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# Synthesis, Characterization, And Anticoagulant Activity Of New Functionalized Biscoumarins

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**Abstract**

Despite their rarity and structural complexity, natural and synthetic biscoumarins have polarized much attention from investigators particularly due to their characteristic activity as anticoagulant agents. In this work, a panel of twelve functionalized biscoumarins was synthesized in two schematic steps; the first one started by condensing various phenol-based derivatives with malonic acid via a Pechmann-type reaction yielding alkyl-substituted 4-hydroxycoumarins herein symbolized as (**E1**-**E12**). The latter compounds were undergone a self-coupling under the influence of methylene iodide to afford the target functionalized biscoumarins, which were symbolized as (**EY1**-**EY12**). The potential of the synthesized biscoumarins as anticoagulant applicants was investigated *in vivo* using rabbit as an animal model. The employed assay was the prothrombin time that was monitored after three and five days of the last oral treatment. The results gathered from this test revealed that the synthesized biscoumarins have a promising anticoagulant activity compared with warfarin as a standard anticoagulant drug, with privileged influence contributed to those substituted at position 7 of the coumarin framework. The authors concluded that the substitution of an alkyl group at that position of the coumarin monomer may intensify the anticoagulant activity of the prepared biscoumarins. Also, this intensity was directly proportionated to the increase in the molecular weight of this alkyl group. Accordingly, the synthesized biscoumarins possessing this property would provide an efficient base for synthesizing new compounds, which have a promising anticoagulant effect.

*Keywords*: Biscoumarin; Prothrombin time; Rabbit; Anticoagulant; Pechmann reaction; Self-coupling.

## 1. Introduction

Since its original isolation via Vogel, coumarin and its based products and compounds have attracted much research concern not only because of their fascinating structural properties but also due to their multifactorial biological activities [1]. Of those, anti-inflammatory, anticoagulant [2], anti-HIV [3], antioxidant [4], antidiabetic [5], anticancer [6], antibacterial [7], and antifungal [8] potentials are widely investigated.

Cardio-cerebrovascular diseases (**CCVDs**) caused by thromboembolism pose a serious threat to human health [9], and they are by far the primary leading cause of death worldwide accounting for about 17.9 million people died in 2015 [9]. For decades, coumarin-based compounds like dicoumarol are being employed as a building precursor of many prophylactic and therapeutic regimes concerning **CCVDs** [10]**.**

Biscoumarins, also known as dimeric coumarins, are an imperative class of biologically active coumarins [11]. Although biscoumarins are characterized by their rarity and structural complexity [12], many investigators have shifted their efforts towards the isolation, synthesis, and exploring the biological activities of these coumarin-phenotypes [13]. Among the explored activities, the anticoagulant potential has acquired priority [14]. To study the anticoagulant effect, Prothrombin time (PT) was conducted in this study because it is commonly used assay method [15]. This test detects the required time in seconds for the clot to being formed as the citrated sample of plasma is mixed with a thromboplastin reagent [16], [17].

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This work aims to synthesize a series of twelve functionalized biscoumarins starting from the condensation of the phenol-based derivative with malonic acid. The potential of the synthesized biscoumarins as anticoagulant applicants was tested *in vivo* utilizing rabbit as an animal model. This was carried out by monitoring the influence of these compounds on the **PT** at the third and fifth days next to the last oral dose.

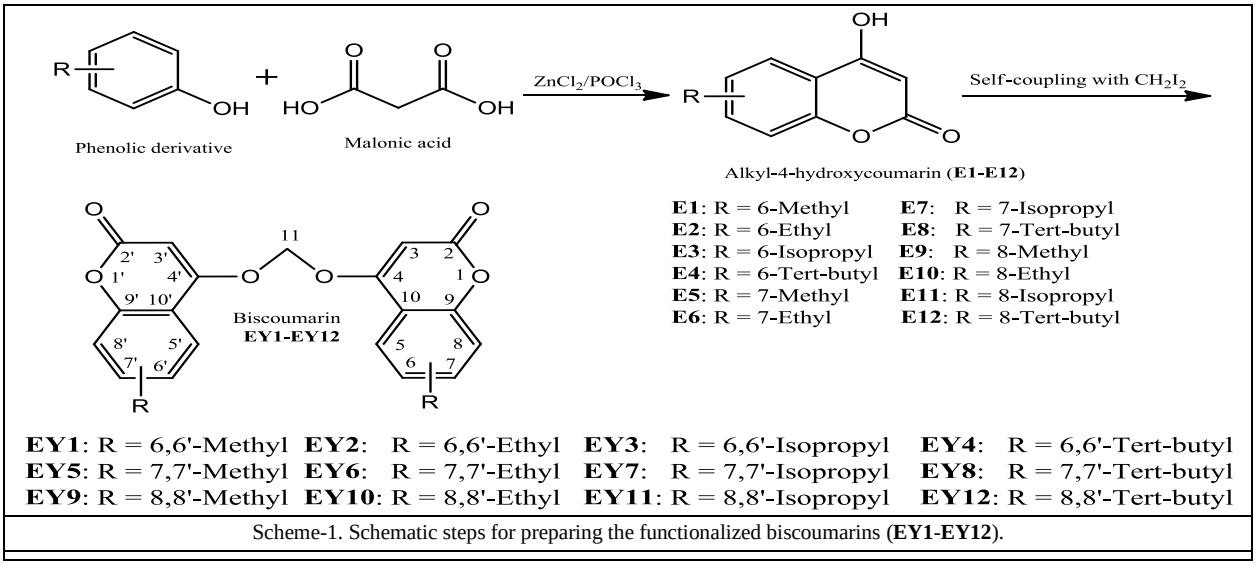
## 2. Experimental

### *2.1. General information*

The chemicals, solvents, and reagents used in the synthesis of the intermediate compounds and target functionalized biscoumarins, and the evaluation of their anticoagulant activity were ordered from several international resources including Labcorp, Scharlau, Sigma-Aldrich, Haihang, and Chem-Lab. The digital melting point apparatus named electrothermal IA9300 was employed to observe the melting points (mp) of the prepared compounds using the one-open capillary tube technique. To trace the synthetic reaction progress and confirm the purity of the resultant compounds, an ascending thin-layer chromatography technique was carried out using Millipore Sigma™ TLC-Silica Gel 60 ($F_{254}$) as a stationary phase and $CHCl_3$: MeOH (3:1) mixture as eluent. The spectrophotometers employed to elucidate the chemical structures of the prepared functionalized biscoumarins were Cary 300 UV-Vis Bio, Bruker-Avance III HD 600MHz (DMSO-$d_6$), and Bruker FTIR-α-ATR.

