3rd International Electronic Conference on Medicinal Chemistry
1-30 November 2017
chaired by Dr. Jean Jacques Vanden Eynde
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Old pharmaceuticals with new applications: the case studies of lucanthone and mitoxantrone

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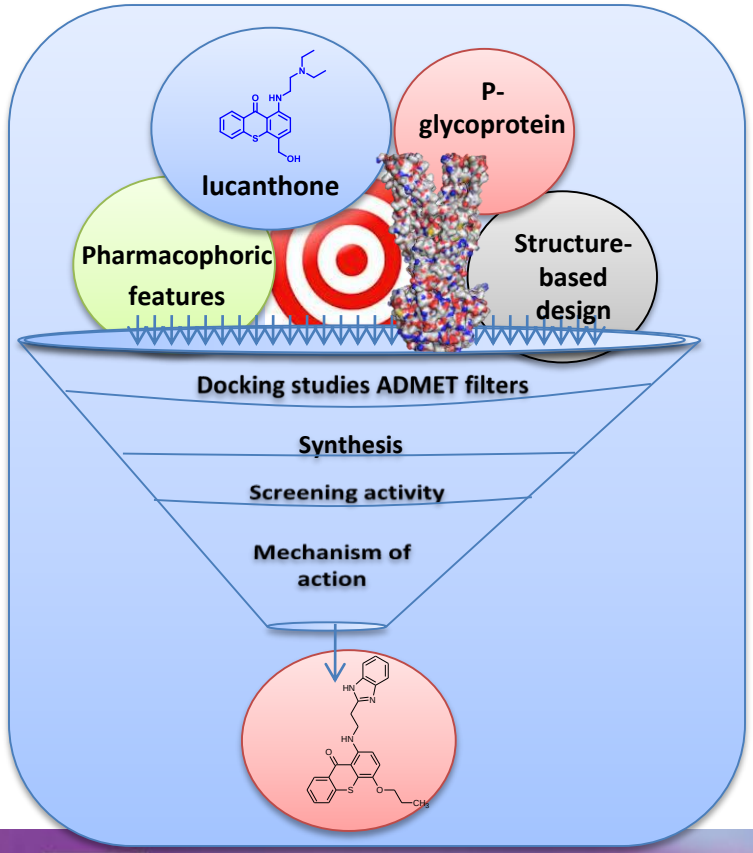
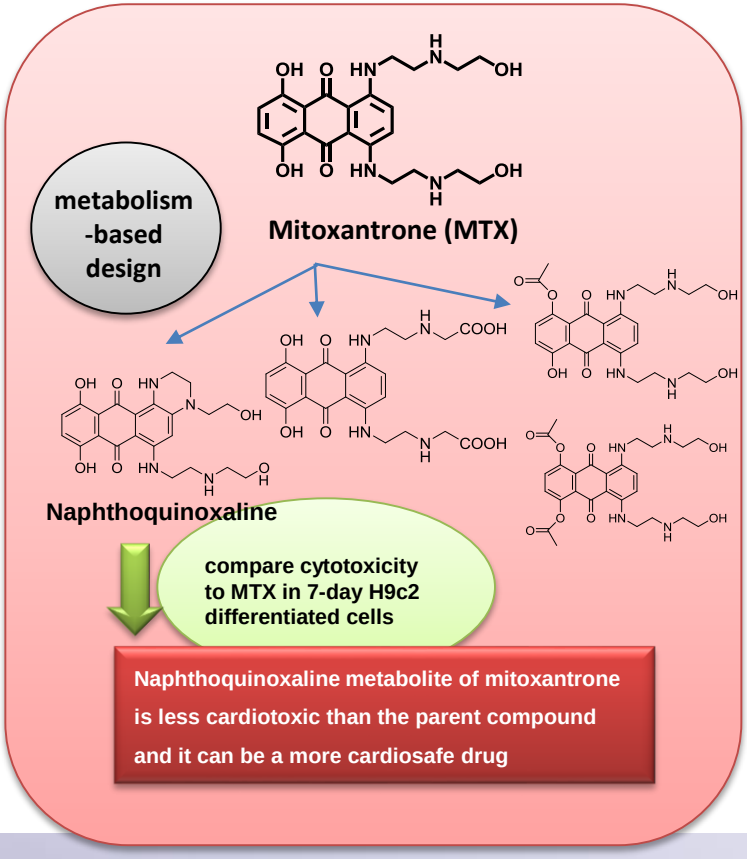
Old pharmaceuticals with new applications: the case studies of lucanthone and mitoxantrone

Graphical Abstract

Design of P-glycoprotein inhibitors with antitumor activity



Are mitoxantrone metabolites responsible for their cardiotoxicity?



Abstract: The recent overview of pharmaceutical companies' R & D programs has been undergoing some changes, especially due to increased immunopharmacology-based treatments. A trend that has also been growing is the search for new activities that may be demonstrated by drugs already used in therapeutics.

