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RESEARCH NOTE

ANTICOAGULANT ACTIVITY OF COUMARIN DERIVATIVES

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Coumarins are natural product compounds and can be found in many plants as secondary metabolites. Chemically, coumarins belong to the subgroup of lactones (Melita *et al*., 2020). There are large numbers of coumarin derivatives, namely, natural and synthetic coumarin, which are associated with various types of biological activities, such as anti-inflammatory, anticancer, antioxidant, anti-HIV, as well as anti-coagulant (Murray *et al*., 1982; Kostova, 2005; Kontogiorgis & Hadjipavlou-Litina, 2005; Yuce *et al*., 2009; Fernanda *et al*., 2015). Coumarins and their derivatives are principal oral anticoagulants and this is attributed to their competitive inhibition effect on vitamin K in the biosynthesis of prothrombin (Beillerot *et al*., 2008; Ozkan *et al*., 2010). Clinically, coumarin derivatives are the precursors of several anticoagulants, particularly warfarin, which is the most commonly used oral anticoagulant medication. All of the above information has been the motivation to embark on the current study. In this context, this study aimed to report the synthesis of some coumarin derivatives, including, 4-aryl-1,2-dihydro-6-(4-hydroxy-2-oxo-2H-chromene-3-yl)-2-oxopyridine-3-carbonitriles and then to evaluate their anticoagulant activities, in comparison to that of commercially anticoagulant drug warfarin.

All reactions were performed under an atmosphere of argon in oven-dried glassware. ^{1}H-NMR spectra were recorded on a 300 MHz NMR spectrometer instrument (Bruker AC 300). Mass spectra were recorded on an AutoSpeq Q VG with an ionization energy of 70ev. 3-Acetyl-Synthesized coumarin 6-(4-hydroxy-2-oxo-2H-chromene-3-yl)-4-(3-nitro-phenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**1)** was obtained by mixing 4-hydroxy-coumarin (1 g, 5mmoL) with 0.75 g (5 mmoL) 3-nitro-benzaldehyde in 20 mL ethanol. In this mixture was added 0.57 mL ethyl acetate and 0.75 g (10 mmoL) ammonium acetate. The mixture was refluxed for 4 h and coumarin **1** was obtained as a yellow precipitate. Coumarine **1** was recrystallized from ethanol. Melting point 223 °C. Yields 72%. IR (KBr, ν cm^{-1}): 3450. 3300. 3030, 2950, 2200, 2000, 1750, 1520, 1340.^{1}H-NMR (300 MHz, DMSO): δ 5.63 (s, 1H, C-H), 7.20-8.23 (m, 9H), 13.33 (s, 1H, OH). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{21}H_{12}N_3O_6$ 402.0681, found 402.0683. Coumarine 4-(3-bromo-phenyl)-6-(4-hydroxy-2-oxo-2H-chromene-3-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**2**) was obtained when 3-Acetyl-4-hydroxy-coumarin (1 g, 5 mmoL) in 20 mL ethanol was mixed with 3-bromo-benzaldehyde (0.95 g, 5 mmoL). In this mixture was added 0.57 mL ethyl acetate and 0.75 g (10 mmoL) ammonium acetate. The mixture was refluxed for 5 hr. Coumarine **2** was obtained as white precipitates and these crystals were recrystallized from ethanol to obtain white crystals. Melting point 215 °C Yields 64%. IR (KBr, ν cm^{-1}): 3450, 3030, 2950, 2240, 1750, 750-800.^{1}H-NMR (300 MHz, DMSO): δ 5.71 (s, 1H, C-H), 7.19-8.20 (m, 9H), 13.50 (s, 1H, OH). HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{21}H_{12}BrN_2O_4$ 434.9903, found 434.9969.

