High-Throughput Screening (HTS) by Immunoassay Tests

Service de **M**arquage **M**oléculaire
et de **C**himie **B**ioorganique
CEA Saclay

Service de **P**harmacologie
et d '**I**mmunologie
CEA Saclay *(J. Grassi)*

Laboratoire de Synthèse Bioorganique
UMR 7514
ULP / CNRS Illkirch

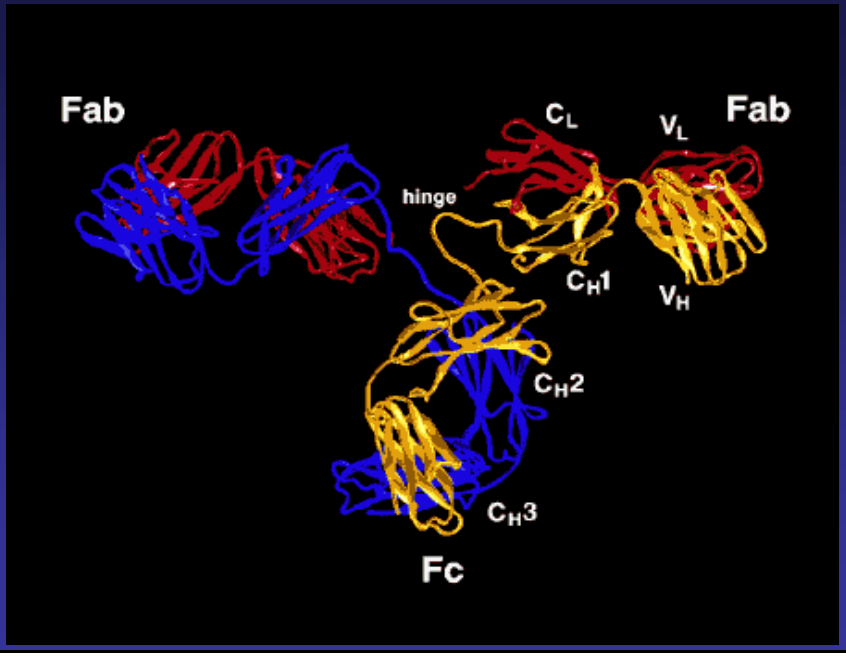
High-Throughput Synthesis and Screening (HTS)

- HT Synthesis of molecules, catalysts, materials ...
- HT Screening for: molecules, macromolecules
  properties (biological, physical)
  (pure compounds or mixtures)

HTS
by immunoassay tests

- H T S for enantioselective catalysts : yields & ee's
- H T S for antioxidants
  protective agents against oxidative stress
  selection of Norbadione A
- Total synthesis of Norbadione A

Structure of an antibody

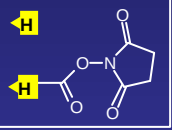


Generation of poly- and monoclonal antibodies (Ab)

Chemistry

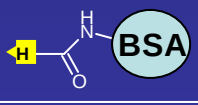
Hapten H

Synthesis of a fonctional derivative of H



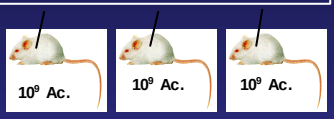
H_2N—BSA

Synthesis of the immunogen



Biology

Injection of the immunogen



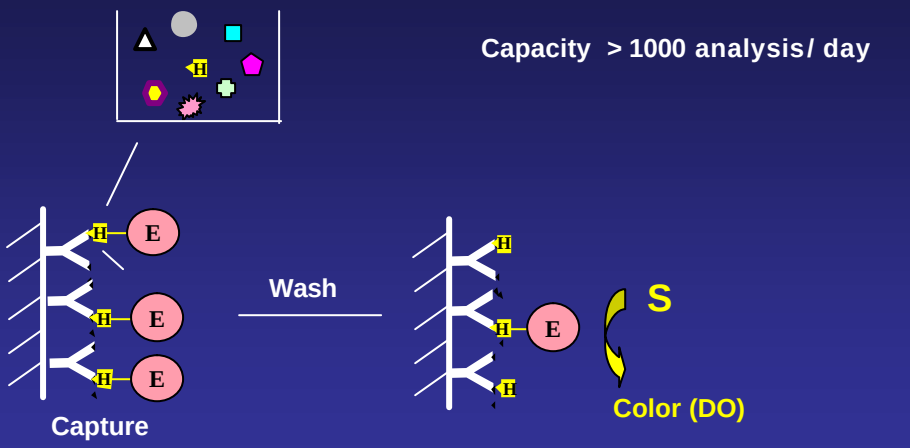
Polyclonal response
Selection of the mouse with the best response

Cell fusion
~ 10^3 hybridomes
selection of anti-H Ab

Multiplication by cloning
~ 10 Monoclonal Ab
anti-H

Competitive EIA (Enzyme ImmunoAnalysis)

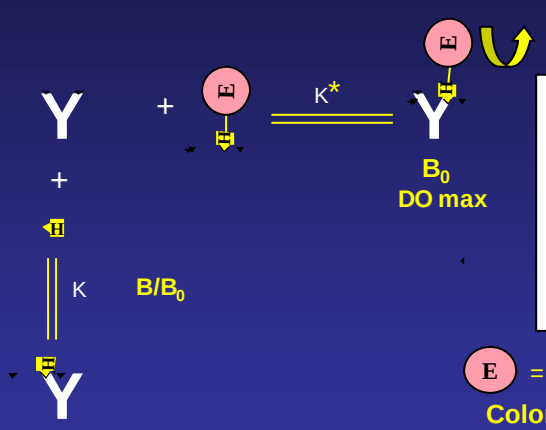
Capacity > 1000 analysis/ day

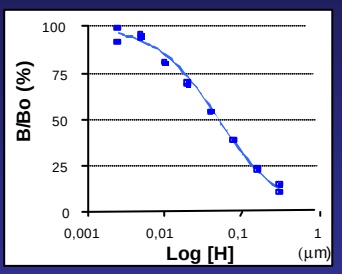


E = Acethylcholinesterase (AChE)

Color (DO): Ellman reagent

Competitive EIA
(Enzyme ImmunoAnalysis)

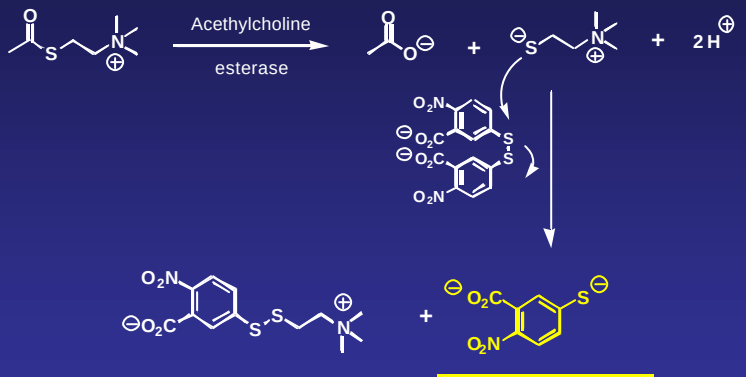




E = Acethylcholinesterase (AChE)

