The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

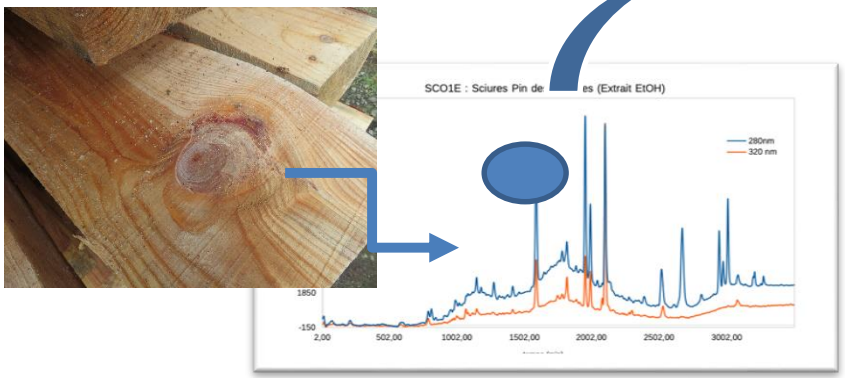
Anti-inflammatory effects of flavonoids derivatives : Investigation of their structure activity relationships

Cynthia Sinyeue [1,4]*, Mariko Matsui [2], Michael Oelgemöller [3], Frédérique Brégier [4], Vincent Chaleix [4], Vincent Sol [4] and Nicolas Lebouvier[1]

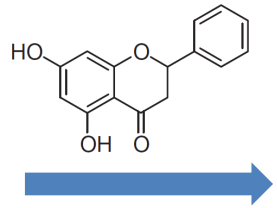
[1] Institut des Sciences Exactes et Appliquées (ISEA) EA7484, Université de la Nouvelle Calédonie, Campus de Nouville, 98851 Nouméa, New Caledonia.
[2] Group Immunity and Inflammation, Institut Pasteur of New Caledonia, 9 Ave Paul Doumer, Noumea 98800, New Caledonia
[3] College of Science and Engineering, Discipline of Chemistry, James Cook University, Townsville, QLD 4811 Australia
[4]Laboratoire Peirene EA 7500, Faculté des Sciences et Techniques, 123, avenue Albert Thomas 87060 Limoges
* Corresponding author: cynthia.sinyeue@etudiant.unc.nc/ sarah.sinyeue@gmail.com

Anti-inflammatory effects of flavonoids derivatives : Investigation of their structure activity relationship

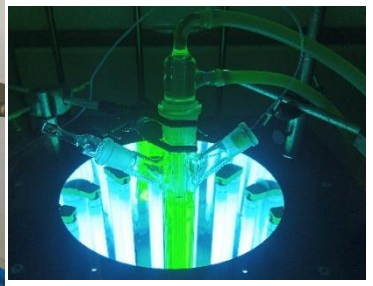
Graphical Abstract



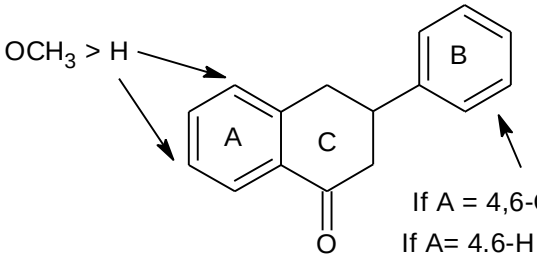
Valorization of forestry co-products



Pinocembrin/ pharmacomodulation

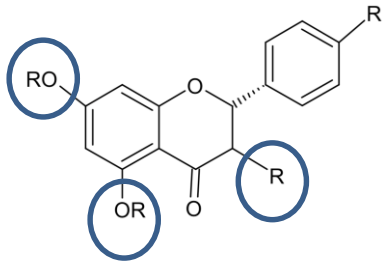


Thermal and photochemical activation



If A = 4,6-OMe then B = 2-COOH > 4-H > 4-OMe
If A= 4.6-H then B = 5-Br 2-OMe, 2-COOH> 4-Cl..

