Novel synthesis of 3-fluoro- and 3,3-difluoro-substituted β-lactams: evaluation as potential antiproliferative and tubulin destabilizing agents

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Introduction

- Combretastatin A-4 (CA-4), a natural product stilbene is a potent microtubule-disrupting agent which binds at the colchicine-binding site of tubulin.
- The design, synthesis and biochemical evaluation of a series of analogues of the microtubule-destabilising agent CA-4 is described.
- The monocyclic β-lactam CA-4 analogues containing halogen substituents at the C-3 position of β-lactam ring were synthesized using the Staudinger reaction[1, 2].
- Previous investigations described two approaches for the construction of 3-fluoro-β-lactams using the ketene-imine condensation or the enolate-imine condensation method [3].
- In the present work, the synthesis of 3-fluoro and 3,3-difluoro substituted β-lactams was developed easily by a convenient microwave assisted Reformatsky reaction using ethyl bromofluoroacetate and ethyl bromodifluoroacetate respectively (Scheme 1).

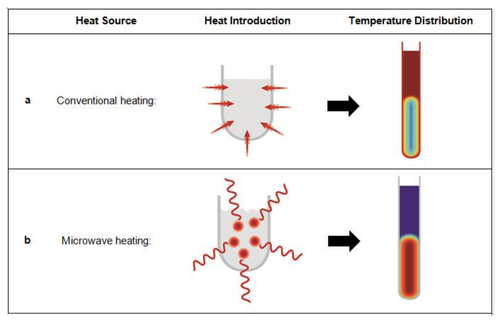
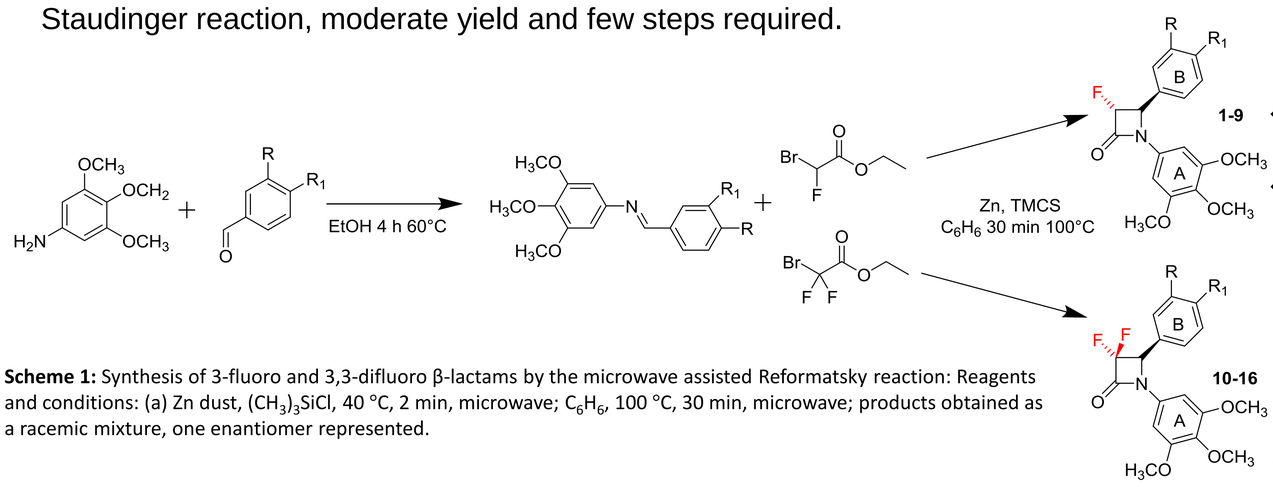


Figure : The difference between the microwave reaction and the conventional reaction[4]

