1st International Electronic Conference on Medicinal Chemistry

2-27 November 2015

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Peptidomimetic inhibitors of ABC transporters

Marie-Emmanuelle Million[1], Ophélie Arnaud[2], Géraldine Agusti[3], Waël Zeinyeh[4], Lucia Gonzalez-Lobato[2], Ali Koubeissi[4], Laurent Ettouati[4,*], Thierry Lomberget[4], Joelle Paris[4], Marc Le Borgne[4] and Pierre Falson[2]

[1] Université de Lyon, Université Lyon 1, Faculté de Pharmacie - ISPB, Département des Sciences du Médicament, 8 avenue Rockefeller, F-69373, Lyon Cedex 8, France ;

[2] Equipe Labellisée Ligue 2014, BMSSI UMR 5086 CNRS/Université Lyon 1, IBCP, 69367 Lyon, France

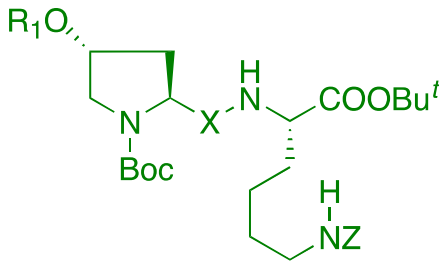
[3] Laboratoire d'Automatique et de Génie des Procédés (LAGEP), UMR-CNRS 5007, Université Claude Bernard Lyon 1, CPE Lyon, Villeurbanne Cedex, France

[4] Université de Lyon, Université Lyon 1, Faculté de Pharmacie - ISPB, EA 4446 Biomolécules Cancer et Chimiorésistances, SFR Santé Lyon-Est CNRS UMS3453 - INSERM US7, 8 avenue Rockefeller, F-69373, Lyon Cedex 8, France

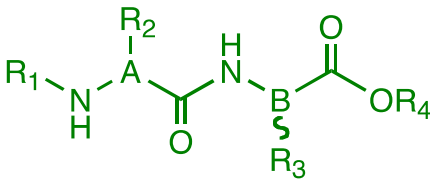
UNIVERSITÉ DE LYON Université Claude Bernard Lyon 1 ISPB Lyon 1 IBCP cnrs B2C

* Corresponding author: laurent.ettouati@univ-lyon1.fr

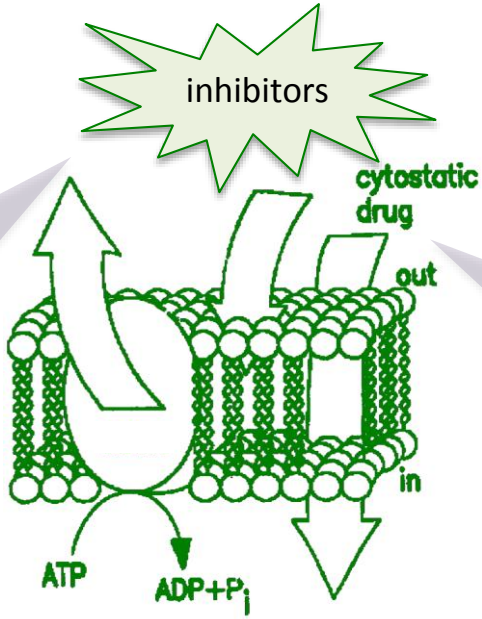
Peptidomimetic inhibitors of ABC transporters



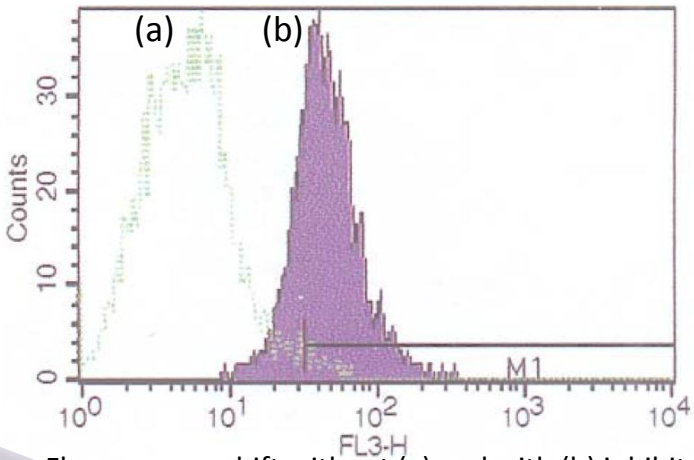
"Hyp" series



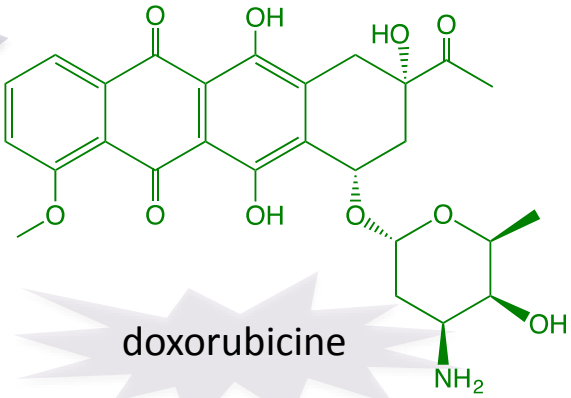
"Aza" series



ABCB1 and ABCG2



Fluorescence shift without (a) and with (b) inhibitor

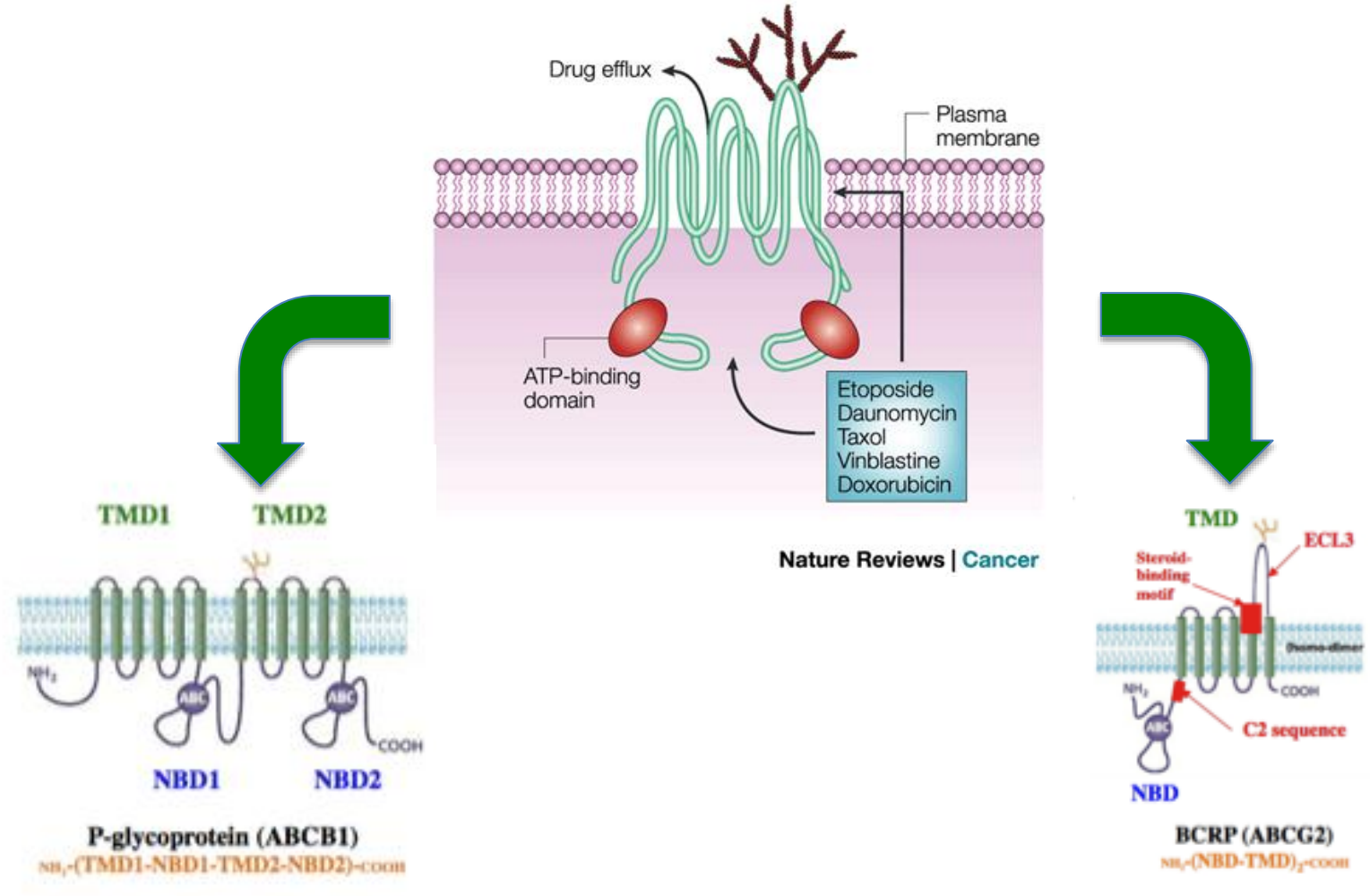


doxorubicine

Adapted from Sarkadi *et al.* US Patent n° 6297216, 02-10-2001

Abstract: The discovery of the physiological role of a great number of peptides (e.g. angiotensin II, neuropeptide Y, enkephalin, gonadotrophin-releasing hormone…) stimulated researchers towards design and synthesis of analogues. Since the last two decades, peptidomimetics have emerged as promising therapeutic agents such as goserelin, cetrorelix, and atazanavir. Structural modifications of the sequence of the native peptides can optimize their biological properties such as bioavailability, plasma half-life, resistance of metabolism and selectivity. Another advantage to develop peptidomimetics as drugs and/or probes can be the control of their conformation. A peptidomimetic with a restricted conformational flexibility can minimize binding to non-target receptors and then enhances the activity at the target receptor or transporter. For many years, our researchers worked on multidrug resistance (MDR) to anticancer and anti-infectious agents. This phenomenon is often associated with over-expression of several proteins belonging to ABC transporters (e.g. ABCB1, ABCG2). Numerous molecules have shown activities on these transporters. Among them, we can list steroids, bivalent ligands, azaheterocycles and short linear hydrophobic peptides. For example, reversin 121, a dipeptide, showed high affinity and specificity for ABCB1. Reversin 121 became the new starting point of our research. From 2005 we developed different series of (aza)peptidomimetic-type ligands of ABC transporters. First, we wish to expose the synthesis work accomplished to reach our selection of molecules and secondly to present our biological data on ABCB1 and ABCG2.
Keywords: reversins; ABCB1; ABCG2; (aza)peptidomimetics

