Synthesis and Biological Evaluation of Novel Substituted Pyrrolyl and Pyrazolyl Oxazolidinone Analogues

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A number of life threatening infections caused by multidrug-resistant Gram-positive pathogens have reached an alarming level in the hospitals and the community.[1,2] Infections caused by these organisms pose a serious challenge to the scientific community and the need for an effective therapy has lead to a search for novel antibacterial agents. Oxazolidinones are a novel class of totally synthetic antimicrobial agents active against Gram-positive pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA),[3,4] vancomycin-resistant *Enterococcus faecium* (VRE),[5] and penicillin-resistant *Streptococcus pneumoniae* (PRSP).[6,7] Linezolid (**1**) is the first member of this class currently launched worldwide, and available in both intravenous and oral formulations. Oxazolidinones have a unique mechanism for inhibiting bacterial protein synthesis at an early phase of translation by binding selectively to the central loop of domain of 23S rRNA of 50S ribosomal subunit. Oxazolidinones show no cross resistance with other classes of protein synthesis inhibitor.[8,9] Linezolid is efficacious in treating skin and soft tissue infections, pneumonia and bacteremia, and it is particularly effective against infections caused by MRSA, VRE, and PRSP.

Recent studies by many research groups have focused on the development novel oxazolidinones based upon the replacement of the morpholine ring moiety of linezolid with various five-membered nitrogen-containing heterocycles (azoles).[10,11] In particular, the 3-cyano derivative **2** (PNU-172576) has excellent *in vitro* and *in vivo* activities. In light of these results, we envisioned the design for synthesis of pyrrolyl and pyrazolyl derivatives **5** having an oxadiazole and alkoxyaminomethyl groups as a bioisostere of cyano group of **2** (PNU-172576). Several potent compounds were studied to find out their pharmacokinetic profiles and *in vivo* efficacies (Figure 1).

The general routes for the synthesis of the pyrrolyl and pyrazolyl oxazolidinone derivatives are outlined in Scheme 1 and 2. The cyano intermediate **3**, which was prepared by a known procedure,[10] was treated with hydroxylamine to give amidoxime **4** in 90% yield. The subsequent cyclization of the amidoxime group with acetic anhydride, trifluoroacetic anhy-

