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Glucose conjugation of coumarin-pyrazoline derivatives as a promising strategy for cancer cell targeting

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ABSTRACT

Two novel series of 3-methyl-4-oxo-7-hydroxy- benzopyranyl [4,3-c][1-*H*] pyrazoline derivatives and their O-glycosylated derivatives at the 7th position of coumarin in the second series were synthesized. The first series compounds were prepared according to knoevenagel reaction by condensing of 2,4-dihydroxybenzaldehyde with ethyl acetoacetate to produce 3-acetyl-7-hydroxycoumarin which reacts with hydrazine hydrate and its four derivatives to produce the final products. The second series compounds were obtained through fusion reaction of glucose pentaacetate with the first series products in the presence of ferric chloride as Lewis acid to yield the O-glycosylated products. The chemical structures of the synthesized compounds were established by analyzing their FTIR, ^{1}H-NMR and ^{13}C-NMR spectra. The two series compounds were bio-assayed to examine theircytotoxic effect against two cancer cell lines, which are MCF-7 and SKG.The O-glycosylation of the synthesized compounds at position 7 of coumarin nucleus resulted in a much lower IC_{50} values than non-glycosylatedones. The best cytotoxic activity among the O-glycosylated compounds attributed to compound IVc with IC_{50} 2.47 µg/mlagainst MCF-7 cancer cell line. The results may indicate the importance of glucose conjugation as a promising strategy for targeting and treating of cancer.

Keywords: cancer, coumarin, glycosylation, pyrazoline, targeting.

HOW TO CITE THIS ARTICLE

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1. INTRODUCTION

Coumarins are important heterocyclic ring system bearing benzopyran-2-one moiety that isolated naturally from plants, and also highly synthesized. They carry much attention from scientists due to their various pharmacological activities as anti-inflammatory, anti-tuberculosis [1], anticoagulant, antibacterial, and anticancer [2].

Pyrazoline, a five member ring of three carbons and two adjacent nitrogen atoms, and its derivatives are widely distributed in nature. They possess various pharmacological properties such as analgesic, antipyretic, anti-diabetic, antidepressant, antimicrobial, and anticancer activities [3][4].

In many studies, a diversity of coumarin-pyrazoline compounds have been synthesized and showed high anti-proliferative activity against a wide range of tumor cell lines. For instance, twoseries of coumarin-pyrazoline hybrids were prepared and tested against 60 cell lines. The results showed a high cytotoxic activity against the tested cell lines especially toward colorectal cell line HCT-116 with an $IC_{50}$of 0.01-2.8µM[5]. In1927,the scientist Oto Warburg reported that the cancerous tissue of diverse origin exhibit a marked demand of glucose and a high rate of anaerobic glycolysis

which was known as Warburg phenomena, hence, insulin independent glucose transporter GLUT-1 and glycolytic enzymes are widely up regulated in a high percentage of human cancers [6][7][8].

In the early of 1990s, the idea of monosaccharide-conjugated analogues of a variety of agents was established. These conjugates showed an improved water solubility, serum stability, and best targeting of their aglycons. For instance, the mannose and glucose phosphotriester conjugates of zidovudine, an anti-retroviral nucleotide analogue, showed an improved antiviral efficacy and enhanced targeting to the CNS[9].Another important example is the glucose conjugate of the anticancer drug ifosfamidethat known as glufosfamide which is developed in an effort to reduce the toxic effects and increase selectivity of its aglycon[10].

Based on the previously mentioned facts, this study aimed to synthesize two series of compounds, the first one consists of five derivatives of 3-methyl-4-oxo-7-hydroxy-benzopyranyl[4,3-c][1-*H*] pyrazoline while the second series consists of their glucose conjugates at position 7 of coumarin nucleus. The*in vitro* cytotoxicity study of the synthesized compounds was studied on two cancer cell lines, which are MCF-7 breast cancer and SKG esophageal cancer due to their high prevalence in Iraq. This study was conducted to test the cytotoxic effect of the synthesized compounds and accordingly to find the influence of glycosylation on their cytotoxic activity.

