chemistry proceedings

MDPI

Proceeding Paper

Synthesis of Aromatic Macrodiolides and Study of Their Antitumor Activity In Vitro †

Ilgam Gaisin [1,*], Ilgiz Islamov [1], Lilya U. Dzhemileva [2] and Usein Dzhemilev [2]

[1] Institute of Petrochemistry and Catalysis, Ufa Federal Research Center, Russian Academy of Sciences, 141 Prospekt Oktyabrya, Ufa 450075, Russia; iislamovi@gmail.com
[2] N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospekt, 47, Moscow 119991, Russia; dzhemilev@mail.ru (L.U.D.); dzhemilev@anrb.ru (U.D.)
* Correspondence: ilgamgaisin.ipcras1@gmail or ilgamgaisin.ipcras1@gmail.com
† Presented at the 27th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-27), 15–30 November 2023; Available online: https://ecsoc-27.sciforum.net/.

Abstract: Based on (5*Z*,9*Z*)-tetradeca-5,9-diene-1,14-dioic acid previously undescribed polyether aromatic macrodiolides were synthesized in good yields (53–67%). The cytotoxicity of the resulting macrocyclic compounds in vitro against tumor Jurkat, K562, conditionally normal Hek293 cell lines and normal fibroblasts was assessment carried out. The ability of the most active macrodiolide to induce apoptosis towards Jurkat cells and influence the cell cycle was studied.

Keywords: 1,5-dienoic compounds; homo-cyclomagnesiation; Grignard reagents; macrodiolides; crown ether

Citation: Gaisin, I.; Islamov, I.; Dzhemileva, L.U.; Dzhemilev, U. Synthesis of Aromatic Macrodiolides and Study of Their Antitumor Activity In Vitro. *Chem. Proc.* **2023**, *14*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2023

1. Introduction

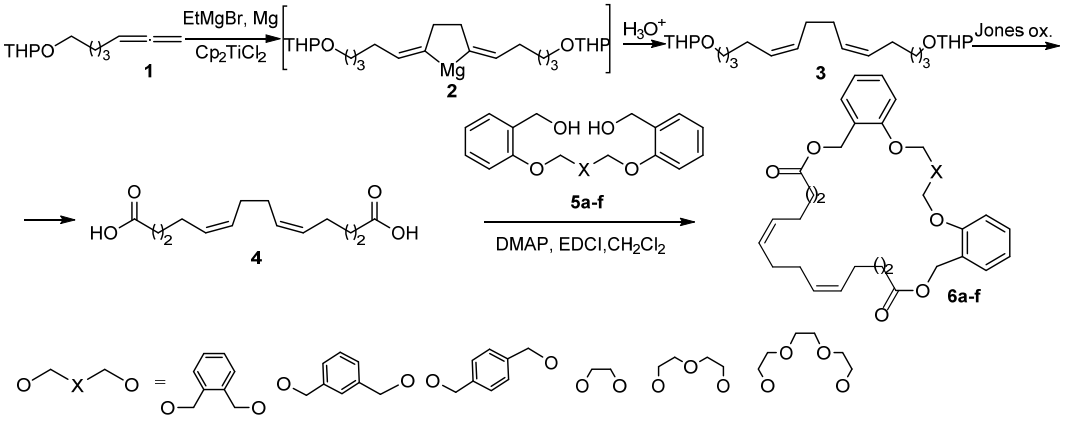
Unsaturated fatty acids, due to their wide variety and outstanding biological activity, are considered by researchers as the basis for the creation of modern drugs. Recent studies conducted in various scientific centers have shown that fatty acids with bis-methylene separated cis, cis double bonds in the structure exhibit antibacterial, antitumor, fungicidal and antimalarial activities [1–3].

Previously, using the original cyclomagnesiation reaction, we developed effective methods for obtaining natural 5*Z*,9*Z*-dienoic fatty acids and their semi-synthetic analogs that exhibit antitumor properties. In development of these studies, new biologically active hybrid molecules and macrocyclic compounds with a 1*Z*,5*Z*-diene fragment in the structure were synthesized [4–7].

This work presents the synthesis of previously undescribed multifunctional macrodiolides and provides preliminary results of an in vitro analysis of the antitumor activity of the resulting macrocyclic compounds.

2. Results and Discussion

To accomplish the tasks set for the synthesis of new polyfunctional macrodiolides, we have preliminarily carried out the synthesis of (5*Z*,9*Z*)-tetradeca-5,9-diene-1,14-dioic acid **4**. Further by the conditions at a molar ratio of reagents [diacid (4):diol (5):DMAP:EDCI = 1:1:0.5:2] with strong dilution in dichloromethane ([5 mM]), new polyether unsaturated macrocyclic compounds **6a–f** were synthesized (Scheme 1).



