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Synthesis and antibacterial activities of new piperidine substituted (5*R*)-[1,2,3]triazolylmethyl and (5*R*)-[(4-F-[1,2,3]triazolyl)methyl] oxazolidinones

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ABSTRACT

A novel series of 5(*R*)-[1,2,3]triazolylmethyl and (5*R*)-[(4-F-[1,2,3]triazolyl)methyl]oxazolidinones having various piperidine group were synthesized and evaluated antibacterial activity against clinically isolated resistant strains of Gram-positive and Gram-negative bacteria. The compound **12a** having *exo*-cyanoethylidene group in the 4-position of piperidine ring was found to be two to threefold more potent than the linezolid against penicillin-resistant *Staphylococcus pneumonia* and *Staphylococcus agalactiae*, and also exhibited reduced MAO-B inhibitory activity.

Multidrug-resistant Gram-positive bacteria are an increasingly pressing problem in clinic. Linezolid (Zybox, **1**), an oxazolidinone antibiotics approved for clinical use orally, represent new class of antibiotic that has been very effective in treating multidrug-resistant Gram-positive pathogens.[1] The action mechanism of oxazolidinone is known for inhibiting of initial stage of protein synthesis which is formation of 70S mRNA initiation complex due to binding of 30S mRNA and 50S ribosome through fMet–tRNA.[2] Linezolid has competes with chloramphenicol and lincomycin because of having similar binding site but has only infrequent cross-resistance between linezolid and chlorampenicol or lincosamicine due to different mechanism.[3] Because linezolid has a novel mechanism of action, linezolid has same activity against many antibiotic-sensitive and antibiotic-resistant bacteria, and is active against methicillin resistant and vancomycin resistant bacteria.[4] Therefore therapeutic usage of linezolid is impressively increased annually worldwide since its approval in 2000. However, linezolid resistant organisms have been developed and treatment failures have been reported.[5] To overcome these unwanted drawbacks, further generation of oxazolidinone antibiotics active against the resistance organisms are still needed and actively studied by several research groups.[6] Recently, we reported that cyanomethylenepiperidinyl oxazolidinones (**2**) showed good antibacterial activity against multidrug-resistant strains including methicilline-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* (MRSE) and vancomycin resistant *enterococci* (VRE).[7] As a continuous effort to improve the potency and broaden the spectrum of the oxazolidinine antibacterial agents, we interested in the influence of substituent variation of piperidine ring on the antibacterial activity of piperidinylphenyl oxazolidinones having triazole heterocycle at the C-5 position of oxazolidinone A-ring. It is reported that the introducing 1,2,3-triazole ring instead of acetamide on the C-5 position of oxazolidinone core enhanced the antibacterial activity and among them, 4-substituted 1,2,3-triazole analogues were known to sustain their antibacterial activities.[8]

Here we describe the synthesis of a novel series of 5(*R*)-[1,2,3]triazolyl and (5*R*)-[(4-F-[1,2,3]triazolyl)methyl]oxazolidinones having various piperidinyl moieties including 4-substituted methyl-3-ene-piperidinyl groups, 4-substituted-*exo*-methylene-piperidinyl, 4-substitutedpiperidinyl, and 4-substituted-3-ene-piperidinyl as a modification of morpholine C-ring of linezolid (Fig. 1), and evaluation of their biological activity against Gram-positive and Gram-negative bacteria clinically isolated strains in South Korea. Also we tested their CYP profile, hERG inhibition, and mitochondrial monoamine oxidase (MAO) inhibition for the evaluation of toxicity.