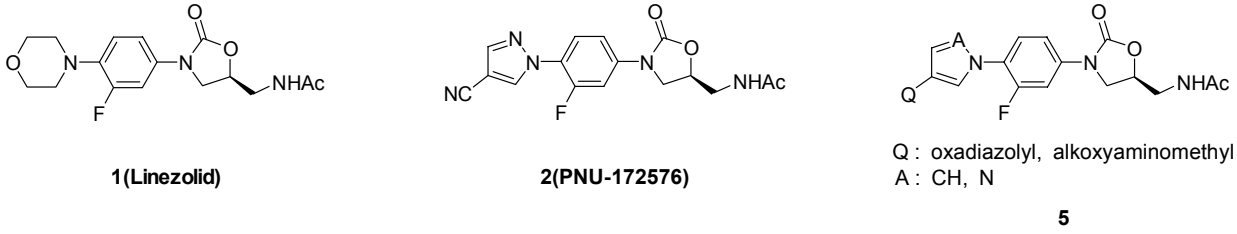
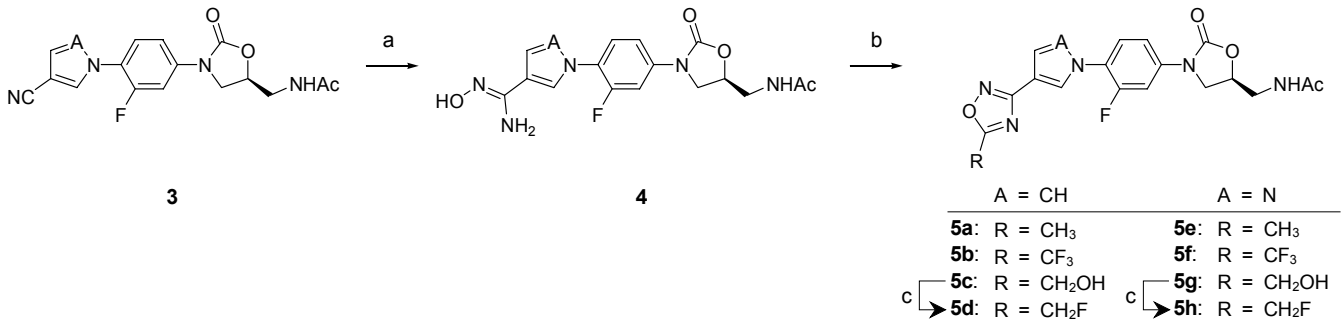
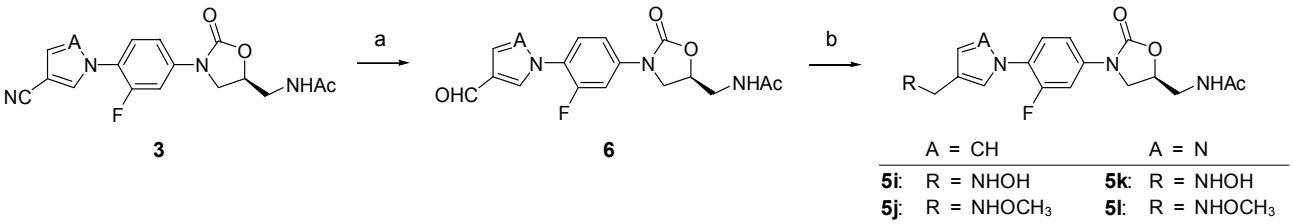


Figure 1. Structures of oxazolidinone antibacterial agents.



Scheme 1. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH; (b) Ac_2O (for **5a** and **5e**) or $(CF_3CO)_2O$ (for **5b** and **5f**) or ClC(= O) CO_2Et (for **5c** and **5g**, and then $NaBH_4$/THF), Pyridine, Toluene; (c) DAST, CH_2Cl_2.



3 **6**

Scheme 2. Reagents and conditions: (a) Raney Ni, HCO_2H; (b) i) $NH_2OH{\cdot}HCl$, K_2CO_3, CH_2Cl_2/MeOH (for **5i** and **5k**) or $NH_2OCH_3{\cdot}HCl$, K_2CO_3, CH_2Cl_2/MeOH (for **5j** and **5l**) ii) Borane-pyridine complex, 10% HCl, MeOH.

dride or ethyl chlorooxoacetate gave the oxadiazole derivatives **5a-h** in 40 ~ 90% yield after chromatographic purification on silica gel.

Fluorination of alcohol (**5c** or **5g**) with diethylaminosulfur trifluoride (DAST) under low temperature condition afforded the corresponding fluorinated product (**5d** or **5h**) in 50 ~ 66% yield.

Alkoxyaminomethyl compounds **5i-5l** were prepared by reductive amination of formyl intermediate **6**, which was afforded from oxidation of **3** with Raney Ni/HCOOH.

Minimum inhibitory concentration (MIC) values were determined by the Mueller-Hinton agar dilution method against Gram-positive strains including MRSA, VRE and PRSP. The activities of the compounds synthesized were compared with linezolid as a reference. *In vivo* efficacy for selected compounds were also evaluated against *S. aureus* infection model in mice. The MIC and ED_{50} values of the pyrroyl and pyrazolyl analogues are presented in Table 1.

