dissolution. This solution was stored in the refrigerator to prepare the working solution as below:

ii. MTT working solution preparation

The working of concentrations for MTT is 0.5 mg/mL according to the procedures. That is 10% v/v of the inventory. A total volume of 24 mL of cell medium and 10 % MTT was using. Cell media was precisely measured in 21.6 mL and assigned to an appropriate flask. The medium and sufficiently homogenized solution was adding to a 2.4 mL MTT stock solution. The 10% MTT cell media was ready for cell lines and 3 hours' incubation time.

iii. Preparation of the chemical concentrations for the cell lines

A sufficient quantity of each substance was dissolved into DMSO for each chemical and standard to obtain a stock solution of 5 mg/mL. After many cell line experiments, when serial dilution was performed at a higher concentration, the concentration of 50 g/mL was optimized for each chemical synthesized and standard. A 990 µL of the drug was measured precisely and a 10 µL of 5 mg/mL was inserted then homogenized in order to get a final 50 µg/mL concentration and a total of 1% of the DMSO concentration. Series of dilutions has been done at: 31.25, 62.5, 125, 250, 500 and 1000 µg/mL. For each, a series of dilutions was performed.

iv. Chemicals application to cell lines

The serial of dilution solutions described above was administered to each well in triplicates in 200 µL portions and allowed to incubate for 48 hours. After the incubation period, the screen photograph took for each under an inverted phase-contrast microscope A 10% MTT media was a replacement. The cells were cultured for 3 hours with the new medium. After 3 hours of incubation, the media was removed, and the wells were rinsed with PBS. Finally, 200 µL sections of DMSO were added to each well and left for 30 minutes before being measured at 630 nm using a plate reader.

2.6. Statistical analysis

The statistical analysis was done by Student's t-test using GraphPad Prism 9.2.0.332. The results were presented as Mean± SD (standard deviation) of triplicates done in the same experiment or an average of three independent experiments (n=3).

3. Results and Discussion

Two Olaparib analogs are successfully designed and synthesized. Thionyl chloride first reacts with the acid to form compound A, as seen in Scheme 1. The reaction type was involving the substitution nucleophile intramolecular mechanism (SNi). On the other hand, the compounds of **B**, **C**, **D1**, and **D2** , that have result from the intermediate reactions included the arrangement of the double bond around the carbon was transformed from a trigonal to a tetrahedral mechanism.

The FT-IR spectrum of compounds **D1** and **D2**. Figure **4a** revealed that the compound **D1** has a new stretching vibration band at 1166, 1371 cm^{-1} due to SO_2 sulphonamide group and disappearing of stretching vibration band of -NH Piperazine. Also showed a band at 1706 cm^{-1} for the stretching vibration of (C=O) cyclic amid the group, 3290 cm^{-1} for the stretching vibration -NH group. Figure 4b shows that the **D2** compound has a new stretching vibration band at 1170, 1365 cm^{-1} due to (SO_2) sulphonamide group, band at 3300 cm^{-1}, and also

disappeared of stretching vibration band of -NH Piperazine. There is a band at 1697 cm^{-1} for the stretching vibration of (C=O) cyclic amid the group, 3113 cm^{-1} for the stretching vibration -NH group. FT-IR spectroscopy is considered a rapid, reliable, and non-destructive tool used ordinarily in drug characterization [15].

One of the very characteristic bands frequency in IR spectroscopy is carbonyl functional and sulphonamide groups [16]. The FT-IR spectroscopy used is primarily to identify and validate the chemical structure of newly synthesized compounds to use as drugs, with the cyclic amid group serving as a key indicator, which is very characteristic of the two new compounds.

