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A matrix-focused structure-activity and binding site flexibility study of quinolinol inhibitors of botulinum neurotoxin serotype A

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ABSTRACT

Our initial discovery of 8-hydroxyquinoline inhibitors of BoNT/A and separation/testing of enantiomers of one of the more active leads indicated considerable flexibility in the binding site. We designed a limited study to investigate this flexibility and probe structure-activity relationships; utilizing the Betti reaction, a 36 compound matrix of quinolinol BoNT/A LC inhibitors was developed using three 8-hydroxyquinolines, three heteroaromatic amines, and four substituted benzaldehydes. This study has revealed some of the most effective quinolinol-based BoNT/A inhibitors to date, with 7 compounds displaying IC_{50} values $\leq$ 1 μM and 11 effective at $\leq$ 2μM in an ex vivo assay.

Abbreviations: BoNTs, botulinum neurotoxins; BoNT/A, botulinum neurotoxin serotype A; HC, heavy chain; LC, light chain; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; SNAP-25, 25 kDa synaptosomal-associated protein; BIG-IV, Botulism Immune Globulin Intravenous (Human); SAR, structure-activity relationship; IC_{50}, 50% inhibitory concentration; MPNHDA, Mouse Phrenic Nerve Hemidiaphragm Assay

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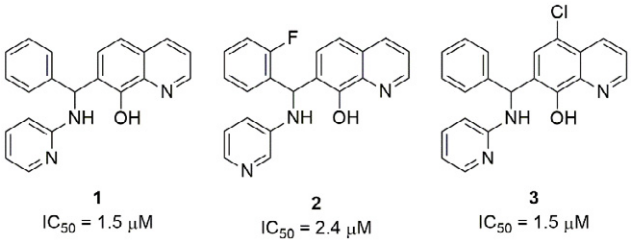

Figure 1. Serving as structural inspiration for this study, compounds **1**, **2**[15] and **3**[16] all showed low μM BoNT/A inhibitory activity in previous enzymatic and *ex vivo* studies.

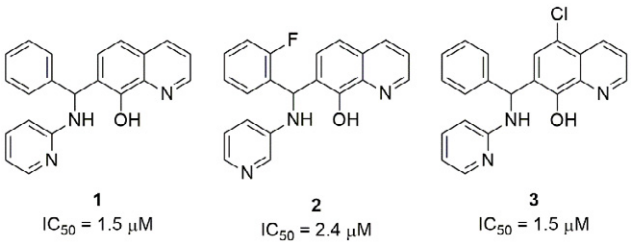

Figure 1. Serving as structural inspiration for this study, compounds **1**, **2**[15] and **3**[16] all showed low μM BoNT/A inhibitory activity in previous enzymatic and *ex vivo* studies.

Botulinum neurotoxins (BoNTs), produced by the anaerobic bacterium *Clostridium botulinum*, are the direct cause of botulism, which is characterized by flaccid muscle paralysis resulting from blockage of acetylcholine release from nerve cells. BoNTs occur as seven immunologically distinct serotypes (A-G) and are some of the most potent toxins found in nature. Recently, a new purported serotype designated as type H has been reported, but this requires experimental confirmation.[1,2] Botulinum neurotoxin serotype A (BoNT/A) is the most common, with a lethal dose (LD_{50}) of less than 1 ng/kg in humans.[3] The high level of toxicity associated with BoNT/A, coupled with its apparent ease of production makes it a dangerous potential biowarfare agent.[4]

Synthesized as 150 kDa protoxins, BoNTs are proteolytically cleaved to form the active holotoxin comprised of a 100 kDa heavy chain (HC) and a 50 kDa light chain (LC) linked via a disulfide bond.[5,6] The HC is responsible for the binding of the toxin to the surface of cholinergic nerve cells, as well as translocation of the LC into the cytosol of the nerve cell.[7-10] The LC is a zinc metalloprotease that cleaves various portions of SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein complex. In BoNT/A, the LC cleaves SNAP-25 (25 kDa synaptosomal-associated protein), preventing acetylcholine release from the synaptic terminals causing flaccid muscle paralysis.[11,12]

