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Synthesis and biological evaluation of aryloxyacetamide derivatives as neuroprotective agents

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ABSTRACT

A series of new aryloxyacetamide derivatives **10a–s** and **14a–m** are designed and synthesized. Their protective activities against the glutamate-induced cell death were investigated in differentiated rat pheochromocytoma cells (PC12 cells). Most compounds exhibited neuroprotective effects, especially for **10m**, **10r**, **14b** and **14c**, which showed potential protection of PC12 cells at three doses (0.1, 1.0, 10 μM). MTT assay, Hoechst 33342/PI double staining, and high content screening (HCS) revealed that pretreatment of the cells with **10m**, **10r**, **14b** and **14c** has significantly decreased the extent of cell apoptosis in a dose-dependent manner. The results of western blot analysis demonstrated these compounds suppressed apoptosis of glutamate-induced PC12 cells via caspase-3 pathway. These compounds can be lead compounds for further discovery of neuroprotective agents for treating cerebral ischemic stroke. Basic structure–activity relationships are also presented.

Stroke is the second commonest cause of death and leading cause of adult disability worldwide. Over two-thirds of stroke deaths worldwide are in developing countries.[1,2] In China, stroke is the second cause for mortality in all diseases. Particularly, ischemic strokes account for 60–80% of these strokes.[3] The pathological mechanisms of ischemic strokes are complex and not fully understood, but it is generally accepted that a pathological release of glutamate from neurons plays a central role in mediating subsequent neuronal cell injury and death.[4] Excessive excitatory transmission can be transformed into an implement of neuronal destruction resulting in CNS disorder, such as cerebral ischemia, hypoxia, autoimmune, Alzheimer's, Parkinson's diseases, and so on.[5]

So far, treatment options for stroke-related brain damage are very limited. Most pharmacological agents have focused on mechanisms that occur in acute stage of stroke, such as restoration of blood flow with antithrombosis and thrombolytic therapy, or reducing the effects of ischemia by neuroprotective therapy. Unfortunately, thrombolytic therapy can only be given to highly selected patients.[6] Therefore, there is an urgent need for effective neuroprotective agents to treat stroke-related brain damage.

Natural products are the single most productive source of lead molecules for development as clinically useful drugs for human disorders.[7] It has been reported that the cinnamon extract has vasodilative, antithrombotic, anti-ulcerous, and antiallergic action.[8] It is believed that the extracts (aqueous and/or organic solvent extraction) would provide different compositions of phytochemicals (such as cinnamic acid, cinnamaldehyde, proanthocyanidins), and these are responsible for the above effect.[9]

Encouraged by these observations and in continuation of our ongoing research program, we designed and synthesized several cinnamide derivatives to explore their neuroprotective properties.[10] We found that cinnamide scaffold often affords neuroprotective compounds (Fig. 1). Especially the compound **NY-308** (**1**), which has the (*E*)-*p*-methoxycinnamoyl moiety, exhibited good neuroprotection in vitro PC12 cells and in vivo rat focal cerebral ischemic animal model.[10,11] Structurally, the (*E*)-*p*-methoxycinnamoyl moiety in **NY-308** was believed to play a very important role in its activity. To further elucidate the structure–activity relationship, we designed and synthesized a novel series of phenoxyacetamide derivatives using $-OCH_2CO-$ connecting bridge as the surrogate replacing –CH=CHCO– moiety of cinnamide. Furthermore, we replaced diphenylmethylpiperazine of **NY-308** with benzylpiperazine and changed substituent groups of benzene rings to find new chemical entities with better neuroprotective activity (Fig. 2).

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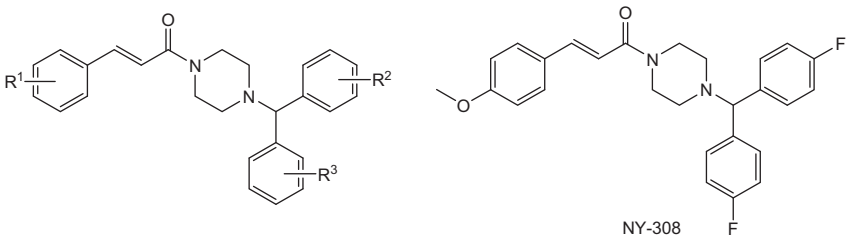


Figure 1. Structures of new cinnamide derivatives.

