Molecular Modeling and Chemical Reactivity of Sanfetrinem and Derivatives

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The indiscriminate use of β-lactams has considerably diminished their efficiency as a result of bacteria developing effective defense mechanisms against them. Recent pharmaceutical research has led to the synthesis of tricyclic β-lactam antibiotics known as "tricyclic carbapenems" or "trinems". In this work, we studied the chemical reactivity, an essential property for antibiotic action, of trinems and found it to be similar to that of cephalosporins. Also, we elucidated the interaction pattern for sanfetrinem and 4β-methoxy trinem and compared it to that for classical β-lactams. The behavior of both trinems was found to be similar to that of penicillin G toward *Staphylococcus aureus* PC1, and that of cephalothin and imipenem toward *Enterobacter cloacae* P99.

Introduction

In 1966, the scientists at Glaxo Wellcome reported a novel class of antibiotics: the tricyclic β-lactams known as "tricyclic carbapenems" or "trinems".[1] The next year, Hanessian et al.[2] developed an efficient synthesis for 4α-methoxy trinem (sanfetrinem, **I**) and some structural variants. Recently, Vilar et al. reported a detailed kinetic study of the interaction between two ethylidene derivatives of tricyclic carbapenems and typical β-lactamases.[3] The results showed both compounds to be highly efficient inactivators for class C enzymes (*E. cloacae* 908R), and also highly stable against class A (TEM-1) and class D (OXA-10) β-lactamases. This is especially interesting as other inhibitors (viz., clavulanic acid and sulbactan) usually exhibit low activity against class C enzymes.[4] However, these compounds are readily hydrolyzed by carbapenem-hydrolyzing β-lactamases of classes A (Nmc A) and B (BcII).

This new compound family was developed with the aim of inhibiting β-lactamases. These enzymes are involved in the defense mechanism used by bacteria to counter the action of the most widely used antibiotics (viz., penicillins and cephalosporins).

Producing a new effective antibiotic is a complex labor-intensive task. Chemically, a potential antibiotic must meet two essential requirements, namely: (a) enzyme−substrate interactions should facilitate the incorporation of the antibiotic, in an appropriate orientation, to the active site of the enzyme; and (b) the antibiotic should exhibit appropriate chemical reactivity. Alkaline hydrolysis has been widely used to rationalize the intrinsic reactivity of β-lactams,[5] which is one of the major requirements for a potential drug or inactivator.

Theoretical chemistry has proved invaluable with a view to accurately interpret the structural and kinetic information available for a wide range of β-lactam antibiotics, as well as to predict the behavior of related substances and designing new antibiotics with specific properties. Much effort has been devoted to elucidating the mechanisms behind the hydrolysis of bicyclic β-lactam antibiotics and their derivatives.[6−10] However, only the vibrational frequencies of sanfetrinem appear to have been examined at the theoretical level.[11] The increasing access to databases containing low-resolution crystallographic structures for all types of β-lactamases, carboxypeptidases, and transpeptidases has facilitated the modeling of enzyme−substrate interactions, rationalizing the specificities of the active site and identifying subtle differences between enzyme−substrate complexes. These Henry−Michaelis complexes are crucial inasmuch as they constitute the starting points for the attack of the serine residue in the enzyme on the carbon atom in the β-lactam carbonyl. This has led to the recent modeling of the interactions of carboxytranspeptidase[12] and class A and C β-lactamases mainly[13−19] with a variety of β-lactams.

In this work, various potential mechanisms for the alkaline hydrolysis of a model trinem were subjected to a comprehensive theoretical study. Two different pathways were considered: (a) cleavage of the β-lactam ring with elimination of the methoxy group by electron rearrangement; and (b) proton transfer to the β-lactam nitrogen. Both processes were started from the tetrahedral intermediate. In addition, the Henry−Michaelis complexes formed by sanfetrinem and its derivative 4β-methoxy trinem (**II**) with class A (*Staphylococcus aureus*) and class C lactamases (*Enterobacter cloacae* P99) were modeled. A comparison of the results with those for effective substrates and inhibitors can help one identify the molecular sites involved with a view to design new compounds with improved antibiotic properties.

Methodology

Calculations on the initial structure, a model tricyclic carbapenem (**A** in Figure 1), together with the structures formed in the reactions, were performed at the B3LYP/6+31G*// B3LYP/6+31G* level within the framework of the density

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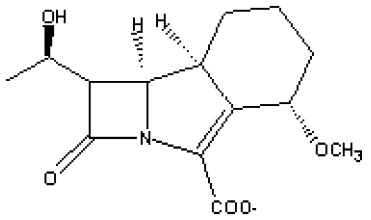
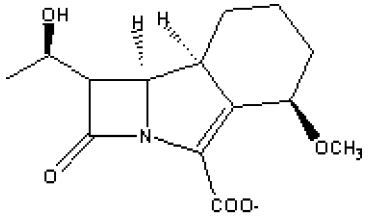


Sanfetrinem (I)



4β–methoxy trinem (II)

functional theory (DFT). DFT methodology considers the effects of electron correlation,[20−22] which makes it particularly attractive here. The absolute necessity of diffuse functions into calculations on anionic systems is accepted.[23] All transition states identified