Color (DO): Ellman reagent

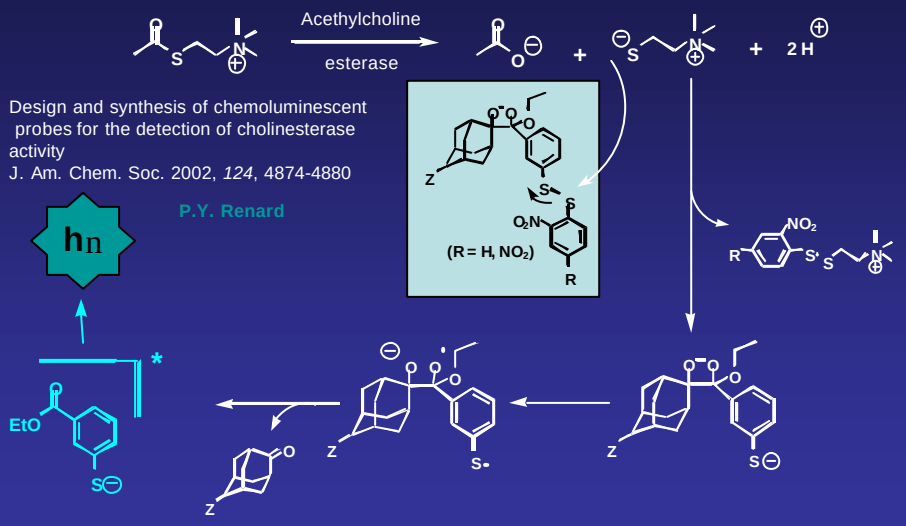
Principle of AChE detection with Ellman reagent



YELLOW COLOR

Reading of the DO

New chemoluminescent probes



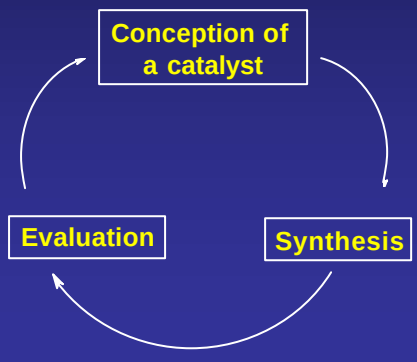
Design and synthesis of chemoluminescent probes for the detection of cholinesterase activity
J. Am. Chem. Soc. 2002, *124*, 4874-4880

