Published in final edited form as:
Bioorg Med Chem Lett. 2006 December 15; 16(24): 6209–6212.

Structural Correlation Between Lipophilicity and Lipopolysaccharide-sequestering activity in Spermine-Sulfonamide Analogs

Mark R. Burns[a], **Scott A. Jenkins**[a], **Nicolas M. Vermeulen**[a], **Rajalakshmi Balakrishna**[b], **Thuan B. Nguyen**[b], **Matthew R. Kimbrell**[b], and **Sunil A. David**[b]

a *MediQuest Therapeutics, Inc., 22322 20th Ave. SE, Bothell, WA 98021*

b *Department of Medicinal Chemistry, University of Kansas, Life Sciences Research Laboratories, 1501 Wakarusa Drive, Lawrence, KS 66049*

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 had previously defined the pharmacophore necessary for small molecules to specifically bind and neutralize this complex carbohydrate. A series of aryl and aliphatic spermine-sulfonamide analogs were synthesized and tested in a series of binding and cell-based assays in order to probe the effect of lipophilicity on sequestration ability. A strong correlation was indeed found, supporting the hypothesis that endotoxin-neutralizing ability involves a lipophilic or membrane attachment event. The research discussed herein may be useful for the design of additional carbohydrate recognizing molecules and endotoxin-neutralizing drugs.

Lipopolysaccharides (LPS) are the predominant structural components of the outer membrane of Gram-negative bacteria.[1,2] Otherwise termed ‘endotoxins’, LPS play a pivotal role in septic shock, a syndrome of systemic toxicity which occurs frequently when the body’s defense mechanisms are compromised.[3–6] Gram-negative sepsis is the thirteenth leading cause of overall mortality[7] and the number one cause of deaths in the intensive care unit,[8] accounting for more than 200,000 fatalities in the US annually.[9] The presence of LPS in the systemic circulation causes an uncontrolled activation of the innate immune system[10,11] leading to the production of numerous inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-1 β (IL-1β), and interleukin-6 (IL-6),[12,13] culminating in the frequently-fatal multiple system organ failure.

The toxic moiety of LPS resides in its glycolipid component called Lipid A,[14] which is composed of a hydrophilic, *bis*-phosphorylated diglucosamine backbone, and a hydrophobic domain of 6 (*E. coli*) or 7 (*Salmonella*) acyl chains[14] (Fig. 1). The pharmacophore necessary for the neutralization of lipid A[15] by small molecules requires two groups protonatable at physiological pH, with an intervening distance of ~ 14 Å, enabling ionic H-bonds between the cationic groups and the lipid A phosphates. In addition, appropriately positioned pendant hydrophobic functionalities are required to further stabilize the resultant complexes via hydrophobic interactions with the polyacyl domain of lipid A (for a recent review, see Ref. 16). In two recent studies of the effect of the hydrocarbon chain length in two series of acylhomospermines, it was shown that C_{16} is the ideal lipophilic substituent, corresponding to maximal affinity, optimal aqueous solubility (and bioavailability), and neutralization potency.[17,18] In building on our earlier work focused upon acylated spermines, this paper examines spermine-sulfonamides composed of various numbers (mono-, di- and tri-substituted) and types of lipophilic groups with a view to correlating such structural modifications on the affinity of binding to LPS and on the potency of abrogating endotoxicity in *in vitro* neutralization

assays. We specifically address several key questions: (i) will spermine-sulfonamides display similar activity to previously studied acylspermines, (ii) what is the relationship between lipophilicity of the substituent hydrophobic groups and binding activity, and (iii) can highly lipophilic aryl groups effectively mimic long hydrocarbon chains?

The analogs described were produced as shown in Scheme 1 (Supplemental Data).

The relative binding affinities of the analogs are reported in Table 1 (*Bis*-substituted analogs were also isolated and showed higher ED_{50} values, for the sake of brevity, representative data on the mono-substituted compounds are shown) as half-maximal effective displacement of probe (ED_{50}) obtained using a high-throughput fluorescence based displacement assay, employing BODIPY-TR cadaverine (BC).[19,20] Polymyxin B (PMB), a decapeptide antibiotic, known to bind and neutralize LPS,[21–24] was used as a reference compound.

As is evident in Table 1, a clear trend towards higher-affinity binding by those analogs with more lipophilic substituents was observed. An especially instructive series was the chloro-substituted phenyl devivatives: phenyl (**3**, 2955 μM), 4-monochloro (**7**, 158 μM), 2,4-dichloro (**8**, 58 μM), 2,4,5-trichloro (**12**, 18.7 μM), and 2,3,4-trichloro (**13**, 9.08 μM).