### *2.2. Synthetic pathway*

The synthetic steps that followed to prepare the functionalized biscoumarins (**EY1**-**EY12**) are illustrated in Scheme-1.




Scheme-1. Schematic steps for preparing the functionalized biscoumarins (**EY1**-**EY12**).

### *2.3. General method for synthesizing alkyl-4-hydroxycoumarins (**E1**-**E12**)*

A mixture of phenol-based derivative (10 mmol), malonic acid (1.04 g, 10 mmol), anhydrous zinc chloride (3 g, 22 mmol), and phosphoryl chloride (4 ml, 43 mmol) was heated for 10 hr using a hot water-bath adapted at 70°C. The reaction mixture was rained as one portion onto a 500 ml crushed ice-$H_2O$ mixture. After a harmonic stirring via glass rod, the separated solid was treated with 30 ml of 10% aqueous $Na_2CO_3$ solution, filtered, acidified, and purified by recrystallizing from the ether [18]. The physicochemical properties and spectral interpretations of the synthesized alkyl-4-hydroxycoumarins (**E1**-**E12**) were closely similar to those recorded by Karia et al [19].

### *2.4. General method for synthesizing 2,2'-bis(alkyl-4-comarinyl-oxy)methylene compounds (Functionalized biscoumarins, **EY1**-**EY12**)*

A mixture of alkyl-4-hydroxycoumarin (5 mmol), anhydrous potassium carbonate (1.38 g, 10 mmol), and methylene iodide (0.2 ml, 2.5 mmol) in dry

acetone was refluxed for 35 hr. The reaction mixture was filtered while hot and evaporated, and the crude was recrystallized from ethanoic acid [20].

**2,2'-Bis(6-methyl-4-comarinyl-oxy)methylene (EY1)**: Pale yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(6-methyl-2*H*-chromen-2-one); mp = 202-205°C; $\lambda_{max}$ (EtOH)=544 nm; %yield=62 (1.13 g); FTIR (ν, stretching, $cm^{-1}$): 3042 (alkene CH), 2913 (alkane CH), 1706 (ester C=O), 1689 (alkene C=C), 1586 (aromatic C=C), 1223 and 1053 (ether C-O-C); $^{1}$H-NMR: δ= 7.68 (2H, s, H-5, H-5'), 7.42 (2H, d, *J*=6 Hz, H-8, H-8'), 7.32 (2H, d, *J*=6 Hz, H-7, H-7'), 6.74 (2H, s, H-11), 5.52 (2H, s, H-3, H-3'), 2.43 (6H, s, $CH_3$-6, $CH_3$-6') ppm; $^{13}$C-NMR: δ= 169.2 (C-4, C-4'), 162.0 (C-2, C-2'), 152.1 (C-9, C-9'), 137.3 (C-6, C-6'), 134.1 (C-7, C-7'), 129.1 (C-5, C-5'), 118.4 (C-10, C-10'), 116.0 (C-8, C-8'), 88.5 (C-3, C-3'), 82.2 (C-11), 22.6 ($CH_3$-C-6, $CH_3$-C-6') ppm.

**2,2'-Bis(6-ethyl-4-comarinyl-oxy)methylene (EY2)**: Pale yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(6-ethyl-2*H*-chromen-2-one); mp = 189-191°C; $\lambda_{max}$ (EtOH)=539 nm; %yield=60 (1.18 g); FTIR (ν, stretching, $cm^{-1}$): 3033 (alkene CH), 2907 (alkane CH), 1705 (ester C=O), 1688 (alkene C=C), 1584 (aromatic C=C), 1222 and 1055 (ether C-O-C); $^{1}$H-NMR: δ= 7.63 (2H, s, H-5, H-5'), 7.43 (2H, d, *J*=6 Hz, H-8, H-8'), 7.36 (2H, d, *J*=6 Hz, H-7, H-7'), 6.72 (2H, s, H-11), 5.50 (2H, s, H-3, H-3'), 2.40 (4H, m, $CH_2$-6, $CH_2$-6'), 1.36 (6H, t, $CH_2C\underline{H}_3$-6, $CH_2C\underline{H}_3$-6') ppm; $^{13}$C-NMR: δ= 169.1 (C-4, C-4'), 162.3 (C-2, C-2'), 152.0 (C-9, C-9'), 137.3 (C-6, C-6'), 134.4 (C-7, C-7'), 129.6 (C-5, C-5'), 118.9 (C-10, C-10'), 116.1 (C-8, C-8'), 88.6 (C-3, C-3'), 82.1 (C-11), 31.2 ($\underline{C}H_2CH_3$-C-6, $\underline{C}H_2CH_3$-C-6'), 16.5 ($CH_2\underline{C}H_3$-C-6, $CH_2\underline{C}H_3$-C-6') ppm.