The synthesis of oxazolidinone derivatives having 1,2,3-triazoles at the C-5 position of oxazolidinone ring were started from the known ketone intermediate **3** as shown in Scheme 1.[9]

The cycloaddition reaction of azide **3** with vinyl acetate or phenyl α–fluorovinyl sulfone[10] gave triazole **4a** and 4-fluoro-1,2,3-triazole **4b** respectively.[11]

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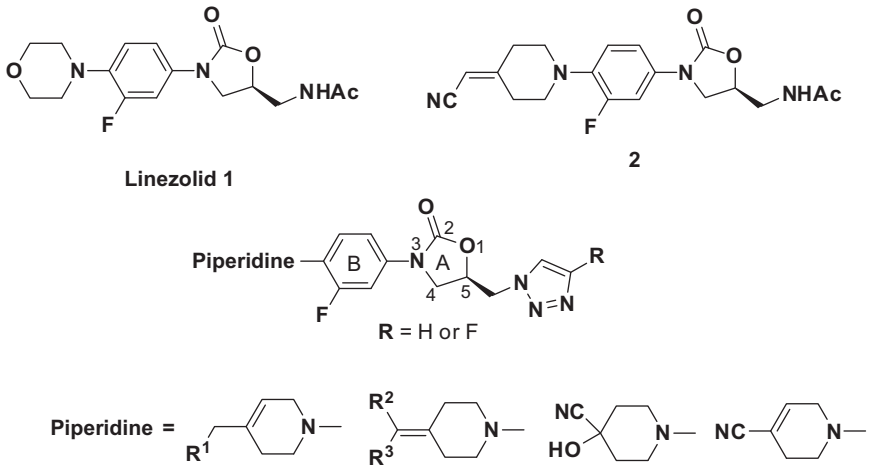
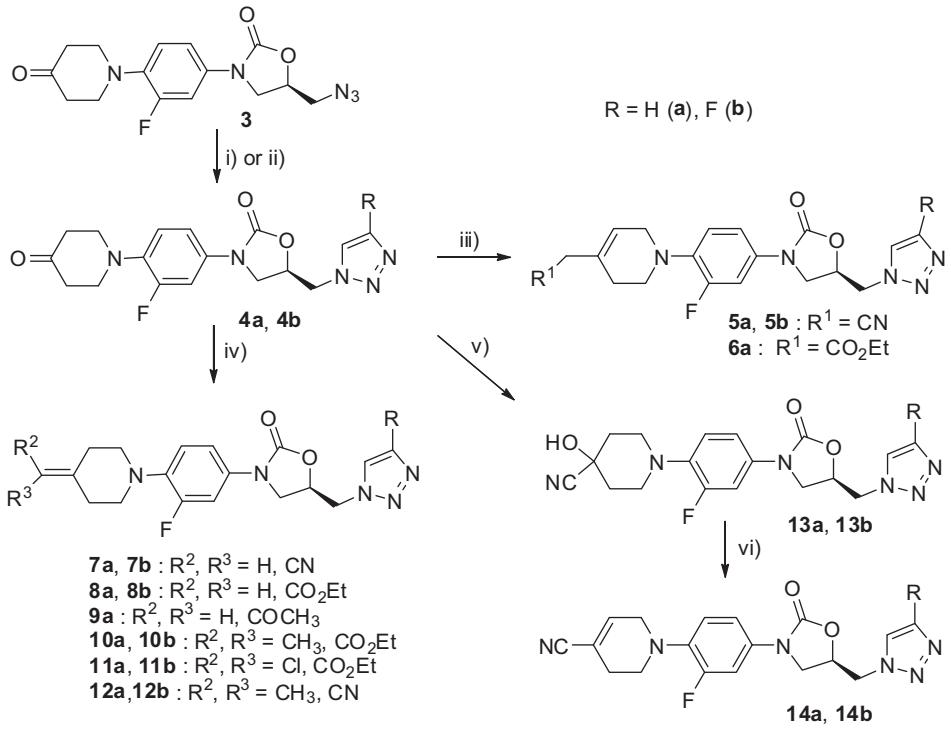


Figure 1. Known oxazolidines and new triazolyl-oxazolidinones bearing piperidine group.



