Synthesis and biological evaluation of 2-azetidinone derivatives with antiproliferative activity in breast cancer and chemoresistant colon cancer

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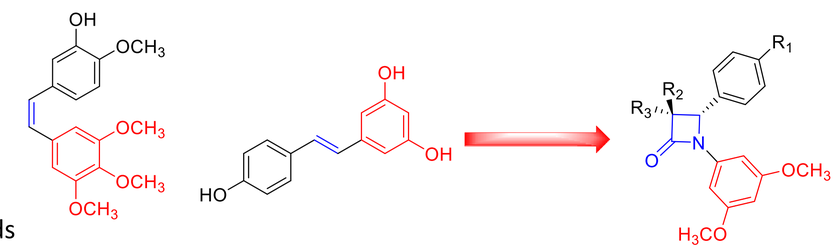
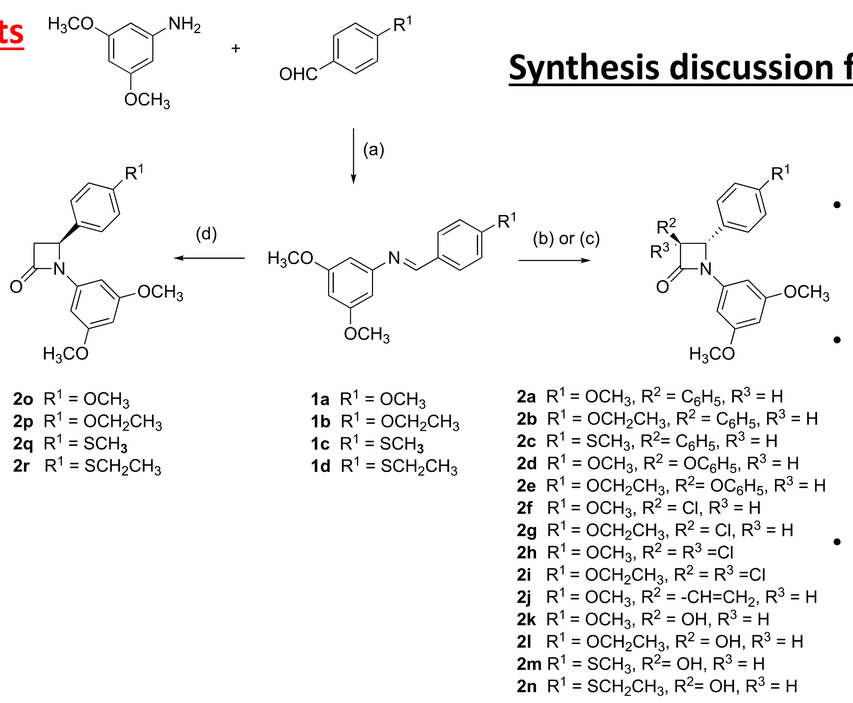
Introduction

- Stilbene-based compounds are widely occurring natural products and demonstrate a range of biological activities [1]
- Combretastatin A-4 (CA-4) shows potent anticancer activity in many human cancer cells together with inhibition of tubulin polymerisation and antivascular effects [2]
- The 3,5-dihydroxyphenyl substitution pattern is characteristic of stilbenes such as well-known natural polyphenolic stilbene resveratrol with reported therapeutic and chemopreventive properties in colorectal and skin cancers [3]
- We have previously investigated the antiproliferative activity of the 1,4-diarylazetidin-2-one (β-lactam) scaffold in MCF-7 breast cancer cell lines and the chemoresistant HT-29 colon cancer cells [4]
- A 3,5-dimethoxy ring A β-lactam compound with comparable activity to the 3,4,5-trimethoxy ring A of CA-4 and β-lactam compounds was previously reported [5]
- In this work, we focused on the design of a panel of 1,4-diaryl-2-azetidinones containing different C-3 and ring B substituents together with the 3,5-dimethoxyphenyl-substituent at N-1 replacing the 3,4,5-trimethoxyphenyl Ring A of the antimitotic combretastatin CA-4
- 3,5-Dimethoxy ring A β-lactams could be promising leads for the development of anti-breast cancer drugs that target tubulin



