Bioorganic & Medicinal Chemistry Letters 24 (2014) 2177–2181

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Successful application of serum shift prediction models to the design of dual targeting inhibitors of bacterial gyrase B and topoisomerase IV with improved in vivo efficacy

Emanuele Perola [a,*], Dean Stamos [a], Anne-Laure Grillot [a], Steven Ronkin [a], Tiansheng Wang [a], Arnaud LeTiran [a,†], Qing Tang [a], David D. Deininger [a], Yusheng Liao [a], Shi-Kai Tian [a,‡], Joseph E. Drumm [a,§], David P. Nicolau [b], Pamela R. Tessier [b], Nagraj Mani [a], Trudy H. Grossman [a,¶], Paul S. Charifson [a]

[a] Vertex Pharmaceuticals Incorporated, 50 Northern Avenue, Boston, MA 02210, United States
[b] Center for Anti-Infective Research and Development, Hartford Hospital, 80 Seymour Street, Hartford, CT 06102, United States

ARTICLE INFO

Article history:
Received 4 March 2014
Accepted 7 March 2014
Available online 19 March 2014

Keywords:
Gyrase B
Topoisomerase IV
Antibacterial
Protein binding
Serum shift
Predictive models

ABSTRACT

A series of dual targeting inhibitors of bacterial gyrase B and topoisomerase IV were identified and optimized to mid-to-low nanomolar potency against a variety of bacteria. However, in spite of seemingly adequate exposure achieved upon IV administration, the in vivo efficacy of the early lead compounds was limited by high levels of binding to serum proteins. To overcome this limitation, targeted serum shift prediction models were generated for each subclass of interest and were applied to the design of prospective analogs. As a result, numerous compounds with comparable antibacterial potency and reduced protein binding were generated. These efforts culminated in the synthesis of compound **10**, a potent inhibitor with low serum shift that demonstrated greatly improved in vivo efficacy in two distinct rat infection models.

© 2014 Elsevier Ltd. All rights reserved.

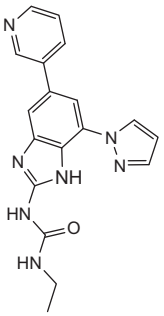
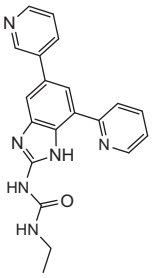
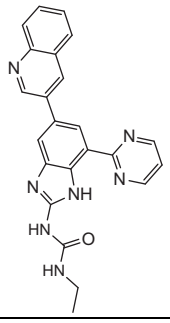
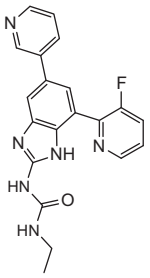
The efficacy of a drug is driven by two main factors: the drug's intrinsic potency and its available concentration at the site of action. One important factor, which affects both the percentage of drug molecules available for interaction with the target and the drug's pharmacokinetic behavior, is binding to plasma or tissue proteins. High levels of protein binding can severely limit the concentration of unbound drug, thus affecting its ability to interact with the intended target. At the same time, binding to plasma or tissue proteins reduces the availability of free drug to the target organs of clearance, thus slowing the elimination process and prolonging the exposure at the site of action.[1] The balance of these two effects ultimately determines the efficacy of the drug, and their relative importance may depend on the drug's mechanism and on the extent of protein binding. The impact of protein binding on antibiotic efficacy has been the object of numerous studies. Trends of reduced efficacy in connection with increased protein binding have been reported for a number of beta-lactams,[2–4] and the importance of considering the unbound concentration rather than the total concentration as a predictor of efficacy has been shown for other antibiotics and antibiotic classes.[5–7] While large amounts of proteins are also present in organs and tissues, the impact of protein binding is usually estimated based on the measured binding to serum proteins.[8]

In the gyrase B/topoisomerase IV antibacterial program conducted at Vertex, the benzimidazole urea lead series was optimized to low nanomolar levels of enzyme potency and mid-to-low nanomolar levels of cell potency against a number of bacterial strains.[9] However, measures of minimal inhibitory concentration (MIC) against *Staphylococcus aureus* in the presence of 50% human serum showed that the effective concentration of the most potent molecules in this class was substantially reduced by protein binding, thus resulting in a significant increase in MIC values. The structures of representative early leads in this class and the corresponding *S. aureus* MIC values in the presence and absence of 50% human serum are reported in Table 1. The ratio between the MIC values measured in the presence and in the absence of 50% human serum will be referred to as 'serum shift' in the remainder of this Letter.

