U.PORTO
FACULDADE DE FARMÁCIA
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CEQUIMED UP

IPATIMUP

Antitumor activity of novel synthethic thioxanthonic derivatives mediating cell cycle arrest and apoptosis in human tumor cell lines

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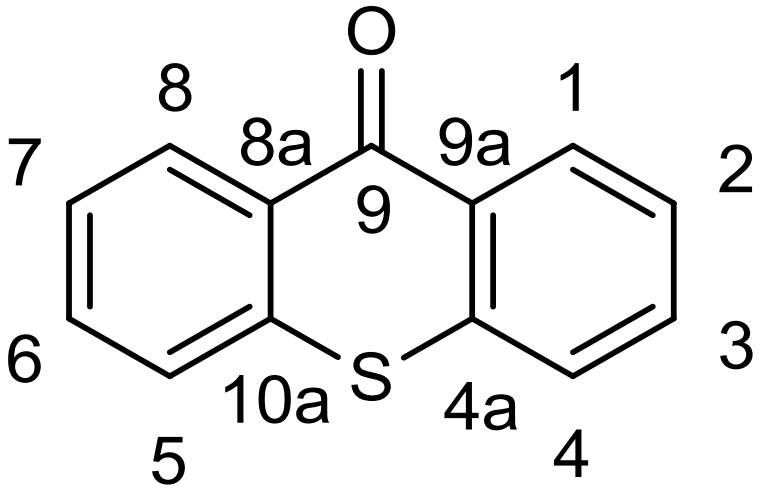
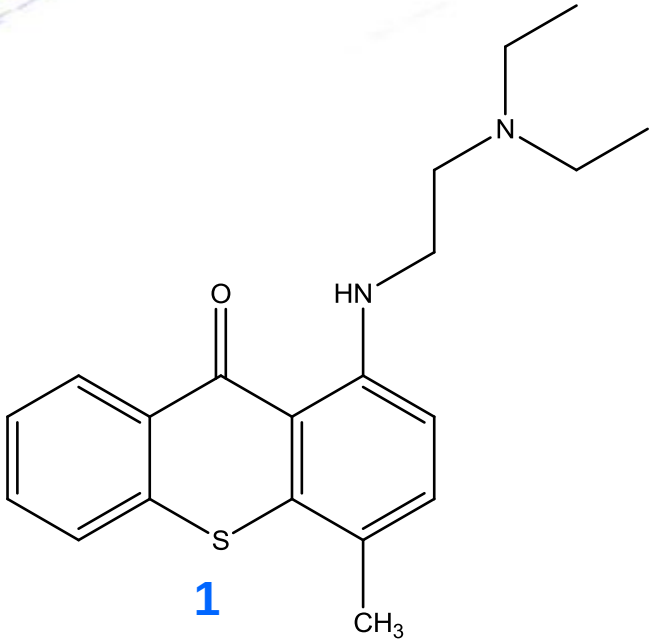
INTRODUCTION



- Antitumor activity
- Antiparasitic activity
- Photoinitiation activity
- P-glycoprotein inhibitory activity

INTRODUCTION

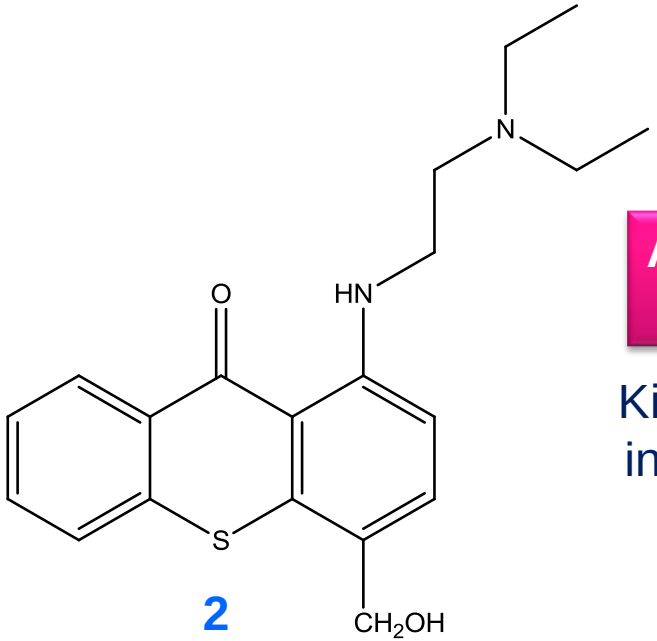
1st Generation



1

Lucanthone

- Clinical trials (*per os*) as antischistosomal agent
- Secondary Effects:
  - nausea, vomiting, cardiovascular and CNS effects
- Not active *in vitro*



2

Hycanthone

- More active
- Oral or IM
- Secondary Effects:
  - anorexia, vomiting, ↑transaminases, icterus

Antischistosomal agents

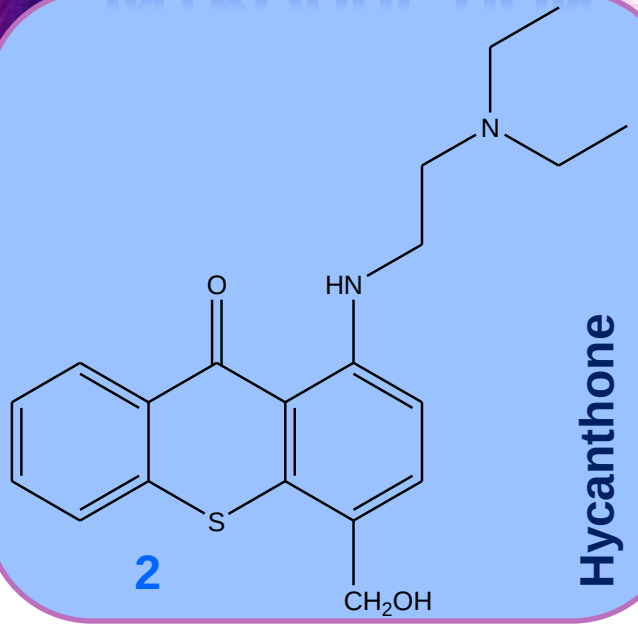
Kill schistosomes by interfering with their nucleic acid synthesis

