Synthesis and Biological Evaluation of Novel 3-Isopropenyl-β-lactams: Heterocyclic Bridged Analogues of Combretastatin A-4 as Novel Antimitotic Agents in Breast Cancer

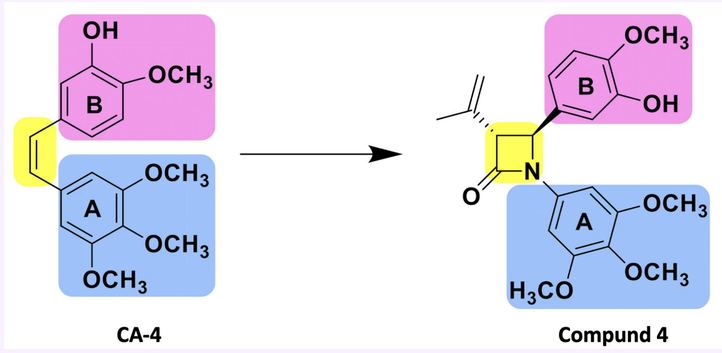
Azizah M. Malebari[1] *, Shu Wang[2], Mary J. Meegan[2]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia; amelibary@kau.edu.sa.

[2]School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Trinity Biomedical Sciences Institute, 152-160 Pearse Street, Dublin 2 DO2R590, Ireland.

Introduction:

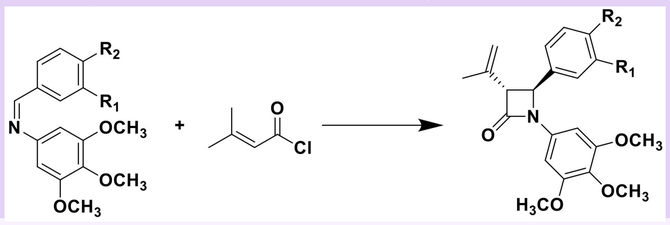
- The use of microtubule-targeted drugs such as taxol is essential in chemotherapeutic treatment of multiple cancer types. [1]
- We have previously investigated the antiproliferative activity of the 1,4-diarylazetidin-2-one (β-lactam) scaffold in MCF-7 breast cancer cell lines [2-3]
- The synthesis of a series of 3-vinyl-β-lactams with potent antiproliferative activity against MCF-7 breast cancer cells has recently been reported [4]
- In this study, we report the synthesis and biological properties of a series of novel 3-isopropenyl-β-lactams (2-azetidinones) which are structurally related to Combretastatin CA-4.
- The 3-isopropenyl-β-lactams in this series contain a 3,4,5-trimethoxyphenyl ring A (required for CA-4), along with different ring B substituents.
- 3-Isopropenyl-2-azetidinones could be promising leads for the development of anti-breast cancer drugs that target tubulin



Results:

Chemistry:

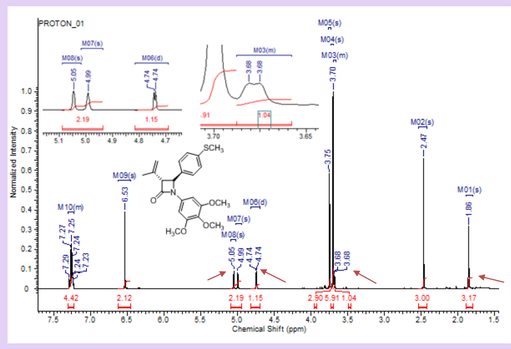
- Synthesis of the 3-isopropenyl-β-lactams was achieved by using appropriate Schiff bases and 3,3-dimethyl acryloyl chloride by Staudinger reaction (Et_3N, DCM, reflux, 5h).
- The products were characterised by IR, ^1H and ^{13}C NMR spectroscopy.



1 R_1=H, R_2=F
2 R_1=H, R_2=Cl
3 R_1=H, R_2=Br
4 R_1=OH, R_2=OCH_3
5 R_1=H, R_2=$N(CH_3)_2$
6 R_1=H, R_2=H
7 R_1=H, R_2=CH_3
8 R_1=H, R_2=OCH_3
9 R_1=H, R_2=OCH_2CH_3
10 R_1=H, R_2=$O(CH_2)_3CH_3$
11 R_1=H, R_2=OPh
12 R_1=H, R_2=OCH_2Ph
13 R_1=H, R_2=SCH_3,
14 naphthalene-1-yl
15 naphthalene-2-yl
16 R_1=NO_2, R_2=OCH_3
17 R_1=H, R_2=NO_2
18 R_1=NH_2, R_2=OCH_3

^1H NMR spectrum of 3-Isopropenyl β-lactam 13:

1- Single signal at δ 1.85 ppm indicate the three methyl group protons at the C3 position of β-lactam ring.
2- Two alkenic methylene protons (H_6) dominated between δ 4.99 and 5.07 ppm and resonated as a mutiplet signal.
3- Multiple signal between δ 3.66 - 3.69 ppm and doublet signal at δ 4.74 ppm indicate the presence of hydrogen at position 3 and 4 of the β-lactam ring respectively, with *J* value of 2.44 Hz indicating the formation of *trans* isomer .