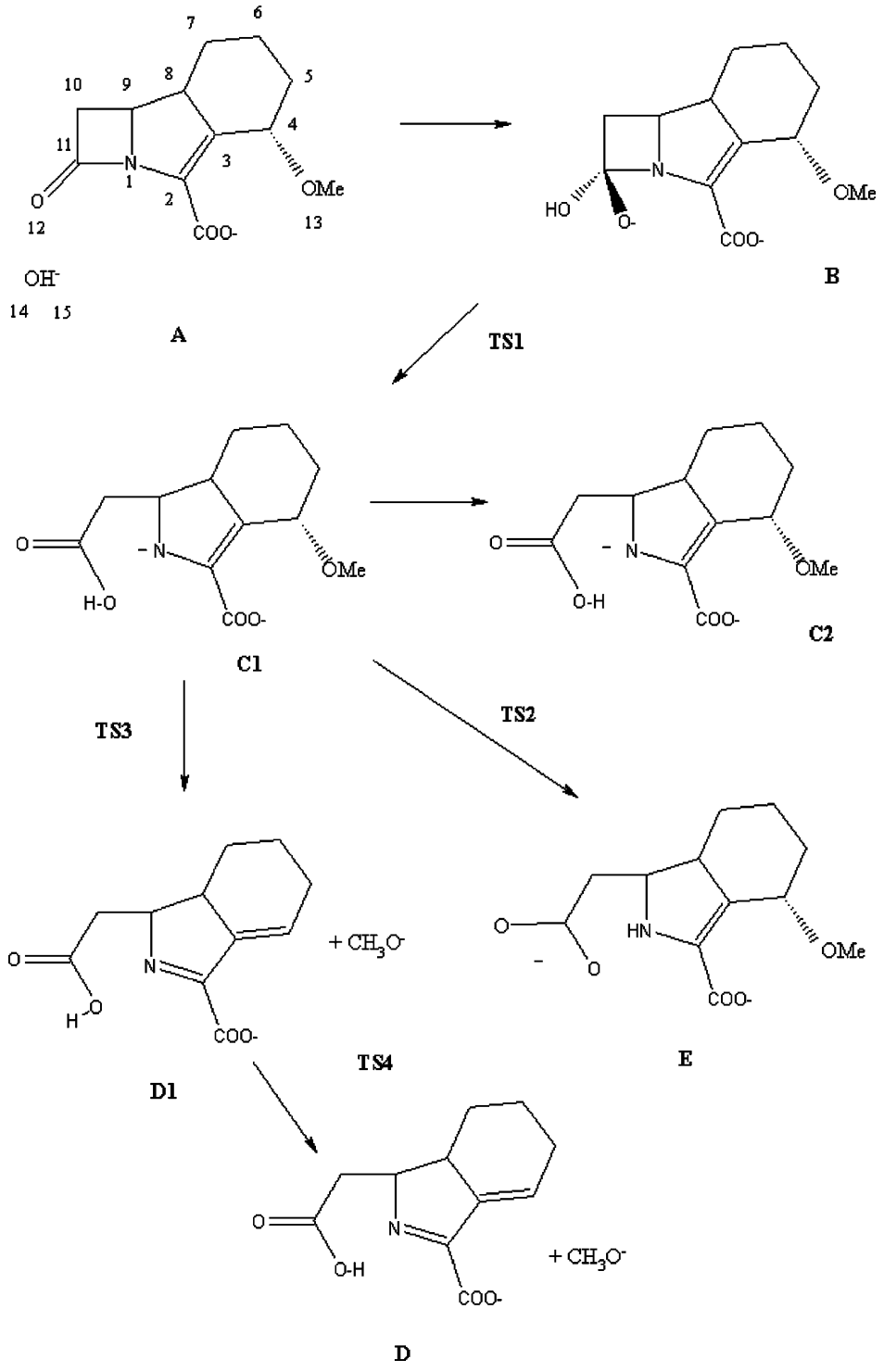


Figure 1. Mechanism of the alkaline hydrolysis of trinem model.

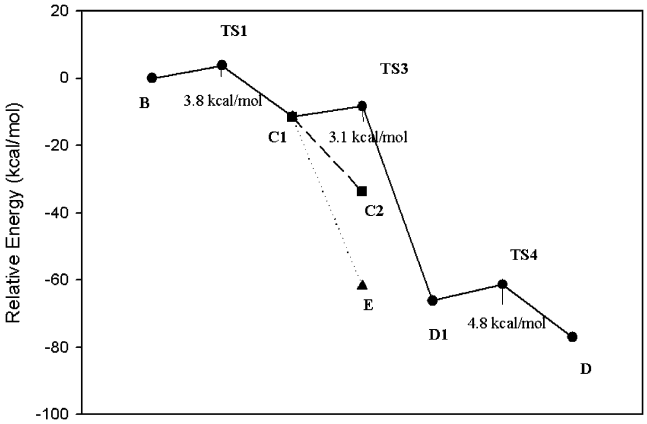


Figure 2. Energy profile for the reaction.

exhibited a single imaginary frequency greater than 100i cm^{-1},[24] and all were confirmed by IRC calculations. All computations were done on SGI Origin 2000 and 200 computers running Gaussian 98 software.[25]

