4th International Electronic Conference on Medicinal Chemistry
1-30 November 2018
chaired by Dr. Jean Jacques Vanden Eynde
sponsored by pharmaceuticals

Biologically-active sulfated steroids: synthesis and state-of-art

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

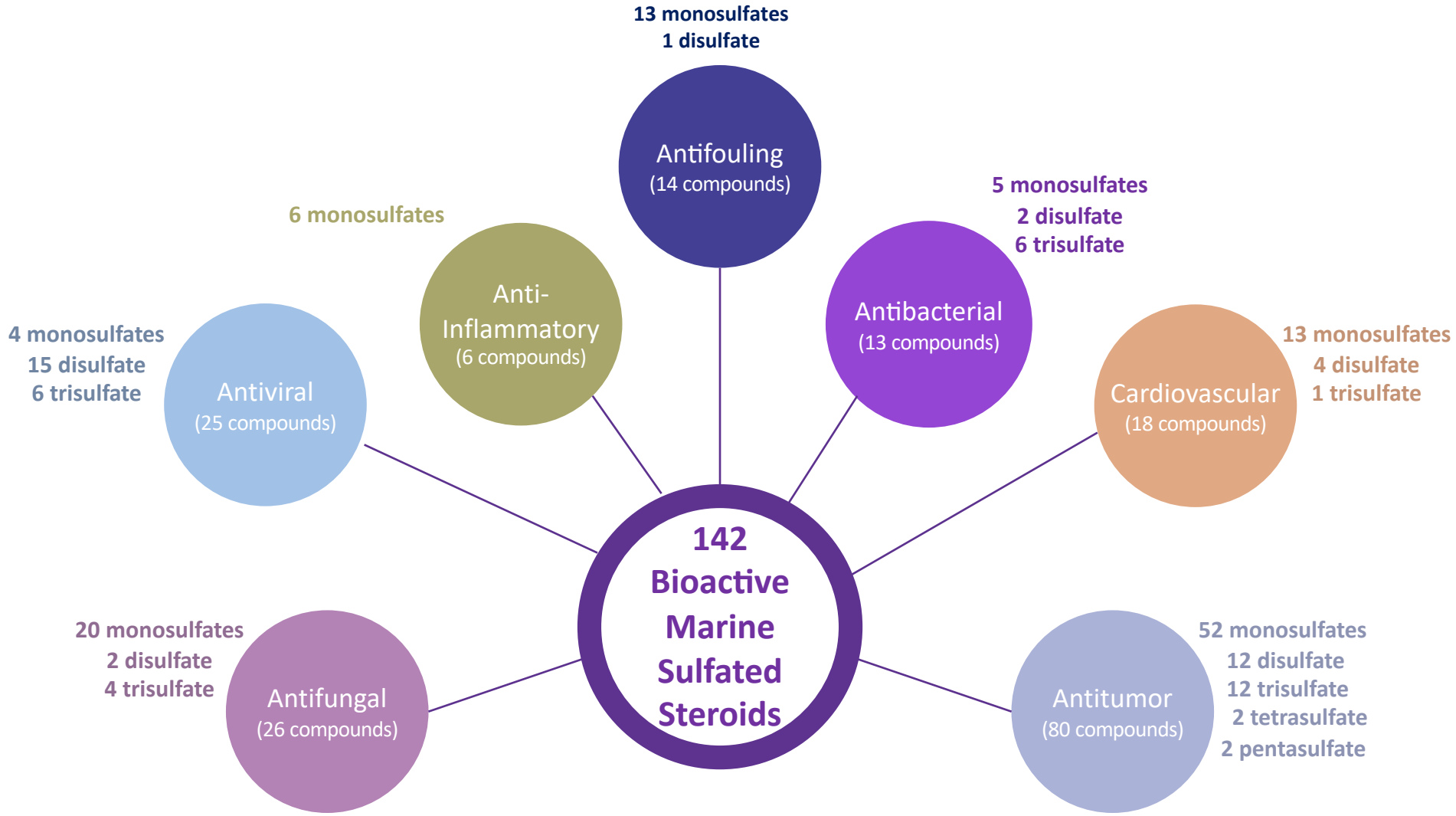
[1] Laboratory of Organic and Pharmaceutical Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[2] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal

* Corresponding author: m_correiadasilva@ff.up.pt

U.PORTO FACULDADE DE FARMÁCIA UNIVERSIDADE DO PORTO

ciimar Centro Interdisciplinar de Investigação Marinha e Ambiental

Biologically-active sulfated steroids: synthesis and state-of-art



Abstract

Several biological activities from nearly 150 marine-derived sulfated steroids have been reported with both pharmacological (antimicrobial, antitumor, cardiovascular and/ or anti-inflammatory activities) and environmental (antifouling activity) applications [1]. Sulfation is used in Nature to avoid toxicity and therefore marine-inspired sulfated steroids could be an interesting strategy for drug discovery. The sulfated aminosterol squalamine, isolated from the internal organs of the dogfish shark, is in phase III of clinical trials as anti-angiogenic drug [2], which evidences the potential of sulfated steroids.

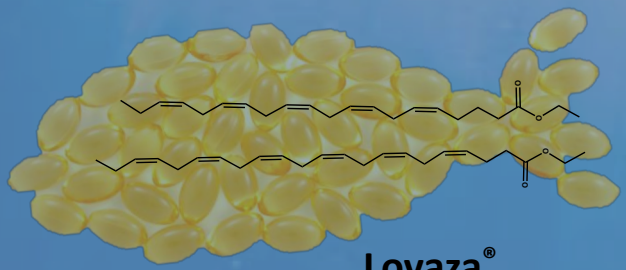
Sulfation of small molecules using sulfur trioxide-amine complexes entails several advantages, such as persulfation, low degradation, and feasibility in the work-up [3]. Moreover, these complexes appear to be suitable for sulfation of alcohol groups present in steroids [4]. In this direction, sulfation of four sterols was achieved using triethylamine-sulfur trioxide adduct in dimethylacetamide under heating, with yields ranging from 3% to 93%. Purification involved insolubilization with diethyl ether followed by several methods to obtain the sulfated derivatives free of inorganic impurities, including dialysis and/ or chromatographic processes. Structure elucidation of these new compounds was established by infrared (IR), nuclear magnetic resonance (NMR) and high resolution mass spectrometry (HRMS). Biological activities will be further studied.

Keywords: Marine; Steroids; Biological Activities; Synthesis

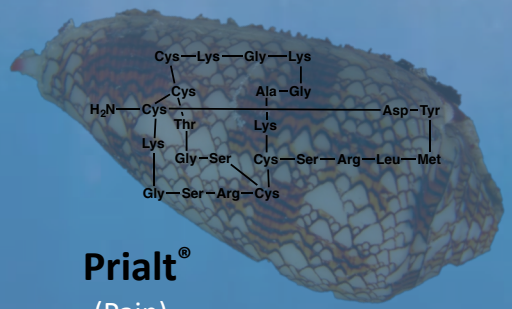
[1] Carvalhal, F., M. Correia-da-Silva, M.E. Sousa, M. Pinto, and A. Kijjoa, Journal of Molecular Endocrinology, 2018, **61**(2) 211-231; [2] NCT02727881 (https://clinicaltrials.gov/ct2/show/NCT02727881, October 15, 2018); [3] Correia-da-Silva, M., E. Sousa, and M.M. Pinto, Medicinal Research Reviews, 2014, **34**(2) 223-79; [4] Al-Horani, R.A., and U.R. Desai, *Chemical Sulfation of Small Molecules - Advances and Challenges.* Tetrahedron, 2010, **66**(16), 2907-2918.

Mayer, A. M.; *et. al,. The FASEB Journal* 2017, *31, 818.1*.

