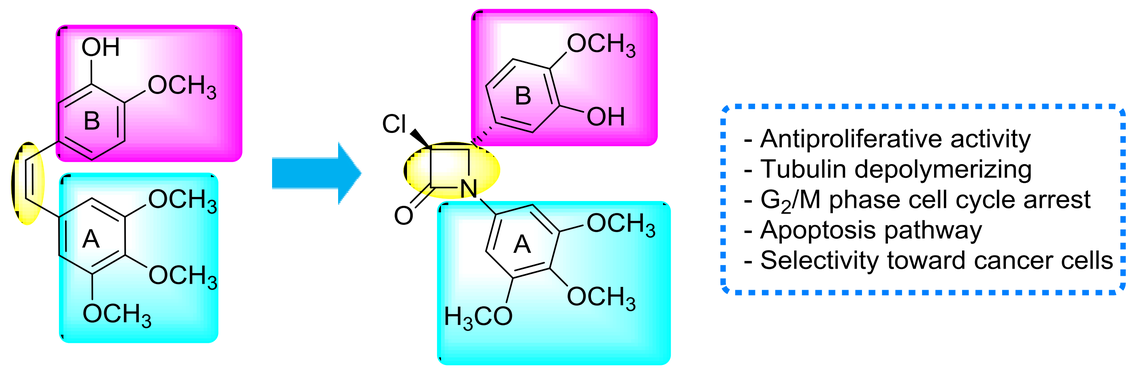
Cyclic bridged analogues of Combretastatin A-4: Design, synthesis and biological evaluation of 3-chloroazetidin-2-ones in breast cancer cells

Azizah M. Malebari[1]*, Shu Wang[2], Darren Fayne[3], Brendan Twamley[4] and 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.
[3]School of Biochemistry and Immunology, Trinity College Dublin, Trinity Biomedical Sciences Institute, 152-160 Pearse Street, Dublin 2 DO2R590, Ireland.
[4]School of Chemistry, Trinity College Dublin, Dublin 2 DO2R590, Ireland.

Introduction

- Antimitotic drugs that target tubulin are among the most widely used chemotherapeutic agents (1) however, the development of multidrug resistance has limited their clinical activity.
- We have previously investigated the antiproliferative activity of the 1,4-diarylazetidin-2-ones (β-lactam) scaffold in breast cancer cell lines (2-4).
- We now report the synthesis and biological properties of a series of novel 3-chloro-β-lactams (2-azetidinone) and 3,3-dichloro-β-lactams which are structurally related to Combretastatin CA-4.

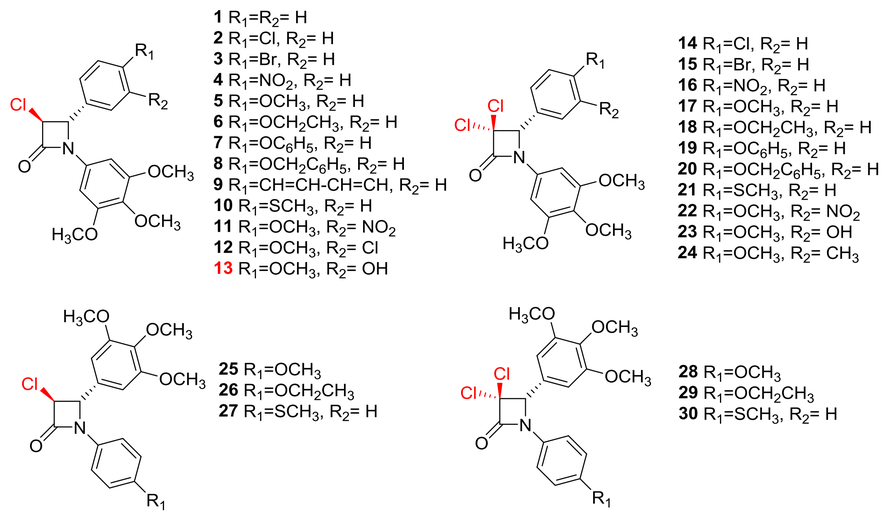


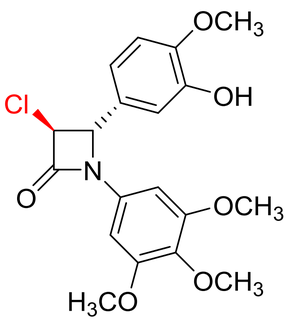
CA-4 **13**

IC_{50}: 17 nM (MCF-7)
32 nM (MDA-MB-231)

