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ORIGINAL PAPER

Minimum requirements of hydrophobic and hydrophilic features in cationic peptide antibiotics (CPAs): pharmacophore generation and validation with cationic steroid antibiotics (CSAs)

Sandeep Sundriyal · Rohit K. Sharma · Rahul Jain · Prasad V. Bharatam

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Abstract Cationic peptide antibiotics (CPAs) are known to possess amphiphilic structure, by virtue of which they display lytic activity against bacterial cell membranes. Naturally occurring antimicrobial peptides contain a large number of amino acid residues, which limits their clinical applicability. Recent studies indicate that it is possible to decrease the chain-length of these peptides without loss of activity, and suggest that a minimum of two positive ionizable (hydrophilic) and two bulky groups (hydrophobic) are required for antimicrobial activity. By employing the HipHop module of the software package CATALYST, we have translated these experimental findings into 3-D pharmacophore models by finding common features among active peptides. Positively ionizable (PI) and hydrophobic (HYD) features are the important characteristics of compounds used for pharmacophore model development. Based on the highest score and the presence of amphiphilic structure, two separate hypothesis, Ec-2 and Sa-6 for *Escherichia coli* and *Staphylococcus aureus*, respectively, were selected for mapping analysis of active and inactive peptides against these organisms. The resulting models not only provided information on the minimum requirement of PI and HYD features but also indicated the importance of their relative arrangement in space. The minimum requirement for PI features was two in both cases but the number of HYD features required in the case of *E. coli* was four while for *S. aureus* it was found to be three. These hypotheses were able to differentiate between active and inactive CPAs against both organisms and were able to explain the experimental results. The hypotheses were further validated using cationic steroid antibiotics (CSAs), a different class of facial amphiphiles with same mechanism of antimicrobial action as that of CPAs. The results showed that CSAs also require similar minimum features to be active against both *E. coli* and *S. aureus*. These studies also indicate that the minimum feature requirements may be conserved for different strains of the same organism.

Keywords Cationic peptide antibiotics · Cationic steroids antibiotics · Amphiphiles · Minimum requirement · Pharmacophore generation · HipHop · Catalyst

S. Sundriyal · R. K. Sharma · R. Jain · P. V. Bharatam (✉)
Department of Medicinal Chemistry,
National Institute of Pharmaceutical Education
and Research (NIPER),
Sector 67, S.A.S.,
Nagar, Punjab 160 062, India
e-mail: pvbharatam@niper.ac.in

R. Jain
e-mail: rahuljain@niper.ac.in

Introduction

The widespread and irrational use of classical antibiotics has led to a stupendous increase in the number of resistant strains of bacteria [1–4]. To further worsen the situation, only three new structural classes of antibiotics, namely the oxazolidinones (linezolid), the streptogramins and the cyclic lipopeptides (daptomycin) have become available on the market in the past 40 years [5–7]. Therefore, it is critical to develop novel, potent and efficacious classes of antimicrobial agents. Cationic peptide antibiotics (CPAs) have shown the potential to represent such a class of antibiotics [8, 9]. Naturally occurring CPAs, which constitute a major component of the innate self-defense system, provide an immediate response to invading microorganisms

and display a broad spectrum of bactericidal and fungicidal activity [10–13].

Most of the known CPAs have an extra positive charge due to the presence of amino acids with a positively ionizable (PI) group in the side chain (most commonly Arg or Lys), and also contain hydrophobic (HYD) amino acid residues. It is now well known that CPAs adopt a particular secondary or tertiary structure, with cationic groups on one face of the molecule and HYD groups on the other side. This segregation of cationic and HYD groups imparts the facial amphiphilicity to the molecule that is responsible for mechanism of action of CPAs [14, 15]. The facial amphiphilicity has been presumed to be essential and responsible for the cell membrane lytic activity of these peptides. Although their exact mode of action is still not completely understood, it is well established that CPAs interact with the cell membrane of susceptible microorganisms, where either their accumulation in the membrane causes increased permeability and loss of barrier function or they cross the membrane to access cytoplasmic targets [16, 17]. For example, mechanistic studies with gramicidin-S have clearly shown that this peptide is extremely membrane active [18]. In order to exert their activity, peptides first interact with, mainly, lipopolysaccharide (LPS) and peptidoglycan and traverse the outer barrier of the microbe. The positively charged CPAs preferentially interact with the anionic phospholipids of the bacterial cell membrane rather than with the neutral mammalian cell membrane, which is made of zwitterionic phospholipids and cholesterol, thus leading to selective action [19–23].

Various models have been proposed to explain the mechanism of disruption of the bacterial cytoplasmic membrane by CPAs, of which the 'Barrel-stave model' and the 'Carpet model' have been widely accepted [24–27]. CPAs have many advantages, including their rapid action, on a variety of microorganisms such as bacteria, fungi and viruses. In addition, the development of resistance to membrane-active CPAs, whose sole target is the cytoplasmic membrane, is not expected because this would require substantial changes in the lipid composition of cell membranes of microorganisms, which they cannot afford. Thus, the unique mechanism of action makes CPA an ideal class of antibiotics, especially in application against resistant bacterial strains. However, there are some serious drawbacks associated with naturally occurring CPAs that limit their practical use. The major problem is their large size, which poses several challenges regarding synthesis, bioavailability, metabolic stability, immunogenicity, route of administration, and high production costs [28]. Also, being membrane active, CPAs are not completely devoid of side effects and exert hemolytic activity. These problems could be alleviated by designing and developing smaller synthetic CPAs, which hopefully would be also devoid of side effects.

Recently, Svendsen et al. reported the synthesis and evaluation of smaller CPAs composed of two to six natural and synthetic amino acids [29, 30]. The motive of the latter studies was to find the minimum requirement of hydrophilic and hydrophobic features that should be present in CPAs in order to exert antimicrobial activity. Basic amino acid residues (Arg and Lys) were used to impart PI features whereas HYD features were imparted by incorporation of various bulky residues (such as indole, substituted and unsubstituted Trp, and aryl rings). The results clearly demonstrated that it is possible to decrease the size of CPAs while retaining antimicrobial activity. From these studies, it was concluded that a minimum of two units of PI (hydrophilic) groups and two units of bulky (hydrophobic) groups are required to exhibit antimicrobial activity. All peptides also showed substantially less hemolytic activity and are thus more selective for prokaryotic cells. However, a few interesting issues arise from these experimental studies. For instance, many peptides fulfilling this minimum requirement were found to be less active or inactive for the same microorganism. This led us to believe that this minimum requirement may be an essential but not sufficient condition for activity. This is understandable as these features should be segregated at two opposite faces of the molecule in order to ensure the amphiphilic structure of the peptide that is important for the antimicrobial mechanism of action. Moreover, it was also observed that a few peptides found active against *Staphylococcus aureus* (Gram positive bacteria) were either inactive or less active against *Escherichia coli* (Gram negative bacteria). This may be due to differing minimum requirements of hydrophilic and hydrophobic features for the two organisms, and thus it would be interesting to analyze such differences among different types of microorganisms. Also, if this concept of minimum requirements is valid for CPAs then logically it should be pertinent to any other class of molecules possessing antimicrobial activity that employ the same mechanism of action.

To study these issues in detail, we decided to translate experimental observations into a computational model that would allow us to determine the minimum requirements for hydrophilic and hydrophobic features within this series of CPAs. If the model shows segregation of PI and HYD features on two opposite faces and is able to predict the activity of test set molecules, it can be considered as valid. However, quantitative estimation of activity in this case was not possible, as the biological data is given as MIC and not as exact IC_{50} values. Thus, it was decided to employ the HipHop module [31] of the software package CATALYST (Accelrys, San Diego, CA), which identifies a set of common chemical features shared by the given set of molecules and their relative alignments to these features, without considering biological activity. Since PI and HYD are the only two important features for antimicrobial

activity, and there is no particular receptor or protein target for CPAs, analysis was restricted to include only PI and HYD features for pharmacophore generation. The dataset was divided into two categories of 'actives' and 'inactives' defined on the basis of their MICs against the organisms *E. coli* and *S. aureus*. In order to study the differences in the minimum requirements for these two organisms, two different hypotheses (or pharmacophores) were generated using 'active' peptides against these two organisms as the training set. To the best of our knowledge such an approach to pharmacophore model generation for membrane active molecules has not previously been reported.

To further examine the proposed models, we selected another class of facial amphiphiles based on cholic acid, which provides three derivatizable -OH groups on the same face. The potential of these compounds as facial amphiphiles was initially recognized by Kahne and coworkers [32], while Savage and coworkers [33–39] have exploited their use as antimicrobials and membrane sensitizers. These are generally known as cationic steroid antibiotics (CSAs), and are found to be active against both Gram negative and Gram positive bacteria. Hence, plausibly, the requirement for minimum PI and HYD features should be the same for both classes of molecules, and a good model should be able to differentiate between active and inactive CPAs and CSAs.