The correlation between lipophilicity and binding was also observed for the aliphatic monosulfonamides: butyl (**1**, >10,000 μM), octyl (**15**, 5.43 μM), decyl (**17**, 3.7 μM), dodecyl (**18**, 2.29 μM), hexadecyl (**19**, 2.34 μM), and octadecyl (**14**, 6.01 μM). Both series display maximal binding affinity leveling off at a chain length of 16. We analyzed the correlation between experimentally determined binding activity for the monosulfonamide spermine analogs, and their calculated lipophilicity (cLogP). As shown in Figure 2, a distinct correlation between these two parameters was observed, with an R^2 value of 0.756 for a first-order exponential fit.

Inhibition of LPS-induced nitric oxide (NO) production (measured as nitrite) by the sulfonamide analogs in murine J774 cells was performed as published previously.[17,25] In parallel we also examined dose-responses in the inhibition of induction of NF-κB (a key transcriptional activator of the innate immune system, leading to uncontrolled cytokine release[25,26]). NF-κB was quantified using human embryonic kidney 293 cells cotransfected with TLR4 (LPS receptor), CD14 and MD2 (co-receptors), available from InvivoGen, Inc., (HEK-Blue™, San Diego, CA) as described elsewhere.[26] A clear relationship between binding affinity and both NO and NF-κB *in vitro* IC_{50} values are evident (Fig. 3). While the dynamic range for the BC displacement assay is very large (nM to mM),[35] linearity for both the NO and NF-κB assays are limiting, resulting in significant deviations from nonlinearity for IC_{50} values above 30 μM (1.5 on the log scale). Indeed, piece-wise linear regressions yielded R values of 0.78 and 0.83 for the NO and NF-κB assays, respectively (Fig. 3).

We have established that NF-κB inhibition is best correlated with a definitive murine model[17,25] of endotoxic shock. We therefore sought to compare the potencies of the high affinity long-chain monosulfonamides with PMB. As shown in Fig. 4, several long-chain monosulfonamide compounds compare very favorably with PMB, the dose-response for **19** being virtually indistinguishable from that of PMB (Fig. 4).

These results confirm that long-chain aliphatic appendages on spermine-like polyamine scaffolds yield the most potent anti-endotoxin compounds as a consequence of imbuing optimal hydrophobic character. Simple cLogP calculations may thus serve as a useful tool in conjunction with detailed *in silico* design and docking experiments[27] to construct focused libraries. As the last group of mostly unexploited drug targets, complex carbohydrates represent an especially challenging group of biomolecules with which to design specific recognition molecules. The complex interplay of hydrogen-bond complementarity, charge and lipophilicity

in interaction with membrane-associated receptors greatly contributes to this challenge. The set of molecules described in this report showcase the role that lipophilicity plays in Lipid A recognition. Given their ease of synthesis, potent LPS-neutralizing effects and activity in a cell-based assay, these spermine sulfonamide analogs may be serve as candidates for animal testing and further preclinical development. A detailed characterization of the endotoxin-sequestering activities of **19** will be reported elsewhere.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported from NIH Grant 1U01 AI054785 (S. David).