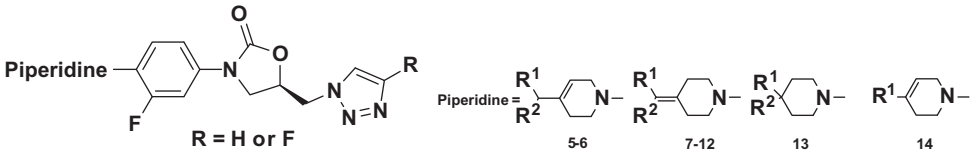
Scheme 1. Reagents and conditions: (i) vinyl acetate, reflux; (ii) 1-(benzenesulfonyl)-1-fluoroethene, toluene, reflux; (iii) $R^1CH_2CO_2H$, toluene, MS, 105 °C; (iv) $R^2R^3CH_2P(=O)(OEt)_2$, NaH, THF or $CNCH_2R^2$, Al_2O_3; (v) TMSCN, $InBr_3$, CH_2Cl_2, 0 °C, then formic acid, rt; (vi) $POCl_3$, pyridine, rt.

A series of 4-substituted-methyl-3-ene-piperidyl (**5a**, **5b**, and **6b**) were prepared from ketone intermediates (**4a**, **4b**) by employing modified Knoevenagel condensation with cyano acetic acid or monomethyl malonate under high temperature. Knoevenagel condensation or Wadsworth–Hornor–Emmons reaction of ketones **4a** and **4b** provided 4-substituted-*exo*-methylenepiperidinyl derivatives (**7**–**12**) in 63–95% yields. The addition reaction of cyanide into carbonyl group with TMSCN in the presence of indium bromide followed by acidic deprotection of TMS group afforded the corresponding cyanohydrin derivatives (**13a**, **13b**). The dehydration of the corresponding cyanohydrin compounds with $POCl_3$ and pyridine gave the cyano(*endo*-methylene)piperidyl derivatives (**14a**, **14b**) in 30% yields.

The all of 5(*R*)-[1,2,3]triazolylmethyl-3-[(3′-fluoro-4′-piperidinyl)phenyl]oxazolidinones (**5a**–**14a**) and 5(*R*)-(4-F-[1,2,3]triazolyl)methyl-3-[(3′-fluoro-4′piperidinyl)phenyl]oxazolidinones (**5b**–**14b**) derivatives[12] were tested for in vitro antimicrobial activity against Gram-positive strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Coagulase negative staphylococci* (MRCS) vancomycin-resistant *Enterococcus faecalis*, (VRE), *Streptococcus pneumonia* (penicillin resistance and intermediate, PRSP), and *Streptococcus agalactiae* (*S.a.*) *betalactamase positive Haemophilus influenzae* {(*H.i.*(+)} clinically acquired in Korea. The minimum inhibitory concentration (MIC) values of produced by the Muller–Hinton agar dilution method[13] for the compounds (**2**, **5**–**14**) are reported in Table 1.

Most of the obtained piperidinyl–oxazolidinone derivatives bearing triazolyl C-5 side chain of oxazolidinone A-ring (**5a**–**14a**) showed good activity comparable or superior to linezolid against clinically isolated Gram-positive strains. Variation of piperidinyl

Table 1
In vitro antibacterial activity of triazolyloxazolidinone derivatives (MIC, μg/mL)[a]



Compd	*MRSA*[b]	*MRCS*[c]	*VRE*[d]	*VRE*[e]	*PRS.p.*[f]	*S.p*[g]	*S.a*[h]	*H.i.(+)* [i]
2	3.12	3.12	3.12	3.12	0.39	0.78	0.78	0.78
5a	3.12	3.12	3.12	3.12	0.39	0.39	1.56	1.56
5b	100	100	100	100	50	25	25	12.5
6a	6.25	6.25	6.25	6.25	1.56	1.56	1.56	3.12
7a	3.12	3.12	3.12	1.56	0.39	0.39	0.78	0.78
7b	50	100	50	50	6.25	50	100	6.25
8a	25	25	25	25	6.25	6.25	12.5	12.5
8b	100	100	100	100	6.25	50	100	6.25
9a	3.12	3.12	3.12	6.25	0.78	0.78	1.56	1.56
10a	12.5	12.5	6.25	6.25	0.78	0.78	1.56	1.56
10b	100	100	100	100	100	100	100	100
11a	25	25	12.5	25	12.5	6.25	6.25	6.25
11b	100	100	100	100	100	50	25	50
12a	1.56	1.56	1.56	1.56	0.19	0.39	0.19	0.39
12b	100	100	100	100	6.25	6.25	12.5	6.25
13a	3.12	3.12	3.12	3.12	0.78	0.78	1.56	1.56
13b	100	100	100	100	6.25	6.25	12.5	6.25
14a	3.12	3.12	3.12	3.12	1.56	1.56	1.56	1.56
14b	100	100	50	50	6.25	50	100	6.26
Linezolid	3.12	3.12	3.12	1.56	0.78	0.78	1.56	0.78

