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Note

Syntheses and activities as trehalase inhibitors of *N*-arylglycosylamines derived from fluorinated anilines

Xuhong Qian,[a,*] Zhibin Li,[b] Zhi Liu,[b] Gonghua Song,[b] Zhong Li[b]

[a]*State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, People's Republic of China*

[b]*East China University of Science and Technology, PO Box 544, 130 Meilong Road, Shanghai 200237, People's Republic of China*

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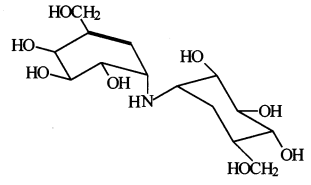
Abstract

Twelve *N*-arylglycosylamines were prepared in a one-pot reaction by treatment of D-glucose, D-galactose, D-mannose or D-xylose with fluorinated anilines in the presence of small amount of hydrochloric acid. The inhibitory activity against porcine trehalase and the fungicidal activity of the title compounds toward *Rhizoctonia solani* have been tested. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Trehalase inhibitors; Porcine trehalase; *Rhizoctonia solani*

Validoxylamine A (**1**) and salbostatin (**2**) are two natural unsaturated dicarba-α,α-trehalose analogues and possess very strong inhibitory activities against a certain insect trehalase.[1,2] The inhibition mechanism is the competitive formation of a trehalase–inhibitor complex involving the imino group in the inhibitor and the carboxyl group in trehalase. The hydroxyl groups in the inhibitor are topologically essential for the strong binding of the inhibitor through hydrogen bonds with the active site of trehalase.[1,3]



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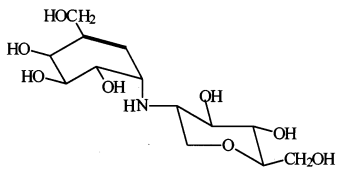

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Although validoxylamine A and salbostatin have strong inhibitory activity against insect trehalase in vitro, they cannot be used commercially as fungicides, because they lack antifungal activity in vivo due to their poor uptake into the cells of fungi.[1,4]

It is reported that when a D-glucopyranose moiety is introduced at C-4′ of validoxylamine A, the new compound, validamycin A, shows potent fungicidal activity toward *Rhizoctonia solani* due to its good uptake into cells of fungi.[4] Consequently, we designed trehalase inhibitors of a new kind (**5a**–**e**) containing the D-glucopyranose moiety in order to increase the antifungal activity in vivo. Because phenyl glycosides are generally accepted as substrates

* Corresponding author. Tel.: +86-21-64252945; fax: +86-21-64253180.

E-mail address: bottle_0@chinaren.com (X. Qian).

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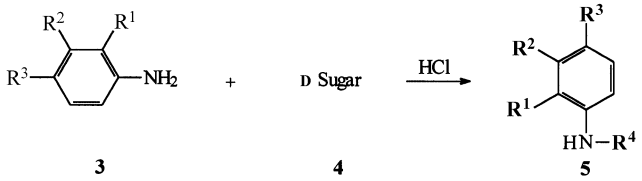
by glycosidases,[5] we tried to introduce a fluorinated phenyl group in the designed compounds, and thus the new compounds can be synthesized easily and should also keep the strong binding ability with the active site of trehalase through F···H hydrogen bonds. In order to elucidate structure–activity relationships in this kind of inhibitor, related compounds containing D-galactose, D-mannose and D-xylose moieties have also been prepared (**5f**–**k**) (Scheme 1).