The crystal structures of the cephalosporinases *Staphylococcus aureus* and *Enterobacter cloacae* 99 (3BLM[26] and 2BLT,[15] respectively) were obtained from the Brookhaven Protein Data Bank.[27] The native enzymes were modeled following a previously defined protocol;[13,28] this was unavoidable for *E. cloacae* because of the absence of crystal water molecules. Subsequently, sanfetrinem and its derivatives were inserted in the active site, and the resulting complexes were reoptimized. Substrate charges were calculated by using Gaussian 98 at the 6-31+G* level; charges were computed with the aid of the ChelpG system[29] normally fitted to the electrostatic potential. The Henry−Michaelis complexes thus obtained were used as the starting conformations for molecular dynamics simulations. Initially, the minimized structures were equilibrated by heating at 300 K for 25 ps, using the shake algorithm for bonds to hydrogen; this was followed by molecular dynamics simulation for 260 ps (using a time step of 1.0 fs) and, finally, full optimization of the simulated structures. All of these computations were performed on a Silicon Graphics Origin 200 R10000 computer, using the AMBER* force field[30,31] as implemented in the Macromodel v. 6.0 software suite.[32]

Electrostatic interactions were computed from PM3 semiempirical calculations[33] for the structures of the Henry−Michaelis complexes, using the software TRITON.[34]

Results and Discussion

Chemical Reactivity. As in other β-lactam systems, the four-membered ring is essentially planar (4.8°); also, the N_1-C_{11} bond length is similar to that obtained from ab initio calculations for other β-lactam systems. Such a bond length is slightly greater than that for azethidin-2-one [viz., 1.395 vs 1.356 Å[35]].

The alkaline hydrolysis of β-lactams involves the nucleophilic attack of hydroxyl ion on the carbonyl group to form a tetrahedral intermediate. Only the attack on the α side, which corresponds to the position of the serine residue in classes A and C β-lactamases,[15,26] was considered here. As can be seen in Figure 1, the result of the nucleophilic attack is a tetrahedral intermediate (**B**) that evolves to **C1**. Subsequently, **C1** undergoes cleavage of the amide bond and yields the end-products **D** and **E**. Figure 2 shows the energy profile for this mechanism, and Table 1 gives the geometric and energetic parameters for the reactants, intermediates, and products involved in the alkaline hydrolysis of the trinem.

The tetrahedral intermediate (**B**) evolves to **C1**. This reaction involves a transition state **TS1** that is 3.8 kcal/mol less stable than the tetrahedral intermediate. Subsequently, **TS1** evolves to compound **C1**, which is 11.4 kcal/mol more stable. These results suggest that the energy for the process is much lower than those for other β-lactams. The small energy barriers encountered are only comparable to those for the cleavage of the C−O bond in oxo-lactam (2 kcal/mol with HF and 0.2 kcal/mol with MP2)[8] and cephalothin (2.5 kcal/mol with AM1[36]).

Starting from **C1**, we examined two different pathways: (a) proton transfer to the β-lactam ring via transition state **TS2** to give compound **E**; and (b) rearrangement of the Δ-2 double bond to the exocyclic position with release of the methoxy group to give compounds **D** and **D1**.

Pathway (a) requires an appropriate orientation in the proton (H_{15}) to be transferred. The $O_{12}C_{11}-O_{14}H_{15}$ dihedral angle and the N_1-H_{15} distance (greater than 4 Å) in **C1** are both inappropriate for the transfer. However, if the dihedral angle is opened to about 180°, then the structure collapses to **E**. **TS2** was identified from HF calculations; optimization of its structure at a constant $O_{14}-H_{15}$ distance provided an energy barrier of 26.8 kcal/mol. The final structure, **E**, was 61.8 kcal/mol more stable than the tetrahedral intermediate.

Pathway (b) is similar to that for cephalosporins[36] with an appropriate leaving group at position 3′. DFT calculations showed the release of the leaving group to involve a transition state, **TS3**, with a low energy barrier (3.1 kcal/mol). The two **D** structures differ in the orientation of the acid group. The energy difference is 11.0 kcal/mol, and the energy barrier from **D1** to **D**, the most stable structure in the series, is 4.8 kcal/mol. The existence of a compound similar to structure **D** was experimentally revealed by Vilar et al.,[3] who found it to be the main hydrolysis product of ethylidene carbapenems. Structure

TABLE 1: Main Geometric Parameters and Relative Energy of Tetrahedral Intermediate, End-products, and Transition States for the Alkaline Hydrolysis of Trinem Model (See Figure 1)

	B	C1	C2	D	D1	E	TS1	TS2[c]	TS3	TS4
N_1-C_{11}[e]	1.688	3.041	2.951	2.984	3.014	3.360	1.962	2.879	3.017	2.785
N_1-H_{15}	2.676	4.144	1.646	1.702	4.082	1.015	2.751	1.410	4.138	2.659
C_4-O_{13}	1.486	1.511	1.499			1.468	1.495	1.495	1.853	
$N_1C_9-C_8C_3$	9.1	29.5	28.7	−20.6	−21.6	27.3	15.1	28.4	−21.4	−22.0
$N_1C_2-C_3C_8$	9.5	13.0	12.8	−11.9	−13.3	3.6	10.4	12.2	−9.7	−13.4
$N_1C_2-C_3C_4$	−179.2	162.4	163.8	159.6	159.4	165.1	176.5	164.0	166.2	159.2
$C_4C_3-C_8C_7$	−56.5	−54.9	−55.8	−22.1	−22.0	−59.7	−55.1	−56.0	−27.7	−21.6
$C_3C_8-C_7C_6$	20.0	29.3	28.7	50.7	50.7	61.0	22.5	27.7	−17.2	50.1
$O_{12}C_{11}-O_{14}H_{15}$	−55.5	−8.1	176.4[b]	177.7	−5.0		−47.3	176.2	−6.4	73.8
energy[a]	0	−11.4	−33.6	−77.1[d]	−66.1[d]	−61.8	3.8	15.4	−8.3	−61.3[d]