**2,2'-Bis(6-isopropyl-4-comarinyl-oxy)methylene (EY3)**: Yellowish powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(6-isoproyl-2*H*-chromen-2-one); mp = 209-211°C; $\lambda_{max}$ (EtOH)=561 nm; %yield=55 (1.16 g); FTIR (ν, stretching, $cm^{-1}$): 3036 (alkene CH), 2867, 2945 (alkane CH), 1702 (ester C=O), 1688 (alkene C=C), 1580 (aromatic C=C), 1228 and 1056 (ether C-O-C); $^{1}$H-NMR: δ= 7.62 (2H, s, H-5, H-5'), 7.43 (2H, d, *J*=6 Hz, H-8, H-8'), 7.35 (2H, d, *J*=6 Hz, H-7, H-7'), 6.74 (2H, s, H-11), 5.53 (2H, s, H-3, H-3'), 2.94 (2H, m, CH-6, CH-6'), 1.24 (12H, d, $CHC\underline{H}_3$-6, $CHC\underline{H}_3$-6') ppm; $^{13}$C-NMR: δ= 169.0 (C-4, C-4'), 162.3 (C-2, C-2'), 152.4 (C-9, C-9'), 142.0 (C-6, C-6'), 134.2 (C-7, C-7'), 126.1 (C-5, C-5'), 119.1 (C-10, C-10'), 116.3 (C-8, C-8'), 88.6 (C-3, C-3'), 82.1 (C-11), 36.1 ($\underline{C}HCH_3$-C-6, $\underline{C}HCH_3$-C-6'), 24.2 ($CH\underline{C}H_3$-C-6, $CH\underline{C}H_3$-C-6') ppm.

**2,2'-Bis(6-*tert*-butyl-4-comarinyl-oxy)methylene (EY4)**: Dark yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(6-*tert*-butyl-2*H*-chromen-2-one); mp = 224-226°C; $\lambda_{max}$ (EtOH)=584 nm; %yield=51 (1.16 g); FTIR (ν, stretching, $cm^{-1}$): 3033 (alkene CH), 2882, 2972 (alkane CH), 1704 (ester C=O), 1688 (alkene C=C), 1583 (aromatic C=C), 1224 and 1055 (ether C-O-C); $^{1}$H-NMR: δ= 7.90 (2H, s, H-5, H-5'), 7.62 (2H, d, *J*=6 Hz, H-7, H-7'), 7.43 (2H, d, *J*=6 Hz, H-8, H-8'), 6.73 (2H, s, H-11), 5.48 (2H, s, H-3, H-3'), 1.49 (18H, s, C-$C\underline{H}_3$-6, C-$C\underline{H}_3$-6') ppm; $^{13}$C-NMR: δ= 169.3 (C-4, C-4'), 162.2 (C-2, C-2'), 153.6 (C-6, C-6'), 152.1 (C-9, C-9'), 134.0 (C-7, C-7'), 126.6 (C-5, C-5'), 119.2 (C-10, C-10'), 116.1 (C-8, C-8'), 88.6 (C-3, C-3'), 82.0 (C-11), 36.2 ($\underline{C}$-$CH_3$-C-6, $\underline{C}$-$CH_3$-C-6'), 32.7 (C-$\underline{C}H_3$-C-6, C-$\underline{C}H_3$-C-6') ppm.

**2,2'-Bis(7-methyl-4-comarinyl-oxy)methylene (EY5)**: Pale yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(7-methyl-2*H*-chromen-2-one); mp = 190-192°C; $\lambda_{max}$ (EtOH)=541 nm; %yield=54 (0.98 g); FTIR (ν, stretching, $cm^{-1}$): 3036 (alkene CH), 2911 (alkane CH), 1705 (ester C=O), 1688 (alkene C=C), 1582 (aromatic C=C), 1225 and 1052 (ether C-O-C); $^{1}$H-NMR: δ= 7.82 (2H, d, *J*=6 Hz, H-5, H-5'), 7.12 (2H, d, *J*=6 Hz, H-6, H-6'), 7.04 (2H, d, *J*=6 Hz, H-8, H-8'), 6.74 (2H, s, H-11), 5.53 (2H, s, H-3, H-3'), 2.44 (6H, s, $CH_3$-7, $CH_3$-7') ppm; $^{13}$C-NMR: δ= 169.2 (C-4, C-4'), 162.0 (C-2, C-2'), 152.1 (C-9, C-9'), 145.7 (C-7, C-7'), 128.2 (C-5, C-5'), 126.1 (C-6, C-6'), 119.3 (C-8, C-8'), 116.2 (C-10, C-10'), 88.5 (C-3, C-3'), 82.1 (C-11), 22.4 ($CH_3$-C-7, $CH_3$-C-7') ppm.

**2,2'-Bis(7-ethyl-4-comarinyl-oxy)methylene (EY6)**: Pale yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(7-ethyl-2*H*-chromen-2-one); mp = 221-223°C; $\lambda_{max}$ (EtOH)=542 nm; %yield=50 (0.98 g); FTIR (ν, stretching, $cm^{-1}$): 3037 (alkene CH), 2876 (alkane CH), 1701 (ester C=O), 1689 (alkene C=C), 1585 (aromatic C=C), 1228 and 1053 (ether C-O-C); $^{1}$H-NMR: δ= 7.86 (2H, d, *J*=6 Hz, H-5, H-5'), 7.25 (2H, d, *J*=6 Hz, H-6, H-6'), 7.12 (2H, s, H-8, H-8'), 6.74 (2H, s, H-11), 5.52 (2H, s, H-3, H-3'), 2.72 (4H, m, $C\underline{H}_2CH_3$-7, $C\underline{H}_2CH_3$-7'), 1.38 (6H, t, $CH_2C\underline{H}_3$-7, $CH_2C\underline{H}_3$-7') ppm; $^{13}$C-NMR: δ= 169.4 (C-4, C-4'), 162.6 (C-2, C-2'), 151.7 (C-9, C-9'), 146.2 (C-7, C-7'), 128.3 (C-5, C-5'), 125.7 (C-6, C-6'), 123.1 (C-8, C-8'), 114.6 (C-10, C-10'), 88.5 (C-3,

C-3'), 82.1 (C-11), 29.6 ($CH_3\underline{C}H_2$-C-7, $CH_3\underline{C}H_2$-C-7'), 16.1 ($\underline{C}H_3CH_2$-C-7, $\underline{C}H_3CH_2$-C-7') ppm.