P.Y. Renard

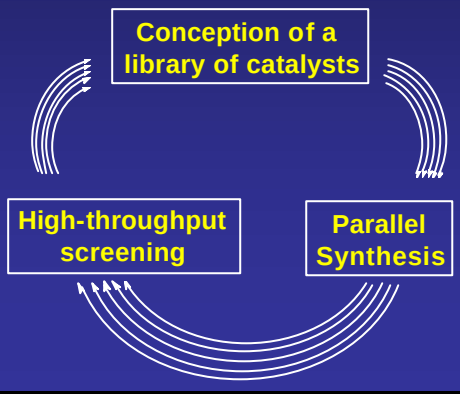
Screening for new catalysts

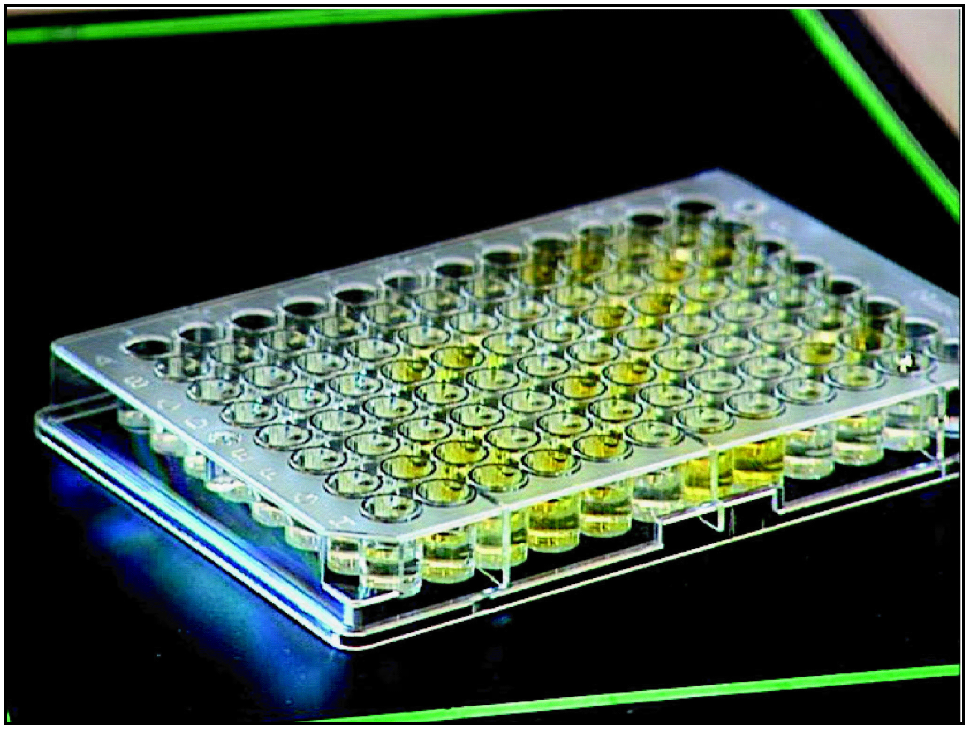
A —— Catalyst ——— B

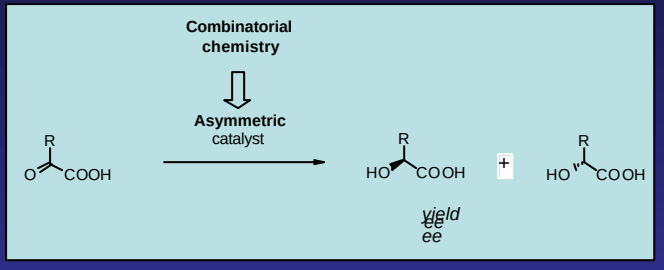
Classical approach

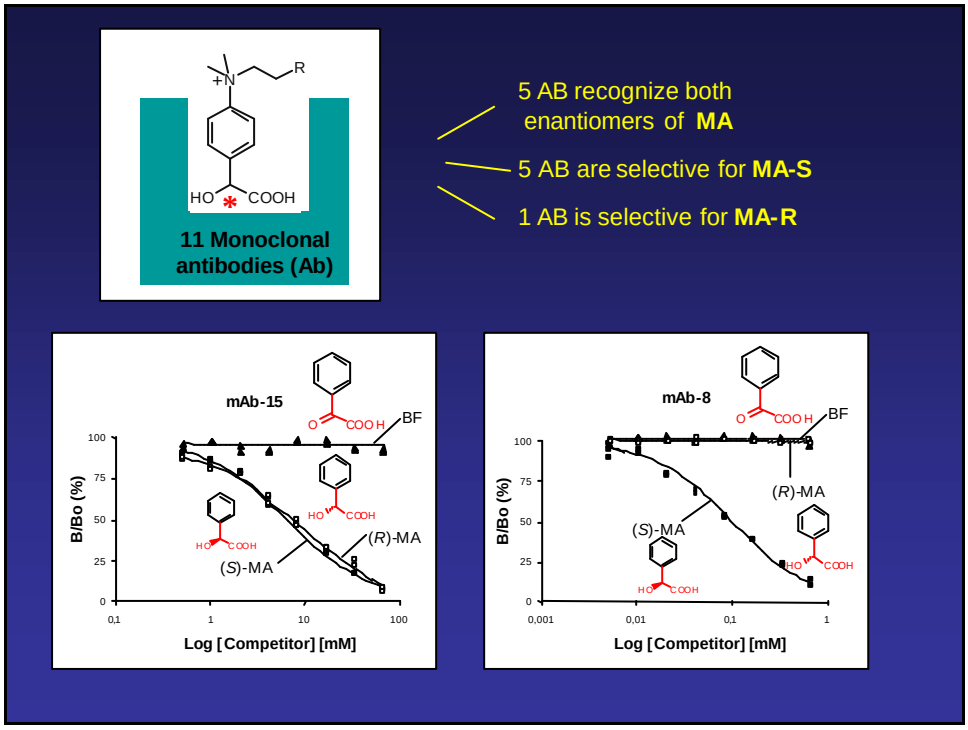


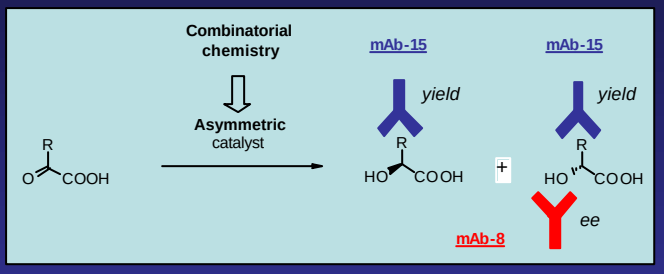
Combinatorial approach



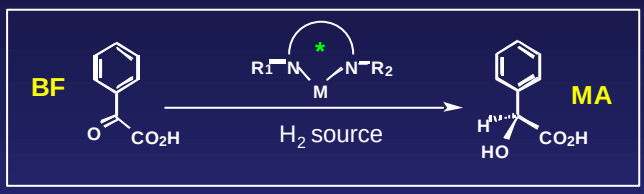



Combinatorial chemistry
Asymmetric catalyst
R
O
COOH
HO
COOH
+
yield
ee
ee


11 Monoclonal antibodies (Ab)
5 AB recognize both enantiomers of MA
5 AB are selective for MA-S
1 AB is selective for MA-R
mAb-15
BF
(R)-MA
(S)-MA
B/Bo (%)
Log [Competitor] [mM]
mAb-8
BF
(R)-MA
(S)-MA
B/Bo (%)
Log [Competitor] [mM]


Combinatorial chemistry
Asymmetric catalyst
mAb-15
yield
mAb-15
yield
mAb-8
ee

Conception catalysts' library

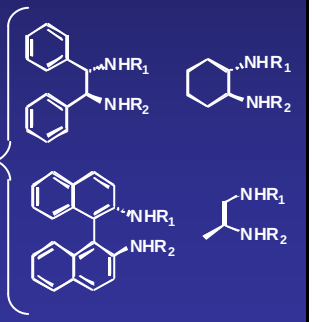


4 M = (2 solvants)
- $[RuCl_2\ (p\text{-cym.})]_2$
- $[RuCl_2\ (p\text{-benz.})]_2$
- $[RhCl_2\ (Cp)]_2$
- $[IrCl_2\ (Cp)]_2$

2 Sources of hydrogen =
- HCOOH/TEA
- KOH/iPrOH

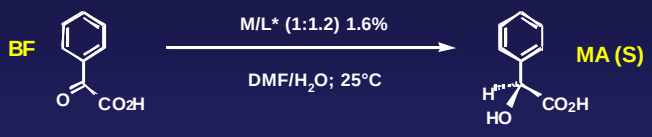
6 Solvants =
- DMF, DMF/H_2O, EtOH, DMSO
- DMF/ EtOH, DMF/DMSO

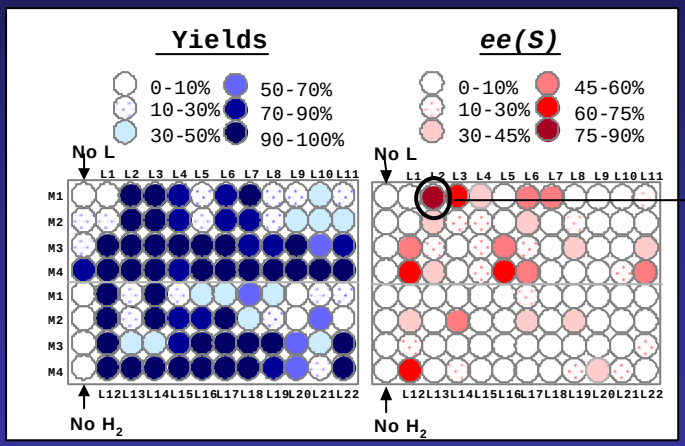
22 Ligands =

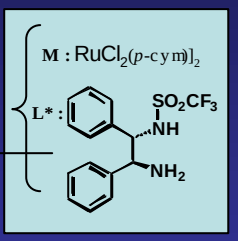


2112 experiments

Screening results







High-Throughput Screening of Enantioselective Catalysts by Immunoassay. **Angew.Chem. Int. Ed.** *2002, 114, 132-135.*

HTS tests for the ee determination

- IR thermography (Reetz and coll. 1999)

- Capillary aray electrophoresis (Reetz and coll. 2000)

- CD-HPLC (Mikami and coll.2001)

- Electrospray ionization with isotopically labeled substrates (Reetz and coll. 1999)

- Immunoassay

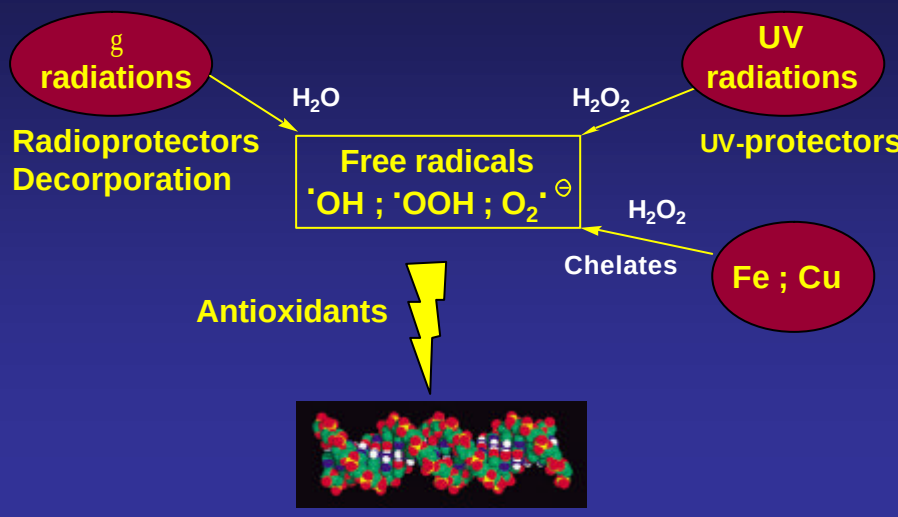
HTS

- H T S for enantioselective catalysts : yields & ee's

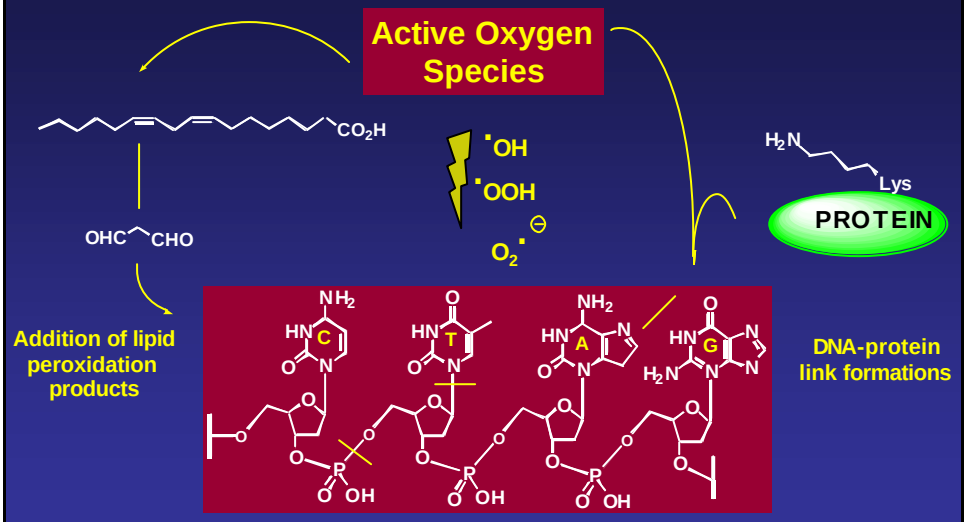
- H T S for antioxidants
  protective agents against oxidative stress
  selection of Norbadione A