Results

Synthesis discussion for β-lactam compounds



2o $R^1 = OCH_3$
2p $R^1 = OCH_2CH_3$
2q $R^1 = SCH_3$
2r $R^1 = SCH_2CH_3$

1a $R^1 = OCH_3$
1b $R^1 = OCH_2CH_3$
1c $R^1 = SCH_3$
1d $R^1 = SCH_2CH_3$

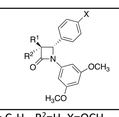
2a $R^1 = OCH_3$, $R^2 = C_6H_5$, $R^3 = H$
2b $R^1 = OCH_2CH_3$, $R^2 = C_6H_5$, $R^3 = H$
2c $R^1 = SCH_3$, $R^2 = C_6H_5$, $R^3 = H$
2d $R^1 = OCH_3$, $R^2 = OC_6H_5$, $R^3 = H$
2e $R^1 = OCH_2CH_3$, $R^2 = OC_6H_5$, $R^3 = H$
2f $R^1 = OCH_3$, $R^2 = Cl$, $R^3 = H$
2g $R^1 = OCH_2CH_3$, $R^2 = Cl$, $R^3 = H$
2h $R^1 = OCH_3$, $R^2 = R^3 = Cl$
2i $R^1 = OCH_2CH_3$, $R^2 = R^3 = Cl$
2j $R^1 = OCH_3$, $R^2 = -CH=CH_2$, $R^3 = H$
2k $R^1 = OCH_3$, $R^2 = OH$, $R^3 = H$
2l $R^1 = OCH_2CH_3$, $R^2 = OH$, $R^3 = H$
2m $R^1 = SCH_3$, $R^2 = OH$, $R^3 = H$
2n $R^1 = SCH_2CH_3$, $R^2 = OH$, $R^3 = H$

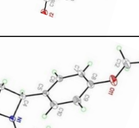
Scheme 1: Synthesis of β-lactams **2a-r.** Reagents and conditions: (a) H_2O, 30 min, 20 °C (85 - 97%). (b) Compounds **2a-2j**: Triethylamine, acid chloride [$C_6H_5CH_2COCl$, $C_6H_5OCH_2COCl$, $ClCH_2COCl$, $Cl_2CHCOCl$, $CH_3CH=CH\text{-}COCl$ or CH_3COOCH_2COCl], toluene, reflux, N_2, 5 h., (11-31%). (c) Compounds **2k-2n** (i) CH_3COOCH_2COCl, toluene, reflux, N_2, 5 h., (11-31%), (ii) $NH_2NH_2.2HCl$,Triethylamine, MeOH, reflux, 4 h. (d) Compounds **2o-2r**: Ethylbromoacetate, Zn dust, TMSCl, 40 °C, 15 min, then 100 °C, 2 min, microwave, C_6H_6, 100 °C, 30 min, microwave; Products obtained as racemic mixtures, one enantiomer illustrated.

- A panel of 3,5-dimethoxyphenyl ring A β-lactam compounds with a variety of β-lactam C-3 substituents was synthesised (Scheme 1).
- β-Lactam compounds **2a-2n** containing an aryl substituent at C-3 were obtained by Staudinger reaction of phenylacetyl chloride with the appropriate imine to afford the racemic products.
- Microwave technology was successfully applied for the Reformatsky synthesis of 3-unsubstituted β-lactams **2o**-**2r** using zinc activation by TMCS, followed by reaction with the appropriate imine and ethyl bromoacetate
- Structures of products were confirmed by ^1H and ^{13}C NMR, IR, HRMS and X-Ray crystallography.