ATP-Binding Cassette transporters family



P-Glycoprotein (P-gp, MDR1, ABCB1)

BCRP (ABCG2)

http://www.nature.com/nrc/journal/v2/n6/pdf/nrc823.pdf
http://www.sigmaaldrich.com/content/dam/sigma-aldrich/life-science/biochemicals0/drug-metabolixm-tech/figure-2.gif

P-gp drugs-mediated efflux

Anticancer
Methotrexate
Amsacrine
Colchicin
Doxetaxel
Etoposide
Imatinib
Ivermectin
Paclitaxel
Teniposide
Topotecan
Vinblastine
Vinorelbine
Vindesine
Vincristine
Doxorubicin
Mitoxantrone
Irinotecan
Daunorubicin

Antibiotics
Azithromycin
Ciprofloxacin
Dactinomycin
actinomycin D
Epirubicin
Levofloxaxin
Mitomycin
Rifanpicin
Sparfloxacin
Tetracyclin
Erythromycin

Cardiovascular
Acebutol
Atorvastatin
Cliprolol
Digitoxin
Digotoxin
Ditiazem
Losartan
Lovastatin
Mibefradil
Phenytoin
Quinidine
Talinolol
Verapamil

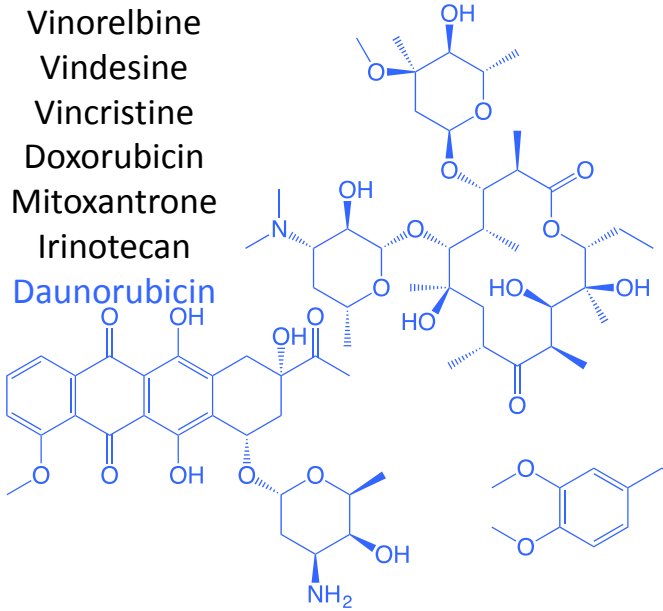
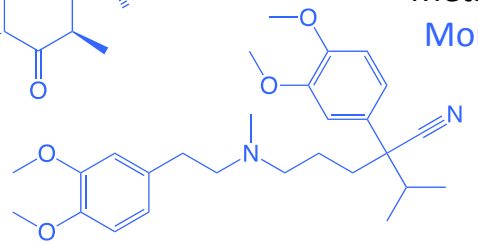
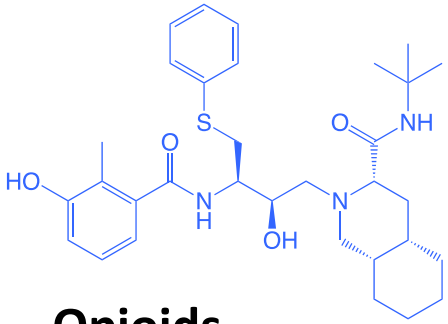
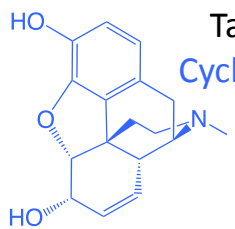
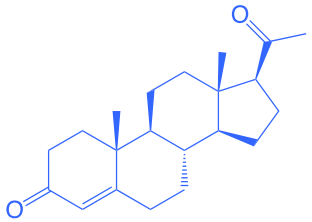
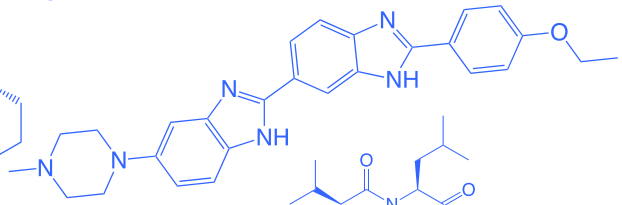
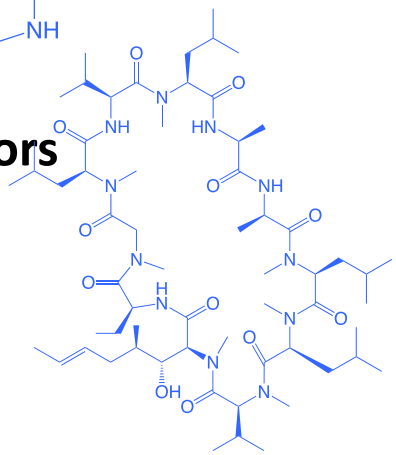
Antiviral
Amprenavir
Indinavir
Lopinavir
Ritonavir
Saquinavir
Zidovudine
Nelfinavir

Hormones
Cortisol
Dexamethasone
Prednisolone
Estradiol
Hydrocortisone
Prednisolone
Progesterone

Others
Cimetidin
Domperidone
Fexofenadine
Mefloquine
Ondansetron
Ranitidin
Terfenadine
Peptides
Phenobarbital
Hoechst 33342

Opioids
Loperamide
Methadone
Morphine

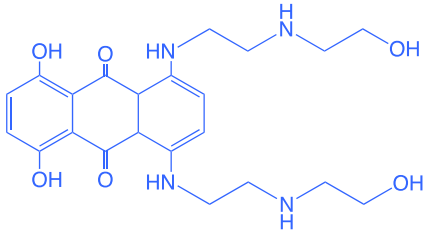
Immunosuppressors
Sirolimus
Tacrolimus
Cyclosporine A

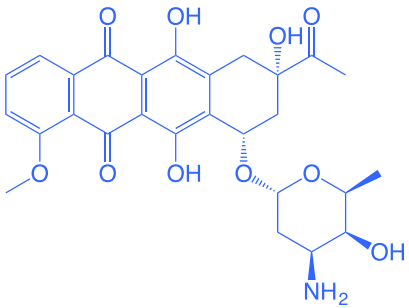
BCRP drugs and physiological compounds-mediated efflux