* Corresponding author. Tel.: +1 617 341 6646.
E-mail address: emanuele_perola@vrtx.com (E. Perola).
† Present address: Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy sur Seine, France.
‡ Present address: Department of Chemistry, University of Science and Technology of China, 96 Jinzhai Road, Hefei, Anhui 230026, China.
§ Retired.
¶ Present address: Tetraphase Pharmaceuticals, 480 Arsenal Street, Watertown, MA 02472, United States.

http://dx.doi.org/10.1016/j.bmcl.2014.03.022
0960-894X/© 2014 Elsevier Ltd. All rights reserved.

Table 1
Structures of representative compounds in the benzimidazole urea class and corresponding antimicrobial activities against *Staphylococcus aureus*, strain ATCC 29213

Compound #	**1**	**2**	**3**	**4**
Structure				
MIC (medium)[a] (μg/ml)	0.125	0.047	0.032	0.031
MIC (serum)[b] (μg/ml)	4	2	2	0.625

[a] Minimal inhibitory concentration measured in standard assay medium.
[b] Minimal inhibitory concentration measured in standard medium + 50% human serum. The experimental protocols for the MIC measurements were previously reported.[9,10]

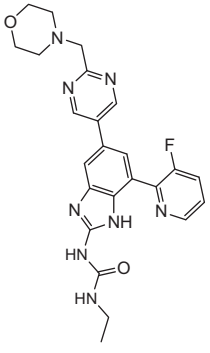
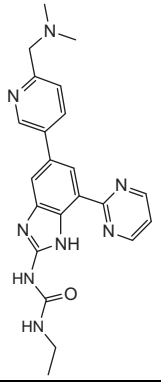
In general, protein binding levels above 95% and serum shifts ranging from 16 to 128-fold were common among these compounds. While the reduced free concentration may be counterbalanced by the protective effect of protein binding, which slows the elimination process and leads to more extended exposure, the free concentration of a drug at the site of infection must be at or above the MIC at least some of the time during treatment to achieve efficacy. Given the degree of protein binding and the levels of exposure observed for our early leads upon IV or oral administration, the doses required to achieve free concentrations above the MIC even for a limited time were exceedingly high, resulting in limited activity in vivo. As an example, compound **2** only achieved 20% survival in rat when dosed intravenously (IV) at 25 mg/kg in a systemic *S. aureus* sepsis model,[11] in spite of its potent in vitro activity and a favorable pharmacokinetic profile. As a point of comparison, a 30% survival rate was achieved in the same model by the marketed antibiotic linezolid, which has inferior rat IV PK, much higher *S. aureus* MIC (1.56 μg/ml) but no serum shift. These results prompted us to explore targeted design approaches to reduce protein binding for this class of compounds.

Binding to serum proteins is governed both by non-specific hydrophobic interactions and by specific interactions with plasma proteins. Due to the importance of both components, the generation of reliable models to predict the fraction of a given drug that is sequestered by serum proteins has been challenging, and attempts to generate predictive models that could be applicable to diverse compound classes have produced mixed results.[12–14] Hydrophobicity is generally believed to play a major role, and an analysis of the available data for marketed drugs shows that drugs with *C*log*P* >4.5 tend to be over 90% protein bound, while for drugs with *C*log*P* >5 protein binding tends to exceed 95% (data not shown). Unfortunately, while high *C*log*P* values are generally associated with high levels of protein binding, lower *C*log*P* values do not always lead to reduced protein binding. Due to the contribution of both specific and non-specific interactions, more polar molecules can still be sequestered in large proportions, only leaving a small fraction available for interaction with the target of choice. Another important factor is the presence of ionizable groups. When molecules of similar log*P* are considered, acidic compounds tend to be more highly protein bound than neutral compounds, which in turn tend to be more highly protein bound than basic compounds and zwitterions.[13] While a *C*log*P* reduction strategy may be successfully applied to lower protein binding in specific cases,[15] a detailed analysis of properties, structural features and existing data for the compound classes of interest is likely to be required to capture the complexity of the process and build reliable protein binding prediction models.

