Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2467–2470
Bioorganic & Medicinal Chemistry Letters

Inhibitors of anthrax lethal factor based upon *N*-oleoyldopamine

Brandon D. Gaddis, Charles M. Rubert Pérez and Jean Chmielewski*

Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA

Received 13 December 2007; revised 12 February 2008; accepted 14 February 2008
Available online 20 February 2008

Abstract—The structural features of an anthrax lethal factor inhibitor, *N*-oleoyldopamine (OLDA, **1**) have been probed. The oleic acid moiety is critical, but, more interestingly, the presence of the double bond and its geometry were found to play an essential role. One compound, **5**, was found to be an uncompetitive inhibitor of lethal factor (LF) with a K_i value of 2.2 μM and a cell-based IC_{50} value of 4.3 μM.

The disease anthrax is caused by the Gram-positive bacteria *Bacillus anthracis* whose virulence results from three secreted proteins: protective antigen (PA), lethal factor (LF), and edema factor (EF).[1] PA binds to the cellular receptor[2,3] and, after furin cleavage,[4,5] forms a heptameric prepore[6] that binds LF and EF[7–10] and transports them into the cytosol of the cell.[11–13] Once in the cytosol, lethal factor, a Zn^{2+}-metalloprotease, cleaves members of the mitogen-activated protein kinase (MAPK)-kinase (MEK) family,[14,15] disrupting signal transduction pathways and causing cell death.[16,17] Currently, the only treatments for post-exposure infection are antibiotics, such as ciprofloxacin and doxycycline, which are effective at treating the bacterial infection,[1] but do not afford protection against the toxins that still persist.[18]

Because LF has been shown to act as a key virulence factor for anthrax pathogenesis,[17] a number of studies have focused on identifying LF inhibitors.[19–32] Some of the most potent LF inhibitors include peptide hydroxamates that are based upon known metalloprotease substrates,[31] a hydroxamate from an SAR study of a lead compound identified through high throughput screening,[32] and a catechin gallate isolated from green tea.[19] The above compounds demonstrated good cell-based activity with IC_{50} values of 4 μM, 210 nM, and <1 μM, respectively. The above compounds, along with a handful of others,[20,27,29] are the few to inhibit LF substrate cleavage in vitro as well as maintain activity in the lethal toxin challenge of macrophages, an important distinction since LF functions in the cytosol.[15] Therefore, there is a need to identify additional compounds that inhibit LF substrate cleavage, and enter and remain active within cells.

Previous work in our lab identified *N*-oleoyldopamine (OLDA) (Fig. 1, **1**) as an uncompetitive inhibitor of LF with a K_i value of 3.0 ± 0.2 μM and almost equipotent activity in the cell-based assay (IC_{50} value of 5.0 ± 0.2 μM). OLDA was found to have low cytotoxicity itself, with complete cell viability up to 30 μM. A

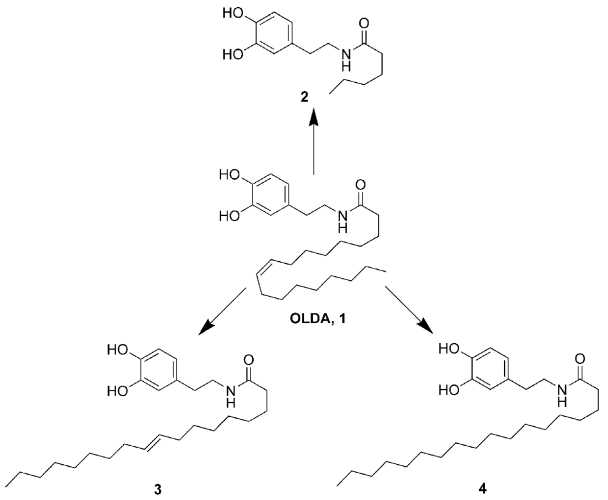

Figure 1. Structures of analogs that probe the importance of length, double bond conformation, and double bond presence in the oleic acid moiety of OLDA (**1**).

Keywords: Anthrax; Uncompetitive inhibition; Lethal factor; Antitoxin.
* Corresponding author. E-mail: chml@purdue.edu

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.02.044

subsequent SAR study showed the importance of the intact catechol for activity.[33] The goal of the current study was to determine the importance of features within the hydrophobic tail of OLDA for inhibition and mechanism of action.