Anticancer

Methotrexate
Imatinib
Ivermectin
Topotecan
Irinotecan
Mitoxantrone

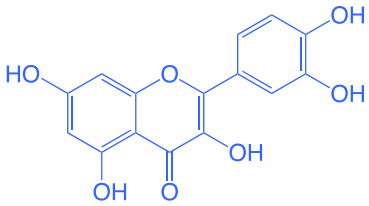


Doxorubicin
Daunorubicin



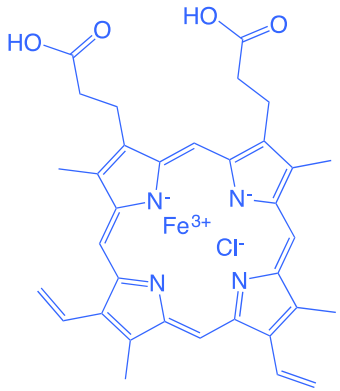
Phytoestrogens

Genistein
Daidzein
Coumestrol
Quercetin



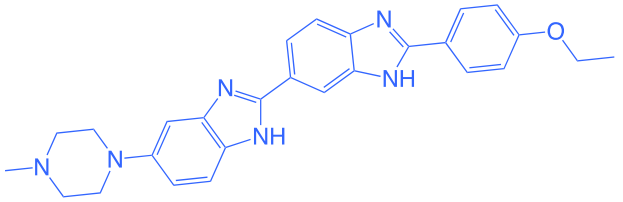
Porphyrins

Pheophorbin
Protoporphyrin IX
Heme
Hemin



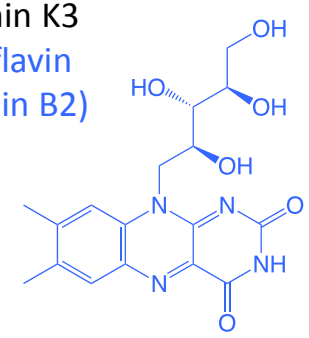
Dyes

Rhodamine 123
Hoechst 33342

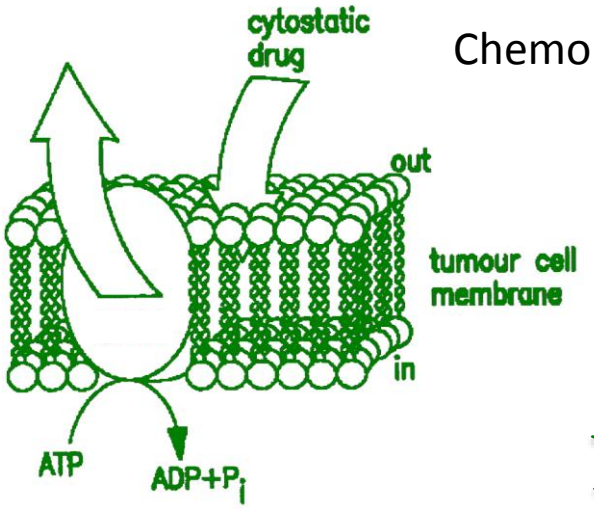


Vitamins

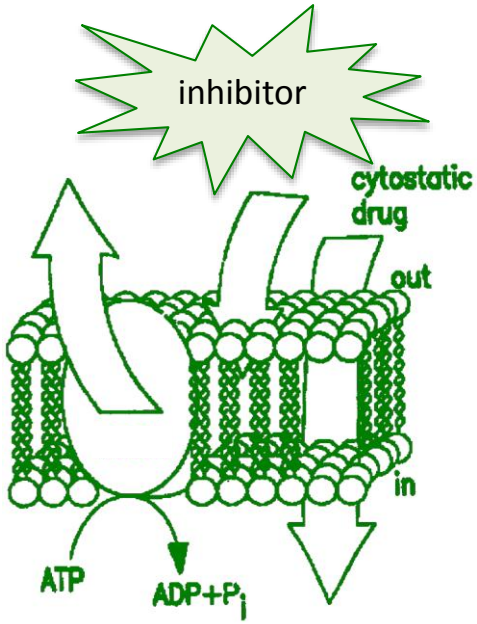
FMN
Biotin
(vitamin B7)
Vitamin K3
Riboflavin
(vitamin B2)



ABC transporter inhibition - solving cancer drug resistance?



Chemoresistance

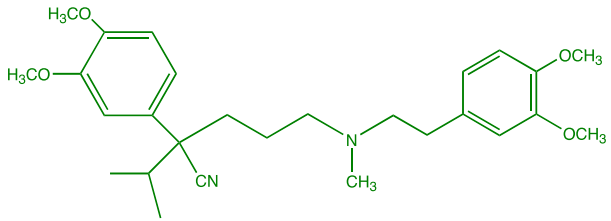


Chemosensitization

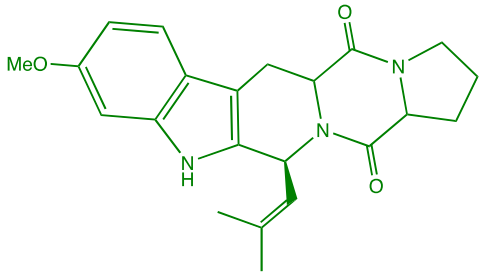
- Design modulators not competing with drug-binding sites
- Specificity towards other ABC transporters
- Elucidate the transport mechanism

Adapted from Sarkadi *et al.* US Patent n° 6,297,216, 02-10-2001

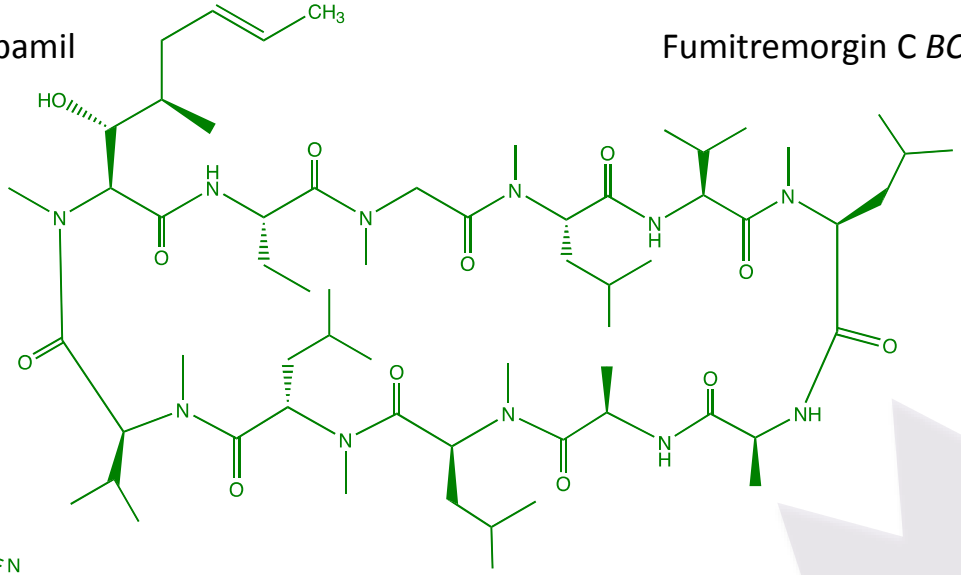
Known P-gp and BCRP transporter inhibitors



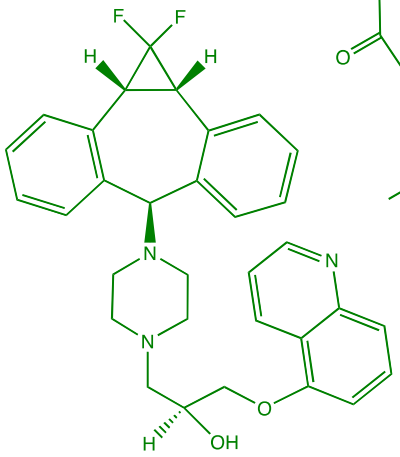
Verapamil



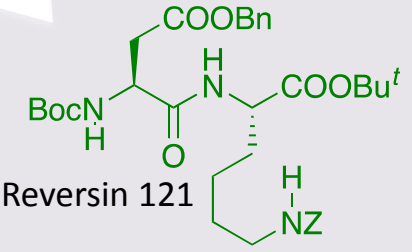
Fumitremorgin C *BCRP selective*



Cyclosporin A



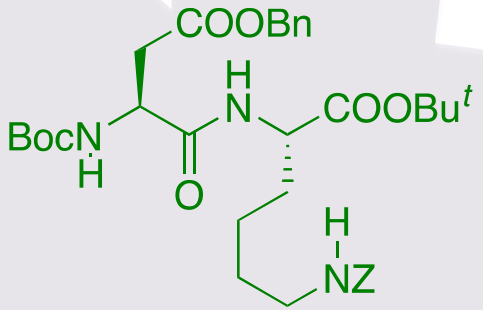
Zosuquidar (LY335979) *P-gp selective*



Reversin 121

Reversins as P-glycoprotein inhibitors

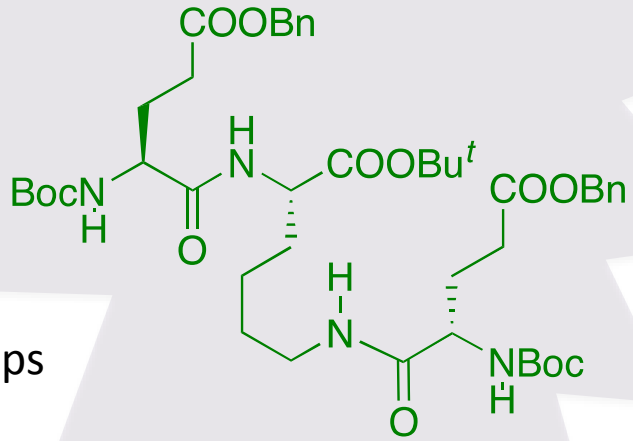
Fully protected
di- or tripeptides



Reversin 121

High affinity to
P-glycoprotein (K_d ≈ 100 nM)

Inhibition of
cytotoxic drug transport
(IC_{50} ≈ 1.5 μM)



Reversin 205

Hydrophobic protecting groups

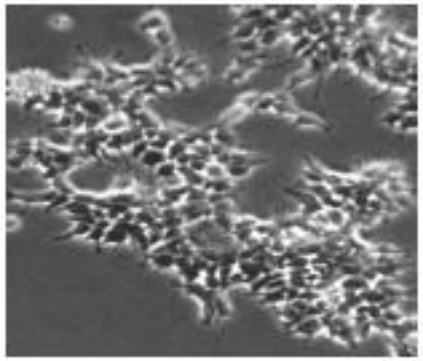
No toxicity
up to 100 mg/kg *in vivo*

Sharom *et al. Biochem. Pharmacol.* ***1999****, 58, 571-86*

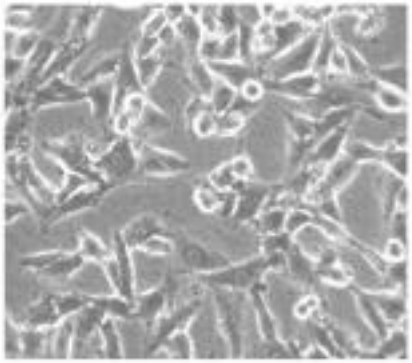
Screening assay

- *In vitro* models

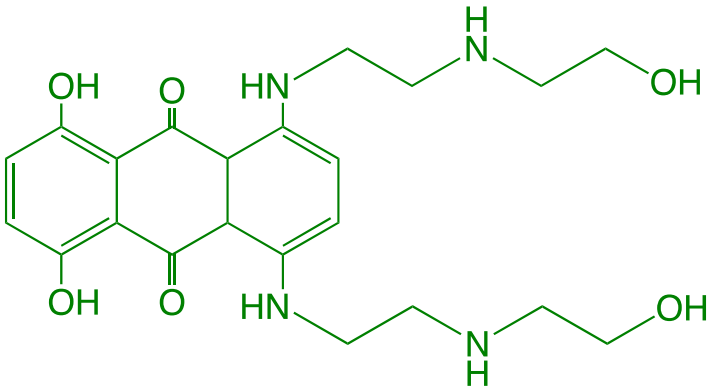
Immortalized
P-gp-transfected
NIH3T3 cells