SAR



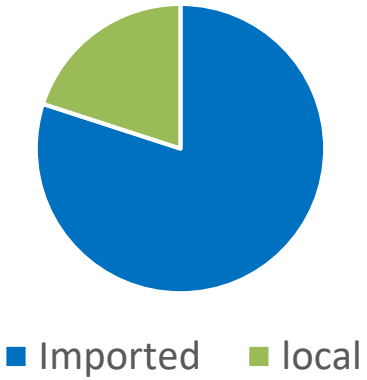
Anti-inflammatory assays

Abstract: Wood is a renewable source of natural molecules such as polyphenols that play an important role in biological processes. An analysis of byproducts from the pine forestry industry shows that flavonoids such as pinocembrin are widely found. Pinocembrin (5,7-dihydroxyflavanone) possesses a variety of biological properties such as anti-tumor, anti-oxidation and particularly anti-inflammatory activities. This flavanone represents an important class of natural products containing a 2-phenyl-benzopyran-4-one skeleton, which can be used as a starting material for the synthesis of bioactive molecules. In order to achieve rapid pharmacomodulations, flavanones can be obtained by a two-step synthesis route. The strategy used in this study starts with the synthesis of the intermediate chalcones by Claisen-Schmidt condensation, followed by an optimized cyclization to obtain the target flavanones. A series of flavanone derivatives was successfully synthesized with different functional groups on the two aromatic rings. Alternative access was considered for the cyclization of two derivatives by a photochemical route. All structures were established and confirmed by ^{1}H, ^{13}C NMR and HR-MS analyses. Anti-inflammatory activities of synthesized compounds were evaluated using an in vitro model of LPS-induced RAW264.7 macrophages and quantification of nitric oxide using the Griess assay. The screening results produced a structure-activity-relationship (SAR) that enabled identification of the structural requirements and essential functional groups for anti-inflammatory properties. This work confirmed flavanones as promising pharmacological candidates, which can be used as platform molecules for the future development of new pharmaceutical compounds.

Keywords: flavanone derivatives ; inflammation ; Structure Activity Relationship ; RAW264.7 ; photoactivation

Introduction

Forestry in New Caledonia



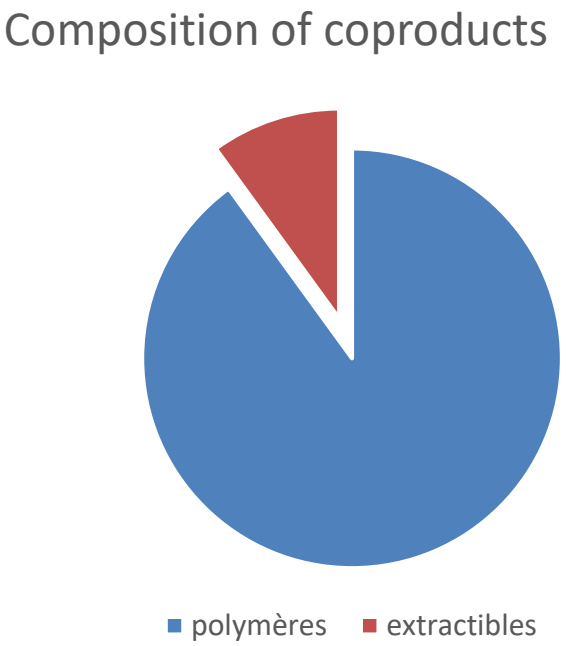
Timber : 19.000 m^3 (3000 m^3 local production)

- Imported species :
  - *Pinus radiata* from New Zeland
  - *Pinus sylvestris* from Europe

- the most exploited: *Pinus caribaea (Caribbean pine)*
  - Introduced in 1960,
  - Accredited for construction: framing, carpentry…

Pinus caribaea

Logging generates huge quantities co-products (40% of the wood mass)

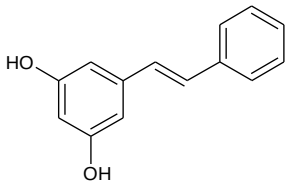


Bark, sawdust and knot of pinus caribaea

New ways of adding value : extractibles of pine sawdust, bark and knots

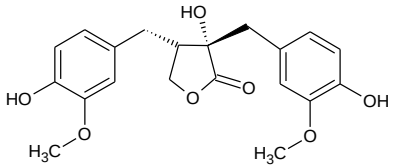
Pinus genus is mainly composed of stilbenes, lignans and flavonoids

- **Stibenes:** anti-fungal properties. Pinosylvin in *P. strobus*, *P. resinosa* and *P. Sylvestris*[1].