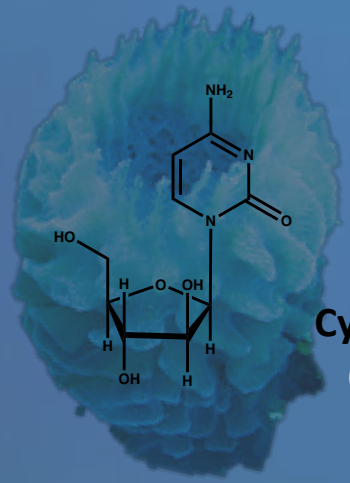
Introduction



Lovaza®
(Hipertriglyceridemia)

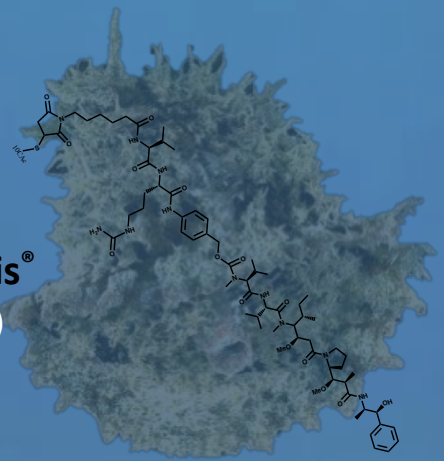


Prialt®
(Pain)



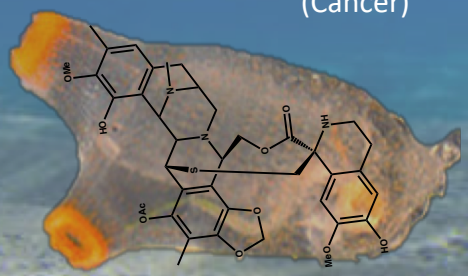
Drugs from Marine World

Cytosar-U®
(Cancer)

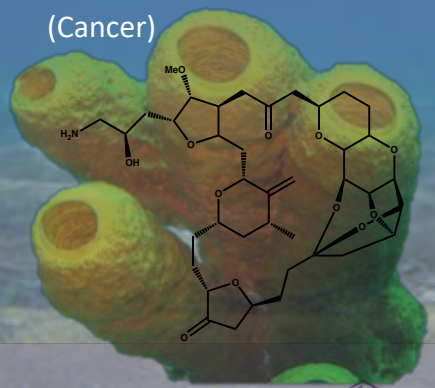


Adcetris®
(Cancer)

Yondelis®
(Cancer)

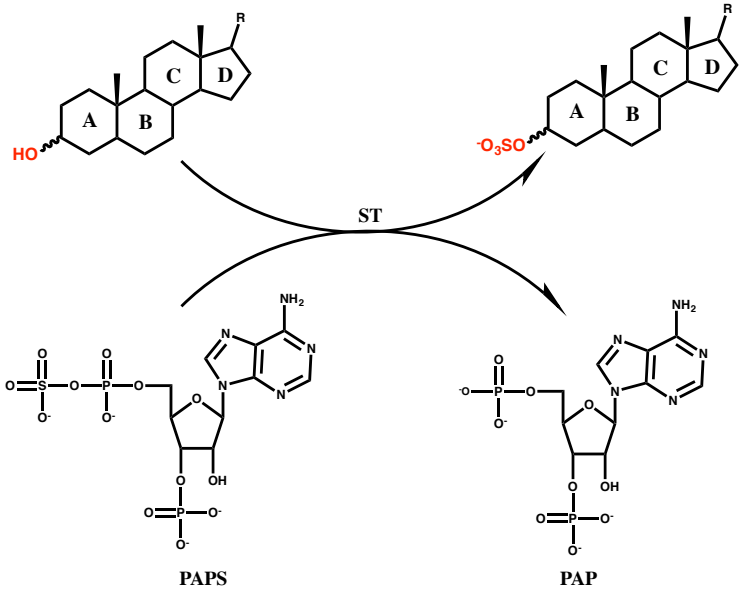


Halaven®
(Cancer)



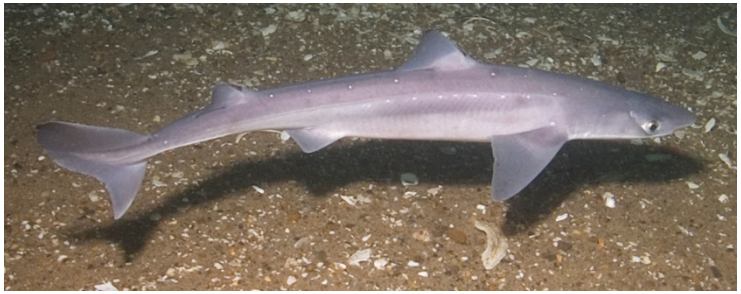
Introduction

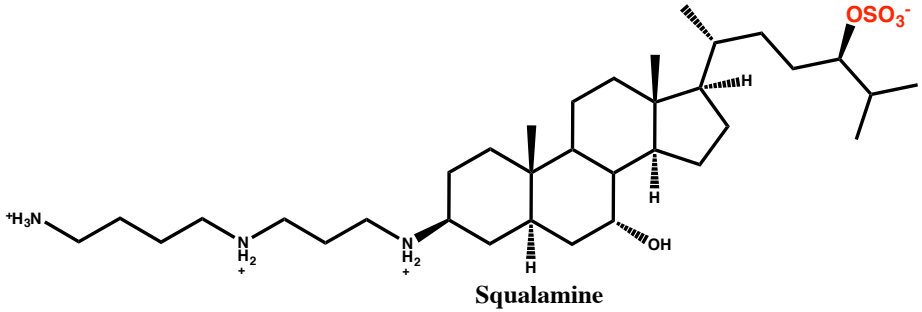
Sulfation in Marine Biota



ST – Sulfotransferases; PAPS – 3`-fosfoadenosine 5´-fosfosulfate; PAP – 3`-fosfoadenosine 5´-fosfate

DETOXIFICATION





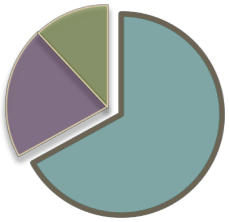
- Sulfated aminosterol derived from the internal organs of the dogfish shark
- Anti-angiogenic drug with a novel intracellular mechanism of action
- Efficacy and safety Phase III clinical trials

Correia-da-Silva, M. *et. al. Med. Res. Rev.* **2014,** *34* (2), 223-79.

Connolly B, *et. al. Ophthalmology Clinics* **2006**, 19**,** 381–91.
NCT02727881 (https://clinicaltrials.gov/ct2/show/NCT02727881)

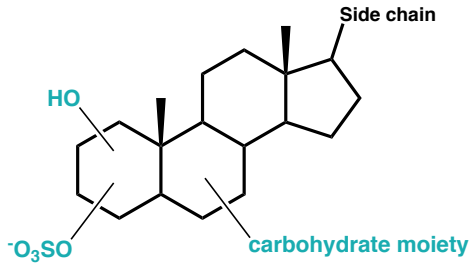
State of art

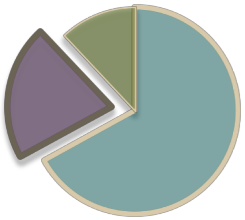
Isolated Bioactive Marine Sulfated Steroids



