1st International Electronic Conference on Medicinal Chemistry

2-27 November 2015

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

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

Ana Rita Neves [1], Emília Sousa [1,2], Madalena Pinto [1,2], and Marta Correia-da-Silva[1,2,*]

[1] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313, Porto, Portugal;

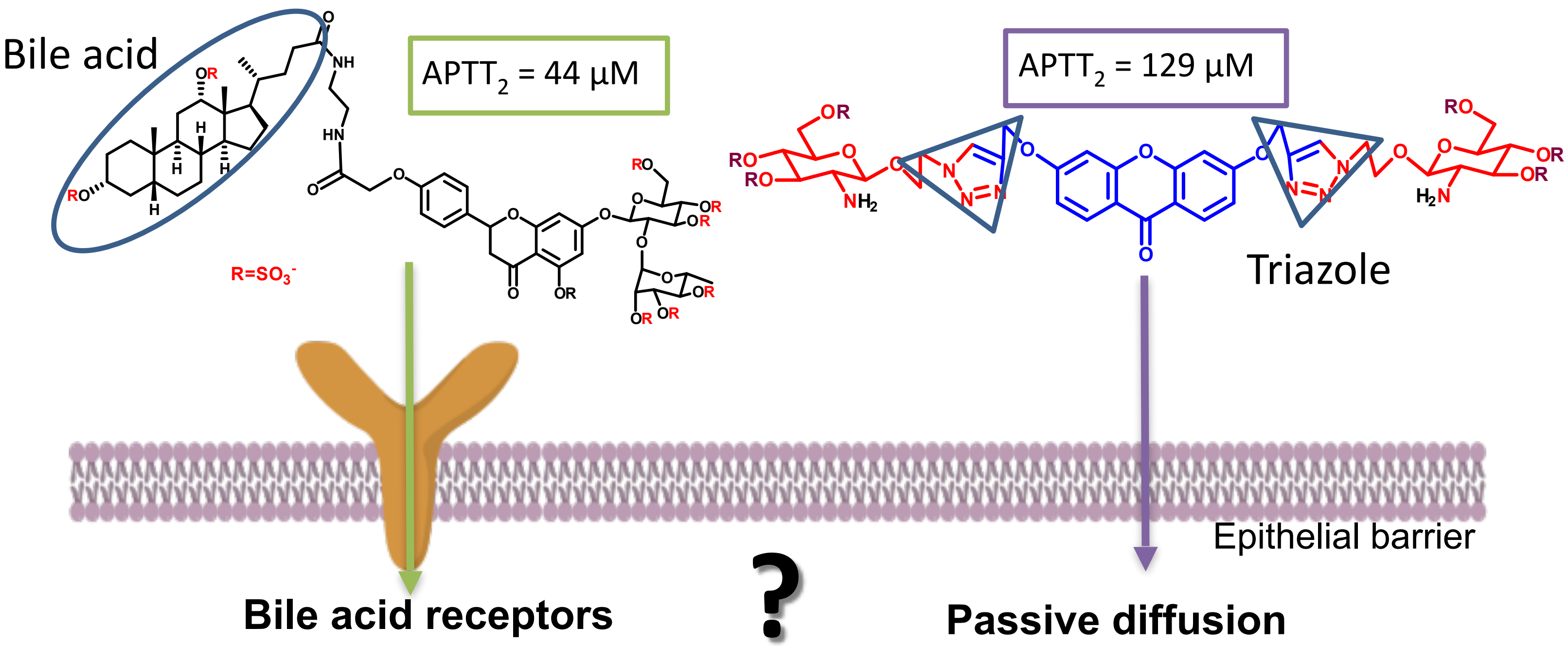
[2] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Universidade do Porto, Rua dos Bragas 289, 4050-123, Porto, Portugal.

* Corresponding author: **m_correiadasilva@ff.up.pt**

U.PORTO

FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

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



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-(1*H*-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 ($APTT_2$) 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

Introduction
Oral bioavailability of drugs

Oral bioavailability plays an important role in drug discovery and development.

Structure optimization

Molecular modification (e.g. prodrugs)

Drug formulation

Excipients or micro and nanoparticles

Rule of 5:

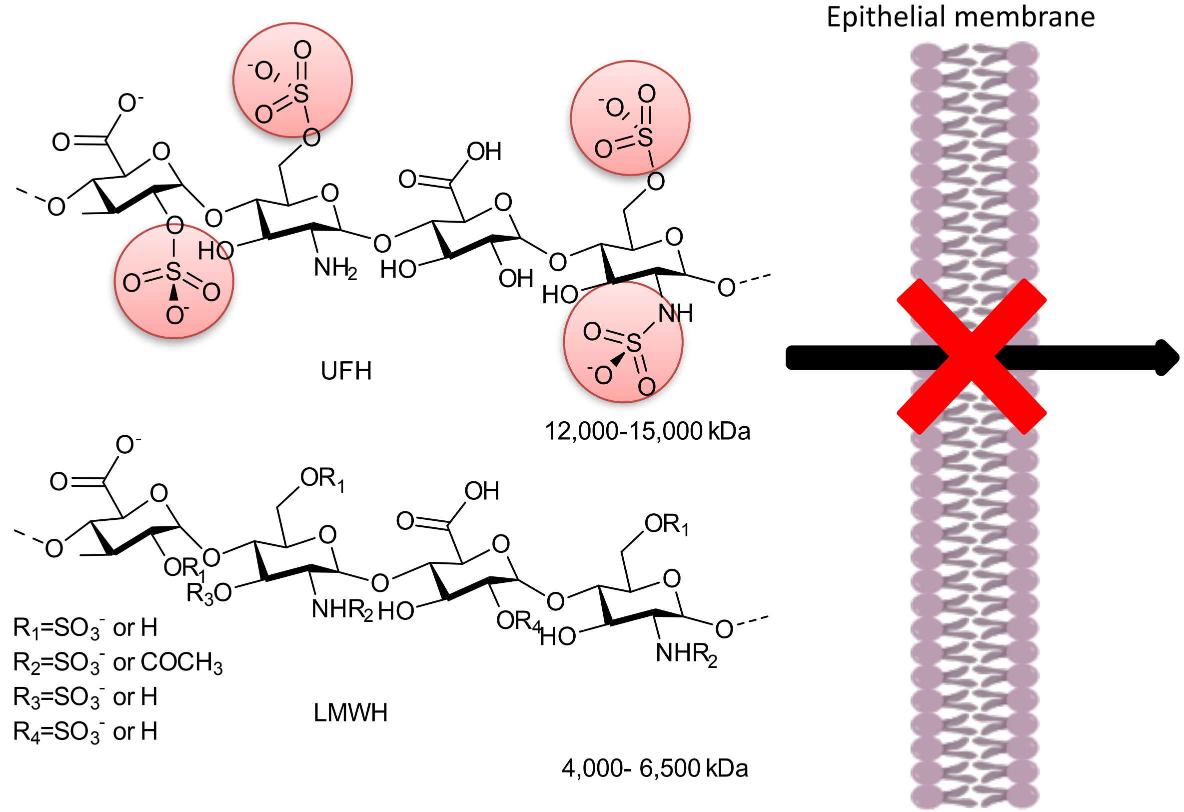
- Molecular Weight ≤500
- CLogP ≤5
- H-bond donor ≤5
- H-bond acceptors (N+O) ≤10

Extensions:

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

Keller *et al.*, *Curr. Opin. Chem. Biol.* **2006**, 10:357–361.

