ORIGINAL ARTICLE

Study on retroaldol degradation products of antibiotic oligomycin A

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Studies of reactivity of antibiotic oligomycin A in various alkaline conditions showed that the compound easily undergoes retroaldol degradation in β -hydroxy ketone fragments positioned in the C7–C13 moiety of the antibiotic molecule. Depending on reaction conditions, the retroaldol fragmentation of the 8,9 or 12,13 bonds or formation of a product through double retroaldol degradation, when the fragment C9–C12 was detached, took place followed by further transformations of the intermediate aldehydes formed. The structures of the obtained non-cyclic derivatives of oligomycin A were supported by NMR and MS methods. NMR parameters demonstrate the striking similarity of the geometry (conformation) of the fragment C20–C34 in the non-cyclic products of retroaldol degradation and the starting antibiotic 1. The compounds obtained had lower cytotoxic properties than oligomycin A for human leukemia cells K-562 and colon cancer cells HCT-116 and lower activity against growth inhibition of model object *Streptomyces fradiae*. It cannot be excluded that the products of retroaldol degradation participate in the biological effects of antibiotic oligomycin A.

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INTRODUCTION

Oligomycin has been recognized as a potent inhibitor of the mitochondrial ATP synthase since 1958.¹ It has been proposed that oligomycins specifically inhibit the proton translocation process associated with the F₀F₁-ATPase in the mitochondria. Recently, it was shown that the subunit c of the F₀ portion of the F₀F₁-ATP synthase is the binding site of oligomycin.² The molecular mechanism by which the antibiotic interacts with mitochondrial F₀F₁-ATPase remains obscure. At present, the main efforts are directed towards the study of subunits of the F₀F₁-ATP synthase as possible oligomycin-binding site(s). Whereas the mechanism of oligomycin interaction with F₀F₁-ATPase is under serious investigations in the past years,^{2,3} relatively little is known about the structural basis for oligomycin A's F₀F₁-ATPase activity and its potential interaction with other targets. Recently, we have prepared a series of oligomycin derivatives modified at the macrolactone ring,^{4,5} at the side chain (at C33),⁶ and also that with the open macrolactone ring.⁷ Studies of structure–activity relationships for the model bacterial strain ATCC-19609, super-sensitive to oligomycin, and cancer cells K-562 and HCT-116 permitted to suggest the existence of the target(s) beyond F₀F₁-ATP synthase that is important for the antitumor potency of oligomycin A. A part of the oligomycin A structure C5–C13 represents a highly reactive moiety containing several β -hydroxy ketone moieties capable

of retroaldol degradation in relatively mild conditions. The aim of this work was to study the possibilities of retroaldol degradation of oligomycin A (1) in various conditions and preparation of novel oligomycin derivatives with the opened macrolactone ring. The identification and biological evaluation of new oligomycin A congeners provide an opportunity to develop a structure–activity relationship among this class of natural products.

RESULTS AND DISCUSSION

The first product (2) of retroaldol degradation of oligomycin A (1) with the intact lactone bond was obtained when 1 was stirred at room temperature with K₂CO₃ and *n*-Bu₄NHSO₄ in CHCl₃ in phase-transfer conditions.⁷ Compound 2 was generated through the initial retroaldol fragmentation of the 8,9 bond. It is worth noting that in this case the retroaldol degradation occurred under mild conditions at room temperature, and the lactone bond was not hydrolyzed (Figure 1).

Earlier in the elucidation of oligomycins structures,⁸ an aldehyde 3a formed from oligomycin A (1) via retroaldol fragmentation of the 12,13 bond followed by the hydrolysis of the lactone bond was isolated. Similar transformation and formation of aldehyde 3b was described for oligomycin B containing an oxo group in position 28. In the same study, aldehyde 3c and its methyl ester were isolated.⁸

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Figure 1 Oligomycin A (1) and the known products of retroaldol degradation of 1.

In this work, we studied the possibilities of oligomycin A degradation in various alkaline conditions. When NaOH was added to the solution of 1 at room temperature in ethanol, compound 4 was formed and was isolated in 12% yield (Scheme 1). The structure of compound 4 was supported by ¹H and ¹³C NMR (Table 1), and also by HR-MS. In THF–methanol solution in the presence of BA(OH)₂ · 8H₂O, oligomycin A gave another retroaldol product 5 in 36% yield (Scheme 2), and the structure 5 was formed through double retroaldol degradation when the C9–C12 fragment was detached. Aldehyde 5 in ethanol in the presence of HCl provided acetal 6.