Monosulfated Steroids

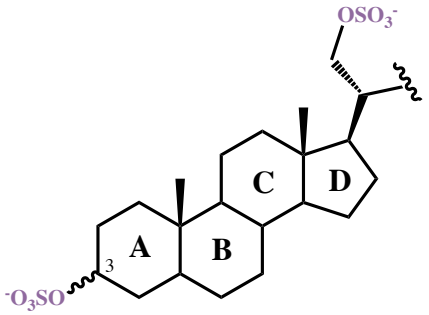
Large structural diversity

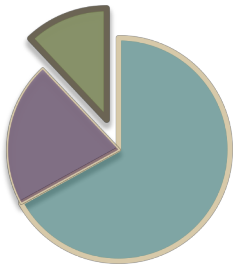




Disulfated Steroids

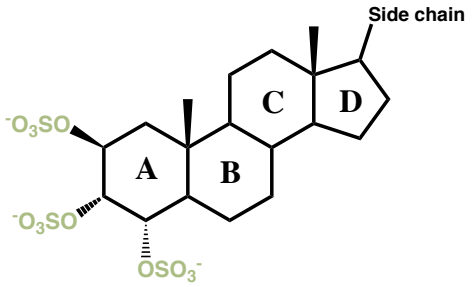
Most common structure





Trisulfated Steroids

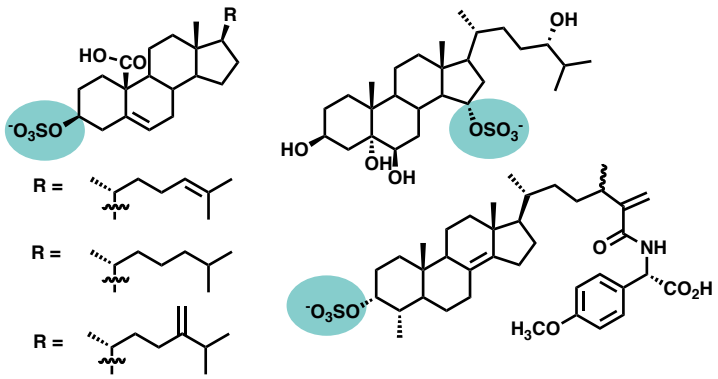
Most common structure



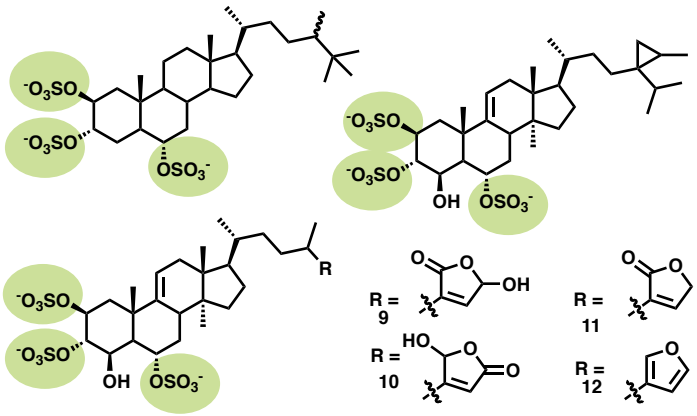
State of art

Antimicrobial Activity – **Antibacterial**

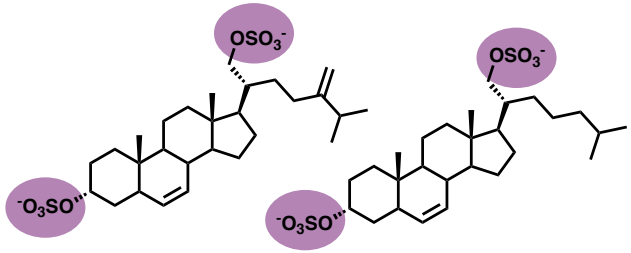
Monosulfated Steroids



Trisulfated Steroids



Disulfated Steroids

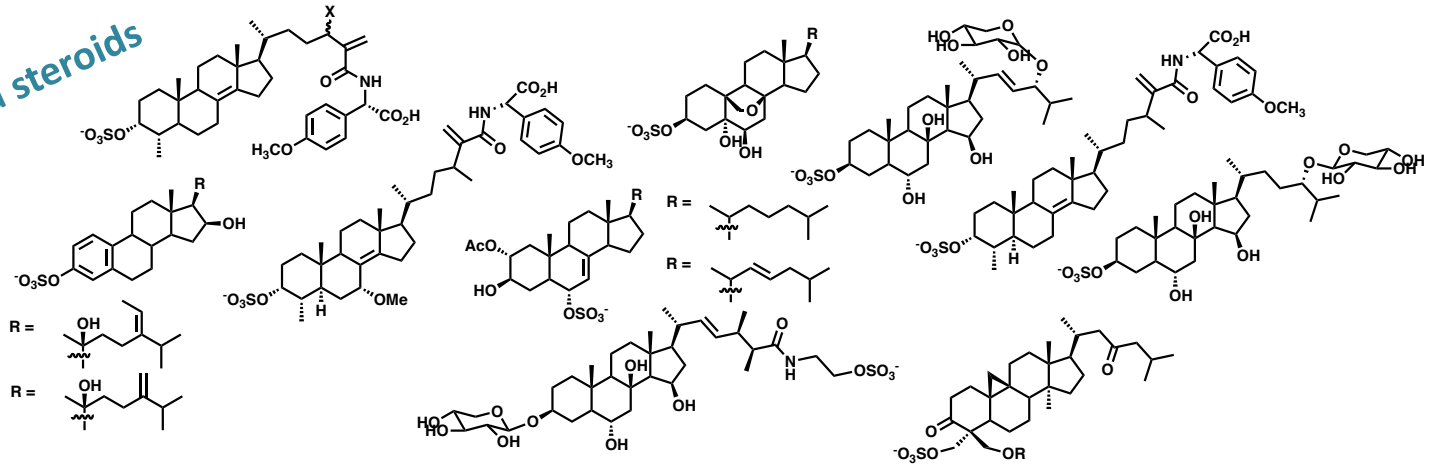


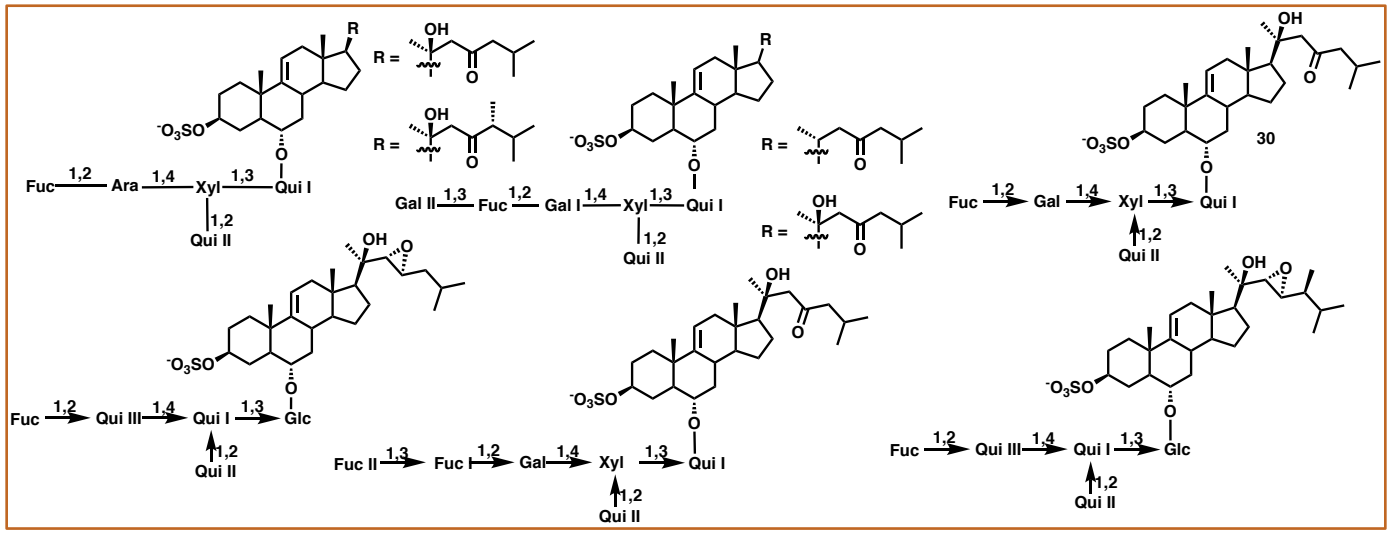
- **Some studies have highlighted the sulfate groups as crucial for activity**