Pharmacophore generation methodology

All computational analyses were conducted on a Silicon Graphics Octane 2 workstation using an IRIX 6.5 operating system. The HipHop module of CATALYST 4.10 [40] was used to generate a set of hypotheses. The dataset was adopted from the work of Svendsen et al. [29, 30], in which they reported a variety of cationic peptides possessing antimicrobial activities against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). For the purpose of pharmacophore development, peptides having MIC≤50 μg/mL were considered as 'actives' while peptides having MICs≥ 200 μg/mL were considered as 'inactives'. The 3-D pharmacophores for *E. coli* and for *S. aureus* were constructed using 11 and 29 active peptides, respectively (Table 1, Fig. 1). Peptide selection comprised structural diversity as representatives of dipeptides, tripeptides, tetrapeptides, pentapeptides and hexapeptides with a variety of unnatural amino acids and different N- and C-terminal groups were included (Table 2). All chemical structures were constructed using the 2D/3D editor of CATALYST 4.10 software. Conformational space for all peptides was explored using CHARMM force fields [41], implemented in the software, and a constraint of 20 kcal mol^{-1} energy threshold above the estimated global minima was used for considering conformers. The 'best conformation genera-

Table 1 Sequence and biological activities of cationic peptide antibiotics (CPAs) reported in [28] and [29]

CPA	Sequence	MIC	
		Escherichia coli (ATCC 25922)	*Staphylococcus aureus* (ATCC 25923)
1	KF-OBzl	>200	>200
2	WR-Obzl	>200	200
3	KW-Obzl	>200	50
4	rW-Obzl	>200	25
5	RF-Obzl	>200	200
6	wrw-Obzl	75	5
7	WRW-OBzl	75	5
8	WRW-NH_2	>200	200
9	ChaR-OBzl	>300	100
10	BipR-OBzl	150	5
11	BipR-OBzlPh	50	5
12	WR-NH-Bzl	>300	100
13	AthR-NHBzl	150	20
14	K(COO)w-OBzl	150	50
15	K(COO)W-OBzl	150	50
16	RW-OBzl	>200	15
17	TbtR-NHBzl	10	2.5
18	TbtR-NH_2	>150	15
19	AR-OBzlPh	300	100
20	wRW-OBzl	75	20
21	kW-OBzl	>200	75
22	WWR-OMe	>200	>200
23	Rw-OBzl	>200	50
24	RWR-NH_2	>200	>200
25	WR-OMe	>200	>200
26	Kw-OBzl	>200	75
27	rw-OBzl	>200	50
28	FtbR-OBzl	200	25
29	BipR-OMe	>300	150
30	BipR-NHBzl	>50	15
31	FR-OBzlPh	100	12.5
32	TbtR-OMe	30	5
33	TbtR-NHiPr	50	7.5
34	FR-OBzl	>200	>200
35	K(CH_2NH)W-OBzl	>300	150
36	GtbR-OBzl	>300	>300
37	AtbR-OBzl	>300	>300
38	WRWR-OMe	>200	>200
39	RWRW-OBzl	50	2.5
40	RWrw-OBzl	75	5
41	RWWR-OMe	>200	75
42	RWWR-NH_2	>200	100
43	WRWR-NH_2	>200	200
44	WRRW-NH_2	>200	200
45	WRWRWR-NH_2	10	7.5
46	RWRWRW-NH_2	5	5
47	WRWRW-NH_2	15	10
48	RWRWR-NH_2	200	25
49	WRWRY-NH_2	100	50
50	Ftb-OMe	>300	>300
51	RRRWWW-NH_2	5	5
52	RWWWRR-NH_2	5	5
53	WWRRRW-NH_2	25	10
54	WRYRW-NH_2	100	50

MIC Minimum inhibitory concentration (μg/mL)

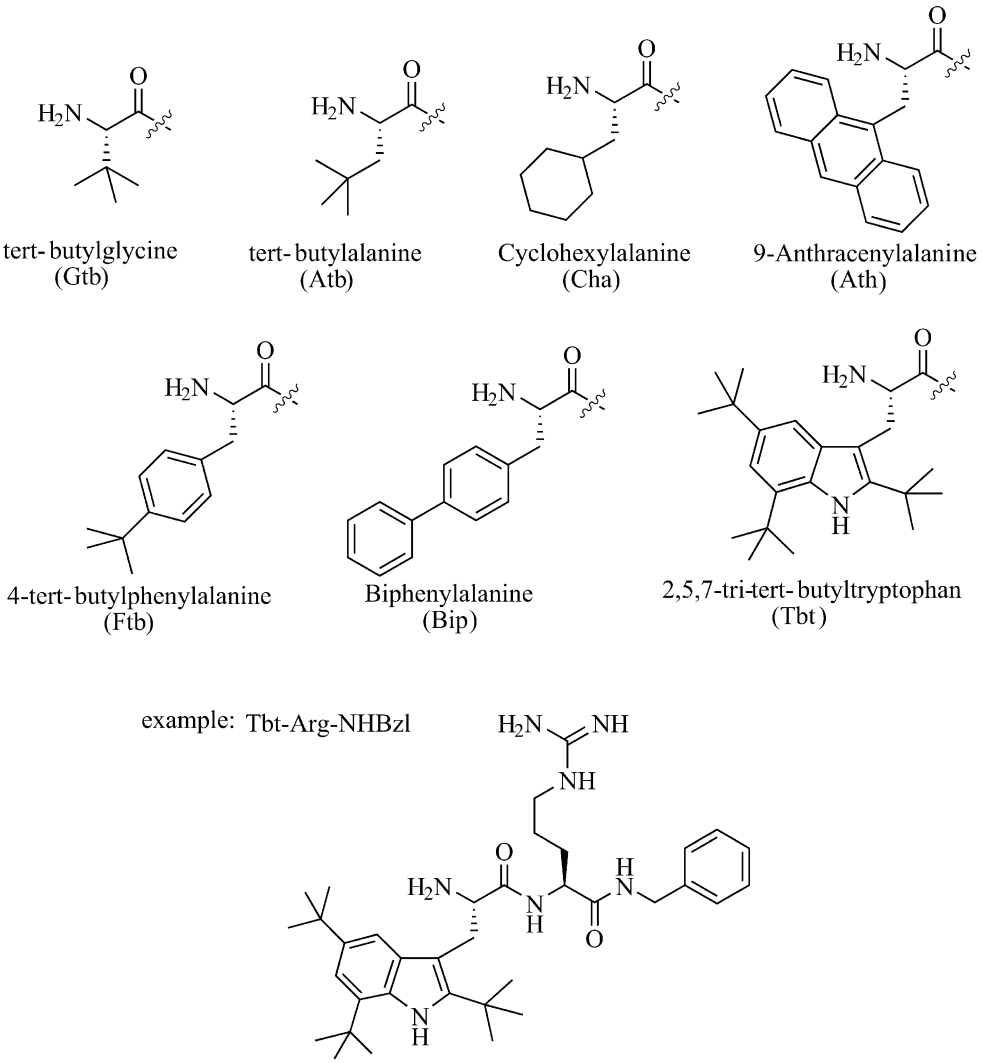

Fig. 1 Structures of various unnatural amino acids incorporated in the cationic peptide antibiotics (CPAs) used for pharmacophore generation

tion' option within CATALYST, which utilizes the poling algorithm [42–44], was employed as a method to generate conformational models, and a maximum of 250 conformers for each molecule was generated to ensure maximum coverage of the conformational space. Among the dataset, peptides with MIC≤5 μg/mL (Table 2) were selected as reference molecules, with Principal=2 and MaxOmitFeat (MOF) = 0, whereas Principal=1 and MOF=1 were used for the remaining peptides. Only PI and HYD features were selected for hypothesis generation, as only these features are critical for exhibiting biological activity. Furthermore, PI definition was customized to include also pyridyl and imidazolyl rings. In the case of *S. aureus*, a minimum of one and a maximum of five PI and HYD features were demanded to generate hypotheses, while in case of *E. coli* a minimum of zero and a maximum of five such features were requested. Default values were used for various other parameters (such as Spacing, Misses, CompleteMisses, Minipoints and MiniSubsetPoints) in the advanced options.

