



PII: S0960-894X(96)00453-2

Trifluoromethyl Alcohol and Ketone Inhibitors of Metallo- β -Lactamases

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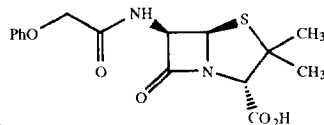
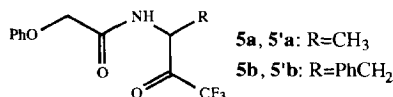
Abstract: α -Amido trifluoromethyl alcohols and ketones were synthesised *via* two independent routes using Ruppert's Reagent (TMS-CF₃) and shown to be the first reported synthetic inhibitors of metallo- β -lactamases.

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The most important mode of resistance to the β -lactam antibiotics occurs *via* their β -lactamase catalysed hydrolysis¹. β -Lactamases have been divided into four classes on the basis of their structures and substrate specificities². The mechanisms and inhibition³ 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 -lactamases⁶, *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 proteases⁷ 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 ketone⁸ 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 Crompton⁹ indicated that simple trifluoromethyl ketones were inhibitors of the *Bacillus cereus* metallo- β -lactamase.

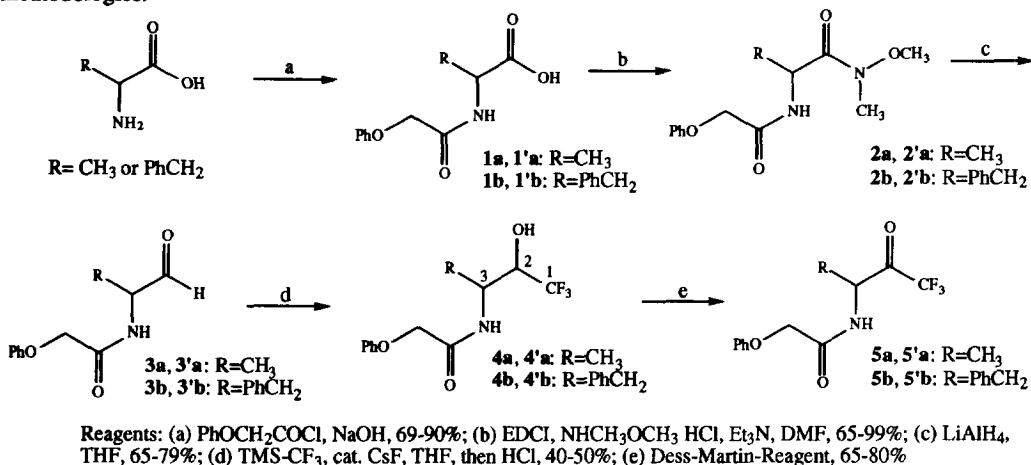
Herein, we report the synthesis and inhibitory activity of α -amido trifluoromethyl ketones against four metallo- β -lactamases. We hoped that the active site zinc-ion would bind the trifluoromethyl ketone in its hydrated form and the nitrogen substituent would mimic the side-chains of β -lactam antibiotics.



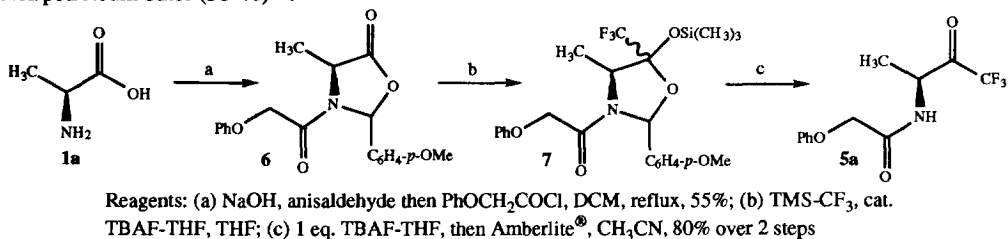
Scheme 1

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. Recently¹² 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.



In the established approach (**Scheme 2**) the requisite α -amido aldehydes were synthesised by reduction of the Weinreb-amides¹⁴ **2** derived from the *N*-phenoxyacetylated amino acids with LiAlH_4 ¹⁵. Addition of TMS-CF_3 to the aldehydes **3** using tetrabutylammonium fluoride trihydrate¹⁶ 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 reagent¹⁷ gave the trifluoromethyl ketones **5**, which were purified by crystallisation from diethyl ether/petroleum ether (30-40)¹⁸.



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	<i>X. maltophilia</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>
4a (2 <i>R</i> , 3 <i>S</i>); (2 <i>S</i> , 3 <i>S</i>)	>5000	300	400
4'a (2 <i>R</i> , 3 <i>R</i>); (2 <i>S</i> , 3 <i>R</i>)	35 \pm 2	700	400
5a (3 <i>S</i>)	1.5 \pm 0.01	300	300
5'a (3 <i>R</i>)	3.0 \pm 0.4	700	500
4b (2 <i>R</i> , 3 <i>S</i>); (2 <i>S</i> , 3 <i>S</i>)	>5000	1000	900
4'b (2 <i>R</i> , 3 <i>R</i>); (2 <i>S</i> , 3 <i>R</i>)	>5000	30	60
5b (3 <i>S</i>)	15 \pm 1	500	530
5'b (3 <i>R</i>)	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 ($\text{M}^{-1}\text{s}^{-1}$)
4'a (2 <i>R</i> , 3 <i>R</i>); (2 <i>S</i> , 3 <i>R</i>)	217 \pm 2	1.1×10^{-3}	5.25
5a (3 <i>S</i>)	44 \pm 2	4.0×10^{-2}	9.1×10^2
5'a (3 <i>R</i>)	11 \pm 0.5	1.0×10^{-2}	9.1×10^2
4b (2 <i>R</i> , 3 <i>S</i>); (2 <i>S</i> , 3 <i>S</i>)	19 \pm 1	4.3×10^{-2}	2.3×10^3
4'b (2 <i>R</i> , 3 <i>R</i>); (2 <i>S</i> , 3 <i>R</i>)	20 \pm 1	3.9×10^{-3}	1.9×10^2
5b (3 <i>S</i>)	6 \pm 0.4	4.3×10^{-2}	7.2×10^3

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.

