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ORIGINAL PAPER

Predicted binding rate of new cephalosporin antibiotics by a 3D-QSAR method: a new approach

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Abstract Antibiotics are chemotherapeutic agents with activity against microorganisms, for example bacteria, fungi, or protozoa, used for the treatment of many types of diseases. Binding of antibiotics to serum proteins in human plasma is a major determinant of their pharmacodynamic and pharmacokinetic behavior and, consequently, can affect their systemic distribution in the body. Here, the predicted binding rates of ceftazidime and 13 other pharmacological agents classified as antibiotics to plasma proteins (percentage fraction bound; PFB) were evaluated by use of 3D-QSAR models. We attempted to establish the contribution of hydrogen bond donor/acceptor and hydrophobic properties supplied by electrostatic fields to the PFB. Significant cross-validated correlation q^2 (0.5–0.7) and the fitted correlation r^2 (0.7–0.97) coefficients revealed that these models have reasonable power to predict the design 19 new antibiotics using ceftazidime as template, these compounds being our suggestion for further studies.

Keywords Percentage fraction bound · Ceftazidime · Computer chemistry · Drug research · Bioorganic chemistry

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Introduction

There are currently many classes of chemical structures which can be regarded as antibiotics: penicillins (natural penicillins, anti-staphylococcal penicillins, aminopenicillins, penicillins effective against pseudomonads), cephalosporins (four generations), carbapenems, monobactams, glycopeptides, aminoglycosides, tetracyclines, macrolides, and relative drugs, etc. [1, 2]. The main problem in antibiotherapy is the development of antibiotic-resistant bacteria, so that design and chemical synthesis are orientated to develop new antibiotic molecules with a broad antibacterial spectrum [3–6]. Cephalosporins have bactericidal activity on both Gram-positive and Gram-negative bacteria, but are not active against enterococci or methicillin-resistant staphylococci [3, 7–9]. Some cephalosporins, for example cefepime (a 4th-generation cephalosporin effective against Gram-positive cocci and Gram-negative bacilli including *Pseudomonas aeruginosa*, extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumonia*, and *Escherichia coli*), ceftazidime (a 3rd-generation cephalosporin), ceftriaxone, and cefotaxime are highly effective in treating meningitis with sensitive microorganisms [10–12].

Ceftazidime, an extremely potent antibiotic, is included in the empirical protocol for treatment of postneurosurgical meningitis due to *P. aeruginosa*, and in combination with vancomycin covers the methicillin-resistant *Staphilococcus aureus* [10–12]. There is currently much interest in developing new antibiotic derivatives starting from ceftazidime. It has an improved range of antimicrobial activity [13]. The important limitations of prescription antibiotics are allergies to one or more antibiotics (mostly piperacillin, ceftazidime, and ticarcillin) developing in about 30% of patients [14–17] and also the binding affinity of these drugs to the serum proteins in the body, one of the important

ADME (absorption, distribution, metabolism, and excretion) properties considered in drug discovery and development [18–20]. Binding of a drug to human plasma proteins is important for its pharmacodynamic and pharmacokinetic behavior and, consequently, affects the systemic distribution of the drug. Basically, the unbound drug molecules contribute to pharmacological efficacy and are also susceptible to metabolic reactions.

Prediction of percentage serum protein binding is more difficult than that of other ADME factors because this is a composite property made up by the sum of interactions with multiple proteins, each with a different affinity [21], and at present the detailed mode of plasma binding for many drugs is not known. An objective of this study was the use of 3D-QSAR models to predict the effect of molecular properties, for example hydrogen bond donor/acceptor property, hydrophobicity, and electrostatic fields, on the extent of serum protein binding of ceftazidime and 13 other pharmacological agents classified as antibiotics (cephalexin, cephalothin, cephapirin, cefnidir, cefepime, cefixime, cefotaxime, cefoxitin, cephpodoxime, cefprozil, ceftibutin, ceftizoxime, cefuroxime), in order to gain new insight into drug-design models. Even if nowadays the importance of drug binding to serum proteins is recognized, very few reports on developing QSAR models to predict the binding affinity to serum proteins have been published [19, 21, 22]. Yet these analogs were not tested in the ALMOND model, a very fast method presented in our study. This encouraged our study in which the molecular properties mentioned above led to powerful 3D-QSAR models in PFB prediction, even though we did not opt for a large number of molecules. The correlation between predicted and observed PFB of antibiotics from this study was compared with other QSAR studies [19, 21] and the power of our 3D-QSAR models was proven. Also, in our attempt to obtain new derivatives with lower binding to protein plasma than ceftazidime, nineteen new ceftazidime derivatives were modelled and their binding to plasma proteins was predicted in accordance with estimated 3D-QSAR models.