We will give examples of antitumor small molecules lead compounds obtained in our research group that arise from two existing drugs, lucanthone and mitoxantrone (MTX). Lucanthone was the antitumor model used to design inhibitors of P-glycoprotein with antitumor activity. Very recently we engaged a project that intend to understand the influence of metabolites in the cardiotoxicity of an antitumor drug, MTX. Studies on cardiotoxicity of a synthetized metabolite, naphthoquinoxaline (NAPHT) revealed that the parent drug, MTX, caused a higher disruption in the energetic pathways in a cardiac model in vitro. Therefore, this metabolite should be regarded as a good option for a safer anticancer therapy since it is less cardiotoxic than MTX.

The case studies presented herein are expected to contribute to a recent trend in drug discovery, with the involvement of old pharmaceuticals.

Keywords: old drugs; lucanthone; mitoxantrone; P-glycoprotein; metabolism

pharmaceuticals

Special Issue "Old Pharmaceuticals with New Applications"

In recent years, we have experienced a surge of interest in **drug repositioning**. There is a trend in finding new uses for existing drugs, especially in diseases where there is an unmet clinical need such as neglected and orphan diseases. Another opportunity is developing novel applications using a **combination of old drugs**.

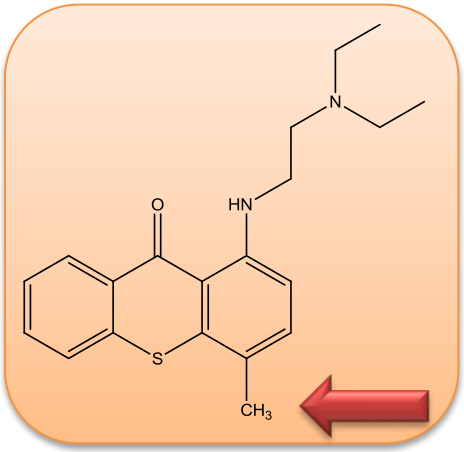
"The most fruitful basis for the discovery of a new drug is to start with an old drug" goes a famous statement from Sir James Black, which has received many adherents this century, not only in finding new applications but also looking for the unexploited potential of old drugs as **starting points for molecular modifications**.

The journal Pharmaceuticals invites both reviews and original articles shedding light on the challenges and opportunities of using old pharmaceuticals in drug discovery. Topics include: **drug repositioning**, **selective optimization of side effects**, **drug metabolites** as sources of new drugs, **old drug combinations**, beyond pharmaceuticals applications.

Case-study: Lucanthone

Lucanthone

Antischistosomal introduced in therapy, in 1945



Withdraw due to mutagenic side effects

Moiety associated to cardiotoxic effects

Phase I dose-escalation study of lucanthone in patients with recurrent malignant gliomas receiving temozolomide

Cancer Sensitizer

APE-1/ BER

APE-1 - apurinic-apyrimidinic endonuclease
BER – base excison repair

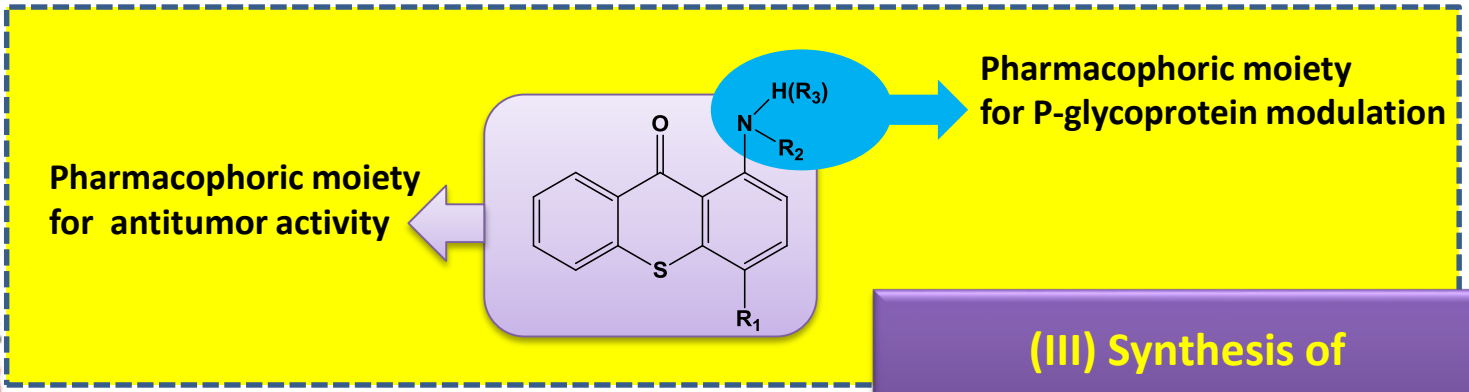
DNA repair systems

MGMT/Direct repair

MGMT - Methyl guanine methyl transferase

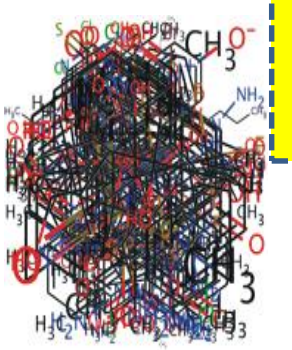
Paiva et al. Current Medicinal Chemistry, 2013, *20,* 2438-2457