Withdrawn due to mutagenicity

Antitumor activity

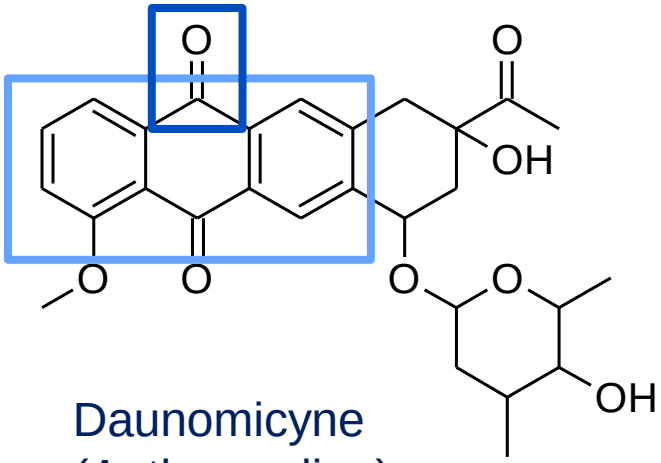
INTRODUCTION

Antitumor agents

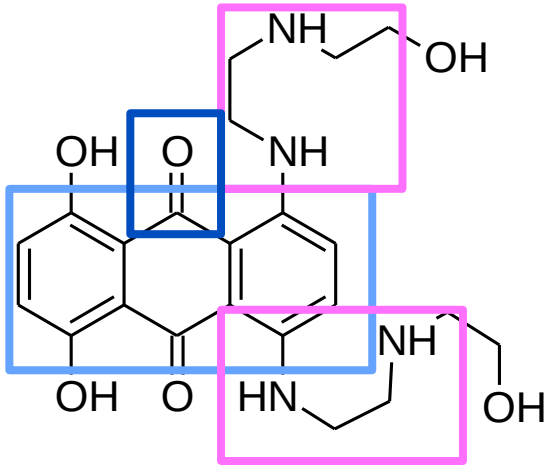


2

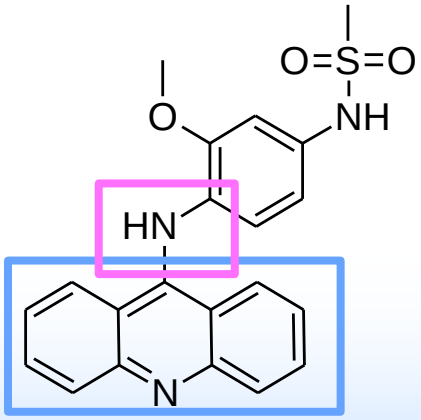
Hycanthone



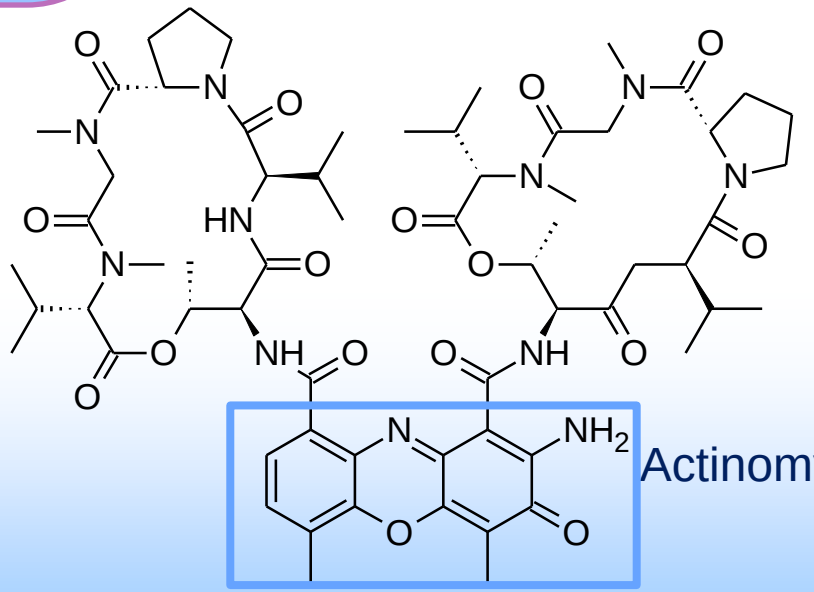
Daunomicyne
(Anthracycline)



Mitoxanthrone
(Anthraquinone)



Ansacrine
(Acridine)



Actinomycin D

Intercalating agents

INTRODUCTION

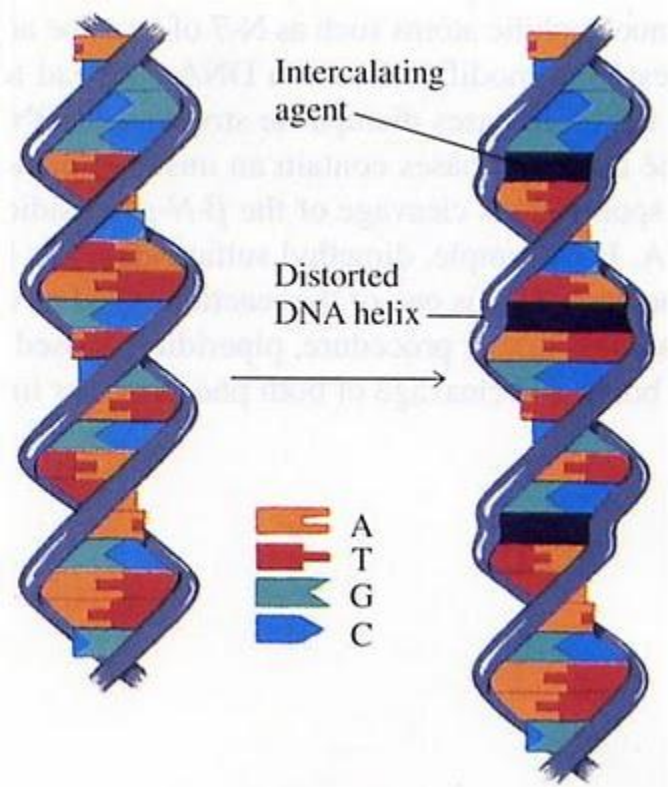
Intercalating agents

- Planar and chromophoric (generally aromatic)
- Planar area ≥ 22 Å (3-4 aromatic rings)

Intercalates between base pairs

Hydrogen bond
Π-stacking
van der Waals

- Terciary DNA structure is distorted (uncoiled)
  - Larger distance between base pairs (3.4 Å→7.8Å)
  - Alteration in sugar-phosphate torsion angles