[a] Relative energies related to tetrahedral intermediate in kcal/mol. These values involve ZPE corrections. [b] In this structure, $O_{12}C_{11}-O_{14}H_{15}$ dihedral angle is constant. In the other case, this structure collapses to **E**. [c] $O_{14}H_{15}$ distance is not optimized. [d] Energy of **D**, **D1**, and **TS4** structures plus CH_3O^-. [e] Bond length in angstroms. Bond and dihedral angles in degrees.

TABLE 2: Intermolecular Distances (Å) between the Heteroatoms in the Minimized *Staphylococcus aureus* PC1 β-Lactam Henry−Michaelis Complexes Modeled[a]

	imipenem[b]	cephalothin[c]	penicillinG[c]	sanfetrinem	4β-methoxy trinem
A Ser70[$^{\alpha}$NH]−β-lactam[CO]	3.42	4.23	3.02	3.11	3.08
B Gln237[$^{\alpha}$NH]−β-lactam[CO]	2.81	3.09	2.73	2.69	2.69
C Ser70[$^{\gamma}$O$^-$]−β-lactam[CO]	3.57	3.29	3.02	2.97	2.93
D Ser130[$^{\eta}$OH]−β-lactam[O_1 (COO^-)]	2.66	3.52	3.29	3.62	3.20
E Lys234[$^{\eta}NH_3^+$]−β-lactam[O_1 (COO^-)]	3.35	2.80	3.23	4.78	2.63
F Gln237[$^{\alpha}$CO]−β-lactam[NH side chain]		3.80	2.89		
G Asn132[$^{\delta}NH_2$]−β-lactam[CO side chain]	2.78	4.94	3.80	2.73	2.89
I Ser235[$^{\beta}$OH]−β-lactam[O_2 (COO^-)]	2.69	2.71	2.66	3.66	2.64
K′ Arg244[$^{\eta 1}$N]−β-lactam[O_2 (COO^-)]	2.67	2.70	2.67	2.65	2.63
L Ser235[$^{\beta}$OH]−β-lactam[O_1 (COO^-)]	3.43	3.21	3.34	2.66	3.40

[a] H and J interactions (see Figure 3) are not possible in PC1 complexes. [b] Reference 40. [c] Reference 13.

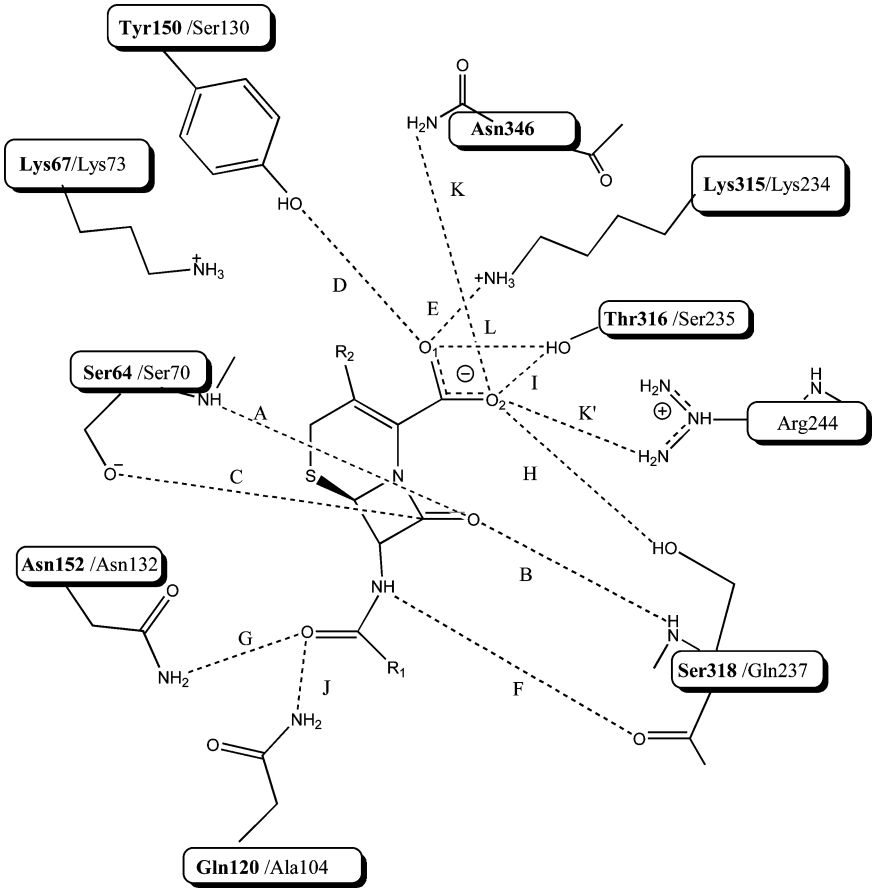


Figure 3. Interactions between a β-lactam (cephalosporin) and the active site amino acids in the class C β-lactamase P99 (boldfaced) and its most common equivalent amino acids in class A β-lactamases. In PC1, there are no H and J interactions due to molecular differences between Gln237 and Ala104 residues (in PC1) and its analogues Ser318 and Gln120 in P99.

