Importance of Efflux Pumps in Modern Drug Design and Discovery

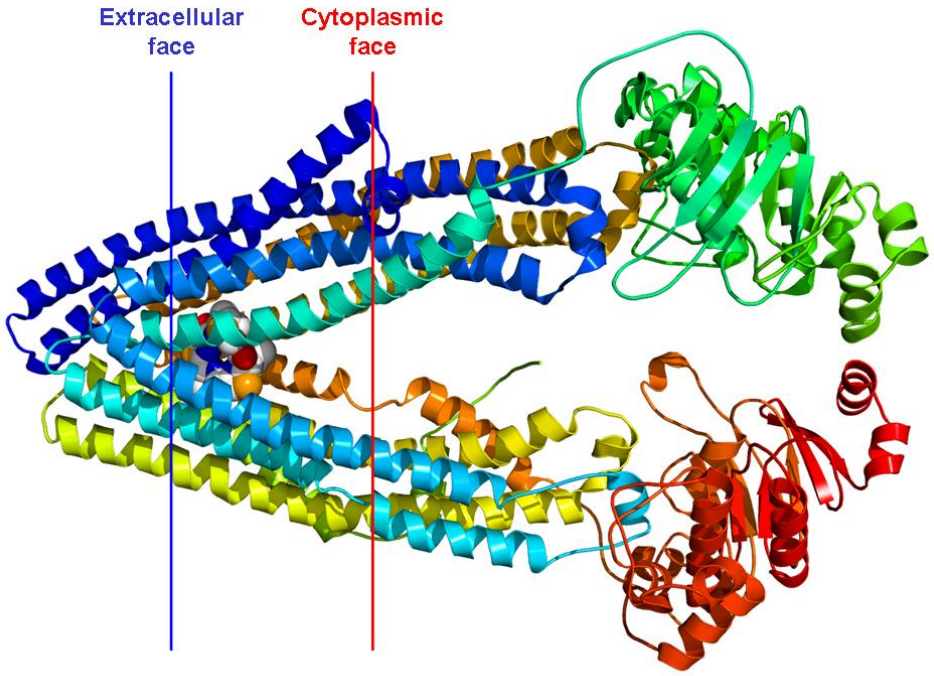
Peter ZUBAC

Efflux pumps

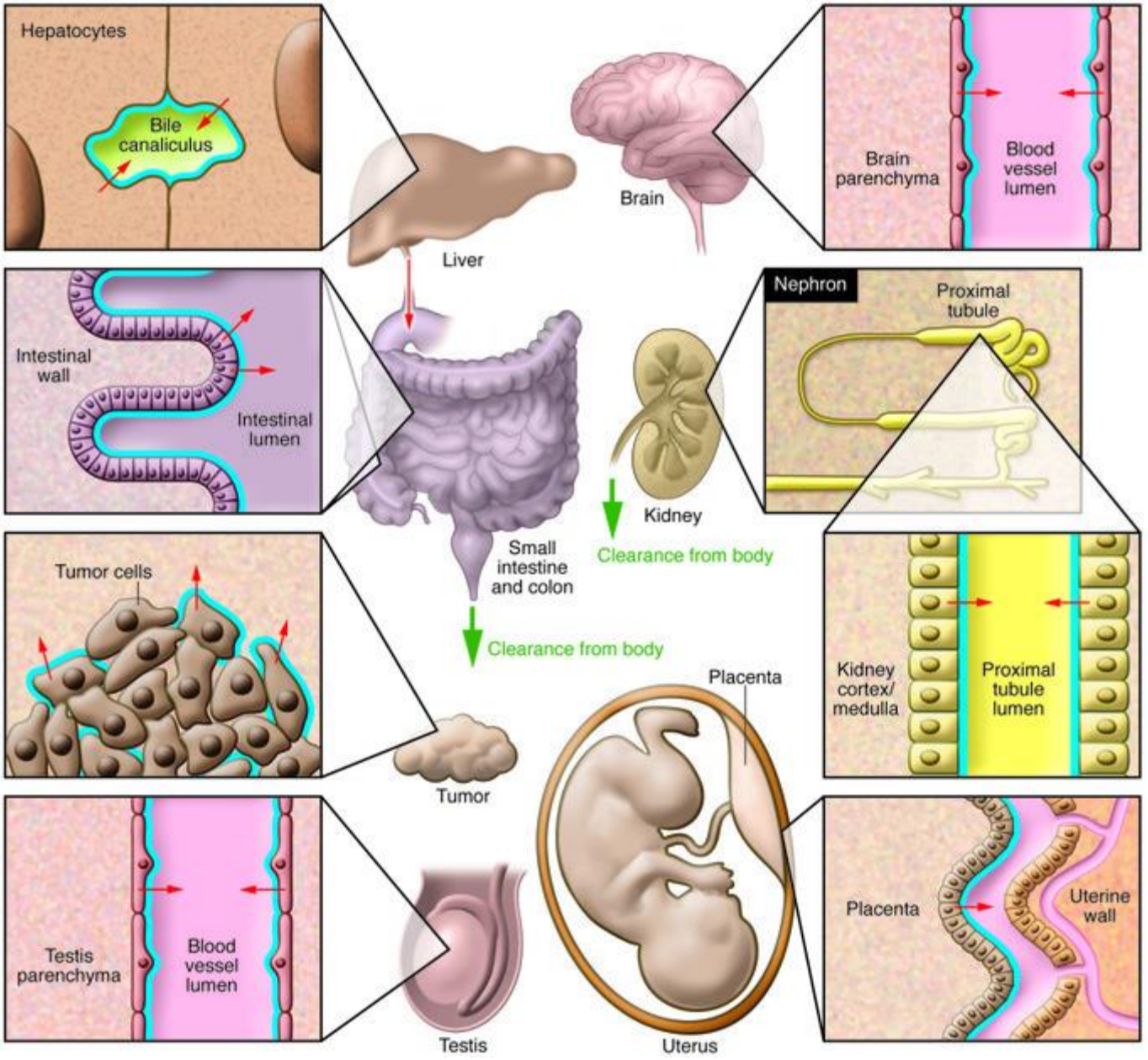
- Group of transmembrane proteins.
- Responsible for active transport of several substrates (direction: from cell out) while utilising energy obtained from hydrolysis of ATP.
- Against the concentration gradient of pumped substrates.
- The main function is to pump out all xenobiotics (xeno = alien, biotics – biologicaly active substrates, that can affect cell function and metabolism).
- Mainy from the „family“ of proteins calledc **A**TP-**b**inding **C**assette (**ABC**-proteins), with several different „subfamilies“, including several „members“.
- The most important and significant is the ABC subfamily B member 1 (ABCB1) **P-glycoprotein**;
- These pumps are the most prevalent farmacokinetic issue in field of modern cancer chemoterapy. They pump out anticancer drugs from the cells, but the core problem is, that tumor (cancer) cells are expriming EP in much higher levels than normal surrounding tissue, causing higher degree of resistance to chemotherapy agents (anticancer drugs) than normal healthy cells.

P-glykoprotein (P-gp)

- other names: ABCB1, **MDR1**, CD243
  ATP-**B**inding **C**assette sub-family **B** member **1**;
- N-terminally glycosylated 170 kDa transmembrane glycoprotein;
- The part of functional barriers like the blood-brain barrier or the blood–testis barrier;
- The case of several anticancer chemotherapy failures (P-glycoprotein was previously known as **M**ulti**D**rug-**R**esistant-**P**rotein **1**);
- Also the case of several drug – drug interactions;



- On the next slide you can see the distribution of P-glycoprotein in normal tissues in the body.
- Of particular importance is also the red arrow pointing the direction in which xenobiotics („alien substrates“) are actively removed from particulate tissues.
- You can clearly see, how important is P-glycoprotein in protecting the fetus against xenobiotics.
- It is also part of the CNS (BBB), liver, testicles, kidneys, intestines, placenta and of course malignant tumors.


