

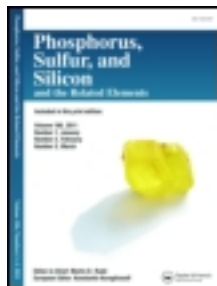
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The Synthesis and Fungicidal Activities of 2,6-Bis[(3-aryl)-s-triazolo[3,4-b]-[1,3,4]thiadiazole-6-yl]pyridines

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The Synthesis and Fungicidal Activities of 2,6-Bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridines

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*In search of better bioactive compounds, a series of novel 2,6-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridines **2** were synthesized in high yields by the cyclization of 3-aryl-4-amino-5-mercapto-1, 2, 4-triazoles **1** with 2,6-pyridine dicarboxylic acid. **2** exhibited good fungicidal activities against *Cerospora beticola* sacc.*

Keywords 3-aryl-4-amino-5-mercapto-1,2,4-triazole; 2,6-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4] thiadiazole-6-yl]pyridines; fungicidal activities; synthesis

INTRODUCTION

s-triazolo[3,4-*b*]-[1,3,4]thiadiazole derivatives possess significant biological activities, such as anti-inflammatory, and antiviral, antifungal, antineoplastic, and antidepressant effects^{1–2} and electrochemical properties.³ They are highly important heterocycles and have been used in the research and development of agrochemicals and in the pharmaceutical chemistry. Most derivatives reported only contain one s-triazolo[3,4-*b*]-[1,3,4]thiadiazole unit in one molecule.^{4–11} Recently, some of the bis[1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole-4-yl]alkanes were reported to possess antibacterial property,¹² and bis[1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole-3-ylmethoxy]phenylenes possess

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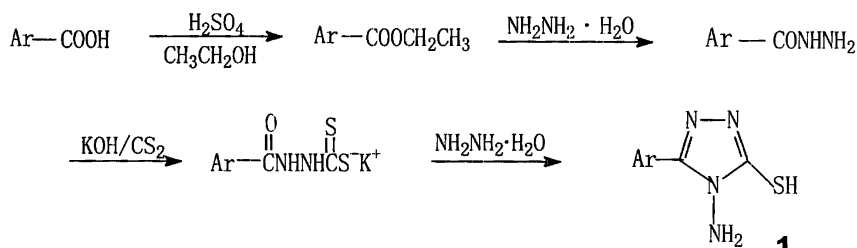
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anticancer activity against a panel of 60 cell lines derived from 7 cancer types, namely lung, colon, melanoma, renal, ovarian, CNS, and leukemia.¹³ Prompted by these observations and in continuation of our search for bioactive molecules, we designed the synthesis of a series of novel 2,6-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridines by the cyclization of 3-aryl-4-amino-5-mercapto-1,2,4-triazoles with 2,6-pyridine dicarboxylic acid. The synthesis, characterization, and results of fungicidal activity screening studies of the newly synthesized compounds are presented in this article.

RESULTS AND DISCUSSION

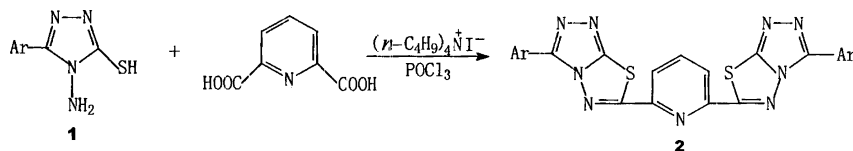
Aroylhydrazides were prepared by the esterification and hydrazinolysis of corresponding aromatic carboxylic acids. The reaction of aroylhydrazides with CS₂/KOH in absolute ethanol gave potassium aroyldithiocarbazates, and then the hydrazinolysis of potassium aroyldithiocarbazates with hydrazine hydrate afforded 3-aryl-4-amino-5-mercapto-1,2,4-triazoles **1** (Scheme 1).



