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SYNTHESIS , CHARACTERIZATION AND STUDY BIOLOGICAL ACTIVITY SOME NEW DERIVATIVES AMIDE AND PYRAZOLE –PYRAZOLINE OF STEROID ANALOGS .

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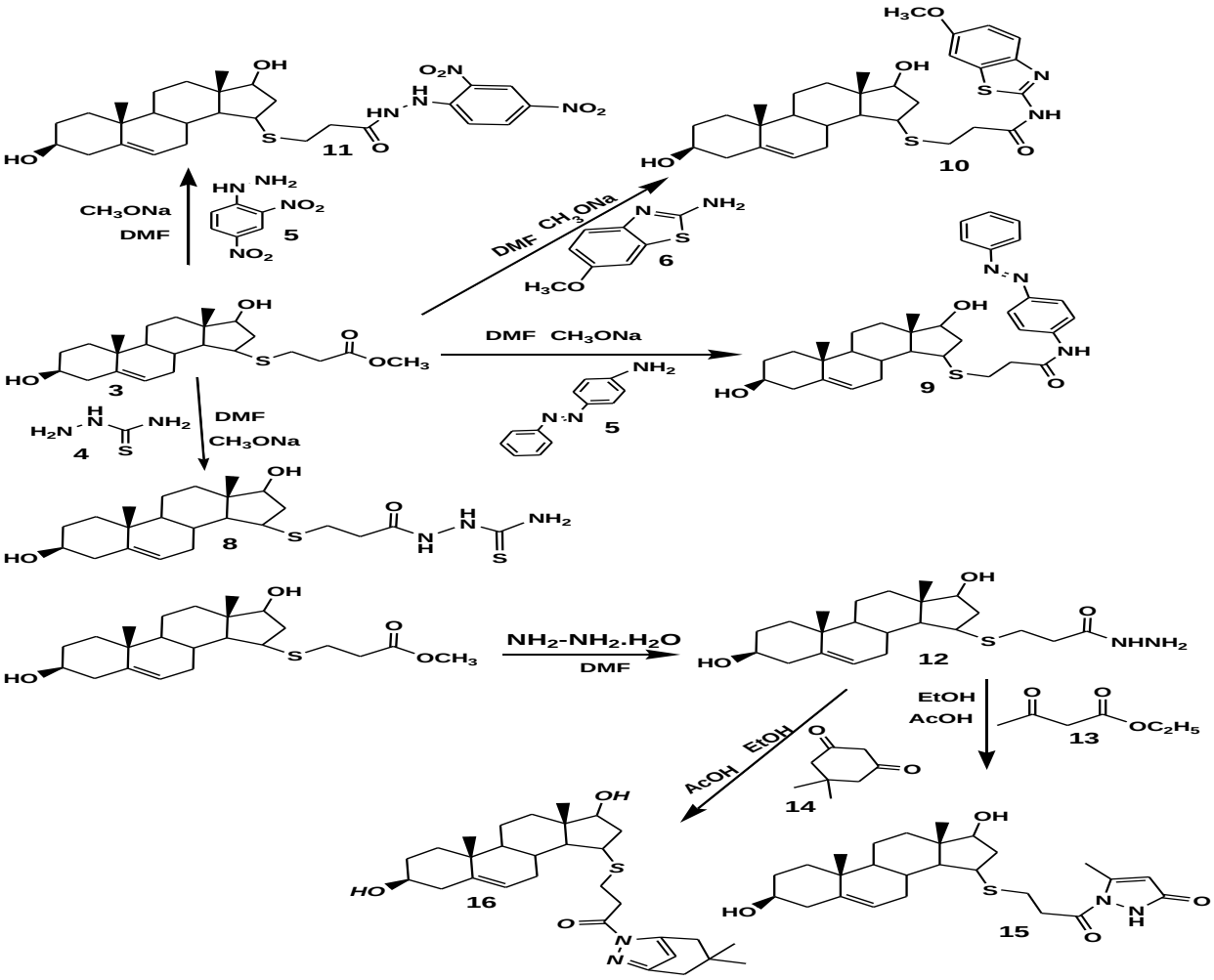
ABSTRACT

The number of steroids and their derivatives possess divers pharmacological activities as drugs for the treatment of a large number of diseases so in this work was suggested prepared two series amides and pyrazoles of steroid analogs (methyl((5-pregnant-3β,17-diol-15-yl) thio) propanoate) . Amides derivatives (6 - 9) prepared directionally after treated the steroid analogs with primary amine substituted in basic medium . Carbo hydrazide steroid derivative (10) was obtained by treated steroid analogs with hydrazine hydrate , this compound use as intermediate for synthesis Pyrazolin (13) as well as Pyrazole (14) .
All the newly synthesized compounds have been identified by IR, ^{1}H NMR, ^{13}CNMR, 2D-NMR (HMBC , HSQC) and C.H.N Analysis .



Scheme (1) synthesis amides and Pyrazole –Pyrazolin of Steroid analogs

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KEYWORDS

Steroids , Pyrazole , Amide , Biological activity .

INTRODUCTION

Steroids are fatty molecules derived from cholesterol, the general composition of which are characterized by four rings of hydrocarbons three hexagonal and the fourth five-ring called cyclopenta[a]phenanthrene [1-4] .

These molecules mediate different physiological activities in the human body [5,6] , including building bones and muscles, maintaining blood volume, reproduction and growth [7] , the high bioactivity of steroid molecules are due to the ability to penetrate the cell membrane and its association with specific hormonal receptors [8,9].

Steroids were used as a treatment for Addison's disease by German chemists after they discovered that the adrenal gland secrete steroid molecules[10,11] .

Nowadays, a large number of steroids and their derivatives are used as a treatment for many diseases, for example as anti-inflammatory[12] , anti-microbial [13], diuretic , brain tumors[14] , cardiovascular[15], autoimmune diseases , osteoarthritis , breast cancer [16] and prostate cancer by inhibition CYP17 enzyme[17,18] fig (1) .

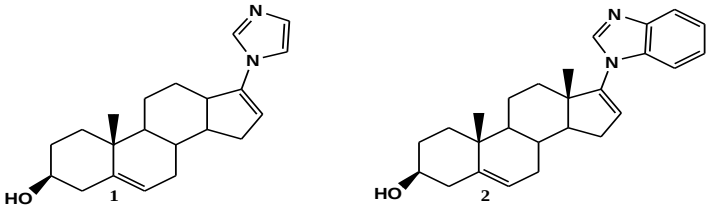

Figure. 1. CYP17 inhibitors

Most of the steroids currently used as a treatment are semi-prepared [19]) which prompted researchers to prepare new derivatives of steroids through a modification in the structure of the skeletonor by conducting an reaction on the side chain of molecules [20] and examined as anti-diseases[21] so in 2015,Al- Masoudi [22]and his group prepared new derivatives of aryl chalcone for pregnenolone by Suzuki reaction and tested as inhibitor for CYP17 , then in 2017 Fan, N. [23] was prepared new derivatives of benzylidene for progesterone and evaluated as anti-breast cancer in cell line MCF-7, as well in 2018 Shi, Y. K [24] and co-workers design and synthesis steroidal pyridine and evaluated it as anti-prostate cancer in cell line PC-3.

2- EXPERIMENTAL SECTION

2-1. Chemistry

The melting points were measured by capillary method ,The IR spectra were recorded on Shimadzu . the NMR (300 MHz) was recorded on Bruker DPX 400 spectrometer in DMSO-d6 using TMS as internal standard reference and chemical shifts are in d ppm , Elemental analyses were performed on Elementar Vario EL III, Carlo Erba 1108 and the chemicals substances required for the work were supplied from commercial sources.

2.2 Chemical Synthesis

2-2-1-General Procedure for synthesis aryl amide of methyl ((5-pregnen-3β,17-diol-15-yl) thio) propanoate .

A steroid analogue (100 mg, 0.24mol) in 25 ml DMF was treated with amine derivatives (0.36 mmol) and sodium methoxide (190 mg, 0.36 mmol) refluxed for (17-26) h. The end of the reaction was monitored by TLC (n-hexane-ethyl acetate) (4:1). After completion of the reaction, the mixture was cooled and added to ice water then extracted with DCM (3 x 20). The organic layer dried and concentrated under reduced pressure ,The product was purified by column chromatography to give the desired amide derivatives .

2-2-1-1- Synthesis ((5-pregnen-3β,17-diol-15-yl) thio) propanoyl)thiosemicarbazide . (6)

Yield: 95 mg (83%) . M.p:110-112 ^{0}C *R.f* = 0.29 , form : light yellow powder

IR (KBr) (cm^{-1}): 3440 (OH) , 3371 and 3301 (NH_2, str) , 2933 (CH-aliph) 1704 (C=O amide).

^{1}H NMR (301 MHz, DMSO) δ 7.27 (s,1H, NH amide) ,7.10 (s,1H, NH Thiamide) , 6.93 (s,2H, NH_2) , 5.29 (t ,1H, H6), 4.56, (br,s , 1H, OH-3) , 3.60 (br, s , 1H , OH-17) , 3.45 (m ,1H, H-17) , 3.26 (m , 1H, HC-3) ,3.08 (m, 2H, H-4) , 2.66 (m, 1H, HC-15) , 2.30 (t, 2H , H22) , 2.11(tr ,2H , H-21), 1.81 - 1.71 (t , H8+7+2) , 1.50 (m , 4H , HC-11+12) , 1.39 (t , 2H ,HC-16) , 1.25 (m , 2H ,HC-14) , 0.96 (t , H9+1) , 0.87 (s , 3H, Me-19) , 0.83 (s, 3H, Me-18) . **^{13}C NMR (76 MHz, DMSO)** δ 185.22 (C=S) , 173.45 (C=O amide), 141.62 (C-5) , 120.89 (C-6) , 80.47 (C-17 OH) , 70.48 (C-3 OH) , 54.40 (C-14) , 50.50 (C-9) , 43.72 (C-13), 42.72 (C-4) , 37.69(C-12) 37.49 (C-1), 36.75 (C-10), 35.11 (C-15) , 31.91 (C-8) , 31.30 (C -7), 31.16 (C2) , 29.48 (C-22), 28.47(C-21), 24.63 (C-16) , 20.52 (C-11), 19.54 (C-19), 14.18 (C-18). **Anal.calc.for** $C_{23}H_{37}N_3O_3S_2$ (467.69) C, 59.07; H, 7.97; N, 8.98; **found** C, 59.16; H, 7.92; N, 9.04 . from 33 mg thiosemacabazide.