Mapping of compounds onto a particular hypothesis was done using the Compare/Fit command within the CATALYST program. There are two options of fitting a molecule to the hypothesis, namely the 'Best fit' method and the 'Fast Fit' method. The 'Fast Fit' method finds the optimum conformer from the already generated conformation space that fits the hypothesis, while the 'Best Fit' method manipulates the first 100 conformers within the specified energy threshold to minimize the distance between the hypothesis and mapped atoms in the molecules. The 'Best Fit' method was used to map all the peptides in this study. Furthermore, a molecule can either be forced to fit all the features using MOF=0 in the Compare/Fit command or it can be given freedom to miss one or two features by selecting MOF=1 or MOF=2, respectively. This allows one to identify a feature of the hypothesis that may be present but is not important for activity, in case a highly active molecule lacks that particular feature. The program maps the particular compound onto the hypothesis accordingly using the conformation space and calculates the 'fit value'. The higher the fit

Table 2 CPAs active against *E. coli* and *S. aureus*, used as the training set for model development, together with the fit values against the hypothesis Ec-2 and Sa-6, respectively

CPA	Sequence	MIC[a]	Confs[b]	Principal[c]	MaxOmitFeat[d]	BestFit-0[e] (ΔE)[f]
E. coli (ATCC 25922)						
11	BipR-OBzlPh	50	251	1	1	NM[g]
17	TbtR-NHBzl	10	251	1	1	3.79 (8.00)
32	TbtR-OMe	30	251	1	1	3.21 (18.2)
33	TbtR-NHiPr	50	251	1	1	4.83 (8.7)
39	RWRW-OBzl	50	251	1	1	5.74 (11.8)
45	WRWRWR-NH_2	10	250	1	1	5.19 (7.9)
46	RWRWRW-NH_2	5	250	2	0	4.55 (11.2)
47	WRWRW-NH_2	15	250	1	1	4.90 (10.3)
51	RRRWWW-NH_2	5	236	2	0	6.00 (7.00)
52	RWWWRR-NH_2	25	241	1	1	4.47 (18.8)
53	WWRRRW-NH_2	25	251	1	1	4.35 (13.8)
						Avg=4.70
S. aureus (ATCC 25923)						
3	KW-OBzl	50	251	1	1	4.57 (0.0)
4	rW-OBzl	25	251	1	1	4.70 (15.6)
6	wrw-OBzl	5	251	2	0	3.88 (13.5)
7	WRW-OBzl	5	251	2	0	3.96 (10.7)
10	BipR-OBzl	5	251	2	0	2.57 (18.8)
11	BipR-OBzlPh	5	251	2	0	4.02 (5.93)
13	AthR-NHBzl	20	251	1	1	3.31 (18.2)
16	RW-OBzl	15	251	1	1	4.55 (18.5)
17	TbtR-NHBzl	2.5	251	2	0	3.61 (12.4)
18	TbtR-NH_2	15	251	1	1	3.49 (10.8)
20	wRW-OBzl	20	251	1	1	3.64 (8.1)
23	Rw-OBzl	50	251	1	1	4.34 (5.8)
27	Kw-OBzl	50	251	1	1	3.96 (5.1)
28	rw-OBzl	25	251	1	1	3.51 (19.4)
30	BipR-NHBzl	15	251	1	1	3.56 (12.3)
31	FR-OBzlPh	12.5	251	1	1	2.31 (9.3)
32	TbtR-OMe	5	251	2	0	3.53 (17.2)
33	TbtR-NHiPr	7.5	251	1	1	3.69 (17.1)
39	RWRW-OBzl	2.5	251	2	0	4.84 (13.3)
40	RWrw-OBzl	5	251	2	0	5.00 (10.1)
45	WRWRWR-NH_2	7.5	250	1	1	4.89 (16.9)
46	RWRWRW-NH_2	5	250	2	0	4.08 (3.8)
47	WRWRW-NH_2	10	250	1	1	3.77 (14.1)
48	RWRWR-NH_2	25	251	1	1	4.70 (4.6)
49	WRWRY-NH_2	50	251	1	1	4.72 (19.6)
51	RRRWWW-NH_2	5	236	2	0	4.24 (19.7)
52	RWWWRR-NH_2	5	241	2	0	4.85 (17.6)
53	WWRRRW-NH_2	10	251	1	1	4.79 (5.4)
54	WRYRW-NH_2	50	212	1	1	2.70 (17.4)
						Avg=3.99

[a] As described in Table 1

[b] Total number of conformations generated for a compound using the 'best conformation generation' option of CATALYST program

[c] Prinicipal=1 means that this compound must map onto the hypothesis generated by the search procedure. Partial mapping is allowed. Principal=2 means that this is the reference compound and chemical features of such a compound must be used to define the initial set of potential hypotheses

[d] MaxOmitFeat=1 means that one feature of the compound may not be mapped on the hypothesis. MaxOmitFeat=0 means that all the features of a compound must map to the hypothesis

[e] This value was generated using MaxOmitFeat = 0 in the 'Compare/Fit' command of CATALYST, and means that the molecule is forced to map all the features. The greater the best fit value, the better the molecule fits to the hypothesis

[f] Energy difference between the best fit conformer and the estimated global minima for the same molecule

[g] No mapping possible

value, the better the compound maps to the given hypothesis. In this case, mapping was done using the 'Best Fit' option with MOF=0 and MOF=1, leading to two different or identical fit values BestFit-0 and BestFit-1, respectively.

Results and discussion

For short cationic peptides (data shown in Table 1), Svendsen and coworkers reported that the requirement for positive charge is fulfilled by the guanidino group of the arginine (R) residue and the free amino group at the N-terminus, while the indole ring of tryptophan (W) and other aromatic rings fulfill the criteria for hydrophobic or bulky groups [29, 30]. In addition, it was also mentioned by the authors that the two units of bulky (hydrophobic) groups may actually have more than two hydrophobic point features. For instance, the indole ring of Trp was counted as one bulky unit but has two aromatic rings as two hydrophobic point features and can be compared to the biphenyl moiety. However, there is no mention about the arrangement of these features in space although it is well documented that an amphiphilic structure is important [30]. Also, the peptides WRWR-NH_2 and WRRW-NH_2 (43 and

44 in Table 1, respectively) both have two Arg and two Trp residues, which fulfills the minimum requirement for two PI and two bulky groups as reported by the authors, but show contradicting high MIC values against both organisms (200 μg/mL against *S. aureus* and >200 μg/mL against *E. coli*, Table 1) [29].

It is known that facial amphiphilicity plays an important role in the mechanism of action of CPAs and is important for their antimicrobial activity. Thus, it is expected that, in the active peptide, PI and HYD features should be arranged in such a way that an amphiphilic structure can be attained, while an inactive peptide might not be able to do so despite fulfilling the minimum requirements. The experimental study also showed that most of the highly active peptides against *S. aureus* were only moderately active or inactive against *E. coli*. This is expected, given the fact that these organisms belong to different classes (one is Gram positive and the other Gram negative) of bacteria, leading to inherent differences between their cell membranes—the site of action of these CPAs. Thus, the minimum requirement of hydrophobic and hydrophilic features may be different for these organisms. Hence, we also aimed to develop separate hypotheses for both *E. coli* and *S. aureus* and to validate these hypotheses with the help of reported activity data.

The HipHop module of CATALYST was employed for pharmacophore (hypothesis) generation, which yielded the top ten hypotheses with the corresponding chemical features and scores (Table 3). The higher the score, the higher the significance of the hypothesis, and the lower the possibility of chance correlation. The top hypothesis for *E. coli* (Ec-1) scored 172.25, while the last hypothesis (Ec-10) scored 152.49. In the case of *S. aureus*, the corresponding values were 409.07 (Sa-1) and 398.67 (Sa-10) as shown in Table 3. For *S. aureus*, all hypotheses showed the presence of three HYD and two PI features. This was also the case with *E. coli*, with the exception of the top two hypotheses (Ec-1 and Ec-2), which showed the presence four HYD features, and the last hypothesis (Ec-10), which showed the presence of only one PI feature. However, it should be noted that all of these hypotheses may not be relevant and one has to identify a valid hypothesis among those generated. We followed three criteria for selecting a relevant hypothesis in both cases: (1) the PI and HYD features of the hypothesis should be at two opposite faces giving amphiphilic structure, since this is the

Table 3 Details of the top ten hypotheses generated against *E. coli* and *S. aureus*