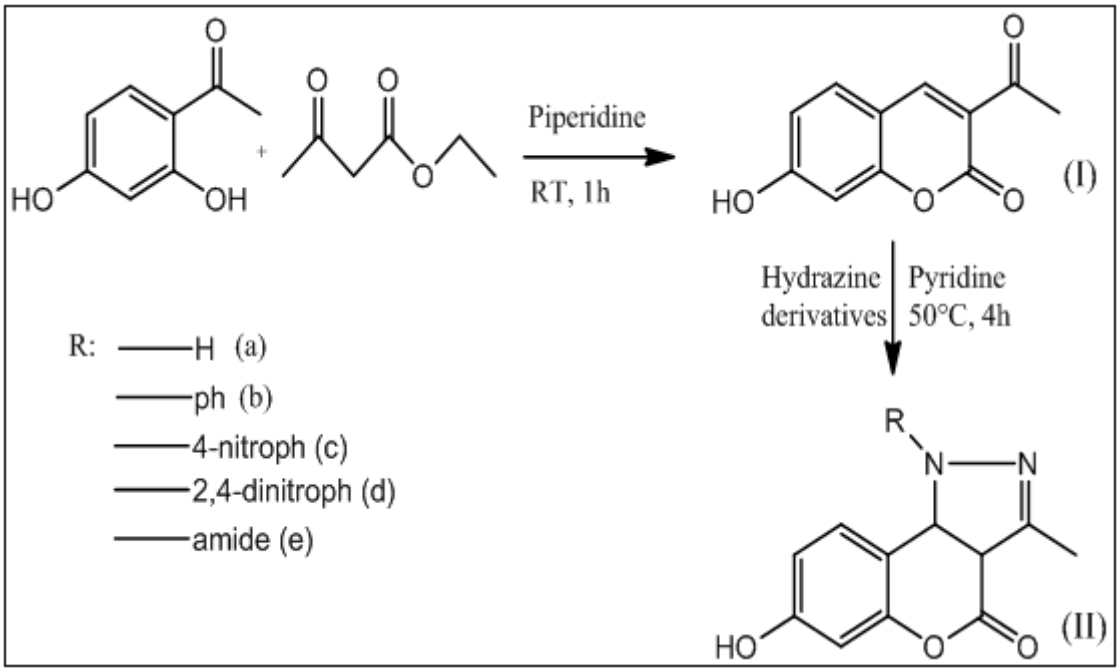
2. MATERIALS AND METHODS

All the starting materials for the synthesis of the two series compounds were purchased from Sigma Aldrich and used without purification.IR spectra of the synthesized products were detectedon Bruker Alpha FTIR-ATR Spectrophotometer (Germany) while their NMR spectra were scanned on Bruker (400MHz) spectrophotometer (Germany) in DMSO-d_6 using tetramethylsilane as an internal standard. Melting points were determined on an electrochemical CIA 9300 melting point apparatus (UK) using open capillary method. Elemental microanalysis was performed on Perkin-Elmer 2400 CHN analyzer (USA) and results were within ± 0.5% of theoretical values. The purity of compounds were checked on TLC plates performed on Merck Silica gel 60 F254 aluminum sheets using iodine vapor as visualizing agent.

Regarding to the cytotoxic city study, the two cell lines MCF-7 and SKG were obtained from the Iraqi Center for Cancer and Medical Genetic Research (ICCMGR) Cell Bank Unit. Cell lines were maintained in Roswell Park Memorial Institute(RPMI)-1640 medium (Sigma/USA) supplemented with 5% calf bovine serum (Capricorn/USA), 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA(Capricorn/USA), reseeded at 50% confluence twice a week, and incubated at 37 °C. MTT stain was supplied from Bioworld/USA.