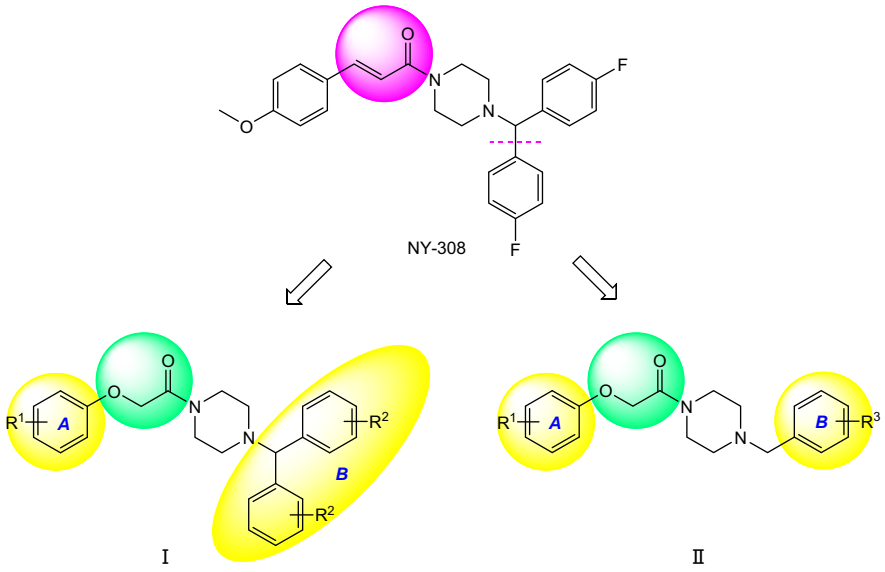


Figure 2. Structure exploration strategy based on compound **NY-308** (**1**).

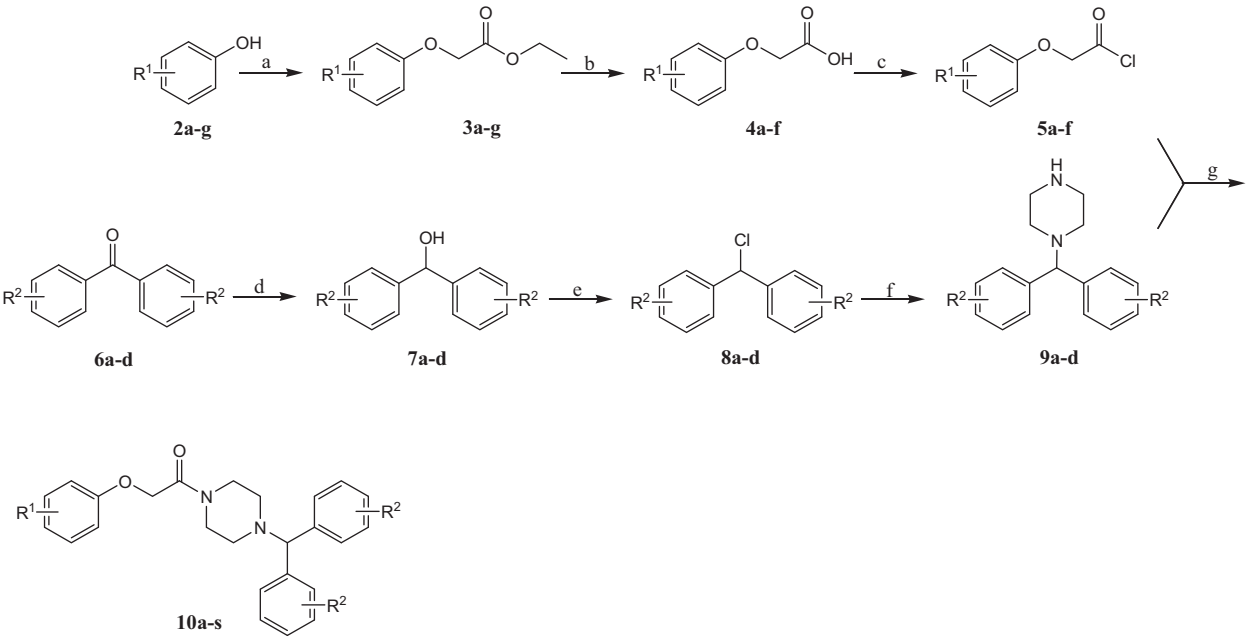
Therefore, a total of 32 target compounds were designed and synthesized. Their in vitro neuroprotective activities in the cell injury induced by glutamate, apoptosis assays, and the inhibition of caspase-3 were evaluated. Herein, the synthesis and preliminary biological evaluation of these compounds were reported.

