Investigation of CA-4 metabolism and related β-lactam analogues in chemoresistant HT-29 colon cancer cells

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Introduction

- Drug resistance is a common cause of the failure of chemotherapeutic agents to achieve cytotoxicity responses in human malignant disease.
- Drug inactivation by metabolism within tumour cells is recognised as an important mechanism of drug resistance [1].
- Glucuronidation is a major route for the metabolic inactivation of many drugs and also endogenous substances.
- Combretastatin-A4 (CA-4) undergoes direct glucuronidation in the presence of UGTs at the meta-hydroxy group of the B-ring and could cause an inherent resistance in HT-29 colon cancer cells [2].

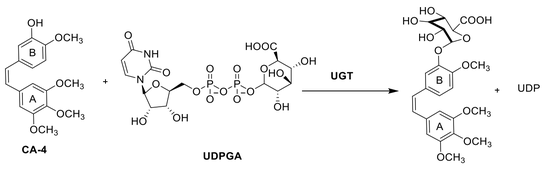


Figure 1 : Glucuronidation reaction of CA-4

Results and Discussion

Comparison of antiproliferative activity between m-hydroxy ring B and deletion of m-hydroxy ring B β-lactam compounds

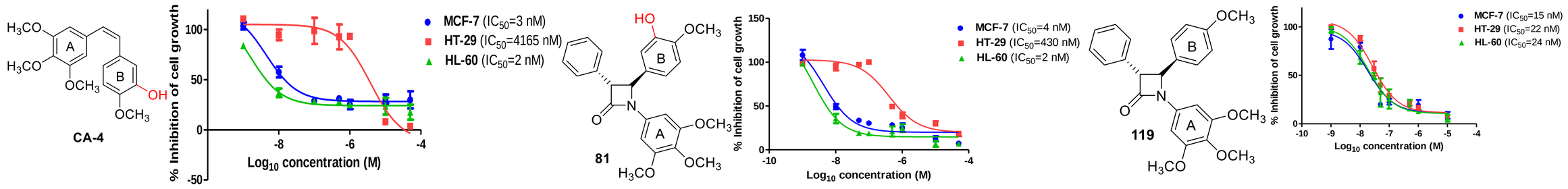


Figure 2 : Antiproliferative effect of **CA-4, 81** and **119** in MCF-7, HT-29 and HL-60 cancer cells.

- The excellent activity of CA-4 in MCF-7 cells in nanomolar range and its resistance in HT-29 cells with IC_{50}= 4 µM is well known
- compound **81** (bearing m-hydroxyphenyl ring B) mimics the same manner of low cytotoxicity as CA-4 in HT-29 cells.
- Compound **119** is an example of deletion of m-hydroxy in ring B that showed the significant improvement of cytotoxicity in HT-29 cells compared to its related m-hydroxyphenyl ring B β-lactam as well as CA-4

Expression level of UGT protein is significantly higher in CA-4 resistant HT-29 cells as compared to CA-4 sensitive MCF-7 and HL-60 cells

- The endogenous level of UGT in CA-4 resistant HT-29 cells was significantly higher when compared to UGT expression levels in CA-4 sensitive MCF-7 and HL-60 cells.
- The apparent abundant expression of UGT in HT-29 cells would confer resistance to CA-4 and derivatives of CA-4 that contain the required phenolic functional groups which facilitate glucuronate conjugation and subsequent inactivation.

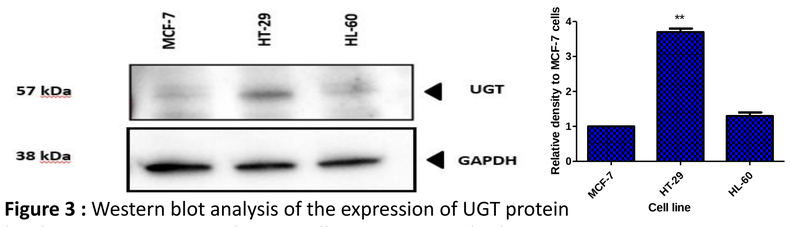
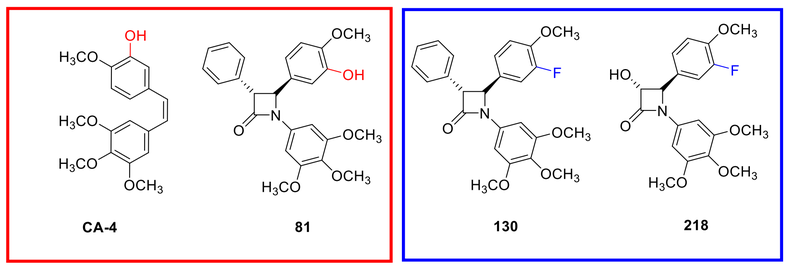


