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Trifluoromethyl Alcohol and Ketone Inhibitors of Metallo-β-Lactamases

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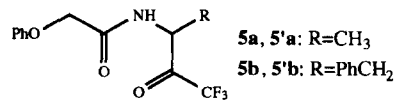
Abstract: α-Amido trifluoromethyl alcohols and ketones were synthesised *via* two independent routes using Ruppert's Reagent ($TMS-CF_3$) and shown to be the first reported synthetic inhibitors of metallo-β-lactamases.

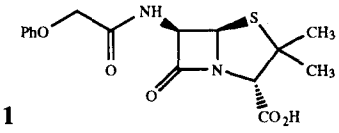
The most important mode of resistance to the β-lactam antibiotics occurs *via* their β-lactamase catalysed hydrolysis[1]. β-Lactamases have been divided into four classes on the basis of their structures and substrate specificities[2]. The mechanisms and inhibition[3] of the Class A, C, and D enzymes, which contain a nucleophilic serinyl residue at their active site, have been extensively studied due to their established clinical importance. In contrast, the Class B enzymes, which require a transition metal-ion for activity, have received relatively scant attention[2,4,5]. Since they are resistant to inhibitors of the serine enzymes including clavulanic acid, the spread of metallo-β-lactamase mediated bacterial resistance to β-lactam antibiotics including carbapenems is of major concern. Two phenazines, isolated from a *Streptomyces*, were reported to exhibit inhibitory activity against two metallo-β-lactamases[6], *via* non specific metal chelation. No synthetic inhibitors of Class B enzymes have been reported in the chemical literature.

Trifluoromethyl ketones are known inhibitors of serine proteases[7] and have also been reported to be inhibitors of the zinc dependent enzyme carboxypeptidase A. In the latter case x-ray analysis of a complex between carboxypeptidase A and a trifluoromethyl ketone[8] revealed the inhibitor bound at the active site in its hydrated form with one hydrate oxygen co-ordinating the zinc ion. A study by Waley and Crompton[9] indicated that simple trifluoromethyl ketones were inhibitors of the *Bacillus cereus* metallo-β-lactamase.

Herein, we report the synthesis and inhibitory activity of α-amido trifluoromethyl ketones against four metallo-β-lactamases. We hoped that the active site zinc-ion would bind the trifluoromethyl ketone in its hydrated form and the nitrogen substituent would mimic the side-chains of β-lactam antibiotics.