The comparison of compounds 4 and 5 and the starting antibiotic 1 revealed that in all these compounds the similar skeleton moiety C20–C34 with methyl groups connected with this fragment. All atoms with the similar numbers within this fragment in compounds 1, 4 and 5 have very close values of chemical shifts of ¹³C and ¹H atoms, and also *J*_{H,H} values. It demonstrates the similarity of the geometry (conformation) of the C20–C34 fragment in compounds 4 and 5 and the starting antibiotic 1 (Table 1). Similar chemical shifts and *J*_{vic} for atoms H13, H14 and CH₃-40 represent the additional support for the presence of 14-C(CH₃)CHO moieties in compounds 4 and 5. Similarly for compounds 2 and 5, parameters of ¹³C and ¹H NMR for atoms C7, C8 and CH₃-37 are very close (fragment 7-CO–CH₂CH₃).

Molecular composition of compound 5 was also supported by the presence of HR-ESI-MS ion (*m/z*: 683.4473 [M + Na]⁺) corresponding to the intramolecular disruptions between C8–C9 and C12–C13 bonds in 1 and splitting off of the fragment C8–C12 and ESI-MS fragmentations. Tandem MS/MS spectra of this compound generated by ESI and collision-induced dissociation multiple reaction monitoring mode showed that low collision energies (50–70 eV) led to the

formation of unstable acyclic fragment ions corresponding to the loss of a fragment of acrylic acid (*m/z*: 487.3 [M + Na]⁺) and sequential loss of H₂O (*m/z*: 469.3 [M + Na]⁺) (Figure 2).

Biology

We studied the antibacterial activity of novel compounds against *Streptomyces fradiae* ATCC-19609 strain that was extremely sensitive to 1 (<0.001 μmol l^{−1} or 0.0005 nmol per disk).⁹ This test system has been validated by us as an adequate model for the analysis of sensitivity to 1.⁹ The derivatives studied with this test system are shown in Table 2. Compounds 2, 4 and 5 had lower antibacterial activity than the parent antibiotic 1.

The cytotoxic potencies of compounds 1, 2, 4 and 5 were determined as described by us.⁶ The values of IC₅₀ were defined as the concentration of a compound that reduced the number of viable cells by 50% (Table 3).

EXPERIMENTAL PROCEDURE

Chemistry

General procedures. Oligomycin A (1) (purity 95%) was produced at Autonomous Non-Commercial Research Center of Biotechnology of Antibiotics BIOAN (Moscow, Russian Federation), using *Streptomyces avermitilis* NIC B62. Fermentation was performed for 8 days at 28 °C in a liquid medium. Isolation and purification were performed by extraction with acetone–hexane mixture followed by crystallization. All other reagents and solvents were purchased from Aldrich (St Louis, MO, USA), Fluka (Buchs, Switzerland) and Merck (Darmstadt, Germany). The solutions were dried over sodium sulfate and evaporated under reduced pressure on a Buchi rotary evaporator at <35 °C. The progress reaction products, column eluates and all final samples

Scheme 1 Retroaldol degradation of oligomycin A (**1**) under the action of NaOH in ethanol at room temperature.