Our first approach to address the problem was the introduction of basic functionalities in our lead molecules. A large number of analogs bearing basic amines were synthesized, and a significant reduction of serum shift was observed in most cases (e.g., see compounds **5** and **6** in Table 2).

However, the introduction of a basic functionality led to less favorable rat IV PK for these compounds, as the more potent analogs designed with this approach exhibited increased clearance. Eventually, it became apparent that a more subtle approach was required to design neutral analogs with reduced protein binding. Aside from the general considerations about the impact of high *C*log*P* values on protein binding, no accurate predictive model was available for compounds of intermediate lipophilicity. We therefore initiated attempts to generate a local model to predict the serum shift of the aminobenzimidazole class using the available data from our gyrase program to derive a training set. The choice to model serum shift instead of protein binding was

Table 2
Structures and *S. aureus* MIC values for two representative benzimidazole ureas containing basic amines

Compound #	**5**	**6**
Structure		
MIC (medium) (μg/ml)	0.125	0.5
MIC (serum) (μg/ml)	0.125	0.125

dictated by two factors: (1) serum shift provides a direct measure of the impact of protein binding on antibacterial activity; (2) at the high levels of protein binding observed for our series, small variations in protein binding lead to relatively large variations in serum shift, thus making measurements of the latter more discriminant. As an example, if compound A is 99.5% protein bound and compound B is 98% protein bound, the free fraction of compound B is 4-fold higher than the free fraction of compound A, and the corresponding serum shift will be 4-fold lower. A 4-fold difference in serum shift can be reliably measured, while the difference between 99.5% and 98% in protein binding is likely within experimental error. Serum shift data was available for 338 compounds sharing the substructure in Figure 1 below.

Since the objective was to build a model for neutral compounds, all the charged compounds (mostly basic amines) were removed, and the initial attempts at model building were made on the remaining set of 202 compounds. Additional experiments had shown that binding to human serum albumin was the dominant cause of the observed serum shift. In our initial approaches we analyzed the correlation between serum shift and global properties such as molecular weight, *C*log*P* and polar surface area. We then attempted to combine *C*log*P*, which gave a weak but tangible signal, with 2D or 3D similarity to warfarin and other known high affinity ligands of albumin. We also built a binary model using support vector machine with different sets of 2D descriptors and we docked our compounds into the benzodiazepine and warfarin binding sites of albumin. Although some of these approaches provided hints on the factors affecting serum shift for this class of compounds, none of them resulted in a convincing model that could bear sufficient predictive power. At that time the two subclasses illustrated in Figure 2 were being further investigated.

A pairwise analysis across the two subclasses showed that compounds carrying a pyrimidine ring at the 7 position of the benzimidazole core (class B) consistently showed a lower serum shift relative to the corresponding 7-fluoropyridines (class A). This realization prompted us to perform a separate analysis for each subclass. Serum shift data was available for 62 compounds in class A and 47 in class B. Given the findings that emerged from the broader

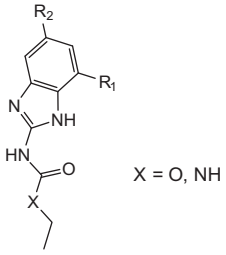


Figure 1. General substructure for compounds in the benzimidazole series with available serum shift data.

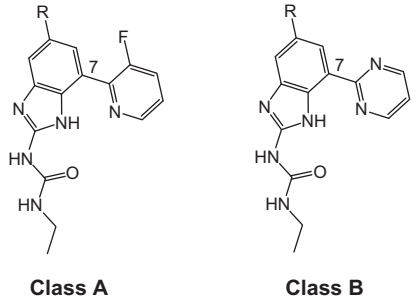


Figure 2. Substructures of the benzimidazole urea subclasses investigated in this study.

set, we focused our analysis on the correlation between serum shift and global properties such as *C*log*P* and polar surface area (PSA). The plots in Figure 3 illustrate the correlation between serum shift and each of these two properties for class A compounds.