Results and discussion

When the binding of antibiotics to plasma proteins predicted on the basis of hydrogen bond acceptor property (model A) was evaluated, at first in the training set all 14 antibiotics were considered. The statistical data q^2 (cross-validated correlation coefficient), r^2 (fitted correlation coefficient), and standard deviation of error calculation (SDEC) were not statistically significant. Because of their negligible effect on the model, the outliers cefepime and cefixime were removed from the initial training set and placed in the testing set, with clear q^2 and r^2 improvement. The statistical data described above as control criteria are presented in Table 1.

In model B, considering simultaneously the effect of hydrogen bond acceptor and hydrophobicity on binding energy of antibiotics to plasma proteins, quite small q^2 and r^2 statistical data were obtained with ten molecules of antibiotics in the training set (Table 1).

One of our objectives was to assess the contribution of electrostatic hydrogen bond donor/acceptor properties to the binding energy of antibiotics to plasma proteins (model C). Initially, the training set comprised 14 molecules. Finally, the QSAR model had 11 molecules after eliminating the outliers cefalexin, cefprozil, and ceftizoxime, which were included into the test set. For the remaining antibiotics, the binding to plasma proteins was predicted. The statistical data used to validate the QSAR model were $q^2 = 0.57$ and $r^2 = 0.98$ (Table 1).

Data presented in Table 1 are emphasized by graphical representations of the correlation between observed and predicted binding of antibiotics to plasma proteins for the significant 3D-QSAR models A and C (Fig. 1a, b).

Also, in Table 2, are presented the PFBs of the antibiotics predicted by use of models A, B, and C and the observed PFB of the antibiotics.

The best correlation between observed and calculated PFB for the antibiotics included in model A (the most significant statistic 3D-QSAR model in our study) seems to be for cephpodoxime ($PFB_{observed} - PFB_{calculated} = -1$), cefprozil ($PFB_{observed} - PFB_{calculated} = 1$), and ceftizoxime ($PFB_{observed} - PFB_{calculated} = -2$). Unsuitable PFB residual values (the difference between $PFB_{observed}$ and $PFB_{calculated}$) were obtained for cefnidir [($PFB_{observed} - PFB_{calculated} = 20$) and cefuroxime ($PFB_{observed} - PFB_{calculated} = -20$). In model B (the statistically poor QSAR model in our study], when the contributions of both descriptors hydrogen bond acceptor and hydrophobicity were considered, poor correlation between observed and

Table 1 Summary of the ALMOND statistical data

Descriptors	Number of molecules in training set	q^2	r^2	SDEC
Hydrogen bond acceptor property (model A)	12	0.70	0.92	5.46
Hydrogen bond acceptor and hydrophobicity properties (model B)	10	0.50	0.96	3.56
Hydrogen bond donor/acceptor and electrostatic properties (model C)	11	0.57	0.98	2.53

q^2 cross-validated correlation coefficient, r^2 fitted correlation coefficient; SDEC, standard deviation of error calculation

