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Synthesis and screening of small molecule inhibitors of anthrax edema factor

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ABSTRACT

The synthesis and development of a novel class of molecules that inhibit anthrax edema factor, an adenylyl cyclase, is reported. These molecules are derived from the initial discovery that histidine and imidazole adducts of the prostaglandin PGE_2 reduce the net secretory response of cholera toxin-challenged mice and act directly on the action of anthrax edema factor, a calmodulin-dependent adenylyl cyclase. The simple enones examined in this letter were prepared by palladium-catalyzed Suzuki reaction.

Anthrax toxin produced by *Bacilus anthracis*, the causative agent of anthrax, consists of three proteins; lethal factor (LF), protective antigen (PA), and edema factor (EF). Working in concert these proteins are responsible for the virulence associated with inhalation anthrax infections, that if not promptly treated, are often fatal.[1] Individually, the three proteins are not toxic, but the combination of PA and LF forms lethal toxin (LT), which causes death when injected into experimental animals.[2] The combination of PA and EF forms edema toxin (ET), which produces tissue edema at the site of infection. Edema and lethal toxins synergize their action against a host's innate immunity. It has been shown that the deletion of the EF or LF gene results in a reduction of virulence of anthrax bacteria.[2] Given that EF is an adenylyl cyclase, edema toxin's role in pathogenesis is thought to impair phagocyte function. For that reason small molecule inhibitors of EF may play a role in the treatment of inhalation anthrax infections, as well as other diseases that involve edema, due to increased cyclic AMP production. Herein we report the synthesis and testing of a series of inhibitors of anthrax edema factor.

The initial lead for the development of the molecules reported here came from the report by Peterson that histidine and imidazole adducts of prostaglandin E_2 (PGE_2) (Fig. 1) reduced the net secretory response of the small intestine of mice challenged with cholera toxin (CT).[3] It was reported that upon in vitro incubation of PGE_2 with either L-histidine or imidazole, a covalent adduct formed, presumably with PGA_2 as the intermediate (Scheme 1). These adducts were found to be responsible for the observed antisecretory activity. Subsequent studies revealed that not only were the imidazole-prostaglandin adducts active against mammalian adenylyl cyclases, but also the edema factor of anthrax ($IC_{50} = 21$ μm). With these derivatives as the lead compounds, an effort has been made to develop novel small molecule inhibitors of EF through the synthesis of a series of related compounds.

With PGE_2-L-histidine, and PGE_2-imidazole in mind, a series of simple enone adducts of imidazole were examined. One problem that had to be considered in the development of new inhibitor molecules was that the initial lead compounds were unstable to elimination of the amine at the β-position with the subsequent reformation of PGA_2 (**6** Scheme 1). While it was understood that the reversible nature of the amine adducts would present a problem, the decision was made to examine such adducts, since they were readily available. Interestingly, a number of these very simple compounds (Fig. 2) exhibited adenylyl cyclase inhibition activity in the same potency range as the PGE_2 imidazole adducts (100–500 μM). In the initial experiments the cyclohexenone adducts appeared to be more active than the five-membered ring compounds. However, these observations were not conclusive, since they may be biased by the fact that the six-membered ring adducts appeared to be more stable to elimination of imidazole than the five-membered ring derivatives.

Because of the labile nature of the amine adducts, the decision was made to examine molecules with the heterocycle attached via a carbon–carbon bond. There were a number of methods that could have been used in the synthesis of such molecules. The addition of the imidazolium cuprate was attempted on cyclohexenone.[4,5]

