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Synthesis and Pharmacological Evaluation of Novel Coumarin Derivatives

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ABSTRACT

The current work focuses on new architecture, synthesis of coumarin-oxadiazole hybrid derivative products as both these (coumarin ring and oxadiazole) have a wide variety of biological behavior, Compounds containing the nucleus of coumarin (2H-1-benzopyran-2-one) are an interesting class of hetero cycles which hold an important role in the field of natural ingredients and synthetic organic chemistry. It has been exciting medicinal chemists to study native coumarins or synthetic analogs for their application for decades. And they can be further modified to synthesize more effective and potent drugs. Compounds have been characterized by spectrophotometry of physicochemical properties and their structures verified by infrared spectroscopy (FTIR) and nuclear magnetic resonance (1H-NMR) Such new derivatives of coumarinyl-oxadiazole was qualified to estimate the lethal dose, anticancer, anticoagulant and antioxidant activity. Their pharmacological properties depend on their pattern of substitution, compound S4F proved significant anticoagulant activity in concentration (50, 100, 200 mg/ml) similar for heparin, and monitor the coagulation effect on plasma, while compound S_4CO give significant anticancer activity against MCF-7 a breast cancer cell. Specific compounds have strong antioxidants with the effective action of radical scavengers; the S4Cl compound with IC_{50} 1.49 is the most potent antioxidant activity note. Basically, all the formulations tested reported satisfactory behavior. The review shows that varieties of coumarin derivatives have synthesized and shown anti-cancer, antioxidant and anti-coagulant potentials. These derivatives synthesis and its biological assay can be further modified in the future to improve the anti-cancer, anti-oxidant and anticoagulant potentials of the versatile coumarin nucleus.

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INTRODUCTION

Cumarin derivatives have a wide range of biological functions. Diverse heterocyclic compounds consisting of coumarins are among the most important groups of heterocycles holding a leading position in synthetic and therapeutic chemistry, leading to their different applications as anti-oxidant, reduce inflammation, bactericidal, antitumor and blood thinner compound (Manojkumar *et al.*, 2009).

In addition to 1,3,4-oxadiazoles display evident anticoagulant (Jeong *et al.*, 2004; Saibara *et al.*, 2003) and anti-cancer properties (Lin *et al.*, 2007; Oza *et al.*, 2012).

Coumarins have attracted researchers to work on this moiety, which is instrumental in the creation of new coumarin compounds, a large spectrum of biological activity and its effectiveness as valuable synthons, while oxadiazoles have a great contribution to the development of heterocyclic chemistry. Numerous oxadiazoles have synthesized and subjected to biological screening; the results have increased their importance because of potential activities and use in different fields of daily life. To date, our research is based on the new synthesis of coumarinyl -1, 3, 4-oxadiazole derivatives, considering it has a large variety of medical and industrial uses.

The novel products prepared from coumarin -3-carboxylic acid through synthesis hydrazide–hydrazine compound (CO-NHN=CH) as an intermediate product. By cyclization of that intermediate to give 1,3,4-oxadiazole ring moiety at 3 position in coumarin ring, necessary to get effective binding with different enzymes and receptors in biological systems eliciting an array of bioactivities spectrum including anticancer (Nasr *et al.*, 2018), anticoagulant (Rishavy *et al.*, 2018), and antioxidant (Al-Majedy *et al.*, 2016).

We prepared this work to synthesis and design four novel coumarin derivatives substituted at site 3 by oxadiazole ring and rationalize the pharmacological activity such as antioxidant, anticoagulant and anticancer to observe clear analysis into the relationship between structure and behavior of these compounds.

Aim of the study

We based the present study on a synthesis of coumarin heterocyclic compounds and then pharmacological study for this derivative such as anticancer, antioxidant and anticoagulant activity, and to provide the development of substituted coumarin nucleus to give a potent, beneficial product.

MATERIALS AND METHODS

Reagents and chemicals

Coumarone -3-carboxylic acid, Sigma–Aldrich German. /. Methanol, sigma–Aldrich, German / conc. Sulphuric acid 99%, Merk, German. / Ethyl acetate, Alpha Chemika, India / Hexane, sigma–Aldrich German. / Hydrochloric acid, Merk German / Absolute ethanol, sigma–Aldrich, German. / Hydrazine hydrate 80%, ALPHA Company, India./ Chloroform, SDFCL, India./Aromatic aldehyde (Benzyaldehyde), 4-chlorobenzyladehyde, 4-floro benzylaldehyde, Merck, Germany /. 4- methoxy benzyldehyde, BDH, England /Acetic anhydride, Merck, Germany/ pyridine, Hayashi Pure Chemical, Japan/ Glacial acetic acid, Thomas baker, India/ Heparin, HAVER (25,000 IU/5ml), Canada/ Blood plasma, From the researcher (Sanaryh.M)/ DMSO Merck, Germany, Ascorbic acid/ Sigma, Aldrich and 1,1-diphenyl-2-picrylhydrazyl (DPPH)/Sigma, Aldrich.

Instrumentation Condition

The FT-IR8400S spectrophotometer(SHIMADZU / Japan) was reported the infrared spectrum as KBr wavelengths ^{1}HNMR (Proton nuclear magnetic resonance) spectra was calculated by a College of Science and Technology – Iran on a Bruker Ultra shield 499 MHz spectrometer (Switzerland) system on Dimethyl - sulphoxide (DMSO-d6). Chemical shifting of hydrogen atoms are measured in proportional parts per million (ppm) relative to the internal norm of tetramethylsilane.

