Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

ROYAL SOCIETY OF CHEMISTRY

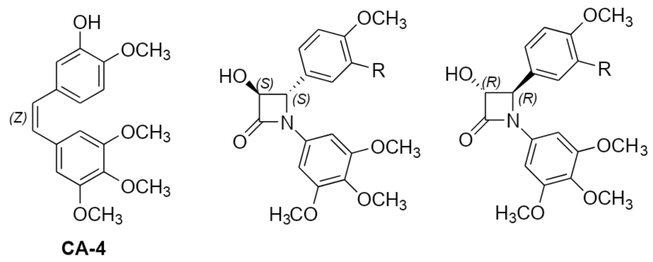
A biocatalytic approach for kinetic resolution toward enantiopure anti-cancer β-lactams using *Candida antarctica* Lipase B

Eavan C. McLoughlin and Niamh M. O'Boyle

The School of Pharmacy and Pharmaceutical Sciences, Trinity College, The University of Dublin, Ireland

INTRODUCTION

The combretazet β-lactams are *cis*-restricted analogues of combretastatin A-4 (CA-4, **Figure 1**), chemically manipulated by insertion of the 2-azetidinone scaffold to enhance their *in vitro* and *in vivo* stability. 3-Hydroxyl combretazets (**Figure 1**) demonstrate excellent anti-proliferative IC_{50} values in sub nanomolar ranges across a panel of cancer cell lines. Their 3*S,4S* enantiomers/eutomers (**Figure 1**) have been isolated using chiral diastereomeric resolution using *N*-Boc-L-proline.[1,2] This approach required large process mass intensities (PMI) of approximately 150,000 kg/kg and produced only modest yields (5-10%) of enantiomers, insufficient for progression toward *in vivo* pre-clinical toxicology studies. Chemoenzymatic kinetic resolution (KR) of enantiomers using lipase *Candida antarctica* lipase B (CAL-B) in comparison offers a sustainable, greener and safer resolution process.



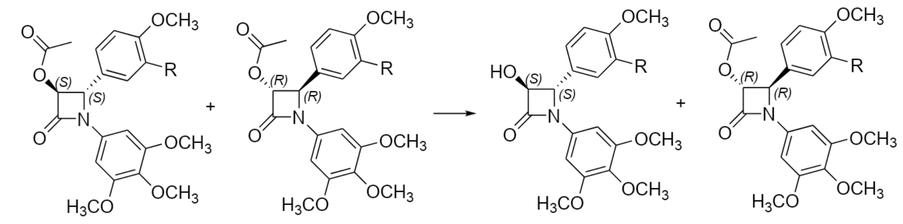
CA-4

Figure 1: Chemical structures of combretastatin A-4 and β-lactam enantiomers (3*S,4S* eutomers and 3*R,4R* distomers)

OPTIMISED KR PROCEDURE

Early experiments resutled in rapid methanolysis and conversion and demonstrated poor *ee.* The most optimal conditions were selected for enantioseparation of 3-hydroxyl 3*S,4S* eutomers **and** 3*R,*3*R* 3-acetoxy distomers (**Scheme 1).**

1. **Reaction quenching *via* filtration at 30-50% conversion/methanolysis.**
   - ✓ Quenching <50 % ↑ *ee* of 3*S,4S* enantiomers.
2. **Reactions carried out using 3 eq of methanol and 1:2 ratio of CAL-B: racemate.**
   - ✓ Reducing methanol from 10 → 3 eq ↓ reaction rate.
   - ✓ Reducing ratio of enzyme: substrate from 1:1 → 1:2 ↓ reaction rate.
     - ✓ ↓ reaction rate ↑ *ee.*
3. **Isolation of unreacted, enantioenriched 3-acetoxy susbtrate *via* LC purification followed by further enantioenrichment in a second KR**.