SCHEME 1

The synthesis of 2,6-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl] pyridines **2** was accomplished in one step with good yields by condensing 3-aryl-4-amino-5-mercapto-1,2,4-triazoles **1** with 2,6-pyridine dicarboxylic acid in the presence of POCl₃ and tetrabutylammonium iodide (Scheme 2, Table I). Because of the poor solubility of **1** and 2,6-pyridine dicarboxylic acid in POCl₃, the yield of **2** is very low. For example, the yield of **2g** was 25.3%. However, where the tetrabutylammonium iodide as a phase transfer catalyst was utilized and the mixture was first stirred for 6 h at 55–60°C, and then refluxed for 10 h at 115–120°C, **2g** was obtained in a 71% yield.

The structures of all compounds **2a–p** were established on the basis of elemental analysis and spectral data. The IR spectral data of



SCHEME 2

compounds **2** showed bands at 1610–1635 cm^{-1} , 1230–1260 cm^{-1} , and 700 cm^{-1} due to C=N, N=N=C, and C-S-C, respectively. The ^1H NMR spectra of **2** exhibited multiple signals in the δ 7.00–8.80 range accounting for hydrogen of aryl group. With compound **2g** as an example, it exhibited multiple signals in the δ 8.78–8.74, 8.52–8.26, and 7.59–7.55 ranges accounting for the 11 hydrogens of phenyl and pyridyl groups. A sharp singlet at δ 2.56 integrating for 6 protons is attributed to the $-\text{CH}_3$ groups. The EI-MS for compounds **2** exhibited molecular ion peaks. With compound **2g** as an example, it showed a strong molecular ion peak M^+ with m/z 507 and 59% relative abundance.

The biological activity of compounds **2** were investigated, and the results showed that they exhibited fungicidal activities, especially against

TABLE I The Preparation of 2,6-Bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridines **2** from 3-Aryl-4-amino-5-mercapto-1, 2, 4-triazoles **1**

Entry	Ar	Condition	Yield (%) ^a	M.P. (°C)
2a	Ph	115–120°C/9.0 h	72	>300
2b	2-Cl-Ph	115–120°C/10 h	56	>300
2c	3-Cl-Ph	115–120°C/11 h	60	>300
2d	4-Cl-Ph	115–120°C/11 h	67	>300
2e	2-CH ₃ -Ph	115–120°C/10 h	56	>300
2f	3-CH ₃ -Ph	115–120°C/11 h	54	>300
2g	4-CH ₃ -Ph	115–120°C/12 h	71	>300
2h	3-Br-Ph	115–120°C/10 h	62	>300
2i	4-Br-Ph	115–120°C/12 h	66	>300
2j	2-I-Ph	115–120°C/11 h	58	>300
2k	3-I-Ph	115–120°C/10 h	63	>300
2l	4-I-Ph	115–120°C/11 h	71	>300
2m	4-OCH ₃ -Ph	115–120°C/13 h	68	>300
2n	4-Pyridyl	115–120°C/11 h	55	>300
2o	3-Pyridyl	115–120°C/12 h	51	>300
2p	2-Furyl	115–120°C/9.0 h	50	>300

^aPurified yields of **2a–2p** 2,6-pyridine dicarboxylic acid.

**TABLE II The Fungicidal Activities of
2,6-Bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]
Pyridines **2** (50 mg/L, Relative Inhibition %)**

Entry	<i>Gibberella</i> <i>zeae</i>	<i>Cerospora</i> <i>beticola sacc</i>	<i>Physalospora</i> <i>piricola</i>	<i>Pellicularia</i> <i>sasakii</i>
2a	20	73	59	15
2b	45	90	78	80
2c	58	91	80	89
2d	70	95	76	84
2e	26	78	65	29
2f	35	74	62	32
2g	39	70	56	25
2h	32	75	45	40
2i	30	80	42	45
2j	28	84	38	32
2k	26	86	41	28
2l	29	80	46	24
2m	32	70	26	33
2n	48	90	70	36
2o	52	88	65	42
2p	38	77	72	51

Cerospora beticola sacc. For example, **2d** showed 95% of *Cerospora beticola sacc* inhibition of in 50 mg/L (see Table II).

