Targeting Tubulin – a Tale of the Development of the Combretazets

Dr. Niamh O'Boyle

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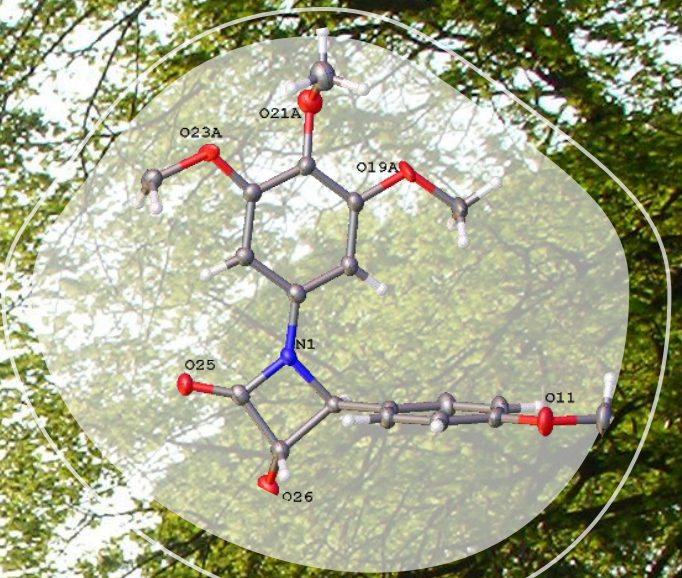
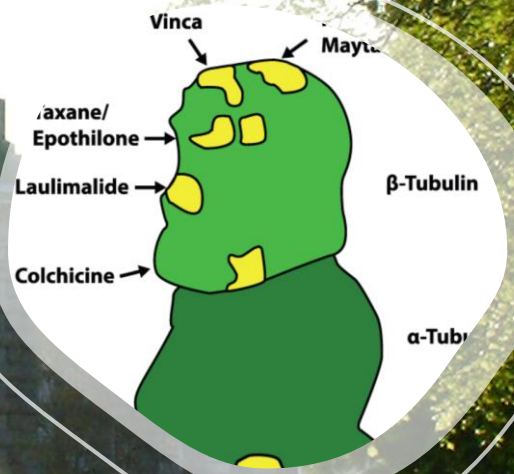




CELL DIVISION

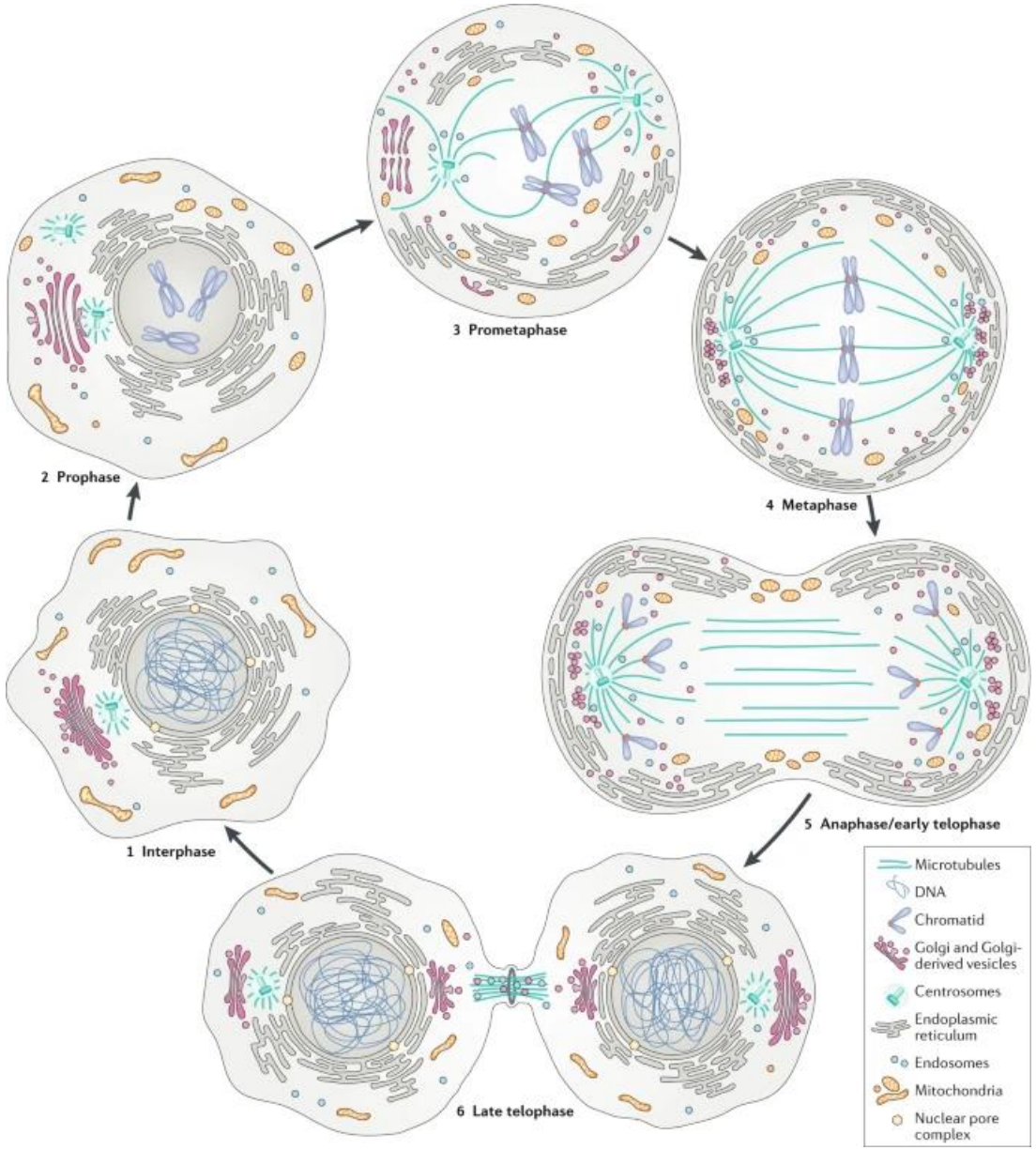
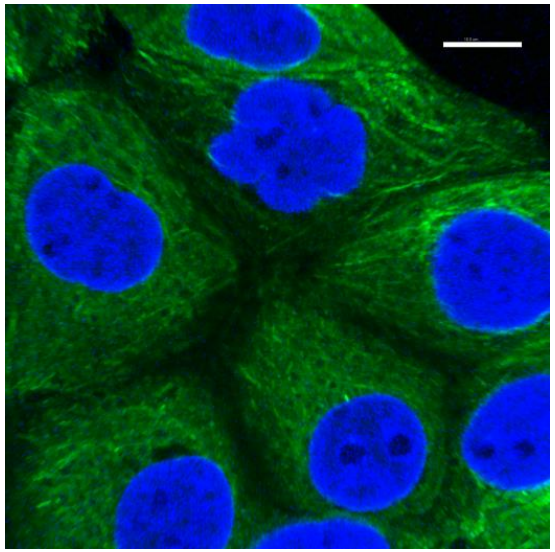
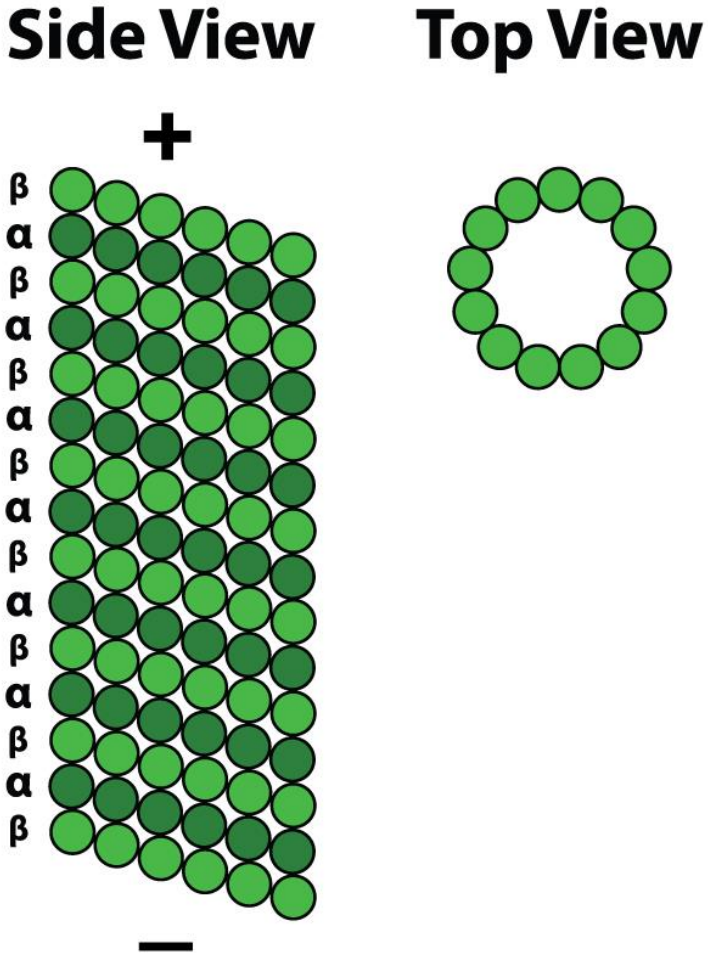