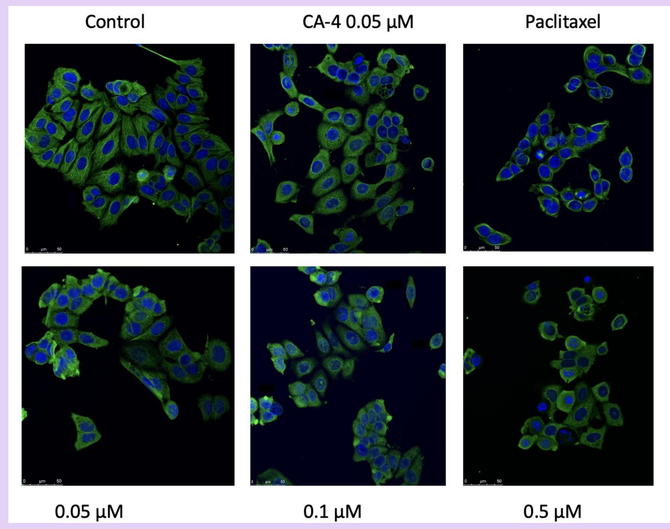
Antiproliferative Activities:

- All β-lactam compounds were screened for their antiproliferative activity in MCF-7 human breast cancer (the table shows values for compounds with IC_{50} less than 200 nM)
- The majority of the target compounds displayed moderate to potent antiproliferative activity
- 3-Isopropenyl-β-lactam **4** with *meta*-hydroxy substitution in ring B showed potent antiproliferative activity with IC_{50} of 10 nM

Compound no.	IC_{50} (μM)
8	0.033
9	0.081
13	0.131
4	0.010
CA-4	0.003

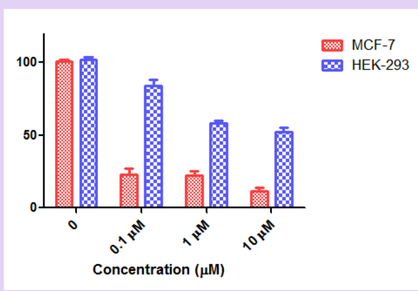
Tubulin disrupting effect by confocal microscopy:

- Treatment with tubulin polymerizer Paclitaxel caused a marked increase in polymerized tubulin.
- Cells treated with β-lactam **4** showed disruption of the microtubule network and inhibition of the polymerization of tubulin in a concentration-dependent manner



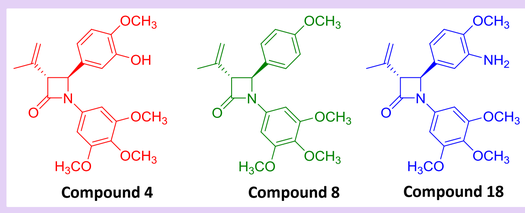
Low cytotoxicity in human non-cancer cells:

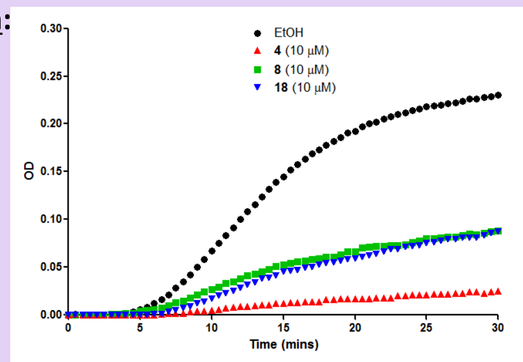
- 3-Isopropenyl-β-lactam **4** demonstrated low cytotoxicity in the non-tumourigenic cell line HEK-293 (normal human embryonic kidney)
- Cell viability of HEK-293T cells was significantly higher than MCF-7 cells at 10, 1 and 0.1 μM concentrations of β-lactam **4**. At concentration of 0.1 μM MCF-7 was inhibited by 80%, four times more than inhibition of HEK-293T cells, thus providing a window of selectivity for compound **4**.



In vitro Inhibition of tubulin polymerization:

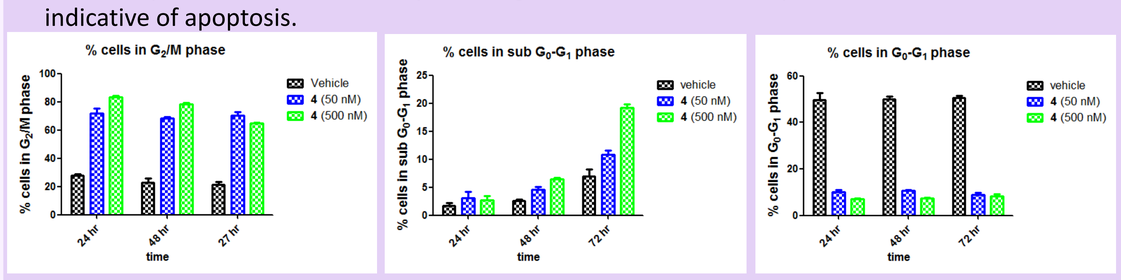
- *meta*-hydroxy substituted β-lactam **4** showed significant inhibition of tubulin polymerization compared to β-lactams **8** and **18**, (S. Nathwani).