Hypothesis	Features	Ranking score[a]	Direct hit (DH) and partial hit (PH)[b]
E. coli (ATCC 25922)			
Ec-1	2xPI, 4xHYD	172.25	DH: 11111111111 PH: 00000000000
Ec-2	2xPI, 4xHYD	161.99	DH: 11111111110 PH: 00000000001
Ec-3	2xPI, 3xHYD	157.80	DH: 11111111111 PH: 00000000000
Ec-4	2xPI, 3xHYD	156.28	DH: 11111111111 PH: 00000000000
Ec-5	2xPI, 3xHYD	156.23	DH: 11111111111 PH: 00000000000
Ec-6	2xPI, 3xHYD	153.93	DH: 11111111111 PH: 00000000000
Ec-7	2xPI, 3xHYD	153.67	DH: 11111111110 PH: 00000000001
Ec-8	2xPI, 3xHYD	153.65	DH: 11111111111 PH: 00000000000
Ec-9	2xPI, 3xHYD	152.61	DH: 11111111110 PH: 00000000001
Ec-10	1xPI, 4xHYD	152.49	DH: 11111111110 PH: 00000000001
S. aureus (ATCC 25923)			
Sa-1	2xPI, 3xHYD	409.07	DH: 10111111111111111111111111111111 PH: 01000000000000000000000000000000
Sa-2	2xPI, 3xHYD	406.39	DH: 11111111111111111111111111111111 PH: 00000000000000000000000000000000
Sa-3	2xPI, 3xHYD	403.28	DH: 11111111110111111111111111111111 PH: 00000000001000000000000000000000
Sa-4	2xPI, 3xHYD	403.02	DH: 11111111111011111111111111111111 PH: 00000000000100000000000000000000
Sa-5	2xPI, 3xHYD	402.20	DH: 11111111111111111111111111111111 PH: 00000000000000000000000000000000
Sa-6	2xPI, 3xHYD	401.40	DH: 10111111111111111111111111111111 PH: 01000000000000000000000000000000
Sa-7	2xPI, 3xHYD	400.30	DH: 11111111111111111111111111111111 PH: 00000000000000000000000000000000
Sa-8	2xPI, 3xHYD	399.82	DH: 11111111111111111111111111111111 PH: 00000000000000000000000000000000
Sa-9	2xPI, 3xHYD	399.80	DH: 11111111111111111111111111111111 PH: 00000000000000000000000000000000
Sa-10	2xPI, 3xHYD	398.67	DH: 11111111110111111111111111111111 PH: 00000000001000000000000000000000

PI Positively ionizable, *HYD* hydrophobic group

[a] The higher the ranking score, the lower the probability of chance correlation. The best hypothesis shows the highest value

[b] Each number in the DH and PH rows corresponds to a molecule used to generate hypothesis (in the same order from right to left as shown in Table 2). DH and PH indicates whether (1) or not (0) a molecule mapped to every feature or all but one feature in the hypothesis, respectively

necessary condition for the activity, (2) if more than one hypothesis fulfills the above criteria, the one with the higher rank should be selected, and (3) inactive peptides should either map the hypothesis poorly or lack one or more features of the hypothesis. Consequently, Ec-2 and Sa-6 were found to match all the above mentioned criteria, and further detailed mapping analysis was carried out using these two hypotheses.

Mapping analysis of CPAs with hypothesis Ec-2

The 'BestFit' values of all the training set molecules for *E. coli* with hypothesis Ec-2, which has two PI features and four HYD features, are given in Table 2. These features are arranged on two opposite faces, thus providing the facial amphiphilicity required for activity. With the exception of peptide 11, all molecules mapped quite well to all the features of the hypothesis, with fit values ranging from 3.21 to 6.00 and an average fit value of 4.70. As expected, the two PI features were mapped by either a free amino group and a guanidino group or both guanidino groups. The mapping of an active CPA (33) with Ec-2 is shown in Fig. 2a, which demonstrates the separation of the PI and HYD features on the two opposite faces together with the distance tolerances among these features. One guanidino group and one free -NH_2 group mapped to the two PI features. The three HYD features were mapped by the substituted indole ring and one by the *i*Pr group at the C-terminus.

Interestingly, the majority of the inactive peptides (MIC≥200 μg/mL) ,when forced to map to all the features (BestFit-0) of hypothesis Ec-2, did not show any mapping (Table 4). Out of the thirty inactive peptides, mapping was not possible with twenty-four, while two of the peptides (22 and 43) showed poor 'BestFit-0' values of 0.52 and 1.62, respectively. Only pentapeptide 48 showed a fit value greater than the average fit value (4.70) of the active peptides. When inactive peptides were provided with the freedom of missing one feature of the hypothesis (MOF=1), all the inactive peptides could also map to the hypothesis except 1, 5, 9, 19, 34, 36 and 37. Although the fit values (BestFit-1, Table 4) improved, the compounds lacked either the PI or HYD feature of the hypothesis. For example, peptide 2 lacked one of the two essential PI features of Ec-2 as shown in Fig. 2b. These results indicate that, for activity against *E. coli*, a peptide should fit all the features of Ec-2 in the given relative position in space with high BestFit-0 values. On the other hand, an inactive peptide does not fulfill this criteria and fits the hypothesis either poorly or partially by missing an important feature of Ec-2. Thus, the developed hypothesis is able to differentiate between active and inactive small CPAs in the given series. However, compounds 38, 41, 42 and 48, which are shown to be inactive experimentally, were found to possess minimum features of Ec-2 and mapped well to the hypothesis. These exceptional results may be due to some other factors, such as self-aggregation of the peptides, leading to a decrease in effective concentration, or these peptides may not be able to attain the required conformation under the given conditions.

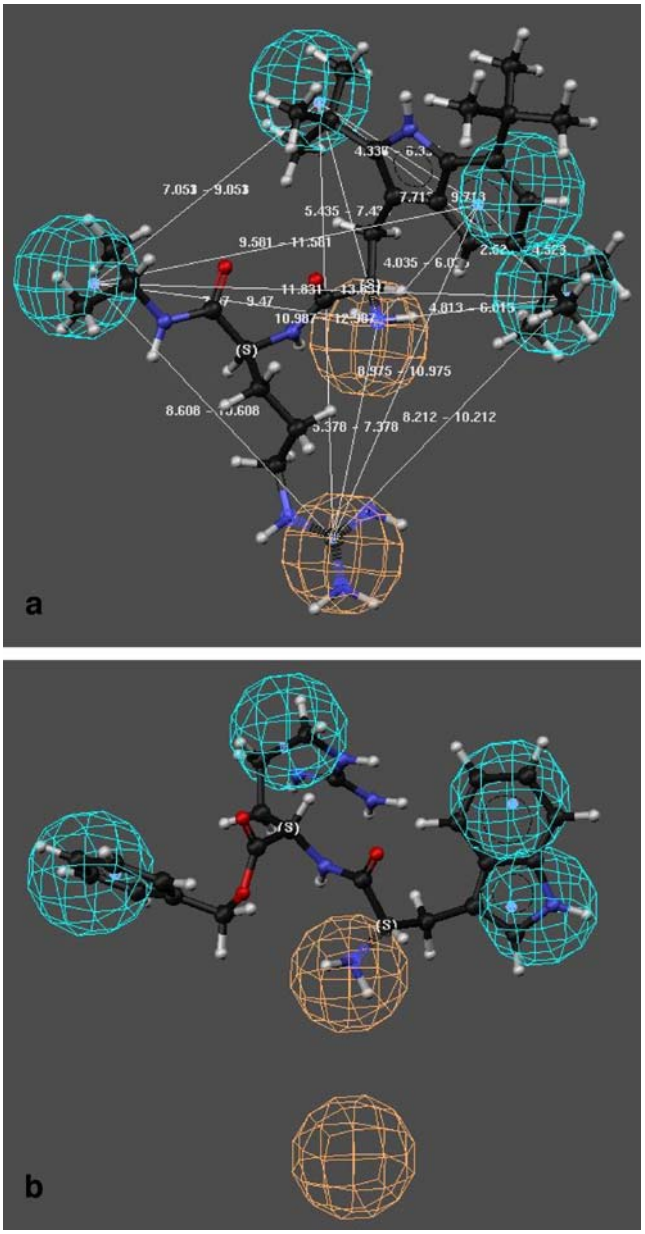

Fig. 2 Example of (**a**) active and (**b**) inactive CPAs against *Escherichia coli* mapped to hypothesis Ec-2. *Cyan-* and *brown-colored spheres* represent HYD and PI features, respectively

Mapping analysis of CPAs with hypothesis Sa-6

Similar to Ec-2, hypothesis Sa-6 shows that, in the case of *S. aureus*, a minimum of two PI features are required, while

Table 4 Inactive CPAs against *E. coli* and *S. aureus*, together with best-fit values against hypotheses Ec-2 and Sa-6