**2,2'-Bis(7-isopropyl-4-comarinyl-oxy)methylene (EY7)**: Yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(7-isopropyl-2*H*-chromen-2-one); mp = 201-203°C; $\lambda_{max}$ (EtOH)=566 nm; %yield=46 (0.97 g); FTIR (ν, stretching, $cm^{-1}$): 3032 (alkene CH), 2856, 2947 (alkane CH), 1702 (ester C=O), 1686 (alkene C=C), 1589 (aromatic C=C), 1223 and 1052 (ether C-O-C); $^1$H-NMR: δ= 7.84 (2H, d, *J*=6 Hz, H-5, H-5'), 7.14 (2H, d, *J*=6 Hz, H-6, H-6'), 7.04 (2H, s, H-8, H-8'), 6.73 (2H, s, H-11), 5.45 (2H, s, H-3, H-3'), 2.94 (2H, m, $C\underline{H}CH_3$-7, $C\underline{H}CH_3$-7'), 1.24 (6H, d, $CHC\underline{H}_3$-7, $CHC\underline{H}_3$-7') ppm; $^{13}$C-NMR: δ= 169.5 (C-4, C-4'), 162.3 (C-2, C-2'), 150.5 (C-9, C-9'), 149.9 (C-7, C-7'), 128.0 (C-5, C-5'), 123.4 (C-6, C-6'), 119.4 (C-8, C-8'), 115.2 (C-10, C-10'), 88.5 (C-3, C-3'), 82.2 (C-11), 35.2 ($CH_3\underline{C}H$-C-7, $CH_3\underline{C}H$-C-7'), 24.9 ($\underline{C}H_3CH$-C-7, $\underline{C}H_3CH$-C-7') ppm.

**2,2'-Bis(7-*tert*-butyl-4-comarinyl-oxy)methylene (EY8)**: Yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(7-*tert*-butyl-2*H*-chromen-2-one); mp = 212-214°C; $\lambda_{max}$ (EtOH)=569 nm; %yield=41 (0.92 g); FTIR (ν, stretching, $cm^{-1}$): 3038 (alkene CH), 2878, 2982 (alkane CH), 1705 (ester C=O), 1688 (alkene C=C), 1588 (aromatic C=C), 1226 and 1054 (ether C-O-C); $^1$H-NMR: δ= 7.90 (2H, d, *J*=6 Hz, H-5, H-5'), 7.38 (2H, d, *J*=6 Hz, H-6, H-6'), 7.30 (2H, s, H-8, H-8'), 6.72 (2H, s, H-11), 5.45 (2H, s, H-3, H-3'), 1.34 (18H, 2, C-$C\underline{H}_3$-7, C-$C\underline{H}_3$-7') ppm; $^{13}$C-NMR: δ= 169.4 (C-4, C-4'), 162.2 (C-2, C-2'), 153.8 (C-7, C-7'), 150.9 (C-9, C-9'), 128.1 (C-5, C-5'), 122.9 (C-6, C-6'), 119.4 (C-8, C-8'), 115.1 (C-10, C-10'), 88.6 (C-3, C-3'), 82.3 (C-11), 36.1 ($CH_3\underline{C}$-C-7, $CH_3\underline{C}$-C-7'), 33.3 ($\underline{C}H_3$C-C-7, $\underline{C}H_3$C-C-7') ppm.

**2,2'-Bis(8-methyl-4-comarinyl-oxy)methylene (EY9)**: Yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(8-methyl-2*H*-chromen-2-one); mp = 196-199°C; $\lambda_{max}$ (EtOH)=562 nm; %yield=51 (0.93 g); FTIR (ν, stretching, $cm^{-1}$): 3034 (alkene CH), 2916 (alkane CH), 1702 (ester C=O), 1681 (alkene C=C), 1580 (aromatic C=C), 1226 and 1051 (ether C-O-C); $^1$H-NMR: δ= 7.76 (2H, d, *J*=6 Hz, H-5, H-5'), 7.42 (2H, t, *J*=6 Hz, H-6, H-6'), 7.33 (2H, d, *J*=6 Hz, H-7, H-7'), 6.72 (2H, s, H-11), 5.52 (2H, s, H-3, H-3'), 2.22 (6H, s, $CH_3$-8, $CH_3$-8') ppm; $^{13}$C-NMR: δ= 169.1 (C-4, C-4'), 162.1 (C-2, C-2'), 152.4 (C-9, C-9'), 134.2 (C-7, C-7'), 127.4 (C-8, C-8'), 127.1 (C-6, C-6'), 121.9 (C-5, C-5'), 118.8 (C-10, C-10'), 89.2 (C-3, C-3'), 81.7 (C-11), 16.9 ($CH_3$-C-8, $CH_3$-C-8') ppm.