The designed compounds (**5a**–**k**) can be readily prepared from the D sugar and fluoro-substituted anilines. Treatment of D sugars with fluoro-substituted anilines in water at 60 °C, in the presence of a small amount of hydrochloric acid, gave the desired products



3 4 5

	R1	R2	R3	R4
a	F	H	F	β-D-glucopyranosyl
b	H	F	F	β-D-glucopyranosyl
c	H	H	F	β-D-glucopyranosyl
d	F	F	F	β-D-glucopyranosyl
e	H	Cl	F	β-D-glucopyranosyl
f	H	F	F	β-D-galactopyranosyl
g	H	F	F	β-D-mannopyranosyl
h	H	F	F	β-D- xylopyranosyl
i	H	Cl	F	β-D-galactopyranosyl
j	H	Cl	F	β-D-mannopyranosyl
k	H	Cl	F	β-D- xylopyranosyl

Scheme 1.

Table 1
Inhibitory activity of compounds **5a**–**k** toward porcine trehalase

Compound	Inhibitory activity (IC_{50}:M)
5a	$>10^{-3}$
5b	3.99×10^{-6}
5c	$>10^{-3}$
5d	2.31×10^{-4}
5e	3.11×10^{-4}
5f	8.11×10^{-4}
5g	$>10^{-3}$
5h	4.69×10^{-4}
5i	$>10^{-3}$
5j	$>10^{-3}$
5k	$>10^{-3}$

in moderate yields and with satisfactory elemental analyses. The structures of **5a**–**k** were confirmed by examination of their ^{1}H NMR spectral data. The β configuration of the imino linkage was confirmed from the value of the of $J_{1,2}$ coupling constant in the sugar ring. For compounds **5a**–**f**, **5h**, **5i** and **5k**, $J_{1,2}$ 8.0–8.8 Hz, indicating that the H-1 and H-2 atoms exist in a trans relation (*a*, *a*) to each other. For compounds **5g** and **5j**, $J_{1,2}$ 2.1 Hz, which indicated that the H-1 and H-2 atoms exist in a cis relation (*a*, *e*) to each other.

Compounds **5a**–**k** were subjected to biological assay for their inhibitory activity against porcine trehalase in vitro by the standard procedure.[6] The inhibitory data of **5b**, **5f**, **5g** and **5h** or **5e**, **5i**, **5j** and **5k** (Table 1) indicated that the compounds having the D-glucose moiety showed the higher activity against the porcine trehalase. Furthermore, the inhibitory data of **5a**–**e** showed that the activity of the compound also depended upon the number and positions of fluorine atoms in the phenyl ring. The combination of fluorine at positions 3 and 4 of the phenyl ring (compound **5b**) worked better than any other combination.

Compounds **5a**–**k** were also screened for their antifungal activity toward *R. solani* by spraying rice seedlings at a concentration of 1000 ppm. A commercial fungicide, validamycin A, was also tested under similar conditions for comparison. Among the tested compounds, **5b** and **5e** showed strong fungicidal activity (90 and 70% respectively) at 1000 ppm, but other compounds showed no obvious activity. All compounds showed lower activity as compared to the standard fungicide validamycin A.

1. Experimental

General methods.—Melting points were determined in an electrothermal apparatus and are uncorrected. IR spectra (KBr disks) were measured with a Nicolet FT-IR-20SX spectrophotometer. Mass spectra were taken on a Hitachi M80 instrument. ^{1}H NMR (500 MHz) spectra were recorded with a Bruker AMX-500 instrument for solutions in $(CD_3)_2CO$, using Me_4Si as internal standard. Elemental analyses were made with an Italian

MOD.1106 analyzer. All reactions were monitored by TLC on aluminium sheets coated with Silica Gel 60 F_{254} (E. Merck).

N-*(Fluoro-substituted phenyl)-β-*D*-glucopyranosylamines* (**5a**–**k**)*: general procedure.*—To a mixture of fluoro-substituted aniline (**3a**–**k**) (10 mmol), D sugar (10 mmol) and water (8 mL) was added 1 mL of 6% HCl solution. The mixture was kept for 10 min at 60 °C and then cooled to 0 °C. The resulting mass was filtered, washed with 2 mL water, dried and recrystallized from EtOH to give **5a**–**k** as white solids. The following compounds were prepared in this manner.