CAZ-1a R^1 = H, R^2 = OCH_3
CAZ-2a R^1 = F, R^2 = OCH_3
CAZ-3a R^1 = CH_3, R^2 = OCH_3
CAZ-4a R^1 = Cl, R^2 = OCH_3
CAZ-5a R^1 = Br, R^2 = OCH_3
CAZ-6a R^1 = H, R^2 = OCH_3
CAZ-7a R^1 = NH_2, R^2 = OCH_3
CAZ-8a R^1 = H, R^2 = OCH_2OCH_3
CAZ-9a R^1 = H, R^2 = OCH_2OCH_3

CAZ-1b R^1 = H, R^2 = OCH_3
CAZ-2b R^1 = F, R^2 = OCH_3
CAZ-3b R^1 = CH_3, R^2 = OCH_3
CAZ-4b R^1 = Cl, R^2 = OCH_3
CAZ-5b R^1 = Br, R^2 = OCH_3
CAZ-6b R^1 = H, R^2 = OCH_3
CAZ-7b R^1 = NH_2, R^2 = OCH_3
CAZ-8b R^1 = H, R^2 = OCH_2OCH_3
CAZ-9b R^1 = H, R^2 = OCH_2OCH_3

Scheme 1: Kinetic resolution of chiral combretazet enantiomers using *Candida antarctica* lipase B and methanol in MTBE. Reactions were carried out under continuous stirring in Quick-Thread Glass Reaction Tube 24x150mm using Radley Carousel 12 Plus Reaction Station™.

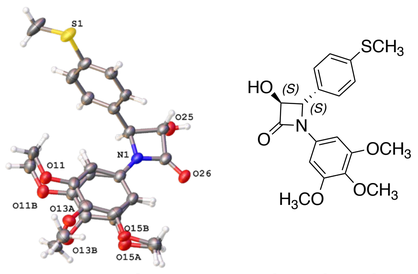


Figure 2: XRD of 3*S,4S* CAZ-6b isolated *via* KR.

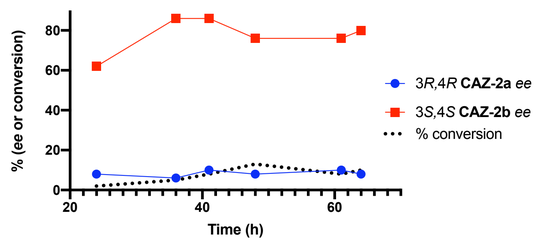


Figure 3: % conversion and *ee* of 3*S,4S* CAZ-2a → CAZ-2b during optimisation reactions (1:1 CAL-B: CAZ-2a and 10 eq of methanol) illustrating higher *ee* between 30-40 hours versus after 60 hours

Table 3: Comparison of *ee* values obtained *via* chiral diastereomeric resolution versus KR. 3*R,4R* enantiomers isolated as 3-acetoxy derivatives and compared to *ee* values of the corresponding 3-hydroxyl enantiomers isolated *via* chiral diastereomeric resolution.

Compound	Ee (%) obtained via diastereomeric resolution	Ee (%) obtained via kinetic resolution
CAZ-1b (*S,S*)	94	74
CAZ-1a (*R,R*)	71	99
CAZ-2b (*S,S*)	78	86
CAZ-4b (*S,S*)	91	81
CAZ-4a (*R,R*)	78	99
CAZ-3b (*S,S*)	66	78
CAZ-3b (*R,R*)	50	68
CAZ-5b (*S,S*)	84	76
CAZ-5a (*R,R*)	85	96
CAZ-6a (*S,S*)	84	81
CAZ-6b (*R,R*)	85	98

Table 1: Optimised CAL-B mediated methanolysis of CAZ-1a - CAZ-8a for isolation of 3*S,4S* 3-hydroxyl eutomers in 62-86% *ee*

	Scale (mmol)	Time	conversion[1]	3R,4R 3-acetoxy yield (s)	% ee_s	3S,4S 3-hydroxyl yield (p)	% ee_p	E[2]
CAZ-1a → CAZ-1b	1	24 h	29%	37%	44	36%	82	16
CAZ-2a → CAZ-2b	1	8 d	22%	29%	18	11%	86	16
CAZ-3a → CAZ-3b	1	3 d	40%	54%	52	26%	70	9
CAZ-4a → CAZ-4b	1	5 d	38%	36%	42	17%	81	14
CAZ-5a → CAZ-5b	1	5 d	38%	36%	42	17%	81	8
CAZ-6a → CAZ-6b	1	4 d	45%	31%	66	34%	76	14
CAZ-7a → CAZ-7b	0.1	3 d	41%	11%	40	8%	62	6
CAZ-8a → CAZ-8b	1	6 d	22 %	29%	24	4.3%	62	9
CAZ-9a → CAZ-9b	1	2 d	43%	34%	66	25%	70	12

[1]measured using ^{1}H NMR at 400 MHz in $CDCl_3$, [2] Enantiomeric ratio (*calculated using ENANTIO online tool),* [s] substrate, [p] product, Reactions were carried out under continuous stirring in Quick-Thread Glass Reaction Tube 24x150mm using Radley Carousel 12 Plus Reaction Station™.