State of art

Antimicrobial Activity – **Antifungal**

Majority are monosulfated steroids



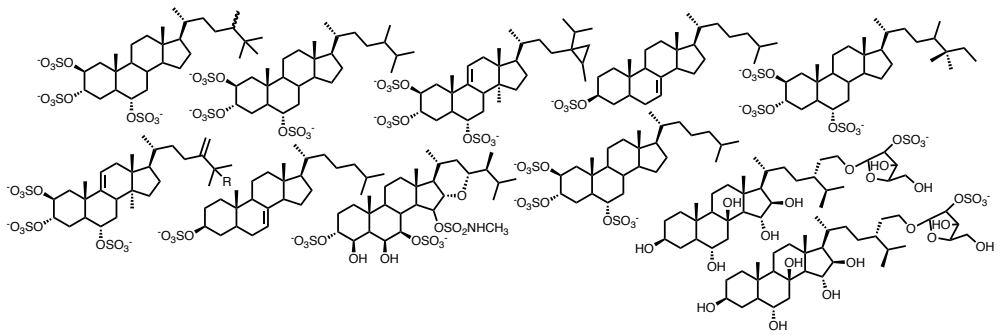


ASTEROSAPONINS

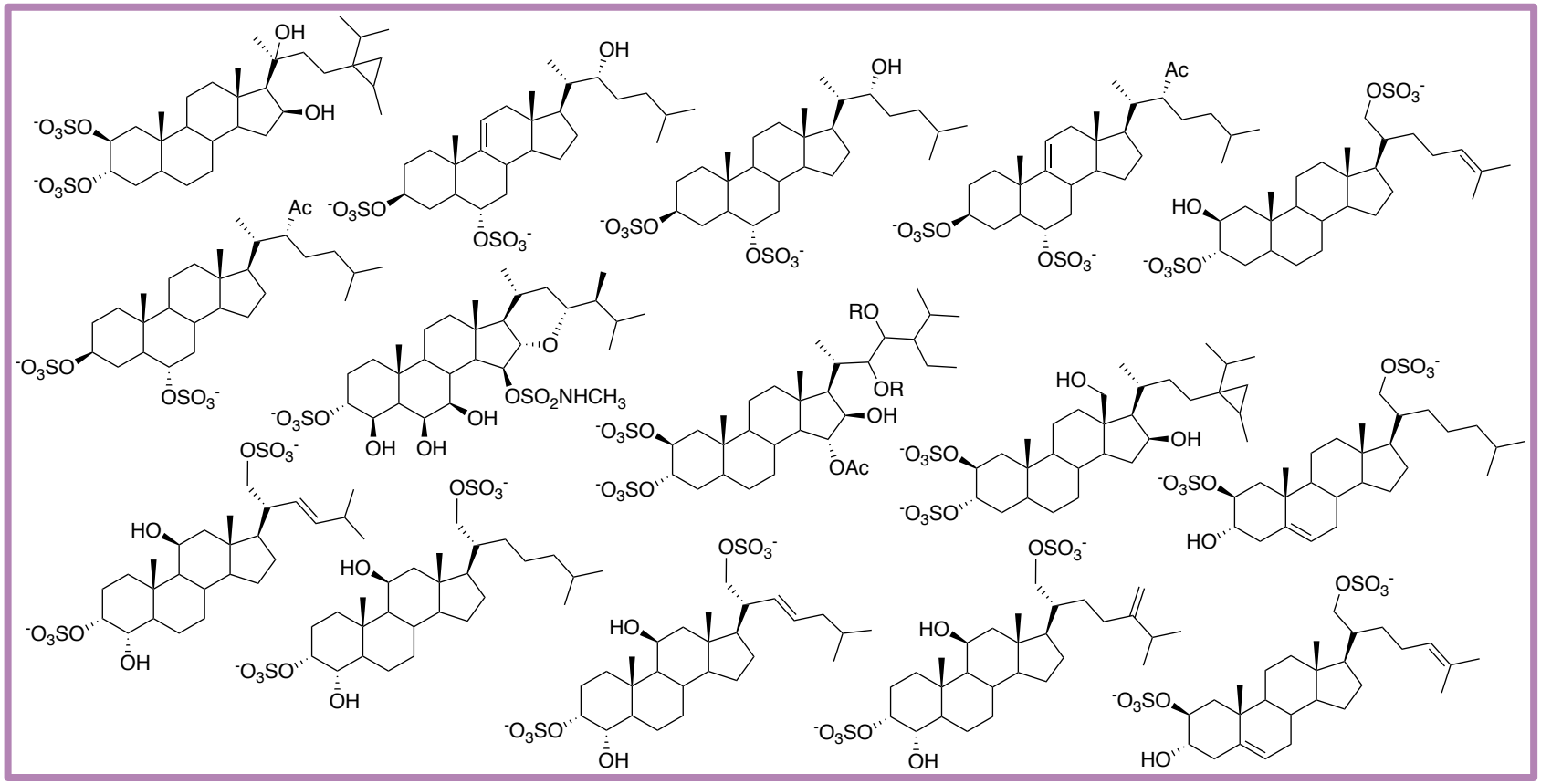
State of art

Antimicrobial Activity

Antiviral



Majority are **disulfated** steroids

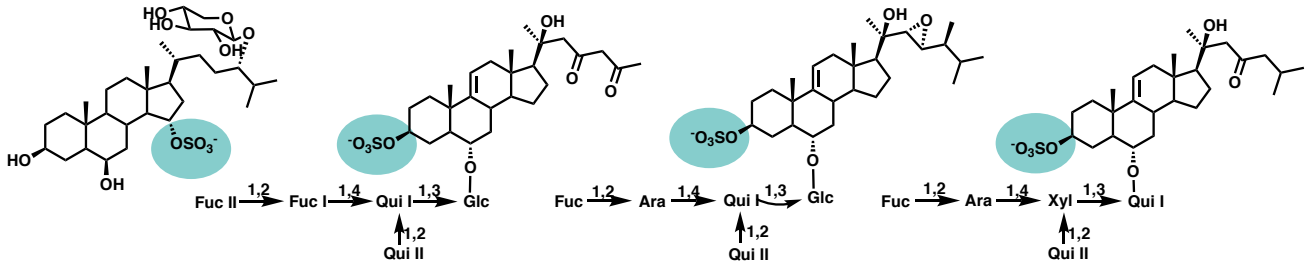


State of art

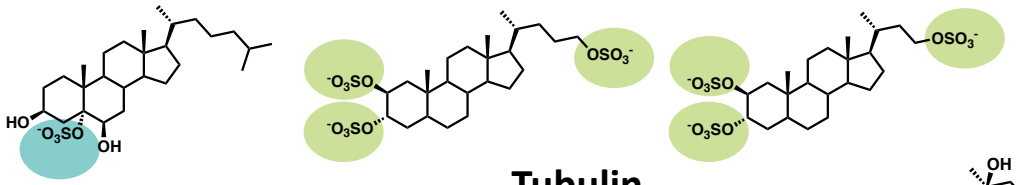
Antitumor Activity - **Targets**

Monosulfate Disulfate Trisulfate

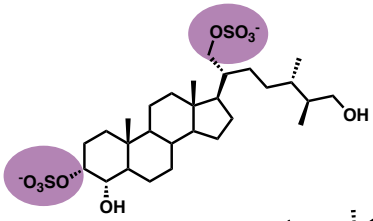
Apoptosis



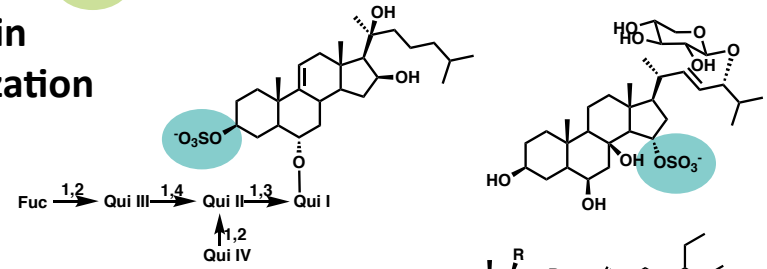
Angiogenesis



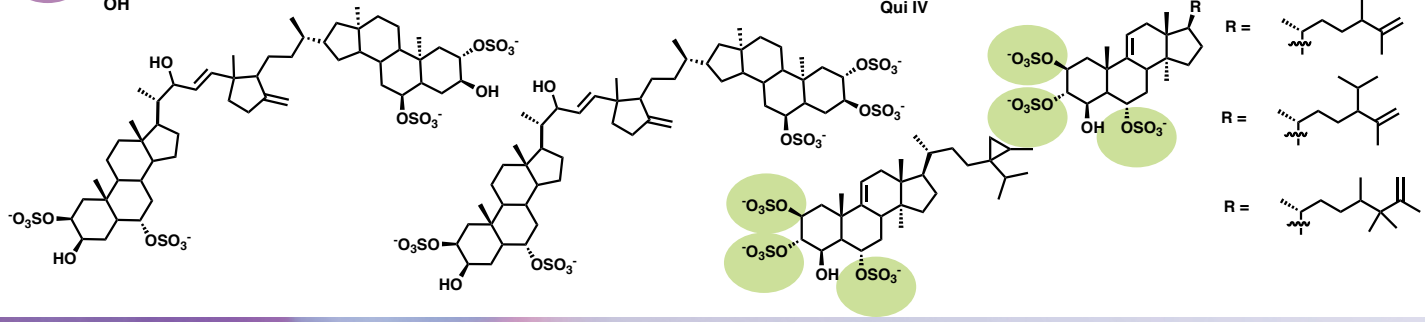
Antiproliferative