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2-2-1-2-SynthesisN(E)-4-(2-phenyldiazenyl)benzenamine ((5-pregnen-3β,17-diol-15-yl) thio) propanamide (7) .

IR (KBr) (cm^{-1}): 3427 (OH) , 2929 and 2854 (CH-aliph) 1724 (C=O amide) , 1550 (N=N), 1600 and 1373 (C=C) .

Yield: 85 mg (62%) . M.p:180-181 ^{0}C *R.f* = 0.42 , form : red powder

^{1}H NMR (301 MHz, DMSO) δ 7.81-7.76 (dd , 2H, H3,5 arom A) , 7.51-7.56 (dd , 2H, H2,6 arom B) , 7.48-7.46 (t , 1H, H4 arom B) , 7.41-7.38 (dd , 2H, H3,5 arom B) , 7.31 (br ,s,1H, NH-amide) , 6.95-6.92 (dd , 2H, H2,6 arom A) , 5.26 (t ,1H, H6), 4.51 (br,s , 1H, OH-3) , 4.06 (s , 1H , OH- , 54.39 (C-14) , 50.50 (C-9) , 43.75 (C-13), 42.71 (C-4) , 38.01(C-12) , 37.50 (C-1), 36.73 (C-10), 35.13 (C-15) , 31.87 (C-8) , 31.15 (C-7+2) , 29.90 (C-21), 29.51 (C-22) , 28.49 (C16) , 20.39 (C-11) , 19.53 (C-19) , 14.17 (C-18).

Anal.calc.for $C_{34}H_{43}N_3O_3S$ (573.79) C, 71.17; H, 7.55; N, 7.32;**found** C, 71.23; H, 7.64; N, 7.39 from 71mg (E)-4-(2-phenyldiazenyl)benzenamine .

2-2-1-3-SynthesisN-(6-methoxybenzo[d]thiazol-2-yl) ((5-pregnen-3β,17-diol-15-yl) thio) propanamide (8) .

IR (KBr) (cm^{-1}): 3392 (OH) , 2935 and 2831 (CH-aliph) 1664 (C=O amide) , 1584 and 1469 (C=C) .

Yield: 90 mg (67%) . M.p:155-157 ^{0}C *R.f* = 0.38 , form : dark yellow powder

^{1}H NMR (301 MHz, DMSO) δ 7.88 (br ,s,1H, NH-amide) , 7.28-7.21 (, 3H, H-arom) , 5.26 (t ,1H, H6), 4.60 (br,s , 1H, OH-3) , 3.80 (s , 1H , OH-17) ,3.73 (s,3H,OMe) 3.60 (m ,1H, H-17) , 3.35 (m , 1H, HC-3 + H_2O) ,3.06 (m, 2H, H-4) , 2.66 (m, 1H, HC-15) , 2.29 (t, 2H , H22) , 2.12(tr ,2H , H-21), 1.78 - 1.65 (t , H8+7+2) , 1.52-1.47 (m , 4H , HC-11+12) , 1.28 (t , 2H ,HC-16) , 1.23 (m , 2H ,HC-14) , 1.10 (t , H9+1) , 0.94 (s , 3H, Me-19) , 0.80 (s, 3H, Me-18) .**^{13}C NMR (76 MHz, DMSO)** δ 172.40 (C=O amide) ,165.17 (C2-benzthiazole) 154.75 (C-OMe) , 147.32 (C3a+ 8a) , 141.64 (C-5) , 120.85 (C-6) , 118.53 (C4-arom) , 113.34 (C5-arom) ,106.01 (C7-arom) , 80.47 (C-17 OH) , 70.50 (C-3 OH) ,56.03 (OMe) ,54.39 (C-14) , 50.52 (C-9) , 43.62 (C-13), 42.72 (C-4) , 37.69(C-12) , 37.51 (C-1), 36.74 (C-10), 34.79 (C-15) , 31.91 (C-8+7) , 31.27 (C-2) , 29.83 (C-22), 28.32 (C-22) , 25.80 (C16) , 20.51 (C-11) , 19.54 (C-19) , 14.18 (C-18). **Anal.calc.for** $C_{30}H_{40}N_2O_4S_2$ (556.78) C, 64.72; H, 7.24; N, 5.03; **found** C, 64.63; H, 7.19; N, 4.97 from 65 mg 6-methoxybenzo[d]thiazol-2-amine.

2-2-1-4-Synthesis N'-(2,4-dinitrophenyl) ((5-pregnen-3β,17-diol-15-yl) thio) propanhydrazide (9) .

Yield: 80 mg (58%) . M.p:184-185 ^{0}C *R.f* = 0.35 , form : orange powder

IR (KBr) (cm^{-1}): 3367 (OH) , 2935 and 2904 (CH-aliph) , 1726 (C=O amide) , 1602 and 1411 (C=C,arom) , 3107 (CH-arom) , 1539 and 1301 (NO_2 group) .

^{1}H NMR (301 MHz, DMSO) δ 8.61 (s,1H,H3-arom) , 8.44-8.42 (d,1H,H5-arom) , 7.77 (br ,s,1H, NH-amide) , 7.75-7.72 (d,1H, H6-arom) , 7.20 (s,1H, NH-arom) , 5.03 (t ,1H, H6), 4.33 (br,s , 1H, OH-3) , 4.80 (s , 1H , OH-17) , 3.37 (m ,1H, H-17) , 3.20 (m , 1H, HC-3 + H_2O) ,3.02 (m, 2H, H-4) , 2.83 (m, 1H, HC-15) , 2.28 (t, 2H , H22) , 2.06(tr ,2H , H-21), 1.51 - 1.46 (t , H8+7+2) , 1.30-1.25 (m , 4H , HC-11+12) , 1.10 (t , 2H ,HC-16) , 1.00 (m , 2H ,HC-14) , 0.71 (t , H9+1) , 0.58 (s , 3H, Me-19) , 0.46 (s, 3H, Me-18) .**^{13}C NMR (76 MHz, DMSO)** δ 172.39 (C=O amide) 17) , 3.76 (m ,1H, H-17) , 3.45 (m , 1H, HC-3) ,3.06 (m, 2H, H-4) , 2.66 (m, 1H, HC-15) , 2.31 (t, 2H , H22) , 2.16(tr ,2H , H-21), 1.75 - 1.71 (t , H8+7+2) , 1.48 (m , 4H , HC-11+12) , 1.23(t , 2H ,HC-16) , 1.13 (m , 2H ,HC-14) , 1.05 (t , H9+1) , 0.95 (s , 3H, Me-19) , 0.85 (s, 3H, Me-18) .**^{13}C NMR (76 MHz, DMSO)** δ 173.44 (C=O amide) ,152.54 (C1-aromB + C4-aromA) , 145.20 (C1-aromA) , 146.49 (C2-arom) , 141.60 (C-5) , 130.43(C4-aromB) , 129.73 (C3,5-aromA) , 125.51 (C3,5-aromB) ,123.66 (C2,6-arom B) , 122.33 (C-6) , 116.65 (C2,6-aromA) , 80.47 (C-17OH), 70.50 (C-3OH) ,148.51 (C2+4-arom) , 142.04 (C1-arom) , 141.63 (C-5) ,132.10(C6-arm) , 129.83 (C5-arom) , 120.83 (C-6) , 119.02 (C3-arom) , 80.47 (C-17 OH) , 70.49 (C-3 OH) , 54.41 (C-14) , 50.53 (C-9) , 43.62 (C-13), 42.72 (C-4) , 37.70(C-12) , 37.52 (C-1), 36.74 (C-10), 34.79 (C-15) , 31.91 (C-8+7) , 31.27 (C-2) , 29.82 (C-22), 28.63 (C-22) , 28.32 (C16) , 20.52 (C-11) , 19.53 (C-19) , 14.16 (C-18).

Anal.calc.for $C_{28}H_{38}N_4O_7S$ (574.69) C, 58.52; H, 6.66; N, 9.75; **found** C, 58.55; H, 6.73; N, 9.78 from 71 mg 2,4-di-Nitro phenyl hydrazine.

2-2-2 - Synthesis ((5-pregnen-3β,17-diol-15-yl) thio) propane hydrazide. (10)

A mixture of methyl ((5-pregnen-3β,17-diol-15-yl) thio) propanoate 650 mg (1.59 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). (3:2) The product mixture was evaporated and allowed to cool. to afford compound The resulting solid was filtered, dried & recrystallized from ethanol to obtain it as a yellow powder 500 mg (76%) M.p:152-153 *R.f* = 0.32.

IR (KBr) (cm^{-1}): 3442 (OH) , 3421 and 3284 (NH_2, str) , 2964 and 2933 (CH-aliph) 1660 (C=O amide) , 1633 (NH, bend), 1633 (C=C) .

