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Electrostatic and structural similarity of classical and non-classical lactam compounds

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Summary

Various electrostatic and structural parameters for a series of classical and non-classical β-lactams were determined and compared in order to ascertain whether some specific β-lactams possess antibacterial or β-lactamase inhibitory properties. The electrostatic parameters obtained, based on the Distributed Multipole Analysis (DMA) of high-quality wavefunctions for the studied structures, suggest that some non-classical β-lactams effectively inhibit the action of β-lactamases. As shown in this work, such electrostatic parameters provide much more reliable information about the antibacterial and inhibitory properties of β-lactams than do structural parameters.

Introduction

β-Lactam antibiotics exert their antibacterial action by inhibiting transpeptidases and carboxypeptidases. These compounds mimic the conformation of the natural substrate of transpeptidases, D-alanyl-D-alanine, by binding to them and ultimately preventing the formation of the bacterial wall [1]. This mechanism of action was originally described by Tipper and Strominger in 1965 [2].

For a β-lactam to successfully compete with D-alanyl-D-alanine at the transpeptidase active site, it should be structurally and electrostatically similar enough to the natural substrate to be recognized by the enzyme; also, it should possess appropriate chemical reactivity in order to facilitate the rapid acylation of the enzyme's active site [3–5].

Bacteria defend themselves against β-lactam antibiotics in various ways one of the most efficient of which involves producing β-lactamases; these are enzymes that act by hydrolysing their β-lactam substrates [6]. Like transpeptidases, many β-lactamases are serine enzymes. Bacterial resistance to antibiotics has so far been addressed in two different ways. One involves developing new molecules such as aztreonam [7] and imipenem [8], which possess antibacterial properties and resist the action of β-lactamases. The other involves obtaining molecules with little or no antibacterial activity but capable of inactivating β-lactamases; such molecules are used in combination with antibiotics possessing antibacterial activity (e.g., clavulanic acid [9] and sulbactam [10] are used with ampicillin, and tazobactam [11] is employed with piperacillin). Inhibitory antibiotics exert their action in different ways; thus, so-called 'suicidal substrates' (e.g., clavulanic acid) bind irreversibly to β-lactamases, whereas third-generation cephalosporins bind reversibly with low turnover rates to these enzymes [12].

Ghosez and co. [13–15] studied aza-γ-lactams (bicyclic imidazolidinones) with a view to identifying molecules with antibacterial properties capable of inactivating β-lactamases. The inhibitory potential of these compounds must be a result of their ability to form especially stable carbamoyl-enzyme complexes. However, the compounds examined exhibited little antibacterial activity [14, 15], probably as a result of the

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low reactivity of the five-membered ring in γ-lactams. Subsequently, Nangia and co. [16-18] used semi-empirical methods to determine various structural parameters for aza-β-lactams including the pyramidality of the lactam nitrogen and charges on the atoms in the diazethidin-2-one ring; they concluded that these compounds must possess both antibacterial and inhibitory properties by virtue of their ability to form carbamoyl-enzyme complexes with β-lactamases.

It is well known, however, that structural parameters (bond distances and charges) in general, and those determined by use of semi-empirical methods in particular, are inappropriate for describing the chemical and antibacterial properties of β-lactams.

Because the initial recognition of the substrate by most enzymes is governed by electrostatic forces, electrostatic parameters could in principle be of interest with a view to studying these processes. Molecules with a similar electrostatic potential distribution can interact with the same receptor [19–28], which can be used to predict whether a given compound will be an effective substrate for a specific enzyme, even if the structure of the enzyme's active site is unknown. This requires using a realistic model of the electrostatic potential distribution for the molecule. Various studies have shown electrostatic similarity between ligands binding to the same active site to be more realistic if Distributed Multipole Analysis (DMA) is used than if only atomic charges are considered [29–31]. In this type of analysis, the distribution of charge around each atom is represented in terms of dipoles, quadrupoles and hexadecapoles calculated from high-quality *ab initio* wavefunctions [32]. This methodology has been applied by Frau and Price to various lactams [29, 33]; they found a direct relationship of antibacterial activity to degree of structural and electrostatic similarity between β-lactam antibiotics and the natural substrate for transpeptidases (D-alanyl-D-alanine) [29].

In recent theoretical work, our group found substitution of the carbon next to the carbonyl group in the β-lactam ring by various heteroatoms (nitrogen, oxygen and sulphur) to yield products with the chemical reactivity required to possess both antibacterial activity and β-lactamase inhibitory capacity [34–37].

In this work, the structure and charge distribution around each atom (as obtained by Distributed Multipole Analysis, DMA, of high-quality wavefunctions) for aza, oxo and β-lactams were determined, and the structural and electrostatic parameters thus obtained were compared with those for β-lactams with well-known antibacterial or β-lactamase inhibitory properties in order to establish their potential as substrates for transpeptidases and/or β-lactamases.

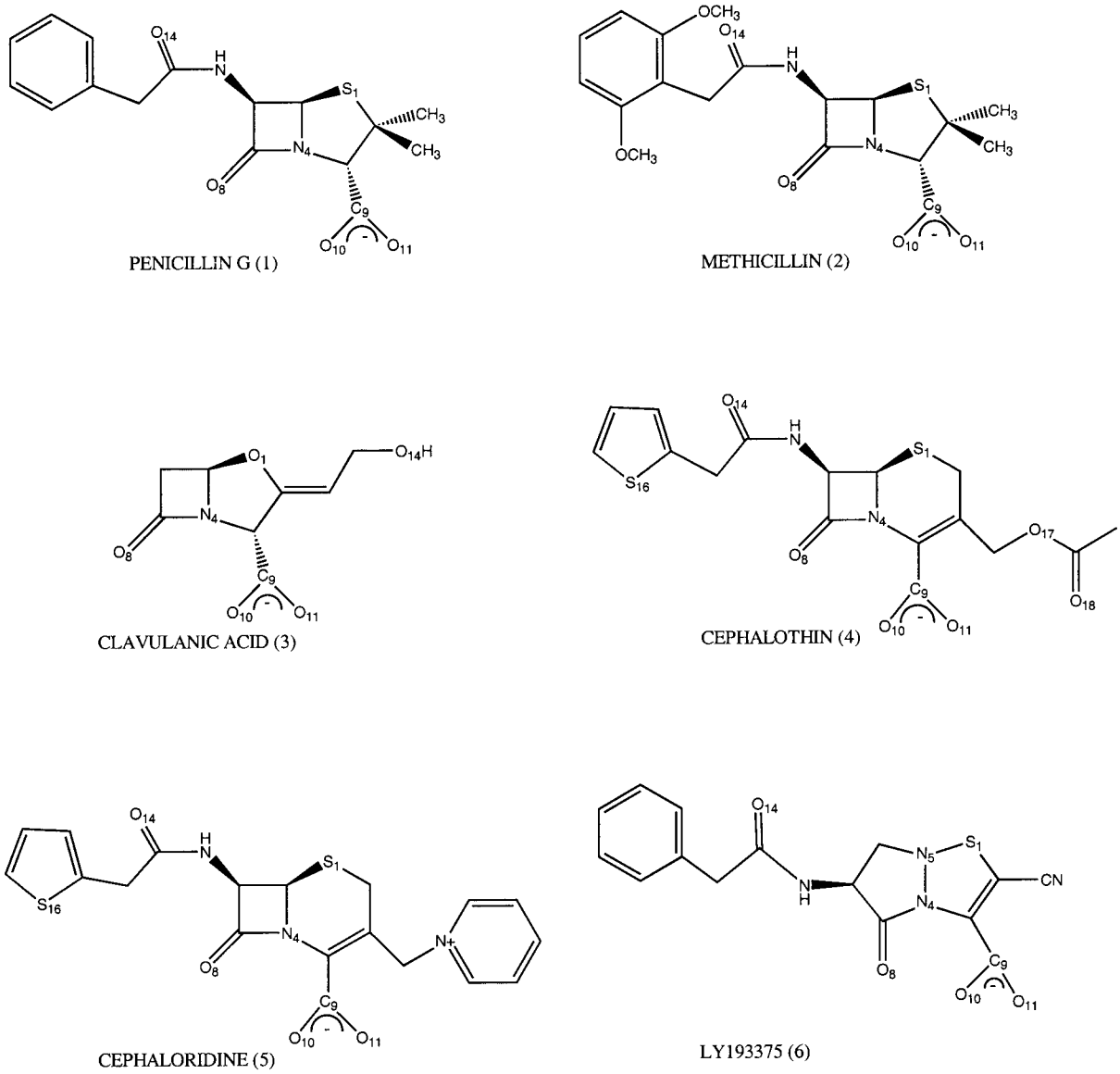
Because the natural substrates for β-lactamases are lactams, which are also appropriate substrates for transpeptidases, the reference structures used were those of members of the penicillin, cephalosporin and β-lactamase inhibitor families (viz. penicillin G, cephalothin and clavulanic acid, respectively). The study was completed by studying other, non-classical γ- and β-lactams.