Introduction
Heparins case study



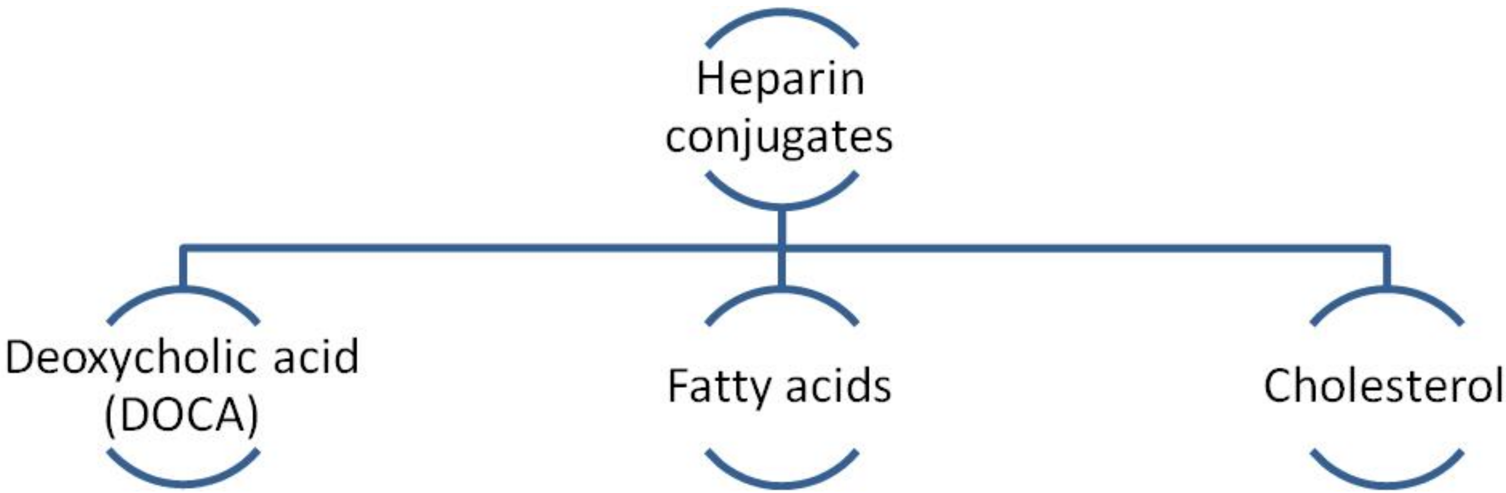
↑ Negative charge

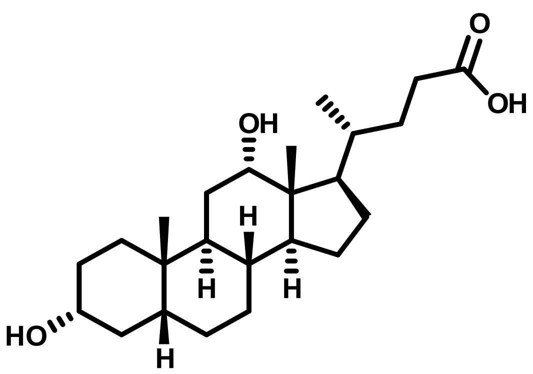
↑ Molecular weight

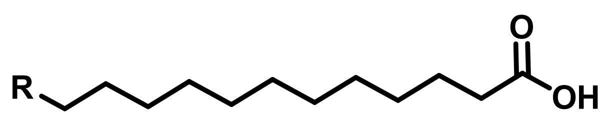
UFH - Unfractioned heparin; LMWH - Low molecular weight heparins

Linhardt, *J. Med. Chem.* **2003**, 46(13):2551-64; Page, *ISRN Pharmacol.* **2013**, 13;

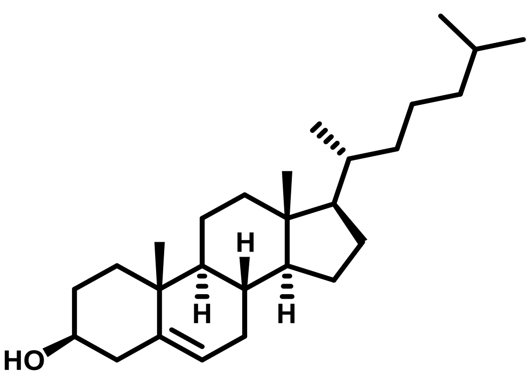
Introduction: Heparins case study
Drug conjugates





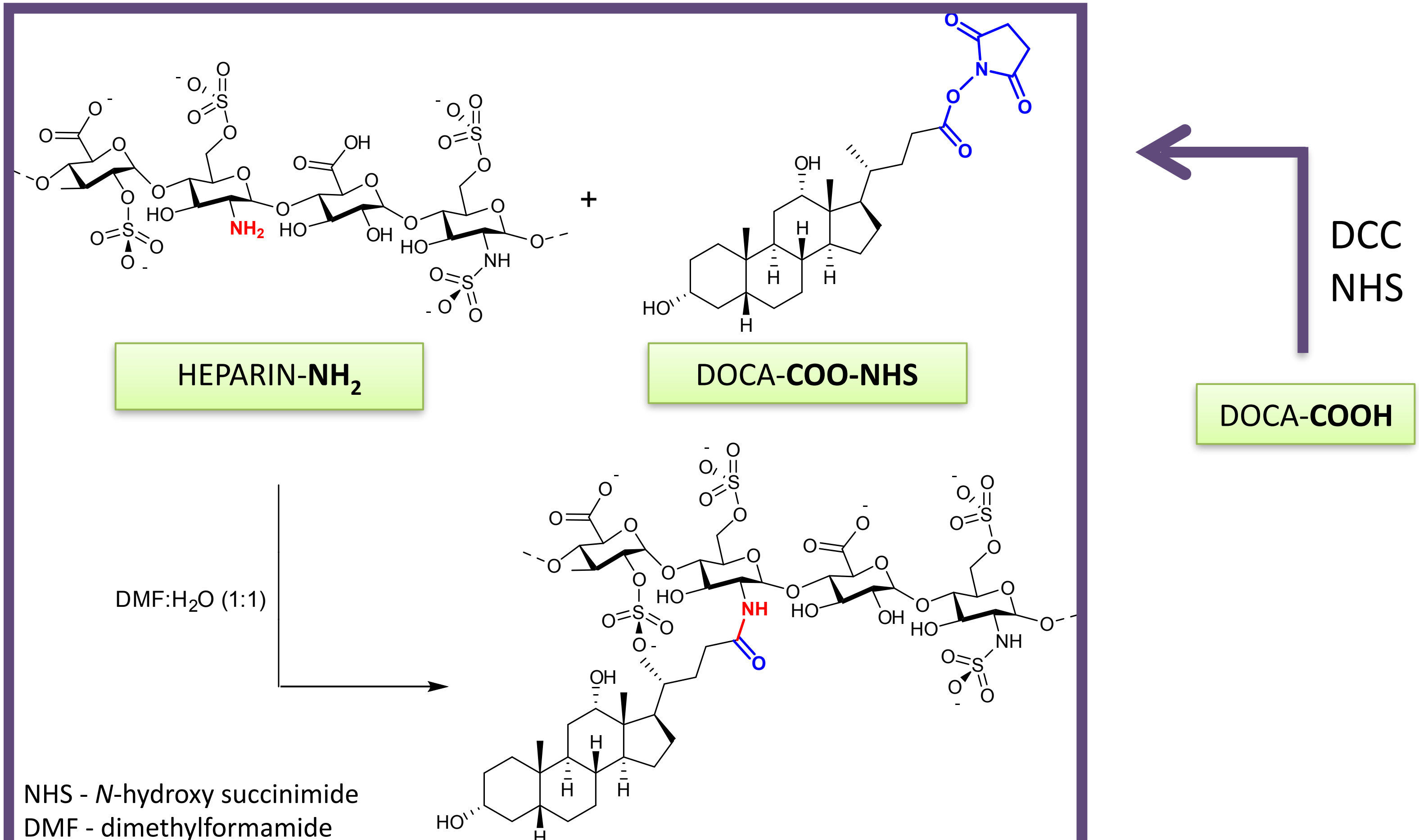


R=H lauric acid
R=CH_2CH_3 myristic acid
R=$(CH_2)_3CH_3$ palmitic acid
R=$(CH_2)_5CH_3$ stearic acid



Kim *et al.*, *J. Controll. Release* **2007**, 120 (1-2), 4-10; Lee *et al.*, *Circulation* **2001**, 104(25):3116-20; Eom *et al.*, *Thromb. Res.* **2010**, 126(3):220-4