^{1}H NMR (301 MHz, DMSO) δ 9.06 (s,1H, NH amide), 6.88 (s,2H, NH_2) , 5.28 (t ,1H, H6), 4.53, (br,s , 1H, OH-3) , 3.72 (br, s , 1H , OH-17) , 3.44 (m ,1H, H-17) , 3.27 (m , 1H, HC-3) ,3.06 (m, 2H, Hb-4) , 2.64 (m, 1H, HC-15), 2.43 (tr ,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) , 1.24 (t , H9+1) , 0.96 (s , 3H, Me-19) , 0.82, (s, 3H, Me-18) . **^{13}C 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.calc.for** $C_{22}H_{36}N_2O_3S$ (408.6) C, 64.67: H, 8.88: N, 6.86: **found** C, 64.59: H, 8.80: N, 6.93

2-2 –3- General Procedure for synthesis Pyrazoline and Pyrazole of ((5-pregnen-3β,17-diol-15-yl) thio) propane hydrazide .

A solution of (0.24mmol) 1,3-di carbonyl compounds in (10 mL) ethanol and 1 ml AcOH stirred for 1h, then the solution of 100 mg (0.24mmol) ((5-pregnen-3β,17-diol-15-yl) thio) propane hydrazide) was added , the reaction mixture

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was refluxed for 7-9 h after complete the reaction by TLC using (n-hexane : ethyl acetate) (3 : 2) the reaction contents were cooled to r.t and the obtained product was filtered, dried and purified by recrystallization from ethanol **.**

2-2-3-1- Synthesis 1,2-dihydro-5-methylpyrazol-3-one ((5-pregnen-3β,17-diol-15-ylthio)propanoyl .

Yield: 92 mg (81%) . M.p:112-114 ^{0}C *R.f* = 0.35 , form : yellow powder

IR (KBr) (cm^{-1}): 3408 (OH) , 2933 and 2866 (CH-aliph) , 1708 (C=O lactam) , 1664 (C=O- amide) ,1631 (C=C , Pyrazoline).

^{1}H NMR (301 MHz, DMSO) δ 9.03 (s, 1H, NH-lactam) , 7.99 (s,1H-H4,Pyrazoline) , 5.28 (t ,1H, H6), 4.63 (br,s , 1H, OH-3), 3.54 (s , 1H , OH-17) , 3.44 (m ,1H, H-17), 3.27 (m , 1H, HC-3), 3.07 (m, 2H, H-4b), 2.65 (m, 1H, HC-15) , 2.31 (s,3H-Me5-pyrazole), 2.29 (tr ,2H , H-21), 2.14(t, 2H , H22), 2.10 (m, 2H, Ha-4) , 1.81- 1.68 (t , H8+7+2), 1.50-1.55 (m , 4H , HC-11 +12), 1.39 (t , 2H ,HC-16), 1.25 (m , 2H ,HC-14), 0.96 (t , H9+1) , 0.83 (s , 3H, Me-19) , 0.70 (

^{1}H NMR (301 MHz, DMSO) δ 9.03 (s, 1H, NH-lactam) , 7.99 (s,1H-H4,Pyrazoline) , 5.28 (t ,1H, H6), 4.64 (br,s , 1H, OH-3), 3.54 (s , 1H , OH-17) , 3.40 (m ,1H, H-17), 3.27 (m , 1H, HC-3), 3.10 (m, 2H, H-4b), 2.63 (m, 1H, HC-15) , 2.31(t, 2H , H22) , 2.21 (s, 2H,H8-dimendone) , 2.14 (s, 2H,H6- dimendone) , 2.01 (tr ,2H , H-21) , 2.10 (m, 2H, Ha-4) , 1.81- 1.67 (t , H8+7+2), 1.50 (m , 4H , HC-11 +12), 1.39 (t , 2H ,HC-16), 1.25 (m , 2H ,HC-14) ,1.00((s,6H-Me7- dimendone) 0.96 (t , H9+1) , 0.83 (s , 3H, Me-19) , 0.70 (s, 3H, Me-18) , **^{13}C NMR (76 MHz, DMSO)** δ 173.48 (C=O amide), 160.65 (C-3 , Pyrazole), 141.60 (C5-Pyrazole) ,140.81 (C-5) , 120.96 (C-6) , 112.77 (C4 – Pyrazole) , 80.49 (C17-OH) , 70.50 (C3-OH) , 54.38(C-14), 50.90 (C-9), 50.51 (C7- dimendone) , 42.72 (C-13), 42.12 (C-4), 37.70 (C-12) , 37.46 (C-1), 36.74(C-10), 35.09 (C-15), 34.32(C8- dimendone) , 32.97(C-8) , 31.89 (C-7), 29.83 (C-2), 29.60 (C-22), 28.33 (C-21),27.73(C7-s, 3H, Me-18) , **^{13}C NMR (76 MHz, DMSO)** δ 173.21 (C=O amide), 170.56 (C=O , lactam), 142.48 (C-5 +C5-Pyrazoline) , 121.09 (C4 – Pyrazoline) , 119.73 (C-6) , 80.49 (C17-OH) , 70.50 (C3-OH) , 54.42(C-14), 50.54 (C-9), 42.73 (C-13), 42.13 (C-4), 37.70 (C-12), 37.51 (C-1), 36.74(C-10), 35.10 (C-15), 31.90 (C-8) , 31.28 (C-7), 29.84 (C-2), 29.62 (C-22), 27.68 (C-21), 25.40 (C-16), 20.51 (C-11), 19.46 (C-19), 14.16 (C-18), 12.17 (C-5-Methyl- Pyrazoline) . **Anal.calc.for** $C_{26}H_{38}N_2O_4S$ (474.66) C, 65.79; H, 8.07; N, 5.90 ; **found** C, 65.85; H, 8.13; N, 5.87 from 0.03 ml Ethyl aceto acetate .

2-2-3-2 - Synthesis 1-pyrazol -1*H* -1-yl -[c,e]3-dimethyl cyclohexyl ((5-pregnen-3β,17-diol-15-ylthio)propanoyl .

Yield: 91mg (74%) . M.p:146-148 ^{0}C *R.f* = 0.44 , form : light yellow powder

IR (KBr) (cm^{-1}): 3425 (OH) , 2931(CH-aliph) , 1697 (C=O amide) , 1620 (C=C) , 1666 (C=N group)

dimendone) , 25.44 (C-16), 20.51 (C-11), 19.46 (C-19), 14.20 (C-18), 12.20 (2Me, C7- dimendone) from 34 mg dimendone . .

Anal.calc.for $C_{30}H_{44}N_2O_3S$ (512.75) C, 70.27; H, 8.65; N, 5.46;**found** C, 70.18; H, 8.78; N, 5.39 .

2-3- Biological methods

We conduct the evaluation experiments for antibacterial activities of compounds against ***E-coli*** , ***Klebsiella*** , ***Salmonella*** and ***Proteus*** bacteria by agar well diffusion method[25] , in this method was prepared culture medium from Muller Hinton agar Poured in petri dishes , after that, well in the center of the culture medium using the pliers and leaved some time for solid , 1 ml of 20 µM from the prepared compounds was added in to the well all medium were placed on the incubated at 37 ^{0}C for 24 h .

3- RESULTS AND DISCUSSION

3-1- Chemical synthesis

New amide derivatives of steroid synthesized by the reaction of steroid analog with primary amine substituted (Thiosemacarbazide, 4-(2-phenyldiazenyl) benzenamine, 2-Amino-6-methoxy benzothiazole and 2,4-Dinitro phenyl hydrazine) in presence sodium methoxide as a catalyst such as in the followed scheme :

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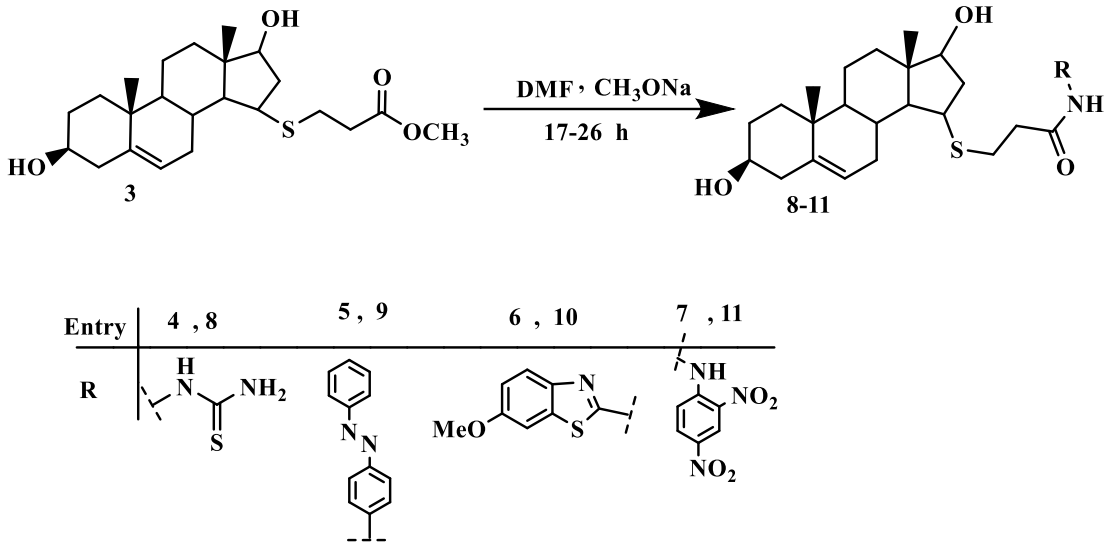


Scheme 1 : Synthesis of amide derivatives

All of the prepared compounds were identified by ^{1}H-NMR &^{13}C-NMR and some of the characterized by 2D-NMR (HSQC , HMBC) .