Design of P-glycoprotein inhibitors with antitumor activity



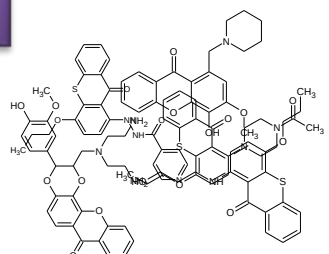
(III) Synthesis of thioxanthones (Tx)

~ 1000 designed thioxanthones (Tx)

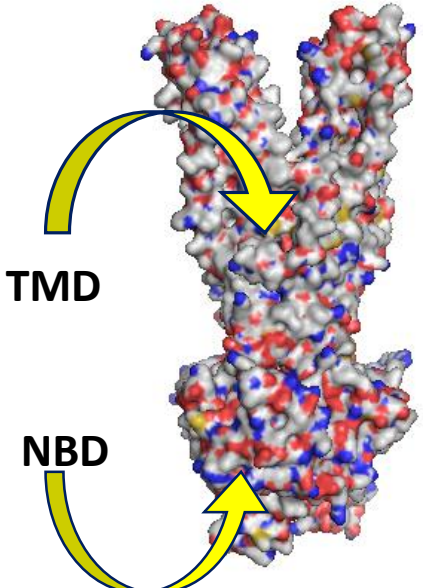


- LogP
- MW
- Amine

Hundreds…



Docking



Molecules with the best scores

Cell growth inhibition (Sulphorhodamine-B assay)

Compound	GI_{50} (K562) (μM)		
TxA1	**1.90**	**±**	**0.15**
Tx141	**3.00**	**±**	**0.48**
Tx34	**3.72**	**±**	**1.47**
TxOH131	**4.38**	**±**	**0.44**
TxOMe	**4.47**	**±**	**1.93**
Tx18	**4.81**	**±**	**4.21**
Tx127	12.98	±	0.36
TxAc	13.57	±	2.96
Tx131	15.57	±	3.15
Tx41	16.22	±	0.48
Tx104	16.50	±	3.06
Tx48	16.99	±	2.33
Tx96	18.13	±	4.35
Tx128	19.23	±	0.98
Tx86	20.96	±	2.08
Tx15	21.47	±	2.61
TxOH	22.73	±	0.64
Tx53	29.79	±	3.02
TxA4	52.95	±	1.47
Tx62	59.45	±	2.77
Tx79	60.58	±	2.01
TxOH1H	74.32	±	7.16
Tx87	92.92	±	3.33
Tx129	104.71	±	7.29
TxA3	H		
Tx54	H		
Tx105	H		
Verapamil	H		
Doxorubicin	0.06	±	1.27

GI_{50} values for new thioxanthonic compounds in K562 (sensitive) cell line

Six new compounds GI_{50} < 10μM

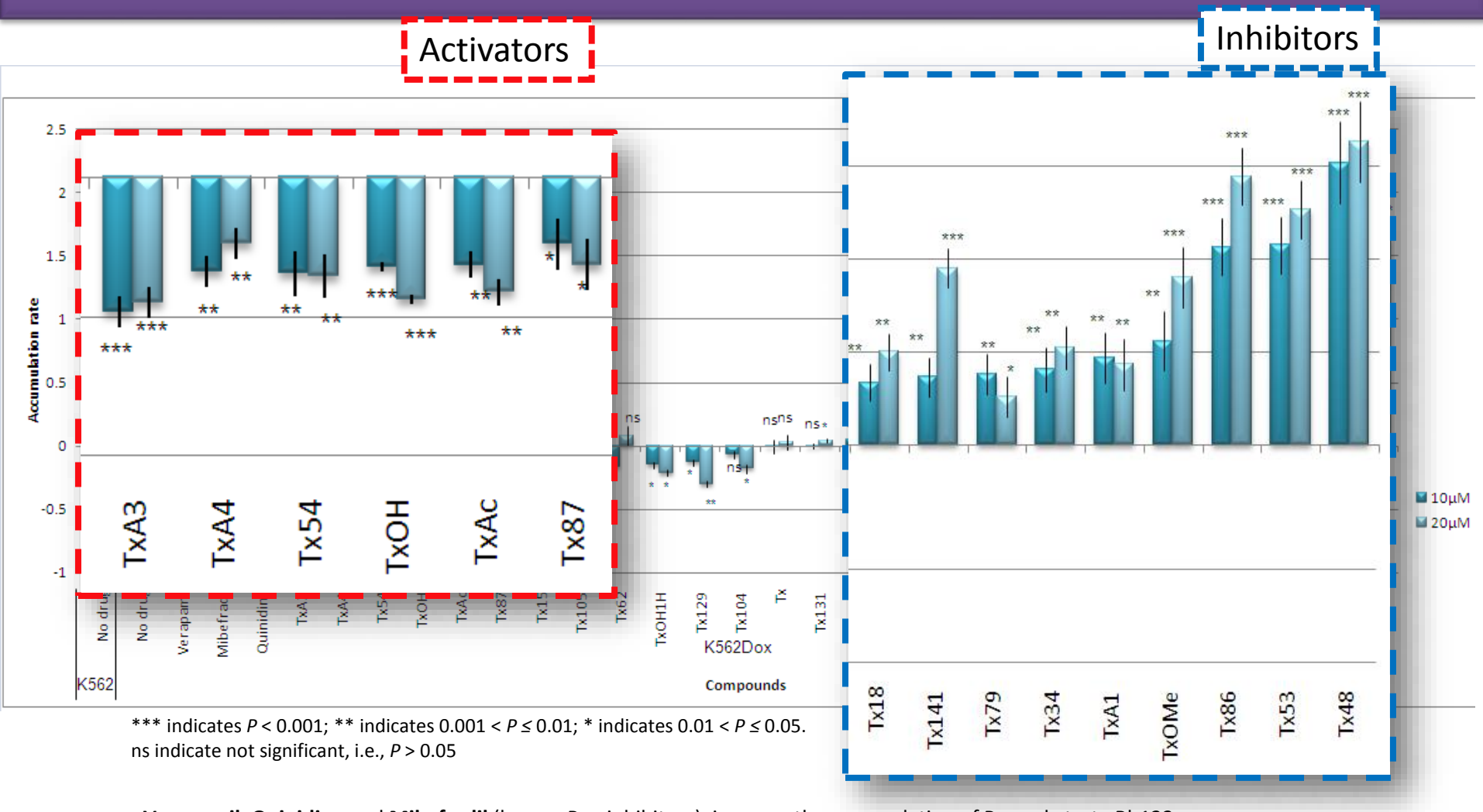
No significant effect on MRC-5 cell line (non-tumor cells, trypan blue)