Figure 3 : Western blot analysis of the expression of UGT protein levels in MCF-7, HT-29 and HL-60 cells using UGT antibody .

Microsomal metabolic stability



Potency in HT-29 cells

IC_{50}: 3 nM (MCF-7 cells)	4 nM (MCF-7 cells)	16 nM (MCF-7 cells)	22 nM (MCF-7 cells)
IC_{50}: 4165 nM (HT-29 cells)	430 nM (HT-29 cells)	9 nM (HT-29 cells)	3 nM (HT-29 cells)

Metabolic stability in HT-29 cells

CL_{int} : 645 µl/min/mg protein	18.1 µl/min/mg protein	0.982 µl/min/mg protein	14.2 µl/min/mg protein
$t_{1/2}$: 2.15 min	76.5 min	1410 min	97.7 min

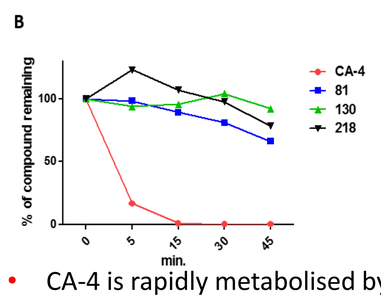


Figure 4 : microsomal metabolic stability for CA-4 and compounds **81**, **130** and **218**

- CA-4 is rapidly metabolised by glucuronidation
- **81** (*m*-hydroxy ring B) demonstrated 66% remaining at 45 min
- **130** and **218** displayed considerable stability toward hepatic enzymes

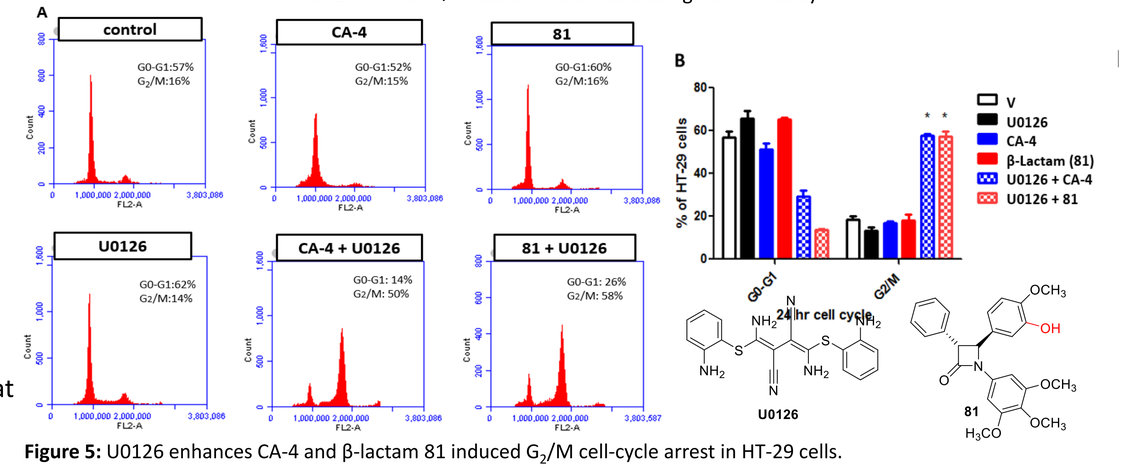


Figure 5: U0126 enhances CA-4 and β-lactam 81 induced G_2/M cell-cycle arrest in HT-29 cells.