Synthetic route for substituted diphenylmethylpiperazine analogs **10a–s** was depicted in Scheme 1. Commercially available substituted aromatic phenols **2a–g** were transformed into the desired intermediates **3a–g**, via reacting with ethyl chloroacetate in acetonitrile. Subsequent hydrolysis of **3a–f** with 10% sodium hydroxide gave carboxylic acids **4a–f**, which were chloridized with oxalyl chloride to give acyl chlorides **5a–f**. The synthesis of other key intermediates **9a–d** commenced with the reduction reactions of benzophenones **6a–d** under sodium borohydride. Subsequent chlorination of benzhydrols **7a–d** with $SOCl_2$, followed by alkylation with anhydrous piperazine, afforded diphenylmethylpiperazines **9a–d**. The target compounds **10a–s** were finally obtained by acylation of various substituted diphenylmethylpiperazines with the corresponding acyl chloride under mild conditions (at RT in acetone, and with triethylamine as base).

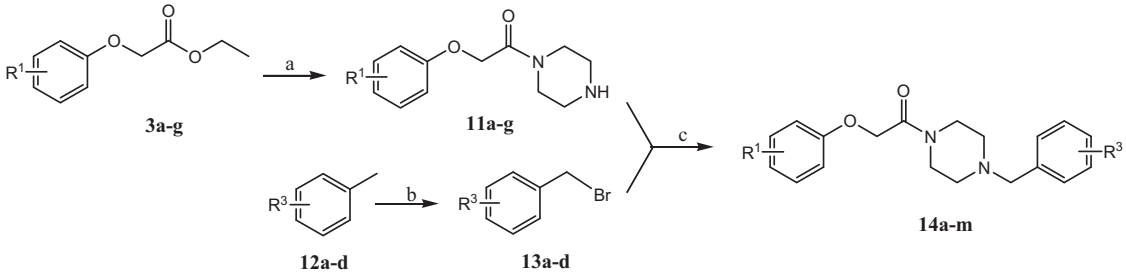
The second series of benzylpiperazine compounds **14a–m** were conveniently synthesized as outlined in Scheme 2. Substituted aryloxy acetyl piperazine intermediates **11a–g**, which were obtained by aminolysis of substituted ethyl aryloxyacetates **3a–g** with anhydrous piperazine, were used to react with benzyl bromides **13a–d** to directly provide the target compounds **14a–m**. The key intermediates benzyl bromides **13a–d**, were prepared by free-radical bromination of commercially available substituted toluenes **12a–d** with little excess of *N*-bromosuccinimide (NBS).

In order to study the potential neuroprotective activities of the title compounds, a preliminary screening was performed investigating neuroprotection on impairment induced by glutamine (Glu) in differentiated PC12 cells,[12,13] as evaluated by MTT assay.[14–16] The results are showed in Table 1. The vast majority of compounds tested exhibited protection of PC12 cells against glutamate-induced cell death, indicating that its bioactivity remained after introduction of the –O–CH_2–CO– connecting bridge into structures. Potency and toxicity were highly sensitive to structural variations. Remarkably, compounds **10j**, **10k**, **10m**, **10r**, **14b**, **14c**, **14f**, **14i**, and **14j** showed good neuroprotective activity for all three test concentrations (0.1, 1.0, 10 μM) (protection >20%). From Table 1, it was found that the cumulative addition of the compounds **10m**, **10r**, **14b**, and **14c** (0.1–10 μM) caused concentration-dependent neuroprotective effects with the maximal effect observed at 10 μM. Compounds **10j**, **10k**, **14d**, and **14j** showed a pattern of increased protection with increasing concentrations (0.1–1 μM) in terms of cell protection. Compounds **10n**, **14f**, **14h** and **14k** were observed to have the highest protection at the lowest concentrations of 0.1 μM (cell protection: 45.45%, 35.28%, 53.34% and 39.86%, respectively). Compounds **10a**, **10b**, **10f**, **10g**, **10i**, **10l**, and **14e** were observed to have the highest protection at the highest concentrations of 10 μM (cell protection: 25.64%, 30.24%, 21.17%, 56.98%, 80.50%, 50.34% and 25.10%, respectively). Though many of derivatives showed interesting neuroprotective effects, unfortunately, some of them possess unexpected cytotoxicity toward PC12 cells. Moreover, some compounds have limited solubility in cell culture medium, which complicates interpretation of these negative results.