Hepatocytes
Bile canaliculus
Liver
Brain
Brain parenchyma
Blood vessel lumen
Intestinal wall
Intestinal lumen
Small intestine and colon
Nephron
Proximal tubule
Kidney
Clearance from body
Tumor cells
Clearance from body
Kidney cortex/ medulla
Proximal tubule lumen
Placenta
Tumor
Testis parenchyma
Blood vessel lumen
Testis
Uterus
Placenta
Uterine wall

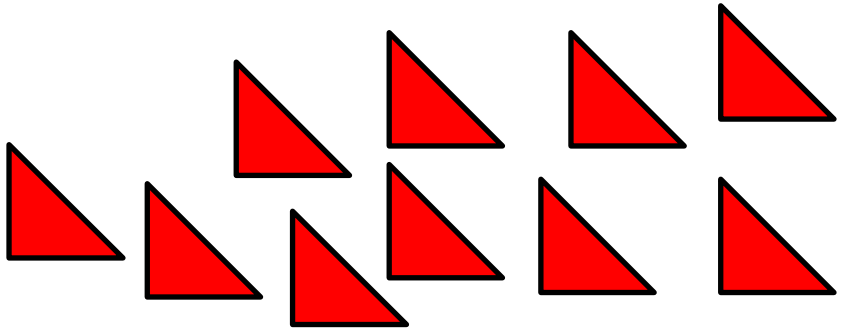
Next animations:

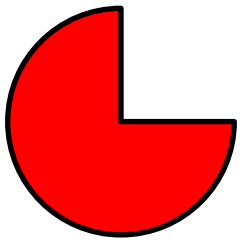
Red triangle = molecule of the pharmaceutical agent (drug)

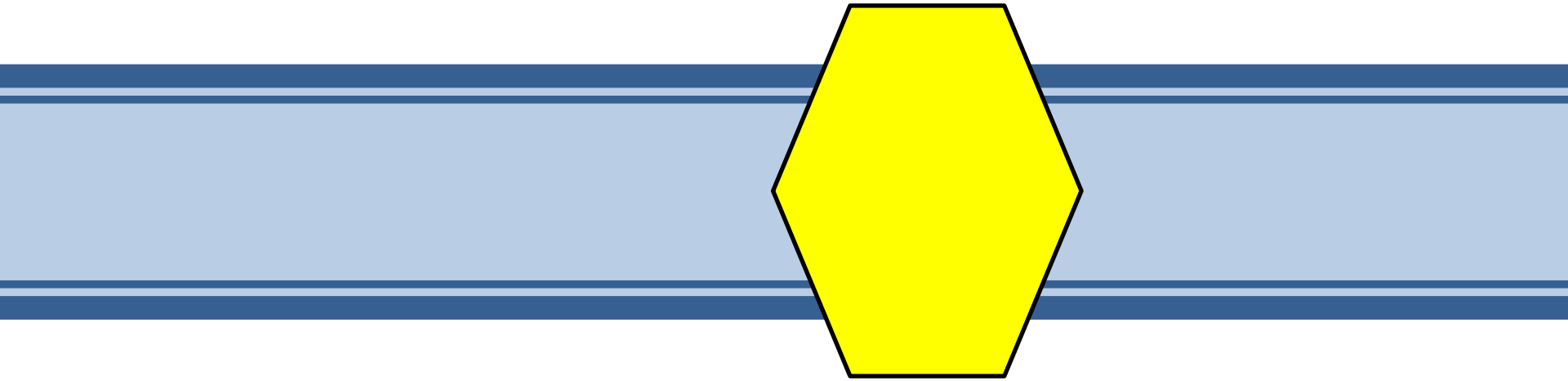
Red circle without triangular part = molecular target of the drug