- Total synthesis of Norbadione A

Protections against the oxidative stress

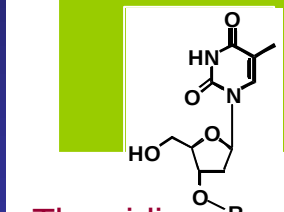


Oxidative Stress

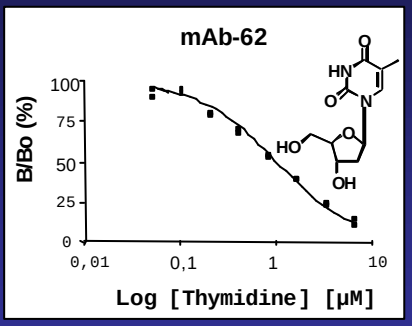


Antibodies' generation

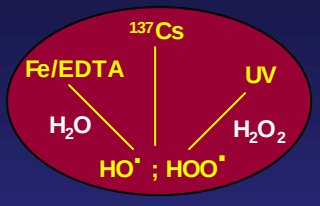
3 monoclonal antibodies

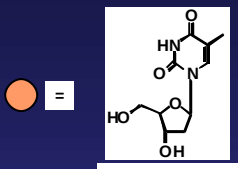


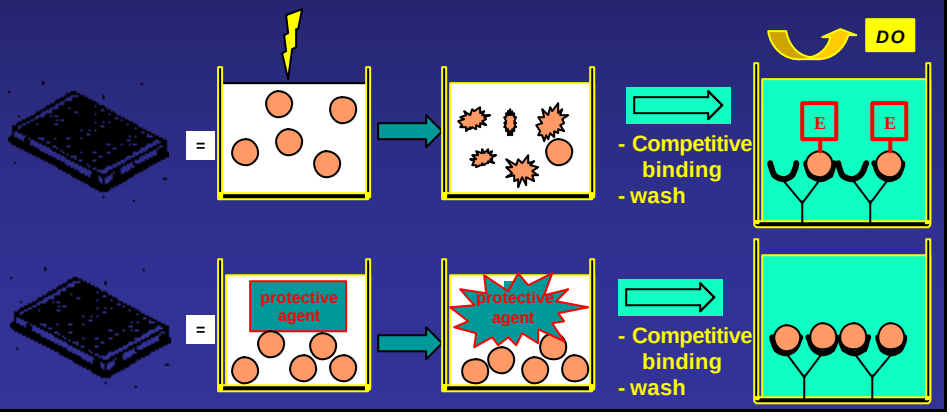
Thymidine



High-throuput sceening of new protective agents against oxidative stress







Strategy for the discovery of new protective agents against oxidative stress

Lead optimisation for the synthesis of analogs

different sources:
Plants, mushrooms, algaes…

Extraction

Synthesis

Libraries of Molecules

in vitro screening antioxidant activity

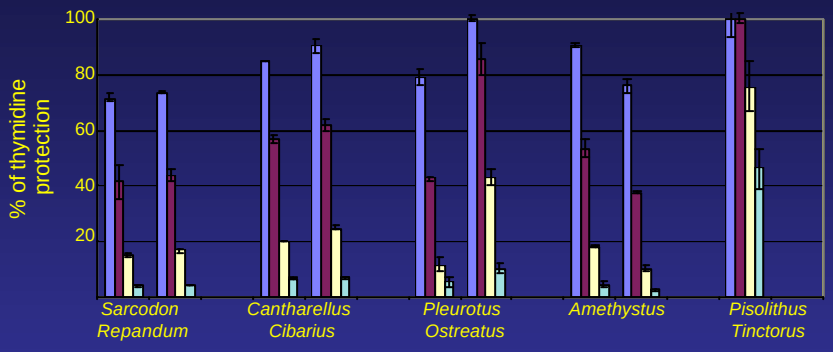
Lead identification

Toxicological evaluations
Physical determinations
Mechanism of action studies

Biological activities:
- anti-inflamatory
-anti-proliferative
-anti-cancer
-anti-aging
- radioprotective

Tests:
Cellular
Tissular
In vivo

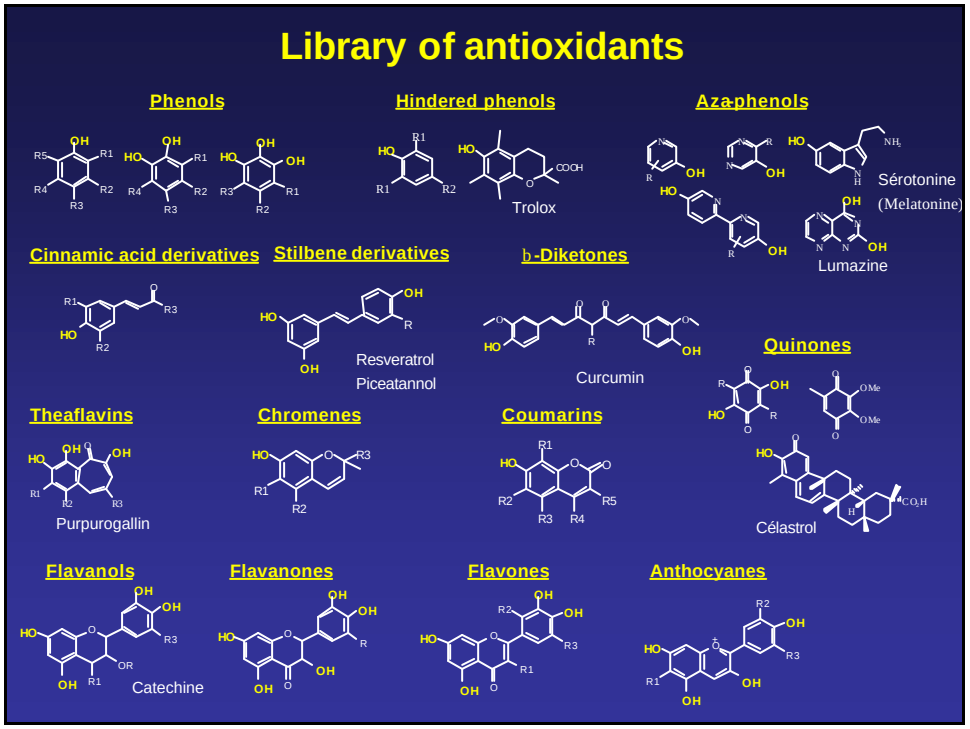
Mushroom extracts – UV degradation tests