were analyzed by TLC on Merck G60F₂₅₄-precoated plates. Reaction products were purified by column chromatography on Merck silica gel 60 (0.04–0.063 mm) dards. The NMR spectra were recorded on Unity + 400 (Varian, Palo Alto, CA, USA; ¹H: 400.0 MHz; ¹³C: 100.6 MHz) and Avance 600 (Bruker, Karlsruhe, Germany; ¹H: 600.0 MHz; ¹³C: 150.9 MHz) spectrometers. The solvent used was CDCl₃, its signals in NMR ¹³C (δ 76.9) and the signal of residual protons in NMR ¹H (δ 7.24) were internal standards. The NMR elucidation was made using homo ¹H, ¹H (COSY) and hetero ¹H, ¹³C (HETCOR, heteronuclear single quantum coherence) 2D correlations as well as ¹H and ¹³C 1D spectra of **1** and its derivatives. HR-ESI-mass spectra were recorded on micrOTOF-Q II Instrument (Bruker Daltonics GmbH, Bremen, Germany). HPLC analyses were performed on a Shimadzu HPLC Instrument (Kyoto, Japan) of the LC-20AD (2-System WAT, Colon Kromasil 100-C18, size 5 μm, 4.6 × 250 mm², injection volume 10 μl, wavelength 230 nm). The concentration of samples was 0.02–0.05 mg ml⁻¹. The systems contained (A) H₂O or (B) acetonitrile. The proportion of acetonitrile varied from 80 to 95% for 15 min and then 95% for 20 min, flow rate 1 ml min⁻¹. Retention time for **1** was 10.46 min. UV spectra were obtained on a UV/visible double-beam spectrometer UNICO (Dayton, NJ, USA) in methanol. IR spectra were recorded on Thermo Nicolet iS10 (Thermo Scientific, Waltham, MA, USA).

Preparation of compound 4. Oligomycin A (**1**) (0.1 g, 0.13 mmol) was dissolved in the mixture EtOH–H₂O (5:1, 15 ml) and NaOH (0.005 g, 0.1 mmol) was added and stirred at room temperature for 1 h. The reaction mixture was analyzed by TLC in CH₂Cl₂–acetone (10:2), and the staining reagent for TLC was anisaldehyde–sulfuric acid in ethanol. The reaction mixture was extracted with EtOAc, washed with water to pH 7.0, dried over Na₂SO₄ and concentrated. The reaction product was purified by column chromatography (silica gel; Merck) in CH₂Cl₂–acetone (20:2) and the purification was repeated in toluene–EtOAc–EtOH (10:5:0.1) to give 0.012 g

(0.015 mmol), 12% of the product **4** as a colorless amorphous powder, retention time 12.93. MW was calculated for C₄₅H₇₄O₁₁ 790.5231, found in HR-ESI-mass spectrum (*m/z*) 813.5144 (M + Na)⁺. UV-spectrum (λ_{max} nm, MeOH), 230 nm. IR v max, cm⁻¹ (film) 3478, 2965, 2935, 2875, 1724, 1457, 1385, 1353, 1317, 1255, 1222, 1192, 1173, 1135, 1093, 1056, 986, 915, 887.

Preparation of compound 5. Oligomycin A **1** (0.1 g, 0.13 mmol) was dissolved in the mixture THF–MeOH (2:1, 15 ml) and BA(OH)₂·8H₂O (0.060 g, 0.19 mmol) was added and stirred at room temperature for 1 h. The reaction mixture was analyzed by TLC in CH₂Cl₂–acetone (10:2), and the staining reagent for TLC was anisaldehyde–sulfuric acid at ethanol. The reaction mixture was extracted with EtOAc, washed with water to pH 7.0, dried over Na₂SO₄ and concentrated. The reaction product was purified by column chromatography (silica gel, Merck) in CH₂Cl₂–acetone (20:2) and the purification was repeated in toluene–EtOAc–EtOH (10:5:0.1) to give yield 36% product **5** as a colorless oil 0.030 g (0.055 mmol), Rt 14.44. MW was calculated for C₃₉H₆₄O₈ 660.4601, found in HR-ESI-mass spectrum (*m/z*) 683.4448 (M + Na)⁺. UV-spectrum (λ_{max} nm, MeOH), 229 nm. IR v max, cm⁻¹ (film) 3478, 2965, 2935, 2875, 1713, 1632, 1458, 1383, 1282, 1224, 1189, 1136, 1096, 1057, 986.

Preparation of acetal 6. The product **5** (0.03 g, 0.045 mmol) was dissolved in EtOH (10 ml) and conc. HCl was added to, pH 2, the mixture and was stirred at room temperature for 24 h. The reaction mixture was analyzed by TLC in toluene–EtOAc–EtOH (10:5:0.1), and the staining reagent for TLC was anisaldehyde–sulfuric acid at ethanol. The reaction mixture was extracted with EtOAc, washed with water to pH 7.0, dried over Na₂SO₄ and concentrated. The reaction product was purified by column chromatography (silica gel; Merck) in toluene–EtOAc–EtOH (10:5:0.1) to give yield 80% product **6** as a colorless oil 0.026 g (0.035 mmol), Rt 15.01. MW was calculated for C₄₃H₇₄O₉ 734.5328, found in HR-ESI-mass spectrum (*m/z*) 757.5172 (M + Na)⁺.