Synthesis

The first series compounds were synthesized as shown in scheme I:



Scheme I: The synthesis of 3-methyl-4-oxo-7-hydroxy-benzopyranyl[4,3-c][1-*H*] pyrazoline derivatives (series I)

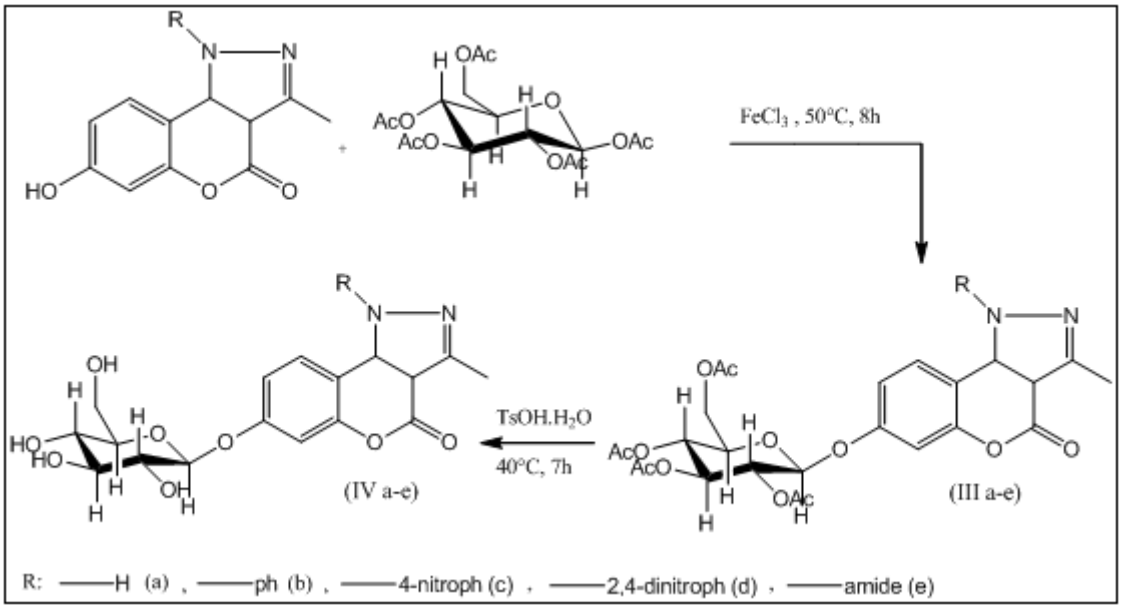
Synthesis of 3-acetyl-7-hydroxycoumarin (I)

To a mixture of 2,4-dihydroxybenzaldehyde (0.05 mole, 6.9 g)and ethyl acetoacetate(0.05 mole, 6.34 ml), a few drops of piperidine was added. The reaction mixture was stirred for one hour at room temperature until 3-acetyl-7-hydroxy coumarin solidify; the compound was filtered and washed with absolute ethanol to achieve clear yellow color; recrystallized from methanol [11].

General procedure for the synthesis of 3-methyl-4-oxo-7-hydroxy- benzopyranyl[4,3-c][1-*H*] pyrazoline derivatives(II a-e)

These compounds were prepared from equivalent mole (0.01 mole) of 3-acetyl-7-hydroxy coumarin (I) with equimole of hydrazine hydrate and its derivatives which are (hydrazine hydrate, phenyl hydrazine, 4-nitrophenyl hydrazine, 2,4-dinitrophenyl hydrazine, and semicarbazide) in minimum amount of pyridine. The reaction mixture was stirred for around 15 minutes, and for 4 hours at 50°C, then left around 1 hour to cool at room temperature; the reaction mixture was poured on to crushed ice. The precipitate was filtered and the product was re-crystallized from absolute ethanol [12][13].

Series II compounds were produced from O-glycosylation of the first series compounds at position 7 of coumarin nucleus and de-acetylation of glucose as shown in scheme II:



Scheme II: Synthesis of 3-methyl-4-oxo-7-O-glycosylated benzopyranyl[4,3-c][1-*H*] pyrazoline derivatives(series II)

General procedure for the synthesis 3-methyl-4-oxo-7-O-acetylated-glycose-benzopyranyl[4,3-c][1-H] pyrazoline derivatives (III a-e)

An equimole(1.2 mmole) of each compound of series II (a-e) was added to a suspension of equimole of β-D-glucose pentaacetatewith anhydrous ferric chloride (10 mole %) in dichloromethane at 50°C. The reaction mixture was stirred for 8 hours in anhydrous conditions; and dried under reduced pressure. The solid was washed with distilled water several times. The product was re-crystalized from methanol [14].