TLC (Analytical thin-layer chromatography) was conducted on silica gel coated plates (Merck 60 F254, 0.25 mm), which were visualized under 254 nm of ultraviolet or iodine mist.

Compounds Synthesis

Methyl 2-oxo-2H-chromene-3-carboxylate (S_1)

In100 ml broad bottom flask linked to a condenser for reflux, coumarin -3-carboxylic acid (2g, 1mol)was solubilized in20ml absolute methanol, and then 3 drops of sulphuric acid were applied and the reflux system heated for 7 hours., cooling the reaction and evaporated the mixture to dryness and the subsequent mixture of reaction extracted with the ethyl acetate, then added 5% bicarbonate of sodium until the solution becomes basic.

The final product, as shown in (Scheme 1), was separated by 25 ml dichloromethane using separating funnel (Manvar *et al.*, 2008).

Off-white needle-like crystals.Production of 75%. M.P = 113°C, RF=0.75 (Ethyl acetate n-Hexane: 3:7); The IR (cm^{-1}): 2933 (Aliphatic, C-H.), 3055 (Aromatic C-H,), 1745.5 (Ester C=O,), 1683.8 (Lactone, C = O.), 1610.53 (Alkene C=C,), 1567.2 (Aromatic C=C,).

2-oxo-2H-chromene-3-carbohydrazide (S_2)

In 100 ml flat bottom round flask, coumarin ester S_1(0.1mol) dissolved in 10 ml ethanol, then added (0.5 mol) hydrazine hydrate (98%), refluxed the mixture for 12hrs, cooled at room temperature, the reaction combination remains precipitate to the next day.

The solid product, as shown in (Scheme 2) filtered separately, and recrystallized with ethanol to give off white glittery crystals. (Manvar *et al.*, 2008). White shiny crystals; yield (65-70) %. M.P = 90-93°C; RF=0.66 (Ethyl acetate n-Hexane: 6:4). The