While there was no clear correlation between serum shift and PSA, there was a clear dependency in this subclass between serum shift and *C*log*P*. A *C*log*P* value of 3.1 provided a good separation between high and low shifters: 79% of compounds with *C*log*P* <3.1 had serum shifts of 8 or less, while 97% of the compounds with *C*log*P* >3.1 had serum shifts of 16 or more. This trend suggested that prospective compounds with *C*log*P* >3.1 would almost certainly be high shifters, while compounds with *C*log*P* <3.1 would likely be low shifters. Figure 4 illustrates the correlation between serum shift and the same two properties for class B compounds.

In this case both *C*log*P* and PSA played a significant role, and the plots clearly indicated that the vast majority of the compounds with serum shift of 4 or less had *C*log*P* <2.1 and PSA >120. A more detailed analysis of the individual data showed that using the two parameters in combination had the potential to minimize both the false positives and the false negatives: 92% of the compounds with *C*log*P* <2.1 and PSA >120 had serum shifts of 4 or less, while 91% of the compounds with *C*log*P* >2.1 and/or PSA <120 had serum shifts of 8 or more. This trend suggested that prospective compounds meeting both criteria would almost certainly be low shifters, while compounds violating at least one of the two would almost certainly be high shifters.

The findings of this analysis were applied to the design of prospective analogs by prioritizing compounds that met the following criteria:

C7-fluoropyridines (class A): *C*log*P* <3.1 (expected serum shift $\leqslant$8)

C7-pyrimidines (class B): *C*log*P* <2.1 and PSA >120 (expected serum shift $\leqslant$4)

This approach, combined with the SAR-driven and structure-based methods previously described for this series,[9] led to the synthesis of compounds with low serum shift and a greater potential to be efficacious in an animal infection model. Overall, 61 additional compounds between the two subclasses were synthesized according to the general procedure in Scheme 1. Additional details on the synthesis of these two subseries can be found in our previous publications.[9,18]

Low nanomolar potencies against gyrase B and topoisomerase IV were generally maintained, and MIC values between 0.016 and 0.25 μg/ml against *S. aureus* were observed for 55 of the 61 compounds. The serum shift values were consistent with the predictions based on the above criteria in 46/61 cases (75.4%). In all the remaining 15 cases the shifts were only 2-fold outside of the predicted range. This method proved especially effective in filtering out compounds with high serum shift, as only two of the compounds predicted to have high shift turned out to have low shift. Overall, the compounds with low shift were predicted with 62% accuracy while the compounds with high shift were predicted with 92% accuracy. Table 3 illustrates the structures of four optimized compounds with their antimicrobial activities both in the absence and in the presence of human serum.

As both models included an upper limit for *C*log*P* of low serum shifters, their application led to the design of compounds with reduced lipophilicity. It has been observed that reducing the lipophilicity of compounds within a series often leads to a reduction in intrinsic clearance, which in turn can result in increased levels of exposure in vivo.[1,19] Such an effect could have been a contributing factor to the desired improvements in efficacy for our series. However, a clear correlation between *C*log*P* and intrinsic clearance for the synthesized compounds could not be established, and no correlation was observed between the same parameter and in vivo exposure upon IV or oral dosing (data not shown).

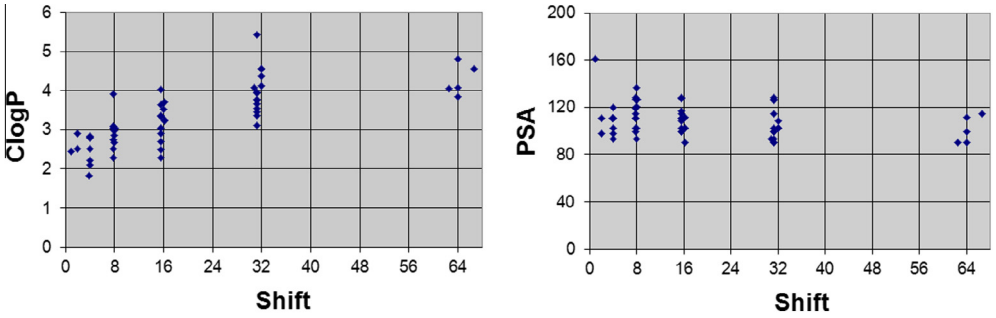


Figure 3. Serum shift versus *C*log*P* and polar surface area (PSA) for compounds in class A. The *C*log*P* calculations were performed using the ClogP program from Daylight.[16] The PSA calculations were performed using an internal implementation of the method described by Ertl and colleagues.[17]

