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*"Drug Resistance Mechanism and Mxodulation"*
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# Modulation of cancer cell multidrug ABC transporters


***10th French-Belgian ABC Meeting***

***UCL, Brussels, Belgium***

*October 19-20, 2012*

# Cancer cell multidrug resistance and ABC transporters

- Cell growth resistance to multiple drugs,

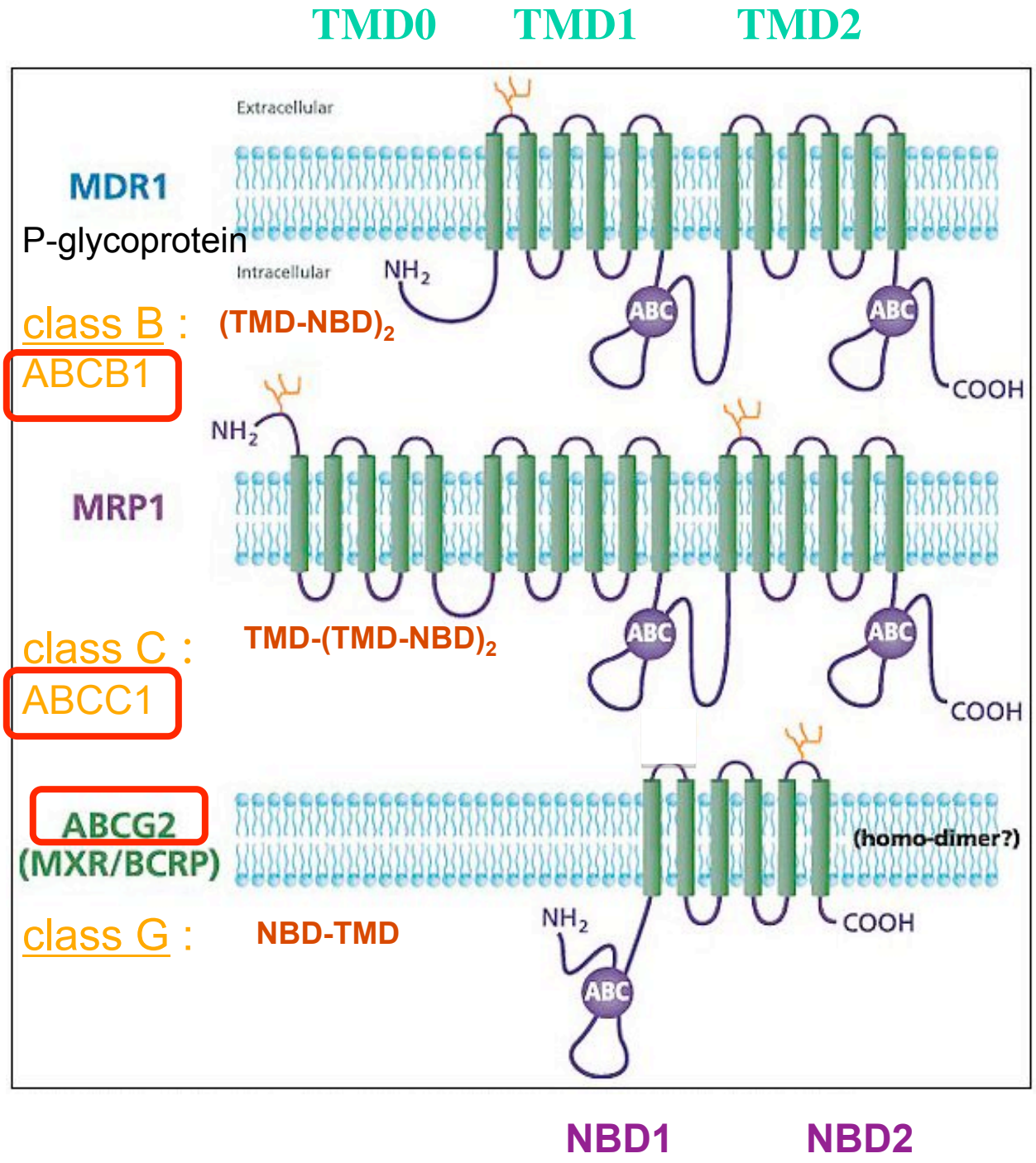
- Low intracellular accumulation of cytotoxic drugs,

- Due to **overexpression of ABC transporters** (P-glycoprotein/ABCB1, MRP1/ABCC1 and/or BCRP/ ABCG2) within plasma membranes.

- Prevented *in vitro* by characteristic inhibitors:
  * verapamil/cyclosporine A / P-glycoprotein
  * MK571/probenecid / MRPs
  * FTC/Ko143 / BCRP

## 3 main multidrug ABC-transporters:

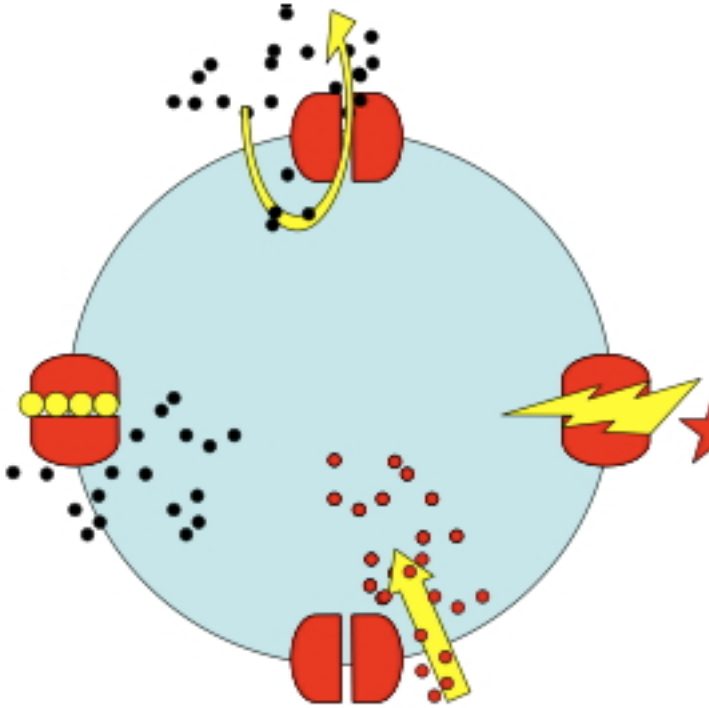
belong to
3 different classes
of the 48 human
ABC proteins



# Different strategies to antagonize MDR cancer cells overexpressing ABCB1, ABCC1 or ABCG2

Inhibit

Using nontransported inhibitors:
ABCB1
ABCG2



Target

Using "**Collateral Sensitivity**" = Achilles'heel
ABCC1

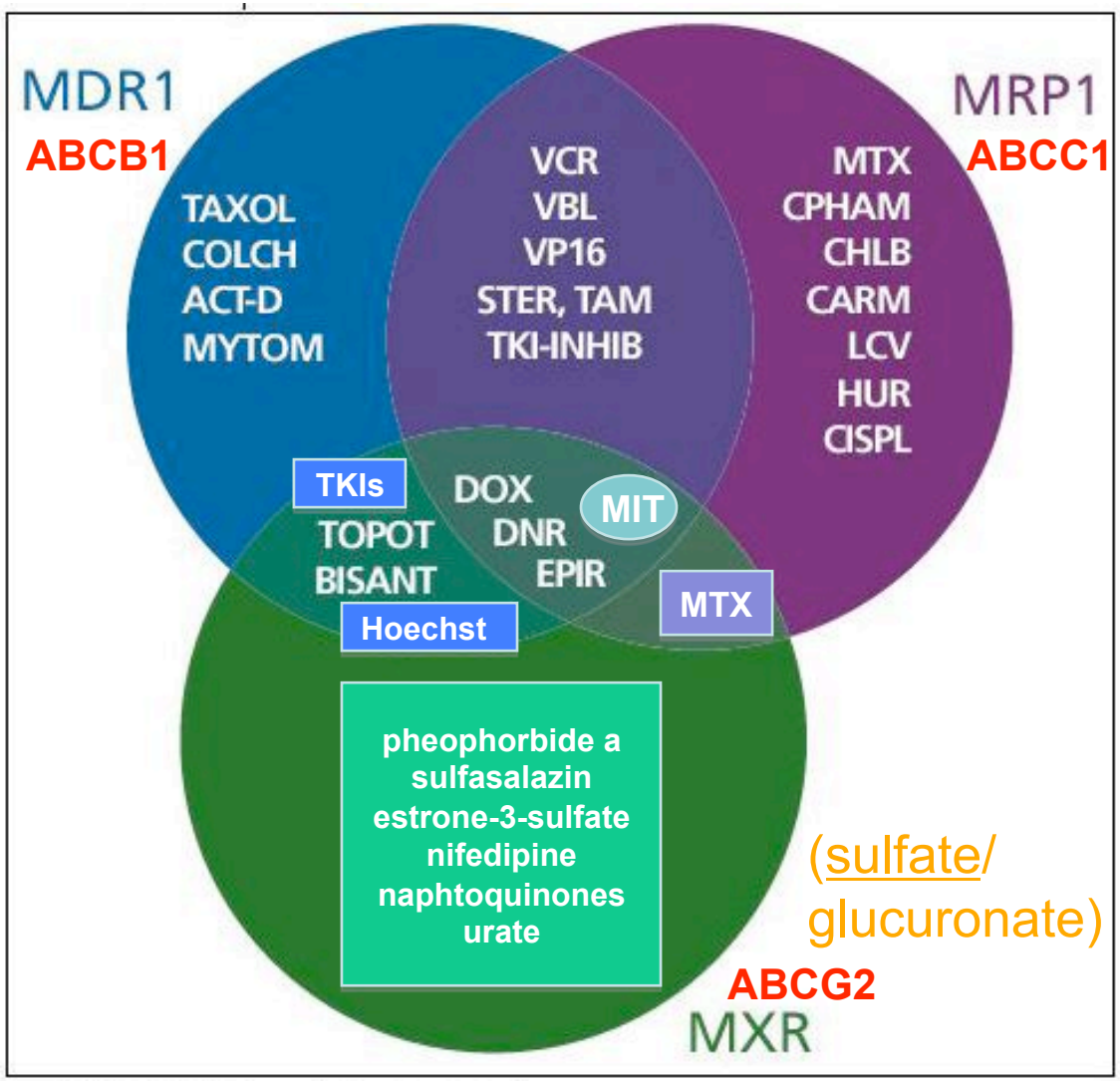
Bypass

Synthesizing nontransported chemotherapeutics
(but generally less active ...)