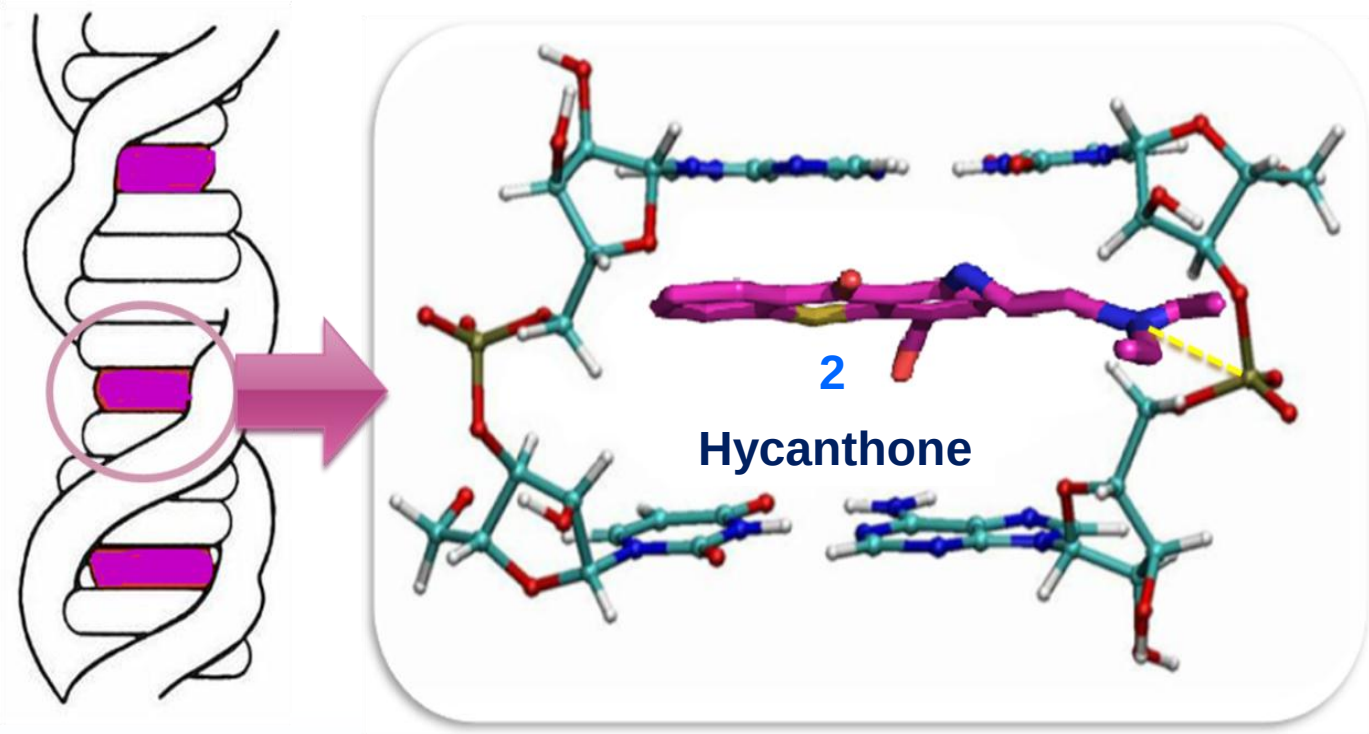
Topoisomerase
DNA/RNA polymerase

Inhibits REPLICATION and TRANSCRIPTION

INTRODUCTION

Antitumor

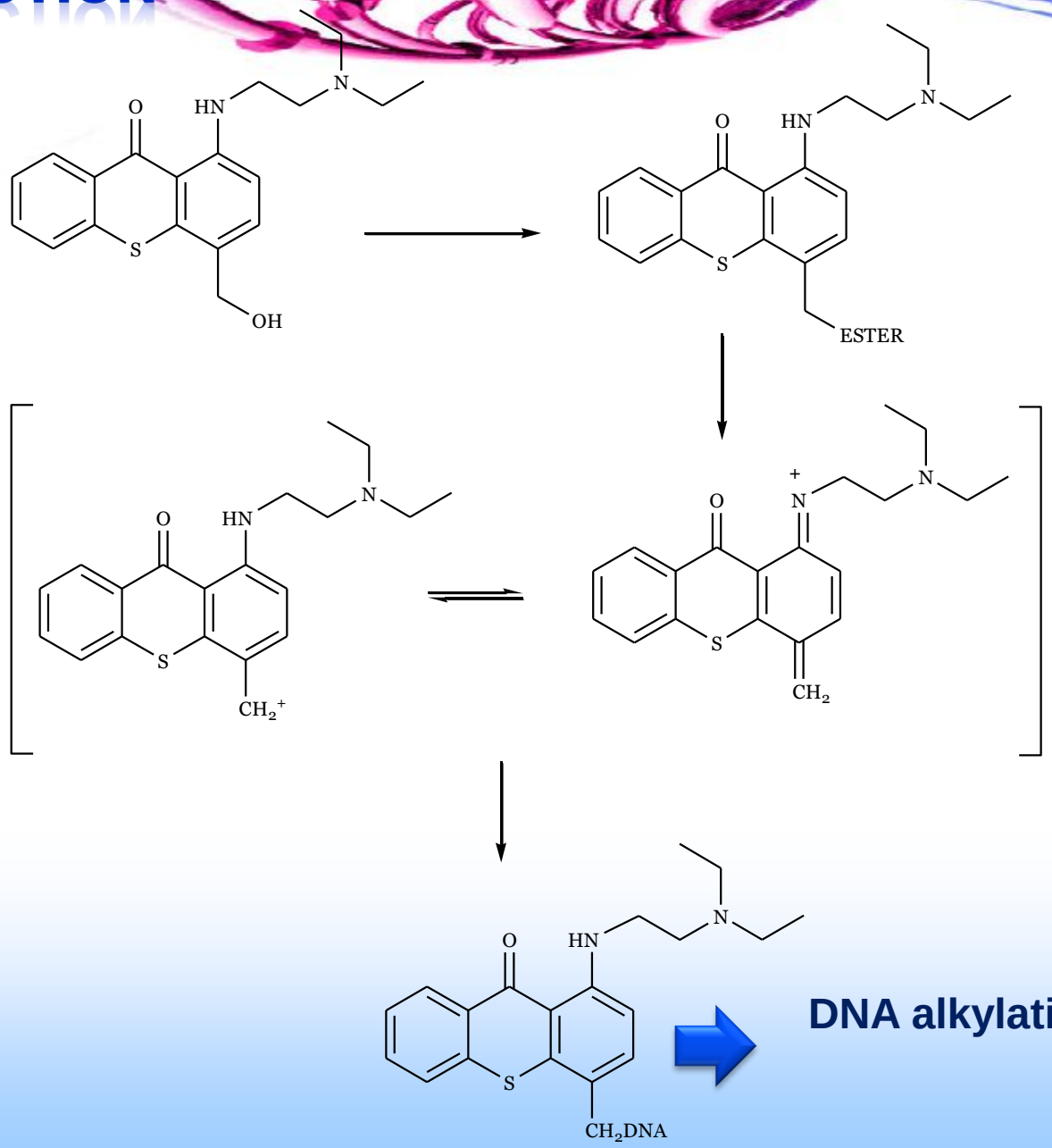
Intercalating agents



- Tricyclic system→ AT=AT intercalation
- Protonated amine→ electrostatic interaction with phosphates in DNA
- C-4 chain outside the groove
- Sulphur atom → large→ helix distortion