Scheme 1 depicts the studied structures, which included the following: penicillin G (1), a major antibacterial agent; methicillin (2), with an axial conformation; clavulanic acid (3), a β-lactamase inhibitor with low antibacterial capacity [38]; cephalothin and cephaloridine (4, 5), two active cephalosporins; LY193375 (6), an active γ-lactam [39, 40]; α- and β-cyclopropylpenams (7, 8), and sanfenitrem (9), the conformations of which are dictated by the presence of additional rings fused to the antibiotic core [41, 42]; an inactive aza-γ-lactam reported by Nangia and co. (10) [43]; and the aza, oxo and thio-β-lactams obtained by replacing the carbon atom close to the carbonyl group in the β-lactam ring with a heteroatom (N, O and S in 11, 12 and 13, respectively).

Methods

The geometry and the wave function of each molecule studied were obtained by ab initio calculations on the structures, that were carried out at the RHF/6-31+G*//RHF/6-31+G* level, which includes polarized and diffuse functions on heavy atoms. The incorporation of diffuse functions is especially relevant in the calculations of anionic systems [44]. These calculations were performed on a SGI Origin 2000 and a SGI Origin 200 computers running the Gaussian 94 program [45].

We compared the structural similarity between the molecules by minimising the root-mean-square (rms) distances between specified pairs of atoms that are expected to be in the same position in the active site. The overlaping sequence includes the carbonyl group, since this group represents the reaction center if the antibiotic is bound to the cell-wall enzimes [6], and the carboxylic group, believed to be essential for antibacterial activity [46]. The amide group in the side chain is necessary in normal penicillins and cephalosporins. However, some β-lactams structures (e.g., imipenem

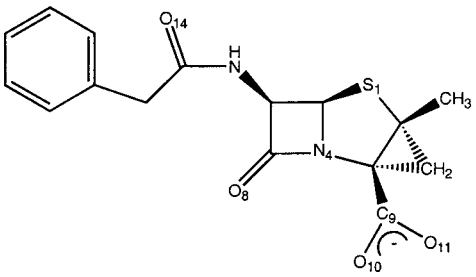


Scheme 1. Structures and numeration of the atoms used in this work. The numeration of the atoms is the usual for penicillins, and it has been adapted to the other structures.

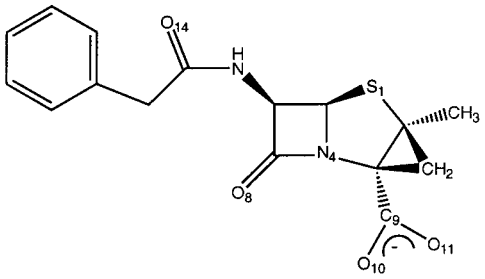
or clavulanic acid) without an acylamino side chain possess antibacterial activity. Finally, the sequence that we used for the structural overlays was the O_8-C_7-N_4-C_3-C_9-($O_{10}O_{11}$), present in all the structures studied. We compared, through the use of rms separations of the atoms of the studied sequence calculated by the Chem3D software package [47], the structural similarity of different molecules with the penicillin, cephalothin (good substrates) and clavulanic acid (a poor substrate, but a good inhibitor of β-lactamases).

However, structural similarity is not the only determinant of the ability to bind in the same site, and within the range of structures that can sterically fit, the electrostatic similarity is likely to be more important than the finer details of steric overlap [29].

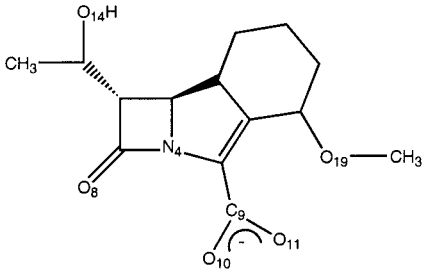
The electrostatic models were derived from the SCF wave function of the molecules. Each wave function was represented by sets of multipoles up to the hexadecapole at each atomic site, obtained by a DMA. These calculations were carried out running the CAD-



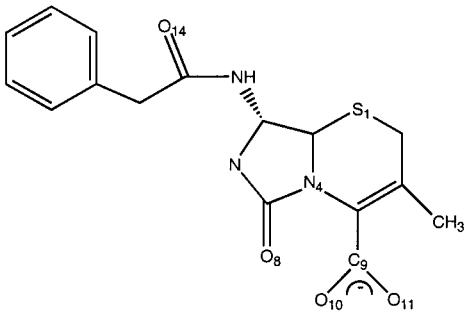
α-CYCLOPROPYLPENAM (7)



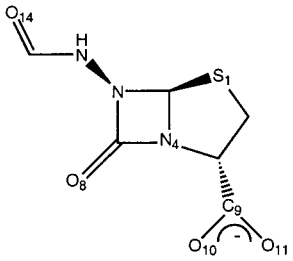
β-CYCLOPROPYLPENAM (8)



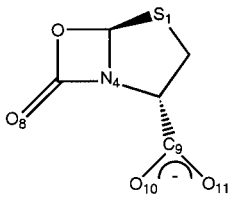
SANFENITREM (9)



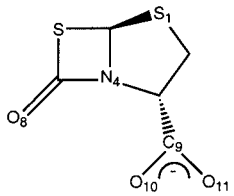
(10)



AZA-β-LACTAM (11)



OXO-β-LACTAM (12)



THIO-β-LACTAM (13)

Scheme 1. Continued.