N-*(2,4-Difluorophenyl)-β-*D*-glucopyranosylamine* (**5a**).—Yield: 2.09 g (72%); mp 156–158 °C; ν_{max} 3310 (NH), 3250 (OH), 1600 and 1570 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 7.20 (td, 1 H, $J_{4',6'}$ 9.1, $J_{5',6'}$ 9.1, $J_{2',6'}$ 5.6 Hz, H-6′), 6.96 (ddd, 1 H, $J_{2',3'}$ 8.7, $J_{3',4'}$ 11.2, $J_{3',5'}$ 2.8 Hz, H-3′), 6.85 (tdd, 1 H, $J_{2',5'}$ 1.5, $J_{3',5'}$ 2.8, $J_{5',6'}$ 9.1 Hz, H-5′), 4.50 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.76 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 12.3 Hz, H-6a), 3.58 (dd, 1 H, $J_{5,6}$ 5.5 Hz, H-6b), 3.41 (m, 2 H, H-2, H-3), 3.20 (m, 2 H, H-4, H-5); EIMS: *m*/*z* (%): 291 ([M$^+$], 2), 129 (100), 109 (24), 101 (73), 82 (47). Anal. Calcd for $C_{12}H_{15}F_2NO_5{\cdot}H_2O$: C, 46.60; H, 5.54; N, 4.53. Found: C, 46.54; H, 5.56; N, 4.55.

N-*(3,4-Difluorophenyl)-β-*D*-glucopyranosylamine* (**5b**).—Yield: 1.34 g (46%); mp 158–160 °C; ν_{max} 3350 (NH), 3260 (OH), 1600 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 7.02 (q, 1 H, $J_{3',5'}$ 9.1, $J_{4',5'}$ 9.1, $J_{5',6'}$ 9.1 Hz, H-5′), 6.65 (ddd, 1 H, $J_{2',3'}$ 12.9, $J_{2',4'}$ 6.9, $J_{2',6'}$ 2.7 Hz, H-2′), 6.49 (d, 1 H, $J_{5',6'}$ 9.0 Hz, H-6′), 4.57 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 3.78 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 12.3 Hz, H-6a), 3.62 (dd, 1 H, $J_{5,6}$ 5.5 Hz, H-6b), 3.45 (m, 2 H, H-2, H-3), 3.35 (m, 2 H, H-4, H-5); EIMS: *m*/*z* (%): 291 ([M$^+$], 5), 142 (100), 129 (42), 113 (40). Anal. Calcd. for $C_{12}H_{15}F_2NO_5{\cdot}H_2O$: C, 46.60; H, 5.54; N, 4.53. Found: C, 46.58; H, 5.52; N, 4.56.

N-*(4-Fluorophenyl)-β-*D*-glucopyranosylamine* (**5c**).—Yield: 1.69 g (62%); mp 155–156 °C; ν_{max} 3320 (NH), 3260 (OH), 1510 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 6.92 (t, 2 H, $J_{2',3'}$ 8.9, $J_{2',4'}$ 8.9, $J_{4',6'}$ 8.9, $J_{5',6'}$ 8.9 Hz, H-3′, H-5′), 6.71 (q, 2 H, $J_{2',3'}$ 8.9, $J_{2',4'}$ 4.5, $J_{4',6'}$ 4.5, $J_{5',6'}$ 8.9 Hz, H-2′, H-6′), 4.60 (d, 1 H, J_1,2 8.8 Hz, H-1), 3.79 (dd, 1 H, $J_{5,6}$ 2.0, J_{gem} 12.3 Hz, H-6a), 3.58 (dd, 1 H, $J_{5,6}$ 5.6 Hz, H-6b), 3.41 (m, 2 H, H-2, H-3), 3.20 (m, 2 H, H-4, H-5); EIMS: *m*/*z* (%): 273 ([M$^+$], 8), 124 (100), 111 (71), 95 (48), 75 (24). Anal. Calcd. for $C_{12}H_{16}FNO_5{\cdot}H_2O$: C, 49.48; H, 6.23; N, 4.81. Found: C, 49.32; H, 6.24; N, 4.81.