INTRODUCTION

Mutagenicity

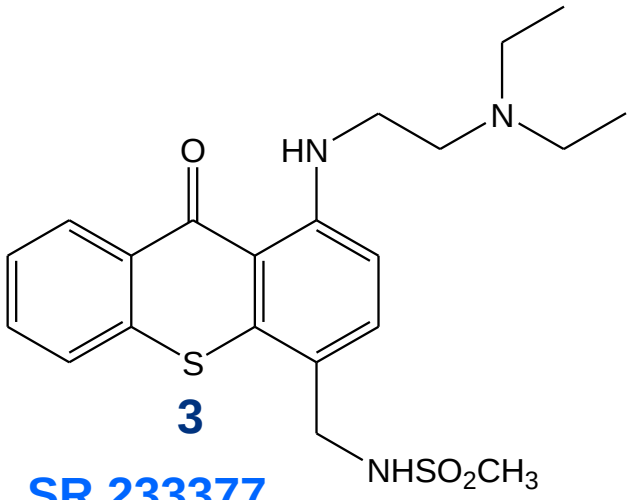


Carbonium ion

DNA alkylation

INTRODUCTION

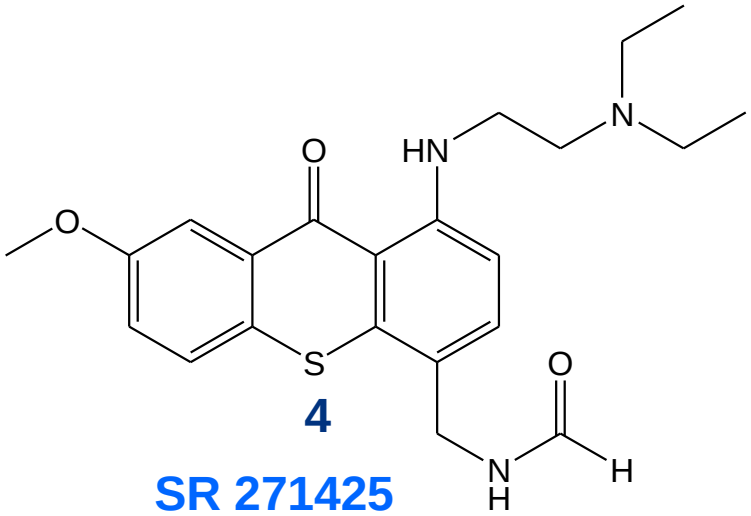
2nd/3th Generation



3

SR 233377

- Colon, pancreatic, mammary adenocarcinoma (*in vitro*)
- Colon, pancreatic, mammary adenocarcinoma (*in vivo*) (IV)
- Clinical trials (I): dose-limiting toxicity
  - Myelotoxicity, QT interval prolongation (life-threatening)



4

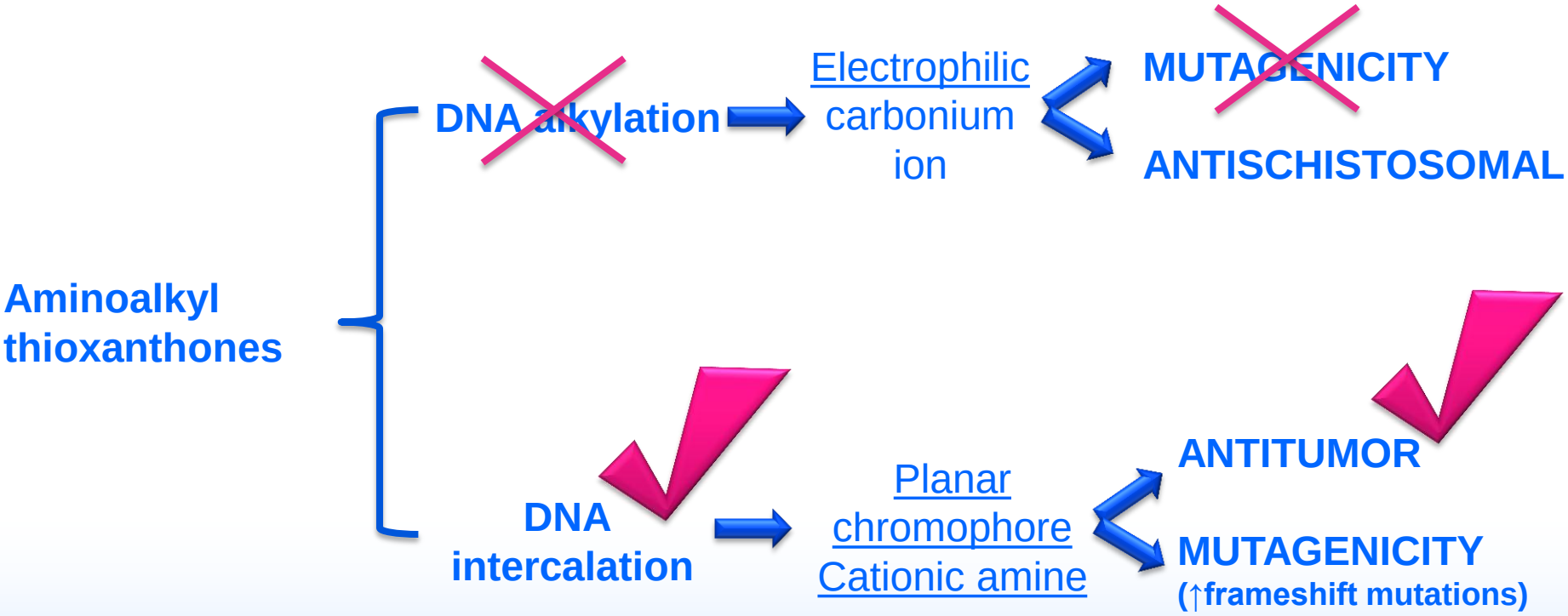
SR 271425

- ↑antitumor activity, broadspectrum
- ↓hepatic toxicity
- Orally active

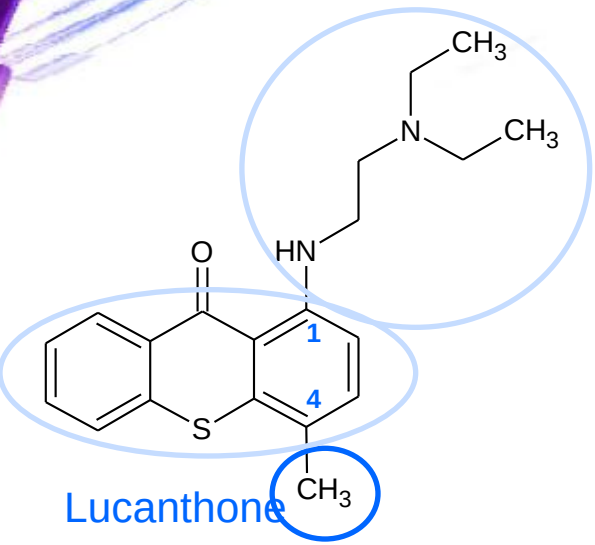
- Clinical trials (I)→ Cardiotoxicity
  QT interval prolongation
  Torsade de Pointes

