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Persulfated Coumarin Glucosides: New Anticoagulant Hybrids

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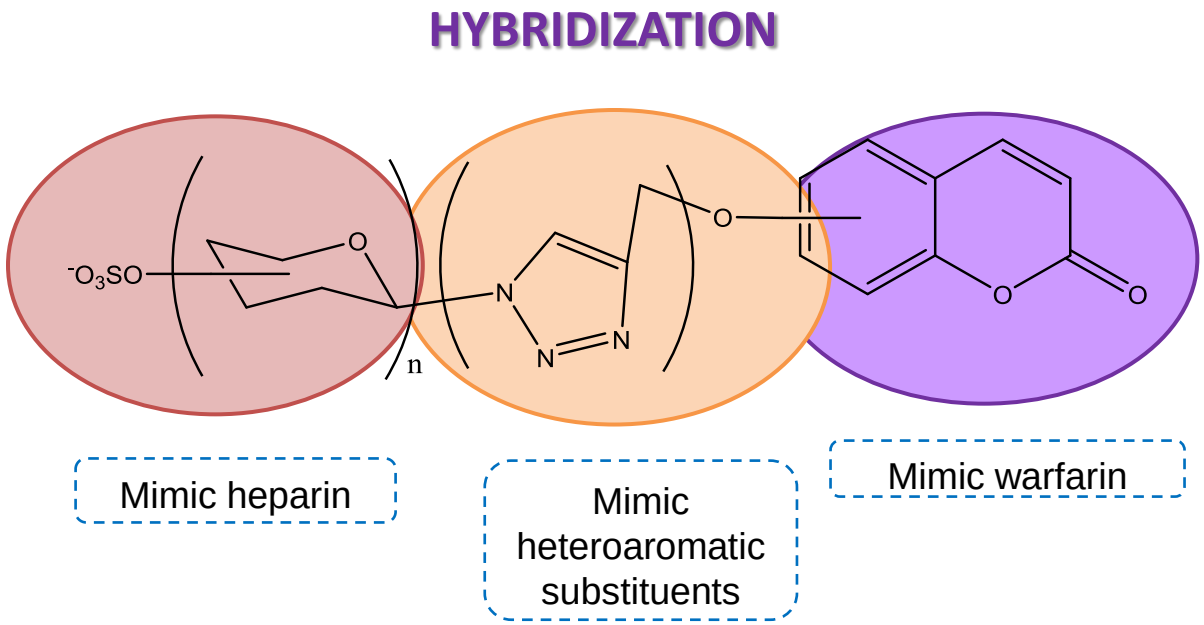
Persulfated Coumarin Glucosides: New Anticoagulant Hybrids

Graphical Abstract



Abstract

Coumarins are a large class of naturally occurring phenolic substances consisting in fused benzene and α-pyrone rings (benzo-α-pyrones). Coumarins are extremely variable in structure, due to different types of substituents in their scaffold, which can influence their biological activity. Coumarin-derivatives possessing a 4-hydroxyl group have been therapeutically used for their orally anticoagulant activity (e.g. warfarin). Nevertheless, being vitamin K antagonists, coumarins have a delayed onset and offset of action and several interactions with many drugs and food. In opposite, heparin, which is a polysulfated polysaccharide, has a short onset and offset of action, due to an effective mechanism of action, but is only active by parenteral route. As a result, it is important to develop effective orally active antithrombotic agents. In this work, a hybridization strategy was planned joining a coumarin scaffold with a heparin-like sugar sulfated moiety. With this approach it is expected to mimetize the sulfated polysaccharide anticoagulants, while adding some hydrophobic character to the resulting molecule to achieve oral bioavailability. Five persulfated triazole and non-triazole linked coumarin glucosides were obtained by microwave irradiation with triethylamine-sulfur trioxide adduct, and their structure elucidation was established by IR and NMR for the first time. A purification procedure involving dialysis with a cellulose membrane was successfully applied to remove water soluble impurities. The anticoagulant activity was measured by the classical clotting times - activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT). The most active compound exhibited an $APTT_2$ of 22×10^{-5} M. In the future, oral bioavailability of this innovative coumarin hybrid will be evaluated.