Current therapies for BoNT intoxication, which include supportive care, primarily mechanical ventilation, and the administration of antitoxins, are impractical in the event of widespread biological attack. Botulism Immune Globulin Intravenous (Human) (BIG-IV), an FDA-approved drug from the California Department of Public Health, is available to treat infant botulism caused by neurotoxin serotypes A and B. An FDA-approved heptavalent equine antitoxin (HBAT) is available for treatment of botulism in adults. Antitoxins of equine origin, however, carry the risk of serum-sickness and are only effective while toxin is in the circulatory system.[13] Once the toxin has entered the nerve cell, antibodies are ineffective. Prophylactic medical countermeasures against botulism do exist with vaccines,[14] but the increasing number of medical disorders being treated with therapeutic BoNT (e.g., Botox) makes vaccination of the general population inadvisable. Therefore, small-molecule therapeutics effective in the neuronal cell are attractive targets for the post-exposure treatment of botulism.

In our original disclosure of BoNT inhibitory activity of the 7-substituted 8-hydroxyquinolines, we tested 55 analogs and found that over half of them, including **1** and **2**, exhibited ≥50% inhibition of BoNT/A at doses ≤50 μM (**Figure 1**).[15] Those compounds contained a wide array of substituent groups attached at C-7, as did the less active or inactive analogs, providing no obvious or definitive structure-activity relationship (SAR) information. Early on, we hypothesized that the binding site of BoNT/A was very accommodating or flexible,[16] a rather widely occurring phenomenon that has recently been reviewed.[17] When we subsequently separated the enantiomers of **3**, one of our more potent compounds, and found them to have essentially equivalent inhibitory activity,[16] that hypothesis became more solidly supported. Recently, Caglič et al. examined 188 8-hydroxyquinoline compounds and found similar levels of activity and reached similar conclusions about the binding site.[18]

After our original observations about the activity of compounds in this class and the flexibility or plasticity of the binding site, we embarked on a detailed study of a limited, focused matrix of analogs in an effort to determine the relevance of substitutions on the 8-hydroxyquinoline ring and compositions of the aldehyde and amine components of the Betti reation[19,20] used to construct these compounds. We report here the results of this study and its implications for further development of this class of BoNT/A inhibitors.

The Betti reaction is a one pot, Mannich-type reaction that involves the condensation of an amine, aldehyde, and phenol. We utilized this chemistry to develop a 3 x 3 x 4 matrix of potential

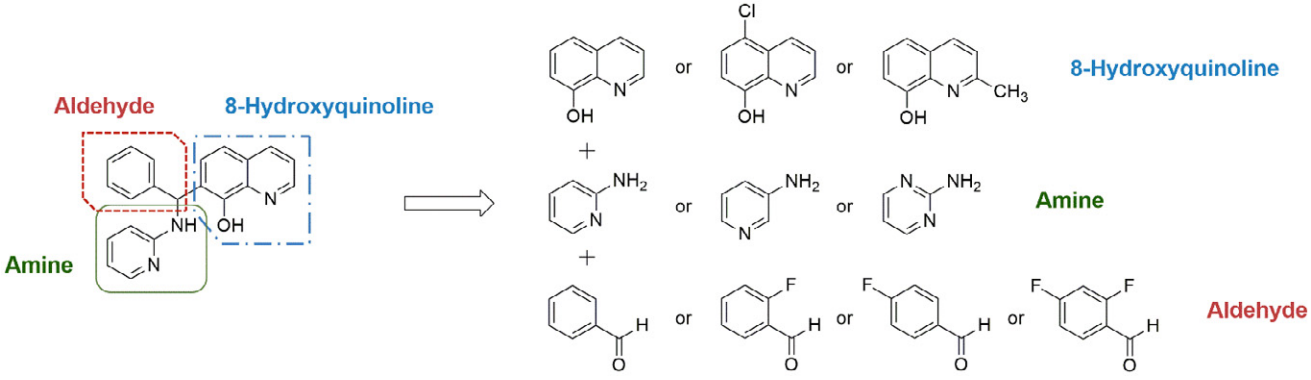

Figure 2. A 3 x 3 x 4 matrix of BoNT/A inhibitors using three substituted 8-hydroxyquinolines, three heteroaromatic amines, and four substituted benzaldehydes.