In probing the role of the hydrophobic tail of OLDA on activity, we first focused on three questions: the need for the full-length oleic acid tail, the preferred geometry of the double bond, and the role of the double bond on activity (Fig. 1). In order to address the first concern, we significantly truncated the tail of OLDA from oleic acid to hexanoic acid (**2**).[34] Compound **2** was synthesized as previously described, and this general synthesis scheme was employed for all of the compounds.[33] This modification led to a complete loss of LF inhibition up to a concentration of 100 μM in a fluorescence assay that monitors the proteolytic cleavage of an optimized LF substrate (LF FRET assay), confirming the importance of the full oleic acid moiety for activity.[35]

The second question that we addressed was the preferred conformation of the double bond in OLDA for LF inhibition. Interestingly, switching the geometry of the double bond from cis to trans (**3**)[34] led to a complete loss of inhibition of LF up to a concentration of 100 μM. This may be due to a preferred conformation of the oleic acid in which the tail may fold back onto itself for LF binding. Third, we replaced oleic acid with stearic acid (**4**)[34] to probe the role of the double bond itself. Again, a complete loss of LF inhibition up to a concentration of 100 μM was observed, perhaps also due to the loss of a preorganized conformation of the hydrophobic chain for binding to LF. An alternative possibility for the lack of activity with compounds **3** and **4** could be potential aggregation of these agents, leading to loss of potency. Recent work has shown that 0.01% Triton X-100 can be used to prevent aggregation of inhibitors in enzymatic assays.[36] Therefore, 0.01% Triton X-100 was employed in the LF FRET assay with compounds **3** and **4**, but, again, no inhibition was demonstrated up to a concentration of 100 μM. These data indicate that aggregation is most likely not the cause for lack of efficacy.

The importance of the distance between the catechol ring and the tail was next investigated. With this in mind, one methylene unit was removed between the aromatic ring and the amide nitrogen of OLDA, yielding compound **5**[34] (Fig. 2). This modification resulted in an inhibitor that is equipotent with OLDA (IC_{50} value of 15 ± 1 μM), indicating that this feature of OLDA can be a point of modification in future libraries. With this in mind, and with the knowledge that hydroxamic acid analogs of OLDA demonstrated potency,[33] we synthesized a hydroxamic acid variant of **5** containing the above truncation between the amide and the phenyl ring (**6**).[34] This modification led to an additional inhibitor of LF, albeit with reduced potency (IC_{50} value of 42 ± 1 μM).

Kinetic studies were undertaken to determine the mechanism of inhibition and K_i values for **5** and **6**. A Lineweaver–Burk analysis of **5** with LF[37] produced data whose

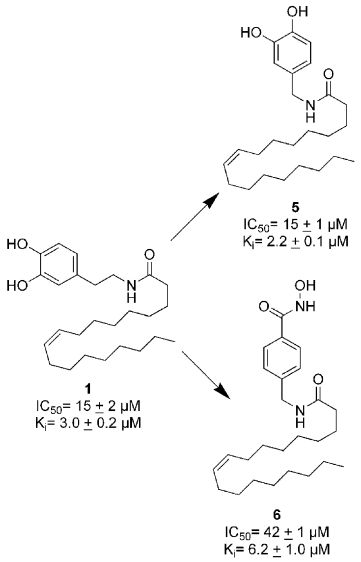

Figure 2. Structures of analogs with a truncation between the amide and phenyl moiety of OLDA (**1**) or within a hydroxamate-containing analog.

lines were parallel (Fig. 3), indicative of uncompetitive inhibition (as did analysis of the non-double reciprocal kinetics data). Compound **5** was found to have a K_i value of 2.2 ± 0.1 μM, a slight improvement as compared to OLDA. Compound **6** was also found to be an uncompetitive inhibitor of LF with a K_i value of 6.2 ± 1.0 μM. An uncompetitive mechanism of inhibition suggests that these inhibitors, along with substrate, may co-occupy LF to produce an inactive ESI complex. Uncompetitive inhibition may seem to be at odds with possible Zn^{2+}-binding of the catechol moiety. It has been demonstrated that Zn^{2+}, however, is not essential for substrate binding to LF; indeed a co-crystal structure of LF (without zinc) and substrate has been obtained.[38] The true nature of this co-occupation awaits structural elucidation.

