ARTICLE

OBC
www.rsc.org/obc

Application of the Barton photochemical reaction in the synthesis of 1-dethia-3-aza-1-carba-2-oxacephem: a novel agent against resistant pathogenic microorganisms

Gholam Hossein Hakimelahi,*[a,b] Pai-Chi Li,[a] Ali A. Moosavi-Movahedi,[c] Jamshid Chamani,[c] Ghadam Ali Khodarahmi,[d] Tai Wei Ly,[a] Famil Valiyev,[a] Max K. Leong,[b] Shahram Hakimelahi,[a] Kak-Shan Shia[b] and Ito Chao*[a]

[a] *Institute of Chemistry, Academia Sinica, Taipei, Taiwan, 115, R. O. C. E-mail: ichao@chem.sinica.edu.tw; Fax: +886-2-27831237; Tel: +886-2-27898530*

[b] *TaiGen Biotechnology, 138 Shin Ming Rd., Taipei, Taiwan 114, R. O. C. E-mail: hosein@taigenbiotech.com.tw; Fax: +886-2-27963606; Tel: +886-2-27901861*

[c] *Institute of Biochemistry-Biophysics, Tehran University, Tehran, Iran*

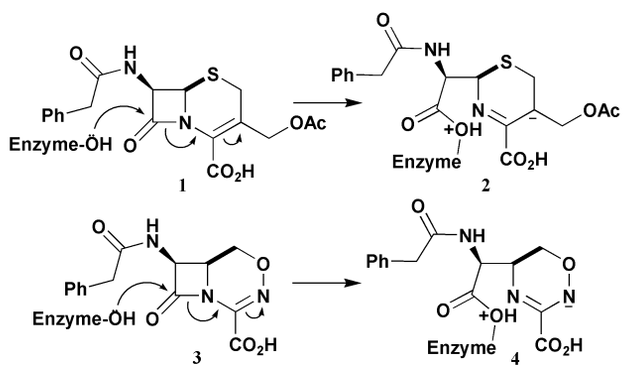
[d] *Department of Medicinal Chemistry, Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran*

Received 23rd April 2003, Accepted 30th May 2003
First published as an Advance Article on the web 12th June 2003

Racemic 7-(phenylacetamido)-1-dethia-3-aza-1-carba-2-oxacephem **3** was synthesized and found to possess antibacterial activity against *Staphylococcus aureus* FDA 209P, *Escherichia coli* ATCC 39188, *Pseudomonas aeruginosa* 1101–75 and *Klebsiella pneumoniae* NCTC 418 as well as the β-lactamase producing organisms *E. coli* A9675 and *P. aeruginosa* 18S-H and the methicillin-resistant organism *S. aureus* 95. Formation of the carbacephem **3** originated from the Barton photochemical reaction in the conversion of **8** to **10**. Intramolecular cyclization of *syn*-oximino β-lactam **10** afforded 7-azido-2-oxa-3-azacephem **11**, which was reduced and acylated to **12**. Enzymatic removal of the methyl group from **12** gave the target molecule **3**.

Introduction

Like penicillins, cephalosporins exert a certain biological activity by acylating serine residues of transpeptidases, where the cross-linking of peptidoglycans does not take place (Scheme 1).[1,2] Bacteria become resistant to antibiotics either through genetic mutations or by acquiring resistant genes from other bacteria.[3] The rapid development and spread of mechanisms of bacterial resistance, however, are making virtually all β-lactam antibiotics obsolete.[3] The main cause of bacterial resistance to β-lactam antibiotics is the β-lactamases, which are related in evolutionary terms to transpeptidases.[4] Bacteria may synthesize the β-lactamase in the presence of frequently used antibiotics. Thus to overcome the destructive action of an inducible β-lactamase, the structure of the β-lactam (*i.e.*, cephalosporin) may be altered to become insensitive to hydrolysis by the β-lactamase (not to be a substrate for β-lactamase) while maintaining its potency as an antibiotic (to be a substrate for transpeptidase).[5] As such, generations of penicillins and cephalosporins were developed.[6]



Scheme 1 Mode of action of β-lactam antibiotics with transpeptidases.

The first generation cephalosporins (*i.e.*, cephalexin and cefadroxil) have excellent activity against gram-positive bacteria but variable activity against gram-negative organisms.[6] The second generation cephalosporins (*i.e.*, cefaclor, cefuroxime axetil, and cefprozil)[7–10] remain fairly active against gram-positive organisms while their gram-negative spectrum is somewhat expanded compared to the first generation cephalosporins.[6] The third generation cephalosporins (*i.e.*, cefixime, cefpodoxime proxetil, and ceftibuten)[7–10] are, generally, less active against gram-positive organisms than the first generation cephalosporins (satisfactory for *streptococci* but inadequate for *staphylococci*). They are, however, extremely active against enteric gram-negative bacteria including *H. influenzae*.[6,8] The fourth generation cephalosporins such as cefepime,[11] with a positively charged quaternary ammonium attached to the dihydrothiazone ring, can penetrate better through the outer membrane of gram negative bacteria and possess a lower affinity than the third generation cephalosporins for certain chromosomal β-lactamases of gram-negative bacilli. Like the third generation agents, cefepime is active against *neisseria* and *H. influenzae*, but has greater activity against ceftazidime-resistant *Pseudomonas aeruginosa* and the gram-negative enterics (*i.e.*, *Enterobacter*, indol-positive *Proteus*, *Citrobacter*, and *Serratia*) possessing inducible chromosomal β-lactamase.

Another new drug which is related to, but differs from, the cephalosporins is loracarbef.[12] It is a carbacephem, which differs from cephalosporins by the substitution of a methylene group for the sulfur atom in the six-membered ring. Carbacephems have remarkable chemical stability that permits structural manipulation that can broaden the antibacterial spectrum of the compound and also facilitate oral absorption. Loracarbef is extremely well absorbed and has a long half-life, permitting twice daily dosing. The drug has been used successfully in patients with streptococcal pharyngitis, otitis media, pneumonia, and cystitis. Unfortunately, it is not active against

DOI: 10.1039/b304167k

Table 1 Minimum inhibitory concentrations (MIC, μg mL^{-1})[a] of carbacephem (±)-**3**, monocyclic-β-lactam (±)-**17**, cephalosporin (**1**),[2,33] cephalothin (**18**),[2,42] cefotaxime (**19**),[34,35] penicillin G (**20**),[36] imipenem (**21**),[37] clavulanic acid (**22**),[32] (±)-**3** + **22** (1 : 1 w/w) and **19** + **22** (1 : 1 w/w) against Microorganisms

	S. aureus		*E. coli*		*P. aeruginosa*		*K. pneumoniae*
β-lactam	FDA 209P	95[b,c]	ATCC 39188	A9675[b]	1101–75	18S-H[b]	NCTC 418
(±)-**3**	0.01 ± 0.00	0.35 ± 0.10	0.08 ± 0.02	0.29 ± 0.10	0.45 ± 0.13	0.71 ± 0.14	0.03 ± 0.01
(±)-**17**	98.7 ± 3.85	>128	>128	>128	93.5 ± 3.33	128	>128
1	0.78 ± 0.11	>128	7.35 ± 0.92	98.6 ± 3.20	128	>128	>128
18	0.31 ± 0.04	34.3 ± 2.03	0.97 ± 0.14	29.5 ± 1.47	7.48 ± 0.31	120	15.31 ± 2.11
19	0.07 ± 0.02	42.8 ± 1.71	0.28 ± 0.03	58.4 ± 1.58	69.6 ± 2.90	> 128	10.61 ± 1.02
20	0.48 ± 0.12	>128	2.78 ± 0.41	>128	128	>128	>128
21	0.02 ± 0.00	14.12 ± 2.10	0.33 ± 0.06	12.1 ± 0.32	5.30 ± 0.25	33.7 ±2.98	0.07 ± 0.02
22	>128	>128	>128	>128	>128	>128	>128
3 + **22**	0.02 ± 0.00	0.66 ± 0.17	0.14 ± 0.01	0.62 ± 0.12	0.92 ± 0.14	1.63 ± 0.20	0.05 ± 0.00
19 + **22**	0.04 ± 0.01	5.10 ± 0.13	0.11 ± 0.02	8.74 ± 0.08	29.0 ± 2.50	37.1 ± 2.30	1.52 ± 0.10