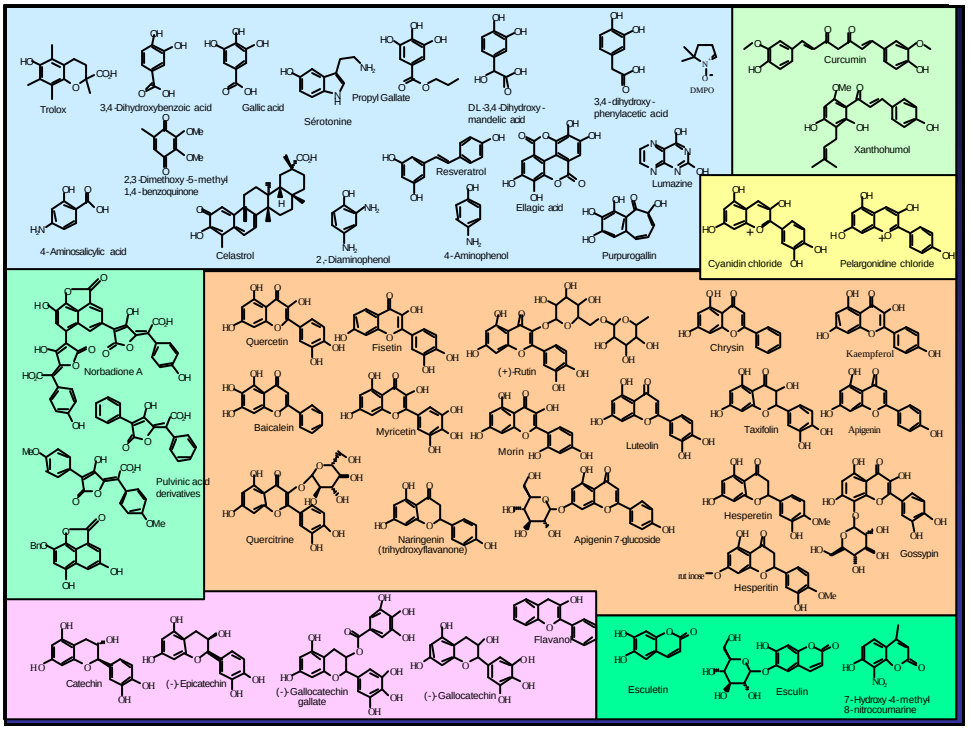


Norbadione A

Extractions :
- MeOH / Acetone / HCl
- CH_2Cl_2 then MeOH

Experimental conditions:
Thymidine (70µM) ; H_2O_2 (5mM) ;
irradiation at 254nm 1.75 J/cm^2
Tp Phosphate 25mM pH 7.4


Library of antioxidants
Phenols
Hindered phenols
Aza-phenols
Trolox
Sérotonine
(Melatonine)
Lumazine
Cinnamic acid derivatives
Stilbene derivatives
b-Diketones
Resveratrol
Piceatannol
Curcumin
Quinones
Theaflavins
Chromenes
Coumarins
Purpurogallin
Célastrol
Flavanols
Flavanones
Flavones
Anthocyanes
Catechine


Trolox
3,4-Dihydroxybenzoic acid
Gallic acid
Sérotonine
Propyl Gallate
DL-3,4-Dihydroxy-mandelic acid
3,4-dihydroxy-phenylacetic acid
DMPO
Curcumin
Xanthohumol
2,3-Dimethoxy-5-methyl 1,4-benzoquinone
Resveratrol
Ellagic acid
Lumazine
4-Aminosalicylic acid
Celastrol
2,-Diaminophenol
4-Aminophenol
Purpurogallin
Cyanidin chloride
Pelargonidine chloride
Norbadione A
Pulvinic acid derivatives
Quercetin
Fisetin
(+)-Rutin
Chrysin
Kaempferol
Baicalein
Myricetin
Morin
Luteolin
Taxifolin
Apigenin
Quercitrine
Naringenin (trihydroxyflavanone)
Apigenin 7-glucoside
Hesperetin
Gossypin
Catechin
(-)-Epicatechin
(-)-Gallocatechin gallate
(-)-Gallocatechin
Flavanol
Esculetin
Esculin
7-Hydroxy-4-methyl 8-nitrocoumarine

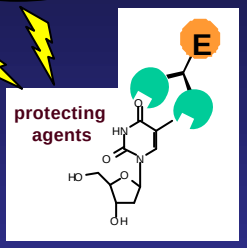
Oxidative stress

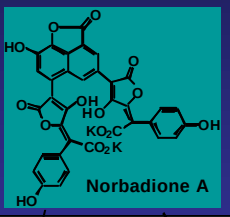
- 70 commercial molecules
- 10 synthetic molecules
- 10 natural extracts

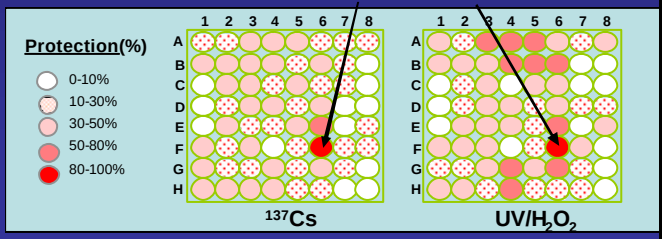
- tested in 4h
- 1 highly efficient molecule found

UV, Fenton

g Radiations





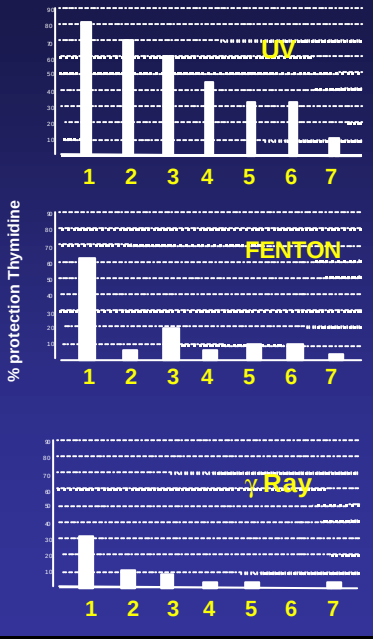


Antioxidative properties

Thymidine 70m mol
UV 254nm 1.75 J/cm²
H_2O_2 5mM in H_2O
antiox. 100m m

Fe_2SO_4 0.35 m mol +
H_2O_2 35 m mol in H_2O

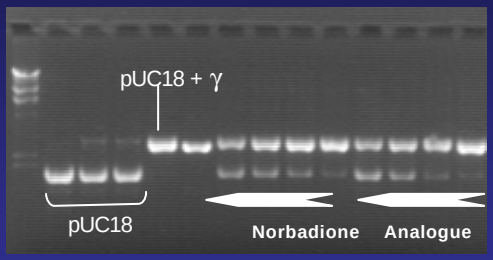
g Ray 3h

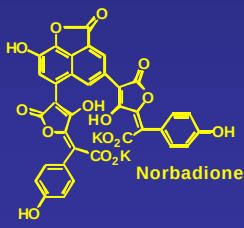


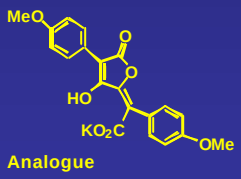
1- Norbadione
2- Quercitine
3- Fisetin
4- Myricetin
5- Catechin
6- 4-hydroxy-4-methyl-8-nitrocoumarine
7- Trolox

DNA protection against γ radiations

Plasmide pUC18, phosphate buffer pH 7,4
Irradiation ^{137}Cs, 30 min. (60Gy)



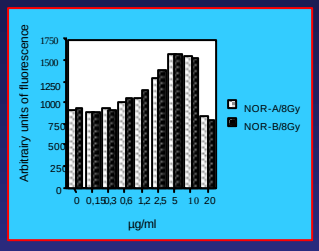


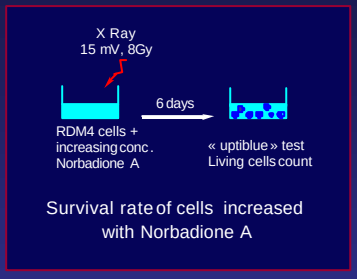


Radio- & chemoprotecting effects of Norbadione A

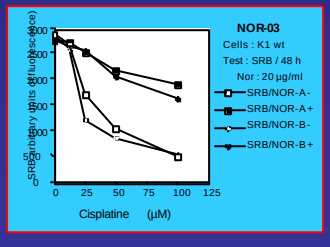
P. Bischoff (Strasbourg)

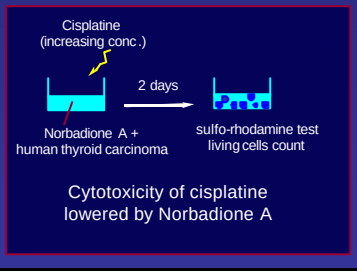
X Rays





Cisplatine



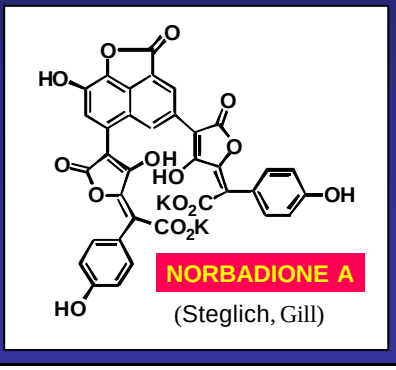


Mushrooms and radioactivity

Bolet bai (*Xerocomus badius*)

- In 1986, radioactive particles from the electrical plan of Tchernobyl contaminated several european countries including several french regions. **Bolet bai ,** a commestible mushroom, contains high concentrations in cesium 137.
- In 1989, Steglich found that cesium 137 is selectively localised in the pigments present on the top of the mushroom in association with **norbadione A**.
- Norbadione A can be extracted in higher quantities from a other mushroom **Pisolithe** (M. Gill).