Keywords: sulfated, coumarins, synthesis, anticoagulant

INTRODUCTION

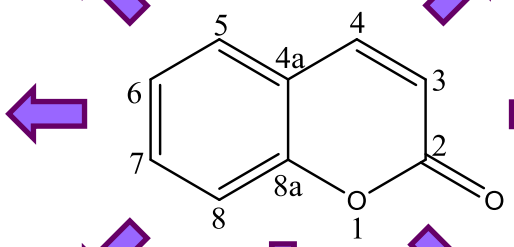
Anticoagulant Therapy

COUMARINS

Anti-inflammatory

Antibacterial

Antihypertensive



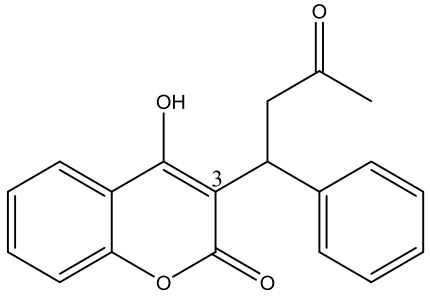
Anticancer

Antifungal

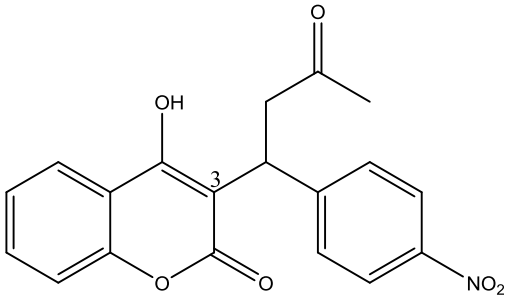
Antiviral

Anticoagulant

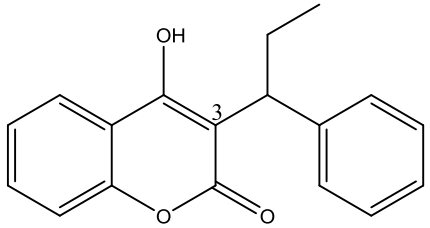
4-hydroxycoumarins



Warfarin



Acenocoumarol



Phenprocoumon

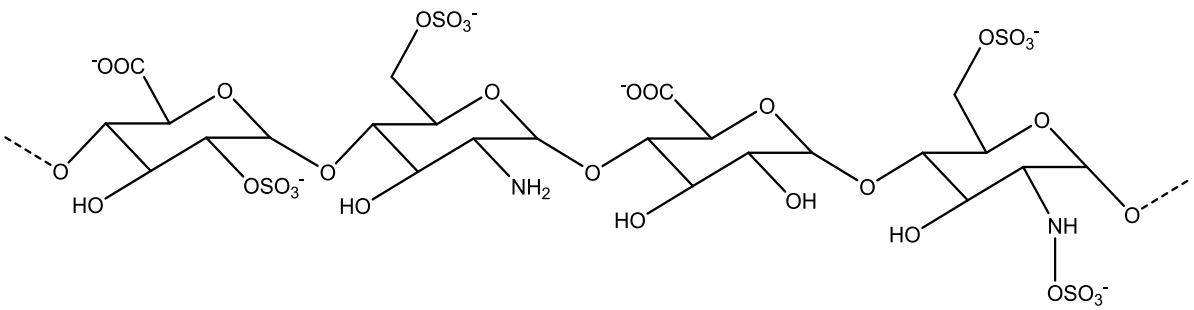
Vitamin K antagonists

Food and drugs interactions

INTRODUCTION

Anticoagulant Therapy

HEPARIN



Thrombin, Factor Xa, and Platetet factors Inhibitor

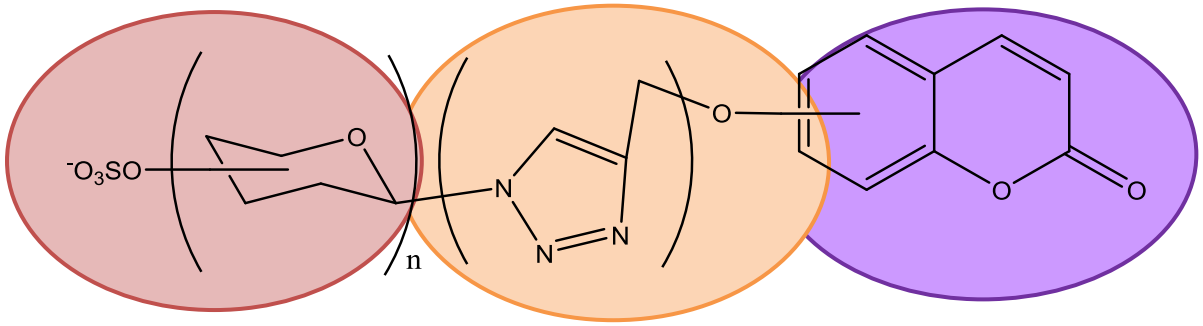
Bovine or porcine sources
Active only by parenteral route

