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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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(54) **Title**: DERIVATIVES OF α-, β- AND γ-CYCLODEXTRIN AND THEIR USE AS ANTI-INFECTIVES

(57) **Abstract**: Alpha-, beta- and gamma-cyclodextrins (CDs), per-substituted at positions 2, 3, and 6 with amino, aminoalkylamino, guanidinoalkylamino, and certain other functional groups, inhibit a biological activity of anthrax lethal toxin (LeTx), staphylococcal α-hemolysin (alpha-HL) toxin, and epsilon-toxin produced by Clostridium perfringens or block the ion current though the transmembrane pores formed by those toxins. Specific compounds useful for treating, preventing, or delaying a disease condition caused by Bacillus anthracis, Staphylococcus aureus, and Clostridium perfringens were identified.

DERIVATIVES OF ALPHA-, BETA-, AND GAMMA-CYCLODEXTRIN AND THEIR USE AS ANTI-INFECTIVES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application 61/482,108 filed on May 3, 2011, the entire content of which is hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to the development of symmetry-based small molecule blockers of pore-forming virulence factors and their use as anti-infectives.

BACKGROUND OF THE INVENTION

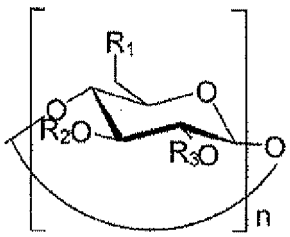
We have previously proposed an approach for the discovery of inhibitors of pore-forming toxins that involves selection of molecules with comparable dimensions and the same symmetry as the target pores and capable of blocking the pores. We suggested that this approach would allow highly efficient identification of leads using orders of magnitude fewer initial screenings of candidate compounds compared to the standard High-Throughput Screening approach employed in pharmaceutical drug discovery. The identification of small molecule drug candidates can be performed faster and significantly cheaper in comparison with the existing industry standards. We have successfully applied this approach to anthrax toxin which plays a key role in anthrax infection. The toxin was disabled using high-affinity blockage of the pore formed by protective antigen (PA) subunit of anthrax lethal toxin (LeTx) by rationally designed low-molecular weight compounds. Guided by the seven-fold symmetry of the PA pore, cyclic molecules of seven-fold symmetry using β-cyclodextrin as a starting molecule were synthesized and tested (Fig. 1). The hydroxyls at positions 2 and 3 form hydrogen bonds and are required to keep the molecule rigid, making the 6-OH group a favorable site for modifications. We successfully tested the discovered inhibitors of anthrax toxin *in vitro* and *in vivo*.[1-6]

SUMMARY OF THE INVENTION

We identified the structural features of the pore blocking cyclodextrin (CD) compounds that affect their ability to inhibit the cytotoxic activities of anthrax lethal toxin, α-hemolysin (α-HL) toxin of *Staphylococcus aureus,* and ε-toxin produced by *Clostridium perfringens* as well as to block the ion current through the channels formed by PA and α-HL in artificial membranes. These toxin targets form trans-membrane pores with sevenfold symmetry[5,7]

The inventor discovered that not only β-CD derivatives, but also certain α- and γ-CDs can be utilized as blockers of pore-forming virulence factors and as anti-infectives. Furthermore, it was also established that cyclodextrins carrying substitute groups at positions 2 and 3, rather than at position 6, can be utilized as blockers of pore-forming virulence factors and as anti-infectives.

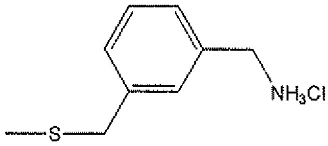
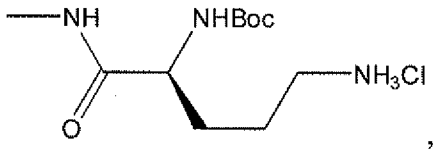
Accordingly, one aspect of the present invention is directed to a compound for use in treating a disease condition caused by a pore-forming toxin, said compound having the general formula:

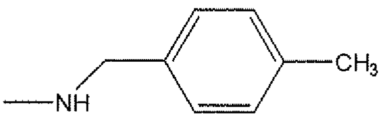
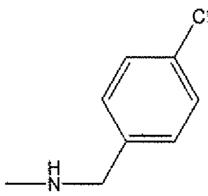
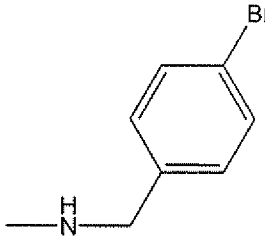


, wherein n = 6, 7, or 8, depending on the geometry of the pore opening; $R_2 = R_3 =$ H, OH, ethylamine, or butylamine; and $R_1 =$ any of the substituents shown in tables 1 and 2.

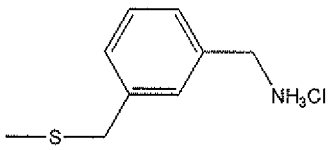
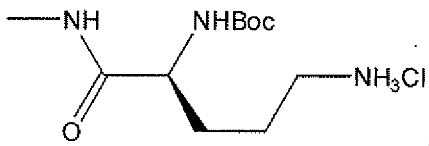
In one preferred embodiment, the pore-forming toxin is α-HL or ε-toxin, and the compound is one in which:

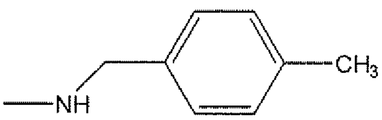
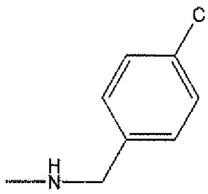
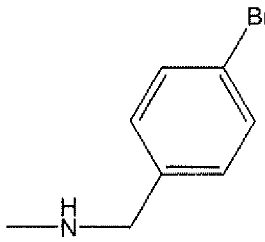
$R_2 = R_3 =$ H, OH, $(CH_2)_2NH_2$ or $(CH_2)_3NH_2$ and

R_1 = OH, NH_2,  ,  ,

 ,  ,  , or

$N(CH_2)_4CH_3$. wherein n = 7, $R_2 = R_3$ = H, OH, $(CH_2)_2NH_2$ or $(CH_2)_3NH_2$ and

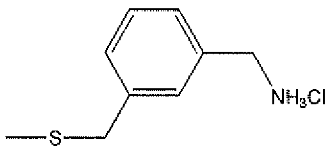
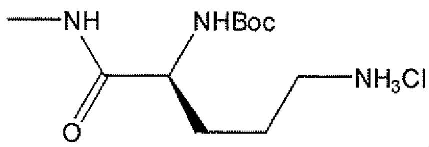
R_1 = OH, NH_2,  ,  ,

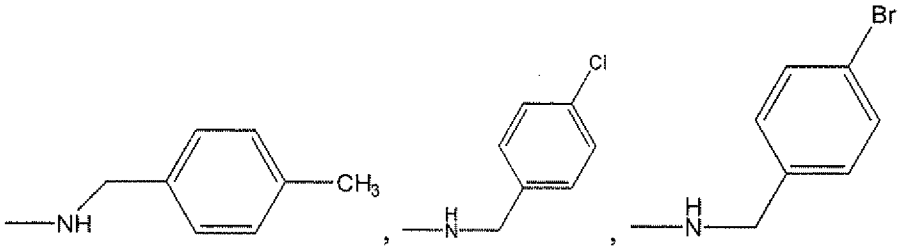
 ,  ,  , or

$N(CH_2)_4CH_3$.