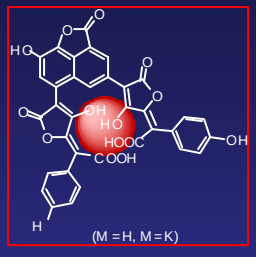
Pisolithe (*Pisolithus tinctorius*)

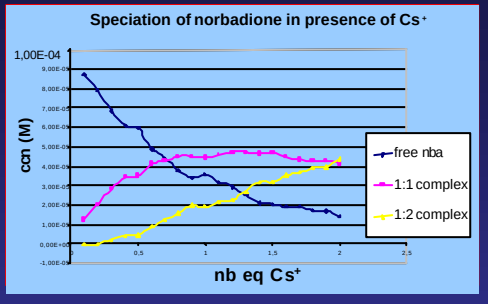


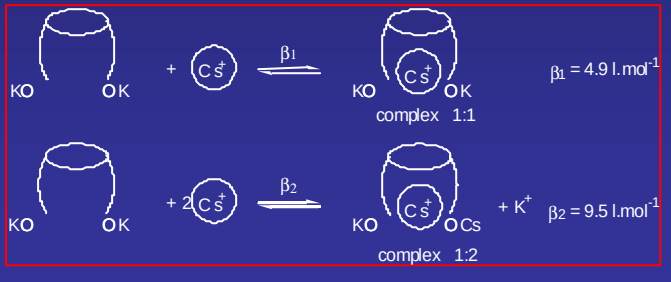
NORBADIONE A

(Steglich, Gill)

Cesium complexation by Norbadione A: mass spectrum studies

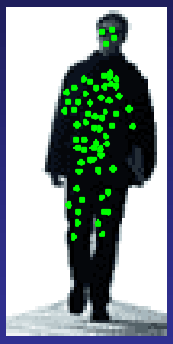






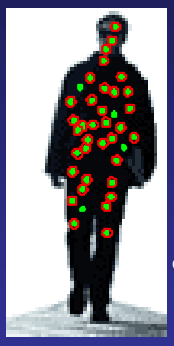
M. Desage
B. Amekraz
C. Moulin

Decontamination by decorporation with a chelating agent



Individual contaminated by the radioelement

Absortion of Norbadione A



Elimination of the Norbadione complexed cesium in the urine and faeces

Norbadione A, a radioprotective agent with a **double mechanim of action**:

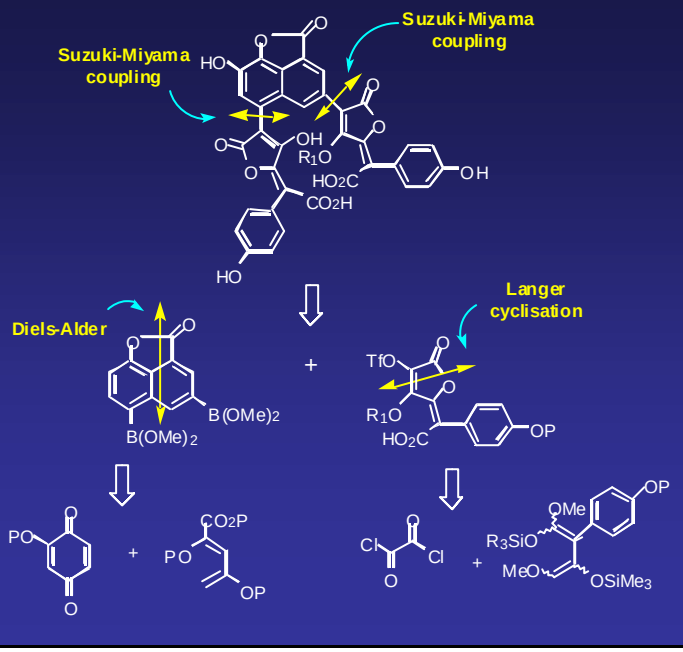
1- detoxification by specific chelation and elimination of ^{137}**Cs** (decorporation)

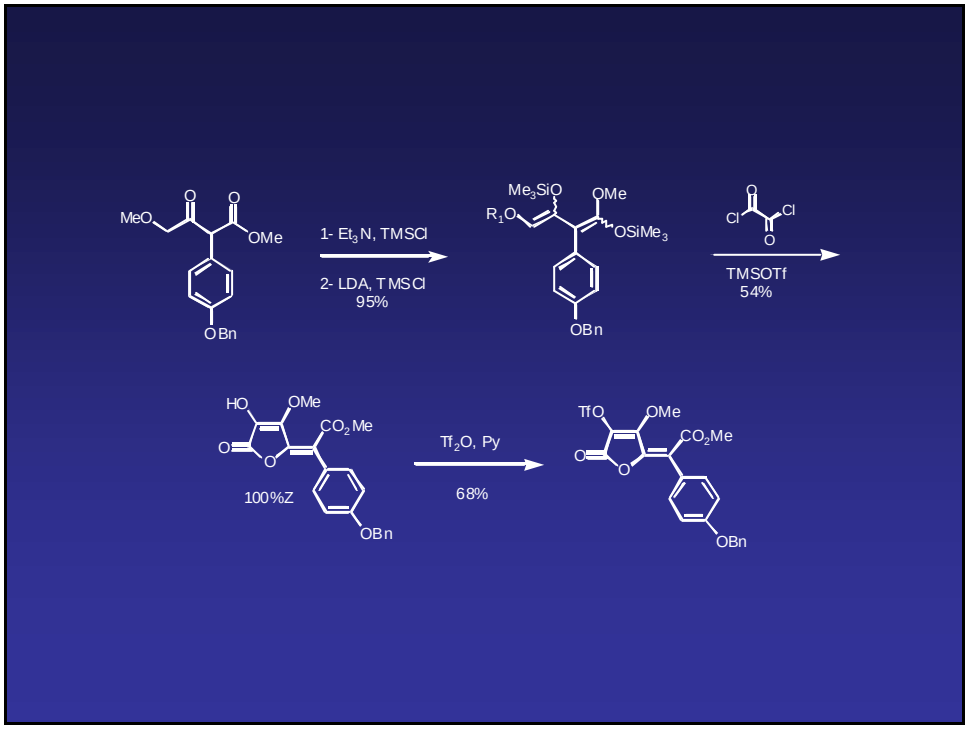
2- antioxidant properties to capture the reactive oxygen species generated by γ Ray emitted from ^{137}**Cs**