CPA	Sequence	MIC[a]	BestFit-0[b] (ΔE)[c]	BestFit-1[d] (ΔE) [c]
E. coli (ATCC 25922)				
1	KF-OBzl	>200	NM[e]	NM
2	WR-OBzl	>200	NM	4.37 (10.8)
3	KW-OBzl	>200	NM	4.81 (2.6)
4	rW-OBzl	>200	NM	4.88 (19.2)
5	RF-OBzl	>200	NM	NM
8	WRW-NH_2	>200	NM	4.58 (9.1)
9	ChaR-OBzl	>300	NM	NM
12	WR-NH-Bzl	>300	NM	4.69 (13.0)
16	RW-OBzl	>200	NM	4.37 (17.7)
19	AR-OBzlPh	300	NM	NM
21	kW-OBzl	>200	NM	4.57 (4.8)
22	WWR-OMe	>200	0.52 (14.1)	4.78 (9.6)
23	Rw-OBzl	>200	NM	4.87 (7.0)
24	RWR-NH_2	>200	NM	4.64 (18.6)
25	WR-OMe	>200	NM	3.36 (11.9)
26	Kw-OBzl	>200	NM	4.66 (6.67)
27	rw-OBzl	>200	NM	4.82 (8.1)
28	FtbR-OBzl	200	NM	3.68 (11.0)
29	BipR-OMe	>300	NM	1.87 (8.2)
34	FR-OBzl	>200	NM	NM
35	K(CH_2NH) W-OBzl	>300	NM	4.90 (11.8)
36	GtbR-OBzl	>300	NM	NM
37	AtbR-OBzl	>300	NM	NM
38	WRWR-OMe	>200	3.83 (10.1)	4.93 (10.0)
41	RWWR-OMe	>200	5.6 (14.7)	5.69 (14.7)
42	RWWR-NH_2	>200	4.20 (9.15)	4.90 (8.1)
43	WRWR-NH_2	>200	1.62 (17.9)	4.83 (11.0)
44	WRRW-NH_2	>200	NM	4.95 (10.4)
48	RWRWR-NH_2	200	NM	5.44 (10.6)
50	Ftb-OMe	>300	NM	2.42 (10.6)
S. aureus (ATCC 25923)				
1	KF-OBzl	>200	NM	3.87 (13.6)
2	WR-OBzl	200	NM	3.70 (14.2)
5	RF-OBzl	200	NM	3.90 (5.6)
8	WRW-NH2	200	3.20 (13.0)	4.00 (4.5)
22	WWR-OMe	>200	2.47 (11.1)	3.99 (9.9)
24	RWR-NH_2	>200	0.98 (10.2)	3.99 (7.8)
25	WR-OMe	>200	0.90 (9.3)	3.63 (17.5)
34	FR-OBzl	>200	NM	3.71 (1.9)
36	GtbR-OBzl	>300	NM	2.56 (9.3)
37	AtbR-OBzl	>300	NM	3.49 (2.3)
38	WRWR-OMe	>200	3.82 (13.6)	3.99 (14.9)
43	WRWR-NH2	200	3.32 (12.9)	3.98 (14.9)
44	WRRW-NH2	200	2.89 (8.45)	4.00 (15.9)
50	FtbR-OMe	>300	1.80 (19.6)	3.68 (9.5)

[a] As described in Table 1

[b, c] As described in Table 2

[d] This value was generated using MaxOmitFeat = 1 in the 'Compare/Fit' command of CATALYST, and means that the molecule was allowed to miss only one feature. The greater the best fit value, the better the molecule fits the hypothesis

[e] No mapping possible

only three HYD point features, arranged closely in space, are required. In this case, the PI and HYD features are also arranged to form an amphiphilic structure. All the peptides map well to the hypothesis, with an average best fit value of 3.99 (Table 2), except peptides 10, 31 and 54, for which this value is less than 3.00. The mapping of an active CPA (3), against Sa-6 is shown in Fig. 3a together with the distance tolerances among various features of Sa-6. Again, the two PI features are mapped by one guanidino and one

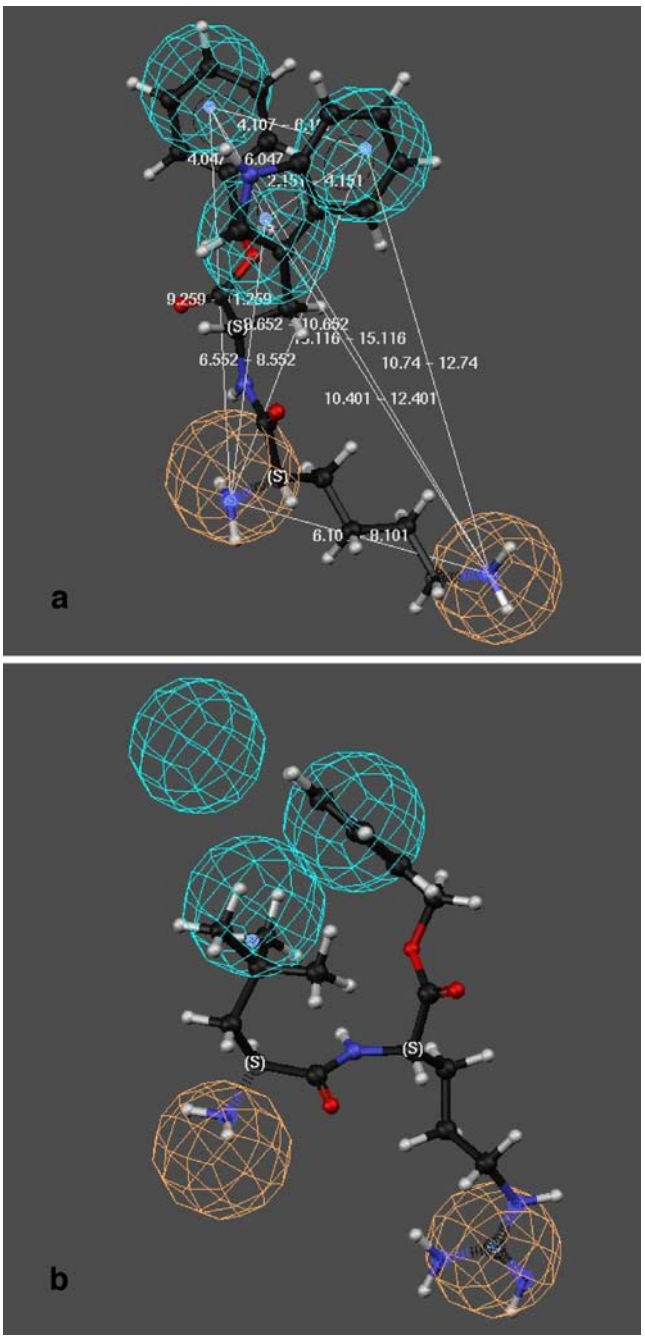

Fig. 3 Example of (**a**) active and (**b**) inactive CPA against *S. aureus* mapped to hypothesis Sa-6. *Cyan-* and *brown-colored spheres* represent HYD and PI features, respectively

Table 5 Cationic steroid antibiotics (CSAs) (Fig. 4) evaluated against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923), together with fit values against hypotheses Ec-2 and Sa-6

CSA	MIC[a]		BestFit-0[b](ΔE)[c]		BestFit-1[d](ΔE)[c]	
	E.coli	*S. aureus*	*E.coli*	*S. aureus*	*E.coli*	*S. aureus*
55	22 (20)	3.1	1.80 (5.1)	4.05 (11.5)	4.77 (11.8)	4.05 (11.5)
56	5.1 (7.0)	1.0	1.21 (14.7)	3.83 (5.8)	4.80 (8.7)	3.96 (14.5)
57	1.4 (2.0)	0.6	4.31 (15.1)	4.48 (12.8)	4.80 (0.0)	4.48 (12.8)
58	80 (140)	8.6	NM[e]	1.83 (14.1)	NM	3.71 (14.2)
59	36 (36)	2.0	NM	0.64 (12.2)	3.58 (10.3)	3.77 (6.9)
60	6.6 (11)	0.55	0.41 (17.2)	3.86 (14.2)	4.77 (2.3)	3.94 (6.6)
61	3.0 (1.5)	0.40	3.01 (4.4)	3.38 (16.3)	4.77 (6.2)	3.97 (16.3)
62	0.31 (1.0)	0.59	2.03 (12.5)	4.44 (18.0)	4.78 (10.2)	4.44 (18.0)
63	1.0	1.8	4.98 (9.6)	4.12 (13.3)	4.98 (9.6)	4.12 (13.3)
64	7.0	4.0	3.68 (10.6)	3.34 (19.2)	4.79 (19.6)	3.97 (19.2)
65	3.5	1.2	5.68 (8.8)	4.30 (8.8)	5.68 (8.8)	4.30 (8.8)
66	60	15	NM	0.12 (16.6)	4.51 (15.5)	3.85 (15.3)
67	30	11	NM	2.80 (9.5)	4.70 (17.1)	3.97 (18.0)
68	23	14	NM	2.04 (15.0)	4.79 (1.3)	3.99 (8.8)
69	>100	>100	NM	NM	1.53 (17.7)	3.75 (13.9)
70	6.6	4.6	NM	4.05 (11.3)	4.82 (8.5)	4.05 (11.3)
71	7.3	2.0	NM	3.13 (14.3)	4.55 (9.3)	3.99 (10.7)

[a] As described in Table 1. Minimum inhibitory concentrations against *E.Coli* (ATCC 10798) are given in parenthesis
[b] As described in Table 2
[c, d] As described in Table 4
[e] No mapping possible

free -NH_2 group, while the HYD features are mapped by the indole ring and the phenyl moiety at the C-terminus.