[a] The lowest concentrations of antibiotics needed for the prevention of visible growth of microorganisms, reported as the average values of duplicate determinations (± standard error) and were obtained by use of an agar dilution method whereby organisms were deposited onto medicated agar plates by the replication device of Steers *et al.*[31] [b] β-Lactamase-producing organism. [c] Methicillin-resistant organism.

S. pneumoniae and β-lactamase producing bacterial species such as *H. influenzae* and *M. catarrhalis*.

Since all gram-negative bacteria possess a chromosomal β-lactamase gene capable of producing an inducible chromosomal β-lactamase that can mediate resistance to the old penicillins and cephalosporins, we designed and synthesized new carbacephem (**3**) and monocyclic β-lactam **17** having an electron sink at the 3-position (see Schemes 1 and 4).

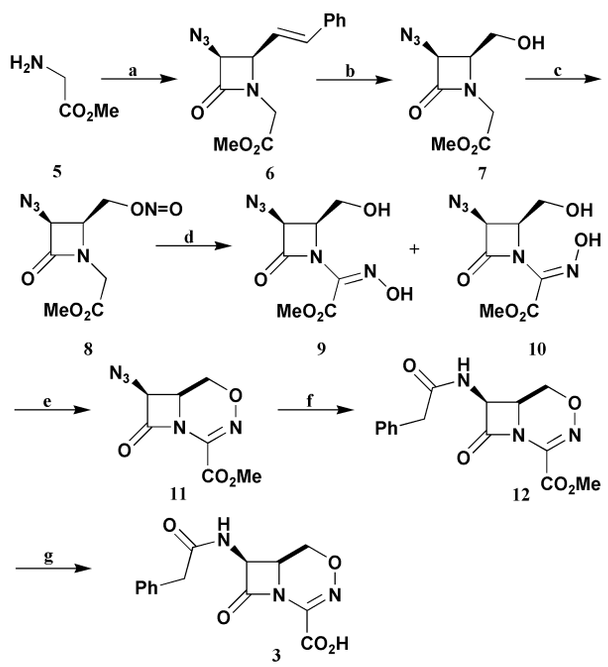
As shown in Scheme 1, ring opening of the β-lactam nucleus occurs when cephalosporins (**1**) react with the enzymes responsible for the cell wall synthesis of bacteria. Consequently, a carbanion at the C-3 position of the acyl–enzyme intermediate **2** will be generated.[13] When the atom at the 3-position of cephalosporins possesses high electronegativity, the susceptibility of the β-lactam ring towards nucleophilic attack by transpeptidases can be enhanced. As such, cephalosporins may exhibit superior antibacterial activity. Antibacterial activity of these novel compounds against *Staphylococcus aureus* FDA 209P, *Escherichia coli* ATCC 39188, *Pseudomonas aeruginosa* 1101–75, *Klebsiella pneumoniae* NCTC 418 as well as the β-lactamase producing organisms *E. coli* A9675, *P. aeruginosa* 18S-H and methicillin-resistant organism *S. aureus* 95 were evaluated and compared to those of cephalosporins and other classical β-lactams. Results from the computational studies suggest that the location of β-lactam binding to the penicillin binding proteins (*i.e.*, D-Ala-D-Ala-peptidase) as well as the solvation effects may be playing roles in profound antibacterial activity of novel 1-dethia-3-aza-1-carba-2-oxacephem **3** relative to those of classical β-lactams (*e.g.* **1**) and monocyclic β-lactam **17** (see Table 1).

Results

Synthesis of 7-(phenylacetamido)-1-dethia-3-aza-1-carba-2-oxacephem (±)-3 (Scheme 2)

Methyl glycinate (**5**) was treated with *trans*-cinnamaldehyde to produce the corresponding Schiff base, which upon reaction with azidoacetyl chloride gave racemic β-lactam **6** in 75% yield. The coupling constant (5.0 Hz) of the two hydrogen atoms on the β-lactam ring indicated the *cis* relationship of the two substituents.[14,15] Ozonolysis of the styryl group in **6**, followed by reductive workup, gave alcohol **7** in 80% yield.[16]

The Barton reaction,[17–19] the intramolecular exchange of NO and H resulting from a photolysis reaction, requires exclusively a six-membered transition state.[20] To meet the requirement for the application of the Barton reaction in β-lactam chemistry, we decided to synthesize nitrite ester **8**. Thus, alcohol **7** was reacted with nitrosyl chloride in pyridine[21] to generate nitrite



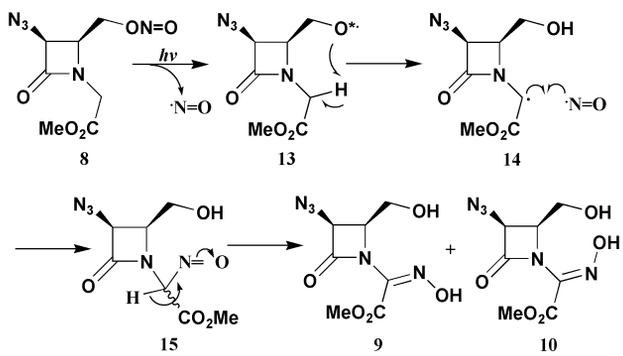
Scheme 2 Synthesis of 1-dethia-3-aza-1-carba-2-oxacephem **3**. *Reagents*: (a) 1. *trans*-PhCH=CHCHO, 2. N_3CH_2COCl, Et_3N; (b) 1. O_3, −75 °C, MeOH, 2. $NaBH_4$, −25 °C; (c) ClNO, pyridine, −20 °C; (d) *hv*, benzene, N_2; (e) DEAD–$P(Ph)_3$, THF; (f) 1. H_2S, Et_3N, 2. $PhCH_2COCl$, pyridine; (g) PLE, phosphate buffer, pH 7.0, acetone.

ester **8** in 55% yield. Compound **8** proved to be stable when protected from light below 0 °C. Photolysis of nitrite ester **8** in dry benzene under a nitrogen atmosphere using a mercury lamp gave *anti*-oximino β-lactam **9** (35% yield) and *syn*-oximino β-lactam **10** (25% yield).[17–19,22a] These two regioisomers exhibited characteristic differences in their IR absorption frequencies of the ester carbonyl and oxime hydroxyl functionalities. Strong intramolecular hydrogen bonding between the ester and the oxime hydroxyl groups in **9**, through a six-membered ring, caused a significant decrease in the absorption frequencies of the ester (C=O; 1738 cm^{-1}) and the hydroxyl (N–OH; 3320 cm^{-1}) groups relative to the corresponding absorptions of **10**, in which the ester and the hydroxyl groups appeared at 1750 and 3620 cm^{-1}, respectively.[22b] Treatment of *syn*-oximino β-lactam **10** with diethyl azodicarboxylate (DEAD) and triphenylphosphine in THF afforded bicyclic β-lactam **11** in 53% yield.[23] A similar reaction with *anti*-oximino β-lactam **9** failed and resulted in the destruction of the β-lactam ring as evidenced by the lack of an IR absorption frequency for the β-lactam carbonyl. The azide group in **11** was reduced by H_2S and Et_3N, and

the resultant amine was acylated with phenylacetyl chloride to 7-(phenylacetamido)-β-lactam **12** in 70% yield.[24] Finally, we demethylated **12** with pig liver esterase (PLE, EC 3.1.1.1) to produce carbacephem **3** in 35% yield.[25]