BoNT/A inhibitors for this SAR study. These 36 compounds were synthesized using the three substituted 8-hydroxyquinolines, three heteroaromatic amines, and four substituted benzaldehydes shown in **Figure 2**.

Small-molecule BoNT/A inhibitors **1** and **2** share the same general structural scaffold, differing only in the placement of the nitrogen in the pyridyl ring and a fluorine substituent. One goal of this study was to determine the importance of these structural features in regard to BoNT/A inhibitory activity. In choosing the starting material for the amine portion of the inhibitors for this SAR study, we selected 2- and 3-pyridyl amines as we previously observed that both components gave rise to potent BoNT/A inhibitors. We also chose to use 2-pyrimidyl amine as we envisioned that an extra nitrogen on this ring might provide additional contacts in the active site and improve solubility and formulation characteristics. As for the aldehyde portion, we wanted to probe the effects of fluorine substitutions on the aromatic ring. In this case, benzaldehyde was chosen as a starting material, along with 2-fluoro, 4-fluoro and 2,4-difluorobenzaldehydes.

In addition to studying the effects of these structural differences on BoNT/A inhibitory activity, we also evaluated changes to the core 8-hydroxyquinoline structure. In our two previous studies, we discovered that compounds **1** and **3**, differing in a hydrogen vs. a chlorine attached at C-5 on the 8-hydroxyquinoline moiety, both inhibit BoNT/A with an IC_{50} of 1.5 μM. Additionally, compounds containing a methyl group at the C-2 position showed promising activity in preliminary (unpublished) studies. With this in mind, we chose 8-hydroxyquinoline as well as the 5-chloro and 2-methyl substituted versions as the last three components in this 3 x 3 x 4 matrix SAR study.

To determine IC_{50} values for the 36 BoNT/A inhibitors, an HPLC-based enzymatic assay was implemented using a full-length recombinant BoNT/A LC in the presence a 17-mer SNAP-25 peptide substrate. This substrate consists of residues 187 to 203 of SNAP-25 and catalytically cleaves the BoNT/A LC between residues Q197 and R198.[21] All 36 compounds were very effective in inhibiting cleavage of the BoNT/A LC, and had IC_{50} values ranging from 0.6 to 12.7 μM, with all but four compounds demonstrating starting IC_{50} values at or below 4.6 μM. Upon first glance, it seems no single compound stands out and no discernible pattern exists. However, interesting general trends across each group are apparent upon taking the average of all IC_{50} values for each starting material type (**Table 1**, for an inclusive list of biological data for each of the 36 individual compounds, see **Table 2**).

Table 1. Average IC_{50} values for starting material components

Inhibitor Moiety	Starting Material	Average IC_{50} (μM)
8-hydroxyquinoline	8-hydroxyquinoline	3.9
	2-methyl-8-hydroxyquinoline	3.9
	5-chloro-8-hydroxyquinoline	1.9
amine	2-aminopyridine	1.8
	3-aminopyridine	3.3
	2-aminopyrimidine	4.6
aldehyde	benzaldehyde	2.9
	2-fluorobenzaldehyde	2.8
	4-fluorobenzaldehyde	2.3
	2,4-difluorobenzaldehyde	4.9

A review of the data for the three different 8-hydroxyquinoline starting materials indicated that the 5-chloro substituted analogs show the highest activity against BoNT/A (average IC_{50} of 1.9 μM), a two-fold increase in activity relative to the unsubstituted 8-hydroxyquinoline and the 2-methyl version, which both have average IC_{50} values of 3.9 μM. Upon examination of the BoNT/A inhibitory activity with respect to the three heteroaromatic amines used in this study, the two pyridine reagents provided most of the active compounds. The 2-amino derivatives have an average IC_{50} of 1.8 μM, the most active of the group. This is in stark contrast to the 2-aminopyrimidine derivatives which have an average IC_{50} of 4.6 μM across the group, approximately 2.5x less potent than the 2-aminopyridine containing compounds.