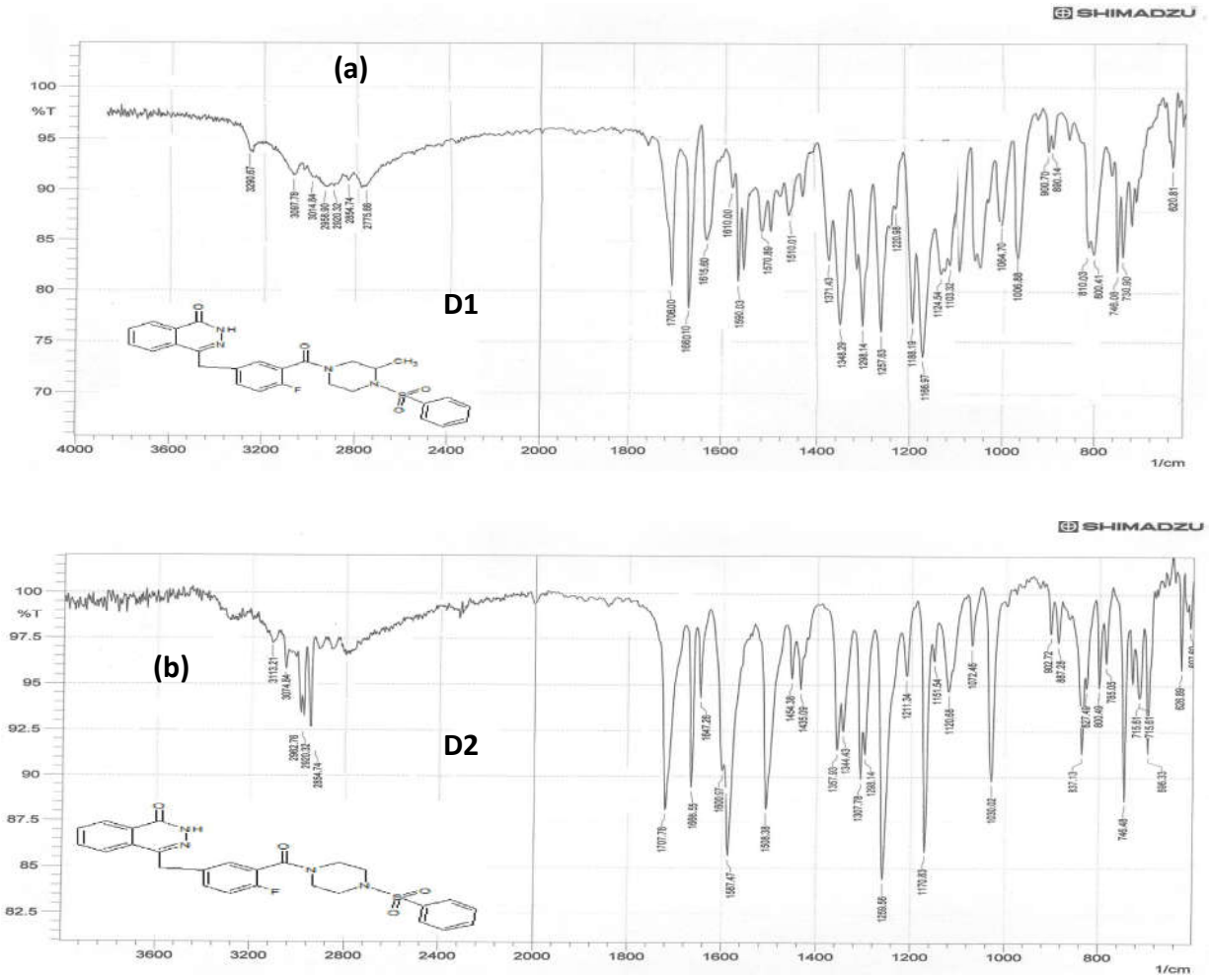


Fig.4: Spectrum of FT-IR: (a) chemical structure of compound D1, (b) chemical structure of compound D2

^{1}H-NMR spectroscopy is considered a fundamental tool that applies a magnetic field to an atomic nucleus and radiofrequency pulses to describe the resonant frequency of that atomic nucleus according to its chemical surroundings [18]. ^{1}H-NMR spectroscopic was used to characterize both compounds **D1** and **D2**. The compound's ^{1}H-NMR spectra **D1** shown in figure 5A and the result signal refer to: 1H-NMR (DMSO-d6): δ,ppm= 1.47 (s, 3H,

CH3), 2.88 (s,2H,CH2), 3.46-4.40 (m, 7H pip.), 7.10-7.78 (m,12H,ArH), 9.37 (s, 1H,). The compound's ^{1}H-NMR spectra **D2** shown in figure 5B and the result signal refer to: ^{1}H-NMR (DMSO-d6): δ, ppm= 1.49 (s, 3H, CH3 Pip.) 2.88 (s, 2H, CH2), 3.47-4.40 (m,7H pip. And 3H of CH3), 7.53 - 7.78 (m, 12H, ArH), 9.39 (s, 1H, NH, pip.), 10.48 (s, 1H, NH sulph.).