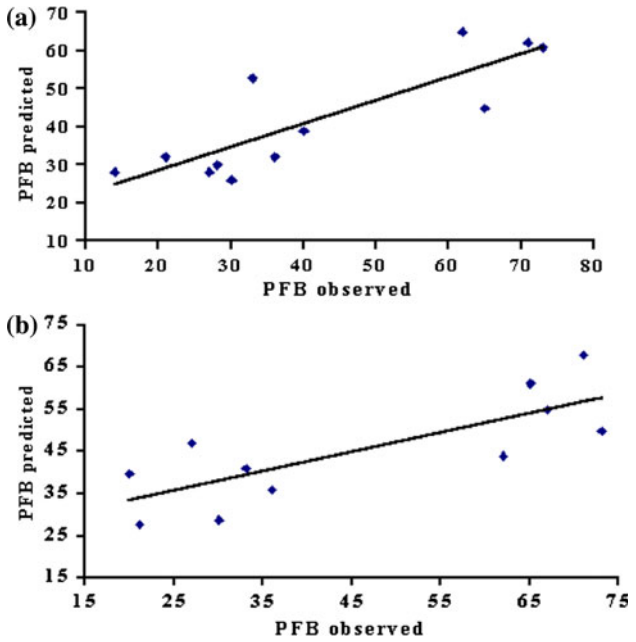


Fig. 1 Correlation between observed and predicted PFB of antibiotics for a training set of plasma proteins. **a** Hydrogen bond acceptor property is regarded as descriptor. **b** Hydrogen bond acceptor/donor and the electrostatic properties are regarded as descriptors

calculated PFB was obtained for cephalothin ($PFB_{observed} - PFB_{calculated} = 27$) and cefoxitin ($PFB_{observed} - PFB_{calculated} = 20$) and just good correlation between $PFB_{observed}$ and $PFB_{calculated}$ was obtained for ceftibutin ($PFB_{observed} - PFB_{calculated} = 1$) and cefotaxime ($PFB_{observed} - PFB_{calculated} = 2$). When the combined contribution of donor/acceptor hydrogen bonding and electrostatic descriptors (model C) was considered for all antibiotics a good correlation between $PFB_{observed}$ versus $PFB_{calculated}$ was observed for cephalothin ($PFB_{observed} - PFB_{calculated} = 3$), cefnidir ($PFB_{observed} - PFB_{calculated} = 4$), cefotaxime ($PFB_{observed} - PFB_{calculated} = 0$), and ceftibutin ($PFB_{observed} - PFB_{calculated} = 1$).

The predicted PFB of antibiotics from models A and C and others studies [19, 21] were compared (Table 3) and we noticed that predicted PFB values are very close (cephalexin $PFB_{predicted} = 28$ (model A), 27.1 [21], 30.38 [19]; cefoxitin $PFB_{predicted} = 61$ (model A), 60 [21], 54.6 [19]; ceftazidime $PFB_{predicted} = 32$ (model A), 33.6 [21]; ceftizoxime $PFB_{predicted} = 30$ (model A), 29.7 [21], 27.42 [19]). With some exceptions, in our study the correlation between observed and predicted PFB of the antibiotics was better, even if our training set contained 12 molecules (cephapirin $PFB_{observed} = 62$, $PFB_{predicted} = 65$ (model A), 69.4 [21], 70.4 [19]; cefotaxime $PFB_{observed} = 36$, $PFB_{predicted} = 32$ (model A), 41.3 [21]; cephpodoxime $PFB_{observed} = 27$, $PFB_{predicted} = 28$ (model A), 32.6 [21]; or cefprozil $PFB_{observed} = 40$, $PFB_{predicted} = 39$ (model A), 23.4 [21], 42.83 [19]). Furthermore, comparing $PFB_{predicted}$ obtained for the antibiotics by use of model C and studies presented above we observed that correlation between predicted and observed PFB of cefnidir, cefixime, and ceftazidime by model C is much better (cefnidir $PFB_{residual} = 4$ (model C), 33.7 [21]), and also for cefixime $PFB_{residual} = 12$ (model C), 30 [21], ceftazidime $PFB_{residual} = -7$ (model C), −12.7 [21], −25.03 [19]).

Modelling of new ceftazidime derivatives with potentially superior antibacterial activity