Similarly, mapping analysis of the inactive peptides was also carried out with Sa-6 (summarized in Table 4). Of the fourteen inactive peptides (MIC≥200 μg/mL), six did not map at all to the hypothesis when forced to map to all the features (BestFit-0, Table 4). Peptides 24 and 25 mapped to all the features but with very poor fit values of less than 1.0, while peptides 22, 44 and 50 also produced best fit values less than the average best fit value (3.99) of the active peptides. An improvement in fit values was observed when these inactive peptides were allowed to miss one feature of the hypothesis (BestFit-1, Table 4). For instance, the inactive CPA 37 missed one HYD feature of the hypothesis Sa-6 (Fig. 3b). Thus, as in the case of *E. coli*, all the peptides inactive against *S. aureus* also fit either poorly or partially to the computational hypothesis Sa-6. Therefore, the given hypothesis Sa-6 is in accordance with the experimental observations of Svendsen and coworkers [29, 30] that two units of a bulky group is a minimum requirement for high antimicrobial activity against *S. aureus*. However, the computational hypothesis (Sa-6) shows that the two bulky groups should actually have a total of three HYD point features. The mapping analysis has also shown that these HYD features may be mapped by two bulky groups or even a single bulky group such as a substituted indole ring. This hypothesis also explains why the stereochemistry or sequence of the constituent amino acids may not be important as long as the overall peptide structure can adopt an amphiphilic structure as predicted by the hypothesis. For example, peptides 6 (wrw-OBzl) and 7 (WRW-OBzl), which have identical sequences but opposite configuration at the chiral centers of the amino acids, exhibited high activity against *S. arueus* (MIC=5 μg/mL) and are also able to map to all the features of the hypothesis with comparable best fit values of 3.88 and 3.96, respectively. Experimentally, it has also been reported that smaller peptides with closely positioned bulky groups are more active than those having distantly situated bulky groups. For example, peptide 16 (RW-OBzl; MIC=15 μg/mL), where Trp and benzyl groups are close together, is more active than peptide 2 (WR-OBzl; MIC=200 μg/mL), which has the opposite sequence. Hypothesis Sa-6 also shows that all three

Table 6 CSAs evaluated against a different strain of *E. coli* (ATCC 10798)

CSA	MIC[a] *E. coli* (ATCC 10798)	BestFit-0[b] (ΔE)[c]
72	85	NM[d]
73	80	NM
74	85	1.41 (15.6)
75	70	NM
76	>100	NM
77	>100	NM
78	85	NM
79	80	NM
80	100	NM
81	5.0	NM
82	140	NM
83	70	NM
84	70	NM
85	28	NM
86	3.0	4.94 (10.0)
87	3.0	3.02 (11.4)
88	12	NM

[a] As defined in Table 1
[b,c] As defined in Table 2
[d] No mapping possible

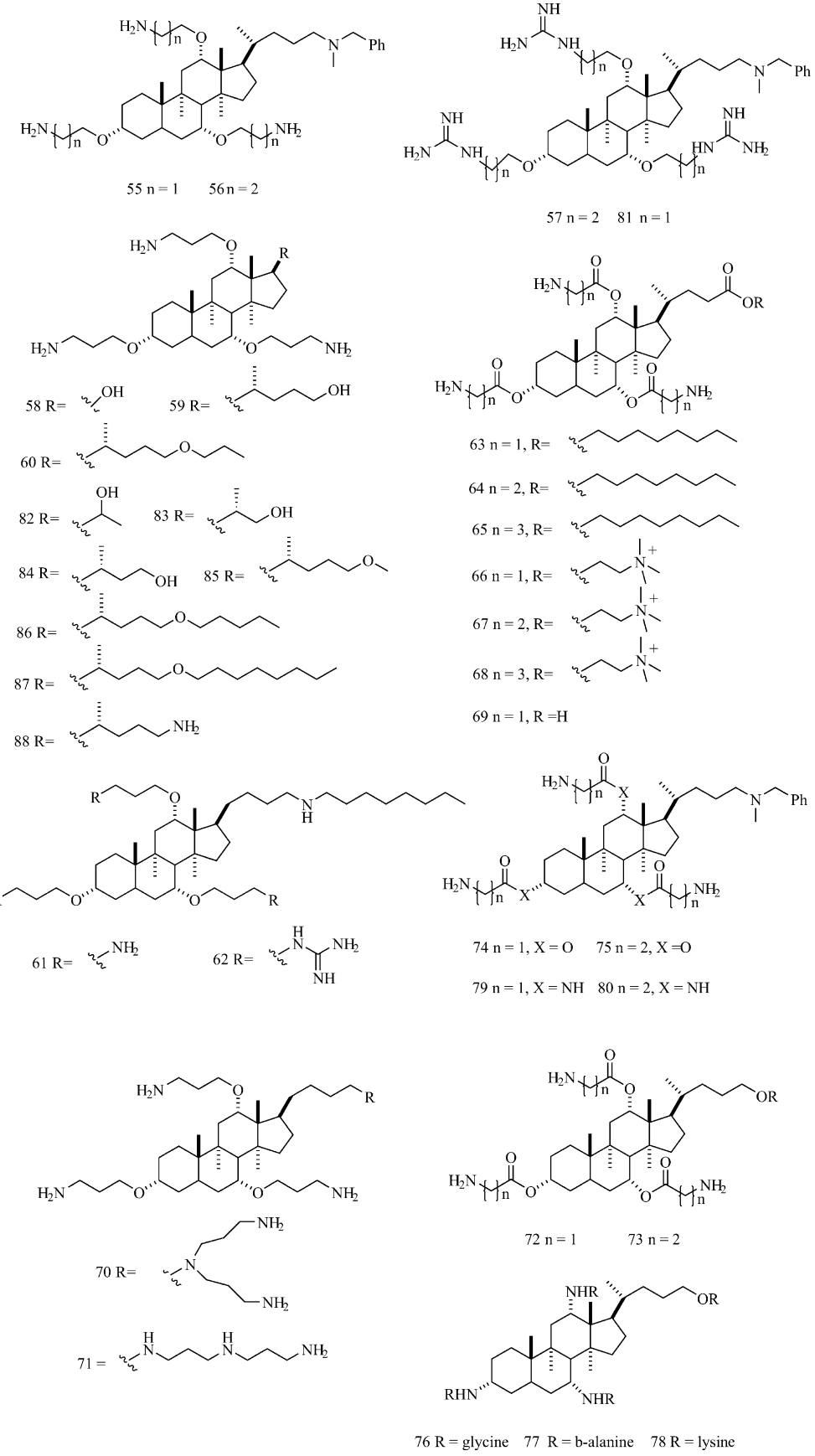

Fig. 4 Structures of the cationic steroid antibiotics (CSAs) used for the validation of pharmacophore models Ec-2 and Sa-6

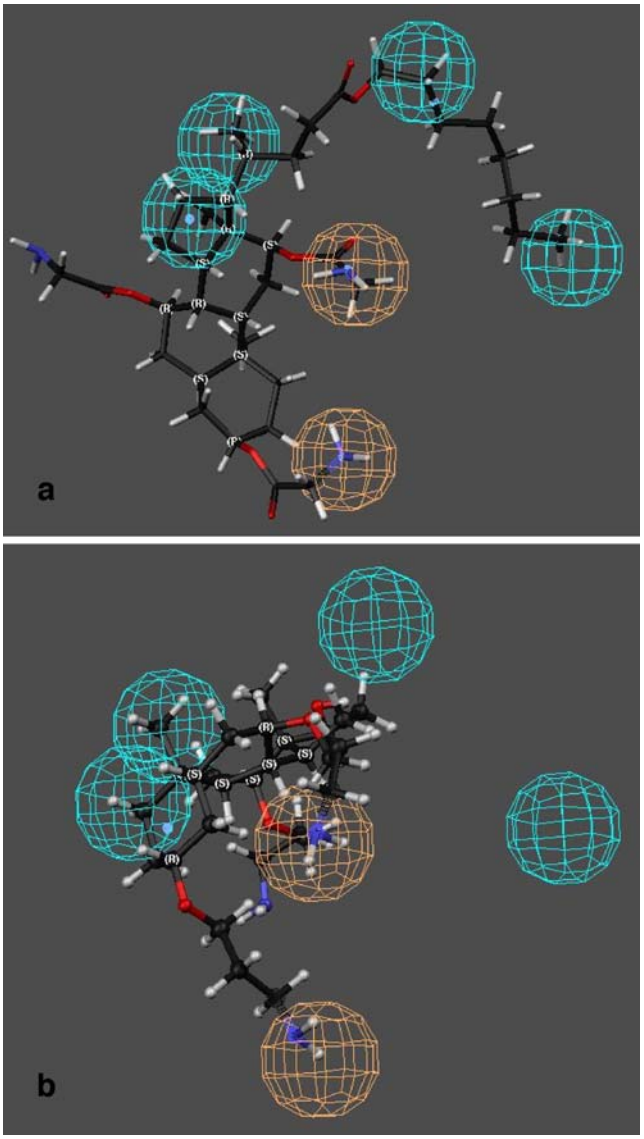

Fig. 5 Example of (**a**) active and (**b**) inactive CSAs against *E. coli* mapped to hypothesis Ec-2. *Cyan*- and *brown-colored spheres* represent HYD and PI features, respectively

HYD groups should be located in close proximity in order to form the amphiphilic structure required for activity. Peptide 16 fulfills this requirement with its closely situated indole and benzyl groups at the C-terminus, whereas peptide 2 failed to map to all features despite containing all the required features in the hypothesis. Hence, the computationally generated hypothesis Sa-6 not only suggests the minimum requirement of hydrophobic and hydrophilic features for activity against *S. aureus*, but also maintains that a particular arrangement in space should be attained for activity.