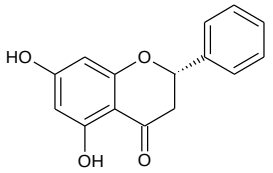
Pinosylvin

- **Lignans:** antitumor, antioxidant and antiviral potentials. Nortrachelogenin in *P. sylvestris*[2] and *P. pinaster*[3].



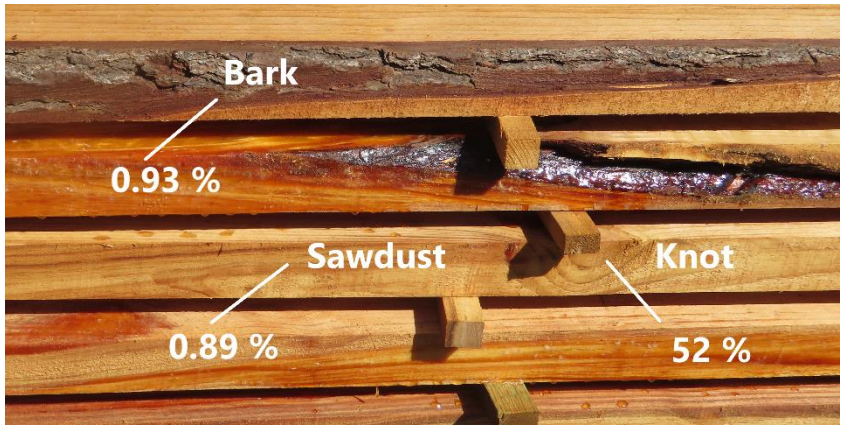
Nortrachelogenin

- **Flavonoids:** antioxidant, anti-inflammatory, anticancer and neuroprotective activities[4,5,6]**.** Pinocembrin and pinobanksin i*n P. pinaster*[3].



Pinocembrin

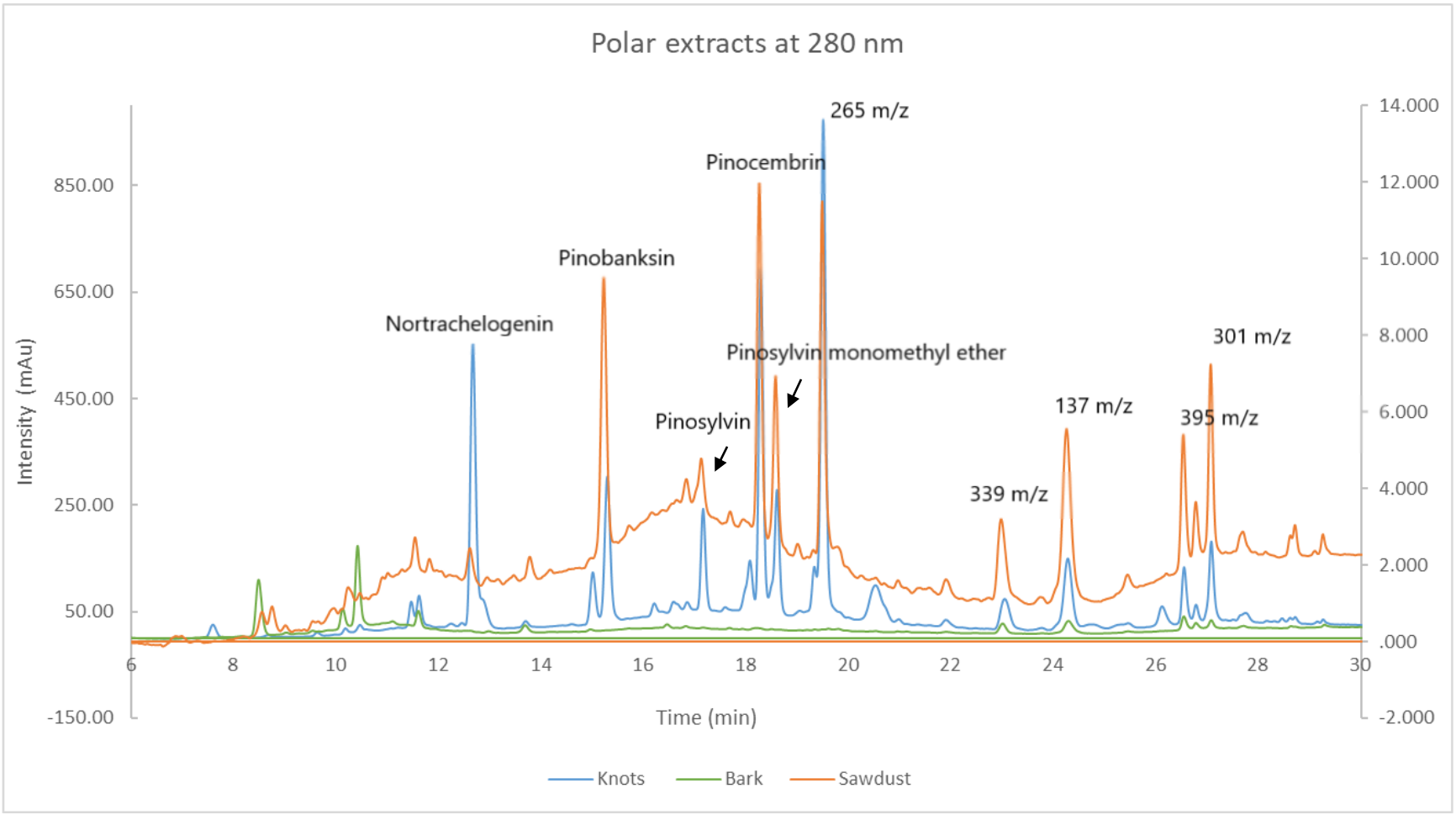
- Biomass was air-dried and ground into fine particle.
- Extractions in cyclohexane (lipophilic extractibles) then in ethanol (hydrophilic extractibles).