unconjugated glutathione/glucuronate/sulfate

# Overlapping patterns for transported **substrates**

>> inhibition of a single transporter not sufficient to fully abolish cell multidrug resistance



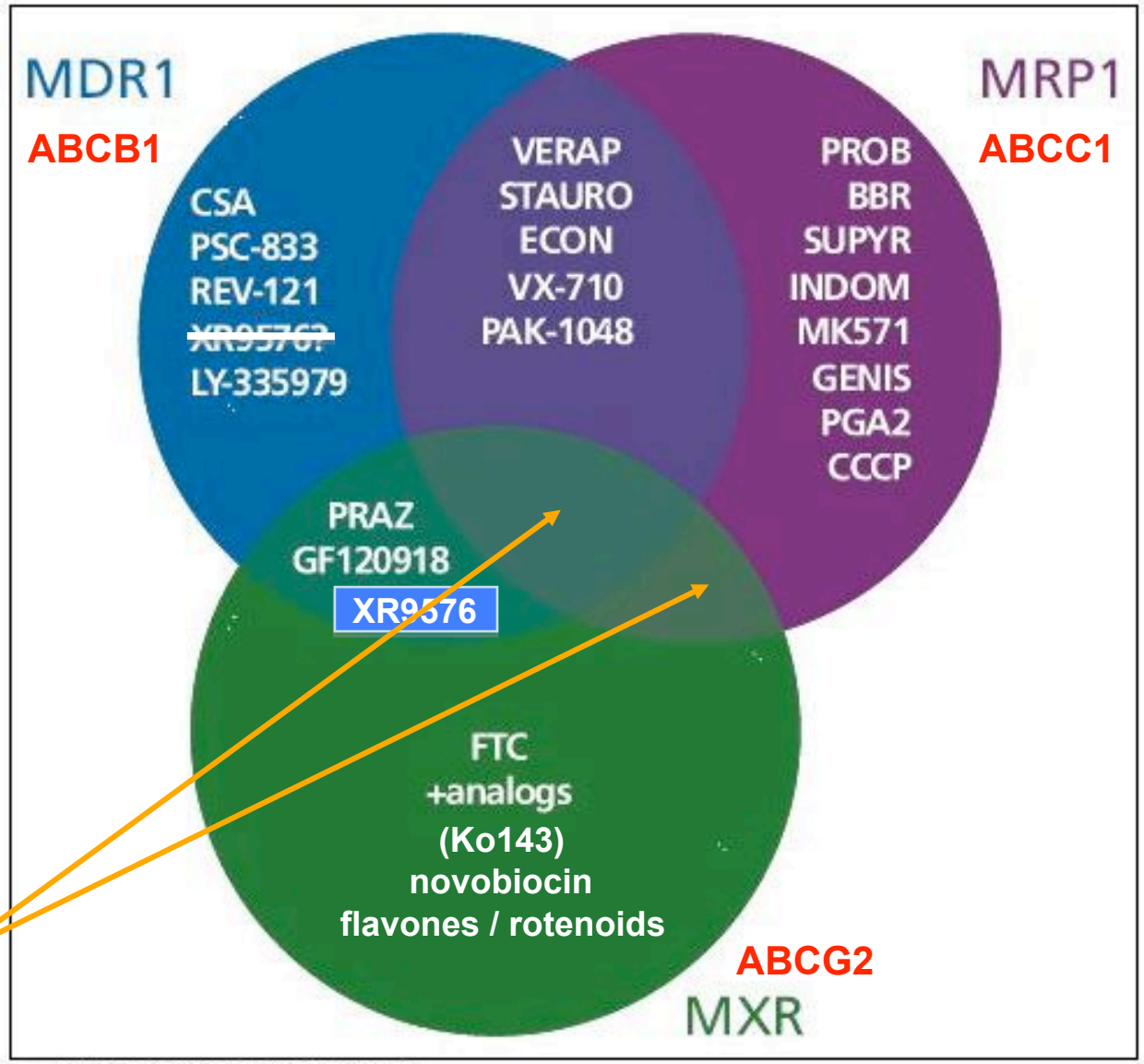

**MDR-substrate anticancer agents.**
*Abbreviations:* VCR: vincristine, VBL: vinblastine, VP-16: etoposide, STER: steroids, TAM: tamoxiphen, TKI-INHIB: tyrosine kinase inhibitors, e.g. STI-571, DOX: doxorubicine or adriamycin™, DNR: daunorubicin, EPIR: epirubicin, MX: mitoxantrone, TOPOT: topotecan, iridotecan, BISANT: bisanthrone, COLCH: colchicin, ACT-D: actinomycin D, MYTOM: mytomycin, TX: methorexate, CPHAM: cyclophosphamide, CHLB: chlorambucil, CARM: carmustine, LCV: leucovorin, HUR: hydroxyruea, CISPL: cisplatin, TAXOL™: paclitaxel

**Very few**

**common**

**inhibitors**

None for the
3 transporters
(even for ABCC1
and ABCG2)

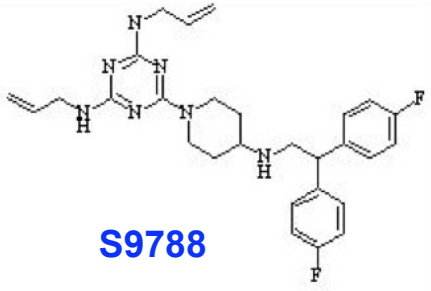



**MDR-modulating agents.**

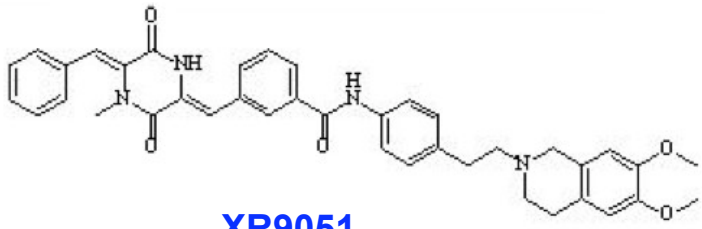
*Abbreviations:* CSA: cyclosporin A, VERAP: verapamil, STAURO: staurosporine, ECON: econazole, PRAZ: prazosine, FTC: fumitremorgin C, PROB: probenecide, BBR: benzbromarone, SUPYR: sulfinpyrazone, INDOM: indomethacine, GENIS: genistein, PGA2: prostaglandine A2, CCCP: chlorocarbonyl cyanide phenylhydrazone.

>> **Specific** inhibitors may be found

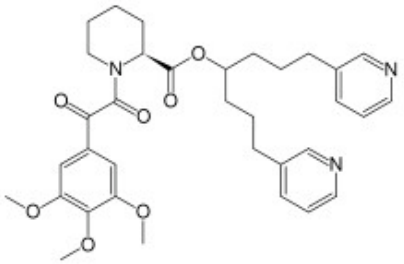
# Third-generation inhibitors against P-glycoprotein



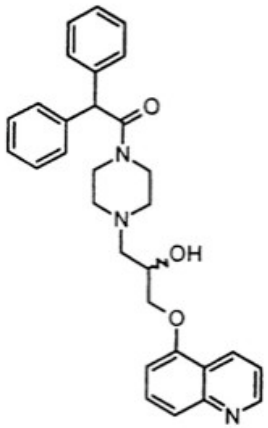
S9788



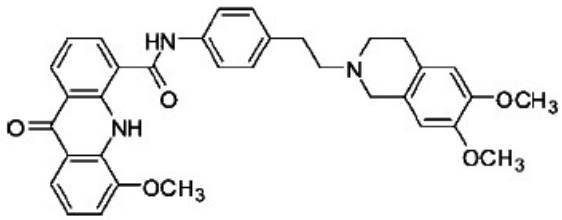
XR9051



VX-710 (Biricodar)



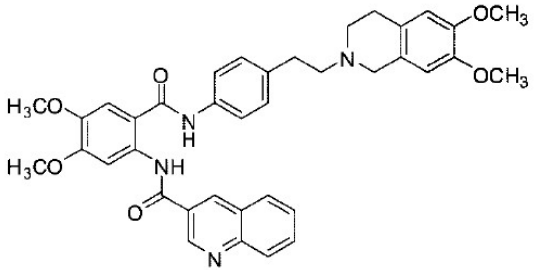
MS-209



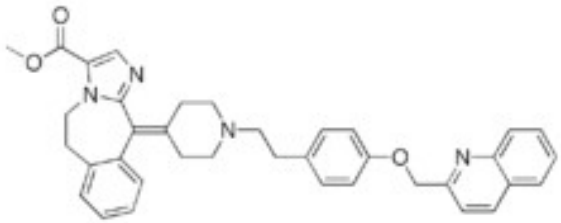
GF120919 (Elacridar)