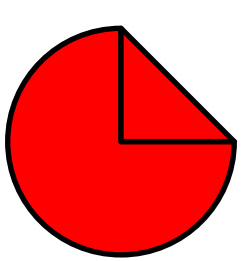
Blue barrier = cell membrane

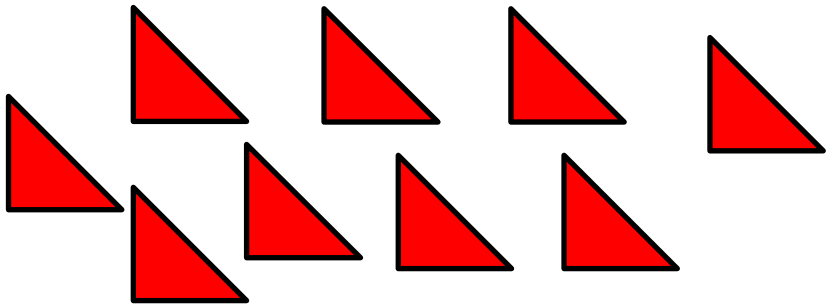
Yellow hexangular object = P-glycoprotein

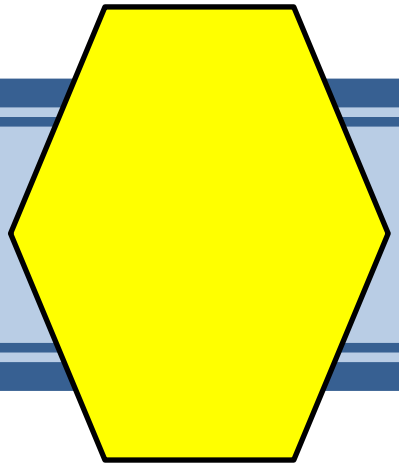




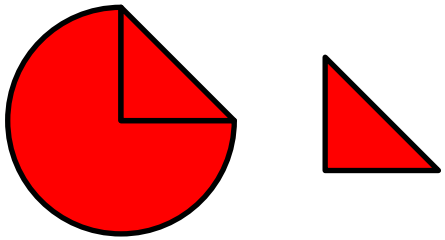








A TP

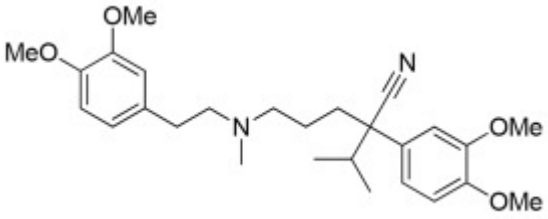


A TP

Substrates of the efflux pumps

- Prazoles (proton pump inhibitors, eg. omeprazole);
- PAD (peroral antidiabetics): DPP-4 inhibitors (gliptins), SGLT2 inhibitors (gliflosins);
- The „blood-thinning“ drugs – peroral direct inhibitors of coagulation (xabans a gatrans);
- Diuretics, beta-blockers, Calcium channel blockers (Cave! verapamil), hypolipidemics (statin-drugs);
- Inhibitors of PDE type 5 (nafils – Viagra);
- Glucocorticoids;
- Antibiotics /macrolides/ (Cave! IVERMECTIN also STRUCTURALLY belongs here), fluoroquinolones, antimycotic agents (azoles), antivirotic medications (HIV-infections - Cave! again REMDESIVIR structurally belongs here);
- CAVE! Cancer chemotherapy agents: nitrogen mustard derivatives, cytostatic alkaloids, terpenes and antibiotics (including semisynthetic derivatives), tyrosine kinase inhibitors (tinibs);
- Immunosuppresants (Cave! cyklosporine), also –limuses!
- NSAIDs (non-steroidal anti-inflammatory drugs): –fenaks and similary drugs;
- Anticonvulsive agents, antipsychotics and antidepressants (SSRI, some others);
- Antihistamines /allergy medications/;
- Opioids (Cave! loperamide a diphenoxylate).

