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Design, Synthesis and Bioactivity Evaluation of Tribactam β Lactamase Inhibitors

Anton Copar,[a] Tadeja Prevec,[a] Borut Anžič,[a] Tomaž Mesar,[a] Lovro Selič,[a] Mateja Vilar[a] and Tom Solmajer[a,b,*]

[a]*Lek d.d., Research and Development, Celovška 135, 1526 Ljubljana, Slovenia*
[b]*National Institute of Chemistry, POB 660, Hajdrihova 19, 1001 Ljubljana, Slovenia*

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Abstract—Known carbapenem compounds with inhibitory effect towards β-lactamase enzymes are formed from bicyclical beta lactam structural scaffolds. On the basis of results from theoretical computational methods and molecular modelling we have designed and developed a synthetic route towards novel, biologically active tricyclic derivatives of carbapenems.

Introduction

Due to their high efficiency and high specificity, β-lactam antibiotics are currently the most widely used antibacterial agents. However, this effectiveness has been seriously compromised by the ability of bacteria to produce β-lactamases, enzymes which hydrolyse the β-lactam ring, thus rendering the drug biologically inactive.

Clinical experience has established that co-administration of a classical β-lactamase-sensitive compound with a specific β-lactamase inactivator represents an efficient strategy in fighting the β-lactamases. Although the few inactivators in clinical use (e.g., clavulanate,[1] sulbactam[2] and tazobactam[3]) readily inactivate most class A enzymes, they are rather inefficient against the class C β-lactamases. It appears that there is no clinically available efficient inactivator of the latter class of enzymes, which represents an increasingly worrying problem.[4]

The clinically used inhibitors of β-lactamases are bicyclic compounds with adjacent five-member ring on the beta lactam core containing a heteroatomic ring atom constituent. Primarily, compounds substituted at positions 3, 4 and 6 of the carbapenem bicycle have been studied and evaluated for activity towards β-lactamases from various bacterial sources and stability against renal dihydropeptidase (Fig. 1).

In order to address the increasing threat of bacterial infections a novel class of tricyclic carbapenem antibiotics (trinems) were developed and an extremely good biological profile was observed by the best compound in the series, sanfetrinem.[5] On the other hand, compounds with 6-substituted methylene penem scaffold, for example, BRL 42715 have shown excellent inhibitory activity towards various classes of β-lactamases.[6]

Our goal was to design and synthesise a tricyclic system in which two structural elements of compounds with inhibitory activity towards the β-lactamase and/or D,D peptidase would be combined. Introduction of an ethylidene bond at position 6 was hypothesised to stabilise the inhibitor–enzyme complex based on the conjugation of the carbonyl group of the beta lactam ring with ethylidene β-orbitals at position 6; in addition to this, a hydrophobic cyclohexane ring in the C3–C4 position of the penem should block the access of the

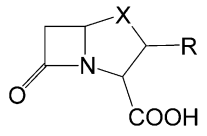

Figure 1. Chemical structures of available inhibitors. (a) clavulanate, X=O, R=CH_2OH; (b) sulbactam X=SO_2; (c) tazobactam X=SO_2, R=CH_2-triazole.

*Corresponding author. Tel.: +386-1-4760-277; fax: +386-1-4760-300; e-mail: tom.solmajer@ki.si

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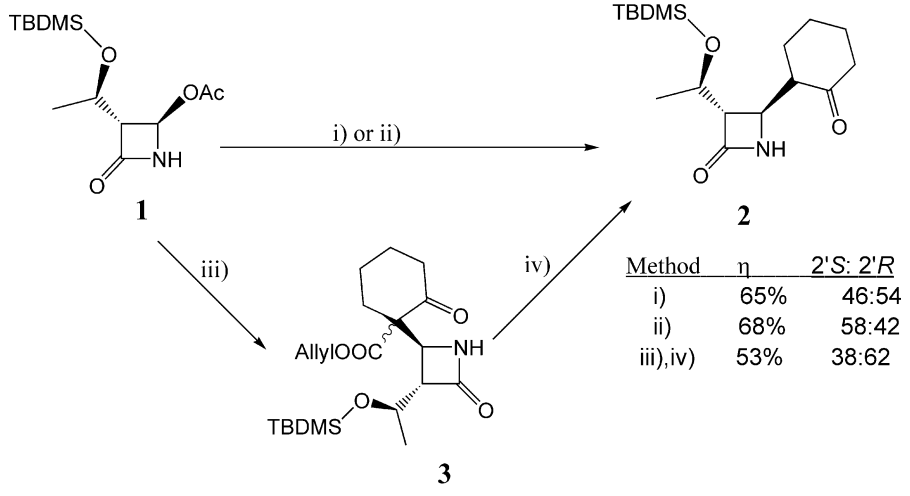
water molecules to the tetrahedral acyl–enzyme complex which is formed in the first step of the enzyme–inhibitor interaction process thus facilitating the stability of the tetrahedral intermediate and preventing the deacylation step.[7,8]