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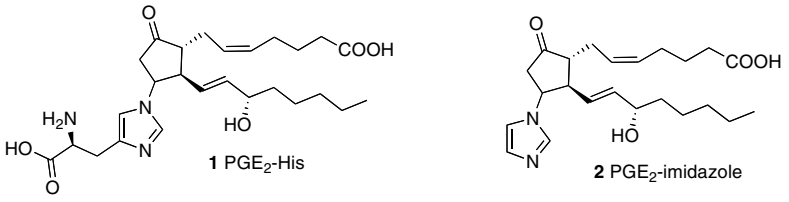
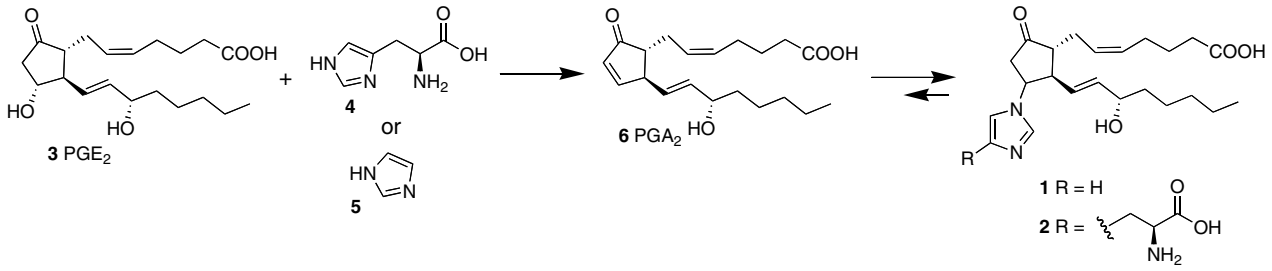


Figure 1.



Scheme 1.

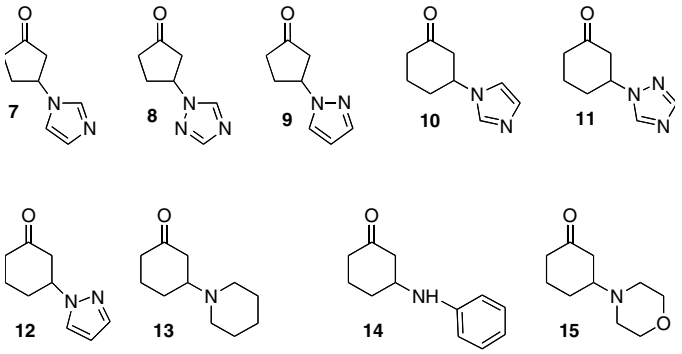
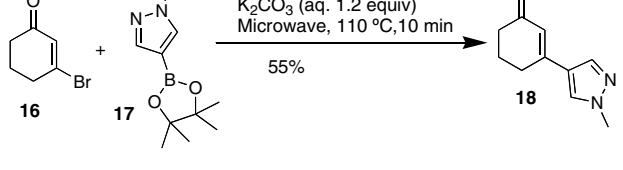


Figure 2.

However our initial use of cuprate chemistry demonstrated that each cuprate addition required its own set of reaction conditions. This precluded it as a method to provide ready access to a number of different derivatives. Attempts to use the Heck reaction also proved problematic. The Heck reaction between cyclohexenone and vinyl or arylbromides often provided mixtures of saturated and unsaturated products. These materials proved to be difficult to separate, and consequently this approach was not considered appropriate for a system where ready access to a variety of compounds was desired.

Despite the initial problems with the Heck reaction, a number of select compounds were synthesized and assayed. We observed that the unsaturated versions of these molecules had inhibitory activities comparable to the saturated adducts. Consequently it was decided to synthesize unsaturated versions of the primary target. This allowed the use of the Suzuki reaction for their synthesis (Scheme 2). For ensembles of the desired molecules, the Suzuki reaction appeared to be a good choice. There are over 500 commercially available boronic acids and esters, and the necessary vinyl bromo enones are available from 1,3-diones in a single step.[6] The ready availability of the two principal starting materials allowed for a variety of different structural types to be synthesized and tested (Table 1).



Scheme 2.

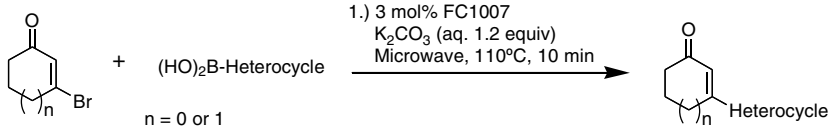
In our initial work we synthesized twenty-four enones. The Suzuki reaction was catalyzed by a variety of different palladium catalysts with FC1007 providing the most consistent results.[7,8] The reactions were conducted under microwave conditions (250 W at 110 °C for 10 min). The yields ranged from low to quite good with no attempt to optimize the reaction conditions. In all cases the samples were purified by column chromatography to remove any metal-based contaminants and other impurities.