References

1. Lüderitz O, Galanos C, Rietschel ET. PharmacolTher 1982;15:383–402.
2. Rietschel ET, Kirikae T, Schade FU, Mamat U, Schmidt G, Loppnow H, Ulmer AJ, Zähringer U, Seydel U, Di Padova F, et al. FASEB J 1994;8:217–225. [PubMed: 8119492]
3. Hurley JC. ClinInfectDis 1992;15:840–854.
4. Hurley JC. Drug Saf 1995;12:183–195. [PubMed: 7619330]
5. Prins JM, van Agtmael MA, Kuijper EJ, van Deventer SJ, Speelman P. JInfectDis 1995;172:886–891.
6. Prins JM, Van Deventer SJH, Kuijper EJ, Speelman P. AntimicrobAgents Chemother 1994;38:1211–1218.
7. Gelfand JA, Shapiro L. New Horizons 1993;1:13–22. [PubMed: 7922386]
8. Gasche, Y.; Pittet, D.; Sutter, P. Outcome and prognostic factors in bacteremic sepsis. In: Sibbald, WJ.; Vincent, JL., editors. Clinical trials for treatment of sepsis. 95. Springer-Verlag: Berlin; p. 35-51.
9. MMWR 1990;39:31–34.
10. Ulevitch RJ. ImmunolRes 2000;21:49–54.
11. Ulevitch RJ, Tobias P. CurrOpinImmunol 1999;11:19–23.
12. Dinarello CA. CurrTopMicrobiolImmunol 1996;216:133–165.
13. Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, Cerami A, Wolff SM, Wilmore DW. NEnglJMed 1988;318:1481–1486.
14. Raetz CRH, Whitfield C. AnnuRevBiochem 2002;71:635–700.
15. David SA, Mathan VI, Balaram P. JEndotoxinRes 1995;2:325–336.
16. David SA. JMolecRecognition 2001;14:370–387.
17. Miller KA, Suresh Kumar EVK, Wood SJ, Cromer JR, Datta A, David SA. JMedChem 2005;48:2589–2599.
18. Burns MR, Wood SJ, Miller KA, Nguyen T, Cromer JR, David SA. BioorgMedChem 2005;13:2523–2536.
19. Wood SJ, Miller KA, David SA. CombChemHighThroughputScreen 2004;7:239–249.
20. Wood SJ, Miller KA, David SA. CombChemHighThroughputScreen 2004;7:733–743.
21. Aoki H, Kodama M, Tani T, Hanasawa K. AmJSurg 1994;167:412–417.
22. Bucklin SE, Lake P, Logdberg L, Morrison DC. AntimicrobAgents Chemother 1995;39:1462–1466.
23. Mayumi T, Takezawa J, Takahashi H, Kuwayama N, Fukuoka T, Shimizu K, Yamada K, Kondo S, Aono K. Shock 1999;11:82–86. [PubMed: 10030792]
24. Stokes DC, Shenep JL, Fishman ML, Hidner WK, Bysani GK, Rufus K. JInfectDis 1989;160:52–57.
25. David SA, Silverstein R, Amura CR, Kielian T, Morrison DC. AntimicrobAgents Chemother 1999;43:912–919.
26. Khownium K, Wood SJ, Miller KA, Balakrishna R, Nguyen TB, Kimbrell MR, Georg GI, David SA. BioorgMedChemLett 2006;16:1305–1308.

27. Guo JX, Wood SJ, David SA, Lushington GH. BioorgMedChemLett 2006;16:714–717.

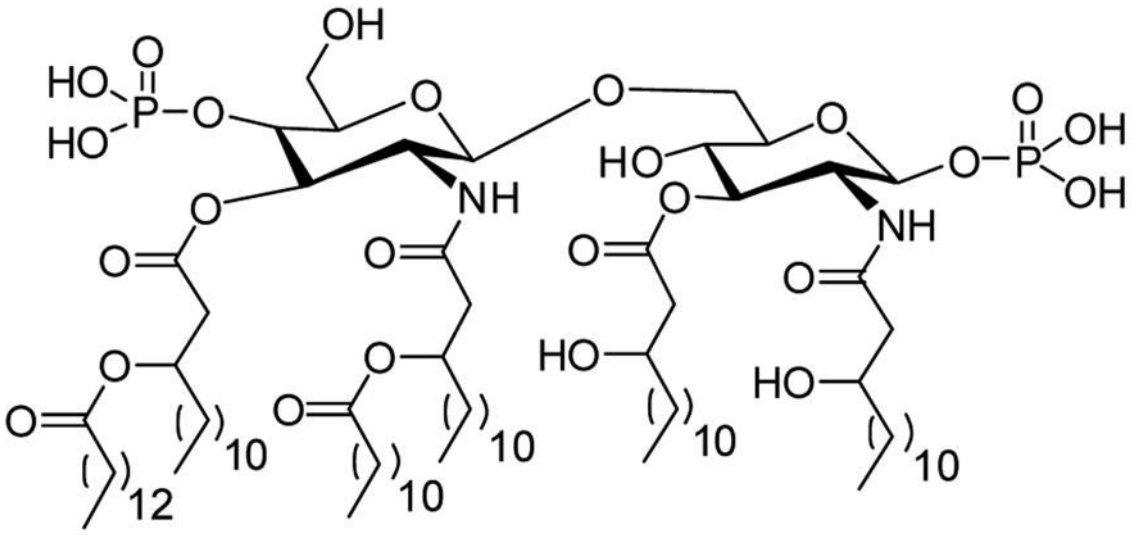


Figure 1.
Structure of Lipid A, the toxic principle of lipopolysaccharide.