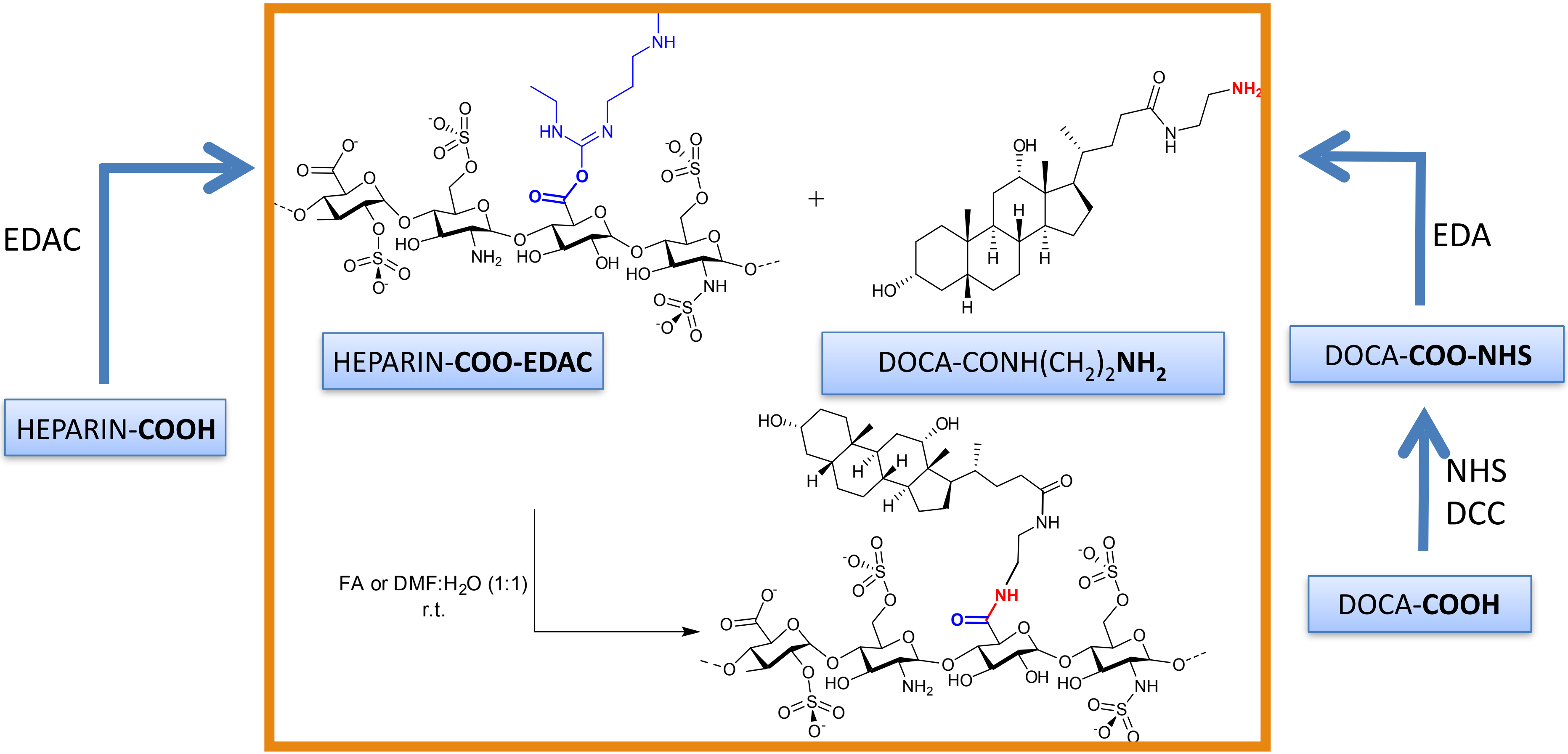
Introduction: Heparins case study
Drug conjugates



NHS - *N*-hydroxy succinimide
DMF - dimethylformamide

Lee *et al.*, *J. Control. Release* **2006**, 111(3):290–8; Eom *et al.*, *Thromb. Res.* **2010**, 126(3):220-24.

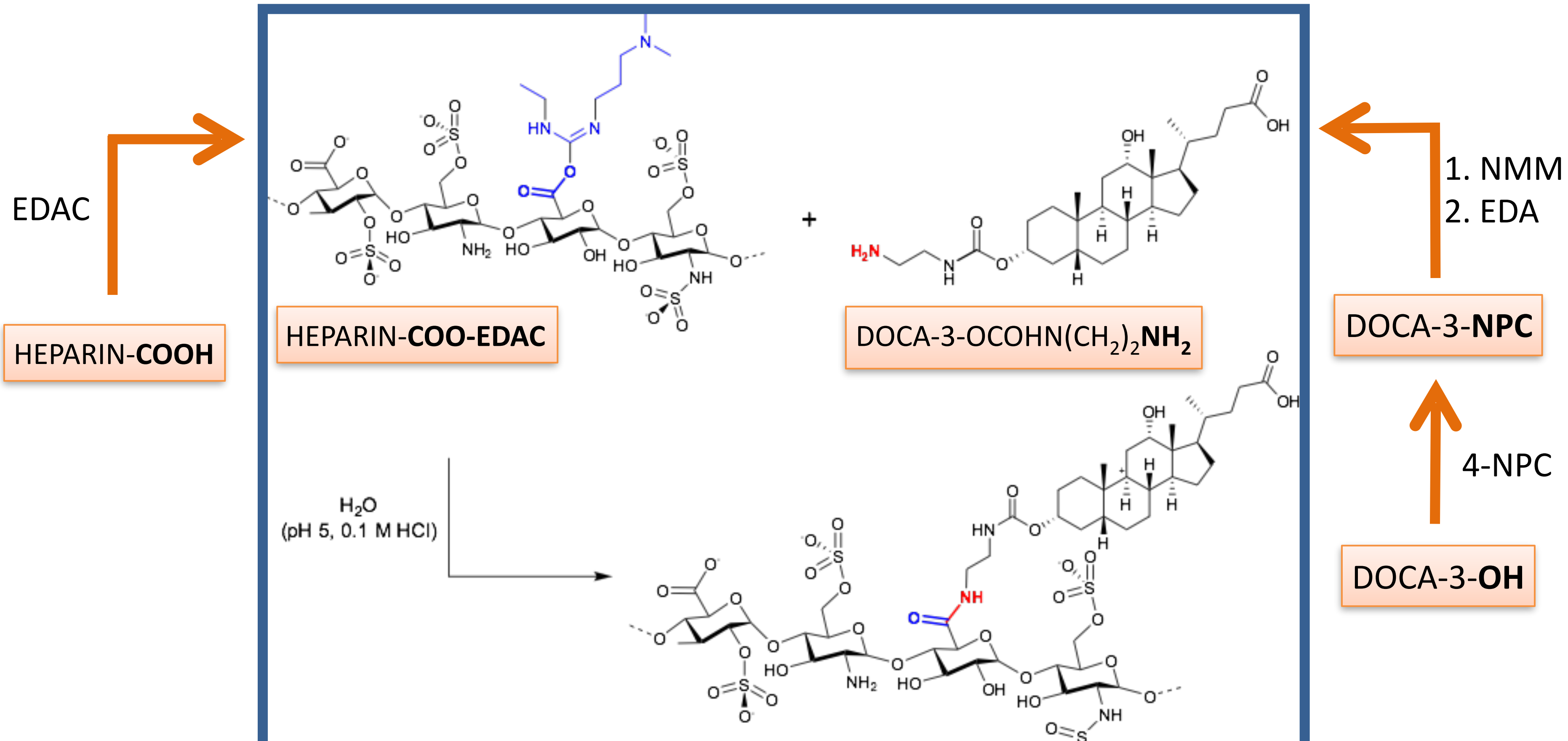
Introduction: Heparins case study
Drug conjugates



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

Park *et al.*, *Langmuir* **2004**, 20:11726-31; Kim *et al.*, *J. Control. Release* **2007**, 120(1-2):4–10; Kim *et al.*, *Bioconjugate Chem.* **2011**, 22:1451–8.

Introduction: Heparins case study
Drug conjugates

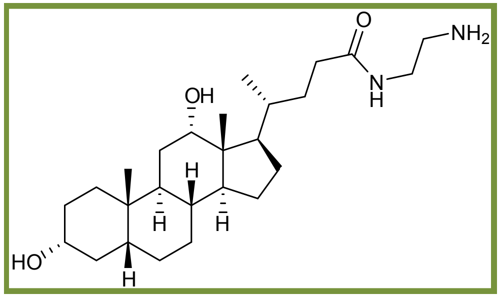


4-NPC - 4-nitrophenyl chloroformate; NMM – 4-methylmorpholine.

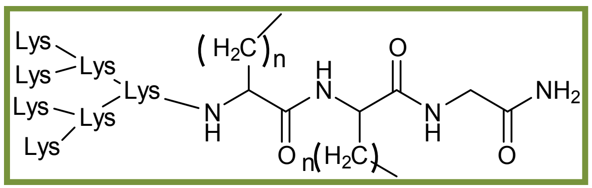
Moon *et al.*, *Macromol. Res.* **2009**, 17(2):79-83; Khatun *et al.*, *Carbohydr. Polym.* **2012**, 90(4):1461-8.

