Synthesis, Characterization and Study biological activity of some New Derivatives of Steroid analogs.

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Abstract. The search includes a new series of hydrazide-hydrazone derivatives [12-14] as well as 1,3,4-Oxidiazole[7,8] for steroid analog (methyl((5-pregnen-3β,17-diol-15-yl)thio)propanoate) were synthesized through ester group with Hydrazine Hydrate in presence DMF to obtained carbohydrazide derivative (4), then the 1,3,4-Oxidiazole are prepared by interaction Benzoic Acid substituted with steroid (4) in presence Phosphorous Oxy Chloride as catalyst, while Hydrazide-hydrazone are prepared by react the steroid (4) with aromatic aldehydes or ketone by catalyzes HOAc. Structures of all the newly synthesized compounds have been elucidated by means of IR, 1H NMR, 13C NMR, 2D (HMBC, HSQC) and C.H.N Analysis.

1-Introduction

Breast cancer is one of the most common cancers among women around the world (1,2) which accounts for about 10% of all cancers and 23% of female cancers (3). It is well known that breast tumors are that depend on the steroid hormone where estrogen receptors play an important role in the development and growth of prostate cancer also the tumor can be produced from cholesterol through stimulation by 27-Hydroxy Cytochrom P450 27A1 (CYP27A1) to production 27-Hydroxy cholesterol (4,5), The therapeutic used to inhibit estrogen receptor are Fulvestrant and Exemstane fig (1) that join to and target ER for degeneration and aromatase inhibitors that inhibit the activity of aromatas (CYP19A1) the enzyme that responsible for the synthesis of estrogens from androgenic majors (6-9).

From the extensive studies found that the breast cancer is a complex disease that requires the use of hybrid drugs are multi-purpose and not one type of medication for treatment will not be effective (10,11). Steroids are one of the most effective pharmaceutical compounds that are used to treat many common ailments (12,13) also heterocyclic compounds and Schiff base (14-17), So the researchers were suggested modification of steroids by linking it with compounds known to have biologic activity for increase the bioactivity of steroids.

From this point in 2011 Gamal A. Elmegeed and co-workers designed groups of steroid compounds bearing a heterocyclic and evaluated as a treatment for breast cancer in cell line MCF-7 (18), then the S.M. M Akram and his groups in 2013 synthesize new derivatives from pyrazoles and triazoles to the pregnenolone and tested against breast cancer (19).

Later in 2015 N.A. Al-Masoudi et al prepared new azo methan derivatives from pregnenolone and evaluate their activity for inhibition of CYP17 hydroxylase enzyme responsible for prostate cancer (20). So in this study we synthesize two series of steroids analog, steroids bearing heterocycles 1,3,4-Oxidiazole as well as hydrazide-hydrazone steroid analog derivatives and evolution as anti breast cancer in cell line MCF-7.
Scheme 1 Synthesis of new hydrazide-hydrazones and 1,3,4-Oxidiazole derivatives.

Figure (1) the therapeutic for breast cancer
2- Experimental Part

2-1. Chemistry
The IR spectra were recorded on Shimadzu. The NMR (300 MHz) was recorded on Bruker DPX 300 spectrometer in DMSO-d6 using TMS as internal standard reference and chemical shifts are in d ppm. The melting points were measured by capillary method. Elemental analyses were performed on Elementar Vario EL III, Carlo Erba 1108.

2.2 Chemical synthesis

2-2-1. Synthesis ((5-pregnen-3β,17-diol-15-yl) thio) propane hydrazide.

A mixture of methyl ((5-pregnen-3β,17-diol-15-yl) thio) propanoate 1000 mg (2.45 mmole) and excess of hydrazine hydrate 80% (5 ml) in DMF (15 mL) was refluxed for 10 hours. The progress of reaction was check by TLC (n-hexane-ethyl acetate). (4:1) The product mixture was evaporated and allowed to cool. to afford compound. The resulting solid was filtered, dried & recrystallized from ethanol.