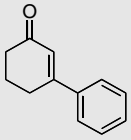
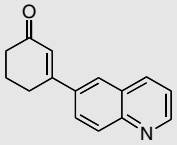
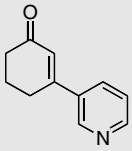
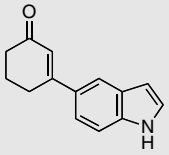
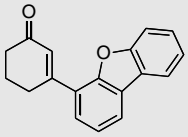
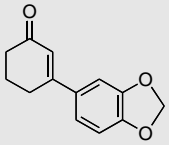
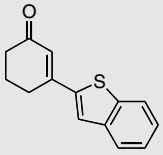
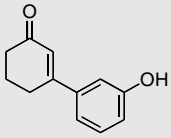
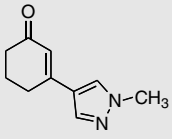
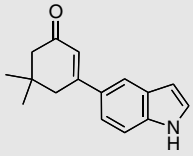
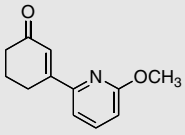
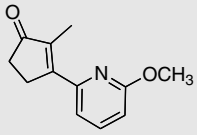
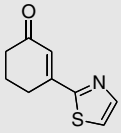
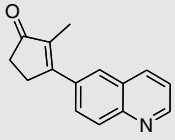
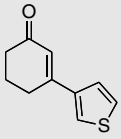
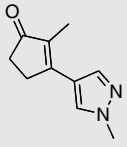
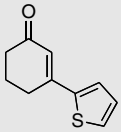
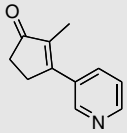
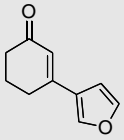
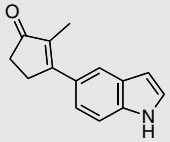
The molecules were tested in whole cells to determine their ability to inhibit the production of cAMP by anthrax edema factor.[9] As can be seen methoxypyridine adducts of 2-methylcyclopentenone (**32** and **39**) had good activity, while the analogous adduct of cyclohexenone (**27**) was not active. The indole adduct of cyclohexenone (**35**) also exhibited comparable activity to the best compounds.

Through this limited study we have established some structure–activity relationships. It was determined that only cyclic ketone and enone adducts are active acyclic versions of active structures such as the imidazole adduct of methylvinyl ketone are not. The ketone functionality appears to be necessary in that molecules where the ketone has been reduced to an alcohol are inactive. Additionally, only aromatic heterocyclic adducts are active. The addition of simple amines did not provide inhibitors.

In summary, through the targeted synthesis of a small collection of compounds moderately active structures were obtained. The Suzuki reaction of β-bromoenones with boronic acids provided the desired compounds for screening. Additional versions of these molecules, including structures with the prostaglandin side chains, are being synthesized and evaluated.

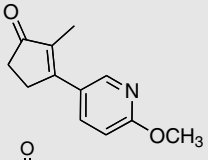
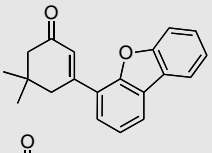
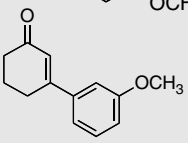
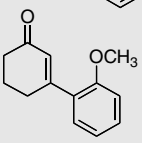
Table 1
Yield and select activity data for enone adducts



Compound	Yield[a] (%)	IC_{50}[b] (μM)	Compound	Yield[a] (%)	IC_{50}[b] (μM)
22 	75	>100	**34** 	88	> 100
23 	54	>100	**35** 	83	56
24 	83	>100	**36** 	85	53
25 	89	*	**37** 	64	>100
26 	29	>100	**38** 	95	>100
27 	89	>100	**39** 	69	30
28 	28	>100	**40** 	78	*
29 	72	>100	**41** 	16	*
30 	83	>100	**42** 	31	*
31 	16	>100	**43** 	92	*

(*continued on next page*)

Table 1 (*continued*)

Compound		Yield[a] (%)	IC_{50}[b] (μM)	Compound		Yield[a] (%)	IC_{50}[b] (μM)
32		92	24	**44**		96	>100
33		88	30	**45**		98	35

[*] IC_{50} was not determined.