INTRODUCTION

DNA binding

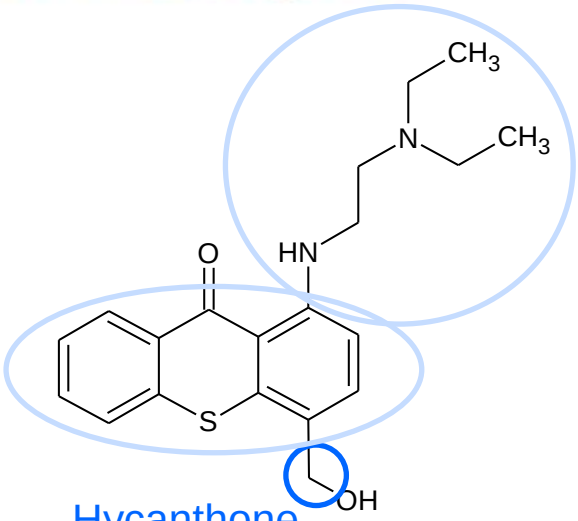


INTRODUCTION



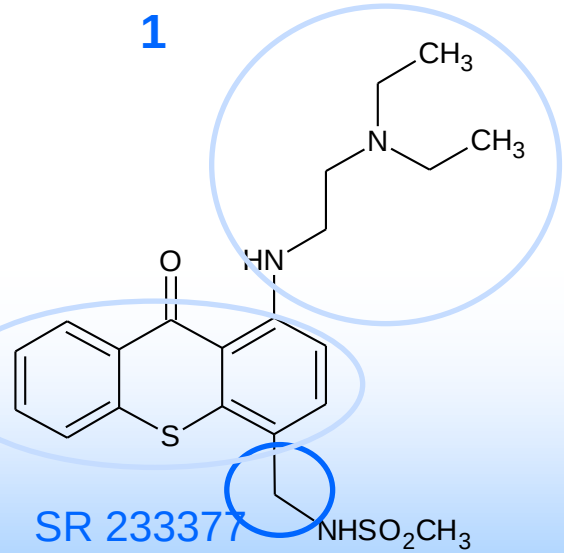
Lucanthone

1



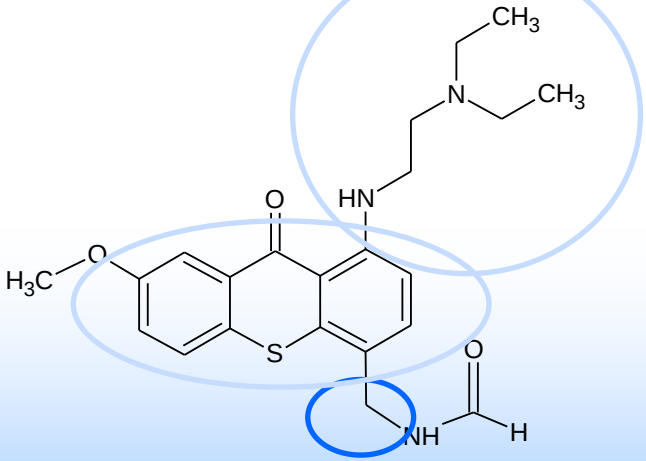
Hycanthone

2



SR 233377

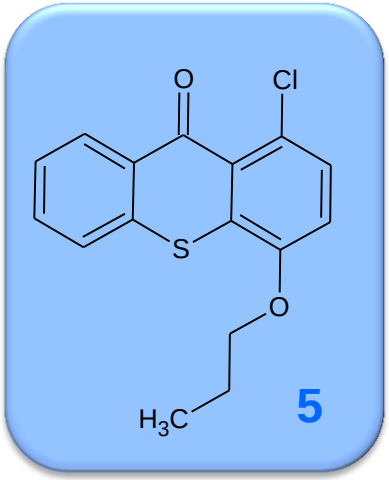
3



SR 271425

4

Thioxanthones investigated as antitumor agents



1-chloro-4-propoxy-9*H*-thioxanthen-9-one

"chemical tool"

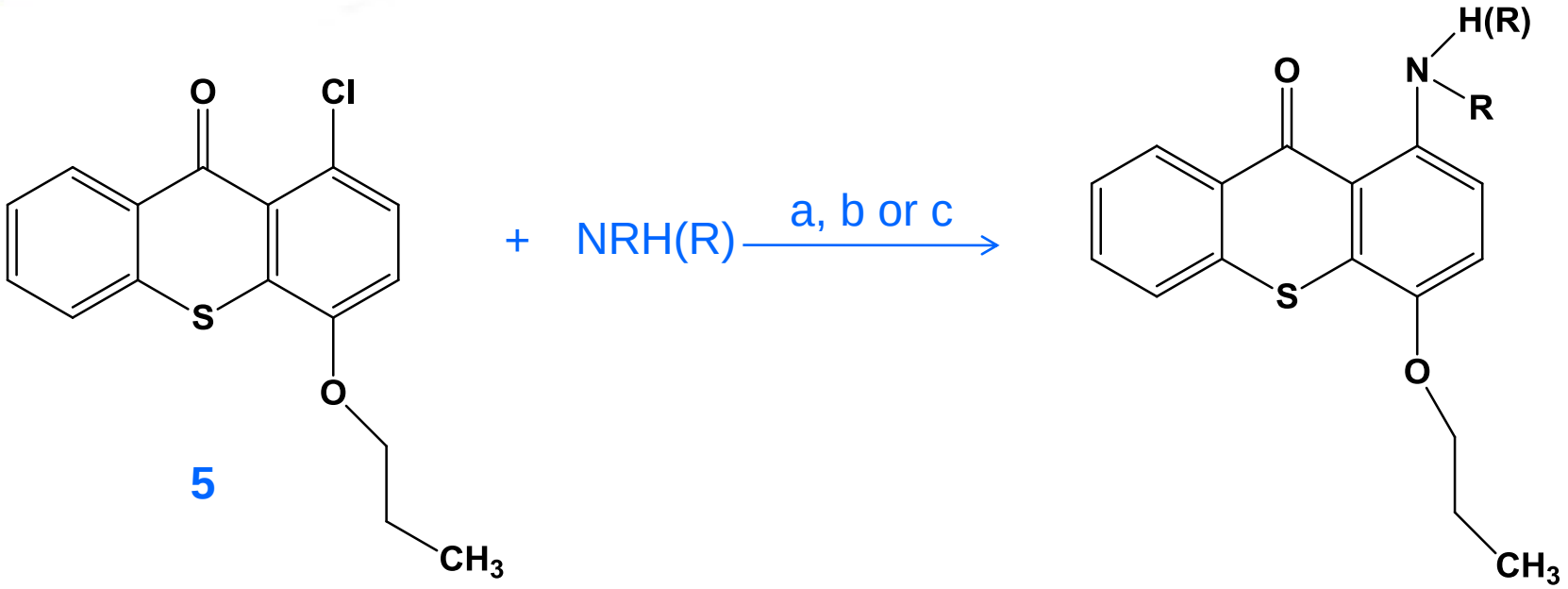
Intercalation

Alkylation/ Mutagenicity

AIMS

◆ To obtain new thioxanthonic derivatives with potent **cell growth inhibitory** effect, more **effective** and **safe**, throught the synthesis of a diversity of aminated thioxanthones by appendance of different primary and secondary amines

PROTOCOL

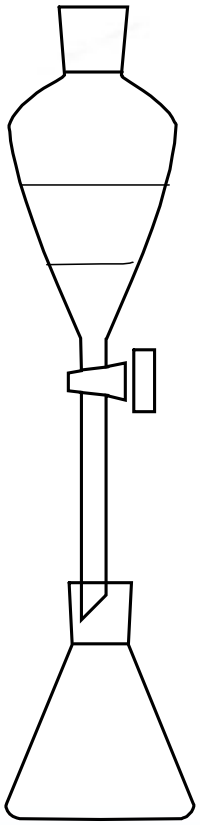


a) Cu_2O, MeOH, 100°C, 2 days
b) Cu_2O, K_2CO_3, MeOH, 100°C, 2 days
c) Cu_2O, K_2CO_3, NMP, MW 205°C, 50 min