Tubulin polymerization



PKCζ inhibition

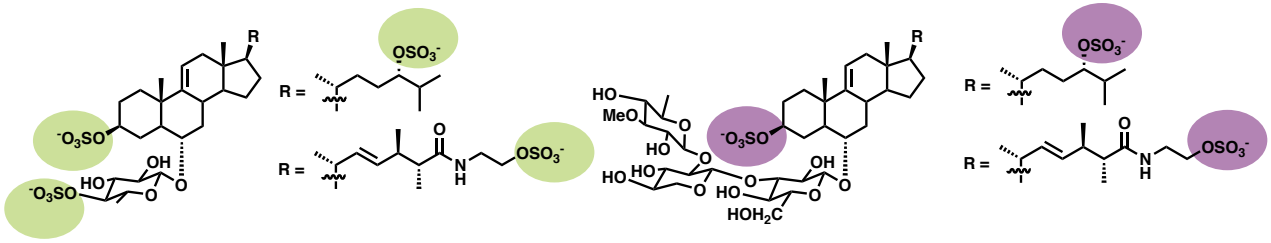


State of art

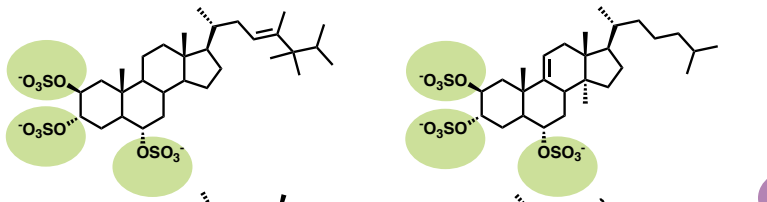
Antitumor Activity - **Targets**

Monosulfate Disulfate Trisulfate

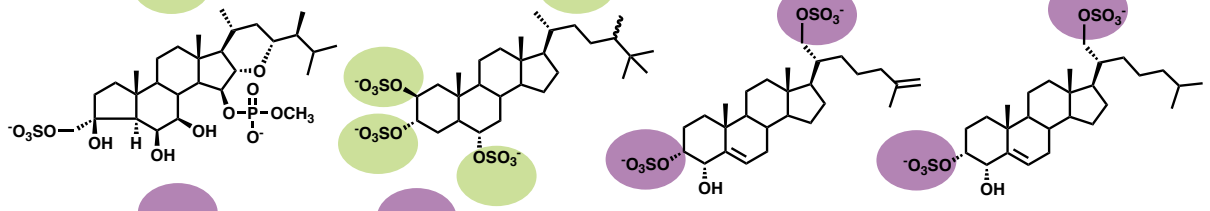
PTK inhibition



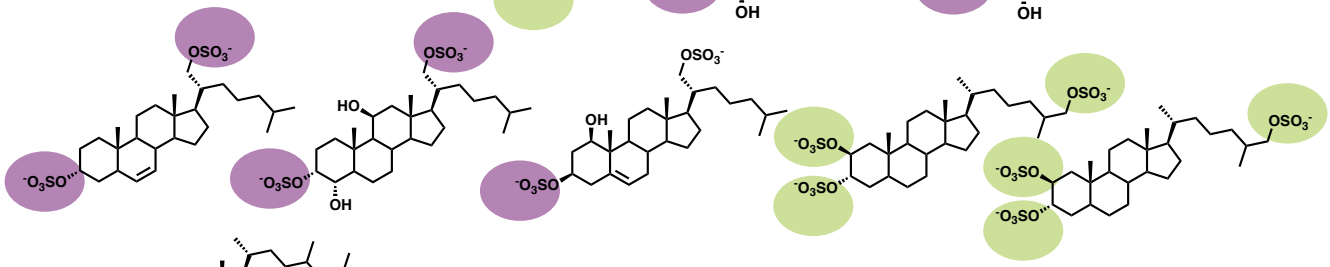
GDPX inhibition



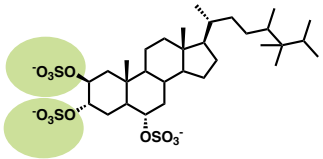
MTP1 inhibition



PXR inhibition



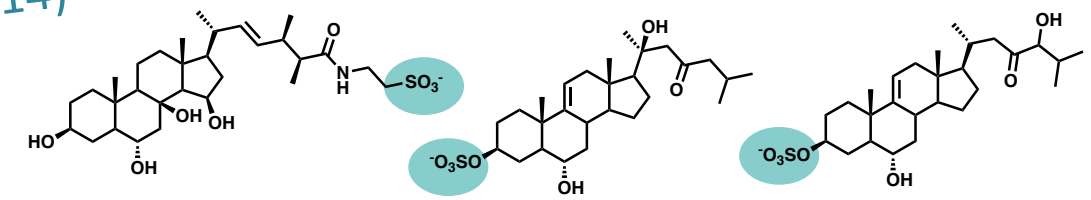
FXR inhibition

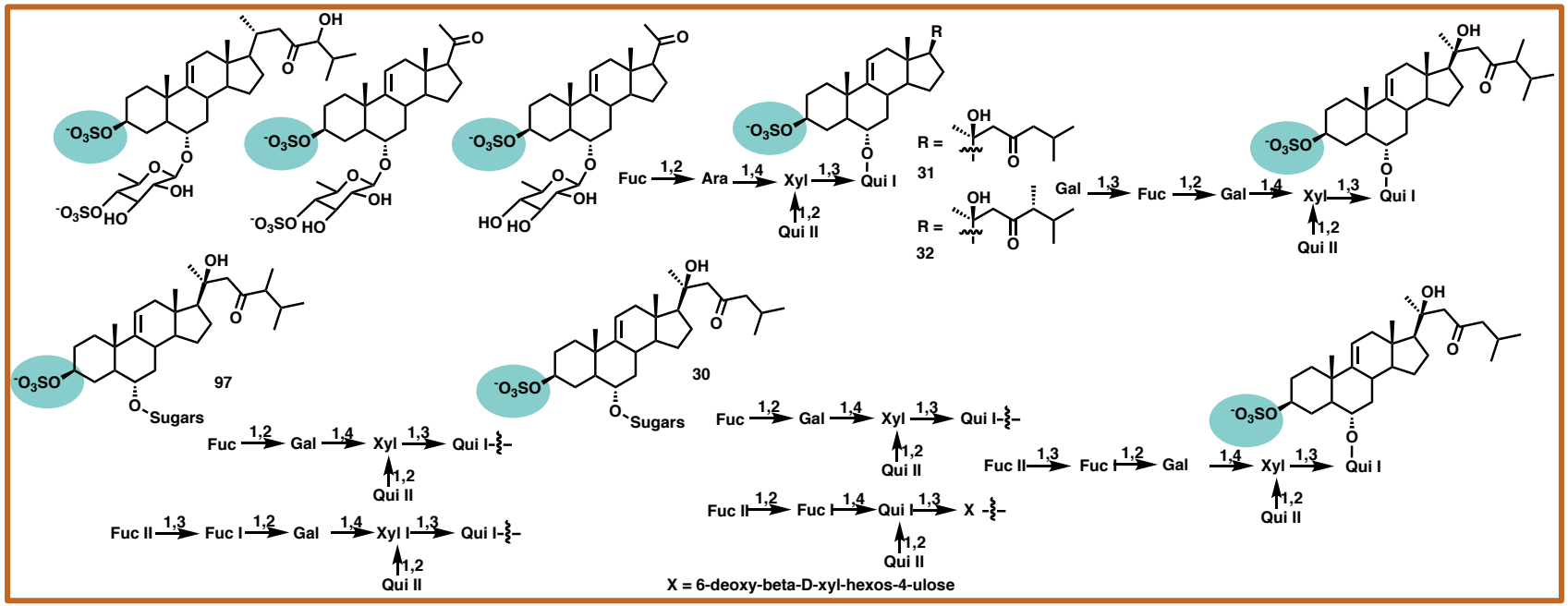


State of art

Cardiovascular Activity – **Hemolytic**

All are **monosulfated** steroids (14)