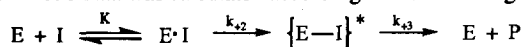
In summary, we have shown that α -amido trifluoromethyl ketones can be synthesised by addition of Ruppert's Reagent to oxazolidin-5-ones more efficiently than by previously known methods. The trifluoromethyl compounds thus obtained are the first reported synthetic inhibitors of different strains of metallo- 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 ZnCl₂. 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 substrates²¹. In the case of the *X. maltophilia* enzyme, the K values were determined by analysing the initial rate of hydrolysis of 100 μ M 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 μ M imipenem as the reporter substrate; k_{+2} and k_{+2}/K were determined as described previously²². 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 previously²². The kinetic data was calculated according to the following established model²²:



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.

Acknowledgements:

This work was supported by the European Community via a Human Capital and Mobility Research Network. We thank the late Stephen G. Waley for inspiration and helpful discussions.

References:

- Frère, J.-M. *Molecular Microbiology* **1995**, *16*, 385-395.
- Waley, S.G. In *The Chemistry of β -Lactams*; Page, M. I., Ed.; Chapman and Hall: London, 1992; pp. 198-228.
- Waley, S.G. In *The Chemistry of β -Lactams*; Page, M. I., Ed.; Chapman and Hall: London, 1992; pp. 229-263.
- Felici, A.; Amicosante, G.; Oratore, A.; Strom, R.; Ledent, P.; Joris, B.; Fanuel, L.; Frère, J.-M. *Biochem. J.* **1993**, *291*, 151-155.
- Carfi, A.; Pares, S.; Duée, E.; Galleni, M.; Duez, C.; Frère, J.-M.; Dideberg, O. *EMBO J.* **1995**, *14*, 4914-4921.
- Gilpin, L. M.; Fulston, M.; Payne, D.; Cramp, R.; Hood, I. *J. Antibiotics* **1995**, *48*, 1081-1085.
- (a) Gelb, M. H.; Svaren, J. P.; Abeles, R. H. *Biochemistry* **1985**, *24*, 1813-1817.
(b) Brady, K.; Wei, A.; Ringe, D.; Abeles, R. H. *Biochemistry* **1990**, *29*, 7600-7607.
- Christianson, D. W.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1986**, *108*, 4998-5003.
- Crompton, I. E. D. Phil. Thesis, Oxford, 1988.
- (a) Kolb, M.; Barth, J.; Neises, B. *Tetrahedron Lett.* **1986**, *27*, 1579-1582. (b) Boivin, J.; El Kaim, L.; Zard, S. Z. *Tetrahedron* **1995**, *51*, 2573-2584. (c) for a review see: McClinton, M.A.; McClinton, D.A. *Tetrahedron* **1992**, *48*, 6555-6666.
- (a) Imperiali, B.; Abeles, R. H. *Tetrahedron Lett.* **1986**, *27*, 135-138. (b) Patel, D. V.; Rielly-Gauvin, K.; Ryone, D. E. *Tetrahedron Lett.* **1988**, *29*, 4665-4668. (c) Edwards, P. D. *Tetrahedron Lett.* **1992**, *33*, 4279-4282. (d) Skyles, J. W. *et. al. J. Med. Chem.* **1992**, *35*, 641-662.
- Walter, M. W.; Adlington, R. M.; Baldwin, J. E.; Chuhan, J.; Schofield, C. J. *Tetrahedron Lett.* **1995**, *36*, 7761-7764.
- Ruppert, I.; Schlich, K.; Volbach, W. *Tetrahedron Lett.* **1984**, *25*, 2195-2198.
- Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815-3818.
- No evidence for reduction of the amide was accrued by 500 MHz ¹H- and ¹³C-NMR analysis.
- (a) Krishnamurti, R.; Bellow, D. R.; Surya Prakash, G. K. *J. Org. Chem.* **1991**, *56*, 984-989.
(b) Ramaiah, P.; Krishnamurti, R.; Surya Prakash, G. K. *Organic Synthesis* **1995**, 232-240.
- Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.
- Silica gel chromatography resulted in low recovery of products 5.
- Hiskey, R. G.; Jung, J. M. *J. Am. Chem. Soc.* **1963**, *85*, 578-582.
- The non-fluorinated analogues of compounds 4a, 4'a, 5a, 5'a, 4b, 4'b, 5b, and 5'b were prepared by known literature procedures¹⁴ and showed no significant activity as inhibitors of the four metallo- β -lactamases tested.
- De Meester, F.; Joris, B.; Reckinger, B.; Bellefroid-Bourguignon, C.; Frère, J.-M.; Waley, S. G. *Biochem. Pharmacol.* **1987**, *36*, 2393-2403.
- Matagne, A.; Misselyn-Baudin, A.-M.; Joris, B.; Epircum, T.; Granier, B.; Frère, J.-M. *Biochem. J.* **1990**, *265*, 131-146.