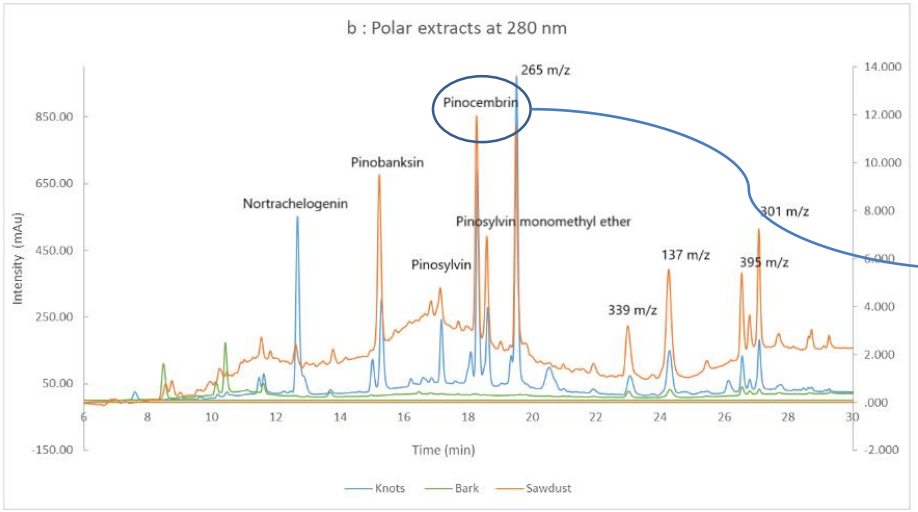
Mass yield of extractibles in bark, sawdust and knots of P. caribaea

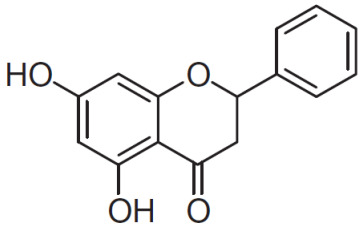
- Analysis of extract: HPLC-UV -MS on column (Zorbax SB-C18 3µm, 100x3,0 mm) with a gradient elution AcCN/H_2O + 0.1% of formic acid.