In this paper, we report the synthesis and biological activity of unsubstituted trinems **14a** and **14b** and their 6-thia derivatives **14c** and **14d**.[9]

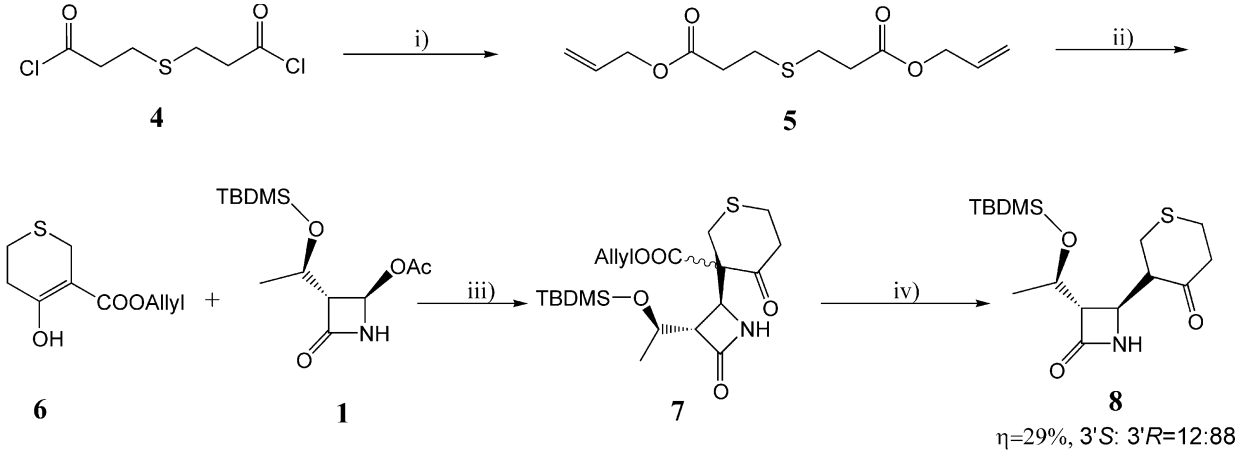
The syntheses of key intermediate **2** are depicted in Scheme 1. Commercially available azetidone **1** reacted with 1-trimethylsilyloxycyclohexene[10] or 4-cyclohex-1-enylmorpholine[11] in the presence of ZnI_2 to give the mixture of 2′-epimers. The least successful approach in terms of yield and assay of the more desirable 2′-*S*-epimer was coupling of azetidone **1** with allyl 2-oxocyclohexanoate in the presence of sodium hydride, and the consequent removal of allyloxycarbonyl group.[12] Epimers were separated by the column chromatography (ethylacetate/hexane 5:1).

The key intermediate for the 6-thia trinems was synthesised as shown in Scheme 2. Allyl 4-hydroxy-5,6-dihydro-2*H*-thiopyran-3-carboxylate (**6**) was prepared from 3,3′-thiopropionyl chloride (**4**) by substitution of chlorine with allyloxy group and subsequent cyclisation. Thiopyran **6** was then coupled with azetidone **1** in the presence of sodium hydride. Allyloxycarbonyl group was removed under standard protocols[12] to give **8** as a mixture of epimers which were separated with column chromatograpy (ether/petroleum ether 3:1) (Scheme 2).