Image from: https://doi.org/10.1038/s41580-019-0208-1

THE TARGET | TUBULIN

- Main constituent of (dynamic) microtubules
- αβ dimeric protein





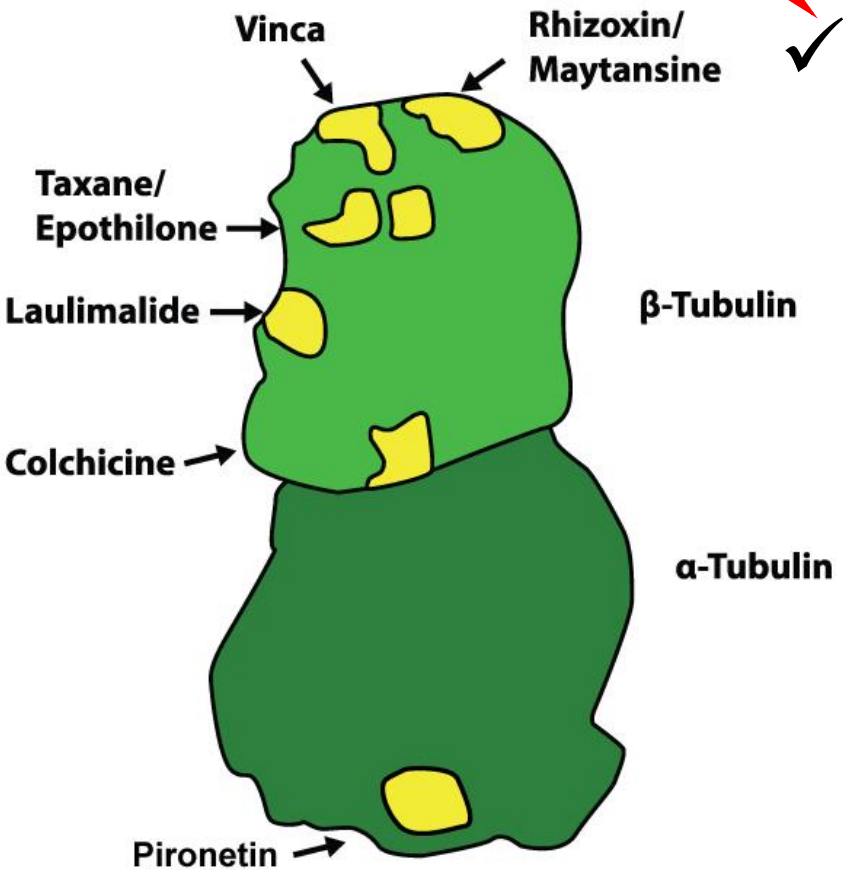
DRUGS TARGETING TUBULIN

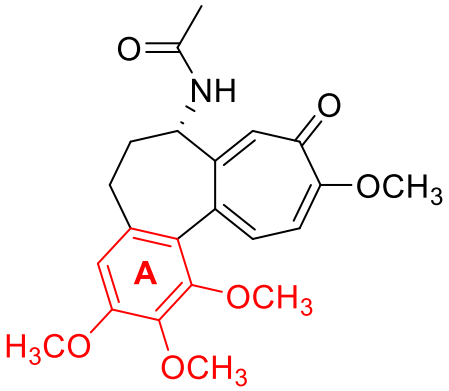
VINCA ALKALOIDS (DEPOLYMERISING)

MONOMETHYL AURISTATIN E (ADC)

DM1 (ADC)

PACLITAXEL (POLYMERISING)





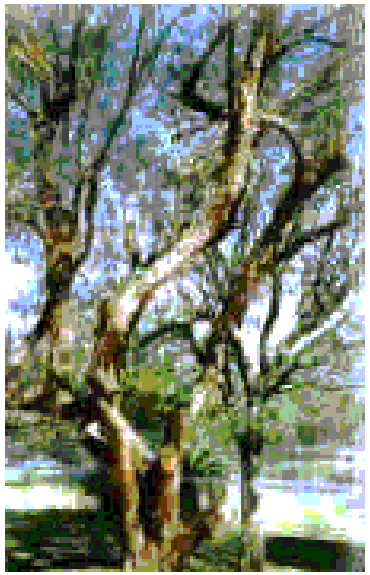
Colchicine

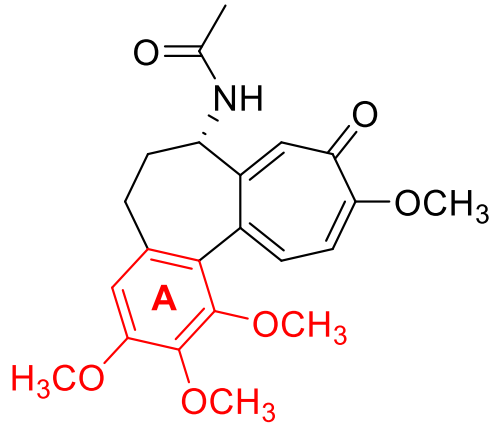
= DRUG RESISTANCE

AUTUMN CROCUS | *COLCHICUM AUTUMNALE*

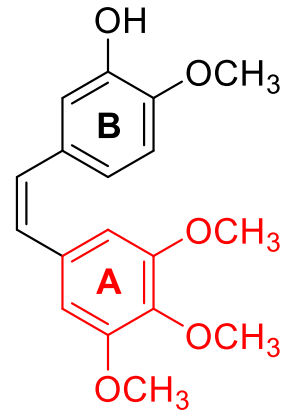
TARGETING THE COLCHICINE-BINDING SITE

- Colchicine – nephrotoxic at therapeutic doses
- Combretastatin A-4
  - Water solubility
  - Isomerisation