Because of the before-mentioned high importance of ceftazidime as cephalosporin agent, a set of 19 new ceftazidime derivatives was created in order to predict smaller PFB by using our above presented 3D-QSAR model A. In designing our new ceftazidime derivatives we followed two strategies: first, we generated more negative electrostatic contacts by adding halogen (F, Cl, Br), hydroxyl, nitro, methoxy, or amide substituents, and second, we enhanced the number of hydrophobic contacts of ceftazidime by adding allyl, ethyl, isopropyl, propyl, and *t*-butyl substituents (Table 4). Chemical structures of ceftazidime derivatives, residual PFB values (the difference between ceftazidime PFB and ceftazidime derivative PFB) and predicted PFB values are presented in Table 4. Following our design strategy, four of the ceftazidime derivatives (derivatives 14 (R^1=Cl, R^2=H, R^3=H, R^4=Cl, $PFB_{res} = -4.98$), 15 (R^1=Cl, R^2=H, R^3=H, R^4=Br, $PFB_{res} = -3.61$), 16 (R^1=Cl, R^2=H, R^3=H, R^4=F, $PFB_{res} = -8.15$), and 18 (R^1=isopropyl, R^2=H, R^3=H, R^4=F, $PFB_{res} = -1.74$)) had PFB values in agreement with our intention. We noticed that the double substitution of the pyridine nucleus with halogens (Cl, Br, F) and with isopropyl (Table 4) leads to a significant decrease in binding to plasma proteins. In contrast, addition of NH_2 (derivative 5 (R^1=NH_2, R^2=H, R^3=H, R^4=H, $PFB_{res} = 35.35$)), HNC=O (derivative 9 (R^1=HNC=O, R^2=H, R^3=H, R^4=H, $PFB_{res} = 35.03$)), or OH (derivative 11 (R^1=OH, R^2=H, R^3=H, R^4=H, $PFB_{res} = 31.94$)) at the pyridine ring significantly increases binding to plasma proteins.

Conclusions

3D-QSAR models can give different kinds of information, for example reliable prediction of protein binding of compounds belonging to a data set and chemical interpretation of the results obtained. In this paper we have reported alignment-independent 3D-QSAR studies, using three QSAR models, of a series of 14 antibiotics already used in clinical practice and of 19 new ceftazidime derivatives. The models were used to elucidate the most important physicochemical properties responsible for the

Table 2 Observed binding to plasma proteins of the antibiotics in the training set and the values predicted by use of 3D-QSAR models

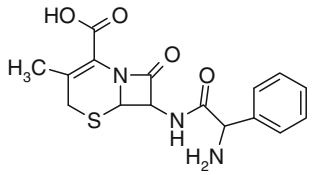
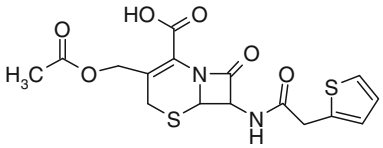
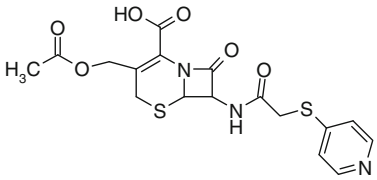
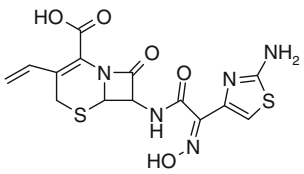
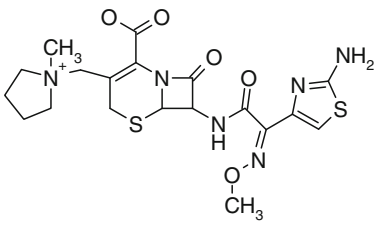
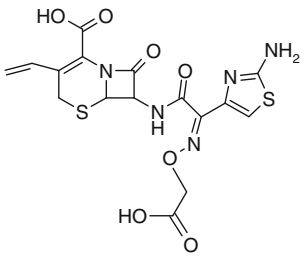
Antibiotic	2D structure	PFB_{obs} [23]	PFB_{pred}		
			Model A	Model B	Model C
Cephalexin		14	28	–	–
Cephalothin		71	62	44	68
Cephapirin		62	65	76	44
Cefnidir		65	45	–	61
Cefepime		20	–	–	40
Cefixime		67	–	–	55