Mechanism of the Barton photochemical reaction (Scheme 3)

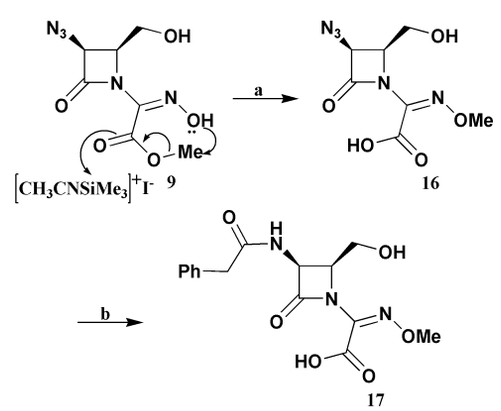
Absorption of light by nitrite ester **8** affords activated alkoxy radical **13** and NO radical. The activated radical abstracts hydrogen, through a six-membered transition state, to furnish carbon radical **14**, which is then captured by NO radical to produce the stereoisomeric oximino β-lactams **9** and **10** through intermediate **15**.



Scheme 3 Mechanism of the Barton photochemical reaction in the synthesis of oximino β-lactams **9** and **10**.

Synthesis of methyl oximino β-lactam (±)-17 (Scheme 4)

Racemic azido β-lactam **9** was treated with trimethylsilyl chloride and NaI in refluxing CH_3CN,[26,27] but only 20% yield of the desired carboxylic acid **16** was obtained and no starting β-lactam **9** was recovered. A possible path for the formation of **16** is depicted in Scheme 4. All attempts, including enzymatic hydrolysis, to gain a high yield of formation of **16** failed and resulted in the recovery or destruction of the starting material. Silylation of the hydroxyl groups in **16** followed by reduction of the azide group with H_2S and Et_3N afforded the corresponding amine. Without isolation, the amine was subsequently treated with phenylacetyl chloride and pyridine to give the corresponding amide **17** in 46% overall yield.[24]



Scheme 4 Synthesis of methyl oximino β-lactam **17**. *Reagents*: (a) 1. TMSCl, NaI, CH_3CN, 2. H_2O; (b) 1. TMSCl, Et_3N, CH_2Cl_2, 2. H_2S, Et_3N, 3. $PhCH_2COCl$, pyridine, CH_2Cl_2.

Solubility and stability of carbacephem (±)-3, monocyclic-β-lactam (±)-17 and cephalothin (18) in water

We found that the solubility in water was 41, 50 and 25 mg mL^{-1} for carbacephem **3**, methyl oximino β-lactam **17** and cephalothin (**18**), respectively; they were stable at physiological pH for 3, 5 and 9 days, respectively. At pH 1.0, the β-lactam ring in **3**, **17** and **18** survived for ~40, ~60 and ~60 min, respectively; yet at pH 12, they were destroyed within ~5 min.

The IR absorption frequency of the carbonyl of a β-lactam can be considered as a measure of its reactivity towards nucleophilic attack.[28,29] β-Lactams **3**, **17** and **18** possessed an IR absorption at 1794, 1782 and 1780 cm^{-1}, respectively. Consequently, highly strained carbacephem **3** is more susceptible to nucleophilic attack than monocyclic β-lactam **17** and cephalothin (**18**).

Table 2 Minimum protective concentrations (MPC, μg mL^{-1})[a] of β-lactams **1**, (±)-**3**, (±)-**17** and **18**–**22** against bacterial β-lactamases

	β-Lactamase of		
β-Lactam	*S. aureus* 95	*E. coli* A9675	*P. aeruginosa* 18S-H
(±)-**3**	97.7 ± 2.30	99.8 ± 1.74	85.8 ± 2.03
(±)-**17**	>128	>128	>128
1	1.35 ± 0.23	3.47 ± 0.51	0.99 ± 0.05
18	4.01 ± 0.30	6.25 ± 0.82	3.70 ± 0.63
19	2.72 ± 0.18	1.12 ± 0.07	1.08 ± 0.22
20	1.85 ± 0.26	2.02 ± 0.39	1.03 ± 0.16
21	30.2 ± 2.94	47.65 ± 3.48	35.6 ± 4.89
22	0.36 ± 0.01	5.20 ± 0.16	3.65 ± 0.14

[a] The average values of duplicate determinations (± standard error) and the ability of compounds to inhibit the hydrolysis of 3-[*E*-(2,4-dinitro)styryl]-(6*R*,7*R*)-7-(2-thienylacetamido)-3-cephem-4-carboxylic acid by β-lactamases from *S. aureus* 95, *E. coli* A9675 and *P. aeruginosa* 18S-H. MPC values, determined by the procedure of O'Callaghan *et al.*,[38] are the lowest concentrations of β-lactams needed to protect the indicator from hydrolysis by β-lactamases under standard test conditions within 40 min. The hydrolysis of indicator was evidenced by a distinct red color.

Biological activity

We carried out screening experiments for the antibacterial activity[30,31] of β-lactams (±)-**3**, (±)-**17**, a mixture of (±)-**3** and clavulanic acid (**22**)[3,32] (1 : 1 w/w) as well as the reference compounds cephalosporin (**1**),[2,33] cephalothin (**18**),[2] cefotaxime (**19**),[34,35] penicillin G (**20**),[36] imipenem (**21**),[37] clavulanic acid (**22**) and a mixture of **19** and **22** (1 : 1 w/w). The experiments were performed *in vitro* against different strains of four pathogenic microorganisms. The results are summarized in Table 1. Furthermore, we tested the β-lactamase inhibitory[38] properties of β-lactams (±)-**3**, (±)-**17** and **18**–**22**. The results are shown in Table 2. The doses used in the above experiments were as high as 128 μg mL^{-1}.

Anticellular activity

Inhibition experiments of the proliferation of human embryonic cells (HEL) and normal fibroblasts (Hef522) by carbacephem (±)-**3** and cephalothin (**18**) were carried out.[47] The toxicity of the tested compounds is expressed as the cytotoxic concentration (μM) required to reduce normal cell growth by 50% (CC_{50}). For carbacephem (±)-**3**, CC_{50} (HEL) = 103.0 μM and CC_{50} (Hef522) = 120.0 μM; yet for cephalothin (**18**), CC_{50} (HEL) > 150 μM and CC_{50} (Hef522) > 150 μM. As such, the newly synthesized carbacephem (±)-**3** was found to be somewhat more toxic than cephalothin (**18**).