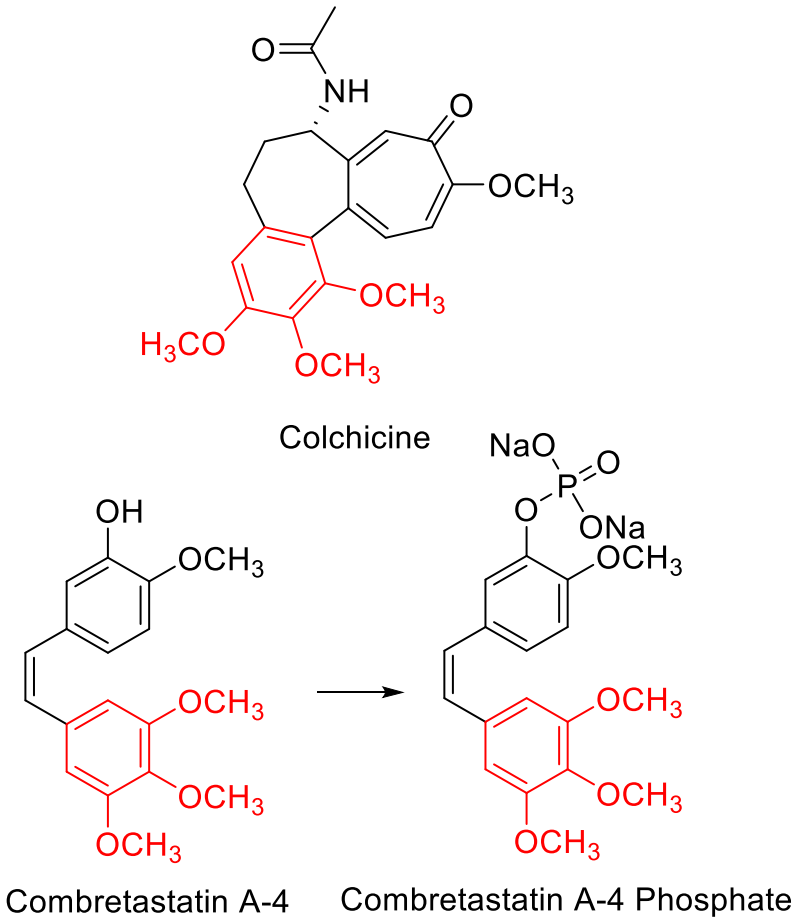
Colchicine



Combretastatin A-4

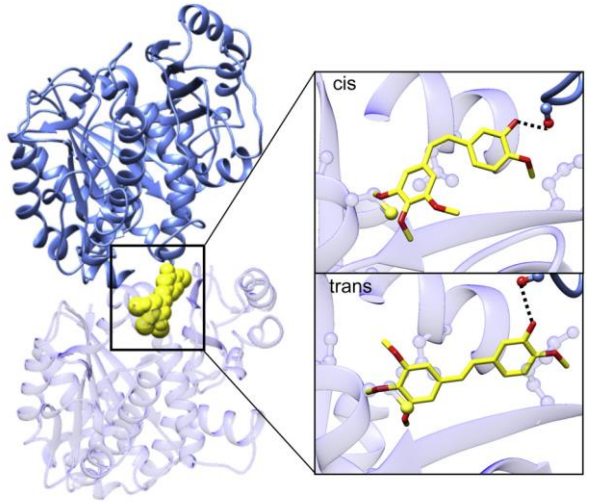
COMBRETASTATIN A-4

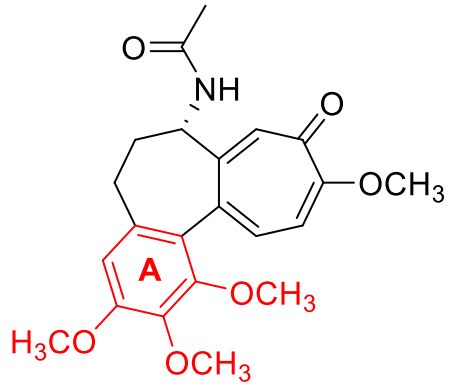
- Water solubility – prodrug formation ✓



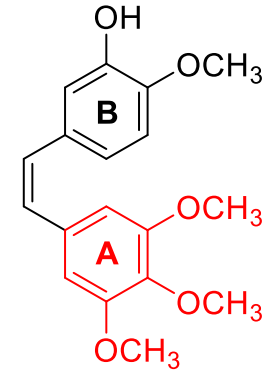
COMBRETASTATIN A-4

- Isomerisation
- The affinity of *cis* CA-4 is predicted to be 10,000-fold larger than that of *trans* CA-4