No

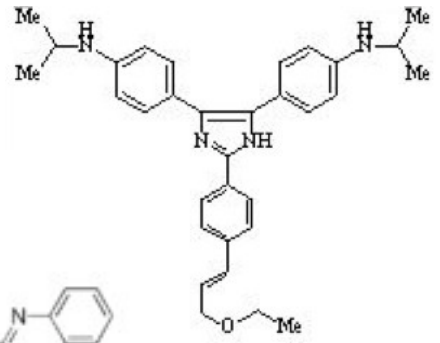
***Inhibitors having reached clinical trials:***



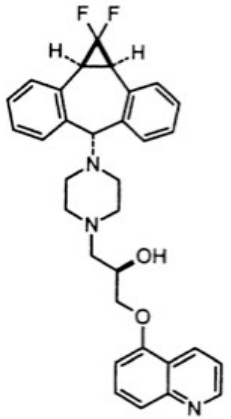
XR-9576 (Tariquidar)



R-101933 (Laniquidar)



OC144-093
ONT-093
(Ontogen)



LY335979
(Zosuquidar)

| Specific for P-glycoprotein | No | ? | ? | YES |
|---|---|---|---|---|

# The half-transporter BCRP / ABCG2 (ABCP / MXR) *[discovered in 1998]*

- Located within plasma membranes,
- Naturally overexpressed in placenta, liver, small intestine and colon, supporting a role in **protection / secretion.**,

- Physiological transport substrates :
  * pheophorbide a (= chlorophyll catabolite) and porphyrins,
  * **urate** in kidney proximal tubule cells (Q141K >> gout),

- Identified as a **marker of stem cells** ("side-population").

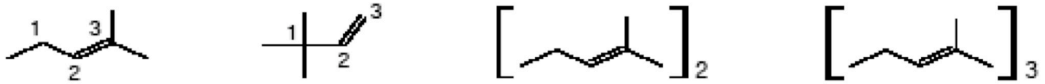
- Overexpressed in many types of tumors,
- Transports mitoxantrone, **methotrexate** and topotecan (and anthracyclines and rhodamine 123 upon R482 hot-spot mutation).

- Since discovered more recently than ABCB1
  >> less inhibitors known.

# Inhibition of ABCG2-mediated mitoxantrone efflux by Flavonoids

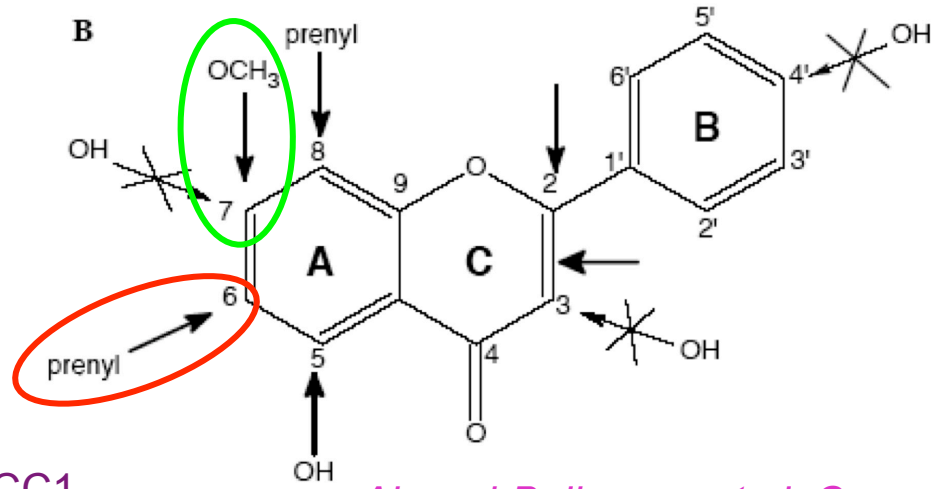
- Mitoxantrone efflux measured by flow cytometry with ABCG2-transfected HEK-293 cells

| Inhibitor substitution | IC 50 (μM) BCRP-R482 | BCRP-T482 |
|---|---|---|
| Flavone | 2.8 ± 0.6 | 1.7 ± 0.4 |
| 3-*OH*-flavone | 8.1 ± 1.9 | 4.9 ± 0.1 |
| 7-*OH*-flavone | 7.1 ± 0.3 | 13.9 ± 1.51 |
| Chrysin (5, 7-diOH-flavone) | 4.6 ± 0.5 | 4.5 ± 0.8 |
| Tectochrysin (5-OH, 7-$OCH_3$-flavone) | 3.0 ± 0.9 | 1.9 ± 0.3 |
| 6-Prenylchrysin | 0.29 ± 0.06 | 3.6 ± 1.9 |
| 6-(1.1-Dimethylallyl)chrysin | 0.78 ± 0.15 | >10 |
| 8-Prenylchrysin | 0.89 ± 0.31 | >10 |
| 8-(1.1-Dimethylallyl)chrysin | 1.4 ± 0.5 | >10 |
| 6-Geranylchrysin | 1.0 ± 0.4 | ND |
| 6-Farnesylchrysin | >10 | ND |
| 6.8-Digeranylchrysin | 2.1 ± 0.5 | ND |
| GF120918 | 0.31 ± 0.14 | 6.9 ± 2.6 |




- High-affinity inhibition by **6-prenylchrysin** and **tectochrysin**
>> natural compounds as potent inhibitors

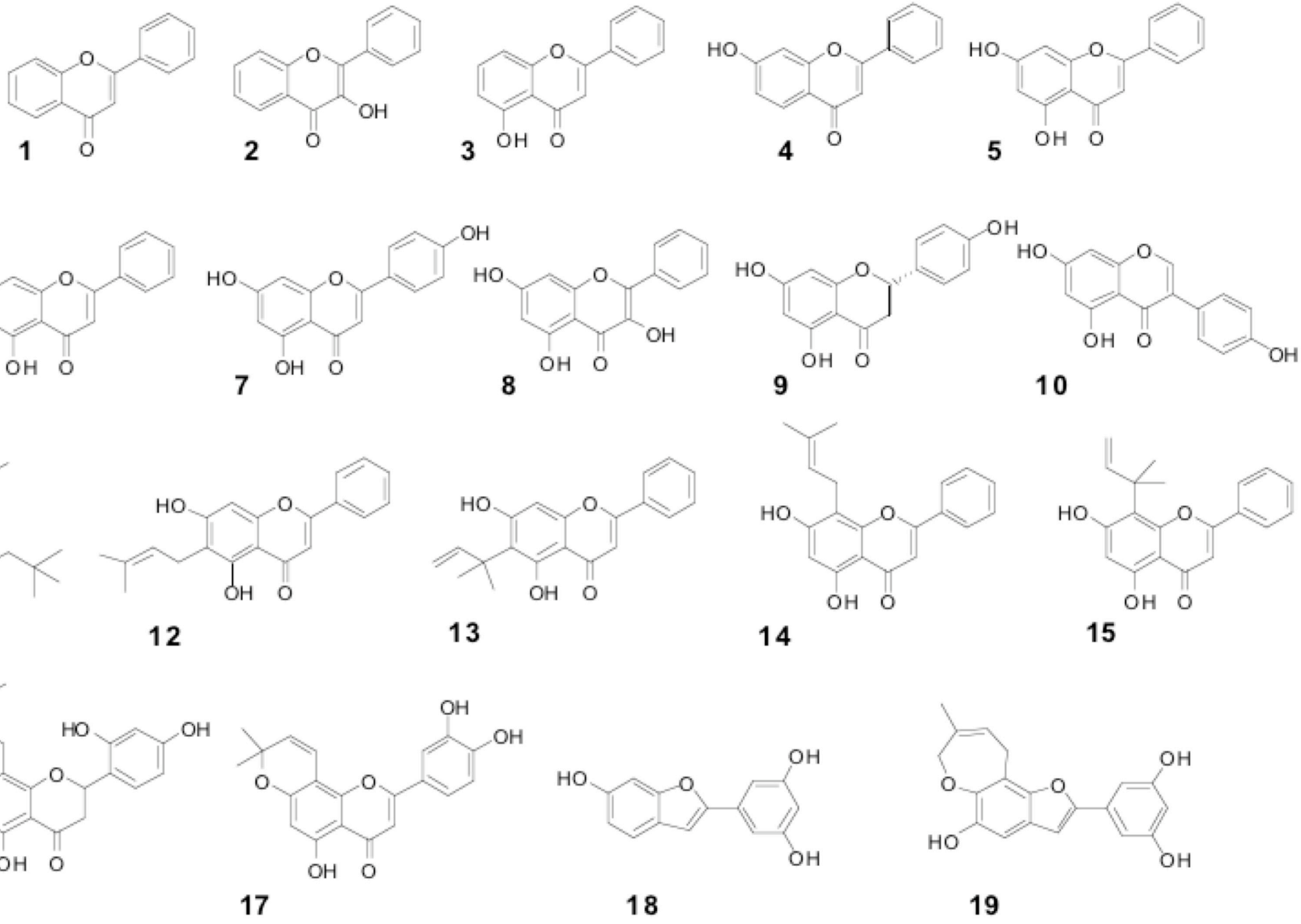
**SARs for flavonoid inhibition of wild-type ABCG2**