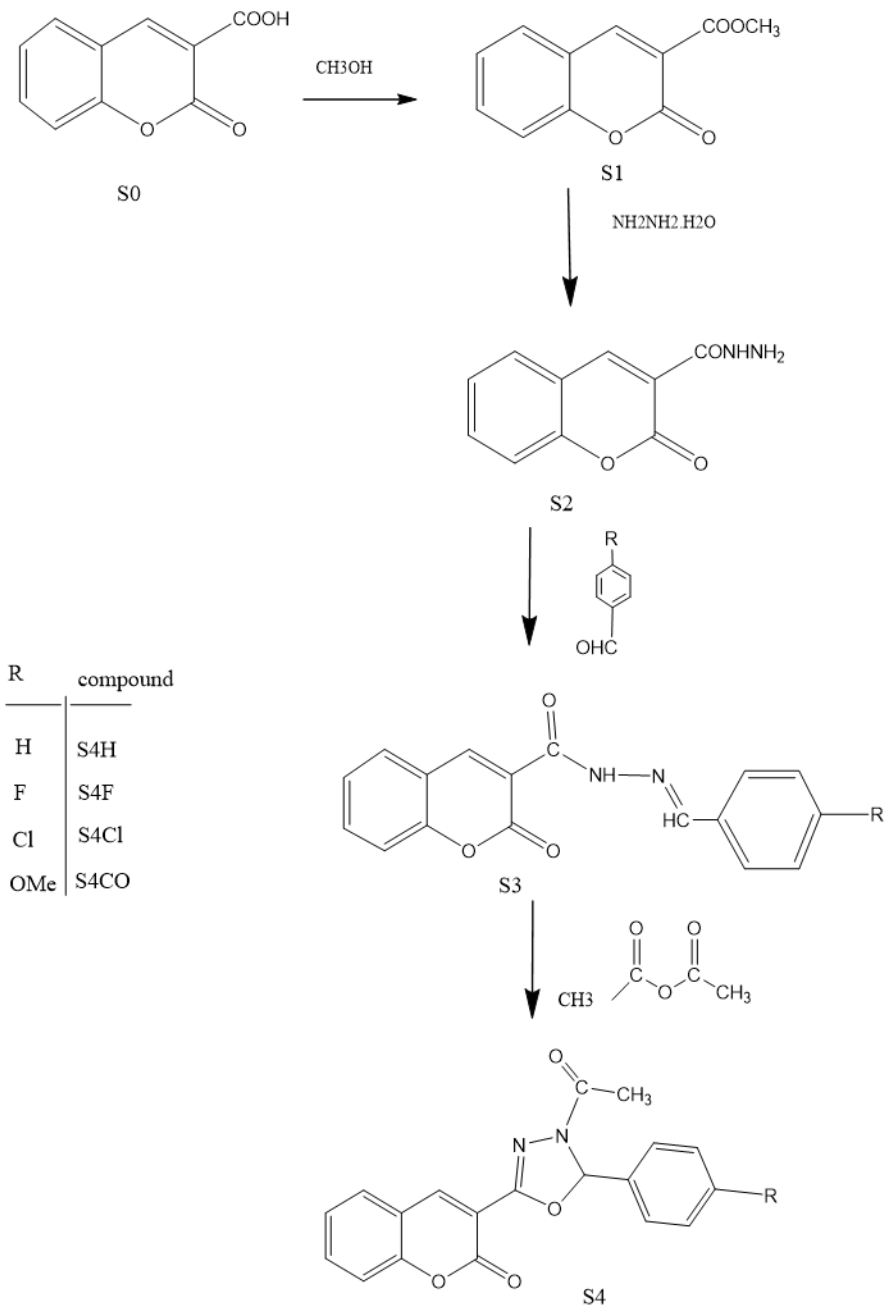


Figure 1: Synthesis of coumarin -1, 3, 4- Oxadiazole derivative

IR (cm^{-1}): 2933 (Aliphatic C-H,), 3043 (Aromatic C-H,), 3386, 3290 (Hydrazide NH-NH_2), 1745 (Lactone C =O), 1614 (C=O,CONH), 1608 (Alkene C=C,), 1573 (Aromatic C=C,).

N'-benzylidene-2-oxo-2H-chromene-3-carbohydrazide synthesis (S_3)

In 100 ml flat bottom round flask, A mixture of S_2 (0.01 mol) and aromatic aldehydes (0.01mol, benzaldehyde, 4-floroubenzaldehyde, 4-chlorobenzenedehyde, and 4-methoxybenzaldehyde was refluxed in absolute ethanol and 2-3 drops of glacial acetic acid, for 4 hours ethanol was evaporated after finalization of the reaction to provide the yellow component recrystallized with ethanol.

All derivatives structures were reported by physical and chemical properties (Color, M.P, as well as TLC system (Ethyl acetate: n-hexane::7:3) and also confirmed by FT-IR spectrometry (Berthomieu and Hienerwadel, 2009; Manvar *et al.*, 2008).

N'-benzylidene-2-oxo-2H-chromene-3-carbohydrazide (S_3H)

Yellowish powder as shown in (Scheme 3) ; M.P.=85°C ; R.F=0.55 ; The IR (cm^{-1}): 3079 (C-H), 2970 (As C-H), 2865 (Sy C-H), 3437 (Secondary amine N-H), 1687 (C=N), 1620 (Amide C=O), 1766

(Lactone C=O), 1483 (C=C), 1269 (C-O).

N'-(4-fluorobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (S_3F)

Pale Yellow powder as shown in (Scheme 4); M.P =137°C; RF=0.5; The IR (cm^{-1}):3079 (C-H), 2970 (As C -H), 2865 (Sy C-H) , 3437 (Secondary amine N-H), 1687 (C=N), 1620 (Amide C=O,), 1766 (Lactone C=O) , 1483 (C=C), 1269 (C-O).

N'- (4-chlorobenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (S_3Cl)

Light yellow powder as shown in (Scheme 5) ; M.P. = 158 ˚C ; RF= 0.62 ; The IR (cm^{-1}): 3079 (C-H), 2970 (As C-H) , 2865 (Sy C-H), 3437 (Secondary amine N-H), 1687 (C=N), 1620 (Amide C=O), 1766 (Lactone C=O), 1483 (C=C), 1269 (C-O).

N'-(4-methoxybenzylidene)-2-oxo-2H-chromene-3-carbohydrazide (S_3CO).

Pale yellow crystal as shown in (Scheme 6); M.P. =143°C ; R F = 0.6; The IR (cm^{-1}): 3079 (C-H), 2970 (As C-H), 2865 (Sy C-H), 3437 (Secondary amine N-H), 1687 (C=N), 1620 (Amide C=O), 1766 (Lactone C=O), 1483 (C=C), 1269 (C-O).

Coumarin1, 3, 4-Oxadiazole derivatives (S_4)

The reaction mixture of S3 compounds (0.5 g,0.01mol) and excess acetic anhydride (10 ml, 0.01 mol) with 4-5 drops of pyridine added in 100 ml round flask, then refluxed for 2 hours.

The mixture of the reaction was left during the night at room temperature when the yellowish-like solid mass was isolated and obtained by filtration and washed with water.

Ethanol recrystallized the product to get the desired product (Aa and Mg, 2015). FTIR spectrometry, 1HNMR (Macomber and Harbison, 1999) confirmed structures of all oxadiazole derivatives and TLC (n-hexane: ethyl acetate/2:8) reported structures.

3-(4-acetyl-5 phenyl-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)-2H- Chromen-2-one (S_4H)

Light yellow powder as shown in (Scheme 7); Yield (28 %)., M.P. =109°C, RF.= 0.4 ; The IR (cm^{-1}): 3113 (Aromatic C-H), 2935(Aliphatic C-H), 1768 (Lacton C= O), 1676 (C= N), 1620 (C= O), 1600 (C= C), 1199 (C-N), 1253 (C-O), 1H NMR (499 MHz, ppm, DMSO-d6): 8.66 ppm (s,1H. Oxa.ring), 2.36 ppm (s, H, CH3) and range 7.26-8.3 ppm (d, t, Ar-H system).