Because LF functions in the cytosol,[15] it is imperative that compounds are not only active in vitro but also in cyto. With this in mind, compound **5** was evaluated in

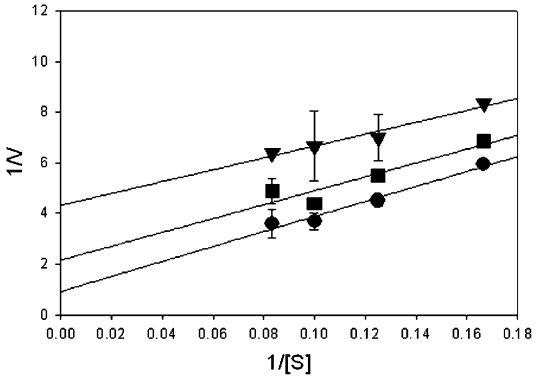

Figure 3. Lineweaver–Burk analysis of compound **5** with LF (12.5 nM): (●) 0 μM **5**, (■) 4.1 μM **5**, (▼) 8.2 μM **5**; pH 7.4, Hepes buffer, 25 °C.

the lethal toxin challenge of macrophages.[39] In this assay, PA, LF, and **5** were allowed to incubate with J774 macrophages for four hours, and cell rescue, as compared to PA and LF alone, was quantified via the MTT assay. Compound **5** was found to have good potency in cyto with an IC_{50} value of 4.3 ± 0.3 μM, thereby providing another agent with anti-toxin and cell-based activity.

In conclusion, the structural features of OLDA necessary for LF inhibition have been probed. The oleic acid tail of OLDA is critical, but, more interestingly, the presence of the double bond and its geometry were found to play an essential role. These studies, in addition to previous work that focused on the catechol ring of OLDA, contribute to our understanding of the importance of the functionality within OLDA for LF inhibition.

Acknowledgments

This work was supported by a grant from the Showalter Foundation. C.M.R.P was supported through an REU in Chemical Biology grant from NSF. The authors are grateful to Mark Lipton for helpful discussions, Andrew Mesecar for PA, and Jenna Rickus for use of the Flex Station II fluorometer.