In another preferred embodiment, the pore-forming toxin is anthrax toxin and the compound is one in which:

n = 6 or 8, and $R_2 = R_3$ = H, OH, $(CH_2)_2NH_2$ or $(CH_2)_3NH_2$ and

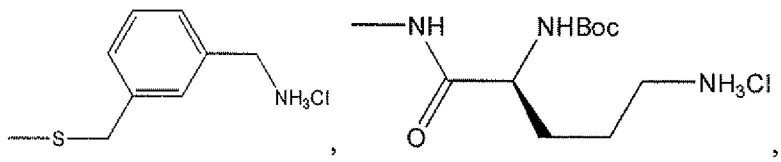
R_1 = OH, NH_2,  ,  ,

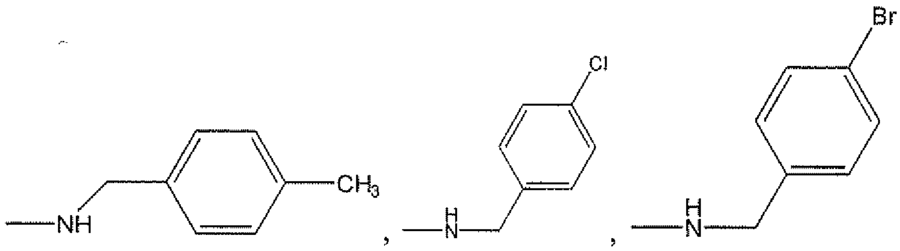


, or $N(CH_2)_4CH_3$.

In yet another preferred embodiment, the pore-forming toxin is anthrax toxin, and the compound is one in which:

$n = 7$, $R_2 = R_3$ = ethylamine or butylamine, and R_1 = OH, NH_2,





, or $N(CH_2)_4CH_3$.

In another preferred embodiment, a compound having a formula of any of the compounds presented in tables 1 and 2 is used for treating a disease condition caused by a pore-forming toxin.

DESCRIPTION OF THE FIGURES

Figure 1. Schematic illustration of β-cyclodextrin molecule in comparison with anthrax PA (left) and staphylococcal α-HL (right) pores.

Figure 2. Protection of RAW 264.7 cells from LeTx action by compound 3.

Figure 3. Channel blocking activity of compounds 1-3.

Figure 4. Protection of rabbit erythrocytes from α-HL action by compound 12.

Figure 5. Multichannel α-HL conductance upon *cis*-addition of CD derivatives.

Figure 6. Activation of ε-prototoxin with trypsin. 10% SDS-polyacrylamide gel stained by Coomassie brilliant blue.

Figure 7. Protection of MDCK cells from ETX-induced cell death by monoclonal antibodies against ETX. Cells were incubated with different concentrations of anti-ETX mAb with or without ε-toxin. Each experimental condition was performed in duplicates. Cell viability was determined by MTS colorimetric assay. Error bars represent standard deviations.

Figure 8. Protection of MDCK cells from ε-toxin-induced cytotoxicity by compound 5105. MDCK cells were incubated with different concentrations of compound 5105 with or without ε-toxin. Each experimental condition was performed in duplicates. Error bars represent standard deviations.

DETAILED DESCRIPTION OF THE INVENTION

Compounds 1-9 and 13 (see Table 1) were tested for their ability to protect mouse macrophage cells from LeTx-induced cell death (Fig. 2). For some of these compounds, their ability to block the ion current though the pores formed by PA in planar lipid membranes (Fig. 3) was also evaluated. First, the activities of per-6-amino derivatives carrying unmodified hydroxyls at positions 2 and 3 (compounds 1-3) were compared with the 2,3-methylated ones (compounds 4-6). None of the methylated derivatives displayed anti-toxin activities while the unmodified compounds 2 and 3 showed activity at low micromolar concentrations. This data demonstrates the importance of the rigidness of the cyclodextrin core provided by free OH groups. In contrast, compound 13 carrying ethylamino groups at positions 2 and 3 displayed anti - LeTx activity at low micromolar concentrations.

The activities of various α-, β-, and γ-cyclodextrin derivatives carrying the same modifications were also investigated. One α-cyclodextrin derivative (compound 7) showed a detectible level of LeTx inhibiting activity, while another α-cyclodextrin derivative (compound 1) demonstrated no such activity. Although not wanting to be bound by a theory, these results could be explained by smaller size or by structural features of α-CDs, which could provide a less favorable spatial orientation of the substituting groups.

All the β- and γ-cyclodextrin derivatives with free OH groups at positions 2,3 displayed anti-LeTx activity in the micromolar range. When structurally similar derivatives of β- and γ-cyclodextrins were compared, it appeared that the γ-CDs displayed comparable activities with the β-CD derivatives (compounds 2 and 3; 8 and 9). Although not wanting to be bound by a theory, these results could be explained by a recent discovery that, in addition to the heptameric pores, PA can form octameric pores having the same eight-fold symmetry as γ-CD.[8]

Channel blocking activity was tested for some of the compounds. The results demonstrate that there is a general correlation between the cytotoxicity inhibition and channel blocking activity. Similar to the results of the cytotoxicity experiments, α-CD derivative (compound 1) exhibited a much lower blocking activity than β- and γ-CD derivatives (compounds 2 and 3) (Fig. 3).

The activities of two α- and γ-CDs (compounds 10 and 12) structurally related to a β-CD derivative (compound 11) which inhibits α-HL as shown previously, were also evaluated.[3] The compounds were tested using a rabbit erythrocyte assay (Fig. 4). Both α- and γ-CDs did not display any activity. Similar results were obtained when the compounds' ability to block ion current though the pores formed by α-HL in planar lipid membranes (Fig. 5) was compared. Although not wanting to be bound by a theory, this difference in the activities of anthrax and staphylococcal toxins could be explained by the fact that in contrast to α-HL, PA can form octameric pores having the same eight-fold symmetry as γ-CD. This data demonstrates that size and conformation as well as the match of the symmetry of a blocking molecule and a pore play important roles in the activity of the inhibitors of pore-forming toxins.

A cell-based assay was developed for the screening of chemical libraries. The procedure includes incubation of target cells with ε-toxin in the presence or absence of tested compounds followed by the colorimetric detection of the cytotoxic effect.

First, ε-toxin was prepared using the optimized protocol to obtain the product with highest yield and activity. The mature ε-toxin was prepared using the optimized conditions and its quality was assessed by 10% SDS-PAGE (Fig. 6).