3-(4-acetyl-5-(4-fluorophenyl)-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)-2H-Chromen-2-one (S_4F)

Very light yellow powder as shown in (Scheme 8), Yield (63%), M.P. =103°C, RF = 0.49 ; The IR (cm^{-1}) : 3113 (Aromatic C-H), 2935 (AliphaticC-H) , 1768 (Lacton C=O), 1676 (C=N), 1620 (C=O) , 1600(C=C),1199(C-N), 1253 (C-O). ^{1}H NMR (499 MHz, ppm, DMSO-d_6): 8.66 ppm (s,1H.Oxa.ring), 2.36 ppm (s, H, CH3) group and rang 7.26-8.3 ppm (d., t. Ar-H system).

3-(4-acetyl-5-(4-chlorophenyl)-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)-2H-Chromen-2-one (S_4Cl)

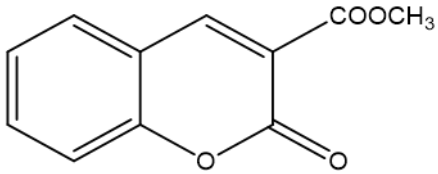
The light yellow powder, as shown in (Scheme 9), Yield (33%), M.P. =162°C, RF=0.05. The IR (cm^{-1}): 3113 (Aromatic C-H), 2935 (Aliphatic C-H), 1768 (Lacton C=O), 1676 (C=N), 1620 (C=O), 1600 (C=C),1199 (C-N), 1253 (C-O).

^{1}H NMR (499 MHz, ppm, DMSO-d_6): 8.66 ppm (s,1H. Oxa. Ring), 2.36 ppm (s, H, CH3) group. and rang 7.26-8.3 ppm (d., t., Ar-H system)

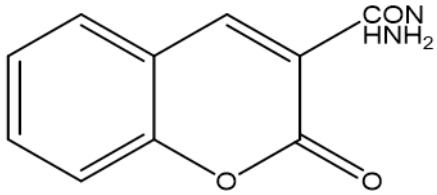
3-(4-acetyl-5-(4-methoxyphenyl)-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl)-2H-chromen-2-one (S_4CO)

White crystals as shown in (Scheme 10), Yield (67%), M.P.=136 ˚C , RF=0.52; The IR (cm^{-1}): 3113(Aromatic C-H), 2935 (Aliphatic C-H), 1768 (Lacton C=O) , 1676 (C=N), 1620 (C=O), 1600 (C=C), 1199(C-N), 1253 (C-O).

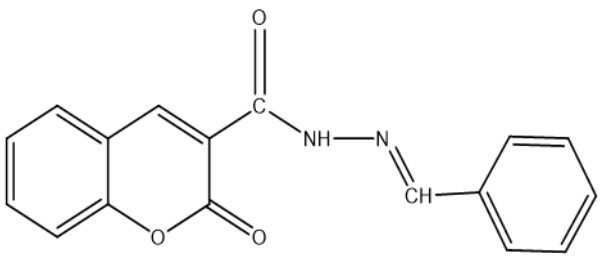
^{1}H NMR (499 MHz, ppm, DMSO-d_6): 8.66 ppm (s, 1H. Oxa. Ring), 2.36 ppm (s, H, CH3) group. Rang 7.26-8.3 ppm (d, t, Ar-HH system)