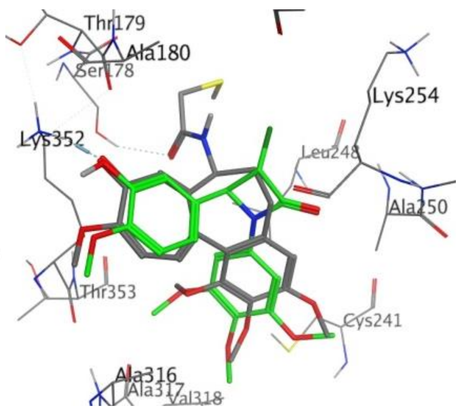
Results

- These β-lactam compounds were evaluated as potential tubulin polymerization inhibitors and for their antiproliferative effects in breast cancer cells.
- A number of the compounds showed potent activity breast cancer cells e.g. compound **13** (3-chloro-4-(3-hydroxy-4-methoxy-phenyl)-1-(3,4,5-trimethoxyphenyl)azetidin-2-one) with an IC_{50} values of **17 nM** and **32 nM** in MCF-7 and MDA-MB-231 breast cancer cells respectively and displayed comparable cellular effects to those of Combretastatin A-4.



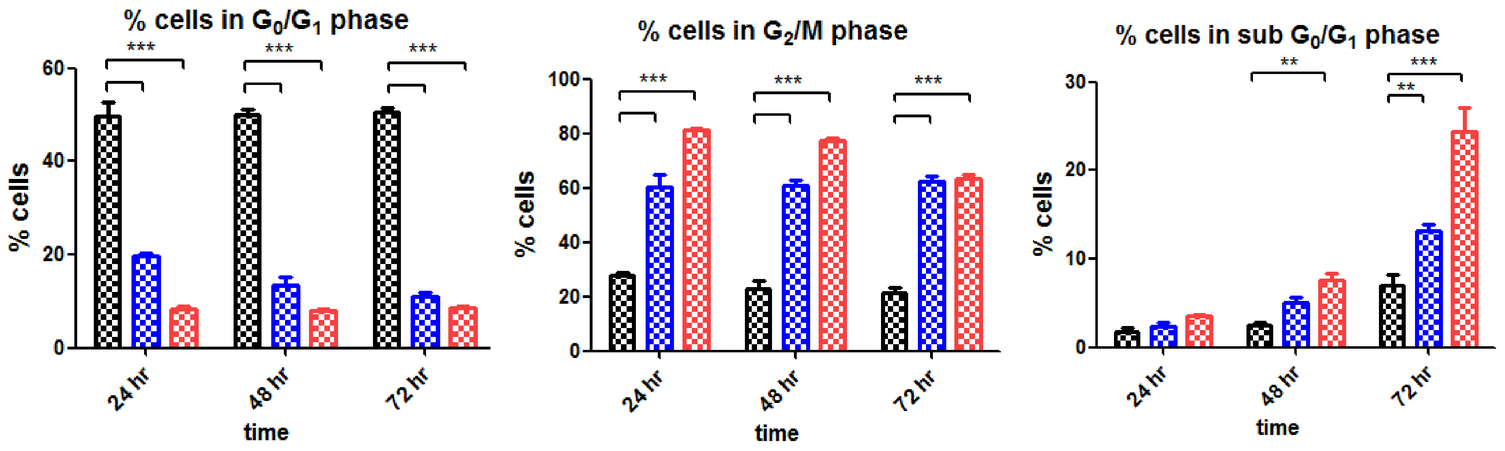


Compound 13



Molecular modelling:

- β-lactams **13** is able to recapitulate the colchicine binding mode which contribute to inhibition of tubulin polymerization.
- the hydroxy *meta*-substituent on the 4-aryl B ring co-locate with the colchicine carbonyl group to form the required HB acceptor (HBA) interaction with Lys β352.
- Also, the 3,4,5-trimethoxyphenyl groups are able to make favourable van der Waals contacts with Val β318 and Cys β241.

