Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 16 (2006) 714–717
Bioorganic & Medicinal Chemistry Letters

Molecular modeling analysis of the interaction of novel bis-cationic ligands with the lipid A moiety of lipopolysaccharide

Jian-Xin Guo,[a] Stewart J. Wood,[b] Sunil A. David[b,*] and Gerald H. Lushington[a,*]

[a]*Molecular Graphics and Modeling Laboratory, University of Kansas, Lawrence, KS 66045, USA*
[b]*Department of Medicinal Chemistry, University of Kansas, Life Sciences Research Laboratories, 1501 Wakarusa Drive, Lawrence, KS 66047, USA*

Received 8 September 2005; revised 30 September 2005; accepted 6 October 2005
Available online 2 November 2005

Abstract—Lipopolysaccharides (LPS), otherwise termed 'endotoxins', are outer-membrane constituents of Gram-negative bacteria and play a key role in the pathogenesis of 'septic shock', a major cause of mortality in the critically ill patient. We have shown that the pharmacophore necessary for optimal recognition and neutralization of LPS by small molecules requires an interaction between two protonatable positive charges separated by a distance of ~14 Å, which corresponds to the distance between two anionic phosphates on the glycolipid component of LPS called lipid A. The in silico binding of a diverse set of compounds with bis-amino, -amidino, -guanidino, and -aminoguanidino functionalities, identified as potential lead scaffolds in a high-throughput screen, with lipid A was explored using molecular docking simulations. A weighted expression for binding affinity was trained relative to experimental ED_{50} measurements, attaining a correlation of $R^2 = 0.66$. Our docking results showed that the electrostatic interaction between ligands and lipid A phosphates dominates the expression and varies little across the series, and other ligand–receptor interactions seem to play a secondary role in governing the observed variations in the relative ligand binding affinity. Further, it appears that the ligand internal energy plays the primary role in differentiating between compound binding affinities which also correlated well with experimental ED_{50} data ($R = 0.77$). Application of this strategy would be useful in the de novo design of highly active endotoxin-sequestering agents.

Gram-negative sepsis is a serious and common clinical problem, and is the primary cause of mortality in the intensive care unit worldwide[1] accounting for some 200,000 fatalities in the US annually.[2] Mortality due to septic shock has essentially remained unchanged at about 45%,[3] reflecting the absence of specific therapy targeting underlying host-response mechanisms. The primary trigger in the pathogenesis of the Gram-negative septic shock syndrome is endotoxin, a constituent of the outer-membrane of all Gram-negative bacteria. Otherwise referred to as lipopolysaccharides (LPS), endotoxins consist of a polysaccharide portion and a glycolipid called lipid A. The structurally highly conserved lipid A[4] (Fig. 1) is the toxic moiety of LPS[5] and sequestration of LPS by molecules designed to bind lipid A is a logical and attractive target for drug development.[6–8]

The bis-anionic, amphiphilic nature of lipid A enables it to interact with a variety of bis-cationic hydrophobic ligands. In our ongoing efforts to develop small molecules that would specifically bind lipid A, we have converged on linear cationic amphipathic molecules possessing terminal, protonatable cationic groups positioned so as to be able to simultaneously interact with the glycosidic phosphates on lipid A,[6–12] as well as appropriately positioned apolar moieties to enable hydrophobic interactions with the polyacyl domain of lipid A. Noteworthy

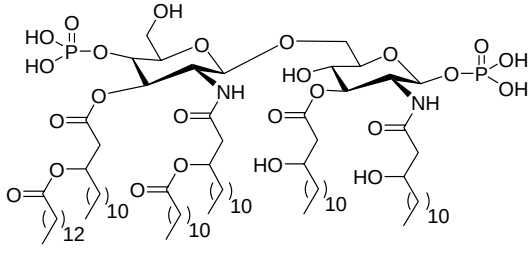

Figure 1. Structure of lipid A, the toxic center of LPS.