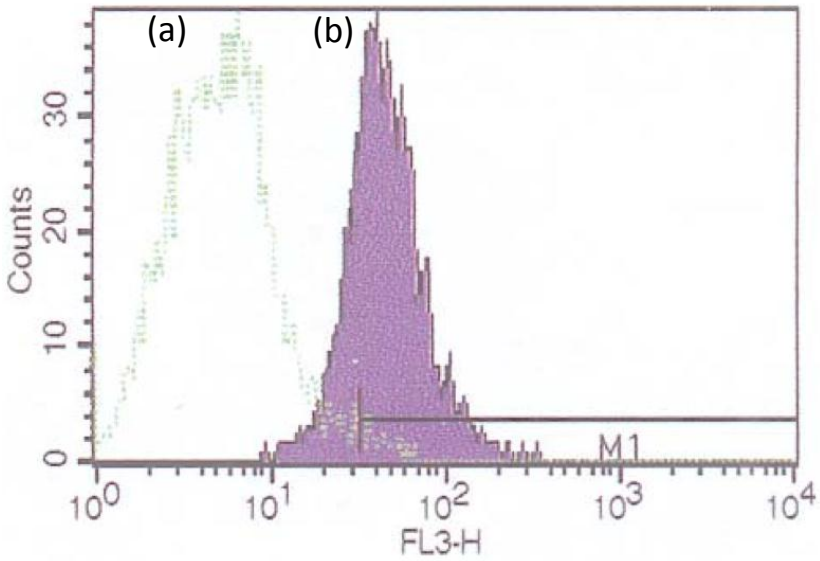
Immortalized
BCRP-transfected
HEK 293 cells



- Intracellular fluorescence quantitation of mitoxantrone by flow cytometry

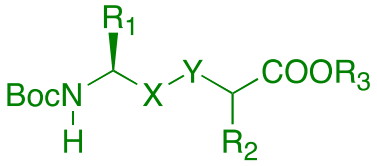


mitoxantrone (MTX)

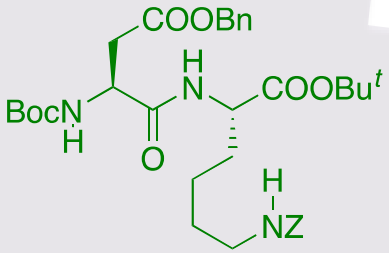


Fluorescence shift without (a) and with (b) inhibitor

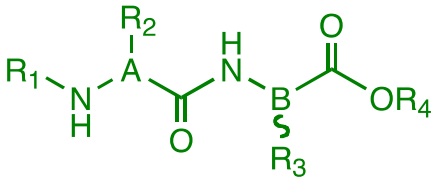
Pharmacomodulation of reversins



X = CH_2, CO ; Y = NH, CH_2

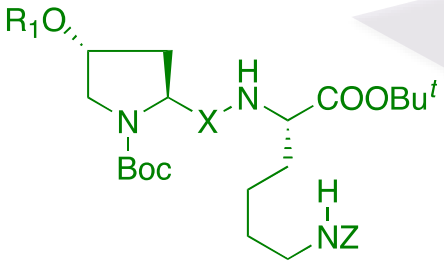


Reversin 121



A ou B = N

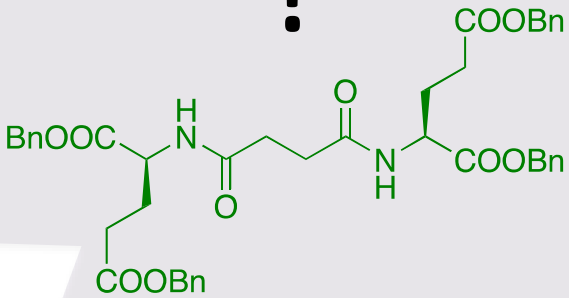
"Aza" series



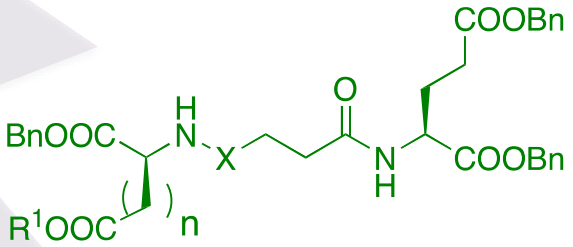
X = CH_2, CO

"Hyp" series

?

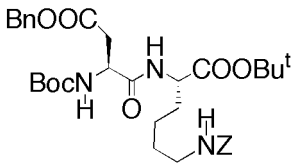


Reversin 213



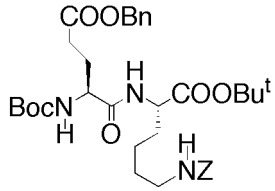
X = CH_2, CO

Koubeissi *et al. Bioorg. Med. Chem. Lett*. **2006**,16, 5700 - Koubeissi *et al. J. Med. Chem*. **2010**,53, 6720-6729