TxOMe induced an S-phase cell-cycle arrest; the six Tx induced a decrease of the G2/M phase

Most Tx derivatives increased cellular apoptosis

H = high

Rh-123 accumulation assay

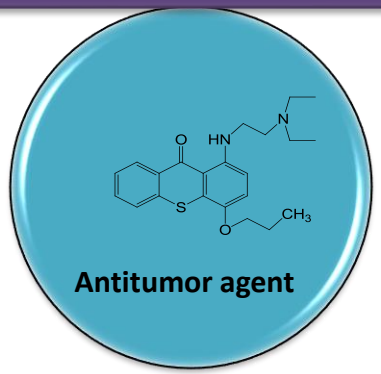


*** indicates $P < 0.001$; ** indicates $0.001 < P \leq 0.01$; * indicates $0.01 < P \leq 0.05$.
ns indicate not significant, i.e., $P > 0.05$

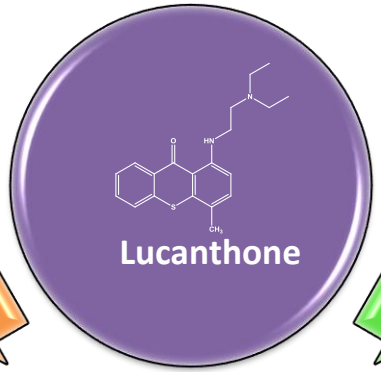
- **Verapamil, Quinidine** and **Mibefradil** (known Pgp inhibitors): increase the accumulation of Pgp substrate Rh123

- **TXA3, TXA4, TX54, TXOH, TXAc, TX87:** effect compatible with **Pgp activation**
- **TX48, TX53, TX86:** effect compatible with **Pgp inhibiton** ~ Quinidine

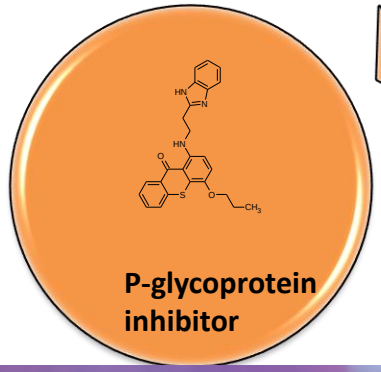
Case-study: Lucanthone



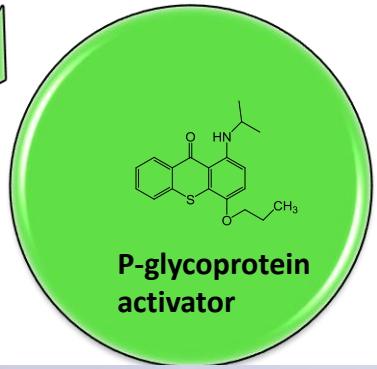
Molecules 2016, 21, 1343



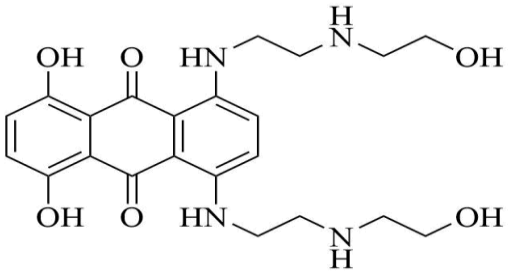
Biochemical Pharmacology 2012, 83 (1), 57–68.