Keywords: Lipopolysaccharide; Endotoxin; Sepsis; Docking; QSAR.
* Corresponding authors. Tel.: +1 785 330 4316; fax: +1 785 330 4332 (S.A.D.); tel.: +1 785 864 1140; fax: +1 785 864 5326 (G.H.L.); e-mail addresses: sdavid@ku.edu; glushington@ku.edu

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.10.025

examples of such molecules displaying potent in vitro and in vivo LPS-sequestering properties are acyl-polyamines.[6–10]

Hypothesizing that a judicious pre-selection of bis-cationic compounds fulfilling the primary pharmacophore criterion of an N ↔ N distance of ~14 Å, and bearing multiple H-bond donor/acceptor atoms on the scaffold would increase the probability of generating high-affinity binders with novel scaffolds, we had performed searches in three-dimensional structure databases which yielded about ~4000 commercially available compounds. Employing a fluorescent probe displacement high-throughput screening method,[13] approximately 400 such compounds were screened in an effort to validate the method by which high-affinity endotoxin binders can be identified.[14] These compounds were chosen for screening based on their commercial availability. We now seek to address the question if high-affinity binders could be predicted computationally, for if this could be accomplished within reasonable error, very large numbers of computationally generated structures could be pre-screened in silico, prior to selective syntheses of novel lead scaffolds. Using a subset of 54 compounds as a training set (see Supplementary data), we report here the application of molecular docking methods. The results indicate that such an approach may indeed be feasible.

The application of in silico molecular design has become an increasingly vital component of modern drug discovery and development. Conventional drug design focuses on predicting molecular interactions with sterically delimited concave protein receptor sites. However, interactions with the convex surface of lipid A present unique challenges due to a greatly increased solvent exposed molecular area, and a much larger spread of translational, rotational, and conformational freedoms of the binding ligand due to the relative absence of firm steric boundaries.

A 3D structure search of commercial compound libraries identified over 4000 molecules with an N–N distance of 15.0 ± 3.0 Å apart. These molecules were screened using a high-throughput fluorescence displacement assay, using BODIPY cadaverine (BC) (5-(((4-(4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-yl)phenoxy)acetyl)-amino) pentylamine HCl) as the displacement probe.[13,14] Concentrations of the molecules causing 50% effective displacement of BC from LPS (ED_{50} values) provided the LPS binding affinity of these molecules. From this high-throughput screen, 54 molecules were selected from the highest affinity binders to perform in silico docking studies.

Each of the 54 molecules studied herein was computationally docked onto the crystal structure of lipid A via the dock module within MOE,[15] using default in vacuo settings. Hydrogen atoms were added to the lipid A crystal structure,[16] and the receptor region was specified via the docking box utility to include the entire backbone region plus part of the acyl chains. Twenty-five different poses were generated for each molecule and scored according to electrostatic and van der Waals interaction terms evaluated according to the MMFF94 force field.[17] The tabu algorithm[18] was used for conformation searching, optimizing the efficiency of the process by imposing restrictions on revisiting space previously explored in prior steps, thus increasing conformational diversity. Structures for the top scoring pose of each ligand have been merged into a single PDB format file available for download at http://www.msg.ku.edu/~msg/BMCL_9624/LipidA+ligands.pdb.

For the sake of brevity, the binding mode of the simplest of ligands tested (NCI10400) will be described (Fig. 2). NCI10400 binds to LPS by interaction with lipid A phosphate groups, whose P ↔ P distance is 13 Å. The AM1[19] energy-minimized conformation of NCI10400 yield a N ↔ N separation of 13.7 Å, a distance slightly longer than the 13.0 Å of the lipid A P ↔ P distance. However, when bound to lipid A, the NCI10400 is found to yield a N ↔ N distance 12.3 Å—very close to the shortest O ↔ O distance (12.6 Å) spanning the two phosphate groups, and slightly shorter than the previously determined pharmacophore distance of 14 Å.[7] A detailed search of over 100 poses for NCI10400 yielded a distributed sample of binding conformations, most of which displayed simultaneous interactions of NCI10400 with both phosphate groups. The root-mean-square-deviation (RMSD) in atomic positions relative to the highest ranked conformation ranged from 0 to 4 Å. The resulting conformations appeared to cluster with a bimodal distribution into two classes, the first of which had relatively small RMSD values (0–1.3 Å), while the latter two clusters had RMSD values larger than 1.76 Å (Fig. 3). Analysis of these classes revealed that in the first class the ligands bound to both phosphates, whereas in the second, the ligands interacted with only one phosphate (data not shown). Given that experimental evidence has been obtained for the requirement of simultaneous ionic H-bond ('salt-bridge') interactions of both protonated cationic groups with the lipid A phosphates,[12] members of the latter class were omitted from further consideration.