General procedure for de-acetylation of glucose and formation of

3-methyl-4-oxo-7-O-glycosylated-benzopyranyl[4,3-c][1-*H*] pyrazoline derivatives (IV a-e)

To a solvent mixture of CH_2Cl_2 / MeOH (9:1), an equimole (0.0005 mole) of each series III (a-e) compounds was dissolved; p-toluene sulfonic acid monohydrate ($TsOH.H_2O$) (one equivalent per acetate) was added and stirred for 7 hours at 40°C. the reaction mixture was treated with aqueous $NaHCO_3$(50 ml of 5%). the organic phase was dried with magnesium sulfate; evaporated under reduced pressureand re-crystalized from ethanol [15].

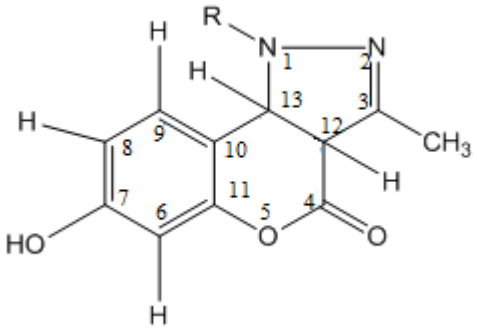
Methodology of in vitro MTT assay of compounds II (a-e) and IV (a-e) in MCF-7 and SKG cell lines

This study was conducted at the Iraqi center for cancer and medical genetic research. SKG esophageal cancer cell line and MCF-7 breast cancer cell line, were obtained from the IRAQ Biotech Cell Bank Unit and 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 50% confluence twice a week, and incubated at 37 °C. The prepared compounds with the positive control (5-fluorouracil) were dissolved in dimethyl sulfoxide (DMSO) at six serial concentrations (500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml, and 15.6µg/ml), the negative control was the cell culture media. To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1×10^4cells/well. After 24h of confluent monolayer was achieved, cells were treated with tested compound. Cell viability was measured after 72 hrs. of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT (and incubating the cells for 1.5 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 followed by 37 °C incubation for 15 min with shaking. The absorbency was determined on a microplate reader at 492 nm (test wavelength)[16]; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated according to the following equation:

(A _ B)/A _ 100, where A is themean optical density of untreated wells, and B is the optical density of treated wells and the IC_{50}(s) (compound concentration that inhibit 50% of cancer cells) were calculated for all synthesized compounds and compared with the standard cytotoxic drug 5-flourouracil (5-FU).Graph pad prism software was used to analyze data through non-linear regression; significant difference with respect to control: * $P<0.05$[16].

3. RESULTS

The spectral data of the prepared compounds are according to the following structure numbering:



3-acetyl-7-hydroxycoumarine (I)
Yield 86.2%, m.p. 232-234°C. IR (ν_{max}): 3203.22(stretch band of OH), 1703.23(C=O of cyclic ester, 1676.95(C=O of ketone), 1575(Ar C=C).

3-methyl-4-oxo-7-hydroxy-benzopyranyl [4,3-c] [1-*H*] pyrazoline (II a)
Yield 45.8%, m.p. 289-290°C. IR (ν_{max}): 3465.98(N-H of pyrazoline), 3200.09(stretch band of OH), 1687.32(C=O of cyclic ester), 1612.04(C=N), 1536.40(Ar C=C); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 2.2 (s, 3H, CH_3), 3.3 (d, 1H attached to C12), δ 4.9 (d, 1H attached to C13), δ 5.9 (s, 1H of OH group), δ 6.4-7.5 (m, 4H, Ar-H); ^{13}C-NMR (DMSO-*d6*100MHz, δ ppm): 17, CH3; 45, C12; 60, C13; 108-161, Ar-carbons; 162.22, C7; 162.53, C=O. Anal. Cal. C, 60.55; H, 4.62; N, 12.84; Found C, 60.59; H, 4.52; N, 12.79.