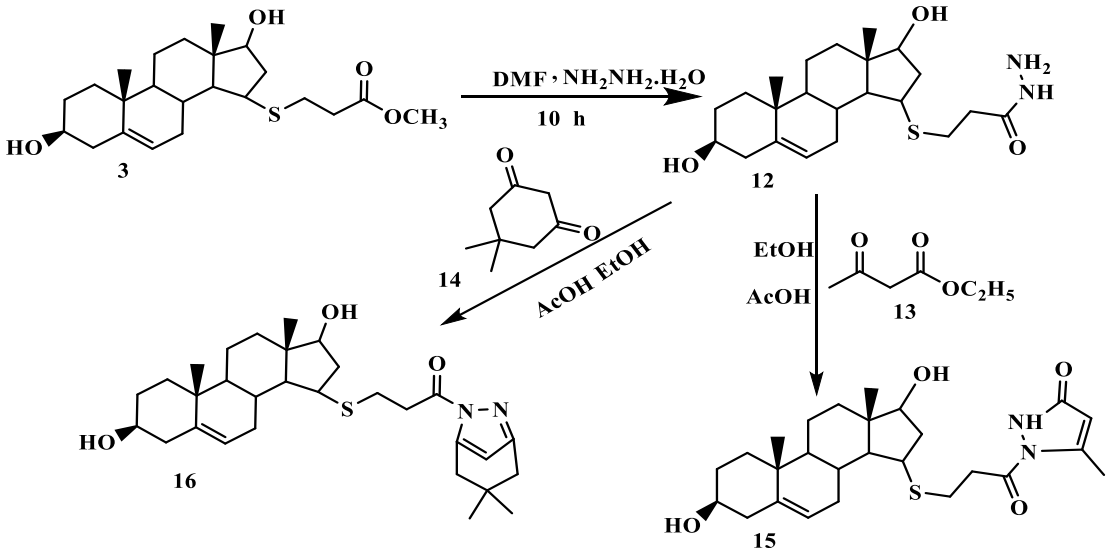
^{1}H-NMR spectrum of amide derivatives show disappear the band of methoxy ester and appear new bands of amide groups and aromatic rings at chemical shift 7.27 , 7.31 , 7.88 and 7.77 ppm for (8-11) respectively , the proton thiamide group of (8) appear at 7.10 ppm and NH_2 at 6.93 ppm .

The protons of aromatic ring appear at range 7.81- 6.92 ppm for nine protons of derivative (9) as shown in Fig. (12) , while the three protons of aromatic ring for derivative (10) appear at chemical shift 7.28 – 6.79 ppm also the three protons of methoxhy group belonging to the same compound appear at 3.80 ppm , finally the three aromatic protons of derivative (11) appear at chemical shift 8.61 – 7.72 ppm the high deshieldinh of H-3 due to the high withdrawing effect of two nitro groups , the proton of amine group of the same compound appear at chemical shift 7.20 ppm ,

The ^{13}C-NMR spectrum for carbonyl group of amide compounds (8-11) it was at range 173.45 , 173.44 , 172.40 , 172.39 ppm respectively , the carbon of thiamide group of derivative (8) appear at chemical shift 185.22 ppm .

Carbon atom of aromatic ring appear at range chemical shift 152.54 – 116.64 ppm for the derivaeive (9) , while the derivaeive (10) appear at chemical shift 165.17 -106.01 ppm as for the methoxy group of the same compound appear at 56.03 ppm , finally the carbon atoms of derivative (11) appear at range of chemical shift 148.51 – 119 .02 ppm .

pyrazoline and pyrazole derivatives synthesized after obtained steroid 12 and reaction it with ethyl aceto acetet and acetyl aceton in presence acetic acid as a catalyst to obtained 15-16 such as in followed scheme :



Scheme 2 : Synthesis of Pyrazolin– Pyrazole derivatives

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^{1}H-NMR spectrum of Pyrazolin **(15)** show two singlet bands at area of down field one back to proton of lactam group at chemical shift 9.03 ppm and other at chemical shift 7.99 ppm to the H-4 of Pyrazolin , while the methyl group at C-5 of the same compound at up field at chemical shift 2.31 ppm .

^{1}H-NMR spectrum of H-4 for Pyrazole **(16)** appear at same chemical shift of derivative **(15)** 7.99 ppm this has been due to the effect of stereo chemistry , at the same time methylene groups of derivative **(16)** appear at chemical shift 2.21 ppm for H-8 and 2.14 for H-6 , while the two methyl groups appear as singlet band at chemical shift 0.96 ppm .

^{13}C-NMR spectrum for carbonyl group of Pyrazolin–Pyrazole derivatives **(15 , 16)** appear at chemical shift 173.21 and 173.48 respectively also the C-3 for two derivatives appear at 170.56 and 160.65 respectively , the C-5 and C-4 for same compounds appear at chemical shift 141.60ppm , (C-5) and 119.73 , 112.77 ppm respectively .

Methyl group at C-5 for compound **(15)** appear at chemical shift 12.17 ppm , while the compound **(16)** show four bands at area of up field one at chemical shift 50.51 for C-7 , 34.32 ppm for C-8 , 25.44 for C-6 and 12.20 for two methyl groups at C-7 .

the other atoms of proton and carbon to the back bone of steroid of all compounds identified as in experimental section .

Among the prepared compounds has been choice the derivative **(11)** for studying the 2D-NMR (HMBC) where they showed correlation between carbonyl group and H-22 from type 1,2 , also the C-4 arom of phenyl moiety showed three correlation two with H3,5-arom from type 1,2 and one with H-6 from 1,3 , finally correlation between C-3 and H-5 from 1,3 as shown in fig. (2) .

Furthermore the HSQC NMR spectra was used to determine protons and carbons to the derivatives **(8)** and **(11)** .

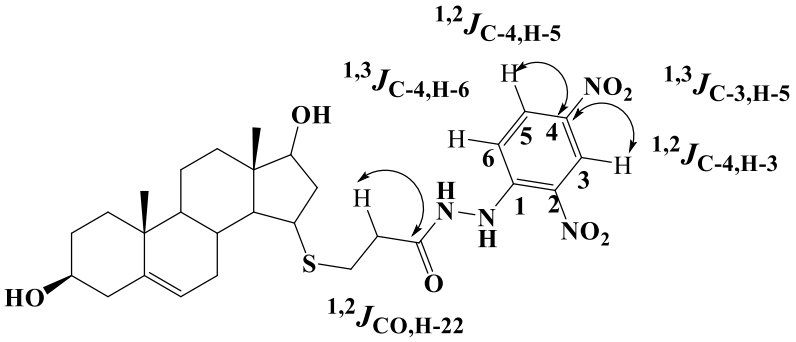

figure (2) HMBC correlation of Compound (11)

3-2- Biological Study

By using Agar well diffusion method the prepared compounds evaluated as anti-bacterial against ***E-Coli*** , ***Protuse*** , ***Klepsilla*** **and** ***Salmonella*** **,** the compound **(12)** do not effect on the all bacterial , whereas the derivative **(16)** have high inhibition activity against ***E-Coli*** **and** ***Protuse*** and mid toward ***Klepsilla*** and ***Salmonella*** the bacterial ***Klepsilla*** and ***Salmonella*** appear resistance toward compound **(15)** and the same derivative appear mid inhibition percent against ***E-Coli*** and ***Protuse*** .

In addition the bacteria ***E-Coli*** appear resistance all amide compounds **(8-11)** , but the bacteria ***Protuse*** appear mid inhibition activity by **(8)** and resistance toward **(9-11) ,** while the bacteria ***Klepsilla*** appear resistance toward **(8-10)** and high inhibition activity with **(11)** , finally the bacteria ***Salmonella*** mid inhibition percent toward **(9,10)** and resistance toward **(8 ,11)** , all results summaries in the table **(3-1)** .

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Table (3-1) percent inhibition of the prepared compounds

NO.compounds	*E-Coli*	*Protuse*	*Klepsilla*	*Salmonella*
8	R	+	+	R
9	R	R	+	+
10	R	R	+	+
11	R	R	++	R
12	R	R	R	R
15	+	R	+	R
16	++	++	+	+

CONCLUSION

From the data obtained of the biological activity can improved the steroid bearing pyrazole cycle **(16)** and evaluation in vitro against prostate and breast carcinoma by cell lines .

ACKNOWLEDGEMENTS

Thank you for Ph.D student lugain in the environmental research Centre , College of Sciences, University of Qadisiyah for help me to conduct biological activity test against anti-bacterial .