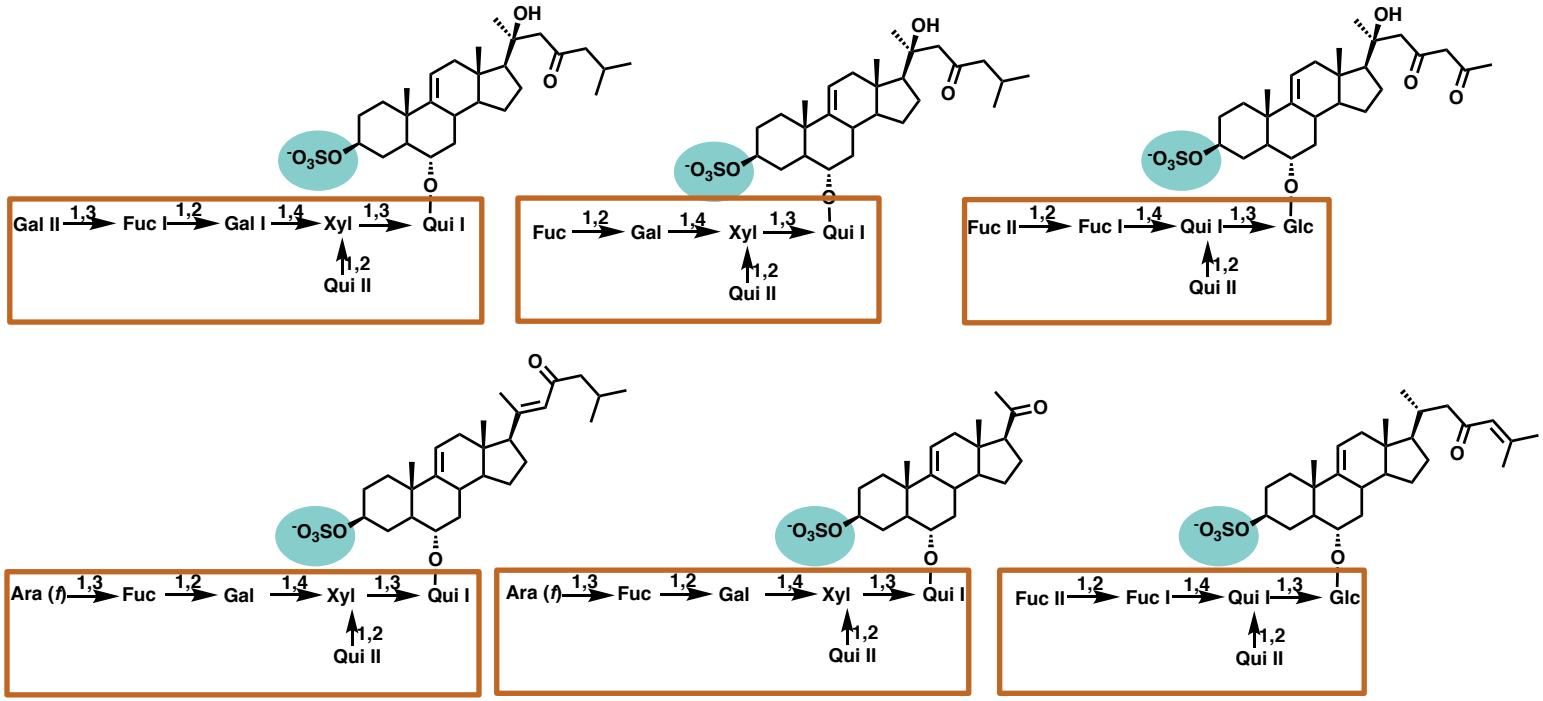


- 8 **asterosaponins** and 3 **monoglycosides**

State of art

Anti-inflammatory Activity

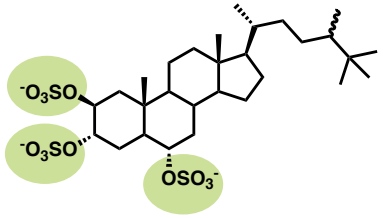
All are **asterosaponins**

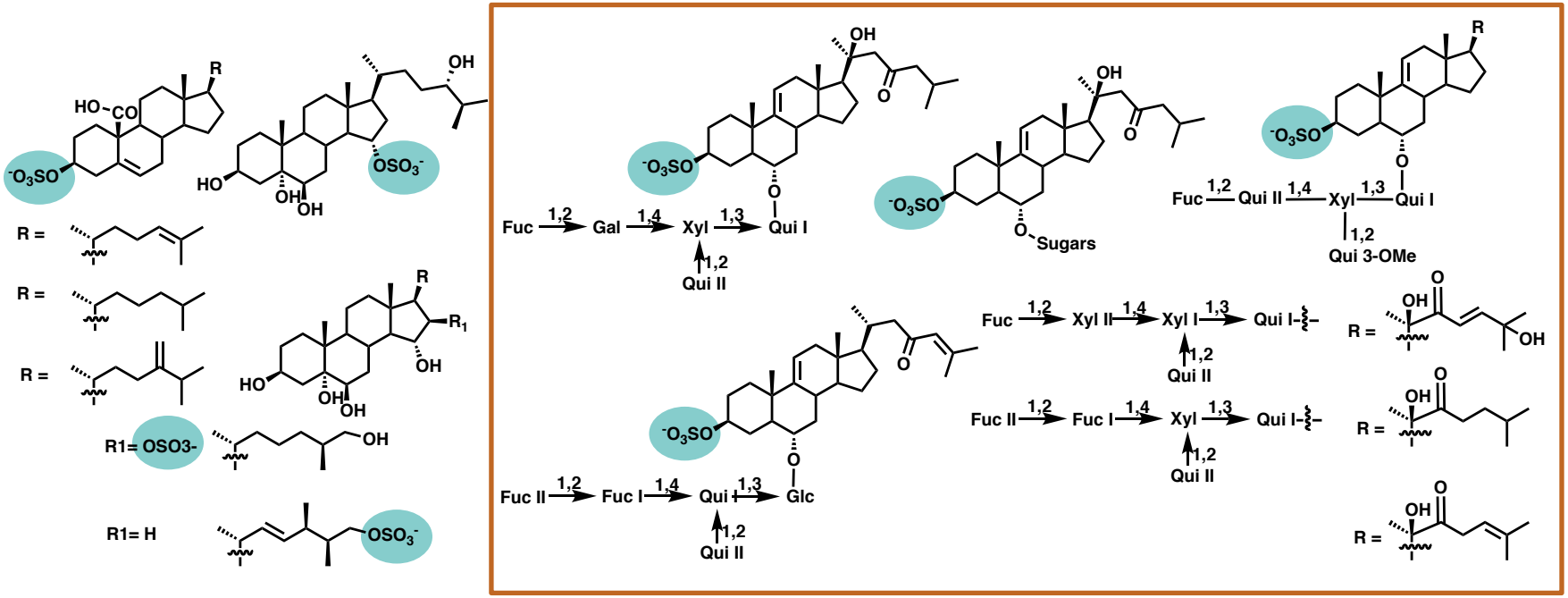


State of art

Antifouling Activity

13 are **monosulfates** and 1 is **trisulfate**





➢ 7 **asterosaponins**

Synthesis

OPPORTUNITY

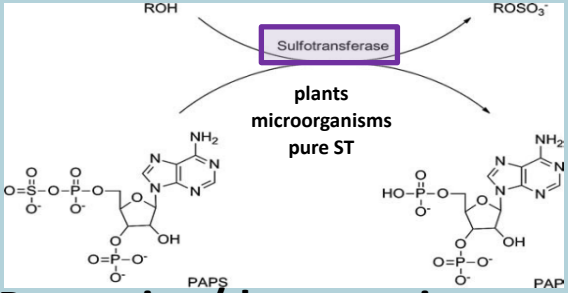
CHALLENGE

A. Monosulfation

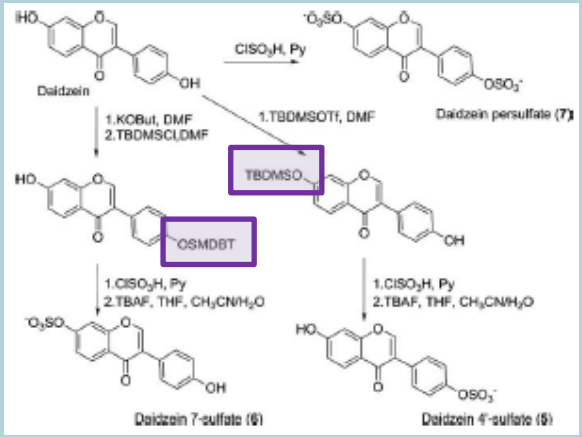
Obtain endogenous/naturally occurring sulfate monoesters

Achieve high regioselectivity in polyfunctional substrates

Biomimetic method



Protection/deprotection



B. Polysulfation

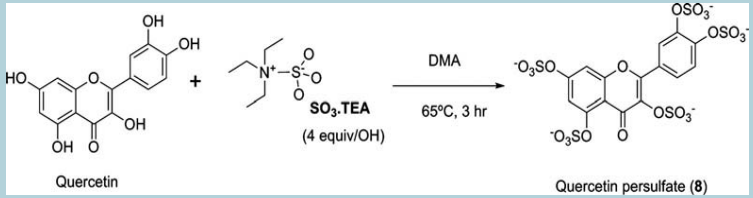
Obtain innovative compounds

Sulfate all hydroxyl groups

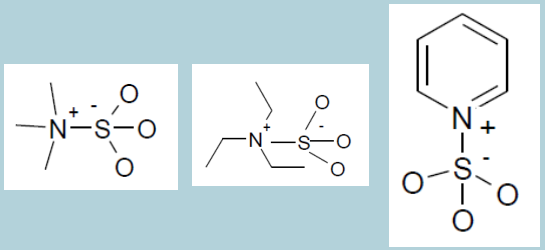
Sulfur trioxide adducts

High degree of substituition

Low degradation



Reactivity of the adduct

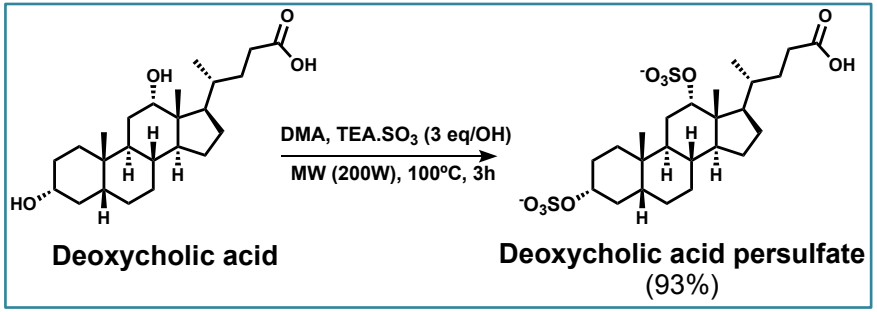


Base strength

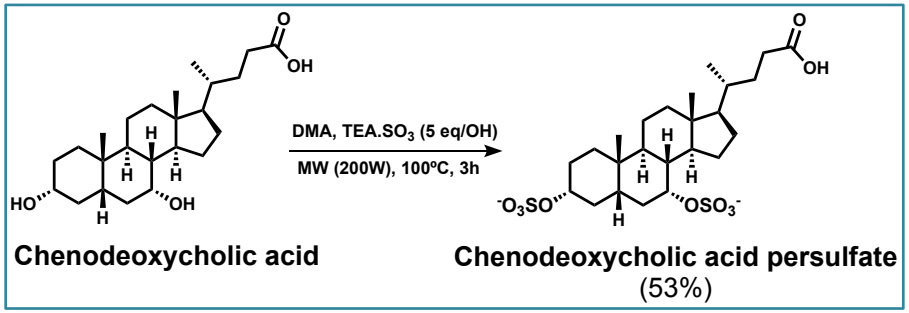
Correia-da-Silva et al
Med. Res. Rev. **2014**,
Volume 34, Issue 2, pages
223–279

Results and discussion: synthesis

Sulfation of di-hydroxysteroids

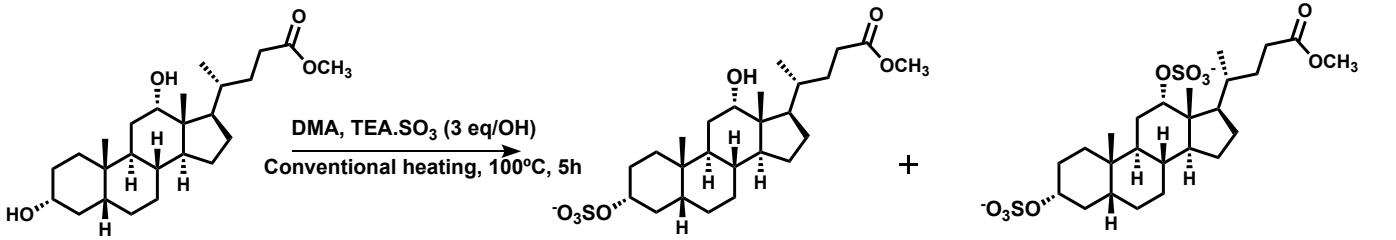


Deoxycholic acid → **Deoxycholic acid persulfate** (93%)



Chenodeoxycholic acid → **Chenodeoxycholic acid persulfate** (53%)

- ✓ MW irradiation allowed to obtain persulfated derivatives with moderate to high yiels



Methyl deoxycholate → **Methyl deoxycholate 3-sulfate** (3%) + **Methyl deoxycholate persulfate** (Not isolated)

- ✓ With conventional heating the C-3 monosulfated derivative was obtained however with low yields

$TEA.SO_3$ - Triethylamine-sulfur trioxide complex; MW – microwave; h- hours; W – Watts.