**2,2'-Bis(8-ethyl-4-comarinyl-oxy)methylene (EY10)**: Yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(8-ethyl-2*H*-chromen-2-one); mp = 209-212°C; $\lambda_{max}$ (EtOH)=567 nm; %yield=49 (0.96 g); FTIR (ν, stretching, $cm^{-1}$): 3037 (alkene CH), 2946 (alkane CH), 1707 (ester C=O), 1685 (alkene C=C), 1583 (aromatic C=C), 1225 and 1054 (ether C-O-C); $^1$H-NMR: δ= 7.74 (2H, d, *J*=6 Hz, H-5, H-5'), 7.43 (2H, t, *J*=6 Hz, H-6, H-6'), 7.31 (2H, d, *J*=6 Hz, H-7, H-7'), 6.70 (2H, s, H-11), 5.53 (2H, s, H-3, H-3'), 2.70 (4H, m, $CH_3C\underline{H}_2$-8, $CH_3C\underline{H}_2$-8'), 1.34 (6H, t, $C\underline{H}_3CH_2$-8, $C\underline{H}_3CH_2$-8') ppm; $^{13}$C-NMR: δ= 169.2 (C-4, C-4'), 162.3 (C-2, C-2'), 151.2 (C-9, C-9'), 134.7 (C-8, C-8'), 128.6 (C-7, C-7'), 126.5 (C-6, C-6'), 120.4 (C-5, C-5'), 119.1 (C-10, C-10'), 89.2 (C-3, C-3'), 81.7 (C-11), 23.9 ($CH_3\underline{C}H_2$-C-8, $CH_3\underline{C}H_2$-C-8'), 15.4 ($\underline{C}H_3CH_2$-C-8, $\underline{C}H_3CH_2$-C-8') ppm.

**2,2'-Bis(8-isopropyl-4-comarinyl-oxy)methylene (EY11)**: Dark yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(8-isopropyl-2*H*-chromen-2-one); mp = 218-220°C; $\lambda_{max}$ (EtOH)=581 nm; %yield=47 (0.99 g); FTIR (ν, stretching, $cm^{-1}$): 3036 (alkene CH), 2854, 2922 (alkane CH), 1703 (ester C=O), 1684 (alkene C=C), 1581 (aromatic C=C), 1227 and 1052 (ether C-O-C); $^1$H-NMR: δ= 7.82 (2H, d, *J*=6 Hz, H-5, H-5'), 7.42 (2H, t, *J*=6 Hz, H-6, H-6'), 7.34 (2H, d, *J*=6 Hz, H-7, H-7'), 6.71 (2H, s, H-11), 5.50 (2H, s, H-3, H-3'), 3.16 (2H, m, $CH_3C\underline{H}$-8, $CH_3C\underline{H}$-8'), 1.25 (12H, d, $C\underline{H}_3CH$-8, $C\underline{H}_3CH$-8') ppm; $^{13}$C-NMR: δ= 169.1 (C-4, C-4'), 162.2 (C-2, C-2'), 148.6 (C-9, C-9'), 145.4 (C-8, C-8'), 127.0 (C-7, C-7'), 125.9 (C-6, C-6'), 121.5 (C-5, C-5'), 119.1 (C-10, C-10'), 89.2 (C-3, C-3'), 81.7 (C-11), 28.5 ($CH_3\underline{C}H$-C-8, $CH_3\underline{C}H$-C-8'), 24.6 ($\underline{C}H_3CH$-C-8, $\underline{C}H_3CH$-C-8') ppm.

**2,2'-Bis(8-*tert*-butyl-4-comarinyl-oxy)methylene (EY12)**: Dark yellow powder; IUPAC name: 4,4'-(methylenebis(oxy))bis(8-*tert*-butyl-2*H*-chromen-2-one); mp = 232-234°C; $\lambda_{max}$ (EtOH)=589 nm; %yield=43 (0.96 g); FTIR (ν, stretching, $cm^{-1}$): 3039 (alkene CH), 2878 (alkane CH), 1701 (ester C=O), 1686 (alkene C=C), 1589 (aromatic C=C), 1225 and 1056 (ether C-O-C); $^1$H-NMR: δ= 7.70 (2H, d, *J*=6 Hz, H-5, H-5'), 7.52 (2H, d, *J*=6 Hz, H-7, H-7'), 7.45 (2H, t, *J*=6 Hz, H-6, H-6'), 6.72 (2H, s, H-11), 5.53 (2H, s, H-3, H-3'), 1.42 (18H, m, $CH_3$C-8, $CH_3$C-8') ppm; $^{13}$C-NMR: δ= 169.0 (C-4, C-4'), 162.3 (C-2, C-2'), 148.9 (C-9, C-

9'), 145.1 (C-8, C-8'), 127.5 (C-7, C-7'), 126.5 (C-6, C-6'), 121.2 (C-5, C-5'), 118.7 (C-10, C-10'), 89.2 (C-3, C-3'), 81.8 (C-11), 36.2 ($CH_3\underline{C}$-C-8, $CH_3\underline{C}$-C-8'), 33.4 ($\underline{C}H_3$C-C-8, $\underline{C}H_3$C-C-8') ppm.

*2.5. Anticoagulant activity*

The capability of the prepared functionalized biscoumarins to counteract the coagulating effect of vitamin K was investigated by detecting their impact on the **PT** in rabbits employing warfarin as a standard drug. Forty-two rabbits weighting 1kg±50g were categorized into groups of three members, restrained in standard cages, and feed equally. In the morning of three consequent days, the members of each marked group were handled orally with 1 mg of the standard drug, selected biscoumarin, or placebo. Since the last dose, the PT assay was initiated in the morning of the 3rd and 5th days. Shortly, 1 ml of the tested mixture consisted of sodium citrate (0.1 ml, 0.1 M), and rabbit blood (0.9 ml) was centrifuged (3000 rpm) at 37°C for 8 min. The supernatant was drawn back by a micropipette and housed in a thermostatic water-bath adjusted at 37°C. To 0.1 ml of the warmed supernatant, a previously prepared and warmed thromboplastin-calcium chloride aqueous solution (0.2 ml) was added. The time was started to report from this moment, and the mixture housed in the thermostatic water-bath with gentle agitating. The clot formation was monitored visually every 5 seconds to quantify the PT of the test compound [21].