Table 2 continued

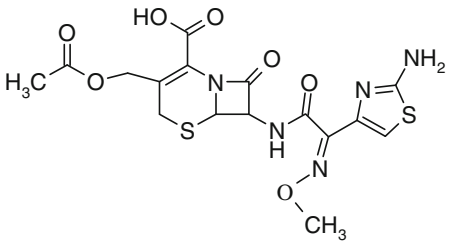
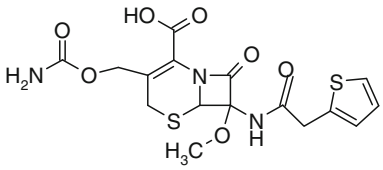
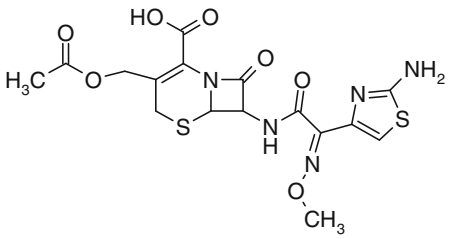
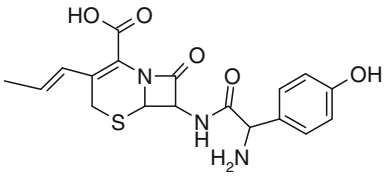
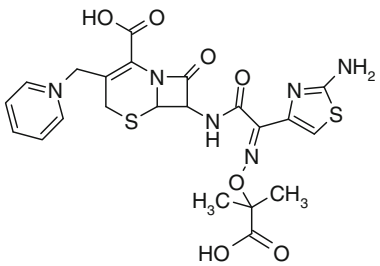
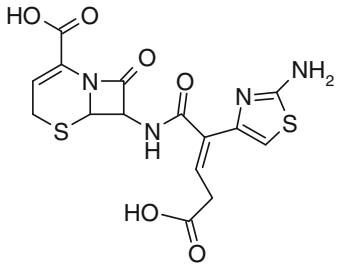
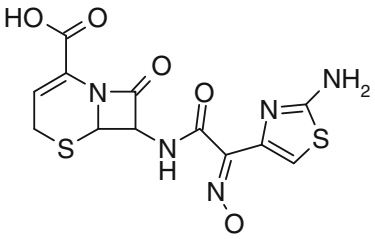
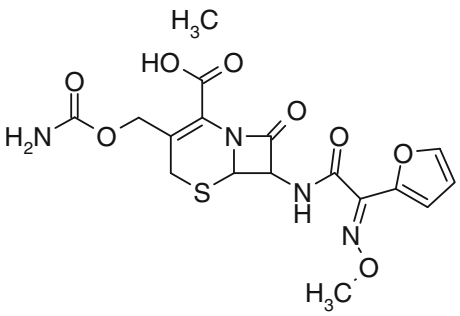
Antibiotic	2D structure	PFB_{obs} [23]	PFB_{pred}		
			Model A	Model B	Model C
Cefotaxime		36	32	34	36
Cefoxitin		73	61	53	50
Cephpodoxime		27	28	34	47
Cefprozil		40	39	36	–
Ceftazidime		21	32	30	28

Table 2 continued

Antibiotic	2D structure	PFB_{obs} [23]	PFB_{pred}		
			Model A	Model B	Model C
Ceftibutin		30	26	29	29
Ceftizoxime		28	30	43	–
Cefuroxime		33	53	42	41

Model A (hydrogen bond acceptor property was used as descriptor), B (hydrogen bond acceptor and hydrophobic properties were used as descriptors), C (electrostatic and hydrogen bond donor/acceptor properties were used as descriptors)

PFB_{obs} PFB obtained from experimental data, PFB_{pred} PFB obtained by QSAR models

binding properties of the antibiotics to plasma protein. In our study hydrogen donor/acceptor and hydrophobic properties supplied by electrostatic fields were considered. Significant PLS results were obtained when the hydrogen bond acceptor (model A) and the simultaneous presence of electrostatic field and hydrogen bond donor/acceptor descriptors (model B) were considered.

Our results suggest that hydrogen bond acceptor property plays a key role in determining the binding rates of antibiotics to plasma proteins, and the simultaneous presence of the descriptors electrostatic field and hydrogen donor/acceptor properties enables good prediction of the binding of the 14 antibiotics studied. Thus, judicious modulation of physicochemical properties, particularly hydrogen bond acceptor, may be very useful for designing new antibiotic drugs. Considering the above set of 19 new potential ceftazidime structures, the established equations could be used to enhance or reduce PFB values in accordance with biological need. It was noticed that double substitution of the pyridine ring with halogens (Cl, Br, F) and with isopropyl significantly reduces binding of the antibiotics to plasma proteins.