All of pyrrolyl derivatives (**5a-5d**, **5i** and **5j**) displayed good antibacterial activity. In contrast, most of pyrazolyl derivatives (**5e-5h** and **5k** except for **5l**) exhibited comparable or slightly lower activity than linezolid. Therefore these findings led us to believe that the electronic character of the five-membered rings (pyrrole versus pyrazole) may be playing a crucial role in the antibacterial activity of these analogues. In particular, compound **5a** with methyl group on the oxadiazole ring demonstrated MIC values 2-8 fold better than those of linezolid in all of the tested organisms including MRSA and VRE. And hydroxymethyl or fluoromethyl compounds (**5c**, **5d**) also showed 16-32 fold excellent activities than linezolid against penicillin-susceptible *Streptococcus pneumoniae*. By comparing activities of methoxyaminomethyl (**5j** and **5l**) with oxadiazole (**5a-5d** and **5e-5h**) as a bioisostere of cyano group, it could be found that non-rigid methoxyaminomethyl derivatives were comparable to that of the corresponding rigid oxadiazolyl derivatives. Compound **5e** (ED_{50} = 5 ~ 8 mg/kg), pyrazolyl analogue having methyl oxadiazole displayed an oral efficacy comparable to that of linezolid (ED_{50} = 4.54 mg/kg), whereas compounds **5a**, **5b** and **5d** showed lower *in vivo* efficacy. Among these analogues, compound **5j**, containing non-rigid alkoxyaminomethyl exhibited excellent *in vitro* activity (MIC = 0.1 ~ 0.8 μg/mL) and *in vivo* efficacy (ED_{50} = 2.75 mg/kg).

The single-dose pharmacokinetic profiles of some active compounds in male Sprague-Dawley rats are summarized in Table 2. After oral administration, the compound **5a** showed better results than the linezolid, particularly outstanding in AUC and $t_{1/2}$.

In conclusions, a series of new pyrrolyl and pyrazolyl oxazolidinones have been synthesized, and their *in vitro* and *in vivo* antibacterial activities were evaluated against Gram-positive organisms including the resistant strains of Staphylococci, Ente-

Table 1. *In vitro* (MIC, μg/mL) and *in vivo* (ED_{50}, mg/kg) antibacterial activity

Compd	R	A	MIC (μg/mL) (microorganism)[a]								ED_{50} (mg/kg)[c]
			S.a 1	S.a 2	MRSA[b]	MSSA[b]	VRE.f[b]	VSE.f[b]	PNSP[b]	PSSP[b]	
5a	CH_3	CH	0.4	0.4	0.8	0.8	0.8	0.2	0.1	0.1	> 10
5b	CF_3	CH	0.4	0.8	1.6	1.6	1.6	0.8	0.4	0.4	> 10
5c	CH_2OH	CH	0.8	0.4	0.8	0.4	0.8	0.2	0.05	0.025	
5d	CH_2F	CH	0.8	0.4	0.8	0.2	0.4	0.2	< 0.025	< 0.025	> 10
5e	CH_3	N	3.1	1.6	3.1	1.6	1.6	1.6	1.6	0.4	5-8
5f	CF_3	N	12.5	12.5	25	12.5	12.5	6.3	12.5	6.3	
5g	CH_2OH	N	3.1	0.8	3.1	1.6	1.6	0.8	1.6	0.8	
5h	CH_2F	N	3.1	3.1	3.1	3.1	1.6	1.6	0.8	0.4	
5i	NHOH	CH	0.8	1.6	3.1	1.6	0.8	0.8	0.2	0.4	
5j	$NHOCH_3$	CH	0.4	0.8	0.8	0.4	0.4	0.4	0.4	0.1	2.75
5k	NHOH	N	3.1	1.6	3.1	1.6	1.6	0.8	0.4	0.4	
5l	$NHOCH_3$	N	0.8	1.6	1.6	0.8	1.6	0.8	0.2	0.1	
1			1.6	1.6	3.1	1.6	1.6	1.6	0.8	0.4	4.54

[a]Organisms: *S.a* 1, *Staphylococcus aureus* SG511; *S.a* 2, *Staphylococcus aureus* 503; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; VRE.f, vancomycin-resistant *Enterococcus faecium*; VSE.f, vancomycin-susceptible *Enterococcus faecium*; PNSP, penicillin-non susceptible *Streptococcus pneumoniae*; PSSP, penicillin-susceptible *Streptococcus pneumoniae*. [b]Supplied from Yonsei University College of Medicine, Seoul, Korea. [c]ED_{50} is the amount of drug required after oral administration (mg/kg/day) to cure 50% of infected mice subjected to a lethal systemic infection of *S. aureus* SG 511.