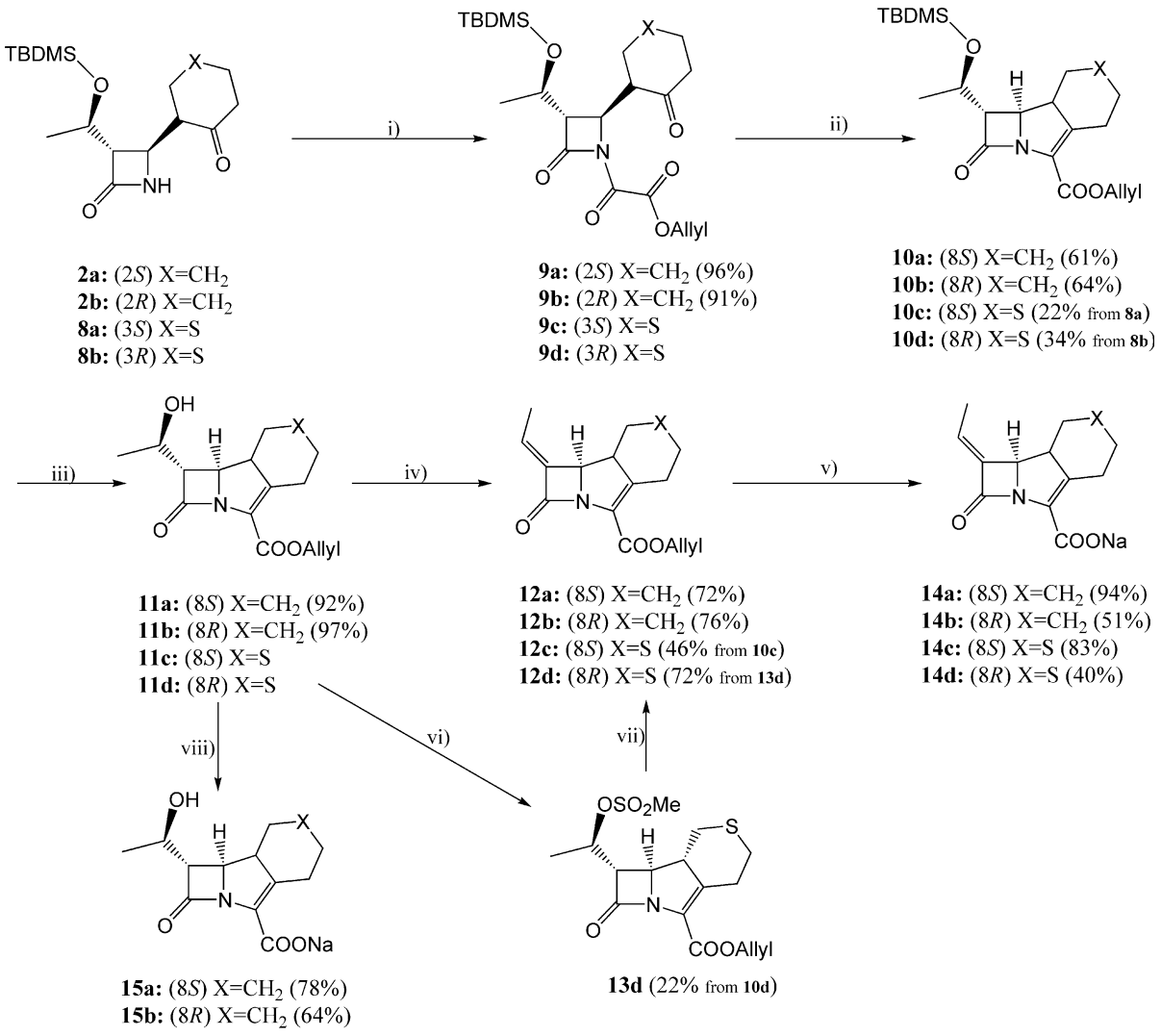
Compounds **2** and **8** were coupled with allyl glyoxylate and consequently transformed into fully protected trinems **10** with oxalimide cyclisation methodology[13] in moderate yields. The silyl protecting group was removed with tetrabutyl ammonium fluoride and acetic acid.[14] Thus, prepared hydroxyethyl trinems **11** were transformed into their 10-ethylidene derivatives **12** via Mitsunobu reaction (diethylazodicarboxylate/triphenylphosphine), **6a** with the exception of compound **11d** which was first converted into mesylate ester **13d** and then treated with 1,8- diazabicyclo[5.4.0]undec-7-ene (DBU)[6a] to give the 10-ethylidene derivative **12d**. Only (*E*)-trinems were isolated upon purification with column chromatography. The structure of these compounds were confirmed with COSY and NOESY NMR experiments. Finally, the target compounds **14** were obtained



Scheme 1. (i) 1-Trimethylsilyoxycyclohexene, ZnI_2, CH_2Cl_2; (ii) 4-cyclohex-1-enyl-morpholine, ZnI_2, CH_2Cl_2; (iii) allyl 2-oxocyclohexanoate; NaH, THF; (iv) $Pd(PPh_3)_4$, PPh_3, NEt_3, HCOOH, CH_2Cl_2.