D was in fact the most stable (43.0 kcal/mol more stable than the reactants), which is consistent with the experimental results.

Based on chemical reactivity alone, the incorporation of a third fused ring to carbapenem introduces some interesting differences. Thus, the system is more labile: the energy barrier for the cleavage of the β-lactam ring is only 3.8 kcal/mol and hence much lower than those for similar systems [e.g., 14.1 kcal/mol for oxo-lactam,[8] 5.9 kcal/mol for aza-lactam,[37] and 8.6 kcal/mol for clavulanic acid (AM1)]. Also, if an appropriate substituent is incorporated, the reaction mechanism is very similar to that for cephalosporins, which can be related to an interesting antimicrobial spectrum.

Complexed Enzyme. The orientation of the trinem at the active site is crucial with a view to predict not only its chemical reactivity, but also the recognition mechanism and various other properties. Coll et al.[39] reported a study on the structural and electrostatic similarity between various β-lactams. As expected, sanfetrinem and cephalothin were found to be very similar in structural (rms 0.197 Å) and electrostatic terms (rms 0.218 Å). One can therefore assume them to act via a docking mechanism similar to those for cephalosporins. To confirm this assumption, we modeled the Henry−Michaelis complexes formed with the class A β-lactamase *Staphylococcus aureus* PC1 and the class C β-lactamase *Enterobacter cloacae* P99. The results allowed us to identify a pattern for the interaction between the enzymes and the carboxyl group in penicillin G, cephalothin, and imipenem, three representative members of the major β-lactam antibiotic families.[13,28,40−42]

Table 2 and Figure 3 show the parameters for the interactions of penicillin G and cephalothin with the active site of *Staphylococcus aureus* PC1. As can be seen, the most salient difference between the two antibiotics in this respect is that the interactions