N-*(2,3,4-Trifluorophenyl)-β-*D*-glucopyranosylamine* (**5d**).—Yield: 1.67 g (54%); mp 188–190 °C; ν_{max} 3310 (NH), 3260 (OH), 1590 and 1570 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 6.88 (qd, 1 H, $J_{3',5'}$ 9.0, $J_{4',5'}$ 9.0, $J_{5',6'}$ 9.0, $J_{2',5'}$ 2.1 Hz, H-5′), 6.64 (tq, 1 H, $J_{2',6'}$ 4.7, $J_{3',6'}$ 2.2, $J_{4',6'}$ 9.0, $J_{5',6'}$ 9.0 Hz, H-6′), 4.60 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 3.79 (dd, 1 H, $J_{5,6}$ 2.1, J_{gem} 12.4 Hz, H-6a), 3.58 (dd, 1 H, $J_{5,6}$ 5.5 Hz, H-6b), 3.45 (m, 2 H, H-2, H-3), 3.38 (m, 2 H, H-4, H-5); EIMS: *m*/*z* (%): 309 ([M$^+$], 3), 147 (100), 119 (47). Anal. Calcd. for $C_{12}H_{14}F_3NO_5{\cdot}H_2O$: C, 44.04; H, 4.93; N, 4.28. Found: C, 43.90; H, 4.90; N, 4.28.

N-*(3-Chloro-4-fluorophenyl)-β-*D*-glucopyranosylamine* (**5e**).—Yield: 2.09 g (68%); mp 186–188 °C; ν_{max} 3310 (NH), 3250 (OH), 1600 and 1500 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 7.05 (t, 1 H, $J_{4',5'}$ 8.9, $J_{5',6'}$ 8.9 Hz, H-5′), 6.85 (s, 1 H, H-2′), 6.67 (dd, 1 H, $J_{4',6'}$ 6.1, $J_{5',6'}$ 8.9 Hz, H-6′), 4.58 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 3.76 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 12.3 Hz, H-6a), 3.62 (dd, 1 H, $J_{5,6}$ 5.5 Hz, H-6b), 3.45 (m, 2 H, H-2, H-3), 3.35 (m, 2 H, H-4, H-5); EIMS: *m*/*z* (%): 309 ([M$^+$ + 2], 33), 307 ([M$^+$], 100), 290 (5), 288 (15), 160 (8), 158 (24). Anal. Calcd. for $C_{12}H_{15}ClFNO_5{\cdot}H_2O$: C, 44.25; H, 5.26; N, 4.30. Found: C, 44.18; H, 5.24; N, 4.32.

N-*(3,4-Difluorophenyl)-β-*D*-galactopyranosylamine* (**5f**).—Yield: 1.69 g (58%); mp 178–179 °C; ν_{max} 3340 (NH), 3250 (OH), 1595 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 7.10 (q, 1 H, $J_{3',5'}$ 9.2, $J_{4',5'}$ 9.2, $J_{5',6'}$ 9.2 Hz, H-5′), 6.76 (ddd, 1 H, $J_{2',3'}$ 12.8, $J_{2',4'}$ 6.8, $J_{2',6'}$ 2.5 Hz, H-2′), 6.61 (m, 1 H, H-6′), 4.63 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.00 (d, 1 H, $J_{5,6}$ 2.0 Hz, H-6a), 3.80 (t, 1 H, $J_{3,4}$ 6.1, $J_{4,5}$ 6.1 Hz, H-4), 3.63–3.75 (m, 4 H, H-2, H-3, H-5, H-6b); EIMS: *m*/*z* (%): 291 ([M$^+$], 5), 143 (19), 142 (100), 129 (30), 115 (23), 113 (25). Anal. Calcd. for $C_{12}H_{15}F_2NO_5{\cdot}H_2O$: C, 46.60; H, 5.54; N, 4.53. Found: C, 46.72; H, 5.53; N, 4.51.