Archives of Toxicology, 2015, 89(10):1783-800.



Case-study: Mitoxantrone (MTX)



- Drug repurposing

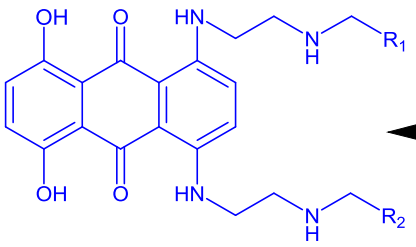
approved in 1987 as antitumor drug and in 2002 for use in multiple sclerosis

MTX-induced cardiotoxicity

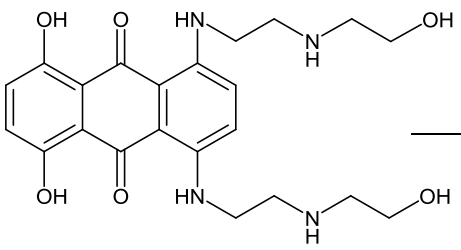
- **Adverse effects**: early and late cardiotoxicity.
- **Cardiotoxicity** affects up to 18% of MTX-treated patients, being multiple sclerosis patients more susceptible.
- **Maximum recommended cumulative doses:**
  - Cancer patients: 140 mg/m^2
  - Multiple sclerosis patients: 100 mg/m^2

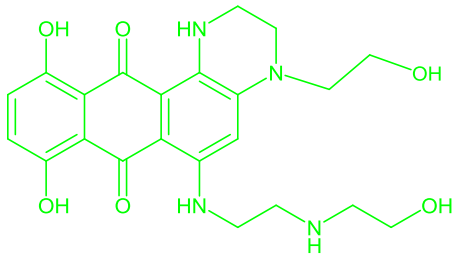
Mechanisms involved in cardiotoxicity: largely unknown.

Synthesis of drug metabolites to study their toxicity



Oxidation





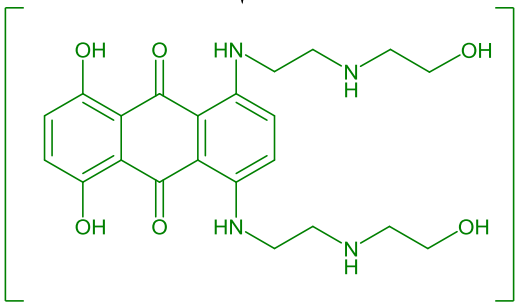
Carboxilic acid metabolites of MTX

Anticancer activity: inactives
Cardiotoxicity: n.a.

Compounds	R_1	R_2
Monocarboxylic acid of MTX	COOH-	CH_2OH-
Dicarboxylic acid of MTX	COOH-	COOH-

MITOXANTRONE (MTX)

Conjugation



. Glutathione
. Glucuronide

MTX conjugates

Anticancer activity: n.a.
Cardiotoxicity: n.a.

Naphthoquinoxaline of MTX

Anticancer activity: active
Cardiotoxicity: n.a.

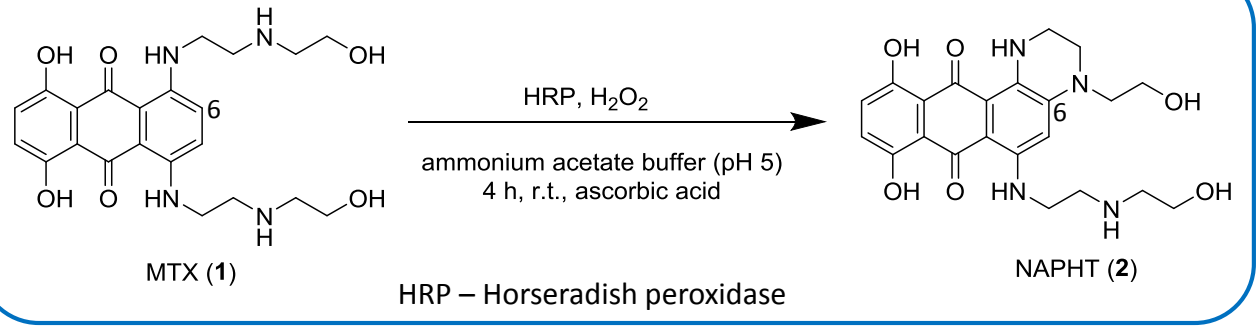
Metabolization S9 and microssomal products of MTX

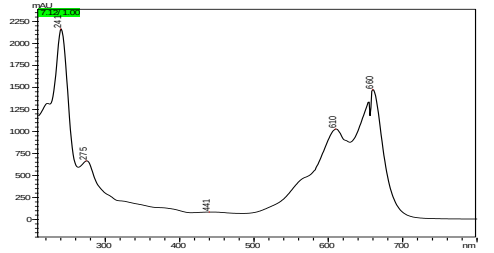
Anticancer activity: active
Citotoxicity (namely cardiotoxicity): higher

A F Reis-Mendes, et al. Current Drug Metabolism, 2015 17(1):75-90.

