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Short communication

The synthesis of 2,5-bis(4-amidinophenyl)thiophene derivatives providing submicromolar-range inhibition of the botulinum neurotoxin serotype A metalloprotease

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ABSTRACT

Botulinum neurotoxins (BoNTs), composed of a family of seven serotypes (categorized A–G), are the deadliest of known biological toxins. The activity of the metalloprotease, light chain (LC) component of the toxins is responsible for causing the life-threatening paralysis associated with the disease botulism. Herein we report significantly more potent analogs of novel, lead BoNT serotype A LC inhibitor 2,5-bis(4-amidinophenyl)thiophene ($K_i = 10.88\ \mu M \pm 0.90\ \mu M$). Specifically, synthetic modifications involved simultaneously replacing the lead inhibitor's terminal bis-amidines with secondary amines and the systematic tethering of 4-amino-7-chloroquinoline substituents to provide derivatives with K_i values ranging from 0.302 μM (±0.03 μM) to 0.889 μM (±0.11 μM).

1. Introduction

Botulinum neurotoxins (BoNTs), a family of seven serotypes (categorized A–G), are secreted by *Clostridia* species *botulinum*, *baratii*, and *butyricum* [1, 2], and are listed among the highest priority of bioterrorism agents [3].

The enzymes are composed of a 100 kDa heavy chain (HC) and a 50 kDa light chain (LC), which are tethered by a reducible disulfide bridge [4,5]. The HC binds to neuronal receptors and releases the LC into the cell cytosol [5–7]. The LC, a zinc^{2+} (Zn^{2+}) metalloprotease, cleaves neuron soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) proteins [6,7]. This proteolytic activity inhibits the release of acetylcholine into neuromuscular junctions, resulting in the disease state botulism [6,7]. The BoNT serotype A LC (BoNT/A LC), which is the target enzyme of this study, cleaves SNARE protein synaptosomal associated protein of 25 kDa (SNAP-25) [6,7], and is known to cause human botulism [8,9].

We have previously reported the discovery of a variety of non-Zn^{2+} chelating BoNT/A LC lead inhibitor chemotypes [10–15], many of which possess terminal di-cationic moieties [11–14]. With respect to di-cationic inhibitors, the synthesis of more potent derivatives of leads possessing bis-amidine and bis-imidazoline functional groups has been described [16–19]. Importantly, the synthetic modification of one of these leads, bis [3-amide-5-(imiazolino)phenyl]terephthalamide-based inhibitor **I** ($K_i = 8.52 \pm 0.53\ \mu M$) [18] (Fig. 1), via the incorporation of terminal $-(CH_2)_3-$4,7-ACQ components, resulted in derivative **II** (Fig. 1), which possesses a $K_i = 0.572\ \mu M \pm 0.041\ \mu M$ [18]. To the best of our knowledge, **II** is the most potent, non-hydroxamic acid-based BoNT/A LC inhibitor reported to date.

As part of an ongoing research program to discover novel BoNT/A LC inhibitor chemotypes for development, a variety of di-amidine

Abbreviations: BoNT/A LC, Botulinum neurotoxin serotype A light chain; 4,7-ACQ, 4-amino-7-chloroquinoline.

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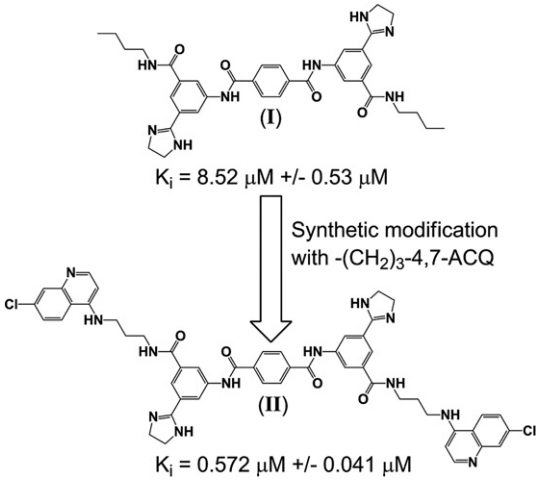


Fig. 1. Adding $-(CH_2)_3-$4,7-ACQ components translated lead BoNT/A LC inhibitor I into nM-range derivatives, II was the most potent of the congeneric series [18].

substituted compounds obtained from the National Cancer Institute's Open Repository were screened. Subsequently, 2,5-bis(4-amidinophenyl)thiophene **1** (NSC 300510) (Scheme 1) was found to provide 78% BoNT/A LC inhibition when tested at 20 μM concentration. Following, **1** was synthesized to ensure purity, and subsequent *in vitro* testing indicated that it possesses a $K_i = 10.88\ \mu M \pm 0.90\ \mu M$ (Table 1).

Interestingly, **1** has previously been shown to possess anti-trypanosomal activity [20, 21], and provided 100 % survival when administered as a single, 320 mg/kg dose during *Trypanosoma rhodesiense* infection [21]. Additionally, **1** has been analyzed for antineoplastic activity, and based on a dosing regimen of three 100 mg/kg injections (administered on day one, and then every fourth day) was non-toxic to mice [22].