The activated ETX was assessed for its cytotoxicity. Only one cell line with a reasonable sensitivity to ε-toxin has been identified - the Madin-Darby canine kidney (MDCK) epithelial cell line, and the concentration of the toxin reducing cell culture viability by 50% (LC_{50}) was 2μg/ml according to the literature. This cell line was utilized for ETX cytotoxicity testing and for the development of a screening assay in a 96-well format. Cytotoxicity was monitored with the use of the MTS bioreduction cell viability assay kit (Promega).

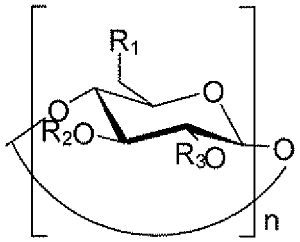
The assay was adapted to a 96-well format. Using anti-ETX monoclonal antibody (mAb) as a toxin inhibitor, it was demonstrated that anti-ETX mAb dose-dependently protected MDCK cells from ε-toxin-mediated cytotoxicity (Fig. 7).

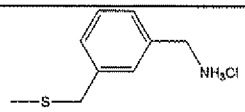
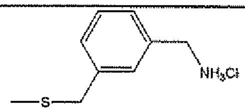
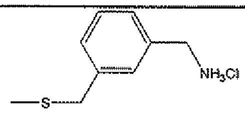
The final protocol developed was successfully utilized for the screening of the library of β-CD derivatives. Over one hundred β-cyclodextrin derivatives at a concentration of 50 μM were screened using the cell-based assay.

Compounds that showed at least 50% inhibition of ε-toxin cytotoxicity at a 50 μM concentration were selected. They were serially diluted and tested to determine the IC_{50} values. Four compounds displayed dose-dependent inhibition of ε-toxin cytotoxicity (Fig. 8, Table 2).

The obtained results demonstrate that the pore blocking cyclodextrin (CD) compounds with the above-described structural features inhibit the cytotoxic activities of anthrax lethal toxin, α-hemolysin (α-HL) toxin of *Staphylococcus aureus*, and ε-toxin produced by *Clostridium perfringens*. Thus, this invention demonstrates that such CD compounds can be used for treating, preventing, or delaying a disease condition caused by *Bacillus anthracis*, *Staphylococcus aureus*, and *Clostridium perfringens*. Compound having a formula of any of the compounds presented in Table 1 and 2 of this application can be used for treating, preventing, or delaying a disease condition caused by a pore-forming toxin. In particular, compounds having a formula of any of the compounds presented in Table 2 of this application are particularly useful for testing, preventing, or delaying a disease condition caused by ε-toxin of *Clostridium perfringens*. The structures of the compounds identified by the inventor is being utilized for the further design, synthesis and testing of a biased library of β-cyclodextrin derivatives.

TABLE 1. Compound Activities



Compound no.	n	R_1	R_2, R_3	Inhibition of cytotoxicity IC_{50} (μM)	Inhibition of trans-membrane conductance IC_{50} (nM)
1	6	$-NH_2$	-H	>100	1200±300
2	7	$-NH_2$	-H	27±6	140±90
3	8	$-NH_2$	-H	12±3	170±50
4	6	$-NH_2$	$-CH_3$	>100	
5	7	$-NH_2$	$-CH_3$	>100	
6	8	$-NH_2$	$-CH_3$	>100	
7	6		-H	45±13	
8	7		-H	0.5±0.2	
9	8		-H	5.4±0.8	
10	6		-H	>100	ND

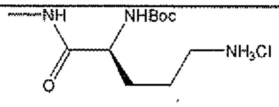
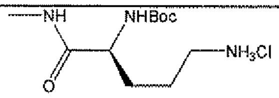
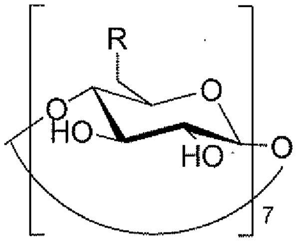
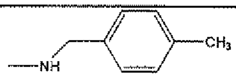
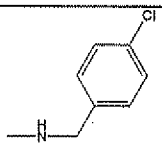
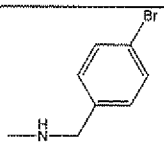
11	7		-H	3.3±2.3	~50
12	8		-H	>100	ND
13	7	-OH	$-(CH_2)_3-NH_2$	4.1±0.4	

TABLE 2. Compound Activities



Compound #	R	Inhibition of ETX cytotoxicity IC_{50} (μM)
5105		20 ± 10
5130		21 ± 9
5137		20 ± 1
5073	$-N-(CH_2)_4-CH_3$	5.6 ± 1.5

REFERENCES

The following references are cited herein. The entire disclosure of each reference is relied upon and incorporated by reference herein.

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2. Karginov VA, Yohannes A, Robinson TM, Fahmi NE, Alibek K, Hecht SM. β-Cyclodextrin derivatives that inhibit anthrax lethal toxin. *Bioorg Med Chem* 2006; 14(1): 33-40.

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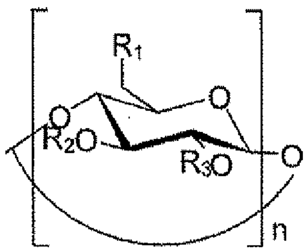
8. Kintzer KF, Thoren KL, Sterling HJ, Dong KC, Feld GK, Tang II, Zhang TT, Williams ER, Berger JM, Krantz BA. The protective antigen component of anthrax toxin forms functional octameric complexes. *J. Mol. Biol.* 2009; 392: 614–629.

CLAIMS

What is claimed is:

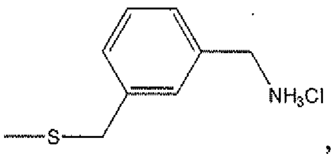
1. A compound for use in the treatment of a disease condition caused by α-HL or ε-toxin, said compound having a formula according to:

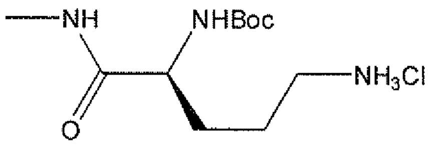


,

wherein n = 7,

$R_2 = R_3$ = H, OH, $(CH_2)_2NH_2$ or $(CH_2)_3NH_2$ and

R_1 = OH, NH_2, 

, 

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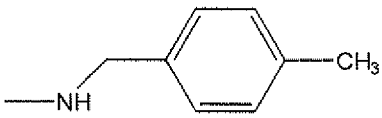
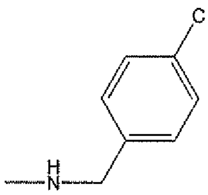
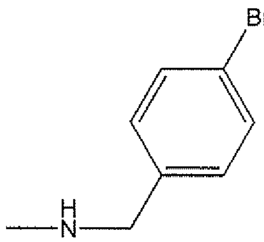


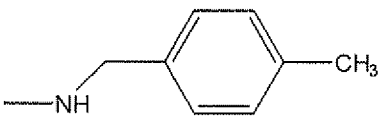
, 

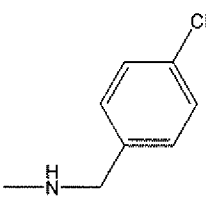
, 

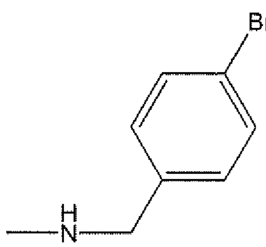
, or

$N(CH_2)_4CH_3$.