N-*(3,4-Difluorophenyl)-β-*D*-mannopyranosylamine* (**5g**).—Yield: 2.38 g (82%); mp 203–204 °C; ν_{max} 3345 (NH), 3270 (OH), 1602 (Ph);

^{1}H NMR (CD_3COCD_3, 500 MHz): δ 7.11 (q, 1 H, $J_{3',5'}$ 9.0, $J_{4',5'}$ 9.0, $J_{5',6'}$ 9.0 Hz, H-5′), 6.78 (ddd, 1 H, $J_{2',3'}$ 12.9, $J_{2',4'}$ 6.8, $J_{2',6'}$ 2.7 Hz, H-2′), 6.61 (m, 1 H, H-6′), 4.93 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 4.03 (d, 1 H, $J_{5,6}$ 2.7 Hz, H-6a), 3.88 (dd, 1 H, $J_{5,6}$ 2.2, J_{gem} 12.2 Hz, H-6b), 3.70–3.74 (m, 2 H, H-2, H-3), 3.63 (t, 1 H, $J_{3,4}$ 9.7, $J_{4,5}$ 9.7 Hz), 3.49 (m, 1 H, H-5); EIMS: *m*/*z* (%): 291 ([M$^+$], 2), 143 (11), 142 (100), 129 (43), 114 (26), 113 (24). Anal. Calcd. for $C_{12}H_{15}F_2NO_5 \cdot H_2O$: C, 46.60; H, 5.54; N, 4.53. Found: C, 46.41; H, 5.52; N, 4.51.

N-*(3,4-Difluorophenyl)-β-*D*-xylopyranosylamine* (**5h**).—Yield: 2.04 g (78%); mp 167–168 °C; ν_{max} 3320 (NH), 3270 (OH), 1580 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 6.94 (q, 1 H, $J_{3',5'}$ 9.1, $J_{4',5'}$ 9.1, $J_{5',6'}$ 9.1 Hz, H-5′), 6.57 (ddd, 1 H, $J_{2',3'}$ 12.9, $J_{2',4'}$ 6.9, $J_{2',6'}$ 2.7 Hz, H-2′), 6.41 (m, 1 H, H-6′), 4.45 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 3.72 (dd, 1 H, $J_{4,5}$ 5.38, J_{gem} 11.4 Hz, H-5a), 3.45 (m, 1 H, H-4), 3.34 (t, $J_{2,3}$ 9.1, $J_{3,4}$ 9.1 Hz, 1 H, H-3), 3.20–3.26 (m, 2 H, H-2, H-5b); EIMS: *m*/*z* (%): 261 ([M$^+$], 4), 260 [M$^+ - 1$, 26], 130 (13), 129 (100), 114 (18), 113 (23). Anal. Calcd. for $C_{11}H_{13}F_2NO_4 \cdot H_2O$: C, 50.58; H, 5.02; N, 5.36. Found: C, 50.32; H, 5.03; N, 5.38.