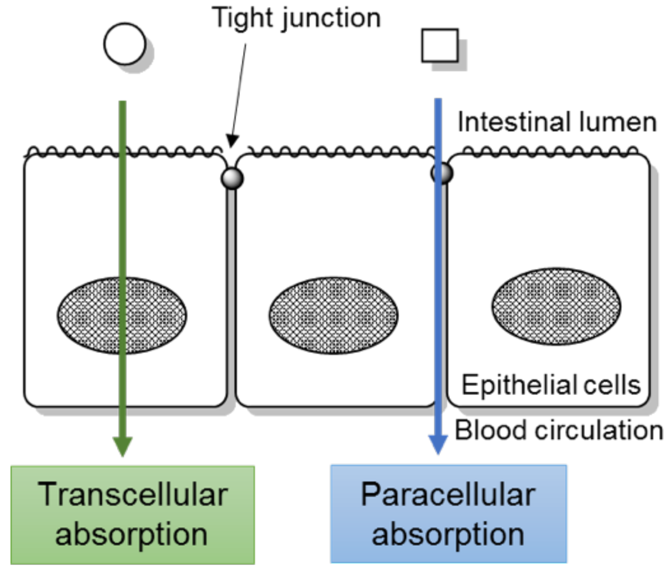
Introduction: Heparins case study
Penetration enhancers

Deoxycholylethylamine

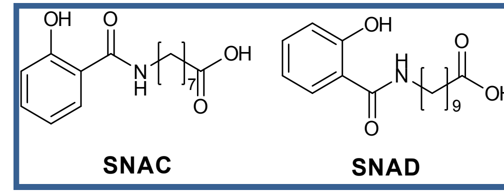


Polycationic lipophilic-core dendrons

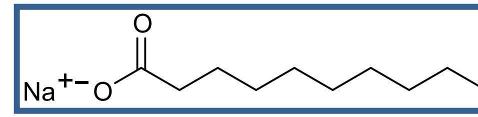




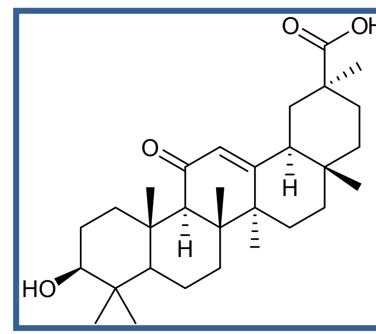
Non-α aminoacids



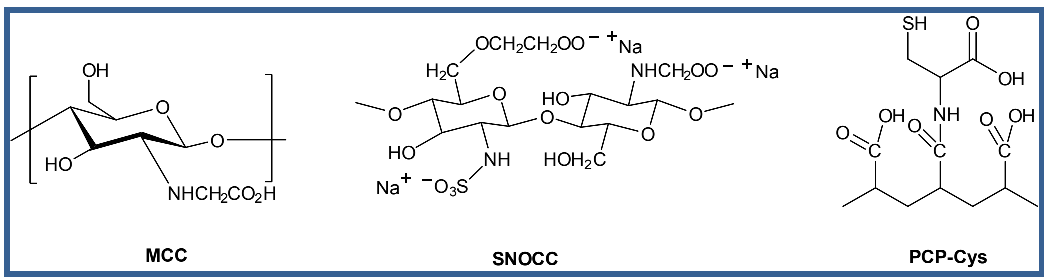
Sodium caprate



18β-Glycyrrhetinic acid



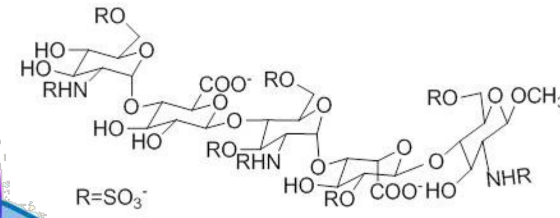
Mucoadhesive polymers



Krug *et al.*, *Biomaterials* **2013**, 34(1):275-82; Motlekar *et al.*, *Drug Dev. Res.* **2006**, 67(2):166-74; Thanou *et al.*, *J. Pharm. Sci.* **2001**, 90(1):38-46; Thanou *et al.*, *J. Controll. Release* **2007**, 117(2):171-8; Bernkop-Schnürch *et al.*, *Adv. Drug Deliv. Rev.* **2005**, 57(11):1569-82; Pineo *et al.*, *Best Pract. Res. Clin. Haematol.* **2004**, 17(1):153-60; Hayes *et al.*, *Biorg. Med Chem.* **2006**, 14 (1):143-52; Lee *et al.*, *J. Crontrol. Release* **2007**, 123(1):39-45.