## 3. Results and discussion

*3.1. Chemical synthesis*

Among various traditional and emerging innovative methods for the synthesis of coumarin-derived products, the Pechmann-type reaction is the most popular one. Many acid phenotypes can catalyze this reaction, such as those having heterogeneous or homogenous character, and those belonging to the organic, inorganic, or Lewis acid-family [22]. In our work, a Pechmann-type reaction catalyzed via anhydrous zinc chloride was employed to condense various phenol-based derivatives with malonic acid affording different alkyl-substituted 4-hydroxycoumarins. In this reaction, Three inter-conventional steps are proposed to be the mechanism of this reaction including trans-esterification, electrophilic assault, and dehydration [23]. To improve the yields of the alkyl-substituted 4-hydroxycoumarins, the work team utilized phosphoryl chloride as a drying agent [24].

In the second step of the synthetic pathway, the resulted alkyl-substituted 4-hydroxycoumarins were attacked nucleophilically the electrophilic carbon of methylene iodide. This coupling reaction was facilitated by three practical issues; the first was the utilization of anhydrous reaction conditions involving the dry solvent and catalyst [25]. The second was improving the nucleophilicity of the phenolic hydroxyl group by deprotonating via potassium carbonate [26]. Finally, the use of methylene iodide as an electrophile-containing molecule instead of methylene chloride since the first reagent affording better-leaving groups than the other [27].

*3.2. Anticoagulant activity*

Since it is essential for life, vitamin K is synthesized and released inside the human body. This essentiality derives from its role as a cofactor of a hepatic enzyme named 2,3-epoxide reductase, which is catalyzed the formation of the proteins S and C as well as the active coagulation factors VII, IX, and X [28]. Many natural and synthetic biscoumarins have exhibited a variable activity as anticoagulant agents through their vitamin K antagonizing role, which is resulted in the inhibition of hepatic 2,3-epoxide reductase and subsequently prolonged the **PT** [29]. To investigate the vitamin K antagonized activity of the prepared functionalized biscoumarins, a **PT**-based assay was executed *in vivo* using rabbits as a test model since they have similar metabolic pathways for coumarins to those witnessed in the human beings [30].

A delicate analysis of the **PT** values, recorded in Table-1 and depicted graphically in Figure-1, showed four conclusive points. The first one is the prepared biscoumarins exhibited a hopeful anticoagulant effect but less than that of the standard drug. The second is the biscoumarins substituted at position 6 (**EY1**-**EY4**) or 8 (**EY9**-**EY12**) showed roughly the same activity, which is less than those substituted at position 7. The third point is the biscoumarins substituted at position 7 (**EY5**-**EY8**) have an anticoagulant activity similar to a higher extent to that of the standard drug. Finally, the anticoagulant activity of the latter group of biscoumarins was related to the size of the substituted alkyl group, where the order of activity increased as the molecular weight of this alkyl group increases [31].

Table 1

The values of **PT** acquired from testing the prepared functionalized biscoumarins as anticoagulant agents.

| Test name | The tested compounds | | | | | | |
|---|---|---|---|---|---|---|---|
| | Warfarin | Placebo | **EY1** | **EY2** | **EY3** | **EY4** | **EY5** |
| **PT** 3rd day | 19.68 ± 1.35 | 13.62 ± 1.25 | 14.33 ± 1.02 | 14.38 ± 1.15 | 14.35 ± 1.26 | 14.42 ± 1.30 | 16.78 ± 0.94 |
| **PT** 5th day | 21.27 ± 1.25 | 13.48 ± 1.18 | 15.48 ± 1.08 | 15.31 ± 1.20 | 15.46 ± 1.28 | 15.58 ± 1.20 | 17.81 ± 1.04 |
| Test name | The tested compounds | | | | | | |
| | **EY6** | **EY7** | **EY8** | **EY9** | **EY10** | **EY11** | **EY12** |
| **PT** 3rd day | 17.09 ± 1.32 | 17.12 ± 0.96 | 17.44 ± 1.24 | 14.37 ± 1.15 | 14.42 ± 1.25 | 14.39 ± 1.21 | 14.52 ± 1.25 |
| **PT** 5th day | 19.02 ± 1.08 | 19.78 ± 1.22 | 19.95 ± 1.10 | 15.43 ± 1.20 | 15.30 ± 1.18 | 15.50 ± 1.32 | 15.64 ± 1.18 |

The outcomes were recorded in the term of **PT** (seconds) ± SD (standard deviation for three test animals).