References and notes

1. Dixon, T. C.; Meselson, M.; Guillemin, J.; Hanna, P. C. *N. Eng. J. Med.* **1999**, *341*, 815.
2. Bradley, K. A.; Mogridge, J.; Mourez, M.; Collier, R. J.; Young, J. A. T. *Nature* **2001**, *414*, 225.
3. Scobie, H. M.; Rainey, J. A.; Bradley, K. A.; Young, J. A. T. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5170.
4. Gordon, V. M.; Klimpel, K. R.; Arora, N.; Henderson, M. A.; Leppla, S. H. *Infect. Immun.* **1995**, *63*, 82.
5. Klimpel, K. R.; Molloy, S. S.; Thomas, G.; Leppla, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10277.
6. Milne, J. C.; Furlong, D.; Hanna, P. C.; Wall, J. S.; Collier, R. J. *J. Biol. Chem.* **1994**, *269*, 20607.
7. Elliott, J. L.; Mogridge, J.; Collier, R. J. *Biochemistry* **2000**, *39*, 6706.
8. Christensen, K. A.; Krantz, B. A.; Collier, R. J. *Biochemistry* **2006**, *45*, 2380.
9. Mogridge, J.; Cunningham, K.; Collier, R. J. *Biochemistry* **2002**, *41*, 1079.
10. Lacy, D. B.; Mourez, M.; Fouassier, A.; Collier, R. J. *J. Biol. Chem.* **2002**, *277*, 3006.
11. Miller, C. J.; Elliot, J. L.; Collier, R. J. *Biochemistry* **1999**, *38*, 10432.
12. Beauregard, K. E.; Collier, R. J.; Swanson, J. A. *Cell. Microbiol.* **2000**, *2*, 251.
13. Gordon, V. M.; Leppla, S. H.; Hewlett, E. L. *Infect. Immun.* **1988**, *56*, 1066.
14. Duesbery, N. S.; Webb, C. P.; Leppla, S. H.; Gordon, V. M.; Klimpel, K. R.; Copeland, T. D.; Ahn, N. G.; Oskarsson, M. K.; Fukasawa, K.; Paull, K. D.; Vande Woude, G. F. *Science* **1998**, *280*, 734.
15. Montecucco, C.; Tonello, F.; Zanotti, G. *Trends Biochem. Sci.* **2004**, *29*, 282.
16. Xu, L.; Frucht, D. M. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 20.
17. Pezard, C.; Berche, P.; Mock, M. *Infect. Immun.* **1991**, *59*, 3472.
18. Borio, L. L.; Gronvall, G. K. *Biosecur. Bioterror* **2005**, *3*, 102.
19. Dell'Aica, I.; Dona, M.; Tonello, F.; Piris, A.; Mock, M.; Montecucco, C.; Garbisa, S. *EMBO Rep.* **2004**, *5*, 418.
20. Forino, M.; Johnson, S.; Wong, T. Y.; Rozanov, D. V.; Savinov, A. Y.; Li, W.; Fattorusso, R.; Becattini, B.; Orry, A. J.; Jung, D.; Abagyan, R. A.; Smith, J. W.; Alibek, K.; Liddington, R. C.; Strongin, A. Y.; Pellecchia, M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9499.
21. Fridman, M.; Belakhov, V.; Lee, L. V.; Liang, F.-S.; Wong, C.-H.; Baasov, T. *Angew. Chem., Int. Ed.* **2005**, *44*, 447.
22. Goldman, M. E.; Cregar, L.; Nguyen, D.; Simo, O.; O'Malley, S.; Humphreys, T. *BMC Pharmacol.* **2006**, *6*, 8.
23. Jiao, G.-S.; Cregar, L.; Goldman, M. E.; Millis, S. Z.; Tang, C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1527.
24. Jiao, G.-S.; Simo, O.; Nagata, M.; O'Malley, S.; Hemscheidt, T.; Cregar, L.; Millis, S. Z.; Goldman, M. E.; Tang, C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5183.
25. Johnson, S.; Jung, D.; Forino, M.; Chen, Y.; Satterthwait, A.; Rozanov, D. V.; Strongin, A. Y.; Pellecchia, M. *J. Med. Chem.* **2006**, *49*, 27.
26. Lee, L. V.; Bower, K. E.; Liang, F.-S.; Wu, D.; Sucheck, S. J.; Vogt, P. K.; Wong, C.-H. *J. Am. Chem. Soc.* **2004**, *126*, 4774.
27. Min, D.-H.; Tang, W.-J.; Mrksich, M. *Nat. Biotechnol.* **2004**, *22*, 717.
28. Numa, M. M. D.; Lee, L. V.; Hsu, C.-C.; Bower, K. E.; Wong, C.-H. *ChemBioChem* **2005**, *6*, 1002.
29. Panchal, R. G.; Hermone, A. R.; Nguyen, T. L.; Wong, T. Y.; Schwarzenbacher, R.; Schmidt, J.; Lane, D.; McGrath, C.; Turk, B. E.; Burnett, J.; Aman, M. J.; Little, S.; Sausville, E. A.; Zaharevitz, D. W.; Cantley, L. C.; Liddington, R. C.; Gussio, R.; Bavari, S. *Nat. Struct. Biol.* **2004**, *11*, 67.
30. Schepetkin, I. A.; Khlebnikov, A. I.; Kirpotina, L. N.; Quinn, M. T. *J. Med. Chem.* **2006**, *49*, 5232.
31. Tonello, F.; Seveso, M.; Marin, O.; Mock, M.; Montecucco, C. *Nature* **2002**, *418*, 386.
32. Xiong, Y.; Wiltsie, J.; Woods, A.; Guo, J.; Pivnichny, J. V.; Tang, W.; Bansal, A.; Cummings, R. T.; Cunningham, B. R.; Friedlander, A. M.; Douglas, C. M.; Salowe, S. P.; Zaller, D. M.; Scolnick, E. M.; Schmatz, D. M.; Bartizal, K.; Hermes, J. D.; MacCoss, M.; Chapman, K. T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 964.
33. Gaddis, B. D.; Avramova, L. V.; Chmielewski, J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4575.
34. *Characterization data for compounds* ***2–6***.
Compound ***2****:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (d, $J = 8.1$ Hz, 1H), 6.75 (s, 1H), 6.54 (d, $J = 8.1$ Hz, 1H), 5.82 (s, 1H), 3.48 (q, $J = 6.6$ Hz, 2H), 2.65 (t, $J = 6.6$ Hz, 2H), 2.16 (t, $J = 7.5$ Hz, 2H), 1.59–1.55 (m, 2H), 1.31–1.23 (m, 4H), 0.88 (t, $J = 6.6$ Hz, 3H); MALDI MS: *m/z* 252.50 ($M+H^+$), 274.42 ($M+Na^+$).
Compound ***3****:* ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.79 (d, $J = 8.1$ Hz, 1H), 6.75 (s, 1H), 6.54 (d, $J = 8.1$ Hz, 1H), 5.65 to 5.52 (m, 2H), 5.37 (s, 1H), 3.48 (q, $J = 6.6$ Hz, 2H), 2.65 (t, $J = 6.6$ Hz, 2H), 2.16 (t, $J = 7.5$ Hz, 2H), 2.05 to 1.99 (m, 4H), 1.59 to 1.55 (m, 2H), 1.29 to 1.27 (m, 20H), 0.88 (t, $J = 6.6$ Hz, 3H); MALDI MS: *m/z* 418.41 ($M+H^+$), 440.30 ($M+Na^+$).
Compound ***4****:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (d, $J = 8.1$ Hz, 1H), 6.75 (s, 1H), 6.54 (d, $J = 8.1$ Hz, 1H), 5.58 (s, 1H), 3.48 (q, $J = 6.6$ Hz, 2H), 2.65 (t, $J = 6.6$ Hz, 2H), 2.16 (t, $J = 7.5$ Hz, 2H), 1.59 to 1.55 (m, 2H), 1.29 to 1.25 (m, 28H), 0.88 (t, $J = 6.6$ Hz, 3H); MALDI MS: *m/z* 420.71 ($M+H^+$), 442.73 ($M+Na^+$).

Compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (d, J = 8.1 Hz, 1H), 6.75 (s, 1H), 6.54 (d, J = 8.1 Hz, 1H), 5.87 (s, 1H), 5.36 to 5.30 (m, 2H), 4.31 (d, J = 5.7 Hz, 2H), 2.16 (t, J = 7.5 Hz, 2H), 2.05 to 1.99 (m, 4H), 1.59 to 1.55 (m, 2H), 1.29 to 1.27 (m, 20H), 0.88 (t, J = 6.6 Hz, 3H); MALDI MS: m/z 404.43 ($M+H^+$), 426.42 ($M+Na^+$).
Compound **6**: ^{1}H NMR (300 MHz, Acetone-d_6) δ 7.89 (s, 1H), 7.77 (d, J = 9.0 Hz, 2H), 7.35 (d, J = 9.0 Hz, 2H), 5.36 to 5.30 (m, 2H), 4.45 (d, J = 4.2 Hz, 2H), 2.16 (t, J = 7.5 Hz, 2H), 2.05 to 1.99 (m, 4H), 1.59 to 1.55 (m, 2H), 1.29 to 1.27 (m, 20H), 0.88 (t, J = 6.6 Hz, 3H); MALDI MS: m/z 431.40 ($M+H^+$), 453.28 ($M+Na^+$).

35. *FRET assay conditions (IC_{50}):* Compounds **2**–**6** (0.6% DMSO) were incubated with 50 nM LF (List Biological Laboratories) in assay buffer (65 μL) for 1 h before addition to 4 μM MAPKKide (List Biological Laboratories) in assay buffer (10 μL). Fluorescent measurements were performed over 10 min (excitation and emission of 485 nM and 590 nM, respectively). Assay buffer consisted of 20 mM Hepes, pH 7.4.
36. Feng, B. Y.; Shoichet, B. K. *Nat. Prot.* **2006**, *1*, 550.
37. Kinetic assay conditions (K_i): Compounds **5** and **6** (1.6% DMSO) were incubated with 12.5 nM LF (List Biological Laboratories) in assay buffer (65 μL) for 1 h before addition to 6–12 μM MAPKKide (List Biological Laboratories) in assay buffer (10 μL). Fluorescent measurements were performed over 10 min (excitation and emission of 485 nM and 590 nM, respectively). Assay buffer consisted of 20 mM Hepes, pH 7.4.
38. Pannifer, A. D.; Wong, T. Y.; Schwarzenbacher, R.; Renatus, M.; Petosa, C.; Bienkowska, J.; Lacy, D. B.; Collier, R. J.; Park, S.; Leppla, S. H.; Hanna, P.; Liddington, R. C. *Nature* **2001**, *414*, 229.
39. *Lethal toxin challenge conditions:* J774A.1 cells were allowed to grow to confluency in 96-well plates before 3 nM PA, compound **5** (0.6% DMSO), and 0.61 nM LF (List Biological Laboratories) were added. Cells were allowed to incubate with the PA/LF/compound mixture for 4 h at 37 °C before MTT was added. Cells were allowed to incubate for an additional 1.5 h at 37 °C before cell viability was determined.