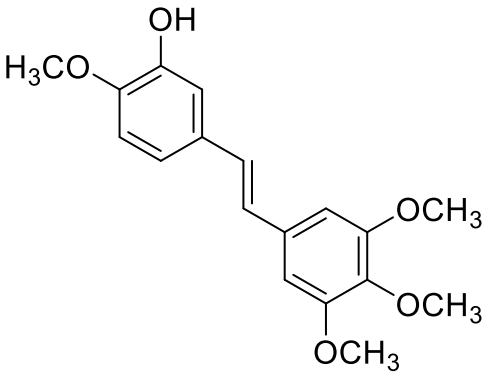




Colchicine



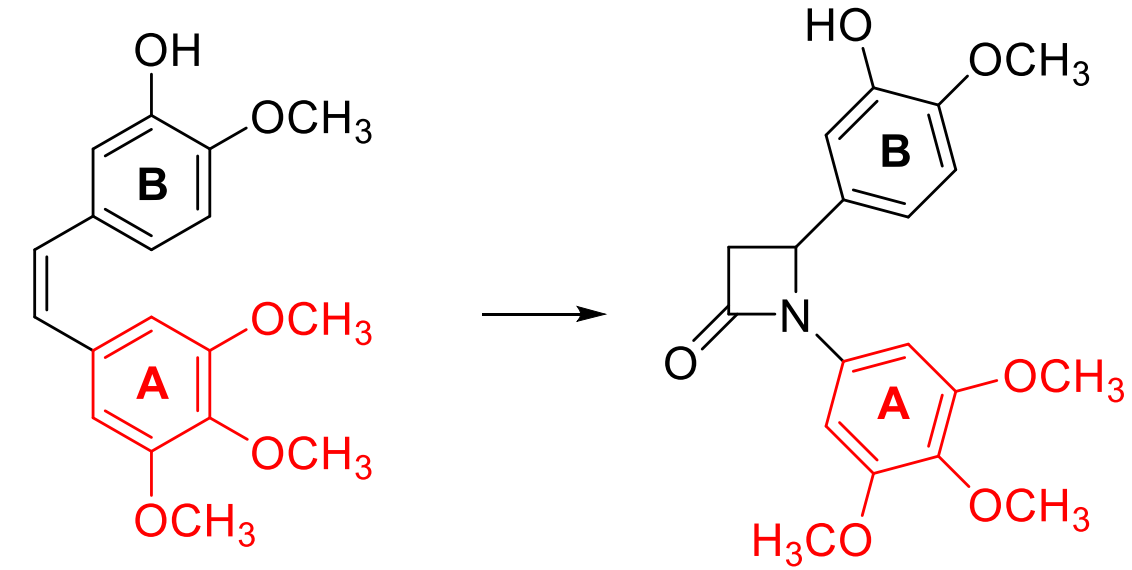
Combretastatin A-4



Trans isomer

CIS RESTRICTION

- β-Lactam (azetidinone) structure

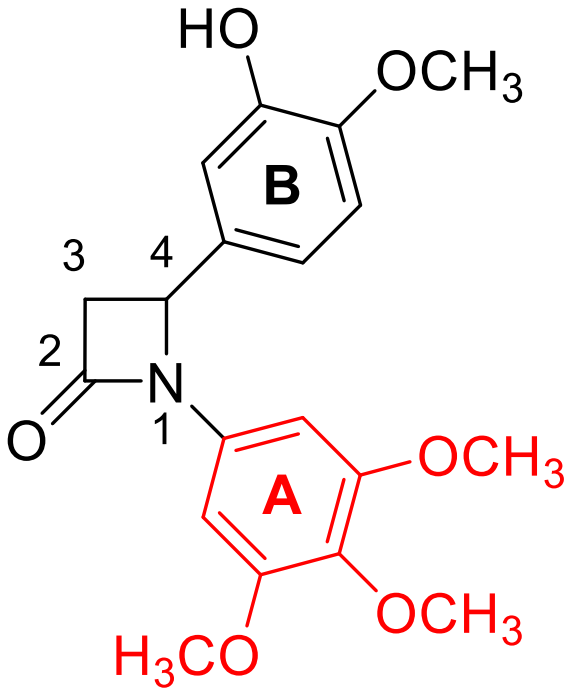


Combretastatin A-4

Combretazets

COMBRETAZETS | STRUCTURE-ACTIVITY RELATIONSHIPS

Position 3:
Hydroxyl
Phenyl



Combretazets

Position 4:
Fluoro
Hydroxy

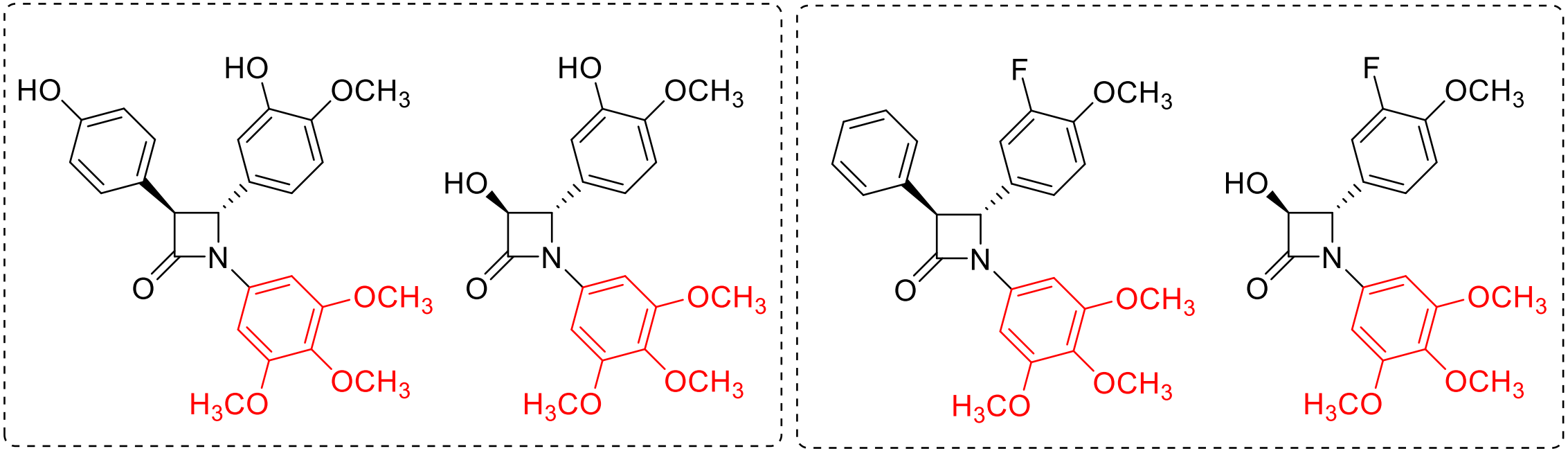
Position 1:
Trimethoxyphenyl A-ring best

Carr et al. Eur. J. Med. Chem. 45 (12; 2010, 5752-5766
O'Boyle et al. J. Med. Chem. 53, (24), 2010, p8569-8584
O'Boyle et al. Eur. J. Med. Chem. 46, (9), 2011, p4595 – 4607
O'Boyle et al. Bioorg. Med. Chem. 19, (7), 2011, p2306-2325
O'Boyle et al. Eur. J. Med. Chem. 62, 2013, p705-721
Greene et al. J. Med. Chem. 59, (1), 2016, p90 – 113
Malebari et al. Eur. J. Med. Chem. 130, 2017, p261-285
Wang et al. Pharmaceuticals, 2019 12(2):56
Malebari et al. Eur. J. Med. Chem. 189, 2020, 112050
Malebari et al. Pharmaceuticals, 14, (11), 2021, 1119

BEST RACEMIC ANALOGUES

Breast cancer (incl. TNBC)

Drug-resistant colon cancer

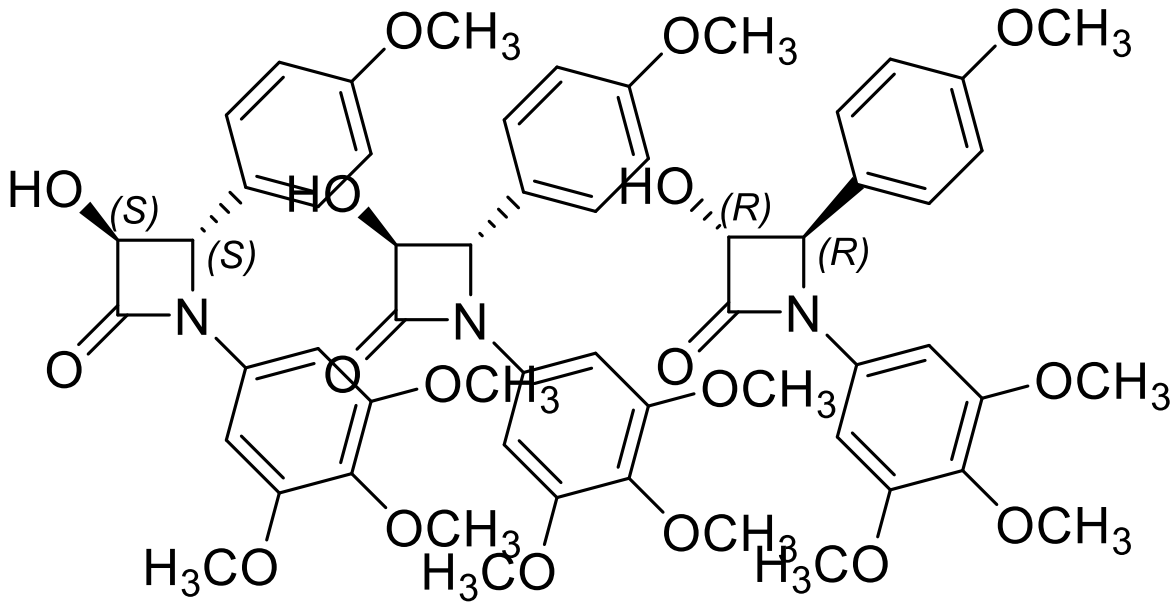


IC_{50} **MCF-7**:	0.8 nM	3 nM	17 nM	22 nM
IC_{50} **HT-29**:	Nd	463 nM	9 nM	3 nM

CA-4 IC_{50} MCF-7: 5.2 nM
CA-4 IC_{50} HT-29: 4165 nM

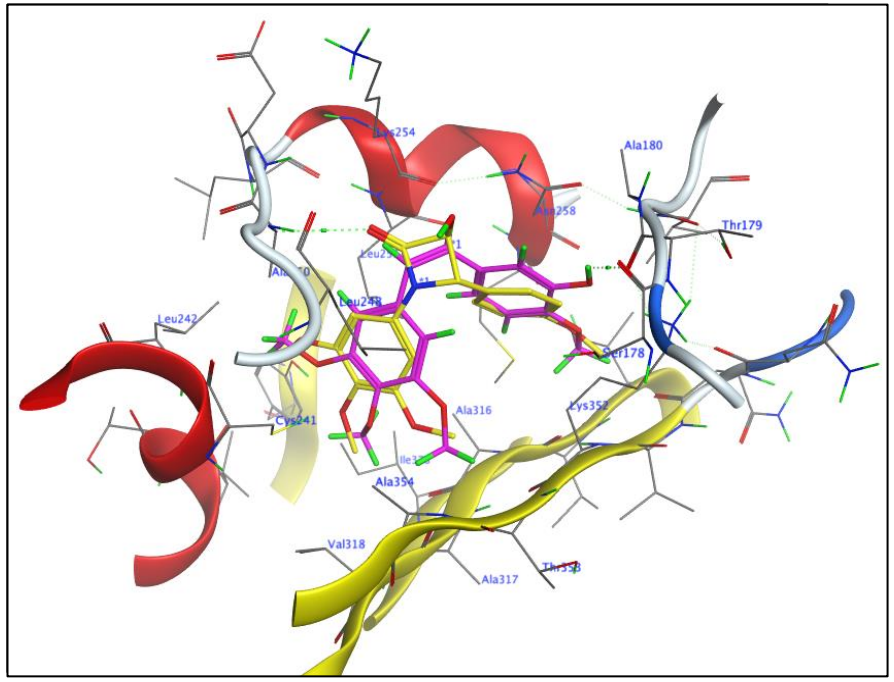
O'Boyle et al. J. Med. Chem. 53, (24), 2010, p8569-8584
Malebari et al. Eur. J. Med. Chem. 130, 2017, p261-285
Malebari et al. Eur. J. Med. Chem. 189, 2020, 112050

WHAT WOULD YOU DO NEXT?