PAC ab initio program suite [48], on an IBM SP2 computer.

The electrostatic potential was calculated from DMAs, using all terms in the multipole expansion up to R^{-5}, within the program ORIENT [49].

The electrostatic potential was examined at fixed distances outside the van der Waals surfaces of the molecules, as defined by the Pauling radii: 1.5 Å for N; 1.4 Å for O; 2 Å for C; and 1.85 Å for S. There was no explicit hydrogen van der Waals radius, since the hydrogen atom is included in a united methyl radius, and polar hydrogen atoms effectively have no radius if involved in hydrogen bonding [30].

A quantitative comparison of electrostatic similarity can be made using the position of the extrema of the electrostatic potential at fixed distances from the molecules. The alignment of the extrema of the same sign and similar magnitude between a ligand and substrate ensures a similar electrostatic potential around the two molecules in the matched regions. Since the electrostatic extrema correspond to regions where strong interactions such as hydrogen bonds with the binding site are possible, some of the extrema of the ligand and the substrate are expected to overlay if they correspond to important van der Waals contacts between the ligands and binding site [29, 30]. The structures studied here are negatively charged at physiological pH, and so we only consider the positions and strengths of the minima in the electrostatic potential energy, at the distance that would be sampled by a hydrogen-bonding proton of the binding site. These minima were determined by minimising the interaction energy of a single positive point charge (radius 0.5 Å) with each molecule, using hard-sphere repulsion between sites with nonzero van der Waals radii, using the ORIENT software package [49]. We compared, through the use of rms separations of the positions of the minima calculated by the Chem3D software package [47], the electrostatic potential of the structures with that of the penicillin, cephalothin and clavulanic acid.

Results and discussion

Scheme 1 shows the structures studied and the numbering system used in this work. Table 1 shows the electrostatic potential minima for each structure as defined by the distance to the nearest atom, in kJ mol^{-1}.

Penicillin G (1). An overall 9 minima that ranged from −200 to −568 kJ mol^{-1} were identified on the electrostatic potential surface for this antibiotic (see Table 1 and Figure 1). The four most important minima on account of their direct relationship to enzyme binding [33] were those near the carbonyl oxygen (*a*), lactam nitrogen (*c*) and carboxyl oxygens (*d* and *e*). Two other, less pronounced, minima (*i* and *j*) were observed around the sulphur atom in the thiazolidine ring, and three more in the side chain, one at O_{14} (*g*) and the other two on both sides of the phenyl group (*k* and *l*). These results are similar to those previously obtained for other penicillins [29].

Methicillin (2). The optimized structure for this compound has the thiazolidine ring in the axial conformation; by contrast, the crystallographic structure [50] has the ring in the equatorial conformation. The equatorial conformation of lactam antibiotics is known to be the active one in most cases, so the structure studied here possesses no antibacterial activity [51, 52]. An overall 7 minima were identified on the potential surface of axial methicillin (see Table 1 and Figure 1), namely: two on the carbonyl group (*a* and *b*), two other on the carboxyl group (*d* and *e*), two more near the oxygens in the side chain (*g* and *k*) and the seventh on the sulphur atom in the thiazolidine ring (*i*). The values for the minima ranged from −206 to −571 kJ mol^{-1}. There were substantial differences from the results for penicillin G including the absence of the minimum corresponding to the lactam nitrogen, one of the minima due to sulphur in the thiazolidine ring and the differential conformation of the carboxyl group. The absence of these minima in the axial conformation of penicillins was previously reported by Frau and Price [29].

Clavulanic acid (3). This compound belongs to the family of β-lactamase inhibitors known as "suicidal substrates". It possesses little antibacterial activity [38] owing to the absence of a side chain and the unfavourable conformation of the carboxyl group imposed by the presence of the double bond in the oxazolidine ring, so it must be used in combination with other antibiotics [53]. Clavulanic acid exhibits 6 electrostatic potential minima (see Table 1 and Figure 2) that differ from those for penicillin G essentially in the position of the minima for the carboxyl group (*d* and *e*) and in that it possesses a single minimum (*i*) close to O_1 (S_1 in the penicillins). In addition, it exhibits a minimum on the carbonyl oxygen (*a*), one

Table 1. Distance to the nearest atom (in Å) and values (in kJ/mol) of the minima of the electrostatic potential in the Van der Waals surface for the different structures.

	O_8 (a/b)	N_4 (c)	O_{10} (d)	O_{11} (e/f)	O_{14} (g/h)	S_1 (i/j)	Ph (k/l)	S_{16} (m)	O_{17} (o)	O_{18} (p)	N_5 (q)	O_{19} (r/s)
Penicillin G (1)	1.90	2.02	1.89	1.89	1.90	2.35/2.35	2.55/2.60	–	–	–	–	–
	−419.30	−541.00	−568.69	−567.97	−335.31	−289.23/−320.69	−200.87/-241.87					
Methicillin	1.89/1.90	–	1.89	1.89	1.89	2.35	2.55	–	–	–	–	–
(2)	−403.90/−390.36		−571.77	−571.72	−367.19	−285.90	−206.77					
Clavulanic acid	1.90	2.26	1.89	1.89	1.90	1.90 (O_1)	–	–	–	–	–	–
(3)	−407.65	−516.26	−576.66	−575.61	−395.02	−331.77						
Cephalothin	1.90	–	1.89	1.89	1.93	2.37/2.35	–	2.36	1.89	1.90	–	–
(4)	−532.57		−565.57	−564.35	−335.7	−321.97/−286.89		−194.01	−331.15	−377.88		
Cephaloridine	1.90	–	1.89	1.90	1.90	2.36/2.35	–	2.59	–	–	–	–
(5)	−273.46		−312.46	−390.45	−178.48	−79.77/−51.76		−56.89				
Ly193375	2.14	2.00	1.90	1.89/1.89	1.89/1.89	2.35	2.55/2.57	–	–	–	2.00	–
(6)	−442.03	−363.07	−466.15	−485.90/−468.32	−340.60/−341.58	−285.68	−240.05/−267.18				−330.46	
α- Cyclopropyl	2.04	2.00	1.89	1.89	1.89	2.35	2.55/3.90	–	–	–	–	–
(7)	−532.48	−390.16	−528.14	−529.55	−375.53	−322.77	−250.77/−370.37					
β-Cyclopropyl	1.90	2.02	1.89	1.89	1.90	2.35/2.35	2.55/2.59	–	–	–	–	–
(8)	−425.82	−540.25	−567.58	−570.84	−335.36	−319.90/−274.10	−199.43/−237.45					
Sanfenitrem	2.04	2.03	1.89	1.89	1.89	–	–	–	–	–	–	1.89/1.89
(9)	−572.12	−544.50	−575.73	−572.12	−335.72							−353.74/−355.59
Structure 10	1.90	–	1.89	1.89	1.90	3.82/2.36	2.55/2.59	–	–	–	–	–
(10)	−504.93		−581.75	−580.98	−331.69	−317.86/−328.34	−194.74/−233.59					
Aza-β-lactam	1.90/1.90	2.02	1.89	1.89	1.98	2.35	–	–	–	–	–	–
(11)	−372.89/−359.54	−541.74	−567.24	−566.27	−422.15	−257.78						
Oxo-β-lactam	1.90/1.80	2.02	1.89	1.89	–	2.42	–	–	–	–	–	–
(12)	−380.02/−374.54	−538.28	−566.10	−564.93		−362.27						
Thio-β-lactam	1.90/3.54	2.03	1.89	1.89	–	2.36	–	–	–	–	–	–
(13)	−383.68/−317.62	−527.63	−568.31	−567.04		−356.42						