Male laboratory mice of the type *swiss albino* aged 12-17 weeks and weighing 130-160 g were used to measure the anticoagulant effect of the synthesized compounds. The animals were housed in stainless steel cages in a room at 23 ± 2 °C under 12 hr dark/light cycles. After arrival, all the mice were fed standard rat chow for 7 days. Mice were given free access to food and water. Then the mice were randomly divided into the 5 following groups: Group I: 4-hydroxycoumarine (*n*=5), Group II: compound 1 (*n*=5), Group III: compound 2 (*n*=5), Group IV: warfarin (*n*=5), Group V: saline solution as control (*n*=5). The synthesized compounds were mixed with a 0.9% NaCl solution. A gastric tube was used for oral administration of prepared - solutions of warfarin (1 mg/kg) and synthesized compounds **1** and **2** (20 mg/kg), respectively. The mice were then allowed to stand for 24 h for the substances to achieve the desired effect

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before we commenced the analysis. The determination of prothrombin time (PT) was done according to the procedure described by Ozkan *et al*. (2010). PT values for the groups are given in Table 1. Plasma PT was prolonged in all groups when compared to control. However, it was significantly higher in the **2** treated and warfarin-treated groups than in the control group (p<0.01). Compound **2**, is a potential candidate as an antithrombotic drug for further elaboration. After injection of synthesized compound **1** in mice, we have seen stereotypical behavior (increased locomotors activity repeated in uninterrupted series). Also, in animals, treated with compound **1**, changes in the intestinal tract were observed which appeared with diarrhea (liquid feces). Another symptom that was observed was the increased secretion of the eye and edema of the eyelids (Figure 1). Mice treated with compound **1** had milky white secretion from the tear duct and increased eyelid edema up to total eye closure. Mice treated with compound **2** had almost identical intestinal symptoms. All other behavioral or visual system-related symptoms were not present in mice treated with compound **2**. The commercial drug, warfarin, had the same symptomatology as that of the group of mice treated with compound **1**, except that the symptoms were not as high. Especially no changes in behavior were recorded. Based on these results as well as the results obtained from Table 1 , we see that these derivatives show *in vivo* anticoagulant potential. All statistical analysis was carried out using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA. USA). Groups of data were compared with a two-tailed t-test. Values of p<0.05 or p<0.01 were regarded as significant or highly significant, respectively. On the other hand, in addition to anticoagulant activity, these derivatives have been associated with toxic effects, which is another problem that requires further study.

CONCLUSION

The PT value of the synthesized compounds **1** and **2** was higher compared to that of warfarin. Compounds **1** and **2** are potentially antithrombotic drug candidates for further elaboration. Furthermore, these derivatives showed satisfactory anticoagulant potential, but this anticoagulant ability was closely related to toxicity. Therefore, our advice is to work on these derivatives by changing their structures to decrease their toxic effects.

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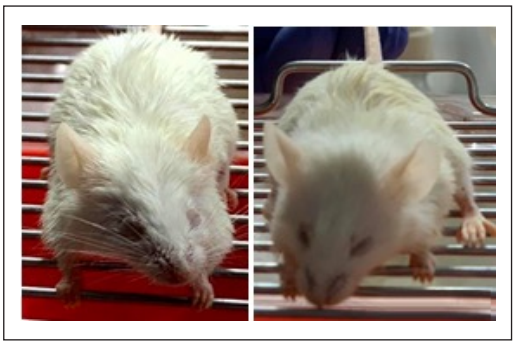

Fig. 1. Some of the toxic effects presented in the tested mice: were edema of the eyelids and increased secretion in the eye.

Table 1. Effect of the coumarins derivatives on prothrombin time of the mice 24 h after single oral administration

	Group I	Group II	Group III	Group IV	Group V
Prothrombin time (PT) (s)	11.30	20.80*	21.30*	14.60*	9.16
SD/SE	0.63/0.30	0.54/0.24	0.38/0.15	0.39/0.16	0.15/0.07

Values are given as mean and standard deviation (SD), standard error (SE). Group I was treated with 4-hydroxycoumarin (20 mg/kg), Group II with compound **1** (20 mg/kg), compound **2** (20 mg/kg), Group IV with warfarin (1 mg/kg), while Group V was treated with saline (control).* Significant difference between control and treated sample: p<0.01; n=5.

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