1-Phenyl-3-methyl-4-oxo-7-hydroxy-benzopyranyl [4,3-c] pyrazoline (II b)
Yield 65.9%; m.p. 226-227°C.IR (ν_{max}): 3324.23(stretch band of OH), 1682.37(C=O of cyclic ester), 1595.70 (C=N), 1504.79(Ar C=C); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 2.3 (s, 3H, CH_3), 3.4 (d, 1H attached to C12), δ 4.2 (d, 1H attached to C13), δ 5.7 (s, 1H of OH group), δ 6.4-7.9 (m, 8H, Ar-H); ^{13}C-NMR (DMSO-*d6* 100MHz, δ ppm): 15, CH_3;49, C12; 59, C13;102-164, Ar-carbons; 155, C7; 161, C=O. Anal. Cal. C, 69.38; H, 4.79; N 9.52; Found C, 69.40; H, 4.74; N, 9.49.

1-[p-Nitrophenyl]-3-methyl-4-oxo-7-hydroxy-benzopyranyl [4,3-c] pyrazoline (II c)

Yield 47.1%, m.p. 277-278°C.IR (ν_{max}): 3313.83(stretch band of OH), 1693.09(C=O of cyclic ester), 1601.60 (C=N), 1497.53(Ar C=C), 1504.31(Asym. vib. of NO_2, 1329.21 (sym. vib. of NO_2); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 1.8 (s, 3H, CH_3), 3.4 (d, 1H attached to C12), δ 4.9 (d, 1H attached to C13), δ 5.9 (s, 1H of OH group), δ 6.6-8.3 (m,

7H, Ar-H); ^{13}C-NMR (DMSO-*d6*100MHz, δ ppm): 16, CH3; 47, C12; 53, C13; 103-152, Ar-carbons; 161, C7; 166, C=O. Anal. Cal. C, 60.18; H, 3.86; N, 12.38; Found C, 60.17; H, 3.81; N, 12.29.

1-[2,4-dinitrophenyl]-3-methyl-4-oxo-7-hydroxy-benzopyranyl [4,3-c] pyrazoline (II d)

Yield 67.6%, m.p. 279-281°C.IR (ν_{max}): 3348.41 (stretch band of OH), 1685.54(C=O of cyclic ester), 1611.95 (C=N), 1583.18(Ar C=C), 1505.66 (Asym. vib. of NO_2, 1326.16(sym. vib. of NO_2); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 1.8 (s, 3H, CH_3), 3.4 (d, 1H attached to C12), δ 4.7 (d, 1H attached to C13), δ 5.7 (s, 1H of OH group), δ 6.7-9.1 (m, 6H, Ar-H); ^{13}C-NMR (DMSO-*d6*100MHz, δ ppm): 17, CH3; 49, C12; 54, C13; 116-152, Ar-carbons; 162, C7; 164, C=O. Anal. Cal. C, 53.13; H, 3.15; N, 14.58; Found C, 53.14, H, 3.13; N, 14.49.

1-Acetamido-3-methyl-4-oxo-7-hydroxy-benzopyranyl[4,3-c] pyrazoline (II e)

Yield 61.3%, m.p. 220-221°C. IR (ν_{max}): 3416.25(N-H of pyrazoline), 3140.34(stretch band of OH), 1686.97(C=O of cyclic ester), 1619.28 (C=O of amide), 1593.04 (C=N), 1501.63(Ar C=C); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 2.2 (s, 3H, CH_3), 3.4 (d, 1H attached to C12), δ 5.2 (d, 1H attached to C13), δ 5.9 (s, 1H of OH group), δ 6.5 (s, 2H, NH_2) δ 6.7-7.9 (m, 3H, Ar-H); ^{13}C-NMR (DMSO-*d6*100MHz, δ ppm): 15, CH3; 54, C12; 59, C13; 102-155, Ar-carbons; 157 (C=O of amide); 160, C7; 162, C=O. Anal. Cal. C, 16.09; H, 4.24; N, 16.09; Found C, 15.99; H, 4.20; N, 15.99.

