Synthesis, Characterization and Antibacterial Evaluation of Some Coumarin Derivatives

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Abstract
Coumarin derivatives have shown different biological activities. New coumarin derivatives (hydrazones and an amide) were synthesized through multisteps reactions. All the synthesized target compounds were characterized by FT-IR spectroscopy and ¹H-NMR analysis. The compounds then evaluated for their anti-bacterial activity by means of Disc-well diffusion against two gram-positive bacteria (Staphylococcus aureus and Streptococcus pneumoniae) and two gram-negative bacteria (E. coli and Pseudomonas aeruginosa). The highest activity was demonstrated by compound k2 which found to be highly active against pseudomonas aeruginosa (containing NO2 group which is a strong electron withdrawing group that creates a localized electron deficient sites within molecules).

Key words: Coumarin, hydrazones, amide, antibacterial, well diffusion method.

Introduction
Heterocyclic chemistry is one of the main parts in organic chemistry and it is growing quickly. In 1998 heterocyclic synthesis accounted about 60 %. However today the heterocyclic synthesis is much bigger diverse fields such as pharmaceuticals, biochemistry materials and others are the areas which new heterocyclic compounds are published (1). The same development is perceived for coumarin. Coumarin is a white crystalline solid with a scent like a new mown-hay. The chemical structure of coumarin consist of an aromatic ring fused to an unsaturated cyclic lactone ring (2). Coumarin (which is referred 1,2-benzopyrone or less commonly, as o-hydroxycinnamic acid-8-lactone) a natural heterocyclic organic aromatic compound, present in a widespread variability of microorganisms and higher plants (3,4), vogel was first one who insulate coumarin in 1820 by extracting from the tonka beans (Dipteryx odorata) specie previously known as Coumarona odorata, later the term coumarin. It has been then identified in a huge number of the plants which belong to numerous diverse families (5).

Coumarin is classified as a member of the benzopyrone family of compounds, all of which consist of a benzene ring joined to a pyrone ring. The benzopyrones may be divided into benzo-α-pyrones (1) to which the coumarins fit and (2) benzo-γ-pyrones named as chromones, of which flavonoids are the main chemical member, the later differs from the earlier only in the position of the carbonyl group in the heterocyclic ring. The various biological activities coumarin derivatives such as anticoagulants and antithrombotics are well known, so that; they are active for the inhibition and treatment of venous and arterial thrombosis (6), a number of coumarin derivatives are also described as antifungal and bacterial agents (7), antiviral and antitumor agents (8), lipid-lowering agents (9), HIV agents (10), antioxidants and lipoygenase inhibitor (11). They have also been found to possess antiproliferative, vasorelaxing activities (12), anti-inflammatory activity, anthelmintic, hypnotic, insecticidal activities, and diuretic properties (13). The coumarins vary highly in their structure, as a result of the different substitution types in their main structure, which can affect their biological activities. The 4-hydroxycoumarin comprises the structural nucleus of many natural products, drugs and pesticides.

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It is the key intermediate for different widely used anticoagulants and rodenticides. As well as drugs used as antithrombotic agents in humans. Coumarin-3-carboxylic acids have anti-tumor and cyto-cell selective effects. 7-hydroxy-4-methylcoumarin could be used as a cardioactive drug by inhibition of calcium influx.

Schiff bases are the compounds carrying imine (–C=N–) functional group, and were first reported by Hugo Schiff. Schiff bases have a significance in medicinal and pharmaceutical fields as a result of a broad spectrum of biological activities such as anti-inflammatory, analgesic, antimicrobial, anticonvulsant, antitubercular, anticancer, antioxidant, anthelmintic, etc. The nitrogen atom of azomethine may be involved in the formation of a hydrogen bond with the active centers of cell constituents and interferes in normal cell processes more. They are very useful as catalysts, intermediates in organic synthesis, pigments, dyes, polymer stabilizers. It was found that the Coumarin moiety was introduced with the Schiff base, the compound synthesized may have some remarkable pharmacological and microbiological activity.

It was found that the Coumarin moiety was introduced with the Schiff base, the compound synthesized may have some remarkable pharmacological and microbiological activity. Amide bond creation is one of the most significant chemical reactions. The occurrence of amides can be seen in natural peptides, proteins, and many amide-bond containing biomolecules as well as in several synthetic compounds with various uses such as pharmaceutically active compounds or synthetic polymers. Amides are of great importance in organic chemistry especially because of their high stability and polarity and their conformational variety. Coumarin contains amide moieties and have drawn considerable amount of interest because of their pharmacological properties and their synthetic derivatives having a significant pharmacological and microbiological activity.