Discussion

We found that the carbacephem (±)-**3**, which possesses an electronegative nitrogen atom at the 3-position, exhibited profound antibacterial activity (Table 1), but low β-lactamase susceptibility (Table 2). Its behavior is very different from that of cephalosporin **1**. On the other hand, methyl oximino β-lactam (±)-**17**, a monocyclic analog of bioactive compound (±)-**3**, was not a good antibacterial agent and did not show any inhibitory property towards β-lactamases. In comparison to (±)-**3**,

imipenem **21** [39–41] was found to be less stable to the β-lactamases in Table 2. As such, **21** showed much less activity relative to (±)-**3** against β-lactamase producing microorganisms (Table 1). In contrast to carbacephem (±)-**3** and imipenem **21**, β-lactams **1** and **18**–**20** are highly susceptible to β-lactamases from *S. aureus* 95, *E. coli* A9675, and *P. aeruginosa* 18S-H (Table 2). As a result, they either showed minute or no activity against the corresponding microorganisms (see Table 1). Although carbacephem (±)-**3** exhibited slightly more cellular toxicity than cephalothin **18**, it possesses much superior antimicrobial activity relative to **18**. Clavulanic acid **22** is a highly potent inhibitor of β-lactamases (Table 2), but shows no activity against microorganisms (Table 1). Therefore, it is used to examine the synergistic effect of binary β-lactams (**3** + **22** and **19** + **22**). Unlike cefotaxime **19**, carbacephem (±)-**3** was not an effective substrate for β-lactamases of different bacterial species (Table 2). Consequently, the β-lactamase inhibitory property of clavulanic acid **22** did not exert any synergistic effect on (±)-**3**; yet it exerted a large synergistic effect on cefotaxime **19** (see Table 1).

Although **3** and **17** were designed with the concept of generating an electron sink at the 3 position, density functional calculation results of β-lactams **1**, **3** and **17**, however, showed that the C=O NPA (natural population analysis) charges are rather similar (0.715, 0.713 and 0.718 for the carbon and −0.564, −0.566 and −0.561 for the oxygen of the carbonyl groups in β-lactams **1**, **3** and **17**, respectively). It remains to be explored how the nitrogen at the 3 position and its neighboring oxygen affect the whole reaction path. Computational structural analysis has shown that the low-energy conformation of **17** is very different from those of **1** and **3**. Unlike the relatively extended forms of bicyclic β-lactams **1** and **3**, monocyclic β-lactam **17** folds up due to two pairs of intramolecular hydrogen bonds (O–H ··· O, N–H ··· O; see Fig. 1). Consequently, monocyclic β-lactam **17** binds to D-Ala-D-Ala-peptidase [42a] in a position different from those of bicyclic β-lactams **1** and **3**. As shown in Fig. 2, the O ··· H distance between the carbonyl of the β-lactam ring in **17** and peptidase reaction site, Ser-62, is 4.958 Å. The corresponding distances in peptidase–**1** and peptidase–**3** are 2.310 and 2.260 Å (Fig. 3), respectively. While hydrogen bond networks between the enzyme and substrates **1** and **3** are similar (Tyr-159, Arg-285, Thr-299, Ser-62 and Thr-301 residues are involved in both peptidase–**1** and peptidase–**3** complexes), the hydrogen bond network of peptidase–**17** (Fig. 2) was found to be very different from those of peptidase–**1** and peptidase–**3** complexes (Fig. 3). It should be noted that the unfolded structure of **17** had also been used in MD simulations, but the binding affinity was found to be inferior to the folded one shown in Fig. 2. As such, monocyclic β-lactam **17** did not exhibit activity against pathogenic microorganisms (see Table 1) presumably due to its failure to approach the active site in the correct orientation. While the shorter MD averaged distances between Ser-62 and the β-lactam carbonyl groups in bicyclic β-lactams **1** and **3** (*ca.* 2.3 Å) correlated with their

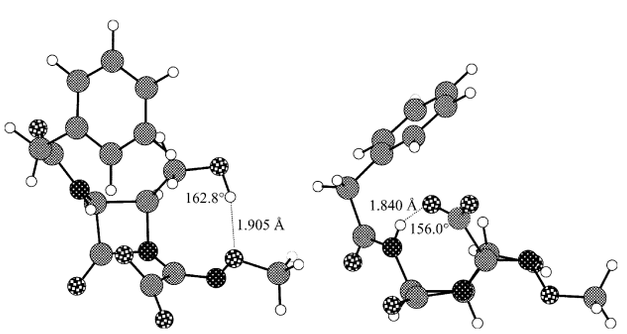


Fig. 1 Top view and side view of monocyclic β-lactam **17** calculated at the B3LYP/6-31G* level. The OH ··· O and NH ··· O hydrogen bonds are shown in the top view (left) and the side view (right), respectively. The numbers show the H ··· O distances as well as the NH ··· O and OH ··· O angles.

Table 3 Solution MD results of D-Ala-D-Ala-peptidase complex with bicyclic β-lactams **1** and **3**

	Difference in binding energy/kJ mol^{-1}	
	1	**3**
<H> 300 K	0.00	−207.47
Av stretch	0.00	−0.61
Av bend	0.00	−1.13
Av torsion	0.00	5.58
Av van der Waals	0.00	9.00
Av electrostatic	0.00	21.96
Av solvation	0.00	−242.28

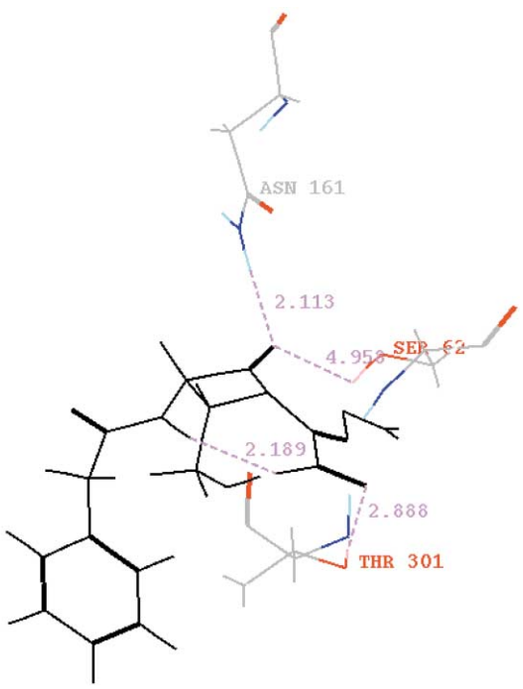


Fig. 2 MD averaged geometry of peptidase–**17** complex. The structure of monocyclic β-lactam **17** is shown in black. The distance between the hydroxyl group of Ser-62 and the carbonyl group of the β-lactam ring is 4.958 Å.

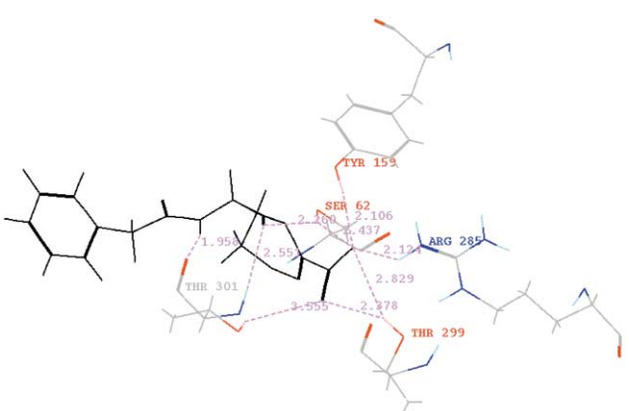


Fig. 3 MD averaged geometry of peptidase–**3** complex. The structure of carbacephem **3** is shown in black. The distance between the hydroxyl group of Ser-62 and the carbonyl group of the β-lactam ring is 2.260 Å.

biological activity, the greater binding affinity of carbacephem **3** relative to cephalosporin **1** (Table 3) correlated with the superior antibacterial property of **3**. Interestingly, as shown in Table 3 the stronger binding affinity of carbacephem **3** was the result of the solvation effect, rather than the non-bonded interactions. Therefore, current preliminary computational exploration of protein–substrate binding indicates that the structure of the drug,

location of the drug binding, and solvation effects could be playing roles in biological activities. A theoretical study on the reaction path is needed to fully elucidate the electronic effect of **3** relative to **1**.