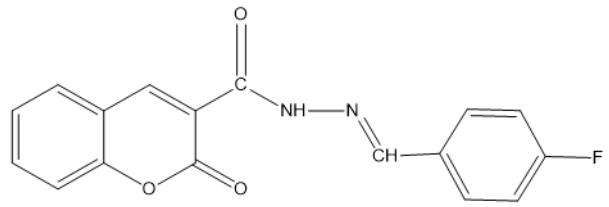
Scheme 1: S1



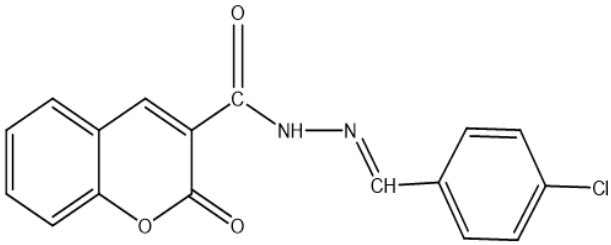
Scheme 2: S2



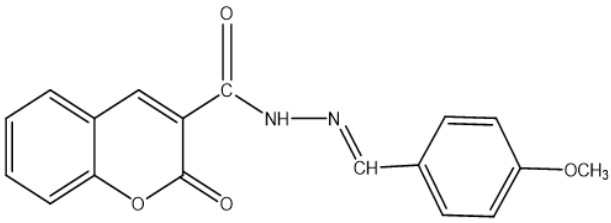
Scheme 3: S3H



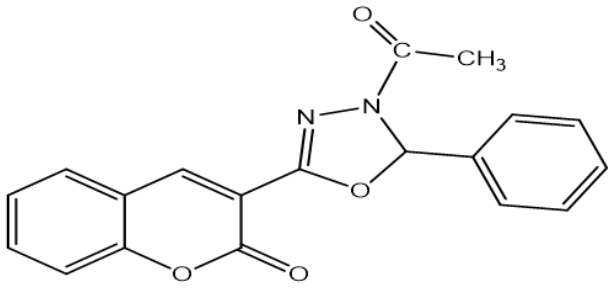
Scheme 4: S3F



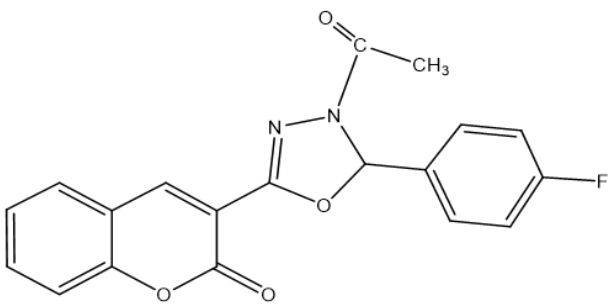
Scheme 5: S3Cl



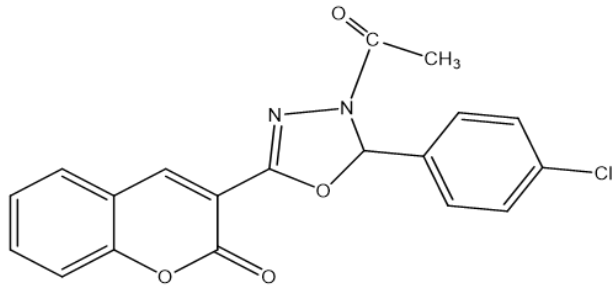
Scheme 6: S3CO



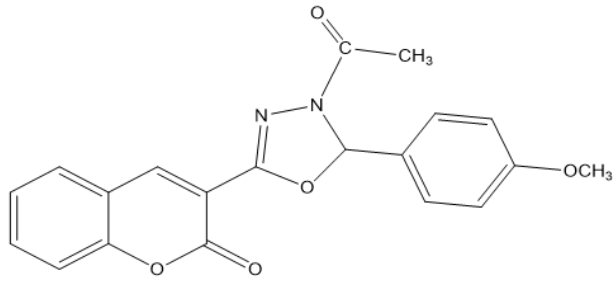
Scheme 7: S4H



Scheme 8: S4F



Scheme 9: S4Cl



Scheme 10: S4CO

Biological assay

LD50 measurement

With a slight adjustment on the general procedure, the median lethal dose (LD_{50}) of four novel synthesis compounds was estimated in mice. Initially, the test required four animals for each tested compound (S_4H, S_4F, S_4Cl, and S_4CO). 100, 200, 300, and 400 mg/kg, gavage for each mouse as the first trial for LD_{50} measurement. We carried out the same concentrations for each tested drugs. We observed no mortality at these concentrations. The second stage then started involves administration of 500, 600, 700, and 800 mg/kg gavage for each mice as a second trial for LD_{50} measurement. The LD_{50} determined for both S4F and Cl but no mortality was noticed for LD_{50} measurements in S_4H and S_4CO coumarin derivatives, so we started the 3^{rd} stage, 900, 1000, 1100, and 1200 mg/kg gavage for each mouse I did conformity investigations for each lethal dose in the four tested compounds. LD_{50} measured according to the following equation (Chinedu *et al.*, 2013)

LD_{50} = [M0+M1] ÷ 2 (M0= Highest calculated dose were no mortality observed)

(M1=Lowest calculated dose were mortality observed)

Antioxidant action

Free radical compound hunting activity of coumarin derivatives (S_4H, S_4F, S_4Cl, S_4CO) measured by DPPH with few modification of dependable procedure, (0.1 mmol) methanol-prepared DPPH solution, (0.1

ml) added to (0.4 ml) specific concentration sample solutions (50,100,150,200,250 μg/ml) and (0.5 ml) methanol solution. Then shake the combination and sit in the dark for 30 minutes at room temperature. Ascorbic acid with the same sample concentrations and the identical procedure used as a positive control. We measured the absorbencies at (516 nanometers) using the spectrophotometer (ChemWell/USA) The lower reaction mixture absorbance indicated a higher free radical scavenging function. The study revealed radical scavenging behavior as the free radical inhibition factor. This inhibition percentage measured by adopting the coming formula,

% inhibition = (AC-AT) ÷ AC

Ac: absorbance of the control (solvent + DPPH without sample)

At: absorbance of the test sample.

These tests were conducted in triplicates and the findings were represented as an average value according (Sanja *et al.*, 2009).

Anticoagulant activity

A soft modification of a familiar procedure. Added (0.1 ml) of 4 coumarin derivatives (S_4H, S_4F, S_4Cl, S_4CO) with a series of concentrations (50, 100, 200) mg/ml, as optimistic control group, heparin (1IU/μl), solvent as control group to (0.9 ml) plasma just incubated at 37 ° C for 10 minutes for the next prothrombin time PT and enabled partial prothrombin time APTT evaluation as directed by the manufacturer For PT calculation (0.2ml) of PT reagent spend 15 min prewarming at 37 ° C applied to the plasma samples and control groups in the cuvette and calculated in seconds. Additionally, APPT assessment, the reagent was prewarmed at 37 ° C for 2 minutes and applied to the samples, added the Ca-solution kit and recorded clotting times. We carried these steps out in triplicate for each samples and control groups (Raposo *et al.*, 2015).