[a] Minimum inhibitory concentration by agar dilution method.
[b] Methicillin-resistant *Staphylococcus aureus* C463.
[c] Methicillin-resistant *Coagulase negative staphylococci*.
[d] Vancomycin-resistant *Enterococcus faecalis* C474.
[e] Vancomycin-resistant *Enterococcus faecium* C803.
[f] penicillin-resistant *Staphylococcus pneumonia* C402.
[g] *Staphylococcus pyogenes* 308A.
[h] *Staphylococcus agalactiae* ATCC 2901.
[i] *Betalactamase positive Haemophilus influenzae*.

group such as exomethylene (**7a**–**12a**), endomethylene (**5**, **6**, **14a**) or saturated (**13a**) piperidinyl group represent a little effect on their antimicrobial activity. Among the screened compounds (**2**, **5**–**14**) the cyanoethylidene compound **12a** showed most potent activity against all tested strains. The *exo*-cyanomethylene compound **7a** twofold higher activity against vancomycin-resistant *Enterococcus faecium* and *Staphylococcus pyogenes* compare to its C-5 acetamide oxazolidinone analogue **2**. Although the variation of piperidine ring have affected a little to the activity, the cyano group on exomethylene type played an important role on the antibacterial activity. However, all of 4-fluoro-[1,2,3]-triazole derivatives (**5b**, **7b**–**14b**) lost the activity against MRSA, MRCS and VRE strains. The replacement of triazole group with 4-fluoro-[1,2,3]-triazole group led to loss of antibacterial activity. The cyanomethylene compound **7b** having 4-fluoro-[1,2,3]-triazole moiety was inactive for all tested strains.

The most active two compounds **7a** and **12a** were profiled cytochrome P450 (CYP) inhibition liabilities[14] involved in drug–drug interaction and also evaluated for hERG channel,[15] MAO-A and MAO-B enzyme activities.[16] And the toxicity profiling data of corresponding 4-F-[1,2,3]-triazol derivatives **7b** and **12b** were also presented for comparison (Table 2).

Both compounds **7a** and **12a** showed low inhibitory activities against hERG and CYP except for the 3A4 inhibition of **12a**. It means that both compound found to have no problems involved in drug metabolism and drug interaction. The compound **7a** exhibits high IC_{50} value (49.5 ± 2.12 μM) against hERG channel which plays a crucial role on cardiac side effects of drugs. And also show high remaining activities for isozymes of CYP 450, indicating that it is not affected in drug metabolism and drug interaction. The potency of MAO-B for **7a** improved 3.5-fold relative to linezolid as shown almost no inhibition, but the inhibition of MAO-A which lead to severe hypertensive crises, increased compare to linezolid.

Although the inhibition of MAO-A still sustained strong, the compound **12a** exhibits 10-fold reduced inhibitory activity against MAO-A relative to compound **7a** and also the inhibitory activity of MAO-B showed threefold decreased compare to linezolid. While the corresponding 4-F-[1,2,3]triazole derivatives **7b** and **12b** showed over 10-fold higher inhibitory activities against hERG channel than their unsubstitiuted trialzoles **7a** and **12a**, they showed almost no inhibition against 1A2 and 2D6. In case of **12b**, all of CYP-450 inhibitory activity is decreased compare to **12a**. Therefore the 4-F-substitution of 1,2,3-triazole ring might be good effect for reducing toxicity induced from CYP-450. The MAO-A activity of **7b** was reduced over 10-fold compare to **7a**.