Conclusions

The application of the Barton photochemical reaction led to the preparation of a new carbacephem antibiotic, (±)-**3**, with high stability toward β-lactamases of different bacterial species. Methyl oximino β-lactam (±)-**17** was also synthesized. This monocyclic β-lactam (±)-**17** did not exhibit significant biological activity. Results from the biological tests as well as the computational studies indicate that the newly synthesized carbacephem (±)-**3** is an excellent substrate for transpeptidases. On the other hand, the reference β-lactams **1** and **18**–**20** can be recognized by both transpeptidases and β-lactamases. Thus, in comparison to (±)-**3**, they showed no significant activity against β-lactamase producing microorganisms. Moreover, imipenem **21** with moderate substrate activity toward β-lactamases exhibited less activity than (±)-**3** against *S. aureus* FDA 209P, *E. coli* ATCC 39188, *P. aeruginosa* 1101–75 *Klebsiella pneumoniae* NCTC 418 as well as the β-lactamase producing organisms *E. coli* A9675, *P. aeruginosa* 18S-H and methicillin-resistant organism *S. aureus* 95.

Experimental

General

For anhydrous reactions, glassware was dried overnight in an oven at 120 °C and cooled in a desiccator over anhydrous $CaSO_4$ or silica gel. Reagents were purchased from Fluka Chemical Co. Solvents, including dry ether and tetrahydrofuran (THF), were obtained by distillation from the sodium ketyl of benzophenone under nitrogen. Other solvents, including chloroform, dichloromethane, ethyl acetate, and hexanes were distilled over CaH_2 under nitrogen. Absolute methanol and ethanol were purchased from Merck and used as received. Melting points were obtained with a Büchi 510 melting point apparatus. Infrared (IR) spectra were recorded on a Perkin-Elmer Paragon 1000 Fourier-Transform spectrophotometer. The wave numbers reported are referenced to the 1601 cm^{-1} absorption of polystyrene. Proton NMR spectra were obtained on a Varian XL-300 (300 MHz) spectrometer. Chloroform-*d* and dimethyl sulfoxide-d_6 were used as solvent; Me_4Si (δ 0.00 ppm) was used as an internal standard. All NMR chemical shifts are reported as δ values in parts per million (ppm) and coupling constants (J) are given in hertz (Hz). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, unresolved multiplet due to the field strength of the instrument; and dd, doublet of doublets. Mass spectra were carried out on a VG 70–250 S mass spectrometer. Microanalyses were performed on a Perkin–Elmer 240-B microanalyzer. Purification on silica gel refers to gravity column chromatography on Merck Silica Gel 60 (particle size 230–400 mesh). Analytical TLC was performed on precoated plates purchased from Merck (Silica Gel 60 F_{254}). Compounds were visualized by use of UV light, I_2 vapor or 2.5% phosphomolybdic acid in ethanol with heating.

Computational details

Molecular mechanics (MM) and molecular dynamic (MD) simulations were carried out with the AMBER* force field using software MacroModel 6.5.[43] Lowest energy structures of the carboxylate anions of compounds **1**, **3** and **17** were found by AMBER* and used in geometry optimization at the B3LYP/6-31G* level to obtain NPA charges.[44] To prepare the peptidase–substrate complexes for solution phase MM and MD calculations, the gas phase structures of **1**, **3** and **17** were optimized with the GB/SA solvation model[45] for water and then their β-lactam rings were superimposed to the corresponding part of cephalothin observed in the active site of D-Ala-D-Ala-peptidase.[42a] Before full geometry optimization of the whole peptidase–substrate complex, the substrate was relaxed in two ways. 1. The substrate was optimized in the peptidase structure fixed in the X-ray structure of D-Ala-D-Ala-peptidase–cephalothin. 2. The substrate was partially optimized with the β-lactam ring fixed, and then fully optimized in the fixed X-ray peptidase structure. Fully optimized structures of the peptidase–substrate complex obtained by both procedures were used as the initial structures of MD simulations and the ones afforded lower averaged energies are shown in Table 3 and Figs. 2 and 3. In the MD simulations, the SHAKE algorithm was used for all bonds to hydrogen atoms[46] in the substrate and the peptidase structure was fixed. The temperature was chosen to be 300 K and the time step was 1.5 fs. The equilibration time was 200 ps, followed by 1000 ps of averaging period. The coordinates of structures were sampled every 2 ps during the MD simulations.

Methyl (3*SR*,4*RS*)-2-(3-azido-2-oxo-4-styryl-1-azetidinyl)-acetate (6)

trans-Cinnamaldehyde (2.66 g, 0.0201 mol) in CH_2Cl_2 (6.0 mL) was added dropwise to a solution of methyl glycinate (**5**, 1.79 g, 0.0201 mol) in CH_2Cl_2 (45 mL) containing anhydrous $MgSO_4$ (3.0 g). The solution was stirred at 0 °C for 3.0 h, then was filtered through Celite. Evaporation afforded a quantitative yield of the corresponding Schiff base, which was dissolved in CH_2Cl_2 (85 mL) and Et_3N (5.02 g, 0.0496 mol) was added. A solution of azidoacetyl chloride (2.40 g, 0.0201 mol) in CH_2Cl_2 (9.0 mL) was added dropwise over 10 min at 25 °C. After 2.0 h stirring, the solution was washed with H_2O (2 × 80 mL), dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure. Purification of the residue by use of column chromatography (CH_2Cl_2 as eluent) gave (±)-**6** (4.32 g, 0.0151 mol) as a foam in 75% yield (Found: C, 58.71; H, 4.90; N, 19.59. $C_{14}H_{14}N_4O_3$ requires C, 58.73; H, 4.93; N, 19.57%); ^{1}H NMR ($CDCl_3$) δ 3.54 (s, 3H, CH_3), 4.31 (AB q, J = 17.5, 2H, CH_2), 4.48 (dd, J = 8.0, 5.0, 1H, HC(4)), 4.81 (d, J = 5.0, 1H, HC(3)), 6.32 (dd, J = 16.1, 8.0, 1H, CH=C), 6.61 (d, J = 16.1, 1H, CHPh), 7.12–7.46 (m, 5H, C_6H_5); IR (CH_2Cl_2) 2100 (N_3), 1765 (β-lactam), 1745 (ester) cm^{-1}; CI–MS: 287 (M^+ + 1).