Yield: 750 mg 75%, yellow powder, M.p:152-153 R.f = 0.32 IR (KBr) (cm-1): 3442 (OH), 3421, 3284 (NH2, str), 1660 (C=O amide), 1633 (NH, bend)

1H NMR (301 MHz, DMSO) δ 9.06 (s,1H, NH amide), 6.88 (s,2H, NH2), 5.28 (t,1H, H6), 4.53, (s, 1H, OH-3), 3.72 (s, 1H, OH-17), 3.44 (m, 1H, H17), 3.27 (m, 1H, HC-3), 3.06 (m, 2H, Hb-4), 2.64 (m, 1H, HC-15), 2.43 (t,2H, H-21), 2.29 (t, 2H, H22), 2.09 (m, 2H, Ha-4), 1.80-1.67 (t, H8+7+2), 1.54 (m, 2H, HC-11), 1.49 (t, 2H, HC-12) 1.39 (t, 2H, HC-16), 1.31 (m, 2H, HC-14), 12.4 (t, H9+1), 0.96 (s, 3H, Me-19), 0.82, (s, 3H, Me-18). 13C NMR (76 MHz, DMSO) δ 170.46 (C=O amide), 141.57 (C-5), 120.94 (C-6), 80.49 (C-17 OH), 70.51 (C-3 OH), 54.41 (C-14), 50.52 (C-9), 43.55 (C-13), 42.71 (C-4), 40.76, 40.49, 40.23, 40.19 (DMSO), 37.68 (C-12) 37.51 (C-1), 36.73 (C-10), 34.66 (C-15), 31.87 (C-8), 31.32 (C-7), 30.90 (C2), 29.83 (C-21), 29.12 (C-22), 28.69 (C-16), 20.51 (C-11), 19.54 (C-19), 14.18 (C-18). Anal. calcd for C12H10N2O3S Mol. Wt: 208.6 C, 64.67 H: 8.88: N: 6.86: found C: 64.59: H: 8.80: N: 6.93

2-2.2- General procedure for the synthesis 1,3,4- oxadiazole derivatives of ((5-pregnen-3β,17-diol-15-yl) thio) propane hydrazide).

A solution of (120 mg 0.29 mmol) of ((5-pregnen-3β,17-diol-15-yl) thio) propane hydrazide), (0.29 mmol) benzoic acid derivatives and 8 mL of POCl3 was refluxed with stirring for 8–10 h, after full conversion by TLC, using n-hexane-ethyl acetate (3:2) as eluents the reaction mixture was cooled and poured over crushed ice. Then the product neutralize with sodium bicarbonate solution, the organic layer extracted by DCM (3 x 20) evaporated, dried and recrystallised with ethanol.

2-2-2.1. Synthesis 15-(2-(5-(2-aminophenyl)-1,3,4-oxadiazol-2-yl) ((5-pregnen-3β,17-diol) ethylthio (7) from 2-Amino benzoic acid (5) 40 mg).

Yield: 85 mg 57%, brown powder, M.p:180-182 R.f = 0.4 IR (KBr) (cm-1): 3425 (OH), 3348 and 3178 (NH3,str), 3101 (CH-arom), 1681 (C=N), 1527 and 1465(C=H arom), 1118(C-O-C) 1H NMR (301 MHz, DMSO) δ 7.87 (d, 1H, H3-arom), 7.59 (m,1H, H5-arom), 7.10 (m,1H, H4-arom) 6.95 (d,1H, H3-arom), 5.26 (m,1H, H6), 4.76 (s, 2H,NH2), 4.08 (s, 1H, OH-3), 3.35 (s, 1H, OH-17), 3.07 (m, 1H, HC-3). 13C NMR (76 MHz, DMSO) δ 162.75 (C5=O arimide), 157.55 (C2-Oxadiazole), 144.85 (C-NH2), 142.18 (C5+Cl-arom), 135.31 (C4-arom), 131.01(C3-arom),126.16(C6-arom),123.19 (C5-arom), 120.60 (C-6), 80.37 (C-17 OH), 67.17 (C-3 OH) 80.50 (C-17 OH), 70.50 (C-3 OH), 55.51 (C-14), 50.55 (C-9), 42.24 (C-13), 41.87 (C-4), 37.85 (C-12), 36.77 (C-1), 35.78 (C-10), 34.30 (C-15), 33.63 (C-8), 31.14 (C-7), 29.74 (C2), 29.47 (C-21), 26.78 (C-22), 25.01 (C-16), 22.12 (C-11), 20.65 (C-19), 14.41 (C-18). Anal. calcd for C28H39N3O4S Mol. Wt: 509.7 C, 68.34; H: 7.71; N: 8.24; found C: 68.25; H: 7.64; N: 8.16
2-2-2.2- Synthesis 15-(2-(5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl) (5-pregnen-3β,17-diol) ethylthio (8) from 3,5-Di nitro benzoic acid (6) 61.4 mg.