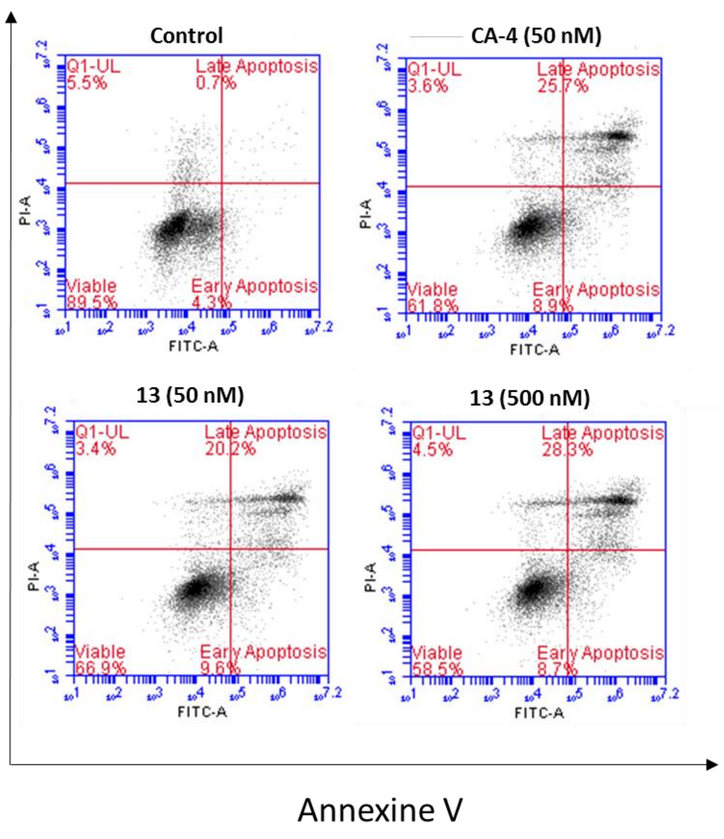


Induction of mitotic arrest of the cell cycle:

- In MCF-7 cells, β-lactams **13** was found to arrest the cell cycle arrest at G2/M phase compared to untreated cells at 24 h.
- A time dependant increase in the percentage of cells in the sub-G1 phase which indicative of apoptosis.
- A corresponding decrease of cells in the G0-G1 phase of the cell cycle was also observed.

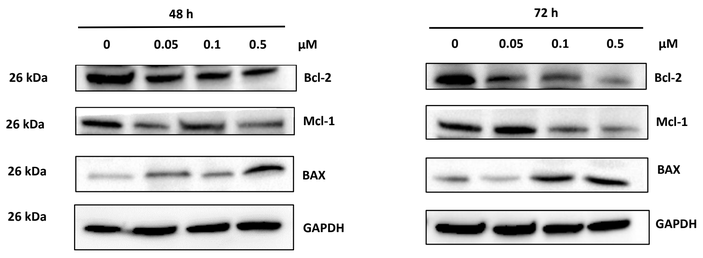
Induction of cellular apoptosis:

- β-lactam **13** induced cell apoptosis (both early and late) in MCF-7 cells at 48 h in a concentration dependent manner as compared to untreated control cells.
- This confirmed that the mode of cell death induced by **13** is apoptosis