Derivatives of I and II were substituted with different functional groups to study of the substituent variability influence on the biological activity and find new chemical entities with better neuroprotective activity. Based on lipophilic and electronic



Scheme 1. Reagents and conditions: (a) $ClCH_2COOEt$, K_2CO_3, CH_3CN; (b) 10% NaOH, EtOH; (c) oxalyl chloride, CH_2Cl_2; (d) $NaBH_4$, EtOH; (e) $SOCl_2$, CH_2Cl_2; (f) anhydrous piperazine, cyclohexane; (g) TEA, acetone.



Scheme 2. Reagents and conditions: (a) anhydrous piperazine, $CHCl_3$; (b) NBS, AIBN, CCl_4, *h.v.*; (c) TEA, CH_3CN.

attributes, these substituents were considered and selected, such as lipophilic electron-withdrawing groups F, Cl and Br; hydrophilic electron-donating groups OCH_3; lipophilic electron-withdrawing groups $COOCH_3$; and lipophilic electron-releasing groups CH_3. For series I, compounds with 2-substituent of *A* moiety were more active than those with 4-substitutent, while 4-substituent of *A* moiety exhibited more neuroprotective effect than 2-substituent for series II. The general structure–activity relationship of these compounds showed that the derivatives having electron-releasing groups were more active than those having electron-withdrawing groups. Among the electron-donating groups, hydrophilic methoxy derivatives were more active than the lipophilic methyl derivatives (cell protection: **10a** > **10c**, **10f** > **10h**, **14j** > **14e**, and **14i** > **14d**). On the other hand, for the different isoelectronic halogens, the lipophilic chloro substituent at the 4-position of *A* ring was more potent and less neurotoxic than the corresponding bromo-substituted derivatives (cell protection: **10p** > **10s**, **14k** > **14m**). Similarly, the lipophilic F-substituent at the 4-position of *B* ring was more potent and less neurotoxic than the corresponding bromo-substituted derivatives (cell protection: **10i** > **10l**). It was worthwhile to note that the compound **10r** exhibited promising neuroprotective effect (cell protection: 77.12% at 10 μM, 47.58% at 1.0 μM, and 26.80% at 0.1 μM, respectively), although it includes bromo substituent.

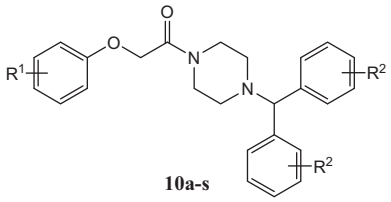
It is interesting to note that both compounds **14b** and **14c** possessing naphthyl moieties attached to the *A* system show a pattern of increased protection with increasing concentrations (0.1–10 μM) in terms of cell protection. On the contrary, the similar trend was not observed for the analogue **10a–d**. Perhaps it was because the rigidity of molecule prevented binding with the receptors.

For further investigation, apoptosis assays and western blot analysis were performed to determine their mechanism of action, at three concentration of 0.1, 1.0 and 10 μM, against glutamate-induced cell death. According to the in vitro biological activities, the compounds **10m**, **10r**, **14b** and **14c** were selected and further studied.