Table 3 Predicted PFB of antibiotics obtained by QSAR models A and C and others studies

Antibiotic	PFB observed [23]	PFB predicted			
		Model A	Model C	Ref. [21]	Ref. [19]
Cephalexin	14	28	–	27.1	30.38
Cephalothin	71	62	68	62	73.21
Cephapirin	62	65	44	69.4	70.4
Cefnidir	65	45	61	31.3	–
Cefepime	20	–	40	27.9	31.02
Cefixime	67	–	55	36	–
Cefotaxime	36	32	36	41.3	37.76
Cefoxitin	73	61	50	60	54.05
Cefpodoxime	27	28	47	32.6	–
Cefprozil	40	39	–	23.4	42.83
Ceftazidime	21	32	28	33.6	46.03
Ceftibutin	30	26	29	12.5	30.97
Ceftizoxime	28	30	–	29.7	27.42
Cefuroxime	33	53	41	41	40.50

The references used for observed and predicted PFB of compounds are presented in brackets. For models A and C presented antibiotics belong to training set

Experimental

Dataset for analysis

The serum protein binding data of 14 pharmacological agents (cephalexin, cephalothin, cephapirin, cefnidir, cefepime, cefixime, cefotaxime, cefoxitin, cephpodoxime, cefprozil, ceftazidime, ceftibutin, ceftizoxime, cefuroxime) used in this study were collected from the literature [23] and are expressed as percentage of the drug binding to total serum proteins (percent fraction bound, PFB). The PFB value ranges were large—from 14 (cephalexin) to 73 (cefoxitin). The names, 2D structures, and corresponding PFB values of the antibiotics are given in Table 2.

Molecular modelling and minimum energy strategy

Three-dimensional structures of the compounds were obtained by use of the Build module from Sybyl 7 software [24]. In the first step, 2D structures of the antibiotics and the additive hydrogen atoms were constructed. These were then automatically changed to the 3D structures of the antibiotics, which were saved in Sybyl specific files.

In our study, the conformation with minimum potential energy of the antibiotics was established using the Maxim 2 minimization routine in Sybyl 7, with Tripos force field [25], conjugate-gradient algorithm [25], and convergence 0.01. During energy minimization, the whole chemical structure of the antibiotics was allowed free movement. After energy minimization, the Gasteiger–Marsili partial charges of the compounds [25] were loaded on the chemical structures from the Sybyl 7 dictionary.

ALMOND strategy

These data were introduced into the ALMOND software package [26, 27] incorporated into Sybyl 7.3, where calculation of the descriptors hydrophobicity, hydrogen bond acceptor (carboxyl-*O* as atom probe)/donor (nitrogen in cationic state as atom probe), and the electrostatic field (water as atoms probe), in succession or in different combinations, was done. It has already been mentioned that the process of binding to plasma proteins is very complex. Thus, in our effort to predict with more accuracy the binding of antibiotics to plasma proteins, three 3D-QSAR models considering the contribution of one or more descriptors presented above were performed, but not all these combinations of atom probes were considered in the final models, some of them being statistically insignificant. The three most significant 3D-QSAR models covering the contribution of carboxyl-*O*, nitrogen in the cationic state, H_2O, and hydrophobicity in different combinations are presented in the "Results and discussion" section (Table 1). Most of the ALMOND variables were set to the default values, for example the grid spacing was equal to 0.5 Å, the smoothing window of the correlograms was set to 0.8, and the size of the correlograms was automatically established by the software.

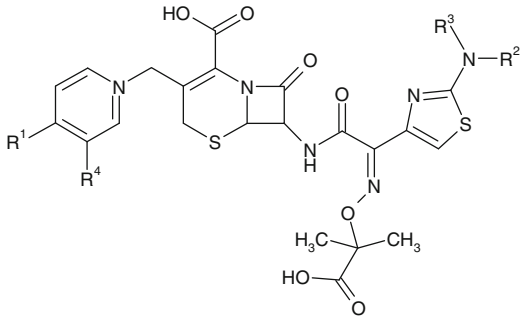
Chemometric analyses

Regression analysis was performed using the partial least squares (PLS) [25, 28] algorithm within Sybyl 7, and q^2 [25] was obtained by the leave-one-out cross-validation technique available in ALMOND. Furthermore, the control criterions r^2 coefficient and SDEC (standard deviation of error calculation) [25] were predicted in the Sybyl ALMOND module by a non-cross-validated method [25, 28]. All calculations were performed on an Intel Core 2 Duo processor-based Linux workstation.