Chromatograms at 280 nm of ethanolic extracts of knots, sawdust and barks of *P. caribaea*



1. Strategy of flavonoids synthesis



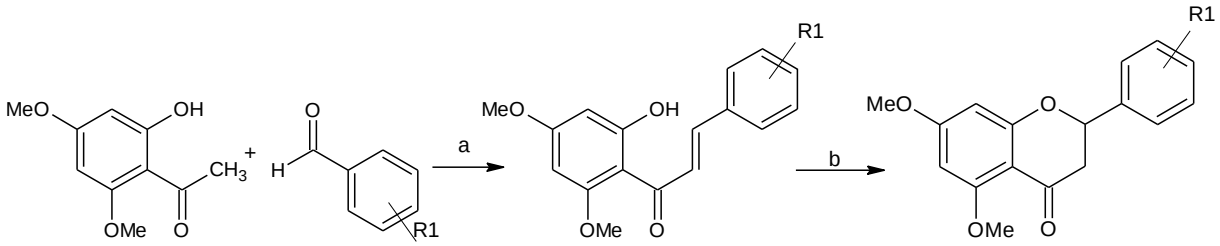


Platform molécules??

Pinocembrin : antioxidant, antibacterial, anticancer and anti-inflammatory potential[7]

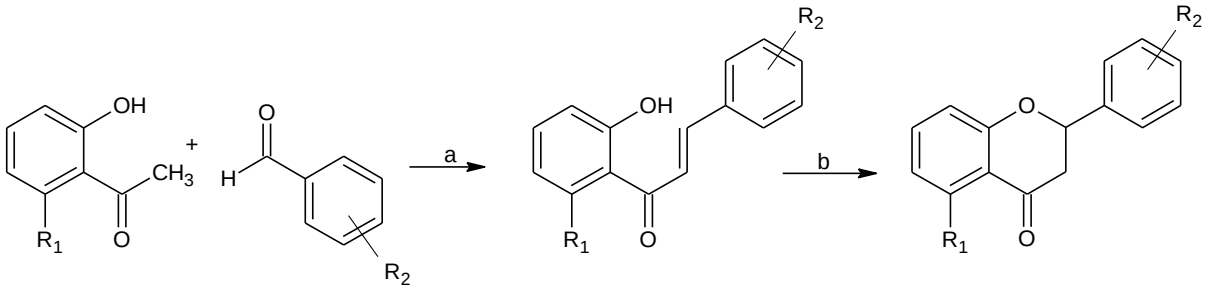
2. Synthesis of flavanones by two steps route

1. Claisen-Schmidt condensation (a : excess of NaOH / MeOH)
2. Intramolecular Mickaël addition (b : NaOAc (6eq) /MeOH).



Series 1

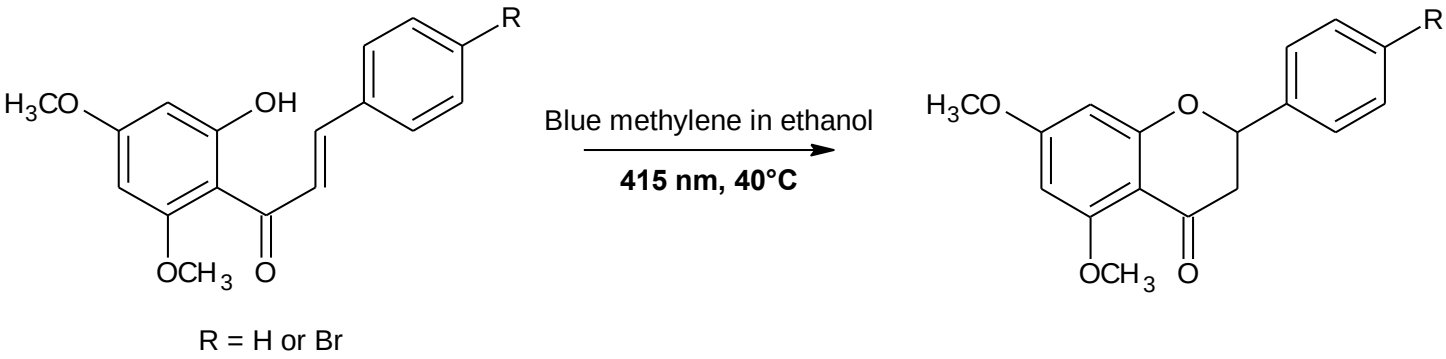
Compounds	R1	Chalcones (3)	Flavanones (4)	
		Yield (%)	Yield (%)	M (m/z)
A	H	27	10	284
B	2-OCH_3	36	79	314
C	3-OCH_3	82	74	314
D	4-OCH_3	92	66	314
E	4-Br	52	70	363
F	4-Cl	23	66	318
G	2-COOH	36	72	328