Table 2: The second kinetic resolution of the double resolution strategy for isolation of 3-acetoxy 3*R,4R* enantiomers using CAL-B in ≧ 98% *ee.*

Compound	*ee* prior to second KR (%)	Time	Conversion	3-acetoxy (s) yield	*ee* (%)
CAZ-1a	32	4 d	52%	30%	99
CAZ-4a	42	5 d	43%	36%	99
CAZ-5a	40	3.5 d	63%	16%	96
CAZ-6a	66	3.5 d	42%	20%	98
CAZ-9a	64	5 d	36%	16%	99

1:1 CAL-B: 3-acetoxy β-lactam, 6 eq of methanol, 0.5 mmol scale. Reactions were carried out under continuous stirring in Quick-Thread Glass Reaction Tube 24x150mm using Radley Carousel 12 Plus Reaction Station™.

Table 4: PMI of chiral resolution versus KR

Chiral diastereomeric resolution					Kinetic resolution				
Total mass required for synthesis	Overall enantiomer yield % (mg)				Total mass required for synthesis	Overall enantiomer yield % (mg)			
3753 – 4753 g	3*S,4S* **CAZ-1b** 7 (25.2)	3*R,4R* **CAZ-1** 9 (32.4)	3*S,4S* **CAZ-2b** 6 (23)	3*R,4R* **CAZ-2** 5(19)	~ 700 mg	3*S,4S* **CAZ-1b** 16-36 (30-130)	3*R,4R* **CAZ-1a** 60 (30)	3*S,4S* **CAZ-2b** 6-16 (6-30)	3*R,4R* **CAZ-2** Nd
PMI (kg/kg)	148,810 -188,492		117,187 – 141,049		PMI (kg/kg)	5-23	23	23-116	Nd

Fold reduction in PMI using KR versus chiral diastereomeric resolution (minimum)	3S,4S CAZ-1b	3R,4R CAZ-1a	3S,4S CAZ-2b	3R,4R CAZ-2
	6470	6470	1010	Nd

CONCLUSIONS

- Isolation of a panel of β-lactam enantiomers in excellent *ee* using a sustainable biocatalytic approach with lipase enzyme CAL-B has been achieved.
- Ring opening reactions of 3-acetoxy β-lactams have not been not observed underpinning chemical stability of β-lactam ring, of relevance for formulation strategies.
- Reduction >6000 fold in PMI using KR versus diastereomeric resolution. KR is a sustainable and greener alternative to chiral diastereomeric resolution.
- Cost effective and reduces requirement for skilled labour.
- Rapid and accessible process proposed for scale up and commercial development of novel anti-cancer combretazet APIs for the treatment of triple negative breast and chemo-resistant colorectal cancers.

ACKNOWLEDGEMENTS

We would like to than the Royal Society of Chemistry Research Enablement Grant (E22-0457725696 for funding this research.

REFERENCES

1. McLoughlin, E.C.; O'Brien, J. E.; Trujillo, C.; Meegan, M. J.; O'Boyle, N. M., Application of 2D EXSY and qNMR Spectroscopy for Diastereomeric Excess Determination Following Chiral Resolution of β-Lactams. *ChemistryOpen* **2022**.
2. McLoughlin, E. C.; Twamley, B.; O'Brien, J. E.; Hannon Barroeta, P.; Zisterer, D. M.; Meegan, M. J.; O'Boyle, N. M., Synthesis by diastereomeric resolution, biochemical evaluation and molecular modelling of chiral 3-hydroxyl b-lactam microtubule-targeting agents for the treatment of triple negative breast and chemoresistant colorectal cancers. *Bioorg Chem* **2023,** *141*, 106877.