Training and test sets in QSAR models

To test the binding of antibiotics to plasma proteins we created three sets in which a variable number of molecules were used for testing. Initially, to validate 3D-QSAR ALMOND models A, B, and C, individual atom probes hydrophobicity, hydrogen bond acceptor (carboxyl-*O* as atom probe)/donor (nitrogen in cationic state as atom probe), and electrostatic field (water as atoms probe), in succession or in different combinations, were used to predict the PFB. Finally, choosing a set of descriptors

Table 4 PFB values for ceftazidime derivatives and the difference between $PFB_{ceftazidime}$ and $PFB_{derivative}$



Derivative	R^1	R^2	R^3	R^4	$PFB_{derivative}$	$PFB_{ceftazidime} - PFB_{derivative}$
Derivative 1	H	CH_3	H	H	53.72	32.72
Derivative 2	H	CH_3	CH_3	H	29.19	8.19
Derivative 3	Cl	H	H	H	23.45	2.45
Derivative 4	Br	H	H	H	24.07	3.07
Derivative 5	NH_2	H	H	H	56.35	35.35
Derivative 6	Allyl	H	H	H	26.31	5.31
Derivative 7	$O{=}CNH_2$	H	H	H	41.25	20.25
Derivative 8	COOH	H	H	H	49.39	28.39
Derivative 9	HNC=O	H	H	H	56.03	35.03
Derivative 10	Isopropyl	H	H	H	25.16	4.16
Derivative 11	OH	H	H	H	52.94	31.94
Derivative 12	*t*-Butyl	H	H	H	25.39	4.39
Derivative 13	Isopropyl	CH_3	CH_3	H	34.27	13.27
Derivative 14	Cl	H	H	Cl	16.02	**−4.98**
Derivative 15	Cl	H	H	Br	17.39	**−3.61**
Derivative 16	Cl	H	H	F	12.85	**−8.15**
Derivative 17	Cl	H	H	OH	42.49	21.49
Derivative 18	Isopropyl	H	H	F	19.26	**−1.74**
Derivative 19	Isopropyl	H	H	Cl	22.88	1.88

Bold letters denote $PFB_{derivative} < PFB_{ceftazidime}$, values which are in agreement with our intention to obtain new ceftazidime derivatives

sufficient to enable accurate validation of the QSAR model (q^2 − (cross-validated r^2) not <0.5, r^2 higher than 0.9), different combinations were considered for different PFB as follows:

1 set A (cefepime and cefixime belonging to the test set and the other 12 antibiotics used for training) assessed with hydrogen bond acceptor property as descriptor;
2 set B (cefepime, cefixime, cefdinir, and cefalexin used for testing, the training set containing the other 10 antibiotics) assessed with hydrogen bond acceptor and hydrophobicity properties as descriptors; and
3 set C (cefalexin, cefprozil, and ceftizoxime belonging to the test set, with the other 11 molecules belonging to training set) assessed with the combination of hydrogen bond acceptor/donor properties and the electrostatic field as descriptors.

Starting from the initial model, model A, containing 14 antibiotics in the training set, and elimination of cefepime and cefixime as outliers, a new 3D-QSAR ALMOND model A was obtained, described by significant improvement of $q^2 = 0.7$, and $r^2 = 0.92$, (Table 1). Further, with cefepime, cefixime, cefdinir, and cefalexin as tested molecules $q^2 = 0.5$, and $r^2 = 0.96$, were obtained for the 3D-QSAR ALMOND model B. Also, starting from initial model C containing 14 antibiotics in the training set, and elimination of cefalexin, cefprozil, and ceftizoxime as outliers a new 3D-QSAR ALMOND model C was obtained, described by significant improvement of $q^2 = 0.57$, and $r^2 = 0.98$ (Table 1).