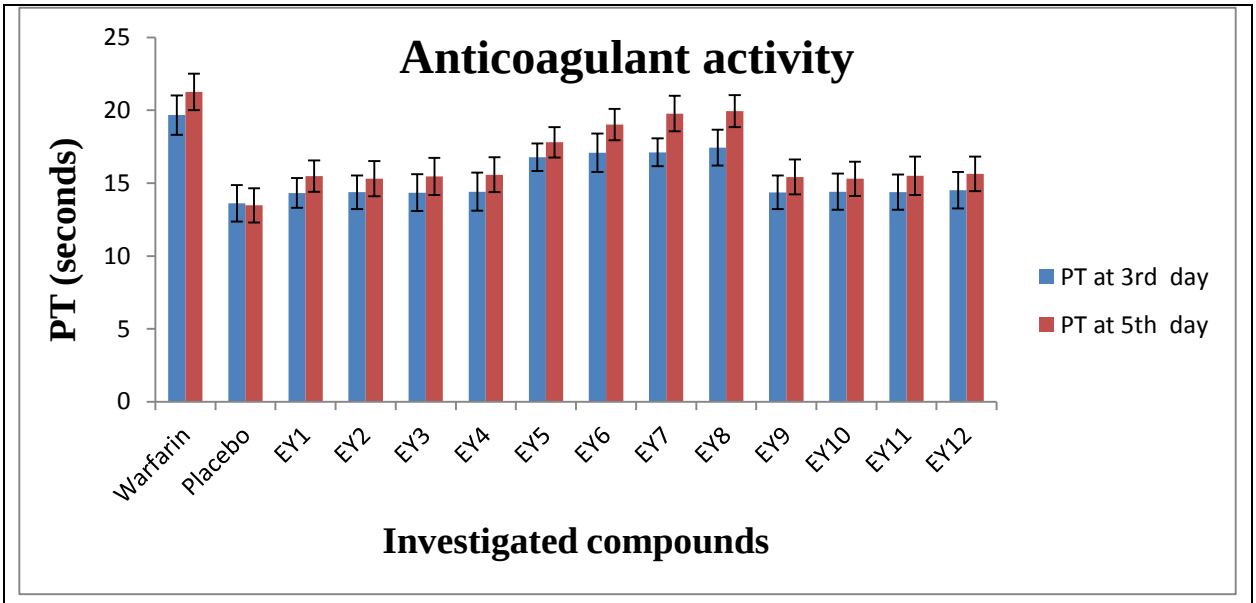



Figure-1. Diagram displayed the anticoagulant activity of the reference drug, placebo, and the synthesized biscoumarins calculated at the third and fifth days next to the last oral dose.

## 4. Conclusions

The synthesis and characterization of a series of twelve functionalized biscoumarins symbolized as (**EY1**-**EY12**) were successfully reported in this work. The results of PT of the synthesized biscoumarins suggested that these compounds have a distinctive anticoagulant effect comparing with warfarin as a standard drug. Also, the biscoumarins functionalized at position 6 (**EY1**-**EY4**) or 8 (**EY9**-**EY12**) exhibited a closely related activity, but their impact was lower than those functionalized at position 7 (**EY5**-**EY8**). Besides, the anticoagulant activity of the latter biscoumarins is directly improved as the molecular weight of the functionalized group is increased. Therefore, these biscoumarins could be considered as a potential scaffold for designing and synthesizing agents that act as potent anticoagulant agents in the prophylaxis and treatment of **CCVDs**.

## 5. Conflicts of interest

There are no conflicts to declare.

## 6. Acknowledgments

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## 7. References

[1] A. Stefanachi, F. Leonetti, L. Pisani, M. Catto, and A. Carotti, Coumarin: A natural, privileged and versatile scaffold for bioactive compounds, vol. 23, no. 2. 2018.
[2] L. Lei, Y. B. Xue, Z. Liu, S. S. Peng, Y. He, Y. Zhang, R. Fang, J. P. Wang, Z. W. Luo, G. M.