Methyl (3*SR*,4*RS*)-2-(3-azido-2-oxo-4-hydroxymethyl-1-azetidinyl)acetate (7)

Ozone was bubbled through a solution of (±)-**6** (2.16 g, 7.55 mmol) in MeOH (75 mL) at −75 °C until KI–starch paper showed excess O_3 (~1 h). The excess O_3 was removed by a stream of nitrogen, $NaBH_4$ (1.33 g, 35.0 mmol) added at −20 °C, and the solution allowed to warm up to 25 °C within 1.0 h. Then 5% aq. HCl solution (10 mL) was added, the mixture concentrated, H_2O (60 mL) added, and extracted with EtOAc (100 mL). The organic layer was then dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure. The crude product was purified by use of column chromatography ($CHCl_3$ as eluant) to give (±)-**7** (1.29 g, 6.03 mmol) as a foam in 80% yield (Found: C, 39.14; H, 4.80; N, 26.08. $C_7H_{10}N_4O_4$ requires C, 39.25; H, 4.71; N, 26.16%); ^{1}H NMR ($CDCl_3$–D_2O) δ 3.25–3.41 (m, 2H, CH_2O), 3.55 (s, 3H, CH_3), 3.70–4.20 (m, 1H, HC(4)), 4.38 (AB q, J = 18, 2H, CH_2), 4.60 (d, J = 4.8, 1H, HC(3)); IR (CH_2Cl_2) 3160 (OH), 2100 (N_3), 1756 (β-lactam), 1740 (ester) cm^{-1}; CI–MS: 215 (M^+ + 1).

Methyl (3*SR*,4*RS*)-2-(3-azido-2-oxo-4-nitrosooxymethyl-1-azetidinyl)acetate (8)

A solution of (±)-**7** (2.50 g, 11.7 mmol) in dry pyridine (35 mL) was kept in a cooling bath at −20 °C and stirred, while nitrosyl

chloride was allowed to distill into the reaction flask until a yellow color persisted in the solution (~20 min). The solution was partitioned between Et_2O (100 mL) and H_2O (100 mL). The organic layer was washed with H_2O (4 × 100 mL), 5% aq. HCl solution (2 × 70 mL), dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure. The crude product was purified by use of column chromatography ($CHCl_3$ as eluant) to give (±)-**8** (1.56 g, 6.42 mmol) as a foam in 55% yield (Found: C, 34.35; H, 3.60; N, 28.99. $C_7H_9N_5O_5$ requires C, 34.57; H, 3.73; N, 28.80%); ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 3H, CH_3), 3.62–3.70 (br, 2H, CH_2O), 3.74–4.20 (m, 1H, HC(4)), 4.36 (AB q, J = 17.7, 2H, CH_2), 4.70 (d, J = 5.0, 1H, HC(3)); IR (CH_2Cl_2) 2100 (N_3), 1772 (β-lactam), 1750 (ester), 1652, 1615 (nitrite) cm^{-1}; CI–MS: 214 ($M^+ + 1 - NO$).

Methyl (3*SR*,4*RS*)-2-(3-azido-2-oxo-4-hydroxymethyl-1-azetidinyl)-2-*anti*-hydroxyiminoacetate (9) and the respective *syn*-hydroxyiminoacetate (10)

A solution of (±)-**8** (2.43 g, 9.99 mmol) in benzene (170 mL) was irradiated by a 450 W medium pressure Hanovia mercury lamp placed inside an ice–water cooled Pyrex immersion well. Nitrogen flow was maintained during photolysis and the reaction temperature was kept near 10 °C by regulating the temperature of the cooling water. After 1.0 h, the solvent was concentrated under reduced pressure and the residue was purified by use of column chromatography (EtOAc as eluant) to give (±)-**9** (0.849 g, 3.49 mmol, 35% yield) and (±)-**10** (0.600 g, 2.46 mmol, 25% yield), respectively. For (±)-**9** (Found: C, 34.33; H, 3.71; N, 28.67. $C_7H_9N_5O_5$ requires C, 34.57; H, 3.73; N, 28.80%); mp 111–113 °C; ^{1}H NMR ($CDCl_3$–D_2O) δ 3.31–3.56 (br, 2H, CH_2O), 3.89 (s, 3H, CH_3), 3.76–4.22 (m, 1H, HC(4)), 4.65 (d, J = 5.0, 1H, HC(3)); IR (CH_2Cl_2) 3200–3320 (OH), 2100 (N_3), 1788 (β-lactam), 1738 (ester), 1640 (C=N) cm^{-1}; CI–MS: 244 ($M^+ + 1$). For (±)-**10** (Found: C, 34.51; H, 3.72; N, 28.77. $C_7H_9N_5O_5$ requires C, 34.57; H, 3.73; N, 28.80%); mp 117–119 °C; ^{1}H NMR ($CDCl_3$–D_2O) δ 3.38–3.61 (br m, 2H, CH_2O), 3.75 (s, 3H, CH_3), 3.72–4.20 (m, 1H, HC(4)), 4.60 (d, J = 4.9, 1H, HC(3)); IR (CH_2Cl_2) 3620, 3230 (OH), 2100 (N_3), 1790 (β-lactam), 1750 (ester), 1605 (C=N) cm^{-1}; CI–MS: 244 ($M^+ + 1$).

Methyl (6*RS*,7*SR*)-7-azido-8-oxo-1,3-diaza-4-oxabicyclo[4.2.0]-oct-2-ene-2-carboxylate (11)

To a solution of **10** (0.250 g, 1.03 mmol) and $(Ph)_3P$ (0.270 g, 1.03 mmol) in THF (8.0 mL) was added diethyl azodicarboxylate (0.210 g, 1.20 mmol). The reaction mixture became red and the color disappeared after 4 min. The mixture was stirred at 25 °C for 24 h and then the solvent was concentrated under reduced pressure. Purification of the residue by use of column chromatography (CH_2Cl_2 as eluant) afforded (±)-**11** (0.124 g, 0.550 mmol) as a foam in 53% yield (Found: C, 37.30; H, 3.21; N, 31.13. $C_7H_7N_5O_4$ requires C, 37.34; H, 3.13; N, 31.10%); ^{1}H NMR ($CDCl_3$) δ 3.67–4.28 (m, 2H, CH_2O), 3.99 (s, 3H, CH_3), 4.28–4.66 (m, 1H, HC(6)), 4.68 (d, J = 5.0, 1H, HC(7)); IR (CH_2Cl_2) 2110 (N_3), 1800 (β-lactam), 1754 (ester), 1598 (C=N) cm^{-1}; CI–MS: 198 ($M^+ + 1 - N_2$).

Methyl (6*RS*,7*SR*)-8-oxo-7-(phenylacetamido)-1,3-diaza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylate (12)

To a solution of (±)-**11** (0.428 g, 1.90 mmol) in CH_2Cl_2 (50 mL) and Et_3N (0.193 g, 1.90 mmol) at −0 °C, H_2S was bubbled for 10 min. After stirring for 1.0 h, nitrogen was bubbled for 30 min. Pyridine (0.20 g, 2.5 mmol) was added at 25 °C followed by phenylacetyl chloride (0.386 g, 2.50 mmol) dropwise over 5.0 min. After stirring for 1.5 h, the solution was washed with water (3 × 40 mL) and brine (50 mL), then treated with $MgSO_4$ (s) and charcoal. Filtration, evaporation, and purification of the residue by use of column chromatography ($CHCl_3$ as eluant) gave (±)-**12** (0.422 g, 1.33 mmol) as a foam in 70% yield (Found: C, 56.71; H, 4.69; N, 13.14. $C_{15}H_{15}N_3O_5$ requires C, 56.78; H, 4.76; N, 13.24%); ^{1}H NMR ($CDCl_3$) δ 3.56–4.21 (m, 2H, CH_2O), 3.60 (s, 2H, CH_2CO), 3.98 (s, 3H, CH_3), 4.51–4.87 (m, 1H, HC(6)), 5.03 (dd, J = 8.0, 5.0, 1H, HC(7)), 6.28 (d, J = 8.0, 1H, NH), 7.31 (s, 5H, C_6H_5); IR (CH_2Cl_2) 3410 (NH), 1798 (β-lactam), 1750 (ester), 1685 (amide), 1600 (C=N) cm^{-1}; CI–MS: 318 ($M^+ + 1$).