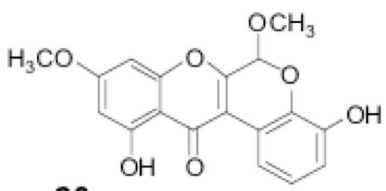

Hydrophobic flavones are **specific** for ABCG2, *versus* ABCB1 and ABCC1

*Ahmed-Belkacem et al. Cancer Res. (2005)*

# Flavone, benzopyrane and benzofurane derivatives



## Boeravinone derivatives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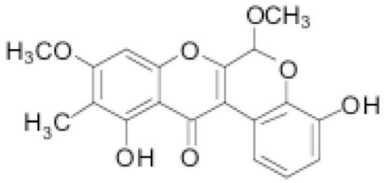
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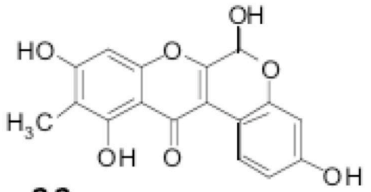
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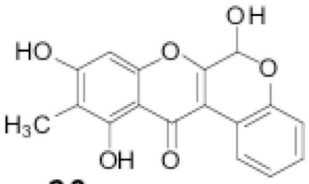
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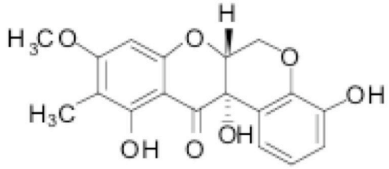
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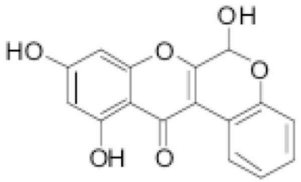
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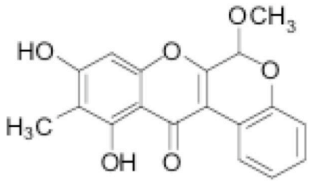
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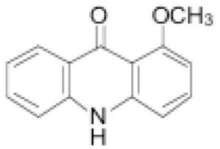
## Acridone derivatives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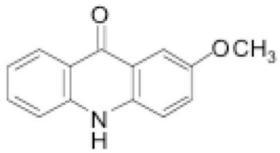
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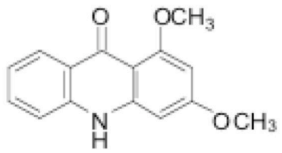
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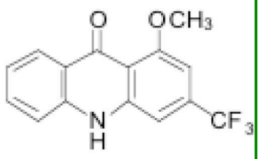
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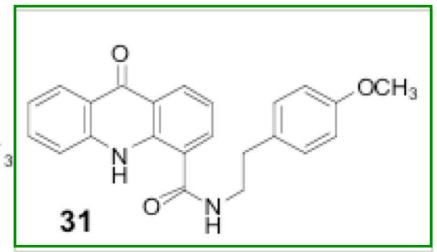
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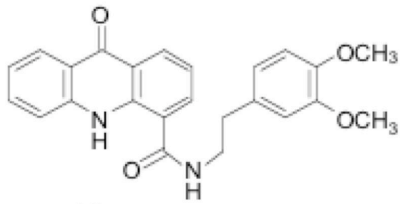
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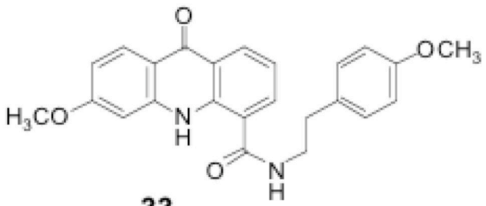
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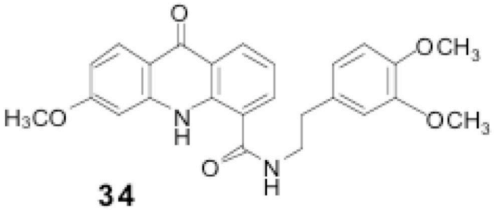
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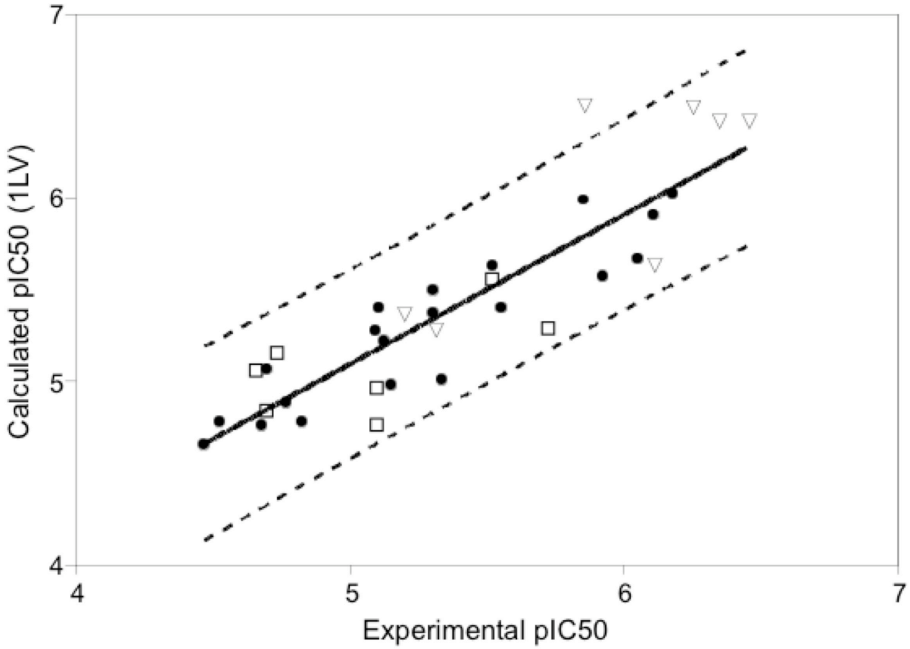



*Nicolle et al. Eur. J. Pharm. Sci. (2009)*

# Pharmacophore molecular modeling

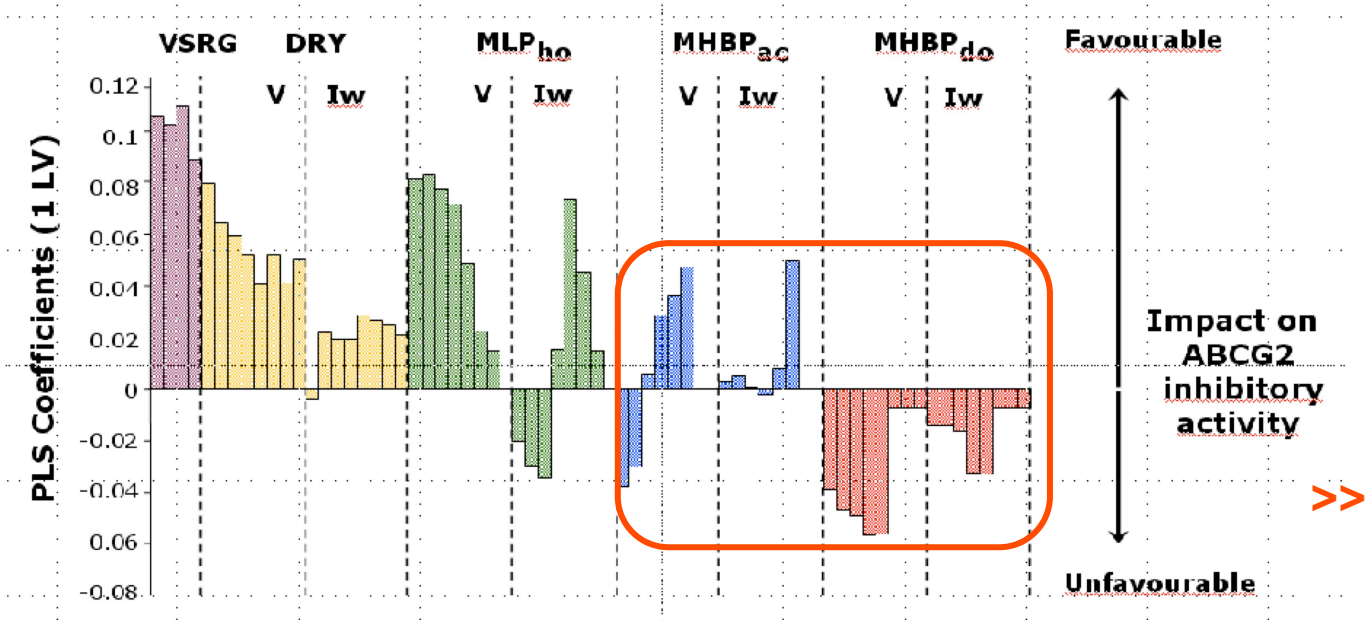
>> good correlation for nearly all compounds

## Descriptors:

- Positive roles of the size, polarisability and hydrophobicity

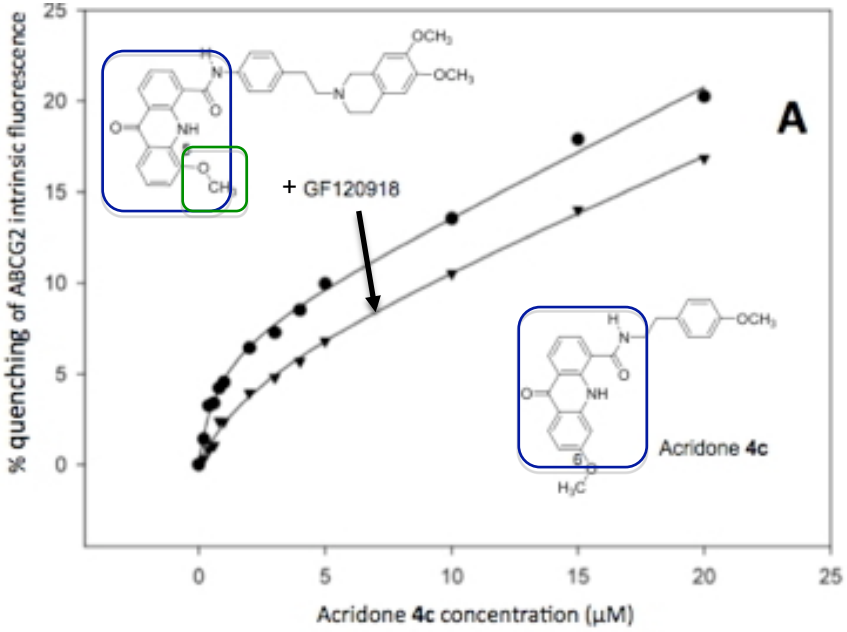
- No clear effect of H-bond acceptor
- Negative effect of H-bond donor

