Design, synthesis and biological evaluation of ansamycin prodrugs as water-soluble Hsp90 inhibitors

Barrie Wilkinson,[1] Alexander Guiblin,[1] Alison McElhinney,[1] Steven J. Moss,[1] Christoph Beckmann,[1] Lesley S. Sheehan, [1] Jean-Philippe Crochard,[1] Andrew J. Kaja,[1] Rose M. Sheridan,[1] Mohammed Nur-E-Alam,[1] Nigel Coates,[1] Matthew A. Gregory, [1] Thomas Greiner,[2] Niko Bausch,[2] Christine J. Martin[1] and Ming-Qiang Zhang[1]

[1]Biotica Technology Ltd, Chesterford Research Park, Little Chesterford, Essex CB10 1XL, United Kingdom
[2]Oncotest GmbH, Am Flughafen 12-14 Freiburg 79108, Germany

biotica

INTRODUCTION

Heat-shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone required for the stability and function of numerous proteins, many of which are necessary for cancer cell growth and survival. Inhibition of Hsp90 causes dysfunction and degradation of these client proteins and therefore tumor inhibition.

Existing polyketide Hsp90 inhibitors suffer from narrow therapeutic windows. For example, the ansamycin polyketide geldanamycin was found to be hepatotoxic in several species and was not soluble in aqueous media. A geldanamycin derivative 17-AAG has an improved therapeutic window but remains poorly soluble. A derivative with increased solubility, 17-DMAG is now in clinical trials. We are interested in developing water-soluble macbecin-based prodrugs as potent Hsp90 inhibitors.

HSP90 BINDING & INHIBITION

Macbecin is an Hsp90 inhibitor with different characteristics from geldanamycin; better water solubility (87 μM *vs* 1.8 μM), a higher affinity to yeast Hsp90 (K_d 0.24 μM *vs* 1.2 μM in isothermal titration calorimetry studies, Figure 1) and a slightly more potent inhibitor of Hsp90 ATPase activity with an IC_{50} ~2 μM (geldanamycin IC_{50} ~7 μM, Figure 2).

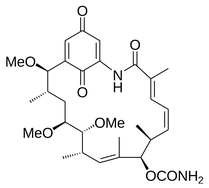

Chart 1. Structure of macbecin

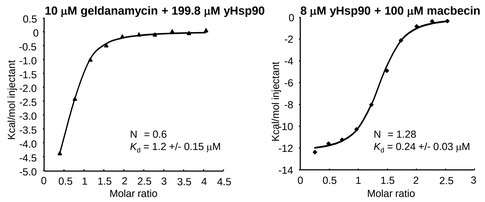

Figure 1. ITC determination of binding to Hsp90

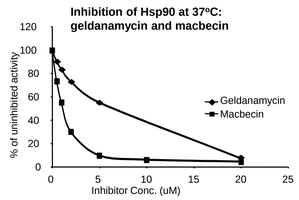

Figure 2. *In vitro* inhibition of Hsp90 ATPase activity by macbecin

CARBAMATE PRODRUGS OF MACBECIN

Macbecin lacks a readily applicable chemical 'handle' for prodrug formation. Our strategy centered on the reduction of the quinone moiety to the hydroquinone form, followed by masking the phenolic group at C18 through a carbamate linkage. A series of prodrugs were synthesised with varying substitutions on the nitrogens of the carbamate. The synthesis of BC274 is shown (Figure 3).

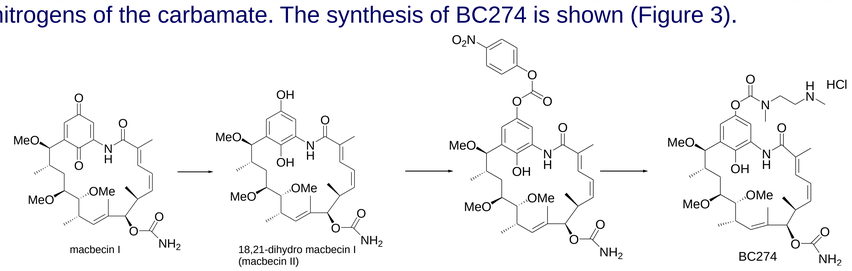

Figure 3. Generation of BC274

SOLUBILITY AND *IN VITRO* CLEAVAGE OF BC274

Introduction of a tertiary amine improved water solubility dramatically. BC274, has a solubility of >350 mM (> 250 mg/mL) in 5% D-glucose, more than 150-fold higher than 17-DMAG (Table 1). It is stable at pH <6.5 but readily converts to macbecin II and then macbecin I at physiological pH, for example in plasma or blood with a $t_{1/2}$ of ~45 min (Table 2).

Compound	Solubility mg/mL
BC274*	>250 mg/mL
17-AAG**	0.1 0.01
17-DMAG**	1.4 0.01

* in 5% D-glucose
** in 50 mM phosphate buffer pH 7.0; Tian et a.,l Bioorg. Med. Chem. 12 (2004) 5317-5329

Table 1. Solubility of ansamycin Hsp90 inhibitors in aqueous solution

Medium	pH	$t_{1/2}$ (min)	R^2
rat blood	6.9	164 / 215	0.87 / -
mouse blood	6.9	197	0.91
phosphate buffer	7.2	64	> 0.99
rabbit blood	7.3	45	0.99
phosphate buffer	7.5	45	> 0.99
phosphate buffer	7.9	25	> 0.99
human plasma	8.3	15/18/19	-

Table 2. Cleavage of BC274 to macbecin I in 66mM phosphate or blood at 37 C. The observed trend demonstrates pH-dependent cleavage.

IN VIVO EFFICACY OF BC274

A pharmacokinetic (PK) study of 17-AAG and BC274 after a single intravenous injection of 25 mg/kg demonstrated that macbecin I is observed in the plasma following administration of BC274. BC274 was determined to have an improved MTTD over macbecin I: 60 mg/kg *vs* 20 mg/kg; similar to 17-AAG – 50 mg/kg. In a xenograft study in mice, at 60 mg/kg, BC274 produced potent inhibition in tumor growth (T/C 30% at day 24 *vs* T/C 36% by 50 mg/kg 17-AAG at day 24) (Figure 5).

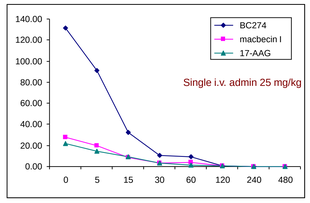

Figure 4. *In vivo* plasma PK calculations for 17-AAG and BC274. The macbecin I observed is generated by cleavage of BC274.

	AUC24h μg/mL*min	T1/2 min	Clearance mL/min	Vz mL
BC274	2149.53	27	0.02	0.73
macbecin I	696.713	72	0.06	5.92
17-AAG	455.517	37	0.09	4.68

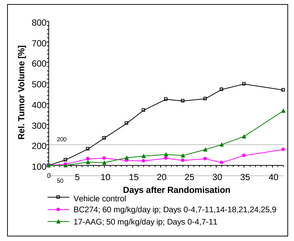

Figure 5. *In vivo* DU145 prostate cancer xenograft in mice, treated with BC274.

SUMMARY

BC274 is a promising candidate for further development with significantly improved water-solubility, coupled with the potent *in vivo* efficacy. The conversion to macbecin is not enzyme-dependent and therefore devoid of any potential issues from metabolic polymorphism.

ACKNOWLEDGMENTS

Oncodesign S.A. (Dijon, France) for PK study; ASI for bioanalysis; Laurence Pearl, Chrisostomos Prodromou and S. Mark Roe for ITC and ATPase studies.