Previous work: our strategy

Mimetize sulfated polysaccharides

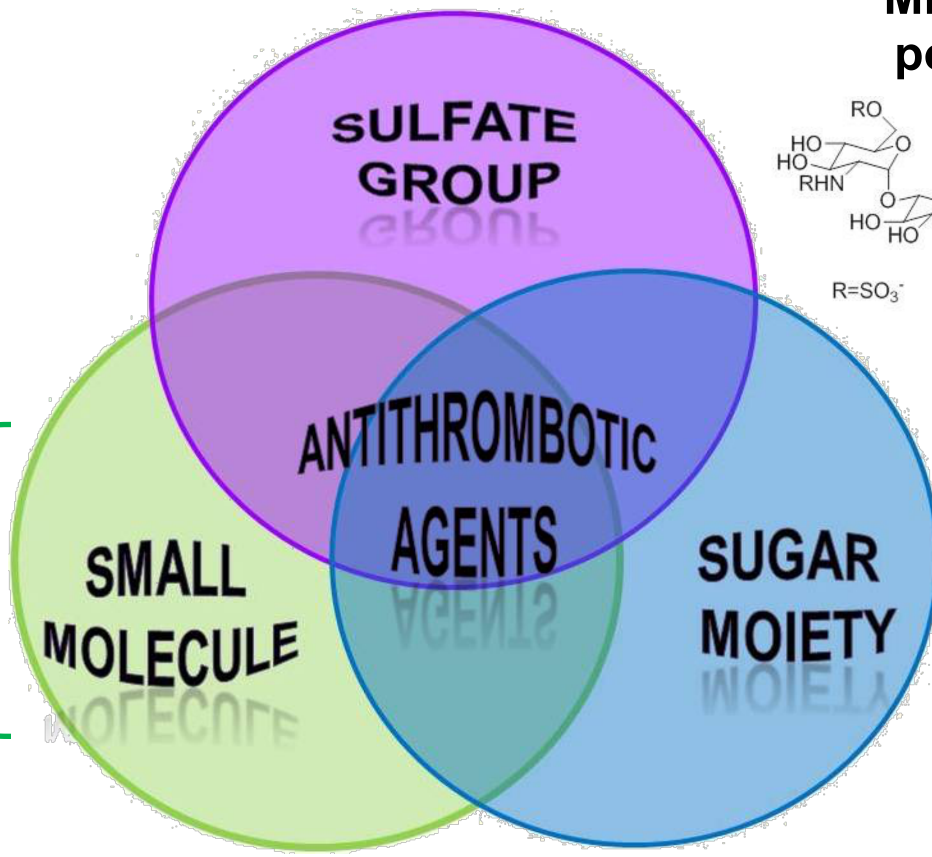


fondaparinux

More hydrophobic

Less polyanionic

Feasible synthesis

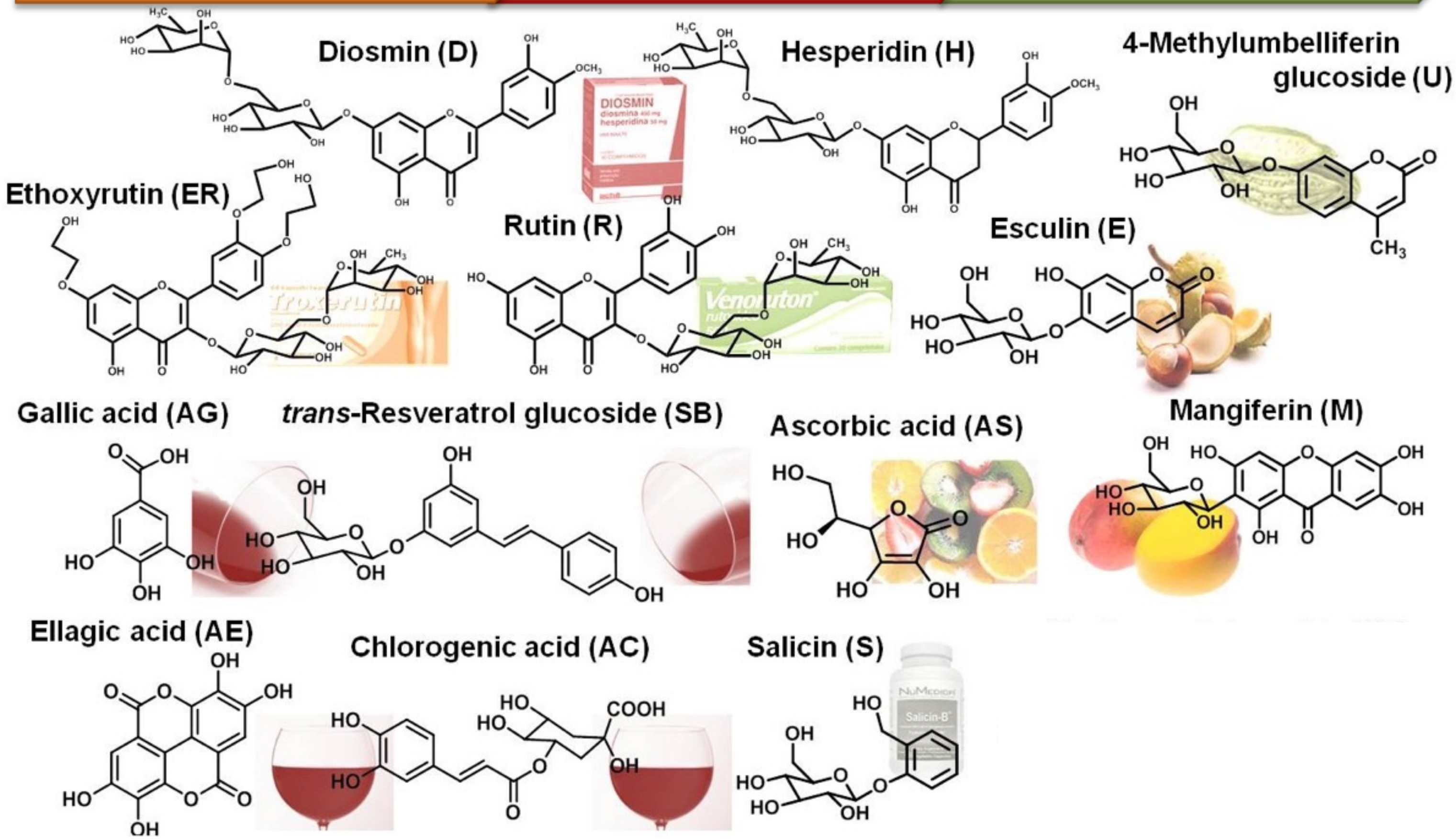


SULFATED OLIGO-PHENOLIC MOLECULES

Correia-da-Silva *et al.*, *J. Med. Chem.* **2011**, 54(1):95-106; Correia-da-Silva *et al., J. Med. Chem.* **2011**, 54(15):5373-84.

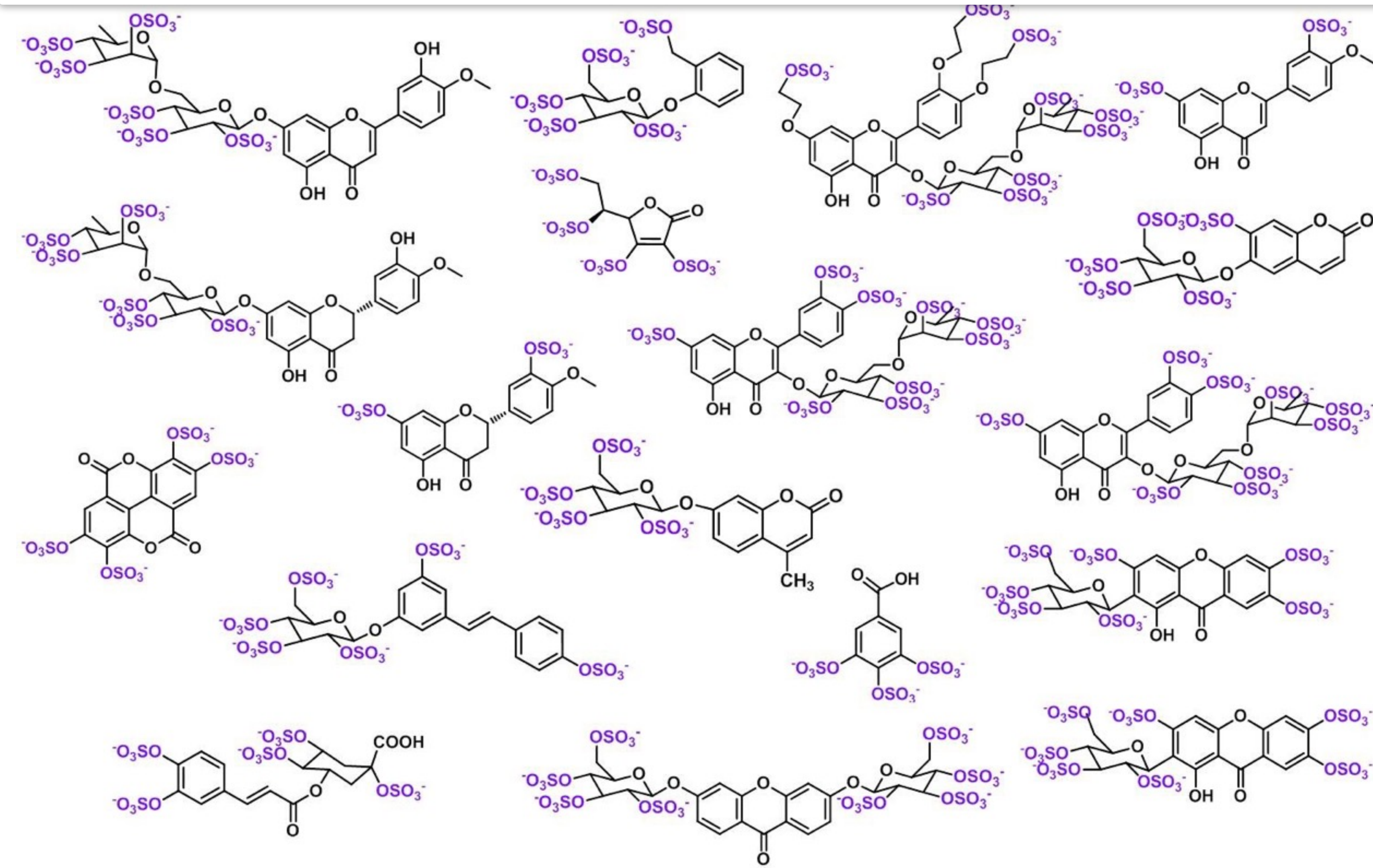
Previous work: old drugs as building blocks for sulfation

OLD DRUGS → CARDIOVASCULAR BENEFITS → NATURAL PRODUCTS



Correia-da-Silva *et al., J. Med. Chem.* **2011**, 54(1):95-106; Correia-da-Silva, M.; *et al. Eur. J. Med. Chem.* 2011, 46, 2347-2358
Correia-da-Silva *et al., J. Med. Chem.* **2011**, 54(15):5373-84.

Previous work: synthesis of polysulfated derivatives



Correia-da-Silva *et al.*, *J. Med. Chem.* **2011**, 54(1):95-106; Correia-da-Silva, M.; *et al. Eur. J. Med. Chem.* 2011, 46, 2347-2358
Correia-da-Silva *et al., J. Med. Chem.* **2011**, 54(15):5373-84.

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

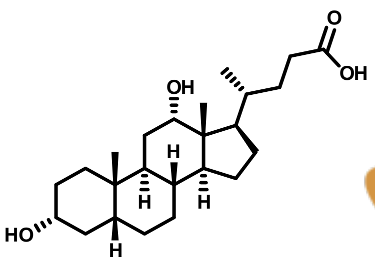
Correia-da-Silva *et al.*, *J. Med. Chem.* **2011**, 54(1):95-106; Correia-da-Silva *et al., J. Med. Chem.* **2011**, 54(15):5373-84.

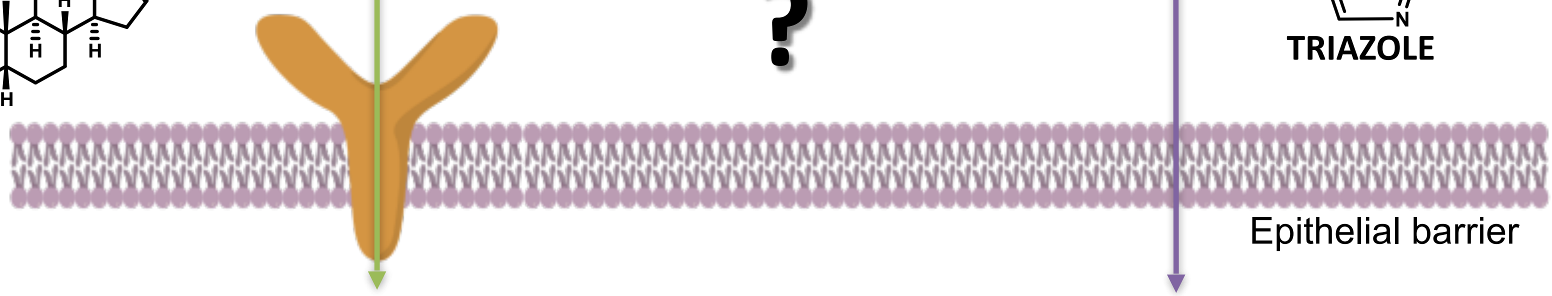
Aims

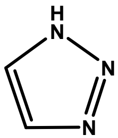
Synthesis of potential orally-active polysulfated compounds