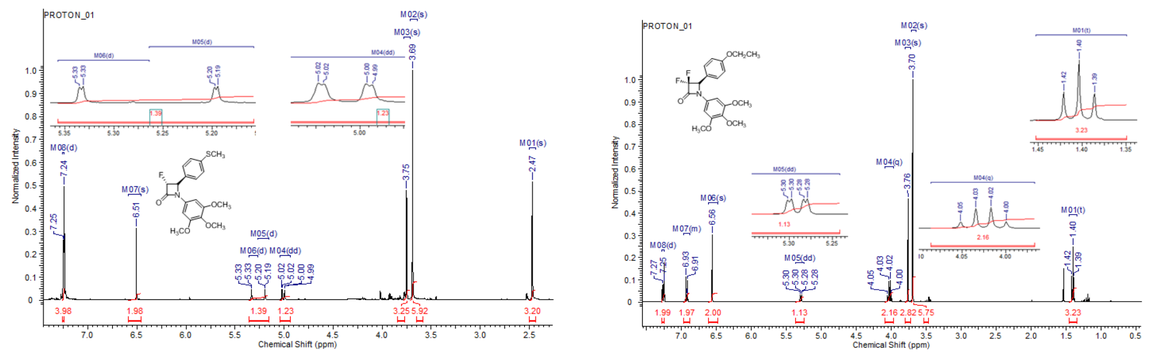
Results

- To the best to our knowledge, this is the first report of this new synthetic approach for 3-fluoro and 3,3-difluoro β-lactams as CA-4 analogues.
- In the present work, the synthesis of 3-fluoro and 3,3-difluoro substituted β-lactams was developed easily by a convenient and applicable method using the Reformatsky reaction assisted by microwave using ethyl bromofluoroacetate and ethyl bromodifluoroacetate, respectively (scheme 1).
- All the 3-fluoro **1-9** and 3,3-difluoro **10-16** β-lactam compounds in this series contain 3,4,5-trimethoxyphenyl ring A with different substituents at phenyl ring B.
- The reaction was successful with short reaction time compared to the conventional Staudinger reaction, moderate yield and few steps required.



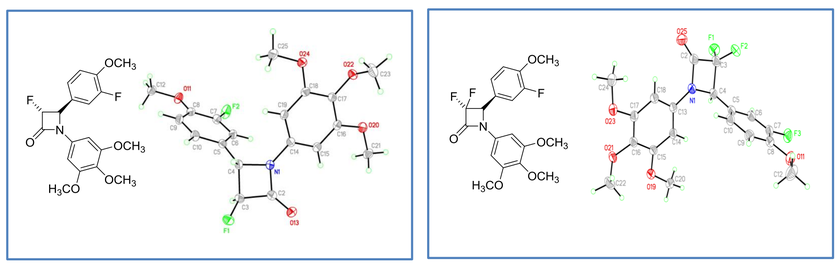
Scheme 1: Synthesis of 3-fluoro and 3,3-difluoro β-lactams by the microwave assisted Reformatsky reaction: Reagents and conditions: (a) Zn dust, $(CH_3)_3SiCl$, 40 °C, 2 min, microwave; C_6H_6, 100 °C, 30 min, microwave; products obtained as a racemic mixture, one enantiomer represented.

^{1}H-NMR spectrum of β-lactams 3 & 13 ($CDCl_3$)

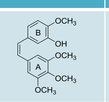
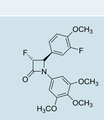


- The doublets at δ 5.00 and δ 5.33 ppm are assigned to the hydrogens on positions 3 and 4 of the β-lactam ring, respectively.
- The coupling constant of 1.21 Hz indicates that the only isomer present in the *trans* form. A doublet doublet signal appears at δ 4.99-5.02 ppm attributed to the proton at position 3 of the β-lactam with a large coupling constant of J=10.71 Hz and 1.24 Hz due to the adjacent fluorine while H4 appears as double doublet at 5.18-5.31 ppm which is due to non-equivalent coupling of J=1.24 and 1.66 Hz (H3 and F at C3).