CONCLUSION

In conclusion, tetrabutylammonium iodide is an efficient catalyst for the synthesis of 2,6-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridines by the reaction of 3-aryl-4-amino-5-mercapto-1, 2, 4-triazoles with 2,6-pyridine dicarboxylic acid. Among all the compounds tested, **2d** showed a high degree of inhibition against *Cerospora beticola sacc.* Hence, compound **2d** stands to be a promising fungicidal agent.

EXPERIMENTAL

Melting points were determined on an X₄ melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Nexus 470 FT-IR spectrophotometer using KBr discs in the range 4000–400 cm^{−1}. ¹H NMR spectra were recorded on a Varian Mercury-Plus 400 NMR spectrometer in CF₃COOD or pyridine-*d*₅ solution using TMS as an internal reference. MS spectra were recorded on a Finnigan Trace

GC-MS spectrometer. Elemental analyses were taken on a Perkin-Elmer-2400-CHN elemental analysis instrument.

The General Procedure for the Preparation of 3-Aryl-4-amino-5-mercapto-1, 2, 4-triazole **1**¹⁴

A mixture of potassium aroyldithiocarbazate (30 mmol) and hydrazine hydrate (80%, 36 mL) was refluxed with stirring for 4–5 h at 120°C. The color of the reaction mixture changed to green, and a homogeneous solution resulted. Acidification with HCl (3 mol/L) resulted in the precipitation of a white solid. The product was filtered, washed with cold water, and recrystallized from ethanol to give the pure compounds **1a–p**.

The General Procedure for the Preparation of 2,6-Bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]-thiadiazole-6-yl]pyridines **2**¹⁵

A mixture of compounds 3-aryl-4-amino-5-mercapto-1,2,4-triazole (2.2 mmol), 2,6-pyridine dicarboxylic acid (1.67 g, 1.0 mmol), the phase transfer catalyst tetrabutylammonium iodide (1.85 g, 0.5 mmol), and POCl₃ (7 mL) was stirred for 4 h at 55–60°C and then refluxed for 9.0–13 h at 115–120°C. Excess POCl₃ was removed under reduced pressure. The concentrated mass was cooled and poured into crushed ice and neutralized with potassium carbonate. The separated solid was filtered, washed with water, washed with ethanol, and then dried. The crude material was recrystallized (ethanol-pyridine), giving the pure products **2a–p**.

2,6-Bis[(3-phenyl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridine (**2a**)

Yellow powder, ¹H NMR (Pyridine-*d*₅, 400 MHz): δ 8.62–8.53 (m, 4H, Ar-H), 8.42–8.21 (m, 4H, Ar-H), 7.79–7.56 (m, 5H, Ar-H); IR (KBr, cm⁻¹): 1612, 1251, 700. MS-EI (*m/z*): 479 (M⁺, 2%), 304 (3%), 146 (51%), 102 (100%). Elemental anal. calcd. for C₂₃H₁₃N₉S₂: C, 57.61; H, 2.73; N, 26.29. Found: C, 57.76; H, 2.62; N, 26.18.

2,6-Bis[(3-*o*-chlorophenyl)-s-triazolo[3,4-*b*]-[1,3,4]-thiadiazole-6-yl]pyridine (**2b**)

Brown powder, ¹H NMR (Pyridine-*d*₅, 400 MHz): δ 8.47–8.39 (m, 4H, Ar-H), 8.31–8.23 (m, 3H, Ar-H), 7.82–7.71 (m, 4H, Ar-H); IR (KBr, cm⁻¹): 1619, 1244, 697; MS-EI (*m/z*): 547 (M⁺, 58%), 549 (M+2, 15%), 357 (38%), 338 (100%), 146 (51%), 102 (49%). Elemental anal. calcd. for C₂₃H₁₁N₉S₂Cl₂: C, 52.56; H, 2.02; N, 22.99. Found: C, 52.43; H, 2.11; N, 22.79.

