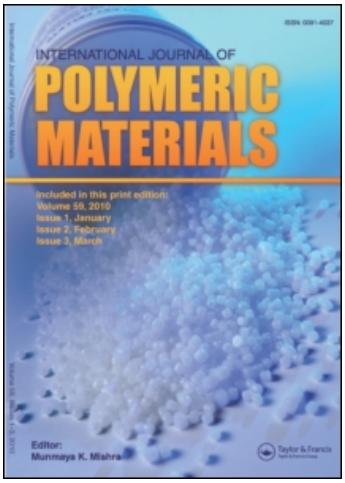

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Maria Campos[a]; Humberto Gonzalez[a]; Gaston Bocaz[a]; Osman Vasquez[a]; Roberto Paredes[a]; Cecilia Parra[a]
[a] Departamento de Polimeros, Facultad de Ciencias Quimicas, Universidad de Concepción, Concepción, Chile

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Design of a Novel Inhibitor of Class A β-lactamases by a Computational Method

MARIA CAMPOS, HUMBERTO GONZALEZ, GASTON BOCAZ, OSMAN VASQUEZ, ROBERTO PAREDES and CECILIA PARRA

Departamento de Polimeros, Facultad de Ciencias Quimicas, Universidad de Concepción, Casilla 3-C, Concepción, Chile

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A new inhibitor bromopenem (6 β-bromo-1-dioxi-penem) for class A β-lactamases has been designed by computational methods. The structure was developed by applying the minimization MM2 of the Alchemy III program and compared with the one of the BRL 42715.

The inhibitor is a penem derivative analogous of the substrate and covalently bound to the reactive serine 70.

Bromopenem has some structural features of the following inhibitors: 6 β-bromo- penicillanic acid, sulbactam and BRL 42715. Its stability energy is 44.74 (Kcal/mol), mostly lower to the other inhibitors studied.

The interaction between bromopenem and the crystal structure of *E. coli* TEM-1 β-lactamase at 1.8 Å is also given, by using the minimization MM2 of the Alchemy III and RasMol programs.

Keywords: Inhibition; β-lactamase; structure; substrate; penem; stability energy

INTRODUCTION

The β-lactamases (penicillin amido β-lactam hydrolases, E.C. 3.5.2.6) have been recognized as the most relevant mechanism against the penicillins and cephalosporins, which are extensively used to overcome serious infectious diseases. These enzymes are produced by an increasingly wide range of bacteria and they vary remarkably in their physicochemical properties. The resistance might be overcome by the use of the β-lactamic antibiotic together with a suitable inhibitor [1].

Six irreversible inhibitors, analogous of the substrate have been studied towards the β-lactamase [2] from *S. flexneri* UCSF-129. This is a pathogenic strain which produces Shigellosis, a serious gut disease especially in the child population [3]. Its β-lactamase is highly resistant to benzylpenicillin and ampinicillin and has a molecular weight [4] of 23.6 KDa. Serine 70 dependent [5] and class A [6] according to Ambler [7]. The residue of Arginine 65 is vital to stabilizes the negative charge of the carboxylic group of C-3 or C-4 from the substrate [8].

The most powerful inhibitor studied was the BRL 42715 [C6-(N_1-methyl-1,2,3 triazolylmethylene) penem] [9]. The MIC fell from 2048 to 2 μg/ml in the presence of 1μg/ml of this inhibitor [1]. However, it is too reactive and hydrolyzes quickly in aqueous solution [10] due to the reactivity of the triazolyl methylene group.

In this work, we design an optimum inhibitor for class A β-lactamases, fitting this inhibitor in the active site peptide of the *S. flexneri* β-lactamase [11] and in the crystal structure of *E. coli* TEM-1 β-lactamase [12] at 1.8 Å by computational methods.