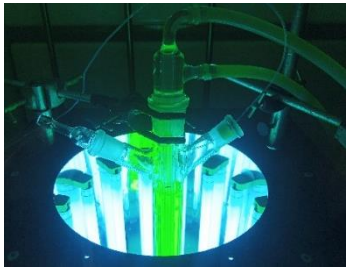
Series 2

Compounds	R_1	R_2	Chalcones (3)	Flavanones (4)	
H	H	H	62	59	224
I	H	4-Cl	86	37	258
J	H	4- OCH_3	56	37	254
K	H	2-COOH	32	81	268
L	H	2-OCH3, 5-Br	84	56	333
M	OCH_3	H	30	9	254

Photochemical activation: Intramolecular Mickaël addition at a specific wavelength.



R = H or Br



- Chalcones 3A et 3E (85 mg, 1eq), blue methylene (10 mg) in 70 mL of ethanol.
- Two irradiation wavelengths have been evaluated : 300 and 415 nm).
- Solutions was deoxygenated by bubbling with purified nitrogen gas in a RMR-600 photochemical reactor with 16 tungsten lamps (100W) and sealed at 40°C.

All products were determined by UV, ^{1}H and ^{13}C NMR, HRMS analysis.

1. Biological assays

In vitro: murine macrophages RAW264.7

Treatment 24h

LPS: inflammatory bacterial component (1µg/mL)

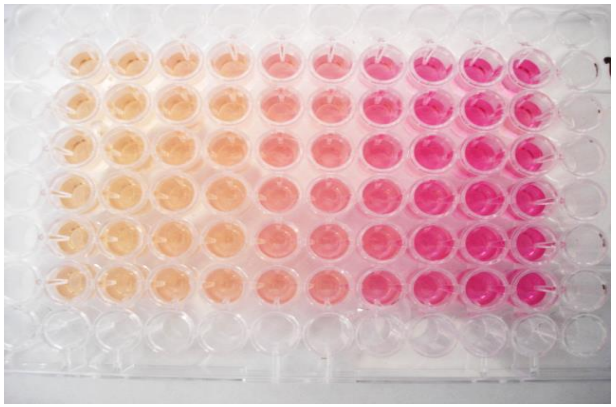
molecules: 2µg/mL (DMSO 0.1%)

Dexamethasone: reference

Measurements (colorimetric assays)

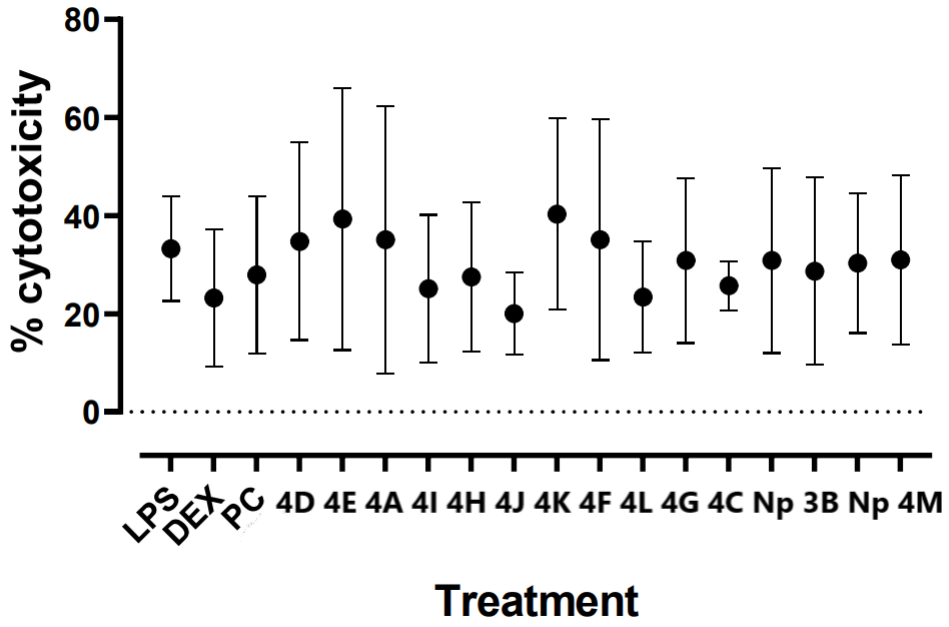
Cytotoxicity: Quantification of Lactate Deshydrogenase (LDH) → % of dead cells