Yield: 80 mg 47%, brown powder, M.p:173-175° R.f = 0.38

IR (KBr) (cm-1): 3425 (OH), 1664 (C=O amide), 7.45 (C=O amide), 1681 (C=N), 1650 (C=N), 1534 (C-N), 1527 (C=C arom), 1483 (C-N), 1458 (C=C arom), 837 (C=O amide), 70.89 (C=O amide), 161.87 (C2=O amide), 148.76 (C3=C5- arom), 140.80 (C-5), 129.37 (C1-arom), 128.21 (C2+C6-arom), 123.22 (C4-arom), 122.58 (C-6), 83.73 (C-17 OH), 70.89 (C-3 OH), 53.86 (C-17 OH), 49.99 (C-9), 43.23 (C-13), 42.70 (C-4), 37.23 (C-12), 36.42 (C-1), 35.10 (C-10), 33.48 (C-15), 31.16 (C-8+C-7), 29.58 (C2), 28.56 (C-21), 25.01 (C-22), 22.94 (C-16), 20.22 (C-11), 19.27 (C-19), 14.39 (C-18).

Analytical for C29H18N4O6S Mol. Wt: 584.68 C, 59.57; H, 6.21; N, 9.58 found C, 59.54; H, 6.30; N, 9.52

2-2-3-General procedure for the synthesis hydrazide- hydrazone derivatives from (5-pregnen-3β,17-diol-15-yl) thio) propane hydrazide.

A substituted aldehyde or ketone (0.24 mmol) and glacial acetic acid (1 ml) in EtOH (15 ml) was stirred for 1 h then a solution of steroid hydrazide (4) (100 mg 0.24 mmol) in (10 ml) EtOH added drop wise a mixture was heated under reflux for 12–17 h. The reaction was monitored by TLC by using n-hexane-ethyl acetate (3:2) as eluents. After cooling, the solid product was collected by filtration and recrystallized from ethanole to give the desired imine derivatives.

2-2-3-1- Synthesis N’-(4-(Dimethylamino)benzylidene) (5-pregnen-3β,17-diol-15-yl) thio) propane hydrazide (12) from 4-Dimethylaminobenzaldehyde (9) 35.7 mg.

Yield: 90 mg 69%, red powder, M.p:142-143° R.f = 0.34

IR (KBr) (cm-1): 3417 (OH), 1658 (C=O amide), 1604 (C=N), 1527 (C=C arom) 1H NMR (301 MHz, DMSO) δ 10.91 (s, 1H, HC=N), 9.77 (s, 1H, NH-amide), 7.24, 7.21 (H3+5 arom), 6.56, 6.53 (H2+6 arom), 5.03 (m, 1H, H-6), 4.36 (s, 1H, OH-3), 3.84 (s, 1H, OH-17), 3.20 (m, 1H, H-17), 3.03 (m, 1H, HC-3), 2.81 (N-Me2, s, 6H), 2.72 (m, 2H, Hb-4), 2.70 (m, 1H, HC-15), 2.28 (tr, 2H, H-21), 1.89 (t, 2H, H22), 1.55 - 1.43 (t, H8+7+2), 1.24 (m, 2H, HC-11), 1.14 (t, 2H, HC-12), 1.10 (t, 2H, HC-16), 0.99 (m, 2H, HC-14), 0.71 (t, H9+1), 0.59 (s, 3H, Me-19), 0.54, (s, 3H, Me-18).

