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Small molecule inhibitors of anthrax lethal factor toxin

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ABSTRACT

This manuscript describes the preparation of new small molecule inhibitors of *Bacillus anthracis* lethal factor. Our starting point was the symmetrical, bis-quinolinyl compound **1** (NSC 12155). Optimization of one half of this molecule led to new LF inhibitors that were desymmetrized to afford more drug-like compounds.

1. Introduction

Aerosolized spores of *Bacillus anthracis* represent one of the most serious bioterrorist threats to the security of the United States. Inhalation of these environmentally stable spores progresses rapidly to a highly fatal systemic infection. In 2001, this category A agent was used in several acts of terrorism in the United States. While ciprofloxacin and doxycycline were effective if administered immediately after suspected contact with *B. anthracis*, there is little doubt that capable terrorists will seek to develop forms of the bacterium resistant to these common antibiotics, especially since mechanisms of resistance are known and resistant alleles may be generated readily. New therapeutic agents based on novel chemical scaffolds are vital to biodefense because they are likely to be effective against both natural and engineered resistant forms of *B. anthracis*, and because there are no pre-existing resistance alleles.

Bacillus anthracis is the cause of the acute and often lethal disease called anthrax.[1] In humans, anthrax is a rare infection acquired by inhalation, ingestion or cutaneous contact with the endospores of *B. anthracis*.[2,3] Anthrax infection has, until recently, been observed mostly in farm animals and in humans who have had direct contact with contaminated animals or animal products. However, for approximately ten years, the Centers for Disease Control (CDC) and public health authorities have been investigating cases of bioterrorism-related anthrax exposure and death in the United States.[4] The CDC recognizes *B. anthracis* as a category A agent of bioterrorism. It is a serious bioterrorism threat because its spores are stable under extreme conditions in the environment, are easily produced and distributed by aerosol (in a powder form), and are highly fatal via inhalation.[5] Penetration of spores via the skin or gastrointestinal tract is generally less dangerous and results in mainly localized disease.

Therapy recommended by the CDC includes the use of ciprofloxacin or doxycycline as well as rifampin, vancomycin, imipenem, clindamycin or chloramphenicol.[6] Beta-lactamases have been found in recent clinical isolates, precluding the use of penicillins for the treatment of anthrax.[6] In addition, researchers have been able to generate ciprofloxacin resistant and doxycycline resistant strains of *B. anthracis* in the laboratory,[7,8] which demonstrates that unscrupulous scientists could engineer drug resistance into strains of the species.

The *B. anthracis* bacterium-secreted endotoxin is comprised of three components: (1) a zinc metallopreotease lethal factor (LF); (2) a calmodulin-activated edema factor adenylate cyclase (EF); and (3) a protective antigen (PA).[9] Although these proteins are independently nontoxic, their concerted action disrupts cell signaling events and can lead to cell death. Importantly, lethal factor combines with protective antigen to form the lethal factor toxin.[10]

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When lethal factor is translocated into the cytoplasm of host target cells, lethal factor cleaves MAP kinase kinases (MAPKKs) and disrupts the signaling pathway mediated by these MAPKKs. NOD-like receptor (NLR) Nlrp1 protein has recently been identified as a physiologically relevant substrate of anthrax lethal toxin,[11–13] and interactions of anthrax lethal factor with protective antigen has been defined by site-directed spin labeling studies.[14]

Lethal factor plays a critical role in all stages of anthrax infection; in the early stages it assists the bacteria by helping evade the host immune system, and in the later stages, when infection is systemic, it targets the epithelial cells to cause vascular barrier dysfunction.[15,16] Zinc metalloprotease enzymes are a well-studied class of enzymes and many are drug targets for which potent inhibitors have been designed.[17] For these reasons, we chose to exploit a lethal factor inhibitor that we discovered in a screening campaign, and to optimize this screening hit for potential use as an antidote for anthrax intoxication. We specifically chose a non-hydroxamic acid inhibitor to increase our chances of finding a drug-like, metabolically stable inhibitor that would allow us to develop a series of compounds with improved pharmacokinetic properties and fewer undesired interactions.