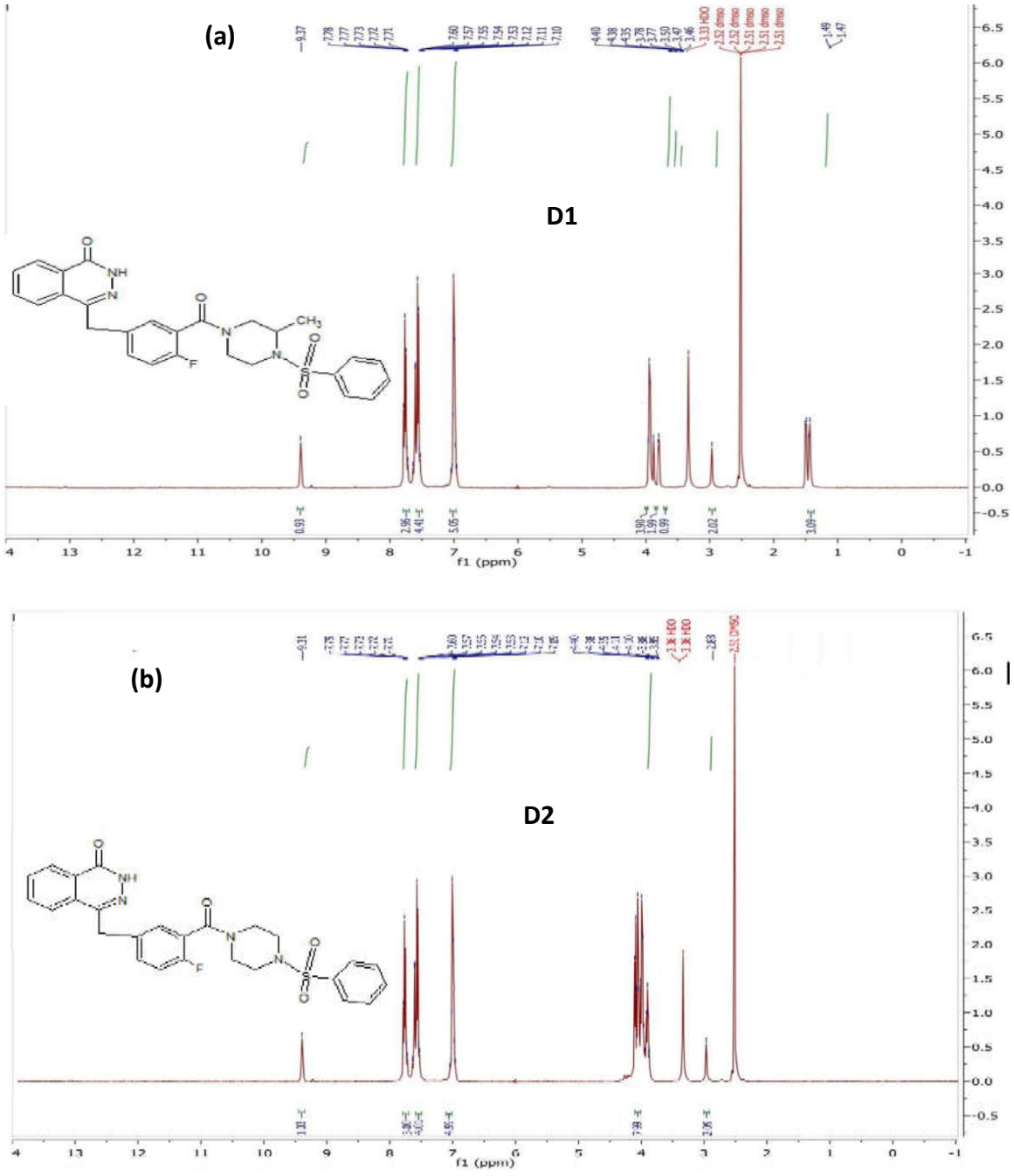


Fig. 5: Characteristic peaks of ^{1}H-NMR: (a) for compound D1 and (b) for compound D2

Mass spectroscopy (MS) is considered a promising analytical tool since it provides the unique molecular structural identification of each component while maintaining a high sensitivity and specificity characterization of the compounds [18, 19]. The mass spectrum of the D1 compound (Figure 6A) shows the molecular ion peak (M+, m/z) =520, and this is identical to the molecular weight of compound D1. The mass spectrum of the D2 compound (Figure 6B) shows the molecular ion peak (M+, m/z) = 473, and that is conformable to the molecular weight of compound D2.