Table 2. Single dose pharmacokinetic parameters[a]

Compd	Dose[b] (mg/kg)	T_{max}[c] (min)	C_{max}[d] (μg/mL)	AUC[e] (μg·min/mL)	$T_{1/2}$[f] (min)
5a	15	180	5.1	4296.7	969.6
5c	15	NA[g]	NA	NA	NA
5d	15	60	4.881	741.9	102.2
5e	15	60	9.2	1809.7	110.7
5j	15	30	5.826	1191.8	54.9
1	15	30	4.431	1340.9	169.5

[a]Each compound was dissolved in 1% CMC solution and orally administered to SD rat. [b]N = 5. [c]Time at which C_{max} achieved. [d]Maximum plasma concentration. [e]The total area under the plasma concentration-time curve form time zero to time infinity. [f]Harmonic mean apparent terminal disposition half-life. [g]NA = Not Absorbed.

rococci and Pneumococci. Some of these analogues exhibited more potent *in vitro* antibacterial activities than those of linezolid. Especially, compound **5j**, containing non-rigid alkoxyaminomethyl as a bioisostere of cyano group on pyrrole ring exhibited excellent *in vitro* activity (MIC = 0.1 ~ 0.8 μg/mL), *in vivo* efficacy (ED_{50} = 2.75 mg/kg) and favorable pharmacokinetics. With these results in hand, further optimization of oxazolidinone analogues for *in vitro* and *in vivo* activities is in progress.

Experimental Section

General. NMR spectra were recorded on a Bruker Avance II 400 (100.63 MHz for ^{13}C and 400.13 MHz for ^{1}H). Chemical shifts are indicated in δ values (ppm) downfield from internal TMS. IR spectra were recorded on a Jasco FT/IR 4100 spectrophotometer. Mass spectra were recorded on a Finnigan LC/MSD. The products were analyzed with a Hypersil Gold C18 column (2.1 × 100 mm, 3.0 μm particles) using the linear gradient condition. The flow rate was 0.2 mL/min, and the eluent was monitored at 260 nm. The mass spectral mode of operation was positive ion electrospray (API-ES).

***N*-((S)-3-{3-Fluoro-4-[3-(*N*-hydroxycarbamimidoyl)-pyrrol-1-yl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide (4):** To a solution of **3** (428 mg, 1.25 mmol) in EtOH (4.12 mL) were added hydroxylamine hydrochloride (435 mg, 6.25 mmol) and $NaHCO_3$ (525 mg, 6.25 mmol). The mixture was heated under reflux overnight. The mixture was allowed to cool to room temperature and then the solvent was evaporated under reduced pressure. The resulting precipitate was collected by filtration, washed with water and dried under vacuum to give the product (424 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (1H, s), 8.28 (1H, dd), 7.73 (1H, dd), 7.62 (1H, dd), 7.50 (1H, s), 7.41 (1H, dd), 7.10 (1H, s), 6.51 (1H, dd), 5.56 (2H, s), 4.79 (1H, m), 4.19 (1H, t), 3.88 (1H, dd), 3.45 (2H, dd), 1.84 (3H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.0, 155.1, 154.0, 152.7, 147.6, 137.7, 125.1, 121.8, 119.4, 114.2, 109.7, 107.7, 106.2, 71.7, 47.2, 41.3, 22.4; IR (KBr) 1737, 1631, 1592, 1527, 1411, 1224, 1201 cm^{-1}