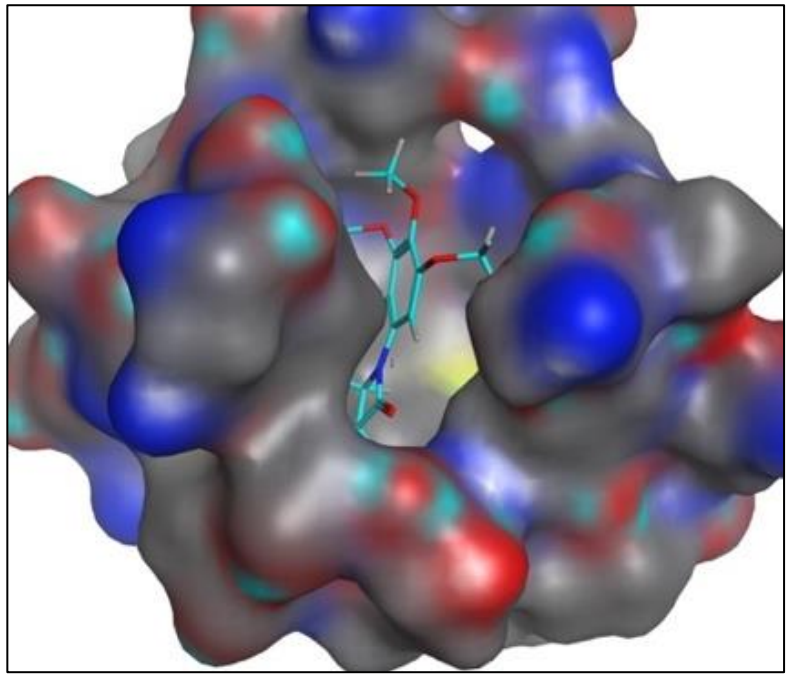


MOLECULAR MODELLING

- Enantiomer 1 (*3S, 4S;* yellow) and enantiomer 2 (*3R, 4R;* cyan) in the colchicine binding site
  - Red: Hydrophilic pocket interactions; blue: hydrophobic pocket interactions



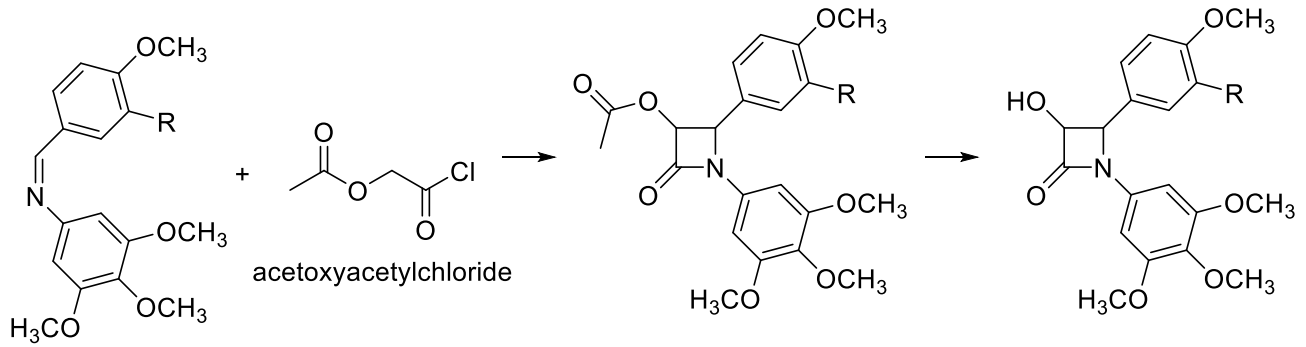
Predicted number of binding interactions: 25
Carbonyl hydrogen bond to Ala-250 and 3-OH pointing out of the colchicine binding pocket



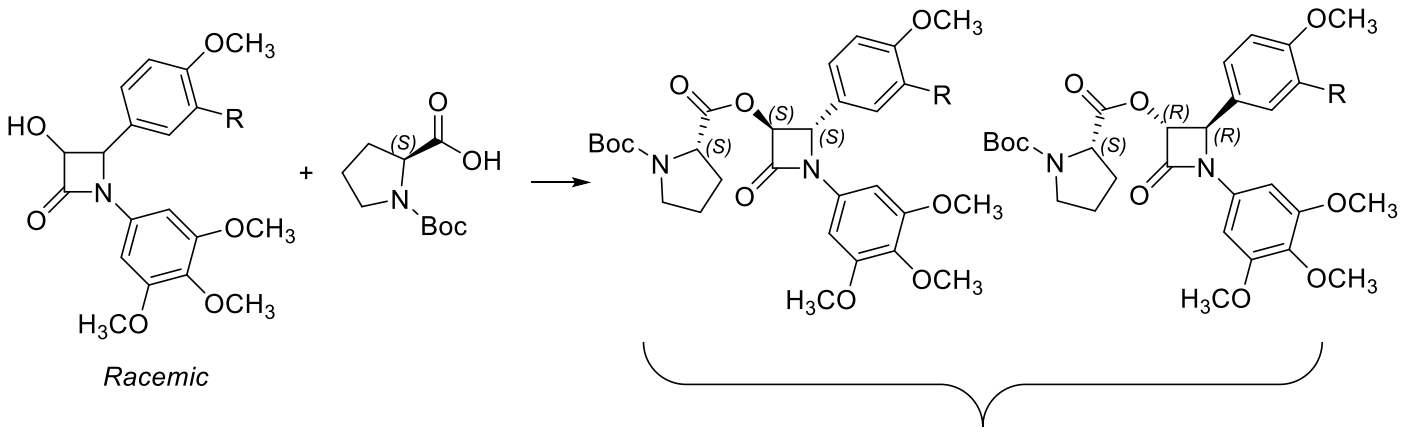
Predicted number of binding interactions: 18
A ring and carbonyl point out of pocket without the critical Ala-250 interaction

PLAN FOR SYNTHESIS

Steps 1 and 2: Beta-lactam formation (2 step - Staudinger reaction followed by removal of acetoxy)

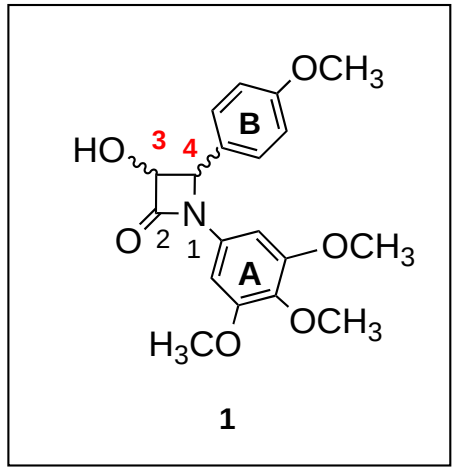


Steps 3 and 4: Derivitisation with Boc-protected proline, separation, and removal of proline

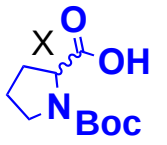


Separate by column chromatography
Remove proline residue by reaction with hydrazine dihydrochloride