Anticancer activity

Inconsiderable modification on approval procedure It was accomplished by using the 96-well flat- bottomed microtitration plate, which involves three stages in this process. We separated the breast cancer cell line from their flasks as they went via trypsinization to the subconfluent monolayer. Added to the falcons 20 ml of culture medium with 10% serum and combined with cells to prepare for cell suspension It poured the suspension of cells into a culture flask a sterile beaker, then using microtitration, plate 96 well, 100 μl of cell suspension can pass to each well using multi micropipette, plates protected with a disinfect adhesive film, lid put on, shake and incubated in 5 percent CO2 incubator for 24 hours at 37 ° Supporting cell association, proliferation and convergence of monolayers. After 24 hours, we assessed cell viability for therapy by eliminating the medium, adding MTT (dye) solution for 100 μl of 2mg / ml and incubating at 37 C for 2 hours.

Solubilize the remaining crystals in the wells after extracting the MTT solution by adding 90 μl of DMSO with mild shaking accompanied by room temperature incubation in a darkened position for 20 minutes (Geraghty *et al.*, 2014).

RESULTS AND DISCUSSION

The synthesis of coumarinyl 1, 3, 4-oxadiazoles from -3-carboxylic acid through several sequential steps (Figure 1) and FTIR confirmed their structures with ^{1}H NMR spectrometry. To achieve the desired heterocycles, the sequence of reaction in the figure1 was followedCoumarin-3carboxylic acid esterification with methanol in the existence of sulfuric acid resulted in coumarin3carboxylic acid methyl ester (S1) characterized by the absence of a wide-band for OH stretching COOH group absorption in coumarin-3 carboxylic acid and appearance of two bands at 2933 cm-1 and 2880 cm-1 attributed to C-H stretching vibration for CH3 group. The key intermediate for the synthesis of substituted one, 3, 4-oxadiazole derivatives is coumarin-3-carboxylic acid hydrazide (S2), which was prepared by reaction of (S1) with hydrazine hydrate (80%). The FT-IR spectrum (S2) showed an absorption band in the region of 3385 cm$^-$ 1 of the NH2 group and 3290 cm-1 of the NH group. The C = O stretching vibration was observed at 1614 cm-^{1} in the amid-group, respect to (S2) with various substituted benzaldehyde in moderate to good yield, to form C=N bond at 1687 cm^{-1}. Then oxidation of S3 derivatives to give (S_4H, S_4Cl, S_4F, S_4OC) by acetyl anhydride to form coumarin -1,3,4-oxadiazole were confirmed using FT-IR spectra showing C-O- C asymmetric and symmetric stretching bands at 1253 and 1199 cm-^{1} respectively. Therefore, the 1676 cm-1 band for the C = N stretching combined with the disappearance of the NH2, NH and C=O amid stretching bands.Oxadiazole has an inductive effect due to the presence of heteroatom in the ring and is known to be a weak base. This consists of two pyridine-like nitrogen that exhibits the character of the conjugated diene form (Bhat *et al.*, 2005).

LD_{50} measurement

As summarized in (Table 1), the median lethal doses were calculated for the newly synthesized coumarin derivatives (S4H, S4F, S4CL and S4Co), as following (1150, 450, 550 and 950) mg/kg, respectively. S4H

Table 1: lethal dose evaluation of coumarin-oxadiazol derivatives

compound	M0	M1	LD_{50} (mg/kg)
S4H	1100	1200	1150
S4F	400	500	450
S4Cl	500	600	550
S4CO	900	1000	950

M0= Highest calculated dose were no mortality observed.
M1= Lowest calculated dose were mortality observed.

and S4CO appear to be the safest compounds with the highest LD50 values. On the other hand, S4F and S4CL appear to be more toxic with the lowest lethal dose. The high levels of LD_{50} for S4F and S4CL appear to be similar to the results observed by Ghate et al. with LD50 more than 1000mg/kg (Ghate *et al.*, 2005).

Antioxidant activity

Various compounds like polyphenols possess impressive antioxidant with potent radical scavenger's activity. In the present study, we use the DPPH scavenging assay to evaluate the capability of the four synthesized coumarin derivatives as antioxidants. As shown in (Figure 2), free radical scavenging activity was expressed as a percentage of radical scavenging (% inhibition). The more potent antioxidant activity notice is the S4Cl compound with IC_{50} 1.49 followed by S4CO compound with IC_{50} 5.29, S4Fcompound with IC_{50} 17.54, and S4H compound with IC50 18.79. We used ascorbic acid as a positive control for antioxidant comparison.

At low concentration, we had observed no significant differences between all tested compounds and standards. Increase dose associated with differenced regarding S4F and S4CO, as shown in (Figure 3).

Actually, all newly synthesized coumarin derivatives expressed considerable radical scavenging or antioxidant activity with IC_{50} range (1.49- 18.79) μg/ml. Our finding came in agreement with Kenchappa et al. they found that the presence of electron-withdrawing functional groups have promising antioxidant activities (Kenchappa *et al.*, 2017).

In general heterocyclic coumarin, molecules have been reviewed by Al-Majedy Y et al. for their radical scavenging activity, a series of coumarin derivatives with different substitutions gave rise to significant free radical scavenging activity making synthesized derivatives promising molecules to act as antioxidants, anticancer and reducing cardiovascular diseases with beneficial role in general health (Al-Majedy *et al.*, 2016).