27 thioxanthonic derivatives were obtained

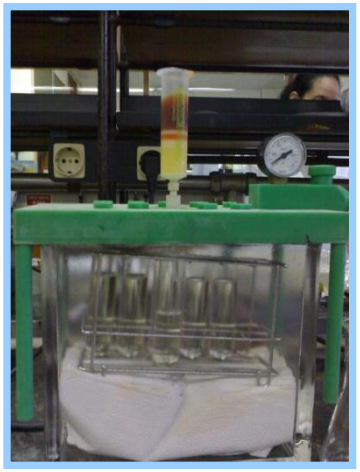
PROTOCOL

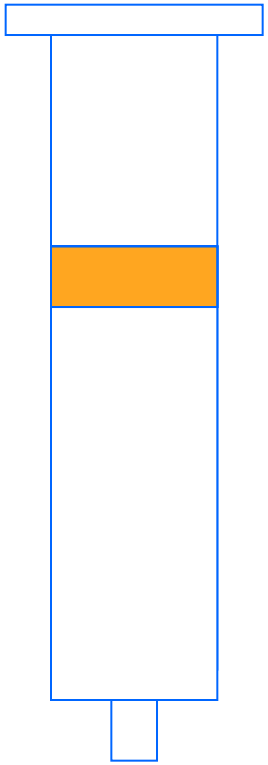
Liquid-liquid extraction



NMP/ether

Flash chromatography using silica columns





→ Amine

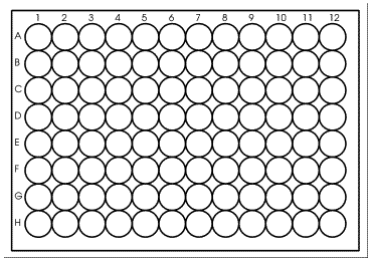
→ **Thioxanthone** (product)

→ Thioxanthone (reagent)

• Structure elucidation (^{1}H NMR, ^{13}C NMR, HMBC, HSQC, elemental analysis)

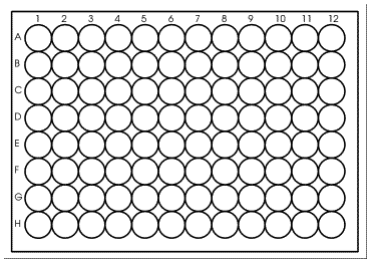
PROTOCOL

Cell growth inhibition



37ºC

24h



37ºC

48h

Cell growth analysed using the **sulphorhodamine B assay**

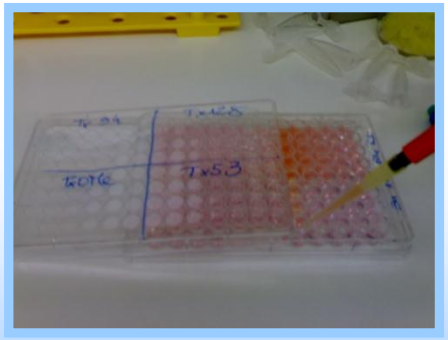
Melanoma (A375-C5)

Breast cancer (MCF-7)

Lung cancer (NCI-H460)

Chronic myelogenous leukemia (K562)

Serial dilutions of test compounds

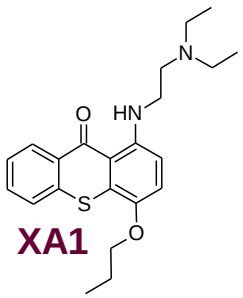


Levels of apoptosis (flow cytometry)

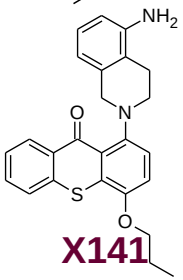
Cell cycle (flow cytometry)