When looking at the aldehyde group, the results are not as straightforward, with the 4-fluoro derivatives showing slightly higher inhibitory activity when compared to the rest of the group. Inhibitors employing benzaldehyde, 2-fluorobenzaldehyde, and 4-fluorobenzaldehyde all have average IC_{50} values ranging from 2.3 to 2.9 μM, roughly twice as potent the 2,4-difluorobenzaldehyde derivatives.

After finding interesting inhibitory trends across these structural groups in our *in vitro* BoNT/A LC protease assay, we wanted to see if these general trends held true in biological systems at the tissue level. We tested our 3 x 3 x 4 matrix of small-molecule inhibitors in an *ex vivo* mouse phrenic nerve hemidiaphragm assay (MPNHDA).[15] Inhibitors were tested at final concentrations of 20 and 2 μM, with the exception of compounds **1**-**3**, which were previously tested at 10 and 2 μM.[15,17] Compounds listed as not effective did not cause a delay in time to 50% loss of twitch tension at a concentration of 20 μM.

Trends similar to those observed in the *in vitro* assay emerged across each group in the *ex vivo* assay. When considering the 8-hydroxyquinoline, the 5-chloro derivative appears to be the most potent (**Table 2**; for ease of comparison, this table sorted by amine and aldehyde groups can be found in **Tables S1** and **S2**, respectively, in the supporting information). This group has the largest number of compounds active at 2 μM and has only one compound that was not effective at 20 μM in the MPNHDA. The unsubstituted 8-hydroxyquinoline and the 2-methyl analogs show similar activity to one another, with three ineffective compounds in each group. These two groups have five compounds combined that are active at 2 μM, the same number as the 5-chloro group alone.

When observing the data across the amine substituents, there is a stark contrast between different starting materials (**Table S1**). Inhibitors containing either 2- or 3-pyridyl groups are all active in this assay. Interestingly, all of the compounds that are not effective at delaying onset of paralysis at 20 μM can be found in the group incorporating the pyrimidine moiety. Data from both the MPNHDA and the BoNT/A protease assay indicate that having two nitrogens in this ring is clearly detrimental to BoNT/A inhibitory activity.

Inhibitors synthesized using 4-fluorobenzaldehyde seem to be the most active in the MPNHDA in the aldehyde group, with all 4-fluoro derivatives showing activity at either 2 or 20 μM (**Table**

S2). Both the benzaldehyde and 2-fluoro groups have three compounds active at 2 μM, as well as two each that do not delay onset of BoNT/A induced paralysis at 20 μM. However, if we remove the pyrimidine containing compounds that are clearly far less active in this assay, the benzaldehyde, 2-fluoro, and 4-fluoro groups all show similar activity, with three compounds each that cause a delay in time to 50% loss of twitch tension at a concentration of 2 μM. The 2,4-difluoro analogs are the least active group, with only one compound active at 2 μM and three ineffective compounds. These results are very consistent with those from the *in vitro* BoNT/A LC protease assay.

Overall, this study has produced some of the most effective quinolinol-based BoNT/A inhibitors to date, with seven compounds displaying submicromolar IC_{50} values. Some of our original observations about the activity of compounds in this series were confirmed and some additional support for our hypothesis about the flexibility of the binding site was developed, with all compounds in this study displaying IC_{50} values ≤ 12.7 μM. As expected, active compounds were found throughout, although certain structural preferences emerged. Inhibitors containing a 2-pyridyl ring were the most active across the group. Interestingly, compounds containing an extra nitrogen in this ring (2-aminopyrimidine) were the least active in this series. All compounds that were not effective in the MPNHDA came from this group. The active site seemed to accommodate fluorine substitutions at the R^3 or R^4 positions, but substitution at both sites proved less active. While the flexibility of the BoNT/A active site does seem to accommodate added steric bulk at the R^1 position, the addition of a methyl group here provides no discernable difference in activity from that of the unsubstituted 8-hydroxyquinoline. However, the 5-chloro substituted compounds were the most active, with **31** and **35** (IC_{50} = 0.6 μM) being the most potent quinolinol-based BoNT/A inhibitors discovered to date. Our current efforts are directed toward further medicinal chemistry exploration of the aldehyde and amine components to determine whether additional improvements can be made in the BoNT/A inhibitory activity profiles.