Table 1 ^{13}C and ^1P NMR spectra of compounds 1, 2, 4 and 5 (δ_{C} , δ_{H} , p.p.m.; $J_{\text{H,H}}$, Hz)

C No.	1			2			4			5		
	δ_{C}	δ_{H}	$J_{\text{H,H}}$	δ_{C}	δ_{H}	$J_{\text{H,H}}$	δ_{C}	δ_{H}	$J_{\text{H,H}}$	δ_{C}	δ_{H}	$J_{\text{H,H}}$
1	165.02	O-CO		165.83	O-CO		170.90	O-CO		165.83	O-CO	
2	122.61	CH 5.86dd	15.6, 0.7	121.81	CH 5.86dd	15.6, 0.9	37.91	CH ₂ 2.67dd ^a , 2.42dd ^b		121.80	CH 5.86dd	15.7, 1.0
3	148.29	CH 6.62dd	15.6, 10.1	149.72	CH 6.73dd	15.6, 9.3	70.42	CH 3.66ddd	10.2, 10.1, 2.3	149.75	CH 6.74dd	15.7, 9.0
4	40.06	CH 2.36tq	10.0, 6.6	39.89	CH 2.41ddq	9.3, 9.0, 6.7	42.75	CH 1.35ddd	10.1, 10.0, 6.4	39.91	CH 2.42m	
5	72.88	CH 3.75dd	10.1, 1.3	73.26	CH 3.75ddd	9.0, 2.6, 2.6 ^c	73.95	CH 3.41dd	10.0, 10.1	73.28	CH 3.75dd	8.8, 2.5
6	46.38	CH 2.70dq	1.3, 7.4	47.44	CH 2.56dq	2.6, 7.2	42.24	CH 1.72dq	10.1, 6.7	47.45	CH 2.57dq	2.5, 7.1
7	220.16 ^d	CO		216.62	CO		104.09	O-C-O		216.58	CO	
8	41.88 ^d	CH 3.59dq	8.6, 6.9	34.66	CH ₂ 2.53dq, 2.41dq ^e		31.18	CH 2.04dq	3.3, 7.0	34.68	CH ₂ 2.54dq; 2.41dq ^f	
9	72.57	CH 3.94dd	8.6, 3.1	173.19	CH 8.00q	1.1	75.24	CH 3.47dd	3.3, 2.8			
10	45.63 ^d	CH 2.74dq	3.0, 7.1	114.44	C _q =		37.85	CH 2.34ddq	3.0, 2.8, 7.4			
11	219.93 ^d	CO		208.25	CO		72.74	CH 4.62d	3.0			
12	82.91	C _q -O		90.14	C _q -O		209.36	CO				
13	72.15	CH 3.89d	1.9	75.90	CH 3.83dd	8.3 ^g , 1.6	204.79	CH 9.63d	1.4	204.66	CH 9.63d	1.4
14	33.41	CH 1.88m		33.62	CH 1.84dq	1.8, 6.9	46.33	CH 2.41m		46.23	CH 2.41m	
15	38.33	CH ₂ 2.17bd; 1.98dt		38.36	CH ₂ 2.15m, 2.04m		33.66	CH ₂ 2.44m; 2.14m		33.56	CH ₂ 2.45m; 2.14m	
16	129.30	CH 5.42ddd	14.8, 10.5, 4.1	129.45	CH 5.48dt	14.6, 7.3	127.30	CH 5.46ddd	15.3, 7.1, 7.1	127.29	CH 5.47ddd	15.1, 7.3, 7.1
17	132.30	CH 6.00ddd	14.7, 10.4, 1.4	132.61	CH 6.00ddt	14.6, 10.3, 1.1	133.22	CH 6.02dd	15.3, 10.4	133.06	CH 6.03ddt	15.1, 10.4, 1.3
18	130.19	CH 5.90dd	14.9, 10.5	129.94	CH 5.92dd	14.8, 10.3	129.70	CH 5.91dd	15.0, 10.4	129.65	CH 5.91dd	15.1, 10.4
19	137.67	CH 5.21dd	14.8, 9.6	137.24	CH 5.31dd	14.8, 8.8	137.96	CH 5.34dd	15.0, 8.7	137.80	CH 5.34dd	15.1, 8.7
20	45.94	CH 1.85m		44.51	CH 1.83m		44.62	CH 1.86m		44.52	CH 1.86m	
21	31.38	CH ₂ 1.52m; 1.35m		31.30	CH ₂ ~1.40m		31.39	CH ₂ 1.44m; 1.39m		31.33	CH ₂ 1.43m; 1.39m	