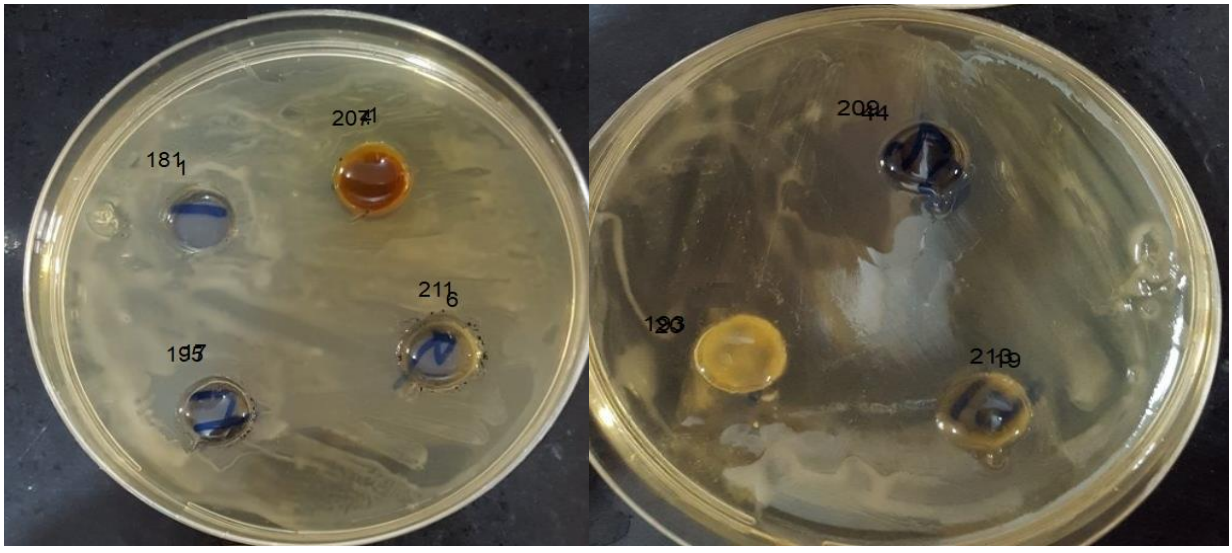

Figure . (3) effect of the prepared compounds against bacteria *Protuse*

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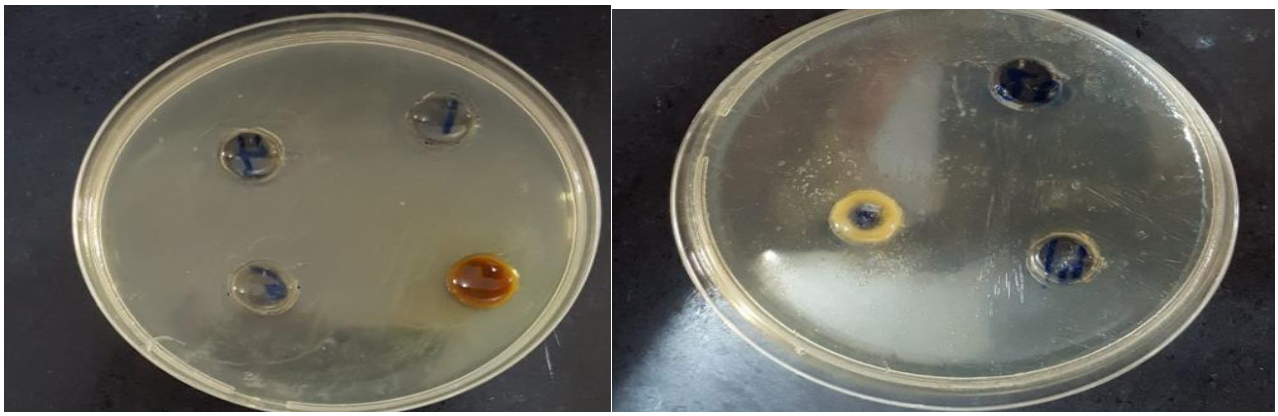

Figure. (4) effect of the prepared compounds against bacteria *Salmonella*

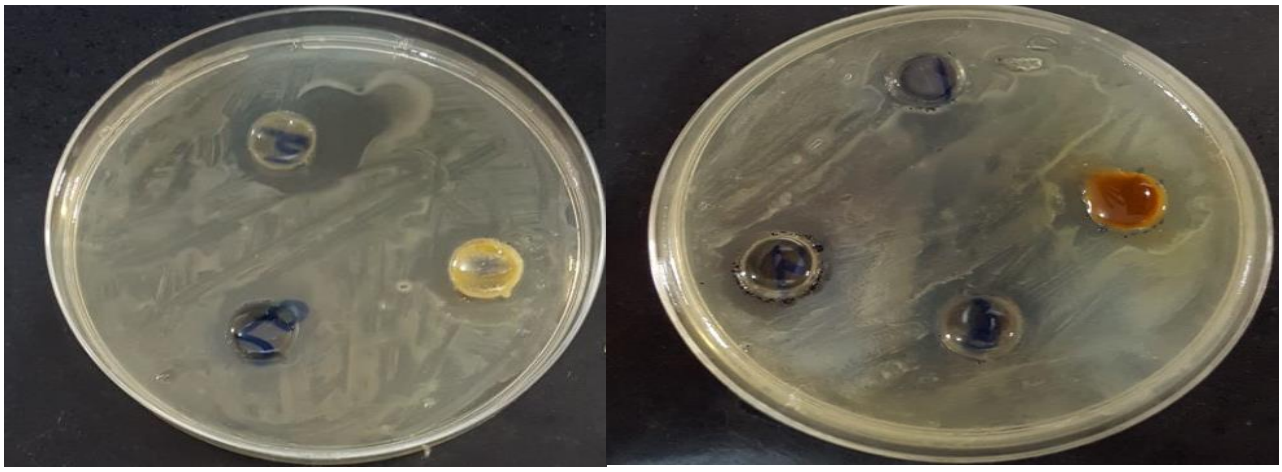

Figure. (5) effect of the prepared compounds against bacteria Klepsi

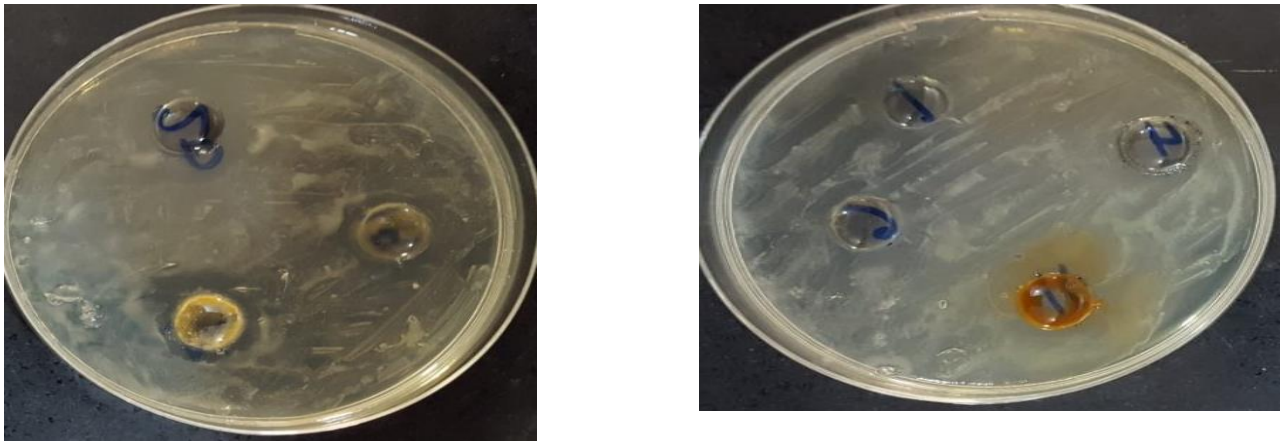

Figure. (6) effect of the prepared compounds against bacteria *E-Coli*

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Appendix

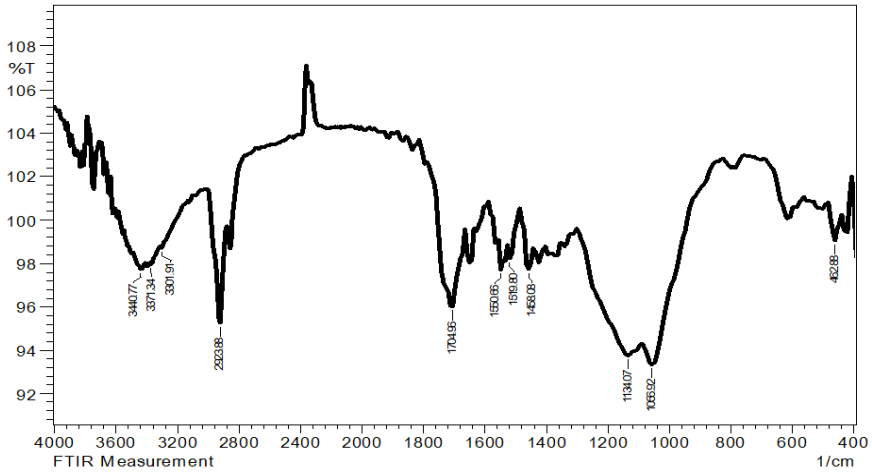

Figure. (7) FT-IR of derivatives (8)

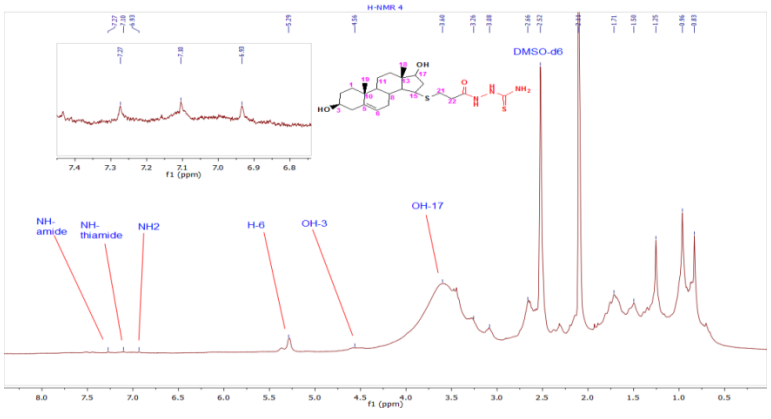

Figure. (8) ^{1}H-NMR of derivatives (8)