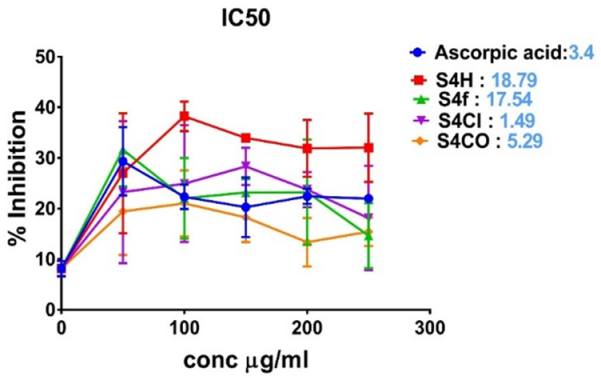


Figure 2: The effect of coumarin derivatives on percent inhibition for IC_{50} assessments at different concentrations. Ascorbic acid as + control

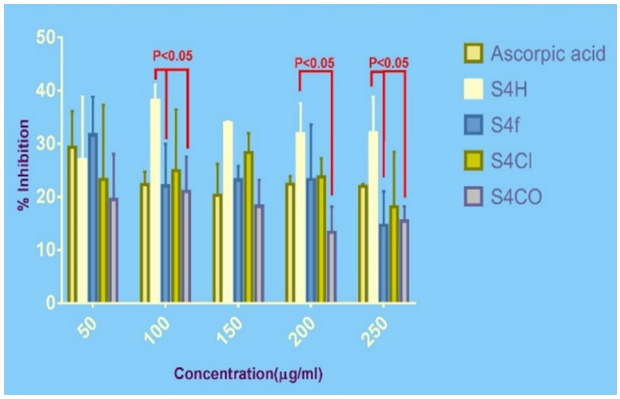


Figure 3: The effect of coumarin derivatives on free radical scavenging activity at different concentrations (% inhibition) associated with group comparison. P<0.05 considered significantly different

Anticoagulant activity

Clinical research suggest that anticoagulants are the top choices for thrombosis disorder prevention and treatment (Rishavy *et al.*, 2018) in the present research, we utilized PT and APTT to check the direct coagulation effect of coumarin derivatives. As shown in (Figure 4) with respect to S4H, 200 mg/ml prolonged coagulation time in the APTT test significantly compared to the control group but remain

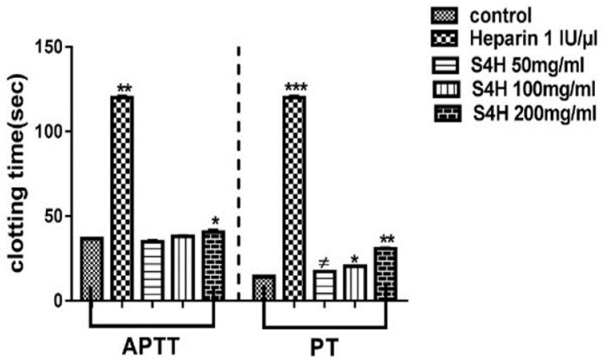


Figure 4: The effect of S4H on prothrombin time and partial thromboplastin time in different concentrations. Different characters (*, **, *, ≠) represent significantly difference between groups in the same test P<0.05**

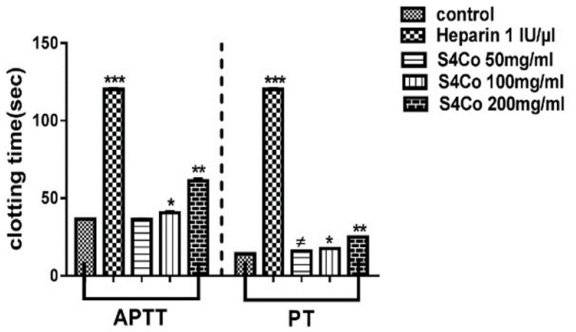


Figure 7: The effect of S4CL on prothrombin time and partial thromboplastin time in different concentrations. Different characters (*, **) represent significantly difference between groups in the same test P<0.05

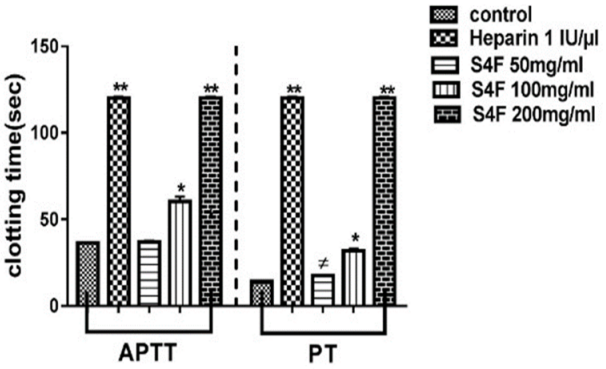


Figure 5: The effect of S4F on prothrombin time and active partial thromboplastin time in different concentrations. Different characters (*, **, *, ≠) represent significantly difference between groups in the same test P<0.05**

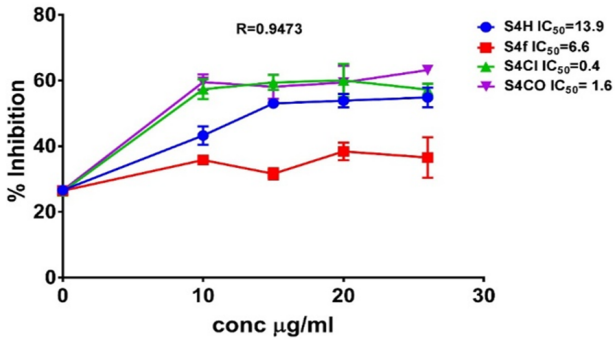


Figure 8: The effect of coumarin derivatives on percent inhibition for IC_{50} measurements at different concentrations