22	30.90	CH ₂ 1.59ddd; ~1.02m		29.95	CH ₂ 1.53m, 1.22m		30.11	CH ₂ 1.52m; 1.20m		30.05	CH ₂ 1.52m; 1.21m	
23	68.95	CH 3.78ddd	9.8, 2.7, 2.4	69.40	CH 3.74ddd	8.0, 5.4, 2.0	69.31	CH 3.70ddd	8.7, 5.0, 2.0	69.41	CH 3.74ddd	
24	35.94	CH 2.11ddq	5.0, 2.2, 6.9	35.34	CH 2.05m		35.41	CH 1.99m		35.45	CH 2.05ddd	
25	76.10	CH 4.91dd	11.4, 5.0	76.17	CH 4.97dd	11.4, 4.9	76.88	CH 4.92dd	11.4, 4.7	76.16	CH 4.97dd	
26	37.61	CH 1.78dq	11.4, 6.6	37.81	CH 1.75dq	11.4, 6.7	37.91	CH 1.66dq	11.4, 6.7	37.82	CH 1.74dq	
27	99.11	O-C-O		99.05	O-C-O		99.13	O-C-O		99.07	O-C-O	
28	25.88	CH ₂ 1.90m; 1.23m		25.85	CH ₂ 1.88m, 1.22m		26.01	CH ₂ 1.88m; 1.22m		25.88	CH ₂ 1.88m; 1.22m	
29	26.41	CH ₂ 2.07m; 1.38m		26.43	CH ₂ 2.10m, 1.36m		26.57	CH ₂ 2.11m; 1.39m		26.45	CH ₂ 2.10m; 1.38m	
30	30.38	CH 1.54m		30.36	CH 1.56m		30.47	CH 1.53m		30.41	CH 1.54m	
31	67.14	CH 3.96dt	10.3, 2.5	67.21	CH 3.97dt	10.3, 2.5	67.26	CH 3.95ddd	10.1, 2.7, 2.5	67.18	CH 3.97ddd	10.4, 2.7, 2.5
32	42.46	CH ₂ 1.55m, 1.25m		42.44	CH ₂ 1.57m, 1.25m		42.43	CH ₂ 1.58m, 1.26m		42.43	CH ₂ 1.58m, 1.26m ^h	
33	64.57	CH 4.00ddq	9.2, 3.1, 6.2	64.67	CH 4.00ddq	9.2, 6.2, 3.0	64.55	CH 4.00ddq	9.0, 3.0, 6.1	64.62	CH 4.01ddq	9.2, 3.1, 6.2
34	24.66	CH ₃ 1.213d	6.2	24.56	CH ₃ 1.202d	6.2	24.79	CH ₃ 1.211d	6.1	24.60	CH ₃ 1.208d	6.2
35	17.84	CH ₃ 1.159d	6.6	16.43	CH ₃ 1.146d	6.7	13.56	CH ₃ 0.999d	6.4	16.43	CH ₃ 1.151d	6.6
36	8.21	CH ₃ 1.047d	7.3	9.36	CH ₃ 1.095d	7.2	11.35	CH ₃ 1.103d	6.7	9.39	CH ₃ 1.100d	7.1
37	13.99	CH ₃ 1.085d	6.9	7.48	CH ₃ 1.016t	7.3	11.89	CH ₃ 1.029d	7.0	7.50	CH ₃ 1.022d	7.2
38	9.16	CH ₃ 1.011d	7.0	5.15	CH ₃ 1.679d	1.1	11.23	CH ₃ 0.832d	7.4			
39	20.92	CH ₃ 1.110s		19.61	CH ₃ 1.320s		27.69	CH ₃ 2.184s				
40	14.39	CH ₃ 0.977d	6.6	13.22	CH ₃ 0.876d	7.0	13.15	CH ₃ 1.079	7.0	13.06	CH ₃ 1.084d	7.0
41	28.42	CH ₂ ~1.35m, ~1.25m		27.70	CH ₂ ~1.37m, ~1.20m		27.73	CH ₂ 1.41m, 1.24m		27.67	CH ₂ 1.39m, 1.23m	
42	11.98	CH ₃ 0.799t	7.4	11.62	CH ₃ 0.797t	7.4	11.70	CH ₃ 0.794t	7.5	11.60	CH ₃ 0.800t	7.3
43	5.97	CH ₃ 0.822d	6.9	5.53	CH ₃ 0.805d	7.0	5.68	CH ₃ 0.761d	7.0	5.55	CH ₃ 0.806d	7.1
44	11.67	CH ₃ 0.950d	6.6	11.63	CH ₃ 0.922d	6.6	11.79	CH ₃ 0.906d	6.6	11.64	CH ₃ 0.927d	6.7
45	11.13	CH ₃ 0.884d	6.9	11.13	CH ₃ 0.872d	6.8	11.20	CH ₃ 0.862d	7.1	11.16	CH ₃ 0.883d	7.0