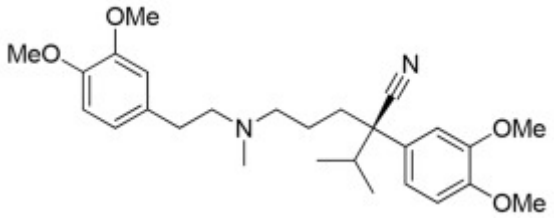
First generation inhibitors



Verapamil (racemate)

Levoverapamil
(used as vasodilator)

Second generation inhibitors



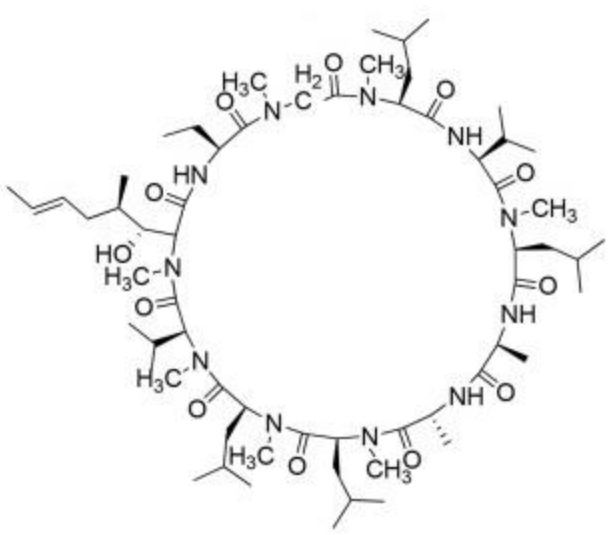
Dextroverapamil
(ineffective as vasodilator)
(effective as P-glycoprotein inhibitor)

Drugs in common use which were found (in addition to their own pharmacodynamic effect) to inhibit P-glycoprotein in vivo.

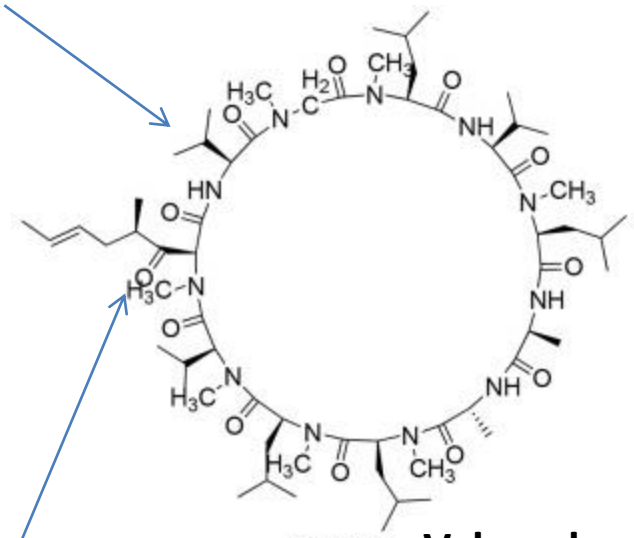
Modifications (stereoisomers, derivatives) of FGIs with lowered or fully absent original pharmacodynamic effect, but with conserved P-glycoprotein inhibitory activity.