X-ray Crystallographic Data *m*-fluorophenyl ring B β-lactams 6 & 14



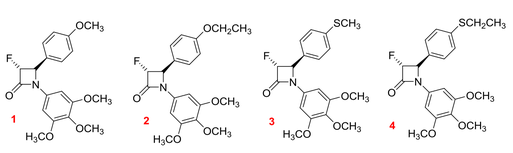
The NCI-60 human tumour cell lines screening:

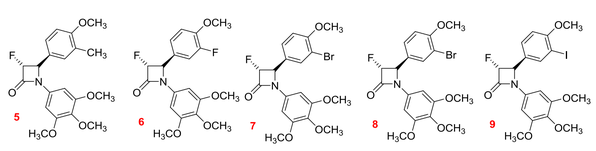
NCI reference number	Compound	Structure	GI_{50} (μM)	TGI_{50} (μM)	LC_{50} (μM)
D-613729	CA-4		0.099	10.3	85.5
D-788819	Lead compound		0.057	20.8	95.4
D-792959	β-lactam 6		0.223	52.4	95.4

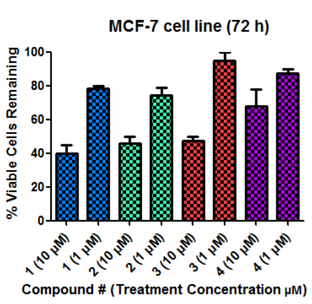
GI_{50} and **LC_{50}** are the mean concentrations required to inhibit the growth and kill 50% of the cells in the assay respectively. *Developmental Therapeutics Program; National Cancer Institute: Bethesda, MD;* http://dtp.cancer.gov/
TGI is the mean concentration required to completely inhibit the growth of all cells. *Developmental Therapeutics Program; National Cancer Institute: Bethesda, MD;* http://dtp.cancer.gov/

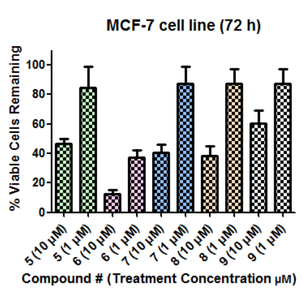
Anti-proliferative activities:

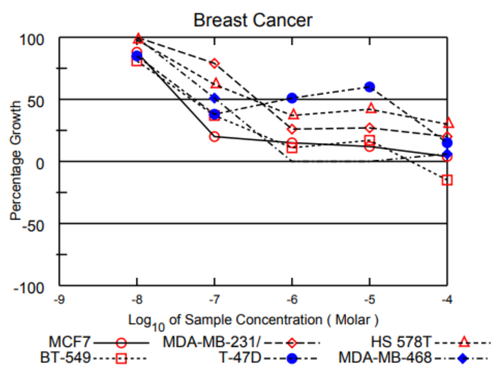
- A preliminary screening was performed for all compounds in MCF-7 cells at two different concentrations: 1 μM and 10 μM.
- All the β-lactams are > 80% viable remaining cells at 1 μM, except for compound **6** with 12.5 and 37% remaining at 10 μM and 1 μM respectively.









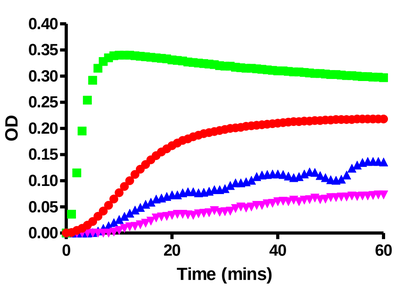


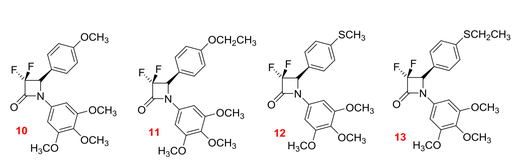
IC_{50} values of β-lactam 6 in different breast cancer cell lines:

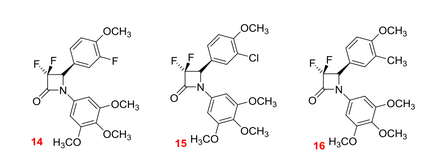
MCF-7 **(0.036 μM)**
MDA-MB-231 **(0.354 μM)**
HS 578T **(0.295 μM)**
BT-549 **(0.050 μM)**
MDA-MB-468 **(0.104 μM)**