Abbreviation: SOC, spin coupling constant J .^aSOC: $J_{\text{H2A,H2B}} = 16.7\text{ Hz}$, $J_{\text{H2A,H3}} = 2.3\text{ Hz}$.^bSOC: $J_{\text{H2A,H2B}} = 16.7\text{ Hz}$, $J_{\text{H2B,H3}} = 10.2\text{ Hz}$.^cOH: $\delta\ 3.07$, $^3J = 2.6\text{ Hz}$.^dReverse assignments of signals are possible.^eSOC: $J_{\text{H6A,H6B}} = 18.1\text{ Hz}$, $J_{\text{H6A,H37}} = J_{\text{H6B,H37}} = 7.3\text{ Hz}$.^fSOC: $J_{\text{H6A,H6B}} = 18.0\text{ Hz}$, $J_{\text{H6A,H37}} = J_{\text{H6B,H37}} = 7.2\text{ Hz}$.^gOH: $\delta\ 2.12$, $^3J = 8.3\text{ Hz}$.^hSOC: $J_{\text{H32A,H32B}} = 14.0\text{ Hz}$, $J_{\text{H32A,H33}} = 3.1\text{ Hz}$, $J_{\text{H32B,H33}} = 9.2\text{ Hz}$, $J_{\text{H32B,H31}} = 2.7\text{ Hz}$.

Scheme 2 Retroaldol degradation of oligomycin A (1) under the action of $\text{Ba}(\text{OH})_2$ in THF–MeOH at room temperature.

Figure 2 ESI-MS fragmentations of compound 5.

UV-spectrum (λ_{max} nm, MeOH), 229 nm. IR ν_{max} , cm^{-1} (film) 3478, 2968, 2931, 2875, 1711, 1651, 1457, 1377, 1272, 1223, 1188, 1097, 1056, 984.

$^1\text{H-NMR}$ (δ_{C} , p.p.m.): 24.58 (C34), 14.33, 11.63, 11.61, 11.15, 9.38, 7.49, 5.53 (eight CH_3 groups); 42.43, 35.14, 34.67, 33.55, 31.38, 30.04, 27.72, 26.44, 25.86 (nine CH_2 groups); 47.45, 44.51, 39.91, 37.81, 36.65, 35.43, 30.39 (seven CH-CH_3 ($-\text{CH}_2-$) groups); 0.24 ($-\text{O-CH-O-}$, C13), 76.16, 73.27, 69.42, 67.16, 64.59 (six CH-O- ($-\text{O-CH-O-}$) groups); 149.73, 136.46, 131.95, 130.19, 129.86, 121.80 (six CH= groups); 216.55 (CO, C7), 165.81 (COO, C1),

Table 2 Growth inhibition of *Streptomyces fradiae* ATCC-19609 strain by 1 and its degraded derivatives 2, 4 and 5^a

Compound	Streptomyces fradiae	
	Concentration (nmol per disk)	Halo diameter (mm)
Oligomycin A (1)	0.001	9.5
2	0.1	9.5
4	1.0	7.5
5	1.0	8.0

^aMean $\pm$ s.d. of three independent measurements.

Table 3 Cytotoxicity (IC_{50} (μM)) of 1 and its derivatives for tumor cell lines^a

Compound	Cell line		
	K-562	HCT-116	MCF7
1	0.16 $\pm$ 0.02 ^a	1.00 $\pm$ 0.20	0.10 $\pm$ 0.01
2 ⁷	0.2 $\pm$ 0.01	3.1 $\pm$ 1.1	ND
4	27.0 $\pm$ 2.8	19.0 $\pm$ 2.2	2.8 $\pm$ 0.28
5	12.5 $\pm$ 1.5	17.0 $\pm$ 2.3	1.40 $\pm$ 0.17
6	35.0 $\pm$ 3.8	46.0 $\pm$ 5.9	4.00 $\pm$ 0.56

Abbreviation: ND, not determined.

^aMean $\pm$ s.d. of 3 independent measurements.

99.05 ($-\text{O-C-O-}$, C27) (three C_q groups); 2 CH_2 : 62.08, 61.86; 2 CH_3 : 15.21, 15.23 (C13-(OCH_2CH_3)₂). Atoms in positions C9–C12, C38 and C39, which are present in oligomycin, are absent in 6.

Biology

Studies of cytotoxicity of compounds and the antibacterial activity. The antibacterial activity of **1** and its degraded derivatives **4**, **5** was determined as the diameter of growth inhibition zone of *S. fradiae* cells around paper discs filled with tested compounds as described previously.⁶

CONCLUSION

Two novel products of retroaldol degradation of oligomycin A were obtained under the action of NaOH in the mixture EtOH–H₂O or BA(OH)₂·8H₂O in the mixture THF–MeOH. These non-cyclic derivatives of **1** with no hydrolyzed lactone bond were characterized by NMR and MS methods. It is worth mentioning that according to NMR data (δ , p.p.m. and J_{vic} values), the conformations of the untouched fragments C20–C34 in compounds **4**, **5** and the starting antibiotic **1** (and also previously described retroaldol product **2**) are similar. However, compounds **2**, **4** and **5** had low activity against *S. fradiae* ATCC-19609 strain, which was extremely sensitive to **1** (<0.001 nmol ml^{−1} or 0.0005 nmol per disk). It suggests that the labile fragment C7–C13 is very important for the interaction with F₀F₁-ATPase. The compounds were not active in spite of the presence of highly conserved the ‘down’ macrolactone part and the side chain C32–C33 with a hydroxyl group hydrogen bonded to the side chain of Glu59 of ATPase.² At the same time, we cannot exclude the possibility of the formation of retroaldol degradation oligomycin products in biological conditions and exclude their role in the biological effects of the antibiotic. The cytotoxic properties of novel compounds are also decreased.

Recently, studies of the high-resolution crystal structure of oligomycin bound to the subunit c of the yeast mitochondrial F₀F₁-ATP synthase revealed that oligomycin binds to the surface of the c ring.² The carboxyl side chain of Glu59, which is essential for proton translocation, forms an H bond with oligomycin via a bridging water molecule. The remaining contacts between oligomycin and subunit c are primarily hydrophobic, and the hydrophobic effect is likely the largest contributor to the binding energy. The hydrophobic face of the oligomycin molecule covers the hydrophobic face of subunit c, and the hydrophilic face of oligomycin is predominantly exposed to the

bulk solvent.² Our compounds **2**, **4** and **5** with the degraded C7–C12 fragment still contain the non-distorted hydrophobic part and the propanol moiety C32–C33(OH)–C34 essential for binding to Glu59. However, structural changes that result from retroaldol degradation in compounds **2**, **4** and **5**, in particular, degradation of the C7–C13 macrolactone hydrophilic moiety, led to the decrease of anti-F₀F₁-ATP synthase activity and significantly altered the interaction with the subunit c of F₀F₁-ATP synthase. These SAR considerations demonstrate the essential role of the hydrophilic moiety of **1** for binding of this drug to its major target.

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