Potential Orally-Active Heparin-Like Compounds: Synthesis and Anticoagulant Activity

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Bile acid receptors

EPHITELIAL BARRIER

Passive diffusion

Triazole

Bile acid

APTT$_2$ = 44 μM

APTT$_2$ = 129 μM
Abstract:

According to World Health Organization, cardiovascular diseases are the first cause of death worldwide. Although health improved in the last decades, lifestyle changes led to an increased incidence of cardiovascular diseases. Currently, the available antithrombotic drugs are associated with significant drawbacks that limit their use and the development of more advantageous drugs with less secondary effects is necessary. A new class of polysulfated small-molecules with anticoagulant and antiplatelet activities was discovered in our group. However, these polysulfated derivatives showed poor antithrombotic efficacy by in vivo oral administration in mice, predicted to be due to poor absorption in the gastrointestinal (GI) tract. The main aim of this work was to improve the oral bioavailability of these compounds. In order to get new optimized analogues two strategies were considered: i) obtaining conjugates with bile acids and ii) introduction of a triazole ring.

Naringin-deoxycholic acid conjugate was obtained through a crosslinking reaction using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro borate (TBTU) as coupling reagent. Triazole linked xanthone glycoside was obtained through a copper(I)-catalyzed alkyne-azide cycloaddition following by O- and N-deacetylation. Sulfation was successfully achieved with triethylamine-sulfur trioxide adduct under microwave irradiation.

The three sulfated derivatives were screened for anticoagulant activity using the three classic clotting times: activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT). All the sulfated compounds prolonged the clotting times and the most active compound was the persulfated naringin-deoxycholic acid conjugate, exhibiting a double concentration value on the APTT (APTT₂) in the micromolar range (around 44 µM). These new optimized analogues with anticoagulant activity are expected to cross the GI tract membranes after oral administration.

Keywords: Bile acid; triazole; flavonoid; sulfates; anticoagulant
Oral bioavailability plays an important role in drug discovery and development.

Rule of 5:
- Molecular Weight ≤500
- CLogP ≤5
- H-bond donor ≤5
- H-bond acceptors (N+O) ≤10

Extensions:
- Polar surface area ≤140 Å or H-bond donors + acceptors ≤12
- Rotatable bonds ≤10

Introduction

Heparins case study

UFH - Unfractioned heparin; LMWH - Low molecular weight heparins

**Introduction: Heparins case study**  
**Drug conjugates**

Heparin conjugates

Deoxycholic acid (DOCA)

Fatty acids

Cholesterol

R=H lauric acid  
R=CH₂CH₃ myristic acid  
R=(CH₂)₃CH₃ palmitic acid  
R=(CH₂)₅CH₃ stearic acid

Introduction: Heparins case study
Drug conjugates

Introduction: Heparins case study
Drug conjugates

DCC - N,N-dicyclohexylcarbodiimide; EDA – ethylenediamine; EDAC - 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; FA – formamide; DMF - dimethylformamide

Introduction: Heparins case study
Drug conjugates

Introduction: Heparins case study
Penetration enhancers

Deoxycholyethlamine

Polycationic lipophilic-core dendrons

Tight junction
Intestinal lumen
Epithelial cells
Blood circulation

Transcellular absorption
Paracellular absorption

Non-α aminoacids
SNAC
SNAD

Sodium caprate

18β-Glycyrrhetinic acid

Mucoadhesive polymers

MCC
SNOC
PCP-Cys

Previous work: our strategy

Mimetize sulfated polysaccharides

More hydrophobic
Less polyanionic
Feasible synthesis

SULFATED OLIGO-PHENOLIC MOLECULES

Previous work: old drugs as building blocks for sulfation

Previous work: synthesis of polysulfated derivatives

Previous work: anticoagulant activity in vitro and in vivo

- In vitro and in vivo anticoagulant activity
- Fast onset of action
- Low toxicity
- Plasma stability

NOT ACTIVE BY ORAL ADMINISTRATION

Aims

**Synthesis of potential orally-active polysulfated compounds**

**Polysulfated oligophenols-DOCA conjugate**

**Glucoside triazole-linked xanthone**

- Bile acid receptors
- Passive diffusion
- Epithelial barrier

**Aims**

Synthesis of potential orally-active polysulfated compounds
Results and discussion
Strategy 1: Conjugation with DOCA

Suitable model to plan antithrombotic derivatives

Results and discussion
Strategy 1: Conjugation with DOCA

DMF – dimethylformamide; NHS – N-hydroxysuccinimide; DCC - N,N-dicyclohexylcarbodiimide; EDA – ethylenediamine. TBTU - 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro borate; TEA – triethylamine
Results and discussion
Strategy 1: Conjugation with DOCA

SO$_3$:Et$_3$N – triethylamine-sulfur trioxide adduct; DMA – dimethylacetamide.
Results and discussion
Strategy 2: Introduction of triazole

Dual anticoagulant and antiplatelet activity

10 Xanthones
Privileged scaffold with several biological activities

APTT$_2 =$ 60 μM

NOT ACTIVE BY ORAL ADMINISTRATION

Results and discussion
Strategy 2: Introduction of triazole

THF/water
Sodium ascorbate
CuSO₄·5H₂O
70°C, MW (500W), 30min

11

12

13 (73%)

R=COCH₃

MeONa/MeOH
r.t., 3h

14 (86%)

R=COCH₃

aq. sol. NaOH 20%
100°C, 4h

15 (36%)

SO₃·TEA (10eq/OH and NH₂)
DMA, MW (200W)
100 °C, 2h

16 (78%)

R=SO₃⁻
Results and discussion
Anticoagulant activity

Dose-dependent effects of polysulfated compounds 8 and 9 on APTT, PT, and TT clotting assays using human pooled plasma, expressed as ratio of clotting time in the presence/absence of compound. \(^a\) clotting time values greater than 180s, \(^b\) clotting time values greater than 120s, \(^c\) clotting time values greater than 240s, * \(P < 0.05\)
Results and discussion
Anticoagulant activity

Dose-dependent effects of polysulfated compound 16 on APTT, PT, and TT clotting assays using human pooled plasma, expressed as ratio of clotting time in the presence/absence of compound. * clotting time values greater than 180s, c clotting time values greater than 240s. * P < 0.05
Successful synthesis of **optimized polysulfated compounds** with the application of **microwave radiation** in copper(I)-catalyzed alkyne-azide 1,4-cycloaddition and sulfation.

Persulfated naringin-DOCA conjugate (8) and triazole-linked xanthone glycoside (16) showed anticoagulant activity and **persulfated naringin-DOCA conjugate** was the most potent anticoagulant sulfated compound synthesized in OUR GROUP.

**FUTURE WORK:**
Test permeability of the optimized polysulfated derivatives.
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