[a] Spectral data for compounds the purification information is available in the supplementary material.

[b] The assay was performed with murine monocyte/macrophage (RAW 264.7) cells. Media was aspirated from the cells and replaced with varying concentrations of PGE_2-imidazole and test inhibitors (100, 50, 10, 5, 1, and 0.1 μM) containing PA and EF and incubated for 4 h at 37 °C in 5% CO_2. Following the incubation period, culture supernatants were removed (extracellular cAMP) and transferred to a 96-well plate for cAMP determination by ELISA.

Acknowledgment

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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.2008.05.059.

References and notes

1. Brachman, P. S. *Anthrax*; Harper & Rowe: New York, 1972.
2. Brossier, F. M.; Weber-Levy, M.; Mock, M.; Sirard, J.-C. *Infect. Immun.* **2000**, *68*, 1781.
3. Peterson, J. W.; King, D.; Ezell, E. L.; Rogers, M.; Gessell, D.; Hoffpauer, J.; Reuss, L.; Chopra, A. K.; Gorenstein, D. *Biochim. Biophys. Acta* **2001**, *1537*, 27.
4. Tatsuta, K.; Miura, S.; Ohta, S.; Gunji, H. *J. Antibiot.* **1995**, *48*, 286.
5. Kirk, K. L. *J. Org. Chem.* **1978**, *43*, 4381.
6. Piers, E.; Nagakura, I. *Sny. Commun.* **1975**, *5*, 193.
7. Wang, Y.; Sauer, D. R. *Org. Lett.* **2004**, *6*, 2793.
8. Sauer, D. R.; Kalvin, D.; Phelan, K. M. *Org. Lett.* **2003**, *5*, 4721.
9. Cell Culture: Murine monocyte/macrophage cells (RAW 264.7) were propagated in T75 flasks containing Dulbecco's modified Eagle's medium (DMEM) (Mediatech, Inc., Herndon, VA) at 37 °C with 5% CO_2. The culture media contained 10% fetal bovine serum (FBS), 100 μg/ml penicillin/streptomycin and L-glutamine.
Cell assay: Murine monocyte/macrophage (RAW 264.7) cells (ATCC, Manassas, VA) were plated at 1×10^6 cells per well in DMEM containing 10% fetal bovine serum (FBS), 5 mL of 100 μg/ml penicillin/streptomycin and 5 mL of 2 mM L-glutamine (Mediatech, Herndon, VA) in 48-well tissue culture plates. The cells were then allowed to adhere to the wells overnight at 37 °C in 5% CO_2. PGE_2-imidazole, Protective Antigen (2.5 μg/mL PA), edema factor (0.625 μg/mL EF) and test inhibitors were reconstituted using sterile water and then diluted to the desired concentrations using DMEM assay media (without phenol red) containing 50 μM of 3-isobutyl-1-methylxanthine (IBMX) (BIOMOL, Plymouth Meeting, PA). Media was aspirated from the cells and replaced with varying concentrations of PGE_2-imidazole and test inhibitors (100, 50, 10, 5, 1, and 0.1 μM) containing PA and EF and incubated for 4 h at 37 °C in 5% CO_2. Following the incubation period, culture supernatants were removed (extracellular cAMP) and transferred to a new 48-well plate for cAMP determination.
cAMP determination: The extracellular cAMP concentration in the supernatants was measured with a cAMP-specific ELISA from Assay Designs, Inc. (Ann Arbor, Michigan) per manufacturer directions.