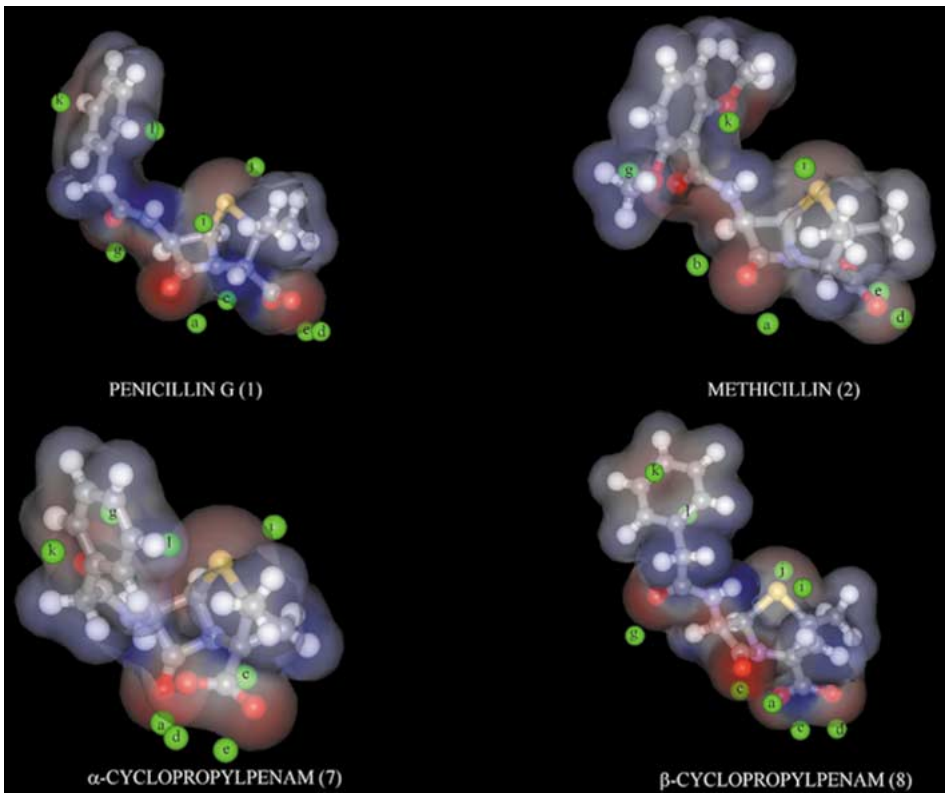


Figure 1. Different minima of the electrostatic potential in the Van der Waals surface (green points) for the penicillin G, methicillin, α-cyclopropylpenam and β-cyclopropylpenam.

other on the lactam nitrogen (*c*) and a third one on the side chain (*g*). According to Frau et al. [33], the most salient interactions of this compound with PC1 β-lactamase in *Staphylococcus aureus* are those that take place via the carboxyl and carbonyl groups; on the other hand, the lactam nitrogen does not interact at all.

Cephalothin (4) and cephaloridine (5). In these compounds, the O_{10}-C_9-C-N_4 dihedral for the minimized structure was altered in order to eliminate the hydrogen bond between one of the carboxyl oxygens and a hydrogen atom in the side chain as this interaction might result in spurious positions and values for some electrostatic potential minima. There were substantial differences in the position of the minima with respect to penicillins. Thus, the minima assigned to the carboxyl oxygens (*d* and *e*) are markedly shifted with respect to those of penicillin G, as a result of the different spatial arrangement of the group, and the minimum associated to the lactam nitrogen is absent (Table 1, Figure 3), probably because of the nearness to the carboxyl oxygens. The minimum associated to the carbonyl group (*a*), those ascribed to S_1 (*i* and *j*) and those assigned to the side chains, viz., on O_{14} (*g*) and S_{16} (*m*), were identified on the potential surface. Cephalothin also shows two minima on O_{17} (*o*) and O_{18} (*p*). The value for the minima are much smaller for cephaloridine (-51 to -312 kJ mol^{-1}) than for cephalothin (-194 to -565 kJ mol^{-1}) by virtue of their difference in electric charge (0 in the former and -1 in the latter).

LY193375 (6). This is an aza-γ-lactam with major antibacterial action [39, 40]. It exhibits minima associated to the carbonyl group (*a*), the lactam nitrogen (*c*), S_1 (*i*) and the carboxyl group (*d* and *e*); the last two are similar to those for the cephalosporins but strongly shifted with respect to the penicillins (see Table 1 and Figure 3). It exhibits additional minima associated to the phenyl group (*k* and *l*), O_{14} (*g* and *h*), the second nitrogen in the γ-lactam ring (*q*) and one more in between O_{11} and the nitrile group (*f*). The potentials of the minima range from -240 to -485 kJ mol^{-1}.