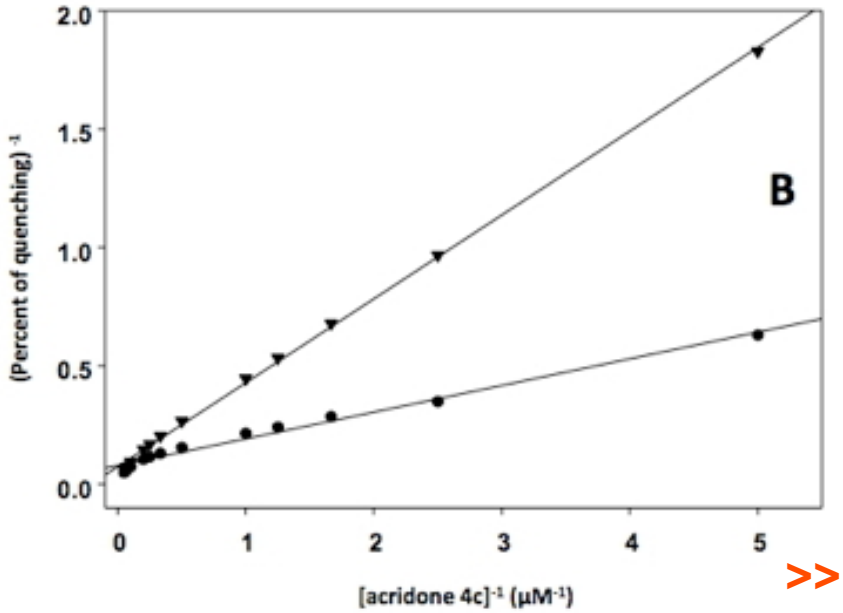
**>> different from ABCB1**




**Specific** inhibitors >> likely bind <u>outside</u> the catalytic transport site

# The ABCG2-specific inhibitory site overlaps the dual site

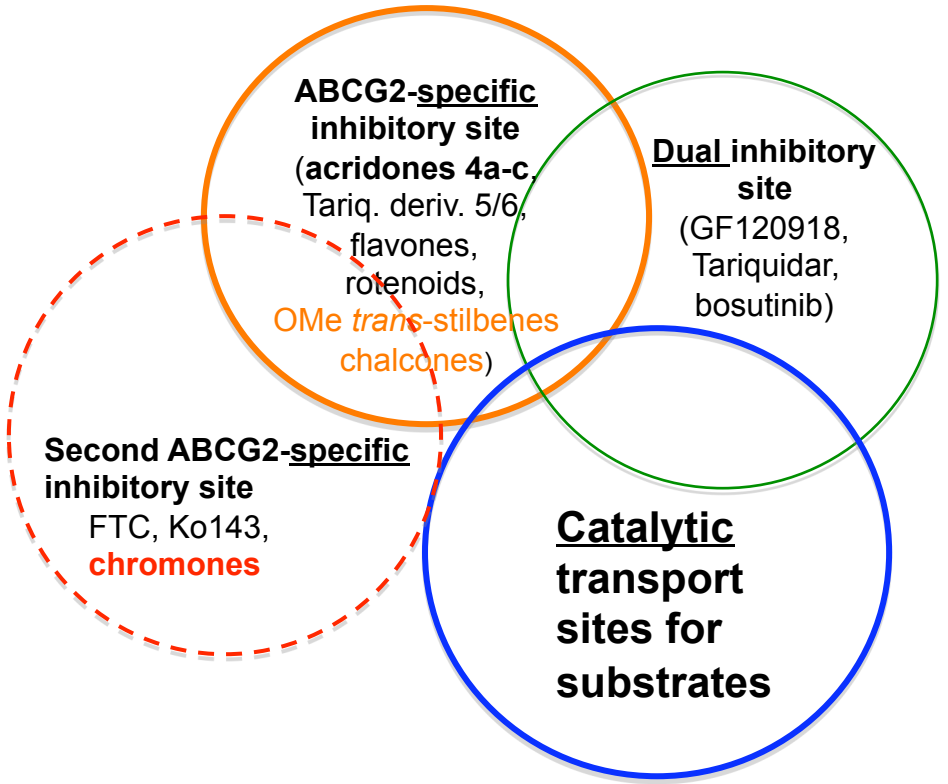



- Acridone 4c binding is prevented by GF120918.
>> both sites are widely overlapping.

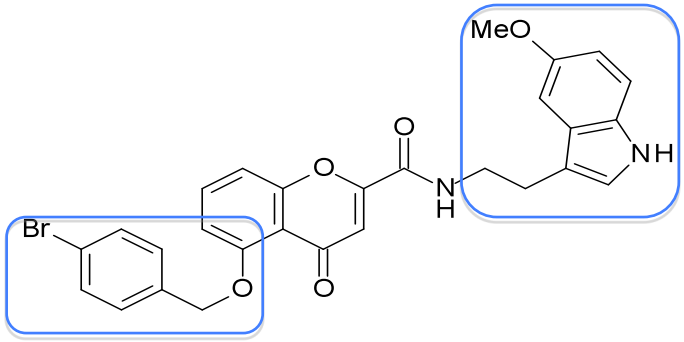
- The specific site of flavonoids is distinct from another specific site related to ATPase inhibition.

- Chromones bind to this FTC/Ko143 site.

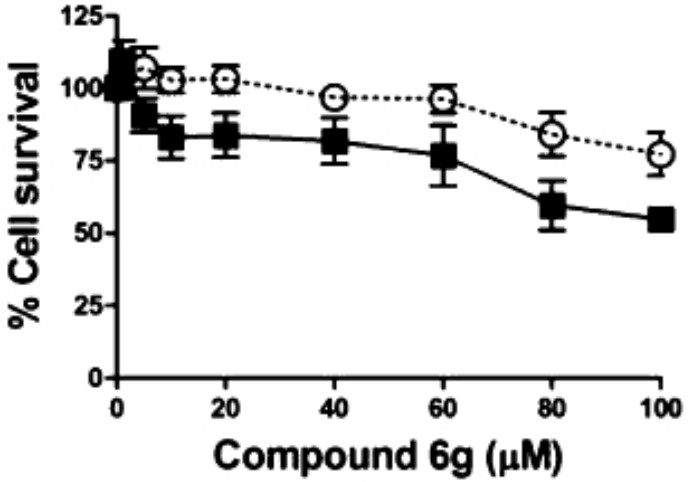



>> Polyspecificity for inhibitors and substrates

# Chromone derivatives: the best candidates for *in-vivo* assays




*Valdameri et al. J. Med. Chem. (2012)*




**Chromone 6g**

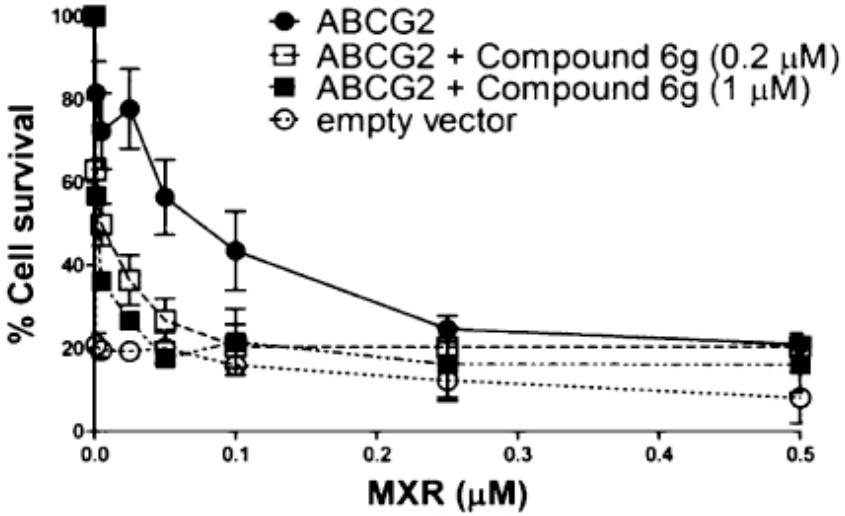
high affinity ($IC_{50}$ = 0.11 μM)

complete, non-competitive, inhibition

and low cytotoxicity ($IG_{50}$ > 100 μM)