Scheme 1. Synthesis of aromatic polyether macrodiolides.

With the aim of studying polyether macrocycles for antitumor activity and assessing their potential clinical applicability, we tested the products for in vitro cytotoxicity, ability to influence the cell cycle and induce apoptosis (Table 1).

Table 1. Cytotoxic activities in vitro of synthesized cyclophanes 6a–f measured on cell cultures (Jurkat, K562, Hek293, and normal fibroblasts) (μM).

Comp.	Jurkat (CC_{50}, μM) *	K562 (CC_{50}, μM) *	Hek293 (CC_{50}, μM) *	Fibrobl. (CC_{50}, μM) *	Selectivity Index	$CC50_{max}/CC50_{min}$
6a	0.21 ± 0.02	0.16 ± 0.03	1.91 ± 0.21	2.98 ± 0.31	0.21–2.98	14.19
6b	0.17 ± 0.02	0.22 ± 0.02	1.86 ± 0.19	2.69 ± 0.26	0.17–2.69	15.82
6c	0.67 ± 0.07	0.41 ± 0.04	2.84 ± 0.28	3.72 ± 0.36	0.41–3.72	9.07
6d	2.12 ± 0.22	2.49 ± 0.24	9.07 ± 0.91	10.11 ± 1.01	2.02–10.11	5.00
6e	2.49 ± 0.24	3.02 ± 0.31	9.51 ± 0.93	11.59 ± 1.19	2.44–11.59	4.75
6f	2.81 ± 0.29	3.18 ± 0.30	9.28 ± 0.93	11.24 ± 1.26	2.74–11.24	4.10
Staurosporin	1.72 ± 0.15	4.35 ± 0.85	8.16 ± 0.88	18.08 ± 2.12	1.72–18.08	10.51

It was shown that macrodiolides 6a,b exhibit the most pronounced cytotoxicity, while the introduction of one, two or three ethylene glycol fragments into macrodiolide molecules instead of the central benzene fragment in the aromatic diol leads to a significant decrease in the cytotoxicity of macrodiolides (6d–f) (Table 1).

To conduct further studies on *Jurkat* cell lines of apoptosis-inducing activity and the ability to influence the cell cycle, the most active macrocyclic compounds 6a–c were selected. As a result, it was established that the synthesized compounds are inducers of apoptosis and help slow down the process of cell division due to a block at the G1/S checkpoint.

3. Materials and Methods

3.1. Chemistry

One-(^{1}H, ^{13}C) and two-dimensional heteronuclear (HSQC, HMBC) NMR spectra were recorded in $CDCl_3$ on Bruker Avance-400 ((400.13 MHz (^{1}H), 100.62 MHz (^{13}C)) instruments. The mass spectra were obtained on an UltraFlex III TOF/TOF (Bruker Daltonik GmbH, Bremen, Germany) operating in linear (TOF) and reflection (TOF/TOF) positive and negative ion modes. S_8 and DCTB (trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenyliden]malononitrile) were used as the matrix. Macrocyclic compounds were synthesized similarly according to the procedure described in the literature [7].

(11*Z*,15*Z*)-8,9,10,13,14,17,18,19,28,33-decahydro-5*H*,22*H*-tribenzo[*c*,*g*,*k*][1,5,10,14]tetraoxacyclooctacosine-7,20-dione (7a). White waxy solid; yield 54%. R_f = 0.55, hexane/EtOAc 5:1. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.59–7.50 (m, 2H), 7.43–7.25 (m, 6H), 7.02–6.91 (m, 4H),

5.38–5.15 (m, 12H), 2.37–1.90 (m, 8H), 1.72–1.65 (m, 4H), 1.65–1.55 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.7, 156.9, 134.9, 131.0, 130.2, 129.9, 128.9, 128.4, 128.3, 124.6, 120.9, 111.9, 68.1, 61.9, 33.4, 27.3, 26.3, 24.6. ESI-MS: calcd. for $C_{36}H_{40}O_6$ + Na^+ $[M + Na]^+$ 591.2717; found 591.2731.