2. The compound according to claim 1, wherein $R_2 = R_3$ = OH, and R_1 =



, 

, 

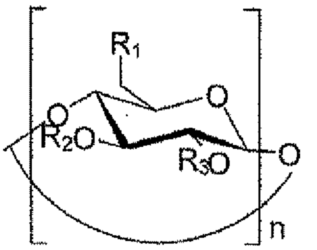
, or $N(CH_2)_4CH_3$.

3. The compound according to claim 1, wherein $R_2 = R_3 = (CH_2)_2NH_2$ or $(CH_2)_3NH_2$.

4. The compound according to claim 2, wherein the disease condition is caused by ε-toxin.

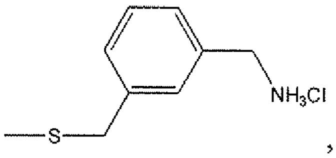
5. The compound according to any one of claims 1-4 having a formula of any of the compounds presented in Table 2.

6. A compound for use in the treatment of a disease condition caused by anthrax toxin, said compound having a formula:



,

wherein n = 6, or 8, $R_2 = R_3$ = H, OH, , $(CH_2)_2NH_2$ or $(CH_2)_3NH_2$ and R_1 = OH, NH_2,



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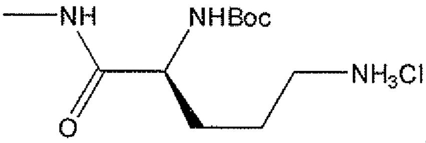

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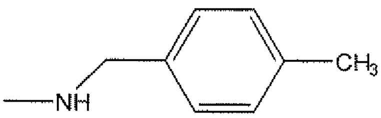

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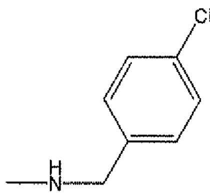
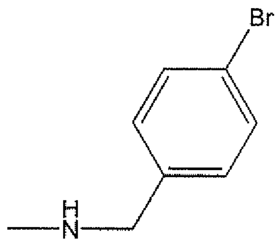
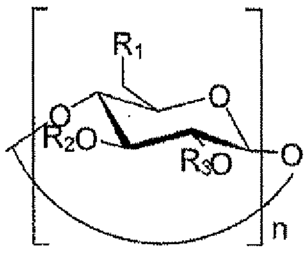


,

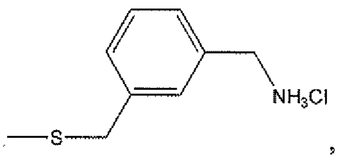
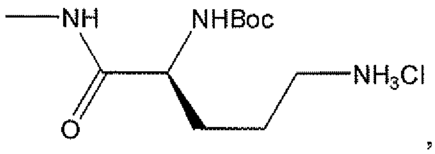
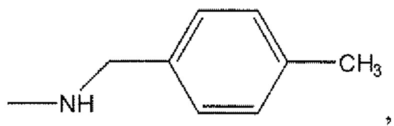
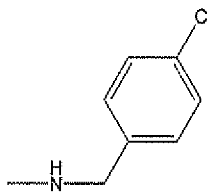
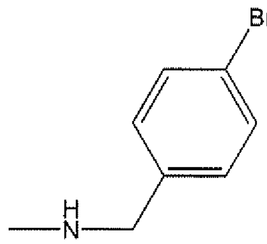


, or $N(CH_2)_4CH_3$.

7. A compound for use in the treatment of a disease condition caused by anthrax toxin, said compound having a formula:



, wherein $n = 7$, $R_2 = R_3 = (CH_2)_2NH_2$ or $(CH_2)_3NH_2$, and

$R_1 = OH, NH_2,$ , , , , , or $N(CH_2)_4CH_3$.