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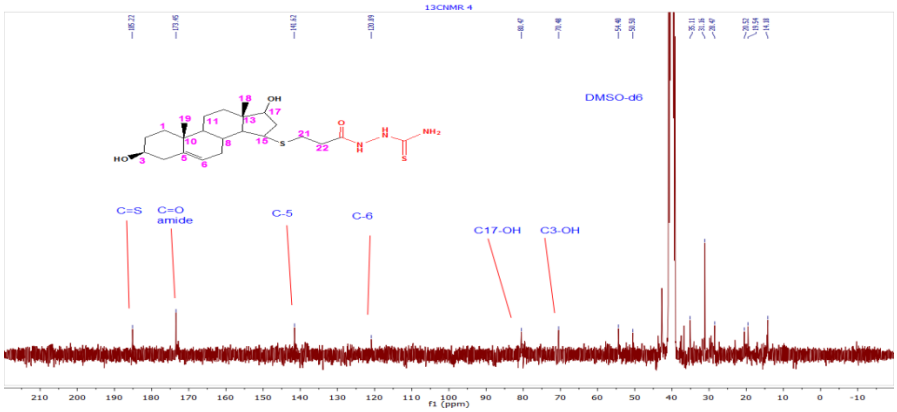

Figure. (9) ^{13} C-NMR of derivatives (8)

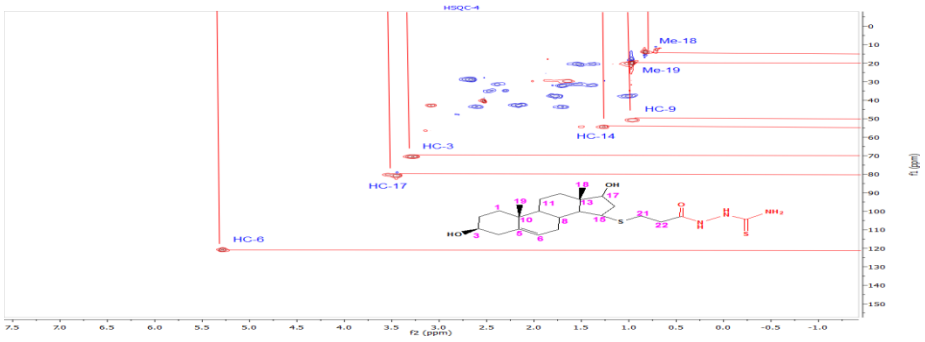

Figure. (10) HSQC of derivatives (8)

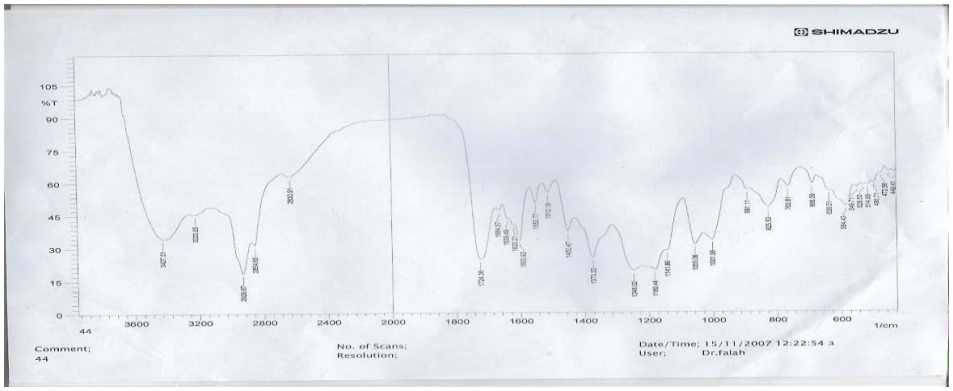

Figure. (11) FT-IR of derivatives (9)

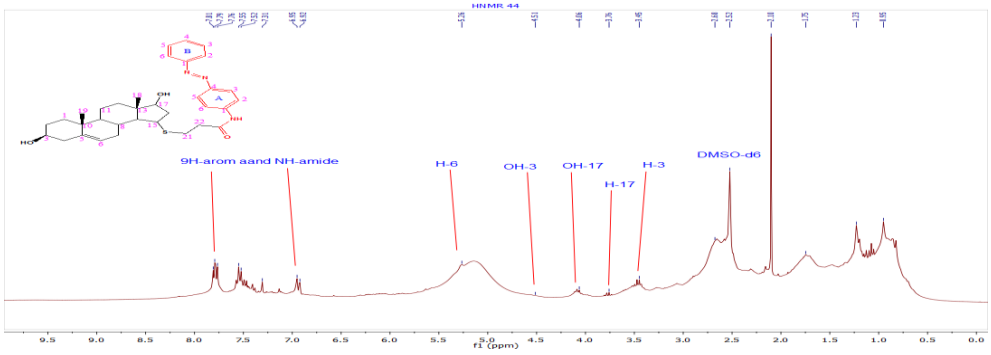

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Figure. (12) ^{1}H-NMR of derivatives (9)

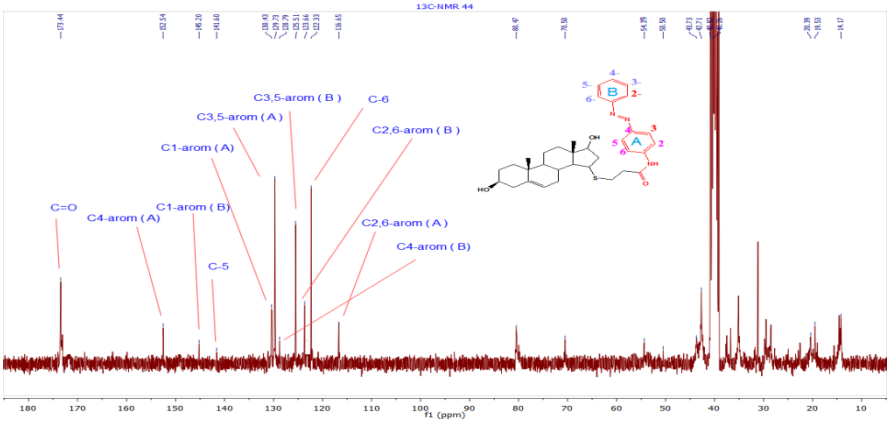

Figure. (13) ^{13} C-NMR of derivatives (9)

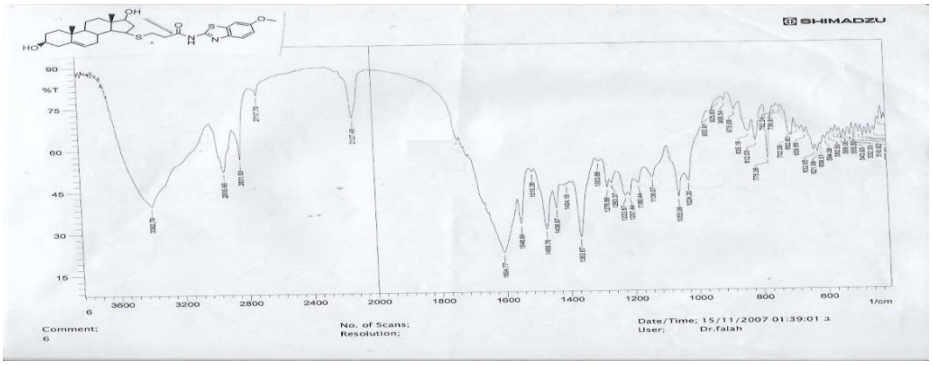

Figure. (14) FT-IR of derivatives (10)

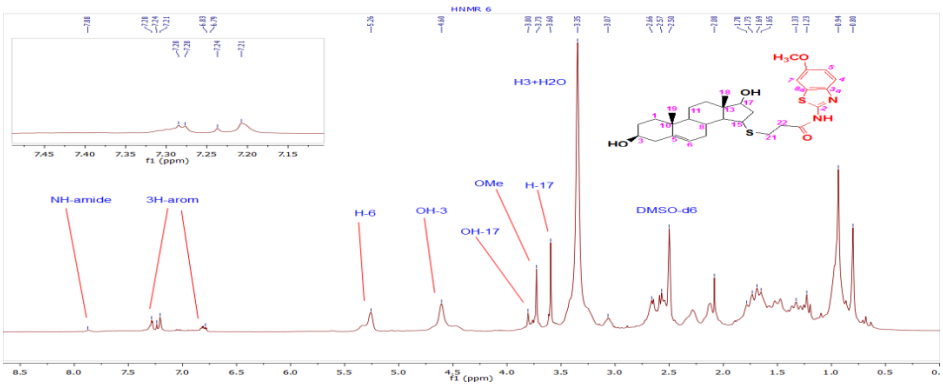

Figure. (15) ^{1}H-NMR of derivatives (10)

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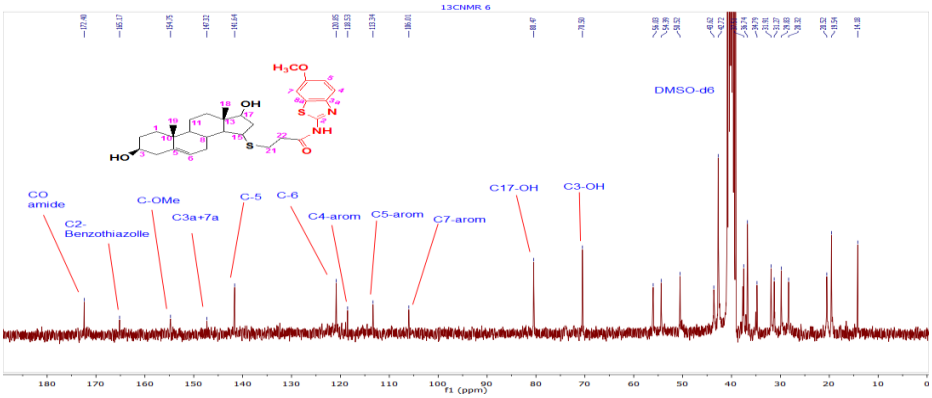