3-methyl-4-oxo-7-O-glycosylated-benzopyranyl[4,3-c][1-H] pyrazoline (IV a)

Yield 59.2%, m.p. 299-300°C.IR (ν_{max}): 3464.92 (N-H of pyrazoline), 3210.21 (stretch band of OH), 1694.45 (C=O of cyclic ester), 1613.11 (C=N), 1537.97(Ar C=C), 1116.77(Asym. C-O-C), 1040.66(Sym. C-O-C); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 2.1 (s, 3H, CH_3), 2.8 (d, 1H attached to C12), δ 4.7 (dd, 1H attached to C13), δ 3.2-5.1(m, 6H of glucose moiety), δ 6.6-7.9 (m, 4H, Ar-H); ^{13}C-NMR (DMSO-*d6*100MHz, δ ppm): 18, CH3; 49, C12; 58, C13; 68-86, glucose-carbons;103, anomeric carbon; 108-161, Ar-carbons; 162.12, C7; 162.58, C=O. Anal. Cal. C, 53.68; H, 5.30; N, 7.37; Found C, 53.71; H, 5.24; N, 7.30.

1-Phenyl-3-methyl-4-oxo-7-O-glycosylated-benzopyranyl[4,3-c]pyrazoline (IV b)

Yield 60.2%; m.p. 233-234°C.IR (ν_{max}): 3241.45 (stretch band of OH), 1691.24(C=O of cyclic ester), 1598.21 (C=N), 1559.25 (Ar C=C), 1150.64 (Asym. C-O-C), 1008.19 (Sym. C-O-C); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 1.7 (s, 3H, CH_3), 2.9 (d, 1H attached to C12), δ 5.5 (d, 1H attached to C13), δ 3.5-5.3 (m, 6H of glucose moiety), δ 6.0 (d, 1H, anomer-H), δ 6.4-8.2 (m, 9H, Ar-H); ^{13}C-NMR (DMSO-*d6*100MHz, δ ppm): 16, CH3; 51, C12; 59, C13; 68-81, glucose-carbons; 100, anomeric carbon; 109-152, Ar-carbons; 160, C7; 164, C=O. Anal. Cal. C, 60.52;H, 5.30; N, 6.14; Found C, 60.53; H, 5.21; N, 6.12.

1-[p-Nitrophenyl-3-methyl-4-oxo-7-O-glycosylated-benzopyranyl [4,3-c] pyrazoline (IV c)

Yield 58.4%; m.p. 282-283°C.IR (ν_{max}): 3243.92 (stretch band of OH), 1683.08 (C=O of cyclic ester), 1589.43 (C=N), 1533.40 (Ar C=C), 1492.90 (Asym. NO_2), 1321.17 (Sym. NO_2),1140.15 (Asym. C-O-C), 1102.11 (Sym. C-O-C); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 1.7 (s, 3H, CH_3), 2.8 (d, 1H attached to C12), δ 5.1 (d, 1H attached to C13), δ 3.0-4.4 (m, 6H of glucose moiety), δ 6.0 (d, 1H, anomer-H), δ 6.8-8.6 (m, 8H, Ar-H); ^{13}C-NMR (DMSO-*d6*100MHz, δ ppm): 15, CH3; 52, C12; 55, C13; 64-78, glucose-carbons; 107.23, anomeric carbon; 107.42-152.25, Ar-carbons; 160, C7; 163, C=O. Anal. Cal. C, 55.09; H, 4.62; N, 8.38; Found C, 55.12; H, 4.55; N, 8.36.

1-[2,4-dinitrophenyl]-3-methyl-4-oxo-7-O-glycosylated-benzo-pyranyl[4,3-c] pyrazoline (IV d)

Yield 60.6%; m.p. 287-288°C.IR (ν_{max}): 3288.07 (stretch band of OH), 1687.83 (C=O of cyclic ester), 1598.15 (C=N), 1531.02 (Ar C=C), 1505.49 (Asym. NO_2), 1326.07 (Sym. NO_2), 1109.68 (Asym. C-O-C), 1002.20 (Sym. C-O-C); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 1.6 (s, 3H, CH_3), 3.1 (d, 1H attached to C12), δ 5.3 (d, 1H attached to C13), δ 3.5-4.9 (m, 6H of glucose moiety), δ 6.3 (d, 1H, anomer-H), δ 7.2-8.8 (m, 7H, Ar-H); ^{13}C-NMR (DMSO-*d6*100MHz, δ ppm): 19,CH3; 54, C12; 59, C13; 66-85, glucose-carbons; 102, anomeric carbon; 114-159, Ar-carbons; 163, C7; 166, C=O. Anal. Cal. C, 50.55; H, 4.06; N, 10.25; Found C, 50.50; H, 3.98; N, 10.18.