CHIRAL RESOLUTION WITH AMINO ACIDS

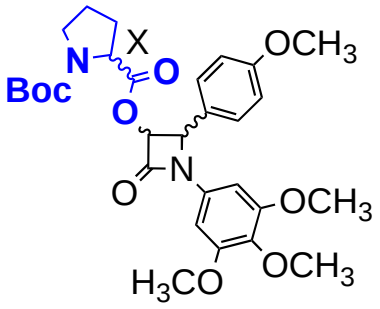


1

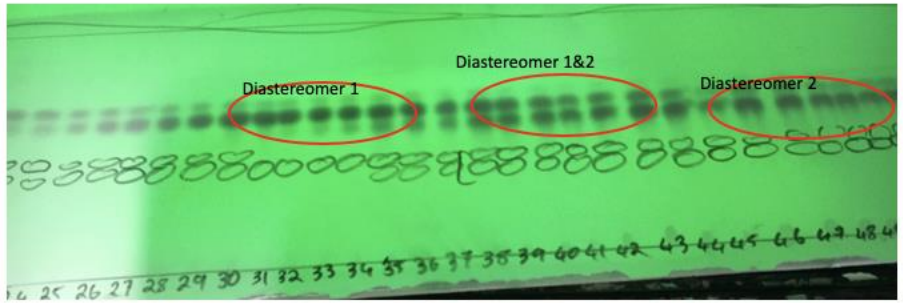


D- proline X= *R*
L- proline X= *S*

a

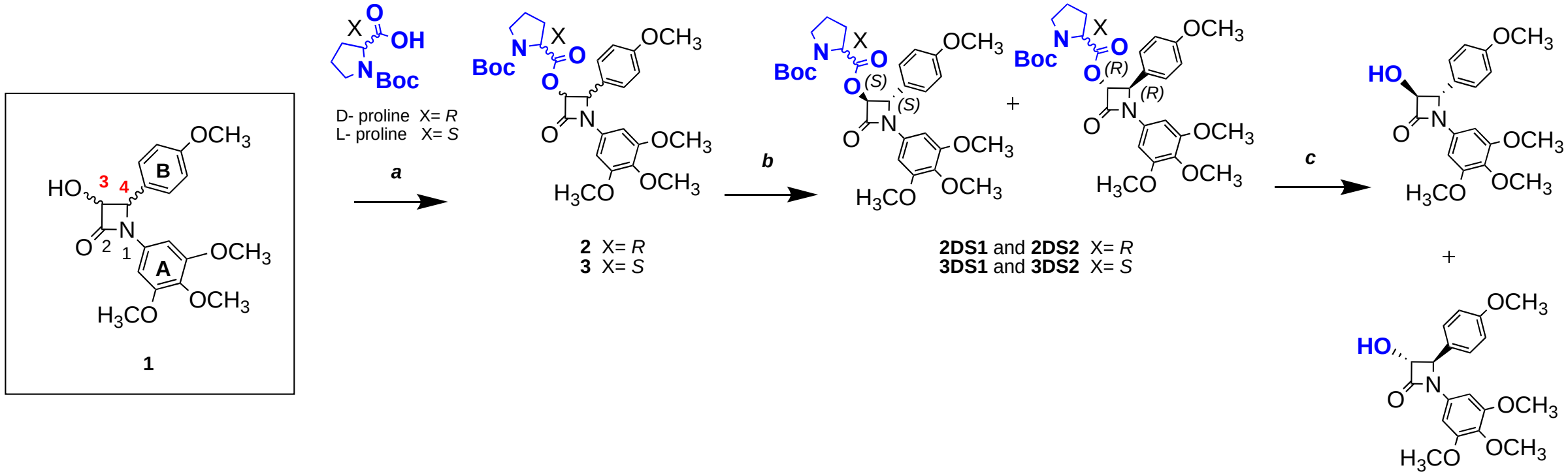


2 X= *R*
3 X= *S*



Scheme 1. Reagents and conditions: **a:** HBTU (2 eq), DIPEA (30 eq), anhydrous MeCN (30 mL), N_2, rt, 24 hr. Yield = 31-77%. **b:** resolution of diastereomers using gravity and gradient elution of MTBE: *n*-hexane (2:8-2:1). **c:** Hydrazine dihydrochloride (5 eq), triethylamine (TEA) (9 eq), methanol (MeOH) (30 mL) at 0 °C, then reflux, 6h.

CHIRAL RESOLUTION WITH AMINO ACIDS



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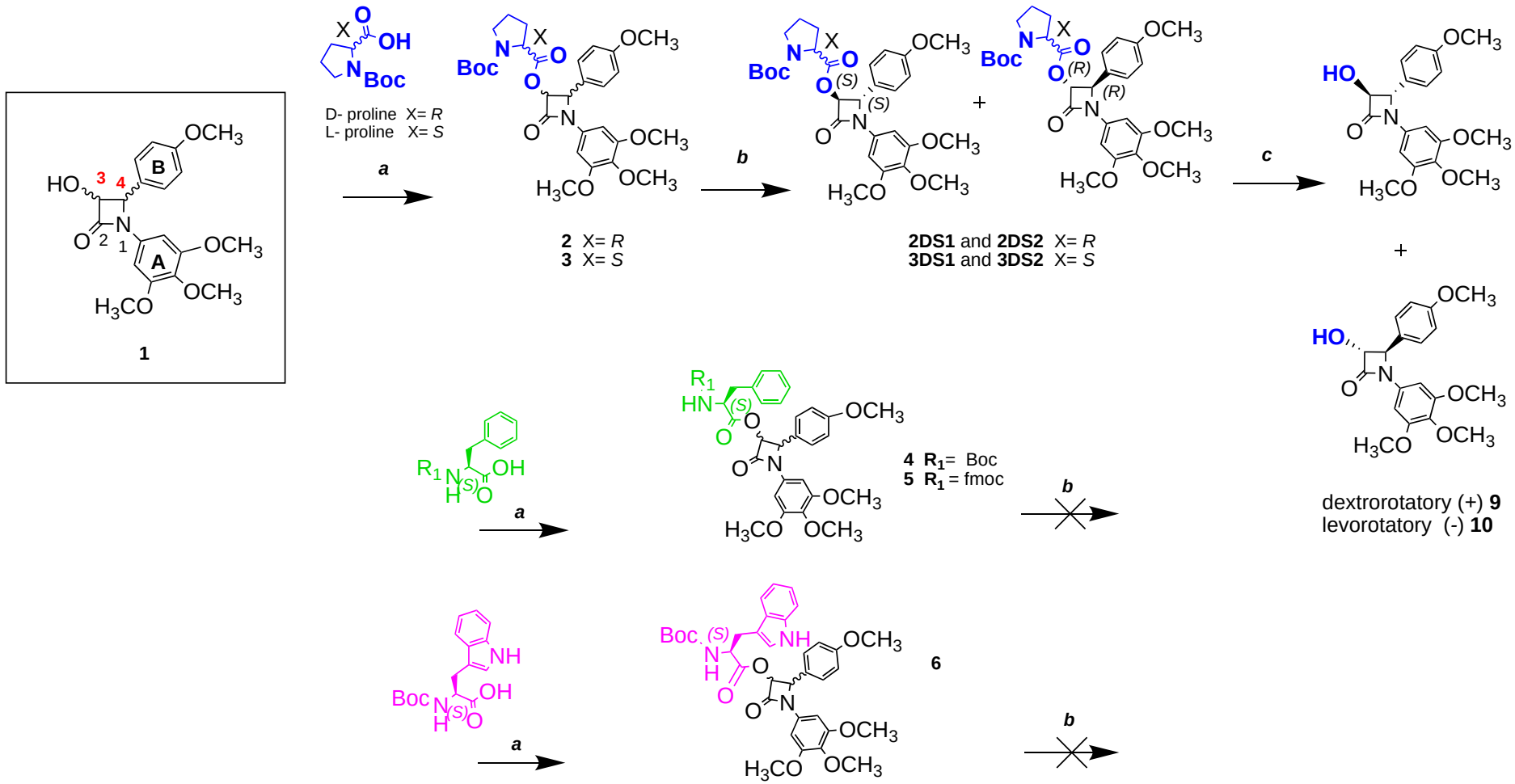
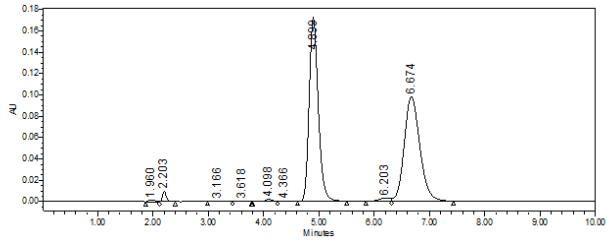
CHIRAL RESOLUTION