8. The compound according to any one of claims 6 or 7 having a formula of any of the compounds presented in Table 1.

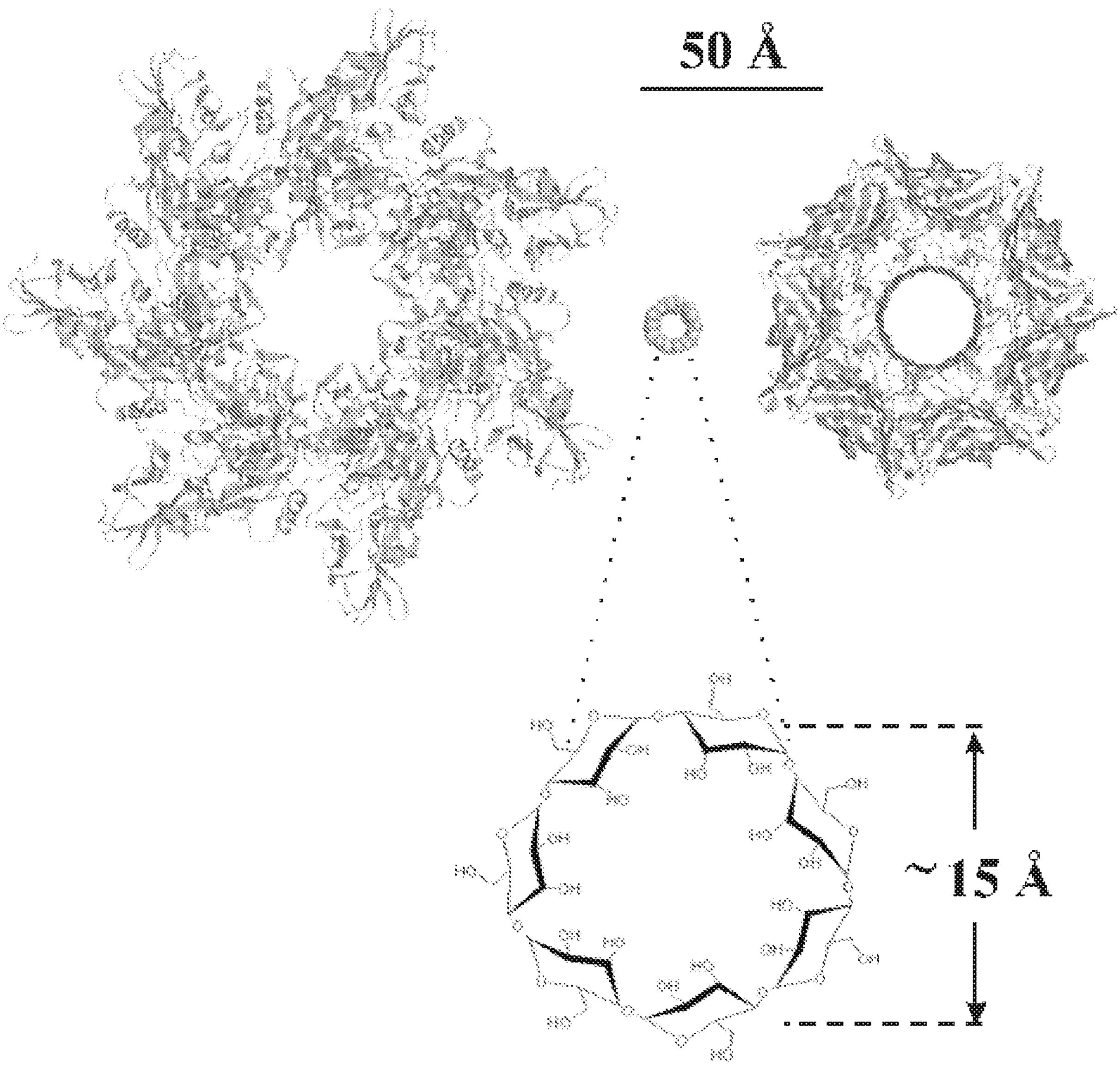
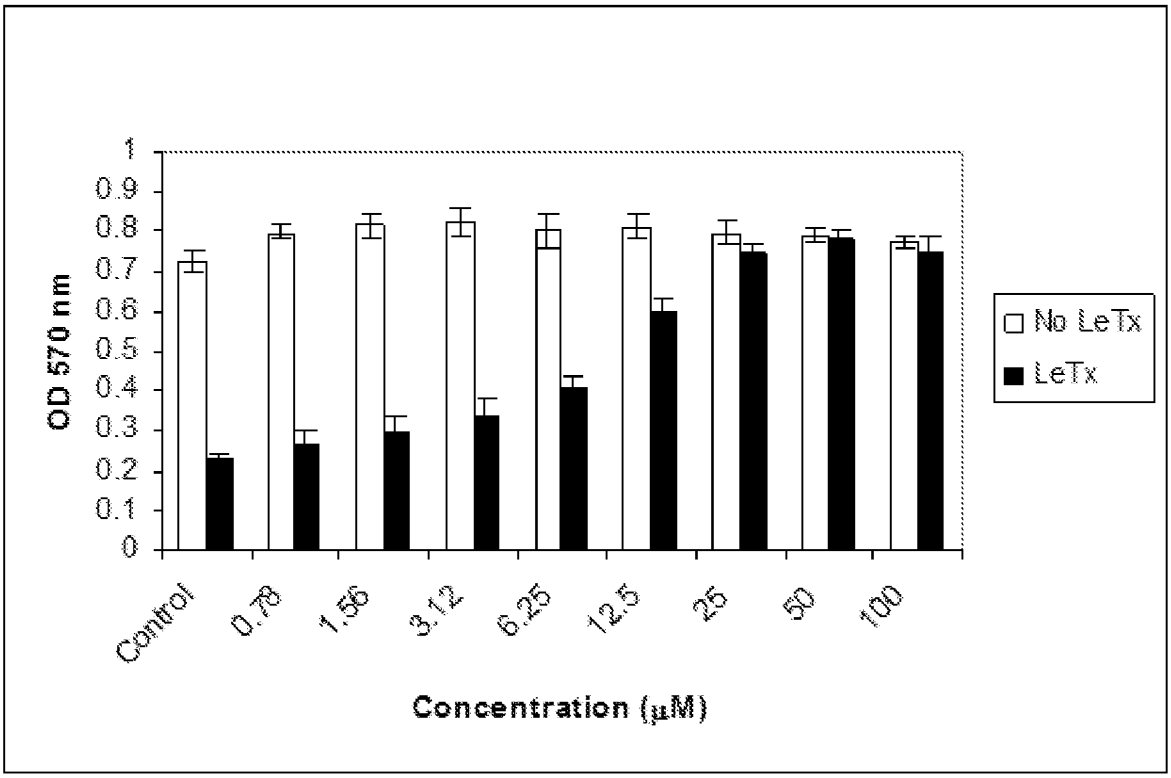


Figure 1



OD 570 nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
0.78
1.56
3.12
6.25
12.5
25
50
100
Concentration (μM)
No LeTx
LeTx

Figure 2

IC_{50} averaged over 3-4 experiments:
Compound 1 – 1.2 ± 0.3 uM
Compound 2 – 0.14 ± 0.09 uM
Compound 3 – 0.17 ± 0.05 uM