A recent report from our laboratory disclosed that two early hits from our investigation of 8-hydroxyquinoline based inhibitors also inhibit BoNT's B, C, E, and F and, intriguingly, display different profiles of inhibition.[22] This work has further expanded the potential for this class of compounds in the

Table 2. IC_{50} and MPNHDA BoNT/A inhibitory data sorted by 8-hydroxyquinoline component[a]

Compound	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)	MPNHDA[b] (μM)
1	H	H	H	H	2-pyridyl	1.5	10[c]

4	H	H	H	H	3-pyridyl	6.2	20
5	H	H	H	H	2-pyrimidyl	2.7	NE[d]
6	H	H	F	H	2-pyridyl	1.2	20
2	H	H	F	H	3-pyridyl	2.4	10[c]
7	H	H	F	H	2-pyrimidyl	1.9	NE[d]
8	H	H	H	F	2-pyridyl	4.1	2
9	H	H	H	F	3-pyridyl	4.6	2
10	H	H	H	F	2-pyrimidyl	1.7	20
11	H	H	F	F	2-pyridyl	3.6	20
12	H	H	F	F	3-pyridyl	4.4	20
13	H	H	F	F	2-pyrimidyl	12.7	NE[d]
14	CH_3	H	H	H	2-pyridyl	0.7	2
15	CH_3	H	H	H	3-pyridyl	1.4	20
16	CH_3	H	H	H	2-pyrimidyl	4.6	NE[d]
17	CH_3	H	F	H	2-pyridyl	1.4	2
18	CH_3	H	F	H	3-pyridyl	0.7	20
19	CH_3	H	F	H	2-pyrimidyl	12.0	NE[d]
20	CH_3	H	H	F	2-pyridyl	1.6	20
21	CH_3	H	H	F	3-pyridyl	0.8	20
22	CH_3	H	H	F	2-pyrimidyl	4.2	20
23	CH_3	H	F	F	2-pyridyl	2.7	20
24	CH_3	H	F	F	3-pyridyl	12.3	2
25	CH_3	H	F	F	2-pyrimidyl	4.3	NE[d]
3	H	Cl	H	H	2-pyridyl	1.5	2[c]
26	H	Cl	H	H	3-pyridyl	3.5	2
27	H	Cl	H	H	2-pyrimidyl	4.5	2
28	H	Cl	F	H	2-pyridyl	1.4	20
29	H	Cl	F	H	3-pyridyl	0.7	2
30	H	Cl	F	H	2-pyrimidyl	4.0	2
31	H	Cl	H	F	2-pyridyl	0.6	20
32	H	Cl	H	F	3-pyridyl	2.3	2
33	H	Cl	H	F	2-pyrimidyl	0.7	20
34	H	Cl	F	F	2-pyridyl	0.7	20
35	H	Cl	F	F	3-pyridyl	0.6	20
36	H	Cl	F	F	2-pyrimidyl	2.2	NE[d]

[a] Assays conducted as described in ref. 15 and the Experimental Procedures section.

[b] Mouse phrenic nerve hemidiaphragm assay. Compounds were tested at 20, 2, and 0.5 μM and data represent lowest effective concentration.

[c] Inhibitors **1**-**3** were also tested at 10 μM.

[d] Not Effective at concentration of 20 μM.

treatment of BoNT intoxication. There has been interest in this class of inhibitor in other laboratories as well,[18] including a detailed computer modeling study by Gómez-Jeria et al.[23] that expanded our earlier examination into the binding of enantiomers of **3**.[16]

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Supplementary Material

Supplementary data associated with this article, including tables of assay data sorted by starting material component, experimental protocols, and characterization data, can be found, in the online version, at http://