In summary, new series of 5(*R*)-[1,2,3]-triazolyl or 5(*R*)-(4-F-[1,2,3]-triazolyl)methyl-3-[(3′-fluoro)phenyl]oxazolidinone derivatives containing various piperidinyl moieties on 4′-position of phenyl ring were designed, synthesized and evaluated for the antibacterial activity against clinically isolated Gram-positive and Gram-negative strains. We observed that the introducing of 4-fluoro-1,2,3-triazole group at C-5 position of oxazolidinone ring showed loss of antibacterial activities. Although the biological activity showed a little differences according to the variation of piperidine moieties, compounds **12a** having *exo*-cyanoethylidene group in the 4-position of piperidine ring was found to be two to threefold more potent than the linezolid against penicillin-resistant *Staphylococcus pneumonia* and *Staphylococcus agalactiae*, and also exhibited reduced MAO-B inhibitory activity.

Table 2
Human hERG channel and CYP inhibition profile of selected compounds

Compd	hERG [a] (IC_{50}, μM)	% Control of CYP-450 (10 μM)[b]				MAO (remaining%, 10 μM)	
		1A2	2D6	2C9	3A4	hMAO-A[c]	hMAO-B[d]
7a	49.50 ± 2.12	55.19	80.87	88.01	32.03	3.54	111.30
7b	2.37 ± 1.16	91.77	117.2	66.57	23.65	50.6	19.5
12a	213.32 ± 32.0	45.73	65.09	61.49	7.45	34.8	74.81
12b	21.00 ± 1.63	92.76	106.35	64.84	21.44	10.2	34.8
Linezolid						6.79	31.41

[a] Human egg.
[b] Values are remained% activities and the mean ± SD of triplicate determinations.
[c] Monoamine oxidase A in human.
[d] Monoamine oxidase B in human.