***N*-((S)-3-{3-Fluoro-4-[3-(5-methyl-[1,2,4]oxadiazol-3-yl)-pyrrol-1-yl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide (5a):** To a solution of **4** (30 mg, 0.08 mmol) in pyridine (1.1 mL) was added acetic anhydride (11 μL, 0.117 mmol). The mixture was heated under reflux overnight and then was allowed to cool to room temperature. The solution was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with 2 *N* HCl and brine, dried and concentrated. Chromatographic purification (ethyl acetate/hexane/methanol = 4:4:1) afforded **5a** (28.4 mg, 89%) of the desired material. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (2H, dd), 7.42 (1H, dd), 7.35 (1H, dd), 7.28 (1H, dd), 7.05 (1H, s), 6.82 (1H, dd), 4.82 (1H, m), 4.11 (1H, t), 3.84 (1H, dd), 3.65 (2H, dd), 2.63 (3H, s), 2.03 (3H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 175.9, 171.3, 164.9, 156.1, 154.2, 153.7, 137.6, 125.1, 124.4, 122.7, 113.8, 112.8, 109.2, 107.3, 72.1, 47.4, 41.8, 23.1, 12.3; IR (KBr) 1739, 1531, 1411, 1225, 1198 cm^{-1}; MS 399 (M^+).

***N*-((S)-3-{3-Fluoro-4-[3-(5-hydroxymethyl-[1,2,4]oxadiazol-3-yl)-pyrrol-1-yl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide (5c):** To a solution of **4** (62 mg, 0.16 mmol) in toluene (1 mL) were added pyridine (40 μL, 0.49 mmol) and ethyl chlorooxoacetate (27.8 μL, 0.25 mmol) at 0 °C. After stirring 30 min, the mixture was allowed to warm to room temperature and stirred for 1 h. Then, the mixture was heated under reflux overnight and was allowed to cool to room temperature. The solution was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with 2 *N* HCl and brine, dried, and concentrated. Chromatographic purification (ethyl acetate/hexane/methanol = 4:4:1) afforded ethylcarboxy intermediate (30.8 mg, 42%). And to a solution of the intermediate (42.3 mg, 0.09 mmol) in THF (1 mL) was added $NaBH_4$ (6.8 mg, 0.18 mmol) at 0 °C. The mixture was stirred for 3 h and then diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried, and concentrated. Chromatographic purification (ethyl acetate/hexane/methanol = 4:4:1) afforded **5c** (16 mg, 43%) of the desired material. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (1H, dd), 7.75 (3H, dd), 7.46 (1H, dd), 7.31 (1H, dd), 6.75 (1H, s), 6.02 (1H, s), 4.77 (2H, s), 4.75 (1H, m), 4.18 (1H, t), 3.79 (1H, dd), 3.44 (2H, dd), 1.85 (3H, s); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 178.4, 170.0, 164.1, 155.3, 154.0, 152.9, 138.4, 125.8, 123.9, 122.7, 114.1, 111.8, 108.5, 106.3, 71.8, 55.0, 47.2, 41.3, 22.4; IR (KBr) 1741, 1531, 1415, 1227, 1199 cm^{-1}; MS 415 (M^+).

***N*-((S)-3-{3-Fluoro-4-[3-(5-fluoromethyl-[1,2,4]oxadiazol-3-yl)-pyrrol-1-yl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide (5d):** To a solution of **5c** (63.8 mg, 0.153 mmol) in CH_2Cl_2 (1 mL) was added diethylaminosulfur trifluoride (DAST) (26.7 μL, 0.202 mmol) in CH_2Cl_2 (3 mL) at -78 °C. The mixture was stirred for 2 h at -78 °C. Stirring was continued for 1 h while