**> very high Therapeutic Ratio (TR) > 1,000**

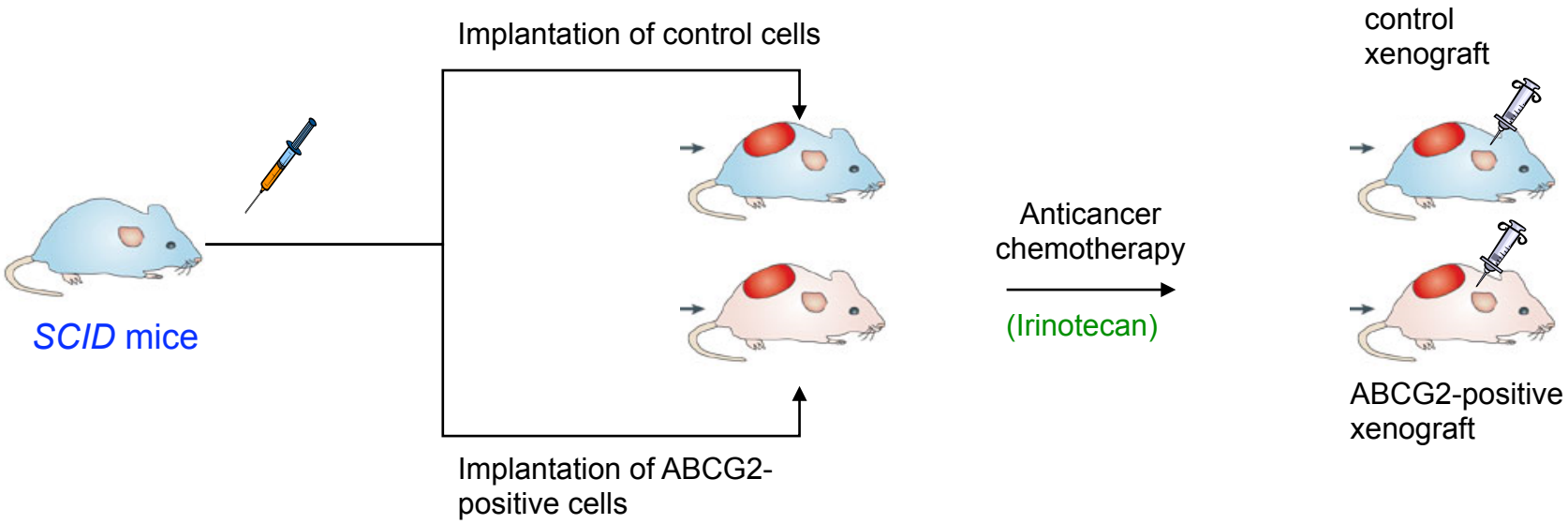
Not transported (only inhibits ATPase activity)

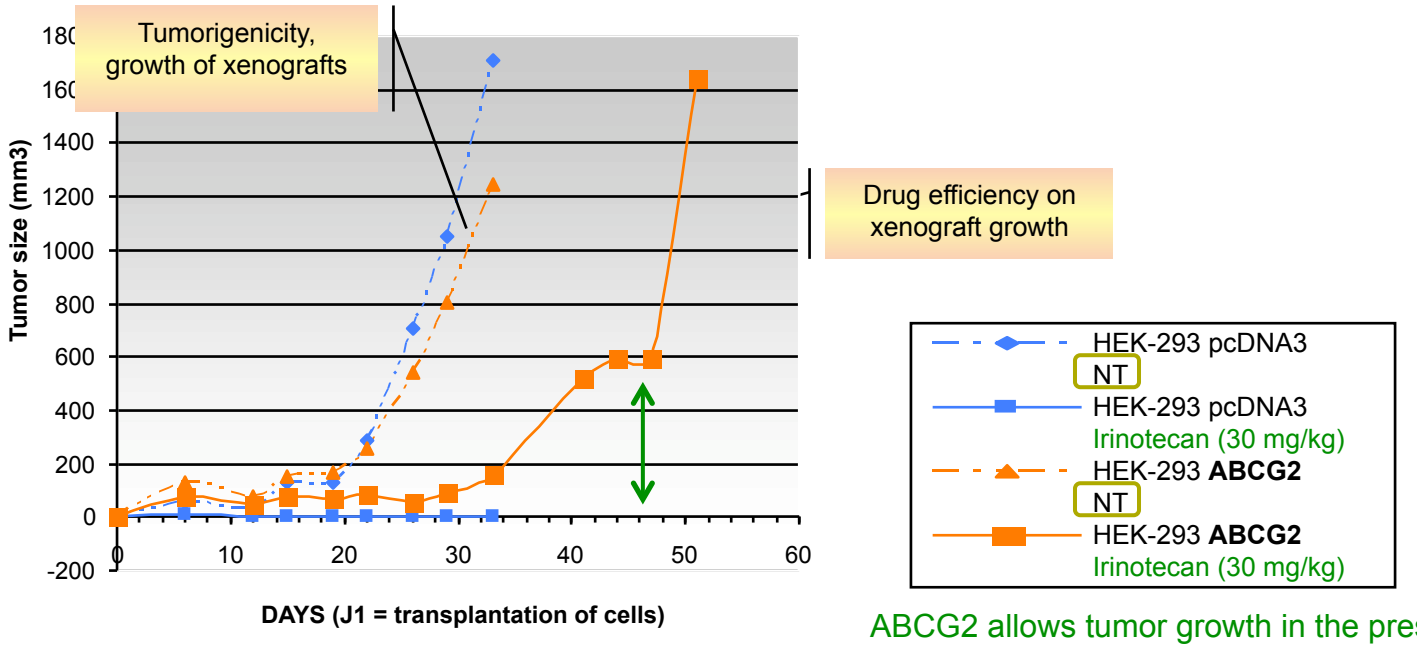



Chemosensitizes resistant cancer cell growth to mitoxantrone or SN-38 (irinotecan metabolite)

**>> suitable for *in-vivo* assays**

# Mouse model with ABCG2-expressing human xenografts

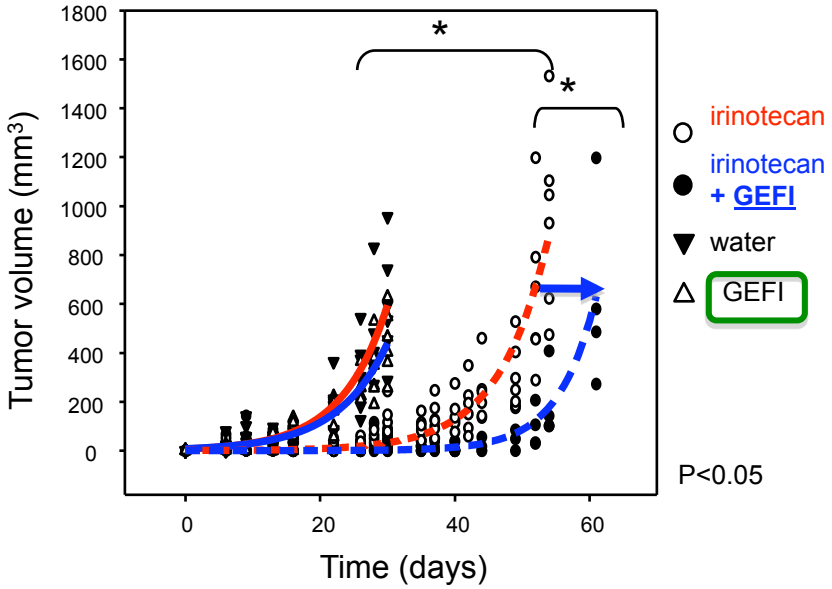


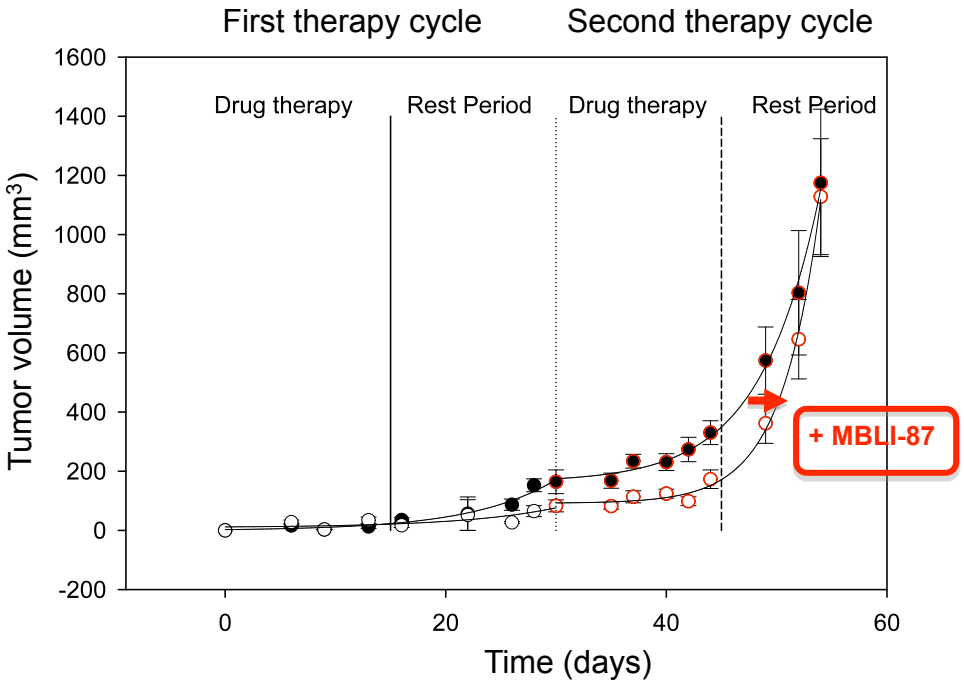




ABCG2 allows tumor growth in the presence of irinotecan, by conferring **chemoresistance**

# *In vivo* chemosensitization of tumor growth to irinotecan by either Gefitinib or acridone 4c (MBLI-87)

*Arnaud et al. Eur. J. Cancer. (2011)*




- no effect of Gefitinib or vector alone on tumor growth
- ABCG2-dependent tumor growth in presence of irinotecan is delayed by Gefitinib

>> MBLI-87 also delayed tumor cell proliferation, **at lower concentration** than Gefitinib

But MBLI-87: low solubility and relatively high cytotoxicity

>> **improve formulation**

>>> use **more potent and less toxic compounds (chromone 6g)**.