Scheme 2. (i) AllylOH, Et_3N; (ii) Na, AllylOH, Et_2O; (iii) NaH, THF; (iv) $Pd(PPh_3)_4$, PPh_3, Et_3N, HCOOH, CH_2Cl_2.



Scheme 3. (i) Allyl oxalyl chloride, Et_3N, CH_2Cl_2; (ii) $P(OEt)_3$, hidrochinon, toluene; (iii) TBAF, AcOH, THF; (iv) PPh_3, DEAD, CH_2Cl_2; (v) PPh_3, $Pd(PPh_3)_4$, sodium ethylhexanoate, CH_2Cl_2/THF; (vi) Et_3N, $MeSO_2Cl$, CH_2Cl_2; (vii) DBU, CH_2Cl_2; (viii) PPh_3, $Pd(PPh_3)_4$, sodium ethylhexanoate, CH_2Cl_2/THF.

by removing the allyl ester group using $Pd(Ph_3P)_4$ in the presence of sodium 2-ethylhexanoate (Scheme 3).[15]

Compounds **14**[16] were sufficiently stable and were initially screened for their inhibitory activity towards the enzyme β-lactamase I from *Bacillus cereus*. This was determined by spectroscopic monitoring of the reduction in hydrolytic inactivation of a chromogenic substrate (nitrocefin) by the enzyme in the presence of a β-lactamase inhibitor. The data obtained for the series of synthesised compounds were compared with results of potassium clavulanate and sulbactam (Table 1).

In order to further test our hypothesis that ethylidene bond at position 10 provides the necessary stability of the acyl–enzyme complex, we have synthesised and tested the compounds **15a** and **15b** in which the ethylidene group in compounds **14a** and **14b** is substituted to hydroxyethyl.[7] Both epimers **15a** and **15b** which were obtained by deallylation from compounds **11a** and **11b**,

Table 1. Inhibitory activity[a] at β-lactamase I from *B. cereus*

Inhibitor	% Inhibition of β-lactam hydrolysis at inhibitor concentration[b]		
	100.0 μmol/L	10.0 μmol/L	1.0 μmol/L
Clavulanate	78	33	9
Sulbactam	77	40	2
14a	61	14	−1
14b	23		
14c	60	19	6
14d	38		
15a	3		
15b	5		

[a]For compounds in which the inhibition at 100.0 μmol/L was less than 50% the inhibitory activity at lower concentrations was not determined.

[b]100 μmol of the enzyme lactamase I from *B. cereus* were added to 100 μmol of solution with inhibitor concentrations in range 1–100 μmol/L and the mixture was incubated in the microtitre plate for 20 min at 25 °C. The aliquot of nitrocefin was added and additional incubation for 60 min followed. The % inhibition of the enzyme was monitored at 490 nm.

Table 2. Inhibitory activity of ethylidene trinems against Class A and Class C β-lactamase

Inhibitor	IC_{50} μmol/L	
	E. coli TEM1[a]	*E. cloacae* 908R[b]
Clavulanate	25	100
14a	39	3.0
14b	120	1.8
14c	32	5.0
14d	100	4.2

[a]TEM 1 concentration was 0.3 μmol. Experimental details as in footnote b of Table 1.
[b]908R concentration was 0.8 μmol.

respectively (Scheme 3), were showing very limited inhibitory activity (Table 1) thus pointing to the pivotal role of the double bond in position 10. This result is in accord with excellent stability of trinem antibiotics towards β-lactamase degradation.[5f]

In Table 2, the results of inhibition by ethylidene trinems **14a**–**14d** against representative Class C β-lactamase from *Enterobacter cloacae* 908R are listed along with data for a benchmark Class A enzyme (*Escherichia coli* TEM1).