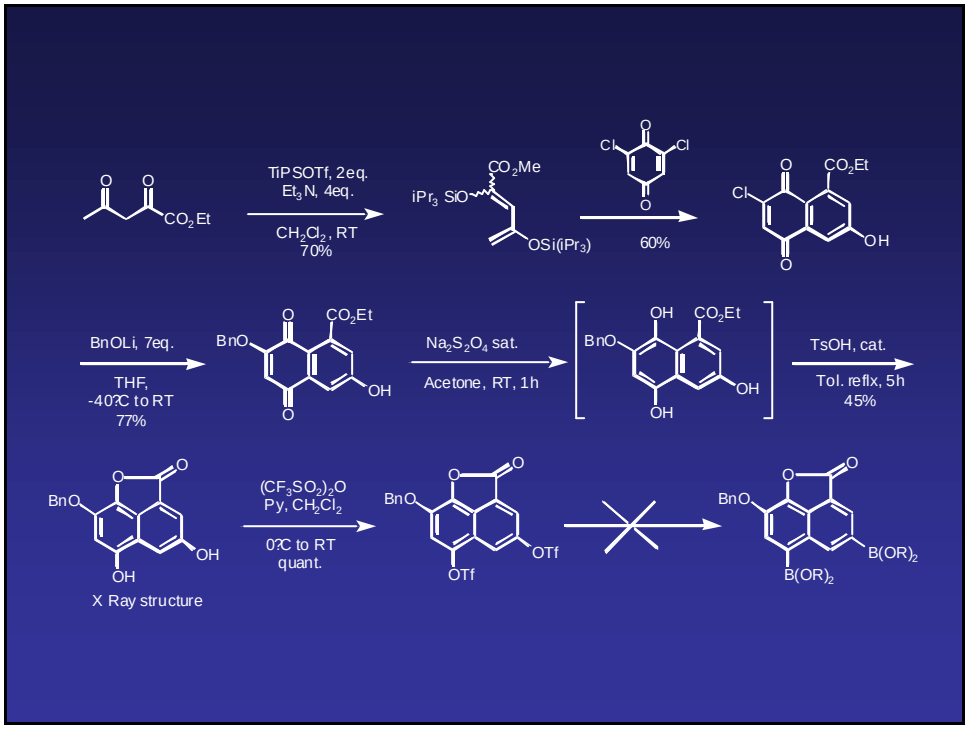
HTS

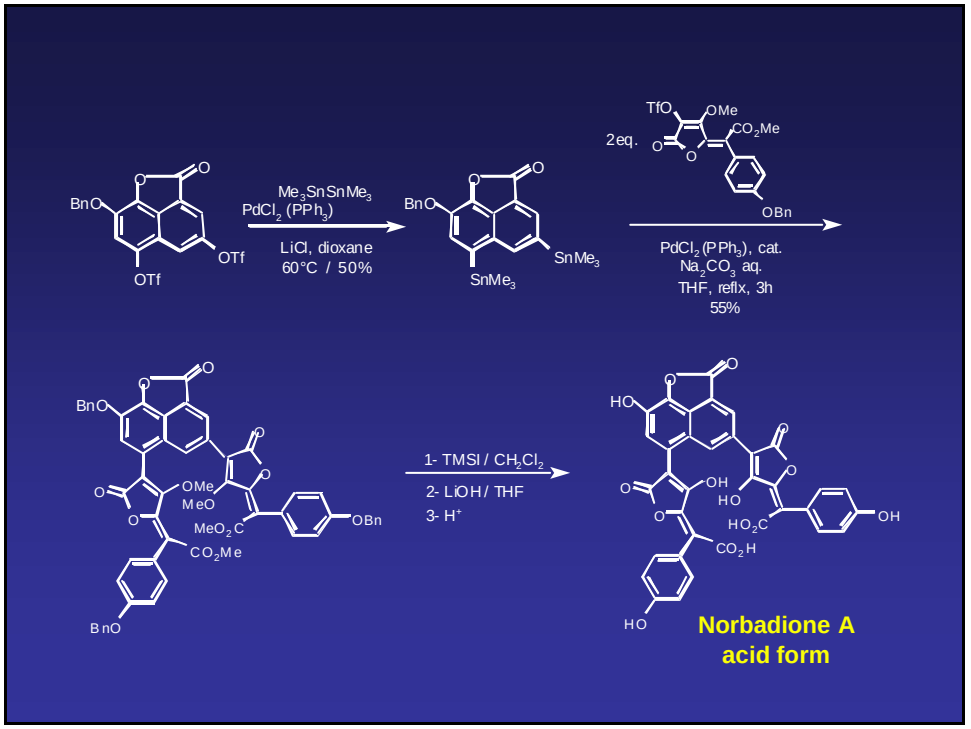
- H T S for enantioselective catalysts : yields & ee's
- H T S for antioxidants
  protective agents against oxidative stress
  selection of Norbadione A
- Total synthesis of Norbadione A

Norbadione A: retrosynthetic scheme




1- Et3N, TMSCl
2- LDA, TMSCl
95%
TMSOTf
54%
100%Z
Tf2O, Py
68%


TIPSOTf, 2eq.
Et3N, 4eq.
CH2Cl2, RT
70%
60%
BnOLi, 7eq.
THF,
-40°C to RT
77%
Na2S2O4 sat.
Acetone, RT, 1h
TsOH, cat.
Tol. reflx, 5h
45%
(CF3SO2)2O
Py, CH2Cl2
0°C to RT
quant.
X Ray structure



Conclusions

- **Two high-throughput screenings (HTS) by immunoassay tests for the selection of :**
  - **catalytic systems**
  - **selection of highly active antioxidant agents**

- **Total synthesis of Norbadione A a potent radioprotective agent selected by HTS immunoassay tests**

Acknowledgments

SMM ➡

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Thierry Le Gall
Marine Lesage
Stephanie Nowaczyk
Alain Valleix
Céline Caussignac

SPI ➡

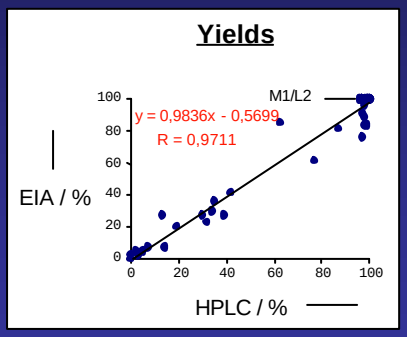
Laure Buscarlet
Jacques Grassi
Philippe Pradelles
Christophe Créminon
Hervé Volland

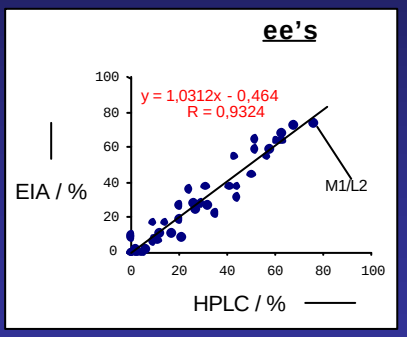
CNRS ➡

Alain Wagner
Barbara Mohar

Validation of the Enzyme Immuno Assays (EIA)

Validation on n = 42 samples





Antioxidant Tests

Radical sources

+ Antioxidant

idem but higher conc. of unaffected target

Target molecule

conc. determination of the unafected target

+ Antioxidant

degradation products

Immunoanalysis

concentration determination of one of the oxidized product

idem but signal inhibition

HPLC UV Abs. Electrophoresis RPE Luminescence

Tests for the evaluation of antioxidant properties of single compounds or mixtures

. TBA method (thiobarbituric acid) :
inhibition of the oxidation of deoxyribose (1959)

. HPLC : inhibition of the hydroxylation aromatic compouds (1984)