Figure. (16) ^{13} C-NMR of derivatives (10)

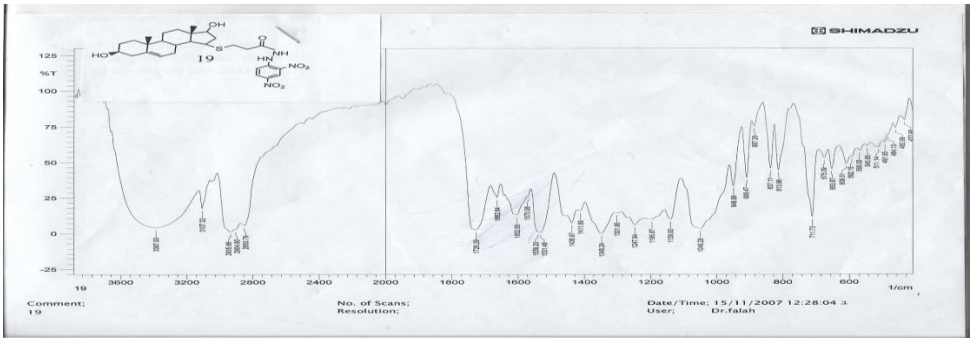

Figure. (17) FT-IR of derivatives (11)

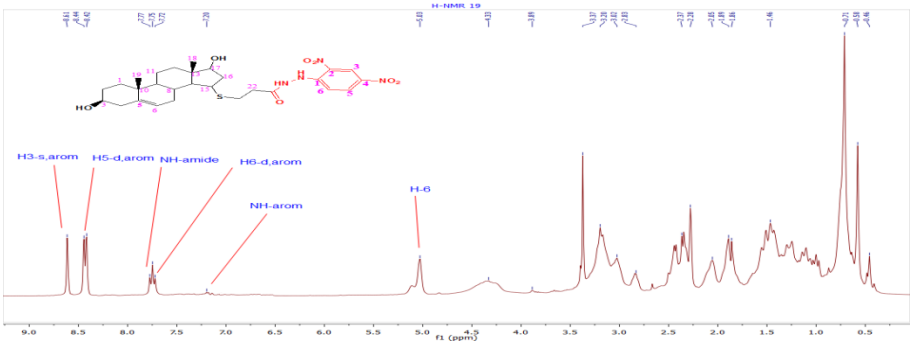

Figure. (18) ^{1}H-NMR of derivatives (11)

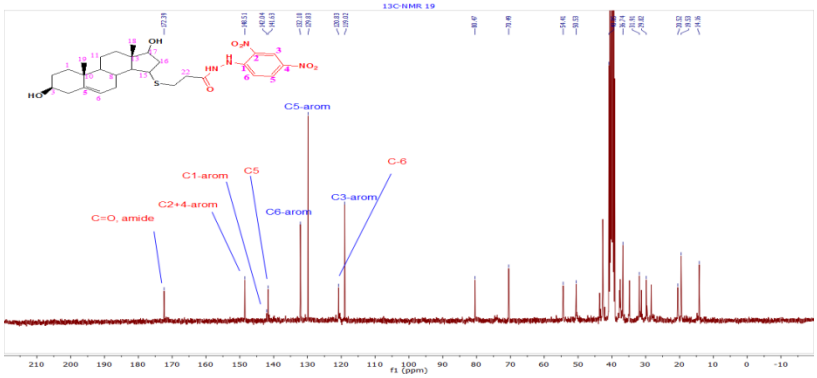

Figure. (19) ^{13} C-NMR of derivatives (11)

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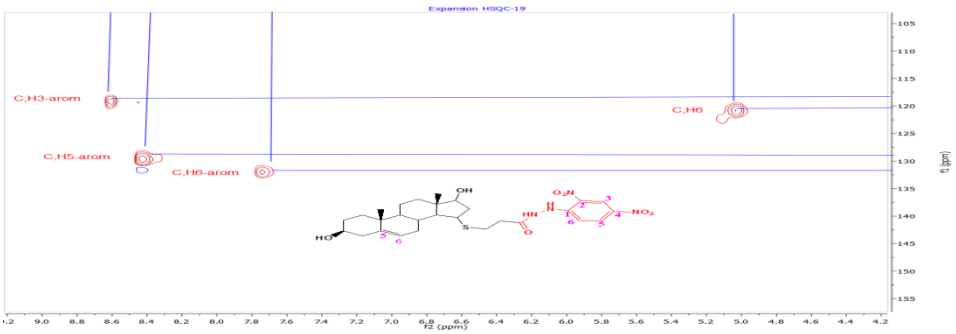

Figure. (20) Expansion of aromatic region HSQC of derivatives (11)

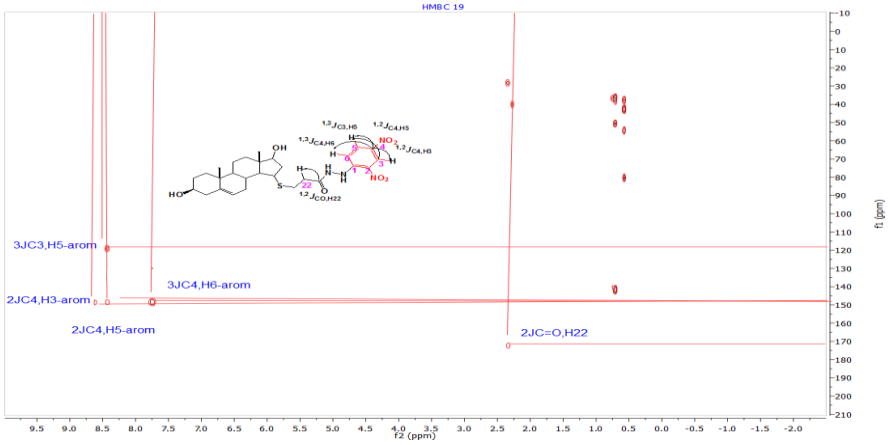

Figure. (21) HMBC of derivative (11)

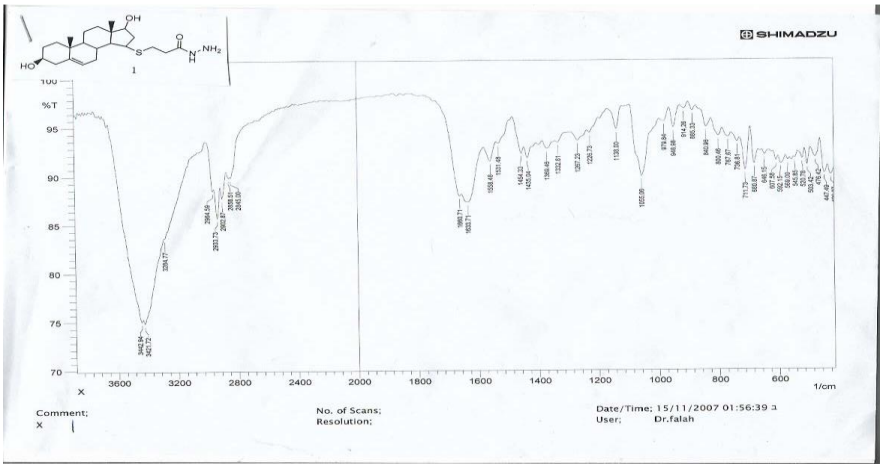

Figure. (22) FT-IR of derivatives (12)

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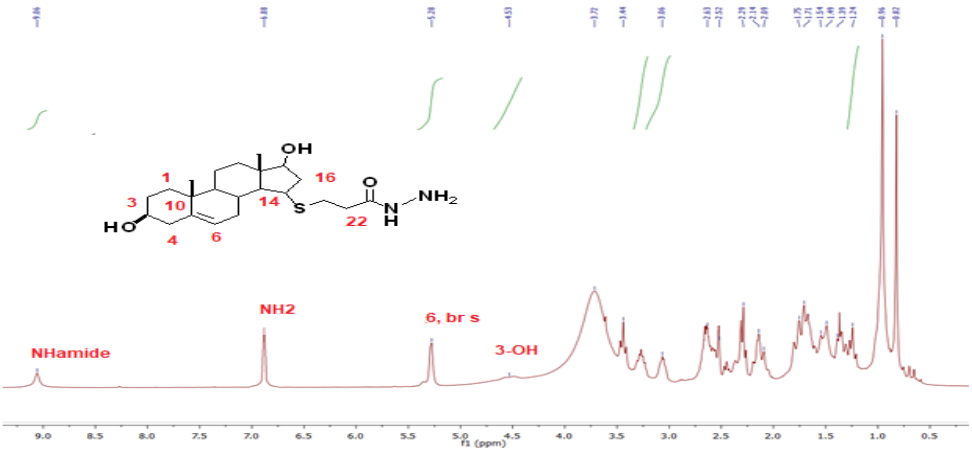

Figure. (23) ^{1}H-NMR of derivatives (12)