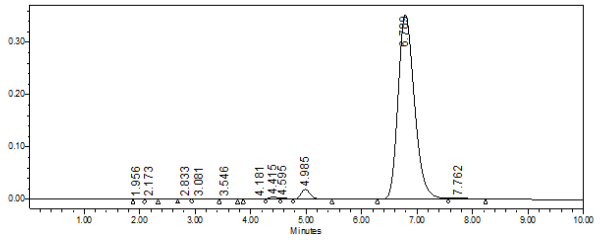


CHIRAL PURITY | STEREOCHEMISTRY

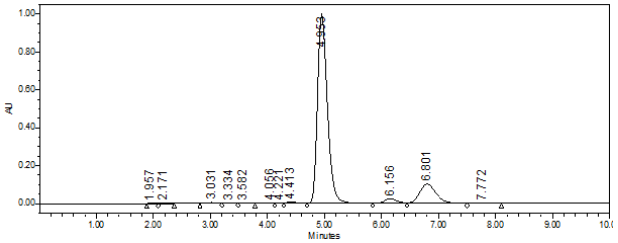
Chiral HPLC data for 01R



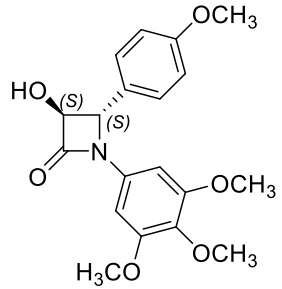
Chiral HPLC data for 01En1

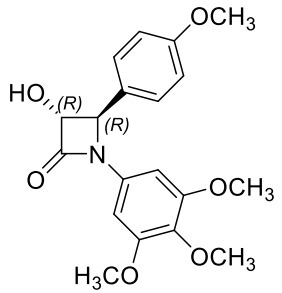


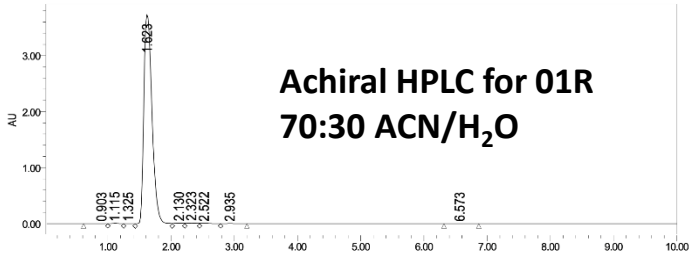
Chiral HPLC data for 01En2



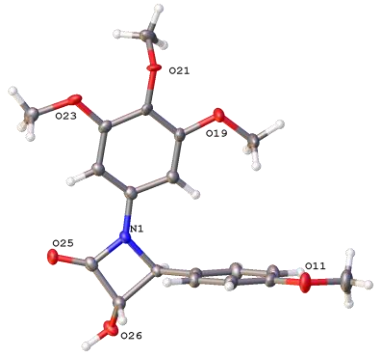
	Major Peak Area (%)	Minor Peak Area (%)	% *ee*
01En1 (*S,S*)	96.9	3.0	94 %
01En2 (*R,R*)	85.4	14.6	71 %

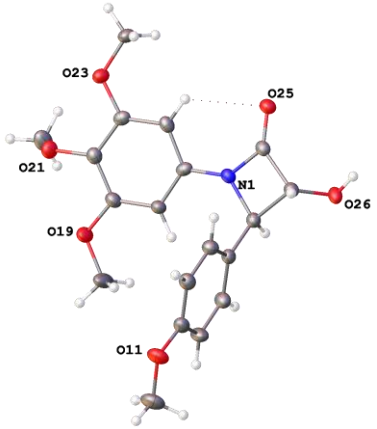






Achiral HPLC for 01R
70:30 ACN/H_2O

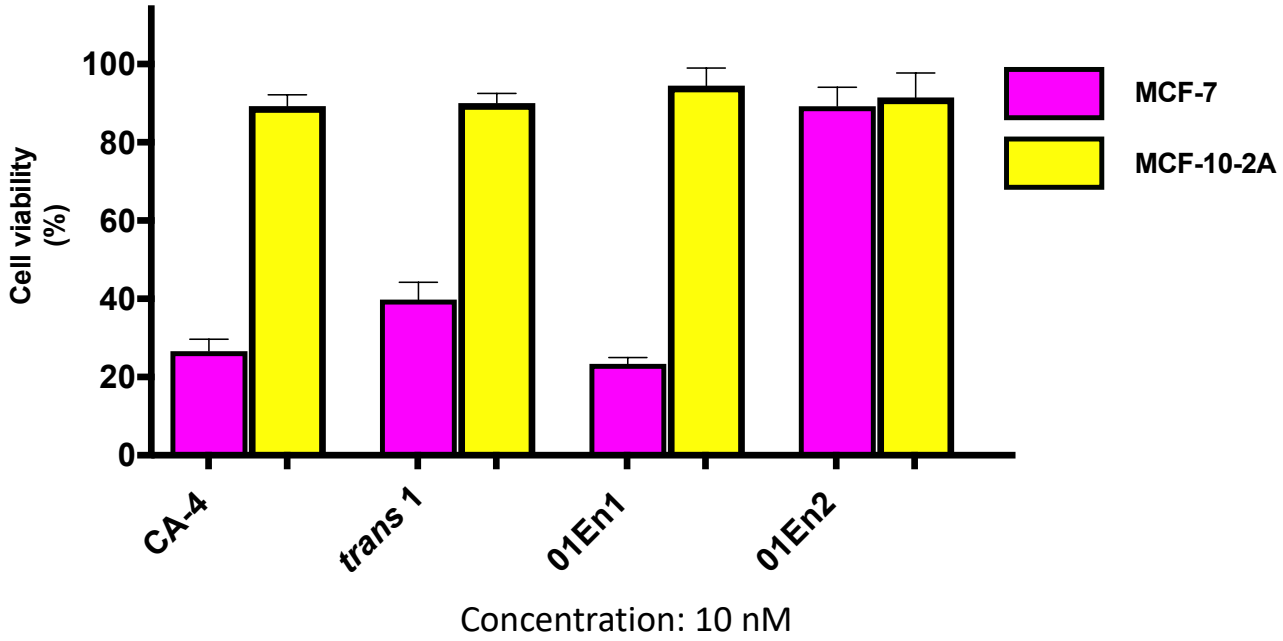




BIOCHEMISTRY | ANTIPROLIFERATIVE ACTIVITY

BIOCHEMISTRY | MCF-10a

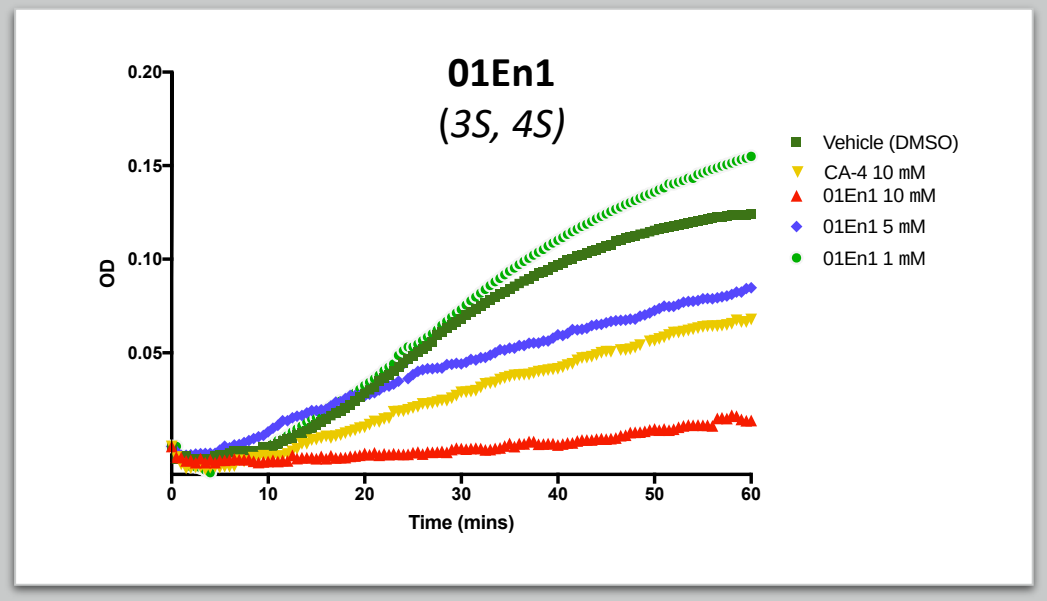
- Human non-cancerous breast epithelial cell line.
- Viability measured by alamarBlue assay.