Development of effective orally active antithrombotic agents

INTRODUCTION

Strategy for the development of new anticoagulant coumarins

Hybridization



Mimic heparin

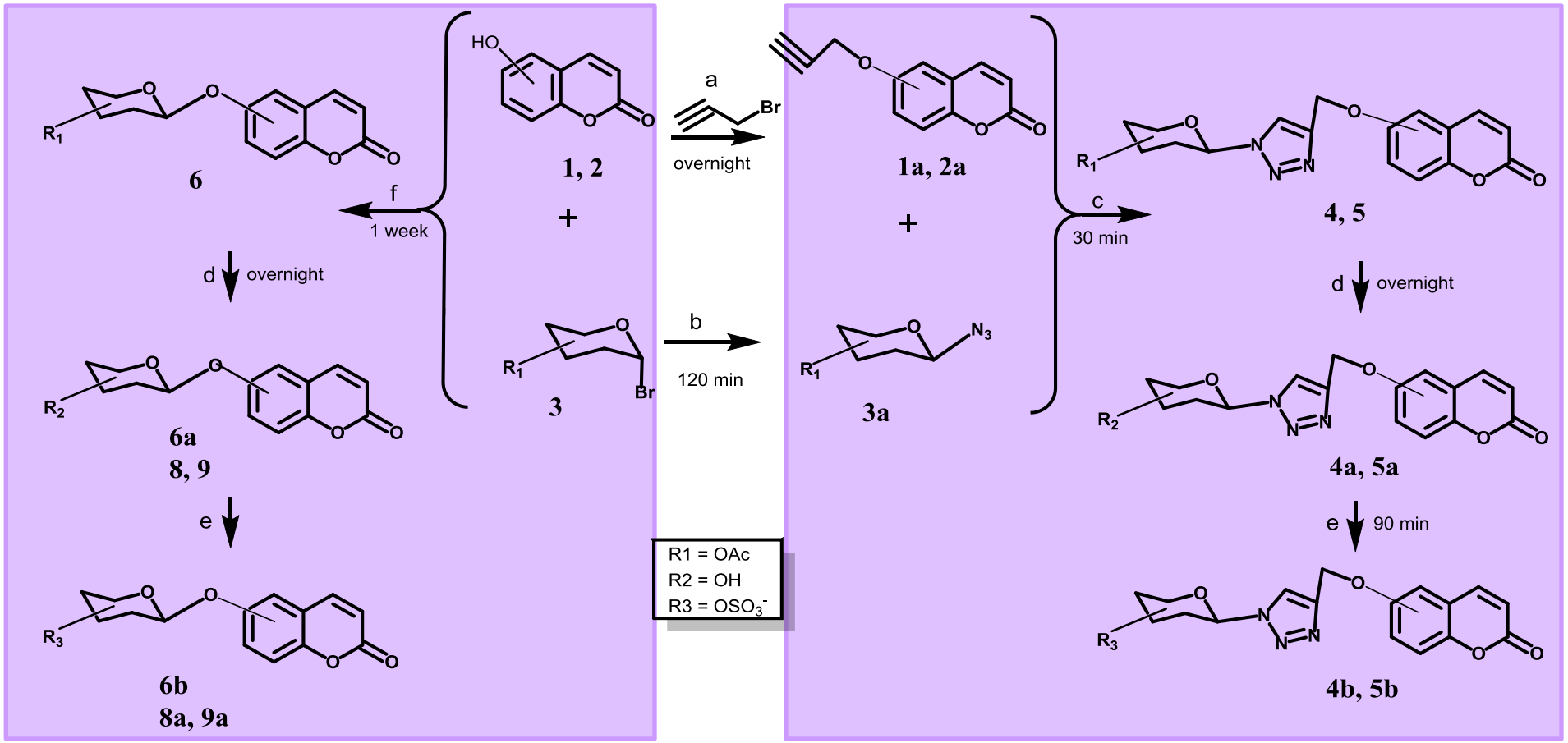
Mimic heteroaromatic substituents

Mimic warfarin

New anticoagulant coumarins with different mechanism of action ?

