
ANTICOAGULANT ACTIVITY OF SUBSTITUTED HYDROXY PROPOXYCOUMARIN DERIVATIVES

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Summary

Coumarin molecule is used as anticoagulant. Other uses like anti-HIV, anti-tumor, anti-hypertensive, anti-arrhythmia, anti-osteoporosis, pain relief increase research of coumarin derivatives. Perkin discovered the synthetic method of coumarin and opened the door in synthetic research. Warfarin is a well known drug of 4-hydroxy coumarin derivative. It is consider that, warfarin drug act as a lead molecule of anticoagulant. To introduce alkylaminohydroxyprooxy side chain in coumarin nucleus changes its anticoagulant activity. Hence we have synthesized substituted coumarin derivatives and performed its anticoagulant activity.

Key words: Warfarin, Coumarin derivative, Anticoagulant

Introduction

Thromboembolism is the combination of thrombosis and its main complication, embolism. When a thrombus occupies more than 75% of surface area of the lumen of an artery, blood flow to the tissue supplied is reduced enough to cause symptoms because of decreased oxygen (hypoxia) and accumulation of metabolic products like lactic acid. More than 90% obstruction can result in anoxia, the complete deprivation of oxygen, and infarction, a mode of cell death [1].

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The symptoms of a thromboembolism depend on the organ or blood vessel that has lost blood supply. Blood clots in an arm or leg may cause pain, swelling, and increased temperature in the affected area. A clot that travels to the lung is called a pulmonary embolus. This condition can cause: chest pain, shortness of breath, rapid heartbeat, known as tachycardia, fainting or death. If a blood clot is formed in the heart, it can travel to almost any organ in the body. This could cause a stroke, which is a type of damage to the brain from lack of blood circulation. In other cases, damage may be done to an arm or leg, or a heart attack or kidney damage may occur. Other areas of the body can also be affected [2].

An anticoagulant is a substance that prevents coagulation; that is, it stops blood from clotting. Anticoagulants were introduced into medical practice more than three decades ago. Extensive use of these drugs in the prevention and treatment of thromboembolic disease has made them one of the most widely used classes of pharmacological agents. Anticoagulant drugs include: Heparin and derivative substances e.g. Low molecular weight heparin and Coumarins (Vitamin K antagonists) e.g. Warfarin [3].

Warfarin acts via inhibition an enzyme vitamin K epoxide reductase, which recycles oxidized vitamin K to its reduced form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII [4]. Reduced vitamin K must be regenerated from the epoxide for sustained carboxylation and synthesis of biologically competent proteins [5]. So we have tried to synthesize coumarin derivative which posse’s anticoagulant activity.

Coumarin derivatives are used as therapeutic anticoagulants and as rodenticides by causing fatal haemorrhage [6]. Because the range between efficient therapy and undue hemorrhagic risk may vary greatly from one patient to another, the need for carefully individualized treatment and frequent observations has long been stressed. However, a summary of recent research findings, along with certain principles, may offer possible explanations for responsiveness to make highly efficient lead with fewer side effects to resist both, coagulopathy as well as hypertension. The primary aim of this present work is
to study pharmacological screening and synthetic aspects of the coumarin ring structure especially its combined analogues profile as an anticoagulant and antihypertensive property. Our first aim is to study anticoagulant activity of synthesized compounds and then work on antihypertensive parameters.

**Materials and Method**

Sterile disposable pricking needle or lancet, stop watch, dry glass capillary tube (narrow diameter 1 top 2 mm, minimum 10 cm long), cotton swab of absorbent cotton, spirit wetted. 70 % v/v ethyl alcohol or 70 % v/v denatured spirit is used as antiseptic.

Blood sample collection and blood analysis: Blood samples were collected in clean dry centrifuge tubes as end of three weeks of treatment after 12hrs fast from retro orbital plexuses under light ether anesthesia and were collected in EDTA tube to prevent clot formation at room temperature.

Determination of clotting time using Lee and white method.

**Experimental:**

We have synthesized a series of 4-substituted coumarin derivatives and performed its pharmacological screening for anticoagulant activity. 4th position is substituted with alkylaminohydroxypropoxy side chain.

Blood was collected from animal by retro orbital plexus method under light anesthetic conditions. Immediately stop watch was started. Dip one end of capillary into blood drop gently without pressure. After every 30 seconds, using stopwatch, break a small piece of capillary. Repeat breaking at regular time intervals, till fibrin thread appears at broken end of capillary tube. Do not pull away the cut pieces ling apart and bristly. Record time interval between pricking finger and first appearance of fibrin thread at the broken ends of capillary tube. That is clotting time of blood.
Statistical analysis: Results are presented as mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey’s test. Data were considered statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$. Statistical analysis was performed using Sigma stat statistical software.

**Result and Discussion**

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<thead>
<tr>
<th>Compound</th>
<th>Bleeding Time</th>
<th>Clotting Time</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Warfarin</td>
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<td>500</td>
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<tr>
<td>BLT 1</td>
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<td>400</td>
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<tr>
<td>BLT 10</td>
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</tbody>
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Warfarin treated (0.1mg/kg p.o.) rats were found to be shown significant increase in bleeding and clotting time as compare to normal healthy rat. Treatment with tested compounds (5ml/kg/day, p.o) also produced significant increase in bleeding and clotting...
time as compared to normal rats. It may be act same as warfarin, but it is confirmed that change in chemical structure of coumarin side chain altered its anti-coagulant activity. Coumarin nucleus is responsible for anticoagulant activity. While side chain play important role in other activity like hypertension, arrhythmia etc. Our first aim is synthesized anticoagulant compound which posses antihypertensive activity. Primary screening for anticoagulant activity is performed while other activity will perform as early as possible.

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References