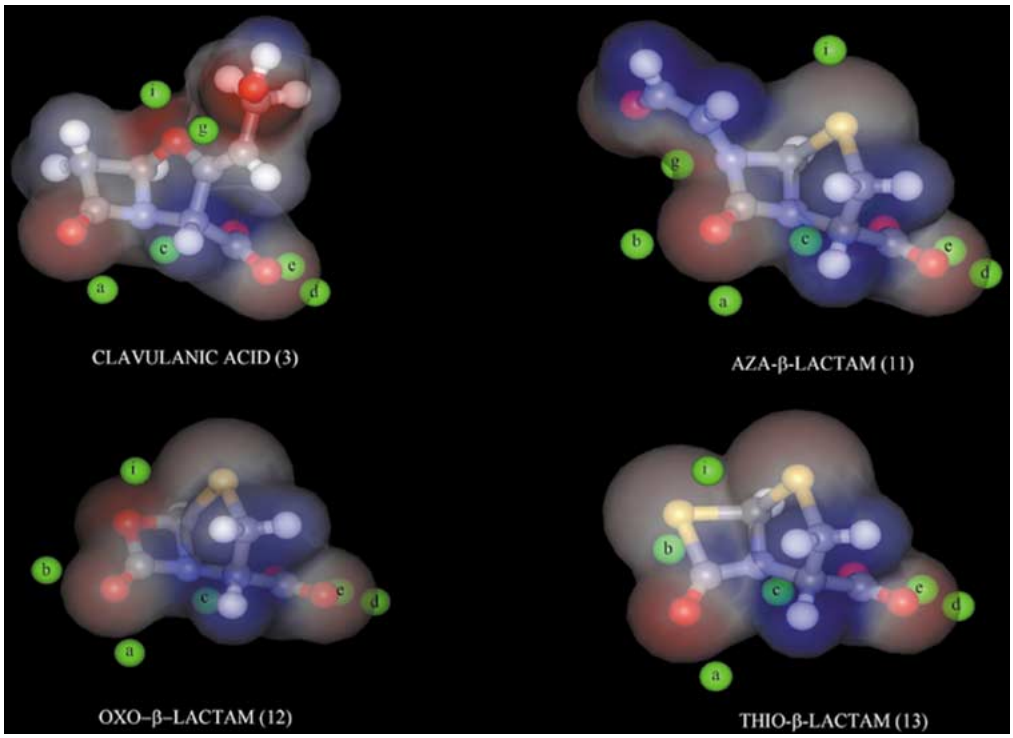


Figure 2. Different minima of the electrostatic potential in the Van der Waals surface (green points) for the clavulanic acid, aza-β-lactam, oxo-β-lactam and thio-β-lactam.

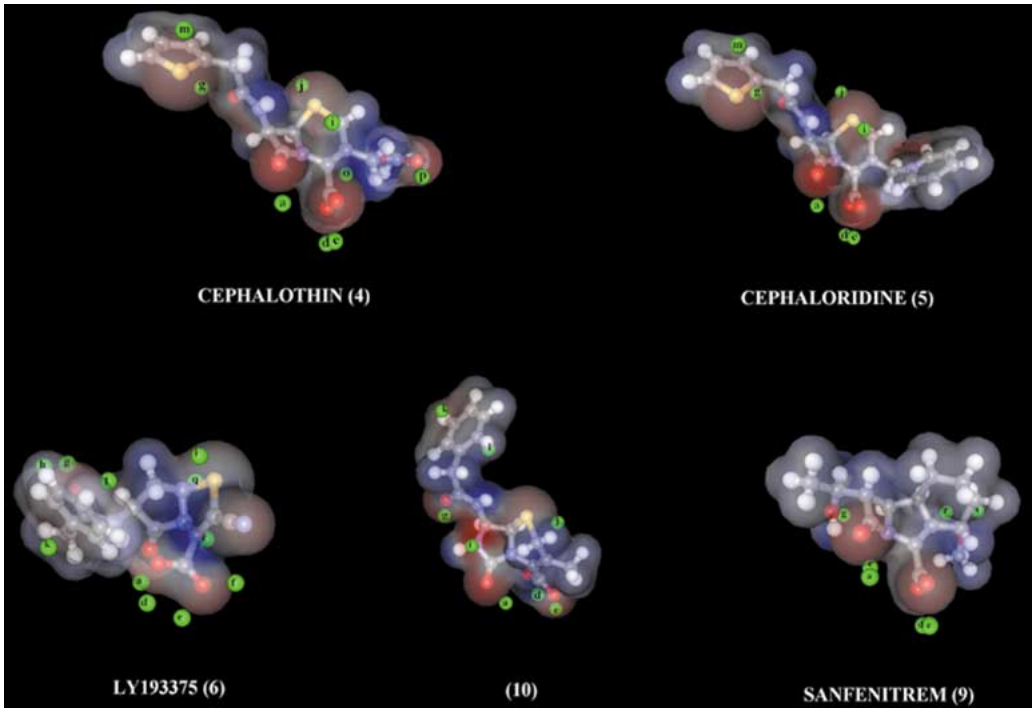


Figure 3. Different minima of the electrostatic potential in the Van der Waals surface (green points) for the cephalothin, cephaloridine, LY193375, structure 10 and sanfenitrem.

α- *and* β-*cyclopropylpenams (7 and 8).* The presence of a cyclopropyl ring bonded to the thiazolidine ring results in a highly strained structure in both compounds. The β isomer exhibits antibacterial properties similar to those of penicillins whereas the α isomer resembles cephalosporins in this respect (e.g., it is resistant to penicillinases) [41]. Similarly to penicillin G, the β isomer exhibits two minima associated to S_1 (*i* and *j*); on the other hand, the α isomer exhibits a single minimum (*i*). The distribution of minima in these structures is illustrated in Figure 1 and Table 1. The differential conformation of the two isomers results in differences between the values of their respective minima. In addition to the previous minima, they exhibit the usual ones on the carbonyl and carboxyl oxygens (*a*, *d* and *e*), one other on the lactam nitrogen (*c*), two more associated to the phenyl group (*k* and *l*), and one assigned to O_{14} (*g*).

Sanfenitrem (9). This trinem is a broad-spectrum antibiotic resistant to β-lactamases [42, 54, 55]. Its distribution of minima (Table 1, Figure 3) is similar to that in cephalosporins, especially as regards the carboxyl group. Unlike them, however, it exhibits no minimum at position 1 but possesses a minimum associated to the lactam nitrogen (*c*) that is absent from the potential surface for cephalosporins. Its surface exhibits two additional minima on O_{19} (*r* and *s*) and one other on O_{14} (*g*). The potential of the minima range from −353 to −575 kJ mol^{-1}. Specially strong (−572.12 kJ mol^{-1}) is the minimum on the carbonyl group (*a*), which is much stronger than in penicillin G (−419.30 kJ mol^{-1}) and cephalothin (−532.57 kJ mol^{-1}).

Structure 10 (10). This compound, which possesses no antibacterial activity, was examined by Nangia and co. [43] and initially proposed by these authors as a potentially effective antibiotic on account of its special chemical reactivity [16-18]. In fact, the compound lacks antibacterial properties. As can be seen from Table 1 and Figure 3, its structure does not possess the minimum for the lactam nitrogen. The conformation of the five-membered ring is C_2(up), by contrast, that in cephalothin and cephaloridine is C_2(down). This, together with the presence of the nitrogen atom in the aza-γ-lactam ring, results in a strong shift in one of the minima associated to S_1 (*i*). In addition, this structure exhibits two minima on the phenyl group in the side chain (*k* and *l*), one other on O_{14} (*g*) (also in the side chain), and the two usual minima on the carbonyl and carboxyl groups. The minimum on the carbonyl group is quite strong (−504.93 kJ mol^{-1}); in fact, it is stronger than in penicillin G (−419.30 kJ mol^{-1}) but not so strong as in cephalothin (−532.57 kJ mol^{-1}).