Validation with cationic steroidal antimicrobials

The computational hypotheses Ec-2 and Sa-6 were further validated on a different class of antimicrobial molecules: cationic steroid antibiotics (CSAs). This class of molecules is also known to possess facial amphiphilicity and to act by the same mechanism as that exhibited by CPAs [33, 39]. Initially, it was decided to select from the literature only those CSAs that had been tested against the same strains

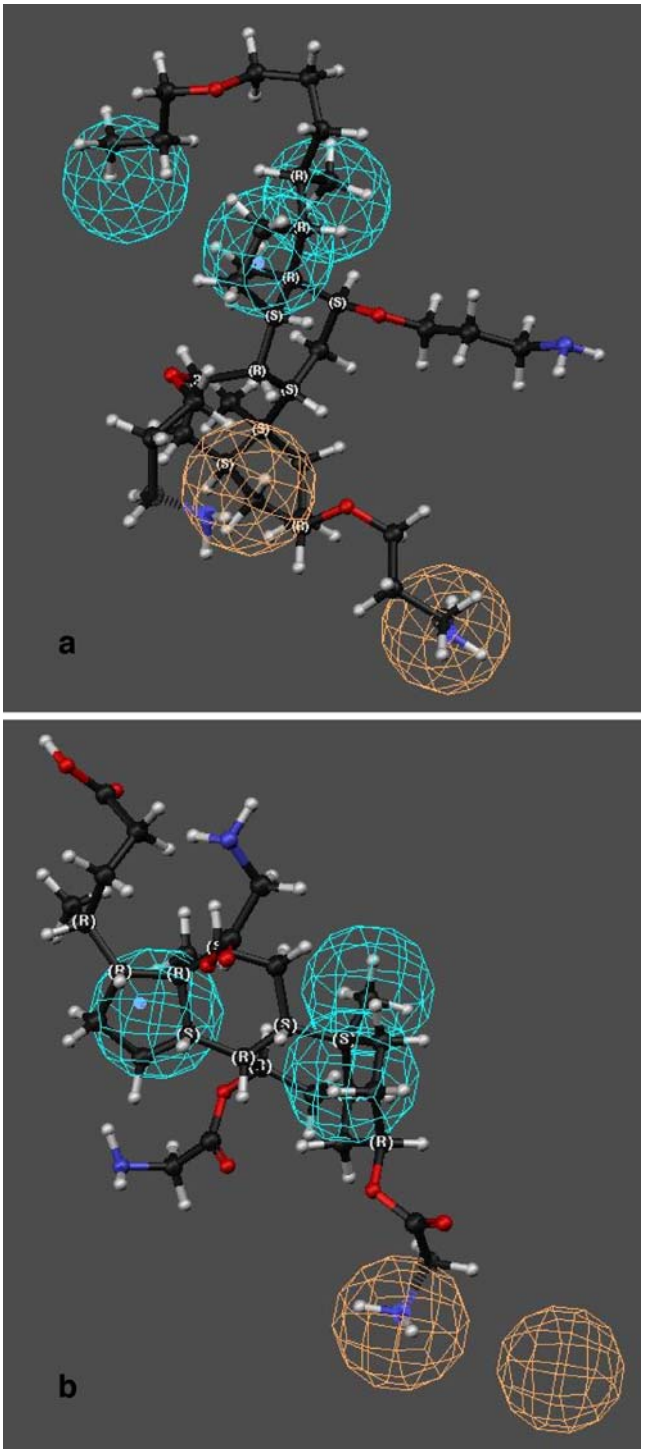

Fig. 6 Example of active (**a**) and inactive (**b**) CSA against *S. aureus*, mapped to hypothesis Sa-6. *Cyan*- and *brown-colored spheres* represent HYD and PI features, respectively

of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) [37–39]. However, the same activity trend was observed for some CSAs tested against a different strain of *E. coli* (ATCC 10798), e.g., CSAs 55–62 shown in Table 5 [34–36]. Thus, activity data of CSAs against the latter strain of *E. coli* were also included to increase the size of the validation set of molecules (Table 6). Accordingly, a total of 34 CSAs were selected from the literature—all reported by the same research group (Tables 5, 6; Fig. 4).

However, since the two separate research groups are involved in the synthesis and biological evaluation of CPAs [29, 30] and CSAs [34–39], the biological assay protocols adopted are different in the two cases. Thus, the definition of 'active' and 'inactive' CSAs is also not expected to be identical to that used for CPAs but should follow the same trend. In the case of CPAs, the activity defined against *E. coli* (ATCC 25922) ranged from 5 μg/mL to 50 μg/mL, i.e., the most active peptide was ten times more potent than the least active in the training set. Thus, since the most highly active CSA in this series had an MIC equal to 0.31 μg/mL against *E. coli* (Table 5), molecules with activity up to 3.1 μg/mL should be considered as active. Interestingly, all the molecules in this range (0.31–3.1 μg/mL; 57, 61, 62, 63, and 65) mapped well to hypothesis Ec-2 as was evident from their BestFit-0 values (Table 5). Along similar lines, the 'inactive' CPAs (MIC≥200 μg/mL) have MICs at least four times higher compared to 'active' CPAs (5–50 μg/mL). Consequently, in the case of CSAs, molecules with MIC≥ 12.4 μg/mL can be labeled as 'inactive'. Hypothesis Ec-2 also predicted the CSAs in this activity range to be inactive as evident from the inability of 58, 59, 66, 67, 68 and 69 to map to Ec-2 (Table 5).

It is interesting to note that two of the four HYD features of Ec-2 are mapped by the long aliphatic chain present at C-17, as evident from the mapping of 63 (Fig. 5a). This is in agreement with the experimental observation that the long hydrophobic substituents at C-17 improve the activity of CSAs against *E. coli*. On the other hand, CSA 58, with no substitution at all at C-17, lacks both side chain HYD features (Fig. 5b) and is thus inactive. The high MIC of 69 (>100 μg/mL) is explained by the fact that the presence of a free -COOH group would partially neutralize the overall positive charge of the molecule. However, according to mapping analyses, the inactivity of 69 is due to the inability of this molecule to conform to the given hypothesis Ec-2, showing no mapping with BestFit-0 and a poor BestFit-1 value (Table 5). It is also possible that both of these factors contribute to making 69 a highly inactive molecule compared to the other molecules in the series. The CSAs with intermediate activity against *E. coli* (60, 64, 70 and 71; MIC between 3.1 and 12.4 μg/mL) showed mixed results when mapped to hypothesis Ec-2. While 64 (MIC=7.0 μg/mL) showed a good fit value of 3.68, 70 (MIC=6.6 μg/mL) and 71 (MIC=7.3 μg/mL) failed to map to the hypothesis, and 60 (MIC=6.6 μg/mL) showed a poor fit value (0.41).

Similarly, mapping of CSAs was performed with hypothesis Sa-6. A 20-fold difference was found between the least active (MIC=2.5 μg/mL) and most active (MIC= 50 μg/mL) CPA in the training set against *S. aureus*. Accordingly, the CSAs within the MIC range of 0.40–8.0 μg/mL may be considered as 'active' against *S. aureus*, while CSAs with MIC≥32 μg/mL may be labeled as 'inactive'. As expected, all the active CSAs (55–57, 59–65, 70, 71) mapped well to hypothesis Sa-6, with fit values of greater than 3.0—the only exception being 59 with a poor BestFit-0 value of 0.64. As observed experimentally, a long hydrophobic chain at C-17 is not critical for activity against *S. aureus*. According to Sa-6, this is because the minimum requirement for HYD features in this case is only three, of which only one maps to the C-17 group. This is clear from the mapping of 60 to hypothesis Sa-6 (Fig. 6a). Again, as is the case with *E. coli*, the inactivity of 69 against *S. aureus* can be explained on the basis of its inability to map to all the features of hypothesis Sa-6 (Fig. 6b). When a poorly active or inactive CSA was allowed to miss one feature, it showed improved fit values with both hypotheses (BestFit-1, Table 5). These results demonstrate that, like CPAs, CSAs also require the same number of minimum HYD and PI features to be active against *E. coli* and *S. aureus*. In