Analytical for C31H24N4O2S Mol. Wt: 539.77 C, 68.98; H, 8.40; N, 7.78; found C, 68.88; H, 8.47; N, 7.85;

2-2-3-2- Synthesis N’-(4-Hydroxy-3-methoxybenzylidene) (5-pregnen-3β,17-diol-15-yl) thio) propane hydrazide (13) from 4-Hydroxy-3-methoxybenzaldehyde (10) 38.8 mg.

Yield: 85 mg 65%, light yellow powder, M.p:200-202° R.f = 0.29

IR (KBr) (cm-1): 3394 (OH), 1681 (C=O amide), 1650 (C=N), 1458 (C=C arom) 1H NMR (301 MHz, DMSO) δ 12.23 (s, br,1H,OH-arom), 11.09, (s, 1H, HC=N), 9.82 (s, 1H, NH-amide), 7.47-7.45 (2H-H5,6-arom), 6.87 (1H,2-arom), 5.27 (m,1H, H-6), 4.70 (s, 1H, OH-3), 3.83 (s, 3H, OCH3), 3.52 (s, 1H, OH-17), 3.37 (m, 2H, H-17+H-3), 3.12 (m, 2H, H-4), 2.83 (m, 2H, H-13), 2.05 (m, 2H, H-15), 1.97 (t, 2H, H-21), 1.54 - 1.37 (t, H8+7+2), 1.21 (m, 2H, HC-11), 1.05 (t, 2H, HC-12), 1.00 (t, 2H, HC-16), 0.96 (m, 2H, HC-14), 0.70 (t, H9+1), 0.61 (s, 3H, Me-19), 0.51, (s, 3H, Me-18)

Analytical for C31H24N2O3S Mol. Wt: 529.44 C, 68.98; H, 8.40; N, 7.78; found C, 68.88; H, 8.47; N, 7.85;
m. 1H, HC-15) , 2.64 ( tr ,2H , H-21 ), 2.15 ( t , 2H , H22) , 1.87 - 1.72 ( t , H8+7+2 ) , 1.52 ( m , 4H , HC-11 + 12) , 1.39 ( t , 2H , HC-16 ) , 1.25 ( m , 2H ,HC-14) , 0.98 ( t , H9+1) , 0.87 ( s , 3H , Me-19 ) , 0.71 , ( s , 3H, Me-18 ). ¹³C NMR (76 MHz, DMSO) δ 173.08 (C=O amide ) , 167.66 ( C=N ) , 151.57 ( C-OH ), 147.69 (C-OCH3), 142.03 ( C-5 ) , 123.94 ( C1-arom ) , 122.11 ( C6-arom ) , 120.31 ( C-6 ) , 115.49 ( C2- arom ) , 113.25 ( C5- arom ) , 79.98 ( C-7 OH) , 70.47 ( C-3 OH ), 56.04 (OCH3) , 54.54 (C-14) , 50.90 (C-9) , 42.61 (C-13) , 42.12 (C-4) , 38.22 ( C-12 ) , 37.48 (C-1), 36.74 (C-10 +15), 31.89 (C-8 ) , 31.62 ( C -7), 29.99 (C2) , 29.61 (C-22) , 27.75 (C-21) , 25.46 (C-16 ) , 20.47 ( C-11), 19.45 ( C-19 ), 14.12 ( C-18 ). Anal.calc.for C₉H₄₂N₂O₅S (40 ) Mol. Wt: 542.73 C, 66.39; H, 7.80; N, 5.16; found , 66.48; H, 7.75; N, 5.22 .