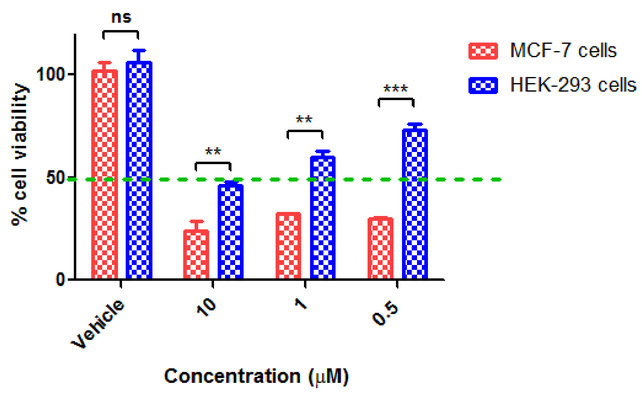
Induction of down regulation of anti-apoptotic protiens:

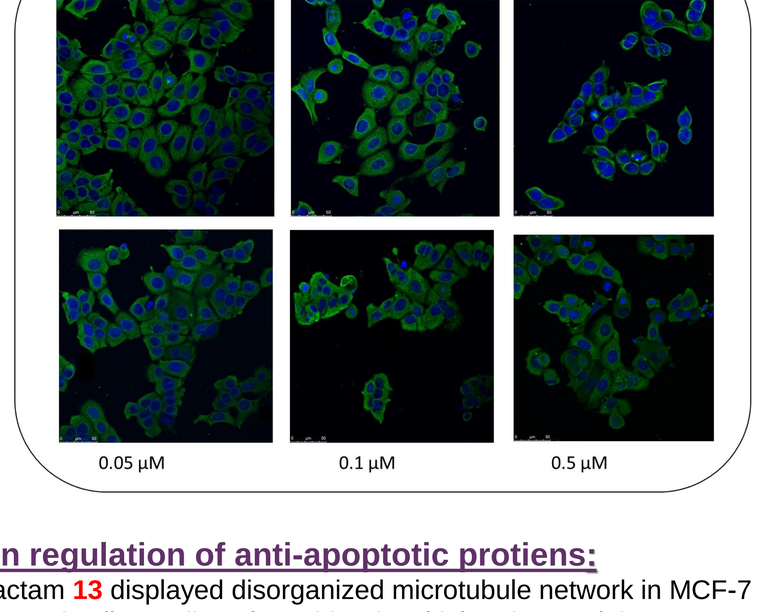
- β-lactam **13** showed a decrease in the expression level of the anti-apoptotic protein Bcl-2 and MCl-1 and correspondingly an up-regulation in expression of the pro-apoptotic protein BAX in time and concentrations dependent manner



Low cytotoxicity in human non-cancer cells:

- The IC_{50} value of **13** was around **10000 nM** in HEK-293 cells which was significantly higher than that observed against the MCF-7 breast cancer cell lines (**17 nM**) which is less toxic to human normal cells, providing a window of selectivity





Down regulation of anti-apoptotic protiens:

- β-lactam **13** displayed disorganized microtubule network in MCF-7 cells and cell rounding alongside of multiple micronuclei
- Depolymerisation and solubilisation of the cell membrane of microtubules was observed in CA-4.
- Hyperpolymerization of tubulin was observed in the paclitaxel.

References

1.Jordan, M., *Mechanism of action of antitumor drugs that interact with microtubules and tubulin.* Current Medicinal Chemistry-Anti-Cancer Agents, 2002. **2**(1): p. 1-17.
2.O'Boyle, N.M., et al., *Synthesis and evaluation of azetidinone analogues of combretastatin A-4 as tubulin targeting agents.* Journal of medicinal chemistry, 2010. **53**(24): p. 8569-8584.
3.Wang, S., et al., *3-Vinylazetidin-2-Ones: Synthesis, Antiproliferative and Tubulin Destabilizing Activity in MCF-7 and MDA-MB-231 Breast Cancer Cells.* Pharmaceuticals, 2019. **12**(2): p. 56.
4.Malebari, A.M., et al., *β-Lactams with antiproliferative and antiapoptotic activity in breast and chemoresistant colon cancer cells.* European Journal of Medicinal Chemistry, 2020: p. 112050.

6th International Electronic Conference on Medicinal Chemistry
1-30 November 2020
sponsored: MDPI *pharmaceuticals*