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11. *Representative spectroscopic data of key intermediates:* **4a** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50–7.44 (dd, 1H), 7.10–7.06 (dd, 1H), 7.00–6.94 (t, 1H), 6.38 (NH, 1H), 4.80–4.77 (m, 1H), 4.06–4.00 (t, 1H), 3.80–3.77 (t, 1H), 3.69–3.63 (m, 2H), 3.67 (t, 4H), 2.62 (t, 4H), 2.03 (s, 3H) ^{13}C NMR ($CDCl_3$, 300 MHz) δ 208.42, 171.59, 154.76, 136.12, 133.79, 120.30, 114.27, 107.76, 72.36, 64.74, 51.22, 48.00, 42.30, 23.51.; (b) **4b** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08–8.05 (m, 1H), 7.65–7.51 (m, 2H), 6.92–6.90 (m, 1H), 5.12–5.01 (m, 1H), 4.81–4.60 (m, 2H), 4.11–4.00 (m, 2H), 3.39–3.35 (m, 4H), 2.64–2.60 (m, 4H).
12. *Representative spectroscopic data of selected compounds which were evaluated biological activity:* ***5a*** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79–7.75 (m, 2H) 7.46 (1H), 7.08–7.05 (m, 1H), 6.97–6.91(t, 1H), 5.91(s, 1H), 5.05 (1H, m) 4.79 (2H), 4.05 (1H), 3.77 (1H), 3.62 (4H), 3.26 (2H), 3.11 (2H), 2.29 (s, 2H); **5b** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79–7.75 (2H) 7.45–7.4 (dd, 1H), 7.07–7.04 (dd, 1H), 6.94–6.88 (t, 1H), 6.15 (1H), 5.72 (s, 1H), 4.77–4.75 (m, 1H), 4.20–4.13 (m, 2H), 4.04–3.98 (m, 1H), 3.76–3.60 (m, 7H), 3.12 (s, 5H), 2.48 (2H); **6a** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (1H), 7.62–7.50 (m, 2H), 6.89 (1H), 5.20 (s, 1H), 5.05 (m, 1H), 4.81 (m, 2H), 4.16 (1H), 3.93 (1H), 3.12 (m, 4H), 2.76 (t, *J* = 5.28 Hz, 2H), 2.53 (t, *J* = 5.28 Hz, 2H); **7a** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (1H), 7.75 (1H), 7.32 (dd, *J* = 14.0 Hz, 1.2 Hz, 1H), 6.97 (dd, *J* = 8.7 Hz, 1.1 Hz, 1H), 6.91 (t, *J* = 9.1 Hz, 1H), 5.19 (s, 1H), 5.08 (m, 1H), 4.79 (2H), 4.16 (t, *J* = 7.1 Hz, 1H), 3.93 (m, 1H), 3.16 (m, 4H), 2.78 (t, *J* = 5.28 Hz, 2H), 2.54 (t, *J* = 5.28 Hz, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 163.70, 157.01, 153.32, 134.55, 125.04, 119.86, 116.37, 114.27, 108.04, 93.90, 70.30, 51.97, 47.34, 35.30, 32.79; **12a** ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (1H), 7.74 (1H), 7.32 (dd, *J* = 14.2 Hz, 2.6 Hz, 1H), 6.97 (dd, *J* = 8.8 Hz, 1.7 Hz, 1H), 6.90 (t, *J* = 9.1 Hz, 1H), 5.09 (m, 1H), 4.80 (2H), 4.16 (1H), 3.93 (1H), 3.12 (m, 4H), 2.78 (t, *J* = 5.4 Hz, 2H), 2.55 (t, *J* = 5.5 Hz, 2H), 2.00 (s, 3H), 1.93 (s, 3H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 157.95, 154.18, 153.38, 136.54, 134.53, 132.45, 125.08, 119.68, 114.31, 107.99, 102.32, 70.34, 51.98, 47.35, 34.15, 29.85, 15.45; **7b** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (1H) 7.62–7.50 (m, 2H), 6.89 (1H), 5.20 (s, 1H), 5.05 (m, 1H), 4.81 (m, 2H), 4.16 (1H), 3.93 (1H), 3.12 (m, 4H), 2.76 (t, *J* = 5.28 Hz, 2H), 2.53 (t, *J* = 5.28 Hz, 2H); **12b** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.04 (m, 1H), 7.52–7.68 (m, 2H), 6.90 (m, 1H), 5.09 (m, 1H), 4.80 (2H), 4.16 (1H), 3.93 (1H), 3.12 (m, 4H), 2.78 (t, *J* = 5.4 Hz, 2H), 2.55 (t, *J* = 5.5 Hz, 2H), 2.00 (s, 3H); **13a** ^{1}H NMR (DMSO, 300 MHz, δ): 8.16 (1H), 7.76 (1H), 7.45–7.37 (1H), 7.14–7.07 (2H), 6.75(–OH), 5.12 (m, 1H) 4.83 (2H), 4.20 (1H), 3.87(1H), 3.22 (m, 2H) 2.93 (m, 2H) 2.08 (m, 2H) 1.93 (m, 2H); **13b** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (m, 1H), 7.45–7.37 (2H), 7.01 (1H) 6.75 (–OH), 4.89 (m, 1H), 4.60 (2H), 3.87 (2H), 3.13 (2H), 2.91 (2H), 2.09 (2H), 1.94 (2H); **14a** ^{1}H NMR ($CDCl_3$, 300 MHz, δ): 7.79–7.74 (2H), 7.33–7.28 (1H), 6.99–6.96 (1H), 6.91–6.85 (1H), 6.67 (s, 1H), 5.08–5.02 (m, 1H), 4.79 (2H), 4.17–4.10 (t, 1H), 3.93–3.88 (1H), 3.40 (2H), 3.25 (2H), 2.08 (2H); **14b** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (m, 1H), 7.62–7.49 (m, 2H), 6.88 (m, 1H), 5.05 (1H), 4.79 (2H), 4.12 (m, 1H), 3.86 (1H), 3.12 (2H), 2.13 (2H), 1.90 (2H).
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