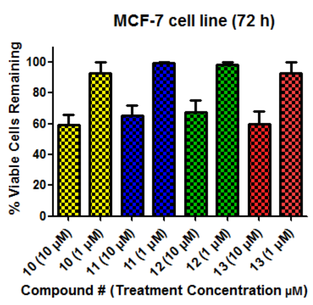
Tubulin polymerization studies:

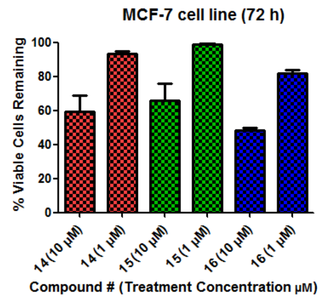
- Tubulin polymerization studies on β-lactam **6** showed significant inhibition of tubulin polymerization (~3 fold reduction) compared to the vehicle





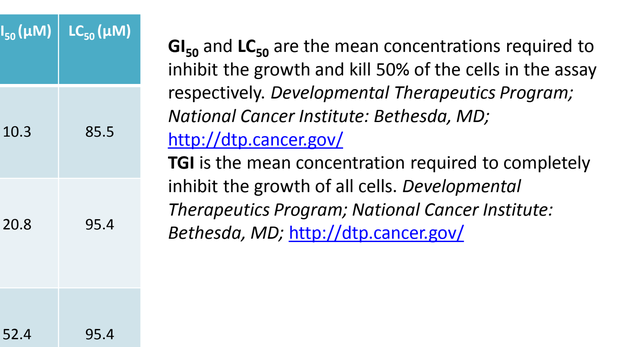






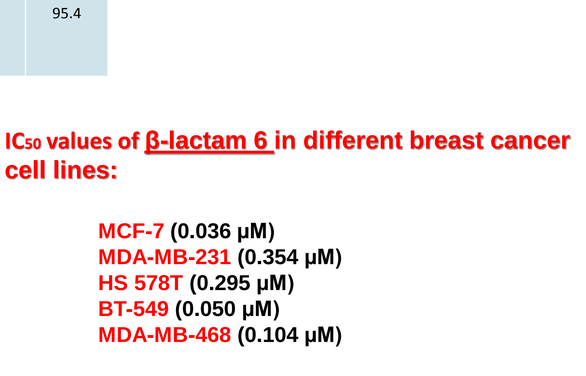
Apoptosis quantification by Annexin V-FITC/PI assay:

- β-lactam **6** 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.



Apoptosis induction by Western blot analysis

- β-lactam **6** showed a decrease in the expression level of survivin and anti-apoptotic protein Bcl-1 and correspondingly an up-regulation in expression of the pro-apoptotic protein BAX and significantly at 72 h for higher concentration tested 0.5 μM (MCF-7).



Conclusion

- We have reported the ring closure of zinc enolate and imine with organozinc reagents in a one-pot fashion to form β-lactam. The required Reformatsky reagents were readily prepared by microwave irradiation TMCS with zinc in benzene for 5 min at 100 °C.
- Addition of this Reformatsky reagent to of the corresponding bromoacetate and the relevant imine followed by further irradiation for 30 min at 100 °C provided the β-lactam in good yields.
- β-lactam **6** induced significant apoptosis in MCF-7 in dose and time dependent manner, and strongly inhibited tubulin assembly exhibiting activity at 3 fold greater than the control.

References

1) T. F. Greene, S. Wang, L. M. Greene, S. M. Nathwani, J. K. Pollock, A. M. Malebari, T. McCabe, B. Twamley, N. M. O'Boyle, D. M. Zisterer and M. J. Meegan, *Journal of Medicinal Chemistry*, 2016, 59, 90-113.
2) A. M. Malebari, L. M. Greene, S. M. Nathwani, D. Fayne, N. M. O'Boyle, S. Wang, B. Twamley, D. M. Zisterer and M. J. Meegan, *European journal of medicinal chemistry*, 2017, 130, 261-285.
3) K. Araki, J. A. Wichtowski and J. T. Welch, *Tetrahedron Letters*, 1991, 32, 5461-5464.
4) https://wiki.anton-paar.com/en/microwave-assisted-synthesis/