*Aza, oxo and thio-*β*-lactams (11, 12 and 13).* These compounds exhibit a distribution of minima similar to that of clavulanic acid, particularly as regards the carboxyl group and lactam nitrogen (see Figure 2 and Table 1). Like clavulanic acid, the three compounds possess a single minimum associated to position 1 (*i*). They exhibit two other minima on the carbonyl group: one at the usual position (*a* in Figure 2) and the other in between the carbonyl oxygen and the heteroatom (N, O or S) (*b*), as well as two more minima on the carboxyl group (*d* and *e*) and one on the lactam nitrogen (*c*). In addition, the aza-β-lactam exhibits a minimum on the side chain (*g*) that is absent from the potential surfaces for the other two compounds as they possess no such chain. The potentials of the minima are similar for the three compounds and range from −257 to −568 kJ mol^{-1}; the thio-β-lactam exhibits the strongest minima on the carbonyl and carboxyl groups. In all three compounds, the minimum on the carbonyl group is much weaker than in penicillin G and cephalothin.

Direct examination of the structures and electrostatic potential minima for the studied structures only provides qualitative information about the structural and electrostatic similarity of the different compounds. In order to derive quantitative values for such similarity, the structural and electrostatic root mean square (rms) for the different structures with respect to penicillin G, cephalothin and clavulanic acid were calculated. The choice of these three compounds as standards was dictated by their being a typical penicillin, cephalosporin and suicidal substrate, the three major types of β-lactam antibiotics, respectively, and also by their being suitable substrates for both transpeptidases and β-lactamases. Structural overlap was examined in the O_8-C_7-N_4-C_3-C_9-($O_{10}O_{11}$) sequence, which mimics the natural substrate for transpeptidases (D-alanyl-D-alanine) [29] and was present in all the studied compounds. Electrostatic overlay was examined in terms of the minimum for the carbonyl group and the two minima for the carboxyl group, which are those pertinent to the antibiotic-enzyme binding and present in all the structures. The minimum on the lactam nitrogen observed in some structures was not considered as it was absent from some others. The results thus obtained are given in Table 2.

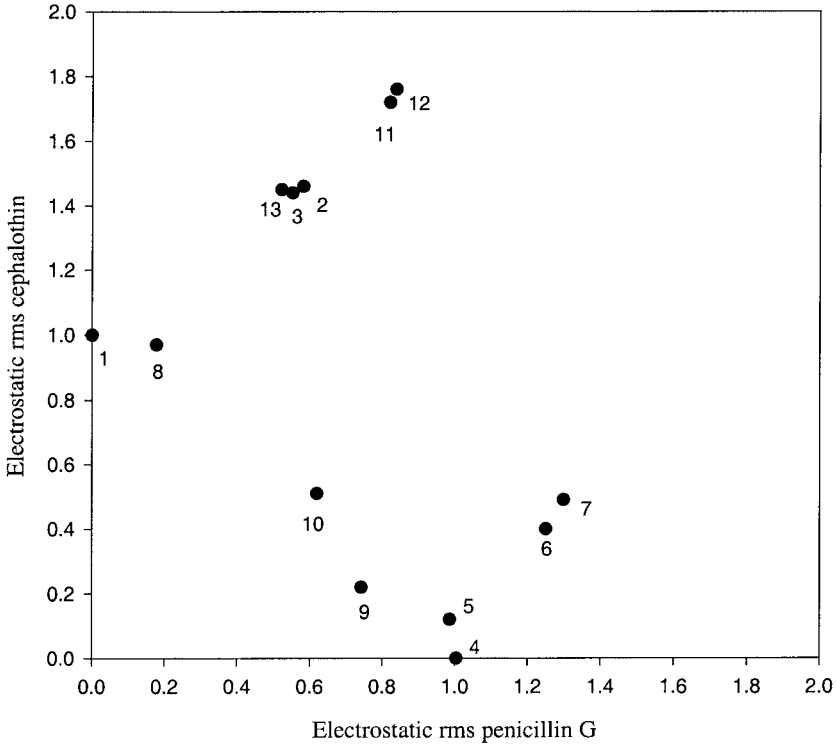


Figure 4. Electrostatic rms of the different structures with respect to cephalothin versus electrostatic rms of the different structures with respect to penicillin G.

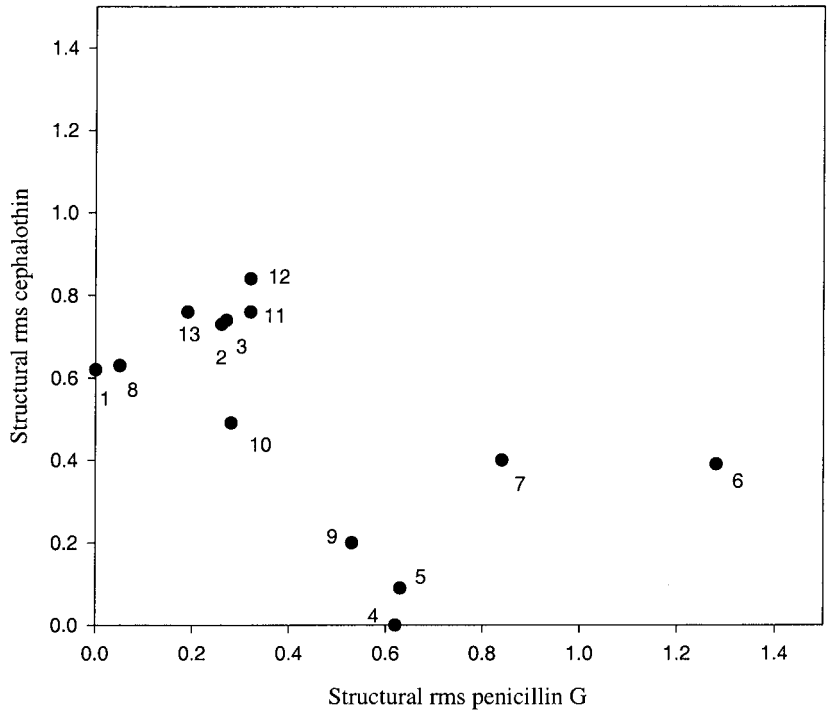


Figure 5. Structural rms of the different structures with respect to cephalothin versus structural rms of the different structures with respect to penicillin G.