Results and discussion: purification

Di-hydroxysteroids

Diethyl ether + 4ºC = Solid

↓

Filtration

↓

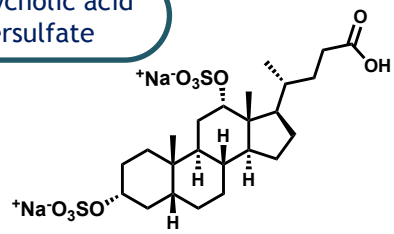
Dissolution in a methanolic solution of NaOH 0.1N

↓

Filtration

↓

Deoxycholic acid persulfate



Diethyl ether + 4ºC = Solid

↓

Filtration

↓

Dissolution in a methanolic solution of NaOH 0.1N

↓

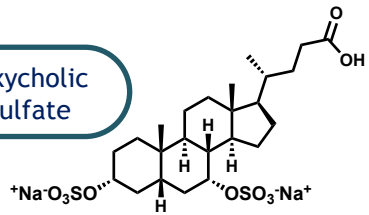
Filtration

↓

Dialysis

↓

Chenodeoxycholic acid persulfate



Diethyl ether + 4ºC = Solid

↓

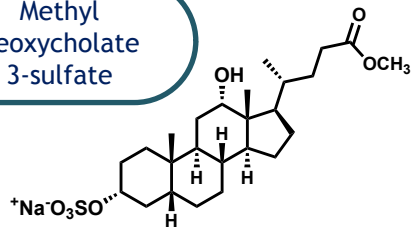
Filtration

↓

Gradient flash chromatography

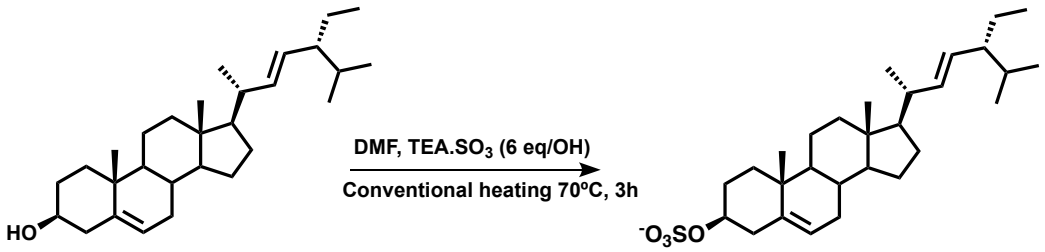
↓

Methyl deoxycholate 3-sulfate



Results and discussion: synthesis

Sulfation of mono-hydroxysteroids



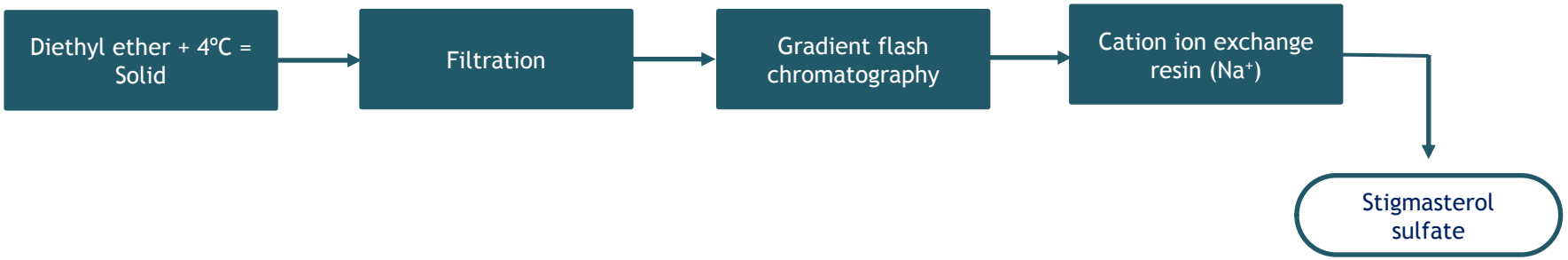
Stigmasterol

Stigmasterol sulfate
(2%)

$TEA.SO_3$ - Triethylamine-sulfur trioxide complex; h- hours.

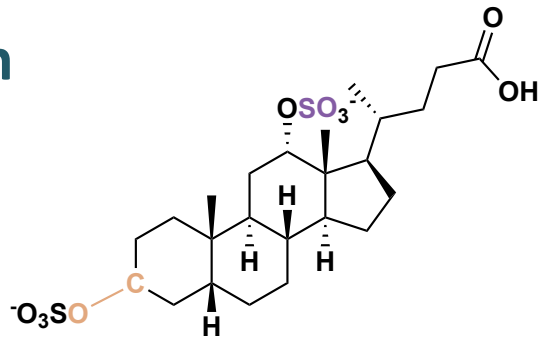
- ✓ With conventional heating the C-3 monosulfated derivative was obtained however with low yields