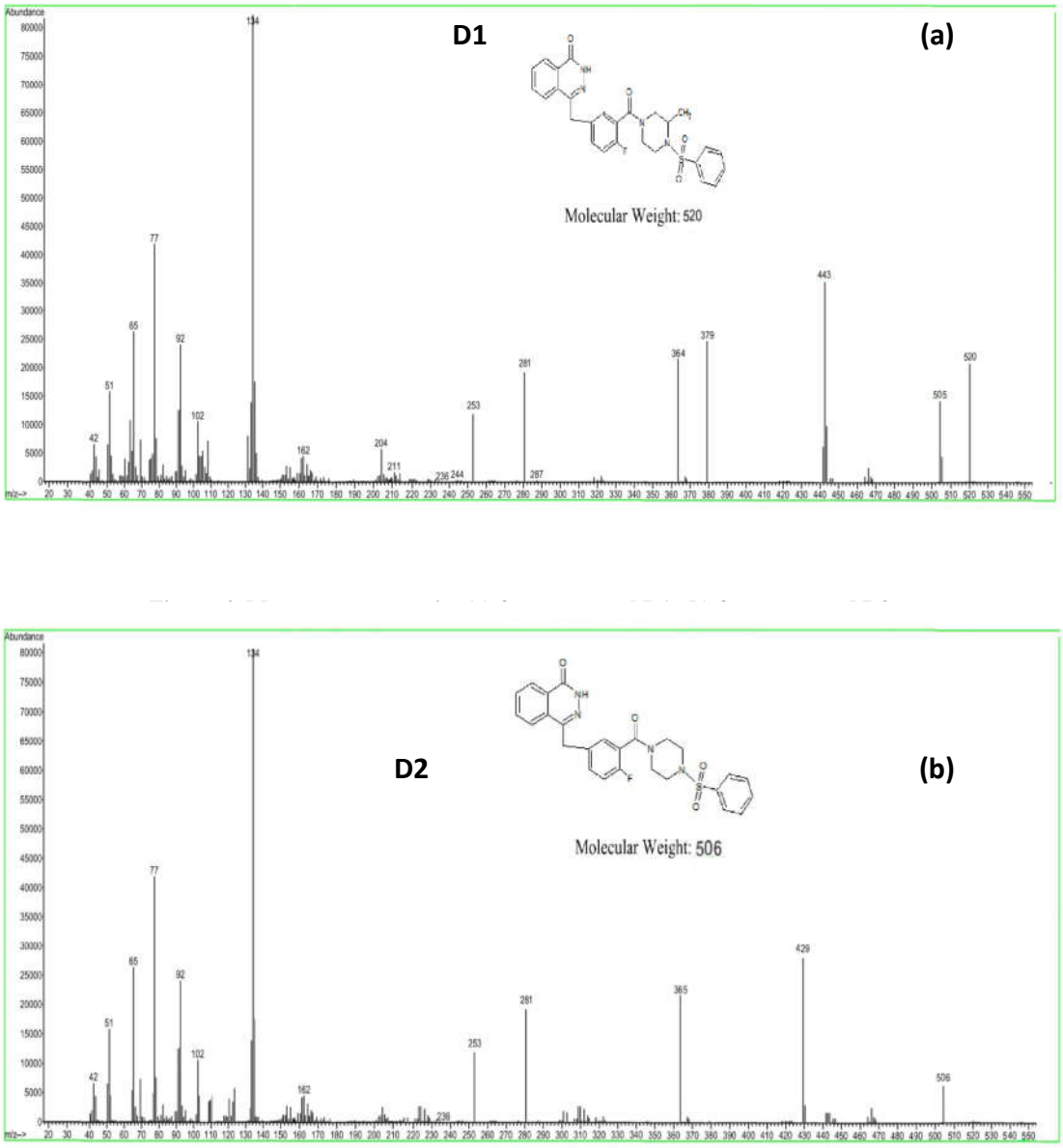


Fig.6: Mass –spectrometric: (a) for compound D1, (b) for compound D2

Several tools, including GOLD (Genetic Optimization of Ligand Docking), supplied by the Cambridge Crystallographic Data Centre (CCDC) (https://www.ccdc.cam.ac.uk/) in version 2020.3.0, as well as other relevant programs, were used to conduct the docking research. PARP-1 is a proto-oncogene identified as the target protein for this research (PDB ID 5DS3). As part of the PARP-1 enzyme complex, Olaparib works as combined with components B, C, D1, and D2 chemicals (figure 10). The following amino acids are designated as binding sites:

1. GLYCINE 863 2. SERINE 904
3. TYROSINE 896 4. ARGININE 878

The analogs compounds of Olaparib are effectively synthesizing. Some of their fitness and interaction properties were investigated and summarized in Table 1. We calculated the percent yield of chemical reactions by dividing the amount of product reaction by the theoretically predicted amount multiplying by 100, which indicates to reaction efficiency of the chemical compound [20].