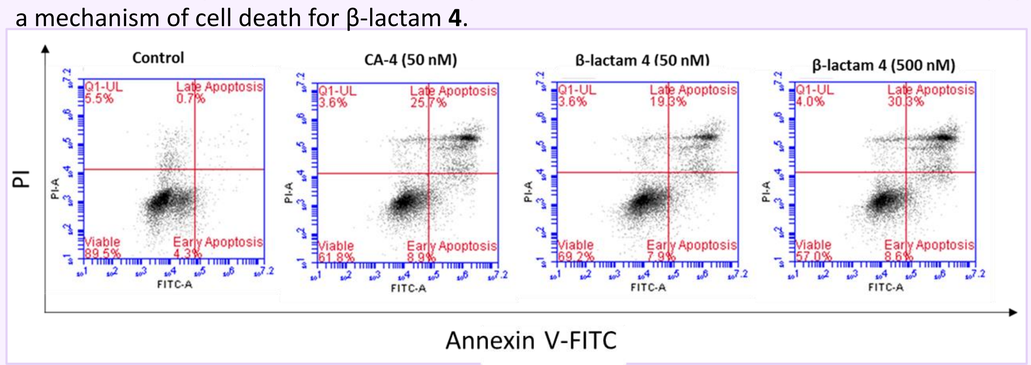
Induction of mitotic arrest of the cell cycle:

- 3-Isopropenyl-β-lactam **4** was found to arrest the cell cycle at G2/M phase compared to untreated cells at 24 h.
- A time dependent increase in the percentage of cells in the sub-G1 phase was observed which is indicative of apoptosis.



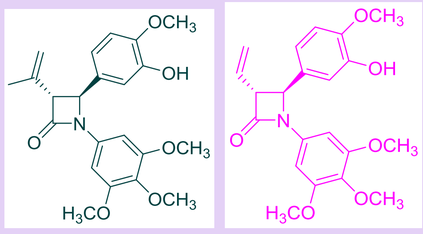
Induction of cellular apoptosis:

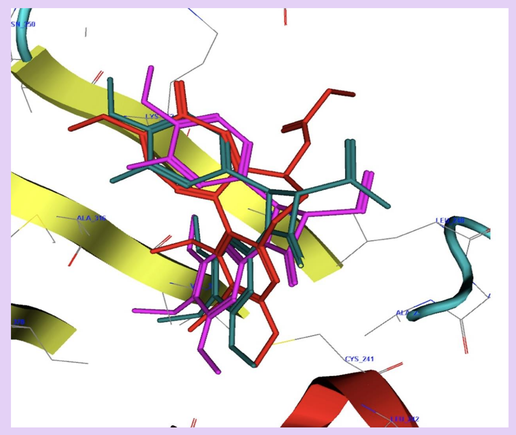
3-Isopropenyl-β-lactam **4** induced cell apoptosis (both early and late) in MCF-7 cells at 48 h in a concentration dependent manner as compared to untreated cells, which indicated that apoptosis is a mechanism of cell death for β-lactam **4**.



Molecular Modelling in the colchicine–binding site:

- The *meta*-hydroxy substituent on the 4-aryl B ring β-lactam is co-located with the colchicine carbonyl group to form the required HB acceptor (HBA) interaction with Lys β352.
- The 3,4,5-trimethoxyphenyl groups are able to make favourable van der Waals contacts with Val β318 and Cys β241.





Overlay of the X-ray structure of tubulin crystallised with DAMA Colchicine (red) (PDB entry 1SA0) on docked solution of β-lactam **4** (green) and 3-vinyl β-lactam (pink)

Conclusion:

- We have designed a series of β-lactams with 3-isopropenyl substitution at C-3 that are structurally related to colchicine and CA-4.
- These 3-isopropenyl-β-lactams selectively modulated the activity of the tubulin protein, resulting in significant cytotoxicity to MCF-7 breast cancer cells and minimum cytotoxic effects to HEK-293 normal cells.
- Molecular modelling studies indicated that these compounds could interact with the colchicine binding site of tubulin, and consequently disrupt tubulin polymerization.

References:

1. McLoughlin, E. C. and N. M. O'Boyle (2020). "Colchicine-Binding Site Inhibitors from Chemistry to Clinic: A Review." *Pharmaceuticals,* **13**(1).
2. Malebari, A. M., et al. (2022). "Synthesis, Characterisation and Mechanism of Action of Anticancer 3-Fluoroazetidin-2-ones." *Pharmaceuticals* 15(9): 1044.
3. Malebari, A. M., et al. (2021). "Synthesis and Antiproliferative Evaluation of 3-Chloroazetidin-2-ones with Antimitotic Activity: Heterocyclic Bridged Analogues of Combretastatin A-4." *Pharmaceuticals* 14(11): 1119.
4. Wang, S., et al. (2019). "3-Vinylazetidin-2-Ones: Synthesis, Antiproliferative and Tubulin Destabilizing Activity in MCF-7 and MDA-MB-231 Breast Cancer Cells." *Pharmaceuticals* 12(2): 56.