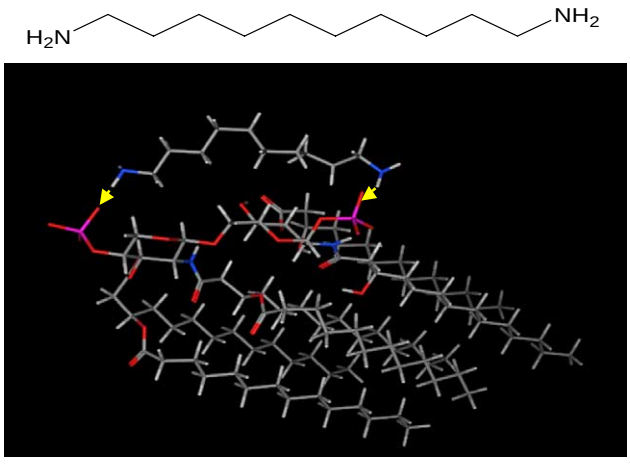

Figure 2. Binding conformation of NCI10400 to lipid A. Ionic H-bonds between the ligand amines and lipid A phosphates are shown in yellow.

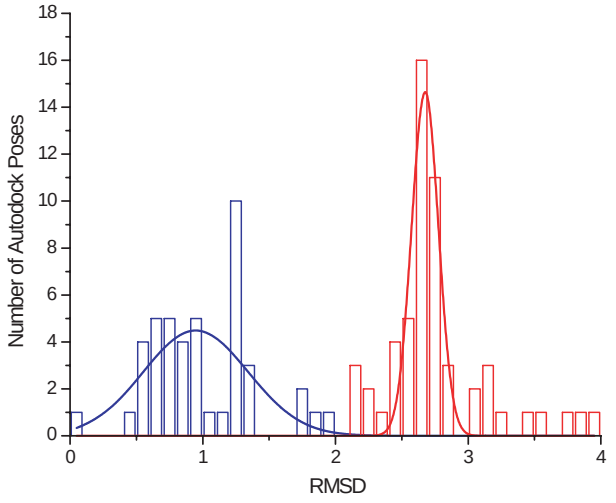

Figure 3. Bimodal distribution of RMSD values of 100 docked conformers of NCI10400. Only the low RMSD conformers (blue bars) show simultaneous ionic H-bonds with the lipid A phosphates as shown in Figure 2.

Given this correlation between experimental results and computational data of the bidentate model, poses resulting from conformational searches among the remaining 53 molecules studied were selectively filtered so as to identify those structures binding to both phosphate groups. Interestingly, we did not uncover any examples in the training set exhibiting H-bonding interactions with the other sites on the lipid A backbone.

Although our predicted enthalpic scores correlated well with experimental ED_{50} data ($R = 0.82$), (Fig. 4), the constituent individual ligand–receptor electrostatic and van der Waals interactions, as is evident from the correlation matrix examining (Fig. 5), do not correlate well. Specifically coefficients relative to experiment are only 0.05 and −0.01, respectively. What this collectively indicates is that: (a) the main electrostatic interaction between ligands and lipid A phosphates dominates

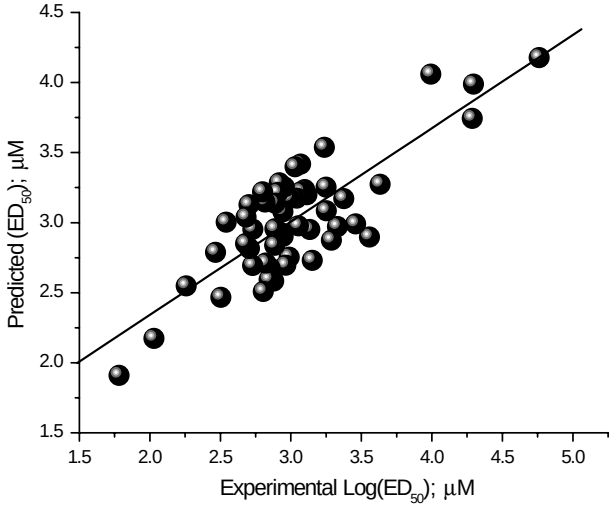

Figure 4. Correlation of experimental and predicted ED_{50} values.

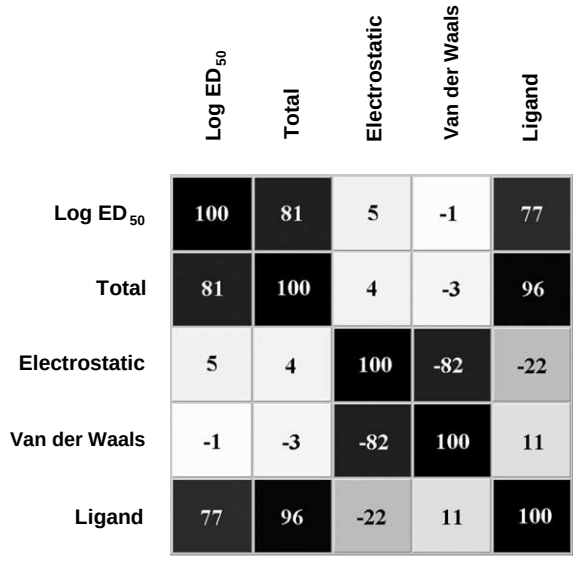

Figure 5. Correlation matrix of $\log(ED_{50})$ versus the total docking score, electrostatic, van der Waals, and internal ligand components of the score. The density of cell shading reflects the strength of correlation.