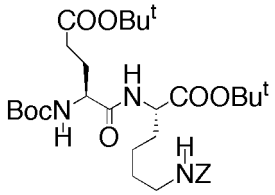


Rev. 121 76±1%

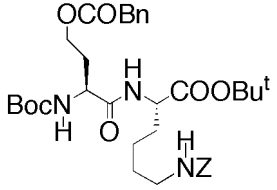
IC_{50}: 1.2 μM



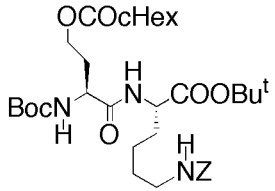
CT1329 63±5%



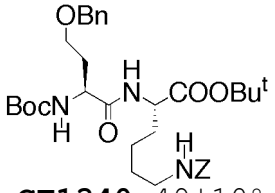
CT1343 7±13%



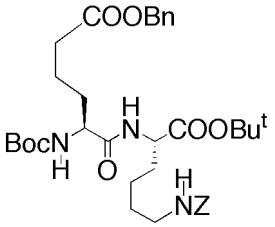
CT1337 59±14%



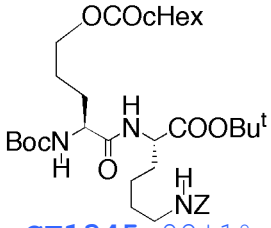
CT1317 61±3%



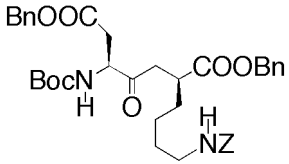
CT1340 49±10%



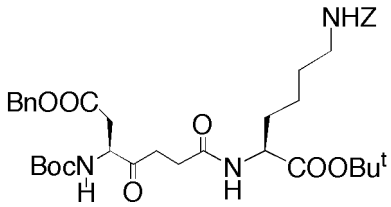
CT1348 97±1%



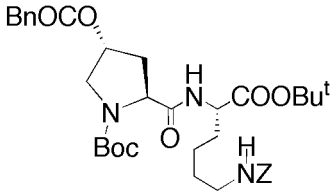
CT1345 82±1%



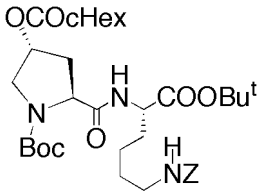
CT1361 35±15%



CT1316 67±7%

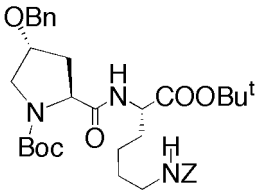


CT1338 68±10%



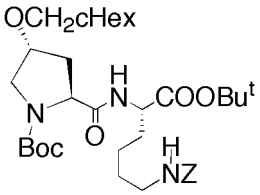
CT1343 105±13%

IC_{50}: 1.6 μM



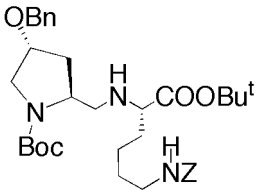
CT1336 99±11%

IC_{50}: 0.6 μM



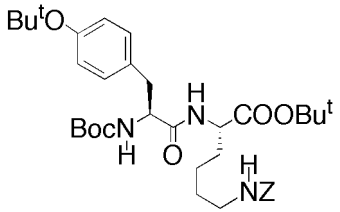
CT1344 77±11%

IC_{50}: 0.6 μM

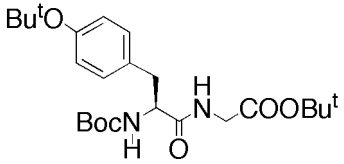


CT1347 106±23%

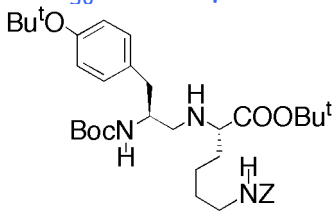
IC_{50} : 0.2 μM



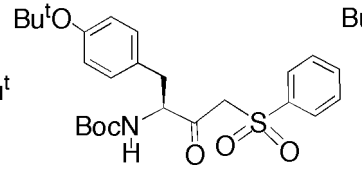
CT1341 34±10%



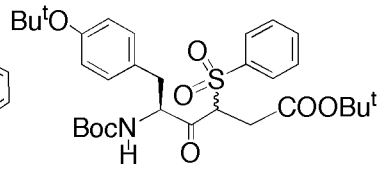
CT1333 93±13%



CT1346 49±5%

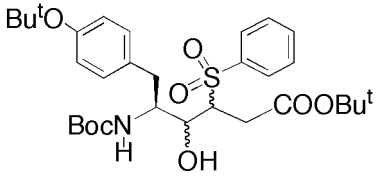


CT1351 49±5%

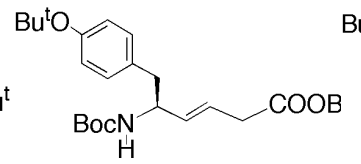


CT1354 106±8

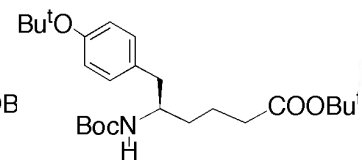
IC_{50}: 1.55 μM



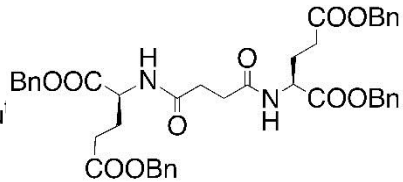
CT1357 104±10%



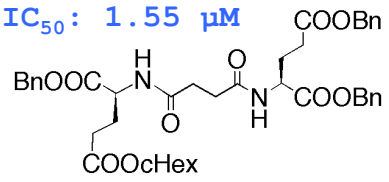
CT1355 71±1%



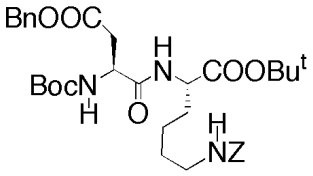
CT1353 78±8%



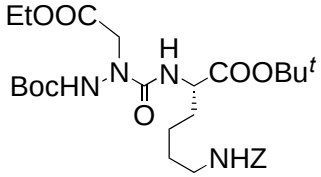
Rev. 213 26±7%



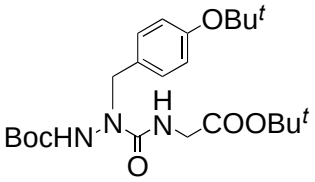
CT1327 29±12%



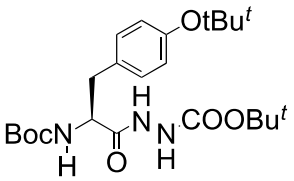
Rev. 121
37±4%



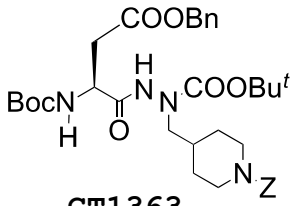
CT1310
5%±7



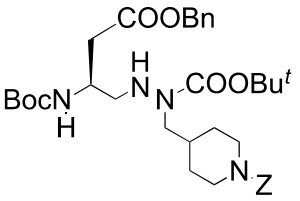