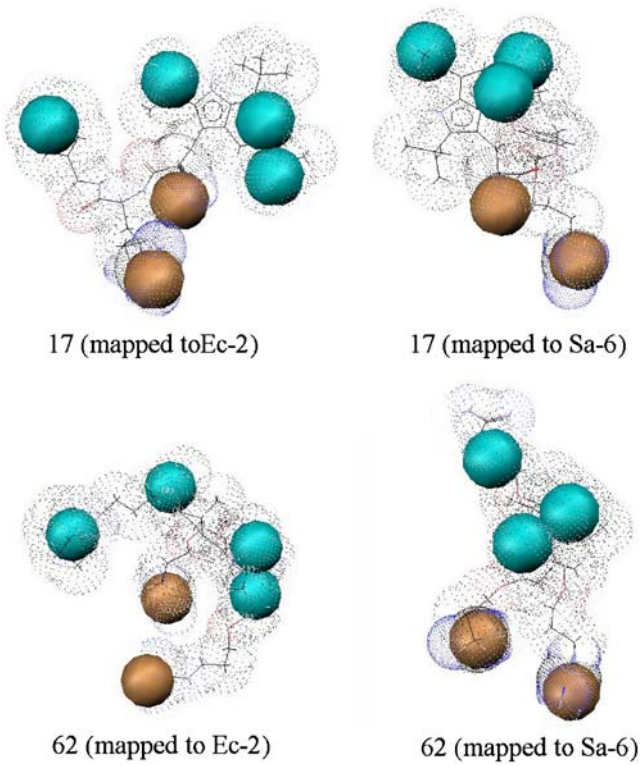

Fig. 7 Van der Waal surface of a representative of the CPA (17) and CSA (62) classes of antimicrobial compounds, mapped to hypotheses Ec-2 and Sa-6

addition, the relative three-dimensional arrangements of the features in both cases are same and explain the experimentally observed differences among active and inactive molecules. To visualize the similarity in the amphiphilic structure of chemically diverse CPAs and CSAs, the van der Waal surface was generated for 17 and 62, together with the features mapped to these molecules (Fig. 7). This figure presents a clear picture of oppositely placed PI and HYD features in the two classes of molecules.

The CSAs evaluated against a different strain of *E. coli* (ATCC 10798) were also tested with Ec-2. Most of the selected CSAs were found to be inactive against the given strain (Table 6) except 86 and 87, both of which have MIC= 3 μg/mL, whereas the activity range for other CSAs was 70–100 μg/mL. Interestingly, as expected, none of the inactive compounds mapped to hypothesis Ec-2, except 74, which also showed a poor fit value of 1.41 (Table 6). On the other hand, active CSAs 86 and 87 showed good fit values of 4.94 and 3.02, respectively. Thus, it can be stated that the requirement for minimum PI and HYD features is conserved among various strains of the same organism (at least in case of *E. coli*), although more experimental data is required to increase confidence in this regard.

Conclusions and future prospects

In conclusion, the minimum requirements for hydrophilic and hydrophobic features contributing to antimicrobial activity of CPAs has been established by employing HipHop-based pharmacophore mapping analysis. Two separate hypotheses, Ec-2 and Sa-6, were generated for Gram negative (*E. coli*, ATCC 25922) and Gram positive (*S. aureus*, ATCC 25923) bacteria, respectively, using reported experimental data. The generated pharmacophores showed separation of PI and HYD features on opposite faces of the molecules, reflecting the presence of amphipathic structure in active CPAs. A minimum of two PI features were found to be sufficient for activity in both cases, although the models differed in the number of HYD features. Mapping analyses showed that all the active peptides were able to adopt the proper conformation to map to the given hypothesis with good fit values. On the other hand, inactive peptides mapped either partially or poorly to the respective hypotheses. The hypotheses were further validated on a set of CSAs, which are reported to act as antimicrobials with a mechanism of action similar to that of CPAs. The hypotheses Ec-2 and Sa-6 were also able to distinguish active and inactive molecules among CSAs. The model Ec-2 could also predict active and inactive CSAs against another strain of *E. coli* (ATCC 10798), indicating that the minimum features required might be conserved among different strains of the same organism.

Hence, it can be stated that hypotheses Ec-2 and Sa-6 indicate the minimum hydrophobic and hydrophilic features required for antimicrobial activity, and they illustrate the importance of amphiphilic structure in this regard. However, it is important to note that these hypotheses can provide only qualitative estimate of activity. They can distinguish active molecules from inactive ones, but cannot predict biological activity in a precise manner. This is obvious as precise activity data is not used for generation of these pharmacophores. Nevertheless, final pharmacophoric models can be used for rapid virtual screening of large libraries of small peptides, peptidomimetics, amphiphilic steroids and other molecules possessing facial amphiphilicity, in order to find potentially novel antimicrobial agents.

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References

1. Neu HC (1992) Science 257:1064–1073
2. Travis J (1994) Science 264:360–362
3. Overbye KM, Barrett JF (2005) Drug Discov Today 10:45–52
4. Levy SB (1998) Sci Am 275:46–53
5. Calza L, Manfredi R, Chiodo F (2004) Expert Opin Pharmacother 5:1899–1916
6. Jacqueline C, Asseray N, Batard E, Mabecque VL, Kergueris MF, Dube L, Bugnon D, Potel G, Caillon J (2004) Int J Antimicrob Agents 24:393–396
7. Wagenlehner FM, Naber KG (2004) Int J Antimicrob Agents 24:39–43
8. Hancock REW (1997) Lancet 349:418–422
9. Tossi A, Sandri L, Giangaspero A (2000) Biopolymers 55:4–30
10. Nicolas P, Mor A (1995) Annu Rev Microbiol 49:277–304
11. Sitaram N, Nagaraj R (2002) Curr Pharm Des 8:727–742
12. Giangaspero A, Sandri L, Tossi A (2001) Eur J Biochem 268:5589–5600
13. Hancock REW, Scott MG (2000) Proc Natl Acad Sci USA 97:8856–8861
14. Shai Y (1999) Biochim Biophys Acta 1462:55–70
15. Hancock REW (1999) Drugs 57:469–473
16. Hancock REW (1998) Trends Biotechnol 16:82–88
17. Duclohier H, Molle G, Spach G (1989) Biophys J 56:1017–1021
18. Zhang L, Dhillon P, Yan H, Farmer S, Hancock REW (2000) Antimicrob Agents Chemother 44:3317–3321
19. Epand RM, Vogel HJ (1999) Biochim Biophys Acta 1462:11–28
20. Hancock REW, Chapple DS (1999) Antimicrob Agents Chemother 43:1317–1323
21. Huang HW (2000) Biochemistry 39:8347–8352
22. Matsuzaki K (1998) Biochim Biophys Acta 1376:391–400
23. Oren Z, Shai Y (1998) Biopolymers 47:451–463
24. Reddy KVR, Yedery RD, Aranha C (2004) Int J Antimicrob Agents 24:536–547
25. Matsuzaki K, Sugislita K, Fshibe N (1998) Biochemistry 37:11856–11863
26. Dagan A, Efron L, Gaidukov L, Mor A, Ginsburg H (2002) Antimicrob Agents Chemother 46:1059–1066
27. Yeaman MR, Yount NY (2003) Pharmacol Rev 55:27–55

28. Hancock REW (1999) Drugs 57:469–473
29. Strom MB, Haug BE, Skar ML, Stensen W, Stiberg T, Svendsen JS (2003) J Med Chem 46:1567–1570
30. Haug BEML, Stensen W, Stiberg T, Svendsen JS (2004) J Med Chem 47:4159–4162
31. Kurogi Y, Guner OF (2001) Curr Med Chem 8:1035–1055
32. Cheng YC, Ho DM, Gottlieb CR, Kahne D (1992) J Am Chem Soc 114:7319–7320
33. Savage PB, Li C, Taotafa U, Ding B, Guan Q (2002) FEMS Microbiol Lett 217:1–7
34. Li C, Peters AS, Meredith EL, Allman GW, Savage PB (1998) J Am Chem Soc 120:2961–2962
35. Li C, Budge LP, Driscoll CD, Willardson BM, Allman GW, Savage PB (1999) J Am Chem Soc 121:931–940
36. Rehman AU, Li C, Budge LP, Street SE, Savage PB (1999) Tetrahedron Lett 40:1865–1868
37. Li C, Lewis MR, Gilbert AB, Noel MD, Scoville DH, Allman GW, Savage PB (1999) Antimicrob Agents Chemother 43:1347–1349
38. Guan Q, Li C, Schmidt EJ, Boswell JS, Walsh JP, Allman GW, Savage PB (2000) Org Lett 2:2837–2840
39. Ding B, Guan Q, Walsh JP, Boswell JS, Winter TW, Winter ES, Boyd SS, Li C, Savage PB (2002) J Med Chem 45: 663–669
40. Catalyst Program, Version 4.10. Accelrys Inc., San Diego, CA
41. Brooks BR, Brucolleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M (1983) J Comput Chem 4:187–217
42. Smellie A, Kahn SD, Teig SL (1995) J Chem Inf Comp Sci 35:285–294
43. Smellie A, Kahn SD, Teig SL (1995) J Chem Inf Comp Sci 35:295–304
44. Smellie A, Teig SL, Towbin P (1995) J Comp Chem 16:171–187