

Structural correlation between lipophilicity and lipopolysaccharide-sequestering activity in spermine-sulfonamide analogs

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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.

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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 mortality⁷ 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-1 β), 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, which is composed of a hydrophilic, bis-phosphorylated diglucosamine backbone, and a hydrophobic domain of 6 (*Escherichia coli*) or

7 (*Salmonella*) acyl chains¹⁴ (Fig. 1). The pharmacophore necessary for the neutralization of lipid A¹⁵ 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 C₁₆ is the ideal lipophilic substituent, corresponding to maximal affinity, optimal aqueous solubil-

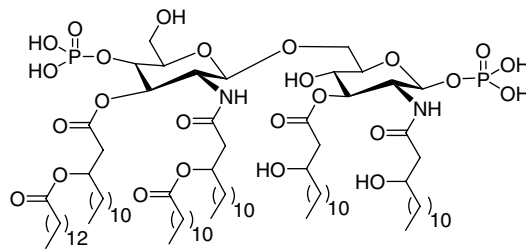


Figure 1. Structure of Lipid A, the toxic principle of lipopolysaccharide.

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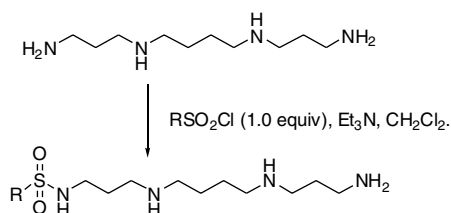
ity (and bioavailability), and neutralization potency.^{17,18} In building on our earlier work focused upon acylated spermines, this paper examines spermine-sulfonamides composed of various types of lipophilic groups with a view to correlating such structural modifications on the affinity of binding 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 ED₅₀ values, for the sake of brevity, representative data on the mono-substituted compounds are shown) as half-maximal effective displacement of probe (ED₅₀) 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 toward higher-affinity binding by those analogs with more lipophilic substituents was observed. An especially instructive series was the chloro-substituted phenyl derivatives: phenyl (**3**, 2955 μM), 4-monochloro (**7**, 158 μM), 2,4-dichloro (**8**, 56 μM), 2,4,5-trichloro (**12**, 19 μM), and 2,3,4-trichloro (**13**, 9 μM).

The correlation between lipophilicity and binding was also observed for the aliphatic monosulfonamides: butyl (**1**, >10,000 μM), octyl (**15**, 5 μM), decyl (**17**, 3 μM), dodecyl (**18**, 3 μM), hexadecyl (**19**, 2 μM), and octadecyl (**14**, 6 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 previous-



Scheme 1. Synthesis of spermine-sulfonamides.

Table 1. Binding affinity of mono-substituted spermine-sulfonamides

Analog	R	Binding ED ₅₀ (μM)
PMB	—	0.31
1	CH ₃ (CH ₂) ₂ CH 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) ₆ CH ₂ —	5
16		4
17	CH ₃ (CH ₂) ₈ CH ₂ —	3
18	CH ₃ (CH ₂) ₁₀ CH ₂ —	3
19	CH ₃ (CH ₂) ₁₄ CH ₂ —	2

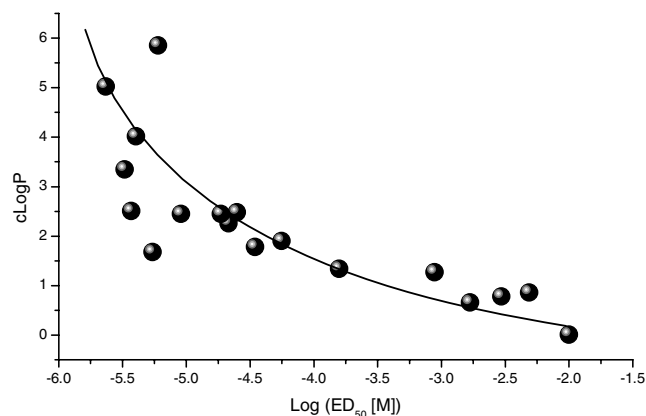


Figure 2. Correlation of binding affinity and lipophilicity in spermine monosulfonamides.

ly.^{17,25} In parallel, we also examined dose-responses in the inhibition of induction of NF- κ B (a key transcriptional activator of the innate immune system, leading to uncontrolled cytokine release)^{25,26}. NF- κ B 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- κ B in vitro IC₅₀ values is evident (Fig. 3). While the dynamic range for the BC displacement assay is very large (nM to mM), linearity for both the NO and NF- κ B assays is limiting, resulting in significant deviations from nonlinearity for IC₅₀ values above 30 μ M (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- κ B assays, respectively (Fig. 3).

We have established that NF- κ B 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 Figure 4, several long-chain monosulfonamide compounds compare very favorably with

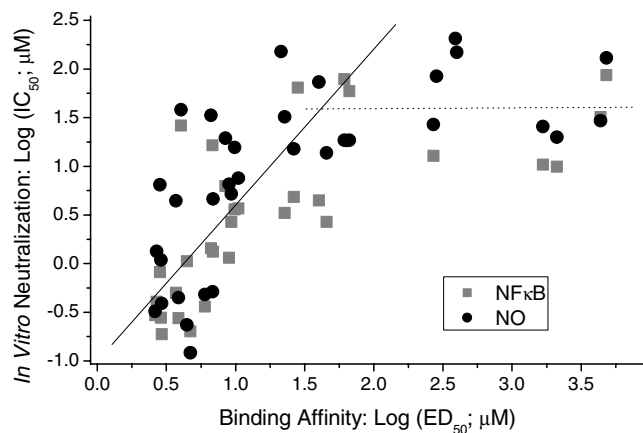


Figure 3. Correlation of binding affinity with NF- κ B and NO inhibition in vitro of the monosulfonamides. Piece-wise linear regression lines show the experimentally useful range of linearity (≤ 100 μ M; solid line).

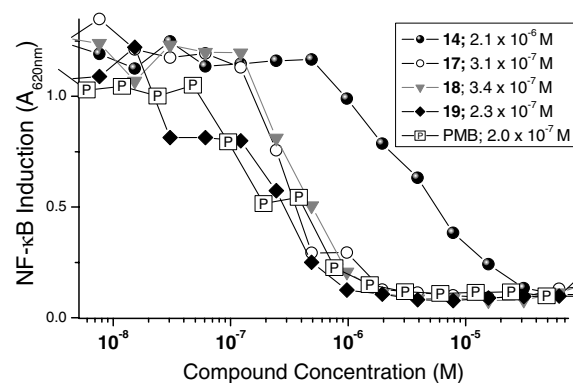


Figure 4. Comparison of NF- κ B inhibition by long-chain sulfonamides and polymyxin B. IC₅₀ values were determined by four-parameter logistic fits.

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 *c*Log *P* calculations may thus serve as a useful tool in conjunction with detailed in silico design and docking experiments²⁷ 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 showcases 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 serve as candidates for animal testing and further preclinical development. A detailed characterization of the endotoxin-sequestering activities of **19** will be reported elsewhere.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bmcl.2006.09.026](https://doi.org/10.1016/j.bmcl.2006.09.026).

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