1. generace iPgp

2. generace iPgp

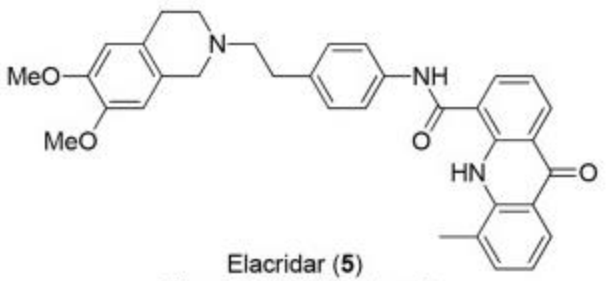


Cyclosporine A

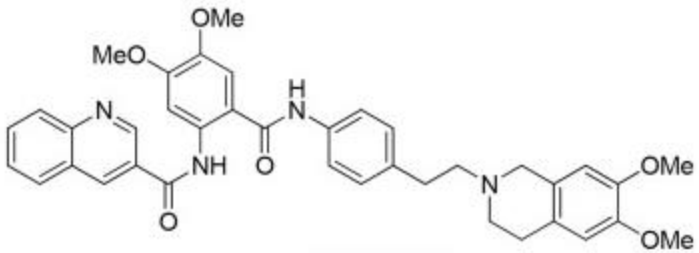


PSC-833 **Valspodar**

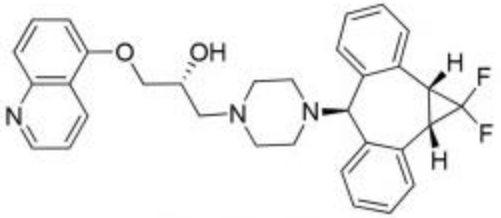
Third generation of P-glycoprotein inhibitors



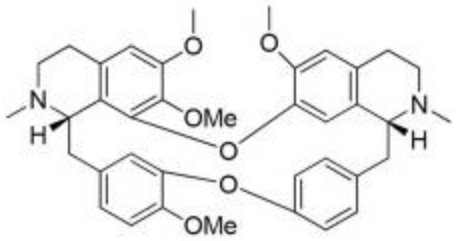
Elacridar (**5**)
(Phase I with Topotecan)



Tariquidar (**6**)
(Phase II with docetaxel)



Zosuquidar (**7**)
(Phase III with daunorubicin and cytarabine)



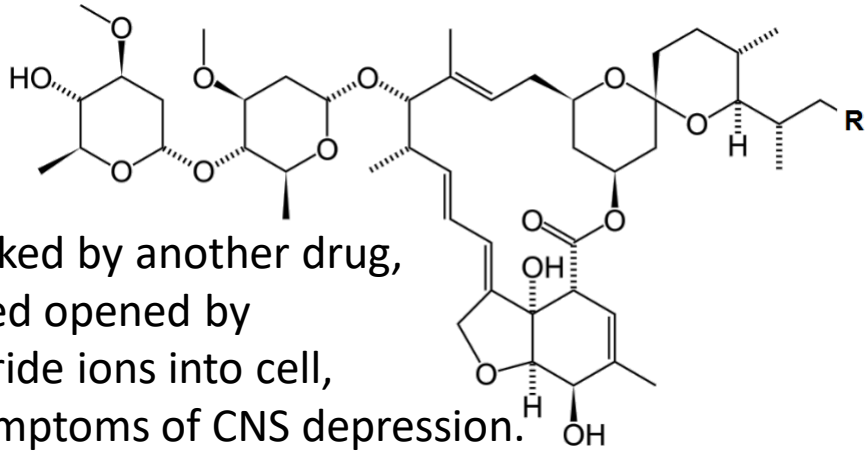
Tetrandrine/ CBT-01 (**8**)
Phase II with Paclitaxel

This generation contains modified structures (predominantly from 2. generation) or „all new“ tricyclic (or more) molecule core with well-known moiety (eg. dimethoxy-isoquinoline /from verapamil/).

Practical aspect of some P-gp drugs (ivermectin)