(6*RS*,7*SR*)-8-Oxo-7-(phenylacetamido)-1,3-diaza-4-oxabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (3)

To a solution of (±)-**12** (0.243 g, 0.766 mmol) in acetone (6.0 mL) was added 0.1 M phosphate buffer solution (pH 7.0, 6.0 mL) containing PLE (30 mg). After stirring for 12 h at 25 °C, the acetone was evaporated and the aq. solution was kept at 10 °C to afford (±)-**3** (0.082 g, 0.27 mmol) as pale yellow crystals in 35% yield (Found: C, 55.32; H, 4.28; N, 13.98. $C_{14}H_{13}N_3O_5$ requires C, 55.44; H, 4.32; N, 13.85%); mp 132–133 °C; ^{1}H NMR ($CDCl_3$–DMSO-d_6–D_2O) δ 3.60–4.12 (br m, 2H, CH_2O), 3.58 (s, 2H, CH_2CO), 4.45–4.83 (m, 1H, HC(6)), 5.11 (d, J = 4.9, 1H, HC(7)), 7.28 (s, 5H, C_6H_5); IR (CH_2Cl_2) 3140–3665 (CO_2H, NH), 1794 (β-lactam), 1715 (C=O), 1680 (amide), 1608 (C=N) cm^{-1}; CI–MS: 304 ($M^+ + 1$).

(3*SR*,4*RS*)-2-(3-Azido-2-oxo-4-hydroxymethyl-1-azetidinyl)-2-*anti*-methoxyiminoacetic acid (16)

To a solution of (±)-**9** (0.485 g, 1.99 mmol) in dry CH_3CN (20 mL) was added anhydrous NaI (0.62 g, 4.0 mmol) and trimethylsilyl chloride (0.436 g, 4.00 mmol). The mixture was heated at reflux temperature for 1.5 h, then H_2O (25 mL) was added and the solution was extracted with EtOAc (40 mL). The organic layer was concentrated under reduced pressure and the residue was purified by use of column chromatography (EtOAc–MeOH (9 : 1) as eluant) to give (±)-**16** (0.0970 g, 0.399 mmol) in 20% yield (Found: C, 34.38; H, 3.60; N, 28.79. $C_7H_9N_5O_5$ requires C, 34.57; H, 3.73; N, 28.80%); mp 125–127 °C; ^{1}H NMR ($CDCl_3$–DMSO-d_6–D_2O) δ 3.29–3.60 (br, 2H, CH_2O), 4.01 (s, 3H, CH_3), 3.81–4.28 (m, 1H, HC(4)), 4.61 (d, J = 5.0, 1H, HC(3)); IR (KBr) 3150–3400 (CO_2H, OH), 2100 (N_3), 1786 (β-lactam), 1705 (C=O), 1620 (C=N) cm^{-1}; CI–MS: 244 ($M^+ + 1$).

(3*SR*,4*RS*)-2-(2-Oxo-3-(phenylacetamido)-4-hydroxymethyl-1-azetidinyl)-2-*anti*-methoxyiminoacetic acid (17)

To a solution of (±)-**16** (0.194 g, 0.798 mmol) in CH_2Cl_2 (25 mL) and Et_3N (0.638 g, 6.29 mmol) was added trimethylsilyl chloride (0.433 g, 3.99 mmol). The mixture was stirred at 0 °C for 1.0 h. Then H_2S was bubbled for 5.0 min into the solution. After stirring at 0 °C for 1.0 h, nitrogen was bubbled for 20 min. Pyridine (0.128 g, 1.60 mmol) was added at 25 °C followed by phenylacetyl chloride (0.247 g, 1.60 mmol) dropwise over 5.0 min. After stirring for 2.0 h, the solution was washed with water (2 × 25 mL), dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure. Purification of the residue by use of flash chromatography (EtOAc–MeOH (8.5 : 1.5)) afforded (±)-**17** (0.123 g, 0.367 mmol) in 46% yield (Found: C, 53.59; H, 4.97; N, 12.68. $C_{15}H_{17}N_3O_6$ requires C, 53.73; H, 5.11; N, 12.53%); mp 131–133 °C; ^{1}H NMR ($CDCl_3$–DMSO-d_6–D_2O) δ 3.31–3.75 (m, 2H, CH_2O), 3.58 (s, 2H, CH_2CO), 3.99 (s, 3H, CH_3), 4.12–4.40 (m, 1H, HC(4)), 5.19 (d, J = 4.9, 1H, HC(3)), 7.26 (s, 5H, C_6H_5); IR (KBr) 3160–3420 (NH, CO_2H, OH), 1782 (β-lactam), 1710 (C=O), 1680 (amide), 1617 (C=N) cm^{-1}; CI–MS: 336 ($M^+ + 1$).

Antibacterial activity tests

The serial broth dilution method was used to study the antibacterial activity of β-lactams (Table 1).[30] The inocula were prepared by use of the heart infusion broth (Difco Laborator-

ies) to make 10^{-4} dilutions of the overnight cultures. Tubes of the seeded antibiotic-containing media were incubated at 37 °C for 20 h. The lowest concentration of the β-lactam that prevented visible growth of microorganisms was then determined.

Toxicity test procedure *in vitro*

Human embryonic cell (HEL) and normal fibroblasts (Hef522) cell lines were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2.0 mM glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycine in a humidified atmosphere with 5% CO_2 and 20% O_2 at 37 °C.[47] After 24 h, carbacephem (±)-**3** and cephalothin (**18**), at various concentrations, were added. The cell numbers of the control cultures as well as those cultures supplemented with the test compounds were determined after 12, 24, 48, 72 and 90 h of growth. The CC_{50} values were estimated from dose–response curves compiled from two independent experiments and represent cytotoxic concentration (μM) required to reduce cell growth by 50% after 90 h incubation.

Acknowledgements

For financial support, we thank the Ministry of Science and Higher Education of Iran, Academia Sinica, Tehran University, and the National Science Council of Republic of China.