the reaction mixture was allowed to warm at room temperature. The mixture was poured into water and extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried, and concentrated. Chromatographic purification (ethyl acetate/hexane/methanol = 4:4:1) afforded **5d** (34.8 mg, 54%) of the desired material. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (1H, dd), 7.82 (1H, dd), 7.74 (2H, dd), 7.46 (1H, dd), 7.31 (1H, dd), 6.78 (1H, s), 5.88 (1H, s), 5.77 (1H, s), 4.75 (1H, m), 4.20 (1H, t), 3.79 (1H, dd), 3.45 (2H, dd), 1.84 (3H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.3, 171.2, 165.2, 156.2, 154.1, 153.7, 137.8, 125.3, 123.8, 113.8, 112.1, 109.3, 107.3, 73.5, 72.2, 61.9, 47.4, 41.9, 29.7, 23.1; IR (KBr) 1732, 1657, 1608, 1531, 1417, 1230 cm^{-1}; MS 417 (M^+).

***N*-{(*S*)-3-[3-Fluoro-4-(3-formyl-pyrrol-1-yl)-phenyl]-2-oxo-oxazolidin-5-yl methyl}-acetamide (6):** To a solution of **3** (1 g, 2.92 mmol) in formic acid (6 mL) was added Raney nickel (2 g) in formic acid (6 mL) at 0 °C. The mixture warmed to 70 °C and stirred for 3 h. The Raney nickel was removed by celite filtration and the filtrate was neutralized with ammonia solution. The solution was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane/methanol = 4:4:1) to afford **6** (820 mg, 81%) of the desired material. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (1H, s), 8.28 (1H, t), 8.27 (1H, dd), 7.68 ~ 7.72 (2H, m), 7.45 (1H, dd), 7.29 (1H, s), 6.68 (1H, dd), 4.77 (1H, m), 4.18 (1H, t), 3.78 (1H, dd), 3.40 ~ 3.45 (2H, m), 1.84 (3H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 185.4, 170.0, 155.5, 154.0, 153.1, 138.9, 131.2, 127.3, 124.9, 122.2, 114.1, 107.8, 106.3, 71.8, 47.2, 41.3, 22.4; IR (KBr) 1735, 1681, 1652, 1525, 1384, 1213 cm1^{-1}

***N*-{(*S*)-3-[3-Fluoro-4-(3-hydroxyaminomethyl-pyrrol-1-yl)-phenyl]-2-oxo-oxazolidin-5-ylmethyl}-acetamide (5j):** To a solution of **6** (785 mg, 2.27 mmol), methoxylamine hydrochloride (285 mg, 3.41 mmol) and potassium carbonate (471 mg, 3.41 mmol) were stirred in methanol/methylene chloride (1:1) (18 mL/18 mL) overnight. And then the resulting precipitate was collected, washed with water and dried under vacuum to give the methoxyimine intermediate (839 mg, 99%). And to a solution of methoxyimine intermediate (8.39 mg, 2.24 mmol) in methanol (18.2 mL) was added borane pyridine complex (987 μL) at 0 °C. The mixture was stirred for 5 min and added 10% HCl (11.3 mL) at 0 °C. Stirring was continued for 15 min while the reaction mixture was allowed to room temperature. The solution was basified with saturated sodium bicarbonate and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane/methanol = 4:4:1) to afford **5j** (783 mg, 93%) of the desired material. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (1H, dd), 7.33 (1H, dd), 7.29 (1H, dd), 6.93 (1H, dd), 6.90 (1H, dd), 6.31 (1H, s), 4.81 (1H, m), 4.05 (1H, dd), 3.99 (1H, s), 3.82 (1H, dd), 3.67 (2H, dd), 3.57 (1H, s), 2.04 (1H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.0, 155.0, 153.9, 152.5, 137.2, 125.0, 123.3, 121.8, 119.8, 114.1, 110.7, 106.3, 71.7, 60.6, 47.6, 47.2, 41.3, 22.4; IR (KBr) 1725, 1654, 1531, 1421, 1230, 1195 cm^{-1}; MS 376 (M^+).

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