Review

Cell PRESS

# Is resistance useless? Multidrug resistance and collateral sensitivity

Matthew D. Hall, Misty D. Handley and Michael M. Gottesman

Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892, USA

Review

2009

*Trends in Pharmacological Sciences* Vol.30 No.10

# Targeting the Achilles' heel of Multidrug Resistant Cancer

Gergely Szakacs[1], Matthew D. Hall[2], Michael M. Gottesman[2], Ahcène Boumendjel[3], Remy Kachadourian[4], Brian J. Day[4], Hélène Baubichon-Cortay[5] and Attilio Di Pietro[5, *]

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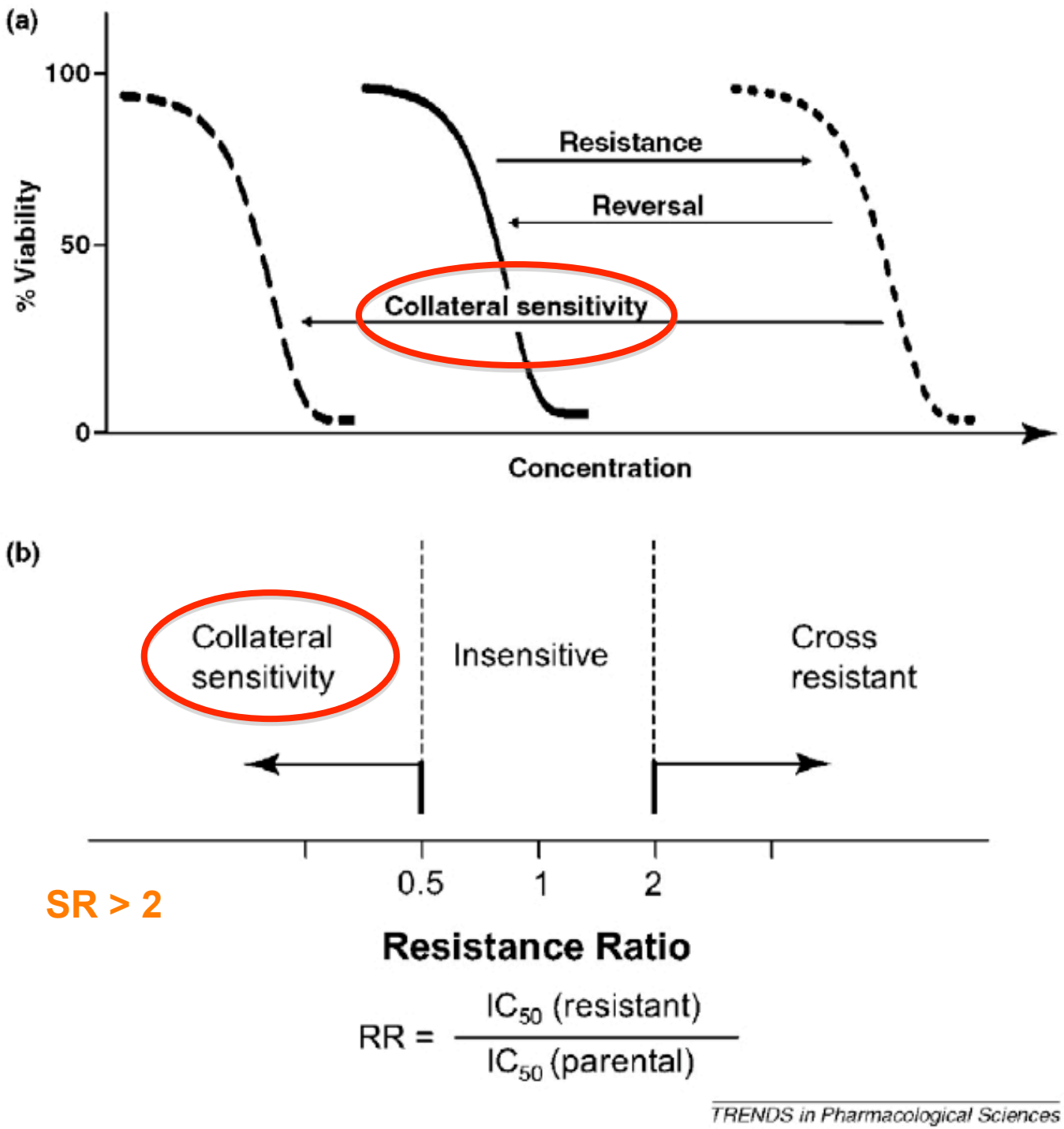
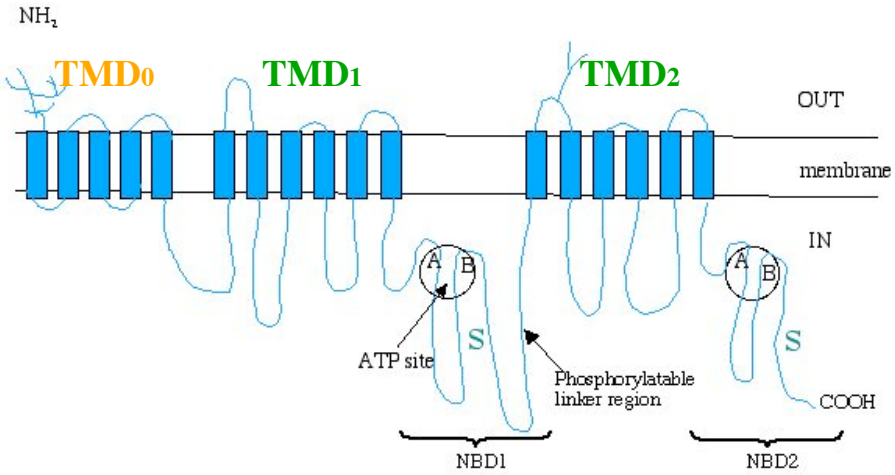
*Review to appear in **Chemical Reviews** (2012/2013)*




TRENDS in Pharmacological Sciences

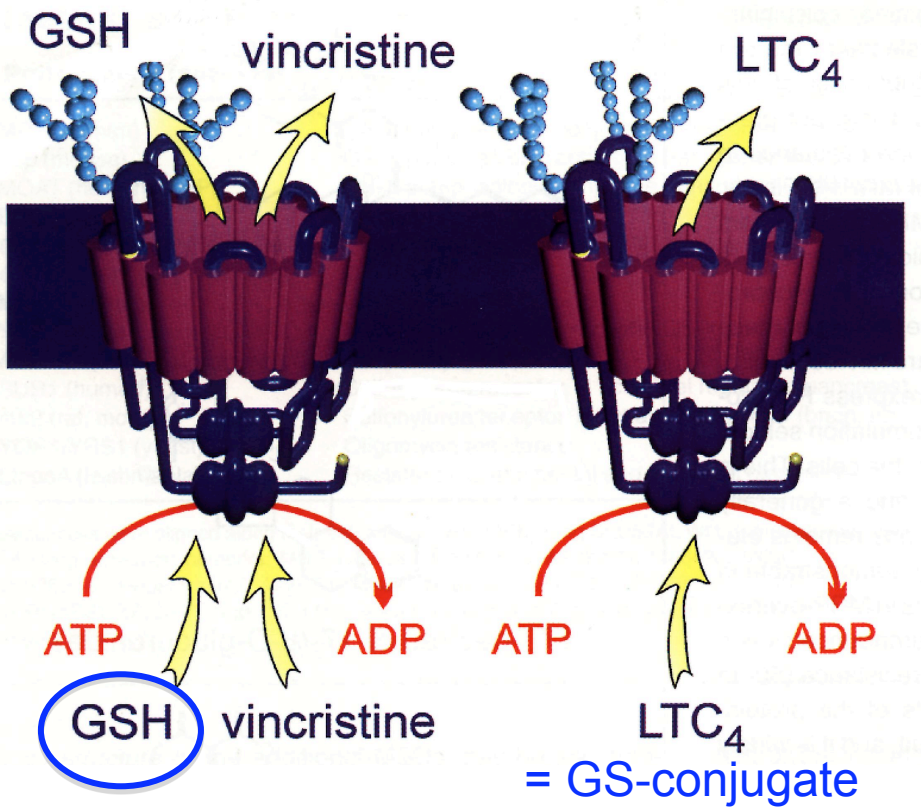
# Schematic structure of MRP1/ABCC1 *[discov. 1992]*



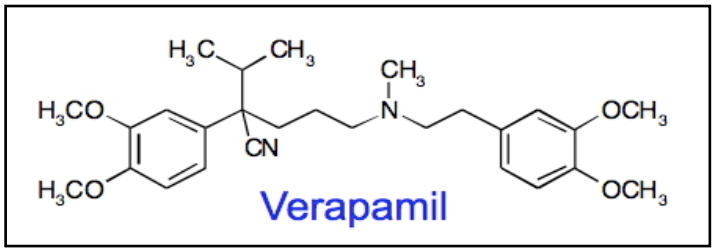

- Additional TMD0

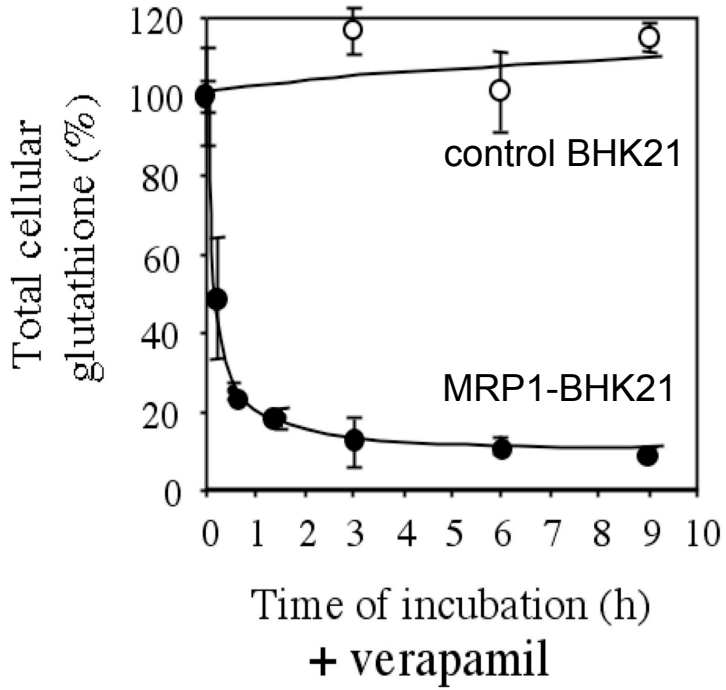
- Physiological role in inflammation: efflux of leukotriene LTC4 from leukocytes