2,6-Bis[(3-*m*-chlorophenyl)-*s*-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridine (2c)

Brown powder, ^1H NMR (Pyridine- d_5 , 400 MHz): δ 8.63–8.59 (m, 2H, Ar-H), 8.52–8.26 (m, 5H, Ar-H), 7.85–7.69 (m, 4H, Ar-H); IR (KBr, cm^{-1}): 1621, 1248, 701; MS-EI (m/z): 547 (M^+ , 100%), 549 ($\text{M} + 2$, 38%), 357 (67%), 338 (69%), 146 (56%), 102 (62%). Elemental anal. calcd. for $\text{C}_{23}\text{H}_{11}\text{N}_9\text{S}_2\text{Cl}_2$: C, 52.56; H, 2.02; N, 22.99. Found: C, 52.68; H, 2.01; N, 22.87.

2,6-Bis[(3-*p*-chlorophenyl)-*s*-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridine (2d)

Brown powder, ^1H NMR (Pyridine- d_5 , 400 MHz): δ 8.59–8.51 (m, 3H, Ar-H), 8.40–8.17 (m, 4H, Ar-H), 7.74–7.60 (m, 4H, Ar-H); IR (KBr, cm^{-1}): 1630, 1254, 702; MS-EI (m/z): 547 (M^+ , 100%), 549 ($\text{M} + 2$, 25%), 357 (42%), 338 (52%), 146 (63%), 102 (74%). Elemental anal. calcd. for $\text{C}_{23}\text{H}_{11}\text{N}_9\text{S}_2\text{Cl}_2$: C, 52.56; H, 2.02; N, 22.99. Found: C, 52.71; H, 2.13; N, 22.82.

2,6-Bis[(3-*o*-methylphenyl)-*s*-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridine (2e)

Pale yellow powder, ^1H NMR (Pyridine- d_5 , 400 MHz): δ 8.73–8.62 (m, 3H, Ar-H), 8.42–8.24 (m, 4H, Ar-H), 7.69–7.51 (m, 4H, Ar-H), 2.53 (s, 6H, 2CH₃); IR (KBr, cm^{-1}): 1628, 1241, 701; MS-EI (m/z): 507 (M^+ , 38%), 317 (52%), 147 (19%), 117 (60%), 115 (100%). Elemental anal. calcd. for $\text{C}_{25}\text{H}_{17}\text{N}_9\text{S}_2$: C, 59.16; H, 3.38; N, 24.83. Found: C, 59.02; H, 3.42; N, 24.95.

2,6-Bis[(3-*m*-methylphenyl)-*s*-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridine (2f)

Yellow powder, ^1H NMR (Pyridine- d_5 , 400 MHz): δ 8.72–8.60 (m, 4H, Ar-H), 8.69–8.38 (m, 5H, Ar-H), 7.59–7.55 (m, 2H, Ar-H), 2.54 (s, 6H, 2CH₃); IR (KBr, cm^{-1}): 1635, 1250, 701; MS-EI (m/z): 507 (M^+ , 42%), 317 (31%), 147 (37%), 117 (51%), 115 (100%), 102 (32%). Elemental anal. calcd. for $\text{C}_{25}\text{H}_{17}\text{N}_9\text{S}_2$: C, 59.16; H, 3.38; N, 24.83. Found: C, 59.23; H, 3.43; N, 24.78.

2,6-Bis[(3-*p*-methylphenyl)-*s*-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridine (2g)

Yellow powder, ^1H NMR (Pyridine- d_5 , 400 MHz): δ 8.78–8.74 (m, 2H, Ar-H), 8.52–8.26 (m, 5H, Ar-H), 7.59–7.55 (m, 4H, Ar-H), 2.56 (s, 6H, 2CH₃); IR (KBr, cm^{-1}): 1632, 1256, 702; MS-EI (m/z): 507 (M^+ , 59%), 317 (49%), 147 (27%), 117 (42%), 115 (100%), 101 (20%). Elemental anal.

calcd. for $C_{25}H_{17}N_9S_2$: C, 59.16; H, 3.38; N, 24.83. Found: C, 59.28; H, 3.39; N, 24.72.

