Novel Botulinum Neurotoxin/A Inhibitors: Active in Both Enzyme and Cell-Based Assays

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**ABSTRACT**

Botulinum neurotoxin (BoNT/A) is the most potent of the botulinum toxins and, due to their lethal nature, are listed under category A (high priority) bioterrorism agents by the Centers for Disease Control and Prevention. BoNT/A is easily produced and may be delivered by aerosol route. Consequently, these toxins represent a serious threat to both military personnel and civilians. Once inhaled into the lung, BoNT/A is taken up by the blood stream, target the peripheral cholinergic nerve endings, and cause death by interrupting autonomic nerve function. Due to the lethality and difficulty of treating intoxication with BoNT/A, new small-molecule inhibitors of these toxins are critically needed. A high-throughput screening (HTS) assay was used to screen the NCI diversity set and NCST 49088 was one of 10 compounds that emerged, inhibiting both the HTS and the secondary HPLC assay.

**RESULTS**

In the present study, a recently synthesized series of BoNT/A inhibitors, based on the structure of NCST 49088 (Mbx 1107) and analogs, Mbx 1107 and 1176, were tested. These compounds displayed significant IC50 values in the enzyme, cell-based and in vivo assays of BoNT/A intoxication. The compounds exhibited half-maximal inhibitory activity in BoNT/A enzymatic assays of 0.17 μM, with varying degrees of specificity and cytotoxicity, when tested against other metalloproteases and for effects on mammalian cells. The compounds demonstrated activity in vivo or chick neuronal cell assays and, importantly, provided protection against BoNT/A intoxication in mice.

**CONCLUSIONS**

These results confirmed earlier studies that highlighted NCST 49088 (Mbx 1107) as a viable lead compound that can inhibits BoNT/A enzymatic activity in enzyme assays and within neuronal cells, a requisite for a botulinum toxin countermeasure. The results also suggested that performing SAR around NCST 49088 is proving to be a useful approach to developing better inhibitors.

**INTRODUCTION**

• Botulinum neurotoxin (BoNT/A) are the most potent of the botulinum toxins (1-2). Because of their lethality and are listed under category A (high priority) bioterrorism agents by the Centers for Disease Control and Prevention. BoNT/A are easily produced and may be delivered by aerosol route (3, 4).

• Botulinum neurotoxic serotypes A–G differ significantly in amino acid sequence, protein substrate, and substrate cleavage sites (5) as well as in the duration of resulting paralysis. BoNT/A is the most toxic of the botulinum toxins and has been used as a biological weapon in four different terrorist incidents (5).

• BoNT/A neurotoxins are zinc metalloproteases that cleave presynaptic proteins (6). The N-termini of these toxins are disulfide bonds (7). The N-termini are required for receptor binding, intracellular trafficking, and release of the catalytic domains (8). The catalytic domains are responsible for cleaving crucial neuronal proteins, including synaptic vesicle proteins, resulting in the inhibition of acetylcholine release and paralysis (9).

• Mbx 1131 was synthesized, and the inhibition of BoNT/A, was assessed in enzyme assays, cell-based assays, and in vivo systems.

**METHODS**

**SUMMARY**

A synthetic chemistry campaign generated over 150 2-12 phenoxyphenyl compounds based on the structure of NCST 49088 (Mbx 1107). These compounds were tested in an enzyme assay, cell-based assays, and in vivo systems. The most potent inhibitors, Mbx 1107 and 1131, also demonstrated significant protection in vivo. These findings suggest that a significant number of these small molecules may be useful as BoNT/A inhibitors.

**REFERENCES**

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