Antiproliferative Activities

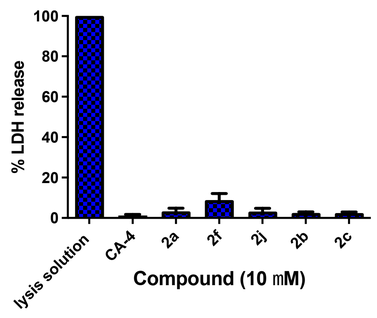
- A small library of structurally diverse 1,4-diaryl-2-azetidinones containing phenyl, chloro, hydroxyl, vinyl and phenoxy substituents at C-3 of β-lactam, together with the 3,5-dimethoxyphenyl-substituent at N-1 replacing the 3,4,5-trimethoxyphenyl Ring A of CA-4 were evaluated.
- Ring A substituents included 4-OCH_3, 4-OCH_2CH_3, 4-SCH_3, 4-SCH_2CH_3.
- Tripodi et al. had previously reported a 3,5-dimethoxy ring A β-lactam compound **2k** which demonstrated comparable activity to the 3,4,5-trimethoxy ring A of CA-4 and β-lactam compounds [5]
- The most potent antiproliferative compounds in the present series with activity in nanomolar range were compounds **2a** (3-phenyl), **2e** (3-phenoxy), **2l** (3-hydroxy), **2m** (3-hydroxy), **2o** (3-unsubstituted), (IC_{50} values in MCF-7 cells 25, 15, 3, 23, 55 nM respectively), together with **2k** (IC_{50} =15 nM).

Compound		MCF-7 IC_{50} (μM)[a]	cLog P[e]
2a	R^1= C_6H_5, R^2=H, X=OCH_3	0.025 ± 0.003	4.416
2b	R^1= C_6H_5, R^2=H, X=OCH_2CH_3	0.055 ± 0.01	4.945
2c	R^1= C_6H_5, R^2=H, X=SCH_3	0.063 ± 0.006	5.056
2d	R^1= OC_6H_5, R^2=H, X=OCH_3	0.054 ± 0.01	4.7015
2e	R^1= OC_6H_5, R^2=H, X=OCH_2CH_3	0.015 ± 0.007	5.2305
2f	R^1= Cl, R^2=H, X=OCH_3	0.680 ± 0.16	3.941
2g	R^1= Cl, R^2=H, X= OCH_2CH_3	0.045 ± 0.01	4.47
2h	R^1= R^2= Cl, X=OCH_3	6.612 ± 2.0	5.124
2i	R^1= R^2= Cl, X= OCH_2CH_3	0.273 ± 0.01	5.653
2j	R^1= C_2H_3, R^2=H, X=OCH_3	0.170 ± 0.07	3.622
2k[5]	R^1= OH, R^2=H, X= OCH_3	0.015 ± 0.007	2.595
2l	R_1= OH, R^2=H, R_2=H, X= OCH_2CH_3	0.003 ± 0.0009	3.124
2m	R^1= OH, R^2=H, X=SCH_3	0.023 ± 0.002	3.235
2n	R^1= OH, R^2=H, X=SCH_2CH_3	0.031 ± 0.006	3.764
2o	R^1= H, R^2=H, X= OCH_3	0.055 ± 0.01	2.858
2p	R^1= H, R^2=H, X= OCH_2CH_3	0.063 ± 0.006	3.387
2q	R^1= H, R^2=H, X=SCH_3	0.244 ± 0.09	3.498
2r	R^1= H, R^2=H, X=SCH_2CH_3	0.123 ± 0.08	4.027
CA-4		0.0039±0.00032	3.323.