Ivermectin

- mixture 80:20 of two B1-avermectins (22,23-dihydroavermectin B1a (R = CH_3) and 22,23-dihydroavermectin B1b (R = H));
- 16-membered macrocyclic lactone with side spirokondensation glycosylated with 2 molecules of L-oleandrose;
- production microorganism: *Streptomyces avermitilis*;
- **mechanismus účinku:** ivermectin binds to glutamate-gated chloride ion channel, „locking" the channel in „open" conformation, allowing unrestricted passage of chloride ions into the cell, causing hyperpolarisation and death of the parasite (protostoma); because animals and humans have this type of ion channels behind the blood-brain barrier (in the CNS) under normal circumstancies is for them ivermectin non-toxic;
- **concurrent use of pharmaceutical agents, that are able to interfere with (inhibit) the function of P-glycoprotein is dangerous**, because small amounts of ivermectin are able to penetrate the brain and spinal cord and there must be a constantly active efflux pump (p-glycoprotein) that continuously pumps out these remains (traces) of ivermectin to achieve normal function of CNS; if these pumps are blocked by another drug, sensitive glutamate-gated ion channels are locked opened by ivermectin, leading to subsequent influx of chloride ions into cell, hyperpolarisation, ataxia, sedation and other symptoms of CNS depression.



Ivermectin (IVM)

- **Strong inhibitors of P-gp, that are able to undego severe interactions with IVM**: verapamil, digoxin, fluoroquinolones, amiodarone, clarithromycine, cyclosporine, dilthiazem, erythromycine, ketoconazole, itraconazole, propaphenone, quinidine, glucocorticoids (eg. dexamethasone)…
- **Risk groups of patients** (interferencing drugs): **patients with hearth disease** (verapamil, digitalis-like drugs, antiarrhytmics, anticoagulants), **patients with psychiatric disorders** (antipsychotics, benzodiazepines), **patients after transplantations** (cyclosporine, -limuses agents, antibiotics /macrolides, fluoroquinolones/, azole antimycotic agents), **patients with AIDS** /HIV/ (anti/retro/virotics, antibiotics, antimycotic agents and others), **patients with asthma / CHOPD / with cystic fibrosis** (glucocorticoids, ATBs, azole antimycotic agents, -limuses agents, antivirotic agents)
- IVM interferes with pharmacokinetics of **dexamethasone** and **other glucocorticoid drugs** (mostly throught P-gp)

Sources

- **What have we learnt thus far from mice with disrupted P-glycoprotein genes?** url: https://www.sciencedirect.com/science/article/pii/0959804996000639
- https://www.sciencedirect.com/science/article/pii/S0223523417304932

European Journal of Medicinal Chemistry 138 (2017) 273–292

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Review article

Natural alkaloids as P-gp inhibitors for multidrug resistance reversal in cancer

Prashant Joshi [a, b], Ram A. Vishwakarma [a, b], Sandip B. Bharate [a, b, *]

[a] Medicinal Chemistry Division, CSIR - Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, India
[b] Academy and Scientific & Innovative Research (AcSIR), CSIR - Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, India

ARTICLE INFO

Article history:
Received 23 February 2017
Received in revised form
19 April 2017
Accepted 23 June 2017
Available online 27 June 2017

Keywords:
P-Glycoprotein
MDR
Cancer
Alkaloids
Pgp inhibitor

ABSTRACT

The biggest challenge associated with cancer chemotherapy is the development of cross multi-drug resistance to almost all anti-cancer agents upon chronic treatment. The major contributing factor for this resistance is efflux of the drugs by the p-glycoprotein pump. Over the years, inhibitors of this pump have been discovered to administer them in combination with chemotherapeutic agents. The clinical failure of first and second generation P-gp inhibitors (such as verapamil and cyclosporine analogs) has led to the discovery of third generation potent P-gp inhibitors (tariquidar, zosuquidar, laniquidar). Most of these inhibitors are nitrogenous compounds and recently a natural alkaloid CBT-01® (tetrandrine) has advanced to the clinical phase. CBT-01 demonstrated positive results in Phase-I study in combination with paclitaxel, which warranted conducting it's Phase II/III trial. Apart from this, there exist a large number of natural alkaloids possessing potent inhibition of P-gp efflux pump and other related pumps responsible for the development of resistance. Despite the extensive contribution of alkaloids in this area, has never been reviewed. The present review provides a comprehensive account on natural alkaloids possessing P-gp inhibition activity and their potential for multidrug resistance reversal in cancer.