Therefore, based on the possibility of repurposing a biologically relevant chemotype, **1** was chosen as a candidate for synthetic modification to provide derivatives with increased BoNT/A LC inhibitory potencies. Specifically, based on the strategy used to increase the inhibitory potency of lead **I** (Fig. 1) [18], it was hypothesized that systematically tethering terminal 4,7-ACQ motifs to **1** with short methylene chains would also provide significantly more potent derivatives.

2. Chemistry

Initially, we attempted to substitute $-(CH_2)_n-$4,7-ACQ components directly onto the terminal amidines of **1**. However, as previously encountered when attempting the same amidine substitution with a different BoNT/A LC inhibitor chemotype [18], prohibitive synthesis and/or degradation during purification were encountered. To circumvent these obstacles, an alternative cationic motif, with the terminal amidines of **1** replaced with secondary amines, provided an efficient synthetic route for the generation of a congeneric series to further test our hypothesis for increasing the inhibitory potency of chemotype **1**.

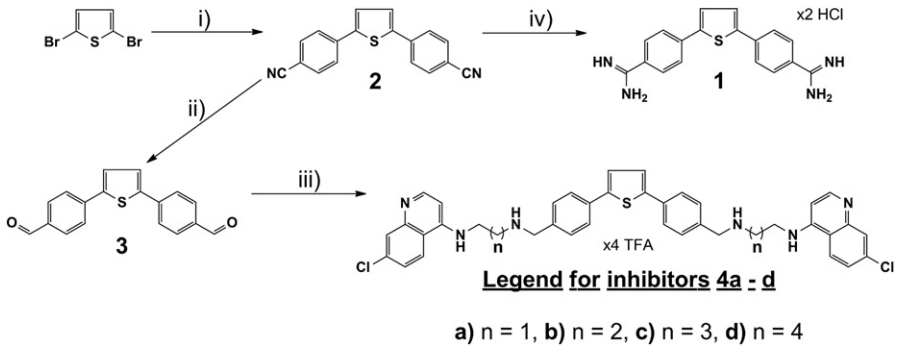
The synthesis of **1** and derivatives **4a**–**d** are outlined in Scheme 1. Key intermediate di-nitrile **2** was obtained in 94% yield by coupling 4-cyanophenylboronic acid and 2,5-dibromothiophene under Suzuki conditions. The synthesis of **1**, to ensure purity (as it was initially obtained from a chemical repository (*vide supra*)), was achieved in two-steps by reacting **2** with LHMDS at room temperature, followed by isolation as an HCl salt (97% yield). Di-aldehyde **3** was prepared in 77% yield via the DIBAH-mediated reduction of **2** at 0 °C. Following, simultaneous reductive amination of **3** and coupling with $NH_2-(CH_2)_n-$4,7-ACQ motifs provided targets **4a**–**d**, which were isolated as TFA salts (yields ranged from 24 to 49%).

Table 1
BoNT/A LC inhibition constants for lead **1** and derivatives **4a**–**d**.

Inhibitor	K_i (μM)
1	10.88 ± 0.90 μM
4a	0.882 ± 0.11 μM
4b	0.302 ± 0.03 μM
4c	0.535 ± 0.60 μM
4d	0.889 ± 0.11 μM

3. BoNT/A LC inhibition

Table 1 provides the K_i values for inhibitors **1** and **4a**–**d** when examined *in vitro* employing a well documented HPLC-based assay for BoNT/A LC inhibition [23–29]. The potencies of the derivatives, in support of our hypothesis, provide further evidence that tethering $-(CH_2)_n-$4,7-ACQ components onto the termini of di-cationic lead BoNT/A LC inhibitors (*i.e.*, such as **I** (Fig. 1)) and **1** (Scheme 1 and Table 1) can significantly improve inhibitory potency. Interestingly, as was encountered when tethering 4,7-ACQ components onto the terminal amides of inhibitor chemotype **I** [18] (Fig. 1), trimethylene linkers also afforded the most significant increase in the inhibitory potency of **1**. Specifically, tethering $-(CH_2)_3-$4,7-ACQ groups onto the secondary amino termini of the 2,5-bis(4-methylaminophenyl) thiophene scaffold (**4b**, Scheme 1) increased inhibitory potency by approximately (approx.) 36-fold versus **1** (Table 1).



Scheme 1. Reagents and conditions. i) 4-CN–PhB$(OH)_2$, $Pd(OAc)_2$, DME, $Na_2CO_{3(aq)}$, MW, 80 °C, 2 h; ii) DIBAH, PhMe, 1 h, 0 °C, Ar; iii) (step 1) $NH_2-(CH_2)_n-$4,7-ACQ, $NaBH_4$, AcOH, $MeOH/CH_2Cl_2$ and (step 2) TFA; iv) (step 1) LHMDS, THF, r.t. and (step 2) HCl/EtOH.