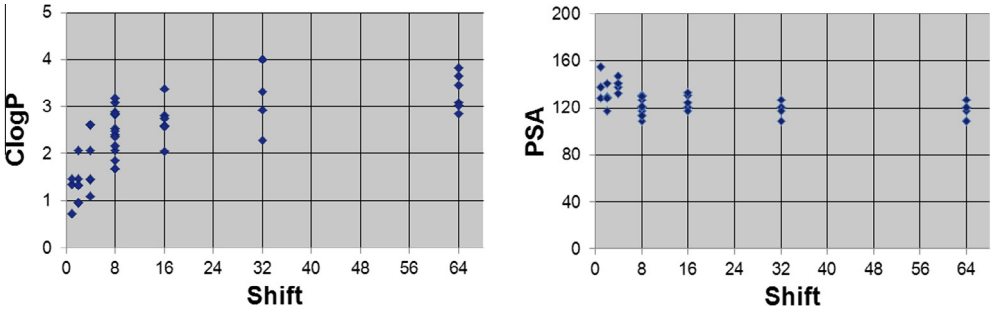
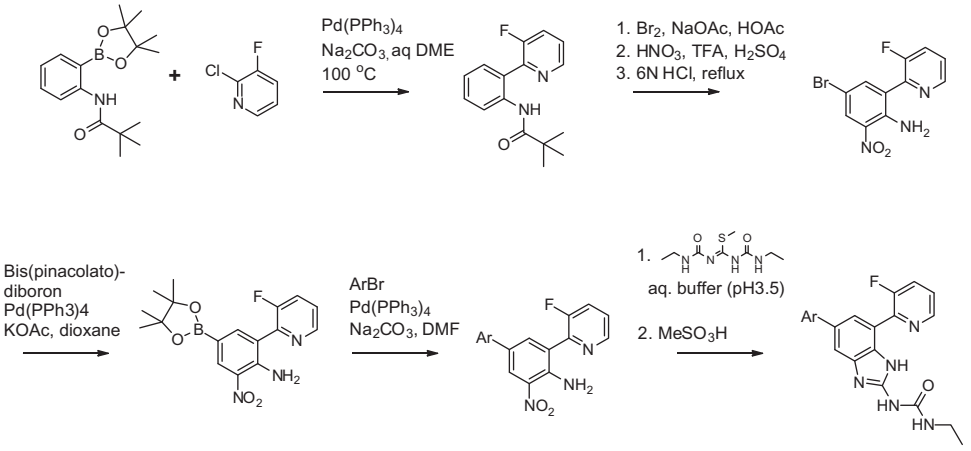


Figure 4. Serum shift versus *C*log*P* and polar surface area (PSA) for compounds in class B.



Scheme 1. General procedure for the synthesis of the compounds in class A. The same scheme applies to class B with the 2-(3-fluoro)pyridyl group at the 7-position of the benzimidazole core replaced by a 2-pyrimidyl group. Ar = aryl group.

Compound **10** had adequate exposure in rat both upon IV and upon oral administration and was evaluated in vivo in two rat infection models. The experimental protocols for these models have been described in our previous publication.[9] A single 30 mg/kg IV dose of compound **10** administered in a *S. aureus* rat thigh infection model caused a reduction in bacterial density of 2.1 logs in CFU (colony forming units) 8 h after dosing, while a 3 mg/kg dose caused a reduction of 1.7 logs. Linezolid caused a reduction of 1.4 logs in CFU when dosed at 30 mg/kg and no measurable reduction when dosed at 3 mg/kg in the same model. Compound **4**, which has an equal *S. aureus* MIC in the absence of serum and comparable IV PK but a 2.5-fold higher serum shift relative to compound **10**, caused a reduction of 1.5 logs in CFU after 6 hours when dosed at 50 mg/kg. As mentioned above, compound **2**, which has an equal *S. aureus* MIC in the absence of serum and slightly better IV PK relative to **10** but an 8-fold higher serum shift (64 vs 8), exhibited modest efficacy in a systemic *S. aureus* infection model when dosed IV at 25 mg/kg, similarly to linezolid. Compound **10** was also dosed orally in a *Streptococcus pneumoniae* rat lung infection model where it achieved a reduction in bacterial density of 5.8 and 3.7 logs in CFU when dosed at 30 and 3 mg/kg, respectively. Linezolid achieved a reduction of 4.5 and 0.8 logs at the same doses, while compound **2** achieved a reduction of 3.0 logs when dosed IV at 38.5 mg/kg in the same model.