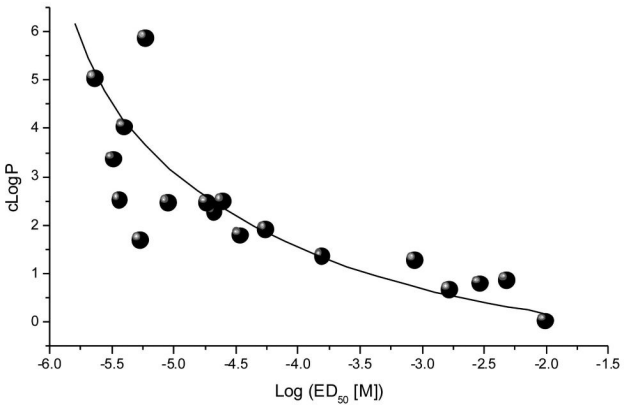


Fig 2.
Correlation of binding affinity and lipophilicity in spermine monosulfonamides

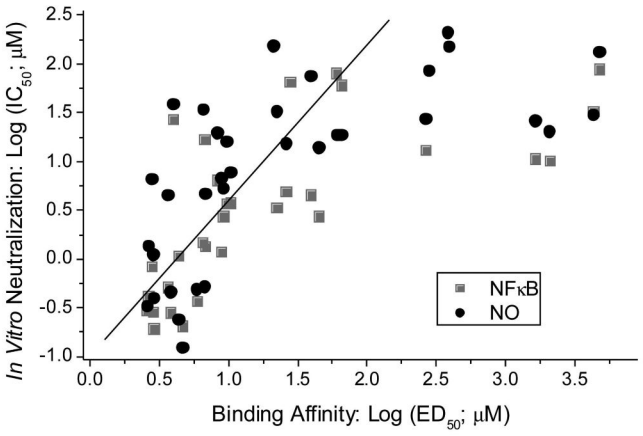


Fig 3.

Correlation of binding affinity with NF-κB and NO inhibition *in vitro* of the monosulfonamides. Piece-wise linear regression lines show the experimentally useful range of linearity (≤ 100 μM; solid line).

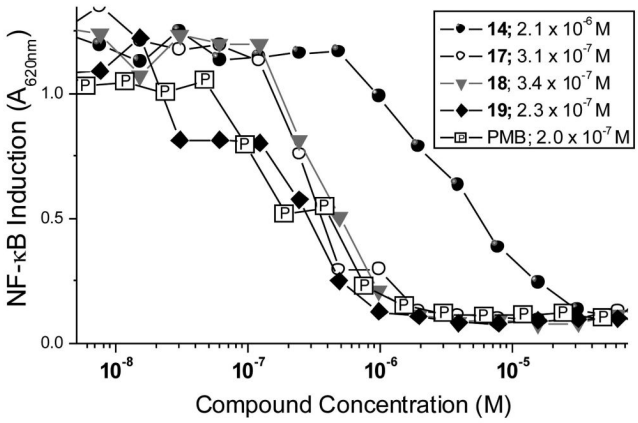
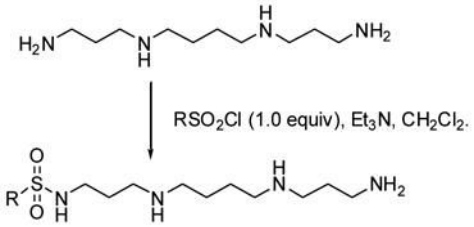


Fig 4.

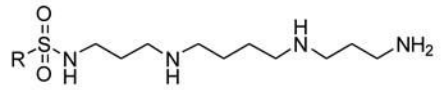
Comparison of NF-kB inhibition by long-chain sulfonamides and polymyxin B. IC_{50} values were determined by four-parameter logistic fits.

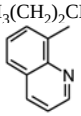
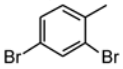
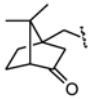
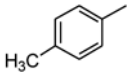
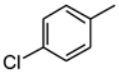
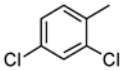
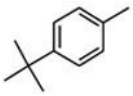
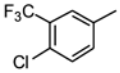
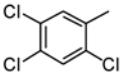
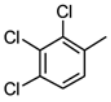
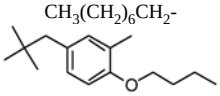


Scheme 1.
Synthesis of spermine sulfonamides.

Table 1

Binding affinity of mono-substituted spermine sulfonamides.



Analog	R	Binding ED_{50} (μM)[a]
PMB	---	0.31
1	$CH_3(CH_2)_2CH_2$-	>10,000
2		4877
3		2955
4		2108
5		1675
6		884
7		158
8		56
9		34
10		25
11		21
12		19
13		9
14	$CH_3(CH_2)_{16}CH_2$-	6
15	$CH_3(CH_2)_6CH_2$-	5
16		4
17	$CH_3(CH_2)_8CH_2$-	3
18	$CH_3(CH_2)_{10}CH_2$-	3
19	$CH_3(CH_2)_{14}CH_2$-	2