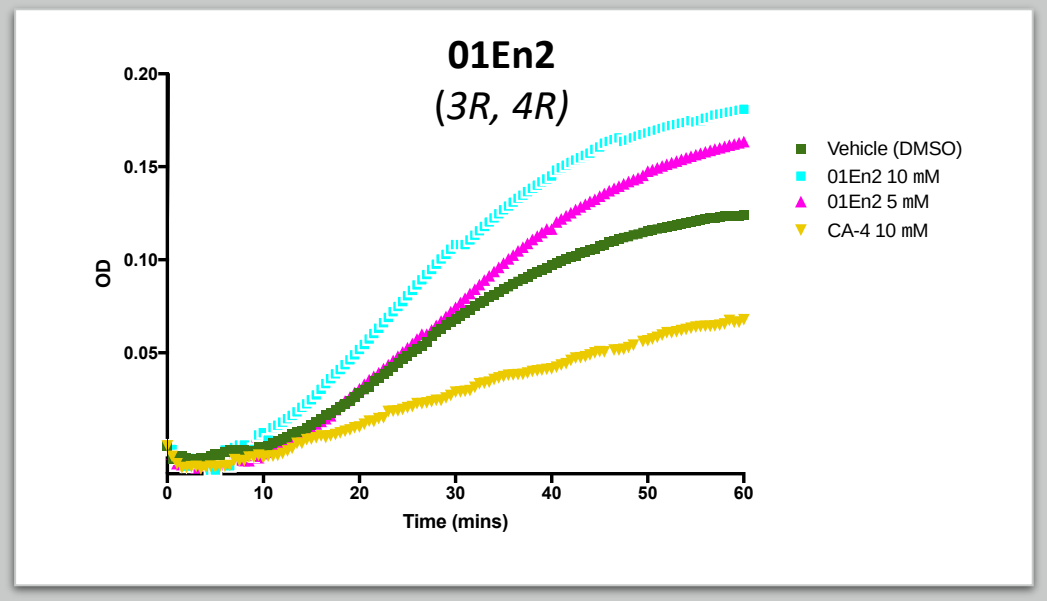


Concentration: 10 nM

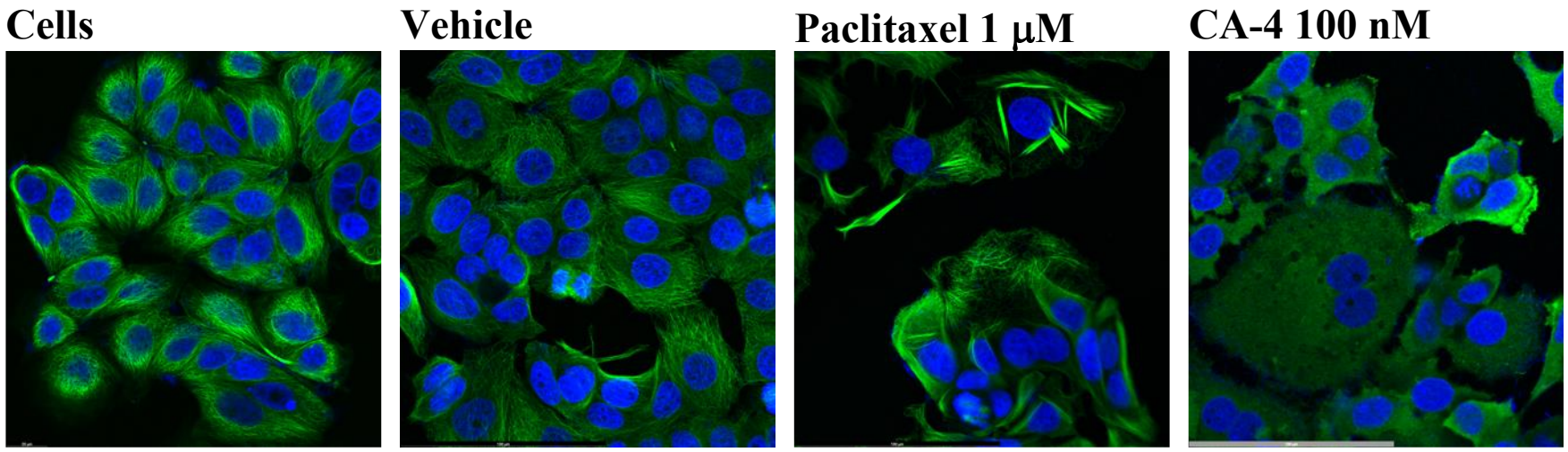
TUBULIN POLYMERISATION

- *In vitro* pure tubulin (porcine)
- Absorbance at 340 nm is proportional to degree of tubulin polymerisation
- CA-4 as positive control



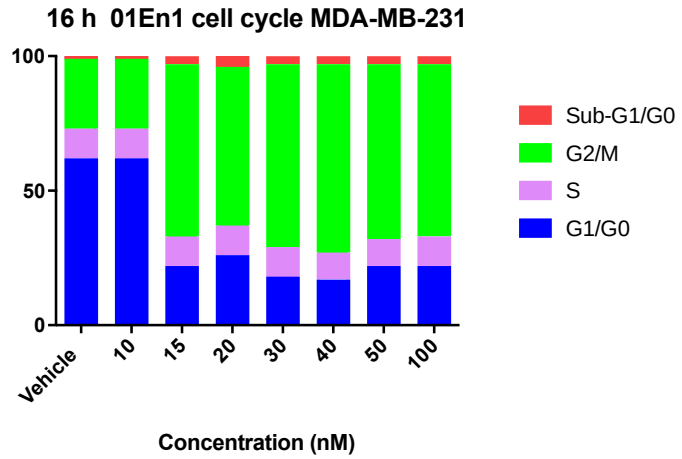


BIOCHEMISTRY | MICROSCOPY



Green: Tubulin (stained with mAb)
Blue: Nuclei (stained with DAPI)

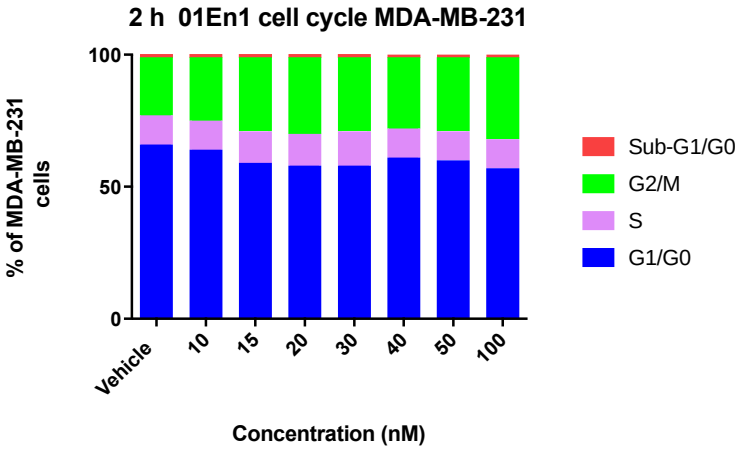
CELL CYCLE

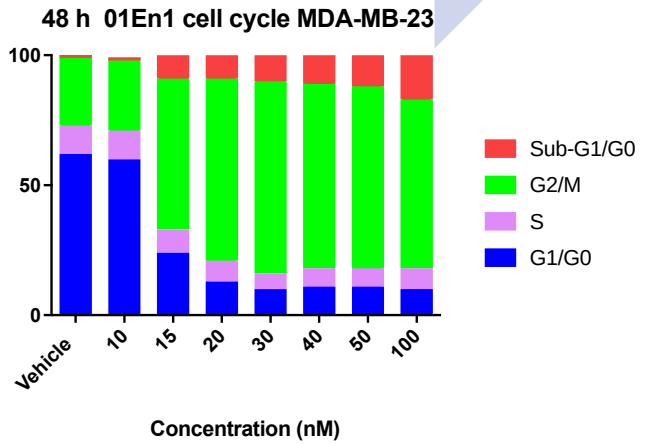


2 hr

16 hr

48 hr





BREAST CANCER

ACKNOWLEDGEMENTS

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Dr. Daniela Zisterer

Dr. Manuel Ruether and Dr. John O'Brien (NMR)

Dr. Brendan Twamley (XRD)

Mr. Conn Power

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