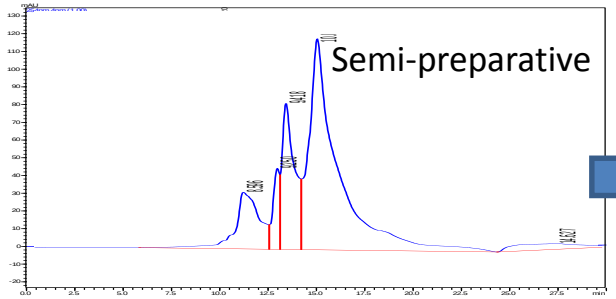
Synthesis of drug metabolites to study their toxicity

Synthesis and purification of MTX-naphthoquinoxaline metabolite

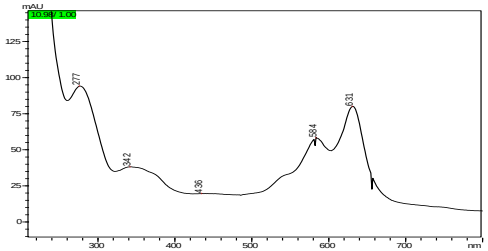




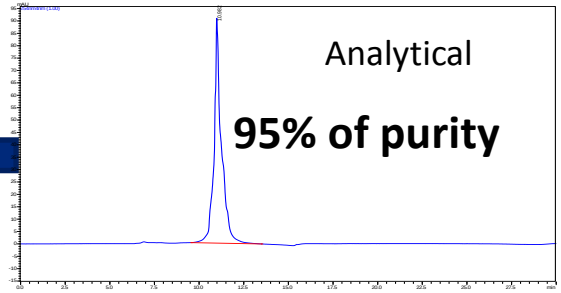
UV-Vis spectra of MTX (**1**)



HPLC chromatogram of NAPHT (**2**) crude



UV-Vis spectra of NAPHT (**2**)



HPLC chromatogram of NAPHT (**2**)

- New chromophore was formed
- HRMS m/z 443.19313

Representative HPLC chromatograms (λ= 254 nm, C18, isocratic system 3:7 of eluent B within 30 min).

Studies on naphthoquinoxaline (NAPHT) cardiotoxicity

- MTX causes higher cellular damage in H9c2 differentiated cells than does NAPHT
- MTX and NAPHT produce mitochondrial dysfunction in differentiated H9c2 cells, although less pronounced for NAPHT
- MTX causes a greater loss of cellular membrane integrity
- MTX caused a more severe lysosome uptake dysfunction
- MTX increased intracellular ATP levels and lactate levels, whereas its metabolite did not change those parameters

3-Methyladenine, an autophagy inhibitor, partially protected against lysosomal uptake dysfunction

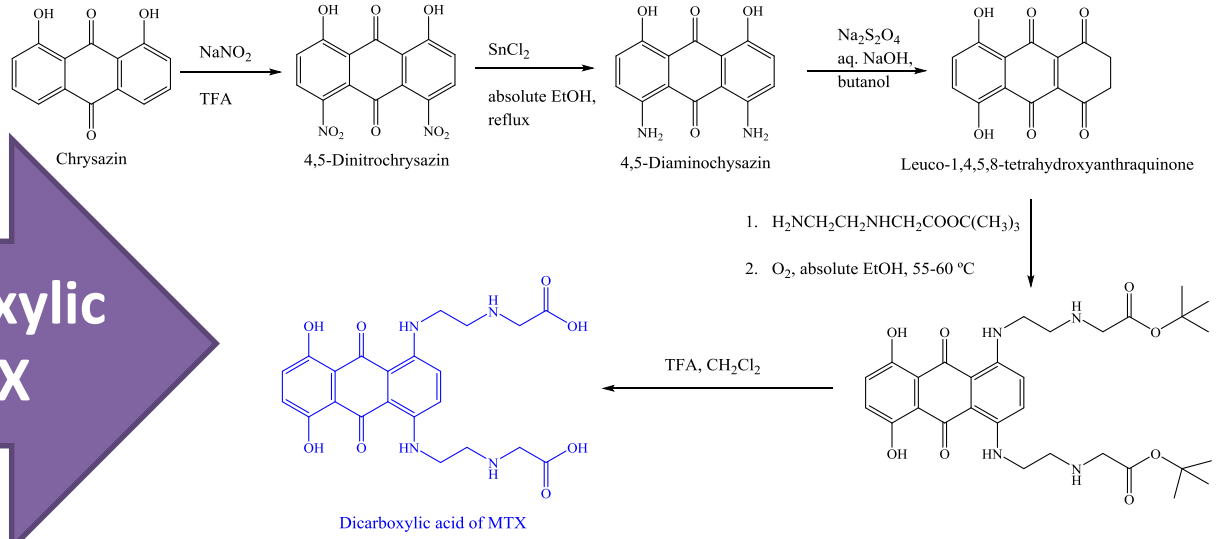
the parent drug, MTX, caused a higher disruption in the energetic pathways in a cardiac model *in vitro*

previous data has shown that NAPHT can have a potential role on MTX anticancer effects