Polysulfated oligophenols-DOCA conjugate

Glucoside triazole-linked xanthone







TRIAZOLE

Bile acid receptors

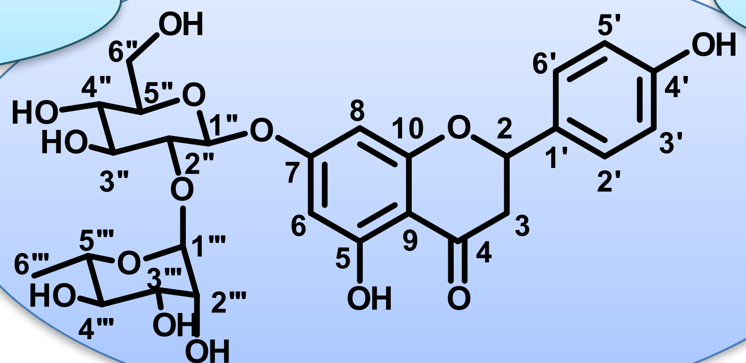
Passive diffusion

Results and discussion
Strategy 1: Conjugation with DOCA

Attenuates mitochondrial dysfunction

Neuroprotective effects

Commercialized as supplement

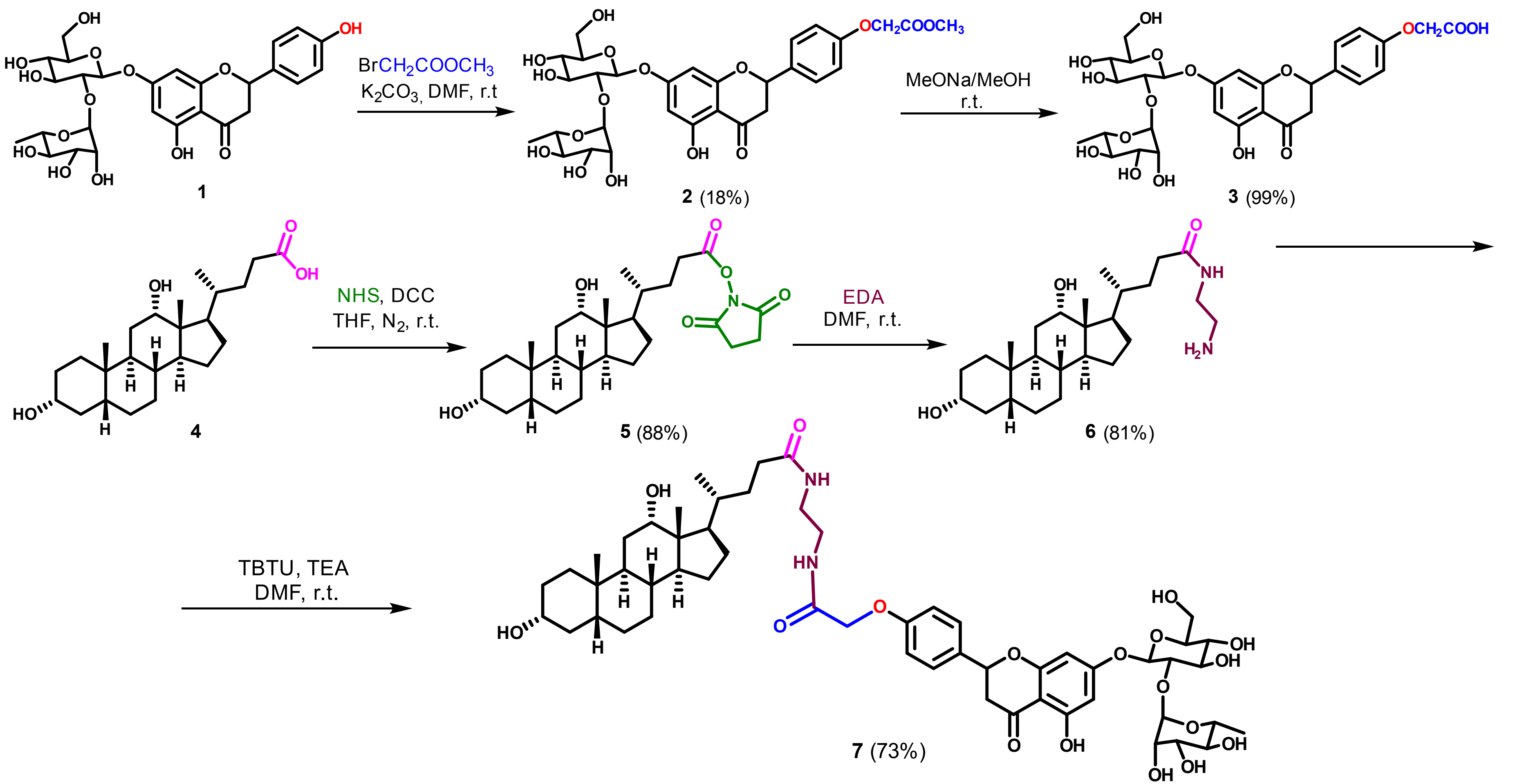


Naringin (1)

Suitable model to plan antithrombotic derivatives

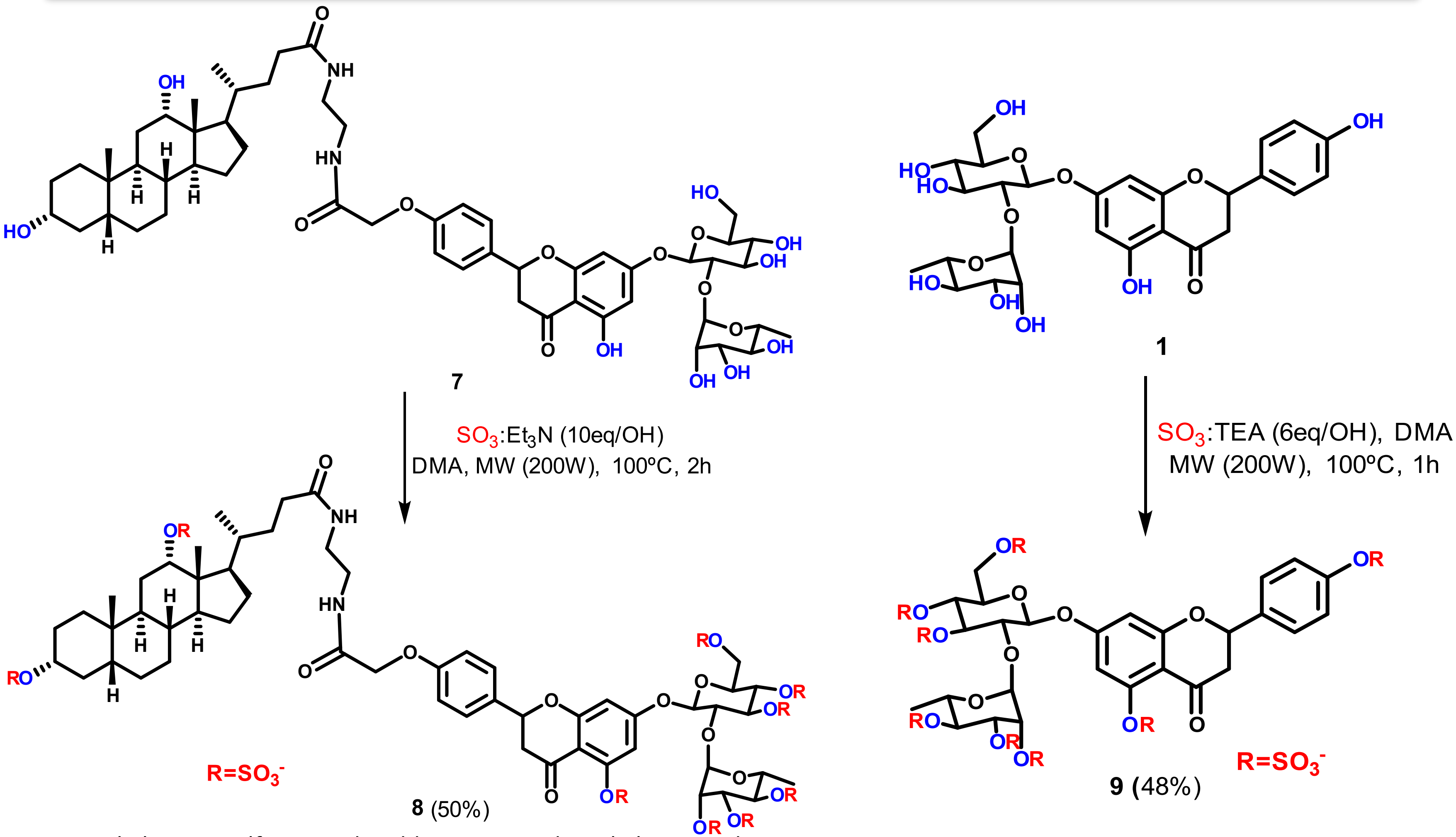
Sachdeva *et al.*, *Pharmacol. Biochem. Behav.* **2014**, 127:101-10; Kandhare *et al.*, *Fitoterapia* **2012**, 83(4):650-59; Choi *et al.*, *Ann. Nutr. Metab.* **2001**, 45(5):193-201.