EXPERIMENTAL

The TRIPOS Alchemy III program was used to compare the created structures by the energy minimization, interfase MM2, which allows to obtain the most stable conformation of the design molecule (Sigma Chemical Company, St. Louis, MO, USA). The RasMol v2.4, a molecular visualization program, was also applied. It reads in a molecule coordinate file and interactively displays the molecule on the screen in a variety of representations and colour schemes supported input file formats include Brook haven Protein Databank (PDB) and Alchemy format. RasMol v2.4 is a molecular graphic program intended for the visualization of proteins and small molecules. (Roger Sayle, Biomolecular Structure Glaxo Research and Development, Greenford, Middlesex, U.K. 1994).

The crystallographic data of *E. coli* TEM-1 β-lactamase was obtained from Jelsch *et al.*[12] and the active site peptide of β-lactamase from *Shigella flexneri* UCSF-129 from Campos *et al.*[11].

RESULTS AND DISCUSSION

A novel inhibitor, Bromopenem (6-β-bromo-1-dioxi-penem) for class A β-lactamases has been designed by computational methods. The structure was

developed by applying the minimization MM2 of the Alchemy III Program to obtain the most stable designed molecule. Figure 1 shows the structure of this inhibitor and compare with the one of the penem BRL 42715 [9], the most powerful studied up to now. However it hydrolyzes quickly [10], a reflection of poor chemical stability *in vitro* test system. It is also unstable in bladder urine at body temperature [13]. Bromopenem is a penem derivative, analogous of the substrate and has some structures features of 6β-bromopenicillanic acid, sulbactam and BRL-42715.

The different values of the stability energy for the studied inhibitors are given in Table I. Bromopenem shows to have an stable molecule geometry, and its molecule's geometry fits perfectly well in the peptide of the active site of β-lactamase from *S. flexneri* UCSF-129 (Fig. 2). It specifically bounds to the serine

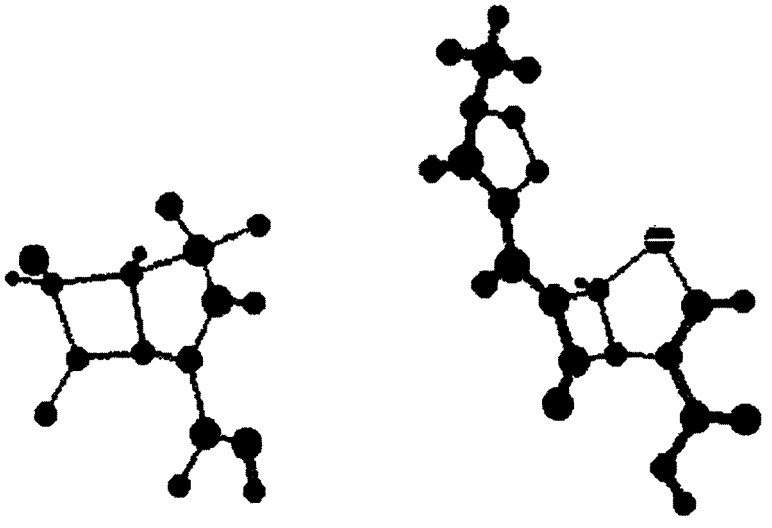

FIGURE 1 Bromopenem structure (left) obtained with the Alchemy III program. The structure of the BRL 42715 (right) is given for comparison. See Color Plate I.

TABLE I Stability energy of the most relevant inhibitors of β-lactamase compared with bromopenem

Inhibitor	*Stability energy Kcal/mol*
Sulbactam	47.06
Clavulanic acid	44.31
Tazobactam	57.95
BRL 42715	53.38
Bromopenem	44.74

in the cleft of the *E. coli* TEM-1 β-lactamase (Fig. 3). This enzyme proved to be nearly identical to the one of *S. flexneri* UCSF-129 [6]. The inhibition mechanism is also observed in Figure 4.

Then bromopenem has the main characteristics to be an optimum inhibitor [14]. The next step will be its synthesis and the microbiologic and pharmacologic assays.