RESULTS

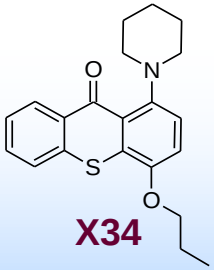
Cell growth inhibition



XA1



X141



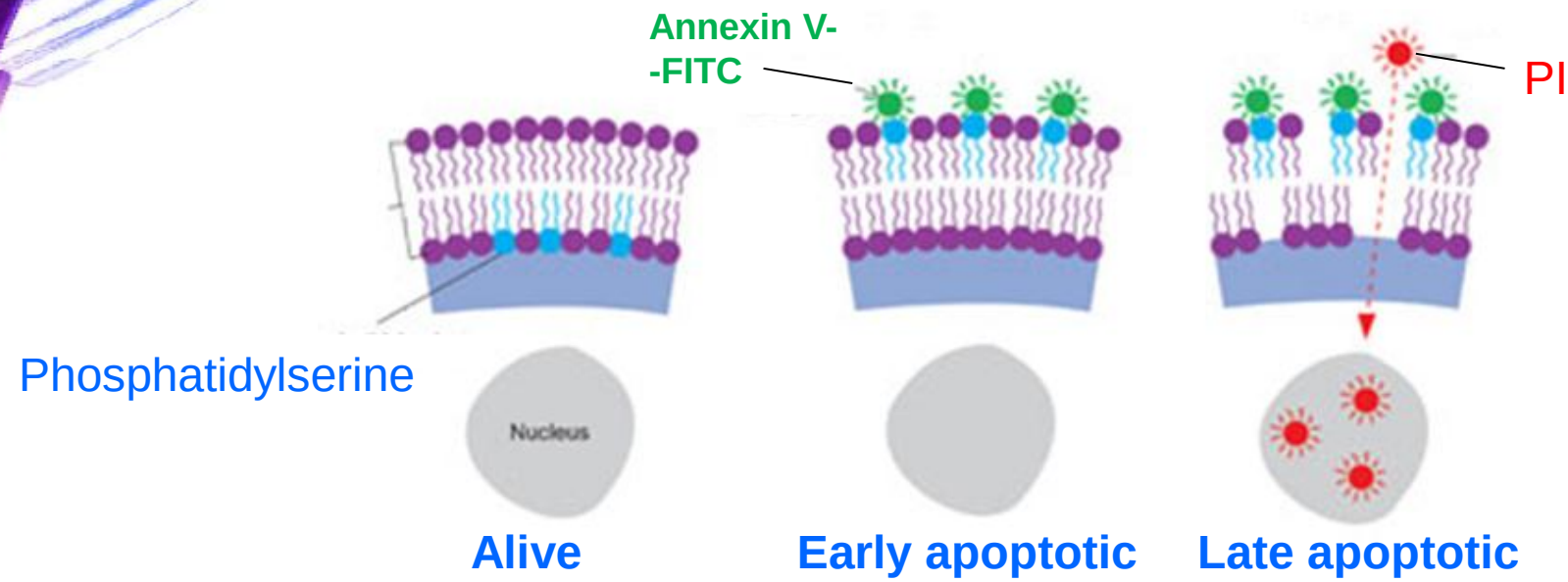
X34

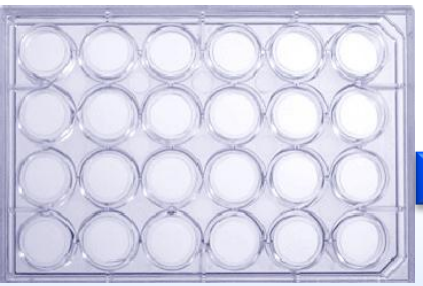
	GI_{50} (µM)											
	K562 (Leukemia)			A375-C5 (Melanoma)			MCF-7 (Breast Cancer)			NCI-H460 (Lung Cancer)		
X104	16.5	±	3.06	H			H			H		
X105	H			81.67	±	2.85	85	±	1	58.33	±	7.17
X127	12.98	±	0.36	H			H			H	±	
X128	19.23	±	0.98	55	±	1.53	65.67	±	1.45	59.33	±	7.88
X129	104.71	±	7.29	H			H			H		
X131	15.57	±	3.15	H			H			H		
X141	3	±	0.48	24.33	±	3.28	34	±	3.06	39.33	±	6.44
X15	21.47	±	2.61	33	±	2.65	33	±	2.31	27.33	±	2.73
X18	4.81	±	4.21	27.33	±	5.33	48	±	3.05	38.67	±	4.41
X34	3.72	±	1.47	13.33	±	0.33	15	±	1	13.33	±	0.67
X41	16.22	±	0.48	73	±	5.5	111	±	11.72	73	±	6.62
X48	16.99	±	2.33	20	±	2.08	28.33	±	2.85	22.67	±	2.52
X53	29.79	±	3.02	60.33	±	1.85	92.67	±	2.6	89.67	±	2.4
X54	H			H			H			H		
X62	59.45	±	2.77	H			H			H		
X79	60.58	±	2.01	H			H			H		
X86	20.96	±	2.08	54.75	±	9.42	70.75	±	6.9	61.25	±	4.12
X87	92.92	±	3.33	H			H			H		
X96	18.13	±	4.35	34	±	4.04	34.33	±	2.96	35	±	5.2
XA1	1.9	±	0.15	8.5			10.6			8.8		
XA3	H			H			H			H		
XA4	52.95	±	1.47	H			H			H		
XAc	13.57	±	2.96	8.23	±	0.34	9.37	±	0.18	8.7	±	0.2
XOH	22.73	±	0.64	15.33	±	0.88	23.33	±	1.45	21	±	1.53
XOH131	4.38	±	0.44	34.33	±	1.86	24.33	±	2.85	23	±	2.52
XOH1H	74.32	±	7.16	34	±	6.51	39	±	3.61	28.67	±	2.19
XOMe	4.47	±	1.93	H			H			H		

→ No significant effect on MRC-5 cell line

PROTOCOL

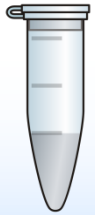
Levels of apoptosis





48h

37ºC



Centrifuge
Ressuspend

Annexin V-FITC

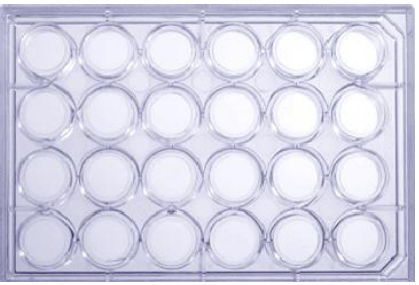
10 min, RT

Propidium iodide

2 min, 4ºC

- 2.0x10^5 K562 cells / mL
- **X34, X141, XA1, XOMe** and **X18** at the concentration of the GI_{50}

PROTOCOL



- $2.0x10^5$ K562 cells / mL
- **X34, X141, XA1, XOMe** and **X18** at the concentration of the GI_{50}

10 min, RT

Centrifuge

Ressuspend in ethanol 70%

30 min

4ºC

Centrifuge

Ressuspend in **Propidium Iodide** working solution

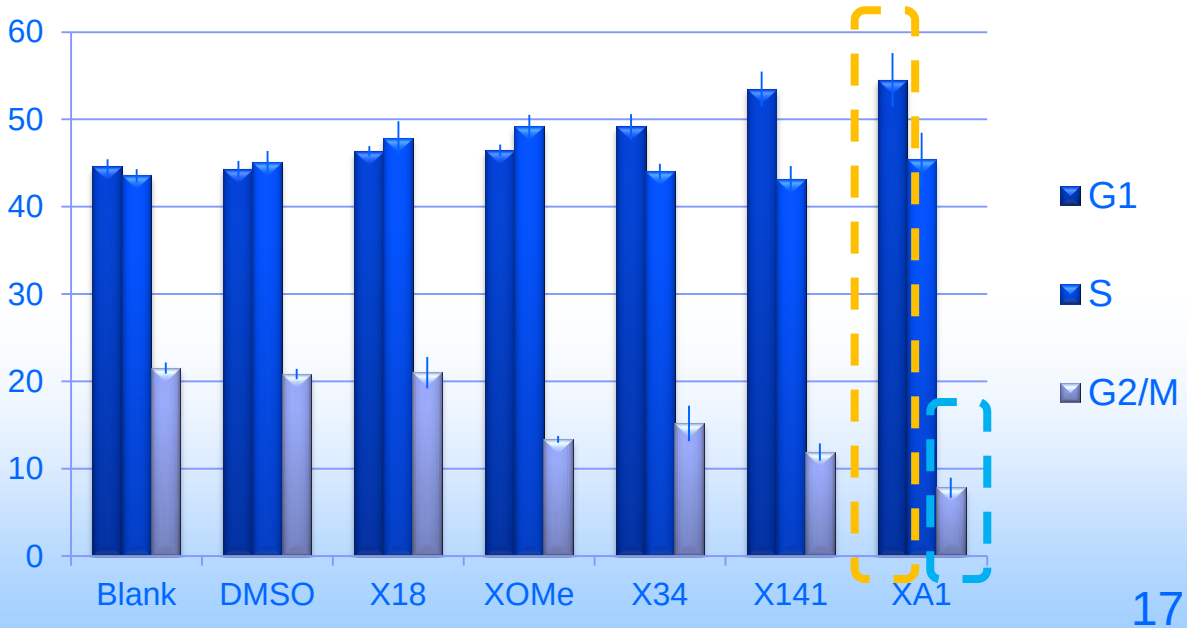
Propidium Iodide working solution = 40µg/ml PI, 100µg/ml RNase in PBS