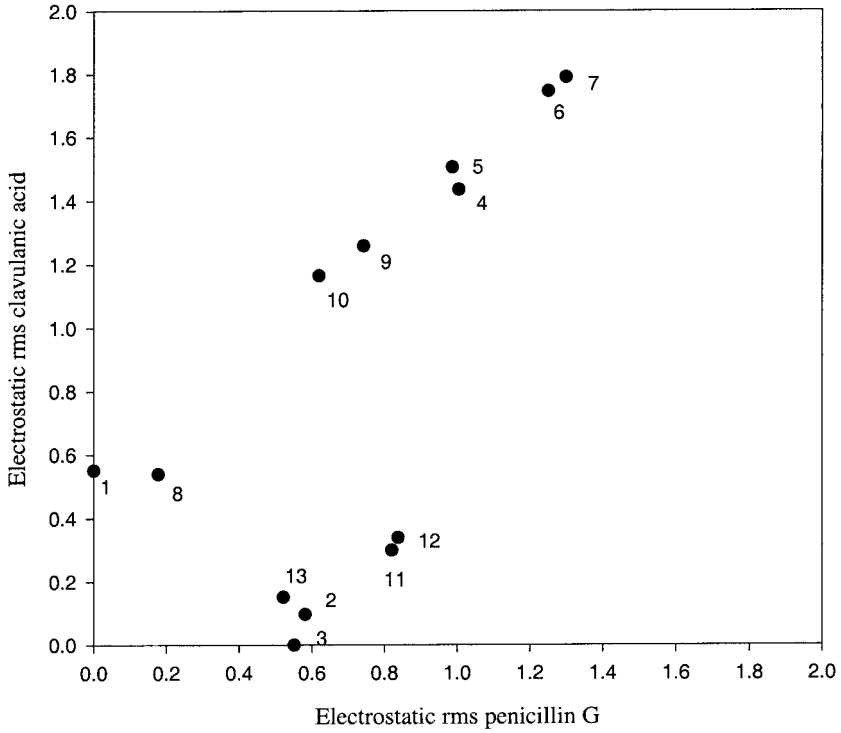


Figure 6. Electrostatic rms of the different structures respect to clavulanic acid versus electrostatic r.m.s. of the different structures with respect to penicillin G.

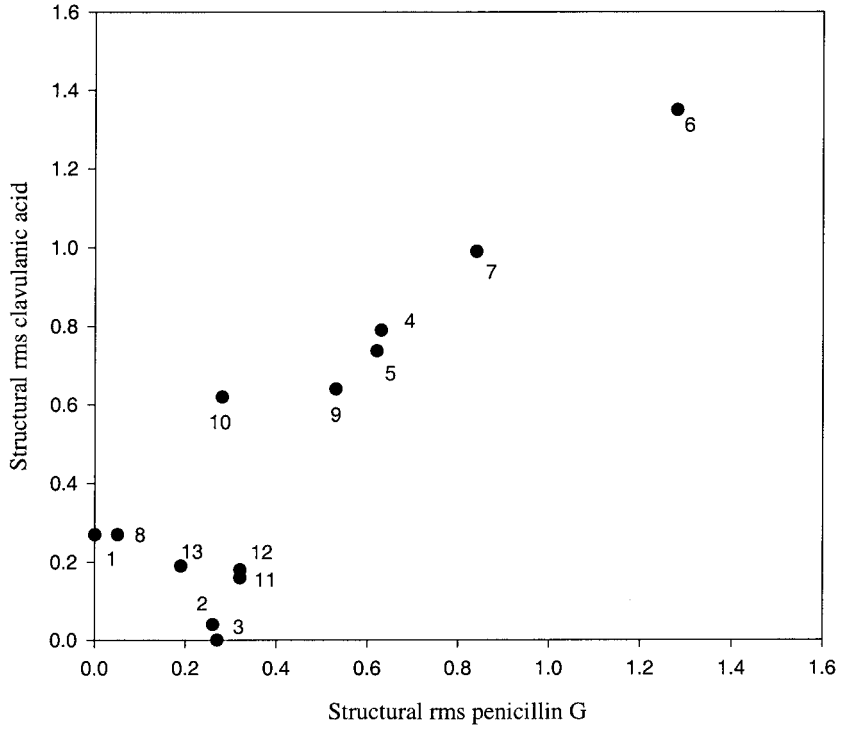


Figure 7. Structural rms of the different structures with respect to clavulanic acid versus structural r.m.s. of the different structures with respect to penicillin G.

Table 2. Electrostatic and structural r.m.s. of the overlays for the different structures respect to the clavulanic acid, penicillin G and cephalothin.

	Antibacterial activity	r.m.s. elec. clav.	r.m.s. elec. pen. G	r.m.s. elec. cephalothin	r.m.s. struct. clav.	r.m.s. struct. pen. G	r.m.s. struct. cephalothin
Penicillin G (1)	High	0.551	0	1.004	0.270	0	0.617
Methicillin (axial) (2)	Non active	0.097	0.581	1.455	0.044	0.263	0.729
Clavulanic acid (3)	Low, inhibitor of β-lactams	0	0.551	1.436	0	0.270	0.737
Cephalothin (4)	High	1.436	1.004	0	0.737	0.617	0
Cephaloridine (5)	High	1.506	0.986	0.124	0.789	0.632	0.089
Ly193375 (6)	High	1.747	1.250	0.397	1.351	1.280	0.391
α-Cyclopropyl (7)	High	1.791	1.299	0.489	0.988	0.844	0.399
β-Cyclopropyl (8)	High	0.540	0.177	0.966	0.273	0.053	0.630
Sanfenitrem (9)	High	1.259	0.742	0.218	0.643	0.529	0.197
Structure 10 (10)	Non active	1.165	0.619	0.508	0.621	0.283	0.491
Aza-β-lactam (11)	–	0.300	0.820	1.725	0.156	0.323	0.844
Oxo-β-lactam (12)	–	0.339	0.837	1.759	0.177	0.317	0.761
Thio-β-lactam (13)	–	0.152	0.521	1.445	0.194	0.189	0.764

Figures 4 and 5 show the electrostatic and structural rms, respectively, for the different compounds against those for cephalothin and penicillin G. As can be seen in Figure 4, the structures cluster in three distinct groups consisting of penicillin-related compounds, *viz.* penicillin G (1) and β-cyclopropylpenam (8), cephalosporin-related compounds, *viz.* structures 4, 5, 6, 7, 9 and 10, and compounds exhibiting marked differences from both penicillins and cephalosporins, *viz.* structures 2, 3, 11, 12 and 13. This distribution is consistent with experimental evidence for the antibacterial properties of the studied compounds. This clear-cut grouping of the compounds is absent from Figure 5, however. Figures 6 and 7 show the electrostatic and structural rms, respectively, of the studied compounds against clavulanic acid and penicillin G. Like Figure 4, Figure 6 exhibits three distinct groups consisting of clavulanic acid-related compounds (structures 2, 3, 11, 12 and 13), penicillin-related compounds (structures 1 and 8) and compounds differing markedly from both clavulanic acid and penicillin G (structures 4, 5, 6, 7, 9 and 10), and including those related to cephalosporins. Compound clustering in Figure 7, which shows structural rms, is much less evident. Based on the previous figures, it is clear that the analysis of the multipole charge distribution provides much more information about antibacterial power than does the mere comparison of structures.