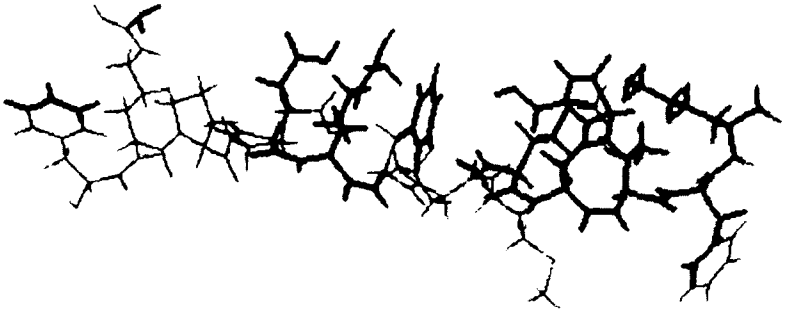

FIGURE 2 Bromopenem (in red) fitted in the active site sequence of the peptide containing the catalytic serine (yellow) of β-lactamase from *S. flexneri* UCSF-129. Its sequence is Arg^{65}-Phe-Pro-Met-Met-Ser^{70}-Thr-Phe-Lys (11). See Color Plate II.

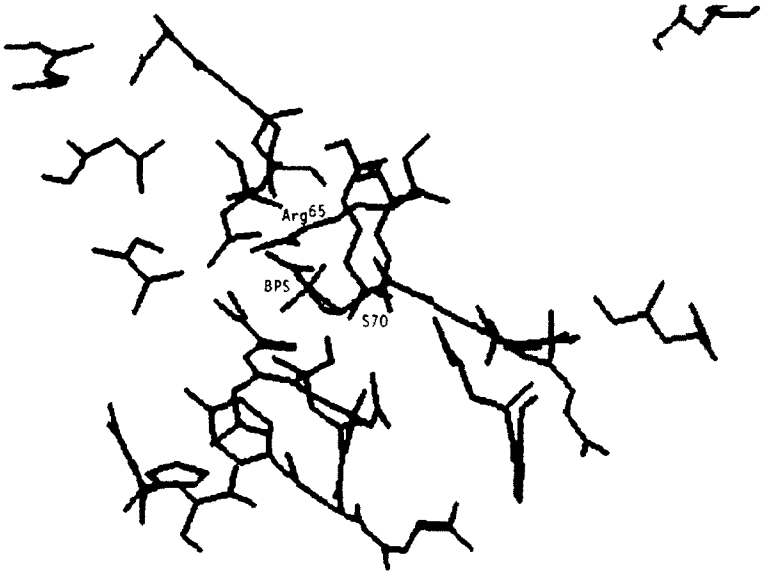

FIGURE 3 Active site crystallographic structure at 1,8 Å of the *E. coli* TEM-1 β-lactamase [12], interacting with the bromopenem. Arg^{65} is in the radius of 4Å around the catalytic $Serine^{70}$. See Color Plate III.

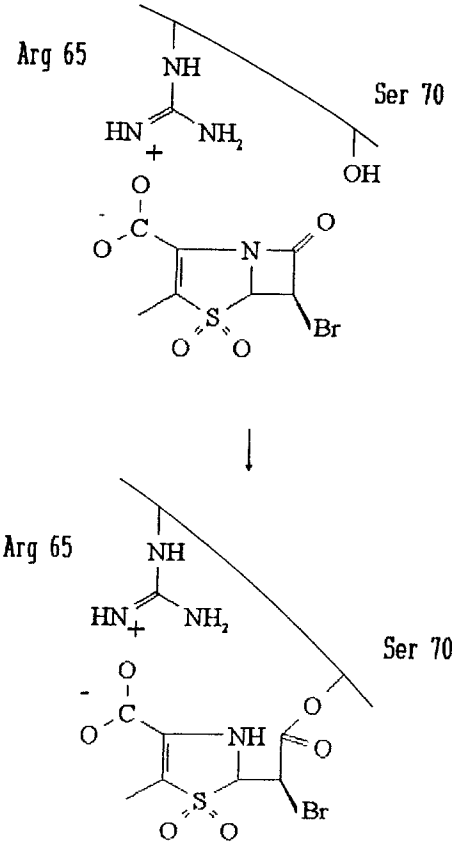

FIGURE 4 Inhibition mechanism of bromopenem of β-lactamase from *S. flexneri* UCSF-129.

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