Modelling of new ceftazidime derivatives with potentially superior antibacterial activity to that of ceftazidime

Taking into account the best correlations between observed and predicted PFB for ceftazidime, 19 molecules derived from ceftazidime were obtained and PFB was evaluated. Molecular modelling of ceftazidime derivatives was performed under the above described procedure. The ceftazidime derivatives were generated using the add atoms and groups from Sybyl data base. The minimum potential energy for ceftazidime derivatives were established using the Maxim 2 minimization routine in Sybyl 7, with Tripos force field [25], conjugate-gradient algorithm [25], and convergence 0.01. During energy minimization, only the ceftazidime skeleton was kept rigid while the specific substituents were allowed free movement. After energy minimization, the Gasteiger–Marsili partial charges of the compounds [25] were loaded on the chemical structures from the Sybyl 7 dictionary.

Prediction of energy of binding to plasma proteins of the obtained derivatives was performed using QSAR models statistically validated during previous phases of the study.

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References

1. Zechini B, Versace I (2009) Rec Pat Antiinfect Drug Discov 4:37
2. Wong DM, Blumberg DA, Lowe LG (2006) Am Fam Physician 74:956
3. Lapis K (2008) Orv Hetil 149:2419
4. Sacha P, Wieczorek P, Hauschild T, Zorawski M, Olszanska D, Tryniszewska E (2008) Folia Histochem Cytobiol 46:137
5. Limban C, Chifiriuc MC, Missir AV, Chirita IC, Bleotu C (2008) Molecules 13:567
6. Mesaros N, Nordmann P, Plesiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Van Bambeke F (2007) Clin Microbiol Infect 13:560
7. Guggenheim M, Zbinden R, Handschin AE, Gohritz A, Altintas MA, Giovanoli P (2009) Burns 35:553
8. Barcenilla Gaite F, Jover Saenz A, Vallverdu Vidal M, Castellana Perello D (2008) Rev Esp Quimioter 21:9
9. Levine DP (2008) J Antimicrob Chemother 62:35
10. Hatipoglu CA, Yildiz E, Koktekir E, Ipekkan K, Karakoc EA, Demiroz AP (2008) Mikrobiyol Bul 42:695
11. Watanabe Y, Akizuki T (2008) J Craniofac Surg 19:1542
12. Hsu HE, Shutt KA, Moore MR, Beall BW, Bennett NM, Craig AS, Farley MM, Jorgensen JH, Lexau CA, Petit S, Reingold A, Schaffner W, Thomas A, Whitney CG, Harrison LH (2009) N Engl J Med 360:244
13. Jacoby GA, Munoz-Price LS (2005) N Engl J Med 352:380
14. Hershkovich J, Broides A, Kirjner L, Smith H, Gorodischer R (2009) Clin Exp Allergy 39:726
15. Halevy S, Grossman N (2008) Isr Med Assoc J 10:865
16. Lugovic Mihic L, Bulat V, Situm M, Cavka V, Krolo I (2008) Coll Antropol 32:153
17. La Shell MS, Tankersley MS (2008) Ann Allergy Asthma Immunol 101:559
18. Gunturi SB, Narayanan R, Khandelwal A (2006) Bioorg Med Chem 14:4118
19. Narayanan R, Gunturi SB (2005) Bioorg Med Chem 13:3017
20. Herve F, Urien S, Albengres E, Duche JC, Tillement JP (1994) Clin Pharmacokinet 26:44
21. Hall LM, Hall LH, Kier LB (2003) J Comput Aided Mol Des 17:103
22. Colmenarejo G (2003) Med Res Rev 23:275
23. Hardman JG, Limbird LE (1996) Goodman & Gillman's the pharmacological basis of therapeutics, 9th edn. McGraw–Hill, New York (appendix)
24. Homer RW, Swanson J, Jilek RJ, Hurst T, Clark RD (2008) J Chem Inf Model 48:2294
25. Sybyl Theory Manual (1988) Tripos Associates Inc, St Louis
26. Pastor M, Cruciani G, McLay I, Pickett S, Clementi S (2000) J Med Chem 43:3233
27. Carosati E, Lemoine H, Spogli R, Grittner D, Mannhold R, Tabarrini O, Sabatini S, Cecchetti V (2005) Bioorg Med Chem 13:5581
28. Waller CL, Oprea TI, Giolitti A, Marshall GR (1993) J Med Chem 36:4152