SYNTHESIS

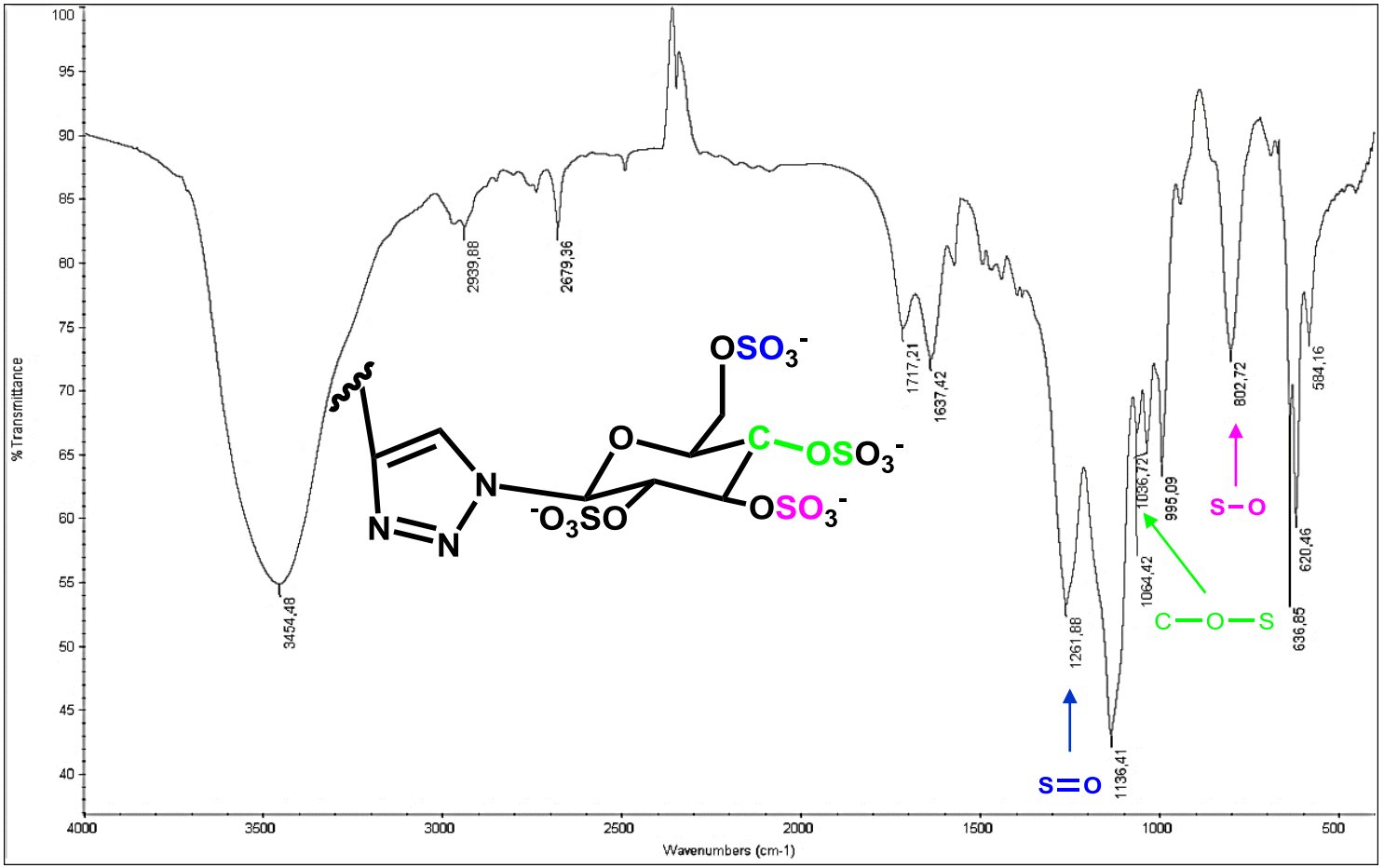
Persulfated (non-)triazole linked coumarin glucoside



(a) TBAHS, $CsCO_3$, 65°C; (b) NaN_3, r.t.; (c) Sodium ascorbate, $Cu_2SO_4.5H_2O$, THF/H_2O, MW, 70°C; (d) NaOMe, MeOH, r.t.; (e) TEA:SO_3, DMA, MW, 100°C; (f) TBAB, K_2CO_3, r.t.