- Recent studies demonstrated that U0126 enhanced Combretastatin A-4 induced cytotoxicity by inhibiting glucuronidation of CA-4 [3].
- U0126 reversed the resistance of CA-4 and also reversed the resistance of the Ring B meta-hydroxylated β-lactam **81** in HT-29 cells.
- G2/M cell-cycle arrest is a known marker of the activity of CA-4 which clearly demonstrated a significant increase of G2/M cell-cycle induced by CA-4 and its related Ring B meta-hydroxylated β-lactam **81** in the presence of U0126 in HT-29 cells.

Evaluation of CA-4 stability in HT-29 cells using different UGT inhibitors

	Inhibitor	Boreneol		Propofol		4-nitrophenol	
		-	+	-	+	-	+
Flufenamic acid	CLin	7.75	4.79	10.0	2.20	19.7	1.11
	$t_{1/2}$	89.4	145	69	314	35.5	623
Mefenamic acid	CLin	10.5	6.59	12.5	2.20	14.8	0.61
	$t_{1/2}$	66.1	105	55.4	316	46.2	1140
CA-4	CLin	21.5	21.2	21.55	15.2	26.1	13.7
	$t_{1/2}$	32.8	32.7	32.25	45.6	25	50

Table 1: Intrinsic clearance (CL_{int}) (µL/min/mg protien) and half-life ($t_{1/2}$) (min) for flufenamic acid, mefenamic acid, CA-4 and **81**in HT-29 cells

- Intrinsic clearance(Cl*int*) (µL/min/million cells) and half-life ($t_{1/2}$) for flufenamic acid, mefenamic acid as positive controls, together with CA-4 in HT-29 cells in the presence or absence of boreneol, propofol and 4-Nitrophenol are shown in table 1.
- CA-4 pretreatment with boreneol indicated weak inhibition
- There is a significant intracellular accumulation of CA-4 treated with propofol compared to CA-4 alone
- 4-nitrophenol produced a significant inhibition of CA-4 metabolism by glucurontrasferase in HT-29 cells.

Figure 6 : Percentage of CA-4 pretreated with different UGT inhibitors remaining in HT-29 cells

- Many different glucuronidation inhibitors for CA-4 were used to evaluate UDP-glucuronyltransferase activity toward CA-4.
- Three different known inhibitors of glucuronidation were used; boreneol, propofol and 4-nitrophenol.
- 4-Nitrophenol was found to be the most potent inhibitor of UGTs activity in HT-29 cells compared to the other inhibitors which used boreneol and propofol
- There is a significant improvement of CA-4 and β-lactam **81** cytotoxicity pretreated with 4-nitrophenol at 6, 12 and 24 h and at higher concentration at 10 and 50 µM.

Figure 7: 4-Nitrophenol inhibits CA-4 and **81** inactivation in HT-29 cells. Cells were treated with different concentration of CA-4 and **81** alone or in the presence of 10 µM of 4-nitrophenol for 6, 12 and 24 h

Conclusion:

- CA-4 resistance mediated by glucuronidation could be inhibited weakly by a broad UGT inhibitor Boreneol, and strongly inhibited by UGT1A9 competitive inhibitor propofol, or UGT1A6 substrate 4-nitrophenol.
- The strategic deletion of the ring B hydroxyl group can produce CA-4 analogues that are equally effective in cancer cells expressing UGTs as compared to those expressing little or undetectable levels of UGTs, offering a simple solution to overcoming resistance associated with glucuronidation of CA-4.

References:

1-Vickers, P.J. et al, Mechanisms of resistance to antineoplastic drugs. In *Developments in cancer chemotherapy*, CRC Press: 2019; pp. 117-152.

2-Malebari, A.M. et al, β-Lactam analogues of combretastatin A-4 prevent metabolic inactivation by glucuronidation in chemoresistant HT-29 colon cancer cells. *European journal of medicinal chemistry* **2017**, *130*, 261-285.

3-Quan, H. et al, 1,4-Diamino-2,3-dicyano-1,4-bis(methylthio)butadiene (U0126) enhances the cytotoxicity of combretastatin A4 independently of mitogen-activated protein kinase kinase. *J Pharmacol Exp Ther* **2009**, *330*, 326-333,