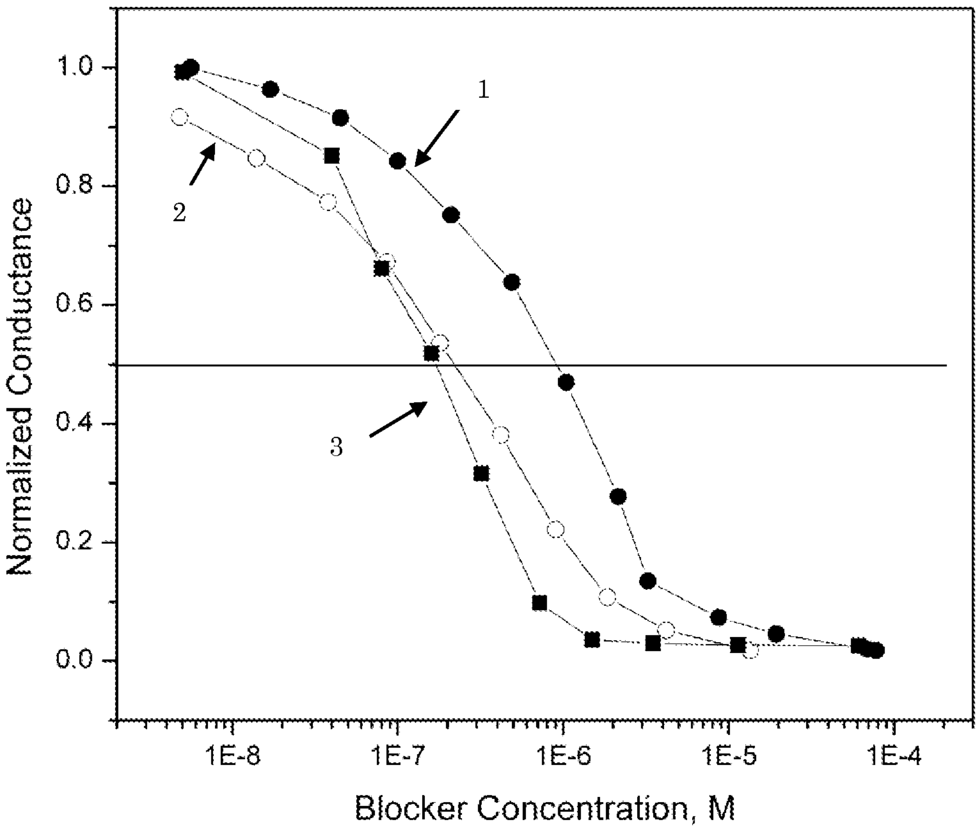


Figure 3

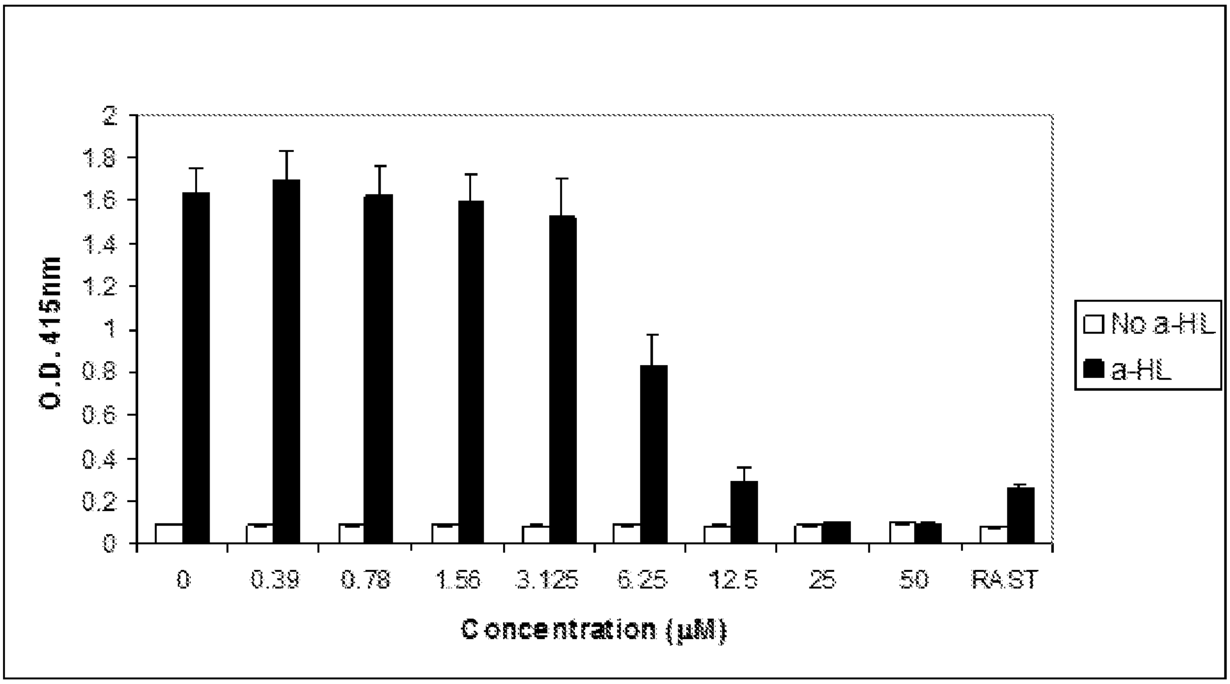


Figure 4

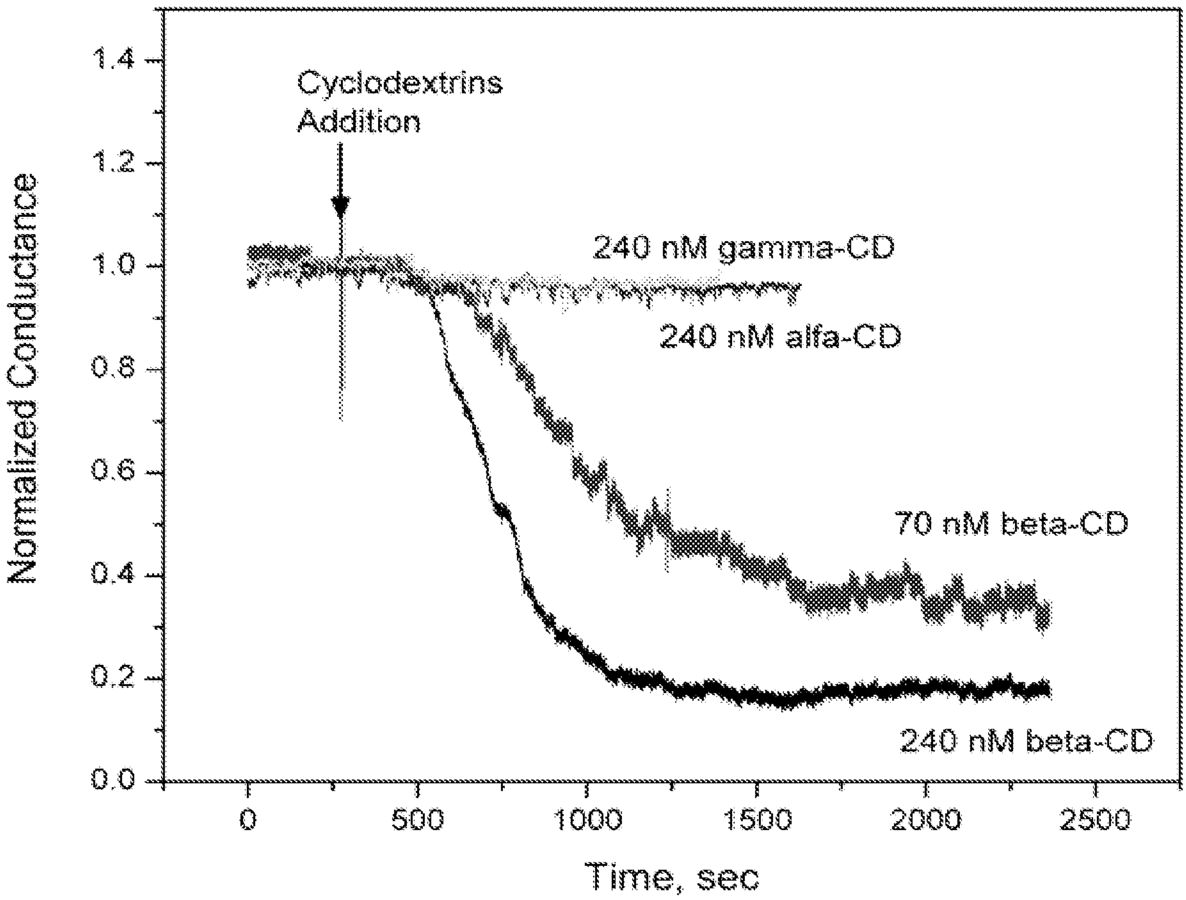


Figure 5

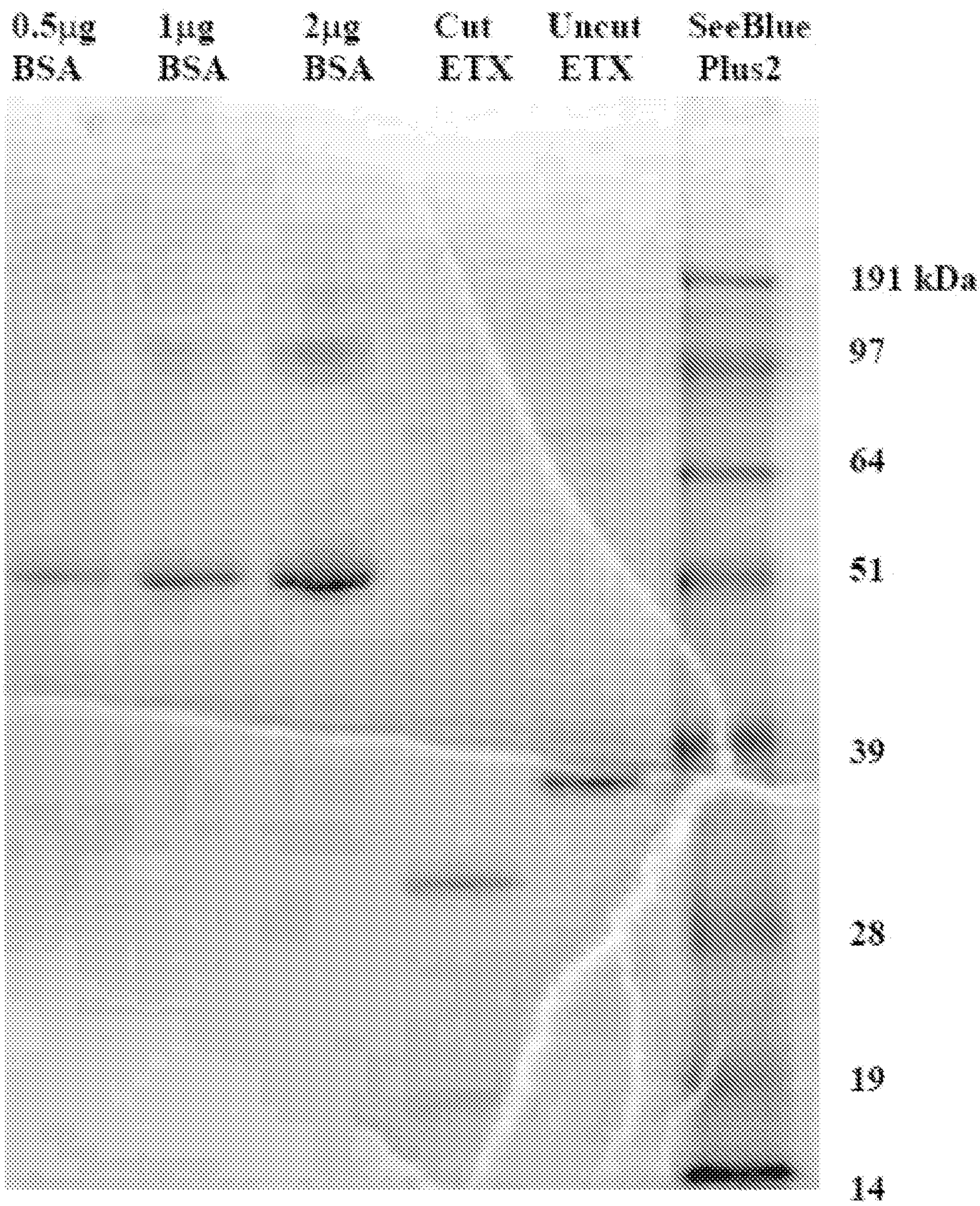

0.5µg BSA
1µg BSA
2µg BSA
Cut ETX