STRUCTURE ELUCIDATION

Persulfated triazole-linked coumarin glucoside

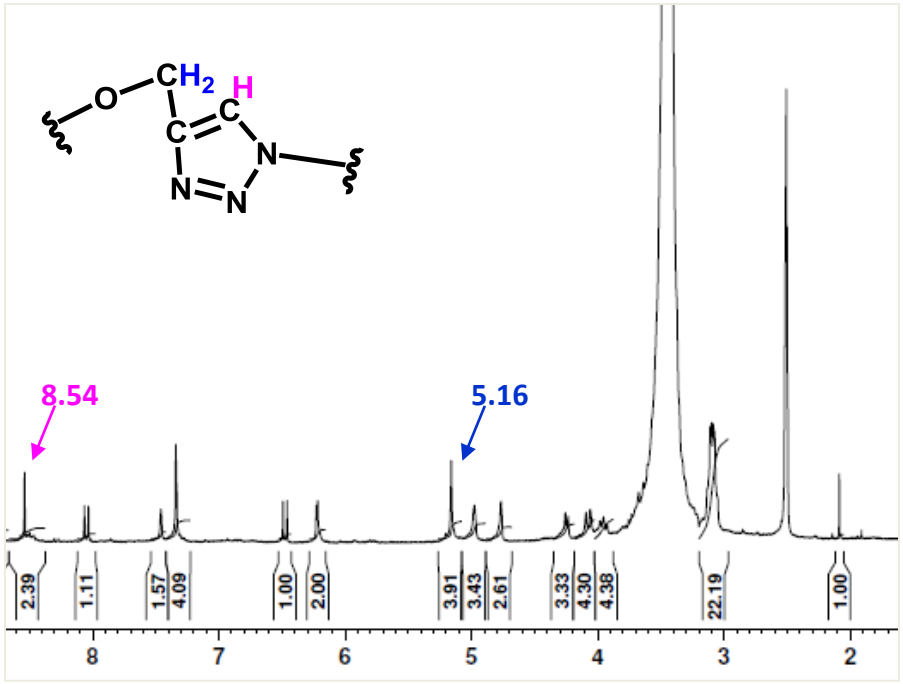


IR spectrum (KBr) of persulfated triazole-linked coumarin glucoside

STRUCTURE ELUCIDATION

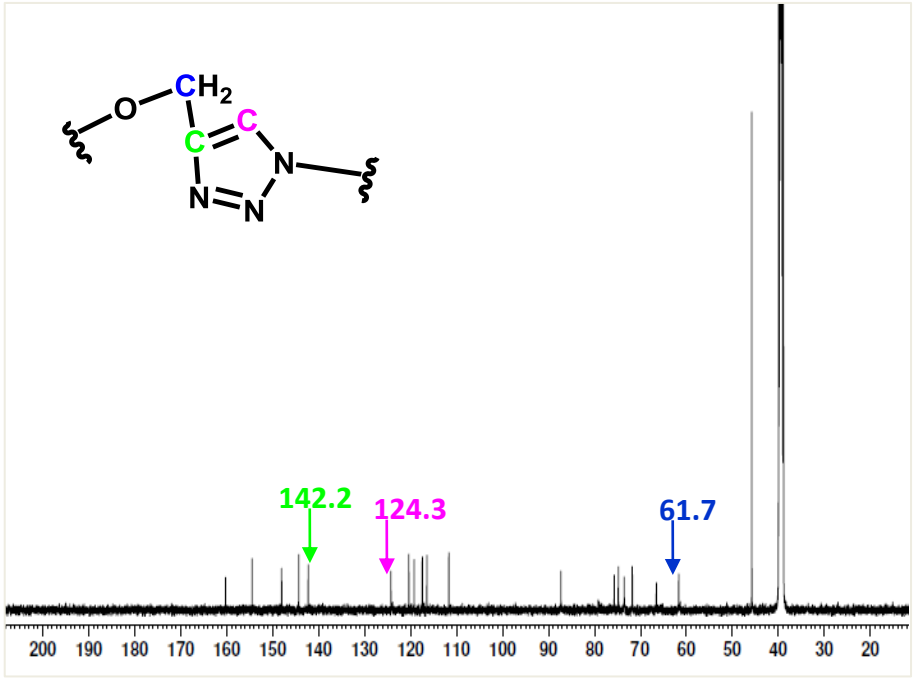
Persulfated triazole-linked coumarin glucoside