[Nitrite NO_2^-]: % of NO production (inflammatory marker) by Griess reaction



Supernatant / Griess assay

2. Cytotoxicity of analogues on LPS-induced RAW264.7

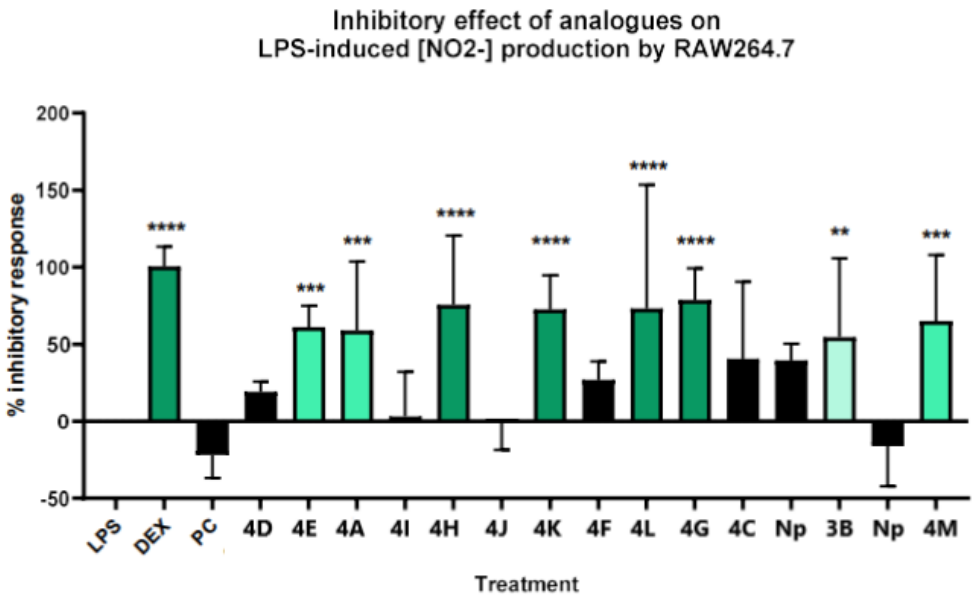


Analogues at 2µg/mL

No difference in cytotoxicity compared to LPS condition

→ **Validation for assays**

3. Inhibitory effect of analogues



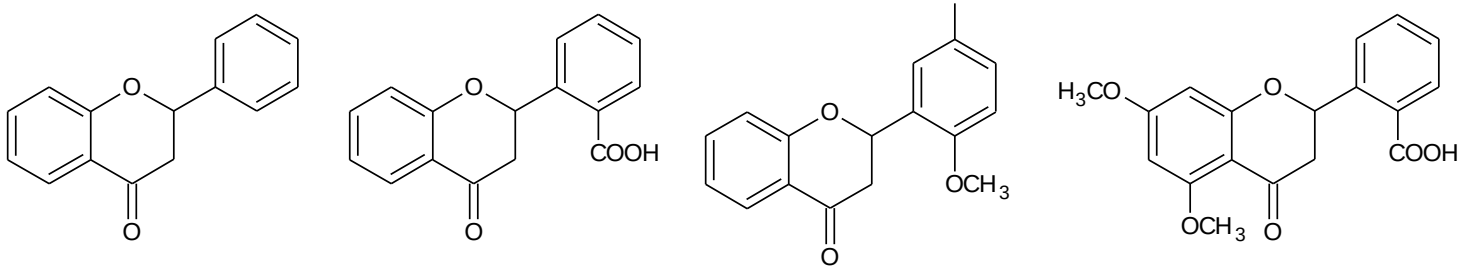
** P<0,005, *** P<0,0005, **** P<0,0001, Np: Not pure