N-*(3-Chloro-4-fluorophenyl)-β-*D*-galactopyranosylamine* (**5i**).—Yield: 2.55 g (83%); mp 165–166 °C; ν_{max} 3340 (NH), 3260 (OH), 1595 and 1500 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 7.11 (t, 1 H, $J_{4',5'}$ 9.1, $J_{5',6'}$ 9.1 Hz, H-5′), 6.97 (dd, 1 H, $J_{2',6'}$ 2.7, $J_{2',4'}$ 6.2 Hz, H-2′), 6.77 (ddd, 1 H, $J_{2',6'}$ 2.7, $J_{4',6'}$ 5.4, $J_{5',6'}$ 9.1 Hz, H-6′), 4.62 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 4.00 (d, 1 H, $J_{5,6}$ 2.2 Hz, H-6a), 3.80 (t, 1 H, $J_{3,4}$ 6.2, $J_{4,5}$ 6.2 Hz, H-4), 3.72–3.77 (m, 3 H, H-3, H-5, H-6b), 3.64 (t, 1 H, $J_{1,2}$ 8.7, $J_{2,3}$ 8.7 Hz, H-2); EIMS: *m*/*z* (%): 309 ([M$^+ + 2$], 3), 307 ([M$^+$], 5), 160 (35), 158 (100), 147 (9), 145 (27), 131 (7), 129 (15). Anal. Calcd. for $C_{12}H_{15}ClFNO_5 \cdot H_2O$: C, 44.25; H, 5.26; N, 4.30. Found: C, 44.29; H, 5.25; N, 4.31.

N-*(3-Chloro-4-fluorophenyl)-β-*D*-mannopyranosylamine* (**5j**).—Yield: 2.42 g (79%); mp 210–211 °C; ν_{max} 3320 (NH), 3250 (OH), 1600 and 1500 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 7.02 (t, 1 H, $J_{4',5'}$ 8.9, $J_{5',6'}$ 9.1 Hz, H-5′), 6.98 (dd, 1 H, $J_{2',6'}$ 2.6, $J_{2',4'}$ 6.2 Hz, H-2′), 6.79 (m, 1 H, H-6′), 4.94 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 4.03 (d, 1 H, $J_{5,6}$ 3.1 Hz, H-6a), 3.89 (dd, 1 H, $J_{5,6}$ 1.7, J_{gem} 12.3 Hz, 1 H, H-6b), 3.69–3.78 (m, 2 H, H-2, H-3), 3.63 (t, 1 H, $J_{3,4}$ 9.7, $J_{4,5}$ 9.7 Hz, H-4), 3.48 (m, 1 H, H-5); EIMS: *m*/*z* (%): 309 ([M$^+ + 2$], 9), 307 ([M$^+$], 23), 160 (32), 158 (100), 147 (17), 145 (53), 131 (9), 129 (20). Anal. Calcd. for $C_{12}H_{15}ClFNO_5 \cdot H_2O$: C, 44.25; H, 5.26; N, 4.30. Found: C, 44.37; H, 5.27; N, 4.29.

N-*(3-Chloro-4-fluorophenyl)-β-*D*-xylopyranosylamine* (**5e**).—Yield: 1.30 g (47%); mp 170–171 °C; ν_{max} 3320 (NH), 3260 (OH), 1585 and 1500 (Ph); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 7.12 (t, 1 H, $J_{4',5'}$ 9.1, $J_{5',6'}$ 9.1 Hz, H-5′), 6.95 (dd, 1 H, $J_{2',6'}$ 2.5, $J_{2',4'}$ 6.1 Hz, H-2′), 6.76 (m, 1 H, H-6′), 4.63 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 3.72 (dd, 1 H, $J_{4,5}$ 5.3, J_{gem} 11.4 Hz, H-5a), 3.63 (m, 1 H, H-4), 3.52 (t, $J_{1,2}$ 9.1, $J_{2,3}$ 9.1 Hz, H-3), 3.38–3.44 (m, 2 H, H-2, H-5b); EIMS: *m*/*z* (%): 279 ([M$^+ + 2$], 3), 278 ([M$^+ + 1$], 10), 277 ([M$^+$], 8), 276 ([M$^+ - 1$], 28), 160 (31), 158 (100), 147 (22), 145 (68), 131 (9), 129 (21). Anal. Calcd. for $C_{11}H_{13}ClFNO_4 \cdot H_2O$: C, 47.58; H, 4.72; N, 5.04. Found: N, 47.39; H, 4.71; N, 5.02.

Acknowledgements

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