β-Cyclopropylpenam (8) exhibits an electrostatic rms against penicillin G of only 0.177, much smaller than that against cephalothin (0.966); on the other hand, α-cyclopropylpenam (7) exhibits the opposite trend, *viz.* an rms of 1.299 against penicillin G and 0.489 against cephalothin. These data are consistent with experimental evidence which suggests that the β isomer acts similarly to penicillins whereas the α iso-

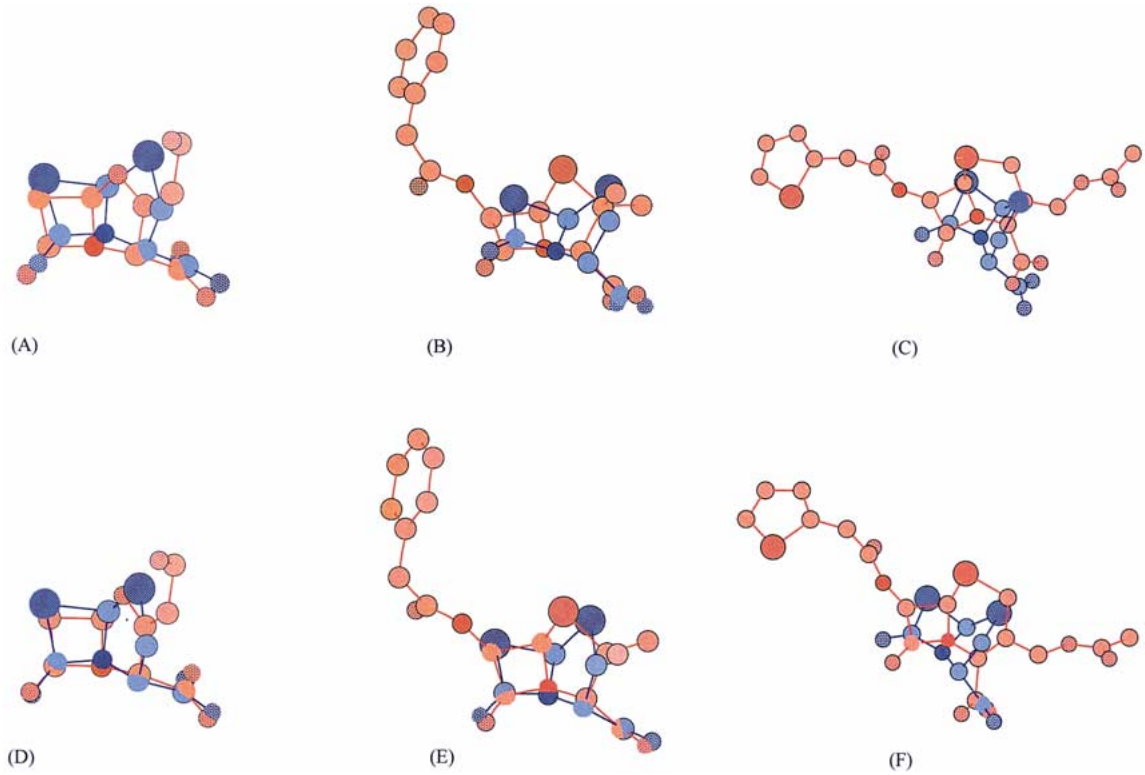


Figure 8. Electrostatic overlay of thio-β-lactam structure (blue) with (A) clavulanic acid, (B) penicillin G and (C) cephalothin, and structural overlay of thio-β-lactam with (D) clavulanic acid, (E) penicillin G and (F) cephalothin.

mer resembles cephalosporins in its action (it is active against gram-negative bacteria but resistant to penicillinases) [41]. Both structures are rather different from that of clavulanic acid (the electrostatic rms is 1.791 for the α isomer and 0.540 for the β one).

The results for LY193375 (6) and the compound 10, which are seemingly very similar, suggest that the former is in fact much more similar to cephalothin than to penicillin G (with electrostatic r.m.s. of 0.397 and 1.250, respectively); on the other hand, the latter is virtually equidistant from cephalothin in Figure 4 (0.619 against the cephalosporin and 0.518 against the penicillin). Although neither compound exhibits especially small values, structure 6 is active whereas structure 10 is not. The origin of this divergence is the differential chemical reactivity of the two compounds that arise from differences in the aza-γ-lactam ring. The structural rms for LY193375 follow the same trend (1.280 against penicillin G and 0.391 against cephalothin); on the other hand, those for the compound 10 are anomalously small (only 0.283 against penicillin G and 0.491 against cephalothin). These last results contradict the experimental fact that structure 10 is inactive, which, again, indicates that structural overlays provide inaccurate predictions of the antibacterial properties of lactams. In fact, the electrostatic rms against clavulanic acid are rather large for both compounds.

Sanfenitrem (9) exhibits significant similarity to cephalothin, with an electrostatic and structural rms of 0.218 and 0.197, respectively; hence, it exhibits similar properties such as a broad spectrum of action and high stability against penicillinases [42]. On the other hand, it is rather different from clavulanic acid (electrostatic r.m.s. 1.259).

Clavulanic acid (3) is markedly different from cephalosporins (electrostatic rms 1.436) and only slightly similar to penicillin (electrostatic r.m.s. 0.551). This accounts for its low antibacterial power, which is a result of its poor ability to bind to the active site of bacterial transpeptidases. The assumption that it possesses an unsuitable conformation to bind to transpeptidases is supported by the fact that its rms are very similar – nearly identical – to those of axial methicillin (2), which is also inactive. This last pos-

sesses an electrostatic rms against clavulanic acid of only 0.097.

The aza, oxo and thio-β-lactams (structures 11, 12 and 13), for which no experimental evidence of antibacterial or inhibitory action on β-lactamases exists, are structurally and electrostatically rather different from penicillin G and cephalothin (their electrostatic rms against penicillin G range from 0.521 to 0.820, and those against cephalothin from 1.445 to 1.759). However, they possess rather small electrostatic rms against clavulanic acid (between 0.152 and 0.339), which suggests that their enzyme binding constants must be similar to those for the acid.

On the other hand, these compounds exhibit adequate chemical reactivity to act as β-lactamase inhibitors [34-37]. Substitution of the carbon atom next to the carbonyl group in the β-lactam ring by a heteroatom (X = N, O or S) facilitates cleavage of the C-X bond by alkaline or enzymatic hydrolysis of the compound and, hence, the formation of carbamoyl-enzyme complexes. The formation of these, especially stable, complexes may inhibit the activity of β-lactamases.

Of the three compounds, the thio-β-lactam (with an electrostatic r.m.s. of 0.152 as compared to 0.300 and 0.339 for the aza and oxo compound, respectively) is that exhibiting the highest electrostatic similarity to clavulanic acid.

Figure 8, which shows the different overlays for the thio-β-lactam, reveals the structural and electronic similarities of this compound to clavulanic acid, penicillin G and cephalothin. As can be seen, the electrostatic and structural overlays are divergent, especially for penicillin G.

In summary, a comparison of electrostatic and structural r.m.s. with experimental evidence of antibacterial activity suggests that the electrostatic r.m.s. is a much more reliable parameter than is the structural r.m.s. with a view to assessing or predicting the ability of the substrate to bind to the enzyme. In this respect, aza, oxo and thio-β-lactams can be expected to be much more similar to clavulanic acid than to cephalosporins and penicillins in their behaviour towards enzymes as they not only exhibit small electrostatic r.m.s. values against the clavulanic acid but also possess chemical reactivity typical of suicidal substrates.

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