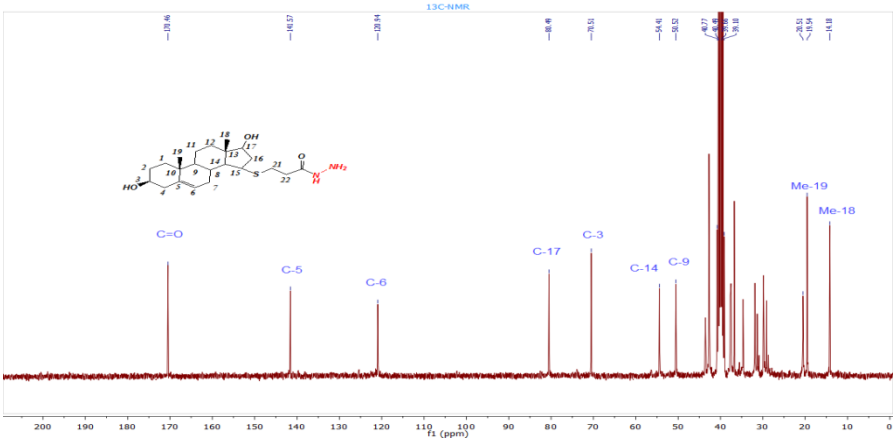

Figure. (24) ^{13} C-NMR of derivatives (12)

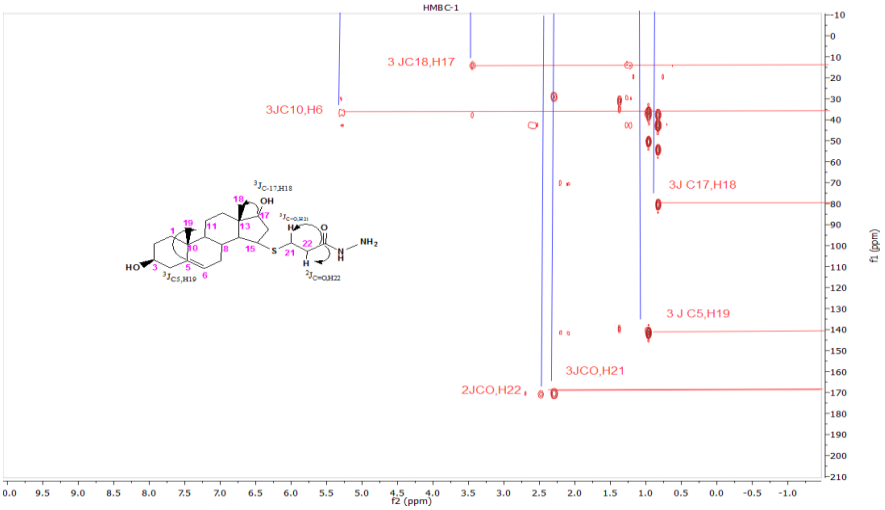

Figure. (25) HMBC of derivative (12)

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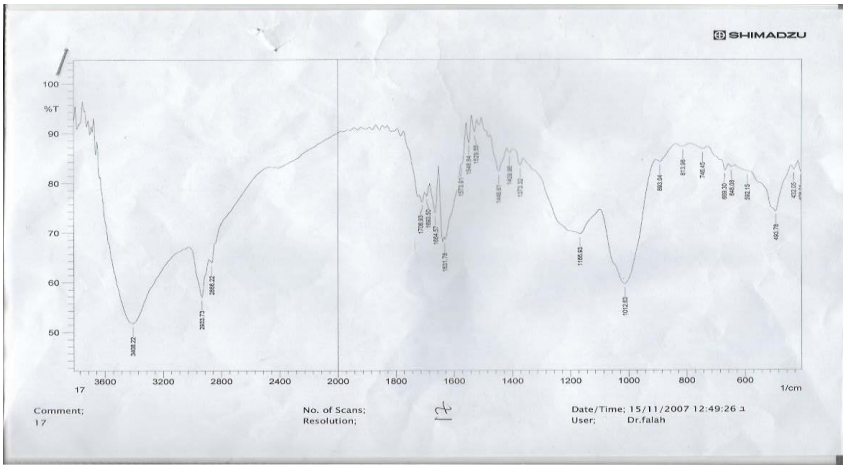

Figure. (26) FT-IR of derivatives (15)

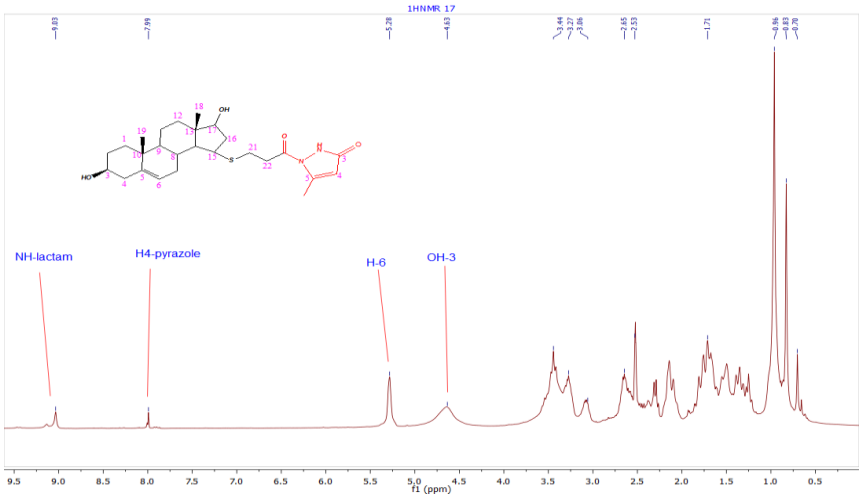

Figure. (27) ^{1}H-NMR of derivatives (15)

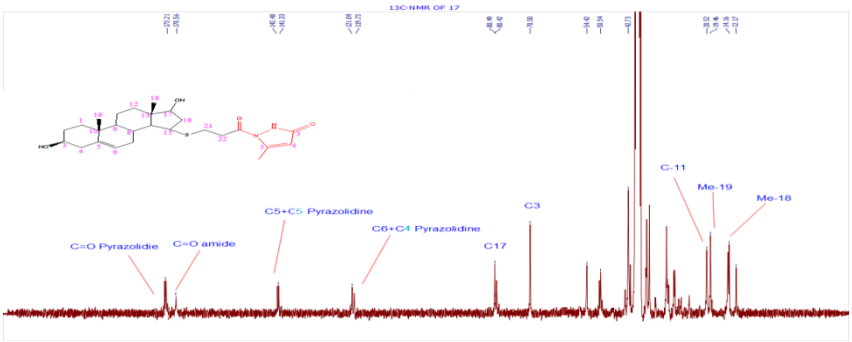

Figure. (28) ^{13} C-NMR of derivatives (15)

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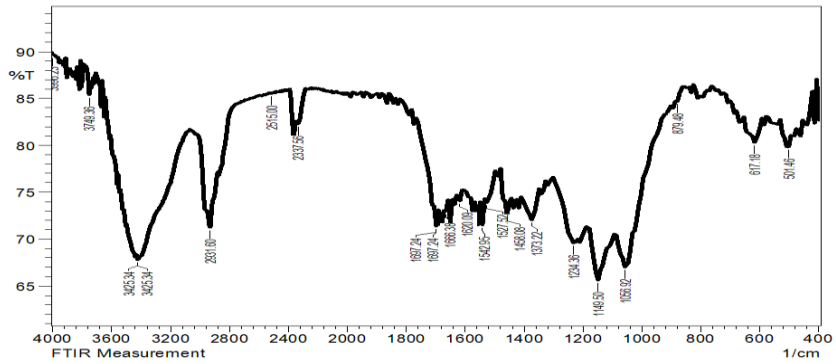

Figure. (29) FT-IR of derivatives (16)

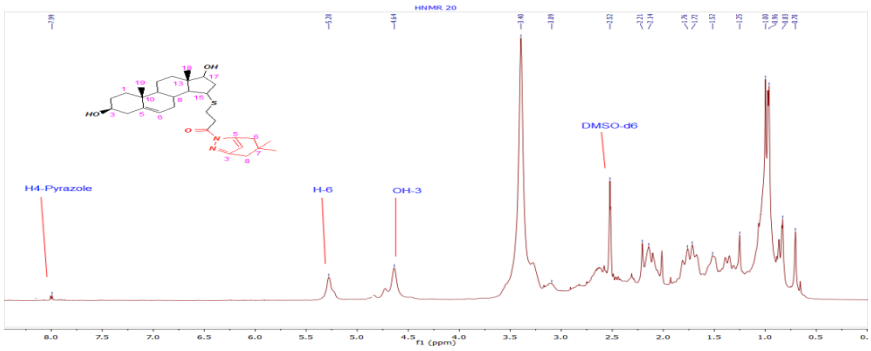

Figure. (30) ^{1}H-NMR of derivatives (16)

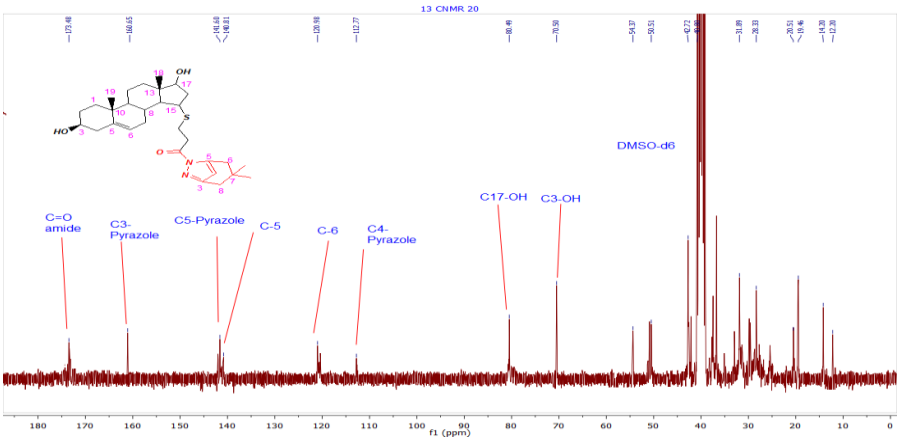

Figure. (31) ^{13} C-NMR of derivatives (16)