References

1 D. J. Waxman and J. L. Strominger, *J. Biol. Chem.*, 1980, **255**, 3964–3976.
2 J. M. Frére, M. Nguyen-Disteche, J. Coyette and B. Joris, in *The Chemistry of β-Lactams*, ed. M. I. Page, Blackie Academic & Professional, New York, 1992, pp. 148–197.
3 G. H. Hakimelahi, K.-S. Shia, C. Xue, S. Hakimelahi, A. A. Moosavi-Movahedi, A. A. Saboury, A. Khalafi-Nezhad, M. N. Soltani-Rad, V. Osyetrov, K.-P. Wang, J.-H. Liao and F.-T. Luo, *Bioorg. Med. Chem.*, 2002, **10**, 3489–3498 and references cited therein.
4 J. R. Knowles, *Acc. Chem. Res.*, 1985, **18**, 97–104 and references cited therein.
5 G. H. Hakimelahi, A. A. Moosavi-Movahedi, S.-C. Tsay, F.-Y. Tsai, J. D. Wright, T. Dudev, S. Hakimelahi and C. Lim, *J. Med. Chem.*, 2000, **43**, 3632–3640.
6 C. A. Gustaferro and J. M. Steckelberg, *Mayo. Clin. Proc.*, 1991, **4**, 1064–1073.
7 L. R. Levine, *Pediatr. Infect. Dis.*, 1985, **4**, 358–360.
8 W. J. Rodrigues and B. L. Wiedemann, *Adv. Pediatr. Infect. Dis.*, 1994, **9**, 125–149.
9 P. Mendelman, M. Del Becarro and S. McLinn, *J. Pediatr. (St. Louis)*, 1992, **121**, 459–465.
10 N. Lowery, G. L. Kearns, R. A. Young and J. G. Wheeler, *J. Pediatr. (St. Louis)*, 1994, **325**, 325–328.
11 W. E. Sanders, Jr., J. H. Tenney and R. E. Kessler, *Clin. Infect. Dis.*, 1996, **23**, 454–461.
12 M. Green and E. R. Wald, *Ann. Allergy Asthma Immunol.*, 1996, **77**, 167–175.
13 W. S. Faraci and R. F. Pratt, *J. Am. Chem. Soc.*, 1984, **106**, 1489–1490.
14 H. B. Kagan, J. J. Basselier and J. L. Luche, *Tetrahedron Lett.*, 1964, 941–948.
15 P. J. Decazes, J. L. Luche and H. B. Kagan, *Tetrahedron Lett.*, 1970, 3661–3664.
16 J. R. Hwu, L.-L. Lai, G. H. Hakimelahi and H. Davari, *Helv. Chim. Acta*, 1994, **77**, 1037–1045.
17 D. H. R. Barton, J. M. Beaton, L. E. Geller and M. M. Pechet, *J. Am. Chem. Soc.*, 1961, **83**, 4076–4083.
18 D. H. R. Barton and J. M. Beaton, *J. Am. Chem. Soc.*, 1961, **83**, 4083–4089.
19 M. Akhtar, D. H. R. Barton, J. M. Beaton and A. G. Hortmann, *J. Am. Chem. Soc.*, 1963, **85**, 1512–1519.
20 P. Kabasakalian and E. R. Townley, *J. Am. Chem. Soc.*, 1962, **84**, 2711–2716.
21 N. Kornblum and E. P. Oliveto, *J. Am. Chem. Soc.*, 1949, **71**, 226–228.
22 (*a*) R. D. Rieke and N. A. Moore, *J. Org. Chem.*, 1972, **37**, 413–418; (*b*) R. M. Silverstein, G. C. Bassler and T. C. Morrill in *Spectrometric Identification of Organic Compounds*, John Wiley & Sons, California, 1981, pp. 95–180.
23 E. Grochowski and J. Jurczak, *Synthesis*, 1976, 682–684.
24 G. H. Hakimelahi and G. Just, *Can. J. Chem.*, 1981, **59**, 941–944.
25 S. Gehanne, M. Giammaruco, M. Taddei and P. Ulivi, *Tetrahedron Lett.*, 1994, **35**, 2047–2048.
26 T. Morita, Y. Okamoto and H. Sakurai, *Tetrahedron Lett.*, 1978, 2523–2526.
27 G. H. Hakimelahi and G. Just, *Helv. Chim. Acta*, 1982, **65**, 1359–1367.
28 R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon and S. L. Andrews, *J. Am. Chem. Soc.*, 1969, **91**, 1401–1407.
29 L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, 2nd edn., Wiley, New York, 1958.
30 T. A. Pursiano, M. Misiek, F. Leitner and K. E. Price, *Antimicrob. Agents Chemother.*, 1973, **3**, 33–39.
31 F. Steers, F. L. Foltz and B. S. Graves, *Antibiot. Chemother.*, 1959, **9**, 307–311.
32 J. R. Hwu, S. Hakimelahi, A. A. Moosavi-Movahedi and S.-C. Tsay, *Chem. Eur. J.*, 1999, **5**, 2705–2711.
33 J. R. Hwu, A. A. Moshfegh, S.-C. Tsay, C. C. Lin, W. N. Tseng, A. Azaripour, H. Mottaghian and G. H. Hakimelahi, *J. Med. Chem.*, 1997, **40**, 3434–3441.
34 F. J. Muhtadi and M. M. A. Hassan, in *Analytical Profiles of Drug Substances*, ed. K. Florey, Academic, New York, 1982, Vol. 11, pp. 139–168.
35 R. Wise, T. Rollason, M. Logan, J. M. Andrews and K. A. Bedford, *Antimicrob. Agents Chemother.*, 1978, **14**, 807–811.
36 J. J. Morris and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 1980, 212–219.
37 H. Kroop, J. G. Sundelof, J. S. Kahan, F. M. Kahan and J. Birnbaum, *Antimicrob. Agents Chemother.*, 1980, **17**, 993–1000.
38 C. H. O'Callaghan, A. Morris, S. M. Kirby and A. H. Shingler, *Antimicrob. Agents Chemother.*, 1972, **1**, 283–288.
39 H. C. Neu and P. Labthavikul, *Antimicrob. Agents Chemother.*, 1982, **21**, 180–187.
40 S. Mitsuhashi, *J. Antimicrob. Chemother.*, 1983, **12**(Suppl. D), 53–64.
41 W. J. Leanza, K. J. Windonger, T. W. Miller and B. G. Christensen, *J. Med. Chem.*, 1979, **22**, 1435–1436.
42 (*a*) J. R. Knox and A. P. Kuzin, *Biochemistry*, 1995, **34**, 9532–9540; (*b*) H. C. Neu, in *The Chemistry of β-Lactams*, ed. M. I. Page, Blackie Academic & Professional, New York, 1992, pp. 101–128.
43 (*a*) F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1990, **11**, 440–467; (*b*) S. J. Weiner, P. A. Kollman, D. A. Case, U. C. Singh, C. Chio, G. Alagona, S. Profeta and P. Weiner, *J. Am. Chem. Soc.*, 1984, **106**, 765–784; (*c*) S. J. Weiner, P. A. Kollman and D. A. Case, *J. Comput. Chem.*, 1986, **7**, 230–252; (*d*) D. Q. McDonald and W. C. Still, *Tetrahedron Lett.*, 1992, **33**, 7743–7746.
44 (*a*) M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A. Liashenko, I. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, M. Head-Gordon, E. S. Reploge and J. A. Pople, *Gaussian 98, Revision A.5*, Gaussian Inc., Pittsburgh, PA, 1998; (*b*) P. Hobenberg and W. Kohn, *Phys. Rev. Sect. B*, 1964, **136**, 864–871; (*c*) W. Kohn and L. J. Sham, *Phys. Rev. Sect. A*, 1965, **140**, 1133–1138; (*d*) A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648–5652; (*e*) C. Lee, W. Yang and R. G. Parr, *Phys. Rev. Sect. B*, 1988, **37**, 785–789.
45 W. C. Still, A. Tempczyk, R. C. Hawley and T. Hendrickson, *J. Am. Chem. Soc.*, 1990, **112**, 6127–6129.
46 (*a*) W. F. van Gunsteren and H. J. C. Berendsen, *Mol. Phys.*, 1977, **34**, 1311–1327; (*b*) H. J. C. Berendsen, J. P. M. Potsma, W. F. van Gunsteren, A. D. DiNola and J. R. Haak, *J. Chem. Phys.*, 1984, **81**, 3684–3690.
47 S. Rai'c-Mali'c, A. Hergold-Brundi'c, A. Nagal, M. Grdisa, K. Paveli'c, E. DeClercq and M. Mintas, *J. Med. Chem.*, 1999, **42**, 2673–2678.