To best understand the impact of serum shift on the in vivo efficacy of these compounds, these results should be viewed in light of their antibacterial activities, serum shift values and rat IV PK parameters (Table 4).

Compounds **2**, **4** and **10** have similar antibacterial potencies against both *S. aureus* and *S. pneumoniae*; compound **2** has slightly better IV PK while **4** and **10** have comparable PK parameters. Linezolid is considerably less potent and has comparable IV PK

Table 3
Structures, calculated properties and *S. aureus* MIC values in the absence and in the presence of human serum for a representative set of optimized compounds in the benzimidazole urea class

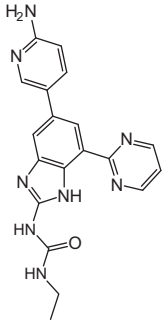
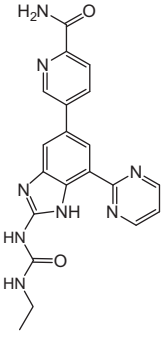
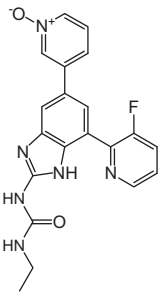
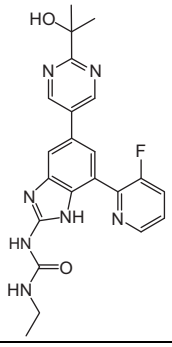
Compound #	**7**	**8**	**9**	**10**
Structure				
Clog*P*	1.73	1.56	2.64	1.96
PSA (Å^2)	134	139	110	129
MIC (medium) (μg/ml)	0.125	0.064	0.5	0.032
MIC (serum) (μg/ml)	0.25	0.25	0.5	0.25

Table 4
Antimicrobial activities, serum shifts and rat IV pharmacokinetic parameters for compounds **2**, **4**, **10** and linezolid

Compound	*S. a* MIC[a]	*S. p* MIC[b]	SS[c]	CL[d]	$t_{1/2}$[e]	*Vss*[f]	AUC[g]
2	0.047	0.004	43	12.5	2.1	1.1	1.6
4	0.031	0.004	20	14	0.7	1.6	1.1
10	0.031	0.004	8	23	1.2	2.1	0.8
Linezolid	1.56	0.4	1	14.5	0.7	1	1.1

[a] Minimal inhibitory concentration versus *S. aureus* (μg/ml).
[b] Minimal inhibitory concentration versus *Streptococcus pneumoniae* (μg/ml).
[c] Serum shift.
[d] Clearance (ml/min/kg).
[e] Half-life (hours).
[f] Volume of distribution (L/kg).
[g] Area under the curve (μg*h/ml). The AUC values are dose-normalized to 1 mg/kg. The actual doses in the PK studies ranged from 2.8 to 9.5 mg/kg.

relative to both **4** and **10**. If protein binding and the consequent serum shift were not a factor, compound **2** should have comparable or slightly better efficacy relative to compounds **4** and **10** and all three compounds should have better efficacy relative to linezolid. Instead, the large serum shift increases the effective MIC of compound **2** and makes it comparable to that of linezolid, which does not experience any shift. The comparable efficacy of compound **2** and linezolid reflects their serum shifted MIC values. The lower serum shift experienced by compound **10** relative to compounds **2** and **4** resulted in a lower effective MIC, which led to its superior in vivo efficacy.

Overall, this study showed the effectiveness of a divide-and-conquer approach that enabled us to derive accurate predictive models for the serum shift of specific subclasses when clear trends could not be identified for the entire class. It also showed that, in some cases, simple local models can have greater predictive value than more sophisticated global models, consistent with a retrospective analysis reported in a previous study.[13] The application of these models contributed to the design of compounds with reduced protein binding and improved in vivo efficacy.