Compounds **14a**–**d** were shown to competitively inhibit this Class C β-lactamase in micromolar range. We note that the Class C β-lactamase enzymes such as *E. cloacae* 908R represent a phenomenon which compromises further therapy by the existing of antibiotic/inhibitor combinations. Cephalosporins were found to be extremely good substrates for Class C β-lactamases encoded by the AmpC gene products.[3] Our design strategy of blocking the water access to the deacylation site in the inhibitor–lactamase enzyme complex by addition of a hydrophobic moiety onto penem scaffold resulted in promising lead compounds which are being further derivatised to increase their potency.[9b]

Conclusions

Sodium(8*S*,9*R*,10*E*)-1-aza-10-ethylidene-11-oxo-tricyclo-(7.2.0.0^{3,8})undec-2-en-2-carboxylate (**14a**) and sodium (8*S*,9*R*,10*E*)-1-aza-10-ethylidene-11-oxo-6-thia-tricyclo-(7.2.0.0^{3,8})undec-2-en-2-carboxylate (**14c**) have been shown to have activity at the enzyme β-lactamase I from *B. cereus* and another Class A β-lactamase (*E. coli* TEM1) comparable to the two reference compounds. The potential shown by compounds **14a**–**d** to inhibit Class C β-lactamase *E. cloacae* 908R as well could be useful in efforts to retain the β-lactam antibiotics activity in spite of the presence of Class C β-lactamases in bacteria.

Acknowledgements

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16. All the compounds have been characterised by routine analytical techniques:
Compound **14a**: mp 222–225 °C dec. (DMF/ether). ^{1}H NMR 300 MHz (δ, ppm, DMSO-d_6): 1.10–2.05 (m, 7H), 1.77

(d, $J=7.1$ Hz, 3H), 2.65–2.80 (dt, $J=10.3$, 5.1 Hz, 1H), 3.54–3.64 (m, 1H), 4.53–4.61 (dd, $J=10.3$, 1.3 Hz, 1H), 6.21–6.30 (dq, $J=7.1$, 1.3 Hz, 1H). IR (ν_{max}, cm^{-1}, KBr): 2933, 2858, 1740, 1592. MS: m/z 232 (M−23)+.

Compound **14b**: mp 215–219 °C (ether). ^{1}H NMR 300 MHz (δ, ppm, DMSO-d_6): 1.10–2.05 (m, 7H), 1.75 (d, $J=7.1$ Hz, 3H), 2.65–2.80 (dt, $J=10.3$, 5.1 Hz, 1H), 3.37–3.43 (m, 1H), 4.53–4.61 (dd, $J=10.3$, 1.3 Hz, 1H), 6.21–6.30 (dq, $J=7.1$, 1.3 Hz, 1H). IR (ν_{max}, cm^{-1}, KBr): 2930, 2865, 1745, 1594. MS: m/z 232 (M−23)+.

Compound **14c**: mp 195–225 °C (DMF/ether). ^{1}H NMR 300 MHz (δ, ppm, DMSO-d_6): 1.80 (dd, $J=7.1$, 1.0 Hz, 3H), 2.34–2.64 (m, 5H), 3.04 (ddt, $J=5.2$, 10.6, 1.0 Hz, 1H), 3.98 (dt, $J=12.6$, 2.6 Hz, 1H), 4.66 (d, $J=10.0$ Hz, 1H), 6.34 (dq, $J=7.1$, 1.8 Hz, 1H). IR (ν_{max}, cm^{-1}, KBr): 3375, 1757, 1606.

Compound **14d**: mp 223–233 °C (DMF/ether). ^{1}H NMR 300 MHz (δ, ppm, DMSO-d_6): 1.76 (d, $J=7.4$, 1H), 1.86–1.98 (m, 1H), 2.41 (dt, $J=12.9$, 2.9 Hz, 1H), 2.48–2.66 (m, 2H), 2.70–3.20 (m, 1H), 3.15–3.25 (m, 1H), 3.96 (dd, $J=14.7$, 2.8 Hz), 4.08–4.12 (m, 1H), 6.18 (dq, $J=7.0$, 1.5 Hz). IR (ν_{max}, cm^{-1}, KBr): 3427, 1752, 1594.