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-3 β ,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, ¹H NMR, ¹³CNMR, 2D-NMR (HMBC , HSQC) and C.H.N Analysis .

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

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 skeleton by conducting a 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 δ 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-3 β ,17-diol-15-yl) thio) propanoate .

A steroid analogue (100 mg, 0.24mmol) 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-3 β ,17-diol-15-yl) thio) propanoyl)thiosemicarbazide . (6)

Yield: 95 mg (83%) . **M.p:**110-112 °C **R_f** = 0.29 , **form** : light yellow powder

IR (KBr) (cm⁻¹): 3440 (OH), 3371 and 3301 (NH₂, str), 2933 (CH-aliph) 1704 (C=O amide).

¹H NMR (301 MHz, DMSO) δ 7.27 (s, 1H, NH amide), 7.10 (s, 1H, NH Thiamide), 6.93 (s, 2H, NH₂), 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). **¹³C 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 (C-2), 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** C₂₃H₃₇N₃O₃S₂ (467.69) C, 59.07; H, 7.97; N, 8.98; **found** C, 59.16; H, 7.92; N, 9.04. from 33 mg thiosemicarbazide.

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 24, No. 3, pp. 37–53, Year2019

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 °C R_f= 0.42 , form : red powder

¹H NMR (301 MHz, DMSO) δ 7.81-7.76 (dd , 2H, H_{3,5} arom A), 7.51-7.56 (dd , 2H, H_{2,6} arom B), 7.48-7.46 (t , 1H, H₄ arom B), 7.41-7.38 (dd , 2H, H_{3,5} arom B), 7.31 (br , s, 1H, NH-amide), 6.95-6.92 (dd , 2H, H_{2,6} 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 C₃₄H₄₃N₃O₃S (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 °C R_f= 0.38 , form : dark yellow powder

¹H 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 + H₂O), 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). **¹³C 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** C₃₀H₄₀N₂O₄S₂ (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 °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 (NO₂ group) .

¹H 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 + H₂O), 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). **¹³C 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). **¹³C 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-aromB), 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 C₂₈H₃₈N₄O₇S (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 (NH₂, str), 2964 and 2933 (CH-aliph) 1660 (C=O amide), 1633 (NH, bend), 1633 (C=C).

¹H NMR (301 MHz, DMSO) δ 9.06 (s, 1H, NH amide), 6.88 (s, 2H, NH₂), 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, H₄), 2.64 (m , 1H, HC-15), 2.43 (tr , 2H , H-21), 2.29 (t , 2H , H₂₂), 2.09 (m , 2H, H_{a-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). **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.calc.for** C₂₂H₃₆N₂O₃S (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

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 24, No. 3, pp. 37–53, Year2019

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-3 β ,17-diol-15-ylthio)propanoyl .

Yield: 92 mg (81%) . M.p:112-114 °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) .

¹H 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 (**¹H 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) , **¹³C 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) , **¹³C 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** C₂₆H₃₈N₂O₄S (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 -1H -1-yl -[c,e]3-dimethyl cyclohexyl ((5-pregnen-3 β ,17-diol-15-ylthio)propanoyl .

Yield: 91mg (74%) . M.p:146-148 °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 C₃₀H₄₄N₂O₃S (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 μ M from the prepared compounds was added in to the well all medium were placed on the incubated at 37 °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 (Thiosemicarbazide, 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 :

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 24, No. 3, pp. 37–53, Year2019

Scheme 1 : Synthesis of amide derivatives

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

$^1\text{H-NMR}$ 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 methoxy 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 $^{13}\text{C-NMR}$ 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 derivate (9), while the derivate (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 acetone in presence acetic acid as a catalyst to obtained 15-16 such as in followed scheme :

Scheme 2 : Synthesis of Pyrazolin- Pyrazole derivatives

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 24, No. 3, pp. 37–53, Year2019

^1H -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 .

^1H -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**) .

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

NO.compounds	<i>E-Coli</i>	<i>Protuse</i>	<i>Klepsilla</i>	<i>Salmonella</i>
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 .

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

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) ¹H-NMR of derivatives (8)

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 24, No. 3, pp. 37–53, Year2019

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

Figure. (10) HSQC of derivatives (8)

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

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

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

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

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

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

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

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

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

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

Figure. (21) HMBC of derivative (11)

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

Al-Qadisiyah Journal Of Pure Science (QJPS)

Vol. 24, No. 3, pp. 37–53, Year2019

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

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

Figure. (25) HMBC of derivative (12)

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

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

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

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

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

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