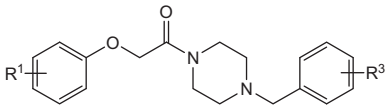
Apoptosis after cerebral ischemia is one of the major pathways that leads to the process of cell death.[17] Therefore, we investigated the anti-apoptotic effects of the compounds **10m**, **10r**, **14b** and **14c** by Hoechst 33342/PI double staining assay using high content screening system.[18,19] Quantitative analysis of the stained cells indicated that pretreatment of the cells with **10m**, **10r**, **14b** or **14c** has significantly decreased the extent of cell apoptosis in a dose-dependent manner. As shown in Table 2, after glutamate insult for 24 h, 43.97% of cultured cells showed typical characteristics of apoptosis. However, under pretreatments of **10m**, **10r**, **14b** and **14c** at three doses (0.1, 1.0 and 10 μM respectively), the apoptotic percentages were significantly reduced (Table 2).

Another important finding, which confirmed the protective effect of these derivatives, was the data obtained from western blot analysis of procaspase-3 and caspase-3. As shown in Figure 3,

Table 1
Neuroprotective effects of all target compounds against glutamate-induced neurotoxicity in PC12 cells



10a-s



14a-m

Compd	R^1	R^2	R^3	Cell viability (%)		
				0.1 μM	1.0 μM	10 μM
10a	R_1-phenyl = 2-naphthyl	4-Methoxy		2.55	5.42	25.64
10b	R_1-phenyl = 2-naphthyl	4-Bromo		4.06	7.87	30.24
10c	R_1-phenyl = 2-naphthyl	4-Methyl		4.75	5.63	10.28
10d	R_1-phenyl = 2-naphthyl	4-Fluoro		[a]	[a]	[a]
10e	2-Methyl	4-Bromo		[a]	[a]	[a]
10f	2-Methyl	4-Methoxy		2.45	3.75	21.17
10g	2-Methyl	4-Fluoro		7.92	8.70	56.98
10h	2-Methyl	4-Methyl		3.24	4.24	11.32
10i	4-Methoxy	4-Fluoro		3.10	5.26	80.50
10j	4-Methoxy	4-Methyl		21.67	42.28	47.50
10k	4-Methoxy	4-Methoxy		59.44	80.67	56.66
10l	4-Methoxy	4-Bromo		3.52	7.06	50.34
10m	2-Methoxy	4-Fluoro		32.03	45.89	79.67
10n	2-Methoxy	4-Bromo		45.45	12.82	6.67
10o	2-Methoxy	4-Methyl		—[a]	—[a]	—[a]
10p	4-Chloro	4-Bromo		4.22	8.01	16.74
10q	4-Chloro	4-Methyl		5.81	9.14	9.20
10r	4-Bromo	4-Fluoro		26.80	47.58	77.12
10s	4-Bromo	4-Bromo		—[a]	—[a]	—[a]
14a	4-Methyl		2-Methoxycarbonyl	—[a]	—[a]	—[a]
14b	R_1-phenyl = 2-naphthyl		2-Methoxycarbonyl	25.55	37.44	49.50
14c	R_1-phenyl = 2-naphthyl		2-Ethoxy	29.77	45.70	59.58
14d	2-Methyl		4-Bromo	10.25	22.14	20.80
14e	2-Methyl		2-Methoxycarbonyl	6.39	9.55	25.10
14f	2-Methyl		4-Ethoxy	35.28	30.65	28.93
14g	4-Methoxy		2-Methoxycarbonyl	—[a]	—[a]	—[a]
14h	2-Methyl		2-Ethoxy	53.34	11.90	8.52
14i	2-Methoxy		4-Bromo	38.00	41.00	20.76
14j	2-Methoxy		2-Methoxycarbonyl	23.20	32.82	23.06
14k	4-Chloro		2-Methoxycarbonyl	39.86	5.15	6.36
14l	4-Bromo		4-Bromo	—[a]	—[a]	—[a]
14m	4-Bromo		2-Methoxycarbonyl	—[a]	—[a]	—[a]
NY-308				71.01	49.66	77.75
Edaravone				33.04[b]		

[a] No activity.
[b] Edaravone—90 μM.