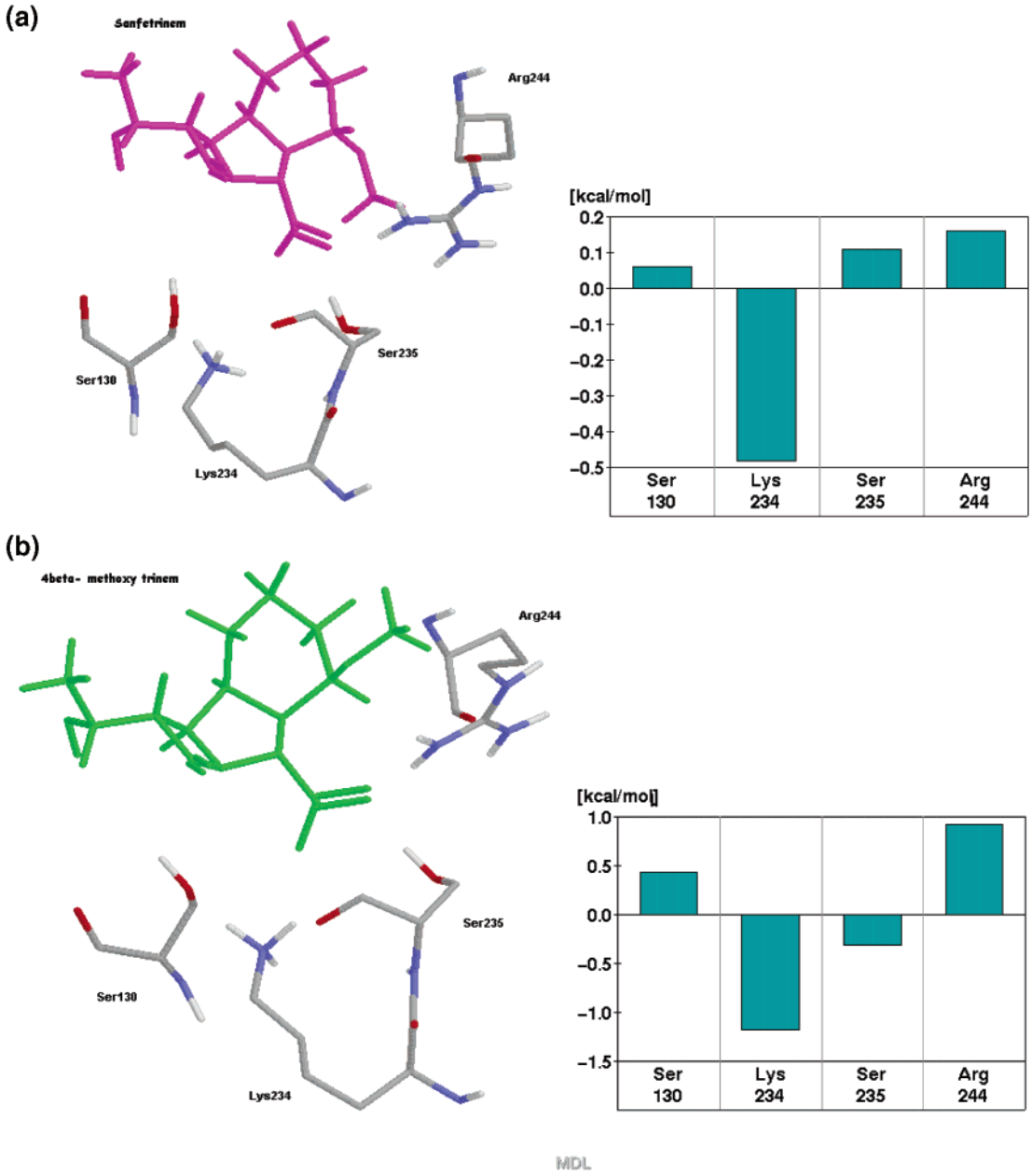


Figure 4. Relative orientations of the sanfetrinem (a) and 4β-methoxy trinem (b) into the *Staphylococcus aureus* PC1 β-lactamase. Histogram figures show the interactions between methoxy oxygen at C_4 and the Ser130, Lys234, Ser235, and Arg244 residues calculated by TRITON software.

of the carbonyl group with the Ser70 and Gln237 residues are weaker in cephalothin than in penicillin G. This causes the substrate to rearrange in such a way that the side chain is much better anchored in penicillin G (see interactions F and G). On the other hand, the interactions of the carboxyl group with the amino acid residues are similar in both antibiotics. Overall, the orientation of the two substrates is different, and penicillin G is more markedly displaced to the inside of the active side than is cephalothin. These results are consistent with the fact that penicillin G is a good substrate for class A β-lactamases. The results for sanfetrinem reveal an interaction pattern similar to those for imipenem and penicillin G, but substantial differences from cephalothin. The presence of the methoxy group prevents the antibiotic from rearranging similarly to imipenem, which results in somewhat weaker D, E, and I interactions. The methoxy group interacts with Arg244, the distance from the oxygen atom in the methoxy group and the nitrogen in the arginine is 4.7 Å, which precludes strong anchoring of the carboxyl group via hydrogen bonding with the Ser130 and Lys234 residues mainly. However, the leaving group in 4β-methoxy trinem possesses a different orientation; there is no steric hindrance to an orientation similar to that of imipenem at the active site. Figure 4a and b shows the relative orientations of the two compounds at the active site and compares the interactions of the methoxy oxygen with the Ser130, Lys234, Ser235, and Arg244 residues. As can clearly be seen, the orientation of the methoxy group in sanfetrinem leads to rather weak interactions relative to 4β-methoxy trinem. The comparison between analogue groups with different orientations should lead to reliance on this methodology. These results suggest that, while the presence of an appropriate leaving group at position 4 is important in terms of chemical reactivity, the group should not be too bulky, otherwise it may hinder anchoring of the substrate to the active site.

The results for *Enterobacter cloacae* P99 were significantly different (see Table 3). Thus, O_1 in the carboxyl groups of penicillin G and cephalothin was found to interact with the $^{\eta}$OH group in Tyr150 (2.66 and 2.65 Å, respectively); on the other

TABLE 3: Intermolecular Distances (Å) between the Heteroatoms in the Minimized *Enterobacter cloacae* P99 β-Lactam Henry−Michaelis Complexes Modeled

	imipenem	cephalothin[a]	penicillinG[a]	sanfetrinem	4β-methoxy trinem
A Ser64[$^{\alpha}$NH]−βlactam[CO]	3.09	3.70	2.80	2.82	2.89
B Ser318[$^{\alpha}$NH]−βlactam[CO]	2.70	2.70	2.61	2.74	2.73
C Ser64[$^{\gamma}$O$^-$]−βlactam[CO]	2.81	2.84	2.65	2.79	2.83
D Tyr150[$^{\eta}$OH]−βlactam[O_1 (COO^-)]	3.45	2.66	2.65	2.68	3.44
E Lys315[$^{\eta}NH_3^+$]−βlactam[O_1 (COO^-)]	2.68	2.69	2.67	2.73	2.66
F Ser318[$^{\alpha}$CO]−βlactam[NH side chain]		2.93	3.01		
G Asn152[$^{\delta}NH_2$]−βlactam[CO side chain]	4.65	2.74	2.76	2.90	2.88
H Ser318[$^{\gamma}$OH]−βlactam[O_2 (COO^-)]	2.66	2.69	4.82	2.66	3.45
I Thr316[$^{\beta}$OH]−βlactam[O_2 (COO^-)]	3.60	3.74	2.63	3.46	3.96
J Gln120[$^{\xi}NH_2$]−βlactam[CO side chain]	5.37	2.74	2.78	2.81	2.82
K Asn346[δNH_2]−βlactam[O_2 (COO^-)]	5.20	5.47	2.85	5.39	5.21
L Thr316[$^{\beta}$OH]−βlactam[O_1 (COO^-)]	2.69	3.45	3.30	2.75	2.66

[a] Reference 28.