CT1335
21%±9



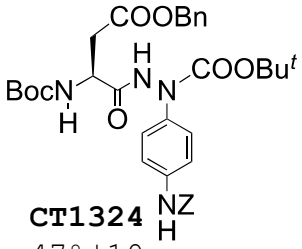
CT1334
19±13



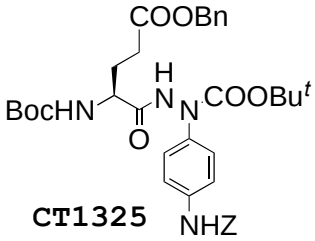
CT1363
20%±1



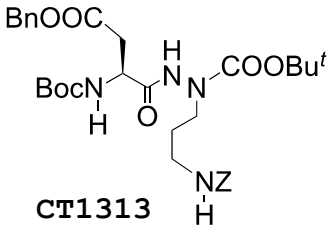
CT1362
20%±1



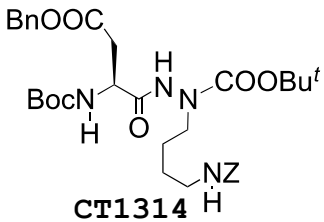
CT1324
47%±10



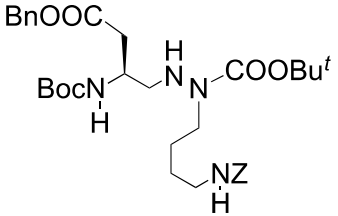
CT1325
65%±1



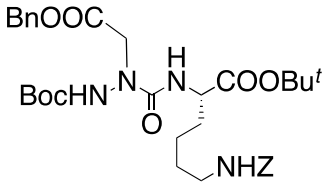
CT1313
22%±11



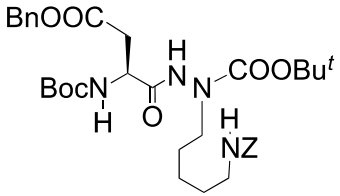
CT1314
23%±1



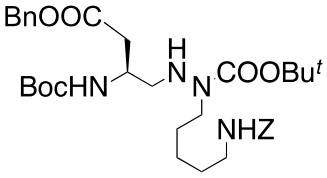
CT1315
25%±4



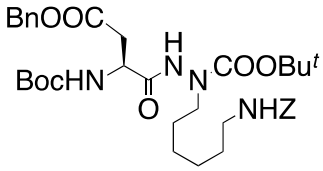
CT1311
43%±4



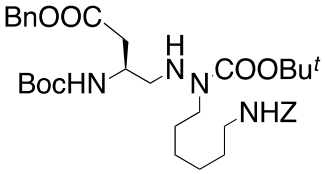
CT1349
63%±12



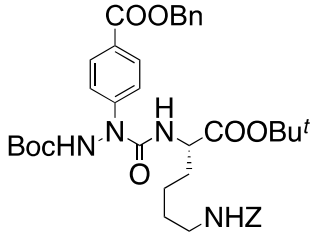
CT1365
79%±21



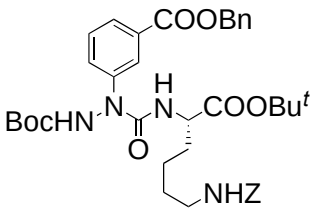
CT1367
60%±10



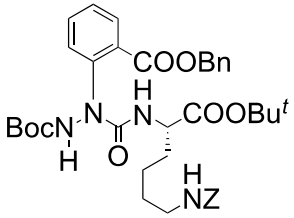
CT1366
87%±27 IC_{50}: 0.9 µM



CT1321
38%±14



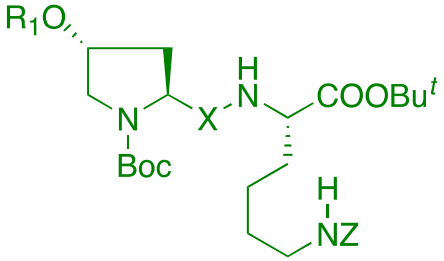
CT1364
112%±26 IC_{50}: 1 µM



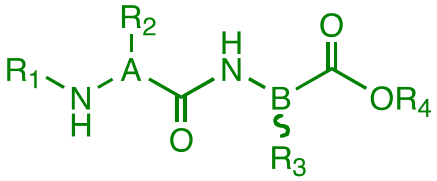
CT1328
112%±6 IC_{50}: 2 µM

J. Paris et al. Patent WO 2010/084292, published Sept 29, 2010

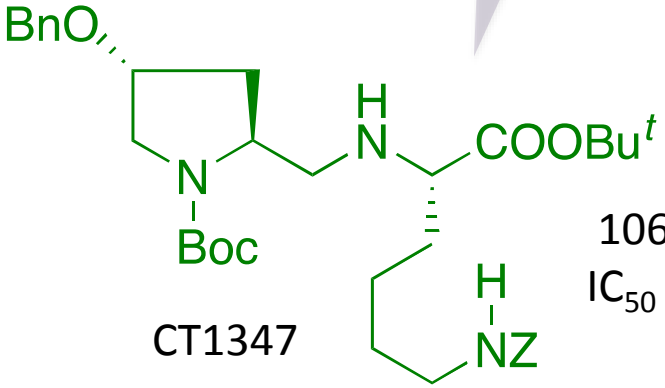
Hits selection



"Hyp" series



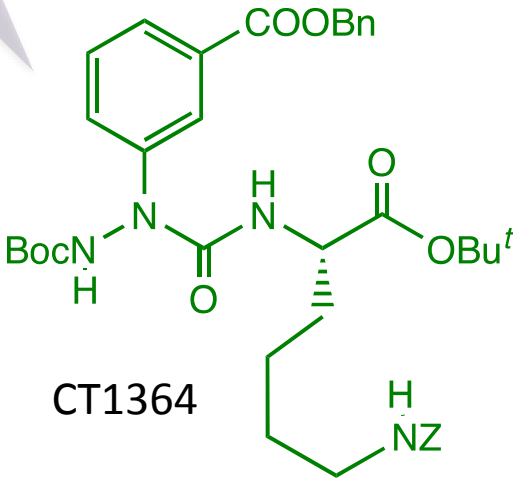
"Aza" series



CT1347

106%±23

IC_{50} 0.2 µM



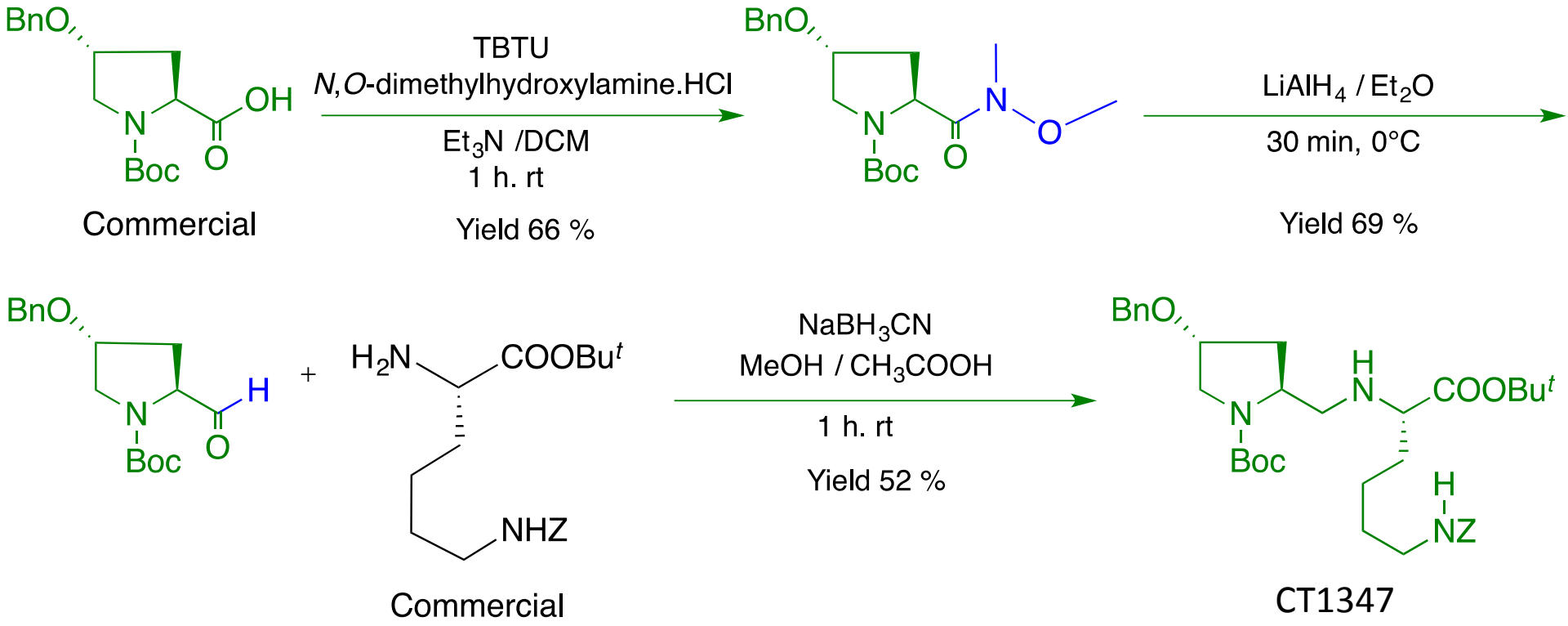
CT1364

112%±26

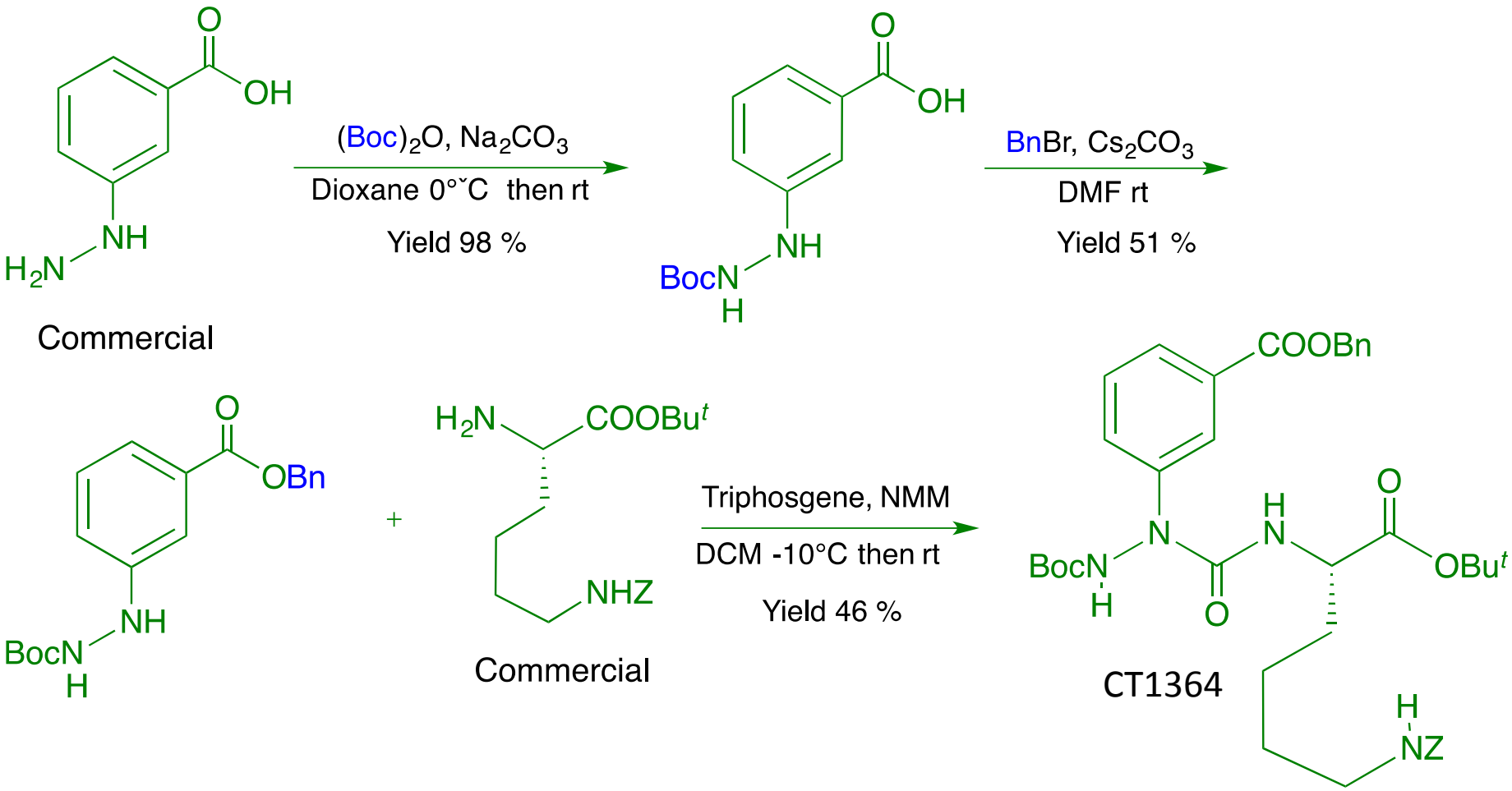
IC_{50} 1 µM

French Patents n° FR09 50450 and FR09 56954

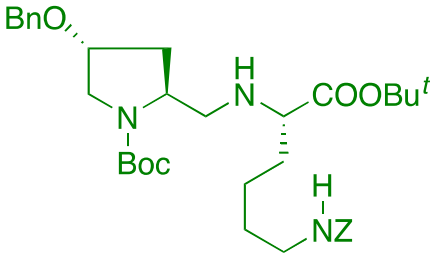
Synthesis of reversin analog CT1347



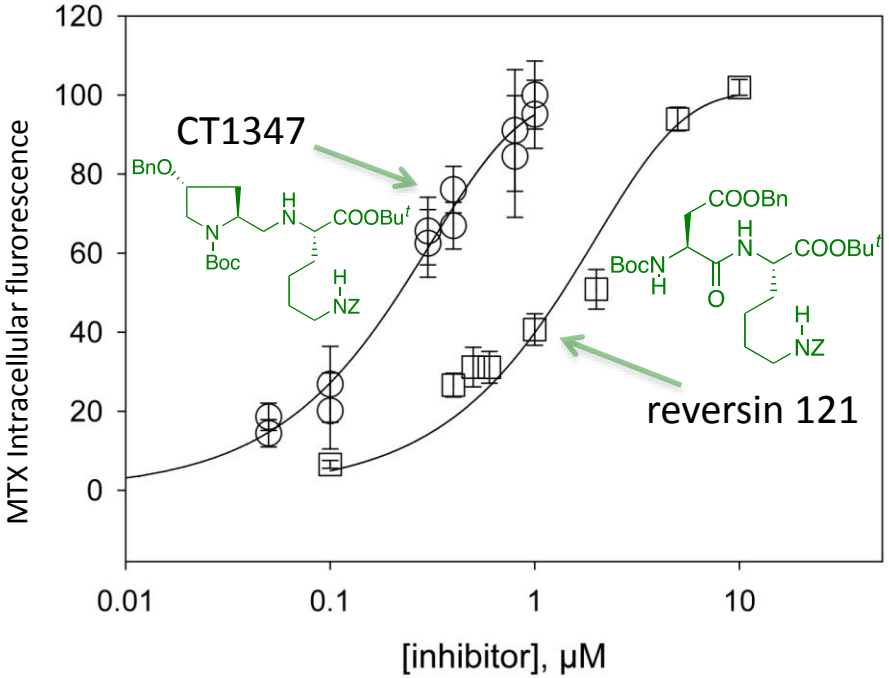
Synthesis of reversin analog CT1364



CT1347 - Biological studies

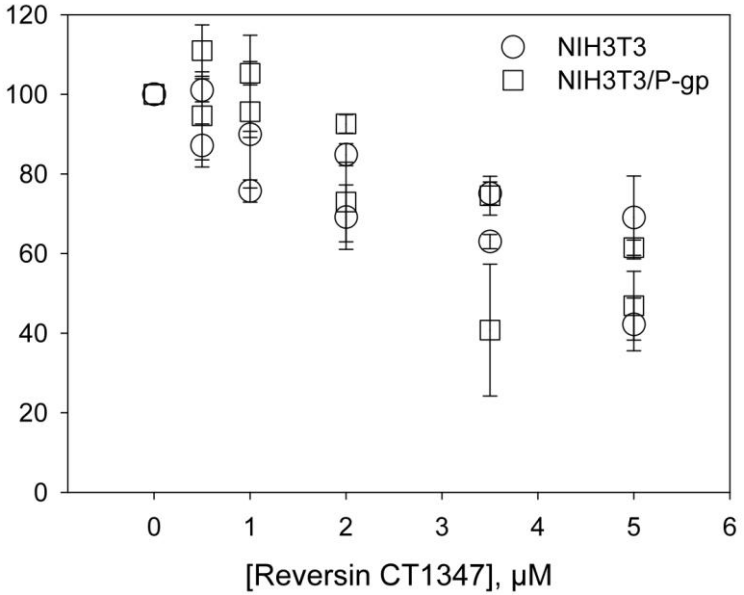


Mitoxantrone inhibition efficacy of P-glycoprotein efflux



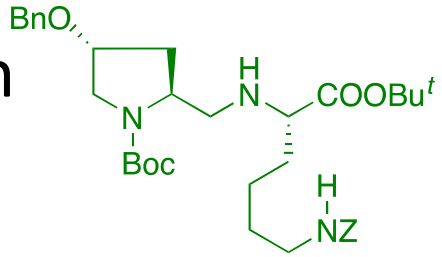
- IC_{50} 0.22 μM
- P-gp selectivity (SI: 2.5)