2-2-3-3- Synthesis N’-(1-(4-Bromophenyl)ethylidene) ((5-pregnen-3β,17-diol-15-yl) thio) propanehydrazone (14) from p-Bromo acetophenon (11) 48 mg.

Yield: 90 mg 63 % , light yellow powder , M.p:170-172 R.f= 0.55

IR (KBr) (cm⁻¹): 3366 (OH) , 1674 (C=O amide ) , 1535 ( C=N ) , 1415 ( C=C arom ) , ¹¹H NMR (301 MHz, DMSO) δ 8.79 (s,1H, NH amide ) , 7.87-7-81 (H3,5 arom) 7.61-7.57 (H2,6-arom ), 5.27 (t ,1H, H6), 4.62, (s , 1H, OH-3 ) , 3.72 ( s , 1H , OH-17 ) , 3.42 ( m ,1H, H7-17 ) , 3.28 ( m ,1H, H-3) , 3.10 ( m ,2H, H-4) , 2.59 ( m, 1H, HC-15), 2.28 ( tr ,2H , H-21) , 2.25 ( s , 3H,MeC=N ) , 2.14 ( t, 2H , H22), 1.76 - 1.72 ( t, H8+7+2 ) , 1.49 ( m , 4H, HC-11 +12) , 1.39 ( t , 2H ,HC-16 ) , 1.25 ( m , 2H ,HC-14) , 0.96 ( t , H9+1) , 0.84 ( s , 3H, Me-19 ) , 0.71 , ( s , 3H, Me-18 ). ¹³C NMR (76 MHz, DMSO) δ 173.56 ( C=O amide ) ,166.54 ( C=N ), 148.66 (C1-arom) 141.62 ( C-5 ) , 131.73 ( C5+2C5- arom ) , 128.52 (C2+C6-arom) , 122.97 (C-Br) , 120.96 (C-6) , 120.96 ( C-7 OH), 70.49 ( C-3 OH ) , 54.44 (C-14) , 50.52 (C-9) , 43.69 ( C-13 ), 42.73 (C-4), 37.71 ( C-12 ) , 37.50 (C-1), 36.74 (C-10) , 35.14 (C-15), 31.91 (C-8 +7) , 31.27 ( C-2) , 29.84 (C-22) , 29.62 (C-21) , 25.47 (C-16 ) , 20.51 ( C-11), 19.53 ( C-19 ), 15.10 (Me-C=N ) , 14.17 ( C-18 ). Anal.calc.for C₉H₄₂BrN₂O₅S Mol. Wt: 589.63 C, 61.11; H, 7.01; N, 4.75; found C, 61.22; H, 7.06 ; N, 4.71 .

2-3-Biological study
2-3-1 Maintenance of cell cultures

MCF-7 were maintained in RPMI-1640 supplemented with 10% Fetal bovine, 100 units/mL penicillin, and 100 µg/mL streptomycin.Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week, and incubated at 37 °C (21).

2-3-2 Cytotoxicity Assays

To determine the cytotoxic effect of synthesize compounds, the MTT cell viability assay was done using 96-well plates. Cell lines were seeded at 1 × 10⁵ cells/well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compounds at different concentration. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT stain and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking [2]. The absorbency was determined on a microplate reader at 492 nm, the assay was performed in triplicate. The
inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

\[ \text{Cytotoxicity} = \frac{A - B}{A} \times 100 \]

Where A is the optical density of control and B is the optical density of Samples.

For visualizing the shape of cells under inverted microscope, 200 μL of cell suspensions were seeded in 96-well micro-titration plates at density 1x10^4 cells mL \(^{-1}\) and incubated for 48 hrs at 37°C. Then the medium was removed and the tested components were added (IC50). After exposure time, the plates were stained with 50 μL of Crystal violet and incubated at 37°C for 15 min; the stain was washed gently with tap water until the dye was removed. The cell observed under inverted microscope at 40 x magnification microscope filed and photographed with digital camera \(^{(22)}\).