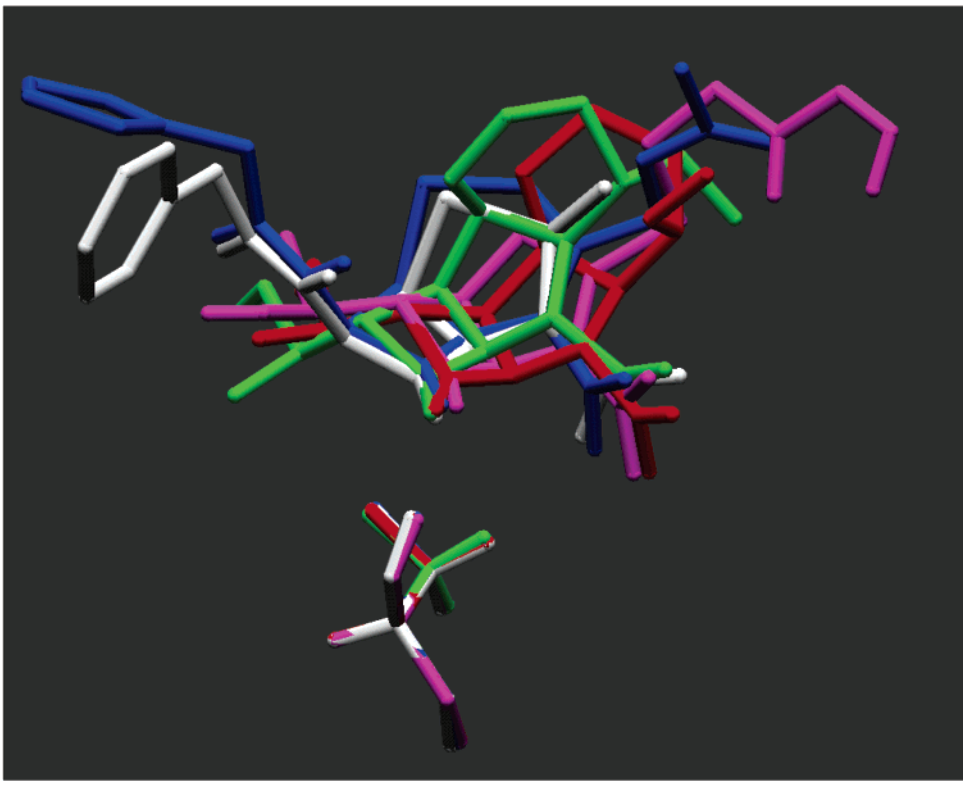


Figure 5. Superposition of active sites in the P99 complexes with imipenem (violet), cephalothin (blue), penicillin G (white), sanfetrinem (green), and 4β-methoxy trinem (red).

hand, O_1 in imipenem interacted with the hydroxyl group in the Thr316 residue (2.69 Å). In all cases, the interaction of $^{\eta}NH_3^+$ with Lys315 was preserved. On the other hand, O_2 in the carboxyl groups of cephalothin and imipenem interacted with Ser318 (2.69 and 2.66 Å, respectively), and also with the W81 water molecule (2.62 Å in both cases); by contrast, O_2 in penicillin G interacted with Thr316 and Asn346 (2.63 and 2.85 Å, respectively) and with W459 (2.71 Å). As can be seen in Table 3, the side chains in both penicillin G and cephalothin bind to the enzyme via virtually identical F, G, and J interactions. Also, the presence of a substrate at the active site causes the amino acids to rearrange with respect to the native enzyme. The position of the carboxyl group in penicillin G and cephalothin considerably decreases the distance between the phenol oxygen and the amine nitrogen (by ca. 0.6 Å) and slightly increases that between the phenol oxygen and the $^{\eta}NH_3^+$ group in Lys67. This arrangement is consistent with the hypothesis of Stoichet et al.,[19] who identified the carboxyl group of the antibiotic as the activator of Tyr150; as a result, this residue can abstract the proton from Ser64, either directly or via Lys67. With imipenem, the Tyr150 residue is too distant from the carboxyl group to interact; this hinders the activation of Ser64 and is consistent with the low k_{cat}/K_M ratio for *Enterobacter cloacae* P99 with this antibiotic (0.6×10^5 M^{-1} s^{-1})[43] relative to penicillin G and cephalothin (4.5×10^6 and 42×10^6 M^{-1} s^{-1}, respectively).[44] This differential pattern, particularly with regards to the carboxyl group, allows one to classify sanfetrinem. The data in Table 3 reveal some similarities with cephalothin and only slight differences from imipenem (Figure 5). On the other hand, the interaction pattern is rather different from that for penicillin G, so sanfetrinem can be expected to anchor to the active site and act as either a good substrate, similarly to the cephalosporin, or an inactivator, similarly to imipenem. The methoxy group exhibits no steric hindrance or interactions with the enzyme in sanfetrinem or 4β-methoxy trinem. Therefore, both are similarly oriented.

The previous results confirm that, despite the similarity between all β-lactamases, subtle differences between them result

in substantial differences with regards to the recognition of various substrates at the active site. It is therefore very important to establish theoretical patterns, allowing one to obtain accurate predictions of the antibiotic potential of new compounds. For sanfetrinem and its derivatives, the results depend on the particular enzyme. Thus, it behaves similarly to penicillin G toward class A β-lactamases, and to cephalothin and imipenem toward class C β-lactamases.

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References and Notes

(1) Géhanne, S.; Piga, E.; Andreotti, D.; Biondi, S.; Pizzi, D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2791.

(2) Hanessian, S.; Griffin, A. M.; Rozema, M. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1857 and references therein.

(3) Vilar, M.; Galleni, M.; Solmajer, T.; Turk, B.; Frère, J. M.; Matagne, A. *Antimicrob. Agents Chemother.* **2001**, *45*, 2215.

(4) Matagne, A.; Dubus, A.; Galleen, M.; Frère, J. M. *Nat. Prod. Rep.* **1999**, *16*, 1.