2,6-Bis[(3-m-bromophenyl)-s-triazolo[3,4-b]-[1,3,4]-thiadiazole-6-yl]pyridine (2h)

Pale yellow powder, 1H NMR (CF_3COOD , 400 MHz): δ 8.79–8.65 (m, 3H, Ar-H), 8.24–8.21 (m, 5H, Ar-H), 7.85–7.74 (m, 3H, Ar-H); IR (KBr, cm^{-1}): 1623, 1239, 703; MS-EI (m/z): 635 (M^+ , 11%), 637 ($M + 2$, 12%), 400 (3%), 382 (35%), 146 (47%), 102 (100%). Elemental anal. calcd. for $C_{23}H_{11}N_9S_2Br_2$: C, 43.34; H, 1.74; N, 19.78. Found: C, 43.42; H, 1.70; N, 19.65.

2,6-Bis[(3-p-bromophenyl)-s-triazolo[3,4-b]-[1,3,4]-thiadiazole-6-yl]pyridine (2i)

Yellow powder, 1H NMR (CF_3COOD , 400 MHz): δ 8.78–8.51 (m, 4H, Ar-H), 8.27–8.24 (m, 4H, Ar-H), 7.92–7.88 (m, 3H, Ar-H); IR (KBr, cm^{-1}): 1617, 1245, 701; MS-EI (m/z): 635 (M^+ , 13%), 637 ($M + 2$, 18%), 400 (7%), 381 (4%), 146 (51%), 102 (100%). Elemental anal. calcd. for $C_{23}H_{11}N_9S_2Br_2$: C, 43.34; H, 1.74; N, 19.78. Found: C, 43.27; H, 1.72; N, 19.91.

2,6-bis[(3-o-iodophenyl)-s-triazolo[3,4-b]-[1,3,4]-thiadiazole-6-yl]-pyridine (2j)

Yellow powder, 1H NMR (CF_3COOD , 400 MHz): δ 8.70–8.59 (m, 5H, Ar-H), 8.26–8.12 (m, 4H, Ar-H), 8.02–7.92 (m, 2H, Ar-H); IR (KBr, cm^{-1}): 1625, 1231, 700; MS (m/z): 731 (M^+ , 6%), 430 (52%), 146 (29%), 129 (15%), 102 (100%). Anal. calcd. for $C_{23}H_{11}N_9S_2I_2$: C, 37.77; H, 1.52; N, 17.24. Found: C, 37.84; H, 1.50; N, 17.18.

2,6-bis[(3-m-iodophenyl)-s-triazolo[3,4-b]-[1,3,4]-thiadiazole-6-yl]-pyridine (2k)

Pale yellow powder, 1H NMR (Pyridine- d_5 , 400 MHz): δ 8.67–8.38 (m, 4H, Ar-H), 8.24–8.12 (m, 4H, Ar-H), 7.85–7.78 (m, 3H, Ar-H); IR (KBr, cm^{-1}): 1621, 1262, 700; MS-EI (m/z): 731 (M^+ , 51%), 675 (7%), 430 (76%), 146 (26%), 129 (6%), 102 (44%), 92 (100%). Anal. calcd. for $C_{23}H_{11}N_9S_2I_2$: C, 37.77; H, 1.52; N, 17.24. Found: C, 37.86; H, 1.67; N, 17.13.