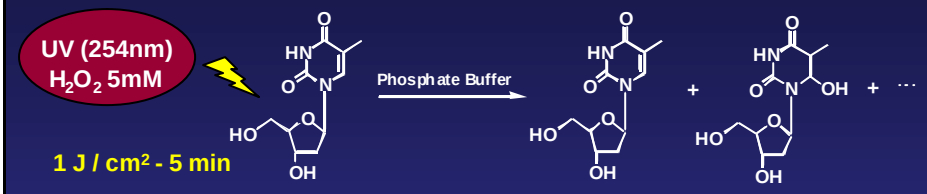
. TRAP method (total peroxyl radical trapping parameter) (1987)

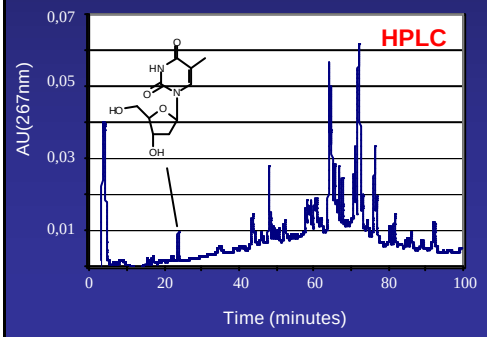
. RPE : inhibition of the DMPO-OH radical generation (1990)

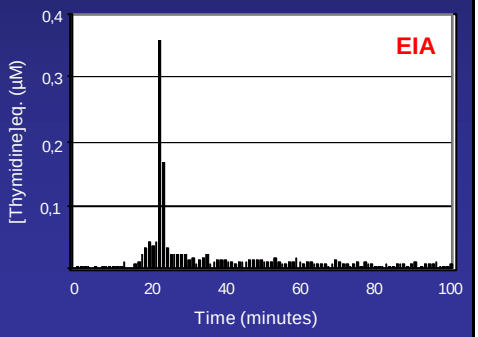
. Randox-TEAC method (Trolox equivalent antioxydant capacity)
: decoloration of the ABTS radical (1993)

. Electrophoresis : inhibition of the split of DNA strand (1993)

UV Degradation



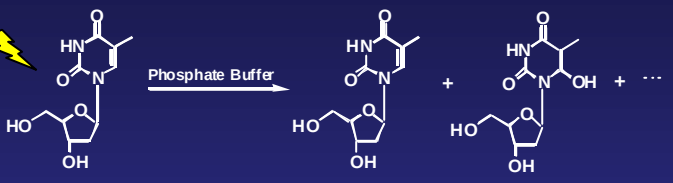


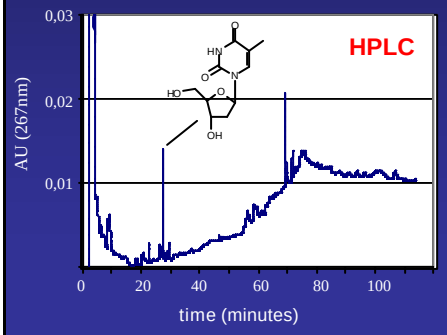


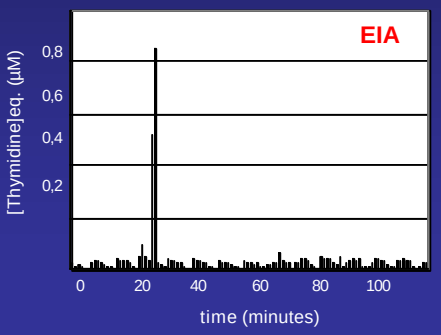
Degradation by Radiolysis

g radiations ^{137}Cs

Irradiation
1h – 150 Gy.



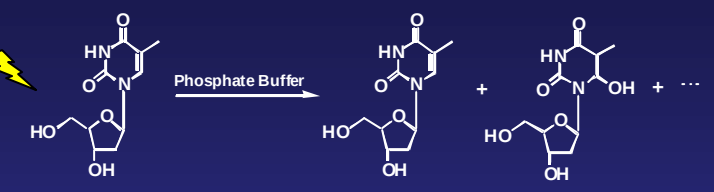


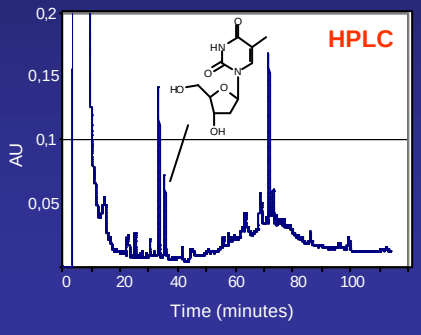


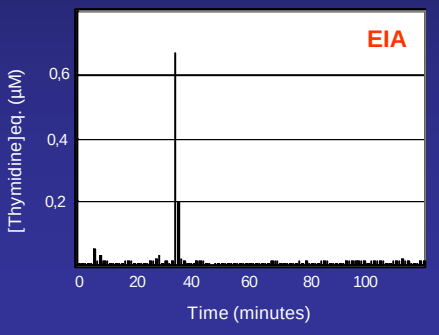
Fenton type degradation

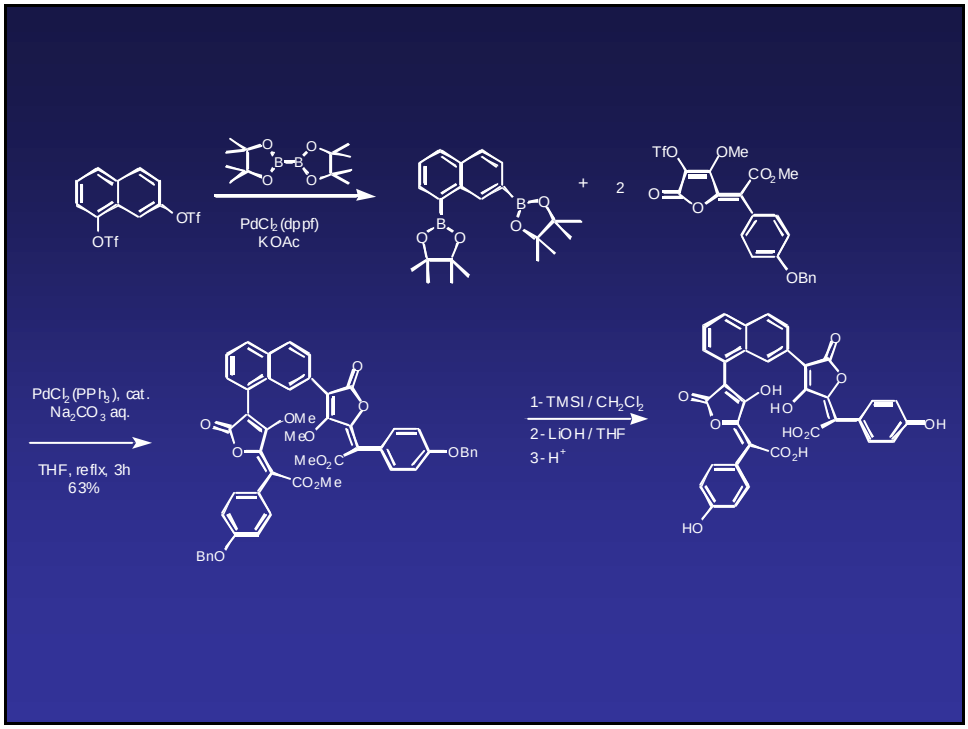
Fenton

Fe/EDTA/H_2O_2
(1:1:100) 5min.








OTf
OTf
PdCl2(dppf)
KOAc
+
2
TfO
OMe
CO2Me
O
O
OBn
PdCl2(PPh3), cat.
Na2CO3 aq.
THF, reflx, 3h
63%
OMe
MeO
MeO2C
CO2Me
O
O
OBn
BnO
1- TMSI / CH2Cl2
2- LiOH / THF
3- H+
OH
HO
HO2C
CO2H
HO
O
O
OH