the expression and varies little across the series, (b) the other ligand–receptor interactions seem to play a little role in governing the observed variations in the relative ligand efficacy. In fact, it appears that the ligand energy ($R = 0.77$) plays the primary role in differentiating the various efficacies. Statistically reweighting these components via regression analysis did not improve the correlation between the calculation and the experimental value, giving the same $R^2 = 0.66$.

In conclusion, exploring the LPS binding modes of a set of 54 primary and secondary bis-amino compounds via molecular docking simulations has allowed us to verify that the binding mode is dominated by interaction with the phosphate groups. We have also determined that within a relatively diverse set of compounds, the ligand internal energy appears to be the primary factor in distinguishing variations in binding efficacy from one ligand to another. This may suggest that the conformational strain induced in forcing the ligand to adhere to a bidentate binding mode may be key to the process. Given a fundamental scaffold that exhibits both a strong bidentate overlap with the lipid A phosphate groups and minimal intra-ligand strain, we believe it would be beneficial to explore further the placement of potential hydrogen bonding donors/acceptors capable of exploiting two available acceptors within the lipid A headgroup, as well as hydrophobic groups suitable for interacting with the acyl chains. A candidate for such a scaffold is the molecule NCI22905, which exhibits both an optimal ED_{50} among this set of compounds as well as the best in silico docking score.

Acknowledgments

This work was supported from NIH Grants 5U01AI-056476 and 1R01 AI50107 (S. David).

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.10.025.

References and notes

1. Gasche, Y. D. P.; Sutter, P. *Outcome and Prognostic Factors in Bacteremic Sepsis*; Springer: Berlin, 1995, p 35.
2. *MMWR* **1990**, *39*, 31.
3. Cross, A. S. a. O. S. *J. Endotoxin. Res.* **1994**, *1*, 57.
4. Rietschel, E. T.; Kirikae, T.; Schade, F. U.; Mamat, U.; Schmidt, G.; Loppnow, H.; Ulmer, A. J.; Zahringer, U.; Seydel, U.; Di Padova, F., et al. *FASEB J.* **1994**, *8*, 8.
5. Galanos, C.; Luderitz, O.; Rietschel, E. T.; Westphal, O.; Brade, H.; Brade, L.; Freudenberg, M.; Schade, U.; Imoto, M.; Yoshimura, H., et al. *Eur. J. Biochem.* **1985**, *148*, 1.
6. Miller, K. A.; Suresh Kumar, E. V.; Wood, S. J.; Cromer, J. R.; Dutta, A.; David, S. A. *J. Med. Chem.* **2005**, *48*, 2589.
7. David, S. A. *J. Mol. Recognit.* **2001**, *14*, 370.
8. David, S. A.; Silverstein, R.; Amura, C. R.; Kielian, T.; Morrison, D. C. *Antimicrob. Agents Chemother.* **1999**, *43*, 912.
9. Burns, M. R.; Wood, S. J.; Miller, K. A.; Nguyen, T.; Cromer, J. R.; David, S. A. *Bioorg. Med. Chem.* **2005**, *13*, 25.
10. Blagbrough, I. S.; Geall, A. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1959.
11. David, S. A. M. V. I. *J. Endotoxin Res.* **1995**, *2*, 325.
12. David, S. A.; Bechtel, B.; Annaiah, C.; Mathan, V. I. *Biochim. Biophys. Acta* **1994**, *1212*, 167.
13. Wood, S. J.; Miller, K. A. *Comb. Chem. High Throughput Screen.* **2004**, *7*, 239.
14. Wood, S. J.; Miller, K. A. *Comb. Chem. High Throughput Screen.* **2004**, *7*, 733.
15. *Molecular Operating Environment (MOE) 2002.03*, Chemical Computing Group.
16. Ferguson, A. D.; Hofmann, E.; Coulton, J. W.; Diederichs, K. *Science* **1998**, *282*, 2215.
17. Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720.
18. Westhead, D. R.; Clark, D. E. *J. Comput. Aid. Mol. Des.* **1997**, *11*, 209.
19. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1995**, *107*, 3902.