Cytoxicity evaluation (MTT assay)

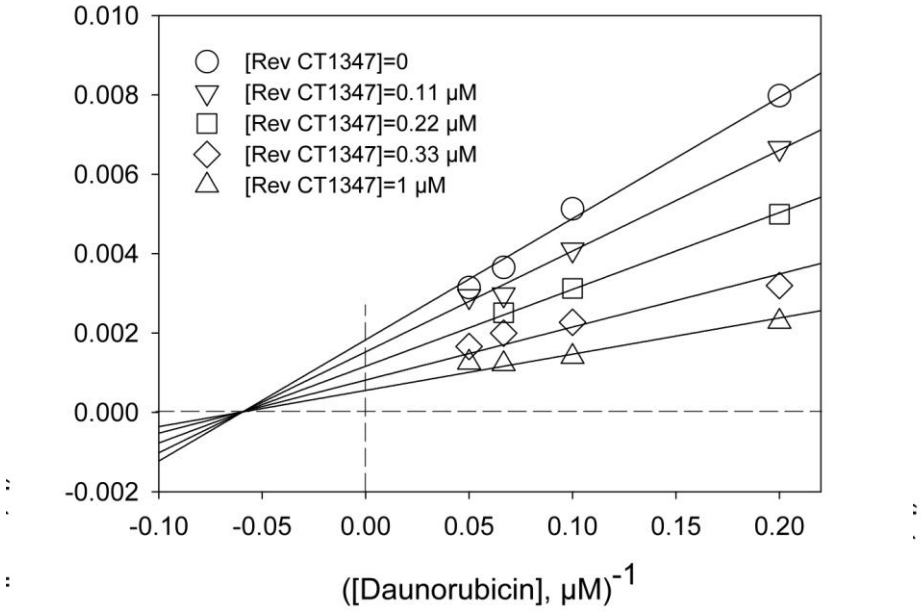


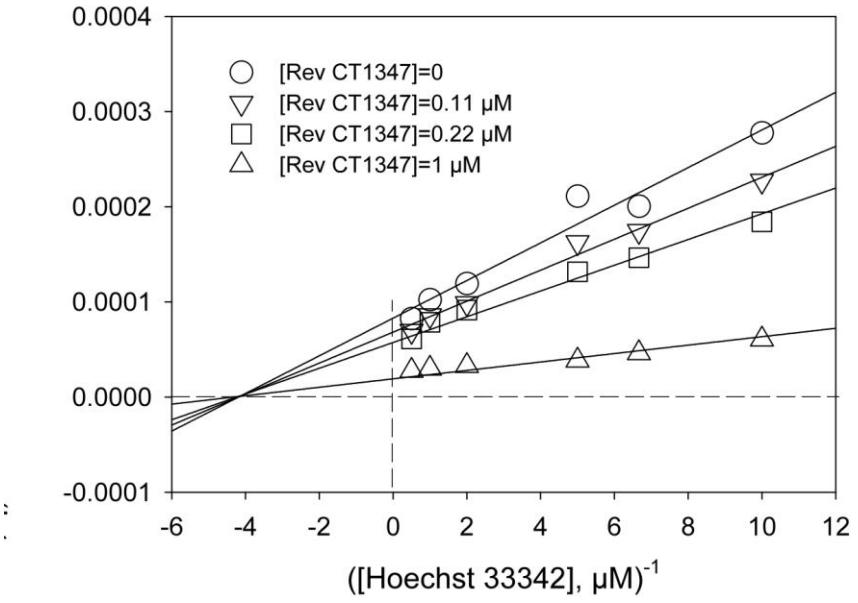
- Acceptable cytoxicity at 5X concentration

CT1347 - Mechanism of inhibition



Drugs accumulation with fixed CT1347 inhibitor concentrations (Lineweaver-Burk plot)

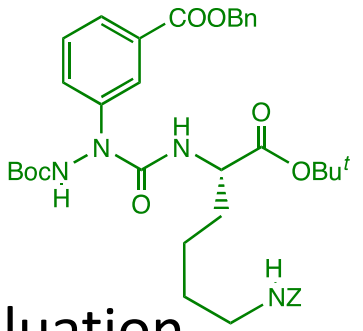




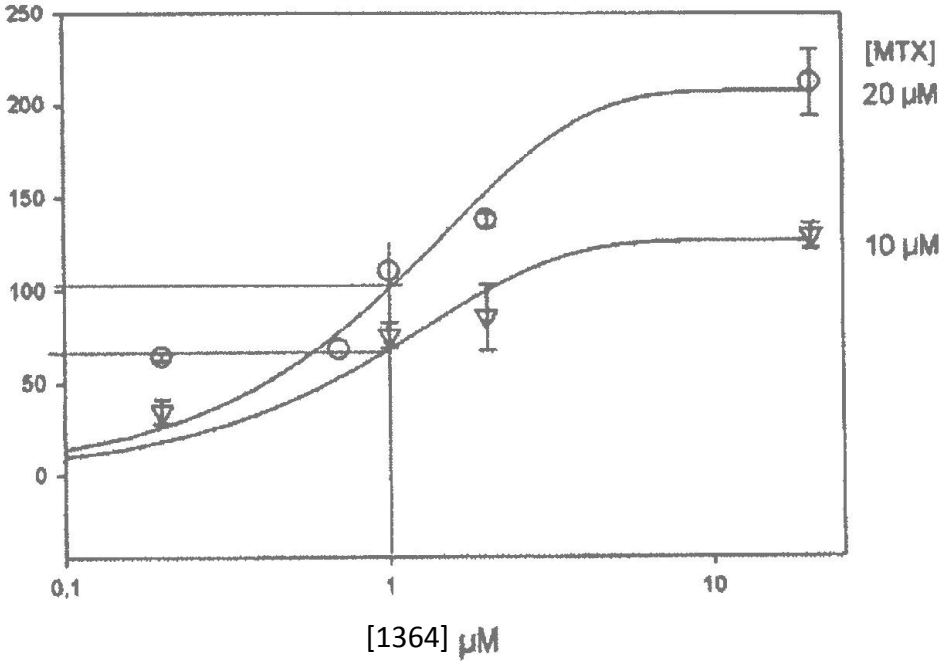
- **Non competitive inhibition** of daunorubicin and Hoechst 33342 on P-glycoprotein

Koubeissi *et al. J. Med. Chem*. **2010**,53, 6720-6729

CT1364 – Biological studies

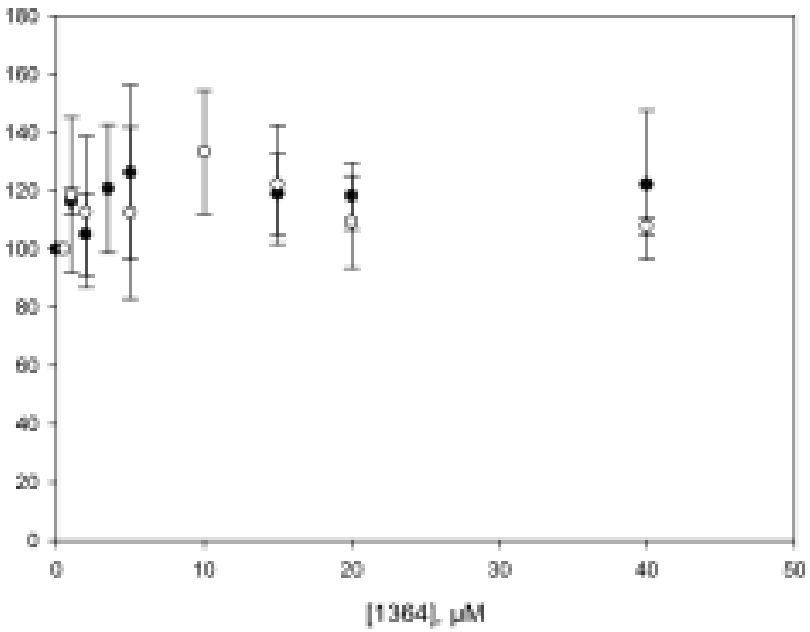


Mitoxantrone inhibition efficacy of BCRP efflux



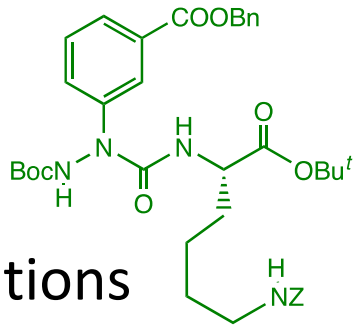
- IC_{50} 1.06 μM
- BCRP selectivity

Cytoxicity evaluation (MTT assay)