Yao, J. W. Zhang, G. Zhang, H. P. Song, and Y. H. Zhang, "Coumarin derivatives from Ainsliaea fragrans and their anticoagulant activity," Sci. Rep., vol. 5, pp. 1–9, 2015, doi: 10.1038/srep13544.
[3] Y. F. Mustafa and N. T. Abdulaziz, "Biological potentials of hymecromone-based derivatives : A systematic review," Syst. Rev. Pharm., vol. 11, no. 11, pp. 438–452, 2020, doi: 10.31838/srp.2020.11.65.
[4] Y. F. Mustafa, E. T. Mohammed, and R. R. Khalil, "Antioxidant and antitumor activities of methanolic extracts obtained from Red Delicious and Granny Smith apples ' seeds," Syst. Rev. Pharm., vol. 11, no. 4, pp. 570–576, 2020, doi: 10.31838/srp.2020.4.84.
[5] A. E. Kummerle, "Coumarin compounds in medicinal chemistry: Some important examples from the last year," Curr. Top. Med. Chem., vol. 18, no. 5, pp. 124–148, 2018, doi: 10.2174/1568026618666180329115523.
[6] M. K. Bashir, Y. F. Mustafa, and M. K. Oglah, "Synthesis and antitumor activity of new multifunctional coumarins," Period. Tche Quim., vol. 17, no. 36, pp. 871–883, 2020.
[7] Y. F. Mustafa, "Synthesis, characterization and antibacterial activity of novel heterocycle, coumacine, and two of its derivatives," Saudi Pharm. J., vol. 26, no. 6, pp. 870–875, 2018, doi: 10.1016/j.jsps.2018.03.010.
[8] E. T. Mohammed and Y. F. Mustafa, "Coumarins from Red Delicious apple seeds : Extraction , phytochemical analysis , and evaluation as antimicrobial agents," Syst. Rev. Pharm., vol. 11, no. 2, pp. 64–70, 2020, doi: 10.5530/srp.2020.2.11.
[9] Y. Ruan, Y. Guo, Y. Zheng, Z. Huang, S. Sun, P. Kowal, Y. Shi, and F. Wu, "Cardiovascular disease ( CVD ) and associated risk factors among older adults in six low-and middle-income countries : results from SAGE Wave 1," BMC Public Health, vol. 18, pp. 1–13, 2018, doi: 10.1186/s12889-018-5653-9.
[10] L. Chen, G. Tang, F. Guo, W. Li, J. Zhang, and B. Liu, "( P )/( M ) -corinepalensin A , a pair of axially chiral prenylated bicoumarin enantiomers with a rare C-5 e C-5 ′ linkage from the twigs of Coriaria nepalensis," Phytochemistry, vol. 149, pp. 140–145, 2018, doi: 10.1016/j.phytochem.2018.02.012.
[11] X. Gao, S. Huang, P. Dong, C. Wang, J. Hou, X. Huo, B. Zhang, T. Ma, and X. Ma, "Horseradish peroxidase (HRP): A tool for catalyzing the formation of novel bicoumarins," Catal. Sci. Technol., vol. 6, no. 10, pp. 3585–3593, 2016, doi: 10.1039/c5cy01682g.
[12] M. Chand, "Solvent free synthesis of biscoumarins," Arch. Org. Inorg. Chem. Sci., vol. 2, no. 3, pp. 204–207, 2018, doi: 10.32474/AOICS.2018.02.000139.
[13] M. K. Oglah, Y. F. Mustafa, M. K. Bashir, and M. H. Jasim, "Curcumin and its derivatives: A review of their biological activities," Syst. Rev. Pharm., vol. 11, no. 3, pp. 472–481, 2020, doi: 10.5530/srp.2020.3.60.
[14] H. Hussain, J. Hussain, A. Al-harrasi, and K. Krohn, "The chemistry and biology of bicoumarins," Tetrahedron, vol. 68, no. 12, pp. 2553–2578, 2012, doi: 10.1016/j.tet.2012.01.035.
[15] P. Meijer, K. Kynde, A. M. H. P. Van Den Besselaar, M. Van Blerk, and T. A. L. Woods, "International normalized ratio (INR) testing in Europe: Between-laboratory comparability of test results obtained by Quick and Owren reagents: A collaborative study from the European Committee for External Quality Assurance Programmes in Laboratory Medici," Clin. Chem. Lab. Med., vol. 56, no. 10, pp. 1698–1702, 2018, doi: 10.1515/cclm-2017-0976.
[16] N. R. Mishra, "Prothrombin Time and International Normalized Ratio in Pediatric Prothrombin Time & International Normalized Ratio in Pediatric Liver Disease," no. December 2013, pp. 2–8, 2017.
[17] E. J. Favaloro, M. Bahraini, and F. Rad, "Standardization of prothrombin time / international normalized ratio (PT / INR)," Int. J. Lab. Hematol., vol. 43, pp. 21–28, 2021, doi: 10.1111/ijlh.13349.
[18] M. M. Abdou, R. A. El-Saeed, and S. Bondock, Recent advances in 4-hydroxycoumarin chemistry. Part 1: Synthesis and reactions, vol. 12, no. 1. King Saud University, 2019.
[19] D. Karia, K. Pancholi, and R. Varu, "Synthetic Advances and Biological Activities of 4-Hydroxy Coumarin Derivatives," Int. J. Pharm. Res. Sch., vol. 2, no. 4, pp. 139–142, 2013.
[20] M. H. A. Elgamal, N. M. M. Shalaby, M. A. Shaban, H. Duddeck, B. Mikhova, A. Simon, and G. Tóth, "Synthesis and spectroscopic investigation of some dimeric coumarin and furanocoumarin Models," Monatshefte fur Chemie, vol. 128, no. 6–7, pp. 701–712, 1997, doi: 10.1007/BF00807602.
[21] L. Lei, Y. Xue, Z. Liu, S. Peng, Y. He, Y. Zhang, and R. Fang, "Coumarin derivatives from Ainsliaea fragrans and their anticoagulant activity," Sci. Rep., vol. 5, no. 13544, pp. 1–9, 2015, doi: 10.1038/srep13544.
[22] Y. F. Mustafa, M. K. Bashir, and M. K. Oglah, "Original and innovative advances in the synthetic schemes of coumarin-based derivatives: A review," Syst. Rev. Pharm., vol. 11, no. 6, pp. 598–612, 2020, doi: 10.31838/srp.2020.6.90.
[23] M. K. Oglah and Y. F. Mustafa, "Curcumin analogs: synthesis and biological activities," Med. Chem. Res., vol. 29, no. 3, pp. 479–486, 2020, doi: 10.1007/s00044-019-02497-0.
[24] M. K. Oglah and Y. F. Mustafa, "Synthesis , antioxidant , and preliminary antitumor activities of new curcumin analogues," J. Glob. Pharma Technol., vol. 12, no. 2, pp. 854–862, 2020.
[25] V. Vahabi and F. Hatamjafari, "Microwave assisted convenient one-pot synthesis of coumarin derivatives via Pechmann condensation catalyzed by FeF3 under solvent-free conditions and antimicrobial activities of the products," Molecules, vol. 19, no. 9, pp. 13093–13103, 2014, doi: 10.3390/molecules190913093.
[26] Y. F. Mustafa, M. K. Oglah, and M. K. Bashir, "Conjugation of sinapic acid analogues with 5- Fluorouracil: Synthesis, preliminary cytotoxicity, and release study," Syst. Rev.

Pharm., vol. 11, no. 3, pp. 482–489, 2020, **doi: 10.5530/srp.2020.3.61**.

[27] M. K. Oglah, M. Kahtan Bashir, Y. Fakri Mustafa, E. T. Mohammed, R. R. Khalil, and Y. F. Mustafa, "Synthesis and biological activities of 3,5-disubstituted-4-hydroxycinnamic acids linked to a functionalized coumarin," Syst. Rev. Pharm., vol. 11, no. 6, pp. 717–725, 2020, **doi: 10.31838/srp.2020.6.106**.

[28] B. Dahlbäck, "Vitamin K-dependent protein S: Beyond the protein C pathway," Semin. Thromb. Hemost., vol. 44, no. 2, pp. 176–184, 2018, **doi: 10.1055/s-0037-1604092**.

[29] K. Kasperkiewicz, M. B. Ponczek, J. Owczarek, and P. Guga, "Antagonists of vitamin K—popular coumarin drugs and new synthetic and natural coumarin derivatives," Molecules, vol. 25, no. 1465, pp. 1–24, 2020, **doi: 10.3390/molecules25061465**.

[30] R. O. Juvonen, F. Novák, E. Emmanouilidou, S. Auriola, J. Timonen, A. T. Heikkinen, J. Küblbeck, M. Finel, and H. Raunio, "Metabolism of scoparone in experimental animals and humans," Planta Med., vol. 85, no. 6, pp. 453–464, 2019, **doi: 10.1055/a-0835-2301**.

[31] F. Annunziata, C. Pinna, S. Dallavalle, L. Tamborini, and A. Pinto, "An overview of coumarin as a versatile and readily accessible scaffold with broad-ranging biological activities," Int. J. Mol. Sci., vol. 21, no. 13, pp. 1–83, 2020, **doi: 10.3390/ijms21134618**.