(14*Z*,18*Z*)-2,6,9,24-tetraoxa-1,7(1,2),4(1,3)-tribenzenacyclopentacosaphane-14,18-diene-10,23-dione (7b). White waxy solid; yield 58%. R_f = 0.54, hexane/EtOAc 5:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53–7.24 (m, 8H), 7.03–6.88 (m, 4H), 5.48–4.94 (m, 12H), 2.33 (t, *J* = 7.4 Hz, 4H), 2.15–1.86 (m, 8H), 1.74–1.59 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.5, 156.6, 137.4, 130.2, 129.9, 129.6, 128.9, 128.8, 126.5, 125.6, 124.8, 120.8, 111.9, 69.8, 61.7, 33.7, 27.2, 26.6, 24.9. ESI-MS: calcd. for $C_{36}H_{40}O_6$ + NH_4^+ $[M + NH_4]^+$ 586.3163; found 586.3187.

(14*Z*,18*Z*)-2,6,9,24-tetraoxa-1,7(1,2),4(1,4)-tribenzenacyclopentacosaphane-14,18-diene-10,23-dione (7c). White waxy solid; yield 67%. R_f = 0.54, hexane/EtOAc 5:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (s, 4H), 7.42–7.21 (m, 4H), 7.00 (t, *J* = 7.4 Hz, 4H), 5.43–5.08 (m, 12H), 2.42–2.27 (m, 4H), 2.15–1.92 (m, 8H), 1.76–1.63 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 173.6, 157.1, 136.6, 130.9, 130.2, 129.9, 129.0, 127.3, 127.3, 124.7, 120.8, 111.9, 69.7, 62.2, 33.6, 27.3, 26.4, 24.8.ESI-MS: calcd. for $C_{36}H_{40}O_6$ + Na^+ $[M + Na]^+$ 591.2717; found 591.2694

(11*Z*,15*Z*)-8,9,10,13,14,17,18,19,28,29-decahydro-5*H*,22*H*-dibenzo[*e*,*y*][1,4,8,23]tetraoxacyclohexacosine-7,20-dione (7d). White waxy solid; yield 53%. R_f = 0.57, hexane/EtOAc 3:1. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.34 (dd, *J* = 13.6, 7.4 Hz, 4H), 7.03–6.93 (m, 4H), 5.49–5.27 (m, 4H), 5.20 (d, *J* = 9.5 Hz, 4H), 4.38 (s, 4H), 2.36–2.26 (m, 4H), 2.13–1.94 (m, 8H), 1.72–1.62 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.6, 156.7, 130.2, 130.1, 129.6, 129.1, 125.0, 121.0, 111.8, 66.9, 61.5, 33.4, 27.5, 26.4, 24.8. ESI-MS: calcd. for $C_{30}H_{36}O_6$ + Na^+ $[M + Na]^+$ 515.2404; found 515.2391.

(11*Z*,15*Z*)-8,9,10,13,14,17,18,19,28,29,31,32-dodecahydro-5*H*,22*H*-dibenzo[b_1,*h*][1,4,7,11,26]pentaoxacyclononacosine-7,20-dione (7e). White waxy solid; yield 60%. R_f = 0.49, hexane/EtOAc 3:1. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.42–7.20 (m, 4H), 7.03–6.85 (m, 4H), 5.47–5.27 (m, 4H), 5.19 (s, 4H), 4.22–4.10 (m, 4H), 3.96 (t, *J* = 4.6 Hz, 4H), 2.33 (t, *J* = 7.2 Hz, 4H), 2.18–1.87 (m, 8H), 1.77–1.59 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 173.5, 157.1, 130.5, 130.3, 129.8, 129.0, 124.8, 120.8, 111.9, 70.1, 68.3, 61.8, 33.6, 27.4, 26.4, 24.8. ESI-MS: calcd. for $C_{32}H_{41}O_6$ + H^+ $[M + H]^+$ 537.2847; found 537.2858

(11*Z*,15*Z*)-8,9,10,13,14,17,18,19,28,29,31,32,34,35-tetradecahydro-5*H*,22*H*-dibenzo[e_1,*k*][1,4,7,10,14,29]hexaoxacyclodotriacontine-7,20-dione (7f). White waxy solid; yield 67%. R_f = 0.38, hexane/EtOAc 3:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31 (dd, *J* = 16.2, 6.8 Hz, 4H), 7.01–6.87 (m, 4H), 5.45–5.29 (m, 4H), 5.19 (d, *J* = 6.1 Hz, 4H), 4.17 (t, *J* = 4.6 Hz, 4H), 3.89 (t, *J* = 4.7 Hz, 4H), 3.77 (s, 4H), 2.40–2.28 (m, 4H), 2.15–1.96 (m, 8H), 1.77–1.63 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$)): δ = 173.5, 157.0, 130.4, 130.2, 129.7, 129.0, 124.6, 120.7, 111.7, 71.1, 69.8, 68.1, 61.8, 33.6, 27.3, 26.5, 24.9. ESI-MS: calcd. for $C_{34}H_{44}O_8$ + Na^+ $[M + Na]^+$ 603.2928; found 603.2943.