In Figure 1 is shown a cartoon that displays a rudimentary understanding of binding, assembly, uptake and mechanism of action for *B. anthracis*. A variety of approaches have been taken to intervene at different points of this process to prevent anthrax toxin function. Human antibodies against PA that block receptor binding[18] and antibodies specific for lethal factor (LF) that interfere with assembly of the toxin components[19] have been shown to prevent lethal toxin induced death in rodents. Soluble TEM8/ATR and CMG2 proteins have also been shown to be effective in cell culture, preventing anthrax toxin receptor binding.[20,21] Hexa-D-arginine, a furin inhibitor that blocks PA cleavage, has been shown to delay anthrax toxin-mediated cell cytotoxicity in vitro and reduce lethal toxin mediated lethality in rats.[22] Dominant-negative PA mutants have been generated, which co-assemble with the wild-type PA protein, preventing translocation of LF or EF across the cell membrane.[23]

Peptides and peptide-based analogs have also been shown to inhibit anthrax toxin. In one approach, polyvalent peptide inhibitors, which block LF or EF association with the PA pre-pore, have been shown to protect rats from lethal toxin.[24] A second peptide approach targeted the LF active site. LF has a deep and long (40 Å) groove, with an overall negative electrostatic potential, contiguous to the active site, that binds peptide substrates and peptide inhibitors.[25,26] Several peptide inhibitors of LF activity have been identified on the basis of consensus sequences of the MAPKKs.[24,26–29] Incorporation of zinc-binding chemical groups into these peptides greatly enhanced their potency.[30]

The LF target is attractive because it is a zinc-dependent metalloprotease enzyme, and there is a wealth of information that has been assembled for the inhibition of this class of enzyme; a crystal structure for a rhodanine derivative complexed with lethal factor has been reported.[31,32] Several research groups have developed small molecule LF inhibitor programs based on either structural information or high-throughput screening hits; a collection of small molecule inhibitors of anthrax lethal factor is shown in Figure 2. The bis-quinoline **1** is the screening hit[33] around which we began our synthetic efforts of hit and lead optimization. Benzopyranone **2**[34] was shown to be an inhibitor of *Botulinum* neurotoxin A in addition to LF. A class of acylhydrazones represented by compound **3** also blocked the cleavage of MEK1 in 293T cells.[35] The use of a hydroxythiopyranone core as a hydroxamic acid replacement led to the synthesis of compound **4**, which represented a class of inhibitor that was more potent than the corresponding hydroxypyrones.[36,37] Furan **5** was one of several compounds discovered in a HPLC-based high throughput screen in which 'fragment-based focusing' was used to enrich the pool of potential inhibitors.[38] Related compound **6** was designed using an iterative NMR-based binding assay of LF inhibitors and confirmed by X-ray crystallography.[32] Chiral sulfonamide **7** is one of the most potent LF inhibitors reported;[15,16] a synthetic route using asymmetric hydrogenation was also reported for this inhibitor.[39] Hydroxamic acids, which are known zinc-binding agents that have been widely used for the inhibition of a variety of metalloproteases, have also been used

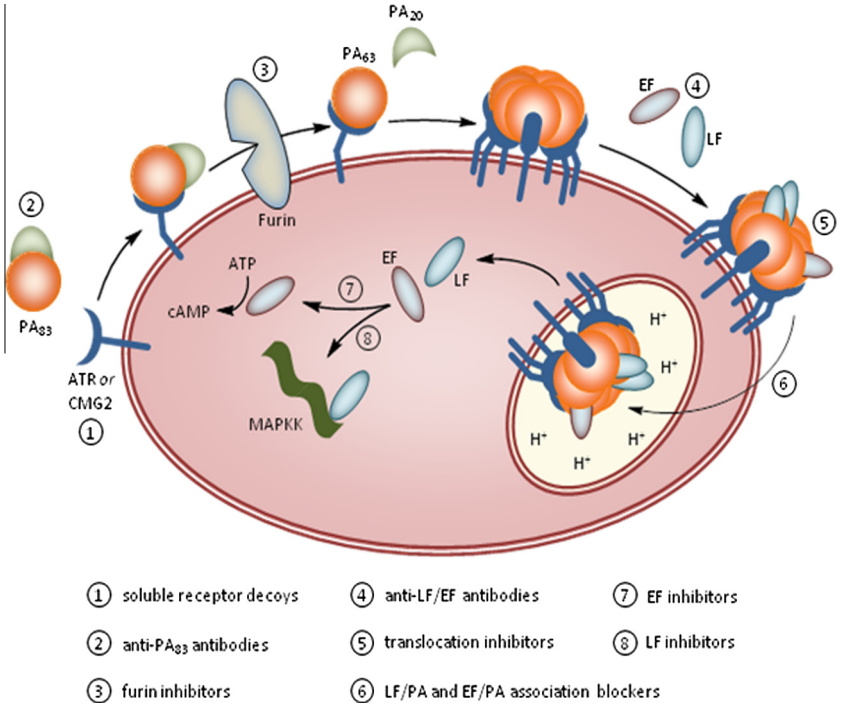

Figure 1. Potential drug targets and points of intervention for the prevention of *Bacillus anthracis* function.