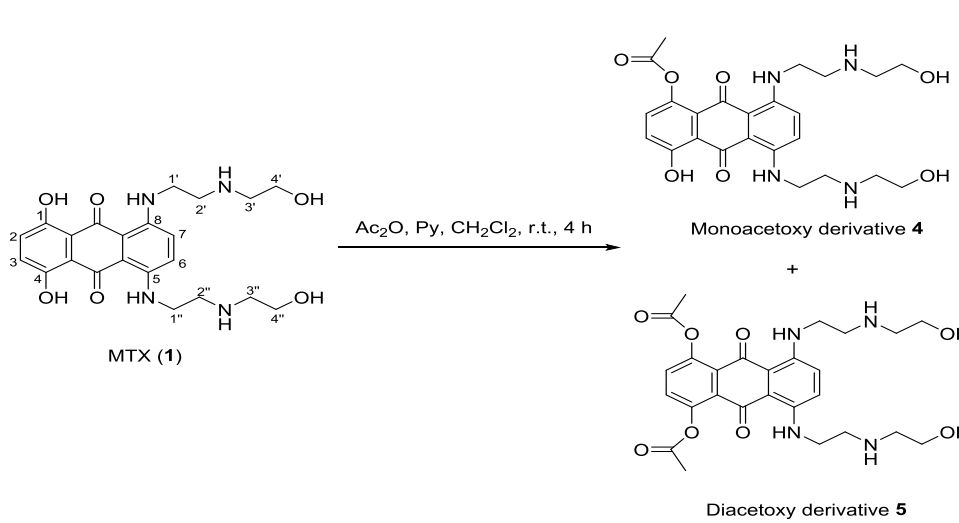
NAPHT can be a more cardiosafe drug in anticancer therapy