Mohd et al. synthesized new coumarin compounds that screened in vitro for their antibacterial activity against the two bacterial strains, *E. coli* (Gram –ve) and *B. cereus* (Gram + ve). N-(4-bromo-2-fluorophenyl)-6,8-dichloro-2-oxo-2H-chromene-3-carboxamide with dichloro substitution showed maximum growth inhibition in comparison with the rest of the compounds against both bacterial strains.

**Materials and Methods**

Coumarin-3-carboxylic acid was bought from Hyper-chem. Company (china). Solvents (dichloromethane, ethyl acetate, ethanol, Hexane, toluene) (AR) and other reagents that used through reaction were bought from commercial sources. The purity of products and monitoring of the reactions were done by thin layer chromatography TLC(GF254, merck- Germany) under UV light (254nm). Two solvent systems were used S1 [(toluene: ethyl acetate: ethanol (3:2:1)] and S2 [(hexane:ethyl acetate:ethanol (3:2:1)] (by trial). Melting points were uncorrected and detected by using Stuart SMP3 melting point apparatus in open capillary tubes. FT-IR spectra were done by thin film technique (υ, cm-1), (Shimadzu FT-IR spectrophotometer, Japan) at College of pharmacy/University of Baghdad. 1H-NMR were done, using Brucker ultra shield model 500 MHz at (University of Tehran, Iran) using DMSO as a solvent.

**Chemical synthesis**

Schemes (1), (2), and (3) show the synthesis of targeted compounds.
Scheme 1. Synthesis of N'-benzylidene-2-oxo-2H-chromene-3-carboxyhydrazide derivatives compounds (H1-H2)

Scheme 2. Synthesis of N,N-diethyl-2-oxo-2H-chromene-3-carboxamide (B).
Scheme 3. Synthesis of N’-(1-(2-oxo-2H-chromen-3-yl) ethylidene) benzohydrazide derivatives compounds (K1,K2).

**Synthesis of coumarin hydrazide 2-oxo-2H-chromene-3-carbohydrazide (A):**

Coumarin-3-carboxylic acid (10 mmol)(1.9g) were dissolved in Dichloromethane (DCM)(60 mL) And stirred at room temperature. Then, HOBt (Hydroxybenzotriazole) (11.84 mmol)(1.6g) was added followed by adding EDCI (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) (12 mmol)(2.3g). The mixture was stirred overnight at room temperature, which was then gradually added to a solution of hydrazine (60 mmol,0.64g) and cyclohexene (1 mL) in DCM (5mL) while the temperature was kept between 0-10°C( using ice bath and check the temperature by thermometer) . The reaction was completed upon the end of addition. The organic layer wash with 15 ml of 5% sodium carbonate to eliminate HOBt and the excess of acid. Elimination of the solvents under reduced pressure gave hydrazide which was recrystallized from methanol.

**Synthesis of final N’-benzylidene-2-oxo-2H-chromene-3-carboxyhydrazide derivatives compounds (Compounds H1-H2)**

Three drops of glacial acetic acid were added to an ethanolic solution of each of the following aldehydes shown in detail in Table 1.1 :

| NO | Name                          | Quantity       | Molecular weight |
|----|-------------------------------|----------------|------------------|
| 1- | 3,5-Dimethoxy-4-hydroxybenzaldehyde | (5 mmole, 0.91g) | 182.17 g/mol     |
| 2- | 4-hydroxy-benzaldehyde         | (5mmole, 0.61g) | 122.12 g/mol     |

Synthesis of coumarin 3 carboxylic amide (2-oxo-2H-chromene-3-carboxamide (B):

Coumarin-3-carboxylic acid (9.9 mmol)(1.9g) were dissolved in DCM (20 mL) stirred at room temperature. Then, HOBt (11.84 mmol)(1.6g) followed by adding of (9.3 mmol)(1.8g) EDCi (9.3 mmol)(1.8g) . The reaction was stirred at room temperature . After that a solution of diethyl amine (19.1 mmol)(1.4g) and cyclohexene (1 mL) in DCM (10 mL) was added gradually to above solution while temperature was kept between 0-10 °C during the addition. The reaction mixture stirred overnight (TLC monitored), the mixture was washed with aqueous solutions of 0.1N HCL (15 mL) and 5% sodium bicarbonate (15 mL) to eliminate the excess of amine and acid. The organic layer was evaporated.
and the precipitate was dried and recrystallized from ethanol. 