2,6-bis[(3-p-iodophenyl)-s-triazolo[3,4-b]-[1,3,4]-thiadiazole-6-yl]-pyridine (2l)

Yellow powder, 1H NMR (CF_3COOD , 400 MHz): δ 8.73–8.42 (m, 4H, Ar-H), 8.11–8.04 (m, 5H, Ar-H), 7.93–7.89 (m, 2H, Ar-H); IR (KBr, cm^{-1}): 1627, 1238, 703; MS-EI (m/z): 731 (M^+ , 5%), 430 (67%), 146 (44%), 129

(9%), 102 (100%). Anal. calcd. for $C_{23}H_{11}N_9S_2I_2$: C, 37.77; H, 1.52; N, 17.24. Found: C, 37.64; H, 1.49; N, 17.32.

2,6-Bis[(3-*p*-methoxyphenyl)-*s*-triazolo[3,4-*b*]-[1,3,4]-thiadiazole-6-yl]pyridine (2m)

Yellow powder, 1H NMR (Pyridine- d_5 , 400 MHz): δ 8.71–8.64 (m, 2H, Ar-H), 8.47–8.18 (m, 5H, Ar-H), 7.69–7.63 (m, 4H, Ar-H), 3.89 (s, 6H, $2OCH_3$); IR (KBr, cm^{-1}): 1616, 1241, 700; MS-EI (m/z): 540 (M^+ , 54%), 334 (84%), 146 (48%), 132 (100%), 101 (58%). Elemental anal. calcd. for $C_{25}H_{17}N_9S_2O_2$: C, 55.65; H, 3.18; N, 23.36. Found: C, 55.74; H, 3.12; N, 23.27.

2,6-Bis[(3-4-pyridyl)-*s*-triazolo[3,4-*b*]-[1, 3, 4]thiadiazole-6-yl]-pyridine (2n)

Yellow powder, 1H NMR (Pyridine- d_5 , 400 MHz): 8.47–8.43 (m, 2H, Ar-H), 8.38–8.35 (m, 3H, Ar-H), 8.24–8.17 (m, 4H, Ar-H), 7.42–7.38 (m, 2H, Ar-H); IR (KBr, cm^{-1}): 1626, 1256, 700; MS-EI (m/z): 481 (M^+ , 100%), 323 (23%), 305 (45%), 146 (16%), 104 (10%). Elemental anal. calcd. for $C_{21}H_{11}N_{11}S_2$: C, 52.38; H, 2.30; N, 32.01. Found: C, 52.27; H, 2.11; N, 32.18.

2,6-Bis[(3-3'-pyridyl)-*s*-triazolo[3, 4-*b*]-[1, 3, 4]thiadiazole-6-yl]-pyridine (2o)

Pale yellow powder, 1H NMR (Pyridine- d_5 , 400 MHz): 8.67–8.51 (m, 3H, Ar-H), 8.42–8.33 (m, 4H, Ar-H), 8.28–8.11 (m, 2H, Ar-H), 7.46–7.32 (m, 2H, Ar-H); IR (KBr, cm^{-1}): 1621, 1242, 701; MS-EI (m/z): 481 (M^+ , 100%), 323 (15%), 305 (71%), 146 (32%), 104 (9%). Elemental anal. calcd. for $C_{21}H_{11}N_{11}S_2$: C, 52.38; H, 2.30; N, 32.01. Found: C, 52.47; H, 2.28; N, 31.87.

2,6-Bis[(3-2-furyl)-*s*-triazolo[3, 4-*b*]-[1, 3, 4]thiadiazole-6-yl]pyridine (2p)

Pale yellow powder, 1H NMR (Pyridine- d_5 , 400 MHz): 8.43–8.25 (m, 3H, Ar-H), 7.24–6.67 (m, 6H, furyl-H); IR (KBr, cm^{-1}): 1617, 1251, 700; MS-EI (m/z): 459 (M^+ , 41%), 312 (4%), 294 (64%), 146 (100%), 93 (31%). Elemental anal. calcd. for $C_{19}H_9N_9O_2S_2$: C, 49.67; H, 1.97; N, 27.44. Found: C, 49.75; H, 2.11; N, 27.33.

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