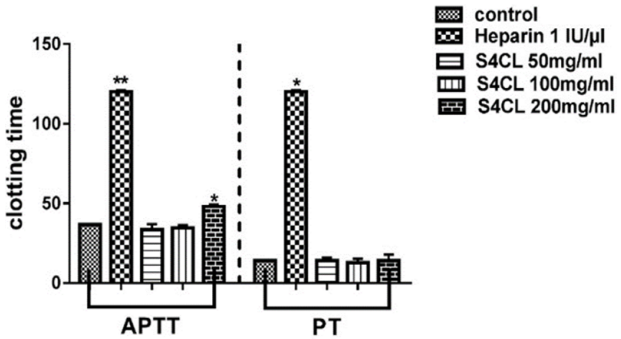


Figure 6: The effect of S4CL on prothrombin time and partial thromboplastin time in different concentrations. Different characters (*, **) represent significantly difference between groups in the same test P<0.05

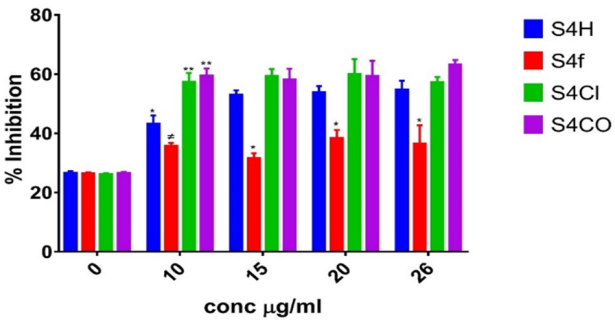


Figure 9: The effect of coumarin derivatives at the different concentrations on the availability of breast cancer cell (% inhibition) associated with group comparison. P <0.05 considered significantly difference

less than the positive control (heparin group) indicating anticoagulant activity. Whilst at low concentrations we watch no significant effect compared to control. To complete the idea about the coagulation effect, we measured PT. The clotting time prolongs with increase dose, such prolongation less than that watch with heparin treated group. We realize a significant prolongation of clotting time with a high dose of S4F coumarin derivative compared to control. An effect seems to be like with heparin in both PT and APTT tests. Low doses of S4F also prolong clotting time in the PT test only. As shown in (Figure 5). Proposition S4Cl has showed in (Figure 6) we observed no significant prolongation of clotting times on low and high doses on PT, except 200mg/kg slightly prolong on the APTT test. Regarding S4CO, significant prolongation of both prothrombin time and APTT in a dose-dependent manner, as shown in (Figure 7) Anticoagulant studies have been conducted to improve prothrombin time (PT and partial thromboplastin active time (APTT) at a different tested does. (Gurupadayya and Balasubramanyam, 2015) reported that the presence of electron with drawl substitution increase the activity against coagulation factor and coumarin derivatives presently available in the clinical field have been the core of anticoagulation therapy (Levine *et al.*, 2004).

Anticancer activity

Assessment of the IC50 values on the(MCF7) breast cancer cell line (Figure 8) revealed with calculated IC_{50} values that compounds (S4Cl 0.4microgram/ml) and (S4CO 1.6 microgram/ml) exhibited high anticancer activity and. Meanwhile, S4f exhibited a high anticancer activity with IC_{50} equal to 6.6microgram /ml. Compound S4h displayed moderate anticancer activity with IC_{50} values equal to 13.9 microgram/ml. When we compared anticancer activity between synthesized four coumarin derivatives, showed significant differences too clear at concentration 10microgram /ml regarding S4CL and S4CO, as shown in (Figure 9). We can relate this result depending on researchers showed that Coumarins could use various mechanisms to exercise their anticancer function; either by suppressing the telomerase enzyme (Adsule *et al.*, 2006), by inhibiting protein kinase activity and by decreasing oncogenic expression or by stimulating caspase-9 mediated apoptosis.

In addition, coumarins can inhibit the proliferation of cancer cells by stopping the progression of cells in G0/G1, G2//M phases (Chen *et al.*, 2012) and hydrazide-hydrazone (CO – NH – N = CH) motherhood play an important role as an antitumor agent (Kumar *et al.*, 2012; Terzioglu and Gürsoy, 2003).

CONCLUSIONS

The study included the synthesis of the new coumarin -1, 3, 4-oxadiazole compounds (S4H, S4F, S4Cl, and S4CO). This combination has been shown to be an extremely useful tool for the development of certain bioactive compounds., Taking into account that derived from 3-carboxylic acid coumarin(S0) and followed by the synthesis series of intermediate, S1, S2, S3H, S3F, S3CL, S3CO. The synthesized compounds S4CL and S4CO were tested as anti-cancer agents against MCF7 breast cancer cell, which gave a significant activity, S4F and S4CO gave good activity against clotting factor (as anticoagulant agents) PT and APTT comparing with heparin, and S4CL, S4CO and S4H which gave significant action against DPPH(as antioxidant agents). A good system for developing and synthesizing recent and more powerful drugs is the structure of such compounds.

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