**Synthesis of N’-(1-(2-oxo-2H-chromen-3 yl)ethylidene)benzoylhydrazide derivatives (K1-K2)**

Three drops of glacial acetic acid were added to an ethanolic solution of (5mmole) Coumarin 3 acetyl placed in a round flask equipped with magnetic stirrer. Both of 2 hydrazides vanalic acid hydrazide (5mmole,0.9g, MW 182.18 g/mol) and P-nitro –benzylhydrazide (5mmole,0.9g, MW 122.12 g/mol) was added separately to above stirred solution. Then reaction mixture was refluxed at 80°C for 2 h. At the end of the reaction (monitored by TLC), 50 mL of cold ice water was added to the crude product. The precipitate formed was filtered, dried and recrystallized from ethanol.

**Antibacterial essay**

Disc-well diffusion method has been performed through the use of the bacterial suspension of (1.5x10⁴ CFU/ml) obtained from McFarland standard of turbidity (number 0.50). Which has been utilized for inoculation by the swabbing of Mueller Hinton Agar (MHA) plates’ surface. The excess liquid has been dried by air under a sterile hood. In every one of the agar plates of the examined bacteria, 4 wells have been made, and (80μl) of every one of the concentrations of the synthesized compound was poured to it. The plates have been incubated for 24h at 37°C. The evaluation of antibacterial activity has been based upon the measurement of the inhibition zone diameter which is formed around the well. 

**Results and Discussion**

The synthesis of A and B was done by reaction of coumarin-3-carboxylic acid with hydrazine hydrate and diethyl amine, respectively, in the presence of coupling agent (EDCI) and catalyst (HOBT). Schiff base products (H1, H2, K1, and K1) were the results of the reactions between Coumarin-3-carboxylic hydrazide with different aldehydes (H1 and H2) or the reactions between coumarin-3-acetyl with two hydrazides (K1 and K2) in ethanol using glacial acetic acid as catalyst.

**Chemistry**

Compound A: (C₁₀H₁₀N₂O₃), Green yellow powder, yield= 86%, M.P: 204-206 ⁰C, Rₛ =0.68 (S1).

FT-IR (5 cm⁻¹): 3319, 3273(NH) str. of hydrazide, 1604 (C=O) str. of amide, 1705 Coumarin (C=O).

Compound H1: (C₁₁H₁₂N₂O₄), Yellowish powder, yield: 82%, M.P: (156-160 ⁰C), Rₛ =0.82(S1).

FT-IR (6 cm⁻¹): 3205 (NH) str. of hydrazide, 3159 (OH) str. (Broad), 3051 Aromatic (C-H) str., 1651 (C=O) str. of amide, 1615 (C=N) str., 1701.22 Coumarin (C=O).

1H-NMR: 11.58ppm (1H,s,-CO-NH), 9.99ppm (1H,s,-OH), 8.89ppm (1H,s,N=C-H), 6.83-8.00ppm (8H,m,Ar-H), 8.32ppm (1H,s,C4-H)

Compound H2: (C₁₁H₁₀N₂O₄), yellowish brown powder, yield = 70% M.P: (170-172⁰C), Rₛ =0.78 (S1).

Compound K1: (C₁₉H₁₂N₂O₅), White powder, Yield: 70%, M.P.: (173 – 174⁰C, Rₛ =0.84(S1)).

FT-IR (6 cm⁻¹): 3255 (NH) str. of hydrazide, 3060 Aromatic (C-H) str., 1660 (C=O) str., 1720 coumarin (C=O), 1519 (NO₂) asndm. str., 1651(C=N) str.

1H-NMR: 2.35ppm (3H,s,-CH₃), 7.45-8.35ppm (8H,m,Ar-H), 8.27ppm (1H,s,C4-H), 11.13ppm (1H,s,-CO-NH).

Compound K2: (C₁₉H₁₀N₂O₅), Yellowish powder, Yield: 82%, M.P: 165-167⁰C, Rₛ =0.80(S1).

FT-IR (6 cm⁻¹): 3322 (NH) str. of hydrazide, 3051 Aromatic (C-H) str., 2939 asymm. str. of CH₃, 2835 symm. str. of CH₃, 1647 (C=O) str. of amide, 1701.22 Coumarin (C=O), 1624 (C=O) str.