3.2. Cell Culturing

Cells (Jurkat, K562, U937, Hek293, Fibroblasts) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences, Novosibirsk, Russia) and cultured according to standard protocols and sterile technique.

3.3. Cytotoxicity Assay

Viability (live/dead) assessment was performed by staining cells with 7-AAD (7-Aminoactinomycin D) (Biolegend). After treatment cells were harvested, washed 1–2 times with phosphate-buffered saline (PBS) and centrifuged at 400g for 5 min. Cell pellets were resuspended in 200 mL of flow cytometry staining buffer (PBS without Ca2+ and Mg2+, 2.5% FBS) and stained with 5 μL of 7-AAD staining solution for 15 min at room temperature in the dark. Samples were acquired on NovoCyteTM 2000 FlowCytometry System

(ACEA) equipped with 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in FL4 channel.

Studies of antitumor activity (induction of apoptosis tests, cell cycle analysis) were carried out following the known procedure [6].

4. Conclusions

As a result of the research, the synthesis of polyether aromatic macrodiolides was carried out in good yields for the first time. Biological studies have shown that the synthesized macrocycles have cytotoxicity against tumor cell lines, are capable of slowing down the cell cycle and can act as inducers of apoptosis.

Author Contributions: Conceptualization, U.D., L.U.D. and I.I.; methodology, validation, and execution of chemistry experiments, I.G. and I.I.; manuscript preparation, L.U.D., U.D. and I.I. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available on request.

Acknowledgments: The work was done within approved plans for research projects at the IPC RAS State Registration No. FMRS-2022-0075. The structural studies of the synthesized compounds were performed with the use of Collective Usage Centre "Agidel" at the Institute of Petrochemistry and Catalysis of RAS.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Makarieva, T.N.; Santalova, E.A.; Gorshkova, I.A.; Dmitrenok, A.S.; Guzii, A.G.; Gorbach, V.I.; Svetashev, V.I.; Stonik, V.A. A new cytotoxic fatty acid (5*Z*,9*Z*)-22-methyl-5,9-tetracosadienoic acid and the sterols from the far Eastern sponge *Geodinella robusta*. *Lipids* **2002**, *37*, 75–80.
2. Mishra, P.M.; Sree, A.; Baliarsingh, S. Antibacterial study and fatty acid analysis of lipids of the sponge *Myrmekioderma granulata*. *Chem. Nat. Compd.* **2009**, *45*, 621–624.
3. Carballeira, N.M. New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents. *Prog. Lipid Res.* **2008**, *47*, 50–61.
4. D'yakonov, V.A.; Dzhemileva, L.U.; Makarov, A.A.; Makarova, E.K.; Khusnutdinova, E.K.; Dzhemilev, U.M. The facile synthesis of the 5Z,9Z-dienoic acids and their topoisomerase I inhibitory activity. *Chem.Commun.* **2013**, *49*, 8401–8403.
5. D'yakonov, V.A.; Tuktarova, R.A.; Dzhemileva, L.U.; Ishmukhametova, S.R.; Dzhemilev, U.M. Synthesis and Anticancer Activity of Hybrid Molecules Based on Lithocholic and (5*Z*,9*Z*)-Tetradeca-5,9-dienedioic Acids Linked via Mono(di,tri,tetra)ethylene Glycol and α,ω-Diaminoalkane Units. *Pharmaceuticals* **2021**, *14*, 84.
6. Dzhemileva, L.U.; D'yakonov, V.A.; Islamov, I.I.; Yunusbaeva, M.M.; Dzhemilev, U.M. New 1Z,5Z-diene macrodiolides: Catalytic synthesis, anticancer activity, induction of mitochondrial apoptosis, and effect on the cell cycle. *Bioorg. Chem.* **2020**, *99*, 103832.
7. D'yakonov, V.A.; Islamov, I.I.; Dzhemileva, L.U.; Makarova, E.K.; Dzhemilev, U.M. Direct synthesis of polyaromatic cyclophanes containing bis-methylene-interrupted *Z*-double bonds and study of their antitumor activity in vitro. *Int. J. Mol. Sci.* **2021**, *22*, 8787.