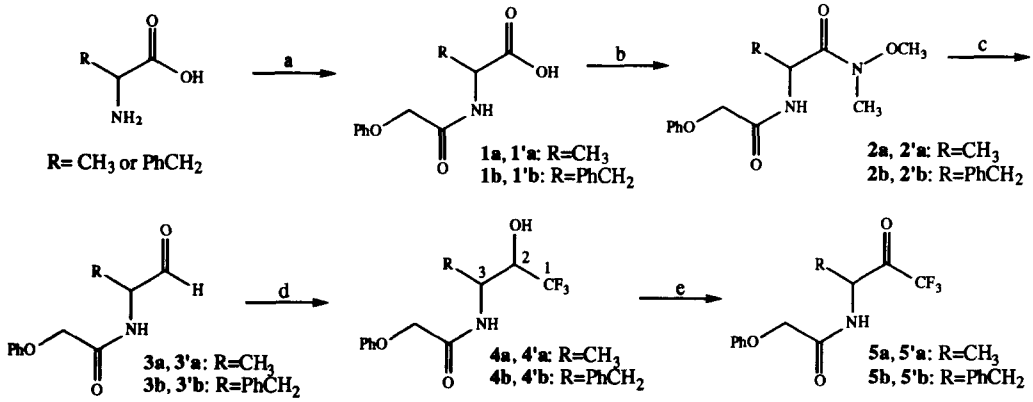
5a, 5'a: R=CH_3
5b, 5'b: R=$PhCH_2$



Scheme 1

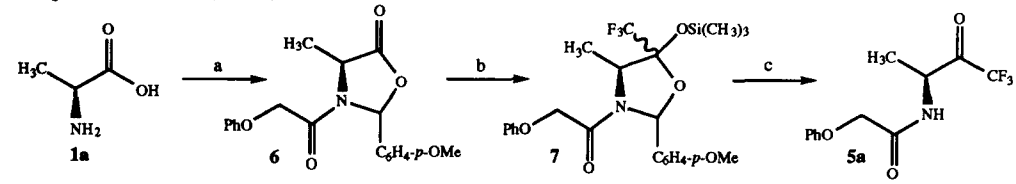
Several syntheses of α-amido trifluoromethyl ketones[10,11] have been reported. Most involve coupling of an α-amino trifluoromethyl alcohol with a carboxylic acid derivative followed by oxidation to give the desired trifluoromethyl ketones. Recently[12] we reported the synthesis of α-carbamoyl trifluoromethyl ketones

based on reaction of (trifluoromethyl)trimethylsilane (TMS-CF_3, Ruppert's Reagent)[13] with oxazolidin-5-ones. Hydrolysis of the adducts gives trifluoromethyl ketones derivatives without the need for oxidation. In order to compare our approach to the established procedure the target compounds **5** were synthesised by both methodologies.



Reagents: (a) $PhOCH_2COCl$, NaOH, 69-90%; (b) EDCI, $NHCH_3OCH_3$ HCl, Et_3N, DMF, 65-99%; (c) $LiAlH_4$, THF, 65-79%; (d) TMS-CF_3, cat. CsF, THF, then HCl, 40-50%; (e) Dess-Martin-Reagent, 65-80%

In the established approach (**Scheme 2**) the requisite α-amido aldehydes were synthesised by reduction of the Weinreb-amides[14] **2** derived from the *N*-phenoxyacetylated amino acids with $LiAlH_4$[15]. Addition of TMS-CF_3 to the aldehydes **3** using tetrabutylammonium fluoride trihydrate[16] resulted in variable yields of the product trifluoromethyl alcohols **4**. Moderate, but reproducible yields were obtained using catalytic amounts of caesium fluoride with sonication. Oxidation of the alcohols **4** using the Dess-Martin periodinane reagent[17] gave the trifluoromethyl ketones **5**, which were purified by crystallisation from diethyl ether/petroleum ether (30-40)[18].



Reagents: (a) NaOH, anisaldehyde then $PhOCH_2COCl$, DCM, reflux, 55%; (b) TMS-CF_3, cat. TBAF-THF, THF; (c) 1 eq. TBAF-THF, then Amberlite®, CH_3CN, 80% over 2 steps

Scheme 3

In our alternative approach (**Scheme 3**)[12], reaction of the sodium salt of alanine with anisaldehyde gave the Schiff base-salt which was subsequently *N*-phenoxyacetylated and cyclised *in situ*[19]. The *N*-phenoxyacetyl substituted oxazolidin-5-one **6** was reacted with TMS-CF_3 to give the silylated adduct **7** in excellent yield. Desilylation using tetra-butylammonium fluoride followed by hydrolysis with strongly acidic ion-exchange resin gave the target molecule **5a** in good overall yield (44% over 3 steps without any silica gel chromatography, compared to 7.5% over 5 steps for the synthesis of **5a** according to **Scheme 2**).

The *N*-phenoxyacetyl-substituted trifluoromethyl ketones and alcohols were tested as inhibitors of

metallo β-lactamases from *Xanthomonas maltophilia* ULA-511, *Aeromonas hydrophilia* AE036, *Bacillus cereus* 569H, and *Pseudomonas aeruginosa* 101 (**Tables 1** and **2**)[20]. Trifluoromethyl ketones derived from *L*-alanine (**5a**), *D*-alanine (**5'a**), *L*-phenylalanine (**5b**) and *D*-phenylalanine (**5'b**) displayed significant inhibition of the enzymes from *X. maltophilia* and *A. hydrophilia*, but were less active against the enzymes from *B. cereus* and *P. aeruginosa*. Interestingly, the C-2 epimeric trifluoromethyl alcohols **4b** and **4'b** also displayed significant inhibitory activity, and in fact **4'b** was the most potent inhibitor of the *B. cereus* and *P. aeruginosa* enzymes.