Results and discussion
Strategy 1: Conjugation with DOCA



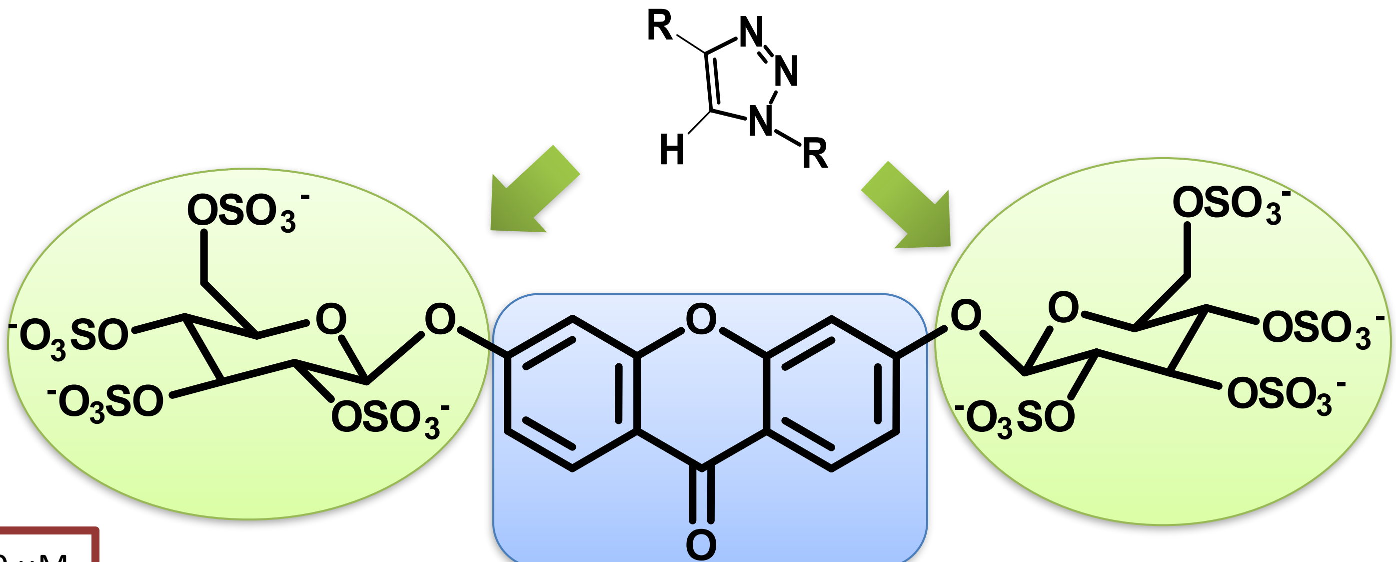
DMF – dimethylformamide; ; NHS – N-hydroxysuccinimide; DCC - *N*,*N*-dicyclohexylcarbodiimide; EDA – ethylenediamine. TBTU - 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro borate; TEA – triethylamine

Results and discussion
Strategy 1: Conjugation with DOCA



SO_3:Et_3N – triethylamine-sulfur trioxide adduct; DMA – dimethylacetamide.