Purification

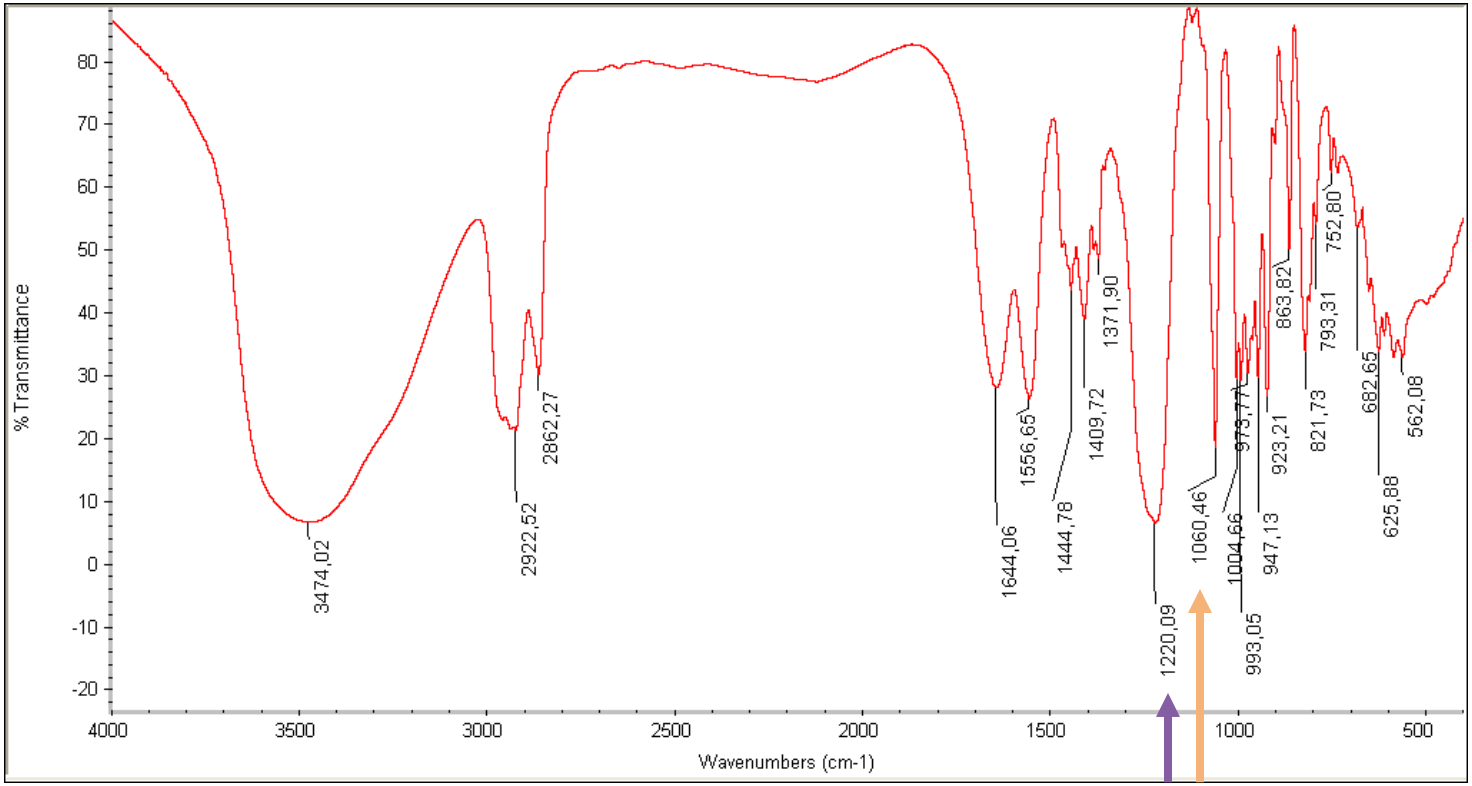


Results and discussion: structure elucidation

Infrared



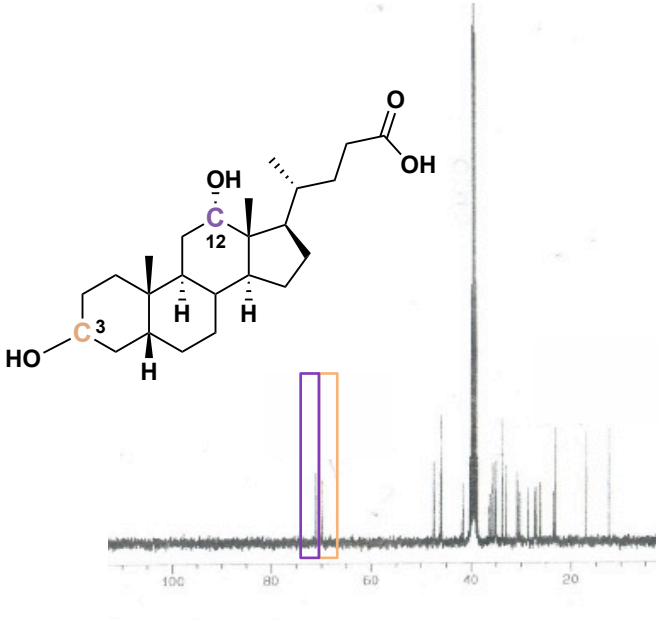
✓ IR spectrum (KBr) of deoxycholic acid persulfate



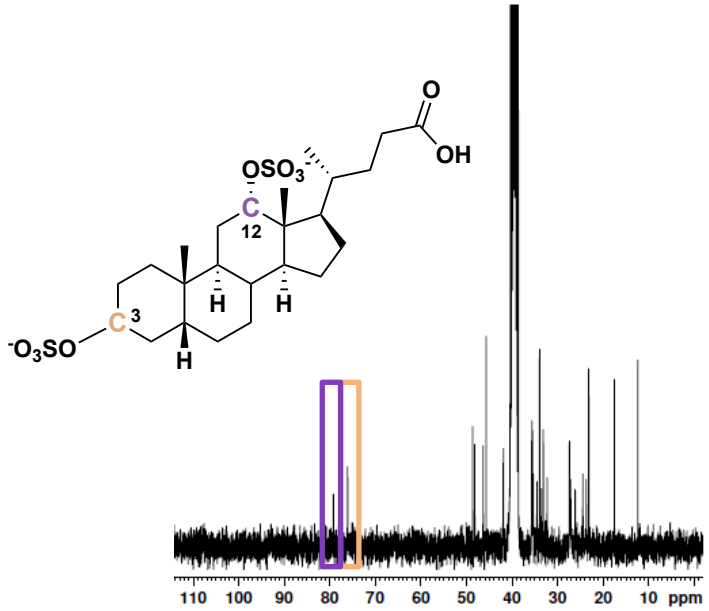
Results and discussion: structure elucidation

Nuclear Magnetic Resonance

✓ ^{13}C (75 MHz) spectrum of deoxycholic acid (DMSO $-d_6$)



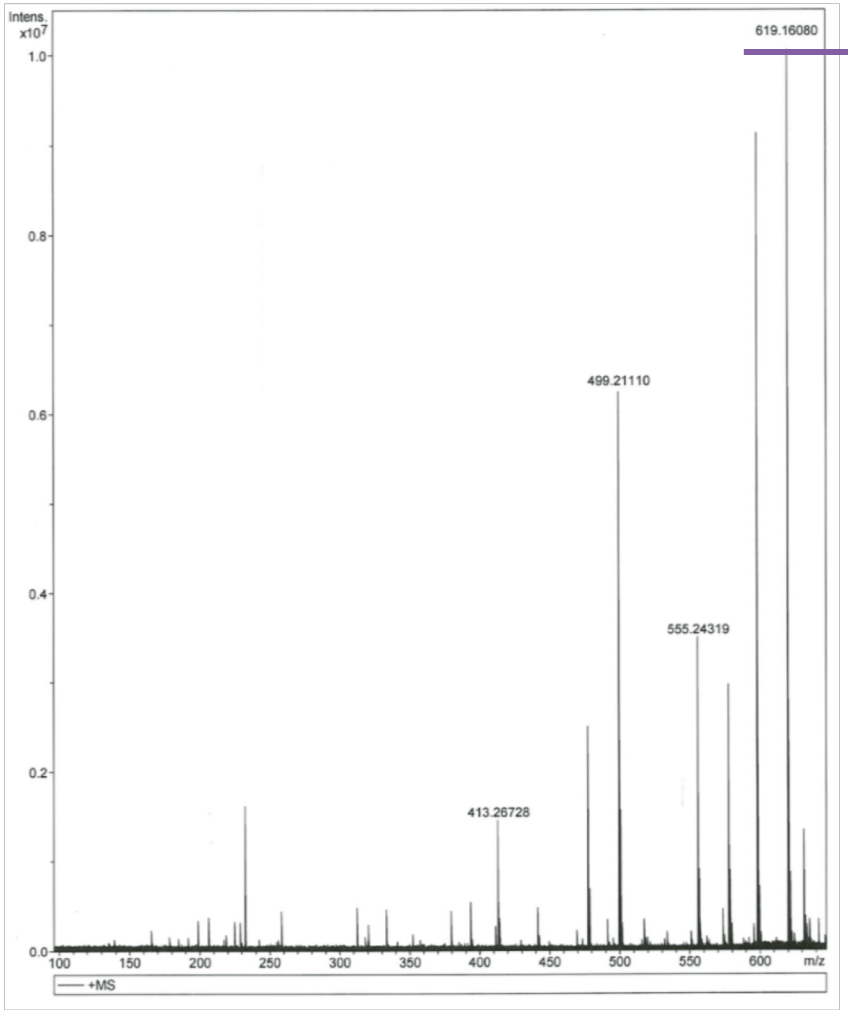
✓ ^{13}C (75 MHz) spectrum of deoxycholic acid persulfate (DMSO $-d_6$)



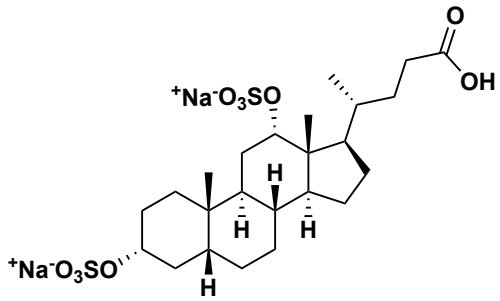
^{13}C	δ (ppm)	
	Deoxycholic acid	Deoxycholic acid persulfate
3	69.9	76.1
12	71.0	79.2

Results and discussion: structure elucidation

High Resolution Mass Spectrometry



HRMS (ESI+) m/z calcd for $C_{24}H_{38}O_{10}S_2Na_3$ 619.15940, **found 619.16080**.



[M+Na] = 619.65

Conclusions

Bioactive marine sulfated steroids: state of art

- ✓ Marine **monosulfated** steroids are more abundant than **disulfated** steroids, which, in turn, are more abundant than **trisulfated** steroids
- ✓ The majority of the isolated marine sulfated steroids belong to the class of sterols and the sulfate group is prevalent at C-3 position.
- ✓ The nontoxicity associated to the sulfate molecules predict the potential of marine-inspired sulfated steroids as novel and safer therapeutic agents

Synthesis of sulfated steroids

- ✓ Synthesis of four C-3 sulfated steroids was accomplished in the presence of triethylamine – sulfur trioxide complex
- ✓ The sulfated steroids were successfully characterized by IR, NMR, and HRMS

Acknowledgments

This work was supported through national funds provided by FCT/MCTES - Foundation for Science and Technology from the Ministry of Science, Technology and Higher Education (PIDDAC) and European Regional Development Fund (ERDF) through the COMPETE Programa Operacional Factores de Competitividade (POFC) programme, under the projects PTDC/MAR-BIO/4694/2014 (reference POCI-01-0145-FEDER-016790; Project 3599–PPCDT), PTDC/AAGTEC/0739/2014 (reference POCI-01-0145-FEDER-016793; Project 9471-RIDTI) and POCI-01-0145-FEDER-028736 in the framework of the programme PT2020. Carvalhal F also acknowledges FCT for the grant PTDC/AAG- TEC/0739/2014-018.