References and notes

1. Barash, J. R.; Arnon, S. S. *J. Infect. Dis.* **2014**, *209*, 183.
2. Dover, N.; Barash, J. R.; Hill, K. K.; Xie, G.; Arnon, S. S. *J. Infect. Dis.* **2014**, *209*, 192.
3. Schantz, E. J.; Johnson, E. A. *Microbiol. Rev.* **1992**, *56*, 80.
4. Arnon, S. S.; Schechter, R.; Inglesby, T. V.; Henderson, D. A.; Bartlett, J. G.; Ascher, M. S.; Eitzen, E.; Fine, A. D.; Hauer, J.; Layton, M.; Lillibridge, S.; Osterholm, M. T.; O'Toole, T.; Parker, G.; Perl, T. M.; Russell, P. K.; Swerdlow, D. L.; Tonat, K. *J. Am. Med. Assoc.* **2001**, *285*, 1059.
5. Montecucco, C.; Schiavo, G. *Q. Rev. Biophys.* **1995**, *28*, 423.
6. Lacy, D. B.; Tepp, W.; Cohen, A. C.; DasGupta, B. R.; Stevens, R. C. Nat. *Struct. Biol.* **1998**, *5*, 898.
7. Black, J. D.; Dolly, J. O. *J. Cell. Biol.* **1986**, *103*, 521.
8. Black, J. D.; Dolly, J. O. *J. Cell. Biol.* **1986**, *103*, 535.
9. Montecucco, C.; Schiavo, G.; Tugnoli, V.; deGrandis, D. *Mol. Med. Today* **1996**, *2*, 418.
10. Montal, M. S.; Blewitt, R.; M., T. J.; Montal, M. *FEBS Lett*. **1992**, *313*, 12.
11. Simpson, L. L. *Annu. Rev. Pharmacol. Toxicol*. **2004**, *44*, 167.
12. Schiavo, G.; Matteoli, M.; Montecucco, C. Neurotoxins Affecting Neuroexocytosis. *Physiol. Rev*. **2000**, *80*, 717.
13. Hibbs, R. G.; Weber, T.; Corwin, A.; Allos, B. M.; El Rehim, M. S. A.; El Sharkaway, S.; Sarn, J. E.; McKee, K. T., Jr. *Clin. Infect. Dis.* **1996**, *23*, 337.
14. Smith, L. A.; Rusnak, J. M. *Crit. Rev. Immunol*. **2007**, *27*, 303.
15. Roxas-Duncan, V.; Enyedy, I.; Montgomery, V. A.; Eccard, V. S.; Carrington, M. A.; Lai, H.; Gul, N.; Yang, D. C.; Smith, L. A. *Antimicrob. Agents. Chemother*. **2009**, *53*, 3478.
16. Cardellina, J. H., II; Vieira, R. C.; Eccard, V.; Skerry, J.; Montgomery, V.; Campbell, Y.; Roxas-Duncan, V.; Leister, W.; LeClair, C. A.; Maloney, D. J.; Padula, D.; Pescitelli, G.; Khavrutskii, I.; Hu, X.; Wallqvist, A.; Smith, L. *ACS Med. Chem. Lett*. **2011**, *2*, 396.
17. Cozzini, P.; Kellogg, G. E.; Spyrakis, R.; Abraham, D. J.; Constantino, G.; Emerson, A.; Fanelli, F.; Gohlke, H. Kuhn, L. A.; Morris, G. M.; Orozco, M.; Pertinhez, T. A.; Rizza, M.; Striffer, C. A. *J. Med Chem.* **2008**, *51*, 6237.
18. Caglič, D.; Krutein, M. C.; Bompiani, K. M.; Barlow, D. J.; Benoni, G.; Pelletier, J. C.; Reitz, A. B.; Lairson, L. L.; Houseknecht, K. L.; Smith, G. R.; Dickerson, T. J. *J. Med. Chem.* **2014**, *57*, 669.
19. Betti, M. *Gazz. Chim. Ital.* **1900**, *30 (II)*, 301.
20. Betti, M. *Gazz. Chim. Ital.* **1900**, *30 (II)*, 310.
21. Schmidt, J. J.; Bostian, K. A *J. Protein Chem.* **1997**, *16*, 19.
22. Montgomery, V. A; Ahmed, S. A.; Olson, M. A.; Mizanur, R. M.; Stafford, R. G.; Roxas-Duncan, R. I.; Smith, L. A. *Toxicon* **2015**, *98*, 12.
23. Gómez-Jeria, J. S.; Reyes-Díaz, I.; Valdebenito-Gamboa, J. *J. Comput. Methods Mol. Des.* **2015**, *5*, 25.

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Graphical Abstract

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