2-2-3 Statistical analysis:

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6. The values were presented as the mean ± SEM of triplicate measurements \(^{(23)}\).

3- Results and discussion

3-1. Chemistry

Steroid analogues methyl ((5-pregnen-3β,17-diol-15-yl) thio) propanoate (3) has been choices as a starting material for synthesis hydrazide-hydrazone as well as 1,3,4-oxidiazole derivatives and evaluated as anti breast cancer. Steroid 3 was treated with hydrazine hydrate to obtain carbohydrazide derivative 4. Later, compound 4 was used as an intermediate step for the preparation of carbohydrazide derivatives \([12-14]\) after its condensation with aldehydes and ketones (eg: 4-(Dimethylamino) benzaldehyde, 4-Hydroxy,3-methoxy benzaldehyde, 1-(4-Bromophenyl)ethanone), using acetic acid as a catalyst in ethanol also was obtained derivatives \([7,8]\) from treated 4 with substituted benzoic acid for instance (3,5-Dinitro benzoic acid, 2-Amino benzoic acid) in presence phosphoryl trichloride as a catalyst according to the scheme 1.
The structures of all compounds were assigned by the $^1$H, $^{13}$C and 2D NMR spectra. The $^1$H NMR spectra of derivative 4 showed chemical shift singlet at 9.06, 6.88 ppm for the protons of amide and amine groups respectively, while the aromatic protons of 7 appear doublet at 7.87 and 6.95 to the (H6, H3) sequentially as well protons of (H5, H4) appear triplet at 7.59 and 7.10 respectively. 

The $^1$H NMR spectra of compound 8 appear high chemical shift for H4 aromatic at 8.16 ppm as a result two nitro groups adjacent while the protons H2 + 6 appear singlet at 7.43 ppm. As shown the $^1$H NMR spectra the protons of amide groups for hydrazone derivatives (12-14) appear at (9.77, 9.82, 8.79) respectively while protons imine groups appear at 10.91 and 11.09 ppm for (12,13) respectively and the protons of methyl group for 14 derivative appear singlet sign at 2.25 ppm.

The protons of aromatic rings for (12-14) derivative appear at range (7.24-6.54), (7.47 – 6.87), (7.87-7.57) respectively as well as appear singlet sign at 2.81 duo to protons of N-Me2 (12), at 12.23 to protons of OH-arom and 3.83ppm to protons of methoxy goup (13). The rest of the protons belonging to the pregen loop were diagnosed in the practical part. In the $^{13}$C-NMR the spectrum showed the disappearance of the band back to the carbonyl group at position 170 ppm for derivative 4 and the emergence of new bands belonging to C = N of the oxodiazole ring at (162.75, 164.39) ppm for C5 and (157.55, 161.87) ppm for C2 to the (7.8) derivatives, at the same time the carbon atoms of azo methane appear at (160.17, 167.66, 166.54) (12-14) respectively in addition to the bands back to carbon atoms of aromatic ring and backbone of pregenen ring which identified in the section part 125.

To further increase accuracy, the some compounds were prepared characterized by

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**Scheme (2)** Reagent and condition: (a) Hydrazine hydrate, DMF 10h reflux (b) substituted benzoic acid, POCl3 8-10 h reflux (c) substituted aldehyde and ketone, EtOH, HOAc, 12-17 h reflux.
2D – NMR (HSQC, HMBC) where the spectrum HMBC of 7 showed the coupling between C-5 of oxidiazole and H-6 of aromatic ring FIG. 2, while the compounds 14 appear coupling between C1 and H2,6 as well coupling between C4 and H3,5 FIG. 3.

![Figure (2)](image1)

**Figure (2)** explain J.C.H correlations in the HMBC of compound (7).

![Figure (3)](image2)

**Figure (3)** explain J.C.H correlations in the HMBC of compound (14).

### 3.2. Biological assays

#### 3.2.1. Evaluation of cytotoxic activity in vitro

When survey the prepared compounds against breast cancer using cell line MCF-7 at concentrations of 6.25, 12.5, 25, 50 and 100 µg/ml, the results exhibited the cytotoxicity of cell ranging from (5-75) % and inhibition of cell began from IC<sub>50</sub> 24.78 to 47.16.