Uncut ETX
SeeBlue Plus2
191 kDa
97
64
51
39
28
19
14

Figure 6

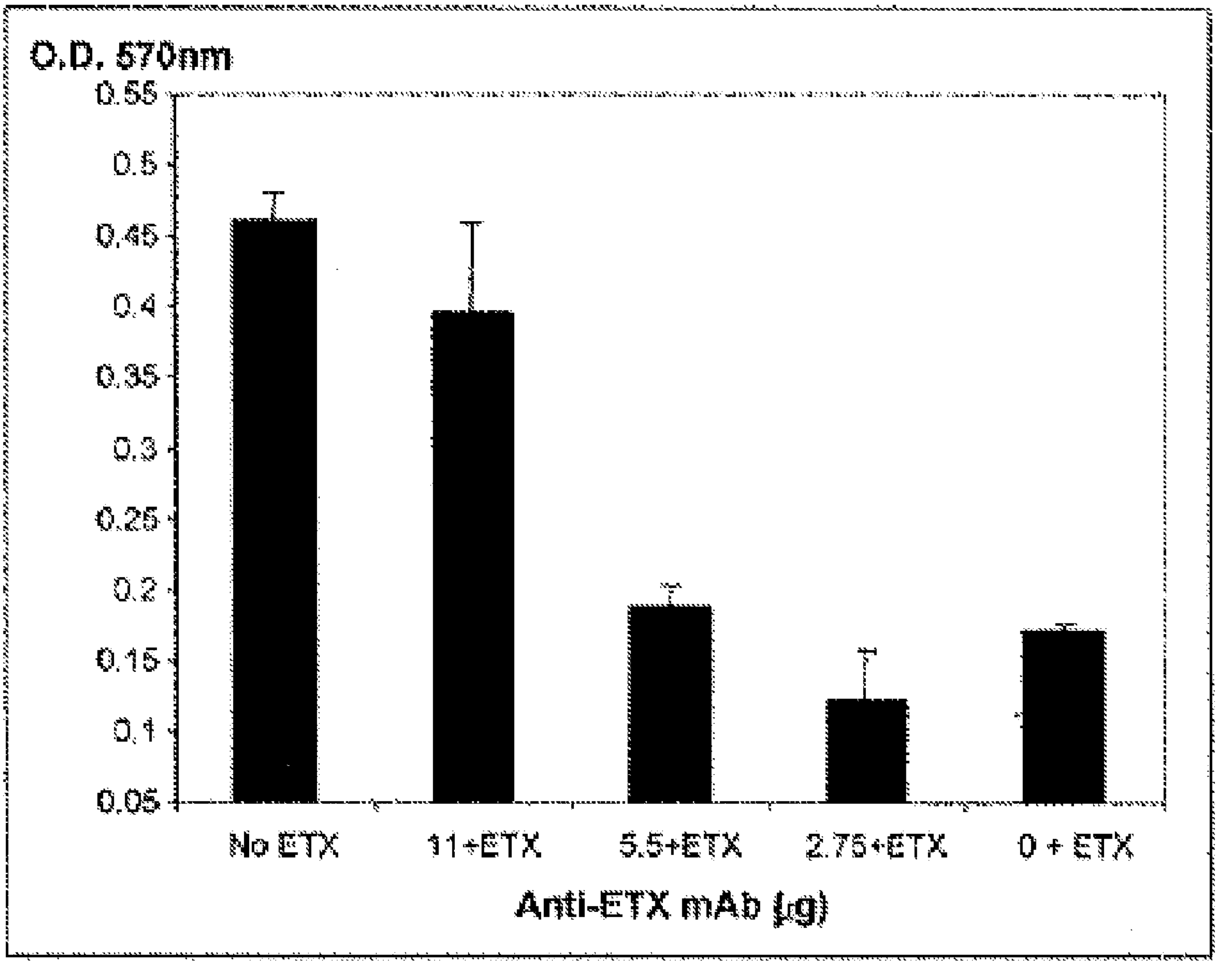

O.D. 570nm
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
No ETX
11+ETX
5.5+ETX
2.75+ETX
0 + ETX
Anti-ETX mAb (μg)