Compound		HT-29 IC_{50} (μM)
2b	R^1= C_6H_5, R^2=H, X=OCH_2CH_3	0.102 ± 0.01
2c	R^1= C_6H_5, R^2=H, X=SCH_3	0.115 ± 0.02
2e	R^1= OC_6H_5, R^2=H, X=OCH_2CH_3	0.012 ± 0.003
2k	R^1= OH, R^2=H, X= OCH_3	0.012± 0.003
2l	R_1= OH, R^2=H, R_2=H, X= OCH_2CH_3	0.008 ± 0.0009
2m	R^1= OH, R^2=H, X=SCH_3	0.0255 ± 0.004
2o	R^1= H, R^2=H, X= OCH_3	0.102 ± 0.01
2p	R^1= H, R^2=H, X= OCH_2CH_3	0.115 ± 0.02
CA-4		4.165 ± 0.100

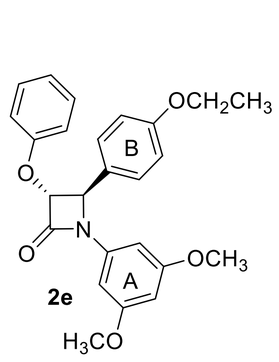
Evaluation of in vitro cytotoxicity via lactate dehydrogenase (LDH) assay

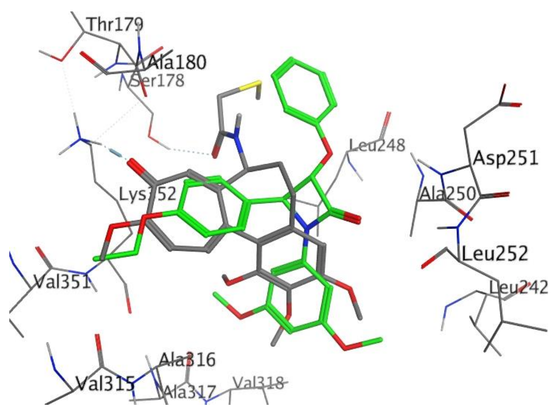
- MCF-7 cells were treated with the most potent compounds and **CA-4** at 10 μM concentration for 24 h period
- The majority of the compounds demonstrated minimal cytotoxicity (<3.5% at 10 μM concentration), apart from compound **2f** (8.9%).



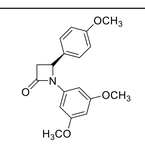
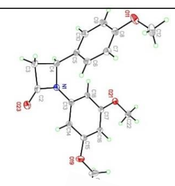
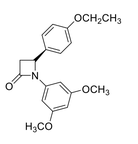
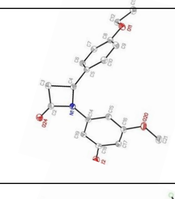
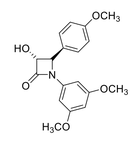
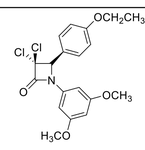
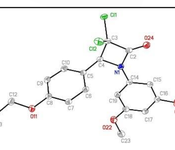
Molecular Modelling in the colchicine binding site (PDB entry 1SA0)

- The best ranked docked pose of compound **2e** showed that methoxy groups at positions 3 and 5 of Ring A in compounds **2e** makes favourable interactions with the hydrophobic residues Val β315, Ala β316, Ala β317, Val β318, Leu β242, Leu β248, Leu β252 and Ala β 250 of the tubulin binding site to confer the required binding stabilisation for these compounds
- It is interesting that the lack of the 4-methoxy substituent of Ring A does not result in loss of activity





X-ray crystal structure of compounds 2o, 2p, 2k and 2i

Compound	Structure	X-Ray representation
2o		
2p		
2k		
2i		

Conclusion

- The inclusion of the 3,5-dimethoxyphenyl substituent at N-1 replacing the 3,4,5-trimethoxyphenyl substitution pattern which is present in the combretastatin-type antimitotic stilbenes has produced novel 2-azetidinone products with potent antiproliferative activity in MCF-7 and similar activity in HT-29 colon cancer cells, indicating structural diversity for C-3 β-lactam substituents.
- The most potent compound identified as the novel compound **2l**
- Low levels of % LDH released were obtained (2-9%) at 10 μM confirming low cytotoxicity for the compounds in the MCF-7 cell line.
- The structural study of these compounds will facilitate further design of more effective and diverse β-lactams for potential development in breast and chemoresistant colon cancer applications.

References

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