Table 2
Effect of some aryloxyacetamide on apoptosis of PC12 cells induced by glutamate

Compd	Rate of apoptotic cells (%)		
	0.1 μM	1.0 μM	10 μM
Control	19.27 ± 2.00		
Glutamate	43.97 ± 2.69[a]		
10m	42.28 ± 3.47	31.04 ± 3.11[b]	19.18 ± 3.64[b]
10r	41.83 ± 3.84	32.18 ± 3.12[b]	24.65 ± 3.18[b]
14b	35.67 ± 3.54[b]	29.27 ± 3.33[b]	21.81 ± 3.38[b]
14c	35.09 ± 3.64[b]	23.73 ± 3.03[b]	16.21 ± 2.04[b]

[a] Significantly different from control group (p <0.05).
[b] Significantly different from glutamate group (p <0.05).

glutamate induced the appearance of cleaved active caspase-3, showing the involvement of caspase-3 in glutamate-induced cell death in PC12 neurons. In the cells pretreated with **10m**, **10r**, **14b** or **14c** at three doses, bands of both procaspase and cleaved (active) caspase-3 were weaker compared to glutamate-treated cells. The compounds **10m** and **14b** significantly inhibited the expression of both procaspase-3 and caspase-3 induced by glutamate in a dose-dependent manner. The procaspase-3 level was not depressed significantly by compounds **10r** and **14c**, while, **10r** and **14c** significantly inhibited the expression of caspase-3 in a dose-dependent manner. The above results demonstrated these compounds inhibited apoptosis of glutamate-induced PC12 cells via caspase-3 pathway.

In summary, we synthesized a series of novel aryloxyacetamide derivatives and studied their protective activities against the glutamate-induced cell death in differentiated PC12 cells. Most target compounds displayed protective effects on the cell viability against the damage caused by glutamate, especially for **10m**, **10r**, **14b** and **14c**, which had significant potency and efficiently inhibited glutamate induced cell apoptosis via caspase-3 pathway. The present findings provided a preliminary pharmacological basis to exploit preventive or therapeutic strategies for stroke and other neurological insults.

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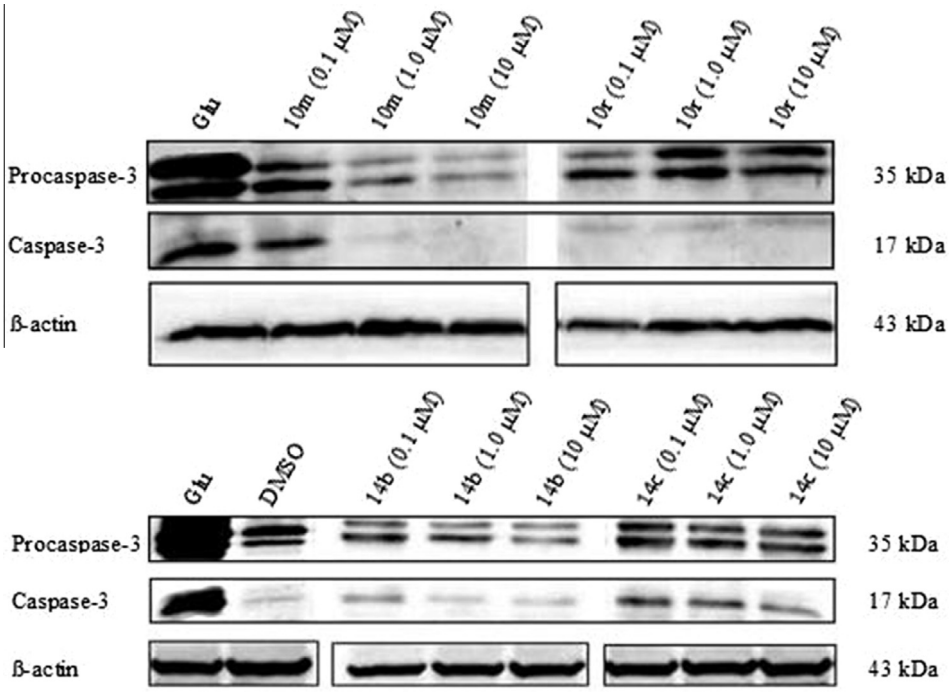

Figure 3. Caspase-3 activity in PC12 cells pretreated with aryloxyacetamide derivatives.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.03.094.

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