Results and discussion
Strategy 2: Introduction of triazole



APTT$_2$ = 60 μM

Dual anticoagulant and antiplatelet activity

10

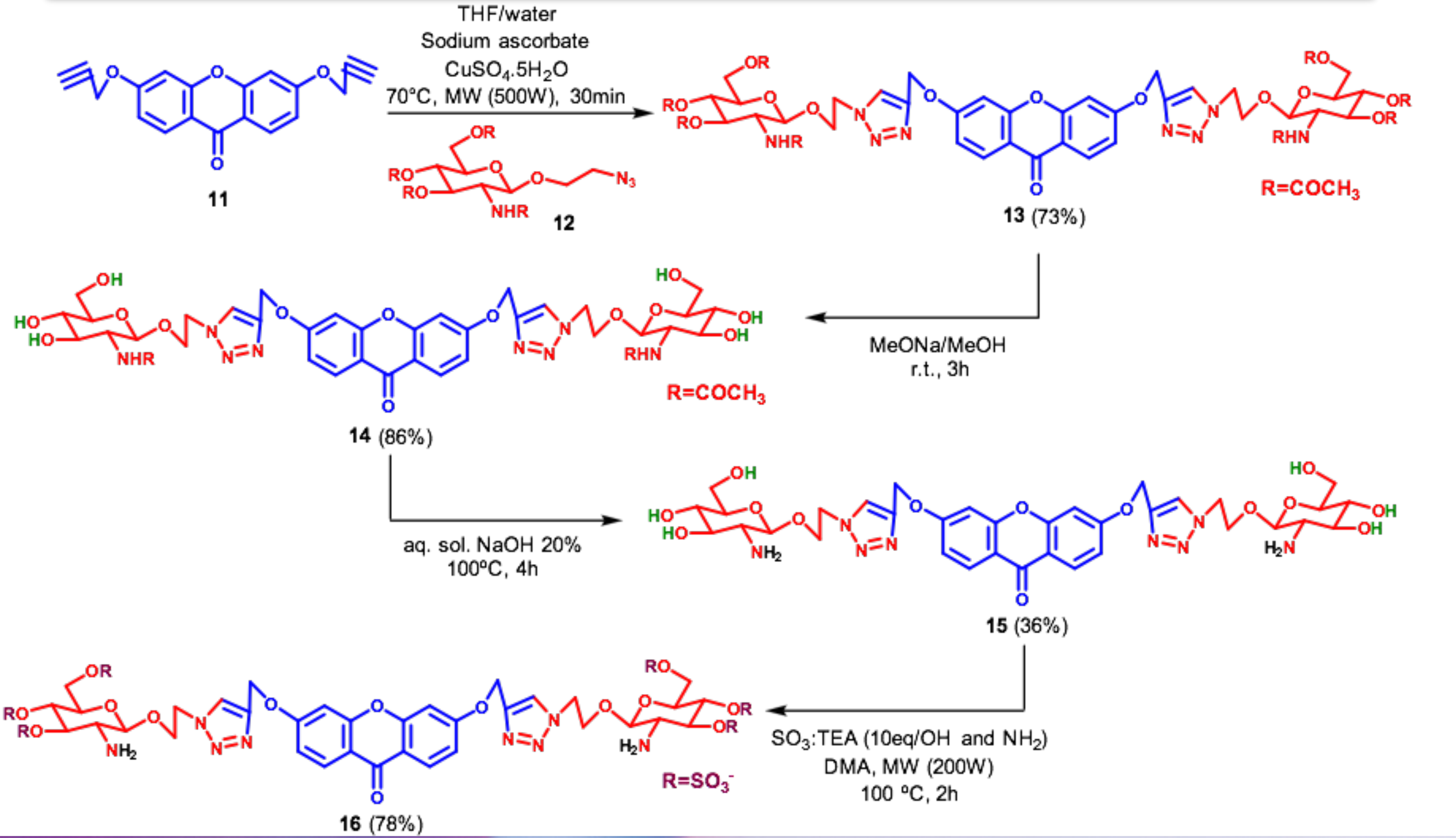
Xanthones

Privileged scaffold with several biological activities

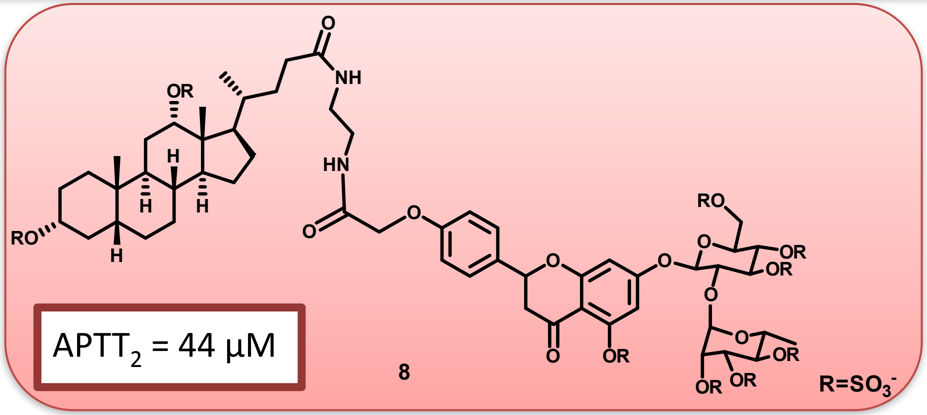
NOT ACTIVE BY ORAL ADMINISTRATION

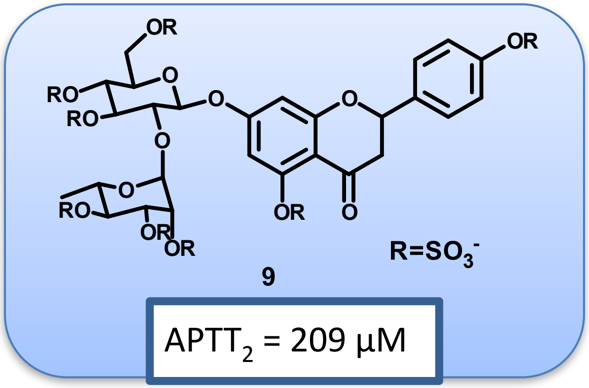
Negi *et al.*, *J. Appl. Chem.* **2013**, *2013*, 9; Klyosov, ACS Symposium Series; American Chemical Society: Washington, DC, **2012**; Correia-da-Silva *et al.*, *J. Med. Chem.* **2011**, 54(15):5373-84.

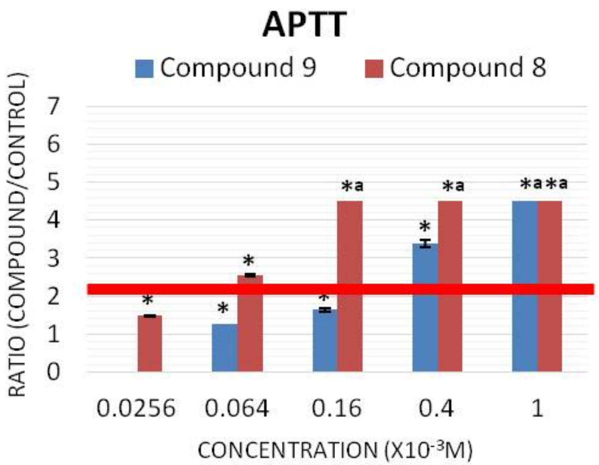
Results and discussion
Strategy 2: Introduction of triazole

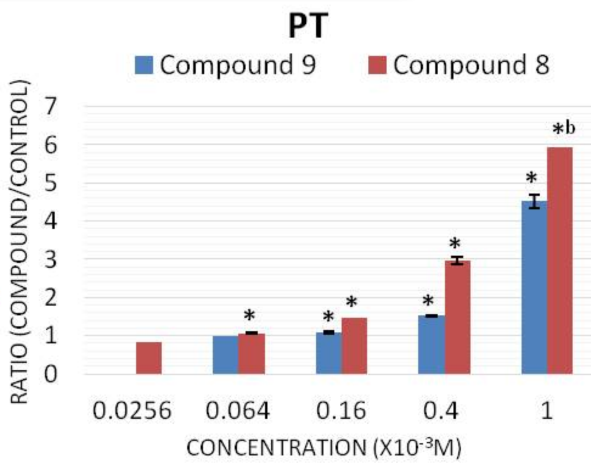


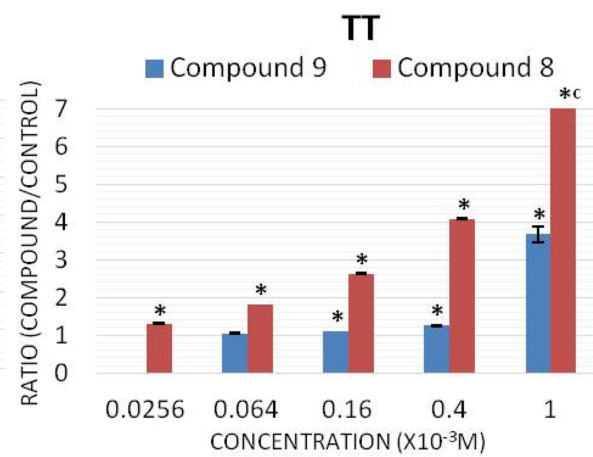
Results and discussion
Anticoagulant activity





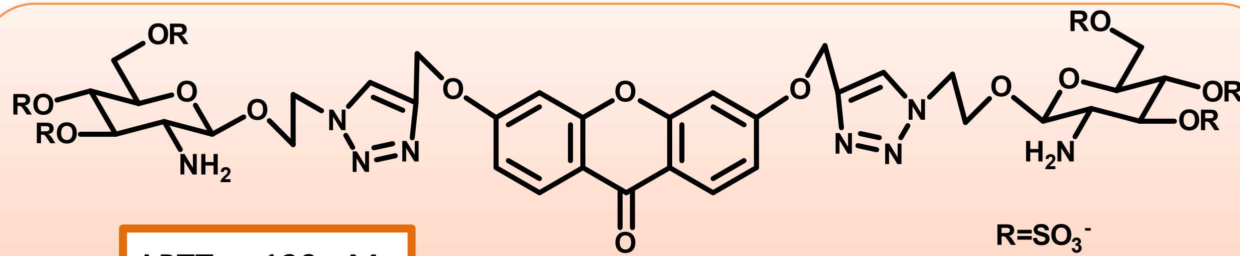






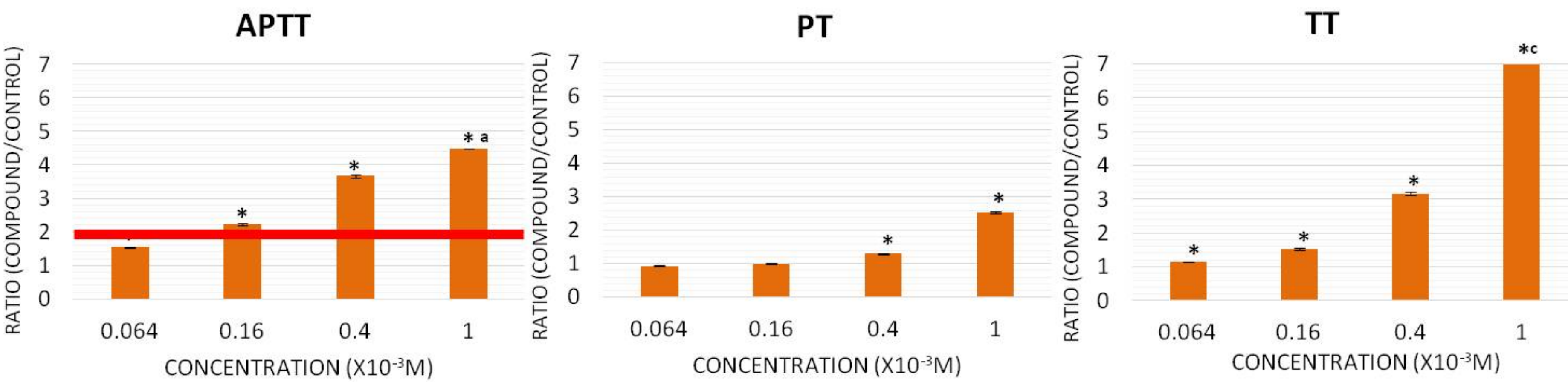
Dose-dependent effects of polysulfated compounds **8** and **9** on APTT, PT, and TT clotting assays using human pooled plasma, expressed as ratio of clotting time in the presence/absence of compound. [a] clotting time values greater than 180s, [b] clotting time values greater than 120s, [c] clotting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

Results and discussion
Anticoagulant activity



APTT$_2$ = 129 μM

16



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. [a] clotting time values greater than 180s, [c] clotting time values greater than 240s, * $P < 0.05$

Conclusions

- 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.

Acknowledgements

COMPETE
PROGRAMA OPERACIONAL FACTORES DE COMPETITIVIDADE

QREN
QUADRO DE REFERÊNCIA ESTRATÉGICO NACIONAL
PORTUGAL 2007.2013

UNIÃO EUROPEIA
Fundo Europeu de Desenvolvimento Regional

CEQUIMED UP

ciimar
Centro Interdisciplinar de Investigação Marinha e Ambiental

FCT
Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR