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SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTI-HIV-1 ACTIVITY OF 1-[ω-(PHENOXY)ALKYL AND -ALKENYL]URACIL DERIVATIVES

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1-[ω-(Phenoxy)alkyl and -alkenyl]uracil derivatives were synthesized via condensation of equimolar amounts of 2,4-*bis*(trimethylsilyloxy)pyrimidine and 1-halo-ω-(phenoxy)alkane or -alkene in order to discover new non-nucleoside inhibitors of HIV-1 reverse transcriptase. Their anti-HIV-1 activity was studied in CEM-cell culture and against HIV-1 reverse transcriptase. It was found that several compounds exhibited marked activity.

Keywords: synthesis, anti-HIV-1 activity, 1[ω-(phenoxy)alkyl and -alkenyl]uracil derivatives.

Beginning in 1995, a combination of drugs, which was called highly active antiretroviral therapy (HAART) [1], was used as the basis for long-term treatment of human immunoviral (HIV) infection. At present, about 50 structurally diverse classes of new HIV non-nucleoside reverse transcriptase inhibitors (NNRTIs) are known. Many of these are characterized by three aromatic rings in the molecule (a typical example is etravirine, which was approved for clinical application in 2008) [2]. The binding confirmation of this class of compounds with HIV reverse transcriptase (RT) was described by a "butterfly-like" model.

In addition, several classes of NNRTIs containing only two aromatic rings joined by a linker (e.g., HI-238 and R100943) [3] are known. They all demonstrated antiviral activity at nanomolar concentrations and had activity profiles against clinically important mutant HIV-1 isolates that were better than those of first-generation NNRTIs. In particular, compound R100943 was prepared during optimization of the etravirine structure. It was found that this inhibitor was capable of closer contact with the surface of the binding site owing to high molecular flexibility. The active conformation of R100943 differed considerably from that of etravirine and was called a "horseshoe" [3]. Furthermore, a study of the binding mechanism of triazole and tetrazole thioacetanilide derivatives (Fig. 1) to HIV-1 RT established that the middle ring was not required for the manifestation of virus-inhibiting properties. It could be replaced by a simple *Z*-alkenyl moiety [4]. It is noteworthy that the aromatic radicals of all 2-ring NNRTIs shown herein interact with an identical set of RT hydrophobic pocket amino acids. Highly conserved residues Phe227 and Trp229 are most important for binding. An examination of the structures of the new generation of highly active NNRTIs showed clearly that small hydrophobic sub-

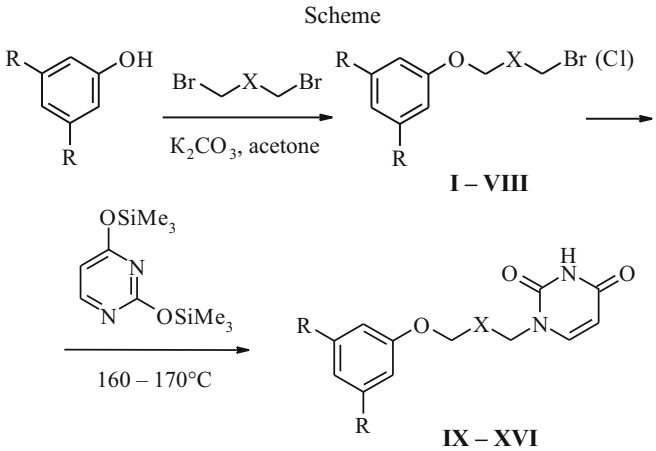

Fig. 1. Non-nucleoside HIV-1 RT inhibitors.

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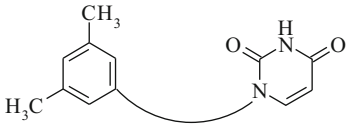


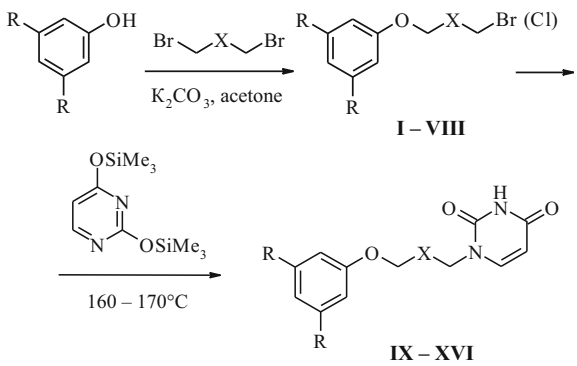
Fig. 2. Structural features of uracil-containing NNRTI.

stituents in the *meta*-positions of one of the terminal aromatic rings facilitated significantly this type of interaction [2].

Thus, an NNRTI molecule according to current thinking should have high conformational lability that provides the capability for reorientation and repositioning within the RT hydrophobic pocket if amino-acid residues are replaced as a result of mutations [2]. Structures containing two π-electron systems separated by a flexible linker, as illustrated in Fig. 2, satisfy fully these requirements. Based on this concept, a series of potential antiviral agents were synthesized. Their structures were based on two aromatic rings joined by an O-containing acyclic moiety. Uracil was selected as one of the aromatic systems because it is a convenient foundation for elaborating molecular constructs. A benzene ring with methyl substituents in the *meta*-positions was used as the second system. In our opinion, this could enhance the binding of these derivatives to the enzyme.

The target uracil derivatives containing various ω-(phenoxy)alkyl and -alkenyl moieties in the 1-position were synthesized in two steps, as shown in the scheme below.

Scheme



Starting 1-bromo-3-(3,5-dimethylphenoxy)propane (**I**, R = CH_3, X = CH_2); 1-bromo-4-(phenoxy)butane [**II**, R = H, X = $(CH_2)_2$]; 1-bromo-4-(3,5-dimethylphenoxy)butane [**III**, R = CH_3, X = $(CH_2)_2$]; 1-bromo-5-(phenoxy)pentane [**IV**, R = H, X = $(CH_2)_3$]; 1-bromo-5-(3,5-dimethylphenoxy)pentane [**V**, R = CH_3, X = $(CH_2)_3$]; and 1-bromo-6-(3,5-dimethylphenoxy)hexane [**VI**, R = CH_3, X = $(CH_2)_4$] in addition to isomeric (*E*)-1-chloro-4-(3,5-dimethylphenoxy)- [**VII**, R = CH_3, X = (*E*)-HC=CH] and (*Z*)-1-chloro-4-(3,5-dimethylphenoxy)but-2-ene [**VIII**, R = CH_3, X = (*Z*)-HC=CH] were prepared by treating phenol or 3,5-dimethylphenol with a four-fold molar excess of α,ω-dibromoalkane or (*E*)- or (*Z*)-1,4-dichlorobut-2-ene in refluxing Me_2CO in the presence of a 1.5-molar excess of freshly calcined K_2CO_3 according to the published methods [5, 6]. With this, the yields of **I** – **VIII** were 54 – 79%. Subsequent condensation of equimolar amounts of bromides **I** – **VI** and 2,4-*bis*(trimethylsilyloxy)pyrimidine at 160 – 170°C for 1.5 h with exclusion of moisture according to the literature methods [7, 8] gave target 1-[ω-(phenoxy)alkyl]uracil derivatives **IX** – **XIV** in yields of 71 – 83%. Condensation of 2,4-*bis*(trimethylsilyloxy)pyrimidine with chlorides **VII** and **VIII** under analogous conditions gave target (*E*)- (**XV**) and (*Z*)-1-[4-(3,5-dimethylphenoxy)but-2-en-1-yl]uracil derivatives (**XVI**) in substantially lower yields of 50 – 54%. This was explained by the tendency of the unsaturated C=C double bond to undergo side reactions under the reaction conditions.

The purity of the products was determined by TLC; the structures, by PMR and ^{13}C NMR spectroscopy. Table 1 presents the physicochemical properties.

EXPERIMENTAL CHEMICAL PART

PMR and ^{13}C NMR spectra were recorded in CCl_4 and DMSO-d_6 with TMS internal standard on a Bruker Avance 400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C). TLC was carried out on Merck TLS Silica gel 60 F_{254} plates (Germany) using EtOAc eluent. Preparative column chromatography used Kieselgel 60 – 200 μm, 60 A (Acros Organics) and $CHCl_3$:MeOH (10:1) eluent. Melting points were measured in glass capillaries on a Mel-Temp3.0 apparatus (Laboratory Devices Inc., USA).

1-Bromo-ω-(phenoxy)alkanes (I – VI) and 1-chloro-4-(phenoxy)but-2-enes (VII and VIII). General method. A suspension of phenol or 3,5-dimethylphenol (0.159 mol), anhydrous K_2CO_3 (33.0 g, 0.239 mol), and α,ω-dibromoalkane or 1,4-dichlorobut-2-ene (0.658 mol) in Me_2CO (200 mL) was refluxed for 24 h. The precipitate was filtered off and washed on the filter with a small amount of Me_2CO. The filtrate was evaporated at reduced pressure. The solid was dissolved in $CHCl_3$ (200 mL) and washed successively with aqueous NaOH solution (2%, 100 mL × 2) and H_2O (100 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated at reduced pressure. The residue was vacuum distilled (oil pump).

1-[ω-(Phenoxy)alkyl]- (IX – XIV) and 1-[4-(3,5-dimethylphenoxy)but-2-en-1-yl]uracil derivatives (XV and XVI). General method. Uracil (1.5 g, 13.38 mmol) in an excess of HMDS was refluxed to produce 2,4-*bis*(trimethylsilyloxy)pyrimidine, which was treated with an equimolar amount of the bromide (**I**-**VI**) or chloride (**VII** or **VIII**), heated with exclusion of moisture at 160 – 170°C for 1 h, and worked up with EtOAc (40 mL) and 2-propanol (10 mL). After 30 min, the resulting mixture was evaporated in vacuo to dryness. The residue was dissolved in $CHCl_3$ (10 mL). The products were separated by column chromatography over silica gel with elution by $CHCl_3$:MeOH (10:1). Fractions containing product were combined and evaporated in vacuo. The residue was recrystallized from 2-propanol:DMF.

1-[3-(3,5-Dimethylphenoxy)propyl]uracil (IX). PMR spectrum (DMSO-d_6), δ, ppm, J (Hz): 1.95 (t, 2H, J 6.3, CH_2); 2.15 (s, 6H, CH_3); 3.77 (t, 2H, J 6.0, NCH_2); 3.87 (t, 2H, J 5.7, OCH_2); 5.48 (d, 1H, J 7.5, H-5); 6.44 (s, 2H, H-2′, H-6′); 6.50 (s, 1H, H-4′); 7.55 (d, 1H, J 8.1, H-6); 11.20 (s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 21.1, 28.0, 45.4, 64.6, 100.9, 112.2, 122.3, 138.6, 145.8, 151.0, 158.4, 163.9.

1-[4-(Phenoxy)butyl]uracil (X). PMR spectrum (DMSO-d_6), δ, ppm, J (Hz): 1.64 (s, 4H, CH_2); 3.67 (t, 2H, J 6.2, NCH_2); 3.88 (t, 2H, J 6.2, OCH_2); 5.51 (dd, 1H, J 7.8 and 2.1, H-5); 6.89 – 6.91 (m, 3H, H); 7.21 (t, 2H, J 8, H-3′, H-5′); 7.62 (d, 1H, J 7.5, H-6); 11.24 (s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 25.3, 25.7, 47.3, 66.8, 100.9, 114.4, 120.5, 129.5, 145.7, 151.0, 158.6, 163.8.

1-[4-(3,5-Dimethylphenoxy)butyl]uracil (XI). PMR spectrum (DMSO-d_6), δ, ppm, J (Hz): 1.63 (s, 4H, CH_2); 2.15 (s, 6H, CH_3); 3.66 (t, 2H, J 6.2, NCH_2); 3.86 (t, 2H, J 6.2, OCH_2); 5.50 (dd, 1H, J 7.8 and 2.1, H-5); 6.46 (s, 2H, H-2′, H-6′); 6.49 (s, 1H, H-4′); 7.61 (d, 1H, J 7.5, H-6); 11.20 (s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 21.1, 25.3, 25.7, 47.2, 66.7, 100.9, 112.2, 122.1, 138.6, 145.7, 151.0, 158.6, 163.8.

1-[5-(Phenoxy)pentyl]uracil (XII). PMR spectrum (DMSO-d_6) δ, ppm, J (Hz): 1.39 (q, 2H, J 5.3, CH_2); 1.63 (q, 2H, J 7.2, CH_2); 1.72 (q, 2H, J 7.2, CH_2); 3.67 (t, 2H, J 7.2, NCH_2); 3.93 (t, 2H, J 6.5, OCH_2); 5.55 (dd, 1H, J 7.7, and 2.1 H-5); 6.89 – 6.92 (m, 3H, H); 7.26 (t, 2H, J 8, H-3′, H-5′); 7.64 (d, 1H, J 7.8, H-6); 11.25 (s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 22.9, 28.6, 28.7, 47.8, 67.5, 101.2, 114.8, 120.8, 129.9, 146.2, 151.4, 159.0, 164.3.

1-[5-(3,5-Dimethylphenoxy)pentyl]uracil (XIII). . PMR spectrum (DMSO-d_6), δ, ppm, J (Hz): 1.37 (q, 2H, J 6.8, CH_2); 1.62 (q, 2H, J 7.2, CH_2); 1.67 (q, 2H, J 7.2, CH_2); 2.21 (s, 6H, CH_3); 3.66 (t, 2H, J 7.1, NCH_2); 3.88 (t, 2H, J 6.4, OCH_2); 5.55 (dd, 1H, J 7.9 and 2.2, H-5); 6.51 (s, 2H, H-2′, H-6′); 6.53 (s, 1H, H-4′); 7.64 (d, 1H, J 7.8, H-6); 11.25 (s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 21.1, 22.5, 28.2, 28.4, 47.4, 66.9, 100.8, 112.1, 122.0, 138.6, 145.7, 151.0, 158.7, 163.8.

1-[6-(3,5-Dimethylphenoxy)hexyl]uracil (XIV). . PMR spectrum (DMSO-d_6), δ, ppm, J (Hz): 1.23 (m, 2H, J 6.6, CH_2); 1.36 (m, 2H, J 6.9, CH_2); 1.53 (m, 2H, J 7.3, CH_2); 1.62 (m, 2H, J 5.4, CH_2); 2.15 (s, 6H, CH_3); 3.59 (t, 2H, J 7.4, NCH_2); 3.83 (t, 2H, J 6.5, OCH_2); 5.47 (dd, 1H, J 8.0 and 1.8, H-5); 6.46 (s, 2H, H-2′, H-6′); 6.48 (s, 1H, H-4′); 7.59 (d, 1H, J 8.1, H-6); 11.17 (s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 21.1, 25.2, 25.6, 28.4, 28.6, 47.4, 67.0, 100.8, 112.1, 122.0, 138.5, 145.7, 151.0, 158.7, 163.8.

(*E*)-1-[4-(3,5-Dimethylphenoxy)butene]uracil (XV). PMR spectrum ($CDCl_3$), δ, ppm, J (Hz): 2.27 (s, 6H, CH_3); 4.38 (dd, 2H, J 5.6 and 0.8, CH_2); 4.51 (dd, 2H, J 4.7 and 1.2, CH_2); 5.71 (d, 1H, J 7.9, H-5); 5.84 – 5.98 (m, 2H, HC=CH); 6.52 (s, 2H, H-2′, H-6′); 6.61 (s, 1H, H-4′); 7.13 (d, 1H, J 7.9, H-6); 9.45 (c, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 21.1, 48.1, 66.8, 101.3, 112.3, 122.3, 127.3, 129.1, 138.6, 145.3, 150.8, 158.2, 163.8.

(*Z*)-1-[4-(3,5-Dimethylphenoxy)butene]uracil (XVI). PMR spectrum (DMSO-d_6), δ, ppm, J (Hz): 2.22 (s, 6H, CH_3); 4.41 (d, 2H, J 6.8, CH_2); 4.70 (d, 2H, J 5.8, CH_2); 5.59 (dd, 1H, J 7.8 and 2.1, H-5); 5.63 (dt, 1H, J 11.0 and 5.7, CH); 5.83 (dt, 1H, J 11.1 and 5.3, CH); 6.57 (s, 3H, H-2′, H-4′, H-6′); 7.62 (d, 1H, J 7.8, H-6); 11.31 (s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 21.5, 45.1, 63.8, 101.7, 112.8, 122.8, 127.6, 130.0, 145.7, 151.3, 158.6, 164.2.

EXPERIMENTAL BIOLOGICAL PART

We used [α-^{32}P]dATP (5,000 Ci/mol) (Izotop, Russia); 2′-deoxyribonucleoside-5′-triphosphate (Promega, USA); and Whatman 3MM cellulose filters (Whatman, Great Britain). All other reagents were as pure as possible and purchased from Sigma-Aldrich and Fluka. Activated DNA was prepared from salmon sperm DNA (Pharmacia Biotech, USA) by treatment with bovine pancreatic DNase (Fermentas, Latvia) as described before [9]. HIV-1 RT was expressed in *E. coli* cells and isolated as before [10].

Cells of eukaryotes were cultivated in a moist atmosphere with CO_2 (5%) in DMEM HeLa cells) or RPMI (CEM or L1210 cells) medium containing fetal calf serum (10%, v/v), L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 μg/mL).

The anti-HIV investigations used the following procedure. CEM cells were inoculated into a 96-well culture plate (~3 × 10^5 cells/mL, 0.1 mL/well), infected with HIV-1 strain III_B (100 $CCID_{50}$/mL), and treated with solutions of the studied compounds in growth medium (0.1 mL/well) at the required dilutions. Formation of HIV-induced gigantic CEM cells was established under a microscope after incubation for 4 d at 37°C.

Cytotoxicity of the tested compounds was determined by dissolution in DMSO at concentration 50 mM. Aliquots were

TABLE 1. Properties of Synthesized Compounds

Compound	R	X	Yield, %	mp, °C	R_f*
IX	CH_3	CH_2	71	89.5 – 91	0.49
X	H	$(CH_2)_2$	74	136 – 137	0.42
XI	CH_3	$(CH_2)_2$	71	151 – 152	0.43
XII	H	$(CH_2)_3$	83	151 – 152.5	0.53
XIII	CH_3	$(CH_2)_3$	80	96 – 98	0.58
XIV	CH_3	$(CH_2)_4$	76	130 – 131.5	0.54
XV	CH_3	(E)-CH=CH	54	124 – 126	0.65
XVI	CH_3	(*Z*)-CH=CH	50	99.5 – 101	0.59

* Eluent EtOAc.

added to uninfected CEM, L1201, or HeLa cells in growth medium until the desired DMSO concentration was reached. The number of vital cells was determined after incubation for 4 d at 37°C by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent and the standard method [11].

Determination of HIV-1 RT activity in the activated DNA system. The standard reaction mixture (20 μL) contained activated DNA (150 μg/mL), HIV-1 RT (0.05 μg), ATP (3 μM), other nucleoside-5′-triphosphates (30 μM each), and [α-^{32}P]dATP (0.02 MBq) in buffer in order to measure HIV-1 RT activity (50 mM Tris•HCl, pH 8.1, 10 mM $MgCl_2$, and 200 mM KCl). The compounds were added to the reaction mixture as DMSO solutions to a final DMSO concentration of 10% in experiments on the inhibitory properties. The control reactions were treated with an analogous volume of pure DMSO. The reaction was initiated by adding RT and incubating for 20 min at 37°C. Then, samples were placed on filters (1 × 1 cm, Whatman 3MM) soaked with EDTA solution (1 μL, 0.5 M). The filters were rinsed of labeled nucleotide not incorporated into DNA (5 × 25 mL, 10% TCA, 5 min each, 25 mL EtOH) and dried in air. Radioactivity absorbed in the filters was measured by the Cherenkov method in an intertechnique SL-4000 Liquid Scintillation Counter. Inhibition constants were calculated by the Dixon method [12].

RESULTS AND DISCUSSION

The properties of target compounds **IX** – **XVI** were studied by determining the inhibitory activity (inhibition constant K_t) against HIV-1 RT and their antiviral activity (inhibiting concentration IC_{50}) in infected CEM-cell culture (Table 2).

TABLE 2. Anti-HIV-1 Activity of 1-[ω-(Phenoxy)alkyl and -alkenyl]uracil Derivatives

Compound	R	X	K_i, μM[a]	IC_{50}, μM[b]
IX	CH_3	CH_2	> 100	> 250
X	H	$(CH_2)_2$	-	> 250
XI	CH_3	$(CH_2)_2$	> 100	40 ± 1.1
XII	H	$(CH_2)_3$	> 100	> 430
XIII	CH_3	$(CH_2)_3$	63 ±	24.4 ± 0.5
XIV	CH_3	$(CH_2)_4$	87.5 ± 1.2	31 ± 0.6
XV	CH_3	(*E*)-CH=CH	> 100	37 ± 0.7
XVI	CH_3	(*Z*)-CH=CH	> 100	> 220.7
Nevirapine			0.86 ± 0.08	0.075 ± 0.004

[a] Inhibition constant, concentration of non-competitive reverse transcriptase inhibitor for which enzyme activity is suppressed by 50%;
[b] Inhibiting concentration, concentration providing virus replication suppression by 50%.

Compounds containing an unsubstituted benzene ring (**X** and **XII**) turned out to be inactive. Compound **IX**, which had the shortest linker of three CH_2 groups, and compound **XVI**, clearly because of considerable conformational differences from the other compounds in the series due to the *Z*-configuration of the double bond, also did not exhibit anti-HIV activity.

An analysis of the activity of **XI**, **XIII**, **XIV**, and **XV** showed that the optimum structure of the linker joining the phenyl and the pyrimidine rings included one O atom and five CH_2 groups. The activity increased slightly upon adding a double bond in the *trans*-configuration to the linker (**XV** vs. **XI**). However, this did not enable unambiguous conclusions about the role of a moiety with increased electron density in this molecular fragment to be drawn.

Investigations of the HIV-1 RT inhibition constants showed that only **XIII** and **XIV** of the all compounds exhibited weak activity.

Cytotoxicity was estimated in cultures of CEM and L1210 lymphoid cells and of the HeLa line (ovary adenocarcinoma). Table 3 presents the results. In general, the manifested activity level was rather low with the exception of **XV**, which demonstrated CTD_{50} of 23 μM in HeLa cell culture.

Thus, we described 1-[ω-(phenoxy)alkyl and -alkenyl]uracil derivatives as new anti-HIV-1 agents. The prerequisites for manifestation of virus-inhibiting properties in this series of compounds were the presence of *meta*-substituents in the benzene ring and a 5 – 7-atom chain separating the aromatic rings (with an optimum of 6 atoms). Further structural modification in order to increase the anti-retroviral activity should be focused on adapting the linker to the amino-acid environment in the HIV RT binding pocket and determining the structure–activity relationship of the pyrimidine moiety.

TABLE 3. Cytostatic Activity of 1-[ω-(Phenoxy)alkyl and -alkenyl]uracil Derivatives

Compound	CTD_{50}, μMa		
	L1210	CEM	HeLa
IX	176 ± 21	198 ± 74	228 ± 30
X	> 250	> 250	132 ± 11
XI	> 250	> 250	144 ± 11
XII	> 250	> 250	≥ 250
XIII	124 ± 2	178 ± 57	187 ± 72
XIV	> 250	> 250	> 250
XV	178 ± 27	162 ± 24	161 ± 10
XVI	146 ± 38	156 ± 31	23 ± 3

[a] Cytotoxic dose, concentration at which cell growth is suppressed by 50%.

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