References and notes

1. Smith, D. A.; Di, L.; Kerns, E. H. *Nat. Rev. Drug Disc.* **2010**, *9*, 929.
2. Merrikin, D. J.; Briant, J.; Rolinson, G. N. *J. Antimicrob. Chemother.* **1983**, *11*, 233.
3. Tawara, S.; Matsumoto, S.; Kamimura, T.; Goto, S. *Antimicrob. Agents Chemother.* **1992**, *36*, 17.
4. Wise, R. *Clin. Pharmacokinet.* **1986**, *11*, 470.
5. Lee, B. L.; Sachdeva, M.; Chambers, H. F. *Antimicrob. Agents Chemother.* **1991**, *35*, 2505.
6. Crandon, J. L.; Banevicius, M. A.; Nicolau, D. P. *Antimicrob. Agents Chemother.* **2009**, *53*, 1165.
7. Scaglione, F.; Mouton, J. W.; Mattina, R.; Fraschini, F. *Antimicrob. Agents Chemother.* **2003**, *47*, 2749.
8. Zeitlinger, M. A.; Derendorf, H.; Mouton, J. W.; Cars, O.; Craig, W. A.; Andes, D.; Theuretzbacher, U. *Antimicrob. Agents Chemother.* **2011**, *55*, 3067.
9. Charifson, P. S.; Grillot, A. L.; Grossman, T. H.; Parsons, J. D.; Badia, M.; Bellon, S.; Deininger, D. D.; Drumm, J. E.; Gross, C. H.; LeTiran, A.; Liao, Y.; Mani, N.; Nicolau, D. P.; Perola, E.; Ronkin, S.; Shannon, D.; Swenson, L. L.; Tang, Q.; Tessier, P. R.; Tian, S. K.; Trudeau, M.; Wang, T.; Wei, Y.; Zhang, H.; Stamos, D. *J. Med. Chem.* **2008**, *51*, 5243.
10. Mani, N.; Gross, C. H.; Parsons, J. D.; Hanzelka, B.; Muh, U.; Mullin, S.; Liao, Y.; Grillot, A. L.; Stamos, D.; Charifson, P. S.; Grossman, T. H. *Antimicrob. Agents Chemother.* **2006**, *50*, 1228.
11. The Staphylococcal neutropenic sepsis model utilized specific-pathogen-free, male Sprague-Dawley rats. Neutropenia was induced by cyclophosphamide intraperitoneal (IP) injections of 150 and 50 mg/kg on day 4 and 1 day prior to bacterial inoculation. Infection was established via an IP injection of 1.5 ml of 109 CFU/ml *Staphylococcus aureus* ATCC 29213. Antibacterial test agents were administered intravenously 1 h after bacterial inoculation and survival was assessed over 120 h.
12. Yamazaki, K.; Kanaoka, M. *J. Pharm. Sci.* **2004**, *93*, 1480.
13. Gleeson, M. P. *J. Med. Chem.* **2007**, *50*, 101.
14. Ma, C. Y.; Yang, S. Y.; Zhang, H.; Xiang, M. L.; Huang, Q.; Wei, Y. Q. *J. Pharm. Biomed. Anal.* **2008**, *47*, 677.
15. Ellsworth, B. A.; Sher, P. M.; Wu, X.; Wu, G.; Sulsky, R. B.; Gu, Z.; Murugesan, N.; Zhu, Y.; Yu, G.; Sitkoff, D. F.; Carlson, K. E.; Kang, L.; Yang, Y.; Lee, N.; Baska, R. A.; Keim, W. J.; Cullen, M. J.; Azzara, A. V.; Zuvich, E.; Thomas, M. A.; Rohrbach, K. W.; Devenny, J. J.; Godonis, H. E.; Harvey, S. J.; Murphy, B. J.; Everlof, G. G.; Stetsko, P. I.; Gudmundsson, O.; Johnghar, S.; Ranasinghe, A.; Behnia, K.; Pelleymounter, M. A.; Ewing, W. R. *J. Med. Chem.* **2013**, *56*, 9586.
16. CLOGP v. 4.1. Daylight Chemical Information Systems.
17. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
18. Charifson, P. S.; Deininger, D. D.; Grillot, A. L.; Liao, Y.; Ronkin, S. M.; Stamos, D.; Perola, E.; Wang, T.; LeTiran, A.; Drumm, J. US Patent 7,495,014 B2; **2009**.
19. Lewis, D. F.; Jacobs, M. N.; Dickins, M. *Drug Discovery Today* **2004**, *9*, 530.