1-Acetamido-3-methyl-4-oxo-7-O-glycosylated-benzo- pyranyl[4,3-c] pyrazoline (IV e)

Yield 59.4%; m.p. 232-233°C.IR (ν_{max}): 3489.03 (N-H), 3398.39 (stretch band of OH), 1683.60 (C=O of cyclic ester), 1618.38 (C=O of amide), 1559.98 (C=N), 1508.55 (Ar C=C), 1134.18 (Asym. C-O-C), 1016.45 (Sym. C-O-C); ^{1}H-NMR (DMSO-*d6* 400MHz, δ ppm): δ 1.6 (s, 3H, CH_3), 3.3 (d, 1H attached to C12), δ 5.4 (d, 1H attached to C13), δ 3.4-4.9 (m, 6H of glucose moiety), δ 6.1 (d, 1H, anomer-H),δ 6.4 (s, 2H, NH_2), δ 6.7-8.3 (m, 3H, Ar-H); ^{13}C-NMR (DMSO-*d6*100MHz, δ ppm): 13, CH3; 52, C12; 57, C13; 59-78, glucose-carbons; 101, anomeric carbon; 107-155.10,

Ar-carbons; 155.89, (C=O of amide); 162, C7; 167, C=O. Anal. Cal. C, 51.06; H, 5.00; N, 9.93; Found C, 51.13; H, 4.99; N, 9.81.

The IC_{50}(concentration that inhibit 50% of cancer cells) of the prepared compounds are summarized in Table I.

Table I: The IC_{50} of prepared compounds in MCF-7 and SKG cell lines

MCF-7 Breast cancer		SKG Esophageal cancer	
Compound	**IC_{50} µg/ml**	**Compound**	**IC_{50}µg/ml**
5-Fluorouracil	35.58	5-Fluorouracil	5.6
IIa	671.0	IIa	10.0
IIb	571.2	IIb	40.4
IIc	143.1	IIc	531.6
IId	413.5	IId	528.6
IIe	64.1	IIe	56.8
IVa	50.6	IVa	8.3
IVb	274.3	IVb	109.7
IVc	2.4	IVc	19.4
IVd	17.6	IVd	14.6
IVe	380.3	IVe	72.8

4. DISCUSSION

Coumarin and pyrazoline containing compounds were both approved to possess cytotoxic activity, so hybridization of such heterocyclic molecules is a new approach to make compounds with a significant cytotoxic activity like the study made by Liu et al, who conjugate coumarin derivatives with 4,5-dihydropyrazole and found a significant cytotoxic effect through inhibition of telomerase enzyme activity[17]. On the other hand, glycosylation is also a new strategy to exaggerate selective cytotoxic effect due to glucose over need and high expression of glucose transpoter-1 (GLUT-1) in cancerous cells[7].

In this study, the synthesis of 3-acetyl-7-hydroxycoumarin which is the row compound for series I was in a very good yield, then its hybridization with hydrazine derivatives to produce series I compounds were also in a good percentage of yield according to literature reviews[3]. The fusion reaction of the previous compounds with β-D-glucose pentaacetate through O-glycosidic linkage at position 7 of coumarin in the presence of anhydrous $FeCl_3$ and CH_2Cl_2 produced good yield productsaccording to Narayanaperumal et al. method [14]. The physical properties and spectrometric analysis data confirmed the identity of the synthesized compounds.

In MCF-7 cell line, the O-glycosylated compounds (IVa-e)possessed a high cytotoxicity activity specially for compound IVc with an IC_{50} 2.4 µg/ml which is even less than IC_{50} of the standard drug 5-FU(Figure I). In SKG cell line, the O-glycosylated compounds (IVa-e) possessed a high cytotoxicity activity especially for compounds IVa and IVc with an IC_{50} 8.3 µg/ml and 19.4 µg/ml respectively (Figure II). The high cytotoxic effect for compound IVc might be attributed to the presence of nitro group which is a good withdrawing group on the phenyl ring which may lead to a better interaction with the target site; such results are compatible with previous cytotoxic city studies which showed an increase in the cytotoxicity percentage of a group of chalcone compounds substituted by nitro group at phenyl ring [18][19], however a mechanistic and simulation docking studies may be needed to understand the cytotoxic effects of such hybrid compounds.