Compound	*X. maltophilia*	*B.cereus*	*P. aeruginosa*
4a (2*R*, 3*S*); (2*S*, 3*S*)	>5000	300	400
4'a (2*R*, 3*R*); (2*S*, 3*R*)	35±2	700	400
5a (3*S*)	1.5±0.01	300	300
5'a (3*R*)	3.0±0.4	700	500
4b (2*R*, 3*S*); (2*S*, 3*S*)	>5000	1000	900
4'b (2*R*, 3*R*); (2*S*, 3*R*)	>5000	30	60
5b (3*S*)	15±1	500	530
5'b (3*R*)	not tested	1000	not tested

Table 1: K_i values (in μM) for competitive inhibition of three metallo β-lactamases

Compound	K (μM)	k_{+2} (s^{-1})	k_{+2}/K ($M^{-1}s^{1}$)
4'a (2*R*, 3*R*); (2*S*, 3*R*)	217±2	$1.1x10^{-3}$	5.25
5a (3*S*)	44±2	$4.0x10^{-2}$	$9.1x10^{2}$
5'a (3*R*)	11±0.5	$1.0x10^{-2}$	$9.1x10^{2}$
4b (2*R*, 3*S*); (2*S*, 3*S*)	19±1	$4.3x10^{-2}$	$2.3x10^{3}$
4'b (2*R*, 3*R*); (2*S*, 3*R*)	20±1	$3.9x10^{-3}$	$1.9x10^{2}$
5b (3*S*)	6±0.4	$4.3x10^{-2}$	$7.2x10^{3}$

Table 2: Kinetic data for the inhibition of the enzyme from *A. hydrophilia*. *Trifluoromethyl* alcohol **4a** did not show any interaction with the enzyme from *A. hydrophilia* up to a concentration of 4 mM.

Competitive inhibition was observed for three of the metallo β-lactamase enzymes tested (**Table 1**). However, in the case of *A. hydrophilia* AE036 irreversible inhibition was observed for both trifluoromethyl ketone and alcohol inhibitors (**Table 2**). The kinetic data indicate that dissimilar mechanisms of inhibition by the trifluoromethyl compounds occur with different Class B enzymes. These will be the subject of further investigations.

In summary, we have shown that α-amido trifluoromethyl ketones can be synthesised by addition of Ruppert's Reagent to oxazolidin-5-ones more efficiently than by previously known methods. The trifluoromethyl compounds thus obtained are the first reported synthetic inhibitors of different strains of metallo-β-lactamases.

Experimental details for kinetic essays:

Analyses with β-lactamases from *X. maltophilia* and *A. hydrophilia* were performed at 30 °C in 30

mM sodium cacodylate buffer, pH 6.5, containing 0.1 mM $ZnCl_2$. For the *B. cereus* β-lactamase II, analyses were performed at 30 °C in 10 mM sodium cacodylate buffer, pH 6.0. For the enzyme from *B. fragilis* all the experiments were performed at 30 °C in 50 mM HEPES, pH 7.5, containing 20% glycerol.

Inhibitory activities were determined in competition experiments using different reporter substrates[21]. In the case of the *X. maltophilia* enzyme, the K values were determined by analysing the initial rate of hydrolysis of 100 μM nitrocefin and benzylpenicillin using Dixon-plots. The kinetic data for the *A. hydrophilia* enzyme was obtained by following the complete curve of hydrolysis using 200 μM imipenem as the reporter substrate; k_{+2} and k_{+2}/K were determined as described previously[22]. For the *B. fragilis* and *B. cereus* enzymes benzylpenicillin was used as a reporter substrate. The hydrolysis of the antibiotics was monitored by following the absorbance variation resulting from the opening of the β-lactam ring, using a Uvikon 860 spectrophotometer equipped with thermostatically controlled cells and connected to a Copam PC88C microcomputer via a RS232C serial interface. The wavelengths and absorbance variations were those described previously[22]. The kinetic data was calculated according to the following established model[22]:

$$E + I \overset{K}{\rightleftharpoons} E{\cdot}I \xrightarrow{k_{+2}} \{E{-}I\}^* \xrightarrow{k_{+3}} E + P$$

E = enzyme; I = inhibitor; K = dissociation constant of the enzyme-inhibitor-complex; k_{+2} = first order acylation rate constant; k_{+3} = first order deacylation rate constant (k_{+3}= 0 for *A. hydrophilia*). k_{+2}/K corresponds to the k_{cat}/K_M value or 'acylation efficiency' of the enzyme.

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