A. Reis-Mendes, et al. Arch Toxicol. 2016, 91(4):1871-1890

Synthesis of drug metabolites to study their toxicity



Synthesis of carboxylic acid derivatives of MTX



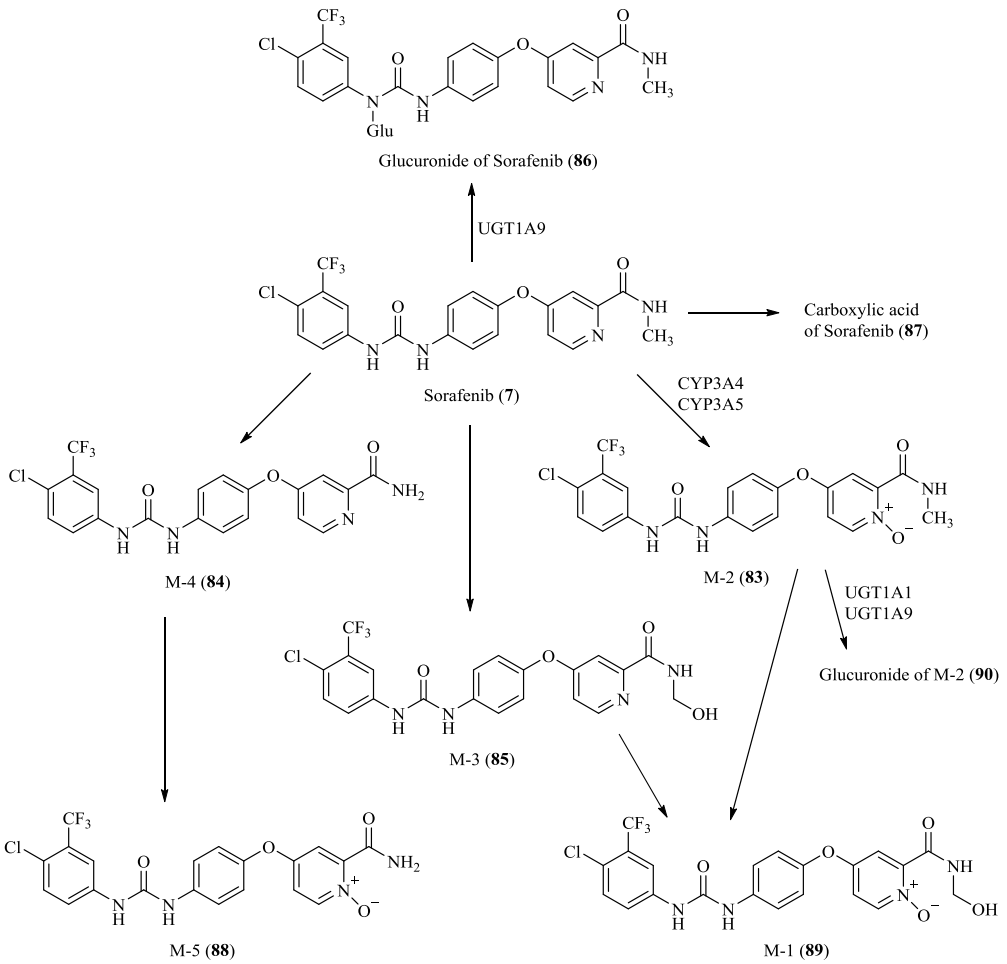
Synthesis of acetoxy derivatives of MTX

Synthesis of drug metabolites to study their toxicity

Opportunities with existing drugs

One of several examples…

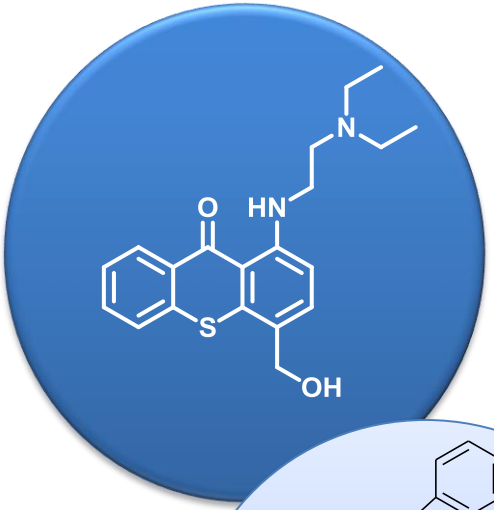
- Sorafenib (**7**) is known to induce acute coronary symptoms including myocardial infarction in 2.9% of patients
- **No assessment of the potential cardiotoxicity of metabolites was done so far, to the best of our knowledge**



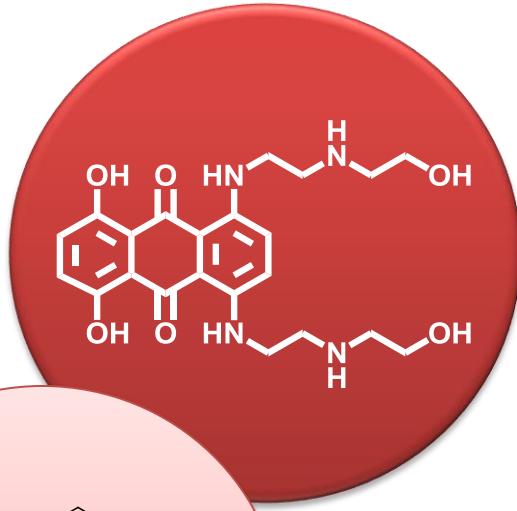
I. Hrynchak, et al. Drug Metabolism Reviews, 2017, 49, 158-196 .

Conclusions

lucanthone

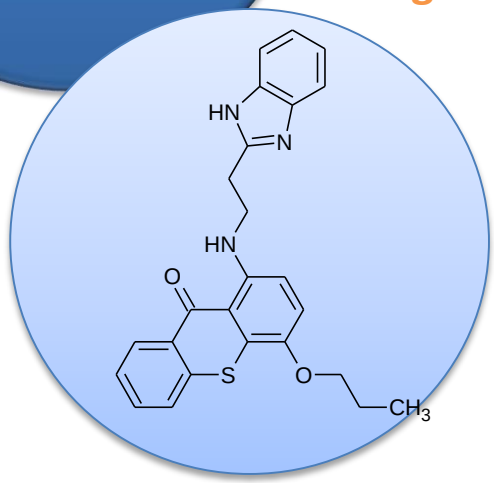


mitoxantrone

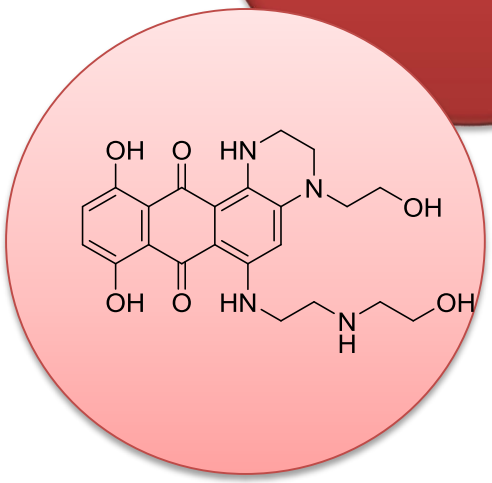


- **Higly active models**
- Surpassed clinical trials
- Examples of drug repurposing

Structure-based design



Metabolism-based design



- **Most active leads**
- **Mee better drugs?**
  To be continued

"The most fruitful basis for the discovery of a new drug is to start with an old drug" Sir James Black

Acknowledgments

This research was partially supported through national funds provided by FCT – Foundation for Science and Technology and European Regional Development Fund (ERDF), in the framework of the programme PT2020 by the Strategic Funding UID/Multi/04423/2013, , the project PTDC/MAR-BIO/4694/2014 (POCI-01-0145-FEDER-016790; 3599-PPCDT) and PTDC/DTP-FTO/1489/2014 (POCI-01-0145-FEDER-016790) in the framework of PT2020, to INNOVMAR (NORTE-01-0145-FEDER-000035, NOVELMAR), supported by NORTE 2020, under PORTUGAL 2020, through ERDF.