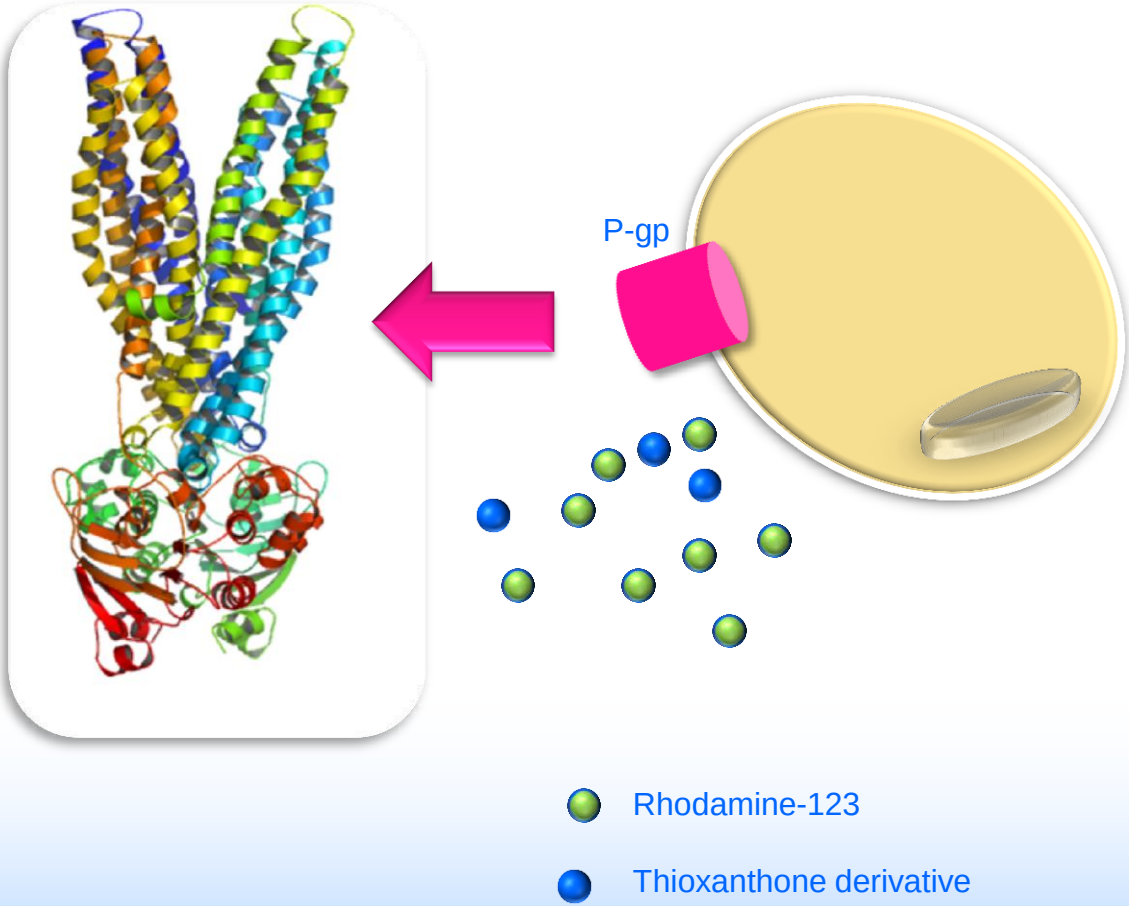
RESULTS

Treatment	Viable	Apoptotic
Blank	95.70%	3.90%
DMSO	92.90%	6.50%
X18	89.10%	9.20%
XOMe	86.40%	13.00%
X34	81.80%	16.80%
X141	81.70%	17.60%
XA1	77.40%	22.10%

↑ apoptosis

G1-phase arrest



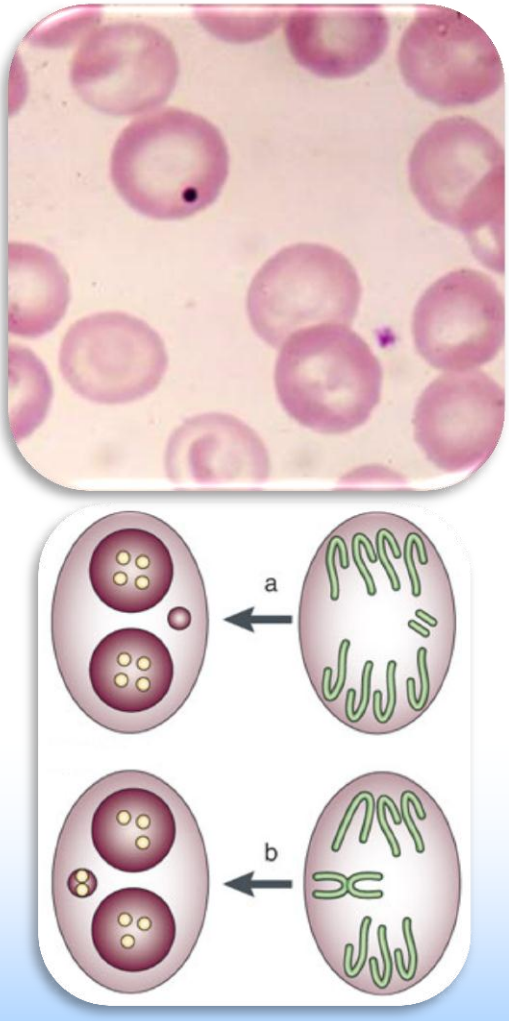


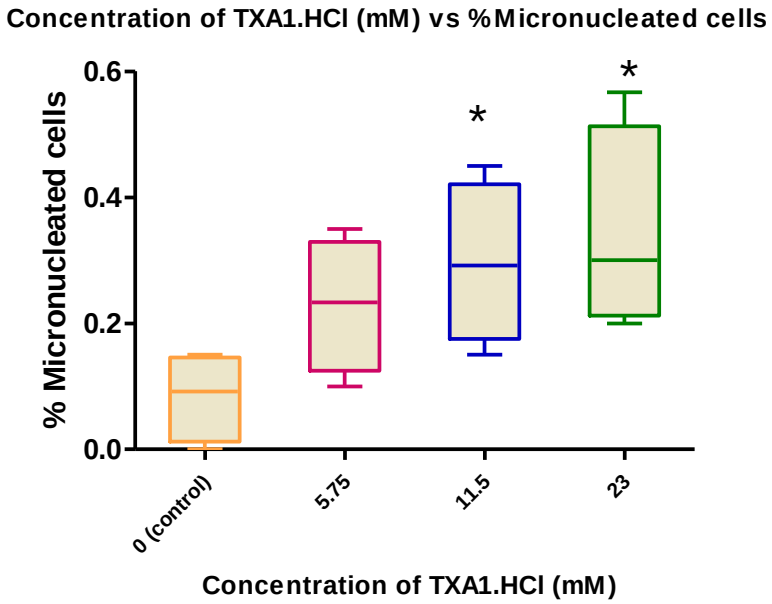
P-glycoprotein (P-gp) inhibition

- Increased rhodamine accumulation in a P-gp overexpressing cell line
- Competitive and noncompetitive inhibitors
- Partially reversed resistance of a P-gp overexpressing cell line to doxorubicin
(submitted for publication)

PROTOCOL

Micronuclei assay*





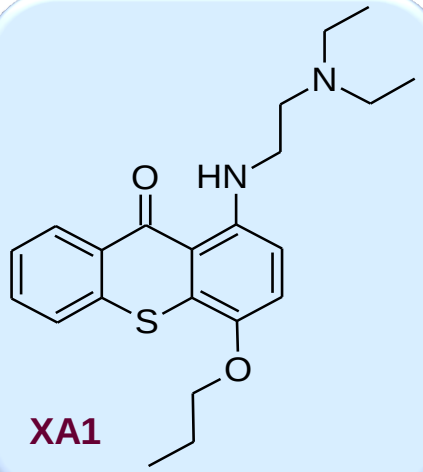
Not genotoxic at the lowest conc. tested

*Ana Sara Gomes, Davide Vieira, Emília Sousa, Carlos Afonso, Madalena Pinto, Félix Carvalho, Alice Santos-Silva, "*Synthesis and genotoxicity evaluation of a thioxanthone derivative*". 4th Meeting Young Researchers of University of Porto (IJUP'11), Porto, Portugal, 17-19 February 2011

CONCLUSION

♦ **Appendance diversity and the introduction of amines to the thioxanthone scaffold was a successful strategy in the discovery of potent possible antitumor agents**

♦ **Both increase in the level of apoptosis and/or cell cycle arrest seem to be implicated in the growth inhibitory effect induced by the new thioxanthonic derivatives**



XA1

NEXT STEP…

Cardiotoxicity?

Intercalation?

Synthesis of XA1 derivatives

ACKNOWLEDGEMENT

Prof.Doutora Emília Sousa
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Prof.Doutora Helena Vasconcelos

CEQUIMED-UP (FFUP)
Cancer Drug Resistance Group(IPATIMUP)