Of the data obtained from the derivatives of steroid analogue most active against cell line MCF-7 was they can be arranged as follows 14 > 7 > 13 > 12 > 8.

It is evident that there is a relationship between the structure and the rate of inhibition where it was found that the hydrazide-hydrazone 14 was more active than the of all compounds and followed 1,3,4-Oxidiazole steroid analogue 7.

This result can be due to the fact that these compounds No (14 and 7) are lipophilic where as the compounds No (13, 12 and 8) are hydrophilic.

### Table 1. The cytotoxic activity in vitro of the newly prepared compounds on cell line MCF-7 cancer.

| Compd No. | IC<sub>50</sub> µg/ml |
|-----------|------------------|
| 7         | 31.51            |
| 8         | 47.16            |
| 12        | 44.19            |
| 13        | 39.44            |
| 14        | 24.78            |
Fig. 4.: Cytotoxicity effect of 14 in MCF-7 cells. IC50=24.78 µg/

Fig. 5.: Cytotoxicity effect of 12 in MCF-7 cells. IC50=44.19 µg/

Fig. 6.: Cytotoxicity effect of 7 in MCF-7 cells. IC50=31.51 µg/ml

Fig. 7.: Cytotoxicity effect of 13 in MCF-7 cells. IC50=39.44 µg/ml

Fig. 8.: Cytotoxicity effect of 8 in MCF-7 cells. IC50=47.16 µg/ml
Figure 9. Inhibition of cell line MCF-7

Figure 10. FT-IR of compounds (4)
Figure 1. $^1$H NMR spectrum of compound(4).

Figure 2. Expansion of $^1$H NMR spectrum of compound(4).
Figure 13. $^{13}$C NMR spectrum of compound (4).

Figure 14. Expansion of $^{13}$C NMR spectrum of compound (4).
Figure 15. HMBC NMR spectrum of compound (4).

Figure 16. (FT-IR) of compounds (7)
Figure 17. $^1$H NMR spectrum of compound (7).

Figure 18. $^{13}$C NMR spectrum of compound (7).
Figure 19. $^{13}$C NMR spectrum of compound (7).

Figure 20. HMBC- NMR spectrum of compound (7).
Figure 21. FT-IR of compounds (8).

Figure 22. $^1$H NMR spectrum of compound(8).
Figure 23. $^{13}$C NMR spectrum of compound (8).

Figure 24. $^{13}$C NMR spectrum of compound (8).
Figure 25. (FT-IR) of compounds (12).

Figure 26. $^1$H NMR spectrum of compound(12).
Figure 27. Expansion of $^1$H NMR spectrum of compound (12).

Figure 28. $^{13}$C NMR spectrum of compound (12).
Figure 29. Expansion of $^{13}$C NMR spectrum of compound (12).

Figure 30. (FT-IR) of compounds (13)
Figure 31. $^1$H NMR spectrum of compound(13).

Figure 32. Expansion of $^1$H NMR spectrum of compound(13).
Figure 3.3. $^{13}$C NMR spectrum of compound (13).

Figure 3.4. Expansion of $^{13}$C NMR spectrum of compound (13).
Figure 35. HMBC- NMR spectrum of compound (13).

Figure 36. HSQC- NMR spectrum of compound (13).
Figure 3.7. FT-IR of compounds (14)

Figure 3.8. $^1$H NMR spectrum of compound(14).
Figure 39. Expansion of $^1$H NMR spectrum of compound (14).

Figure 40. $^{13}$C NMR spectrum of compound (14).
Figure 41. Expansion of $^{13}$C NMR spectrum of compound (14).

Figure 42. HMBC-NMR spectrum of compound (14).

Figure 43. HSQC-NMR spectrum of compound (14).
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