^{1}H NMR



^{1}H NMR spectrum (300 MHz) of persulfated triazole-linked coumarin glucoside (DMSO –d_6)

^{13}C NMR



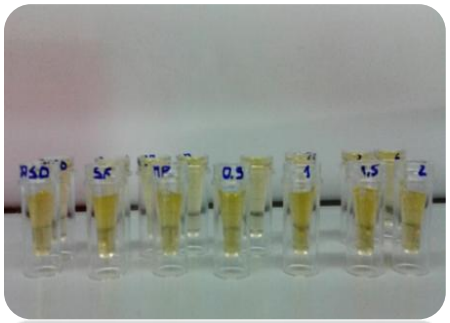
^{13}C NMR spectrum (75.47 MHz) of persulfated triazole-linked coumarin glucoside (DMSO –d_6)

BIOLOGICAL ACTIVITY

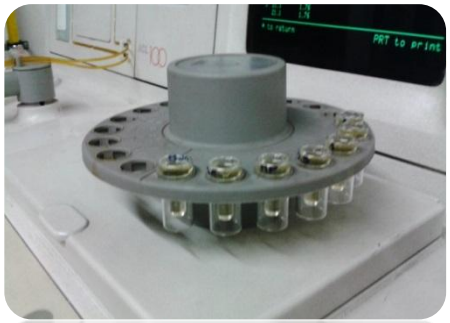
Anticoagulant activity

The anticoagulant activity was measured by the classical clotting times: activated partial thromboplastin time (**APTT**), prothrombin time (**PT**), and thrombin time (**TT**) - in five different concentrations

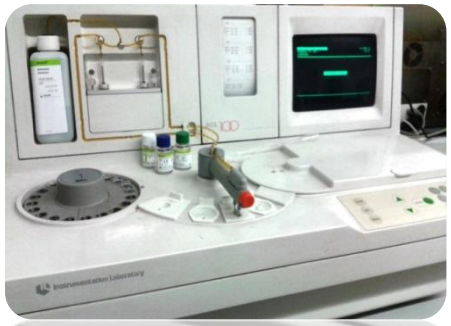
Citrated normal human plasma mixed (1:1) with sample solution (50 µL) was incubated for 2 min at 37°C



Plasma mixed with compound solution



Sample place cuvette rotor



ACL-100

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Correia-da-Silva M, Sousa E, Duarte E, Marques F, Carvalho F, Cunha-Ribeiro LM, Pinto M, *J. Med. Chem.,* **2011**, 54, 5373-5384.

BIOLOGICAL ACTIVITY

Anticoagulant activity

The **sulfated derivatives (4b, 5b, 6b, 8a, and 9a) showed anticoagulant properties** in a dose-dependent manner.

All non-sulfated parent compounds were **inactive** in all clotting times.

APTT was the most sensitive test to the presence of these persulfated coumarins. Although warfarin affects the APTT, the APTT is less sensitive to warfarin then is the PT, which is not the case of these new coumarins.

The double concentration for APTT of the most potent derivative was 22×10^{-5} M.

CONCLUSIONS

In this work **four different molecular modifications** were applied to the coumarin scaffold.

Twelve **new coumarin derivatives** were synthesized.

The anticlotting activity of the **five sulfated derivatives** was evaluated.

The new **sulfated coumarin hybrids** showed **anticlotting** profile different from warfarins.

ACKNOWLEDGMENTS

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(POCI-01-0145-FEDER-016793)