Figure 7

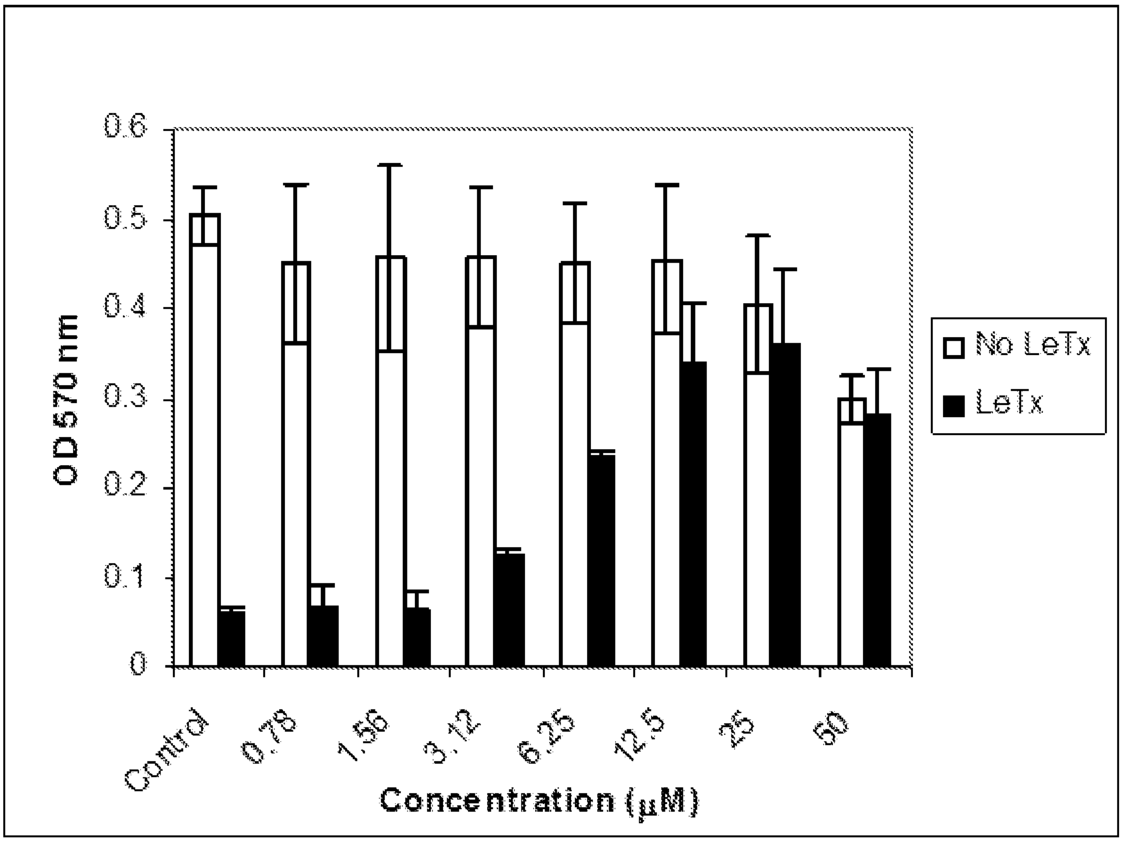

0.6
0.5
0.4
0.3
0.2
0.1
0
OD 570 nm
Control
0.78
1.56
3.12
6.25
12.5
25
50
Concentration (μM)
No LeTx
LeTx

Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/36397

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/04; A61K 31/715; C08B 30/18 (2012.01)
USPC - 514/58
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) -A01N 43/04; A61K 31/715; C08B 30/18 (2012.01)
USPC -514/58

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 536/46 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest, Google Patents, Google Scholar, WIPO (cyclodextrin, anthrax, disease, formula, hemolysin, toxin, beta, hydroxy, acid, anti-infective, peroxide, gamma, alpha, PA, blockers, staphylococcus, clostridum,OOH)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0005343 A1 (HECHT, et al.) 01 January 2009 (01.01.2009) entire document, especially para[0022]-[0023], [0030]	1 ------------ 2-5
Y	US 2006/0199785 A1 (FAHMI, et al.) 07 September 2006 (07.09.2006) entire document, especially para[0020]-[0021], [038]; Table 2, pg. 32	2-5
Y	US 2007/0149435 A1 (KOENIG, et al.) 28 June 2007 (28.06.2007) entire document, especially para[0185], [0186], [0196]	2, 4, 5/2,4
Y	US 2008/0234182 A1 (KARGINOV) 25 September 2008 (25.09.2008) entire document, especially para[0005]-[0007], [0083]; Table 3, pg 9	4, 5/4
A	Gu, et al. Prolonged Residence Time of a Noncovalent Molecular Adapter, B-Cyclodextrin, within the Lumen of Mutant a-Hemolysin Pores, J. Gen. Physiol., Vol. 118, November 2001, 481 -493.	1-5

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 2012 (18.07.2012)	28 SEP 2012

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/36397

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of:
Box NO III. Observations where unity of invention is lacking

Group I: claims 1-5, drawn to a compound for use in the treatment of a disease condition caused by alpha-HL or E-toxin, said compound having a formula pictured.

Group II: claims 6-8, drawn to a compound for use in the treatment of a disease condition caused by anthrax toxin, said compound having a formula pictured.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I do not include the inventive concept of a compound for use in the treatment of a disease condition caused by anthrax toxin, as required by Group II.

The inventions of Groups II do not include the inventive concept of a compound for use in the treatment of a disease condition caused by alpha-HL or E-toxin, as required by Group I.

The inventions of Groups I and II share the technical feature of a compound for use in the treatment of a disease condition caused by microbial pore-forming toxin. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2009/0005343 A1 to Hecht et al. (hereinafter 'Hecht'). Hecht discloses a compound for use in the treatment of a disease condition caused by alpha-HL (Claim 4, a method for treating a bacterial or viral infection; para[0030], staphylococcal alpha-hemolysin). Further, Hecht discloses a compound for use in the treatment of a disease condition caused by anthrax toxin (Claim 4, a method for treating a bacterial or viral infection; para[0019]-[0020], protective antigen of anthrax toxin). As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Groups I and II is the specific beta-cyclodextrin formulae recited therein. The inventions do not share a special technical feature, because Hecht teaches a compound of Claim 1 having a formula according to: [pictured structure] wherein n = 7, R2=R3=H, R1 = NH2 (Fig. 1, compound AmbetaCD, para[0022]-[0023]). Without a shared special technical feature, the inventions lack unity with one another.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.