- Also transports a number of drugs, such as vincristine



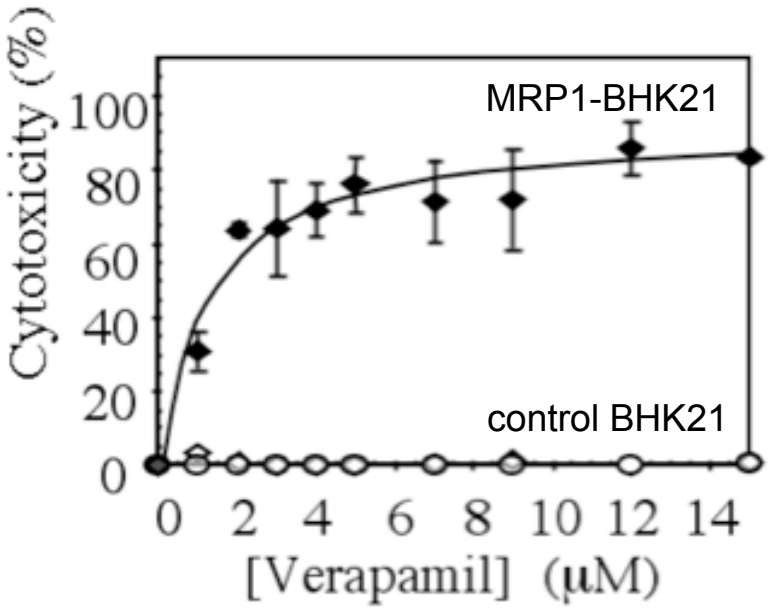
# MRP1 is modulated by hydrophobic compounds such as Verapamil




Fast and massive MRP1-mediated **GSH efflux** induced by Verapamil

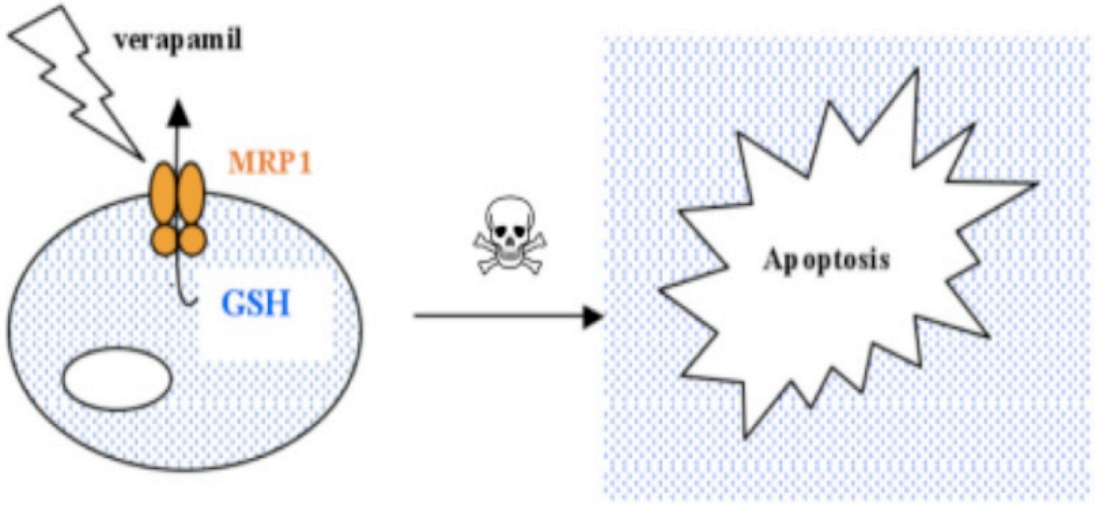
*Trompier et al. Cancer Res. (2004)*




**Selective cytotoxicity** identified as **apoptosis** (PS, caspases)

*Lauriane DURY, Short Talk, Saturday 10:40*

# Summary of verapamil-induced collateral sensitivity




1) Verapamil binds to MRP1 (competitively to the drug site ? Is it transported ?)
1) It promotes a massive and fast GSH efflux through MRP1
2) Only the *S*-verapamil enantiomer is active [*Perrotton et al. J. Biol. Chem. (2007)*]
3) Role of ROS ? amplified effects upon GSH efflux ?
4) This induces a selective apoptosis of MDR cells expressing MRP1 (transfected BHK-21, or SCLC drug-selected H69AR) **>> *in vivo* experiments** on xenografts

**>> New potential therapeutic strategy:**

- **targeting** cancer cells >> limited side effects,
- new alternative, especially **after chemotherapy failure**.

Since Verapamil is known to be *cardiotoxic*

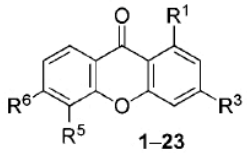
**>> HTS of chemical libraries > new classes** of apoptogenic compounds,

**>> Xanthones and Flavones**.

# Structure-activity relationships of xanthones to promote GSH efflux and cytotoxicity

*Lorendeau et al. ChemMedChem. (2011)*

**Table 1.** Structures of the xanthones studied and net GSH efflux induced in BHK-21-MRP1 cells.




1–23

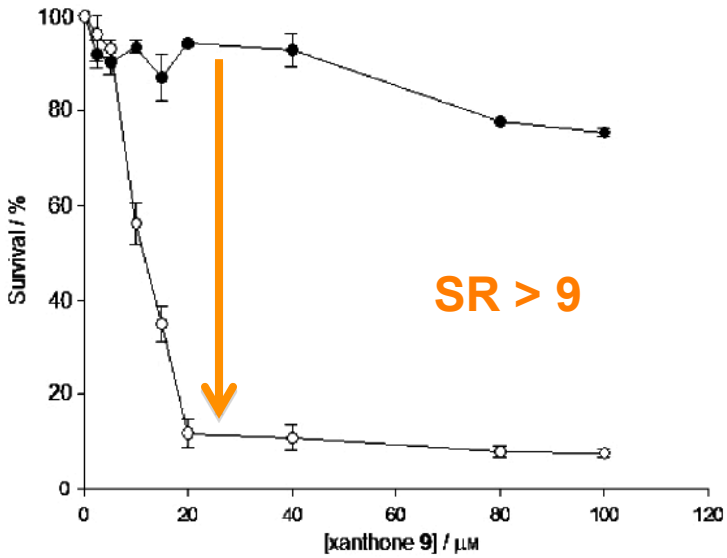
| Compd | $R^1$ | $R^3$ | $R^5$ | $R^6$ | Clog *P* | Efflux [%][a] |
|---|---|---|---|---|---|---|
| 1 | OH | H | H | H | 3.60 | 0 |
| 2 | OH | H | OMe | H | 3.59 | 15 |
| 3 | OH | H | H | Me | 3.10 | 2 |
| 4 | OH | H | Me | H | 4.10 | 2 |
| 5 | OH | Me | H | H | 4.10 | 27 |
| 6 | OH | Me | OMe | H | 4.09 | 41 |
| 7 | OH | OH | OMe | H | 3.01 | 46 |
| 8 | OH | OH | H | H | 3.06 | 52 |
| 9 | OH | OH | H | OMe | 3.01 | 82 |
| 10 | OH | OH | H | Me | 3.55 | 65 |
| 11 | OH | Me | H | Me | 4.60 | 14 |
| 12 | OH | OMe | H | H | 3.65 | 28 |
| 13 | OH | OH | OH | OH | 1.84 | 1 |
| 14 | OH | OH | OH | H | 2.43 | 29 |
| 15 | OH | OH | H | OH | 2.43 | 43 |
| 16 | OH | *O*-prenyl | H | OMe | 5.29 | 12 |
| 17 | *O*-prenyl | *O*-prenyl | H | OMe | 6.46 | 36 |
| 18 | OH | OMe | H | OMe | 3.59 | 66 |
| 19 | OMe | OMe | H | OMe | 3.06 | 13 |
| 20 | OH | *O*-Bz | H | OMe | 3.36 | 6 |
| 21 | OMe | *O*-Bz | H | OMe | 4.83 | 45 |
| 22 | OH | OH | H | $NH\text{-}COCF_3$ | 3.22 | 75 |
| 23 | OH | OH | H | $NH_2$ | 1.86 | 70 |
| (±)-verapamil | | | | | – | 75 |

[a] GSH efflux determined at 20 μM.

**Table 2.** Cytotoxicity of selected xanthones on NCI-H69 (sensitive) and H69AR/MRP1 (resistant) cells.

| Compd | $IC_{50}$ [μM][a] H69AR/MRP1 | NCI-H69 |
|---|---|---|
| 1 | ≫100 | ≫100 |
| 6 | ≫100 | ≫100 |
| 7 | 24±2.32 | ≫100 |
| 8 | 33±0.02 | ≫100 |
| 9 | 11±0.44 | >100 |
| 10 | 26±0.67 | ≫100 |
| 15 | 51±0.39 | >100 |
| 18 | >100 | ≫100 |
| 21 | >100 | >100 |
| 22 | 54±0.07 | >100 |
| 23 | >100 | ≫100 |
| (±)-verapamil | 15±0.35 | ≫100 |

[a] Values represent the mean ±SD of $n=3$ experiments.



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("Sandwich PhD")

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