1H-NMR: 2.33ppm (3H,s,CH₃), 3.84ppm (3H,s,-O-CH₂), 6.88-7.85ppm (7H,m,Ar-H), 8.2ppm (1H,s,C4-H), 9.7ppm (1H,s,OH), 10.57ppm (1H,s,-CO-NH).

**Antibacterial evaluation**

The antibacterial activities of the synthesized derivatives (B,H1,H2,K1,K2) were measured using disc well diffusion method technique, tested on gram +ve and gram -ve bacteria, Using amoxicillin as standard. DMSO was used as a solvent and as a control, as shown in the following Table (2) .
Table 2. The antibacterial activity of the target compounds (B, H1-H2, K1, K2).

| Compound NO | Conc. µg/ml | pseudomonas aeruginosa | Staphylococcus aureus | streptococcus pneumoniae | E.coli |
|-------------|-------------|------------------------|-----------------------|------------------------|--------|
|             |             | Inhibition Zone (mm)    |                       |                        |        |
| B           | 10³         | 15                     | -----                 | -----                  | 12     |
| H1          | 10³         | 14                     | -----                 | -----                  | 10     |
| H2          | 10³         | 12                     | 6                     | 10                     | 15     |
| K1          | 10³         | 10                     | 6                     | ----                   | 12     |
| K2          | 10³         | 22                     | ----                  | ----                   | 14     |
| Amoxicilin  | 10³         | 10                     | 23                    | 20                     |        |

(-)= No activity, slightly active: (Inhibition zone in between 5-10 mm), moderately active: (Inhibition zone in between 10-15 mm), and highly active (Inhibition zone more than 15 mm) (35)

Discussion

Compounds A, B were synthesized by the reaction of coumarin 3 carboxylic acid with hydrazine hydrate (NH₂·H₂O) or amines. This reaction involves several parts of mechanism. The first part involves two stages that include conversion of the carboxyl group into a good leaving group. The first stage include acid base reaction resulted in the transfer of proton from carboxylic acid to the EDCI, the second stage involves attacking of RCO2 which is formed from the first stage on the EDCI (conjugate acid).

The second part includes the addition of HOBt that is supposed to work by primarily reacting with the O-acylsourea to give the OBT active ester, which improves the reactivity of the “activated ester” by encouraging/ stabilizing the method of the amine through hydrogen bonding. On the other hand, HOBt can produce by-products, thus it catalyses the creation of diazetidine.

The third part involves formation of hydrazide/ amides that is due to the last procedure which is the removal of the leaving group (water soluble urea) (36,37).

Scheme 1. Mechanism of the synthesis of coumarin 3 carboxylic hydrazide and amide. When R group is NH₂ The whole structure is hydrazide but if R group is alkyl, aryl or H group the whole structure is Amide.
The final compounds (H1, H2, K1 and K2) were synthesized by the reaction of Coumarin-3-carboxylic hydrazide with different aldehydes and coumarin-3-acetyl (as ketone) with two hydrazides by using glacial acetic acid as a catalyst. Aldehydes or ketones react with primary amine of hydrazide to form imines in a two-step reaction.

Regarding the first step of the reaction; the amine adds to the carbonyl group to form carbinolamine. Carbinolamine then undergoes dehydration to give N-aryl-substituted imine. The steps of the reaction are catalyzed by the addition of a few drops of acid.

Among the synthesized derivatives, compounds (B and H1) show moderate activity against pseudomonas aeruginosa. Furthermore, compounds (H2 and K1) show slight activity against *Staphylococcus aureus*, compound K2 show moderate activity against *E. coli*. The highest activity was demonstrated by compound K2 which found to be highly active against *Pseudomonas aeruginosa* (due to the presence of Nitro functional group which is strong electron withdrawing group) which was found to be highly active against *Pseudomonas aeruginosa*.

**Conclusion**

New coumarin hydrazones and amide derivatives were synthesized successfully. Their chemical structure were characterized using IR spectroscopy and ^1^H NMR. The antibacterial activity of target compound (B,H1,H2,K1,K2) was evaluated using Disc-well diffusion. The highest activity was demonstrated by compound K2 (ionized containing NO2 which is a strong electron withdrawing group) which was found to be highly active against *Pseudomonas aeruginosa*.

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