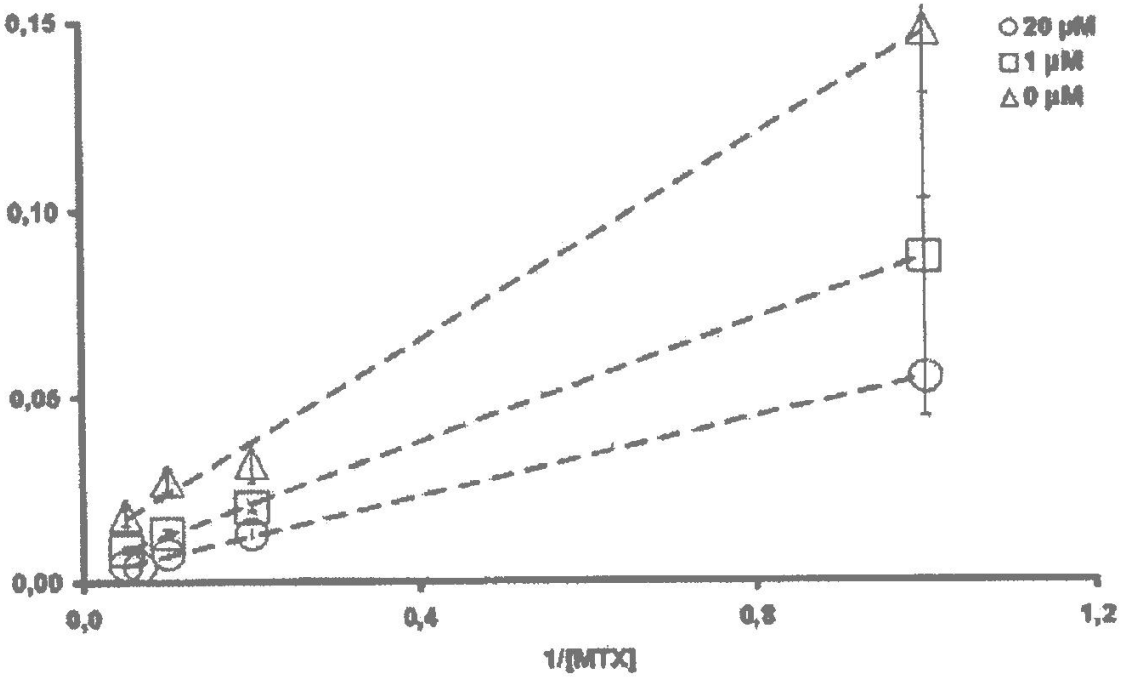


- No cytoxicity at 40X concentration

CT1364 - Mechanism of inhibition

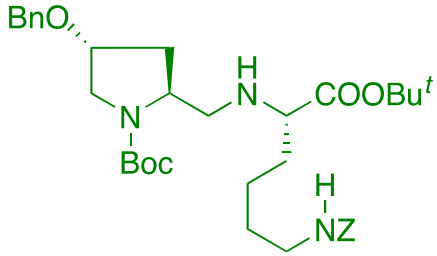


Drugs accumulation with fixed CT1364 inhibitor concentrations (Lineweaver-Burk plot)

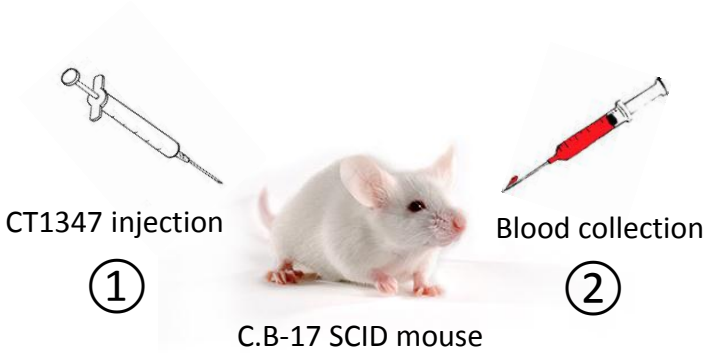


- **Non competitive inhibition** of mitoxantrone on BCRP

CT1347 – *In vivo* studies



Plasma concentration after intraperitoneal injection in mice

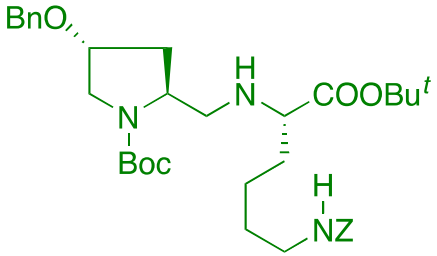


③ Plasma assay after 1 hour

Injected dose	# mouse	ng/ml
10mg/kg	1	20.53
10mg/kg	2	783
10mg/kg	4	171
100mg/kg	5	293
100mg/kg	6	4467
100mg/kg	7	2012

- High concentration variability

CT1347 – *In vivo* studies



CT1347+doxorubicine toxicity assessment protocol

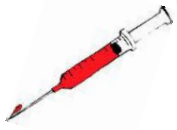
+ doxorubicine
2 mg/kg

SCID mouse

21-day observation

Tolerance → CT1347

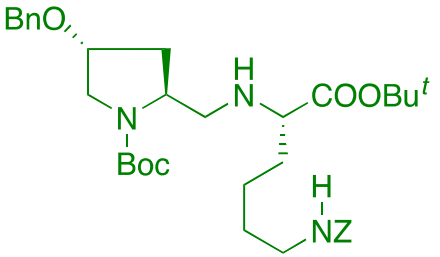
Intolerance

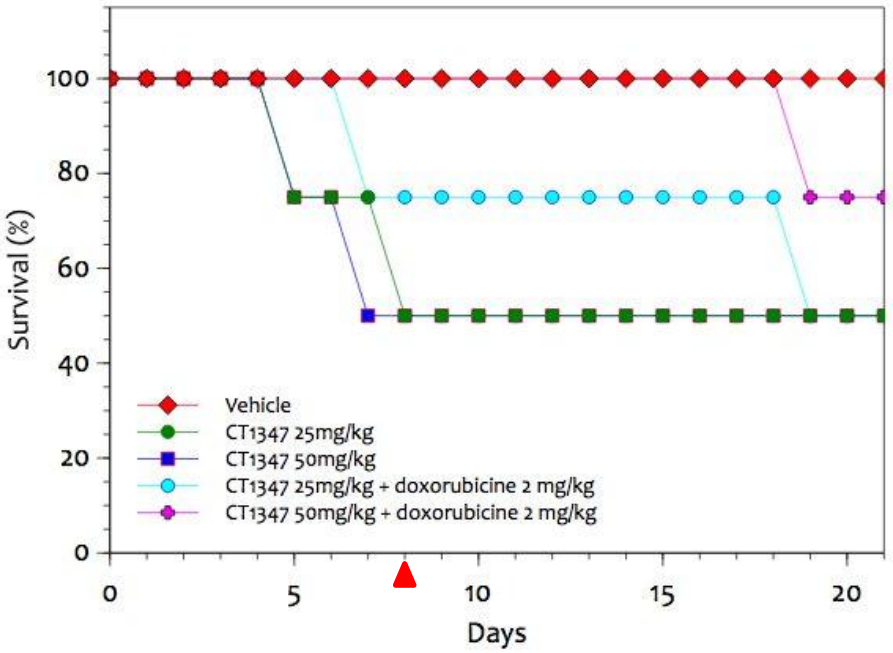


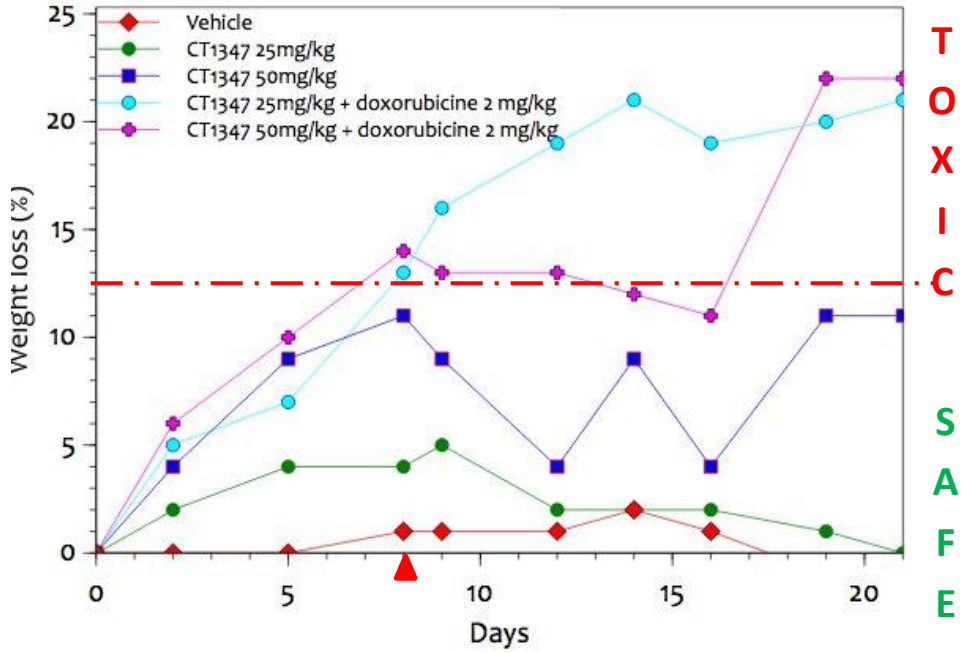
Blood collection
(retro-orbital)

- CT1347 plasma
- doxorubicine plasma
- Liver function
- Blood count

CT1347 – *In vivo* studies







CT1347+doxorubicine combinations were toxic => lower doses have to be tested

▲ - Four-fold decrease of CT1347 dosage for 50mg/kg groups (⇨12.5mg/kg) at day 8

Conclusions

- We have exposed the synthesis works accomplished to reach our selection of molecules and presented our biological data obtained with CT1347 and CT1364 reversins on ABCB1/P-gp and ABCG2.

- *In vivo* studies on CT1347 reversin showed toxicity to mice with a combined treatment of doxorubicine 2 mg/kg and CT 1347 25 mg/kg reversin.

- Further *in vivo* studies with lower CT1347 concentration must be carried out as well as *in vivo* studies with selective ABCG2 inhibitor CT1364 reversin.

Acknowledgments

ISPB Faculté de Pharmacie de Lyon

Université de Lyon
Lyon Science Transfert

Région RhôneAlpes