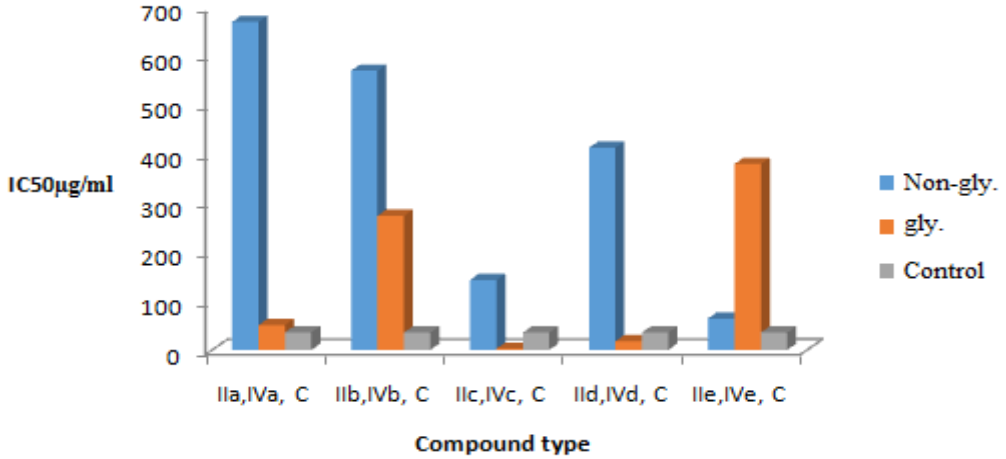


Figure I: Comparison of IC_{50}(s) between non-gly., gly., with controlin MCF-7

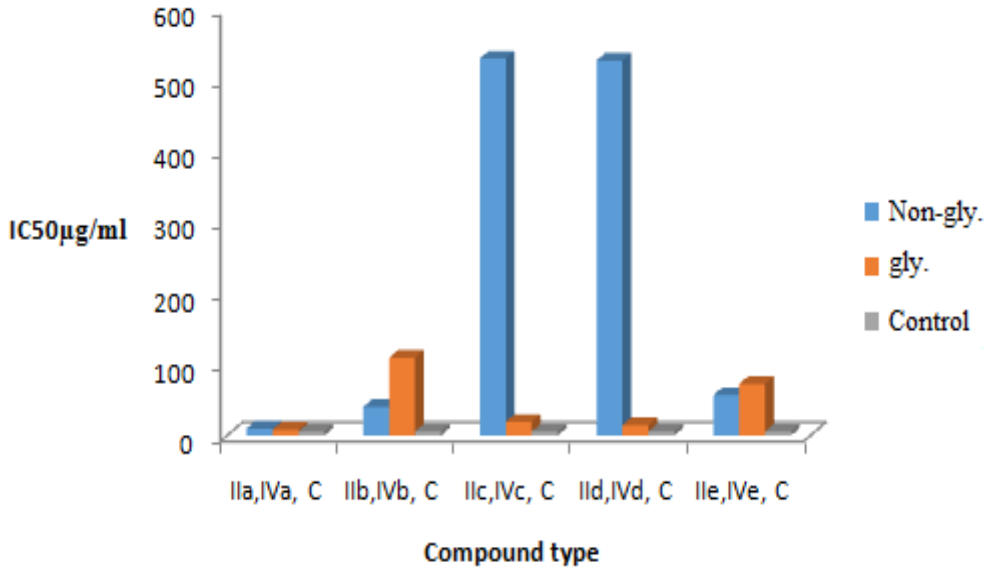


Figure II: Comparison of IC_{50}(s) between non-gly., gly., with controlin SKG

CONCLUSION

Coumarin-pyrazoline containing compounds became an important motif of interest in pharmaceutical chemistry because of its highly pharmacological activities especially as anticancer activity, and O-glycosylation of such conjugates with β-D-glucose was found to increase their cytotoxic activity such as in compound IVcas cancerous cells consume more glucose than normal cells. This strategy may be applied to many other compounds with cytotoxic activity to potentiate their effect through high selectivity with less side effects.

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