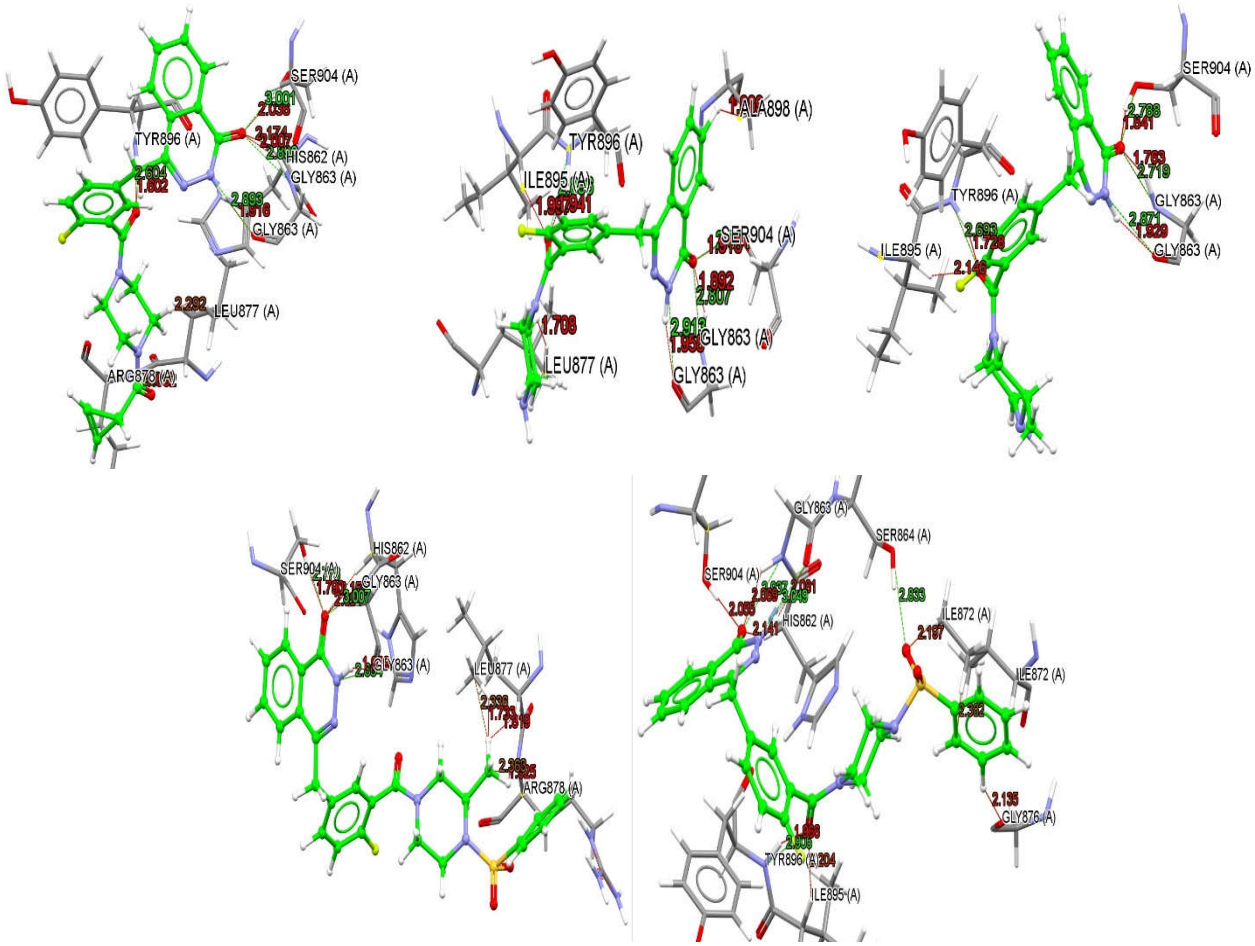


Fig.9: Interactions of chemical compounds syntheses with Olaparib. Docking the structures of the Olaparib (a), compound B (b), compound C (c), compound D1 (d), and compound D2 (e). PARP-1's crystal structure in combination with Olaparib (PDB code: 5DS3) and compounds B, C, D1, and D2 as a result of which the stick model, and PARP-1 is shown as a space fill representation

Table 1: The design *in silico*, docking, and the number of binding sites of Amino Acid Sequence

Ligand	A.A. (H-Bond)	A.A. (other interaction)	Fitness ppl
OLAPARIP	ARG 878, GLY 863 (2), SER 904	LEU 877, ARG 878, TYR 896, GLY 863, SER 904, HIS 862	111.28
B	GLY 863 (2), SER 904, TYR 896	GLY 863 (2), SER 904, ALA 898, TYR 896, ILE 895, LEU 877	96.35
C	TYR 896, GLY 863 (2), SER 904	ILE 895, TYR 896, GLY 863, SER 904	96.83
D1	GLY 863 (2), SER 904	SER 904, HIS 862, GLY 863 (2), ARG 878, LEU 877 (3)	104.09
D2	SER 864, GLY 863 (2), TYR 896	TYR 896, ILE 895, ILE 872 (2), GLY 876, GLY 863 (2), HIS 862, SER 904	115.8

A significant number of researchers used the MDCK cell line to evaluate the toxicity profile of the pick-out drugs [21-25]. The incubation time decreased to 48 hours rather than 72 hours, while the incubation period increased to 48 hours rather than 24 hours. The concentrations of the produced compounds administered to the cells, on the other hand, were optimized. Starting at the maximum 1000 µg/mL concentration and reaching the optimum of high concentration 31.25 µg/mL, the serial dilution of 31.25 g/mL was mathematically determined to provide representative curves, and it was approved. The coefficients of correlation of curves are very high. The half-maximal inhibitory concentrations (IC50) are mainly seen in the middle of the curves to eliminate any drift. The IC_{50} measured the effectiveness of a chemical substance created to impede the growth of cells. The cell lines were purposefully selected, accurately identifying ways breast cancer is considered the first cancer in American women. According to invasive and noninvasive breast cancer in the US 2019, the American Cancer Society has concluded 268,600 new cases of invasive breast cancer, and 62,930 new instances of noninvasive breast cancer diagnosed [26]. Of importance, toxicity evaluation of the newly synthesized compounds must be performed on noncancerous cells to properly assess their selectivity for cancer cells, It is worthy to mention that the most main factor induced cancer in general is imbalance of oxidative stress that affected by many negative factors such as containments (chemical subsistences, Mycotoxins and pesticides) [26- 31]. Researchers used the MDCK cell line to conduct cell viability tests, availability, and they chose the MDCK cell lines [22, 23, 26]. Due to this, the synthesized Olaparib analogs tested on the body cells seen for activity and toxicity profile assessed. Table 2 summarizes the findings of this current study and describes the biological impact of chemically generated compounds on malignant and normal cells. Classification of each chemical and cell to critically evaluate the data observed in this study:

- The Olaparib standard has greats inhibitory concentration (IC50) for the MCF-7 cell line. Compound D1, on the other hand, has the lowest IC50 value (1.0685-times lower than Olaparib standard). Finally, Compound D2 exhibits IC50 values that are in the center of the range.

- For the MDCK cell line, the IC50 for compound D1 has the lowest value, followed by compound D2, then Olaparib standard. On the other hand, compound D1 has (0.985-times the IC50 value) for the Olaparib standard against the cancer cell line.
- For the MDCK cell line, the Olaparib possessed the highest IC50 value, which means they were less toxic for compound D1 and compound D2 to have higher cytotoxicity profiles than the Olaparib standard. In Summary: compound D1 has better activity and lower cytotoxicity than compound D2 in the MDCK cell line.
- The MCF-7 cell lines contain the most intriguing discoveries. The IC50 value for compound D1 is the lowest, indicating more cytotoxic action than the Olaparib reference compound. Compound D2, on the other hand, has the intermediate values for IC_{50}. Finally, the Olaparib standard has the highest IC_{50} value.

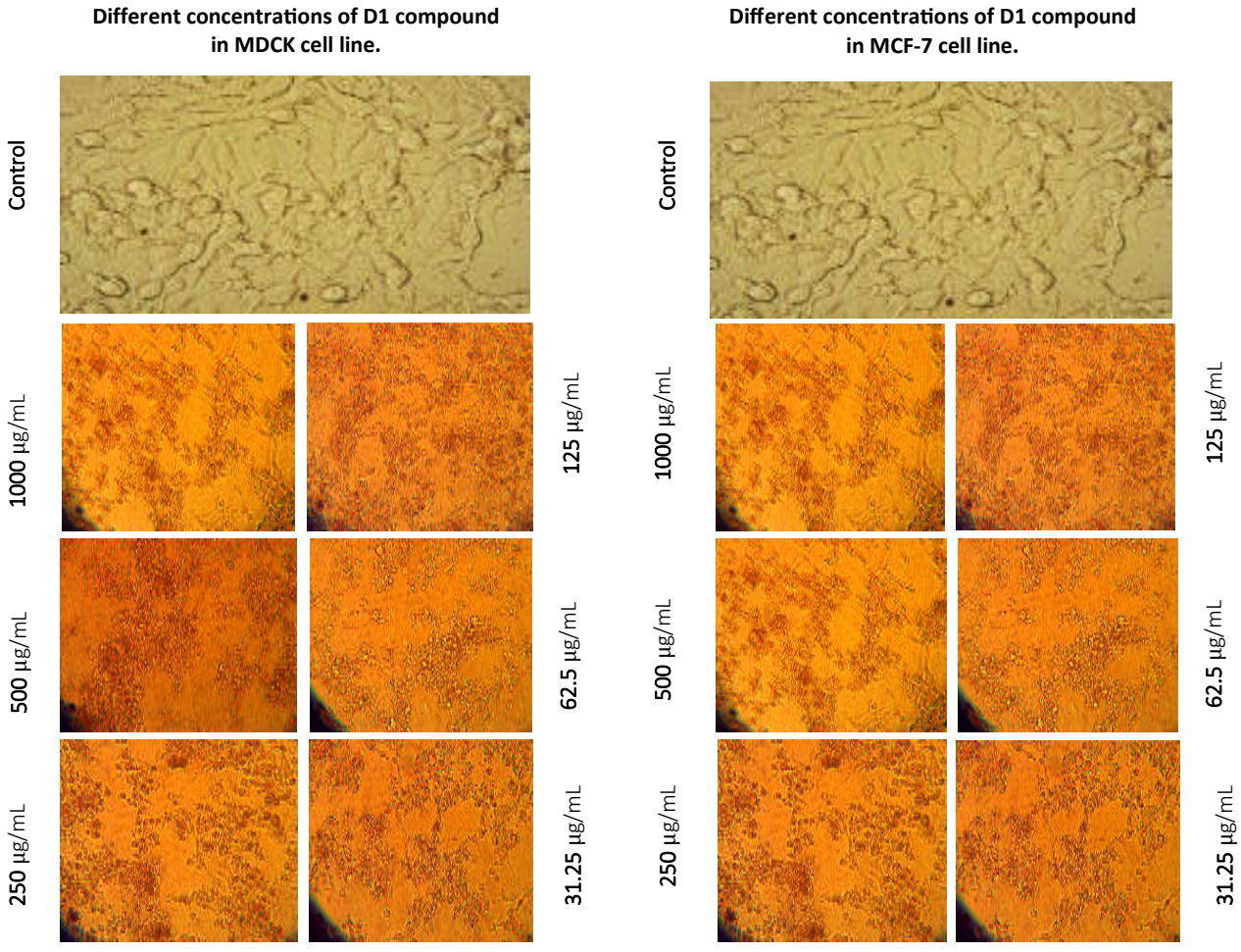


Fig.7: Morphological changes of MDCK and MCF-7, an observation was made using an inverted phase-contrast microscope at 20 X magnification. Exposure to different concentrations of D1 compound µg/mL, after 48 hours of incubation

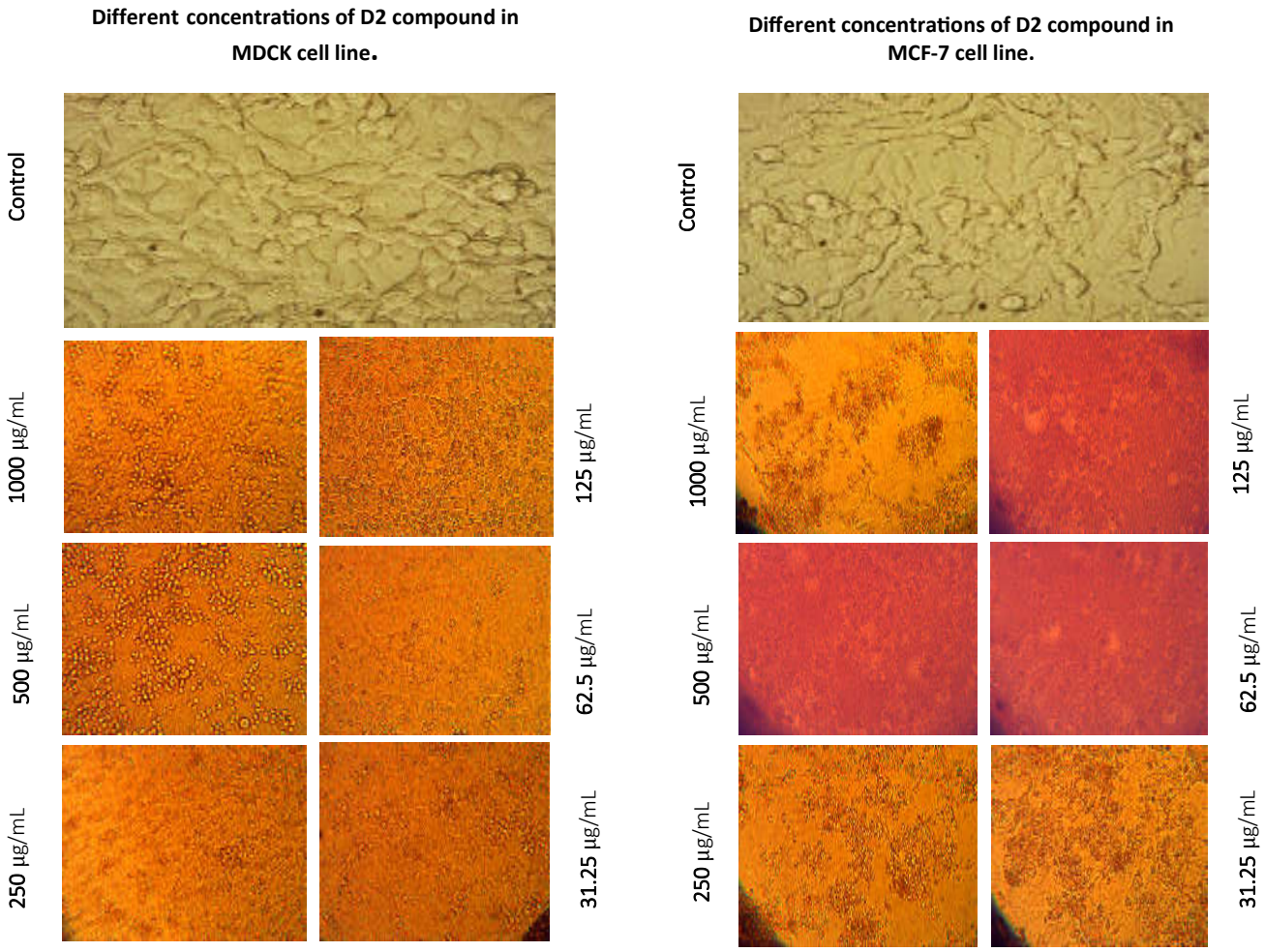


Fig.8: Morphological changes of MDCK and MCF-7, an observation was made using an inverted phase-contrast microscope at 20 X magnification. Exposure to different concentrations of D2 compound µg/mL, after 48 hours of incubation