% inhibitory response compared to LPS condition

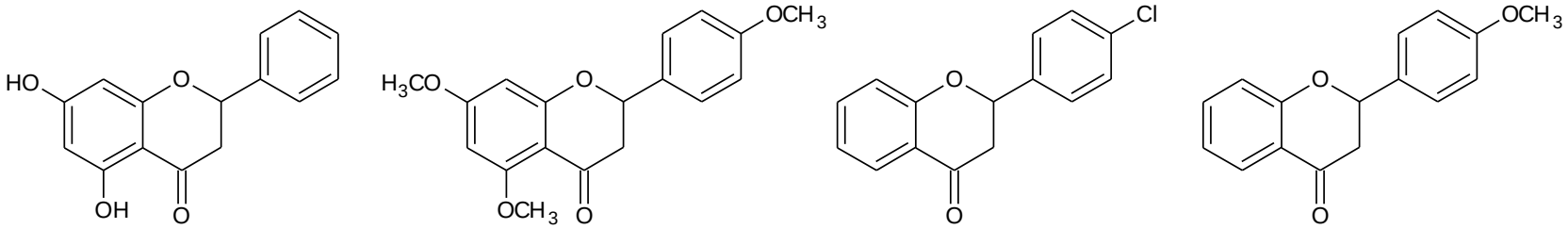
- Dexamethasone DEX
  - Inhibitory effect confirmed
- Pinocembrine PC at 2µg/mL
  - No effect (IC_{50}: 240 µg/mL)
- Analogues at 2µg/mL
  - **Inactive to very significant effect**

1. Analysis of Structure Activity Relationships (SAR)

More active : 4H, 4K, 4L, 4G

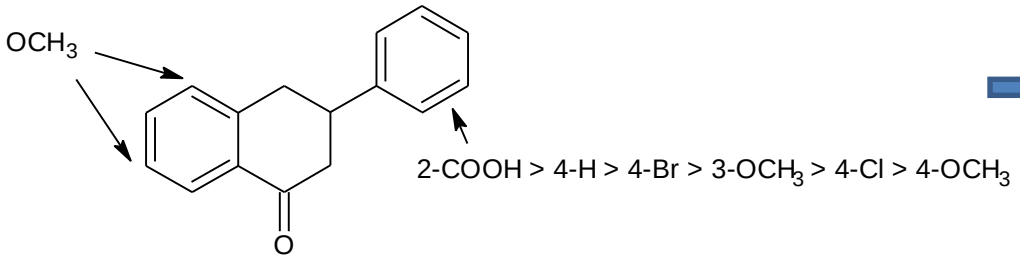


Inactive : PC, 4D, 4I, 4J



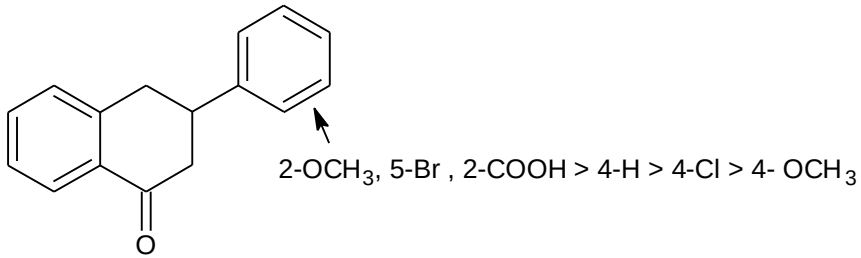
2. Discussions of Structure Activity Relationships (SAR)

Series 1 : 4G > 4A, 4E > 4C > 4F > 4D



Carboxylic group at 2-position on the B-ring : highest inhibition capacity

Series 2 : 4L, 4H, 4K > 4M > 4I > 4J



Carboxylic group at 2-position and 2-OCH_3, 5-Br on the B-ring are the most active analogues

For the first cycle : 4H > 4M > 4A

The addition of methoxy group in the A-ring decrease the activity.

Conclusions

- Caribbean pine wood is composed of bioative molecules as pinocembrin,
- 13 flavanones derivatives were synthesized with variable anti-inflammatory activities,
- SAR: COOH groups at its 2-position on the B-ring shows the highest inhibition capacity on LPS-induced NO production whereas the addition of methoxy group on the A-ring decrease the anti-inflammatory potential.

Perspectives

- Synthesis of new flavanones according to first SAR results,
- Enhancing conditions of photochemical activation,
- Further study of pharmacological mechanisms,
- Studying of hemisynthesis pathways from pinocembrin (platform molecules).

Acknowledgments

References

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