(5) Coll, M. Hidrólisis alcalina y características estructurales y electrostáticas de compuestos γ y β-lactámicos no clásicos. Doctoral Thesis, Universidad de las Islas Baleares, España, 1999.

(6) Frau, J.; Donoso, J.; Muñoz, F.; García Blanco, F. *J. Comput. Chem.* **1992**, *13*, 681.

(7) Frau, J.; Donoso, J.; Muñoz, F.; Vilanova, B. *Helv. Chim. Acta* **1996**, *79*, 353.

(8) Coll, M.; Frau, J.; Vilanova, B.; Donoso, J.; Muñoz, F.; García Blanco, F. *J. Phys. Chem A* **1999**, *103*, 8879.

(9) Pitarch, J.; Ruiz-López, M.; Pascual-Ahuir, J. L.; Silla, E.; Tuñon, I. *J. Phys. Chem. B* **1997**, *101*, 3581.

(10) Coll, M.; Frau, J.; Vilanova, B.; Donoso, J.; Muñoz, F. *Chem. Phys. Lett.* **2000**, *326*, 304.

(11) Kim, M. S.; Park, W. K.; Park, S. C. *J. Mol. Struct. (THEOCHEM)* **2003**, *630*, 265.

(12) Grail, B. M.; Payne, J. W. *J. Mol. Recognit.* **2002**, *15*, 113.

(13) Vilanova, B.; Donoso, J.; Frau, J.; Muñoz, F. *Helv. Chim. Acta* **1999**, *82*, 1274.

(14) Juteau, J. M.; Billings, E.; Knox, J. R.; Levesque, R. C. *Protein Eng.* **1992**, *5*, 693.

(15) Lobkovsky, E.; Moews, P. C.; Liu, H.; Zhao, H.; Frere, J. M.; Knox, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11257.

(16) Lobkovsky, E.; Billings, E. M.; Moews, P. C.; Rahil, J.; Pratt, F.; Knox, J. R. *Biochemistry* **1994**, *33*, 6762.

(17) Oefner, C.; D'Arcy, A.; Daly, J. J.; Gubernator, K.; Charnas, R. L.; Heinze, I.; Hubschwerlen, C.; Winkler, F. K. *Nature* **1990**, *343*, 284.

(18) Tsuchida, K.; Yamaotsu, N.; Hirono, S. *Drug Des. Discovery* **1999**, *16*, 145.

(19) Beadle, B. M.; Trehan, I.; Focia, P. J.; Shoichet, B. K. *Structure* **2002**, *10*, 413.

(20) Parr, R. G.; Yang, W. *Density-functional theory of atoms and molecules*; Oxford University Press: Oxford, 1989.

(21) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372.

(22) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098.

(23) Madura, J. D.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1986**, *108*, 2517.

(24) McIver, J. W.; Komornicki, J. *J. Am. Chem. Soc.* **1972**, *94*, 2625.

(25) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Baroni, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; González, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.9; Gaussian, Inc.: Pittsburgh, PA, 1998.

(26) Herzberg, O. *J. Mol. Biol.* **1991**, *217*, 701.

(27) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F., Jr.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *J. Mol. Biol.* **1997**, *112*, 535.

(28) Fenollar-Ferrer, C.; Frau, J.; Donoso, J.; Muñoz, F. *Proteins: Struct., Funct., Genet.* **2003**, *51*, 442.

(29) Breneman, C. M.; Wiberg, K. B. *J. Comput. Chem.* **1990**, *11*, 361.

(30) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S. J.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765.

(31) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230.

(32) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caulfield, C.; Chang, G.; Henddickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

(33) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *209*, 209.

(34) Prokop, M.; Damborsky, J.; Koca, J. *Bioinformatics* **2000**, *16*, 845.

(35) Frau, J.; Donoso, J.; Muñoz, F.; Vilanova, B. *Helv. Chim. Acta* **1997**, *80*, 739.

(36) Frau, J.; Donoso, J.; Muñoz, F.; García Blanco, F. *J. Comput. Chem.* **1993**, *12*, 1545.

(37) Coll, M.; Frau, J.; Vilanova, B.; Donoso, J.; Muñoz, F.; García Blanco, F. *J. Phys. Chem. B* **2000**, *104*, 11389.

(38) Frau, J.; Donoso, J.; Muñoz, F.; García Blanco, F. *Helv. Chim. Acta* **1994**, *77*, 1557.

(39) Coll, M.; Frau, J.; Vilanova, B.; Donoso, J.; Muñoz, F. *J. Comput.-Aided Mol. Des.* **2001**, *15*, 819.

(40) Vilanova, B.; Coll, M.; Frau, J.; Muñoz, F.; Donoso, J. *Helv. Chim. Acta* **2001**, *84*, 3366.

(41) Fenollar-Ferrer, C.; Frau, J.; Vilanova, B.; Donoso, J.; Muñoz, F. *J. Mol. Struct. (THEOCHEM)* **2002**, *578*, 19.

(42) Fenollar-Ferrer, C.; Frau, J.; Donoso, J.; Muñoz, F. *Chem. Biodiversity*, accepted.

(43) Galleni, M.; Amicosante, G.; Frere, J. M. *Biochem. J.* **1988**, *255*, 123.

(44) Dubus, A.; Ledent, P.; Lamotte-Brasseur, J.; Frère, J. M. *Proteins: Struct., Funct., Genet.* **1996**, *25*, 473.