Table 2: The inhibitory concentrations (IC_{50}) values for the cell lines and chemical synthesis compounds (D1 and D2) that synthesized in this study

Cell Lines Types	IC_{50} of Olaparib Standard	IC_{50} of Compound D1	IC_{50} of Compound D2
MCF-7	68.951 µg/mL	64.517µg/mL	65.497µg/mL
MDCK	**6.648 mg/mL**	**5. 975mg/mL**	**6. 349mg/mL**

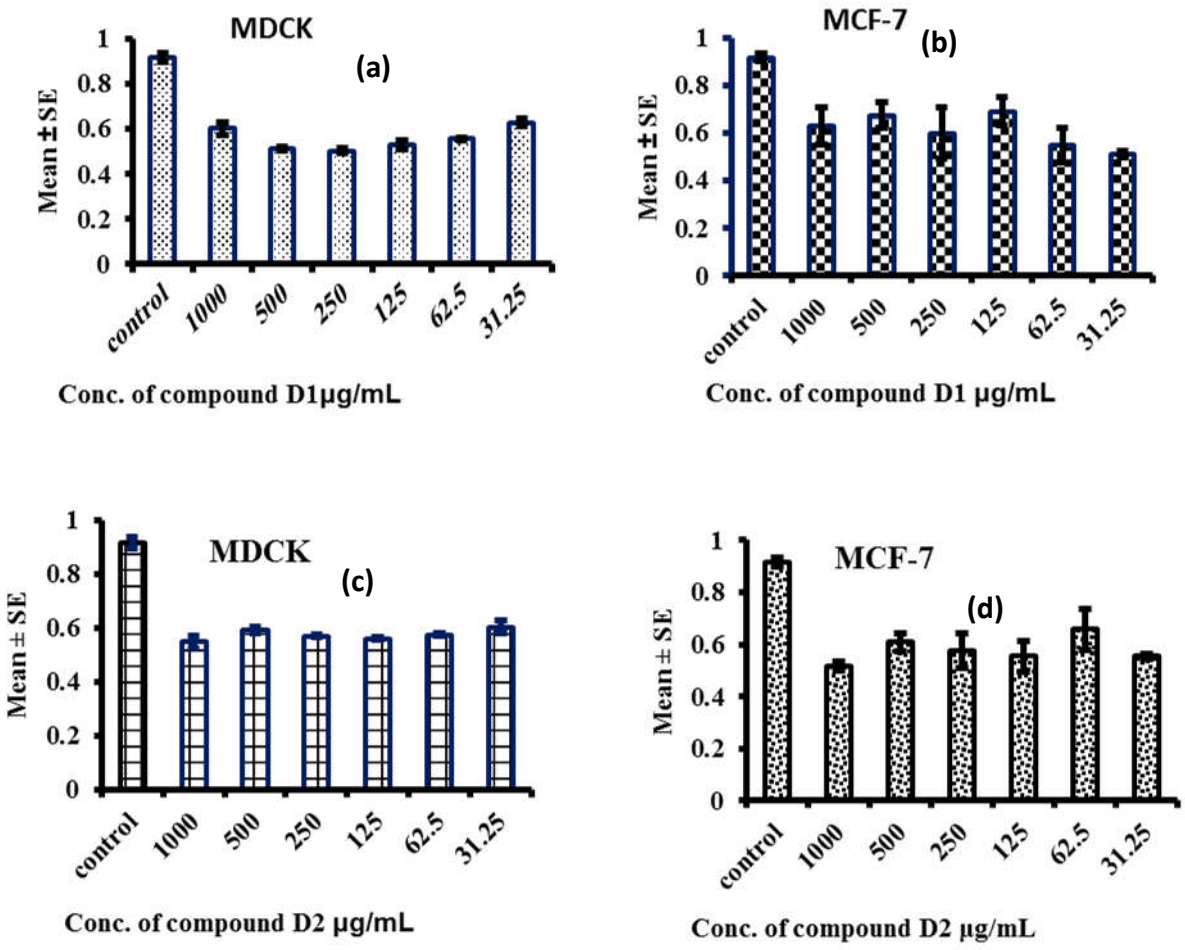


Fig.9: MDCK and MCF-7 cytotoxicity for different concentration of D1 and D2 compounds (Data are reported as mean ± SE)

4. Conclusions

In this study, two of the synthesized compounds were discovered to exhibit biological activity. The authors concluded that the two newly synthesized compounds have moderate antineoplastic activity against the MCF-7 cancer cell line and a good safety profile when tested against the MDCK normal kidney cell line.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgments

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